



Flagellate protist abundance in phytotelmata of *Aechmea distichantha* Lem. (Bromeliaceae) in the upper Paraná river basin

Gisele Silva Costa Duarte, Geziele Mucio Alves, Fernando Miranda Lansac-Tôha, Luiz Felipe Machado Velho and Fábio Amodêo Lansac-Tôha*

Núcleo de Pesquisa em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brazil.
*Author for correspondence: fabio@nupelia.uem.br

ABSTRACT. In some Bromeliaceae species the leaf sheaths form a tank or phytotelma, accumulating water and organic detritus, conditions that allow the establishment of different communities. Thus, this study evaluated the relationship between diameter and volume of water in the tank of the bromeliad *Aechmea distichantha* Lem. and density of flagellates found in the phytotelmata, as well as the influence of the hydrological periods and proximity of sampled strata on the phytotelma patterns of abundance distribution. Samplings were conducted in two hydrological periods in 2010. In each sampling, four plants were collected from three different strata, classified according to the distance from the river level. A one-way ANOVA identified significant differences in flagellate density between hydrological periods. The influence of the water volume in the phytotelmata on the flagellate density was evidenced by regression analyses, as well as the lack of relationship between the density and perimeter of the tank. Moreover, the distance of the strata from the river level did not influence the pattern of flagellate abundance. Thus, our results suggested that the hydrological period and the volume of water in the tanks of *Aechmea distichantha* are determinant on the abundance of heterotrophic flagellate in the phytotelmata.

Keywords: Phytotelma, protozoa, bromeliad, rocky walls.

Abundância de protozoários flagelados em fitotelmata de *Aechmea distichantha* Lem. (Bromeliaceae) na bacia do alto rio Paraná

RESUMO. Em algumas Bromeliaceae as bainhas foliares formam uma cisterna ou fitotelma, onde ocorre acúmulo de água e detritos orgânicos, que permitem o estabelecimento de diferentes comunidades. Assim, este estudo avaliou a relação entre o diâmetro e volume de água no tanque de *Aechmea distichantha* Lem. e a abundância de flagelados encontrados nestes fitotelmata, bem como a influência do período hidrológico e a proximidade dos estratos amostrados sobre os padrões de distribuição desse atributo. As coletas foram realizadas em dois períodos hidrológicos no ano de 2010. Foram coletadas quatro plantas em três estratos diferentes de acordo com a distância em relação ao rio. Foram identificadas diferenças significativas entre a densidade dos flagelados e os períodos hidrológicos. Além disso, verificou-se a influência do volume de água presente nos fitotelmata sobre a densidade dos flagelados, bem como a ausência de relação entre a densidade desses protistas e o perímetro do tanque das bromélias. A distância dos distintos estratos em relação ao rio não influenciou a abundância dos flagelados. Assim, os resultados sugerem que o período hidrológico bem como o volume de água presente nos tanques de *Aechmea distichantha* atuam como um fator determinante sobre a abundância da comunidade de flagelados heterotróficos nos fitotelmata.

Palavras-chave: Fitotelma, protozoa, bromélia, paredões rochosos.

Introduction

The family Bromeliaceae features a high richness and biodiversity in the Atlantic Forest (MARTINELLI et al., 2008), with approximately 3,000 species which underwent an extensive adaptive radiation (LUTHER, 2006). This Neotropical-endemic family is characterized by terrestrial, epiphytic plants, small-sized with simple leaves arranged in a rosette (BENZING, 2000; LEME; MARIGO, 1994).

In some species, the so-called tank bromeliads, the leaf sheaths form a tank or phytotelma, accumulating water and organic detritus (BENZING, 2000; LEME; MARIGO, 1994), being this storage promoted by the overlapping of the leaves. The tanks capture, in addition to rainwater, leaves that are decomposed and consumed by organisms. In this way, a phytotelma favors the colonization for many species, supplying nutrients and refuge for plankton organisms and insect larvae, which interact and form a complex food web (NGAI; SRIVASTAVA, 2006).

Bromeliads grow near freshwater environments, which enable the exchange of organisms between these aquatic systems. However, the species composition of the bromeliad tanks seems to be characterized by a high level of endemism, being considerably different from the fauna found in nearby environments (LOPEZ et al., 2009). The absence of organisms in common between the tanks and nearby environments may be explained by the high level of specialization developed by the phytotelma fauna over time (LOPEZ et al., 2009). According to these authors, the tanks may suffer the effect of small islands, where the low volume of water stored restricts most of the biotic communities. The presence of invertebrates and microorganisms inhabiting bromeliad tanks allows the supply of organic nutrients required for the survival and growth of these plants under stressful conditions, such as water shortage, and low availability of nitrogen and phosphorus in the environment.

Furthermore, the phytotelmata, like carnivorous plants, tree holes, and tanks of bromeliads, provide an excellent opportunity for studying and understanding ecological processes in tropical and temperate communities (KITCHING, 2000, 2001). Given the above, the theory of island biogeography proposed by MacArthur and Wilson (1967) presents relevant considerations likely to be applied to bromeliads, once the tanks of these plants can be considered as isolated islands colonized by many species. Regarding this theory, Townsend et al. (2006) highlighted the direct relationships between island size and species diversity and between distance of an island from a dispersal source of organisms and species diversity.

In general, there is a great diversity and abundance of microorganisms in phytotelmata of bromeliads, among them, bacteria (HAGLER et al., 1993), fungi (RUIVO et al., 2005, 2006) and ciliate (BUOSI, personal communication), but none addressed the composition and abundance of flagellates in these environments. In the upper Paraná river basin, samplings undertaken on the left bank of the river. Flagellate protozoa have small size and high metabolic rate (FENCHEL, 1982), which gives an important role in the rapid cycling of nutrients, mineralization of organic compounds, and production and transference of organic matter in aquatic environments (AZAM et al., 1983; POMEROY, 1974). These organisms make up a morpho- and physiologically diverse group, with all basic trophic strategies observed among auto- and heterotrophic eukaryotes, only behind bacteria,

among all organisms' groups, in the diversity of ways used to obtain energy (SLEIGH, 2000).

In this way, this study aimed to evaluate the density of heterotrophic flagellate associated with phytotelmata of *Aechmea distichantha* (Bromeliaceae). For this, we examined the relationship between the diameter of the bromeliad tank and the density of flagellate found in the phytotelma, as well as the influence of hydrological period and proximity of sampled strata on the rocky walls in relation to the river. The study tested the following hypotheses: (i) the major changes in the flagellate community are temporal, i.e., determined by the hydrological periods; (ii) the community density is higher at lower volumes of water in the tanks; (iii) the density is greater in the phytotelmata of bromeliads closer to the river, at the lower stratum of the rocky walls, than in the plants located at the upper stratum (farther from the river level); (iv) spatially, the increased perimeter of the tank increases the density of the flagellate community.

Material and methods

Study area

This study was developed in the last free flowing stretch of the upper Paraná river, between the mouth of Parapanema river and the mouth of Ivinheima River, about 200 km upstream of Itaipu reservoir (TAKEDA et al., 2002). Samplings were held in rocky walls on the left bank of the river, near the Field Station of Nupélia, in the municipality of Porto Rico, Paraná State (Figure 1). The left bank of the Paraná river features convex-top hills that confer a high elevation to this side of the river, besides several rocky walls formed by sedimentary rocks of the Cretaceous period and sparse flooding areas (SOUZA FILHO; STEVAUX, 2004). These rocky walls allow the establishment of diverse plant species, including epiphytic plants like the bromeliads.

Aechmea distichantha (Figure 2) has the leaves arranged in a rosette, enabling the water storage. Its leaves are about 30-100 cm, with alternation of generations, either sexual or asexual. The exposure to different environmental conditions makes the bromeliad to present variations in the size and number of leaves (CAVALLERO et al., 2009). According to these authors, the shaded individuals are taller and have larger diameters, while individuals exposed to the sun may have more leaves and a larger tank, storing larger amounts of water.

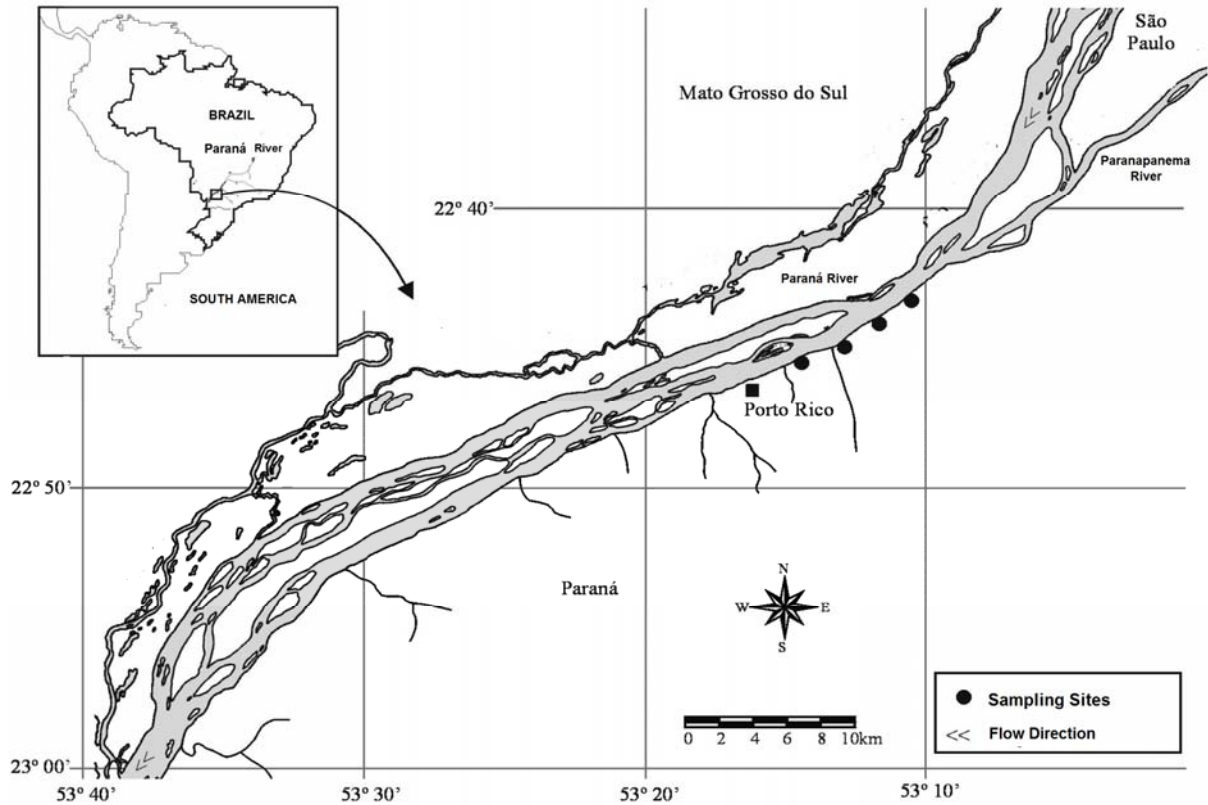


Figure 1. Study area with location of the sampling sites on the left bank of the Paraná river

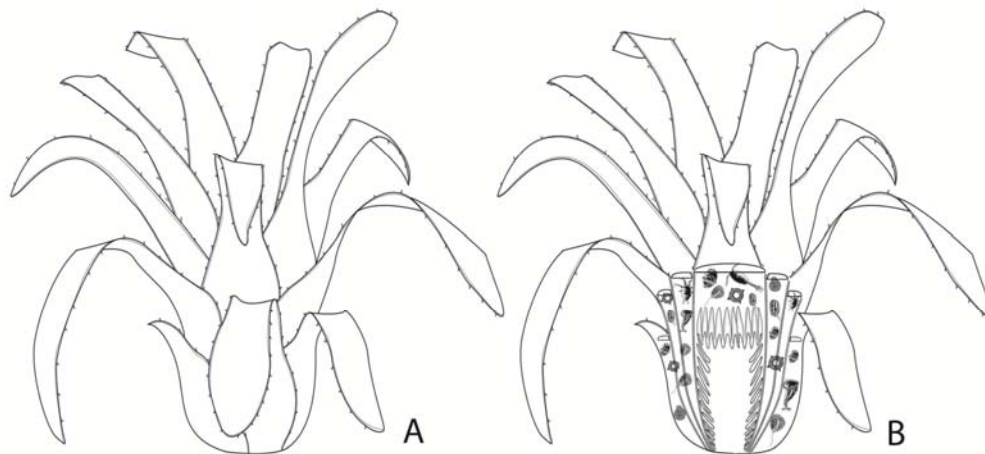


Figure 2. Schematic drawing of a bromeliad showing the overlapping of the leaves (A) and a longitudinal section of a tank bromeliad showing the community of organisms present in the phytotelma (B).

Aechmea distichantha is widely distributed throughout South America, found in Southern forests of Brazil, Bolivia, Paraguay, Uruguay, and in Northern Argentine. Populations of this species may be terrestrial or epiphytic in deciduous or semideciduous forests (SMITH; DOWNS, 1979).

Sampling

Samplings were conducted in two hydrological periods (rainy and dry) in 2010, by collecting 72

individuals of *Aechmea distichantha* from the rocky walls on the left bank of the Paraná river. At each sampling, we collected four individuals from three different strata classified according to the distance from the river level, as follows: lower stratum ($h \leq 3$ m), middle ($h > 3$ and ≤ 7 m) and upper ($h > 7$ m) (Figure 3).

Each collected plant was georeferenced considering the stratum and distance from the river level, by using a GPS device.

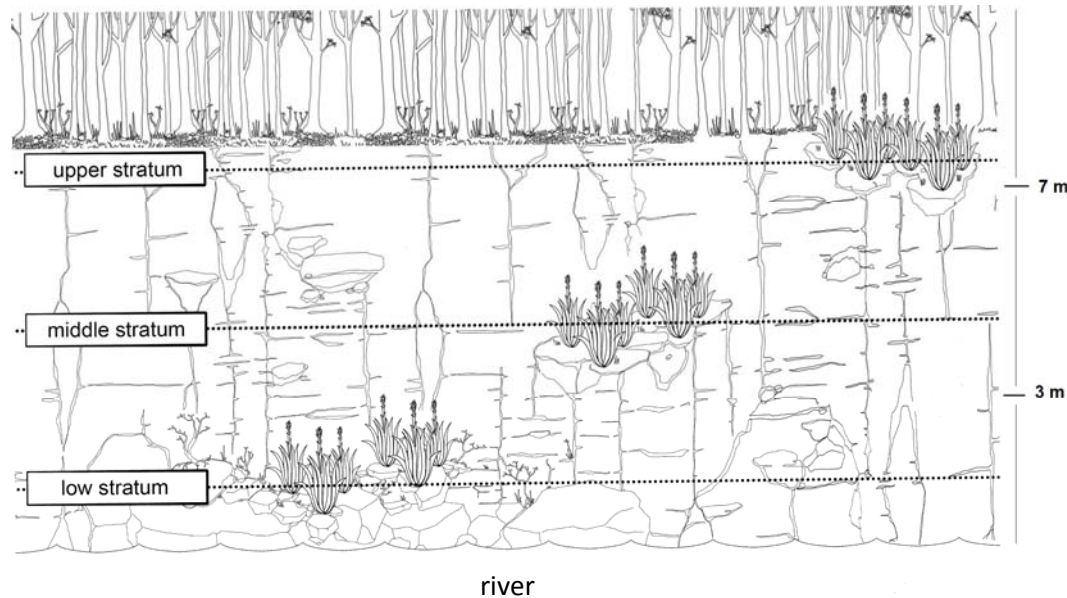


Figure 3. Rocky wall on the left bank of the Paraná river, highlighting the three sampled strata.

The plants were removed manually from the wall, using work gloves and knife to protect against the spines. Then the plants were placed in labeled plastic bags to be taken to the Field Station of Nupélia.

Rainfall data were provided by the climatologic station of Nupélia, located near the Field Station. Only the accumulated rainfall data of the week immediately prior to the samplings was used.

Laboratory analysis

In the lab, the plants were inclined down into a tray to remove all the water in the tanks, and the plants were also washed with 500 mL of distilled water. In this way, the volume in the tanks was calculated by subtracting 500 mL from the resulting volume after washing. Also we measured the perimeter of the tanks.

The flagellate samples were fixed with a solution made up by formaldehyde, alkaline lugol and thiosulfate (SHERR; SHERR, 1993). To determine the density and biomass, semi-permanent glass slides were prepared by filtering samples through a black filter (Nucleopore/Watchman 0.8 μm) and stained with DAPI (fluorochrome 4'-6-diamidino-2-phenylindole at 0.001%) for 15 minutes in the dark (PORTER; FEIG, 1980). After this, the slides were stored in refrigerator for 24 hours. Then, the flagellates were quantified by counting on each slide 300 cells or 100 fields, chosen randomly, with an epifluorescence microscope (Zeiss Axiophot), final magnification of 1000x. The organisms were counted under UV light and using a blue filter, adopting the criterion of low fluorescence and greenish color for heterotrophic flagellates, and reddish color for autotrophic ones. The

individuals were measured with a micrometer eyepiece, in order to determine the cell volume, through the cell dimensions and approximate geometric shapes (WETZEL; LIKENS, 1991), and the carbon content by means of the expression: $1 \mu\text{m}^3 = 167 \text{ fg C}$ (FENCHEL, 1982).

For the qualitative analysis of fresh samples, in addition to the traditional analysis under microscope, we also prepared cultures in Petri dishes, from adding crushed rice grains and distilled water. In this way, the population growth of heterotrophic flagellates allowed a better visualization and identification.

Data analysis

A factorial ANOVA (SOKAL; ROLHF, 1991) was used to test the differences of flagellate density between sampling periods in the different strata, with seasons and distance from the river as factors, being the significance level set at $p < 0.05$. The ANOVA assumptions were previously tested by Shapiro-Wilk for normality, and Levene's test for homoscedasticity.

In order to check the effects of tank perimeter and volume of water stored in the bromeliads on the density, a linear regression was employed (ZAR, 2009). Data of abundance (density and biovolume) and volume of water were previously log transformed. These tests were employed using Statistica 7.1 software (STATSOFT INC., 2005).

Results

Throughout the study period, there was no outstanding pattern in the rainfall indices. The rainy months were February and October, and dry

months, March, April and November. Higher values of rainfall were observed in October, when we performed two samplings (13.2 and 26.8 mm). So, this was considered the rainiest month, with a total of 40 mm. In April, no rainfall was verified in the week prior the sampling (Figure 4).

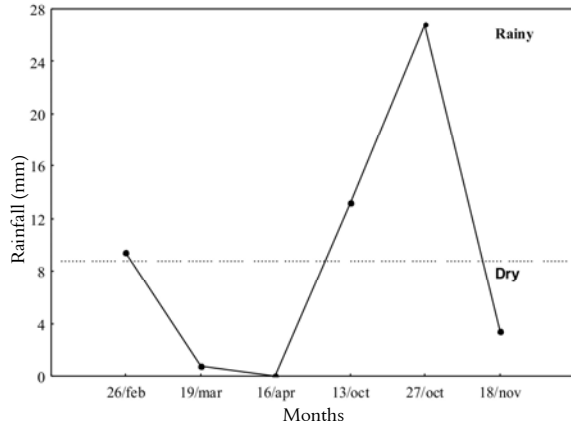


Figure 4. Rainfall data in the sampling periods.

Seventeen infrageneric taxa were identified, distributed into six orders. Euglenida was the most representative with ten taxa (Table 1).

Table 1. Fauna survey of flagellate protozoa in the phytotelmata of the bromeliad *Aechmea distichantha*.

Order	Species
Chromulinales	<i>Goniomonas</i> sp.
	<i>Monas</i> sp.
Cryptomonadida	<i>Chilomonas</i> sp.
	<i>Anisonema acinus</i> Dujardin
	<i>Distigma</i> sp.
Euglenida	<i>Entosiphon sulcatum</i> (Stein) Dujardin.
	<i>Heteronema</i> sp.
	<i>Menoidium</i> sp.
	<i>Notosolenus</i> cf. <i>canellatus</i> Skuja
	<i>Peranema trichophorum</i> (Ehrenberg) Stein
	<i>Petalomonas</i> sp.1
	<i>Petalomonas</i> sp. 2
	<i>Euglenideo</i> não identificado
	<i>Bodo saltans</i> Ehrenberg
	<i>Bodo</i> sp.
Kinetoplastea	<i>Hexamita</i> sp.
Pelobiontida	<i>Glenodinium</i> sp.
Dinoflagellida	

According to ANOVA results, the flagellate density was significantly different over time, with higher values in the dry period (Figure 5).

No significant difference was found between the values of flagellate density in the different sampled strata (rainy period: $F(2;6) = 3.89$; $p = 0.08$; dry period: $F(2;6) = 0.34$; $p = 0.72$).

The regression analysis indicated a strong and positive relationship between density and biovolume data of flagellate protozoa in the phytotelmata (Figure 6). For further analysis, only density data were considered.

It was registered an influence of the volume of water in the tank on the density of flagellates. The increase in water volume led to a lower density of these protozoa (Figure 7).

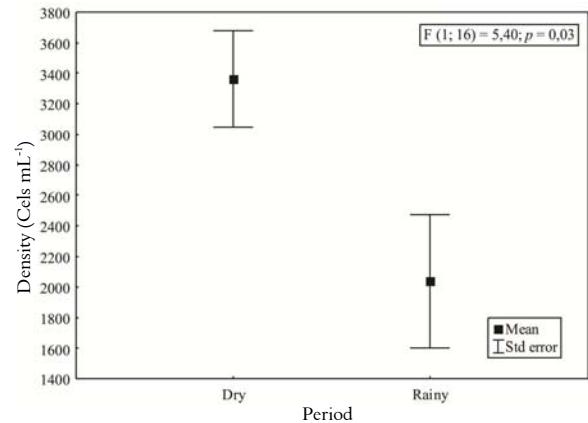


Figure 5. Variation in the density of flagellate protozoa in the study periods.

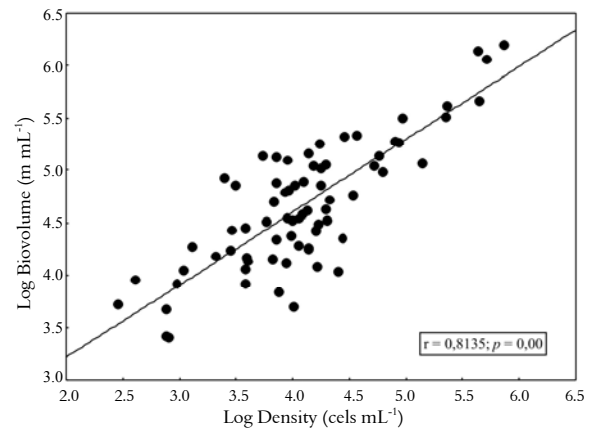


Figure 6. Relationship between density and biovolume of flagellate protozoa in the phytotelmata.

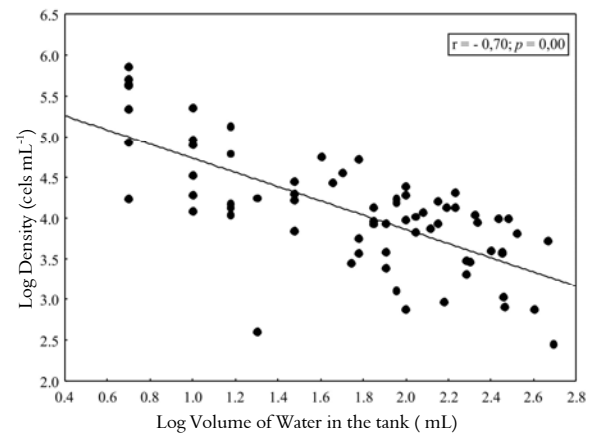


Figure 7. Relationship between the volume of water in the bromeliad tank and density of flagellate protozoa.

A regression analysis between density and perimeter of the tank evidenced a lack of relationship between these variables (Figure 8).

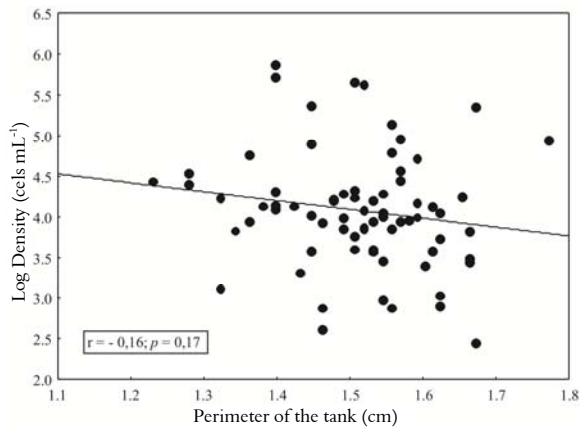


Figure 8. Relationship between the perimeter of the tank and the density of flagellate protozoa.

Discussion

The scarcity of studies on the ecology of heterotrophic flagellates associated with phytotelmata of *Aechmea distichantha* (bromeliad) hinders drawing comparisons between the community structure in this microcosm. In general the phytotelmata are poorly studied regarding the fauna composition. Most of the studies performed in these microecosystems is related to the bromeliad macrofauna. The bromeliad tanks also represent a site for oviposition and larval development for anurans, and several orders of insects and also a drinker and foraging site for small mammals and birds (LEME; MARIGO, 1993; ROCHA et al., 2004). Moreover, some researches with microorganisms had mainly investigated bacteria and fungi (ALBUQUERQUE; LOPES, 1976; ANDRADE, 1956; HADEL; CARVALHO, 1988; PITTENDRIGH, 1948).

The composition of heterotrophic flagellates registered in bromeliad tanks was represented by species identified in other inland aquatic environments (KISS et al., 2009), and in the upper Paraná river floodplain, in samples of plankton and of organisms associated with aquatic macrophytes (CAMARGO; VELHO, 2010; PEREIRA et al., 2010). Free-living heterotrophic Euglenida, highlighted by the highest richness in the present study, were also representative in the aforementioned studies. Also, studies on marine sediment from Australia, registered 29 species from nine genera of Euglenida, with little evidence of endemism since most of the species had already been registered in other habitats (LEE, 2006).

Colorless Euglenida (heterotrophic) are usually abundant in water enriched with organic matter, where they feed on bacteria and organic solutes (SANDERS, 1991). Camargo and Velho (2010),

evaluating the patterns of composition and richness of flagellate protozoa in tropical streams, identified Euglenida as the most representative order, contributing with 65% of total of species. This author considered that the great success of these organisms in the studied streams may be assigned to the preference to environments rich in organic matter, with high concentration of ammonia and high biochemical oxygen demand.

The lower density values in the rainy period for the plants sampled in the different strata suggest a dilution effect on the community of these protozoa. This can be explained by the great input of water in the rainy period, increasing the volume of water in the bromeliads. This observation was evidenced by the inverse relationship between density and volume of water in the tank, as verified by the regression analysis. Buosi (personal communication), examining ciliate protozoa in tanks of *Aechmea distichantha* from the same rocky wall, suggested that the higher density of these organisms in samples with low volume of water can be attributed to the great development of some populations at the expense of the growth of sensitive species, once the samples with low volume of water contained few dominant species at high densities. On the other hand, other studies found distinct results with other organisms, like Chironomidae, where the density in the phytotelmata of the bromeliads increased along with the rainfall and volume of water observed in the tanks (SODRÉ et al., 2010).

Furthermore, the lack of significant difference in the density of flagellates of the different strata indicates that the distance from the river level did not have any influence on the patterns of density of these protozoa. We expected that the bromeliads at the lower stratum had a greater influence from the river on the flagellate abundance, so that this proximity could favor the dispersal of these organisms as also the development of bromeliads at the lower stratum.

The absence of relationship between the perimeter of the tank and the flagellate density diverges from observed in other study on invertebrates associated with bromeliads (ARAÚJO; COSTA, 2007). According to Richardson (1999), larger bromeliads contain a larger amount of detritus that may support a higher density of fauna (JENKINS et al., 1992; RICHARDSON, 1999; RICHARDSON et al., 2000). In the same way, Araújo and Costa (2007) affirmed that larger plants have more resources and water, allowing housing a greater quantity of organisms, pointing out that the abundance may be related to the increased area for colonization.

Nevertheless, the increased perimeter of the tank and consequently the enlarged capacity to store water in the different periods, and the increase in the number of niches, may have favored the development of predator of flagellates. In this way, most of the sampled plants with a considerable volume of water in the tanks had a high density of microcrustaceans and rotifers (Amadeo, personal communication), potential predators of flagellates. Thus, the presence of these predators may have caused the lack of relationship between the perimeter and density of flagellates.

Conclusion

Our results indicated that the distance of the different strata from the river level did not influence the pattern of flagellate abundance. Also, the results suggested that the hydrological period and the volume of water in the tanks of *Aechmea distichantha* work as determining factors on the abundance of heterotrophic flagellates in these microhabitats. In this way, the hypotheses i and ii are validated, which assumed that the major changes in the community of flagellates are temporal and determined by water availability. In relation to the sampled strata, the proximity to the river did not affect the density of flagellate, rejecting thus the third hypothesis. Although we could not detect any significant relationship between the perimeter of the tank and the abundance of the community, also rejecting the fourth hypothesis, we still suggest the accomplishment of further studies to complement the investigations and the knowledge about the community structure of heterotrophic flagellates associated with bromeliads.

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