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JOÁS DA SILVA BRITO

Padrões de diversidade e co-ocorrência de assembleias de libélulas (Odonata; Insecta) na Amazônia Brasileira

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PADRÕES DE DIVERSIDADE E CO-OCCORRÊNCIA DE ASSEMBLEIAS DE LIBÉLULAS (ODONATA; INSECTA) NA AMAZÔNIA BRASILEIRA

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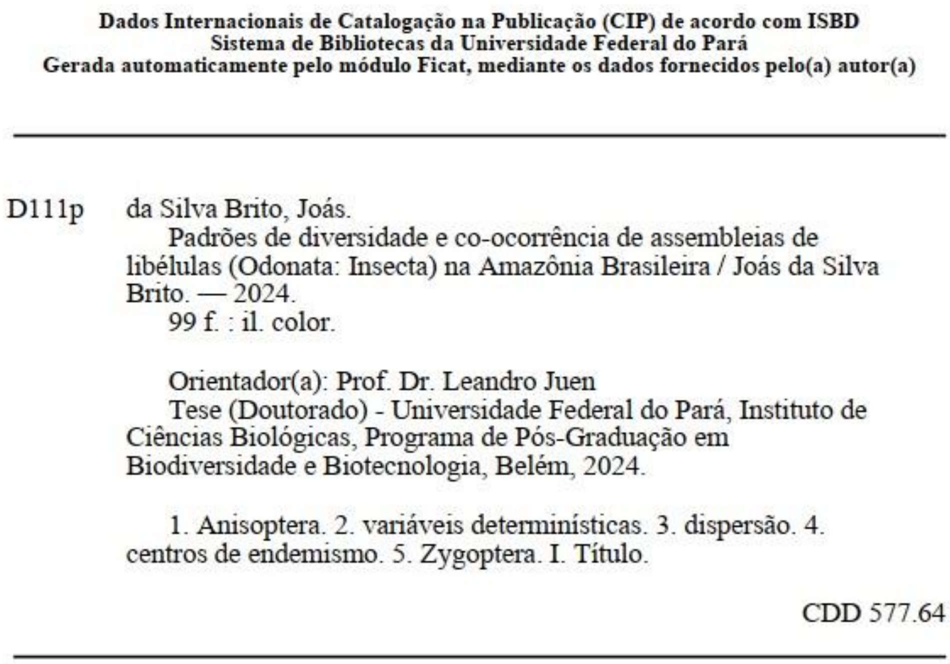

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"*A imaginação muitas vezes nos leva a mundos que nunca sequer existiram. Mas sem ela não vamos a lugar algum.*" (Carl Sagan)

Sumário

Resumo Geral

Riachos possuem características únicas, com uma grande dependência da vegetação ripária e a configuração dendrítica hierárquica das bacias de drenagens, variação longitudinal nas condições físicas, permitem a manutenção de uma alta biodiversidade, desproporcional à área que ocupam na superfície terrestre. Os insetos aquáticos são uns dos principais componentes bióticos nos sistemas aquáticos de riachos, atuando como predadores e presas nas redes tróficas, bem como na ciclagem de nutrientes. Entretanto, as alterações de natureza humana, relacionadas principalmente à exploração de recursos naturais para a obtenção de energia, produção de alimentos, e exportação de *commodities*, vem alterando profundamente os sistemas aquáticos, alterando as condições de micro-hábitat para esses organismos. Esse cenário é ainda mais preocupante para a Amazônia, uma vez que a biodiversidade desse bioma é altamente subestimada. Uma das maneiras de mitigar os impactos das modificações humanas é a implementação de áreas protegidas, que visam manter as florestas e os sistemas aquáticos nelas inseridos, mantendo assim a biodiversidade, aliando com uso sustentável dos recursos naturais. A ordem Odonata, composta por insetos alados popularmente chamados de libélulas, é um dos grupos de insetos aquáticos mais utilizados no monitoramento ambiental, principalmente devido às diferenças ecológicas e fisiológicas entre as suas duas subordens neotropicais, Anisoptera e Zygoptera. Assim, os principais objetivos da presente tese foram investigar quais os principais preditores abióticos e bióticos de comunidades de libélulas em riachos da Amazônia Brasileira, avaliando os padrões de diversidade e de co-ocorrência em áreas protegidas de diferentes categorias de proteção. Para atingir os objetivos a tese foi dividida em três capítulos:

1) No primeiro capítulo buscamos avaliar a importância relativa de processes de dispersão e nicho sobre comunidades de libélulas distribuídas ao longo de seis centros de endemismo na Amazônia Brasileira. Além disso, buscamos investigar o papel exercido por traços funcionais relacionados à dispersão sobre as distribuições latitudinais e longitudinais das espécies de libélulas;
2) No segundo capítulo investigamos a diversidade de libélulas em áreas protegidas de uso sustentável, traçando comparação com riachos amostrados fora dessas áreas. Além disso, investigamos as principais variáveis preditoras, fossem elas ambientais ou espaciais;
3) No terceiro capítulo investigamos padrões de co-ocorrência, e quais variáveis ambientais influenciavam esses padrões, da família de Zygoptera Calopterygidae, amostradas em riachos dentro e fora de áreas protegidas.

Ao todo utilizamos dados bióticos e abióticos amostrados em 280 riachos da Amazônia Brasileira, variando de primeira à terceira ordens, utilizando o método de coleta de varredura por áreas fixas. Nossos resultados indicaram que as diferenças já conhecidas entre as subordens de libélulas se refletiram nos padrões encontrados. Na escala biogeográfica, Anisoptera apresentou influência relativa conjunta de processos de dispersão e nicho, ao passo que Zygoptera mostrou forte influência de processos de dispersão, algo que se refletiu também nos resultados dos traços funcionais de dispersão, podendo ser um indício de limitação de dispersão imposto pelos grandes rios Amazônicos. Considerando as comunidades de libélulas amostradas em áreas protegidas, Zygoptera demonstrou ser um grupo mais sensível às modificações humanas que se refletem nas condições limnológicas e estruturais dos riachos. Também verificamos que algumas áreas protegidas abordadas podem estar perdendo sua capacidade de manter a diversidade local e integridade de hábitat. Foram verificadas mais co-ocorrências negativas significativas dentro de áreas protegidas do que em áreas adjacentes, o que pode ser um indicativo de que as primeiras estão mantendo redes tróficas mais complexas, considerando que avaliamos espécies de uma mesma família, mais próximas evolutivamente. Assim, os capítulos que compõe a presente tese demonstraram a importância da investigação dos principais processos estruturantes das comunidades de libélulas, relacionados à dispersão ou condições ambientais, e como tal arcabouço teórico pode ser aplicado na ecologia da conservação.

Palavras-chave: Anisoptera; biomonitoramento; Calopterygidae; dispersão; centros de endemismo; variáveis determinísticas e estocásticas; Zygoptera

Introdução Geral

Os sistemas aquáticos de água doce são únicos, devido primariamente a sua estruturação dendrítica e hierárquica, que varia desde poucos centímetros a metros (no caso de riachos) até dezenas de metros (no caso dos grandes rios) (Tonkin et al. 2018; Allan et al. 2021). Ao longo da organização hierárquica desses sistemas ocorre intensa variação em características estruturais, como largura e profundidade dos canais, estruturação das margens, cobertura vegetal, e entrada de luz solar, o que influencia a produtividade primária (Vannote et al. 1980; Dosskey et al. 2010; Tonkin et al. 2018; Dala-Corte et al. 2019). Devido a essa variação de condições locais e de paisagem ao longo do percurso desses sistemas, eles são capazes de suportar uma alta diversidade de espécies aquáticas, disproporcional ao tamanho que eles ocupam na superfície da Terra (Tonkin et al. 2018). Espécies de insetos aquáticos, peixes e plantas aquáticas ocupam diferentes porções desses sistemas aquáticos, sendo filtradas primariamente pelas condições abióticas, mas também pelas relações intra e inter específicas (Tonkin et al. 2018; Allan et al. 2021). Variáveis abióticas relacionadas à estruturação física, por exemplo, têm grande influência sobre as condições de microhábitat (ex., temperatura local, tipos de substrato, sítios de reprodução), atuando como filtros ambientais, filtrando quais espécies são capazes de sobreviver e estabelecer suas populações nesses locais (Hutchinson 1959; Dala-Corte et al. 2019). As relações bióticas também têm um grande papel na distribuição das espécies ao longo desses sistemas, estando relacionadas às características funcionais de cada uma (ex., tamanho corporal total, proporções corporais, comportamentos, fisiologia), e a presença de potenciais competidores, o que influencia fortemente os padrões de co-ocorrência (Abrams 1983). Fatores abióticos importantes são as condições em escala biogeográfica, como presença de regiões montanhosas, grandes corpos de água e regiões de transição entre diferentes biomas (Carvalho et al. 2022; Pelinson et al. 2022). Tais condições em macroescala tem papel de limitadores de dispersão das espécies, e por vezes podem até mascarar os efeitos locais de variáveis ambientais, por exemplo, levando a uma grande dificuldade de se diferenciar os principais preditores das comunidades aquáticas (Pelinson et al. 2022).

Na Amazônia Brasileira, os fatores abióticos em escala biogeográfica, como a presença de grandes rios, tem profundo papel modelador sobre as comunidades aquáticas (Silva et al. 2005; Brasil et al. 2018; Alves-Martins et al. 2019), e mesmo sobre as comunidades de vertebrados terrestres (Ribas et al. 2012). Esses grandes corpos hídricos, cujas margens por vezes estão separadas por quilômetros de distância, tem profundo impacto sobre a dispersão de espécies aquáticas, como invertebrados e peixes, criando assim os chamados centros de endemismo Amazônicos (Silva et al. 2005). Por vezes as condições ambientais de ambas as margens dos rios são adequadas para o estabelecimento das populações das mesmas espécies, entretanto, devido à presença de um grande rio, as espécies não conseguem transpor essa barreira, e consequentemente ficam restrita a um centro de endemismo (Silva et al. 2005; Ribas et al. 2012; Brasil et al. 2018; Carvalho et al. 2022). Em escalas mais locais e regionais, os fatores abióticos tem uma atuação mais forte, pois têm impacto mais direto sobre as comunidades aquáticas, moldando e filtrando aquelas espécies que poderão sobreviver e estabelecer suas populações (Hutchinson 1959; Brasil et al. 2020). Nesse contexto, as modificações de origem humana, relacionados à agricultura, pecuária, mineração, urbanização e exploração madereira, podem ter forte impacto sobre esses fatores abióticos dos sistemas aquáticos, alterando variáveis limnológicas, estruturação física das margens e cobertura vegetal, conectividade entre as manchas de hábitat, impactando a diversidade aquática (Tonkin et al. 2018).

Os insetos aquáticos são importantes componentes das redes tróficas em sistemas aquáticos de riachos, atuando como presas e predadores (Calvão et al. 2020), bem como na ciclagem de nutrientes (Colas et al. 2017). Possuem uma alta diversidade taxonômica e funcional nesses sistemas justamente por possuírem diversas características morfológica, fisiológicas e ecológicas que permitem a sobrevivência e estabelecimento de suas populações nesses sistemas (Brasil et al. 2020). Alterações ambientais que modifiquem as condições microclimáticas e de conectividade tem profunda influência sobre esses organismos, haja vista que eles possuem distintas respostas a tais modificações. Alguns gêneros das ordens Ephemeroptera, Plecoptera e Trichoptera, por exemplo, estão intimamente associados a ambientes altamente florestados, que apresentam maior quantidade de oxigênio dissolvido na água, condições estáveis de acidez e condutividade (Shimano & Juen 2016; Lima et al. 2022; Rivera-Peréz et al. 2022). Assim, qualquer alteração ambiental, relacionada à remoção de matas ciliares, ou entrada de poluentes, altera as condições limnológicas e físicas desses ambientes, influenciando as espécies mais sensíveis, enquanto pode favorecer outros grupos taxonômicos (Brasil et al. 2020). As subordens de libélulas Anisoptera e Zygoptera (Odonata) também possuem características morfológicas e fisológicas distintas, que fazem com que apresentem respostas distintas às condições ambientais, e também às alterações disruptivas de natureza humana (Miguel et al. 2017). As libélulas da subordem Anisoptera possuem maior tamanho corporal, e são geralmente classificadas como heliotérmicas (necessitam de alta incidência de luz solar para regular a temperatura corporal) ou endotérmicas (são capazes de controlar a temperatura corporal), estando mais relacionadas a ambientes ensolarados, que geralmente são mais abertos (Corbet 1999; Oliveira-Junior et al. 2015). Por outro lado, as libélulas da subordem Zygoptera possuem menor tamanho corporal, corpos mais delgados, e geralmente são conformadoras termais, necessitando de condições mais estáveis de temperatura, mais comuns em locais com maior cobertura vegetal (Corbet 1999; Oliveira-Junior et al. 2015). Tais diferenças para libélulas também se refletem em seus padrões de dispersão, que se deve principalmente aos aspectos fisiológicos e características morfológicas funcionais, tais como tamanho corporal, asas e proporções das asas, aspectos dos abdomens e tórax (Brasil et al. 2018; O'Malley et al. 2020; Wootton 2020). A presença de grandes rios, funcionando como barreiras à dispersão das espécies de libélulas, tem influências diferentes sobre ambas as subordens, com o signal de limitação à dispersão mais fraco para Anisoptera, e mais forte sobre Zygoptera (Juen & De Marco 2012; Brasil et al. 2018; Alves-Martins et al. 2019). Nesse contexto, fatores mais estocásticos, como dispersão através de bancos de macrófitas, eventos hidrológicos como secas prolongadas, teriam uma atuação maior sobre os padrões de dispersão das comunidades de libélulas (Juen & De Marco 2012), mas com efeitos diferentes sobre ambas as subordens de libélulas.

As subordens de libélulas são modelos eficientes para estudos ecológicos que objetivam investigar a influência de fatores abióticos relacionados ao ambiente e espaço, para avaliar padrões de das comunidades e seus principais preditores (Brasil et al. 2020; Bried et al. 2022). As respostas e resultados obtidos aumentam o conhecimento sobre a distribuição das espécies, diminuindo as lacunas relacionadas às suas identidades, variação temporal e espacial, e mesmo suas distribuições geográficas (Brasil et al. 2021). Considerando que os sistemas naturais da Amazônia Brasileira estão sob intensa pressão das atividades humanas, a aplicação desses conhecimentos ecológicos teóricos dentro de uma perspectiva de conservação da biodiversidade é fundamental (Brasil et al. 2020). O uso de bioindicadores, como as subordens de libélulas, auxilia no processo de compreensão do que está impactando as comunidades aquáticas, quais são as variáveis ambientais mais influenciadas pelas modificações humanas, e como isso leva à diminuição da biodiversidade (Brasil et al. 2020). Com essas informações em mãos, os tomadores de decisão podem avaliar onde aplicar os recursos destinados às questões de conservação da biodiversidade, sejam elas relacionadas a fiscalização, aplicação de multas, políticas sociais, ou mesmo a criação de novas áreas protegidas.

Assim, a presente tese é composta por três capítulos que possuem objetivos distintos mas complementares: 1) investigar padrões biogeográficos das comunidades de libélulas em riachos da Amazônia Brasileira, relacionados primariamente a processos de dispersão e nicho, e também avaliar a importância de traços funcionais das espécies para seus *ranges* latitudinais e longitudinais; 2) investigar padrões de diversidade (abundância de indivíduos, composição e riqueza de espécies) de libélulas em riachos amostrados dentro e fora de áreas protegidas de uso sustentável no estado do Acre, na Amazônia Brasileira; e 3) analisar padrões de co-ocorrência de espécies da família Calopterygidae (Zygoptera) em riachos amostrados dentro e fora de áreas protegidas no estado do Pará, na Amazônia Brasileira. Em todos os capítulos da tese buscamos avaliar como os fatores abióticos moldavam as comunidades de libélulas, de maneira mais téorica no primeiro capítulo, e mais aplicada à ecologia da conservação nos capítulos dois e três.

O primeiro capítulo intitulado ***Odonata responses to dispersal and niche processes differ across Amazonian endemism regions*** está atualmente na segunda rodada de revisão, pelos revisores anônimos, na revista internacional *Insect Conservation and Diversity*. O segundo capítulo intitulado ***Main drivers of dragonflies and damselflies (Insecta; Odonata) metacommunities in streams inside protected areas in the Brazilian Amazon*** foi aceito para publicação na revista internacional *Environmental Monitoring and Assessment* no dia 12/02/2024. O terceiro capítulo entitulado ***Co-occurrence and ecology of Calopterygidae species (Insecta; Odonata; Zygoptera) in streams in Amazonian protected areas*** está atualmente na primeira rodada de revisão, pelos revisores anônimos, na revista internacional *Ecological Entomology*.

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1º Capítulo: ODONATA RESPONSES TO DISPERSAL AND NICHE PROCESSES DIFFER ACROSS AMAZONIAN ENDEMISM REGIONS

Abstract

Niche and dispersal-based processes govern freshwater communities, such as aquatic insects, shaping their distribution and establishment in the environment. So, we aimed to address the relative influence of niche and dispersal-based processes on Odonata diversity in Amazonian freshwater systems, and the influence of species dispersal functional traits on their longitudinal and latitudinal ranges. We used Dispersal-Niche Continuum Index to address the relative importance of niche and dispersal-based processes for Odonata communities across six Amazonian endemism regions. Moreover, we addressed the influence of Odonata dispersal functional traits on their longitudinal and latitudinal ranges through generalized linear models. We found that more distant regions had lower values of pairwise Dispersal-Niche Continuum, mainly for Zygoptera, corroborating our first hypothesis. Moreover, Zygoptera also presented the lowest absolute values of Dispersal-Niche Continuum and Anisoptera presented a joint influence of niche and dispersal-based processes, agreeing with our second hypothesis. Only Zygoptera presented a significant association between dispersal functional traits and latitudinal and longitudinal ranges, corroborating our third hypothesis. Our results indicated a prevalence of dispersal-related processes for Zygoptera, which can be explained by the presence of massive geographical barriers, such as the rivers, and their narrow physiological and ecological tolerance. Moreover, dispersal-related traits demonstrated significant influence on longitudinal ranges of Zygoptera, a scenario that the presence of the rivers may explain. A better understanding of the prevalent predictors of the species and how their dispersal ability governs their distribution has conservational implications.

Keywords: Anisoptera, PER-SIMPER, DNCI, river barriers, dispersal-related traits, environmental filtering, Zygoptera

Introduction

One of the most important goals of freshwater ecology is understanding how deterministic (niche-based) and stochastic (dispersal-based) processes influence the distribution of species in aquatic systems (Hubbell, 2001; Leibold *et al.,* 2004; Juen & De Marco, 2012; Siqueira *et al.,* 2015; Brasil *et al.,* 2018; Alves-Martins *et al.,* 2019a). Moreover, the deterministic paradigm is related to niche properties and how the biological communities respond to the environmental conditions (Chase, 2007; Landeiro *et al.,* 2011; Datry *et al.,* 2016; Brasil *et al.,* 2018). So, the species morphological and physiological characteristics would define their adaptation to specific environmental conditions, as well as their tolerance range (Wang *et al.,* 2022). Furthermore, the stochastic paradigm is related to random processes, such as birth/death and speciation/extinction rates, biogeographical history, and dispersal (Hubbell, 2001; Leibold *et al.,* 2004; Leibold & Chase, 2018). Dispersal is one of the most important processes in shaping species distribution, related to their abilities to move effectively from one site to another, and these abilities are also related to physiological and morphological characteristics that define how far the species would disperse across the landscapes (Hubbell, 2001; Leibold *et al.,* 2004).

Niche and dispersal have been considered the most important drivers for several aquatic taxa, such as Chironomidae (Kotzian *et al.,* 2020), Ephemeroptera (Shimano *et al.,* 2021), Trichoptera (Cruz *et al.,* 2022), and fishes (Palheta *et al.,* 2021). However, it is important to underline that these processes are not mutually exclusive because of their gradient nature, and so the biological communities can be driven at a greater or lesser extent by each one. Several analytical procedures are used to address such processes, such as null models and variance partitioning, but since niche and dispersal occur in a gradient, often the environmental variables may be spatially structured, making difficult to disentangling their relative importance (Cottenie *et al.*, 2003; Cottenie, 2005; Alahuhta & Heino, 2013; Vilmi *et al.*, 2021; Pelinson *et al.*, 2022). Moreover, both processes are scale-dependent, and the variation in broad and fine scale variables related to topography (mountainous vs. flatlands), altitudinal gradients (high vs medium vs low), the presence of natural barriers (mountains and massive rivers), and geographical distances also impact the dispersal movements of the species, making it challenging to address the individual importance of regional and local processes (Alahuhta & Heino, 2013; Heino *et al.*, 2015; Tonkin *et al.*, 2018; Maxwell *et al.*, 2021).

Historically, the massive Amazonian rivers have shaped the distribution of bats, birds, primates, and aquatic insects, mainly as spatial barriers to their dispersal displacements (Wallace, 1854; Ribas *et al.*, 2012; Brasil *et al.*, 2018; Alves-Martins *et al.*, 2019a; Shimano *et al.*, 2021; Carvalho *et al.*, 2022). This theory of isolation by rivers predicts that these aquatic systems provoked historical vicariance processes, resulting in different subsets of species along the Amazon biome, called endemism regions (da Silva *et al.*, 2005; Juen & De Marco, 2012; Brasil *et al.*, 2018). The endemism regions are important for understanding species distribution because their spatial extension and the isolation promoted by the large Amazon rivers make them good models for biogeographic studies(da Silva *et al.*, 2005). Moreover, they present one of the most common way to address the relative importance of niche and dispersal-based processes on the distribution of the species, considering the different influences on a wide variety of taxa (passive vs. active dispersers) (Alves-Martins *et al.*, 2019a; Carvalho *et al.*, 2022). Altitudinal gradients were more important than the rivers for the beta diversity of bats across the Amazon (Carvalho et al., 2022). The probable explanation is the effective dispersal ability of these taxa. Nevertheless, Ribas *et al.*, (2012) observed the historical importance of the barriers imposed by the rivers to diversify the avifauna in the Amazon. So, the degree of the relative importance of the presence of rivers to drive species distribution varies according to the target organisms, spatial scales, and historical aspects (González-Trujillo *et al.*, 2020).

The order Odonata (Insecta) is an ancient insect group with a strictly aquatic larval stage and flying adult phase (Stoks & Córdoba-Aguilar, 2012; McCauley, 2013; Mendes *et al.*, 2017; Brasil *et al.*, 2018; Alves-Martins *et al.*, 2019a). The intrinsic characteristics of its suborders, Anisoptera and Zygoptera, make them interesting models for assessing the influence of niche and dispersal-based processes (Corbet & May, 2008; Córdoba-Aguillar, 2008; Carvalho *et al.*, 2018; Dalzochio *et al.*, 2018; Oliveira-Junior & Juen, 2019; Alves-Martins *et al.*, 2019a). Anisoptera species can endure a high amplitude of environmental variance due to their broader range of ecological and physiological tolerance

(De Marco *et al.*, 2015). Moreover, the dispersal capacity of these species allows them to disperse further, traversing landscapes to reach other available habitats (Bastos *et al.,* 2021). Environmental characteristics, mainly related to the sunlight incidence and vegetation cover, are considered the main local environmental conditions driving their distribution (Carvalho *et al.* 2013; Borges *et al.* 2021; Calvão *et al.* 2022), considering that most of Anisoptera species are considered heliothermics (Corbet & May, 2008; Oliveira-Junior *et al.*, 2015). Furthermore, the strong flight capacity of Anisoptera allows them to cross long distances, with some species even managing to cross the ocean (Troast *et al.*, 2016), and their body size (generally larger than Zygoptera) allows them to endure the high variation of environmental conditions. Zygoptera species are strongly associated with specific environmental conditions, such as low temperature amplitude, high vegetation cover, and the presence of wood debris provided by the riparian forest; any modification of those conditions threatens their presence and survival (Oliveira-Junior *et al.,* 2019; Dalzochio *et al.,* 2018; Veras *et al.,* 2020; Veras *et al.,* 2022). Moreover, the species in this group are considered poor dispersers, compared to Anisoptera, due to some intrinsic morphological characteristics (e.g., long abdomens and less stronger muscle complex) and narrow tolerance to the amplitude of variation of the environment (De Marco *et al.*, 2015; Bastos *et al.*, 2021). Therefore, due to these characteristics, the Odonata suborders are good models to address the relative importance of niche and dispersal-based processes in ecological studies. However, considering that most of these processes occur in a gradient, well-known methodological frameworks, such as variance partitioning, would not allow addressing the individual importance of niche and dispersal-based processes (Heino *et al.,* 2015; Tonkin *et al.,* 2018; Vilmi *et al.*, 2021).

To address these methodological issues, Gibert & Escarguel (2019) developed the PER-SIMPER method, which is based on the Similarity Percentage (SIMPER; Clarke, 1993) to address the relative importance of niche and dispersal processes. The PER-SIMPER allows a qualitative identification of the predominant process (niche, dispersal-based or shared influence) driving two sets of communities, based on the species incidence or abundance matrix (Gibert & Escarguel, 2019). However, since niche and dispersal-based processes occur in a gradient, often presenting a shared influence on communities, a qualitative approach, such as PER-SIMPER, falls in the same problem from classic methodologies, such as the variance partitioning (Vilmi *et al.* 2021). Because of this, Vilmi *et al* (2021) proposed the Dispersal-Niche Continuum Index (DNCI), which aims to address the continuum of processes driving the communities in a quantitative way, and has been applied on a variety of taxa, such as macroinvertebrates (Vilmi *et al.* 2021), fleas and their mammals hosts (Gibert *et al.* 2021), and montane small mammals (Wen *et al.* 2022).

Thus, using the PER-SIMPER/DNCI framework with the analysis of dispersal functional traits, the objective of the present study was to investigate the relative importance of dispersal and niche processes on communities of Anisoptera and Zygoptera among the Amazonian endemism regions. We predicted that i) in regions more distant to each other the dispersal-based processes are the main drivers explaining the differences between the communities of both groups, considering the biogeographical scale. Besides, as both Anisoptera and Zygoptera present different dispersal rates and environmental tolerance, we predicted ii) the first would show a shared influence of dispersal and niche, while the second would present the prevalence of dispersal influence. Finally, we also predicted iii) Zygoptera dispersal functional traits (e.g., morphological characteristics) would be associated more markedly to the longitudinal ranges than with latitudinal variation; and Anisoptera species, due to their broader ecological tolerance, would not present any association with none of them.

Material and Methods

Study area

The study encompassed Odonata collected from 182 streams belonging to six endemism regions in the Brazilian Amazon (Belém = 47; Guiana = 24; Inambari = 48; Rondônia = 19; Tapajós = 17; Xingu = 27) (Fig. 1). The Belém endemism region is limited by Tocantins River in the southwest; Guiana region is limited by Amazonas and Negro rivers; Inambari has the Amazonas and Madeira rivers as its boundaries; Rondônia is limited by Madeira and Tapajós rivers; Tapajós limited by Tapajós and Xingu rivers; and Xingu by Xingu and Tocantins rivers. The endemism division is one the best ways to understand the biodiversity patterns in the Amazon since there is a possible influence of isolation by rivers, creating endemism spots (Wallace, 1954; da Silva *et al.,* 2005; Brasil *et al.,* 2017), and since the massive rivers can work as barriers, likely to influence the Odonata distribution (Juen & De Marco, 2012).

There are a mix of climate types according to Köppen classification (Peel *et al.,* 2007), varying from *Af*-type (tropical rainforest climate) in Inambari region to *Aw*-type (tropical wet and dry climate) in the Xingu region and *Am*-type in Belém endemism region. Moreover, the annual rainfall ranges from 1.400 to 1.700 mm, with a mean temperature of 25° (Juen *et al.*, 2007), with two undefined seasons: dry and rainy (Peel *et al.,* 2007). The land use in the sampled regions encompasses monoculture as oil palm *Elaeis guineensis* Jacq. and soybean *Glycine max* L. plantation (Cunha *et al.,* 2015; Brasil *et al.,* 2017; Carvalho *et al.,* 2018), urbanization and traditional activities (Monteiro-Júnior *et al.,* 2016; Faria *et al.,* 2017) and mining entrepreneurship (Fearnside, 1989; Luiza-Andrade *et al.,* 2020), which can cause environmental disruption, bringing positive or negative influences on the stream-dwelling biota.

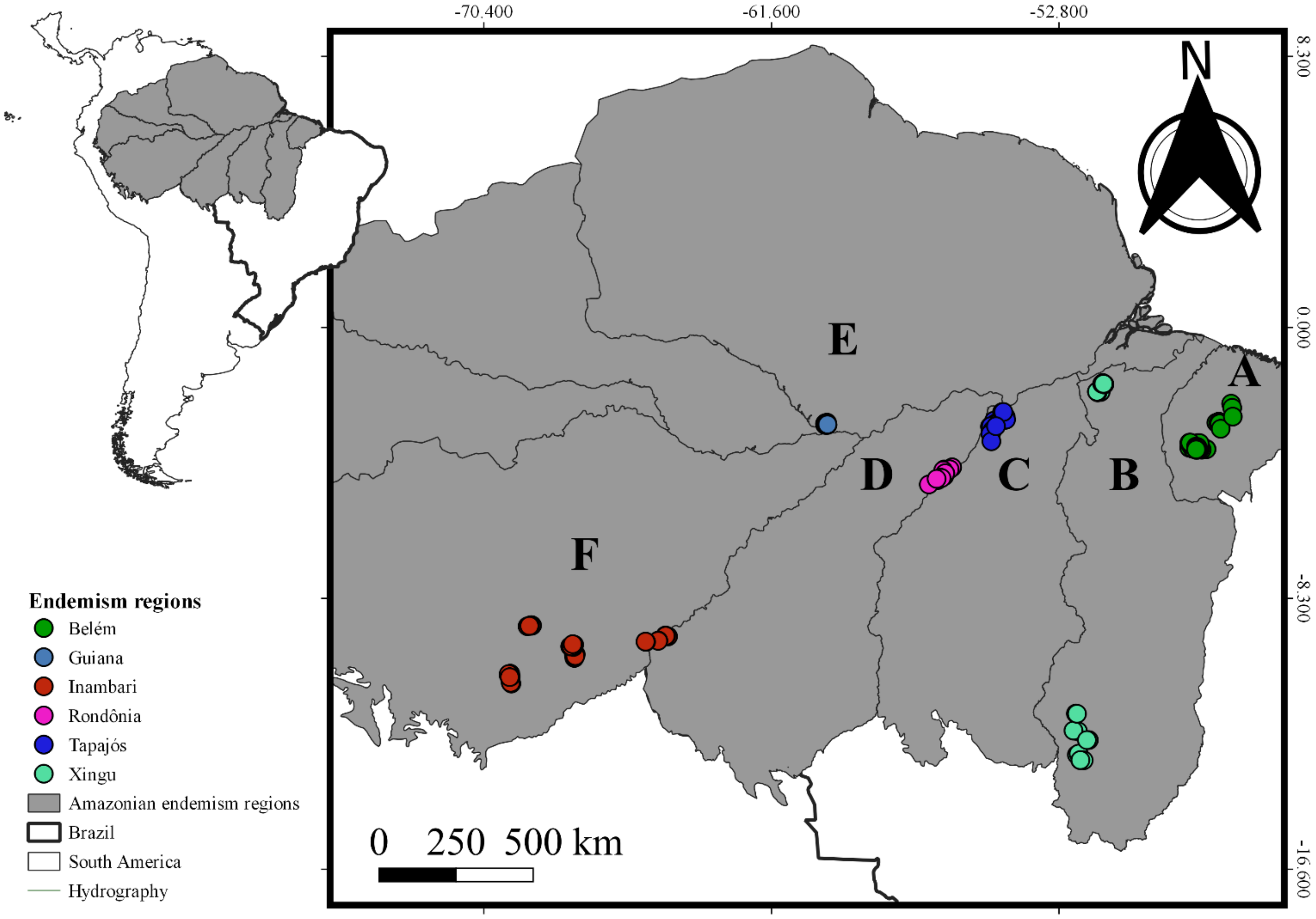

Fig. 1. Geographical location of each endemism region with their respective streams, in the Brazilian Amazon. A = Belém region; B = Xingu region; C = Tapajós region; D = Rondônia region; E = Inambari region; F = Guiana region.

Sampling of biological material

We sampled during the drier season to ensure that a major part of the species from both Odonata suborders, Anisoptera and Zygoptera, could be collected (Corbet & May, 2008; Oliveira-Junior *et al.*, 2015; Monteiro-Júnior *et al.*, 2016). Moreover, each stream was our sampling unit. Thus, the streams were divided into 150 m, and a collector stood for 60 minutes spotting specimens from both suborders, using an entomological hand net (diameter: 40 cm; depth: 65 cm; aluminium handle: 90 cm) to catch the specimens. According to previous studies (May, 1976; Corbet & May, 2008), the different species of the suborders of Odonata, with distinct thermoregulatory requirements, are most active when the sunlight reaches the bed of stream, which usually occurs between 10 hr and 14 hr, our sampling period (Batista *et al.*, 2021). After the collection, the specimens were stored in paper envelopes and immersed into an alcohol-70% solution. In the laboratory, the specimens were prepared according to Lencioni protocol (2005, 2006), identified using specific taxonomic keys (Garrison, 1990; Lencioni, 2005; Lencioni, 2006; Garrison *et al.*, 2006; Garrison *et al.,* 2010; Pessacq, 2014), and when necessary, they were sent to specialists to solve identification issues. Finally, after the identification procedures, the specimens were deposited in the Zoology Museum Collection at the Federal University of Pará (Mendoza-Penagos *et al.,* 2022).

Morphological data

Morphological measurements were obtained following previous studies on Odonata species (Pereira *et al.,* 2019; Bastos *et al.,* 2020; Oliveira-Junior *et al.,* 2021). The first step was to select ten individuals from each species without severe damage in any structure. In the situation where ten individuals were not available, all specimens were measured. We measured nine body parts from individuals of both suborders: Total body length; Fore wing length; Fore wing width; Hind wing length; Hind wing width; Abdomen length; Abdomen width; Thorax length and Thorax width.

All the metric information was taken from the right side of each specimen to prevent differences derived from fluctuating asymmetry issues. In addition, only males were measured due to the lack of taxonomic information about females and to avoid sexual dimorphism (Bastos *et al.,* 2021). A digital calliper was used to measure the morphological metrics, and to minimize possible errors in the measurement process, each metric was taken three times, and the mean value was used. Despite high Pearson multicollinearity among some metrics, we kept the ones already used in previous studies (Johansson, 2003; Hassall *et al.,* 2008; Hassall *et al.,* 2009; Bastos *et al.,* 2020; Wonglersak *et al.,* 2020) and others that presented low collinearity (total body length, abdomen length and abdomen width).

Additionally, we calculated some indices relating to the proportions of morphological characteristics: I) relative fore wing length, which is the ratio of fore wing length/total body length, where higher values indicate more effective fliers (McCulloch *et al.,* 2017; Arce *et al.,* 2021); II) thorax volume (TV), calculated from the following formula π radius of the thorax 2*4/3 (Resende *et al.,* 2021; Turlure *et al.,* 2010), a measurement flight and dispersal capacity; III) wing stroke (WS), taken from the following calculus (fore wing length*fore wing width), also related to flight capacity; IV) wing loading (WL), from the ratio TV/WS, a factor that influences the thrust generated by wings' beats (Villalobos-Jiménez & Hassall, 2019); and finally V) wing thorax ratio, from the calculus FWL^2/TV, to address the allometry of the body (Resende *et al.,* 2021).

Statistical procedures

We sampled Anisoptera and Zygoptera in streams from six endemism regions in the Brazilian Amazon. Each stream was our sampling unit. To test our hypotheses i) and ii), we applied the recently proposed PER-SIMPER/DNCI framework for calculating the main drivers (niche-based vs dispersal-based processes) of the Odonata communities across the endemism regions in the Brazilian Amazon (Gibert & Escarguel, 2019; Vilmi *et al*., 2021). PER-SIMPER verifies if the taxa distribution is primarily driven by niche-based or dispersal-based processes. Moreover, the incidence/abundance dataset is permuted under three main null-models, corresponding to the three main assembly hypotheses (niche-based and dispersal-based influence, and shared influence). The first permutational model constrains the rows of the dataset, generating the null SIMPER profile expected if the species distribution is driven only by the availability of niches and their breadth (niche-based processes). On the other hand, the second permutational model constrains the columns of the dataset, generating the null SIMPER profile expected if the species distribution is determined only by their dispersal abilities/spatial constraints (dispersal-based processes). Finally, the third permutational model constrains both rows and columns, considering simultaneously niche-based and dispersal-based processes (shared influence). For more details, please read Corentin & Escarguel (2019) and Vilmi *et al.* (2021). Although PER-SIMPER has some advantages, such as the requirement of only a species-by-site binary matrix and the application to any number of local communities (>2), due to the often-shared influence of niche and dispersal-based processes on the communities, its qualitative nature fails in disentangling the relative influence of both processes.

To deal with these caveats, Vilmi *et al.* (2021) proposed the Dispersal-Niche Continuum Index (DNCI), built on the PER-SIMPER, aiming to address the relative influence of niche and dispersal processes quantitatively. The basics of the test is: positive DNCI (>0) indicates that niche-based processes are dominant; negative DNCI values (<0) indicate the dominance of dispersal-based processes; and values closer to 0 indicate a shared influence of both processes (Vilmi *et al*., 2021). Besides, the larger the absolute value (far from 0), the stronger is the dominance of the processes. The DNCI can estimate the most dominant processes driving the community assembly without environmental or spatial data, and it allows the comparisons of processes among different datasets (Vilmi *et al.*, 2021). Besides, the DNCI method relies on the distribution of taxa, which can better clarify the relative importance of niche and dispersal-based processes shaping the community structure (Chase *et al.*, 2011; Vilmi *et al.*, 2021). Moreover, considering the occurrence of all taxa at least one site, some areas did not meet the necessary number of sites to be used in the PER-SIMPER/DNCI analyses (>5 sites per region) (Gibert & Escarguel, 2019). So, we removed Guiana and Rondônia regions from Anisoptera dataset since they did not present the sufficient number sites. Moreover, for Zygoptera, as all the six regions presented the sufficient number of sites (>5 per region), they were maintained in the analyses.

We used the package DNCImper (Gibert *et al.*, 2020) implemented in the R computational environment (version 4.3.1). To test the hypothesis i) we used the function *DNCI_multigroup*, which compares the pairwise dissimilarity values among the communities from the different endemism areas. This function is based on *DNCI_ses* (for 2 group analysis) and PER-SIMPER function E index output. The three distributions of E index, corresponding to the three hypotheses: individual or shared influence of niche and dispersal-based processes, are used to compute the DNCI index. If DNCI is significantly lower than 0, dispersal is the main driver; if DNCI is significantly higher than 0, thus niche is the main driver; and if DNCI presents values closer to 0, there is a shared influence of niche and dispersal-base processes (Vilmi *et al.* 2021). Besides, to test the hypothesis ii) we applied the function *DNCI_ses_overall_symmetrized*, in which the DNCI values are calculated by subsampling the largest group to the size of the smallest one (symmetrize = TRUE). For all the analytical procedures for calculating the DNCI values, we set the number of iterations of 100. To address possible significative differences between Anisoptera and Zygoptera absolute DNCI values we apply an independent t-test, following the assumptions of independence among the samples and variance homogeneity. When those assumptions were not met, we applied the t-test version for heterogeneous variances (Zar, 2010).

To test hypothesis iii) (species latitudinal and longitudinal ranges ~ dispersal functional traits), we performed generalized linear models (GLMs; Zuur *et al.,* 2009) using the Gaussian distribution. We also applied the VIF to address multicollinearity among the dispersal proxies, and we maintained the variables whose score was equal or lower than five (Borcard *et al.,* 2018; Miguel *et al.,* 2022). First, we calculated the latitudinal and longitudinal ranges of each species as the mean between the maximum and minimum values of the points of occurrence (Rohde *et al.,* 1993; Miguel *et al.,* 2022). After that, we built null models (~1), additive and individualized models with one variable at once. Then, we selected the models through the AIC method (Akaike Information Criterion; Burham & Anderson, 2002), choosing the models with the lowest values. Finally, only the models validated by observing the residuals were maintained through the simulated envelope method (Moral *et al.,* 2017). All the analytical procedures were carried in the R computational environment (R Core Team, 2023), using the interface RStudio (version 1.524), and are summarized in Figure 2.

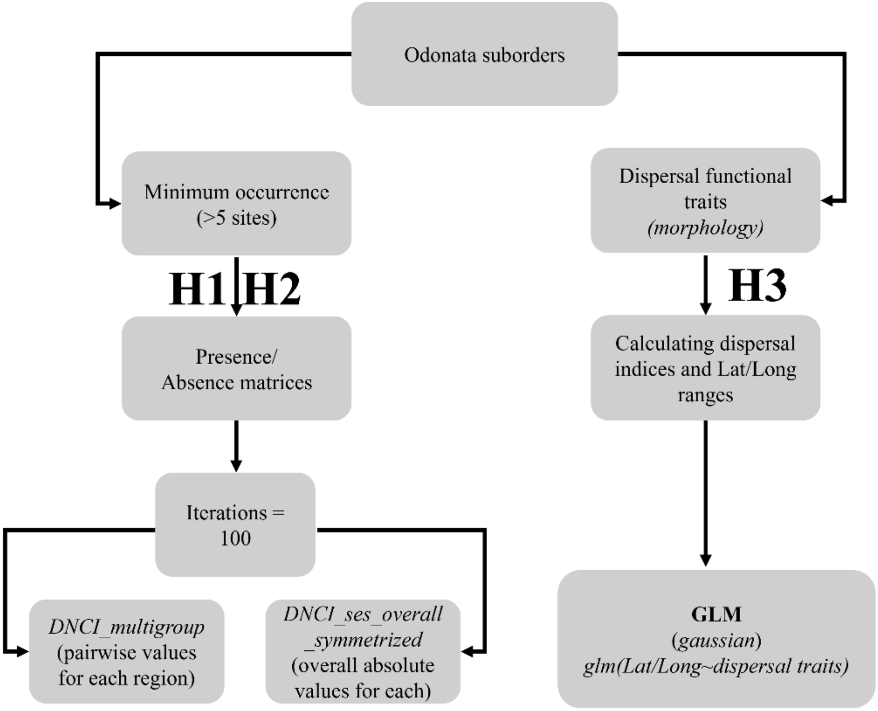

Fig. 2. Diagram of the analytical framework showing the statistical steps used in the study. DNCI = Dispersal-Niche Continuum Index; GLM = generalized linear models; Lat = latitudinal ranges; Long = longitudinal ranges.

Results

Description of Odonata communities

A total of 3.215 individuals of Odonata were sampled, belonging to 171 species within nine families. From this, 408 individuals of Anisoptera were collected, belonging to 63 species and three families, with *Erythrodiplax fusca* (Rambur, 1842) as the most abundant (n = 52), followed by *Fylgia amazonica* Kirby, 1889 (n = 37) and *Erythrodiplax maculosa* (Hagen, 1861) (n = 32). Anisoptera presented 46 exclusive species, belonging to only one interfluve regions. For this group, Inambari region presented the highest number of exclusive anisopteran species (n = 22), followed by Tapajós (n = 11), and Guiana presented only one exclusive species. For Zygoptera, 2.807 individuals were collected, belonging to 106 species and six families, with the most abundant species being *Chalcopteryx rutilans* (Rambur, 1842) (n = 331), followed by *Mnesarete aenea* (Selys, 1853) (n = 258) and *Argia infumata* Selys, 1865 (n = 153). Zygoptera presented 53 exclusive species, occurring in only one endemism regions. Similarly, to Anisoptera, Inambari region showed the highest number of exclusive zygopteran species (n = 18), followed by Xingu (n = 17), and Tapajós presented the lowest (n = 2). The overall distribution of Odonata species across the endemism regions are summarized in table S1 (Supplementary material).

Relative influence of niche and dispersal-based processes

The overall DNCI values for Anisoptera was 0.289+1.499 (mean±standard deviation), demonstrating a shared influence of niche and dispersal-based processes on its communities across the four endemism regions used for this suborder (Table 1; Fig. 3a). Moreover, the pairwise comparisons among the four endemism regions for this suborder indicated a shared influence of niche and dispersal-based processes, with values closer to the zero threshold (Table 1; Fig. 3a). Besides, the overall DNCI values for Zygoptera was -13.566+2.818, demonstrating dispersal as the main driver of its communities across the six endemism regions used for this suborder (Table 1; Fig. 3b). Moreover, the pairwise comparisons indicated a strong influence of dispersal-based processes, with values more distant from zero (Table 1; Fig. 3b).

Taxa	**DNCI**	**CI.DNCI**	**S.DNCI**

For Anisoptera, the pairwise comparison between Inambari and Xingu showed the lowest value (Table 1), while the comparison between Belém and Xingu showed the highest (Table 1). These results did not corroborate our predictions since the regions more distant to each other (Belém and Inambari) did not present the highest or lowest values. For Zygoptera, the pairwise comparison between the regions more distant to each other (Belém with Inambari, Guiana, Rondônia and Tapajós) showed the lowest DNCI values, corroborating the prediction that dispersal is the main process shaping the communities of this suborder rather than niche-based processes. Moreover, the comparison between Anisoptera and Zygoptera overall DNCI values indicated significative difference, with dispersal-based processes being more predominant for Zygoptera (independent t-test = 47.627; df = 2089.5; $p < 0.001$) (Fig. 4).

Table 1. Pairwise DNCI (Dispersal-Niche Continuum Index) for Anisoptera and Zygoptera suborders across the six interfluve regions in the Brazilian Amazon. CI.DNCI = Confidence Interval associated to the DNCI; S.DNCI = Variance associated to the DNCI.

ANISOPTERA			
Belém-Inambari	0.06032521	1.468.395	0.7341974
Belém-Tapajós	1.42591120	1.109.540	0.5547698
Belém-Xingu	1.69121408	1.553.173	0.7765866
Inambari-Tapajós	-1.31522966	2.164.550	1.0822750
Inambari-Xingu	-1.95237449	1.994.911	0.9974553
Tapajós-Xingu	-1.03978715	2.100.871	1.0504356
ZYGOPTERA			
Belém-Guiana	-15.358.629	1.827.372	0.9136859
Belém-Inambari	-13.014.299	1.516.376	0.758188
Belém-Rondônia	-19.235.594	3.005.079	15.025.395
Belém-Tapajós	-15.278.139	2.764.124	13.820.618
Belém-Xingu	-11.354.635	1.801.320	0.9006601
Guiana-Inambari	-15.690.715	2.255.832	11.279.161
Guiana-Rondônia	-29.165.081	3.448.088	17.240.439
Guiana-Tapajós	-14.164.334	1.772.292	0.8861461
Guiana-Xingu	-26.431.238	3.961.968	19.809.842
Inambari-Rondônia	-8.768.693	2.728.827	13.644.134
Inambari-Tapajós	-5.720.159	1.792.571	0.8962854
Inambari-Xingu	-7.617.333	1.685.854	0.8429268
Rondônia-Tapajós	-7.298.322	1.897.632	0.9488159

Rondônia-Xingu	-7.205.326	2.112.524	10.562.622
Tapajós-Xingu	-7.196.461	1.988.610	0.994305

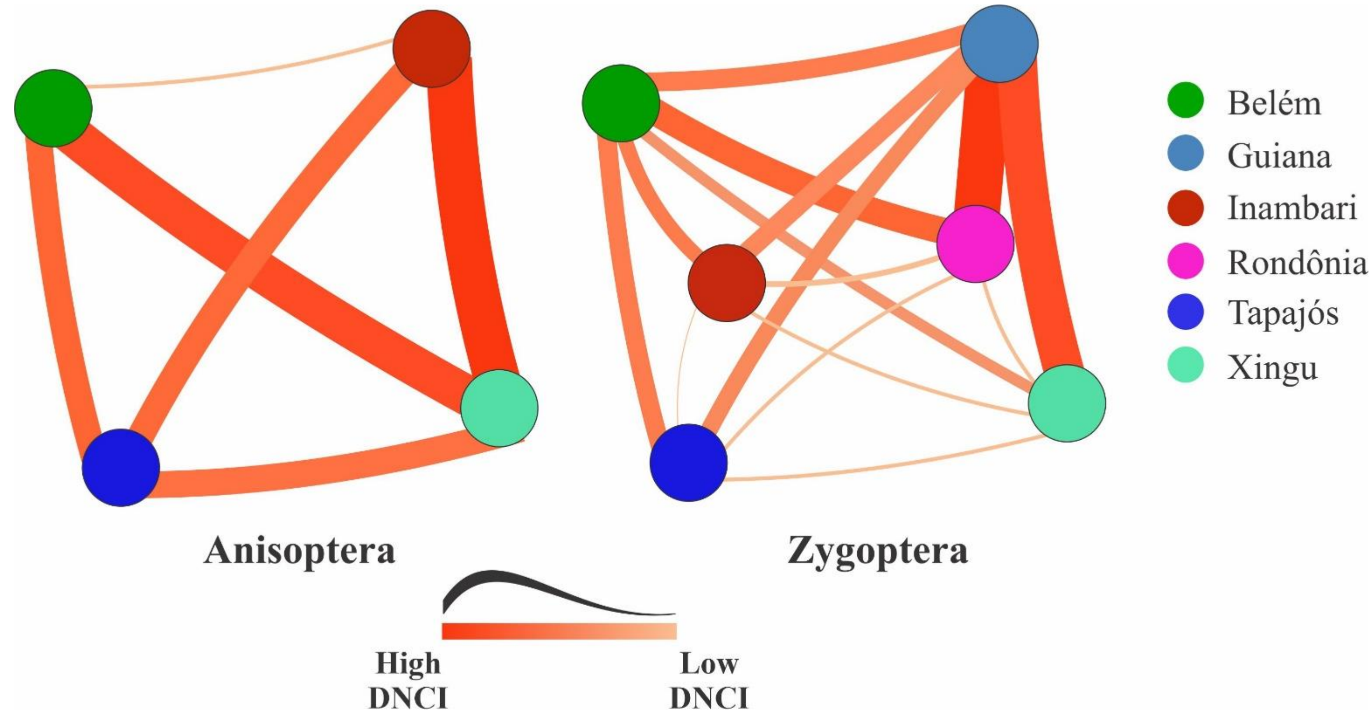

Fig. 3. Network plots representing the pairwise Dispersal-Niche Continuum Index (DNCI) among the six endemism regions across the Brazilian Amazon, for Anisoptera and Zygoptera suborders. Wider and darker lines indicate the prevalence of dispersal-based processes, while narrower and lighter lines indicate the prevalence of niche-based processes.

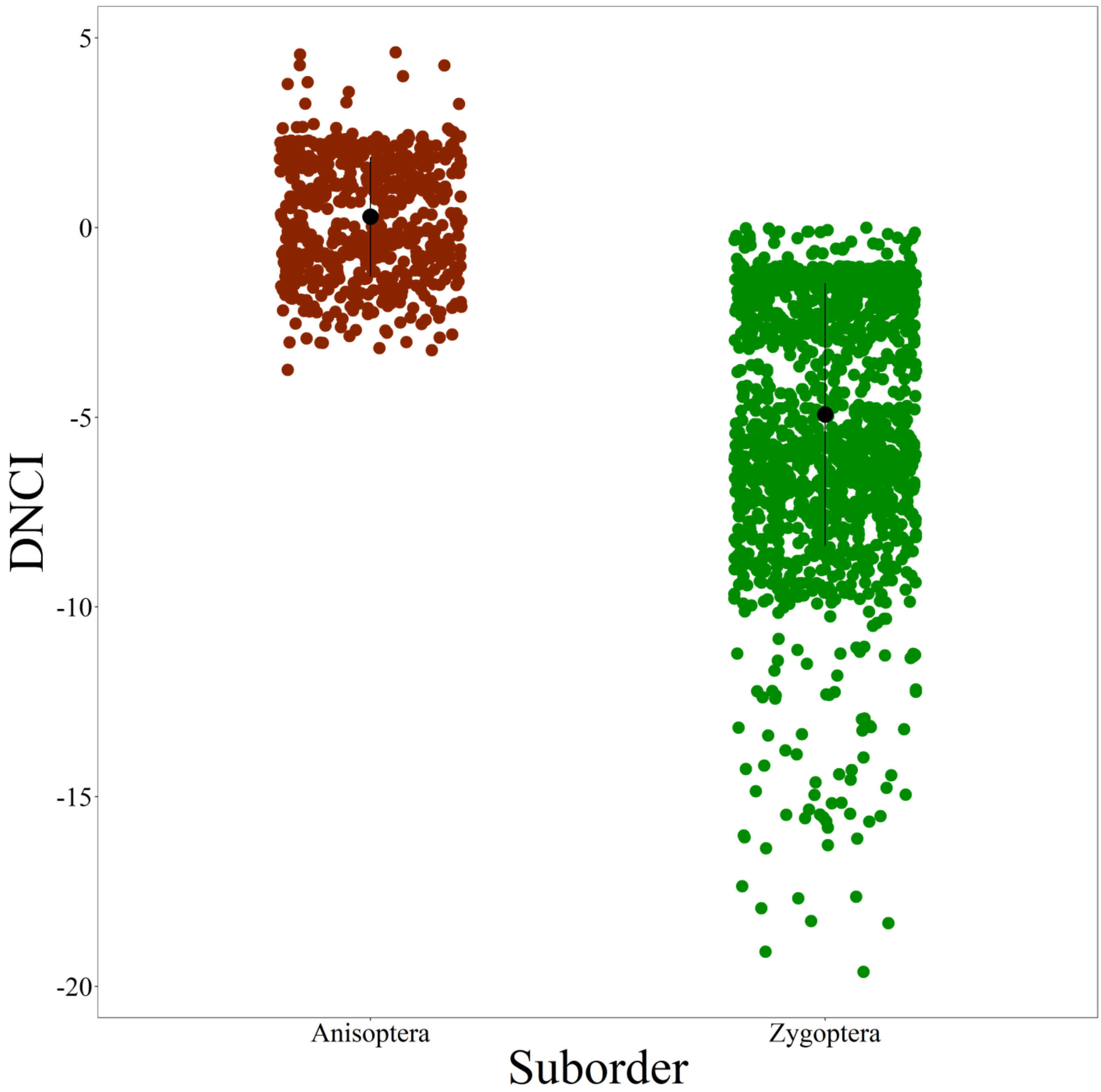

Fig. 4. Boxplots showing the overall Dispersal-Niche Continuum Index (DNCI) for Anisoptera and Zygoptera suborders across the six endemism regions in Brazilian Amazon. The overall DNCI values were computed based on 100 iterations.

Influence of dispersal traits on latitudinal and longitudinal ranges of Odonata

Regarding the influence of dispersal functional traits of Odonata species on their latitudinal and longitudinal ranges, there were significant relationships only for Zygoptera species. Moreover, we identified positive relationships between latitudinal and longitudinal ranges and wing loading

(WL; Fig. 5A, 5B), and a negative relationship between longitudinal range and abdomen width (AW; Fig. 5C) (Table 2).

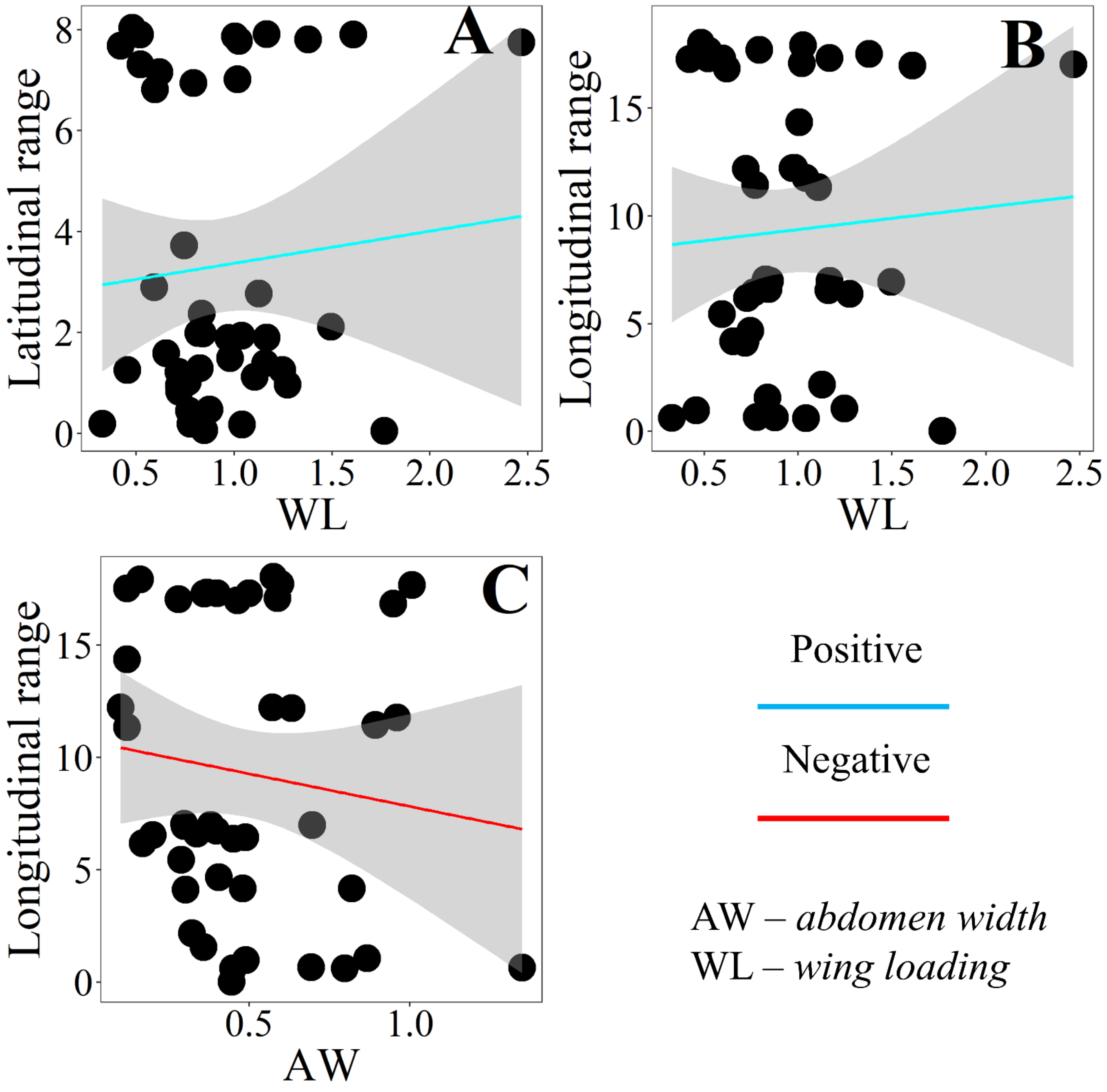

Fig. 5. Results from Generalized Linear Models (GLMs), with the relation between dispersal functional traits of Zygoptera species along their latitudinal and longitudinal ranges.

Table 2. Results from Generalized Linear Models (GLMs), with the response variables and the significative dispersal functional traits of Zygoptera suborder. AW = abdomen width; Std. error = Stardand error; WL = wing loading.

	Estimated	**Std. error**	**t**	**p-value**

ZYGOPTERA				
Latitudinal range				
WL	0.01768	1.17540	0.015	0.001
Longitudinal range				
AW	-1.702	1.557	-1.093	0.001
WL	0.3911	2.4682	0.158	0.001

Discussion

Our hypothesis i) was partly corroborated since areas closer and more distant to each other presented a mixture of pairwise values, with all of them negative but varying in intensity. We corroborated hypothesis ii) as all absolute pairwise values for Zygoptera communities were negative and lower than those from Anisoptera communities. Finally, our hypothesis iii) was also corroborated as only Zygoptera species presented dispersal functional traits influencing significantly their latitudinal and longitudinal ranges. Our results revealed a shared influence of dispersal and niche-based processes on Anisoptera, and a profound influence of dispersal-related processes on Zygoptera, but with variation in absolute values. Furthermore, this difference in absolute values would be expected given the difference in ecological, physiological, and morphological characteristics of both groups, even though they are evolutionarily close.

Our study area encompasses six endemism areas in the Brazilian Amazon, with different historical processes, mainly associated with the rivers dynamics (Wesselingh & Salo, 2006). The riverine configuration in Amazon was highly influenced by the rise of the Andes, which changed the drainage patterns and shaped the evolutionary history of several taxa in this biome (Silsby, 2001). According to Juen and De Marco (2012), the influences of this tectonic event would generate important biogeographical patterns, mainly on taxa, such as dragonflies and damselflies, that were already in the Amazon before the Amazon river existed in its current shape. Zygoptera species presented the lowest absolute negative values of DNCI pairwise, indicating a strong signal of dispersal processes, which we took as a proxy for dispersal limitation. The presence of the massive rivers creates a strong geographical barrier to the dispersal of species from this suborder (Juen & De Marco, 2012; Brasil *et al.*, 2018). For example, the widest section of the Tapajós river, presents more than 14km, a massive barrier to the dispersal of Zygoptera species, with slender bodies and low amount of flight muscles (Wootton, 2020). Zygoptera species from genera *Argia*, *Chalcopteryx* and *Epipleoneura* present such morphological conditions, being more associated with environments with intermediate to high vegetation cover, which might indicate pristine conditions. So, considering the broad scale of the study, the geographical distance among the available habitats can present such harsh conditions (e.g., high variation in air temperature and vegetation cover) that these species cannot endure while dispersing (Heino *et al.*, 2015; Pires *et al.*, 2018), being more restricted to their endemism region. Those results picture a scenario of prevalence of dispersal limitation for Zygoptera, which can be explained by the biogeographical scale, encompassing a wide range of geographical distance and probably spatial-structured environmental conditions (Pires *et al.*, 2018).

On the other hand, Anisoptera species presented values closer to the zero threshold, indicating a shared influence of dispersal and niche processes. Anisoptera species present more robust bodies, with stronger flight muscles and high tolerance to the variation in local environmental conditions, characteristics that allow their effective dispersal throughout the landscapes to reach other available sites (Juen & De Marco, 2012; Outomuro & Johansson, 2019). Because of their vagility and ecophysiological tolerance, Anisoptera species could more easily cross geographical barriers, such as mountains, rivers, and even oceans (Troast *et al.*, 2016), enduring harsh conditions during the displacements. Moreover, two intrinsic Amazonian factors can explain such patterns: 1) the possible passive transportation of Odonata larvae by macrophyte banks, and 2) the presence of meanders that straightened the river course, which can cause translocation of terrestrial portions from both margins, enabling an exchange of fauna (Juen & De Marco, 2012). Moreover, those processes can explain the weaker influence of isolation by rivers on Anisoptera species.

Our results for the relationship between the latitudinal and longitudinal ranges and the dispersal functional traits of Zygoptera can also be explained through the importance of the massive rivers as physical barriers to their dispersal. Previous studies (Johansson, 2003; Hassall *et al.*, 2008; Outomuro & Johansson, 2019) have been focused on the influence of latitudinal on different biological aspects of Odonata species and families, usually finding the temperature gradient as an important component of the latitudinal range shaping Odonata distribution. Considering the different thermal groups that compose Odonata (thermal conformers, heliothermics, and endothermic), the temperature gradient can shape their distributions, broaden the distribution of the species able to control their inner temperature, and constrain the distribution of those more associated with steady local environmental conditions. In the present study, we used the longitudinal ranges as dependent variables, different from the most common approach, and the massive rivers across the endemism regions could be a strong barrier to the longitudinal dispersal displacements of the small Odonata species, mainly the Zygoptera species. Outomuro & Johansson (2019) found a significant association between proportionally smaller hindwing pterostigma of Libellulidae species and the longitudinal ranges, with males presenting larger longitudinal ranges. So, considering that some species present gliding to disperse, this is an expected result. However, other results, such as the positive and significant association between broader and shorter wings and the longitudinal ranges were not expected since these dispersal morphological traits are more related to less efficient flights (Outomuro & Johansson, 2019). Because of these conflicting results, it is hard to make robust predictions about the importance of longitudinal ranges as predictor or response variables related to the dispersal morphological traits. Furthermore, in our study, we considered the massive rivers as important barriers to the dispersal of Odonata species, mainly those from the Zygoptera suborder.

Generally, as we found in the study, Odonata communities are structured by niche and dispersal-based processes (Brasil *et al.,* 2018; Alves-Martins *et al.,* 2019; Bried *et al.,* 2023). So, addressing the relative influence of these predictors is one of the most common starting points to disentangle the most important predictors of the communities. Here, we applied the DNCI methodological framework on Amazonian dragonflies and damselflies communities for the first time, aiming to unravel the influence of both processes. Furthermore, this new methodological tool can help advance our understanding of the underlying processes shaping the freshwater communities, comparing these new results with previous studies. Previous studies conducted at a biogeographical scale with Odonata in Brazilian Amazon found that Zygoptera species are by far the ones most influenced by the presence of massive rivers (Juen & De Marco, 2012; Brasil *et al.,* 2018; Alves-Martins *et al.*, 2019)..

Conclusion

Our results indicated that dispersal-based processes were predominant for Zygoptera communities. The presence of massive water bodies can explain the patterns found for these taxa, something already pictured in previous studies in the Brazilian Amazon using Odonata as model organisms. Regarding the lack of an accentuated influence of dispersal or niche-based processes for Anisoptera species, the more efficient flight rates, and bigger body sizes, when compared to Zygoptera, can allow these species to disperse throughout the landscapes, diminishing the influence of the isolation by rivers, resulting in a shared influence of both processes.

The influence on latitudinal ranges can be related to the temperature gradients, even though the sampling sites are located only in the Amazon, not spreading to other biomes. Moreover, the influence on longitudinal ranges may be associated with dispersal limitation due to the presence of the massive Amazonian rivers. Considering the distances between the margins, the smaller Zygoptera species cannot disperse across the endemism regions, being restricted to their original region.

Furthermore, our final results leave open the likelihood, in case of predominance of dispersal-based processes, of either dispersal limitation or populational surplus (mass-effect dynamics). Although we inferred dispersal limitation for Zygoptera in the study, because of their ecophysiological constraints, future studies could apply this analytical framework along with connectivity and landscape variables to get a more robust overview about their distribution. Future studies could address a broader range of species and their dispersal functional traits based on finer morphological measurements (e.g., weight mass of each thorax, abdomen, wings, head, etc), to understand their influences on latitudinal and longitudinal gradients deeply. Additionally, sampling more species along the landscapes could shed light on the role played by these also important dispersal traits. Finally, this could improve our understanding over longitudinal ranges, either as a factor or a response variable, since there are few systematic studies about this important variable that may have conservational implications.

Authors contributions

Joás Silva Brito: Conceptualization (lead); visualization(lead); investigation (supporting); formal analysis (lead); writing–original draft (lead); writing–review and editing(lead); **Karl Cottenie:** Conceptualization (equal); visualization(supporting); formal analysis (equal); writing–original draft (equal); writing–review and editing(equal); **Gabriel Martins Cruz:** Investigation (supporting); Writing - Original Draft Preparation (supporting); writing–original draft (supporting); **Lenize Batista Calvão:** Investigation (supporting); Writing - Original Draft Preparation (supporting); writing–original draft (supporting); **José Max Barbosa Oliveira-Junior:** Investigation (supporting); Writing – Original Draft Preparation (supporting); writing–original draft (supporting); **Fernando Geraldo Carvalho:** Investigation (supporting); Writing–original draft (supporting); **Leandro Schlemmer Brasil:** Investigation (supporting); Writing – Original Draft Preparation (supporting); writing–original draft (supporting); **Karina Dias-Silva:** Investigation (supporting); Writing - original draft (supporting); **Rafael Costa Bastos:** Investigation (supporting); Writing–original draft (supporting); **Bethânia Oliveira Resende:** Investigation (supporting); Writing–original draft (supporting); **Victor Rennan Santos Ferreira:** Investigation (supporting); Writing – original draft (supporting); **Lisandro Juno Soares Vieira:** Writing – original draft (supporting); **Thaísa Sala Michelan:** Writing – original draft (supporting); **Leandro Juen:** Conceptualization (lead); visualization(lead); Investigation (lead; supporting); formal analysis (equal); writing–original draft (lead); writing–review and editing (lead).

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Conflict of interest statement

The authors declare no conflict of interest.

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2º Capítulo: MAIN DRIVERS OF DRAGONFLIES AND DAMSELFLIES (INSECTA; ODONATA) METACOMMUNITIES IN STREAMS INSIDE PROTECTED AREAS IN THE BRAZILIAN AMAZON

Abstract

The evaluation of environmental and spatial influence in freshwater systems is crucial for the conservation of aquatic diversity. So, we evaluated communities of Odonata in streams inside and outside sustainable use areas in the Brazilian western Amazon. We predicted that these streams would differ regarding habitat integrity and species α and β diversity. We also predict that environmental and spatial variables will be important for both suborders, but with more substantial effects on Zygoptera species, considering their nature of forest-specialist. The study was conducted in 35 streams, 19 inside and 16 outside sustainable use areas. The streams outside presented high species richness, abundance, and number of exclusive forest-specialist species from Zygoptera and higher scores of habitat integrity. In contrast, one sustainable use area presented the lowest values of these metrics. Besides, we found that environmental and spatial variables were significantly associated to Zygoptera species composition, but not with Anisoptera, which can be explained by their cosmopolitan nature. Our results indicated that an interplay between environmental and spatial processes determines the structure of the metacommunities of Zygoptera. The less effective dispersal rates and narrow ecological tolerance of Zygoptera species make them more influenced by local conditions and dispersal limitation, and more sensible to habitat modifications. We highlight the importance of improving the local management of the sustainable use areas by environmental agencies, mainly on areas that are losing their capacity to maintain the aquatic fauna, and implementation of social policies toward traditional people.

Keywords: Anisoptera, Freshwaters, α and β-diversity, Species sorting, sustainable use area, Zygoptera

Introdução

Conservation of freshwater systems is a critical challenge for the current ecological research field (Oliveira-Junior & Juen, 2019; Dias-Silva et al., 2021; Brasil et al., 2021b). Riverine ecosystems are unique due to their dendritic nature embedded within the terrestrial matrices and the longitudinal distribution of the energy, resulting in high aquatic species diversity (Vannote et al., 1980; De Marco et al., 2014; Tonkin et al., 2018). Moreover, human activities can change the physical organization of these systems, bringing several effects on the aquatic fauna (Oliveira-Junior et al., 2015; Dalzochio et al., 2018; Sganzerla et al., 2021). Activities such as mining exploitation, cash crop, cattle grazing, and urbanization can fragment the riverine network, and change the physical and environmental conditions at local and landscape scales (Monteiro-Júnior et al., 2015; Cunha & Juen, 2020; O'Malley et al., 2020; Faria et al., 2021). Environmental disturbances can strongly influence the establishment and survival of the local fauna, diminishing their niche breadth, and resulting in local extinctions in the most severe scenarios (O'Malley et al., 2020; Brasil et al., 2021a; Bastos et al., 2021).

One way to mitigate the influences of human activities on natural forests is the creation of conservation units and protected areas, aiming to safeguard biodiversity (Hansen & De Fries, 2007; Brasil et al., 2021b; Dias-Silva et al., 2021). The Brazilian legislation recognizes two main groups (Brasil et al., 2021b): 1) Strictly Protected Areas - areas where there cannot be human interference, and 2) Sustainable Use Protected Areas (hereafter SUA) - which the sustainable multiple uses of forest resources are released. The main purposes of conservation units are maintaining biodiversity in general, protecting threatened species, and promoting sustainable development (Federal Law 9.985, July/2000; Brasil et al., 2021b; Dias-Silva et al., 2021). Therefore, while conducting ecological studies, targeting conservational purposes, the researchers usually expect to find higher integrity levels and species diversity inside these protected areas. The RESEX (extractive reserve) is the least restrictive category from the SUA. Within extractive reserves, traditional people can sustainably exploit natural resources (Law 9.985/2000). However, they cannot exploit those resources in a predatory way for industrial purposes (Federal Law 9.985/2000), and the local management by public environmental agencies is crucial for ensuring the legal use of those areas. Furthermore, these areas with different conservation statuses provide a unique opportunity to study ecological processes. For instance, ecological niche models illustrated the importance of conservation units in the Amazon for the turtle (Fagundes et al., 2018), fishes (Frederico et al., 2018), dragonflies and damselflies (Brasil et al., 2021b) biodiversity. Thus, the effectiveness of these sustainable use areas in maintaining biodiversity in freshwater systems relies on better local management by environmental agencies, in conjunction with policies toward traditional people and scientific research carried inside these areas.

Among the aquatic diversity, the insects stand as good indicators of environmental health since they participate in the trophic network as predators and preys, and present different responses to environmental changes (Siqueira et al., 2015; Luiza-Andrade et al., 2020; Faria et al., 2021; Brasil et al., 2021a; Lima et al., 2022). Dragonflies and damselflies (Odonata suborder) are efficient groups to address the effectiveness of conservation units, because of the different ecological and physiological requirements, providing distinct responses to environmental changes and spatial constraints (Brasil et al., 2018; Alves-Martins et al., 2019; Silva et al., 2021). The Anisoptera suborder (dragonflies) is typically composed of species considered effective dispersers due to their flight ability, bigger body size and ecological and physiological tolerance, which allow them to disperse more easily and to endure suboptimal conditions (Corbet & May, 2008; Oliveira-Junior & Juen, 2019; Wootton, 2020). On the other hand, the Zygoptera suborder (damselflies) encompasses smaller Odonata species, with slender bodies, defined in several studies as poor dispersers (May, 1979; Corbet & May, 2008; Oliveira-Junior & Juen, 2019), widely influenced by local environmental conditions and spatial constraints. Any modification on the riparian vegetation increases the input of sediments in the bed of the streams, changes the availability of allochthonous materials and the environmental stability (Carvalho et al., 2013; Calvão et al., 2016; Mendes et al., 2018; Brito et al., 2021; Borges et al., 2021), diminishing the species richness and abundance of Zygoptera. Additionally, the geographical distance among the available sites can prevent the dispersal of Zygoptera species due to their physiological restrictions, not allowing them to endure extreme conditions throughout the landscapes (Brasil et al., 2018; Alves-Martins et al., 2019). The characteristics described above of both suborders make them useful bioindicators to address the health of aquatic systems, where Anisoptera would be more connected to open sites, natural or with some degree of human-made alteration, and Zygoptera more associated to forested habitats, with high vegetation cover (Carvalho et al., 2013; Oliveira-Junior et al., 2015). Additionally, there is a need to address the importance of conservation units in maintaining freshwater biodiversity, many of these areas were created without considering aquatic biodiversity and its specificities (Leal et al. 2020; Dias-Silva et al. 2021). So, the use of such robust indicators as Odonata species in conservation units is crucial for building our knowledge about their importance for freshwater fauna.

Thus, the present study evaluated the relative importance of environmental and spatial variables on metacommunities of Odonata (Anisoptera and Zygoptera) and investigated the possible differences regarding the diversity of species and habitat integrity in streams inside and outside sustainable use protected areas (SUA). Considering the expected differences between the areas, we predicted (i) streams located inside SUA would have higher diversity (α-diversity and β-diversity) and exclusiveness of species, mainly of Zygoptera species, and higher habitat integrity scores, for that we will use streams from four distinct areas, two inside the Sustainable use Area and two outside . Moreover, we predict ii) streams located outside the SUA would present a lower contribution to β-diversity than streams located inside SUA; and Zygoptera species would present the highest contribution to β-diversity in streams inside SUA, and Anisoptera would contribute more for the β-diversity in streams outside SUA. Finally, we expect iii) variables associated with physical structuring features (e.g., habitat integrity, channel width and canopy cover) and broad-scale spatial components would strongly be related to the Zygoptera species composition due to their low dispersal capacity, when compared to Anisoptera, and forest-specialist nature. So, considering the distance among the sampled areas, we also expected a strong influence of spatial components on communities from the most distant areas, and it would be a prevalence of species-sorting perspective (environmental filtering processes) rather than mass-effect (increased dispersal among closer sites).

Material and Methods

Study area

We sampled 35 stream reaches in the municipalities of Assis Brasil, Senador Guiomard, Sena Madureira, and Porto Acre, all belonging to the state of Acre, in the Brazilian Amazon during August 2019 (Fig. 1). The vegetation in the region is mostly classified as a dense and open ombrophilous forest (Acre, 2018). According to the Köppen classification (Peel et al., 2007), the climate is "*Af*": a tropical rainforest with approximately 312 mm of rainfall, relative air humidity between 80% - 90%, and a high mean temperature (24.5 ºC – 32 ºC) (Acre, 2018).

In the present study, the SUA streams are situated in two extractive reserves (Federal law 9.985/2000, Decree 4.340/2002), in which the natural resources are explored by the traditional people. Although the SUA belongs to protected areas according to the Brazilian legislation, they are still under some pressure from human activities (e.g., urbanization, monocultures, crop cash and pasture). The SUA Chico Mendes (CM) has a total area of approximately 931.542 ha (10º 06'11'' S, 67º 56'13'' W), located on the triple border between Brazil, Bolivia, and Peru, and is characterized by the extraction of rubber from the rubber tree *Hevea brasiliensis* (Willd. ex A. Juss.) Müll.Arg. (Federal law 99.144, March 12th, 1990). The SUA Cazumbá-Iracema (CZ) has an area of 754.987 ha (09º 01'00'' S, 70º 11'00'' W), where the main livelihood activities are the cultivation of açaí palm *Euterpe oleracea* Mart. and hunting (Acre, 2010).

Additionally, the streams outside SUA belong to municipalities Senador Guiomard (SG) and Porto Acre (PAc), which are not embodied in the conservation units' zones and have a much higher impact of human activities such as agriculture, pasture, and urbanization. Together, the populations of both municipalities surpass 38.000 inhabitants, and most of these populations perform activities related to cattle grazing and agriculture (IBGE, 2020). Overall, the study area encompassed about 20.5% of the state of Acre.

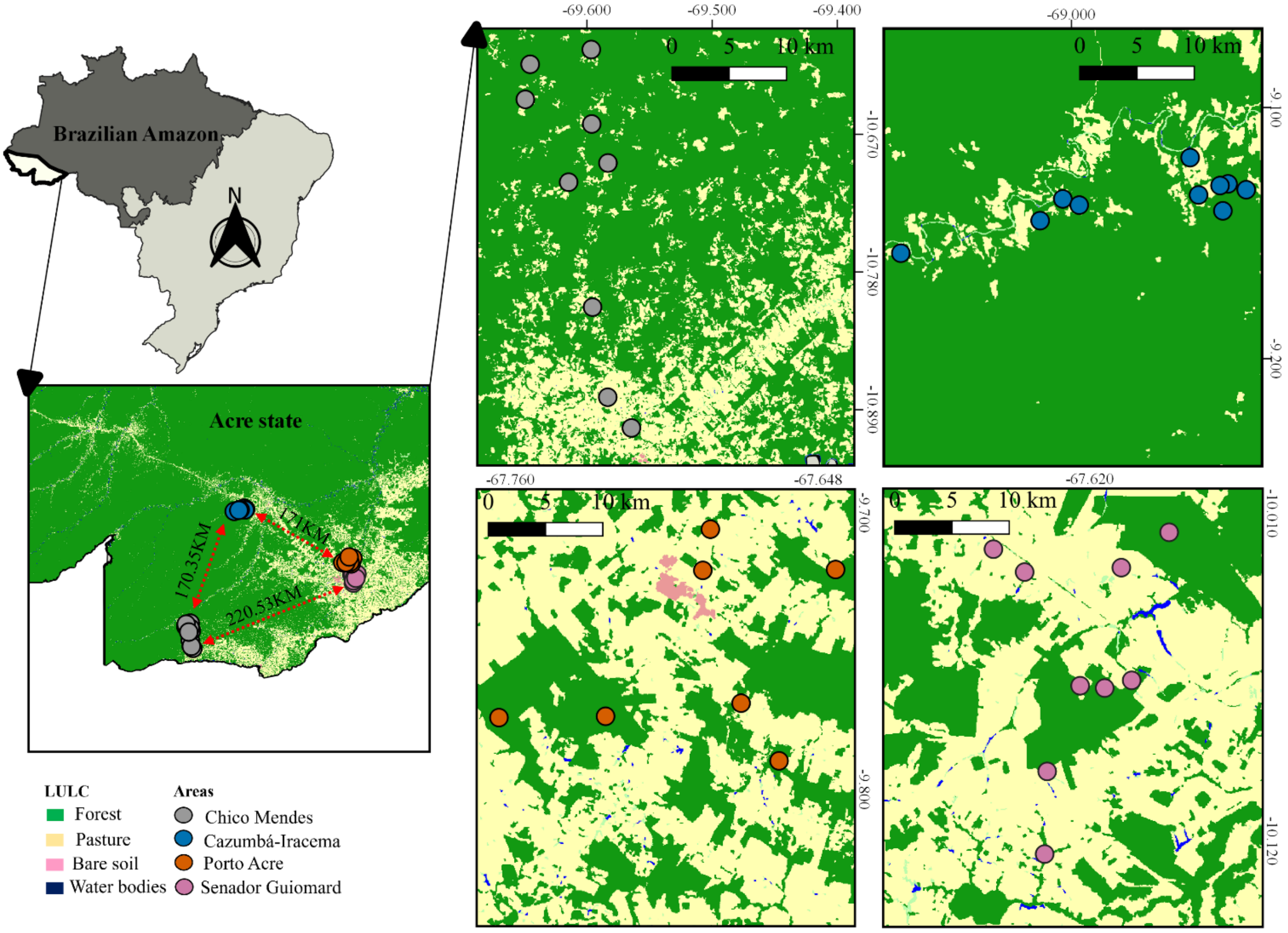

Fig. 1 Location of 35 sampling sites within the study area in sustainable use areas Chico Mendes and Cazumbá-Iracema, and the municipalities out of sustainable use areas Senador Guiomard and Porto Acre, Acre state, Brazil. LULC = Land use and land cover.

Biological sampling

We sampled the biological data in each stream using the well-known scanning methodology already applied in previous studies (Oliveira-Junior et al., 2015; Calvão et al., 2022; Bastos et al., 2021). Following this methodology, each stream was divided into 150 m, with ten subsections of 15 m, in which Odonata adults were sampled using an entomological net (diameter: 40 cm, depth: 65 cm, aluminum handle: 90 cm), in the warmest period of the day (10 am – 2 pm) when they perform their most frequent daily activities (e.g., territorialism, breeding, foraging, ovipositing) (Batista et al., 2021). Afterward, the specimens were stored in paper envelopes according to the standardized methodology of the Lencioni (2005, 2006). The laboratory routines for identification encompassed the use of taxonomic keys (Lencioni, 2005, 2006; Garrison et al., 2006, 2010), and, when necessary, the aid of specialists. After all the procedures, the biological vouchers were deposited at the collection of the Universidade Federal do Pará.

Environmental and spatial variables

We measured four categories of environmental variables:

i) Limnological variables: water temperature (wtemp: ºC), pH, conductivity (cond: mS/m-1), dissolved oxygen (do: mg/L) (Table 1), measured with a multiparameter probe at three equidistant points along the channel of the streams (downstream – middle – upstream) (Monteiro-Júnior et al., 2016).

ii) Air variables: air temperature (at: ºC) and humidity (hum: %), measured with a thermohygrometer of the streams (Table 1). These variables are important in determining species composition in both Odonata suborders (Carvalho & Nessimian, 1998; Mendes et al., 2018; Mendes et al., 2019).

iii) Physical variables: percentage of canopy cover (canopy: %), channel depth (depth: cm), channel width (width: cm) and Habitat Integrity Index (HII) proposed by Nessimian et al. (2008) (Table 1). HII provides scores from 0 to 1, where closer to 0 indicates a degraded or modified environment, and closer to 1 indicates a well-preserved environment. The HII is largely used in ecological studies in Amazonian freshwater systems (Oliveira-Junior et al., 2015; Monteiro-Júnior et al., 2015; Luíza-Andrade et al., 2020; Bastos et al., 2021).

iv) Landscape variables: altitude (m) and the distance to the nearest patch (dnp: m) (Maxwell et al., 2021). Altitude is related to the variation in water flow, a variable that might influence the selection of oviposition sites by the females (Palacino-Rodriguez et al., 2020), and the distance to the nearest patch might be related to the dispersal of the dragonflies and damselflies, varying from near to farther patches. The first was measured through the GPS information collected on the field, which we used into Shuttle Radar Topography Mission and inserted into QGIS software to obtain altitudinal values. We took the values of dnp using Google Earth satellite images (Maxwell et al., 2021).

The spatial dataset was generated through spatial eigenfunction analysis (Borcard et al., 2004; Dray et al., 2006; Legendre & Legendre, 2012), based on the Principal Coordinates of Neighbor Matrices (hereafter PCNM; Dray et al., 2006) applied to a Euclidean distance matrix of the geographical coordinates . This analytical process was performed in the R computational environment (R Core Team, 2023), and we used *pcnm*, *chooseNC*, *nb2listw* and *moran.randtest* functions, built in the packages *adespatial* (Dray et al., 2023) and *spdep* (Bivand & Wong, 2018). Moreover, the first eigenvectors (PCNM1, PCNM2, PCNM3) represented broad-scale variation, while the last represented small-scale variation (PCNM20, PCNM21, PCNM23) (Borcard et al., 2004; Maxwell et al., 2021).

Table 1 Minimum, maximum, mean and standard deviation (SD) values of the environmental variables of the streams (air, limnological, physical structuring and landscape) collected in the streams sampled in the four areas, Acre, Brazil.

Environmental variables	**Code**	**Min**	**Max**	**Mean**	**SD**

Air temperature (°C)*	at	21.7	34.7	29.62	2.819
Altitude (m)	Altitude	149	316	206	49.787
Channel depth (cm)*	depth	8	40.47	17.91	6.600
Channel width (m)	width	0.605	28.8	7.850	7.670
Conductivity (mS/cm)	cond	0	10.41	2.379	3.382
Dissolved oxygen (mg/L)*	do	29.43	121.53	73.81	22.577
distance to the nearest patch (km)	dnp	0.36	8.140	2.667	1.815
Habitat Integrity Index*	HII	0.29	0.97	0.6277	0.189
Humidity (%)	hum	0.34	83	48.04	35.315
Percentage of canopy cover (%)	canopy	82.81	99.6	91.9	4.840
Potential of hydrogen	pH	5.040	8.933	6.853	1.125
Water temperature (°C)	wt	14.13	28.7	22.73	3.760

Statistical analysis

We standardized each stream as our sampling sites, where abiotic and biotic data were collected. We verified the possible effects of multicolinearity among the environmental variables using the Variance Inflation Factor (VIF) with the *vif* function, from *faraway* package (Faraway, 2016), in R computational environment. We applied the procedure carried out by Heino et al. (2017), which consists in sequentially removing the variables scoring higher than a pre-established value (we adopted a threshold of 3) until all the remaining VIF values are smaller than the threshold (Zuur et al., 2010; Heino et al., 2017). Although the VIF is a robust method to deal with multicolinearity, after the analysis, we always considered the variables most important biologically for Odonata.

We carried out for both environmental and spatial datasets an independent forward selection to retain the most important variables for the suborders of Odonata (Blanchet et al., 2008), using *forward.sel* function, in *adespatial* package. Basically, this method selects a subset of predictor variables, relying on two stopping criteria: i) the p-value of $\alpha = 0.05$ or lower and ii) the adjusted R^2, calculated using all dataset variables (Blanchet et al., 2008; Borcard et al., 2018). Air temperature, dissolved oxygen, depth of the channels and HII were maintained for both Anisoptera and Zygoptera, along with PCNM1 and PCNM2 spatial filters, both as broad-scale variables.

To test our first prediction, we carried out a student t-test to address significant differences regarding α-diversity (species richness) of both suborders and HII scores at the coarser level, in this case, between the levels of conservation (inside vs outside SUA), using *t.test* function from *car* package. Due to its reliability as a tool to address the local environmental quality, and to predict the aquatic insect communities in freshwater systems (Brasil et al. 2020), we used HII as the main independent variable in the univariate analytical procedures. Moreover, considering that the four areas may have some particularities, we carried out a one-way Anova to verify significant differences regarding α-diversity and HII scores among the four areas of study, using the *aov* function, from *vegan* package. We checked the main assumptions when running the student t-test and one-way anova analyses: 1) the independence among the sampling sites, 2) normal distribution and 3) homogeneity of variances (Zar, 2010). When any significant result for one-way anova was found, we applied the Honestly Significant Difference Tukey test (HSD Tukey) to assess the individual differences (Zar, 2010), using *TukeyHSD* function, built in *stats* package (R Core Team, 2023). When the student t-test and one-way anova assumptions were not met, we applied the t-test with separate variances and the non-parametric Kruskal-Wallis test, respectively. We carried out a Permutational Multivariate Variance Analysis (PERMANOVA; Anderson, 2001) to assess significant differences between the levels of conservation and among the four areas regarding the species composition of both suborders, using *adonis2* function, from *vegan* package, with 9999 permutations and Bray–Curtis matrix of dissimilarity. Then, we applied a Permutational Analysis of Multivariate Dispersions (PERMDISP; Anderson, 2006) to address possible differences regarding the community heterogeneity (β-diversity) between the levels of conservation and among the areas (Anderson & Walsh, 2013), through *betadisper* function, in *vegan* package.

For our second prediction we calculated the local contribution (LCBD) and species contribution (SCBD) to β-diversity following the routine proposed by Legendre and de Cáceres (2013), using the composition data of both Anisoptera and Zygoptera. For the species composition we applied a Hellinger-transformation and *beta.div* function from *adespatial* package (Legendre & de Cáceres, 2013; Brasil et al., 2021a; Bomfim et al., 2023). We used 999 permutations to obtain the total sum of squares (SStotal), from which we calculated the total beta diversity (BDtotal), LCBD and SCBD. To test possible significant differences of means of LCBD between the levels of conservation we carried out student t-test. When the assumptions were not met, we applied the student t-test for separate variances.

Considering the third prediction, to determine the relative influence of environmental and spatial variables on the species composition of Anisoptera and Zygoptera, we applied the variance partitioning procedure using *varpart* function from *vegan* package (Legendre & Legendre, 2012; Borcard et al., 2018). This method is routinely used in ecology to address the relative importance of environmental and spatial variables on metacommunities. The *varpart* function partition the metacommunity structure in four fractions: pure environmental importance (E | S), pure spatial importance(S | E), shared importance (E ∩ S) and the residuals (U) (unexplained variance) (Peres-Neto et al., 2006). Additionally, to test the significative influence of environmental and spatial components on species composition, we carried out a distance-based redundancy analysis (db-RDA; Borcard et al., 2018), most suitable to non-euclidian dissimilarity indices, applying the Bray-Curtis distance as a measure of dissimilarity on the log-transformed species abundance matrices (Borcard et al., 2018). Prior to db-RDA, the environmental variables selected by *forward.sel* function were standardized (except HII), using *decostand* function, from *vegan* package, to prevent higher influence of a single variable. The function *capscale* from *vegan* package was used to carry out the db-RDA. Moreover, the procedure provides a Pseudo-F value as a measure of the significance of the overall analysis, and we tested the significance through a normal anova procedure (Borcard et al., 2018). As we used dissimilarity matrices are response variables (Bray-Curtis), the db-RDA was also applied to partition the variation into the pure components of environment, space, and their shared contribution to the composition of Anisoptera and Zygoptera. To test the significance of each component, an anova permutation test for the db-RDA was performed with 999 permutations.

All the analytical procedures were conducted in the R computational environment (R Core Team, 2023), version 4.3.1, in the RStudio interface. For building the graphical information, we used ggplot2 package (Wickham, 2016). The main analytical procedures are summarized in the flowchart (Fig. 2).

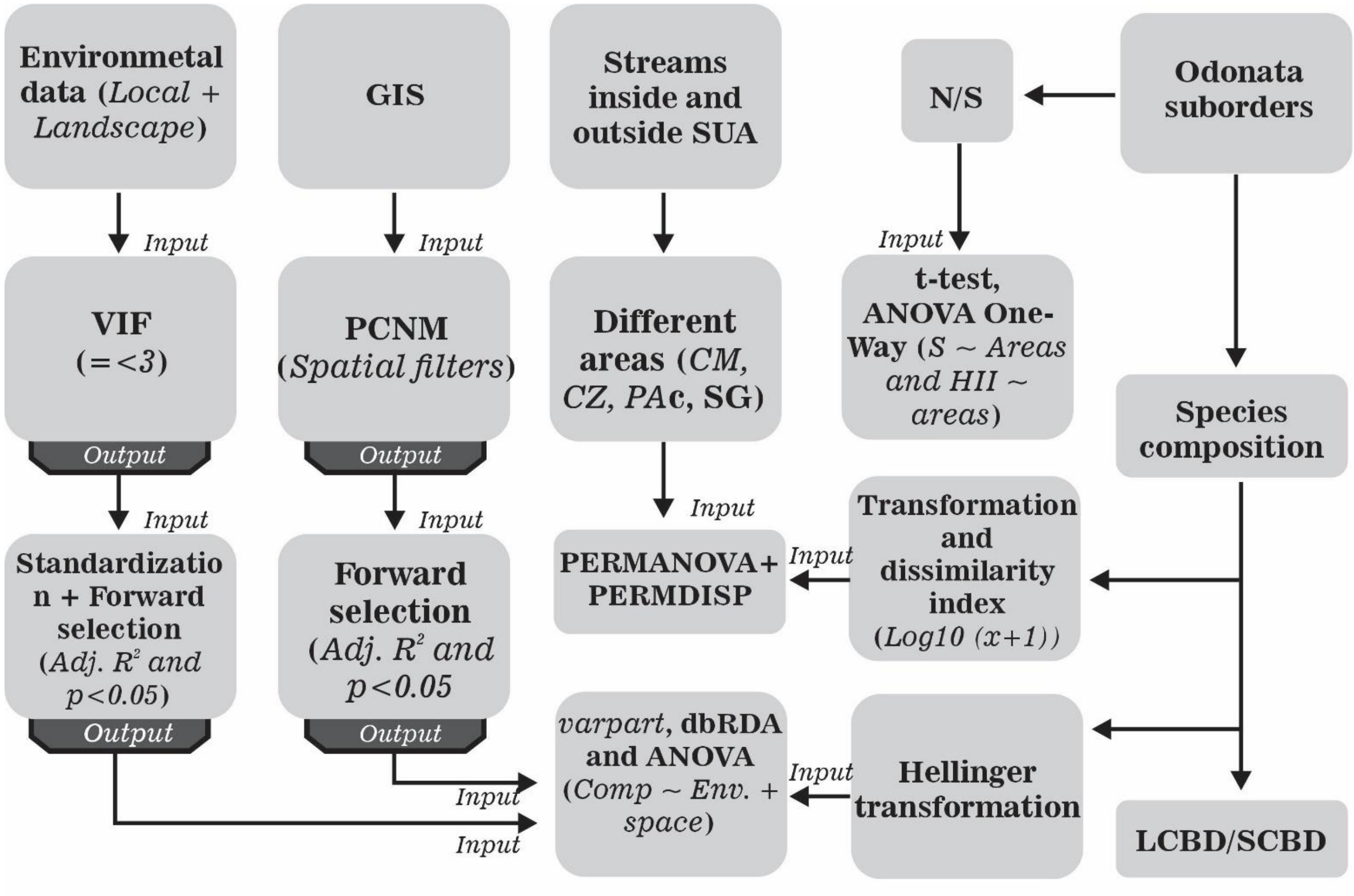

Fig. 2 Schematic flowchart presenting the main analytical procedures used in this study. Adj. R² = Adjusted R²; CM = Chico Mendes area; Comp. = species composition; CZ = Cazumbá-Iracema area; db-RDA = distance-based redundancy analysis; Env. = environmental data; GIS = geographical information system; LCBD = Local Contribution to the Beta Diversity; N/S =

abundance and species richness of Anisoptera and Zygoptera; PAc = Porto Acre municipality; PCNM = Principal Coordinates of Neighbor Matrices; PERMANOVA = Permutational Analysis of Variance; PERMDISP = Permutational Analysis of Multivariate Dispersions; SCBD = Species Contribution to the Beta Diversity; SUA = sustainable use areas; SG = Senador Guiomard area; VIF = Variance Inflation Factor.

Resultados

Biological overview

A total of 561 individuals of Odonata were sampled, belonging to nine families and 64 species (Table S1). We collected 22 species of Anisoptera encompassing 76 specimens (13.5% of the total Odonata), the most abundant genus was *Oligoclada* (n = 20), followed by *Perithemis icteroptera* (Selys in Sagra, 1857) (n = 8), followed by *Erythrodiplax basalis* (Kirby, 1897) (n = 4) and *Perithemis thais* Kirby, 1898 (n = 4). On the other hand, 49 species of Zygoptera were identified from 485 individuals (86.2% of the total Odonata). *Heteragrion bariai* De Marmels, 1989 (n = 73) was the most abundant species, followed by *Mnesarete cupraea* (Selys, 1853) (n = 51), *Heteragrion bickorum* Daigle, 2005 (n = 43) and *Epipleoneura venezuelensis* Rácenis, 1955 (n = 35). Additionally, we recorded for the first time the Zygoptera species *Drepanoneura loutoni* von Ellenrieder & Garrison, 2008 in Brazil, occurring in eight sites.

Relationship between Odonata α and β-diversity, areas, and levels of conservation

We did not find significant differences in species richness and abundance of Anisoptera between the levels of conservation (Table 2). Similarly, we did not find significant results for species richness and abundance of Zygoptera (Table 2). HII also did not differ between the levels of conservation (Table 2). Nevertheless, we did find significant differences among the four areas. CM and SG areas differed, regarding habitat integrity (Table 2; Fig. 3a), with the first scoring the lowest HII, and the second the highest. There was a significant difference regarding the species richness of Zygoptera (Table 2) between PAc and CZ (Tukey p = 0.004), and SG and PAc (Tukey p = 0.008) (Fig. 3b), where CZ presented, on average, 49 more species than PAc, and SG 41 more species than PAc. Additionally, we also found significant differences regarding abundance of Zygoptera between PAc and CZ (Tukey p = 0.003) (Fig. 3c; Table 2). However, we did not find any significant difference regarding the species richness and abundance of Anisoptera among the areas (Table 2). So, conservation levels were a categorization too coarse to allow us to find any significant association; on the other hand, the finer results from each area provided significant results.

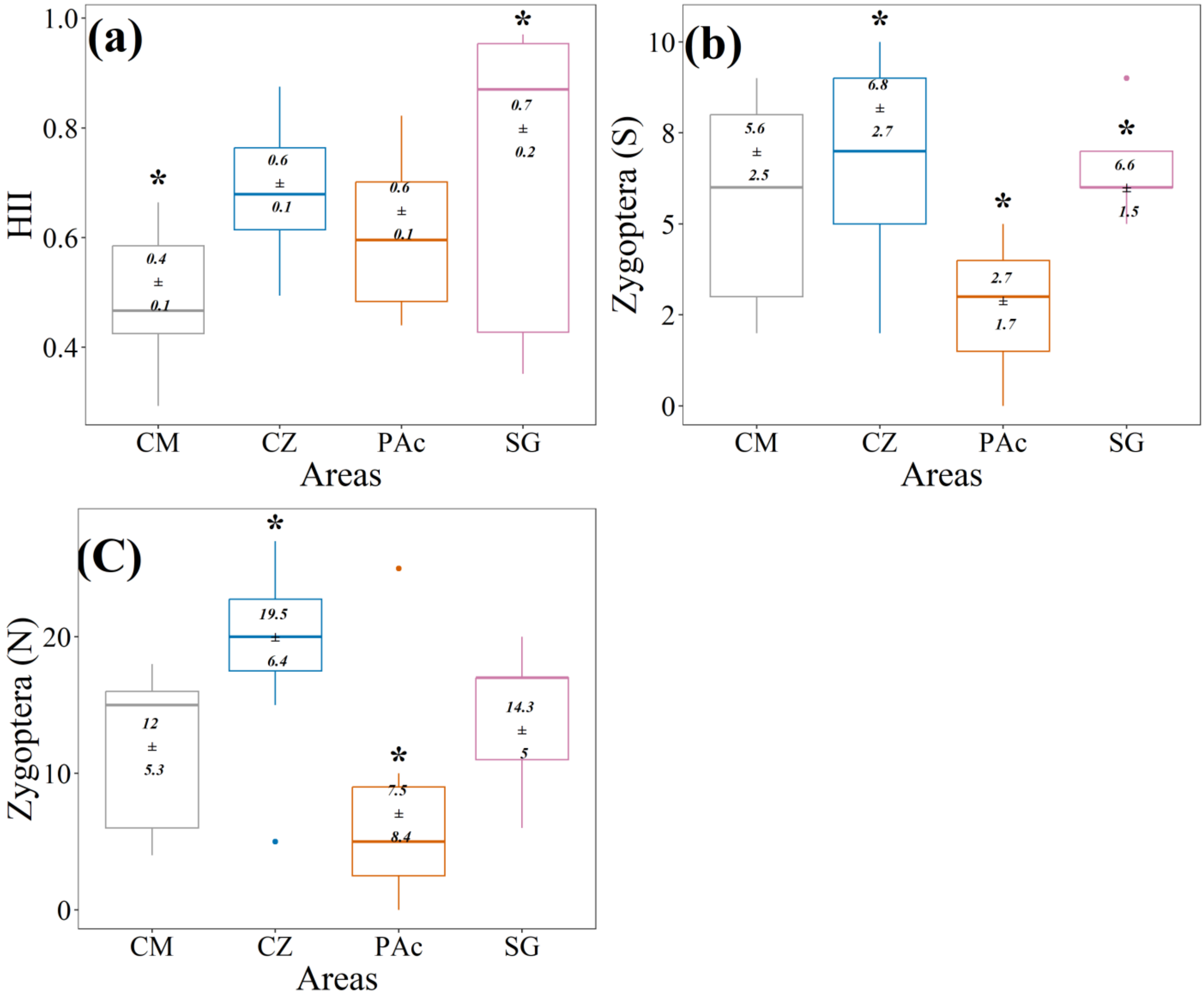

Fig. 3 Habitat Integrity Index (HII) (a), species richness (Zygoptera (S)) (b), and abundance of Zygoptera (Zygoptera (N)) (c) of the four sampling areas. CM = sustainable use area of Chico Mendes; CZ = sustainable use area of Cazumbá-Iracema; PAc = out of sustainable use area of Porto Acre; SG = out of sustainable use area of Senador Guiomard. Asterisks indicate areas with significant differences.

Table 3 Results of the PERMANOVA and PERMDISP applied between the levels of conservation (inside and outside Sustainable Use Areas) and among the four areas (Senador Guiomard, Porto Acre, RESEX Chico Mendes and RESEX Cazumbá-Iracema). HII = Habitat Integrity Index; N = abundance of individuals; S = species richness. F= Pseudo-F; R^2 = R-squared; df = degree of freedom

PERMANOVA	**F**	**R^2**	**df**	**p-value**
Anisoptera				
Levels of conservation	1.673	0.048	1	0.106

Four areas	2.365	0.186	3	**0.002***
Zygoptera				
Levels of conservation	4.192	0.112	1	**0.001***
Four areas	3.352	0.244	3	**0.001***
PERMDISP	**F**		**df**	**p-value**
Anisoptera	0.619		3	0.607
Zygoptera (SG-PAc)	6.719		3	**<0.001***

Considering the species composition of both suborders, we found a significant difference in Anisoptera composition among the areas, but not between the levels of conservation (Table 3). We did not find difference regarding the heterogeneity of the areas (Table 3). We also found a significant difference in Zygoptera composition among the areas, with PERMDISP indicating that SG is more heterogeneous than PAc (Table 3). Additionally, we found significant difference between the levels of conservation, but no difference regarding the heterogeneity of the groups (Table 3). Thus, those results partly did not corroborate our first hypothesis since SG presented the highest integrity level and the highest number of exclusive species of Anisoptera (n = 5), and mainly Zygoptera (n = 10). Only in conjunction, CM and CZ areas presented a slightly higher number of exclusive Zygoptera species (n = 12).

Table 3 Results of the PERMANOVA and PERMDISP applied between the levels of conservation (inside and outside Sustainable Use Areas) and among the four areas (Senador Guiomard, Porto Acre, RESEX Chico Mendes and RESEX Cazumbá-Iracema). HII = Habitat Integrity Index; N = abundance of individuals; S = species richness. F= Pseudo-F; R² = R-squared; df = degree of freedom

PERMANOVA	**F**	**R²**	**df**	**p-value**
Anisoptera				
Levels of conservation	1.673	0.048	1	0.106
Four areas	2.365	0.186	3	**0.002***
Zygoptera				
Levels of conservation	4.192	0.112	1	**0.001***
Four areas	3.352	0.244	3	**0.001***
PERMDISP	**F**		**df**	**p-value**
Anisoptera	0.619		3	0.607
Zygoptera (SG-PAc)	6.719		3	**<0.001***

The total beta diversity (BDtotal) was 0.805 and SStotal was 27.401. The LCBD results indicated that streams inside and outside SUA had similar contribution to the β-diversity (Fig. 4a and 4b), but the student t-test did not show any significant difference between the levels of conservation (t = -1.974; df = 33; p = 0.056). The LCBD results for streams outside SUA indicated a BDtotal of 0.821 and SStotal of 12.319. Moreover, considering streams outside SUA, females from the Anisoptera genus *Oligoclada* contributed the most to the β-diversity (SCBD = 0.119), followed by Zygoptera species *H. bariai* (SCBD = 0.082) and females from the Zygoptera genera *Hetaerina* (SCBD = 0.079) and *Ischnura* (SCBD = 0.078). The LCBD results for streams inside SUA showed BDtotal of 0.717 and SStotal of 12.909. Besides, streams inside SUA presented only Zygoptera species contributing the most to β-diversity: *H. bickorum* (SCBD = 0.096), *M. cupraea* (SCBD = 0.094), *H. bariai* (SCBD = 0.082) and *D. janirae* (SCBD = 0.072).

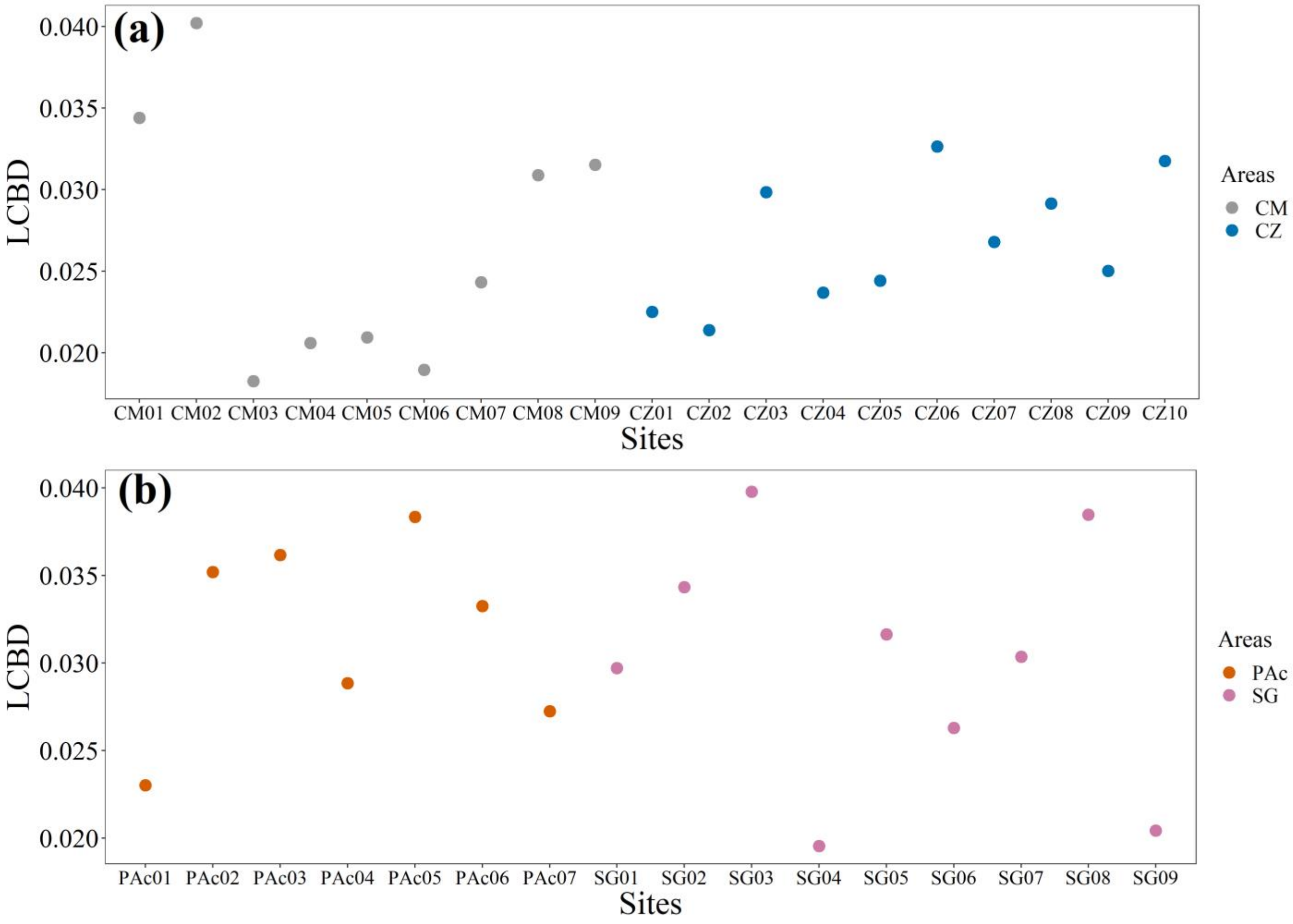

Fig. 4 Results of the local contributions to β-diversity (LCBD) for the streams sampled in all four areas. CM = sustainable use area of Chico Mendes; CZ = sustainable use area of Cazumbá-Iracema; PAc = out of sustainable use area of Porto Acre; SG = out of sustainable use area of Senador Guiomard.

Main drivers of the Odonata communities

The variance partitioning applied on the species composition of Anisoptera indicated that environment and space explained 7% and 6% of the total explanation, respectively (Fig. 5a).The two axes of the db-RDA explained 37.33% of the total variance for species composition of Anisoptera (Fig. 5a). However, the db-RDA indicated no influence of environmental and spatial variables on the species composition of Anisoptera (F = 1.040; p = 0.222; F = 1.126; p = 0.069), with no visible clustering of the streams (Fig. 6a).

The variance partitioning for species composition of Zygoptera indicated that environment explained 4%, space explained 9%, and their interaction explained 4% (Fig. 5b). Moreover, the two axes of the db-RDA explained 57.37% of the total variance for species composition of Zygoptera (Fig. 6b). Besides, differently from Anisoptera, the db-RDA indicated that both environmental and spatial variables were significantly associated to the species composition of Zygoptera (F = 1.213; p = 0.017; F = 1.961; p = 0.001, respectively). Both spatial filters, dissolved oxygen and HII were the variables that drove the most the species composition of this suborder (Fig. 6b). Species such as *H. bickorum* and *D. janirae* were more associated with pcnm2, mainly in CZ sites (Fig. 6b); sites from SG and PAc were more influenced by dissolved oxygen, HII and pcnm1, with species from the genus *Argia* being more associated with these variables (Fig. 6b). So, the results indicated a significative joint influence of spatial, limnological, and physical structuring influence on Zygoptera, and no influence on Anisoptera, agreeing partly with our predictions.

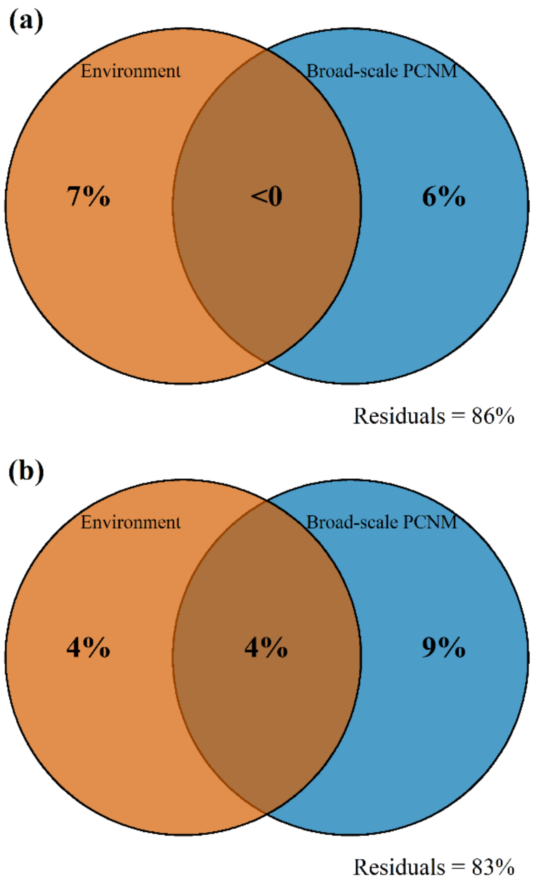

Fig. 5 Variance partitioning diagram showing the fractions of explained variance of Anisoptera (a) and Zygoptera (b) metacommunities structure by environmental and broad-scale spatial drivers (PCNM).

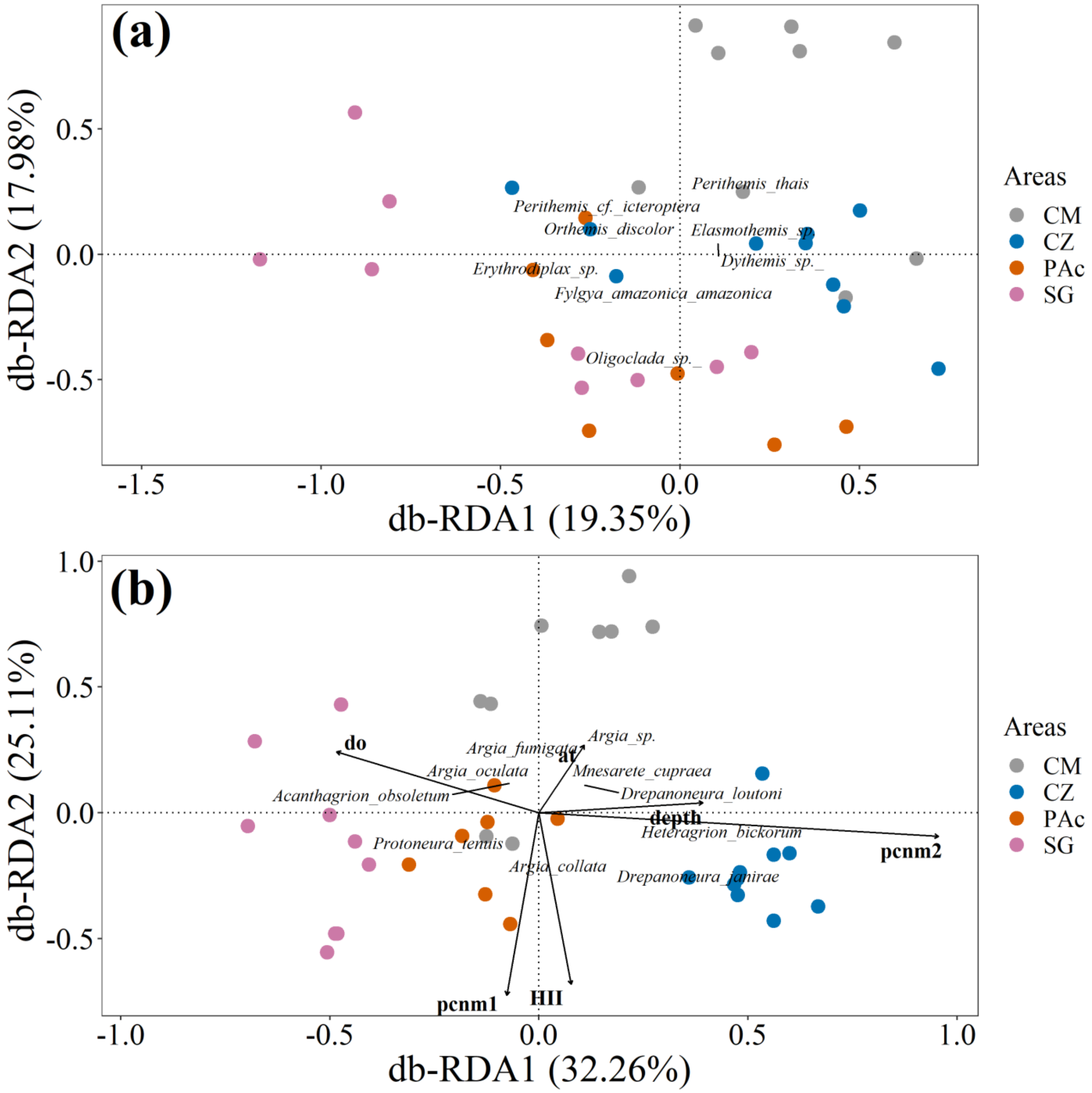

Fig. 6 Ordination of sites based on the composition of Anisoptera (a) and Zygoptera (b), with the axes explanation from distance-based redundancy analysis (db-RDA) and with the variables significantly related to the Zygoptera communities. at = air temperature (°C); depth = depth of the channel (cm); do = dissolved oxygen (mg/L); HII = Habitat Integrity Index; pcnm01 and pcnm02 = broad-scale spatial drivers. We plotted the Anisoptera ordination only for showing the overall pattern since there was no significant relationship of this group with environmental or spatial variables.

Discussão

Our results indicated that CM streams may be losing their integrity levels and capacity to preserve the local diversity of sensitive species, due to the high number of Anisoptera species and lowest integrity scores. This scenario disagrees partly with our first prediction. Moreover, we found no significant difference between the contribution to β-diversity of streams inside and outside SUA, with LCBD being similar, partly not corroborating our second hypothesis. Additionally, only one Anisoptera genus contributed the most to the β-diversity in streams outside SUA, and only Zygoptera species contributed the most to the β-diversity in streams inside SUA, corroborating our second prediction partly. We can also infer that environmental and spatial variables presented a joint importance for communities of Zygoptera, but not for Anisoptera. Besides, environmental variables related to air temperature, limnology and physical structure of the habitats, and the broad-scale spatial filters, were the most important for Zygoptera species, corroborating our third hypothesis.

Sites belonging to CZ area presented activities more related to açaí plantation and small pasture activities, less intense when compared to the activities carried inside CM area (e.g., rubber tap process and intense pasture activities) (personal observations). Moreover, the streams inside CZ did not present cattle trampling near their margins, which can explain the high abundance and species richness of Zygoptera, compared to CM streams, whose margins were more affected by the presence of cattle. Besides, a similar pattern was found regarding the HII scores, where CZ scored the second highest mean value, only below SG scores. The absence or less intense influence of human activities, related to agriculture, pasture and trails, in CZ streams may explain the strong difference between this area and CM area. The activities observed in the field (e.g., agriculture, pasture, trails) modify the surroundings of the streams, increasing the channel run-off and silting, unbalancing the chemical composition of the water, diminishing the vegetation cover and the provision of allochthonous materials to the channel (Rodrigues et al., 2019; O'Malley et al., 2020; Bastos et al., 2021). For the species *C. rutilans* and genus *Heteragrion*, the presence of wood debris from the riparian vegetation is crucial for ovipositing behavior, as well as for other daily activities (e.g., territorialism, foraging, mating) (Resende & De Marco Junior, 2010; Carvalho et al., 2018; Bastos et al., 2021). However, contradicting our prediction, SG streams (outside SUA) presented the highest HII scores, which might explain the importance of dissolved oxygen in these sites since preserved habitats often present more vegetation cover and physical stability, crucial for keeping high levels of dissolved oxygen (Calvão et al., 2016; O'Malley et al., 2020; Dala-Corte et al., 2020). For Zygoptera species, mainly composed by conformer species (whose body temperatures vary with the environment), environmental stability provided by high-integrity sites is crucial, due to the low range of variation of local conditions such as temperature and canopy cover.

Several ecological studies using the LCBD found either negative or positive relationship between this metric and human activities (Heino et al., 2017; Leão et al., 2020). In the present study, there was no difference between streams inside and outside SUA, with these sites contributing similarly to the overall β-diversity. This might indicate some degree of similarity between the areas inside and outside SUA, mainly among CM and the areas outside SUA (SG and PAc). Furthermore, Heino & Grönroos (2017) found a negative relationship between both metrics, which may indicate the sites with unique species usually would have low species richness. As discussed by López-Delgado et al. (2020), the use of LCBD should be considered cautiously, always considering preserving streams that have high species richness, and high ecological uniqueness. The high contribution of Zygoptera species for the β-diversity in streams outside SUA may also agree present some suitable conditions to preserve the diversity of species of this suborder. However, *H. bariai* are more associated to shaded sites, with more vegetation cover and stable conditions, usually present in preserved areas. This may indicate that some streams outside SUA have some of these conditions and so can maintain the populations of this species. Moreover, only Zygoptera species contributed significantly to β-diversity in streams inside SUA. Species from the genera *Heteragrion* and *Drepanoneura* are usually associated to dense vegetation cover, mainly because of the provision of allochthonous material to the stream and environmental stability (Ellenrieder & Garrison, 2008; Brito et al., 2021; Calvão et al., 2022), conditions found mainly in CZ streams.

In general, spatial variables may influence Anisoptera and Zygoptera differently (Juen & De Marco, 2012; Brasil et al., 2018; Oliveira-Junior et al., 2019). Anisoptera species are well-known effective dispersers, with morphological and physiological adaptations to overcome environmental harshness while dispersing (Corbet & May, 2008; Juen & De Marco, 2012; Dalzochio et al., 2018). In this study, we did not find evidence that space restricted the distribution of the Anisoptera species since no PCNM spatial filter was significant for their composition. On the other hand, the significant importance of broad-scale spatial variables for Zygoptera species indicates dispersal constraints, and the geographical location of the areas may explain these results. To reach suitable sites the species must endure the environmental variation and should have the physiological apparatus to disperse effectively (Leibold et al., 2004). For strictly aquatic tolerant invertebrates, such as Chironomidae, the space would not constrain their dispersal due to their watercourse dispersal, following the channel of the streams, and they would be able to endure harsh environmental conditions while dispersing (Kotzian et al., 2020; Maxwell et al., 2021). However, for more sensible taxa, such as adults of Zygoptera species, the dispersal gets more difficult because of the distance among the available sites, and due to intrinsic morphological characteristics. Zygoptera species present slender bodies, with high surface-to-volume ratio, making them susceptible to the high amplitude of variation of the environmental conditions (e.g., air temperature) during the dispersal movements (Oliveira-Junior et al., 2015). So, this may explain the influence of the spatial filter PCNM2 on CZ streams, which are more distant from the other areas, indicating dispersal constraints for Zygoptera species at broad scale. On the other hand, the closeness between SG and PAc can explain the lack of fine-scale spatial influence for both suborders.

The continuum between mass-effect and species-sorting is usually the most common driver in structuring metacommunities (Leibold et al., 2004; Heino, 2013; Cunha & Juen, 2020). Although in aquatic systems, the species-sorting mechanism is generally the most common, the spatial variables related to the geographical location of the sites must be considered since are linked to species dispersal restrictions and their ability to track the most suitable environmental conditions (Tonkin et al., 2018). Thus, human-induced modifications can change these dynamics by removing the vegetation surrounding the streams and forest corridors , reducing the connectivity among sites, and changing the local environmental features (Mendes et al., 2019; O'Malley et al., 2020; Cruz et al., 2022). The environmental influence on Zygoptera highlight the prevalence of niche processes as important local drivers, but the accentuated effect of spatial variables calls attention to restrictions imposed by the landscape fragmentation and the location of the sites on their species (Brasil et al., 2017; Cruz et al., 2022). Moreover, even though environmental variables can shape the distribution of Anisoptera species, the effective dispersal rates of this suborder generate directional patterns more independent from the environmental conditions and may somehow mask their influences (Leibold et al., 2004), which can explain the no significant results for this group in this study.

Conclusion

Our results indicated a lower diversity of Zygoptera species and habitat integrity in Chico Mendes SUA, which underlines the loss of its capacity to maintain the local diversity of species and habitat integrity. Moreover, we found that communities of Zygoptera were primarily influenced by environmental and spatial variables, with a greater influence of the latter. The use of the metacommunity approach in conservation units of sustainable use is compelling since human activities can hugely influence the exchange of individuals of both suborders among the available sites, change the environmental stability and consequently determine the relative influence of the abiotic variables on the communities of aquatic species. Moreover, we highlight that sustainable use areas need closer management by public agencies, in our study mainly Chico Mendes SUA. Most importantly, the policymakers must implement social policies favouring traditional people. Due to extreme scenarios of poverty, in some severe situations, the only way those people have to ensure their families' survival is to explore these sustainable use areas through activities out of the scope of the current legislation. Therefore, implementing social policies would directly influence the rational exploration of natural resources in sustainable use areas.

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Author contributions

Conceptualisation: Joas Silva Brito, Karl Cottenie and Leandro Juen; Data collection: Rafael Costa Bastos and Victor Rennan Santos Ferreira; Methodology: Joas Silva Brito, Rafael Costa Bastos and Victor Rennan Santos Ferreira; Formal analysis and investigation: Joas Silva Brito, Rafael Costa Bastos and Leandro Juen; Writing–original draft preparation: Joas Silva Brito, Karl Cottenie and Leandro Juen; Writing–review and editing: Leandro Schlemmer Brasil, Rafael Costa Bastos, Victor Rennan Santos Ferreira, Gabriel Martins Cruz, Diego Viana Melo Lima, Lisandro Juno Soares Vieira and Thaísa Sala Michelan; Resources: Leandro Juen, Lisandro Juno Soares Vieira and Thaísa Sala Michelan; Supervision: Karl Cottenie and Leandro Juen.

Declarations

Ethical Responsibilities of Authors All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Availability of data and material All data generated or analyzed during this study are included in this published article.

Ethics statement Ethical review and approval was not required for the study in accordance with the local legislation and institutional requirements. Ethical approval for research on invertebrates is under the permanent license SISBIO 11841-6.

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3º Capítulo: CO-OCCURRENCE AND ECOLOGY OF DAMSELFLIES OF CALOPTERYGIDAE SPECIES (ODONATA: ZYGOPTERA) IN STREAMS IN AMAZONIAN PROTECTED AREAS

Abstract

Interspecific competition and environmental filtering can lead to niche overlap, influencing species co-occurrence in freshwater systems, mainly of those closely related taxa. We evaluated co-occurrence patterns of damselflies from the Calopterygidae family in streams inside and outside protected areas, examining the impact of abiotic variables on species composition and co-occurrence. This family exhibits high diversity and abundance in the tropics, along with remarkable behavioral traits, closely associated with local conditions. Our hypothesis was that streams within protected areas would show more significant co-occurrences and less random associations, while the opposite would be observed in streams outside these areas. We also predicted a stronger influence of physical structuring variables on the composition of the Calopterygidae species. We collected 369 individuals of ten Calopterygidae species, in 98 streams in four protected areas in the Brazilian Amazon. Negative co-occurrences were observed within protected areas, with more random co-occurrences outside. Furthermore, we found a joint significant influence of limnological and physical structuring variables on the composition of Calopterygidae species, although these variables had no significant impact on species co-occurrence. Our findings suggest that protected areas play a crucial role in maintaining more heterogeneous ecological networks because of their more heterogeneous nature. Additionally, the evolutionary proximity of our model organisms likely explains the observed co-occurrence patterns since they would present more ecological similarities. While our results underscore the importance of protected areas in preserving diverse ecological networks, further studies on the basic biology of Calopterygidae species are necessary to facilitate more robust inference.

Keywords: interspecific competition; environmental filtering; niche overlap; conservation units; freshwater systems; rubyspot damselflies

Introduction

Interspecific competition stands out as a paramount ecological force shaping the species distribution within natural communities (Abrams, 1983). The ability of species to access available resources is contingent on various factors, including morphological and physiological characteristics, as well as their interactions with other organisms (Connell, 1978; Connor & Simberloff, 1979). The most efficient competitors would be able to explore these resources more efficiently, limiting the access of the other species. The limiting similarity hypothesis predicts that species that are more ecologically similar would compete more intensely to access a given resource (MacArthur & Levins, 1967; Abrams, 1983). Besides, in such a scenario, the likelihood of co-occurrence would occur only if there were some niche differentiation or specialization among competing species (Bode et al., 2011; Guterres et al., 2019; Oliveira-Junior et al., 2021). Furthermore, environmental filtering introduces additional complexity to our understanding of how species exploit available resources (Hutchinson, 1959). Environmental features, such as air and water temperature, humidity, dissolved gases in water, and sunlight exposure,) can select the species that can occur in each area. Species with appropriate physiological and morphological characteristics are favored, while those with inadequate characteristics are outcompeted (MacArthur & Levins, 1967; Guterres et al., 2019).

In the context of environmental filtering processes, the species could coexist in a scenario of niche complementarity, where they occupy partially distinct niches (MacArthur & Levins, 1967; Mason et al., 2011). Moreover, while occupying different niches, the species would enhance the availability of ecosystem services (Mason et al., 2011). However, in a scenario of complete niche overlap, where species share the same resources, competition intensifies and the most efficient competitor would dominate access to those resources (Connell, 1978; Abrams, 1983).

Throughout their evolutionary pathways, species have developed physiological and morphological characteristics in response to both biotic interactions and abiotic factors (Silvertown, 2004; Oliveira-Junior et al., 2021). Consequently, they would adapt to the new abiotic conditions or biotic interactions. Environmental template naturally fluctuates over time, opening and closing niche occupation opportunities for the species (Connell, 1978). However, since the advent of agriculture and, more recently, the Industrial Revolution, humankind has increased its ability to change the environmental balance (Allan et al., 2021). Considering the changes induced by human interventions, such as physical and chemical modifications, interspecific relationships among species may be disrupted. These modifications can either expand or contract the realized niche of species(Sévêque et al., 2020).

One of the most prevalent strategies to mitigate disruptive environmental changes is the establishment of protected areas (Restello et al., 2020; Brasil et al., 2021; Dias-Silva et al., 2021). In Brazilian legislation, the protected areas range from fully protected categories to those that allow sustainable use by the local communities (Brasil et al., 2021). The main objectives of protected areas are maintaining the environmental heterogeneity, protecting the water sources, and preserving the biodiversity of a given area (Abell et al., 2007; Chowdhury et al., 2023). Typically, the implementation of protected areas are focused primarily on terrestrial environments and vertebrate fauna, overlooking the crucial role of freshwater systems (e.g., rivers, streams, ponds, lakes) in maintaining ecosystem services (Chowdhury et al., 2023). Despite the focus on terrestrial environments and biodiversity, protected areas can indirectly safeguard freshwater systems through their buffer zones (Brito et al., 2023). Nevertheless, the importance of freshwater systems must be considered in the first stages of discussions, because they offer innumerous ecological services, provided mainly by aquatic fauna (insects, fish, amphibians), the physical structuring components (riparian vegetation protecting water sources), and the maintenance of diverse ecological networks (Leal et al., 2020; Brasil et al., 2021; Dias-Silva et al., 2021; Brito et al., 2023).

Calopterygidae (Odonata: Zygoptera) stands out as one of the most extensively sampled damselfly families in Amazonian streams. In the Brazilian legal Amazon occurs 28 species of *Hetaerina* and *Mnesarete* (hereafter referred to as Calopterygidae), the only Calopterygidae genera that occur in this biome (De Marco et al., 2023). Moreover, their species are typically found near the stream channel while perched on branches and twigs of trees and bushes (Garrison, 1990; Monteiro Junior et al., 2014; Oliveira-Junior et al., 2019). The taxonomic distinction between both genera is unclear, and phylogenetic relationships show that *Mnesarete* is polyphyletic and nested within Hetaerina (Standring et al., 2022). Therefore, differences between both genera rely mainly on body coloration and wing pigmentation (Garrison, 2006). *Mnesarete* species have green or red metallic bodies and hyaline wings, while Hetaerina species have red metallic bodies and ruby spots in wing bases (Garrison et al., 2010). Calopterygidae species exhibit a set of diverse behaviors, mainly related to territorialism, foraging, and mating tactics, making them valuable models for exploring intra- and interspecific competition patterns (Córdoba-Aguillar, 1995; Córdoba-Aguillar & Cordero-Rivera, 2005; García-Monsalve et al., 2021).

Considering that Calopterygidae species are evolutionary closer to each other, the behavioral characteristics of these species, due to phylogenetic inertia, might indicate different patterns of co-occurrence due to similarity in resources exploration (e.g., oviposition sites, perching points) (Standring et al., 2022). Hetaerina and Mnesarete occupy streams with intermediate preservation conditions (Carvalho et al., 2018; Brito et al., 2021) and well-preserved environments (Monteiro Junior et al., 2015; Calvão et al., 2016) in Brazilian Amazon, indicating their ability to endure harsh conditions even in less heterogeneous environments. Most ecological studies encompassing only Calopterygidae species address predominantly behavioral patterns related to territorialism, mating, breeding, and foraging processes, while co-occurrence patterns remain understudied. The similar evolutionary pathways of these species could lead to negative co-occurrence patterns, given the limiting similarity (Abrams, 1983). Positive co-occurrence could also occur in a scenario of high availability of resources. Still, it is likely that for evolutionary close taxa the negative pattern can be the most common. Furthermore, the use of these species as indicators can provide insights into the biotic conditions within protected areas and sites outside these areas, offering valuable information on whether they contribute to maintaining more heterogeneous ecological networks.

The main goal of this paper was to investigate co-occurrence patterns in Calopterygidae species, as well to evaluate the influence of abiotic conditions on both co-occurrence patterns and species composition of the family in streams situated inside and outside protected areas. Considering the ecological context of Calopterygidae species in the Amazon, we formulated the following hypotheses: I) inside protected areas, we hypothesized a prevalence of either negative or positive co-occurrences (checkerboard distributions) in streams. This expectation stems from the typically higher environmental heterogeneity within protected areas, fostering more intricate ecological networks. Additionally, the ecological similarity among Calopterygidae species, such as their common exploration of resources like twigs, wood debris, and reeds overhanging stream channels, may contribute to these co-occurrence patterns; II) Due to increased human intervention in impacted streams, resulting in different degrees of disturbances and resource availability for Calopterygidae species, we anticipated a random distribution as the predominant pattern in these sites; and III) We expected that physical structuring variables, such as channel width and depth, canopy cover, along with habitat integrity, would exert more influence on Calopterygidae species composition than limnological variables. Furthermore, we predicted a significant impact of environmental variables on the co-occurrence patterns of Calopterygidae species.

Material and methods

Study location

We sampled 98 streams in the western of Pará state, in the Brazilian Amazon, from 2021 to 2023 (Figure 01), with 63 located inside and 35 outside four protected areas, belonging to different preservation categories. The Parque Nacional da Amazônia (hereafter PARNA AM) belongs to the category of national parks, within which only a handful of activities are authorized, mainly related to scientific expeditions, management, and educational activities (ICMBio, 2021a; Brito et al., 2023). PARNA is in the Itaituba municipality, on the left bank of Tapajós River, within approximately 1.066.208 hectares. Thus, we sampled 17 streams inside PARNA and 11 outside its area. The Parque Nacional do Jamanxim (hereafter PARNA JX) also belongs to the category of national parks (ICMBio, 2021b) and is located in Itaituba and Trairão municipalities, with a total area of 851.754 hectares. We sampled 15 streams inside the Jamanxim area and 13 outside its area. The Reserva Biológica Nascentes da Serra do Cachimbo (hereafter REBIO SC) belongs to the category of biological reserve, aiming to house vegetal and animal species with significative importance for scientific knowledge (ICMBio, 2009). The Serra do Cachimbo area spans over Altamira and Novo Progresso municipalities, with a total area of 342.477 hectares. We sampled 18 streams inside the Serra do Cachimbo area, and we could not sample streams outside because of logistic issues. The Floresta Nacional de Saracá-Taquera (hereafter FLONA ST) belongs to the sustainable use category of national forest, within which some human activities conducted by traditional people and authorized companies can be carried out (ICMBio, 2001). This national forest area spans over three municipalities (Faro, Oriximiná, and Terra Alta), with a total area of approximately 429.600 hectares. We sampled 13 streams inside Saracá-Taquera area, and 11 either outside or close to human activities conducted inside its area.

The predominant vegetation in our study locations is classified as dense ombrophilous forest, interspersed with primary and secondary forests, and some portions present savanna-like and grassland phytophysiognomies (ICMBio, 2009, 2021). The region's climate is classified as tropical rain- forest with a dry and rainy season (Peel et al., 2007), with the annual rainfall varying from 1.500 to 2.000mm, and mean temperature around 26°C (ICMBio, 2001, 2009, 2021a, 2021b). The protected areas, although under federal protection, conducted by IBAMA (Instituto Brasileiro de Meio Ambiente e Recursos Naturais) and ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade) environmental branches, suffer enormous pressure from human activities, such as illegal farmlands and mining fields, an increasing urbanization process, fishing and hunting, and the presence of dirty roads (ICMBio, 2021a). So, these activities, the lack of more management personnel to conduct a closer oversight, and the large size of these protected areas make it difficult to understand if they are fulfilling their purposes of conserving and maintaining the original forests and their biodiversity and ecological networks.

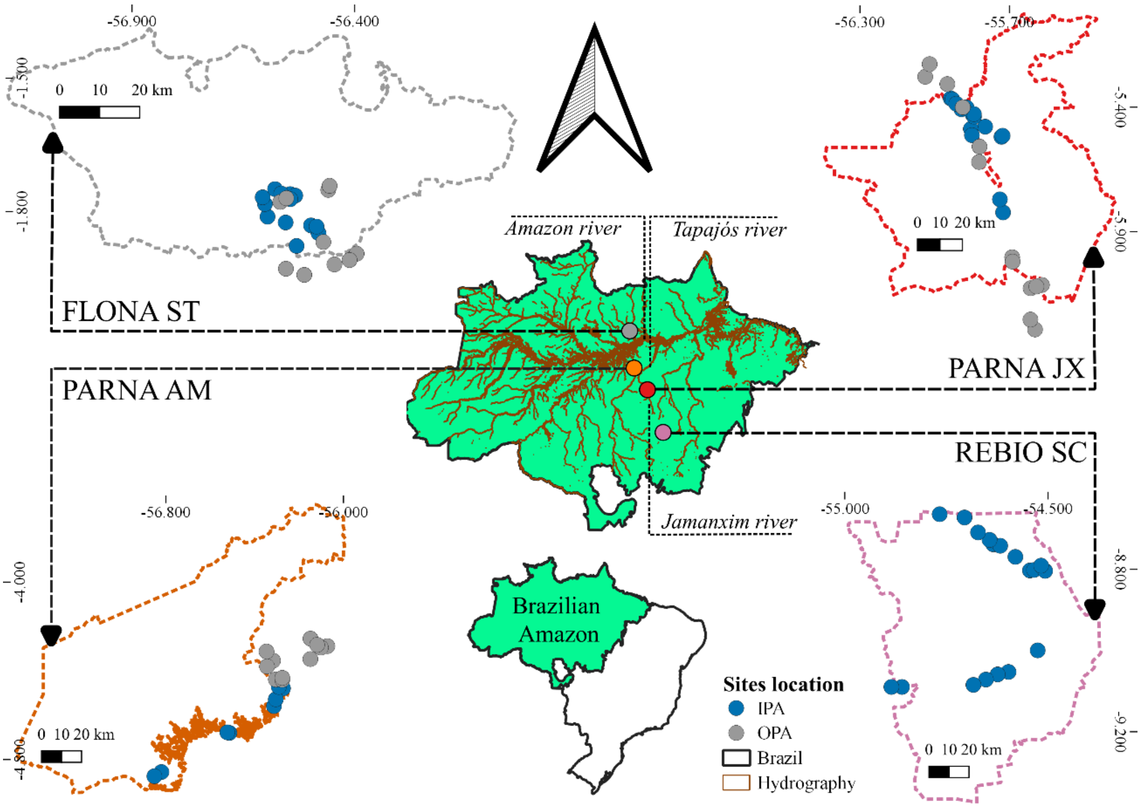


Figure 01. Map with the geographical distribution of the four protected areas, and the 98 sampling sites located inside (IPA) and outside (OPA) their boundaries, in Pará state, Brazilian Amazon. FLONA ST = Flona Saracá-Taquera; PARNA AM = Parque Nacional da Amazônia; PARNA JX = Parque Nacional do Jamanxim; REBIO SC = Reserva Biológica Nascentes da Serra do Cachimbo.

Environmental variables

We measured four limnological variables and three physical variables, from downstream to upstream direction, before the biological sampling, to ensure the high-quality measurement of water variables. The limnological variables encompassed: dissolved oxygen (mg/L), conductivity (mSm−1), potential of hydrogen (pH), and water temperature (°C). The physical variables encompassed the depth and width of the streams (cm and m, respectively) and canopy cover (%). We also applied the Habitat Integrity Index (HII; Nessimian et al., 2008; Brasil et al., 2020), which addresses the riparian vegetation completeness. We measured the limnological variables through a multiparameter probe, the physical variables using a centimeter-graded PVC pipe and a convex densiometer, and the HII through the visual measurement of riparian vegetation aspects.

Calopterygidae sampling

The biological sampling procedures of Calopterygidae species (Figure 2A, 2B, 2C, 2D, and 2E) were conducted during the dry season in the Amazon biome, mainly between 10 am to 02 pm, avoiding rainy days considering the ecophysiological constraints of these taxa (Corbet, 1999; De Marco &Peixoto,

2004; Oliveira-Junior et al., 2019). In each stream, we defined a 150-m stretch (downstream to headstream direction), divided into 10 sections of 15 meters, within which we performed the collection procedures using the fixed-areas protocol (Cezário et al., 2020; Batista et al., 2021). We standardized one researcher performing the sampling procedures for one hour in each stream, using an entomological net (diameter: 40 cm; depth: 65 cm), attached to a 90-cm aluminum pole), spending at least six minutes in each section (Cezário et al., 2021). After the collection, we kept the Calopterygidae species in paper envelopes and immersed them in a plastic container with pure acetone for analysis (Lencioni, 2006). The identification processes were conducted at the Laboratório de Ecologia e Conservação at Universidade Federal do Pará applying specific taxonomic keys, and when needed, the aid of specialists was requested (Garrison, 1990; Garrison, 2006, Lencioni, 2006; Garrison et al., 2010).

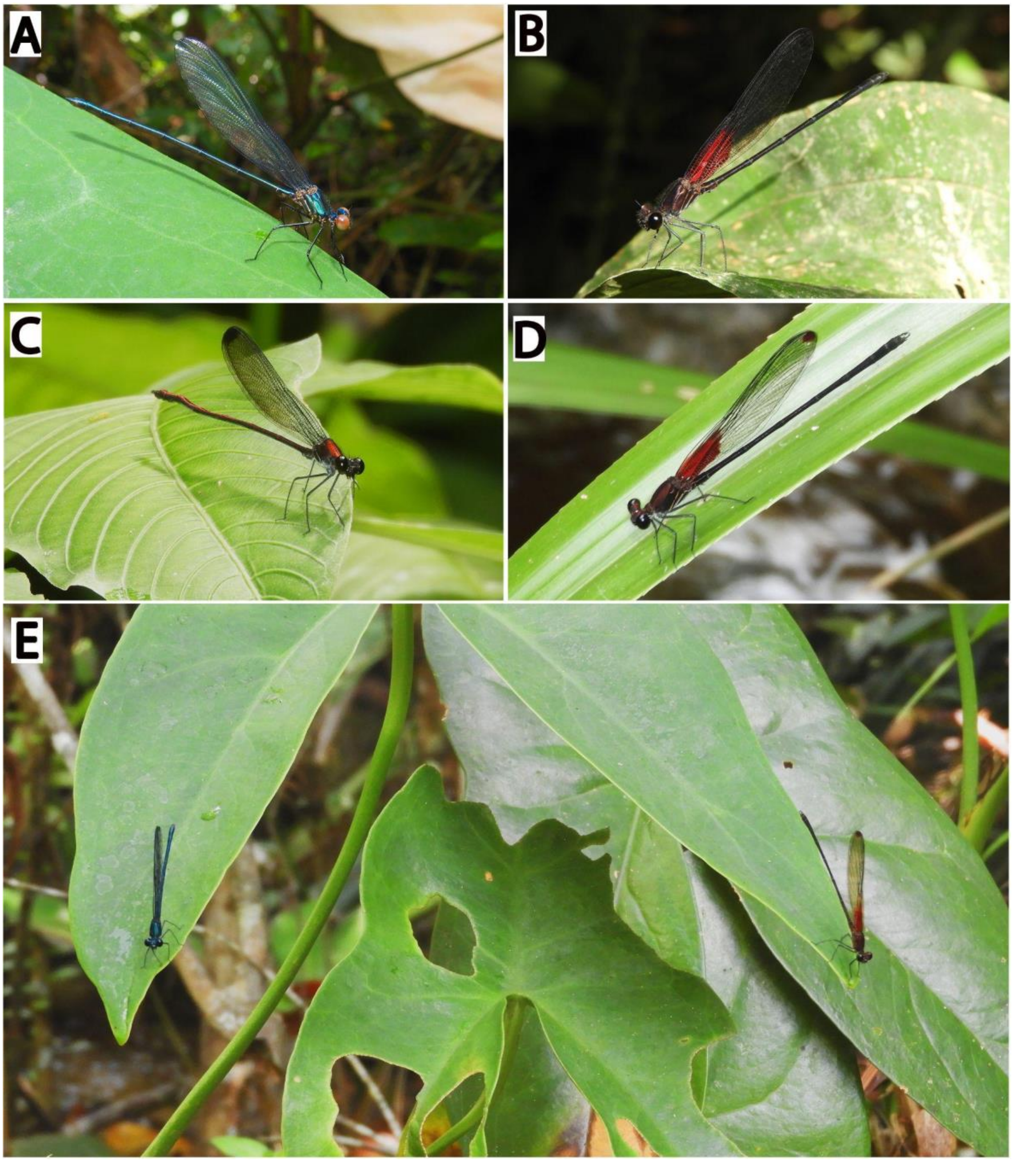


Figure 2. Amazonian Calopterygidae species representing the overall morphological characteristics. (A) *Mnesarete williamsoni* Garrison, 2006, (B) *Hetaerina amazonica* Sjöstedt, 1918, (C) *Mnesarete cupraea* (Selys, 1853), (D) *Hetaerina indeprensa* Garrison, 1990, (E) *M. williamsoni* (left) and *H. indeprensa* (right) co-occurring at the same spot. Photos: Mendoza-Penagos C.C.

Data analyses

We considered each stream as our sampling unit. The incidence of Calopterygidae species was quantified for both categories inside and outside protected areas. Besides, for each one, we tested if each pair of species presented a significative positive, negative, or random co-occurrence pattern.

To test hypotheses I and II, we addressed the patterns of co-occurrence among the Calopterygidae species through probabilistic models using the package *cooccur*, using the function *cooccur* on presence/absence matrices (Griffith et al., 2016), in the R program (R Core Team, 2023). The probabilistic model of the species co-occurrence measures occurrence based on the number of sites where a pair of species occur. Moreover, the observed and expected co-occurrence is compared by multiplying the occurrence probabilities of species pairs by the number of sampling sites (Griffith et al., 2016). So, the analysis evaluates the presence of significative pairwise patterns of species co-occurrence, estimating the likelihood of each pair of species occurring less (P_{lt}) or more (P_{gt}) often than expected if their distribution were randomly associated (Griffith et al., 2016). Besides, if the species pair presents – P_{lt}, it means they have a significative negative co-occurrence; if the species pair presents + P_{gt}, they have a significative positive co-occurrence.

We addressed the overall patterns of morphological characteristics of Calopterygidae species using a Principal Components Analysis (PCA; Borcard et al., 2018). We used mean values of nine morphological measurements gathered from Ferreira et al (2023): total body length (TBL); fore wing length and width (FLW and FWW); hind wing length and width (HWL and HWW); abdomen length and width (AL and AW); and thorax length and width (TL and TW). We used a covariance matrix since all the morphological characteristics are at the same measurement scale (mm). Moreover, we used Broken-Stick criterium to retain the most important components. To facilitate our assessment of the overall patterns, we classified Calopterygidae species into three classes regarding their co-occurrences: high (>8 significative co-occurrences), low (<3 significative co-occurrences), and medium (<8 and >3 significative co-occurrences) co-occurrence rates. Finally, we built biplots to graphically present the overall patterns for species presenting high, low, or medium co-occurrences. Although the PCA does not test hypotheses, because of its heuristic nature, it aims to represent the total variation using a reduced number of principal components, providing clues about overall patterns (Borcard et al., 2018).

Considering the multicolinearity issue, we applied a Pearson correlation on the environmental dataset, with variables presenting a correlation > 0.50 being removed, always maintaining those that are more important ecologically for Calopterygidae species, according to the literature (Córdoba et al., 2009; Guillermo-Ferreira & Del Claro, 2011). The remaining variables were used in the further analysis as predictor variables. After that, to test hypothesis III, we ran a redundancy analysis (RDA; Borcard et al., 2018) to address the influence of limnological, physical structuring, and habitat integrity variables on Calopterygidae species composition. The RDA was performed on the abundance matrix by applying the Hellinger transformation and standardizing the predictor variables (Borcard et al., 2018). We ran the RDA for all the areas without separating inside or outside. Additionally, to test the influence of environmental variables on the Calopterygidae species co-occurrence, we first ran a PCA (Borcard et al., 2018) to summarize the environmental gradient and applied the Broken-Stick criteria to select the PCA axis. Then, we related the selected axes to the abundance of each species using linear regression (Zar, 2010; Guterres et al., 2019; Oliveira-Junior et al., 2021). The regression slope coefficients (b) and the standard errors of the coefficients were used to calculate the values of t, where t = $bx - by$. The x and y values represent the pairs of species considered in the analysis. Thus, these values were used to generate a species co-occurrence matrix as a function of environmental gradient. We used a Mantel test to evaluate the degree of correlation between the co-occurrence matrix and the triangular matrix of species pairs (Mantel, 1967; Legendre & Legendre, 2012). The Mantel test was also applied for all the areas, with no separation between inside and outside protected areas. We set a significance level 0.05 for co-occurrence, redundancy analysis, and Mantel test.

All the analytical procedures were performed in R Computational Environment (R Core Team, 2023), version 4.3.1 with *cooccur*, *vegan*, and *ggplot2* packages, available in the Comprehensive R Archive Network (R Core Team, 2023).

Results

Biotic overview

We sampled 369 individuals of Calopterygidae species, distributed into ten species, corresponding to two genera *Hetaerina* and *Mnesarete*. Most of the species occurred in streams located inside and outside protected areas, with the first having 215 individuals, and the second having 154 individuals. *Mnesarete cupraea* presented the highest number of individuals (n = 91), followed by *Hetaerina auripennis* (n = 84), *Hetaerina laesa* (n = 81), and *Hetaerina amazonica* (n = 39). *Hetaerina indeprensa* occurred only in streams outside protected areas. Moreover, the remaining species presented less than 39 individuals (Table I).

Table I. Distribution of Calopterygidae species across streams located inside and outside protected areas, in Pará state, Brazilian Amazon. FLONA ST = Flona Saracá-Taquera; PARNA AM = Parque Nacional da Amazônia; PARNA JX = Parque Nacional do Jamanxim; REBIO SC = Reserva Biológica Nascentes da Serra do Cachimbo.

	PARNA JX		**PARNA AM**		**FLONA ST**		**REBIO SC**		**Total**
Species	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	
Hetaerina amazonica Sjöstedt, 1918	3	7	16	0	2	9	2	0	39
Hetaerina auripennis (Burmeister, 1839)	17	8	2	16	0	0	41	0	84
Hetaerina indeprensa Garrison, 1990	0	4	0	0	0	6	0	0	10
Hetaerina laesa Hagen *in* Selys, 1853	2	0	26	5	13	25	10	0	81
Hetaerina moribunda Hagen *in* Selys, 1853	0	2	0	0	0	0	2	0	4
Hetaerina rosea Selys, 1853	0	0	1	1	0	0	4	0	6
Mnesarete aenea (Selys, 1853)	5	0	0	1	1	1	18	0	26
Mnesarete astrape De Marmels, 1989	0	0	0	0	6	6	0	0	12
Mnesarete cupraea (Selys, 1853)	14	40	23	13	0	0	1	0	91
Mnesarete smaragdina (Selys, 1869)	6	10	0	0	0	0	0	0	16
Total	47	71	68	36	22	47	78	0	379

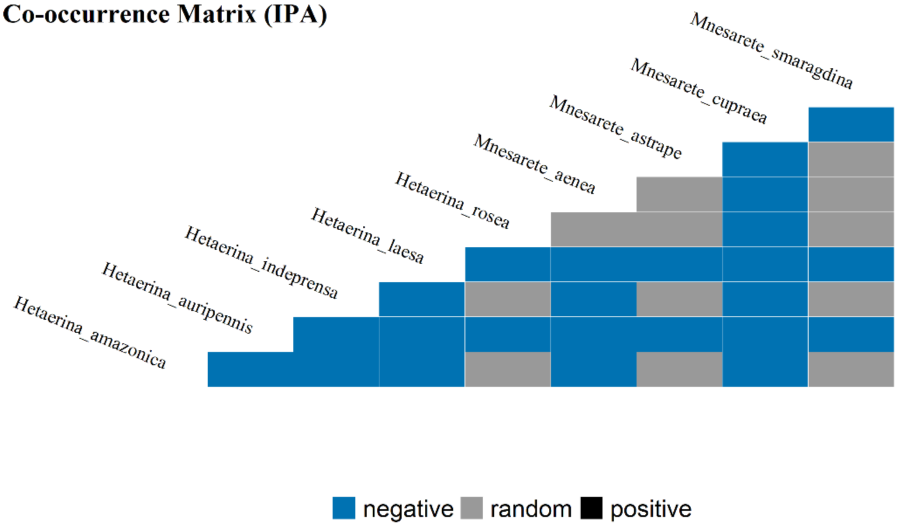
* In Serra do Cachimbo, only streams inside the protected area were sampled.

Co-occurrence and morphometric patterns of the Calopterygidae species

The co-occurence analysis for streams inside protected areas showed 36 species combinations, with six pairs (16.67%) being removed because the expected co-occurrence was lower than 1, and 30 pairs were analyzed. Overall, we found only significative negative (24) and random co-occurrences, with *H. auripennis* and *M. cupraea* presenting the highest number of significative (-) co-occurrences with other Calopterygidae species (Figure 3A).

On the other hand, outside protected areas showed 36 species combinations, from which 14 pairs were removed (38.89%) since the expected co-occurrence was lower than 1, and 22 pairs were analyzed. Overall, we found only negative and random occurrences, with *H. laesa* presenting the highest number of significative (-) associations with other species (Figure 3B). None of the areas presented positive co-occurrence associations. All values from *cooccur* analyses are available in tables S1 and S2 (Supplementary material). The Broken-Stick criterium retained the first component of PCA, which explained 94.66% of the total variation (Figure 4). Variables related to the length of abdomen, fore and hind wing, and total body length contributed the most to the overall pattern, mainly on species presenting high negative co-occurrences (Figure 4). The general output of PCA is available in table S3 (Supplementary material).

(A) Co-occurrence Matrix (IPA)



(B) Co-occurrence Matrix (OPA)

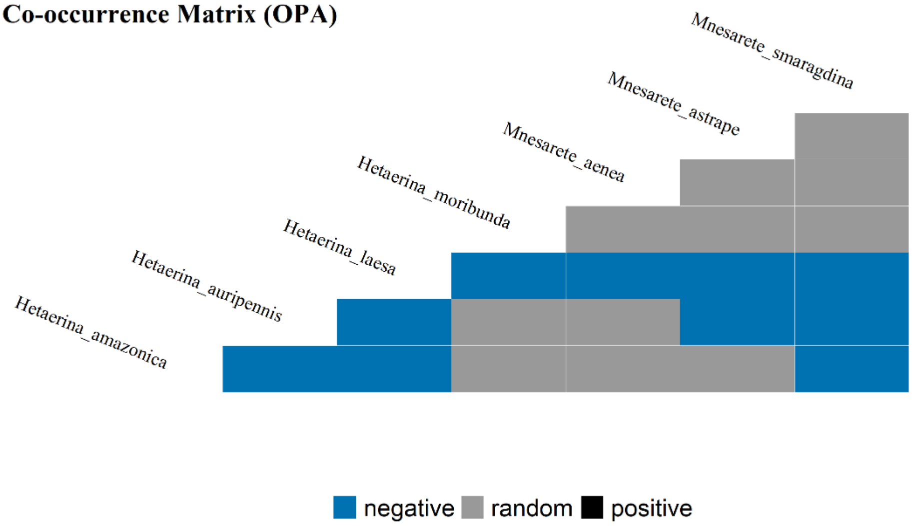


Figure 3. Lower triangle heat map of the co-occurrence patterns of Calopterygidae species in streams (A) inside (IPA) and (B) outside (OPA) protected areas. No positive co-occurrence was found.

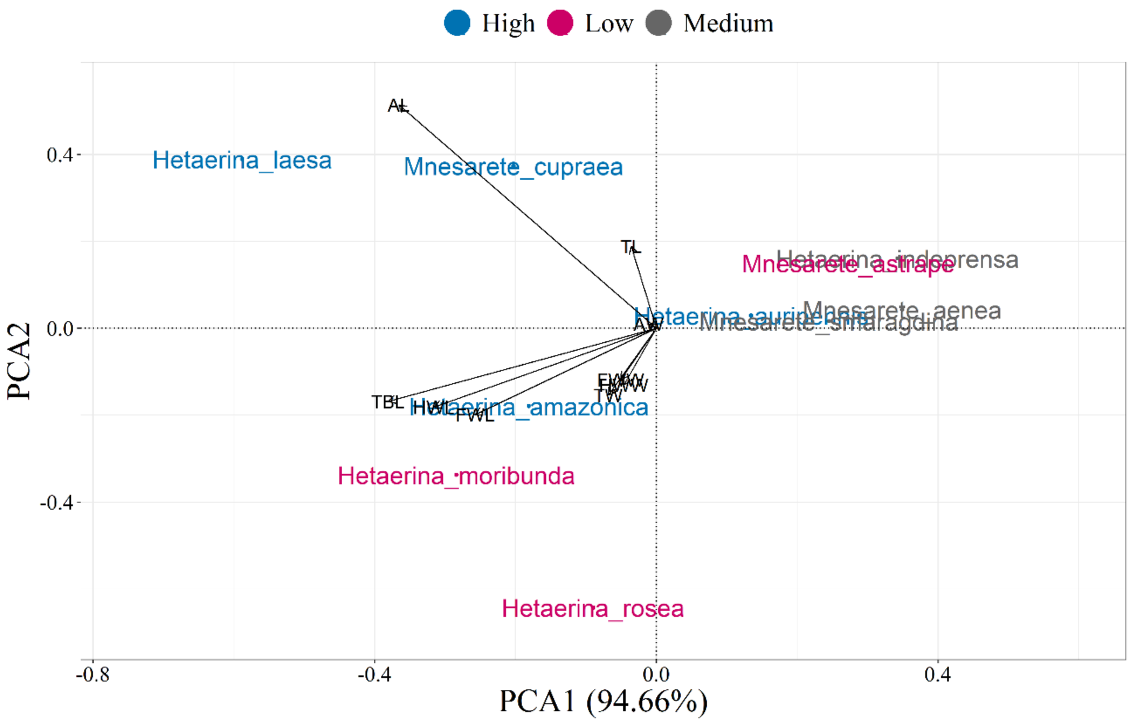


Figure 4. Biplot of the results of the Principal Component Analysis (PCA) showing the representativity of morphometric characteristics for the overall variation of Calopterygidae species presenting high, low, or medium negative co-occurrence.

Abiotic influences on Calopterygidae species composition and co-occurrence

The first two axes of the RDA represented 74.23% of the total variation, with the first axis contributing 52.80 %, and the second with 21.93% (Figure 5). Moreover, physical structuring and water variables contributed the most to the Calopterygidae species composition (Figure 5). The water variable pH presented the most significative influence on the first axis, associated with *Hetaerina moribunda*, *Hetaerina rosea*, *Mnesarete aenea* and *Mnesarete smaragdina* (Figure 5). Besides, physical structuring variables, such as depth and width, joint with dissolved oxygen, and conductivity, also presented a significative influence on the mentioned species but a weaker influence on *Hetaerina amazonica*, *Hetaerina indeprensa*, *Hetaerina auripennis*, and *Mnesarete cupraea* (Figure 5). Canopy cover and HII presented influence on *Hetaerina laesa* and *Mnesarete astrape* (Figure 5). The Mantel test between the random matrices generated by the coexistence index and the triangular matrix of species versus species revealed that co-occurrence was unrelated to environmental variables (r = 0.336, P = 0.087).

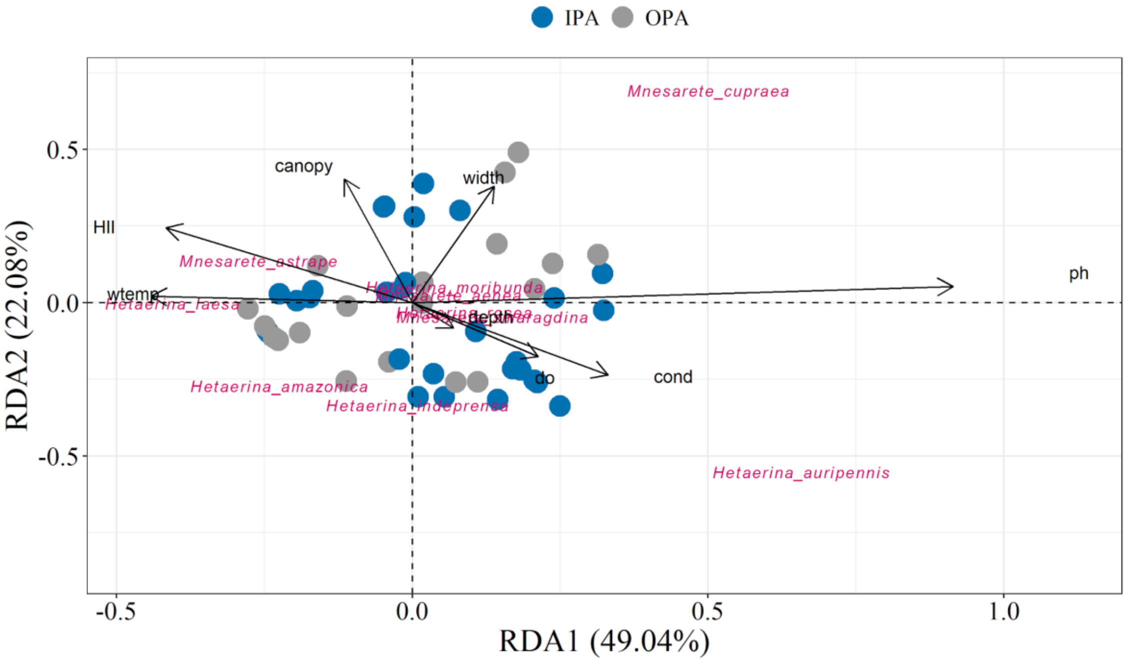


Figure 5. Triplot of the relationship between environmental variables and the Calopterygidae species composition, evaluated by the redundancy analysis (RDA) with two selected axes. Canopy = canopy cover (%); cond = water conductivity; depth = channels depth (cm); do = dissolved oxygen (mg/L); HII = Habitat Integrity Index; IPA = inside protected areas; OPA = outside protected areas; pH = potential of hydrogen; width = channels width (m); wtemp = water temperature (°C).

Discussion

Our two first predictions that IPA streams would have more significant co-occurrences (positive or negative) of Calopterygidae species whereas OPA streams have more random co-occurrences were corroborated. Our heuristic approach indicated that the length of the abdomen and wings and total body length contributed the most to the overall pattern. Finally, a joint significative influence of limnological and physical structuring components structured the species composition of Calopterygidae, corroborating partly the third prediction. However, we did not find a correlation between the co-occurrence Calopterygidae species and the environmental gradient. Considering that evolutionarily close taxa generally also present ecological similarities, those similarities could provide insights into their co-occurrence patterns and on the importance of abiotic variables. Furthermore, considering the more heterogeneous nature of preserved streams, the availability of these resources could explain the relationship between positive or negative co-occurrence patterns for this Odonata family.

The theory of limiting similarity predicts that a pair of species with identical realized niches could not coexist (Abrams, 1983), which could lead to a negative co-occurrence where one species is present in a given site but the others are not. In our study, we found more negative co-occurrences in streams inside protected areas, which could be more heterogeneous when compared to those outside these areas. Protected areas usually maintain more heterogeneous microhabitats, capable of maintaining more complex ecological networks and providing more resources to be explored by the local fauna (Thomas et al., 2015; Cooke et al., 2023). Moreover, the presence of more resources to be explored can lead to strong inter-specific competition, mainly among those taxa evolutionarily closer (Burns & Strauss, 2011), providing a scenario of more negative co-occurrences. In headwater streams, the riparian zone provides most of the physical heterogeneity, such as wood debris, branches, and twigs, valuable resources to be used as perching points for Calopterygidae males (Garrison, 1990; Guillermo-Ferreira & Bispo, 2012), which leads to intense interspecific competition since the access to these resources means access to the females (Garrison, 1990; Pestana et al., 2018). Considering the availability of more resources, we could expect less niche overlap among the species. However, we built our predictions on the fact that our model organisms would be evolutionarily closer. So, Calopterygidae species would present ecological similarities related to the reliance on the same environmental resources needed to perform their daily activities (e.g., mating, breeding, territorialism and foraging). Branches and twigs hanging over the channel of the streams are important physical structuring resources given the need of their species to perform courtship behaviors, in a common display of their rubyspots, in the case of *Hetaerina* species (García-Monsalve et al., 2021). Removing this vegetation leads to a decrease in the provision of these resources and alters the microclimatic conditions. Moreover, this can diminish species populations, filtering those more resistant and removing those more sensible (Monteiro-Júnior et al., 2014; Calvão et al., 2016; Dala-Corte et al., 2019). These changes can also modify the intrinsic characteristics of the species, such as coloration and morphometric variables (Novella-Fernandez et al., 2023).

The coloration in Calopterygidae species is crucial for mating behaviors (Córdoba et al., 2005; Guillermo-Ferreira & Del Claro, 2011), indicating to the females which one of the available males are healthy. So, the human-made modifications surrounding the streams can have negative influences on the reproductive processes of these species. Moreover, these changes can also filter species according to their morphological characteristics, such as total body length and wings aspects, usually favoring bigger species (Pereira et al., 2019). Although we did not test human influences on morphometric characteristics of Calopterygidae species, the heuristic approach indicated an overall pattern regarding the important contribution of length of abdomen and wings, and total body length, to species that presented the highest number of negative co-occurrence. These variables are related mainly with the access to oviposition substrates and flight maneuverability, some aspects of the species behaviors that might be influenced by human-made modifications (Pereira et al., 2019; Resende et al., 2021). However, we cannot go any further since we did not test significative differences concerning Calopterygidae morphometric variables between inside and outside protected areas.

The streams outside the protected areas presented the lowest number of significative negative co-occurrences, and more random associations. Generally, those streams would present altered riparian vegetation, with less provision of allochthonous resources to their channels (Dala-Corte et al., 2019). As the provision of these resources shrinks, other factors can enhance the local environmental heterogeneity, such as the presence of aquatic plants (De Marco et al., 2014). Aquatic plants occur in major abundance in open sites, presenting a high incidence of sunlight reaching the water, which helps the growth of these organisms (Kovalenko et al., 2012, Fares et al., 2020). Their emersed structures (e.g., leaves and stolons) can also work as perching points for Odonata (Brito et al., 2020) and in places with these plants, Calopterygidae species can perform their behavior activities. Personal observations made by the authors found species, such as *M. cupraea*, *M. aenea* and *Hetaerina* species, perching in leaves of aquatic species, performing oviposition, mating, and guarding behaviors. The intermediate disturbance hypothesis (Connell, 1978) predicts that intermediate levels of disturbance could enhance the local diversity. Moreover, these disturbances could also mean more microhabitats available to the species, diminishing negative interspecific competition (Connell, 1978). None of the *Mnesarete* species presented negative or positive significative co-occurrences between them in more open site (more altered), meaning that somehow these sites provide enough resources to prevent a niche overlap. This scenario was different when compared to the streams inside the protected areas, where we found more significative negative co-occurrences among most of the species of *Hetaerina* and *Mnesarete*, indicating at the very least niche overlap.

In our study, physical structuring variables and limnological variables presented significative influence on the Calopterygidae species composition. The HII is considered a proxy for the completeness of the riparian vegetation (Brasil et al., 2020), the vegetation surrounding the small streams. The riparian vegetation provides physical structuring support to the banks of the streams, maintaining their stability (Dala-Corte et al., 2019; Allan et al., 2021), prevents the excessive input of sediments during heavy rainfalls (Gregory et al., 1991), doing so maintain stable the limnological variables, and provides physical structures (e.g., twigs and branches) and woody debris to the channel of the streams. Thus, those structures are usually used by males of Calopterygidae to perform thermoregulation, foraging, mating, and breeding behaviors. Males of *Hetaerina* species, such as *Hetaerina vulnerata*, *Hetaerina americana*, and *Hetaerina cruentata* aggregate in a competitive display and courtship rituals to entice the females, called lek mating systems (Córdoba et al., 2009; Guillermo-Ferreira & Del Claro, 2011), and physical structure components provided by the surrounding vegetation are used as perching points. However, a previous study found that although the presence of physical structuring components may be used as perching points by the *Hetaerina* males, it did not significatively increase the number of females visits (Córdoba et al., 2009). So, we can infer that those physical structuring components are important in providing perching points for the performance of thermoregulation, foraging, and territorialist behaviors among the males. Still, when it comes to increase the number of females visits, no significative influence is detected. We did not detect any significative influence of the environmental gradient on Calopterygidae species co-occurrence, differently from previous studies conducted in the Brazilian Amazon with Odonata (Oliveira-Junior et al., 2021) and water striders (Guterres et al., 2019). One of the explanations could be the low number of species (S = 10, N = 369), which decreased the number of possible co-occurrences, and consequently influenced the correlation analysis. The aforementioned studies approached these correlations using a higher taxonomic level (order/suborder), so having more available species and individuals provides robustness to the analytical procedures regarding the correlation between species co-occurrences and environment gradient. Therefore, although we did not find significative results for this correlation, we did find for Calopterygidae species composition, which is a robust biotic metric.

Although our results are compelling, mainly about the co-occurrence patterns in different degrees of integrity, some caveats must be discussed. Firstly, most studies surveying basic behavioral knowledge about Calopterygidae species were conducted with different species than those we sampled. For example, *Mnesarete pudica* was studied by two different studies in Brazil (Guillermo-Ferreira & Bispo, 2012; Pena-& Guillermo-Ferreira, 2020), aiming to address behavioral patterns related to reproductive aspects. *Hetaerina cruentata* was also the target of a study in Colombia (García-Monsalve et al., 2021), aiming to address the demographic and behavioral aspects of this species. A general study about Calopterygidae species was carried out in Mexico (Córdoba-Aguillar et al., 2009), also with different species than those we have sampled (*Hetaerina americana*, *Hetaerina cruentata*, *Hetaerina titia*, and *Hetaerina vulnerata*). So, to discuss our results and because of the lack of populational studies about the Amazonian Calopterygidae species, we had to make congener inferences since species sharing similar evolutionary history would present ecological similarities (Burns & Strauss, 2011). However, although similar evolutionary histories may translate in ecologically similar species, a handful of examples for Calopterygidae species slightly move away from this pattern. For example, Guillermo-Ferreira & Del-Claro (2011) found that *H. rosea*, a species we sampled, did not display the well-known lek mating system as other *Hetaerina* species. So, we highlight the need for more populational studies aiming at the behavioral aspects of Amazonian Calopterygidae species, mainly those more abundant. Furthermore, those studies could shed light on the co-occurrence patterns of this taxa and allow us to make stronger inferences about those patterns. Regarding the co-occurrence patterns in general, the specialized literature underlines that negative co-occurrences could also be explained by processes other than mutualistic interactions (Bell, 2005; Dallas et al., 2019). Although this is true, we can minimize these noises by applying a holistic approach, such as addressing the influence of abiotic variables on species co-occurrences and composition (Guterres et al., 2019; Oliveira-Junior et al., 2021). This can ensure we are addressing the overall patterns and enable more robust inferences.

Secondly, our study approached only the species incidence and abundance of Calopterygidae, not going further and addressing functional traits related to morphological characteristics, for example (Guterres et al., 2019; Oliveira-Junior et al., 2021). Previous studies addressed such patterns for Odonata suborders (Oliveira-Junior et al., 2021) and water striders (Guterres et al., 2019) using morphological traits and co-occurrence methodologies. Moreover, these approaches revealed compelling results, such as more non-random co-occurrences and morphological divergences of damselfly species in streams located inside preserved environments (Oliveira-Junior et al., 2021), demonstrating the robustness of using both approaches together. Although the same could be applied to our study, it is important to highlight that the mentioned studies evaluated co-occurrence patterns at the order/suborder taxonomic levels, which means they had a wider number of available taxa than ours, and consequently more different responses (positive, negative, and random co-occurrences) could be found. Future studies could shed light on whether the responses at low taxonomic levels (family, genera), using functional traits, are as robust as those at high levels are (order, suborder).

Conclusion

Our results indicated that streams inside protected areas presented the highest number of significative negative co-occurrence associations of Calopterygidae species, and streams outside these areas presented more random co-occurrences. Generally protected areas are more heterogeneous, capable of providing more resources to be explored by the local fauna. However, we evaluated evolutionarily close taxa, belonging to the same family, because of their ecologically similarity, there would exist a higher likelihood to occur more significative negative co-occurrences. Although we did not test differences of morphometric variables of Calopterygidae species found inside and outside protected areas, our heuristic approach showed insightful results, open avenues for future studies aiming to address these species aspects. Furthermore, a joint influence of limnological, physical structuring, and habitat integrity variables was strongly associated to the Calopterygidae species composition, showing that the environmental heterogeneity was important for these taxa. However, maybe because of the low number of Calopterygidae species, there was no correlation between co-occurrence and environmental gradient. We highlight a need for more studies about the basic biology of Amazonian Calopterygidae species to ensure we could perform stronger inferences about their co-occurrence patterns.

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Authors contribution

Project design: JSB, LJ. Data collection: ECS, GMC, VRSF, RCB, JSM. Identification of species: JSB, VRSF, RCB, CCMP, FGC. Data analysis: JSB, LJ. Writing of the manuscript: JSB, ECS, GMC, VRSF, RCB, JSM, CCMP, FGC, JMBO-J, KDS, TSM, LFAM, LC, LJ. Funding acquisition: LC, LJ.

Conflict of interest statement

The authors declare no conflicts of interest.

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Conclusão Geral

Nossos resultados indicaram que processos de dispersão e nicho apresentaram uma importância compartilhada para Anisoptera, enquanto que um forte sinal de dispersão foi detectado para Zygoptera. A presença dos grandes rios Amazônicos tem um impacto profundo sobre a distribuição das espécies, principalmente sobre aquelas que possuem taxas de dispersão mais baixas, quando comparadas aquelas que são capazes de se dispersar mais efetivamente. Anisoptera e Zygoptera possuem diferentes requerimentos ecológicos e fisiológicos, além de características morfológicas que as fazem diferir em relação às suas taxas de dispersão. Os padrões de diversidade investigados em riachos dentro e fora de áreas protegidas mostraram que apesar de sob intensa pressão de atividades humanas, essas áreas ainda são capazes de manter a diversidade de espécies de libélulas, principalmente de Zygoptera. Entretanto, conforme foi mostrado no segundo capítulo da tese, as áreas protegidas de uso sustentável já mostram indícios de perda de diversidade e de integridade de hábitat, o que chama atenção para um monitoramento mais de perto, por parte dos órgãos ambientais responsáveis. Além disso, aliando esse monitoramento a políticas de cunho social é muito importante. As áreas protegidas também parecem manter as mais complexas redes de interações entre as espécies, conforme investigamos no terceiro capítulo, onde o maior número de co-ocorrências significativas ocorreu dentro dessas áreas. Os dois últimos capítulos da tese demonstraram também que o conjunto de variáveis ambientais, limnológicas e de estruturação física, são importantes preditores das assembleias de libélulas nos riachos Amazônicos, e o desbalanceamento nessas variáveis pode desencadear resultados diferentes para ambas as subordens de libélulas. Daí a importância de abordagens holísticas, investigando diferentes aspectos das assembleias de libélulas, objetivando diminuir as lacunas de conhecimentos, serem aplicadas na conservação da biodiversidade aquática.

Atividades Complementares

Durante o desenvolvimento da minha tese de doutorado, realizei atividades paralelas, relacionadas à co-orientação de alunos de graduação, monitoria em disciplinas da pós-graduação, expedições de campo para coleta de dados, revisão de artigos científicos para revistas nacionais e internacionais, e escrita de artigos paralelos.

Durante os quatro anos de doutorado co-orientei três alunos de graduação em projetos relacionados ao uso de insetos aquáticos como bioindicadores da qualidade ambiental, envolvendo estudo de limiares ecológicos, medidas morfométricas e trabalhos em micros-cosmos para avaliação de ecologia básica desses grupos.

Também atuei como monitor da disciplina de estatística univariada e multivariada, oferecida pelo Programa de pós-graduação em Ecologia, auxiliando os alunos na utilização dos programa estatísticos utilizados em sala de aula, e na aplicação dos testes estatísticos dentro de fenômenos biológicos e ambientais.

Participei de diversas expedições de campo, dentro de projetos com as agências públicas de fomento à pesquisa, bem como dentro de projetos com entes da iniciativa privada. Tais atividades foram importantes para diminuir nossas lacunas de conhecimento sobre a diversidade aquática na Amazônia Brasileira, bem como para o desenvolvimento de monografias, dissertações e teses pelos alunos do Laboratório de Ecologia e Conservação e laboratórios parceiros. Além da parte científica, tais expedições foram extremamente valiosas devido ao contato com as populações tradicionais que ocupam a Amazônia Brasileira. Elas possuem conhecimentos populares extremamente valiosos, e sem a participação delas a execução dos projetos seria bem mais difícil.

Fui convidado ao longo dos quatro anos de doutorado como revisor de artigos científicos para revistas nacionais e internacionais, dentro das temáticas de ecologia de populações e comunidades, sendo teóricas ou aplicadas à ecologia da conservação.

Ao longo do doutorado publiquei 15 artigos científicos, sendo quatro como autor principal e 11 como co-autor, além de desenvolver ideias de artigos paralelos que ainda não foram totalmente polidas a ponto de serem submetidas, dentro de um artigo, a uma revista científica. Na classificação de Qualis da CAPES, 11 artigos estão na classe A+ (73.3%) e quatro como B+ (26.7%). Para mais informações sobre minhas publicações científicas disponibilizo os *links* do meu currículo Lattes e *ResearchGate* abaixo:

Lattes - http://lattes.cnpq.br/6013054034235293

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