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Ciliates and trophic state: A study in five adjacent urban ponds in Mexico City

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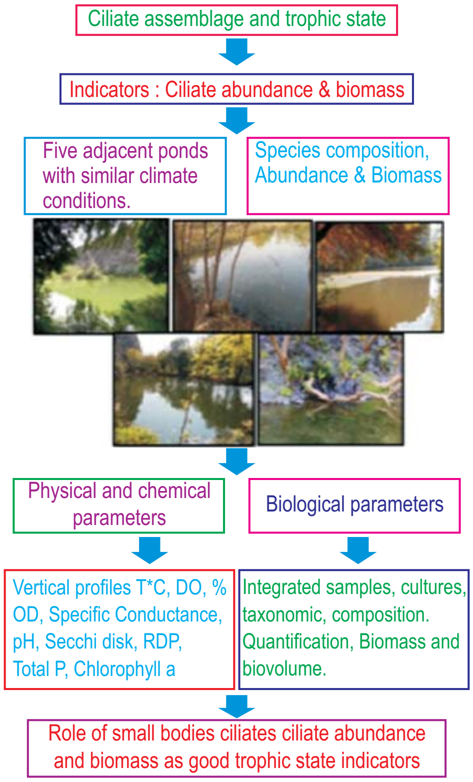
Abstract

Aim: The ciliated protozoa are components of the microbial food web. A relationship between species richness, number, and size of ciliates, and the trophic state has been recognized in some works. The aim of this study was to determine how the trophic differences affect the protozooplankton composition, abundance, and biomass considering the similarity in location and climatic conditions in five adjacent shallow urban water bodies in Mexico City with differences in trophic state.

Methodology: Monthly protozooplankton samplings were conducted from January to December 2015 and measured water temperature, dissolved oxygen, specific conductance, pH and Secchi disk, RDP, TP, and chlorophyll a. Integrated column samples for ciliates were obtained and taxonomic richness, species density, and biomass by size class calculated using in vivo, stained and Lugol fixed samples. ANOVA was applied for environmental data comparison, and cluster analyses were carried out using environmental and biological data. A redundancy analysis was used to explain the variation of the ciliates biomass related to physicochemical conditions.

Results: Ciliate densities and biomass varied accordingly with the trophic state and showed higher values in the more productive ponds, and lower values in the other ponds. Oligotrichia and Prostomatea were the dominant taxonomic groups. Eastern Quarry ponds trophic state varied from oligotrophic to near hypertrophic and abundance and biomass variation of planktonic ciliates followed a trophic gradient, showing higher values in more eutrophic systems. Ciliates in the range body size of 30-50 µm dominated the biomass in the ponds. An unexpected result was the low species number and densities of the small size ciliates (mainly Scuticociliatida) that often are important in eutrophic conditions.

Interpretation: The relationship between the trophic state and the abundance and biomass of plankton ciliates was confirmed. The size fraction < 20 µm was not important, and the 30-50 µm body size ciliates were dominant even in the most productive ponds.



Introduction

Urban lakes are ecosystems that suffered from pollution and eutrophication (Schueler y Simpson 2003, Nasseli-Flores 2008). Eutrophication causes significant changes in the system components, especially in plankton communities favoring the increase of primary production and the emergence and dominance of potentially toxic organisms such as cyanobacteria (Sommaruga, 1995).

The ciliated protozoa are components of the microbial food web and they have some ecological functions: they are important consumers of bacteria (Chróst *et al.,* 2009) and phytoplankton grazers (Lischke *et al.,* 2016). They feed on autotrophic picoplankton (Buholce *et al.,* 2015), and transfer energy to higher trophic levels, for example to the rotifers, cladocerans, and copepods (Adrian and Schneider-Olt, 1999). It has been suggested that ciliates may enhance nutrient availability for phytoplankton (Xu and Cronberg, 2010). Recent studies show that ciliates are a significant part of the diet of the larvae of different fish species (Zingel *et al.,* 2012).

Relationship between species richness, number and size of ciliates, and trophic state was long known (Beaver and Crisman 1982; Beaver and Crisman 1989). This relationship has been found in other studies, though not always in the same way. While some authors believe that the importance of microbial webs is higher in oligotrophic environments (Weisse, 1991, Macek *et al.,* 2006) others argue that in highly productive environments where large filter feeders like *Daphnia* are absent, small size grazers, including ciliates, can become important (Sommaruga, 1995). When fish predation pressure on zooplankton is very high, small size filter feeders may be the main phytoplankton grazers in the systems (Lischke *et al.,* 2016).

In this paper, composition and abundance of protozooplankton were compared in five shallow lakes (ponds) located in an urban ecological reserve (Reserva Ecologica del Pedregal de San Angel, REPSA). The water bodies are found in the grounds of an old mine of asphalt called the Eastern Quarry (Cantera Oriente, in Spanish). These ponds are fed by several springs and are found adjacent. Despite their closeness and sharing the same source of water, the waterbodies have differences in their trophic status that are observable to the naked eye.

The aim of this study was to determine how the trophic differences affect protozooplankton composition, abundance, and biomass considering the similarity in location and weather conditions.

Materials and Methods

Study area : The Eastern Quarry is a part of the REPSA (Ecological Reserve of Pedregal of San Angel) and is located on the campus of the National Autonomous University of Mexico (UNAM) of Mexico City (19º 19' 14'' N, 99º 10' 23'' W to 19º 18' 50'' N, 99º 10' 16'' W). REPSA vegetation dominance corresponds to high elevation desert scrub, and the main species is *Pittocaulon praecox* (Palo loco). The substrate is mostly volcanic rock (Peralta-Higuera and Prado-Molina 2009).

The Eastern Quarry is an area of approximately 206,000 m^2. In 1970 the extraction of rock began on this site, but at some time the drilling reached the level of groundwater and the water began to sprout, forming small ponds. Rock extraction no longer continued and in 1996 the UNAM joined this area to the REPSA (Lot, 2007). Thereafter, a restoration process began and exotic plants were introduced. Currently, in Eastern Quarry there are four shallow lakes ($Z < 1.5$ m) comprising an area of 11,906.45 m^2 (14.36 % of the total area of the reserve zone) (Ortiz-Perez *et al.,* 2007) (Fig. 1). These water bodies are supplied by several springs that flow from the rock walls surrounding the area (Hernandez- Martinez, 2007).

Monthly samplings were conducted from January to December 2015. Sampling sites were the central point of four ponds: North, Center, South and Regulation and the puddle formed by one of the springs that fill them. At each site vertical profiles (every 0.2 m) were measured in the water column of the

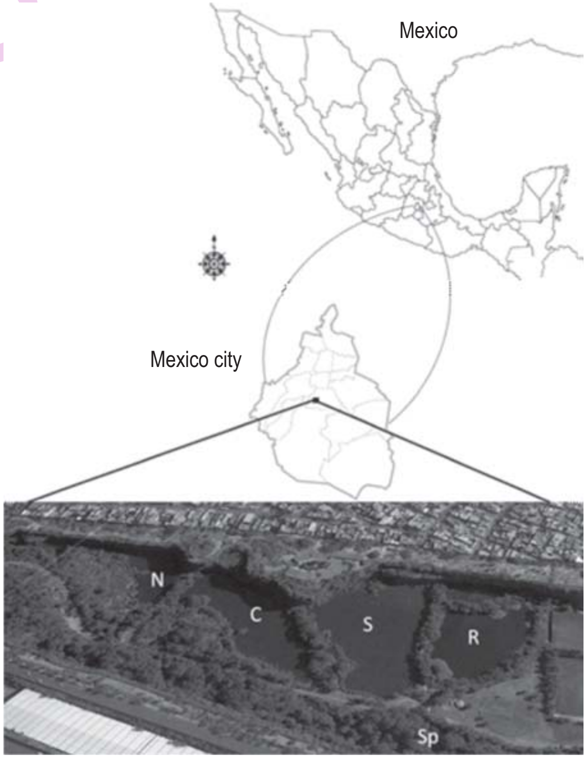


Fig. 1 : Map of Eastern Quarry with sampling stations. N:north; C: center; S: south; R: regulation; Sp: spring. Photo from Google Earth

following variables: water temperature, dissolved oxygen concentration, D.O. saturation percentage and specific conductance at 25°C using a Multi-probe YSI (Cincinnati, USA) model 85. The pH was measured with a Conductronic (Puebla, Mexico) model pH10 potentiometer. Secchi disk visibility was measured using a 0.20 m diameter black and white disk.

Integrated samples of the water column in each pond were taken using an acrylic tube sampler (26 mm diameter). Duplicate 50 ml samples were obtained for ciliates. One sample was maintained in dark and temperate conditions for *in vivo* observation of organisms. The other samples were fixed with 1% acid Lugol (Finlay and Guhl 1992). Fresh plastic containers (500 ml) were used for Reactive Dissolved Phosphorous (RDP) samples. These samples were transported to the laboratory at 4°C. For chlorophyll *a* determination, fresh samples of 50 ml were collected in polypropylene tubes and a variable volume (5 to 30 ml) filtered in situ using Whatman ® GF / F glass fiber filters with the help of a manual vacuum pump.

RDP was measured by the ascorbic acid spectrophotometric method (APHA 2012). Total phosphorus was measured by the alkaline digestion method (Valderrama,1981), and organic phosphorus was calculated form the difference between total and reactive P Chlorophyll *a* concentration was obtained using a Turner Designs® Model 10-AU fluorometer and the cold extraction method (4° C) with 90% acetone following Arar and Collins (1997). The ciliates samples were observed *in vivo* and using vital staining dyes (Lee *et al.* 2000). Some ciliate species were cultured using filtered lake water and Chalkley medium with wheat grains. Silver staining techniques were applied for selected species (Lynn, 1992).

Lugol fixed samples were counted in a Sedgwick–Rafter chamber (1.0 ml) (Finlay and Guhl 1992). A minimum of 100 organisms of the most abundant species (Wetzel and Likens 2001) were counted with a Zeiss microscope model K7 (160X).

The taxonomic keys used for ciliate identification were Foissner and Berger (1996); Foissner *et al*. (1991, 1992, 1994, 1995, and 1999), and Lynn and Small (2000).

At least 20 individuals of each species were measured in vivo for biomass calculation, using a calibrated micrometer. The shape of each species was approximated to the nearest geometric form according to Sun and Liu (2003). Biovolume was transformed into biomass assuming a specific density of 1.0.

For comparison of the environmental variables between the lakes, ANOVA analyses were performed. A hierarchical cluster analysis was applied using environmental data from the ponds. Pearson r was used as similarity index and the intra-groups cluster method (IBM SPSS ver. 19, 2010). Log (n+1) transformed total abundances were also compared using ANOVA. Ciliate community composition in the ponds was compared using the similarity Bray-Curtis Index and grouped by the simple linkage hierarchical method with the PAST statistical package (ver. 3.6 2015).

Redundancy analysis was applied to explain the variation of the ciliates biomass in relation to physicochemical conditions (CANOCO Program ver. 4.5.1)

Results and Discussion

Environmental conditions : Water temperature, conductivity, and pH did not show significant differences (P> 0.05) among the ponds of the Eastern Quarry (table 1). Water temperature fluctuated between 13.3 and 21°C, conductivity from 377 to 491 µS cm^{-1} and the pH conditions were from neutral (6.8) to alkaline (9.7).

Oxygen saturation showed significant differences (P < 0.05) between ponds. South and Center ponds with averages of 195 and 216 % were different from the North (142 %) and Regulation (158 %) ponds and from the Spring (89%).

Secchi disk transparency ranged from 0.15 m in the South pond to 1.3 m for Regulation pond and showed an inverse relationship with chlorophyll a values. Regulation Pond and Spring, with the higher average transparency values (1.15 and 0.65 m) had the lowest chlorophyll a mean concentration (14 and 7 µg l^{-1}). The South pond showed the minimum average value of transparency (0.35 m) but the maximum mean value of chlorophyll a (188 µg l^{-1}), followed by the Center (0.45 m and 164 µg l^{-1}). The North pond had intermediate values of transparency (0.82 m) and also a moderate value of chlorophyll a (56 µg l^{-1}) (Table 1).

The RDP behavior also had an inverse relationship with chlorophyll *a*. The most productive water bodies (South and Center) presented the lower mean concentrations of RDP (0.03 and 0.05 mg l^{-1}). RDP concentration in the Spring was the higher (0.12 mg l^{-1}) and the Regulation pond also had a high average (0.1 mg l^{-1}). The North pond was in the middle with a mean of 0.08 mg l^{-1}. When ANOVA was applied to RDP data the Center and South ponds were different (P<0.05) from the other three ponds. RDP consume by phytoplankton may explain the inverse relationship of this nutrient with chlorophyll a concentrations. Our data also show that Spring water source to the ponds provides significant RDP concentrations and may a factor that causes the observed eutrophic conditions.

A hierarchical cluster analysis (Fig. 2) was carried out using the environmental variables data of the five water bodies. Two clearly separated groups were formed. The first includes the Center, South and North ponds, which shared high levels of chlorophyll *a* and dissolved oxygen saturation, low transparency, and low RDP concentrations; this group corresponds to eutrophic ponds. In the second group the Regulation pond and Spring were

Table 1 : Variation range and mean values of physical and chemical variables in the five ponds of Eastern Quarry

	North	Center	South	Regulation	Spring
Temperature(°C)	13.3 – 18.5(16.4)	14.7 – 19.8(17.5)	16.4 - 21(18.72)	16.3 – 19.5(17.7)	16.1 – 17.8(17)
pH	6.8 – 8.25 (7.7)	8.7 – 9.7 (8.9)	8.1 – 9.6 (9.2)	6.9 – 8.3 (7.8)	6.9 – 8.1 (7.3)
Conductivity(µS cm^{-1})	377 - 480(436)	381 – 472(426)	382 – 422(406)	390 – 452(418)	396 – 491(445)
% Oxygen Sat.	75 – 223(142)	103 – 267(195)	130 – 260(216)	96 – 237(158)	66 – 128(89)
Secchi disk(m)	0.45 – 1.10(0.82)	0.35 -0.60(0.45)	0.15 – 0.55(0.35)	1.00 – 1.30(1.15)	0.50 – 1.00(0.65)
Chlorophyll *a*(µg l^{-1})	13 – 114(56)	74 – 274(164)	58 – 468(188)	6 – 24(14)	2 – 36(7)
P – dissolved(mg l^{-1})	0.06 – 0.1(0.08)	0.01 – 0.09(0.05)	0.01 – 0.07(0.03)	0.09 – 0.15(0.1)	0.1 – 0.15(0.12)
P-total(mg l^{-1})	0.08-0.29(0.17)	0.19-0.72(0.42)	0.19-1.27(0.53)	0.10-0.17(0.14)	0.12-0.16(0.14)
P-organic(mg l^{-1})	0.02-0.22(0.08)	0.03-0.33(0.13)	0.10-0.97(0.17)	0.00-0.06(0.03)	0.00-0.03(0.02)

Table 2 : List of ciliate species recorded in the lakes of the Eastern Quarry. ++ indicates new records to the area

CiliophoraDoflein, 1901 [Ciliata Perty, 1852; Infusoria Bütschli, 1887] (Adl *et al.,* 2005)

Gymnostomatea Butschli, 1887
Actinobolina sp. Kahl ++
Askenasiavolvox Blochmann++
Litonotus lamella (Muller)
Trachelophyllum pusillum Claparéde & Lachmann ++
Hypotrichea Stein, 1859
Aspidisca cicada (Muller)
Euplotes sp. Ehrenberg ++
Stylonychia mytilus Complex
Heterotrichea Stein, 1859
Balantidium pellucidum Eberhard ++
Metopus es (Muller)
Spirostomum teres Claparéde & Lachmann
Protostomatea Schewiakoff, 1896
Bursellopsis sp. Corliss ++
Coleps hirtus (Muller)
Oligotrichea Bütschli, 1887
Halteria grandinella (Muller)
Pelagohalteria cirrifera (Kahl)
Limnostrombidium sp. Krainer ++
Cyrtophorida Fauré-Fremiet in Corliss, 1956
Chilodonella uncinata (Ehrenberg)
Chlamydonellopsis plurivacuolata (Blatterer & Foissner)
Phascolodon vorticella Stein ++
Trithigmostoma cucullulus (Muller)
Peniculida Fauré-Fremiet in Corliss, 1956
Lembadionlucens (Maskell)
Paramecium bursaria (Ehrenberg)
Scuticociliatida Small, 1967
Cyclidium glaucoma(Muller)
Dexiotricha granulosa (Kent)
Hymenostomatida Delage and Hérouard, 1896
Cinetochilum margaritaceum (Ehrenberg)
Glaucoma scintillans Ehrenberg
Tetrahymena pyriformis (Muller)
Urocentrum turbo (Muller)
Peritricha Stein, 1859
Cothurnia annulata Stokes
Epistylis pygmaeum Ehrenberg ++
Hastatella sp. (Stiller)++
Pelagovorticella mayeri ++
Vorticella campanula Ehrenberg
Vorticella microstoma Complex
Litostomatea Small and Lynn, 1981
Monodinium sp Fabre-Domergue ++
Paradileptus elephantinus Kahl++

joined because they share low values of chlorophyll a, moderately oxygen saturation, lower pH values, but higher concentrations of RDP; this means a lower trophic state.

Taxonomic composition of planktonic ciliates : Thirty-five ciliate morphospecies were observed in the plankton samples from Eastern Quarry ponds (Table 2). Species richness was as follows: North Lake 23 species, Center Lake 26, South pond 25, 13 species in the Regulation pond and just seven in the Spring. Bagatini et al. (2013) in a study conducted in 13 water bodies in urban and rural areas of San Paulo, Brazil, found 20.6 as the average number of species of ciliates in every lake and a range between 3 and 66. The average value of lakes in Brazil is similar to the most productive lakes in the Eastern Quarry.

Machado-Velho *et al.* (2013) identified 35 species of ciliates in two climatic periods in a eutrophic urban lake located in the city of Maringa, State of Parana, Brazil. In contrast, Buholce *et al.* (2015) found only 13 taxa in a study conducted in two ponds and two urban reservoirs in the city of Riga, Latvia, corresponding to the temperate zone of the world. Esquivel *et al.* (2016) in a tropical, deep and large lake (Catemaco, Mexico) identified 28 species of planktonic ciliates. The species richness in water bodies of Eastern Quarry is in the middle of the observed range found in other urban and natural bodies of water in different climatic regions.

Aladro-Lubel *et al.* (2007) found 75 species of ciliates in the same study site. However, in their research samples were

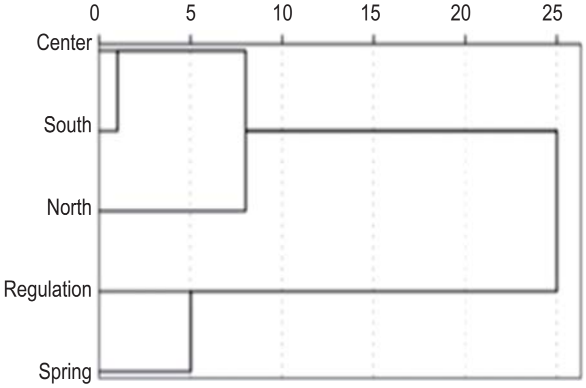


Fig. 2 : Hierarchical clustering of the aquatic bodies in the Eastern Quarry based on the log transformed data of the environmental variables. Similarity: 1- r Pearson

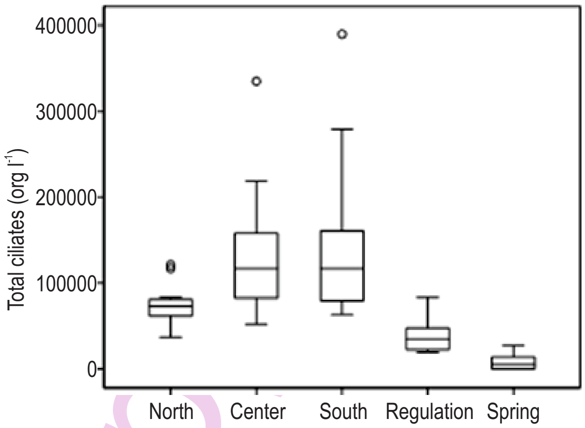


Fig. 3 : Box and whisker plot of the ciliate total abundance in the Eastern Quarry ponds

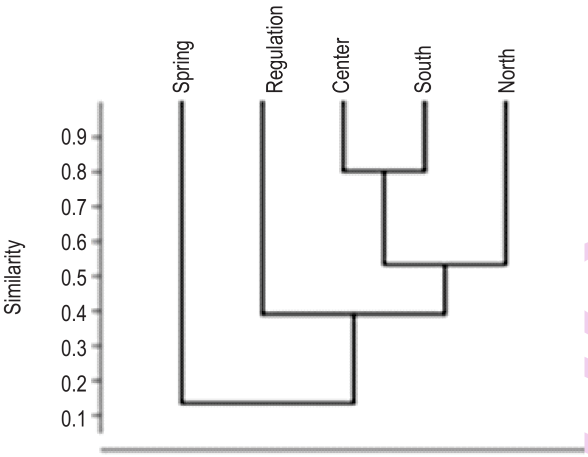


Fig. 4 : Cluster analysis (Bray-Curtis Similarity Index, Hierarchical cluster) of Eastern Quarry ponds using ciliate species abundances

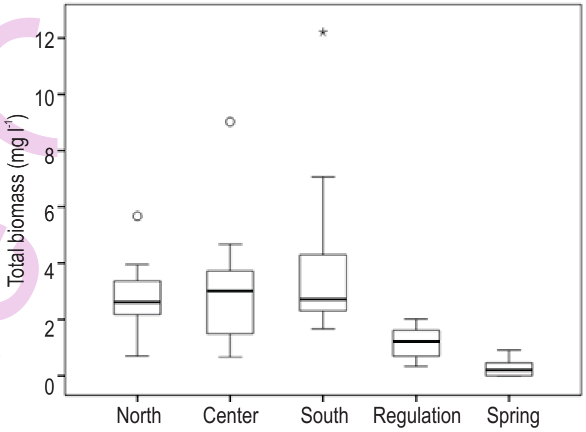


Fig. 5 : Box and whisker plot of ciliate total biomass (mg l^{-1} wet weight) in the five water bodies of the Eastern Quarry

taken from the littoral area of the ponds and channels, and from aquatic plants and sediment. The higher diversity of studied habitat may explain the greater species richness obtained by them. However, in our study 13 species not found previously were observed. Most of these species are true planktonic, as *Limnostrombidium* sp., *A. volvox*, *Bursellopsis* sp., *P. vorticella*, *Monodinium* sp., *P. mayeri*, and *Hastatella* sp., but other were associated with the littoral area. *E. pygmaeum* was observed as epibiont on rotifers and cladocerans, as described by Gilbert and Schröder (2003)

Species abundances : The species present in the four ponds and Spring were *Halteria grandinella, Coleps hirtus, Limnostrombidium* sp. and *Pelagohalteria cirrifera*. Overall, *Limnostrombidum sp*. showed the highest average abundances (North 16 ind m l^{-1}, Center 23 ind m l^{-1}, South 28 ind m l^{-1}). It was the dominant species in the Regulation pond (15 ind m l^{-1}) and the Spring (3 ind m l^{-1}). *C. hirtus* dominated the abundance in the Center (30 ind m l^{-1}) and South (40 ind m l^{-1}) ponds, whereas *H. grandinella* was the most abundant species in North (average 21 ind m l^{-1}).

These species are the most common in the plankton of water bodies in different parts of the world. For example, *H. grandinella* has a wide distribution in water bodies of Brazil (Bagatini *et al.,* 2013). Also in many lakes of the temperate region, the genus *Halteria* is usually present with significant abundances (Buholce *et al.*, 2015). *Pelagohalteria cirrifera* (formerly *Halteria cirrifera*) is equally dominant and widely distributed in lakes of different latitudes. *Limnostrombidum* (formerly *Strombidium*), another Oligotrichea genus, has been also found in many sites (Beaver and Crisman 1982; Machado-Velho *et al.* 2013).

These three species belong to the Oligotrichea group (Foissner *et al.,* 1999) that is usually the most abundant in plankton biomass in the lakes (Beaver and Crisman 1989, Buholce et al. 2015). Prostomatea (which includes *C. hirtus*) is also of high importance in the protozooplankton abundance in many water bodies (Machado-Velho *et al.,* 2013).

In the ponds of Eastern Quarry, the Oligotrichea represented the largest percentage of the total number of ciliates found in the 5 sampling sites, providing from 65 % in the North to about 40 % in the Center and South ponds. The Protostomatea (represented only by *C. hirtus*) contributed between 25 and 30 % of total abundance in Center and South ponds. The other 10 taxonomic groups contributed globally with about 30 % of the total abundance, with the exception of the Regulation pond where their contribution was slightly higher (35 %).

Peritricha were the group with the largest number of species (6). However, their abundances were not very high in any case. *Vorticella campanula,V. microstoma* and *Pelagovorticella mayeri* were the main representatives of this group. *Epystilis pygmaeum* was observed as epibiont on *Moina micrura*, a cladoceran species found in North and Center ponds.

Total abundances of ciliates : The variation of the total abundance of ciliates between ponds was remarkable (Fig. 2). The most productive ponds (South and Center) had the highest range: 68000-390 000 org l^{-1} in the South and 52000-335000 org l^{-1} in the Center. North pond had intermediate range (37000-121000 org l^{-1}) and Regulation 19000-83000 org l^{-1}; the lowest range was found in Spring (0-27000 org l^{-1}).

Beaver and Crisman (1982) found the following values of total abundance of ciliates in subtropical Florida lakes in agreement with their trophic status in org ml^{-1} (mean ± SD): oligotrophic 10.4 ± 5.4; mesotrophic 27.5 ± 7.7; eutrophic 55 ± 7.6 and 66 ± 199 hypertrophic. Values found in the Eastern Quarry ponds were: 148 ± 96 org m l^{-1} (mean ± SD) in the South, and 137 ± 78 org m l^{-1} in the Center. North pond had intermediate values (74 ± 21 org m l^{-1}) and Regulation 38 ± 19 org m l^{-1}; lowest numbers was counted in Spring (8 ± 10 org m l^{-1}). Taking into consideration the above, the trophic state of Eastern Quarry ponds based on ciliate numbers would be as follows: Spring is oligotrophic, Regulation pond mesotrophic, North, Center and South ponds, eutrophic, but the last two with values very close to those of the hypertrophic conditions. Chlorophyll a mean concentration and Secchi disk measures also support this point of view. The result of the cluster analysis of the ponds using the data of environmental variables indicated the same differences.

The ANOVA applied to compare the total ciliate abundances between lakes showed that North, Center and South ponds mean values were higher and different (P<0.05) than those from the Regulation pond and Spring as can be appreciated in Fig. 3. Likewise, a hierarchical cluster of the lakes using ciliates abundance, and the Bray–Curtis similarity index (Fig. 4), grouped the three most eutrophic lakes (South, Center and North) and in a

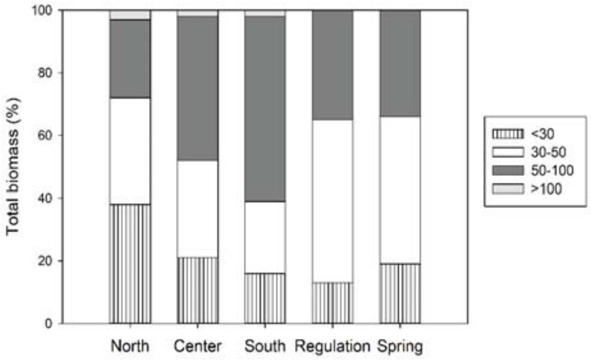


Fig. 6 : Percentage contribution of ciliate size intervals to the total biomass in each water body

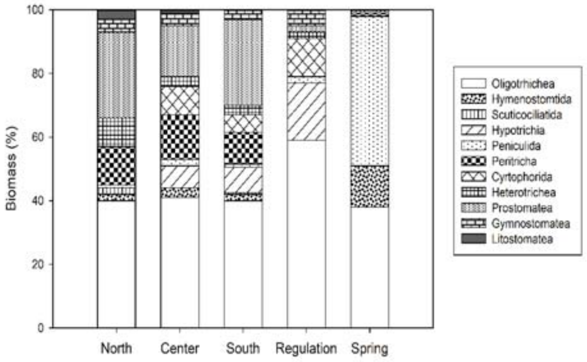


Fig. 7 : Biomass percentage contribution by taxonomic group in each pond

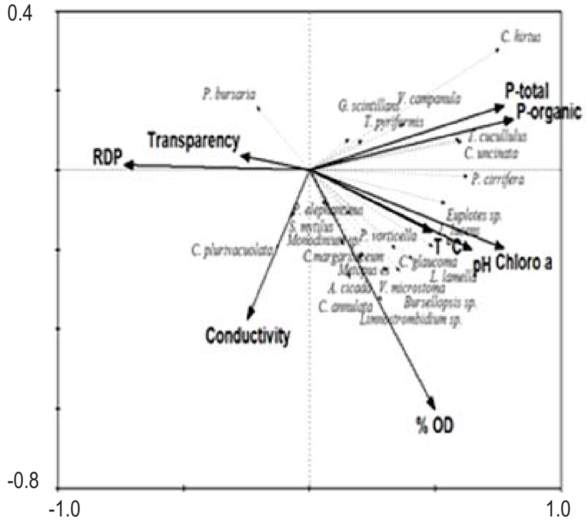


Fig. 8 : Redundancy analysis of planktonic ciliates species biomass and physico-chemical variables

different group the other two water bodies, with the lower trophic state. A cluster analysis of lakes using environmental data (Table 1) was coincident and confirm the similarity of eutrophic conditions in the Center and South ponds, as well, in the North. Regulation pond and Spring had a variable but a lower trophic state.

Size variation : Size variation of species had a particular behavior. Only three species (*H. grandinella*, *Cyclidum glaucoma* and *Cinetochilum margaritaceum*) had a size ≤ 30 µm. The size of 15 species was between 30 and 50 µm, and also 15 species were found between 50 and 100 µm. Finally, only two species were larger than 100 µm (*Spirostomum teres* and *Lembadion* sp.)

Beaver and Crisman (1989b) observed a replacement of large body ciliate species (mainly from the group of Oligotrichea) by small-body size species (mainly Scuticociliatida) as trophic state increases. The results of this study do not agree with this, as the number of species of the small size found in all ponds was very low (3). As occurred in Latvian urban and rural lakes (Buholce *et al.* 2015) species in the range size of 30-50 µm dominate in the North (11 sp.), South (11) and Regulation (6) ponds and in the Spring (4) whereas the range of 50-100 µm predominated in Center pond (12 sp.) and occupied the second place in importance in North (8), South (11), Regulation (6) and in the Spring (2).

Biomass : Ciliate biomass in the ponds varied according to the trophic state (Fig. 5). The South and Center ponds, the more eutrophic water bodies, had the highest median biomass (wet weight) values (3.0 mg l^{-1} in North and 2.8 mg l^{-1} in South pond) followed by the North pond (2.6 mg l^{-1}). In contrast, median biomass values in the Regulation pond (1.6 mg l^{-1}) and Spring (0.7 mg l^{-1}) were much lower. Biomass fluctuation in Center and South ponds was highly variable reaching maximum values of 9.6 mg l^{-1} in North and 12.2 mg l^{-1} in South.

Biomass behavior in the ponds agrees with the statement that ciliate biomass increases with the trophic state (Beaver and Crisman 1982; Zou *et al.,* 2013). Some authors proposed that the increase in nutrient availability favors higher abundance of bacteria and phytoplankton, both important food resources for planktonic ciliates (Machado-Velho *et al.,* 2005; Buholce *et al.,* 2015).

Percentage of biomass per ciliate size intervals : The contribution to the biomass of each size intervals of ciliates is shown in Fig 5. The 30-50 µm size class contribution to biomass was the higher in North (56 %) Center (55 %) and Regulation (55 %) ponds and in the Spring (68 %). In the South pond, the range size of 50-100 µm formed the main portion of the biomass (48.5 %), barely surpassing the 30-50 µm range (47.5 %). The next size range was 50-100 µm that varied from 48.5 % in the South to 24% in the Spring. The range below 30 µm represented 11% in the North and only 3.8 % in the Spring. Ciliates >100 µm contributed less than 5% in all ponds except in the North where they contributed 7.5% of the biomass. The relation of biomass to body size of the ciliate species did not agree with the results of Beaver and Crisman (1989b) because in Eastern Quarry the small size class was not important for biomass in any water body.

Percentages of biomass by taxonomic groups : Oligotrichea provides the main portion of ciliate biomass in the four ponds. The contribution was around 40% in North, Center and South ponds and up to 60% in the Regulation pond. In the Spring, with the lowest trophic status, Peniculid (*Paramecium bursaria*) constitute the largest percentage of the biomass, followed by Oligotrichea (*H. grandinella* and *Limnostrombidium* sp.). Prostomatea (*C. hirtus*) was the second contributor to biomass in North and South (about 30%) ponds but lower in the Center pond (less than 20 %).This group was not important in Regulation pond and it was absent in Spring (Fig.6)

Peritricha contribution was about 10 % in the North, Center and South ponds, but negligible in the Regulation and Spring. Cyrtophorida contributed slightly less than 10% of the biomass in the South, Center and Regulation, while Heterotrichea accounted for 7 % in the North and <5 % in the South and Regulation ponds.

Hypotrichea contribution was higher in the Regulation pond (15 %) and lower in the Center (7 %) and South (6%); Gymnostomatea only contributed between 3.75 (North, Center and South) and 5.75 in the Regulation pond. A remarkable fact is that Scuticociliatida had not a significant biomass in any of the most productive lakes (South and Center) and there was a visible contribution (2%) only in the North pond. This differs from that found by Beaver and Crisman (1982) who found that small-body ciliates (<30 µm), mainly belonging to Scuticociliatida, were prevalent at high trophic states.

Environment-species relationship: In the Redundancy analysis (RA) the first two axes explained the 79.7% of the ciliate biomass variation (Axis 1: 65.3%, axis 2: 14.4%). Most species were related with trophic state associated variables as chlorophyll *a*, % O.D., pH and total an organic phosphorous, as well as temperature (Fig. 8). Many ciliate species related with these conditions but *P. vorticella*, *Lembadion* sp., *Bursellopsis* sp., and *Limnostrombidium* sp. are notorious because they are important phytoplankton and picoplankton grazers. Other species also related with eutrophic conditions were mainly bacterivorous as *V. microstoma*, *C. glaucoma*, *A. cicada*, *C. annulata*, and *Euplotes* sp. Other species, as *C. hirtus*, *V. campanula*, *T. pyriformis*, and *G. scintillans* were specially related with high values of both total and organic phosphorous and could be also considered as eutrophic conditions indicators. On the other hand, *C. plurivacuolata*, related with conductivity, and *P. bursaria*, present in transparent water (Spring), could be indicators of better trophic conditions.

Ciliate species composition in the ponds of the Eastern Quarry showed significant similarities with other freshwater bodies in very different latitudes in the world. The dominant groups (Oligotrichea, Prostomatea) are also common in many other aquatic ecosystems. Eastern Quarry ponds trophic state varied from oligotrophic to near hypertrophic and abundance and biomass variation of planktonic ciliates was according with this gradient, showing higher values in more eutrophic water bodies. Medium size ciliates (30-50 µm) provided most of the biomass, while very small (≤ 30 µm) or very large (>100 µm) species provided a low percentage. A major difference in the Eastern Quarry ponds was the low participation of the group of Scuticociliatida, both in abundance and biomass of all ponds. This group has been observed in high numbers in eutrophic conditions, but this did not happen in the eutrophic lakes studied.The abundance and biomass of planktonic ciliates in these water bodies were suitable signals of the trophic state differences.

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