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Ecological thresholds of Odonata larvae to anthropogenic disturbances in neotropical savanna headwater streams

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Abstract We evaluated whether ecological thresholds could be detected along gradients of disturbances by using larval Odonata genera. Morphological, physiological, and behavioral differences between Odonata suborders may be reflected in different thresholds to anthropogenic disturbance. Therefore, we hypothesized that larval Zygoptera genera would have narrower ecological thresholds in response to increased levels of stream disturbance and would be considered sensitive to anthropogenic disturbances, and the opposite for larval Anisoptera genera, which would have wider ecological thresholds and would be considered tolerant to anthropogenic disturbances. We assessed 30 larval Odonata genera collected from 186 headwater stream sites in the Neotropical Savanna. Threshold Indicator Taxa Analysis detected ecological thresholds in seven Odonata genera (*Argia*, *Brechmorhoga*, *Cacoides*, *Gomphoides*, *Phyllocycla*, *Progomphus and Hetaerina*) revealing them as robust bioindicators (purity and reliability ≥ 0.85). Most Zygoptera were associated with less-disturbed sites and most Anisoptera were associated with

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more-disturbed sites, but not all genera corresponded to this pattern. Therefore, we recommend using Odonata larvae at the genus-level, versus the suborder level, for constructing improved biomonitoring tools and obtaining more accurate impact assessments of neotropical stream sites.

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 degraded aquatic ecosystems worldwide (Dudgeon, 2010; Reid et al., 2019; Higgins et al., 2021). Moreover, such activities decreased biodiversity in freshwater ecosystems, almost four times more than in terrestrial ecosystems (Reid et al., 2019). In streams, because of interacting hydrological and morphological factors, there is an additive synergistic effect of those activities, which magnifies their potential individual impacts and requires more comprehensive management solutions and conservation efforts (Birk et al., 2020; Herlihy et al., 2020).

For example, ecological thresholds are points or zones where changes in an environmental condition can be observed (Huggett, 2005), and result in marked changes in biological assemblages, such as a sharp increase or decrease (Baker & King, 2010) in frequency of occurrence or abundance of taxa. Knowledge of ecological thresholds can help us evaluate the effects of anthropogenic changes in natural ecosystems, mainly because the disturbed areas may represent new environmental conditions outside of 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 assemblage composition (Baker & King, 2010). Knowing the ecological thresholds of species aids us in detecting change points before they become irreversible, facilitating possible interventions (e.g.,

stream rehabilitation, river basin conservation) and establishing compensatory or mitigating measures (Huggett, 2005; Baker & King, 2010; King & Baker, 2014).

Increased human activities can cause nonlinear changes in aquatic communities because of different ecological thresholds of each species along environmental gradients (Baker & King, 2010). Such changes have been reported for neotropical fish (Brejão et al., 2018; Dala-Corte et al., 2020; Cantanhêde et al., 2021; Martins et al., 2021) and macroinvertebrate assemblages (Mendes et al., 2017, 2019; Castro et al., 2018; Giehl et al., 2019; Firmiano et al., 2021; Guterres et al., 2021). However, information gaps persist regarding the ecological processes that drive these nonlinear responses. This is because the ecological thresholds of most aquatic insect species 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 Odonata order of insects, commonly known as dragonflies and damselflies, are efficient tools for assessing and monitoring anthropogenic disturbances in terrestrial and aquatic ecosystems (Corbet, 1980; Mendes et al., 2017; Miguel et al., 2017; Oliveira-Junior & Juen, 2019; Silva et al., 2021a, b). Odonata adults have been reported responsive to deforestation and siltation (Dalzochio et al., 2018; Mendes et al., 2019, 2020), urbanization (Monteiro Júnior et al., 2015; Brito et al., 2021; Sganzerla et al., 2021), land-use changes (Samyways & Steytler, 1996; Calvão et al., 2018; Carvalho et al., 2021; Dolný et al., 2021; Ishak et al., 2021; Resende et al., 2021; Manu et al., 2022; Ribeiro et al., 2022), climate change (Gómez-Tolosa et al., 2021), and hydropower plant construction (Klein et al., 2018). 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). Over the past twenty years, studies about the use of neotropical Odonata in assessing environmental impacts have 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. Information about ecological thresholds in neotropical streams related to the loss of riparian vegetation cover and physical habitat conditions is available only

for Odonata adults (Rodrigues et al., 2016; Mendoza-Penagos et al., 2021).

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

Based on reported differences between Odonata suborders, which may reflect different ecological thresholds, we aimed to identify those thresholds along disturbance gradients measured at multiple spatial extents. We hypothesized that larval Zygoptera genera would have narrower ecological thresholds in response to increased levels of stream disturbance and would be considered sensitive to anthropogenic disturbances. On the other hand, we hypothesized the opposite for larval Anisoptera genera, which would have wider ecological thresholds and would be considered tolerant to anthropogenic disturbances.

Methods

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) (Fig. 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).

Sampling of Odonata larvae

We sampled once in each hydrological unit 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. In each hydrological unit, we sampled 40 stream sites previously selected following a probabilistic sampling design detailed in Macedo et al. (2014b). We used a GRTS (Generalized Random-Tessellation Stratified) approach, whereby sites were selected randomly but spatially balanced to minimize their proximity. This approach is based on converting all stream segments in a two-dimensional drainage network into a one-dimensional line from which sites were selected (Stevens & Olsen, 2004). Sites were selected in R (R Core Development Team, 2016) by using the *Spsurvey* library (Kincaid, 2009) with a minimum distance of 1 km between them, and by ensuring approximately equal numbers of first, second, and third-order sites. 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 (Castro et al., 2018; Callisto et al., 2019; Agra et al., 2021; Firmiano et al., 2021), as well as across the USA (Herlihy et al., 2020). Probabilistic study designs allow samples from a relatively small number of sites to be used for inferring results to the entire sampled stream network (e.g., Jimenez-Valencia, 2014; Silva et al., 2017, 2014; Martins et al., 2021).

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 a Surber type sampler (250 μm mesh, 0.09 m² area), following a zigzag trajectory across the transects (left, center, and right). In the laboratory, the samples were washed and the sorted organisms were stored in 70% alcohol, identified, and then deposited in the reference collection of the Institute of Biological Sciences at the Federal University of Minas Gerais (Callisto et al., 2021). Odonata larvae were identified under a stereoscopic microscope and using taxonomic keys (Costa et al., 2004; Neiss & Hamada, 2014; Pessacq et al., 2018) and taxonomic reviews available for each taxon. We identified larvae to genus because this taxonomic rank has been considered sufficient to evaluate the responses of Odonata larvae to anthropogenic disturbances in Brazilian streams (Dalzochio et al., 2018; Oliveira-Junior & Juen, 2019; Mendes et al., 2020; Pires et al., 2020a).

Environmental gradients

To assess disturbance gradients at multiple spatial extents, we calculated three indices developed by Ligeiro et al. (2013): Local Disturbance Index (LDI), Catchment Disturbance Index (CDI), and Integrated Disturbance Index (IDI). The LDI is calculated based on eleven observations assessing the presence and proximity of anthropogenic disturbances at the site and riparian vegetation (such as buildings; channel revetment; pavement; roads; pipes; trash and landfill; parks and lawns; row crop agriculture; pasture; logging and mining) (Peck et al., 2006; Kaufmann et al., 2022). The CDI is calculated by adding the % of land use, weighted by the potential for degradation that each has on aquatic ecosystems ($CDI = 4 \times \%$ urban $+ 2 \times \%$ agriculture $+ \%$ pasture). 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 ($IDI = [(LDI/5)^2 + (CDI/300)^2]^{1/2}$. The LDI, CDI, and IDI scores increase with increasing disturbance (Ligeiro et al., 2013).

Data analyses

To assess the ecological thresholds of larval Odonata genera, we performed a Threshold Indicator Taxa Analysis (TITAN) (Baker & King, 2010) with each index, i.e., LDI, CDI and IDI. 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 of taxa exhibiting 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 its frequency of occurrence and abundance increased with the disturbance index score. On the other hand, the Z- means the taxon was considered sensitive, and its frequency of occurrence and abundance decreased with the disturbance index score (Baker & King, 2010). Two criteria should be observed to compute z scores: include only taxa occurring in at least three sites (i.e., occurrence) and having abundances of at least five individuals (i.e., abundance), to minimize uncertainty in change point estimates and ensure interpretable z scores, as recommended by Baker and King (2010). 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. Other studies (Giehl et al., 2019; Valente-Neto et al., 2021) have used reliability scores different from 0.95 and obtained satisfactory results with TITAN analysis. We performed TITAN analysis in Version 2.4.1 (R Core Development Team, 2016) with TITAN2 (Baker et al., 2020).

Results

Among the 186 stream sites sampled, we recorded Odonata larvae at 180. In total, we sampled 3209 Odonata larvae, belonging to 30 genera and ten families. Retaining only taxa that occurred in at least three sites and with at least 5 individuals, our Odonata larvae data were reduced to 1320 individuals in 68 stream sites. Those individuals occurred in four families (Calopterygidae, Coenagrionidae, Gomphidae and Libellulidae) and seven genera (*Argia*, *Brechmorhoga*, *Cacoides*, *Gomphoides*, *Phyllocycla*,

Progomphus, *Hetaerina*), including two Zygoptera genera and five Anisoptera genera.

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

Discussion

Titan results

We identified significant ecological thresholds for seven larval Odonata genera to disturbance gradients along three spatial extents in Neotropical Savanna headwater stream sites. Our hypothesis was

partially corroborated. We expected that all Zygoptera genera would be sensitive, but all Anisoptera genera would be tolerant. However, TITAN classified *Brechmorhoga* (Anisoptera: Libellulidae) as sensitive. The other genera belonging to Anisoptera, *Cacoides*, *Phyllocycla*, *Progomphus* (all belonging to Gomphidae), were considered tolerant, as expected. Generally, Zygoptera larvae are associated with less-disturbed sites and Anisoptera larvae are associated with more-disturbed sites (Mendes et al., 2015; Silva et al., 2021a, b; Ribeiro et al., 2022). However, some genera do not correspond to this pattern, as we found in this study, with both Odonata suborders including sensitive and tolerant genera that can be used to detect and monitor changes in environmental conditions.

Regarding the anthropogenic disturbance indices (IDI, CDI, LDI), the higher the 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 these indices can be considered as being associated with

Table 1 Change points (zenv.cp), purity, and reliability of larval Odonata genera in response to disturbance gradients in Brazilian Cerrado stream sites. In bold, the genera with purity and reliability greater than 85% and $p < 0.05$

| Indices	Genus (suborder)	zenv.cp	maxgrp	obsiv.prob	Purity	Reliability
Integrated Disturbance Index (IDI)	*Cacoides* (Anisoptera)	**0.27**	**Z+**	**0.03**	**0.98**	**0.9**
	Gomphoides (Anisoptera)	**0.56**	**Z-**	**0.02**	**0.9**	**0.93**
	Phyllocycla (Anisoptera)	**0.52**	**Z+**	**0**	**0.99**	**0.95**
	Progomphus (Anisoptera)	**0.13**	**Z+**	**0.01**	**0.94**	**0.92**
	Brechmorhoga (Anisoptera)	**0.56**	**Z-**	**0.02**	**0.94**	**0.93**
	Hetaerina (Zygoptera)	**0.6**	**Z+**	**0**	**0.97**	**0.97**
	Argia (Zygoptera)	**0.19**	**Z-**	**0**	**0.99**	**0.98**
Catchment Disturbance Index (CDI)	*Cacoides* (Anisoptera)	**153.06**	**Z+**	**0**	**0.99**	**0.94**
	Gomphoides (Anisoptera)	174.43	Z-	0.15	0.76	0.55
	Phyllocycla (Anisoptera)	**37.18**	**Z+**	**0.03**	**0.95**	**0.88**
	Progomphus (Anisoptera)	**51.71**	**Z+**	**0.03**	**0.94**	**0.91**
	Brechmorhoga (Anisoptera)	55.51	Z-	0.02	0.93	0.85
	Hetaerina (Zygoptera)	133.7	Z+	0.05	0.85	0.76
	Argia (Zygoptera)	**22.39**	**Z-**	**0**	**0.98**	**0.97**
Local Disturbance Index (LDI)	*Cacoides* (Anisoptera)	1.12	Z-	0.15	0.65	0.49
	Gomphoides (Anisoptera)	0	Z-	0.07	0.76	0.55
	Phyllocycla (Anisoptera)	**1.74**	**Z+**	**0.01**	**0.95**	**0.94**
	Progomphus (Anisoptera)	**0.28**	**Z+**	**0.02**	**0.91**	**0.87**
	Brechmorhoga (Anisoptera)	0.73	Z-	0.02	0.77	0.83
	Hetaerina (Zygoptera)	**0.61**	**Z+**	**0.03**	**0.95**	**0.89**
	Argia (Zygoptera)	**0.68**	**Z-**	**0**	**0.94**	**0.96**
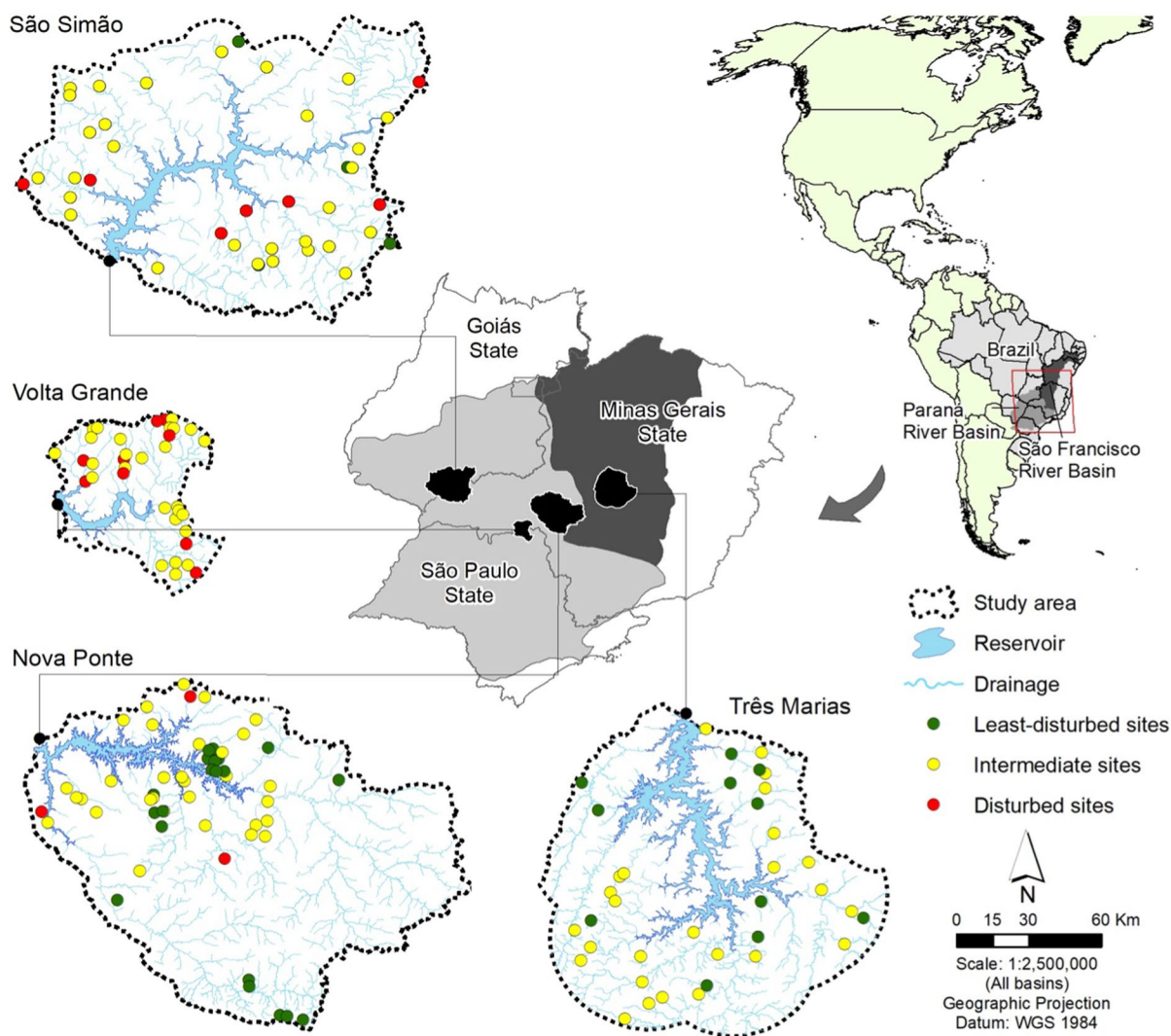
Fig. 1 Sampled sites in four Brazilian Cerrado hydrological units, Brazil. Green circles represent least-disturbed sites, yellow circles represent intermediately disturbed sites, and red circles represent disturbed sites, based on the Integrated Disturbance Index (IDI; Ligeiro et al., 2013)

less-disturbed streams. *Brechmorhoga* larvae generally occur in substrates formed by rock or stone, shading, and moving water (Assis et al., 2004; Pires et al., 2020a, b), conditions generally observed in least-disturbed streams. These requirements are associated with their larval morphology, which is a dorsoventrally flattened body 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 assume that *Brechmorhoga* larvae might indicate

least-disturbed conditions in neotropical headwater streams.

Although *Argia* was considered sensitive to the IDI, their 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 streams draining oil palm plantations (Mendes et al., 2019). Adults responded negatively to forest-loss (Rodrigues et al., 2016; Carvalho et al., 2018; Brito et al., 2020), but were also found in deforested (Juen et al., 2014; Brito et al., 2021) and altered

Fig. 2 Genera change points identified by TITAN in response to disturbance index scores 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 genera relative to disturbance indexes (IDI, CDI, and LDI)
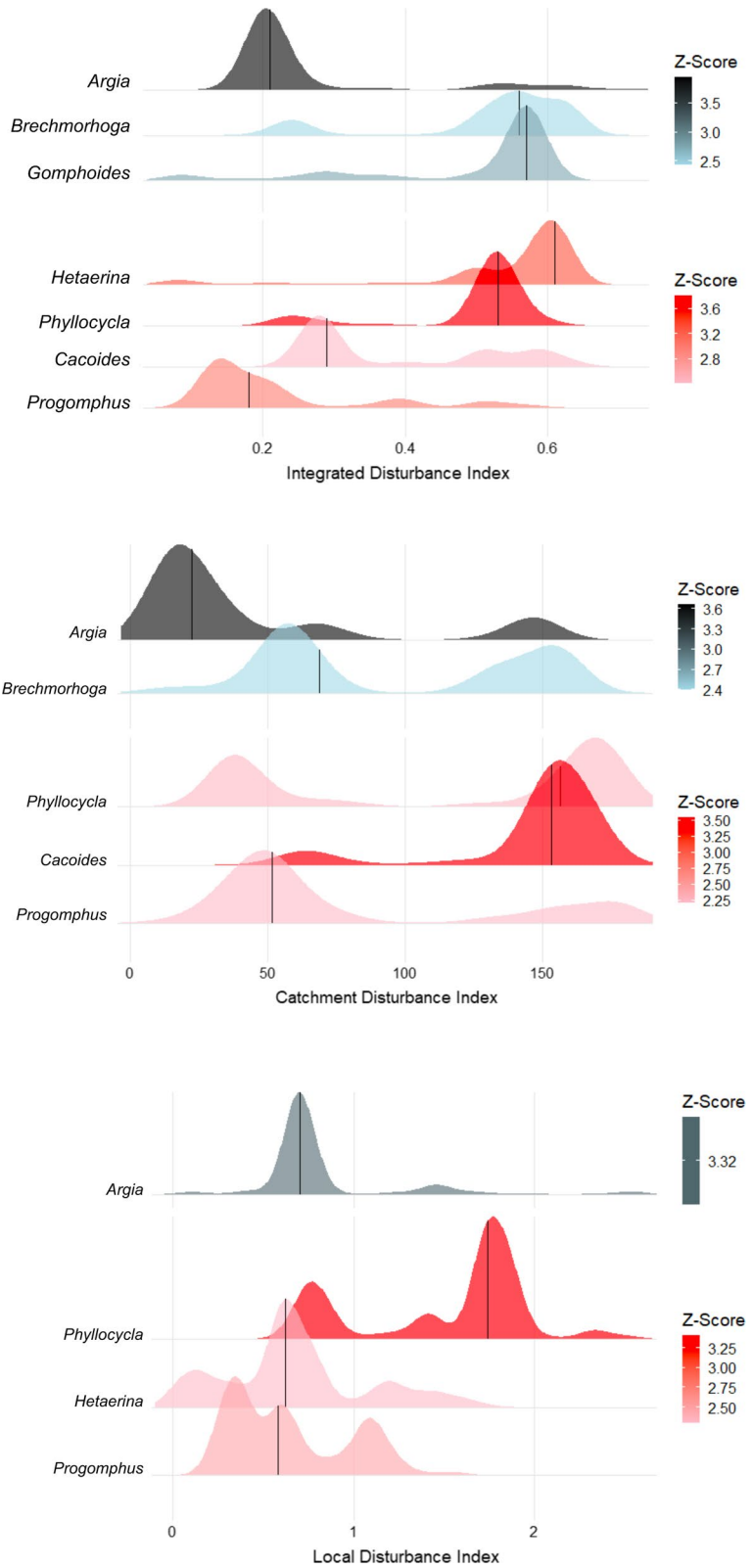
environments (Carvalho et al., 2021; Pereira-Moura et al., 2021). *Argia* is one of the most speciose genera among the Coenagrionidae, with over 130 described species worldwide and almost 50 occurring in Brazil (Vilela et al., 2018). These different species probably have different ecological requirements, which explains their occurrences across several types of aquatic environments (Rodrigues et al., 2016; Pires et al., 2020a). Although most species of *Argia* adults occur in areas with better environmental quality (Gómez-Tolosa et al., 2022), for larvae we suggest that the identification to genus-level should be used with caution in evaluating the effects of anthropogenic changes in freshwater environments. This reinforces the importance of increasing our knowledge about ecological thresholds of different groups that may be used as bioindicators in monitoring programs or to develop ecological hypotheses that facilitate obtaining robust information of some determined area and disturbances.

On the other hand, genera tolerant to increased IDI, CDI, LDI scores can be associated with more-disturbed stream sites, such as the Gomphidae genera (*Cacoides*, *Phyllocycla*, *Progomphus*). Larvae of those genera are burrowers and commonly associated with streambed sand and fine sediments (Carvalho & Nessimian, 1998; Assis et al., 2004), and narrower widths of riparian vegetation [< 0.5 m for *Cacoides* and 15-5 m 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, adult Gomphidae genera were also not associated with changes in environmental condition (Mendoza-Penagos et al., 2021) which can be related to the difficulty of sampling adults or measuring their ecological requirements.

In addition, *Hetaerina* was considered tolerant to LDI and IDI indices. *Hetaerina* have several habitat preferences and general habitat behaviors, with species occupying temperate and tropical forests (Paulson, 2006, Standring et al., 2022), which can explain why they have a tolerance to disturbance indices of aquatic environments. Larvae of this genus also have generalist behaviors, such as clinger or climbers,

and can be found in areas with roots, macrophytes and debris or in actively eroding areas (Carvalho & Nessimian, 1998). Like *Argia*, *Hetaerina* is a speciose genus (39 species of *Hetaerina* and 24 of *Mnesarete*, which now belongs to *Hetaerina*) (Standring et al., 2022), which is reflected in different ecological requirements and, thus, in different tolerances to environmental disturbances.

Response of Odonata larvae to anthropogenic disturbance

Similar to our findings, other studies with Odonata larvae demonstrated relationships to anthropogenic disturbances (Valente-Neto et al., 2016; Mendes et al., 2017; Ribeiro et al., 2022), particularly associated with reduced riparian vegetation (Pires et al., 2020a; Mendes et al., 2021; Silva et al., 2021b). Odonata larvae, mostly Zygoptera, show narrower ecological thresholds in relation to environmental changes resulting from anthropogenic pressures, such as reduced riparian vegetation (Mendes et al., 2015; Pires et al., 2020a; Silva et al., 2021b), and poorer 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) as a consequence of this biological homogenization.

Why use Titan and biological responses

Despite collecting 30 genera, we identified ecological thresholds for seven. The low abundances, expected for rarer genera (Pompeu et al., 2021), affect the accuracy of genera abundance estimates in the local pool available for TITAN (Valente-Neto et al., 2021), which is a limitation of this analysis. However, considering that until now, there have been no studies of larval Odonata ecological thresholds for the neotropical region (Gómez-Tolosa et al., 2021), our findings are important and potentially useful. Moreover, these seven Odonata genera may be used as practical surrogates of macroinvertebrate assemblages to evaluate anthropogenic disturbances in Neotropical Savanna streams.

We suggest using as many larval Odonata genera as possible for indicating anthropogenic disturbances. Because neotropical ecosystems have high environmental complexity and beta diversity (Leal et al., 2018; Firmiano et al., 2021), it is unwise to choose a single genus to serve as an indicator across such a diverse geographical region (Gómez-Tolosa et al., 2021). In addition, our sampled area (45,180 km^2) incorporated large portions of two large river basins (São Francisco and Paraná). Therefore, caution must be employed when generalizing the ecological threshold of one genus to another (Huggett, 2005), even within the same suborder, because it is unlikely they will respond similarly.

Regardless of these limitations, we believe that using ecological thresholds of Odonata larvae can provide effective and sensitive tools to assess anthropogenic disturbances in aquatic ecosystems. Using ecological thresholds allowed us to identify the level of disturbance that affects the abundance of certain genera, making them robust biological indicators (King & Baker, 2014). Although environmental disturbance variables can be somewhat easily measured, they only reflect anthropogenic pressures and stressors. However, biological indicators synthesize multiple pressures and stressors, making them critically important scientific indicators of ecosystem services that are of greater concern to the public than land-use index scores or water quality and physical habitat measures (Hering et al., 2010; Schinegger et al., 2016; Herlihy et al., 2020; Oliveira et al., 2020; Lemm et al., 2021).

Conclusions and management implications

This is the first use of TITAN for assessing ecological thresholds for Odonata larvae in the neotropical region (Gómez-Tolosa et al., 2021). However, limitations to the knowledge of the ecological requirements of Odonata larvae remain. We highlight the necessity for greater sampling effort to detect relatively rare taxa (Silva et al., 2014; Hughes et al., 2012; Pompeu et al., 2021) and to 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 because most species have no larval identity so far.

Our study provides information about the ecological thresholds of seven larval Odonata genera. We highlight that *Argia* (Zygoptera: Coenagrionidae) and *Brechmorhoga* (Anisoptera: Libellulidae) were considered sensitive to local and regional anthropogenic disturbances, whereas *Cacoides*, *Phyllocycla* and *Progomphus* (Anisoptera: Gomphidae) were considered tolerant. Despite the known general pattern of Odonata sensitivity to environmental disturbances at the suborder (Silva et al., 2021b) or family levels (Šigutová et al., 2019, Mendoza-Penagos et al., 2021), some genera do not follow this pattern because of their specific ecological requirements. These differences reinforce the importance of identification to the lowest possible taxonomic level. In addition, families such as Coenagrionidae and Libellulidae have most of the described species, which can also affect the observed response patterns.

In summary, both 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 here. Ecological threshold 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. These field protocols will support environmental managers to assess and identify the consequences of biological homogenization and facilitate basin rehabilitation efforts in Neotropical Savanna headwater streams.

Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflicts of interest.

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