Ecology and community structure of ciliated protists in two alkaline–saline Rift Valley lakes in Kenya with special emphasis on *Frontonia*

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Ciliated protist assemblages of the shallow soda lakes Bogoria and Nakuru in Kenya were studied weekly during the short rainy season in October and November 2008 to identify their taxonomic composition and possible interactions with abiotic and biotic factors. Overall, 22 ciliate morphotypes were detected. *Cyclidium glaucoma* was the most abundant, whereas *Frontonia* sp., *Condylostoma* sp. and *Holophrya* sp. dominated in terms of biovolume. Further, the assignment of ciliates to nutritional modes indicated that the abundance of bacterivorous ciliates was not related to bacterial abundance, most likely because of the very high bacterial food concentrations (83.0×10^6 cells mL^{-1} on average). The abundance of *Frontonia* sp. was positively correlated with chlorophyll *a* in Lake Bogoria, but not in Lake Nakuru. Morphometric measurements of *Frontonia* sp. indicated significant intraspecific differences in mean cell length, i.e. 116.1 ± 2.3 vs. 139.0 ± 2.7 μm in Lake Bogoria and Lake Nakuru, respectively. Sequences of the 18S SSU rRNA, however, turned out to be identical for individuals of the two lakes. Phylogenetic relationships of the subclass Peniculia based on the 18S rRNA genes revealed that *Frontonia* from these lakes rather clustered with *Apofrontonia* and *Paramecium* than with other *Frontonia* species, indicating that the genus *Frontonia* is paraphyletic. With the exception of...
C. glaucoma and Euplotes moebiusi, the ciliate taxa from the two lakes could be identified only down to the genus level. We assume that these taxa are still not yet described and thus highlight the unique character of these ecosystems and the need for more studies.

KEYWORDS: Frontonia; pelagial; eukaryotic microbial community; Bogoria; Nakuru

INTRODUCTION

Alkaline–saline tropical lakes have a limited species inventory, although they are among the most productive ecosystems in the world with productivity rates in excess of 10 g C m$^{-2}$ day$^{-1}$ (Oduor and Schagerl, 2007a). The high productivity presumably reflects elevated ambient temperatures, high incident solar radiation throughout the year and unlimited access to inorganic carbon (Melack and Kilham, 1974). Floristic and faunistic surveys have revealed that the biological diversity and community structure of most alkaline–saline lakes are controlled primarily by salinity and the degree of environmental stability (Hecky and Kilham, 1973). Some lakes in the Gregorian rift valley in Kenya are outstanding examples of alkaline–saline ecosystems. The lakes show unique features, such as a diverse avian fauna, which has considerable economic and scientific value (Schagerl and Oduor, 2008).

Food webs of alkaline–saline lakes often differ from those of other freshwater systems in that zooplankton and fish are either lacking or are represented by only a few taxa, whereas alkaliphilic bacteria and Archaea are present in extremely high numbers ranging between $1.0 \times 10^2$ and $2.0 \times 10^9$ cells mL$^{-1}$ (Yasindi et al., 2002; Rees et al., 2004). Moreover, in a comprehensive survey on the food web of Lake Nakuru, Vareschi and Jacobs (Vareschi and Jacobs, 1984) reported that the cyanobacterium *Arthrospira fusiformis* accounted for up to 95% of the biomass of primary producers.

Protists have been shown to be some of the major planktonic components of the microbial assemblage in most temperate lakes (e.g. Sonntag et al., 2006). Compared with such lakes, alkaline–saline tropical lakes remain largely understudied in terms of ciliate assemblages and taxonomy, although a few studies have demonstrated the occurrence and high biomass of planktonic ciliates (e.g. Finlay et al., 1987; Yasindi et al., 2007). For Lake Nakuru some ciliate taxa, in addition to other biota such as rotifers, copepods, chironomids, fish and the lesser flamingo, are among the most important group of herbivores feeding on phytoplankton, cyanobacteria and flagellates (Finlay et al., 1987; Yasindi et al., 2002).

In this study, we focused on the abundance and distribution of the ciliate assemblages in the two alkaline–saline Lakes Bogoria and Nakuru with respect to changes in abiotic and biotic factors over short time intervals. We sampled the lakes weekly during the short rainy season from October through November 2008 when notable changes in physico-chemical and biological conditions are expected. We hypothesized that ciliate abundance was closely related to potential food resources (bacteria, phytoplankton and cyanobacteria). Furthermore, we hypothesized that the ciliate species inventory, as illustrated by the detailed study of *Frontonia* sp., differed from that of temperate lakes and that ciliate morphotypes were represented by different genotypes in the two lakes.

METHOD

Study sites

The endorheic lakes Bogoria and Nakuru are located in the equatorial region about 150 km apart in the eastern branch of the Great African rift valley in Kenya (Fig. 1). Both lakes are wetlands of international importance and are listed Ramsar sites. Dissolved inorganic carbon in the form of bicarbonate and carbonate is found in concentrations up to 1000-fold higher than in typical freshwater systems. Sodium is the dominant cation, whereas Mg$^{2+}$ and Ca$^{2+}$ are detectable only in traces (Oduor and Schagerl, 2007a, Jirsa et al., 2012).

Lake Bogoria ($00^015^0 N, 36^006^0E$) is a shallow lake ($\bar{z}_{\text{max}} = 10$ m) situated in the Bogoria Game Reserve at 975 m above sea level covering an area of $\sim 34$ km$^2$. The catchment area is affected by tectonic activity with some hot springs and geysers occurring along the lake shore. Furthermore, four small tributaries, Emsos, Waseges, Ndolaita and Loboi, discharge into the lake seasonally (Harper et al., 2005). Lake Bogoria is saline (38.3 ± 1.2‰) with high concentrations of total phosphorus beyond 5 mM L$^{-1}$ (Oduor and Schagerl, 2007b). It has a low total nitrogen to phosphorus ratio, characteristic for water bodies supporting the growth of cyanobacteria such as *Anabaenopsis arnoldii* and *Anabaenopsis abijatae*. 
The mean chlorophyll \( a \) concentration is usually high (up to 650 \( \mu \text{g} \text{L}^{-1} \) on average), although it sometimes decreases to as low as 75 \( \mu \text{g} \text{L}^{-1} \) (Schagerl and Oduor, 2008). *Arthospira fusiformis* dominates the biomass of the autotrophic plankton (Oduor and Schagerl, 2007a,b). Zooplankton comprises three rotifer species (*Brachionus dimidiatus*, *Brachionus plicatilis*, *Hexarthra jenkinae*) and a chironomid of the genus *Paratendipes* (Harper et al., 2003; Burian et al., 2012). The lesser flamingo *Phoeniconaias minor* is the main metazoan grazer of *A. fusiformis* in the lake (Harper et al., 2003).

Lake Nakuru (00°23’S, 36°05’E) is a shallow lake with a long-term mean depth of 1 m (Williams, 1996), covering an area of \( \approx 40 \) km\(^2\) and is situated in the Nakuru National Park at an altitude of 1759 m above the sea level. Three seasonal rivers (Njoro, Makalia and Enderit), the Baharini springs and water from the Nakuru town sewage treatment plant discharge into the lake. The lake area and depth vary substantially depending on the prevailing weather conditions including dry and rainy seasons (Schagerl and Oduor, 2008). The variation in water volume results in large fluctuations of physico-chemical parameters. For example, salinity ranges between 10 and 120‰ (Williams, 1996) and daily temperature fluctuations result in strong diurnal cycles of stratification and mixing (Melack and Kilham, 1974).

High solar radiation and adequate nutrient supply support the growth of primary producers resulting in a super-saturation of dissolved oxygen (17.2 ± 7.9 mg L\(^{-1}\)) in the uppermost 50 cm layer during the day (Oduor and Schagerl, 2007a,b). Most of the time, the plankton is dominated by *A. fusiformis*, *B. dimidiatus*, *B. plicatilis* and *H. jenkinae* and the copepod *Lovenula africana* (Vareschi and Jacobs, 1984). The lesser flamingo *P. minor* and the greater flamingo *Phoenicopterus ruber* dominated among hundreds of bird species (Owino et al., 2001), but the population

moved to other lakes like Lake Oloidien recently. Due to its large biomass, *A. fusiformis* is the main food of the flamingos and of the only fish species *Oreochromis alectius grahami*, which is the prey for piscivorous birds such as the white pelican *Pelecanus onocrotalus* (Vareschi and Jacobs, 1984).

**Field sampling**

Water samples were collected weekly from October through November 2008 at about 0.3 m depth using a 2-L Ruttner sampler. Three sites were considered in each lake, i.e. in the south (S), central (C) and north (N) section (Fig. 1). Physico-chemical parameters, namely water temperature, dissolved oxygen saturation and concentration, salinity, conductivity (corrected to 25°C) and pH were measured *in situ* with appropriate sensors (WTW Multiline P4). Additionally, the Secchi depth was determined by using a black and white Secchi disc (20 cm in diameter).

Water for chlorophyll *a* analyses was collected in 1-L plastic bottles and stored in the dark in a cooling box at 4°C. For the determination of bacterioplankton abundance, phytoplankton and cyanobacterial biovolume, subsamples of 50 mL was preserved with formalin (3% final conc.) in plastic vials. For the determination of ciliate abundance, 300 mL raw water were poured into clean 500-mL plastic bottles containing freshly prepared Bouin’s fixative (5% final conc.). For the single-cell molecular analyses, 3 L raw water was concentrated by filtration through a 50-µm sieve. This larger mesh-size reduced clogging of the net by the dense cyanobacteria but was small enough to capture the target ciliate *Frontonia* sp. The material retained was transferred into 50-mL plastic vials and immediately preserved with Lugol’s iodine solution (Auinger et al., 2008). Additionally, samples for the analysis of zooplankton composition and abundance were collected by using a 100-µm net and preserved with formalin (4% final conc.) in 100-mL plastic bottles. All samples were transported to the laboratory at Egerton University, Njoro, Kenya and stored appropriately until further processing.

**Laboratory procedures**

Chlorophyll *a* concentration was determined spectrophotometrically (Pharmacia Biotech Novaspec II). First, lake water was vacuum filtered through BM/C filters (Ederol; pore size 0.45 µm). The filters were homogenized in 90% acetone in a tissue grinder after deep-freezing overnight at −20°C to assist cell burst. After 12 h, the extract was centrifuged and the supernatant measured. Chlorophyll *a* concentration was calculated according to Talling and Driver (Talling and Driver, 1961). Quantitative and qualitative analyses of phytoplankton and cyanobacteria were made by inverted microscope (Nikon Diaphot) at ×400 magnification according to Utermöhl (Utermöhl, 1958). Bacterioplankton data were provided by Gruber et al. (in preparation), after staining according to the method of Noble and Fuhrman (Noble and Fuhrman, 1998).

Ciliates were concentrated by settling the Bouin’s preserved samples in 500-mL graduated cylinders for 48 h before the supernatant (~250 mL) was removed by siphoning; the remaining 50 mL was stored in plastic vials for further processing. For qualitative and quantitative analyses, 1 mL of the concentrated sample was filtered onto a gridded cellulose nitrate membrane filter (Sartorius; pore size 1.2 µm) and processed according to the quantitative protargol staining method of Montagnes and Lynn (Montagnes and Lynn, 1993). The ciliates were enumerated using an inverted microscope (Zeiss Axiovert 200) and a minimum of 300 cells was counted per filter. For categorization of ciliate size, the ciliates were considered spheres and their size expressed as equivalent spherical diameter (ESD). Counts comprised observations of the whole filter area at ×200 total magnification for large (>50 µm, ESD) or rare ciliates and three randomly selected square counting grids at ×400 total magnification for small ciliates (<50 µm, ESD). The ciliates were identified at up to ×1000 magnification based on the keys of Foissner et al. (Foissner et al., 1994, 1999) and Small and Lynn (Small and Lynn, 1985).

Ciliate cell biovolumes were calculated assuming standard geometric shapes (Lynn et al., 1991) using measurements from at least 30 individuals. Identified ciliates were further categorized into four groups based on their feeding modes (Yasindi et al., 2002), i.e. bacterivores, algaevores, carnivores and parasites. Bacterivores included *Cristigera* sp., *Cyclidium glaucoma*, *Vorticella* sp., *Euplotes* spp. and *Halteria* sp. *Frontonia* sp. was included into the algivore category, although it mainly feeds on cyanobacteria in these lakes (Burian et al., 2012). Further, carnivores included *Acineria* sp., *Condylostoma* sp., *Rimaletus* sp., *Holophrya* sp., *Limonotus* sp., *Monodinium* sp., *Stphaeophrya* sp. and *Spaluidium* sp. Finally, parasitic ciliates comprised the single taxon *Trichodina* sp. (As and Basson, 1989).

Zooplankton abundance was determined by counts from 1-mL subsamples in a Sedgwick-Rafter counting chamber. Identification and counting were done at ×400 magnification with an inverted microscope (Motic BA 400) by scanning at least 10 squares randomly.

Single-cell PCR according to Auinger et al. (Auinger et al., 2008) was carried out at the Research Institute for Limnology at Mondsee, Austria. Briefly, single ciliates assigned to *Frontonia* were identified morphologically,
isolated with a drawn micropipette and rinsed in 400 μL Lugol’s iodine (2 g KI L⁻¹ and 1 g I₂ L⁻¹). Sodium thiosulfate solution (3.1 g Na₂S₂O₃ × 5H₂O L⁻¹) was added to overcome PCR inhibition caused by the Lugol’s iodine solution. For DNA amplification, the broad eukaryotic SSU rDNA targeting forward primer Sogin2f (5’-AGGGTTGATCCTGGAG-3’) and the reverse primer Proto5r (5’-GACGGCGGTGTGTCGA-3’) were applied. Each 50 μL PCR mixture contained 1.25 μL Taq polymerase (Qiagen), 5 μL 10x PCR buffer, 3 mM MgCl₂, 200 nM of each primer, 200 μM of deoxyribonucleoside triphosphate, 21.75 μL distilled, autoclaved water and 20 μL liquid containing the template DNA. PCR was carried out in an Eppendorf Mastercycler starting with a denaturation step of 3 min at 94°C, followed by 35 cycles including denaturation (94°C for 45 s), annealing (52°C for 60 s), elongation (72°C for 90 s) and a final extension step of 5 min at 72°C. In all cases, an additional negative control was taken from the remaining 400 μL after the last washing step to check for contamination by free DNA. The PCR products were checked on a 1% agarose gel, quantified (Spectrophotometer Nano Drop ND-1000) and commercially sequenced (GENOME express, Grenoble). The sequences were assembled using the program DNA Baser 2.75 and submitted to the BLAST search program of the National Center for Biotechnology Information (NCBI) to find closely related sequences. Sequences were aligned using the ‘CLUSTAL W’ option (Thompson et al., 1997) in the BioEdit 7.0.9 sequence analysis software (Hall, 1999). Where necessary, alignments were subsequently manually processed and corrected. Phylogenetic analysis based on 1034 positions was conducted using the computer software Molecular Evolutionary Genetics Analysis (MEGA) version 4 (Tamura et al., 2007). The neighbour joining method using the Kimura-2-Parameter with 1000 bootstrap replicates was used to calculate the phylogenetic tree.

**Statistical analysis**

For an overview of correlations between abiotic and biotic factors, we used random forest models (Breiman, 2001) to predict the frequency of specific ciliates based on the frequency of other organisms and physico-chemical properties of the lakes. Subsequently, we calculated the correlation between the predicted frequencies and the measured frequencies using the statistical package R (http://www.r-project.org). Additionally, we estimated the importance of each parameter using the random forest importance measurement (Breiman, 2001). For testing correlations between distinct factors, we used correlation analyses and descriptive statistical methods (software package SigmaStat).

**RESULTS**

**Physico-chemical and biological parameters**

Between Lake Bogoria and Lake Nakuru, no significant differences (paired t-test, P > 0.05) were detected for pH and oxygen concentration. For temperature, conductivity, salinity and Secchi depth, we found significant differences (t-test, P < 0.05; Table I). Chlorophyll a concentration in the two lakes was significantly different (t-test, P < 0.05); bacterial abundance was not (t-test, P > 0.05). The chlorophyll a concentration in Lake Bogoria ranged from 80 to 1650 μg L⁻¹, whereas in Lake Nakuru, it ranged from 83 to 380 μg L⁻¹. The cyanobacterial biovolume was about two-fold higher in Lake Bogoria than in Lake Nakuru and rotifer abundance was within the same order of magnitude (Table I).

Within Lake Bogoria, oxygen concentration was significantly different at site BN when compared with the other sites (P < 0.05) and salinity differed between sites BC and BN (P < 0.05). In Lake Nakuru, pH and salinity at site NS were significantly different when compared with the other sites (P < 0.05) and conductivity differed among all three sites. Other physico-chemical and biological parameters did not vary significantly between sites within the lakes.

**Table I: Summary of physico-chemical parameters (mean values ± SD), chlorophyll a, cyanobacterial biovolume, bacterial and rotifer abundance in lakes Bogoria and Nakuru**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lake Bogoria</th>
<th>Lake Nakuru</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>10.02 ± 0.07</td>
<td>10.08 ± 0.07</td>
<td>n.s.</td>
</tr>
<tr>
<td>Dissolved oxygen (mg L⁻¹)</td>
<td>23.9 ± 7.6</td>
<td>17.2 ± 7.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>29.8 ± 1.3</td>
<td>23.5 ± 7.6</td>
<td>**</td>
</tr>
<tr>
<td>Conductivity (mS cm⁻¹)</td>
<td>58.7 ± 4.1</td>
<td>27.9 ± 1.6</td>
<td>**</td>
</tr>
<tr>
<td>Salinity (‰)</td>
<td>38.3 ± 1.2</td>
<td>17.0 ± 0.9</td>
<td>**</td>
</tr>
<tr>
<td>Secchi depth (m)</td>
<td>0.29 ± 0.08</td>
<td>0.38 ± 0.07</td>
<td>*</td>
</tr>
<tr>
<td>Chlorophyll a (μg L⁻¹)</td>
<td>362 ± 330</td>
<td>186 ± 82</td>
<td>*</td>
</tr>
<tr>
<td>Cyanobacterial biovolume (mm³ L⁻¹)</td>
<td>160</td>
<td>90</td>
<td>–</td>
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<tr>
<td>Bacterioplankton (×10⁶ cells mL⁻¹)</td>
<td>76.6 ± 18.5</td>
<td>103.8 ± 45.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Rotifers (×10⁶ ind. L⁻¹)</td>
<td>6.6 ± 2.6</td>
<td>7.9 ± 2.8</td>
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</table>

Significance levels (t-test) between the lakes are n.s. = not significant, * = P < 0.05, ** = P < 0.01, ‘−’ = not determined.
Ciliate assemblage, abundance and total biovolume

In total, we identified 22 ciliate morphotypes from both lakes (Table II). Overall, 10 morphotypes occurred in Lake Bogoria, 19 in Lake Nakuru and 7 were detected in both lakes. *Cyclidium glaucoma* in both lakes and *Euplotes moebiusi* in Lake Nakuru were the only species that were conclusively identified in contrast to all other taxa that could be identified only down to the genus level by means of the available literature and identification keys. For some species, we highlight characteristics distinguishing them from their closest congeners (counts and measurements from protargol-stained specimens) (Fig. 2).

Table II: Relative abundance and biovolumes of ciliate morphotypes detected in lakes Bogoria and Nakuru from November through December 2008 with additional list of ciliate taxa detected in other studies in Lake Nakuru.

<table>
<thead>
<tr>
<th>Ciliate Morphotype</th>
<th>Lake Bogoria</th>
<th>Lake Nakuru</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>O         RA (%)</td>
<td>BV (%)</td>
</tr>
<tr>
<td>Acineria sp.</td>
<td>p         0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Actinobolina sp.</td>
<td>p         1.9</td>
<td>0.5</td>
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<tr>
<td>Aspidisca cicada</td>
<td></td>
<td></td>
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<tr>
<td>Aspidisca sp.</td>
<td></td>
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<tr>
<td>Chilodonella sp.</td>
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<tr>
<td>Chlamydodon sp.</td>
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<tr>
<td>Cinetoehdon sp.</td>
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<tr>
<td>Gastrocirrhus sp.</td>
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<tr>
<td>Condylostoma sp.</td>
<td></td>
<td></td>
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<tr>
<td>Cristigera sp.</td>
<td></td>
<td></td>
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<tr>
<td>Cyclidium glaucoma</td>
<td>p         73.4</td>
<td>1.7</td>
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<tr>
<td>Dileptus sp.</td>
<td></td>
<td></td>
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<tr>
<td>Diophrys sp.</td>
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<tr>
<td>Epaklexia sp.</td>
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<tr>
<td>Euplotes moebiusi</td>
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<td></td>
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<tr>
<td>Frontonia sp.</td>
<td>p         16.3</td>
<td>58.5</td>
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<tr>
<td>Halteria sp.</td>
<td></td>
<td></td>
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<tr>
<td>Holophrya sp.</td>
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<tr>
<td>Lagynophrya sp.</td>
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<tr>
<td>Litonotus sp.</td>
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<tr>
<td>Loxodes sp.</td>
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<tr>
<td>Metopus sp.</td>
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<tr>
<td>Monodinium sp.</td>
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<tr>
<td>Papillorhabdos sp.</td>
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<tr>
<td>Paradoileptus sp.</td>
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<tr>
<td>Phascocirrhus sp.</td>
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<tr>
<td>Pleuronema sp.</td>
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<td>Prorodon sp.</td>
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<tr>
<td>Rimaleptus sp.</td>
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<tr>
<td>Spatidium sp.</td>
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<tr>
<td>Sphaerophrya sp.</td>
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<tr>
<td>Stichotricha sp.</td>
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<tr>
<td>Strobilidium sp.</td>
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<tr>
<td>Stylocometes sp.</td>
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<td>Stylophenchus sp.</td>
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<tr>
<td>Trachelus sp.</td>
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<tr>
<td>Trachelophyllum sp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichodina sp.</td>
<td>p         1.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Vorticella sp.</td>
<td>p         &lt;0.01</td>
<td>n.d.</td>
</tr>
<tr>
<td>Others (un-identified)</td>
<td>p         &lt;0.01</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

p, present; a, absent; RA, relative abundance (% on total); BV, biovolume (% on total); O, occurrence, n.d., not determined, empty fields indicate that the species was not found in our study.

aThe values are averages calculated from 21 samples in each lake. Superscripts on taxon names in the first column indicate data sources (Lake Nakuru): this publication.
bOyoo-Okoth et al. (2011).
cYasindi et al. (2002).
dFinlay et al. (1987).
Lake Bogoria: *Cyclidium glaucoma* was the most abundant species followed by *Frontonia* sp. accounting for 73.4 and 16.3% of the ciliate community in the lake, respectively (Table II). Both taxa reached highest abundance in November at a time when high cyanobacterial and heterotrophic bacterial biomass was observed. Large ciliates, notably *Condylostoma* sp. and *Frontonia* sp. accounted for most of the ciliates biovolume—38% and 59%, respectively (Table II). For all other species detected, abundance and biovolume accounted for less than 10%.

Some important morphometric and taxonomic characteristics of selected ciliate morphotypes include: *Condylostoma* sp. (size 345/130 mm, 65–70 ciliary rows, 14–16 macronuclear nodules), *Frontonia* sp. (size 116.1 × 66 mm, 95–130 ciliary rows, 2 contractile vacuoles each with 2–4 excretory pores) and *Rimaleptus* sp. (size 95 × 37 μm, 2 macronuclear nodules with a single micronucleus in between, distinct tail).

Lake Nakuru: There was high temporal and spatial variation in the ciliate assemblage. Small ciliates such as *Cyclidium* spp. were most abundant. *Halteria* sp., *Acineria* sp., *Euplotes* spp. and *Holophrya* sp. also occurred in high numbers occasionally, while others such as *Actinobolina* sp., *Chlamydomon* sp., *Monodinium* sp., *Spatidium* sp., *Sphaerophrya* sp., *Stylonychia* sp., *Trachelus* sp. and *Vorticella* sp. were rare (Table II). However, large ciliates (ESD > 50 μm) including mainly *Frontonia* sp. and *Holophrya* sp. accounted for most of the ciliate biovolume. *Euplotes* spp. occasionally also contributed significantly to the biovolume at high abundance. In total, these three morphotypes accounted for 90% of the total ciliate biovolume in the lake (Table II).

Some important morphometric and taxonomic characteristics of selected ciliate morphotypes include: *Acineria* sp. (size 58 × 20 μm), *Frontonia* sp. (size 139.0 × 80 μm), two *Euplotes* species, i.e. *E. moebiusi* (size 52 × 32 μm) and *Euplotes* sp. (44 × 24 μm, 6–7 dorsal ridges, 4 transverse cirri), and *Holophrya* sp. (size 87 × 60 μm, 60 ciliary rows).

Biotic interactions

*Frontonia* sp. was the only abundant ciliate taxon closely linked to the variables measured. We found a good prediction performance ($R = 0.32$, $P < 0.001$) with the random forest estimated parameters, chlorophyll $a$ concentration and Secchi depth being most important for the frequency of this ciliate. Further, the abundance of *Frontonia* sp. was positively correlated to the chlorophyll $a$ concentration (Spearman’s correlation, $r_s = 0.46$, $n = 40$) and with cyanobacterial biomass ($r_s = 0.34$, $n = 40$). Except for *Frontonia*, only the abundance of the much rarer *Acineria* sp. was significantly correlated to bacterial abundance ($R = 0.48$, $P < 0.001$).
The distribution of the main bacterivorous ciliates Cyclidium spp. and the main algivorous ciliate Frontonia sp. indicated a counterrate during the whole sampling period. This was most pronounced at site BS, where the abundance of Cyclidium spp. initially decreased by roughly 50% but, after the population of Frontonia sp. collapsed, Cyclidium spp. increased again. However, the total bacterivorous ciliate biovolume was not related to bacterial abundance ($P > 0.05$, $n = 41$) even though abundance maxima of bacterivorous ciliates coincided with abundance minima of bacteria. The abundances of carnivorous ciliates and potential prey (Cyclidium spp. and Halteria sp.) were not correlated ($r_s = 0.06$, $n = 41$). Nevertheless, at high abundances of carnivorous ciliates, the abundance of prey ciliates was generally low. Trichodina sp. was detected in Lake Nakuru (in high densities at times) as was the fish Oecichthys alcalicus grahami, on which it parasitizes. Trichodina was found freely floating in the water.

Population heterogeneity of Frontonia sp.

Despite the presumably similar physico-chemical conditions in the lakes (except for conductivity and salinity), the mean size of the individuals of Frontonia sp. in Lake Bogoria was significantly smaller than that in Lake Nakuru with mean lengths 116.1 ± 2.3 vs. 139.0 ± 2.7 μm, respectively ($t$-test, $P < 0.05$, $n = 38$). Surprisingly, the 1115 base-pair fragment of the SSU rRNA gene did not differ between cells originating from both lakes. Moreover, the Frontonia morphotypes were not closely associated with the other so far characterized Frontonia species according to molecular data (Fig. 3). Phylogenetic analysis revealed that both Frontonia populations clustered basally to the genus Paramecium together with Frontonia didieri and Apofrontonia dohrni. The Frontonia sequences have been submitted to Genbank (accession numbers FN667825-FN667830).

**DISCUSSION**

Physico-chemical and biological parameters

The physico-chemical variables recorded in Lake Nakuru such as pH, temperature and conductivity in our study are similar to those observed by Yasindi et al. (Yasindi et al., 2002) and Oduor and Schagerl (Oduor and Schagerl, 2007a,b) except for dissolved oxygen concentration which was comparatively low in the study of Yasindi et al. (Yasindi et al., 2002), i.e. 2.4–3 mg L$^{-1}$ probably due to the method of analysis (i.e. Winkler’s titration versus use of oxygen electrode probes) and temporal variations, especially for Lake Nakuru. Temperature was strongly influenced by the time of sampling, while conductivity and salinity fluctuate with changes in water level as a result of changing seasons (Vareschi, 1984).

Ciliate communities and taxonomic composition

There is much debate over the pattern of protist distribution, particularly with respect to biogeography and habitat characteristics (Finlay, 2002; Foissner, 2007). Micro-diversity, i.e. molecular or ecophysiological variation below the species level, recently accelerated this discussion (e.g. Boenigk et al., 2005; Pfandl et al., 2009). On the other hand, ciliate assemblages in various aquatic ecosystems may also reflect population dynamic responses to resource levels and biotic interactions.

Within the 2-month period, we found a total of 22 ciliate morphotypes, with 19 morphotypes occurring in Lake Nakuru but only 10 in Lake Bogoria. Oyoo-Okoth et al. (Oyoo-Okoth et al., 2011) recorded only four ciliate species in Lake Nakuru in a study covering a whole year (Table II). This low species number in their study may be due to methodological bias as the method for collection and preservation of zooplankton used (i.e. 33.5-μm net and fixation with 4% neutralized formalin) may not have been appropriate for ciliates. In their detailed study, Yasindi et al. (Yasindi et al., 2002) reported 33 ciliate taxa from Lake Nakuru over an investigation period of 8 months. Finlay et al. (Finlay et al., 1987) identified 21 morphotypes in Lake Nakuru including 9 morphotypes that were also observed in our study in that lake. Taken together, our study and the studies by Finlay et al. (Finlay et al., 1987), Yasindi et al. (Yasindi et al., 2002) and Oyoo-Okoth et al. (Oyoo-Okoth et al., 2011) identified 39 ciliate morphotypes to genus level and 3 to species level. The methods used in identification of some of the morphotypes did not involve live observations. Therefore, important taxonomic features such as movement, number and location of contractile vacuoles etc. could not be determined and made definite identification impossible.

Even though the ciliate morphotypes detected in the lakes investigated belong to well-known genera, most of the species except for C. glaucoma and E. moebiusi did not match available species descriptions. For instance, Frontonia sp. is similar to E. microstoma (Roque, 1961), but differs in size, number of postoral kineties (5–6 vs. 3–4) (Roque, 1961) and number of excretory pores (2–4 vs. 1–2 per contractile vacuole). Recently, Frontonia anatolica was described from the bottom sediment of the soda Lake Van in Turkey (Yildiz and Senler, 2013). The Frontonia sp. found in our systems differs from E. anatolica.
in size, number of somatic kineties (95–130 vs. 110–120) and number of excretory pores (2–4 vs. 1 per contractile vacuole). The number of validly described *Frontonia* species and the current status of taxonomy in the genus are still under debate and may require the inclusion of additional morphometric data.
Rimaleptus sp. from Lake Bogoria is similar to Rimaleptus similis in having on average 30 ciliary rows. Rimaleptus similis was isolated from terrestrial habitats (soil) of different biogeographical regions including Shimba Hills in Kenya, Costa Rica and Australia (Vďačný and Foissner, 2012). However, the Rimaleptus species from Lake Bogoria differs from R. similis in size (95 x 37 µm vs. 245 x 43 µm on average), body morphology (distinct tail vs. rounded posterior end) and habitat (terrestrial vs. limnetic).

Condylostomids have been described from both freshwater and saline inland waters (e.g. Dragesco, 1960; Alekperov, 1984). The identification of Condylostoma species is difficult because relatively few taxonomic features are available. The cell shape and size are usually highly variable and their infraciliature is often unclear (Foissner et al., 1999). Currently, seven Condylostoma species are known from saline inland waters or from freshwater, i.e. C. tardum, C. nigra, C. vonx, C. kazimovi, C. luteum, C. reichi and C. patulum. Condylostoma sp. found in Lake Bogoria (also recorded in Lake Nakuru, Yasindi et al., 2002) is similar to Condylostoma magnus in terms of body shape and the general appearance of the buccal area. However, it has more somatic kinetics (about 70 vs. 50 in C. magnus). Additional observations from living material and appropriately stained specimens will be necessary for conclusive identification and/or description (Ong’ondo et al. in preparation).

Finlay et al. (Finlay et al., 1987) detected two Euplotes species in Lake Nakuru; one of which was identified as Euplotes moebiusi. Likewise, we identified two Euplotes species, i.e. E. moebiusi and a second species that is similar to Euplotes affinis. However, Euplotes sp. differs from E. affinis in size (44 x 24 vs. 38 x 26 µm), dorsal surface (6–7 vs. 5 longitudinal ridges), number of transverse cirri (4 vs. 5) and shape of the macronucleus (C-shape vs. 3-shape).

The different numbers of taxa and the generally strong fluctuations in taxon abundances within the different studies in Lake Nakuru raise the question of temporal dynamics and required sampling intervals to resolve such dynamics. Our observations indicate that the change in the ciliate community composition and abundance is pronounced and may not be captured adequately when samples are collected over long time intervals. We therefore applied autocorrelation analyses (Supplementary data, Tables S1–SIII) based on the time series data for ciliate abundance in our study (weekly sampling) as well as for the monthly collected data provided by Yasindi et al. (Yasindi et al., 2002). As expected, the correlation coefficients decreased for increasing sampling intervals. For sampling intervals of more than 2 weeks, abundances were hardly correlated for both data sets. Thus, weekly sampling intervals are certainly ideal but intervals of up to 2 weeks seem to mostly capture ciliate dynamics in the lakes investigated.

Population heterogeneity of Frontonia sp.

Frontonia is widely distributed in tropical East African lakes (Dragesco and Dragesco-Kernéis, 1991; Yasindi et al., 2007), in subtropical and temperate water bodies (Finlay et al., 1988; Beaver and Crisman, 1989) and in marine environments (e.g. Fan et al., 2011). Large intraspecific size differences even between individuals of a single population have been reported as a common phenomenon for this taxon (e.g. Fan et al., 2011), although the mean size of individuals is usually not that different between populations. However, in our study the mean sizes of the specimens from both lakes differed significantly. We therefore considered this taxon to be promising for molecular analysis. The phylogenetic analysis based on single cells provided evidence that the two populations belong to the same species despite the difference in cell size. Ciliates are likely transferred between the two lakes by the frequent movement of flamingos and other birds (Foissner, 2006). The cell size difference of the two populations may have therefore been caused by different environmental conditions, especially salinity, which is roughly twice as high in Lake Bogoria as in Lake Nakuru (Table I). The high salinity in Lake Bogoria leads to increased osmotic pressure which may result in smaller ciliate cells. Irrespective of these findings, the two populations may be differently adapted to salinity, which was, however, not the focus of this study. The different size and a potentially different osmotic adaptation between these two obviously closely related populations seem promising for studying intraspecific variants in geographically close habitats and possibly even for studying allopatric speciation in progress due to a certain separation by habitat characteristics. Interestingly, phylogenetic analyses indicated that the Frontonia species from our study lakes clustered with Apofrontonia and Paramecium rather than with other Frontonia species. Based on its morphological characters, this ciliate undoubtedly belongs to the genus Frontonia, suggesting that the genus is non-monophyletic (Fig. 2g). This is consistent with the findings in other studies where E didieri was placed within the Paramecium–Apofrontonia clade, rather than into the Frontonia clade (e.g. Fokin et al., 2006; Gao et al., 2008). At present, the genus Frontonia is highly under-sampled considering the number of validly described species and a proper elucidation of phylogenetic relationships among the Peniculia needs much wider taxonomic coverage (Fokin et al., 2006).
Dynamics of biotic interactions

Two mechanisms regulating the abundance and biomass are generally recognized in aquatic ecosystems: bottom-up control by resources and top-down control by predators (Wiackowski et al., 2001). Commonly, protist abundance is linked to the availability of resources within an ecosystem, thereby influencing the species composition and distribution (e.g. Sanders and Wickham, 1993).

The bottom-up control of ciliates is determined by food availability including phytoplankton, bacterioplankton and heterotrophic nanoflagellates (Andrushchyshyn et al., 2006), whereas top-down control is usually imposed by metazooplankton predation (e.g. Gaedke and Wickham, 2004). In our study, we found ciliate abundance to be poorly correlated with abiotic and biotic factors except for the algivorous Frontonia sp. As feeding interactions seem to be important and basically allowed for the prediction of the abundance of Frontonia sp., ciliate feeding modes needed to be considered separately.

Bacterivorous ciliates: Bacterivorous ciliates such as Cristigera sp., C. glaucoma and Halteria sp. are considered to be among the most important grazers on bacterioplankton in both marine and freshwater systems (e.g. šimek et al., 2000). The unexpected finding that the total biovolume of the bacterivorous ciliates was not correlated with bacterial abundance may be attributed to the exceptionally high abundance of bacterioplankton in these lakes, i.e. 103.8 x 10⁶ cells mL⁻¹ in Lake Nakuru and 76.6 x 10⁶ cells mL⁻¹ in Lake Bogoria (Table I; Gruber et al., in preparation). Even though bacterial abundance was somewhat lower when Cyclidium spp. and other bacterivorous ciliates were abundant, the bacterial abundance never decreased below 50.0 x 10⁶ cells mL⁻¹. Posch et al. (Posch et al., 2001) demonstrated that the ingestion rates of C. glaucoma increased with rising bacterial abundance up to 30.0 x 10⁶ cells mL⁻¹, then food concentrations became satiating. Our results imply that food concentrations were always satiating for Cyclidium spp. in these lakes, consequently they were not bottom-up controlled.

Algivorous ciliates: The algivorous ciliates, specifically Frontonia sp., seemed to be the only ciliates that were, at least to some extent, bottom-up controlled. Frontonia spp. are typically omnivorous ciliates that are known to feed upon chrysophytes, cryptophytes, chlorophytes and diatoms (Dias and D’Agusto, 2006). In accordance with earlier studies in these lakes (e.g. Burian et al., 2012), Frontonia spp. consumed the cyanobacterium A. fusiformis. Especially in the southern section of Lake Bogoria, the increase in abundance of Frontonia sp. was positively correlated to the chlorophyll a concentration indicating a pronounced increase in cyanobacterial biomass due to a bloom of A. fusiformis in November. The relative isolation of this lake area due to a distinct headland affects the stratification and mixing regime leading to the somewhat different plankton dynamics from that recorded in the rest of the lake. The importance of vertical stratification and mixing depth of the rift valley lakes has been suggested to be a major controlling variable for planktonic community composition, community changes and perhaps biomass levels as well (Talling and Lemoalle, 1998).

Carnivorous ciliates: The high abundance of carnivorous ciliates in both lakes during periods when small ciliates were abundant indicates bottom-up control. Acineria sp., Holophrya sp., Litonotus sp., Monodinium sp., Spathidium sp. and Trachelus sp. may feed on small ciliates such as Cyclidium spp. and Halteria sp. (Yasindi et al., 2002) and rotifers (Finlay et al., 1987). Sphaerophrya sp. either feeds as a predator or parasitizes other ciliates (Foissner et al., 1999), at times, it was observed attached with its tentacles to Euplotes sp. cells. However, the role of Sphaerophrya sp. in regulating pelagic protozoan communities is still unknown (Foissner et al., 1999). Further, this is the first report from Lake Nakuru of the parasitic taxon Trichoidea sp. which is most likely parasitic on the fish Oreochromis alcalicus grahami.

CONCLUSIONS

Due to high bacterial abundances, bottom-up control of bacterivorous ciliates was weak or absent. Even for algivorous taxa, bottom-up control was weak. The ciliate assemblage was composed of morphotypes which are presumably not described to date and specifically not known from the comparatively better investigated temperate lakes. This does not necessarily reflect a biogeographic restriction but rather ecophysiological adaptations to the alkaline–saline conditions of the tropical rift valley lakes. This finding is further supported by the fact that the populations of Frontonia from both lakes, although significantly differing in size, have the same SSU sequence.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

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