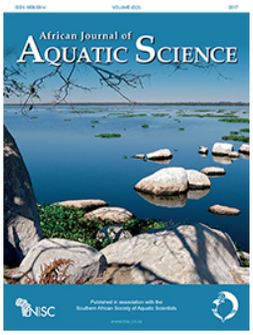

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Plankton composition, biomass, phylogeny and toxin genes in Lake Big Momela, Tanzania

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Lake Big Momela, one of the East African soda lakes in Northern Tanzania characterised by highly saline-alkaline conditions, making them inhospitable to a range of organisms, although supporting massive growths of some adapted planktonic microorganisms that serve as food for birds, such as Lesser Flamingo. The temporal dynamics of plankton, with an emphasis on cyanobacteria, were examined in 2007 using morphological traits and ribosomal genetic markers (16S and 18S rRNA). Cyanobacterial genes encoding for hepatotoxins (*mcyE* and *ndaF*) were also screened. Rotifers and copepods dominated the zooplankton, whereas cyanobacteria, such as *Anabaenopsis elenkinii* and *Arthrospira fusiformis* dominated the phytoplankton community, and these being related to representatives in other East African soda lakes. The cyanobacteria community also showed distinct seasonal patterns influenced by environmental parameters, mainly salinity, pH and nitrate. Significant positive correlations were found between phytoplankton abundance and nitrate concentrations (r = 0.617, p = 0.033). No signals of the hepatotoxin synthetase genes *mcyE* and *ndaF* were retrieved from cyanobacteria during the whole year. In general, our data illustrate the presence of rich planktonic communities, including some unique and potentially endemic cyanobacteria.

Keywords: cyanotoxin, limnology, plankton diversity, soda lakes

Introduction

Soda lakes are extreme ecosystems with high pH (typically pH 9–12), because of high levels of CO_3^{2-} ion (Jones et al. 1998). Many soda lakes also contain high concentrations of sodium chloride and other dissolved salts, making them saline or hypersaline as well (Jones et al. 1998). Despite their apparent inhospitability, soda lakes are often highly productive ecosystems (Oduor and Schagerl 2007). Life in soda lakes is often dominated by prokaryotes, i.e. bacteria and archaea, however, a rich diversity of eukaryotic algae, protists and fungi have also been encountered (Lanzén et al. 2013; Luo et al. 2013). The availability of dissolved inorganic nutrients may lead to permanent or seasonal algal blooms, the photosynthesis of which may rise to above 10 g C fixed m^{-2} day^{-1}, more than ten-fold higher than the global average for freshwater ecosystems (Vareschi 1982; Oduor and Schagerl 2007).

The photoautotrophic cyanobacterial blooms play an important role as a primary energy source for life in soda lakes (Vareschi 1978, 1982; Oduor and Schagerl 2007). Particularly relevant is the occurrence of the filamentous non-heterocystous species *Arthrospira fusiformis* ((Voronikhin) Komárek and Lund 1990), formerly *Spirulina platensis* (Geitler 1925) (Ballot et al. 2004, 2005; Kaggwa et al. 2013; Kihwele et al. 2014; Schagerl et al. 2015), the filamentous heterocystous *Anabaenopsis* and several unicellular cyanobacteria from the genera *Synechococcus* or *Chroococcus* (Zavarzin et al. 1999; Girma et al. 2012). Under favourable conditions, *A. fusiformis* may sporadically comprise up to 98% of the phytoplankton biomass in the water column, thereby often being the main primary producer in the African soda lakes, (e.g. Vareschi 1978, 1982; Kaggwa et al. 2013; Schagerl et al. 2015). In Lake Big Momela concentrations of cyanobacteria have been observed to range from 0.7–2 million filaments per litre under non-bloom conditions (Kaaya et al. 2007) and up to 150 million filaments l^{-1} under bloom conditions (Lugomela et al. 2006).

Wild animals avoid using Lake Big Momela for drinking water, but it serves as a feeding refuge for huge flocks of birds, particularly the Greater Flamingo *Phoenicopterus ruber* (Linnaeus 1758) and Lesser Flamingo *Phoeniconaias minor* (Geoffroy, 1798) (see e.g. Vareschi 1978; Vonshak 1997; Krienitz and Kotut 2010; Krienitz et al. 2016). While grazing and filtering the water of this lake, the flocks of Lesser Flamingo consume phytoplankton and benthic microalgae, of which cyanobacteria represent the main part of their food (Krienitz and Kotut 2010; Krienitz et al. 2016). However, besides being a nutritious food source for Lesser Flamingo, some cyanobacteria are also

notorious toxin producers. These secondary metabolites have been proposed to cause mass mortalities of Lesser Flamingo in the East Africa region (Ballot et al. 2002, 2004, 2005; Lugomela et al. 2006). For instance, cyanobacteria in Kenyan alkaline lakes may produce the hepatotoxin microcystin and the neurotoxin anatoxin, and thereby constitute a potential health risk for wildlife dependent on cyanobacteria as a food source (Ballot et al. 2004, 2005).

Microcystin concentrations recorded in various Kenyan soda lakes varied from 12 µg microcystin–LR equivalents g^{-1} DW: (dry weight) in Lake Sonachi to 4.6 mg microcystin–LR equivalents g^{-1} DW in Lake Nakuru (Ballot et al. 2004, 2005). However, at the same time neither microcystin nor anatoxins were detected in other similar lakes in the region, such as Lake Elmenteita (Ballot et al. 2004), and Lakes Bogoria and Nakuru (Straubinger-Gansberger et al. 2014). These findings suggest that toxin production varies between locations, possibly because of control of the microcystin or anatoxin biosynthesis by biotic or abiotic factors, or indeed, by the type of cyanobacteria that dominate in each season, including their life stage (Ballot et al. 2004).

The occurrence of cyanobacteria dominated by *A. fusiformis*, and a subsequent mass mortality of flamingos were reported during the dry season 2004 in the Tanzanian Lakes Big Momela, Manyara and Embakai (Lugomela et al. 2006). An association between these events was proposed, although the production of cyanotoxins was not examined. More recently, the presence of three microcystin variants (MC-LR, MC-YR and MC-RR) was detected in tissues from flamingos at Lake Manyara, with high concentrations in liver than kidney, lungs and heart tissues, pointing to the presence of toxic cyanobacteria in this environment during the dry season (Nonga et al. 2011).

Besides the potential importance that phytoplankton, particularly cyanobacteria, have on the primary production and health of the food chain in African soda lakes, their presence, abundance, genetic identity, phylogenetic relationships, toxin production, and the regulation of biotic and abiotic factors, are still far from being elucidated. A phenotypic (morphological) and genotypic (genetic) examination was therefore done using Lake Big Momela as a model to determine the identity and monthly variations of phytoplankton populations, with emphasis on cyanobacteria, over one full year, including the regulation by specific ecological factors. Screening for the presence of cyanotoxin encoding genes (microcystin and nodularin synthetase genes) was also performed.

Material and methods

Study site and sampling

Lake Big Momela, 9.7 km wide, is the largest of a series of seven shallow saline post-volcanic Momela Lakes located close to one another in Arusha National Park, Tanzania. These lakes are highly alkaline with a pH ranging from 9.3 to 10.4 (Hecky and Kilham 1973). The principal sources of 'new' water entering the Momela Lakes are precipitation and underground recharge. Sampling was done once per month throughout 2007 at the centre of Lake Big Momela, reached by boat, at latitude 3°13′22.08″ S, 36°54′33.48″ E (Figure 1). The lake lies at approximately 1 460 m above sea level and it is in mostly shallow, with a maximum depth of approximately 30 m (Hecky and Kilham 1973). The rainy seasons in the area occur in March–May and October–December, and the dry season in June–September and January–February.

Environmental parameters

Water temperature, salinity, pH and dissolved oxygen were measured *in situ* using a Horriba-U10 (Japan) multiprobe water quality meter at various depths down to 8 m. Water samples for analysis of inorganic nutrient concentrations were collected at the surface by using a 1.5 l Niskin bottle and filtered through glassfibre filters (GFF) using a peristaltic pump. Filtered samples were stored in 50 ml acid-cleaned plastic vials and kept frozen at –20 °C until analysed. Analyses for nitrite (NO_2^-), nitrate (NO_3^-) and phosphate (PO_4^{3-}) were done using a spectrophotometer following the method of Parsons et al. (1989). Prior to analysis, the samples intended for NO_3^- analyses were treated with HCl to adjust the pH to 7 (APHA 1992).

Plankton composition, cell counts and biomass quantification

Phytoplankton and zooplankton were collected by towing a 20-µm mesh plankton net for a distance of approximately 20 m. For morphological characterisation, the samples were immediately fixed in formalin in the field to a final concentration of 4% and kept in dark glass bottles until examined. Their composition was determined by light microscope, following descriptions by Prescot (1978); Anagnostidis and Komárek (1985, 1988); Komárek and Anagnostidis (1986); Ballot et al. (2004, 2008); Dadheech et al. (2013); Kaggwa et al. (2013) for phytoplankton, and by Shiel (1995) for zooplankton. Plankton samples for genetic characterisation were stored at −20 °C until analysed.

A Niskin bottle was used to collect 200 ml surface water samples for plankton abundance determinations, which were fixed with 0.5% Lugol's solution, followed by 2% formalin and stored in dark glass bottles for analysis in the laboratory. Plankton counts were performed under light microscope using a Sedgewick-Rafter cell (Woelkerling et al. 1976), whereas phytoplankton biomass was determined by measuring Chlorophyll *a* levels. Water samples collected from the surface for Chlorophyll *a* determination were filtered *in situ* onto 0.45 µm pore size 47 mm diameter Whatman Membrane Filters and Chlorophyll *a* was extracted in 10 ml of 90% acetone for 24 hours at 4 °C. After extraction, the samples were vortexed and centrifuged at 5 000 rpm for 10 minutes before being decanted into clean test tubes. Chlorophyll *a* was determined using a Shimadzu (Japan) spectrophotometer as described by Parsons et al. (1989).

DNA extraction, 16S rRNA-DGGE fingerprinting and gel band sequencing

Prior to the DNA extractions, the phytoplankton samples were centrifuged for biomass concentration, and freeze-dried for storage in desiccators. DNA was then extracted using GenElute Plant Genomic DNA Mini prep kit (Sigma-Aldrich Sweden AB, Sweden). The quality and

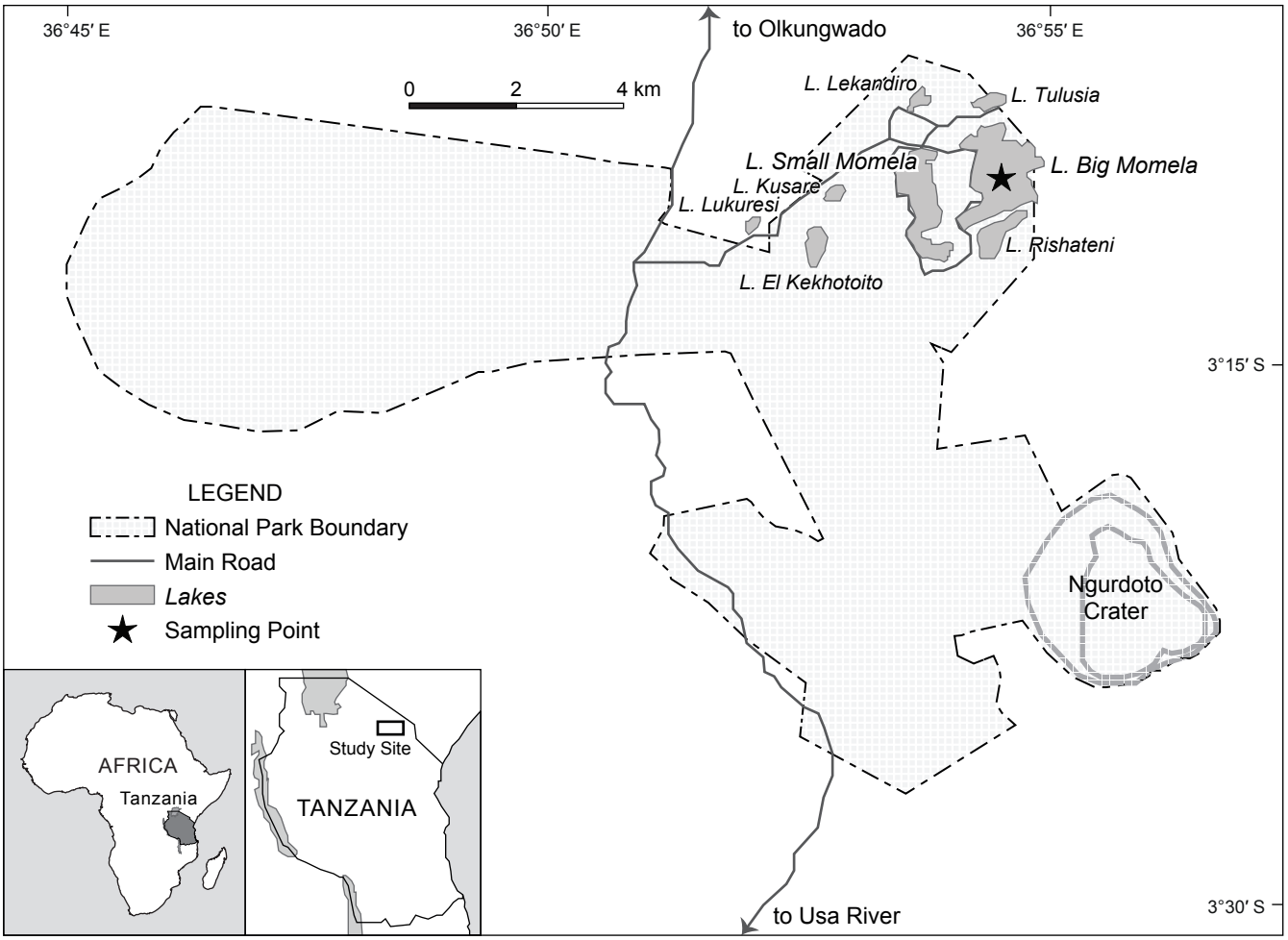

Figure 1: Map of Arusha National Park (dotted line), Tanzania, showing locations of East African rift valley soda lakes, including Lake Big Momela, in its north-eastern corner

quantity of DNA was determined using a nano-drop spectrophotometer (Nano Drop Technologies, USA). The presence and quality of the DNA were also verified by agarose gel electrophoresis. Extracted DNA was stored at −20 °C until further analysis.

PCR amplifications of ribosomal genes from cyanobacteria (16S rRNA) and eukaryotes (18S rRNA) were performed using a hot-start *Taq* DNA polymerase (QIAGEN, Germany). The cyanobacterium specific 16S rRNA genes were amplified using the primers CYA106F (with a 40-nucleotide GC clamp at the 5' end), CYA781Ra and CYA781Rb (Nübel et al. 1997), which amplify a fragment of 675 base pairs. The 18S rRNA genes were amplified using the eukaryotic universal primer set Euk1A and Euk516r-GC (Díez et al. 2001), which amplify a fragment of 560 base pairs. The PCR conditions comprised a denaturation step of 95 °C for 15 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min (16S rRNA) and 56 °C for 45 sec (18S rRNA), extension at 72 °C for 1 min, and a final extension at 72 °C for 30 min as the last cycle. The PCR products obtained were examined by agarose gel electrophoresis.

DGGE analyses were carried out using a Dcode system (Bio-Rad, USA) to separate the PCR amplified products further. DGGE was performed at 75 V for 16 h in 0.75 mm thick 6% polyacrylamide gels (acrylamide/bisacrylamide; ratio 37.5: 1) submerged in 1X TAE buffer (40 mM Tris, 40 mM acetic acid, 1 mM EDTA; pH 7.4) at 60 °C, as described by Uku et al. 2007. Linear gradients of denaturing agents of 45–75% for the cyanobacterial 16S rRNA gene fragments, and of 45–70% for the eukaryotic 18S rRNA gene fragments were used. After electrophoresis the gel was stained in 1X TAE buffer containing SYBR gold nucleic acid stain (1: 10 000 dilution; Molecular Probes, Life Technologies, Sweden), the bands were visualised and the images documented using a Chemidoc system using Quantity 1 software (Bio-Rad, USA). The dominant DGGE bands located along the gels were then excised using sterilised razor blades, re-suspended in 20 µl Milli-Q® water and stored overnight at 4 °C. PCR re-amplifications were performed using the eluted DNA from excised bands as templates, using the same primers and conditions as given above, except that no GC clamp was added to the primers. The re-amplified PCR products were purified using GFX DNA/PCR extraction kit and were sequenced (DNA Technology, Denmark) with the corresponding forward primer.

Eukaryotic phytoplankton and cyanobacteria phylogenetic analysis

The sequences generated for both eukaryotic phytoplankton (18S rRNA genes) and cyanobacteria (16S rRNA genes) were separately aligned in Bioedit using ClustalW (Tom Hall, Ibis Therapeutics, Carlsbad, USA) and manually corrected. All sequences were subjected to searches using the basic local alignment search tool (BLAST) at www.ncbi.nlm.nih.gov/blast (Altschul et al. 1997), and the closest relatives obtained from the GenBank were included in the subsequent phylogenetic analysis. The sequences generated are available in GenBank, accession numbers JF907014 - JF907026, and KJ736758 - KJ736759, for the 16S rRNA sequences, and JF907027 - JF907035 for the 18S rRNA sequences.

Cyanobacterial hepatotoxin synthase genes analysis

To screen for the presence of specific toxin genes among cyanobacteria present in Lake Big Momela, the same genomic DNA extracted for composition identity analyses, as described above, was used for PCR amplification of both microcystin and nodularin synthetase genes, using the hot start *Taq* DNA polymerase (Qiagen, Germany) and the hepatotoxin specific primers HEPF and HEPR (Jungblut and Neilan 2010). These primers amplify a fragment of 472 base pairs. The PCR programme comprised a denaturation step of 95 °C for 15 min, followed by 35 cycles of denaturation at 92 °C for 20 sec, annealing at 52 °C for 30 sec, extension at 72 °C for 1 min and a final extension at 72 °C for 30 min during the last cycle.

Statistical analysis

Statistical analyses were done for temporal and covariations, as described by Zar (1999), using GraphPad Instant software t_m 1992–2003 version 3.06. Prior to analysis, the software was tested for normality, to determine whether to use a parametric test, if it passed the normality test, or a non-parametric test, in the absence of a normal distribution. When comparing two variables the *t*-test was used for normally distributed data, and the unpaired Mann-Whitney test for those lacking normal distribution, whereas the Pearson *r* correlation test was used to test correlations between the two variables. Here, *p*-values of less than 0.05 ($p < 0.05$) were considered significantly different.

Results

Environmental parameters

The potential environmental drivers, such as temperature, water transparency, salinity, pH and dissolved oxygen, and the levels of inorganic nutrients (nitrite, nitrate and phosphate) were determined in parallel to the monthly plankton samplings (Figure 2). Generally, the water temperature ranged from 28.3 °C in February to 19.5 °C in August, whereas salinity ranged from 18.0‰ in January to 25.0‰ at the end of the year (Figure 2a). The pH values were constantly high, at around 10.0, whereas dissolved oxygen levels at the surface were highest (12.0 mg l^{-1}) in February and lowest (6.70 mg l^{-1}) in June. Water transparency (Secchi depth), was lowest (34.0 cm) in January and highest (100 cm) in November (Figure 2a). No significant differences were found between the dry and rainy seasons for water temperature ($t = 1.36$, $p = 0.10$), salinity ($t = 1.61$, $p = 0.46$), pH ($t = 0.14$, $p = 0.089$) and Secchi depth ($t = 1.593$, $p = 0.647$). However, dissolved oxygen levels were significantly higher during the dry season than in the rainy season ($t = 1.160$; $p = 0.0003$). Nitrate concentrations ranged markedly from a maximum of 1.1 µmol l^{-1} in February to a minimum of 0.4 µmol l^{-1} in April, whereas nitrite varied at lower levels of 0.2 to 0.5 µmol l^{-1} (Figure 2b). Phosphate levels were high and stable (5.3 to 5.9 µmol l^{-1}) throughout the year. There were no significant differences in concentrations of nitrate, nitrite and phosphate between the dry and rainy seasons.

Vertical distribution patterns in environmental parameters down to 8 m are shown in Figure 3. The pH values were comparatively constant, whereas the dissolved oxygen levels decreased with depth from an average 8.5 mg l^{-1} at the surface to anoxia at 6 m. Water temperature decreased moderately with depth, from 23.7 at the surface to 21.6 at 8 °C m, whereas the salinity values increased with depth, from 21.1 at the surface to 30.4 at 8 m. No clear pattern or variations in the vertical profiles of the environmental parameters were observed between seasons.

Taxonomic and phylogenetic analyses of plankton

A varied community of planktonic organisms was apparent in Lake Big Momela. The phytoplankton community was ubiquitous and phenotypical analyses revealed a dominance of members of two filamentous cyanobacterial phylotypes: the heterocystous *Anabaenopsis elenkinii* (Nostocaceae) and the spirally shaped non-heterocystous species *Arthrospira fusiformis* (Oscillatoriaceae) (Figure 4). The *A. fusiformis* showed two main morphological variations, one composed of densely coiled spirals, the other of loose spirals (Figures 4a and b, respectively). The *A. elenkinii* were composed of short spirally twisted trichomes with rounded cells and terminal spherical heterocyts (Figure 4c). Other cyanobacteria observed occasionally during the year were small unicellular *Synechococcus* sp. (cylindrical cells, typically 2–3 times longer than wide) and *Synechocystis* sp. composed of coccoid cells in clusters.

A detailed molecular characterisation based on 16S rRNA/DGGE fingerprint band patterns, revealed a total of 25 distinct bands (phylotypes) related to cyanobacteria within the community sampled. Figure 5 illustrates the phylogenetic reconstruction of all 16S rRNA cyanobacterial sequences and their placement and relationship within the three major cyanobacterial orders: Nostocales (filamentous heterocystous), Oscillatoriales (filamentous non-heterocystous) and Chroococcales (unicellular or colony forming), in accordance with our phenotypic analyses. The 16S rRNA/DGGE analyses also revealed that within the Nostocales clade, the sequence Lake Momela 19 and 22 (JF907024 and KJ736759) clustered with sequences of the species *Anabaenopsis elenkinii,* isolated from other soda lakes (Lake Sonachi, Kenya, and Lake Texcoco, Mexico) (Ballot et al. 2008), with Like Momela 22 showing a 99% similarity to the *A. elenkinii*. Furthermore, four sequences, Like Momela 4, 5, 6 and 7 (JF907015, JF907016, JF907017 and JF907018) formed a distinct clade closely related

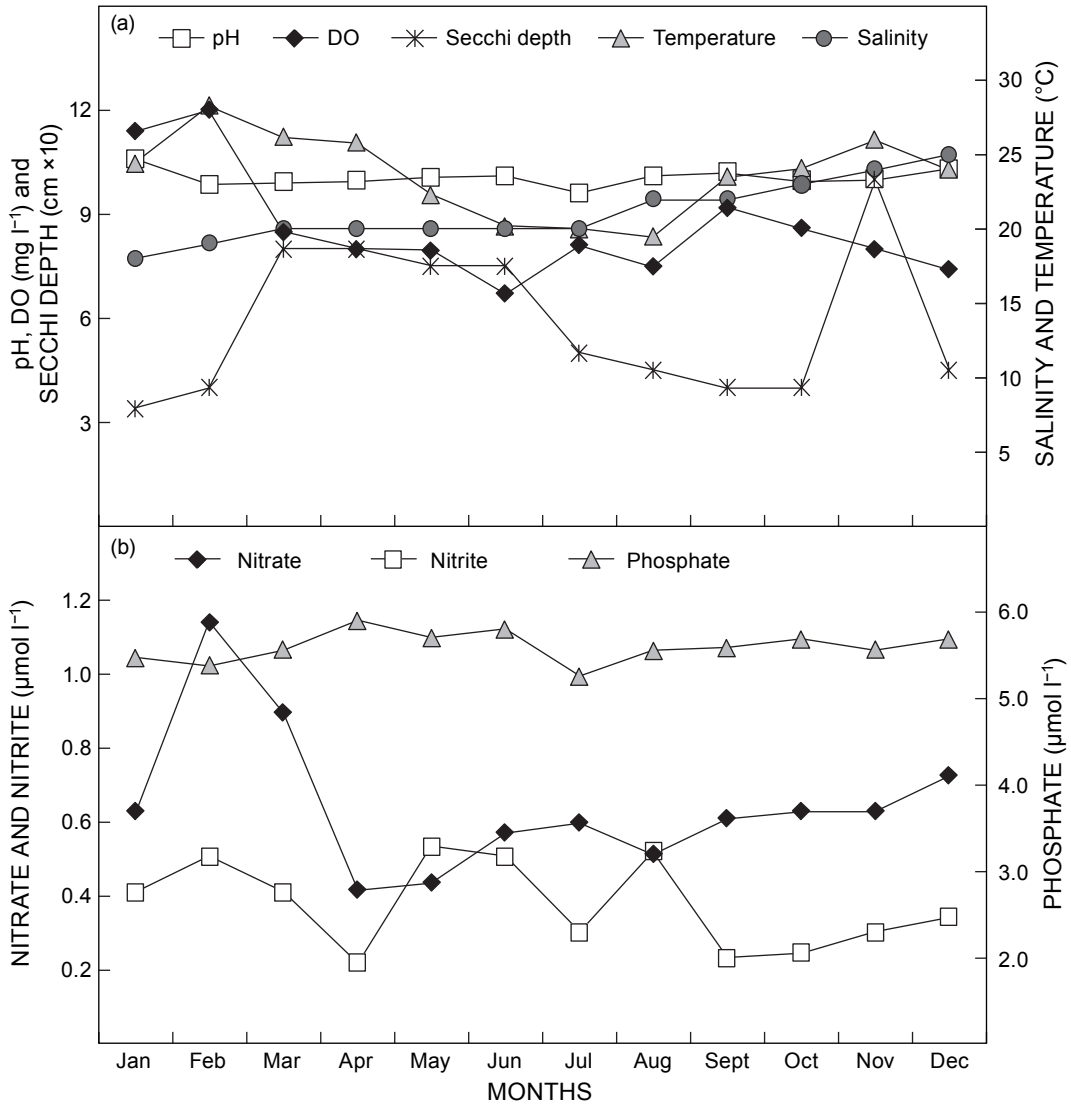

Figure 2: Seasonal variability in (a) physical environmental parameters and (b) key-nutrient levels in surface waters of Lake Big Momela during 2007

to a sequence of *Anabaenopsis abijatae* retrieved from a Kenyan soda lake, Lake Simbi (Ballot et al. 2008). The latter cluster may partly be associated with the recently described *Cyanospira rippkae* subcluster, of which sequences were retrieved from hyper-alkaline lakes in the Republic of Chad (Sili et al. 2011). Furthermore, the sequence Like Momela 1 (JF907014) was closely related (97% sequence similarity) to strains belonging to *Anabaena bergii* (e.g. AF160256) but also to strains of *Aphanizomenon ovalisporum* (*Chrysopsorum ovalisporum*) (Stüken et al. 2009; Cirés and Ballot 2016). In addition, the three sequences, Lake Momela 10, 15 and 20 (JF907019, JF907022 and KJ736758, respectively), formed a distinct clade separated from all the other sequences identified within the Order Nostocales (Figure 5).

In the Order Oscillatoriales, the two sequences, Lake Momela 12 and 23 (JF907021 and JF907026, respectively), clustered (99% similarity) with *Arthrospira fusiformis* sequences typically retrieved in several Kenyan soda lakes (AY575923 from Lake Elmentetia), but also with *A. indica* retrieved from a freshwater Indian lake (AY575932 from Lake Anasagar) (Ballot et al. 2004). Finally, within the Chroococcales clade, two sequences, Lake Momela 13 and 17 (KJ736757 and JF907023, respectively) clustered with *Aphanothece* sp. GSP132-4 (98% similarity), isolated from a hypersaline environment (Kirkwood et al. 2008), and with *Synechococcus* sp. PCC 8806, whereas Lake Momela 11 and 21 (JF907020 and JF907025) clustered with *Synechocystis minuscula* isolates (KT354193, KM 019989 and KJ746516; unpublished) (Figure 5).

Apart from prokaryotic cyanobacteria, morphological analysis revealed the presence of eukaryotic phytoplankton species, particularly members of the green algal genus *Picocystis* (Prasinophytes, Chlorophyta) and few diatoms of the genus *Navicula*. The zooplankton community was also well represented, for instance by the copepod

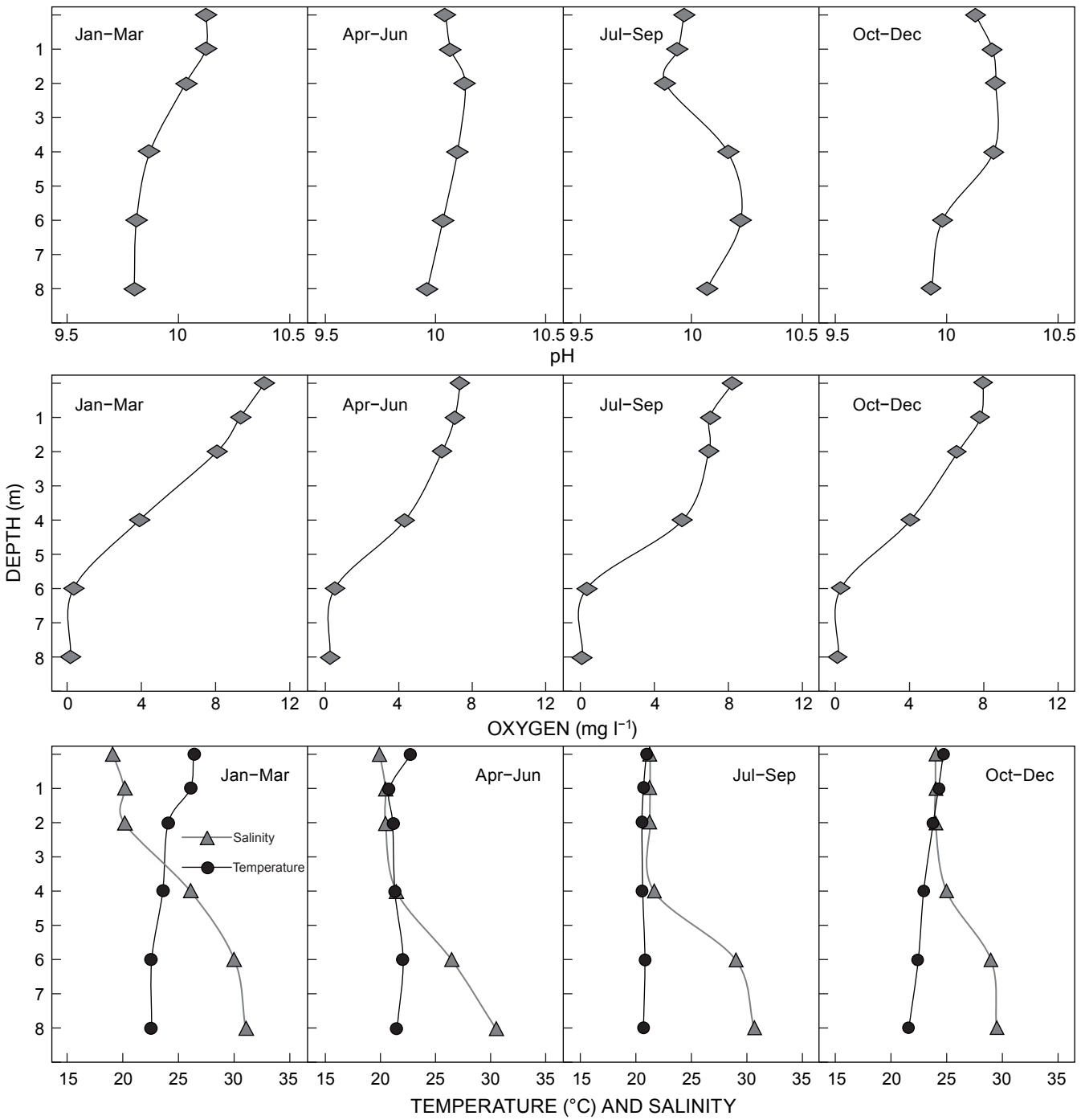

Figure 3: Vertical profiles of mean values of environmental parameters in Lake Big Momela in 2007

Hemidiaptomus spp. and the rotifer *Branchionus* spp. The 18S rRNA-DGGE fingerprint-band-pattern analysis revealed a total of 11 distinct bands (phylotypes) for eukaryotes representing phytoplankton and zooplankton (Table 1). These correlated positively to the morphological observations. The sequence Euk5 (JF907031) and Euk6 (JF907032) were closely related to the chlorophyte *Picocystis salinarum*, albeit at 94–98% sequence similarity with sequence HM990669 retrieved from other East African soda lakes (Krienitz et al. 2012). This species has recently been reported to outcompete *Arthrospira* at the highest salinity and turbidity levels (Schagerl et al. 2015). Although the band sequence Euk4 (JF907030) was related to a cultured isolate of the haptophyte *Isochrysis galbana* (99% similarity), the sequence Euk8 (JF907034) and Euk9 (JF907035) were related to the copepod *Hemidiaptomus* (99% sequence similarity). Finally, four sequences Euk1, 2, 3 and 7 (JF907027, JF907028, JF907029 and JF907033,

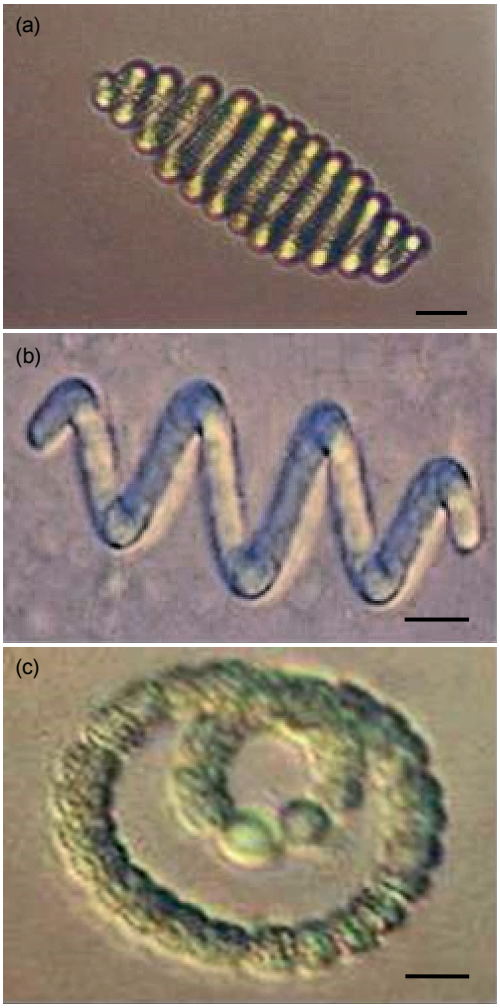

Figure 4: Photomicrographs showing (a & b) the two morphotypes of *Arthrospira fusiformis* and (c) *Anabaenopsis elenkinii* encountered in Lake Big Momela

respectively) were related to the rotifer *Brachionus plicatilis* showing a 97–99% sequence similarity.

Plankton composition and abundance

As indicated above, the plankton community in Lake Big Momela was highly variable, being dominated by filamentous cyanobacteria of the genera *Anabaenopsis* (family Nostocaceae) and *Arthrospira* (family Oscillatoriaceae) (Table 2). A considerably lower abundance of unicellular cyanobacteria, the chlorophyte members of the genus *Picocystis* and the diatom *Navicula* spp. were also recorded.

Distinct temporal shifts among the phytoplankton, and in particular cyanobacteria populations, were observed in Lake Big Momela in 2007 (Table 2). The data illustrated a higher abundance of the heterocystous cyanobacterium *Anabaenopsis elenkinii* between December and May, with a maximum of up to 14 750 filaments l^{-1} in February. The *A. elenkinii* dominance was succeeded by *Arthrospira fusiformis* from March to August, with a maximum of 2 725 filaments l^{-1} in March, the period with moderate water salinity and less turbidity. Both *A. elenkinii* and *A. fusiformis* coexisted from March to May, then decreased in abundance until below detection limits during September to October, the period with the highest salinity and more turbid waters, as recorded in this study (Table 2, Figure 2). During their absence, other phytoplankton species, such as the unicellular cyanobacterium *Synechococcus*, together with eukaryotic chlorophytes of *Picocystis* and diatoms of the genus *Navicula*, increased their biomass in the lake. Furthermore, the zooplankton community was represented by the copepods *Hemidiaptomus* spp. from July to November and by the rotifers *Brachionus* spp., the latter being present through most of the year, except in January and February (Table 2).

Phytoplankton abundance corresponded with Chlorophyll *a* levels, which ranged from 137 to 2 615 mg l^{-1} in November and February, respectively. Chlorophyll *a* levels correlated positively with the phytoplankton abundance patterns (r = 0.853, p = 0.0008). In addition, there was a significant positive correlation between the levels of Chlorophyll *a* and of nitrite and nitrate (r = 0.617, p = 0.033). However, there was no significant correlation of Chlorophyll *a* with any other environmental parameters tested.

Cyanobacterial hepatotoxin synthase genes

The potential for hepatotoxin production by cyanobacteria was examined by screening for genes involved in the biosynthesis of microcystin (encoded by *mcyE)* and nodularin (encoded by *ndaF)*. PCR amplification using primers for these two genes was negative in all cyanobacteria samples from Lake Big Momela throughout the entire sampling year, suggesting the presence of non-toxic strains.

Discussion

In recent decades, soda lakes around the world have attracted research attention, in particular to reveal the causes of extensive mortalities inflicted on animals feeding on microbes inhabiting these unique inland waters. Besides the recognised ecological importance that phytoplankton, and in particular cyanobacteria, play in food chains of African soda lakes, e.g. lesser flamingo, cyanobacteria are also identified as a potential cause of such bird mortalities, because of their potential toxin production. The toxin producers and toxins, as well as the regulatory effect that environmental factors (along temporal scales) exert on their dynamics in Tanzanian soda lakes are, however, still largely unknown.

Planktonic composition and abundance

The current study identifies and verifies both a ubiquity and high abundance of cyanobacterial genera in Lake Big Momela, with the species *Anabaenopsis elenkinii* and *Arthrospira fusiformis* being major representatives within the planktonic community. For the first time, the genetic composition and phylogeny of phyto- and zooplankton have been explored among microbes in Lake Big Momela, extending our knowledge by giving a more comprehensive view of this unique ecosystem. Overall, however, our findings corroborate data from other East African soda lakes (Vareschi 1978, 1982; Ballot et al. 2004, 2008; Kaaya et al. 2007; Dadheech et al. 2013; Kaggwa et al. 2013; Krienitz et al. 2016).

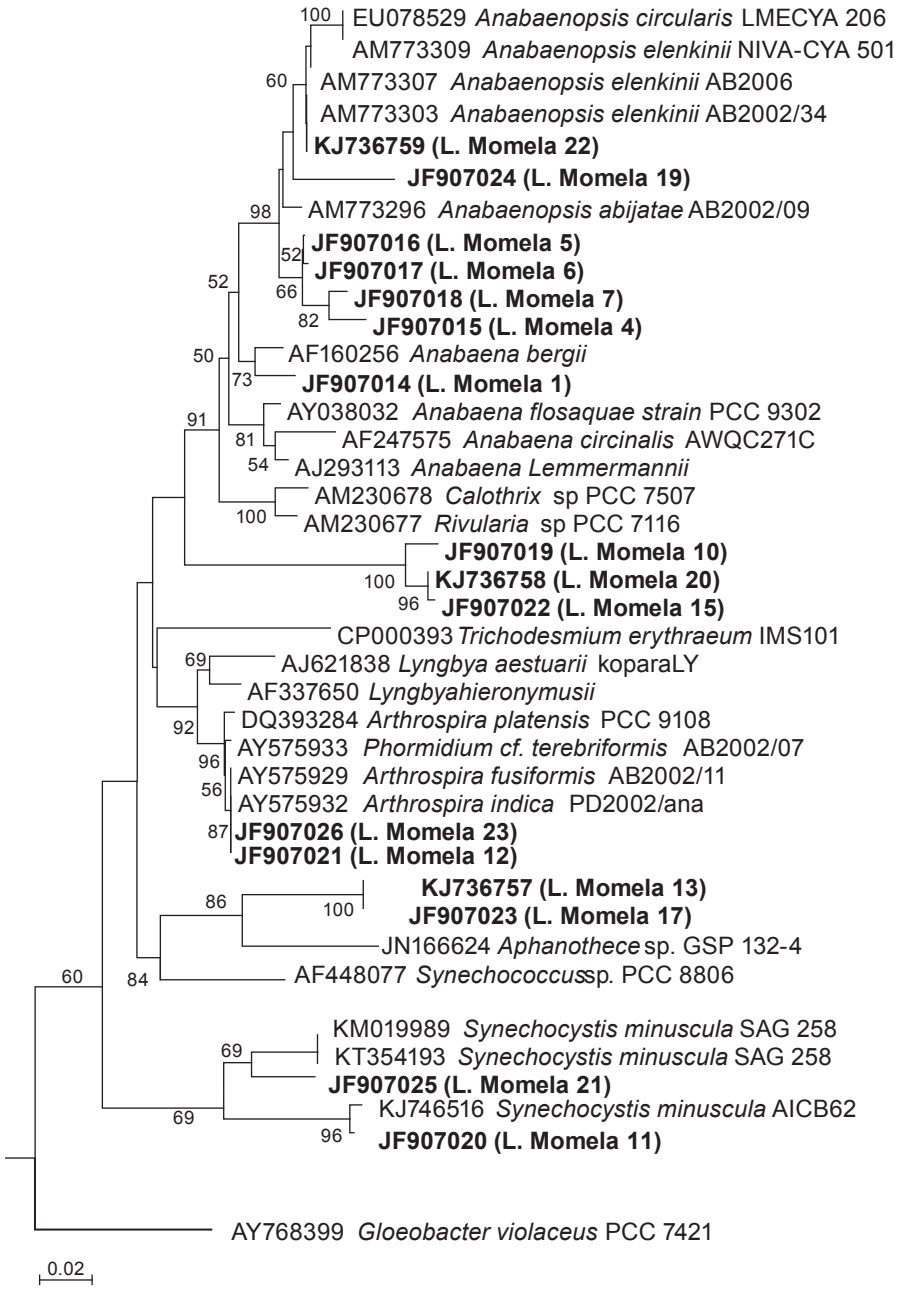

Figure 5: Phylogenetic affiliations of partial 16S rRNA gene sequences of plankton composition of the sequences retrieved from Lake Big Momela, in 2007. Tree constructed from the 16S rRNA based DGGE sequences in bold in this figure

Environmental conditions of the water in Lake Big Momela were fairly stable over the entire year, showing only slight temporal variations, with the exception of dissolved oxygen, which showed some significant temporal variations. The partially stable but extreme ecosystem of Lake Big Momela is considered a major reason for its overall low biodiversity, because it offers conditions above and beyond the tolerance threshold levels of most of the larger aquatic organisms, such as fish (Melack 1979; Vareschi 1982). However, the low organism competition in soda lakes does allow the development of massive blooms, ranging from a few well-adapted phytoplankton organisms, such as the cyanobacteria genera *Arthrospira* and *Anabaenopsis* (Vareschi 1978, 1982; Kaaya et al. 2007), to some eukaryotic phytoplankton and zooplankton. The restricted biodiversity is a phenomenon that may be attributed not only to the lack of competition but also, perhaps equally importantly, to the supreme adaptive capabilities of certain cyanobacteria, as reflected by their richness in a variety of major extreme global ecosystems, both terrestrial and aquatic (Whitton and Potts 2000). The stable conditions in Lake Big Momela, attributed to the lack of water inflow (Jones et al. 1998), besides precipitation and underground water recharge, may be yet another factor stimulating the development of

Table 1: Closest match of the 18S rRNA-DGGE band sequences with public databases for eukaryotic phytoplankton and zooplankton species from Lake Big Momela in 2007

DGGE band No.	Closest match (Accession number)	% identity	Source (location)	Reference
Euk 1 (JF907027)	*Brachionus plicatilis* (AY218118/9)	97	Freshwater	Giribet et al. 2004
Euk 2 (JF907028)	AY218118/9 (*Brachionus plicatilis*)	99	Freshwater	Giribet et al. 2004
Euk 3 (JF907029)	AY218118/9 (*Brachionus plicatilis*)	95	Freshwater	Giribet et al. 2004
Euk4 (JF907030)	KC888110/11 strain RCC1348/1353 (*Isochrysis galbana*)	99	Culture in seawater media	Bendif et al. 2013
Euk5 (JF907031)	(HM990664/9) *Picocystis* sp. KR 2010/4	94	Soda lake	Krienitz et al. 2012
Euk6 (JF907032)	HM990664/9 *Picocystis* sp. KR 2010/4	98	Soda lake	Krienitz et al. 2012
Euk7 (JF907033)	AY218118/9 *Brachionus plicatilis*	99	Freshwater	Giribet et al. 2004
Euk8 (JF907034)	(JX945122) *Hemidiaptomus gurneyi canaanita* isolate Hgurcan_118	99	Inland water	Marrone et al. 2013
Euk9 (JF907035)	JX945122 *Hemidiaptomus gurneyi canaanita* isolate Hgurcan_118	93	Inland water	Marrone et al. 2013

Table 2: Phytoplankton biomass (Chl *a*) and abundances of the cyanobacteria *Anabaenopsis elenkenii* and *Arthrospira fusiformis*, other phytoplankton (occasionally unicellular cyanobacteria and diatoms), and the zooplankters *Hemidiaptomus* sp. and *Brachionus* sp. in Lake Big Momela in 2007

Category	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Total chlorophyll *a* (mg m^{-3})	995	2 615	2 433	935	1 440	1 048	820	492	582	306	137	1 216
Anabaenopsis elenkenii *	4 850	14 750	2 700	1 550	625	0	0	0	0	0	100	8 000
Arthrospira fusiformis *	0	0	2 725	1 225	487	125	63	12.5	0	0	37.5	337
Other phytoplankton †	98	74	412	341	375	690	432	340	574	310	408	235
Hemidiaptomus sp. †	0	0	0	0	0	0	0.3	0.7	0.3	0.3	0.3	0
Brachionus sp.†	0	0	5.3	1.7	6	2	1	0.3	0.7	0.7	13.3	4.7

† = cells ml^{-1}, * = filaments ml^{-1}

the cyanobacterial blooms in this lake. Typically, aquatic ecosystems that support massive growths of the cyanobacterium *Arthrospira* are stable alkaline-saline waters of high temperature, irradiance and eutrophic conditions (Whitton and Potts 2000). The temperatures recorded in Lake Big Momela during 2007 were within the optimal temperature ranges (25–28 °C) for growth of cyanobacteria in nature (Whitton and Potts 2000). Other factors that may contribute to their dominance may be their ability to adjust osmotic and cellular mechanisms regulating their internal pH levels (Vonshak and Tomaselli 2000). Blooms of *Arthrospira* have also earlier been attributed to their efficient energy and nutrient self-support of carbon (via photosynthesis) and for *Anabaenopsis* also of atmospheric nitrogen (via N_2 fixation) (Vareschi 1978, 1982; Kaaya et al. 2007; Kaggwa et al. 2013). In addition, both genera possess gas vacuoles (Miklaszewska et al. 2012), which assist in buoyancy regulation and improve their ability to harvest light energy and carbon in surface waters as well as nutrients in deeper waters (Walsby 1978, 1994).

An important feature of the cyanobacteria community in Lake Big Momela was the distinctly different seasonal distribution pattern over the year of the two dominating cyanobacteria genera. This is in contrast to the situation in other soda lakes, with a constant presence of these cyanobacteria communities over the year (Vareschi 1978, 1982; Kaaya et al. 2007). Besides the small variations found in physico-chemical parameters in Lake Big Momela over the year, the cyanobacteria community's seasonality may be regulated by more subtle conditions and reflect changing environmental conditions. This was confirmed in a previous study showing that sodium (salinity), pH and turbidity influence pattern in phytoplankton taxa of the soda lakes; with *Arthrospira* being out competed at high turbidity and salinity (Schagerl et al. 2015). For instance, the higher abundance of the nitrogen fixing *Anabaenopsis elenkinii* observed in February in Lake Big Momela, correlated with higher water temperatures, dissolved oxygen and nitrate concentrations, which are probably a consequence, not a reason, for its dominance over *Arthrospira* (e.g. Irwin et al. 2006; Dolman et al. 2012). The increase in water nitrogen concentrations from nitrogen fixation by *Anabaenopsis elenkinii* may subsequently promote growth of non-N_2 fixing cyanobacteria, primarily *Arthrospira fusiformis* (Grant 2006), but also the unicellular genera *Synechococcus* and *Synechocystis* present in Lake Big Momela.

The prevalence and distinct seasonal patterns of *Arthrospira* and *Anabaenopsis* in Lake Big Momela contrast

with a previous report on Lakes Nakuru and Bogoria, in which these two cyanobacteria genera coexisted throughout the year (Kaggwa et al. 2013). In Lake Nakuru *Arthrospira* abundance peaked during September to November, whereas in Lake Big Momela it peaked in March, with *Anabaenopsis* dominating in December to February and low abundances of both *Arthrospira* and *Anabaenopsis* occurring from September to October. To what extent these patterns are permanent or inter-annual variations in Lake Big Momela now should be examined. If they are stable, cyanobacterial patterns may potentially act as predictors for the migration of flamingos between East African soda lakes (Harper et al. 2003). For instance, high abundance of *Anabaenopsis* in Lake Big Momela may deter Lesser Flamingo showing feeding preference towards chlorophytes and euglenophytes. The *Anabaenopsis* filaments form large and slimy colonies that may block their feeding filtration system (Krienitz and Kotut 2010). However, *Anabaenopsis*is an important food source for flamingo in Lakes Elmentaita, Nakuru and Simbi, Kenya (Krienitz et al. 2016). Such seemingly contradicting results may suggest the presence of distinct *Anabaenopsis* morphotypes in the various soda lakes. Moreover, the current data also suggest that the monospecific occurrence of *Arthrospira fusiformis* in soda lakes of the East African Rift Valley (Vareschi 1978), may no longer exist (Krienitz and Kotut 2010; Kaggwa et al. 2013). This in turn may be of concern with regard to the survival of Lesser Flamingo, or may lead to shifts in their dietary preferences.

Genetic composition and phylogeny of the planktonic community

Studies of microbial populations in soda lakes have traditionally relied on microscopy (Zavarzin et al. 1999) and, although of immense importance, this technology is limited by its relatively low resolution, potentially hindering identification of the often poorly studied unique microbial populations of soda lakes. It is also known that the overall plankton morphology may vary from lake to lake, depending on local conditions, potentially contributing to some taxonomic confusion. The polyphasic approach used here, based on a combination of morphological characters and genetic analyses, allow a more detailed exploration of the microbial biodiversity of a natural ecosystem. Molecular methods were only recently introduced to examine the diversity in soda lakes (e.g. Ballot et al. 2004; 2008; Krienitz et al. 2016). Data obtained have revealed a high genetic diversity of both prokaryotic and eukaryotic microorganisms, and a species richness often rivalling that of freshwater ecosystems (Wang 2011). Soda lakes contain an unusually high proportion of alkaliphilic microorganisms with a low genetic similarity to known fresh water or marine species, even including low overlap to microbial communities in other soda lakes with different abiotic conditions of pH and salinity (Xiong et al. 2012; Lanzén et al. 2013). Recent data suggest the existence of a rich endemic microbial community, potentially unique for each individual alkaline lake. Using the 16S rRNA gene as a molecular marker, together with morphological analysis, the ubiquity and dominance of cyanobacteria species in Lake Big Momela was revealed, this being composed of representatives of both filamentous heterocystous and non-heterocystous, plus some unicellular, species. These results highlight the importance of Cyanobacteria in Lake Big Momela's productivity.

Cyanobacterial 16S rRNA sequences retrieved from Lake Big Momela clustered primarily with sequences retrieved from Kenyan soda lakes (e.g. Lake Nakuru, Lake Sonachi and Lake Elmenteita) (Ballot et al. 2004, 2008), suggesting a highly related cyanobacteria biodiversity on a wider regional scale basis. This close genetic relationship is probably driven by their geographic proximity and the similar conditions offered. The existence of potentially unique Nostocales clades in Lake Big Momela illustrates the presence of hitherto unknown phylotypes in this ecosystem. However, endemism in Lake Big Momela may not yet be claimed, as the collected genetic information on cyanobacterial populations in other soda lakes around the world is still too scarce (Ballot et al. 2004; Wani et al. 2006; Xiong et al. 2012; Lanzén et al. 2013). That may for instance be the case for the sequences related to *A. abijatae,* here forming part of one of the sister clusters composed of members of the genus *Cyanospira* (Sili et al. 2011). In this phylogenetic relationship, *A. abijatae* seems distantly related to the rest of the *Anabaenopsis* spp., suggesting that the current sequences might belong to the *Cyanospira rippkae* sub cluster sequences retrieved from hyperalkaline lakes in Chad (Sili et al. 2011).

Genetic analyses further showed that some of the sequences retrieved here clustered with those of the Australian *Anabaena bergii* (AF160256), which earlier was reported to belong to *Aphanizomenon ovalisporum* (*Chrysosporum ovalisporum)*, even though some studies have described them as morphologically and genetically distinct types (Stüken et al. 2009; Ballot et al. 2011). Lately, however, Cirés and Ballot (2016) provided insight into the taxonomy and clustering of these two genera, indicating a misidentification of *Anabaena bergii* sequence AF160256, suggesting a geographically widespread distribution *of Aphanizomenon* from the tropics to temperate areas, with *C. ovalisporum* occurring in tropical, subtropical and Mediterranean areas. In this context, the Lake Big Momela sequence JF907014 may belong to *C. ovalisporum,* suggesting a tropical habitat for this species.

Furthermore, clades identified within the Oscillatoriales verified the abundance of the non-N_2 fixing *Arthrospira* phylotypes in Lake Big Momela and their relationship to those in Kenyan soda lakes and in some Indian freshwater lakes (Ballot et al. 2004). In contrast, several unicellular Chroococcales identified in Lake Big Momela clustered with cultured/uncultured species earlier retrieved from both marine and freshwater environments, but not with any from soda lakes. These findings suggest the existence of unique Chroococcales clades in Lake Big Momela. Notably, a high degree of endemism for members of Chroococcales in other extreme aquatic environments has been implied, such as hot springs, hypersaline environments and even in soda lakes of East Africa (Kirkwood et al. 2008; Dadheech et al. 2013).

Using the 18S rRNA eukaryotic gene as a molecular marker, phylogenetic analysis of microbes in Lake Big Momela showed the highest similarity to prasinophyte members of the genus *Picocystis* and to sequences earlier

retrieved from East African soda lakes (Krienitz et al. 2012). Moreover, the Lake Big Momela sequences, which were related to the rotifer *Brachionus* plicatilis, showed only a distant relationship (95–99% similarity) to species from inland water environments, such as those from euryhaline strains (King and Zhao 1987; Giribet et al. 2004). The copepod sequences retrieved showed similarity to the *Hemidiaptomus* spp. recovered from the western Mediterranean Sea (Marrone et al. 2013). Together, apart from the commonly reported cyanobacteria species, these genetic data confirm earlier findings (Krienitz et al. 2012; Luo et al. 2013; Lanzén et al. 2013; Schagerl et al. 2015; Krienitz et al. 2016), and suggest the existence of a 'hidden' diversity of eukaryotic plankton in the East African soda lakes. The affiliation of the eukaryotic microorganisms retrieved in the current study suggests either exclusiveness of Lake Big Momela or a paucity of information on soda lake eukaryotic microorganisms in general, hence calling for further investigations.

Analysis of cyanobacterial toxin encoding genes

The lack of amplification signals from genes coding for the toxins microcystin and nodularin in cyanobacteria samples from Lake Big Momela, strongly suggests that the cyanobacterial strains present, at least in 2007, were unable to produce these toxins. However, this may not exclude the existence of some toxic strains with low cell numbers, e.g. those below the detection limit of the methods used in the current study, or alternatively that the existing toxin gene sequences (of distant hosts) are too different to be recognised by the genetic primers used here. However, the current study corroborates previous reports from other soda lakes, such as Lake Elmenteita, in which *A. fusiformis* did not produce any cyanotoxin (Ballot et al. 2004). On the other hand, production of the toxins microcystin–YR and anatoxin-a was apparent in *A. fusiformis* from Lake Bogoria, whereas *A. fusiformis* from Lake Nakuru produced only anatoxin-a. One explanation for the discrepancies between soda lakes in their toxin production may be the existence of subtle genetic differences reflected in morphological differences, such as the different trichome configurations noted in *A. fusiformis* (Bai and Seshadri 1980; Hindák 1985; Kebede 1997). Moreover, according to Hindák (1985), Lake Bogoria, was in the past dominated by a 'type H-variant' of *Arthrospira,* which is not found today. Such findings suggest, that strain variants have evolved in soda lakes over time, e.g. among cyanobacteria that may either possess or lack toxin genes (Ballot et al. 2004).It is also possible that unknown secondary metabolites toxic to Lesser Flamingo may be produced by some cyanobacteria in Lake Big Momela.

By combining morphological and genetic analyses, it is clear that Lake Big Momela is dominated by two prominent cyanobacteria within the genera *Anabaenopsis* (Nostocales) and *Arthrospir*a (Oscillatoriales). It is also apparent that these cyanobacteria are not constantly present in the microbial community, as earlier reported, including in other soda lakes, but show distinct seasonal variations, probably controlled by physico-chemical parameters inherent to this soda lake. The close phylogenetic relationship of the dominant cyanobacteria to counterparts retrieved from other East African soda lakes suggests the existence of 'pan-soda lake' populations. However, some potentially specific phylotypes of *Anabaenopsis* and *Arthrospira* were also retrieved from Lake Big Momela, as well as some low abundance phylotypes from within the unicellular Chroococcales. The absence of cyanobacterial toxic genes, earlier found in these dominant cyanobacteria (Ballot et al. 2004, 2005; Straubinger-Gansberger et al. 2014), suggests that these genes are not permanent traits. The molecular markers used also identified eukaryotic microorganisms (zoo- and phytoplankton) and revealed their distinct presence in the planktonic community of Lake Big Momela. Because the data point to potential endemism among the microbes found, additional examinations in time and space in East African soda lakes (and other geographical areas) are warranted, so as to understand fully the extent of planktonic populations in these extreme aquatic ecosystems.

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