Abundance of planktonic ciliates in a cascading reservoirs of the Paranapanema River, Brazil

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ABSTRACT. The present study aimed to investigate the spatial and temporal distribution patterns of density and biomass of the planktonic ciliates, in a reservoir serie of the Paranapanema River (22º 18' - 23º 35' S; 49º 30' - 52º 50' W). We intend to test the hypothesis that, along reservoirs serie, there is a decrease of abundance from river towards the dam. Samplings were carried out in seven reservoirs, during 2001, in two distinct hydrological periods, at subsurface of the lacustrine region, using a Van Dorn bottle (5 liters). Aliquots from one liter were obtained and fixed with a fixing solution compounded by alkaline lugol, formaldehyde and sodium tiosulfate. Obtained values for ciliates abundance (density and biomass) in different reservoirs (0.4 to 16.6 cells.mL^{-1}; 0.23 to 20.7 μg.C.L^{-1}, respectively) were inferior to results found in eutrophic environments, but similar to those registered in oligo-mesotrophic environments. Analysis of the abundance distribution results, along reservoirs serie, did not corroborate the hypothesis of decreasing density and biomass from upstream to dowstream, due to an expectancy of a possible oligotrophication downriver. In relation to different functional groups of ciliates, it was verified the dominance of Oligotrichida, mainly small sized individuals (<30 μm).

Key words: protozoans, ciliates, abundance, plankton, cascading reservoirs.

RESUMO. Abundância de ciliados planctônicos em reservatórios em cascata do rio Paranapanema, Brasil. O presente estudo teve como objetivo investigar os padrões de distribuição espacial e temporal da densidade e biomassa dos ciliados planctônicos em reservatórios em cascata do rio Paranapanema (22º 18' - 23º 35' S; 49º 30' - 52º 50' W). Pretende-se testar a hipótese de que, ao longo da série de reservatórios, observa-se um decréscimo da abundância desses organismos no sentido montante-jusante. Para tal, foram coletadas amostras em sete reservatórios, no ano de 2001, em dois períodos hidrológicos distintos, à sub-superfície da região lacustre, com o auxílio de uma garrafa de Van Dorn de 5 litros. Alíquotas de um litro foram obtidas e sua fixação realizada com solução fixadora composta de lugol alcalino, formol e tiossulfeto de sódio. Os valores obtidos para a abundância (densidade e biomassa) de ciliados nos diferentes reservatórios (0,4 a 16,6 células.mL^{-1}; 0,23 a 20,7 μg.C.L^{-1}, respectivamente) foram inferiores aos resultados encontrados em ambientes eutróficos, porém similares àqueles registrados em ambientes oligo-mesotróficos. A análise dos resultados de distribuição da abundância ao longo da série de reservatórios não corroborou a hipótese preconizada de uma diminuição de densidade e biomassa no sentido montante-jusante, em função da expectativa de uma possível oligotrofização rio abaixo. Em relação aos diferentes grupos funcionais de ciliados, foi constatada a dominância dos Oligotrichida, principalmente os de menor porte (< 30 μm).

Palavras-chave: protozoários, ciliados, abundância, plâncton, reservatórios em cascata.

Introduction

The traditional concept of classical food chain in aquatic environments has been recently supplemented through the new paradigm of microbial loop (Laybourn-Parry, 1994), which suggests that bacteria and protozoans have primary paper in production, transferring and maintenance of organic matter in pelagic region (Azam *et al.*, 1983). This concept, jointly to scientific and technological advances of microbial ecology through the advent of epifluorescence microscopy (Caron, 1983; Hobbie *et al.*, 1997), brings on the development of new studies on these communities during last three decades.

Protozoans are the principal bacteria consumers and, in turn, they are predated by large sized zooplankton (Sherr and Sherr, 1988, Laybourn-Parry, 1992). Many times, they constitute in dominant trophic link through picoplanktonic and nanoplanktonic production is transferred to high trophic levels (Hwang and Health, 1997). Among protozoans, ciliates are potential nanoflagelate predators, and they can control its abundance (Weisse, 1991). Although, according to Gifford (1985), ciliates have been found feeding on preys of different sizes, from bacteria to organisms of their own size.

In relation to biotic factors that can potentially influence ciliates abundance, studies on pelagic food chains have demonstrated the effect of resource availability, in case that increasing nutrients concentration brings about an increasing algal production and biomass with subsequent increase in bacteria, flagellates and ciliates abundance (Gasol and Vaqué, 1993; Gasol *et al.*, 1995; Burns and Schallenberg, 1996; Hobbie *et al.,* 1999; Samuelsoon *et al.*, 2002).

Besides biotic factors, abiotic ones, as temperature, light and oxigen concentration in water column, have important roles in seasonal succession, in protozooplankton composition and abundance (Laybourn-Parry, 1992).

In Brazil, several reservoirs have been built in last three decades. Serie building of these ecosystems imply in formation of a complex pattern of interactions, that can be percept along longitudinal axis of the dammed river, which generates physical, chemical and biological alterations. Studies have also shown the influence of upstream reservoirs on downstream ones.

According to SDC (Serial Discontinuity Concept), proposed by Ward and Stanford (1983), building of reservoirs serie causes a discontinuity of physical and biological characteristics along main river. However, some surveys show the influence of upstream reservoirs on downstream ones, evidencing alterations as oligotrophication, caused by reduction of phosphorus concentration, decrease in turbidity, due to reduction of organic matter, and decrease of primary production (Straskraba, 1990; Ney, 1996).

Several researches have been developed on planktonic community, although, studies on protozoans, which play a primal function in ecosystem metabolism, have been, in tropical region and specially in Brazil, systematically neglected. Besides that, studies that evaluate the effect of serie reservoirs building on structure and dynamics of planktonic ciliates assemblage are inexistent.

The present study aimed to investigate the spatial and temporal distribution patterns of density and biomass of the planktonic ciliates, in a reservoir serie of the Paranapanema River, as well as identify biotic (bacteria, heterotrophic nanoflagellates, phytoplankton and zooplamkton) and abiotic factors intervening on determine those patterns. More specifically, considering the oligotrophication process in reservoir serie, and the remarkable relation between protozoans abundance and the productivity of aquatic environments, related on literature, we intend to test the hypothesis that, along reservoirs serie, there is a decrease of abundance from river towards the dam.

Material and methods

Study area

Samples were collected in seven reservoirs of Paranapanema River (PR/SP) with variable areas, morphometrics and age (Figure 1). Most of them have the main use of electric power generation. Some characteristics of these reservoirs are presented in Table 1.

Table 1. Some characteristics of the seven reservoirs in the Paranapanema River.

	Age (year)	Reservoir level (m)	Area (Km²)
Chavantes	35	474	242,4
Salto Grande	47	385	8,4
Canoas II	5	364	22,51
Canoas I	6	351	30,85
Capivara	30	334	515
Taquaruçu	13	284	40,8
Rosana	19	258	220

Sampling and preservation

Collects were carried out at subsurface, in lacustrine region of the reservoirs, during July (dry season) and November (rainy season) from 2001.

Water for ciliate and abiotic factors analyses, as well as for phytoplankton, bacteria and heterotrophic nanoflagellate (HNF) community was sampled in Van Dorn bottle (5L). Water aliquots were stored in separated flaskes for HNF mensuration and enumeration (100mL), phytoplankton (100mL), bacteria (50mL) and ciliates (1.5mL). Samples for physical, chemical and chlorophyll-*a* analyses were maintained in plastic flasks (5L).

Zooplankton organisms (rotifers, cladocerans and copepods) were collected using a motorized pump and plankton net (68 μm) to filter 1000L of water per sample.

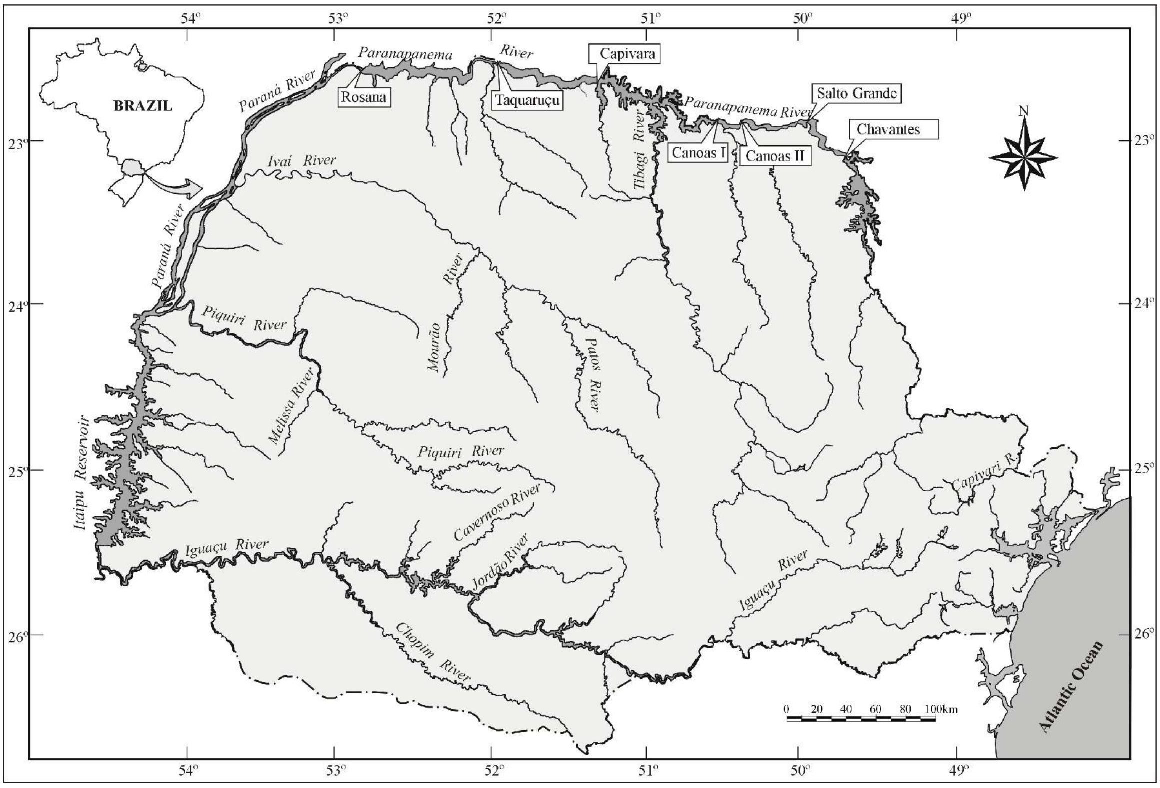

Figure 1. Location of serie cascading reservoirs in the Paranapanema River.

Ciliate and HNF preservation were done using fixing solution compounded by formaldehyde, alkaline lugol and tiosulfate (Sherr and Sherr, 1995). Phytoplankton was preserved in acetic lugol and bacteria and zooplankton in formaldehyde (4%), buffered with calcium carbonate.

Mensuration of physical and chemical variables

Following abiotic variables were determined at field: water temperature (ºC), dissolved oxygen (mg/L) (Oriba Oxymeter) and pH (portable pHmeter).

Dissolved organic carbon (ppm) (Carbon Analyzer Schimadzu TOC 5000), chlorophyll-*a* (mg/L) (Golterman *et al.*, 1978), turbidity (NTU) (turbidimeter) and total phosphorus (total-P) (μg/L) and total nitrogen (total-N) (μg/L) (Mackereth *et al.*, 1978) concentrations were determined in laboratory.

Counting and biomass estimative of different communities

Ciliates

For ciliates enumeration and biomass estimative, a variable volume of each sample (between 50 and 1000mL) was dyed with Rose Bengal, maintained in sedimentation chambers for a time (in hours) equivalent to three times the chamber height (cm), afterwards, quantified in Olympus CK40 inverse microscope, with magnitud of 400 x. For biomass estimative, organisms were measured and their biovolume (μm^3) calculated from approximated geometrical shape. Carbon content (μg/L) was estimated using 110 fg C factor (Weisse, 1991).

Bacterioplankton

Bacterioplankton has its density and biomass determined from subsamples (0.1mL) filtering in a black filter Nucleopore/Whatman with 0.2 μm pore size, previously dyed with approximately 1mL of 0.1% fluorocromo 4,6'-diamidino-2-fenil-indole (Dapi), during 15 minutes, in the dark. Afterwards, filters were mounted in slides, stored in refrigerator for 24 hours, and then stored in freezer in –8ºC until counting on microscopic. Bacteria were quantified in 1000 X magnification in epifluorescence microscope (Zeiss Axiophot) and their biovolume were determined through equation proposed by Fry (1990): $v=(\pi/4).w^2(l-w/3)$, where: v=cell volume; l=length and w=width. To convert biovolume in biomass, it was considered that $1\mu m^3=3.5 \times 10^{-13}gC$ (Bjornsen, 1986).

Heterotrophic nanoflagelates

For estimative of HNF density and biomass, we used the same protocol of filtering, slides mounting and quantification described for bacterioplankton, however, subsamples between 5 and 50 mL of water were filtered in black filter Nucleopore/Whatman with 0.8 μm pore size, and counted at least 300 cells or 100 fields per sample.

HNF quantification was made under UV light and the differentiation among those and autotrophic flagellates, under blue light, using as criterious the low fluorescence and HNF greenish color, contrasting to reddish or orangy color of autotrophic flagellates.

Simultaneously to counting, individuals were measured using micrometricals oculars, to determine cell volume, through cell dimensions and approximated geometrical shapes (Wetzel and Likens, 1991), and the carbon content, where $1\mu m^3$=167 fg C, according to Fenchel (1982).

Phytoplankton

Phytoplankton density was estimated in inverse microscope, after previous sedimentation in Utermöhl chambers (Utermöhl, 1958). Calculations were done according to APHA (1985). Phytoplankton counting was done until obtaining 100 individuals of most abundant species. Results were expressed in individuals (cells, colonies or filaments) per mililiter.

Zooplankton

To quantify zooplankton organisms, three subsamples per sample were done, using Hensen-Stempell pipette. Approximately 80 individuals were counted by subsample (2.5mL), according to methodology suggested by Botrell *et al.* (1976). Density was expressed in number of organisms/m^3.

Statistical treatment

To evaluate which environmental factors (biotics and abiotics) would be involved in determining abundance distribution patter, along cascading reservoirs, Pearson correlations were done between ciliate density and biomass and abiotic and biotic variables analyzed. For this analysis, raw data were log transformed, to balance variances.

Results

Ciliate density values varied between 0.43 and 16.6 $ind.mL^{-1}$. Higher densities, in both hydrological periods, were registered in Canoas I Reservoir, followed by Rosana. On the other hand, lower densities, during dry season, were observed in Salto Grande and Capivara Reservoirs, and during rainy season, in Salto Grande, Capivara and Taquaruçu (Figure 2).

Considering the reservoir position, in Paranapanema River, we verified that, in general, there was an increase in density toward middle of the reservoir seri, with higher abundance values in Canoas I Reservoir, and following a decrease, and once again, an increase in the end cascade. In general, higher densities occurred during dry season (Figure 2).

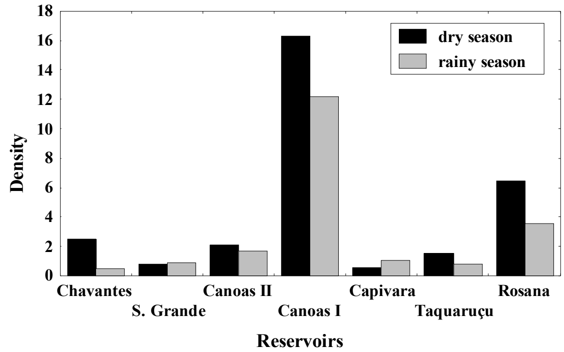

Figure 2. Total ciliates density ($cells.mL^{-1}$) registered in seven reservoirs during dry and rainy seasons.

Higher ciliates biomass values were also registered in Canoas I Reservoir, in both hydrological periods, followed by Canoas II and Chavantes Reservoirs, during dry season. Reduced biomass values were observed in Salto Grande, Taquaruçu, Chavantes and Capivara Reservoirs during rainy season, and Capivara Reservoir during dry season (Figure 3).

Considering Canoas I Reservoir, where occurred higher values of density and biomass, we observed an inverse pattern. In other words, we found higher density values during dry season and higher biomass during rainy season. These results were related to the ciliates body size registered during both hydrological periods, considering that lower values were observed during dry season and higher ones during rainy season.

In relation to biomass values, considering reservoir position, we verified, in general, a similar pattern to that registered for density, with higher values at the middle of the cascade.

Considering different ciliates groups, we observed, during dry season, that Oligotrichida had higher densities in all reservoirs, except for Salto Grande. This ciliate group was most abundant in Canoas I, followed by Rosana and Taquaruçu, and it was the only one registered in Capivara in this season. In this same hydrological period, Hymenostomatida stand out in Canoas I and Rosana Reservoirs (Figure 4).

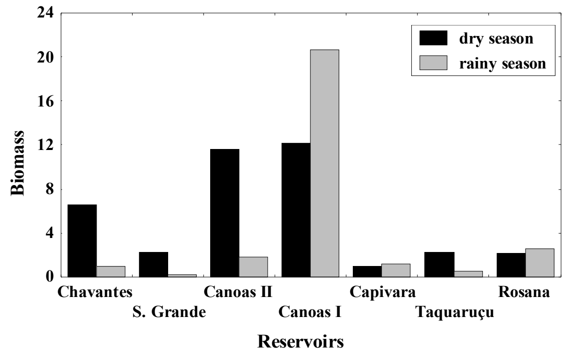

Figure 3. Total ciliates biomass (μgC^{-1}) registered in the seven reservoirs during dry and rainy seasons.

During rainy season, although Oligotrichida had been dominant in some reservoirs (Chavantes and Taquaruçu), Colpodea group dominated in Canoas I, and Hymenostomatida in Rosana, Salto Grande and Capivara. In this hydrological period, higher densities were also registered in Canoas I, due to higher abundances of Colpodea, Hymenostomatida and Oligotrichida (Figure 4).

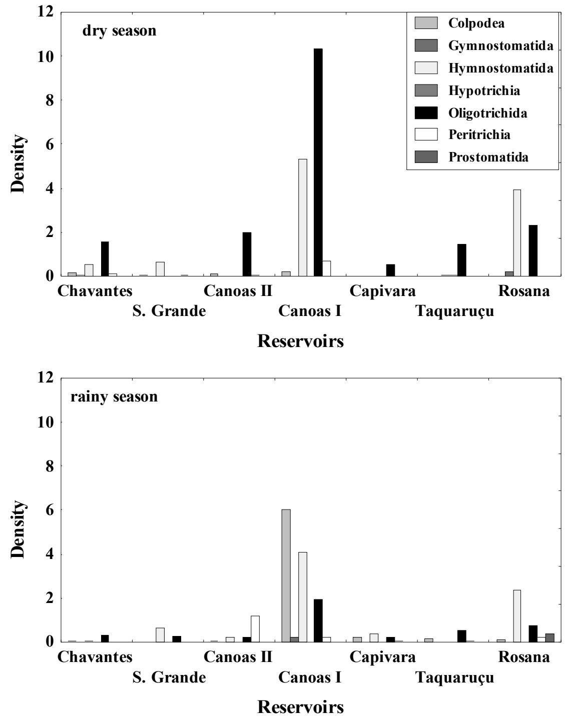

Figure 4. Density (cells.mL^{-1}) of different ciliate groups in seven reservoirs during dry and rainy seasons.

For biomass, during dry season, Oligotrichida were the most representative in most of reservoirs, especially in Canoas II and Canoas I, and this group was the only one to occur in Capivara. Hymenostomatida presented higher biomass values in Chavantes, Salto Grande and Rosana. We must emphasize that, considering the density values, during this hydrological period, Oligotrichida were the dominant group in Chavantes. Nevertheless, presenting low biomass (Figure 5). This fact can be related to individuals size registered for this group in this reservoir.

During rainy season, the biomass values registered in most of reservoir were lower to those registered during dry season, except for Canoas I, where Colpodea reached a biomass approximately of 20 μgC.L^{-1} (Figure 5).

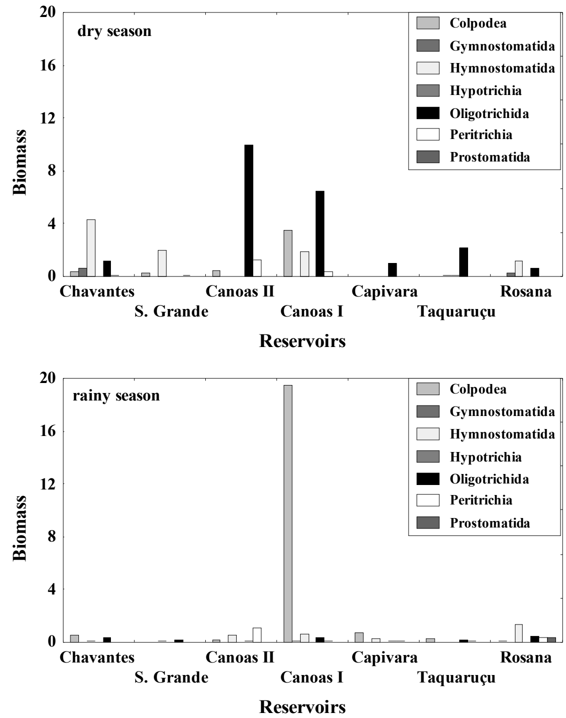

Figure 5. Biomass (μgC.L^{-1}) of different ciliate groups in seven reservoirs during dry and rainy seasons.

Mean size of ciliates cells were greater, in most of reservoirs, during dry season, except for Canoas I. Greater sizes occurred, in this period, in Canoas II, Capivara, Salto Grande and Taquaruçu (Figure 6). These results showed that, although higher densities and biomass had been registered in Canoas I (Figures 2 and 3), ciliates mean size was greater in Canoas II.

Results from Pearson Correlation carried out to evaluate the influence of biotic and abiotic factors on density and biomass of ciliates community evidenced that only rotifers correlation ($p=0.02$; $r=0.61$) and Moinidae correlation ($p=0.49$; $r=0.52$) were significant.

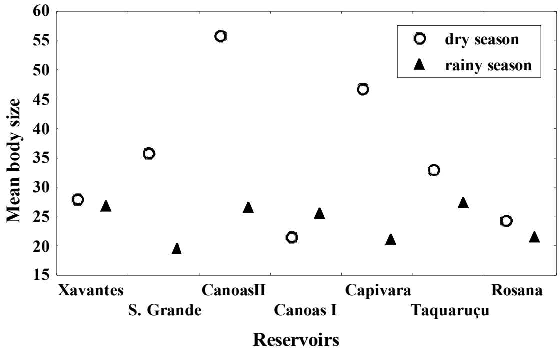

Figure 6. Ciliates mean size (μm) registered in seven reservoirs during dry and rainy seasons.

Discussion

Values obtained for ciliates abundance in different studied reservoirs (0.43-16.6 cells.mL^{-1}; 0.23-20.7 μgC.L^{-1}) were inferior to results found in eutrophic environments (Beaver and Crisman, 1982; James *et al.*, 1995), but similar to those registered in oligo-mesotrophic environments (Pace, 1982; Taylor and Heynen, 1987; James *et al.*, 1995). In this way, the abundance results of planktonic ciliates suggest that these seven reservoirs investigated in Paranapanema river present characteristics of environments with low to moderate trophic status.

Results analysis of abundance distribution along reservoirs serie did dot corroborate the hypothesis of decreasing density and biomass from upstream to dowstream, due to an expectancy of a possible oligotrophication downriver.

These results can be related to the concept proposed by Barbosa *et al.* (1999) about cascading reservoirs, the CRCC (Cascading Reservoir Continuum Concept). This concept shows the existing connectivity among several dammings, considering the number of reservoirs in serie, the nutrients input from watershed, water outflow depth, reservoir residence time and reservoir morphometry.

Thus, a river can present cascading reservoir serie but this does not mean that there will be a remarkable influence from upstream reservoir on dowstream ones. In case of Paranapanema River, the presence of several tributaries, for example, brings about nutrients input in distinct reservoir regions, overlaying the oligotrophication effect arisen from nutrients settlement in lacustrine region of different reservoirs in serie.

Other aspect that must be considered would be the different soil uses in watershed of Paranapanema river, taking into account farming and cattle raising activities frequently contribute to an expressive input of organic and inorganic resources for aquatic environments of the watershed.

In relation to different functional groups of ciliates, the Oligotrichida dominance, as observed in this study, is a common pattern registered in plankton of different aquatic environments, in several regions of the world (Sime-Ngando and Hartman, 1991; Macek, 1994; James *et al.,* 1995; 1998; Zingel *et al.*, 2002). Besides this group, Hymenostomatida has also been registered as important among planktonic ciliates (Beaver and Crisman, 1982; Zingel *et al.*, 2002).

The dominance of small sized ciliates (<30 μm) has been verified as a common pattern among planktonic ciliates community (Beaver and Crisman, 1982; Gates and Lewg, 1984; Taylor and Heynen, 1987). According to Fenchel (1987), predominance of small sized organisms in plankton can be explained by their high metabolic activity due to the need to win water viscosity as well as to keep in water column. In this way, considering the oxygen diffusion inside the cell needs to reach all the parts of the cell, large sized organisms, in plankton, could not maintain a high metabolic rate, in function of oxygen claim (Fenchel 1987).

Considering the results derived from the Pearson Correlation, they were not expected. Especificaly, several surveys have evidenced a remarkable relation between ciliate abundance and the resources consumed by them (bacteria and algae) (Gates and Lewg, 1984; Zingel *et al.*, 2002). On the other hand, direct correlations were observed between ciliates and zooplankton organisms (rotifers and Moinidae, which regularly compete with ciliate to the same resources (Sime-Ngando and Hartmann, 1991). Ciliates present wide food range, including algae, bacteria, detritus, flagellates and even other ciliates, thus, results suggest that correlation analyses did not succeed in detect a determined food resource as responsible to its abundance distribution.

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