UNIVERSIDADE FEDERAL DO PARÁ INSTITUTO DE CIÊNCIAS BIOLÓGICAS PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

KENNED DA SILVA SOUSA

Odonata diversity and ecological thresholds in Amazonian protected areas

Belém - Pará 2024

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Dissertação apresentada ao Programa de Pós-Graduação em Ecologia da Universidade Federal do Pará, como requisito parcial para obtenção do título de Mestre em Ecologia. Área de concentração: Ecologia. Linha de Pesquisa: Ecologia de Comunidades e Ecossistemas

Orientadora: Prof^a. Dr^a. Karina Dias da Silva

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Dados Internacionais de Catalogação na Publicação (CIP) de acordo com ISBD Sistema da Bibliotecas da Universidade Federal do Pará Gerada automaticamente pelo módulo Ficat, mediante os dados fornecidos pelo(a) autor(a)

S7250 Sousa, Kenned da Silva. Odonata diversity and ecological thresholds in Amazonianprotected areas / Kenned da Silva Sousa. — 2022.

35 f. : il. color.

Orientador(a): Prof. Dr. Karina Dias da Silva Dissertação (Mestrado) - Universidade Federal do Pará, Instituto de Ciências Biológicas, Programa de Pós-Graduação emEcologia, Belém, 2022.

1. Ambientes aquáticos. 2. Biodiversidade. 3. Integridadeambiental. 4. Jamanxim. 5. Libélulas. I. Título.

CDD 574.524

KENNED DA SILVA SOUSA

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Dissertação apresentada ao Programa de Pós-Graduação em Ecologia do convênio da Universidade Federal do Pará e Embrapa Amazônia Oriental, como requisito para obtenção do título de Mestre em Ecologia pela Comissão Julgadora composta pelos membros:

Comissão Julgadora

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Aprovado em: 02/07/2024 Local de Defesa: <u>https://meet.google.com/rbt-wmwz-uje</u>

Dedico essa dissertação a minha mãe Raimunda Vitória e as minhas irmãs Kenny e Kelly

AGRADECIMENTOS

Agradeço de todo o meu coração aos meus familiares que me acompanharam nessa jornada, em especial minha mãe, Raimunda Vitória, que sempre compreendeu a importância dos estudos e me incentivou a trilhar esse caminho acadêmico, fazendo o possível e o impossível para que eu continuasse a estudar. Às minhas irmãs, Kenny e Kelly, que sempre me deram apoio emocional e seguraram minha mão nos momentos difíceis; aos meus sobrinhos, Antônio Carlos e Vitória Luz *les amours de ma vie*; ao meu pai, Kaká, por todos os ensinamentos e apoio; e ao meu padrasto, Sandro Homar, que sempre esteve disposto a cuidar e ajudar toda a minha família. Vocês são minha base, e todas as minhas conquistas dedico a vocês.

Sou imensamente grato à minha orientadora, Karina Dias da Silva, que me orienta nesses quase sete anos. Ela se tornou mais do que uma mentora, tornou-se uma amiga, uma mãe para todos os seus orientandos, cuidando, aconselhando e, principalmente, apoiando e incentivando nosso crescimento profissional e pessoal. O pesquisador que me tornei, devo a você.

Agradeço a todos os meus amigos que estão comigo desde a época da graduação: Mean Girls, Anderson, Kesley, Paulo, Gustavo, Iluany, Isadora e Gleyce. Às Baby Princess, Milena, Adélia, Fernanda e Natália. À Fernanda Alexandre por me aturar e não desistir de mim quando eu só atrapalhava durantes as identificações. A todos os meus amigos do LEIA-X, o grupo de pesquisa mais incrível e minha segunda casa, sempre serei grato a essa enorme família. A todos os amigos que fiz em Belém do LABECO e LABEV durante a temporada que passei lá, em especial a Arianny, minha irmã baiana, que fez o tempo em Belém ser mais leve. E a todos aqueles que, durante essa jornada, tiraram um tempinho para jogar conversa fora, brincar e tomar uma cervejinha, tornando o momento mais leve durante esse período do mestrado.

Também Agradeço ao Joás, Rafael, Cristian, Everton, José Max, Gabriel, Lilian, Thaísa, Leandro que se dispuseram a contribuir para que ele fosse concluído com êxito.

E por fim agradeço à Universidade Federal do Pará – UFPA pelo ensino público e de qualidade, ao IABS, CECAV, ICMBio e VALE, em especial ao Projeto Ferruginosas, pela bolsa concedida durante o mestrado. Ao programa de Pós-graduação em Ecologia – PPGECO, pelo suporte e infraestrutura oferecidos para o desenvolvimento e conclusão da minha pesquisa. Ao LEIA-X o grupo de pesquisa da qual faço parte e não conseguiria fazer o mestrado se não fosse com vocês e ao LABECO por ter me acolhido e dado um espeço para estudar em Belém.

Essa é boa!!!

Diversidade de Odonata e limiares ecológicos em áreas protegidas da Amazônia

RESUMO

O estabelecimento de Unidades de Conservação (UCs) tem como objetivo preservar a biodiversidade, mas essas áreas estão sob grande pressão antropogênica, principalmente de exploração madeireira, mineração e pecuária. Diante desse contexto, nosso estudo avalia a importância da área protegida (Parque Nacional) e da integridade do habitat usando o Índice de Integridade do Habitat (HII) para a conservação das assembleias de Odonata. Nossa hipótese é que: 1) os locais dentro do parque nacional teriam valores mais altos no HII e maiores métricas de diversidade de Zygoptera (diversidade de espécies, abundância, proporções e composição) em comparação com os valores dos locais fora do parque; e 2) considerando a integridade do habitat dos riachos, Anisoptera e Zygoptera servem como indicadores, com o primeiro associado a valores mais baixos e o segundo a valores mais altos. Foram amostrados 25 riachos (dentro e fora) do Parque Nacional Jamanxim, identificando 43 espécies de Odonata, incluindo 16 Anisoptera e 27 Zygoptera. Os riachos fora do parque nacional abrigaram o maior número de espécies exclusivas de Anisoptera e Zygoptera. Os modelos lineares revelaram uma correlação negativa significativa entre a integridade do habitat e a abundância de Anisoptera. Além disso, foram observadas diferenças significativas na abundância de Anisoptera entre os riachos dentro e fora do parque nacional, juntamente com uma heterogeneidade distinta na composição de espécies de Anisoptera. A diminuição da abundância de Anisoptera com o aumento da integridade do habitat pode ser atribuída à sua preferência por áreas abertas para termorregulação. Por outro lado, a resposta positiva das espécies de Zygoptera à integridade do habitat ressalta sua dependência de ambientes mais conservados, melhorando nossa compreensão de suas exigências ecológicas. Esses resultados reforçam a importância e a eficiência da resposta às mudanças ambientais, usando Odonata como bioindicador da qualidade ambiental e integrando a ordem em programas de monitoramento aquático juntamente com o HII, que fornece uma medida direta e objetiva da perturbação ambiental.

Palavras-chave: Ambientes aquáticos, Biodiversidade, Integridade ambiental, Jamanxim, Libélulas.

Odonata diversity and ecological thresholds in Amazonian protected areas

ABSTRACT

The establishment of Conservation Units (CUs) aims to preserve biodiversity, yet these areas are under great anthropogenic pressure, particularly from logging, mining and cattle ranching. In light of this context, our study assesses the importance of the protected area (National Park) and habitat integrity using the Habitat Integrity Index (HII) for conserving Odonata assemblages. Our hypothesis is that 1) the sites inside the national park would have higher HII scores and greater Zygoptera diversity metrics (species diversity, abundance, proportions and composition) compared to the scores of the sites outside the park; and 2) considering the habitat integrity of the streams, Anisoptera and Zygoptera serve as indicator, with the former associated with lower scores and the latter with higher scores. We sampled 25 streams (both inside and outside) of the Jamanxim National Park, identifying 43 species of Odonata, including 16 Anisoptera and 27 Zygoptera. Streams outside the national park harbored the highest number of exclusive Anisoptera and Zygoptera species Linear models revealed a significant negative correlation between habitat integrity and Anisoptera abundance only. Moreover, significant differences in Anisoptera abundance were observed between streams inside and outside the national park, along with distinct heterogeneity in Anisoptera species composition. The decrease in Anisoptera abundance with increasing habitat integrity may be attributed to their preference for open areas for thermoregulation. Conversely, the positive response of Zygoptera species to habitat integrity underscores their reliance on more conserved environments, enhancing our understanding of their ecological requirements. These results reinforce the importance and efficiency of responding to environmental changes, using Odonata as a bioindicator of environmental quality and integrating the order into aquatic monitoring programs alongside the HII, which provides a straightforward and objective measure of environmental disturbance.

Keywords: Aquatic environments, Biodiversity, Dragonflies, Environmental integrity, Jamanxim.

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Este manuscrito foi elaborado e formatado de acordo com as normas da revista Neotropical Entomology disponíveis em: <u>https://link.springer.com/journal/13744</u>

Odonata diversity and ecological thresholds in Amazonian protected areas

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Abstract

The establishment of Conservation Units (CUs) aims to preserve biodiversity, yet these areas are under great anthropogenic pressure, particularly from logging, mining and cattle ranching. In light of this context, our study assesses the importance of the protected area (National Park) and habitat integrity using the Habitat Integrity Index (HII) for conserving Odonata assemblages. Our hypothesis is that 1) the sites inside the national park would have higher HII scores and greater Zygoptera diversity metrics (species diversity, abundance, proportions and composition) compared to the scores of the sites outside the park; and 2) considering the habitat integrity of the streams, Anisoptera and Zygoptera serve as indicator, with the former associated with lower scores and the latter with higher scores. We sampled 25 streams (both inside and outside) of the Jamanxim National Park, identifying 43 species of Odonata, including 16 Anisoptera and 27 Zygoptera. Streams outside the national park harbored the highest number of exclusive Anisoptera and Zygoptera species Linear models revealed a significant negative correlation between habitat integrity and Anisoptera abundance only. Moreover, significant differences in Anisoptera abundance were observed between streams inside and outside the national park, along with distinct heterogeneity in Anisoptera species composition. The decrease in Anisoptera abundance with increasing habitat integrity may be attributed to their preference for open areas for thermoregulation. Conversely, the positive response of Zygoptera species to habitat integrity underscores their reliance on more conserved environments, enhancing our understanding of their ecological requirements. These results reinforce the importance and efficiency of responding to environmental changes, using Odonata as a bioindicator of environmental quality and integrating the order into aquatic monitoring programs alongside the HII, which provides a straightforward and objective measure of environmental disturbance.

Keywords: Aquatic environments, Biodiversity, Environmental integrity, Dragonflies, Jamanxim.

Introduction

The main objective behind the creation of Conservation Units (CU) is to conserve biodiversity (Brasil, 2000). Despite this noble objective, our understanding of biodiversity distribution, or which species are preserved or occur in these areas remains insufficient (Oliveira et al. 2017). These pieces of information are essential not only for the management planning of the unit but also for reducing knowledge gaps and proposing even more effective conservation strategies (Frank and Schäffler 2019). In addition, these areas can bring valuable information for the development and assessment of lists of endangered species. In Brazil, however, the discourse on conservation often clashes with regional development agendas, leading to widespread deforestation driven by activities such as logging, construction of hydropower plants, mining exploitation, and cattle ranching (Pedlowski et al. 2005; Coy and Klingler 2014; Castello 2021). Regrettably, even the most restricted protected areas in the Amazon fall victim to these anthropogenic pressures (Imazon 2022).

The lack of knowledge about the distribution and biological requirements of the species becomes particularly concerning when considering invertebrates, which provide a crucial role in ecosystem functioning beyond the pollination processes (Cardoso et al. 2011; Rhodes 2018; Noriega et al. 2018). Knowledge of Brazilian biodiversity remains limited both inside and outside conservation units (Oliveira et al. 2017). Within protected areas, the lack of knowledge about small water bodies and their biodiversity is still incipient (Monteiro-Junior et al. 2016; Dias-Silva et al. 2021). Studies examining the efficacy of conservation units in preserving the beta diversity of waterbugs (Dias-Silva et al. 2021), highlight the need for strategies beyond political and economic realms (e.g., on the basis of conservation policies). Research conducted in an Amazonian national park suggests that even streams outside protected areas may benefit from the buffer effect of adjacent conservation zones, underscoring their broader ecological significance (Brito et al. 2023). Thus, there is a pressing need for further studies within protected areas to elucidate species identification, distribution in aquatic systems, and the efficacy of conservation measures in preserving these systems.

Freshwater ecosystems, renowned for their sensitivity, are considered to be the most threatened habitats in the world, facing challenges related to biodiversity loss, water quality degradation, and landscape persistence (Allan 2004; Dudgeon 2006; Leal et al. 2020). The integrity of small streams (up to 3rd order) is particularly compromised due to their direct reliance on the surrounding landscape (Budnick et al. 2019). Deforestation along riparian vegetation zones profoundly alters these ecosystems, disrupting energy dynamics (from allochthonous to autochthonous), destabilizing banks, and introducing sediment and pollutants entering the stream channel and loss of local climate stability (Dala-Corte et al. 2020; Paula et al. 2022). These factors alone or in synergy cause a decrease in environmental heterogeneity and ecosystem quality, resulting in the loss of aquatic biodiversity (Faria et al. 2017).

Among aquatic communities, Odonata insects are excellent indicators of ecosystem integrity due to their rapid response to anthropogenic impacts, even those more subtle (Miguel et al. 2017; Oliveira-Júnior and Juen 2019). Odonata comprises the suborders Anisoptera, Zygoptera and Anisozygoptera (this one exclusive for some regions in Southern Asia), which exhibit distinct ecophysiological and habitat requirements (Corbet 1999; Corbet and May 2008; Oliveira-Junior et al. 2015). While most Zygoptera thrive in well-shaded environments with little environmental modification, most Anisoptera succeed in open environments, even those with some environmental modification (De Marco et al. 2015). The patterns of modification in the abundance, richness and composition of Odonata assemblages, in relation to environmental modifications, are already well known (Alves-Martins et al. 2024). Another important aspect of Odonata biology addresses the ecological thresholds exhibited by some species due to their different responses to environmental gradients (e.g., air temperature, canopy cover, habitat integrity) (Miguel et al. 2017; Faria et al. 2021). Then work on thresholds, would enable discussion of the environmental gradient that is supported by the community (Miguel et al. 2017), as well as thinking about strategies for restoration or mitigation of environmental impacts. In addition, assessing which species can be considered sensitive or tolerant to changes in the environment will assist in environmental quality assessments, in the development of monitoring protocols and also in extension actions, scientific dissemination and environmental education.

Our study aims to underscore the importance of a protected area (a Brazilian national park) in conserving the diversity of Amazonian Odonata species. We hypothesize that 1) streams inside the protected areas have higher diversity metrics of Zygoptera (species richness, abundances, proportions and composition), when compared to Anisoptera; 2) streams outside the protected area show the inverse pattern observed streams inside, regarding Anisoptera; 3) habitat integrity is the most important environmental variable explaining the Odonata assemblage structure and presents significative influence on Anisoptera and Zygoptera diversity metrics; and 4) distinct ecological thresholds exist for Anisoptera and Zygoptera in response to habitat integrity gradient, particularly among Zygoptera species associated to high habitat integrity.

Material and methods

Study area

The study encompasses 25 streams situated within (n = 12) and outside (n = 13) the boundaries of the Jamanxim National Park (5°52′20″ S, 55°59′0″ W), established in 2006, spanning an area of approximately 858,860.00 hectares. The points sampled are located between the municipalities of Trairão and Itaituba in the southwest of the state of Pará, Brazil (Figure 1). The region presents a tropical rainy climate with dry winters (December to June) according to the

Köppen and Geiger classification (1928). The sampling sites comprised small streams ranging from 1st to 3rd order (according to Strahler's 1957 classification), within a climate classified as *Am*. Data collection occurred between September and October 2022, corresponding to the dry season in the region, facilitating stream access and ensuring greater stability for aquatic communities.



Figure 1: Map of the study area of the points sampled inside and outside the Jamanxim National Park, Pará state, Brazil.

Biological sampling procedures

Biological sampling of Odonata specimens were carried out in each stream between 10:00 AM to 02:00 PM, coinciding with the peak activity period of these organisms (Oliveira-Junior et al. 2019b; Batista et al. 2021). Within each stream, a 150 m stretch was delimited, subdivided into 10 sections of 15 m each, sampled from the downstream to the upstream portions (Peck et al. 2006; Monteiro-Junior et al. 2016). We applied the fixed-area scan procedure (Batista et al. 2021) using an entomological net (diameter: 40 cm; depth: 65 cm) affixed to a 90-cm aluminum pole (Figure 2). The specimens were stored in paper envelopes and immersed in a container with acetone P.A. (pure for analysis) for up to 12 hours, following Lencioni (2006) protocols. The taxonomic identification was conducted at the Laboratory of Ecology

and Conservation at Universidade Federal do Pará, utilizing specific identification keys (Garrison et al. 2006, 2010; Lencioni 2005, 2006), supplemented by consultation with specialists whenever possible.



Figure 2. Representation of the division of the sections following the delineation of the 150-meter stream reach. (A) Marking segments with a tape measure, (B) employing the active sweeping collection method with nets (C) storing collected individuals in parchment paper to prevent damage to identification structures, (D) individual identification cards.

Abiotic sampling procedures

Four limnological variables were measured in each stream using specific sensors: conductivity (mS /cm), dissolved oxygen (mg/L and %) and pH. Additionally, we also measured variables related to the physical structure of the streams, including depth (cm) and width (m) of the channel and canopy cover (%). Depth and width were measured using a metric PVC pipe, while the canopy cover was assessed using a spherical densiometer (Bastos et al. 2021). We employed the Habitat Integrity Index (HII; Nessimian et al. 2008; Brasil et al. 2020b) to assess the physical conditions within and

surrounding each stream. Comprising 12 weighted items, which include characteristics such as the structure of the riparian vegetation, the width of the riparian forest and the frequency of gullies. The HII ranges from 0 (highly degraded) to 1 (highly preserved), and has been widely utilized in Amazonian streams across different taxonomic groups (Oliveira-Junior et al. 2015; Enríquez-Espinosa et al. 2020; Bastos et al. 2021).

Statistical analyses

Each stream sampled in the present study represents a sampling unit. To describe the relative importance of the environmental variables in shaping the overall pattern, we conducted Principal Components Analysis (PCA; Borcard et al. 2018). Prior to carrying out the PCA, all variables, except pH, were standardized (Borcard et al. 2018). To determine which axes to retain, we applied the Broken-Stick criteria (Borcard et al. 2018).

To assess possible differences in habitat integrity, species richness, abundance proportion of species richness and abundance between streams inside and outside the Jamanxim National Park, we carried out student T tests (Zar 2010). Species richness and abundance proportions of Odonata communities have been utilized in previous ecological studies in Brazilian biomes (Oliveira and Juen 2019; Ribeiro et al. 2021) to gauge their responses to environmental modification gradients, highlighting the robustness of Zygoptera and Anisoptera proportions in reflecting environmental gradients on both groups (Oliveira-Junior and Juen 2019; Ribeiro et al. 2021). In cases where assumptions of normality and homogeneity of variances were violated, we conducted the student t test for heterogeneous variances (Zar 2010).

Furthermore, we employed generalized linear mixed models (GLMM; Zuur et al. 2009) to evaluate the effects of the habitat integrity on Anisoptera and Zygoptera species richness, abundance and their proportions, with stream category (inside and outside the national park) treated as a random variable. Poisson distribution was used for counting biological metrics (abundance and species richness), with logarithmic transformation (log10 +1) applied in cases of overdispersion detected to normalize the response variable (Zuur et al. 2009). For proportions of abundance and species richness, we applied the binomial distribution and quasi-binomial distribution in cases of overdispersion detected (Zuur et al. 2009), with assumptions checked using the '*DHARMa*' package (Hartig and Hartig 2017).

A Permutation Multivariate Analysis of Variance (PERMANOVA; Anderson 2001) was conducted to explore differences in Odonata species composition between streams inside and outside the national park, with significance determined through Monte Carlo randomizations using 9999 permutations (Anderson 2001). Additionally, Multivariate Dispersion Analysis (PERMDISP; Anderson 2006) was performed to assess the homogeneity of variance between assemblages of both categories (inside and outside the national park).

Finally, we applied Threshold Indicator Taxa Analysis (TITAN) (Baker and King 2010) to identify the ecological thresholds of Odonata assemblages and species in relation to the HII. This analysis, based on the Indicator species index (IndVal), which estimates the specificity (relative abundance) and fidelity (relative frequency) (Dufrêne and Legendre 1997) of the species, and associates the taxa with continuous environmental scores, creating confidence limits and identifying positive (Z+) and negative (Z-) points of change in the taxa according to the variable tested (Baker and King 2010). For this analysis, we considered only the taxa that occurred in five or more streams and with five or more individuals, given that taxa with low frequencies do not provide accurate estimates of the z score (Baker and King 2010). We used the bootstrap procedure (n = 1000) to calculate the confidence limits (5–95%) of the change point of each taxon along the environmental gradient, which was also used to evaluate the quality of the response of each taxon by measuring purity and reliability (Baker and King 2010). Purity is the proportion of the directions (z +or z -) of the change point response among the bootstrapping replicates that are similar to the observed response, while reliability is estimated by the proportion of change points obtained in the bootstrapping in which the Ind-Val scores have significant p values (Baker and King 2010). In this analysis, the taxa were considered tolerant or sensitive if they had purity values ≥ 0.95 and reliability ≥ 0.95 .

All the analytical procedures were conducted in R software (R Core Team 2022; version 4.3.2), using packages '*car*' (Fox and Weisberg 2020), '*vegan*' (Oksanen et al. 2020), '*TITAN2*' (Baker and King 2010). Graphs we generated using the '*ggplot2*' package (Wickam 2016).

Results

Biological overview

A total 530 of individuals and 43 species of Odonata were sampled, comprising 16 from the Anisoptera suborder and 27 from the Zygoptera suborder. The predominant Anisoptera family was Libellulidae, encompassing 15 species, while Aeshnidae was represented by only one species (Table 1). Among the Zygoptera family, Coenagrionidae predominantly with 14 species, followed by Calopterygidae, with seven species (Table 1). The national park Streams located inside the national park presented 205 individuals and 23 species, five species of Anisoptera and 18 species of Zygoptera.The national park Streams located outside the national park presented 325 individuals and 36 species, 15 species of Anisoptera and 21 species of Zygoptera the national park. These streams harbored the highest number of exclusive Anisoptera n= 11 and Zygoptera n= 9 species (Figure 3).

 Table 1: Species and abundance collected in streams inside and outside the Jamanxim National Park, Pará state, Brazil.

 LC: Least Concern ND: Data Deficient.

Subordors/Familias/Spacios		Abundance		
Suborders/Families/Species		Status		
ANISOPTERA	Inside park	Outside park	Total	
Aeshidae				
Staurophlebia wayana Geijskes, 1959	2	0	2	LC
Libellulidae				
Diastatops intensa Montgomery, 1940	0	2	2	LC
Elasmothemis cannacrioides (Calvert, 1906)	0	1	1	LC
Erythrodiplax basalis (Kirby, 1897)	0	18	18	LC
Erythrodiplax sp.	0	1	1	-
Fylgia amazonica amazonica Kirby, 1889	1	5	6	LC
Macrothemis sp.	0	1	1	-
Micrathyria sp.	0	1	1	-
Oligoclada abreviata abreviata Rambur, 1842	2	11	13	LC
Oligoclada monosticha Borror, 1931	0	2	2	LC
Oligoclada rhea Ris, 1911	0	1	1	DD
Oligoclada risi Geijskes, 1984	0	2	2	LC
Oligoclada walkeri Geijskes, 1931	3	3	6	LC
Orthemis concolor Ris, 1919	0	2	2	LC
Perithemis lais Perly, 1834	1	3	4	LC
Perithemis thais Kirby, 1889	0	4	4	LC
ZYGOPTERA				
Calopterygidae				
Hetaerina amazonica Sjöstedt, 1918	3	7	10	LC
Hetaerina auripennis Burmeister, 1839	17	8	25	LC
Hetaerina laesa Hagen in Selys, 1853	1	0	1	LC
Hetaerina moribunda Hagen in Selys, 1853	0	2	2	LC
Mnesarete aenea (Selys, 1853)	5	0	5	LC
Mnesarete cupraea (Selys, 1853)	14	40	54	LC
Mnesarete smaradgina (Selys, 1869)	6	10	16	LC
Coenagrionidae				
Acanthagrion apicale Selys, 1876	0	17	17	LC
Acanthagrion ascendens Calvert, 1909	0	1	1	LC
Argia collata Selys, 1865	69	13	82	LC
Argia infumata Selys, 1865	0	1	1	LC
Argia oculata Hagen, 1865	8	18	26	LC
Argia tinctipennis Selys, 1865	2	37	39	LC
Epipleoneura lamina Williamson, 1915	33	13	46	LC

Epipleoneura metallica Racenis, 1955	3	55	58	LC
Epipleoneura westfalli Machado, 1986	0	5	5	LC
Neoneura confundens Wasscher & Van't Bosch, 2013	0	6	6	DD
Neoneura denticulata Williamson, 1917	5	0	5	LC
Neoneura luzmaria De Marmels, 1989	0	6	6	LC
Phoenicagrion flammeum Selys, 1876	0	3	3	LC
Protoneura tenuis Selys, 1860	4	0	4	LC
Dicteridadae				
Heliocharis amazona Selys, 1853	1	3	4	LC
Megapodagrionidae				
Heteragrion bariai De Marmels, 1989	0	8	8	LC
Heteragrion ictericum Williamson, 1919	1	6	7	LC
Heteragrion silvarum Sjöstedt, 1918	5	0	5	LC
Perilestidae				
Perilestes solutus Williamson & Williamson, 1924	1	0	1	LC
Polythoridae				
Chalcopteryx rutilans Rambur, 1842	17	9	26	LC
Total	205	325	530	



Figure 3. Venn diagram illustrating the exclusivity and overlap of Odonata species between streams located outside and inside and Jamanxim National Park, Pará state, Brazil.

Environmental influence on Odonata communities

The first two axes of the PCA explained 53.87% of the total variance concerning the environmental variables (Table 1; Figure 4). Dissolved oxygen (mg/L), HII and pH contributed negatively to the formation of the first axis (Table 2; Figure 3). For the second axis, air temperature, depth and width of the channels were the ones more important, contributing positively to it (Table 2; Figure 3).

Table 2. Results of the Principal Components Analysis (PCA) performed on the environmental variables, in Jamanxim National Park, Pará state, Brazil. air.temp = air temperature; cond = water conductivity; do_mgl = dissolved oxygen (mg/L); depth and width of the channels; canopy = canopy cover; HII = Habitat Integrity Index; pH = potential of hydrogen.

	PCA1	PCA2
Temp	0.586	0.592
Cond	-0.409	0.198
DO_mgl	-0.730	0.222
Depth	0.114	0.750
Width	0.074	0.906
Wood	-0.059	-0.391
Canopy	-0.614	0.115
ни	-0.778	0.039
рН	-0.688	0.260
%	32.13	20.79
Broken-Stick	2.82	2.51
Eigenvalues	2.05	1.82



Figure 4. Principal Components Analysis (PCA) was applied to the environmental variables measured inside and outside the Jamanxim National Park, Pará state, Brazil.

The GLMM revealed responses of Anisoptera abundance, Zygoptera abundance, and Zygoptera species richness to the habitat integrity gradient (Table 3). Furthermore, there was a negative association observed between Anisoptera abundance and the habitat integrity gradient (Table 3 and Figure 5A), whereas positive relationships were identified between Zygoptera abundance and species richness with habitat integrity (Table 3 and Figures 5B and 5C).

 Table 3. Results of General Linear Mixed Model (GLMM) evaluating the effects of habitat integrity index (HII) on the abundance, species richness and proportions of Anisoptera and Zygoptera, in Jamanxim National Park, Pará state, Brazil.

Parameters	Estimate	Std. error	p-value
Anisoptera			
Abundance	-2.783	0.589	>0.001
Zygoptera			
Abundance	1.837	0.363	>0.001
Species richness	1.441	0.725	0.047



Figure 5. Relationships between the abundance (N) and species richness (S) of Anisoptera (A) and Zygoptera (B and C) in relation to the habitat integrity index (HII) of the streams sampled in the national park of Jamanxim, Pará state, Brazil.

There were significant differences only regarding the Anisoptera abundance when we compared streams inside and outside the national park (t_{homogeneous variance} = 2.194; df = 12.689; p = 0.047). On average, streams outside the national park presented an additional five individuals of Anisoptera than streams located inside of it (Figure 6).



Figure 6. Boxplots illustrating the differences in Anisoptera abundance (N) between streams inside and outside the Jamanxim National Park, Pará state, Brazil.

There was a significant difference of heterogeneity between treatments concerning the species composition of Anisoptera (Pseudo-F = 4.381; df = 1; p = 0.047) (Figure 7A). However, no difference in species composition was observed (Pseudo-F = -0.078; df = 1; p = 0.941). On the other hand, we found significant differences regarding the species composition of Zygoptera between treatments (Pseudo-F = 3.035; df = 1; p = 0.003) (Figure 7B). Unlike Anisoptera, no significant results were observed regarding the heterogeneity of assemblages between treatments (Pseudo-F = 2.370; df = 1; p = 0.137).



Figure 7. Principal Coordinates Analysis (PCoA) applied to the species composition of Anisoptera (A) and Zygoptera (B) samples in the Jamanxim National Park, Pará state, Brazil.

The TITAN analysis revealed that only Zygoptera species exhibited a significant response to the habitat integrity gradient, at the community level, taxa with positive responses (Z+) demonstrated an increase at 0.60 and 0.80 (Figure 8A). In addition, three species showed a positive association (Z+) (Figure 8B).



Figure 8. Threshold indicator Analysis (TITAN) is applied to the community level (A) and species levels (B). The analysis retained only the positive responses (Z+) from Zygoptera species.

Discussion

In this study our hypotheses were partly supported. Concerning the first hypothesis, only Anisoptera abundance showed significant variation between streams inside and outside the Jamanxim National Park. While there were no differences in Anisoptera species composition, notable dissimilarities were observed in Zygoptera composition between streams inside and outside the national park. Regarding the second hypothesis, there was a difference only in Anisoptera abundance between the treatments. Finally, only Zygoptera species exhibited a response to the habitat integrity gradient, partially aligning with our hypotheses and consistent with previous research.

In Brazil, the Sistema Nacional de Unidades de Conservação – SNUC (Law 9885 of July 18, 2000) categorizes conservation units into two main groups: Sustainable Use Units (SUs) aimed at balancing nature conservation with the sustainable use of natural resources, and a Full Protection Units (FPUs), where only indirect use of natural resources is allowed. Considering that the Jamanxim National Park falls within the FPU group, it was expected that points within the park would exhibit values closer to the maximum value of the habitat integrity index (HII) compared to points outside the park. However, no significant difference in HII values was observed between treatments within (0.762±0.088) and outside (0.637±0.187; mean±standard deviation) the park. Notably, the highest HII value was recorded outside the park (0.907), with the highest value within the park being 0.875. Furthermore, out of the total of 15 UAs within the park, nine presented HII values below 0.8 (Supplementary Material), which according to the literature, may indicate an intermediate state of environmental degradation (Cabette et al. 2017).

The relatively low values of HII within the Jamanxim National Park observed in the study may be attibuted to three primary factors. Firstly, the park is effectively bisected by BR-163, which serves as a significant conduit for regional production flow, connecting the northern and southern regions of the country (Rodrigues and Nahum 2023). Secondly, there is an approximately 7,098 hectares area within the central region of part that is not part of the park, where anthropogenic activities such as pasture and monoculture were observed. Notably, these activities encroach upon the park boundaries in some instances (Supplementary Material). Finally, six points within the park (or 40% of the total) are tributaries of the Arari River, the main tributary of the Jamanxim River, which sustains several riparian families through fishing, river transportation, hunting, and recreation.

This complex landscape may complicate the evaluation of the park's effectiveness in maintaining Odonata communities within the framework used in this study. While no significant difference in species richness between samples

inside and outside the park was observed, differences in species composition were noted, consistent with previous studies. The decrease in Anisoptera abundance with increasing HII may be attributed to the necessity of many Anisoptera species for open areas conducive to thermoregulation. In the Amazonian forest, streams of first order with lower HII often feature more open canopy or narrower riparian vegetation, facilitating activities and metabolism favored by heliothermic Anisoptera species (Oliveira-Júnior and Juen 2019; Miguel et al. 2017; Bastos et al. 2021; Calvão et al. 2023). This pattern aligns with findings from other studies on Odonata in Amazonian streams (Monteiro Junior et al. 2013), as well as in streams in areas subjected to intensive livestock grazing and agriculture (Carvalho et al. 2018). This response also can be attributed to variations of the degrees of alteration in aquatic environments and their adjacent habitats, such as the loss of riparian vegetation and siltation leading to a change from organic substrates in channels, among other factors. These modifications create conditions to promote the successful colonization of Anisoptera species, which are typically more adaptable in open areas (Monteiro-Junior et al. 2013; Calvão et al. 2018; Carvalho et al. 2018).

Compositional variation analysis revealed high diversity in Anisoptera across areas inside and outside the park, reflecting their dispersal capacity and colonization of open or disturbed environments (Clausnitzer et al. 2009). Conversely, Zygoptera exhibited no richness and abundance differences inside and outside the park but displayed composition variations, suggesting the occupation of sites by species with distinct requirements and tolerances. Thus, species sensitive to alteration are present in streams with vegetation, and more generalist species occupy streams with some degree of alteration (Juen et al. 2014; Miguel et al. 2017).

The positive response of Zygoptera species to habitat integrity may suggest their dependency one more conserved environments, advancing our understanding of these species needs. Among the species that showed a positive response to habitat integrity were the individuals of *Hetaerina auripennis* (Calopterygidae). This may have occurred due to the specificity of organic substrates (Assis et al. 2004). What can explain this increase of species in places with good habitat conditions, *Argia collata* (Coenagrionidae), *Epipleoneura lamina* (Coenagrionidae), this species so far is distributed by the Amazon biome, and other studies describe its occurrence in well-shaded streams with small widths and depths and the presence of organic substrate (Pessacq et al. 2014; Carvalho-Soares et al. 2022). Representatives of the family Coenagrionidae are classified as endophytic in terms of oviposition, requiring organic substrates for oviposition and the presence of perches for males that can spend many hours at mating sites (Calvão et al. 2021; Corbet and May 2008).

The results of the study reinforce the importance of riparian vegetation for the success of indicator species and highlight their potential use as bioindicators of environmental quality, particularly since at values above 0.65 of the habitat integrity index. This approach may be useful in aquatic monitoring programs given the ease of capturing these insects and the use of the HII, such as the MONITORA program carried out by ICMBio in partnership with researchers from

different institutions in the country's Conservation Units (Brasil et al. 2020). It is worth noting that conservation actions focused on aquatic ecosystems directly benefit terrestrial ecosystems (Leal et al. 2020). This is especially important considering the applications of Systematic Conservation Planning - SCP (Kirkpatrick 1983), which selects areas for conservation based on the ability of a given region to sustain the ecological niche of the largest possible number of species, commonly referred to as the principle of complementarity (Adams et al. 2019). Furthermore, we suggest that future studies evaluating the effectiveness of protected areas consider other aspects of communities such as phylogenetic, functional, and/or ecological interactions diversity. These aspects may reflect intrinsic evolutionary processes, as well as community stability (Eisenhauer et al. 2023; Tylianakis et al. 2010; Valdovinos 2019).

Acknowledgment

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (process: 428961/2018-5) through the project "Diminuindo as lacunas Lineanas e Wallaceanas da biota aquática na Amazônia"; by Fundação Amazônica de Amparo a Estudos e Pesquisa do Pará – FAPESPA, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (processes: 068/2020 and 2019/25445-1), through the project "Padrões de distribuição da biodiversidade aquática no Estado do Pará" and part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. We are grateful to ICMBio and the Monitora Program that allowed the collection of the data at the Amazônia National Park. JSB thanks CNPq for the doctoral scholarship in Brazil (process: 141113/2020-0) and LJ, LC and JMBOJ are grateful to CNPq for productivity grants (grants 304710/2019-9, 304403/2021-0, and 307808/2022-0, respectively).

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Supplementary Material



Figure 9. Map showing the HII values of the points sampled inside and outside the Jamanxim National Park.