Seasonal development of cyanobacteria and microcystin production in Ugandan freshwater lakes

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Abstract: This study pioneered investigation on seasonal development of phytoplankton and potential microcystin (MC)-producing cyanobacteria and MC concentrations in freshwater lakes in Uganda. During one year (May 2007-April 2008) chemical and physical characteristics, the phytoplankton composition and the MC concentrations were recorded monthly in a hypertrophic crater lake (Lake Saka), in shallow eutrophic lakes (Lakes Mburo, George, Edward), and in Lake Victoria (Murchison Bay, Napoleon Gulf). Throughout the study period, cyanobacteria (composed of the genera *Anabaena*, *Aphanocapsa*, *Chroococcus*, *Cylindrospermopsis*, *Microcystis*, *Planktolyngbya*, *Planktothrix*) dominated and always contributed >50% to total phytoplankton biovolume. All samples from all sampling sites were found to contain MC. Samples from Lake Saka had the maximum MC concentration (10 µg L^{-1}) in July 2007. The minimum concentration (0.02 µg L^{-1}) was recorded in Lake George (Kahendero) in the months of May 07, June 07, January 08 and April 08. At the sampling sites in the other three lakes intermediate MC concentrations (0.1 - 2.5 µg MC-LR eq. L^{-1}) were recorded. For all sampling sites highly significant positive linear relationships between the total MC concentration and *Microcystis* cell numbers were obtained. Relating the total MC concentrations to *Microcystis* cells revealed a >100-fold variation in the average MC contents per cell between lakes. While *Microcystis* from Lake George showed the lowest MC cell quotas (0.03-1.24 fg $cell^{-1}$) *Microcystis* from Lake Saka consistently showed maximum MC cell contents (14-144 fg $cell^{-1}$). It is concluded that at all sites MC production is due to the occurrence of *Microcystis*, however between sites the populations differ consistently and independently of the season in their average MC content per cell.

Keywords: Blue-green algae, Cyanotoxins, *Microcystis*, Phytoplankton, Water monitoring

1. Introduction

During the last decades cyanobacteria in freshwater have been of general awareness due to their ability to produce various hepatotoxic and neurotoxic substances. It is generally agreed that the hepatotoxic microcystin (MCs) are probably the most abundant toxins produced by cyanobacteria in freshwater (1). While the number of taxa that has been found to produce MCs is constantly increasing, the MC-producing genera that are of major importance in phytoplankton have already been identified during the nineties: *Anabaena*, *Microcystis*, and *Planktothrix* (2, 3).

In a recent paper, we could show that cyanobacteria contribute significantly to the phytoplankton of freshwater lakes in Uganda while other algal groups like diatoms, green algae, and cryptomonads are of a relatively minor importance (4). We further concluded that in Uganda the genus *Microcystis* is favoured under more shallow, eutrophic conditions which is in correspondence to the general theory on how abiotic and biotic factors govern phytoplankton associations that have been defined since 1984 (5). In contrast to *Microcystis* other genera known to produce MCs were found in lower abundance, e.g. *Anabaena* was abundant in Lake Victoria and one of the Crater Lakes while *Planktothrix* was not observed.

However, in this earlier study we were unable to monitor the phytoplankton community during different seasons. This issue is of relevance as usually dry seasons with precipitation minima and wet seasons with maximum precipitation have been correlated with changes in phytoplankton composition. Usually precipitation varies annually from 30 mm to 132 mm in Kasese, western Uganda and 60 mm to 184 mm in

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Kampala, the L. Victoria basin (Meteorological Department, Entebbe). This also results in annual changes of water temperature by 5°C in Kasese and 3°C in Kampala. The highest air temperatures are usually recorded during the dry season in February (33°C in Kasese and 29°C in Kampala). During the rainy season (from March to May and August to November), the phytoplankton in shallow lakes will be affected directly by a reduced air temperature (2.5°C in Kasese and 3°C in Kampala), reduced light availability in the water column as well as increased terrestrial run-off. In deeper lakes such as Lake Victoria, the mixing regime will change, as a higher stability of the water column has been described during the dry season June to July (6). These physical changes have a significant effect on phytoplankton community composition. For example, (7) reported highest cell densities of *Anabaena* in Lake Victoria (Mwanza Gulf) in the dry period from November - January when the water was calm and *Anabaena* accumulated on the surface. In Lake Victoria, typically after the first algal maximum that occurred between July and August and that is composed of diatoms, the second and broader algal maximum occurs between November and January and it is chiefly composed of cyanobacteria (8). In contrast, in shallow lakes much less seasonality in phytoplankton composition is observed.

This study investigated the seasonal development of phytoplankton and potential MC-producing cyanobacteria and MC concentration in five lakes in Uganda. During one year (May 2007-April 2008), chemical and physical characteristics, the phytoplankton composition and the MC concentrations were recorded. The data on phytoplankton and MC concentrations are of importance towards creating awareness and guidance for designing water safety plans for Uganda to achieve the UN millennium development goals.

2. Materials and methods

2.1 Description of the study sites

Six sites were selected from five freshwater lakes of varying limnological characteristics (Fig. 1): 1) Station in the middle of L. Saka (N0°41.670', E30°14.667'), mean depth of 3.6 m. L. Saka is a small crater lake (1.4 km^2) at an altitude of 1520 m. 2) Lake George (228.5 km^2) part of the western rift valley at an altitude of 914 m, Kahendero (N0°03.004', E30°03.439'), mean depth of 1.8 m. This site was hydrologically separated from the main basin of the lake due to the formation of three large islands. 3) Lake Edward-Katwe (0°09.198'S, 29°53.056'E) which is a fish landing site (mean depth of 3.2 m). Originally Katwe was a salt mining area. It is located in a bay at the northeastern shoreline where the Kazinga channel and the river Nyamugasani are inflowing (close to Mweya Lodge in Queen Elisabeth National Park). 4) Lake Mburo, in the centre of the lake (S0°38.513', E30°56.869'), mean depth of 2.8 m. L. Mburo inhabits hippopotami and other wildlife. The site was selected in order to keep the influence from the animals and the inflowing river Rinzi to a minimum

Two sites were selected from the northern part of L. Victoria: 5) Murchison Bay (N0°15.461', E32°38.481'), Kampala, mean depth of 5.5 m. 6) Napoleon Gulf (N0°24.177', E33°14.756'), Jinja, mean depth of 17.8 m, it is also close to the Source of River Nile. Both sites were located inshore and considered pilot zones for the Lake Victoria Environmental Management Project (LVEMP).

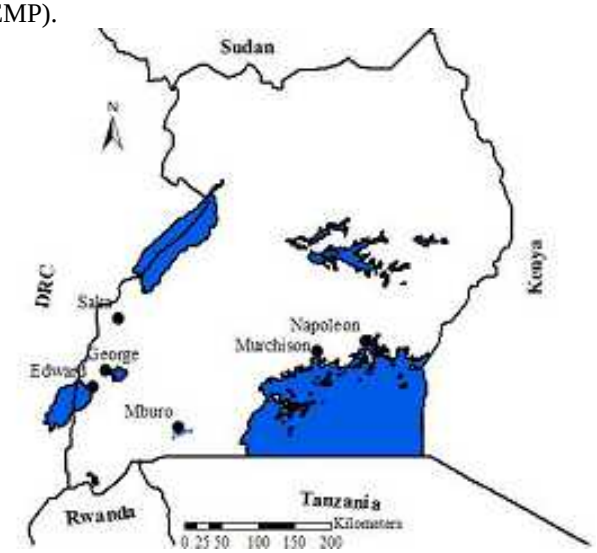

Fig. 1 Map of Uganda showing the six sampling sites (black circles)

2.2 Sampling and limnological characteristics

Depth integrated water samples were taken monthly from May 2007 until April 2008 (Fig. 1). A two-litre horizontal van Dorn sampler was used for sampling the water column at a one-meter interval. Samples were filtered using 47mm diameter GF/C filters (Whatman, Kent, Great Britain). The filters were stored frozen for the analysis of chlorophyll a. Filtrates were

again filtered through 47mm diameter membrane filters (0.45 µm, Millipore Corporation, Bellerica, United States) for the analyses of dissolved nutrients: soluble reactive phosphorus (SRP), nitrate (NO_3-N) and ammonia (NH_4-N). These were determined using the ammonium molybdate method (9), the sodium-salycilate method (10) and the indophenol blue method (11), respectively. Total phosphorus (TP) was determined as SRP subsequent to persulphate digestion from aliquots of the samples.

2.3 Phytoplankton community and abundance

Chlorophyll a analysis was based on hot ethanol extraction (12). Algae were counted by the inverted microscope technique from Lugol fixed samples as described in (13). Cyanobacteria were identified according to the descriptions by (14-16). For each sample 400 specimen of the dominant phytoplankton genera were counted at 400-fold magnification. Most of the genera were counted as single cells (cyanobacteria: *Aphanocapsa, Anabaena, Chroococcus, Merismopedia, Microcystis*; diatoms: *Nitzschia* and unidentified centric diatoms, green algae, desmids, cryptomonads). Filamentous cyanobacteria were counted as single filaments *(Aphanizomenon, Cylindrospermopsis, Planktolyngbya*, and *Planktothrix*).

In general the genera *Aphanizomenon* and *Cylindrospermopsis* were reliably identified by the formation of solitary filaments composed of elongated cells showing clear constrictions at the attaching cell walls. In contrast to *Aphanizomenon* containing heterocytes located between the cells the genus *Cylindrospermopsis* only had long and conical terminal heterocytes. *Planktolyngbya* could be undoubtedly discriminated by single filaments of lowest diameter (mean±1SE 2.4±0.03 µm), composed of tightly attached cells and embedded in a colourless sheath. In contrast *Planktothrix* had much wider straight filaments without a visible sheath and no constrictions at the tightly attached cells (17). From each genus cells/filaments dimensions from ten specimens were measured to calculate the biovolume according to standard geometric shapes (9, 18).

2.4 Microcystin analysis

A volume of 250 to 2400 mL of integrated water samples depending on the algal concentration were filtered using 47mm GF/C filters and the filters were dried at 50°C. Phytoplankton collected on filter was extracted in 1.5 mL of 50% methanol (v/v) and MCs were extracted following (19). The mixture was agitated for 60 minutes at 250rpm and centrifuged at 15000 × *g* for another 10 minutes. Clear supernatants were pipetted into new reaction tubes and the extraction procedure was repeated twice. Extracts were concentrated to dryness at 30°C using a vacuum centrifuge (Eppendorf AG, Hamburg, Germany). The residues were resuspended in 350 µL of 50% methanol mixed, centrifuged at 15000 × *g* for 10 minutes and the clear supernatants were analysed by high performance liquid chromatography-diode array detection (HPLC-DAD) on a LiChrosper 100, octydecyl silane, 5-µm LiChroCART 250-4 cartridge column (Merck, Darmstadt, Germany). A linear gradient of aqueous acetonitrile containing 0.05% trifluoroacetic acid (TFA) was used at a flow rate of 1 mL^{-1} increasing from 30% acetonitrile to 70% acetonitrile in 42 minutes (20). MCs were quantified at 240 nm and the concentration of all MC variants was determined as concentration equivalents of [D-MeAsp, D-Mdha]-MC-LR (Cyanobiotech GmbH, Berlin, Germany). The concentration of MC-LR was calculated from the regression curve y = 1885.3x – 6.8775, (R^2=0.99), where y was ng of MC-LR and x was mAU recorded at 240 nm.

HPLC fractions identified as MC were collected manually and concentrated to dryness. The collected fractions were re-dissolved in 10 µL of 100% methanol. 1 µL of each sample was then loaded onto a steel template (CellPath, Newtown Powys, Great Britain) and 1 µL of matrix solution (2,5-dihydroxy-benzoic acid (DHB) solublized in 50% acetonitrile, 0.03 % TFA) was loaded immediately and the mixture left to dry. Samples were then analysed by matrix-assisted laser desorption/ionisation voyager elite time-of-flight mass spectrometry (MALDI-TOF), (PerSeptive BioSystems, Framingham MS, USA) as described in (21, 22). Identification of particular MCs was by the post source decay (PSD) fragment analysis as described in (19, 23).

3. Results

3.1 Limnological characteristics

The limnological parameters characterising either eutrophic or hypertrophic state varied significantly among the six sampling sites (Table 1). Averaged over the study period TP concentrations varied fourfold and chlorophyll a concentrations varied tenfold between the lakes ($p<0.0001$, Kruskal Wallis One Way ANOVA on Ranks). Lake Saka, the only Crater Lake sampled in this study had the highest TP (193 ± 15.3 (mean ± 1SE) and chlorophyll a concentration (192 ± 17.6) resulting in low transparency (0.3 ± 0). Surprisingly the relatively low pH 7.9 was not in accordance with the highest primary production. Lakes George (Kahendero), Edward (Katwe) and Mburo also showed high TP concentrations, 117.3 ± 15.2, 110.9 ± 19 and 101.7± 13.3, respectively that accordingly resulted in high chlorophyll a concentrations (70.3 - 94.9 µg L^{-1}) characteristic of a hypertrophic state with pH 8.5 - 10.4. The two sampling sites in Lake Victoria (Murchison Bay and Napoleon Gulf) showed the lowest TP concentrations resulting in relatively low chlorophyll a concentrations, highest transparency and intermediate pH 7.8 - 9.9.

3.2 Cyanobacterial composition

In total 36 genera of phytoplankton were identified that belonged to six classes (cryptomonads, cyanobacteria, diatoms, dinoflagellates, euglenoids and green algae). The total phytoplankton community composition will be described in detail elsewhere. Only four classes (cryptomonads, cyanobacteria, diatoms and green algae) contributed to ≥ 5% of total biovolume (Fig. 2). The total biovolume ranged from 83 mm^3 L^{-1} in Lake Saka, 433 mm^3 L^{-1} in Lake George (Kahendero) to 47 mm^3 L^{-1} in Lake Victoria (Murchison Bay). The lowest biovolume would have been recorded in Lake Victoria (Napoleon Gulf) at an average of 5 mm^3 L^{-1} in July 2007. However in June 2007 extremely high cell numbers of *Anabaena* were recorded. In summary, cyanobacteria dominated during the whole study period and always contributed >50% to phytoplankton biovolume.

Cyanobacterial biovolume ranged from 427 mm^3 L^{-1} (maximum) in Lake George (Kahendero) to 3.2 mm^3 L^{-1} (minimum) in Lake Victoria (Napoleon Gulf). Seven cyanobacterial genera were identified each contributing to ≥ 5% of total cyanobacterial biovolume (Fig. 3). However, not all the genera were present in all the samples. In samples from lakes George (Kahendero), Edward (Katwe) and Mburo, filamentous cyanobacteria (*Planktolyngbya*) dominated and co-occurred with *Anabaena*, *Aphanocapsa*, *Chroococcus* and *Microcystis*. The genus *Cylindrospermopsis* was abundant and contributed to the total biovolume of cyanobacteria in Lake Mburo. In contrast to lakes George (Kahendero), Edward (Katwe) and Mburo, *Planktothrix* contributed 24 - 92% to the cyanobacterial biovolume in Lake Saka. In contrast to *Cylindrospermopsis* and *Planktothrix*, *Microcystis* appeared throughout the sampling period in all the lakes. *Anabaena* occurred consistently in Lake Victoria (Murchison and Napoleon) ranging in biovolume from 1.0 $mm^3 L^{-1}$ to 50 $mm^3 L^{-1}$. We recorded the highest *Microcystis* biovolume in samples from Lake George (Kahendero) (273 $mm^3 L^{-1}$) and the lowest (0.3 $mm^3 L^{-1}$) in samples from Lake Victoria (Napoleon).

In summary, three genera of potentially microcystin-producing cyanobacteria (*Anabaena*, *Microcystis* and *Planktothrix*) were identified. This indicated that the phytoplankton in all the five lakes had the potential for microcystin production (Table 2).

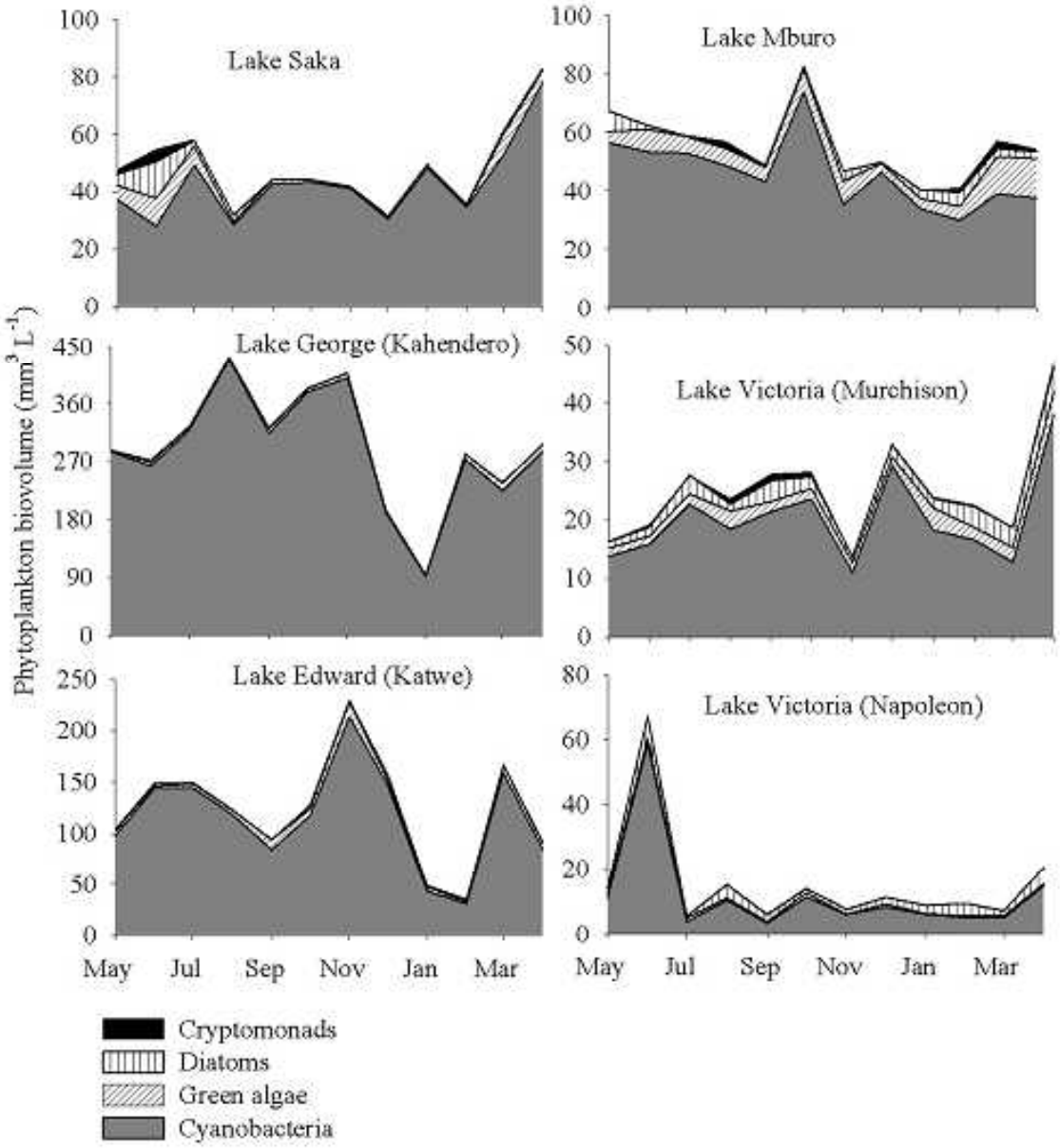

Fig. 2 Phytoplankton composition at the six sampling sites from May 2007 to April 2008. Note that the scales on the y-axis differ between sites

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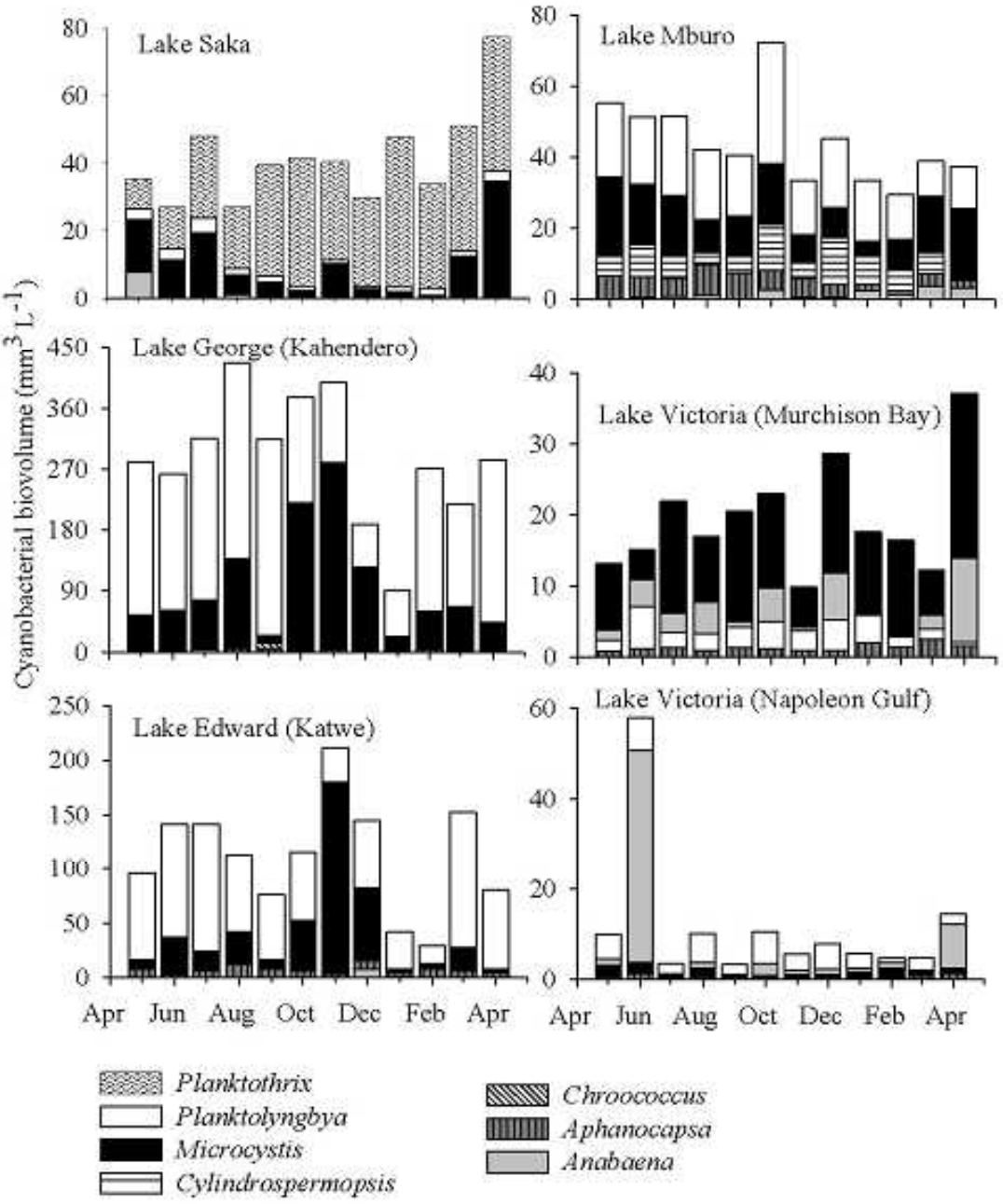

Fig. 3 Cyanobacteria composition at the six sampling sites from May 2007 to April 2008. Note that the scales on the y-axis differ between sites

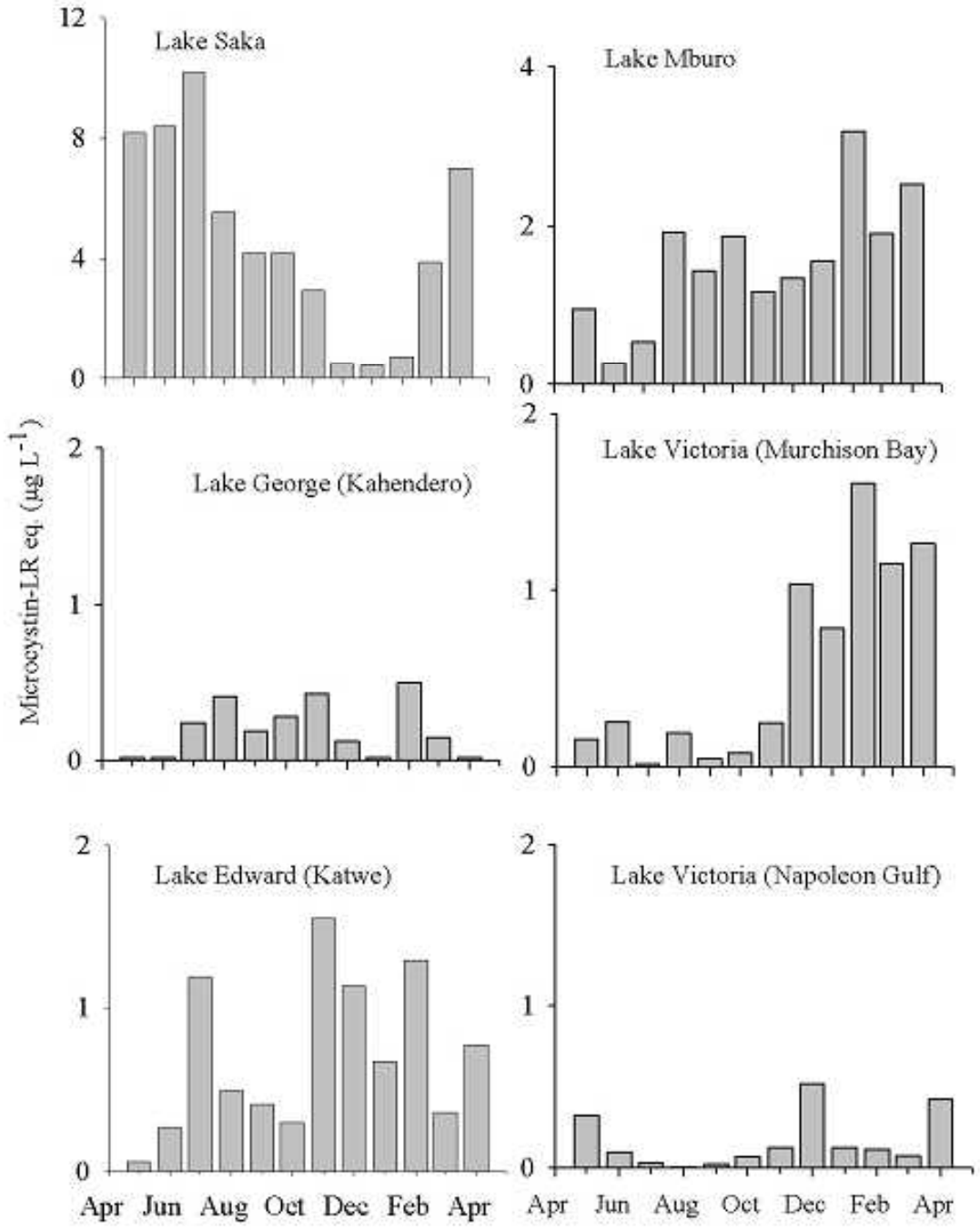

Fig. 4 Microcystin concentrations (µg MC-LR eq.) at the six sampling sites from May 2007 to April 2008. Note that the scales on the y-axis differ between sites.

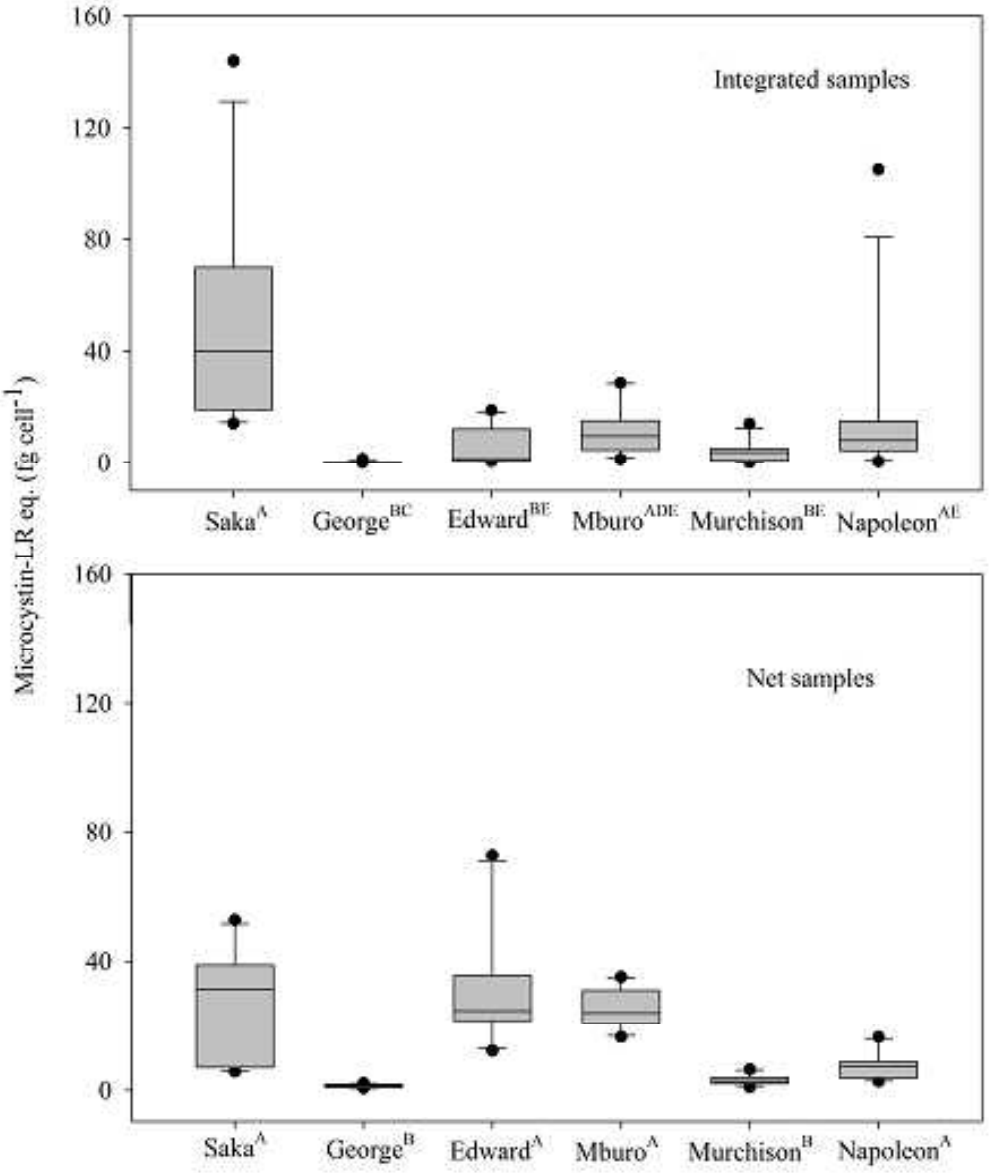

Fig. 5 MC cell quotas of *Microcystis* sp. at the six sites from May 2007 to April 2008. The lower and upper borders of each box indicate the 25th and 75th percentiles; the line inside the box represents the median; the whiskers indicate the 10th and 90th percentiles and the black dots are the outliers. N = 12 per sampling site for both net and integrated samples. Superscripts indicate homogeneous subsets ($p < 0.05$) after testing the overall significance differences (Kruskal Wallis, One Way ANOVA)

Tab. 1 Limnological characteristics (min-mean±SE-max) during the sampling period, May 2007 to April 2008. For all characteristics, sample size = 12

	Secchi depth (m)	Chlorophyll-a (μgL^{-1})	pH	Temperature (°C)	Total Phosphorus (μgL^{-1})	SRP (μgL^{-1})	Nitrate (μgL^{-1})	Ammonia (μgL^{-1})
	min-mean±SE-max	min-mean±SE-max	min-mean±SE-max	min-mean±SE-max	min-mean±SE-max	min-mean±SE-max	min-mean±SE-max	min-mean±SE-max
Saka	0.2-0.3±0-0.5	86.6-192±17.6-273	7.6-7.9±0.1-8.7	20.7-22.4±0.4-24.4	99-193.0±15.3-301	2.0-10.0±2.8-33.0	11.1-65.4±11.3-113	0.0-17.1±4.9-51.0
George	0.2-0.2±0-0.4	30.0-94.9±1.8-177	9.8-10.4±0.1-10.9	24.3-26.8±0.3-29.3	52-117.3±15.2-243	2.0-6.8±1.3-16.0	11.1-86.4±12.0-167	3.0-11.3±1.6-19.0
Edward	0.3-0.4±0-0.6	36.5-70.3±7.1-122	9.7-9.9±0-10.2	25.4-27.2±1.2-29.0	60-110.9±19.0-293	2.0-8.3±3.3- 41.0	11.1-65.5±9.3-122	0.0-8.6±2.1-26.0
Mburo	0.3-0.4±0-0.5	31.3-77.9±8.3-129	7.5-8.5±0.2-9.5	24.2-23.8±1.2-26.2	36-101.7±13.3-206	1.0-7.1±1.4 -16.0	44.4-94.7±9.8-160	1.0-15.5±5.2-69.0
Murch†	0.5-0.6±0-0.7	30.0-64.5±5.3-98.3	7.8-8.7±0.2-9.6	24.3-25.8±1.0-27.4	35-87.2±11.8-172	0.0-5.6±0.0 - 14.0	0.0-78.6±24.4-325	0.0-21.8±7.0-79.0
Napo†	1.2-1.4±0-1.6	7.8-19.4±3.4-47.0	8.8-9.5±0.1-9.9	25.5-26.7±0.7-28.0	30-49.3±5.5-90	2.0 - 7.7±1.7 - 21.0	0.0-33.7±15.7-175	2.0-12.3±2.0-24.0

Min = Minimum; Max = Maximum; SRP = Soluble reactive phosphorus; SE = Standard error; Murch† = Murchison Bay, L. Victoria; Napo† = Napoleon Gulf, L. Victoria.

Tab. 2 Proportion (percentage) of the possible microcystin producing cyanobacteria in the five lakes from May 2007 to April 2008. Sample size = 12.

	Anabaena	*Microcystis*	*Planktothrix*
	Min- Mean ± SE-Max	Min-Mean ± SE-Max	Min-Mean ± SE-Max
Lake Saka	0.0-0.8 ± 0.6-7.7	2.3-18.3 ± 4.0-39.5	61-82 ± 4-98
Lake George (Kahendero)	0.0-0.8 ± 0.4-5.2	94.8-99.2 ± 0.4-100	nd
Lake Edward (Katwe)	0.0-2.6 ± 1.1-10.4	89.6-97.4 ± 1.1-100	nd
Lake Mburo	0.0-3.8 ± 0.9-9.3	52.9-64.3 ± 1.8-74.9	nd
Lake Victoria (Murchison)	15.0-27.1 ± 2.0-42.0	742.0-2.9 ± 2.0-85.0	nd
Lake Victoria (Napoleon)	53.4-66.5 ± 3.6-93.2	93.2-33.5 ± 3.6-46.6	nd

nd = not detected.

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Tab. 3 Microcystin variants retention time, their percentage composition (in parentheses) and frequency of occurrence underneath for each site from May 2007 to April 2008. Number of samples per site = 24.

	MC1	MC2	MC3	MC4	MC5	MC6	MC7	MC8	MC9	MC10	MC11
M+H	1024	1038	1063	1031	1045	995	nd	1031	1045	1024	1031
	[Asp^3]MC-RR	MC-RR	Unknown	[Asp^3]MC-YR	MC-YR	MC-LR		[Asp^3]MC-RY	MC-RY	Unknown	Unknown
Saka	14.0-14.5	15.2-15.5	16.0	17.7	18.0-18.9	19.0-19.9		21.0-21.7	23.1-23.9	24.7	
	(0.8±0.6)	(58.7±4.1)	(3.2±2)	(0.4±0.2)	(17.3±3.5)	(4.4±1.3)		(8.0±2.8)	(7.0±1.3)	(0.1±0.07)	
	3.7	22.4	2.8	4.7	15.0	14.0		15-0	18.7	3.7	
George		15.4						21.7-21.8	23.1-23.4		
		(1.6±1.6)						(12.0±4.4)	(78.0±6.6)		
		3.2						25.8	71.0		
Edward	14.1-14.3	15.1-15.4		17.0	18.6	19.9		21.6-21.8	23.0-23.2	24.0-25.0	27.8-27.9
	(0.6±0.2)	(8.3±1.7)		(0.3±0.1)	(1.8±0.5)	(0.03±0)		(5.9±4.3)	(81.1±4.5)	(1.0±0.2)	(0.3±0.1)
	7.2	19.3		10.8	13.3	1.2		3.6	27.7	7.2	9.6
Mburo	14.0-14.4	15.2-15.5		17.3-17.5	18.3-18.7	19.0-19.9	20.9	21.6-21.7	23.1-23.2	24.0-24.2	27.9
	(2.6±0.9)	(46.9±2.8)		(0.8±0.2)	(12.9±1.5)	(1.6±0.4)	(1.8±0.4)	(2.2±0.5)	(27.7±3.5)	(2.6±0.4)	(0.4±0.1)
	7.9	12.9		7.3	12.4	7.9	9.0	10.7	13.5	11.2	7.3
Murch†		15.1-15.4	16.3-16.4		18.0	19.3-19.9			23.0-23.5	24.3-24.4	
		(3.9±1.9)	(7.8±3.0)		(2.9±1.4)	(12.4±2)			(48.8±6.3)	(6.7±1.7)	
		18.1	8.4		7.2	22.9			25.3	18.1	
Napo†		15.3-15.7	16.3-16.4		18.0-18.8	19.2-19.8		21.1	23.3	24.6	
		(3.9±1.9)	(82.1±6.9)		(4.7±2.4)	(0.5±0.3)		(0.5±0.5)	(2.1±2.1)	(2±2)	
		11.8	61.8		11.8	5.9		2.9	2.9	2.9	
RT	14.0-14.5	15.1-15.7	16.0-16.4	17.0-17.7	18.0-18.9	19.0-19.9	20.9	21.0-21.8	23.0-23.6	24.0-24.9	27.8-27.9
	(0.7±0.2)	(23.0±2.1)	(15.5±2.8)	(0.3±0.1)	(6.6±0.9)	(3.3±0.6)	(0.3±0.09)	(4.8±1.2)	(40.5±3.2)	(2±0.5)	(0.1±0.02)
	4.7	16.1	6.0	5.2	11.4	9.7	3.3	9.1	21.5	8.9	4.1

M+H = molecular weight; nd = molecular weight not determined; MC = microcystin variant; RT = retention time in minutes; Murch† = Murchison; Napo† = Napoleon.

3.3 Microcystin net production

All samples from all sampling sites were found to contain MC. HPLC-DAD analyses documented the occurrence of eleven MC structural variants that showed an unequivocal match with the spectrum of MC-RR, MC-YR and MC-LR as available from the spectrum library. All fractions identified as MC by HPLC were collected and analysed by means of MALDI-TOF MS for their molecular weight (24). The following variants could be undoubtedly identified by their retention time, their mass and by spiking using MC-RR, YR, LR standards: MC1, [Asp^3]-MC-RR (M+H 1024), MC2, MC-RR (M+H 1038), MC4, [Asp^3]-MC-YR (M+H 1031), MC5, MC-YR (M+H 1045), MC6, MC-LR (M+H 995), MC8, [Asp^3]-MC-RY (M+H 1031), MC9, MC-RY (M+H 1045). [Asp^3]-MC-RY and MC-RY have been identified by LC-MS^2 recently (5). The following MC variants were considered unknown: MC3 (M+H 1063), MC7, MC10 (M+H 1024), MC11 (M+H 1031), (Table 3).

The sampling sites differed significantly in the relative abundance of all the MC variants (Chi square test, $p<0.01$). For example MC-RR and [Asp^3]-MC-RR were most frequent in L. Saka, L. Mburo and L. Edward and in Murchison Bay. In contrast MC-RY and [Asp^3]-RY were dominant at all sites except Napoleon Gulf. (Table 4). Surprisingly in the samples from Napoleon Gulf an unknown MC3 (M+H 1063) variant occurred most frequently. Taking all sampling sites together MC-RY was most frequently occurring, followed by MC-RR and MC-YR. Notable MC-LR only occurred in 11% of all the samples.

Tab. 4 Relative frequency of occurrence of each of the MC variants (Table 3) in the 24 samples obtained from each lake (n=24). For each variant the hypothesis of random distribution of the MC variants was rejected based on the Chi-square statistic (P).

MC Name	Saka	Geo†	Edw†	Mbu†	Mur†	Nap†	In Total	P
Asp-MC-RR	17	0	25	58	0	0	17	<0.01
MC-RR	100	4	67	96	63	17	58	<0.01
unknown	13	0	0	0	29	88	22	<0.01
Asp-MC-YR	21	0	38	54	0	0	19	<0.01
MC-YR	67	0	46	92	25	17	41	<0.01
MC-LR	63	0	4	58	79	8	35	<0.01
unknown	0	0	0	67	0	0	11	<0.01
Asp-MC-RY	67	33	13	79	0	4	33	<0.01
MC-RY	83	92	96	100	88	4	77	<0.01
unknown	17	0	50	17	33	4	20	<0.01
unknown	0	0	33	54	0	0	15	<0.01

Geo† = George, Edw† = Edward, Mbu† = Mburo, Mur† = Murchison Bay, L. Victoria; Nap† = Napoleon Gulf, L. Victoria

In general, the contribution of each MC variant to the total MC concentration was closely related to the frequency of occurrence. For example, MC-RR contributed on average >50% to the total MC in Lakes Saka and Mburo. MC-RY contributed >50% to the total MC in Lakes George and Edward and in Murchison Bay. The MC observed in Napoelon was dominated by the unknown MC3 variant. The phytoplankton further differed significantly in the production of MC in total (calculated as MC-LR equivalents) ($p<0.0001$, Kruskal Wallis One Way ANOVA on Ranks). On average, the MC concentrations were 28-fold higher in Lake Saka (4.7±0.9 µg L^{-1}) when compared with the mean (0.2±0.1 µg L^{-1}) MC concentration measured in L. George (Kahendero) (Fig. 4). Samples from Lake Saka had the maximum MC concentration (10 µg L^{-1}) in July 2007. The minimum concentration (0.02 µg L^{-1}) was recorded in Lake George (Kahendero) in the months of May 07, June 07, January 08 and April 08. At the sampling sites in the other three lakes intermediate MC concentrations (0.1 - 2.5 µg MC-LR eq. L^{-1}) were recorded. Within the lakes, the total MC concentration varied seasonally from 12 fold to 30 fold.

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For all sampling sites highly significant positive linear relationships between the total MC concentration and *Microcystis* cell numbers were obtained (Table 5). For other potentially MC-producing taxa (*Anabaena* and *Planktothrix*) occurring in Lakes Saka, George, Edward only marginal significant relationships (with a low explained variation) between cell numbers and MC concentrations were found. In contrast significant relationships were observed for the sites in L. Mburo, Murchison bay and Napoelon Gulf. However, as we were unable to detect genes involved in MC production of any other taxa than *Microcystis* in the same habitats (4), we consider this relationship as due to the co-occurrence of these taxa.

Tab. 5 Linear regression output of *Microcystis* cells versus microcystin concentration for each lake from May 2007 to April 2008

	Sample size	r^2	Equation of the slope
Lake Saka	24	0.97	$y = 3.18\times10^{-8}x + 0.01$
Lake George	24	0.87	$y = 1.12\times10^{-9}x - 0.0009$
Lake Edward	24	0.66	$y = 2.38\times10^{-8}x + 0.0005$
Lake Mburo	24	0.94	$y = 2.11\times10^{-8}x - 0.0009$
Lake Victoria (Murchison)	24	0.86	$y = 2.64\times10^{-9}x + 0.0009$
Lake Victoria (Napoleon)	24	0.87	$y = 6.33\times10^{-9}x + 0.0001$

Both integrated and net samples were combined (n = 24). y is the MC concentration (µg MC-LR eq. mL^{-1}) and x is *Microcystis* cell concentration (Cells mL^{-1}). $p < 0.001$in all sites.

Relating the total MC concentrations to *Microcystis* cell numbers revealed a >100-fold variation in the average MC contents per cell between lakes. While *Microcystis* from Lake George (Kahendero) consistently showed the lowest MC cell quotas (0.03-1.24 fg $cell^{-1}$) *Microcystis* from Lake Saka consistently showed maximum MC cell contents (14 -144 fg $cell^{-1}$). While in general the between site variation was found reduced in plankton net samples the ranking of average MC contents per cell between lakes was not affected (Fig. 5).

It is concluded that at all sites MC production is due to the occurrence of *Microcystis*, however between sites the populations differ consistently and independently of the season in their average MC content per cell.

4. Discussion

4.1 Natural and human induced eutrophication

The shallow lakes George, Edward, Mburo are all of a hypertrophic state with TP and chl a values > 100 µg L^{-1} and 70µg L^{-1}, each. The high trophy is probably natural as those lakes are shallow, located closely to the Ruwenzori Mountain where massive run offs and nutrients are introduced via several rivers (Nyamwamba, Rukoli, Mubuku, Ruimi, Nyamugasani, Ishasha, Rutshuru, Rwindi and Rinzi) particularly during the rainy season. In addition abundant wildlife contributes to eutrophication. (24) reported that hippopotami introduce large amounts of excrements that cause considerable nutrient loading. Hippopotami also occur in L. Edward and George and particularly the bulls use the excrements as territorial marks. In contrast Lake Saka located at the Northern end of the Crater lakes does not contain wildlife, however has been eutrophied by agricultural activities and fish stocking for decades (25). The same authors reported highest dissolved oxygen maxima 15 mg L^{-1} (max 180% saturation) during the period of the dry season.

Compared with Lake George, Edward and Mburo the pH in L. Saka was found to be relatively low (pH 7.9) which is corresponding to the pH 7.7 as observed from January 1995 – January 1998 by (25). Since rather low oxygen concentrations have been reported at a depth of 10 m in the southern part of the lake it is possible that a low redox potential also counteracts the pH increase due to a highest primary production in the surface layer. As for Lake Saka, a significant human induced eutrophication comparable to bays of Lake Victoria due to human activities has been observed. For example, studies on Winam Gulf, Kenya (26), Nyanza Gulf, Kenya (27), Mwanza Gulf, Tanzania (28), Murchison Bay, Napoleon Gulf and Fielding Bay, Uganda (29) all reported the trend in nutrient enrichment. However,

either natural or human-induced eutrophication both finally resulted in a dominance of cyanobacteria.

4.2 Seasonal and spatial variation in phytoplankton composition

Over the whole study period, total phytoplankton biovolume was found to be relatively stable, and cyanobacteria never contributed less than 53 % to the phytoplankton in total. Only at the station in Lake Victoria, Napoleon Gulf a distinct maximum of *Anabaena* in June was recorded. In this study Napoleon Gulf was the deepest sampling site (18 m) and the dry weather conditions might have favoured the growth of *Anabaena* at the expense of the diatoms. Buoyant cyanobacteria, like *Anabaena* and *Microcystis* are found in higher cell numbers during the dry season when compared with the wet season (8). In general, in Kampala there are seasons of minimum rainfall from January-February and June-July (Meteorological Department, Entebbe) contributing to a higher degree of stratification in the water column. Buoyant cyanobacteria are expected to have a selective advantage compared with non-motile algae under stratifying conditions (8).

In contrast to the seasonal phytoplankton biovolume the composition of cyanobacteria was more variable, for example phytoplankton of Lake Saka was dominated by *Planktothrix* spp. during December – February, while *Microcystis* was more abundant during the rest of the year. For unknown reasons *Planktothrix* was recorded in Lake Saka only. There have been generally rather few records of the occurrence of *Planktothrix* from the tropical climatic zone. Up to date *Planktothrix* has been reported to occur in another tropical lake in Cameroon (30). The genus *Planktothrix* is more frequently and dominates phytoplankton composition in deep and shallow lakes within the northern temperate climatic zone.

Interestingly *Planktothrix* occurring in this area is morphologically indistinguishable from *P. agardhii* occurring in Europe and Asia (31). It is likely that *Planktothrix* in L. Saka is *P. pseudagardhii* that is known to grow under higher temperature regimes when compared with *P. agardhii* (31). Consequently, while in most freshwater lakes in Uganda *Planktothrix* seems to be out competed by other genera (*Planktolyngbya*, *Microcystis* and *Anabaena*), it is still able to dominate in habitats of a more specific physical-chemical characteristic. Both habitats, Lake Saka and Yaounde Municipal Lake in Cameroon are relatively small lakes, shallow and anoxic in the lower layers (25, 30) The observation that *Planktothrix cf. ornata* living at the chemocline of a sulphate-rich karstic lake in Spain can sustain high sulphide concentrations found around the chemocline in the lake may provide a hint on specific ecological adaptations of this species in tropical Africa.

When compared with the phytoplankton in Lake Saka the genus *Planktolyngbya* replaced *Planktothrix* in the shallow hypertrophic lakes George, Edward and Mburo. In contrast to *Planktothrix*, *Microcystis* and *Anabaena* the genus *Planktolyngbya* belongs to the group of the Pseudanabaenaceae and never forms gas vesicles (32). Since the genus *Planktolyngbya* grows in rather thin filaments, probably no compensation of sinking losses is required. *Planktolyngbya* is a typical member of the group of shade-adapted species (5) resulting in the so-called third stable state to which shallow lakes in Europe may develop. It is known that together with *Planktothrix agardhii*, *Limnothrix redekei*, *Pseudanabaena limnetica*, *Planktolyngbya contorta* can form the so-called association “S” that is characterised by highly light deficient conditions. In Lakes George, Edward and Mburo co-occurring *Microcystis* sp. can escape these light-limiting conditions by vertical migration to the surface.

In contrast to the shallow lakes Mburo, George and Edward the genus *Anabaena* is much more abundant at stations of Lake Victoria. While *Anabaena flos-aquae* are also capable of vertical migration the higher proportion of *Anabaena* might be due to the prevalence of N-limiting conditions.

4.3 Effects of phytoplankton composition on microcystin production

For all lakes the abundance of *Microcystis* was significantly related to MC production. In contrast MC production was negatively related to the abundance of *Planktothrix* in Lake Saka and to the abundance of

Anabaena in Lake Victoria in Napoleon Gulf and Murchison Bay. We reported previously that in the same lakes we were unable to amplify genotypes of the *mcy*E gene encoding microcystin synthesis that are indicative of the genera *Anabaena* or *Planktothrix* (4). In contrast in the same study the occurrence of *mcy*E and *mcy*B genotypes assigned to the genus *Microcystis* showed a perfect correspondence with MC production. In addition nine strains of Planktothrix sp. were isolated from Lake Saka in April 2008 and analysed for MC production as described (33). None of the strains were found to contain MCs and/or the *mcy*E/*mcy*B gene part of the *mcy* gene cluster (R. K., unpublished results).

Surprisingly, the average MC *Microcystis* cell quotas differed significantly between populations (Fig. 5). In general environmental conditions such as light availability and nitrogen availability have been shown to increase MC production in *Microcystis*. For example (34) reported a linear increase in MC content per cell of *Microcystis* strain PCC7806 from 40–80 fg cell^{-1} under light conditions from 10-100 µmol m^{-2} s^{-1}. (35) observed a variation in MC content per cell of Microcystis strain MASH 01-A19 from 0.052-0.116 fmol cell^{-1} under nitrogen limiting and nitrogen-replete conditions. Typically, environmental factors have been shown to modulate MC production per cell up to 5-fold (36), while larger variation (up to 30-fold) at 30°C vs 12.5°C has been reported in exceptional cases only (37).

In this study the average MC contents differed between populations by 17-175 folds in integrated samples and 2.5-23 folds in plankton net samples. The range of variation substantially increases the variation observed for single strains under variable conditions in the laboratory. Consequently it is possible that genetic differences between populations such as the variable proportion of *mcy* genotypes contributes to the between site variation that is observed. In order to test this hypothesis phytoplankton from Lakes George and Mburo was extracted for DNA at four sampling dates and analysed for the proportion of mcy genotypes following exactly the quantitative real-time PCR (Taq nuclease assay, TNA) protocol that has been described (38). In general the Microcystis cells that were counted in the microscope correlated significantly with the Microcystis cell number as estimated by the TNA (n=16, R^2=0.70). The average proportion of *mcy* genotypes in Lake George (1.14±0.13 (SE)% and 5.62±0.38% in integrated and net samples, each) was 17-fold lower in integrated samples and 7-fold lower in net samples when compared with the average *mcy* proportion in Lake Mburo (19.6±4.8% and 40.5±7.4 % in integrated and net samples, each). This difference in *mcy* genotype proportion compares with the average difference in cellular MC content both in integrated samples (0.3±0.1 vs 11.7±2.6 fg cell^{-1} of Microcystis in Lake George and Lake Mburo, each) and in net samples (1.2±0.1 vs 24.4±1.9 fg cell^{-1} in Lake George and Mburo, each). It is concluded that the variation in average MC content of *Microcystis* that is observed between lakes could be explained by the between site differences in *mcy* genotype proportion. Notably the variation in average MC content that was observed within sites could not outweigh this between site variations in MC content.

5. Conclusion

The finding that *Microcystis* is a consistent MC producer has important implications for water monitoring. By counting *Microcystis* cells under the microscope, *Microcystis* cell numbers can be used as a proxy to predict MC concentrations in surface water. Since a relatively minor variation both during dry and rainy seasons has been found worst case MC concentrations could be calculated from cell numbers using the maxima of cellular MC quotas as reported for each sampling site. This approach is considered mostly reliable as the microscopical enumeration technique is well established and the maintenance of technically sophisticated equipment is avoided.

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