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Use of the Zygoptera/Anisoptera Ratio (Insecta: Odonata) for Habitat Alteration Assessment in Cerrado Streams

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All relevant data are within the paper and its Supporting Information files.

Abstract. Natural landscapes of Latin America, such as the Cerrado biome, are increasingly changing due to conflicting development models between economic growth and biodiversity conservation. In cases of total or partial suppression of natural vegetation, more sunlight reaches the streams, leading to changes in Odonata assemblages. Due to their thermoregulation characteristics, the proportion of the suborder Anisoptera tends to increase whereas the suborder Zygoptera will decrease, as this suborder is more sensitive to habitat loss. We assessed whether the proportions of individuals and species richness of Zygoptera and Anisoptera changed due to environmental quality loss in Cerrado stream habitats. Also, we assessed the performance of ratios using genus and family level. We conducted our study at 18 streams in Mato Grosso State, Brazil. We sampled Odonata communities and measured the environmental quality of each stream using the Habitat Integrity Index. To assess the relationship between the environmental quality of the streams and the Odonata ratios, we performed generalized linear models with the beta distribution family. The models showed that the loss of environmental integrity caused Zygoptera to decrease and Anisoptera to increase. In addition, we found that *Acanthagrion*/Zygoptera and *Argia*/Zygoptera ratios showed a strong relationship with habitat integrity, being plausible alternatives for use in monitoring programs. We conclude that the Zygoptera/Anisoptera ratio is a good indicator of environmental quality for the Cerrado biome and therefore makes for a suitable tool for citizen science programs in which no taxonomic expertise is required.

Key words. Dragonfly, aquatic insects, bioindicators, Habitat Integrity Index

Introduction

The Cerrado is the second largest biome in South America and covers about 22% of the Brazilian territory. It comprises a great environmental heterogeneity and represents one of the most biodiverse regions in the world (Oliveira-Filho & Ratter, 2002). Some studies estimate that the Cerrado hosts 160,000 species of plants, fungi, and animals (Dias, 1992; Ratter et al., 1997). Of these, 44% of the plants, 9.5% of the mammals, 3.4% of the birds, 17% of the reptiles, and 28% of the amphibians are endemic species (Klink & Machado, 2005).

Despite the importance of the Cerrado for maintaining biodiversity, its natural landscapes are constantly replaced by agrosystems, threatening thousands of organisms that live in this biome. This results from a conflicting development model between economic growth and the conservation of biodiversity and ecosystems (Pompeu, 2022; Santana & Simin, 2022). Some of the consequences of removing totally or partially the vegetation, especially in the riparian zones, are erosion along the margins of streams and subsequent silting processes (Neres et al., 2015). Also, the absence of a forest canopy causes more sunlight to reach the streams, leading to higher air and water temperatures. This, in turn, reduces water oxygenation (Martins et al., 2017) and the availability of leaves (food and shelter resources for aquatic organisms; Brasil et al., 2014) due to increased microorganism activity (Martínez et al., 2014). As a response to anthropogenic modifications, some studies suggest that generalist species replace habitat-specialist species that depend on unaltered environments (Smith et al., 2023). For these specialist species, conservation policies are crucial for saving them from extinction (Batista, 2010).

Gallery forests reduce the exposure of streams to sunlight and maintain a comparatively mild and stable microclimate in their surroundings. These environmental characteristics favor small-sized organisms of Odonata, such as many Zygoptera species, which are ectothermic and depend on heat exchange by convection to maintain a suitable body temperature (Corbet & May, 2008; De Marco et al., 2015). On the other hand, canopy loss can favor large species that depend on direct exposure to sunlight. This is the case in most species of the suborder Anisoptera, which are endothermic and capable of maintaining body temperature by flapping their wings and by hemolymph circulation (May, 1979). Moreover, Anisoptera are more tolerant of higher temperatures than Zygoptera (Bota-Sierra et al., 2022), although the critical thermal limits of both suborders may be modified by the state of the habitat (Castillo-Perez et al., 2022). These thermoregulatory characteristics have an evolutionary origin and make organisms of the order Odonata important bioindicators of small streams' health in Cerrado biomes (Calvão et al., 2018). Furthermore, they are consistent with evidence that unaltered and shaded streams harbor more individuals and species of Zygoptera. Conversely, altered environments, where deforestation is more intense, present more species and individuals of Anisoptera. Such patterns have been found in the Cerrado (Calvão et al., 2018; De Marco et al., 2015), the Amazon (Oliveira-Junior & Juen, 2019), and in Atlantic Forest (Ribeiro et al., 2021).

In Brazil, the government environmental agency ICMBio (Chico Mendes Institute for Biodiversity Conservation) uses the Zygoptera/Anisoptera ratio for monitoring the environmental quality of streams in conservation units of the Amazon rainforest (Brasil et al., 2020a). ICMBio is also interested in learning whether this ratio could be applied in other Brazilian biomes. Thus, in this study, we tested whether the proportion of Zygoptera and Anisoptera is efficient to measure the environmental integrity of streams in the Cerrado. We expected that, with an increase in habitat integrity, the proportion of species richness and abundance of Zygoptera will increase and, consequently, the proportion of species richness and abundance of Anisoptera will decrease (such as in Amazonian streams (Oliveira-Junior & Juen, 2019). In addition, we expected the Zygoptera/Anisoptera ratio to be robust enough, compared with finer levels of taxonomic resolution, to reflect the response of Odonata to habitat degradation.

Materials and methods

Study area

Our study was conducted at 18 streams in the Cerrado biome, in the municipalities of Nova Xavantina and Nova Nazaré in the eastern parts of Mato Grosso State in the midwest region of Brazil (Fig. 1). The climate of this region has two well-defined seasons, a rainy (October to March) and a dry (April to September) one. Considering historic data from 1995 to 2005 of two meteorological stations in the study region, the annual precipitation during this time averaged 1,447 mm and the mean temperature was 25.5°C. Data from Nova Xavantina were obtained at eight streams and published by Calvão et al. (2018), whereas data from Nova Nazaré were published by Carvalho et al. (2013) and represent collections made at ten streams.

Historical sampling units

In total, 18 first- to third-order streams up to three meters wide were included in the study (Fig. 1). Each was considered a sampling unit and was visited once in the dry season. More details on stream characteristics can be found in Calvão et al. (2018) and Carvalho et al. (2013).

Environmental integrity of streams

For all streams, a protocol of environmental quality analysis named Habitat Integrity Index (HII) (Nessimian et al., 2008) was applied (Calvão et al., 2018; Carvalho et al., 2013). This index is composed of twelve items that describe the environmental conditions of the streams. It is based on the type of land use in the surroundings of the stream, the width and state of the riparian zone or gallery forest, and the structural conditions of the streambed. At the end of the analysis, the index quantifies the environmental integrity of each stream with a value that varies from zero to one. Low values, closer to zero, represent altered streams, with low environmental integrity. High values, closer to one, represent more preserved areas, with high environmental integrity. The HII considered stretches of 100 meters each that were used for the collection of Odonata.

The HII was first used in Brazil by Nessimiam et al. (2008) on Amazonian streams. Thereafter, it was widely applied in Cerrado biomes to quantify the environmental quality of streams and proved to be as efficient as it was at Amazonian streams (Brasil et al., 2020b).

Collection and identification of Odonata

The collections of Odonata by Carvalho et al. (2013) and Calvão et al. (2018) were made out during the dry season, between April to July 2010 and April to August 2011, respectively. For this, they demarcated a linear transect of 100 m per stream and collected all the observed adults of Odonata using an entomological net. The adult life stage of Odonata was chosen because it proved to be useful for environmental monitoring studies (Gómez-Tolosa et al., 2021; Oertli, 2008; Šigutová et al., 2023). Collections were made within 60 minutes, between 11.00 and 14.00 h, which corresponds to the period when adults of Odonata are most active (Calvão et al., 2018). Both Carvalho et al. (2013) and Calvão et al. (2018) stored the collected specimens following Lencioni's (2006) protocol and identified them using taxonomic keys and illustrated, specialized guides (Borror, 1942; Garrison et al., 2006; Lencioni, 2005, 2006). They deposited the specimens in the James Alexander Ratter scientific collection at the Mato Grosso State University (UNEMAT), Nova Xavantina, Mato Grosso, Brazil.

Data analysis

Since our data were adopted from these two previous studies, we evaluated the sampling coverage for Anisoptera, Zygoptera, and the most abundant genera. To answer whether the proportion of Zygoptera and Anisoptera was adequate to evaluate the environmental quality of Cerrado streams, we performed two generalized linear models using the beta distribution family. We generated one model for species richness and one for abundance as response variables. In both instances, the habitat integrity index was the predictor variable. We calculated the Zygoptera richness ratio by dividing the number of Zygoptera species by the total number of Odonata species. For abundance, we divided the number of Zygoptera individuals by the total abundance of Odonata. These calculations were performed for each stream. For each model, we calculated Nagelkerke's R^2. We did not calculate the proportion for species richness and abundance of Anisoptera, as it would be equal to 1—richness (or abundance) of Zygoptera. Therefore, the effects for Anisoptera would be exactly the inverse of those found for Zygoptera (Supplementary Material 4).

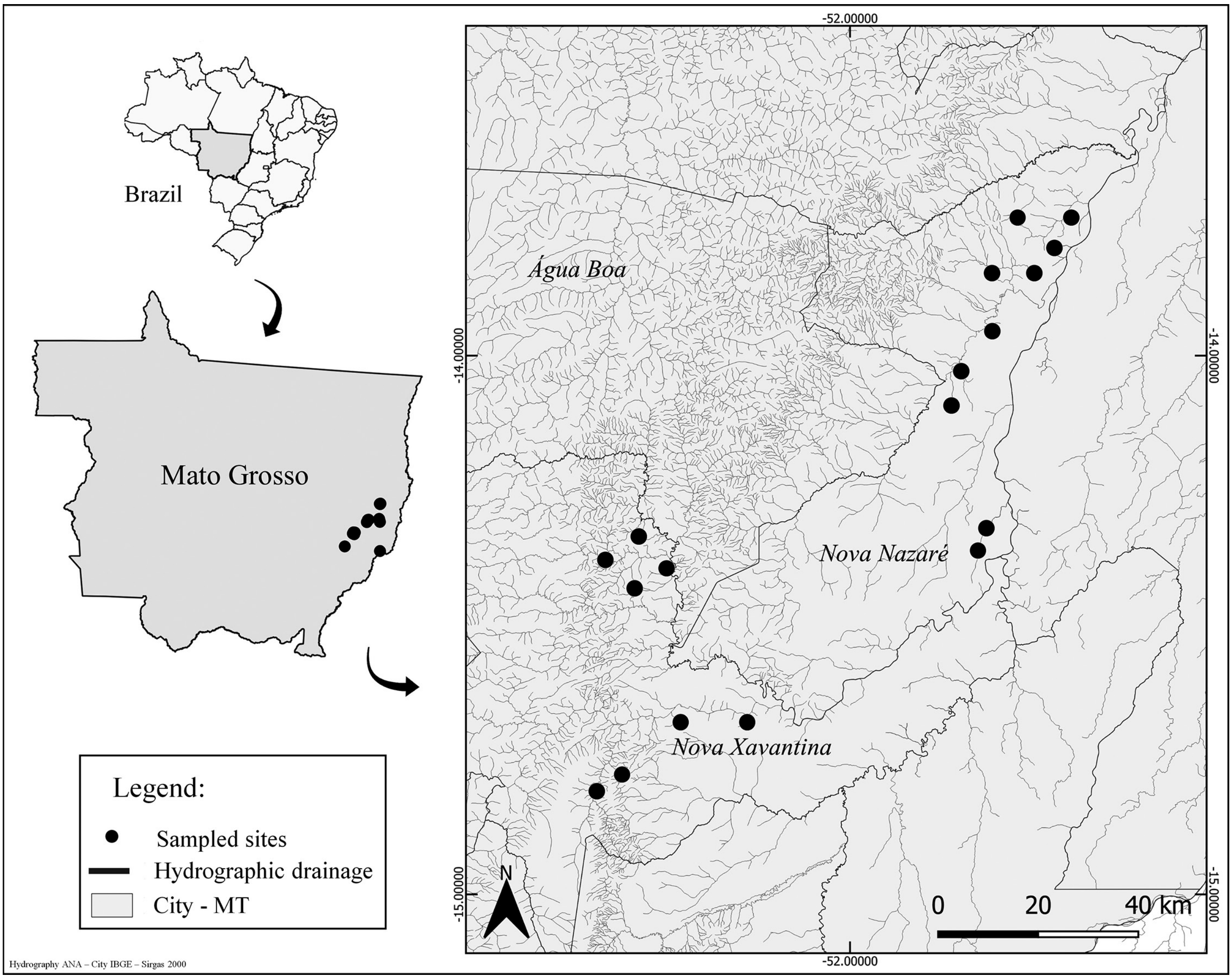

Figure 1. Streams sampled in the Cerrado biome in Mato Grosso (MT), Brazil. Data for the Nova Xavantina and Nova Nazaré streams were adopted from Calvão et al. (2018) and Carvalho et al. (2013), respectively.

Table 1. Beta regression models describing the influence of habitat integrity (as habitat integrity index, HII) on the proportion of Zygoptera and Anisoptera in the Cerrado biome, Brazil. SE: standard error; R^2: Nagelkerke's R^2; statistically significant effects ($p < 0.05$) in bold.

Ratio	Metric	Estimate	SE	z-value	p-value	R²
Zygoptera ratio	Richness	-2.348	0.574	4.091	**<0.001**	0.488
	Abundance	3.175	0.902	3.519	**<0.001**	0.407
Erythrodiplax/ Anisoptera	Richness	0.113	0.397	0.284	0.781	0.004
	Abundance	-0.789	0.788	-1.002	0.335	0.053
Coenagrionidae/ Zygoptera	Richness	-0.735	0.351	-2.096	0.056	0.196
	Abundance	-1.831	0.661	-2.772	**0.016**	0.311
Calopterygidae/ Zygoptera	Richness	0.977	0.386	2.532	**0.025**	0.263
	Abundance	1.854	0.658	2.819	**0.014**	0.318
Acanthagrion/ Zygoptera	Richness	-3.772	0.833	-4.530	**0.001**	0.532
	Abundance	-1.288	0.551	-2.339	**0.036**	0.224
Argia/ Zygoptera	Richness	4.524	0.971	4.659	**<0.001**	0.562
	Abundance	1.946	0.764	2.547	**0.024**	0.273
Hetaerina/ Zygoptera	Richness	0.726	0.773	0.939	0.365	0.048
	Abundance	0.347	0.429	0.810	0.433	0.036

To analyze whether the Zygoptera/Anisoptera ratio is robust enough to reflect differences in the pattern of Odonata response to habitat degradation, we compared the performance of Zygoptera/Anisoptera with ratios at a finer scale (i.e., at the family and genus levels) following the proposals of Šigutová et al. (2019) and Gómez-Tolosa et al. (2022). To do so, we identified the family and genera with the greatest abundance of individuals and highest species richness for all the assemblage. Then, for each stream, we calculated the abundance (or species richness) of these families (or genera) and divided this value by the total abundance (or species richness) of the suborder to which each family (or genus) belongs. Posteriorly, we used these values to construct generalized linear models using the beta-inflated distribution family, and the habitat integrity index was the predictor variable. Finally, we compared these models with the models obtained using the Zygoptera/Anisoptera ratio using Nagelkerke's R^2. A higher R^2 means that the model explains a larger proportion of the variance (Nagelkerke, 1991).

For all these models, we verified their goodness of fit with residuals vs. linear predictor plots and half-normal plots of residuals (Supplementary Material 5). We used a significance level of $\alpha = 0.05$.

All analyses were performed in R 4.2.3 software (R Core Team, 2023). The iNext package (Hsieh et al., 2016) was used to perform sample coverage curves; the betareg package (Cribari-Neto & Zeileis, 2010) for the beta distribution models; and the gamlss packages (Rigby & Stasinopoulos, 2005) for the beta-inflated distribution models and the calculation of their R^2.

Results

The streams had HII values ranging from 0.118 to 0.963, forming a gradient of environmental integrity. Thus, our study area had streams that ranged in status from well preserved (values closest to 0) to greatly altered (values closest to 1).

In total, 1,594 individuals of 47 genera and 92 species were collected. In the study, 642 individuals belonged to Anisoptera, represented solely by the family Libellulidae (17 genera and 45 species). The most abundant Anisoptera genus was *Erythrodiplax* (374 individuals). This genus had also the highest species richness of Anisoptera (11 species), and the most abundant Anisoptera species were *Erythrodiplax basalis* (Kirby, 1897) (239 individuals), *Erythrodiplax fusca* (Kirby, 1897) (86 individuals), and *Diastatops obscura* (Fabricius, 1775) (86 individuals). In the case of Zygoptera, 954 individuals were captured, which belonged to five families, 20 genera and 47 species: Calopterygidae (two genera and eight species), Coenagrionidae (15 genera and 36 species), Dicteriadidae (one genus and one species), Perilestidae (one genus and one species), and Polythoridae (one genus and one species). The most abundant Zygoptera genera were *Acanthagrion* (10 species and 292 individuals), *Argia*, (eight species and 293 individuals), and *Hetaerina* (five species and 120 individuals). The most abundant species of this suborder were *Acanthagrion gracile* (Rambur, 1842) (107 individuals), *Acanthagrion truncatum* Selys, 1876 (109 individuals), and *Argia tinctipennis* Selys, 1865 (104 individuals). Additionally, sampling coverage was close to 100% for Anisoptera, Zygoptera, and the most abundant genera (*Erythrodiplax*, *Acanthagrion*, *Argia* and *Hetaerina*) (Fig. 2).

We corroborated our hypothesis that streams with high environmental integrity host high proportions of species (Nagelkerke's $R^2 = 0.4874$) and abundance (Nagelkerke's $R^2 = 0.4069$) of Zygoptera (Table 1, Fig. 3). The opposite was observed for Anisoptera, which occur in high proportions of species and abundance at streams with low environmental integrity (Table 1).

As for Anisoptera, Libellulidae was the only family recorded, for which reason we did not compute a ratio at family level. Nevertheless, we used the genus *Erythrodiplax* to perform an analysis at genus level. We found that neither using abundance nor species richness to calculate the *Erythrodiplax*/Anisoptera ratio resulted in a statistically significant relationship with the HII (Table 1). For Zygoptera, we performed the analysis with the families Coenagrionidae and Calopterygidae, and the genera *Acanthagrion, Argia*, and *Hetaerina*, as they presented the highest species richness and abundance. Most of the ratios showed a relationship with the HII (except *Hetaerina*/Zygoptera and species richness Coenagrionidae/Zygoptera) (Table 1). Only the models using species richness to calculate the *Acanthagrion*/Zygoptera and *Argia*/Zygoptera ratios showed a higher proportion of explained variance (R^2) than the Zygoptera ratio (Table 1).

Discussion

Our analyses provide strong evidence that the proportion of species richness and abundance of the suborders Zygoptera and Anisoptera reflect the environmental quality of streams in Cerrado biomes. They show that the suborder Anisoptera is strongly related to anthropomorphized environments. Conversely, Zygoptera is strongly associated with preserved environments that feature a riparian zone surrounding the streams and

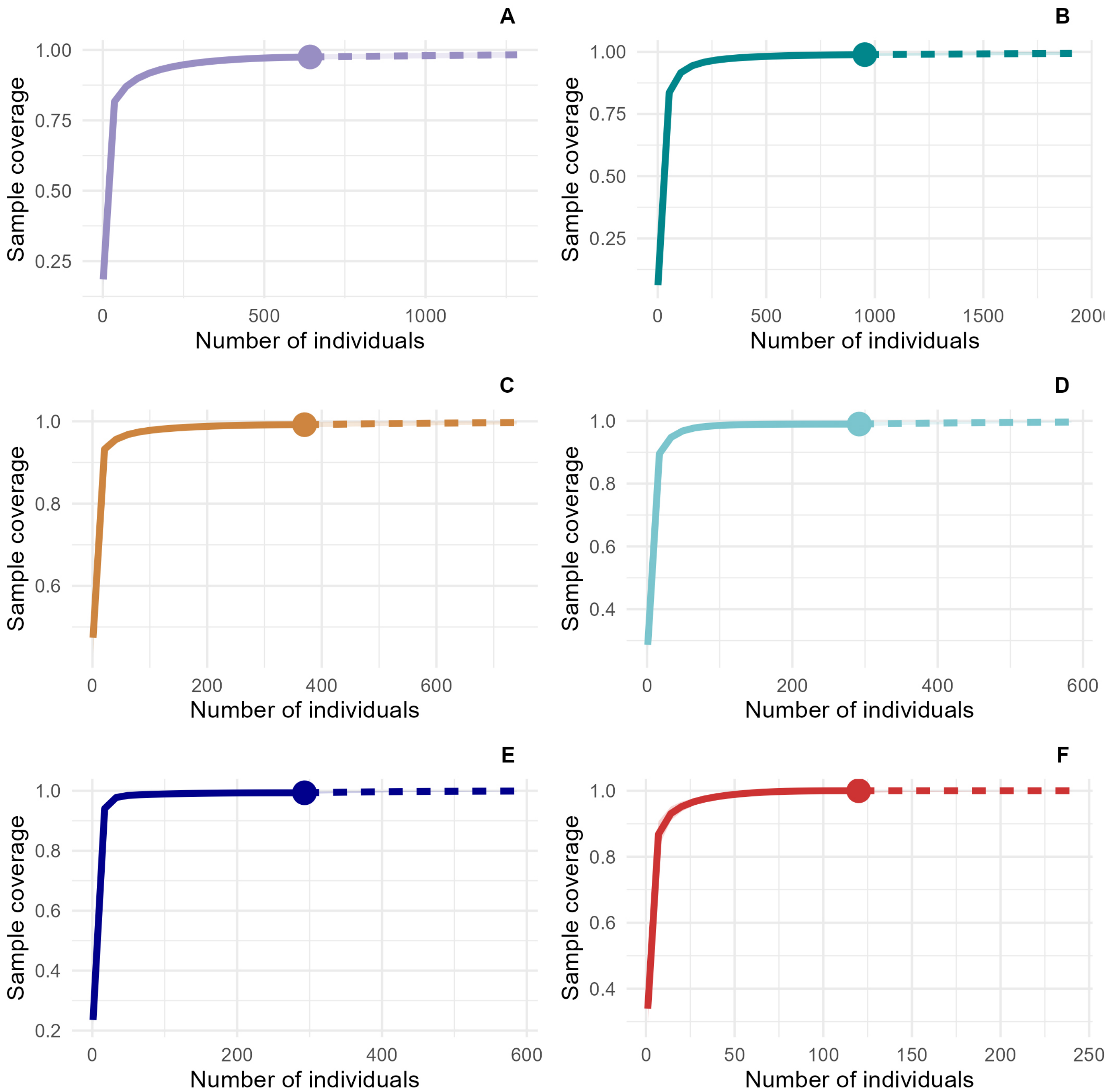

Figure 2. Sample coverage curves for suborders and most abundant genera of Odonata in the Cerrado biome, Brazil. A – Anisoptera, B – Zygoptera, C – *Erythrodiplax*, D – *Acanthagrion*, E – *Argia*, and F – *Hetaerina*.

thus provide better living conditions for members of this suborder. Our findings support studies conducted in other Brazilian biomes, such as in Amazon Rainforest (Oliveira-Junior & Juen, 2019) and the Atlantic Forest (Ribeiro et al., 2021). These studies revealed that the proportion of Zygoptera and Anisoptera was a good indicator of the environmental quality of streams, suggesting that it could be used in other biomes, such as the Cerrado.

Other authors have demonstrated the relationship between environmental integrity, measured by the HII, and Odonata communities at Cerrado streams, but they considered absolute values of species richness and abundance (e.g., Brasil et al., 2014; Calvão et al., 2018; Carvalho et al., 2013; De Marco et al., 2015; Juen et al., 2014). Here, we demonstrated for the first time the strong relationship between the proportions of species richness and abundance of Odonata communities with the environmental quality of Cerrado streams. This method was first proposed by Oliveira-Junior & Juen (2019) and it is already used in Amazonian streams for monitoring the environmental quality of habitats (Brasil et al., 2020a). Our findings support these studies and confirm that this method is useful for assessing habitat alterations in Cerrado streams as well.

Despite the above-mentioned evidence, it is important to acknowledge that this metric is not universally regarded as a reliable environmental impact indicator. In Indonesia, Zygoptera proportions were a good indicator of more richly forested areas (Dolný et al., 2012). However, in a comprehensive review of the usefulness of these ratios for assessing the state of tropical streams, Coenagrionidae/other Zygoptera and Libellulidae/other Anisoptera ratios proved more effective than the Zygoptera/Anisoptera one (Šigutová et al., 2019). This emphasizes the significance of examining both community structures and specificities at a regional scale so that the most appropriate indicator can be employed. For instance, studies conducted by Gómez-Tolosa et al. (2022) in Mexico and Mendoza-Penagos et al. (2021) in the Brazilian Amazon utilized family and genera level for analysis. In ours, we demonstrated the robustness of the Zygoptera/Anisoptera ratio in capturing shifts within Odonata communities caused by habitat degradation. Furthermore, within the Zygoptera suborder, we identified that alternative ratios based on species richness (such as *Acanthagrion*/Zygoptera and *Argia*/Zygoptera) could serve as effective monitoring instruments. However, it is worth noting that these particular genera are recognized for their exceptional diversity within the Neotropical regions (Garrison et al., 2010), potentially posing challenges to their integration into citizen science initiatives. This underscores the importance of this study, in that it proved that the evidence derived from the Amazon Rainforest (Oliveira-Junior & Juen, 2019) and Atlantic Forest (Ribeiro et al., 2021) could be not robust enough for this biome. Moreover, the Cerrado naturally possesses both non-forested areas (such as fields and scrub areas) (Brasil et al., 2021; Vilela et al., 2016), and gallery forests. Therefore, it is crucial to test the efficacy of Zygoptera/Anisoptera ratios in all these landscapes, as the accuracy of this indicator may vary between them. Because our study was conducted in areas with gallery forests, this ratio at least appears to be safe for use for streams in such landscapes.

The proportion of Zygoptera and Anisoptera represents a relatively easy tool for monitoring the environmental quality of streams and it has been successfully used in Brazil, in the Amazon biome. Our study demonstrated that this method can also be successfully used at Cerrado streams as an indicator of environmental quali-

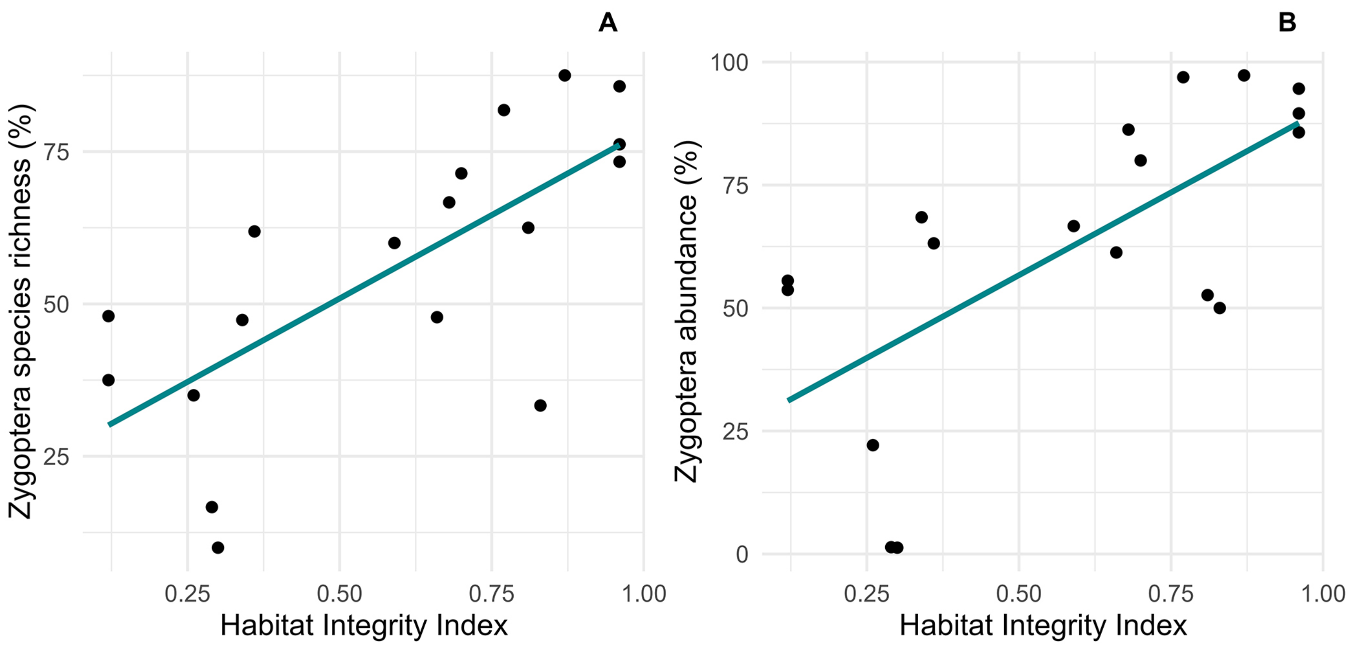

Figure 3. Relationship between habitat integrity (as habitat integrity index, HII) and the proportion of Zygoptera richness (a) and abundance (b) in the Cerrado biome, Brazil.

ty. Although a linear relationship between environmental integrity and the proportions of Zygoptera (positive) and Anisoptera (negative) has been evidenced here, it is not suitable to provide a fixed threshold for monitoring. However, like in other biomes, when Cerrado streams exhibit higher proportions of Zygoptera, these indicate a higher level of preservation, whereas a higher proportion of Anisoptera suggests a more altered condition. Furthermore, abundance-based monitoring of Zygoptera and Anisoptera is a method that can be easily used by people unfamiliar with species-level identification. Thus, we recommend this method for agencies responsible for quality-monitoring Cerrado streams. Follow-up studies are planned in the Cerrado in which the Zygoptera/Anisoptera ratio will be compared between raw adult observations and occurrences filtered by local recruitment or reproductive success (such as exuviae and immatures). This selectively assembled "resident" subset would contribute to a comprehensive evaluation of this tool (Šigutová et al., 2023).

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Supplementary material

Supplementary material 1. Anisoptera (Libellulidae) assemblage in the Cerrado biome in Mato Grosso (MT), Brazil.

Supplementary material 2. Zygoptera assemblage in the Cerrado biome in Mato Grosso (MT), Brazil.

Supplementary material 3. Habitat Integrity Index of the streams sampled in the Cerrado biome in Mato Grosso (MT), Brazil.

Supplementary material 4. Beta regression models describing the influence of the habitat integrity on the proportion of Anisoptera in the Cerrado biome, Brazil.

Supplementary material 5. Goodness of fit of the models that evaluate the influence of the state of habitat on the Zygoptera/Anisoptera ratio in the Cerrado biome in Mato Grosso (MT), Brazil.