Seasonal and spatial dynamics of a ciliate assemblage in a warm-monomictic Lake Alchichica (Puebla, Mexico)

Dinámica temporal y espacial de la comunidad de ciliados en un lago monomóctico-cálido Alchichica (Puebla, México)

Miroslav Macek¹, ², Dana Peštová¹, ³, María Elena Martínez Pérez¹

¹ Universidad Nacional Autónoma de México campus Iztacala, Av. de los Barrios 1, los Reyes Iztacala, Tlaíhapeantla, 54090 Edo. México, México
² Biology Centre, Institute of Hydrobiology, Academy of Sciences of the Czech Republic, Na sádkách 7, 370 05 České Budějovice, Czech Republic
³ Masaryk University Brno, Faculty of Science, Dept. Botany and Zoology, Kotlárská 2, 611 37 Brno, Czech Republic.
mirek@campus.iztacala.unam.mx

ABSTRACT

The distribution of the ciliate assemblage was surveyed along a depth gradient in the maar crater, athalassohaline, warm monomictic Lake Alchichica (Puebla, Mexico) from June 2003 to October 2006 (monthly). DAPI staining was employed to count ciliates while the Quantitative Protargol Staining was used for their identification. Peritrichs often numerically dominated the ciliate assemblage; a maximum of 54 cells ml⁻¹ (Rhabdostyla sp.) was observed in the surface layer at the end of the mixing period, during the development of diatoms (Cyclotella alchichicana), cyanobacterial (Nodularia sp.) bloom and its decay. Minute spirotrichs (particularly Halteria grandinella) and a haptorid, Belonophrya pelagica occasionally dominated the epilimnion while mixotrophic Euplotes cf. daidaleos and Pelagothrix sp. were important round the oxycline along with haptorids, particularly Phialina sp. Scuticociliates Cyclidium glaucoma, Uronema nigricans, and anaerobic ciliates Isocyclidium globosum and Caenomorpha sp. dominated within the hypolimnetic assemblages.

Key words: Ciliates, athalassohaline lake, anoxic.

RESUMEN

De junio de 2003 a diciembre de 2006 se estudió mensualmente la distribución de la comunidad de ciliados en el gradiente de profundidades del Lago Alchichica (Puebla, México), un lago cráter, tipo maar, atalassohalino de comportamiento monomóctico-cálido. La tinción de DAPI fue empleada para el recuento de los ciliados y la tinción de protargol cuantitativa para su identificación. Frecuentemente los ciliados peritrichos dominaron numéricamente la comunidad. Al final del periodo de estratificación y durante el desarrollo de las diatomeas (Cyclotella alchichicana) y el florecimiento y posterior decaimiento de las cyanobacterias filamentosas (Nodularia sp.), se observó un máximo de 54 cél. ml⁻¹ de Rhabdostyla sp. en la superficie del lago. Los espirotricos pequeños (particularmente Halteria grandinella) y los haptóridos como Belonophrya pelagica, dominaron ocasionalmente en el epilimnion, mientras que los ciliados mixotróficos: Euplotes cf. daidaleos y Pelagothrix sp. estuvieron asociados a los haptóridos grandes, particularmente a Phialina sp. y fueron importantes alrededor de la oxicina. Los escuticociliados: Cyclidium glaucoma, Uronema nigricans y los ciliados anaerobios: Isocyclidium globosum y Caenomorpha sp. dominaron en el hipolimnion.

Palabras clave: Ciliados, lago atalassohalino, anoxia.
INTRODUCTION

The importance of ciliates in the pelagic marine microbial loop (sensu Azam et al., 1983) as well as in freshwater environments of a moderate trophic status has been demonstrated (Pace, 1982; Gates, 1984; Müller, 1989; Šimek et al., 1990; Müller et al., 1991). Many authors have shown that an effective feeding and rapid growth of ciliates were detected when they could explore rich food-patches such as phase borders where food particles are accumulated (the thermocline or oxycline). It has been repeatedly documented that a peak in protozoa distribution temporarily coincided also with the decomposition of primary production (Taylor & Heynen, 1987; Weisse et al., 1990; Macek et al., 1994; Šimek et al., 1995; James et al., 1995). Generally, a maximum of the ciliate distribution was found in the metalimnion or below it (reviewed in Beaver & Crisman 1989; Fenchel et al., 1990; Sherr et al., 1991; Macek et al., 1994; Šimek et al., 1995), apparently related to microaerobic processes. If an oxycline did not occupy the same position as a thermocline, additional ciliate maxima could be found there and/or in the layer above the water body floor.

More information is available from shallow water bodies with light conditions, which are sufficient to provide both oxygenic and anoxygenic photosynthesis (Berninger et al., 1986; Finlay et al., 1996; Macek et al., 2001). Species of genera Loxodes and Spirostomum were related to nitrate and/or sulphate microbial reduction (Finlay et al., 1983; Guel & Finlay 1993; Foissner et al., 1995) while others of Metopus and Caenomorpha, were found to be strictly anaerobic (Foissner et al., 1992; Decamp & Warren 1997).

However, Foissner et al. (1999) doubted the existence of typical anaerobic-pelagic (hypsolimnion) species. Apart from peaks of the above mentioned ciliates, the presence of anaerobic ciliates was accepted as a result of migration of benthic species. The typical anaerobes (genus Caenomorpha but also scuticociliates) possess hydrogenosomes instead of mitochondria; moreover, their metabolic pathways need co-metabolism with symbiotic microbes (Esteban et al., 1993; Fenchel & Finlay, 1995). On the other hand, there is evidence that the “symbiont” acquisition could be provided via ingestion (van Hoek et al., 2000).

In the present study we have tried to provide more comprehensive information on the seasonal ciliate assemblage development throughout the water column including an anoxic layer of crater Lake Alchichica (Puebla, Mexico), from June 2003 to December 2006.

MATERIAL AND METHODS

Study site and sampling. We conducted the study in the large and deep maar-crater Lake Alchichica (Puebla, Mexico) with extended hypolimnetic anoxia classified as tropical, warm and monomictic (Filinov et al., 2006). Alchichica is located at 19° 24' N; 97° 24' W at an altitude of 2340 m.a.s.l. Mean and maximum depths are 40.9 and 62 m, respectively, surface area being 2.3 km²; salinity averaged 8.5 g L⁻¹. The climate of the region is dry-temperate, with a mean annual temperature of 12.9 °C and annual precipitation less than 400 mm.

The only sampling station was located above the maximum depth; samples from 5 to 12 depths were taken monthly (within 12:00 to 15:00) using a Niskin (USA) or IHE (Czech Republic) sampler. The sampling depths were decided according to in situ measured temperature and dissolved oxygen concentration, DO, which were assessed by means of a Hydrolab DS4/SVR4.

Fixation. Ciliates were fixed with acid Lugol’s iodine, decolourised with thiosulfate this is fate and post-fixed with 2% formalin (Sherr & Sherr 1993), or eventually with 7% (v/v) Bouin’s fixative (Montagnes & Lynn 1987). Total picoplankton including autotrophic picoplankton, APP was fixed with 2% formalin (Straškrabová et al., 1999).

Quantification and identification of microorganisms. Ciliates were counted in the DAPI-stained samples (Porter & Feig 1980) and identified using a quantitative protargol staining (QPS) on cellulose-esters membranes (1.2 μm) according to Skibbe’s (1994) modification of the Montagnes & Lynn (1987) method. Foissner et al. (1999) key, as well as the literature cited therein, was used for ciliate identification; however, higher taxa are presented according to Adl et al. (2005).

Autotrophic picoplankton (APP), particularly picocyanobacteria, were evaluated in the samples concentrated on polycarbonate membranes (0.2 μm) and mounted in an immersion oil via autofluorescence (epifluorescence microscope Leica, Germany) using the CY3 filter-set (green excitation and red observation-light); the commonly used yellow fluorescence of APP upon AO (Acridine orange) was not sufficiently intense.

Data analysis. For the ciliate stratification analysis, all data were used; in order to determine the annual pattern of development, weighted mean-numbers were calculated within the whole water column, and for the top 20 m and the below 35 m layers, representing the epilimnion and the anoxic hypolimnion during the stratification period, respectively.

RESULTS

A total of about 40 taxa of ciliate have been identified to genus and, when possible, to species level, covering nearly all higher taxa of Ciliophora (important species listed in Table 1). Apart from several abundant taxa present during the whole sampling period and distributed throughout the water column (scuticociliates, peritrichs and spirotrichs), most of the present taxa appeared in low numbers during most of the study period.
Average ciliate abundances in the whole water column ranged from undetectable numbers in January 2005 to 26 cells ml\(^{-1}\) in September 2006 (Fig. 1). The highest water column means of ciliates were observed in the late stratification period (September to November); when the assemblage was almost exclusively dominated by scuticociliates, however, mainly in the oxycline-anoxic layers (Figs. 1, 2). An absolute maximum of 54 cells ml\(^{-1}\) was observed in the surface in May 2004, when peritrichs colonizing *Nodularia* sp. filaments dominated the assemblage. During August to October, local maxima of over 40 cells ml\(^{-1}\) were found, as mentioned above, in micro-aerobic-anoxic layers. Near bottom ciliate numbers reached the maximum of 40 cells ml\(^{-1}\) in September 2005.

Peritrichs were the numerically dominant group in Alchichica (Fig. 2), contributing nearly 40% to the total numbers throughout the study period. They were characterised by small-sized taxa (<40 μm), either single-celled, non-attached (*Pelagovorticella natans* (Fauré-Fremiet, 1924) Jankowski, 1985, and *Vorticella aquadulcis* complex) or sessile *Rhabdostyla* sp. and unidentified *Vorticella* sp. Peritrichs contributed frequently >70% to the total ciliate abundance in the epilimnion. When *Rhabdostyla* sp. absolutely dominated the ciliate assemblage, it colonised a centric diatom, *Cyclotella alchichicana* Oliva et al., 2006 and/or filamentous cyanobacteria, *Nodularia* sp. (peaking March to May 2004 in epilimnion). Nearly all filaments of cyanobacteria were colonized, as well as diatoms, which frequently possessed ciliates at both sides of their centric cell. Around the oxycline, peritrichs abundances were of the similar value as those of spirotrichs; they were observed neither abundant in the anoxic hypolimnion nor around the oxycline.

Scuticociliates were the most abundant group in 2005 (68%) and throughout the study they formed 27% of the sampling average. Scuticociliates were found generally around the oxycline and in anoxic layers (Fig. 2), mainly represented by minute ciliates *Uronema nigricans* (Müller, 1786), *Cyclidium glaucoma* (Müller, 1773) and *Cinetochilum margaritaceum* Perty, 1852. They were also characteristic for the near-bottom ciliate assemblage, especially from August to October, when an anaerobic *Isocyclidium globosum* Esteban, Finlay & Embley, 1993 was observed (particularly in 2005).

Minute spirotrichs were observed to be distributed throughout the water column, and they formed about 10% of the total ciliate numbers (Fig. 2). Average sampling mean was about 15.0%, however, *Halteria grandinella* (Müller, 1773) Dujardin, 1841 solely contributed to 12.5%. Moreover, very small *Halteria* sp. and

![Figure 1. Dissolved oxygen concentrations, DO and total ciliate numbers in Lake Alchichica (upper panel); mean ciliate numbers in the whole water column, the top 20 m and bottom (lower panel).](image-url)
Rimostrombidium sp. (with globoid macronucleus) were found. Typical stratification of temperature, dissolved oxygen and ciliate numbers are presented in Figure 3.

Among haptorids, Belonophrya pelagica André, 1914 (Fig. 4b-d), Monodinium sp. (Fig. 4e) and Mesodinium acarus Stein, 1862 were the most abundant species. However, from the point of view of biomass, Phialina sp. (Fig. 4a) was the most important, followed by Lacrymaria sp. and Pelagolacrymaria sp. They were concentrated in thermocline-oxycline layers and round the anoxic boundary, accompanied by mixotrophic hypotrich, Euplotes cf. daidaleos Diller & Kowaris, 1966 (Fig. 5a-f). In addition to these groups, some very large ciliates, generally observed in micro-aerobic or anoxic conditions, particularly Holosticha kessleri Kessler (Wrzesniowski, 1877), Uroleptus rattulus Stein, 1859 (Fig. 5g) and Chaetospira sp. (Fig. 6h-j) showed very important contribution to the ciliate biomass. A minute Litonotus sp. was also found (Fig. 4f, g).

Among strictly anaerobic ciliates, Isocyclidium globosum (Esteban, Finlay & Embley, 1993) and a minute Caenomorpha sp. (Fig. 6a-d) were the most abundant; Epalxella sp. and Trimyema sp. were found in considerable numbers at the end of the stratification period (November 2003 and 2006).
Ciliates in Lake Alchichica

DISCUSSION

Ciliate abundance and species richness found in Alchichica did not markedly differ from those reported from the oligo- to mesotrophic environment (Gates, 1984; Taylor & Heynen, 1987; Beaver & Crisman, 1989; James et al., 1995; Weisse & Müller, 1998; Foissner et al., 1999; Callieri & Bertoni, 1999; Callieri et al., 2002; Carrick, 2005; Macek et al., 2006). Although the annual-average ciliate abundances varied nearly two-fold, the ciliate dynamics apparently followed a similar annual pattern.

Generally, the highest ciliate abundances (>10 cells ml\(^{-1}\)) were found during the late stratification period (mainly scuticociliates) while the ciliates were scarce during the mixing period (December through February); local maxima were observed in the stratification onset in May 2004 as the result of the attached peritrichous ciliate peak in the epilimnion, during the *Nodularia* sp. bloom.

Such dynamics differed from the known patterns in template dimictic water bodies, where ciliate maxima were generally concentrated to the metalimnion, reflecting either spring (following mixing) or summer phytoplankton peak in the epilimnion (e.g., Carrick, 2005; Macek et al., 1994; Šimek et al., 1990, 1995; Weisse & Müller, 1998). Numerically, free-swimming either omnivorous or algivorous ciliates, followed by peritrichs dominated.

In Alchichica, low ciliate abundances during the mixing period coincided with increasing APP numbers throughout the water column. During this period, the contribution of the different ciliate groups was distributed only within peritrichs and sprotrichs. The lake mixing was followed by the important peritrichous ciliate peak whenever the ciliates found sufficient abundance of phytoplankters to be colonised: filamentous cyanobacteria *Nodularia* sp. and/or diatoms *Cyclotella alchichicana* throughout the water column, and *Chaetoceros elmorei* Boyer mainly in the thermocline. *Rhabdostyla* sp. and unidentified minute *Vorticella* sp. contributed for about 25% and 20% as a sampling mean in numbers and biomass, respectively. Typical pelagic colonial peritrichous genera such as *Epistylis* or *Zoanthella* from oligotrophic-mesotrophic environment (Macek et al., 1994; Müller et al., 1991; Salbrechter & Arndt, 1994; Foissner et al., 1999) were not found; however, *Pelagovorticella natans* and *Vorticella aquadulcis* complex were observed throughout the water column penetrating below the oxycline. The contribution of peritrichs to the total biomass was very high (compare, Macek et al., 1994, 2000; Lugo et al., 1998 with Salbrechter & Arndt, 1994; Weisse & Müller, 1998; Wille et al., 1999; Callieri et al., 2002, Macek, 2002; Macek et al., 2006).

The dominance of peritrichs, sprotrichs and scuticociliates was found a common feature in freshwater systems and marine environments (e.g., James et al., 1995; Šimek et al., 1995). Minute (< 20 µm) picoplanktivorous heterotrophic sprotrichs such as oligotrichs (*Rimostrombidium brachykinetum* Krainer, 1995 and *R. humile* Petz and Foissner, 1992) and sprotchotrichs (*Halteria* spp.) dominated within bacterivorous ciliates in freshwater environments, at least during summer stratification period (Šimek et al., 2000; Carrick 2005). Additionally, the peaks of *H. grandinella* and *Halteria* sp. have frequently been located at the food-rich and/or above bottom-layers (Foissner et al., 1999; Macek, 2002; Macek et al., 1996; Salbrechter & Arndt, 1994; Šimek et al., 1995, 1996, 2000; Stabell, 1996; Weisse & Müller, 1998).
The predominance of large mixotrophic, fine- to coarse filter feeding ciliates was reported for oligotrophic lakes and coastal waters (Beaver & Crisman, 1989; Šimek et al., 1996; Macek et al., 2001, 2006). Large oligotrichs (like Rimostrombidium lacustris Petz and Foissner, 1992 or a mixotrophic R. velox Jankowski, 1978) were found in lakes with a well-distinguished pelagic zone (cf., Taylor & Heynen; 1987; Müller et al., 1991; Salbrechter & Arndt, 1994; Weisse & Müller, 1998) but they were rather scarce in Alchichica. On the other hand, during the late stratification period, the peak of microaerobic/anoxic and/or mixotrophic species were found. The distribution of mixotrophic Euplotes cf. daidaleos and Pelagotrich sp. followed on a very fine way the oxygen depletion below the thermocline, which dropped down 30 m. Euplotes cf. daidaleos frequently dominated in the oxycline while Pelagotrich sp. was repeatedly observed in low numbers throughout the water column. Its occurrence in anoxic water layers with sufficient light was in concordance with published data on the role of its symbionts in the ciliate distribution (Berninger et al., 1986; Finlay et al., 1996; Foissner et al., 1999). A mixotrophy might support its growth in nearly anoxic environment such as it was observed either for prostomes (Finlay et al., 1996) or strombidiids (Macek et al., 2001); however, during August (2006), Euplotes sp. was found without an apparent presence of zoochlorelae.

Figure 4. Ciliates found around the oxycline of Lake Alchichica (protargol staining): Phialina sp. (a); Belonophrya pelagica with contracted (b, c) and extended tentacles (d); Monodinium sp. (e); Litonotus sp. (f, g)
Bacterial abundance in Alchichica was high enough to maintain the populations of scuticociliates (Peštová et al., 2008). Scuticociliates were typically connected with phytoplankton decay (Müller et al., 1991; Šimek et al., 1995, 1996) following the summer peak, even though they were also found in very low numbers in the water column in the above timberline lakes (cf., Weisse & Müller, 1998; Callieri & Bertoni, 1999; Wille et al., 1999; Macek et al., 2002, 2006). In Alchichica, apparently, their peaks did not directly follow phytoplankton maxima, measured by Chl a, but could be related to the phytoplankton decay. Particularly, Cinetochilum margaritaceum was observed above the thermocline, Uronema nigricans around the oxycline.
meanwhile *Cyclidium* and related genera penetrated into the anoxic hypolimnion. It is in concordance with data by Bernard & Fenchel (1996), which proved that *Uronema marina* (synonymic with *U. nigricans*) was not able to grow without the oxygen. A minute *Isocyclidium globosum* found at the lake floor, covered with symbiotic bacteria (Fig. 6f), had been first reported in a karstic lake (Esteban et al., 1993).

Other anaerobic ciliates, particularly *Trimyema* sp. and *Caenomorpha* sp. were less important even though they dominated at the lake floor occasionally. Due to a peculiar quality of anoxic water in Alchichica, we are lacking good protargol impregnated preparations but we could observe fluorescence of bacterial/archaeal symbionts of *Caenomorpha* sp. (Fig. 6d). Large anaerobic ciliates such as genera *Loxodes* or *Spirostomum*, were not found (Guhl & Finlay 1993; Macek et al., 1994, 2000).

Raptorial haptorids could be found frequently in oligotrophic waters (Lugo et al., 1998; Macek et al., 1994, 2000); also in Alchichica, we found regularly the heterotrophic gymnostomes *Mesodinium acarus* and *Monodinium* sp. (Fig. 4e) throughout the year but normally in low numbers. Other gymnostomes were
present in elevated numbers, particularly *Belonophrya pelagica* (Fig. 4b-d) or biomass, particularly *Phialina* sp. (Fig. 4a).

Among undoubtedly algivorous species, only prostomes of the genus *Prorodon* were found in Alchichica but in negligible frequency. In comparison with other reported data, *Balanion plancintonicum* Foissner, Berger and Kohmann, 1994 and *Histiobalantium* spp. that are typical for oligo- to mesotrophic low-alkaline environments (Müller et al., 1991; Salbrechter & Arndt 1994; Šimek et al., 1995; Macek et al., 1996; Weisse & Müller 1998; Wille et al., 1999) were not found.

Figure 6. Ciliates found below the oxycline of Lake Alchichica: *Caenomorpha* sp. in DAPI staining (a-d) with stained macronucleus, MA, autofluorescing ingested prokaryots in foos vacuoles, FV and symbiotic prokaryots (SP); *Isocyclidim globosum* in protargol staining (e) and with fluorescently labelled bacterial symbionts (f); *Pelagothrix* sp. (g) and *Chaetospira* sp. (h-j) in protargol staining

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