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NATAN GUILHERME DOS SANTOS

Suscetibilidade do zooplâncton ao uso do solo, às aplicações de agrotóxicos e vinhaça: análise da diversidade funcional e taxonômica

São José do Rio Preto, SP
2024

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Susceptibility of zooplankton to land use, pesticide and vinasse applications: analysis of functional and taxonomic diversity

Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Biodiversidade, junto ao Programa de Pós-Graduação em Biodiversidade do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de São José do Rio Preto.

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CoAdvisor Prof. Dr. Evaldo Luiz Gaeta Espíndola

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São José do Rio Preto
08 de março de 2024

Dedico esta tese a minha mãe, a minha irmã e ao meu pai,
pelo apoio diário durante a minha trajetória de vida e
por sempre serem o suporte as minhas decisões e escolhas.

EPÍGRAFES

Andar com fé eu vou que a fé não costuma faiá (Gilberto Gil)

Don't stop me, don't stop me Have a good time good time (Queen)

All you need is love, love.
Love is all you need (The Beatles)

Se a educação sozinha não transforma a sociedade, sem ela tampouco a sociedade muda (Paulo Freire)

Não diga que a canção está perdida
Tenha fé em Deus, tenha fé na vida
Tente outra vez (Raul Seixas)

We are the champions, my friends and we'll keep on fighting till the end (Queen)

Sou feliz, alegre e forte
Tenho amor e sorte
Aonde quer que eu vá (Marisa Monte)

Quem tem um amigo tem tudo
Se o poço devorar, ele busca no fundo
(Emicida)

Você é o único representante
Do seu sonho na face da terra (Emicida)

Ser você é a única forma de viver

Tenho sangrado demais
Tenho chorado pra cachorro
Ano passado eu morri
Mas esse ano eu não morro (Belchior)

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RESUMO

DOS SANTOS, N.G. **Suscetibilidade do zooplâncton ao uso do solo, às aplicações de agrotóxicos e de vinhaça: análise da diversidade funcional e taxonômica.** 2024. Tese de Doutorado, Programa de Pós-Graduação em Biodiversidade, Universidade Estadual Paulista, Instituto de Biociências, Letras e Ciências Exatas, São José do Rio Preto, São Paulo, Brasil.

O uso e manejo do solo são um dos principais impactos às comunidades aquáticas e terrestres na atualidade. Dentre os diferentes usos do solo, os usos para plantações e pastagens são um dos mais impactantes, uma vez que utilizam agroquímicos, como inseticidas, herbicidas e fertilizantes, além da vinhaça, empregada como fertilizante natural nos cultivos de cana de açúcar no Brasil. Esses pesticidas e vinhaça podem ser carreados aos corpos aquáticos e impactar as comunidades aquáticas e os parâmetros da água. Diante disso, o objetivo principal dessa tese de doutorado foi avaliar a suscetibilidade do zooplâncton ao uso do solo, às aplicações de agrotóxicos e à vinhaça utilizando análises da diversidade funcional e taxonômica. Para atingir esse objetivo foram realizados estudos que resultaram na elaboração de 4 artigos com 3 abordagens, sendo elas: uma revisão de cienciometria, análise de dados coletados em campo e dois experimentos de mesocosmos. O primeiro artigo teve como objetivo apresentar um panorama global dos estudos sobre os efeitos do uso da terra sobre as comunidades zooplanctônicas de água doce, por meio da ciênciometria e de uma síntese dos principais conhecimentos produzidos em artigos publicados sobre o tema. Após a seleção, chegamos a um total de 80 artigos, revelando que este número tem aumentado ao longo do tempo, especialmente nos EUA, Polónia, Canadá, China, Argentina e Brasil. O uso da terra para plantações, pastagem e urbanização tem demonstrado ter um impacto negativo nas comunidades de zooplâncton. A presença de vegetação natural circundante em ambientes aquáticos tem demonstrado contribuir para uma maior diversidade taxonômica e funcional do zooplâncton. O segundo artigo teve como objetivo avaliar o efeito dos parâmetros da água de oito reservatórios inseridos em uma paisagem agropastoril sobre a estrutura funcional do zooplâncton. Os modelos mistos aditivos generalizados revelaram que o pH e o nitrogênio total são variáveis chave para a estruturação funcional do zooplâncton. No terceiro artigo foi realizado um experimento com simulações realísticas de uso e manejo do solo com a construção de mesocosmos. Este estudo teve como objetivo verificar os efeitos do uso e manejo do solo em pastagem extensiva, pastagem intensiva e cana-de-açúcar sobre a estrutura taxonômica e funcional do zooplâncton e analisar quais parâmetros da água estruturam o zooplâncton sob a influência de cada tipo de uso do solo. Nos usos do solo para pastagem intensiva e cana de açúcar houve uma redução da diversidade de Shannon e da riqueza funcional entre o início e o final do experimento. O pH foi o parâmetro mais importante na estruturação das comunidades zooplanctônicas, sendo a única variável estruturante presente em todos os tratamentos. O quarto artigo teve como objetivo avaliar os efeitos do fipronil, do 2,4-D e da vinhaça, isoladamente ou em misturas, sobre a diversidade do zooplâncton e identificar os parâmetros da água que estruturam a comunidade. Foi realizado um experimento em mesocosmos com seis tratamentos e três réplicas: controle (C), 2,4-D (D), fipronil (F), fipronil e 2,4-D (M), vinhaça (V) e todos os compostos (MV). Após a aplicação dos compostos, a riqueza de espécies, a diversidade de Shannon-Wiener, a riqueza e a dispersão funcional foram significativamente mais baixas nos tratamentos F, M, MV e V do que nos tratamentos C e D durante o experimento. Os parâmetros estruturais da água foram diferentes para cada tratamento. No tratamento C, a temperatura e a clorofila foram as variáveis estruturantes. No tratamento D, a turbidez e o pH foram as principais variáveis. A clorofila a foi a principal variável estruturante no tratamento F, enquanto o pH e a condutividade elétrica foram as variáveis estruturantes nos tratamentos MV e V, respectivamente. Os resultados desta tese destacam os impactos deletérios do uso e manejo do solo para pastagens e cana de açúcar e da aplicação de inseticida e vinhaça. Além disso, dependendo do tipo de uso do solo ou da aplicação de pesticida e de vinhaça os parâmetros da água que estruturam o zooplâncton serão diferentes, destacando o pH e a condutividade elétrica como os mais frequentes. As práticas agrícolas, uso e manejo do solo e aplicações de pesticidas ou vinhaça, reduzem a diversidade taxonômica e funcional do zooplâncton contribuindo para a perda de funções ecossistêmicas. Portanto, novas práticas sustentáveis de manejo e uso do solo precisam ser adotadas para diminuir os efeitos negativos sobre as comunidades aquáticas e, consequentemente, contribuir para a manutenção e conservação da biodiversidade aquática e das funções ecossistêmicas.

Palavras-chave: Impactos Ambientais. Paisagem. Inseticida. Herbicida. Cladocera. Copepoda. Rotifera.

ABSTRACT

DOS SANTOS, N.G. **Susceptibility of zooplankton to land use, pesticide and vinasse applications: analysis of functional and taxonomic diversity**. 2024. Doctoral thesis, Postgraduate Program in Biodiversity, Universidade Estadual Paulista, Instituto de Biociências, Letras e Ciências Exatas, São José do Rio Preto, São Paulo, Brazil.

Land use and management is one of the most important impacts on aquatic and terrestrial communities today. Among the different land uses, sugarcane crpops and pastures are one of the most impactful because they use agrochemicals such as insecticides, herbicides and fertilizers, as well as vinasse, which is used as a natural fertilizer in sugarcane crops in Brazil. These pesticides and vinasse can be carried into water bodies and affect aquatic communities and water parameters. Against this background, the main objective of this thesis was to assess the sensitivity of zooplankton to land use, pesticide applications and vinasse using functional and taxonomic diversity analyses. To achieve this objective, studies were carried out that resulted in the preparation of 4 articles with 3 approaches: a review of scientometrics, analysis of data collected in the field and two mesocosm experiments. The aim of the first article was to provide a global overview of studies on the effects of land use on freshwater zooplankton communities, using scientometrics and a synthesis of the main findings in articles published on the subject. After selection, we arrived at a total of 80 articles, showing that this number has increased over time, especially in the USA, Poland, Canada, China, Argentina and Brazil. Land use for cropland, pasture and urbanization have been shown to have a negative impact on zooplankton communities. The presence of surrounding natural vegetation in aquatic environments has been shown to contribute to greater taxonomic and functional diversity of zooplankton. The second article aimed to evaluate the effect of water parameters of eight reservoirs inserted in an agropastoral landscape on the functional structure of zooplankton. The generalized additive mixed models revealed that pH and total nitrogen are key variables for the functional structuring of zooplankton. In the third article, an experiment with realistic simulations of land use and management was carried out using mesocosms. The aim of this study was to verify the effects of land use and management in extensive pasture, intensive pasture and sugarcane on the taxonomic and functional structure of zooplankton and to analyze which water parameters structure zooplankton under the influence of each type of land use. Intensive pasture and sugarcane land use showed a reduction in Shannon's diversity and functional richness between the beginning and the end of the experiment. pH was the most important parameter in structuring zooplankton communities, being the only structuring variable present in all treatments. The fourth article aimed to evaluate the effects of fipronil, 2,4-D and vinasse, alone or in mixtures, on zooplankton diversity and to identify the water parameters that structure the community. A mesocosm experiment was conducted with six treatments and three replicates: control (C), 2,4-D (D), fipronil (F), fipronil and 2,4-D (M), vinasse (V) and all compounds (MV). After application of the compounds, species richness, Shannon-Wiener diversity, richness and functional dispersion were significantly lower in the F, M, MV and V treatments than in the C and D treatments during the experiment. The structural parameters of the water were different for each treatment. In treatment C, temperature and chlorophyll were the structuring variables. In treatment D, turbidity and pH were the main structuring variables. Chlorophyll a was the main structuring variable in treatment F, while pH and electrical conductivity were the structuring variables in treatments MV and V, respectively. The results of this work highlight the detrimental effects of land use and management for pasture and sugarcane, as well as the application of insecticides and vinasse. In addition, depending on the type of land use or application of pesticides and vinasse, the water parameters that structure the zooplankton will be different, with pH and electrical conductivity being the most common. Agricultural practices, land use and management, and pesticide or vinasse applications reduce the taxonomic and functional diversity of zooplankton, contributing to the loss of ecosystem functions. Therefore, new sustainable land use and management practices need to be adopted to reduce the negative impacts on aquatic communities and thus contribute to the maintenance and conservation of aquatic biodiversity and ecosystem functions.

Keywords: Environmental Impact. Landscape. Insecticide. Herbicide. Cladocera. Copepoda. Rotifera.

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feeding type trait was different between land uses (*F* = 3.62 ; *P* = 0.01). Abbreviation defined as follows: clad, cladoceran; cop, copepod; rot, rotifer.

Appendix C: Figure 3. Density of the zooplankton community according to the trophic group (carnivore, herbivore and omnivore) observed in different types of land uses (citrus, pasture and sugarcane). No difference was observed between the different types of land uses (*F* = 3.08 ; *P* = 0.078).

Appendix C: Figure 4. Density of the zooplankton community according to habitat occupied in an environmental aquatic (limnetic, littoral and littoral-limnetic) observed in different types of land uses (citrus, pasture and sugarcane). No difference was observed between the different types of land uses (*F* = 0.92 ; *P* = 0.50).

Chapter 3 – How does soil management for pasture and sugarcane affect zooplankton and its relationship with water structuring parameters?

Figure 1. A) View of the area where the experiment was conducted. Each plot represents a sampling unit with a mesocosm in the center. EP = Extensive Pasture; IP = Intensive Pasture and SC = Sugarcane Crop. The black lines indicate the separation of each treatment. B) View of the area of each mesocosm placed in the center of each sampling unit.

Figure 2. Water parameters responsible for structuring taxonomic diversity in each treatment. A) Structuring variables in the EP treatment. B) Structuring variables in the IP treatment. C) Structuring variables in the SC treatment.

Figure 3. Water parameters responsible for structuring functional diversity in each treatment. A) Structuring variables in the EP treatment. B) Structuring variables in the IP treatment. C) Structuring variables in the SC treatment.

Chapter 4 – Using a mesocosm approach to evaluate the effects on the zooplankton community of applying fipronil, 2,4-D and vinasse and to assess the relationship between application and water parameters.

Figure 1. Effects of treatments on diversity indices over time. A) Effect on species richness B) Effect on Shannon-Wiener diversity C) Effect on functional richness D) Effect on functional dispersion. The points represent the mean, and the vertical lines of the points represent the standard deviation.

Figure 2. Structuring water parameters of taxonomic and functional diversity indices for each treatment. A) Structuring water parameters for treatment C. B) Structuring water parameters for treatment D. C) Structuring water parameters for treatment F.

Figure 3. Structuring water parameters of taxonomic and functional diversity indices for each treatment. A) Structuring water parameters for treatment M. B) Structuring water parameters for treatment MV. C) Structuring water parameters for treatment V.

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index between treatments at the beginning and end of the experiment. EP = Extensive Pasture; IP = Intensive Pasture and SC = Sugarcane Crop.

Table 3 - Comparison of the average index values between the beginning and the end of the experiment. - = indicates a decrease and + an increase. Bold P values indicate significant differences.

Table S1 - List of the species and the functional traits of the zooplankton community that were found in the experiment.

Table S2 - Parameters used to select the best linear mixed models (GLMM) for taxonomic and functional diversity indices. Model combinations were based on the analysis of the full model (including all water variables). Collinearity between water variables was checked. The water variables that were collinear were removed from the full model. Subsequently, the variables that showed an influence (P value) on the indices were combined with each other. Then, each variable was analyzed individually. To select the best models, only homoscedastic and linear models (Dharma function), models with significant variables and the lowest AICC value were considered. temp = temperature; do = dissolved oxygen; turbidity; ec = electrical conductivity; cla = chlorophyll a; tn = total nitrogen and tp = total phosphorus.

Chapter 4 – Using a mesocosm approach to evaluate the effects of application of fipronil, 2,4-D and vinasse on the zooplankton community and its relationship with water parameters.

Table 1 - Mean and standard deviation (±) of the environmental variables per treatment at the beginning (day 0), in the middle (day 14) and at the end (day 74) of the experiment. C = control treatment with no pesticide or vinasse application; F = fipronil insecticide application; D = 2,4-D herbicide application; M = combined insecticide and herbicide application; V = vinasse application; and MV = combined insecticide, herbicide and vinasse application.

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SUMMARY

1 STRUCTURE OF THE THESIS

This thesis is organized into a general introduction, which contains the main information about the studies of land use, pesticides and vinasse on ecological communities in Brazil. That led to the thesis development, followed by 5 chapters, a general conclusion and the next steps. The first 4 chapters are divided into 1 published article (chapter 2) and 3 manuscripts (chapters 1, 3 and 4). The first one has already been submitted. Chapters 3 and 4 are manuscripts written in a scientific journal format that are intended to be published. The fifth chapter describes the main scientific activities carried out during the PhD formation. The last section is a general conclusion of the thesis and the next step for the future.

The first chapter is a scientometric review that aims to provide an overview of studies on the effects of land use on the freshwater zooplankton community, considering geographical, temporal and ecological aspects.

The second chapter is a published article (Dos Santos et al., 2023). This paper evaluated what were the main water parameters in the structure of the diversity and functional composition of zooplankton. This study was carried out in 8 reservoirs inserted in agropastoral landscape in Brazil. The pH and total nitrogen were the structuring variables in these environments.

The third chapter shows the implementation of an experiment that represents, under realistic conditions, the main land uses in Brazil: extensive pasture, intensive pasture and sugarcane crops. These land uses are one of the main economic activities in Brazil and have caused serious damage to Brazilian biodiversity. The effects of these land uses on the diversity and composition of zooplankton, both functionally and taxonomically, were analyzed. Also, the main water variables that structure the zooplankton in these land use types were identified.

The fourth chapter describes the isolated and mixed effects of insecticide, herbicide and vinasse on the functional and taxonomic diversity of zooplankton using a mesocosm experiment. The insecticide and vinasse isolated and mixed treatments caused a reduction in functional and taxonomic richness, Shannon diversity and functional dispersion. We also identified the main structuring water variables for each type of pesticide and vinasse.

The fifth chapter describes the main academic activities carried out during the doctorate, including scientific dissemination and teaching, co-supervision, participation in and organization of scientific events, publications and awards.

Finally, in the general conclusion and next steps, the conclusion of the doctoral thesis and new studies that should be carried out are presented.

2 GENERAL INTRODUCTION

2.1 Impact of land use on communities

Throughout human history, anthropogenic actions have caused several changes to native biomes, one of the most important being the reduction and/or fragmentation of natural landscapes, which has resulted in the loss of biodiversity worldwide (NEWBOLD et al., 2015, 2016A; MORRISON; BROSI; DIRZO; 2020). These changes are driven by high population growth, which has led to increased demand for food and housing (ROSA, 2020; WILLIAMS; BATES; NEWBOLD, 2020; SCHNEIDER; ZABEL; MAUSER, 2022). The impacts of the Anthropocene have resulted in the conversion of natural landscapes to agricultural and urbanized landscapes (TILMAN et al., 2011; TSCHARNTKE et al., 2012; LIU et al., 2022; SCHNEIDER; ZABEL; MAUSER, 2022). These changes are altering the abiotic and biotic characteristics of all ecosystems around the world, resulting in the loss of taxonomic and functional diversity of diverse biological communities (LIU et al, 2022, PAQUETTE et al., 2022). Currently, the expansion of land use and management for agricultural activities and urbanization over natural areas is increasing every year, especially in Brazil (OGURA et al., 2022a; LUCAS; CALDARELLI; VENTURA, 2023), which as a developing country is experiencing economic growth and has agribusiness as one of its main economic activities.

There are myriad abiotic changes and impacts resulting from land use change and management. The increase in deforestation associated with land use change has led to an increase in greenhouse gas emissions, which has contributed to increased global warming (ROLANDO et al., 2017; EL-MAGD et al., 2024). In addition, changes in land use have increased the amount of organic matter and sediment in lakes and streams over the years, resulting in the siltation of these environments (WILD; GÜCKER; BRAUNS, 2019; CHEN et al., 2023; WU et al., 2023; BIBENTYO et al., 2024). Furthermore, loss of riparian vegetation increases nutrient inputs and temperature of aquatic environments, leading to altered trophic interactions of aquatic communities (MOLINA-NAVARRO et al., 2018; REIS; BUNN; ALBRECHT; 2022). On the other hand, increases in nutrients and dissolved organic matter may benefit some species of phytoplankton and macrophytes, whose abundance increases as they assimilate the nutrients introduced into the aquatic environment (ZHANG et al., 2022a).

Understanding the effects of abiotic changes caused by land use is essential for the conservation of biodiversity. By identifying the conditions under which abiotic variables lead to greater biodiversity in terms of species taxonomy and function, it is possible to design environmental conservation laws and projects.

Several studies have assessed the impacts of land use on terrestrial communities, such as the impacts on terrestrial vertebrate diversity, which contributes to the loss of ~15% of terrestrial vertebrate species and ~14% of their native habitat, potentially resulting in the global extinction of 556 to 818 vertebrate species (CHAUDHARY; MOOERS, 2018, SEMENCHUK et al., 2022). With increased habitat loss due to changes in land use, it is estimated that approximately 1,700 species of birds, amphibians and mammals will be extinct by 2070 (POWERS, JETZ, 2019). In addition, the conversion of land from natural vegetation to agricultural and/or urban areas promotes the migration of vertebrate species to new habitats, leading to an intensification of competitive interactions with endemic species (BAJARU et al., 2020; MAYANI-PARÁS et al., 2021; POLAINA et al., 2021; STRONA; BRADSHAW, 2022). Another factor that impacts terrestrial communities is the intensification of land use, which influences the fluctuation of terrestrial animal populations, with deforestation being one of the main factors responsible for reducing bat population stability and pastureland for bird population (BLÜTHGEN et al., 2016). Moreover, herbivory rates of terrestrial invertebrates decrease with increasing intensity of land use near forest areas and in pasture areas (NEFF et al., 2023). Changes in land use, such as the conversion of areas from pasture to croplands, have been associated with changes in the composition and structure of small mammal communities (TORRE et al., 2015; GRAHAM et al., 2019). Thus, changes in land use, whether through conversion of land, intensification of use, or loss of habitat, have resulted in the loss of terrestrial biodiversity worldwide (NEWBOLD et al., 2015, 2016B; OUTHWAITE; MCCANN; NEWBOLD, 2022).

In addition to impacts on terrestrial communities, some studies have shown the effects of land use on aquatic communities, particularly fish and macroinvertebrate communities (ITO et al., 2020; CABRERA et al., 2023). Land use intensification affects trophic interactions and the functioning of aquatic ecosystems, reducing the number of interactions between species but increasing the efficiency of energy transfer (COLLYER et al., 2023). In addition, deforestation causes limnological changes, such as increases in temperature and nutrients, and consequently alters trophic interactions between species and within fish guilds (REIS; BUNN; ALBRECHT, 2022). Other studies have shown that land use conversion from pasture to sugarcane has caused a decrease in the functional diversity of fish and macroinvertebrates (ZENI; HOEINGHAUS; CASATTI, 2017; LIU et al., 2022). Thus, land use has led to a loss of aquatic biodiversity and a homogenization of aquatic ecosystems, especially on fish and macroinvertebrate communities. Studies on the effects of land use on zooplankton are still scarce (BALSEIRO et al., 2023; CASTILHO-NOLL et al., 2023) and it is necessary to find a global overview to identify the state of the art of these studies. It is expected that the majority of these studies will be conducted in developed countries with high publication rates and that promote environmental stewardship. Therefore, the study of the effects of land use on other aquatic communities is essential to understand these effects on the different biological communities that exist and, consequently, to propose public policies to mitigate these effects.

Historically, there has been an increase in land use for agricultural areas to grow different crops such as soy, rice, oranges, and sugarcane, which are essential raw materials for different products used for human food. In addition, the use of pastureland is one of the most important forms of land use worldwide, as raising livestock provides meat for the population (WRIGHT et al., 2015, LAL et al. 2021). The use of land for planting and pasture alters the characteristics of the soil and the communities associated with it (DAMIAN et al., 2021; 2023), as well as affecting nearby water bodies, since crops use different chemicals to maximize production, which are transported to aquatic environments (FREITAS et al., 2023; CORAPI et al., 2023; CABRERA et al., 2023). Thus, cropland and pasture are among the main forms of land use that cause various impacts on terrestrial and aquatic communities, leading to changes in species composition and extinction and loss of biodiversity (PEARSON et al., 2018; BRAUER; BEHEREGARAY, 2020).

Brazil suffers from intense deforestation and uncontrolled land use, with intensive agricultural practices being one of the main activities that directly and indirectly affect the biodiversity of aquatic and terrestrial environments (TILMAN, 1999; NORTON; REID; YOUNG, 2013; DUDLEY; ALEXANDER, 2017; TURCOTTE, et al., 2017). In Brazil, soybeans, corn and sugarcane predominate, the latter being one of the most important crops in the country for ethanol production (MARTINELLI et al., 2010). The increased stimulus for the production of this fuel has led to a demand for the use of arable land, as well as the conversion of pastureland into sugarcane crops (BORDONAL et al., 2018). According to recent studies, this measure prevents the deforestation of native areas of the Brazilian biome and, consequently, allows the maximization of the ecosystem services provided by these areas (ALKIMIM; SPAROVEK; CLARKE, 2015; OLIVEIRA et al., 2019). However, the effects of pasture conversion to sugarcane on aquatic communities are still unknown. It is known that sugarcane crops intensively use fertilizers, vinasse, and pesticides such as insecticides and herbicides, which contaminate aquatic environments (KHATRI; TYAGI, 2015; SCHIESARI, L.; CORRÊA, 2016) and directly affect aquatic communities. In addition, extensive pastures have been converted to intensive pastures to maximize livestock production and avoid deforestation of native areas (MACDONALD et al., 2017; OLIVEIRA et al., 2019). This has involved the application of fertilizers to the soil and/or the use of highly productive grasses as a food source for animals (DE OLIVEIRA SILVA et al., 2017; MACDONALD et al., 2017). The runoff of fertilizers and vinasse to aquatic environments in intensive pasture and sugarcane, increases the concentration of nutrients and consequently the phytoplankton community can have a greater abundance (ZHANG et al., 2024; DORY et al., 2024). On the other hand, the intensification of the pasture areas will not be sufficient to avoid the pressure on the natural areas (KREIDENWEIS et al., 2018). However, the effects of these different types of pastures on communities, especially aquatic communities, are still unknown. It is expected that in pasture and sugarcane environments, total nitrogen and total phosphorus (nutrients) will be the structuring variables because of fertilizer and vinasse application.

One of the most important existing aquatic communities that has been little studied is the zooplankton. Zooplankton organisms have a short life cycle and therefore respond rapidly to various environmental changes (MIN et al., 2021; MUÑOZ-COLMENARES; SORIA; VICENTE, 2021; RAJEWICZ et al., 2023). The zooplankton community plays a fundamental role in the functioning of the aquatic system, since it is responsible for the transfer of energy between the producing organisms and the other consuming trophic levels, acting as an essential link in trophic interactions (WETZEL, 2001). In addition, these organisms are highly sensitive to environmental changes, such as climate and landscape changes (DAS CANDEIAS et al., 2022; BOMFIM et al., 2023). Their importance can also be seen in the role of some species in controlling the phytoplankton community through herbivory, mediating the possible risks of eutrophication (AMORIM et al., 2019; BUTTS; MOODY; WILKINSON, 2022; TERESHINA et al., 2023), as well as in the consumption of organic matter through nutrient cycling in aquatic ecosystems (HE; WANG, 2020; JOHNSTON et al., 2022). However, most studies on zooplankton relate to the effects of predators and macrophytes, and how communities are affected by environmental changes caused by eutrophication and salinization (DOS SANTOS et al., 2020; IGLESIAS, 2020; CASTILHO-NOLL et al., 2023). Thus, the effects of land use on the zooplankton community are still poorly understood (BALSEIRO et al., 2023; CASTILHO-NOLL et al., 2023).

Studies on the effects of land use on zooplankton have yielded inconsistent results. Some have reported that the zooplankton community is not affected by changes in land use, but by water parameters such as pH, dissolved oxygen, temperature and nutrients (ALLEN et al., 1999; MSITELI-SHUMBA; KATIVU; HULOT, 2017; LOEWEN et al., 2019). Some studies have shown that in preserved environments, temperature and chlorophyll are structuring variables of the zooplankton community (LI et al., 2019; GOMES et al., 2020). In addition, other studies have shown that the presence of predators and macrophytes are factors that structure the zooplankton community more than land use (GELINAS; PINEL-ALLOUL, 2008; BATTES et al., 2019). On the other hand, other studies have shown that community structure is explained by the land use in which the aquatic environments are inserted (HOFFMANN; DODSON, 2005; DODSON et al., 2007; DRINAN et al., 2013; BEAVER et al., 2014). However, these studies do not specify the different land uses and how each type affects the community. Few studies differentiate between different types of land use, such as types of pastures, cropland, and urbanization, and compare the impact of different types of land use on zooplankton communities (RUMES; EGGERMONT; VERSCHUREN, 2011; GILBERT et al., 2017; Li; Li, 2023; FLORES-MENDEZ et al., 2024). The zooplankton community in aquatic environments where the environment is managed has lower taxonomic and functional diversity than communities in environments where the environment is preserved (PAQUETTE et al., 2022; WANG et al., 2023). On the other hand, the zooplankton community in lakes with an agricultural environment has a higher biomass and density than communities with a preserved environment (ZHANG et al., 2022b). Few studies have identified which water parameters structure the zooplankton community under the influence of different land uses. It is expected that chlorophyll a will be a structuring variable of the zooplankton community in preserved environments or without the application of pesticides, since chlorophyll a is a parameter to infer the quantity of phytoplankton, which is the food resource of zooplankton. Therefore, studying the effects of land use and management and identifying the structuring variables is essential to improve the understanding of these effects and, consequently, to develop more sustainable policies and actions to maintain and conserve the limnological features that provide better water quality and greater aquatic biodiversity.

Among the ecological metrics for community structure studies, the concept and classical measure of taxonomic diversity, represented by the number of species and their relative contributions, have proven ineffective in representing the structure and functioning of communities (CIANCIARUSO; SILVA; BATALHA, 2009). Recently, the functional diversity approach has been considered as a new ecological metric for these studies, but it is still little explored for the zooplankton community (BARNETT; FINALY; BEISNER, 2007; VOGT; PERES-NETO; BEISNER, 2013; BOMFIM et al., 2023). This ecological approach is more efficient in detecting community responses to environmental changes than classical diversity measures, as it results from the study of specific functional traits of each species, reflecting the functioning of the ecosystem as a whole (TILMAN et al., 1997, MOUILLOT et al., 2013). Thus, combining both metrics is a way to understand how communities respond to changes in ecosystems and to identify which metrics are most effective in predicting specific environmental impacts.

Understanding the effects of land use and management practices, such as sugarcane cropland and pastures, on aquatic communities is essential to identify their impacts and, consequently, to provide food for thought in developing more sustainable and productive forms of land use and management. More sustainable and productive agricultural practices reduce impacts on terrestrial and aquatic communities and consequently contribute to increased food security. Thus, this work contributes directly and indirectly to Goals 2 (hunger and sustainable agriculture) and 13 (action on global climate change) of the 17 Sustainable Development Goals (SDGs) of the United Nations (UN), as set out in the UN Agenda 2023 for Sustainable Development (ONU, 2015). In addition, Goals 14 (Life Below Water) and 15 (Life on Land) are also covered (ONU, 2015), as this dissertation examines the main impacts of land use on aquatic communities and how water characteristics can influence these communities. Fresh water is an essential source of life for terrestrial organisms, and its use is becoming increasingly inaccessible and expensive under deteriorating conditions.

2.2 Impact of pesticides and vinasse on communities

In order to prevent pests and correct soil characteristics, and thus maximize agricultural yields, various types of agricultural inputs such as insecticides, herbicides and fertilizers are used on croplands (PELINSON et al. 2023, SCHULZ et al. 2024). Especially in Brazil, vinasse, a liquid residue from ethanol production, is also used as a natural fertilizer (CHRISTOFOLETTI et al. 2013, DA SILVA et al., 2013). There are numerous brands and active ingredients of agricultural inputs that have different physical and chemical properties, and therefore different levels of toxicity and impact (DA SILVA PINTO et al., 2021a; COTTA et al., 2023). In addition to impacting terrestrial communities, these compounds can be transported to aquatic environments and cause various effects on aquatic communities, such as behavioral and physiological changes at the population level or a reduction in diversity and richness at the community level (DA SILVA PINTO et al., 2021b, SILVA et al., 2021). Understanding the effects caused by these agricultural inputs is essential for the conservation of aquatic biodiversity and the promotion of new bio-organic products for use in soil management and use.

Brazil is one of the main countries in the world in terms of pesticide consumption, as its economy is based on agricultural activities. In addition, in recent years, especially in the period 2016 to 2022, Brazil had a government with anti-environmental policies that allowed the regulation of more than 400 agrochemicals, some of which are banned in several European countries (ABESSA; FAMÁ; BURUAEM, 2019, CARNEIRO, 2022). During this period, the indiscriminate use and consumption of agrochemicals may have increased, as well as the rate of deforestation.

Among the many types of pesticides regulated in Brazil, the insecticide fipronil and the herbicide 2,4-D are among the most widely used in sugarcane crops (ALBUQUERQUE et al., 2016). Fipronil is an insecticide that acts as a neurotoxin, inhibiting gamma-aminobutyric acid (GABA) ionotropic neureceptors in the central nervous system, and is classified as highly toxic in Brazil (CETESB, 2018; MAPA, 2020). Some studies have shown various effects on terrestrial and aquatic non-target species. For example, a decrease in the bee population was observed in areas where fipronil was applied compared to areas where this insecticide was not applied (FARDER-GOMES et al., 2021). Behavioral and physiological changes have also been observed in insects, such as habitat exploration behavior and neuromuscular changes (ROSA et al., 2023). Various effects of this insecticide have been reported in several laboratory studies with different aquatic organisms. For example, fipronil causes changes in respiration rate and oxidative stress in amphibians, leading to high mortality (GRIPP et al., 2017; FREITAS et al., 2022). In addition, the acute and sublethal effects of fipronil have resulted in physiological and histopathological changes in freshwater mussels (ARSLAN; GÜNAL, 2023). These effects have been reported in various aquatic populations, but the effects of this insecticide on the taxonomic and functional diversity of the zooplankton community are not yet known.

2,4-D is a herbicide whose active ingredient is 2,4-dichlorophenoxyacetic acid. In high concentrations, 2,4-D acts by altering the growth and cell division of weeds, causing plant death (OGURA et al. 2022b). This herbicide is classified as extremely toxic by the National Health Surveillance Agency (MAPA, 2020). When applied to the soil, 2,4-D can alter the microbial community, increasing the diversity of rhizospheric fungi in the soil (AGUIAR et al., 2020). On the other hand, laboratory studies have shown that 2,4-D causes changes in mitochondrial function, oxidative status, and fish behavior (THIEL et al., 2020; VIANA et al., 2022). In addition, some studies have shown that this herbicide can alter the predator avoidance behavior of zooplankton and also reduce the growth rates of some cladoceran species (ANDRADE et al., 2018). However, the effects on different community metrics, such as taxonomic and functional diversity index and species richness, are still scarce.

The insecticide fipronil and the herbicide 2,4-D can be applied sequentially to sugarcane plantations. In this way, the two compounds can be carried into the aquatic environment, resulting in a more harmful synergistic effect on aquatic communities (COTTA et al., 2023; GALLO et al. 2023; GOULART et al. 2024). An experimental study has shown that when these two pesticides are applied together, they are detected by the fish species *Danio rerio*, which avoids contaminated areas in search of areas free of pesticides (MOREIRA et al., 2022). The same fish shows changes in its homeostasis when these two pesticides are applied at concentrations recommended for sugarcane crops (VIANA et al., 2022). On the other hand, the isolated effects of fipronil and 2,4-D on the biomass of the tropical amphipod *Hyalella meinerti* are more pronounced than when they are mixed (DA SILVA PINTO et al., 2021a). In addition, the vast majority of studies evaluating the effects of pesticides on zooplankton analyze the effects at the population level, examining only a few zooplankton species, mainly cladocerans (SILVA et al., 2021; GALLO et al., 2023). These studies attempt to determine the effects of the use of insecticides and herbicides that are transported to aquatic environments associated with agricultural areas (HANAZATO, 1991; RELYEA, 2005, 2009; HAYASAKA et al., 2012; GERG et al., 2013, MOREIRA et al., 2017). There are few studies evaluating the effects of pesticides on the zooplankton community as a whole and their impact on aquatic ecosystems. It is therefore important to study the effects of these compounds in isolation and in mixtures to identify potential synergies on community metrics such as diversity indices.

In addition to the effects of pesticides, there are the effects of fertilizers, which are used to enrich soils with nutrients and improve crop production (CHRISTOFOLETTI et al., 2013). However, the irrational and inappropriate use of fertilizers contributes to the increase of the primary community in aquatic environments near crops. This leads to excessive growth of algae and aquatic macrophytes (WAISER; ROBARTS, 1997; GRACE et al., 2019), which affects the limnological parameters and trophic interactions of these environments (VILLAMAGNA; MURPHY, 2010). In addition to the application of fertilizers, the use of vinasse as a natural fertilizer has recently increased in Brazil (CHRISTOFOLETTI et al., 2013, MORAES et al. 2014). Vinasse is a liquid residue from ethanol production that has chemical properties such as high concentrations of nutrients, which helps in the growth and increase of sugarcane production (CHRISTOFOLETTI ET AL., 2013; DA SILVA et al., 2013). In addition, the physiological and chemical characteristics of vinasse include high electrical conductivity and the amount of organic matter, as well as various metals (DA SILVA PINTO et al., 2021; COTTA et al., 2023; FREITAS et al. 2023). The high concentrations of nutrients can flow into aquatic ecosystems and increase their primary productivity, causing eutrophication (MARTINELLI; FILOSO, 2008). Moreover, when vinasse is discharged into aquatic environments, the concentration of dissolved oxygen in the aquatic environment can decrease significantly due to the high concentration of organic matter (CHRISTOFOLETTI et al., 2013, FREITAS et al., 2023). In this way, several negative effects can occur on aquatic communities, especially on the zooplankton community. However, the effects of vinasse on the zooplankton community are still unknown.

Fipronil, 2,4-D and vinasse are widely used in sugarcane crops in Brazil, which is the main type of land use and management for agricultural activities in the country. As such, these compounds can be found together in Brazilian water bodies and cause numerous adverse effects (COTTA et al., 2023; GIROTTO et al., 2023). Some studies have demonstrated the effects on aquatic communities, especially on macroinvertebrate communities and on populations of tadpoles and aquatic invertebrates such as *Hyalella* and *Ceriodaphnia* (DA SILVA PINTO et al., 2021a; SILVA et al. 2021; GIROTTO et al., 2023). In general, these studies have shown that fipronil and vinasse, alone or in mixtures, are more harmful than 2,4-D, causing rapid mortality and consequently reducing species diversity and abundance (MOREIRA et al. 2020, COTTA et al. 2023). However, the effects of these compounds, alone or in mixtures, on the zooplankton community, especially on taxonomic and functional diversity indices, are still unknown.

Therefore, the analysis of the effects of land use and the application of insecticides, herbicides and vinasse, alone or in mixtures, on the zooplankton community, using taxonomic and functional diversity metrics, is essential for progress in the assessment of the ecological risk generated by these effects on the functioning of ecosystems. This thesis has made it possible to advance studies on the functional and taxonomic diversity of zooplankton associated with the effects of changes in the agro-ecosystem, such as land use with different intensities of pasture and sugarcane and the use of pesticides and vinasse. The results of this thesis will provide a more detailed understanding of the effects of these practices on aquatic ecosystems, which will contribute to the development of new sustainable practices and environmental protection laws.

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11 GENERAL CONCLUSION AND THE NEXT STEPS

General conclusion and the next steps

In this thesis, the main information on studies on the effects of land use on the zooplankton community around the world was highlighted (article 1), as well as the main water variables that structure the zooplankton community under the influence of agropastoral landscapes, pesticides and vinasse (articles 2, 3 and 4). In addition, we found that land use and management for intensive pasture and sugarcane crops, uses of extreme importance for the Brazilian economy, affect the taxonomic and functional diversity of zooplankton (article 3). We also analyzed how the application of fipronil, 2,4-D and vinasse, alone or in mixtures, affects the zooplankton community and water parameters (Article 4). This work has therefore contributed to a better understanding of the effects of land use and the application of agrochemicals and vinasse on zooplankton.

Chapter 1 provided an overview of studies on the effects of land use on freshwater zooplankton communities and a synthesis of the main results presented in articles on this topic. A total of 80 articles were identified. The number of studies has increased over time, particularly in the USA, Poland, Canada, China, Argentina and Brazil. Land use for cropland, pasture and urbanization has been shown to have a negative impact on zooplankton communities. The presence of surrounding natural vegetation in aquatic environments has been shown to contribute to greater taxonomic and functional diversity. The least studied are the effects of land use for mining and the groups of testaceous amoebae, ciliates and flagellates. Different landscape metrics have been used, which may be related to different environmental policies in different countries. We highlight the main gaps so that studies on the effects of land use can advance and contribute to environmental policies for the maintenance and conservation of aquatic biodiversity. Thus, the results of Chapter 1 confirm our hypotheses 1 and 2.

In Chapter 2, the effect of water parameters of eight reservoirs in an agropastoral landscape on the functional structure of zooplankton was analyzed. The results showed that there was a greater functional richness and diversity of traits in alkaline environments with lower nitrogen concentrations. The study indicates that pH and TN are key variables for the functional structure of zooplankton communities in an agropastoral landscape. Thus, this chapter has partially confirmed our hypothesis 3 by demonstrating that pH, in addition to total nitrogen, is a key variable in structuring the zooplankton community of reservoirs under agropastural.

In Chapter 3, the effects of land use were studied through a realistic simulation of land use: extensive pasture, intensive pasture and sugarcane crops. In the mesocosms inserted in the intensive pasture and sugarcane landscapes, there was a change in the functional and taxonomic composition of zooplankton, resulting in more tolerant species and a significant reduction in the taxonomic and functional richness of zooplankton. In addition, temperature, pH and dissolved oxygen were the structuring variables in aquatic environments in extensive pastures, while pH and electrical conductivity were the structuring variables in intensive pastures. In aquatic environments in sugarcane crops, temperature and pH were the main water variables. pH was the most important parameter in structuring zooplankton communities, being the only structuring variable present in all treatments. Therefore, this chapter confirmed our hypothesis 4 by showing that the zooplankton of aquatic environments in intensive pastures and sugarcane fields have lower taxonomic and functional diversity than those in extensive pastures. However, this chapter did not confirm our hypothesis 5 because the structuring variables were not phosphorus and total nitrogen for the intensive pasture and sugarcane environments and chlorophyll in the extensive pasture environments.

Chapter 4 evaluated the effects of fipronil, 2,4-D and vinasse, alone or in mixtures, on zooplankton diversity and identified the water parameters that structure the community. A mesocosm experiment was conducted with six treatments: control (C), 2,4-D (D), fipronil (F), fipronil and 2,4-D (M), vinasse (V), and all compounds (MV). After application of pesticides and vinasse, species richness, Shannon-Wiener diversity, richness and functional dispersion were significantly lower in treatments F, M, MV and V than in treatments C and D throughout the experiment. In treatment C, temperature and chlorophyll were the structuring parameters, while in treatment D, turbidity and pH were the main variables. Chlorophyll a was the main structuring variable in treatment F, while pH and electrical conductivity were the structuring variables in treatments MV and V, respectively. These results showed that the application of fipronil and vinasse, alone or in a mixture, had detrimental effects on the zooplankton community. Therefore, the results of this chapter confirmed hypothesis 6. However, hypothesis 7 was partially confirmed. As each application had a set of structuring variables, as for the control treatment we had temperature and chlorophyll (according to our hypothesis), but for the 2,4-D application turbidity and pH were the structuring variables. For the fipronil and vinasse treatments, total phosphorus and nitrogen were not the key variables as hypothesized, but rather conductivity and pH.

Chapter 5 presents the entire academic career, including the publication of scientific articles and the teaching of three undergraduate courses, one of which was written by the student.

In this way, this thesis highlights the extreme need for new legislative measures to allow the use of pesticides and vinasse. Furthermore, in order to mitigate the effects of land use, it is necessary to understand the best size of riparian buffer around aquatic environments, especially lentic ones, in order to protect aquatic communities, especially zooplankton. In this way, new legislative measures can be developed to protect, conserve and maintain the functioning of aquatic environments and aquatic biodiversity in Brazil. For example, the use of land for intensive pasture and sugarcane crops, and the application of vinasse and fipronil have harmful effects on the zooplankton community, and new sustainable forms of management and use should be studied to reduce the impact on aquatic communities.

Zooplankton is a community that stands out in the interconnections of aquatic food webs. A wide variety of zooplankton species are able to exploit available resources by performing specific functions in trophic chains and in the ecosystem as a whole. These functions include their ability to filter phytoplankton and to feed on organic matter, thus performing the important function of recycling organic matter and making nutrients available to the higher links in the food chains of aquatic ecosystems.

In this sense, our studies highlight the importance of considering this microscopic community and the detrimental effects of conventional land use practices on them, as they represent a group of organisms of great importance for maintaining ecosystem functions and the overall balance of aquatic environments.

Finally, in order to publicize the results of this thesis, manuscripts will be submitted for publication. Subsequently, scientific dissemination will be necessary to make the knowledge generated by this thesis available to the general public. To this end, once the data have been published in scientific journals, we intend to create posts and videos for dissemination in social media, such as the Unesp/Ibilce PPG Biodiversity Instagram, and try to publish the main results in local newspapers.