Temporal dynamics and environmental predictors on the structure of planktonic testate amoebae community in four Neotropical floodplains

Rodrigo Leite Arrieira, Leilane Talita Fatoreto Schwind, Claudia Costa Bonecker and Fábio Amodéo Lansac-Tóha

State University of Maringa, Nupélia, Maringá, Paraná, Brazil

ABSTRACT
Understanding the environmental factors that control community structure has become a major focus of ecological research in recent decades. Here, we aimed to analyze the structure of planktonic testate amoebae community and the impact of environmental variables on the diversity of planktonic species in four floodplains of Brazil (Amazonian, Araguaia, Paraná, and Pantanal) over two hydrological periods (2011 and 2012). We hypothesized that biological diversity (richness, abundance, and diversity) of the testate amoebae community is higher during drought periods. Samples were collected from the subsurface of the limnetic region of 72 lakes in the four floodplains during both drought and flood periods in both years. We identified 109 species, belonging to 11 families. Diffugiiidae and Arcellidae exhibited higher species composition and abundance. ANOVA results showed noticeable temporal variation in testate amoebae community structure. We confirmed that the highest richness, abundance, and diversity were primarily recorded during drought periods, with significant differences being documented among floodplains and across the two hydrological periods. Multiple regression analysis also indicated that testate amoebae diversity is related to the productivity of the environments in the Amazonian, Araguaia, and Paraná floodplains. Depth of lakes and phosphorus appeared to be limiting factors in the Paraná and Araguaia floodplains, while dissolved oxygen limited species diversity in the Pantanal floodplain. Our results highlight that testate amoebae community exhibit the greatest biological diversity during drought periods, while species diversity is influenced by the environmental conditions (primarily productivity) of each floodplain.

KEYWORDS
Protist; Arcellinida; zooplankton; diversity; testate amoebae

Introduction
Identification of the processes that drive the assembly of communities is becoming one of the main objectives of ecological studies in recent decades (Rohde 2011). Increased understanding of broad-scale diversity is essential to determine the mechanisms that control diversity at different scales (Gaston & Blackburn 2000). Floodplains exhibit high species diversity (Tundisi & Matsumura-Tundisi 2008). These aquatic environments are characterized by heterogeneous river habitat microsystems that have great functional and structural complexity (Tockner et al. 2000; Ward et al. 2002). These ecosystems have seasonal effects, marked by the flood pulse, which is considered to be the
main force that influences the functioning of these ecosystems (Ward & Tockner 2001). Therefore, floodplains are excellent model systems for investigating potential factors that regulate the organization of aquatic communities.

Changes to the water level lead to alterations of several environmental variables, such as productivity variables (Arrieira et al. 2016), which, in turn, affect the aquatic communities (Junk et al. 1989; Neiff 1990). Consequently, fluctuations to the water level facilitate the development of high species diversity in floodplains (Rocha & Thomaz 2004; Lansac-Tôha et al. 2009). However, species diversity may be influenced by the environmental characteristics of each region, due to differences in environmental factors and the strength of biotic interactions, both of which influence the physiology and behavior of organisms (Gering et al. 2003). These factors determine species richness and promote the replacement of species composition (Simões et al. 2013). Thus, the environmental characteristics of floodplains may serve as a good predictor on the structure of aquatic communities including those of testate amoebae (Neiff 1996). These organisms occupy a variety of trophic roles in the food chain, ranging from decomposers to consumers (Gimenes et al. 2004; Jassey et al. 2013). Furthermore, testate amoebae quickly respond to changing environmental conditions, showing that environmental variability influences community structure (Schonborn 1992).

Although several studies have been conducted in the Brazilian floodplains, knowledge about the diversity and ecology of testate amoebae remains limited. Most studies on testate amoebae have been conducted in the Paraná River floodplain (e.g. Velho et al. 2000, 2003; Lansac-Tôha et al. 2004, 2014; Alves et al. 2010, 2012; Schwind et al. 2016). Other studies on testate amoebae diversity are still at the early stages in other Brazilian floodplains, including the Amazon, Araguaia, and Pantanal (Machado et al. 2015; Patterson et al. 2015; Vieira et al. 2015). Thus, studies investigating the community structure of testate amoebae in floodplains could enhance taxonomic knowledge and characterize the main predictors of these communities.

Here, we aimed to analyze temporal variability in the structure of planktonic testate amoebae community and identify how environmental variables influence species diversity in four floodplains of Brazil. We hypothesized that biological diversity (richness, abundance, and diversity) of the testate amoebae community is higher during periods of drought, when isolation of the lakes and greater impact of environmental conditions (e.g. primary productivity) would increase effects on the community. We also predicted that the species diversity would be influenced by the distinct environmental conditions of each floodplain.

**Methods**

**Study sites**

The study sites (Figure 1) used in this investigation are located in four major floodplains in Brazil: Amazonian (3°02′–3°34′S; 59°38′–60°50′W), Araguaia (12°49′–13°25′S; 50°28′–50°43′W), Pantanal (18°46′–19°34′S; 56°58′–57°46′W), and Paraná (22°35′–22°50′S; 53°05′–53°40′W).

The Amazonian floodplain is composed of a complex network of lakes, and covers an area of 350,000 km². It has the largest river basin (6.1 × 10⁶ km²) and greatest discharge volume in the world (6.3 × 10¹² m³/yr; Melack & Hess 2010). The Araguaia floodplain is elongated in shape, divided into three segments (Upper, Middle, and Lower), the last of which is located next to the confluence of the Tocantins River. The Middle Araguaia floodplain has a mean discharge of 6,420 m³/s. Precipitation ranges between 1,300 mm/yr in the Upper Araguaia to 2,000 mm/yr in the Lower Araguaia (Latrubesse & Stevaux 2002; Aquino et al. 2008). The Pantanal floodplain is one of the largest continuous wetlands in the world, and covers an area of 140,000 km². This ecosystem is separated into 10 different sub-regions due to edaphic, hydrological, and biogeographical variations (Hamilton et al. 1995). The meandering and anastomosing rivers, lakes, and small temporary channels connect lake waters with nearby rivers during floods (Carvalho 1986). The Paraná River is the main river of the Plata basin, which was formed by the joining of the Grande and Paranaiba rivers. It has the tenth
largest discharge volume in the world ($5 \times 10^8$ m$^3$/yr) and has a $2.8 \times 10^6$ km$^2$ drainage area. This river has a wide anastomosing main channel, numerous secondary channels, lakes, and tributary rivers, and includes the Ivinhema and Baía rivers (Agostinho et al. 2001).

**Sampling design**

Water samples were obtained from the subsurface of the limnetic region: (1) in 16 lakes located between the Solimões and Amazon Rivers, during October 2011 (drought period) and May 2012 (flood period); (2) in 18 lakes located in the Araguaia River floodplain, during November 2011 (drought period) and May 2012 (flood period); (3) in 18 lakes located in the Paraguay and Miranda River floodplains, during August 2011 (drought period) and March 2012 (flood period); and (4) in 20 lakes located in the Paraná, Baía, and Ivinhema Rivers of the Upper Paraná River floodplain, during September 2011 (drought period) and February 2012 (flood period). We collected a total of 144 samples (72 samples x 2 periods). More detailed characterization of study sites is available in Supplemental Table S1.

For each sample, 500 L water were filtered through a plankton net with 68 μm mesh, using a motorized pump. We chose the 68 μm mesh to sample the widest range of planktonic community possible and this bias was standardized for all samples. This study is part of a larger project, which involves sampling of other planktonic communities such as rotifers, cladocerans, and copepods. A sample fraction was collected from the net, transferred into polyethylene-labelled vials, and fixed.
with 4% formaldehyde solution buffered with calcium carbonate. The samples were stained with Rose Bengal. Only living testate amoebae with a cytoplasm stained by the dye were counted and identified to the species level.

Testate amoebae abundance was determined using a Sedgewick–Rafter counting chamber placed under an optical microscope at a magnification of 400× (Olympus CX31). Counting was performed using sets of three sequential sub-samples obtained by a Hensen–Stempel pipette. Samples of 7.5 ml were used to count the testate amoebae; at least 50 individuals were counted per sample. Samples were fully quantified when the minimum number of individuals per sample was not achieved (Bottrell et al. 1976). Total abundance was expressed as individuals per cubic meter (ind/m³).

We measured the environmental variables at the same sampling point from which water samples were obtained: water temperature (°C), dissolved oxygen concentration (mg/L) (portable oxygen meter, YSI 550A, YSI, Inc., http://www.ysi.com), depth of lake at the sampling site (m), water transparency (Secchi disk), turbidity (NTU), conductivity (mEq/L), total nitrogen (µg/L), ammonia (µg/L), total phosphorus (µg/L), phosphate (µg/L), pH (portable pH meter, DM-2, DigiMed, http://www.digimed.ind.br), and chlorophyll-a (µg/L). Total nitrogen was quantified by the persulfate method, which involves the oxidation of all nitrogenous compounds to nitrate-N. Samples were reduced to nitrite-N in the presence of cadmium using a flow-injection system (Mackereth et al. 1978), and the concentration of the ion was determined spectrophotometrically. Total phosphorus concentration was determined using an orthophosphate reaction and subsequent spectrophotometric measurement of absorbance at 660 nm (Golterman et al. 1978). The concentration of chlorophyll-a was quantified by extraction with 90% acetone, and absorbance was measured in a spectrophotometer at 663 nm (Golterman et al. 1978).

Data analysis

We performed a principal components analysis (PCA) to establish the differential environmental characteristics of the studied floodplains. The data used for this analysis were previously log-transformed (x + 1), with the exception of pH. The Broken-Stick model was used as the selection of the significant axes (Jackson 1993), and the significance of the axes was verified by Analysis of Variance (ANOVA, Sokal & Rohlf 1991). These statistical analyses were performed using the ‘vegan’ package version 3.2.1 (Oksanen et al. 2015) in R version 3.0.2 software (R Core Team 2015).

Species diversity was estimated using the Shannon Index (H′; Pielou 1975). Two-way ANOVA (Sokal & Rohlf 1991) was used to investigate differences in richness, diversity, and abundance of testate amoebae among floodplain lakes and across the two hydrological periods, with α = 0.05 being set as the significance threshold. The analyses considered the hydrological periods and sampled lakes, as well as the interaction between them. The Fisher’s test was used to compare significant differences a posteriori. Assumptions of normality and homoscedasticity (homogeneity of variance) were previously tested through Shapiro-Wilk and Levene’s tests, respectively.

The relationship between species diversity and the environmental variables in each floodplain was assessed by multiple regression (Sokal & Rohlf 1991). For this analysis, a stepwise backward selection procedure was performed to produce a parsimonious model. This procedure included all available factors (independent variables), and progressively excluded non-significant factors (p < 0.05), to derive the simplest model with the most representative variables. After setting the complete model, the variables without a significant relationship were removed from the model, to obtain a model that only contained statistically significant parameters. The data employed were log-transformed. Assumptions of linearity, normality, homoscedasticity, and independence were tested. These analyses were carried out using Statistica Software 7.0 (Statsoft Inc. 2005).
Results

Characterization of the environmental variables

The mean measured values of environmental variables of the floodplains during drought and flooding are shown in Table 1. The PCA results (Figure 2) indicated distinct characteristics between the environmental variables in each floodplain during both hydrological periods. The PCA 1 axis explained 36% of environmental variability, while the PCA 2 axis explained 14.8% of environmental variability, totaling 50.8% when both axes were combined during the drought period. The following associations were observed for PCA axis 1: Amazonian and Araguaia floodplains showed positive correlations with water transparency and depth; Pantanal and Paraná floodplains showed negative correlations with turbidity, chlorophyll-a, total phosphorus, and phosphate. For PCA axis 2 Paraná and Araguaia floodplains showed positive correlations with water transparency, dissolved oxygen, and chlorophyll-a, whereas Pantanal and Amazonian floodplains showed negative correlations with conductivity, water temperature, and pH (Figure 2a).

PCA 1 axis explained 46.25% of environmental variability, while PCA 2 axis explained 17.75% of environmental variability, totaling 64% when combining both axes during the flooding period. PCA axis 1 indicated that the Pantanal floodplain was positively correlated with depth and transparency, whereas the Amazonian floodplain was negatively correlated with turbidity, chlorophyll-a, temperature, and total nitrogen (Figure 2b).

Composition and structure of testate amoebae community

We identified 109 testate amoebae species belonging to 11 families (Supplemental Table S2). Diffugiiidae had the highest number of species (50), followed by Arcellidae (24), Lesquereusiidae (14), Centropyxidae (13), Hyalospheniidae (two species), Heleoperidae (one species), Phryganellidae (one species), Plagiopyxidae (one species), Trigonopyxidae (one species), Euglyphidae (one species), and Trinematidae (one species).

The testate amoebae community exhibited higher richness during drought in most of floodplains, with the highest richness being detected in the Pantanal floodplain. During flooding, the lowest testate amoeba richness was detected in the Amazonian floodplain. The ANOVA results (Figure 3) indicated significant differences for species richness interactions among the four floodplains and across the two hydrological periods ($F = 4.72; p < 0.01$).

Most floodplains had a higher abundance of organisms during the drought period, with the exception of the Paraná floodplain, where abundance was higher during the flooding period. The Amazonian floodplain had the highest abundance of all four floodplains during the drought period. During the flooding period, the lowest abundance was observed in the Amazonian floodplain. The ANOVA results (Figure 4) indicated significant results to species abundance interaction between floodplains and hydrological periods ($F = 7.70; p < 0.01$).

The most abundant species in each floodplain are shown in Figure 5, with Cucurbitella dentata f. quinquiloculata being the most abundant in Amazonian floodplain (102,838 ind/m$^3$), Cucurbitella madagascariensis in the Araguaia floodplain (1,260 ind/m$^3$), Centropyxis aculeata in the Pantanal floodplain (9,409 ind/m$^3$), and Diffugia pseudogramen in the Paraná floodplain (31,408 ind/m$^3$; Figures 5 and 6).

The testate amoebae community also showed higher species diversity in most of floodplains during the drought period, with the exception of the Pantanal floodplain. The highest species diversity was detected in the Pantanal floodplain, whereas the lowest species diversity was detected in the Araguaia floodplain. During the flooding period, the lowest species diversity was detected in the Araguaia floodplain. The ANOVA results (Figure 7) indicated significant differences in the interaction of species diversity with the four floodplains and two hydrological periods ($F = 5.11; p < 0.01$).
Table 1. Mean measured values and standard deviation (SD) of environmental variables in the floodplains during drought (2011) and flooding (2012) periods.

| Environmental variables       | Amazonian Drought | Amazonian Flooding | Araguaia Drought | Araguaia Flooding | Pantanal Drought | Pantanal Flooding | Paraná Drought | Paraná Flooding |
|-------------------------------|-------------------|--------------------|------------------|-------------------|------------------|------------------|----------------|----------------|
| Water temperature (°C)        | 32.37 ± 1.78      | 31.94 ± 0.43       | 29.64 ± 0.49     | 28.98 ± 0.79      | 28.96 ± 1.65     | 20.98 ± 0.96     | 28.13 ± 0.51   | 23.43 ± 0.48   |
| Dissolved oxygen (mg/L)       | 89.54 ± 3.05      | 23.28 ± 8.86       | 75.67 ± 1.66     | 30.67 ± 1.39      | 53.71 ± 2.32     | 38.53 ± 1.54     | 69.55 ± 1.21   | 85.99 ± 8.03   |
| Water transparency (m)        | 0.38 ± 0.19       | 1.14 ± 0.23        | 0.53 ± 0.14      | 1.27 ± 0.53       | 0.50 ± 0.09      | 1.74 ± 0.48      | 0.86 ± 0.04    | 0.79 ± 0.02    |
| Depth (m)                     | 1.22 ± 0.76       | 12.61 ± 1.13       | 2.06 ± 0.68      | 4.77 ± 0.82       | 2.06 ± 0.11      | 3.02 ± 0.88      | 2.71 ± 0.64    | 2.35 ± 0.83    |
| Turbidity (NTU)               | 72.08 ± 4.89      | 7.09 ± 0.38        | 29.18 ± 1.63     | 9.40 ± 0.50       | 19.92 ± 0.95     | 4.96 ± 0.28      | 21.17 ± 1.10   | 22.56 ± 1.63   |
| Conductivity (mEq/L)          | 59.79 ± 39.73     | 53.28 ± 8.32       | 39.13 ± 6.98     | 40.34 ± 4.40      | 81.23 ± 3.42     | 86.04 ± 2.98     | 36.78 ± 1.04   | 35.65 ± 1.53   |
| pH                            | 6.31 ± 0.89       | 7.23 ± 1.01        | 6.91 ± 0.20      | 6.39 ± 0.23       | 6.71 ± 0.40      | 7.60 ± 0.28      | 6.15 ± 0.30    | 6.94 ± 0.34    |
| Total nitrogen (µg/L)         | 2579 ± 147.0      | 698.1 ± 28.0       | 1272 ± 22.7      | 776.9 ± 40.2      | 1081 ± 58.3      | 1078 ± 32.4      | 895 ± 22.8     | 1181 ± 29.5    |
| Ammonia (µg/L)                | 36.82 ± 3.59      | 15.96 ± 6.91       | 30.20 ± 2.55     | 8.23 ± 0.48       | 33.49 ± 2.65     | 21.40 ± 5.61     | 11.92 ± 0.50   | 19.11 ± 1.60   |
| Total phosphorus (µg/L)       | 113.8 ± 5.42      | 22.5 ± 7.22        | 81.0 ± 18.7      | 23.5 ± 6.26       | 54.91 ± 1.60     | 60.28 ± 3.33     | 69.41 ± 2.28   | 46.28 ± 1.72   |
| Phosphate (µg/L)              | 14.80 ± 4.75      | 8.14 ± 0.33        | 12.49 ± 3.55     | 7.79 ± 2.80       | 17.35 ± 0.75     | 26.08 ± 1.36     | 14.05 ± 4.56   | 12.01 ± 6.53   |
| Chlorophyll-a (µg/L)          | 48.52 ± 2.95      | 2.78 ± 0.19        | 18.31 ± 7.29     | 5.91 ± 3.55       | 10.27 ± 3.52     | 4.90 ± 0.35      | 16.97 ± 0.91   | 10.70 ± 0.55   |
Figure 2. Principal components analysis ordination showing the environmental differences in each floodplain during drought (a) and flooding (b) periods. Environmental variables: Chl = chlorophyll-a; Cond = conductivity; DO = dissolved oxygen; PO₄, phosphate; TP, total phosphorus; Temp = water temperature; Transp, water transparency; Turb = turbidity.

Figure 3. Species richness recorded during the drought and flooding periods in the four floodplains. Symbol = richness average; bar = standard error; letters = represents statistically significant differences at $p < 0.05$.
Figure 4. Abundance of organisms recorded during the drought and flooding periods in the four floodplains. Symbol = abundance average; bar = standard error; letters = represents statistically significant differences at $p < 0.05$.

Figure 5. Most abundant testate amoeba species in each floodplain.

Figure 6. Light microscopy images of the most abundant species in each floodplain: (A) *Cucurbitella dentata f. quinquilobata* (Amazonian floodplain), (B) *Cucurbitella madagascariensis* (Araguaia floodplain), (C) *Centropyxis aculeata* (Pantanal floodplain), and (D) *Diffugia pseudogramen* (Paraná floodplain).
The variables related to the productivity of environments (total nitrogen, ammonia, total phosphorus, phosphate, chlorophyll- $a$, and turbidity) were selected by multiple regression analysis (Table 2) as the main predictors of testate amoebae diversity in the Amazonian, Araguaia, and Paraná floodplains. In the Amazonian and Araguaia floodplains, species diversity was negatively affected by depth and total phosphorus. In the Pantanal floodplain, species diversity was negatively affected by dissolved oxygen. The equations of the multiple regression models, as well as the percentage of explanation for data variability, are presented in Table 2.

**Discussion**

Our results indicate that changes to environmental variables were associated with how the hydrological periods influenced the environmental characteristics of each floodplain, as evidenced by the PCA results. The hydrologic regime causes major changes to the environmental variables of the aquatic environments (Thomaz et al. 2007). Hydrological dynamics directly and indirectly influence the structuring of aquatic communities, including biotic interactions and species distribution (Dunson & Travis 1991; Schwind et al. 2016). Thus, the considerable changes in environmental variables related to floodplains and the hydrological periods should have a strong influence on the testate amoebae community.

Diffugiiidae and Arcellidae had the highest species richness and abundance. These testate amoeba families are considered to be major planktonic species in floodplains (Dábès 1995; Landa & Mourgués-Schurter 2000; Velho et al. 2004; Arrieira et al. 2015a). The high abundance of Cucurbitella dentata f. quinquilobata (Amazonian floodplain), Cucurbitella magadascariensis (Araguaia floodplain), and Cucurbitella luteoventosa (Pantanal floodplain) and Cucurbitella luteoventosa (Paraná floodplain) are indicative of the presence of these species in the floodplains studied.

**Table 2.** Contents of the multiple regression model between testate amoebae diversity (response variable) and environmental variables (explanatory variables) in each floodplain; $R^2$ indicates the explanatory ability of the model; $t$ corresponds to the value of the t-test parameter; $p$ indicates the significance of parameters ($\alpha = 0.05$). Div = testate amoebae diversity; D = depth; TN = total nitrogen; PO4 = phosphate; Chl-a = chlorophyll- $a$; NH4 = ammonia; TP = total phosphorus; DO = dissolved oxygen; Turb = turbidity.

| Floodplain  | Model equation                             | $R^2$ | $t$   | $p$    |
|-------------|--------------------------------------------|-------|-------|--------|
| Amazonian   | $\log_{10}(\text{Div}) = 1.14 - 0.04 \times \log_{10}(\text{D}) + 0.01 \times \log_{10}(\text{TN}) - 0.01 \times \log_{10}(\text{TP}) + 0.05 \times \log_{10}(\text{PO4})$ | 0.37  | 5.04  | <0.01  |
| Araguaia    | $\log_{10}(\text{Div}) = 2.27 - 0.21 \times \log_{10}(\text{D}) + 0.06 \times \log_{10}(\text{Chl-a}) + 0.01 \times \log_{10}(\text{NH4}) - 0.01 \times \log_{10}(\text{TP})$ | 0.42  | 6.30  | <0.01  |
| Pantanal    | $\log_{10}(\text{Div}) = 3.03 - 0.02 \times \log_{10}(\text{DO})$ | 0.43  | 3.30  | <0.01  |
| Paraná      | $\log_{10}(\text{Div}) = 2.55 + 0.02 \times \log_{10}(\text{Turb}) + 0.01 \times \log_{10}(\text{DO}) + 0.01 \times \log_{10}(\text{TN}) + 0.06 \times \log_{10}(\text{PO4})$ | 0.58  | 3.95  | <0.01  |

**Figure 7.** Species diversity recorded during the drought and flooding periods in the four floodplains. Symbol = diversity average; bar = standard error; letters = represents statistically significant differences at $p < 0.05$. 
floodplain), and *Difflugia pseudogramen* (Paraná floodplain) might be linked to their spherical shell morphology (Lansac-Tôha et al. 2014). Similar results were obtained by Velho et al. (2003), who found that the predominance of spherical and hemispherical testate amoebae was related to their greater capacity to adapt to the limnetic region of floodplains lakes. The highest abundance of *Centropyxis aculeata*, (Pantanal floodplain) could be attributed to shell compression, which is regarded as an adaptation of these organisms. This characteristic could minimize its resistance to water as well as facilitating longer floatation in the water column (Lampert & Somer 1997).

The highest average richness, abundance, and diversity were observed in most floodplains during the drought period. During this period, floodplain lakes are shallow, and are subject to inputs of seston and nutrients to the water column (Carvalho et al. 2001; Roberto et al. 2009). This nutrient input into the aquatic environment leads to an increase in primary productivity of plankton (Bonecker et al. 2013). Consequently, these factors could promote an increase in the biological diversity of the testate amoebae community during drought.

In contrast, the lowest average biological diversity during flooding might be related to the homogenizing effect of the flood pulse, which promotes dilution of water bodies (Thomaz et al. 2007). As a result, biological diversity of the testate amoebae community declines (Costa et al. 2011). Multiple regression analyses showed that lower species diversity was negatively related to the water level of the Amazon and Araguaia floodplains, and might be due to the homogenizing effect. Consequently, an increase in the water level promoted lower testate amoebae diversity.

The multiple regression results also indicated a predominant relationship between the variables related to productivity (chlorophyll-α, total nitrogen, and total phosphorus) as the main predictors of testate amoebae diversity in the Amazonian, Araguaia, and Paraná floodplains. Previous studies on aquatic environments have suggested that ecosystem productivity is directly linked to the availability of food resources, from which protozoan communities benefit (Auer et al. 2004; Bastidas-Navarro & Modenutti 2007). Food resource availability is considered to be the predominant environmental filter in the organization of the testate amoebae community in floodplains (Arrieira et al. 2015b). Moreover, the current study verified that total phosphorus is a limiting environmental factor, based on the negative effects between species diversity and total phosphorus in the Amazonian and Araguaia floodplains. These results support those obtained by Mieczan (2012), in which phosphorus was one of the environmental factors that restricted the occurrence of these protozoa in aquatic environments.

An indirect contribution was observed for species diversity with phosphate (Amazonian and Paraná floodplains) and ammonia (Araguaia floodplain). Higher concentrations of phosphate and ammonia may favor the occurrence of bacteria in aquatic environments, because these organisms are able to absorb these soluble ions, which are excreted by zooplankton (Pinto-Coelho et al. 1997; Torres et al. 2007). As a result, the higher bacterial biomass leads to an increase in the supply of food resources, as they represent important food items in the diet of many testate amoebae (Gilbert et al. 2000; Mieczan 2009).

Another indirect relationship was observed between species diversity and dissolved oxygen in the Pantanal floodplain. Oxygen depletion in water might be related to the increased decomposition of organic matter, which is favored by the presence of large macrophyte biomass and the climatic conditions found in the lakes of the Pantanal floodplain, a process locally known as the *Dequada* process (Hamilton et al. 1995). The areas colonized by macrophytes promote a higher diversity of testate amoebae (Dabés & Velho 2001; Lansac-Tôha et al. 2009; Arrieira et al. 2015a) because macrophyte stands provide a large number of ecological niches (Souza 2005) and, therefore, offer a greater availability food resources to testate amoeba species.

Our results confirmed the hypothesis that the highest richness, abundance, and diversity of planktonic testate amoebae community predominantly occurred during drought periods. The greater abundance of organisms might also be related to the morphological adaptations of the species found in these aquatic environments.
The environmental variables related to primary productivity appeared to be important for testate amoebae diversity, with more productive environments being associated with higher species diversity due to the greater availability of food resources. Other environmental variables, such as phosphate, ammonia, and dissolved oxygen, were indirectly related, operating as environmental filters on testate amoebae diversity in floodplain lakes.

Acknowledgements

We appreciate comments by Dra. Geziele M. Alves, Dra. Juliana D. Dias, Dr Luiz F. M. Velho, Dra. Paulina M. M. Barbosa, Dr Daniel J. G. Lahr, and two anonymous reviewers who helped us to improve this paper. The authors are also grateful to National Program for Research in Biodiversity (Sisbiota Brazil), Nucleus of Research in Limnology, Ichthyology and Aquaculture (Nupélia), and State University of Maringa for the logistic and financial support. We also thank the National Council of Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES), and Araucaria Foundation for their research fellowships.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Agostinho AA, Thomaz SM, Nakatani K. 2001. A planície de inundação do alto rio Paraná: aspectos físicos, biológicos e socioeconómicos [The Upper Paraná River floodplain: physical, biological and socioeconomic]. Maringá: Eduem.
Alves GM, Velho LFM, Simões NR, Lansac-Tôha FA. 2010. Biodiversity of testate amoebae (Arcellinida and Euglyphida) in different habitats of a lake in the Upper Paraná River floodplain. Eur J Protistol. 46:310–318.
Alves GM, Velho LFM, Costa DM, Lansac-Tôha FA. 2012. Size structure in different habitats from a lake in the upper Paraná River floodplain. Eur J Protistol. 48:169–177.
Aquino S, Latrubesse EM, Souza-Filho EES. 2008. Relações entre o regime hidrológico e os ecossistemas aquáticos da planície aluvial do rio Araguaia [Relations between the hydrological regime and aquatic ecosystems of the floodplain of the Araguaia River]. Acta Sci Biol Sci. 30:361–369.
Arrieira RL, Alves GM, Schwind LTF, Lansac-Tôha FA. 2015a. Local factors affecting the testate amoeba community (Protozoa: Arcellinida; Euglyphida) in a Neotropical floodplain. J Limnol. 74:444–452.
Arrieira RL, Schwind LTF, Bonecker CC, Lansac-Tôha FA. 2015b. Use of functional diversity to assess determinant assembly processes of testate amoebae community. Aq Ecol. 49:561–571.
Arrieira RL, Schwind LTF, Bonecker CC, Lansac-Tôha FA. 2016. Environmental factors exert predominant effects on testate amoeba metacommunities during droughts in floodplains. Austral Ecol. doi:10.1111/aecc.12423.
Auer B, Elzer U, Arndt H. 2004. Comparison of pelagic food webs in lakes along a trophic gradient and with seasonal aspects: influence of resource and predation. J Plankton Res. 26:697–708.
Bastidas-Navarro M, Modenutti B. 2007. Efecto de la estructuración por macrófitas y por recursos alimentarios en la distribución horizontal de tecamebas y rotíferos en un lago andino patagónico [Structuring effect of macrophytes and food resources in the horizontal distribution of testate amoebae and rotifers in a Patagonian Andean lake]. Rev Chil Hist Nat. 80:345–362.
Bonecker CC, Simões NR, Minte-Vera CV, Lansac-Tôha FA, Velho LFM, Agostinho AA. 2013. Temporal changes in zooplankton species diversity in environmental changes in alluvial valley. Limnológica. 43:114–121.
Bottrell HH, Duncan A, Gliwicz ZM, Gryjek E, Hezig A, Hillbricht-Ilkowska A, Kurawa H, Larsson P, Weglenska T. 1976. A review of some problems in zooplankton production studies. Norw J Zool. 24:419–456.
Carvalho NO. 1986. Hidrologia da Bacia do Alto Paraguai [Hydrology of the Upper Paraguay River Basin]. In: Symposium on natural and socioeconomic resources of the Pantanal (UFMS, Corumbá, 1984), Brasília: Embrapa; p. 43–49.
Carvalho P, Bini LM, Thomaz SM, Oliveira LG, Robertson B, Tavechio WLG, Darwish AJ. 2001. Comparative limnology of South-American lakes and lagoons. Acta Sci Biol Sci. 23:265–273.
Costa DM, Alves GM, Velho LFM, Lansac-Tôha FA. 2011. Species richness of testate amoebae in different environments from the upper Paraná river floodplain (PR/MS). Acta Sci Biol Sci. 33:263–270.
Dabés MBGS. 1995. Composição e descrição do zooplâncton de 5 (cinco) lagos marginais do rio São Francisco, Pirapora, Três Marias, Minas Gerais-Brasil [Zooplankton composition and description of five marginal lakes of the São Francisco River, Pirapora, Três Marias, Minas Gerais, Brazil]. Rev Bras Biol. 55:831–845.
Dabés MBGS, Velho LFM. 2001. Assemblage of testate amoebae (Protozoa, Rhizopoda) associated to aquatic macrophytes stands in a marginal lake of the São Francisco river floodplain, Brazil. Acta Sci Biol Sci. 23:299–304.
Roberto MC, NF Santana, Thomaz SM. 2009. Limnology in the Upper Paraná River floodplain: large-scale spatial and temporal patterns, and the influence of reservoirs. Braz J Biol. 69:717–725.

Rocha RRA, Thomaz SM. 2004. Variação temporal de fatores limnológicos em ambientes da planície de inundação do alto rio Paraná (PR/MS-Brasil) [Temporal variation of limnological factors in the Upper Paraná River floodplain habitats]. Acta Sci Biol Sci. 26:261–271.

Rohde K. 2011. Latitudinal gradients in species diversity: why are there so many species in the tropics? [Internet]. [cited 2016 Feb 24]. Available from: http://krohde.wordpress.com/article/latitudinal-gradients-in-species/

Schonborn W. 1992. The role of protozoan communities in freshwater and soil ecosystems. Acta Protozool. 31:11–18.

Schwind LTF, Arrieira RL, Dias JD, Simões NR, Bonecker CC, Lansac-Tôha FA. 2016. The structure of planktonic communities of testate amoebae (Arcellinida and Euglyphida) in three environments of the Upper Paraná River basin, Brazil. J Limnol. 75:78–89.

Simões NR, Dias JD, Leal CM, Braghin LSM, Lansac-Tôha FA, Bonecker CC. 2013. Floods control the influence of environmental gradients on the diversity of zooplankton communities in a neotropical floodplain. Aquat Sci. 75:607–617.

Sokal RR, Rolhff FJ. 1991. Biometry: the principles and practice of statistics in biological research. New York: WH. Freeman and Company.

Souza MBG. 2005. Tecamebas (Protozoa Rhizopoda) associadas às macrófitas aquáticas da bacia do rio Jequitinhonha: Parque Estadual do Rio Preto e Parque Estadual do Grão Mogol, MG [Testate amoebae (Protozoa Rhizopoda) associated with macrophytes of the Jequitinhonha River Basin: State Park of Preto River and State Park of Grão Mogol, MG, Brazil]. Rev Unimontes Cient. 2:129–142.

Statsoft Inc. 2005. Statistica for Windows (data analysis software system), version 7.1. Tulsa: Statsoft Inc.

Thomaz SM, Bini LM, Bozelli RL. 2007. Floods increase similarity among aquatic habitats in river-floodplain systems. Hydrobiologia. 579:1–13.

Tockner K, Malard F, Ward JV. 2000. An extension of the flood pulse concept. Hydrol Process. 14:2861–2883.

Torres IC, Resck RP, Pinto-Coelho RM. 2007. Mass balance estimation of nitrogen, carbon, phosphorus and total suspended solids in the urban eutrophic, Pampulha reservoir, Brazil. Acta Limnol Bras. 19:79–91.

Tundisi JG, Matsumura-Tundisi T. 2008. Limnologia [Limnology]. São Paulo: Oficina de Textos.

Velho LFM, Lansac-Tôha FA, Bonecker CC. 2000. On the occurrence of testate amoebae (Protozoa, Rhizopoda) in Brazilian inland waters. II. Families Centropyxidae, Trigonopyxidae and Plagiopyxidae. Acta Sci Biol Sci. 22:365–374.

Velho LFM, Lansac-Tôha FA, Bini LM 2003. Influence of environmental heterogeneity on the structure of testate amoebae (Protozoa, Rhizopoda) assemblages in the plankton of the upper Paraná river floodplain, Brazil. Int Rev Hydrobiol. 88:154–166.

Velho LFM, Bini LM, Lansac-Tôha FA. 2004. Testate amoeba (Rhizopoda) diversity in plankton of the Upper Paraná River floodplain, Brazil. Hydrobiologia. 523:103–111.

Vieira LCG, Padial AA, Velho LFM, Carvalho P, Bini LM. 2015. Concordance among zooplankton groups in a near-pristine floodplain system. Ecol Indic. 58:374–381.

Ward JV, Tockner K. 2001. Biodiversity: towards a unifying theme for river ecology. Freshw Biol. 46:807–819.

Ward JV, Tockner K, Arscott DB, Clare C. 2002. Riverine landscape diversity. Freshw Biol. 47:517–539.