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Planktonic ciliates in a hypertrophic pond: Functional role and importance

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Abstract

Taxonomical composition and abundance of the planktonic ciliate assemblage in a Lake Tezozomoc, a hypertrophic pond located into an urban park in Mexico City, was investigated along the rainy season (May to October). The aims of the study were to know the main trophic roles and ecological significance of ciliates in a highly productive environment. A low number of taxa (27) and a wide abundance fluctuation (104-387 cil ml^{-1}) were found. The most abundant species (up to 162 cil ml^{-1}) was *Halteria grandinella*, an oligotrich ciliate that graze on bacteria and picoplankton, but also several big body sized species that feed on pico and nanoplankton were abundant. Sudden temporal changes in species dominance occurred. Ciliate biomass was very high and fluctuated widely (1.6-88 10^{6} µm^{3} ml^{-1}) being dominated by the >50 µm size fraction that mainly included the pico and nanoplankton feeders. Ciliates are a very important component in the plankton of hypertrophic lakes and their main control factor seems to be the grazing by big-body size *Daphnia* species.

Key words

Protists, Picoplankton, Seasonal fluctuation, Eutrophic conditions

Introduction

The role of ciliates as an important component of the microbial loop in freshwaters is widely recognized (Wiackowsi *et al*., 2001). Ciliates are a significant trophic link in energy transfer from heterotrophic (bacteria) and autotrophic picoplankton to the higher consumers (Zingell *et al*., 2007). Some ciliate species can graze on heterotrophic nanoflagellates and also on nanophytoplankton. On the other hand, ciliates are an essential food source for rotifers, cladocerans and copepods (Jack and Gilbert, 1997). Also some fish larvae, for example the guppy (*Poecilia reticulata*) larvae, can use ciliates as food in their early life stages (Lair *et al*., 1994).

Ciliates can reach elevated densities in hypertrophic water bodies (Nakano *et al*., 1998), where high chlorophyll *a* concentrations and productivity values are commonly present (Sommaruga and Robarts, 1997); small (<30 µm) bacterivorous species are usually dominant (Beaver and Crisman, 1989). Nonetheless, in hypertrophic environments there are also huge quantities of other food sources for ciliates: autotrophic picoplankton, heterotrophic flagellates, phytoplankton and other ciliates. The metazooplankton of hypertrophic lakes is usually very scarce (Sommaruga, 1995) so predation on ciliates is diminished; consequently the role of ciliates in controlling the growth of the smaller plankton size fractions (Nakano *et al*., 1998) and other larger fractions, as the nano and microplankton, is greater.

In the present work we studied the taxonomic composition and temporal fluctuation of the planktonic ciliate assemblage in Lake Tezozomoc, Mexico City, a small and hypertrophic urban pond. A discussion about the taxonomic composition, density and biomass contribution of the different ciliate trophic groups and their size classes is presented, as well as their relationship with some environmental conditions. The study followed the uncommon seasonal succession of zooplankton that took place along the rainy season (May to October) of the 2004 year. First, ciliates and small bodied rotifers were prevalent, followed by arise and growth of a copepod species belonging to the *Acanthocyclops robustus* complex, a predator cyclopoid and latter by *Daphnia exilis*, a big-body cladoceran species, together with the small *Moina macrocopa*. At the end of the study crustacean zooplankton disappeared and the lake returned to the dominance of ciliates and rotifers.

During the studied period, phytoplankton was dominated by several species of small Chlorophyte, as *Selenastrum minimum* and *Monoraphidium caribeum*, as well as the nanophytoflagellate *Chlamydomonas globosa*. Usually, *Microcystis* spp. are the most common phytoplankton species in the lake, but they were scarce along this period.

Materials and Methods

Study area: The Tezozomoc Urban Park is located at the north area of Mexico City (19° 29' 05" N and 99° 12' 36" W, 2250 m a.s.l.) and it comprises an area of 27 ha. It is situated very close to the boundary between Distrito Federal and the State of Mexico.

The lake is a small artificial pond with an area of 17 000 m^2 and volume of 33 000 m^3. The mean depth is about 1.0 m and the maximum depth reaches 2.10 m. The lake is filled with secondary treated water from the waste water treatment plant "El Rosario" by means of a continuous flow of 6 l s^{-1}. After the water stayed in the lake, it is stored and latter used for irrigation of the green areas in the park. The high nutrients loads at the treated water, as well as the excretions of numerous aquatic birds inhabiting the lake, are the main factors promoting the hypertrophic water conditions.

Climate is temperate subhumid, with a rainy summer. The rainy season lasted from May to October. The mean annual temperature is 17.5°C. The warmest month is May (19.9°C average monthly temperature) and the coldest December and January (14.7-14.8°C). The average annual precipitation is 836.5 mm (García, 2004), Besides several different water birds species, a population of the exotic fish *Poecilia reticulata* (guppy) is found in the lake (Elías-Fernandez *et al*., 2006).

Sample collection and procedures: Twelve bi-weekly samplings were made from May to October, 2004. This period correspond to the rainy season in Mexico City (Garcia, 2004). Three sampling stations (influent area, central area and effluent area) were selected in the limnetic zone of the lake (Fig. 1). Sampling took place between 10:00 and 13:00 hr. At each station the following environmental variables were measured throughout the water column: water temperature, specific conductivity (K_{25}) and dissolved oxygen concentration by a YSI model 85 multiparameter sonde (Yellow Spring Instruments, Ohio, USA). pH using a Conductronic model pH10 potenciometer (Conductronic Co, Puebla, Mexico). From the surface level (0.25 m) 100 ml water samples were obtained and carried to the laboratory for chlorophyll *a* concentration measurements using the cold (4°C) methanol extraction method and a HACH/DREL 2000 spectrophotometer (HACH Co., California, USA). P-PO_4 and N-NO_3 was measured by PhosVer 3 and NitraVer 5 methods and a HACH/DREL 2000 water quality lab.

Samples of planktonic ciliates were also obtained at 0.25 m depth using 600 ml plastic jars. 300 ml were fixed immediately with 1% acidified Lugol solution (Finlay and Guhl, 1992). The other 300 ml were carried to the lab for *in vivo* observation of ciliates (Foissner, 1991). When necessary, the protargol staining was applied (Foissner, 1992). For ciliate species identification the works of Foissner and Berger (1996), Foissner *et al*. (1999) and Foissner *et al*. (1991, 1992, 1994, 1995) were used. For ciliate quantification a Sedwick-Rafte counting chamber and a Zeiss K7 microscope (160X) were employed. At least 100 cells of the dominant species were counted to obtain a mean confidence interval of ±20% (Wetzel and Likens, 2001). Twenty cells of each species were measured using a calibrated micrometer. Ciliate biovolume was estimated by multiplying the numerical abundance of each species by the cell volume estimated by the cell dimensions and geometric shape (Beaver and Crisman, 1982).

Statistical analysis: An ANOVA analysis was applied to look for differences (p=0.05) between sampling stations. A Pearson correlation coefficient was calculated to find the relationship between total ciliate densities, chlorophyll *a* and total biovolume.

Results and Discussion

Physichal and chemical conditions: Table 1 shows the environmental conditions fluctuation along the study. Water temperature at the surface ranged between 19.1 and 23°C. The hottest months in Mexico City are May, June and July. Precisely, it was at the end of July when highest temperatures was measured. On the other hand, lowest temperatures were present at the end of September, when the cold season began (Table 1).

Specific conductivity (K_{25}) varied between 620 and 874 µS cm^{-1}. Higher values were found at the beginning of the rainy season (May-June), when the lake was concentrated. During the course of the season, a dilution process of the lake water occurred and lower values were observed at the end of the study.

pH values were high and very homogeneous along the study, ranging between 9 and 10. The only exception was the sampling at the end of July, when pH decreased to 6.6-8.5. Dissolved oxygen concentrations in the surface level showed a wide fluctuation (3.0-19.8 mg l^{-1}). Higher values were present from May to August. From September, when weather was colder, oxygen

Table - 1: Range of physic-chemical variables for different sampling period in Lake Tezozomoc

Variables	May 27th-July 5th	July 22nd	August 4th-October 27th
Temp (°C)	20.1-23.0	19.9-20.3	19.1-22.3
K_{25} (µS cm^{-1})	730-874	810-830	620-820
pH	9.3-10.0	6.6-8.5	8.9-9.8
D.O. (mg l^{-1})	4.5-13.9	4.0-9.8	3.0-19.8
$P\text{-}PO_4$ (mg l^{-1})	2.1- 3.3	6.5-6.8	1.6-3.5
$N\text{-}NO_3$ (mg l^{-1})	2.1-5.6	1.2- 4.2	0.5-1.4

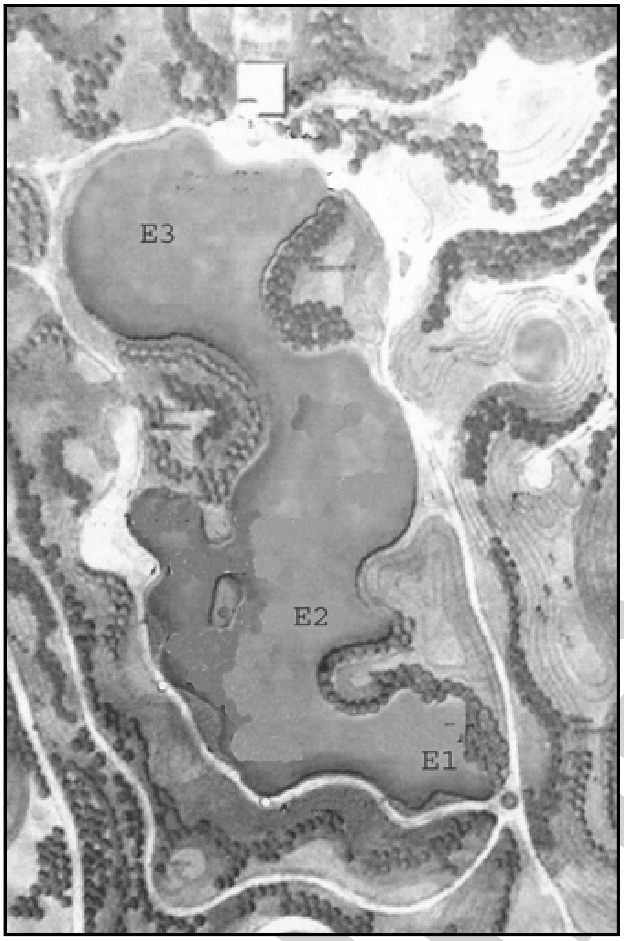

Fig. 1: Lake Tezozomoc map showing the sampling stations

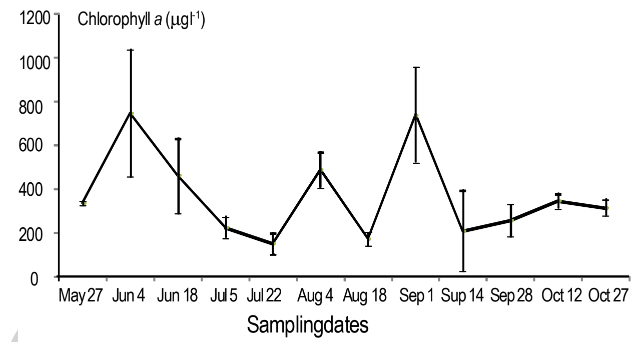

Fig. 2: Temporal chlorophyll *a* fluctuation in Tezozomoc. Mean ± Standard Deviation

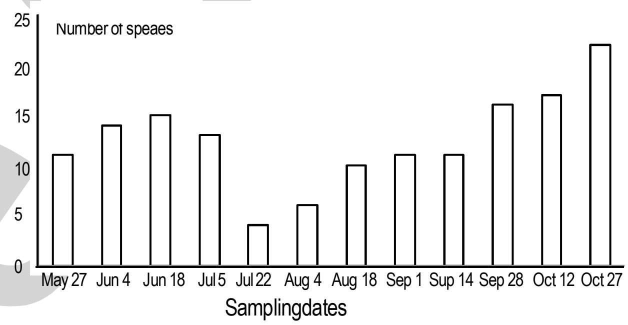

Fig. 3: Temporal variation of ciliate species richness in Lake Tezozomoc

concentrations were lower, probably associated with reduced photosynthetic rates.

Nutrient concentrations were very high in Lake Tezozomoc. The soluble reactive $P\text{-}PO_4$ varied between 1.6 and 6.8 mg l^{-1} as $P\text{-}PO_4$. The maximum value was measured at the end of July and most of the time fluctuated between 1.6 and 3.2 mg l^{-1} as $P\text{-}PO_4$.

Nitrogen as nitrates ($N\text{-}NO_3$) was higher between May and the early August. From the middle of August to October, $N\text{-}NO_3$ concentrations decreased to 1.0-0.5 mg l^{-1}.

In the July 22th sampling, very different environmental conditions were found. pH values varied from acidic to moderately alkaline, OD values were low and $P\text{-}PO_4$ concentrations strongly increased. Additionally, the lowest chlorophyll *a* concentrations were present. These particular conditions could be associated to the presence of high numbers of *Daphnia exilis* that graze intensely on phytoplankton. The algal reduction, reflected by the lowest chlorophyll a concentration, explains the low values of pH and OD and the increase in $P\text{-}PO_4$.

Lake Tezozomoc is hypertrophic. Chlorophyll *a* concentrations were high most of the time and showed a wide fluctuation (154-738 µg l^{-1}). As mentioned above, the lowest value was found at the end of July, while the early June and September had the highest concentration (Fig. 2).

Ciliate composition and abundance: Twenty seven ciliate taxa were identified in the lake (Table 2). Ciliate assemblage was a mixture of true planktonic species and litoral or particle-associated species. An ANOVA analyses, using total ciliate

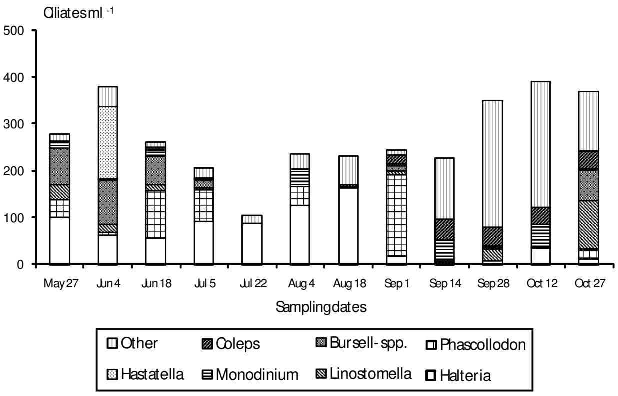

Fig. 4: Temporal fluctuation of total ciliate density and the most abundant species densities in Lake Tezozomoc

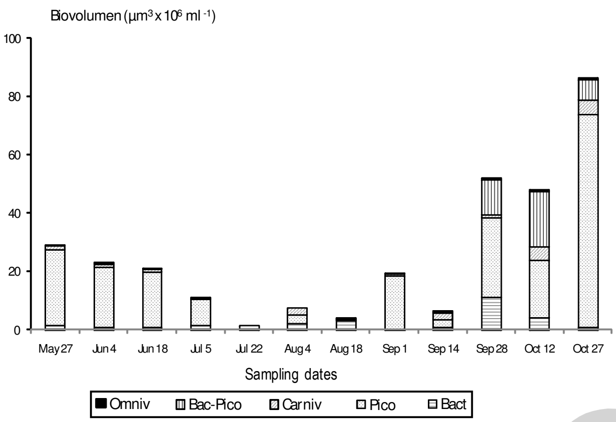

Fig. 5: Contribution of the different ciliate trophic groups to the biovolume along the studied period

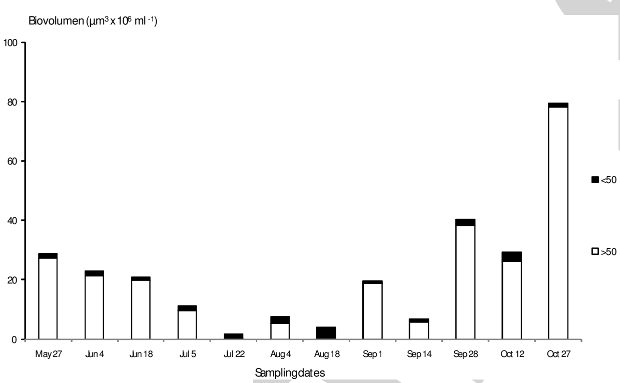

Fig. 6: Temporal variation of biovolume composition by ciliate size fractions

densities showed no significant differences between the three sampling sites ($p>0.05$, $F= 2.31$) therefore data from the three sites was joined.

Temporarily, ciliates variation was as follows (Fig. 3): from May to the early July, species richness varied between 11 and 15 species and high cell densities (>200<378 cil ml^{-1}) were counted. On July 22nd sampling, species richness (4 species) and densities (104 cil ml^{-1}) were the lowest. From August to October there were a recovery in species numbers and densities reaching the maximum values (22 species, >200<387 cil ml^{-1}) at the end of the study.

A shift in the species dominance was also observed. *Halteria grandinella, Linostomella vorticella, Bursellopsis* spp. and *Phascolodon vorticella* were numerically dominant along the first four samplings. *Hastatella* sp. was dominant in June but latter it suddenly disappeared

At the end of July, when minimum values of species richness and density were present, *H. grandinella* and *Epistylis pygmaeum* (mainly as epibiont on rotifers) were the dominant species. Most other species were absent.

Finally, from August to October an increase in both variables was observed. In September the picoplankton feeder *P. vorticella* reached a peak. At the end of the rainy season maximum numbers of species and high densities were observed. *L. vorticella*, *Coleps hirtus*, *Cinetochilum margaritaceum* and *Paramecium aurelia* dominated (Fig. 4).

Ciliates biomass as biovolume ($\mu m^3 l^{-1}$) fluctuated strongly from 1.6×10^9 $\mu m^3 l^{-1}$ (July 22nd) to 88×10^9 $\mu m^3 l^{-1}$ (October27th) (Fig. 5). Picoplanktivorous ciliates biomass was higher most of the time. Only two sampling dates were dominated by bacterivorous ciliate biomass: July 22nd and August 18th. On September 14th a significant contribution of bacterivorous and carnivorous species was observed.

Biomass values were higher from the end of September to October. All the time ciliate species larger than 50 µm were the most important biomass contributors. As mentioned before, July 22nd was the exception because only small size bacterivorous species were observed (Fig. 6).

Hypertrophic conditions in Lake Tezozomoc are strongly coincident with other works. It is a shallow water body with a high external nutrient loading, elevated pH values and a wide fluctuation in dissolved oxygen concentrations, from supersaturation conditions to very low values (Barica, 1980; Sommaruga and Robarts, 1997). Biological conditions also correspond to those of hypertrophy: high and fluctuating chlorophyll *a* concentrations, sudden collapses of phytoplankton and low abundances of crustacean zooplankton (Alvarez-Cobelas and Jacobsen, 1992; Sommaruga and Robarts, 1997).

In the plankton of Lake Tezozomoc ciliates are an important component. As has been found in other hypertrophic lakes (Wiackowski *et al*., 2001; Nakano *et al*., 1998), the number of ciliate taxa was low. This fact could be related to the numerous environmental conditions present in hypertrophic environments that can have a high selective pressure for many species. Usually, a simplification of trophic food webs in hypertrophic environments is observed and strong oscillations in the abundance of many components of the food web are also common (Sommaruga and Robarts, 1997). These sudden fluctuations were observed in some

Table - 2: List of ciliate species found in Lake Tezozomoc

Prostomatida
Actinobolina wenrichii (Wang and Nie 1933)
Coleps hirtus (Nitzsch, 1827)
Haptorida
Monodinium balbianii (Fabré-Domergé 1818)
Pleurostomatida
Litonotus fasciola (Wrze' sniowski 1870)
Trachelophyllum pusillum (Perty, 1852)
Hipotrichia
Aspidisca cicada (O.F. Müller 1876)
Oxytricha fallax (Stein, 1859)
Oligotrichia
Halteria grandinella (Müeller 1773) Dujardin, 1841
Limnostrombidum pelagicum Kahal 1932 Krdiner 1995
Colpodea
Bursellopsis nigricans (Lauterborn, 1894)
Bursellopsis sp.
Linostomella Vorticella Ehrenber, 1833
Colpoda steini (O.F. Müller 1773)
Peritrichia
Carchesium polypinum (Linnaeus 1758)
Pelagovorticella mayeri (Fauré-Fremiet, 1920) Jankowski 1980
Vorticella convallaria Linaeus, 1758
Vorticella microstoma (Ehrenberg, 1830)
Hastatella sp.
Epystilis pygmaeum (Ehrenberg, 1838)
Scuticociliatida
Cinetochilum margaritaceum (Ehrenberg, 1831)
Cyclidium glaucoma (O.F. Müller, 1773)
Hymenostomata
Paramecium aurelia (O.F. Müller, 1773)
Paramecium caudatum (Ehrenberg, 1833)
Tetrahymena pyriformis (Ehrenberg, 1830)
Prostomatida
Phascollodon vorticella (Stein, 1859)
Suctorida
Podophrya fixa (Ehrenberg, 1838)
Sphaerophrya soliformis (Lauterborn, 1908)

cases for the total number of ciliates and for some ciliate species in Tezozomoc.

Ciliate types and densities have been related with the lakes trophic status by Beaver and Crisman (1982). According with them, ciliate density is higher in eutrophic conditions, and the small size (<30 µm) bacterivorous species, mainly belonging to the Scuticociliated group are the dominant. In the present work ciliate densities (272 ± 88.5 cil ml^{-1}) were higher than those found by Beaver and Crisman (155 ± 60.9) in hypertrophic subtropical lakes of Florida, USA. Lake Tezozomoc can be considered as a subtropical water body. The higher densities in Tezozomoc could be related with the abundant food resources along the study. Nakano *et al*. (1998) found up to 3500 cil ml^{-1} in a hypertrophic pond in Japan.

Tezozomoc ciliates size composition was different from Beaver and Crisman proposal. Large-bodied taxa (>50 µm), mainly picoplankton and nanoplankton feeders, represented the major biomass proportion along the study. Some scuticociliates, for example *Cyclidium glaucoma*, were sometimes abundant but, due to the small size, did not contribute notoriously to biomass. As has been observed in several temperate lakes (Simek *et al*., 2000), in Tezozomoc the most important bacterivorous group was the Oligotrichia, being *Halteria grandinella* the dominant species. Simek *et al*. (2000) mention a case when oligotrichs are not the vorticella prevalent bacterivorous; it could be found when metazooplankton abundace is high and peritrich ciliates attached to other organisms (cyanobacteria, algae or zooplankton) are abundant. This situation was observed in Tezozomoc in July 22nd when the cladocera *D. exilis* reached the highest density, the copepod *A. robustus* was also present, and ciliates were the lowest. The bacterivorous epibiont peritrich *Epystilis pygmaeum* was observed attached on organisms of different rotifer species, especially on *Brachionus* spp., as has been presented by Gilbert and Schroeder (2003). This could be a strategy to reduce metazoan predation (Simek *et al*., 2000). *H. grandinella* was also abundant in this sampling date and its characteristic "jumping" response could be the way to explain it resistance to predation effects. According to some studies, the jumping response is an effective escape reaction against predation by rotifers but not so much against *Daphnia* or cyclopoid copepods (Jürgens *et al*., 1999; Jack and Gilbert, 1997). Our results only partially support this point of view.

As Zingel *et al*. (2007) has observed, ciliates can be the dominant grazers on pico and nanoplankton in highly eutrophic lakes. But in these study ciliates less than 30 µm body size prevailed. On the opposite, Tezozomoc had a big body size (>50 µm) ciliate dominance. Most of this big species were picoplankton and nanoplankton feeders, and some of them were capable to graze on bigger size particles (30 µm diatoms). The elevated densities and big cell size of this species are the explanation for the almost permanent dominance of this size category on ciliate biomass. Only in two dates (July 22nd and August 18th) the biomass of bacterivorous surpassed that of picoplankton and nanoplankton grazers. In both cases *H. grandinella* biomass was the major part of bacterivorous biomass. In our work we considered *Halteria* as bacterivorous but this species is also an important autotrophic picoplankton feeder (Simek *et al*., 1995), grazing small cyanobacteria. Consume of autotrophic and heterotrophic picoplankton by *Halteria*, especially when is present in high densities, could be very important for water bodies, as was shown by Simek *et al*. (2000) in two reservoirs. In Tezozomoc, *Halteria* grazing on picoplankton could be important mainly from May to August. *Halteria* was the most abundant ciliate along the study and its average densities (0-162 cil ml^{-1}) were comparable with those found in other hypertrophic environments as the Furuike Pond, Japan (25-389 cil ml^{-1}) and the Poppelsdorfel Weihers Pond, Germany (10-289 cil ml^{-1}) as reported in Simek *et al*. (2000) .

Temporal fluctuation of total ciliate densities in Lake Tezozomoc was notorious, as is common in eutrophic environments (Wiackowski *et al*., 2001). Higher ciliate densities were present when crustacean zooplankton was scarce or absent. On the other hand, the lowest density coincided with the presence of high numbers of *Daphnia exilis*. The negative effect of crustacean zooplankton on ciliates, especially by *Daphnia*, has been observed commonly (Jurgens, 1994; Jurgens and Jeppessen, 1999). The negative impact of *Daphnia* on ciliates can follow two ways: a direct grazing effect and an indirect effect through food availability reduction. In Tezozomoc the lowest chlorophyll *a* values and ciliate densities coincided with the maximum number of *Daphnia*. Then, it seems that ciliates were affected by both ways. Density variations of *A. robustus* copepod had not and evident effect on ciliates, but some species could be consumed.

Ciliate biomass as biovolume was huge. Dominance of large bodied species together with high densities explains these values. Our values are much higher than those observed in other hypertrophic lakes but near to the ciliate biomass in lake Vortsjaer (Estonia), a shallow and eutrophic water body (Zingel, 1999).

It is concluded that ciliates are an important component in the planktonic food web of the hypertrophic Lake Tezozomoc. As has been stated by other authors, they are the major grazers on autotrophic and heterotrophic picoplakton but also consume significant numbers of nanoplanktonic cells and also microphytoplankton (Ueno *et al*., 2005). Usually, ciliates contributed with a very important portion of the zooplankton biomass in Tezozomoc. When crustacean zooplankton is present, ciliates seem to be an important trophic link for energy transfer between pico and nanoplankton and microzooplankton. Hypertrophic waters can be very propitious environments for the growth of several species of ciliates because food resources are diverse and abundant. As has been stated in other water bodies, grazing by *Daphnia* could be a main control factor for planktonic ciliates growth.

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