Universidade Federal de São Carlos
Centro de Ciências Biológicas e da Saúde
Programa de Pós-Graduação em Ecologia e Recursos Naturais

Karime de Araujo Paina

Diversidade taxonômica, funcional e genética da comunidade zooplanctônica do Reservatório do Lobo (Broa, SP-Brasil), em diferentes escalas temporais

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais do Centro de Ciências Biológicas e da Saúde da Universidade Federal de São Carlos, como parte dos requisitos para obtenção do título de Doutora em Ciências: área de concentração em Ecologia e Recursos Naturais.

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Folha de Aprovação

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“Hoje me sinto mais forte
Mais feliz, quem sabe
Só levo a certeza
De que muito pouco sei
Ou nada sei...”

Renato Teixeira e Almir Sater, “Tocando em frente”

Resumo

Nos tempos atuais, têm sido muito frequentes as preocupações com diversos aspectos das questões ambientais, especialmente as que se relaciona com mudanças climáticas. Nesse sentido, estudar e mensurar a biodiversidade dos ecossistemas aquáticos é, sem dúvida, uma das muitas maneiras de pensarmos e agirmos em prol da conservação ambiental. Os ecossistemas aquáticos dulcícolas, ambientes de grande importância para a manutenção do nosso planeta, têm sofrido nas últimas décadas as consequências das atividades antrópicas. Dentre esses ambientes, especialmente os reservatórios merecem destaque, pois desempenham um papel importante no que se refere aos impactos na biodiversidade local e aos benefícios à espécie humana, de diversas maneiras. Neste trabalho, buscou-se analisar como as mudanças climáticas podem impactar os ecossistemas aquáticos, atingindo especialmente as comunidades zooplanctônicas, por meio do estudo de um reservatório tropical do sudeste brasileiro. Realizamos uma análise histórica das últimas cinco décadas, com o objetivo de verificar como as alterações climáticas recentes vêm impactando a dinâmica destas comunidades ao longo dos anos. Também, com enfoque nestes importantes grupos que estão próximos da base da cadeia trófica, buscamos analisar e mensurar a diversidade destes organismos por meio de atributos e análises funcionais. Para caracterizar a comunidade zooplanctônica, buscamos classificar, por meio de ferramentas moleculares, os três grupos mais importantes: Rotifera, Cladocera e Copepoda. Foram realizadas mensalmente coletas da comunidade zooplanctônica, por um período de dois anos, de maio/2019 a abril/2021, no Reservatório do Lobo-Broa. Os dados coletados nesse período recente foram agrupados aos do levantamento histórico dos últimos cinquenta anos, ou seja, desde o início das atividades de estudos desse ambiente. Para as análises de diversidade, as espécies de zooplâncton foram caracterizadas de acordo com seus atributos funcionais e agrupadas a partir de suas funcionalidades no ecossistema, entre Rotifera, Cladocera e Copepoda. Para uma maior compreensão de toda a comunidade zooplanctônica, baseada em análises moleculares, o DNA de cada espécie foi extraído e sequenciado, para análise do marcador molecular COI – *barcoding,* com a finalidade da construção de uma biblioteca de dados moleculares para o zooplâncton, já que essas informações ainda são escassas para ambientes aquáticos brasileiros.

Abstract

Nowadays, concerns about various aspects of environmental issues have become very frequent, especially those related to climate change. In this sense, studying and measuring the biodiversity of aquatic ecosystems is, without a doubt, one of the many ways we can think and act in favor of environmental conservation. Freshwater aquatic ecosystems, environments of great importance for the maintenance of our planet, have suffered the consequences of human activities in recent decades. Among these environments, especially reservoirs deserve to be highlighted, as they play an important role in terms of impacts on local biodiversity and benefits to the human species, in several ways. In this work, we sought to analyze how climate change can impact aquatic ecosystems, especially affecting zooplankton communities, through the study of a tropical reservoir in southeastern Brazil. We carried out a historical analysis of the last five decades, with the aim of verifying how recent climate changes have impacted the dynamics of these communities over the years. Also, focusing on these important groups that are close to the base of the trophic chain, we seek to analyze and measure the diversity of these organisms through attributes and functional analyses. To characterize the zooplankton community, we sought to classify, using molecular tools, the three most important groups: Rotifera, Cladocera and Copepoda. Collections of the zooplankton community were carried out monthly, for a period of two years, from May/2019 to April/2021, in the Lobo-Broa Reservoir. The data collected in this recent period were grouped with those from the historical survey of the last fifty years, that is, since the beginning of study activities in this environment. For diversity analyses, zooplankton species were characterized according to their functional attributes and grouped based on their functionality in the ecosystem, among Rotifera, Cladocera and Copepoda. For a greater understanding of the entire zooplankton community, based on molecular analyses, the DNA of each species was extracted and sequenced, for analysis of the molecular marker COI – barcoding, with the purpose of building a library of molecular data for zooplankton, already that this information is still scarce for Brazilian aquatic environments.

Sumário

Apresentação da tese

Esta tese de doutorado foi organizada na forma de capítulos, os quais foram elaborados conforme as normas de formatação dos periódicos de submissão. Os três capítulos têm como objeto de estudo o Reservatório do Lobo-Broa, localizado no estado de São Paulo, entre os municípios de Itirapina e Brotas. A tese contém, ainda, uma contextualização que tem por objetivo introduzir os assuntos tratados ao longo da pesquisa. Uma breve descrição dos três capítulos se encontra abaixo:

Capitulo 1. Variation during 50 years in the zooplankton community of a tropical reservoir in response to climate variability

Este capítulo abordou uma análise histórica dos estudos com a comunidade zooplanctônica já realizados no Reservatório do Lobo-Broa, desde o ano de 1972, além de uma análise recente, de 2019 a 2021, com o objetivo de estudar os impactos das mudanças climáticas sobre os principais grupos zooplanctônicos que compõem o reservatório.

Capítulo 2: Functional diversity of the zooplankton community in a shallow tropical reservoir

O objetivo deste capítulo foi analisar a comunidade zooplanctônica do Reservatório do Lobo-Broa na perspectiva da diversidade funcional. Para isso, as espécies de Rotifera, Cladocera e Copepoda foram agrupadas em grupos funcionais, a partir das características (traços funcionais). Com isso, foi possível uma análise mais aprofundada da comunidade, com base em suas características e adaptações ao ambiente.

Capítulo 3: DNA barcoding of the zooplankton community of the Brazilian tropical reservoir Lobo-Broa.

Este capítulo teve por objetivo a identificação molecular, por meio de DNA *barcoding* (COI) de algumas espécies da comunidade zooplanctônica, para dar suporte à identificação taxonômica e para a criação de um banco de dados moleculares.

Contextualização

Introdução

O reservatório do Lobo-Broa está localizado na região centro-oeste do estado de São Paulo, entre os municípios de Brotas e Itirapina e inserido na sub-bacia hidrográfica do Ribeirão do Lobo. Ele é afluente do rio Jacaré-Guaçu, o qual é tributário do rio Tietê. Sua rede hidrográfica é formada pelos tributários principais Ribeirão do Lobo e Rio Itaqueri, além dos Córregos do Geraldo, das Perdizes, do Limoeiro, da Água Branca e o Ribeirão da Onça, sendo que somente este último não flui diretamente na represa (Argenton, 2004). Trata-se de um ambiente de grande importância em sua região, tendo sido amplamente estudado nas últimas quatro décadas, com diversos enfoques dentro da Limnologia (Mansano, 2012). Muitos dos trabalhos relacionados à Limnologia no Brasil na década de 1970 têm este reservatório como um dos primeiros a serem estudados no país e um dos mais importantes (Matsumura-Tundisi e Tundisi, 1976; de Afonso, 1978; Angelini; Petrere, 2000). Além da sua importância ecológica para a região, o reservatório possui uma grande relevância para a comunidade local no sentido de lazer e abastecimento urbano (Argenton, 2004).

Diversas pesquisas foram desenvolvidas no Reservatório do Lobo-Broa nos últimos anos objetivando o estudo de diferentes grupos, como os relacionados com comunidades fitoplanctônicas, bacterioplâncton, piscicultura, ecotoxicologia aquática e, principalmente, grupos zooplanctônicos (Rietzler et al, 2002; Tundisi et al., 2004; Lucinda, 2007; Tundisi e Matsumura-Tundisi, 2014; Gatti-Junior, 2015; Tundisi et al., 2015). Especificamente, a comunidade zooplanctônica local foi amplamente conhecida nos últimos quarenta anos, dado o grande número de trabalhos e listas de grupos de zooplâncton que se encontra na literatura sobre esse ambiente (e.g. Rocha e Matsumura-Tundisi, 1976; Matsumura-Tundisi e Tundisi, 1976; Rocha e Matsumura-Tundisi, 1984; Bini et al., 1997; Tundisi e Matsumura-Tundisi, 2014).

Embora haja na literatura um número vasto de informações sobre a comunidade zooplanctônica do Reservatório do Lobo-Broa, há uma lacuna de conhecimento no que se refere aos atributos funcionais das espécies já conhecidas nesse ambiente. Assim, a análise de diversidade funcional aborda características importantes das espécies e, com isso, é possível diagnosticar a estrutura das comunidades que compõem o ecossistema (Mcgill et al., 2006; Smith et al., 2013). Altos valores de diversidade funcional podem estar relacionados à otimização dos recursos disponíveis e de equilíbrio ecossistêmico (Sobral e Cianciaruso, 2012).

De modo geral, para a comunidade zooplanctônica há poucos estudos sobre diversidade funcional. A falta de estudos básicos sobre a biologia desses organismos pode ser uma justificativa, uma vez que com informações mais detalhadas e precisas sobre as espécies é possível explorar um grande número de suas características funcionais. Medidas melhores e mais amplas de diversidade funcional seriam possíveis, o que explicaria ainda mais as funções ecológicas dos organismos e sua relação com os ecossistemas (Paina e Melão, 2019).

Os organismos zooplanctônicos representam o principal grupo de consumidores primários na cadeia trófica aquática. Possuem uma grande variedade de traços funcionais e estratégias ecológicas que influenciam em outros níveis tróficos. Portanto, descrever as características funcionais destes organismos contribui para o conhecimento dos diversos padrões que atuam em diferentes ecossistemas e o quanto e como estão relacionados com padrões ambientais (Litchman et al., 2013). Conhecer as mudanças nos aspectos funcionais das comunidades e sua composição é fundamental para a compreensão de padrões ecológicos que moldam o ecossistema (Petchey e Gaston, 2006).

Estudos ecológicos classificavam, até então, a Represa do Lobo como um ecossistema mesotrófico (Leite e Espíndolola, 2002). Porém alguns estudos nas últimas décadas sinalizam uma preocupante mudança nas características deste sistema, o que pode significar um processo de eutrofização do reservatório (Argenton, 2004; Tundisi e Matsumura-Tundisi, 2014). Essa mudança pode estar associada ao tipo de uso e ocupação de sua bacia hidrográfica, uma vez que têm crescido nos últimos anos a exploração desse ambiente para fins residenciais e atividades de lazer (Argenton, 2004). Neste sentido, é importante uma abordagem que possibilite a relação entre a composição do ambiente e as mudanças provocadas na ocorrência e abundância das espécies.

Pela necessidade de uma investigação integrativa dos ecossistemas, a identificação molecular se torna mais uma ferramenta de grande importância. Ao longo dos últimos anos, houve um avanço científico nas técnicas moleculares, o que permitiu que muitas divergências taxonômicas fossem sanadas, para diversos grupos de organismos (Hebert et al., 2003; Adamowicz, 2015). Especialmente para grupos zooplanctônicos, há diversos trabalhos recentes que enfatizam o sucesso na identificação por ferramentas moleculares, em diversas localidades (Baek et al., 2016; Elías-Gutiérrez et al., 2018; Zhang, et al., 2018). Porém, para o Brasil, ainda há um pequeno número de estudos com esse enfoque (Silva et al., 2014; Castilho et al., 2015; Rocha et al., 2019).

Em face de lacunas de conhecimento ainda existentes sobre a diversidade de espécies zooplanctônicas no Brasil, devido às possíveis incertezas taxonômicas desses grupos, além da eficiência de análises com DNA *Barcoding* e diversidade funcional como valiosas ferramentas de estudo, o presente estudo buscou investigar a divergência genética e funcional da comunidade zooplanctônica do Reservatório do Lobo-Broa. A correta identificação das espécies é crucial em estudos ecológicos que visem o entendimento dos ecossistemas e a preservação da biodiversidade.

Justificativa

Buscando uma maior compreensão da dinâmica e funcionamento dos ecossistemas aquáticos, é importante conhecer todos os processos que influenciam a distribuição das espécies, tanto em escalas menores quanto em macroescalas (Ricklefs, 1987). Frequentemente se tem buscado investigar a diversidade biológica dos organismos por meio de diversas teorias ecológicas de comunidades, as quais abordam padrões espaciais e temporais, tais como as teorias da sucessão ecológica (Gleason, 1927) e da biogeografia de ilhas (Macarthur e Wilson, 1967). Considerando as características e a complexidade de cada ambiente, têm surgido diversas maneiras de se estudar a biodiversidade, com enfoque múltiplo, considerando que as muitas medidas de biodiversidade existentes são, na maioria, complementares (Magurran, 2004; Buckland et al., 2005).

Alguns índices clássicos de diversidade, como Shannon, Simpson e Margalef são muito utilizados em análises biológicas, porém são falhos na predição da estrutura e funcionamento dos ecossistemas. Isso ocorre pelo fato de que consideram somente a representação do número de espécies, mas não captam as respostas dessas espécies às pressões do ambiente (Cianciaruso et al., 2009). Há, porém, diferentemente dessas métricas, outra forma de medida que considera aspectos funcionais, já que aborda características como as comportamentais, fisiológicas, morfológicas e o ciclo de vida dos indivíduos das espécies. A essas características denomina-se “traços funcionais” das espécies (Violle et al., 2007; Webb et al., 2010; Litchman et al., 2013).

Na análise da diversidade funcional, a partir dos traços funcionais dos indivíduos, as espécies podem ser classificadas em grupos funcionais, sendo que as mesmas se relacionam diretamente entre si pelas funções que desempenham no ecossistema. Cada espécie identificada pode possuir um número extenso de traços funcionais. No caso da comunidade zooplanctônica, muitos dos traços identificados podem não estar disponíveis para exploração (Barnett et al., 2007; Litchman et al., 2013). A partir das características das espécies, do ambiente e do objetivo do estudo, pode-se definir os traços a fim de se conhecer os grupos funcionais, ou os agrupamento de espécies que respondem de maneira equivalente às características ambientais (Mcgill et al., 2006; Litchman et al., 2013).

Representantes de uma parcela muito importante dos ecossistemas aquáticos, as espécies zooplanctônicas foram objeto, até o presente, de poucos estudos relacionados a traços funcionais, diferentemente de outros grupos biológicos (Barnett e Beisner, 2007; Sodré, 2014; Silva, 2015). Como são bons indicadores ambientais, as condições do ecossistema local podem atuar como filtros para organismos zooplanctônicos, propiciando a coexistência de grupos com características funcionais equivalentes (Webb et al., 2002; Sobral e Cianciaruso, 2012).

Com o crescente uso de ferramentas moleculares, tem sido possível solucionar muitas dúvidas relacionadas à taxonomia de espécies de modo geral, assim como de organismos aquáticos. Dentre as ferramentas utilizadas, a análise de DNA *Barcoding* se destaca por sua eficácia no que se refere a identificação (Adamowicz, 2015). A região de DNA *Barcoding* se refere a um segmento do gene correspondente à enzima mitocondrial citocromo *c* oxidase subunidade I (COI), com tamanho aproximado de 650 pares de bases para animais (Hebert, et al., 2003; Valentini et al., 2009). No que se refere a estudos ecológicos, esta região é considerada bastante importante, sendo amplamente utilizada como ferramenta de identificação e parâmetro para delimitação de espécies em um grande número de animais (Hebert et al., 2003; Hebert e Gregory, 2005; Joly et al., 2014).

O Reservatório do Lobo-Broa é um corpo de água bastante estudado, de diversas perspectivas, inclusive com grande enfoque em comunidades zooplanctônicas (Barbosa et al, 1984; Bini, et al, 1997; Rietzler et al, 2002; Tundisi e Matsumura-Tundisi, 2014). Porém não há estudos relacionados a trabalhos moleculares nesse reservatório, assim como há poucos estudos com análises moleculares de grupos planctônicos no Brasil.

Objetivos

Esta tese teve por objetivo a análise histórica, da diversidade taxonômica, funcional e genética da comunidade zooplanctônica do reservatório do Lobo-Broa, ao longo de um período de tempo. Para tanto, realizou-se:

- A investigação da flutuação populacional da comunidade zooplanctônicas com enfoque em uma variação temporal, a partir da análise de compilação histórica de dados de cinquenta anos do reservatório.
- A identificação de traços e grupos funcionais, a fim de analisar índices de diversidade funcional da comunidade zooplanctônica de um ponto amostral do reservatório, em um período de vinte e quatro meses.
- A identificação da comunidade zooplanctônica por meio de técnicas de *barcoding,* com o marcador molecular COI.

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Capítulo 1. Zooplankton community composition variation over 50 years on a tropical reservoir

Abstract

Temporal variation of zooplankton was studied in the Lobo-Broa Reservoir, southeastern Brazil, which ongoing changes in the rainfall regime in recent decades. We analyzed data from two different time scales: referring to the last five decades, and those obtained monthly for a period of two years (2019-2021), analyzing zooplankton, phytoplankton, and limnological variables. We found 219 taxa in the long temporal sequence and 63 taxa in recent monthly samplings, for the zooplankton community. The variations between the dry and rainy periods were not significant when analyzed within each year, but were different when compared between the years. Ordering analysis (NMDS) pointed to clear inter-annual differences between the 1970s -1990s and after 2010, and the beta diversity calculated for the years suggests one-off inter-annual turnover effects. It is possible to affirm that the dynamics of the zooplankton community underwent marked changes in the sampled period, possibly due to the nutrient inputs from the upstream basin, resulting in a slight eutrophication process with reflections on the zooplankton community, and in the less rainy years, the effects were most evident.

Keywords: Seasonality, diversity, long-term study, climate changes.

1. Introduction

Reservoirs are artificial lentic environments built for single or multiple services (Sen, 2021) that can influence the global and local hydrological cycle (Ogilvie, 2019). Nevertheless, these water bodies can be of great importance in many environmental processes (St. Louis et al., 2000; Ogilvie, 2019). Most reservoirs were constructed for multiple purposes, such as electric power generation, flood control, irrigation, urban supply, aquaculture, and recreation (ICOLD, 1988). As a result, they are constantly exposed to several influences that can cause significant changes in their biota composition and dynamics. With the growth of the human population, especially in developing countries, an increasing volume of water will be required for the demands of the agricultural, industrial, energy, and environmental sectors. Therefore, it is important to understand all biological and ecological processes that occur in reservoirs for the maintenance of services and the good quality of the system (Hansen et al., 2020).

Regarding the reservoir biota dynamics, the zooplankton represents the main group of primary consumers, and it has a wide variety of ecological strategies that influence other trophic levels, since the producers (e.g. phytoplankton, cyanobacteria) to other consumers (other invertebrates, fish), which are linked whether in the bottom-up or in the top-down control. Therefore, the understanding of the temporal dynamics of these organisms contributes to the knowledge of the different patterns of dynamics that occurs in aquatic ecosystems and, consequently, to the biodiversity preservation (Bonecker et al., 2013). A set of ecological factors can influence the zooplankton biodiversity in reservoirs (Picapedra et al., 2020), such as direct and indirect effects of rainfall; input of nutrients strongly influenced by the characteristic and the use of the soil and the environment around the basin; temperature changes, among others. Finally, studies on the zooplankton community are extremely important to understand the interaction of these organisms in the trophic chains and how different factors alter the functioning of communities.

Many studies have focused on the zooplankton dynamics in Brazilian reservoirs during periods of high and low rainfall (Santos-Wisniewski & Rocha, 2007), but generally for short-term annual cycles (Sartori et al., 2009; Perbiche-Neves and Nogueira, 2010, 2013). Few studies, both in Brazil and globally, have investigated zooplankton over longer temporal scales, such as up to 10 years (Picapedra et al., 2020). Long-term studies on zooplankton in reservoirs remain scarce (e.g., Horn & Horn, 1995; Hart, 2004; Beaver et al., 2018), despite the fact that they provide valuable information for understanding the relationships between the environment and changes in species occurrence and abundance over extended time periods. Such information is essential for minimizing or mitigating problems related to water quality (Tundisi and Matsumura-Tundisi, 2003).

The effects of interannual changes in zooplankton may be linked to climatic variations, such as precipitation and temperature, leading to a shift in predominant trophic groups. Large filter feeders, such as daphnid cladocerans and calanoid copepods, may be replaced by smaller organisms such as bosminid cladocerans and generalist rotifers (Picapedra et al., 2020). Changes in rainfall patterns due to climatic changes and increased nutrient input from anthropic sources can affect both bottom-up and top-down control, with direct impacts on the phytoplankton community and ichthyofauna (Marcogliese et al., 1989; Starling & Rocha, 1990; Starling, 1993; Arcifa et al., 1997). However, a long-term study of Lake Biwa in Japan demonstrated that higher trophic levels were not influenced by eutrophication in some cases (Hsieh et al., 2011).

The rise in global mean temperature has an impact on precipitation patterns, altering hydrological cycles, which in turn impacts aquatic ecosystems, potentially leading to the dominance of cyanobacteria (Hsieh et al., 2011; Tundisi et al., 2015). During periods of drought, the residence time in reservoirs tends to increase, leading to blooms (Tundisi et al., 2015). Furthermore, changes in rainfall volume can exacerbate nutrient effects, leading to the growth of phytoplankton.

Our objective was to conduct a long-term analysis of zooplankton in the Lobo-Broa Reservoir located in southeastern Brazil. Ecological studies until the 2000s classified the reservoir as a mesotrophic ecosystem (Leite and Espíndola, 2002). However, subsequent studies in the following decades have indicated changes in the reservoir's characteristics with an undesirable increase in eutrophication (Argenton, 2004; Tundisi and Matsumura-Tundisi, 2014), which may be associated with the type of land use and occupation in the watershed. This study aims to understand the interannual variations that have occurred in the zooplankton community of the Lobo-Broa reservoir over a long (50 years) and short (2 years) period of time, and to identify the factors influencing these variations.

2. Methodology

2.1. Study area

The Lobo-Broa reservoir is located in the state of São Paulo, Brazil. It has a maximum length of 7.5 km, an average depth of 3.0 m and a surface area of 6.8 km (Rocha & Matsumura-Tundisi, 1984). The region has a humid subtropical climate – with dry winters and hot summers (Cwa), according to the Köppen climate classification, and annual rainfall of 1,300 to 1,600 mm (Köppen, 1936).

The reservoir is polymictic and has a volume of 22 million m^3, and its residence time is less than 25 days. Although it is relatively small and shallow, the reservoir was originally built for electricity generation in 1936 (Tundisi & Matsumura-Tundisi, 2016). It is an environment of great multiple importance to the local community.

2.2. Data collection

The present study was based on two data series: i) recent series – monthly time variation considering two consecutive seasonal cycles, between 2019 and 2021; ii) Historical series – interannual temporal variation over five decades (since 1972).

2.2.1. Samples for the recent series

Samplings were carried out monthly between May 2019 and April 2021, at a sampling site in the lentic region of the reservoir and close to the dam (22º 10’ 43.5”S, 47º 53’ 44.5”W) (Fig. 1).

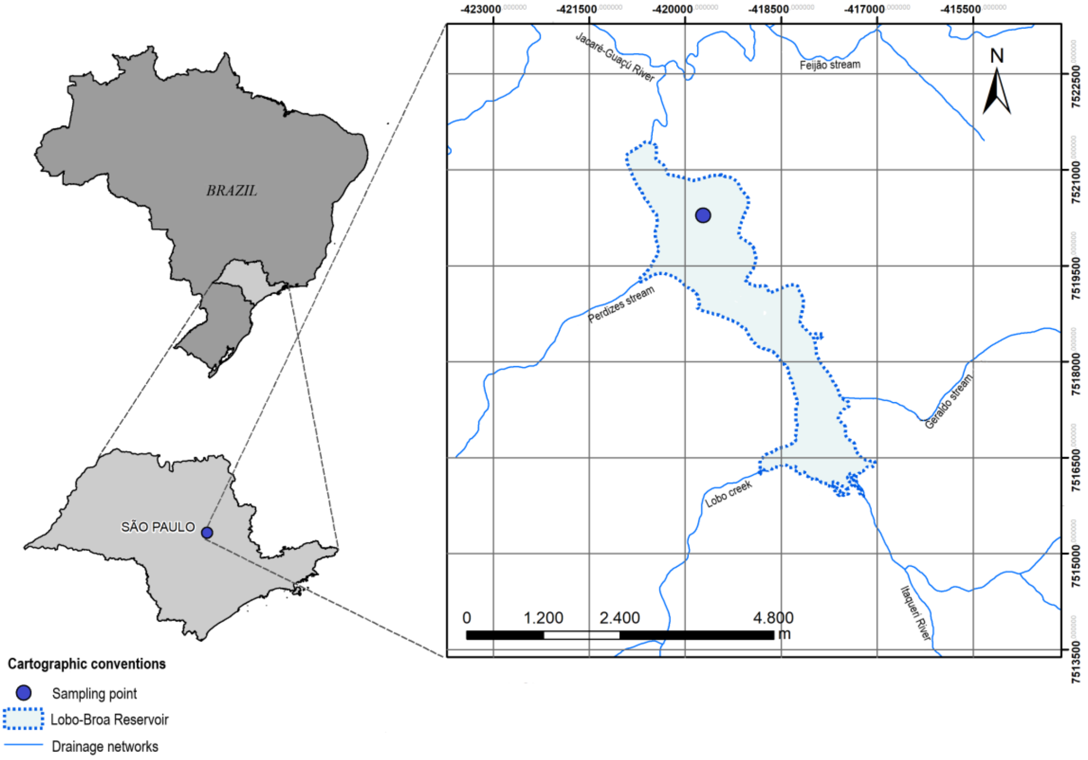

Fig. 1. Map of the study area in the Lobo - Broa Reservoir and its tributaries rivers, with emphasis on the sampling point.

Environmental variables were measured during each sampling event, including water transparency using a Secchi disk, water temperature, pH, and conductivity using a Horiba U50 multiparameter probe. The concentration of suspended solids, both organic and inorganic, was quantified using a gravimetric method (Cole, 1975), where samples were filtered through 45mm GF-C glass fiber micro filters with a 1.2 µm porosity. The micro filters were previously calcined in a muffle furnace at 450°C and weighed using a precision micro analytical scale. Chlorophyll a concentration was obtained using the extraction method with 90% acetone (Golterman et al., 1978), with readings taken on a spectrophotometer at wavelengths of 663 nm and 750 nm.

Rainfall data between 1970 and 2021 was collected from the DAEE-São Paulo for the Graúna station, which is located 7 km away from the Lobo-Broa Reservoir. For zooplankton sampling, vertical hauls were conducted using a conical plankton net with a mesh size of 45µm and opening by 7 meters along the water column. A volume of 500 L was filtered per sample, and the samples were preserved in buffered 4% formaldehyde for later identification in the laboratory.

The taxonomic identification of zooplankton species was based on the following references: Koste (1986, 1987), Segers (1995), and Nogrady (2002) for Rotifera; Smirnov (1974), Elmoor-Loureiro (1997), Sinev (2001), and the website Cladóceros do Brasil (https://cladocera.wordpress.com/) for Cladocera; and Reid (1985), Perbiche-Neves (2011), and Perbiche-Neves et al. (2015) for copepods. For qualitative analysis, a stereoscopic microscope (with magnification up to 50x) and an optical microscope (with magnification up to 1000x), both equipped with a millimeter eyepiece, were used. For quantitative analysis based on population density (individuals.m-3), subsamples were counted in a Sedgewick-Rafter chamber under an optical microscope for Rotifera and nauplii, and in checkered acrylic chambers under a stereoscopic microscope for Cladocera and Copepoda.

2.2.2. Obtaining data for the historical series

We conducted an extensive search for scientific papers on the Lobo-Broa reservoir in Brazil published between 1970 and 2022. The databases used were: Scielo (https://www.scielo.org/), Clarivate-Web of Science (https://www.isiwebofknowledge.com), Google Scholar e Scopus (https://www.scopus.com). The following search terms were used: “Brazilian Reservoir” OR “Lobo Reservoir” OR “Broa Reservoir” Lobo-Broa Reservoir” OR “Carlos Botelho Reservoir” OR “Itirapina Reservoir” AND Zooplankton AND Cladocera AND Copepods AND Rotifera AND Phytoplankton.

The studies that were not initially found in the search but were cited in the papers identified in the search were also included in the analysis. In addition, searches were conducted in the repositories of major Brazilian universities and research institutes to obtain dissertations and theses related to the Lobo-Broa reservoir. Based on the literature review and the data collected between 2019-2021, tables were organized to summarize the presence/absence and abundance of zooplankton and phytoplankton, as well as the values of the limnological variables.

2.3. *Data analysis*

The data were categorized into inter-annual categories (historical series), which were further studied in detail for 24 months (recent series), and into intra-annual categories (dry and rainy periods). Environmental variables associated with zooplankton data were ordered spatially and temporally using a principal component analysis (PCA), with data transformed using LogX+1, except for pH. The data were standardized using Pearson's correlation, which is appropriate for variables in different units. Collinear variables that were positively correlated were removed. The statistical analysis was performed using R 4.1.2 software (R Core Team, 2019), and the ggplot2 package was used to create graphs. The significance level between environmental and biotic parameters was analyzed using the Monte Carlo test, with a significance level set at $p \leq 0.5$.

Zooplankton data were analyzed and classified into three categories: Rotifera, Cladocera, and Copepods. The analysis was explained based on their use as either recent series or historical series. Species richness, alpha diversity using the Shannon-Wiener index (H´), and the components of beta diversity were calculated using the Vegan package (for alpha diversity) and the Tidyverse, Betapart, and Reshape2 packages (for beta diversity) in the R 4.1.2 software (R Core Team, 2019).

To analyze interannual and intra-annual variations in community structure, we used PERMANOVA with 1000 free permutations to compare the effects of years (2019, 2020, and 2021), periods (dry and rainy), and even years*periods, with a significance level of 0.05. Additionally, a non-metric multidimensional scaling analysis was performed using the Vegan package in R 4.1.2 (R Core Team, 2019). Analysis of variance (ANOVA) and betadisper were conducted to verify the heterogeneity of data, primarily intra and interannual. The abundance of organisms transformed into Bray Curtis dissimilarity was used with the Betapart package in R 4.1.2 (R Core Team, 2021). Graphics were created using the Ggplot2 package in R 4.1.2 software (R Core Team, 2019). Taxa such as Bdelloidea, nauplii, and copepodites of Cyclopoida and Calanoida were not included in the non-metric multidimensional scaling (NMDS) analysis because they are not species, but rather a grouping of orders due to the inability to identify them at the species level. To assess the spatial pattern of beta diversity and overall community dissimilarity in this study, we used a partitioning measure for the Bray-Curtis dissimilarity index.

To find for the effects of limnological variables on zooplankton community abundance, a distance-based redundancy analyzes (db-RDA) was performed with 1,000 permutations, also using the Vegan and Betapart packages to transform abundance into Bray-

Curtis dissimilarity, in R 4.1.2 software (R Core Team, 2019). All data, with the exception of pH, were transformed into LogX+1, and filtering of collinear variables was done with VIF.

Beta diversity was calculated to help understand possible changes over time or not in the zooplankton community for data collected on a more refined time scale, between 2019 and 2021. Beta diversity analysis was performed for the phytoplanktonic and zooplanktonic communities. The similarity or dissimilarity of the composition of the communities over the years studied was measured using the Sørensen index. The beta diversity pattern was assessed using the abundance-based dissimilarity indices model from Baselga (2017). Abundance-based dissimilarity can be broken down into two components: I. Balanced variation in abundance, when individuals of one species in one location are completely replaced by individuals of another species in another location, or for this work in question, in another campaign sample (turnover); and II. Abundance gradients, when a species is absent from one location or sampling campaign to another (nestedness, or nesting) (Anderson, 2011; Baselga, 2010).

For the analysis of long temporal variation, the presence and absence data (binary) were also analyzed with NMDS, and the Bray-Curtis index was used to obtain dissimilarity matrices. Species with only one occurrence were removed. For phytoplankton, presence and absence data of the classes Chlorophyceae, Cyanobacteria, Trebouxiophyceae, Bacillariophyceae, Coscinodiscophyceae, Cryptophyceae, Dinophyceae, Euglenophyceae, Xanthophyceae and Zygnemaphyceae were used. Abundance data in the long temporal range were analyzed with generalized linear models, due to the greater variation in abundance and the non-linearity to use parametric statistics.

3. *Results*

3.1 Recent time series

3.1.1 Limnological variables and rainfall

In the PCA analysis used for the temporal ordering of the limnological variables, the first two components explained 55% of the data variance (see Fig. 2). The raw data of the environmental variables analyzed can be found in Table 1 of the supplementary material (S1).

The environmental variables were separated into the dry and rainy periods in both components. During the dry period, the variables with the highest correlation were chlorophyll and turbidity, as well as several months in 2020 with less rainfall (refer to Fig. 3). The rainy season showed positive correlations with conductivity, temperature, pH, depth, and transparency.

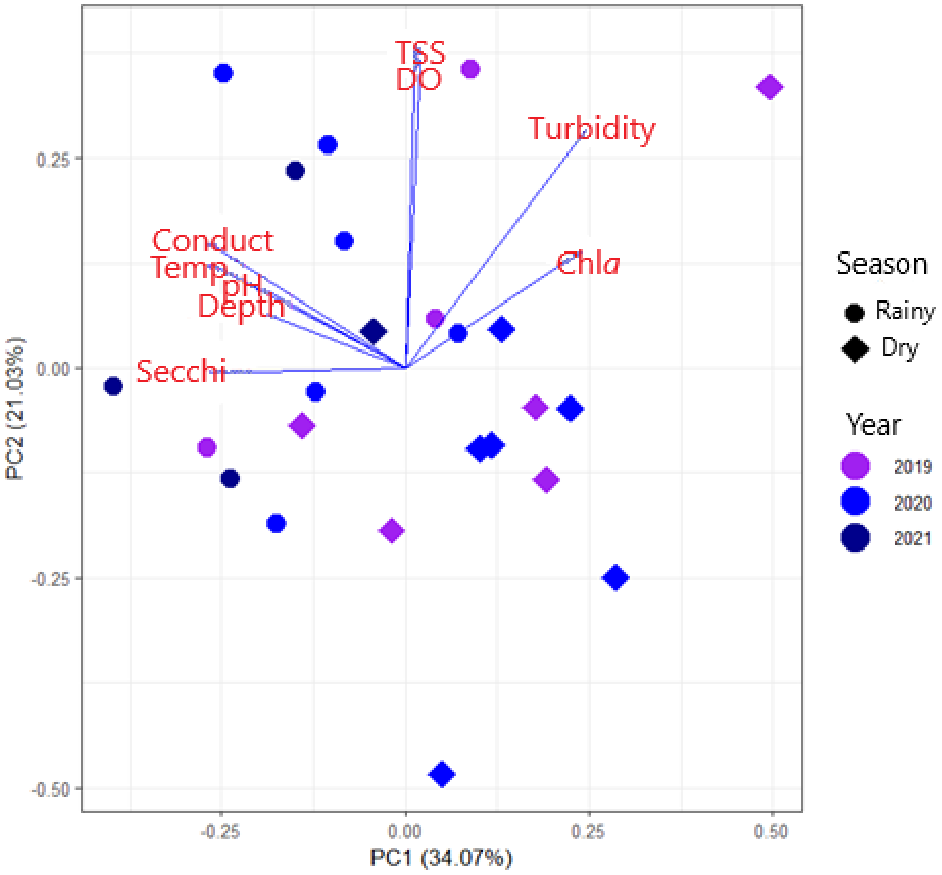

Fig. 2. Principal Component Analysis (PCA) for the temporal ordering of environmental variables over 24 months, between the years 2019 and 2021, also considering the dry and rainy months.

Rainfall showed variable patterns between 1970 and 2021. Considering the average annual rainfall for the region, 1500 mm, many years throughout the historical series had low average annual rainfall. For the 2-year sampling period, seven years were below the average (2007, 2008, 2009, 2012, 2013, 2019 and 2020), with 2007 being the year with the lowest rainfall. Including these years, over the 50 years of rainfall data, nine years had lower-than-average annual rainfall (1978, 1983, 2007, 2008, 2009, 2012, 2013, 2019, and 2020). In contrast, six years presented atypical rainfall higher than the average, for example, in the years (1975, 1981, 1982, 1990, 1994, 2003, 2010, 2011) the accumulated annual rainfall was up to 1700 mm. Periods of intense drought and excessive rainfall higher than the mean were observed every five years from mid-2000 onwards, and previously, based on these data, oscillations were close to every 10 years.

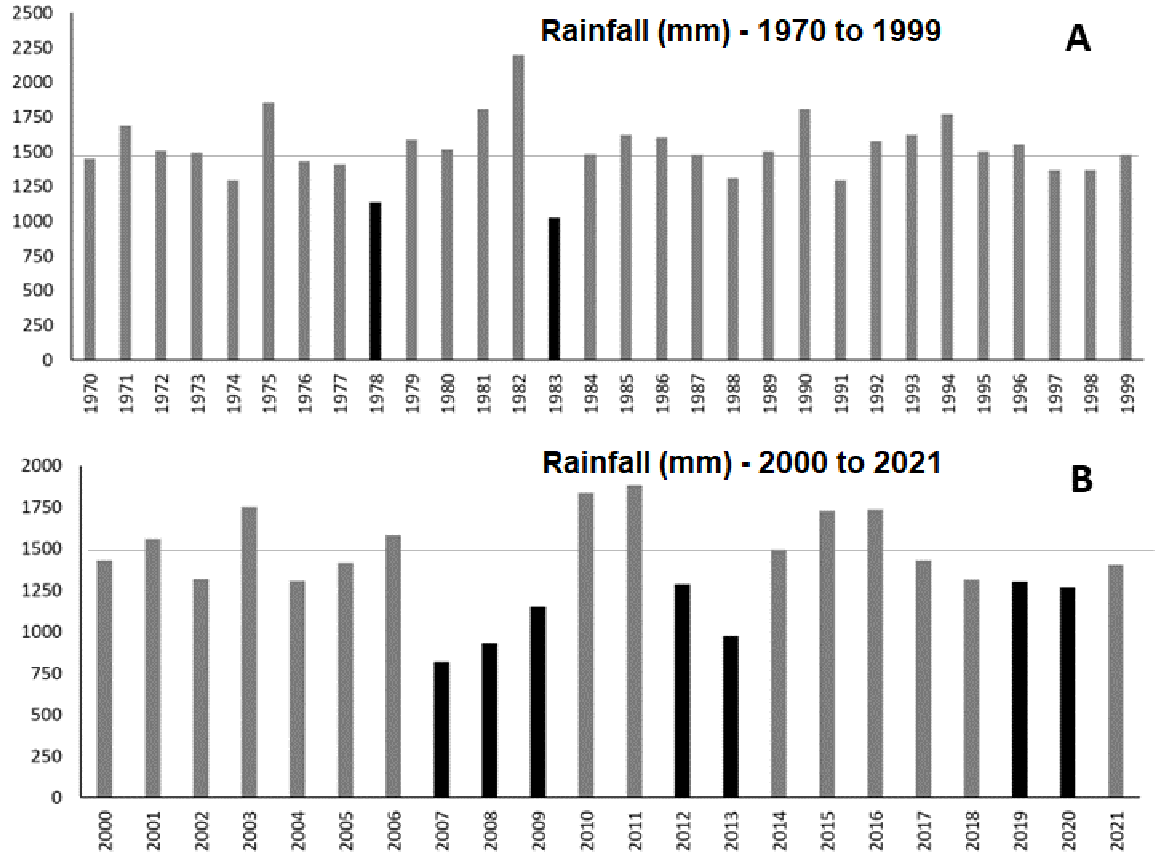

Fig. 3. Rainfall variation in the present study: A. 1970-1999, B. 2000-2020. Black bars indicate years with rainfall below the approximate annual average of 1500 mm.

3.1.2 Zooplankton richness and diversity

The number of taxa found in the 24 sampling months for the three studied groups, Rotifera, Cladocera, and Copepoda, was 63. Among the three groups, Rotifera had the highest species richness, followed by Cladocera and Copepoda. The supplementary material – S2 provides a list of all the taxa. Zooplankton species richness of the three groups varied from 21 to 36 taxa during the 24 months of sampling (Table 1). There was no clear relationship between species richness and the months with higher or lower rainfall, but the diversity and evenness indices were generally high in most of the sampled months, indicating that the zooplankton community underwent species alternation, particularly during periods with low rainfall and in months that differed from the typical rainfall patterns for the region.

Tab. 1. Shannon (H') diversity indexes variation, Pielou (J') equitability, and species richness (S) for the zooplanktonic community between May 2019 and April 2021.

Season	H'	J'	S
May/19	2.79	0.92	21
June/19	2.65	0.76	33
July/19	2.42	0.74	26
August/19	2.89	0.83	33
September/19	1.94	0.55	34
October/19	1.78	0.56	24
November/19	2.6	0.74	34
December/19	1.74	0.51	31
January/20	2.59	0.79	26
February/20	2.67	0.78	30
March/20	2.56	0.8	24
April/20	2.52	0.77	26
May/20	2.2	0.71	22
June/20	2.47	0.73	30
July/20	2.39	0.7	32
August/20	2.22	0.71	23
September/20	2.46	0.79	21
October/20	2.37	0.74	25
November/20	1.88	0.55	31
December/20	2.82	0.82	31
January/21	2.09	0.68	22
Febreary/21	2.9	0.83	33
March/21	2.56	0.75	30
April/21	2.51	0.7	36

Considering the beta diversity of the total zooplankton community, the sampling period exhibited a low nestedness value, likely due to species loss. A higher turnover/replacement value was observed at the beginning of the driest months in 2019 and the wettest months in 2020 and 2021. This pattern was also observed for the phytoplankton community during these rainfall periods. The dissimilarity of the total zooplankton community for the 63 taxa was low (0.24), with beta partitioning indicating that the low dissimilarity may have resulted from a balance in species abundance variation. Rotifera exhibited a low similarity (0.27), resulting in a less distinct assemblage. This low distinction occurred differentially in 2019 and 2021 during the rainy and dry seasons, respectively. Cladocera exhibited low dissimilarity for both the rainy and dry periods, with species composition overlapping practically throughout the collection period. For Copepoda species, nesting predominated during the period and there was low dissimilarity in relation to the rainy period (0.16). The months with the highest rainfall exhibited high values of beta diversity, especially in 2019 and 2021 (Fig. 4).

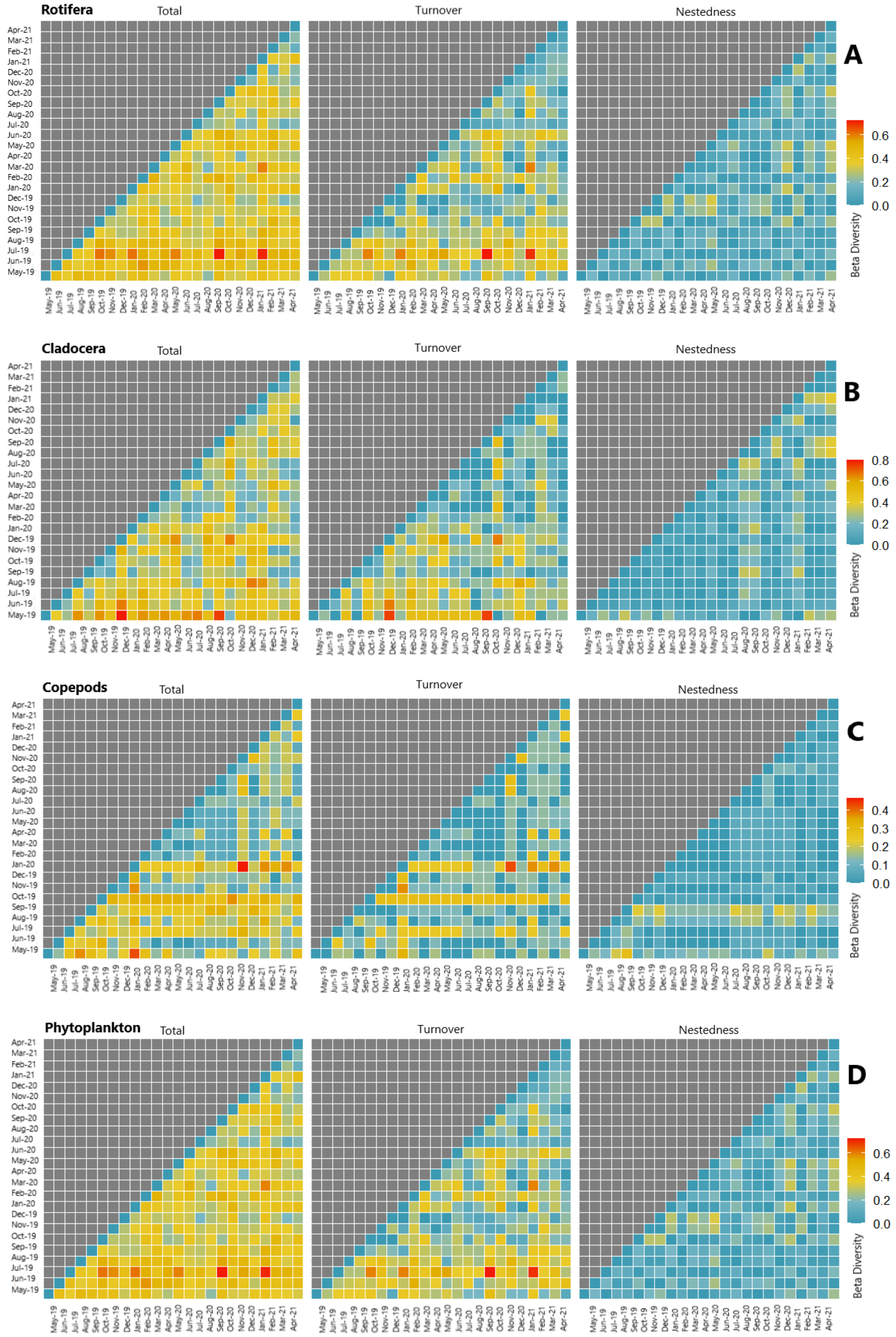

Fig. 4. Representation of the components of total Beta diversity, turnover and nestedness for zooplankton and phytoplankton communities: A: Rotifera, B: Cladocera, C: Copepods and D: Total phytoplanktonic community.

3.1.3 Seasonal and interannual variation of zooplankton

ANOVA did not show a significant variation in dissimilarity (betadisper) between years and periods for the total zooplankton community (years: F=1.52, p=0.23; periods: F=1.00, p=0.99) and for the groups separately (Rotifera, Cladocera, and Copepoda), making it valid for analysis using NMDS. NMDS results indicated low or no intra-annual variation between dry and wet periods, but clear differences between years for all zooplankton categories (Figure 5 A-F). This finding was supported by PERMANOVA (Table 2), which showed an almost significant difference (p=0.06) for the total zooplankton and Rotifera in the interaction between periods and years, suggesting that within each year, there may be a more intense effect of the periods, particularly during atypical rainfall, mainly below the annual average.

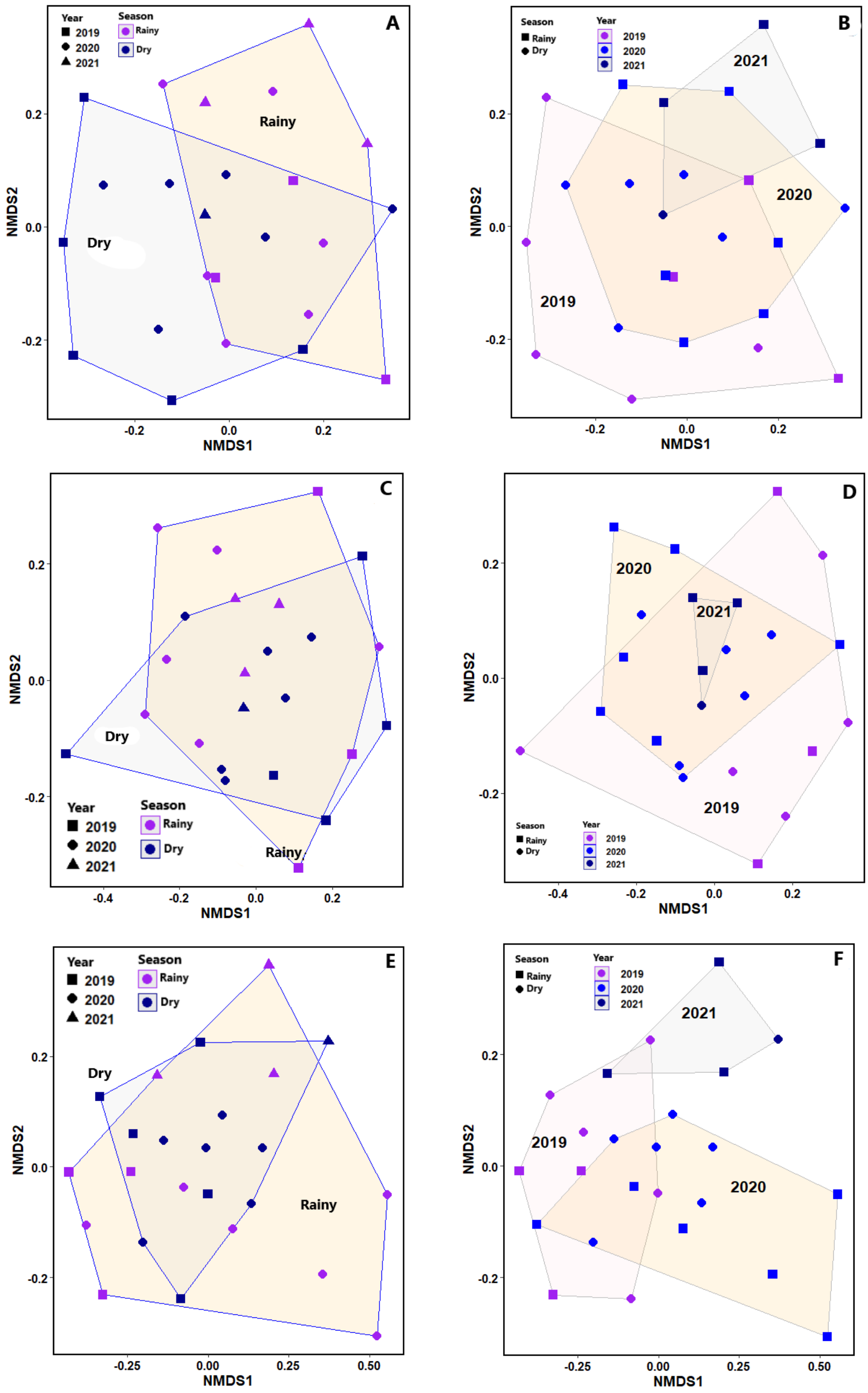

Fig. 5. Biplot of NMDS analysis based on abundance and dissimilarity the three-zooplankton groups, A and B: Rotifera, without Bdelloidea; C and D: Cladocera; E and F: Copepods.

Tab. 2. ANOVA on abundance dissimilarity (beta-disper) and PERMANOVA values for zooplankton community: Rotifera, Cladocera and Copepods. In bold with * the significant differences between the factors.

	Rotifera		Cladocera		Copepoda	
ANOVA	F	p	F	p	F	p
Year	0,33	0,71	0,33	0,71	0,38	0,68
Season	0,16	0,68	0,16	0,68	0,84	0,36
PERMANOVA	R2	p	R2	P	R2	p
Year	0,14	**0,03***	0,26	**0,00***	0,19	**0,01***
Season	0,03	0,39	0,02	0,61	0,02	0,61
Year: Season	0,12	0,06	0,06	0,53	0,09	0,25

3.1.4. Relationship of zooplankton with limnological variables and phytoplankton

In the db-RDA analyses, the lowest percentage of explanation was found for the first two components for the rotifers (42%), copepods (49%), and cladocerans (54%). Unlike Cladocera and Copepods (Figure 6B, C), rotifers (Figure 6A) showed a similar temporal ordering trend, with the months of the dry period of the year 2019 being positively correlated mainly with turbidity, chlorophyll-*a*, and with the classes Euglenophyceae, Dinophyceae, and Charophyceae. Collections in the remaining months of the three years were associated with rain, transparency, depth increase, temperature and conductivity, and also with the classes Ochrophyceae and Cryptophyceae.

For micro crustaceans, the effect of increasing the abundance mainly of cyanobacteria and chlorophyll-*a* in the dry months of the year 2019, as opposed to the rainy months mainly in 2020 with temperature, precipitation, transparency and Ochrophyceae, was evident. Dinophyceae and Bacillariophyceae also followed the upward trend in the dry months of 2019.

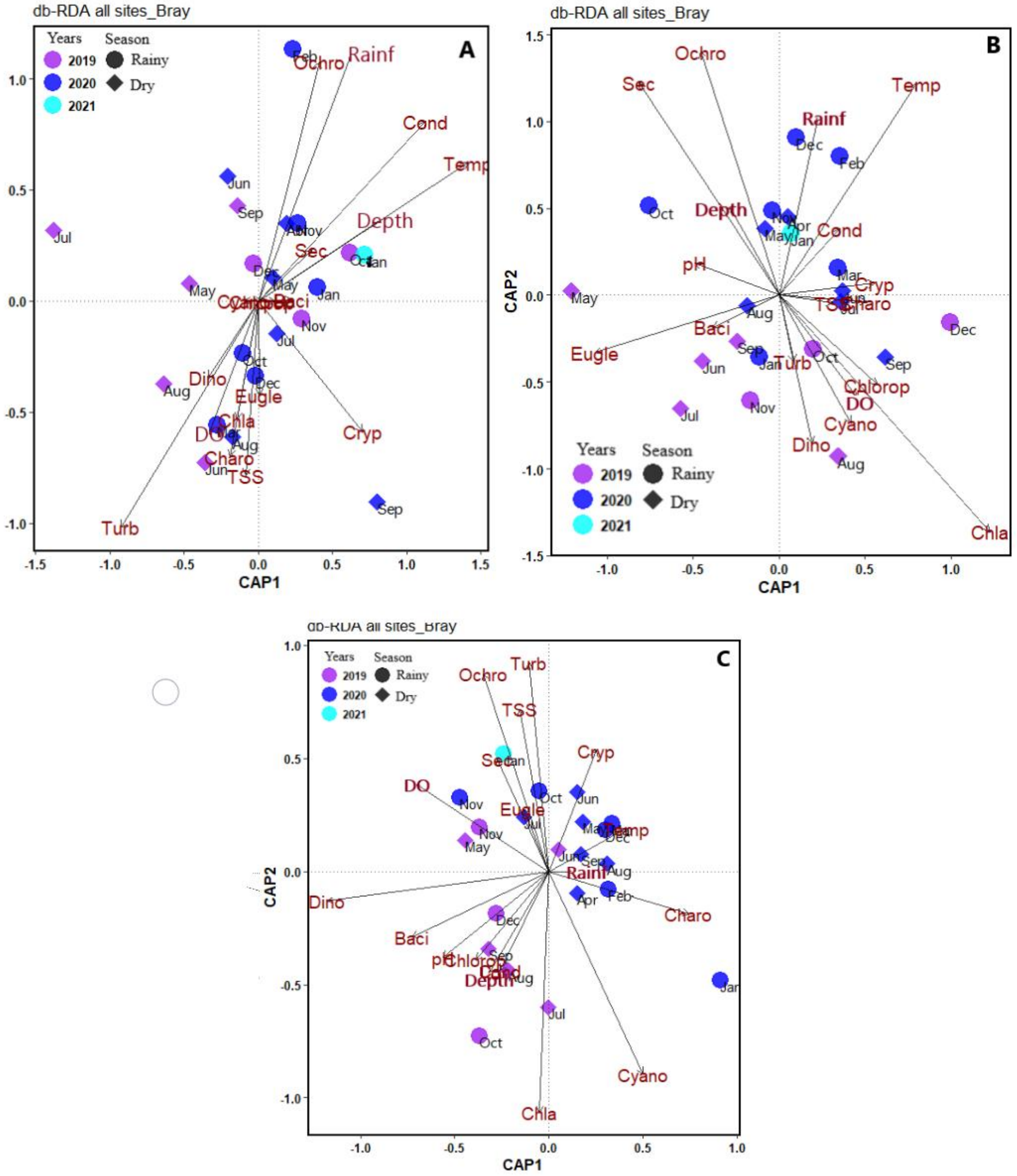

Fig. 6 Distance-based redundancy analysis (dbRDA), showing the relationships of zooplankton groups: A-Rotifera, B-Cladocera and C-Copepoda, with environmental variables and abundance of phytoplanktonic classes. Explanations of the components: A: CP1: 28% and CP2: 14%; B: CP1: 34% and CP2: 20%; C: CP1: 27% and CP2: 22%).

3.2. Zooplanktonic Community variation during 50 years

For the long time series, 35 studies from 1972 onwards were considered, with data from the recent time series. For the three groups of the zooplankton community, Rotifera, Cladocera and Copepoda, 222 taxa present in the Lobo-Broa Reservoir were identified.

The NMDS analysis carried out indicate fluctuations in the composition of the zooplankton community over the years. For Rotifera, the differences in two groups were evident, one with the years 1970 to 2011, and another with the years 2015 to 2021. For Cladocera, it was possible to identify a group in the 1970s, and another containing the years from 2011 onwards, 1983 being the intermediate year between them. For Copepoda the temporal patterns were a less evident, but in the same way as to the other groups, with the 1970s. Analyzing the composition of phytoplankton throughout this period of study, we can observe that there was the presence of distinct groups of species in almost all the years sampled: a more evident group between the years up to 2006, another apparently intermediate group between the years of 2015 to 2019/20, and a third similar group from 2020 onwards.

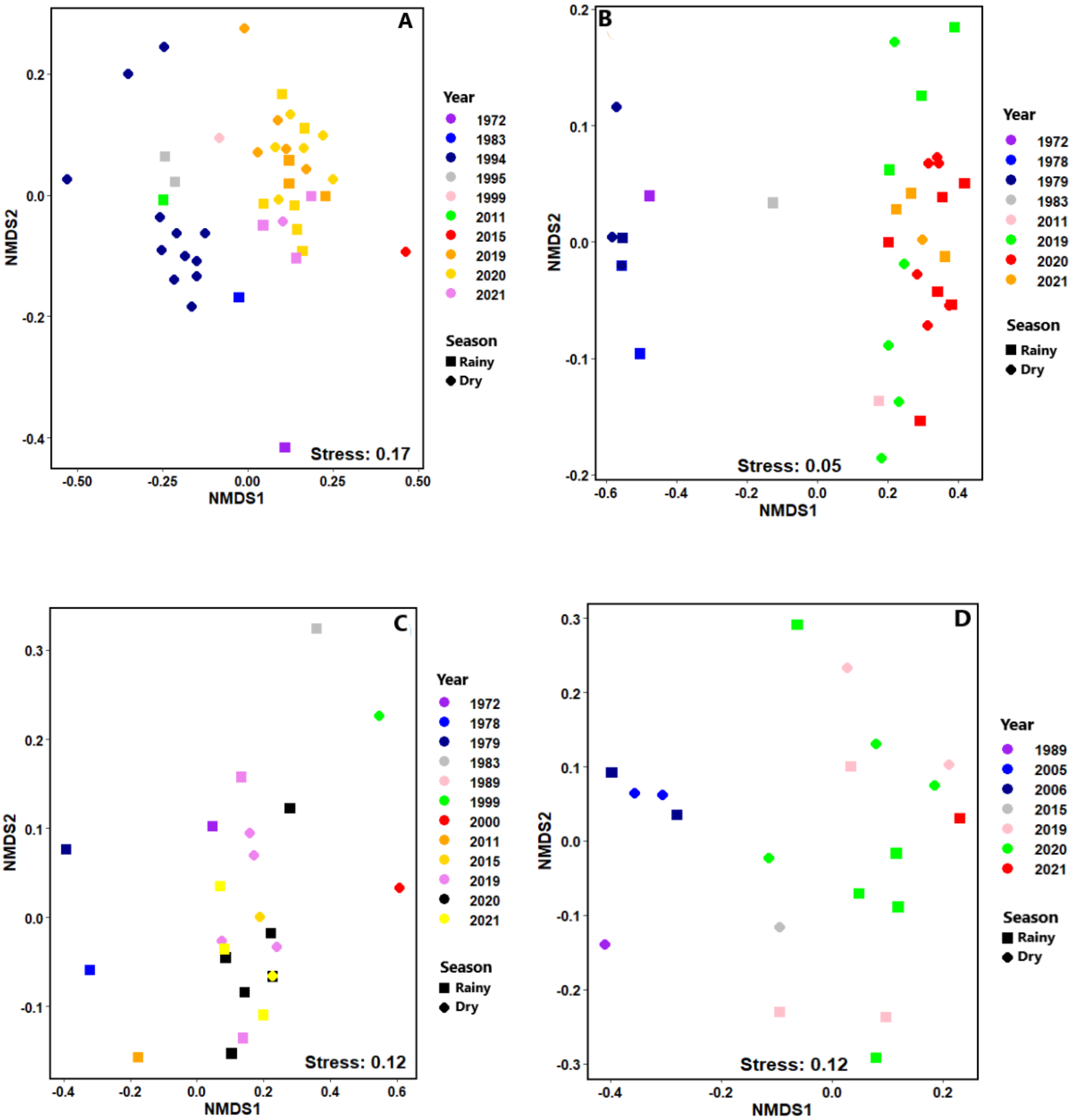

Fig. 7. NMDS analyzes for zooplankton groups: Rotifera (A), Cladocera (B), Copepods (C) and for phytoplankton species (D).

The beta diversity results showed high values (0.93) and a high rate of species replacement, with a turnover index of 0.97. There was, when comparing some moments of the analyzed period, a gradual loss of species (0.42), (Fig. 8). Observing individually each zooplanktonic group over the last decades in the reservoir, the beta diversity indices were very similar for the three groups, with 0.95 for Copepoda, 0.94 for Rotifera, and 0.92 for Cladocera. Such as for the total community, the groups individually followed the same pattern, showing a high turnover rate (0.90 for Cladocera, 0.89 for Copepoda, and 0.88 for

Rotifera), especially Cladocera, reinforcing the fact that the reservoir has undergone significant changes in recent years, leading to changes in species composition between the different periods sampled. In Cladocera, and Copepoda this change is evident when comparing the last years of our temporal analysis, which refer to the present study, with the first years sampled in the reservoir. The phytoplankton community also showed high beta diversity over the studied decades (0.93). There was a change in the composition of phytoplankton species between the samples analyzed (turnover of 0.82), especially when we relate the first decades of study of the Lobo-Broa reservoir (the 70s to the end of the 90s) with the last observed years (after 2014).

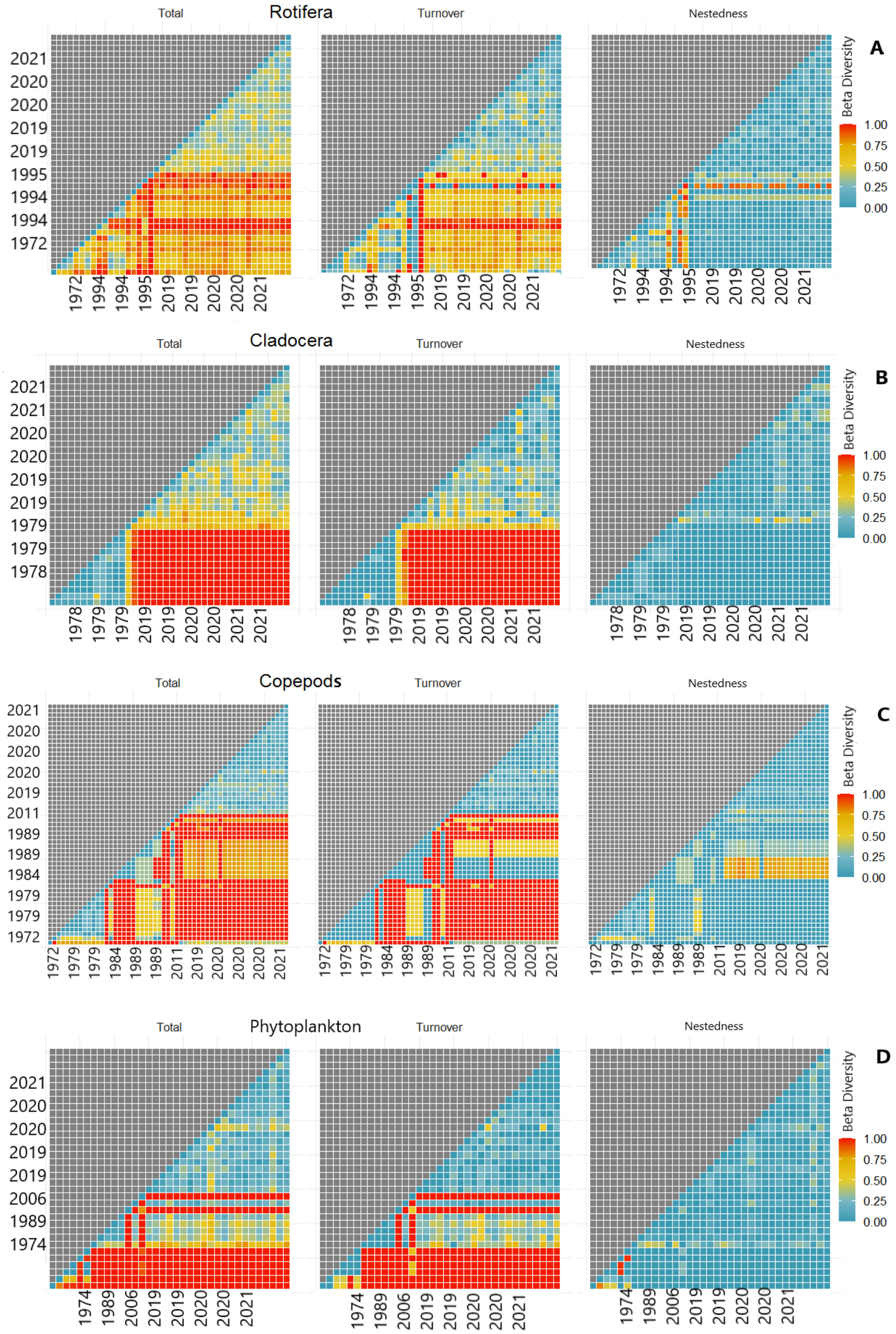

Fig. 8. Representation of the components of total Beta diversity, turnover and nestedness of zooplankton and phytoplankton communities from historical data: A: Rotifera; B: Cladocera; C: Copepoda and D: Phytoplanktonic community.

4. Discussion

The Lobo-Broa reservoir has experienced periods of intense drought and excessive rainfall occurring every 5 to 10 years. However, in years with less rainfall during the wet season, these months were grouped with the driest months. Studies have shown different results regarding the trophic condition of the reservoir, with most authors suggesting that anthropic activities, such as land use and occupation, have altered the trophic status of the reservoir in recent years (Leite & Espíndola, 2002; De-Carli et al., 2019; Da Silva-Anjinho et al., 2021). The fluctuations in trophic status may be related not only to chlorophyll-*a*, but also to the presence of algal blooms, particularly cyanophytes, especially during years with below-average rainfall.

However, some studies suggest that there is a non-continuous and gradual process of eutrophication at the Broa Reservoir. According to Tundisi & Matsumura-Tundisi (2016), the low water retention time of the reservoir (<25 days) appears to be sufficient in controlling the biogeochemical cycles and phosphorus concentration in the water. This can be attributed to the shallow depth of the reservoir and the action of the wind (approximately 10 ms-1), which maintains a well-oxygenated water column and reduces the concentration of inorganic phosphate in the water, thus preventing eutrophication and assimilation of incoming nutrients (primarily phosphorus) into the reservoir. Chalar & Tundisi (2001) support this idea, noting that the reservoir has low sediment retention of P (13.0%), Al (9.9%), Fe (9.9%), Mn (1.4%), and Ca (traces), owing to high vertical dynamics (resuspension and background release) and a short residence time.

In 2014, which was one of the years with the lowest accumulated annual rainfall during the study period, a bloom of the invasive species *Cylindrospermopsis raciborskii* was observed for the first time with such intensity, along with high levels of chlorophyll-a and nutrients. This bloom was caused by an increase of up to 2°C in the average water temperature during the winter period, in addition to a lower volume of rainfall during the summer (30% less than the annual average), according to Tundisi et al. (2015). Consequently, the residence time was increased from less than 20 days to 60 days, in order to maintain the necessary volumes for energy production.

The seasonal cycle of phytoplankton composition in reservoirs is influenced by rainfall, rain and wind action, and changes in the zooplankton community, as reported by

Perbiche-Neves et al. (2007, 2016). Significant variations occurring every 50 years in zooplankton community have been identified within 10-20 year cycles in reservoirs, according to Lazareva (2010). Long-term investigations of lentic environments suggest cycles of eutrophication and de-eutrophication, as well as a rise in temperature after the 1990s in the temperate region, as reported by Lazareva (2010) and Hsieh et al. (2011).

Our analysis indicates that tropical reservoirs appear to be regulated by factors resulting from non-seasonal variations, such as rainfall. Although there was a noticeable difference in the limnological variables between the dry and rainy periods, the same cannot be said for the zooplankton, which exhibited no significant intra-annual variations. These findings reject the hypothesis that zooplankton variations occur seasonally. However, when analyzing annual cycles or comparing just the dry and rainy periods, the differences are clear (Santos-Wisniewsky & Rocha, 2007; Portinho et al., 2016). In larger time scales, interannual variation becomes more important. This pattern for tropical systems, where seasonal variations are less pronounced than interannual variations, was also reported in a long-term study of the phytoplankton community (Rugema et al., 2019). We found significant differences between years and in the year*period interaction, which reinforces the effects of years with atypical climate variability, particularly in relation to rainfall. This variability has implications for the stability of the water column, nutrients, and cyanobacterial blooms.

Statistical analyses (PERMANOVA, NMDS, db-RDA) confirmed that interannual exchanges and substitutions occurred in the zooplankton community, likely due to limnological variables and rainfall. These changes were evident in beta diversity, with higher values observed during years of lower rainfall (in terms of both total and turnover) and corresponding shifts in phytoplankton. These trends were most pronounced in 2014 and 2020. Interestingly, microcrustaceans appeared to be more sensitive to rainfall than other groups, such as phytoplankton and rotifers. This may be linked to the dominant phytoplankton class and their palatability.

Although there have been fluctuations in the composition of the zooplankton community over the years, no replacement of larger filter feeders by smaller ones has been observed. High abundances of taxa such as *Daphnia gessneri* and some large calanoids such as *Argyrodiaptomus furcatus* were observed. Even though bosminid cladocerans have also been found in considerable abundance, the values are lower than in hypereutrophic reservoirs

(Ghidini et al., 2009). Larger filter-feeding crustaceans have reproductive and ecological cycles associated with the phytoplankton in the reservoir, with oscillations between *Argyrodiaptomus furcatus* and *Notodiaptomus iheringi* (Rietzler et al., 2002), which are dominant species in the Lobo-Broa Reservoir (Rocha & Matsumura- Tundisi, 1984).

In the recent temporal scale sampled, the differences between the dry and rainy periods were found to be minimal when compared to the interannual scale, which is contrary to findings in other studies conducted in the same reservoir, but with a focus on abundance rather than composition (Matsumura-Tundisi et al., 1989) and in other studies (Santos-Wisniewsky & Rocha, 2007; Portinho et al., 2016). In the Lobo-Broa Reservoir, the seasonal variation of species is characterized by two abundance peaks: one in the rainy season (summer) and the other in the windy season (winter). The seasonal cycle of zooplankton biomass is likely a typical case for small reservoirs with a volume of up to 506 x 10-3m (Matsumura-Tundisi et al., 1989; Gozdziejewska & Kruk, 2022).

The summer months of November 2020 and February 2021 were atypical with low rainfall, below the period averages. However, this exceptionally dry period did not affect the dissimilarity of the zooplankton, as revealed by the dbRDA analysis. On an interannual scale, it appears that the accumulated annual precipitation may have an influence on zooplankton changes. These changes may be related to the accumulated drought events that have occurred in recent decades, affecting various physical and chemical variables (Nadai & Henry, 2009; Chaparro et al., 2011; Brito et al., 2018). Based on the observed rainfall data, the years of lowest rainfall seemed to occur every ten years until mid-2000, after which they started occurring every five years. This fact may have contributed to fluctuations in the zooplankton community, as it is a climate effect that is becoming increasingly frequent. Global climate changes, particularly those associated with greenhouse gas emissions, can impact reservoir dynamics due to rising air temperatures and changes in precipitation patterns (Dias, 2018).

Studies have shown that climate change has a direct impact on regional and specific patterns, affecting the functioning of reservoirs, particularly when daily regulation is analyzed in detail. Reservoir functioning is sensitive to changes in seasonal distribution and flow extremes. Increased flow during the flood season leads to significant increases in the amount of water spilled and accelerates the time when the reservoir reaches normal storage levels. Changes in water flow, particularly during extreme dry years in the impounding phase, can result in reduced total flood rates and average water storage levels during the dry season. (Qin et al., 2020; Huntington et al., 2018).

The present study is pioneering in Brazil in analyzing a long temporal dataset of almost 40 years with refined data for zooplankton in reservoirs. The results indicate that interannual variation is more important than within the same year in terms of changes in species composition. Overall, the changes in species composition observed in the study suggest the action of environmental filters on the reservoir in recent decades. These filters include the gradual increase in trophic status, which is related to, among other factors, periods of below-average rainfall and the input of nutrients exceeding assimilation capacity.

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Supplementary material

S1. Table of environmental variables for the Lobo-Broa Reservoir, from May 2019 to April 2021.

Month	Year	Season	Chl*a* (mg/L)	TSI	Secchi (meters	Turbidity	Conduct. (µS/cm)	Temperature	pH	D.O (mg/L)	TSS (mg.L)	Depth (meters)	Rainfall (mm^3)
May	2019	Dry	5,443	57	1,7	19	18	22,48	7,16	7,22	3,67	7,7	24,5
June	2019	Dry	25,69	72	1,2	21,1	19	20,32	6,7	7,51	3,9	7,8	12,5
July	2019	Dry	17,42	68	1,2	18,6	19	18,78	6,77	21,1	4,31	7,7	25,1
August	2019	Dry	60,31	81	0,65	40	19	21,28	6,55	20,71	8,14	7,65	13,6
September	2019	Dry	14,97	67	1,3	8,3	25	22,68	7,51	6,96	6,23	7,8	124,1
October	2019	Rainy	24,88	72	1,4	2,6	25	26,5	6,79	6,61	4,67	10,4	220
November	2019	Rainy	18,72	69	0,9	23,4	25	25,51	6,65	7,76	6,13	7,8	309,1
December	2019	Rainy	21,77	70	0,8	31,4	23	27,7	6,93	16,6	10,1	7,6	340,2
January	2020	Rainy	12,63	65	1	14,6	25	28,45	6,86	7,18	5,3	8,1	459,6
February	2020	Rainy	16,98	68	1,3	5,8	23	28,92	6,93	6,65	3	8,25	451,1
March	2020	Rainy	18,51	69	0,9	15,9	18	27,22	6,88	7,1	10,4	7,8	147,3
April	2020	Dry	14,59	66	1	9,2	19	24,63	6,74	4,75	0,2	7,6	6,5
May	2020	Dry	18,51	69	0,95	19	18	21,4	5,79	6,42	5,6	7,7	23,7
June	2020	Dry	12,63	65	1,06	21,1	19	21,53	5,96	7,83	8,7	7,8	59
July	2020	Dry	16,55	68	1,2	18,6	19	21,65	6,85	8,47	4	7,7	4,5
August	2020	Dry	10,89	64	1,2	16	19	21,33	6,88	6,61	9,3	7,4	51,3
September	2020	Dry	21,45	70	1,2	16,7	19	22,51	6,77	9,51	6,4	7,65	3,8
October	2020	Rainy	9,144	62	1,1	15,9	21	25,27	6,93	8,62	13,6	9,6	139,7
November	2020	Rainy	6,967	59	1,48	23,4	23	26,1	6,94	8,91	7,9	7,9	66,4
December	2020	Rainy	10,45	63	2,18	31,4	26	28,8	7,02	8,88	10,1	8,4	373,9
January	2021	Rainy	16,77	68	1,43	14,6	24	27,19	7,05	9,46	9,2	8,4	173,9
February	2021	Rainy	3,919	53	2,05	0,3	21	28,2	6,68	8,96	4,4	7,8	69,1
March	2021	Rainy	6,314	58	1,86	0,8	28	26,68	6,93	8,23	5,4	9,12	105,4
April	2021	Dry	18,07	69	1,65	9,2	23	23,33	6,76	13,7	6,6	7,55	14,7

S2. List of taxa from the zooplankton community of the Lobo-Broa Reservoir included in this study.

Phylum: Arthropoda
  Subphylum: Crustacea
    Class: Branchiopoda
      Order: Cladocera
        Family: Bosminidae
          Bosmina coregoni Baird, 1857
          Bosmina freyi De Melo and Hebert, 1994
          Bosmina hagmanni Stingelin, 1904
          Bosmina tubicen Brehm, 1953
          Bosminopsis deitersi Richard 1895
        Family: Chydoridae
          Acroperus tupinamba Sinev & Elmoor-Loureiro, 2010
          Alona guttata Sars, 1862
          Alona ossiani Sinev, 1998
          Alona yara Sinev & ElmoorLoureiro, 2010
          Camptocercus australis Sars, 1896
          Chydorus globosus Baird, 1843
          Chydorus eurynotus Sars, 1901
          Euryalona orientalis Daday, 1898
          Kurzia longirostris (Daday, 1898)
        Family: Daphniidae
          Ceriodaphnia cornuta G.O. Sars, 1885
          Ceriodaphnia cornuta rigaudi Richard, 1894
          Ceriodaphnia cf. *laticaudata* P.E. Müller, 1867
          Ceriodaphnia silvestrii (Daday, 1902)
          Ceriodaphnia cf. *quadrangula* (O.F.Müller, 1785)
          Daphnia ambigua Scourfield, 1947
          Daphnia gessneri Herbst, 1967
          Daphnia leavis Birge 1879
          Scapholeberis armata (Herrick, 1882)
          Simocephalus semisserratus (Koch, 1841)
        Family: Ilyocryptidae
          Ilyocryptus spinifer Herrick 1882
          Ilyocryptus sordidus (Liévin, 1848)
        Family: Macrotricidae
          Macrothrix flabelligera Smirnov, 1992
        Family: Moinidae
          Moina minuta Hansen (1899)
        Family: Sididae
          Diaphanosoma birgei Korínek, 1981
          Diaphanosoma brachyurum (Liévin, 1848)
          Diaphanosoma spinulosum Herbst 1975

Phylum: Arthropoda
  Subphylum: Crustacea
    Class: Copepode
      Order: Calanoida
        Family: Diaptomidae

Argyrodiaptomus furcatus (Sars 1901)
Argyrodiaptomus furcatus f. *exilis* (Dussart 1986)
Diaptomus furcatus Sars G.O,1901
Notodiaptomus iheringi (Wright 1935)
Notodiaptomus spiniger (Brian, 1926)
Notodiaptomus spinuliferus (Dussart, 1985)

      Order: Cyclopoida
        Family: Cyclopidae

Acanthocyclops robustus (Sars G.O 1863)
Ectocyclops rubescens Brady, 1904
Elaphoidella bidens (Schmeil, 1894)
Eucyclops robustus (Sars, G.O,1863)
Eucyclops cf. *ensifer* Kiefer, 1936
Macrocyclops albidus (Jurine, 1820)
Mesocyclops longisetus (Thiébaud, 1912)
Mesocyclops meridianus (Kiefer, 1926)
Metacyclops mendocinus (Wierzejski, 1892)
Microcyclops anceps anceps (Richard, 1897)
Paracyclops fimbriatus (Fischer, 1853)
Paracyclops chiltoni (Thomson G.M., 1883)
Thermocyclops decipiens (Kiefer, 1929)
Thermocyclops minutus (Lowndes, 1934)
Tropocyclops prasinus meridionalis (Kiefer, 1931)

- **Phylum:** Rotifera
  - **Class:** Eurotatoria
    - **Subclass:** Monogononta
      - **Order:** Collothecaceae
        - **Family** Collothecidae
          - *Collotheca campanulata* Dobie, 1849
          - *Collotheca ornata* Ehrenberg,1832
          - *Collotheca ornata* f. *coruta*
      - **Order:** Flosculariaceae
        - **Family** Conochilidae
          - *Conochilus coenobasis* Skorikov,1914
          - *Conochilus unicornis* Rousselet,1892
        - **Family** Filinidae
          - *Filinia longiseta* Ehrenberg, 1834
          - *Filinia pejleri* Hutchinson, 1964
        - **Family** Flosculariidae
          - *Ptygura* cf. *libera* Myers,1934
          - *Sinantherina semibulata* (Thorpe, 1893)
        - **Family** Hexarthridae
          - *Hexarthra longicornicula* Turner,1987
          - *Hexarthra intermedia bralisiensis* Hauer, 1953
          - *Hexarthra mira* Hudson,1871
        - **Family:** Testudinellidae
          - *Testudinella amphora* Hauer,1938
          - *Testudinella emarginula* Stenroos, 1898
          - *Testudinella patina* Hermann,1783
          - *Testudinella parva* Ternetz, 1892
        - **Family** Trochospharidae
          - *Horaella thomassoni* Koste, 1973
      - **Order:** Ploima
        - **Family:** Asplanchnidae
          - *Asplanchna* sp. Gosse, 1850
          - *Asplanchna priodonta* Gosse, 1850
          - *Asplanchna sieboldii* (Leydig, 1854)
        - **Family:** Brachionidae
          - *Anuraeopsis fissa* Gosse, 1851
          - *Anuraeopsis navicula* Rousselet, 1911
          - *Brachionus calyciflorus* (Pallas, 1766)
          - *Brachionus falcatus* Zacharias, 1898
          - *Brachionus quadridentatus mirabilis* Daday, 1897
          - *Brachionus mirus laticaudatus* Paggi, 1973
          - *Brachionus mirus mirus* (Daday, 1905)
          - *Brachionus quadridentatus* Skorikov, 1894
          - *Kellicottia bostoniensis* (Rousselet,1908)
          - *Keratella americana* Carlin, 1943
          - *Keratella cochlearis* (Gosse, 1851)
          - *Keratella lenzi* (Hauer, 1953)
          - *Keratella quadrata* (Müller, 1786)
          - *Platyias leloupi* Gillard, 1967
          - *Platyias quadricornis* (Ehrenberg, 1832)
          - *Platyias quadricornis quadricornis* (Daday, 1905)
          - *Plationus patulus macracanthus* (Ehrenberg, 1832)

Family: Dicranophoridae

Aspelta circinator (Gosse 1886)
Dicranophorus prionacis Harring & Myers, 1928
Dicranophorus epicharis Harring & Myers, 1928
Dicranophorus luetkeni Bergendal,1892
Dicranophorus robustus Harring & Myers, 1928

Family: Epiphanidae

Epiphanes senta Müller, 1773

Family: Euchlanidae

Euchlanis dilatata Hauer, 1930
Euchlanis meneta Myers,1930
Euchlanis proxima Myers, 1930
Euchlanis triqueta Ehrenberg, 1838
Manfredium eudactilota Gallagher, 1957

Family: Gastropodidae

Ascomorpha ecaudis Perty, 1850
Ascomorpha tundisii Segers & Dumont, 1995
Ascomorpha ovalis (Bergendahl, 1892)
Gastropus stylifer (Imhof, 1891)

Family: Lecanidae

Lecane ludwigii (Eckstein, 1883)
Lecane lunaris (Ehrenberg, 1832)
Lecane papuana (Murray, 1913)
Lecane agilis Bryce, 1892
Lecane bulla bulla Gosse, 1851
Lecane bifurca Bryce, 1892
Lecane braziliensis Segers,1993
Lecane broaensis Segers &Dumont, 1995
Lecane clara Bryce,1892
Lecane closterocerca Schmarda,1859
Lecane cornuta Müller,1786
Lecane costatoides
Lecane curvicornis Murray,1913
Lecane decipiens Murray, 1913
Lecane flexilis Gosse,1886
Lecane furcata Murray, 1913
Lecane hamata Stockes,1896
Lecane hornemanni Ehrenberg,1834
Lecane inermis Bryce, 1892
Lecane laticaudata
Lecane leontina Turner,1892
Lecane ludwigii Eckstein,1883
Lecane mitis Harring &Myers, 1926
Lecane monostyla Daday,1897
Lecane ohioensis Herrick, 1885
Lecane pusilla Harring, 1914
Lecane pyriformis Daday,1905
Lecane quadridentata Ehrenberg,1830
Lecane signifera Jennings,1896
Lecane stichaeta Jennings,1896
Lecane subtilis Harring & Myers, 1926
Lecane uenoi Yamamoto,1951
Lecane ungulata Fadeev,1925

Family: Lepadellidae
Colurella uncinata (Müller, 1773)
Colurella tesselata Glascott, 1893
Colurella uncinata bicuspidata Ehrenberg,1832
Lepadella dactyliseta (Stenroos, 1898)
Lepadella patella patella (Müller, 1786)
Lepadella elongata Koste,1992
Lepadella neglecta Dumont, 1995
Lepadella benjamini Harring,1916
Lepadella closterocerca Schmarda,1859
Lepadella costatoides Segers,1992
Lepadella cristata Rousselet,1893
Lepadella dactyliseta (Stenroos,1898)
Lepadella minuta Weber &Montet, 1918
Lepadella patella Müller,1773
Lepadella quadricarinata Stenroos,1898
Lepadella triba Myers, 1934
Lepadella triptera Ehrenberg,1832
Squatinella lamellaris Bory de St. Vicent, 1826

Family: Lindiidae
Lindia torulosa Dujardin, 1841

Family: Mytilinidae
Mytilina ventralis Ehrenberg,1830

Family: Notommatidae
Cephalodella hoodi Gosse, 1886
Cephalodella mucronata Myers, 1924
Cephalodella cf. *exigua* Gosse, 1886
Cephalodella eva (Gosse, 1887)
Cephalodella forficula Ehrenberg, 1830
Cephalodella gibba Ehrenberg, 1830
Eothinia elongata Ehrenberg,1832
Monommata actices Myers,1930
Monommata arndti Remane, 1933
Monommata maculata Myers,1930
Notommata cerberus Gosse,1886
Notommata cyrtopus Gosse,1886
Notommata pachyura Gosse,1886
Notommata saccigera Ehrenberg,1830
Rousseletia corniculata Harring,1913
Taphrocampa selenura Gosse, 1887

Family: Proalidae
Proales decipens Ehrenberg, 1832
Proales fallaciosa Wulfert, 1937

Family: Scaridiidae
Scaridium bostjani Daems & Dumont, 1974
Scaridium elongatum Segers, 1996

Family: Synchaetidae
Ploesoma truncata (Levander, 1894)
Polyarthra libera Myers,. 1934
Polyarthra vulgaris Carlin,1943
Synchaeta stylata Wierzejski, 1893

Family: Trichocercidae

Trichocerca bicristata Gosse,1887
Trichocerca bidens Lucks,1912
Trichocerca braziliensis Murray,1913
Trichocerca inermis Linder,1904
Trichocerca jenningsi Voigt, 1957
Trichocerca longiseta Schrank,1802
Trichocerca myersi Hauer,1931
Trichocerca montana Hauer, 1956
Trichocerca porcellus Gosse,1851
Trichocerca pusilla Jennings, 1903
Trichocerca rattus Muller, 1776
Trichocerca scipio Gosse, 1886
Trichocerca simillis (Wierzejski, 1893)
Trichocerca stylata Gosse,1851
Trichocerca tigris Müller, 1786
Trichotria tetractis Ehrenberg,1830

Family Trichotriidae

Macrochaetus altamirai Arevalo, 1918
Macrochaetus collnsi collnsi Gosse,1867

Class: Bdelloidea
Order: Philodinida
Family Philodinidae

Dissotrocha acuelata acuelata Ehrenberg, 1832

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Capítulo 2. Functional diversity of the zooplankton community in a shallow tropical reservoir

Abstract

Currently almost all kinds of freshwater ecosystems are subjected to constant anthropogenic interference, which significantly affect the species dynamics. In this study, we evaluated the functional diversity of the zooplankton community according to seven functional traits in the well-studied tropical Brazilian Lobo-Broa Reservoir. We studied temporal trends with 24 monthly samplings between May 2019 and April 2021 using the following indices: functional richness (FRic), evenness (FEve), and divergence (FDiv). We hypothesize that changes in environmental drivers, especially in rainfall volumes, affect the functional composition of the zooplankton community, what was partially demonstrated by this work. Our data showed low functional richness and a direct relationship between the variation of diversity indices and the environmental conditions of dry and rainy periods along the year, supported by multivariate analysis associating few variables as chlorophyll-*a*, temperature, dry period in winter months with rainfall and atypical dry period in summer, with the groups of species pointed by functional cladogram. Some functional traits, such as habitat preference, feeding habits, response to predation and body size were more determinant than others for the functional composition of zooplankton species.

Keywords: Functional traits; environmental variables; freshwater; seasonal variation.

Introduction

In recent decades, human actions have changed processes in the dynamics of ecosystems and species, and these changes are evident in aquatic ecosystems (e.g. Nogueira et al., 2021). Aquatic ecosystems are directly impacted by anthropic activities, resulting in changes in relevant communities, such as the zooplanktonic (Papa and Briones, 2014). Zooplankton is a diverse community in species number and functionality, and is a key component in aquatic ecosystems that, quickly respond to changes from several sources (Lomartire et al, 2021). In an attempt to assess environmental changes in aquatic ecosystems, some indices of species richness and diversity have currently been widely used (e.g., Zhao et al, 2019; Hipsey et al, 2020).

In ecological studies of zooplankton, one of the objectives is to analyze patterns of diversity, richness, and uniformity in communities (e.g. Nogueira et al 2021). Biodiversity has been expressed, for many decades, through indices based on richness and abundance (e.g. Whittaker 1972; Purvis and Hector 2000; Lougheed and Chow-Fraser 2002). Although these indices have been constantly studied and adapted, some other components related to diversity need to be better explored and clarified. Many studies with zooplankton communities are carried out from the point of view of taxonomic diversity, but, in recent decades, the need and interest in carrying out studies with the functional approach has emerged (e.g. Barnet 2007; Braghin et al, 2018; Lopes et al, 2019).

Functional diversity indices are measured from the trait’s selection, which are classified at the individual level, if possible. The traits can determine the individual's role or its response to changes in the environment (de Bello et al, 2010). Functional traits reflect the morphological, physiological, behavioral, and ecological characteristics of the individuals, and affecting their growth, reproduction, and survival (Violle et al. 2007).

For the zooplankton community, many functional characteristics can be chosen as traits such as feeding, growth, reproduction, and survival. In addition, some characteristics are associated with the patterns of the species, such as morphological, physiological, and behavioral characteristics (Violle 2007; Litchman et al, 2013). Some features describe the aptitudes of the organism and reflect on the niche sizes these organisms can occupy in ecosystems. However, some characteristics are considered as key traits, such as feeding, reproduction capacity, and mortality, because they are not only related to fitness but can direct or indirectly affect the ecological processes (Rizo et al, 2017).

Functional diversity can be measured by different ways (e.g. Bragin et al., 2018; Mwagona et al., 2018). One way to give robustness for the diversity analyzes is the use of complementary indices, such as functional richness, functional uniformity, and functional divergence (Villéger et al., 2018). Functional richness (FRic) represents the functional space that species occupy in the community. High FRic values mean high functional dissimilarity within the community species. FEve is a value-weighted by species abundance and demonstrates the regularity with which species occupy the functional space, and this index is related to Pielou Equitability (J'). A community with high FEve values has more homogeneous characteristics combinations. Finally, functional divergence (FDiv) is the index of the deviation of the species from their mean distance to the centroid of the space occupied by the community, weighted by the abundance of each species (Villéger et al. 2008). The analysis of these diversity indices as a whole provides a more complete approach to the functionality of a given community in an ecosystem (Mouchet et al. 2010). Each community has different degrees of similarity and, consequently, has different responses to environmental filters, climate change, and anthropic actions. The environmental conditions can directly reflect on the functional response of a species within a community (Hebert el al. 2016). These different responses can be better understood by analyzing each index (Villéger et al. 2008). In this sense, it is important to have an individualized approach to ecosystems, considering their composition and everything that influences them.

Reservoirs and other freshwater ecosystems are constantly experiencing anthropogenic disturbances, as they generally provide services to local people (Augustine, 2009). These disturbances can also reflect on the life quality of the human population, since the health functioning of the ecosystem is directly related to the functions and dynamics of the species that compose it (Violle et al. 2007; Hansen et al., 2020).

To investigate the functionalities of the freshwater zooplankton community, a well-studied, small and shallow Brazilian reservoir was sampled monthly along two years, focusing especially on temporal variations between dry and rainy periods. We aimed to understand the relationship and impact of environmental factors on the different diversity indices that can be important drivers to favorable conditions for the maintenance of these species. There was an atypical dry period during the rainy season in summer in these years, and we hypothesize that changes in environmental drivers, especially in rainfall volumes, as expected by climatic changes, connected to other variables, affect the functional composition of the community.

Methods

Study area

Samplings were carried at one site in the deeper and lentic region of the Lobo-Broa Reservoir, close to the dam (22º 10’ 43.5”S, 47º 53’ 44.5”W) (Fig. 1). The reservoir is located in the municipality of Itirapina, state of São Paulo, Brazil. It has a maximum length of 7.5 km, an average depth of 3.0 m and a surface area of 6.8 km^2 (Rocha & Matsumura-Tundisi, 1984). The region has a humid subtropical climate, with dry winters and rainy summers (Cwa), according to the Köppen climate classification, and annual rainfall of 1,300 to 1,600 mm. (Köppen, 1936).

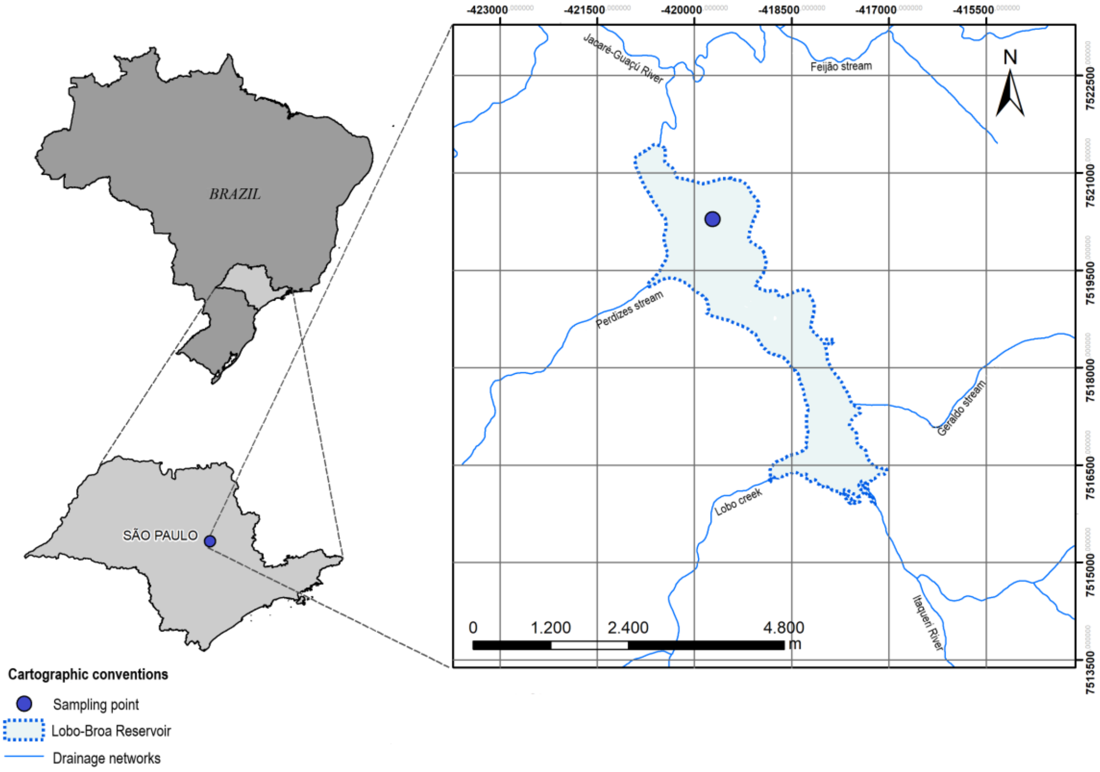


Fig. 1. Map of the study area in the Lobo - Broa Reservoir, with the sampling site (blue circle) located close to the dam.

Field sampling

Zooplankton was sampled monthly between May 2019 and April 2021, using vertical hauls along 7 meters in the water column with a conical plankton net of 45µm mesh size, filtering 500 L per sample. The samples were fixed in buffered 4% formaldehyde for later laboratory identification.

Zooplankton identification was carried out according to the specialized bibliography, as follows: Rotifera with Koste (1986, 1987), Segers (1995), and Nogrady (2002); Cladocera with Smirnov (1974), Elmoor-Loureiro (1997), Sinev (2001) and the website Cladóceros do Brasil (https://cladocera.wordpress.com/); and copepods using Reid (1985), Neves (2011), and Perbiche-Neves et al. (2015).

For the qualitative analyses, a stereoscopic microscope Zeiss Stemi 305 (magnification of up to 50 x) and an optical microscope Olympus CS (magnification of up to 1000 x) were used, both with a millimeter eyepiece. For quantitative analysis based on the population density (individuals.m^{-3}), subsamples were counted in a Sedgewick-Rafter chamber under an optical microscope, in the case of Rotifera, and checkered acrylic chambers under a stereoscopic microscope, for Cladocera and Copepoda. At least 200 individuals were counted per sample, and the final density was expressed in individuals.m^{-3}.

Characterization of environmental predictors of the Lobo - Broa Reservoir

The environmental variables measured in each sampling were: water transparency using a Secchi disk; and water temperature, pH, dissolved oxygen, and conductivity using a Horiba U50 multiparameter probe. The concentration of suspended solids (total, organic and inorganic) was quantified by the gravimetric method (Cole, 1975), by filtering determined volumes of samples, using 45mm GF-C glass fiber micro filters (1.2 µm porosity), previously calcined at 450ºC, and weighing the microfilters on a precision micro analytical scale (five digit analytical balance). Chlorophyll-*a* concentration values were obtained from the extraction method with 90% acetone (Golterman et al., 1978), whose readings were performed in a spectrophotometer, at wavelengths of 663 nm and 750 nm. Rainfall data were obtained from information provided by DAEE-São Paulo, Brazil for the Graúna station, located 7 kilometers from the Lobo-Broa Reservoir.

Species functional traits

To analyze the species of Rotifera, Cladocera, and Copepoda, we selected seven functional traits: [1]Body size; [2]Habitat selection**:** littoral and pelagic; [3]Feeding type: Rotifera classified as predator, filtering and sucking; Cladocera as filtering and scraping, and Copepoda classified as filtering and raptorial; [4]Trophic group: Herbivore and Omnivore; [5]Type of reproduction: sexual or asexual; [6]life span: short or long; [7]Predator escape response: pausing, reduced swimming, rapid swimming, jumping, pausing and jumping.

The traits were chosen based on the characteristics of the species selected from some studies (Strickler 1975; Paggi 1978; Bonecker et al. 1998; Chang 2003; Barnett et al. 2007; Joko 2011; Litchman et al. 2013; Perbiche-Neves et al. 2015; Braghin et al. 2018; Coelho and Henry 2021; Braghin et al. 2021). The table of functional traits selected for the sampled species is in Table S1 of the Supplementary Material.

Data analysis

To measure the diversity of the community from different taxonomic and functional perspectives, in addition to diversity indices, classical indices were calculated, such as Shannon-Wiener (H') and Pielou Equitability (J').

To compare the taxonomic diversity (species richness) with the functional richness, a correlation test between taxonomic species richness and functional richness was performed.

To analyze the effects of the limnological variables on the zooplankton abundance, four distance-based redundancy analyses (db-RDA) were performed using 1,000 permutations. Four analyses were made previously to verify the strong effect of species with high total abundances for Rotifera, even transforming in dissimilarity distances, and in this way two analyses for this group and one for each, Cladocera and Copepoda, were conducted. The zooplankton abundance was transformed in dissimilarity using Bray-Curtis distance. The Vegan and Betapart packages for R software were used.

The functional components were calculated from a community matrix and a functional trait matrix. For the rank of the species in the multivariate trait space it was used a Principal Coordinate Analysis – PCoA (Laliberté and Legendre 2010) of the functional trait matrix, and transformed into a Gower distance matrix (Gower 1966). From the matrices, Funcional Richnesse (FRic), Functional Evenness (FEve), and Funcional Divergence (FDiv) were calculated. All analyzes were performed with the RStudio software (R Core Team 2020), using the “FD”, “cluster”, and “vegan” packages (Laliberté and Legendre 2010, Maechler et al. 2013, Oksanen et al. 2017).

Results

Taxonomic composition and Functional groups

We found 58 taxa of the zooplankton community, among Rotifera, Cladocera and Copepoda (Supplementary file S1, Table 1). From the matrix of functional traits, a matrix of dissimilarity between species was generated by Gower distance. The Gower index ranges from 0-1, and the higher the value, the greater the dissimilarity between species. To confirm the reliability of the dendrogram concerning the original similarity matrix, a Pearson correlation test was performed, where the Cophenetic Correlation Coefficient – CCC (Sokal and Rohlf, 1962) found was $r^2 = 0.8986472$. This value indicates a satisfactory fit between the graphical representation and the dissimilarity matrix. From the functional traits attributed to the species, the community was grouped into five functional groups (Fig. 2).

The first group-G1: Composed of Copepods Calanoida and Cyclopoida, sexually reproducing organisms, long-lived, with coastal and pelagic habits, and that feed by capture and suspended particles. As a predatory escape response, this group has strategies pausing/jumping and rapid swimming. The G2 Group was composed of Cladocera, all pelagic, filter feeders, and herbivores. They have a short lifespan, reproduce by parthenogenesis, and as a response to predation they use escape reduced swimming, rapid swimming and jumping which is characteristic of each genus. The G3 Group consists of Rotifera, mostly Brachionidae, which were grouped together, separately from other rotifers exclusively because they have a pelagic habit. G4 group was composed of rotifers from coastal habitats and feeding habits by filtration. Finally, the G5 Group was composed of rotifers, both littoral and limnetic, which feed on filtering, sucking and predation.

Is important to highlight that within the G1 group, composed of Copepods Calanoida and Cyclopoida, it is still possible to observe a separation, distancing Calanoida exclusively from two subdivisions of Cyclopoida: coastal ones that have rapid swimming in response to predation and those that occupy pelagic regions and respond to predation with both rapid swimming and pausing and jumping. The G2 group, composed of cladocerans, also showed a distinct composition, with species closer in terms of body size pattern and type of predatory escape response. For the G5 group, composed of rotifers, there was also a gap between pelagic and littoral species.

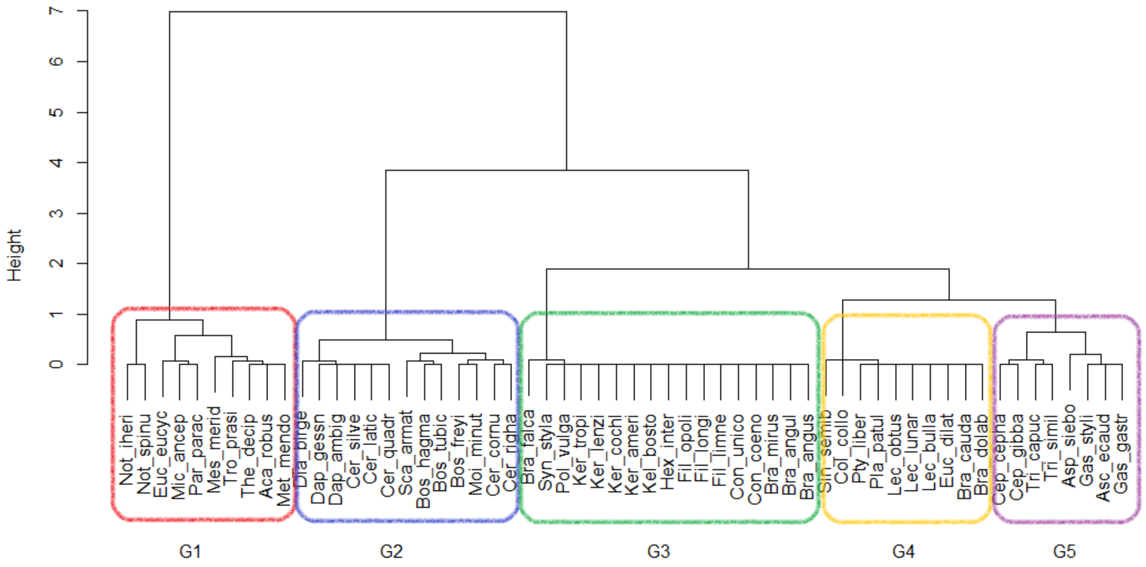


Fig. 2. Cladogram for functional groups (G1 to G5) of the zooplanktonic species organized by a similarity matrix, from the functional traits. G1 grouped copepods, G2 cladocerans, G3 brachionid rotifers, G4 coastal and filtering rotifers, and G5 littoral and limnetic predator rotifers.

Diversity indices

All the diversity indexes in general showed variation between dry and rainy periods, and the effect of an atypical dry summer was also visible (Tab. 1). The richness value presented refers to the number of species in each sampling, while the FRic value represents the number of functional entities that make up the sampling instant (Tab. 1). FRic ranged from 17 to 31 entities throughout the study. FEve is the Equity value of the community, which showed little discrepancy over the sampled period, and remained low. The FDiv values reflect how the community differs from each other, based on their functional attributes, and demonstrate high values (Tab. 1).

Table 1. Values of diversity indices (species richness, Shannon-Wiener (H´), Pielou (J´), FRic, FEve, FDiv) for each sampling campaign during 24 months in the Lobo-Broa Reservoir, also including the hydrological period corresponding to each sampling.

Sampling Period	Hydrological period	Richness	Shannon (H')	Pielou (J')	FRic	FEve	FDiv
May/19	Dry	17	2.59	0.91	5.11	0.34	0.78
Jun/19	Dry	27	2.27	0.68	17.8	0.30	0.73
Jul/19	Dry	21	2.05	0.67	14.6	0.26	0.85
Aug/19	Dry	28	2.63	0.79	24.5	0.32	0.80
Sep/19	Dry	30	2.14	0.63	25.3	0.27	0.78
Oct/19	Rainy	20	1.49	0.49	9.6	0.38	0.83
Nov/19	Rainy	29	2.31	0.68	21.0	0.31	0.74
Dec/19	Rainy	26	1.51	0.46	20.2	0.22	0.78
Jan/20	Rainy	21	2.22	0.73	7.50	0.36	0.82
Feb/20	Rainy	25	2.37	0.73	17.40	0.36	0.77
Mar/20	Rainy	19	2.23	0.76	18.1	0.43	0.82
Apr/20	Dry	21	2.29	0.75	20.4	0.43	0.83
May/20	Dry	18	1.90	0.65	16.9	0.51	0.70
Jun/20	Dry	25	2.18	0.67	20.3	0.44	0.67
Jul/20	Dry	26	2.23	0.68	19.2	0.41	0.69
Aug/20	Dry	18	2.00	0.69	16.8	0.30	0.81
Sep/20	Dry	17	2.22	0.78	18.11	0.45	0.81
Oct/20	Rainy	20	2.03	0.67	18.5	0.24	0.79
Nov/20	Rainy	29	1.59	0.47	11.2	0.32	0.74
Dec/20	Rainy	26	2.70	0.83	18.6	0.31	0.80
Jan/21	Rainy	17	2.09	0.74	11.1	0.40	0.79
Feb/21	Rainy	28	2.71	0.81	10.0	0.20	0.80
Mar/21	Rainy	25	2.27	0.70	19.4	0.41	0.76
Apr/21	Dry	31	2.16	0.62	21.8	0.31	0.76

The correlation between taxonomic diversity (richness) and functional richness was significant (p-value: 0.003228) (Fig. 3).

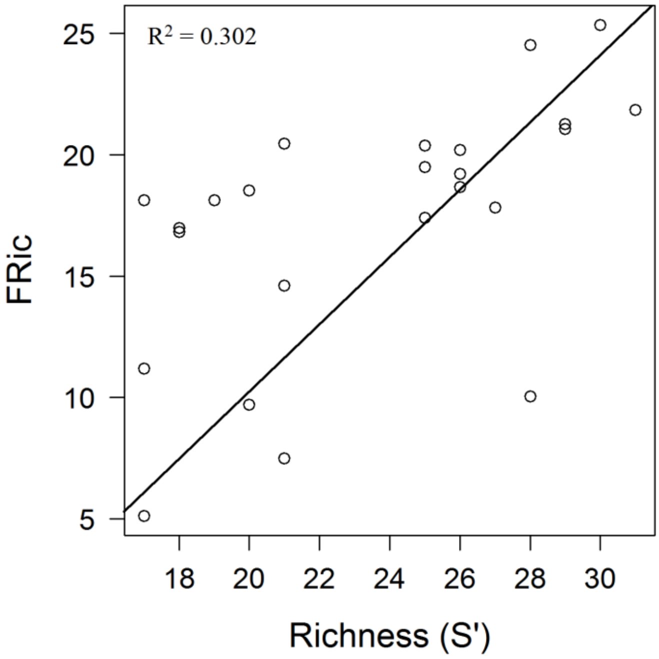


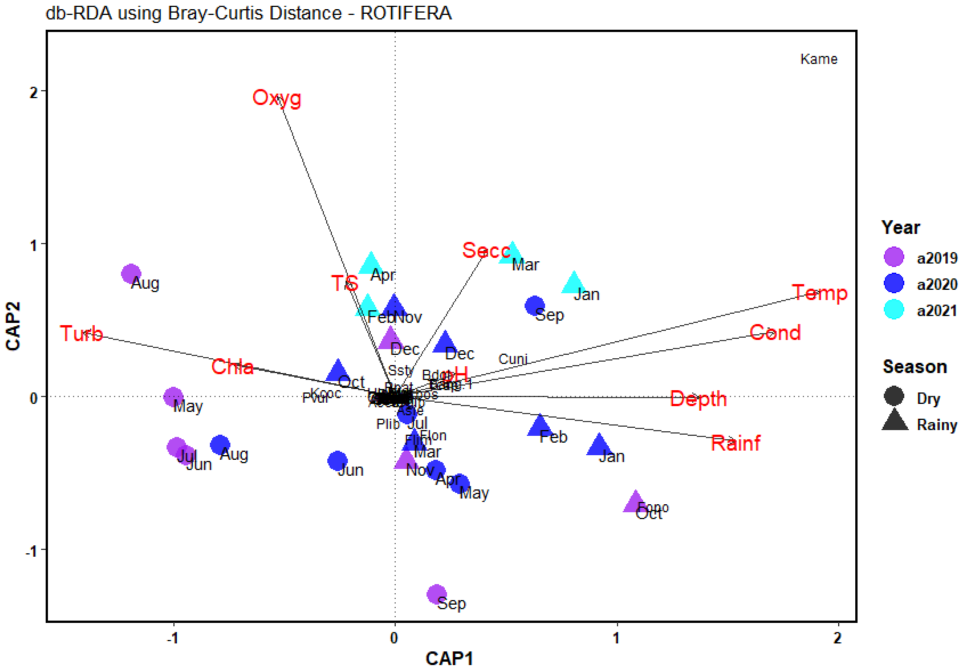
Fig. 3. Correlation test between taxonomic species richness and functional richness

We performed four db-RDA analyses (Fig. 4), one for each microcrustacean group (Cladocera, with explanation of 45% in the first axis and 20% in the second, and Copepoda with explanation of 52% in the first axis and 23% in the second) and two for Rotifera, because to the high abundance of some species in this last group, one analysis with all species (explanation of 29% in the first axis and 19% in the second), and another without *Keratella americana* (with total abundance higher than 1 million organisms), *Filinia opoliensis* and *Keratella cochlearis* (these two last with up than 300,000 ind.m^{-3}) (explanation of 26% in the first axis and 20% in the second). These analyses allowed us to identify the several functional groups and the influence of environmental variables.

For all the Rotifera species (Fig. 4A), *K. americana* and *F. opoliensis* were positively related with variables from rainy period (temperature, conductivity, depth and rainfall). Excluding these two species plus *K. cochlearis*, the analysis allowed to identify species associated to the both seasons (Fig. 4B). *Filinia longiseta*, *A. sieboldi*, *P. vulgaris* were correlated with dry period and turbidity and chlorophyll, in contrast to the rainy period with rainfall, conductivity and temperature increase with *P. libera*, *K. bostoniensis*, *B. angularis*, *B. dolabratus* and *C. unicornis*.

For cladocerans (Fig 4C), besides the rainy and dry seasons samplings were less separated, three main groups were formed. Again, chlorophyll-*a*, turbidity and oxygen were associated with dry season and *C. cornuta* plus *C. silvestrii*. Temperature was correlated in the atypical dry summer with *B. hagmanni* and *B. freyi*, and transparency and pH with *D. gessneri*, *B. tubicen* and *B. freyi*, also in dry months.

Copepods were clearly separated within years in three groups (Fig. 4D), being *T. decipiens* correlated with the dry summer of 2020, temperature and rainfall, *T. prasinus* with dry 2021, transparency and conductivity, and *N. iheringi* with 2019, turbidity, pH, chlorophyll and oxygen. All the values of the environmental variables of our study are in Supplementary file S1, Table 2.



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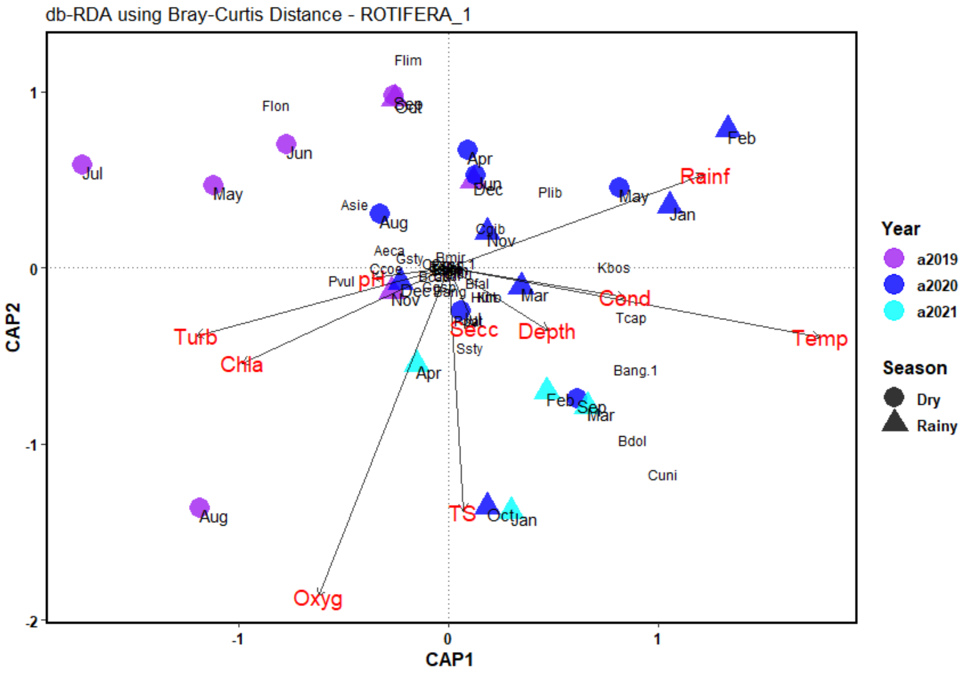
db-RDA using Bray-Curtis Distance - ROTIFERA_1
CAP1
CAP2
Year
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Flon
Plib
Asie
Aeca
Ccog
Pvul
Kbos
Tcap
Ssty
Bang.1
Bdol
Cuni
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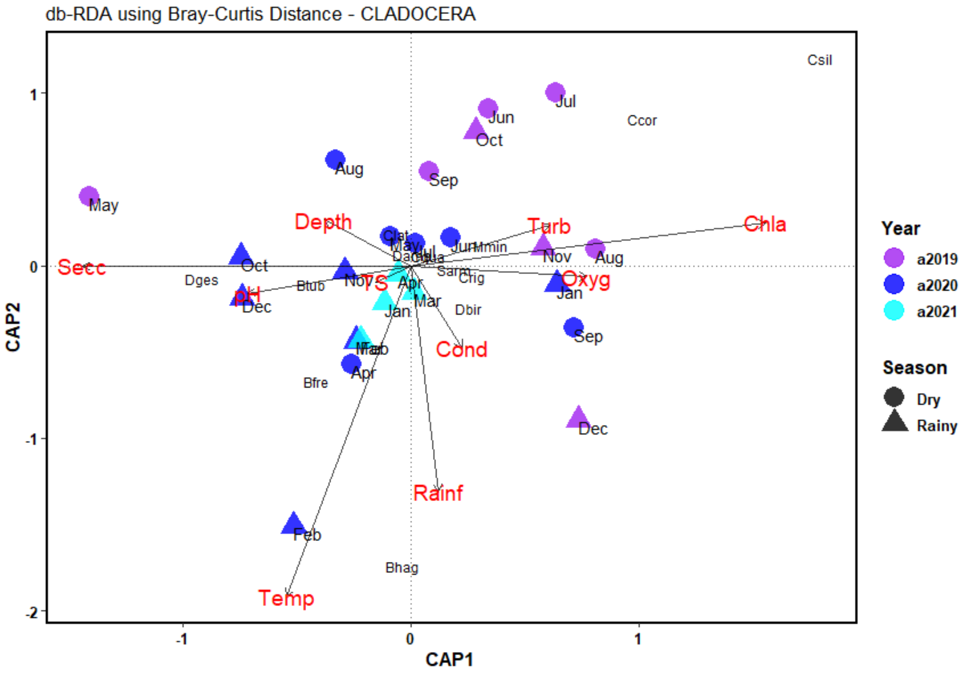
B


db-RDA using Bray-Curtis Distance - CLADOCERA
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Year
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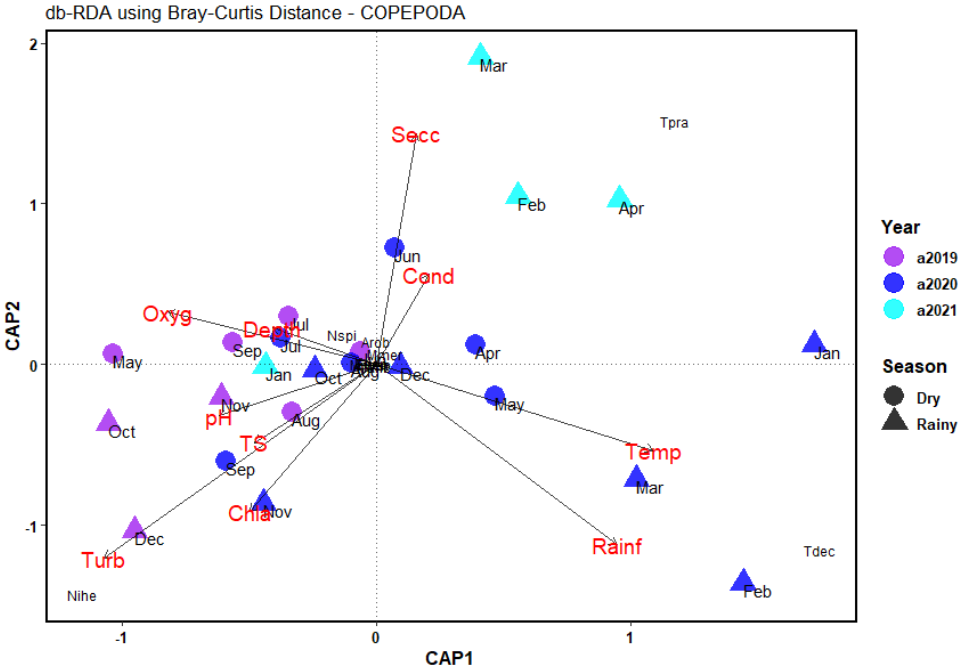

D

Fig. 4. Distance-based redundancy analysis (dbRDA) for the relationship between environmental variables, the zooplankton community represented by abundance transformed in Bray Curtis distance, and the twenty-four sampling campaigns. A. All the species of Rotifera. B. Rotifera without three most abundant species (*K. americana*, *K. cochlearis* and *F. opoliensis*). C. Cladocera. D. Copepoda. Explanations of the components are in the text. For the abbreviatures of species see Supplementary files S1, Table 1.

Discussion

Taxonomic composition and Functional groups

The community analysis showed a greater richness of species for Rotifera, followed by Cladocera and Copepoda, totalizing with 58 taxa found for all groups. Other studies previously carried out in the reservoir already highlight the predominance of rotifers (*e.g* Rocha and Tundisi, 1984; Tundisi and Matsumura-Tundisi, 2011), and is a common trend in reservoirs (Nogueira, 2001; Mwagona et al., 2018).

Our results show that in the assembly of the functional groups the key traits (habitat, predatory scape response, feeding type and body size) were determinants in clustering the zooplankton species. In one of the pioneering studies on the functional diversity of zooplankton, Litchman et al. (2013) highlighted the influence of the key traits on the functional approach of these organisms and other traits. A key trait such as body size, for example, can influence zooplankton herbivore, depending on what types and sizes of cyanobacterial particles are available in that niche. In our study the composition of the functional groups formed for Cladocera and Copepoda was strongly influenced by the body size trait, since there are large variations in size between these microcrustaceans, including at the genus level.

For Cladocera and Copepoda, another key trait in the grouping was the responsiveness to predation. The taxa were distinctly grouped as taxa that have a fast-swimming capacity, such as large daphnid cladocerans and Cyclopoida copepods, and those that respond to escape with other movements, such as jumping, slow swimming movement, and immobility in front of the predator. The latter was composed of bosminids and other smaller cladocerans and calanoid copepods. Responsiveness to predation is an important key trait because it directly influences the survival, lifetime, and reproduction of species (Ohman 1988, Diniz et al, 2021).

For Rotifera, in general, the traits that influenced the determination of grouping were body size and habitat. Key traits such as habitat and trophic group are considered determinants for the entire zooplankton community, regardless of the group in question (Litchman et al 2013, Hebert et al 2017, Josué et al 2019).

Diversity indices

The taxonomic diversity index followed the same response pattern as the functional diversity index, and in the months in which greater or lesser functional diversity was highlighted, this was confirmed by the taxonomic index. The positive correlation found between Richness and FRic reinforces this result. Other studies with zooplankton communities also found results that corroborate this correlation (e.g. Braghin 2018; Coelho and Henry 2021; Ribeiro et al. 2022).

The functional approach to zooplankton communities in tropical environments and in Brazil is recent (Braghin et al, 2018; Lopes et al, 2019; Paina and Melão, 2019; Braghin et al, 2021; Coelho and Henry 2021; Elmoor-Loureiro et al., 2022), as many of the studies already carried out deal with temperate environments (Gomes et al, 2019). Although in the last two decades, important studies have emerged, there are still gaps that need to be filled, especially for reservoirs. Studies already carried out in tropical reservoirs has already established the importance of the functional approach for characterizing the community and consequently maintaining the dynamics of the ecosystem (Gomes et al 2019; Fintelman-Oliveira et al 2022). Tropical environments are always susceptible to external factors and environmental changes. Therefore, measuring biodiversity considering these information gives a more realistic pattern of the dynamics in the environment as a whole and between species (Sodré & Bozelli, 2019; Fintelman-Oliveira et al 2022).

Diversity and environmental components

Diversity indices remained stable throughout the study period. In some sampled months, there was an emphasis on variations in diversity indices and a relationship with environmental components, especially with chlorophyll-*a*, and precipitation values. Analyzing the ecological indices H' and J', the lowest values were found, in October/19, December/19, May/20, and November/20. These low values are related to high values of chlorophyll-*a* in the first three months and a low value of chlorophyll-*a* in the last month. The results revealed by the db-RDA analysis confirmed this relationship among chlorophyll-*a*, rain, and turbidity associated to the dry season including winter months, or the atypical summer dry period in summer, when normally the average rainfall is higher.

Conclusion

This study joins recent ones carried out in tropical shallow reservoirs, including in Brazil, which bring a functional approach. We brought information about the importance of this approach, and how relevant characteristics for the species should be considered, with environmental characteristics. The intrinsic information on the species and on the dynamics of the aquatic ecosystem provide an ecological overview of the complete system. We observed that changes in the indices of environmental indicators reflects the functionality of the species and its role in the ecosystem.

Therefore, more studies with a functional approach are necessary especially in tropical regions, due its rich biodiversity. More studies considering functional aspects of the zooplankton community should be encouraged, as a result of which a full knowledge of the dynamics of the organisms inserted therein will be achieved.

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Compliance with ethical standards: The authors declare that they have no competing interest.

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Supplementary Material

Table S1. List of species and their functional traits found in 24 monthly samplings, from May 2019 to April 2021, in the Lobo-Broa Reservoir (Brazil)

	Body size (µm)	Habitat	Feeding type	Trophic group	Life span	Reproduction	Predatory escape response
Bosmina freyi	426	Pelagic	Filtering-Cla	Herbivore	Short	Asexual	Reduced swimming
Bosmina hagmanni	301	Pelagic	Filtering-Cla	Herbivore	Short	Asexual	Reduced swimming
Bosmina tubicen	301	Pelagic	Filtering-Cla	Herbivore	Short	Asexual	Reduced swimming
Ceriodaphnia cornuta	438	Pelagic	Filtering-Cla	Herbivore	Short	Asexual	Rapid swimming
Ceriodaphnia righaudi	412	Pelagic	Filtering-Cla	Herbivore	Short	Asexual	Rapid swimming
Ceriodaphnia laticaudata	679	Pelagic	Filtering-Cla	Herbivore	Short	Asexual	Rapid swimming
Ceriodaphnia silvestrii	755	Pelagic	Filtering-Cla	Herbivore	Short	Asexual	Rapid swimming
Ceriodaphnia quadrangula	712	Pelagic	Filtering-Cla	Herbivore	Short	Asexual	Rapid swimming
Daphnia ambigua	673	Pelagic	Filtering-Cla	Herbivore	Short	Asexual	Rapid swimming
Daphnia gessneri	597	Pelagic	Filtering-Cla	Herbivore	Short	Asexual	Rapid swimming
Diaphanosoma birgei	507	Pelagic	Filtering-Cla	Herbivore	Short	Asexual	Jumping
Moina minuta	386	Pelagic	Filtering-Cla	Herbivore	Short	Asexual	Reduced swimming
Scapholeberis armata	685	Pelagic	Filtering-Cla	Herbivore	Short	Asexual	Reduced swimming
Notodiaptomus iheringi	968	Pelagic	Suspension-Cop	Herbivore	Long	Sexual	Pausing and jumping
Notodiaptomus spinuliferus	1266	Pelagic	Suspension-Cop	Herbivore	Long	Sexual	Pausing and jumping
Acanthocyclops robustus	1123	Pelagic	Raptorial-Cop	Omnivore	Long	Sexual	Rapid swimming
Eucyclops sp	1054	Littoral	Raptorial-Cop	Omnivore	Long	Sexual	Rapid swimming
Mesocyclops meridianus	937	Pelagic	Raptorial-Cop	Omnivore	Long	Sexual	Pausing and jumping
Metacyclops mendocinus	1145	Pelagic	Raptorial-Cop	Omnivore	Long	Sexual	Rapid swimming
Microcyclops anceps	723	Littoral	Raptorial-Cop	Omnivore	Long	Sexual	Rapid swimming
Paracyclops sp	839	Littoral	Raptorial-Cop	Omnivore	Long	Sexual	Rapid swimming
Thermocyclops decipiens	795	Pelagic	Raptorial-Cop	Omnivore	Long	Sexual	Rapid swimming
Tropocyclops prasinus	507	Pelagic	Raptorial-Cop	Omnivore	Long	Sexual	Rapid swimming
Ascomorpha ecaudis	167	Pelagic	Sucking-Rot	Omnivore	Short	Asexual	Reduced swimming
Asplanchna sieboldi	1135	Pelagic	Predation-Rot	Omnivore	Short	Asexual	Reduced swimming
Brachionus angularis	104	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Brachionus caudatus	223	Littoral	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Brachionus dolabratus	158	Littoral	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Brachionus falcatus	411	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Brachionus angustus	143	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Brachionus mirus	133	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Cephalodella gibba	178	Littoral	Sucking-Rot	Omnivore	Short	Asexual	Reduced swimming
Cephalodella sp	97	Littoral	Sucking-Rot	Omnivore	Short	Asexual	Reduced swimming
Collotheca sp	1200	Littoral	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Conochillus coenobasis	121	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Conochillus unicornis	157	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Euchlanis dilatata	176	Littoral	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Filinia limnetica	177	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Filinia longiseta	189	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Filinia opoliensis	143	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Gastropus stylifer	203	Pelagic	Sucking-Rot	Omnivore	Short	Asexual	Reduced swimming
Gastropus sp	210	Pelagic	Sucking-Rot	Omnivore	Short	Asexual	Reduced swimming
Hexarthra intermedia	298	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Kellicottia bostoniensis	117	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Keratella americana	179	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Keratella cochlearis	112	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Keratella lenzi	123	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Keratella tropica	157	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Lecane bulla	128	Littoral	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Lecane lunaris	109	Littoral	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Lecane obtusa	79	Littoral	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Plationus patulus	112	Littoral	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Polyarthra vulgaris	153	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Ptygura libera	310	Littoral	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Sinantherina semibulatta	585	Littoral	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Synchaeta stylata	257	Pelagic	Filtering-Rot	Omnivore	Short	Asexual	Reduced swimming
Trichocerca capucina	278	Littoral	Sucking-Rot	Omnivore	Short	Asexual	Reduced swimming
Trichocerca similis	299	Littoral	Sucking-Rot	Omnivore	Short	Asexual	Reduced swimming

Table S2. Environmental variables of the Lobo_Broa Reservoir during the study collection months

Month	Year	Season	Chl*a* (mg/L)	TSI	Secchi (meters	Turbidity	Conduct. (µS/cm)	Temperature	pH	D.O (mg/L)	TSS (mg.L)	Depth (meters)	Rainfall (mm^3)
May	2019	Dry	5,443	57	1,7	19	18	22,48	7,16	7,22	3,67	7,7	24,5
June	2019	Dry	25,69	72	1,2	21,1	19	20,32	6,7	7,51	3,9	7,8	12,5
July	2019	Dry	17,42	68	1,2	18,6	19	18,78	6,77	21,1	4,31	7,7	25,1
August	2019	Dry	60,31	81	0,65	40	19	21,28	6,55	20,71	8,14	7,65	13,6
September	2019	Dry	14,97	67	1,3	8,3	25	22,68	7,51	6,96	6,23	7,8	124,1
October	2019	Rainy	24,88	72	1,4	2,6	25	26,5	6,79	6,61	4,67	10,4	220
November	2019	Rainy	18,72	69	0,9	23,4	25	25,51	6,65	7,76	6,13	7,8	309,1
December	2019	Rainy	21,77	70	0,8	31,4	23	27,7	6,93	16,6	10,1	7,6	340,2
January	2020	Rainy	12,63	65	1	14,6	25	28,45	6,86	7,18	5,3	8,1	459,6
February	2020	Rainy	16,98	68	1,3	5,8	23	28,92	6,93	6,65	3	8,25	451,1
March	2020	Rainy	18,51	69	0,9	15,9	18	27,22	6,88	7,1	10,4	7,8	147,3
April	2020	Dry	14,59	66	1	9,2	19	24,63	6,74	4,75	0,2	7,6	6,5
May	2020	Dry	18,51	69	0,95	19	18	21,4	5,79	6,42	5,6	7,7	23,7
June	2020	Dry	12,63	65	1,06	21,1	19	21,53	5,96	7,83	8,7	7,8	59
July	2020	Dry	16,55	68	1,2	18,6	19	21,65	6,85	8,47	4	7,7	4,5
August	2020	Dry	10,89	64	1,2	16	19	21,33	6,88	6,61	9,3	7,4	51,3
September	2020	Dry	21,45	70	1,2	16,7	19	22,51	6,77	9,51	6,4	7,65	3,8
October	2020	Rainy	9,144	62	1,1	15,9	21	25,27	6,93	8,62	13,6	9,6	139,7
November	2020	Rainy	6,967	59	1,48	23,4	23	26,1	6,94	8,91	7,9	7,9	66,4
December	2020	Rainy	10,45	63	2,18	31,4	26	28,8	7,02	8,88	10,1	8,4	373,9
January	2021	Rainy	16,77	68	1,43	14,6	24	27,19	7,05	9,46	9,2	8,4	173,9
February	2021	Rainy	3,919	53	2,05	0,3	21	28,2	6,68	8,96	4,4	7,8	69,1
March	2021	Rainy	6,314	58	1,86	0,8	28	26,68	6,93	8,23	5,4	9,12	105,4
April	2021	Dry	18,07	69	1,65	9,2	23	23,33	6,76	13,7	6,6	7,55	14,7

Capítulo 3. DNA barcoding for the zooplankton community in a Brazilian tropical Reservoir

Abstract

Analyses with molecular tools are increasingly used in the world, but in Brazil there are few studies for freshwater zooplankton. For the molecular identification of organisms, DNA barcoding is strongly recommended for the zooplankton community as for other aquatic organisms. For species identification, however, a library of specific reference sequences is required, according which gene was used. In recent decades, several databases of zooplankton sequences have been deposited. In the present work, we seek to start a library of barcoding sequences for the zooplankton community in the Lobo-Broa Reservoir, in Brazil, one of the oldest studied reservoirs in this country. Therefore, the inclusion of a molecular approach in this environment may help in the future to monitor the quality of the reservoir's ecosystem. For barcoding analysis, the COI haplotype – Cytochrome Oxidase subunit I, of mitochondrial origin, was used. Cladocera, Copepoda, and Rotifera taxa were identified firstly morphologically and after molecularly. Samples were obtained from monthly collections as a part of a reservoir-monitoring project. Sixty-three records of genetic sequences were obtained, 59 for microcrustaceans and 4 for rotifers. Among them, we sequenced the most abundant species found in the reservoir, such as important daphnids and bosminids, representatives of the Cyclopinae and Diaptomidae families, and Brachionidae. The taxa were aligned with the closest sequences available in the databases. This work shows gaps in the availability of COI reference sequences for Neotropical zooplankton. We highlight the use of this identification tool for the success of future works, especially in Brazil, which contains a large biodiversity that is not fully known.

Keyworks: biodiversity, identification, molecular, database, freshwater

Introduction

The zooplankton community is one of the most important components of aquatic ecosystems, in terms of biomass (Bonecker et al., 2007). They make up the base of the food chain in ecosystems and are considered good bioindicators (Neves et al, 2015; Fenoiva et al., 2022). Zooplanktonic organisms are a link to important ecological processes, interfering in top-down and bottom-up processes (Chouvelo et al., 2019; Ke et al., 2022). In this sense, there must be a growing search for studying these organisms from different perspectives.

The classical taxonomic identification of the zooplankton community has been well-established over the years in different countries (e.g. Reid, 1985; Koste, 1986; Koste, 1987; Smirnov, 1974 Roff, 1978; Elmoor-Loureiro, 1997; Perbiche-Neves et al., 2015). However, there are still many gaps to be unraveled regarding the identification and delimitation of species, especially for some more complex groups that require taxonomic reallocation and further studies (Sinev, 2021; Kotov et al., 2021; Krivenkova et al., 2022). In addition, the morphological identification of zooplankton requires the ability to verify very subtle morphological characteristics, which can bring difficulties and serious errors if the identification is not carried out by a truly specialized or well-informed person. The fact that zooplankton taxonomy is constantly being revised shows that constant identification confusion or misinformation can occur (Makino, 2017). To solve the existing gaps and corroborate taxonomic identification, molecular tools have been increasingly necessary. With the advancement of biotechnology and the emergence of new techniques, molecular studies are more accessible in the recent years (Harper, 2021).

Molecular studies for the identification of organisms began in the last twenty years. After the creation of a consortium of researchers, the BOLD (Barcode of Life Data) studies and the availability of molecular data for different organisms under study were intensified (Ratnasingham and Hebert, 2007). The DNA barcode is an interesting identification method because it uses short fragments of a conserved region of DNA (Hebert, 2003a,b).

For the zooplankton community, since the first studies, the use of DNA barcode has already proved to be efficient and can complement the taxonomic identification (Elías-Gutiérrez et al. 2008; García-Morales and Elías-Gutiérez 2013). However, these studies are still scarce concerning the information available in databases, since many important species of zooplankton have not yet been described with molecular tools. Still, most studies focus on temperate regions and marine environments. Therefore, there is a gap of information on species available in the database for freshwater environments in tropical regions (Makino et al., 2017; Xiong et al., 2020). The majority of genetic sequence records of the zooplankton community come researcher from Mexico, followed from Canada (*e.g* Costa et al., 2007) Elías-Gutiérrez et al., 2008; Andrade-Sossa et al., 2020), as these countries have two large barcode research centers and are responsible for the development of some barcode protocols (Hebert et al., 2003a,b; Ivanova et al., 2006a,b; Prosser et a., 2013; Elías-Gutiérrez et al., 2018)

For freshwater ecosystems in Brazil, this gap is even greater. There are few works with the zooplankton community DNA in the country, the vast majority of them with some daphnid species (Santos-Silva el al., 2014; Castilho et al. 2015; Abreu et al., 2021). DNA barcoding can potentially be a very useful tool for freshwater zooplankton monitoring programs, but reference DNA sequences must be available in searchable genetic databases beforehand, at least for applications on a regional (Elías-Gutiérrez et al. 2008; García-Morales and Elías-Gutiérrez, 2013).

In this study, we developed an initial DNA barcode library for the zooplankton of Lobo-Broa reservoir. The preference for the cytochrome c oxidase I COI region is because these haplotypes are often used in the other studies on the molecular taxonomy of freshwater zooplankton (e.g., Bekker et al. 2016; Mills et al. 2016). Furthermore, regional COI reference sequence libraries have been successfully developed for Mexican freshwater microcrustaceans (Elías-Gutiérrez et al. 2008), and rotifer (García-Morales and Elías-Gutiérrez, 2013). This present study is the first DNA-barcode work of a reservoir zooplankton community in Brazil, adding sequences in the online libraries, useful for future studies analyzing the molecular data. This work reveals a shortage of COI reference sequences for zooplankton and underscore the urgency of developing COI barcode libraries for Neotropical species on a global scale.

Materials and Methods

Study area

Sampling was carried out from May 2019 to April 2021, in the Lobo-Broa Reservoir, located in the central-west region of the state of São Paulo, Brazil, at a sampling point in the lentic region of the reservoir and close to the dam (22º 10' 43.5”S, 47º 53' 44.5”W) (Fig. 1).

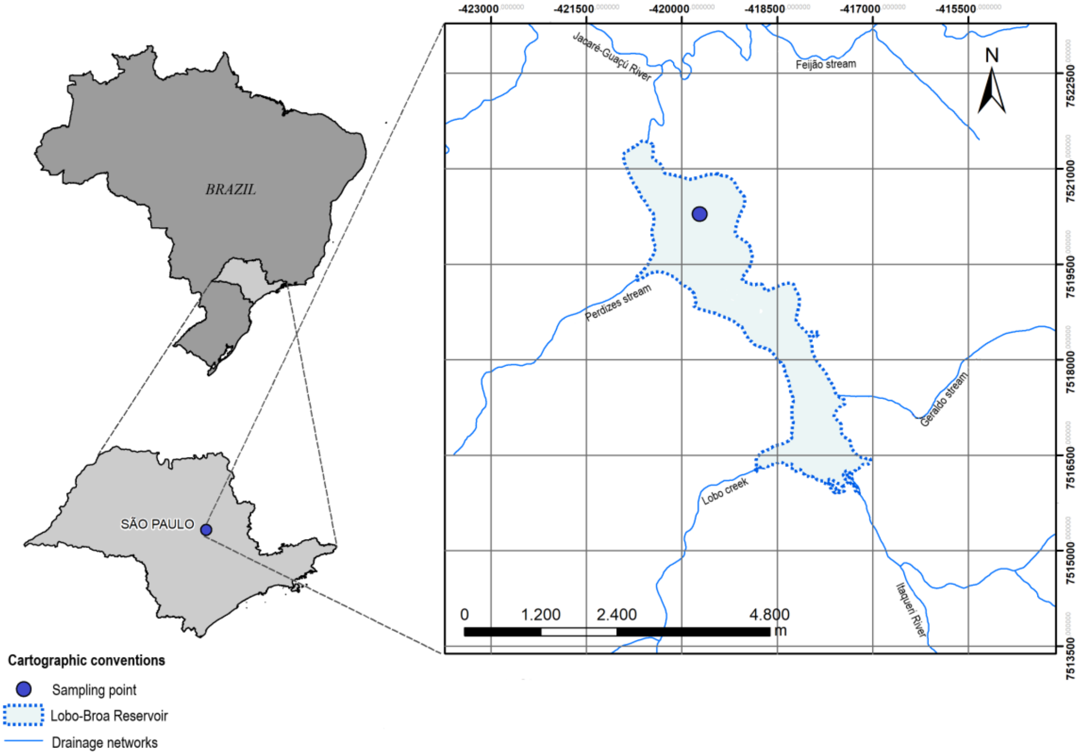

Figure 1. Map of the study region in the Lobo-Broa Reservoir and its tributaries, with emphasis on the sampled point (blue circle) in the deeper portion of the reservoir.

Sample and morphological analysis

The samples were obtained by vertical hauls with a conical plankton net of 48 µm mesh size, and the material was fixed in 97% ethanol, replacing all water came with sample. In the laboratory, specimens were sorted from the samples and stored in tubes with 97% ethanol for the DNA extraction. One specimen was stored per tube. The taxonomic identification of the zooplankton community was carried out with specialized literature: Rotifera was based on Koste (1986, 1987), Segers (1995), and Nogrady (2002), Cladocera with Smirnov (1974), Elmoor-Loureiro (1997), and the website Cladóceros do Brasil (https://cladocera.wordpress.com/), and finally Copepoda with Reid (1985), Neves (2011), and Perbiche-Neves et al. (2015). Detailed analyses were performed with the aid of optical microscope and the scanning electron microscopy JEOL-JSM-6010PLUS/LA, located at El Colegio de la Frontera Sur (ECOSUR, Chetumal Unit, Mexico). Some images of specimens in this study can be seen in figure S1 of the Supplementary Material.

DNA extraction, amplification, and sequencing

DNA extraction and PCR reactions were carried out at El Colegio de la Frontera Sur (ECOSUR, Chetumal Unit, Mexico). For DNA extraction, the following protocols were used: Glass Fiber Plate DNA Extraction - CCDB: Canadian Centre for DNA Barcoding (Ivanova et al., 2006a,b) and Quick-Start Protocol DNeasy® Blood & Tissue Kit – QIAGEN®, according to manufacturer's guidance.

Polymerase chain reactions (PCRs) had a total volume of 12.5μl, containing 02 µL of DNA extract, 6.25 µL of 10% trehalose, 02 µL of ddH$_2$O, 1.25 µL of 10X PCR Buffer for Platinum Taq (Invitrogen™), 0.625 µL of 50mM MgCl$_2$, 0.125 µL each of primer 10 µM, 0.0625 µL of 10 mM dNTPs and 0.06 µL Polymerase Platinum Taq (Invitrogen™). A 658 bp segment of COI was amplified using ZplankF1_t1 and ZplankR1_t1 primers, according to Proseer et al. (2013).

PCR reactions were cycled at the initial denaturation at 94°C for 1 min, followed by five cycles of 94°C for 30 sec, annealing at 45-50°C for 40 sec, and extension at 72°C for 1 min, followed by 30-35 cycles of 94°C for 30 sec, 51-54°C for 40 sec, and 72°C for 1 min, with a final extension at 72°C for 10 min, followed by an indefinite hold at 4°C. PCR products were visualized on 2% agarose gels (E-Gel 96 Invitrogen), and positive PCR products were selected for sequencing bidirectionally at Eurofins Scientific in Louisville, Kentucky.

Data analysis

The sequences were aligned using MUSCLE in the MEGA 6.0 program, according to Tamura et al. (2013), where they were also analyzed for stopcodon detection. The evaluation of the saturation signal in the sequences was performed using the DAMBE5 program (Xia, 2013). Uncorrected p-distance and Kimura 2-Parameters (K2P) were calculated in MEGA 6.0, according to Tamura et al. (2013). Intragroup and intergroup divergence values were also calculated using MEGA 6.0 (Tamura et al., 2013). A distance tree was obtained by the Neighbor Joining method with a non-parametric bootstrap analysis with 1000 replicates.

Alignment with other sequences for comparison purposes was performed using sequences available in the databases Barcode of Life Database (BOLD, boldsystems.org) and GenBank. We performed a Basic Local Alignment Search Tool (BLAST alignment) to find regions of local similarity between sequences of identified taxa with COI reference sequences for zooplanktonic organisms. All sequences were uploaded to the Barcode of Life Database

(BOLD, boldsystems.org), in project BROA. Paratype vouchers will be deposited in the Reference Museum of Zoology of the University of São Paulo-USP.

Results

Were obtained 63 genetic sequences for all groups. The COI sequences obtained are 658 base pairs (bp) in size. The absence of stop codons and the high similarity with sequences of species of the same family in GenBank guarantee that the amplified product consists of the functional COI region of the organisms. The Neighbor-Joining (NJ) method (Fig. 2).

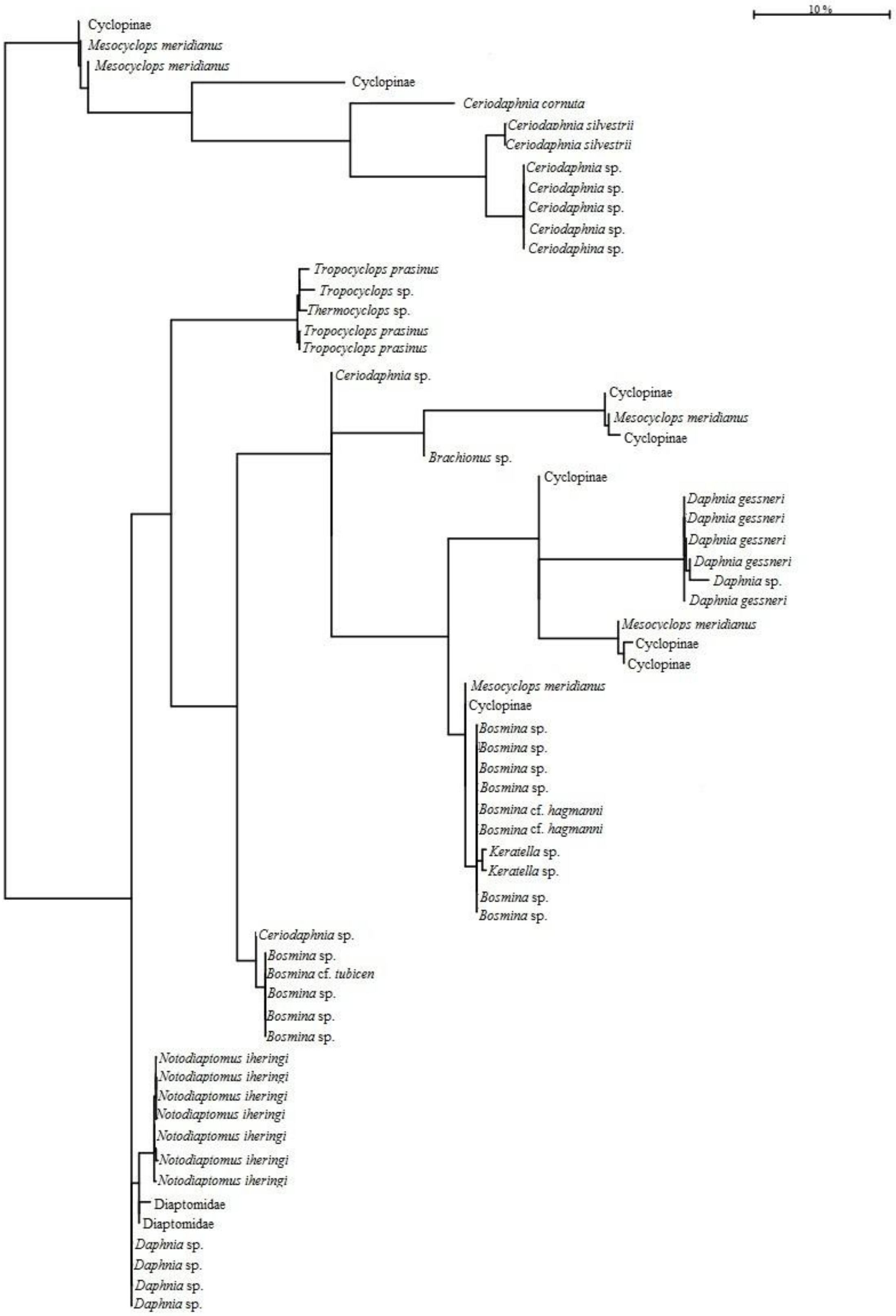

Fig. 2. Simplified ID tree inferred using the NJ method, using 63 sequences for microcrustaceans. All positions containing gaps have been eliminated.

From the sequences obtained, we obtained molecular data on zooplanktonic organisms at the family, genus and species level. For Copepoda, sequences of specimens identified at the level of the Cyclopinae and Diaptomidae families were obtained. At the level of genus and species, we obtained the following results: *Mesocyclops meridianus, Tropocyclops* sp., *Tropocyclops prasinus, Thermocyclops* sp. e *Notodiaptomus iheringi.* For Cladocera, sequences of *Ceriodaphnia* sp., *Ceriodaphnia silvestrii*, *Ceriodaphnia cornuta*, *Daphnia* sp., *Daphnia gessneri*, *Bosmina* sp., *Bosmina* cf. *tubicen* e Bosmina cf. *hagmanni* were obtained. Among Rotifera genetic sequences of *Brachionus* sp. and *Keratella* sp were obtained.

Of the species identified in our study, only a few Daphnidae matched a high percentage with database sequences: *Ceriodaphnia silvestrii* (99.7% with *Ceriodaphnia dubia*); *Daphnia gessneri* (97.32%), a register from Argentina; *Ceriodaphnia cornuta* (99.61%). Despite the percentual above 97%, the data are not enough to confirm the interspecific alignment of our data with those of the banks. In the alignment of Rotifera species, *Brachionus* sp. It had its highest percentage of alignment with a species from Spain (87.55%), which is too close a value to corroborate any information. The specimens of *Keratella* sp, had a good percentage of alignment (above 97%) with Mexican specimens. For Copepoda, there were no sequence records in the database to compare the *Notodiaptomus iheringi*, an impportant species in freshwater environments. This species was aligned with another genus, at a percentage around 80%. In the same way, for *Mesocyclops meridianus*, in which all alignment attempts proved to be too far for genus delimitation, being below 90%. BLAST Alignment results are listed in Table 2.

Table 2. The results of Nucleotide BLASTn search showing COI reference sequence for the Lobo-Broa Reservoir zooplanktonic species.

	BLASTn search result			
Taxa in the present study	Identity (%)	Descripton	Locality	ID GenBank
Diaptomidae	81,96%	*Epishura chankensis*	Russia	KR704407.1
Notodiaptomus iheringi	81,95%	*Boeckella brasiliensis*	Argentina	DQ356546.1
Notodiaptomus iheringi	81,95%	*Boeckella brasiliensis*	Argentina	DQ356546.1
Notodiaptomus iheringi	81,95%	*Boeckella brasiliensis*	Argentina	DQ356545.1
Notodiaptomus iheringi	81,95%	*Boeckella brasiliensis*	Argentina	DQ356546.1
Diaptomidae	81,84%	*Memocalanus sp.*	Russia	KU247732.1
Mesocyclops meridianus	87,61%	*Mesocyclops edax*	Mexico	MN567207.1
Mesocyclops meridianus	88,13%	*Cyclopoida sp.*	Mexico	KC616898.1
Notodiaptomus iheringi	81,96%	*Epishura chankensis*	Russia	KR704407.1
Notodiaptomus iheringi	81,96%	*Epishura chankensis*	Russia	KR704407.1
Daphnia gessneri	97,01%	*Daphnia gessneri*	Argentina	AY323071.1

Ceriodaphnia silvestrii	99,70%	*Ceriodaphnia dubia*	Brasil	KY659308.1
Ceriodaphnia silvestrii	99,70%	*Ceriodaphnia dubia*	Mexico	KC617530.1
Ceriodaphnia sp.	99,70%	*Ceriodaphnia dubia*	Mexico	KC617530.1
Ceriodaphnia cornuta	99,61%	*Ceriodaphnia cf. rigaudi*	Brasil	KU315489.1
Bosmina cf.hagmani	85,08%	*Eubosmina huaronensis*	Mexico	KC617511.1
Bosmina cf hagmani	85,08%	*Eubosmina huaronensis*	Mexico	KC617511.1
Daphnia gessneri	96,85%	*Daphnia gessneri*	Argentina	AY323071.1
Bosmina cf tubicen	85,08%	*Eubosmina huaronensis*	Mexico	KC617511.1
Bosmina cf hagmani	85,08%	*Eubosmina huaronensis*	Mexico	KC617511.1
Ceriodaphnia silvestrii	99,70%	*Ceriodaphnia dubia*	Brasil	KY659308.1
Ceriodaphnia silvestrii	99,70%	*Ceriodaphnia dubia*	Brasil	KY659308.1
Ceriodaphnia silvestrii	96,08%	*Ceriodaphnia dubia*	Brasil	KY659308.1
Daphnia gessneri	96,85%	*Daphnia gessneri*	Argentina	AY323071.1
Bosmina cf tubicen	85,08%	*Eubosmina huaronensis*	Mexico	KC617511.1
Ceriodaphnia sp.	99,70%	*Ceriodaphnia dubia*	Brasil	KY659308.1
Cyclopinae	87,22%	*Cyclopoida sp*	Mexico	KC617549.1
Ceriodaphnia silvestrii	96,08%	*Ceriodaphnia dubia*	Brasil	KY659308.1
Ceriodaphnia silvestrii	99,70%	*Ceriodaphnia dubia*	Brasil	KY659308.1
Bosmina cf hagmanni	85,08%	*Eubosmina huaronensis*	Mexico	KC617511.1
Bosmina sp.	85,08%	*Eubosmina huaronensis*	Mexico	KC617511.1
Daphnia gessneri	97,32%	*Daphnia gessneri*	Argentina	AY323071.1
Daphnia gessneri	96,69%	*Daphnia gessneri*	Argentina	AY323071.1
Ceriodaphnia silvestrii	99,70%	*Ceriodaphnia dubia*	Brasil	KY659308.1
Bosmina sp.	85,08%	*Eubosmina huaronensis*	Mexico	KC617511.1
Mesocyclops meridianus	88,13%	*Cyclopoida sp*	Mexico	KC616898.1
Mesocyclops meridianus	89,06%	*Cyclopoida sp.*	Mexico	KC616894.1
Tropocyclops prasinus	81,60%	*Tropocyclops prasinus*	Mexico	MZ517346.1
Cyclopinae	88,13%	*Cyclopoida sp*	Mexico	KC616898.1
Cyclopinae	88,13%	*Cyclopoida sp*	Mexico	KC616898.1
Cyclopinae	88,13%	*Cyclopoida sp.*	Mexico	KC616898.1
Tropocyclops prasinus	80,62%	*Tropocyclops prasinus*	Mexico	MZ518005.1
Tropocyclops sp.	81,66%	*Tropocyclops prasinus*	Mexico	MZ518057.1
Mesocyclops meridianus	88,13%	*Cyclopoida sp*	Mexico	KC616898.1
Tropocyclops sp.	80,00%	Cyclopidae	Canadá	MG514580.1
Mesocyclops meridianus	87,42%	*Cyclopoida sp.*	Mexico	KC616898.1
Thermocyclops sp.	81,63%	*Tropocyclops prasinus*	Mexico	MZ517346.1
Tropocyclops prasinus	82,00%	*Tropocyclops prasinus*	Mexico	MZ518005.1
Daphnia gessneri	97,32%	*Daphnia gessneri*	Argentina	AY323071.1
Bosmina sp.	85,05%	*Eubosmina huaronensis*	Mexico	KC617511.1
Keratella sp.	98,64%	*Keratella americana*	Mexico	JX216612.1
Cyclopinae	87,22%	*Cyclopoida sp*	Mexico	KC616898.1
Cyclopinae	87,22%	*Cyclopoida sp*	Mexico	KC616898.1
Brachionus sp.	87,55%	*Brachionus quadridentatus*	Espanha	KY749412.1
Keratella sp.	97,58%	*Keratella americana*	Mexico	KC617511.1
Bosmina sp.	85,05%	*Eubosmina huaronensis*	Mexico	KC617511.1
Bosmina sp.	85,05%	*Eubosmina huaronensis*	Mexico	KC617511.1
Bosmina sp.	85,05%	*Eubosmina huaronensis*	Mexico	KC617511.1
Bosmina sp.	85,05%	*Eubosmina huaronensis*	Mexico	KC617511.1
Diaptomidae	81,84%	*Memocalanus sp.*	Russia	KU247732.1
Ceriodaphnia sp.	99,70%	*Ceriodaphnia dubia*	Brasil	KY659308.1
Ceriodaphnia sp.	99,70%	*Ceriodaphnia dubia*	Brasil	KY659308.1

Discussion

This study produced molecular biology data to identify some of the main genera of Cladocera and Copepoda for tropical environments (Rosa et al, 2021; Macedo et al., 2022; Elmoor-Loureiro, 2022). The difficulty in extracting DNA from Rotifera, due to its small size, reflects the low success of molecular in some experiments (Oh et al, 2020). Our goal is to join our data with zooplankton sequences to increase the information in the databases.

ID tree inferred

The genetic distance value, established at approximately 3%, found for DNA Barcoding, is considered one of the parameters for species delimitation in a large number of groups of animals (Hebert and Gregory, 2005). Other authors have shown that this distance value of 3% for the COI region described by Hebert et al. (2003) can be applied to crustaceans for species-level delimitation and emphasized the importance, in ecological work, of a database of DNA Barcoding sequences for this group (Costa et al, 2007). The low percentage of alignment between the studied species and the database data is not enough to confirm molecular identification. It is necessary, in cases like this, when there is little information, that the studies are integrative with the taxonomy (Kasapidis et al., 2018; Bucklin et al., 2021).

Within this percentage of species limitation, only our Daphnid and a species of rotifer found similarity with data from the bank. This control took place with data from Brazil, Argentina, and Mexico. Mexico is one of the countries that currently updates the zooplankton genetic database the most, so it was expected that our data would align with the few available sequences (e.g Valdez-Moreno et al., 2021; Andrade-Sossa et al., 2021; Elías-Gutiérrez et al., 2021). This reinforces an information gap in other countries where daphnids are abundant, especially in tropical countries (Vasquez et al., 2021)

The gap from molecular data to Copepoda data in tropical aquatic ecosystems is evident, as there is increasing information from many marine species data (Rajthilak et al., 2021, Dennis et al., 2021). Copepoda data were more difficult to obtained, maybe associated to intra-specific variability on their populations. The same appointment can be highlighted for rotifers, small organisms that have few DNA information available (Oh et al, 2020). Our copepod data aligned to a low percentage with sequences from Russia, for Diaptomids that are not endemic to our tropical region. Thus, the accompaniment takes place between species that are very distant evolutionarily due to the existing gap in the data.

The results found here are pioneer in contribute to the molecular biology increase in the Neotropical region, especially in Brazil, since studies like this are scarce and very important to monitoring, for example (Santos-Silva el al., 2014; Castilho et al. 2015; Abreu et al., 2021). The most abundant organisms in the studied reservoir were different to the deposited sequences in GenBank, suggesting that more studies are necessary, probably species complexes are involved.

Zooplankton fauna therefore change with geographical regions, which would be the reason why the COI barcode library established for Mexican freshwater zooplankton, for example, has been used as reference to other regions in the world (Richter et al, 2020). In addition the zooplankton fauna can change according to regional environmental conditions, including the topology (Obertegger et al. 2014).

This study points to important sequences of zooplankton barcoding for a reservoir in Brazil. This data can be used to rapid identification and monitoring of water quality in lakes, as also as for phylogenetic studies wishing to identify its relations and evolutions patterns for the main zooplankton species.

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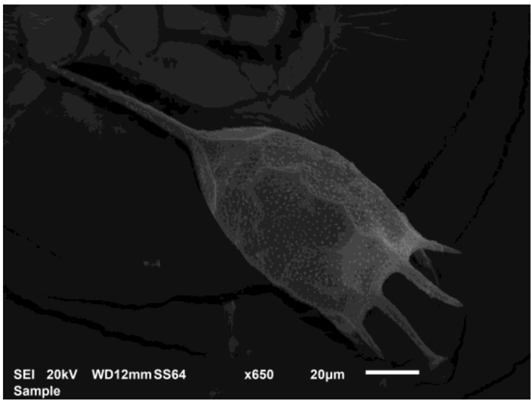
Supplementary Material

S1. Scanning electron microscopy images of some representatives species from Broa-Lobo zooplankton community.



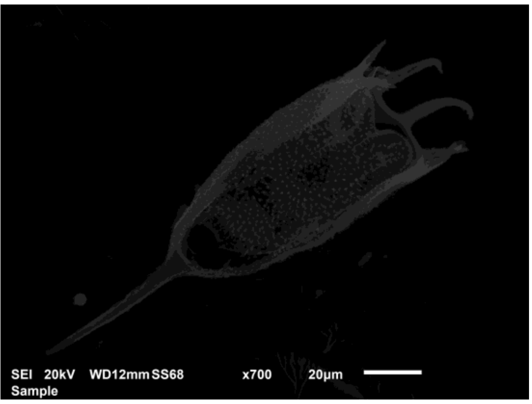


Fig. S1a: *Keratella americana*. Dorsal view. **Fig. S1b:** *Keratella americana* ventral view

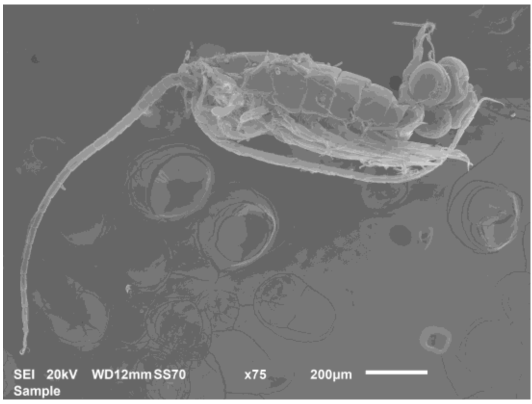


Fig. S1c: Calanoid female. Side view.

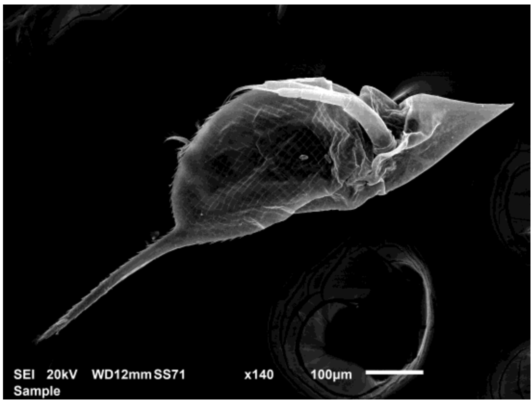


Fig. S1d: *Daphnia gessneri*. Side view

Considerações Finais

O Reservatório do Lobo-Broa é uma referência em estudos limnológicos no Brasil por se tratar de um dos primeiros ecossistemas dulcícolas a ser estudado no país, sendo uma das raízes da história da limnologia brasileira. Nas últimas décadas, diversos pesquisadores analisaram, com diferentes objetivos, a comunidade planctônica do Lobo-Broa e, no que se refere ao zooplâncton, diferentes abordagens podem ser encontradas na literatura referente a esse ambiente. Vários pontos ao longo do reservatório foram amostrados, sendo possível uma análise panorâmica de sua composição, considerando todos os dados já obtidos. Com isso, o primeiro capítulo desta tese buscou contribuir para o conhecimento desse ecossistema, dada sua importância no cenário limnológicos brasileiro, ao realizar um compilado de dados e informações das últimas cinco décadas de estudo no reservatório, além de uma observação pontual dos últimos dois anos, evidenciando as flutuações existentes.

As flutuações da comunidade zooplanctônica e fitoplanctônica se dão, em maior parte, por consequência não somente da sazonalidade, mas também de fatores externos como regimes atípicos de chuvas e ações antrópicas. As análises do compilado dos dados evidenciaram que houve uma flutuação populacional nas últimas cinco décadas, em todos os grupos zooplanctônicos, assim como para o fitoplâncton. Além disso, o reservatório tem passado por oscilações em seu grau de trofia, o que é atribuído, possivelmente, às atividades antrópicas e uso e ocupação do entorno da região.

Os dados evidenciaram que os anos que sofreram efeito de variabilidades climáticas atípicas, como excesso ou ausência de chuvas, foram aqueles em que as flutuações dentro dos grupos zooplanctônicos foram mais evidentes, especialmente para Cladocera e Rotifera. Os dados ainda mostraram que, embora tenha havido flutuações na composição da comunidade em alguns períodos, estas não foram suficientes para impulsionar a substituição de grandes grupos, como filtradores de maior porte para os de menor porte. No que se refere à análise pontual dos últimos anos, os dados mostram que as alterações nos volumes das chuvas podem ter impactado na distribuição da comunidade, uma vez que houve meses de chuvas excessivas acima do esperado, assim como meses de secas atípicas, resultado de variações climáticas dos anos anteriores. Neste sentido, os dados apontam que variações interanuais são mais determinantes em relação às sazonais, no que se refere à composição de espécies zooplanctônicas. Dados como esse chamam a atenção para a influência que filtros ambientais podem exercer sobre ecossistemas ao longo de determinados períodos de tempo, como consequência das alterações climáticas que têm sido cada vez mais acentuadas em nosso planeta. A comunidade zooplanctônica, estando próxima da base das cadeias tróficas aquáticas, reflete claramente os impactos dessas alterações e, portanto, estudos que analisem sua composição, distribuição e diversidade são de grande importância.

Índices e características funcionais foram utilizados neste estudo como métricas para se avaliar a diversidade da comunidade zooplanctônica do Reservatório do Lobo-Broa. Uma vez que no Brasil, assim como em ecossistemas tropicais em geral, a abordagem funcional é relativamente recente, este estudo contribuiu consideravelmente para o conhecimento das características e funcionalidades de espécies zooplanctônicas. Algumas características funcionais foram mais determinantes nos agrupamentos das espécies, tais como habitat, resposta de escape predatório, tipo de alimentação e tamanho corporal. A característica tamanho corporal influenciou consideravelmente a composição dos grupos funcionais formados especialmente por Cladocera e Copepoda, pelo fato de haver variações de tamanho entre os microcrustáceos, inclusive entre espécies do mesmo gênero. Além disso, esse traço funcional influencia diretamente na alimentação, determinando tipo e tamanho de partículas ingeridas pelo zooplâncton. A capacidade de resposta à predação foi também um traço determinante por estar diretamente relacionada à sobrevivência, reprodução e tempo de vida de Cladocera e Copepoda. Os dados demonstraram que, para Rotifera, tamanho corporal e habitat são os traços mais determinantes.

Os índices de diversidade funcional responderam de modo equivalente aos índices de diversidade taxonômica e, em relação aos fatores ambientais, os índices de diversidade funcional estão diretamente ligados às variáveis que se relacionam com precipitação, e as variações nos índices funcionais ocorreram conforme a diferenciação entre períodos secos e chuvosos, sendo que em períodos de chuva observou-se maiores valores de índices de diversidade. Considerando períodos atípicos de chuva e seca, essas variações podem ocorrer de maneiras mais extremas, portanto este estudo mostra que considerar a influência das características funcionais para as medidas de biodiversidade é importante para o monitoramento e preservação dos ecossistemas aquáticos tropicais.

Os ecossistemas aquáticos tropicais possuem uma rica biodiversidade, e isso se estende à comunidade zooplanctônica. Dentre as diversas ferramentas disponíveis para estudos sobre a composição de um ecossistema, as análises moleculares têm se mostrado extremamente eficientes e cada vez mais acessíveis, diante dos avanços científicos das últimas décadas. Apesar disso, no que se refere à comunidades zooplanctônicas de ambientes tropicais, e especialmente para o Brasil, há ainda uma lacuna de informações oriundas de análises moleculares. Este trabalho buscou contribuir para o início de um construção de biblioteca de dados genéticos da comunidade zooplanctônica do Reservatório do Lobo-Broa, obtendo um total de 63 sequências genéticas para os três grupos mais comumente conhecido dentre zooplâncton, com o marcador molecular COI. A comparação das porcentagens de divergências genéticas deste trabalho com as dos bancos de dados revelou uma lacuna de informações, pois não há disponibilizadas sequências genéticas de gêneros e espécies com porcentagens próximas, o que dificulta a confirmação molecular. Diante disso, este trabalho destaca a importância de abordagens complementares, como a ferramenta molecular tendo o respaldo da identificação taxonômica. Dentro dos microcrustáceos, Cladocera é o grupo com o maior número de sequências genéticas disponíveis para comparação / alinhamento com os dados deste trabalho. Dentre o grupo Copepoda, ainda há uma grande lacuna, sendo que a porcentagem de alinhamento com sequências dos bancos de dados não chega a 90% e um padrão parecido ocorre com o grupo Rotifera. Os organismos zooplanctônicos mais abundantes do Reservatório do Lobo-Broa tiveram porcentagem de divergência genética relativamente alta em comparação com espécimes de outras localidades, o que reforça a necessidade de um maior investimento em análises moleculares e construção de banco de dados para a região Neotropical, especialmente para o Brasil.

Um maior número de dados moleculares disponíveis em banco de dados contribui para estudos ecológicos em ecossistemas aquáticos, uma vez que auxilia na identificação de espécies, composição, monitoramento da saude do ambiente, assim como em estudos ecológicos mais aprofundados da comunidade zooplanctônica. Com os avanços científicos, estudos moleculares tem se tornada cada vez mais acessíveis, sendo que este trabalho trouxe importantes contribuições para essa linha de pesquisa com enfoque nas comunidades zooplanctônicas de ecossistemas aquáticos tropicais brasileiros.