Received: 2 February 2024 | Accepted: 19 September 2025

DOI: 10.1111/een.70036

ORIGINAL ARTICLE

Ecological Entomology

# Abiotic drivers of co-occurrence and diversity patterns of Calopterygidae species in Amazonian protected freshwaters

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**Funding information**
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 428961/2018-5; Fundação de Amparo à Pesquisa do Estado de São Paulo, Grant/Award Numbers: 068/2020, 2019/25445-1; Fundação Amazônia Paraense de Amparo à Pesquisa; CNPq, Grant/Award Numbers: 141113/2020-0, 304403/2021-0, 304710/2019-9; CAPES, Grant/Award Number: 88881.690205/2022-01

**Associate Editor:** Christopher Hassall

## Abstract

1. Interspecific competition, environmental conditions and spatial factors can shape species co-occurrence patterns in freshwater habitats, especially among closely related taxa. We investigated the diversity and co-occurrence patterns of damselflies from the Calopterygidae family in streams located inside protected areas (IPAs) and outside protected areas (OPAs) in the Brazilian Amazon, assessing the influence of abiotic variables. We expected that IPA streams would show greater environmental heterogeneity, stronger co-occurrences and fewer random associations than OPA streams. We also predicted that environmental variables would have a stronger influence on species diversity and co-occurrence patterns than spatial predictors.
2. A total of 359 individuals representing 10 Calopterygidae species were collected from 98 streams across 4 PAs. Contrary to our expectations, OPA streams showed higher environmental heterogeneity, potentially related to human disturbances. Negative co-occurrence prevailed in both IPA and OPA streams, but was more pronounced in IPA sites. These patterns may reflect ecological similarity among Calopterygidae species, particularly in resource use. Canonical analyses revealed a stronger influence of broad-scale spatial variables on diversity and co-occurrence than initially predicted, with two major Amazonian rivers likely acting as dispersal

barriers. Limnological and physical habitat variables were also significant predictors, likely related to the availability of exploitable resources.
3. Future research should extend this approach to other damselfly families and aquatic groups and examine the role of morphological traits in mediating species interactions.

**KEYWORDS**
aquatic systems, broad-winged damselflies, conservation units, environmental filtering, Odonata, spatial constraints

## INTRODUCTION

Interspecific competition is a paramount ecological force shaping 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 interactions with other organisms (Connell, 1978; Connor & Simberloff, 1979). The most efficient competitors would be able to exploit these resources more efficiently, thereby restricting access to other species. The limiting similarity hypothesis explains this dynamic and predicts that ecologically similar species will compete more intensely for a given resource (Abrams, 1983; MacArthur & Levins, 1967). In this context, niche differentiation and species specialisation play a major role in determining the likelihood of co-occurrences (Bode et al., 2011; Guterres et al., 2019; Oliveira-Junior et al., 2021).

However, the presence of environmental filtering introduces additional complexity to our understanding of species co-occurrences (Hutchinson, 1959). For instance, in freshwater environments, species respond differently to a range of abiotic variables, including air and water temperature, humidity, dissolved gases, sunlight exposure and physical habitat structure (Calvão et al., 2016; Carvalho et al., 2018). Species possessing appropriate physiological and morphological characteristic adaptations are favoured, while those lacking such traits are competitively displaced (Guterres et al., 2019; MacArthur & Levins, 1967).

Over evolutionary time, species have developed physiological and morphological characteristics in response to both biotic and abiotic factors (Oliveira-Junior et al., 2021; Silvertown, 2004). However, since the advent of agriculture and, more recently, the Industrial Revolution, humankind has increased its ability to alter the environment (Allan et al., 2021). Under these anthropogenic changes, such as physical and chemical modifications, interspecific relationships among species may be disrupted. These modifications can either expand or contract the realised niche of species, also altering the environmental heterogeneity (Sévêque et al., 2020). According to Allan et al. (2021), more heterogeneous habitats are those that provide a greater range of microhabitats for local biota, thereby promoting species co-occurrence. In headwater streams, the availability of fallen wood trunks, leaf banks and roots, provided by the riparian vegetation, influences the heterogeneity of the environment, favouring the co-occurrence of different species (Allan et al., 2021). Such conditions are more likely in well-preserved areas, where riparian vegetation remains intact (Dala-Corte et al., 2020; Dosskey et al., 2010).

To mitigate these anthropogenic disruptions, one of the most prevalent strategies is the establishment of protected areas (PAs) (Brasil et al., 2021; Dias-Silva et al., 2021; Restello et al., 2020). Under Brazilian legislation, PAs range from fully protected categories to those that allow sustainable use by the local communities or companies (Brasil et al., 2021). Their primary objectives include maintaining environmental heterogeneity, protecting the water sources and preserving biodiversity and ecological interactions (Abell et al., 2007; Chowdhury et al., 2023). However, discussions about the implementation of PAs focus 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). Although PAs can indirectly safeguard freshwater systems through buffer zones (Brito et al., 2023), conservation planning has not yet incorporated the importance that freshwater ecosystems represent to biodiversity. These systems provide invaluable ecological services, supported by aquatic fauna (e.g., insects, fish, amphibians), physical structures (e.g., riparian vegetation) and diverse ecological networks, including species co-occurrence (Brasil et al., 2021; Brito et al., 2023; Dias-Silva et al., 2021; Leal et al., 2020). For example, PAs are important for the co-occurrence of populations of species of spotted cats (Nagy-Reis et al., 2017) and snakes (França & Araújo, 2007). Moreover, well-preserved riparian vegetation strips in PAs were important for the co-occurrence patterns of crab communities (Rivera-Pérez et al., 2024). Similarly, forested streams are linked to non-random co-occurrence patterns in dragonflies and damselflies, underscoring the role of habitat integrity (Oliveira-Junior et al., 2021).

Among these, the family Calopterygidae (Odonata: Zygoptera) stands out as one of the most extensively sampled damselfly families in Amazonian streams. In the Brazilian Amazon, 28 species of the genera *Hetaerina* and *Mnesarete* occur (hereafter referred to as Calopterygidae), representing the only Calopterygidae genera in the biome (De Marco, 2023). These species are typically observed near the stream channels, perching on branches and twigs of trees and bushes (Garrison, 1990; Monteiro Junior et al., 2014; Oliveira-Junior et al., 2019). Their diverse behaviours, mainly related to territorialism, foraging and mating tactics, make them valuable models for exploring intra- and interspecific competition (Córdoba-Aguilar, 1995; Córdoba-Aguilar & Cordero-Rivera, 2005; Ferreira et al., 2023; García-Monsalve et al., 2021).

Given their evolutionary closeness, Calopterygidae species may exhibit distinct co-occurrence patterns due to phylogenetic inertia and shared resource use (e.g., oviposition sites, perching points) (Standring et al., 2022). Studies in the Brazilian Amazon have documented these species in both intermediately preserved (Brito et al., 2021; Carvalho et al., 2018) and well-preserved streams (Calvão et al., 2016; Monteiro Junior et al., 2015), yet their co-occurrence patterns remain unexplored. While most research focuses on their behaviour (e.g., Córdoba-Aguilar, 1995; García-Monsalve et al., 2021), their spatial distribution suggests widespread occurrence across Amazonian endemism centres, highlighting the role of historical dispersal processes (Brito et al., 2024). Theoretically, their ecological similarity could lead to negative co-occurrence (due to limiting similarity) or positive co-occurrence (in resource-rich environments). Thus, Calopterygidae species offer a compelling model for investigating co-occurrence patterns, with implications for assessing biotic conditions in and outside protected areas (OPAs).

The primary goal of this study was to investigate co-occurrence patterns in Calopterygidae species and evaluate the influence of abiotic variables (environmental and spatial predictors) on co-occurrence and species composition patterns in streams located inside and outside PAs. Based on the ecological context of Calopterygidae species in the Amazon and the characteristics of our study sites, we proposed five operational hypotheses: First, we expected that streams within PAs would exhibit higher environmental heterogeneity due to greater availability of allochthonous resources from riparian vegetation (e.g., wood debris, leaves and roots). Second, we predicted that, due to greater human-driven disturbances and altered resource availability, broadening and narrowing species' realised niche, streams OPAs would show random co-occurrence patterns, reflecting human-driven disturbances and altered niche dynamics. In contrast, in our third hypothesis, streams inside protected areas (IPAs) were anticipated to display structured patterns (mainly negative co-occurrences, reflecting a checkerboard distribution among species). This expectation was grounded in the presumed higher environmental heterogeneity of PAs and the ecological similarity among Calopterygidae species, which rely on similar microhabitats. Fourth, we hypothesised that physical-structuring variables (channel width and depth, canopy cover, and habitat integrity) would exert more influence on Calopterygidae species composition than spatial variables. Physical-structuring variables are associated with the provision of allochthonous resources that Calopterygidae species use during their daily activities (foraging, breeding, mating, territorialism). Finally, we expected environmental variables to outweigh spatial predictors in shaping co-occurrence patterns, as local conditions likely dominate Calopterygidae ecology.

## MATERIALS AND METHODS

### Study location

We sampled 98 streams in the western part of Pará state, in the Brazilian Amazon, from 2021 to 2023 (Figure 1), with 63 located inside and 35 outside four PAs, 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 authorised, mainly related to scientific expeditions, management and educational activities (Brito et al., 2023; ICMBio, 2021a). PARNA AM is located in the Itaituba municipality, on the left bank of the Tapajós River, within approximately 1.066.208 ha. Thus, we sampled 17 streams inside PARNA AM 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 on the right bank of the Tapajós River located in Itaituba and Trairão municipalities, with a total area of 851.754 ha. We sampled 15 streams inside the PARNA JX 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 significant 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 ha. 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 authorised 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 ha. We sampled 13 streams inside the 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, 2021a, 2021b). The region's climate is classified as tropical rainforest with a dry and rainy season (Peel et al., 2007), with the annual rainfall varying from 1.500 to 2.000 mm and mean temperature of around 26°C (ICMBio, 2001, 2009, 2021a, 2021b). The PAs, 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, increasing urbanisation, 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 PAs make it difficult to understand if they are fulfilling their purpose of conserving and maintaining the original forests and their biodiversity and ecological networks.

### Calopterygidae sampling

The biological sampling procedures of Calopterygidae species (Figure 2a–e) were conducted during the dry season in the Amazon biome, mainly between 10:00 AM and 2:00 PM, avoiding rainy days

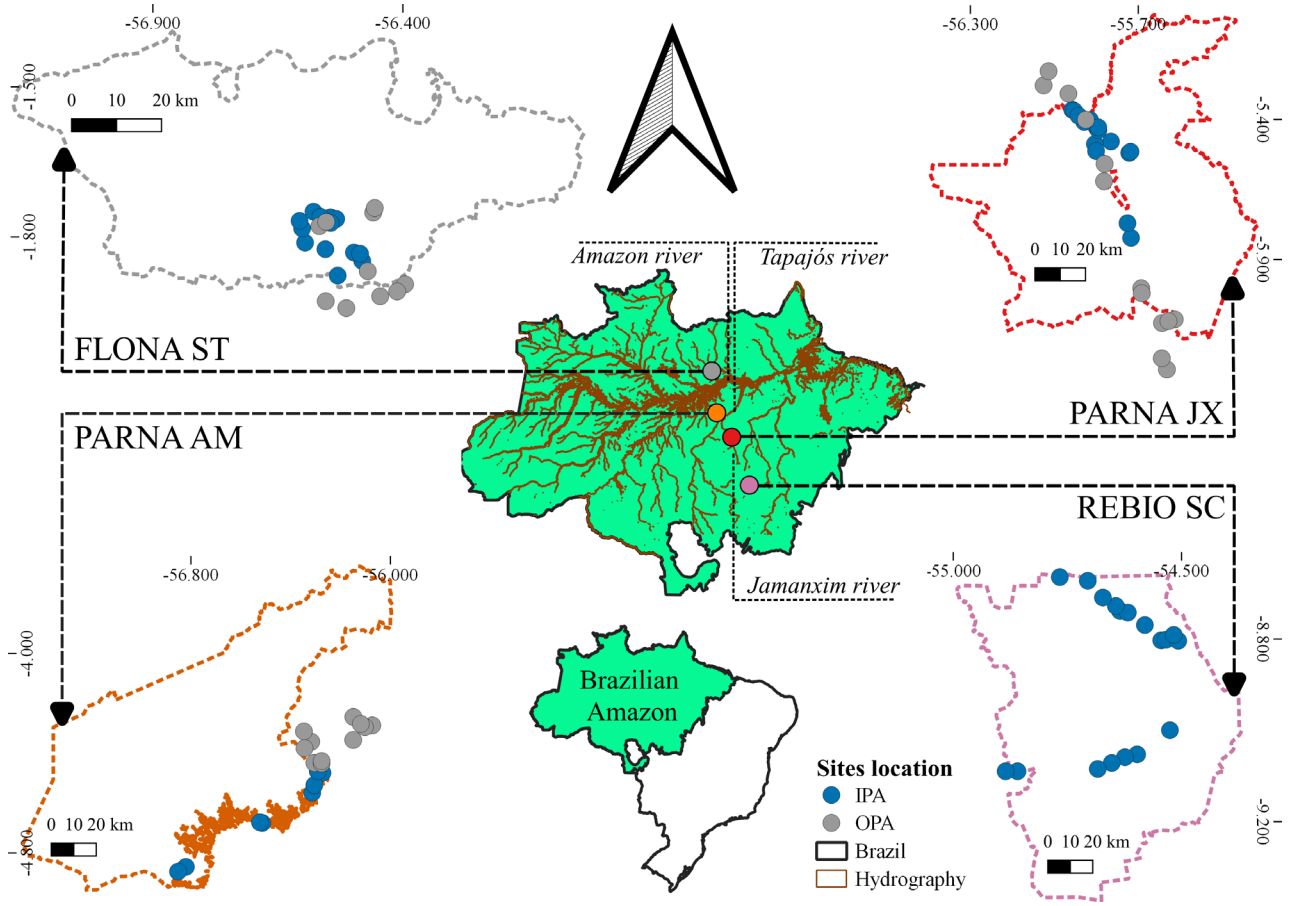


**FIGURE 1** Map with the geographical distribution of the four protected areas, and the 98 sampling sites located inside (IPA) and outside (OPA) their boundaries, in Pará state, Brazilian Amazon. FLONA ST, Flona Saracá-Taquera; PARNA AM, Parque Nacional da Amazônia; PARNA JX, Parque Nacional do Jamanxim; REBIO SC, Reserva Biológica Nascentes da Serra do Cachimbo.

considering the ecophysiological constraints of these taxa (Corbet, 1999; De Marco & Peixoto, 2004; Oliveira-Junior et al., 2019). The Calopterygidae species belong to the heliothermic group, that relies totally on solar irradiation to warm their bodies up to start their daily activities (breeding, mating, flying, foraging, ovipositing) (Ferreira et al., 2023; Mendes et al., 2015). So, the collection procedure during the warmest part of the day assures the sampling of the species from this family. In each stream, we defined a 150-m stretch (from downstream to headstream), divided into 10 sections of 15 m, within which we performed the collection procedures using the fixed-areas protocol (Batista et al., 2021; Cezário et al., 2021). We standardised one researcher performing the sampling procedures for 1 h in each stream, using an entomological net (diameter: 40 cm; depth: 65 cm), attached to a 90 cm aluminium pole, spending at least 6 min 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, 2005). 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, 2006; Garrison et al., 2010; Lencioni, 2005).

## Environmental variables

We measured four limnological variables and three physical variables 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; Brasil et al., 2020; Nessimian et al., 2008), which addresses the completeness of riparian vegetation. We measured the limnological variables through a multiparameter probe, the physical variables using a centimetre-graded PVC pipe and a convex densiometer, and the HII through the visual measurement of riparian vegetation aspects.

## Spatial variables

We generated spatial filters to address the possible influence of these predictors on the species composition and species co-occurrences of

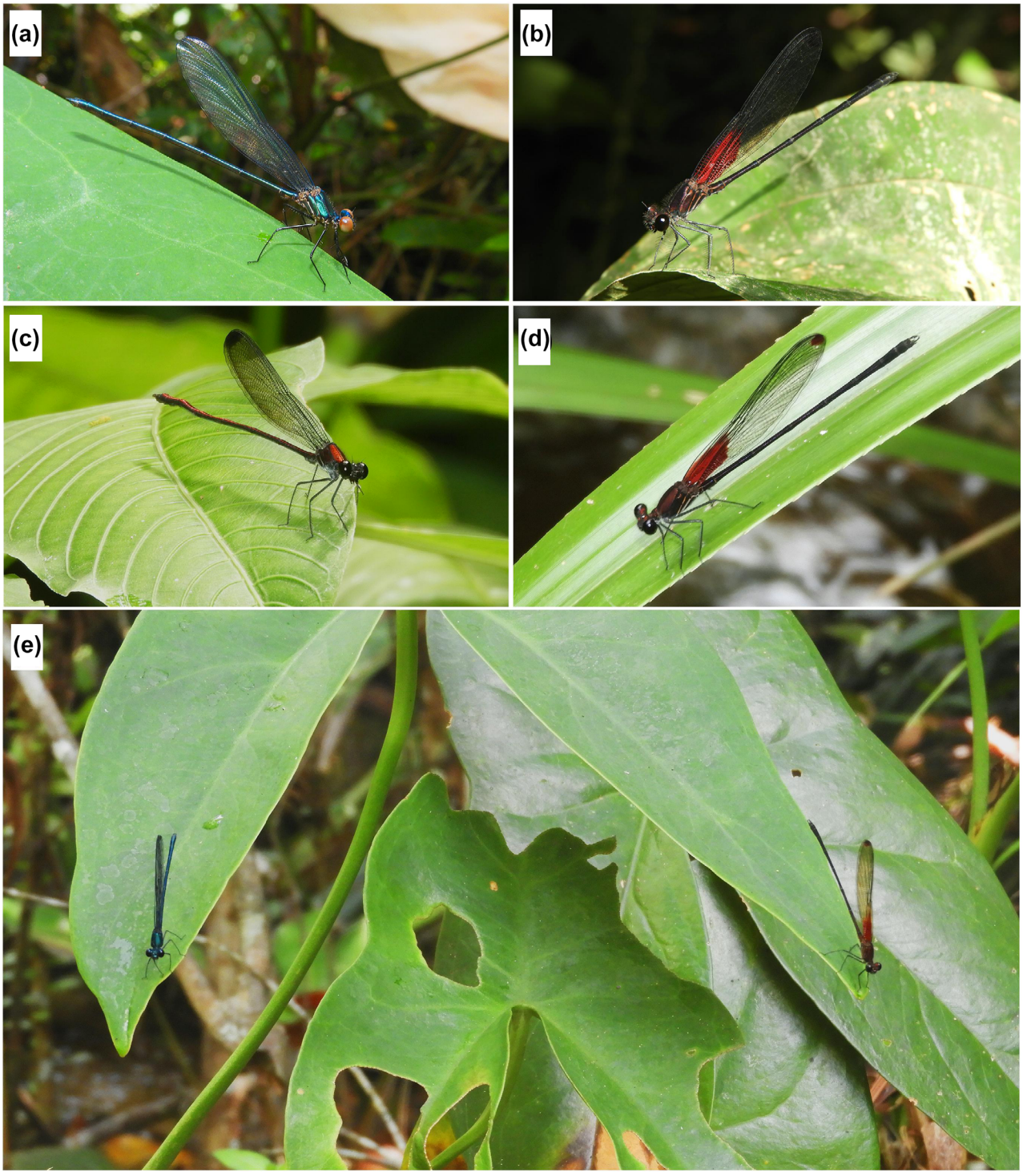


**FIGURE 2** Amazonian Calopterygidae species belonging to the two neotropical genera *Mnesarete* and *Hetaerina*. (a) *Mnesarete williamsoni* Garrison, 2006, (b) *Hetaerina amazonica* Sjöstedt, 1918, (c) *Mnesarete cupraea* (Selys, 1853), (d) *Hetaerina indeprensa* Garrison, 1990, (e) *M. williamsoni* (left) and *H. indeprensa* (right) co-occurring at the same spot. Photos: Mendoza-Penagos, C. C.

Calopterygidae. Spatial features are well-known predictors for the Zygoptera suborder in Amazonian freshwaters (Brasil et al., 2018; Brito et al., 2024; Juen & De Marco, 2012), mainly associated with the dispersal constraints of the species and the presence of geographic barriers, such as the massive rivers. 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 Neighbour 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., 2025) and spdep (Bivand & Wong, 2018). Moreover, the first five eigenvectors (PCNM1, PCNM2, PCNM3, PCNM4 and PCNM5) were retained as positive and significant, representing broad-scale variables (Borcard et al., 2004; Maxwell et al., 2021). Finally, we applied a forward selection by using the *forward.sel.par* function (Blanchet et al., 2008), associating the spatial filters with the Calopterygidae species composition. After that, all the spatial filters were retained except for PCNM5.

## Data analysis

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

To test the Hypothesis I, we applied a PERMANOVA and PERMDISP (Permutational Analysis of Variance and Permutational Analysis of Multivariate Dispersions, respectively; Anderson, 2001, 2006). PERMANOVA tests whether there are significant differences among two or more groups, while the PERMDISP is associated with the detection of dispersion heterogeneity within each tested group (Anderson, 2001, 2006). PERMANOVA and PERMDISP were conducted with a significance of $\alpha = 0.05$, defined by a Monte Carlo test with 9999 permutations, and were performed based on the matrix of Euclidean distances with the raw environmental data standardised (Borcard et al., 2018). Before all the analytical procedures, we applied a Pearson correlation and did not detect multicollinearity between the variables (Pearson-$r$ < 0.05).

To test Hypotheses II and III, 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). As input data, we used species by site matrices, one for streams IPAs and another for those OPAs. Additionally, we removed those streams without any occurrence of species. The probabilistic model of the species co-occurrence measures occurrence based on the number of sites where a pair of species occurs. 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 significant pairwise patterns of species co-occurrence, estimating the likelihood of each pair of species occurring less (Plt) or more (Pgt) often than expected if their distribution were randomly associated (Griffith et al., 2016). Thus, if Plt <0.05, two species have a negative co-occurrence, and if Pgt <0.05, then there is positive co-occurrence between the two species. The ecological meaning of the positive or negative results is, for the first, aggregation of the species, and segregation for the second one, and can be related to niche overlap or limiting similarity.

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) (Table S3): total body length (TBL); fore wing length and width (FWL 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 the Broken-Stick criterion to retain the most important components. To facilitate our assessment of the overall patterns, we classified Calopterygidae species into three discretionary classes regarding their co-occurrences: high (>8 significant co-occurrences), low (<3 significant co-occurrences) and medium (<8 and >3 significant 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).

To test Hypothesis III, we ran a variance partitioning based on partial redundancy analysis (pRDA; Borcard et al., 2018) to address the influence of environmental variables (limnological, physical structuring and habitat integrity) and spatial filters (PCNM retained by the forward selection) on Calopterygidae species composition. The variance partitioning is a common method used to address the relative importance of a set of predictors on species composition, and the pRDA is a constrained ordination that tests the influence of one set of variables while controlling for others. The pRDA was performed on the abundance matrix by applying the Hellinger transformation using ANOVA 9999 permutations (Borcard et al., 2018). We ran the RDA for all the areas without separating inside or outside.

Additionally, to test Hypothesis III regarding the influence of environmental variables on Calopterygidae species co-occurrence, we summarised the environmental gradient using a PCA (Borcard et al., 2018). We retained axes according to the Broken-Stick criterion and related them to species abundance through linear regressions (Guterres et al., 2019; Oliveira-Junior et al., 2021; Zar, 2010). For each species, the regression slope coefficients ($b$) and their standard errors were obtained, and pairwise comparisons were made by calculating $t$-values ($t = bx - by$), where $x$ and $y$ represent two species being compared. These $t$-values quantify the differences in how species pairs respond to the environmental gradient, that is, the degree of similarity or divergence in their environmental relationships. The set of all pairwise $t$-values was used to construct a triangular matrix of species pairs, which represents the co-occurrence structure as a function of the environment. The same procedure was applied to the spatial filters (PCNM retained after forward selection), which were incorporated directly into the analytical framework as explanatory variables, enabling the assessment of their contribution to species co-occurrence. Finally, we used a Mantel test to evaluate the degree of correlation between the co-occurrence matrix derived from environmental gradients or spatial filters and the triangular matrix of species pairs (Legendre & Legendre, 2012; Mantel, 1967).

**TABLE 1** Distribution of Calopterygidae species across streams located inside and outside protected areas, in Pará state, Brazilian Amazon (see footnote "a").

| Species | PARNA JX | | PARNA AM | | FLONA ST | | REBIO SC | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | 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 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 10 |
| *Hetaerina laesa* Hagen *in* Selys, 1853 | 2 | 0 | 26 | 5 | 13 | 25 | 2 | 0 | 73 |
| *Hetaerina moribunda* Hagen *in* Selys, 1853 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *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 | 67 | 68 | 36 | 22 | 41 | 78 | 0 | 359 |

Abbreviations: 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.
[a]In REBIO SC, only streams inside the protected area were sampled.

All the analytical procedures were performed in R Computational Environment (R Core Team, 2023), available in the Comprehensive R Archive Network (R Core Team, 2023). For building the visuals, we used the *ggplot2* function from the ggplot package (Wickham, 2016).

## RESULTS

### Biotic overview

We sampled 359 individuals of Calopterygidae species, distributed into 10 species, corresponding to two genera, *Hetaerina* Hagen in Selys, 1853 and *Mnesarete* Colwey, *1934*. Most of the species occurred in both classes, inside and outside the PAs, with the first having 215 individuals, and the second having 144 individuals. *Mnesarete cupraea* (Selys, 1853) presented the highest number of individuals ($n = 91$), followed by *Hetaerina auripennis* (Burmeister, 1839) ($n = 84$), *Hetaerina laesa* Hagen in Selys, 1853 ($n = 73$) and *Hetaerina amazonica* Sjöstedt, 1918 ($n = 39$). *Hetaerina indeprensa* Garrison, 1990 occurred only in streams IPAs, while *Hetaerina moribunda* Hagen in Selys, 1853 occurred only in streams OPAs. Moreover, the remaining species presented less than 39 individuals (Table 1; please see footnote "a").

### Overview of environmental heterogeneity

The PERMANOVA/PERMDISP framework indicated that environmental heterogeneity was different among streams located inside and outside (Pseudo-$F = 8.022$; $R^2 = 0.07$; $p < 0.001$), and that this difference was regarding the variance in heterogeneity inside each category of streams ($F = 5.218$; $p = 0.024$). The distance to the centroid was higher in streams OPAs (2.372) than in those located IPAs (1.748), meaning that streams from the latter presented higher environmental heterogeneity when compared to the former.

### Co-occurrence and morphological patterns of the Calopterygidae species

The co-occurrence analysis for streams IPAs showed 36 species combinations, with 6 pairs (16.67%) being removed because the expected co-occurrence was lower than 1, and 30 pairs were analysed. Overall, we found only significant negative (24) and random co-occurrences, with *H. amazonica* and *M. cupraea* presenting the highest number of significant (−) co-occurrences with other Calopterygidae species (Figure 3a).

On the other hand, OPAs showed 36 species combinations, from which 15 pairs were removed (41.66%) since the expected co-occurrence was lower than 1, and 21 pairs remained to be analysed. Overall, we found only negative and random occurrences, with *H. amazonica* and *H. auripennis* presenting the highest number of significant (−) associations with other species (Figure 3b). None of the areas presented positive co-occurrence associations. Only the species that present significant results are displayed in Figure 3a,b. All the raw values from *cooccur* outcomes, either negative, positive or random, are available in Tables S1 and S2. The non-significant results are highlighted in bold in Supporting Information.

The Broken-Stick criterion retained the first component of PCA, which explained 96.02% of the total variation (Figure 4). TBL, fore wing length, and thorax width were the variables that contributed the most to the overall pattern presented by the PCA. The general output of PCA is available in Table S4.

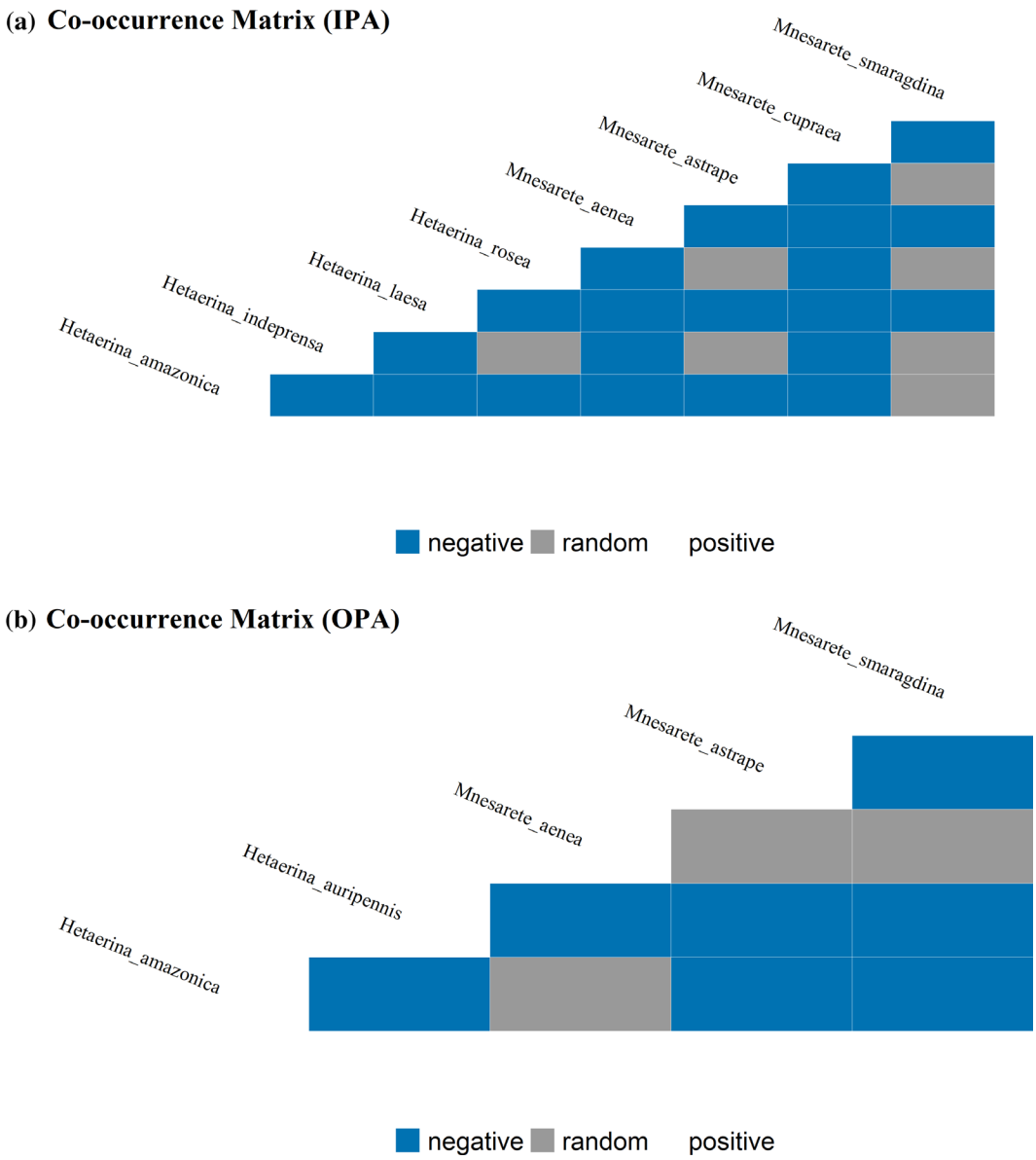


**FIGURE 3** Lower triangle heat map of the co-occurrence patterns of Calopterygidae species in streams (a) inside (IPA) and (b) outside (OPA) protected areas. No positive co-occurrence was found.

## Abiotic influences on Calopterygidae species composition and co-occurrence

The first two axes of the RDA represented 63.54% of the total variation, with the first axis contributing 39.57%, and the second with 23.97% (Figure 5). The variance partitioning indicated that both environmental and spatial variables explained 5% of the total variance, with their interaction also explaining 5% of the total variance. Moreover, the unexplained variance was 85%.

Air temperature, pH and HII, with spatial filters PCNM3 and PCNM4, were the predictors that contributed the most to the Calopterygidae species composition (Figure 5). Air temperature and HII presented the most significant influence on species *H. amazonica*, *H. laesa* and *Mnesarete astrape*, while pH was more associated with *Mnesarete smaragdina* (Figure 5). Besides, the PCNM3 and PCNM4 were the most important spatial filters associated with the Calopterygidae species, mainly *H. moribunda*, *Hetaerina rosea* and *M. smaragdina* (Figure 5).

Considering the PCA applied to the environmental variables to test their influence on Calopterygidae co-occurrences, the Broken-Stick criterion selected the first two components (Comp.1 and Comp.2).

The Mantel test between the coexistence index and the triangular matrix of species pairs showed that co-occurrence was not significantly related to Comp.1 ($r = 0.336$, $p = 0.087$). In contrast, co-occurrence was significantly related to Comp.2 ($r = 0.504$, $p = 0.035$), which was primarily associated with depth of the channel and water conductivity (Table S3). This indicates that differences in species co-occurrence patterns were significantly associated with the

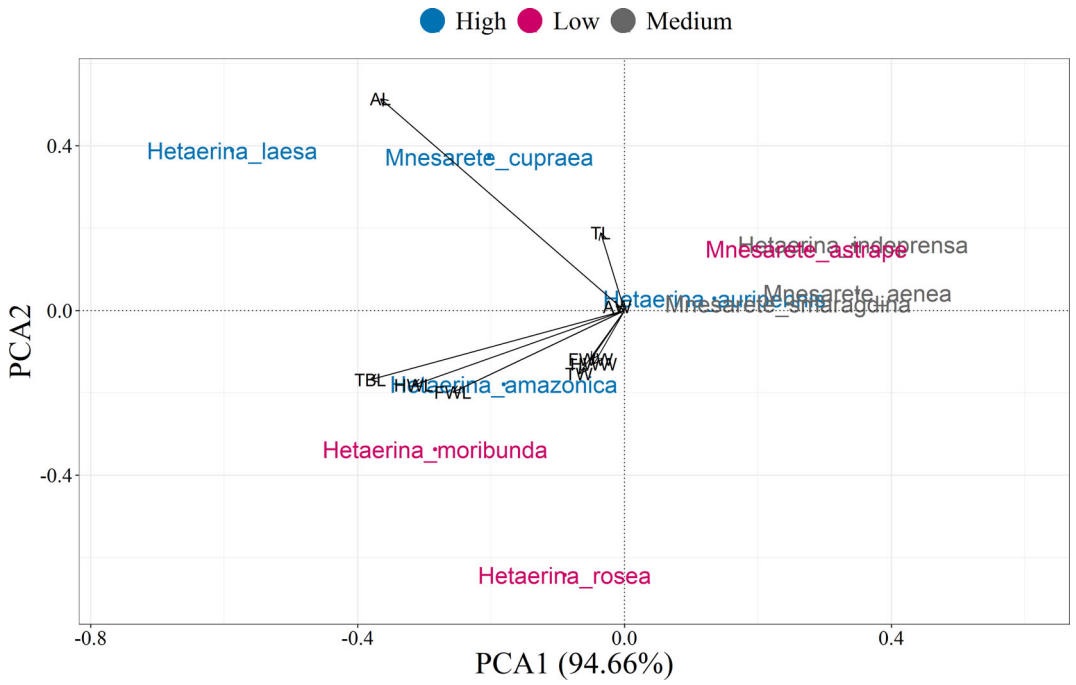


**FIGURE 4** Biplot of the first two axes derived from the principal component analysis (PCA) showing the representativity of morphological characteristics for the overall variation of Calopterygidae species presenting high, low or medium negative co-occurrence.

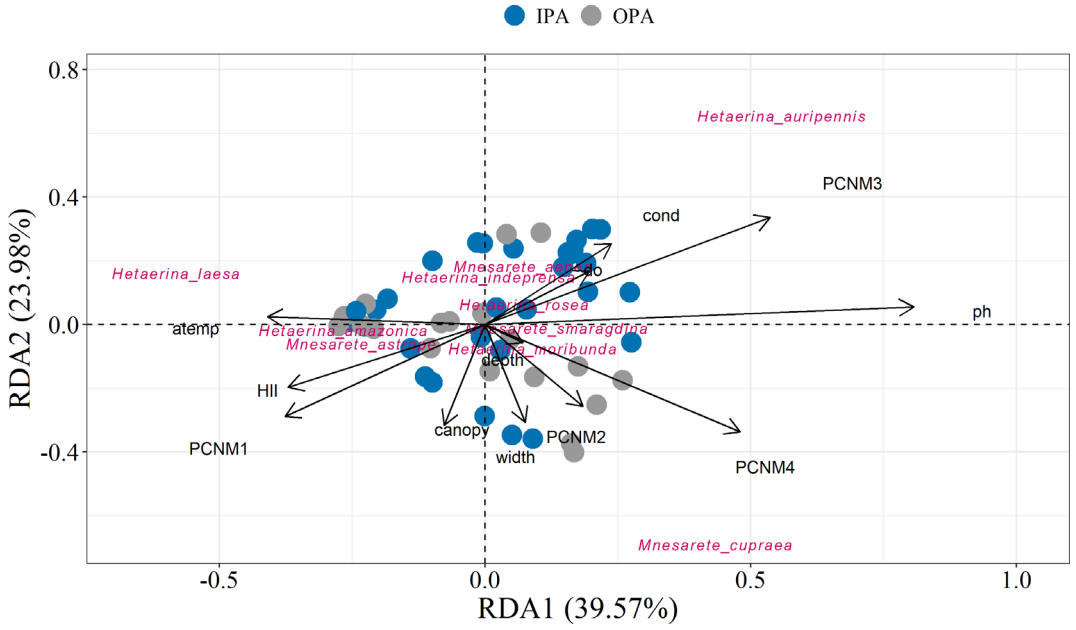


**FIGURE 5** Triplot of the relationship between environmental and spatial variables and the Calopterygidae species composition, evaluated by the partial redundancy analysis (pRDA) with two selected axes. Canopy, canopy cover (%); cond, water conductivity; depth, channel depth (cm); do, dissolved oxygen (mg/L); HII, Habitat Integrity Index; IPA, inside protected areas; OPA, outside protected areas; PCNM, spatial filters generated by the Principal Coordinates of Neighbourhood Matrices; pH, potential of hydrogen; width, channel width (m); wtemp, water temperature (°C).

environmental gradients captured by Comp.2. Additionally, a significant relationship was found for the spatial component, but only for the spatial filter PCNM3 ($r = 0.901$, $p = 0.002$), highlighting the influence of broad-scale spatial predictors on Calopterygidae species distributions.

## DISCUSSION

Our first hypothesis, that streams IPA would be more environmentally heterogeneous than those OPA, was not supported, as OPA streams exhibited greater environmental heterogeneity, likely due to multiple

anthropogenic disturbances. Similarly, our second hypothesis, which predicted stronger and less random species co-occurrence patterns in IPA streams, was not supported. Instead, both IPA and OPA streams showed predominantly negative and random co-occurrence patterns, partially supporting our third hypothesis regarding the prevalence of negative associations. Contrary to our fourth hypothesis, spatial predictors had the strongest influence on Calopterygidae species diversity, followed by limnological and physical habitat variables. Spatial factors also emerged as more important than environmental variables in explaining co-occurrence patterns.

The strong spatial influence on species distribution likely arises from two key mechanisms. First, major biogeographical barriers, particularly large Amazonian rivers, act as effective dispersal filters, limiting gene flow and promoting spatial structuring. Second, the Calopterygidae species examined are closely related evolutionarily and likely share similar ecological niches, leading to competitive exclusion dynamics. This pattern aligns with the theory of limiting similarity (Abrams, 1983), which suggests that species with highly overlapping niches cannot stably coexist, often resulting in spatial segregation where only one species occupies a given site. In our study, we found more negative co-occurrences in streams inside and OPAs. However, our results regarding environmental heterogeneity are in line with what has been found in recent studies (Cruz et al., 2022; Franco et al., 2023; Veras et al., 2024).

Consistent with previous research, our study confirms the strong influence of spatial factors on Odonata communities in Amazonian freshwater systems (Brasil et al., 2018; Brito et al., 2024; Juen & De Marco, 2012). While damselflies generally exhibit more restricted dispersal capabilities than dragonflies due to their slender bodies and smaller flight muscles, we found that even the relatively robust Calopterygidae—characterised by stronger flight musculature and broader wing bases—showed significant responses to broad-scale spatial predictors. This pattern likely reflects historical biogeographical processes, particularly the Andean uplift (Wesselingh & Salo, 2006), which fundamentally reshaped the Amazonian drainage network and consequently influenced the distribution of ancient lineages like Odonata. The Amazon's massive river systems (e.g., Amazonas, Tapajós) appear to impose substantial dispersal barriers, even for volant insects. Despite observing widespread species distributions across our study area, the persistent effect of spatial variables on diversity and co-occurrence patterns suggests important dispersal limitations. We propose two Amazon-specific mechanisms that may explain these patterns: (1) passive larval dispersal via floating macrophyte banks (facilitated by their epiphytic oviposition strategy) and (2) river meandering processes that periodically connect opposite banks through channel straightening, enabling cross-river faunal exchange (Juen & De Marco, 2012). These unique hydrological features may help maintain connectivity while simultaneously creating the spatial structuring we observed.

The prevalence of negative co-occurrences in protected-area streams likely stems from their lower environmental heterogeneity, which reduces resource variety and intensifies interspecific competition. This is particularly evident among evolutionarily closely related taxa (Burns & Strauss, 2011), where niche overlap would naturally lead to competitive exclusion. Interestingly, even in the more heterogeneous streams OPAs (which theoretically offer greater resource diversity), we still found evidence of strong interspecific competition, suggesting that increased resource availability alone may not completely mitigate competitive interactions among these species' occurrences.

The greater environmental heterogeneity observed OPAs likely results from multiple anthropogenic pressures. Human activities such as agriculture, pasture expansion and urbanisation alter key physical variables including stream width, vegetation cover and dissolved gas concentrations (Deacon et al., 2018; Franco et al., 2023; Hill et al., 2016; Veras et al., 2024). In contrast, PAs maintain more stable and homogeneous conditions (Seiferling et al., 2014). Interestingly, the intermediate disturbance levels in unprotected areas, where some native vegetation persists despite human impacts, appear to create greater habitat heterogeneity. This pattern aligns with the intermediate disturbance hypothesis (Connell, 1978), as partial vegetation retention coupled with moderate disturbance generates diverse microhabitats that may reduce interspecific competition.

Such disturbed but vegetated sites often support abundant aquatic plant growth, particularly where increased sunlight penetration occurs (De Marco et al., 2014; Fares et al., 2020; Kovalenko et al., 2012). These plants provide important structural elements—including emergent leaves and stolons—that serve as perching substrates for Odonata (Brito et al., 2021). Our field observations confirm that Calopterygidae species (e.g., *M. cupraea*, *Mnesarete aenea* and various *Hetaerina* spp.) actively use these aquatic plants for key behaviours including oviposition, mating and territorial guarding. Furthermore, the ecological plasticity within Calopterygidae, ranging from habitat specialists (e.g., *H. amazonica*) to generalists (e.g., *H. auripennis*, *H. laesa*, *M. aenea*) that tolerate wider environmental conditions (Brito et al., 2023; Veras et al., 2024), likely contributes to the observed distribution patterns across disturbance gradients.

Environmental factors, particularly limnological and physical-structuring variables, significantly influenced Calopterygidae species diversity and co-occurrence patterns. The riparian zone serves as a critical source of physical heterogeneity, providing essential microhabitat features such as wood debris, branches and twigs that function as perching substrates for territorial males. This is especially evident for species like *Hetaerina vulnerata*, *Hetaerina americana* and *Hetaerina cruentata*, which utilise these structures for their characteristic lek mating systems—where males display competitive aggregations to attract females (Córdoba-Aguilar et al., 2009; Guillermo-Ferreira & Del-Claro, 2011). Beyond supporting reproductive behaviours, riparian vegetation plays additional key ecological roles. It stabilises stream banks, reducing excessive sediment input that could alter channel morphology, while simultaneously maintaining the physical integrity of the aquatic habitat. These combined functions help explain why channel dimensions (width and depth) emerged as particularly important predictors of Calopterygidae co-occurrence patterns in our study. The vegetation's dual role in providing both biological resources (perching sites) and physical habitat maintenance underscores its fundamental importance in shaping damselfly community structure.

While our study did not explicitly examine anthropogenic impacts on Calopterygidae morphology or its relationship to co-occurrence patterns, heuristic analysis revealed that body length and wing morphology significantly correlated with species showing the most frequent negative co-occurrences. These morphological traits directly influence critical ecological functions, including oviposition site access and flight performance (Pereira et al., 2019; Resende et al., 2021), which may be particularly susceptible to human-induced habitat changes. However, without comparative morphological data between protected and unprotected areas, we cannot definitively establish these relationships, highlighting an important avenue for future research.

While our findings provide valuable insights, several caveats warrant discussion. First, existing behavioural studies of Calopterygidae predominantly focus on species not included in our sampling. For instance, research on *Mnesarete pudica* in Brazil (Guillermo-Ferreira & Bispo, 2012; Pena-Firme & Guillermo-Ferreira, 2020) and *H. cruentata* in Colombia (García-Monsalve et al., 2021) examined reproductive and demographic traits, while broader studies in Mexico (Córdoba-Aguilar et al., 2009) investigated different *Hetaerina* species than those in our study. Given this taxonomic gap and the paucity of population studies on Amazonian Calopterygidae, we relied on congeneric inferences based on evolutionary relationships (Burns & Strauss, 2011). However, we recognise that even closely related species may exhibit behavioural divergence. Notably, *H. rosea* (a sampled species) lacks the characteristic lek mating system observed in other *Hetaerina* species (Guillermo-Ferreira & Del-Claro, 2011), underscoring the need for species-specific behavioural studies of Amazonian Calopterygidae, particularly abundant taxa. Such research would clarify co-occurrence patterns and strengthen ecological interpretations. Regarding co-occurrence analysis, we acknowledge that negative associations may arise from processes beyond competitive interactions (Bell, 2005; Dallas et al., 2019). Nevertheless, our holistic approach, incorporating abiotic influences on species composition (Guterres et al., 2019; Oliveira-Junior et al., 2021), helps distinguish true ecological patterns from potential artefacts, yielding more robust inferences.

Beyond Calopterygidae and Amazonian streams, our findings have broader relevance for freshwater community assembly and biodiversity conservation. The predominance of spatial structuring over environmental heterogeneity mirrors patterns reported for other aquatic insects, fishes and benthic invertebrates in both temperate and tropical systems (Cruz et al., 2022; Deacon et al., 2018; Firmiano et al., 2020; Heino & Tolonen, 2017; Hill et al., 2016). This underscores the key role of dispersal limitation and historical biogeographic processes in shaping local assemblages, even for volant taxa such as Odonata. From a conservation perspective, our results suggest that maintaining landscape connectivity is as critical as safeguarding environmental quality within PAs, since dispersal barriers can constrain biodiversity persistence and recovery. Although focused on Amazonian streams, the ecological mechanisms identified here are broadly applicable, offering a general framework for understanding how spatial, environmental and biotic processes interact to structure communities across ecosystems.

## CONCLUSION

Our results revealed that streams within PAs exhibited lower environmental heterogeneity compared to those outside protected zones, a pattern likely driven by multiple human land-use activities. We observed predominantly negative co-occurrences in both area types, along with some random associations. Given that we studied evolutionarily closely related taxa, their ecological similarity may explain the prevalence of negative co-occurrence patterns due to competitive interactions. While our study did not directly compare morphological differences in Calopterygidae species between protected and unprotected areas, our heuristic approach yielded insightful results that merit further investigation. We found that species composition and co-occurrence patterns were strongly influenced by both environmental factors (limnological and physical-structuring variables) and broad-scale spatial constraints, highlighting the combined importance of niche processes and geographical limitations. These findings underscore the need for additional studies on the basic biology of Amazonian Calopterygidae species to strengthen ecological interpretations of their co-occurrence patterns. Furthermore, extending this research to other Odonata families—particularly forest-associated groups like Coenagrionidae and Heteragrionidae—and other aquatic groups, could provide valuable insights into how PAs maintain species co-occurrence networks.

## AUTHOR CONTRIBUTIONS

**Joás Silva Brito:** Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; formal analysis; data curation. **Everton Cruz Silva:** Investigation; writing – review and editing. **Gabriel Martins Cruz:** Writing – review and editing; investigation. **Victor Rennan Santos Ferreira:** Writing – review and editing; data curation; investigation. **Rafael Costa Bastos:** Writing – review and editing; data curation; investigation. **Josinete Sampaio Monteles:** Investigation; writing – review and editing. **Cristian Camilo Mendoza-Penagos:** Data curation; writing – review and editing. **Fernando Geraldo Carvalho:** Writing – review and editing; data curation. **José Max Barbosa Oliveira-Junior:** Writing – review and editing. **Karina Dias-Silva:** Writing – review and editing. **Thaisa Sala Michelan:** Writing – review and editing. **Luciano Fogaça de Assis Montag:** Writing – review and editing. **Lilian Casatti:** Writing – review and editing; investigation; supervision; resources; validation. **Leandro Juen:** Conceptualization; investigation; writing – original draft; writing – review and editing; validation; formal analysis; supervision; resources; methodology.

## ACKNOWLEDGEMENTS

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (process: 428961/2018-5) through the project 'Diminuindo as lacunas Linneanas e Wallaceanas da biota aquática na Amazônia'; by Fundação Amazônica de Amparo a Estudos e Pesquisa do Pará (FAPESPA), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (processes: 068/2020 and 2019/25445-1), through the project 'Padrões de distribuição da biodiversidade

aquática no Estado do Pará' and partly by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) (Finance Code 001). We are grateful to ICMBio and the Monitora Program that allowed the collection of the data in the protected areas. JSB thanks CNPq for a doctoral scholarship in Brazil (process: 141113/2020-0) and also thanks CAPES for a scholarship at the University of Guelph, Ontario, Canada (88881.690205/2022-01). We thank CNPq for the productivity research grants to LC (304403/2021-0) and LJ (304710/2019-9). We thank Dr Rhainer Guillermo-Ferreira for providing the first pictures of Calopterygidae species. We also thank Dr Erlane José Cunha and Dr Alana Patrícia Meguy Guterres for their help during the analytical procedures, and Dr Lenize Batista Calvão for her help during the taxonomic identification process. We are also grateful to the fieldwork teams of Laboratório de Ecologia e Conservação (LABECO) and Laboratório de Ecologia de Produtores Primários (ECOPRO). We extend our gratitude to the local people who helped us access the remote areas and provided advice about the local peculiarities. The Article Processing Charge for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614).

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.cnp5hqchd (Brito et al., 2025).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Table S1.** Co-occurrence patterns found in streams inside protected areas; results with the 'thresh' set as FALSE, to show all the results, including the non-significant.

**Table S2.** Co-occurrence patterns found in streams outside protected areas; results with the 'thresh' set as FALSE, to show all the results, including the non-significant.

**Table S3.** Mean values of the Calopterygidae species' morphological measurements. All variables were used in the principal components analysis (PCA).

**Table S4.** General overview of principal components analysis (PCA). Morphological variables that contributed the most to the retained axis are highlighted in bold.

**How to cite this article:** Brito, J.S., Silva, E.C., Cruz, G.M., Ferreira, V.R.S., Bastos, R.C., Monteles, J.S. et al. (2026) Abiotic drivers of co-occurrence and diversity patterns of Calopterygidae species in Amazonian protected freshwaters. *Ecological Entomology*, 51(2), 235–249. Available from: https://doi.org/10.1111/een.70036