

# Effects of a Landscape Gradient on the Diversity of Odonates in the Legalamazonia Zone of the Brazilian State of Maranhão

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# Abstract

The various ecosystems of the Amazon biome play a vital role in the maintenance of biodiversity, as well as providing important ecosystem services at local and global levels. Small-bodied generalists include a number of important groups, such as the insects of the order Odonata. Given their sensitivity to environmental alterations, odonates are also valuable bioindicators of environmental quality. In this context, the present study evaluated the distribution of the diversity of adult odonates in the Legal Amazonia zone of the Brazilian state of Maranhão, to verify which environmental variables are the most important drivers of the structure of the local odonate communities. To test these predictions, we sampled 27 streams along a landscape gradient located in the hydrographic basin of the Pindaré River in the Legal Amazonia zone of the Brazilian state of Maranhão. The results of the study indicated that alterations in the environment are reflected in the odonate diversity recorded at each site, with the assemblages of degraded environments having a predominance of more generalist species. However, some of these species are threatened by the loss of habitats, primarily denser vegetation. However, such species are threatened by habitat loss, particularly dense vegetation. Despite this, the discovery of *Erythrodiplax unimaculata* in Maranhão highlights ongoing biodiversity revelations. The study underscores the urgent need for continuous monitoring to mitigate biodiversity loss in the face of widespread environmental disturbances in the region.

# Introduction

The Amazon Forest is home to a vast diversity of species, accounting for more than 10% of the planet's terrestrial biodiversity. Many of these taxa are endemic to this biome or are even unknown to science but are being threatened with extinction due to the frequent and constant shifts in land use (Arantes et al. 2017; Jézequel et al. 2021). This biodiversity supports an ample variety of ecosystem services (Carvalho et al. 2021), including pollination, biological control, and the fragmentation and cycling of organic material in the trophic web (Chagnon et al. 2015). These services contribute to the protection and maintenance of the soil, climatic equilibrium, and the availability of freshwater (Ellwanger et al. 2020). Despite the importance of the Amazon Forest and its ecosystem services, large tracts of forest have already been cleared (Flores et al. 2024). Deforestation in the Amazon is driven primarily by the need for land for cattle ranching, while the associated processes of urbanization and industrialization accentuate the threats to the integrity of this biome (Lundberg and Abman 2021; Acheampong et al. 2019; Rueda et al. 2019).

The integrity of stream environments is highly dependent on the configuration of the surrounding matrix, and processes such as deforestation, burn-off, habitat fragmentation, the implantation of monocultures, and urbanization all represent significant threats (Zeni et al. 2019). This is because these processes often involve the reduction or complete suppression of the riparian vegetation, resulting in an increase in ambient temperatures, the destabilization of the stream margins, and an increase in the input of sediments into the channel. These environmental alterations, in turn, affect the quality of the water, including its turbidity, pH, and dissolved oxygens concentrations (Ning et al. 2021; Smith and Schindler

2009). As a consequence, the fauna that is dependent on these environments (Silva-Araújo et al. 2020) tends to undergo a gradual and progressive loss of species richness and abundance, with associated shifts in the species composition over the gradient of land use from natural habitats toward urbanization (Cunha et al. 2015).

Aquatic macroinvertebrates have often been used as a diagnostic tool for the monitoring of the quality of aquatic environments (Shimba and Jonah 2016; Sumudumali and Jayawardana 2021; Couceiro et al. 2021). One group of macroinvertebrates, in particular, the odonates (Insecta, Odonata) is prominent here, due to its diversity and ample distribution in aquatic systems (Kalkman et al. 2008). In addition, odonates have a double life cycle, with aquatic larvae and terrestrial/aerial adults (Balzan 2012). As these different life stages occupy distinct environments, they constitute an extremely useful tool for the detection of changes occurring in both aquatic environments and the surrounding terrestrial matrix (Oliveira-Junior and Juen 2019; Mendoza-Penagos et al. 2021). For example, as the odonates of the suborders Anisoptera and Zygoptera tend to exploit distinct freshwater biotopes, their relative contribution to the insect assemblage will be defined by their ecophysiological adaptations (Oliveria-Júnior and Juen 2019). As this pattern of variation is extremely systematic, the Odonata is the order used most widely in ecological studies and environmental monitoring, due to its potential for the relatively accurate diagnosis of environmental impacts across landscape gradients (Miguel et al. 2017; Dalzochio et al. 2020; Deacon and Samways 2021).

Despite the recent advances in the research on odonates, many knowledge gaps persist, including accurate information on the distribution of many species (Ferreira, et al. 2023). These knowledge gaps include Linnean shortfalls (deficits in taxonomy and systematics), Wallacean shortfalls (deficits in the data on geographic distributions), and Hutchinsonian shortfalls, which refer to deficits in the data on the species' tolerance of abiotic conditions (Hortal et al. 2015). The Legal Amazonia zone of the Brazilian state of Maranhão represents a geographic knowledge gap for the order Odonata, which emphasizes the need for further research, given the twin threats of climate change and burgeoning deforestation (Barbosa dos Santos et al. 2024).

In the present study, we examine the distribution of the diversity of adult odonates in the Legal Amazonia zone of the Brazilian state of Maranhão and verify which environmental variables are the most important of the structure of the region's odonate communities. We predicted that (i) environmental integrity is related systematically to the species richness and abundance of adult odonates, given that more heterogeneous environments provide these organisms with more resources, and (ii) the odonate species composition is related systematically to environmental variables and the physicochemical characteristics of the water (canopy cover, width and depth of the stream, pH, and dissolved oxygen concentrations), which are linked to the environmental integrity of each stream.

## **Material and methods**

### **Study area**

The present study focused on 27 streams located within the hydrographic basin of the Pindaré River, in the Legal Amazonia zone of the Brazilian state of Maranhão (Fig. 1). These streams are distributed within a landscape that has undergone significant alterations provoked by human activities, in particular, the implantation of pasture for cattle ranching. These impacts began in the 1960s and 1970s, with the logging of large areas of forest, which were converted into pasture, and the establishment of settlements (Celentano et al. 2018). The habitat characteristics of the areas of permanent preservation that remain in place along this environmental gradient are extremely heterogeneous. In most of these areas, land is predominantly converted to pasture for cattle raising (Nicasio et al. 2019).

The hydrographic basin of the Pindaré River extends over 720 km (Martins and Oliveira, 2011), with relatively flat to slightly undulating relief, slopes of up to 25°, and a mean altitude of 350 m above sea level (Muniz 2008; INMET 2022). The local climate is hot and humid, with mean annual temperatures of between 24° C and 26° C, and a dry season between June and November (Correia Filho et al. 2011). Annual precipitation is generally between 1400 mm and 1800 mm, with relative humidity ranging from 32–63% (Muniz 2008; INMET 2021).

## **Sample design and the collection of odonate specimens**

The specimens of adult odonates were collected between June and September 2021, which is the peak of the dry season, that is, the period with the lowest precipitation. The biotic and abiotic characteristics of each stream were described based on data collected within a 100-meter stretch that was delimited on each of the 27 study streams, with each stretch being subdivided into 20 segments of 5 meters (Batista et al. 2021). In each segment, specimens of adult odonates were collected using an entomological net with a diameter of 40 cm, following the fixed-area sweep method proposed by Juen and De Marco (2011) and Juen et al. (2014). The mean sampling time at each sampling point was one hour, with the samples being collected always between 10 h and 14 h, to ensure the adequate sampling of all the groups that were active at this time of day (Batista et al. 2021).

The specimens collected at each site were placed in cambric paper envelopes and then immersed in acetone p.a. for 24 hours (Zygoptera) or 48 hours (Anisoptera), following the approach of Lencioni (2006). The appropriate taxonomic keys were used to identify the odonate species, i.e., Garrison and von Ellenrieder (2015), Garrison et al. (2006), and Lencioni (2005; 2006). The conservation status of each species was determined by consulting the IUCN (International Union for Conservation of Nature) red list and the Brazilian list of threatened species (IUCN 2023) and Ministério do Meio Ambiente (MMA). The material collected during the study was deposited in the scientific collections of the Laboratory of Ecology and Conservation at the Federal University of Pará (UFPA) in Belém, to guarantee free access, and resolve persistent taxonomic uncertainties.

## **Description of the environment**

The Habitat Integrity Index (HII) developed by Nessimian et al. (2008) was used to evaluate the abiotic characteristics of each study stream. This protocol is made up of 12 items – (i) the pattern of land use adjacent to the riparian vegetation, (ii) the width of the riparian vegetation and (iii) its degree of

preservation, (iv) the condition of the riparian forest within a radius of 10 m from the stream, (v) the type of sediment and presence of retention structures in the stream channel, (vi) the structure and degradation of the stream margins, (vii) the characteristics of the stream bed in terms of its substrate, (viii) aquatic vegetation, and (ix) debris, and (x) the configuration of rapids, (xi) pools, and (xii) meanders. The value of the HII ranges from 0 to 1, and the higher the value, the greater the integrity of the stream.

At each stream, we measured the width and depth of the water and estimated the canopy cover, given that these environmental variables may influence the composition of the odonate assemblage (Hendry and Taylor 2004). The stream width and depth were measured at three points within the 100-meter sample stretch of each stream, using a surveyor's tape, for the calculation of the mean parameter. The canopy cover was estimated using the Canopy app (version 1.0.4) cellphone application, which processes images of a canopy to calculate the percentage of cover. For this, photographs of the canopy were taken at three transversal points (the center and both margins) at the two extremities and in the middle of the 100-meter sample stretch of each stream, with the camera facing directly upward from a height of one meter above the surface of the water (Veras et al. 2024).

As the physicochemical parameters of the water may also influence the presence of odonates at the different stages of their life cycle (Nelson et al. 2020), we measured the pH and the Dissolved Oxygen (DO) concentration of the water using a Horiba U-50 multiparameter probe. These data were collected at the three standard points (the center and extremities) of the 100-meter sample stretch of each stream.

## Data analysis

For analysis, each stream was considered to be a sampling unit (SU), with the study encompassing a total 27 SUs. The efficiency of the sampling procedures for the capture of the odonate diversity of the study area was evaluated using rarefaction curves. The richness of morphospecies/species was estimated for each stream using the first-order Jackknife estimator, with a 95% confidence interval (Krell 2004).

A Redundancy Analysis (RDA) was used to evaluate the influence of the environmental variables on the abundance of odonate species, with the Hellinger transformation being applied to the species matrix to reduce the effect of the most abundant species. The environmental variables were also standardized to reduce the potential influence of the different scales of measurement, with each variable being assigned to an equivalent scale. We then applied a simple linear regression to verify which variables influenced the odonate species richness and abundance (Zar 1999).

A Principal Coordinates Analysis (PCoA) was run, using the HII to represent the gradient in environmental variation among the SUs. For this, the Euclidean distance was used as a measure of dissimilarity in the environmental data. All the analyses were run in *RStudio* (version 4.2.1), using the following packages: *ggplot2* (Wickham 2016), *ggrepel* (Slowikowski et al. 2024), *gridExtra* (Auguie, 2017), *ggplotify* (Tang et al. 2016), *iNEXT* (Hsieh et al. 2016), *ggthemes* (Arnol, 2024), *adespatial* (Dray et al. 2023), and *vegan* (Oksanen et al. 2017).

## Results

An environmental gradient with intense anthropogenic impacts was observed among the 27 study streams, related primarily to the presence of planted pastures, which reach the stream margins in many cases (see supplementary material). A total of 437 odonate specimens were collected during the present study (Table 1), representing the suborders Anisoptera and Zygoptera, distributed in three families, 22 genera, and 29 species/morphospecies. The suborder Anisoptera was the most abundant, with 255 individuals, 17 species, and three morphospecies, while the most diverse genus was *Erythrodiplax*, with five species, the most abundant being *Erythrodiplax basalis*, with 132 individuals. The suborder Zygoptera, in turn, was represented by 182 individuals, six species and three morphospecies, with *Ishnura capreolus* Hagen, 1861 being the most abundant zygopteran species, with 112 individuals.

Table 1

The odonate species/morphospecies collected from 27 streams in the Pindaré basin in the Legal Amazonia zone of western Maranhão, Brazil. LC = Least Concern, NE = Not Evaluated. The asterisk (\*) indicates a new species record for the state of Maranhão.

Species	Species abbreviations	IUCN classification	ICMBio classification	Reference	Number of specimens
<b>ZYGOPTERA</b>					
<b>Calopterygidae</b>					
<i>Hetaerina sanguinea</i> Selys, 1853	H. san	LC	LC	Lozano, 2021	40
<b>Coenagrionidae</b>					
<i>Acanthagrion</i> sp. 1	A. sp1	NE	NE	–	9
<i>Argia oculata</i> Hagen in Selys, 1865	A. ocu	LC	LC	Bota-Sierra & Sandoval, 2021	14
<i>Argia</i> sp. 1	A. sp1	NE	NE	–	1
<i>Ischnura capreolus</i> Hagen, 1861	I.cap	LC	LC	Undefined	112
<b>Protoneurinae</b>					
<i>Epipleoneura metallica</i> Rácenis, 1955	E. met	LC	LC	von Ellenrieder, 2009	2
<i>Epipleoneura</i> sp. 1	E. sp1	NE	NE	–	1
<i>Epipleoneura westfalli</i> Machado, 1986	E. wes	LC	LC	Mauffray & Tennessen, 2020	2
<i>Neoneura sylvatica</i> Hagen, 1886	N. syl	LC	LC	Lozano, 2021	1
<b>ANISOPTERA</b>					
<b>Libellulidae</b>					
<i>Argyrothemis argentea</i> Ris, 1909	A. arg	LC	LC	von Ellenrieder, 2009	12
<i>Dasythemis esmeralda</i> Ris,	D. esm	LC	LC	Lozano, 2021	8

Species	Species abbreviations	IUCN classification	ICMBio classification	Reference	Number of specimens
1910					
<i>Diastatops obscura</i> Fabricius, 1775	D. obs	LC	LC	Lozano, 2021	30
<i>Dythemis</i> sp. 1	D. sp1	NE	NE	–	1
<i>Erythemis carmelita</i> Williamson, 1923	E. car	LC	LC	von Ellenrieder, 2009	4
<i>Erythemis peruviana</i> Rambur, 1842	E. per	LC	LC	Paulson, 2017	1
<i>Erythrodiplax basalis</i> Kirby, 1897	E. bas	LC	LC	Undefined	132
<i>Erythrodiplax media</i> Borror, 1942	E. med	LC	LC	Lozano, 2021	26
<i>Erythrodiplax umbrata</i> Linnaeus, 1758	E. umb	LC	LC	Paulson, 2017	1
* <i>Erythrodiplax unimaculata</i> De Geer, 1773	E. uni	LC	LC	Lozano, 2021	2
<i>Macrothemis heteronycha</i> Calvert, 1909	M. het	LC	LC	von Ellenrieder, 2009	2
<i>Miathyria marcella</i> Selys in Sagra, 1857	M. mar	LC	LC	Paulson, 2017	1
<i>Micrathyria</i> sp. 1	M. sp1	NE	NE	–	8
<i>Oligoclada abbreviata</i> Rambur, 1842	O. abb	LC	LC	von Ellenrieder, 2009	5
<i>Orthemis discolor</i> Burmeister, 1839	O. dis	LC	LC	Undefined	2
<i>Pantala</i> sp. 1	P. sp1	NE	NE	–	1
<i>Perithemis lais</i> Perty, 1834	P. lais	LC	LC	von Ellenrieder, 2009	5

Species	Species abbreviations	IUCN classification	ICMBio classification	Reference	Number of specimens
<i>Perithemis mooma</i> Kirby, 1889	P. moo	LC	NE	Lozano, 2021	2
<i>Uracis imbuta</i> Burmeister, 1839	U. imb	LC	LC	Lozano, 2021	1
<i>Zenithoptera lanei</i> Santos, 1941	Z. lan	LC	LC	Lozano, 2021	11
Total abundance					437
Total species richness					29

Overall, 29 species were recorded in the present study, compared with an estimated species richness of 42 (Fig. 2). The sampling efficiency was thus 69.1% (observed  $S$ /estimated  $S$ ), which may indicate that greater sample coverage is needed. This implies that further research would be necessary to provide a more accurate estimate of the odonate diversity of the study area based on a more comprehensive sampling effort.

In the Redundancy Analysis (Fig. 3), the first two axes explained 66.17% of the variation in the data (44.27% on axis 1 and 22.00% on axis 2). The variables depth, width, HII, and canopy cover of the streams were all associated positively with axis 1 and correlated negatively with the pH and DO of the streams. Axis 2 was correlated positively with the canopy cover and HII, while the pH, width, and depth of the streams correlated negatively with axis 2 (Fig. 3).

*Epipleoneura metallica* Rácenis, 1955, *Ischnura capreolus* Hagen, 1861, *Argia oculata* Hagen in Selys, 1865, *Epipleoneura westfalli* Machado, 1986, and *Micrathyria* sp. 1 Kirby, 1889 were all associated positively with the selected axis 1 variables (depth, width, HII, and canopy cover). By contrast, *Erythrodiplax basalis* Kirby, 1897, *Erythrodiplax media* Borror, 1942, and *Diastatops obscura* Fabricius, 1775 were strongly related with the streams associated negatively with the pH and DO.

The simple linear regression (Fig. 4) revealed a positive relationship between the HII and odonate species richness and abundance ( $b = -3.056$ ;  $r^2 = 0.2188$ ;  $p = 0.01$ ), whereas the other variables (DO, canopy cover, pH, and stream width and depth) were not related significantly ( $p > 0.05$ ). The PCoA indicated that the study streams are arranged within an environmental gradient, within a landscape influenced by profound anthropogenic pressures.

## Discussion

The results of the present study indicate that the study streams are arranged along an environmental gradient of major impacts, which reflect the diversity of the specimens collected. The HII and the

morphological characteristics of the channel, such as the canopy cover, and the depth and width of the streams were important determinants of the variation in diversity, with higher values having a positive effect on species richness and abundance. The results of the analysis indicate that the environmental impacts observed in the study streams result in profound alterations of the odonate species richness and abundance, which is consistent with our hypotheses and the findings of Oliveira-Júnior et al. (2017).

The HII values indicate that the land use in the vicinity of the study streams favors the diversity and abundance of generalist species. Generalist species are more resistant to environmental alterations, allowing them to survive under critical conditions of degradation within an environmental gradient of reduced vegetation cover and land use for cattle rearing (Robinson and Strauss 2020). The disturbances observed in the present study may be causing a reduction in the presence of species that are dependent on more integral environments, and that require more resources and better-quality habitats to survive (Datto-Liberato et al. 2024), given that odonate species have different adaptations and mechanisms of thermoregulation (Corbet, 1999). In fact, some previous studies have also found that odonate species richness and abundance were greater in more open and exposed areas (Monteiro Júnior et al. 2013; Silva et al. 2010; Dolný et al. 2021).

In addition to habitat integrity (HII), the channel morphology (canopy cover, width, and depth) and physicochemical parameters of the water, such as the pH, also had a positive relationship with odonate species richness and abundance. Broader and shallower streams tend to be more exposed to direct sunlight, and typically undergo greater fluctuations in temperature than deeper and narrower channels, leading to modifications in the physicochemical parameters of the water, which affect the local biota (Davies-Colley and Quinn 1998; Caissie 2006). In addition to the direct influence of shading on the temperature of the stream, the canopy cover tends to reduce the erosion of the margins, by protecting the ground from the direct impact of the rain, thereby influencing the configuration of the channel (Mohammad and Adam 2010). Given this, the modification of the riparian vegetation, irrespective of the cause, may alter the natural thermal conditions of the stream and the surrounding ecosystem (De Paula et al. 2021).

*Erythrodiplax basalis* was the most abundant odonate species in the study streams (Table 1). This species is highly resistant to environments with high levels of disturbance and a more open canopy, with more exposure to the sun, as well as an ample geographic range (Ferreira et al. 1979). The species of the suborder Anisoptera were more abundant, overall, than those of the Zygoptera, which may reflect the larger number of more generalist anisopteran species, which tend to prefer more disturbed environments, with less vegetation cover (Mendoza-Penagos et al. 2021). These species also have a greater flight capacity and are able to forage more efficiently for prey (Rüppell 1989), due primarily to their larger body size, which enables them to cover larger areas in search of resources (Grabow and Rüppell 1995).

The species of the suborder Zygoptera were also influenced by the quality of the riparian vegetation and canopy cover, with species richness and abundance being found in streams with greater environmental

integrity (Calvão et al. 2022). Previous studies have shown that zygopterans are more dependent than anisopterans on the climatic characteristics and the temperature of the streams (Mendes et al. 2015; Rocha et al. 2023; Miguel et al. 2017). In addition to canopy cover, parameters such as the width and depth of the stream also influence the occurrence of zygopterans (Mendes et al. 2019) and have an important influence on the structure of the communities of these species. When these parameters are altered, they tend to compromise the species diversity found in these environments (Mendes et al. 2018).

The integrity and conservation of the study streams were relative low, in general. Despite this overall lack of environmental conservation and often critical levels of degradation, these streams play an important role in the maintenance of local biodiversity, given that they enhance the regional connectivity of the local odonate assemblages (Simon and Travis 2010). This is an extremely important consideration, given that many species have a reduced dispersal capacity, due to their ecophysiological characteristics (Lima et al. 2021).

While none of the species identified in the present study are considered to be under any risk of extinction (Table 1), some taxa, in particular the most ecologically sensitive, are threatened principally by the loss of habitat with denser vegetation. One other important finding of the present study was the first record of the anisopteran *Erythrodiplax unimaculata* De Geer, 1773 from the Brazilian state of Maranhão, which is an important addition to the knowledge of the state's odonate fauna.

The findings of the present study indicate clearly that the streams of the Pindaré basin in western Maranhão are undergoing an extreme process of degradation resulting from anthropogenic impacts and inadequate land use, above all, the suppression of the riparian vegetation for the establishment of cattle pasture, which also affects the physicochemical properties of the water. The odonate species richness recorded in the present study was similar to that detected in previous studies based on similar sampling effort, even though most of this richness was composed of generalist species. The majority of the generalist species recorded here were members of the suborder Anisoptera, which are generally well adapted to and tolerant of disturbed environments. These findings reinforce the need for the monitoring of these areas in order to mediate the loss of biodiversity, in particular the disappearance of the species that are dependent on environments with greater vegetation cover. New studies will be necessary to reduce the knowledge gaps related to the taxonomy and systematics of the region's odonate fauna, covering additional variables and expanding the study area.

## Declarations

### Conflict of Interest:

The authors declare no competing interests.

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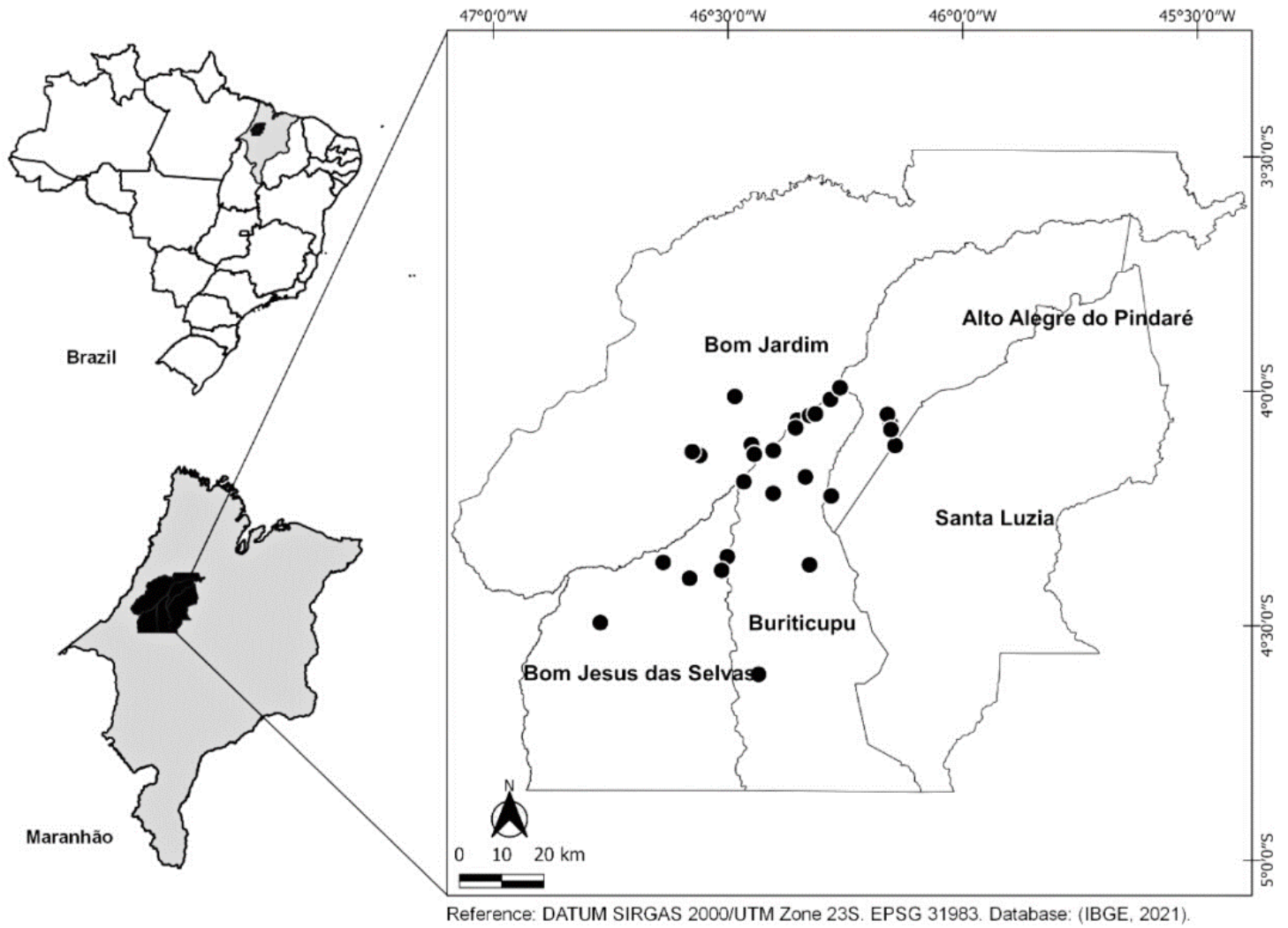
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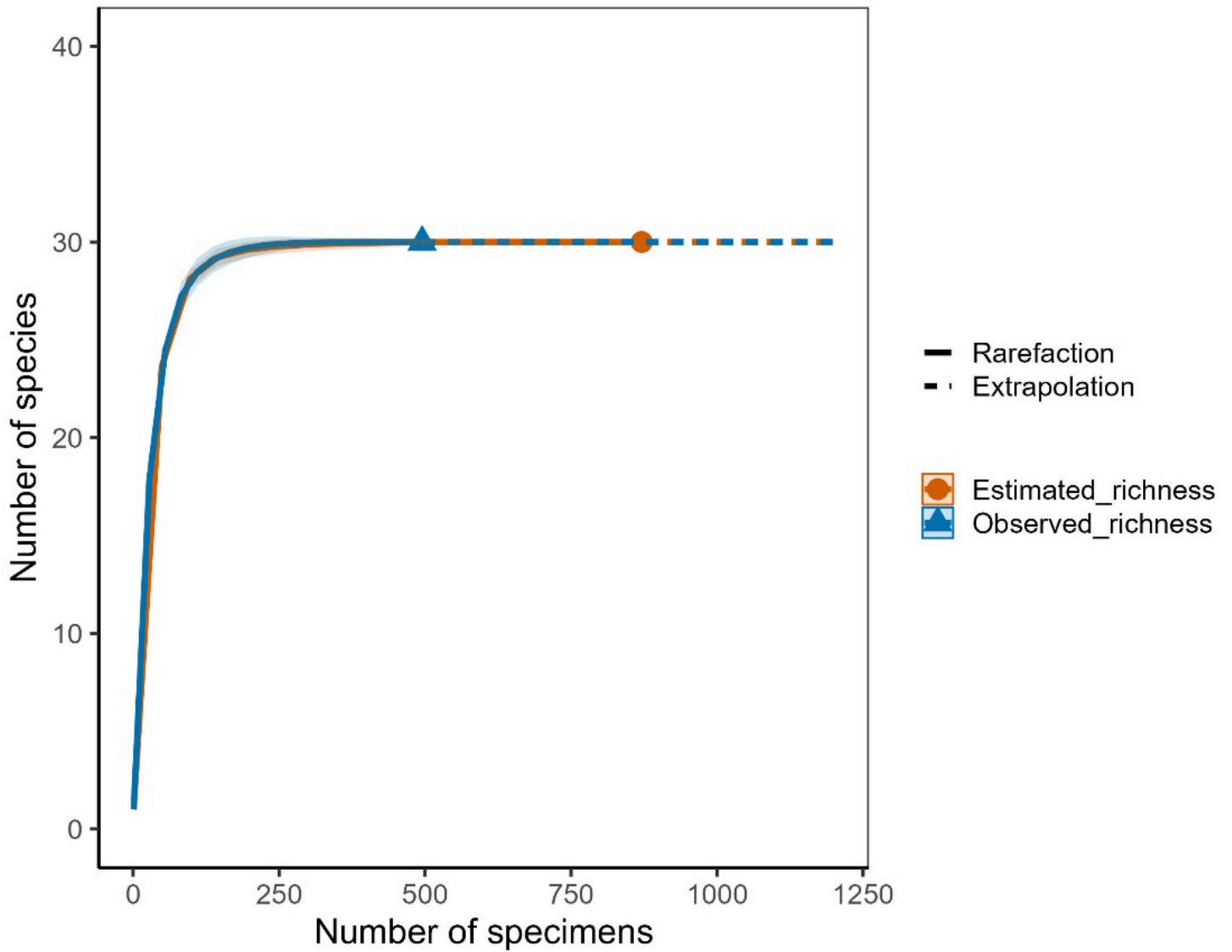
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## Figures



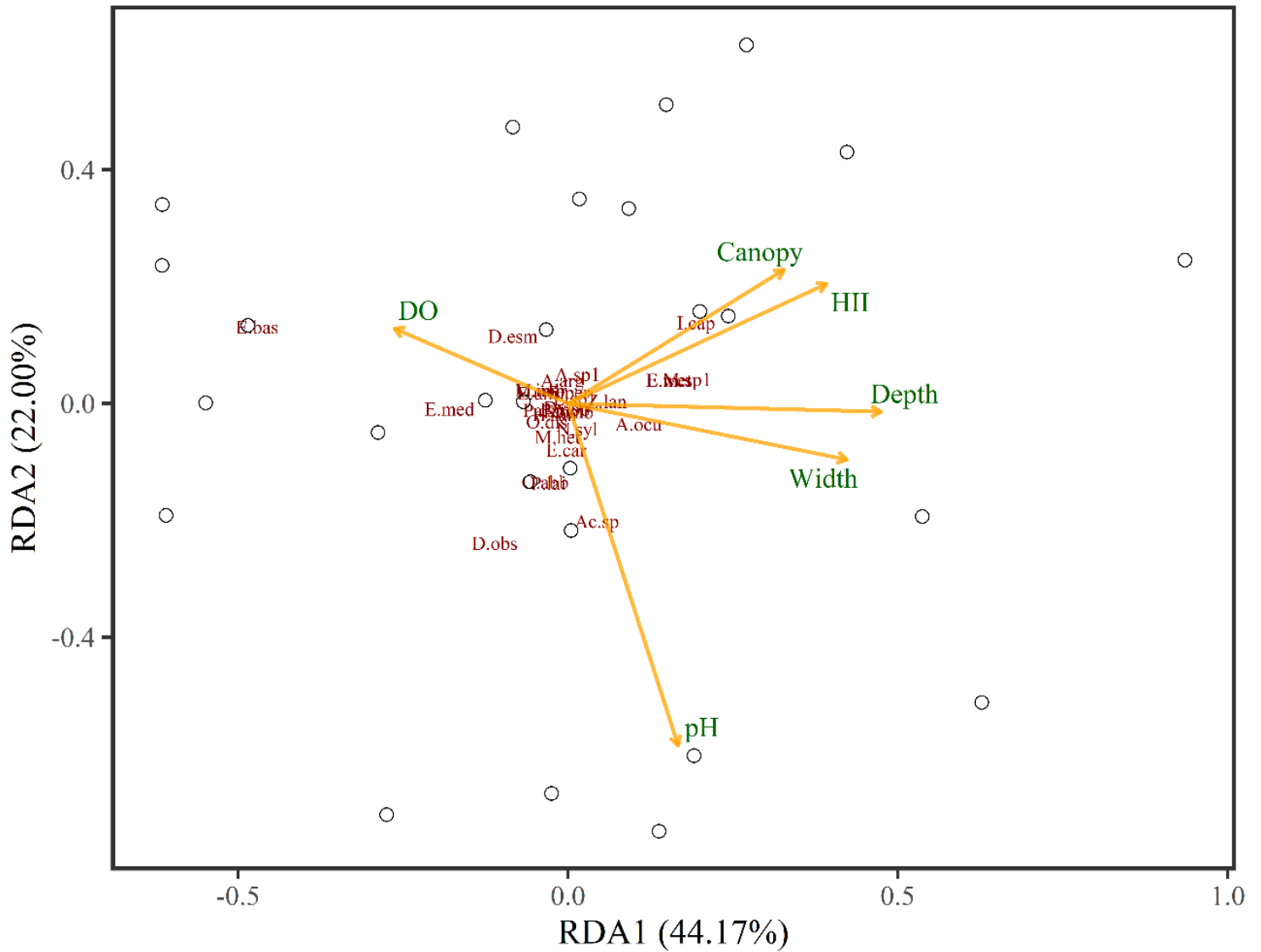
**Figure 1**

Distribution of the 27 study streams (black dots), located in the hydrographic basin of the Pindaré River, in Maranhão state, northern Brazil. The detailed map shows the five municipalities that make up the study area.



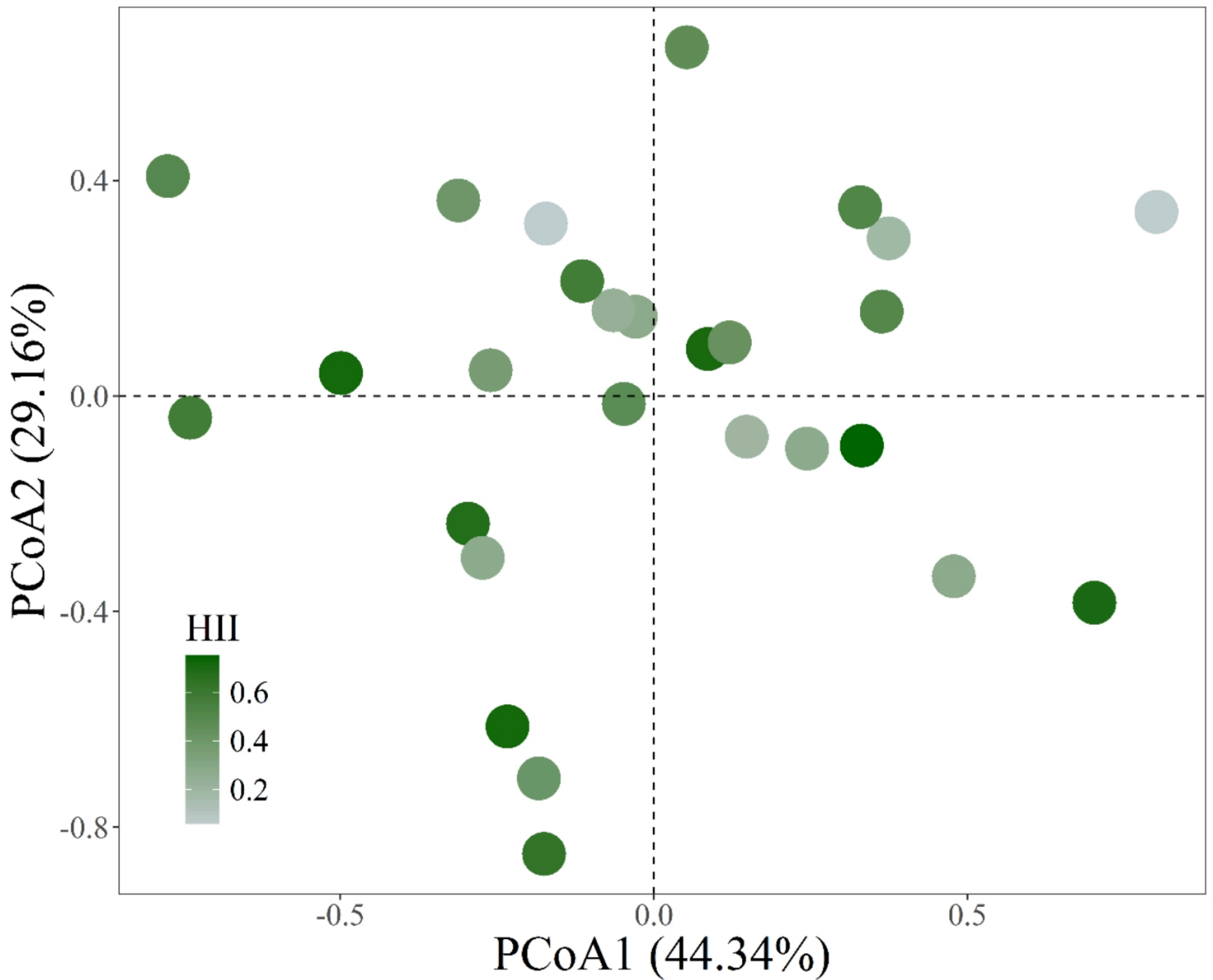
**Figure 2**

Rarefaction curves for the estimated and observed odonate species richness recorded in the present study in the Legal Amazonia zone of western Maranhão, Brazil. The blue line represents the observed species richness, and the orange line, the estimated richness.



**Figure 3**

Plot of the Redundancy Analysis (RDA) employed to evaluate the influence of the environmental variables on the characteristics of the odonate communities (E. bas, E. med, Z. lan, D. obs, O. dis, E. per, U. imb, E. umb, E. car, P. moo, E. met, M. mar, M. het, E. uni, D. esm, A. arg, H. san, P. lai, Pa. sp., Ac. sp., E. sp. 1, O. abb, N. syl, A. sp. 1, I. cap, A. ocu, Dy. sp., E. wes, M. sp. 1) in the study streams in the Legal Amazonia zone of western Maranhão, Brazil.



**Figure 4**

The plot of the Principal Coordinates Analysis (PCoA), showing the landscape gradient of the study streams, with the darker green circles (= streams) representing higher levels of environmental integrity, and the paler shades of green representing greater degradation of the environment.

## Supplementary Files

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