Abundance, biomass and size structure of planktonic ciliates in reservoirs with distinct trophic states.

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ABSTRACT: Abundance, biomass and size structure of planktonic ciliates in reservoirs with distinct trophic states. The objective of this study was to investigate the distribution patterns of density and biomass, as well as analyze the variation in the body size structure of the planktonic ciliate community in three reservoirs (Paraná State) with distinct trophic status, in two distinct hydrological periods (dry and rainy seasons). Samplings were performed in July (dry) and November (rainy) 2001, in the lacustrine region of the reservoirs Iraí (eutrophic), Rosana (mesotrophic) and Chavantes (oligotrophic). In each reservoir, 1-liter samples were taken from the subsurface and the mixing layer, and immediately preserved. The samples were quantified in inverse microscopy. To estimate biomass values, organisms were measured and their biovolume (mm^3) calculated from their approximate geometric shape. Carbon content (mg C. L^{-1}) was estimated using 110 fgC mm^{-3} factor. Higher abundance values were observed in Iraí Reservoir and lower ones in Chavantes Reservoir. In general, ciliates were more abundant at the surface in all reservoirs. Seasonally, a significant difference in density was observed only for Iraí Reservoir. Oligotrichia and Hymenostomatida were frequently dominant in the different reservoir samplings, layers and periods. Analysis of ciliate community size structure showed that in the three studied reservoirs the community was dominated by small-sized individuals (‹40mm). Nevertheless, large-sized individuals were best represented in the oligotrophic reservoir. Results suggest that trophic status constituted a preponderant factor in determining patterns of spatial and temporal variation in the density, biomass and body size of the planktonic ciliates.
Key words: protozooplankton, ciliates, abundance, body size, trophic state.

RESUMO: Abundância, biomassa e estrutura de tamanho de ciliados planctônicos em reservatórios com distintos graus de trofia. O objetivo deste estudo foi investigar os padrões de distribuição da densidade e biomassa, bem como analisar as variações na estrutura de tamanho celular da comunidade de ciliados planctônicos em três reservatórios do Estado do Paraná, com distintos graus de trofia, em dois períodos hidrológicos distintos. As coletas foram realizadas em julho (estiagem) e novembro (chuva) de 2001, na região lacustre, dos reservatórios do Iraí (eutrófico), Rosana (mesotrófico) e Chavantes (oligotrófico). Em cada reservatório, amostras de 1 litro foram tomadas à sub-superfície e camada de mistura, e imediatadmente fixadas. As amostras foram quantificadas em microscópio invertido. Para a estimativa dos valores de biomassa, os organismos foram medidos e seu biovolume (mm^3) calculado a partir da forma geométrica aproximada. O conteúdo de carbono (mg C. L^{-1}) foi estimado utilizando-se o fator de 110 fg C mm^{-3}. Maiores valores de densidade e biomassa foram observados no reservatório do Iraí e os menores no reservatório Chavantes. Em geral, os ciliados foram mais abundantes à superfície. Temporalmente, diferenças representativas da densidade foram observadas apenas para o reservatório Iraí. Freqüentemente, Oligotrichia e Hymenostomatida foram os grupos dominantes em todos os reservatórios, profundidades e períodos de estudo. A análise da estrutura de tamanho das assembléias de ciliados evidenciou que, nos três reservatórios, a comunidade foi dominada por indivíduos de menor tamanho (‹40mm). Entretanto, indivíduos de maior porte foram melhor representados no reservatório oligotrófico.

Os resultados sugerem que as condições de trofia dos reservatórios constituíram-se no fator preponderante na determinação dos padrões de variação espacial e temporal da densidade, biomassa e tamanho celular dos ciliados planctônicos.
Palavras-chave: Protozooplâncton, ciliados, abundância, tamanho do corpo, grau de trofia.

Introduction

Since the Pace & Orcutt (1981) studies, several surveys have indicated that ciliates constitute a significant portion of the microzooplankton community (Beaver & Crisman, 1990) and an important link in the food web, performing a key energy flow function (Wickham, 1995). Moreover, because of their small size and high metabolic rate, ciliates play a substantial role in nutrient regeneration in the water column (Pace & Orcutt, 1981). They also respond to low organic pollution levels, as well as other physical, chemical and biotic alterations, indicating ecological changes in aquatic ecosystems (Paerl et al., 2003).

Studies have shown that the structure and composition of ciliate assemblages are significantly altered with the increase in eutrophication. Among a variety of lake types, ciliates are consistently an abundant component of the planktonic community, and their abundance apparently increases with the increase in trophic state (Bettez et al., 2002; Samuelsson et al., 2002; Auer et al., 2004; Xu et al., 2005).

In Brazil, reservoirs have been subjected to high levels of nutrient input due to the rapid development in industrial and farming production, as well as population growth, which increases sewage discharge from urban areas. This process directly affects the social and economic component of hydric resources (Mehner & Benndorf, 1995), endangering the water quality and its multiple uses such as water supply, fishing resources and sailing. Consequently, it is necessary to apply more efficacious rapid-response management measures, which require predictive studies.

Several surveys have been developed on plankton communities in Brazilian reservoirs. However, few of them have broached the heterotrophic components of the microbial food web. We emphasize Barbieri & Godinho-Orlandi (1989a and b), Hardoim & Heckman (1996), Bossolan & Godinho (2000), Gomes & Godinho (2003), Arantes et al. (2004) and Regali-Seleghin & Godinho (2004), which have considered the ecological features of the ciliate assemblages.

In this study we investigated the patterns of spatial and temporal variation in the density, biomass and size structure of the ciliate assemblages in three sub-tropical reservoirs with distinct trophic states.

Material and methods

Study area

The present study was developed in three reservoirs characterized by distinct trophic states, considering chlorophyll-a, total phosphorus concentrations and Secchi disc. Chavantes (23º 07‘ 00” S; 49º 44‘ 00” W) and Rosana (22º 36‘ 08” S; 52º 49‘ 41” W) reservoirs are located in the Paranapanema River (bordering Paraná and São Paulo states) (Fig. 1), with oligotrophic and oligo-mesotrophic features, respectively, and are mainly used for electric power generation. Iraí Reservoir (25º 25‘ 10” S; 49º 06‘ 49” W), located in the Iguaçu River, in the metropolitan region of Curitiba (Paraná State), is used essentially for water supply (Fig. 1). Because of its location, it receives high nutrient input, which determines the eutrophic condition of this environment.

Measurement of limnological variables

Samples for physical and chemical analysis and chlorophyll-a measurements were collected in the lacustrine region, from the subsurface and mixing layer, in two distinct periods — July (dry season) and November (rainy season) 2001 (Tab.I) — using

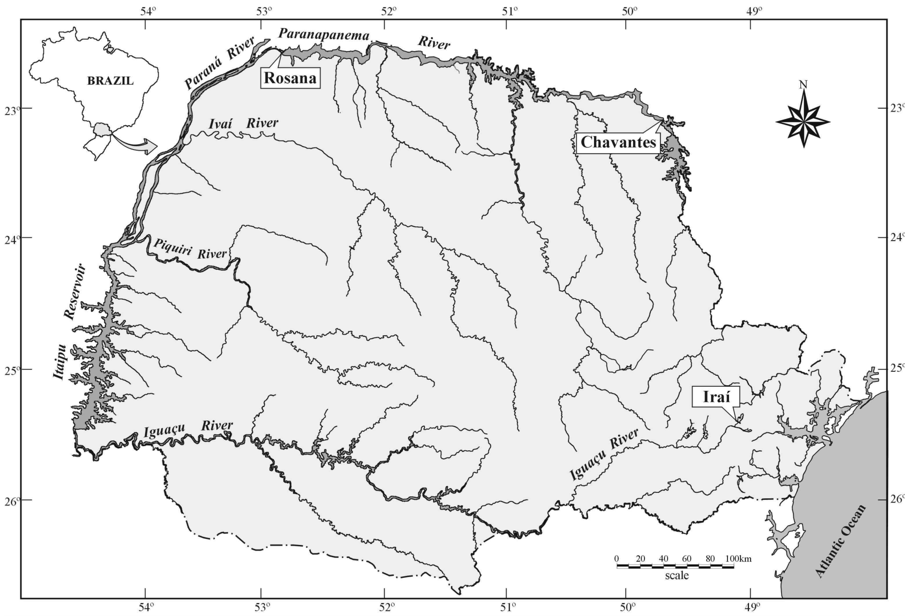

Figure 1: Hydrographic map of the Paraná State with the location of studied reservoirs.

Table I: Sampling depths in the studied reservoirs during dry and rain season.

Reservoirs	**Dry season**		**Rain season**	
	Surface	**ZMix**	**Surface**	**ZMix**
Chavantes	0,5 m	42 m	0,5 m	15 m
Rosana	0,5 m	24 m	0,5 m	12 m
Iraí	0,5 m	7 m	0,5 m	5 m

a Van Dorn sampler (one collection per period). The following abiotic variables were determined: water column transparency (Secchi disc); water temperature and dissolved oxygen (Horiba oxymeter); pH and conductivity (digital potenciometer - Digimed); alkalinity (Mackereth et al., 1978); nitrate (FIA - Zagatto et al., 1981); orthophosphate, total phosphorus and nitrogen, dissolved phosphorus (Mackereth et al., 1978); dissolved organic carbon (Carbon Analyser - Schimadzu TOC 5000). In order to analyze the dissolved nutrient fraction and determine suspended material (Teixeira et al., 1965) and chlorophyll–a concentrations (Golterman et al., 1978), samples were filtered in Whatman GF/C filters.

Protozoan quantification and biomass estimation

Samples (1 liter) for ciliate abundance determination were collected (abiotic parameter sampling occurred simultaneously) using a Van Dorn sampler and preserved using a mixture of two fixatives (alkaline lugol and formalin) and tiosulfate (Sherr & Sherr, 1993).

To estimate ciliate density and biomass, a variable volume from each sample (between 50 and 500mL) was dyed with Rose Bengal, maintained in sedimentation chambers for a period (in hours) equal to chamber height (cm) multiplied by 3 (Margalef, 1983). Later, ciliates were quantified and measured in inverse microscopy (400x magnification) and their biovolume calculated from their approximate geometric shape. Carbon content (mg. L^{-1}) was estimated using $1mm^3$=110 fg C (Weisse, 1991).

Data analysis

Principal components analysis (PCA) was used to summarize changes in abiotic variables over space and time. Significant differences in physical and chemical variables, observed among the reservoirs, were tested using a one-way ANOVA (Null Model). Aiming to evaluate the influence of biotic and abiotic factors on the abundance of ciliate assemblages, ciliate density and biomass were correlated (using Pearson's product-moment correlation) with the scores of the PCA axes (which synthesized abiotic variables), biomass and density of bacteria and heterotrophic nanoflagellates, and chlorophyll-a concentrations. The data set relative to the bacteria and flagellates is presented and discussed in Pereira et al. (2005) and Pagioro et al. (2005).

Results

Physical and chemical variables

Some physical, chemical and morphometric characteristics of the reservoirs are shown in Table II.

Table II: Minimum, maximun and mean values of some physical, chemical and biological water and, morphometric parameters of Iraí, Rosana and Chavantes reservoirs.

Variables/ Reservoirs		Irai	Rosana	Chavantes
pH	Min	6.86	6.91	6.26
	Max	6.98	7.74	7.59
	mean	6.91	7.43	7.03
Electrical Conductivity (mS/cm)	Min	47.5	58.2	52.4
	Max	51.1	60.0	58.6
	mean	49.6	59.4	55.9
Turbidity (NTU)	Min	6.6	3.84	0.82
	Max	30.9	6.60	1.98
	mean	18.4	4.88	1.36
Secchi disc (m)	Min	1.40	1.95	4.40
	Max	0.5	2.25	5.90
	mean	0.95	2.10	5.15
Total Phosphorus (mg/L)	Min	53.38	9.88	6.63
	Max	55.22	10.83	7.75
	mean	54.30	10.35	7.19
Total Nitrogen (mg/L)	Min	821	433	274
	Max	1483	519	338
	mean	1152	476	306
Chlorophyll-a (mg/L)	Min	71.2	3.4	0.7
	Max	82.9	4.9	1.5
	mean	77.1	4.2	1.1
Dissolved oxygen (mg/L)	Min	3.58	7.0	5.35
	Max	7.96	8.33	8.28
	mean	6.52	7.38	6.88
Area (km^2)		14.5	220	400
Depth (m)		8.5	26	100
Residence Time (days)		425	18.5	418

The first two PCA axes explained, cumulatively, 57% and 73.5% of total data variability. Conductivity and nitrate were positively correlated to axis 1, while turbidity, total phosphorus, total dissolved phosphorus, total nitrogen and total seston were negatively correlated to this axis. Total alkalinity presented negative correlation and orthophosphate positive correlation with axis 2 (Fig. 2a). Considering sample units, axis 1 distinguished them on a spatial scale, separating the samples from Iraí

Reservoir (negatively correlated) from those obtained in Chavantes and Rosana reservoirs (positively correlated) to this axis (Fig. 2b).

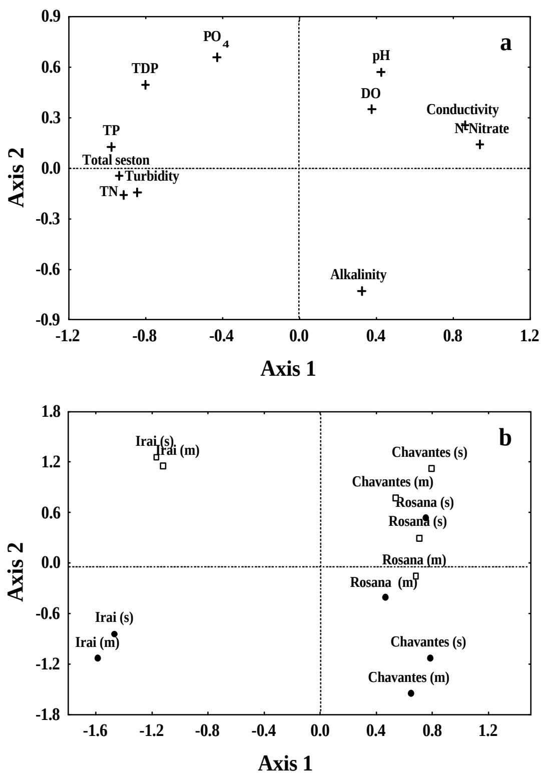

Figure 2: Resulting scores from PCA realized upon matrix of abiotic data. a) limnological variables; b) sample units. Filled circles represent samples obtained during dry season and empty squares, during rainy season. (s)=sub-surface; (m) = mixing layer.

Axis 2, also differentiated sample units on a temporal scale, mainly in Iraí and Chavantes reservoirs (Fig. 2b), distinguishing rainy season samples (positively correlated) from dry season samples (negatively correlated) to this axis.

In summary, PCA axis 1 differentiated Iraí Reservoir samples, characterized by higher values of total phosphorus, total nitrogen, seston and turbidity, from Chavantes and Rosana reservoir samples, characterized by higher values of nitrate and conductivity. On the other hand, axis 2 distinguished, in general, rainy season samples, from Iraí and Chavantes reservoirs (with higher pH and orthophosphate values), from those obtained during the dry season, which presented higher alkalinity values.

The one-way ANOVA (Null Model) revealed the existence of significant differences among reservoirs when we used PCA axis 1 scores (I.O.=194.20; p=0.004) (mainly ordered in function of different forms of nitrogen and phosphorus) and chlorophyll-a (I.O.=473.93; p=0.000). Significant differences were not observed when we used axis 2 scores (I.O.=0.090; p=0.907). These results indicate that nutrients and phytoplankton productivity were the main variables responsible for the statistical differences observed among the reservoirs.

Ciliate density and biomass

Higher ciliate density values were observed in Iraí Reservoir (7.21×10^3 to 4.71×10^4 cells.L^{-1}), intermediate values in Rosana Reservoir (1.54×10^3 to 3.89×10^3 cells.L^{-1})

and lower ones in Chavantes Reservoir (0.2 X 10^3 to 2.85 X 10^3 cells.L^{-1}). Seasonal differences were verified mainly in Iraí Reservoir, where a significant decrease in values occurred in both column layers during the rainy season. In general, ciliates were more abundant at the subsurface layer in all reservoirs during both seasons, except for Chavantes Reservoir during the rainy season (Fig. 3). Considering the contribution of different groups to total density, Oligotrichia and Hymenostomatida were, in general, dominant in all reservoirs, layers and seasons (Fig. 4).

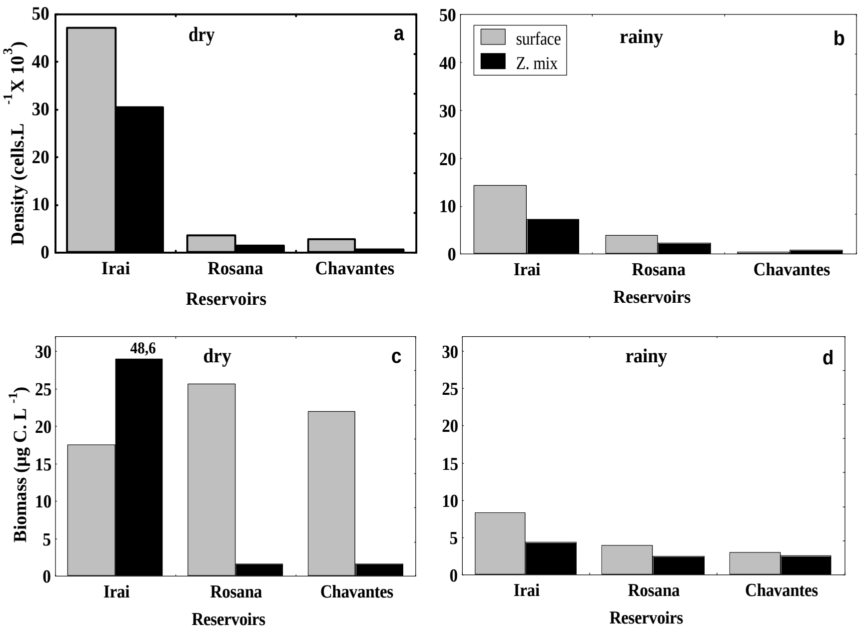

Figure 3: Density values (cells.L^{-1}), a and b; and biomass (mgC.L^{-1}), c and d, from planktonic ciliates in the three reservoirs, during dry and rainy seasons.

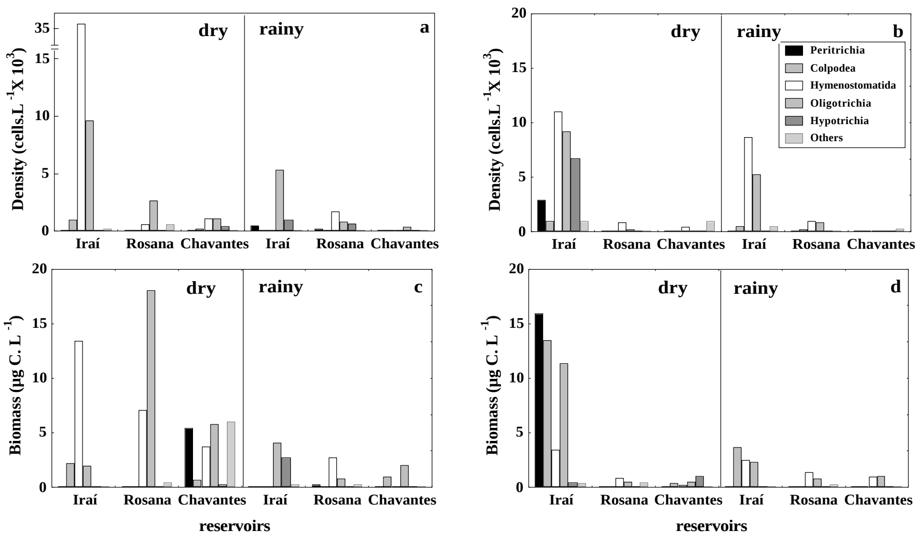

Figure 4: Density (cells.L^{-1}) (a = sub-surface and b = mixing layer) and biomass values (mgC.L^{-1}) (c=sub-surface and d=mixing layer) of planktonic ciliates in the three reservoirs, during dry and rainy seasons.

Biomass distribution results (Fig. 3) showed a pattern similar to observed for density, with higher values recorded in Iraí Reservoir (7.14 to 47.87 $mgC.L^{-1}$), intermediate values observed in Rosana Reservoir (1.88 to 25.67 $mgC.L^{-1}$) and lower ones in Chavantes Reservoir (1.97 to 21.94 $mgC.L^{-1}$). Considering the vertical biomass distribution, higher values were also verified at the surface, except for Iraí Reservoir during the dry season. Seasonal differences were evident for all reservoirs, with a remarkable biomass decrease occurring during the rainy season.

Oligotrichia and Hymenostomatida were also predominant as regards their biomass. In addition, Peritrichia was important at the subsurface layer of Chavantes Reservoir and in the mixing layer of Iraí Reservoir during the dry season, Colpodea showed a representative contribution in Iraí Reservoir, and Hypotrichia dominated in the mixing layer of Chavantes Reservoir during the dry season, and was important at the subsurface of Iraí Reservoir in the rainy period (Fig.4).

Relationships between environmental variables and ciliate abundance

Correlation results showed that ciliate density values were directly related to PCA axis 1 ($r=-0.8803$; $p=0.00015$), represented mainly by nutrient availability (Tab.1), as well as phytoplankton abundance (chlorophyll-a) ($r=-0.8986$; $p=0.00007$), bacteria density ($r=-0.7948$; $p=0.002$) and biomass ($r=-0.8368$; $p=0.00068$), and HNF density ($r=-0.6568$; $p<0.02031$) and biomass ($r=-0.7519$; $p=0.00479$). On the other hand, although correlation was found between ciliate density and biomass, biomass results did not present significant correlation either with the other community data or with the abiotic variables synthesized by the PCA axes.

Trophic state and size structure of ciliate assemblages

Ciliate mean size, obtained for different reservoirs, showed an inverse tendency with the increase in the trophic conditions (Fig. 5). Thus, higher mean size values were, in general, observed in Chavantes Reservoir and lower ones in Iraí Reservoir. In relation to vertical distribution, ciliates had larger mean size in deeper layers (mixing layer), of Iraí and Chavantes reservoirs. In Rosana Reservoir, differences in ciliate mean size were not verified among the analyzed depths.

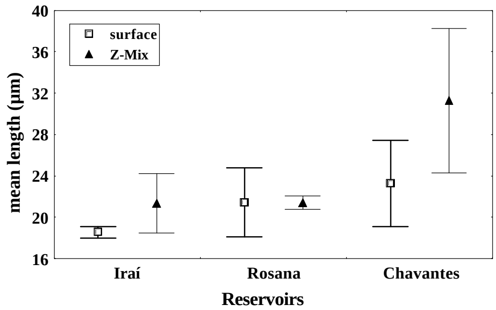

Figure 5: Ciliates mean size observed in subsurface and mixing layers of the studied reservoirs.

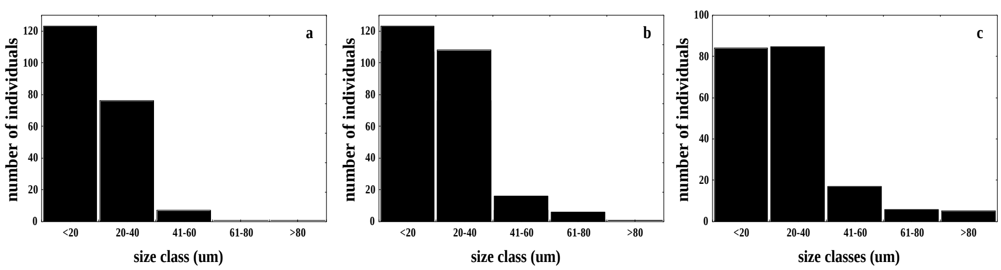

Figure 6: Size structure of ciliate assemblages in a) Iraí, b) Rosana and c) Chavantes reservoirs.

Analysis of ciliate size structure showed that the community was dominated by small-sized individuals (‹40mm) in all reservoirs (Fig. 6). However, when comparing the reservoirs, differences in the contribution of the distinct size classes were observed. In Iraí Reservoir, individuals smaller than 20mm clearly dominated, while in Rosana and Chavantes reservoirs, there was an expressive increase in individuals between 20 and 40mm. The occurrence of individuals larger than 60mm was representative only for Rosana and Chavantes reservoirs, and individuals larger than 80mm occurred only in Chavantes Reservoir (Fig. 6).

Discussion

Results obtained in this study showed that ciliate abundances (density and biomass) were higher in the reservoir with higher trophic state. Significant increase in the mean density and biomass of protozoa with the increasing trophic state of lakes has been reported elsewhere (Hwang & Heath, 1997; Burns & Schallenberg, 1998; Auer &. Arndt, 2004; Kalinowska, 2004), while oligotrophic environments have been typically characterized by low ciliate densities (Beaver & Crisman, 1989).

In the present study, we observed that, in general, ciliates were more abundant at the subsurface layer. Similar results have been recorded in other studies in the temperate (Taylor & Heimen, 1987; Sime-Ngando & Hartmann, 1991) and tropical region (Arantes et al., 2004). Nevertheless, according to Gates & Lewg (1984), ciliate abundance increases with depth. James et al. (1995) investigated an oligotrophic temperate lake, and observed higher density and biomass values in the metalimnium. Other studies carried out in Brazilian reservoirs showed greater ciliate densities and biomass at the bottom (Barbieri & Godinho-Orlandi, 1989a; Gomes & Godinho, 2003). Gomes & Godinho (2003) suggested that it was probably due to greater bacteria concentration in this layer. Bossolan & Godinho (2000), studying the ciliate community of Infernão Lake, also found high density values at the bottom, but only in the dry period; whereas in the rainy season, no differences between surface and bottom layers were observed.

In relation to seasonal variation, our data also diverge from the results obtained in other surveys developed in Brazil (Barbieri & Godinho-Orlandi, 1989; Gomes & Godinho, 2003), which recorded greater density and biomass values in stratified water conditions during the wet-warm season, and also from some studies performed in the temperate region (Taylor & Reynen, 1987; Riemann & Christoffersen, 1993; James et al., 1995), which suggested that this pattern is a consequence of nanoplankton and microzooplankton population dynamics. Nevertheless, in a study developed in tropical region (Hecky & Kling, 1981), high protozoan abundance coincided with water column mixture phases.

Oligotrichia was frequently dominant in relation to the density and biomass of the ciliate community in the three studied reservoirs, followed by Hymenostomatida, independent of sampling depth, season and trophic state. Oligotrichia dominance is a pattern commonly recorded in plankton from different worldwide aquatic environments (Sime-Ngando & Hartman, 1991; Laybourn-Parry, 1992; James et al., 1995; Burns & Schallenberg, 1998; Zingel et al., 2002). Hymenostomatida has also been recorded as an important group among planktonic ciliates (Beaver & Crisman, 1982; Zingel et al., 2002).

The positive correlation observed between ciliate density and nutrient concentrations, chlorophyll-a, bacteria and HNF suggests that greater environmental productivity strongly influenced the increase in ciliate abundance in the studied environments. According to Mathes & Arndt (1994) and Gates & Lewg (1984), positive biotic correlation and the correlation between ciliate and nutrient concentrations suggest that food resource availability prevails in determining the abundance distribution of these organisms. Although predation pressure on the ciliate community certainly occurs, several studies have shown that trophic state is one of the main forces influencing ciliate distribution, in such a way that nutrient levels determine the density of these organisms (Beaver & Crisman, 1982; Sola et al., 1996; Hwang & Health, 1997; Bettez et al., 2002; Samuelsson et al., 2002; Auer et al., 2004).

Small-sized ciliate dominance (‹40mm) has been verified as a common pattern among planktonic ciliate communities (Beaver & Crisman, 1982; Gates & Lewg, 1984; Taylor & Heynen, 1987). Although Thiel (1981) apud Peters (1993) emphasizes that body size increases according to the increase in food resource availability, we observed a tendency of decrease in mean size with the increase in reservoir trophic state. Thus, in the studied reservoirs, ciliates seem lunge more in reproduction than in individual growth, under conditions of high food resource availability.

In summary, the results suggest that the trophic state of reservoirs is a preponderant factor in determining patterns of spatial and temporal variation in planktonic ciliate abundance. In this way, Iraí Reservoir, with high nutrient availability, which determines higher phytoplankton and bacteria abundance, propitiated greater development of planktonic ciliate populations. On the other hand, Chavantes Reservoir, the most oligotrophic, had lower abundance values of these protozoa.

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