



Structure and ecological aspects of zooplankton (Testate amoebae, Rotifera, Cladocera and Copepoda) in highland streams in southern Brazil

Estrutura e aspectos ecológicos do zooplâncton (Testate amoebae, Rotifera, Cladocera e Copepoda) em rios de montanha no sul do Brasil

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Abstract: Aim: This study aimed to characterize the zooplankton structure in highland streams in southern Brazil, Pelotas River sub-basin, as well as the respective relationships with the environmental conditions in the different seasons. **Methods:** Zooplankton was collected using a plankton net (68 µm mesh) and concentrated into a volume of 50 ml for later analysis in the laboratory. Sampling occurred during the winter (August 2013), spring (November 2013), summer (February 2014) and autumn (May 2014). **Results:** Rotifers and testate amoebae were the predominant groups. Mean abundance, diversity and evenness varied considerably between the different seasons. The variability of zooplankton was correlated to environmental conditions (conductivity, suspended solids, pH, temperature and dissolved oxygen). **Conclusions:** The seasonal variation of the zooplankton community made it possible to elucidate some questions involving the ecology of these organisms in highland streams in southern Brazil. These unique ecosystems warrant further research and monitoring efforts, including studies of their hydrological patterns, biological diversity and adaptive mechanisms of the zooplankton community.

Keywords: driving variables; mountain; Pelotas River sub-basin; potamoplankton; taxonomic composition.

Resumo: Objetivo: Este estudo teve como objetivo caracterizar a estrutura do zooplâncton em rios de montanha no sul do Brasil, sub-bacia do rio Pelotas, bem como, as respectivas relações com as condições ambientais nas diferentes estações do ano. **Métodos:** O zooplâncton foi coletado usando uma rede de plâncton (malha de 68 µm) e concentrado em um volume de 50 ml para posteriormente ser analisado em laboratório. As amostragens ocorreram durante o inverno (agosto de 2013), primavera (novembro de 2013), verão (fevereiro de 2014) e outono (maio de 2014). **Resultados:** Os rotíferos e as amebas testáceas foram os grupos predominantes. Os valores médios de abundância, diversidade e equitabilidade variaram consideravelmente entre as diferentes estações do ano. A variabilidade do zooplâncton foi correlacionada com as condições ambientais (condutividade, sólidos em suspensão, pH, temperatura e oxigênio dissolvido). **Conclusão:** A variação sazonal da comunidade zooplanctônica possibilitou elucidar algumas questões que envolvem a ecologia desses organismos em rios de montanhas no sul do Brasil. Esses ecossistemas únicos merecem maiores esforços de pesquisa e monitoramento, incluindo estudos dos seus padrões hidrológicos, diversidade biológica e mecanismos adaptativos da comunidade zooplanctônica.

Palavras-chave: variáveis condutoras; montanha; sub-bacia do rio Pelotas; potamoplâncton; composição taxonômica.



1. Introduction

The highlands of southern Brazil contain remarkable freshwater resources, whose quality, chiefly in the headwater areas, is mostly acceptable (Buckup et al., 2007). These areas are of great biological importance (Winckler-Sosinski et al., 2009; Mazzoni et al., 2014; Braun et al., 2014). Several drainage basins have their headwaters in the highlands of southern Brazil; the largest is the Pelotas River, a tributary of the Uruguay River.

Water bodies of mountains are physically rigorous and ecologically demanding habitats for planktonic organisms, mostly because of a low buffering capacity and low nutrient levels (Psenner, 2002; Williamson et al., 2008). In addition, the high elevation leads to increased exposure to ultraviolet radiation as well as a shortened growing season that aggravates plankton populations due to both temperature and light limitations (Williamson et al., 2008). The extreme environmental conditions of these ecosystems lead to a relatively simple food web, which reacts more quickly and more sensitively to environmental changes than other environments, making mountain environments one of the most sensitive indicators of environmental change (Sommaruga, 2001; Kumar et al., 2012).

Ecological research on Brazilian highland water bodies is rare. Moreover, scientific knowledge of zooplankton diversity in these environments in southern Brazil is almost nonexistent. This is due to the exceptional topography, which restricts these montane ecosystems to regions which are difficult to access, and also because studies of these ecosystems are still in the early stages or have received little attention (Santos-Wisniewski et al., 2002; Eskinazi-Sant'Anna et al., 2011; Moreira et al., 2016). Consequently, determining the factors that control the abundance, diversity and distribution of zooplankton can be an important step to understand the ecology of rivers in these regions.

In lotic environments, zooplankton is found at low densities and is dominated by protozoans, small cladocerans, rotifers and copepoda nauplii (Paggi & Paggi, 2007; Perbiche-Neves et al., 2012). The dynamics of river zooplankton are often considered to be controlled by transport of populations (Perbiche-Neves et al., 2012; Lucena et al., 2015; Picapedra et al., 2017), although the factors regulating their spatial occurrence are not completely understood. Factors related to the transport and locomotion of plankton (hydrological factors: discharge, residence time) should be taken into consideration since plankton

swimming capacity is inefficient and these factors act directly on development and reproduction (Hynes, 1970). In addition, there is also the influence of physical (light, temperature), chemical (nutrient concentration) and biotic factors (competition and predation) (Reynolds, 1997; Viroux, 2002; Thorp & Mantovani, 2005; Williamson et al., 2008), while seasonal variation is dependent on the hydrological regime (precipitation) (Matsumura-Tundisi et al., 2015). Therefore, this study aimed to characterize the zooplankton structure (abundance, species richness, diversity and evenness) in highland streams of the Pelotas River sub-basin, as well as the respective relationships with the environmental conditions during the different seasons.

2. Material and Methods

2.1. Study area

Our study area is located on the border between the states of Santa Catarina and Rio Grande do Sul, Southern Brazil (Figure 1). The climate is temperate (*cfb*) type, with annual average temperature around 14-16 °C, rainfall ranges from 1500 - 2100 mm and there is no dry period, frosts are frequent during the year and there is sporadic snow (Brasil, 2006; Buckup et al., 2007). The area has an average height of around of 830-1,350 m, with many lotic ecosystems, forming narrow meander valleys, with a predominance of ritral zone in watercourses. The geological substrate is of basaltic origin (Hasenack et al., 2009) and vegetation comprises Mixed Rain Forest, interspersed with Deciduous Forest and grassland. Currently, the landscape is altered due to the expansion of farming and forestry of exotic species *Pinus eliotii* Engelm, *Pinus taeda* L. and *Eucalyptus* spp. (Buckup et al., 2007).

This study was conducted in Pelotas River and in six of its tributaries (Contas, Loucos, Invernadinha, Silveira, Inferninho and Cerquinha rivers), belonging to the Uruguay basin. This basin is composed of several sub-basins, among these, the sub-basin of the Pelotas River. The Pelotas River is a tributary of the Uruguay River. It starts at approximately 1,822 m altitude in the São Joaquim National Park region (created by federal decree n° 50,922 of July 1961), is 437 km long and has a drainage area of 13,222 km² (Brasil, 2006).

2.2. Sampling and analysis

Zooplankton samples were taken in four quarterly collections: winter (August 2013), spring (November 2013), summer (February 2014) and

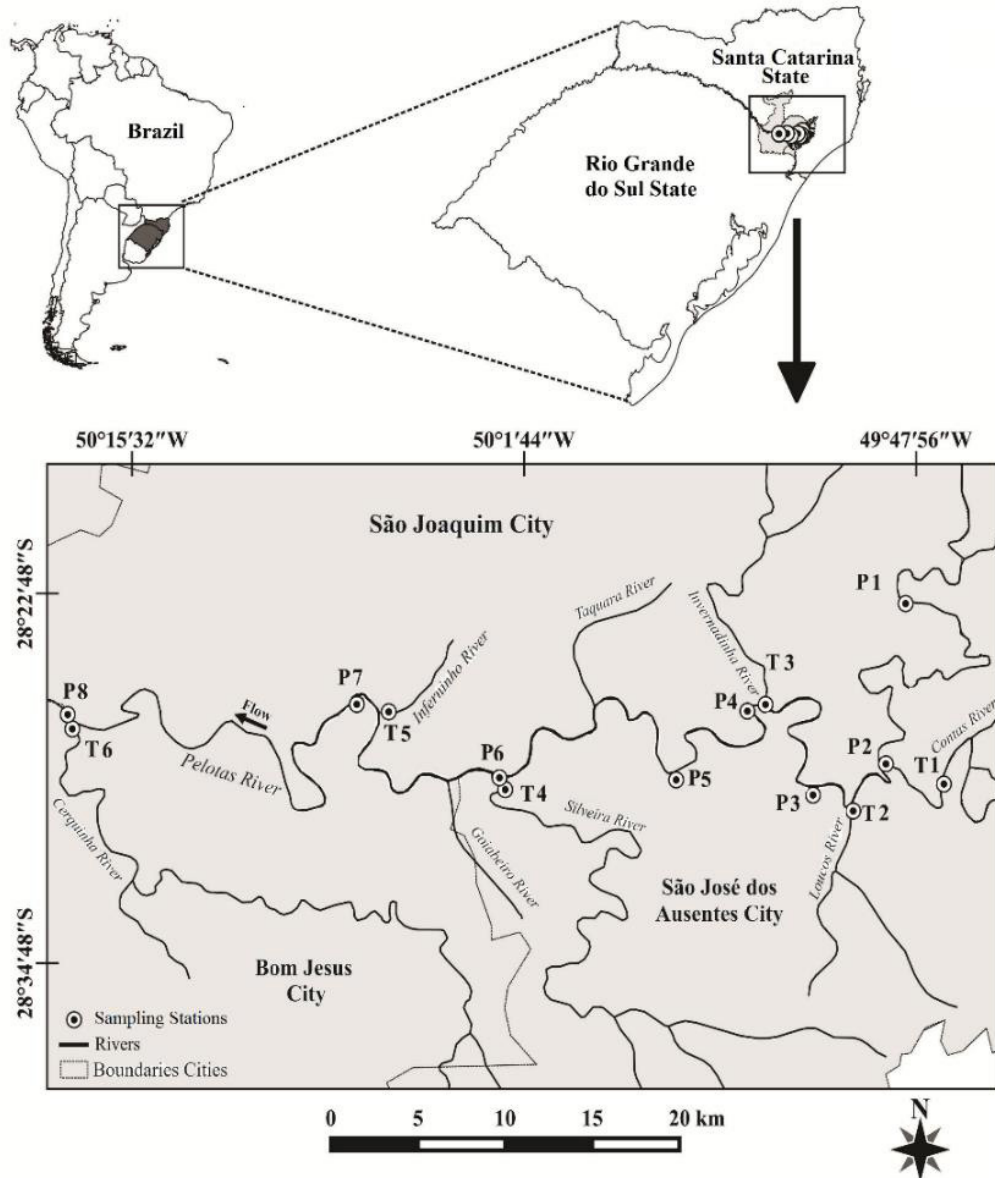


Figure 1. Study area and location of sampling sites in the Pelotas River sub-basin, states of Santa Catarina and Rio Grande do Sul.

autumn (May 2014). We sampled 8 sites located in the bed of the Pelotas River and at a site located in the mouth of each one of its tributaries: Contas, Loucos, Invernadinha, Silveira, Inferninho and Cerquinha rivers, totaling 14 sampling sites (Figure 1). Some features of the streams are shown in Table 1.

Zooplankton was collected in the middle of the rivers using a suction pump to draw 600 liters of water per sample, which were filtered through a conical-cylindrical net (68 μm). The collected material was placed in a

polyethylene bottle (500 ml), labeled and fixed in 4% formaldehyde solution buffered with sodium borate (Na_3BO_3). The following environmental variables were measured: water current velocity ($\text{m}\cdot\text{s}^{-1}$; mechanical flow meter), water transparency (cm^{-1} ; Secchi disk), water temperature ($^{\circ}\text{C}$; mercury bulb thermometer), dissolved oxygen ($\text{mg}\cdot\text{L}^{-1}$; portable oxymeter), electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$; portable conductivity meter), pH (portable pH meter), turbidity (NTU; portable turbidimeter), suspended solids ($\text{mg}\cdot\text{L}^{-1}$; Apha, 2005) and chlorophyll-*a* ($\mu\text{g}\cdot\text{L}^{-1}$; Golterman et al., 1978).

Table 1. Features of the sampled sites in seven streams of the Pelotas River sub-basin, states of Santa Catarina and Rio Grande do Sul.

| Sites | Stream | Longitude (W) | Latitude (S) | Altitude (m) | Width (m) | Depth (m) |
|-------|--------------|---------------|--------------|--------------|-----------|-----------|
| P1 | Pelotas | 49°48'19" | 28°23'07" | 1,085.8 | 57.4 | 3.05 |
| P2 | Pelotas | 49°49'02" | 28°28'11" | 971.4 | 73.7 | 1.5 |
| P3 | Pelotas | 49°51'23" | 28°29'18" | 958.2 | 90.3 | 4.1 |
| P4 | Pelotas | 49°53'54" | 28°26'38" | 935.1 | 58.1 | 3.45 |
| P5 | Pelotas | 49°56'38" | 28°28'57" | 908.9 | 80.1 | 2.6 |
| P6 | Pelotas | 50°02'13" | 28°28'48" | 869.8 | 80.6 | 1.75 |
| P7 | Pelotas | 50°07'49" | 28°25'54" | 837.5 | 89.6 | 1.45 |
| P8 | Pelotas | 50°17'37" | 28°27'01" | 773.6 | 82.8 | 3.95 |
| T1 | Contas | 49°47'10" | 28°29'17" | 1,003 | 64.5 | 3.35 |
| T2 | Loucos | 49°50'17" | 28°29'50" | 962.4 | 16.5 | 0.5 |
| T3 | Invernadinha | 49°53'23" | 28°26'24" | 927.4 | 21.8 | 2.05 |
| T4 | Silveira | 50°02'11" | 28°28'48" | 865.8 | 19.3 | 2.85 |
| T5 | Inferninho | 50°06'34" | 28°26'37" | 844 | 16.9 | 0.5 |
| T6 | Cerquinha | 50°17'37" | 28°27'07" | 777.4 | 26.6 | 0.9 |

In the laboratory, samples were concentrated to 50 ml and 10 sub-samples were taken with a Hensen-Stempel pipette (2.5 ml), evaluating 25 ml from each sample in total, and counted in a Sedgewick-Rafter chamber, using an Olympus CX 41 microscope, at a 10× to 100× magnification range. For the analysis of testate amoebae, only organisms with protoplasm stained with Rose Bengal were identified, assuming that the organisms were alive when the samples were taken. The total density was expressed in terms of individuals per m⁻³.

The identification was based on the following literature: Vucetich (1973), Koste (1978), Reid (1985), Segers (1995), Elmoor-Loureiro (1997; 2010) and Souza (2008).

2.3. Data processing

We carried out a Principal Component Analysis (PCA) to determine the different environmental characteristics between the seasons. The data used for this analysis were previously log-transformed ($x + 1$), except for pH. The selection of the significant axes used the Broken-Stick criteria (Jackson, 1993). The axes significance was verified by Analysis of Variance (ANOVA) (Sokal & Rohlf, 1991).

Species richness (number of species), Shannon diversity index (H') and evenness (E) were calculated from the sample data for each site (Magurran, 1988). To investigate the differences in the zooplankton structure between seasons, the abundance, richness, diversity and evenness of zooplankton communities were evaluated by ANOVA, with level of significance of $p < 0.05$. Normality and homoscedasticity (homogeneity of variance) were previously checked by the Shapiro-Wilk and Levene tests, respectively.

The relationship between environmental variables and zooplankton species was explored by means of a Canonical Correspondence Analysis (CCA) (Ter Braak, 1986). For this analysis the most common species ($\geq 25\%$ occurrence) in the samples were used. The data used for this analysis were previously log-transformed ($x + 1$). The statistical significance of eigenvalues and species-environment correlations for the axes generated by the CCA were tested with the Monte Carlo method based on 999 permutations (Legendre et al., 2011), with level of significance of $p < 0.05$.

Statistical analyses were performed in the statistical environment R version 3.0.2 (R Development Core Team, 2011) using the Vegan R package version 2.0-6 (Oksanen et al., 2012).

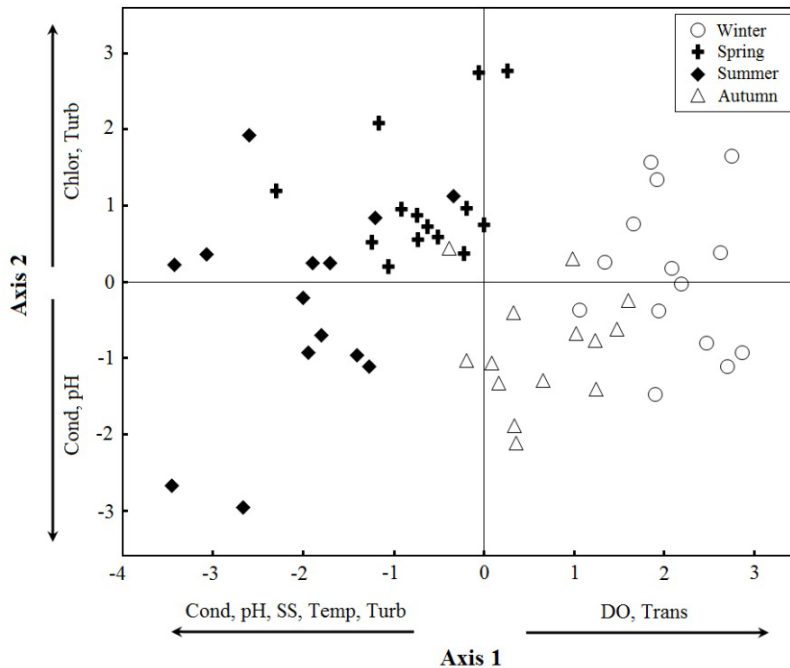
3. Results

3.1. Environmental variables

The environmental variables changed with differential responses for the different seasons (Table 2). The first two PCA axes were significant ($p < 0.05$) and explained 47.7% (axis 1, 31.3%; axis 2, 16.4%) of the environmental variability. The following environmental associations were shown: Axis 1, positive correlation to dissolved oxygen and water transparency in the winter and autumn; negative correlation to electrical conductivity, pH, suspended solids, water temperature and turbidity in the spring and summer. Axis 2, positive correlation to chlorophyll-*a* and turbidity in the spring and summer; negative correlation to electrical conductivity and pH in the winter and autumn (Figure 2).

Table 2. Mean values (\pm standard deviation) of environmental variables during the seasons in the Pelotas River sub-basin.

| Environmental variables | Winter | Spring | Summer | Autumn |
|---|--------------------|--------------------|---------------------|--------------------|
| Chlorophyll-a ($\mu\text{g.L}^{-1}$) | 0.4 (± 1) | 0.7 (± 0.7) | 0.6 (± 0.8) | 0.4 (± 0.5) |
| Dissolved oxygen (mg.L^{-1}) | 10.2 (± 0.3) | 7.5 (± 0.5) | 7.7 (± 0.7) | 8.8 (± 0.6) |
| Electrical conductivity ($\mu\text{S.cm}^{-1}$) | 19.6 (± 3.8) | 22.4 (± 4.8) | 28.4 (± 11.4) | 23.3 (± 4.7) |
| pH | 6.2 (± 0.1) | 6.3 (± 0.1) | 6.6 (± 0.3) | 6.7 (± 0.2) |
| Suspended solids (mg.L^{-1}) | 6.1 (± 9.9) | 4.5 (± 2.2) | 79.4 (± 38.1) | 4.6 (± 2.8) |
| Turbidity (NTU) | 6 (± 2.2) | 8.2 (± 2.7) | 8 (± 4.5) | 5.6 (± 2.6) |
| Water temperature ($^{\circ}\text{C}$) | 11.9 (± 0.7) | 23.9 (± 1.3) | 25.2 (± 2) | 13.8 (± 1.3) |
| Water transparency (m^{-1}) | 1.7 (± 0.8) | 1.3 (± 0.7) | 1.2 (± 0.7) | 1.7 (± 0.8) |
| Water velocity (m.s^{-1}) | 0.8 (± 0.4) | 0.6 (± 0.3) | 0.7 (± 0.3) | 0.8 (± 0.5) |

**Figure 2.** Principal Component Analysis (PCA) ordination showing the environmental differences for each season in the Pelotas River sub-basin (abbreviations: Trans, water transparency; DO, dissolved oxygen; SS, Suspended solids; Chlor, chlorophyll-*a*; Cond, electrical conductivity; Temp, water temperature; Turb, turbidity).

3.2. Zooplankton community

The zooplankton community was composed of 97 species. Rotifera were the most species-rich group (46 species), followed by testate amoebae (38 species), Cladocera (12 species) and Copepoda (1 species). Testate amoebae were represented by 8 families: Diffugiidae was the richest with 15 species. The rotifers were distributed in 14 families, of which Brachionidae (13 species) and Lecanidae (8 species) were species-richest. Among microcrustaceans, cladocerans were represented by 4 families, Chydoridae was the richest family, with 7 species, and copepods were represented by only one species of the family Cyclopidae. The most frequent taxa of zooplankton ($\geq 25\%$ occurrence) were: Testate amoebae, *Arcella dentata*, *A. hemisphaerica*

gibba, *A. megastoma*, *A. vulgaris*, *Centropyxis aculeata*, *C. constricta*, *C. eornis*, *Diffugia cylindrus* and *Diffugia* sp.; Rotifera, *Kellicottia bostoniensis*, *Keratella cochlearis*, *Platyias quadricornis*, *Euchlanis dilatata*, *Lecane bulla*, *L. lunaris*, *Lepadella ovalis*, *L. patella*, *Cephalodella* sp., *Dissotrocha* sp., *Trichocerca similis*, *Trichotriia tetractis* and *Filinia opoliensis*; Cladocera, *Bosmina hagmanni* and *Alona glabra*; Copepoda, young stages (nauplii and copepodids) (Table 3).

The mean abundance values of the zooplankton community changed during the seasons, especially testate amoebae ($F_{(3;52)} = 3.74$; $p = 0.016$), rotifers ($F_{(3;52)} = 4.06$; $p = 0.011$) and copepods ($F_{(3;52)} = 4.54$; $p = 0.006$). Testate amoebae and rotifers contributed the most to the total abundance of the zooplankton

Table 3. Composition, frequency of occurrence (FO %) and mean abundance (ind. m⁻³) of zooplankton during the seasons in the Pelotas River sub-basin, with abbreviation (Ab.) of taxa used in CCA.

| Zooplankton | FO | Winter | Spring | Summer | Autumn | Ab. |
|--|------|--------|--------|--------|--------|-----|
| Testate amoebae | | | | | | |
| ARCELLIDAE | | | | | | |
| <i>Arcella costata</i> Ehrenberg, 1847 | 17.8 | 2.1 | 1.4 | 1.5 | 0.9 | |
| <i>Arcella dentata</i> Ehrenberg, 1830 | 41.1 | - | 12.4 | 7.4 | 2.4 | Ade |
| <i>Arcella gibbosa</i> Pénard, 1890 | 3.6 | - | 0.5 | - | - | |
| <i>Arcella hemisphaerica gibba</i> Deflandre, 1928 | 41.1 | - | 27.3 | 9.5 | 1.8 | Ahe |
| <i>Arcella megastoma</i> Pénard, 1902 | 67.9 | 0.9 | 26.7 | 62.8 | 8.3 | Ame |
| <i>Arcella vulgaris</i> Ehrenberg, 1830 | 96.4 | 11.4 | 73.5 | 57.1 | 39.9 | Avu |
| CENTROPYXIDAE | | | | | | |
| <i>Centropyxis aculeata</i> (Ehrenberg, 1838) | 92.9 | 16 | 82.2 | 106 | 231.8 | Cac |
| <i>Centropyxis cassis</i> (Wallich, 1864) | 3.6 | 1.9 | - | - | - | |
| <i>Centropyxis constricta</i> Ehrenberg, 1841 | 60.7 | 14.1 | 10.5 | 10.4 | 17.6 | Cco |
| <i>Centropyxis discooides</i> Pénard, 1902 | 3.6 | - | 1.4 | - | - | |
| <i>Centropyxis ecornis</i> (Ehrenberg, 1841) | 46.4 | 15.7 | 5.6 | 15.5 | 2.1 | Cec |
| <i>Centropyxis gibba</i> (Deflandre, 1929) | 16.1 | 6.4 | - | - | - | |
| <i>Centropyxis marsupiformis</i> Deflandre, 1929 | 3.6 | - | - | - | 2.1 | |
| DIFFLUGIIDAE | | | | | | |
| <i>Cucurbitella dentata quinquelobata</i> Gauthier-Lièvre & Thomas, 1960 | 5.4 | 0.7 | - | - | - | |
| <i>Diffugia achlora</i> Pénard, 1902 | 1.8 | - | 0.5 | - | - | |
| <i>Diffugia acuminata</i> Ehrenberg, 1838 | 12.5 | 2.9 | 5.1 | 1.5 | 1.2 | |
| <i>Diffugia capreolata</i> Pénard, 1902 | 3.6 | - | 0.3 | 0.3 | - | |
| <i>Diffugia corona</i> Wallich, 1864 | 8.9 | - | 3.2 | 1.2 | - | |
| <i>Diffugia cylindrus</i> (Thomas, 1953) | 46.4 | 1.4 | 15.4 | 12.2 | 16.7 | Dcy |
| <i>Diffugia distenda</i> (Pénard, 1899) | 1.8 | - | 0.3 | - | - | |
| <i>Diffugia elegans</i> Pénard, 1890 | 1.8 | 0.2 | - | - | - | |
| <i>Diffugia globulosa</i> (Dujardin, 1837) | 1.8 | - | - | 0.3 | - | |
| <i>Diffugia gramen</i> Pénard, 1902 | 19.6 | 1.2 | 3.7 | 0.6 | - | |
| <i>Diffugia minuta</i> Rampi, 1950 | 3.6 | 0.5 | - | - | - | |
| <i>Diffugia oblonga</i> Ehrenberg, 1838 | 12.5 | - | 0.3 | 2.1 | 2.1 | |
| <i>Diffugia pyriformis</i> Perty, 1849 | 16.1 | 4.8 | 2.2 | - | - | |
| <i>Diffugia</i> sp. | 26.8 | 3.1 | 8.8 | 0.3 | 1.5 | Dsp |
| <i>Diffugia urceolata</i> Carter, 1864 | 1.8 | - | - | 0.3 | - | |
| EUGLYPHIDAE | | | | | | |
| <i>Euglypha acanthophora</i> (Ehrenberg, 1841) | 3.6 | - | 0.2 | 0.3 | - | |
| <i>Euglypha denticulata</i> Brown, 1912 | 8.9 | - | 0.2 | 0.6 | 0.6 | |
| <i>Euglypha filifera</i> Pénard, 1890 | 3.6 | 0.2 | - | - | 0.3 | |
| <i>Euglypha laevis</i> (Perty, 1849) | 5.4 | 0.7 | 0.2 | - | - | |
| LESQUEREUSIDAE | | | | | | |
| <i>Lesquereusia spiralis</i> (Ehrenberg, 1840) | 48.2 | 6 | 4.9 | 3.3 | 6.8 | Lsp |
| PARAQUADRULIDAE | | | | | | |
| <i>Quadrulella symmetrica tubulata</i> (Gauthier-Lievre, 1953) | 1.8 | 0.2 | - | - | - | |
| TRIGONOPYXIDAE | | | | | | |
| <i>Cyclopyxis impressa</i> (Daday, 1905) | 12.5 | - | - | 2.1 | 3.3 | |
| <i>Cyclopyxis kahli</i> (Deflandre, 1929) | 8.9 | - | 4.1 | 0.6 | - | |
| <i>Cyclopyxis</i> sp. | 3.6 | 0.5 | - | - | - | |
| TRINEMATIDAE | | | | | | |
| <i>Trinema lineare</i> Pénard, 1890 | 5.4 | - | 0.2 | 0.6 | - | |
| Rotifera | | | | | | |
| BRACHIONIDAE | | | | | | |
| <i>Anuraeopsis navicula</i> Roussetlet, 1910 | 5.4 | 0.7 | - | - | - | |
| <i>Brachionus calyciflorus</i> Pallas, 1766 | 1.8 | 0.2 | - | - | - | |
| <i>Brachionus caudatus</i> Barrois & Dadai, 1894 | 1.8 | - | - | 0.3 | - | |
| <i>Brachionus dolabratus</i> Haring, 1914 | 5.4 | 0.5 | - | - | 1.5 | |

Table 3. Continued...

| Zooplankton | FO | Winter | Spring | Summer | Autumn | Ab. |
|---|------|--------|--------|--------|--------|-----|
| <i>Brachionus falcatus</i> Zacharias, 1898 | 1.8 | 0.7 | - | - | - | |
| <i>Brachionus forficula</i> Wierzejski, 1891 | 8.9 | - | - | 1.8 | 0.6 | |
| <i>Brachionus leydigi</i> Cohn, 1862 | 1.8 | - | - | 0.3 | - | |
| <i>Kellicottia bostoniensis</i> (Rousselet, 1908) | 28.6 | 17.4 | 1.9 | 4.2 | 0.6 | Kbo |
| <i>Keratella americana</i> Carlin, 1943 | 5.4 | 1.2 | 0.3 | - | - | |
| <i>Keratella cochlearis</i> (Gosse, 1851) | 25 | 4.5 | 2.7 | - | 0.9 | Kch |
| <i>Keratella lenzi</i> Hauer, 1953 | 3.6 | - | 0.7 | - | - | |
| <i>Keratella tropica</i> (Apstein, 1907) | 7.1 | 0.2 | 0.3 | - | 0.9 | |
| <i>Platyias quadricornis</i> (Ehrenberg, 1832) | 44.6 | 0.2 | 9.2 | 18.5 | 2.1 | Pqu |
| EUCHLANIDAE | | | | | | |
| <i>Dipleuchlanis propatula</i> (Gosse, 1886) | 10.7 | - | - | 6.6 | 0.6 | |
| <i>Euchlanis dilatata</i> Ehrenberg, 1832 | 44.6 | 2.1 | 3.3 | 20.5 | 5.1 | Edi |
| FLOSCULARIIDAE | | | | | | |
| <i>Ptygura</i> sp. | 3.6 | 0.2 | 0.3 | - | - | |
| GASTROPODIDAE | | | | | | |
| <i>Ascomorpha ecaudis</i> Perty, 1850 | 1.8 | 0.2 | - | - | - | |
| HEXARTHRIIDAE | | | | | | |
| <i>Hexarthra mira</i> (Hudson, 1871) | 1.8 | - | - | - | 0.3 | |
| LECANIDAE | | | | | | |
| <i>Lecane bulla</i> (Gosse, 1851) | 71.4 | 0.2 | 43.9 | 143.5 | 31.6 | Lbu |
| <i>Lecane ludwigi</i> (Eckstein, 1883) | 12.5 | 0.7 | 0.2 | 0.6 | 0.3 | |
| <i>Lecane luna</i> (Müller, 1776) | 8.9 | 0.2 | - | 2.4 | 0.3 | |
| <i>Lecane lunaris</i> (Ehrenberg, 1832) | 85.7 | 2.4 | 68.6 | 195.8 | 84.8 | Llu |
| <i>Lecane quadridentata</i> (Ehrenberg, 1830) | 3.6 | 0.5 | - | - | - | |
| <i>Lecane signifera</i> (Jennings, 1896) | 5.4 | - | 0.3 | 0.6 | - | |
| <i>Lecane</i> sp. | 1.8 | - | 0.2 | - | - | |
| <i>Lecane stenroosi</i> (Meissner, 1908) | 1.8 | - | 1.2 | - | - | |
| LEPADELLIDAE | | | | | | |
| <i>Colurella</i> sp. | 1.8 | - | - | 0.3 | - | |
| <i>Lepadella ovalis</i> (Müller, 1786) | 71.4 | 35.5 | 21.8 | 10.7 | 2.4 | Lov |
| <i>Lepadella patella</i> (Müller, 1773) | 51.8 | 3.3 | - | 58.3 | 30.1 | Lpa |
| <i>Lepadella</i> sp. | 1.8 | 0.2 | - | - | - | |
| NOTOMMATIDAE | | | | | | |
| <i>Cephalodella</i> sp. | 58.9 | - | 12.3 | 19.1 | 6.3 | Csp |
| <i>Monotommata</i> sp. | 1.8 | - | 0.5 | - | - | |
| <i>Notommata</i> sp. | 3.6 | 0.2 | 0.2 | - | - | |
| PHILODINIDAE | | | | | | |
| <i>Dissotrocha aculeata</i> (Ehrenberg, 1832) | 5.4 | 0.7 | - | - | - | |
| <i>Dissotrocha</i> sp. | 83.9 | 26 | 17.6 | 8.6 | 5.7 | Dis |
| SYNCHAETIDAE | | | | | | |
| <i>Polyarthra dolichoptera</i> Idelson, 1925 | 3.6 | 0.7 | - | - | - | |
| <i>Polyarthra vulgaris</i> (Carlin, 1943) | 8.9 | 2.6 | - | 1.5 | - | |
| TESTUDINELLIDAE | | | | | | |
| <i>Pompholyx triloba</i> Pejler, 1957 | 3.6 | - | - | 2.1 | - | |
| <i>Testudinella patina</i> (Hermann, 1783) | 14.3 | 0.7 | 0.8 | 1.2 | - | |
| TRICHOCERCIDAE | | | | | | |
| <i>Trichocerca bidens</i> (Lucks, 1912) | 7.1 | 1.4 | - | - | - | |
| <i>Trichocerca cylindrica</i> (Imhof, 1891) | 3.6 | - | - | - | 1.5 | |
| <i>Trichocerca similis</i> (Wierzejski, 1893) | 30.4 | 1.4 | 0.3 | 2.1 | 5.1 | Tsi |
| TRICOTRIIDAE | | | | | | |
| <i>Macrochaetus sericus</i> (Thorpe, 1893) | 14.3 | 1 | - | 4.8 | 0.3 | |
| <i>Trichotriia tetractis</i> (Ehrenberg, 1830) | 50 | 0.2 | 12.6 | 5.7 | 5.1 | Tte |
| TROCHOSPHERIDAE | | | | | | |
| <i>Filinia opoliensis</i> (Zacharias, 1898) | 58.9 | 207.1 | 4.4 | 3.3 | 6.8 | Fop |
| <i>Filinia terminalis</i> (Plate, 1886) | 1.8 | 0.2 | - | - | - | |

Table 3. Continued...

| Zooplankton | FO | Winter | Spring | Summer | Autumn | Ab. |
|--|------|--------|--------|--------|--------|-----|
| Cladocera | | | | | | |
| BOSMINIDAE | | | | | | |
| <i>Bosmina</i> cf. <i>freyi</i> Melo & Hebert, 1994 | 8.9 | - | 1.7 | 42.9 | - | |
| <i>Bosmina hagmanni</i> Stingelin, 1904 | 30.4 | 10.2 | 1.9 | 2.7 | - | Bha |
| <i>Bosminopsis deitersi</i> Richard, 1895 | 16.1 | - | 1.4 | 9.8 | 0.3 | |
| CHYDORIDAE | | | | | | |
| <i>Acroperus tupinamba</i> Sinev & Elmoor-Loureiro, 2010 | 10.7 | - | 1.4 | 0.6 | 0.6 | |
| <i>Alona glabra</i> Sars, 1901 | 30.4 | - | 4.2 | 1.8 | 3.3 | Agl |
| <i>Alona yara</i> Sinev & Elmoor-Loureiro, 2010 | 1.8 | - | 0.3 | - | - | |
| <i>Alonella dadayi</i> Birge, 1910 | 5.4 | - | 0.5 | 0.3 | - | |
| <i>Chydorus</i> sp. | 1.8 | 0.2 | - | - | - | |
| <i>Coronatella poppei</i> (Richard, 1987) | 3.6 | - | 3.9 | - | - | |
| <i>Picripleuroxis similis</i> (Vávra, 1900) | 1.8 | - | 1.2 | - | - | |
| DAPHNIDAE | | | | | | |
| <i>Daphnia gessneri</i> (Herbst, 1967) | 3.6 | - | - | 0.6 | - | |
| MACROTHRICIDAE | | | | | | |
| <i>Macrothrix laticornis</i> (Jurine, 1820) | 1.8 | - | 0.2 | - | - | |
| Copepoda | | | | | | |
| Nauplii | 76.8 | 7.1 | 23.3 | 6.6 | 9.2 | Nau |
| Copepodite | 39.3 | 0.5 | 4.1 | 2.1 | 3 | Cop |
| CYCLOPIDAE | | | | | | |
| <i>Thermocyclops decipiens</i> (Kiefer, 1929) | 5.4 | - | - | 0.3 | 0.6 | |

community, with copepods being the least abundant group in the community, represented mainly by the younger stages (nauplii and copepodids) (Figure 3A; Table 3). Significant differences in species richness between the seasons were recorded only for cladocerans ($F_{(3;52)} = 8.6$; $p < 0.001$). Richness values were driven by the significant contribution of testate amoebae and rotifer species across the seasonal variation (Figure 3B). Data on zooplankton community diversity indicated that there was variation in the composition of the species throughout the seasons, driven mainly by testate amoebae ($F_{(3;52)} = 4.38$; $p = 0.008$), rotifers ($F_{(3;52)} = 4.06$; $p = 0.011$) and cladocerans ($F_{(3;52)} = 6.41$; $p < 0.001$) (Figure 3C). Finally, the equitability of the community also showed variation between seasons, testate amoebae ($F_{(3;52)} = 5.29$; $p = 0.002$), rotifers ($F_{(3;52)} = 4.97$; $p = 0.004$) and cladocerans ($F_{(3;52)} = 5.2$; $p = 0.003$) (Figure 3D).

3.3. Environmental variables and their relationship with the zooplankton community

The Canonical Correspondence Analysis (CCA) summarized 70% of total data variability on the first two axes (axis 1, 55.3%; axis 2, 14.7%). The species-environment correlation in the first two axes were significant in the Monte Carlo permutation test (axis 1, $p = 0.001$; axis 2, $p = 0.005$). Some taxa were associated with environmental variables:

(i) *Arcella vulgaris*, *A. megastoma*, *Centropxyxis aculeata*, *Diffflugia cylindrus*, *Platytias quadricornis*, *Euchlanis dilatata*, *Lecane bulla*, *L. lunaris*, *Lepadella patella*, *Trichocerca similis* and *Trichotriia tetractis* were positively correlated with water velocity, electrical conductivity, suspended solids and pH; (ii) *Centropxyxis constricta*, *C. ecornis* and *Filinia opoliensis* were positively correlated with dissolved oxygen and water transparency, and negatively correlated with water temperature, turbidity and chlorophyll-*a*; (iii) *Arcella dentata*, *A. hemisphaerica gibba*, *Cephalodella* sp., *Alona glabra* and copepodids of copepods were positively correlated with water temperature, turbidity and chlorophyll-*a*, and negatively correlated with dissolved oxygen and water transparency; (iv) *Diffflugia* sp., *Lesquereusia spiralis*, *Kellicottia bostoniensis*, *Keratella cochlearis*, *Lepadella ovalis*, *Dissotrocha* sp., *Bosmina hagmanni* and nauplii of copepods were negatively correlated with water velocity, electrical conductivity, suspended solids and pH (Figure 4).

4. Discussion

Our results showed that local environmental conditions were important to structure the zooplankton community in the Pelotas River sub-basin. In general, the zooplankton structure responded differently during the seasons.

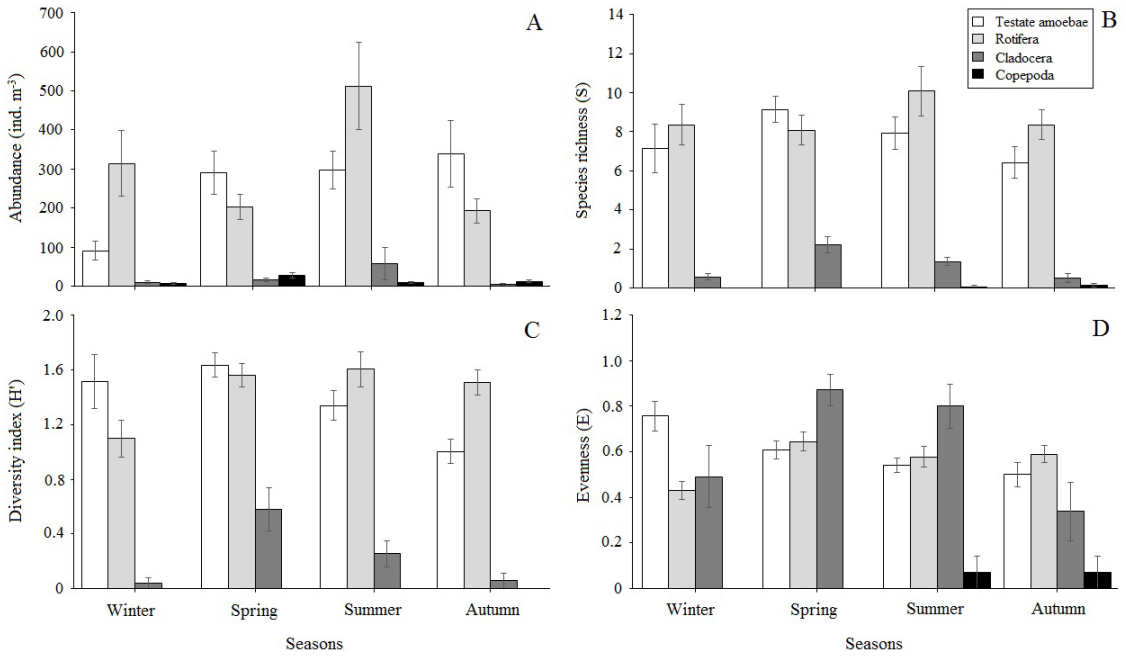


Figure 3. Mean values (\pm standard error) of (A) abundance, (B) species richness, (C) diversity and (D) evenness of Testate amoebae, Rotifera, Cladocera and Copepoda during the seasons in the Pelotas River sub-basin.

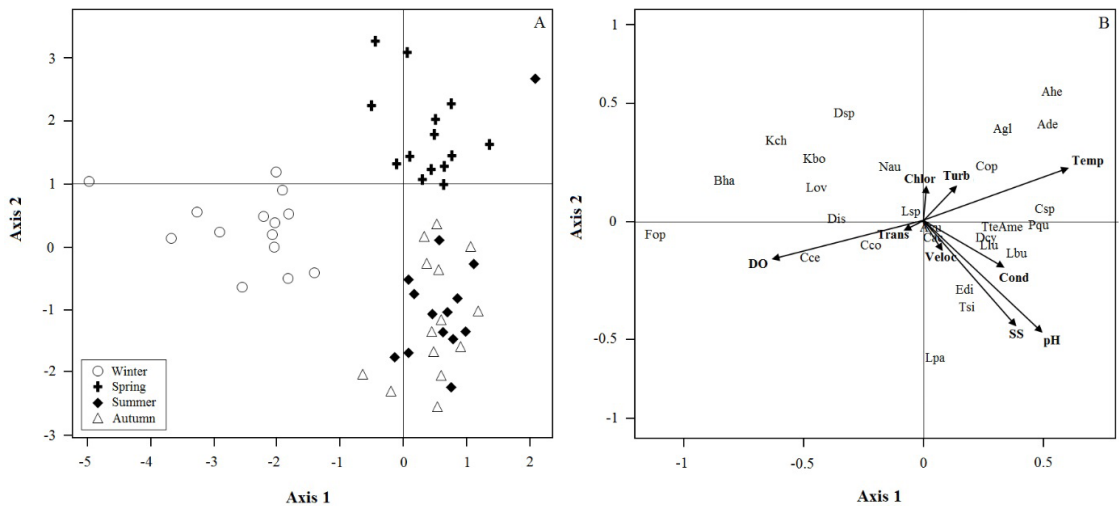


Figure 4. Canonical Correspondence Analysis (CCA). (A) Correlation of the environments and seasons in the Pelotas River sub-basin; (B) Correlation of zooplankton taxa and environmental variables (Abbreviations: Trans, water transparency; DO, dissolved oxygen; SS, Suspended solids; Chlor, chlorophyll-*a*; Cond, electrical conductivity; Temp, water temperature; Turb, turbidity; Veloc, water velocity). See Table 3 for abbreviation of zooplankton taxa used in CCA.

The environmental conditions did not cause evident variability between seasons in terms of species richness (except for cladocerans), but this was clearly important for the variability in abundance, diversity and evenness of the communities.

The zooplankton of Pelotas River was dominated by Neotropical or cosmopolitan species, with an invasive species, the rotifer *Kellicottia bostoniensis*,

also being found. The low species richness in the zooplankton community compared to other Neotropical rivers (e.g., Lansac-Tõha et al., 2009; Matsumura-Tundisi et al., 2015; Picapedra et al., 2017) is probably related to a number of factors acting simultaneously, with geographic isolation being only of these factors. According to Tavernini et al. (2009), there is a linear decrease in species richness

among the zooplankton as altitude increases. Also, according to the Theory of Island Biogeography (Rosindell & Phillimore, 2011), the more isolated the habitat, the lower the probability of colonization by other species, especially for zooplankton species that are passively dispersed (Adamowicz et al., 2009; Moreira et al., 2016).

Rotifers constituted the group with the highest species richness and population density in the Pelotas River, a recurrent pattern in tropical freshwater environments (e.g., Lansac-Tôha et al., 2009; Perbiche-Neves et al., 2012; Matsumura-Tundisi et al., 2015; Lucena et al., 2015; Picapedra et al., 2017). The Rotifera families with the most species were Brachionidae and Lecanidae. Brachionidae is considered one of the most important taxa of freshwater zooplankton, whose species usually have planktonic habit, while Lecanidae is related to the benthic and littoral zones, especially in places rich in vegetation, occurring in plankton only as occasional migrants (Lucena et al., 2015). These organisms are crucial in continental food webs because they occupy the niche of small filter feeders and transfer the energy of the producers to higher trophic levels (Almeida et al., 2009). The success of rotifer populations in freshwater environments is due to its limnetic origin, its ability to adapt to changes in the water's physical-chemical variables and the resistance of several taxa to conditions of hypoxia and anoxia. Parthenogenetic reproduction, a short life cycle and the ability to form resistant cysts also favors the group's success (Lansac-Tôha et al., 2009; Almeida et al., 2009).

Regarding testate amoebae, the families richest in species were Diffugiidae, Arcellidae and Centropyxidae. The predominance of these families is also reported in other studies in various aquatic habitats (Lansac-Tôha et al., 2014; Maia-Barbosa et al., 2014; Matsumura-Tundisi et al., 2015; Rosa et al., 2017; Picapedra et al., 2018). Although testate amoebae are associated with sediment and littoral vegetation, they are considered common in plankton, being transported to the water column with the suspension of sediment caused by water turbulence (Alves et al., 2012; Lansac-Tôha et al., 2014; Picapedra et al., 2018). Moreover, the presence of gas vacuoles to float and low density of the shells enable the persistence of the organisms in the plankton (Lansac-Tôha et al., 2014; Schwind et al., 2016).

In relation to microcrustaceans, there was predominance of the family Chydoridae for cladocerans. Most families of cladocerans are

typically planktonic, with species having swimming appendages that make them independent from the substrate, while the majority of littoral and benthic species are found in the family Chydoridae, with morphological adaptations that allow individuals to live in the interstitial environment, generally being common in rivers with high current flow (Dole-Olivier et al., 2001; Lansac-Tôha et al., 2009; Debastiani-Júnior et al., 2016). Nevertheless, some authors state that these microcrustaceans are not well adapted to the unstable conditions of rivers and prefer sites with lentic waters (Shiozawa, 1991; Viroux, 2002). Among the copepods, most individuals found were at a young stage (nauplii and copepodids). This predominance of young stages of copepods was also reported by other authors in lotic environments (e.g., Dole-Olivier et al., 2001; Nogueira et al., 2008; Lansac-Tôha et al., 2009; Perbiche-Neves et al., 2012; Paranhos et al., 2013) and is often related to three factors: (i) predation of adult forms by planktivorous fish; (ii) limited food sources due to increased discharge from the river carrying food resources downstream; (iii) limited reproduction due to difficulty in finding a sexual partner. Additionally, copepods rarely develop morphological adaptations in response to the hydrological instability of rivers, and the species with cylindrical body shape, like Cyclopoida, are common in rivers, as they are adapted for locomotion between the sediment particles (Dole-Olivier et al., 2001).

The variability of the zooplankton structure (mainly abundance, diversity and evenness) in streams can be attributed to its natural variability and to its temporal heterogeneity regarding physical and chemical patterns of the ecosystem (Thorp & Mantovani, 2005). Supporting our findings, the CCA showed a strong association of environmental variables with the zooplankton taxa during the seasons. Some zooplankton taxa (*Arcella hemisphaerica gibba*, *A. dentata*, *Cephalodella* sp., *Alona glabra* and copepodids of copepods) were associated with higher temperatures during the spring. A drop in temperature during the winter possibly resulted in decreased metabolic rates of zooplankton and consequent low feeding rates in contrast to spring. Temperature is an environmental factor likely to significantly influence the growth, reproduction and succession of planktonic organisms (Loiterton et al., 2004; Arrieira et al., 2015; Arrieira et al., 2016). The low temperatures measured in winter could limit the growth of aquatic organisms, such as testate amoebae and

their food, resulting in less abundance possibly due to temperature variability.

This study has revealed some dependence between densities of some rotifer species and values of conductivity. As its values increased, a decrease was observed in densities of *Keratella cochlearis*, *Kellicottia bostoniensis*, *Dissotrocha* sp. and *Lepadella ovalis*, whereas the densities of *Euchlanis dilatata*, *Lecane lunaris*, *L. bulla* among others increased. Bielanska-Grajner & Gładysz (2010) suggests that most species and forms of rotifers tolerate a wide range of conductivity, and only a small group of taxa avoid waters with a high conductivity.

The results also show that pH was linked to several species, especially species of littoral rotifers (e.g. *Lecane bulla*, *L. lunaris*, *Lepadella patella*, *L. ovalis*, *Platylas quadricornis*, *Trichocerca similis* and *Trichotriia tetractis*). This supports the view that rotifers are generalists and play an important role in the colonization of these extreme environments based on their broad pH tolerance (Deneke, 2000), especially acid pH environments. According to Berzins & Pejler (1987), rotifers found in acidic waters are often non-planktonic or semi-planktonic. The low pH values (mean pH 6.4 ± 0.3) observed in the water of Pelotas River sub-basin reinforce the role of the altitudinal aquatic ecosystems as early warning ecosystems for natural or anthropogenic impacts (Thompson et al., 2009; Eggermont et al., 2010). Low pH values in the water can occur when intense decomposition of organic matter takes place (Moreira et al., 2016). In addition, mountainous water bodies are also very sensitive to atmospheric deposition of pollutants, and acidification is a very common effect of chemical deposition (Camarero et al., 2009; Murphy et al., 2010).

The predominance of rotifers and testate amoebae may be related to higher suspended solid values during the summer and autumn. In some cases, feeding on suspended particles may be more important for zooplankton species than the consumption of live biomass (Melão & Rocha, 2006). Additionally, organic compounds or bacteria that are a suitable source of nutrients for zooplankton may adhere to the surface of suspended solids making digestion of the particles beneficial for zooplankton (Lind & Davaloslind, 1991). On the other hand, suspended solids had a negative effect on some species (e.g. *Keratella cochlearis*, *Kellicottia bostoniensis*, *Dissotrocha* sp. and *Bosmina hagdmani*). The suspended material in water scatters and absorbs light and creates a turbid environment. Changes in turbidity affect aquatic organisms at different

trophic levels, but through different mechanisms. Filter-feeding zooplankton are affected through mechanical disturbance of the filtering apparatus, clogging the filtering appendages, making them less efficient and reducing the beat rate of the filtering appendages due to gut fullness, even if the material filling the gut is not food (Claps et al., 2011; Jonsson et al., 2011). Further, assimilation efficiency may decrease when food is mixed with suspended solids (Arruda et al., 1983). All these mechanisms may result in reduced growth and fitness in zooplankton even if phytoplankton are abundant in the water (Jonsson et al., 2011).

In conclusion, clear correlations were found between environmental conditions and the zooplankton community. The conductivity, suspended solids, pH, temperature and dissolved oxygen, were identified as possible factors governing the seasonal variability of zooplankton in the Pelotas River sub-basin. Thus, this study suggests that the broad range of environmental conditions found in this study plays a substantial role in determining the abundance, diversity and evenness of zooplankton. In addition, the results obtained here revealed the importance of such small humid areas for aquatic biodiversity and the need to expand our understanding of the adaptations and interactions of altitudinal tropical aquatic communities.

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