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PROTOZOOPLANKTON DYNAMICS, URBANIZATION, AND AGRICULTURE: A GEOENVIRONMENTAL APPROACH TO WATER QUALITY IN AQUACULTURE PONDS

DINÂMICA DO PROTOZOOPLÂNCTON, URBANIZAÇÃO E AGRICULTURA: UMA ABORDAGEM GEOAMBIENTAL DA QUALIDADE DA ÁGUA EM PISCICULTURAS

DINÁMICA DEL PROTOZOOPLANCTON, URBANIZACIÓN Y AGRICULTURA: UN ENFOQUE GEOAMBIENTAL DE LA CALIDAD DEL AGUA EN ESTANQUES DE ACUICULTURA

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ABSTRACT

The aim of the study was to document the composition, abundance, and density of free-living protozoa in aquaculture pond water, the impact of urbanization and agriculture, as well as to assess the potential use of these organisms as bioindicators of water quality. The study was conducted in 20 aquaculture farms, with sample collections taken during both hydrological seasons: the rainy season (from September 2021 to April 2022) and the dry season (from May to August 2022). For qualitative sample composition, horizontal and vertical drags were performed at the water surface of the ponds, while each quantitative sample was obtained using a plankton net (50 µm mesh). The abundance and density of protozoa were presented as (Ind mL^-1). Free-living protozoa were found in the water of 55% (11/20) of the aquaculture farms. Eight species of protozoa, all from the phylum Ciliophora, were identified: Paraenchelys terricola, Apospathidium terricola, Spirostomum teres, Linostomella vorticella, Halteria grandinella, Sphaerophrya magna, Paramecium bursaria, and P. caudata. The most abundant species were P. terricola (106.26 Ind mL^-1 in the rainy season and 105.09 Ind mL^-1 in the dry season) and A. terricola (82.15 Ind mL^-1 in the rainy season and 85.63 Ind mL^-1 in the dry season). This study represents a preliminary survey of protozooplankton in aquaculture ponds, contributing significantly to the understanding of the diversity and distribution of free-living protozoa. Although the results

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did not show substantial differences in abundance among sampling points, free-living protozoa are valuable tools for analyzing environments impacted by varying degrees of pollution. Further studies on the dynamics and distribution of aquatic microorganism communities will continue to expand knowledge of the taxonomic diversity and ecology of these species, particularly in impacted environments.

Keywords: Aquatic Microbiology. Biomonitoring. Protozooplankton. Water Pollution.

RESUMO

O objetivo do estudo foi documentar a composição, a abundância e a densidade de protozoários de vida livre na água de viveiros de piscicultura, o impacto da urbanização e da Agricultura, além de verificar a possibilidade de utilização desses organismos como bioindicadores da qualidade da água. O estudo foi desenvolvido em 20 pisciculturas e foram realizadas coletas amostrais nas duas estações hidrológicas, chuvosa (meses de setembro/2021 a abril/2022) e seca (meses de maio a agosto/2022). Para a composição das amostras qualitativas, foram realizados arrastos horizontais e verticais na superfície da água dos viveiros, enquanto cada amostra quantitativa foi obtida em rede de plâncton (malha de 50 µm). A abundância e a densidade dos protozoários foram apresentadas em (Ind mL-1). Foram encontrados protozoários de vida livre na água de 55% (11/20) das pisciculturas. Foram identificadas 8 espécies de protozoários todas do filo Ciliophora, sendo elas: Paraenchelys terrícola, Apospathidium terrícola, Spirostomum teres, Linostomella vorticella, Halteria grandinella, Sphaerophrya magna, Paramecium bursaria e P. caudata. As espécies com maior abundância foram P. terricola (106,26 Ind mL-1 na estação chuvosa e 105,09 Ind mL-1 na estação seca) e A. terrícola (82,15 Ind mL-1 na estação chuvosa e 85,63 Ind mL-1 na estação seca). Este estudo representa um levantamento preliminar do protozooplâncton em viveiros de piscicultura, sendo uma importante contribuição para o conhecimento da diversidade e distribuição de protozoários de vida livre. Embora os resultados obtidos não mostrem grande diferenciação de abundância entre os pontos amostrais, os protozoários de vida livre representam importantes ferramentas para análise de ambientes afetados por diferentes graus de poluição. No entanto, haverá continuidade nos estudos sobre a dinâmica e distribuição das comunidades de microrganismos aquáticos para ampliar o conhecimento da diversidade taxonômica e ecologia das espécies, principalmente em ambientes impactados.

Palavras-chave: Biomonitoramento. Microbiologia Aquática. Poluição Aquática. Protozooplâncton.

RESUMEN

El objetivo del estudio fue documentar la composición, la abundancia y la densidad de protozoos de vida libre en el agua de estanques de acuicultura, así como el impacto de la urbanización y de la agricultura, además de evaluar la posibilidad de utilizar estos organismos como bioindicadores de la calidad del agua. El estudio se desarrolló en 20 pisciculturas y se realizaron colectas de muestras durante las dos estaciones hidrológicas: lluviosa (septiembre/2021 a abril/2022) y seca (mayo a agosto/2022). Para la composición de las muestras cualitativas, se realizaron arrastres horizontales y verticales en la superficie del agua de los estanques, mientras que cada muestra cuantitativa se obtuvo mediante una red de plancton (malla de 50 µm). La abundancia y densidad de los protozoos se presentaron en Ind mL^{-1}.Se encontraron protozoos de vida libre en el agua del 55% (11/20) de las pisciculturas. Se identificaron 8 especies de protozoos, todas del filo Ciliophora, siendo ellas: Paraenchelys terrícola, Apospathidium terrícola, Spirostomum teres, Linostomella vorticella, Halteria grandinella, Sphaerophrya magna, Paramecium bursaria y P. caudata. Las especies con mayor abundancia fueron P. terrícola (106,26 Ind mL^{-1} en la estación lluviosa y 105,09 Ind mL^{-1} en la estación seca) y A. terrícola (82,15 Ind mL^{-1} en la estación lluviosa y 85,63 Ind mL^{-1} en la estación seca).Este estudio representa un levantamiento preliminar del protozooplancton en estanques de acuicultura, siendo una importante contribución al conocimiento de la diversidad y distribución de los protozoos de vida libre. Aunque los resultados obtenidos no muestran una gran diferenciación de abundancia entre los puntos muestreados, los protozoos de vida libre representan herramientas importantes para el análisis de ambientes afectados por distintos grados de contaminación. Sin embargo, se continuará con los estudios sobre la dinámica y distribución de las comunidades de microorganismos acuáticos para ampliar el conocimiento de la diversidad taxonómica y la ecología de las especies, especialmente en ambientes impactados.

Palabras clave: Biomonitorización. Microbiología Acuática. Contaminación Del Agua. Protozoplancton.

1 INTRODUCTION

Fish farming is an important branch of aquaculture, which in turn is one of the fastest-growing segments of animal production in Brazil; since the 2000s, it has shown an average annual production growth of approximately 4.9% (Mattos et al., 2021). In 2021, the country reached a fish production volume of 841 thousand tons (Peixe BR, 2022). Brazil has stood out globally in the production of food derived from aquaculture, largely due to its water availability, favorable climate, and the natural occurrence of aquatic species that meet both zootechnical and market interests (Valenti et al., 2021). The state of Rondônia is the largest producer of native fish in Brazil, accounting for a total of 57.2 thousand tons of fish produced in 2022 (Peixe BR, 2023).

The state of Rondônia produced more than 90 thousand tons of fish in 2022; however, mainly due to market saturation and sanitary issues, there have been successive reductions in production volume. Given this market scenario, water quality is a constant concern in fish farming, since poor water quality can lead to reduced productive performance and fish mortality (Sant’Anna et al., 2012). In this context, fish farming ponds function as artificial aquatic ecosystems, where abiotic and biotic conditions can be partially manipulated (Dantas Filho et al., 2023).

The pollution of surface waters is a global environmental problem. Among impacted aquatic environments, such as rivers, streams, and lakes, fish farming ponds can also be highlighted. These represent artificial environments with high concentrations of organic matter. In addition, these cultivation systems receive significant volumes of effluents from industrial, agricultural, and domestic sources (Dantas Filho et al., 2023). Most aquatic organisms can respond directly to changes in the physical, chemical, and biological profile of water (Zhong et al., 2017). Among aquatic organisms used as bioindicators, free-living ciliated protozoa have gained prominence in recent decades due to their sensitivity to environmental changes (Nunes et al., 2015). These organisms are part of the protozooplankton in fish farming ponds.

Protozoa are unicellular, eukaryotic organisms, which may be heterotrophic or mixotrophic (Grott et al., 2022). They have short life cycles and are sensitive to environmental changes (Xu et al., 2015; Sikder & Xu, 2020). In addition, they are key components of microbial communities and play an essential role in food web functioning, serving as prey for higher trophic levels (Xu et al., 2014; Sikder et al., 2019). Free-living protozoa are abundant in a wide variety of aquatic and soil environments, occurring either as free-swimming forms or associated with biotic and abiotic surfaces, and they have a high capacity to adapt to different environmental conditions. According to Echavez and Leal

(2021), some protozoan species can rapidly respond to physicochemical changes in water by forming resistant cysts, which act as mechanisms of protection and dispersal. This behavior likely explains their wide geographic distribution, making them suitable for use in biomonitoring aquatic ecosystems (Shi et al., 2012; Cabral et al., 2017).

The diversity of protozoa is still underestimated in artificial aquatic ecosystems. However, in recent years, advances in morphological and molecular analyses, along with changes in the proposed phylogenetic systematics of eukaryotes, have contributed to a better understanding of this group (Grot et al., 2016). In the classification proposed by Katz and Grant (2014), protozoa are included in the groups Amoebozoa, Excavata, and SAR (Stramenopiles, Alveolata, and Rhizaria). Ciliates (Alveolata: Ciliophora) comprise a diversity of approximately 8,000 described species. However, it is estimated that about 89% of the existing diversity has not yet been identified (Grott et al., 2022), highlighting the importance of further studies, especially considering the ecological relevance of these organisms in aquatic ecosystems. Moreover, due to their global distribution, high abundance, rapid growth rates, short generation times, ease of collection, and the fact that they are separated from their environment only by a cell membrane, protozoa have been widely used to assess environmental quality and the ecological status of water bodies (Liu et al., 2014).

Few studies have been conducted on protozooplankton in fish farming ponds in Brazil. However, it is already known that an increase in protozoan populations in aquaculture waters is an indicator of contact with urban sewage and surface runoff of wastewater from agricultural areas (Debastiani et al., 2016). To date, these invertebrates have been quantified only for environmental biomonitoring purposes; that is, there is no specific legislation regulating protozooplankton levels in water for fishing and aquaculture activities. In the state of Rondônia, no survey of protozoan fauna in fish farming waters has yet been conducted.

Given the importance of this context, the aim of this study was to document the composition, abundance, and density of free-living protozoa in fish farming ponds, assess the impacts of urbanization and agriculture, and evaluate the potential use of these organisms as bioindicators of water quality.

2 MATERIAL AND METHODS

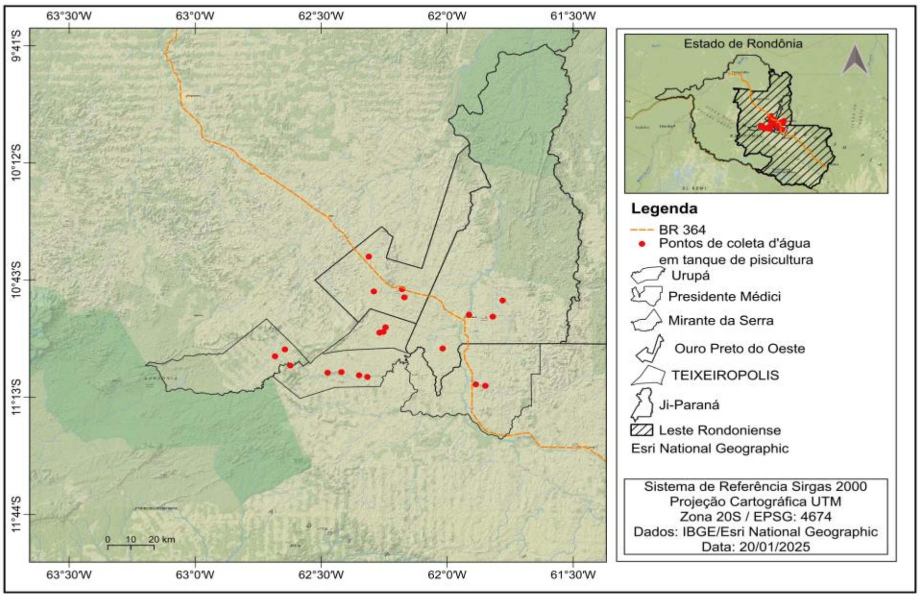
2.1 STUDY AREA AND CLIMATOLOGICAL CONDITIONS

The study was conducted in 20 fish farms located in the municipalities of Ji-Paraná, Ouro Preto do Oeste, Presidente Médici, Urupá, Teixeirópolis, and Mirante da Serra, in the state of Rondônia, Brazil (Figure 1). Sampling collections were carried out during the two Amazonian hydrological seasons: the rainy season (September 2021 to April 2022) and the dry season (May to August 2022).

On average, the fish farms visited adopted a semi-intensive production system (up to 0.6 kg/m²/year with one annual production cycle). These farms covered up to 3 hectares of water surface area, distributed among semi-excavated ponds with individual areas not exceeding 0.5 hectares and an average depth of 1.60 m. These ponds were used for the grow-out phase of tambaqui (Colossoma macropomum). It is worth noting that the fish farms in this region had their ponds constructed in riverbeds, that is, in the lowest parts of the terrain.

Figure 1

Location of fish farms in the municipalities of the State of Rondônia, Brazil



Source: prepared by the authors.

2.2 QUALITATIVE AND QUANTITATIVE ANALYSES OF CILIATED PROTOZOA IN FISH FARM WATER

The study was conducted using a completely randomized design in a 20 × 3 × 3 scheme (20 fish farms, 3 ponds, and 3 replicates per pond). The 20 fish farms selected for data collection were defined based on the availability of the Technical Assistance and Rural Extension Agency (EMATER) of the state of Rondônia, and all farms were commercially active.

Three water samples were collected from three different sampling points. These sampling points included the supply channel and the water column within the ponds. For this study, the approach suggested by Costa et al. (2016) was adopted, in which ponds are interconnected: the supply reservoir provides water to the first pond, and subsequently, water from one pond supplies the next. Thus, the water in the last pond has passed through all preceding ponds.

For qualitative sample composition, horizontal and vertical hauls were performed at the surface of the pond water. Quantitative samples were collected using a plankton net (50 µm mesh) with the aid of a graduated bucket. This study focused on free-living protozoa; samples were immediately stored in polyethylene terephthalate bottles, kept at 7 °C in cooler boxes, and transported to the laboratory.

It is important to note that protozoa were observed without the use of fixatives to avoid alterations in their morphological characteristics. Analyses were carried out using a trinocular stereoscopic microscope (Sigma, USA) at 10× magnification, equipped with a digital camera. The images obtained were captured using a professional camera (Canon Rebel T8i EF-S 18–55 mm lens). To support interpretation, photomicrographs were analyzed using the Olympus Stream image analysis software.

For protozoan analysis, aliquot replicates of 200 mL of water were immediately corrected with 30% alcohol. Samples were concentrated by sedimentation, and the supernatant was discarded. Protozoa were counted and identified in triplicate using 1 mL Neubauer chambers under microscopic observation. Identification was based on Patterson (1996), the “Protist Information Server,” and “Microscope,” along with specialized literature on the morphology of the identified families. The following references were used: Bick (1972), Corliss (1979), Lee et al. (1985), Dragesco and Dragesco-Kernéis (1986), Foissner et al. (1991, 1992, 1994, 1995, 1999), Krainer (1991), Foissner and Berger (1996), and Bagatini et al. (2013).

Protozoan abundance was presented at the taxonomic levels of species, family, order, class, and phylum, while density was expressed as individuals per mL (Ind mL^{-1}) across the different seasons (rainy and dry).

2.3 STATISTICAL ANALYSES

To determine protozoan variation, counts were performed twice during different seasons (rainy and dry), and identifications were carried out by the same individual observer. Abundance was expressed as mean counts for each site, with the standard deviation calculated between the two counts. The means of the different hydrological seasons were compared using Student’s t-test at a 5% significance level.

Following these analyses, Pearson correlation coefficients were calculated to assess the relationship between protozoan population variations and the proximity of fish farming ponds to urbanized areas and agricultural lands (such as soybean, corn, and coffee crops). The information regarding proximity to urban areas was obtained by measuring distances using ArcGIS 2021 Q4 Release software. All statistical analyses were performed using RStudio (Development Core Team), version 3.5.3.

3 RESULTS AND DISCUSSION

In this study, among the twenty fish farming units monitored in the Central-Eastern Mesoregion of Rondônia, eleven (55%) showed the occurrence of free-living ciliated protozoa belonging to the phylum Ciliophora. A total of eight species, seven families, six orders, and five classes were identified, revealing a significant taxonomic diversity within a geographic area characterized by mixed land use, anthropogenic pressures, and seasonal hydrological dynamics. According to the studies by Antipa (1977), the distribution pattern of these species reinforces the idea that fish farming ponds, although anthropogenic environments, can represent ecologically complex and heterogeneous systems.

This pattern suggests that local factors play a more significant role in determining the distribution of zooplankton species than simple geographic variation. For example, the study by Jeelani et al. (2018) in the Kashmir Valley, India, observed higher densities in summer than in winter, a period associated with greater organic matter availability. Thus, these organisms are more influenced by local environmental characteristics, such as habitat type, presence of aquatic vegetation, depth, water temperature, and nutrient availability, than by their position along a longitudinal gradient.

Ciliated protists are used as bioindicators and represent a promising and ecologically relevant approach for monitoring water quality, especially in contexts where traditional analyses do not fully capture environmental impacts. The high sensitivity of these organisms to physicochemical variations and their rapid reproductive response allow for a more dynamic and integrated assessment of the cumulative effects of aquatic pollution, whether in eutrophic environments, such as streams impacted by organic pollution, or in oligotrophic systems, where the presence of certain genera may indicate environmental stress (Américo-Pinheiro, Torres, Ferreira, 2017).

The occurrence of such biological diversity may reflect the occupation of multiple ecological niches within fish farming ponds, influenced by local physicochemical variables and other factors that converge to create favorable conditions for the establishment of ciliated microorganisms.

Table 1 presents a diversity of species distributed across different taxonomic orders, highlighting the ecological richness of these water bodies. The composition of the ciliate community reflects the direct influence of local environmental conditions, often modulated by factors such as land use and occupation, proximity to agricultural and urban areas, and the climatic seasonality typical of the region. The spatial distribution of these organisms, particularly species such as Paramecium bursaria, Spirostomum teres, and Halteria grandinella, is associated with environments exhibiting different levels of anthropogenic disturbance, acting as bioindicators of water quality at a microterritorial scale (Dias et al., 2020; Mostafa et al., 2023).

The occurrence of this taxonomic richness in aquaculture production systems can be interpreted as a reflection of a complex interaction between nature and society, in which factors such as water management, type of feed applied, production intensity, and proximity to agricultural areas directly influence biotic composition. In this context, ciliates become key organisms for microecological landscape research, as their presence and abundance reflect the pressures and conditions imposed on aquatic ecosystems (Américo-Pinheiro; Torres; Ferreira, 2017).

The identified species, Paraenchelys terricola, Apospathidium terricola, Spirostomum teres, Linostomella vorticella, Halteria grandinella, Sphaerophrya magna, Paramecium bursaria, and Paramecium caudatum (Table 1), represent different trophic strata that compose the aquatic landscape.

Table 1

Free-living ciliated protozoa identified in fish farming ponds in Rondônia, Brazil

Phylum	Class	Order	Family	Species
Ciliophora	Gymnostomatea	Pseudoholophryida	Pseudoholophryidae	Paraenchelys terricola (Foissner, 1984)
Ciliophora	Gymnostomatea	Spathidiida	Spathidiidae	Apospathidium terricola (Foissner, Agatha & Berger, 2002)
Ciliophora	Heterotrichea	Heterotrichida	Spirostomidae	Spirostomum teres (Claparède & Lachmann, 1859)
Ciliophora	Heterotrichea	Heterotrichida	Condylostomatidae	Linostomella vorticella Aescht, 1999 (Ehrenberg, 1833)
Ciliophora	Oligotrichea	Halteriida	Halteriidae	Halteria grandinella (Müller, 1773) Dujardin, 1841

Ciliophora	Kinetofragminophora	Suctorida	Podophryidae	Sphaerophrya magna Claparède & Lachmann, 1860 (Maupas, 1881)
Ciliophora	Ciliatela	Peniculida	Parameciidae	Paramecium bursaria (Ehrenberg, 1831) Focke, 1836
Ciliophora	Ciliatela	Peniculida	Parameciidae	Paramecium caudatum (Ehrenberg, 1833)

Source: prepared by the authors.

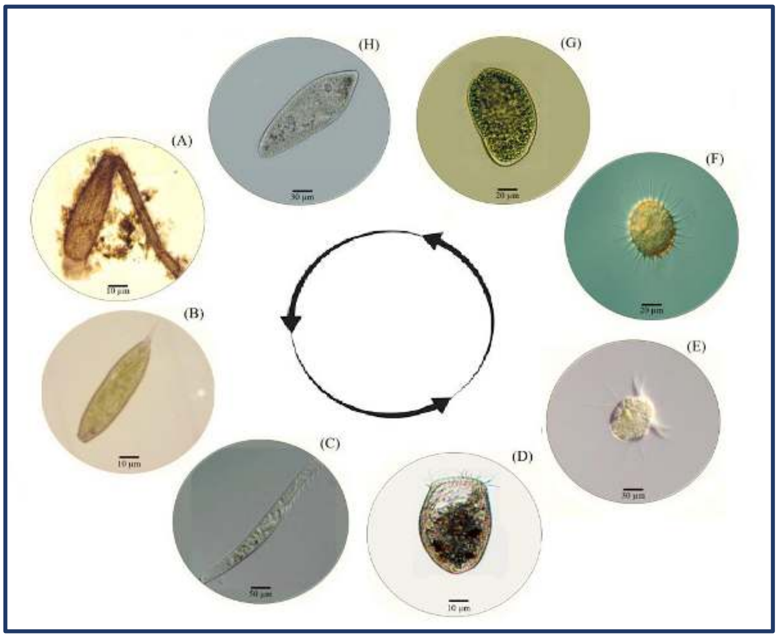
The ecological adaptability of protozoa is reflected in their presence even under conditions of variation in pH, dissolved oxygen, temperature, and nutrient concentration, reinforcing the importance of interdisciplinary approaches that integrate ecological data and geographic studies. Vďačný and Tirjaková (2007) reported, for the first time, the occurrence of Paraenchelys terricola in the Holarctic biogeographic region, in Slovakia. This finding demonstrates the wide geographic distribution of this species under climatic conditions very different from those of the Amazon, supporting the idea that local conditions are complex and important factors influencing the presence or absence of microorganisms.

The elongated and cylindrical shape of Spirostomum teres (Figure 02-C) and the bristles of Halteria grandinella (Figure 02-E) play a crucial role in adaptation to the aquatic environment. These morphological differences reflect adaptations that allow the occupation of distinct ecological niches and the coexistence of species within the same environment. Species such as Paraenchelys terricola (Figure 02-A) and Apospathidium terricola (Figure 02-B), belonging to the class Gymnostomatea, possess specific characteristics that enable them to thrive in environments with variations in water quality, acting as bioindicators. Spirostomum teres (Figure 02-C), often associated with good water quality conditions, also serves as an indicator, while Paramecium, according to Américo-Pinheiro, Torres, and Ferreira (2017), can also be considered a bioindicator, even in environments with higher concentrations of organic matter.

The identified species were Paraenchelys terricola, Apospathidium terricola, Spirostomum teres, Linostomella vorticella, Halteria grandinella, Sphaerophrya magna, Paramecium bursaria, and Paramecium caudatum (Figure 02).

Figure 2

Photomicrographs of free-living ciliated protozoan species



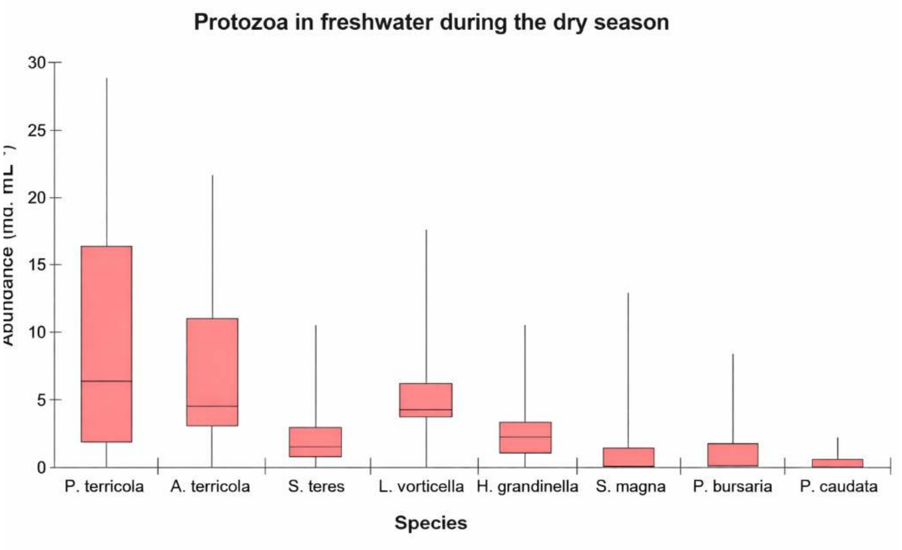
Note: Paraenchelys terricola (A), Apospathidium terricola (B), Spirostomum teres (C), Linostomella vorticella (D), Halteria grandinella (E), Sphaerophrya magna (F), Paramecium bursaria (G), and Paramecium caudatum (H).
Source: prepared by the authors.

Regarding protozoan abundance during the dry season (Figure 03), the species P. terricola showed the highest abundance, while P. caudatum exhibited the lowest abundance in this season. Specifically, P. terricola recorded 106.26 individuals per mL^{-1}, followed by A. terricola with 82.15 individuals per mL^{-1}. During the dry period, P. terricola reached an abundance of 115.59 individuals per mL^{-1}, with A. terricola ranking second at 85.63 individuals per mL^{-1}. Both species, P. terricola, and A. terricola, stood out across the two analyzed seasons, with higher abundance during the dry period. In contrast, P. caudatum showed the lowest abundance in both seasons.

Aquatic protozoa play an essential role in food webs by preying on bacteria while also serving as a link between bacteria and secondary producers. In addition, these organisms can consume algae, cyanobacteria, and other protozoa, contributing to the dynamics of organic matter. Their sensitivity to environmental changes, combined with ease of collection, makes them effective indicators for environmental monitoring, including wastewater. They have also been investigated for biological control, particularly in relation to algal and cyanobacterial blooms (Regali-Seleghin; Godinho; Matsumura-Tundisi, 2010).

Figure 3

Abundance (Ind. mL-1) and species variation of free-living ciliated protozoa identified during the dry season in fish farming ponds in Rondônia, Brazil



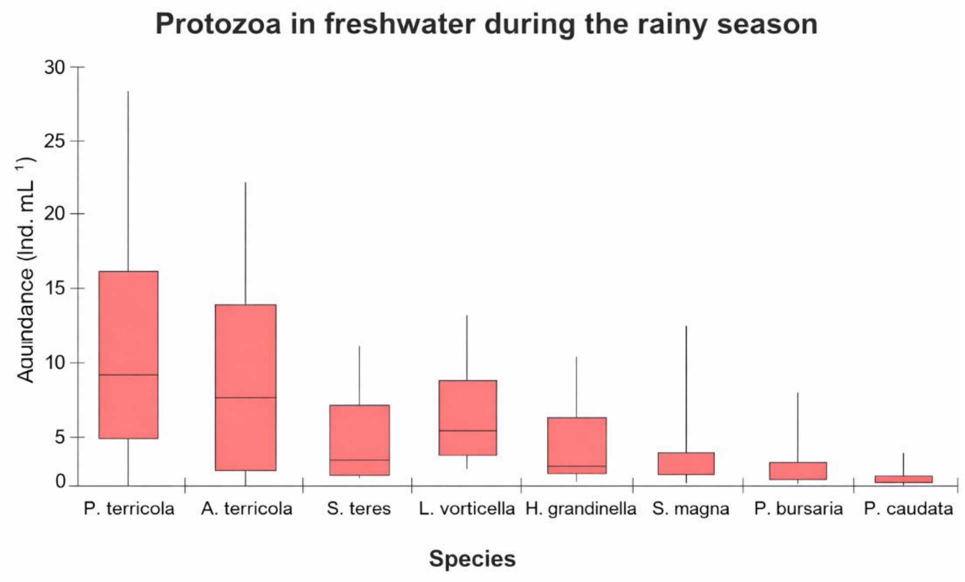
Source: prepared by the authors.

Given the functions performed by protozoa in the aquatic environment, it is possible that species with lower abundance in fish farming ponds are more sensitive to elevated levels of organic matter. These levels may be influenced by natural variables, such as water temperature, as well as by anthropogenic factors, such as the feed used in fish production.

Figure 04 shows the seasonal abundance of protozoa during the rainy period. A higher density of organisms can be observed during the dry season, likely resulting from lower water flow and, consequently, higher concentrations of dissolved nutrients in the ponds. This pattern is consistent with observations made by Sharma et al. (2025) and Iskaros et al. (2025) in large river basins such as the Nile, where hydrological seasonality directly influences nutrient availability and the growth of planktonic communities. In the Amazonian context, where rainfall defines productive and ecological cycles, variation in the composition of ciliate communities reflects flood and drought regimes and their implications for water quality.

Figure 4

Abundance (Ind. mL ⁻¹) and species variation of free-living ciliated protozoa identified during the rainy season in fish farming ponds, Rondônia, Brazil

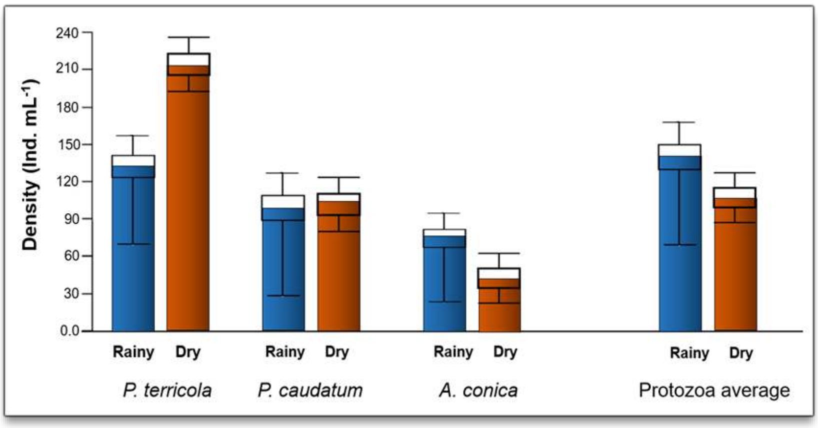


Source: prepared by the authors.

Regarding seasonal variation, the dry and rainy seasons are illustrated in Figure 05, which presents the density (Ind. mL⁻¹) of free-living ciliated protozoa identified in the water of fish farming ponds in inland Rondônia, Brazil.

Figure 5

Density (Ind. mL ⁻¹) of free-living ciliated protozoa identified in fish farming ponds, Rondônia, Brazil



Note: If different letters (a, b) are present between hydrological seasons, they indicate statistically significant differences according to Student’s t-test ($p < 0.05$).
Source: prepared by the authors.

The density of the most abundant species was observed for P. terricola, and A. terricola. For P. terricola, density in the dry season (115.90 Ind. mL⁻¹) was significantly higher than in the rainy season (106.26 Ind. mL^{-1}) ($p < 0.05$), indicating a relevant seasonal variation for this species. In contrast, for A. terricola, there was no statistically significant difference between seasons (82.15 and 85.63 Ind. mL^{-1}, respectively), suggesting that its density is less influenced by seasonal changes.

As pointed out by El-Tohamy et al. (2024), the presence of sensitive species such as Spirostomum teres is associated with ecological integrity, whereas more tolerant species, such as Sphaerophrya magna, are indicators of environments impacted by organic matter and nutrients derived from fertilizers or urban effluents.

These results suggest that although some species may vary with seasonality, the overall ciliate protozoan community does not exhibit significant fluctuations between seasons, which may indicate resilience of the ciliate protozoan community to seasonal environmental changes in these ponds (Buosi et al., 2014).

The dispersion analysis (Figure 06) shows that protozoan populations tend to maintain, on average, about 50% of their density during the dry season compared to the rainy period, indicating a marked reduction in the abundance of these organisms during drought conditions. This pattern suggests a sensitive response of protozoa to reduced water availability, likely associated with decreased habitat complexity and lower availability of trophic resources.

In Figure 06, there is a clear replacement of more sensitive species by more tolerant forms. This pattern was validated by Dias et al. (2020), who demonstrated the value of the saprobic index in detecting qualitative changes in ciliate communities as a function of organic load. The predominance of bacterivorous and omnivorous species in impacted environments, such as Linostomella vorticella, supports the hypothesis that land-use changes directly influence the functional composition of the community.

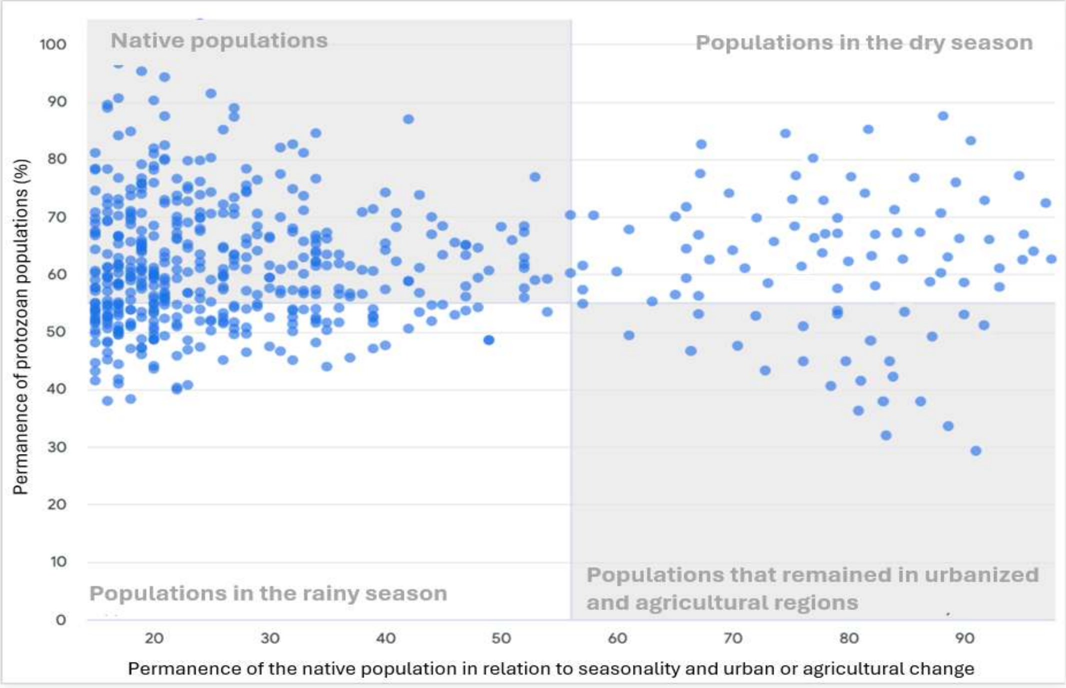
Additionally, it is observed that, on average, only about 20% of protozoan populations are maintained in environments subjected to urbanization or agricultural use when compared to preserved areas. This finding indicates a significant ecological impact resulting from habitat conversion, suggesting that the ecological integrity of protozoan communities is substantially compromised by anthropogenic land-use changes.

Despite this general trend, the relative positions of populations in the dispersion plots show significant temporal variations, particularly under drought conditions and in anthropized areas. Such fluctuations may indicate ecological replacement processes, in which sensitive species are progressively replaced by taxa more tolerant to new environmental conditions (Luiz; Pinto; Scheffer, 2012; Lobato Júnior; Araújo, 2015).

This dynamic can be interpreted as a reflection of specific ecological adaptations or as a response to environmental pressures arising from physical and chemical stressors. Protozoa with greater ecological resilience tend to establish and dominate less favorable environments, whereas more ecologically demanding species may experience population decline or local extinction (Araújo; Costa, 2007). This highlights the importance of protozoa as sensitive bioindicators of environmental degradation, especially in aquatic systems subjected to intense anthropogenic pressures.

Figure 6

Dispersion analysis of the abundance of native populations from preserved areas compared to the persistence of populations in urbanized or agricultural areas



Source: Prepared by the authors.

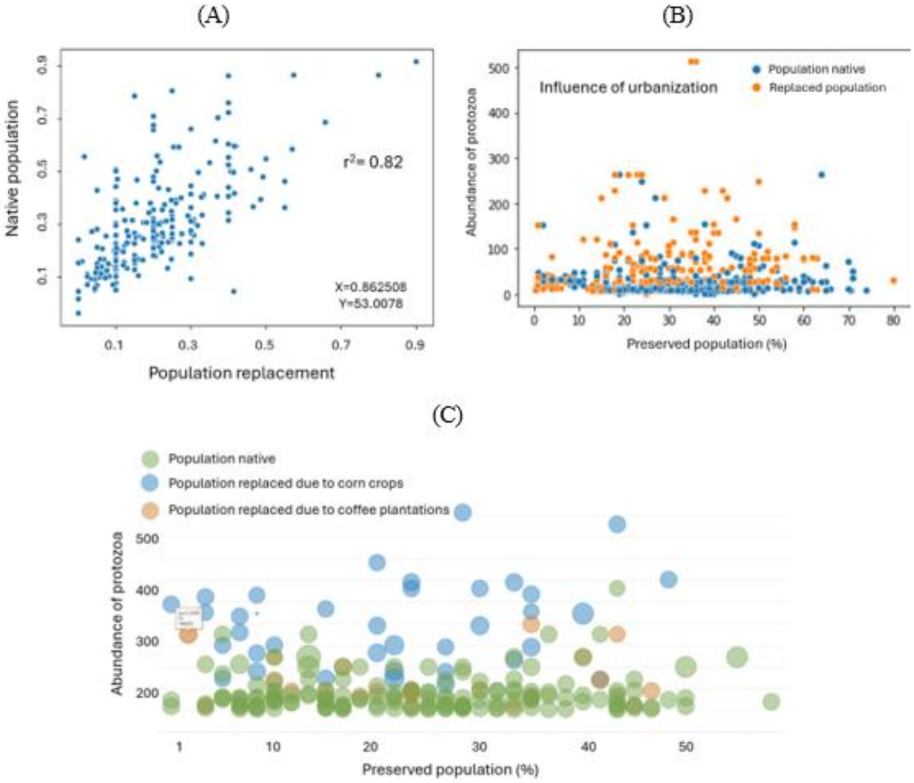
The integrated analysis (Figure 07) presents the dynamics of ciliate protozoan populations in environments subjected to anthropogenic pressure, especially from urbanization and agricultural activities. The three graphs shown in this figure provide important insights into species replacement and changes in the structure of microbial communities in response to land use and land cover changes.

In Figure 07A, a statistically significant correlation is observed between the reduction of native populations and the concurrent increase in replacement populations. This pattern suggests a compensatory response of the communities, reflecting ecological reorganization due to the loss of sensitive species and the subsequent establishment of more tolerant, opportunistic taxa adapted to new environmental conditions. Urbanization and agriculture promote changes in hydrological regimes, nutrient availability, and the physicochemical composition of water, creating favorable niches for species adapted to degraded environments (Lobato Júnior; Araújo, 2015).

The analysis of Figures 07B and 07C, which compare urbanized areas and areas under direct agricultural influence (particularly near corn and coffee crops), reveals critical thresholds of ecological replacement. When more than 40% of the original community composition is replaced, the environment can be considered significantly urbanized. In agricultural areas, impacts become evident with approximately 20% replacement of original populations. These results highlight the high sensitivity of ciliate protozoa to anthropogenic changes, reinforcing their potential as bioindicators of ecological alterations (Dellamatrice; Monteiro, 2014; Piccoli et al., 2016).

Figure 7

Correlation analysis of ciliate protozoan populations in urbanized and agricultural areas, Rondônia, Brazil



Note: Correlation analysis (A) and dispersion of native and replacement populations in urbanized areas (B) and agricultural areas, proximity to corn and coffee crops (C).
Source: Prepared by the authors.

Figure 07 therefore provides a comprehensive overview of the cumulative impacts of anthropogenic activity on ciliate protozoan communities. The replacement of native species by more resistant forms raises important questions regarding the underlying ecological mechanisms, the functional consequences for trophic networks and ecosystem services, as well as the most effective conservation strategies to mitigate such losses.

Free-living ciliates have received increasing attention in the scientific literature due to their recognized sensitivity to environmental disturbances, especially changes in the physicochemical parameters of water (Zhong et al., 2017). These organisms occupy lower trophic levels in aquatic food webs and serve as food for secondary consumers, playing a key role in nutrient cycling and in structuring microbial communities (Sikder et al., 2019).

Moreover, ciliate protozoa exhibit broad ecological plasticity, occurring in a wide range of aquatic and edaphic habitats, either as free-living forms or attached to biotic and abiotic surfaces. Their ability to form resistant cysts allows them to withstand extreme environmental variations and promotes dispersal, contributing to their wide geographic distribution and effectiveness as sentinel organisms in biomonitoring programs (Cabral et al., 2017; Jeelani et al., 2018).

Their high abundance, rapid growth rates, short generation time, ease of sampling, and direct interaction with the environment through the plasma membrane make protozoa valuable tools for assessing ecological water quality across a wide range of ecosystems (Américo-Pinheiro, Torres & Ferreira, 2017). In Brazil, notable studies on taxonomic and ecological surveys of freshwater protozoa include those by Dias et al. (2008), Pauleto et al. (2009), Velho et al. (2013), Bonatti et al. (2016), Debastiani et al. (2016), Segovia et al. (2016), and Souza et al. (2019), which also highlight seasonal and spatial variation patterns often associated with toxicity in aquaculture environments.

Echavez and Leal (2021), in a biomonitoring study conducted in Lake Maracaibo, Venezuela, observed significant ecotoxicological effects of Cr^{3+}, Cr^{6+}, Cd^{2+}, Pb^{2+}, and Ni^{2+} on ciliate protozoa of the genera Paraenchelys and Halteria. Using the lethal concentration parameter for 50% of the population (LC_{50}) across two hydrological seasons (dry and rainy), the authors identified differences in toxicity patterns between periods, inferring that these variations result from distinct adaptations induced by multiple exposures to metal contaminants combined with prevailing physicochemical conditions.

The observed tolerance levels reinforce the idea that ciliate protozoa are among the most susceptible microorganisms to trace metals in lentic ecosystems and, therefore, can serve as potential microbiological early-warning indicators for detecting environmental contamination.

The correlation analysis presented in Figure 07 clearly highlights the positive association between the density of saprobic indicator protozoa and the degree of anthropization in the studied areas. This finding is consistent with the discussion by Garcia et al. (2016) regarding groups of ciliates as effective bioindicators for environmental monitoring, especially in tropical and neotropical environments.

Additionally, it is important to emphasize the potential of these organisms as tools for integrated ecological assessment, given their rapid response to environmental changes, wide distribution, and ease of sampling. These characteristics make ciliate protozoa ideal candidates for inclusion in continuous environmental monitoring programs in tropical aquaculture systems (Garcia et al., 2016; Wang et al., 2024).

Further deepen the spatial analysis by relating protozoan distribution to landscape typology. The replacement of sensitive species by resistant ones in urbanized or agricultural areas indicates a strong correlation between spatial transformation processes and ecological changes in aquatic environments. As discussed by Regali-Seleghim, Godinho, and Matsumura-Tundisi (2011), this replacement can be interpreted, from a geographical perspective, as a biological marker of landscape degradation and fragmentation of natural systems.

Among studies on freshwater protozoa in Brazil that stand out for species surveys are those by Dias et al. (2008), Pauleto et al. (2009), Colzani and Alves (2013), Velho et al. (2013), Bonatti et al. (2016), Debastiani et al. (2016), Segovia et al. (2016), and Souza et al. (2019), which demonstrated that seasonal and spatial variations indicate toxicity in aquaculture environments. Echavez and Leal (2021) conducted biomonitoring in Lake Maracaibo, in northwestern Venezuela, identifying ecotoxicological effects of Cr^{3+}, Cr^{6+}, Cd^{2+}, Pb^{2+}, and Ni^{2+} on free-living ciliate protozoa of the genera Paraenchelys and Halteria. To determine which genera were suitable as bioindicators of water quality, the lethal concentration for 50% of the protozoan population (LC_{50}) was evaluated using samples from both rainy and dry seasons.

Based on this previous study, it can be understood that differences in toxicity patterns are likely the result of various adaptive responses of protozoa, possibly induced by multiple sources, levels, and events of exposure to trace metal contamination, in addition to the physicochemical conditions prevailing across the two hydrological seasons. The observed tolerance levels suggest that ciliate protozoa are highly susceptible to trace metals and can be used as potential microbiological indicators providing early warning in contamination studies of lentic environments.

It is important to emphasize that the approach adopted in this research significantly contributes to the field of environmental geography by integrating ecological data with spatial landscape analysis. Due to their short generation time, sensitivity to physicochemical changes, and wide distribution, ciliate protozoa are key elements for diagnostic and monitoring studies in agricultural frontier areas, where conflicts between conservation and production intensify, including the presence of pollutants such as microplastics in fish farming ponds (Regali-Seleghim; Godinho; Matsumura-Tundisi, 2010; Bebianno et al., 2025).

4 CONCLUSIONS

In the context of geographic sciences, protozoa act here as tools for interpreting territorial processes. Their distribution and abundance make it possible to map, at a micro-scale, the environmental pressures exerted by anthropogenic activities. This bioindicator perspective allows the identification of critical zones of environmental vulnerability, supporting public policies for integrated water resource management and territorial planning based on ecological evidence. The results obtained reinforce the need for continuous monitoring of the microbiological quality of water in fish farming ponds, as well as qualitative and quantitative analyses of the planktonic community. Although the results do not show major differences among sampling points, free-living protozoa represent important tools for analyzing environments affected by different degrees of pollution. Furthermore, the monitoring carried out showed that proximity to urban areas increases populations of ciliate protozoa, in contrast to agricultural activity, which reduces these populations in fish farming pond waters.

Thus, the research developed here not only validates the applicability of protozoa as bioindicators but also contributes to the development of a critical and scientifically grounded environmental cartography, which may be used to support sustainable land-use practices, water management, and territorial planning in the Rondônia Amazon. Therefore, further research on this topic is necessary to continue advancing studies on the dynamics and distribution of aquatic microorganism communities, expanding knowledge of taxonomic diversity and species ecology, especially in impacted environments.

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