Brazilian Journal of Microbiology 45, 1, 143-151 (2014)
ISSN 1678-4405

Research Paper

Protozoans bacterivory in a subtropical environment during a dry/cold and a rainy/warm season

Karina F. Hisatugo, Adrislaine S. Mansano, Mirna H.R. Seleghim

Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos, São Carlos, SP, Brazil.

Submitted: September 18, 2012; Approved: April 01, 2013.

Abstract

In aquatic ecosystems, bacteria are controlled by several organisms in the food chain, such as protozoa, that use them as food source. This study aimed to quantify the ingestion and clearance rates of bacteria by ciliates and heterotrophic nanoflagellates (HNF) in a subtropical freshwater reservoir (Monjolinho reservoir - São Carlos - Brazil) during one year period, in order to verify their importance as consumers and controllers of bacteria in two seasons, a dry/cold and a rainy/warm one. For this purpose, *in situ* bacterivory experiments were carried out bimonthly using fluorescently labeled bacteria with 5-(4,6 diclorotriazin-2yl) aminofluorescein (DTAF). Although ciliates have shown the highest individual ingestion and clearance rates, bacterivory was dominated by HNF, who showed higher population ingestion rates (mean of 9,140 bacteria h^{-1} mL^{-1}) when compared to ciliates (mean of 492 bacteria h^{-1} mL^{-1}). The greater predation impact on bacterial communities was caused mainly by the small HNF (< 5 μm) population, especially in the rainy season, probably due to the abundances of these organisms, the precipitation, trophic index state and water temperature that were higher in this period. Thus, the protozoan densities together with environmental variables were extremely relevant in determining the seasonal pattern of bacterivory in Monjolinho reservoir.

Key words: bacteria, grazing, ciliates, nanoflagellates, microbial food web.

Introduction

In aquatic systems, protozoans are seen as significant portion of the microzooplankton community and an important link in the food web. In aquatic systems microbial loop (Pomeroy *et al.*, 2007), protozoans play an important role as bacterial predators, acting as key components in energy flow. Predation by protozoa (top-down control) is considered to be one of the main bacterial community modifying and regulatory factors in aquatic ecosystems, able to cause direct impacts on bacterial production, biomass, structure, morphology, physiology, taxonomy and diversity (Pernthaler, 2005; Corno *et al.*, 2008; Bell *et al.*, 2010).

Despite the importance of protozoan predation on bacteria, few studies have been conducted in tropical and subtropical aquatic food webs, especially with regard to top-down control mechanisms and its seasonal patterns (±. Tsai *et al.*, 2011, 2012). Most studies are from *in vitro* experiments (*e.g.* Gasol *et al.*, 2002), that often do not necessarily reflect the real natural environment conditions, or analyze only the food webs classic components (phytoplankton and mesozooplankton) without focus on their microbial components (*e.g.* Pagano, 2008).

Considering the need for more studies directed to the microbial trophic interactions in subtropical systems, this study aimed to quantify the ingestion and clearance rates of bacteria by ciliates and heterotrophic nanoflagellates in a subtropical freshwater reservoir (Monjolinho reservoir - São Carlos - Brazil) during one year period, in order to verify their importance as bacterial consumers and controllers in the dry/cold and a rainy/warm season.

Materials and Methods

Study site

This study was carried out at Monjolinho reservoir, a eutrophic environment formed by the damming of Monjolinho river and located in an urbanized area of São Carlos

Send correspondence to Karina F. Hisatugo. Ecology and Evolutionary Biology Department, Federal University of São Carlos, 13565-905 São Carlos, SP, Brazil. E-mail: k_hisatugo@yahoo.com.br.

city (22° 00' S and 47° 54' W), São Paulo, Brazil (Figure 1).The reservoir, is at 816 m above sea level, has an area of 47,157 m^2, volume of 73,251 m^3 and 3 m of maximum depth. The climate is subtropical, Cwa (Köppen, 1931), with dry winter (April to September) and rainy summer (October to March), with maximum summer precipitation higher than or equal to ten times the precipitation of the driest month. The reservoir retention time during the dry season is 22.9 days and 2.1 days during the rainy season (Nogueira and Matsumura-Tundisi, 1994). The reservoir is highly unstable and turbulent due to its short retention time, small size, low depth, precipitation and wind, which affect not only the phytoplankton and mesozooplankton (Nogueira and Matsumura-Tundisi, 1996), but also the protozooplanktonic and bacterioplanktonic communities (Regali-Seleghim and Godinho, 2004). This instability often causes sediment resuspension, which increases the amount of nutrients in the water column and favors the occurrence of algal blooms at the end of the dry season until the middle of the rainy season (Regali-Seleghim and Godinho, 2004).

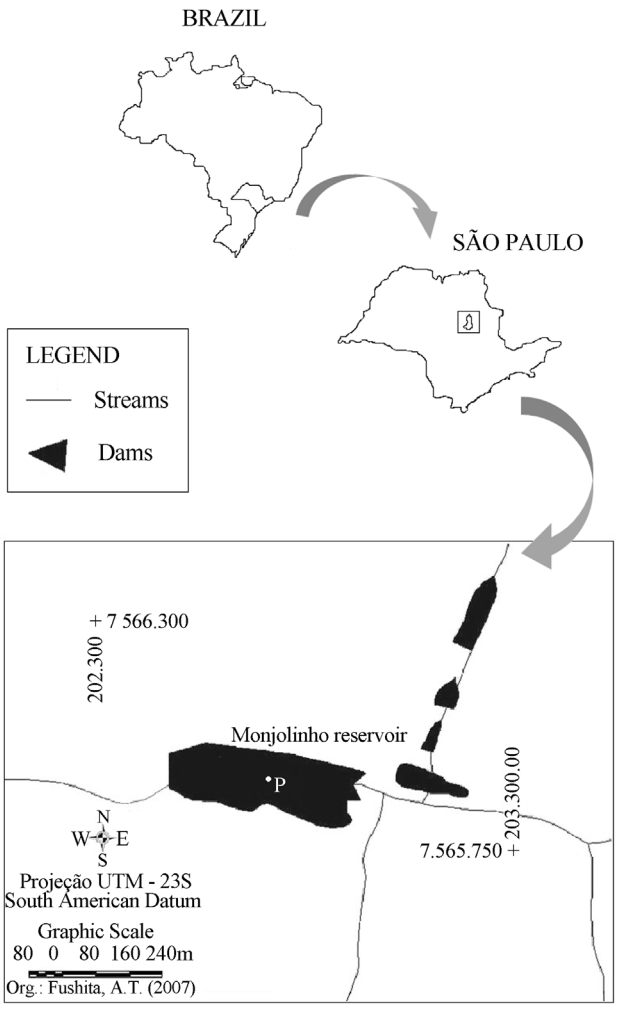

Figure 1 - Location of Monjolinho reservoir and sampling station (P). Modified from Santos (2009).

Sampling and organisms density analyzes

During 2009, grazing experiments were carried out in a central station of the reservoir (47° 52' W and 21° 59' S) (Figure 1) every two months, totalizing six surveys. At the study site, pH, dissolved oxygen (mgO_2 L^{-1}), temperature (°C) and electrical conductivity (μScm^{-1}) of water surface were measured with a multiparameter probe (Horiba U-10). Water transparency (m) was measured with Secchi disc. The reservoir trophic state index (TSI) was calculated according to Carlson (1977) modified by Toledo *et al.* (1983). The concentration of organic material in the water was determined by gravimetric technique using GF/C (Whatman®) filters (Teixeira *et al.*, 1965).

The planktonic ciliates density was estimated from three 200 mL surface water samples (replicates) fixed, in the field, in snap-cap flasks with a mercuric chloride ($HgCl_2$) saturated solution and stained with bromophenol blue 0.04% (Pace and Orcutt, 1981). At the laboratory, after sedimentation and removal of supernatant, the remaining material (concentrated) of each flask was quantified in three Sedgwick-Rafter chambers of 1 mL under optical microscope (100x magnification) (Margalef, 1969). During counts, the ciliates were identified at genus and species level according to the literature (Foissner and Berger, 1996; Foissner, 1999).

The bacteria and nanoflagellate densities were estimated from three 100 mL water samples (replicates) stored in dark flasks and fixed with neutral formaldehyde (2% final concentration). At the laboratory, the samples were stained with 4',6'-diamidino-2-phenylindol (DAPI) (Porter and Feig, 1980) and filtered through 0.2 μm and 1 μm pore size black polycarbonate membranes (Nuclepore®) for bacteria and total nanoflagellates (TNF), respectively (Sherr *et al.*, 1993). The organisms were quantified and measured under epifluorescence microscope (Olympus BHS-313) with UV light filter set, at 1250x magnification. The autotrophic nanoflagellates (ANF) were quantified and measured under the same microscope using a blue light filter set. The number of heterotrophic nanoflagellates (HNF) was obtained by the difference between the number of TNF and ANF. The bacteria and nanoflagellate densities were calculated according to Jones (1979). Due to the difficulty in identifying the nanoflagellates at the epifluorescence microscope, they were only classified into three length classes: Class I (< 5.0 μm); Class II (5-10 μm) and Class III (> 10.0 μm).

Bacterivory experiments

Before the bacterivory experiments, total bacterioplankton density in the reservoir was estimated from the samples, as described above, and it resulted 106.0 x 10^4 cells mL^{-1}, in average. Bacterivory experiments were performed in situ with Escherichia coli bacteria fluorescently labeled (FLB) using 5- (4,6 diclorotriazin-2yl) aminofluo-

rescein (DTAF) (Sherr *et al.*, 1987). As determined by Sherr *et al.* (1989), to evaluate ciliates bacterivory, in a screw cap bottle containing 500 mL of water reservoir FLB were added in a rate of 5% of the total bacterioplankton density (which corresponded here to 5.3 x 10^4 FLB mL^{-1}). To evaluate HNF bacterivory, FLB were added in another bottle in a rate of 30% of the total bacterioplankton density (which corresponded here to 3.2 x 10^5 FLB mL^{-1}). Different FLB concentrations were used for ciliates and HNF in order to allow a clear visualization of bacteria inside the protozoans. After FLB addition, the bottles were incubated and subsamples of 50 mL were taken after 0, 3, 6, 10, 20 and 30 min. The subsamples were immediately fixed with 10 μL of alkaline lugol solution 0.5%, 0.5 mL of formalin buffered with borax, and 20 μL of sodium thiosulfate solution 3% to clear the Lugol color (Sherr and Sherr, 1993).

Twenty milliliters of the fixed subsamples were stained with DAPI, filtered on 0.8 μm size pore black polycarbonate membranes and analyzed under epifluorescence microscope (Olympus BHS-313) with UV light filter sets, in order to locate the protozoa, and blue light filter, for counting FLB inside the ciliates and HNF vacuoles. The relation FLB $cell^{-1}$ was plotted separately for ciliates and HNF on graphics by the incubation time, and the FLB uptake rate (FLB $cell^{-1}$ h^{-1}) for each group of organism was obtained by the linear regression of the graphic curve. The individual ingestion rate (bact $cell^{-1}$ h^{-1}) was obtained by multiplying the uptake rate by the ratio between the environment bacterioplanktonic density and the density of FLB added to the bottles. The individual clearance rates (nL $cell^{-1}$ h^{-1}) was obtained by dividing the uptake rate by the concentration of FLB nL^{-1} in the sample. In order to obtain the ingestion of the population per hour (bact mL^{-1} h^{-1}), the individual rates were multiplied by the total number of individuals of the analyzed taxon mL^{-1} (Sherr and Sherr, 1993).

Statistical analyzes

The possible differences in environmental variables found for dry and rainy seasons were analyzed by Student's *t*-test and Fisher's *f*-test with $\alpha = 0.05$. Potential relationships among the different environmental variables, densities, ingestion and clearance rates were tested by Spearman's correlation test (r_s) with $p < 0.05$. The Principal Component Analysis (PCA) was used as a method of ordination of the correlations among physical, chemical and biological water variables. The statistical analyzes were performed with the software XL Stat Pro 2008.

Results

Physical and chemical variables (Table 1) presented significant differences between dry (April to September) and rainy (October to March) seasons, except for precipitation and organic material. The trophic state index pointed the reservoir as a mesotrophic environment during the dry period and a eutrophic environment during the rainy period.

Figure 2 shows, through the principal component analysis (PCA), the distinction between the sampled months, defining the seasonal pattern with a rainy (November, January and March) and dry (May, July and September) period. The dry season was marked by higher values of oxygen concentrations, pH, water transparency and conductivity. The rainy season was marked by higher values of temperature, trophic state index (TSI), precipitation and organic material.

The densities of ciliates, HNF and bacteria (Table 2) presented no significant differences between dry and rainy seasons. However, specifically during May (dry season), it was observed lower densities of ciliates and HNF (4 cells mL^{-1} and 47 cells mL^{-1}, respectively), whereas higher densities were observed during the rainy season (October to March), mainly in January (16 cells mL^{-1} for ciliates and 633 cells mL^{-1} for HNF).

In dry and rainy seasons, the ciliate group with highest density was Oligotrichida with maximum value in March (Figure 3). During the dry season this group accounted for 23% of the total density of ciliates, while in the rainy season it accounted for 31%. The predominant ciliate species was *Halteria grandinella* (Oligotrichida), which corresponded to 12% of the total ciliate density in the reservoir. The second predominant species was *Halteria* sp.

Table 1 - Values of physical and chemical variables of Monjolinho reservoir during the rainy/warm (November, January, March) and dry/cold (May, July, September) seasons. TSI (Trophic State Index); OM (Organic Material).

Period	pH	Temperature (°C)	Dissolved Oxygen (mg L^{-1})	Conductivity (μS cm^{-1})	Transparency (cm)	Precipitation (mm)	OM (mg L^{-1})	TSI
November	4.0	23.7	6.3	42.0	45.0	250.0	2.3	56.0
January	5.3	23.7	5.3	46.0	38.0	330.0	2.6	57.6
March	4.8	25.6	6.5	44.0	68.0	305.0	6.8	54.2
May	5.0	15.3	4.0	50.0	145.0	35.0	5.0	49.8
July	6.3	19.3	6.7	33.0	110.0	75.0	1.9	51.8
September	4.5	23.8	6.5	68.0	135.0	130.0	2.2	53.8
AnnualMean	4.9	21.9	5.8	47.1	90.1	187.5	3.4	53.7

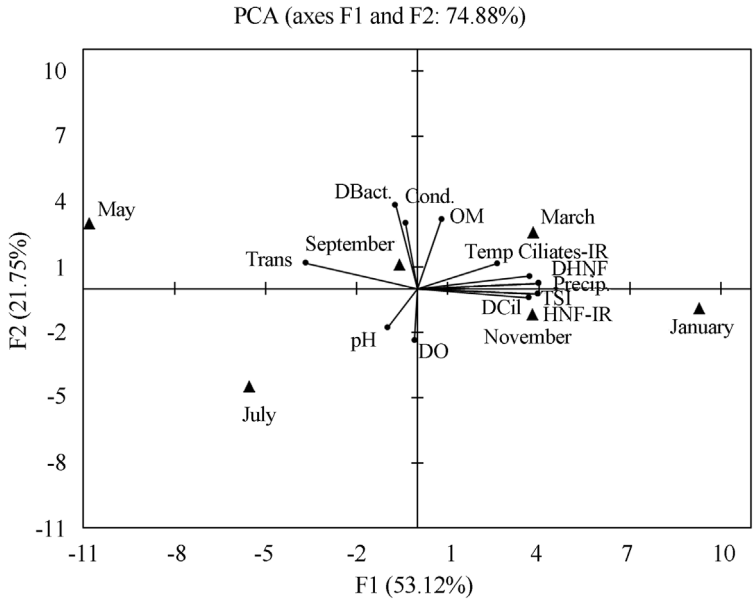

Figure 2 - PCA ordination diagram with the environmental variables registered during the studied period. DO (Dissolved Oxygen); Trans (Water Transparency); Temp (Temperature); OM (Organic Material); TSI (Trophic State Index); Precip. (Precipitation); Cond. (Conductivity); DBact. (Density of Bacteria); DCil. (Density of Ciliates); DHNF (Density of Heterotrophic Nanoflagellates); Ciliates-IR (Ciliates Population Ingestion Rate); HNF-IR (Heterotrophic Nanoflagellates Population Ingestion Rate).

(Oligotrichida; 10%), followed by *Tintinnidium* sp. (Oligotrichida; 9.6%), *Chilodontopsis depressa* (Nassulida; 7%) and *Euplotes* sp. (Hypotrichia; 5%).

Among HNF, those < 5 μm predominated in the reservoir, especially during the rainy season (October to March) (Figure 4). During this season, they corresponded to 48% of the total HNF density in November, 55% in January and 52% in March. During the dry season, there was a different trend among HNF: those between 5 and 10 μm predominated in July (45% of the total HNF quantified in this month) and HNF > 10 μm predominated in September (39%).

HNF population ingestion rates (Table 3) presented significant differences between the dry and rainy periods. The highest HNF population ingestion rate (27,583 bacteria h^{-1} mL^{-1}) was observed in January (rainy period), while the lowest (1,939 bacteria h^{-1} mL^{-1}) was observed in May (dry period). The ciliates population ingestion rates were also higher in the rainy period (Table 3), what can be seen through PCA diagram (Figure 2).

Significant differences among ingestion and clearance rates of ciliates and HNF were also found. Considering population ingestion rates, HNF ones were higher than those of ciliates in all months; however, individually, the ingestion and clearance rates of ciliates (annual mean of 44 bacteria cell $^{-1}$ h^{-1} and 164 nL cell $^{-1}$ h^{-1}, respectively) were higher than those of HNF in all months (annual mean of 23 bacteria cell $^{-1}$ h^{-1} and 83 nL cell $^{-1}$ h^{-1}, respectively) (Table 3).

HNF population ingestion rates were positively correlated with precipitation, TSI and the density of HNF and ciliates, and negatively correlated with water transparency. Ciliates population ingestion rates were also positively correlated with precipitation and HNF density. The density of HNF correlated positively with precipitation and TSI, while the density of ciliates correlated positively with TSI (Table 4).

Discussion

In Monjolinho reservoir, the summer precipitation and the algal blooms that start at the end of the dry season probably produced inputs of nutrient and suspended solids in the system, decreasing the water transparency in the rainy/warm season, when the system became eutrophic, as already was reported to this environment (Santos *et al.*, 2011). The precipitation also causes an increase of suspended inorganic particles in the water column as well as the resuspension of benthic organisms, including some protozoa such as *Tintinnidium* sp. (which contain inorganic material composing their lorica), raising their densities, what can partially explains the highest abundance of HNF, bacteria and ciliates observed in plankton during the rainy period (Table 2). This is corroborated with the positive correlations observed between precipitation and the density of ciliates and HNF (Table 4).

The higher densities of protozoa in this season were also associated with the increasing eutrophication of the reservoir, in view of the positive correlations found between the densities of protozoa (ciliates and HNF) and the trophic state index. According to Pereira *et al.* (2005), eutrophication increases significantly the organisms abundance, affecting in a strong way especially the variation

Table 2 - Ciliates, heterotrophic nanoflagellates (HNF) and bacteria densities (cells mL^{-1}), mean densities (cells mL^{-1}) and standard deviations (SD) in Monjolinho reservoir during the rainy/warm (November, January, March) and dry/cold (May, July, September) seasons.

Months	Ciliates (cells mL^{-1})				HNF (cells mL^{-1})				Bacteria (cells mL^{-1} x10^6)			
Replicates	1	2	3	Mean ± SD	1	2	3	Mean ± SD	1	2	3	Mean ± SD
November	13.6	14.5	15.4	14.5 ± 0.9	416.0	374.0	695.0	495.0 ± 174.4	2.5	3.0	2.0	2.5 ± 0.5
January	14.0	11.5	22.5	16.0 ± 5.7	230.0	748.0	921.0	633.0 ± 359.5	2.0	2.4	3.0	2.5 ± 0.4
March	14.6	9.9	11.3	12.0 ± 2.4	148.0	731.0	621.0	500.0 ± 309.7	2.0	3.5	4.5	3.3 ± 1.2
May	4.0	4.5	2.8	3.7 ± 0.8	43.0	18.0	79.0	47.0 ± 30.6	3.0	4.0	3.0	3.3 ± 0.5
July	2.8	8.1	7.7	6.2 ± 2.9	473.0	382.0	555.0	470.0 ± 86.5	2.0	2.1	1.9	2.0 ± 0.1
September	16.6	10.5	15.0	14.0 ± 3.1	523.0	400.0	556.0	493.0 ± 82.2	2.5	3.0	2.5	2.6 ± 0.2
Annual mean	11.0	9.8	12.4	11.0 ± 2.6	305.5	442.1	571.1	439.0 ± 173.8	2.3	3.0	2.8	2.7 ± 0.5

Table 3 - Ciliates and heterotrophic nanoflagellates (HNF) population ingestion (bact h^{-1} mL^{-1}), individual ingestion (bact cell $^{-1}$ h^{-1}) and clearance (nL cell $^{-1}$ h^{-1}) rates in Monjolinho reservoir during the rainy/warm (November, January, March) and dry/cold (May, July, September) seasons.

Months	Ciliates			HNF		
	Individual ingestion rates (bact cell $^{-1}$ h^{-1})	Individual clearance rates (nL cell $^{-1}$ h^{-1})	Population ingestion rates (bact h^{-1} mL^{-1})	Individual ingestion rates (bact cell $^{-1}$ h^{-1})	Individual clearance rates (nL cell $^{-1}$ h^{-1})	Population ingestion rates (bact h^{-1} mL^{-1})
November	30.0	120.0	436.0	19.0	75.0	9,281.0
January	50.0	200.0	797.0	44.0	175.0	27,583.0
March	64.0	193.0	766.0	13.0	41.0	6,703.0
May	48.0	146.0	182.0	41.0	125.0	1,939.0
July	34.0	171.0	213.0	9.0	47.0	4,406.0
September	40.0	153.0	558.0	10.0	38.0	4,927.0
Annual mean	44.0	164.0	492.0	23.0	83.0	9,140.0

patterns of HNF. Another factor that raises the organisms abundances in aquatic systems is temperature, once, according to Gillooly *et al.* (2001), it increases the communities' metabolism and the reproductive rates of the organisms. Although no significant correlations were found in the present study between the protozoan densities and temperature, higher temperature values characterized the rainy season (Figure 2).

All these factors probably contributed to make the mean density of HNF in the reservoir higher than the estimated by Sanders *et al.* (1992), who consider 300 cell mL^{-1} the limit value for nanoflagellates populations in aquatic systems. Nevertheless, the mean density of HNF found in the present study was according to the values found by Araújo and Costa (2007) in Brazilian subtropical environments.

The environmental conditions possibly affected also the composition of HNF in the reservoir. The predominance in July and September (Figure 4) of flagellates larger than 3 to 4 μm, may be related to the mesotrophic conditions of the system and the algal blooms, since they are

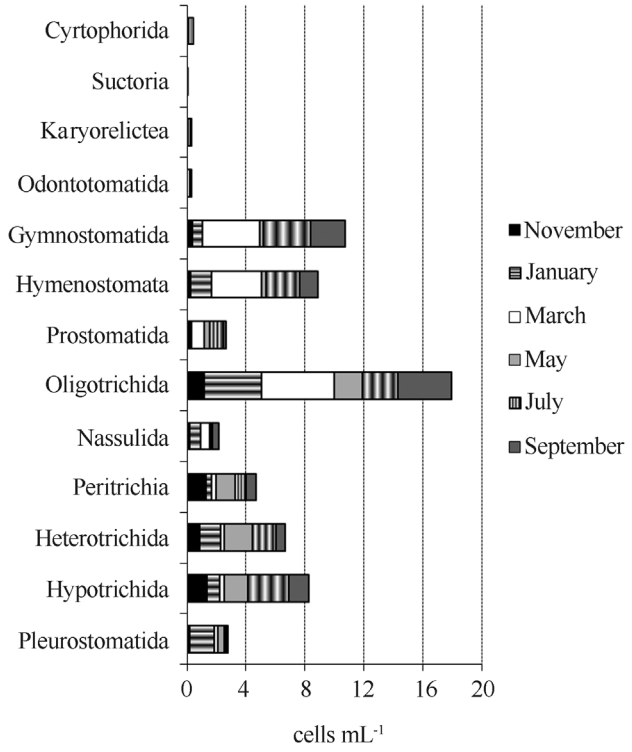

Figure 3 - Density of the taxonomic groups of ciliates found in Monjolinho reservoir during the rainy/warm (November, January, March) and dry/cold (May, July, September) seasons.

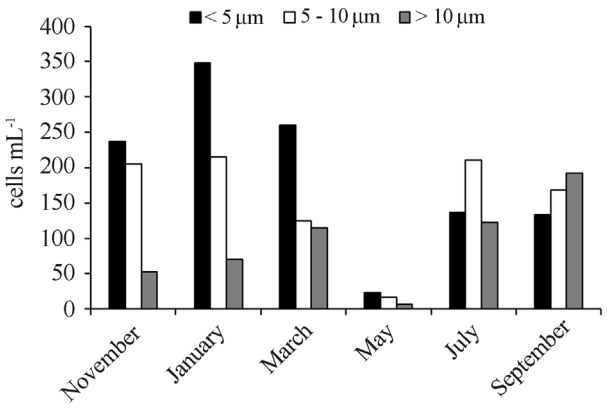

Figure 4 - Densities of different size classes of HNF found in Monjolinho reservoir during the rainy/warm (November, January, March) and dry/cold (May, July, September) seasons.

Table 4 - Spearman's correlation coefficients among the protozoan densities, population ingestion rates and the environmental variables in Monjolinho reservoir. Temp (Temperature); DO (Dissolved Oxygen), Cond. (Conductivity); Trans.(Water Transparency); Precip. (Precipitation); TSI (Trophic State Index); OM(Organic Material); DCil. (Density of Ciliates); DHNF (Density of Heterotrophic Nanoflagellates); DBact. (Density of Bacteria); Ciliates-IR (Ciliates Population Ingestion Rate); HNF-IR (Heterotrophic Nanoflagellates Population Ingestion Rate); $p < 0.05$.

Variables	pH	Temp.	DO	Cond.	Trans.	Precip.	TSI	OM	DCil.	DHNF	DBact.	Ciliates-IR
pH	-											
Temp.	-0.49	-										
DO	0.11	0.38	-									
Cond.	-0.25	0.14	-0.46	-								
Trans.	0.02	-0.34	0.02	0.42	-							
Precip.	-0.14	0.66	-0.02	-0.08	**-0.88**	-						
TSI	-0.25	0.52	-0.11	-0.14	**-0.94**	**0.94**	-					
OM	-0.14	0.23	-0.55	0.25	-0.08	0.31	0.14	-				
DCil.	-0.31	0.46	-0.11	0.02	-0.82	0.82	**0.94**	-0.08	-			
DHNF	-0.14	0.66	-0.02	-0.08	**-0.88**	**1.00**	**0.94**	0.31	0.82	-		
DBact.	-0.35	0.26	-0.40	0.61	0.44	-0.08	-0.26	0.79	-0.35	-0.08	-	
Ciliates-IR	-0.08	0.75	0.05	0.14	-0.71	**0.94**	0.82	0.25	0.77	**0.94**	0.00	-
HNF-IR	-0.25	0.52	-0.11	-0.14	**-0.94**	**0.94**	**1.00**	0.14	**0.94**	**0.94**	-0.26	0.82

Values in bold are significantly different from 0 with significance level $\alpha = 0.05$.

known to ingest larger preys than bacteria (Gonzalez, 1999) and the phytoplankton was possibly their main prey source in this period. In all other months, HNF < 5 μm were the most abundant and possibly the main responsible for predation on bacteria. The predominance of HNF < 5 μm as major bacterial consumers is expected, since they are a dominant planktonic class, formed mainly by bacterivorous organisms (Sherr and Sherr, 2002). The grazing pressure of small HNF (2-3 μm size) on bacteria was also higher than that of larger HNF in the study of Tsai *et al.* (2011).

The mean density of ciliates, as well as the predominant groups and species of ciliates found in the reservoir were similar with other subtropical environments (Araújo and Costa, 2007; Pauleto *et al.*, 2009). According to Gilbert (1994), the success of *Halteria grandinella* and *Halteria* sp. in aquatic systems are associated to their jumping ability as a strategy to escape from cladocerans and rotifers predation. Another important factor is the wide diet of this genus (they feed on several trophic levels organisms: bacteria, algae, flagellates, etc), that may be a selective advantage, when compared to specialized bacterivorous or algivores ciliates, and results in the widespread occurrence of *Halteria* in freshwater plankton (Jürgens and Simek, 2000). The genus *Halteria* and other Oligotrichida species, such as *Tintinnidium* sp., are frequently reported in Brazilian aquatic environments (*e.g.* Araújo and Costa, 2007; Pauleto *et al.*, 2009) and, according to Simek *et al.* (2000), are responsible for the most part of ciliates bacterivory in aquatic systems. Due to their higher density they were possibly the main bacterivorous ciliates also in Monjolinho reservoir during the studied period.

The higher individual ingestion and clearance rates of ciliates (Table 3) than HNF are explained by their morphology and size. Ciliates are, in general, larger than the nanoflagellates and have their feeding apparatus surrounded by cilia (Lee and Kugrens, 1992), which, together with the constant water flow, favors the ingestion of large amounts of bacteria (Fenchel, 1980). So, ciliates can be more efficient than HNF on preying bacteria in some systems, as pointed by some authors (Kisand and Zingel, 2000; Zingel *et al.*, 2006). In the reservoir, the positive correlation observed between the density of HNF and ciliates population ingestion rates suggests that HNF population may being also controlled by ciliates predation, as already reported in literature (*e.g.* Johansson *et al.*, 2004). Thereby, bacterial community control by ciliates occurs not only directly, but also indirectly, in a microbial cascade.

Despite the importance of ciliates, their higher population predation efficiency does not occur in all eutrophic environments, but only in those where bacterial densities are enough to maintain the bacterivorous ciliate populations higher than HNF populations (Kisand and Zingel, 2000). Indeed, in Monjolinho reservoir, despite the higher individual rates of ciliates, HNF dominated the ingestion of bacteria in terms of population (Table 3), causing a more expressive impact on bacterial community, due to their higher abundance in the reservoir when compared to ciliates. Individual bacterivory rates higher for ciliates than HNF but population rates higher for HNF were also found by several authors (Sanders *et al.*, 1989; Ichinotsuka *et al.*, 2006; Tsai *et al.*, 2007). Additionally, in the study of Sanders *et al.* (1989) nanoflagellates population ingestion rates accounted for 79% of the total bacterivory in a freshwater eutrophic environment.

The higher abundances of protozoans during the rainy season explain the higher population ingestion rates, especially of HNF. Similar seasonal patterns with higher protozoans grazing rates during summer periods were also found by other authors in subtropical environments (Tsai *et al.*, 2011, 2012).

Besides density, other factors, such as TSI, nutrients, light, precipitation and temperature can also affect the organisms feeding behavior and top-down relationships (Hall *et al.*, 2004; Unrein *et al.*, 2007; Hoekman, 2010). It is known that in some temperate environments, besides the predation pressure on bacteria, bottom-up forces are important in controlling bacterial communities (Muylaert *et al.*, 2002), especially during the colder periods of the year (Solic *et al.*, 2009). In Monjolinho reservoir, this also happened. Despite the higher predation pressure on bacteria in the summer, during the dry/cold season (when the predation pressure by protozoa was lower) the bacterial densities did not raised, suggesting that in this period, characterized by mesotrophic conditions, the bottom-up control could be acting more efficiently in regulating the bacterial assemblages. The study performed by Santos *et al.* (2011) in the reservoir showed that during the dry period the system is indeed nutritionally poorer, reinforcing this bottom-up hypothesis.

In the subtropical reservoir studied, results showed that the environmental variables in interaction with the substrate sources and the density of organisms of the food web structure were extremely relevant in determining the seasonal oscillation of bacterivory. Bacteria were more strongly regulated by protozoan predation (top-down) during the rainy/warm season, characterized by a higher trophic level. Among protozoans, small HNF impacted more bacterial community structure by predation effect.

Acknowledgments

We thank Darci C.D. Javaroti, Luciana H. Hayashi and Henrique C. Alves for laboratory assistance and FAPESP (Foundation for Research Support of São Paulo-Process n° 2008/03545-0) for the scholarship awarded to Karina Ferreira Hisatugo.

References

Araújo MFF, Costa IAS (2007) Comunidades microbianas (bacterioplâncton e protozooplâncton) em reservatórios do semi-árido brasileiro. Oecol Bras 11:422-432.

Bell T, Bonsall MB, Buckling A, Whiteley AS, Goodall T, Griffiths RI (2010) Protists have divergent effects on bacterial diversity along a productivity gradient. Biol Lett 6:639-642.

Carlson RE (1977) A trophic state index for lakes. Limnol Oceanogr 22:261-269.

Corno G, Caravati E, Callieri C, Bertoni R (2008) Effects of predation pressure on bacterial abundance, diversity, and size-structure distribution in an oligotrophic system. J Limnol 67:107-119.

Fenchel T (1980) Suspension feeding in ciliated protozoa: Functional response and particle size selection. Microb Ecol 6:1-11.

Foissner W (1999) *Identification and ecology of limnetic plankton ciliates*. Informationsberichte, Munich.

Foissner W, Berger H (1996) A user friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. Freshwater Biol 35:375-482.

Gasol JM, Pedrós-Alió C, Vaqué D (2002) Regulation of bacterial assemblages in oligotrophic plankton systems: results from experimental and empirical approaches. Antonie Leeuwenhoek 81:435-452.

Gilbert JJ (1994) Jumping behavior in the oligotrich ciliates *Strobilidium velox* and *Halteria grandinella* and its significance as a defense against rotifer predators. Microb Ecol 27:189-200.

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. Science 293:2248-2251.

Gonzalez J (1999) Bacterivory rate estimates and fraction of active bacterivores in natural protist assemblages from aquatic systems. Appl Environ Microbiol 65:1463-1469.

Hall SR, Leibold MA, Lytle DA, Smith VH (2004) Stoichiometry and planktonic grazer composition over gradients of light, nutrients, and predation risk. Ecology 85:2291-2301.

Hoekman D (2010) Turning up the heat: Temperature influences the relative importance of top-down and bottom-up effects. Ecology 91:2819-2825.

Ichinotsuka D, Ueno H, Nakano SI (2006) Relative importance of nanoflagellates and ciliates as consumers of bacteria in a coastal sea area dominated by oligotrichous *Strombidium* and *Strobilidium.* Aquat Microb Ecol 42:139-147.

Johansson M, Gorokhova E, Larsson U (2004) Annual variability in ciliate community structure, potential prey and predatorsin the open northern Baltic Sea proper. J Plankton Res 26:67-80.

Jones JG (1979) A guide to methods for estimating microbial numbers and biomass in fresh water. Scient Public Biol Ass 39:112.

Jürgens K, Simek K (2000) Functional response and particle size selection of *Halteria* cf. *grandinella*, a common freshwater oligotrichous ciliate. Aquat Microb Ecol 22:57-68.

Kisand V, Zingel P (2000) Dominance of ciliate grazing on bacteria during spring in a shallow eutrophic lake. Aquat Microb Ecol 22:135-142.

Köppen W (1931) *Grundriss der Klimakunde*. De Gruyter, Berlin.

Lee R, Kugrens P (1992) Relationship between the flagellates and the ciliates. Microbiol Mol Biol Rev 56:529-542.

Margalef R (1969) Counting. *In*: Sournia, A. (ed).*Phytoplankton Manual.* United Nation Educational, Scientific and Cultural Organization, Paris, p. 92.

Muylaert K, Van der Gucht K, Vloemans N, De Meester L, Gillis M, Vyverman W. (2002) Relationship between bacterial community composition and bottom-up *vs.* top-down variables in four eutrophic shallow lakes. Appl Environ Microbiol 68:4740-4750.

Nogueira MG, Matsumura-Tundisi T (1994) Limnology of a shallow artificial system (Monjolinho reservoir-São Carlos-SP). Rev Bras Biol 54:147-159.

Nogueira MG, Matsumura-Tundisi T (1996) Limnology of a shallow artificial system (Monjolinho reservoir-São Carlos-SP): planktonic population dynamics. Acta Limnol Bras 8:149-168.

Pace ML, Orcutt Jr JD (1981) The relative importance of protozoans, rotifers and crustaceans in a freshwater zooplankton community. Limnol Oceanogr 26:822-830.

Pagano M (2008) Feeding of tropical cladocerans (*Moina micrura*, *Diaphanosoma excisum*) and rotifer (*Brachionus calyciflorus*) on natural phytoplankton: effect of phytoplankton size-structure. J Plankton Res 30:401-414.

Pauleto GM, Velho LFM, Buosi PRB, Brão AFS, Lansac-Tôha FA, Bonecker CC (2009) Spatial and temporal patterns of ciliate species composition (Protozoa: Ciliophora) in the plankton of the Upper Paraná River floodplain. Braz J Biol 69:517-527.

Pereira DG, Velho LFM, Pagioro TA, Lansac-Tôha FA (2005) Abundância de nanoflagelados heterotróficos no plâncton de reservatórios com distintos graus de trofia. Acta Sci Biol Sci 27:43-50.

Pernthaler J (2005) Predation on prokaryotes in the water column and its ecological implications. Nature 3:537-546.

Pomeroy LR, Williams PJI, Azam F, Hobbie JE (2007) The microbial loop. Oceanography 20:28-33.

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943-948.

Regali-Seleghim MH, Godinho MJL (2004) Peritrich epibiont protozoans in the zooplankton of a subtropical shallow aquatic ecosystem (Monjolinho Reservoir, São Carlos, Brazil). J Plank Res 26:501-508.

Sanders RW, Caron DA, Berninger UG (1992) Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-ecosystem comparison. Mar Ecol Progr Ser 86:1-14.

Sanders RK, Porter KG, Bennett SJ, DeBiase AE (1989) Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. Limnol Oceanogr 34:673-687.

Santos MG (2009) *Decomposição aeróbia de Myriophyllum aquaticum (Vell.) Verdc. e caracterização limnológica na bacia hidrográfica do rio do Monjolinho (São Carlos, SP, Brasil).* São Carlos, Brasil, 135 p. (M.Sc. Dissertation. Departamento de Hidrobiologia. UFSCar).

Santos MG, Cunha-Santino MB, Júnior IB (2011) Alterações espaciais e temporais de variáveis limnológicas do reservatório do monjolinho (*campus* da UFScar). Oecol Aust 15:682-696.

Sherr BF, Sherr EB, Fallon RD (1987) Use of monodispersed fluorescently labeled bacteria to estimate in situ protozoan bacterivory. Appl Environ Microbiol 53:958-965.

Sherr BF, Sherr EB, Pedrós-Alió C (1989) Simultaneous measurement of bacterioplankton production and protozoan bacterivory in estuarine water. Mar Ecol Prog Ser 54:209-219.

Sherr EB, Caron DA, Sherr BF (1993) Staining of heterotrophic protists for visualization via epifluorescence microscopy.*In*: Kemp, P.F.; Sherr, B.F.; Sherr, E.B.; Cole, J.J. (Eds). *Hand-*

book of methods in Aquatic Microbial Ecology.Lewis Publisher, Boca Raton, USA, 777 p.

Sherr EB, Sherr BF (1993) Protistan grazing rates via uptake of fluorescently labeled prey. *In*: Kemp, P.F; Sherr, B.F; Sherr, E.B. (Eds).*Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publishers, Boca Raton, USA, 695-701.

Sherr EB, Sherr BF (2002) Significance of predation by protists in aquatic microbial food webs. Antonie van Leeuwenhoek 81:293-308.

Simek K, Jürgens K, Nedoma J, Comerma M, Armengol J (2000) Ecological role and bacterial grazing of *Halteria* spp.: small freshwater oligotrichs as dominant pelagic ciliate bacterivores. Aquat Microb Ecol 22:43-56.

Solic M, Krstulovic N, Vilibic I, Bojanic N, Kuspilic G, Sestanovic S, Santic D, Ordulj M (2009)Variability in the bottom-up and top-down controls of bacteria on trophic and temporal scales in the middle Adriatic Sea. Aquat Microb Ecol 58:15-29.

Teixeira C, Tundisi JG, Kutner MB (1965) Plankton studies in a mangrove environment. II. The standing stock and some ecological factors. Boletim do Instituto Oceanografico 24:23-24.

Toledo AP, Talarico M, Chinez SJ, Agudo EG (1983) A aplicação de modelos simplificados para a avaliação do processo da eutrofização em lagos e reservatórios tropicais. Congresso Brasileiro de Engenharia Sanitária e Ambiental, Santa Catarina, SC, p.1-34.

Tsai AY, Chiang KP, Chan YF, Lin YC, Chang J (2007) Pigmented nanoflagellates in the coastal western subtropical Pacific are important grazers on *Synechococcus* populations. J Plankton Res 29:71-77.

Tsai AY, Gong GC, Sanders RW, Chen WH, Chao CH, Chiang KP (2011) Importance of bacterivory by pigmented and heterotrophic nanoflagellates during the warm season in a subtropical western Pacific coastal ecosystem. Aquat Microb Ecol 63:9-18.

Tsai AY, Gong GC, Hung J (2012) Seasonal variations of viral- and nanoflagellate- mediated mortality of heterotrophic bacteria in the coastal ecosystem of subtropical Western Pacific. Biogeosciences Discuss 9:17235-17261.

Unrein F, Massana R, Alonso-Sáez L, Gasol JM (2007) Significant year-round effect of small mixotrophic flagellates on bacterioplankton in an oligotrophic coastal system. Limnol Oceanogr 52:456-469.

Zingel P, Agasild H, Noges T, Kisand V (2006) Ciliates are the dominant grazers on pico- and nanoplankton in a shallow, naturally highly eutrophic lake. Microb Ecol 53:134-142.