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Article

The role of spatial and environmental variables in shaping aquatic insect assemblages in two protected areas in the transition area between Cerrado and Amazônia

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Abstract: The distribution of aquatic insects of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) can be influenced by factors such as water quality, habitat integrity and biogeography. The present study evaluated the structure of EPT assemblages in streams in the Cerrado, a global biodiversity hotspot. Samples were collected from 20 streams in two protected areas: Parque Estadual do Mirador (10 streams) and Parque Nacional da Chapada das Mesas (10 streams). A total of 1987 specimens were collected, representing 46 taxa of EPT. The two study areas did not differ significantly in taxonomic richness of EPT genera (t = -1.119, p = 0.279) and abundance of individuals (t = 0.268, p = 0.791) but did differ in genus composition (Pseudo-F = 2.088, R2 = 0.103, p = 0.015) and environmental variables (Pseudo-F = 2,282, R^2 = 0.112, p = 0.014). None of the tested environmental variables were correlated with the community but a spatial filter captured an effect of the spatial distribution of streams. The region of the study is located in MATOPIBA, which is the last agricultural frontier of the Cerrado. Therefore, it is important that there is police and monitoring so that the "Parque Estadual do Mirador" and the "Parque Nacional da Chapada das Mesas" continue to play their role in conserving biodiversity in the future.
Keywords: *Biogeography, Conservation units, EPT, Stream ecology.*

O papel das variáveis espaciais e ambientais na formação de assembleias insetos aquáticos em duas áreas protegidas na área de transição entre Cerrado e Amazônia

Resumo: A distribuição de insetos aquáticos das ordens Ephemeroptera, Plecoptera e Trichoptera (EPT) pode ser influenciada por fatores como qualidade da água, integridade do habitat e biogeografia. O presente estudo avaliou a estrutura das assembleias do EPT em riachos do Cerrado, um *hotspot* de biodiversidade global. Foram coletadas amostras em 20 riachos em duas áreas protegidas: Parque Estadual do Mirador (10 riachos) e Parque Nacional da Chapada das Mesas (10 riachos). Um total de 1987 espécimes foram coletados, representando 46 táxons de EPT. As duas áreas de estudo não diferiram significativamente na riqueza taxonômica dos gêneros EPT (t= -1,119; p= 0,279) e abundância de indivíduos (t= 0,268; p= 0,791), mas diferiram na composição do gênero (Pseudo-F= 2,088, R^2= 0,103; p= 0,015) e variáveis ambientais (Pseudo-F= 2,282; R^2= 0,112; p= 0,014). Nenhuma das variáveis ambientais testadas foi correlacionada com a comunidade, mas um filtro espacial capturou um efeito da distribuição espacial dos riachos. A região do estudo está localizada em MATOPIBA, que é a última fronteira agrícola do Cerrado. Portanto, é importante que exista fiscalização e monitoramento para que o "Parque Estadual do Mirador" e o "Parque Nacional da Chapada das Mesas" continuem desempenhando seu papel na conservação da biodiversidade no futuro.
Palavras-chave: *Biogeografia, Ecologia de riachos, Unidades de Conservação, EPT.*

Introduction

The spatial distribution of species can be influenced by environmental, spatial and biogeographic characteristics (Leibold et al. 2004, 2010; Crisp et al. 2011), the combination of which is important for understanding how communities are distributed across a landscape and the patterns and mechanisms involved (Presley et al. 2010). Areas of transition, such as between forest-dominated regions (Amazonia) and savannas (Cerrado), are known as ecological tension zones (Marimon et al. 2014; Marques et al. 2019). Species composition tends to vary in such tension zones because of the paradoxical influence of two biogeographically distinct ecosystems located in juxtaposition (Marimon et al. 2014). So, in this transition areas it is expected that biogeographic characteristics play an important role in explaining the distribution of the species (Juen et al. 2017).

Distribution patterns of local communities of aquatic insects of the orders Ephemeroptera, Plecoptera, and Trichoptera (hereafter EPT) are influenced by environmental, spatial, and biogeographic characteristics, however the relative role of these predictors vary depending on the systems. For example, habitat structure in-stream, water quality and habitat integrity (Rosenberg & Resh 1993; Thorp et al. 2006; Allan & Castillo, 2007). The spatial configuration of communities reflects interactions between biotic characteristics, such as dispersal capacity, and abiotic factors, including the presence of geographic barriers, which act at larger spatial scales (Chase & Myers 2011; Dale & Fortin 2014; Vellend et al. 2014). In addition to the types of predictors and spatial scale, species richness and composition, as well as the abundance of individuals, stand out as good descriptive metrics of communities (Juen et al. 2014). This is because species richness can serve as a proxy for alpha diversity (local scale) and species composition as a proxy for beta diversity (differences in species composition among sites) (Jost 2007), while abundance can reflect variation in conditions and resources that influence population size (Tokeshi 1993) and all aspects of the aforementioned diversity are more dynamic in the transition zones between biomes (Ferro & Morrone 2014).

Some transition areas in the Neotropical region are experiencing strong and rapid land-use changes, with negative consequences for biodiversity (Gardner et al. 2013). It is the case of the deforestation arc between Amazonia and Cerrado (Brando et. al 2013), particularly in the MATOPIBA region (Spera et al. 2016). It is critical to understand the distributional patterns of biodiversity in these transition areas to conserve them (Marques et al. 2019). Considering that protected areas are a cornerstone of biodiversity conservation, it is urgent to know the patterns of aquatic biodiversity in protected areas located in tropical transition ecosystems.

In this context, the present study evaluated the structure of EPT communities in two protected areas in the state of Maranhão, Northeast Brazil, an transition area between Amazonian and Cerrado systems: Parque Estadual do Mirador (PEM) and Parque Nacional da Chapa das Mesas (PNCM), with the former being located further from Amazonia and the latter, which is located more centrally in the Cerrado. Since the studied streams are located within strictly protected areas, and thus subjected to minimal anthropogenic impact, it is assumed that the principal mechanisms determining differences in the composition of genera will be geographic distance among streams (spatial autocorrelation) and biogeography, because regions closer to the Amazon will vary in composition due to the more intense dynamics of transition regions. Thus, the present study aimed to test the hypothesis that the two protected areas would not differ in the richness of genera and abundance of individuals but would differ in the composition of genera.

Material and methods

1. *Study area*

The present study focused on two protected areas in the Brazilian state of Maranhão: Parque Estadual do Mirador (PEM) and Parque Nacional da Chapada das Mesas (PNCM) (Fig. 1). Parque Estadual do Mirador was created by Maranhão State decree number 641, of June 20 1980, with an initial area of 450,838 ha, and was subsequently expanded by Law nº. 8.958 of May 8 2009, to a total area of 766,781.00 ha (Maranhão 2009). It includes parts of the municipalities of Mirador, Grajaú, and São Raimundo das Mangabeiras, and is located between the headwaters of the Itapecuru and Alpercartas rivers (06º26'01" S, 44º53'58" W). The predominant vegetation of PEM is Cerrado *sensu lato* a (Conceição & Castro 2009), while the main vegetation types of the studied region are areas of cerradão with the presence of buritizais (growths of Buriti palm trees). The climate is sub-humid to humid (*Aw* in the Köppen classification system), with annual precipitation ranging 1,200 – 1,400 mm, mean maximum temperatures ranging 31.4ºC – 33.0ºC, and mean minimum temperatures ranging 19.5ºC – 21.0ºC (ICMBIO 2016).

Parque Nacional da Chapada das Mesas (7º02'39.6" S, 047º26'28.0" W) was created on December 12, 2005, with a total area of approximately 160,000 ha. It is in the municipalities of Estreito, Carolina, and Riachão, within an area dominated by sandstone formations, which vary in altitude from 250 m in valleys to approximately 524 m on plateaus (MMA 2007). The park has more than 400 springs in its interior that supply the city of Carolina and three important hydrographic basins (Parnaíba, Tocantins and São Francisco). Its main watercourses are the Farinha River in the north and the Itapecuru River in the south (MMA 2016).

The climate of PNCM is tropical humid (*Aw* in the Köppen classification system) with high temperatures throughout the year. There are two well-defined seasons: a dry season from May to October, and a rainy season from November to April. The mean annual temperature is 26.1°C, with minimum temperatures ranging from 25.2°C in January to 27.8°C in September, and maximum temperatures of approximately 36°C in July and August. Annual precipitation ranges 1,250 – 1,500 mm (MMA 2007).

It should be noted that both PEM and PCNM are located in the last agricultural frontier of the Brazilian Cerrado known as MATOPIBA, an acronym derived from the abbreviations of the states of Maranhão, Tocantins, Piaui and Bahia (Spera et al. 2016). To determine anthropic impacts, ICMBio recommends monitoring the fauna and environmental conditions of streams within protected areas (Brasil et al. 2020).

2. *Collection of immature insects*

Specimens of immature insects were collected in May 2018. A total of 10 streams were sampled in each protected area by selecting a 50-m stretch and dividing it into five 10-m segments. All the different microhabitats in which aquatic insects are typically found, including leaf litter, rocks, trunks, macrophytes, and roots, within each segment were examined systematically (adapted from Couceiro et al. 2012).

Insects were collected using an aquatic entomological hand-net (known in Brazil as a "rapiché") with a 1-mm mesh and manually using a pair of tweezers. Each 10-m segment of each stream was sampled for 15 min. Sampling was replicated three times in each segment.

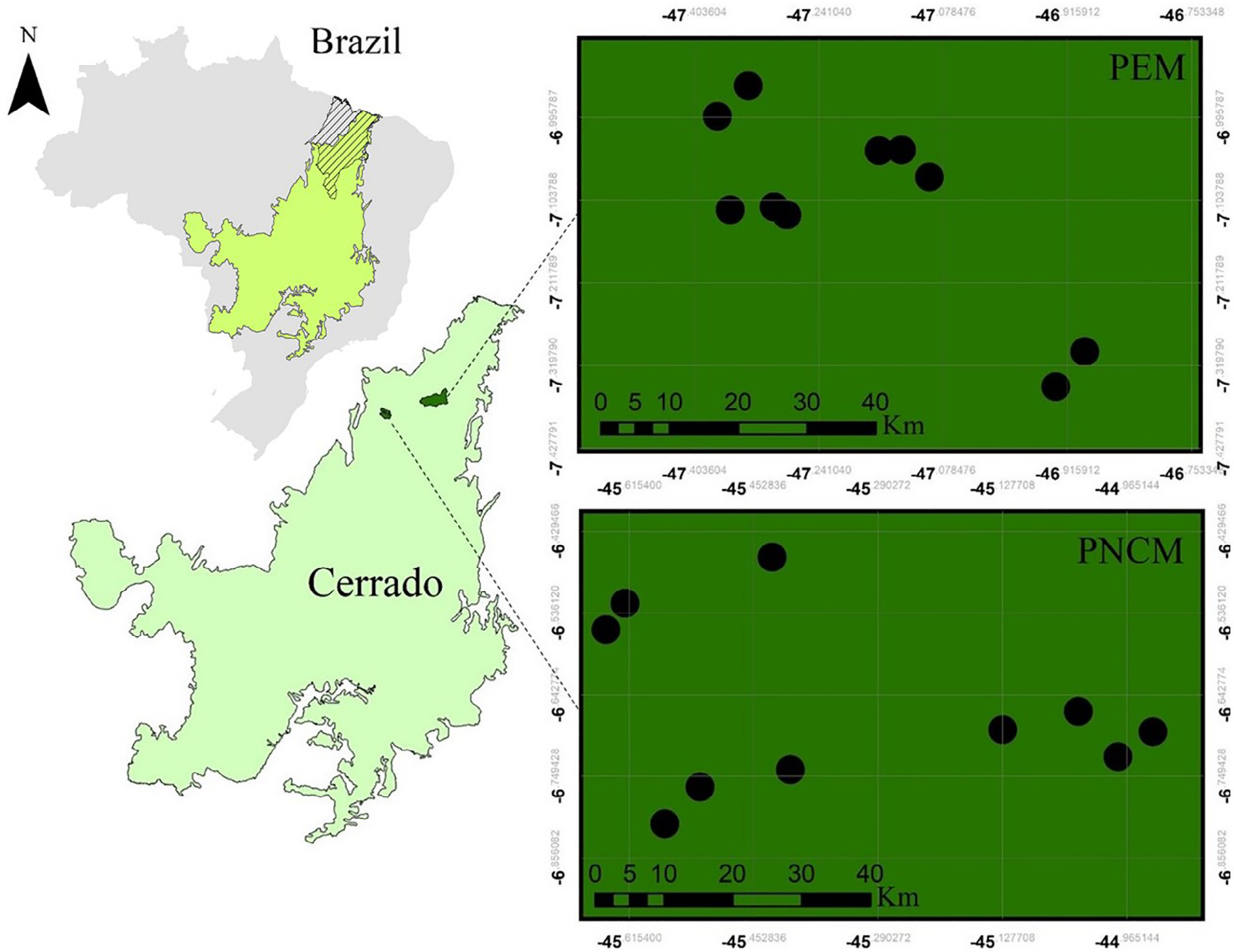

Figure 1. Distribution of the samples (black circles) of aquatic insects in two protected areas of the Brazilian Cerrado, the Parque Nacional da Chapada das Mesas (PNCM) and Parque Estadual do Mirador (PEM).

An initial field screening was undertaken to separate nymphs from the substrate (predominantly leaf, macrophyte, stone and root) with the aid of trays and tweezers. Insect specimens and substrate samples were stored in 80% alcohol in plastic bags and taken to the Laboratório de Entomologia Aquática (LEAq) of the Centro de Estudos Superiores da Universidade Estadual do Maranhão (CESC-UEMA) for further sorting and identification. In the laboratory, substrate samples were washed with water using an entomological sieve, and the nymph specimens separated using a white tray, tweezers and a Stemi DV4 stereomicroscope (Zeiss).

After sorting, the specimens were identified to genus using identification keys for EPT genera, including Costa et al. (2006), Mugnai et al. (2010), Dominguez et al. (2006), Falcão et al. (2011), Salles et al. (2014), Hamada & Silva (2014), and Pes et al. (2014). The specimens were then deposited in the LEAq collection at CESC-UEMA in Caxias, Maranhão, Brazil.

3. *Environmental predictors*

Nine environmental predictors were measured for each stream: width (1), depth (2), environmental integrity (3), pH (4), electrical conductivity (5), temperature (6), dissolved oxygen (7), stream discharge (8) and current velocity (9). Temperature, pH, conductivity, and dissolved oxygen were measured at three segments per stream using an Asko® multiparameter probe.

Current velocity and discharge were estimated using the approach of Craig (1987), with velocity being estimated by the formula: $V = \sqrt{2gD}$, where V = water velocity, g = gravity (9.8 m/s^2), and D = difference in the passage time at points D1 and D2 (D2-D1). Discharge was estimated by the formula: Discharge = stream width x stream depth x water velocity. These measurements were made at each sampling segment (five per stream) and averaged per stream.

Environmental integrity was evaluated for each stream using the Habitat Integrity Index (HII) of Nessimian et al. (2008). The HII varies from 0 (completely altered environments) to 1 (intact habitats) and has been widely used in ecological studies of aquatic insects in Brazil (Juen et al. 2014). All environmental variables are available in Supplementary 1.

4. *Spatial predictors*

Geographic coordinates of all streams were used to calculate spatial filters using a Euclidean distance matrix calculated with the "vegdist" function of the "vegan" package (Dray et al. 2016).

Spatial filters were calculated by Principal Coordinates of Neighbour Matrices (PCNM) with the minimum distance from the connectivity network being used as truncation distance (Dray et al. 2016). This technique makes it is possible to determine if there are spatial predictors structuring the distribution of communities. Other geographic and biotic processes (such as population growth, geographic dispersal, differential fertility or mortality, social organization, or competition dynamics) also can promote spatial autocorrelation and be captured by PCNM (Griffith and Peres-Neto 2006). Geographic coordinates were obtained using a Garmin Etrex handeld GPS. The PCNMs were calculated using the "pcnm" function of the "vegan" package (Oksanen et al. 2018). Subsequent selection of PCNMs was done using the "forward.sel" function of the "adhesive" package (Dray et al. 2016) in the program R.

5. *Data analysis*

Each stream was considered a sampling unit for data analysis, thus there was a total of 20 sampling units with 10 located in PNCM and 10 in PEM. The hypothesis that there would be no significant difference in genus richness between the two protected areas was tested by the Student's *t*-test using the "*t.test*" function of the basic "*stats*" package (R Core Team 2017). Richness of genera and abundance of individuals were the response variables and protected area was the categorical predictor with two levels, PNCM and PEM. Assumptions of normality and homogeneity of variance were tested, with the *t*-test for unequal variances being applied whenever these assumptions were not satisfied (Zar 2013).

Multivariate Permutational Analysis of Variance (PERMANOVA) was used to test the hypothesis that there was a significant difference in genus composition between the two protected areas (Anderson & Walsh 2013). This analysis was based on a matrix of genus composition and abundance data with Bray-Curtis distance as the response variable and protected area (PNCM or PEM) as the predictor variable. Significance was then determined by a Monte Carlo test with 9999 permutations (Anderson 2001; Anderson & Walsh 2013). A second PERMANOVA was run on the matrix of environmental variables using Euclidian distance as the response variable and protected area (PNCM or PEM) as the predictor variable, to determine whether significant differences existed between the protected areas in the set of environmental variables. The "bray" method was used to calculate Bray-Curtis distances in the "vegdist" function of the "vegan" package, while Euclidian distances were calculated using the "euclidean" method (Oksanen et al. 2018).

Two ordinations were constructed to graphically demonstrate differences in genera and environmental conditions between the two areas: Principal Coordinate Analysis (PCoA) using Bray Curtis distance for species composition with abundance data; and Principal Component Analysis (PCA) using Euclidean distance (Legendre & Legendre 1998) for standardized environmental variables

Given the requirement that there must be a minimum of 10 samples for each predictor used in models (Gotelli & Ellison 2004), and that the present study had 20 samples, variables needed to be selected (Forward Selection, Dray et al. 2016) to minimize residuals and produce more robust models.

Results

1. *Community structure*

A total of 1987 specimens were collected representing 46 genera and 15 families of the orders Ephemeroptera, Plecoptera and Trichoptera. The most common genera were *Anacroneuria*, *Smicridea*, *Leptonema*, and *Helicopsyche*, and the rarest were *Callibaetis*, *Macunahyphes*, *Fittkaulus*, *Homothraulus*, and *Massartella*, which were recorded only once each (Table 1).

2. *Description of environmental and spatial predictors*

None of the studied streams had an extremely low HII (<0.5), and so they all can be considered preserved or minimally altered (HI = 0.762 ± 0.084, mean ± standard deviation). The environmental variables differed significantly between the two study areas (Pseudo-F = 2.282, R2 = 0.112, p = 0.014). The first two axes of the PCA explained 58.4% of this environmental variation and partially separated the streams of PNCM and PEM (Fig 2). Forward selection did not select any good environmental predictors for community distribution. Six spatial filters were created to correct for spatial autocorrelation, with only PCNM 3 being selected as important for determining the spatial distribution of communities (PCNM 3: R^2= 0.092; F= 1.821; p= 0.021) (The graphical representation of the PCNM 3 in available in Supplementary 2).

3. *Differences in diversity patterns between protected areas*

Taxonomic richness of EPT genera did not differ significantly between study areas (t = -1.119, p = 0.2795), nor the abundance of individuals (t = 0.268, p = 0.791). A significant difference was found between the protected areas in the composition of EPT genera (Pseudo-F = 2.088, R^2 = 0.103, p = 0.015). The first two axes of the PCoA explained 50% of the variation in composition and partially separated streams of PNCM and PEM (Fig 3). Six genera were exclusive to PEM, nine genera were exclusive to PNCM and 31 occurred in both (Fig. 4).

Table 1. Genera of the orders Ephemeroptera, Plecoptera and Trichoptera collected in the streams of the Parque Nacional da Chapada das Mesas (PNCM) and Parque Estadual do Mirador (PEM).

Ordem	Família	Gênero	PNCM	PEM
Ephemeroptera	Baetidae	*Americabaetis* Kluge, 1992	12	0
		Callibaetis Eaton, 1881	1	0
		Criptonympha Lugo-Ortiz & McCafferty, 1998	1	1
		Waltzoyphius McCafferty & Lugo-Ortiz, 1995	3	1
		Zelusia Lugo-Ortiz & McCafferty, 1998	21	14
	Caenidae	*Caenis* Stephens, 1835	2	0
	Euthyplociidae	*Campylocia* Needham & Murphy, 1924	6	52
	Leptohyphidae	*Amanahyphes* Salles & Molineri, 2006	39	1
		Leptohyphes Eaton, 1882	1	2
		Macunahyphes Dias, Salles & Molineri, 2005	1	0
		Traverhyphes Molineri, 2001	1	3
		Tricorythodes Ulmer, 1920	12	2
	Leptophlebiidae	*Farrodes* Peters, 1971	55	32
		Fittkaulus Savage & Peters, 1978	1	0
		Hagenulopsis Ulmer, 1920	22	2
		Homothraulus Demoulim, 1955	0	1
		Hydrosmilodon Flowers & Dominguez, 1992	31	12
		Massartella Lestage, 1930	0	1
		Microphlebia Savage & Peters, 1983	0	2
		Miroculis Edmunds, 1963	19	43
		Paramaka Savage & Domínguez, 1992	10	3
		Simothraulopsis Demoulin, 1966	39	9
		Tikuna Savage, Flowers & Peters, 2005	3	0
		Ulmeritoides Traver, 1959	5	6
Plecoptera	Perlidae	*Anacroneuria* Klapálek, 1909	333	327
Trichoptera	Helicopsychidae	*Helicopsyche* Siebold, 1856	67	22
	Odontoceridae	*Marilia* Müller, 1880	7	8
	Hydropsychidae	*Leptonema* Guérin-Méneville, 1843	19	73
		Macronema Pictet, 1836	27	10
		Macrostemum Kolenati, 1859	10	21
		Smicridea McLachlan, 1871	96	150
		Synoestropsis Ulmer, 1905	0	8
	Hydroptilidae	*Flintiella* Agrisano,1995	0	8
		Hydroptila Dalman, 1819	3	0
		Metrichia Ross, 1938	2	3
		Neotrichia Morton, 1905	1	7
	Hidrobiosidae	*Atopsyche* Banks, 1905	2	0
	Glossosomatidae	*Mortoniella* Ulmer, 1906	0	10
	Leptoceridae	*Nectopsyche* Müller, 1879	5	16
		Oecetis McLachlan, 1877	17	13
		Triplectides Kolenati, 1859	10	8
		Gênero A Pes, 2005	6	39
	Philopotamidae	*Chimarra* Stephens, 1829	43	36
	Polycentropodidae	*Cernotina* Ross, 1938	14	63
		Cyrnellus Banks, 1913	6	18
		Polyplectropus Ulmer, 1905	7	0
TOTAL	**15**	**46**	**958**	**1029**

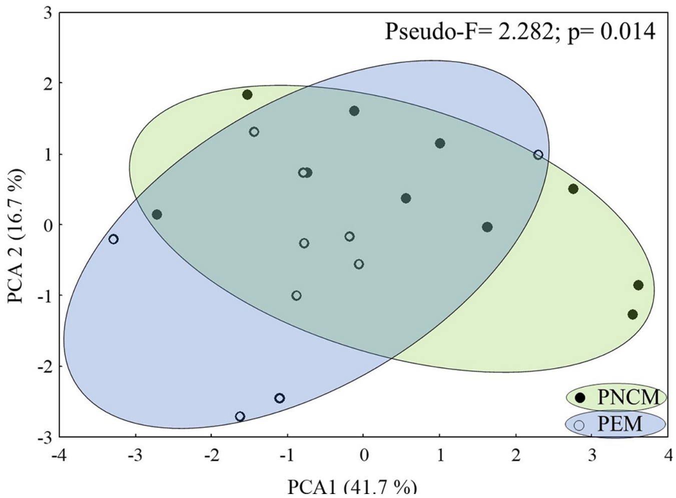

Figure 2. Ordination of the environmental variables in the streams of the Parque Nacional da Chapada das Mesas (PNCM) and Parque Estadual do Mirador (PEM).

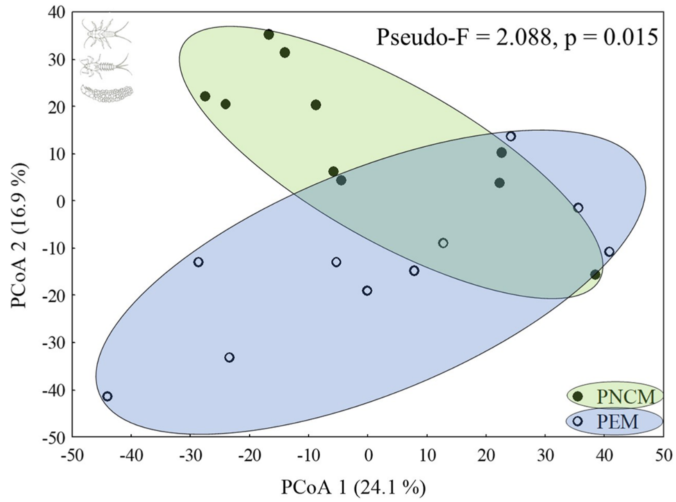

Figure 3. Ordination of the Ephemeroptera, Plecoptera and Trichoptera genera recorded in the streams of the Parque Nacional da Chapada das Mesas (PNCM) and Parque Estadual do Mirador (PEM).

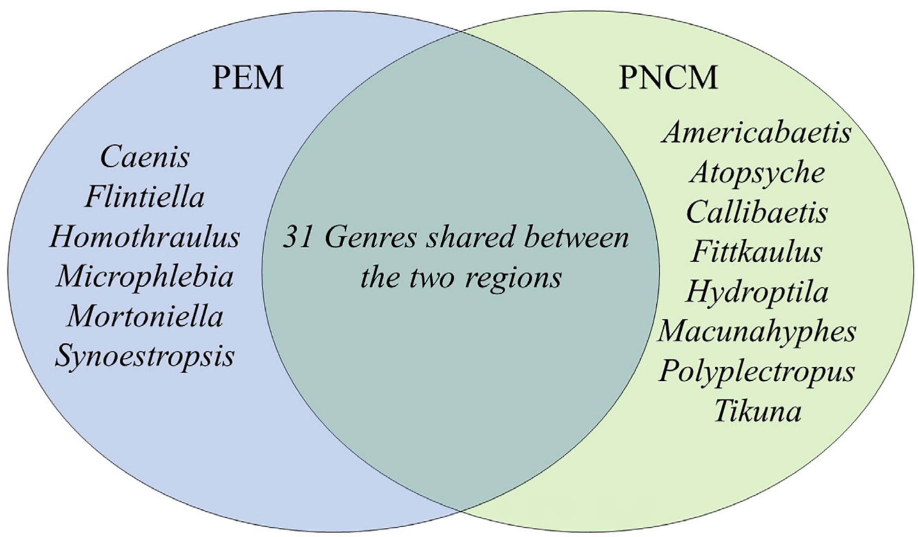

Figure 4. Diagram showing the genera collected exclusively in Parque Nacional da Chapada das Mesas (PNCM) and Parque Estadual do Mirador (PEM), and the genera shared by these two protected areas. PNCN is closest to the Amazon and PEM is in the most distant region.

Discussion

The hypothesis that the two protected areas would not differ in the richness of genera and abundance of individuals, but would differ in genus composition, was supported. Spatial distance and intrinsic characteristics, such as proximity to other biomes, appear to be the principal factors associated with the difference in taxonomic composition of the communities of the two areas. Parque Estadual do Mirador is further from Amazonia, whereas PNCM and is located more centrally within the Cerrado. This difference in the composition of communities of aquatic insects between the Cerrado-Amazonia transition and the central Cerrado is consistent with the findings of previous studies (Nogueira & Cabette 2011; Shimano et al. 2013; Juen et al. 2017).

Mass effect and neutral dynamics are the two ecological mechanisms most used to explain the spatial structuring of communities (Leibold et al. 2010). On a regional scale, such as the present study, Heino & Mykrä (2008) also found evidence of the importance of space in structuring aquatic insect communities, but in this case it was in a temperate region. Under a similar spatial configuration, Brasil et al. (2018) found the composition of adult odonate communities, a group of aquatic insects with greater dispersion potential than EPT, to be biogeographically congruent among different regions. Although integrity measured by the Habitat Integrity Index (Nessimian et al. 2008) has been the most important environmental predictor for EPT in Cerrado streams (Brasil et al. 2013; Brasil et al. 2014; Souza et al. 2011; Pereira et al. 2012; Nogueira et al. 2011), it varied little in the present study.

The association between EPT communities and environmental conditions has been well documented (Rosenberg & Resh 1993; Thorp et al. 2006; Wiens & Donoghue 2004). In the present study, none of the evaluated environmental variables were related to EPT communities. It seems likely that this situation reflects the fact that the studied stream environments were well-preserved or only lightly impacted (mean ± standard deviation HII = 0.76 ± 0.08). Water quality tends to be good and these environmental conditions vary little in preserved streams compared to anthropized streams (Martins et al., 2017). However, good water quality does reinforce the importance of the protected areas, given that previous studies have shown that deterioration of water quality in impacted areas adjacent to protected areas leads to changes in the communities found in anthropized streams (Faria et al. 2017; Montag et al. 2018). Decreased water quality due to anthropogenic impacts creates filters for the distribution of the different species (Martins et al. 2017), when there are anthropized.

The exclusive genera of PNCM and PEM may be a biogeographical signal. These results reinforce the importance of PNCM and PEM for aquatic biota conservation, especially the aquatic insects. The number of protected areas is currently declining worldwide (Ferreira 2014), and the Cerrado is the biome most impacted by agribusiness in Brazil (Lahsen 2016). Therefore, it is important that there are police and monitoring so that the "Parque Estadual do Mirador" and the "Parque Nacional da Chapada das Mesas" continue to play their role in conserving biodiversity in the future.

Supplementary material

The following online material is available for this article:

Table S1 - The complete presentation of environmental and spatial data for all streams in the two regions.

Figure S1 - Graphical representation of the spatial filter distribution by streams in the two regions.

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Conflicts of Interest

The authors have no conflict of interest.

Author Contributions:

Derise de Assunção Barbosa: Contribution to data collection; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Leandro Schlemmer Brasil: Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content; Substantial contribution in the concept and design of the study.

Carlos Augusto Silva de Azevêdo: Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content: substantial contribution in the concept and design of the study.

Lucas Ramos Costa Lima: Contribution to data collection; Contribution to data analysis and interpretation; contribution to critical revision, adding intellectual content; Substantial contribution in the concept and design of the study

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