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Assemblage of immature Odonata (Insecta, Anisoptera) in streams of the Mato Grosso do Sul State: spatial implications

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ABSTRACT. This study investigated the assemblages attributes (composition, abundance, richness, diversity and evenness) and the most representative genera of Odonata, Anisoptera at Água Boa and Perobão Streams, Iguatemi River basin, Brazil. Both are first order streams with similar length that are impacted by riparian forest removal and silting. Quarterly samplings were conducted from March to December 2008 in the upper, intermediate and lower stretch of each stream. The Mantel test was used to check the influence of spatial autocorrelation on the Odonata composition. Spatial variations in the composition were summarized by the Principal Coordinates Analysis (PCoA) using Mantel test residuals. The effects of spatial correlation on richness and abundance were investigated by the spatial correlogram of Moran's I coefficients. The most representative genera in each stream were identified by the Indicator Value Method. The spatial variations in the attributes of the assemblages were assessed using analysis of variance of null models. We collected 500 immature individuals of 23 genera and three families. Among the attributes analyzed only the composition and abundance showed significant spatial differences, with the highest mean abundance found in the Perobão Stream. *Miathyria* and *Zenithoptera* were the indicator genera of the Água Boa Stream and *Erythrodiplax*, *Libellula*, *Macrothemis*, *Progomphus* and *Tramea* were the indicator genera of the Perobão Stream.

KEYWORDS. Aquatic invertebrates, Iguatemi River basin, lotic environments, odonatofauna.

RESUMO. Assembleia de imaturos de Odonata (Insecta, Anisoptera) em riachos sul-matogrossenses: implicações espaciais. Este trabalho investigou atributos de assembleias (composição, abundância, riqueza, diversidade e equitabilidade) e os gêneros mais representativos de Odonata, Anisoptera nos riachos Água Boa e Perobão, bacia do rio Iguatemi, MS, Brasil. Os riachos são de primeira ordem, apresentam extensão similar e são impactados pela remoção da mata ripária e assoreamento. As amostragens foram realizadas trimestralmente de março a dezembro/2008 nos trechos superior, intermediário e inferior de ambos os riachos. O teste de Mantel foi utilizado para verificar a influência da autocorrelação espacial sobre a composição de Odonata. Variações espaciais na composição foram sumarizadas através da Análise de Coordenadas Principais (PCoA) utilizando-se os resíduos do teste de Mantel. Os efeitos da correlação espacial na riqueza e abundância foram investigados através do correlograma espacial dos coeficientes do I de Moran. Para identificar os gêneros mais representativos em cada riacho foi utilizado o Método do Valor Indicador. As variações espaciais dos atributos das assembleias foram avaliadas por meio de análises de variância de modelos nulos. Foram coletados 500 indivíduos imaturos distribuídos em 23 gêneros e três famílias. Dentre os atributos analisados, apenas a composição e abundância apresentaram diferenças espaciais significativas, sendo o maior valor médio do último atributo registrado no riacho Perobão. *Miathyria* e *Zenithoptera* foram os gêneros indicadores do riacho Água Boa e *Erythrodiplax*, *Libellula*, *Macrothemis*, *Progomphus* e *Tramea* do Perobão.

PALAVRAS-CHAVE. Invertebrados aquáticos, bacia do rio Iguatemi, ambientes lóticos, odonatofauna.

Insects are widely found in freshwater environments (Souza & Costa, 2006) where they live associated with vegetation, water surface film, air-water interface and sediment, acting in the food chain as scavengers, herbivores or predators (Merrit & Cummins, 1996). Among them, Odonata is usually recorded since this order encompasses approximately 5,860 species (popularly known as dragonflies), with high richness recorded for the Neotropics (Kalkman *et al.*, 2008) including 828 species identified in Brazil (Souza *et al.*, 2007; Buzzi, 2013).

Odonata adults are terrestrial, mainly diurnal, active fliers and show complex territorial and sexual behaviors (Borror & Delong, 1988; Souza *et al.*, 2007). In turn, immature individuals are longitudinally distributed along water bodies according to their ecological requirements (Assis *et al.*, 2004; Remsburg & Turner, 2009) in search of food, oxygenated water and refuge (Corbet, 1980). These organisms are important functional components of aquatic ecosystems, with essential role in the food chain because they are predators at all life stages (Corbet, 1999; Carchini *et al.*, 2007) and are preyed upon by fish, amphibians and reptiles (Souza & Costa, 2006). In addition to that, once immature individuals present long larval period, they are often used as environmental indicators (Osborn, 2005; Kalkman *et al.*, 2008; Simaika & Samways, 2011).

Several studies have shown the wide distribution of immature Odonata in streams where the composition and distribution of these insects are influenced by the interaction among physical, chemical, structural and hydrological variables (Gibbs *et al.*, 2004; Maltchik *et al.*, 2010; Raio *et al.*, 2011; Che Salmah *et al.*, 2012; Gómez-Anaya *et al.*, 2013). Thereby, changes in the water body characteristics can strongly influence the spatial and temporal distribution patterns of the odonatofauna (Buss *et al.*, 2002, 2004; Silveira *et al.*, 2007).

Although freshwater ecosystems are subjected to unprecedented levels of human impacts (Abell, 2002; Saunders *et al.*, 2002; Abell *et al.*, 2008), an anthropized landscape still supports a great diversity of Odonata (Koch *et al.*, 2014). Specifically for the suborder Anisoptera, studies have shown that richness is higher in environments with lower vegetation cover (Carvalho *et al.*, 2013; Juen *et al.*, 2014) and their larvae exhibit considerable resistance to disturbed habitats (Kalkman *et al.*, 2008). Thus, environmental changes as removal of riparian forest can positively affect most species of Anisoptera since the majority is generalist and adapted to sites with higher solar incidence (Ferreira-Peruquetti & De Marco, 2002; Remsburg & Turner, 2009).

The Brazilian odonatofauna is still poorly known and most studies address the south and southeast regions (De Marco & Vianna, 2005). For the Mato Grosso do Sul State (MS), information on this group is concentrated in the Pantanal region and mainly deal with taxonomic aspects (Longfield, 1929; Santos, 1944; Souza *et al.*, 1999, 2002; Heckman, 2006, 2008; Souza & Costa, 2006; Pessacq & Costa, 2007; Dalzochio *et al.*, 2011a,b). Specifically for the Iguatemi River basin, studies on odonatofauna are nonexistent, despite this river be one of the major tributaries of the Paraná River in the southern of Mato Grosso do Sul.

Considering the adaptability of Anisoptera in colonizing impacted aquatic environments, two first order streams of this basin showing similar length, geographic position and anthropogenic stressors (riparian forest removal and silting) where select to answer de following question: Are the streams also similar with respect to Anisoptera assemblages' attributes? To answer that, we investigated whether the composition, abundance, richness, diversity and evenness of Odonata Anisoptera assemblages are influenced by spatial variations, represented by the streams Água Boa e Perobão. Additionally, we evaluated which genera are more representative of the spatial (streams) components.

MATERIAL AND METHODS

Study area. The Água Boa and Perobão Streams are first order streams with 6.0 and 5.3 km long, respectively. They are located in the southern of Mato Grosso do Sul State and are part of the Iguatemi River basin, a right bank tributary of the Upper Paraná River. Fragments of the original vegetation remaining in the study area correspond to the Atlantic Forest biome (semideciduous forest). The region's climate is tropical of altitude with two distinct periods related to rainfall dynamics: a rainy period (October to March) and a dry period (April to September) with annual mean temperature between 21°C and 28°C and rainfall between 1,000 and 1,500 mm (Godoy, 1986; Campos, 2001).

The studied streams have their headwaters at the municipality of Japorã and flow through rural areas on the right bank of the Iguatemi River (Fig. 1). The Perobão Stream (located at 23°49'25.75"S; 54°26'43.54"W and 23°48'4.06"S; 54°26'25.36"W) has stretches with clay and sandy/rocky bottom, width ranging from 2.0 to 10.0 m and depth between 5.0 to 50.0 cm. Slow water flow is observed only in the upper stretch while the other ones have riffles and pools.

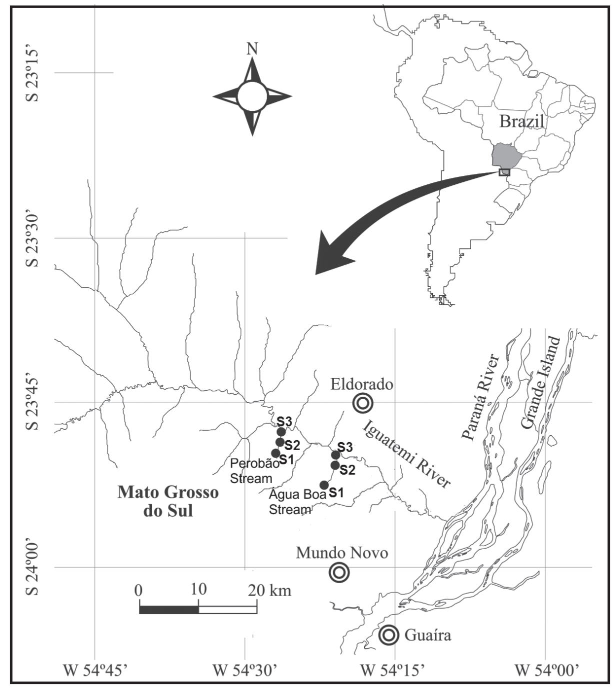

Fig. 1. Location of Perobão and Água Boa Streams, Iguatemi River basin, Brazil, and ampled sites at each stream (S1, upper stretch; S2, intermediate; S3, lower stretch).

The Água Boa Stream (located at 23°52'43.39"S; 54°21'55.59"W and 23°50'9.33"S; 54°20'57.27"W) presents alternating stretches with clay, sandy and rocky bottom with width ranging from 2.0 to 6.0 m and depth between 12.0 to 90.0 cm. The water flow is slow in the upper stretch and submerged aquatic vegetation is abundant. The intermediate stretch has riffles, backwaters and pools while the lower one is a straight channel with rapid water flow.

These habitats are subjected to intense human pressure. The replacement of native vegetation by agriculture and pasture is a remarkable characteristic of the regional occupation history (Súarez & Petrere Jr., 2003). This process included the reduction of most of the original riparian vegetation. Currently, the vegetation is restricted to sparse trees and pasture grass is the predominant component. The soil is sandy and frequently impacted by gully erosion, intensifying the input of sediments to the streams; besides that, rural populations indiscriminately use the streams water for livestock, which aggravates the silting process due to animal trampling (personal observation).

Samplings. Samplings were conducted quarterly from March to November 2008 in three stretches (upper, intermediate and lower) of Água Boa and Perobão Streams.

For a better representativeness of odonatofauna at each stretch, we sampled the water surface, aquatic vegetation and sediment. Samples of the water surface were taken using a conical-cylindrical net (0.5 mm mesh) submerged to 10 cm deep and hauled against the water flow for about two meters, performing five hauls in each stretch (area = 2.6 m^2). To collect the individuals associated with the aquatic vegetation, we used a sieve (3.0 mm mesh size), which was introduced into the vegetation, raised and agitated, totaling five consecutive repetitions (area = 0.98 m^2). For the capture of individuals in the sediment we used two gears: i) a D-net with 3.0 mm mesh size for the capture of the epifauna, which was dragged on the sediment in an approximate area of 1.0 m near marginal vegetation (area = 0.32 m^2); ii) a Petersen type grab sampler for the capture of infauna, which was thrown on the sediment twice in a row, near the marginal region (area = 0.0415 m^2). To optimize the sampling, we also included Odonata (from different substrates in the streams) caught along with fish samplings (not used in this study) through electrofishing (two dip nets and a portable generator - TOYAMA 1600, 220V, DC). The length of each stretch sampled by this methodology was established according to Fitzpatrick *et al.* (1998). A single pass of dip nets was used to take immature Odonata in each stretch. At the end of each stretch it were installed blocking nets (10.0 x 2.0 m; 5.0 mm mesh size) to capture the material carried by the flow. The use of these sampling methods is justified on the basis of differences in equipment efficiency on different substrates (Alves & Strixino, 2003; Juen *et al.*, 2007).

In the laboratory, samples were washed through a set of sieves with different mesh sizes (2.0, 1.0 and 0.5 mm). Samples from the water surface, marginal vegetation, sediment and electrofishing were sorted using a transilluminated tray and placed in labeled vials and preserved in 70% alcohol. Immature individuals of Odonata were identified to the lowest possible taxonomic level, based on Carvalho & Calil (2000), Costa *et al.* (2004), Lencione (2005) and Souza *et al.* (2007).

Data analysis. The spatial distribution of immature Odonata was investigated for composition, abundance, richness, diversity and evenness, assessing whether such assemblage attributes vary between Água Boa and Perobão Streams. In order to standardize the numerical abundance, the results were expressed in individuals/10 m^2. Richness, diversity and evenness were obtained according to Pielou (1969). Genera richness (S) was defined as the number of captured genera.

The influence of spatial correlation on the odonatofauna composition was analyzed by the Mantel Test using the PCord 5.0 software (Mccune & Mefford, 1997). This method was used to test the hypothesis that the distances of the composition matrix are independent of the distance between the points of the geographic distance matrix. For the construction of similarity matrices, we used geographical coordinate data, converted into decimal degrees (Euclidean distance) and genera presence-absence data (Bray-Curtis distance). The Mantel test significance was evaluated based on Monte Carlo statistics after 9,999 permutations, with significance level of $p < 0.05$. As the Mantel test was significant, we use the residual of this test on a Principal Coordinate Analysis (PCoA - Bray-Curtis distance) which ordinated the streams based on composition similarity, controlling the effect of autocorrelation. The axes retained for interpretation were those showing the highest percentages of explanation. Patterns of odonatofauna composition were analyzed by trends in the ordination presented by the scores of the axes. We used the Past 2.17 software (Hammer *et al.*, 2001) to determine the attributes of Odonata assemblages and to run the PCoA.

Likely species composition, richness data are frequently spatially autocorrelated, typically leading to inflation of Type I error in statistical tests (Legendre, 1993; Diniz-Filho *et al.*, 2003; Dormann, 2007). Abundance data is also pointed out as a spatially structured variable (Fortin, 1999; Bourque & Desrochers, 2006). Thus, we evaluated the effects of spatial correlation on richness and abundance using the spatial correlogram of Moran's I coefficients (Diniz-Filho *et al.*, 2003) calculated for eight distance classes. The statement of positive and significant autocorrelation at short distances was the indicator of spatial structuration in the data. The effects of the spatial autocorrelation on diversity and evenness were inferred based on Moran's I results for species richness and abundance since such index are originated from simultaneous association of the richness and abundance. The Moran's I analysis was performed using the software SAM V.4.0 (Rangel *et al.*, 2010).

To check for significant spatial differences in the odonatofauna assemblage atributes we used the PCoA axes' scores and the values of abundance, richness, diversity and evenness to perform the ANOVA null models (Gotelli & Graves, 1996; Gotelli & Entsminger, 2007). In this analysis, each attribute was the dependent variable and the streams (Água Boa and Perobão) were the independent variables. In the ANOVAs we used the EcoSim software (Gotelli & Entsminger, 2007) and entailed significant differences at $p < 0.05$ after 10,000 randomizations.

To identify the most representative genera (indicator) of each stream we used the Indicator Value Method - IndVal (Dufrêne & Legendre, 1997). This method combines information on the abundance and occurrence of a genus at one site generating an indicator value for each genus. The calculation of IndVal was conducted from data of relative abundance of each genus. When the randomization of the original matrix (Monte Carlo test; 10,000 permutations) indicated significant IndVal ($p < 0.05$), the indicator genera were analyzed considering the variation patterns between streams. To calculate the IndVal we used the PCord 5.0 software (Mccune & Mefford, 1997).

RESULTS

We collected 500 individuals of 23 genera and three families. Libellulidae was the most representative family, both in richness (12 genera) and number of individuals (290 individuals) followed by Gomphidae (eigth genera and 196 individuals) and Aeshnidae (three genera and 14 individuals). The Água Boa and Perobão Streams contained 20 and 16 genera, respectively. The taxa *Cyanogomphus* Selys, 1873, *Gynacantha* Rambur, 1842, *Miathyria* Kirby, 1889, *Perythemis* Hagen, 1861, *Triacanthagyna* Selys, 1883, *Zenithoptera* Selys, 1869 and *Zonophora* Selys, 1854 were exclusive to the Água Boa Stream and *Gomphoides* Selys, 1854, *Libellula* Linnaeus, 1758 and *Micrathyria* Kirby, 1889 were exclusive to the Perobão Stream (Tab. I).

The similarity matrix of Odonata assemblages was significantly correlated with the matrix of geographical distances of the streams (Mantel test: $R = 0.32459$; $p < 0.05$), indicating that the odonatofauna of each stream is spatially dependent. In this way, the composition was evaluated by the PCoA based on the Mantel Residual Test. The first axis of the PCoA explained 89.9% of data variability and so it was the only one analyzed. The ordination showed a clear spatial segregation (Fig. 2). This result was confirmed by ANOVA that evidenced significant differences in Odonata composition between the streams for the axis 1 (OI=1894.3; $p_{ob\ s} \geq p_{esp} = 0.0001$).

Regarding autocorrelation on assemblage attributes, we did not observe positive autocorrelation at short distance classes (914 m) for richness (Moran's $I = 0.01$; $p = 0.626$) and abundance (Moran's $I = 0.067$; $p = 0.313$). Considering the spatial variation, the ANOVA revealed that only the mean values of abundance showed significant differences between the streams (Fig. 3).

The IndVal revealed that *Miathyria* and *Zenithoptera* were the indicator genera of the Água Boa Stream, while

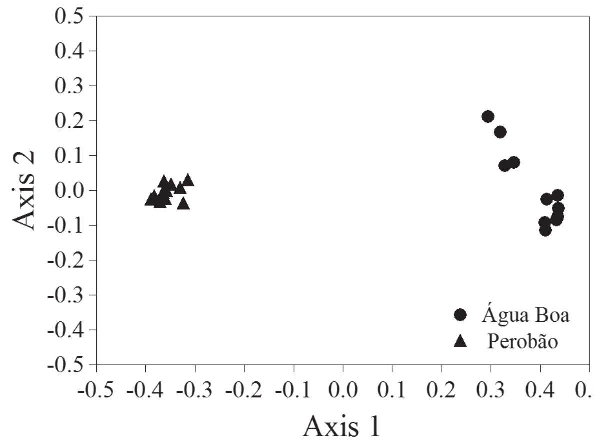

Fig. 2. Scatter plot of the scores of the first two PCoA axes.

Erythrodiplax Brauer, 1868, *Libellula*, *Macrothemis* Hagen, 1868, *Progomphus* Selys, 1854 and *Tramea* Hagen, 1861 were the indicator genera of the Perobão Stream (Tab. II).

DISCUSSION

The suborder Anisoptera presents a wide geographical distribution and makes up most of the Odonata genera occurring in Brazil (Buzzi, 2013). Libellulidae has been identified as the most diverse and numerically expressive family of this suborder (Costa & Oldrini, 2005; De Marco & Vianna, 2005; Ramírez, 2010) which is consistent with our results. The diversity and abundance of

Tab. I. Composition, number of individuals (N) and relative abundance (%) of the imature Odonata sampled at Água Boa and Perobão Streams, Iguatemi River basin, Brazil.

TAXA	Água Boa		Perobão	
	N	%	N	%
Aeshnidae				
Coryphaeschna Williamson, 1903	02	1.4	05	1.4
Gynacantha Rambur, 1842	06	4.3	0	0.0
Triacanthagyna Selys, 1883	01	0.7	0	0.0
Gomphidae				
Aphylla Selys, 1854	02	1.4	08	2.2
Cyanogomphus Selys, 1873	02	1.4	0	0.0
Erpetogomphus Selys, 1858	05	3.6	02	0.6
Gomphoides Selys, 1854	0	0.0	01	0.3
Phyllocycla Calvert, 1948	06	4.3	38	10.5
Phyllogomphoides Belle, 1970	03	2.2	01	0.3
Progomphus Selys, 1854	06	4.3	111	30.7
Zonophora Selys, 1854	11	7.9	0	0.0
Libellulidae				
Brechmorhoga Kirby, 1894	09	6.5	12	3.3
Elasmothemis Westfall, 1988	25	18.0	06	1.7
Erythrodiplax Brauer, 1868	02	1.4	23	6.4
Gynothemis Calvert *in* Ris, 1909	05	3.6	03	0.8
Libellula Linnaeus, 1758	0	0.0	24	6.6
Macrothemis Hagen, 1868	01	0.7	38	10.5
Miathyria Kirby, 1889	12	8.6	0	0.0
Micrathyria Kirby, 1889	0	0.0	02	0.6
Orthemis Hagen, 1861	02	1.4	17	4.7
Perythemis Hagen, 1861	01	0.7	0	0.0
Tramea Hagen, 1861	05	3.6	70	19.4
Zenithoptera Selys, 1869	33	23.7	0	0.0
TOTAL	139	100	361	100

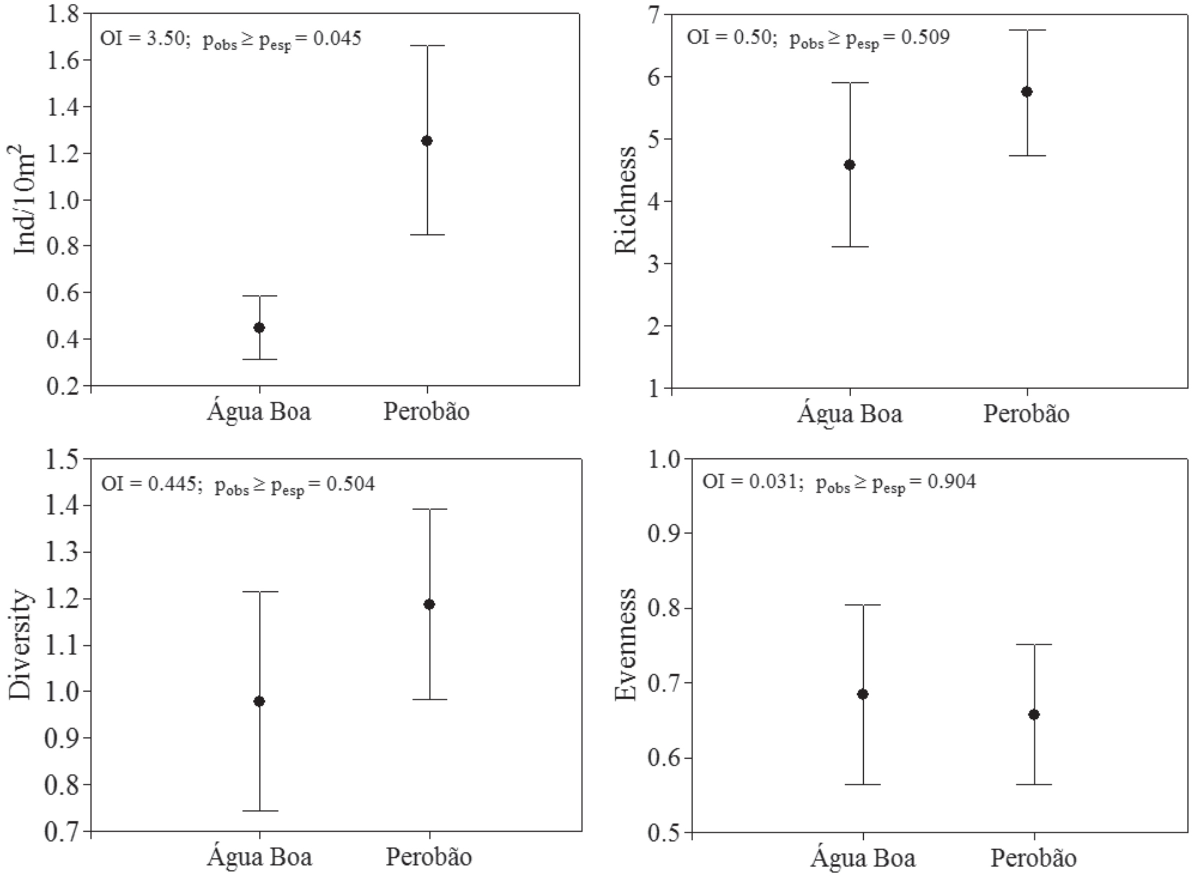

Fig. 3. Mean values of abundance, richness, diversity and evenness of the immature Odonata sampled at Água Boa and Perobão Streams, Iguatemi River basin, Brazil. Means are represented by circles and standard error by vertical bars.

Tab. II. Summary of the Indicator Value Method: relative abundance, relative frequency and indicator value for each stream. Only the species with significant values are shown. Bold values indicate significant indicator values ($p < 0.05$, Monte Carlo test).

Taxa	Relative abundance		Relative frequency		Indicator value	
	Água Boa	Perobão	Água Boa	Perobão	Água Boa	Perobão
Miathyria	100	0	36	0	**36**	0
Zenithoptera	100	0	36	0	**36**	0
Erythrodiplax	6	94	18	50	1	**47**
Libellula	0	100	0	58	0	**58**
Macrothemis	3	97	9	58	0	**57**
Progomphus	4	96	27	83	1	**80**
Tramea	6	94	27	58	2	**55**

this family have been commonly associated with structural characteristics and conservation of the habitats where they occur. Immature individuals of this group preferably inhabit aquatic environments with reduced or absent riparian vegetation, being often found in open areas (Kalkman *et al.*, 2008; Juen *et al.*, 2014).

Moreover, studies have reported the dominance of Libellulidae immature in streams near pastures, with degraded riparian forests or in silted streams (Cortezzi *et al.*, 2009; Dalzochio *et al.*, 2011a). Our results confirm this pattern since most of the stretches sampled in both streams are shallow and the surroundings consist mainly of grass pastures and sparse fragments of riparian vegetation. Combined with the adaptability to human environments, another factor associated with the representativeness of this family is the large body size of adults compared to other families of this suborder, which therefore increases the dispersal ability (Kalkman *et al.*, 2008; Dalzochio *et al.*, 2011a).

Among the 23 genera identified only 12 were common to both streams, which reflect a spatially distinct composition pattern. This variation in the composition between the streams can be attributed to different factors. Firstly, to local and regional historical colonization events, which according to Oliveira *et al.* (1997), are the main responsible for the community characteristics, particularly its composition. Secondly, to physical and structural characteristics of the streams, particularly the nature of the substrate, which can control the distribution of immature Odonata (Juen *et al.*, 2007).

Spatial and/or temporal changes in the substrate are often observed in streams, particularly considering the physical structure, organic composition, stability and heterogeneity (Ribeiro & Uieda, 2005). As a result, we can observe a mosaic of microhabitats (Kikuchi & Uieda, 1998), which allow the establishment of Odonata species with different modes of life, such as swimmers, clingers, climbers, sprawlers, burrowers and sprawler-burrowers (Carvalho & Nessimian, 1998). The heterogeneous environmental structure of the Perobão Stream, with lentic stretches (upper reaches), backwater, rapids and pools (intermediate and lower stretches) and varied substrate (clay and sand/pebbles) should provide greater number of micro-habitats for colonization by Odonata, justifying their higher abundance at this stream. Probably, these structural features particularly favored the populations of *Progomphus* and *Tramea*, which together accounted for more than 50.0% of catches in Perobão. The predominance of these genera among Odonata was also found in other Brazilian regions (Kikuchi & Uieda, 2005; Figueiredo *et al.*, 2013; Pires *et al.*, 2013).

Although having different ecological requirements and habits, *Progomphus* and *Tramea* along with *Erythrodiplax*, *Macrothemis* and *Libellula* were the indicator genera of the Perobão Stream. *Progomphus* is mainly found in lotic environments, presents burrowing habit adapted to dig and burrow in streams' sandy beds (Assis *et al.*, 2004; Costa *et al.*, 2004; Ramírez, 2010). On the other hand, *Tramea* and *Erythrodiplax* occur in lentic habitats and have sprawling habit, wherein the first colonizes sediments mainly composed of clay, sand and stones, whereas the second preferably dwells densely vegetated sites (Carvalho & Nessimian, 1998; Costa *et al.*, 2004; Figueiredo *et al.*, 2013). *Libellula* occurs in lotic environments, colonizing debris and presents sprawling-burrowing habit, while *Macrothemis*, also with sprawling-burrowing habit, occurs both, in streams with rapid waters and streams with low current flow dwelling sandy and clay substrates (Carvalho & Nessimian, 1998). Therefore, the successful establishment of these genera should be related mainly to the mosaic of microhabitats found at the Perobão Stream, as described above.

Beyond that, *Zenithoptera* and *Miathyria* were the indicator taxa of the Água Boa Stream. It differs from the Perobão Stream by presenting a high amount of submerged vegetation in lentic reaches, which must determine the colonization of *Zenithoptera*, *Elasmothemis* Westfall, 1988 and *Miathyria*, as these genera are known to occupy mainly lentic sites with sandy substrate and abundant submerged vegetation (Franco & Takeda, 2002; Costa *et al.*, 2004). Although *Elasmothermis* was not classified as an indicator of the Água Boa Stream, it was among the most abundant taxa in this stream.

Finally, the results pointed out that among the assemblages attributes investigated, only the composition and abundance of Anisoptera were different among the studied streams. Once structural variables of habitats are characterized as an important tool to explain the distribution of immature Odonata, future work addressing the association of the odonatofauna with such variables may contribute to achieve more accurate answers about the patterns found herein.

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