UNIVERSIDADE FEDERAL DE LAVRAS

LARISSA DE FÁTIMA RIBEIRO SILVA

TAXONOMIC AND FUNCTIONAL STRUCTURE OF ODONATA LARVAE ASSEMBLAGES IN NEOTROPICAL SAVANNA HEADWATER STREAMS

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Prof. Dr. Marcel Gustavo Hermes
Advisor
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ESTRUTURA TAXONÔMICA E FUNCIONAL DE ASSEMBLEIAS DE LARVAS DE ODONATA EM RIACHOS DE CABECEIRA NO CERRADO

Thesis presented to the Universidade Federal de Lavras as a partial requirement of the Graduate Program in Entomology (Entomology concentration) to obtain the degree of Doctor.

Approved in March 31st 2022.

Dr. Marcos Callisto UFMG
Dr Paulo Pompeu UFMG
Dra.Thais Giovannini Pellegrini UFLA
Dr. Leandro Schlemmer Brasil UFPA
Dra. Silvia Vendruscolo Milesi URI

Prof. Dr. Marcel Gustavo Hermes
Advisor
Prof. Dr. Marcos Callisto
Co-Advisor

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To my parents Elci and Ézio,

my limitless source of encouragement, strength and love.

I dedicate

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This acknowledgment is also a short story about how the project that culminated in this thesis happened. When I talked to Prof. Marcel about my doctorate project, I mentioned my wish to work with Odonata larvae ecology. Even though Marcel is a specialist on wasp phylogeny, he gave me the freedom and autonomy to work with dragonflies, and for this, I am so grateful.

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“*Hapinnes is only real if shared*”.

“Dragonflies are insects that people notice”.

(Michael L. May)

ABSTRACT

Freshwater ecosystems have been subjected to an increased and continuous process of degradation, mainly due to anthropogenic pressures. Advances in research for assessing and monitoring these ecosystems have not been performed in sufficient time to prevent or reduce the loss of aquatic biodiversity. In this context, it is necessary and urgent to understand and measure how biological assemblages respond to these human pressures. Like other aquatic insects, larval Odonata assemblage structure and functions are closely associated with the environmental conditions of freshwater ecosystems. Therefore, changes in these conditions affect the structure and function of Odonata assemblages, which makes them excellent indicators of environmental condition. However, studies addressing the responses of Odonata larvae to anthropogenic stressors are still scarce, while knowledge about Odonata adults is relatively more advanced and well-established. Given the above, the general aim of this thesis was to evaluate the taxonomic, structural and functional responses of Odonata larvae to a gradient of environmental conditions in Brazilian Savanna headwater streams. This thesis is divided into three chapters. Chapter 1 evaluates the distribution patterns of the two larval Odonata suborders along a gradient of riparian vegetation disturbance. Chapter 2 describes how anthropogenic stressors affect larval Odonata taxonomic and functional structure. Chapter 3 identifies the ecological thresholds of larvae Odonata genera along a gradient of environmental disturbance. We assessed the responses of Odonata larvae to gradients of disturbance considering traditional ecological structure approaches, such as richness and abundance, as well as complementary functional approaches, such as traits and ecological thresholds. We found that, like adults, Zygoptera larvae are more sensitive to changes in environmental conditions, being more dependent on the presence of riparian vegetation, good water quality, and heterogeneous in-stream substrate. Zygoptera genera have specific traits related to these conditions and narrower ecological thresholds and, therefore are more often found in reference condition streams. On the other hand, Anisoptera larvae are more tolerant to anthropogenic pressures, including removal of riparian vegetation, increased erosion and siltation, and higher levels of total dissolved solids and electrical conductivity. Anisoptera genera have specific traits and wider ecological thresholds, and were found in intermediate or degraded streams. I conclude that anthropogenic disturbances, mostly related to the removal or reduction of riparian vegetation, affect larval Odonata assemblages in the Neotropical Savanna. I expect that the products generated by this thesis will support the use of Odonata larvae as assessment and monitoring tools for headwater stream ecosystems.

Key-words: Anisoptera. Cerrado streams. Ecological responses. Zygoptera.

RESUMO

Os ecossistemas de água doce têm sido submetidos a um acentuado e contínuo processo de degradação, principalmente devido a pressões antrópicas. Os avanços nas pesquisas para avaliação e monitoramento desses ecossistemas não têm sido realizados em tempo hábil para prevenir ou reduzir a perda de biodiversidade aquática. Nesse contexto, é necessário e urgente compreender e mensurar como as assembleias biológicas respondem a essas pressões antrópicas. Assim como outros insetos aquáticos, a estrutura de assembleias de larvas de Odonata está intimamente associada às condições ambientais de ecossistemas aquáticos. Mudanças nessas condições afetam a estrutura e composição de assembleias de Odonata, o que as torna excelentes indicadores de integridade ambiental. No entanto, estudos abordando as respostas de larvas de Odonata a estressores antropogênicos ainda são escassos, enquanto o conhecimento sobre adultos de Odonata é relativamente mais avançado e bem estabelecido. Diante do exposto, o objetivo geral desta tese foi avaliar as respostas taxonômicas, estruturais e funcionais de larvas de Odonata frente a um gradiente de condições ambientais em riachos de cabeceira no Cerrado. Esta tese foi dividida em três capítulos, com os objetivos de: 1- Avaliar os padrões de distribuição de larvas das subordens de Odonata ao longo de um gradiente de integridade de vegetação ripária; 2- Compreender como os estressores antropogênicos afetam a estrutura taxonômica e funcional das assembleias de larvas de Odonata; 3- Identificar os limiares ecológicos dos gêneros de larvas de Odonata em um gradiente de integridade ambiental. Avaliamos as respostas de larvas de Odonata frente a gradientes de perturbação considerando abordagens ecológicas tradicionais de estrutura, como riqueza e abundância, bem como abordagens funcionais complementares, como *traits* e limiares ecológicos. Descobrimos que, assim como os adultos, as larvas de Zygoptera foram mais sensíveis às mudanças nas condições ambientais, sendo mais dependentes da presença de vegetação ripária, boa qualidade de água e substrato heterogêneo. Os gêneros de Zygoptera possuem *traits* específicos relacionados a essas condições e limiares ecológicos mais estreitos e, portanto, são encontrados em riachos preservados e em condições de referência. Por outro lado, larvas de Anisoptera são mais tolerantes a pressões antrópicas, incluindo remoção da vegetação ripária, aumento de erosão e assoreamento, e maiores teores de sólidos totais dissolvidos e condutividade elétrica. Os gêneros de Anisoptera possuem *traits* específicos e limiares ecológicos mais amplos, e foram encontrados em riachos intermediários ou degradados. Concluí que distúrbios antropogênicos, principalmente relacionados à remoção ou redução da vegetação ripária, afetam as assembleias de larvas de Odonata no Cerrado. Como perspectivas futuras, espero que os produtos gerados nesta tese apoiem o uso de larvas de Odonata como ferramentas de avaliação e monitoramento de riachos de cabeceira.

Palavras-chave: Anisoptera. Respostas ecológicas. Riachos do Cerrado. Zygoptera.

SUMMARY

FIRST PART

1. GENERAL INTRODUCTION

Dragonflies and damselflies are insects of the order Odonata and have been interested researchers since Linnaeus, in 1793 (MAY, 2019). Since then, “Odonatology”, the study dedicated to Odonata, has made significant progress in many areas such as taxonomy, morphological and molecular systematics, and behavioral and evolutionary ecology (BRIED; SAMWAYS, 2015). In the Neotropical region, over the last ten years, the number of ecological studies using Odonata adults for environmental assessments have been increasing significantly, especially in Brazil (GÓMEZ-TOLOSA et al., 2021).

Odonata have characteristics that make them efficient in environmental assessment and monitoring studies of freshwater ecosystems, such as an aquatic larval stage and a terrestrial adult stage, thus representing a link between aquatic and terrestrial ecosystems (BRIED; SAMWAYS, 2015; KALKMAN et al., 2008; MAY, 2019; OERTLI, 2008). Besides, Odonata assemblages respond to changes in the physical and chemical quality of water and in physical habitat structure at different taxonomic levels, such as suborder (OLIVEIRA-JUNIOR; JUEN, 2019; RIBEIRO; JUEN; RODRIGUES, 2021), family (MENDOZA-PENAGOS; CALVÃO; JUEN, 2021), or genera and species levels (DALZOCHIO et al., 2018; DE MARCO; BATISTA; CABETTE, 2015; MENDES; BENONE; JUEN, 2019). Finally, Odonata may be considered as a flag taxon due to their being charismatic insects, arousing the interest of the general population and decision-makers (BRIED; SAMWAYS, 2015; KALKMAN et al., 2008; MAY, 2019; OERTLI, 2008).

Most assessment and monitoring studies with Odonata have been focused on adults (BRIED; SAMWAYS, 2015; GÓMEZ-TOLOSA et al., 2021; MIGUEL et al., 2017), mainly because their taxonomy is relatively well known and the cost of fieldwork and time employed to identify the species is short (GÓMEZ-TOLOSA et al., 2021). However, several studies concluded that both larval and adult stages are affected by anthropogenic disturbances (LUKE et al., 2017; MENDES et al., 2017; VALENTE-NETO et al., 2016). In fact, larval stages may result in a more detailed evaluation (GÓMEZ-TOLOSA et al., 2021), because as larvae they can live from months to years in aquatic environments (CORBET, 1980) and have a limited dispersion, which makes them more vulnerable to local changes (VALENTE-NETO et al., 2016). Nevertheless, studies using the larval stage are still scarce or based on a limited set of data, which makes it difficult to make predictions and generalizations.

The general aim of this thesis was to evaluate the taxonomic and functional structure of Odonata larvae assemblages in a gradient of environmental conditions in headwater streams of the Brazilian Cerrado. This thesis was divided into three chapters. Chapter 1 evaluates the distribution patterns of the two larval Odonata suborders along a gradient of riparian vegetation disturbance. Chapter 2 describes how anthropogenic stressors affect larval Odonata taxonomic and functional structure. Chapter 3 identifies the ecological thresholds of larval Odonata genera along a gradient of environmental disturbance. Chapter 1 was published in Hydrobiologia (2021) and chapter 2 was published in Ecological Indicators (2021). Chapter 3 was submitted to Ecological Indicators (April 2022) and is under review.

I tested three main hypotheses. 1) Odonata larvae display the same patterns as Odonata adults to anthropogenic disturbances (Chapter 1). 2) 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 larval Odonata genera traits (Chapter 2). 3) Zygoptera genera would have narrower ecological thresholds and earlier change points in response to reduced riparian vegetation, increased water temperature, decreased dissolved oxygen, and decreased environmental heterogeneity, whereas Anisoptera genera would have wider ecological thresholds and later change points (Chapter 3).

2. THEORETICAL BACKGROUND

2.1 Streams and freshwater ecosystems

Streams are complex and dynamic ecosystems. This complexity results from several factors, such as climate, physical and chemical habitat (e.g. luminosity, temperature, current velocity, dissolved oxygen, nutrients, sediment particle sizes, among others), and biology (e.g. organisms and their ecological interactions) (ALLAN; CASTILLO, 2007). As open ecosystems, nutrients and energy are constantly being imported and exported into these environments. Consequently, what enters in a headwater or any other site of a stream will affect downstream sites (VANNOTE et al., 1980). This means that streams are ecosystems highly influenced by their surrounding landscapes, for example, by the presence or absence of riparian vegetation, as well as by the natural and anthropogenic conditions in their catchments (HYNES, 1975; OMERNICK, 1987) and even by downstream stressors (CALLISTO et al., 2019a; OLIVEIRA-JUNIOR et al., 2017).

Natural riparian vegetation provides a series of services, such as filtering polluted runoff water; regulating microclimatic conditions; reducing siltation, erosion and soil loss; maintaining biomass and carbon storage; protecting biological and genetic diversity and pollinators; and controlling pests; in addition to acting as biodiversity corridors. Thus, the preservation of riparian vegetation affects directly freshwater ecosystems, contributing to the maintenance of aquatic biodiversity, as well as to the quality and quantity of water (CALLISTO et al., 2019a; GREGORY et al., 1991; RIIS et al., 2020). Therefore, anthropogenic activities in landscapes affect stream water, sediments, and riparian vegetation, thereby initiating a complex cascade of changes, resulting in more disturbed ecosystems (ALLAN, 2004).

Despite the importance of freshwater ecosystems for human livelihoods and well-being, these ecosystems have been degraded continuously and unprecedentedly (FAGHIHINIA et al., 2021; MAASRI et al., 2021). Overexploitation of freshwaters, inappropriate land use for agriculture and pasture, uncontrolled deforestation, indiscriminate use of fertilizers and biocides, domestic and industrial pollution, fragmentation, degradation and destruction of habitats are some of the most persistent threats to freshwater ecosystems worldwide (DUDGEON, 2010; HIGGINS et al., 2021; REID et al., 2019). Recently, new threats have emerged, such as climate heating, non-native species invasions, hydropower, and microplastic pollution, among others (FAGHIHINIA et al., 2021; REID et al., 2019). In streams, specifically, it is worrying because these human effects often occur in combination, culminating in cumulative stressors, which increase the potential impacts on aquatic environments and require more complex management solutions and conservation efforts (BIRK et al., 2020; REID et al., 2019).

Given this, studies that evaluate stream conditions and loss of aquatic biodiversity have become increasingly necessary in recent years worldwide, especially in the neotropics (ALLAN, 2004). However, gaps and shortfalls in knowledge about species identity (Linnean shortfall) and distribution (Wallacean shortfall) compromise descriptions of existing biodiversity in a timely manner to preserve it or to make accurate predictions about how biodiversity may change in the future. In addition, these gaps lead to incorrect identification of ecological and evolutionary processes and inefficient use of limited conservation resources (HORTAL et al., 2015). Therefore, it is essential to complement traditional approaches based on taxonomy and structure with ecological approaches based on functional traits or ecological thresholds to improve our understanding of the anthropogenic impacts on aquatic environments and how the biological communities respond to them.

2.2 Traits and ecological thresholds

Traditional ecological structure approaches, such as species richness and diversity, have long been used for assessing how biological assemblages change with anthropogenic disturbances (SAITO et al., 2020; SAITO; SIQUEIRA; FONSECA-GESSNER, 2015). Although it seems intuitive to think that an assemblage with more taxa has high diversity, simple taxonomic diversity or richness doesn't consider that taxa have different evolutionary histories and ecological characteristics. Consequently, taxa responses to environmental impacts, in different freshwater systems, are unlikely to be the same (MAGURRAN, 2004).

In fact, even within phylogenetically related taxonomic groups, such as species belonging to the same order, different species do not have the same response to anthropogenic disturbances (e.g., FIRMIANO et al., 2021; GIEHL et al., 2019; MARTINS et al., 2021; RODRIGUES et al., 2016). On the other hand, the use of functional trait-based approaches considers ecological or evolutionary differences among species (SAITO et al., 2020; SAITO; SIQUEIRA; FONSECA-GESSNER, 2015). This approach can be used not as a substitute, but as a complement for traditional ecological approaches (LARSON et al., 2021; POFF, 1997).

Traits can be defined as any feature that affects the fitness of organisms indirectly via their effects on growth, reproduction, and survival (VIOLLE et al., 2007) and have been divided into four types: life-history, morphology, physiology, and behavioral/mobility traits. When traits are used to refer to ecological or environmental preferences of organisms, they should be included in physiological or behavioral traits (CARVALHO et al., 2017; MARTINI et al., 2021).

The use of traits in an index or assessments is a useful tool for understanding why some taxa persist in environments subject to anthropogenic disturbances, when others do not (POFF, 1997; POFF et al., 2006; WEBB et al., 2010). Environments with similar abiotic factors select species with a set of similar traits and, thus determine the structure of the local community or assemblage, as proposed by the concept of “The habitat templet” (TOWNSEND; HILDREW, 1994). These abiotic factors act as environmental filters, in which only species with a certain set of traits can persist, and associated with different spatial and temporal scales, these filters influence the abundance and distribution of species (POFF, 1997).

The main advantage of the use of functional trait-based approaches is providing predictability and generality by identifying general biodiversity patterns along environmental gradients, such as anthropogenic pressures in freshwaters and across wider spatial extents such as hydrological units and river basins (MCGILL et al., 2006; POFF, 1997). These approaches have been successfully used to assess the effects of anthropogenic disturbances on aquatic macroinvertebrate assemblages in Brazil (e.g., CASTRO; DOLÉDEC; CALLISTO, 2018; FIRMIANO et al., 2021; MARTINS et al., 2021; NICACIO et al., 2020; SAITO; SIQUEIRA; FONSECA-GESSNER, 2015; SGANZERLA et al., 2021).

In fact, biological traits are often closely related to a narrow range of environmental conditions in freshwater ecosystems. In a case of new environmental conditions outside those experienced by local species in evolutionary time, such as environmental gradients caused by anthropogenic disturbances, the entire biological community may be altered (BAKER; KING, 2010). These responses are related to the concept of ecological thresholds.

Ecological thresholds can be defined as points or zones in which changes in one environmental condition to another can be observed (HUGGETT, 2005). The ecological community threshold is related to the frequency and/or abundance of species, which can increase or decrease according to changes in a given environmental variable along an environmental gradient, being that the change in the environmental variable results in a disproportionate change in community structure (KING; BAKER, 2014).

Knowledge of ecological thresholds can be useful to detect critical levels of environmental alteration and biodiversity loss before they become irreversible, to identify traits of taxa that increase their fitness, to assess changes in ecosystem function, and to establish compensatory or mitigating measures (BAKER; KING, 2010; HUGGETT, 2005; KING; BAKER, 2014). Despite these advantages, the use of ecological thresholds of species or assemblages of most aquatic insect groups are either poorly understood or have yet to be identified (GIEHL et al., 2019). Several studies in Brazil using ecological thresholds have focused on land use and cover types (FIRMIANO et al., 2017), removal of vegetation in riparian zones (BRITO et al., 2020; FIRMIANO et al., 2017, 2021; GIEHL et al., 2019; RODRIGUES et al., 2016), and water quality (FIRMIANO et al., 2017; GIEHL et al., 2019).

2.3 Odonata

Odonata is the order of insects whose representatives are popularly known as dragonflies (Anisoptera) and damselflies (Zygoptera). The adults draw attention due to their elegant and fast flight, colorful wings, and conspicuous reproductive behavior, making dragonflies and damselflies a charismatic group (MAY, 2019). Odonata exhibit a complex life cycle, with an abrupt ontogenetic change in morphology and ecology and life stages occurring in different ecosystems, with aquatic larvae and terrestrial adults (STOKS; CRDOBA-AGUILAR, 2012). In both stages, they are predators (CORBET, 1980), which confer to Odonata important roles in trophic cascades and transport of materials and energy at the interface of aquatic and terrestrial ecosystems (MAY, 2019), making them potential biological control agents of mosquito larvae – including potential disease vectors – in aquatic ecosystems and in the adjacent terrestrial environment (CHOO et al., 2021; SAHA et al., 2012).

Among other insects, Odonata is a small order, with 6.345 existing species (PAULSON; SCHORR; DELIRY, 2022), although it is recognized that there are still more species to be described (DIJKSTRA et al., 2013). The order is divided into three distinct suborders: Anisozygoptera restricted to mountain regions of Bhutan, Nepal, China, North Korea, and Japan, and Zygoptera and Anisoptera with worldwide distribution (BYBEE et al., 2021; DIJKSTRA et al., 2013). The suborders Zygoptera and Anisoptera, both in the larval and adult stages, differ in many important aspects of their morphology, physiology, behavior, and ecological requirements, being even antagonistic.

Zygoptera species usually have a slender and smaller body size and ecothermic thermoregulation (thermal conformers or heliothermic), which means that their body temperature is determined by the environment temperature (CORBET; MAY, 2008; DE MARCO; RESENDE, 2002; MAY, 1976). Their reduced body sizes directly influence their ability to disperse, allowing them to fly only short distances (CORBET; MAY, 2008). Due to these restrictions imposed by their body size and thermoregulation (DE MARCO; RESENDE, 2002), most Zygoptera species require specific environmental conditions, such as the presence of riparian vegetation, low solar incidence, and adequate temperature ranges.

On the other hand, Anisoptera species usually have a larger body size, with higher metabolic rates and endothermic thermoregulation, which means that they can generate and store heat in their body and control the hemolymph circulation to facilitate thermoregulation. Due to their larger body sizes, they have a high dispersion capacity, being able to travel great distances flying (CORBET; MAY, 2008). Anisoptera species can use low-quality resources due to their lesser environmental specificity, being considered opportunistic and tolerant (FRENCH; MCCAULEY, 2018; MENDES; CABETTE; JUEN, 2015; OLIVEIRA-JUNIOR; JUEN, 2019; PIRES; SAHLÉN; PÉRICO, 2021).

These ecophysiological differences between Zygoptera and Anisoptera determine the pattern of distribution and structure of adult Odonata assemblages in neotropical streams (DE MARCO; RESENDE, 2002; OLIVEIRA-JUNIOR; JUEN, 2019), which is relatively well established and used for the assessment or monitoring of aquatic or terrestrial ecosystems. Mostly, Zygoptera species are usually associated with habitats with higher environmental integrity, and presence of natural riparian vegetation, while Anisoptera species are usually associated with altered or disturbed habitats (OLIVEIRA-JUNIOR; JUEN, 2019).

Regarding the differences between larvae, Zygoptera have more delicate and elongated bodies and breathe through delicate external caudal lamellae at the end of their abdomens, (NEISS; HAMADA, 2014). They can be sprawlers, climbers, burrowers, clingers, or swimmers, which are associated with their preference for substrate, usually gravel, stones and/or vegetation, such as leaves, macrophytes and roots (ASSIS; CARVALHO; NESSIMIAN, 2004; CARVALHO; NESSIMIAN, 1998). Anisoptera larvae have more robust and cylindrical body and breathe through rectal tracheal gills in the rectum (NEISS; HAMADA, 2014), and often bury themselves in sediments, such as organic debris, sand or mud (ASSIS; CARVALHO; NESSIMIAN, 2004; CARVALHO; NESSIMIAN, 1998).

However, contrary to adults, there is a huge knowledge gap regarding the distribution and structure of larval Odonata assemblages (BRIED; SAMWAYS, 2015; GÓMEZ-TOLOSA et al., 2021; MIGUEL et al., 2017). Odonata larvae assemblages may show a significant decline in their abundance and richness, in addition to changes in composition, in streams with anthropogenic disturbances, compared to adults (LUKE et al., 2017; MAY, 2019). Besides, the use of Odonata larvae has advantages, such as limited dispersion capacity, which makes them more vulnerable to local changes in environmental conditions (JUEN; FEEST, 2019; VALENTE-NETO et al., 2016), and easier field sampling (OERTLI, 2008), making Odonata larvae promising for demonstrating associations among environmental conditions of freshwater ecosystems.

3 GENERAL MATERIAL AND METHODS

3.1 The Neotropical Savanna

The Neotropical Savanna, or Cerrado, refers to seasonal savanna vegetation, ranging from grassy ("campo limpo") and slightly brushy ("campo sujo") to scattered tree vegetation ("Cerrado *sensu stricto"*) and semideciduous forest ("Cerradão") in Brazil and central South America (BATALHA, 2011; WANTZEN, 2003), which are mostly associated with the relative intensities of alternating rainy and dry seasons (BATALHA, 2011). Cerrado streams are characterized by low ion concentrations and weakly buffered pH, which are consequences of the ion poverty of the soils and of the seasons, with intense flows during the rainy season, and low flows during the dry season (WANTZEN et al., 2006).

The Cerrado is considered a global biodiversity hotspot (MYERS et al., 2010), and has extreme ecological importance due to its high richness of endemic fauna and flora (COLMAN et al., 2022). Furthermore, the Cerrado contributes to one of the largest freshwater reserves in South America, the Aquífero Guarani (WANTZEN et al., 2006), as well as to eight of the sixteen major river basins in Brazil, which are essential water resources in Brazil (COLMAN et al., 2022; STRASSBURG et al., 2017).

Despite its importance, the Cerrado 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). Recent projections show that this remaining natural vegetation cover tends to continue to decrease significantly, mainly in regions with large farms (COLMAN et al., 2022). In this context and in view of the importance of the Cerrado for maintaining biodiversity and water resources versus the rate and extent of its degradation (WANTZEN et al., 2006), ecological assessment studies are urgently needed (SILVA et al., 2017).

3.2 Study Area

This thesis research was conducted in headwater streams belonging to the hydrological units of the hydroelectric power plant reservoirs of Nova Ponte (Araguari River basin), Volta

Grande (Grande River basin), São Simão (Paranaíba River basin), and Três Marias (São Francisco River basin) located in the Brazilian Cerrado biome, in Minas Gerais state (Fig.1). The hydrological units (SEABER; KAPINOS; KNAPP, 1987) were defined as the drainage areas within 35 km upstream of each of these four hydroeletric reservoirs (CALLISTO et al., 2014a). In addition, other headwater streams located in Serra da Canastra National Park (Paraná River basin) and Serra do Salitre (Paraná River basin) were sampled to ensure that sites minimally affected by human influences were sampled (MARTINS et al., 2018). One sample was taken in the low flow at each site in each of the hydrological units during the years 2010 to 2014 (September 2010 in Três Marias, September 2011 in Volta Grande, September 2012 in São Simão, September 2013 in Nova Ponte, and April and May 2014, in Serra da Canastra National Park and Serra do Salitre).

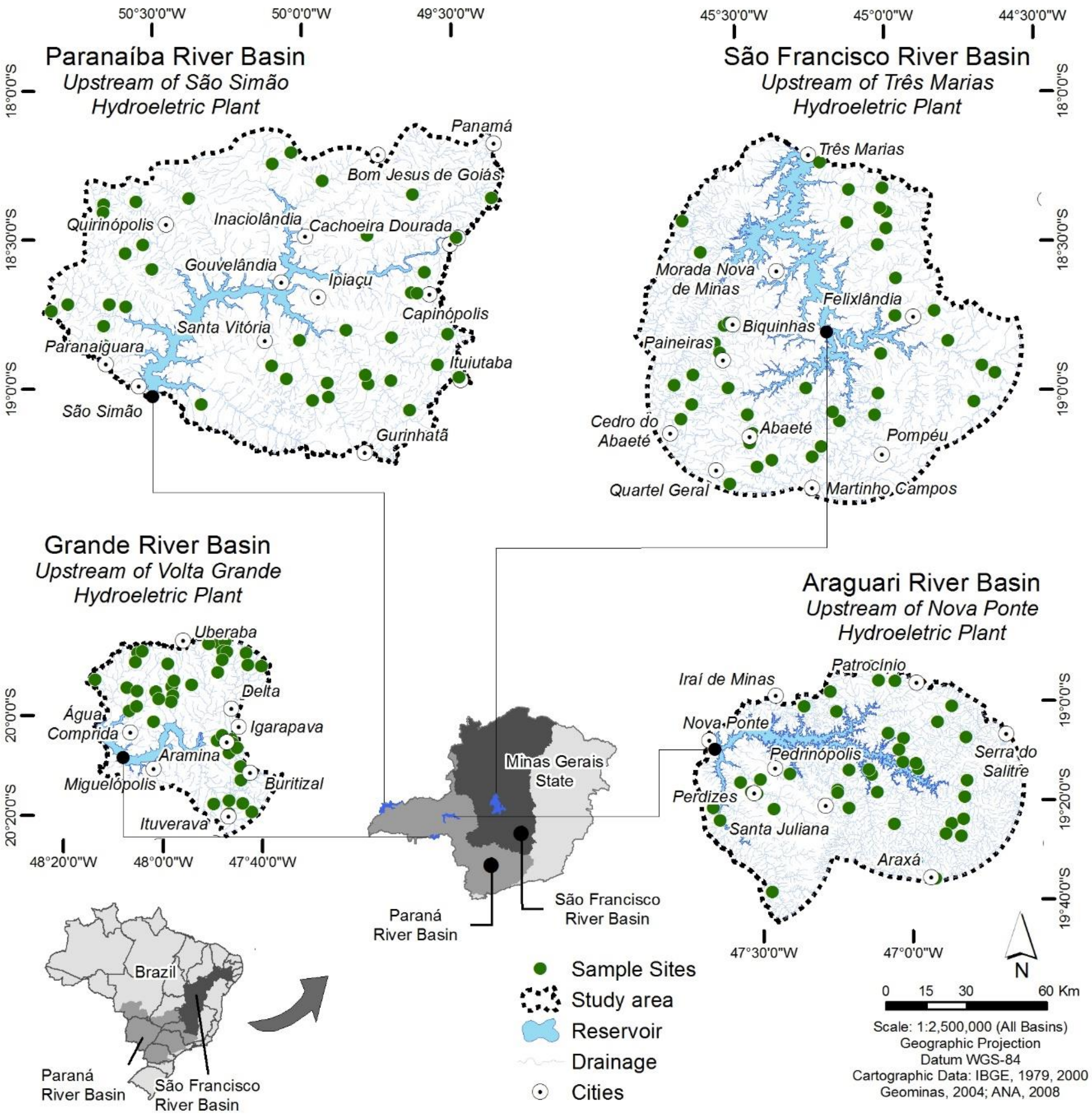


Figure 1: Locations of the stream sites and hydrological units in São Simão, Três Marias, Volta Grande and Nova Ponte.
Source: Laboratório de Ecologia de Bentos, UFMG.

3.3 Sampling Design

In each hydrological unit, we sampled 40 stream sites, except in Serra da Canastra National Park and Serra do Salitre, where we sampled additionally 26 stream sites. These stream sites were selected following a random and probabilistic sampling design (MACEDO et al., 2014a), following the methodology used by the US Environmental Protection Agency (US-EPA) (OLSEN; PECK, 2008) and translated, adapted, and validated for the Brazilian Cerrado (CALLISTO et al., 2014b). This methodology allows the impartial selection of stream sites that were representative of the region and has been successfully used in previous Neotropical Savanna studies (e.g. AGRA et al., 2021; CALLISTO et al., 2019b; CARVALHO et al., 2017; CASTRO; DOLÉDEC; CALLISTO, 2018; FIRMIANO et al., 2017).

The sampling design consists of establishing a potential area of sampling within a buffer of 35 km from the boundaries of each reservoir, using a drainage area map (1:100,000 scale) (MACEDO et al., 2014a). Then, headwater streams 1st to 3rd order were randomly chosen (STRAHLER, 1957), which are streams capable of being walked across by an adult with median height (KAUFMANN et al., 1999). The sampled sites exhibited a wide disturbance gradient, from minimally disturbed sites with high dissolved oxygen and low nutrient concentrations, sites with intermediate levels of human-altered land (pasture, row crops) to highly disturbed urban sites with poor water quality and physical habitat conditions (MACEDO et al., 2014b; SILVA et al., 2017) (Fig. 2).

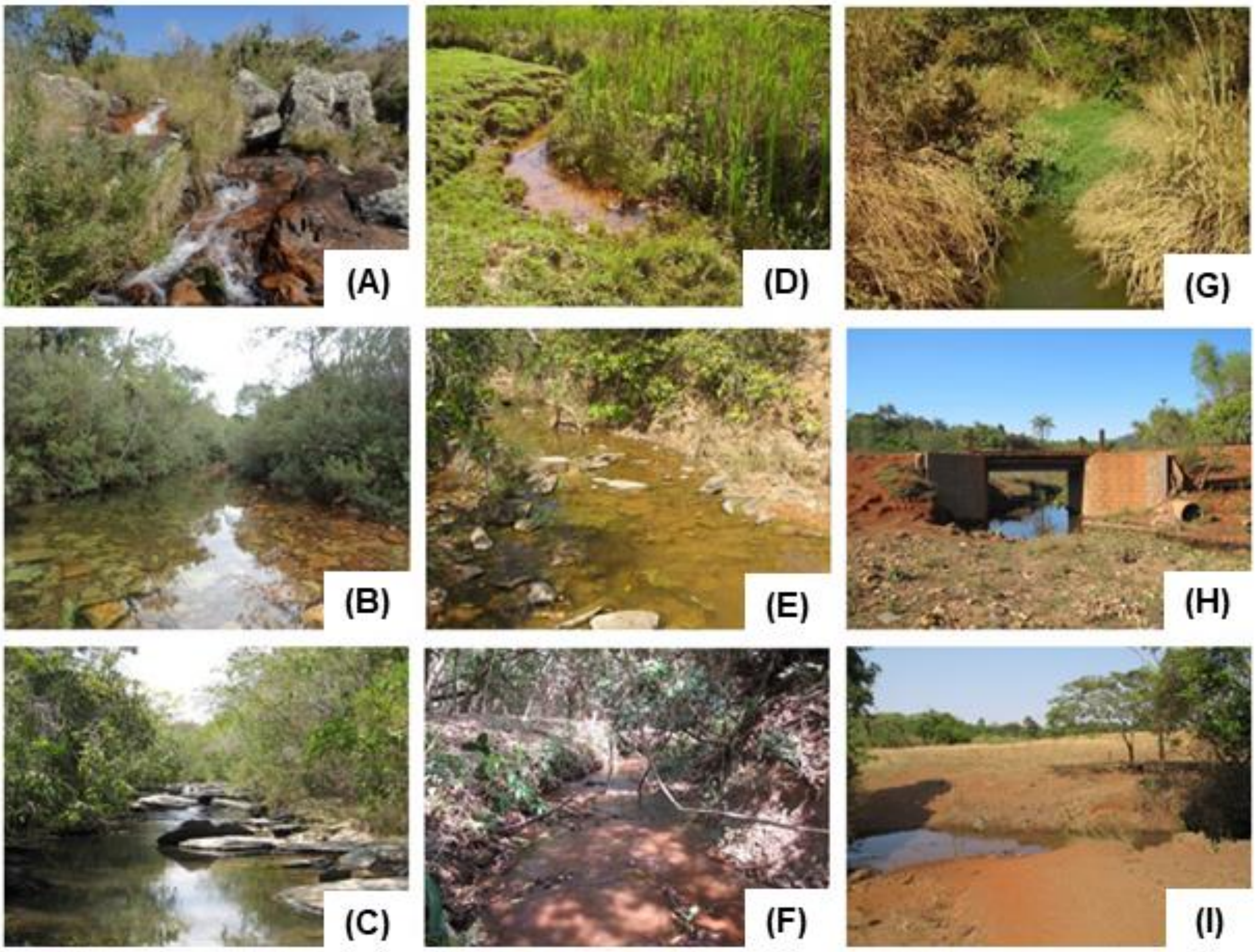


Figure 2: Examples of sampling sites in reference (A-C), intermediate (D-F) and highly disturbed conditions (G-I).
Source: Laboratório de Ecologia de Bentos, UFMG.

3.4 Sampling and Identification of the Odonata larvae

The length of the stream site was determined by the mean of the wetted width multiplied by 40 (PECK et al., 2006), respecting a minimum of 150 m. Each site was divided equally into 11 equidistant transects, designated A to K. The first subsample was randomly selected in transect A and the other followed a systematic zigzag trajectory (left, center, and right) (Fig. 3). The sampling was performed by disturbing bottom sediments and catching benthic macroinvertebrates in a kick-net downstream (Fig. 4 A). The kick-net had a 30cm opening and 250µm mesh, and sampled an area of 0,09 m^2. Then, each sample was placed in a plastic bag and fixed with 50 mL of buffered formol.

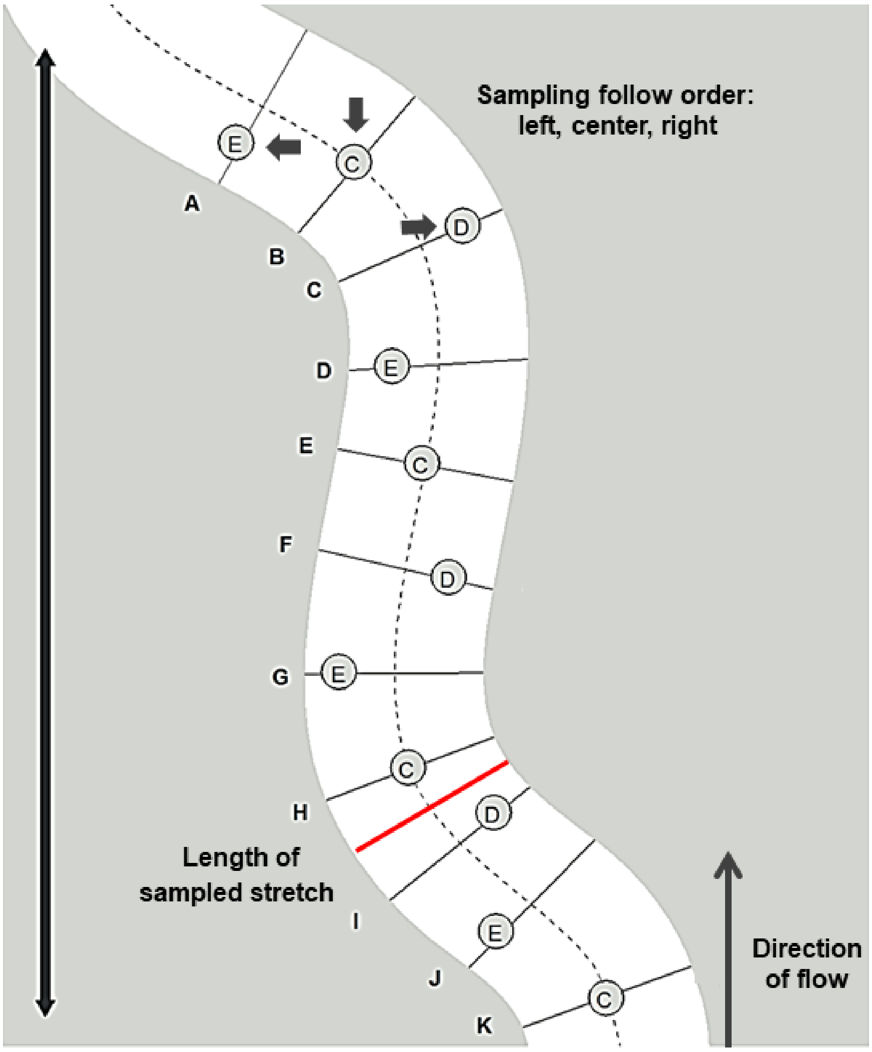


Figure 3: Schematic representation of definition of sample stretch and the sampling of Odonata larvae.
Adapted from Peck et al. (2006).

The samples were taken to the “Laboratório de Ecologia de Bentos” at Universidade Federal de Minas Gerais, where they were washed on a 250 µm sieve mesh and sorted on trays over a lightbox (CALLISTO et al., 2021) (Fig. 4 B and C). The Odonata larvae were separated, taken to “Laboratório de Sistemática e Biologia de Insetos” at Universidade Federal de Lavras and identified under a Leica S8 APO stereomicroscope to genus using taxonomic keys (COSTA; SOUZA; OLDRINI, 2004; NEISS; HAMADA, 2014; PESSACQ; MÚZON; NEISS, 2018) as well as descriptions and reviews available for each taxon (Fig. 4 D). After identifying, the larvae were deposited in the reference collection of the Instituto de Ciências Biológicas at Universidade Federal de Minas Gerais (CALLISTO et al., 2021).

Figure 4: Sample processing and larvae identification. A) Sampling with kick-net collector downstream; B) Sediment washing over sieves at laboratory; C) Sorting on trays over a lightbox; D) Identifying larvae.
Sources: A, B, C - Callisto et al. (2021); D – author.

3.5 Environmental Metrics

Environmental metrics at each stream site were described in terms of channel dimensions and morphology, substrate size and composition, riparian vegetation cover and structure, and anthropogenic disturbance, following the protocol for assessment of physical habitats in streams (CALLISTO et al., 2014b; OLSEN; PECK, 2008) (Fig. 5). In addition, water quality was assessed by measuring temperature (°C), electrical conductivity (µS cm^{-1}), pH, turbidity (NTU), and total dissolved solids (mg L^{-1}) with a multi-probe (YSI, 650 MDS, model 6920). The concentrations of total nitrogen (mg L^{-1}) and dissolved oxygen (mg. L^{-1}) were determined in the laboratory following the APHA (2005). This procedure allowed us to calculate 250 environmental metrics (KAUFMANN et al., 1999) which were chosen according to specific objectives of each chapter**.**

Revisado por (iniciais): ________

Habitat Físico: SEÇÃO TRANSVERSAL DO CANAL/ZONA RIPÁRIA - RIACHOS

IDENTIFICAÇÃO DO PONTO (ID): ____________________ DATA: ___/___/______ TRANSECTO: ☐A ☐B ☐C ☐D ☐E ☐F ☐G ☐H ☐I ☐J ☐K Canal Lateral ☐

INFORMAÇÕES DO SUBSTRATO DA SEÇÃO TRANSVERSAL

	Dist. M. Esq. XX.XX m	Prof. XXX cm	Código Tam. clas.	Imersão 0-100%	Tipo B / F	Obs.
ESQ						
C.ESQ						
CENT						
C.DIR						
DIR						

CÓDIGOS DE CLASSES - TAMANHO DO SUBSTRATO

		Imersão (%)
(F) FÍSICO	RL = Rocha (Lisa) - (Mais larga que um carro)	0
	RR = Rocha (Rugosa) - (Mais larga que um carro)	0
	CO = Concreto/Asfalto	
	ML = Matacão Largo (1000 até 4000 mm) - (Caixa d'água até um carro)	
	MT = Matacão (250 até 1000 mm) - (Bola de basquete até caixa d'água)	
	BL = Bloco (64 até 250 mm) - (Bola de tênis até bola de basquete)	
	CG = Cascalho Grosso (16 até 64 mm) - (Jabuticaba até bola de tênis)	
	CF = Cascalho Fino (2 até 16 mm) - (Joaninha até jabuticada)	
	AR = Areia (0.06 até 2 mm) - (Arenosa - até o tamanho de Joaninha)	100
	FN = Finos (Silte / Argila / Lama - Não arenosa)	100
	AC = Argila consolidada (Hardpan) - Substrato Fino consolidado, firme	0
(B) BIOL	BF = Banco de Folhas (e Galhos Pequenos)	
	SF = Serrapilheira Fina (Materia organica particulada)	
	MA = Macrófitas	
	AL = Algas	
	RT = Raízes Finas da Mata Ciliar	
	MD = Madeira - (qualquer tamanho)	
	OT = Outro (escreva comentário abaixo)	

MEDIDAS DA MARGEM

	Ângulo da margem 0 - 360	Margem escavada Dist. (m)	Obs.
Esquerda			
Direita			
Largura molhada XXX.X m			
Largura das barras de canal XX.X m			
Largura do leito sazonal XXX.X m			
Altura do leito sazonal XX.X m			
Altura da incisão XX.X m			

Cód. Tam. Class F (Marcar apenas se o transecto for 100% biológico

ABRIGO PARA PEIXES/ OUTROS

0 = Ausente (0%)
1 = Esparso (<10%)
2 = Médio (10-40%)
3 = Denso (40-75%)
4 = Muito denso (>75%)
(circule uma opção)

	Cobertura no canal	Obs.
Algas Filamentosas	0 1 2 3 4	
Plantas Aquáticas	0 1 2 3 4	
Pedaços de Madeira >0.3 m (GRANDE)	0 1 2 3 4	
Pedaços de Madeira/Arbustos <0.3 m (PEQ.)	0 1 2 3 4	
Árvores vivas ou raízes	0 1 2 3 4	
Banco de Folhas	0 1 2 3 4	
Vegetação pendurada =<1 m da Superfície	0 1 2 3 4	
Margem Escavada	0 1 2 3 4	
Matacão	0 1 2 3 4	
Estruturas Artificiais	0 1 2 3 4	

MEDIDAS DA COBERTURA DO DOSSEL

DENSIÔMETRO (0-17Max)

		Obs.			Obs.
Centro a montante			Centro a direita		
Centro a esquerda			Esquerda		
Centro a jusante			Direita		

Obs.	Comentário

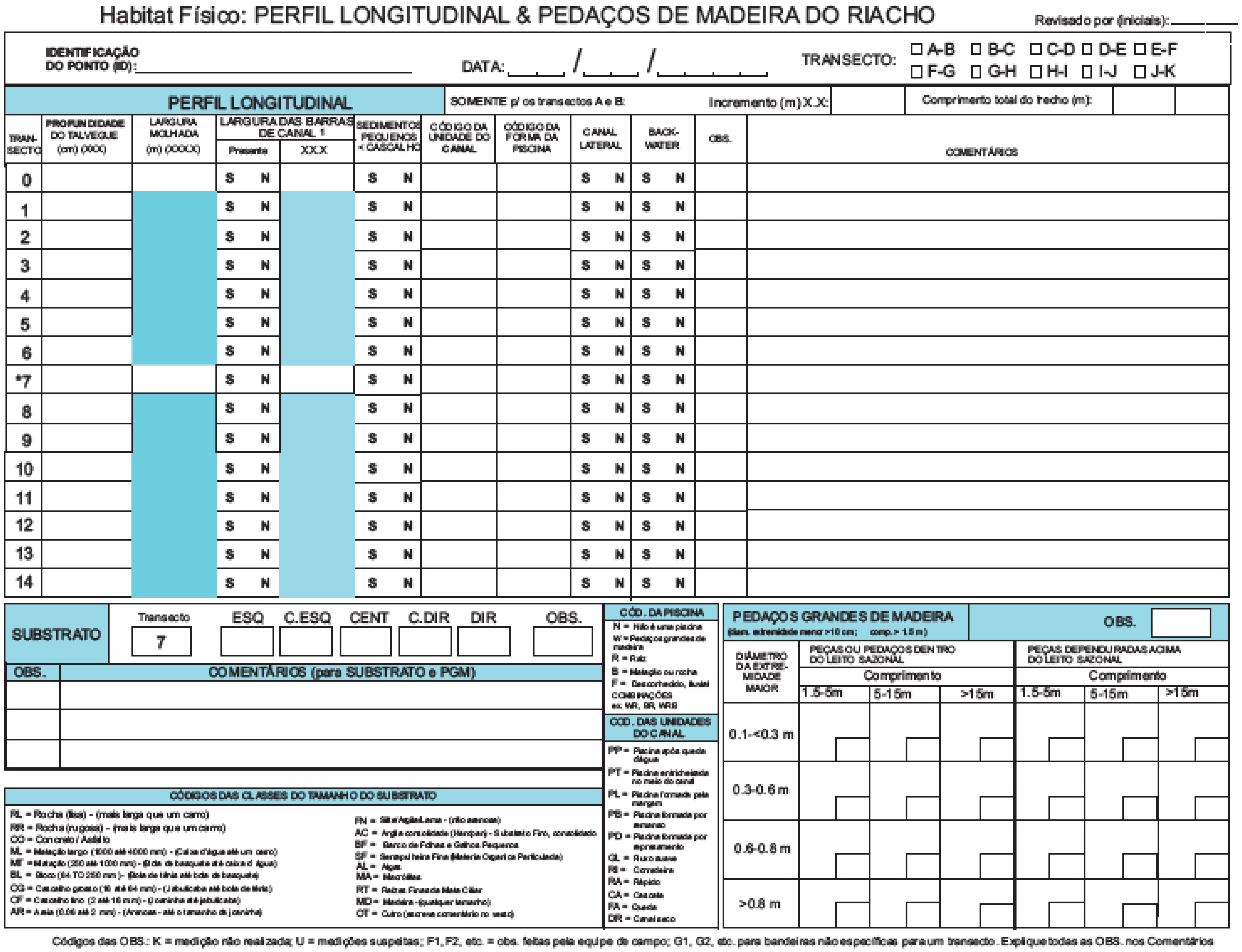
ESTIMATIVAS VISUAIS DA ZONA RIPÁRIA

0 = Ausente (0%)
1 = Esparso (<10%)
2 = Médio (10-40%)
3 = Denso (40-75%)
4 = Muito Denso (>75%)

COBERTURA VEG. DA ZONA RIPÁRIA	Margem Esquerda	Margem Direita	Obs.
	Dossel (>5 m altura)		
Árvores GRANDES (DAP >0.3 m)	0 1 2 3 4	0 1 2 3 4	
Árvores PEQUENAS (DAP <0.3 m)	0 1 2 3 4	0 1 2 3 4	
	Sub-bosque (0.5 até 5 m altura)		
Arbustos lenhosos & mudas	0 1 2 3 4	0 1 2 3 4	
Ervas sem tronco lenhoso & gramíneas	0 1 2 3 4	0 1 2 3 4	
Vegetação Rasteira (<0.5 m altura)			
Arbustos lenhosos & mudas	0 1 2 3 4	0 1 2 3 4	
Ervas sem tronco lenhoso & gramíneas	0 1 2 3 4	0 1 2 3 4	
Solo sem cobertura vegetal ou serrapilheira	0 1 2 3 4	0 1 2 3 4	

INFLUÊNCIA HUMANA	0 = Ausente P = >10 m C = < 10 m B = Na margem		
	Margem Esquerda	Margem Direita	Obs.
Muro/dique/Canalização gabião/barramento	0 P C B	0 P C B	
Construções	0 P C B	0 P C B	
Estrada calçada ou cascalhada	0 P C B	0 P C B	
Rodovia/Ferrovia	0 P C B	0 P C B	
Canos (Captação/descarga)	0 P C B	0 P C B	
Entulho/Lixo	0 P C B	0 P C B	
Parque/Gramado	0 P C B	0 P C B	
Plantações de Grãos	0 P C B	0 P C B	
Pastagem/ campo de feno	0 P C B	0 P C B	
Silvicultura/ desmatamento	0 P C B	0 P C B	
Mineração	0 P C B	0 P C B	

Códigos Obs: K = Amostra não coletada; U = Amostra suspeita F1, F2, etc. = obs. feita pela equipe de campo. Explique todas as observações na seção de comentários.



Habitat Físico: PERFIL LONGITUDINAL & PEDAÇOS DE MADEIRA DO RIACHO

Revisado por (iniciais):____

IDENTIFICAÇÃO DO PONTO (ID):______________ DATA: ___ / ___ / ______ TRANSECTO: ☐ A-B ☐ B-C ☐ C-D ☐ D-E ☐ E-F ☐ F-G ☐ G-H ☐ H-I ☐ I-J ☐ J-K

PERFIL LONGITUDINAL

SOMENTE p/ os transectos A e B: Incremento (m) X.X: ______ Comprimento total do trecho (m): ______

TRANSECTO	PROFUNDIDADE DO TALVEGUE (cm) (XXX)	LARGURA MOLHADA (m) (XXX.X)	LARGURA DAS BARRAS DE CANAL 1		SEDIMENTOS PEQUENOS < CASCALHO	CÓDIGO DA UNIDADE DO CANAL	CÓDIGO DA FORMA DA PISCINA	CANAL LATERAL	BACK-WATER	OBS.	COMENTÁRIOS
			Presente	XX.X							
0			S N		S N			S N	S N		
1			S N		S N			S N	S N		
2			S N		S N			S N	S N		
3			S N		S N			S N	S N		
4			S N		S N			S N	S N		
5			S N		S N			S N	S N		
6			S N		S N			S N	S N		
*7			S N		S N			S N	S N		
8			S N		S N			S N	S N		
9			S N		S N			S N	S N		
10			S N		S N			S N	S N		
11			S N		S N			S N	S N		
12			S N		S N			S N	S N		
13			S N		S N			S N	S N		
14			S N		S N			S N	S N		

SUBSTRATO	Transecto	ESQ	C.ESQ	CENT	C.DIR	DIR	OBS.
	7						

OBS.	COMENTÁRIOS (para SUBSTRATO e PGM)

CÓDIGOS DAS CLASSES DO TAMANHO DO SUBSTRATO

RL = Rocha (lisa) - (mais larga que um carro)
RR = Rocha (rugosa) - (mais larga que um carro)
CO = Concreto / Asfalto
ML = Matacão largo (1000 até 4000 mm) - (Caixa d'água até um carro)
MT = Matacão (250 até 1000 mm) - (Bola de basquete até caixa d'água)
BL = Bloco (64 TO 250 mm) - (Bola de tênis até bola de basquete)
CG = Cascalho grosso (16 até 64 mm) - (Jabuticaba até bola de tênis)
CF = Cascalho fino (2 até 16 mm) - (Joaninha até jabuticaba)
AR = Areia (0.06 até 2 mm) - (Arenoso - até o tamanho de joaninha)
FN = Silte/Argila/Lama - (não arenoso)
AC = Argila consolidada (Hardpan) - Substrato Fino, consolidado
BF = Banco de Folhas e Galhos Pequenos
SF = Serapilheira Fina (Matéria Orgânica Particulada)
AL = Algas
MA = Macrófitas
RT = Raízes Finas da Mata Ciliar
MD = Madeira - (qualquer tamanho)
OT = Outro (escreva comentário no verso)

CÓD. DA PISCINA

N = Não é uma piscina
W = Pedaços grandes de madeira
R = Raiz
B = Matacão ou rocha
F = Desconhecido, fluvial
COMBINAÇÕES ex: WR, BR, WRB

CÓD. DAS UNIDADES DO CANAL

PP = Piscina após queda d'água
PT = Piscina entrincheirada no meio do canal
PL = Piscina formada pela margem
PB = Piscina formada por remanso
PD = Piscina formada por represamento
GL = Fluxo suave
RI = Corredeira
RA = Rápido
CA = Cascata
FA = Queda
DR = Canal seco

PEDAÇOS GRANDES DE MADEIRA (diam. extremidade menor >10 cm; comp. > 1.5 m) OBS.

DIÂMETRO DA EXTREMIDADE MAIOR	PEÇAS OU PEDAÇOS DENTRO DO LEITO SAZONAL			PEÇAS DEPENDURADAS ACIMA DO LEITO SAZONAL		
	Comprimento			Comprimento		
	1.5-5m	5-15m	>15m	1.5-5m	5-15m	>15m
0.1-<0.3 m						
0.3-0.6 m						
0.6-0.8 m						
>0.8 m						

Códigos das OBS.: K = medição não realizada; U = medições suspeitas; F1, F2, etc. = obs. feitas pela equipe de campo; G1, G2, etc. para bandeiras não específicas para um transecto. Explique todas as OBS. nos Comentários

Figure 5: Part of the Protocol that has been adapted and validated for Neotropical Savanna headwater streams and used for accessing environmental metrics.
Source: Callisto et al. (2014b).

4 GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

My findings indicated that Odonata larvae followed the pattern of distribution and abundance shown by adults in a gradient of environmental conditions in headwater streams in the Neotropical Savanna. Zygoptera larvae exhibited higher richness in sites with increased levels of canopy cover by riparian vegetation. On the other hand, Anisoptera larvae showed higher abundance and richness in sites surrounded by decreased levels of riparian vegetation shading. Thus, such metrics can be used for assessing environmental impacts related to riparian vegetation. (Chapter 1).

The trait-based approach indicated that sites in the 'least disturbed conditions' (reference) select Zygoptera larvae with sets of traits distinct from those of Anisoptera, which occur in intermediate or highly disturbed conditions. Zygoptera genera (*Perilestes*, *Allopodagrion*, *Heliocharis*, *Argia*, *Epipleoneura*, *Mnesarete*/*Hetaerina*, *Psaironeura*) have traits such as elongated body shapes, caudal lamellae respiration, conforming thermoregulation, and endophytic oviposition. Such traits favor Zygoptera larvae assemblages in conditions similar to reference streams, including denser riparian vegetation, good water quality, and diverse flows and substrate. Therefore, they could be considered more sensitive to changes in those conditions. On the other hand, Anisoptera genera (*Gomphoides*, *Archaeogomphus*, *Macrothemis*, *Brechmorhoga*, *Gynothemis*, *Phyllocycla*) have traits such as cylindrical body shapes, internal gill respiration, endothermic thermoregulation, and burrowing behavior. Those traits facilitate the presence of Anisoptera larvae assemblages in intermediate or disturbed stream sites, characterized by riparian deforestation, increased erosion and siltation, and higher levels of total dissolved solids and conductivity. The use of Odonata larval traits can be a useful tool for assessing and monitoring anthropogenic impacts in the Brazilian Savanna (Chapter 2).

The approach using ecological thresholds highlighted seven larvae Odonata genera (*Argia*, *Brechmorhoga*, *Cacoides*, *Gomphoides*, *Mnesarete/Hetaerina*, *Phyllocycla* and *Progomphus)*, revealing them as robust bioindicators for environmental metrics related to human disturbances. In this way, Odonata larvae genera may indicate streams least or most disturbed, explaining the process of why human disturbances alter the composition of larvae living in headwater streams (Chapter 3).

I conclude that anthropogenic disturbances, mostly related to the removal or reduction of riparian vegetation cover, affect the composition, structure and traits of Odonata larvae assemblages in the Brazilian Savanna. I expect that the knowledge produced in this thesis will help support the use of Odonata larvae as an efficient and promising tool for the assessment and monitoring of headwater stream ecosystems in Brazil and other tropical regions. Also, I highlighted the importance of expanding the ecological approaches with traits and thresholds to other genera of Odonata not recorded in this study, or even looking for their relationship with other environmental metrics, but using the larval stage. Finally, I hope that the temporal gap between the knowledge about the use of Odonata larvae as a practical and efficient tool for assessing and monitoring freshwaters and the incorporation of this knowledge into decision making can be shortened.

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SECOND PART

ARTICLES

6 ARTICLE 1

Hydrobiologia
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PRIMARY RESEARCH PAPER

A matter of suborder: are Zygoptera and Anisoptera larvae influenced by riparian vegetation in Neotropical Savanna streams?

Larissa F. R. Silva · Diego M. P. Castro · Leandro Juen · Marcos Callisto · Robert M. Hughes · Marcel G. Hermes

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Abstract Initial Odonata larval distributions are primarily influenced by adult females at the moment of oviposition. However, after oviposition, the larvae are strongly associated with environmental conditions. In the case of both adults and larvae, anthropogenic disturbances that change these conditions may alter the composition and structure of Odonata assemblages. Therefore, based on the differing environmental requirements of Zygoptera and Anisoptera adults and larvae, together with their morphological and physiological differences, we suspected differing riparian preferences of larvae and adults for each suborder. We evaluated the richness and abundance of Odonata larvae. We hypothesized that Zygoptera larvae would have greater richness and abundance in streams with canopy shading, lower temperature ranges, and high physical habitat heterogeneity. On the other hand, Anisoptera larvae would be more abundant in streams without canopy cover. We sampled 186 headwater stream sites in the Neotropical Savanna along an anthropogenic disturbance gradient and used a model selection approach to test our hypotheses, correlating environmental metrics with Odonata larval richness and abundance. We found

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L. F. R. Silva (✉) · M. G. Hermes
Laboratório de Sistemática e Biologia de Insetos, Setor de Zoologia Comparada, Departamento de Biologia, Universidade Federal de Lavras, CEP 37200-900 Lavras, Minas Gerais, Brazil
e-mail: larissamg05@hotmail.com

M. G. Hermes
e-mail: marcelhermes@ufla.br

D. M. P. Castro · M. Callisto
Laboratório de Ecologia de Bentos, Departamento de Genética, Ecologia e Evolução, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, CP 486, CEP 31270-901
Belo Horizonte Minas Gerais Brazil
e-mail: diegobioufla@gmail.com

M. Callisto
e-mail: callistom@ufmg.br

L. Juen
Laboratório de Ecologia e Conservação, Instituto de Ciências Biológicas, Universidade Federal do Pará, Av. Perimetral, 2-224, CEP 66077-830 Belém, Pará, Brazil
e-mail: leandrojuen@ufpa.br

R. M. Hughes
Amnis Opes Institute, 2895 SE Glenn Street, Corvallis, OR 97333, USA
e-mail: hughes.bob@amnisopes.com

R. M. Hughes
Department of Fisheries, Wildlife, & Environmental Sciences, Oregon State University, Nash 104, Corvallis, OR 97331, USA

higher richness of Zygoptera larvae in shaded sites with canopy cover > 5 m high, whereas bare ground without riparian vegetation was important for Anisoptera richness and abundance. Our results indicated that Odonata larvae follow the same distribution, richness and abundance patterns as adults. Anthropogenic disturbances related to the removal or reduction of riparian vegetation can favor Anisoptera over Zygoptera larval assemblages in streams. Preserving riparian canopy cover is needed to maintain the richness of Zygoptera larvae in Neotropical Savanna streams.

Keywords Cerrado streams · Damselflies · Dragonflies · GLM · Immatures · Odonata

Introduction

Insects show macroecological patterns related to thermal tolerance, body size, niche range, and dispersion capacity—all of which determine species distributions. For example, body size is related to dispersion capacity and regulated by temperature (Oliveira-Junior et al., 2017; Rocha-Ortega et al., 2020). Although insects can grow and mature over wide temperature ranges (from 10 to 20°C to maximum 40 to 50°C) (Neven, 2000; Dixon et al., 2009), in general, those with smaller body sizes live at higher temperatures, whereas those with larger body sizes can persist at lower temperatures (Chown & Gaston, 2010). When conditions like temperature are adverse, it is their dispersion capacity that allows insects to change habitat locations (Rocha-Ortega et al., 2020; Firmiano et al., 2021).

Adult Odonata respond differently to anthropogenic disturbances, according to Adult Anisoptera and Zygoptera respond differently to anthropogenic disturbances. Those differences result from a combination of physiological (e.g., thermoregulation), ecological (e.g., niche range and dispersion capacity), and morphological (e.g., body size) factors (Mendes et al., 2015; Oliveira-Junior & Juen, 2019). Restrictions imposed mainly by body size and thermoregulation determine the distribution and structure of adult Odonata assemblages in Neotropical streams (De Marco et al., 2015; Oliveira-Junior & Juen, 2019). Headwater streams with lower temperatures and dense canopy cover favor adult Odonata assemblages composed predominantly by Zygoptera (De Marco et al., 2015; Oliveira-Junior et al., 2017; Oliveira-Junior & Juen, 2019). On the other hand, Anisoptera species tend to dominate in larger streams that are less shaded (Vannote et al., 1980) and in streams where riparian vegetation has been removed (De Marco et al., 2015). In heavily degraded streams, even Anisoptera species cannot survive (Monteiro Júnior et al., 2015). This pattern of adult Odonata suborders is well established for the neotropics (De Marco et al., 2015; Oliveira-Junior & Juen, 2019).

Because larvae are strongly associated with environmental conditions, the anthropogenic disturbances that change these conditions may alter the composition and structure of Odonata larval assemblages (Souza et al., 2015; Luke et al., 2017; Mendes et al., 2019; Pires et al., 2020). Odonata larvae have some advantages over adults as bioindicators, including limited dispersion capacity, which makes them more susceptible to local changes (Valente-Neto et al., 2016) and easier field sampling (Oertli, 2008). Also, the larvae clearly differ in their morphologies. Zygoptera larvae have more delicate bodies and breathe through relatively delicate external lamellae (Corbet, 1980). Anisoptera larvae have more robust bodies, breathe through rectal tracheal gills (Corbet, 1980), and possess morphological structures that help defend them against predators (De Marco et al., 2015; Mendes et al., 2019).

Based on the different environmental requirements of Zygoptera and Anisoptera adults, we evaluated the distribution patterns of Odonata larvae along a gradient of riparian disturbance in Neotropical Savanna streams. We hypothesized that Odonata larvae would display the same patterns as Odonata adults. We expected that Zygoptera would have higher taxa richness and proportional abundances in sites with minimal riparian disturbance, considerable canopy shading, lower temperature range, and high physical habitat heterogeneity. Conversely, we expected that Anisoptera larvae would have higher taxa richness and proportional abundances in sites with less canopy cover or without riparian vegetation.

Methods

Study area and survey design

We studied 160 randomly selected 1st to 3rd order (Strahler, 1957) stream sites in the Nova Ponte, Volta Grande, São Simão and Três Marias hydrological units (Fig. 1; Table S1). The sampled sites exhibited a wide disturbance gradient, from minimally disturbed sites with high dissolved oxygen and low nutrient concentrations, sites with moderate levels of human-altered land (pasture, row crops) to highly degraded urban sites with poor water quality and physical habitat conditions (Macedo et al., 2014; Silva et al., 2017). We sampled each of the hydrological units in September (2010 in Três Marias, 2011 in Volta Grande, 2012 in São Simão, and 2013 in Nova Ponte), ensuring that samples were all taken in the low flow

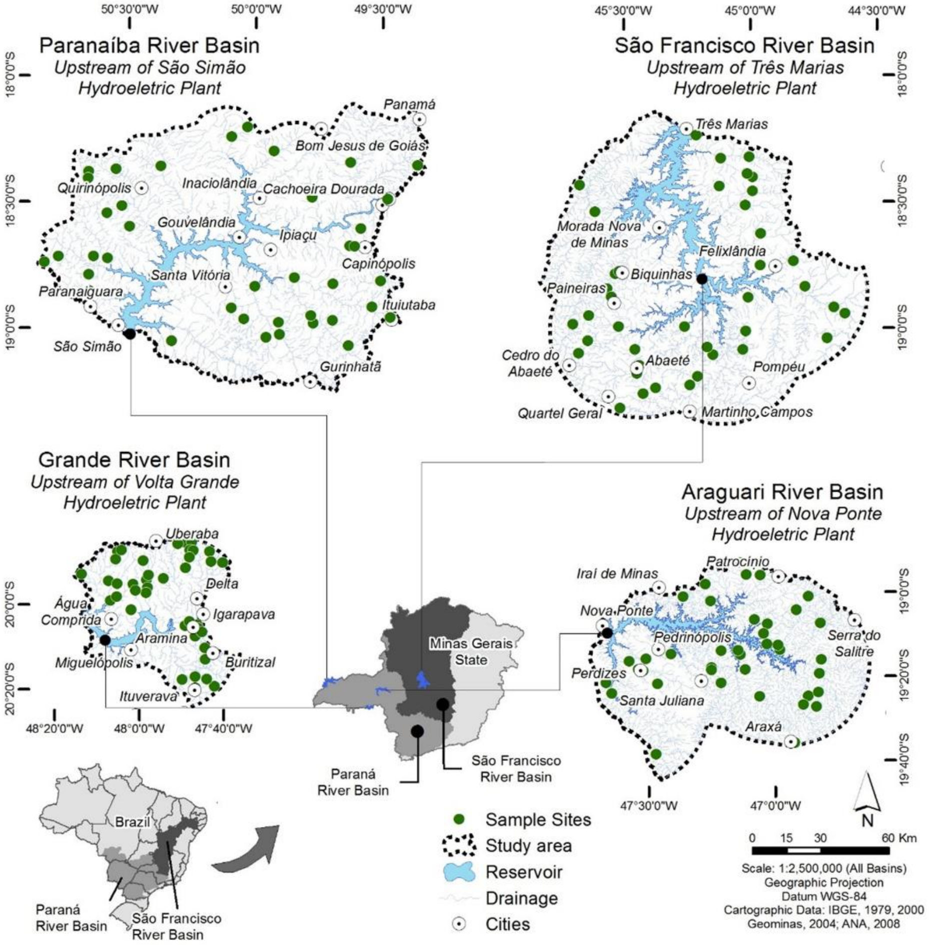

Fig. 1 Locations of the Cerrado stream sites and hydrological units

season. An additional set of 26 reference sites were sampled in April and May 2014, in Serra da Canastra National Park and Serra do Salitre. Those sites differed by exhibiting minimal human catchment disturbance, absence of direct anthropogenic disturbances at the sites, and the presence of native riparian vegetation (Martins et al., 2018).

Sampling of Odonata larvae

In each hydrological unit, we sampled 40 stream sites. Site selection followed a probabilistic sampling design, according to the methodology used by the U.S. Environmental Protection Agency (Peck et al., 2006; Callisto et al., 2014). This design allows the impartial selection of sampling sites representing the region as a whole, avoiding bias in site selection and spatial autocorrelation (Silva et al., 2018).

The length of each site was 40 times its mean wetted width, respecting a minimum of 150 m, and divided into 11 equidistant transects. Odonata larvae were sampled with a kick-net sampler (500 µm mesh, 0.09 m^2 area), following a zigzag trajectory across the transects (left, center, and right). The samples were fixed in 4% formalin. In the laboratory, samples were washed, stored in 70% alcohol, processed and then deposited in the reference collection of the Instituto de Ciências Biológicas of the Universidade Federal de Minas Gerais.

Odonata larvae were examined under a stereoscopic microscope and identified to genus using taxonomic keys (Costa et al., 2004; Neiss & Hamada, 2014; Pessacq et al., 2018) as well as descriptions and reviews available for each taxon. We identified larvae to genus because the larval stages of most species are not described (von Ellenrieder, 2009) and the genus level is sufficient to evaluate the responses of Odonata assemblages to anthropogenic disturbances (Mendes et al., 2019).

Environmental metrics

Environmental metrics at each site were described in terms of local riparian vegetation cover based on Stoddard et al. (2005) and Peck et al. (2006). This protocol assesses metrics related to physical habitat structure, near-stream anthropogenic stressors, and riparian vegetation structure and cover. We selected 11 metrics related to riparian vegetation (Kaufmann et al., 1999), and also calculated local, catchment, and integrated disturbance indices (Ligeiro et al., 2013), totaling 14 predictor variables as described below (Table S2). These metrics have been successfully used in previous studies of these sites (Firmiano et al., 2017; Castro et al., 2018; Silva et al., 2018).

Canopy cover was determined at each of the 11 transects in each site. Four canopy cover measurements were taken: upstream, downstream, right, and left at mid-channel. For this, we used a hemispherical convex densiometer, in which readings potentially varied from zero (without canopy cover) to 17 (maximum canopy cover). The measurements were made at 0.3 m above the water surface, observing the 17 intersection points of the grid on the densiometer. If the reflection of a tree or tall branch or leaf overlaps any intersection points, that specific intersection is counted as having coverage. The mid-channel measurements are used to estimate the canopy coverage over the channel (XCDENMID).

Riparian vegetation structure was determined through visual estimation, with a semi-quantitative assessment of the type and amount of riparian vegetation. Observations were made at both margins in each of the 11 cross-sections, 5-m upstream, 5-m downstream and 10-m landward, creating a 10 × 10 m plot on each side of the stream. Within this plot, we divided riparian vegetation into three layers: canopy ($>$ 5 m high), understory (0.5–5 m high), and ground-cover ($<$ 0.5 m high). In each layer, we determined the class of covered area in large trees ($>$ 0.3 m diameter at breast height) and small trees ($<$ 0.3 m). These data were used to calculate: mean woody canopy cover (XC), mean woody understory cover (XM), mean woody ground layer cover (XG) and bare ground cover (XGB) (Kaufmann et al., 1999).

To assess local and catchment anthropogenic disturbances in each site, we calculated three indices: Local Disturbance Index (LDI), Catchment Disturbance Index (CDI), and Integrated Disturbance Index (IDI) based on Ligeiro et al. (2013).

Data analyses

We calculated ten biological metrics for each site: total Odonata richness and abundance (combining the two suborders); Anisoptera richness and abundance; Zygoptera richness and abundance; proportional richness and abundance of Anisoptera; and proportional richness and abundance of Zygoptera (Table S3). The combined statistical analysis of the two suborders may lead to misinterpretations about what affects these assemblages, which can be avoided if each suborder is analyzed separately (Oliveira-Junior et al., 2015; Mendes et al., 2017).

To explore our hypothesis that riparian vegetation condition affects the distribution of Odonata larvae along a gradient of anthropogenic disturbance, we used a model selection approach (Burnham & Anderson, 2002). First, we tested for Spearman correlations among environmental metrics (Fig. S1), eliminating those that were highly correlated ($r > 0.7$) and retaining those that are the most ecologically relevant. In this step, we retained seven environmental metrics (Table 1). We used our selected variables as predictor variables with each biological metric in generalized linear models (GLMs). For count biological metrics (i.e., richness and abundance), we used a Poisson distribution. Using our most parameterized model, we evaluated a possible overdispersion in each analysis. Once overdispersion was detected, standard errors were corrected using a quasi-Poisson error structure. We next used a binomial or quasi-binomial distribution model for the proportional richness of Anisoptera versus the proportional richness of Zygoptera, and the proportional abundance of Anisoptera versus the proportional abundance of Zygoptera. We then constructed models based on all possible additive variable combinations followed by a model selection procedure using backward elimination to obtain the most parsimonious model. The selected model significance was tested by an Analysis of Deviance (Chi-squared for Poisson or quasi-Poisson distributions and F-test for binomial or quasi-binomial distributions). We checked model residuals using the DHARMa package (Hartig, 2018). All analyses were performed in R version 3.4.3 (R Core Team, 2017), using the Vegan (Oksanen et al., 2019) and lme4 (Bates et al., 2015) packages.

Results

We collected and identified a total of 3209 Odonata larvae, distributed along 30 genera and ten families. Fifteen hundred individuals, eight genera and six families belonged to Zygoptera; 1709 individuals, 22 genera and four families belonged to Anisoptera (Tables S4, S5). After initial model selection, the candidate metrics IDI, LDI, XCDENMID, XM and XG were excluded because none of them were significantly related to the biological metrics.

For total Odonata larval richness, no model was statistically significant. However, when testing Zygoptera and Anisoptera richness separately, models showed a positive relation between Zygoptera and mean canopy cover (XC) ($x^2 = 8.954$, $df = 174$,

Table 1 Environmental metrics used in the generalized linear models (GLM)

Acronyms	Environmental metrics' name	Meaning of environmental metrics	References
IDI	Integrated Disturbance Index	LDI & CDI	Ligeiro et al. (2013)
LDI	Local Disturbance Index	All types of riparian anthropogenic disturbance	Ligeiro et al. (2013)
XCDENMID	Canopy cover midstream	% vegetation covers the middle of the channel	Kaufmann et al. (1999) and Peck et al. (2006)
XGB	Bare ground cover	% exposed soil	Kaufmann et al. (1999) and Peck et al. (2006)
XC	Mean canopy cover	Woody vegetation > 5 m high	Kaufmann et al. (1999) and Peck et al. (2006)
XM	Mean understory cover	Woody vegetation 0.5 to 5 m high	Kaufmann et al. (1999) and Peck et al. (2006)
XG	Mean ground-layer cover	Woody ground cover < 0.5 m high	Kaufmann et al. (1999) and Peck et al. (2006)

$N = 175$, $P = 0.003$) and a positive relation for Anisoptera and bare ground cover (XGB) ($x^2 = 14.206$, $df = 174$, $N = 175$, $p < 0.001$) (Fig. 2).

For total Odonata larval abundance, a model showed a positive relation with bare ground cover ($x^2 = 122.53$, $df = 174$, $N = 175$, $P = 0.004$). However, when testing Zygoptera and Anisoptera abundance separately, no model was statistically significant for Zygoptera, but Anisoptera showed a positive relation with bare ground cover ($x^2 = 95.312$, $df = 174$, $N = 175$, $P = 0.001$) (Fig. 3).

For Zygoptera proportional taxa richness, a model showed a negative relation with bare ground cover ($x^2 = 14.966$, $df = 174$, $N = 175$, $p < 0.001$) and a positive relation with mean canopy cover ($x^2 = 6.255$, $df = 174$, $N = 175$, $P = 0.005$) (Fig. 4 A). Reverse relationships were observed for Anisoptera proportional taxa richness with bare ground cover ($x^2 = 14.966$, $df = 174$, $N = 175$, $p < 0.001$) and mean canopy cover ($x^2 = 6.255$, $df = 174$, $N = 175$, $P = 0.005$) (Fig. 4 B). No model was statistically significant for proportional individual abundances of Anisoptera or Zygoptera.

Discussion

We found congruences between the patterns of distribution, richness and abundance shown by larvae in this study with those documented for Zygoptera and Anisoptera adults in the literature. Our results corroborate the prediction that Zygoptera larvae have greater richness in streams with minimal human disturbances of riparian vegetation canopy cover. We also corroborate the prediction that Anisoptera larvae have greater richness and abundance in streams with less canopy cover or without riparian vegetation, indicated by bare ground cover.

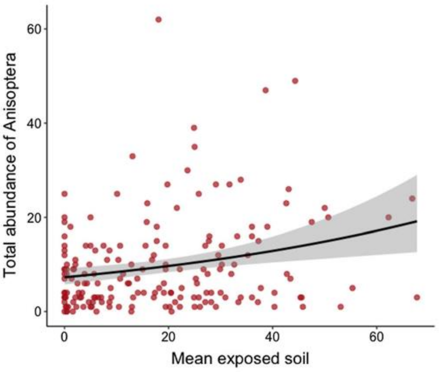

Fig. 3 Poisson GLM relation between mean exposed soil versus Anisoptera total abundance. The dark line indicates predicted values; dots indicate observed values, and the grey band indicates 95% confidence intervals

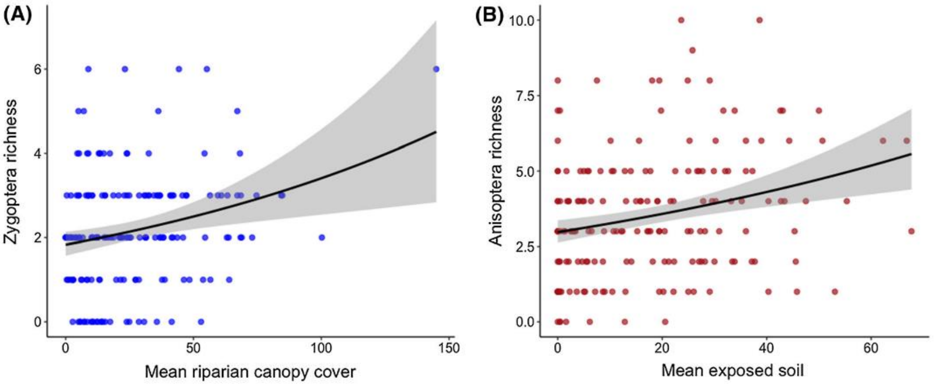

Fig. 2 Poisson GLM relationships between mean riparian canopy cover versus Zygoptera richness (**A**) and mean exposed soil versus Anisoptera richness (**B**). The dark line indicates predicted values; dots indicate observed values, and the grey band indicates 95% confidence intervals

richness and abundance of Zygoptera (Table S3). The combined statistical analysis of the two suborders may lead to misinterpretations about what affects these assemblages, which can be avoided if each suborder is analyzed separately (Oliveira-Junior et al., 2015; Mendes et al., 2017).

To explore our hypothesis that riparian vegetation condition affects the distribution of Odonata larvae along a gradient of anthropogenic disturbance, we used a model selection approach (Burnham & Anderson, 2002). First, we tested for Spearman correlations among environmental metrics (Fig. S1), eliminating those that were highly correlated ($r > 0.7$) and retaining those that are the most ecologically relevant. In this step, we retained seven environmental metrics (Table 1). We used our selected variables as predictor variables with each biological metric in generalized linear models (GLMs). For count biological metrics (i.e., richness and abundance), we used a Poisson distribution. Using our most parameterized model, we evaluated a possible overdispersion in each analysis. Once overdispersion was detected, standard errors were corrected using a quasi-Poisson error structure. We next used a binomial or quasi-binomial distribution model for the proportional richness of Anisoptera versus the proportional richness of Zygoptera, and the proportional abundance of Anisoptera versus the proportional abundance of Zygoptera. We then constructed models based on all possible additive variable combinations followed by a model selection procedure using backward elimination to obtain the most parsimonious model. The selected model significance was tested by an Analysis of Deviance (Chi-squared for Poisson or quasi-Poisson distributions and F-test for binomial or quasi-binomial distributions). We checked model residuals using the DHARMa package (Hartig, 2018). All analyses were performed in R version 3.4.3 (R Core Team, 2017), using the Vegan (Oksanen et al., 2019) and lme4 (Bates et al., 2015) packages.

Results

We collected and identified a total of 3209 Odonata larvae, distributed along 30 genera and ten families. Fifteen hundred individuals, eight genera and six families belonged to Zygoptera; 1709 individuals, 22 genera and four families belonged to Anisoptera (Tables S4, S5). After initial model selection, the candidate metrics IDI, LDI, XCDENMID, XM and XG were excluded because none of them were significantly related to the biological metrics.

For total Odonata larval richness, no model was statistically significant. However, when testing Zygoptera and Anisoptera richness separately, models showed a positive relation between Zygoptera and mean canopy cover (XC) ($x^2 = 8.954$, $df = 174$,

Table 1 Environmental metrics used in the generalized linear models (GLM)

Acronyms	Environmental metrics' name	Meaning of environmental metrics	References
IDI	Integrated Disturbance Index	LDI & CDI	Ligeiro et al. (2013)
LDI	Local Disturbance Index	All types of riparian anthropogenic disturbance	Ligeiro et al. (2013)
XCDENMID	Canopy cover midstream	% vegetation covers the middle of the channel	Kaufmann et al. (1999) and Peck et al. (2006)
XGB	Bare ground cover	% exposed soil	Kaufmann et al. (1999) and Peck et al. (2006)
XC	Mean canopy cover	Woody vegetation > 5 m high	Kaufmann et al. (1999) and Peck et al. (2006)
XM	Mean understory cover	Woody vegetation 0.5 to 5 m high	Kaufmann et al. (1999) and Peck et al. (2006)
XG	Mean ground-layer cover	Woody ground cover < 0.5 m high	Kaufmann et al. (1999) and Peck et al. (2006)

N = 175, P = 0.003) and a positive relation for Anisoptera and bare ground cover (XGB) (x^2 = 14.206, df = 174, N = 175, $p < 0.001$) (Fig. 2).

For total Odonata larval abundance, a model showed a positive relation with bare ground cover (x^2 = 122.53, df = 174, N = 175, P = 0.004). However, when testing Zygoptera and Anisoptera abundance separately, no model was statistically significant for Zygoptera, but Anisoptera showed a positive relation with bare ground cover (x^2 = 95.312, df = 174, N = 175, P = 0.001) (Fig. 3).

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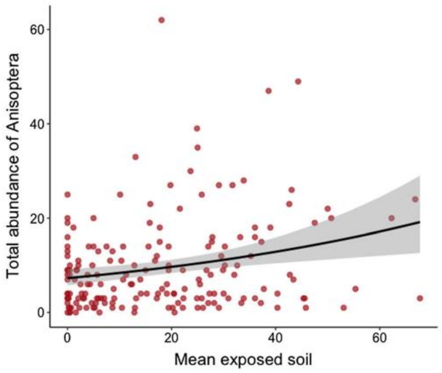

Fig. 3 Poisson GLM relation between mean exposed soil versus Anisoptera total abundance. The dark line indicates predicted values; dots indicate observed values, and the grey band indicates 95% confidence intervals

Discussion

We found congruences between the patterns of distribution, richness and abundance shown by larvae in this study with those documented for Zygoptera and Anisoptera adults in the literature. Our results corroborate the prediction that Zygoptera larvae have greater richness in streams with minimal human disturbances of riparian vegetation canopy cover. We also corroborate the prediction that Anisoptera larvae have greater richness and abundance in streams with less canopy cover or without riparian vegetation, indicated by bare ground cover.

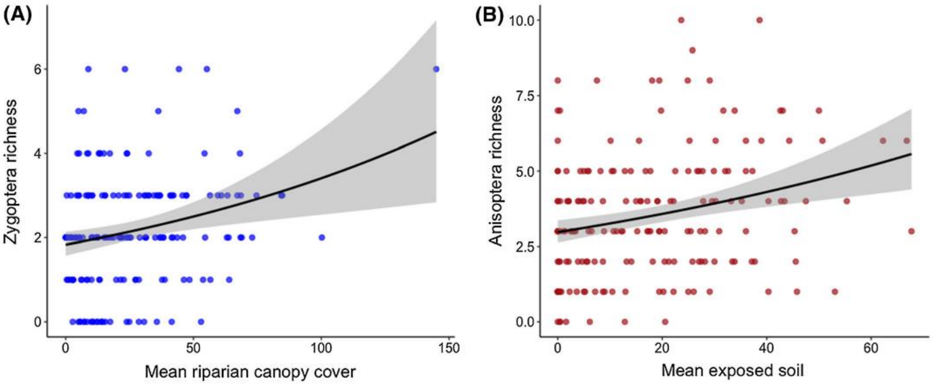

Fig. 2 Poisson GLM relationships between mean riparian canopy cover versus Zygoptera richness (**A**) and mean exposed soil versus Anisoptera richness (**B**). The dark line indicates predicted values; dots indicate observed values, and the grey band indicates 95% confidence intervals

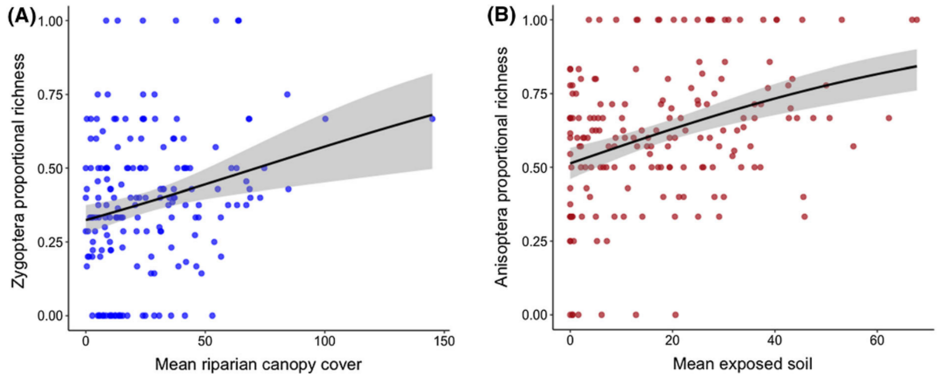

Fig. 4 Binomial GLM relationships between mean riparian canopy cover versus Zygoptera proportional richness (**A**) and mean exposed soil versus Anisoptera proportional richness (**B**). The figures for mean exposed soil versus proportional Zygoptera taxa richness and mean riparian canopy cover versus proportional Anisoptera taxa richness are not shown because they are inversely proportional. The dark line indicates predicted values; dots indicate observed values, and the grey band indicates 95% confidence intervals

Initial Odonata larval distribution is related to adult females' choices of ovipositing location (Corbet, 1980). There is strong selective pressure for oviposition in habitats where the larval survival rate is high, and for this, these habitats must match the ecological requirements, habitats, and behavior of the larvae (Buskirk & Sherman, 1985). After oviposition, larval establishment in the habitat depends on the morphological and behavioral traits of the species as well as on environmental features (Corbet, 1980). The species traits are related mainly to breathing, feeding, and refuge (Corbet, 1980) whereas key environmental features are water quality (Oertli, 2008; Mendes et al., 2015; Souza et al., 2015), substrate composition (Assis et al., 2004; Pires et al., 2020), and riparian vegetation (Remsburg & Turner, 2009; Mendes et al., 2019; Pires et al., 2020). Although oviposition is a female choice, the presence of larvae in the water guarantees that they are living there or nearby (Luke et al., 2017) and, therefore, are being influenced by site environmental conditions. In addition, larval stages are more restrictive because they tend to be much less mobile than adults (Oertli, 2008) and consequently more susceptible to environmental conditions.

In our study, anthropogenic disturbance in the riparian vegetation affected Odonata assemblages more than various anthropogenic disturbances in the local area and catchment, as evaluated by IDI and LDI. This is likely because water quality conditions and site vegetation structure affect Odonata larvae more than other types of environmental disturbances (Luke et al., 2017; Pires et al., 2020).

Zygoptera and Anisoptera larvae have physiological (Neiss & Hamada, 2014) and ecological (Mendes et al., 2017) differences that drive their different responses to riparian vegetation structure. For Zygoptera larvae, riparian vegetation is important because higher density and canopy cover provides greater stream shading and moderated water temperatures (Samways & Steytler, 1996; Peck et al., 2006). Waters with lower temperatures and higher dissolved oxygen provide better conditions for larval survival (Jooste et al., 2020). Zygoptera larvae breathe mostly using their caudal lamellae (Ramirez, 2010), which requires higher levels of dissolved oxygen in the water. Although we did not test for differences in water temperature and dissolved oxygen directly, these variables are affected by riparian vegetation canopy cover, and consequently, the assemblage of Zygoptera larvae may also be affected.

Besides breathing, larval Zygoptera use riparian vegetation for hunting, as predator refuges, and crawling out of the water to emerge as adults (Jooste et al., 2020). Some larvae hold onto overhanging pieces of vegetation, branches or leaves, that hang submerged in the stream, and remain motionless. The combination of vegetation and motionlessness provides refuge against visual aquatic and aerial predators. At the same time, it provides camouflage to facilitate capturing their prey (Sesterhenn et al., 2013). In short, removal of riparian vegetation and open canopies lead to low richness and abundance of Zygoptera adults and larvae (Mendes et al., 2015; Oliveira-Junior et al., 2019).

Unlike Zygoptera, Anisoptera larvae breathe with rectal gills in the internal abdominal cavity, filling and emptying the abdomen with water (Ramirez, 2010). The gill physiology increases capture of dissolved oxygen (Kohnert et al., 2004), facilitating survival in waters with low dissolved oxygen concentrations. Moreover, most Anisoptera larvae live buried or semi-buried in sandy and muddy substrates (Carvalho & Nessimian, 1998). This substrate type is correlated with erosion, frequently caused by removal of riparian vegetation (Wood & Armitage, 1997). Removal of riparian vegetation also may lead to an increase in stream macrophyte biomass (Fares et al., 2020), which is an important predictor of Anisoptera larvae occurrence (Juen et al., 2007). The presence of thermoreceptors in Libellulidae larvae (Rebora et al., 2007) suggests why Anisoptera larvae can remain in unshaded aquatic environments and why bare ground cover was a significant predictor for this suborder. French & McCauley (2018) evaluated the importance of canopy cover for Anisoptera larvae and adults and found no effect on larval survival. Thus, dense canopy cover could reduce adult oviposition, thereby affecting larval distribution.

The amount of riparian vegetation is important for Odonata adults because of their different thermoregulation mechanisms (De Marco et al., 2015; Oliveira-Junior & Juen, 2019). In general, Zygoptera species have smaller body sizes and are known as “thermal conformers”, that is, their body temperature is directly influenced by air temperature (May, 1976; De Marco & Resende, 2002). Most of them cannot survive near streams where there is little or no riparian vegetation because the shading is less, the solar incidence is increased and temperatures oscillate more, causing overheating and desiccation (Oliveira-Junior et al., 2017; Oliveira-Junior & Juen, 2019). On the other hand, Anisoptera species are larger and endothermic, that is, they generate and store heat in the body and control the circulation of hemolymph to facilitate thermoregulation (May, 1976; De Marco & Resende, 2002). They do not depend on the presence of riparian vegetation to control their body temperature and can thrive such environments without dessication (Oliveira-Junior et al., 2017; Oliveira-Junior & Juen, 2019). Previous studies concluded that adult Zygoptera assemblages were bioindicators for the loss of riparian vegetation (Carvalho et al., 2013; Monteiro Júnior et al., 2015; Oliveira-Junior et al., 2017). In our study, the positive correlation between Zygoptera larvae and mean canopy cover indicated that they were also highly sensitive to removing riparian vegetation, following the pattern observed for adults. The opposite is true for Anisoptera larvae, which were tolerant to riparian vegetation removal, similarly to adults (Oliveira-Junior et al., 2017).

Also, it is important to recognize that the proportion of each suborder in larval Odonata assemblages could be used for assessing environmental impacts, without necessarily identifying individuals to genus or species levels. The same was true for the proportion of each suborder in adult Odonata assemblages (Oliveira-Junior et al., 2017; Oliveira-Junior & Juen, 2019). This makes the proportions of Odonata suborders useful for Neotropical biomonitoring programs, including those implemented by citizen scientists (França et al., 2019), because the two suborders are easily identified by eye.

For conservation purposes, it is important to determine the thresholds of riparian vegetation loss affecting the assemblages of Odonata larvae in Neotropical streams. Anisoptera can persist in streams with riparian vegetation width < 15 m, whereas Zygoptera genera require riparian vegetation widths > 15 m (Pires et al., 2020). Our study indicated that riparian vegetation height was also important because canopy cover > 5 m was the metric that best explained larval Zygoptera richness and abundance. However, for larval Anisoptera, riparian vegetation height had no significance because they benefited from bare ground cover. This suggest that vegetated riparian buffers at least 5 m high and 15 m wide might suffice for sustaining Zygoptera taxa, despite somewhat disturbed catchments.

Conclusions

Odonata larvae followed the pattern of distribution and abundance shown by adults and were driven by the degree of riparian vegetation, especially canopy cover.

Zygoptera larvae exhibited higher richness and proportional richness in streams with increased levels of canopy cover. On the other hand, Anisoptera larvae showed higher abundance, richness, and proportional richness in streams surrounded by bare ground and decreased levels of riparian vegetation canopy cover. We conclude that anthropogenic removal or reduction of riparian vegetation can affect Odonata larval assemblages in Cerrado streams. Therefore, such metrics can be used for assessing environmental impacts related to riparian vegetation. Although it is not known exactly how much Neotropical riparian vegetation cover should be preserved so that it does not affect aquatic macroinvertebrate assemblages (Dala-Corte et al., 2020), for Odonata we have this information. We know that areas with reduced riparian vegetation have higher larval Anisoptera richness. On the other hand, streams with canopy cover < 5 m high should have their riparian vegetation preserved to maintain Zygoptera larval richness.

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Declarations

Conflict of interest All authors declare that they have no conflict of interest.

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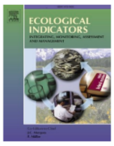

Original Articles

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

Larissa F.R. Silva[a,*], Diego M.P. Castro[b,f], Leandro Juen[c], Marcos Callisto[b],
Robert M. Hughes[d,e], Marcel G. Hermes[a]

[a] *Universidade Federal de Lavras, Setor de Zoologia Comparada, Departamento de Biologia, Laboratório de Sistemática e Biologia de Insetos, CEP 37200-900 Lavras, Minas Gerais, Brazil*
[b] *Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Genética, Ecologia e Evolução, Laboratório de Ecologia de Bentos, Av. Antônio Carlos 6627, CP 486, CEP 31270-901 Belo Horizonte, Minas Gerais, Brazil*
[c] *Universidade Federal do Pará, Instituto de Ciências Biológicas, Laboratório de Ecologia e Conservação, Av. Perimetral, 2-224, CEP 66077-830 Belém, Pará, Brazil*
[d] *Amnis Opes Institute, 2895 SE Glenn Street, Corvallis, OR 97333, USA*
[e] *Oregon State University, Department of Fisheries, Wildlife, & Conservation Sciences, Nash 104, Corvallis, OR 97331, USA*
[f] *Universidade Federal de Lavras, Departamento de Ecologia e Conservação, CEP 37200-900 Lavras, Minas Gerais, Brazil*

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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

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* Corresponding author.
E-mail addresses: larissa.silva9@estudante.ufla.br (L.F.R. Silva), diegobioufla@gmail.com (D.M.P. Castro), leandrojuen@ufpa.br (L. Juen), callistom@ufmg.br (M. Callisto), hughes.bob@amnisopes.com (R.M. Hughes), marcelhermes@ufla.br (M.G. Hermes).

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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 m^2 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.

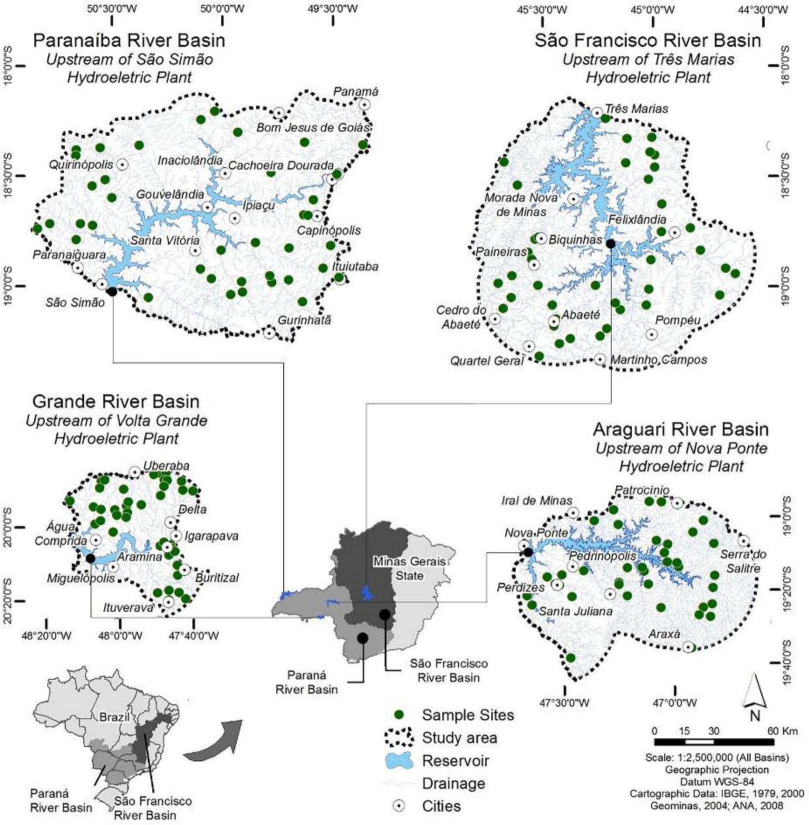

Fig. 1. Locations of sample sites in four hydrological units in the neotropical savanna, Brazil.

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 ($CDI = 4 \times \%$ urban $+ 2 \times \%$ 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

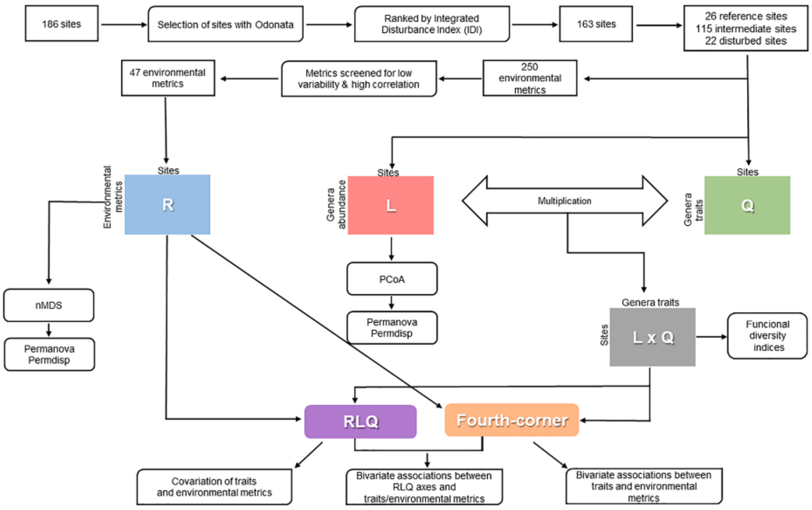

Fig. 2. Schematic diagram presenting the methodological design of site selection and statistical analyses used in this study. R = an environmental characteristics table, L = a taxa abundance table, Q = a traits table.

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

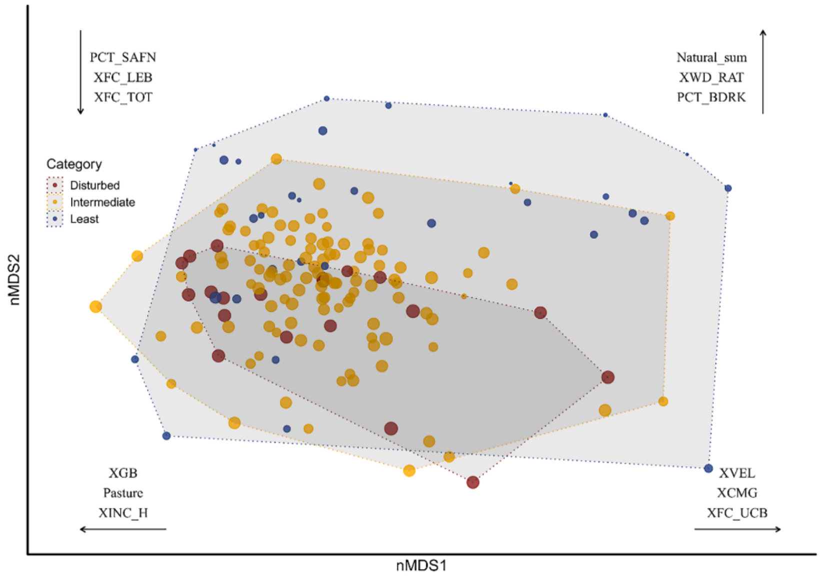

Fig. 3. Non-metric Multidimensional Scaling (nMDS) ordination of environmental variables (PCT_SAFN: sand + fine sediments , XFC_LEB: mean leaf litter cover, XFC_TOT: mean total fish cover, natural_sum: sum of natural catchment cover, XWD_RAT: mean of width/depth ratio, PCT_BDRK: % bedrock, XGB: mean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

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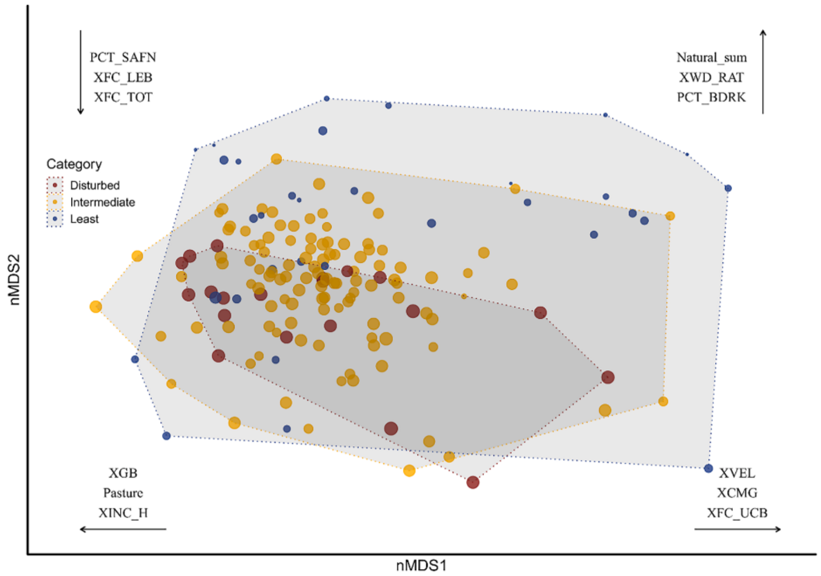

Fig. 3. Non-metric Multidimensional Scaling (nMDS) ordination of environmental variables (PCT_SAFN: sand + fine sediments , XFC_LEB: mean leaf litter cover, XFC_TOT: mean total fish cover, natural_sum: sum of natural catchment cover, XWD_RAT: mean of width/depth ratio, PCT_BDRK: % bedrock, XGB: mean 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 $\chi^2 = 0.33522$, $p = 0.8457$, diversity $\chi^2 = 1.4451$, $p = 0.4855$, evenness $\chi^2 = 0.35127$, $p = 0.8389$, dispersion $\chi^2 = 0.55284$, $p = 0.7585$, specialization $\chi^2 = 1.7584$, $p = 0.4151$, originality $\chi^2 = 0.42996$, $p = 0.8066$, 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

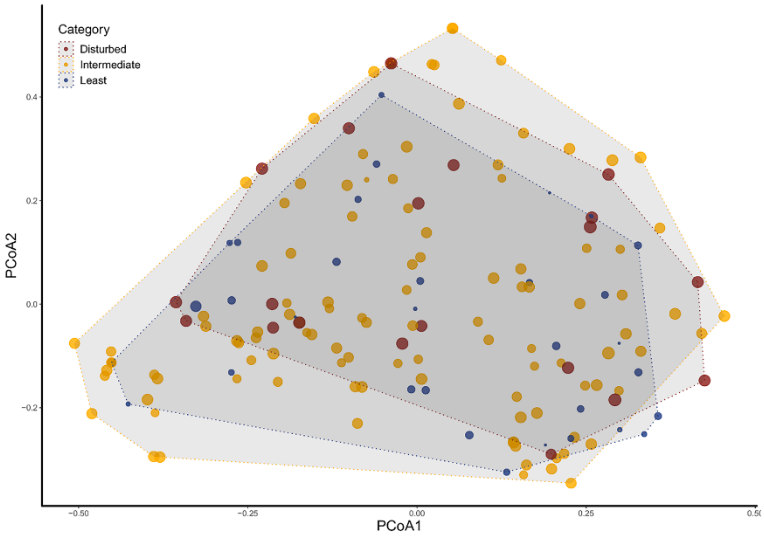

Fig. 4. Principal Coordinate Analysis (PCoA) of the composition of genera.

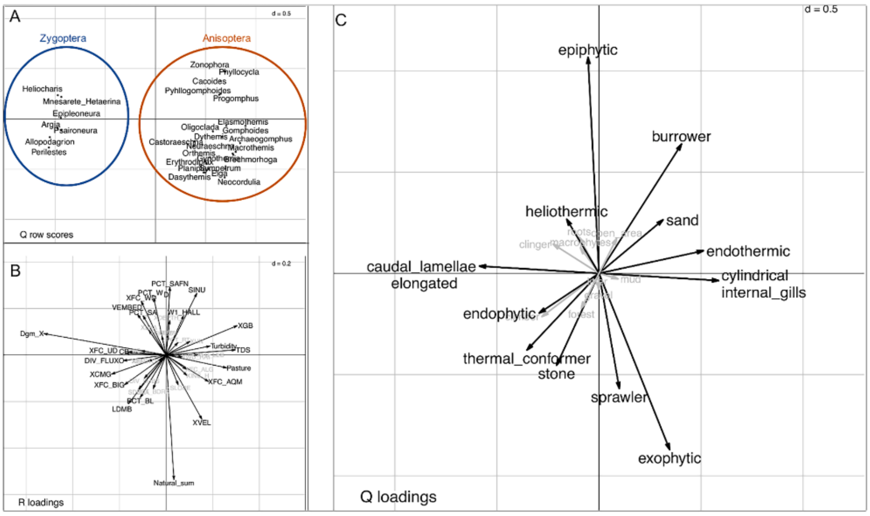

Fig. 5. Axis 1 and axis 2 of the RLQ analysis of 186 sites in the neotropical savanna. (A) larval Odonata scores; (B) environmental variable scores; (C) trait scores. (D) indicates figure grid scale.

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*,

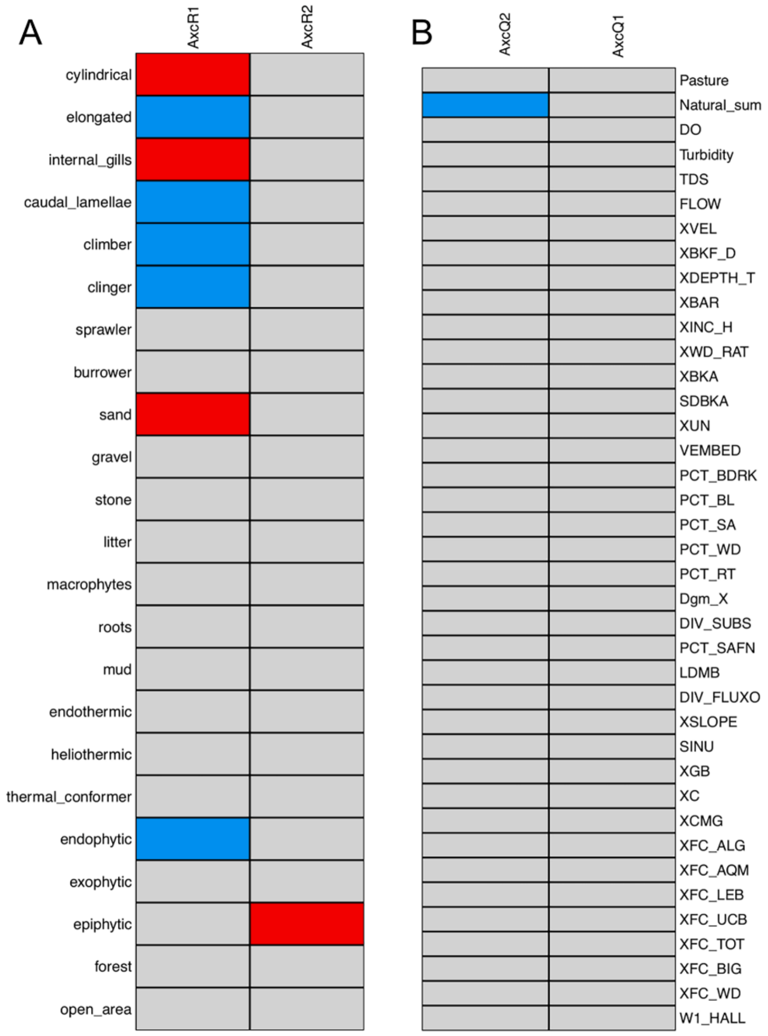

Fig. 6. Significant relationships (P-adjusted < 0.05) between (A) the RLQ environmental axes and individual traits and (B) between the RLQ trait axes and environmental variables. Red indicates positive correlations between factors and blue negative correlations. Non-significant relationships are labeled 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.,

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8 ARTICLE 3

Ecological thresholds of Odonata larvae to anthropogenic disturbances in Neotropical Savanna headwater streams

Larissa F. R. Silva[1], Diego M. P. Castro[2], Leandro Juen[3], Marcos Callisto[2], Robert M. Hughes[4,5], Marcel G. Hermes[1]

[1] Universidade Federal de Lavras, Setor de Zoologia Comparada, Departamento de Biologia, Laboratório de Sistemática e Biologia de Insetos, CEP 37200-900, Lavras, Minas Gerais, Brazil, larissamg05@hotmail.com, +55 (35) 988676926; marcelhermes@ufla.br

[2] Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Genética, Ecologia e Evolução, Laboratório de Ecologia de Bentos, Av. Antônio Carlos 6627, CP 486, CEP 31270-901, Belo Horizonte, Minas Gerais, Brazil, diegobioufla@gmail.com; callistom@ufmg.br

[3] Universidade Federal do Pará, Instituto de Ciências Biológicas, Laboratório de Ecologia e Conservação, Av. Perimetral, 2-224, CEP 66077-830, Belém, Pará, Brazil, leandrojuen@ufpa.br

[4]Amnis Opes Institute, 2895 SE Glenn Street, Corvallis, Oregon, 97333, USA

[5]Oregon State University, Department of Fisheries, Wildlife, & Conservation Sciences, Nash 104, Corvallis, Oregon, 97331, USA, hughes.bob@amnisopes.com

*Corresponding author: larissamg05@hotmail.com

Abstract

Odonata is an efficient tool for assessing and monitoring anthropogenic disturbances in aquatic and terrestrial ecosystems. However, information about how anthropogenic disturbances affect the ecological thresholds of Odonata is still lacking, especially for larvae. Ecological thresholds are defined here as an increase or decrease in occurrence frequency or abundance of genera of Odonata larvae. We evaluated whether ecological thresholds could be detected along gradients of spatial scales of different disturbances using larvae Odonata genera. We hypothesized that genera larvae of Zygoptera would have narrower ecological thresholds in response to an increase in streams disturbance levels and would be considered sensitive to anthropogenic disturbances. On the other hand, we hypothesized the opposite for genera larvae of Anisoptera, which would have wider ecological thresholds and would be considered tolerant to anthropogenic disturbances. We sampled 30 larvae Odonata genera at 186 headwater stream sites in the Neotropical Savanna – Brazilian Cerrado. To assess gradients of spatial scales of different disturbances we used three indexes, Integrated Disturbance Index, Catchment Disturbance Index, and Local Disturbance Index. We used Threshold Indicator Taxa Analysis (TITAN) to assess larval Odonata ecological thresholds and classified Odonata genera as sensitive or tolerant. TITAN detected ecological thresholds in seven Odonata genera (*Argia*, *Brechmorhoga*, *Cacoides*, *Gomphoides*, *Phyllocycla*, *Progomphus*, *Mnesarete/Hetaerina*) revealing them as robust bioindicators (purity and reliability ≥ 0.85). We expected that only genera larvae of Zygoptera would be considered sensitive, while genera larvae of Anisoptera would be considered tolerant. However, TITAN classified *Brechemorhoga* (Anisoptera: Libellulidae) as sensitive. The other genera belonging to Anisoptera, *Cacoides* (Anisoptera: Gomphidae), *Phyllocycla* (Anisoptera: Gomphidae), *Progomphus* (Anisoptera: Gomphidae), were considered tolerant as expected. Despite there being a certain pattern of Odonata response at the suborder level, Zygoptera larvae are associated with less-disturbed streams and Anisoptera larvae are associated with more-disturbed streams, there are some genera that do not correspond to this pattern. In this way, both Odonata suborders included sensitive and tolerant genera that can be used to detect and monitor changes in environmental conditions.

Keywords: Anisoptera**,** ecological responses, environmental gradients, TITAN, Zygoptera.

1. Introduction

Freshwater ecosystems provide a wide range of ecosystem services for human well-being, including water consumption, sanitation, irrigation, and hydropower (Faghihinia et al., 2021; Maasri et al., 2021). Nevertheless, human activities such as overexploitation of freshwater environments, land use for agriculture and pasture, domestic and industrial pollution, among others, have changed and degraded aquatic ecosystems worldwide (Dudgeon, 2010; Higgins et al., 2021; Reid et al., 2019). Moreover, the activities mentioned drive increased biodiversity losses in freshwater ecosystems, almost four times greater than in terrestrial ecosystems (Reid et al., 2019). In streams, specifically, due to hydrological and morphological factors, there is a synergetic effect of these activities, which magnifies their potential impacts and requires more comprehensive management solutions and conservation efforts (Birk et al., 2020).

Ecological thresholds are points or zones where changes in an environmental condition to another can be observed (Huggett, 2005), resulting in marked changes in biological assemblages, such as a sharp increase or decrease in occurrence frequency or abundance of taxa (Baker and King, 2010). Knowledge of ecological thresholds can be useful for evaluating the effects of anthropogenic changes in environmental gradients, mainly because these new gradients may represent new environmental conditions outside those experienced by species in evolutionary time. So, sensitive species with narrow ecological thresholds may not survive, whereas tolerant species with wide ecological thresholds tend to increase or predominate, altering the assemblage's composition (Baker and King, 2010). Knowing the ecological thresholds of species allows the detection of change points before they become irreversible, facilitating possible interventions (e.g., stream rehabilitation, river basin conservation) and establishing compensatory or mitigating measures (Baker and King, 2010; Huggett, 2005; King and Baker, 2014).

Increased human activities can cause nonlinear changes in aquatic communities because of species ecological thresholds along environmental gradients (Baker and King, 2010). Such changes have been reported for fish (e.g. Brejão et al., 2018; Cantanhêde et al., 2021; Dala-Corte et al., 2020; Martins et al., 2021) and macroinvertebrate (e.g. Castro et al., 2018; Firmiano et al., 2021; Giehl et al., 2019; Guterres et al., 2021; Mendes et al., 2019, 2017) assemblages. However, information gaps persist regarding the ecological processes that drive these nonlinear responses. This is because the ecological thresholds of most aquatic insect species or assemblages are either poorly understood or have still to be identified (Giehl et al., 2019), as is the case for Odonata (Gómez-Tolosa et al., 2021).

The order of insects Odonata, commonly known as dragonflies and damselflies, are efficient tools for assessing and monitoring anthropogenic disturbances in aquatic and terrestrial ecosystems (Corbet, 1980; Mendes et al., 2017; Miguel et al., 2017; Oliveira-Junior and Juen, 2019; Silva et al., 2021a, 2021b). Odonata adults have been reported responsive to deforestation and siltation (Dalzochio et al., 2018; Mendes et al., 2020, 2019), urbanization (Brito et al., 2021; Monteiro Júnior et al., 2015; Sganzerla et al., 2021), land-use changes (Calvão et al., 2018; Carvalho et al., 2021; Resende et al., 2021; Ribeiro et al., 2022), climate change (Gómez-Tolosa et al., 2021), and hydropower plant construction (Klein et al., 2018). Over the past twenty years, studies about the use of Odonata in assessing environmental impacts, mainly in the Neotropics, have been sharply increased (Gómez-Tolosa et al., 2021). However, information on the ecological thresholds of Odonata species is still lacking (Gómez-Tolosa et al., 2021), especially for larvae. There is information about ecological thresholds related to the loss of riparian vegetation cover and physical habitat conditions described for neotropical streams only for adults (Rodrigues et al., 2016; Mendoza-Penagos et al. 2021).

Due to the morphological, physiological, and even behavioral differences between the larvae of the two suborders, Zygoptera and Anisoptera, Odonata constitutes an interesting group to study ecological thresholds. For example, Zygoptera larvae have more delicate and elongated bodies and breathe through delicate external caudal lamellae at the end of their abdomens (Neiss and Hamada, 2014). Regarding behavior, they can be sprawlers, climbers, burrowers, clingers, or swimmers, which are associated with their preference for substrate, usually gravel, stones, and/or vegetation, such as leaves, macrophytes, and roots (Assis et al., 2004; Carvalho and Nessimian, 1998). These characteristics give Zygoptera larvae a greater environmental specificity, which makes them more sensitive to changes in the aquatic environment (Silva et al., 2021a). On the other hand, Anisoptera larvae have more robust and cylindrical bodies and breathe through rectal tracheal gills in the rectum (Neiss and Hamada, 2014), and often bury themselves in sediments, such as organic debris, sand, or mud (Assis et al., 2004; Carvalho and Nessimian, 1998). So, they are often more tolerant of changes in the aquatic environment (Silva et al., 2021a).

Based on the antagonism between the larvae suborders of Odonata, which will likely reflect in different ecological thresholds, we aimed to identify those thresholds along gradients of spatial scales of different disturbances. We hypothesized that genera larvae of Zygoptera would have narrower ecological thresholds in response increase in streams disturbance levels and would be considered sensitive to anthropogenic disturbances. On the other hand, we hypothesized the opposite for genera larvae of Anisoptera, which would have wider ecological thresholds and would be considered tolerant to anthropogenic disturbances.

2. Methods

2.1 Study area

We sampled 186 headwater stream sites from 1st to 3rd order (Strahler, 1957) in the Neotropical Savanna (Brazilian Cerrado biome). Of these, 160 stream sites were located in the Nova Ponte, Volta Grande, São Simão, and Três Marias hydrological units (drainage areas within 35 km upstream of four major hydropower reservoirs) (Figure 1). The sites exhibited a gradient of environmental conditions, from minimally disturbed sites with high dissolved oxygen and low nutrient concentrations, sites with moderate levels of human-altered land (pasture, row crops) to highly degraded urban sites with poor water quality and physical habitat conditions (Macedo et al., 2014a; Silva et al., 2017). The other 26 stream sites were located in Serra da Canastra National Park and Serra do Salitre, in Nova Ponte hydrological unit, to ensure that minimally disturbed sites were included in our study. Those sites differed by exhibiting minimal human impacts and the presence of native riparian vegetation (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.

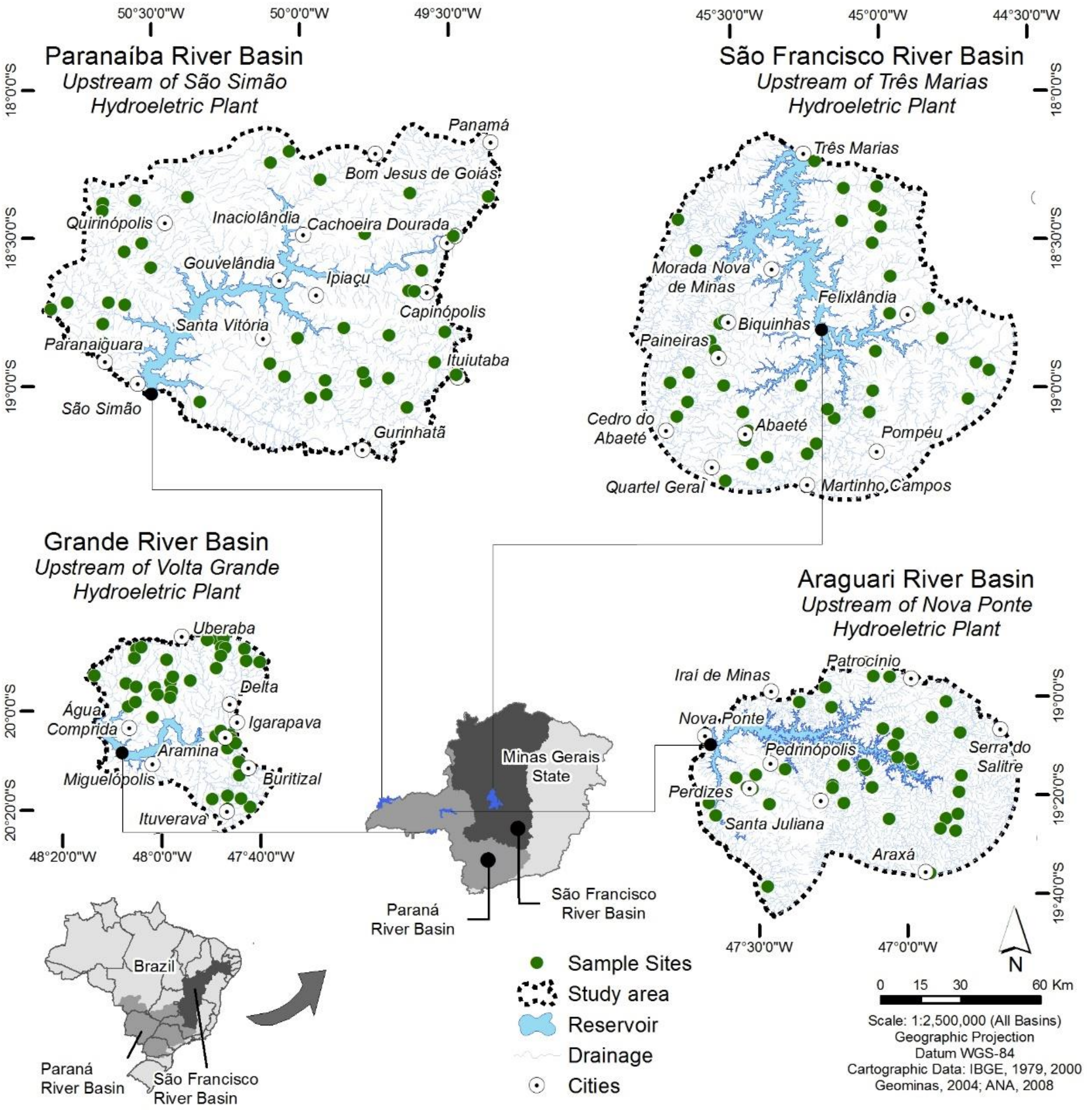


Figure 2:Sampled sites in four Brazilian Cerrado hydrological units, Brazil.

2.2 Sampling of Odonata larvae

In each hydrological unit, we sampled 40 stream sites previously selected following a probabilistic sampling design as described in Macedo et al. (2014b), according to the US Environmental Protection Agency (US-EPA) (Olsen and Peck, 2008). This procedure allows a spatially balanced probabilistic selection of sites that are representative of the region and has been successfully used in previous Neotropical Savanna studies (e.g., Agra et al., 2021; Callisto et al., 2019; Castro et al., 2018; Firmiano et al., 2021), as well as across the conterminous USA (Herlihy et al., 2020).

The length of each site was 40 times its mean wetted width, with a minimum of 150 m, and then divided into 11 equidistant transects. We sampled Odonata larvae with Surber type sampler (250 μm mesh, 0.09 m2 area) through kick-net method, following a zigzag trajectory across the transects (left, center, and right). The samples were washed, stored in 70% alcohol, processed, identified, and then deposited in the reference collection of the Institute of Biological Sciences at the Federal University of Minas Gerais (Callisto et al., 2019). Odonata larvae were identified under a stereoscopic microscope and using taxonomic keys (Costa et al., 2004; Neiss and Hamada, 2014; Pessacq et al., 2018) and taxonomic reviews available for each taxon. We identified larvae to genus-level because this taxonomic rank has been considered sufficient to evaluate the responses of Odonata larvae assemblages to anthropogenic disturbances in Brazilian streams (Dalzochio et al., 2018; Mendes et al., 2020; Oliveira-Junior and Juen, 2019; Pires et al., 2020a). In addition, studies show great congruence between responses of larvae species within a genus, since species of the same genus have morphological and ecological similarities. Reinforcing, therefore, that the resolution to genus level is reliable to assess environmental gradients (Mendes et al., 2017; Valente-Neto et al., 2016).

2.3 Environmental gradients

To assess along gradients of spatial scales, we calculated three indexes developed by Ligeiro et al. (2013): Integrated Disturbance Index (IDI), Catchment Disturbance Index (CDI), and Local Disturbance Index (LDI). The LDI is calculated from eleven observations of the presence and proximity of anthropogenic disturbances at the site and riparian vegetation (such as building; channel revetment; pavement; roads; pipes; trash and landfill; parks and lawns; row crop agriculture; pasture; logging and mining) (Kaufmann et al. In Review; Peck et al., 2006). The CDI is calculated by adding the %s of land use, weighted by the potential for degradation that each has on aquatic ecosystems (CDI = 4 x % urban + 2 x % agriculture + % pasture) (Ligeiro et al., 2013). The IDI is the Euclidian distance between the site and the origin of the disturbance plane formed by the LDI and CDI, applying the Pythagorean theorem (IDI = [(LDI/5)2 + (CDI/300)2]1/2) (Ligeiro et al., 2013).

2.4 Data analyses

To assess the ecological thresholds of larval Odonata genera, we performed Threshold Indicator Taxa Analysis (TITAN) (Baker and King, 2010) with each one index. The TITAN identifies abrupt changes in the frequency of occurrence and abundance over environmental condition gradients by combining change-point analysis (nCPA) and indicator species analysis (IndVal). TITAN responses are evaluated for purity and reliability, given by the IndVal scores at each change point by bootstrap and resampling. Purity corresponds to consistency in the response direction, and reliability to the frequency of a strong response magnitude. The results are standardized as Z scores, which determine the threshold at which the taxa exhibit strong positive (sum z+) or negative (sum z-) changes in their frequency of occurrence and abundance. The Z+ means the taxon was considered tolerant, and their frequency of occurrence and abundance increased with the environmental metric. While, the Z- means the taxon was considered sensitive, and their frequency of occurrence and abundance decreased with the environmental metric (Baker and King, 2010). Two criteria should be observed to compute z scores: include only taxa occurring in at least three sites and having abundances of at least five individuals. Although we sampled 186 sites and identified 30 genera, only 68 sites met those criteria. The association of taxa with environmental scores was tested using 1000 bootstraps and 100 permutations and was considered significant if the IndVal score was <0.05, and purity and reliability scores were >0.85. In addition, other studies (e.g., Giehl et al., 2019; Valente-Neto et al., 2021) have also used purity and reliability scores different from 0.95 and obtained satisfactory results with TITAN analysis. We performed TITAN analysis in Version 4.4.1 (R Core Development Team, 2016) with TITAN2 (Baker et al., 2020).

3 Results

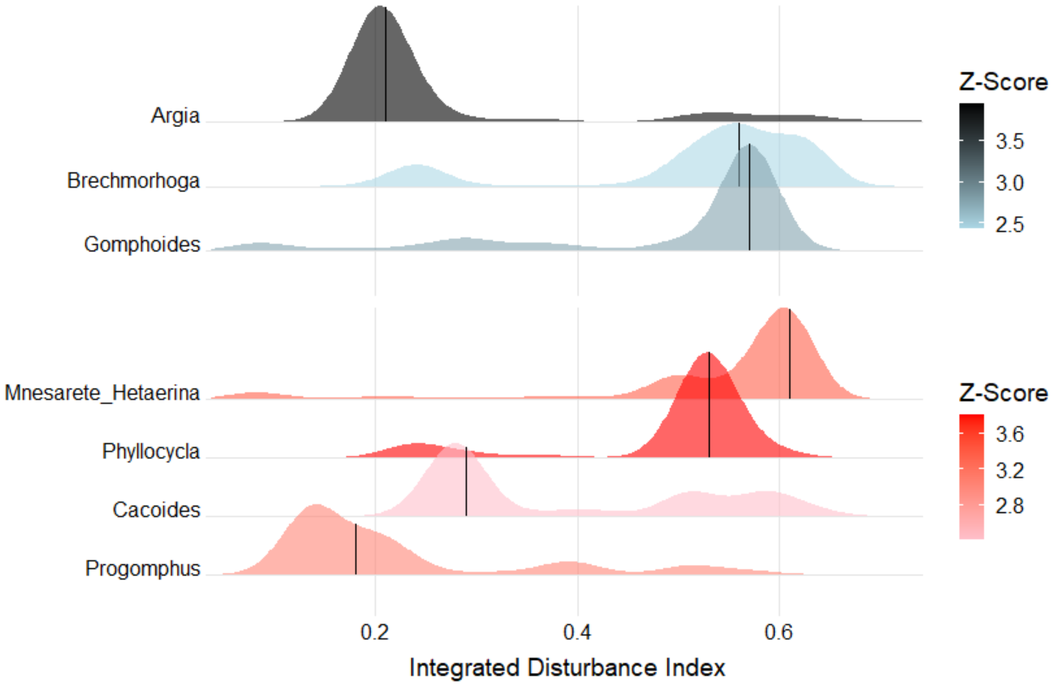
We sampled 3209 Odonata larvae, distributed among 30 genera and ten families. Of these, 1500 individuals, eight genera and six families belonged to Zygoptera; and 1709 individuals, 22 genera and four families were Anisoptera. Retaining only taxa with ≥3 occurrences and abundances ≥5, our larval Odonata data were reduced to 1320 individuals, four families (Calopterygidae, Coenagrionidae, Gomphidae and Libellulidae), and seven genera (*Argia*, *Brechmorhoga*, *Cacoides*, *Gomphoides*, *Phyllocycla*, *Progomphus*, *Mnesarete_Hetaerina*). These included two Zygoptera genera and five Anisoptera genera (Supplementary Material Table 2).

TITAN detected changes in ecological thresholds in seven larvae Odonata genera with purity and reliability ≥ 0.85 for these three indices (Table 1, Figure 2). Regarding their response to the IDI, *Brechmorhoga* and *Argia* were sensitive (Z-) and *Cacoides*, *Phyllocycla, Progomphus* and *Mnesarete/Hetaerina* tolerant (Z+). For CDI, *Argia* was sensitive (Z-) and *Cacoides*, *Phyllocycla* and *Progomphus* were tolerant (Z+). And for LDI, *Argia* was sensitive (Z-) and *Phyllocycla, Progomphus* and *Mnesarete/Hetaerina* were tolerant (Z+) (Table 1, Figure 2).

Table 1: Change points (zenv.cp), purity, and reliability of larvae Odonata genera in response to indexes related to disturbances gradients in Brazilian Cerrado stream sites. In bold, the genera with purity and reliability greater than 85%.

Indexes	Taxa	zenv.cp	maxgrp	obsiv.prob	purity	reliability
Integrated Disturbance Index (IDI)	*Cacoides*	**0.27**	**Z+**	**0.03**	**0.98**	**0.9**
	Gomphoides	**0.56**	**Z-**	**0.02**	**0.9**	**0.93**
	Phyllocycla	**0.52**	**Z+**	**0**	**0.99**	**0.95**
	Progomphus	**0.13**	**Z+**	**0.01**	**0.94**	**0.92**
	Brechmorhoga	**0.56**	**Z-**	**0.02**	**0.94**	**0.93**
	Mnesarete_Hetaerina	**0.6**	**Z+**	**0**	**0.97**	**0.97**
	Argia	**0.19**	**Z-**	**0**	**0.99**	**0.98**
Catchment Disturbance Index (CDI)	*Cacoides*	**153.06**	**Z+**	**0**	**0.99**	**0.94**
	Gomphoides	174.43	Z-	0.15	0.76	0.55
	Phyllocycla	**37.18**	**Z+**	**0.03**	**0.95**	**0.88**
	Progomphus	**51.71**	**Z+**	**0.03**	**0.94**	**0.91**
	Brechmorhoga	55.51	Z-	0.02	0.93	0.85
	Mnesarete_Hetaerina	133.7	Z+	0.05	0.85	0.76
	Argia	**22.39**	**Z-**	**0**	**0.98**	**0.97**
Local Disturbance Index (LDI)	*Cacoides*	1.12	Z-	0.15	0.65	0.49
	Gomphoides	0	Z-	0.07	0.76	0.55
	Phyllocycla	**1.74**	**Z+**	**0.01**	**0.95**	**0.94**
	Progomphus	**0.28**	**Z+**	**0.02**	**0.91**	**0.87**
	Brechmorhoga	0.73	Z-	0.02	0.77	0.83
	Mnesarete_Hetaerina	**0.61**	**Z+**	**0.03**	**0.95**	**0.89**

	Argia	**0.68**	**Z-**	**0**	**0.94**	**0.96**


Argia
Brechmorhoga
Gomphoides
Mnesarete_Hetaerina
Phyllocycla
Cacoides
Progomphus
0.2
0.4
0.6
Integrated Disturbance Index
Z-Score
3.5
3.0
2.5
Z-Score
3.6
3.2
2.8

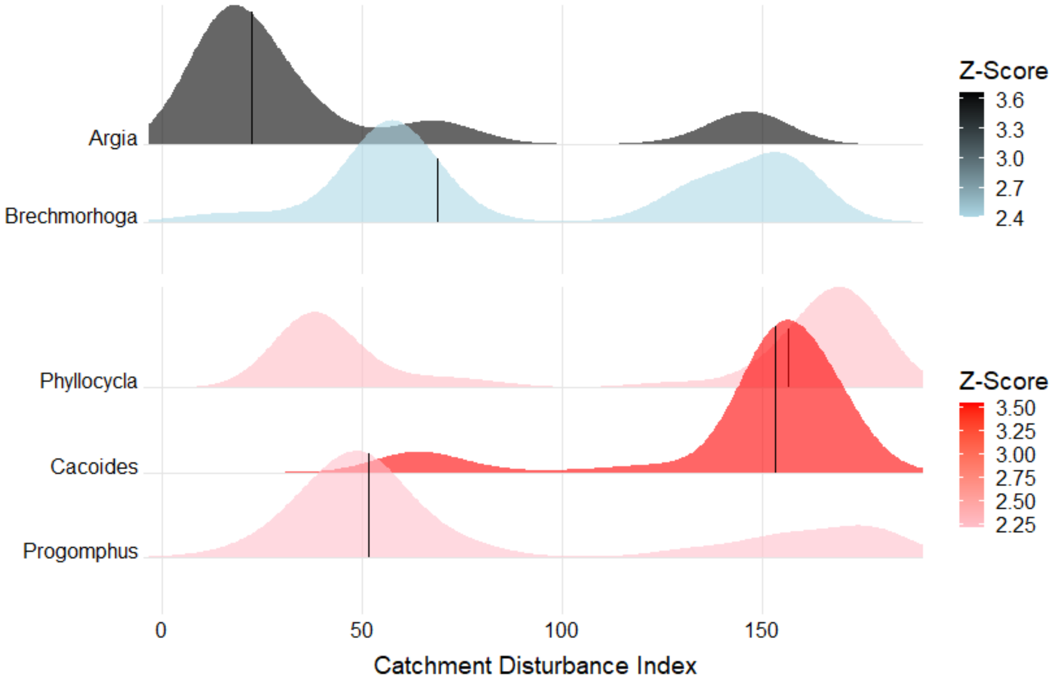

Argia
Brechmorhoga
Phyllocycla
Cacoides
Progomphus
0
50
100
150
Catchment Disturbance Index
Z-Score
3.6
3.3
3.0
2.7
2.4
Z-Score
3.50
3.25
3.00
2.75
2.50
2.25

Figure 3: Genera change points of genera identified by TITAN in response to indexes related to disturbances gradients in Brazilian Cerrado stream sites. Z score indicates the maximum genus change point. Blue or gray indicates declining (Z−) and red indicates increasing (Z+) abundance and frequency of occurrence of Odonata's genera relative to indexes IDI, CDI, and LDI.

4 Discussion

41. Response of larvae Odonata genera to TITAN

We identified ecological thresholds for seven genera of Odonata larvae along gradients of spatial scales of different disturbances in Neotropical Savanna headwater stream sites. However, our hypothesis was partially corroborated. We expected that genera larvae of Zygoptera would be considered sensitive, while genera larvae of Anisoptera would be considered tolerant. However, TITAN classified *Brechemorhoga* (Anisoptera: Libellulidae) as sensitive and for robust reasons. The other genera belonging to Anisoptera, *Cacoides* (Anisoptera: Gomphidae), *Phyllocycla* (Anisoptera: Gomphidae), *Progomphus* (Anisoptera: Gomphidae), were considered tolerant as expected. Despite there being a certain pattern of Odonata response at the suborder level, Zygoptera larvae are associated with less-disturbed streams and Anisoptera larvae are associated with more-disturbed streams (Mendes et al., 2015; Silva et al., 2021b, 2021a), there are some genera that do not correspond to this pattern, as found in this study. In this way, both Odonata suborders included sensitive and tolerant genera that can be used to detect and monitor changes in environmental conditions.

Regarding the anthropogenic disturbance indexes (IDI, CDI, LDI), the higher the IDI score of a site, the more it differs from the ideal reference condition in relation to no disturbance inside the stream channel, in the riparian zone, and in the catchment (Ligeiro et al., 2013). Consequently, genera sensitive to theses indexes can be considered as being associated with less-disturbed streams. *Brechmorhoga* larvae generally occur in stream substrates formed by rock or stone, shading, and moving water (Assis et al., 2004; Pires et al., 2020a, 2020b), conditions of least-disturbed streams. These requirements are associated with their morphology, which is a flattened body dorsoventrally with the dorsal spines of the abdomen developed as a keel, which facilitate their adherence and occurrence in areas with riffles (Assis et al., 2004; Pires et al., 2020a). Thus, we assumed that *Brechmorhoga* larvae might indicate less disturbed streams, at least in neotropical headwater streams.

Despite *Argia* also was considered sensitive to IDI, *Argia* larvae have already been registered in areas with different vegetation types (Assis et al., 2004; Mendes et al., 2019; Pires et al., 2020a), even in areas of oil palm trees (Mendes et al., 2019). Adults responded negatively to forest loss (Brito et al., 2020; Carvalho et al., 2018; Rodrigues et al., 2016), but were also found in deforested (Brito et al., 2021; Juen et al., 2014) and altered environments (Carvalho et al., 2021; Pereira-Moura et al., 2021). *Argia* is one of the most speciose genera among the Coenagrionidae, with over 130 described species worldwide and almost 50 occurring in Brazil (Vilela et al., 2018). These different species probably have different ecological requirements, which explains their occurrences across several types of aquatic environments (Pires et al., 2020a; Rodrigues et al., 2016). Thus, we suggest that, in this case, genus-level taxonomy should be used with caution in evaluating the effects of anthropogenic changes in aquatic environments.

On the other hand, genera tolerant to these indexes (IDI, CDI, LDI), can be considered as being associated with more-disturbed streams, such as the genera of Gomphidae, *Cacoides*, *Phyllocycla*, and *Progomphus.* Larvae of those genera are burrowers and commonly associated with streambed sand and fine sediments (Assis et al., 2004; Carvalho and Nessimian, 1998), and narrower widths of riparian vegetation (< 0.5m for *Cacoides* and 15-5m for *Phyllocycla* and *Progomphus*) (Pires et al., 2020a). Thus, streams where riparian vegetation was removed or reduced, facilitating sediment inputs, tend to benefit those genera, which might be associated with more-disturbed streams. However, *Phyllocycla* larval ecology is still poorly known, with gaps mostly in how they respond to anthropogenic disturbances (Araújo et al., 2020), as well as *Cacoides* and *Progomphus*. In fact, adults of genera belonging to

Gomphidae also were not associated with changes in environmental integrity (Mendoza-Penagos et al., 2021) which be related to the difficulty to sample adults or their ecological requirements.

42. Response of Odonata larvae to anthropogenic disturbance

Similar to our findings, other studies with Odonata larvae demonstrated relationships to anthropogenic disturbances (Mendes et al., 2017; Ribeiro et al., 2022; Valente-Neto et al., 2016), particularly reduced riparian vegetation (Mendes et al., 2021; Pires et al., 2020a; Silva et al., 2021b). Odonata larvae, mostly Zygoptera, show narrower ecological thresholds in relation to environmental changes due to anthropogenic pressures (e.g. reduced or removal of riparian vegetation Mendes et al., 2015; Pires et al., 2020a; Silva et al., 2021b, higher water quality Mendes et al., 2018; Silva et al., 2021a). Tolerant genera tend to become more abundant, whereas sensitive genera disappear. As a result, taxonomic and functional diversities decrease until the assemblage becomes homogeneous, losing ecological functions (Mendes et al., 2019; Pires et al., 2021). Because Odonata larvae are predators, changes result in reduced predation rates (Corbet, 1980).

43. Sampled size X TITAN

Despite 30 genera sampled, we identified ecological thresholds for seven. The low abundances, expected for rarer genera, affect the accuracy of genera abundance estimates in the local pool available for TITAN (Valente-Neto et al., 2021). In addition, neotropical ecosystems have high environmental complexity and beta diversity, so it is impossible to choose a single genus that works as a good indicator across a large geographical region and multiple river basins (Firmiano et al., 2021; Gómez-Tolosa et al., 2021). Caution must be employed when generalizing the ecological thresholds of one genus to a range of other genera (Huggett, 2005), even in the same suborder, because they are all unlikely to respond similarly. Therefore, we suggest using as many as possible of these larval Odonata genera as indicators of anthropogenic disturbances.

5 Conclusions and management implications

Ours is the first use of TITAN for assessing ecological thresholds for larvae Odonata in the neotropical region (Gómez-Tolosa et al., 2021). However, limitations to the knowledge of the ecological requirements of larval Odonata remain. We highlight the need for greater sampling effort to detect relatively rare taxa (Pompeu et al., 2021) and assess other Odonata genera and their ecological thresholds. Furthermore, it would be useful to determine ecological thresholds for Odonata genera/species, which requires greater taxonomic knowledge.

Our study provides information about the ecological thresholds of seven larvae Odonata genera. We highlight that *Argia* (Zygoptera: Coenagrionidae) and *Brechmorhoga* (Anisoptera: Libellulidae) were considered sensitive to local and regional anthropogenic disturbances, while *Cacoides* (Anisoptera: Gomphidae), *Phyllocycla* (Anisoptera: Gomphidae), *Progomphus* (Anisoptera: Gomphidae) were considered tolerant. Despite there being a certain pattern of Odonata response at the suborder level, some genera do not correspond to this pattern, which reinforces the importance of identification at the lowest possible taxonomic level.

In summary, both Odonata suborders, Zygoptera and Anisoptera, include sensitive and tolerant genera that can be used to assess and monitor the effects of anthropogenic disturbances on freshwater streams, as we demonstrated. Ecological thresholds information about Odonata larvae may be useful in the future in simplified protocols for monitoring aquatic environments, in addition to being used for complementing or integrating taxonomic and functional approaches.

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