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ANA CAROLINA ENRÍQUEZ ESPINOSA

O efeito da heterogeneidade ambiental sobre as mudanças na comunidade de Ephemeroptera em riachos da Amazônia oriental

Belém
2020

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Dissertação apresentada ao Programa de Pós-Graduação em Ecologia, do convênio da Universidade Federal do Pará e Embrapa Amazônia Oriental, como requisito parcial para obtenção do título de Mestre em Ecologia.
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Ao meu melhor exemplo e pilar da
minha vida: Minha mãe!
Dedico.

Dedico esta disertación a mi mejor
ejemplo y quien es el pilar de mi vida:
¡mi mamá!

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“The lifetime of a human being is measured by decades, the lifetime of the Sun is a hundred million times longer. Compared to a star, we are like *mayflies*, fleeting ephemeral creatures who live out their lives in the course of a single day”

— Carl Sagan

O efeito da heterogeneidade ambiental sobre as mudanças na comunidade de Ephemeroptera em riachos da Amazônia oriental

RESUMO

A biodiversidade pode ser definida como a variabilidade entre organismos vivos dos complexos ecológicos dos quais são componentes. O estudo da diversidade beta (variação na composição de espécies entre as comunidades) e diversidade funcional (variação das características funcionais das espécies entre as comunidades e funções ecossistêmicas) das comunidades nos ecossistemas aquáticos pode ajudar a entender como essas comunidades funcionam. Os Ephemeroptera são insetos aquáticos considerados bioindicadores devido a sua sensibilidade às alterações ambientais. Variações na composição das comunidades podem ser causadas por diferentes tipos de mudanças ambientais, e uma delas é a mineração. O objetivo do estudo foi avaliar se existem diferenças em vários aspectos da biodiversidade (diversidade – riqueza e abundância de gêneros-, diversidade beta, diversidade funcional e diversidade beta funcional) da ordem Ephemeroptera entre riachos preservados e alterados por mineração. Portanto, a dissertação foi dividida em dois capítulos: 1) Mining effects and lower turnover of Ephemeroptera (Insecta) in Brazilian streams of Eastern Amazon; e, 2) Redução da heterogeneidade ambiental e aninhamento funcional de Ephemeroptera (Insecta) em riachos da Amazônia Oriental. Este estudo foi realizado na Flona de Carajás, e as amostragens foram realizadas em três campanhas de coleta durante a estação seca (2015 a 2017). Foram amostrados 24 riachos divididos em dois tratamentos: “preservados” e “alterados”, e registramos no total 2.259 indivíduos de Ephemeroptera (32 gêneros). Os resultados do capítulo 1 demonstraram que as atividades de mineração possivelmente levaram ao aumento de manganês e ferro nos riachos alterados, e que as variáveis ambientais influenciaram as comunidades de Ephemeroptera. Os resultados de diversidade beta demonstraram maior turnover para riachos preservados. Isso indica que a substituição de espécies em riachos preservados tem uma limitação espacial, provavelmente ligada à sensibilidade dos organismos às variações ambientais dos riachos alterados. No capítulo 2 observamos um maior aninhamento funcional de Ephemeroptera em riachos alterados e maior turnover funcional em riachos preservados. O maior aninhamento pode levar a homogeneização funcional e perda de espécies. Também observamos que o aumento de ferro e turbidez proporcionou aumento de gêneros com a característica funcional brânquia opercular, e que o aumento de manganês total proporcionou diminuição de gêneros filtradores. Dessa forma, indicamos que as diferenças ambientais entre os riachos alterados e preservados levaram a variação na composição das características funcionais dos organismos, indicando potencial efeito negativo em aspectos funcionais e taxonômicos de Ephemeroptera em áreas de mineração.

Palavras-chave: *Alteração antropogênica, características funcionais, diversidade beta, diversidade funcional, insetos aquáticos*.

The effect of environmental heterogeneity over the changes in the Ephemeroptera community in the eastern Amazon streams

ABSTRACT

Biodiversity can be defined as variability between living organisms from the ecological complexes of which they are components. The study of beta diversity (variation in species composition between communities) and functional diversity (variation of functional characteristics from the species between communities and ecosystem functions) of communities in the aquatic ecosystems can help to understand how these communities work. Ephemeroptera are aquatic insects considered bioindicators due to their sensitivity to environmental changes. Variations in the composition of communities can be caused by different types of environmental changes, one of which is mining. The mainobjective of the study was to evaluate the differences in various aspects of biodiversity (diversity – richness and abundance taxa-, beta diversity, functional diversity and beta functional diversity) from the order Ephemeroptera between preserved and altered streams by mining. For this, the dissertation was divided into two chapters: 1) Mining effects and lower turnover of Ephemeroptera (Insecta) in Brazilian streams of Eastern Amazon; and, 2) Reduction of environmental heterogeneity and functional turnover of Ephemeroptera (Insecta) in streams in the Eastern Amazon. This study was carried out at "Flona de Carajás", and sampling was carried out in three collection campaigns during the dry season (2015 - 2017). Twenty-four streams were sampled, divided into two treatments: "preserved" and "altered", and a total of 2,259 Ephemeroptera individuals (32 táxons) were recorded. The results of Chapter 1 shows that mining activities possibly led to an increase in manganese and iron in altered streams, and the environmental variables can influence Ephemeroptera comunities. The results of beta diversity demonstrated a greater turnover for preserved streams. This indicates that the replacement of species in preserved streams has a spatial limitation, probably linked to the sensitivity of the organisms to the environmental variations of the altered streams. In chapter 2 we observed a greater functional nesting of Ephemeroptera in altered streams and a greater functional turnover in preserved streams. Greater nesting can lead to functional homogenization and loss. We also observed that the increment in iron and turbidity provided an increase in genera with the functional characteristic opercular gills, and, the augment in total manganese provided a decrease of the filtering taxa. Thus, we indicate that the environmental differences between the altered and preserved streams led to a variation in the composition of the functional characteristics of the organisms, indicating a potential negative effect over functional and taxonomic aspects of the Ephemeroptera order in mining areas.

Keywords: *Anthropogenic alteration, aquatic insects, beta diversity, functional diversity, traits.*

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1. INTRODUÇÃO GERAL

A biodiversidade pode ser definida, segundo Whittaker (1960), como: 1) a riqueza de espécies de um local (unidade espacial definida) ou comunidade particular, ou de um determinado estrato ou grupo de organismos de um site (diversidade alfa, α); 2) a magnitude da mudança na composição das comunidades (biológicas ou substituição de espécies) ou o grau de diferenciação entre comunidades, em relação a um gradiente ambiental complexo ou padrões ambientais (diversidade beta, β) (Whitakker, 1960, Magurran, 2004);e 3) a diversidade de espécies de um número de amostras ou de comunidades para algum grupo de ambientes, que são combinados de tal forma que o valor da diversidade (gama-diversidade, γ) é o resultado tanto do α diversidade como da β diversidade dessas amostras (Calderón-Patrón et al., 2012).

A diversidade beta é um conceito chave na ecologia de comunidades para entender o funcionamento dos ecossistemas (Schemera et al., 2017) pois têm grande importância nos padrões ecológicos para entender a ecologia e biogeografia, além de quantificar e valorizar a diversidade biológica (Legendre et al., 2005). É uma medida de dissimilaridade biológica entre ambientes. Tais dissimilaridades podem ser causadas principalmente por limitações de dispersão, geralmente autocorrelacionadas no espaço, e por diferenças no ambiente físico (Costa e Melo, 2008).

É possível avaliar a biodiversidade através da diversidade funcional. Ela é definida como o valor, o intervalo, a distribuição ou a abundância relativa das características funcionais (atributos) dos organismos que constituem um ecossistema (Díaz et al., 2007). Podem ser quantificados por diferentes componentes e métricas relacionadas com a função que as espécies desempenham no ecossistema (Tilman, 2001, Cadotte et al., 2011, Albert et al., 2012). As características funcionais das espécies são mensuráveis e freqüentemente usadas para definir alguma característica biológica dos organismos, sua função dentro dos ecossistemas, ou sua relação direta com o meio ambiente (Poff et al., 2006, McGill et al., 2006, Schemera et al., 2017). E isso permite ter melhor compreensão da estrutura e organização das comunidades ao longo de gradientes de alterações ambientais de origem antrópica (Violle et al., 2007).

A chave para entender a relação entre diversidade de espécies e diversidade funcional, e como os dois componentes afetam o ecossistema, é determinar como os fatores bióticos e abióticos influenciam nessas medidas de diversidade (Hempson et al., 2015). Portanto, a influência das variáveis bióticas e abióticas poderia afetar a relação entre a diversidade de espécies e a diversidade funcional (Cadotte et al., 2011) da ordem Ephemeroptera.

Em geral, a biota aquática reflete a integridade ecológica desses ecossistemas e são excelentes instrumentos de pesquisa de interesse conservacionista (Shimano et al., 2015). Dentre essa fauna, os macroinvertebrados aquáticos são componentes importantes na ecologia dos ecossistemas aquáticos, e são utilizados na avaliação de qualidade ambiental (Rosemberg e Resh, 1993), por serem capazes de responder de diferentes formas (diminuição da diversidade taxonômica e funcional) aos impactos antrópicos frente às alterações ambientais (Salles et al., 2004, Domínguez et al., 2006, Shimano et al., 2011, Helson e Williams, 2013).

A ordem Ephemeroptera é um dos grupos de insetos aquáticos mais diversos em ecossistemas aquaticos (Domínguez et al., 2006), composta atualmente por cerca de 4000 espécies, constitui o grupo mais antigo dentre os insetos alados (Salles et al., 2004, Domínguez, e Fernández, 2009). No Brasil, atualmente esta ordem está composta por 10 famílias, 81 gêneros e 409 espécies (Salles e Boldrini, 2019)

As ninfas de Ephemeroptera compõem um importante elo na cadeia alimentar (Salles et al. 2004) como consumidores primários de algas e perifíton e como alimento de consumidores secundários, como peixes e outros insetos (Brittain, 1982, Shimano et al., 2011). Os integrantes desta ordem são obrigatoriamente anfibióticos, com imaturos aquáticos e adultos terrestres (Salles et al., 2004). Enquanto as ninfas de Ephemeroptera exibem uma variedade de estratégias alimentares (podem ser filtradoras, raspadoras, fragmentadoras, coletoras ou até mesmo predadoras) e vivem de algumas semanas a poucos anos, os adultos não se alimentam, possuem as peças bucais atrofiadas e têm um curto período de vida, que em alguns casos não chega a mais de duas horas (Salles et al., 2004).

Assim, os insetos aquáticos são considerados organismos modelos para estudos com diversidade β, pois respondem as mudanças das condições ambientais em diferentes escalas, desde microhabitats até bacias hidrográficas (Baptista et al., 2014). Estes insetos também podem ser descritos através de suas características (atributos) funcionais relacionadas a ciclos de vida, fisiologia (respiração), comportamento alimentar, dispersão e capacidade de reprodução (Feio e Dolédec, 2012).

Às duas principais causas que afetam a diversidade beta são a diferença nas condições ambientais e a distância geográfica, os quais são fatores importantes na composição das assembléias de macroinvertebrados (Costa e Melo (2008)). Por outro lado, os padrões de diversidade funcional e diversidade taxonômica das comunidades de macroinvertebrados aquáticos respondem a diferentes alterações ambientais (Schemera et al., 2017), o que pode ser explicado devido a que as espécies são sensíveis à filtragem ambiental através de suas características de resposta e, portanto, a filtragem ambiental tem um efeito indireto nas características funcionais resposta, o que altera a diversidade funcional (Schemera et al., 2017). Portanto, os impactos decorrentes de atividades de mineração em igarapés, podem afetar as características ambientais (quantitativas e qualitativas), e influenciar nas comunidades de macroinvertebrados bentônicos, tanto taxonômica como funcionalmente (Bozelli et al., 2000, Mesa et al., 2013).

Sabe-se que as alterações ambientais (impactos) afetam a diversidade dos insetos aquáticos, levando à simplificação ou homogeneização dos hábitats (Castro et al., 2018). Na Amazônia brasileira os impactos decorrentes de natureza antrópica têm sido cada vez mais recorrentes, principalmente em ecossistemas aquáticos (igarapés) (Monteiro, 2005). Porém, esses sistemas estão sendo submetidos aos diversos impactos antrópicos (alteração hidrológica, contaminação, enriquecimento com nutrientes – erosão, sedimentação, desmatamento da vegetação ripária, assoreamento, alteração biológica), os quais são considerados como a principal ameaça para a deterioração da sua integridade ecológica (Callisto et al., 2001). As atividades de mineração ocasionam a queda de qualidade da água e perda da biodiversidade em função da desestruturação do ambiente físico, químico e alteração da dinâmica natural das comunidades aquáticas (Goulart e Callisto, 2003, Shimano et al., 2015).

Locais alterados geralmente possuem baixa diversidade de espécies e elevada diversidade beta funcional, restritos a organismos mais tolerantes (Callisto, 2001), o que produz a reestruturação das comunidades com o decrescimento ou desaparecimento de espécies sensíveis (Barbola et al., 2011). Portanto, o presente estudo visou realizar uma avaliação das diferenças que os gêneros da ordem Ephemeroptera apresentam enquanto a sua riqueza e abundância de gêneros, diversidade beta e diversidade beta funcional, comparando igarapés preservados (controle) dos igarapés sob influência de mineração (alterados), na espera de resultados ou ferramentas novas que permitam avaliar os efeitos de atividades antrópicas (no caso: mineração) sobre as assembleias estudadas.

Esta dissertação foi subdividida em dois capítulos: 1) Mining effects and lower turnover of Ephemeroptera (Insecta) in Brazilian streams of Eastern Amazon; 2) Redução da heterogeneidade ambiental e aninhamento funcional de Ephemeroptera (Insecta) em riachos da Amazônia Oriental. Testamos as seguintes hipóteses: Capítulo 1: a) Testamos as seguintes hipóteses: a) as atividades de mineração atuam como um agente perturbador do meio ambiente e reduzem a variação ambiental dos riachos; b) os riachos alterados apresentam valores mais baixos de diversidade beta; e, c) as variáveis ambientais influenciam a riqueza e abundância de Ephemeroptera .Capítulo 2: a) Riachos preservados terão maior diversidade funcional e diversidade beta funcional de Ephemeroptera do que os riachos alterados, baseado na predição de que atividades de mineração reduzem a heterogeneidade ambiental (microhábitats), e a disponibilidade de recursos e, portanto, decresce a diversidade funcional e diversidade beta funcional das ninfas, e b) Há uma relação positiva entre maiores valores de dissimilaridade em termos de diversidade beta e características funcionais dos gêneros Ephemeroptera, e maior dissimilaridade ambiental e espacial (distância espacial) dos riachos avaliados.

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3. SESSÃO 1

Mining effects and lower turnover of Ephemeroptera (Insecta) in Brazilian streams of Eastern Amazon

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Mining effects and lower turnover of Ephemeroptera (Insecta) in Brazilian streams of Eastern Amazon

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3.1 ABSTRACT

Environmental alterations with high impact on the aquatic organisms can be driven by mining activities. Our objectives were to analyze the effects of mining activities on environmental heterogeneity of the eastern Amazon streams, and in beta diversity (and components) of Ephemeroptera communities. We tested the hypotheses: a) mining activities reduces the environmental variation of streams; b) altered streams have lesser beta diversity; and c) environmental variables influence the richness and abundance of Ephemeroptera. The sampling was performed in the Carajás National Forest during three years in dry seasons. Streams were sampled near the extraction of iron ores (16), and in pristine areas (8). The beta diversity was estimated considering occurrence and abundance approaches. We record 2,259 individuals and 32 genera. The assessment of altered environments compared to the preserved environments showed that mining activities possibly led to an increase in total manganese and total iron. Environmental variables influenced Ephemeroptera communities, and the results of beta diversity components demonstrated higher turnover of Ephemeroptera in preserved streams, indicating that species substitution is higher in preserved streams than altered streams. These results indicated that the substitution of species in preserved streams has a spatial limitation, probably linked to the sensitivity of the organisms to the environmental variations of the mentioned variables, which occur in altered environments.

Keywords: aquatic insect, conservation, freshwater, environmental heterogeneity, land use.

3.2 INTRODUCTION

The environmental heterogeneity of habitat plays a key role in the structure and functioning of aquatic communities, and promotes higher variation on physical and chemical conditions of the habitat, strongly influencing which the composition, abundance and distribution of organisms at the local level (Juen and De Marco 2011; Brasil et al. 2020a). Since the distribution of organisms is limited by their ecophysiological habitat needs (eg. limits of physical conditions temperature, depth, width and flow of the stream, types of food resources, etc.), complex habitats promote a wide range and availability of resources and physical refuges (niches or microhabitats), and increase the likelihood of harboring greater species diversity (Cardinale 2011; Heino et al. 2013; Seiferling et al. 2014; Richards 2018) than more simplified habitats or homogeneous habitats (Downes et al. 2000). More homogeneous locations would favor the occurrence of more generalist species, with larger niche amplitude and more tolerant to changes (Callisto et al. 2001; Richmond et al. 2005; Brasil et al. 2014; Cardoso et al. 2015). In addition to influencing species richness, habitat complexity can also affect the variability in species occurrence between communities (beta diversity patterns) within a given area (Magurran 2004; Anderson et al. 2006; Anderson et al. 2010), because it allows the coexistence of species with different ecological tolerances to environmental conditions (Juen and De Marco 2011).

Anthropogenic disturbances produce environmental changes that affect the diversity of aquatic macroinvertebrate communities and may cause negative impacts on aquatic ecosystems, resulting in degradation of water quality, homogenization of their biotas (habitat simplification), causing a decrease in beta diversity (Krynak and Yates 2018; Krynak et al. 2019). In the Brazilian Amazon, impacts resulting from anthropic activities have been increasingly recurrent, especially in aquatic ecosystems (streams, creeks) (Monteiro 2005; Helson 2013). Moreover, these ecosystems are one of the most threatened in the world due to human dependence and use (Costas et al. 2018; Baker et al. 2019).

Environmental alterations with high impact on benthic community can be driven by mining activity worldwide (Callisto and Esteves 1998, Callisto et al. 1998, Armitage et al. 2007). These activities lead to soil exploitation and the devastation of riparian vegetation (Shimano et al. 2015), which directly and indirectly influences aquatic macroinvertebrate fauna, destabilizing the soil and favoring the erosion process (sedimentation of the waterbed) (Martins et al. 2014). Such factors exert influence and have a direct effect on different structural components of the benthic community, such as abundance, diversity, richness or metrics related to sensitive species (Armitage et al. 2007, Costas et al. 2018). Mining activities cause a decline in water quality and loss of biodiversity due to the disruption of the physical environment and a synchrony in trait composition indicating environmental and functional homogenization (Feio et al. 2015), chemical and alteration of the natural dynamics of aquatic communities (biological) (Goulart and Callisto 2003; Shimano et al. 2013).

In this context, aquatic macroinvertebrates are used in environmental quality assessment (Rosenberg and Resh 1993), because they are able to respond in different ways to anthropic impacts due to the different morphological, physiological and behavioral strategies in the face of environmental changes (Salles et al. 2004; Domínguez et al. 2006; Domínguez and Fernández 2009; Shimano et al. 2011; Helson 2013). Among aquatic insect groups, Ephemeroptera nymphs occur in a wide variety of habitats, being highly diverse in rocky substrate lotic systems and fast-flowing waters (Domínguez et al. 2006). They are widely used bioindicators because of their sensitivity to changes occurring in aquatic ecosystems, especially at the microhabitat level (Rosenberg and Resh 1993; Callisto et al. 2001; Ligeiro et al. 2014; Dedieu et al. 2015; Shimano & Juen 2016; Firmiano et al. 2016; Luiza-Andrade et al. 2017). There are species with different levels of tolerance to environmental changes, as they live and feed on sediments where pollutants tend to accumulate (Ward and Holmes 1995; Rosenberg and Resh 1993; Buss et al. 2002, 2004; Ligeiro et al. 2014). In Brazil, this order is currently composed of 10 families, 81 genera and 409 species (Salles and Boldrini 2019).

In Brazil the number of studies on the effects of abiotic variables, anthropic changes and heterogeneity (habitat integrity) in benthic communities rised the increase and intensity of anthropic action in environments (Callisto et al. 2001; Bispo et al. 2004; Bispo et al. 2006; Souto et al. 2011; Monteiro-Júnior et al. 2013; Brasil et al. 2014; Dedieu et al. 2014; Martins et al. 2014; Oliveira – Junior et al. 2015; Firmiano et al 2016; Cunha and Juen 2017; Luiza-Andrade et al. 2017; Castro et al. 2018; Brasil et al. 2020b). These studies and others worldwide have pointed out that changes in the land use or anthropogenic impacts produce a decrease or loss of beta diversity in aquatic invertebrate communities (Feio et al. 2015, Castro et al. 2018, Krynak and Yates 2018, Krynak et al. 2019).

Based on the anthropogenic impacts in aquatic streams, the main objective of this study was to evaluate the effects of mining activities on the environmental heterogeneity and on the Ephemeroptera communities (richness, abundance, composition, and beta diversity) in Amazonian streams. We tested the following hypotheses: a) mining activities act as a disturbing agent of the environment, and reduces the environmental variation of streams, b) altered streams have lower values of beta diversity, and c) environmental variables influence the richness and abundance of Ephemeroptera.

3.3 METHODS

4. MATERIALS AND METHODS

4.1.1 Study area

The study was conducted in the Carajás National Forest (Flona de Carajás), a federal conservation unit created in 1998 located in southeastern Pará, occupying part of the cities of Parauapebas, Canaã dos Carajás and Água Azul do Norte (Bezerra 2017) (Fig. 1). The native vegetation cover of the region consists of the Dense Ombrophylous Forest (Montana, Submontana) and Open Ombrophila (Submontana) phytophysiognomies, with regions with high rainfall and canopy trees that can exceed 50 m in height in the first typology, in addition to an abundance of palm trees or vines found in the second typology (ICMBio 2016). On a smaller scale, we highlight the occurrence of seasonal deciduous herbaceous shrub vegetation that occurs on the ferruginous outcrops, known as “savanna-steppe vegetation” or “canga vegetation” (ICMBio 2016). This “canga” vegetation (Rizzini 1979; Mesquita 1983; Viana et al. 2016; Mota et al. 2018) houses a type of open vegetation consisting mainly of herbs and shrubs associated with distinct iron-rich conglomerates.

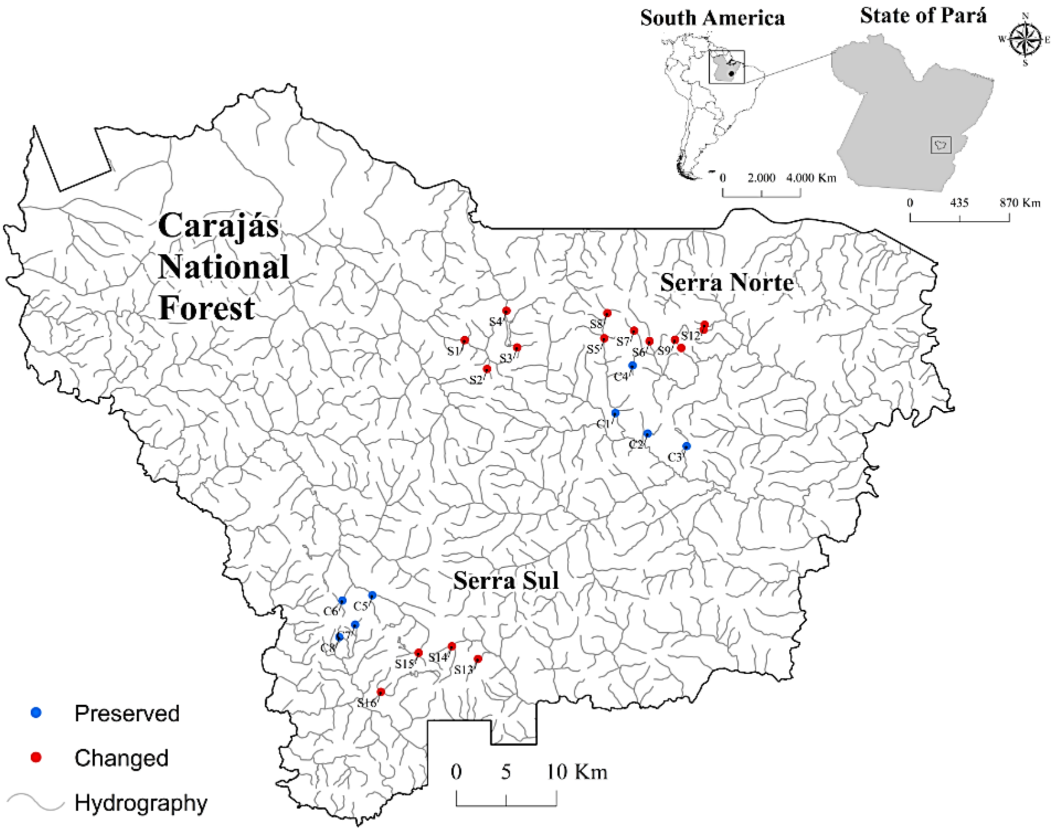

Fig. 1 Geographic location of the streams sampled in the Carajás National Forest (Flona de Carajás), Southeast of Pará State, Brazil. The sampling points are shown in the lower figure, divided into (A) Serra Norte and (B) Serra Sul.

In addition to having large iron deposits, it also naturally includes minerals such as manganese, nickel, copper and gold (Tolbert et al. 1971; Viana et al. 2016; Souza-Filho et al. 2019). A mosaic formed by different phytophysiognomies (Cleef and Silva 1994; Mota et al. 2015) characterizes the “canga”. Therefore, the canga comprises a peculiar flora with a high number of endemic species. Plants growing there are adapt to extreme conditions of acid and nutrient-poor soils (Nunes et al. 2015), high concentrations of heavy metals (Schettini et al. 2018), high temperatures and strong seasonality, with a clearly defined dry season (Mota et al. 2015). Geologically, this region is called Carajás Formation and is composed of banded iron formations (BIF) represented by jaspilites, with mafic rocks located above and below it. Andesites, basalts, vulcanoclastic materials and gabbro are also present (Grainger 2008; Souza-Filho et al. 2019). The climate is montane or Amazonian serrano type, classified as Aw type according to Köppen (Alvares 2013). The dry season occurs between May and October (average rainfall <60 mm in the driest months – IBAMA et al., 2003) and the rainy season between November and April, as well as annual average temperatures around 21 to 22ºC, in contrast to 25 to 26ºC (Souza-Filho et al. 2019) in the areas between Marabá and Parauapebas.

This area was discovered in 1967 by United States Steel Company, which, in association with the Brazilian State-owned Vale do Rio Doce Company (CVRD), obtained the right to explore iron ore in the Carajás region in 1974. In 1977, CVRD took control of the venture in the Carajás region. In 1987, CVRD was granted by the Federal Senate the real right of indefinable and non-transferable use of 411,948.87 hectares of Union domain, adjacent to the mineral province of Carajás in the current city of Parauapebas. With the privatization of Vale do Rio Doce Company in 1997, FLONA de Carajás was created the following year in a space granted to the company in order to make the continuity of the direct use of mineral resources and the sustainable exploitation of natural resources compatible, according to the creation decree, including Art. 2 and Art. 3 of Decree No. 1,298, of October 27, 1994 (National Forest Regulations) (ICMBio 2016). Thus, the data were collected through the campaigns of the Integrated Monitoring Program of Bioindicators in the Carajás National Forest, which aims to mitigate the impacts of mining activity on biodiversity, within the scope of the Federal Environmental Licensing (IBAMA – Brazilian Institute of Environment and Renewable Natural Resources) of the Vale S. A. Carajás Mining Complex. It is also important to note that the extraction company adopts all environmental controls (industrial and sanitary effluent treatment stations, dams and sediment containment sumps), necessary to mitigate the environmental impacts caused by the mining activity.

4.1.2 Sampling

Biological data were collected in three samplings, carried out during the period of least precipitation in the study area, called drought or dry season. The first sampling (C-1) took place from

September to October 2015, the second sampling (C-2) was held from October to November 2016, and the third sampling (C-3) took place in October 2017. 24 streams were sampled. In the study 24 streams were sampled, 16 of which are under the influence of mining (altered) and eight streams located far from the effect of the plant (preserved) (Fig. 1). In the study 24 streams were sampled, 16 of which are under the influence of mining (altered) and eight streams located far from the effect of the plant (preserved) (Fig. 1). In each stream, a 100 meters fixed section was demarcated, divided into 10 sections of 10 meters (referred as A-B, B-C… J-K), composing 10 transections subdivided into 20 longitudinal sections of five meters (20 segments) (Shimano et al. 2015; 2018). The Ephemeroptera individuals were collected using a rapiché (18 cm circumference and 0.05 mm mesh) and the specimens found were fixed in 85% alcohol (Shimano et al. 2018), quantified and identified to the genera taxonomic level (Salles et al. 2004; Domínguez et al. 2006;). Such limitation in identification is because most streams specimens correspond to immature individuals (larvae), and do not have complete development of the genitalia, which is essential for the identification (Shimano 2015; Shimano et al. 2018).

4.1.3 Environmental variables

The environmental dataset was composed of 10 environmental (limnological), represents for electrical conductivity, chemical oxygen demand, total iron, total phosphorus, total manganese, nitrate, dissolved oxygen, pH, water temperature (°C) and turbidity. Temperature and pH were measured in the field using a multiparameter probe. The other variables were obtained in laboratory analysis by collecting water samples in each stream sampled. All the variables are measured three times along the channel, therefore, in the analysis each stream is represented by the average of these three measurements.

4.1.4 Data analysis

Prior to analysis with abiotic data we performed a correlation test with the limnological variables. None of the variables presented more than 70% correlation among them. Higher correlation values occurred between chemical oxygen demand and nitrate (55%), total iron and manganese (61%) and turbidity and total iron (43%).

To evaluate the limnological variation among the streams we performed a Principal Component Analysis (PCA) using correlation matrix (Clarke and Gorley 2006). The limnological variables were previously standardized in order to reduce the possible effects of outliers. Here we did not include chemical oxygen demand, total manganese and water temperature. As a criterion for selecting the number of axes for interpretation, the “Broken-stick” was used. To determine how much each variable contributed to the formation of the axes, the sample scores, variable scores, and loadings were estimated. To test if mining activities reduces the environmental variation of streams, we performed a Multivariate Permutational Analysis of Variance (PERMANOVA) on PCA axes (Clarke and Gorley 2006).

The beta diversity was estimated by two indexes, the Jaccard’s (presence-absence matrix) and Bray-Curtis (abundances matrix), using Permutational Multivariate Dispersion Analysis (PERMIDISP, Anderson et al. 2006). We estimate the turnover and nestedness components of beta diversity as described by Legendre (2014). Differences in the total beta diversity and both the species turnover and nestedness components between preserved and altered streams were tested using a multivariate permutational analyses of variance (PERMANOVA). To view the differences in species composition and beta diversity patterns between preserved and altered streams, we performed a principal coordinate analyses (PCoA).

To verify whether there is an effect of the environmental variables on Ephemeroptera richness and abundance, multiple regressions were performed. To identify the main variables controlling Ephemeroptera distribution, we used the stepwise variable selection model. The assumptions of normality and homogeneity were followed and the level of significance used was $\alpha = 0.05$.

All analyzes were performed using the statistical program R (R Development Core Team 2019). Principal Component Analysis were estimated using prcomp function of vegan package (Oksanen et al. 2019); multivariate permutational analysis of variance were estimated using adonis function of vegan package; beta diversity and their components were estimated using betadisper function of vegan package and beta.div.comp function of adespatial package (Dray et al. 2020).

4.2 RESULTS

4.2.1 Environmental variation

The limnological variables that least varied among the samplings were total iron (mean 0.44 ± 0.55 SD), total phosphorus (mean 0.02 ± 0.03 SD), total manganese (mean 0.09 ± 0.22 SD), nitrate (mean 1.05 ± 2.26 SD), dissolved oxygen (mean 5.93 ± 0.91 SD) and pH (mean 6.74 ± 0.63 SD). Conductivity (mean 38.09 ± 49.8 SD), chemical oxygen demand (mean 26.83 ± 3.53 SD), water temperature (mean 20.13 ± 8.42 SD) and turbidity (mean 6.27 ± 11.60 SD) showed higher range among the samplings.

The Principal Component Analysis (PCA) results represented 39% of total data variability in the first two axes (Fig. 2). The higher correlations between the first axis and the limnological variables was total iron (R= -0.66) and turbidity (R= -0.61). The second axis was positively correlated to pH (R= 0.61) and negatively with nitrate (R= -0.62). Differences were detected between preserved and altered streams in the gradients formed by the PCA (PERMANOVA, Pseudo-F= 2.515; p= 0.001*), with altered streams showing greater values of total iron, turbidity and total manganese. However, it is possible to observe in the figure that streams altered by mining proved to be more scattered on the graph, indicating that it is more heterogeneous.

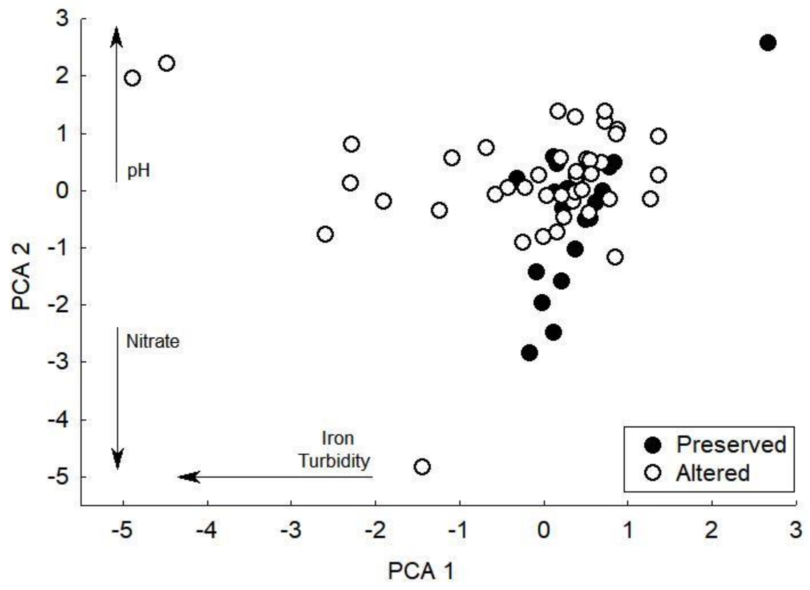

Fig. 2 PCA ordering for environmental variables in preserved and altered by mining streams, FLONA Carajás. Full circles = preserved; Open circles = altered.

4.2.2 Ephemeroptera communities and beta diversity

In the study area, 32 genera and 2.259 individuals of the order Ephemeroptera were registered. From all genera, 28 were common to both treatments (Supplementary material). The occurrence of three genera differed between treatments, with *Crytonympha* Lugo-Ortiz & McCafferty, 1998, *Leptohyphodes* Ulmer, 1919 and *Spiritiops* Lugo-Ortiz & McCafferty, 1998, in altered streams, and *Leentvaria* Demoulin, 1966 in preserved streams. The genera *Miroculis* Edmunds 1963 had the higher abundance (n=639) followed by *Farrodes* Peters, 1971 (n=347). (Supplementary material).

The total beta diversity of Ephemeroptera (F-ratio=1.1377, p-value=0.268) did not differ between the treatments (mean distance to centroid: preserved = 0.500; altered = 0.546) (Table 1). However, when partitioning beta diversity, turnover and nestedness responses varied between the species abundance and occurrence approaches. The turnover considering abundances (F-ratio= 1.886, p-value= 0.249; mean distance to centroid: preserved = 0.232; altered= 0.233), the nestedness with abundances (F-ratio= 1.002, p-value= 0.346; mean distance to centroid: preserved= 0.280; altered= 0.350) and with presence-absence (F-ratio= -0.103, p-value= 0.966; mean distance to centroid: preserved= 0.495; altered= 0.496) did not differ between treatments. However, turnover differed between the treatments considering presence-absence (F-ratio=4.2053, p-value=0.005, Table 1) and was higher in preserved sites (mean distance to centroid: preserved = 0.209; altered = 0.207).

For presence-absence data the PCoA ordering demonstrated 68% of the total percent variation explained by axes 1 and 2 (Fig. 3A), and for abundance data 89% (Fig. 3B). Both ordinations showed overlaid points of the streams.

Table 1. Permutational multivariate analyses of variance (PERMANOVA) for the total beta diversity, turnover and nestedness components in Ephemeroptera among streams.

	F	***P***
Total beta diversity	1.138	0.268
Turnover		
Presence-absence	4.205	**0.005***
Abundance	1.886	0.249
Nestedness		
Presence-absence	-0.103	0.966
Abundance	1.002	0.346

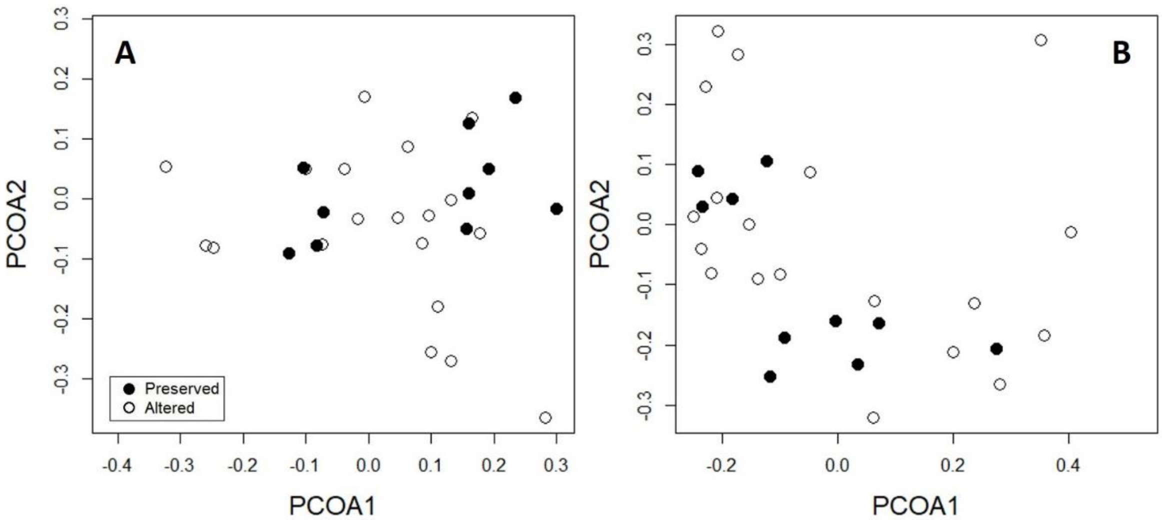

Fig. 3 PCoA ordering for the Ephemeroptera presence and absence matrix (Jaccard index) (A) and PCoA ordering for the Ephemeroptera abundance matrix (Bray-Curtis index) (B). Full circles = preserved; Open circles = altered.

4.2.3 Community and environmental variables relation

The environmental variables were important to determine Ephemeroptera abundance and richness (Table 2) as expected. Environmental variables explained, 18% and 32% of the variations in abundance and richness, respectively. Of the ten tested variables, three were selected by the model for abundance and four were selected for richness (Table 2), the same three (water temperature, total manganese, and total phosphorus) were repeated in both models.

Table 2. Result of forward stepwise regression analyses between environmental variables and richness and abundance of Ephemeroptera in preserved and altered by mining streams, FLONA Carajás. *significative results.

	Beta	t(59)	p-level
Abundance			
Intercept		8.546	0.000*
Water temperature	-0.339	-2.822	0.006*
Total manganese	-0.167	-1.393	0.169
Total phosphorus	-0.133	-1.125	0.265
Model	R^2= 0.183; $F_{(3,59)}$= 4.434; p<0.007*		
Genera richness			
Intercept		11.217	0.000*
Water temperature	-0.369	-3.303	0.002*
Total manganese	-0.281	-2.548	0.014*
Total phosphorus	-0.169	-1.549	0.127
Conductivity	-0.138	-1.255	0.215
Model	R^2= 0.322; $F_{(4,58)}$= 6.893; p< 0.001*		

4.3 DISCUSSION

4.3.1 Environmental variation

Our results demonstrated limnological differences between preserved and altered streams by mining, however, contrary to what was expected, altered streams seems to be more heterogeneous, refuting the hypothesis that mining activities reduces the environmental variation of streams. The assessment of altered environments compared to the preserved environments showed that mining activities possibly led to an increase in elements related to mining activities, such as total manganese and total iron, and, thus, when altered streams with different levels of alterations are evaluated together, it can cause an increase in the variance of environmental variables, which may explain the greater variation in altered treatment. The higher heterogeneity does not always indicate good environmental conditions. Baker et al. (2019), for example, mention that the increase in nutrients and other limnological variables, including manganese, are indicators of serious organic pollution. This pollution can reduce the concentrations of dissolved oxygen and favors taxa of macroinvertebrates highly tolerant to live in habitats with lower environmental quality. According to Brasil et al. (2014) and Baker et al. (2019), environmental variation can change aquatic communities.

From another perspective, a fact that could explain the higher values of iron and manganese in several streams in the study, is that they are all located in a typical area of canga formations (Tolbert et al. 1971; Dittman 2010; Clements 2016). Studies indicates that iron and manganese are common ions of these formations in this region, and that, when they have high concentrations, they can affect some of the functions of aquatic macroinvertebrates (Johnson et al. 2015).

4.3.2 Ephemeroptera communities and beta diversity

The Ephemeroptera composition was very similar between preserved and altered streams, with 29 common genera among them, and beta diversity results confirmed these similarities and did not vary between treatments (preserved and altered). However, the results of beta diversity components (presence-absence data), demonstrated higher turnover of Ephemeroptera in preserved streams, indicating that species substitution is higher in preserved streams than altered streams.

It is possible that the aquatic macroinvertebrates that live in the streams of the study area are adapted to high quantity of heavy metals, natural condition of the area, and are tolerant to the presence of these ions, since they share an evolutionary history together, which could explain the similar beta diversity between treatments. On the other hand, the streams under mining influence still have riparian forest, which may have buffered the major effects of the alteration. So, the similarity of total beta diversity could be explained because there are sets of benthic organisms that inhabit streams unaffected by profound and irreversible changes in habitat, or by lasting pollution, and show relatively high resistance or resilience (Bradt et al. 1999; Woodward et al. 2002).

Despite beta diversity was similar between treatments, the results demonstrated higher turnover of Ephemeroptera in preserved streams. The replacement of some species by others can be a result of environmental, spatial, and/or temporal restrictions (Baselga 2010, Qian et al. 2005). Patterns of turnover over nestedness indicate that all preserved streams are contributing to the regional species pool. And the maintenance of this pool is mediated by species dispersibility (Soininen et al. 2007).

Whereas the study field is inserted in a large forest area within a conservation unit, as well as a long evolutionary period, we believe that the distances between the hydrological connections of these aquatic ecosystems may not be large enough to hinder dispersion between the sampled sites. Dispersion between isolated aquatic ecosystems can be as effective as connected aquatic systems (Beisner et al. 2006). Many genera of Ephemeroptera have small bodies and have life history strategies that allows them to travel long distances (Salles et al. 2004). In addition, individuals of the order Ephemeroptera can disperse by two forms, in the immature stage, due to behavioral drift, and in the adult stage, by flying (Brittain 1982) improving dispersibility.

Contrary to our results, Wright & Ryan (2016) found differences in macroinvertebrates communities between mining altered and preserved streams in Australia. Still, according to the authors, of the insects, the ephemeropterans, especially Leptophlebiidae, were most sensitive to metal pollution. However, in tropical streams, the genera of Leptophlebiidae are generally the most common and abundant, such as *Farrodes* and *Miroculis*, the most abundant genera in our study. These genera are already known for being generalist and tolerant (Baptista et al. 2006; Brasil et al. 2013).

4.3.3 Community and environmental variables relation

Corroborating our last hypothesis, environmental variables were important to determine Ephemeroptera abundance and richness, indicating that if mining activities can change environmental variables, they also can change the role community. In the best model, water temperature, total manganese, total phosphorus and conductivity were the main variables that affecting the abundance and richness of Ephemeroptera. According to Shimano and Juen (2016), water temperature increases can favor the occurrence of more resistant genera of Ephemeroptera, which, in turn, can change the diversity metrics. Besides, elevated water temperatures accelerate insect life cycles (Couceiro et al. 2006). Phosphorus is the main limiting factor of productivity and the main responsible for the artificial eutrophication of aquatic environments (Esteves 1998), and leads to loss of sensitive species. Manganese, as a heavy metal, may affect macroinvertebrates indirectly through alteration of habitat conditions, trophic relationships or directly through water contamination, and showed deleterious effects on macroinvertebrate communities at Igun Reservoir resulted in reduced species richness and changes in the community compositions (Ehikhamele and Ogbogu 2016). Finally, electrical conductivity is an important factor that structure insect communities of lotic habitats and was the most important factor acting in streams of Cerrado, whereas rivers with large conductivity values had a higher genera richness and abundance (Godoy et al. 2016).

Hill et al. (2019) analyzing the compositional variation of beta diversity of the different facets, (taxonomic, functional, and phylogenetic) found that diversity was largely determined by local environmental variables, and that the taxonomic richness was most strongly explained by environmental variables. Rocha et al. (2018) studying the multiple facets of macroinvertebrate beta diversity, also found influence of environmental variables on beta diversity components, indicating, one more time, the importance of environmental variables to aquatic biota. In the Eastern Amazon, Miguel et al. (2017), when testing the effects of environmental changes on the stream Odonata communities, also reports that the variation in species composition is one of the most efficient metrics to be used in monitoring studies to detect anthropogenic changes.

Therefore, our results show that even environmental variables influencing Ephemeroptera communities, and with significant differences between treatments, beta diversity was similar, but, with a significant species turnover only for preserved streams. This indicates that the substitution of species in preserved streams has a spatial limitation, probably linked to the sensitivity of the organisms to the environmental variations of the variables mentioned above, which occur in altered environments.

4.3.4 Authors contributions

L. Juen conceived the ideas and designed the methodology; Y. Shimano collected the data; A.C.E. Espinosa, Y. Shimano and B. Dunck analyzed the data; A.C.E. Espinosa, Y. Shimano and B. Dunck led the writing of the manuscript; L. Juen, A.C.E. Espinosa, Y. Shimano, S. Rolim, L. Maioli, and B. Dunck contributed critically to the drafts and gave final approval for submission.

4.3.5 Compliance with ethical standards

Conflict of interest: this work conforms to the Italian legal requirements including those relating to conservation and welfare. The authors declare that they have no conflict of interest.

4.4 LITERATURE CITED

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4.5 SUPPLEMENTARY MATERIAL

S1. Abundance of Ephemeroptera genera in preserved and altered sampling areas – Flona de Carajás.

Genera	Preserved	Altered	Total Abundance
Amanahyphes Salles & Molineri, 2006	3	14	17
Americabaetis Kluge, 1992	14	29	43
Askola Peters, 1969	18	13	31
Aturbina Lugo-Ortiz & Mccafferty, 1996	18	19	37
Baetodes Needham & Murphy, 1924	7	12	19
Brasilocaenis Malzacher, 1986	12	59	71
Caenis Malzacher, 1986	1	13	14
Callibaetis Eaton, 1881	2	6	8
Callibaetoides Cruz, Salles & Hamada, 2013	10	16	26
Campylocia Needham & Murphy, 1924	59	92	151
Cloeodes Traver, 1938	40	35	75
Cryptonympha Lugo-Ortiz & McCafferty, 1998	-	2	2
Farrodes Peters, 1971	130	217	347
Fittkaulus Savage & Peters, 1978	4	1	5
Hagenulopsis Ulmer, 1920	49	95	144
Hydrosmilodon Flowers & Domínguez, 1992	5	23	28
Leentvaaria Demoulin, 1966	1	-	1
Leptohyphes Ulmer, 1919	3	21	24
Leptohyphodes Ulmer, 1919	-	1	1
Miroculis Edmunds, 1963	243	396	639
Paracloeodes Day, 1955	3	12	15
Paramaka Savage & Domínguez, 1992	48	44	92
Simothraulopsis Demoulin, 1966	4	16	20

Genera	Preserved	Altered	Total Abundance
Spiritiops Lugo-Ortiz& Mccafferty, 1998	-	1	1
Terpides Demoulin, 1966	35	23	58
Thraulodes Ulmer, 1920	8	4	12
Traverhyphes Molineri, 2001	9	37	46
Tricorythodes Ulmer, 1920	22	7	29
Tricorythopsis Traver, 1958	1	10	11
Ulmeritoides Traver, 1959	54	33	87
Waltzoyphius Lugo-Ortiz& Mccafferty, 1995	2	5	7
Zelusia Lugo-Ortiz & Mccafferty, 1998	64	134	198
Total	**869**	**1390**	**2259**

4.6 SUBMITTED:

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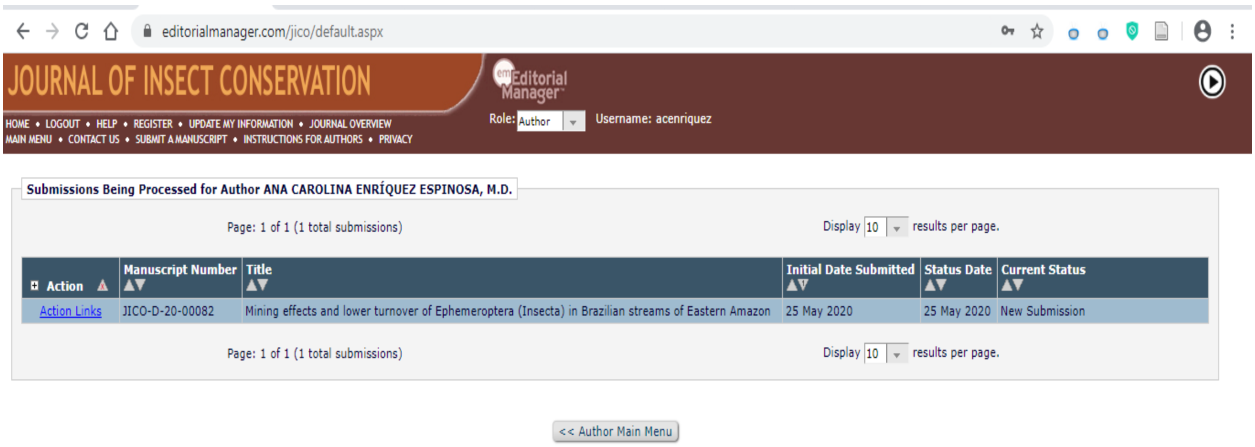

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5. SESSÃO 2

Redução da heterogeneidade ambiental e aninhamento funcional de Ephemeroptera (Insecta) em áreas de mineração na Amazônia Oriental

A segunda sessão desta dissertação foi elaborada e formatada conforme as normas da publicação científica *Hydrobiologia*, disponível em: https://www.springer.com/journal/10750/submission-guidelines

Redução da heterogeneidade ambiental e aninhamento funcional de Ephemeroptera (Insecta) em áreas de mineração na Amazônia Oriental

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5.1 RESUMO

O objetivo deste estudo foi avaliar se os efeitos da mineração podem afetar a diversidade funcional e a diversidade beta funcional de Ephemeroptera em riachos preservados e alterados. Testamos as hipóteses que riachos preservados terão maior diversidade funcional e diversidade beta funcional do que os riachos alterados, e que há uma relação positiva entre a diversidade beta funcional e a dissimilaridade ambiental e espacial dos riachos avaliados. Foram amostrados 8 riachos preservados e 16 alterados. Utilizamos cinco características funcionais e a diversidade funcional foi avaliada pelo RaoQ, e a diversidade beta funcional pelo índice de Sorensen. Avaliamos as relações entre o ambiente e as características funcionais por análise RLQ. Nossos resultados não mostraram diferenças na diversidade funcional entre os distintos tratamentos. Porém, indicaram maior aninhamento funcional em riachos alterados, o que pode levar à uma homogeneização funcional e perda de espécies sensíveis. Observamos que o aumento de ferro e turbidez proporcionou aumento de gêneros com a característica funcional brânquia opercular, e o aumento de manganês total proporcionou diminuição de gêneros da guilda trófica filtrador. Dessa forma, indicamos que as diferenças ambientais entre os riachos levaram a variação na composição das características funcionais dos organismos, indicando potencial efeito negativo da mineração sob os gêneros de Ephemeroptera.

Palavras chave: *Alteração antropogênica, características funcionais, diversidade beta, diversidade funcional.*

ABSTRACT

The aim of this study was to evaluate whether the effects of mining can affect the functional diversity and the functional beta diversity of Ephemeroptera in preserved and altered streams. We tested the hyphotesis that preserved streams will have greater functional diversity and beta functional diversity than altered streams, and that there is a positive relationship between functional beta diversity and the environmental and spatial dissimilarity of the streams evaluated. Eight preserved and 16 altered streams were sampled. We used five functional characteristics and the functional diversity was assessed by RaoQ, and the functional beta diversity by the Sorensen index. We evaluated the relationship between the environment and the functional characteristics by RLQ analysis. Our results did not show differences in the functional diversity between the different treatments. However, they indicated greater functional nesting in altered streams, which can lead to functional homogenization and loss of sensitive species. We observed that the increase in iron and turbidity provided an increase in genera with opercular gills, and an increase in total manganese provided a decrease in genera of the trophic filtering guild. Thus, we indicate that the environmental differences between the streams led to a variation in the composition of the functional characteristics of the organisms, indicating a potential negative effect of mining over the Ephemeroptera genera

Keywords: *Anthropogenic alteration, beta diversity, functional diversity, traits.*

5.2 INTRODUÇÃO

Os estudos da diversidade funcional têm relacionado os aspectos da biodiversidade que influenciam o funcionamento do ecossistema e manutenção de processos ecológicos, e incorpora a diversidade de características fenotípicas (morfológicas e fisiológicas) e ecológicas nas comunidades bióticas (Petchey & Gaston, 2006, Cadotte et al., 2011). O estudo da diversidade funcional também permite uma melhor compreensão da estrutura e organização das comunidades (Petchey & Gaston, 2006, Mason & De Bello, 2013) ao longo de gradientes de degradação antrópica (Violle et al., 2007) ou perturbação (Cadotte et al., 2011).

Nesse contexto, a diversidade funcional é considerada uma ferramenta para avaliar as diferenças das características funcionais entre as comunidades (Di Battista et al., 2016), e estima as diferenças entre os organismos diretamente através de suas características funcionais (Díaz e Cabido, 2001, Tilman, 2001; Petchey e Gaston, 2006). Assim, as características funcionais de um organismo podem ser quantificadas por diferentes componentes e métricas relacionadas com a função que as espécies desempenham no ecossistema (Poff et al., 2006, McGill et al, 2006, Schemera et al., 2017), geralmente medida em nível individual e usada comparativamente entre espécies (McGill et al., 2006).

Por outro lado, a dissimilaridade funcional avalia as mudanças nas características funcionais das espécies entre as comunidades no espaço ou no tempo e, portanto, leva em consideração a similaridade nas características ecológicas e evolutivas das espécies (Swenson, 2011, Swenson et al., 2012). A dissimilaridade funcional pode resultar de dois padrões distintos: *turnover* e aninhamento (Baselga, 2010, Baselga & Orme, 2012, Baselga, 2012, Baselga & Leprieur, 2015). A análise da decomposição da diversidade beta funcional pode aumentar ainda mais a compreensão dos processos de montagem das comunidades (Legendre, 2014).

Os macroinvertebrados bentônicos são organismos que participam do metabolismo de ecossistemas aquáticos como principais componentes da cadeia trófica, uma vez que participam da ciclagem de nutrientes, reduzem o tamanho de partículas orgânicas (por exemplo, fragmentadores), facilitando a ação de micro-decompositores, como bactérias, fungos e leveduras (Ward et al., 1995, Callisto & Esteves, 1995) e transporte de matéria orgânica a jusante (Whiles & Wallace, 1997). Baseado em vários estudos, o estudo da ecologia funcional de grupos de macroinvertebrados aquáticos pode ser avaliada usando todas as características biológicas (Bady et al., 2005, Peru & Dolédec, 2010), mas, principalmente, características relacionadas aos hábitos alimentares (Cummins, 1973, Nhiwatiwa, et al., 2009, Shimano et al., 2012, Brasil et al., 2014), tamanho e hábitos alimentares (Pavoine & Dolédec, 2005), e relação de locomoção e substrato (Heino, 2005, Heino et al., 2008).

Grupos funcionais de macroinvertebrados bentônicos constituem uma boa ferramenta em programas de biomonitoramento (Rosenberg & Resh et al., 1993, Hering et al., 2004) em funções das respostas de suas espécies à degradação ou mudança ambiental (Shimano et al., 2011, 2013, Brasil et al., 2014), e a sua sensibilidade a mudanças ambientais em habitats aquáticos, em particular no nível de micro-habitat (Péru e Dolédec, 2010; Luiza-Andrade et al., 2017). Estes organismos também podem ser avaliados funcionalmente em relação à diversidade de grupos funcionais de alimentação, conhecido como FFGs (*Funcional Feeding Groups* - Guilda trófica) em ecossistemas lóticos (Cummins & Klug, 1979, Cummins et al., 2005, Shimano et al., 2012).

Distúrbios antropogênicos causam alterações na estrutura da comunidade de macroinvertebrados aquáticos, como por exemplo, na riqueza de espécies (Astorga et al., 2011; Ligeiro et al., 2013; Cunha et al., 2015; Brasil et al., 2019), na composição de espécies (Faria et al., 2017), abundância de indivíduos (Paiva et al., 2017), diversidade beta (Cunha e Juen, 2017; Brasil et al., 2017) e na diversidade funcional (Péru e Dolédec, 2010, Brasil et al., 2014, Fengzhi, et al., 2015, Dedieu, et al., 2015).

Os ecossistemas aquáticos podem ser afetados por vários tipos de distúrbios antropogênicos, no entanto, um dos impactos ambientais que tem maior incidência de alteração nas comunidades dos organismos aquáticos é a mineração (Martins et al. 2014). De forma geral, as atividades de mineração ocasionam exploração do solo e a devastação de vegetação ripária (Callisto et al., 2001, Shimano et al., 2015), que influencia direta e indiretamente a fauna de invertebrados aquáticos. Dentre os macroinvertebrados aquáticos, os efemerópteros geralmente estão presentes em todos os tipos de riachos e microhábitats bentônicos e mostram alta diferenciação morfológica e ecológica entre gêneros (Domínguez et al., 2006). Portanto, os indivíduos da ordem Ephemeroptera têm níveis variados de tolerância à poluição (Lewis, 1974), mas geralmente são considerados organismos sensíveis e requerem água com boas condições para sobreviver.

Dessa maneira, o presente estudo visou avaliar se as atividades de mineração podem alterar a diversidade funcional e beta funcional das ninfas da ordem Ephemeroptera. Para isso avaliamos a diversidade funcional e diversidade beta funcional entre riachos preservados e alterados pelas atividades de mineração, além das variáveis ambientais e sua a relação com as características funcionais desses táxons. Testamos as hipóteses: a) Riachos preservados terão maior diversidade funcional e diversidade beta funcional de Ephemeroptera do que os riachos alterados, baseada na predição de que atividades de mineração reduzem a heterogeneidade ambiental (microhábitats), e diminuiem a disponibilidade de recursos e, portanto, decrescem a diversidade funcional e diversidade beta funcional das ninfas, e b) Há uma relação positiva entre maiores valores de dissimilaridade em termos de diversidade beta e características funcionais dos gêneros Ephemeroptera, e maior dissimilaridade ambiental e espacial (distância espacial) dos riachos avaliados. Finalmente, esperamos que os resultados deste estudo indiquem o potencial de diversidade funcional e medidas beta funcionais, como métricas que nos permitam avaliar os efeitos das atividades de mineração nos gêneros de Ephemeroptera.

5.3 MATERIAIS E MÉTODOS

5.3.1 Área de estudo

A área de estudo está localizada no estado de Pará (sudeste), entre os municípios de Parauapebas, Canaã dos Carajás e Água Azul do Norte (Bezerra, 2017), e forma parte da Floresta Nacional de Carajás ou “Flona de Carajás” (Figura 1). A Flona de Carajás é subdividida em duas serras, Serra Norte e Serra Sul (Zappi, 2017). A vegetação característica constitui-se pelas fitofisionomias de Floresta Ombrófila Densa (Montana, Submontana) e Ombrófila Aberta (Submontana), além de ter abundância alta de palmeiras ou cipós, e intercalada com um tipo de vegetação herbáceo-arbustiva caducifolia sazonal, que ocorre sobre os afloramentos ferruginosos, conhecida como “vegetação de canga” (ICMBio, 2016).

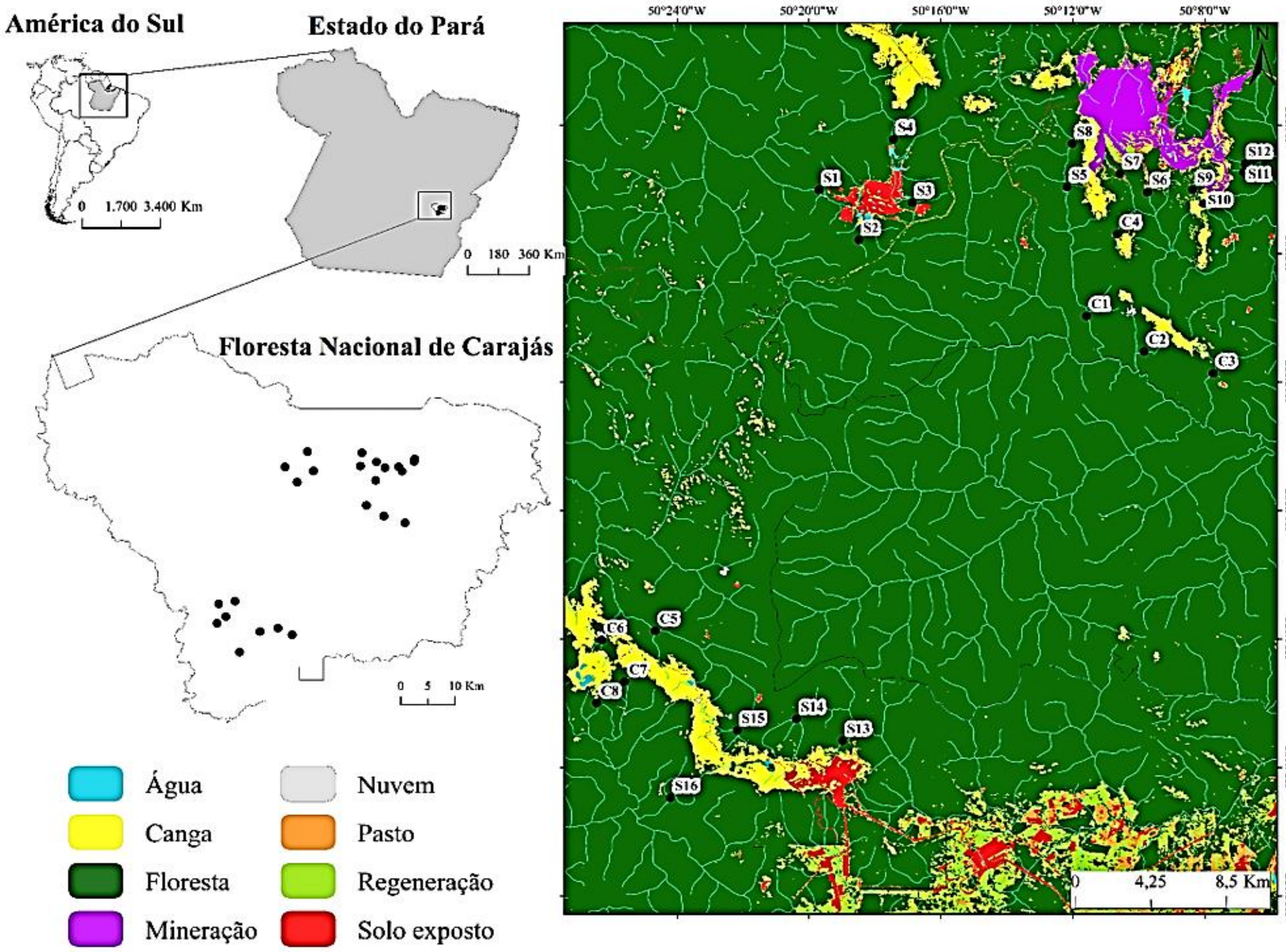

Figura 1 Localização geográfica dos riachos amostrados na Floresta Nacional de Carajás (Flona de Carajás), Sudeste do Estado do Pará, Brasil. Os pontos de amostragem são mostrados na figura inferior, sendo separados em (A) Serra Norte e (B) Serra Sul. Os pontos de cor azul representam os riachos preservados e os pontos de cor vermelha os riachos alterados.

A canga possui um tipo de vegetação aberta formada principalmente por ervas e arbustos associados a distintos conglomerados ricos em ferro, e outros minerais como manganês, níquel, cobre e ouro (Viana et al., 2016; Souza-Filho et al., 2019). O clima é do tipo montano ou serrano amazônico, classificado como o tipo Aw de acordo com Köppen (Alvares, 2013). A estação seca ocorre entre maio e outubro (precipitação média <60 mm nos meses mais secos - IBAMA et al., 2003) e estação chuvosa entre novembro e abril, assim como temperaturas médias anuais em torno de 21 a 22 ºC (seca), em contraste com 25 a 26 ºC (chuva) (Souza-Filho et al., 2019).

5.3.2 Amostragem

Um total de 24 pontos (riachos) foram amostrados na Serra Norte e Serra Sul da Flona de Carajás, durante três campanhas de coleta: campanha 1, realizada de setembro a outubro de 2015, campanha 2 realizada de outubro a novembro de 2016, e a campanha 3 efetivada em outubro de 2017. Na Serra Norte foram amostrados 16 riachos, dos quais 12 foram catalogados como alterados e quatro riachos localizados distantes do efeito da usina como preservados. Na Serra Sul, oito riachos foram amostrados sendo classificados em quatro alterados e quatro preservados (Figura 1).

Em cada riacho amostrado foi demarcado um trecho fixo de 100 metros, dividido em 10 secções de 10 metros, compondo um total de 10 transecções subdivididas em 20 secções longitudinais de 5 metros (20 segmentos) (Shimano et al., 2018). Os indivíduos foram coletados usando um rapiché (coador com circunferência de 18 cm e malha de 0,05 mm) e fixados em álcool 85%, quantificados e identificados até ao nível taxonômico de gênero (Salles et al., 2004; Domínguez et al., 2006). Tal limitação na identificação se deve ao fato de que os espécimes coletados correspondem a indivíduos imaturos (larvas), e não apresentam completo desenvolvimento da genitália, caráter essencial para a identificação (Shimano, 2015, Shimano, et al., 2018).

5.3.3 Variáveis ambientais

Para avaliar a variação ambiental, usamos as dez variáveis limnológicas as quais foram escolhidas previamente no capitulo 1, após utilizar uma matriz de correlação para diminuir a multicolinearidade durante a realização das análises estatísticas. Temperatura e pH foram medidas em campo usando uma sonda multiparâmetro, e as variáveis, condutividade elétrica, demanda química de oxigênio, ferro total, fósforo total, manganês total, nitrato, oxigênio dissolvido e turbidez, foram obtidas em análise laboratorial mediante coleta de amostras de água em cada igarapé amostrado.

5.3.4 Características Funcionais (traits)

Os efemerópteros são insetos aquáticos que vivem em habitats de água doce e são organismos obrigatoriamente anfibióticos, com imaturos aquáticos (ninfas) e adultos terrestres alados. As ninfas de Ephemeroptera exibem uma variedade de estratégias alimentares (podem ser filtradoras, raspadoras, fragmentadoras, coletoras ou até mesmo predadoras) compondo a cadeia trófica dos ecossistemas aquáticos. Como imaturos, podem viver de algumas semanas a poucos anos, enquanto que os adultos não se alimentam, possuem as peças bucais atrofiadas e têm um curto período de vida, que em alguns casos não chega a mais de duas horas (Domínguez et al., 2006).

A estrutura da comunidade de macroinvertebrados bentônicos depende de vários fatores, como qualidade da água, tipo de substrato, tamanho de partícula de sedimento, fluxo de água, disponibilidade de matéria orgânica do sedimento, concentração de oxigênio e condições ambientais em torno do curso de água (Buss et al., 2004). Algumas morfoespécies de efemerópteros têm requisitos muito restritos em relação à sua tolerância à variação ambiental, por exemplo ao oxigênio dissolvido, pH, tipo de substrato, tamanho e fluxo e temperatura da água, devido à sua sensibilidade à poluição e à fragmentação de habitats (Rosenberg e Resh 1993).

Consequentemente, eles são morfologicamente adaptados ao ambiente aquático, e suas adaptações branquiais podem ser usadas para classificação e identificação de várias funções documentadas: Respiração (Beaver 1990, Eriksen & Maeur 1990); Osmorregulação (Wichard 1979; Wichard et al. 2002); Locomoção, Natação (Kluge et al., 1984); Circulação de água (Notestine 1994); Proteção (Cobertura branquial – Opérculo) (Notestine 1994).

Uma medida morfológica importante dos efemerópteros é a cabeça por ser usada para alimentação e também por ser uma medida estável em insetos (Goncalves et al., 2003), o que permite sua comparação entre gêneros. Da mesma forma, o mesonoto (tergito do segundo segmento torácico) das ninfas é importante no momento da identificação taxonômica (último estágio antes do desenvolvimento como indivíduos adultos, onde as tecas alares são desenvolvidas), sendo uma medida estável em ninfas maduras (Flowers e De La Rosa, 2010).A classificação dos grupos tróficos funcionais relacionados ao ecossistema foi baseada na literatura (Merritt e Cummins, 1996, Cummins et al., 2005, Shimano et al., 2012, Cruz, Salles e Hamada, 2013, Brasil et al., 2014, Dedieu et al., 2015), considerando o tipo de alimento.

Para a análise das respostas das características funcionais (avaliar mudanças), foram selecionadas cinco características relacionadas à ecologia (duas variáveis qualitativas) e morfologia dos insetos (três variáveis quantitativas) as quais são importantes nas funções de respiração, hábitos alimentares, proteção, resiliência (Tabela 1), e que tem importância para a permanência dos efemerópteros no ambiente.

Para realizar a matriz de características funcionais dos gêneros de Ephemeroptera, as características qualitativas foram baseadas na literatura (Tabela 1), e as características quantitativas (Tabela 1) foram medidas em laboratório. As características funcionais morfológicas foram medidas em pelo menos dois indivíduos de cada gênero, escolhendo as ninfas em fase madura ou também conhecidas como “ninfas maduras”. As medições dos indivíduos foram feitas para cada local de amostragem (riacho), e realizadas com o auxílio de um estéreomicroscópio (lupa) de marca LEICA, conectada a um computador que recebe as imagens de cada indivíduo, convertendo-as em fotos, através do programa LAS (Leica Apliccation Software). O programa LAS permite obter as medidas necessárias para as características morfológicas escolhidas. A medida foi padronizada em milímetros (mm) para todas as características (Tabela 1).

Usando o programa LAS, tiramos a foto de cada indivíduo, e as medições foram registradas compondo a matriz de características morfológicas (Material Suplementar – Tabela I).

Tabela 1. Características funcionais (traits) dos gêneros de Ephemeroptera.

Tipo	**Característica funcional (trait)**	**Quantitativa**	**Qualitativa**	**Categoria**	**Função**	**Referências**
Ecologia	Tipo de alimentação - Grupo Funcional (FFG)		x	Coletor Filtrador Raspador Predador Cortador	Balance na cadeia alimentar	Merritt e Cummins, 1996, Cummins et al., 2005, Shimano et al., 2012, Cruz, Salles e Hamada, 2013, Brasil et al., 2014, Dedieu et al., 2015.
	Presença de brânquia opercular		x	Sim Não	Resiliência em habitats, respiração proteção	Domínguez et al., 2006, Zhou & Zheng., 2010. Salles et al., 2018.
Morfologia	Tamanho da cabeça	x		Comprimento Largura	Alimentação - aproveitamento dos recursos alimentares de cada hábitat.	Merritt e Cummins, 1996, Goncalves et al, 2003, Shimano et al., 201, Domínguez et al., 2006, Salles et al., 2018.
	Tamanho do mesonoto (tergito do segundo segmento torácico)	x		Largura	Ciclo de vida (desenvolvimento da teca alar) - tamanho da ninfa	Domínguez et al., 2006, Flowers e De La Rosa, 2010, Salles et al., 2018.

5.4 ANÁLISES ESTATÍSTICAS

Baseado nas análises estatísticas usadas no capitulo 1, realizamos uma correlação de Pearson e uma Análise de Componentes Principais (PCA) das variáveis limnológicas. A correlação de Pearson (> 0,7) foi feita a fim de excluir as variáveis limnológicas das análises de diversidade evitando problemas associados à colinearidade dos dados (Juen et al., 2016, Luiza-Andrade et al., 2017). Usando a correlação de Pearson foram escolhidas 10 variáveis limnológicas condutividade elétrica, demanda química de oxigênio, ferro total, fósforo total, manganês total, nitrato, oxigênio dissolvido, pH, temperatura e turbidez para utilização nas análises.

Para avaliar a variação ambiental dos riachos foi realizada uma Análise de Componentes Principais (PCA) usando as variáveis obtidas através da correlação de Pearson. As variáveis ambientais foram previamente padronizadas buscando diminuir os possíveis efeitos dos *outliers* (Clarke e Gorley, 2006). Para seleção do número de eixos para interpretação foi usado o critério de "Broken-stick". Para determinar o quanto cada variável contribuiu para a formação dos eixos, foram estimados os scores das amostras, scores das variáveis e os *loadings*. Para avaliar os agrupamentos formados usamos uma Análise Permutacional Multivariada de Variância (PERMANOVA) (Clarke e Gorley, 2006). Foi avaliada também a heterogeneidade ambiental entre os tratamentos através de um teste multivariado de homogeneidade das dispersões (PERMDISP, Anderson et al., 2006) (α <0,05) calculado a partir da distância do centroide dos scores gerados na PCA.

A diversidade funcional foi estimada usando a Entropia quadrática de Rao (Rao 1982) ou RaoQ, na qual as distâncias entre os pares de taxa são calculadas primeiro usando a matriz de distancias, e então, integrando a abundância relativa (RaoQabu) ou riqueza (RaoQcomp, composição) de táxons (Rao, 1982, Petchey e Gaston, 2006, Pavoine et al., 2009, Péru e Dolédec, 2010). A matriz de distância entre as características funcionais dos gêneros foi calculada utilizando uma modificação da distância de Gower, o coeficiente de distância para variáveis mistas proposto por Pavoine et al. (2009). As diferenças dos valores de RaoQabu e RaoQcomp entre os riachos e os distintos tratamentos (preservado/alterado) foram testadas por Teste de T.

A diversidade beta funcional foi estimada pelo índice de Sørensen (bsor, Legendre & Legendre 1998), baseado nos métodos sugeridos por Dunck et al. (2015, 2019). Foi utilizada uma matriz de presença-ausência derivada da uma matriz quantitativa contendo 27 gêneros de Ephemeroptera e um número amostral de 24 amostras (três campanhas de amostragem). Utilizamos o índice de Sørensen (bsor; Legendre e Legendre, 1998) para calcular a dissimilaridade funcional na composição de gêneros entre todos os pares de riachos. A matriz de distância para os traços funcionais dos gêneros foi calculada utilizando o coeficiente de distância para variáveis mistas proposto por Pavoine et al. (2009). Essa matriz de distância foi transformada em um dendrograma usando o método de agrupamento (UPGMA). Esse dendrograma foi utilizado em conjunto com a matriz presença-ausência de gêneros para calcular a dissimilaridade funcional através do índice de Sørensen (bsor) adaptado às características funcionais (Melo, 2013).

A diversidade beta funcional (bsor) foi particionada em dois componentes que explicam a dissimilaridade obtida por turnover (bsim) e a dissimilaridade baseado nas diferenças na riqueza (aninhamento ou bnes), e esta análise foi realizada de acordo com Melo (2013). Assim, o componente de turnover é obtido calculando o índice de dissimilaridade de Simpson (bsim), enquanto o componente resultante do aninhamento (bnes) é a diferença entre bsor e bsim. Testes t pareados foram usados para avaliar as diferenças entre as médias de bsim e bnes. Para realização desse teste, as matrizes triangulares foram previamente transformadas em vetores para que fosse possível a comparação.

A dissimilaridade ambiental entre os riachos foi calculada usando a distância euclidiana após centralizar cada variável por sua média e escalar cada variável por seu desvio padrão (Legendre e Legendre, 1998). A distância espacial entre todos os pares de amostras foi obtida calculando a distância euclidiana usando uma matriz de coordenadas geográficas (distância fluvial). A relação entre as dissimilaridades ambientais, espaciais (distâncias geográficas) e a diversidade beta funcional dos riachos foi testada através de teste de Mantel parcial com 999 permutações (Mantel, 1967, Legendre e Legendre, 2012).

Para identificar as relações entre o ambiente e as características funcionais (traits) foi realizada uma análise RLQ (Dolédec et al., 1996, Dray et al, 2014). Esta análise é um método multivariado que se fundamenta na ordenação de três matrizes que incluem as variáveis ambientais (R), a abundância das espécies (L) e suas características funcionais ou traits (Q) (Borcard et al., 2018). Primeiro, foi realizada a análise de correspondência (CA) a partir das abundâncias (logaritmizadas) dos gêneros (L), fornecendo uma ordenação simultânea de amostras e táxons. Depois, uma análise de componentes principais (PCA) foi realizada usando variáveis ambientais escolhidas usando correlação de Pearson (R) padronizadas mediante o comando "*standardize*" para homogeneização das escalas das diferentes unidades usadas para mensurar cada variável (Clarke e Gorley, 2006). Em seguida, a partir das características funcionais (Q) foi feita uma análise de "Fuzzy correspondence analysis" (FCA, Chevenet et al., 1994). A análise RLQ (Dolédec et al., 1996) foi combinada com o análise Fourth-corner (Legendre et al. 1997), como proposto por Dray et al. (2014), utilizada para testar por permutações quais variáveis ambientais influenciaram as características funcionais dos gêneros de Ephemeroptera, usando o Modelo 2 e o Modelo 4 (Legendre et al. 1997; Dray et al. 2014; Luiza-Andrade et al. 2017).

Todas as análises foram executadas no software R (R Development Core Team, 2016). O pacote *FD* (Laliberté, Legendre e Shipley, 2014) foi usado para calcular RaoQ, os pacotes *ade4* (Chessel et al., 2004) e *picante* (Kembel et al., 2010) foram utilizados para construir a matriz de distância funcional e o dendrograma funcional, o pacote *betapart* (Baselga et al., 2013) e *CommEcol* (Melo, 2013) foram usados para o cálculo de bsor (Oksanen et al., 2013), o pacote *vegan* para o teste de Mantel (Oksanen et al. 2016), e o pacote *ade4* para análise de RLQ (Dray e Dufour 2007).

5.5 RESULTADOS

Em geral, durante as três campanhas de amostragem, foram amostrados 2.259 indivíduos pertencentes a 32 gêneros da ordem Ephemeroptera. Do total de gêneros, 27 foram utilizados para a análise da diversidade funcional e beta funcional, sendo escolhidos dois indivíduos aleatórios por gênero, de acordo com seu último estágio larval ("ninfa madura") antes de se tornarem adultos, representando um total de 2.153 indivíduos (Tabela 2). Em relação à abundância de indivíduos por tratamento, o grupo alterado apresentou maior abundância mostrando um total de 1.330 indivíduos, e contrastando com o grupo preservado que apresentou 823 indivíduos. O gênero *Miroculis* Edmunds, 1963 (n=639) foi o mais abundante, seguido pelo gênero *Fittkaulus* Savage & Peters, 1978 (n=347), e pelos gêneros *Zelusia* Lugo-Ortiz & Mccafferty, 1998 (n=198), *Cloeodes* Traver, 1938 (n=151), *Hydrosmilodon* Flowers & Domínguez, 1992 (n=144), que apresentaram maior frequência nos riachos de forma geral. Já para o gênero *Farrodes* Peters, 1971 foi registrado apenas dois indivíduos nos riachos alterados (Tabela 2).

Tabela 2. Lista de gêneros usados para estimar a diversidade funcional e beta funcional.

Gênero	**Abundância/Tratamento**		
	Alterado	**Preservado**	**Total**
Amanahyphes Salles & Molineri, 2006	14	3	17
Americabaetis Kluge, 1992	29	14	43
Askola Peters, 1969	13	18	31
Baetodes Needham & Murphy, 1924	19	18	37
Brasilocaenis Malzacher, 1986	12	7	19
Caenis Malzacher, 1986	59	12	71
Callibaetis Eaton, 1881	13	1	14
Callibaetoides Cruz, Salles & Hamada, 2013	6	2	8
Campylocia Needham & Murphy, 1924	16	10	26
Cloeodes Traver, 1938	92	59	151
Farrodes Peters, 1971	2	0	2
Fittkaulus Savage & Peters, 1978	217	130	347

Hagenulopsis Ulmer, 1920	1	4	5
Hydrosmilodon Flowers & Domínguez, 1992	95	49	144
Leptohyphes Ulmer, 1919	21	3	24
Miroculis Edmunds, 1963	396	243	639
Paracloeodes Day, 1955	12	3	15
Paramaka Savage & Domínguez, 1992	44	48	92
Simothraulopsis Demoulin, 1966	16	4	20
Terpides Demoulin, 1966	23	35	58
Thraulodes Ulmer, 1920	4	8	12
Traverhyphes Molineri, 2001	37	9	46
Tricorythodes Ulmer, 1920	7	22	29
Tricorythopsis Traver, 1958	10	1	11
Ulmeritoides Traver, 1959	33	54	87
Waltzoyphius Lugo-Ortiz& Mccafferty, 1995	5	2	7
Zelusia Lugo-Ortiz & Mccafferty, 1998	134	64	198
Total Geral	**1330**	**823**	**2153**

5.5.1 Variação ambiental

A análise de Componentes Principais (PCA) em seu primeiro eixo apresentou um percentual de explicação de 19%, e as variáveis ambientais Manganês Total (MangTot) e Ferro Total (FerroTot) contribuíram negativamente para a formação do eixo 1 e apresentaram maior variação (Figura 2, Tabela 4). Houve diferenças entre os dois tratamentos dos riachos (PERMANOVA, Pseudo-F= 2,515; p= 0,001*), mostrando uma maior relação das variáveis Manganês Total (MangTot) e Ferro Total (FerroTot) nos riachos alterados. Porém não foi observada diferença na heterogeneidade dos ambientes (PERMIDISP: F= 0,755; p = 0,388) (Figura 2, Tabela 3).

Tabela 3. Análise de Componentes Principais (PCA) das variáveis ambientais de riachos preservados e alterados na Flona de Carajás, Pará – PA, Brasil. As variáveis associadas a variação ambiental estão em negrito, considerando uma relação superior a 0.5.

Variáveis ambientais	Eixo 1	Eixo 2
Condutividade Elétrica	-0.01	0.07
Demanda Química de Oxigênio	-0.24	-0.57
Ferro Total	**-0.63**	0.21
Fosforo Total	0.12	0.04

Variáveis ambientais	Eixo 1	Eixo 2
Manganês Total	**-0.51**	0.19
Nitrato	-0.21	-0.62
Oxigênio Dissolvido	0.1	0.12
pH	0.24	0.28
Temperatura	-0.3	0.12
Turbidez	-0.26	0.31
Autovalor	1.90	1.68
Broken-stick	2.92	1.92
% de Explicação	19	16

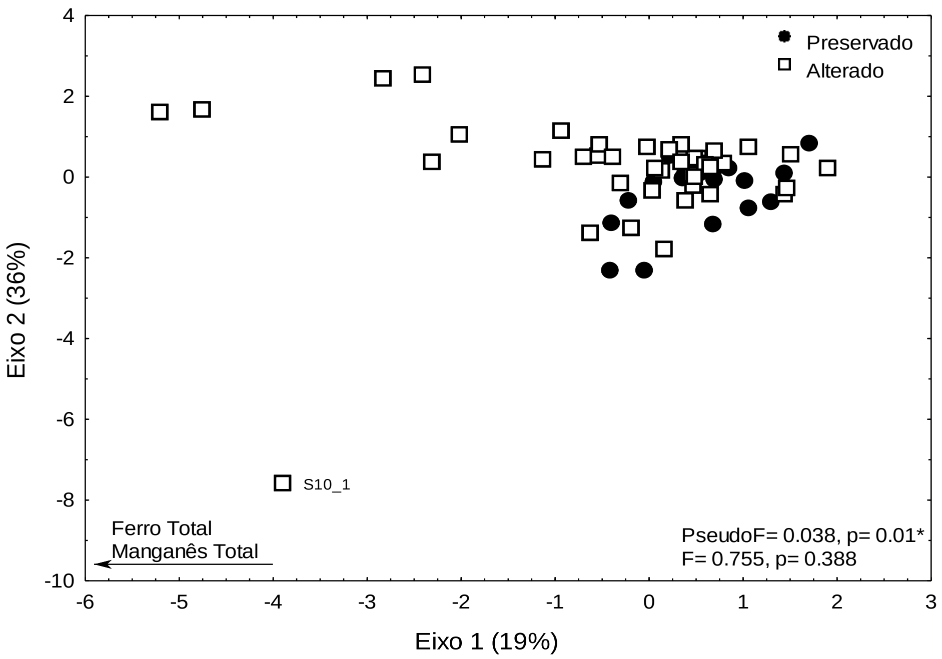

Figura 2 Análise de Componentes Principais (PCA) das variáveis ambientais. Os círculos da cor preta correspondem aos pontos de amostragem dos igarapés preservados e os quadrados indicam os pontos de amostragem dos igarapés alterados, Flona de Carajás, Pará – PA, Brasil.

5.5.2 Diversidade funcional

Os valores de diversidade funcional, estimados pelo índice RaoQ para abundância (RaoQabu), variaram de 0 (min) a 0,15 (máx), com valores médios para ambientes preservados de 0,087 e para ambientes alterados de 0,091. Os valores de RaoQabu não diferiram entre os tratamentos (t = 0,520, df = 61, p = 0,60,). Os resultados da diversidade funcional considerando a composição (RaoQcomp), variou de 0 (min) a 0,15 (máx), com valores médios de 0,103 para ambientes preservados e de 0,108 para ambientes alterados. Os valores de RaoQabu não diferiram entre os tratamentos (t = 0,530, df = 61, p = 0,59). Deve-se mencionar que ambos resultados foram semelhantes.

5.5.3 Diversidade beta funcional

A diversidade beta funcional apresentou valores que variaram de 0 a 0,78, com média de 0,31 (± 0,14 DP) para bsor, média de 0,10, (±0,10 DP) para bsim, e média de 0,21 (± 0,17 DP) de bnes. A diversidade beta funcional (bsor) foi distinta entre os riachos preservados e alterados (t= 95,153, df= 1952, p= <0,001), e foi maior nos riachos alterados (Figura 4). Os resultados da diversidade beta funcional demonstraram que o componente de turnover (bsim) foi menor que o componente aninhamento (bnes) (t = 42,677, df = 1952, p = <0,001) (Figura 3).

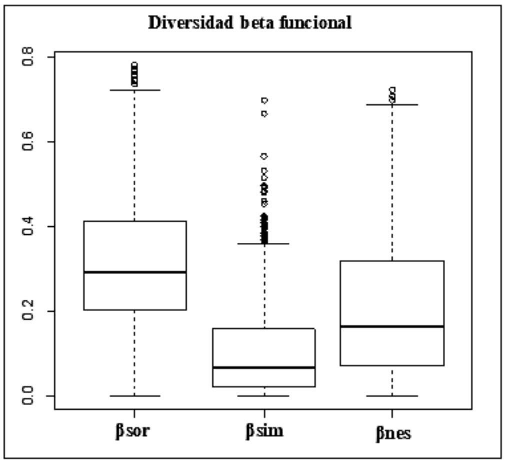

Figura 3 Diversidade beta funcional dos gêneros de Ephemeroptera para as três campanhas de amostragem. A linha no meio dentro de cada boxplot indica o valor da média de cada tratamento, as linhas superior e inferior do boxplot indicam os quartiles, as linhas ponteadas indicam a variação dos dados, e as bolinhas indicam os valores overloading. Significado: βsor: diversidade beta total, βsim: turnover, βnes: aninhamento.

Em riachos preservados os valores de diversidade beta funcional em variaram entre 0 (min) e 0,72 (máx), média 0,30 (± 0,14 DP) para bsor, média de 0,10 ((± 0,09 DP) e 0,20 (± 0,16 DP) de bnes, em em riachos alterados a diversidade beta funcional variou entre 0 (min) e 0,77 (máx), com média de 0,32 (± 0,14 DP) de bsim e 0,21 (± 0,17 DP) de bnes. Avaliando cada tratamento isoladamente, tanto os riachos preservados (t = 15,774, df = 230, p = 0,001, Figura 4A), quanto os riachos alterados (t = 12.387, df = 779, p = 0,001, Figura 4B) apresentaram maior aninhamento funcional.

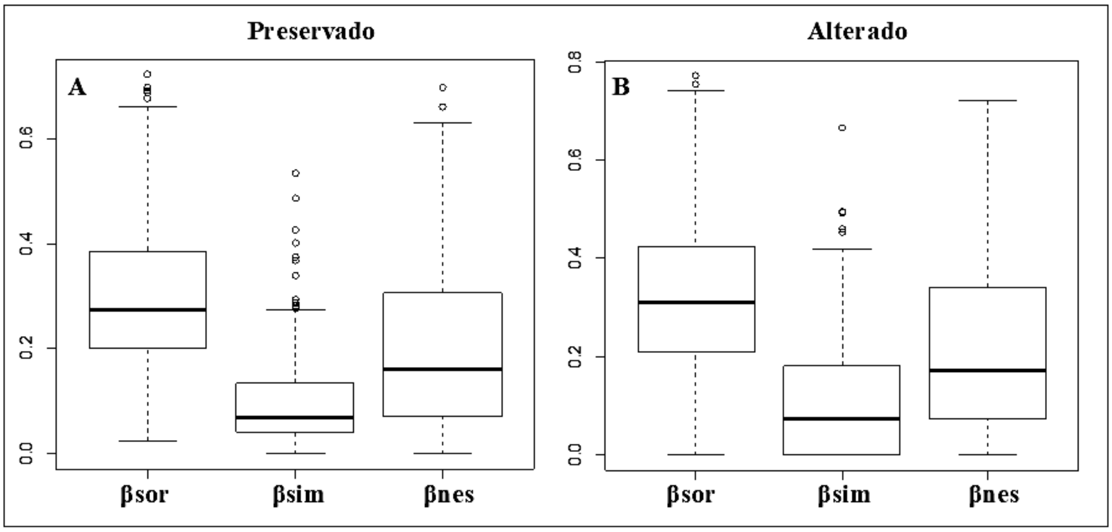

Figura 4 Componentes da beta diversidade funcional (Bsim e Bnes) entre os riachos com tratamentos alterados e preservados. A linha no meio dentro de cada boxplot indica o valor da média de cada tratamento, as linhas superior e inferior do boxplot indicam os quartiles, as linhas ponteadas indicam a variação dos dados, e as bolinhas indicam os valores overloading. A: Preservado, B: Alterado. Significado: βsor: diversidade beta total, βsim: turnover, βnes: aninhamento.

O teste de Mantel parcial não confirmou uma correlação positiva entre a diversidade beta funcional, variação ambiental e a variação espacial em todas as campanhas (Campanha 1 - ambiental: R = 0,425, p = 0,017, espacial: R = -0,020, p = 0,566; Campanha 2 - ambiental: R = 0,066, p = 0,06, espacial: R = -0,086, p = 0.855; Campanha 3 - ambiental: R = 0,53, p = 0,005, espacial: R = -0,033, p = 0,639).

5.5.4 RLQ

Os dois primeiros eixos do RLQ apresentaram autovalores de 0,07 e 0,02 para os eixos 1 e 2, respectivamente, representaram 63% e 21% da coinertia (Figura 5A, Tabela 4). O primeiro eixo foi correlacionado negativamente com turbidez e pH e positivamente com sólidos dissolvidos totais, ferro total, nitrato, oxigênio dissolvido e manganês total. O eixo 2 está correlacionado positivamente à demanda bioquímica de oxigênio e temperatura (Figura 5A, Tabela 4). Para as características funcionais, o primeiro eixo foi correlacionado com a guilda trófica coletor, e o segundo eixo mostrou correlações com os outros traits (tc1: tamanho de comprimento da cabeça; gt=guilda trófica, filtrador, brânquia opercular, brânquia não opercular) (Figura 5B, Tabela 4). Os testes de permutação Modelo 2 e 4 não mostraram relações significativas entre as variáveis ambientais e as características funcionais (Modelo RLQ 2, r= 0,122, p = 0.173; Modelo RLQ 2, r= 0,122, p = 0.078) (Tabela 4).

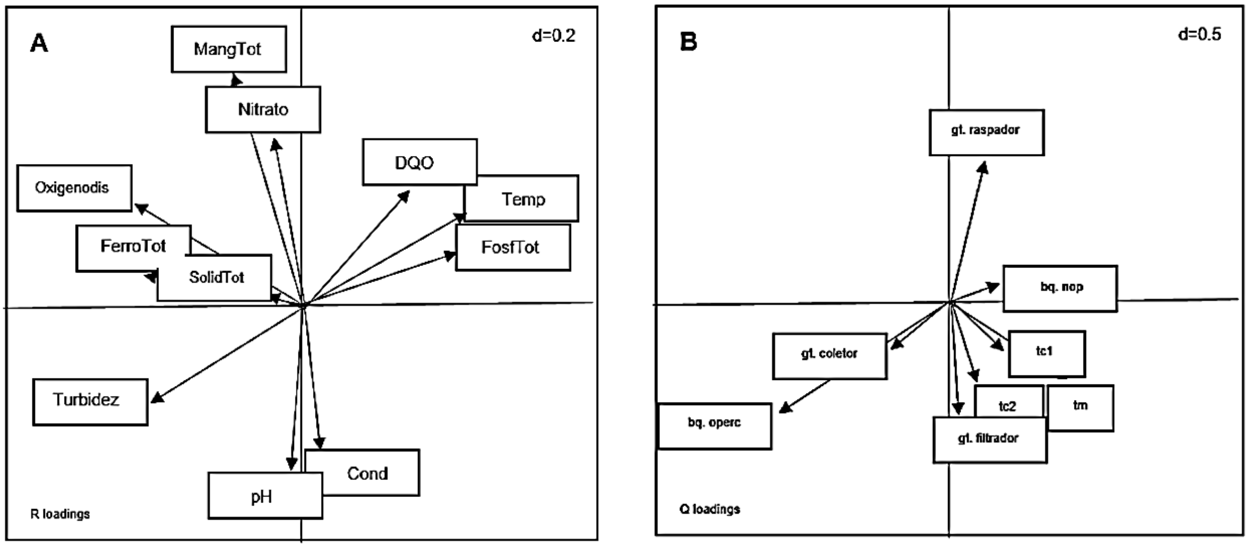

Figura 5 Ordenação dos valores do RLQ das variáveis ambientais (A) e as características funcionais (B). Variáveis ambientais: condutividade elétrica (Cond), demanda química de oxigênio (DQO), ferro total (Ferrotot), fósforo total (Fosftot), manganês total (Mangtot), nitrato, oxigênio dissolvido (Oxigendis), temperatura (Temp), sólidos totais (SolidTot), pH e turbidez. Características funcionais: tc1 (tamanho de comprimento da cabeça), tc2 (tamanho da largura da cabeça), tm (tamanho do mesonoto), gt=guilda trófica, raspador, coletor, filtrador, brânquia opercular, brânquia não opercular.

Já a análise de Fourth-corner demonstrou relações significativas das variáveis ambientais com as características funcionais, sendo positiva (aumento da variável ambiental produz aumento na caraterística funcional) para relação brânquia opercular/ferro total e brânquia opercular/turbidez (r= 0,115; p= 0.016), e negativa (aumento da variável ambiental produz aumento na caraterística funcional) para as interações brânquia não opercular/ferro total, brânquia não opercular/turbidez (r= -0,115; p= 0.016) e guilda trófica – filtrador/manganês total (r= -0,075, p= 0,051) (Tabela 4, Figura 6). Na Tabela 5 foram incluídos apenas os valores significativos das relações entre as variáveis ambientais e características funcionais. Todas as relações possíveis entre as variáveis ambientais e características funcionais são mostradas na Tabela II do Material Suplementar.

Tabela 5 Análise RLQ – Fourth-corner - Ephemeroptera para as três campanhas de amostragem. bqop: brânquia opercular, bnop: brânquia não opercular, gt: guilda trófica.

RLQ	**Porcentagem de explicação (%)**	
	Eixo1	**Eixo2**
Autovalor	0.077	0.025
Covariância	0.279	0.16
Correlação	0.142	0.096
R/RLQ	1.466	0.675
L/RLQ	0.142	0.311

RLQ	Porcentagem de explicação (%)	
	Eixo1	**Eixo2**
Q/RLQ	2.607	0.757
Modelos	**r**	**p<0.05**
Modelo 2	0.122	0.173
Modelo 4	0.122	0.078
Fourth-corner	**r**	**p<0.001**
bqop/ferro total	0.09	0.053
bqop/turbidez	0.115	**0.016***
bnop/ferro total	-0.09	0.053
bnop/turbidez	-0.115	**0.016***
gt-filtrador/manganês total	-0.075	0.051

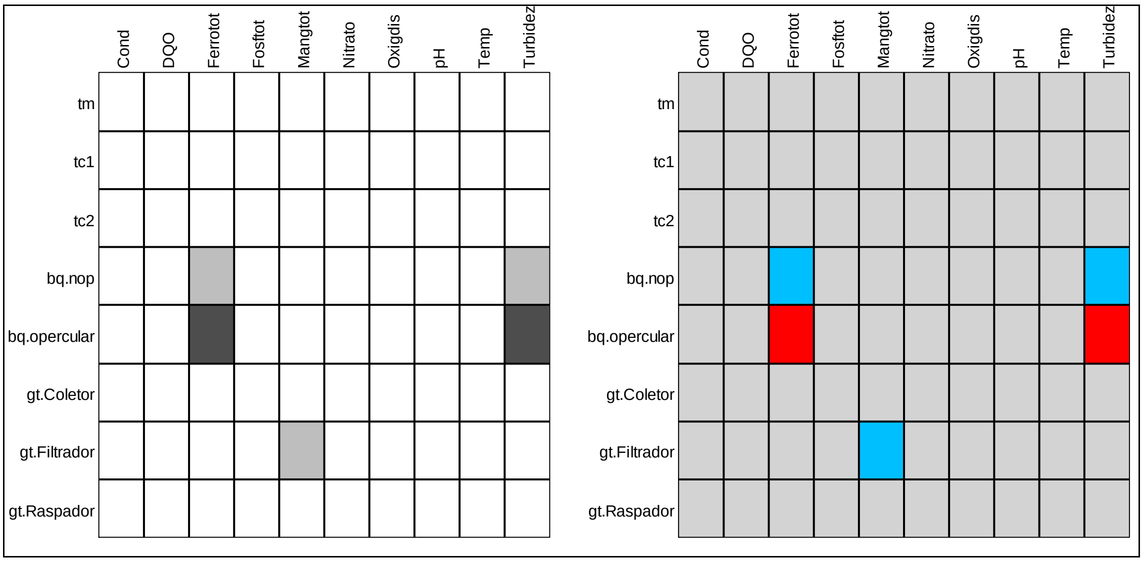

Figura 6 Resultados da análise Fourth-corner com as relações entre as variáveis ambientais e as características funcionais. Células cinza e preta mostram as relações significativas, células vermelhas indicam relações significativas positivas, e células azuis indicam relações significativas negativas.

5.6 DISCUSSÃO

Nossos resultados indicaram que ambientes alterados por mineração no período estudado (entre anos de 2015 a 2017) apresentaram diferenças nas características limnológicas quando comparados com os riachos preservados, principalmente com maiores valores de manganês e ferro. Nosso estudo também demonstrou que a diversidade funcional dos indivíduos de Ephemeroptera não diferiu entre os tratamentos avaliados, o que não corroborou nossa hipótese “a”. Porém, quando avaliada a diversidade beta funcional, verificamos aninhamento funcional nesses riachos.

Sabe-se que o aninhamento e padrões causados pelo turnover não são mutuamente exclusivos (Lennon et al. 2001, Baselga 2010). Os padrões observados de maior aninhamento funcional pode indicar que as características funcionais das assembleias dos riachos nessa área de mineração são um subconjunto das características funcionais de assembléias mais ricas (Baselga 2010; Villéger et al. 2013) adjacentes à essa área.

Nesse contexto podemos deduzir que na área de estudo, as atividades de mineração potencialmente produzem efeitos negativos na composição funcional das assembleias de Ephemeroptera. Aninhamento é um padrão observado que se refere à perda ordenada de organismos em uma assembleia (Patterson e Atmar, 1986; Wright et al. 1998). Estudos têm apontado que as comunidades que mostram um padrão aninhado de distribuição de gêneros, apresentam espécies comuns que podem ocorrer em todas as comunidades locais, enquanto espécies raras ou menos frequentes tendem a ocorrer apenas nas mais diversas (Soininen, 2008; Soininen e Köngäs, 2012). A perda gradual de espécies e de características funcionais que gera um padrão aninhado é influenciada por muitos fatores que podem levar à extinção de espécies ou colonização seletiva (Atmar e Patterson, 1993), e estes resultados mostraram que as comunidades na nossa área de estudo possivelmente estão sob os efeitos de alguma alteração antrópica.

Nossos resultados de aninhamento funcional indicaram que os riachos estão perdendo variabilidade de características funcionais das espécies, e que as comunidades estão se tornando mais homogêneas funcionalmente. A homogeneização funcional pode ser definida como uma maior similaridade funcional devido ao estabelecimento de espécies com características semelhantes ("trabalhos" similares no ecossistema) e à perda de espécies não redundantes para determinar a função (Olden, 2006). Isso é considerado um processo de alteração ecológica, porque a homogeneização funcional produz a troca de espécies sensíveis pelas espécies tolerantes, o que produz um declínio da diversidade, e pode alterar vários aspectos processos e serviços ecossistêmicos (Olden et al., 2011; Petsch, 2018).

Nesse contexto, é necessário que nessas áreas tenha prioridade para a conservação, para que não sejam perdidas mais espécies e características funcionais locais (Gianuca et al., 2017). As atividades de exploração de minérios devem manter pontos preservados com melhores estratégias para que a manutenção da biodiversidade seja mantida.

Alguns estudos evidenciaram que o aumento da dissimilaridade ambiental e espacial podem aumentar a diversidade beta funcional em comunidades aquáticas distintas (Dunck et al. 2016). Porém nossos resultados não demonstraram que o aumento da distância espacial ou aumento na dissimilaridade ambiental favoreceu maior diversidade beta funcional, como indicado para algumas comunidades aquáticas (Heino e Soininen 2010). Possivelmente a variação ambiental, que foi pequena entre os riachos, apenas para manganês e ferro, não proporcionou variação da diversidade beta funcional. Como a distribuição de organismos bentônicos em ecossistemas lóticos está fortemente relacionada à corrente da água, qualidade e disponibilidade de alimentos, tipo de substrato, e às variáveis limnológicas como oxigênio dissolvido (Palmer et al., 1994; Townsend et al., 1997), acredita-se que estes resultados seriam distintos se estas variáveis de habitat apresentassem grande variação entre os riachos alterados e preservados no nosso estudo.

Nossos resultados (RLQ) demonstraram que a característica coletor é fortemente influenciada pela disponibilidade de nutrientes, e que o aumento da turbidez pode prejudicar negativamente sua distribuição. A presença de brânquia opercular pode proporcionar melhor estabelecimento em ambientes alterados. Zhou & Zheng (2010) indicaram que as ninfas dos efemerópteros estão morfologicamente adaptadas e suas adaptações branquiais têm várias funções, como por exemplo, para a respiração (Eriksen & Maeur, 1990), e para proteção das brânquias inferiores (cobertura branquial – brânquia opercular), como, por exemplo, nos indivíduos da família Caenidae (Notestine, 1994). Os gêneros *Amanahyphes, Brasilocaenis, Caenis; Leptohyphes*, *Tricorythodes, Traverhyphes, Tricorythopsis*, apresentaram essa característica (brânquia opercular) e, portanto, podem se estabelecer melhor em riachos alterados com águas mais paradas, como em nosso estudo, ambientes com sedimentação ou maior turbidez, do que os gêneros que não tem este tipo de brânquia.

Nossos resultados também demonstraram que o aumento de manganês total causou a diminuição de gêneros filtradores. Algumas ninfas de Ephemeroptera alimentam-se de detritos e, portanto, são altamente sensíveis a alterações no substrato e a qualquer material tóxico que entre na água (Landa e Soldán, 1995). A presença de metais tais como ferro e o manganês são indicadores de poluição orgânica (Baker et al., 2019) e podem ser tóxicos para os organismos aquáticos. Nossos resultados confirmaram o efeito negativo que estas variáveis podem proporcionar na diminuição de organismos sensíveis a essa alteração.

Concluimos que os impactos de mineração podem ser observados em nível de características funcionais, principalmente na variação (beta) entre comunidades de ambientes sob distintos impactos ambientais, e na relação de características funcionais com as variáveis limnológicas. As análises realizadas durante o presente estudo demostraram que a diversidade beta funcional e seus componentes foram ferramentas que permitem demonstrar os efeitos de origem antrópica sobre a composição e as características funcionais dos gêneros de Ephemeroptera. Enfatizamos que a proteção das áreas consideradas mais preservadas deve ser priorizada, visto que foi possível perceber homogeneização funcional das comunidades avaliadas.

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5.9 MATERIAL SUPLEMENTAR

Tabela I. Matriz de medidas morfológicas (quantitativas) e ecológicas (qualitativas) dos géneros de Ephemeroptera utilizadas nas análises de dados. nop: não opercular.

		Características funcionais			
Gênero	**Tamanho do mesonoto (mm)**	**Tamanho do comprimento – cabeça (mm)**	**Tamanho de largura da cabeça (mm)**	**Brânquia opercular e não opercular**	**Guilda Trófica**
Amanahyphes	1.0927	1.0639	1.2916	opercular	Coletor
Americabaetis	1.7641	1.6960	1.3551	nop	Coletor
Askola	1.5268	1.7863	1.7789	nop	Raspador
Baetodes	1.8086	1.7966	1.6062	nop	Raspador
Brasilocaenis	1.0979	0.7180	1.0089	opercular	Raspador
Caenis	1.5424	0.6335	1.1103	opercular	Raspador
Callibaetis	2.6752	2.4865	1.6135	nop	Coletor
Callibaetoides	1.3321	0.9958	1.2417	nop	Coletor
Campylocia	5.0676	3.5543	3.9238	nop	Coletor
Cloeodes	1.5588	1.7956	1.3045	nop	Raspador
Farrodes	1.2613	1.6843	1.7962	nop	Raspador
Fittkaulus	1.2350	1.8333	1.7538	nop	Filtrador
Hagenulopsis	1.1781	1.5836	1.5489	nop	Raspador
Hydrosmilodon	2.8275	3.4915	3.9132	nop	Filtrador
Leptohyphes	1.5827	1.2812	1.7736	opercular	Coletor
Miroculis	1.4309	1.8704	2.0258	nop	Raspador
Paracloeodes	2.4072	1.2634	1.8363	nop	Coletor
Paramaka	2.5768	2.9620	3.3622	nop	Filtrador
Simothraulopsis	2.2461	2.7761	2.4526	nop	Raspador
Terpides	1.5304	1.3502	1.6671	nop	Filtrador
Thraulodes	2.2046	2.0062	2.4583	nop	Raspador
Traverhyphes	2.3971	1.1882	1.7749	opercular	Coletor
Tricorythodes	1.6765	0.9332	1.3513	opercular	Coletor
Tricorythopsis	2.2291	1.1860	1.8027	opercular	Coletor
Ulmeritoides	1.3773	1.3085	1.6575	nop	Filtrador
Waltzoyphius	1.5969	1.4910	1.1333	nop	Coletor
Zelusia	1.3818	0.8606	1.0305	nop	Coletor

Tabela II. Análise Fourth-corner - Relações entre as variáveis ambientais e as características funcionais calculadas para os gêneros de Ephemeroptera.

Item	Variável ambiental	Característica funcional	Observados	DP observado	Alteração	p
1	Cond	tm	0.023	0.76	two-sided	0.461
2	DQO	tm	0.011	0.20	two-sided	0.863
3	Ferrotot	tm	-0.011	-0.33	two-sided	0.738
4	Fosftot	tm	0.012	0.45	two-sided	0.655
5	Mangtot	tm	-0.030	-0.96	two-sided	0.335
6	Nitrato	tm	-0.048	-1.51	two-sided	0.134
7	Oxigdis	tm	-0.002	-0.05	two-sided	0.966
8	pH	tm	0.061	1.80	two-sided	0.061
9	Temp	tm	-0.002	-0.07	two-sided	0.948
10	Turbidez	tm	-0.011	-0.33	two-sided	0.742
11	Cond	tc1	0.020	0.45	two-sided	0.638
12	DQO	tc1	0.019	0.44	two-sided	0.666
13	Ferrotot	tc1	-0.042	-0.96	two-sided	0.329
14	Fosftot	tc1	0.046	1.09	two-sided	0.247
15	Mangtot	tc1	-0.067	-1.57	two-sided	0.113
16	Nitrato	tc1	-0.042	-0.94	two-sided	0.328
17	Oxigdis	tc1	0.010	0.24	two-sided	0.815
18	pH	tc1	0.021	0.48	two-sided	0.638
19	Temp	tc1	0.025	0.56	two-sided	0.574
20	Turbidez	tc1	-0.051	-1.16	two-sided	0.225
21	Cond	tc2	0.028	0.69	two-sided	0.488
22	DQO	tc2	0.010	0.19	two-sided	0.853
23	Ferrotot	tc2	-0.014	-0.34	two-sided	0.735
24	Fosftot	tc2	0.038	0.94	two-sided	0.339
25	Mangtot	tc2	-0.063	-1.55	two-sided	0.126
26	Nitrato	tc2	-0.050	-1.20	two-sided	0.233
27	Oxigdis	tc2	0.013	0.33	two-sided	0.748
28	pH	tc2	0.023	0.55	two-sided	0.587
29	Temp	tc2	0.006	0.14	two-sided	0.891
30	Turbidez	tc2	-0.028	-0.66	two-sided	0.498
31	Cond	opercular	0.004	0.11	two-sided	0.919
32	DQO	opercular	-0.042	-0.87	two-sided	0.387
33	Ferrotot	opercular	0.090	1.85	two-sided	0.053
34	Fosftot	opercular	-0.063	-1.30	two-sided	0.186
35	Mangtot	opercular	0.027	0.56	two-sided	0.625
36	Nitrato	opercular	-0.018	-0.36	two-sided	0.735
37	Oxigdis	opercular	0.039	0.79	two-sided	0.437
38	pH	opercular	0.022	0.45	two-sided	0.657
39	Temp	opercular	-0.054	-1.09	two-sided	0.284
40	**Turbidez**	**opercular**	**0.116**	**2.39**	**two-sided**	**0.016**
41	Cond	nop	-0.004	-0.11	two-sided	0.919

42	DQO	nop	0.042	0.87	two-sided	0.387
43	Ferrotot	nop	-0.090	-1.85	two-sided	0.053
44	Fosftot	nop	0.063	1.30	two-sided	0.186
45	Mangtot	nop	-0.027	-0.56	two-sided	0.625
46	Nitrato	nop	0.018	0.36	two-sided	0.735
47	Oxigdis	nop	-0.039	-0.79	two-sided	0.437
48	pH	nop	-0.022	-0.45	two-sided	0.657
49	Temp	nop	0.054	1.09	two-sided	0.284
50	**Turbidez**	**nop**	**-0.116**	**-2.39**	**two-sided**	**0.016**
51	Cond	Coletor	0.015	0.43	two-sided	0.677
52	DQO	Coletor	0.000	0.00	two-sided	1.000
53	Ferrotot	Coletor	0.035	0.97	two-sided	0.344
54	Fosftot	Coletor	-0.032	-0.91	two-sided	0.382
55	Mangtot	Coletor	0.055	1.58	two-sided	0.114
56	Nitrato	Coletor	-0.017	-0.49	two-sided	0.636
57	Oxigdis	Coletor	-0.022	-0.62	two-sided	0.545
58	pH	Coletor	0.045	1.26	two-sided	0.209
59	Temp	Coletor	-0.010	-0.27	two-sided	0.792
60	Turbidez	Coletor	0.078	1.49	two-sided	0.142
61	Cond	Raspador	-0.015	-0.37	two-sided	0.733
62	DQO	Raspador	0.106	1.64	two-sided	0.096
63	Ferrotot	Raspador	-0.024	-0.57	two-sided	0.579
64	Fosftot	Raspador	0.080	1.52	two-sided	0.127
65	Mangtot	Raspador	0.032	0.79	two-sided	0.463
66	Nitrato	Raspador	0.008	0.21	two-sided	0.840
67	Oxigdis	Raspador	0.057	1.34	two-sided	0.181
68	pH	Raspador	0.012	0.27	two-sided	0.800
69	Temp	Raspador	0.027	0.62	two-sided	0.541
70	Turbidez	Raspador	-0.094	-1.88	two-sided	0.050
71	Cond	Filtrador	0.007	0.17	two-sided	0.872
72	DQO	Filtrador	-0.045	-1.11	two-sided	0.269
73	Ferrotot	Filtrador	-0.022	-0.52	two-sided	0.613
74	Fosftot	Filtrador	-0.004	-0.03	two-sided	0.974
75	Mangtot	Filtrador	-0.075	-1.89	two-sided	0.052
76	Nitrato	Filtrador	-0.023	-0.52	two-sided	0.614
77	Oxigdis	Filtrador	-0.023	-0.56	two-sided	0.584
78	pH	Filtrador	-0.017	-0.43	two-sided	0.669
79	Temp	Filtrador	0.023	0.56	two-sided	0.585
80	Turbidez	Filtrador	-0.013	-0.29	two-sided	0.787

6. CONCLUSÃO GERAL

Apesar de haver vários estudos sobre a ecologia das ninfas de efemerópteros, ainda existem muitas lacunas no conhecimento relacionadas à taxonomia, diversidade, padrões de composição de assembléias e diversidade funcional dessa ordem.

Consideramos que a avaliação da diversidade beta, diversidade funcional e da beta funcional são boas ferramentas para prever a influência de diferentes variáveis ambientais sobre os gêneros de Ephemeroptera, uma vez que ajudam a prever a organização das comunidades e determinar se essas assembléias podem estar sendo afetadas por fatores externos, como atividades antrópicas.

Concluímos que houve diferenças nas comunidades de ninfas de Ephemeroptera entre os riachos preservados e alterados, e que a homogeneização funcional e a perda de gêneros em riachos alterados, foram substituídos por gêneros mais generalistas.

7. APÊNDICE I

7.10 NORMAS PARA PUBLICAÇÃO NA JOURNAL OF INSECT CONSERVATION

7.10.1 Author Guidelines & Manuscript Template

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

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Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink "Submit online" on the right and upload all of your manuscript files following the instructions given on the screen.

Please ensure you provide all relevant editable source files. Failing to submit these source files might cause unnecessary delays in the review and production process.

Additional request

Upon submission, the e-mail addresses of all authors will be requested. At the end of the submission process, the corresponding author will receive an acknowledgement e-mail and all co-authors will be contacted automatically to confirm their affiliation to the submitted work.

Title Page

Please use this template title page for providing the following information.

The title page should include:

The name(s) of the author(s)

A concise and informative title

The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country

A clear indication and an active e-mail address of the corresponding author

If available, the 16-digit ORCID of the author(s)

If address information is provided with the affiliation(s) it will also be published.

For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

For life science journals only (when applicable)

Trial registration number and date of registration

Trial registration number, date of registration followed by "retrospectively registered"

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Declarations

All manuscripts must contain the following sections under the heading 'Declarations'.

If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

To be used for non-life science journals

Funding (information that explains whether and by whom the research was supported)

Conflicts of interest/Competing interests (include appropriate disclosures)

Availability of data and material (data transparency)

Code availability (software application or custom code)

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

To be used for life science journals + articles with biological applications

Funding (information that explains whether and by whom the research was supported)

Conflicts of interest/Competing interests (include appropriate disclosures)

Ethics approval (include appropriate approvals or waivers)

Consent to participate (include appropriate statements)

Consent for publication (include appropriate statements)

Availability of data and material (data transparency)

Code availability (software application or custom code)

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

Please see the relevant sections in the submission guidelines for further information as well as various examples of wording. Please revise/customize the sample statements according to your own needs.

Text Formatting

Manuscripts should be submitted in Word.

Use a normal, plain font (e.g., 10-point Times Roman) for text.

Use italics for emphasis.

Use the automatic page numbering function to number the pages.

Do not use field functions.

Use tab stops or other commands for indents, not the space bar.

Use the table function, not spreadsheets, to make tables.

Use the equation editor or MathType for equations.

Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX.

LaTeX macro package (Download zip, 188 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Important note:

All authors are requested to use the continuous line numbering function for their manuscripts.

Scientific style

Please always use internationally accepted signs and symbols for units (SI units).

Nomenclature: Insofar as possible, authors should use systematic names similar to those used by Chemical Abstract Service or IUPAC.

References

Citation

Cite references in the text by name and year in parentheses. Some examples:

Negotiation research spans many disciplines (Thompson 1990).

This result was later contradicted by Becker and Seligman (1996).

This effect has been widely studied (Abbott 1991; Barakat et al. 1995a, b; Kelso and Smith 1998; Medvec et al. 1999, 2000).

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.

Reference list entries should be alphabetized by the last names of the first author of each work. Order multi-author publications of the same first author alphabetically with respect to second, third, etc. author. Publications of exactly the same author(s) must be ordered chronologically.

Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. https://doi.org/10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:

Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

Article by DOI

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. https://doi.org/10.1007/s001090000086

Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Dissertation

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Always use the standard abbreviation of a journal's name according to the ISSN List of Title Word Abbreviations, see

ISSN LTWA

If you are unsure, please use the full journal title.

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

Tables

All tables are to be numbered using Arabic numerals.

Tables should always be cited in text in consecutive numerical order.

For each table, please supply a table caption (title) explaining the components of the table.

Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.

Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Artwork and Illustrations Guidelines

Electronic Figure Submission

Supply all figures electronically.

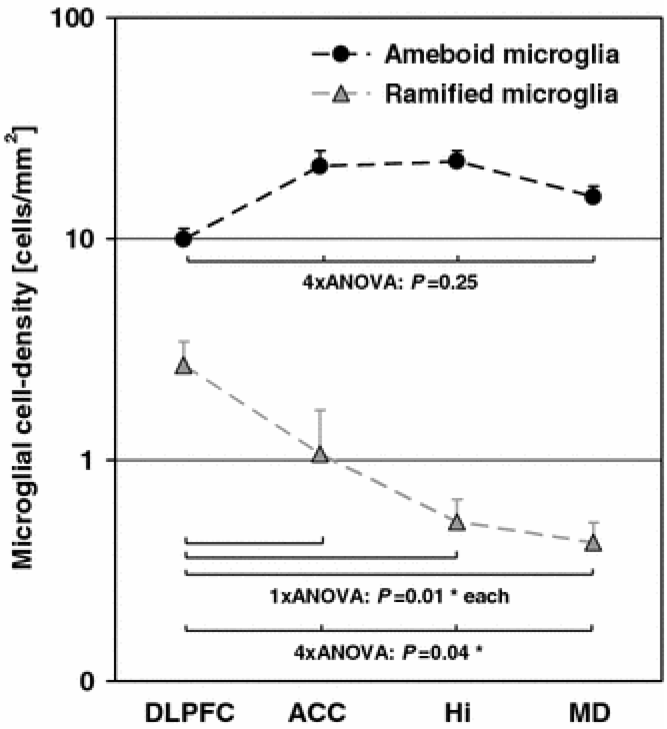
Indicate what graphics program was used to create the artwork.

For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.

Vector graphics containing fonts must have the fonts embedded in the files.

Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

Line Art



Definition: Black and white graphic with no shading.

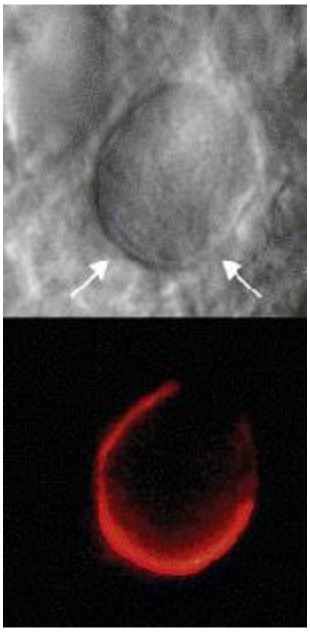
Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.

All lines should be at least 0.1 mm (0.3 pt) wide.

Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.

Vector graphics containing fonts must have the fonts embedded in the files.

Halftone Art

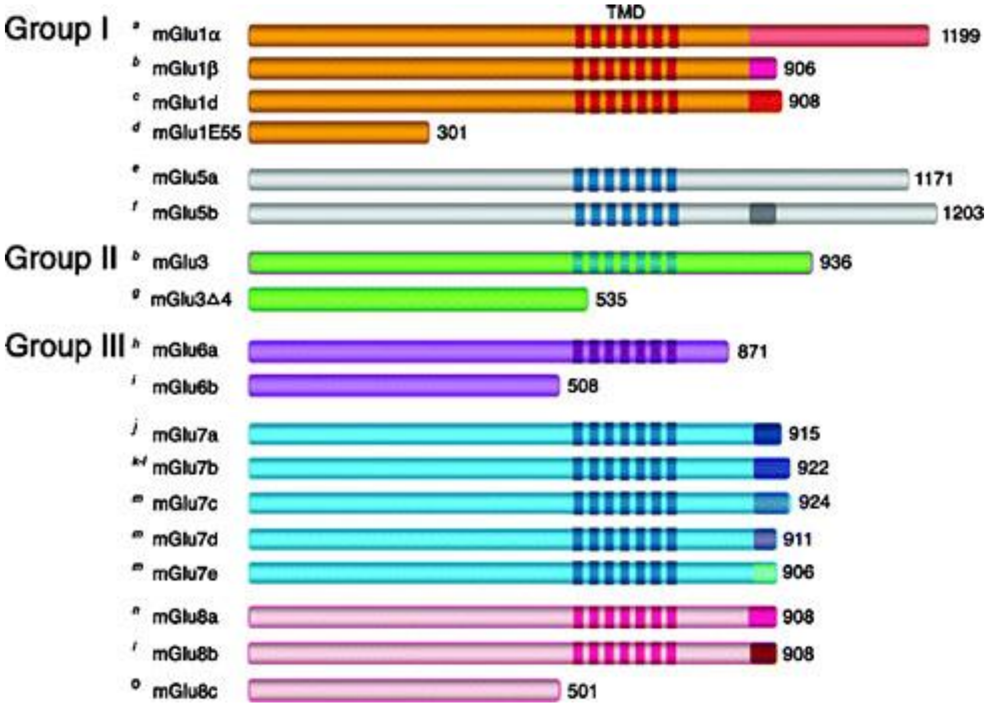


Definition: Photographs, drawings, or paintings with fine shading, etc.

If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.

Halftones should have a minimum resolution of 300 dpi.

Combination Art



Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.

Combination artwork should have a minimum resolution of 600 dpi.

Color Art

Color art is free of charge for online publication.

If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.

If the figures will be printed in black and white, do not refer to color in the captions.

Color illustrations should be submitted as RGB (8 bits per channel).

Figure Lettering

To add lettering, it is best to use Helvetica or Arial (sans serif fonts).

Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).

Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.

Avoid effects such as shading, outline letters, etc.

Do not include titles or captions within your illustrations.

Figure Numbering

All figures are to be numbered using Arabic numerals.

Figures should always be cited in text in consecutive numerical order.

Figure parts should be denoted by lowercase letters (a, b, c, etc.).

If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures,"A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

Figure Captions

Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.

Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.

No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.

Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.

Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

Figure Placement and Size

Figures should be submitted separately from the text, if possible.

When preparing your figures, size figures to fit in the column width.

For large-sized journals the figures should be 84 mm (for double-column text areas), or 174 mm (for single-column text areas) wide and not higher than 234 mm.

For small-sized journals, the figures should be 119 mm wide and not higher than 195 mm.

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In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)

Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)

Any figure lettering has a contrast ratio of at least 4.5:1

Electronic Supplementary Material

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Before submitting research datasets as electronic supplementary material, authors should read the journal’s Research data policy. We encourage research data to be archived in data repositories wherever possible.

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Supply all supplementary material in standard file formats.

Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.

To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

Audio, Video, and Animations

Aspect ratio: 16:9 or 4:3

Maximum file size: 25 GB

Minimum video duration: 1 sec

Supported file formats: avi, wmv, mp4, mov, m2p, mp2, mpg, mpeg, flv, mxf, mts, m4v, 3gp

Text and Presentations

Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.

A collection of figures may also be combined in a PDF file.

Spreadsheets

Spreadsheets should be submitted as .csv or .xlsx files (MS Excel).

Specialized Formats

Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

Collecting Multiple Files

It is possible to collect multiple files in a .zip or .gz file.

Numbering

If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.

Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.

Name the files consecutively, e.g. “ESM_3.mpg”, “ESM_4.pdf”.

Captions

For each supplementary material, please supply a concise caption describing the content of the file.

Processing of supplementary files

Electronic supplementary material will be published as received from the author without any conversion, editing, or reformatting.

Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

The manuscript contains a descriptive caption for each supplementary material

Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

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Authors should refrain from misrepresenting research results which could damage the trust in the journal, the professionalism of scientific authorship, and ultimately the entire scientific endeavour. Maintaining integrity of the research and its presentation is helped by following the rules of good scientific practice, which include*:

The manuscript should not be submitted to more than one journal for simultaneous consideration.

The submitted work should be original and should not have been published elsewhere in any form or language (partially or in full), unless the new work concerns an expansion of previous work. (Please provide transparency on the re-use of material to avoid the concerns about text-recycling ('self-plagiarism').

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These guidelines describe authorship principles and good authorship practices to which prospective authors should adhere to.

Authorship clarified

The Journal and Publisher assume all authors agreed with the content and that all gave explicit consent to submit and that they obtained consent from the responsible authorities at the institute/organization where the work has been carried out, before the work is submitted.

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All authors whose names appear on the submission

1) made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work;

2) drafted the work or revised it critically for important intellectual content;

3) approved the version to be published; and

4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

* Based on/adapted from:

ICMJE, Defining the Role of Authors and Contributors,

Transparency in authors' contributions and responsibilities to promote integrity in scientific publication, McNutt at all, PNAS February 27, 2018

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All authors are requested to include information regarding sources of funding, financial or non-financial interests, study-specific approval by the appropriate ethics committee for research involving humans and/or animals, informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals (as appropriate).

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8. APÊNDICE II

8.1 NORMAS PARA PUBLICAÇÃO NA REVISTA HYDROBIOLOGIA

8.1.1 Instructions For Authors

GENERAL

Hydrobiologia publishes original articles in the fields of limnology and marine science that are of interest to a broad and international audience. The scope of Hydrobiologia comprises the biology of rivers, lakes, estuaries and oceans and includes palaeolimnology and −oceanology, taxonomy, parasitology, biogeography, and all aspects of theoretical and applied aquatic ecology, management and conservation, ecotoxicology, and pollution. Purely technological, chemical and physical research, and all biochemical and physiological work that, while using aquatic biota as test−objects, is unrelated to biological problems, fall outside the journal's scope.

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Authors are encouraged to place all species distribution records in a publicly accessible database such as the national Global Biodiversity Information Facility (GBIF) nodes (www.gbif.org) or data centers endorsed by GBIF, including BioFresh (www.freshwaterbiodiversity.eu)

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There are four categories of contributions to Hydrobiologia:

1. Primary research papers generally comprise up to 25 printed pages (including tables, figures and references) and constitute the bulk of the output of the journal. These papers MUST be organized according to the standard structure of a scientific paper: Introduction, Materials and Methods, Results, Discussion, Conclusion, Acknowledgements, References, Tables, Figure captions.

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1. LaTeX macro package (zip, 182 kB)

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Please use no more than three levels of displayed headings.

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Abbreviations should be defined at first mention and used consistently thereafter.

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References will follow the styles as given in the examples below, i.e. journals are NOT abbreviated (as from January 2003), only volume numbers (not issues) are given, only normal fonts are used, no bold or italic.

2. Engel, S. & S. A. Nichols, 1994. Aquatic macrophytes growth in a turbid windswept lake. Journal of Freshwater Ecology 9: 97−109.

3. Horne, D. J., A. Cohen & K. Martens, 2002. Biology, taxonomy and identification techniques. In Holmes, J. A. &A. Chivas (eds), The Ostracoda: Applications in Quaternary Research. American Geophysical Union, Washington DC: 6−36.

4. Maitland, P. S. & R. Campbell, 1992. Fresh Water Fishes. Harper Collins Publishers, London.

5. Tatrai, I., E. H. R. R. Lammens, A. W. Breukelaar & J. G. P. Klein Breteler, 1994. The impact of mature cyprinid fish on the composition and biomass of benthic macroinvertebrates. Archiv fr Hydrobiologie 131: 309−320.

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1. Definition: Black and white graphic with no shading.

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5. Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

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