Original Articles

Functional responses of Odonata larvae to human disturbances in neotropical savanna headwater streams

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ABSTRACT

Headwater streams are facing increasing disturbances from human pressures worldwide, thus better knowledge about bioindicators, particularly aquatic insect responses to various pressures and stressors, are urgently needed. Multiple trait-based approaches consider species attributes filtered by the environment, allowing them to persist in ecosystems under environmental pressures. Because this approach has been minimally explored in Odonata larvae, we aimed to understand how anthropogenic stressors structure Odonata larval assemblages in neotropical savanna streams by using multiple trait-based approaches. We hypothesized that a set of stressors–such as reduced substrate heterogeneity, poor water quality, natural land cover converted to pasture and agriculture, and reduced local riparian canopy cover–select Odonata functional traits. We collected 3209 Odonata larvae from 186 neotropical savanna headwater stream sites and used 39 environmental variables and seven traits in 23 categories related to their functional roles in Odonata genera. To assess associations between trait categories and environmental variables, we applied RLQ and fourth-corner statistical analyses. We found strong relationships between environmental variables and sets of Odonata biological traits that were separated into two main groups. Zygoptera genera (*Perilestes, Allopodagrion, Heliocharis, Argia, Epipleoneura, Mnesarete/Hetaerina, Psaironeura*) have elongated body shapes, caudal lamellae respiration, conforming thermoregulation, and endophytic oviposition. Such traits favor assemblages in conditions similar to reference streams, including denser riparian vegetation, good water quality, and diverse flows and substrate. Therefore, they are more sensitive to changes in those conditions. On the other hand, Anisoptera genera (*Gomphoides, Archaeogomphus, Macrothemis, Brechmorhoga, Gynothemis, Phyllocycla*) have cylindrical body shapes, internal gill respiration, endothermic thermoregulation, and burrowing behavior. Those traits facilitate their survival in intermediate or disturbed stream sites, characterized by riparian deforestation, increased erosion and siltation, and higher levels of total dissolved solids and conductivity. Therefore, using Odonata larval traits can be a valuable tool for assessing and monitoring anthropogenic impacts on neotropical savanna streams.

1. Introduction

Freshwater ecosystems are greatly affected by multiple human activities that modify and affect landscapes and waterscapes with the aim of benefiting human economies (Limburg et al., 2011). The growing human demands for water and the uncontrolled exploitation of natural resources over time, inappropriate uses of land for agriculture and livestock, deforestation, several types of domestic and industrial

pollution, destruction and/or degradation of natural habitats, hydropower dams, and non-native species introductions have all degraded aquatic ecosystems (Dudgeon, 2010; Higgins et al., 2021; Reid et al., 2019). Such pressures have therefore degraded the quality and quantity of aquatic ecosystem services and aquatic biodiversity worldwide (Dudgeon, 2010; Higgins et al., 2021; Romero et al., 2021).

In Brazil, the Cerrado biome (Neotropical Savanna) is considered a global biodiversity hotspot (Myers et al., 2010) and has been degraded for over 200 years (Strassburg et al., 2017). This historical degradation, which involved converting native land cover to agriculture, pasture, and urbanization, has been increasing with the expansion of the agricultural frontier. Consequently, the Cerrado currently has the smallest proportion of remaining natural vegetation cover (19.5%) of all Brazilian biomes, losing almost 20% of its natural vegetation cover since 1980 (Mello et al., 2020). Considering that Cerrado headwaters contribute to eight of the sixteen major river basins in Brazil, it is urgently necessary for decision-makers to reconcile Cerrado conservation and human well-being based on science (Mello et al., 2020; Ruaro et al., 2021).

Responses to environmental changes in freshwater ecosystems in the Cerrado have been reported for different taxonomic groups of benthic macroinvertebrates assemblages (Castro et al., 2018; Dala-Corte et al., 2020; Firmiano et al., 2021; Guimarães-Souto et al., 2021; Ruaro et al., 2016). Even within the same order, different taxa respond differently to the same pressures, including Ephemeroptera (Firmiano et al., 2017) and Hemiptera- Nepomorpha (Giehl et al., 2019). When we evaluate species within the same taxonomic group, we can understand why some species are resistant to environmental changes while others disappear, which is one of the major challenges for conservation ecology (Powney et al., 2015). The use of trait-based approaches may help identify those characteristics that allow some species to resist anthropogenic pressures (Berger et al., 2018; Dolédec and Statzner, 2010; Firmiano et al., 2021). Those traits include morphological, physiological or phenological features that directly or indirectly improve an individual's fitness or performance (Violle et al., 2007) and facilitate identifying general patterns that improve predictability of responses to anthropogenic disturbance (McGill et al., 2006).

Despite the importance of Odonata as bioindicators (e.g., Mendes et al., 2017; Miguel et al., 2017; Oliveira-Junior and Juen, 2019; Ribeiro et al., 2021), most studies have used traditional ecological approaches based on taxonomic richness and composition (Mendes et al., 2020). In Brazil, studies that include multiple trait-based approaches have been used recently (e.g., Dalzochio et al., 2018; Mendes et al., 2020; Pereira et al., 2019; Pires et al., 2020), but there is still a gap in applying knowledge of Odonata larval traits. The use of multiple trait-based approaches has the advantage of considering the different ecological requirements between species (Saito et al., 2015), which is fundamentally important in larval Odonata studies because its suborders show morphological, physiological, and behavioral differences. For example, Zygoptera larvae have cylindrical bodies with fragile structures in the distal portion of the abdomen called caudal lamellae, used in breathing (Ramirez, 2010; Suhling et al., 2015). They can be sprawlers, climbers, burrowers, clingers, or swimmers (Assis et al., 2004; Carvalho and Nessimian, 1998; Dalzochio et al., 2018). On the other hand, Anisoptera larvae have more robust and cylindrical or flattened bodies, breath through internal rectal gills (Ramirez, 2010; Suhling et al., 2015), and often partially bury themselves in sediments (Assis et al., 2004; Carvalho and Nessimian, 1998; Dalzochio et al., 2018). All those traits are related to three main requirements for larval survival: breathing, feeding, and predation refuge; therefore, they determine the distribution and structure of Odonata assemblages in streams (Corbet, 1980).

Given the advantages of using multiple traits and because it is an underexplored approach for assessing responses of Odonata larvae to anthropogenic changes, we aimed to understand how anthropogenic stressors affect the traits and taxonomic and functional structure of larval Odonata assemblages in Cerrado streams. We assumed that anthropogenic pressures in catchment land use and cover, riparian

canopy cover, physical habitat, and water quality would result in local environmental stressors, which would select for Odonata genera with a specific combination of traits. Thus, we hypothesized that a set of stressors (reduced substrate heterogeneity, reduced water quality, alteration of natural land cover to pasture and agriculture, reduced local riparian canopy cover) would select certain sets of traits (Table 1).

Table 1
Predictions or trait category responses to anthropogenic stressors. (+) indicates an increase in the frequency of the trait category; (-) indicates a decrease in the frequency of the trait category with the increasing stressor.

Stressor	Trait category	Trait response	Explanation effect/trait
Reduced substrate heterogeneity	Larval body shape (Cylindrical; elongated)	+ Cylindrical - Elongated	More robust and cylindrical body shape is favored in microhabitats with higher proportion of stones and gravel substrate. (Pires et al., 2020)
	Larval behavior (Burrower; climber; clinger; sprawler)	- Sprawler - Climber + Burrower - Clinger	Fine sediments homogenize the substrate, favoring burrower larvae and excluding larvae that depend on other types of substrates. (Dalzochio et al., 2018; Mendes et al., 2020)
Reduced water quality	Larval respiration (Caudal lamellae; rectal gills)	+ Rectal gills - Caudal lamellae	Higher water temperature resulting from reduced riparian vegetation decreases dissolved oxygen. Rectal gills allow these larvae to survive because of active water exchange. (Kohnert et al., 2004)
Alteration of natural land cover to pasture and agriculture	Larval preference for substrate (Gravel; litter; macrophytes; mud; roots; sand; stone)	+ Sand + Gravel + Stone + Litter - Macrophytes - Roots + Mud	Increased sedimentation & substrate homogenization substrates exclude larvae that depend on macrophytes. (Mendes et al., 2020)
	Adult habitat preference (Forest; open area)	- Forest + Open area	Favor species adapted to open areas. (De Marco and Resende, 2002; Oliveira-Junior and Juen, 2019; Paulson, 2006)
Reduced local riparian canopy cover	Thermoregulation (Endothermic; heliothermic, thermal conformer)	+Endothermic - Heliothermic - Thermal conformer	Endothermic species can control their body temperature. (Corbet and May 2008; May 1976)
	Oviposition (Endophytic; epiphytic, exophytic)	- Endophytic + Exophytic - Epiphytic	Exophytic oviposition does not require riparian vegetation. (Dalzochio et al., 2018; Paulson, 2006)

2. Methods

2.1. Study area

The study was conducted in Cerrado headwater streams. A total of 160 sites from 1st to 3rd order (Strahler, 1957), belonging to the Nova Ponte, Volta Grande, São Simão, and Três Marias hydrological units, were randomly selected following a probabilistic sampling design, according to the methodology used by the U.S. Environmental Protection Agency (Callisto et al., 2014; Olsen and Peck, 2008) (Fig. 1). The hydrological units were defined as drainage areas < 35 km from one of four major hydropower reservoirs, all subject to a wide disturbance gradient, as demonstrated in previous studies (e.g., Callisto et al., 2019; Castro et al., 2018; Firmiano et al., 2021; Silva et al., 2018). To ensure that minimally disturbed sites were included as reference sites, another 26 sites, located in Serra da Canastra National Park and Serra do Salitre were sampled (Martins et al., 2018). We sampled during the September low flow season from 2010 to 2014: 2010 in Três Marias, 2011 in Volta Grande, 2012 in São Simão, 2013 in Nova Ponte, 2014 in Serra da Canastra National Park and Serra do Salitre.

2.2. Collection and identification of Odonata larvae

The length of each site was 40 times its mean wetted width, with a minimum of 150 m, and divided into 11 equidistant transects. We sampled Odonata larvae with a kick-net (250 μm mesh, 0.09 m² area), following a zigzag trajectory across the transects (left, center, and right). This methodology has been successfully used in previous Cerrado stream studies (e.g. Castro et al., 2018; Firmiano et al., 2021; Silva et al., 2018). The samples were washed, stored in 70% alcohol, processed, identified, and then deposited in the reference collection of the Instituto de Ciências Biológicas at the Universidade Federal de Minas Gerais. Odonata larvae were identified under a stereoscopic microscope and using taxonomic keys (Costa et al., 2004; Neiss and Hamada, 2014; Pessacq et al., 2018) as well as descriptions and reviews available for each taxon. Some morphological structures are essential for identifying Odonata larvae to species. However, those structures are incomplete or absent in early larval stages (Neiss and Hamada, 2014). Therefore, we identified all individuals only to genus to avoid erroneous identifications.
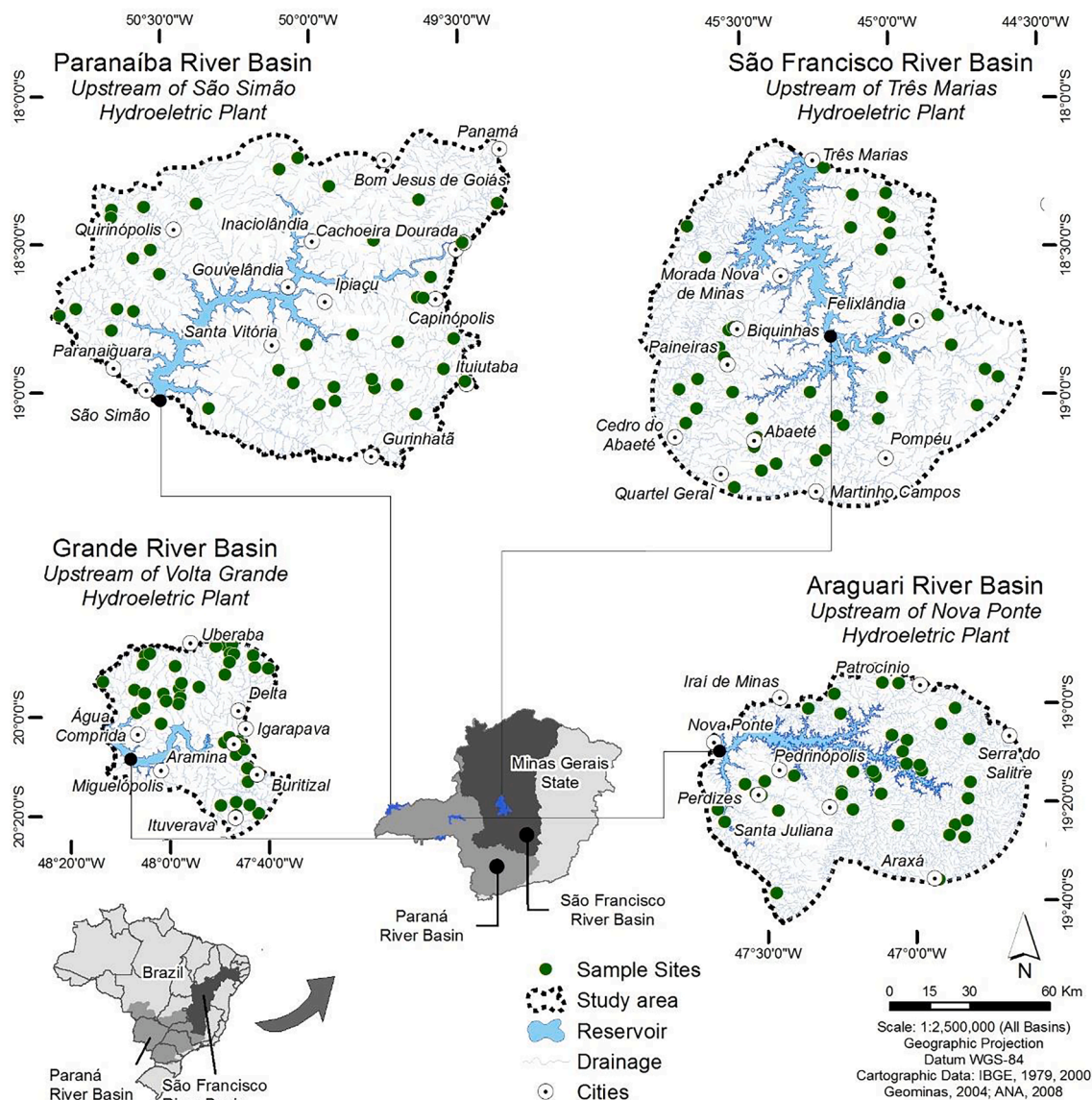

Fig. 1. Locations of sample sites in four hydrological units in the neotropical savanna, Brazil.

2.3. Environmental variables

Environmental variables at each site were described in terms of catchment land use and cover, local riparian canopy cover, physical habitat structure, and water quality. Land use was characterized through use of satellite images provided by Landsat and fine-resolution images from Google Earth. Physical habitat structure variables included substrate heterogeneity (habitat hydromorphology, substrate size, flow regime, instream habitat cover) (Callisto et al., 2014;. Water quality was assessed by measuring temperature (°C), electrical conductivity (μ S cm^{-1}), pH, turbidity (NTU), and total dissolved solids (mg L^{-1}) with a multimeter probe (YSI, 650 MDS, model 6920). In addition, the concentrations of total nitrogen (mg L^{-1}) and dissolved oxygen (mg L^{-1}) were determined in our laboratory through use of chilled water samples from the sites following APHA (2005). To assess local and catchment anthropogenic disturbances in each site, we calculated the Integrated Disturbance Index (IDI) based on Ligeiro et al. (2013b). The IDI is the Euclidian distance between the site and the origin of the disturbance plane formed by two other indexes, the LDI and CDI, applying the Pythagorean theorem (IDI = $[(LDI/5)^2 + (CDI/300)^2]^{1/2}$. The LDI is calculated from eleven observations of the presence and proximity of anthropogenic disturbances (Peck et al., 2006) and the CDI is calculated by adding the % of land uses, weighted by the potential for degradation that each has on aquatic ecosystems (CDI = 4 × % urban + 2 × % agriculture + % pasture) (Ligeiro et al., 2013b).

This procedure allowed us to calculate 250 environmental variables. After screening, those variables having low variability (over 90% with little or no variance) or being highly correlated with each other (r > 0.5) were removed. Whenever a high correlation between two variables was detected, the metric indicated in the literature (Dalzochio et al., 2018; Oliveira-Junior et al., 2019; Oliveira-Junior and Juen, 2019; Pires et al., 2020) as important for Odonata was retained for subsequent analysis. Finally, we retained a set of 39 environmental variables (Supplementary Material Tables 1 and 2).

2.4. Odonata traits

We selected seven traits distributed in 23 categories that are associated with species morphology, behavior, and life history strategies for analyzing the functional structure of the Odonata assemblages (Table 1, Supplementary Material Table 3), based on studies carried out in the Neotropics (Dalzochio et al., 2018; Mendes et al., 2020; Pereira et al., 2019; Pires et al., 2020).

2.5. Data analyses

To assess the gradient of environmental disturbance in the sites, we first categorized the sites into least-disturbed (IDI < 0.21), intermediate (IDI > or = 0.21 and < or = 0.63) , and disturbed (IDI > 0.63) as described in Castro et al. (2018). Then we performed a Non-metric Multidimensional Scaling (nMDS) ordination using Euclidean distance matrices followed by an "EnvFit" to show which variables were most related to each axis. In addition, we performed a Permutational Multivariate Analysis of Variance (PERMANOVA) and a Permutational Analysis of Multivariate Dispersion (PERMIDISP) to confirm the difference between the environmental metrics of disturbance classes. We performed a Principal Coordinate Analysis (PCoA) using the Bray-Curtis distance matrix and the IDI as a *bubble* variable to assess the variation in genera composition. Finally, we performed a PERMANOVA and a PERMIDISP to confirm the difference between the Odonata genera composition of the disturbance classes. We performed the analyses in R (R Core Development Team, 2016) with *ggplot2* (Wickham, 2016), *ape* (Paradis et al., 2004) and *vegan* (Oksanen and Guillaume Blanchet, 2017) (Fig. 2).

To assess associations between trait categories and environmental variables, we applied RLQ and fourth-corner analyses, as recommended by Dray et al., 2014. RLQ produces three tables: an environmental characteristics table (R), a taxa abundance table (L), and a traits table (Q). RLQ aims to identify the main co-structures between traits and environmental characteristics weighted by taxa abundances (Dolédec et al., 1996) and provides classification scores to summarize the joint
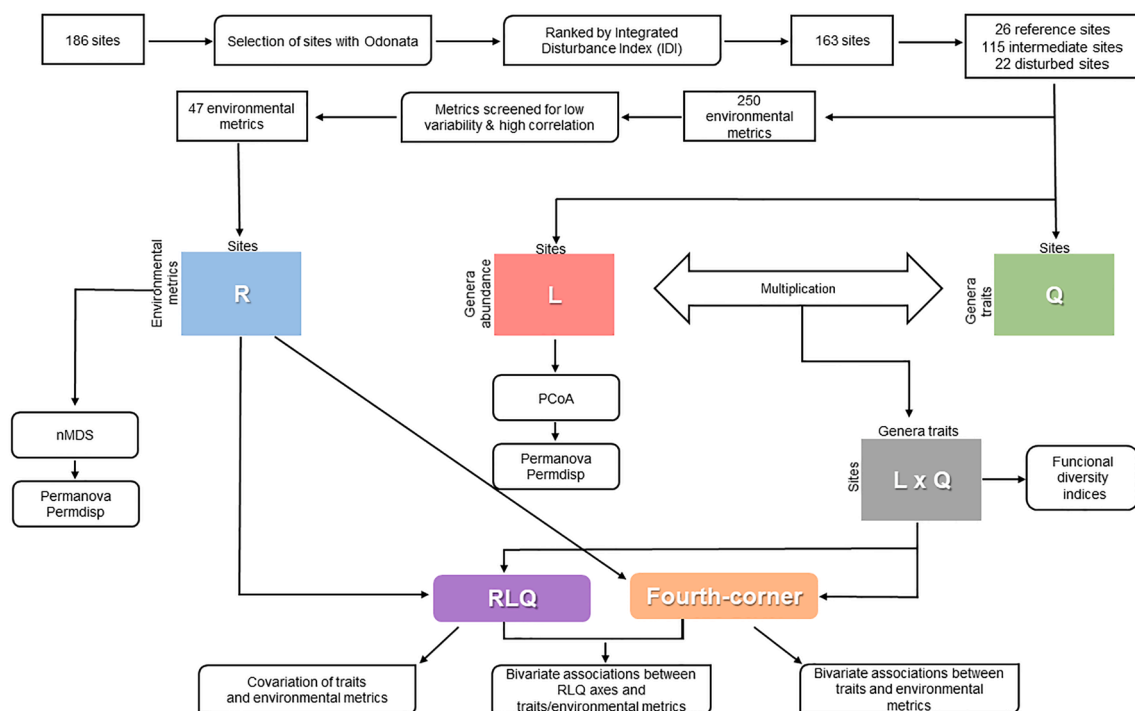

Fig. 2. Schematic diagram presenting the methodological design of site selection and statistical analyses used in this study. R = an environmental characteristics table, L = a taxa abundance table, Q = a traits table.

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structure between the three tables. Fourth-corner analysis primarily tests the relationships between individual characteristics and the environment (that is, one characteristic and one environmental variable at a time) (Dray et al., 2014). Local environmental variables were standardized (mean = 0 and standard deviation = 1) before running all analyses. The overall significance was further assessed via a global Monte-Carlo test using 9999 random permutations of the table rows of R (sites, model 2) and the rows of Q (species, model 4). A combination of RLQ and fourth-corner analyses was used to evaluate the significance of associations between traits and combinations of environmental variables identified by RLQ. RLQ analysis summarizes multivariate structures, but it does not provide significance tests, whereas fourth-corner analysis only tests the significance of bivariate associations and does not consider covariations among traits or among environmental variables. Combining the associations between RLQ axes and traits/environmental variables markedly improves the interpretation of RLQ and fourth-corner results (Dray et al., 2014). This complementary approach has been extensively used in previous studies, including for Cerrado headwater streams (e.g. Castro et al., 2018; Firmiano et al., 2021; Martins et al., 2021). Significance was tested using a permutation procedure with model 6, which is a combination of models 2 (permutation of sites) and 4 (permutation of genera). We used 9999 permutations and the false discovery rate adjustment (FDR) method to correct P-values for multiple-test comparisons (Dray et al., 2014) (Fig. 2). We performed these analyses in R (R Core Development Team, 2016) with *vegan* (Oksanen and Guillaume Blanchet, 2017) and *ade4* (Chessel et al., 2004) packages.

We also calculated six functional diversity indices using the relative abundance of genera in each trait category (Brandl et al., 2016; Laliberté and Legendre, 2010; Mouillot et al., 2013; Villéger et al., 2008). Functional richness is the amount of functional space, or niche, filled by genera in the assemblage. Functional evenness is the uniformity of genera distribution abundance in the filled niche space. Functional divergence indicates how abundance is distributed along a functional trait axis, within the volume of trait niche space occupied by genera, Functional dispersion is defined as the dispersion or spread of the genera in the niche space, Functional specialization is the mean distance of a genus from the rest of the genera pool in niche space. Functional originality indicates the exclusivity of a genus in the functional space occupied by a given assemblage. First, we performed an analysis of variance (ANOVA) for each index between classes of disturbances. Because there was no homogeneity of variances, a Kruskal-Wallis test was carried out for each index.

3. Results

The nMDS ordination based on the Euclidean distance matrix on environmental variables showed a significant difference among disturbance classes (pseudo − F $_{(2,160)}$ = 7.3762, p < 0.0001) (Fig. 3). However, PERMDISP (F $_{(2,160)}$ = 10.344, p < 0.0001) showed that differences were significant only between least-disturbed × intermediate (p < 0.0001) and least-disturbed × most-disturbed (p = 0.025) classes. The differences between intermediate × most-disturbed were not significant (p = 0.778). The least-disturbed sites had a positive relationship with increasing levels of the sum of natural cover types (Natural_sum), channel width/depth (XWD_RAT), and total bedrock (PCT_BDRK). Least-disturbed sites also were negatively related with increased amounts of sand + fines (PCT_SAFN), mean leaf litter (XFC_LEB), and mean total shelter (XFC_TOT). The intermediate and most-disturbed sites were positively related with mean exposed soil (XGB), pasture, and mean incision height (XINC_H), but negatively related to increased current velocity (XVEL), mean total woody riparian vegetation cover (XCMG), and mean undercut banks (XFC_UCB) (Fig. 3).

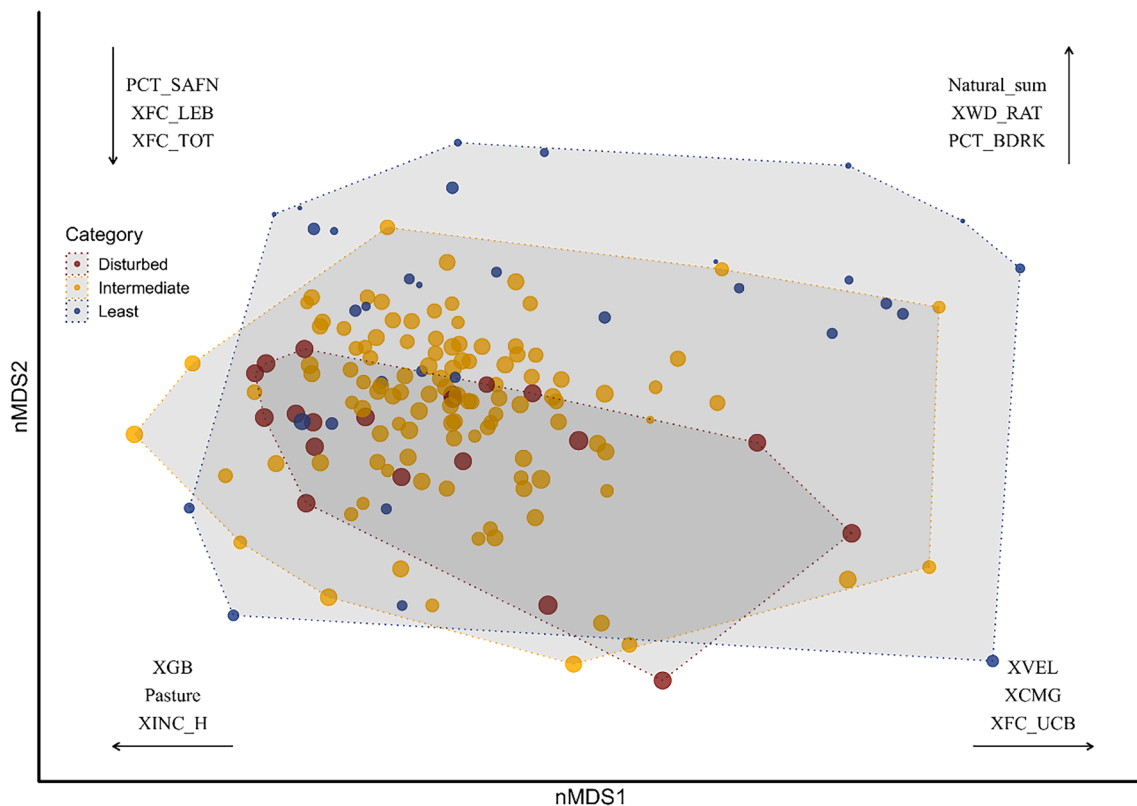We collected a total of 3209 Odonata larvae: 1500 Zygoptera were

Fig. 3. Non-metric Multidimensional Scaling (nMDS) ordination of environmental variables (PCT_SAFN: sand + fine sediments , XFC_LEB: mean leaf litter cover, XFC_TOT: mean total fish cover, natural_sum: sum of natural catchment cover, XWD_RAT: mean of width/depth ratio, PCT_BDRK: % bedrock, XGB: mean exposed soil, XINC_H: mean incision height, XVEL: current velocity, XCMG: mean total woody riparian cover, XFC_UCB: mean undercut bank).

represented by six families and eight genera; 1709 Anisoptera were composed of 22 genera in four families. The PCoA ordination based on the Bray-Curtis distance matrix showed a significant difference among the Odonata composition (pseudo – F $_{(1,161)}$ = 3.709, p < 0.0001) (Fig. 4). However, the PERMDISP (F $_{(2,160)}$ = 0.2928, p = 0.7465) confirmed a difference between the composition but not among the disturbance classes. Of the 30 genera sampled, one occurred only in the least-disturbed sites and five only in intermediate sites. The least-disturbed and intermediate sites shared nine genera, intermediate and most-disturbed sites shared one, and least-disturbed and most-disturbed shared one. Thirteen genera occurred in all classes of disturbance (Supplementary Material Fig. 1).

The global RLQ test revealed a significant relationship between genera abundance and environmental variables (model 2, p = 0.0001), as well as genera abundance and biological traits (model 4, p = 0.029). The cross-variance between traits and environmental variables was summarized by the first two RLQ axes (79.2% and 12.9% for axis 1 and 2, respectively). These axes were responsible for 71% of the variability of the environmental variables and 96% of the variance of the traits table (Fig. 5).

Regarding the first RLQ axis, we observed a positive relationship between *Perilestes*, *Allopodagrion*, *Heliocharis*, *Argia*, *Epipleoneura*, *Mnesarete/Hetaerina*, and *Psaironeura* with higher mean substrate size (Dgm_X), higher mean total cover (XCMG), higher flow diversity (DIV_FLUXO), and large shelters (XFC_BIG). Those genera are mainly elongated with caudal lamellae respiration, thermal conformers, and have endophytic oviposition. On the other quadrant of the axis, we observed a positive relationship between *Gomphoides*, *Archaeogomphus*, *Macrothemis*, *Brechmorhoga*, *Gynothemis*, and *Phyllocycla* with higher exposed soil (XGB), higher total dissolved solids (TDS), higher % pasture (Pasture) and higher water conductivity. Those genera have cylindrical bodies with internal gills, endothermic thermoregulation, and burrower behavior (Fig. 5).

Regarding the second RLQ axis, we observed a positive relationship between *Neocordulia*, *Planiplax*, *Elga*, *Dasythemis*, and *Sympectrum* with more natural land cover (Natural_sum) and greater bed stability

(LDBM). Those genera are exophytic ovipositors and thermal conformers with sprawling behavior and a preference for stony substrate. At the other end of that axis, we found a positive relationship between *Zonophora*, *Phyllocycla* and *Cacoides* with greater % sand + fine sediments (PCT_SAFN). Those genera are mainly burrowers, heliothermic and epiphytic ovipositors (Fig. 5).

The fourth-corner test revealed no significant bivariate relationship. Therefore, we assessed the relationships between individual traits and the two RLQ environmental axes and individual environmental variables and the two RLQ trait axes by combining both RLQ and fourth-corner analysis. The first environmental axis (AxcR1, combination of environmental variables) was significantly positively correlated with genera having cylindrical bodies, internal gills, and a preference for sand substrate. That axis was negatively correlated with elongated bodies, caudal lamellae, climber and clinger behavior, and endophytic oviposition (Fig. 6A). The second environmental axis (AxcR2, combining the RLQ trait axes and environmental variables) was significantly positively correlated with organisms having epiphytic oviposition. The second RLQ trait axis (AxcQ2, combination of traits) was negatively correlated with total natural cover (Fig. 6B).

None of the functional indices calculated concerning disturbance classes was significant (Functional richness χ^2 = 0.33522, p = 0.8457, diversity χ^2 = 1.4451, p = 0.4855, evenness χ^2 = 0.35127, p = 0.8389, dispersion χ^2 = 0.55284, p = 0.7585, specialization χ^2 = 1.7584, p = 0.4151, originality χ^2 = 0.42996, p = 0.8066, Supplementary Material Fig. 2).

4. Discussion

We found strong relationships between environmental variables and certain sets of Odonata larval biological traits, separated into two main groups according to the two suborders, and all our predictions were corroborated (Table 1). The most important traits for structuring Zygoptera assemblages were body shape and larval respiration, thermoregulation, and oviposition, and those traits were associated with environmental variables characteristic of least-disturbed sites. On the
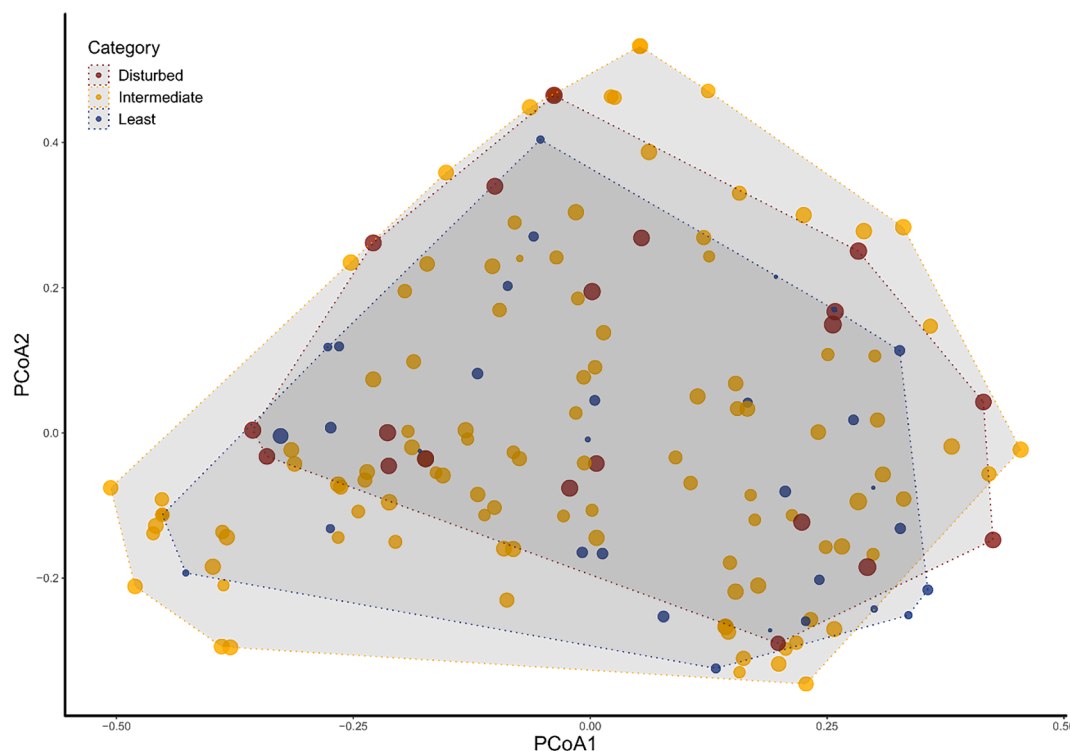

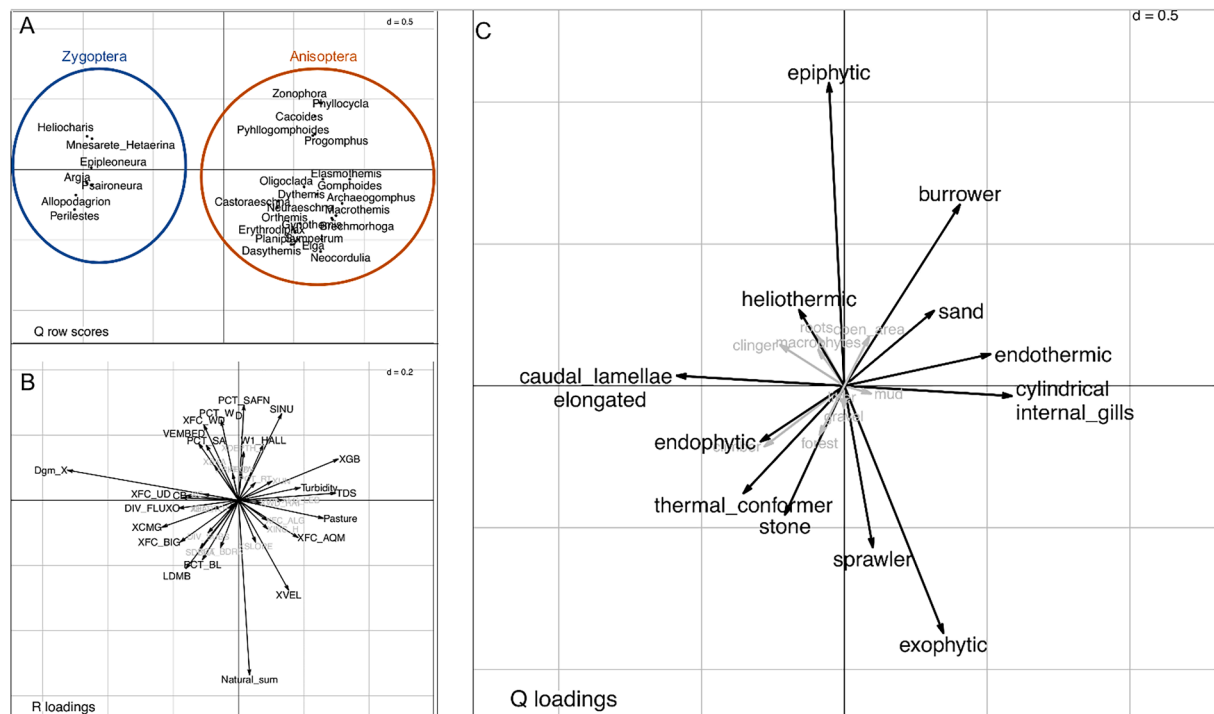**Fig. 4.** Principal Coordinate Analysis (PCoA) of the composition of genera.

Fig. 5. Axis 1 and axis 2 of the RLQ analysis of 186 sites in the neotropical savanna. (A) larval Odonata scores; (B) environmental variable scores; (C) trait scores. (D) indicates figure grid scale.

other hand, the most important traits for structuring Anisoptera assemblages were body shape, respiration, larval behavior, thermoregulation, and oviposition, and those traits were associated with intermediate to most-disturbed sites. Therefore, we assume that the evaluated stressors acted as environmental filters of more than one trait simultaneously, for both Zygoptera and Anisoptera genera.

The Zygoptera genera *Perilestes*, *Allopodagrion*, *Heliocharis*, *Argia*, *Epipleoneura*, *Mnesarete/Hetaerina*, and *Psaironeura* have elongated bodies and caudal lamellae respiration, and are thermal conformers and endophytic ovipositors. Those traits were related to environmental characteristics present in the least-disturbed sites, such as greater mean substrate size, flow diversity, mean total riparian vegetation cover, and larger sized shelters, making those traits indicators of sites experiencing less anthropogenic pressure. For example, Zygoptera larvae have elongated body shapes that favor fixation to the substrate (Pires et al., 2020). This type of morphology minimizes the hydraulic stress because of closer contact with the substrate (Salles and Ferreira-Júnior, 2014) and currents will not dislodge those individuals. However, this depends on higher flow diversity in the site and microhabitats with low current velocities, which are ideal for these larvae (Pires et al., 2020). The higher the site flow and substrate diversity, the greater the chance the site provides conditions supporting Zygoptera traits (Agra et al., 2021).

Zygoptera larvae with caudal lamellae respiration, such as *Perilestes*, *Allopodagrion*, *Heliocharis*, *Argia*, *Epipleoneura*, *Mnesarete/Hetaerina*, and *Psaironeura*, are directly related to places with good water quality and with preserved riparian vegetation canopy cover. Trees and vegetation on the riverbank provide shade and reduce water temperatures, which increases dissolved oxygen concentration (Riis et al., 2020). These conditions facilitate survival of Zygoptera genera that breath through caudal lamellae and require higher concentrations of dissolved oxygen (Jooste et al., 2020; Ramirez, 2010). Furthermore, these genera are thermal conformers and their presence was directly related to higher total riparian vegetation cover. Thermal conformers cannot produce heat to control their body temperatures. They have smaller body sizes and higher thermal conductance, resulting in heat exchange with the environment by convection. Their body temperatures are the same as

those in their environments (Corbet and May 2008; May 1976). Likewise, removal of riparian vegetation negatively affects their adults, because they are even more subject to overheating and desiccation because of greater fluctuations in air temperatures (De Marco Júnior et al., (2015; Oliveira-Junior and Juen, 2019). Besides being important for thermoregulation, woody riparian vegetation is important for endophytic oviposition (Pereira et al., 2019), primarily in woody surfaces above the water (Paulson, 2006).

On the other hand, the Anisoptera *Gomphoides*, *Archaeogomphus*, *Macrothemis*, *Brechmorhoga*, *Gynothemis*, and *Phyllocycla* larvae have cylindrical bodies, internal gills, endothermic thermoregulation and burrower behavior. Those traits were related to environmental characteristics present in intermediate and most-disturbed sites, with higher conductivity and total dissolved solids, higher % pasture in the catchment, higher % exposed soil, and higher stream substrate embeddedness. This indicates that those genera are resistant to anthropogenic pressures and good indicators of disturbed sites. For example, those larvae can survive in waters with higher total dissolved solids and higher water conductivity because of their ability to osmoregulate through their internal rectal gills, which have enzymes in their epithelial cells capable of balancing ions (D'Amico et al., 2004; Khodabandeh, 2007; Rychła et al., 2011). Higher concentrations of total dissolved solids and conductivity often indicate higher concentrations of domestic and industrial wastewater (Rusydi, 2018).

The Anisoptera, *Gomphoides*, *Archaeogomphus*, *Macrothemis*, *Brechmorhoga*, *Gynothemis*, and *Phyllocycla*, were found in sites with higher percentages of catchment pasture, and associated with the thermoregulation trait. The adults are endothermic. In other words, they can produce and store heat in their bodies and control the circulation of the hemolymph to facilitate thermoregulation independent of the air temperature (Corbet and May 2008; May 1976). Therefore, they can survive where there is little or no riparian vegetation canopy (De M arco et al., 2015; Oliveira-Junior and Juen, 2019). Stream sites where the natural riparian vegetation was removed and replaced by pasture and agriculture tend to benefit Odonata larvae with burrowing behavior, such as *Gomphoides*, *Archaeogomphus*, *Macrothemis*, *Brechmorhoga*, *Gynothemis*,
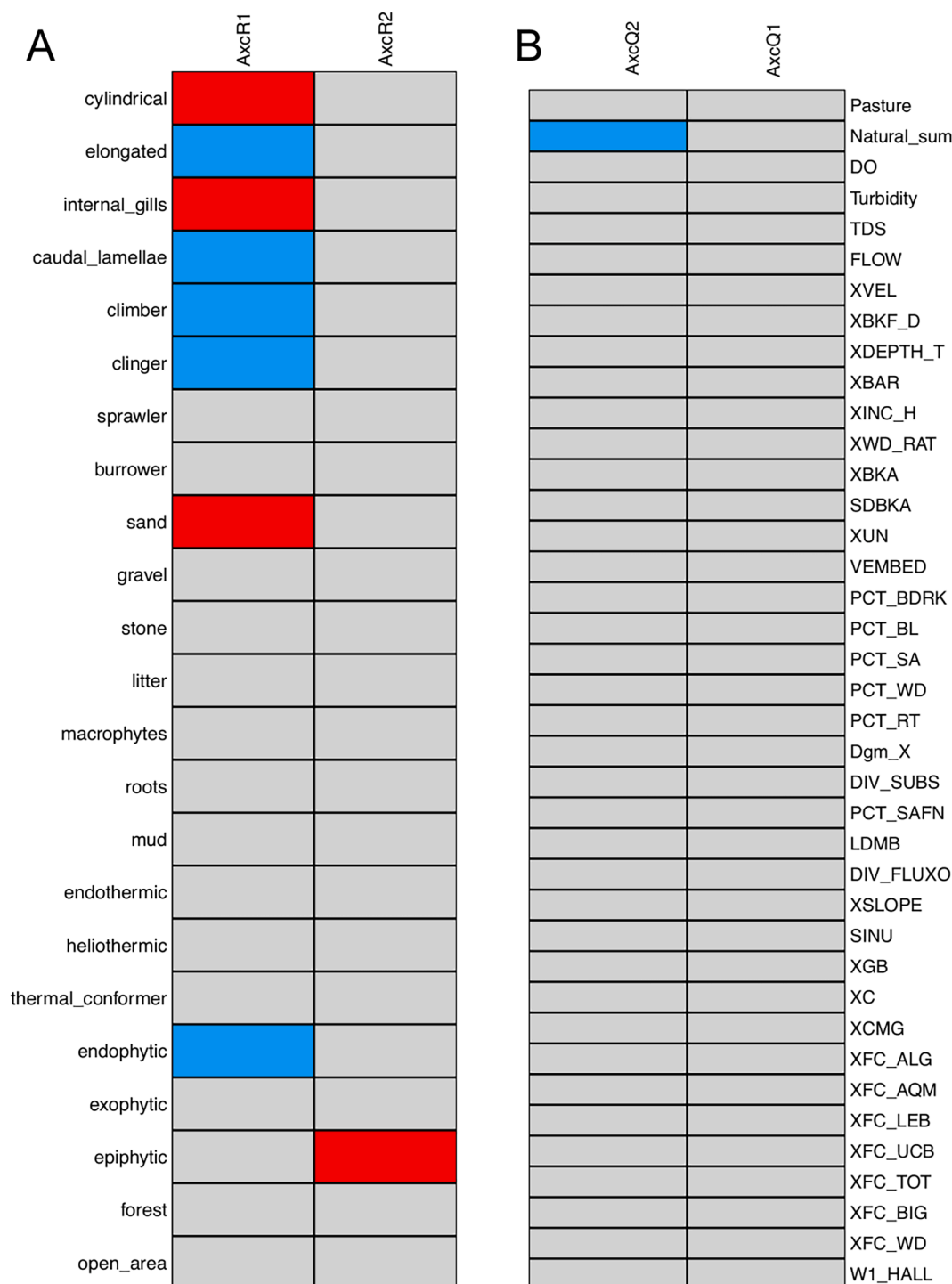
Fig. 6. Significant relationships (P-adjusted < 0.05) between (A) the RLQ environmental axes and individual traits and (B) between the RLQ trait axes and environmental variables. Red indicates positive correlations between factors and blue negative correlations. Non-significant relationships are labeled in grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and *Phyllocycla*. In streams lacking riparian vegetation, fine sediments on stream beds is higher (Wood and Armitage, 1997), which favors those larvae that live buried or semi-buried in fine sediments, sand or mud (Carvalho and Nessimian, 1998).

The multiple trait-based approaches helped us confirm that Odonata larvae have characteristics that respond to human modification of Cerrado headwater streams, making them good indicators of the effects of anthropogenic disturbances on streams. The environmental variables acted as filters on these traits, limiting the occurrence of sensitive genera

and facilitating the occurrence of tolerant ones (Pereira et al., 2019). However, simpler indicators, such as total functional richness, diversity, evenness, dispersion, specialization, and originality failed to do so. We believe these results from the same processes that make measures of taxonomic richness, diversity, evenness, and specialization relatively insensitive measures of disturbance. For example, sensitive taxa respond to low levels of disturbance before tolerant taxa, but at moderate levels of disturbance the sensitive taxa are replaced by tolerant taxa, thereby resulting in no change–or even increased–total taxa richness (Brito et al.,

2020; Davies and Jackson, 2006; Oliveira-Junior et al., 2017). Those same responses affect total taxonomic diversity, evenness, and specialization, which is why such metrics alone have tended to be less frequently employed in rigorous biomonitoring programs (Chen et al., 2019; Silva et al., 2017; Stoddard et al., 2008). Furthermore, total richness and biodiversity estimates at a site are very much functions of natural environmental conditions (Hawkins et al., 2000; Moya et al., 2011), sampling effort (Cao et al., 2002; Li et al., 2001), habitat types sampled (Ligeiro et al., 2020; Silva et al., 2016), and the number of individuals identified (Cao et al., 2002; Ligeiro et al., 2013a). Therefore, we recommend caution when using any functional or taxonomic biodiversity metric for making bioassessments.

5. Conclusions

Our results indicated that stream sites in intermediate conditions or highly disturbed by anthropogenic stressors selected Anisoptera larvae with sets of traits distinct from those of Zygoptera larvae occupying stream sites in least disturbed (reference) conditions. Zygoptera larval traits were more related to least- disturbed stream sites, so they are more sensitive to environmental stressors, whereas Anisoptera larval traits were more related to intermediate or highly disturbed streams, so they are more tolerant. Therefore, using Odonata larvae traits can be a useful tool for assessing and monitoring anthropogenic impacts in Cerrado streams. We assume that knowledge of Odonata suborder traits and their bioindicator uses will be important for scientists, citizen scientists, decision-makers, and policy directed toward improved management of tropical river basins.

CRediT authorship contribution statement

Larissa F.R. Silva: Conceptualization, Writing – original draft, Writing – review & editing. **Diego M.P. Castro:** Conceptualization, Formal analysis, Writing – review & editing. **Leandro Juen:** Conceptualization, Writing – review & editing. **Marcos Callisto:** Resources, Conceptualization, Writing – review & editing. **Robert M. Hughes:** Conceptualization, Writing – review & editing. **Marcel G. Hermes:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.108367.

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