



## Spatial and temporal distribution of free-living protozoa in aquatic environments of a Brazilian semi-arid region

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### ABSTRACT

Free-living protozoa organisms are distributed in aquatic environments and vary widely in both qualitative and quantitative terms. The unique ecological functions they exhibit in their habitats help to maintain the dynamic balance of these environments. Despite their wide range and abundance, studies on geographical distribution and ecology, when compared to other groups, are still scarce. This study aimed to identify and record the occurrence of free-living protozoa at three points in Piancó-Piranhas-Açu basin, in a semi-arid area of Rio Grande do Norte (RN) state, and to relate the occurrence of taxa with variations in chlorophyll *a*, pH and temperature in the environments. Samples were collected in the Armando Ribeiro Gonçalves Dam, from two lentic environments upstream and a lotic ecosystem downstream. Sixty-five taxa of free-living protozoa were found. The Student's t-test showed significant inter-variable differences ( $p < 0.05$ ). Similar protozoan species were recorded under different degrees of trophic status according to chlorophyll *a* concentrations, suggesting the organisms identified are not ideal for indicating trophic level. We hypothesize that food availability, the influence of lentic and lotic systems and the presence of aquatic macrophytes influenced protozoan dynamics during the study period.

**Keywords:** Planktonic microorganisms, reservoirs; biodiversity.

### Distribuição espacial e temporal de protozoários de vida livre em ambientes aquáticos de uma região semiárida brasileira

### RESUMO

Os protozoários de vida livre são organismos amplamente distribuídos em ambientes aquáticos tanto em termos qualitativos como quantitativos. Eles possuem funções ecológicas únicas em seus habitats e ajudam a manter, através delas, o equilíbrio na dinâmica desses ambientes. Apesar do seu variado leque funcional e sua abundância, os estudos que abordam sua ecologia e distribuição geográfica, quando comparados a outros grupos, ainda são escassos. Diante disso o presente trabalho teve por objetivo registrar a ocorrência e identificar os protozoários de vida livre de três pontos da Bacia Hidrográfica Piancó-Piranhas-Açu, localizada no semiárido potiguar, bem como relacionar a ocorrência dos táxons catalogados com as variações de clorofila *a*, pH e temperatura dos ambientes em questão. As amostras foram coletadas em dois ambientes lênticos à montante e um ambiente lótico à jusante da

Barragem Armando Ribeiro Gonçalves. Registrou-se a presença de 65 táxons de protozoários de vida livre, dos quais 29 foram identificados em nível de espécie. O teste-t de Student aplicado no estudo demonstrou diferenças significativas ( $p < 0,05$ ) entre as variáveis. Foram encontradas espécies semelhantes de protozoários sob diferentes graus de trofia, de acordo com as concentrações de clorofila *a*, sugerindo que os organismos identificados não podem ser considerados bons indicadores de nível trófico. Sugere-se que a disponibilidade de nutrientes, a influência dos regimes lótico e lêntico e a presença de macrófitas aquáticas influenciaram a dinâmica dos protozoários no período estudado.

**Palavras-chave:** Microrganismos planctônicos, reservatórios, biodiversidade.

## 1. INTRODUCTION

Clean water is becoming increasingly scarce, directly affecting the quality of life of persons worldwide. In some parts of the world, particularly in semiarid regions, water-related issues are compounded by its scarcity, which occurs due to prolonged, periodic drought. Reservoirs are often constructed in an attempt to mitigate this problem. By contrast, this practice has produced several changes in the biodiversity of aquatic ecosystems. Despite these impacts, it is important to consider that benefits resulting from reservoir construction are directly reflected in the well-being of individuals, in addition to economic and social aspects (Tundisi et al., 2008).

The dynamics of artificial reservoirs are similar to those of natural aquatic environments. Both are potential sites for the proliferation, growth and development of diverse biological communities, such as animals, plants and microorganisms. Included in these groups are free-living protozoa. They consist of three taxonomic groups: Ciliophora (ciliates), Mastigophora (flagellates) and sarcordina (heliozoa and amoebas). All three belong to the plankton family and are collectively known as protozooplankton. They are considered cosmopolitan, because they are found from pole to pole (Laybourn-Parry et al., 2000).

Free-living protozoa are unicellular, heterotrophic, and may also contain mixotrophic organisms. They usually feed on bacteria, algae, and even zooplankton, depending on their size, and play a vital ecological role in aquatic environments (Sherr and Sherr, 2002). Protozooplankton is a key element in the food chain, since it controls the populations of various organisms. According to Lehrter et al. (1999) and Irigoien et al. (2005), protozoa can consume up to 100% of daily phytoplankton production. Heterotrophic flagellates are able to consume the entire bacterial production of an aquatic environment (Fenchel, 1986; Berninger et al., 1991). Moreover, they participate in the carbon cycle, and are a fundamental part of the "microbial loop" (Azam et al., 1983). This term derives from the fact that these organisms are situated in one strategic trophic level, allowing them to transfer energy (carbon and other nutrients) acquired from lower trophic levels, through the ingestion of organisms such as algae and bacteria, moving to higher trophic levels as they are ingested by other organisms (Xu et al., 2008; 2005; Finlay and Esteban, 1998).

Free-living protozoan organisms have been used as bioindicators and biomonitors of pollution (Corliss, 2002). This is because they are highly sensitive to environmental changes, given that they are formed by a single cell composed of fragile membranes. Hydrological cycles, increased nutrients in water bodies, physical and chemical changes and phytoplankton blooms interfere in the dynamics of this community, thereby affecting the entire trophic network.

Despite the ecological importance of protozooplankton to freshwater environments, studies on the dispersion and dynamics of this group are scarce when compared to other species, such as algae and zooplankton. Thus, this investigation aimed to record the

occurrence and identify freshwater free-living protozoa in aquatic ecosystems of a semiarid area of RN, an approach that proposes to establish a relationship between their distribution and the environmental variations observed.

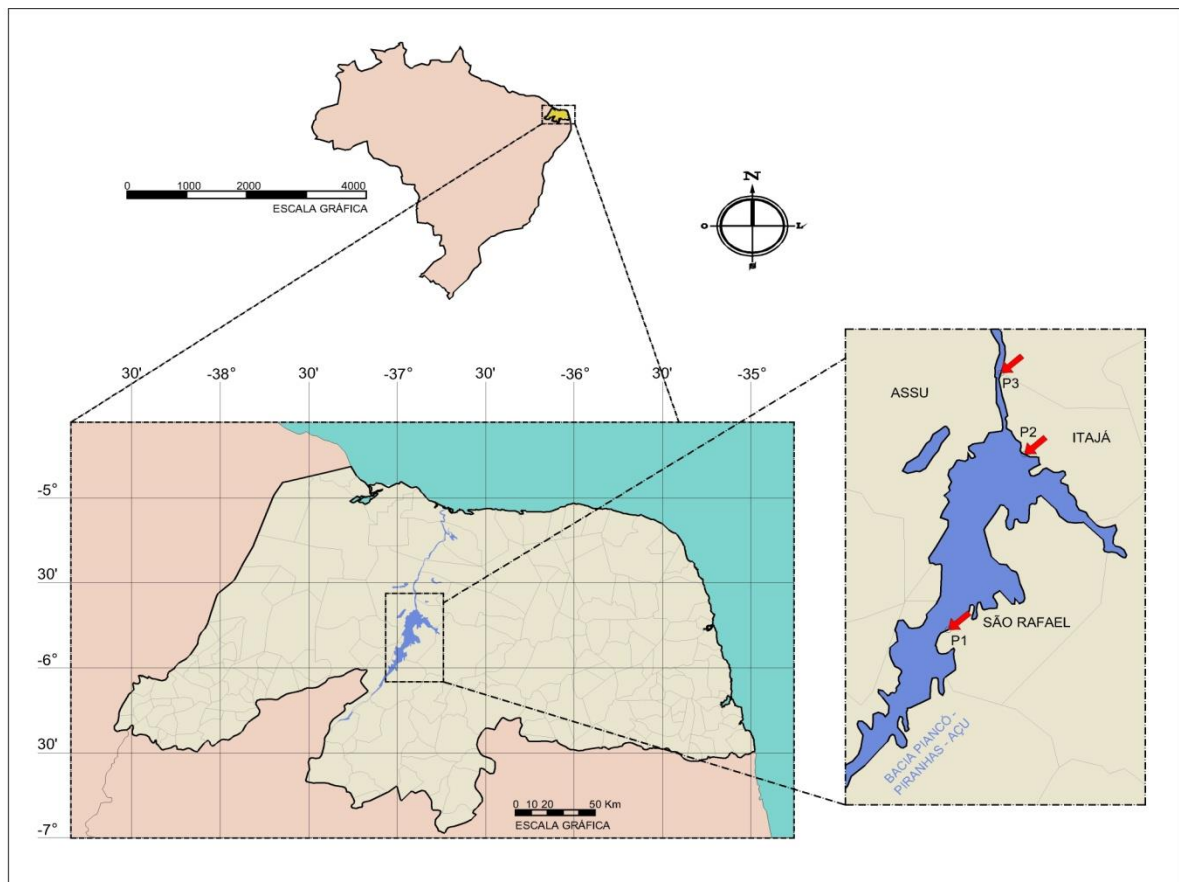
## 2. MATERIAL AND METHODS

### 2. Study Area

The Piancó-Piranhas-Açu River Basin has a total drainage area of 43,681.50 km<sup>2</sup> in the states of Paraíba (PB) and Rio Grande do Norte (RN). The latter contains the Armando Ribeiro Gonçalves Dam (05 ° 40 '9:48' S and 36 ° 52 '44.34 "W) with a storage capacity of 2.4 billion cubic meters of water. This reservoir is socially and economically important and it is estimated that 500,000 people depend on it for their water supply. Nevertheless, many points along this system are eutrophic due to human activities.

#### 2.1. Sampling and determination of variables

Sampling points (Figure 1) were determined according to their proximity to the cities of São Rafael (P1), Itajá (P2) and Assu (P3). P1 and P2 are lentic, located upstream of the dam and P3 is a lotic system, located downstream from the reservoir. Water samples for protozoa identification were collected between May 2010 and March 2011. Three of the nine samples were obtained at the end of the rainy season (ERS) and four in the dry season (DS) of 2010. The other two collections occurred during the rainy season (RS) of 2011 with samples filtered through 68µm and 10µm nets.



**Figure 1.** Piancó-Piranhas-Açu river hydrographic basin and sampling stations P1, P2 and P3.

Protozoa were analyzed without the use of fixatives, in order to better preserve morphological characteristics and make identifications more accurate. Taxonomic identification was based on Foissner and Berger (1996), Mitchell (2003), Patterson (1996), and the "Protist Information Server".

The variables pH and temperature were measured using a multi-parametric analyzer. Chlorophyll *a* concentration followed the procedure recommended by Jespersen and Christoffersen (1988). Significant differences were found between data from different periods using Student's t-test for independent variables ( $p < 0.05$ ).

### 3. RESULTS AND DISCUSSION

Means of physical, biological and chemical variables (pH, temperature and chlorophyll *a*) are described in Table 1. Data refer to the end of the rainy season (ERS) and dry season (DS) of 2010 and the rainy season (RS) of 2011, at three sampling stations. Variables were tested in the environment, showing variations between the study periods. In general, there was a significant difference among them ( $p < 0.05$ ), except for temperatures at the end of the 2010 rainy season and the 2011 rainy season in P2. There were no significant differences in P3 between pH in the dry and rainy seasons, or between temperature at the end of the 2010 rainy season and 2011 rainy season.

**Table 1:** Averages of physical and chemical variables of P1, P2 and P3.

Period / Year	pH	Temperature (°C)	Chlorophyll <i>a</i> ( $\mu\text{g L}^{-1}$ )	Sampling Points
ERS (2010)	8,01	29,5	49,14	P1
DS (2010)	8,48	28,2	54,95	
RS (2011)	9,38	29,8	30,47	
ERS (2010)	8,69	30,2	42,71	P2
DS (2010)	8,65	27,9	38,36	
RS (2011)	9,52	31,0	24,78	
ERS (2010)	6,98	31,9	12,56	P3
DS (2010)	8,11	30,9	15,13	
RS (2011)	8,00	31,9	<i>not measurable</i>	

**Note:** ERS: End of rainy season; DS: dry season; RS: rainy season.

Water temperature was the most constant parameter, exhibiting few significant differences. The lowest averages were recorded in the dry season and the highest during the rainy season. There were significant differences in pH values between lentic and lotic environments. In the lentic environment, pH was always basic, with averages above 8.0, and a peak of 9.52. In the lotic environment, we found averages ranging from 6.98 to 8.0. The highest values in the three environments occurred during the rainy season (January and February/2011).

P1 showed the largest mean concentration of chlorophyll *a*, reaching a maximum of  $54.95 \mu\text{g L}^{-1}$ . The lowest averages were found in P3, which had low concentrations in the rainy season, precluding their measurement. According to Nurnberg (1996), chlorophyll *a* concentrations indicate the degree of trophic status. We therefore surveyed all eutrophic points during all collection periods, with the exception of the rainy season in P3, where

chlorophyll *a* concentration was so low that it could not be measured. Thus, the more eutrophic environments, based on criteria and values proposed by this author, were the lentic sampling stations, especially in P1.

Environmental variables such as temperature, pH and chlorophyll *a* are factors that can significantly interfere in the dynamics of aquatic ecosystems by modifying the structure and function of microbial communities (Liu and Leff, 2002). According to Galvez-Cloutier and Sanchez (2007), chlorophyll *a* indicates algal biomass and is positively related to the phosphorous concentration of an environment, indicating the trophic state of an aquatic ecosystem. Aquatic communities can change quantitatively and qualitatively in response to eutrophication (Biudes and Camargo, 2006) In the present study, the environment has a high level of trophic status considering the results of Cunha et al. (2009) and Eskinazi-Sant'Anna et al. (2007) in the same watershed. Nevertheless these characteristics did not significantly interfere in protozoan distribution.

We cataloged 65 protozoan taxa (Table 2). Twenty-nine were identified in 36 species and genera. Of this total, 29 belong to the phylum Ciliophora (ciliates), 17 to Mastigophora (flagellates) and 19 to the phylum Sarcodina (heliozoa and amoebae, which are naked, and testacea). In all environments we identified three phyla of organisms throughout the study period. Occurrence of ciliates was similar at the three sampling stations during all collection periods (25 taxa in P1 and 24 taxa in P2 and P3). Mastigotes and Sarcodina revealed differences in their qualitative distributions between lentic (P1 and P2) and lotic (P3) collection points. We cataloged 17 flagellates, 10 of which were observed in P1 and P2. Sarcodina were represented by 8 and 9 taxa in P1 and P2, respectively, while 16 representatives of this group were present in P3.

Irrespective of environment, sampling period and the significant difference between some of the parameters evaluated, flagellate organisms stood out in terms of sample density. This pattern has been observed in research aimed at quantifying these organisms (Araújo and Godinho, 2008; Weitere and Arndt, 2003). However, we recorded differences in the taxonomic composition of flagellates between lentic and lotic systems. This occurs primarily due to the presence of the phytoflagellates *Trachelomonas sp*, *Euglena sp* and *Phacus sp* in the lotic environment. They did not occur in the lentic stretch. These organisms, recognized as important components of the periphyton group (Algarte et al., 2006; Ekhtor, 2010), comprise a periphytic community, since the collection point near the shore is surrounded by a variety of aquatic plants, as well as submerged grasses. Chemical and physical parameters such as pH, temperature and chlorophyll *a*, may not have been responsible for the establishment of this community, as reported in similar spatial dispersion studies (Rodrigues and Bicudo, 2004).

Most other flagellate taxa occurred in all environments and seasons, indicating that neither physical nor chemical variables influenced the permanence of these organisms in terms of quality, a fact that occurred with *Ansiomonas sp*, *Bodo sp*, *Entosiphon sulcatum*, *Entosiphon sp*, and *Notosolensus Pentalomonas sp*. Similarly, Weitere and Arndt (2003) noted that the flagellate composition of a river in Germany remained unchanged despite physical and chemical changes. Patterson and Lee (2000) reported that species of the genus *Bodo* and *Pentalomonas* have a cosmopolitan character, belonging to the most frequently reported phylum Mastigophora in studies conducted in various parts in the world. This ubiquity of flagellates is most likely related to their wide range of adaptations, especially with regard to feeding versatility. Studies show that these organisms can feed on viruses and even cyanobacteria (Perntaler, 1996), as well as consume dissolved carbon. This qualitative stability of flagellates hinders the classification of these organisms, cataloged in the present study as indicators of trophic levels in aquatic environments.

**Table 2:** Seasonal and temporal dispersion of Protozoa.

Espécies	P1	P2	P3	ERS/ 2010	DS/ 2010	RS/ 2011	Species	P1	P2	P3	ERS/ 2010	DS/ 2010	RS/ 2011
<b>Ciliophora</b>							<b>Cont.</b>						
<i>Apsiktrata gracilis</i>			x		x		<i>Euglena clavata</i>			x	x	x	x
<i>Aspidisca sp</i>	x	x	x	x	x	x	<i>Mastigela polyvaculata</i>			x			x
<i>Aspidisca aculeata</i>	x			x			<i>Notosolensus</i>	x	x	x	x	x	x
<i>Aspidisca cicada</i>	x	x	x	x	x	x	<i>Pentalomonas</i>	x	x	x	x	x	x
<i>Aspidisca costata</i>	x	x	x	x	x	x	<i>Peranema sp</i>	x	x	x	x	x	x
<i>Cinetochilum sp</i>	x	x	x	x	x	x	<i>Phacus orbiculares</i>			x		x	x
<i>Coleps elongatus</i>	x	x	x	x	x	x	<i>Synura sp.</i>		x	x			x
<i>Coleps hirtus</i>	x	x	x	x	x	x	<i>Trachelomonas sp</i>	x		x		x	x
<i>Coleps sp</i>	x				x		<i>Trachelomonas armata</i>			x	x	x	x
<i>Colpidium sp</i>	x	x			x	x	<b>Sarcodia</b>						
<i>Cinetochilum sp</i>	x	x	x	x	x	x	<i>Actinosphaerium eichornii</i>	x	x	x	x	x	x
<i>Coleps elongatus</i>	x	x	x	x	x	x	<i>Ameba sp</i>	x	x	x	x	x	x
<i>Cyclidium sp.</i>	x	x	x	x	x	x	<i>Amoeba radiosa</i>	x	x	x	x	x	x
<i>Cyclidium glaucoma</i>	x	x	x	x	x	x	<i>Arcella vulgaris</i>	x	x	x	x		x
<i>Dileptus sp</i>		x		x			<i>Centropyxis aculeata</i>	x	x	x	x	x	x
<i>Euplotes sp</i>	x	x	x	x		x	<i>Centropyxis ecornis</i>	x	x	x	x		x
<i>Halteria sp</i>	x	x	x		x	x	<i>Diffugia sp1</i>	x	x	x	x	x	x
<i>Litonotus sp.</i>	x	x	x	x	x	x	<i>Diffugia sp2</i>		x		x	x	x
<i>Mesodinium sp.</i>	x	x	x	x	x	x	<i>Diffugia acuminata</i>	x				x	x
<i>Paramecium sp.</i>	x	x	x	x	x	x	<i>Diffugia corona</i>	x			x		
<i>Paramecium caudatum</i>	x	x	x	x	x	x	<i>Diffugia spiralis</i>			x		x	x
<i>Stentor sp.</i>		x	x	x	x		<i>Diffugia gramen</i>			x	x	x	x
<i>Stentor roeselii</i>	x	x			x	x	<i>Hedriocistys sp</i>			x		x	
<i>Stylonychia sp</i>	x		x		x		<i>Euglypha sp1</i>			x		x	
<i>Tachysoma sp</i>	x	x	x	x	x		<i>Euglypha sp2</i>			x			x
<i>Trachelophylum sp</i>	x	x	x	x	x	x	<i>Euglypha acantophora</i>			x		x	x
<i>Vorticella campânula</i>	x	x	x	x	x	x	<i>Euglypha alveolata</i>	x	x	x		x	x
<i>Vorticella nartans</i>			x	x			<i>Euglypha filifera</i>			x	x		x
<i>Vorticella sp</i>	x	x	x	x	x	x	<i>Trinema sp</i>	x	x	x	x	x	x
<b>Mastigophora</b>													
<i>Ansiinema sp</i>	x	x	x	x	x	x							
<i>Bodo sp</i>	x	x	x	x	x	x							
<i>Cryptomonas sp</i>			x			x							
<i>Chilomonas sp</i>	x	x	x	x	x	x							
<i>Chroomonas sp</i>	x	x	x		x	x							
<i>Entosiphon sulcatum</i>	x	x	x	x	x	x							
<i>Entosiphon sp</i>	x	x	x	x	x	x							
<i>Euglena sp</i>			x	x	x	x							

**Note:** P1: Sampling point 1; P2: Sampling point 2; P3: Sampling point 3; ERS/2010: End of rainy season 2010; DS/2010: dry season 2010; RS/2011: rainy season 2011; x: occurrence of these protozoa during the period mentioned.

The distribution of taxa belonging to the phylum Ciliophora was very similar in all three environments. Differences were observed in the genera *Aspidisca* and *Cinetochilum Cyclidium*, especially *Aspidisca cicada* species, *Aspidisca Cyclidium costata* and *glaucoma*, which were prominent in all samples, regardless of environment and collection period. These organisms are relatively small (20-30 µm) and were identified as dominant by Beaver and Crisman (1989) in eutrophic systems in Florida. The authors also emphasized that among these ciliates, those belonging to the order Scuticociliatida stood out in eutrophic environments, while larger ciliates from the group Oligotrichida were associated with more oligotrophic environments. The present investigation did not evaluate the possibility of associating oligotrichia to oligotrophic environments, since other members of this group such as *Stylonychia sp* and *dumbbells sp* were present in moments of high chlorophyll *a* concentrations (dry season).

Thus, the occurrence of ciliates and flagellates seems more related to food availability than the trophic level of the environment, given that these environments are conducive and admittedly different in terms of algae, bacteria and flagellates (Sodré-Neto and Araújo, 2008), which serve as food for protozoa. Species such as *Aspidisca cicada*, *Coleps hirtus*, *Stentor Roselli* and *Paramecium caudatum*, which displayed widespread occurrence regardless of the period, may feed on algae, flagellates, bacteria and even smaller zooplankton (Foissner and Berger, 1996).

The phylum sarcodina exhibited significant differences in taxonomic composition, and the genera *Diffugia*, *Arcella* and *Centropyxis* were the most frequent in qualitative terms. This corroborates research reporting these genera as among the most common in aquatic environments (Lansac-Toha et al., 2000)

Only one representative of the heliozoa group was recorded: *Actinosphaerium eichhornii*. This species is globally distributed and may occur in environments with different characteristics. Specimens were found in all environments and collections, confirming that the organism is resilient and adapts to physical and chemical changes. Previous research has shown similar results for this organism (Takamura et al., 2000), recording its presence in eutrophic and mesotrophic lakes, indicating the degree of environmental trophic status has not changed its dispersion. In addition, Leonov (2010) identified these organisms in environments with acidic pH up to 4.3, while in the present study they occurred at pH levels up to 9.52. This shows that neither trophic level nor pH are regulators for the presence of this species.

We observed large differences in spatial arrangement and slight variations between testate amoebae with regard to the temporal dispersion. These organisms are commonly reported in lentic and lotic environments, but in this study, the lotic environment (P3) exhibited twice the number of taxa, compared to the lentic (P1 and P2). Moreover, some investigations (Lansac-Toha et al., 2000) have shown they may be more common or even dominant in lotic environments.

Studies conducted in tropical environments (Araújo, 2008) reported an association between testate amoebae and macrophytes. This may explain their higher frequencies in the lotic environment, where these plants were abundant on its banks. Some species of testate amoebae, such as *Diffugia spiralis*, *Diffugia gramen*, *Hedriocystis sp*, *Euglypha filifera*, *Euglypha acantophora*, *Euglypha sp1* and *Euglypha sp2* occurred exclusively in the lotic system. However, except for two species of *Euglypha*, they all occurred in every station, despite their diverse and sometimes contrasting characteristics, during both the rainy and the dry periods. This confirms that their presence is not dependent on the trophic level of the environment.

#### 4. CONCLUSIONS

The identifications will contribute towards an understanding of the geographical distribution of these species, since records of these organisms in these environments are extremely scarce.

Despite variations observed between physical, chemical and biological variables in each environment and during climatic periods, there were no significant differences in the distribution of taxa, indicating these variables were not decisive in the occurrence of these organisms in the study sites and periods.

Similar protozoan species were observed in different degrees of trophic according to chlorophyll *a* concentrations. This suggests the organisms are not ideal for indicating the trophic level of environments, in relation to the studied parameters.

The lentic and lotic regimens and the presence of aquatic macrophytes have influenced the protozoan dynamics during the study period. Thus, further research is needed to identify the variables determining the dynamics of this environment.

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