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Cyanobacteria and cyanobacterial toxins in three alkaline Rift Valley lakes of Kenya—Lakes Bogoria, Nakuru and Elmenteita

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For decades frequent mass mortalities of Lesser Flamingos (Phoeniconaias minor Geoffroy) have been observed at alkaline-saline Kenyan Rift Valley lakes. To estimate the potential influence of toxic cyanobacteria on these mass deaths, the phytoplankton communities were investigated in Lakes Bogoria, Nakuru and Elmenteita. Cyanobacterial toxins were analyzed both in the phytoplankton from the three lakes and in isolated monocyanobacterial strains of Arthrospira fusiformis, Anabaenopsis abijatae, Spirulina subsalsa and Phormidium terebriformis. Lake Bogoria was dominated by the cyanobacterium A. fusiformis. In L. Nakuru and L. Elmenteita the phytoplankton mainly consisted of A. fusiformis, A. abijatae and Anabaenopsis arnoldii, and in L. Nakuru an unknown Anabaena sp. was also found. Furthermore, this is the first time A. abijatae and the unknown Anabaena sp. have been found in Kenyan lakes. Phytoplankton wet weight biomass was found to be high, reaching 777 mg L^{-1} in L. Bogoria, 104 mg L^{-1} in L. Nakuru and 202 mg L^{-1} in L. Elmenteita. Using HPLC, the cyanobacterial hepatotoxins microcystin-LR, -RR -YR, -LF and -LA and the neurotoxin anatoxin-a were detected in phytoplankton samples from L. Bogoria and L. Nakuru. Total microcystin concentrations amounted to 155 μg microcystin-LR equivalents g^{-1} DW in L. Bogoria, and 4593 μg microcystin-LR equivalents g^{-1} DW in L. Nakuru, with anatoxin-a concentrations at 9 μg g^{-1} DW in L. Bogoria and 223 μg g^{-1} DW in L. Nakuru. In L. Elmenteita phytoplankton, no cyanobacterial toxins were found. A. fusiformis was identified as one source of the toxins. The isolated strain of A. fusiformis from L. Bogoria was found to produce both microcystin-YR (15.0 μg g^{-1} DW) and anatoxin-a (10.4 μg g^{-1} DW), whilst the A. fusiformis strain from L. Nakuru was found to produce anatoxin-a (0.14 μg g^{-1} DW). Since A. fusiformis mass developments are characteristic of alkaline-saline lakes, health risks to wildlife, especially the Arthrospira-consuming Lesser Flamingo, may be expected.

INTRODUCTION

The alkaline-saline Lakes Bogoria, Nakuru and Elmenteita are situated in the Kenyan Rift Valley, a part of the Gregory Rift which stretches from Tanzania to Ethiopia. Since the early decades of the 20th century, numerous studies ranging from the geology to the ecology and biology of these lakes have been carried out [e.g. (Jenkin, 1936; Talling and Talling, 1965; Vareschi, 1978; Melack, 1988; Schlüter, 1993; Owino *et al.*, 2001)]. Lakes Bogoria, Nakuru and Elmenteita are characterized by mass

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developments of filamentous cyanobacteria including *Arthrospira fusiformis* (Vorochinin) Komárek (syn. *Spirulina fusiformis* Voronichin) and *Anabaenopsis* spp. (Vareschi, 1982; Hindák, 1985; Melack, 1988). The blooms of *Arthrospira* are the main diet of the Lesser Flamingo, *Phoeniconaias minor* Geoffrey, which inhabit the alkaline Rift Valley lakes in high numbers (Vareschi, 1978; Owino *et al.*, 2001). It has been estimated that an adult Lesser Flamingo consumes up to about 72 g dry weight (DW) of cyanobacteria per day, mostly in shallow lake areas (Vareschi, 1978).

Mass mortalities of flamingos during recent decades have led to a decline in populations in the Kenyan Rift Valley (Wanjiru, 2001). Infections of mycobacteria, causing avian tuberculosis, and poisoning by heavy metals and pesticides have been thought to be major contributors to the mass mortalities (Kairu, 1996; Nelson *et al.*, 1998; Kock *et al.*, 1999). A further possible contribution to these mass deaths is by cyanobacterial toxicosis. Worldwide, cyanobacterial blooms and mats are increasingly recognized as sources of potent toxins (cyanotoxins) (Carmichael, 1997; Codd *et al.*, 1999). Recent studies by Krienitz *et al.* (Krienitz *et al.*, 2003) have shown that microcystins (cyanobacterial hepatotoxins) and anatoxin-a (cyanobacterial neurotoxin) are present in cyanobacterial mats of the hot springs at the shore of L. Bogoria. The same toxins have been detected in livers from dead Lesser Flamingos, collected at L. Bogoria and L. Nakuru (Ballot *et al.*, 2002). The presence of hot spring cyanobacterial cells and cell fragments in stomach contents and fecal pellets confirm that the flamingos ingest toxic cyanobacteria whilst drinking and washing at the hot springs (Krienitz *et al.*, 2003). There is also a lack of knowledge about possible changes in the cyanobacterial compositions of the lakes due to the invasion of potentially toxic species and how this is influenced by physicochemical conditions.

Arthrospira, one of the dominant species in the alkaline lakes, is regarded as non-toxic. However, several investigations have indicated a possible toxicity of *Arthrospira* (Gilroy *et al.*, 2000; Iwasa *et al.*, 2002). A toxic strain of *A. fusiformis* producing microcystin-YR and anatoxin-a was isolated from L. Sonachi, Kenya (Ballot *et al.*, 2004). This paper is an attempt to evaluate changes in the cyanobacterial communities of Lakes Bogoria, Nakuru and Elmenteita and the occurrence of cyanobacterial toxins. To identify possible sources for toxin production, isolated strains of *A. fusiformis, Anabaenopsis abijatae* Kebede et Willén, *Spirulina subsalsa* Oersted and *Phormidium terebriformis* (Agardh ex Gomont) Anagnostdis & Komárek from Lakes Bogoria, Nakuru, and Elmenteita were also investigated.

METHOD

Site description

The three alkaline-saline lakes, Lake Bogoria, Lake Elmenteita and Lake Nakuru are located in the Kenyan part of the Eastern Rift Valley. The three lakes have no surface outlet. Table I summarizes some geomorphological features of the lakes.

Lake Bogoria, formerly known as Lake Hannington, is located north of the Equator in a harsh climatic place. The area around the lake is still volcanically active. Many boiling springs and fumaroles occur along the lake shore and discharge fresh to moderately alkaline and saline water (Cioni *et al.*, 1992). Two small freshwater streams are situated at the southern end of the lake.

Lake Nakuru is situated in Nakuru National Park around 65 km south of L. Bogoria. The Lamudiac-, Njoro-, Makalia- and Nderit Rivers, the Baharini springs and a small alkaline spring at the southern end flow into the lake. In addition the lake receives mechanically and biologically treated wastewater from two sewage treatment plants in the nearby town of Nakuru.

Lake Elmenteita is situated ~30 km south of L. Nakuru. The lake is fed by several small rivers (the Mbaruk, Chamuka and Kariandus). Hot springs at the southern end of the lake discharge slightly alkaline and saline water (Mwaura and Moore, 1991).

Measurements and sampling

The present study was carried out from June 2001 to September 2002 inclusive. The sampling point at L. Bogoria (BO) was at the western shore (00°13.833′N, 36°05.556′E). At L. Nakuru, two sites were chosen for sampling, near the inflow of the Njoro River (Cormorant

Table I: Geographical position, altitude, surface area, catchment area and depth of Lakes Bogoria, Nakuru and Elmenteita (Vareschi, 1982; Melack, 1988; Mwaura and Moore, 1991; Schlüter, 1993)

	Lake Bogoria	Lake Nakuru	Lake Elmenteita
Geographical position	00°15′N	00°20′S	00°27′S
	36°05′E	36°05′E	36°15′E
Altitude above sea level (m)	963	1759	1782
Surface area (km^2)	34	40	~20
Depth (m)	10	0–4.5	0.3–3.1
Catchment area (km^2)	Unknown	~800	500
Rainfall (mm)	500–1000	~880	600–1200

point) (NC) (00°19.583′S, 036°05.325′E) and at the foot of the Baboon Cliffs (NB) (00°21.887′S, 036°03.465′E). At L. Elmenteita (EL), the sampling point was at the eastern shore (00°27.345′S, 036°15.333′E).

Conductivity, pH and salinity were measured directly in the lakes with a WTW Multiline P4 meter (Wissenschaftlich Technische Werkstätten Weilheim, Germany). Water transparency was measured with a Secchi disc (Ø 20 cm). Samples for the determination of total nitrogen (TN) and total phosphorus (TP) were taken a few centimetres below the water surface. Laboratory analysis was carried out within 3 h from the time of collection. For TN and TP, Nanocolor tube tests (two replicates each) and a field photometer (Nanocolor 300 D; Macherey-Nagel GmbH Düren, Germany) were used. The detection limits were 36 μM (TN) and 0.32 μM (TP).

For quantitative phytoplankton analysis, 125 mL water subsamples were removed from samples taken from the lake surface and fixed with formaldehyde. For qualitative phytoplankton analysis, 5 L of lake water were concentrated with a plankton net (20 μm mesh size) and fixed with formaldehyde (final concentration 1% v/v). The acidic Lugol's solution was unsuitable for preservation of highly alkaline samples.

For cyanotoxin analysis, up to 1 L of lakewater from the surface was filtered through glass fibre filters (0.45 μm pore size; Whatman GF/C, Whatman International Ltd, Maidstone, England) using a vacuum pump. The filters were air-dried, packed in aluminium foil and stored in the dark at room temperature for ~4 weeks during the field trip until further analysis in Germany and Scotland. The filtrate was passed through a tC18 Sep-Pak Plus cartridge (Waters Corporation, Milford, USA) to recover the dissolved extracellular cyanotoxin fraction.

A strain of *A. fusiformis* from L. Bogoria, *A. fusiformis*, *A. abijatae*, *S. subsalsa* and *P. terebriformis* from L. Nakuru and *A. fusiformis* and *A. abijatae* from L. Elmenteita were isolated and cultivated in Bourrelly's solution (Hegewald *et al.*, 1994), modified by the addition of 0.3 g Na_2CO_3 and 15 g NaCl per litre. For cyanotoxin analysis, a volume representing ~500 mg DW of cyanobacterial biomass was filtered through glass fibre filters, air dried and stored in the dark at room temperature.

Microscopy

Phytoplankton taxa were counted in sedimentation chambers (Hydro-Bios Apparatebau GmbH Kiel, Germany) using a compound microscope (Eclipse TS 100; Nikon Corporation, Tokyo, Japan) according to Utermöhl (Utermöhl, 1958). Phytoplankton biomass was calculated by geometrical approximations using the computerized counting programme Opticount (Hepperle, 2000). The specific density of phytoplankton cells was calculated as 1 g cm^{-3}. For calculation of cyanotoxin concentrations, the DW of all cyanobacteria was used. For DW estimations, 10% of the wet weight biomass was calculated according to Ruttner (Ruttner, 1938). The total mass of the filtered material could not be used for calculation because of the high amount of suspended inorganic material in the samples.

Cyanotoxin analysis

Microcystins

Filtered samples were extracted by adding 10 mL of 70% v/v aqueous methanol, followed by ultrasonication for 15 min (Ultra Turrax T 25; Janke & Kunkel GmbH & Co. KG—IKA-Labortechnik, Staufen, Germany) and constant shaking for 24 h on an orbital shaker. Filter material and phytoplankton debris were removed by centrifugation at 11 600 *g* for 5 min. The supernatant was evaporated to dryness at 30°C under constant nitrogen flow. The residuals including the toxins were redissolved in 1 mL 75% v/v methanol (Fastner *et al.*, 1998). 50 μL of this solution were used for analysis by high performance liquid chromatography with photodiode array detection (HPLC–PDA; Waters Corp., Milford, USA) and matrix assisted laser desorption/ionization time of flight mass spectroscopy (MALDI–TOF) (Pflugmacher *et al.*, 2001). The enriched tC18 Plus Sep-Pak cartridges were eluted with 90% v/v methanol. The eluates were blown to dryness with nitrogen and the residue dissolved in 500 μL 100% methanol for HPLC–PDA analysis. The detection limit for cell-bound microcystins was 1 μg g^{-1} of dry cyanobacterial material and for dissolved microcystins in the range of 1 μg L^{-1} by HPLC–PDA.

The methanolic extracts analysed by HPLC–PDA were diluted with MilliQ water to a methanol concentration below 10% v/v. These were analysed by ELISA using antibodies raised against microcystin-LR (Metcalf *et al.*, 2000). The ELISA detection limits for cell-bound microcystins and dissolved (extracellular) microcystins were 1 ng g^{-1} and 1 ng L^{-1}, respectively.

Standards for calculation were microcystin-LR (MC-LR; gravimetric standard), and dhb-microcystin-LR provided by G. A. Codd (University of Dundee); microcystin-LA (MC-LA); microcystin-RR (MC-RR), microcystin-LF (MC-LF), microcystin-LW (MC-LW) from Alexis Corporation Biochemicals Grünberg, Germany; and microcystin-YR (MC-YR) from Calbiochem Novabiochem GmbH Bad Soden, Germany.

Anatoxin-a

Analysis of anatoxin-a was performed using HPLC–PDA (Waters Corp., Milford, USA) at a wavelength of 227 nm on a reverse phase column μBondapak C18

(Waters Corp., Milford, USA) over 15 min according to Harada *et al.* (Harada *et al.*, 1989) with a mobile phase of acetonitrile and water (10:90 v/v) containing 0.05% trifluoro acetic acid (TFA) and an injection volume of 25 μL. HPLC calibration was carried out using anatoxin-a from Alexis Corp. (Grünberg, Germany). 50 μL of the same solution was used for MALDI–TOF analysis.

RESULTS

Physicochemical data

The physical conditions of all three lakes were characterized by low water transparencies. The Secchi depths in all lakes never exceeded 0.3 m on all measuring dates. The pH values always exceeded 9.8 in all three lakes. With few exceptions water temperatures were generally above 20°C (Figure 1). Salinity values showed clear differences between the lakes. The highest values were measured at L. Bogoria, whereas L. Elmenteita showed the lowest salinity (Figure 1). Measured TP concentrations were: 0.17–1.08 mM in L. Bogoria, 0.08–1.29 mM in L. Nakuru, and 0.02–0.25 mM in L. Elmenteita (Figure 1). TN concentrations ranged from 0.04 to 0.59 mM in L. Bogoria, from 0.05 to 0.81 mM in L. Nakuru, and from 0.04 to 0.61 mM in L. Elmenteita (Figure 1).

Phytoplankton community

The phytoplankton community in the three lakes was dominated by cyanobacteria. *Cryptomonas* sp. (Cryptophyceae) and the diatoms *Navicula* spp. and *Nitzschia* spp. were present in the samples, mostly in low numbers. The Chlorophyceae were represented by coccoid picoplankton and flagellates including *Dunaliella* sp. (Table II).

In L. Bogoria, *Arthrospira fusiformis* was the dominant cyanobacterial species accounting for >97% of the cyanobacterial biomass (Figure 2). In all samples, coccoid cyanobacteria including *Synechococcus* spp. and *Synechocystis* sp. were present at low biomass. Other cyanobacteria found in small amounts were *Spirulina subsalsa*, *Spirulina subtilissima* Kütz., *Phormidium* sp. and *Oscillatoria* sp. (Figure 2).

As in L. Bogoria, in L. Nakuru cyanobacteria were the dominant group (Table II). *Arthrospira fusiformis* was the main representative at Cormorant Point. The only

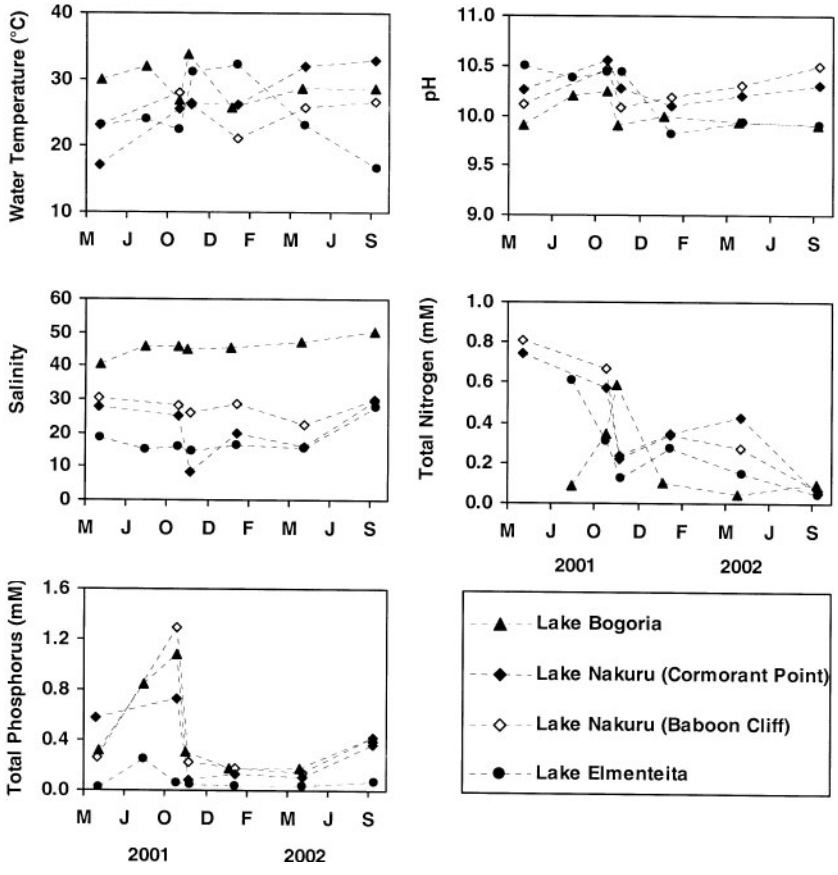

Fig. 1. Physico-chemical conditions in Lakes Bogoria, Nakuru and Elmenteita from June 2001 to September 2002.

Table II: Range of biomass (wet weight) in mg L^{-1} of the main phytoplankton groups in Lakes Bogoria, Nakuru and Elmenteita from June 2001 to September 2002

Phytoplankton groups June 2001–September 2002 wet wt (mg L^{-1})	Lake Bogoria	Lake Nakuru (Cormorant Point)	Lake Nakuru (Baboon Cliff)	Lake Elmenteita
Cyanophyceae	61.1–769.1	5.3–77.5	20.6–96.4	14.1–196.7
Cryptophyceae	n.d.–0.5	0.1–7.1	1.1–46.2	0.5–14.1
Bacillariophyceae	<0.1–0.4	n.d.–1.2	n.d.–1.3	n.d.–1.8
Chlorophyceae	<0.1–7.8	<0.1–1.5	0.1–8.3	0.4–2.8
Total biomass	62.2–777.1	6.7–80.3	25.2–104.3	17.5–202.0

n.d., not detected.

exception was June 2001, when a bloom of a so far unknown *Anabaena* sp. dominated. Other dominant taxa with a lower biomass were *Anabaenopsis abijatae* and *Anabaenopsis arnoldii* Aptekarj. Coccoid cyanobacteria including *Synechocystis* sp. and *Synechococcus* sp. were always present (Figure 2).

At Baboon Cliff, L. Nakuru, the cyanobacterial community changed from an *A. abijatae* dominated community to one dominated by *A. fusiformis*. *A. arnoldii* was always present at low biomass (Figure 2).

In L. Elmenteita, the cyanobacterial community changed from one dominated by *A. abijatae* in June 2001

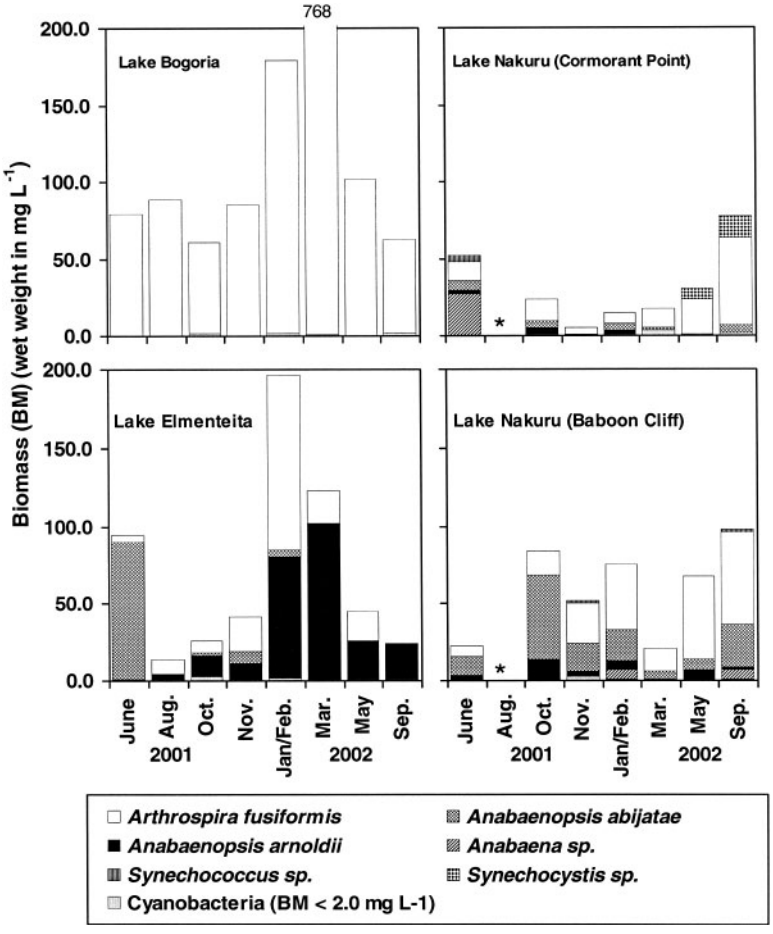

Fig. 2. Cyanobacterial community in Lakes Bogoria, Nakuru and Elmenteita from June 2001 to September 2002. *, not sampled.

to one dominated by *A. fusiformis* and *A. arnoldii* in August 2001. However, in the sample from September 2002, *A. fusiformis* and *A. abijatae* were not present and only *A. arnoldii* was found. *Synechococcus* sp. and *Synechocystis* sp. were present in all samples, *Spirulina subtilissima*, *Spirulina subsalsa* and *Pseudanabaena* sp. in some of the samples (Figure 2).

Cyanotoxins

Microcystins and anatoxin-a were detected by HPLC–PDA in all seston samples from L. Bogoria and L. Nakuru and their molecular masses confirmed by MALDI–TOF. Microcystins were also consistently detected by ELISA. Two different microcystin structural variants (microcystin-LR and -RR) were identified in L. Bogoria samples and five variants (microcystin-LR, -RR, -YR, -LF and -LA) in L. Nakuru samples (Figure 3).

Total microcystin concentrations varied between 16 and 155 μg microcystin-LR equivalents g^{-1} DW in L. Bogoria samples and from 130 to 4593 μg microcystin-LR equivalents g^{-1} DW in L. Nakuru samples (Figure 3). Anatoxin-a concentrations ranged from 0.3 to 9 μg g^{-1} DW in Lake Bogoria and from 5 to 223 μg g^{-1} DW in L. Nakuru (Figure 4). Neither microcystins nor anatoxin-a were detected in cyanobacterial samples from L. Elmenteita by HPLC–PDA, nor by ELISA in the case of microcystins (Figures 3 and 4). In all samples from the three investigated lakes, extracellular microcystins and anatoxin-a were below the detection limit of 1 μg L^{-1}.

Microcystin and anatoxin-a were also detected in a cultivated strain of *A. fusiformis* (AB2002/10) from L. Bogoria. The concentrations found were 15.02 μg microcystin-YR g^{-1} DW and 10.38 μg anatoxin-a g^{-1} DW. In an *A. fusiformis* strain from L. Nakuru (AB2002/04) only anatoxin-a was produced (0.14 μg anatoxin-a g^{-1} DW). No cyanobacterial toxins were detected in strains of *A. fusiformis* and *A. abijatae* from L. Elmenteita and in strains of *A. abijatae*, *S. subsalsa* and *P. terebriformis* from L. Nakuru (Table III).

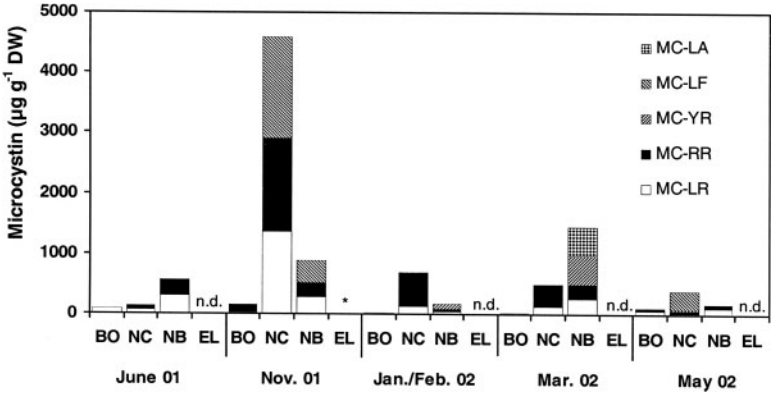

Fig. 3. Microcystins in Lakes Bogoria, Nakuru and Elmenteita in the samples from June 2001 to May 2002. BO, L. Bogoria; NC, L. Nakuru Cormorant Point; NB, L. Nakuru Baboon Cliff; EL, L. Elmenteita; n.d., not detected; *, not sampled.

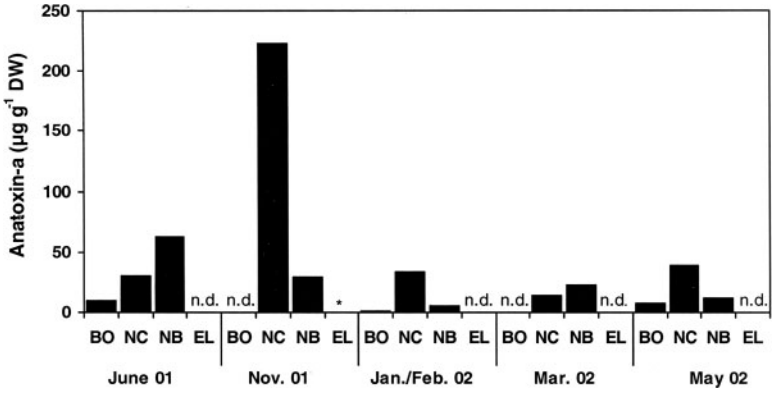

Fig. 4. Anatoxin-a in Lakes Bogoria, Nakuru and Elmenteita in the samples from June 2001 to May 2002. For abbreviations see Figure 3.

Table III: Cyanobacterial toxins in cultured strains of cyanobacteria from Lake Bogoria, Lake Nakuru and Lake Elmenteita

Cultured cyanobacterial strains	Cyanobacterial toxins	
	Microcystin (μg g^{-1} DW)	Anatoxin-a (μg g^{-1} DW)
Lake Bogoria		
Arthrospira fusiformis (AB2002/10)	15.02	10.38
Lake Nakuru		
Arthrospira fusiformis (AB2002/04)	n.d.	0.14
Anabaenopsis abijatae (AB2002/14)	n.d.	n.d.
Spirulina subsalsa (AB2002/06)	n.d	n.d
Phormidium terebriformis (AB2002/07)	n.d	n.d
Lake Elmenteita		
Arthrospira fusiformis (AB2002/01)	n.d	n.d
Anabaenopsis abijatae (AB2002/18)	n.d	n.d

n.d., not detected.

DISCUSSION

Cyanobacterial communities and influencing factors

Arthrospira sp. has been described as the dominant cyanobacterial species in studies concerning alkaline lakes in East Africa (Melack, 1979; Vareschi, 1982). In our study *Arthrospira fusiformis* was the dominant phytoplankton species at L. Bogoria, accounting for at least 97% of the cyanobacterial biomass. In L. Nakuru and L. Elmenteita besides *A. fusiformis*, *A. arnoldii* and *A. abijatae* were found with changing biomasses and dominances. This was also found with the unknown *Anabaena* sp. from L. Nakuru (Figure 2). The occurrence of *A. abijatae* and *Anabaena* sp. in Kenyan lakes has not been mentioned in earlier studies. *A. abijatae* was first found in the Ethiopian Lake Abijata and described by Kebede and Willén (Kebede and Willén, 1996). According to investigations of Jenkin (Jenkin, 1936), Lakes Nakuru and Elmenteita were dominated by *Arthrospira* sp. in the late 1920s. During a five year study, carried out at L. Nakuru from 1972 to 1977, a bloom of '*Spirulina platensis*' (= *Arthrospira fusiformis*) which persisted for about two years changed to one dominated by *A. arnoldii* and picoplanktonic cyanobacteria (Vareschi, 1982). In L. Elmenteita, the disappearance of dominant *Arthrospira* and *Anabaenopsis* blooms was mentioned by Melack (Melack, 1988).

The phytoplankton biomasses measured in L. Nakuru during this study (6.7–80.3 mg wet weight L^{-1}) were considerably lower than those of ~430–1700 mg L^{-1} measured in the 1970s by Vareschi (Vareschi, 1982) in the same lake. At that time the highest biomasses occurred during almost monocyanobacterial blooms of *A. fusiformis*. Changes in the phytoplankton composition were associated with a decrease in biomass (Vareschi, 1982). From Lakes Bogoria and Elmenteita no comparable biomass data are available.

Melack (Melack, 1988) suggested that shifts in salinity which exceeded physiological tolerance were possible causes for the changes in species composition. However, Vareschi (Vareschi, 1982) and Kebede (Kebede, 1997) have shown that *Arthrospira* can tolerate a wide range of salinities. Kebede (Kebede, 1997) tested the growth of isolated strains of *A. fusiformis* from Lake Chitu, Ethiopia in laboratory experiments over a wide range of salinities (using additions of 13–88 g L^{-1} $NaHCO_3$, NaCl or Na_2SO_4). The specific growth rate declined with increasing salinity, but growth did not stop. A wide salinity tolerance is also supported by our own observations. Lake Bogoria with high salinities up to 47 was always dominated by *Arthrospira* blooms, similar to L. Nakuru at Cormorant Point (Figure 2). There, rapid changes in salinity can occur due to inflow from the Njoro River. At the Baboon Cliff area of L. Nakuru and at Lake Elmenteita with their more stable conditions and lower salinities compared with L. Bogoria, a more variable cyanobacterial community was observed.

Besides salinity, other factors may influence the dominance of the main cyanobacterial species. Low Secchi depths (<0.3 m) in all three lakes indicate narrow euphotic zones. The low transparency is caused by the high population density of the phytoplankton and the suspended inorganic material. This material may result from river inflow and sediment disturbance by wading flamingos or through wind action. *A. fusiformis*, *A. abijatae* and *A. arnoldii* possess gas vacuoles, which enable them to form freefloating colonies (Jeeji-Bai *et al.*, 1977; Kebede and Willén, 1996; Tomaselli, 1997). The ability to float and to control vertical location in the water column is an important factor in very turbid water (Walsby, 1994).

Temperature and nutrient loading have also been considered as important environmental factors that influence cyanobacterial dominance (Paerl, 1996). The growth physiology and rates of bloom-forming cyanobacteria, including potentially toxigenic members, are optimal around 25°C or higher (Robarts and Zohary, 1987). From laboratory cultivation of *Arthrospira* it is known that the optimal temperature is in the range of 35–38°C (Vonshak, 1997). The water temperatures measured (25.8–33.7°C) in L. Bogoria, (17.1–32.1°C) in L. Nakuru, and (22.5–32.3°C) in L. Elmenteita are near this optimal range for *Arthrospira*. Vareschi (Vareschi, 1982) and

Melack (Melack, 1988) have described similar water temperatures for L. Nakuru and L. Elmenteita.

Cyanobacterial water blooms are associated worldwide with high nutrient concentrations and eutrophication (Paerl, 1996; Oliver and Ganf, 2000). The high mean TP concentrations (0.07–0.48 mM) and the high mean TN concentrations (0.2–0.4 mM) indicate the hypertrophic status of all three lakes (OECD, 1982). Similar TP concentrations were measured by Talling and Talling (Talling and Talling, 1965) and Vareschi (Vareschi, 1982) in L. Nakuru and L. Elmenteita. Several sources of nutrients exist. At the shore of L. Bogoria numerous hot springs and geysers are active (Cioni *et al.*, 1992). We found a TP content of 0.65 μM in one of the hot spring effluents during the present study. At L. Elmenteita the hot springs are used for the personal hygiene of the local population causing a further input of nutrients into the lake (personal observations). At L. Nakuru, the main source of nitrogen and phosphorus is the discharge of effluents from the Nakuru town sewage treatment plant. In November 2001 high concentrations of TP (0.16 mM) and TN (0.43 mM) occurred in the effluent from the sewage treatment plant. Vareschi (Vareschi, 1982) reported a mean concentration of TP for L. Nakuru of 0.32 mM. Inappropriate agricultural practices and rapid losses of woodland and forests due to increased human settlement has led to an increase in soil erosion and nutrient loads in the catchment areas of the lakes (Mwaura and Moore, 1991; Shivoga, 2001). The high nutrient levels in the lakes can also be explained by the lack of an outflow and the resulting accumulation of nutrients. Especially, a low TN:TP ratio below 29 has been suggested as a major factor favouring cyanobacterial dominance (Smith, 1983). In our study the TN:TP ratio was always below 4. In contrast, other studies have found little evidence that low TN:TP ratios support cyanobacterial dominance (Jensen *et al.*, 1994, Scheffer *et al.*, 1997). According to Reynolds (Reynolds, 1992) the TN:TP ratio is insignificant if the nutrient concentrations exceed those limiting cyanobacterial growth, which is most likely the case in all three investigated lakes.

Cyanobacterial toxins

Our investigation revealed that the cyanobacterial communities in Lakes Bogoria and Nakuru produce microcystins and anatoxin-a. Freshwater, brackish and marine waters worldwide are known for cyanobacterial bloom and toxin production (Sivonen, 1996; Codd *et al.*, 1999). To our knowledge, this is the first evidence of microcystins and anatoxin-a in L. Bogoria and L. Nakuru. Although the cyanobacterial communities in L. Elmenteita and L. Nakuru show taxonomic similarities, it is not clear without further research why the toxins were not detected in samples from L. Elmenteita.

Two microcystin variants (MC-LR and -RR) were found in variable amounts in L. Bogoria and five variants found in L. Nakuru (MC-LR, -RR, -YR, -LA and -LF). Worldwide more than 60 structural variants of microcystins have been isolated (Codd *et al.*, 1999; Sivonen and Jones, 1999). In field samples microcystin concentrations ranged from 0.02 to 14 700 μg g^{-1} DW (Fastner *et al.*, 2001). The microcystin-LR equivalents of up to 155 μg g^{-1} DW in L. Bogoria samples and up to 4600 μg g^{-1} DW in those from L. Nakuru are substantial and could present acute health risks if such material was ingested e.g. by wildlife. Microcystins are mainly reported from blooms of *Microcystis*, *Anabaena*, *Oscillatoria* and *Planktothrix* (Sivonen, 1996; Codd *et al.*, 1999).

The anatoxin-a concentrations detected in L. Bogoria (up to 9 μg g^{-1} DW) and L. Nakuru (up to 223 μg g^{-1} DW) show a similar range to those reported worldwide. Anatoxin-a is mainly reported from blooms of *Anabaena*, *Oscillatora*, *Phormidium*, and with rarer associations with *Cylindrospermum* and *Aphanizomenon* (Sivonen, 1996; Codd *et al.*, 1999).

A. fusiformis was identified as one source of microcystins and anatoxin-a in L. Bogoria and L. Nakuru. In a cultured strain of *A. fusiformis* from L. Bogoria we detected microcystin-YR and anatoxin-a, and anatoxin-a in a strain from L. Nakuru (Table III). These are important findings, because the genus *Arthrospira/Spirulina* is regarded as non-toxic (Ciferri, 1983; Jassby, 1988). Several members of this genus are widely used in mass culture as a source of food, animal feed, and specific chemicals in subtropical and tropical countries (Vonshak, 1987, 1997). Toxicity studies in mice with *Spirulina maxima* from Mexico did not show any toxic effect (Salazar *et al.*, 1998). However, Iwasa *et al.* (Iwasa *et al.*, 2002) have related human liver injury in a 52 year old Japanese person to the intake of *Spirulina*, thus indicating a possible *Spirulina*-associated hepatotoxicity. Analysis of commercial *Spirulina*-based human dietary supplements (tablets, capsules) for microcystins by a combined HPLC–ELISA method gave positive results (0.15–2.12 μg g^{-1} DW) (Gilroy *et al.*, 2000). Whether these commercial products contained cyanobacterial cells/cell products in addition to those of *Spirulina* was not investigated. A toxic strain of *A. fusiformis* producing microcystin-YR and anatoxin-a was also isolated from L. Sonachi, Kenya (Ballot *et al.*, 2004). However, whether the *Arthrospira/Spirulina* assemblages in L. Bogoria and Nakuru are major or minor producers of the microcystins and anatoxin-a detected is undetermined.

A. abijatae and *A. arnoldii* may also be sources for the production of cyanobacterial toxins. In cultures of

A. abijatae from L. Nakuru and L. Elmenteita investigated in this study, no microcystins and anatoxin-a were found (Table III). Lanaras and Cook (Lanaras and Cook, 1994) have reported microcystin in bloom material from Lake Porto Lagos, Greece. The bloom was not monocyanobacterial, but was dominated by *Anabaenopsis milleri* Voronichin, raising the possibility that members of the genus *Anabaenopsis* may produce microcystins.

Another potential source of the cyanobacterial toxins in L. Bogoria could be the inflow of hot spring water carrying toxic cyanobacteria into the lake. Krienitz *et al.* (Krienitz *et al.*, 2003) found microcystins (up to 835 μg g^{-1} DW) and anatoxin-a (up to 18 μg g^{-1} DW) in mats of hot spring cyanobacteria at this location. These mats are mainly formed by *P. terebriformis*, *Oscillatoria willei* Gardner, *S. subsalsa* and *Synechococcus* spp. (Hindák, 2001; Krienitz *et al.*, 2003). Parts of the mats may become detached, both naturally and due to disturbance and incidental grazing by flamingos during drinking, and be washed into the lake. Small quantities of hot spring cyanobacteria were observed in the phytoplankton community of L. Bogoria, never exceeding 0.0002 g DW L^{-1} or 3% of the total biomass. The calculated fraction of microcystins due to the hot spring cyanobacteria is at maximum 11.5% and in the case of anatoxin-a less than 1% of the same toxins found in the lake. In L. Nakuru, small amounts of *Phormidium* sp., *Oscillatoria* sp., *S. subsalsa* and *Synechococcus* sp. were also detected. Microcystins and anatoxin-a have been identified in cyanobacterial mat samples consisting of *Oscillatoria* sp. and *Phormidium* sp. (Edwards *et al.*, 1992; Mez *et al.*, 1997). However, in this study with cultivated *Phormidium* and *S. subsalsa* strains from L. Nakuru, no cyanobacterial toxins were found (Table III).

Cyanotoxins can have significant ecological impacts on aquatic food webs in lakes and cause serious health problems (Christoffersen, 1996; Carmichael, 1997; Codd *et al.*, 1999). Krienitz *et al.* (Krienitz *et al.*, 2003) described ways by which Lesser Flamingos at L. Bogoria ingested microcystins and anatoxin-a from hot spring cyanobacterial mats. The microcystins and anatoxin-a produced by the phytoplanktonic cyanobacterial community in L. Bogoria and L. Nakuru may have a further impact on the flamingo populations at both lakes. The daily feeding rate of an adult bird is about 72 g DW of cyanobacteria (Vareschi, 1978). At L. Bogoria, their potential daily ingestion of microcystins can therefore be estimated at between 1150 and 11 160 μg and of anatoxin-a between 22 and 670 μg. For L. Nakuru, the potential daily intake, based on the samples analysed, could be higher: between 9350 and 330 700 μg microcystins and 370–16 060 μg anatoxin-a. Although no bird LD_{50} values for orally applied microcystins exist, calculations based on the LD_{50} for MC-LR in mice [5000–10 900 μg kg^{-1} (Fawell *et al.*, 1994; Yoshida *et al.*, 1997)] estimate the quantity of toxic cyanobacteria consumed in L. Bogoria by a 2 kg body weight Lesser Flamingo which shows an acute lethal effect to be between 127 and 1920 g DW. This is around 1.8–27 times the daily feeding rate of 72 g for an adult Lesser Flamingo (Vareschi, 1978). Also, as other microcystins were found in the phytoplankton samples, the necessary amount of cyanobacteria causing a lethal effect is probably much lower. In L. Nakuru the flamingos would have to ingest between 7.4 and 874 g cyanobacteria to show lethal effects when only considering MC-LR.

The mouse oral LD_{50} for anatoxin-a is between 5000 and 10 000 μg kg^{-1} body weight (Fitzgeorge *et al.*, 1994). Similarly, as there are no LD_{50} data for flamingos, mouse toxicity data were used for calculations. To show acute toxic effects at L. Bogoria a Lesser Flamingo (2 kg body weight) would have to ingest between 1075 and 66 700 g of cyanobacterial DW, at L. Nakuru between 45 and 4000 g of cyanobacterial DW which is around 15–926 times and 0.6–56 times the daily ingestion rate of 72 g DW at L. Bogoria and L. Nakuru, respectively. Chronic health effects are to be expected at lower amounts for repeated ingestion of toxic cyanobacterial cells. Investigations of livers taken from dead Lesser Flamingos at L. Bogoria and L. Nakuru revealed microcystin concentrations between 0.21 and 0.93 μg MC-LR equ. g^{-1} fresh wt and anatoxin-a concentrations between 1.06 and 5.82 μg g^{-1} fresh wt (Ballot *et al.*, 2002).

The high amounts of cyanotoxins in the flamingo feed and livers therefore imply that cyanotoxins ingested with the daily food can have chronic effects on health and contribute to the mass mortalities of Lesser Flamingos in the Kenyan Rift Valley.

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