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Protozooplankton and its relationship with environmental conditions in 13 water bodies of the Mogi-Guaçu basin - SP, Brazil

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Abstract: Protozooplankton is an important component of the aquatic microbial food webs and its composition, density, and distribution reflect the chemical, physical, and biological aspects of the environment. Considering the scarce literature on freshwater protozoans in Brazil and on protozoan ecology in subtropical environments, we listed the ciliates and amoebae taxa found in 13 water bodies in São Paulo State and analyzed their abundance in relation to the environmental variables. We collected two samples in each environment, fixed immediately with mercuric chloride and stained with bromophenol blue. After microscopical analysis, 74 protozoan genera were identified and the Ciliophora were dominant in the majority of the environments. The Stichotrichia, represented mostly by the genus *Halteria*, occurred in all environments, and was the dominant subclass in five of them. The canonic correspondence analysis of the most frequent genera and the environmental variables showed that nitrite and nitrate were the variables that better explained the distribution of *Limnostrombidium*, *Urotricha*, and *Vorticella*. The densities of the genera *Halteria*, *Coleps*, and of the species *Cinetochilum margaritaceum* were positively affected by increasing concentrations of dissolved oxygen, particulate phosphate, conductivity, and temperature. *C. margaritaceum* were also negatively affected by increasing concentrations of nitrite and nitrate. Considering that we made only one sampling in each environment, the richness was high compared to the mean diversity of lakes in the São Paulo State. The Diogo Lake, located in an ecological reserve, was the richest one, confirming the need of more research on the biodiversity of more preserved environments.
Keywords: *ciliates, amoebae, plankton, environmental variables, freshwater.*

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Resumo: O protozooplâncton é um componente importante da rede trófica microbiana de ambientes aquáticos e sua composição, densidade e distribuição refletem os aspectos físicos, químicos e bióticos do ambiente. Considerando a escassa literatura sobre protozoários de água doce no Brasil e sobre sua ecologia em ambientes subtropicais, inventariamos os táxons de ciliados e amebas em 13 corpos d'água do Estado de São Paulo e analisamos a variação na abundância dos gêneros/espécies de maior incidência em relação às variáveis ambientais. Coletamos duas amostras por ambiente, fixando-as com cloreto de mercúrio e corando-as com azul de bromofenol para posterior quantificação e identificação em microscópio ótico. Identificamos 74 gêneros de ciliados e amebas, e os Ciliophora dominaram na maioria dos ambientes. A subclasse Stichotrichia ocorreu em todos os ambientes, predominando em cinco deles, especialmente pela ocorrência o gênero *Halteria*. A Análise de Correspondência Canônica mostrou que as concentrações de nitrito e nitrato são as principais variáveis que explicam a distribuição dos gêneros *Limnostrombidium*, *Urotricha* e *Vorticella*. O aumento na concentração de oxigênio dissolvido, condutividade, temperatura e concentração de fosfato particulado afetou positivamente a densidade dos gêneros *Halteria* e *Coleps* e da espécie *Cinetochilum margaritaceum*, que foi ainda influenciada negativamente pelo aumento nas concentrações de nitrito e nitrato. Considerando-se que foi realizada apenas uma coleta, a riqueza de espécies foi alta quando comparada à média de taxa encontrada para corpos d'água do Estado de São Paulo. O ambiente mais rico, Lagoa do Diogo, localiza-se em uma estação ecológica, confirmando a necessidade de mais pesquisas sobre a diversidade em ambientes menos impactados.
Palavras-chave: *ciliados, amebas, plâncton, variáveis ambientais, água doce.*

Introduction

Protozoans can control microbial populations and also serve as food items for organisms of higher trophic levels, in addition, they act as important remineralizers and nutrient recyclers in aquatic environments (*e.g*. Beaver & Crisman 1989a). Despite their cosmopolitan distribution, they are not evenly distributed, but live in microhabitats that reflect physical, chemical, and biotic environmental aspects (Lee et al. 1985).They can be excellent biological indicators, especially due to their small size, short generation times, stress sensibility, ease of sampling, and occurrence in many types of environments (Cairns Junior et al. 1993).

Despite their ecological importance and the possibility of using the species as important tools to evaluate the degree of environmental impact caused by human activity, the protozoans have not been studied enough and the data about their diversity and distributions are scarce, especially in Asia and South America (Lévêque et al. 2005).

Studies of aquatic systems are focused on larger organisms and the number of species in Brazilian continental aquatic communities is still imprecise and difficult to estimate. Among the difficulties we can highlight the great number of hydrographic basins never surveyed; insufficient infrastructure for samplings and number of researchers, the dispersion of information that are often difficult to access, and the need for taxonomic revision for many groups (Agostinho et al. 2005).

In Brazil, studies focusing on protists started around 1910 and since the 1980s have increased (Godinho & Regali-Seleghim 1999). In São Paulo State, 75 freshwater environments have been analyzed until 2011 and 471 different protozoan *taxa*, distributed in 218 genera and 304 species, were recorded (Regali-Seleghim et al. 2011).

Since it is important to monitor the biodiversity to quantify human impacts in freshwater environments, aiming to improve their conservation (Lévêque et al. 2005), and considering the scarce data on freshwater protozoans from Brazil, we characterized the ciliate and sarcodine communities occurring in 13 water bodies of São Paulo State, which had not been studied previously, and analyzed the fluctuations of the most important genera in relation to the environmental variables.

Material and Methods

1. Studied sites

Two samples (replicates) were collected from each of the 13 shallow freshwater environments (3 m of maximum depth) located at the Mogi-Guaçu Water Resources Management Unit (UGRHI) from December 15th to 20th in 1999 (Table 1).

The Mogi-Guaçu UGRHI (Figure 1) has a catchment area of 14,653 km^2 composed by urban, industrial and rural regions. These last ones are used for livestock, poultry farming and agricultural activities that are predominantly cultures of sugar cane, coffee, citrus, corn and cotton. The main agro-industrial sectors are the sugar and alcohol, vegetable oils, beverages and cellulose and paper industries (COMPANHIA... 2001).

The region also has an important conservation area called Jataí Ecological Station, located in the municipality of Luis Antônio, where one of the water bodies was sampled, the Diogo Lake (DL). Among the other environments, the Paço Municipal Lake (PML), the Praça Basílio Ceschin Pond (PBC), the Urban Lake of Santa Cruz da Conceição (UL), the Prainha Pond (PP), the Elektro Reservoir (ER), the Araras Municipal Lake (LMA) and the David Reservoir (DR) are urban aquatic environments. The other sites are located within rural properties: Barro Preto Pond (BPP), Cabras Pond (CP), Ivo Carotini Lake (ICL), the Fazenda Aurora Reservoir (FAR) and São Geraldo Reservoir (SGR). Table 1 shows the cities locations and dates of sampling for each environment.

2. Collection and fixation of samples

Water samples were collected with a bucket from de edge of the water bodies. For protozoan analysis, we immediately fixed replicates of 200 ml aliquots with a saturated solution of mercuric chloride and stained with bromophenol blue (Pace & Orcutt 1981). The samples were concentrated by sedimentation and the supernatant was rejected. Protozoans were counted and identified in triplicates in 1 mL Sedgwick-Rafter chambers under an optic microscope (100 to 200x magnification).

For species identification, we used the following references: Bick (1972), Corliss (1979), Curds (1969), Dragesco & Dragesco-Kernéis (1986), Edmondson (1959), Foissner et al. (1991, 1992, 1994, 1995, 1999), Foissner & Berger (1996), Kahl (1930-35), Krainer (1991), Kudo (1977), Lee et al. (1985), Page (1976) and Pennak (1953). The protozoans were separated in groups according to the classification proposed by Lynn (2008) for ciliates and Adl et al. (2005) for Amoebozoa, Centrohelida and Rhizaria.

3. Physical and chemical variables

The pH, dissolved oxygen (mg O_2 L^{-1}), temperature (°C), and electrical conductivity (μS cm^{-1}) of water samples were measured with a multiparameter probe (Horiba U-10). The dissolved organic phosphate (DOP) concentration was obtained by the difference between dissolved inorganic phosphate concentration (DIP) and total dissolved phosphate concentration quantified according to

Table 1. Location and sampling date for each studied water body.

Site	City	Coordinates	Feature	Sampling date
Paço Municipal Lake (PML)	Jaboticabal	21° 15' 23.19" S and 48° 18' 27.40" W	Urban	18/12/1999
Ivo Carotini Lake (ICL)	Águas de Lindóia	22° 28' 32.36" S and 46° 37' 32.65" W	Rural	21/12/1999
Araras Municipal Lake (AML)	Araras	22° 21' 39.43" S and 47° 23' 1.24" W	Urban	21/12/1999
Urban Lake (UL)	Santa Cruz da Conceição	22° 8' 3.00" S and 47° 27' 35.63" W	Urban	15/12/1999
Cabras Pond (CP)	Guatapará	21° 29' 52.35" S and 48° 2' 10.44" W	Rural	16/12/1999
Praça Basílio Ceschin Pond (PBC)	Águas da Prata	21° 56' 3.60" S and 46° 42' 56.82" W	Urban	20/12/1999
Prainha Pond (PP)	Pitangueiras	21° 0' 30.12" S and 48° 13' 9.96" W	Urban	18/12/1999
Barro Preto Pond (BPP)	Guatapará	21° 29' 52.35" S and 48° 2' 10.44" W	Rural	16/12/1999
Diogo Lake (DL)	Luiz Antônio	21° 37' 25.57" S and 47° 48' 36.75" W	Ecological Station	16/12/1999
Elektro Reservoir (ER)	Pirassununga	21° 55' 34.94" S and 47° 22' 3.19" W	Urban	15/12/1999
Fazenda Aurora Reservoir (FAR)	Santa Cruz das Palmeiras	21° 48' 46.36" S and 47° 12' 37.62" W	Rural	15/12/1999
David Reservoir (DR)	Santa Cruz das Palmeiras	21° 49' 35.94" S and 47° 14' 44.19" W	Urban	15/12/1999
São Geraldo Reservoir (SGR)	Sertãozinho	21° 7' 37.70" S and 48° 2' 42.67" W	Rural	18/12/1999

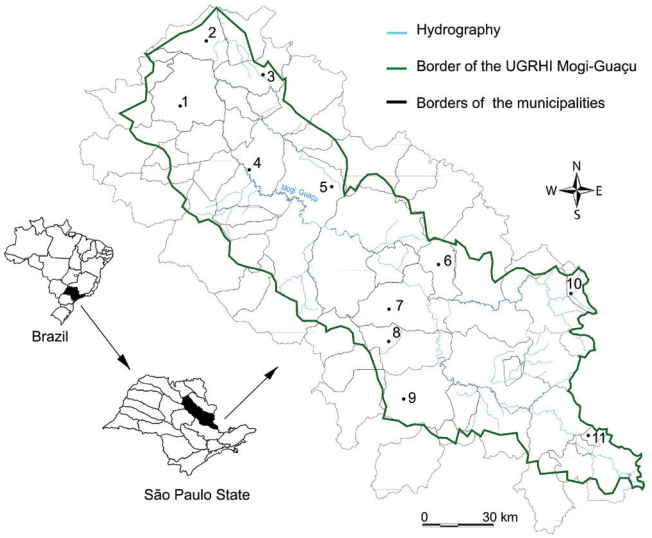

Figure 1. The Mogi-Guaçu Water Resources Management Unit (UGRHI Mogi-Guaçu). The numbers represent the municipalities sampled: 1 Jaboticabal, 2 Pitangueiras, 3 Sertãozinho, 4 Guatapará, 5 Luís Antônio, 6 Santa Cruz das Palmeiras, 7 Pirassununga, 8 Santa Cruz da Conceição, 9 Araras, 10 Águas da Prata, 11 Águas de Lindóia. (http://www.sigrh.sp.gov.br/sigrh/basecon/r0estadual/sintese/images/ugrhi09.pdf - modified).

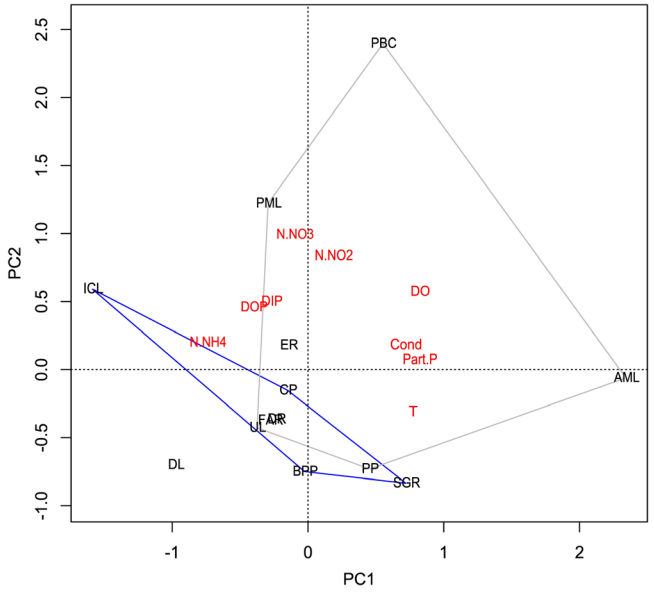

Figure 2. Diagram of Principal Component Analysis (PCA) applied to environmental variables. Proportion explained by axis: 31.5% (PC1) and 25.5% (PC2). Sites are connected by type: urban (gray line), rural (blue line) and ecological station (just site DL).

Strickland & Parsons (1960). We quantified the total nitrogen and total phosphorus according to Valderrama (1981); nitrate ($N\text{-}NO_3$) according to Mackereth et al. (1978); nitrite ($N\text{-}NO_2$) according to Bendchreider & Robinson (1952, cited in Golterman et al. 1978) and ammonium ($N\text{-}NH_4$) according to Koroleff (1976). The particulate phosphate (Part.P) was calculated by the difference between total phosphorus and total dissolved phosphate.

4. *Statistical analysis*

All statistical tests were conducted in the R computing environment (R Development Core Team 2009), with statistical significance set at $p < 0.05$. The Spearman rank correlation coefficient (ρ) was used to test linear correlations. For multivariate analyses, the environmental variables were standardized by Z-score transformation and the pH was withdrawn from the analysis due to its high linear correlation with Dissolved Oxygen ($\rho=0.84$, p-value < 0.001). An ordination diagram resulting from the Principal Component Analysis (PCA) was used to summarize the environmental variables differences among the studied sites. The optimal model for the Canonical Correspondence Analysis (CCA) was selected by the Akaike Information Criterion (AIC). In the CCA diagram, only the genera with higher frequency (occurring at least in six environments) were used.

Results

1. *Physical and chemical analyses: differences between the studied sites*

The diagram of the PCA (Figure 2) with the abiotic variables summarizes the relationships of these variables in the different studied sites, which are grouped according to the environment type: rural, urban, or conservation unit. The first axis of the PCA explained 31.4% of the variation and is mainly associated with dissolved oxygen, total particulate phosphorus, temperature, and conductivity.

The second axis of the PCA (Figure 2), which explains 25.5% of the environmental variation among the samplings (sites) is mainly associated with dissolved phosphate (organic and inorganic), nitrite and nitrate. Among the samples that had the lowest scores for this axis are the ones from the DL (–0.70) and the BPP (–0.75), in which we found the greatest richness of morphotypes: 73 and 71, respectively. These environments, unlike the other two that had the lowest scores for axis 2, the SGR (–0.83) and the PP (–0.73), also had negative scores for axis 1.

2. *Protozooplankton community structure*

We identified 69 species belonging to 50 genera, and other 24 genera were not identified to the species level, totalizing 74 genera in the 13 environments (Appendix 1). The protozoa were separated into four major groups: Ciliophora, Amoebozoa, Rhizaria and Centrohelida (Heliozoa). Amoebozoa and Rhizaria make up the artificial group of amoebas.

When the identification at lower taxonomical level was not possible, we separated the specimens by morphotypes. The morphotype was used as richness unit, however, similar morphotypes from different environments were not compared. Thus, the sum of the richness of the 13 environments should not be considered as an estimate of total richness.

Once the material was fixed, the identification of some naked amoebae was limited, since many taxonomic characteristics of this protozoan group are related to their locomotion (Page 1976). This rendered difficult the separation between the major groups Rhizaria and Amoebozoa, therefore these morphotypes were assigned as "naked amoebae" in figures and tables.

Among the 13 environments, the Diogo Lake was the richest (73 taxa), whereas the Elektro reservoir presented the lowest richness (3 taxa) (Table 2). Although not significantly different, the mean richness of rural environments (excluding the ecological station) was higher (27.4 morphotypes) than mean richness of urban environments (15 morphotypes).

In number of taxa, the Ciliophora dominated most of the environments, except São Geraldo Reservoir, where the numbers of taxa of amoebae and Ciliophora were the same (Table 2). In the plankton of Praça Basílio Ceschin Pond (PBC), Araras Municipal Lake (AML) and Elektro Reservoir (ER) there were no amoebae.

Table 2. Protozoan richness (morphotypes) and density (cell. mL^{-1}) for the studied environments: BPP (Barro Preto Pond), CP (Cabras Pond), DL (Diogo Lake), ICL (Ivo Carotini Lake), AML (Araras Municipal Lake), PP (Prainha Pond), PML (Paço Municipal Lake), UL (Urban Lake), PBC (Praça Basílio Ceschin Pond), DR (David Reservoir), ER (Elektro Reservoir), FAR (Fazenda Aurora Reservoir) and SGR (São Geraldo Resevoir). X is the mean value for the environments.

	Richness				Density (ind.mL^{-1})			
	Total	**Cil.**	**Ameboid**	**Heliozoa**	**Total**	**Cil.**	**Ameboid**	**Heliozoa**
LBP	71	57	14	0	16.02	15.17	0.85	0
CP	28	24	2	2	12.57	11.57	0.95	0.05
DL	73	66	7	0	3.33	3.12	0.21	0
ICL	18	17	1	0	18.80	16.99	1.81	0
AML	6	6	0	0	51.91	51.91	0	0
PP	28	26	2	0	10.48	6.18	4.30	0
PML	22	14	8	0	9.91	4.16	5.75	0
UL	10	9	1	0	30.68	2.03	28.65	0
PBC	12	12	0	0	10.20	10.20	0	0
DR	24	23	1	0	5.44	5.42	0.02	0
ER	3	3	0	0	0.60	0.60	0	0
FAR	4	3	1	0	84.93	61.10	23.83	0
SGR	16	8	8	0	5.29	5.14	0.15	0
X	24.23	20.62	3.46	0.15	20.01	14.89	5.12	0.00

Ciliophora was also more abundant in most environments, except for the Prainha Pond (PP), where its density was similar to amoebae, and for the Paço Municipal (PML) and Urban (UL) lakes, where amoebae was more abundant. The total density of protozoans varied from 596 ind.L^{-1}, in the Elektro Reservoir (ER), to 84.93 × 10^3 ind.L^{-1}, in the Fazenda Aurora Reservoir (FAR) (Table 2).

Except for the Diogo Lake, the sum of the three dominant taxa corresponded to more than 50% of the total protozooplankton in the environments (Table 3). The genus *Halteria* occurred among the dominant species in 11 environments. Amoebae species dominated numerically in the Urban Lake (*Pseudodifflugi*a sp), in the Paço Municipal Lake (genus *Mayorella*) and in the Prainha Pond.

Among the groups found, 18 in total, only Stichotrichia was present in all 13 environments (Table 4) and was predominant in five of them (Figure 3), especially by the occurrence of *Halteria*. The Stichotrichia, along with subclasses Choreotrichia and Oligotrichia, belongs to the class Spirotrichea (Lynn 2008). These two subclasses and Prorodontida (Class Prostomatea in the current classification and Prostomatida according to Foissner et al. (1999)) occurred in 10 environments, whereas Peritrichia occurred in 12.

Despite the wide distribution of Peritrichia, it occurred among the three most important groups in the plankton of only four environments (Figure 3), and made up from 1.1% (Cabras Pond) to 90%, (Elektro Reservoir) of the protozooplankton community.

3. Influence of environmental variables on protozooplankton

The most abundant genera/species that occurred in more than six environments were also analyzed in relation to the nine environmental variables through CCA. The best CCA model, according to the Akaike Information Criterion, was the complete model (Figure 4) and was significant (p = 0.037). The proportion of the variation in the distribution of the genus/species explained by the axis 1 of the CCA was 39.8%, whereas the axis 2 explained 25.5%. The species-environment correlation was high for the first two axes of CCA: 0,993 and 0,989.

The CCA showed that the genera *Pseudodifflugia* and *Mesodinium* were positively influenced by the orthophosphate concentration and negatively affected by conductivity, particulate phosphate, dissolved oxygen, and temperature. The forms of nitrogen did not affect the distribution of these genera.

The concentrations of nitrite and nitrate are the main variables that explain the distribution of *Enchelys*, *Rimostrombidium*, *Paradileptus*, and, especially, *Limnostrombidium*, *Urotricha*, and *Vorticella*, which occur also in environments with higher concentrations of orthophosphate.

The increase in the concentration of dissolved oxygen, particulate phosphate, conductivity, and temperature affects positively the density of the genera *Coleps* and *Halteria* and the species *Cinetochilum margaritaceum* (Ehrenberg, 1831) Perty, 1849. This species is negatively affected by increase on nitrite and nitrate concentrations. Since DO is strongly related with pH, it was assumed that these variables have similar influences on the distribution of the protozoan genus/species in this work.

Discussion

In our work, it was not possible to make a reliable characterization of the environments since they were sampled only once, but it was possible to show that there were differences among the samples and that it likely reflects some differences in the catchment area and in the human activities around the environments, as there were a separation among rural and urban environments in the PCA, and it also influenced protozoan community.

Among the samples with lowest scores for the axis 2 (related with dissolved phosphate, nitrite and nitrate) of the PCA are the ones from DL and BPP that presented the greatest richness of morphotypes. Our results showed higher richness in samples (environments) with lower concentration of dissolved phosphate and nitrogen forms, what differs from the results obtained by Buosi et al. (2011) that showed increasing richness of ciliate community in response to nutrients amendment in Brazilian aquatic environment. Beaver & Crisman (1989b), also found, for 30 Florida (subtropical) lakes, that ciliate species richness is positively related to lake productivity, and the richest lake (hypereutrophic) had 24.5 species. Our contrasting results may be due to the unique sampling for each environment or to other variables than phosphate and nitrogen influencing the productivity of the environments, since the BPP is a rural environment and DL lies within a conservation area and probably suffers less anthropic influence.

The richness of morphotypes in the analyzed environments in UGRHI-Mogi-Guaçu was higher than the mean found in other water

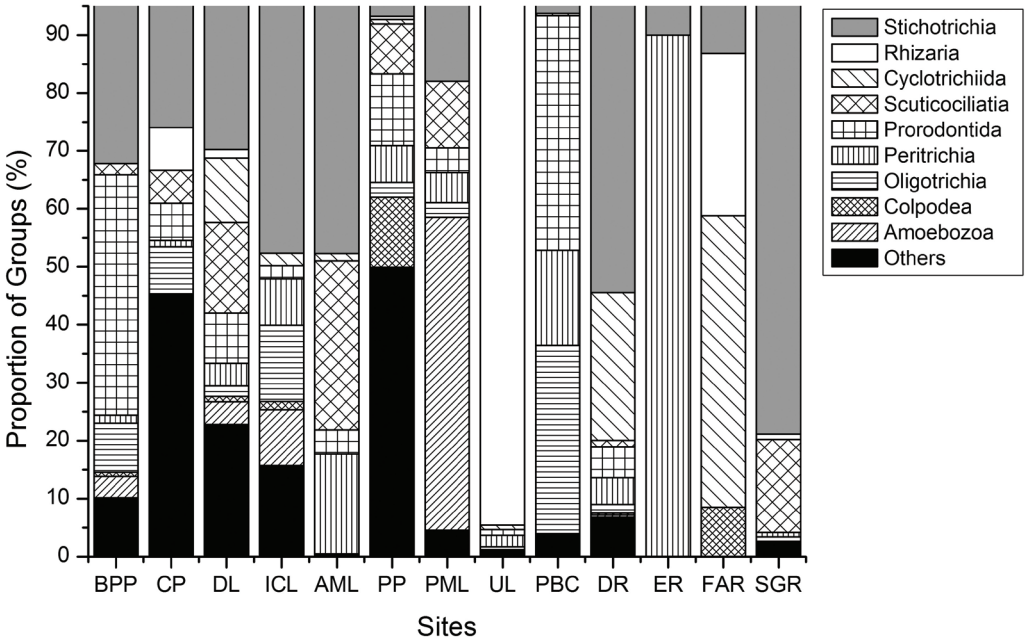

Figure 3. Major groups and their relative abundances in the 13 studied environments. BPP (Barro Preto Pond), CP (Cabras Pond), DL (Diogo Lake), ICL (Ivo Carotini Lake), AML (Araras Municipal Lake), PP (Prainha Pond), PML (Paço Municipal Lake), UL (Urban Lake), PBC (Praça Basílio Ceschin Pond), DR (David Reservoir), ER (Elektro Reservoir), FAR (Fazenda Aurora Reservoir) and SGR (São Geraldo Reservoir).

Table 3. Dominant taxa in the 13 studied environments and their relative abundances. Σ= sum of relative abundances.

		Dominant taxa		
	Σ	**1**	**2**	**3**
Ivo Carotini Lake	70.4%	*Halteria cirrifera* (47.6%)	*Limnotrombidium* sp (13.1%)	*Arcella* sp (9.6%)
Praça Basílio Ceschin Pond	72.6%	*Limnostrombidium* sp (32.5%)	*Urotricha globosa* (20.6%)	*U.* cf. *agilis* (19.5%)
Barro Preto Lake	71.9%	*Urotricha* cf. *agilis* (34.6%)	*Halteria grandinella* (28.8%)	*Limnostrombidium viride* (8.5%)
Cabras Pond	51.6%	*Halteria* cf *grandinella* (25.5%)	*Tintinnidium* cf. *semiciliatum* (17.3%)	*Rimostrombidium humile* (8.8%)
Diogo Lake	45.6%	*Halteria grandinella* (24.3%)	*Mesodinium pulex* (11.1%)	*Cinetochilum margaritaceum* (10.2%)
Prainha Pond	57.2%	"Naked amoebae" (40.5%)	*Urotricha* sp (8.7%)	*C. margaritaceum* (8%)
Paço Municipal Lake	71.7%	*Mayorella* cf. *limacis* (40.7%)	*Halteria grandinella* (17.8%)	*Mayorella bicornifrons* (13.3%)
David Reservoir	77%	*Stichotricha secunda* (42.8%)	*Askenasia volvox* (24.1%)	*Halteria* sp. (10.1%)
Elektro Reservoir	100%	*Vorticella aquadulcis* (85%)	*Halteria grandinella* (10%)	*Epistylis* sp. (5%)
São Geraldo Reservoir	95.3%	*Halteria grandinella* (78.3%)	*C. margaritaceum* (16.1%)	*Pseudodifflugia* sp (0.9%)
Urban Lake	96.2%	*Pseudodifflugia sp.* (93.4%)	*Campanella sp.* (1.6%)	*Halteria sp.* (1.2%)
Araras Municipal Lake	94%	*Halteria sp.* (47.7%)	*C. margaritaceum* (29.2%)	*Vorticella mayeri* (17.2%)
Fazenda Aurora Reservoir	91.5%	*Mesodinium* sp. (50.3%)	*Pseudodifflugia sp.* (28.1%)	*Halteria sp.* (13.1%)

Table 4. Protozoan groups found and the number of environments (N.E.) in which they occurred.

Group	N.E.	Group	N.E.	Group	N.E.
Stichotrichia	13	Scuticociliatia	8	Hymenostomatia	2
Peritrichia	12	Peniculia	7	Pleurostomatida	2
Prorodontida	10	Colpodea	6	Armophorea	1
Choreotrichia	10	Cytphoria	3	Amoebozoa	7
Oligotrichia	10	Heterotrichia	3	Rhizaria	6
Cyclotrichiida	8	Karyorelictea	3	Centrohelida	1
Haptorida	8	Suctoria	3	"Naked amoebae"	5

bodies from São Paulo State. According to Regali-Seleghim et al. (2011), the richness of taxa was between 17 and 20, on water bodies that have been analyzed just once in Biota/FAPESP. Regali-Seleghim (2001) found a maximum of 58 taxa in one sampling in a shallow water body, the Monjolinho lake in São Carlos, after a study of 1 year and six months with monthly sampling. Gomes & Godinho (2003) have related 28 taxa of Sarcodina and Ciliophora to the eutrophic lake Monte Alegre, Ribeirão Preto – SP, during a period of one year.

Considering the sampling effort for each environment and the relative high richness found in some of them, we highlight the necessity of this kind of study in poorly explored environments. Agostinho et al. (2005) reports that preservation of fauna and flora

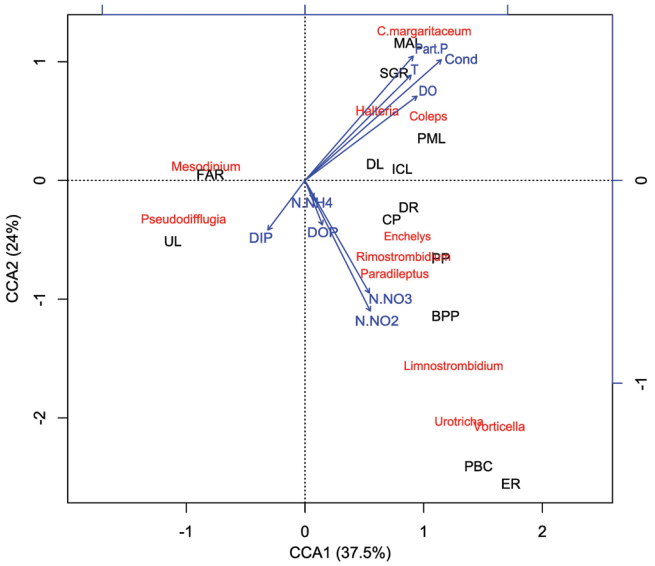

Figure 4. Canonical Correspondence Analysis (CCA) ordination diagram showing the relationships between more frequent (i.e. present in six or more sites) protozoan genera and environmental variables. The unique species of the genus *Cinetochilum* was *C. margaritaceum.*

has been the main reason to the establishment of the major protected areas in Brazil on the last decades. Several environments from these areas include water bodies and wetlands, but their fauna and flora, aquatic and terrestrial, have been little studied or surveyed. Protected areas in which aquatic organisms have been intensely inventoried show the importance of these efforts for biodiversity conservation (Agostinho et al. 2005).

Despite the high richness found, comparatively to other studies, all the environments presented relatively low densities (Table 2). Beaver & Crisman (1989a) found that the number of ciliate in oligotrophic lakes is below 10 ind.mL^{-1}, whereas more productive lakes exhibit greater abundance. In Brazilian reservoirs, however, lower ciliate cell concentrations are recorded in eutrophic environments: in Iraí reservoir (Paraná) ciliate density ranged from 7.2 to 47.1 ind.mL^{-1} (Velho et al., 2005), in São Paulo State, protozoan densities ranged from 3.60 to 389 ind.mL^{-1} in Monte Alegre Lake (Gomes & Godinho 2003) and in Monjolinho lake, ciliate densities ranged from 6.21 to 98.07 ind.mL^{-1}, and amoebae from 0.54 to 22.46 ind.mL^{-1} (Regali-Seleghim 2001).

Concerning the dominant ciliates in this work, several are frequently reported in Brazilian aquatic environments, such as *Halteria grandinella* (Müller, 1973) Dujardin, 1841, *Cinetochilum margaritaceum Limnostrombidium* sp., *Mesodinium* sp., *Urotricha* spp. and *Vorticella* spp. (e.g. Barbieri & Godinho-Orlandi 1989, Buosi et al. 2011, Dias et al. 2008, Gomes & Godinho 2003, Mansano et al. 2013, Pauleto et al. 2009, Regali-Seleghim et al. 2011), and have widespread geographic distribution in Brazil (Foissner et al. 1999, Šimek et al. 2000).

The abundance of amoebae was lower than ciliates considering most environments. Despite some authors claim that amoebas are poorly known in freshwater plankton (e.g. Laybourn-Parry 1992), studies have shown the importance of testate amoebae in plankton of some Brazilian environments (Alves et al. 2010, Bini et al. 2003, Costa et al. 2011, Lansac-Tôha et al. 2007, Velho et al. 2003). Arndt (1993), in a review about planktonic groups from freshwater, claims that amoebae have been underestimated on limnological studies because of methodological problems, even though rarely they could be as abundant as, or even more abundant than ciliates.

The amoeba of the genus *Mayorella* was predominant in the PML. The predominance of amoebas could be related to eutrophication, since *Mayorella* has been specially associated to cyanobacteria grazing in freshwater and saltwater environments (Cook et al. 1974, Laybourn-Parry et al. 1987). This environment presented high score to PCA axis 2 (Figure 2), related to dissolved phosphate and nitrate, which are the main nutrients associated to eutrophication of aquatic environments (Kratzer & Brezonik 1981, Toledo Junior et al. 1983).

Among the main protozoa, the genus *Halteria* is noteworthy, especially because of the species *H. grandinella*. Researches about the protozoa composition in Brazilian water bodies - Lobo Reservoir (São Carlos, SP), Rio Grande Reservoir (São Paulo, SP), Ilha Solteira Reservoir (Ilha Solteira, SP), reservoirs in the basin of Piranhas-Assu River (Rio Grande do Norte state), Lake Monte Alegre (Ribeirão Preto, SP) and Monjolinho Reservoir (São Carlos, SP) - found that *H. grandinella* was among the most frequent and abundant species in these environments (Araújo & Costa 2007, Barbieri & Godinho-Orlandi 1989, Gomes & Godinho 2003, Mansano et al. 2013, Regali-Seleghim 1992, 2001).

The *Halteria* spp. dominance could be due to characteristics such as wide diet and effective escape from predation. According Jürgens & Šimek (2000), *Halteria* spp feeds on organisms belonging to several trophic levels (bacteria, nanoprotists, algae, debris), what can be a selective advantage compared to specialized ciliates, resulting in a wide occurrence and, in most cases, dominance of *Halteria* spp. in freshwater plankton. In addition, its ability to jump could improve their chances of survival in the environment. According to Gilbert (1994) and Jack & Gilbert (1997), the jumper habit is an effective strategy to escape from predatory by cladocerans and rotifers.

The *Halteria* genus was responsible for the higher frequency of Stichotrichia in the environments. This subclass, along to Choreotrichia and Oligotrichia, represented by *Rimostrombidium* and *Limnostrombidium*, belongs to Oligotrichida group, according to Foissner et al. (1999). Oligotrichida are common in the communities of oligotrophic to hypereutrophic sub-tropical lakes throughout the annual cycle (Beaver & Crisman 1990, Laybourn-Parry 1992). In studies conducted in subtropical water bodies by Regali-Seleghim et al. (unpublished data) in UGRHI Pardo, the taxonomic group with greater abundance was Oligotrichida, followed by Prostomatida, Hymenostomata (subdivided into Hymenostomatia, Peniculia and Scuticociliatia on the current classification), and Gymnostomatea (Haptorida on the current classification). Beaver & Crisman (1982, 1990) found predominance of Oligotrichida, Scuticocilatida and Haptorida in sub-tropical Florida lakes and the lacustrine protozooplankton have a significant haptorid (Gymnostomatea), peritrich (Laybourn-Parry 1992), and scuticociliate (within Hymenostomata) component (Beaver & Crisman 1989a).

Whereas a major part of researches about protozoa ecology uses the separation into groups proposed by Foissner et al. (1991, 1992, 1994, 1995), which brings together the genera *Halteria, Limnostrombidium,* and *Rimostrombidium* into *Oligotrichida*, our CCA showed that these genera, specially *Halteria*, respond to environmental variables on different ways. Although it would be necessary a greater number of sampling points to more robust interpretation of genera distribution, considering the 13 points and their abiotic differences, it was possible to make same inferences about the distribution of main genera in relation to the environmental variables, and the CCA was significant. Increasing total sampling points would improve interpretation of protozoan distribution, but

fewer samples do not invalidate statistical analyses, only restricts the interpretation and extrapolation of data.

In our work, *Limnostrombidium, Rimostrombidium, Urotricha* and *Vorticella* were more affected by inorganic nitrogen variation. Buosi et al. (2011) found a slight increase in *Limnostrombidium* sp. density in treatments enriched with phosphorus and nitrogen. Furthermore, nitrite and nitrate, which are easily assimilated by phytoplankton, may indirectly affect the distribution of *Limnostrombidium* spp, since many species of this genus shows mixotrophy (Laybourn-Parry et al. 1990), *Urotricha* spp, which is a herbivorous genus (Weisse & Frahm 2001), and *Vorticella spp*, which occurs specially in eutrophic environments and many are algae and cyanobacteria epibionts (Bick 1972, Laybourn-Parry et al. 1990).

The genera *Halteria* and *Coleps,* and *C. margaritaceum* were positively influenced by the concentration of dissolved oxygen, conductivity, temperature and particulate phosphate, and poorly or negatively related to nitrogen forms. *H. grandinella* and *C. margaritaceum* are more abundant in environments poor in nitrogen compounds, but with higher amounts of organic matter (Bick 1972). Since these ciliates were influenced by DO, they might also have been influenced by pH, due to the strong correlation between these variables. This correlation could be indicative of higher photosynthetic rates in the environments, since the release of oxygen by phytoplankton is associated with the consumption of carbon dioxide during photosynthesis, what may increase the pH of the environment. Mansano et al. (2013) reported positive correlation among *H. grandinella* density and DO, but no correlation with pH.

Regarding to the pH, *C. margaritaceum* was more abundant in more alkaline environments and *Limnostrombidium* and *Rimostrombidium* predominated in environments with lower pH values. These results corroborate those described by Mieczan (2007).

The genus *Coleps* and *C. margaritaceum* were found in similar environments. The species *Coleps hirtus* feeds on *C. margaritaceum*, avoids high concentrations of ammonium (Foissner et al. 1999) and has optimum growth at low concentrations of nitrite and nitrate (Bick 1972).

Besides the different responses of the most commons genera/species to the environmental variables, this work showed that although all the environments studied belong to one basin, they were different in composition of species, reflecting differences in nutrient concentration of the water bodies and possibly in the land use, since we found lower mean richness in urban environments. Galbraith & Burns (2010) suggest that differences in taxonomic structure of ciliate and phytoplankton communities might also be predicted by the land use and vegetation cover in the catchment. Our results corroborate the need of more research in more preserved environments.

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Appendix 1. Protozoan species list for the 13 water bodies from the UGRHI Mogi-Guaçu. The numbers represent the density (ind.mL^{-1}) of each taxon and the numbers between brackets represent the number of morphotypes.

	PML	SGR	BPP	DL	CP	PP	DR	ER	PBC	ICL	UL	AML	FAR
Ciliophora													
Intramacronucleata													
Armophorea Lynn, 2004													
Armophorida Jankowksi, 1964													
Caenomorpha cf. *uniserialis* Levander, 1894				0.01									
Colpodea Small & Lynn, 1981													
Bursariomorphida Fernández-Galiano, 1978													
Bursaridium pseudobursaria (Faure-Fremiet, 1924) Kahl, 1927							0.02						
Bursaridium sp			0.03										
Bryometopida Foissner, 1985													
Thylakidium sp Schewiakoff, 1893			0.03	0.03		0.02				0.06			
Colpodida de Puytorac et al., 1974													
Colpoda sp			0.02										
Maryna sp						0.52							
Mycterothrix sp										0.21			
Cyrtolophosidida Foissner, 1978													
Cyrtolophosis mucicola Stokes, 1885			0.02			0.73							7.22
Cyrtolophosis sp			0.01										
Litostomatea Small & Lynn, 1981													
Haptoria Corliss, 1974													
Cyclotrichiida Jankowski, 1980													
Askenasia volvox Kahl, 1930							1.31		0.04				
Mesodinium pulex (Claparède & Lachmann) Stein, 1867				0.37									
Mesodinium sp						0.08	0.08			0.42	0.24	0.68	42.73
Haptorida Corliss, 1974 (syns. Spathidiida)													
Actinobolina sp			0.04										
Chaenea sp			0.02										
Didinium chlorelligerum Kahl, 1935					0.17								
Didinium sp				0.02									
Enchelydium sp			0.01										
Enchelyodon lasius Stokes, 1885			0.01										
Enchelyodon sp											0.02		
Enchelys gasterosteus Kahl, 1926			0.06	0.05	0.03	0.04	0.02			0.17			
Enchelys sp			0.03										
Gymnostomatida não identificado				0.01									
Lacrimaria olor (Müller, 1786) Bory, 1924				0.02									
Lagynophrya sp										0.13			
Monodinium balbianii Fabre-Domergue, 1888			0.19	0.02					0.16				
Paradileptus elephantinus (Svec, 1897) Kahl, 1931				0.01	0.87	0.02	0.02		0.12				
Paradileptus sp.											0.18		
Phialina pupula Müller, 1773				0.01									
Trachelius ovum (Ehrenberg, 1831) Ehrenberg, 1838				0.01									
Pleurostomatida Schewiakoff, 1896													
Amphileptus pleurosigma (Stokes, 1884) Foissner, 1984				0.01									
Amphileptus sp				0.01									
Litonotus sp				0.01						0.04			
Oligohymenophorea de Puytorac et al., 1974													
Hymenostomatia Delage & Hérouard, 1896													
Glaucoma frontata (Stokes, 1886) Kahl, 1931										0.06			
Tetrahymena sp			0.01										
Hymenostomatia				0.01									
Peniculia Fauré-Fremiet in Corliss, 1956													
Disematostoma tetraedricum (Faure-Fremiet, 1924) Kahl, 1931			0.02										
Frontonia leucas (Ehrenberg, 1834) Ehrenberg, 1838				0.02									
Frontonia sp										0.03		0.24	
Lembadion cf. *bullinum* (Müller, 1786) Perty, 1849				0.01									
Lembadion lucens (Maskell, 1887) Kahl, 1931				0.04			0.05						
Lembadion sp			0.02										
Marituja pelágica Gajewskaja, 1928						0.06							
Paramecium bursaria (Ehrenberg, 1831) Focke, 1836			0.34										
Paramecium cf. *aurelia-komplex* Müller, 1773				0.03									
Paramecium cf. *putrinum* Claparède & Lachmann, 1859					0.02								

Appendix 1. Continued...

	PML	SGR	BPP	DL	CP	PP	DR	ER	PBC	ICL	UL	AML	FAR
Paramecium putrinum Claparède & Lachmann, 1859				0.02									
Paramecium sp			0.04				0.03						
Stokesia vernalis Wenrich, 1929				0.05	1.06	0.04							
Urocentrum turbo (Müller, 1786) Nitzsch, 1827			0.04	0.07			0.11						
Peritrichia Stein, 1859													
Campanella sp	0.04			0.02						1.51	0.49		
Carchesium pectinatum Zacharias, 1897						0.24	0.19						
Carchesium sp					0.06						0.12		
Epistilys sp								0.03					
Pseudovorticella monilata (Tatem, 1870) Foissner & Schiffmann, 1974					0.08								
Telotrochia de *Campanella* sp				0.03									
Telotrochia de *Epistylis* sp				0.01									
Telotrochidium sp			0.04										
Trichodina sp	0.02												
Vorticella aquadulcis-komplex								0.51					
Vorticella campanula Ehrenberg, 1831			0.02	0.02									
Vorticella mayeri Fauré-Fremiet, 1920												8.93	
Vorticella spp	0.46	0.04	0.16	0.03 (3)		0.42	0.06		1.68				
Vorticelid				0.02									
Scuticociliatia Small, 1967													
Cinetochilum margaritaceum (Ehrenberg, 1831) Perty, 1849	1.14	0.85	0.31	0.34	0.72	0.84	0.06					15.15	
Ctedoctema acanthocryptum Stokes, 1884				0.15									
Cyclidium sp						0.06							
Scuticuciliatia				0.03 (2)									
Phyllopharyngea de Puytorac et al., 1974													
Cyrtophoria Fauré-Fremiet in Corliss, 1956													
Chlamydodon sp			0.02										
Pseudochilodonopsis fluviatilis Foissner, 1988				0.01									
Trithigmostoma srameki Foissner, 1988				0.02									
Trithigmostoma steini (Blochmann, 1895) Foissner, 1988									0.02				
Suctoria Claparède & Lachmann, 1858													
Podophrya sp			0.07			0.04							
Sphaerophrya magna Maupas, 1881				0.01									
Staurophrya sp						0.42							
Free-swimming of suctoria				0.03									
Prostomatea Schewiakoff, 1896													
Prorodontida Corliss, 1974													
Balanion sp						0.02							
Bursellopsis nigricans nigricans (Lauterborn, 1894) Foissner, Berger & Schaumburg, 1999				0.01		0.08							
Bursellopsis truncata (Kahl, 1927) Corliss, 1960				0.01									
Coleps hirtus cf. *viridis* Ehrenberg, 1831				0.05									
Coleps sp	0.27		0.83			0.27	0.07			0.03		2.17	
Holophrya discolor Ehrenberg, 1834	0.05		0.18		0.11								
Holophrya sp										0.18			
Pelagothrix sp							0.08						
Urotricha armata Kahl, 1927	0.02			0.02									
Urotricha cf. *agilis* Stokes, 1886			5.54	0.03		0.02	0.03		1.99				
Urotricha cf. *armata* Kahl, 1927					0.02								
Urotricha cf. *faurei* Dragesco, Iftode & Fryd-Versavei, 1974				0.17									
Urotricha globosa Schewiakoff, 1892									2.1	0.21			
Urotricha matthesi matthesi Krainer, 1995					0.62								
Urotricha spp	0.08		0.1		0.05	0.91	0.11		0.04		0.3		
Spirotrichea Bütschli, 1889													
Choreotrichia Small & Lynn, 1985													
Codonella sp										1.1			
Rimostrombidium caudatum Kahl, 1932						0.18	0.08						
Rimostrombidium humile (Penard, 1922) Petz & Foissner, 1992	0.02		0.04	0.05	1.11	0.06							
Rimostrombidium spp	0.02	0.01	0.03	0.03	0.11		0.04		0.1		0.17		
Tintinnidium cf. *semiciliatum* Sterki, 1879					2.17								
Tintinnidium sp										1.38			
Oligotrichia Bütschli, 1887/1889													

Appendix 1. Continued...

	PML	SGR	BPP	DL	CP	PP	DR	ER	PBC	ICL	UL	AML	FAR
Limnostrombidium cyst	0.01												
Limnostrombidium spp									3.32	2.47			
Limnostrombidium viride (Stein, 1867) Krainer, 1995	0.24	0.04	1.36	0.06	1.02	0.27	0.08				0.15		
Stichotrichia Small & Lynn, 1985													
Halteria chlorelligera Kahl, 1932		0.03											
Halteria cirrifera Kahl, 1935										8.95			
Halteria grandinella (Müller, 1973) Dujardin, 1841	1.76	4.14	4.62	0.81	3.21			0.06	0.62				
Halteria sp						0.69	0.55				0.36	24.74	11.15
Holosticha monilata Kahl, 1928				0.01									
Hypotrichidium conicum Ilowaisky, 1921									0.02				
Stichotricha secunda Perty, 1849						0.02	2.33						
Stichotricha sp	0.02		0.17	0.05									
Strongylidium sp			0.03										
Uroleptus cf *musculus* (Kahl, 1932) Foissner, Blatterer, Berger & Kohmann, 1991				0.01									
Uroleptus sp				0.02			0.02						
Stichotrichia not identified			0.33 (3)	0.09 (6)	0.05 (2)		0.06 (1)						
Postciliodesmatophora Gerassimova & Seravin, 1976													
Heterotrichea Stein, 1859													
Heterotrichida Stein, 1859													
Linostomella vorticella Foissner, Berger & Schaumburg, 1999			0.03				0.02						
Stentor muelleri Ehrenberg, 1832						0.11							
Loxodida Jankowski, 1980													
Loxodes sp			0.01			0.02				0.04			
Ciliophora not identified	0.01 (1)	0.03 (2)	0.34 (16)	0.14 (8)	0.09 (4)								
Amoebozoa Lühe, 1913, emend. Cavalier-Smith, 1998													
Mayorella bicornifrons Bovee, 1970	1.32												
Mayorella cf. *limacis* Bovee, 1970	4.03												
Arcella vulgaris Ehrenberg, 1830			0.51	0.12	0.02		0.02						
Arcella sp										1.81			
Thecate amoeba		0.01											
Polychaos timidum Bovee, 1972				0.01									
Amoeba diminuta Bovee, 1972			0.08										
Rhizaria													
Cercozoa Cavalier-Smith, 1998 (insertae sedis)													
Pseudodifflugia cf. *fascicularis* Penard, 1902				0.02	0.93								
Pseudodifflugia cf. *gracilis* Schlumberger, 1845				0.03									
Pseudodifflugia sp		0.05				0.06					28.65		23.83
Naked amoeboid	0.4 (6)	0.09 (6)	0.26 (12)	0.03 (3)		4.24 (1)							
Eukaryota (insertae sedis)													
Centrohelida Kühn, 1926													
Sphaerastrum fockei West, 1901					0.03								
Astrodisculus radians Stern, 1924					0.02								