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Experimental studies on freshwater zooplankton in Brazil: a scientometric analysis from 1978 to 2023

Estudos experimentais sobre zooplâncton de água doce no Brasil: uma análise cienciométrica de 1978 a 2023

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Abstract: Aim: This study aims to contribute to elucidating the importance of experimental research on freshwater zooplankton communities in Brazil through a scientometric analysis. **Methods:** Web of Science, SciELO, and Scopus databases were used to compile 249 scientific papers published between 1978 and 2023. The extracted data included the year of publication, geographical location, experiment characteristics (type, scale, duration), species or taxa studied, and research topics. Quantitative and qualitative syntheses were performed to provide a systematic understanding of experimental studies on freshwater zooplankton. **Results:** The analysis revealed a significant increase in the number of experimental papers over time, with a higher concentration of studies in the Southeastern region of Brazil. Laboratory experiments were conducted with greater frequency than field experiments, mainly microcosms. Predation, chemical pollutants, and eutrophication emerged as frequently addressed research topics. On the other hand, certain topics, such as metapopulation dynamics and microplastics, were relatively underrepresented in the literature. Despite the crucial role of experimental research on freshwater zooplankton in advancing ecological understanding in Brazil, there is an unequal distribution of these studies across the country, indicating the need for investment and more researchers prepared and interested in studying with experiments in certain regions. Addressing the gaps identified in this review, such as metapopulation dynamics and dispersion, along with emerging threats like invasions, microplastics, pathogens, cumulative stressors, emerging contaminants, and nanomaterials, will be essential for generating scientific knowledge to inform effective management strategies to preserve freshwater zooplankton biodiversity amidst ongoing environmental changes. **Conclusions:** This review underscores the importance of expanding experimental research across

diverse regions and underexplored topics to enhance our ecological understanding and better manage freshwater zooplankton biodiversity in Brazil.

Keywords: laboratory experiment; field experiment; duration of experiments; systematic review.

Resumo: Objetivo: Este estudo tem como objetivo contribuir para elucidar a importância da pesquisa experimental sobre a comunidade zooplanctônica de água doce no Brasil através de uma análise cienciométrica. **Métodos:** As bases de dados Web of Science, SciELO e Scopus foram utilizadas para compilar 249 trabalhos científicos publicados entre 1978 e 2023. Os dados extraídos dos artigos foram o ano de publicação, localização geográfica, características do experimento (tipo, escala, duração), espécies ou táxons estudados e os temas de pesquisa. Sínteses quantitativas e qualitativas foram realizadas para fornecer uma compreensão sistematizada dos estudos experimentais sobre zooplâncton de água doce. **Resultados:** A análise revelou um aumento significativo no número de publicações experimentais ao longo do tempo, com uma maior concentração de estudos na região Sudeste do Brasil. Experimentos de laboratório, principalmente microcosmos foram realizados com mais frequência do que experimentos de campo. Predação, poluentes químicos e eutrofização surgiram como os temas de pesquisa frequentemente abordados. Por outro lado, certos tópicos, como dinâmicas de metapopulação e microplásticos, foram relativamente sub-representados na literatura. Apesar da pesquisa experimental sobre o zooplâncton de água doce no Brasil desempenhar um papel crucial no avanço da compreensão ecológica, há uma distribuição desigual desses estudos pelo país, indicando a necessidade de investimentos e mais pesquisadores preparados para trabalhar com experimentos em certas regiões. Abordar as lacunas identificadas nesta revisão, como dinâmicas de metapopulação e dispersão, juntamente com ameaças emergentes como invasões, microplásticos, patógenos, estressores cumulativos, contaminantes emergentes e nanomateriais, será essencial para gerar conhecimento científico que informe estratégias de manejo eficazes para preservar a biodiversidade do zooplâncton de água doce em meio às mudanças ambientais em curso. **Conclusões:** Esta revisão destaca a importância de expandir a pesquisa experimental por diversas regiões e tópicos pouco explorados para melhorar nossa compreensão ecológica e melhor gerenciar a biodiversidade do zooplâncton de água doce no Brasil.

Palavras-chave: experimento em laboratório; experimento em campo; duração do experimento; revisão sistemática.

1. Introduction

Experimental studies offer a possibility to test fundamental ecological and evolutionary theories (Connon et al., 2012) across varying degrees of realism in both artificial systems and natural habitats. The experimental approach has been a consistent trend for decades and can help us develop a more sensible relationship with freshwater ecosystems (Hasler, 1964, Odum, 1984). In experimental settings, environmental and biological variables can be controlled, facilitating the generation of robust scientific evidence. These experimental manipulations provide invaluable insights into the causalities between environmental factors, individuals, and populations on structure and dynamics across different levels of biological organization (Srivastava et al., 2004). Experiments with diverse organisms at various spatial and temporal scales have been fundamental to developing different aspects of ecology. These include the distribution (Grime, 1965) and conservation (Tinya et al., 2023) of plants, coastal marine management and conservation (Castilla, 2000), the search for general ecological principles (Borer et al., 2014) and threats of global change (Knapp et al., 2024).

Observational approaches have been a primary source of knowledge on freshwater zooplankton in Brazil, as indicated by Castilho-Noll et al. (2023). Their comprehensive review spanning 121 years reveals critical aspects of zooplankton studies in the country. Most of these investigations have focused on ecological and taxonomic research. Long-term studies have played a pivotal role in enhancing our understanding of various biological and ecological concepts, including global climate changes (Diniz et al., 2023), environmental impacts (Carneiro et al., 2003; Bonecker et al., 2020; Josué et al., 2021), invasive species (Palazzo et al., 2023), predator-prey relationships (Arcifa et al., 2015), and biodiversity (Garrido et al., 2003; Lansac-Tôha et al., 2014; Maia-Barbosa et al., 2014). The review highlights that cladocerans are the most extensively studied organisms in diverse environments, followed by copepods and rotifers. In contrast, protoplankton groups received comparatively less attention (Castilho-Noll et al., 2023).

Over the past century in Brazil, freshwater zooplankton has been used in experimental investigations to test theories (Matsumura-Tundisi et al., 1990) and explore the intricate link between biodiversity and ecosystem processes (Gazonato Neto et al., 2014; Dib et al., 2020). Qualitative and quantitative syntheses, such as scientometric analyses (Souza et al., 2018), systematic reviews (Macêdo et al., 2021), and meta-analyses (Vieira et al., 2015), have been extensively employed to provide a comprehensive overview or nuanced insights into various experimental research topics (Melo et al., 2006). In this context, this research's primary objective is to comprehend the significance of zooplankton experimentation thoroughly. This will be achieved through a systematic review that aims to quantify the temporal evolution of experimental studies, shedding light on the dynamic landscape of research endeavors over time.

This review is mainly based on Castilho-Noll et al. (2023), aiming to expand our understanding of the temporal and spatial dynamics of experimental freshwater zooplankton studies in Brazil. It will identify and highlight the principal topics investigated and delineate areas that have yet to be explored, thereby guiding future research avenues in this crucial ecological domain.

2. Material and Methods

The database used in this review was an update of the ones used in Castilho-Noll et al. (2023) and Elmoor-Loureiro et al. (2023). These authors conducted the research based on papers published from January 1900 to August 2021 and indexed in SciELO (2024), Web of Science (2024), and SciVerse Scopus (2024). The following keywords were employed: "Cladocer*" OR "Rotifer*" OR "Copepod*" OR "Protoplankton" OR "Ciliates" OR "Flagellates" OR "Testate Amoebae" OR "Zooplan*ton" AND "Freshwater" AND "Brai*l". The asterisk (*) was used to encompass all linguistic variations of words. A total of 1,014 papers were identified, from which 201 experimental studies were selected based on the following criteria: (i) exclusion of duplicate articles found in different databases; (ii) inclusion of only experimental studies, excluding reviews, opinions, observational, or theoretical studies. The screening process was conducted by reading the titles and abstracts of the published experimental studies. We used the same methodology described above to update the database and include the years 2022 and 2023 and we founded 46 experimental publications on freshwater zooplankton. Therefore, we identified 249 experimental studies on zooplankton for quantitative and qualitative synthesis.

We identified (i) the year of publication, (ii) location (we used the affiliation of the first author since most experimental studies occur in laboratories of institutions, universities, and companies), (iii) characteristics of the experiment (type of experiment - microcosm, artificial mesocosm, natural mesocosm, *in situ*, volume of experiments – liters), and experiment duration – Days), (iv) species or taxa which the experiment's response variable was obtained and (vi) research topics. Since many studies have multiple objectives, categorizing "research topics" in experimental studies refers to the primary objective addressed in the study rather than a particular one. For example, studies investigating the impact of toxicity of cyanotoxins on zooplankton structure were categorized under the "Eutrophication" topic. Regarding the type of experiment, and definitions of experimental characteristics, four categories were defined: microcosm, artificial mesocosm, natural mesocosm, and *in situ*. In mesocosm experiments manipulations are conducted within laboratories under controlled environmental conditions (artificial) or in the field (natural mesocosm), typically carried out in experimental units ranging in volume from 1 to 100 liters (Srivastava et al., 2004). Microcosm experiments involve manipulations conducted within laboratories under controlled environmental conditions, typically carried out in experimental units with a volume of less than one liter) (Srivastava et al., 2004). *In situ* experiments refer to manipulations conducted within the natural environment. Additionally, the volume data were standardized to liters and the duration of the experiment to days to facilitate accurate comparison and synthesis of data from diverse studies, which often report measurements in varying units. This standardization ensures consistency and enhances the reliability of the analyses. The analyzer was responsible for converting these values to ensure consistency and reliability in the analysis. Some of the information described above may not be found in certain publications and is reported here as 'data unreported.'

We used a simple linear regression analysis (ANOVA, Sokal & Rohlf, 1995) to assess the temporal trend in the number of papers. The number of papers was the response variable and the publication year was the explanatory

variable. The assumptions of the analysis (normality and homoscedasticity) were tested before the ANOVA. The significance considered was $p < 0.05$. The temporal trend of experimental studies was examined for the research topic over time through a Heatmap, a graphical representation of data where the individual values contained in a matrix are represented as colors. The analyses were performed using R software version 4.3.2 (R Core Team, 2023). We used WordCloud (2024) to generate word clouds with species or taxa.

3. Results

The first experimental publication on freshwater zooplankton in Brazil occurred in 1978 (Figure 1a). Simple regression analysis revealed a significant positive temporal trend in the number of experimental papers ($R^2 = 0.70$, $p < 0.001$). Since 2000, there has been an increase in the number of experiments published in scientific journals (Figure 1a). The papers were from 16 of the 27 Brazilian states. The Southeast region of Brazil contributed 63.85% of the total number of 159 papers, with emphasis on the states of São Paulo (40%; N = 101 papers), Rio de Janeiro (12.85%; N = 32), and Minas Gerais (10.44%; N =26) (Figure 1b). An exception in the regions was the state of Espírito Santo, which had no recorded papers. The states of Rio Grande do Sul (11.65%; N =29) and Paraná (8.03%; N =20) in the south region also stand out for the number of papers. No records of experimental papers with freshwater zooplankton have been identified in the states of Amapá, Tocantins, Acre, Alagoas, Ceará, Goiás, Maranhão, Piauí, Rondônia, Roraima, and Sergipe (Figure 1b).

Regarding location, experiments conducted in the laboratory were more frequent, accounting for 85.54% (N = 213), compared to those conducted in the field, which accounted for 14.46% (N = 36) (Figure 2a). Additional analyses revealed some characteristics of the experimental approaches (Figure 2b-g). Most experiments employed the microcosm approach (59.6%; N = 149 number of papers). Experiments in artificial mesocosms were the second most utilized type (16.8%; N = 42), followed by *in situ* experiments (4.4%; N = 11) and natural mesocosms (4.4%; N = 11). Thirty-seven (14.8%) papers did not inform the type of approach (Figure 2b). The median duration of microcosm experiments was five days (max. 365 days and min. 1 hour). In comparison, artificial mesocosms lasted 15 days (max. 75 days and min. one day), natural mesocosms 11 days (max. 182 days and min. 0.48 days), and *in situ* experiments 25 days (max. 120 and min. two days) (Figure 2c). The median volume of microcosm experiments was 0.05 L (max. 1 L and min. 0.001 L) (Figure 2d). In comparison, the median volume of artificial mesocosms was 46 L (max. 1.08×10^6 L and min. 0.1 L) (Figure 2e), the median volume of natural mesocosms was 6.115×10^3 L (max. 3.1×10^{10} L and min. 3.0×10^3 L) (Figure 2f), and the median volume of *in situ* experiments was 0.05 L (max. 1.5 L and min. 0.01 L) (Figure 2g). Of the 249 manuscripts analyzed, 37 did not provide information on the experimental approaches.

The number of papers published within the 15 research topics identified between 1978 and 2023 are in Table 1. The first topics experimentally analyzed were predation and life cycle, which commenced in 1978 (Figure 3). Chemical

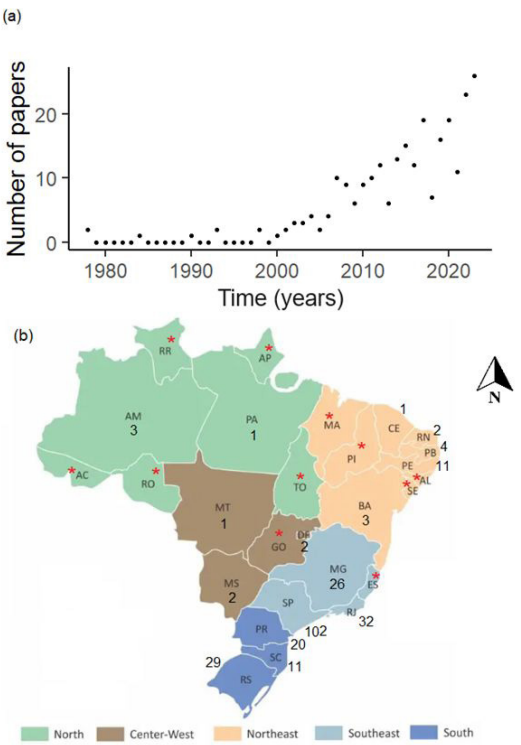

Figure 1. Temporal (a) and spatial (b) distribution of 249 studies published with freshwater zooplankton in Brazil from 1978 to 2023. SP = São Paulo, MG = Minas Gerais, PR = Paraná, RJ = Rio de Janeiro, RS = Rio Grande do Sul, PE = Pernambuco, SC = Santa Catarina, PB = Paraíba, AM = Amazonas, DF = Distrito Federal, RN = Rio Grande do Norte, BA = Bahia, PA = Pará, MT = Mato Grosso, MS = Mato Grosso do Sul, AP = Amapá, ES = Espírito Santo, TO = Tocantins, AC = Acre, AL = Alagoas, CE = Ceará, GO = Goiás, MA = Maranhão, PI = Piauí, RO = Rondônia, RR = Roraima, SE = Sergipe. Red asterisks = no experimental publication found.

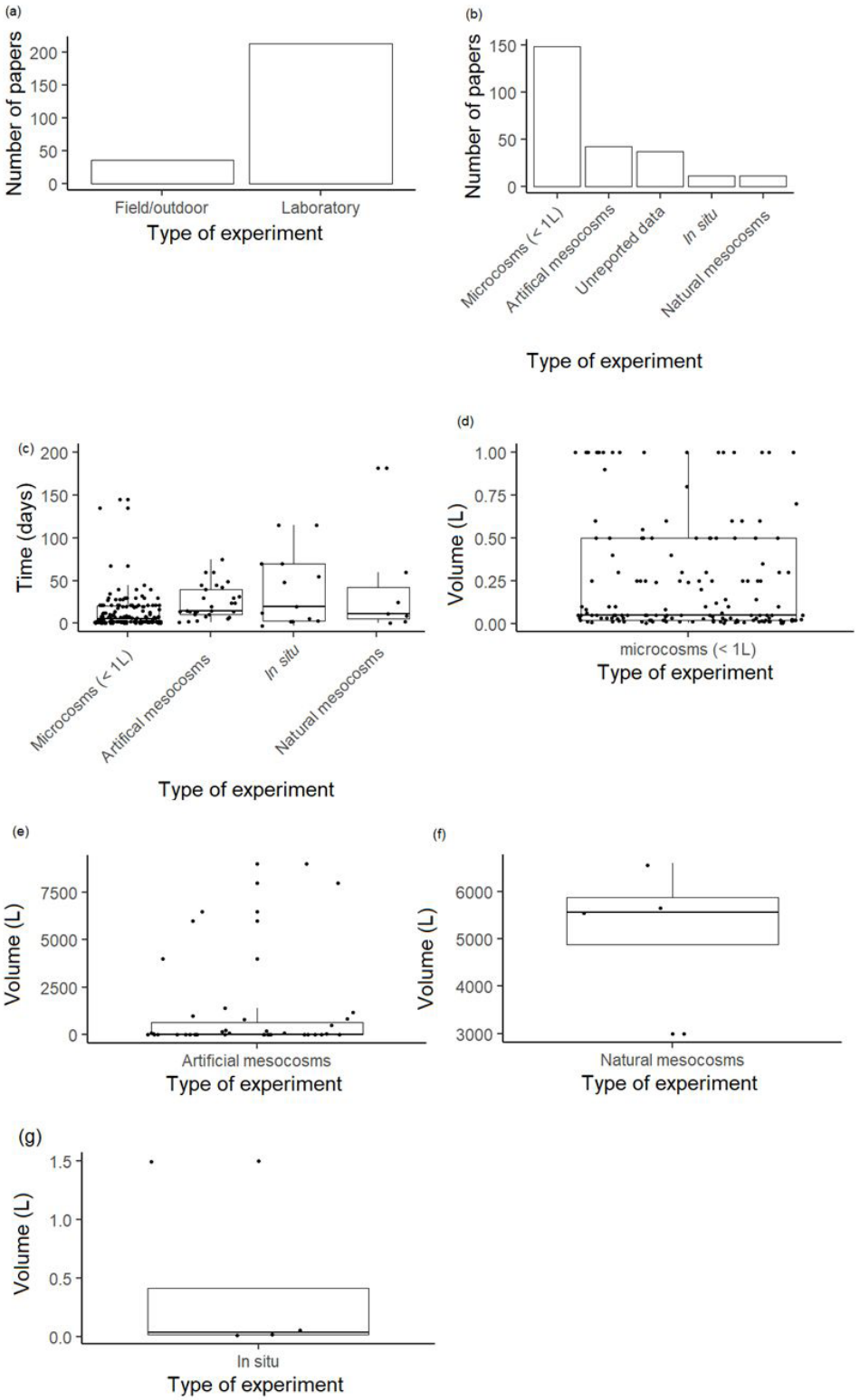

Figure 2. Characteristics of experiments carried out with zooplankton in Brazil. (a) The number of experimental studies with freshwater zooplankton conducted in the field or laboratory (1978 to 2023); (b) The number of studies conducted in each type of experimental approach; (c, d) Jitter boxplots showing the distribution of a set of data (median, minimum value, maximum value, first quartile, third quartile, and outliers) related to the (c) time (i.e., experiment duration in days - For better visualization, the 365-day values are not shown in the graph), and volume used in (d) microcosms, (e) artificial mesocosms, (f) natural mesocosms, and (g) *in situ* in experimental studies with freshwater zooplankton.

pollutants (30.12%; N = 75 number of papers), predation (13.65%; N = 34), and eutrophication (10.44%; N = 26) were the three most frequently addressed topics in experimental manipulations with freshwater zooplankton, particularly from the 2000s onwards (Figure 3). Another experimental research topic that has stood out in the last seven years is resting eggs (9.23%; N = 24). Notably, studies on metapopulation or community themes (1.20%; N = 3), dispersion (1.20%; N = 3), invasion (0.80%; N = 2), microplastics (0.80%; N = 2), and pathogens (0.40%; N = 1) are relatively less common (Figure 3). The three main tags in the word cloud are *Daphnia similis* Claus,1876 (18.07%; N = 45), *Daphnia magna* Straus, 1820 (15.66%; N = 39), and *Ceriodaphnia silvestrii* Daday, 1902 (11.24%; N = 28) (Figure 4).

Table 1. Topics studied in experimental publications with freshwater zooplankton in Brazil (1978 to 2023).

Research topic	Number of papers
Other chemical pollutants	76
Predation	34
Eutrophication	26
Zooplankton recolonization/resting eggs	25
Water/environment vs organisms	23
Pesticides	19
Life cycle	12
Herbivory	10
Trophic cascade	8
Climate changes	7
Dispersal	3
Metapopulation or community	3
Microplastics	2
Pathogens	1
Invasion	1

4. Discussion

Experimental research allowed ecologists to manipulate variables to understand and quantify a wide range of issues encompassing at least 15 research topics in studies of freshwater zooplankton in Brazil (1978 to 2023). We recorded the earliest paper in 1978 (Bertollo, 1978), which focused on the biological aspects of the Brazilian rotifer *Asplanchna* sp. Since 2000, there has been an increase in the number of experimental studies on freshwater zooplankton published in scientific journals. The geographical distribution of these papers is predominantly in the Southeast region, particularly in São Paulo, Rio de Janeiro, and Minas Gerais states. Laboratory experiments were more frequent, with chemical pollutants, predation, and eutrophication being the most frequently addressed research topics. Such findings complement a recent review of freshwater zooplankton in Brazil between 1900 and 2021 (Castilho-Noll et al., 2023) and highlight the main gaps and current challenges in experimental research on freshwater zooplankton in Brazil.

Despite the increasing number of papers, the distribution of these studies is not uniform across

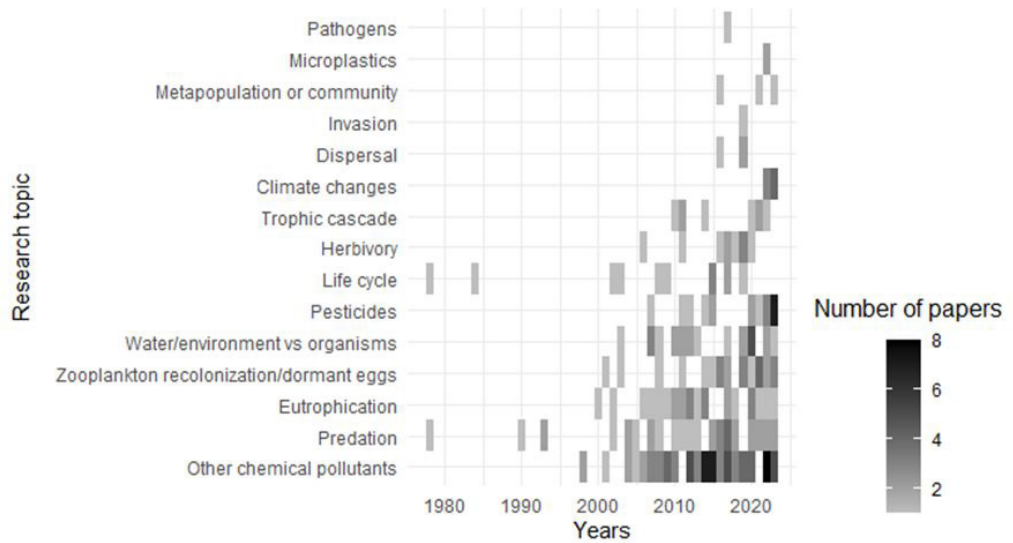

Figure 3. Temporal trend of topics in experimental research with freshwater zooplankton in Brazil. The topics on the y-axis were arranged chronologically as they appeared in papers from 1978 to 2023. Lighter colors indicate fewer studies conducted for a particular year, while darker colors indicate more conducted studies.

Figure 4. Word cloud of key species (words) used in the experimental studies with freshwater zooplankton in Brazil (1978 to 2023). Species that appeared most frequently are shown more prominently.

Brazil. This uneven distribution can be attributed to three main factors. Firstly, the higher concentration of zooplankton experts affiliated with universities and research institutions, particularly those with well-established postgraduate courses (https://sucupira.capes.gov.br/sucupira/), naturally drives a more extensive research possibility that results in a greater focus on experimental studies in the Southeast region. Secondly, limnology is a relatively recent science in Brazil that initially focused on observational studies in the field, and not all researchers conduct experiments. Experimenting is always a challenge, especially when it involves many variables to be controlled. Our data corroborate this explanation because fewer publications were observed for larger-scale experiments. Thirdly, stable funding sources, such as state financing agencies (FAPs), can provide crucial financial support for infrastructure, attracting human resources and fostering research initiatives (McManus et al., 2020; Stegmann et al., 2024).

In contrast, no papers were observed from states including Espírito Santo, Amapá, Tocantins, Acre, Alagoas, Ceará, Goiás, Maranhão, Piauí, Rondônia, Roraima, and Sergipe. Several Brazilian states need enhanced governance capacities and increased research investments (Stegmann et al., 2024). This geographic concentration of research is also evident globally in animal biodiversity, with research being more prevalent in countries of the Northern Hemisphere that boast larger economies and greater research investment (Titley et al., 2017). The combination of a lack of investment in infrastructure, researchers unable to carry out experiments, and insufficient funding could pose challenges to experimental research in these regions. This geographically unbalanced development can limit the advancement of knowledge in zooplankton ecology at the national level. For instance, in invasion ecology, areas that receive little study produce imbalanced knowledge, hindering the field's growth due to a lack of understanding of specific invasion mechanisms present in certain habitats (Pyšek et al., 2008).

Laboratory experiments were more frequent than field experiments. They allow control of all variables, systematic manipulation of one or two variables, and greater ease of both replication within the experiment and repetition. The ecosystems depend on many species and environmental variables and laboratory models can be useless and unrealistic (Diamond, 1983). Field experiments are more naturalistic and provide higher ecological validity than lab experiments. However, the lack of controls

and, in some cases, physical structural complexity and suitable study locations for studies make it challenging to repeat or replicate (Connon et al., 2012). In these experiments, it is easy to lose control of variables. There are many examples of uncontrolled population growth leading to issues like algal blooms, the invasion of predators such as dragonflies, or birds frequenting the experimental units, either preying on or fertilizing them. While this natural variation in some cases may affect treatments equally or occur randomly, it introduces uncertainty in interpreting results. This significantly reduces our confidence in replicating the findings. In addition, we observed that the predominant use of the microcosm approach, also a trend towards experimental studies with phytoplankton in Brazil (Machado et al., 2023), along with variations in experiment duration and volume, highlights the diverse methodologies employed in experimental studies of freshwater zooplankton in Brazil.

The median duration of experiments using microcosms, artificial mesocosms, natural mesocosms, and *in situ* methods was five days, 15 days, 11 days, and 25 days, respectively. Microcosm experiments, with a median duration of five days, are characterized by relatively short time frames of ecotoxicological studies assessing and predicting the effects of an increasing number of chemical stressors on zooplankton species, including the toxicity of cyanotoxin blooms (Sotero-Santos et al., 2007), metal (e.g. Fe, Mn, Zn, Ni, Cd, and Pb) (Lattuada et al., 2009), hormones (Torres et al., 2015), and pesticides (Pitombeira de Figueirêdo et al., 2022; Palma-Lopes et al., 2023). The duration of the experiments varied considerably, ranging from a minimum of 1 hour to a maximum of 365 days for microcosms, illustrating the flexibility in experimental design within this category. Such variability may reflect the specific research questions being addressed, ranging from short-term responses to perturbations to longer-term ecological interactions. In comparison, *in situ* experiments, with a median duration of 25 days, provide a longer observational window, enabling researchers to study more prolonged ecological phenomena (Faria & Cardoso, 2017).

Regarding experiment volume, microcosm experiments had a median volume of 0.05 L, the median volume of artificial mesocosms was 46 L, the median volume of natural mesocosms was 6.115×10^3 L, and the median volume of *in situ* was 0.05 L. The substantial volume range highlights the diverse scales at which ecological experiments are conducted. Microcosms allow the conduct of experiments at a fine scale, facilitating controlled manipulation of variables within a limited space (Connon et al., 2012). In contrast, artificial mesocosms and natural mesocosms involve larger volumes, potentially providing a more realistic representation of ecosystem dynamics at a broader scale (Castilho-Noll & Arcifa, 2007). Differences found in comparative studies of hatching resistance eggs and their probable interference in population dynamics suggest the need for caution when transferring laboratory results to the field (Cáceres & Schwalbach, 2001).

Thirty-seven of the 249 analyzed manuscripts did not explain the experimental approaches employed. This lack of transparency impedes a comprehensive understanding of the methodologies employed in these studies. This is an excellent example of why researchers should strive for more thorough documentation of experimental details to enhance the reproducibility and comparability of the experimental studies on freshwater zooplankton in Brazil. The correct and detailed description of experiments, a necessary step for their efficient analysis, interpretation, and sharing of results, is a fundamental part of the formalization and practice of science (Soldatova & King, 2006).

Papers published within the 15 research topics reveal some notable trends in experimental research involving freshwater zooplankton. Predation, the first topic experimentally analyzed (Bertollo, 1978), remains a topic of interest in current research. Over the years, there has been a notable shift in the focus to experimental manipulations with chemical pollutants (Rosa et al., 2001; Nogueira et al., 2022) and eutrophication (Ferrão-Filho et al., 2000; Kozlowsky-Suzuki & Bozelli, 2002; Soares et al., 2010) emerging as the most frequently addressed topics, particularly since the 2000s. This trend suggests a growing recognition of the importance of understanding the impacts of anthropogenic activities on freshwater zooplankton in Brazil. There has been also a notable increase in experimental studies focusing on resting eggs (Crispim & Watanabe, 2001; Santangelo et al., 2015), indicating their ecological significance and potential implications for population dynamics and community.

Despite significant advances, critical gaps remain in our understanding of freshwater zooplankton ecology. Research areas such as metapopulation dynamics and dispersion, along with emerging threats like invasions, microplastics, pathogens,

cumulative stressors, emerging contaminants, and nanomaterials (Reid et al., 2019), are still relatively underrepresented in the literature. These topics warrant further investigation to elucidate their potential ecological impacts and to inform management strategies aimed at preserving freshwater zooplankton biodiversity amidst ongoing environmental changes.

The word cloud analysis provides additional insights into the species or taxa most studied in experimental research. *D. similis*, *D. magna*, and *C. silvestrii* emerge as the dominant taxa, highlighting their significance as model organisms in zooplankton research in Brazil. This suggests a focus on these species not just due to their ecological importance but also mainly due to their ease of culturing and well-established experimental protocols of ecotoxicology studies. For instance, since the early 1940s, the use of *D. magna* as a model organism in ecotoxicology studies has been scientifically established worldwide owing to its short reproductive cycle and highly sensitive response to external toxicants affecting fecundity (Ebert, 2022).

In conclusion, this review highlights the importance of experimental research in deepening our understanding of freshwater zooplankton in Brazil and contributing to its future development. Our findings suggest a necessity for enhanced governance capacities and increased investments in research in several Brazilian states. We reinforce the need to incorporate metapopulation dynamics, and dispersion, along with emerging threats like invasion, microplastics, and pathogens into experimental studies of freshwater zooplankton in Brazil. Furthermore, coordinated experiments between different researchers and institutions carried out simultaneously with standardized methodology can contribute to answering robust questions related to zooplankton ecology.

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