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EPHEMEROPTERA NA MATA ATLÂNTICA: TENDÊNCIAS ALTITUDINAIS E CONGRUÊNCIA TAXONÔMICA ENTRE NINFAS E IMAGOS

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Dissertação apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais (PPGERN) como parte dos requisitos para obtenção do título de Mestre em Ecologia e Recursos Naturais

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RESUMO

Os ecossistemas de água doce e sua biodiversidade têm sido profundamente impactados pelas atividades antrópicas. Nesse cenário, programas de biomonitoramento voltados à mitigação desses efeitos e à conservação desses ambientes têm ganhado destaque. Esta dissertação avaliou a utilização de insetos da ordem Ephemeroptera como indicadores ecológicos, estando estruturada em dois capítulos. O primeiro capítulo apresenta uma revisão sistemática da literatura sobre a relação entre altitude e diversidade de Ephemeroptera. Os resultados evidenciaram variações metodológicas e regionais significativas entre os estudos, além de uma tendência geral de redução na riqueza taxonômica com o aumento da altitude. A análise de 74 artigos científicos revelou uma concentração geográfica dos estudos nas regiões Paleártica e Neotropical, em altitudes mais baixas e com abrangência espacial limitada. Observou-se ainda uma escassez de estudos focados exclusivamente em Ephemeroptera, indicando lacunas importantes para futuras pesquisas. O segundo capítulo avaliou a fauna de Ephemeroptera em riachos de primeira a terceira ordem na Mata Atlântica, bem como a congruência taxonômica entre os estágios de ninfa e imago, a fim de verificar sua aplicabilidade como substitutos em programas de biomonitoramento. Foram amostrados 40 riachos, e observou-se maior riqueza e abundância de ninfas em comparação as imagos. A congruência entre os dois estágios foi baixa e não significativa, assim como suas correlações com variáveis ambientais, sugerindo que ninfas e imagos respondem de formas distintas às condições ecológicas. Por outro lado, dentro de um mesmo estágio, medidas de abundância e incidência mostraram-se intercambiáveis. Os resultados reforçam a importância de abordagens integradas que combinem diferentes métricas e estágios de vida, visando aumentar a precisão e a eficácia dos estudos ecológicos e de biomonitoramento.

ABSTRACT

Freshwater ecosystems and their biodiversity have been profoundly affected by anthropogenic activities. In this context, biomonitoring programs aimed at mitigating these impacts and conserving these environments have gained increasing prominence. This dissertation examines the use of insects from the order Ephemeroptera as ecological indicators and is structured in two chapters. The first chapter presents a systematic review of the literature on the relationship between altitude and Ephemeroptera diversity. The results revealed significant methodological and regional variation among studies, as well as a general trend of decreasing taxonomic richness with increasing altitude. The analysis of 74 scientific articles showed a geographic concentration of research in the Palearctic and Neotropical regions, primarily at lower elevations and with limited spatial coverage. Additionally, there was a notable lack of studies focusing exclusively on Ephemeroptera, highlighting key gaps for future research. The second chapter assessed Ephemeroptera assemblages in first- to third-order streams within the Atlantic Forest, as well as the taxonomic congruence between the nymph and imago stages, to evaluate their suitability as surrogates in biomonitoring programs. A total of 40 streams were sampled, revealing higher richness and abundance in nymphs compared to imagos. The congruence between the two life stages was low and not statistically significant, as were their correlations with environmental variables, indicating that nymphs and imagos respond differently to ecological conditions. Conversely, within the same life stage, measures of abundance and incidence were found to be interchangeable. These results underscore the importance of integrated approaches that combine multiple metrics and life stages to enhance the accuracy and effectiveness of ecological and biomonitoring studies.

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LISTA DE ABREVIATURAS E SIGLAS

CBSP	Carlos Botelho State Park
CNP	Caparaó National Park
EPT	Ephemeroptera, Plecoptera and Trichoptera
EXT	Information extracted from the articles included in the scientometric analysis
GAM	Generalized Additive Models
GRI	Information derived from a global study
HII	Habitat Integrity Index
MG	Minas Gerais State
PA	Protected Area
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PERMANOVA	Permutational Multivariate Analysis of Variance
SP	São Paulo State
SPSP	Serra do Papagaio State Park
SVN	Santa Virgínia Nucleus
UC	Unidade de Conservação
WoS	Web of Science

SUMÁRIO

INTRODUÇÃO GERAL

A ordem Ephemeroptera representa um grupo de insetos com longa história evolutiva (Da-Silva and Salles 2024). Sua origem remonta ao período Carbonífero, conforme indicam registros fósseis de táxons relacionados (Sartori and Brittain 2015). Trata-se de uma das ordens de insetos que possuem pelo menos um estágio do ciclo de vida em ambiente aquático, vivendo quase exclusivamente nesse habitat (Dijkstra et al. 2014). Seus representantes se caracterizam, entre outros fatores, por possuírem um estágio de ninfa aquática dominante, com tempo médio de desenvolvimento entre três e seis meses, além de dois estágios alados: o subimago, exclusivo da ordem, e o imago, fase terrestre e adulta associada à reprodução (Edmunds and McCafferty 1988a; Da-Silva and Salles 2024).

Mais comumente encontradas em ambientes lóticos, especialmente cursos d'água de segunda e terceira ordem, as ninfas desempenham importantes serviços ecossistêmicos (Mariano and Costa 2014; Jacobus et al. 2019). Táxons escavadores, por exemplo, são fundamentais para processos de bioturbação e bioirrigação (Chaffin and Kane 2010). Além disso, muitos indivíduos servem como hospedeiros de espécies comensais e parasitas, bem como alimento para diversos predadores (Grzybkowska et al. 2016; Aguilera et al. 2022). Características como ampla distribuição, alta diversidade, mobilidade limitada e facilidade de amostragem tornam os Ephemeroptera excelentes bioindicadores no monitoramento da qualidade da água.

Atualmente, a ordem está amplamente distribuída, ausente apenas na Antártida e em algumas ilhas oceânicas, somando cerca de 3.330 espécies, organizadas em 40 famílias e 440 gêneros (Sartori and Brittain 2015). A região Neotropical é a mais diversa para o grupo, com quase 900 espécies registradas (Jacobus et al. 2019). Na América do Sul, são conhecidas 765 espécies, distribuídas em 14 famílias e 117 gêneros (Ephemeroptera da América do Sul, 2025).

No Brasil, 474 espécies válidas já foram registradas, pertencentes a 10 famílias e 83 gêneros, com estimativas que apontam para mais de 10.000 espécies ainda desconhecidas no país (Cardoso et al. 2015; Salles et al., 2025).

Grande parte dessa diversidade ocorre em biomas tropicais úmidos, como a Mata Atlântica, um dos seis biomas brasileiros. Em sua configuração original, essa formação florestal cobria cerca de 1,6 milhão de hectares ao longo da costa atlântica do Brasil, além de porções do Paraguai e da Argentina (Marques et al. 2021). Caracterizada por formações vegetais diversas e elevada riqueza de espécies, muitas delas endêmicas, a Mata Atlântica é hoje um dos ecossistemas mais ameaçados do planeta, com apenas 11,6% de sua cobertura original remanescente, distribuída em fragmentos isolados (Mittermeier et al. 2011; Scarano and Ceotto 2015; Vitória et al. 2019).

Nesse cenário, as Unidades de Conservação (UCs) destacam-se como estratégia central para preservação remanescente, combinando proteção da biodiversidade com desenvolvimento sustentável (Stolton 2010). No Brasil, a Mata Atlântica concentra cerca de 60% das 3.119 UCs do país (Painel de Unidades de Conservação Brasileiras, 2025). Essa concentração é estratégica, já que o bioma abriga ecossistemas e espécies emblemáticas, algumas ameaçadas de extinção, como o muriqui-do-norte, o mico-leão-dourado, o palmito-juçara e a araucária. Além da proteção passiva, essas áreas também assumem funções socioeconômicas, contribuindo com as comunidades locais, fomentando o turismo, além de mitigar as mudanças climáticas (Watson et al. 2014). Dentre as formações protegidas por UCs, as áreas de altitude apresentam grande relevância ecológica. Embora cubram apenas 25% da superfície terrestre, na forma montanhas, colinas e planaltos, esses ambientes possuem uma biodiversidade desproporcionalmente elevada (Körner 2007; Elsen et al. 2018). No contexto da Mata Atlântica, esse padrão se manifesta nas formações montanhosas da Serra do Mar e da Serra da Mantiqueira, com altos níveis de endemismo, provisão de serviços ecossistêmicos essenciais, além de funcionarem como corredores climáticos (Rylands et al. 1996; Cardoso Da Silva et al. 2004; Werneck et al. 2011). Assim, investigar a diversidade e o papel ecológico de grupos bioindicadores, como Ephemeroptera, em áreas montanhosas da Mata Atlântica, representa um caminho promissor para o aperfeiçoamento de estratégias de conservação e gestão de recursos hídricos associados ao bioma.

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CHAPTER 1

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Altitude and Aquatic Insects: A Scientometric Review of Ephemeroptera Research and Biodiversity Trends

RESUMO

A altitude é uma variável ambiental chave que influencia fatores ecológicos e a biodiversidade em ecossistemas naturais. Em organismos aquáticos, a riqueza taxonômica tipicamente declina com o aumento da altitude; no entanto, estudos sobre Ephemeroptera sugerem variabilidade nesse padrão. Para melhor entender essa relação e identificar lacunas de conhecimento para pesquisas futuras, conduzimos uma revisão bibliográfica usando artigos do banco de dados Web of Science, buscando com termos específicos em três idiomas. Além disso, extraímos dados de riachos de um estudo global sobre insetos aquáticos. Dos 228 artigos identificados, 74 atenderam aos nossos critérios de seleção. Os periódicos mais relevantes foram Freshwater Biology (n = 11) e Hydrobiologia (n = 5). Geograficamente, a maioria dos estudos concentrou-se nas regiões Paleártica (40,5%) e Neotropical (29,7%), com Argélia (n = 6) e Eslováquia (n = 4) sendo as mais estudadas na primeira, e Equador (n = 8) e Argentina (n = 8) na segunda. A maioria dos estudos concentrou-se na influência de fatores ambientais na composição e diversidade da comunidade (n = 35). Apenas cerca de 30% dos artigos examinaram exclusivamente Ephemeroptera, com ênfase no estágio de ninfa e na taxonomia em nível de espécie. A maioria dos estudos foi conduzida em áreas de amostragem relativamente pequenas (<50 pontos de coleta) e em altitudes abaixo de 3.000 m. Nossa análise revelou uma relação significativa entre altitude e biodiversidade de Ephemeroptera, com a riqueza de espécies e gêneros, bem como a abundância geral, geralmente diminuindo com o aumento da altitude.

ABSTRACT

Altitude is a key environmental variable influencing ecological factors and biodiversity in natural ecosystems. In aquatic organisms, taxonomic richness typically declines with increasing altitude; however, studies on Ephemeroptera suggest variability in this pattern. To better understand this relationship and identify knowledge gaps for future research, we conducted a literature review using articles from the Web of Science database, searching with specific terms in three languages. Additionally, we extracted stream data from a global study on aquatic insects. Out of 228 identified articles, 74 met our selection criteria. The most relevant journals were *Freshwater Biology* (n = 11) and *Hydrobiologia* (n = 5). Geographically, most studies were concentrated in the Palearctic (40.5%) and Neotropical (29.7%) regions, with Algeria (n = 6) and Slovakia (n = 4) being the most studied in the former, and Ecuador (n = 8) and Argentina (n = 8) in the latter. The majority of studies focused on the influence of environmental factors on community composition and diversity (n = 35). Only about 30% of the articles examined Ephemeroptera exclusively, with an emphasis on the nymph stage and species-level taxonomy. Most studies were conducted in relatively small sampling areas (<50 collection points) and at altitudes below 3,000 m. Our analysis revealed a significant relationship between altitude and Ephemeroptera biodiversity, with species and genus richness, as well as overall abundance, generally decreasing with increasing elevation.

KEYWORDS

High Elevations; Freshwater Ecosystems; Mayfly; Species Richness; Mountain Streams; Aquatic Insects.

1 INTRODUCTION

The relationship between altitude and biodiversity has been studied since the 18th century, with Alexander von Humboldt pioneering the study of vegetation across different regions of the world (West, 2021). Since then, numerous studies have further explored this relationship, highlighting environmental changes associated with increasing altitude, such as reductions in available land area, atmospheric pressure, and temperature, as well as an increase in solar radiation (Sarmiento, 1987; Blumthaler et al., 1997; Peacock, 1998; Körner & Paulsen 2010). These variations also affect aquatic ecosystems, influencing temperature, oxygenation, the concentration of suspended solids, and water flow (Jacobsen, 2008). These factors, in turn, impact gas exchange and the dispersal of organisms inhabiting these environments (MacDonald & Coe 2007; Theodoropoulos et al., 2017), as well as the distribution and diversity of species, with a general trend of decreasing species richness at higher altitudes (Wolda, 1987; Rahbek, 1995; Füreder et al., 2006; Sekar et al., 2024). However, this relationship varies across taxonomic groups (McCain & Grytnes 2010). For instance, fish species richness generally declines with increasing altitude (Bistoni & Hued 2002; Jaramillo-Villa et al., 2010). In contrast, patterns for benthic macroinvertebrates are less clear, with some studies reporting greater species richness at intermediate altitudes (Henriques-Oliveira & Nessimian 2010), while others indicate a continuous decline with altitude (Suren, 1994; García-Ríos et al., 2020). Additionally, some studies have found no significant relationship between altitude and aquatic insect diversity (Siri et al., 2022; Torrejon et al., 2022).

Ephemeroptera have a life cycle characterized by an aquatic immature stage and two winged stages, the subimago and the imago (Da-Silva & Salles 2024). They are notable for their ecological importance and sensitivity to environmental changes (Ramulifho et al., 2020). Their aquatic immature stages play a crucial role in nutrient cycling and energy flow in lotic ecosystems (Mariano & Costa 2014; Dijkstra et al., 2014; Jacobus et al., 2019). Regarding altitude, a global pattern of decreasing species richness with increasing elevation has been observed, and Ephemeroptera generally follow this trend (Miserendino & Pizzolán 2001; Sartori & Brittain 2015; Grigoropoulou et al., 2023; Pohe et al., 2024). However, some studies report different patterns, with greater species richness at higher altitudes (Lang & Reymond 1993; Jiang et al., 2013; Vilenica et al., 2018).

Our aim was to provide a comprehensive overview of the relationship between altitude and Ephemeroptera by reviewing the literature to identify publication patterns and examine the main research objectives. Additionally, we aimed to map the locations and characteristics of the study areas, highlight the most commonly used methodologies, and identify gaps that could guide future research. We also assessed the effects of altitudinal variation on the taxonomic richness and abundance of Ephemeroptera, contributing to a more detailed understanding of this ecological relationship.

2 MATERIAL AND METHODS

To understand the effects of altitude on Ephemeroptera, we conducted a scientometric analysis, which quantitatively examines scientific production (Leydesdorff, 2001; Mingers & Leydesdorff 2015). To identify published studies relating Ephemeroptera to altitude, we searched the Web of Science database (WoS - https://www.webofscience.com/). WoS is a bibliographic database that has indexed publications since 1900, thereby offering a substantially broader temporal coverage than other databases, such as Scopus, which only began indexing in 1966 (Falagas et al. 2008). The search was conducted between July 24, 2024, and January 15, 2025, using the terms "Ephemeroptera" and "Mayfl*" combined with variations of "watercourse" in Portuguese, English, and Spanish, along with the term "altitude." We applied the Boolean operator AND and used the "Topical" search type (Figure 1). The search deadline was December 2024.

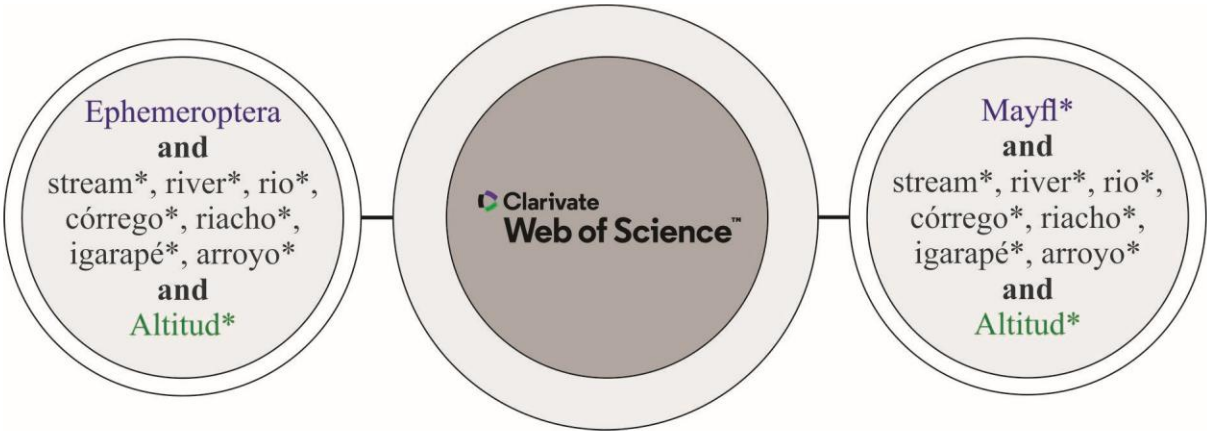

Figure 1. Flowchart detailing the search and selection criteria for scientific articles on the effects of altitude on Ephemeroptera in the Web of Science database.

2.1 CRITERIA FOR INCLUSION OR EXCLUSION OF ARTICLES

Each article was carefully examined based on its title, abstract, objectives, and results. For our analysis, we selected studies that met the following criteria: (1) included representatives of Ephemeroptera; (2) featured at least two collection sites with altitudinal variation between them; (3) focused on a stream as the study area; and (4) were scientific articles, excluding books, book chapters, and other non-article publications.

2.2 DATA EXTRACTED FROM ARTICLES

To achieve our objectives, we extracted the following information from the selected articles: (i) year of publication; (ii) journal of publication; (iii) country and biogeographical region of the study (determined based on the area described in the Methodology); (iv) study objectives, categorized as follows: diversity and composition *vs*. environmental factors (factors influencing community diversity and composition); relationships between altitude and community structure (effects of altitude on species composition and interactions within communities); geographical distribution and species composition (spatial analysis of species and regional comparisons); environmental changes and community responses (community responses to environmental changes such as pollution, climate change, and land-use modifications); (v) keywords; (vi) altitudinal variation of collection sites; (vii) taxonomic group analyzed (Ephemeroptera; EPT - Ephemeroptera, Plecoptera, and Trichoptera community; aquatic insects - Ephemeroptera and other aquatic insect orders; other groups - Ephemeroptera and additional non-insect taxa); (viii) taxonomic level analyzed (order, family, genus, or species); (ix)

Ephemeroptera life stage examined (nymph or imago); and (x) number of collection sites (categorized into the following intervals: 1-25, 26-50, 51-100, 101-500, >500).

2.3 DATA ANALYSIS

To analyze the frequency of keywords in the articles, we used the online tool WordClouds (https://www.wordclouds.com/). The tool generated a word cloud in which the size of each word corresponds to its frequency, ranging from 0 to 1 (from the lowest to the highest occurrence).

To assess the relationship between altitude and Ephemeroptera, we used two data sets: (1) EXT—composed of information extracted from the articles included in the scientometric analysis, which consisted of 1,230 streams for species richness, 253 streams for genus richness, and 335 streams for abundance; and (2) GRI—derived from the global study by Grigoropoulou et al. (2023), from which we extracted data that met the same selection criteria as the first set, resulting in 740 streams for species richness and 59,042 streams for genus richness.

Using these two data sets separately and combined, we applied Generalized Additive Models (GAM; *gam* function from the *mgcv* package) to examine the relationship between altitude and richness or abundance. In the models, we used a negative binomial distribution and included the article of origin of the data as a random factor. Additionally, we employed Moving Average Models (using the *mutate* function from the *dplyr* package) to identify trends and capture variations in Ephemeroptera richness and abundance along the altitudinal gradient. All analyses were conducted in RStudio (RStudio Team, 2020).

3 RESULTS

3.1 GENERAL INFORMATION

We identified 228 articles published in the Web of Science that investigated the relationship between Ephemeroptera and altitude. Of these, 154 were excluded based on the pre-established criteria, resulting in 74 articles for analysis (Appendix 1).

The first publication on this subject dates back to 1948. Between 1948 and 1999, 12 articles were published, while from 2000 to 2024, there was a significant increase in the number of studies, with 62 articles published (Figure 2). During this period, the year with the highest number of publications was 2008 (n = 7), followed by 2015 (n = 6). From 2010 onwards, there was a slight increase in the annual average number of publications, with 2.85 articles per year, compared to the period from 2000 to 2009, when the average was 2.4 articles per year.

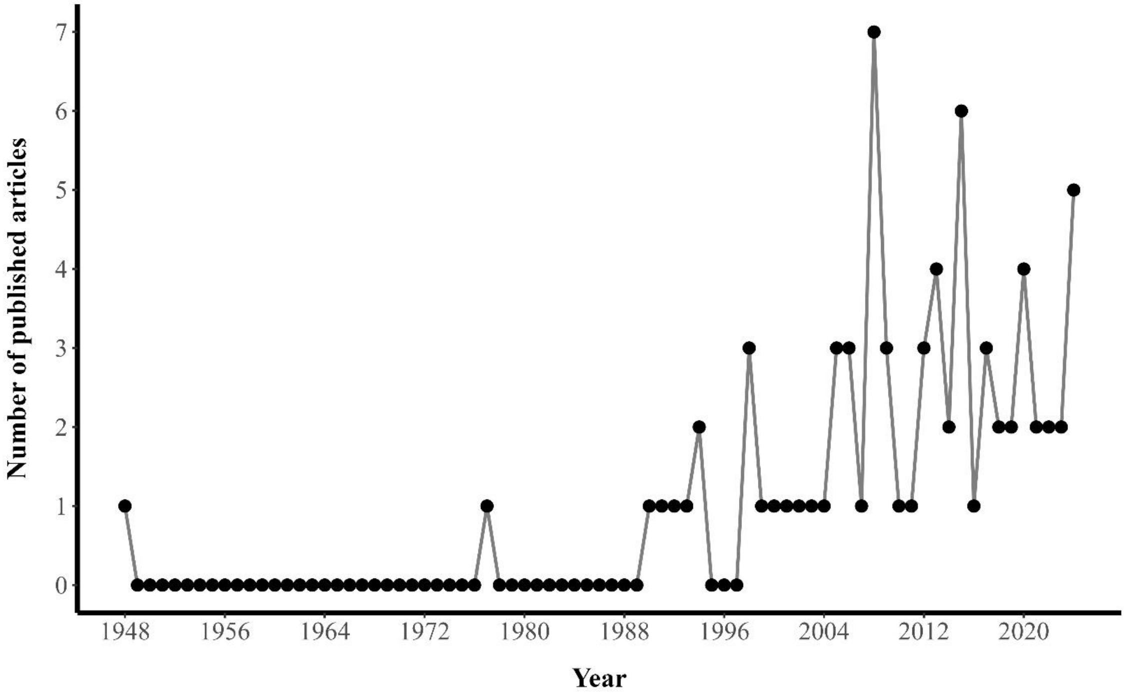

Figure 2. Variation in the number of articles published during the analyzed period (1948–2024).

A total of 44 scientific journals published articles on the subject. Of these, only 13 journals published more than one article, while the remaining 31 journals contributed a single publication each. The journals with the highest number of publications addressing the relationship between altitude and Ephemeroptera were *Freshwater Biology* (n = 11) and *Hydrobiologia* (n = 5) (Figure 3).

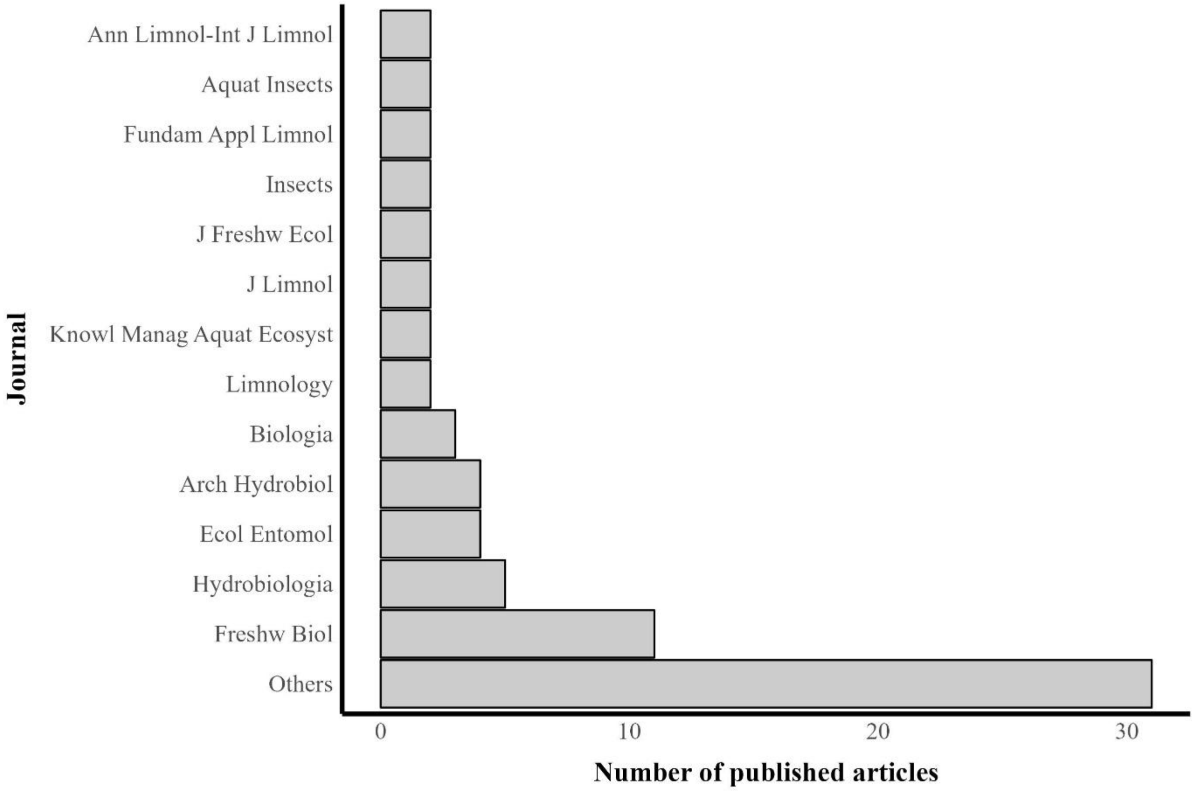

Figure 3. Variation in the number of articles published in scientific journals during the analyzed period (1948–2024).

3.2 LOCATION AND BIOGEOGRAPHICAL CONTEXT

The majority of studies were conducted in countries within the Palearctic (n = 30; 40.5%; Figure 4a) and Neotropical (n = 22; 29.7%) regions. In the Palearctic, the most frequently studied countries were Algeria (n = 6; Figure 4b) and Slovakia (n = 4), while in the Neotropical region, the most commonly studied countries were Ecuador (n = 8), Argentina (n = 8), and Peru (n = 4). The Nearctic (n = 3; 4%) and Afrotropical (n = 2; 2.7%) regions had the fewest published articles, with studies from the United States (n = 3), Uganda (n = 1), and Kenya (n = 1). No studies were found in the Oceanic or Antarctic regions.

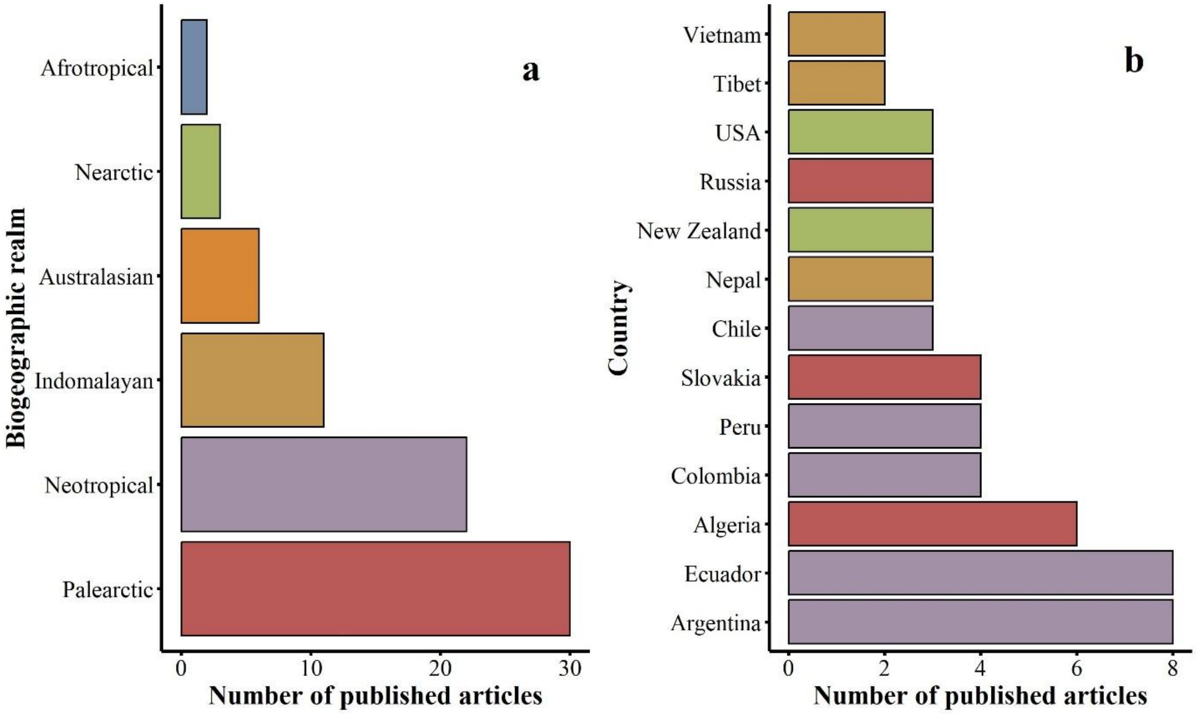

Figure 4. Geographical distribution of articles published by region and country during the analyzed period (1948–2024).

3.3 STUDY APPROACH

In the studies analyzed, the most frequently used keyword was "Stream" (28 occurrences), followed by "Invertebrates" (21 occurrences), "Ephemeroptera" (18 occurrences), "Impacts" (17 occurrences), "Biodiversity" (16 occurrences), and "Altitude" (13 occurrences) (Figure 5).

Figure 5. Word cloud depicting the frequency of keywords in articles published during the analyzed period (1948–2024).

Among the objectives, the majority of studies aimed to analyze the influence of various environmental factors on the diversity and composition of organisms (n = 35) (Figure 6). The next most common objectives were to examine the relationship between altitude and community structure (n = 14) and to investigate the geographical distribution and composition of species (n = 14). Finally, the responses of communities to environmental changes were the focus of 11 studies.

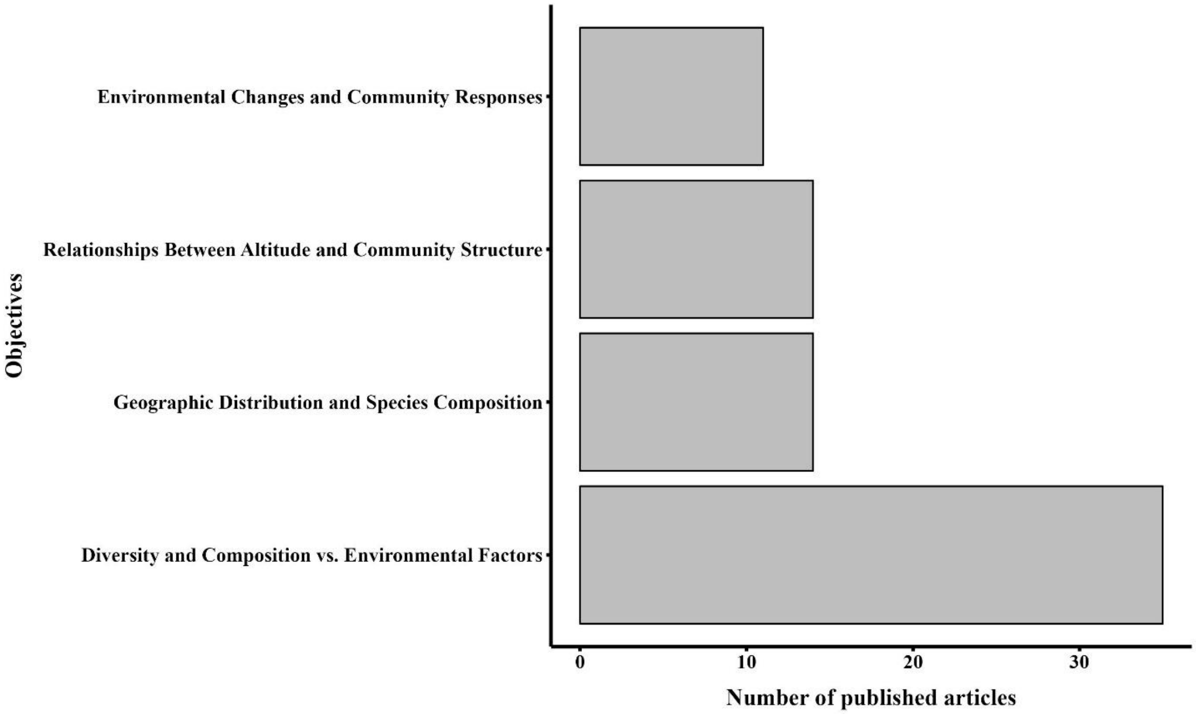

Figure 6. Distribution of the objectives addressed in articles published during the analyzed period (1948–2024).

3.4 TAXONOMY AND BIOLOGICAL ASPECTS

Half of the articles analyzed addressed Ephemeroptera in conjunction with macroinvertebrates and other animal groups, such as birds and fish (n = 37; Figure 7a). Among the studies that considered other orders of aquatic insects (n = 15), seven focused on the EPT group, which includes Plecoptera and Trichoptera, while the remaining eight examined other insect orders. In total, only 22 of the 74 articles analyzed focused exclusively on Ephemeroptera.

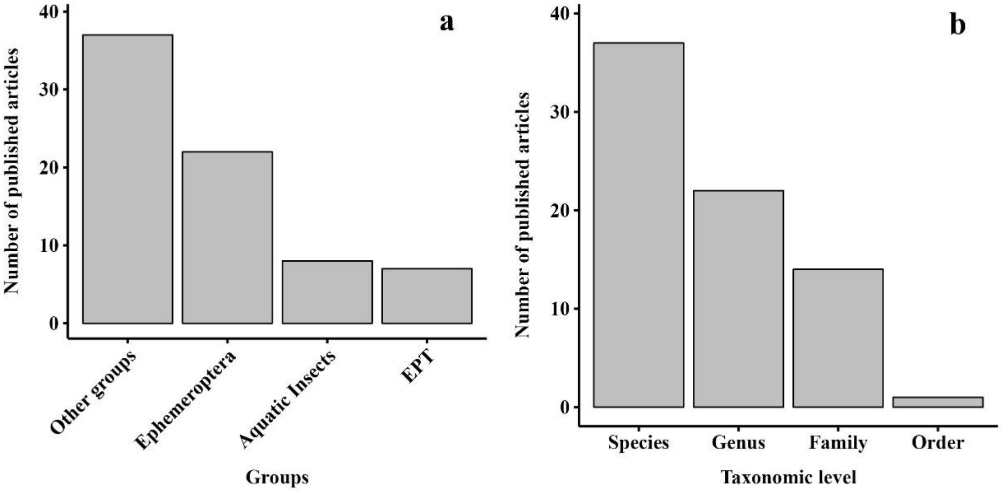

Figure 7. Taxonomic groups and levels studied in the articles published during the analyzed period (1948–2024).

Most studies focused on more specific taxonomic levels, such as species (n = 37) and genus (n = 22; Figure 7b). In contrast, broader taxonomic levels, such as family (n = 14) and order (n = 1), were addressed less frequently. The nymph stage was studied in all the articles (Figure 8), while the imago stage was not examined in isolation but only in conjunction with the nymph stage (n = 3).

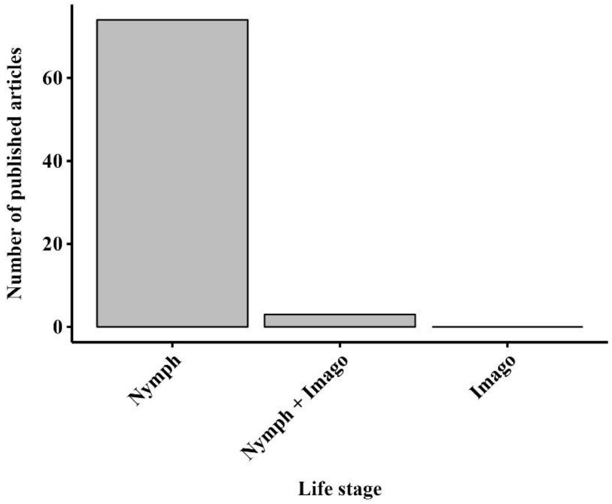

Figure 8. Life cycle stages of Ephemeroptera covered in the articles published during the analyzed period (1948–2024).

3.5 SAMPLING DATA

Most of the studies analyzed used a maximum of 50 collection points (n = 57; Figure 9), while only 17 studies included more than 50 points. Among these, two studies exceeded 500 collection points, with 2,647 and 2,206 points, respectively. The altitudes of the sampled sites ranged from 0 to 5,062 meters, with an average altitude of 731 meters at the lowest sites and 1,972 meters at the highest.

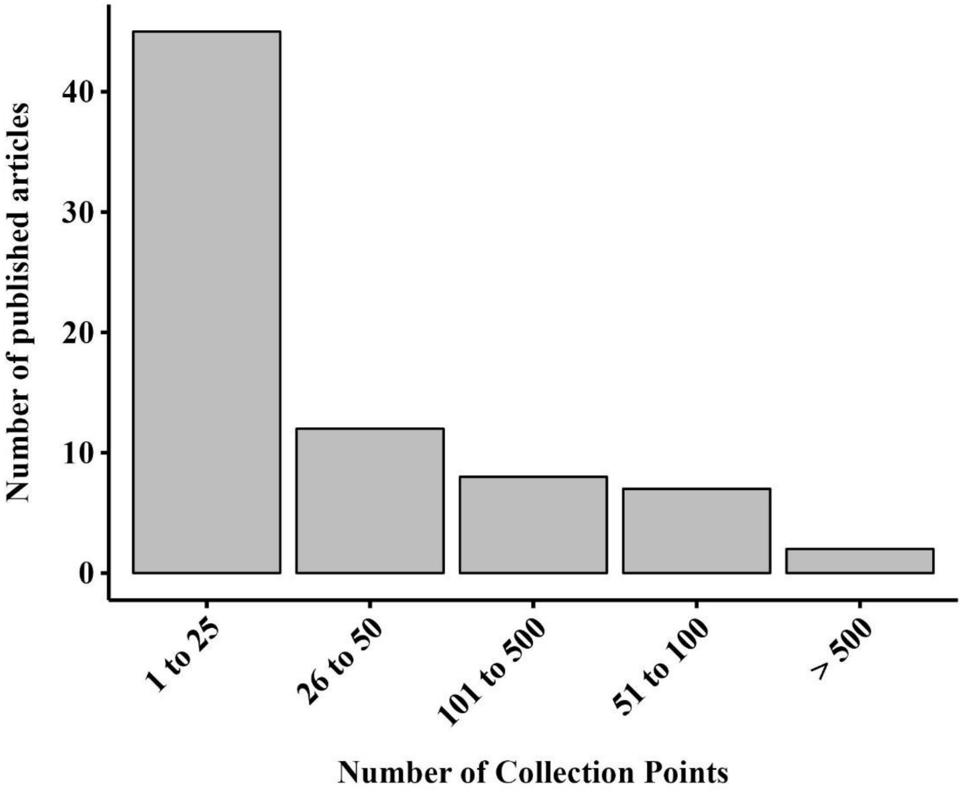

Figure 9. Variation in the number of collection points in articles published during the analyzed period (1948–2024).

3.6 ALTITUDINAL VARIATION AND ASSEMBLAGE STRUCTURE

The individual analysis of each article revealed varying patterns. Most studies found a negative relationship between altitude and species richness (51.7%), genus richness (51.9%), and abundance (56.3%; Figure 10). For species richness, the least frequent patterns were null (13.8%) and positive (13.8%), while for genus richness, the least common pattern was positive (11.1%). Regarding abundance, no studies reported a null relationship.

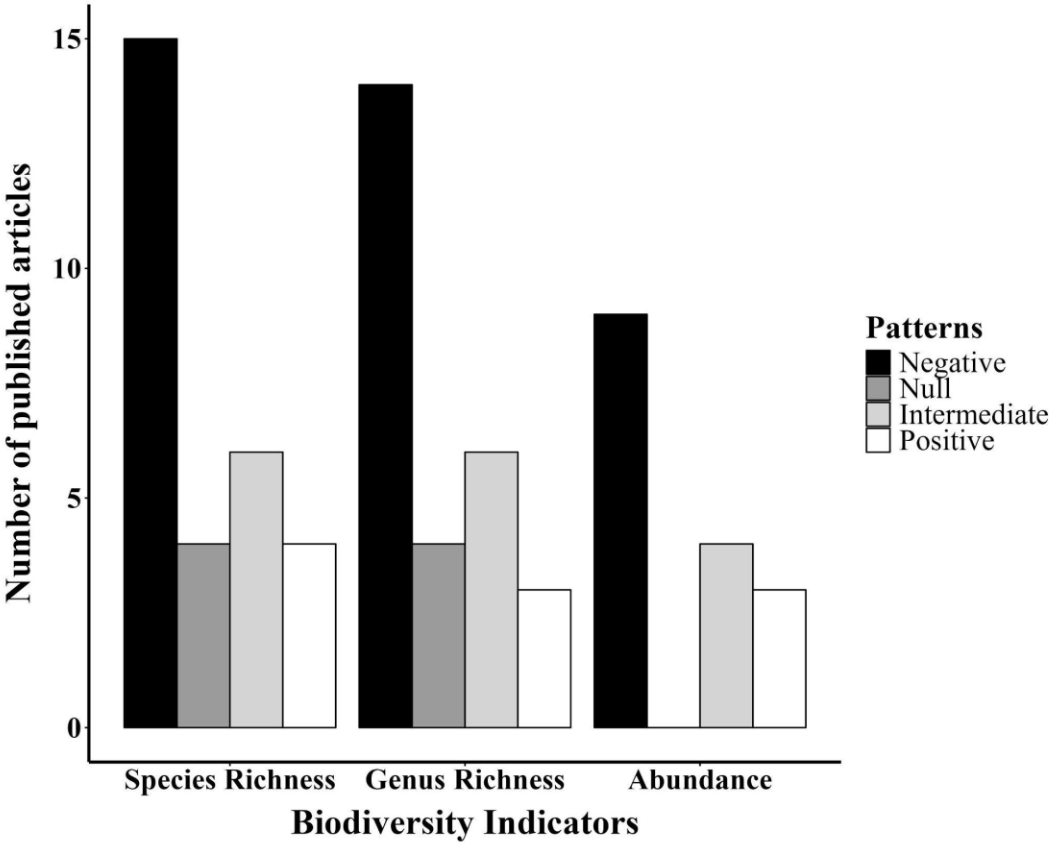

Figure 10. Relationship between the number of articles published and the biodiversity indicators assessed during the analyzed period (1948–2024).

Using two Ephemeroptera databases, we analyzed abundance and taxonomic richness data along an altitudinal gradient ranging from 0 to 5,062 meters. In the dataset extracted from the articles used in scientometrics (EXT), species richness ranged from 0 to 38, genus richness ranged from 0 to 14, and abundance ranged from 0 to 3,661 individuals. In the dataset from Grigoropoulou et al. (2023; GRI), species richness ranged from 0 to 20, while genus richness ranged from 0 to 25.

In both datasets, altitude had a significant, non-linear effect on Ephemeroptera species richness (Appendix 2). The different Generalized Additive Models (GAM) explained between 10.9% (EXT; Fig. 11a) and 19.7% (GRI; Fig. 11b) of the variation in species richness along the altitudinal gradient. The GAM models accounted for between 15% (EXT) and 20.2% (GRI) of the variability in the data. Analysis using moving average models revealed that, for the EXT and EXT + GRI datasets, the highest average species richness values were recorded between 2,500 and 3,000 meters (Fig. 11d; Fig. 11f), while altitudes above 3,000 meters exhibited the lowest values. In the GRI dataset, which had an altitudinal range from 0 to 1,751 meters, the highest average species richness was observed at altitudes up to 1,000 meters (Fig. 11e).

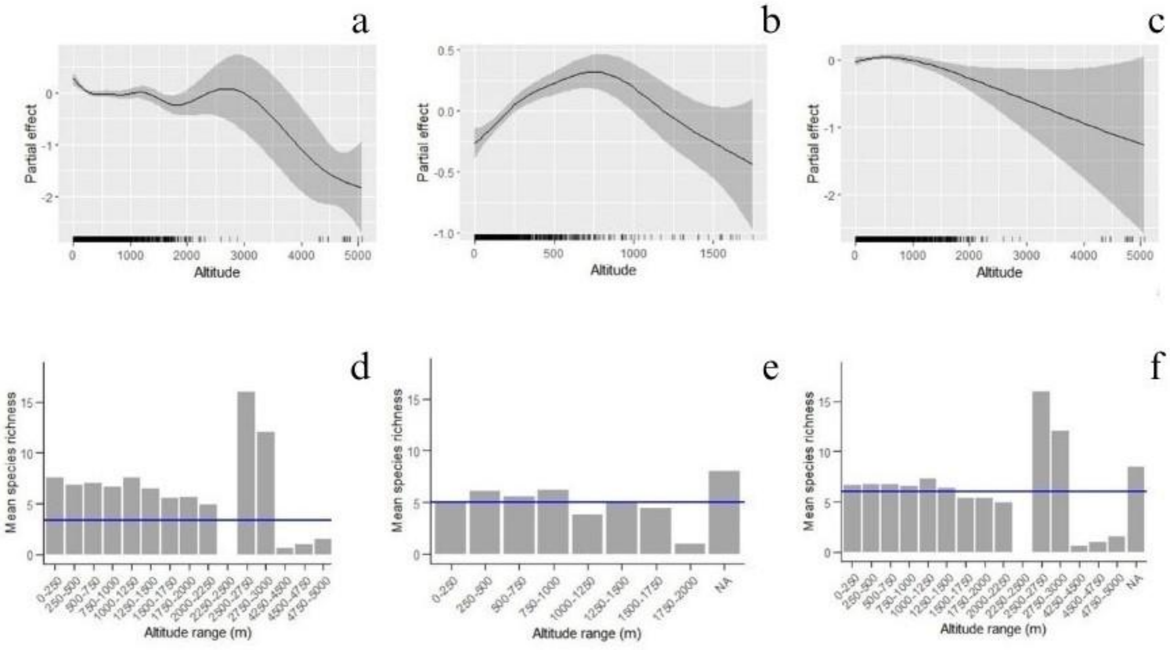

Figure 11. Relationship between species richness and altitude based on Generalized Additive Models (GAM).

In all the databases used, altitude had a significant, non-linear effect on the richness of Ephemeroptera genera (Appendix 2). The different Generalized Additive Models (GAM) explained between 9.6% (EXT; Figure. 12a) and 18.8% (GRI; Figure. 12b) of the variation in genus richness along the altitudinal gradient. The GAM models accounted for between 12.5% (EXT) and 20.4% (GRI) of the variability in the data. Analysis using moving average models revealed that, for all datasets, the two highest peaks in average genus richness occurred between 2,500 and 3,000 meters and between 1,250 and 1,500 meters (Figure. 12d-f), while altitudes above 3,750 meters showed lower values.

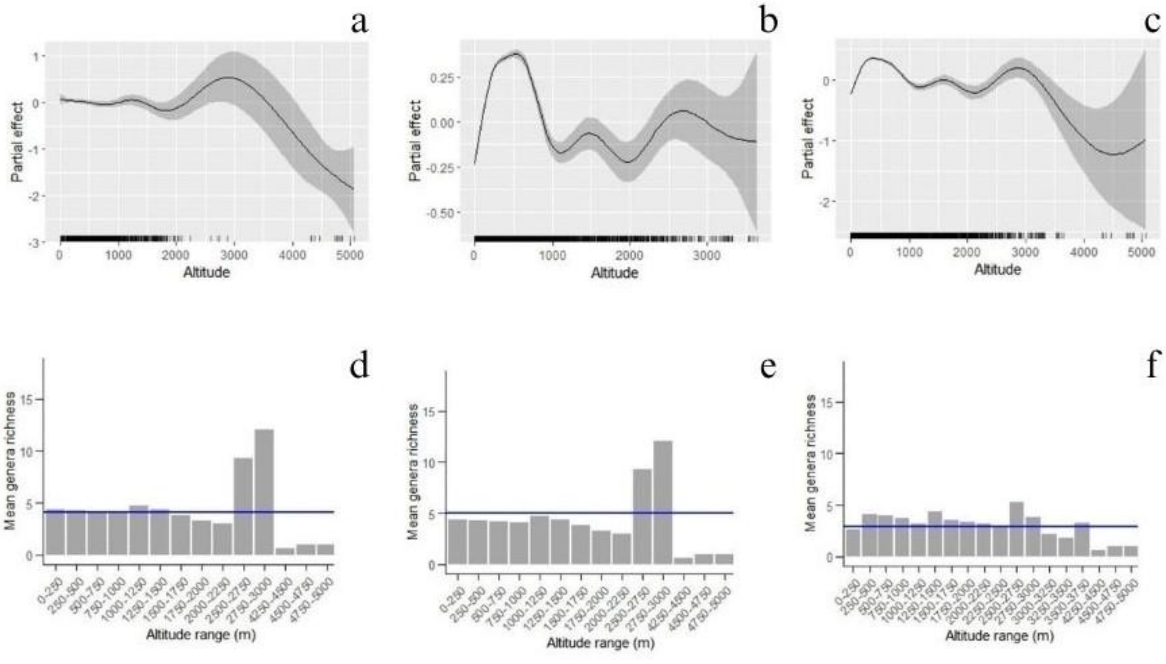

Figure 12. Relationship between altitude and Ephemeroptera genus richness based on Generalized Additive Models (GAM).

Altitude had a significant, non-linear effect on Ephemeroptera abundance (Appendix 2). The Generalized Additive Model (GAM) explained 38.7% (Figure. 13a) of the variation in Ephemeroptera abundance along the altitudinal gradient and accounted for 53.6% of the variability in the data. Analysis using the moving average model revealed that the highest average abundance was recorded between 0 and 250 meters, with a peak between 2,250 and 2,750 meters, while altitudes above 4,000 meters exhibited lower values (Figure. 13b).

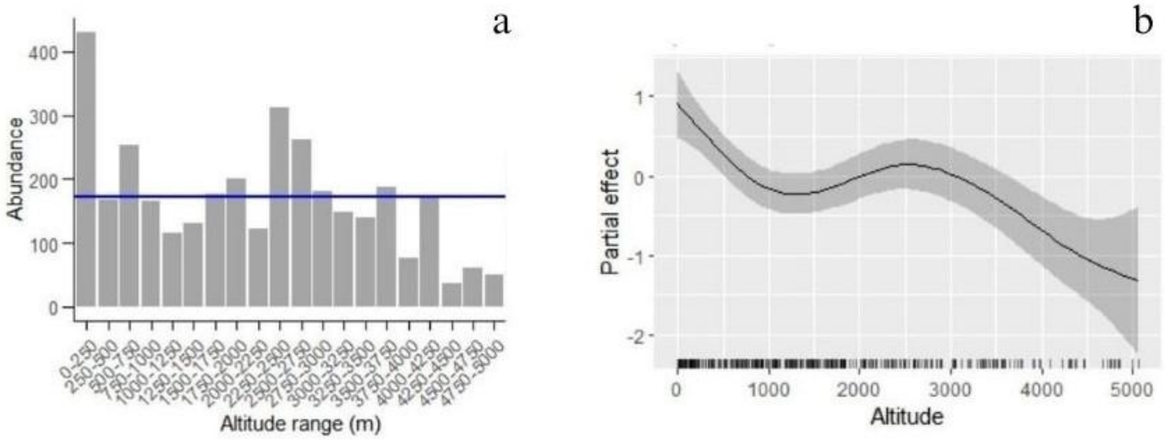

Figure 13. Relationship between altitude and Ephemeroptera abundance based on Generalized Additive Models (GAM).

4 DISCUSSION

4.1 GENERAL INFORMATION

Since the 2000s, there has been a significant increase in the number of publications examining the relationship between Ephemeroptera and altitude. This growth reflects a heightened interest in the distribution of this order across different altitudinal gradients and in the mechanisms that influence its structuring. The scarcity of publications prior to 1999 may be attributed not only to a lack of resources but also to the predominant focus on other environmental variables, such as water quality and land use, which were central themes in aquatic ecology throughout much of the 20th century (De Mendoza & Catalan 2010). The gradual increase in the annual average of publications post-2010 also coincides with a growing awareness of the vulnerability of mountain streams to environmental pressures and global climate change, as highlighted by Jacobsen (2008) and Lewin et al. (2015).

The increasing number of publications highlights the consolidation of this topic across various scientific journals, particularly *Freshwater Biology* and *Hydrobiologia*, which account for the largest share of publications. Both journals are recognized as leading references in the ecology of aquatic ecosystems, covering not only studies on Ephemeroptera but also on other taxonomic groups such as macroinvertebrates, fish, bryophytes, and diatoms (Dominguez & Ballesteros Valdez, 1992; Ormerod et al., 1994; De Castro et al., 2017). In addition, these journals publish research spanning various aspects of aquatic biology, including ecology, genetics, and physiology (Sylvestre & Bailey, 2005; Rostgaard & Jacobsen, 2005; Loayza-Muro et al., 2013). This thematic diversity reflects their capacity to address both specific topics related to the ecology of Ephemeroptera (e.g. Espinosa et al., 2023) and broader themes concerning aquatic ecosystems.

In contrast, journals with a more specialized focus, such as *ZooKeys* and *Austral Entomology*, contain fewer publications on this subject. This can be attributed to their narrower scope, which often restricts their focus to specific geographical regions or less interdisciplinary topics. This distinction underscores the central role of generalist journals in fostering broad, integrative studies on the relationship between altitude and aquatic biodiversity.

4.2 LOCATION AND BIOGEOGRAPHICAL CONTEXT

Publications on Ephemeroptera have been predominantly concentrated in the Neotropical and Palearctic regions, a pattern that can be attributed to the vast geographical extent of these areas, their rich biodiversity, and the availability of mountainous aquatic environments. The Palearctic region, encompassing all of Europe, North Africa, and parts of Asia and the Middle East, has a longstanding tradition of ecological research and substantial investment in scientific studies, particularly in European countries. Similarly, the Neotropical region, which includes all of South and Central America and part of North America (Olson et al., 2001), is notable for hosting major mountain ranges, such as the Andes, which provide ideal conditions for biodiversity studies along altitudinal gradients (Vetaas, 2021).

In contrast, regions such as Australasia and Sub-Saharan Africa are less represented, possibly due to logistical constraints, under-sampling, or limited research funding. This geographical disparity underscores the need to expand research efforts into understudied areas, such as Tropical Africa and parts of East Asia, where Ephemeroptera diversity remains poorly documented (Bauernfeind & Soldan, 2012). In these regions, research often focuses on more homogeneous, low-altitude environments, emphasizing environmental variables other than altitude due to the absence of significant mountain ranges (Jacobsen & Dangles, 2017; The World Factbook, 2024).

4.3 STUDY APPROACH

The analysis of keywords revealed key insights into the predominant themes and approaches of the studies examined. The high frequency of the terms *Stream* and *Invertebrates* reflects the research context, indicating that most studies were conducted in stream environments with a primary focus on aquatic invertebrates (Oliveira-Junior et al., 2022). Similarly, the prevalence of the terms *Biodiversity* and *Impacts* as keywords suggests that many studies aimed to assess the effects of various environmental factors on stream biodiversity (Wang et al., 2023). Aquatic invertebrates are widely used as bioindicators of water quality and ecosystem health, with Ephemeroptera being particularly common bioindicators in studies on lotic environments (Restello et al., 2024). The frequent occurrence of the terms *Ephemeroptera* and *Altitude* directly reflects the scope of this study, which seeks to understand how altitude influences the distribution and diversity of this order. Other studies investigating the relationship between environmental variables and biological groups have also identified keywords directly related to their research focus as among the most frequent (Wang et al., 2023). This underscores the importance of carefully selecting keywords when indexing scientific articles, as it facilitates information retrieval in databases and enhances the visibility of research.

The objectives of the analyzed studies align closely with the trends observed in the most frequent keywords. Most studies focused on assessing the influence of environmental factors on the structuring of aquatic invertebrate communities. This approach is consistent with existing literature, which highlights the role of both internal factors (e.g., physical and chemical characteristics of streams) and external factors (e.g., land use and climate change) in shaping these communities (De Castro et al., 2017; Min & Kong 2020; Brasil et al., 2020; Fathi et al., 2022). Among the various environmental factors examined, altitude stands out due to its indirect influence on a range of ecological variables at local and regional scales (Körner, 2007; Jacobsen, 2008). For instance, altitudinal variation affects temperature, water oxygenation, nutrient availability, and water flow, all of which play a crucial role in determining the composition and diversity of aquatic communities (Calapez et al., 2017; Bonacina et al., 2023). Furthermore, the presence of studies investigating how these communities respond to environmental changes underscores the importance of understanding anthropogenic impacts on aquatic ecosystems. This topic has gained increasing attention in recent decades, particularly in light of growing human pressures such as deforestation, pollution, and alterations to water regimes (Jaureguiberry et al., 2022).

4.4 TAXONOMY AND BIOLOGICAL ASPECTS

Although the analyzed articles focus on the order Ephemeroptera, most also examine the group alongside other organisms, particularly benthic macroinvertebrates. This integrated approach is widely employed in ecological studies due to the characteristics of macroinvertebrates, such as the simplicity of sampling equipment, ease of sample processing, and the low mobility of these organisms (Kenney et al., 2009). Consequently, macroinvertebrates are frequently used to assess water quality, as they respond rapidly to environmental changes and serve as reliable bioindicators (Ruaro et al., 2016; Orozco-González & Ocasio-Torres, 2023). In the case of Ephemeroptera, nymphs are often studied in association with representatives of the orders Plecoptera and Trichoptera, forming the group known as EPT, which is widely used in biomonitoring studies due to its sensitivity to environmental changes (Souza et al., 2024). In most of the studies analyzed, organisms were identified at the genus or species level, reflecting advances in the development of identification keys for Ephemeroptera across different regions (e.g. Edmunds, Jr et al., 1963, 1976; Macan, 1979; Elliot et al., 1988; Domínguez et al., 2006; Bauernfeind & Soldan, 2012; Salles et al., 2018). These tools have facilitated more precise identification, contributing to the increasing quantity and refinement of research. However, in regions where knowledge of the order remains limited, such as Australasia, the Indomalayan realm, and certain Neotropical countries, studies are often restricted to broader taxonomic levels, such as family or order (Barber-James et al., 2008; Sartori & Brittain, 2015). This variation in taxonomic resolution can significantly influence results, as it affects the accuracy of ecological analyses (Valente-Neto et al., 2016). Additionally, the higher number of studies identifying organisms at finer taxonomic levels may reflect a bias introduced by the keywords used in our search, given that distinguishing macroinvertebrates at the genus or species level requires advanced technical expertise (Hauer & Resh 2017). Furthermore, the identification of Ephemeroptera individuals can be hindered by the loss of key morphological structures during collection, particularly in environments with adverse conditions or when inappropriate sampling methods are used (Salles et al., 2018; Brito et al., 2018).

Most of the analyzed articles focused on the nymph stage, while relatively few examined the adult stage. This imbalance is partly due to the difficulty of locating adult Ephemeroptera individuals, as well as the limited taxonomic knowledge of certain genera and species at this stage, leading to a preference for nymphs in taxonomic and biological studies (Burian, 2019). Nymphs play essential ecological roles in aquatic ecosystems and represent the dominant stage of the life cycle (Jacobus et al., 2019; Da-Silva & Salles, 2024). In contrast, adults are short-lived and primarily serve a reproductive function (Da-Silva & Salles, 2024). Dias et al. (2011) highlight the frequent lack of association between nymph and adult stages in taxonomic descriptions, which are often based on only one of these life stages. This study supports the findings of Shimano et al. (2013), who also identified a significant number of articles focusing exclusively on the nymph stage.

4.5 SAMPLING DATA

Although most studies analyzed fewer than 50 collection sites, the predominance of studies with smaller sample areas may be attributed to financial and time constraints, which are key factors in determining sample sizes in biological research (Eckblad 1991). Additionally, there may be a tendency to conduct studies on a smaller spatial scale, focusing on local rather than regional patterns that encompass broader geographical areas. The choice of study scale can directly influence the observed results, a phenomenon known as *scale dependence* (Hobbs 2003; Mod et al. 2020).

The studies analyzed exhibited a broad altitudinal range (0–5,062 m), with mean values ranging from 731 to 1,972 m and a median of 1,392 m. This variation can be attributed to the heterogeneity of the altitudinal gradients examined across different studies. For example, Dos Santos et al. (2018) evaluated extensive altitudinal gradients (1–4,320 m), while Odhiambo et al. (2024) focused on more restricted altitudinal ranges (1,786–2,149 m). A notable proportion of the articles analyzed were conducted in environments at altitudes above 3,000 m (20.2%), classified as “high altitude” (Jacobsen & Dangles, 2017). This altitudinal range is commonly studied in mountain aquatic environments due to its extreme conditions and its relevance as a model for investigating the impacts of climate change and anthropogenic activities (Füreder et al., 2006). However, the majority of studies were conducted at intermediate (1,500–3,000 m) or low (1–1,500 m) altitudes, likely due to the logistical challenges of working in high-altitude regions. Researchers collecting data in these areas often face obstacles such as respiratory and physiological issues, difficulties in transporting equipment, and unpredictable weather conditions (Rivera-Ch et al., 2008). This uneven distribution of studies along the altitudinal gradient underscores the need to expand research into underexplored regions, particularly those between

3,000 and 4,500 m, where there are fewer sampling points. Additionally, few studies have conducted long-term sampling (Scheibler et al., 2014; Fathi et al., 2022). Future research should consider not only the extent of the altitudinal gradient but also the temporal resolution of sampling in order to capture more comprehensive and representative ecological patterns.

4.6 ALTITUDINAL VARIATION AND ASSEMBLAGE STRUCTURE

Analysis of the patterns observed in the studies revealed varying relationships between altitude and the Ephemeroptera metrics tested. The majority of studies (51.7%) indicated a negative correlation between altitude and species richness, genus richness (51.9%), and abundance (56.3%), suggesting that higher-altitude environments tend to have lower diversity and fewer individuals of these organisms. This trend can be explained by the more extreme environmental conditions at higher altitudes, such as lower temperatures, reduced food availability, and limited land area (Birrell et al., 2020). However, less frequent patterns—such as null, intermediate, or positive relationships—were also observed, highlighting the complexity of interactions between altitude and biodiversity. These divergent results can be attributed to local variations in altitudinal gradients, such as specific microclimates or differences in habitat structure (Chiu et al., 2020). Moreover, factors like environmental heterogeneity and local adaptation may enable some species to thrive at high altitudes, challenging the general expectation of decreasing biodiversity with increasing altitude (Jacobsen, 2003).

The considerable variation in the patterns observed between altitude and Ephemeroptera in the studies evaluated underscores the importance of employing an integrated approach to data analysis. By using Generalized Additive Models (GAM) and moving average models, more robust and consistent trends were identified. The results revealed that altitude significantly affects the taxonomic richness and abundance of Ephemeroptera. For abundance (EXT) and species and genus richness data from the GRI database, the highest values were recorded at lower altitudes. These streams typically have higher temperatures (Jacobsen, 2020), which lead to increased primary productivity and higher decomposition rates (Raich et al., 1997; Dodds et al., 2019). These factors contribute to a greater availability of food resources, such as detritus and periphyton, which are essential for the predominantly herbivorous Ephemeroptera nymphs (Sartori & Brittain, 2015). Consequently, warmer environments with a higher supply of organic matter support greater species diversity. In contrast, at higher altitudes, environmental conditions become more extreme, limiting the presence of only specialist organisms that are adapted to such conditions (Jacobsen et al., 2003).

When we conducted the analyses using the EXT databases and the combined EXT+GRI databases, the average species and genus richness was found to be higher at intermediate altitudes (2,500–3,000 m). At these intermediate altitudes, environmental disturbances, such as variations in temperature and nutrient levels, occur at moderate intensities, creating conditions conducive to the coexistence of a greater diversity of genera (Connell, 1978; Siegloch et al., 2008; Souza et al., 2011). This pattern aligns with the Intermediate Disturbance Hypothesis, which posits that moderate levels of environmental disturbance can enhance biological diversity by reducing interspecific competition without completely eliminating less competitive species. Furthermore, the observed pattern can also be partially explained by the mid-domain effect, a biogeographic concept that suggests overlapping distribution ranges of species with different tolerance ranges are more likely to occur in the intermediate regions of an altitudinal gradient (Colwell & Lees, 2000; Colwell et al., 2004). Some Ephemeroptera species have the ability to occupy a wide range of altitudes (Edmunds, Jr. et al., 1976; Domínguez et al., 2006; Bauernfeind & Soldan, 2012). This altitudinal plasticity can be attributed to the adaptation of these species to varying environmental conditions, allowing them to thrive in both low- and high-altitude environments.

Although altitude showed statistical significance, the relatively low deviance explained by the GAM models suggests that other environmental factors also play a crucial role in the observed variation in the distribution of Ephemeroptera. In addition to altitude, variables such as pH, water temperature, flow velocity, dissolved oxygen, and light availability are key determinants in the distribution of aquatic organisms (Vannote et al., 1980; Beschta, 1997; Tadesse et al., 2004; Kalny et al., 2017; Garner et al., 2017; Hamid et al., 2020). These factors often interact with each other, creating specific conditions that can either favor or limit the presence of species at different altitudinal ranges. For instance, water temperature, which decreases with increasing altitude, directly affects the metabolic rate of organisms and the availability of food resources, such as detritus and periphyton (Bonacina et al., 2023). Similarly, dissolved oxygen, which tends to increase in higher-altitude environments due to lower temperatures, can be a critical factor for species adapted to these conditions (Calapez et al., 2017). Flow velocity and pH also influence the structure of aquatic communities, affecting processes such as larval dispersal and habitat colonization (Calapez et al., 2017). Therefore, while altitude remains an important factor, its influence should be considered in conjunction with other environmental variables to provide a more comprehensive understanding of the mechanisms that regulate the distribution of Ephemeroptera along altitudinal gradients.

5 CONCLUSIONS

This study offers a comprehensive analysis of the relationship between altitude and Ephemeroptera diversity, revealing important patterns while also identifying significant gaps in current knowledge. The underrepresentation of certain biogeographical regions, such as the Oceanic and Afrotropical zones, and the absence of Ephemeroptera in Antarctica, point to the need for further exploration in these neglected areas. Additionally, the predominant focus on the nymph stage and the limited number of streams sampled underscore the importance of broadening both the geographical scope and the ecological contexts in future research. Expanding the range of altitudinal gradients, examining other life stages, and incorporating a wider variety of habitats would provide a more holistic understanding of the factors shaping Ephemeroptera distribution and biodiversity.

When analyzed separately, most studies indicated a negative relationship between altitude and measures of richness (species and genus) and abundance, suggesting that higher altitude environments tend to have lower diversity. However, the GAM and moving average models revealed divergent patterns, with peaks in richness and abundance observed at both low and intermediate altitudes. These findings reinforce the complexity of the interactions between environmental factors and Ephemeroptera assemblages, highlighting the need for a more nuanced understanding of how altitude, along with other ecological variables, influences the distribution and diversity of these organisms.

These results emphasize the importance of considering a broader range of environmental variables (e.g., pH, temperature, water velocity, and dissolved oxygen) to gain a more comprehensive understanding of the effects of altitudinal gradients on Ephemeroptera communities. By accounting for these additional factors, we can better elucidate the complex interactions that shape the distribution and diversity of Ephemeroptera along altitudinal gradients.

6. CONSIDERAÇÕES FINAIS

O presente capítulo apresentou uma análise abrangente da relação entre a diversidade de insetos da ordem Ephemeroptera e a variável ambiental “altitude”, identificando padrões recorrentes nos estudos. A baixa representatividade de regiões biogeográficas como Oceania e Afrotropical evidencia a necessidade de expandir os esforços de pesquisa para essas áreas ainda pouco exploradas. Além disso, a predominância de estudos focados no estágio ninfal e o número limitado de pontos de amostragem reforçam a importância de ampliar a cobertura geográfica e ecológica. A maioria dos trabalhos analisados indicou uma relação negativa entre altitude e riqueza ou abundância de Ephemeroptera, sugerindo menor diversidade em altitudes elevadas. No entanto, análises complementares com modelos GAM e médias móveis revelaram picos de riqueza e abundância em altitudes baixas e intermediárias. Dessa forma, este trabalho contribui para o avanço da ecologia de comunidades aquáticas ao sintetizar padrões ecológicos e lacunas na literatura, oferecendo subsídios para futuras pesquisas que visem uma compreensão mais ampla dos fatores que moldam a distribuição e a diversidade de Ephemeroptera em ambientes de diferentes altitudes.

STATEMENTS AND DECLARATIONS

Conflicts of interest The authors declare that they have no conflicts of interest relevant to the content of this article.

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CHAPTER 2

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Ephemeroptera Diversity in Neotropical Streams: Do Immatures and Adults Reflect the Same Patterns?

RESUMO

Atividades antrópicas têm gerado impactos significativos nos ecossistemas de água doce, tornando o biomonitoramento uma ferramenta fundamental. No entanto, sua aplicação enfrenta limitações. Como alternativa, utilizam-se substitutos, indicadores que representam atributos ecológicos difíceis de mensurar. Em ambientes lóticos, Ephemeroptera destacam-se como bioindicadores. Apesar do uso de ninfas, sua identificação é dificultada pela perda de estruturas frágeis, enquanto imagos, com morfologia mais preservada, apresentam potencial como substitutos. Nesse contexto, este estudo caracterizou a fauna de Ephemeroptera em áreas de Mata Atlântica e avaliou a congruência taxonômica entre ninfas e imagos, bem como sua relação com variáveis ambientais. Foram amostrados 40 riachos de primeira a terceira ordem, nos quais foram medidas oito variáveis ambientais. Ninfas foram coletadas com rede entomológica de malha 500 µm, e imagos, por armadilhas Malaise e Pensilvânia. Os organismos foram identificados até o gênero e preservados em álcool 80%. A fauna foi caracterizada por riqueza e abundância, e a congruência entre estágios avaliada pelo Teste de Randomização de Procrustes, utilizando eixos da Análise de Coordenadas Principais (PCoA) com dissimilaridades de Bray-Curtis e Sørensen. Foram coletados 3.067 indivíduos. A riqueza taxonômica de ninfas foi semelhante entre os estados (SP: 7 famílias, 25 gêneros; MG: 6 famílias, 25 gêneros), enquanto a de imagos foi menor (SP: 5 famílias, 13 gêneros; MG: 4 famílias, 7 gêneros). A congruência foi significativa dentro dos estágios, mas não entre eles. As correlações com variáveis ambientais foram geralmente baixas, com algumas significativas. Diferenças ecológicas e funcionais entre estágios de vida e suas respostas ambientais podem explicar a baixa correlação entre eles e com o ambiente. Os resultados indicam que medidas de abundância e incidência são intercambiáveis, mas ninfas e imagos não são substituíveis entre si. Assim, abordagens integradas, combinando métricas e estágios de vida aumentam a precisão e eficácia dos estudos.

ABSTRACT

Anthropogenic activities have caused significant impacts on freshwater ecosystems, making biomonitoring an essential tool for their assessment. However, its implementation faces several challenges. As an alternative, surrogates, indicators representing ecological attributes that are difficult to measure, are often employed. In lotic environments, Ephemeroptera are widely recognized as effective bioindicators. Although nymphs are commonly used, their identification is often hindered by the loss of fragile morphological structures, whereas imagos, which possess more stable morphological features, show potential as surrogates. This study characterized the Ephemeroptera fauna in the Atlantic Forest and evaluated the taxonomic congruence between nymphs and imagos, as well as their relationships with environmental variables. Forty streams ranging from first to third order were sampled, with eight environmental variables measured at each site. Nymphs were collected using a 500 µm mesh entomological net, while imagos were captured using Malaise and Pennsylvania traps. Specimens were identified to the genus level and preserved in 80% ethanol. Faunal composition was characterized based on taxonomic richness and abundance, and congruence between life stages was assessed using the Procrustes Randomization Test on Principal Coordinates Analysis (PCoA) axes derived from Bray-Curtis and Sørensen dissimilarity matrices. A total of 3,067 individuals were collected. Taxonomic richness of nymphs was similar between states (SP: 7 families, 25 genera; MG: 6 families, 25 genera), whereas richness of imagos was lower (SP: 5 families, 13 genera; MG: 4 families, 7 genera). Significant congruence was observed within life stages, but not between them. Correlations with environmental variables were generally weak, although some reached statistical significance. Ecological and functional differences among life stages and their distinct environmental responses likely explain the low correlation between stages and with environmental factors. These findings suggest that abundance and incidence metrics are interchangeable within stages, but nymphs and imagos should not be treated as substitutes.

Therefore, integrated approaches combining multiple metrics and life stages enhance the precision and effectiveness of biomonitoring studies.

KEYWORDS

Aquatic Insects; Biomonitoring; Mayfly; Taxonomic Congruence; Freshwater Ecosystems.

1 INTRODUCTION

Increasing environmental pressures resulting from anthropogenic activities, such as pollution, habitat degradation, and climate change, have significantly impacted lotic ecosystems at both global and local scales (Jaureguiberry et al. 2022). These pressures have led to marked declines in species diversity and abundance across various habitats in recent decades (Sánchez-Bayo and Wyckhuys 2019; Almond et al. 2020). In this context, biomonitoring has emerged as a crucial tool for diagnosing and mitigating environmental impacts (Chowdhury et al. 2023). However, the high variability in the composition and distribution of aquatic communities, coupled with the need for detailed taxonomic identification, makes these programs resource-intensive, requiring substantial time and effort (Heino 2010). In megadiverse countries, these challenges are further exacerbated by a shortage of taxonomic experts, limited financial resources, and declining investments in scientific research (Kauffmann-Zeh 1999; Gibney 2015; Petersen 2021).

A promising approach to addressing these challenges is the use of surrogates, indicators that represent ecological attributes that are difficult to measure directly (Andréfouët et al. 2012). These surrogates may include indicator taxa, higher taxonomic levels, or even environmental variables (Lovell et al. 2007). To be considered effective, such proxies must be cost-effective, logistically feasible, and ecologically reliable in capturing biological patterns (McGeogh 1998).

Freshwater ecosystems are among the most threatened globally and remain underexplored in terms of their potential for the use of surrogates in biodiversity conservation (Stewart et al. 2018; Brumm et al. 2021). Aquatic insects are essential components of these systems, playing key roles in processes such as nutrient cycling, organic matter decomposition, and the regulation of other organism populations (Balian et al. 2008; Suter and Cormier 2014). Among these insects, Ephemeroptera are particularly notable due to their wide distribution and high sensitivity to environmental changes in both lotic and lentic environments (Dijkstra et al. 2014; Jacobus et al. 2019). As such, they are widely employed as bioindicators in biomonitoring programs (Jacobus et al. 2019).

Despite their ecological importance, the use of Ephemeroptera nymphs in ecological studies is constrained by operational limitations, as their identification relies on delicate morphological structures, such as legs and gills, that are frequently damaged or lost during field collection and transport to the laboratory (Salles et al. 2018; Brito et al. 2018). Consequently, the involvement of taxonomic specialists and the use of specific collection techniques are often required, increasing both the cost and processing time before results can be obtained. In contrast, imagos (adult stage) can be more readily identified based on well-preserved structures such as wings and genitalia, making them potential substitutes for nymphs in biodiversity assessments and biomonitoring studies (Domínguez et al. 2006). This potential can be evaluated by examining taxonomic congruence between life stages, particularly through the correlation of richness, composition, and diversity patterns between nymphs and adults (Heino 2010; Gioria et al. 2011).

Studies investigating taxonomic surrogates in freshwater ecosystems have examined a range of biological groups, including macroinvertebrates, amphibians, fish, and macrophytes (Mendes et al. 2017; Carneiro et al. 2019; Martins et al. 2022). Research assessing taxonomic congruence between adult and immature stages within the same insect order, such as Odonata and Trichoptera, has reported low correlations (Valente-Neto et al. 2016a; Anacléto et al. 2023). In contrast, studies focusing on Ephemeroptera have demonstrated high congruence (up to 99%) between genera and morphospecies in nymphs collected in northern Brazil. Furthermore, when analyzed in the context of the broader macroinvertebrate community, Ephemeroptera exhibit strong congruence, underscoring their potential as effective surrogates in ecological studies (Brito et al. 2018; Martins et al. 2022; Valente-Neto et al. 2025).

In this context, the aim of this study was to conduct a comprehensive characterization of the Ephemeroptera fauna at both the nymph and imago stages, using measures of species richness, abundance, and community composition. Additionally, taxonomic congruence between the nymph and imago stages was evaluated at the genus level, along with an assessment of their relationships with environmental variables relevant to the group. Given the characteristics of the Ephemeroptera life cycle and the distinct habitats occupied by the two stages (aquatic for nymphs and terrestrial for imagos), it is likely that each stage is subjected to different environmental pressures. Consequently, community composition is expected to be shaped by distinct ecological filters, potentially resulting in low taxonomic congruence between life stages.

2 MATERIAL AND METHODS

2.1 STUDY AREA

In 2023 and 2024, we sampled 40 streams located within four Protected Areas (PA) in the Atlantic Forest biome: Carlos Botelho State Park (CBSP) and the Santa Virgínia Nucleus of Serra do Mar State Park (SVN) in the state of São Paulo (SP), and Caparaó National Park (CNP) and Serra do Papagaio State Park (SPSP) in the state of Minas Gerais (MG) (Figure X; Appendices 3 and 4). The Atlantic Forest is one of Brazil's six biomes and originally covered approximately 1.6 million hectares (Marques et al. 2021). It comprises a mosaic of vegetation types with high ecological heterogeneity and harbors a rich diversity of flora and fauna, including many endemic species (Mittermeier et al. 2011; Vitória et al. 2019). Today, only 11.6% of its original cover remains, mostly in the form of isolated forest fragments, due to pressures such as deforestation, land-use change, climate change, and the introduction of exotic species (Marques et al. 2021). In this context, protected areas play a strategic role in preserving the remaining biodiversity and fostering sustainable development (Stolton 2010).

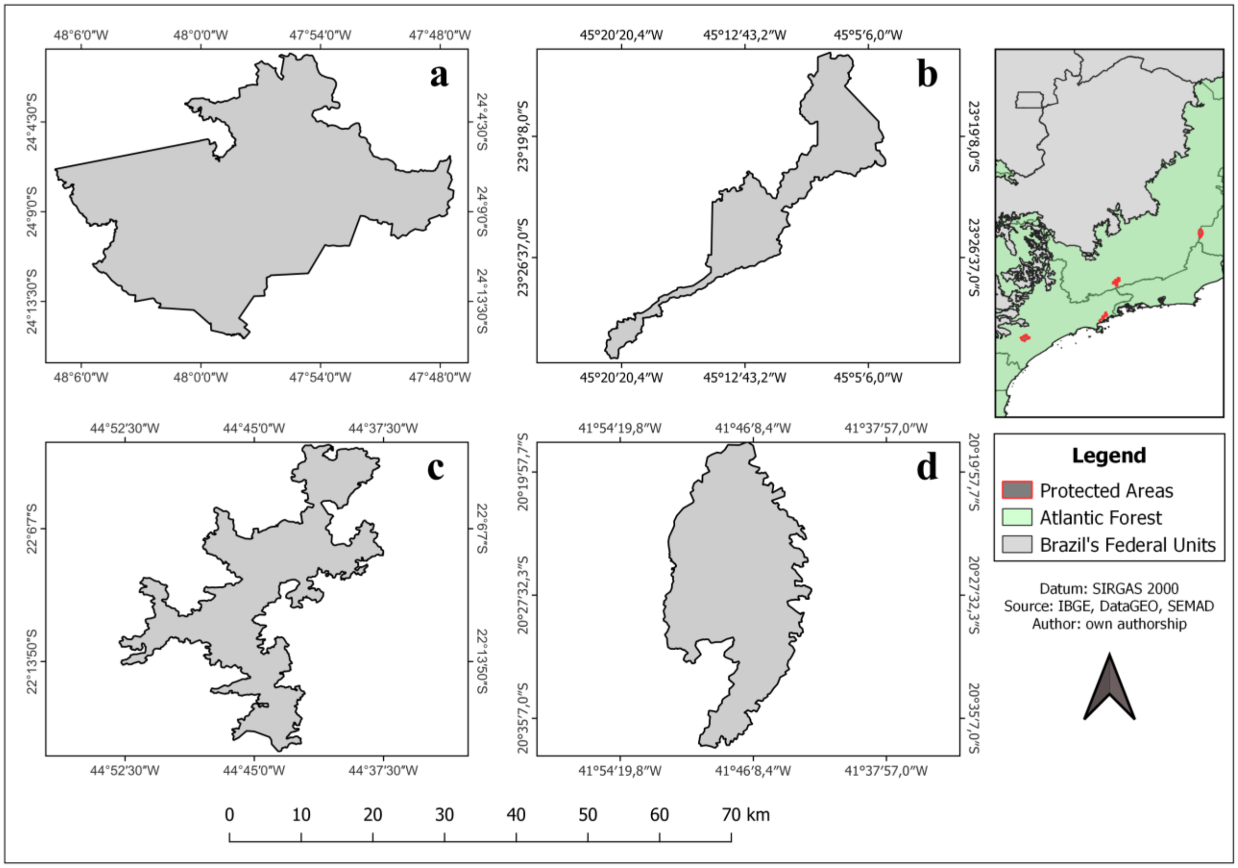

Figure 14. Study area showing four Protected Areas in Brazil: (a) CBSP and (b) SVN in São Paulo; (c) SPSP and (d) CNP in Minas Gerais.

Located in the state of São Paulo, the Carlos Botelho State Park (CBSP) spans altitudes ranging from 20 to 940 meters, with vegetation predominantly composed of Ombrophilous Forest and Montane Shrubland (Instituto Florestal, 2008). The regional climate ranges from hot and humid with no dry season (Cfa) to humid temperate with no dry season (Cfb), with average annual temperatures between 17°C and 22°C (Antunes et al. 2013; Cardoso Cláudio et al. 2020).

The Santa Virgínia Nucleus (SVN), situated in the Serra do Mar range, lies at elevations between 800 and 1,200 meters and has an average annual temperature of approximately 21°C (De Almeida and Carneiro 1998). The predominant vegetation includes Montane and High Montane

Ombrophilous Dense Forest, Cloud Forest, and Altitude Forest, as well as areas of Steppe, Altitude Grasslands (Campos de Altitude), Low Restinga, and Mangroves (Instituto Florestal, 2008).

Located along the border between the states of Minas Gerais and Espírito Santo, the Caparaó National Park (CNP) encompasses elevations from 859 to 2,892 meters, including Pico da Bandeira, the third highest peak in Brazil (Zornosa-Torres et al. 2020). Average annual temperatures range between 19°C and 22°C. The park contains a variety of ecosystems, including Dense Ombrophilous Forest, Semideciduous Seasonal Forest, Montane Forest, and Altitude Grasslands (Campos de Altitude) (ICMBio, 2015).

The Serra do Papagaio State Park (SPSP) features undulating terrain, with elevations around 1,800 meters. The predominant vegetation includes Dense Ombrophilous Forest, High Montane Forest, Montane Forest, and Clear Fields (Campos Limpos) (Instituto Florestal, 2009). The climate is tropical mesothermal, with average annual precipitation exceeding 1,500 mm, primarily concentrated between October and March. Temperatures in the region range from 0°C to 30°C (Menezes et al. 2018).

2.2 DATA SAMPLING

Ten first to third-order streams (Strahler, 1957; Appendices 3 and 4) were selected within each Protected Area (PA), maintaining a minimum distance of 1 km between them along an altitudinal gradient. For sampling, a 150-meter stretch was established in each stream, subdivided into 11 transects spaced at regular intervals of 15 meters (Hughes and Peck, 2008).

2.3 ENVIRONMENTAL VARIABLES

Eight environmental variables were measured in each stream. Altitude and geographical coordinates were recorded at the midpoint of each stream. Depth (cm) and width (m) were measured using a ruler, while water velocity (m/s) was assessed following the methodology described by Craig (1987) at each of the 11 transects. Dissolved oxygen concentration (mg/L) was measured with an oximeter, pH with a pH meter, and temperature (°C) at three points along the stream (0 m, 75 m, and 150 m). Additionally, the Habitat Integrity Index (HII) was evaluated according to Nessimian et al. (2008). This index assesses 12 characteristics of the stream and its surrounding environment, yielding a score ranging from 0 to 1 that reflects increasing levels of environmental integrity.

2.4 COLLECTION OF NYMPHS AND IMAGOS

Ephemeroptera nymphs were collected using a "D"-type entomological net (500 µm mesh), systematically applied along the 11 transects arranged in a zigzag pattern over the 150-meter stretch of each stream, following the protocol described by Hughes and Peck (2008). At each sampling point, an area of 900 cm^2 of the stream substrate was disturbed, allowing organisms to be dislodged and captured by the net. In the field, the collected material was washed and pre-sorted to separate insects with fragile morphological structures, such as legs and gills, which are susceptible to damage during transport (Chen et al. 2017). Adult Ephemeroptera (imagos) were collected using flight interception methods, employing Malaise traps and

Pennsylvania-model light traps, both installed transversely over each stream. The traps were operated for a standardized period at each sampling site to maximize the representativeness of the adult specimens collected.

2.5 DATA ANALYSIS

Each stream was treated as a sampling unit. Accordingly, all immature specimens collected across the 11 subsamples within each stream were pooled to determine total abundance. All statistical analyses were performed using RStudio software (R Core Team, 2022), employing the packages “vegan” (Oksanen et al., 2022), “readxl” (Wickham & Bryan, 2023), “dplyr” (Wickham et al., 2023), and “extrafont” (Chang, 2023).

Environmental variables were compiled into an abiotic data matrix and subjected to Principal Component Analysis (PCA) to explore multivariate patterns and characterize the environmental conditions of the protected areas. Prior to analysis, the data were standardized using the scale function. The PCA was based on a Euclidean distance matrix (calculated with the vegdist function) and conducted through classical multidimensional scaling (cmdscale function). For graphical representation, a two-dimensional PCA plot was generated, with vectors indicating the contribution of individual environmental variables.

Ephemeroptera assemblages were characterized in terms of abundance and taxonomic richness, with separate matrices constructed for nymphs and imagos according to life stage. Compositional patterns were analyzed using Principal Coordinates Analysis (PCoA), based on Bray–Curtis dissimilarities for abundance data and Sørensen’s index for occurrence data (presence/absence). Abundance data were log-transformed [log(1 + x)] to reduce the influence of extreme values. Samples with no taxon records (i.e., zero-sum rows) were excluded prior to calculating the Bray–Curtis dissimilarity matrix. PCoA ordinations were obtained using the cmdscale function. Sørensen dissimilarity was calculated from a binarized version of the abundance matrix, using the vegdist and cmdscale functions. To identify the taxa that contributed most strongly to the observed compositional gradients, correlations between ordination axes and individual taxa were also calculated. Finally, differences in assemblage composition among protected areas (PAs) were tested using Permutational Multivariate Analysis of Variance (PERMANOVA), performed with the adonis2 function.

2.5.1 TAXONOMIC CONGRUENCE

Congruence between nymphs and imagos was assessed using both abundance and presence/absence data, as well as the environmental variables measured across the protected areas (PAs). From the abundance matrices, Bray–Curtis and Sørensen dissimilarity matrices were generated, and the scores of the first two PCoA axes were extracted using the cmdscale function. To evaluate congruence between multivariate patterns, a Procrustes analysis was performed using the procrustes function, and statistical significance was tested via permutation using the protest function with 5,000 permutations (Peres-Neto and Jackson 2001; Figure 15). The comparisons included: (i) Ephemeroptera assemblages vs. environmental variables, (ii) nymphs vs. imagos and (iii) Bray–Curtis vs. Sørensen dissimilarities. The strength of the correlation was expressed by the r coefficient, with values greater than 0.7 interpreted as strong congruence (Heino 2010).

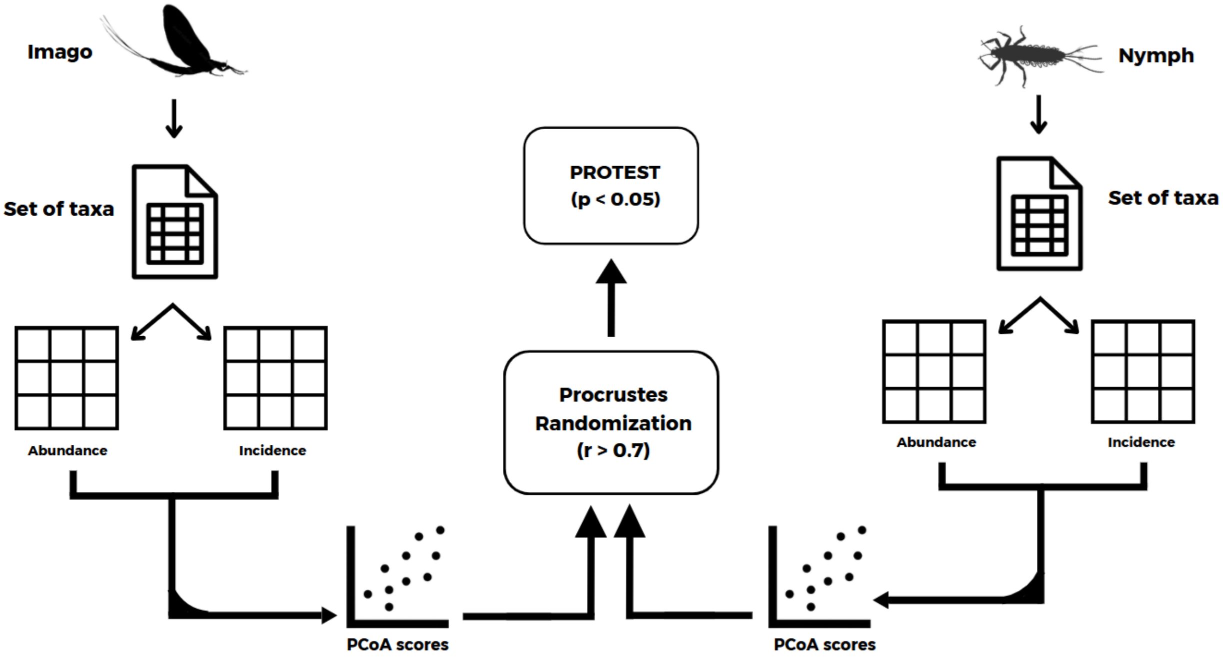

Figure 15. Flow diagram illustrating the analytical steps employed to assess the congruence between biotic and environmental data through Principal Coordinates Analysis (PCoA) and Procrustes analysis.

3 RESULTS

3.1 ENVIRONMENTAL DATA

The streams located in the states of São Paulo and Minas Gerais generally exhibited similar environmental conditions, with low variability among them (Appendix 5). The mean values for depth, width, and water velocity were 20.0 ± 7.8 cm, 2.2 ± 1.2 m, and 21.8 ± 9.3 cm/s, respectively. The streams were well-oxygenated (8.4 ± 0.8 mg/L), with near-neutral pH values (7.1 ± 0.5) and an average temperature of 16.5 ± 1.3°C. Environmental integrity, as measured by the Habitat Integrity Index, averaged 0.82 ± 0.10. Lastly, the streams in Minas Gerais were situated at significantly higher elevations (mean = 1,576 ± 253 m) compared to those in São Paulo (mean = 811 ± 206 m).

Principal Components Analysis (PCA) revealed that the first two axes accounted for 66.3% of the total variation in the environmental dataset (Figure 16). The first axis, which explained 36.7% of the variation, was positively associated with depth ($r = 0.71$), width ($r = 0.57$), water velocity ($r = 0.71$), and dissolved oxygen ($r = 0.83$). In contrast, altitude ($r = –0.59$) and environmental integrity ($r = –0.52$) were negatively correlated with this axis. The second axis was negatively correlated with all variables except dissolved oxygen ($r = 0.39$), with stronger negative correlations observed for depth ($r = –0.62$), water velocity ($r = –0.66$), altitude ($r = –0.66$), and pH ($r = –0.72$).

The PCA ordination showed a clearer clustering of samples from SVN and CBSP (both located in São Paulo) on the right side of the first axis, whereas samples from the parks in Minas Gerais exhibited greater dispersion along both axes.

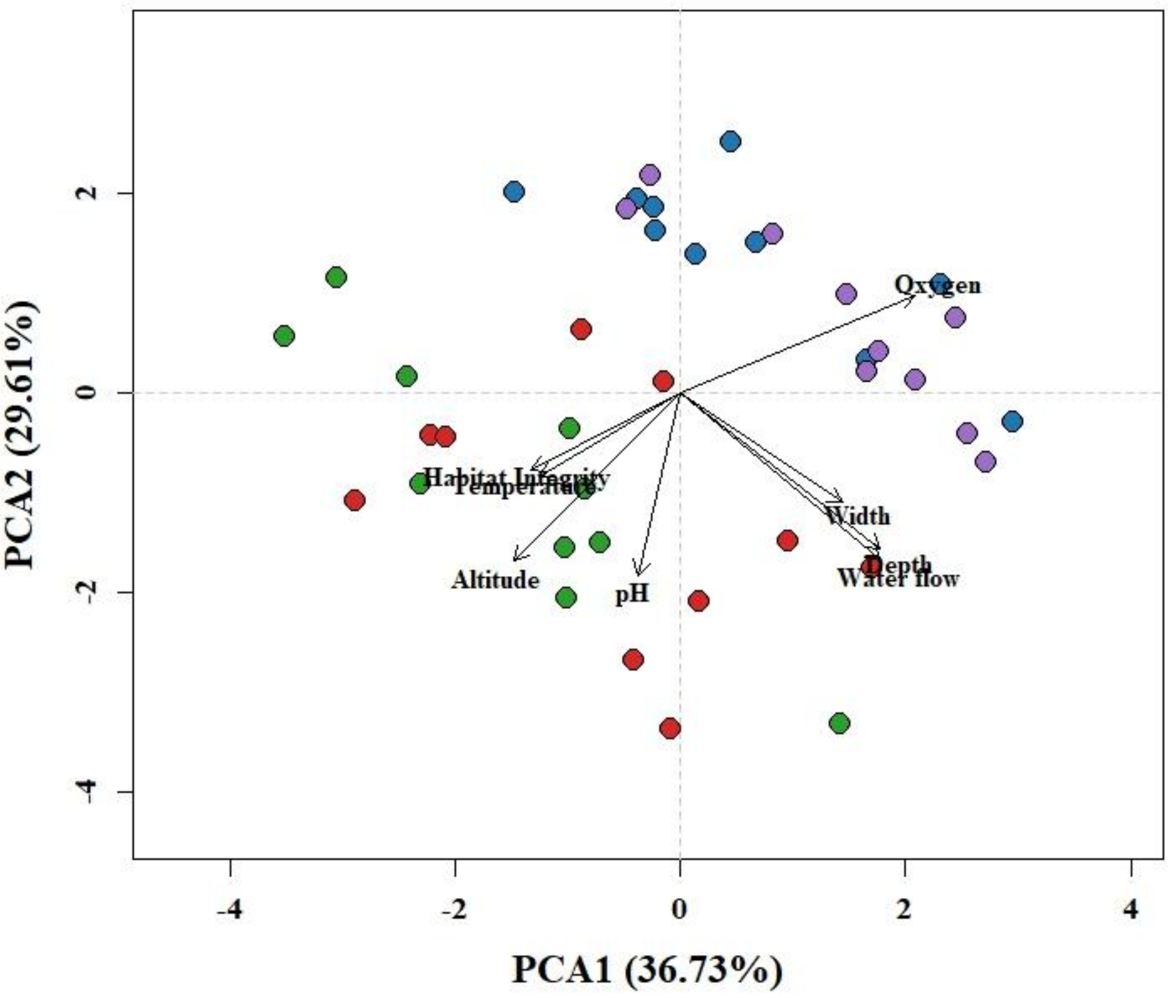

Figure 16. PCA of standardized abiotic variables showing sampling sites ordination. Points colored by site: blue = CBSP, purple = SVN, green = SPSP, red = CNP.

3.2 COMPOSITION OF THE EPHEMEROPTERA ASSEMBLAGES

A total of 3,067 Ephemeroptera individuals were collected from streams in the states of São Paulo (*n* = 1,443) and Minas Gerais (*n* = 1,624). Most specimens were nymphs, accounting for 61% of the total. In São Paulo, imagos were more abundant than nymphs (*n* = 811 and *n* =

632, respectively), whereas the opposite pattern was observed in Minas Gerais, with a predominance of nymphs ($n = 1{,}250$) over imagos ($n = 374$).

Nymph taxonomic richness was similar between the two states (São Paulo: 7 families, 25 genera; Minas Gerais: 6 families, 25 genera). In contrast, imago richness was lower in both states, with five families and 13 genera recorded in São Paulo, and four families and seven genera in Minas Gerais.

In São Paulo, the most abundant nymph families were Leptophlebiidae ($n = 258$), Baetidae ($n = 175$), and Leptohyphidae ($n = 106$), with the genera *Leptohyphodes* ($n = 87$), *Massartella* ($n = 81$), and *Americabaetis* ($n = 59$) being particularly prominent (Appendices 6 and 7). Among the imagos, the dominant families were Leptohyphidae ($n = 626$) and Leptophlebiidae ($n = 156$), primarily represented by *Traverhyphes* ($n = 386$) and *Leptohyphodes* ($n = 174$).

In Minas Gerais, the most abundant nymph families were the same as those recorded in São Paulo: Leptophlebiidae ($n = 584$), Baetidae ($n = 387$), and Leptohyphidae ($n = 174$). The dominant genera included *Cloeodes* ($n = 147$), *Miroculis* ($n = 130$), and *Askola* ($n = 119$; Appendices 8 and 9). For the imagos, Leptophlebiidae ($n = 170$) and Euthyplociidae ($n = 91$) were the most abundant families, with *Ulmeritus* ($n = 135$) and *Campylocia* ($n = 91$) as the dominant genera.

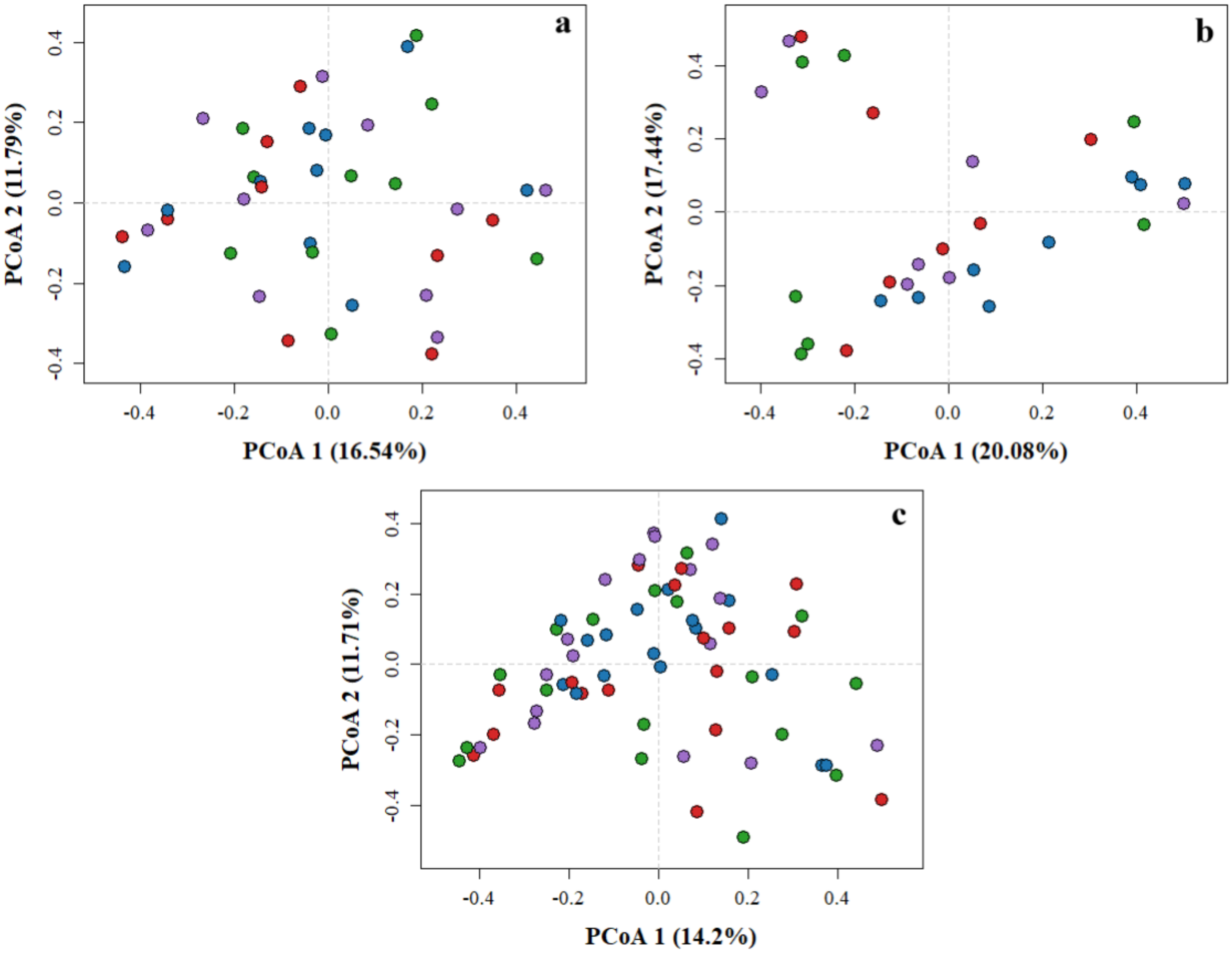

Figure 17. PCoA ordinations of Ephemeroptera assemblages based on Bray–Curtis dissimilarities: (a) nymphs, (b) imagos, and (c) combined life stages.

In general, Principal Coordinates Analysis (PCoA) based on Bray–Curtis dissimilarity revealed a relatively low proportion of variation explained by the first two axes, with a maximum of 37.52% for the imago assemblages (Figure 17b) and a minimum of 25.91% when both life stages were combined (Figure 17c). For the nymph assemblages, the first two axes explained 28.33% of the variation (Figure 17a). When nymphs and imagos were analyzed separately, the taxonomic composition showed a clear distinction among streams from different protected areas. In contrast, combining both life stages resulted in a greater overlap among the streams, indicating reduced differentiation across PAs.

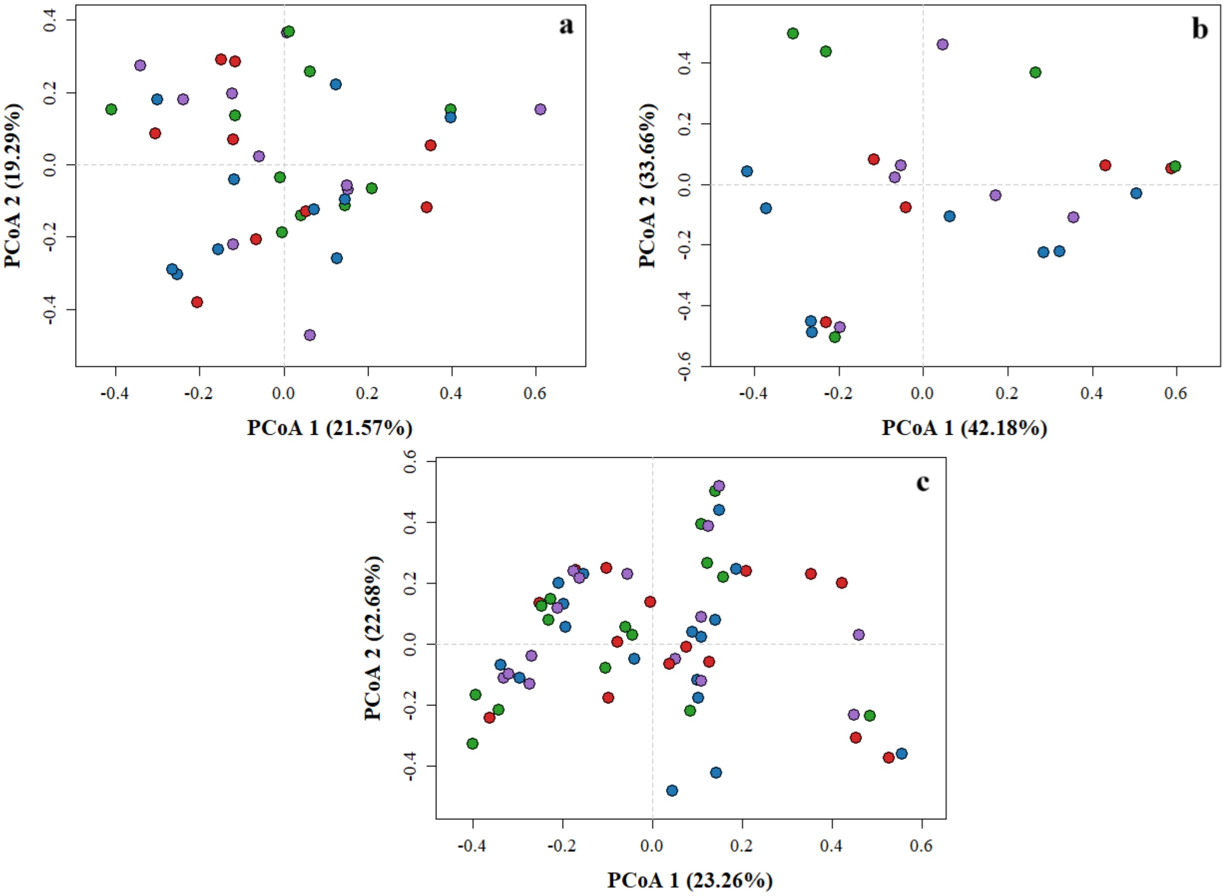

Figure 18. PCoA ordinations of Ephemeroptera assemblages based on Sørensen dissimilarities: (a) nymphs, (b) imagos, and (c) combined life stages.

The PCoA based on Sørensen dissimilarity explained more than 40% of the variation across all life stage scenarios. Specifically, 75.84% of the variation was explained for the imago assemblages (Figure 18b), and 45.94% when both life stages were combined (Figure 18c). For nymph assemblages, the first two axes accounted for 40.86% of the variation (Figure 18a). Similar to the results based on abundance data, the presence–absence approach revealed greater separation among streams when life stages were analyzed separately, whereas combining both stages led to increased overlap among sites.

The results of the Permutational Multivariate Analysis of Variance (PERMANOVA) indicated significant differences in the structure of Ephemeroptera assemblages among the protected areas. Based on abundance data and Bray–Curtis dissimilarity, statistically significant differences were observed for all three assemblage combinations: nymphs ($F_{3,35} = 3.02$, $p = 0.001$), imagos ($F_{3,25} = 2.38$, $p = 0.001$), and combined stages ($F_{3,64} = 2.88$, $p = 0.001$). Similarly, analyses based on incidence data using Sørensen dissimilarity also revealed significant differences among the PAs for all combinations: nymphs ($F_{3,35} = 3.69$, $p = 0.001$), imagos ($F_{3,25} = 5.81$, $p = 0.001$), and combined stages ($F_{3,64} = 4.71$, $p = 0.001$). These results demonstrate consistent differences in assemblage composition across protected areas.

3.3 TAXONOMIC CONGRUENCE

Correlations between biological assemblages and abiotic variables were generally low, although some were statistically significant (Figure 19). In Minas Gerais, environmental variables showed a moderate and statistically significant association with imago assemblages (Bray–Curtis: $r = 0.516$, $p < 0.05$; Sørensen: $r = 0.566$, $p = 0.062$), but exhibited weak and non-significant correlations with nymphs (Bray–Curtis: $r = 0.126$, $p = 0.788$; Sørensen: $r = 0.143$, $p = 0.741$). Conversely, in São Paulo, correlations were stronger and statistically significant for nymphs (Bray–Curtis: $r = 0.434$, $p < 0.05$; Sørensen: $r = 0.431$, $p < 0.05$), but low and non-significant for imagos (Bray–Curtis: $r = 0.265$, $p > 0.05$; Sørensen: $r = 0.246$, $p > 0.05$).

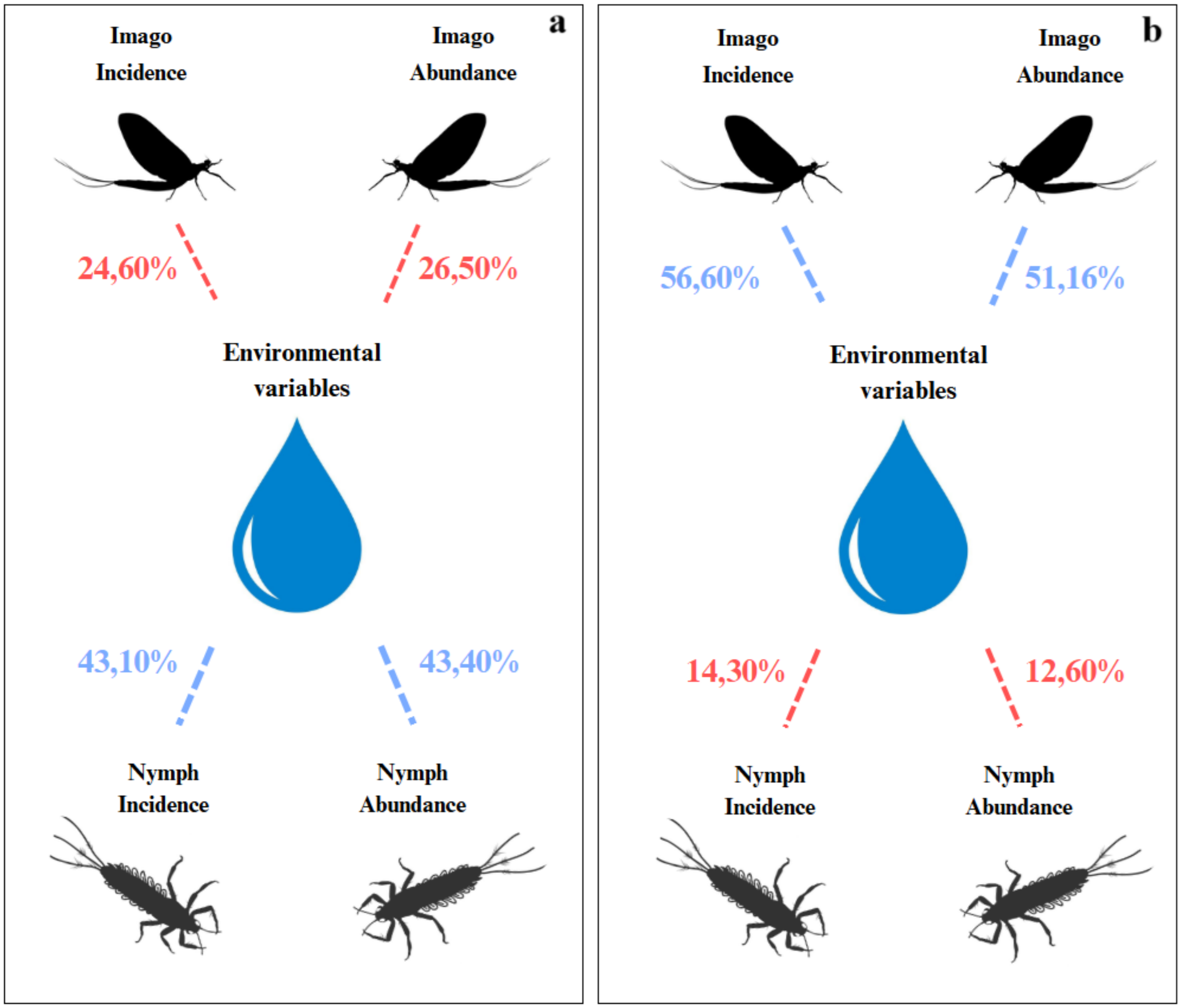

Figure 19. Procrustes analysis showing congruence between Ephemeroptera assemblages (nymphs and imagos) and environmental variables in protected areas of (a) São Paulo and (b) Minas Gerais.

In both states, congruence between abundance and incidence measures was high and statistically significant within the same life stage: immatures (São Paulo: $r = 0.9639$, $p < 0.05$; Minas Gerais: $r = 0.9599$, $p < 0.05$) and adults (São Paulo: $r = 0.9632$, $p < 0.05$; Minas Gerais: $r = 0.9702$, $p < 0.05$; Figure 20). In contrast, comparisons between life stages (nymphs vs. imagos), whether using the same dissimilarity measure (Bray-Curtis or Sørensen) or cross-comparing abundance and incidence data, showed low and non-significant congruence, indicating substantial compositional differences between life stages ($p > 0.05$).

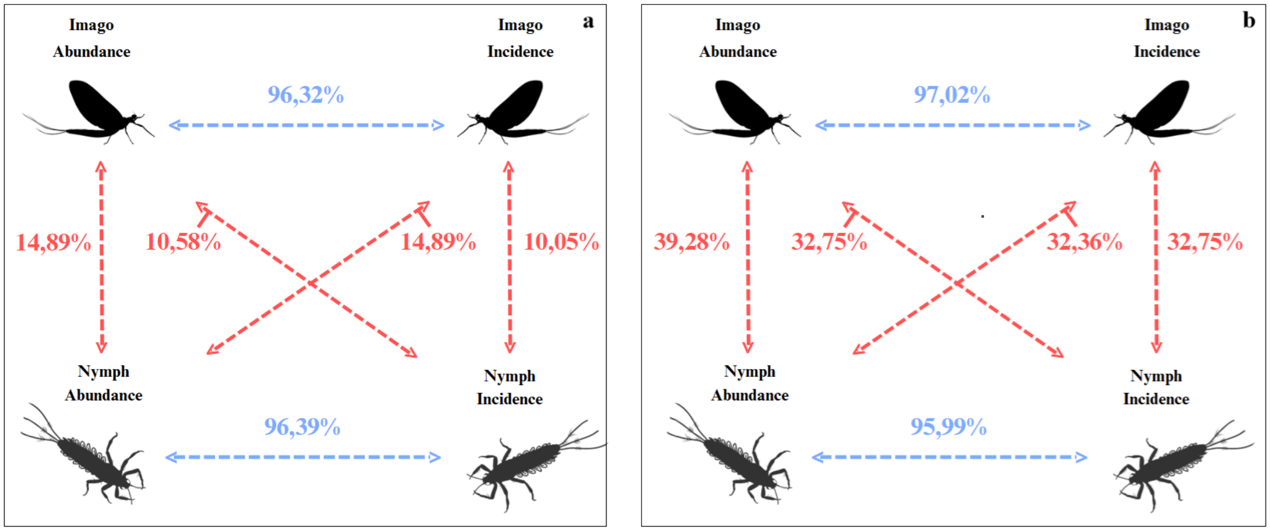

Figure 20. Procrustes analysis showing congruence between nymphs and imagos, and Bray–Curtis and Sørensen dissimilarities in protected areas of (a) São Paulo and (b) Minas Gerais.

4 DISCUSSION

4.1 ENVIRONMENTAL CHARACTERIZATION

The Atlantic Rainforest exhibits high environmental heterogeneity, comprising a mosaic of regions with distinct characteristics (Lins-e-Silva et al. 2021). Nevertheless, streams in São Paulo and Minas Gerais share similar physical attributes, likely due to the shared geological formations of the Serra do Mar and Serra da Mantiqueira mountain ranges (De Almeida and Carneiro 1998). Additionally, the presence of well-preserved riparian vegetation contributes to the structural homogeneity of these aquatic systems by limiting local variability in physical parameters (Langendoen et al. 2009; Krzeminska et al. 2019).

Conversely, the streams exhibited differences in chemical parameters, altitude, and environmental integrity. Altitude, for instance, exerts a direct influence on water temperature and dissolved oxygen concentrations, which generally decrease at higher elevations due to reduced atmospheric pressure (Jacobsen 2008). Variations in water pH were also observed between the states, shaped by both biological processes and the underlying geological composition (Hamid et al. 2020). In São Paulo, the predominance of acidic rocks and base-poor, highly leached soils contributes to conditions favoring acidification (Instituto Florestal, 2008). By contrast, the soils in Minas Gerais are enriched with buffering compounds, such as carbonates, which promote a higher pH (ICMBio, 2015; Instituto Estadual de Florestas, 2009). These findings suggest that, despite the physical similarity of the streams, local geochemical processes generate significant environmental gradients with the potential to influence the structure of aquatic communities.

4.2 COMPOSITION OF THE EPHEMEROPTERA ASSEMBLAGES

The greater abundance of nymphs compared to imagos observed in both states can be attributed to both biological and methodological factors. Nymphs remain for long periods in the aquatic environment, which increases their detectability and their ability to reflect local environmental conditions (Sartori and Brittain 2015; Ramulifho et al. 2020). On the other hand, adults emerge for short periods and are more mobile, which makes it difficult to capture them efficiently with standardized traps. In addition, laboratory studies have shown that imago emergence rates are often low, with less than half of nymphs completing this stage (Finlay 2001; Rowsey, 2015; Smith et al. 2019), which contributes to the under-representation of adults in samples. For these reasons, the use of nymphs is widely prioritized in biomonitoring programs, since their stage is more accessible and more informative about environmental quality and allows for greater standardized collection effort (Shimano et al. 2013). Even so, the inclusion of images can complement the assessment of regional biodiversity.

The predominance of Leptophlebiidae, Baetidae, and Leptohyphidae across both states and life stages highlights a consistent pattern in the structuring of Ephemeroptera assemblages within the studied regions. These families are characterized by broad distributions and high ecological plasticity, enabling them to colonize a wide range of habitats and substrate types (Domínguez et al. 2006; Salles et al. 2014). Moreover, they are among the most abundant families and encompass the greatest species richness across Brazilian biomes, underscoring their representativeness in regional inventories (Salles et al. 2025). Comparable patterns have been documented in various studies conducted in the Atlantic Forest, where these families often dominate Ephemeroptera assemblages in both pristine environments and those subject to varying degrees of disturbance (Siegloch et al. 2012; Do Amaral et al. 2019; Schmitt et al. 2020; Restello et al. 2024).

Although the family-level composition was similar between the states, pronounced differences emerged at the genus level, underscoring the influence of local environmental factors on assemblage structure. In São Paulo, *Leptohyphodes* and *Massartella* were more abundant; these genera are typically associated with higher-altitude streams characterized by rocky substrates and moderate temperatures—conditions indicative of well-preserved mountainous environments (Domínguez et al. 2006; Souto et al. 2021; Hernández Cortes 2023). Consequently, these genera hold potential as indicators of ecological integrity in lotic systems. Conversely, the predominance of *Cloeodes* and *Miroculis* in Minas Gerais likely reflects their broader distribution and greater environmental tolerance. Specifically, *Cloeodes* is commonly found in pools with stony substrates, while *Miroculis* exhibits trophic plasticity, enabling it to exploit diverse food resources (Brasil et al. 2013; Schmitt et al. 2020). This contrast between specialist and generalist taxa highlights the critical importance of genus-level analyses for detecting subtle environmental gradients and enhancing the sensitivity of bioindicators in biomonitoring programs.

4.3 TAXONOMIC CONGRUENCE

4.3.1 EPHEMEROPTERA x ENVIRONMENTAL VARIABLES

Congruence between *Ephemeroptera* assemblages and environmental variables was generally weak, albeit statistically significant in some instances. This low correspondence suggests that the measured abiotic factors explain only a limited portion of the variation observed in taxonomic composition. Such patterns are common in tropical stream ecosystems, where assemblage structure is influenced by a complex interplay of environmental variables, disturbance history, biotic interactions, and stochastic processes (Connell 1978; Rezende et al. 2014; Zhou et al. 2020). Previous studies have similarly reported weak associations between macroinvertebrate communities and environmental variables in tropical regions, even across well-defined degradation gradients (Brito et al. 2018; Martins et al. 2022; Valente-Neto et al. 2025). Moreover, the restricted set of environmental parameters measured may partly account for the low correlation values, particularly if these variables do not directly capture the ecological requirements of the focal taxa. Consequently, these findings underscore the need to incorporate more integrative environmental variables—such as habitat heterogeneity, riparian connectivity, and land-use history—to enhance the predictive power of ecological models (Brasil et al. 2020; Ramulifho et al. 2020).

4.3.2 ABUNDANCE x INCIDENCE

The high congruence observed between abundance and incidence data for both *Ephemeroptera* life stages indicates that these metrics capture similar ecological information regarding assemblage structure. This finding holds important practical implications, suggesting that incidence data can serve as effective proxies for quantitative data in environmental monitoring. Utilizing incidence measures can reduce costs, sampling effort, and logistical complexity without sacrificing the ecological sensitivity of bioindicators (Martins et al. 2022). This trend is supported by previous studies: Shimano et al. (2018) demonstrated strong congruence across taxonomic levels and sampling intensities in *Ephemeroptera*, while Buchner et al. (2019) and Valente-Neto et al. (2016) reported high correspondence between qualitative and quantitative metrics in various macroinvertebrate groups. Therefore, adopting binary metrics may represent a practical, cost-effective, and ethically sound alternative for biomonitoring programs, especially in resource-limited regions.

4.3.3 IMAGOS x NYMPHS

The congruence between imagos and nymphs was low and not statistically significant, regardless of the metric applied. This pattern likely reflects the distinct functional and ecological roles of the two life stages, which occupy different habitats and are influenced by different environmental filters throughout their life cycle (Domínguez et al. 2006; Sartori and Brittain 2015; Valente-Neto et al. 2016). Nymphs are primarily affected by aquatic variables such as temperature, pH, and dissolved oxygen, whereas adults are more influenced by terrestrial factors including vegetation cover, landscape connectivity, and the availability of suitable microhabitats for reproduction (Harper and Peckarsky 2006; Arce et al. 2023). Moreover, the greater dispersal capacity of adults compared to nymphs can produce divergent spatial distribution patterns, complicating direct comparisons between stages (Bilton et al. 2001; Domínguez et al. 2006). Similar discrepancies have been reported in other aquatic insect groups (Valente-Neto et al. 2016; Anacléto et al. 2023), suggesting caution in using a single life stage as a universal surrogate. Instead, integrating data from both stages can provide a more comprehensive understanding of environmental conditions, thereby enhancing the reliability of ecological assessments, especially in ecologically complex tropical systems.

5 CONCLUSIONS

This study provides important practical insights for designing biomonitoring programs in tropical regions. The high congruence between abundance and occurrence metrics within each life stage of Ephemeroptera suggests that both approaches capture similar community patterns and can be used interchangeably. This methodological flexibility is particularly advantageous in situations with logistical or budgetary constraints, allowing for simplified protocols that maintain data consistency and ecological relevance. Conversely, the low congruence between nymphs and imagos indicates that these life stages should not be treated as direct substitutes. Monitoring programs relying solely on imagos, despite their relative ease of sampling, risk overlooking key variations in community structure present in the immature stages. Therefore, the choice of life stage and metric should be carefully aligned with the specific goals and required sensitivity of the assessment. Additionally, the weak correlations observed between biological composition and environmental variables highlight the necessity of incorporating broader environmental descriptors—such as habitat heterogeneity and riparian connectivity—to gain a more complete understanding of ecological integrity. Ultimately, integrated approaches that combine multiple metrics, life stages, and environmental variables will enhance the accuracy and effectiveness of ecological assessments and conservation strategies.

6 CONSIDERAÇÕES FINAIS

Os resultados permitiram uma caracterização abrangente da fauna de *Ephemeroptera* em unidades de conservação da Mata Atlântica. Apesar das semelhanças nas condições ambientais entre os riachos, a composição taxonômica diferiu significativamente entre os locais, evidenciando variações espaciais nas assembleias. As análises multivariadas confirmaram padrões de diferenciação entre os pontos de amostragem, especialmente quando os estágios de vida foram considerados separadamente. As análises de congruência revelaram alta correlação dentro do mesmo estágio de vida, mas baixa entre ninfas e imagos, indicando que cada estágio responde de forma distinta às variáveis ambientais. Esses resultados reforçam a importância de abordagens integradas que considerem múltiplos estágios para a identificação de padrões em estudos ecológicos. Em conjunto, os achados destacam a complexidade das assembleias aquáticas e a necessidade de estratégias analíticas que integrem diferentes variáveis e perspectivas para a compreensão das dinâmicas ecológicas em ecossistemas lóticos.

STATEMENTS AND DECLARATIONS

Conflicts of interest The authors declare that they have no conflicts of interest relevant to the content of this article.

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7 APPENDICES: ADDITIONAL DATA

Appendix 1. List of analyzed articles relating altitude and insects of the order Ephemeroptera.

Title	Year	Journal	doi
The Fauna of Four Streams in the Black Mountain'District of South Wales	1948	Journal of Animal Ecology	https://doi.org/10.2307/1609
Preliminary observations of the aquatic insects of the Smoky Mountains: altitudinal zonation in the spring	1977	Hydrobiologia	https://doi.org/10.1007/BF00023352
Benthic community structure and the effect of rotenone pscicide on invertebrate drift and standing stocks in two Papua New Guinea streams	1990	Archiv Fur Hydrobiologie	10.1127/archiv-hydrobiol/119/1990/35
Diversity of stream-living insects in northwestern Panamá	1991	Journal of the North American Benthological Society	https://doi.org/10.2307/1467605
Altitudinal replacement of Ephemeroptera in a subtropical river	1992	Hydrobiologia	10.1007/BF00005624
Stream macroinvertebrate communities in the island of Tenerife	1993	Archiv Fur Hydrobiologie	10.1127/archiv-hydrobiol/128/1993/209
Altitudinal trends in the diatoms, bryophytes, macroinvertebrates and fish of a Nepalese river system	1994	Freshwater Biology	https://doi.org/10.1111/j.1365-2427.1994.tb01128.x
Macroinvertebrate communities of streams in western Nepal: effects of altitude and land use	1994	Freshwater Biology	https://doi.org/10.1111/j.1365-2427.1994.tb01129.x
Influence of hypolimnetic hydropeaking on the distribution and population dynamics of Ephemeroptera in a mountain stream	1998	Freshwater Biology	https://doi.org/10.1046/j.1365-2427.1998.00353.x
Use of both benthic and drift sampling techniques to assess tropical stream invertebrate communities along an altitudinal gradient, Costa Rica	1998	Freshwater Biology	https://doi.org/10.1046/j.1365-2427.1998.00311.x
Winter macroinvertebrate communities in two montane Wyoming streams	1998	The Great Basin Naturalist	https://www.jstor.org/stable/41713058
Occurrence of the mayfly family Teloganodidae in northern New South Wales	1999	Australian Journal of Entomology	https://doi.org/10.1046/j.1440-6055.1999.00081.x
Testing large-scale hypotheses using surveys: the effects of land use on the habitats, invertebrates and birds of Himalayan rivers	2000	Journal of Applied Ecology	https://doi.org/10.1046/j.1365-2664.2000.00537.x
Macroinvertebrate assemblages in Andean Patagonian rivers and streams: environmental relationships	2001	Hydrobiologia	https://doi.org/10.1023/A:1017519216789

Macroinvertebrate drift in Amazon streams in relation to riparian forest cover and fish fauna	2002	Archiv fur Hydrobiologie	-
Altitudinal changes in diversity of macroinvertebrates from small streams in the Ecuadorian Andes	2003	Archiv Fur Hydrobiologie	10.1127/0003-9136/2003/0158-0145
Relationship between macroinvertebrate fauna and environmental variables in small streams of the Dominican Republic	2004	Water Research	10.1016/S0043-1354(03)00406-8
Respiration rate of stream insects measured in situ along a large altitude range	2005	Hydrobiologia	10.1007/s10750-005-4165-7
Ecology of leaf pack macroinvertebrate communities in streams of the Fraser River Basin, British Columbia	2005	Freshwater Biology	https://doi.org/10.1111/j.1365-2427.2005.01373.x
Macroinvertebrate community response to natural and forest harvest gradients in western Oregon headwater streams	2005	Freshwater Biology	https://doi.org/10.1111/j.1365-2427.2005.01363.x
Structure and spatial variability of mayfly (Ephemeroptera) communities in the upper Hron River basin	2006	Biologia	10.2478/s11756-006-0089-6
Zoobenthic communities of inlets and outlets of high altitude Alpine	2006	Hydrobiologia	https://doi.org/10.1007/s10750-005-1812-y
Macroinvertebrate assemblages in conditions of low-discharge streams of the Cerova´ vrchovina highland in Slovakia	2006	Limnologica	https://doi.org/10.1016/j.limno.2006.07.002
Vulnerability of alpine stream biodiversity to shrinking glaciers and snowpacks	2007	Global Change Biology	https://doi.org/10.1111/j.1365-2486.2007.01341.x
Response of macroinvertebrate and diatom communities to human-induced physical alteration in mountain streams	2008	River Research and applications	https://doi.org/10.1002/rra.1110
Community structure of Ephemeroptera in Siberian streams	2008	Entomological Science	https://doi.org/10.1111/j.1479-8298.2008.00279.x
Land use and the ecology of benthic macroinvertebrate assemblages of high-altitude rainforest streams in Uganda	2008	Freshwater Biology	https://doi.org/10.1111/j.1365-2427.2007.01925.x
Macroinvertebrate communities of non-glacial high altitude intermittent streams	2008	Freshwater Biology	https://doi.org/10.1111/j.1365-2427.2007.01867.x
Spatial and temporal patterns in the aquatic insect community of a hig altitude Andean stream (Mendoza, Argentina)	2008	Aquatic Insects	https://doi.org/10.1080/01650420701880974
Aquatic insect faunas and communities of a mountain stream in Sapa Highland, northern Vietnam	2008	Limnology	https://doi.org/10.1007/s10201-008-0250-8
Short-term colonization patterns of macroinvertebrates in alpine streams	2008	Fundamental and applied limnology	10.1127/1863-9135/2008/0171-0075
Cross-Eurasian and altitudinal distribution of lotic mayflies – species with wider altitudinal ranges have narrower geographical distribution	2009	Annales de Limnologie-International Journal of Limnology	https://doi.org/10.1051/limn/2009024
The Rapoport effect is detected in a river system and is based on nested organization	2009	Global Ecology and Biogeography	https://doi.org/10.1111/j.1466-8238.2009.00466.x

Physical and chemical differences in karst springs of Cantabria, northern Spain: do invertebrate communities correspond?	2009	Aquatic Ecology	https://doi.org/10.1007/s10452-008-9170-2
Spatial and Temporal Patterns of Macroinvertebrate Communities in the Du River Basin in Northern Vietnam	2010	Journal of Freshwater Ecology	https://doi.org/10.1080/02705060.2010.9664413
Downstream changes in spring-fed stream invertebrate communities: the effect of increased temperature range	2011	Journal of limnology	10.3274/JL11-70-S1-10
Responses of benthic macroinvertebrate communities to altitude and geology in tributaries of the Sepik River (Papua New Guinea): the influence of taxonomic resolution on the detection of environmental gradients	2012	Freshwater Biology	https://doi.org/10.1111/j.1365-2427.2012.02839.x
Estructura de la fauna béntica en corrientes de los Andes colombianos	2012	Revista Colombiana de Entomología	-
Analysis of benthic macroinvertebrates and biotic indices to evaluate water quality in rivers impacted by mining activities in northern Chile	2012	Knowledge and Management of Aquatic Ecosystems	https://doi.org/10.1051/kmae/2012027
Ultraviolet-B-driven pigmentation and genetic diversity of benthic macroinvertebrates from high-altitude Andean streams	2013	Freshwater Biology	https://doi.org/10.1111/fwb.12161
Longitudinal patterns of macroinvertebrate communities in relation to environmental factors in a Tibetan-Plateau river system	2013	Quaternary International	https://doi.org/10.1016/j.quaint.2013.02.034
Biodiversity of stream insects in the Malaysian Peninsula: spatial patterns and environmental constraints	2013	Ecological Entomology	https://doi.org/10.1111/een.12013
Contrasting taxonomical and functional responses of stream invertebrates across space and time in a Neotropical basin	2013	Fundamental and applied limnology	http://dx.doi.org/10.1127/1863-9135/2013/0501
The effect of environmental factors on the mayfly communities of headwater streams in the Pieniny Mountains (West Carpathians)	2014	Biologia	https://doi.org/10.2478/s11756-014-0334-3
Temporal and altitudinal variations in benthic macroinvertebrate assemblages in an Andean river basin of Argentina	2014	Journal of Limnology	http://dx.doi.org/10.4081/jlimnol.2014.789
Influence of selected environmental factors on macroinvertebrates in mountain streams	2015	Open Life Sciences	https://doi.org/10.1515/biol-2015-0008
Landscape composition as a determinant of diversity and functional feeding groups of aquatic macroinvertebrates in southern rivers of the Araucanía, Chile	2015	Latin American Journal of Aquatic Research	https://doi.org/10.3856/vol43-issue1-fulltext-16
Diversity and composition of macroinvertebrate assemblages in high-altitude Tibetan streams	2015	Inland Waters	10.5268/IW-5.3.818
Distribution of Ephemeroptera, Plecoptera, and Trichoptera assemblages in relation to environmental variables in headwater streams of Mongolia	2015	Environmental Earth Sciences	https://doi.org/10.1007/s12665-013-2968-9
Altitudinal distribution limits of aquatic macroinvertebrates: an experimental test in a tropical alpine stream	2015	Ecological Entomology	https://doi.org/10.1111/een.12232

Spatial distribution and functional feeding groups of aquatic insects in a stream of Chakrashila Wildlife Sanctuary, Assam, India	2015	Knowledge and Management of Aquatic Ecosystems	https://doi.org/10.1051/kmae/2015028
The altitudinal limit of Leptohyphes Eaton, 1882 and Lachlania Hagen, 1868 (Ephemeroptera: Leptohyphidae, Oligoneuriidae) in Ecuadorian Andes streams: searching for mechanisms	2016	Aquatic Insects	https://doi.org/10.1080/01650424.2015.1109128
Environmental factors influencing the composition and distribution of mayfly larvae in northern Algerian wadis (regional scale)	2017	Revue d'Écologie	https://hal.science/hal-03532796
Analysis and prediction of the spatial distribution of EPT (Ephemeroptera, Plecoptera, and Trichoptera) assemblages in the Han River watershed in Korea	2017	Journal of Asia-Pacific Entomology	10.1016/j.aspen.2017.03.024
Landscape variables influence taxonomic and trait composition of insect assemblages in Neotropical savanna streams	2017	Freshwater Biology	https://doi.org/10.1111/fwb.12961
Cold/Warm stenothermic freshwater macroinvertebrates along altitudinal and latitudinal gradients in Western South America: A modern approach to an old hypothesis with updated data	2018	Journal of Biogeography	https://doi.org/10.1111/jbi.13234
Mayfly (Ephemeroptera) assemblages of a Pannonian lowland mountain, with first records of the parasite Symbiocladius rhithrogenae (Zavrel, 1924) (Diptera: Chironomidae)	2018	Annales de Limnologie-International Journal of Limnology.	https://doi.org/10.1051/limn/2018023
Thirty years after: an update to the mayflies composition in the Tafna basin (Algeria)	2019	Zoosymposia	http://dx.doi.org/10.11646/zoosymposia.16.1.6
Diversity and distribution patterns of benthic insects in streams of the Aurès arid region (NE Algeria)	2019	Oceanological and Hydrobiological Studies	https://doi.org/10.1515/ohs-2019-0004
Distribution patterns of benthic macroinvertebrate communities based on multispatial-scale environmental variables in the river systems of Republic of Korea	2020	Journal of Freshwater Ecology	https://doi.org/10.1080/02705060.2020.1815599
Does elevation influence mayfly emergence timing? A case study using New Zealand's endemic ephemeropteran fauna	2020	Ecological Entomology	https://doi.org/10.1111/een.12848
How altitudinal gradient affects the diversity and composition of benthic insects in arid areas streams of northern East Algeria?	2020	Biologia	https://doi.org/10.2478/s11756-019-00326-8
Macroinvertebrate diversity patterns in tropical highland Andean rivers	2020	Austral Entomology	10.23818/limn.39.44
Growth rates of mayflies (Ephemeroptera) reared in the field differed under contrasting temperatures	2021	Austral Entomology	https://doi.org/10.1111/aen.12550

Ephemeroptera (Mayflies) Assemblages and Environmental Variation along Three Streams Located in the Dry-Hot Valleys of Baima Snow Mountain, Yunnan, Southwest China	2021	Insects	https://doi.org/10.3390/insects12090775
Diversity, phenology and distribution of mayfly larvae (Ephemeroptera) along an altitudinal gradient in two permanent Wadis of Algeria	2022	Oriental Insects	https://doi.org/10.1080/00305316.2021.1904022
Spatiotemporal variation in macroinvertebrate community composition along the stressor gradients in rivers of a middle-eastern basin	2022	International Journal of Environmental Science and Technology	https://doi.org/10.1007/s13762-022-04094-y
Checklist, distribution, diversity, and rarity of mayflies (Ephemeroptera) in Slovakia	2023	ZooKeys	10.3897/zookeys.1183.109819
Inventory and pattern of distribution of mayflies (Insecta, Ephemeroptera) in the Draa river basin, southern Morocco	2023	Alpine Entomology	10.3897/alpento.7.96436
Macroinvertebrate metrics and lipid profiles as potential indicators of land use influence in a high altitude tropical highland stream (Sagana River Basin, Kenya)	2024	Ecological Indicators	https://doi.org/10.1016/j.ecolind.2024.111848
Phenotypic plasticity of a Baetid mayfly larvae (*Baetis rhodani*) at sites with high levels of deposited fine sediment	2024	Ecological Entomology	10.1111/een.13321
Stream Sentinels - Mayfly Diversity, Land Use, and Conservation in Algeria's Djurdjura Mountains	2024	Aquatic Conservation: Marine and Freshwater Ecosystems	https://doi.org/10.1002/aqc.70025
Species Richness and Similarity of New Zealand Mayfly Communities (Ephemeroptera) Decline with Increasing Latitude and Altitude	2024	Insects	https://doi.org/10.3390/insects15100757
Elevation transition of aquatic insects closely matches a thermal feature in the Yungas of Northwestern Argentina	2024	Journal of Mountain Science	https://doi.org/10.1007/s11629-023-8245-9

Appendix 2. Statistical results of the GAM model for each biodiversity indicator based on data extracted from the analyzed articles.

Data set	Model	Taxon	Term	edf	χ^2	p-value	R^2	Deviance	n
EXT	Richness	Species	s(Altitude)	1.54	6.45	0.018	0.109	15.0%	2,457
			s(Articles)	24.40	329.66	< 0.001			
	Richness	Genus	s(Altitude)	1.06	5.51	0.021	0.096	12.5%	2,301
			s(Articles)	21.94	228.79	< 0.001			
	Abundance	-	s(Altitude)	2.93	10.39	0.030	0.387	53.6%	335
			s(Articles)	14.43	417.13	< 0.001			
GRI	Richness	Species	s(Altitude)	8.72	3792.50	< 0.001	0.115	18.6%	58,818
			s(Articles)	13.14	855.60	< 0.001			
	Richness	Genus	s(Altitude)	8.63	4772.00	< 0.001	0.196	18.2%	58,818
			s(Articles)	13.66	5232.00	< 0.001			
EXT + GRI	Richness	Species	s(Altitude)	8.35	3433.00	< 0.001	0.187	23.7%	61,275
			s(Articles)	41.52	4080.00	< 0.001			
	Richness	Genus	s(Altitude)	8.70	4650.00	< 0.001	0.200	19.0%	61,119
			s(Articles)	35.00	5571.00	< 0.001			

Appendix 3. Geographic coordinates and altitude of sampling points in streams of São Paulo (points 01–10: CBSP; points 11–20: SVN).

Protected Areas	**Collection Points**	**Altitude (m)**	**Geographic Coordinates**	
			South (S)	**West (W)**
CBSP	**01.** Bonito Stream	729	24°3'44"	47°56'48.56
	02. Segundo Água Stream	780	24°4'4.22"	47°57'32.63"
	03. Teveeira Stream	755	24°4'7.27"	47°59'12.73"
	04. Pedras Stream	763	24°5'27.22"	47°59'43.50"
	05. Serra Stream	62	24°11'34.59"	47°55'5.28"
	06. Água da Vaca Stream	713	24°9'49.67"	47°58'57.64"
	07. Queixada Stream	711	24°9'16.90"	47°59'13.94"
	08. Bonito River	762	24°8'29"	47°59'40.64"
	09. Preto River	764	24°7'39.65"	47°59'35.40"

	10. São João Miguel Stream	752	24°6'32.03"	47°59'17.59"
SVN	**11.** Veado Stream	988	23°19'8.85"	45°5'2.55"
	12. Tributary of Casa Cumbuca	1000	23°19'30.02"	45°5'13.79"
	13. Barro Branco River	941	23°20'38.70"	45°7'44.77"
	14. Base Bridge Stream	921	23°20'7.12"	45°8'40.57"
	15. Paçada Pito Trail Stream	910	23°18'48.61"	45°7'12.32"
	16. Pau de Bala Stream	900	23°19'24.50"	45°7'52.15"
	17. Olho D'água Trail Stream	909	23°20'24.39"	45°8'57.13"
	18. Pedra Redonda Stream	844	23°23'5.81"	45°10'46.49"
	19. Km 74 Stream	972	23°23'15.06"	45°10'0.70"
	20. Pandorf Bridge Stream	1040	45°7'38.74"	23°17'34.93"

Appendix 4. Geographic coordinates and altitude of sampling points in streams of Minas Gerais (points 01–10: SPSP; points 11–20: CNP).

Protected Areas	Collection Points	Altitude (m)	Geographic Coordinates	
			South (S)	West (W)
SPSP	**01.** Alojamento Stream	1654	22°8'52.907"	44°44'2.017"
	02. Bifurcação Stream	1693	22°9'31.751"	44°44'26.029"
	03. Fazenda Stream	1671	22°10'49.079"	44°43'49.774"
	04. Coelhos Stream	1715	22°11'24.831"	44°44'44.386"
	05. Andreas Stream	1668	22°12'16.300"	44°44'4.357"
	06. Cerca Stream	1722	22°8'26.862"	44°44'12.897"
	07. Dores Stream	1656	22°8'21.007"	44°44'1.755"
	08. Buracão Stream	1706	22°8'34.760"	44°25'3.633"
	09. Alagado Stream	1718	22°8'53.552"	44°42'30.211"
	10. Zé Stream	1641	22°8'6.406"	44°43'22.464
CNP	**11.** Vale Verde Waterfall Stream	1331	20°25'8.886"	41°50'44.304"

12. José Pedro River	2113	20°24'51.433"	41°49'33.153
13. Preto River	1423	20°30'6.169"	41°49'8.511"
14. São Domingos Stream	1944	20°28'19.253"	41°49'41.533"
15. Ponte Waterfall Stream	1640	20°29'26.238"	41°49'17.230"
16. Pinheiros Stream	1380	20°30'29.804"	41°48'27.121"
17. Condomínio Stream	1268	20°31'6.869"	41°48'24.714"
18. Casa da Dona Aparecida Stream	1135	20°32'23.265"	41°48'2.991"
19. Pedra Vista Stream	1245	20°32'46.075"	41°47'36.552"
20. Dona Adriana Stream	1200	20°33'40.402"	41°47'59.881"

Appendix 5. Summary of abiotic variables measured in streams from São Paulo and Minas Gerais states. Values are presented as mean ± standard deviation.

Variable	**São Paulo State**	**Minas Gerais State**
Depth (cm)	20 ± 7.81	19.90 ± 8.38
Width (m)	2.28 ± 1.07	2.17 ± 1.30
Flow velocity (cm/s)	21.64 ± 8.97	21.94 ± 9.58
Dissolved oxygen (mg/L)	8.95 ± 0.47	7.85 ± 0.38
pH	6.83 ± 0.32	7.47 ± 0.48
Temperature (ºC)	15.73 ± 0.77	17.18 ± 1.27
Altitude (m)	810.8 ± 205.8	1576.15 ± 252.94
Environmental Integrity	0.78 ± 0.08	0.86 ± 0.10

Appendix 6A. Abundance of Ephemeroptera nymphs identified to genus or family level across ten sampling points of the CBSP.

Nymphs	Streams										Total
	1	2	3	4	5	6	7	8	9	10	
Baetidae											0
Americabaetis							1				1
Cloeodes				1							1
Paracloeodes	2	1									3
Waltzoyphius			1	1			1				3
Zelusia	1		1				1		1	1	5
Caenidae											0
Caenis			1								1
Euthyplociidae											0
Campylocia	1	8	9	2		3	2		4		29
Leptohyphidae	1		1		1	1			2		6
Leptohyphodes						13	6		3		22
Traverhyphes					1	1					2
Tricorythodes						1				1	2
Leptophlebiidae			1			1	1	1	4	1	9
Farrodes			3			2				1	6
Hagenulopsis	1		2	3	1	1		1	2		11
Hylister	1		1			3		1	6		12
Massartella	3	5	4	5		3	8	7	1	5	41
Miroculis	1										1
Thraulodes	19		2	2			2		3		28
Ulmeritoides		12		1			1				14
Polymitarcyidae											0
Asthenopus	6										6
Total	36	26	26	15	3	29	23	10	26	9	203

Appendix 6B. Abundance of Ephemeroptera imagos identified to genus or family level across ten sampling points of the CBSP.

Imagos	**Streams**									**Total**
	1	**2**	**3**	**5**	**6**	**7**	**8**	**9**	**10**	
Baetidae			1			1			1	3
Caenidae										0
Caenis			1							1
Euthyplociidae										0
Campylocia					2					2
Leptohyphidae	4				17		2	0		23
Leptohyphes									10	10
Leptohyphodes	12			9	47	5	14		44	131
Traverhyphes	13				167					180
Tricorythopsis	7									7
Leptophlebiidae	1									1
Askola									1	1
Massartella	1	1	1	15		2	4	2	2	28
Total	38	1	3	24	233	8	20	2	58	387

Appendix 7A. Abundance of Ephemeroptera nymphs identified to genus or family level across ten sampling points of the SVN.

Nymphs	Streams										Total
	11	12	13	14	15	16	17	18	19	20	
Baetidae	13	1	2	9	1			3		1	30
Americabaetis	6	2	2	4	8	16	20				58
Apobaetis	2	1					1				4
Baetodes					3	6					9
Callibaetis	7										7
Cloeodes							1				1
Cryptonympha					1						1
Paracloeodes	6				4					1	11
Waltzoyphius	2	1		3				6		3	15
Zelusia	12		1	2	4	2	5				26
Caenidae											0
Caenis									1		1
Euthyplociidae											0
Campylocia	6	13						1	1	1	22
Leptohyphidae					1					1	2
Haplohyphes								1			1
Leptohyphodes	31	11			20	1		1		1	65
Traverhyphes								3		1	4
Tricorythopsis										2	2
Leptophlebiidae	10	1	1			1	3		1		17
Farrodes	34				4		7				45
Hagenulopsis	5		2		4	6	2				19
Hylister			1		1	2					4
Massartella	12	7	1	4	1	5	4			6	40
Miroculis	5		1								6
Thraulodes						2					2

Ulmeritoides	1			1	1						3
Melanemerellidae											0
Melanemerella	4			1	2						7
Polymitarcyidae											0
Asthenopus										27	27
Total	156	37	11	24	55	41	43	15	3	44	429

Appendix 7B. Abundance of Ephemeroptera imagos identified to genus or family level across ten sampling points of the SVN.

Imagos	**Streams**										**Total**
	11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	
Baetidae		1		1	1	2	3	3			11
Caenidae											0
Caenis									1		1
Euthyplociidae											0
Campylocia			1				4	5	1		11
Leptohyphidae	5		15	2		1		1			24
Leptohyphodes		1			24	13	3	2			43
Traverhyphes		2				1		203			206
Tricorythodes								2			2
Leptophlebiidae	6	1	2		2	5		4			20
Askola								4			4
Farrodes							1				1
Hylister		4									4
Massartella	4						1	1			6
Miroculis					4	28	4	41	12		89
Thraulodes								2			2
Total	15	9	18	3	31	50	16	268	14	0	424

Appendix 8A. Abundance of Ephemeroptera nymphs identified to genus or family level across ten sampling points of the SPSP.

Nymphs	**Streams**										**Total**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Baetidae	1		12	43	5	4	3	8			76
Americabaetis		3	18	17							38
Baetodes					1						1
Cloeodes		2		110	1			2			115
Cryptonympha				4							4
Paracloeodes		6		8			7			8	29
Tupiara					3						3
Waltzoyphius		29	1		1						31
Zelusia					3			1			4
Caenidae											0
Euthyplociidae											0
Campylocia	4	3	1		1	11	10	47		5	82
Leptohyphidae	7	7	10	2	3						29
Leptohyphodes				5	8	10	1		6		30
Traverhyphes					8						8
Tricorythodes		64	16	1	3					8	92
Tricorythopsis				3							3
Leptophlebiidae	4	4	5		2	18	21	51	1	8	114
Askola		27	2	8	6	15	10	29		8	105
Farrodes		1	1		9	19	8			1	39
Hagenulopsis		1	3								4
Hermanella										1	1
Hylister				1		1	1			1	4
Massartella	4	1	2					14		6	27
Miroculis		1	4	15	12	14	8	39		9	102
Thraulodes				10		3	9				22

Ulmeritoides					1			13			14
Melanemerellidae											0
Melanemerella						11	4			1	16
Total	20	149	75	227	67	106	82	204	7	56	993

Appendix 8B. Abundance of Ephemeroptera imagos identified to genus or family level across ten sampling points of the SPSP.

Imagos	**Streams**										**Total**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Baetidae	3		7		8	4				5	27
Baetodes		5					46				51
Caenidae											0
Euthyplociidae											0
Campylocia	14	30		36		4		1		6	91
Leptohyphidae										1	1
Leptohyphodes				9	2			2			13
Leptophlebiidae		1	1	7		6	7				22
Askola	2	1		1	3	4	2	1		2	16
Farrodes				8							8
Massartella	2				1						3
Ulmeritus					99		4	16			119
Total	21	37	8	61	113	18	59	20	0	14	351

Appendix 9A. Abundance of Ephemeroptera nymphs identified to genus or family level across ten sampling points of the CNP.

Nymphs	Streams										Total
	11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	
Baetidae	2		5	1	1		10	2			21
Baetodes	21					1			2		24
Camelobaetidius	4			1				2	1		8
Cloeodes	14			1	7	5	1	2		2	32
Paracloeodes								1			1
Caenidae											0
Caenis						1					1
Euthyplociidae											0
Campylocia	1					2	1				4
Leptohyphidae	3		1	1		2	1				8
Leptohyphodes						2					2
Traverhyphes					1	1	2				4
Leptophlebiidae	2		3	7		9	11	3	1	5	41
Askola					1		7	1		5	14
Farrodes	1						1	1			3
Hagenulopsis				1							1
Hermanella							2				2
Hylister									1	1	2
Massartella	3		6	6	19	6	2	1		2	45
Miroculis			10		2	1	7		7	1	28
Thraulodes	1		1			2	2	8	2		16
Total	52	0	26	18	31	32	47	21	14	16	257

Appendix 9B. Abundance of Ephemeroptera imagos identified to genus or family level across ten sampling points of the CNP.

Imagos	**Streams**										**Total**
	11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	
Baetidae			1	6							7
Caenidae											0
Euthyplociidae											0
Leptohyphidae											0
Leptohyphodes			2					12			14
Leptophlebiidae											0
Askola			1								1
Massartella			1								1
Total	0	0	5	6	0	0	0	12	0	0	23