On the Ecology, Toxicology, and Phylogeny of Cyanobacteria in Murchison Bay of Lake Victoria, Uganda

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Dissertation presented for the degree of
Doctor Scientiarum

Department of Biology
Faculty of Mathematics and Natural Sciences
University of Bergen
2008

Norsk institutt for vannforskning

ISBN 978-82-308-0594-7
Bergen, Norway 2008-05-22
Printed by Allkopi Tel: +47 55 54 49 40

Acknowledgements

This thesis has been a part of the project "Harmful blue-green algal blooms in developing countries – A threat to development?", funded by the Norwegian Research Council under the UTISØR program. It started as a cooperation between Norwegian Institute for Water Research (NIVA), the University of Bergen (UoB) and the Makerere University in Uganda, but became even more national (University of Oslo, UoO) and international (Leibniz Institute for Freshwater Ecology and Inland Fisheries, IGB) over time. My working place has been at NIVA in Oslo, but I have also lived and worked in Uganda and Germany during the work with this thesis. In addition to enter the interesting world of cyanobacteria, I have learned and experienced so much more along the way.

First of all, I want to express my sincere gratitude to my supervisors, for guidance, cooperation and support, and for our travels to Uganda. I am grateful to Anne Lyche-Solheim for her intensive work to make this project come true, and for including me as a PhD student. Thank you for your everlasting enthusiasm and encouragements and for our many fruitful discussions. I am grateful to Bente Edvardsen for her inspiring enthusiasm and many advises and for being a very caring person; at work and in private life. Thank you for including me in your group at UoO and for patiently guiding me into the world of molecular biology. I am also grateful to Petter Larsson, my supervisor at UoB for his sincere interest in this project and for our many discussions. I have appreciated your enthusiasm for Lake Victoria and I am glad I got the opportunity to share the "life in U-experience" with you. Finally, I am deeply grateful to Thomas Rohrlack for coming to NIVA and for taking over as my main supervisor. Thomas, thank you for your interest in this project, for sharing your great knowledge in the field of cyanobacteria, for always being there, always having time and answers to my questions, for all your encouragements, all your invaluable support and for being such a kind person!

Pål Brettum is warmly thanked for doing the phytoplankton counting originally planned to be carried out in Uganda. I am deeply grateful to Randi Skulberg for patiently learning me isolating techniques and algal culture work and for sharing her knowledge with me, and for providing excellent technical assistance in every way and at every possible occasion. I also want to thank Robert Ptacnik for helping me with statistics, Kai Sørensen for providing help with light data in Murchison Bay, and Susanne Schneider for proofreading my thesis. I especially want to express my gratitude to Olav Skulberg for being an inspiring teacher and a never ending source of knowledge. Ingvild, Kjetil, and the helpful staff at Bioportalen (UoO) are thanked for all the support with the phylogenetic analyses. To my office mate and fellow cyano-PhD-companion Camilla; this PhD life has been our common challenge and adventure. Thank you for all our discussions and talks, all our field trips and out of work activities. Thank you for being my encouraging spirit and for our great friendship. I also want to thank my colleges at NIVA for making a good working environment; the people on my group, the "coffee-break group", the other PhD students, the lab-staff, Erik and Ingar….I warmly acknowledge NIVA for always finding creative solutions for needed postponement of my work. I also appreciate the possibility to get involved in so many other projects at NIVA during my time as a PhD student.

I am so grateful to my colleges and friends in Uganda for all their support in my field work and for making my "life in U" to such a great experience. Thank you for all those happy times on the boat in Murchison Bay, with "happy-clappy-hjerte-smerte-music" on the radio and interesting discussions. A long list of names, a great THANK YOU: Semyalo, Odong, Santa,

Susan, Madena, Betty, Dr. Kizito, Dr. Myingo-Kezimbira, Dr. Bujarujali, Dr. Nils Kirsh, the staff at the Bergen Office. I also want to thank my “Muzungu” friends, Aase Marie and Rune, for nice times in U.

I am grateful to Dr. Claudia Wiedner at IGB for inviting me to Germany and for being my co-supervisor. I appreciate your encouragements and helpful supervision in my work with molecular methods and phylogeny. I also acknowledge the external funding from the Kompetenz Zentrum Wasser Berlin and Veolia. Anke, Martin and Monika in the Molecular Ecology group at IGB are thanked for making a good laboratory environment. I also want to thank all my colleges and friends at IGB for making the remote German forest to such a nice place to work and live. I am grateful to Dr. Jutta Fastner at the Federal Environmental Agency in Berlin for kindly providing MALDI-data.

Dear Andreas, thank you for our collaborations, for our many scientific discussions and unscientific talks, for all your encouragements, for all your help and support, and for always being there for me!

To all my great friends; thank you for all those good times and for the various out of work activities and for your continuous support.

I want to thank my brother and his girls for all their support and encouragements, and for all the fun we have together!

Finally, I want to thank my parents for always being there for me, for always supporting me, for being so caring and encouraging and for always showing great interest in whatever I do in my life.

Oslo, 2008

Sigrid Haande

Abstract

One of the major threats to Lake Victoria is eutrophication and an increasing proliferation of cyanobacteria. Many cyanobacterial species have the ability to produce toxic compounds (cyanotoxins), which can cause considerable hazards for animal and human health. Thus, blooms of toxin-producing cyanobacteria receive increased attention when developing in drinking water supplies and inland waters used for recreational activities. Murchison Bay is a 30 km long embayment in the north western part of Lake Victoria, and is divided in a semi-enclosed inner part and a wider outer part by narrows about 5 km out in the bay. The capital city of Uganda, Kampala, is situated close to Murchison Bay, which serves as the drinking water supply for the population of the city and the surrounding areas. The bay is influenced by local pressures like urban pollution, erosion, flooding, and wetland degradation and is the recipient of both industrial and municipal wastes, sewage effluents and surface runoff from the city.

The aims of this study have been to promote the knowledge on the eutrophication and proliferation of cyanobacteria in Lake Victoria, to assess the possible cyanotoxin (microcystin) production and to describe the morphological, genetic and chemical diversity of cyanobacteria from Lake Victoria and other East-African water bodies. The sampling of Murchison Bay was carried out from November 2000 to March 2004. The study showed that there was a heavy loading of nutrients to Murchison Bay and there were high concentrations of total phosphorous (>90µg/L) and total nitrogen (>1100µg/L) in the inner part of the bay. There was a rapid decrease in conductivity and nutrient concentrations from the innermost part of the bay to the outer part of the bay. We found that surface seiches caused considerable water exchange with the main lake and thereby mediated the eutrophication in the Inner Murchison Bay. The bay is a more dynamic system than first recognized and the rapid transport of nutrients to the open lake indicates that Murchison Bay contributes to the eutrophication process of Lake Victoria. The phytoplankton community was dominated by a variety of cyanobacterial species and diatoms. The proportion of N-fixing species like *Anabaena* sp. was higher in the outer part of the bay whereas *Microcystis* sp. was more abundant in the inner part of the bay. There were microcystins (MC-RR, -LR, -YR) present in Murchison Bay, on average 1.1 µg L^{-1} in the inner part of the bay and 0.6 µg L^{-1} in the outer part of the bay. Based on probability analysis, *Microcystis aeruginosa* was identified as the main microcystin producer. Several cyanobacterial strains of *M. aeruginosa* and *Cylindrospermopsis raciborskii* were isolated from Murchison Bay and other lakes in the East African region and these strains were morphologically, genetically and chemically characterized. Phylogenetic analyses showed that the East-African strains of *M. aeruginosa* were closely related to other strains of *M. aeruginosa* of different geographical regions, whereas the phylogenetic analyses comparing *C. raciborskii* strains from Uganda and Germany to strains from other continents, revealed that strains from the same continent were more closely related to each other than the strains originating from different continents. Some of the strains of *M. aeruginosa* were microcystin producing, and none of the *C. raciborskii* strains were producing cylindrospermopsin.

Two water works are situated at the shores of the Inner Murchison Bay. Water from different steps in the purification process were analysed, and microcystins were not detected. There is, however, a risk for exposure to microcystins for those using the lake water directly as drinking water and increased awareness of cyanobacterial blooms in Murchison Bay is needed.

List of Acronyms and Abbreviations

Adda	3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid
Chl-*a*	Chlorophyll-*a*
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
ITS	internal transcribed spacer
ITS-L	long internal transcribed spacer between 16S and 23S rDNA genes
LC-MS	liquid chromatography mass spectrometry
LD_{50}	Lethal Dose, The amount of a material, given all at once, which causes the death of 50 % of a group of test animals
MALDI-TOF-MS	matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MC-LR	leucine and arginine in the positions of X and Z of microcystin
MC-RR	arginine and arginine in the positions of X and Z of microcystin
MC-YR	tyrosine and arginine the positions of X and Z of microcystin
NO_3-N	nitrate
NRPS	non-ribosomal peptide synthetase
PAR	photosynthetic active radiation
PC	phycocyanin
PC-IGS	phycocyanin – intergenic spacer
PCR	polymerase chain reaction
PKS	polyketide synthase
PO_4-P	phosphate
PS	peptide synthase
rDNA	ribosomal deoxyribonucleic acid
TN	total nitrogen
TP	total phosphorous
tRNA	transfer ribonucleic acid
UNESCO	United Nations Educational, Scientific and Cultural Organization
WHO	World Health Organization

List of papers

The thesis is based on the following papers, referred in the text by their Roman numbers:

I Larsson P, Haande S[*], Luyiga S[*], Semyalo R[*], Kizito YS, Miyingo-Kezimbira A, Brettum P, Lyche-Solheim A, Odong R, Asio SA, Jensen KH
Surface seiches mediate pollution in a eutrophic bay of Lake Victoria.
[*]Contributed equally
(Submitted)

II Haande S, Rohrlack T, Brettum P, Edvardsen B, Lyche-Solheim A, Larsson P
Phytoplankton dynamics and cyanobacterial dominance in Murchison Bay of Lake Victoria (Uganda) in relation to environmental conditions
(In manuscript)

III Haande S, Rohrlack T, Brettum P, Ptacnik R, Edvardsen B, Lyche-Solheim A, Larsson P
On the occurrence of microcystin producing cyanobacteria in Murchison Bay of Lake Victoria (Uganda)
(Submitted)

IV Haande S, Ballot A, Rohrlack T, Fastner J, Wiedner C, Edvardsen B (2007) Diversity of *Microcystis aeruginosa* isolates (Chroococcales, Cyanobacteria) from East-African water bodies.
Archives of Microbiology. 188, 1-15

V Haande S, Rohrlack T, Ballot A, Røberg K, Skulberg R, Beck M, Wiedner C
Genetic characterisation of *Cylindrospermopsis raciborskii* isolates (Nostocales, Cyanobacteria) from Africa and Europe.
Harmful Algae. DOI 10.1016/j.hal.2008.02.010

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1 Introduction

Cyanobacteria have long been recognized as an important component of the phytoplankton community in Lake Victoria (East Africa) and blooms of cyanobacteria have been reported from Lake Victoria ever since the beginning of the last century (Ostenfeld, 1908). Over the last five decades, Lake Victoria has experienced severe eutrophication, and mass occurrence of cyanobacteria has become a common phenomenon (e.g. Ochumba and Kibaara, 1989; Hecky and Bugenyi, 1992; Gophen et al., 1995; Lung'ayia et al., 2000; Kling et al., 2001; Krienitz et al., 2002). Many cyanobacterial species have the ability to produce toxic compounds (cyanotoxins), which can cause hazards for animal and human health (Krienitz et al., 2003; Bell and Codd, 1994; Kuiper-Goodman et al., 1999; Codd et al., 2005a). Thus, cyanotoxins especially pose a health risk when they appear in drinking water supplies and inland waters used for recreational activities. A recent UNESCO survey concludes that the overall knowledge on the occurrence of cyanobacteria and cyanobacterial blooms in large parts of Africa is poor (Codd et al., 2005b). Till now, only a few investigations on the presence of cyanotoxins have been conducted in Lake Victoria (Krienitz et al., 2002; Sekandende et al., 2005). The close proximity between society and nature in the Lake Victoria region (Fig. 1) lead to the recognition that more knowledge on toxic cyanobacterial blooms is of major importance.

Figure 1 Gaba landing site at the shores of Murchison Bay, Lake Victoria

The intention of this thesis has therefore been to promote the knowledge on the eutrophication and proliferation of cyanobacteria in Lake Victoria, to assess the possible cyanotoxin (microcystin) production and to describe the morphological, genetical and chemical diversity of cyanobacteria from Lake Victoria and other East-African water bodies. Our study has mainly been conducted in Murchsion Bay, a shallow embayment in the north-western part of Lake Victoria, close to the capital city of Uganda, Kampala. The bay serves as a drinking water supply for Kampala, but is also as a recipient of both industrial and municipal wastes, sewage effluents and surface runoff from the city and is shown to be strongly eutrophicated.

1.1 Cyanobacteria

Cyanobacteria, also known as blue-green algae, blue-green bacteria, cyanoprokaryots and cyanophytes, are photosynthetic prokaryotes comprising a single phylogenetic group within the domain *Bacteria* (Castenholz, 2001). They have photosystems I and II and use water as an electron donor during photosynthesis, leading to the production of oxygen. Several cyanobacteria can also perform anoxygenic photosynthesis using only photosystem I if electron donors such as hydrogen sulphide are present (Madigan et al., 2003). Cyanobacteria have a long evolutionary history and documented fossil records date back to about 3500 million years ago (Schopf, 2000). However, the earliest DNA-biomarker evidence suggests that cyanobacteria appeared more recently, about 2600 million years ago (Hedges et al., 2001). It is widely accepted that ancient cyanobacteria evolved oxygenic photosynthesis and played a major role in the transformation from an anoxic to an oxic atmosphere (Schopf, 2000). Cyanobacteria are found in almost every conceivable habitat. They are most abundant in aquatic habitats as part of the plankton, and some can be found tightly or loosely attached to surfaces of plants, rocks and sediments. Cyanobacteria are also important in many terrestrial environments and they can live in soils or on rocks and form symbiotic associations with plants, fungi and animals (Whitton and Potts, 2000). Under favorable environmental conditions, mass occurrences of planktonic cyanobacteria can evolve. Cyanobacteria have developed a wide ecological tolerance to temperature, light, salinity, alkalinity, and possess many characteristics and adaptations that explain their world wide distribution and success. Cyanobacteria show considerable morphological diversity. They may be unicellular or have cells arranged in colonies and some form filaments in single trichomes or filaments with false or true branching. Some cyanobacteria have the ability to produce two types of specialized cells: (1) heterocysts, which provide the site for N_2 fixation and thereby counteract nitrogen demand under conditions of nitrogen deficiency, and (2) akinetes, which are resting cells that

allow the species to survive unfavourable growth conditions. Many species of cyanobacteria possess gas vesicles, enabling them to regulate their buoyancy and to maintain a certain vertical position in the water column in response to physical and chemical factors (Reynolds, 1987; Walsby, 1994). A number of chemical, physical and biological factors and their interactions influence the cyanobacterial abundance and success in a water body (Hyenstrand et al., 1998). Generally, cyanobacterial mass occurrences are known to develop in eutrophic and hypertrophic water bodies (Paerl, 1996). Cyanobacteria have high nutrient affinity and it is widely accepted that the availability of nitrogen and phosphorous are important factors in enhancing cyanobacterial growth (Mur et al., 1999). Other chemical factors such as micronutrients, and dissolved organic carbon concentrations may also have effects on cyanobacterial growth (Paerl et al., 2001). The most important physical factors influencing the growth of cyanobacteria include light, temperature, water turbulence, and lake stratification and mixing (Mur et al., 1999). Lake morphometry and water residence time also indirectly influence cyanobacterial growth. Grazing, competition, parasitism, and other microbial interactions are biological factors either causing loss of biomass or influence the growth of cyanobacteria. In temperate regions of the world, most cyanobacterial blooms develop during summer when the light intensity is high and the temperatures are favourable. In tropical areas, where the annual solar radiation and temperatures are relatively constant, cyanobacteria can grow at any time of the year (Oliver and Ganf, 2000).

1.2 Cyanobacterial toxins

Cyanobacteria are known for their ability to produce a wide range of potent toxins (for a review see Sivonen and Jones, 1999), which can cause considerable hazards for aquatic ecosystems, domestic as well as wild animals, and human health (Christoffersen, 1996; Krienitz et al., 2003; Bell and Codd, 1994; Kuiper-Goodman et al., 1999). Cyanobacterial toxins are associated with blooms and scums of planktonic species or mats and biofilms of benthic and littoral species. Cyanobacterial strains of the same species are found to be either producing or non-producing with regard to a specific cyanobacterial toxin (Sivonen and Jones, 1999). The cyanotoxins are to a large extent cell bound, but can also occur in the water phase after extracellular release (Lawton et al., 1994). Cyanobacterial toxins are currently grouped into classes according to their toxicological properties (Sivonen and Jones, 1999; Codd et al., 2005a).

1.2.1 Hepatotoxins

The best known cyanotoxins are the cyclic heptapeptides microcystins. These compounds were first isolated from *Microcystis aeruginosa* and thus named microcystins (Carmichael et al., 1988). Microcystins share the common structure cyclo-(D-alanine-L-X-D-erythro-β-methylaspartic acid -L-Z-Adda-D-glutamate-*N*-methyldehydroalanine) in which X and Z are variable L amino acids (Fig. 2). Structural variance can occur in all seven positions, with positions 2(X) and 4(Z) as the most variable, and till now, more than 80 different microcystins have been identified (Welker and von Döhren, 2006). The known microcystin producing species belong to the planktonic and benthic cyanobacterial genera *Microcystis*, *Anabaena*, *Planktothrix*, *Anabaenopsis*, *Nostoc* and *Phormidium* and the terrestrial genus *Hapalosiphon* (for reviews see Sivonen and Jones, 1999; Codd et al., 2005a). The biosynthesis gene clusters for microcystin have been sequenced for the genera *Microcystis* (Tillet et al., 2000), *Planktothrix* (Christiansen et al., 2003) and *Anabaena* (Rouhiainen et al., 2004).

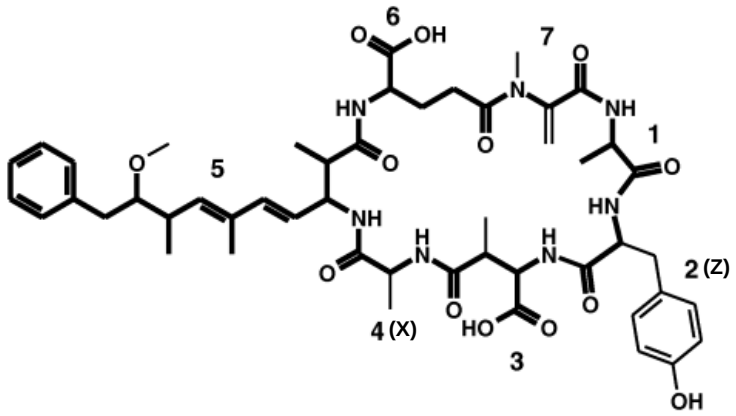

Figure 2 General structure of microcystin (Tillett et al., 2000), the variable L-amino acid residues at positions 2 and 4 are indicated by X and Z, respectively.

The other group of hepatotoxins comprises the cyclic pentapeptides nodularins (Rinehart et al., 1988) with six known structural variants (Codd et al., 2005a). They have only been characterized from *Nodularia* (Sivonen and Jones, 1999) and the biosynthesis gene clusters for nodularin have recently been sequenced (Mofitt and Neilan, 2004).

The microcystins and nodularins inhibit the eukaryotic serine- and threonine-specific protein phosphatase 1 and 2A that are important for cellular metabolism (Honkanen et al., 1990; MacKintosh et al., 1990; Yoshizawa et al., 1990). Microcystins and nodularins can act as tumour promoters (Carmichael, 1997), and the nodularins are also carcinogenic (Ohta et al.,

1994). The microcystins and nodularins can cause major liver damage due to the ability to enter into hepatocytes and cause lethal intrahepatic haemorrhage, liver necrocis and destruction of parenchymal cells of the liver (Carmichael, 1992; 1994). Different microcystin variants exhibit different hepatotoxicities. In general, the microcystins have LD_{50} values (intraperitoneal mouse) that can vary from 25 to ~1000 μg kg^{-1} body weight (Carmichael, 1997; Sivonen and Jones, 1999; Codd et al., 2005a). The WHO has provided a provisional guideline value for the maximum allowable concentration of microcystin (MC-LR) of 1μg L^{-1} in drinking water and 10 μg L^{-1} in bathing water (WHO, 1998).

1.2.2 Neurotoxins

The neurotoxins include anatoxin-a, and homoanatoxin-a, anatoxin-a(s) and saxitoxins (Sivonen and Jones, 1999; Codd et al., 2005a). The anatoxin-a, and homoanatoxin-a, are alkaloids and are postsynaptic, cholinergic neuromuscular blocking agents. Anatoxin-a(s) is a guanidine methyl phosphate ester and inhibits acetylcholinesterase. The saxitoxins are alkaloids which block sodium channels, and about 20 structural variants are known in cyanobacteria (Codd et al., 2005a). Anatoxin-a has been found in blooms of *Anabaena*, *Oscillatoria*, *Aphanizomenon*, *Cylindrospermum*, *Planktothrix* and *Raphidiopsis* (Sivonen, 1996; Codd et al., 1999; Sivonen and Jones, 1999; Namikoshi et al., 2003; Gugger et al., 2005a), and homoanatoxin-a has been found in blooms of *Planktothrix* (Skulberg et al., 1992). Anatoxin-a(s) has been found in *Anabaena* (Matsunaga et al., 1989; Henriksen et al., 1997; Onodera et al., 1997) and saxitoxins have been found in *Anabaena*, *Aphanizomenon*, *Planktothrix*, *Cylindrospermopsis* and *Lyngbya* (Sivonen and Jones, 1999; Codd et al., 2005a).

1.2.3 Cytotoxins

The cyclic alkaloid cylindrospermopsin is an inhibitor of protein synthesis causing injury of kidneys, heart, thymus, spleen, and intestine in mammals (Hawkins et al., 1997; Falconer et al., 1998). It is also a potent hepatotoxin (Hawkins et al., 1985), it can cause DNA double-strand break and chromosome loss (Humpage et al., 2000; Shen et al., 2002) and it is carcinogenic (Falconer and Humpage, 2001). Till now, three variants of cylindrospermopsin are known; cylindrospermopsin, deoxy-cylindrospermopsin and 7-epi-cylindrospermopsin (Ohtani et al., 1992; Banker et al., 2000; Li et al., 2001a). Humpage and Falconer (2003) have proposed a guideline value of 1 μg L^{-1} cylindrospermopsin in drinking water. The known cylindrospermopsin producing species belong to the cyanobacterial genera

Cylindrospermopsis, *Aphanizomenon*, *Anabaena*, *Raphidiopsis* and *Umezakia* (Hawkins et al., 1985; Harada et al., 1994; Banker et al., 1997; Li et al., 2001a; Preussel et al., 2006; Spoof et al., 2006).

1.2.4 Skin irritants and gastrointestinal toxins

Aplysiatoxin, debromoaplysiatoxin, and lyngbyatoxin are produced by marine cyanobacteria (*Lyngbya*, *Schizothrix*, *Oscillatoria*) and cause skin irritation and are tumour promoters (Codd et al., 2000). Lipopolysaccharide endotoxins are widely produced by cyanobacteria as an important constituent of the cell wall and may contribute to inflammatory and gastrointestinal incidents (Codd et al., 2000).

1.2.5 Other cyanobacterial bioactive peptides

Cyanobacteria can produce a diverse range of secondary metabolites, both non-toxic and toxic compounds (Namikoshi and Rinehart, 1996), and over 600 peptides and peptidic metabolites have been described from various taxa (for a review see Welker and von Döhren, 2006). A major part of the known secondary metabolites are oligopeptides and they are synthesized nonribosomally by large enzyme complexes known as NRPS or NRPS/PKS using the thio-template mechanism (Marahiel et al., 1997; Dittmann et al., 2001). Welker and van Döhren (2006) grouped the cyanobacterial peptides according to molecular structure into seven classes, the aeruginosins, microginins, anabaenopeptins, cyanopeptolins, microviridins and cyclamides and the previously described microcystins/nodularins. Concerning a specific oligopeptide, both producing and non-producing individuals of the same cyanobacterial species co-exist in nature (Vezie et al., 1998; Kurmayer et al., 2004).

1.2.6 Toxicity and harmful effects of cyanobacterial toxins

Mass developments of toxin producing cyanobacteria can have severe impact on the food web of lakes (Christoffersen, 1996). Laboratory experiments have shown that cyanotoxins have inhibited or reduced growth of different phytoplankton groups (Kirpenko, 1986; Bagchi et al., 1990; Babica et al., 2006) and macrophytes (Pflugmacher et al., 1998; Babica et al., 2006) and caused behavioural changes and higher mortality of zooplankton organisms such as cladocerans and copepods (Riehmann and Christoffersen, 1993; DeMott et al., 1991; DeMott and Moxter, 1991; Rohrlack et al., 1999). Acute effects of cyanotoxins are also found in fish, including liver damage, disturbed ionic regulation, behavioural changes and mortality (Tencalla et al., 1994; Bury et al., 1995; Mohamed et al., 2003). Mass mortalities of wild and

domestic birds have been related to cyanobacterial blooms and scums (Yoo et al., 1995; Matsunaga et al., 1999; Henriksen et al., 1997; Krienitz et al., 2003). Cyanobacterial toxins have also caused several incidents of wild and domestic animal poisoning, often lethal (for a review see Kuiper-Goodman et al., 1999). Cyanobacterial toxins present health hazards to humans through contaminated drinking water and food, in addition to recreational exposure to cyanobacteria (Bell and Codd, 1994; Codd et al., 1999; Kuiper-Goodman et al., 1999; Ibelings and Chorus, 2007). Thus, cyanobacterial toxins may pose a health risk particularly when they appear in raw water sources of poorly equipped drinking water plants, and there are reports on incidents where dissolved microcystins in surface waters have passed through conventional water treatment plants (Jochimsen et al., 1998; Lahti et al., 2001). Cyanobacterial toxins are linked to incidents of different human illnesses, including skin and eye irritation, allergy-like symptoms, gastro-enteritis and hepatoenteritis caused by acute exposure to toxins (Carmichael and Falconer, 1993; Kuiper-Goodman et al., 1999). A severe outbreak of hepato-enteritis among an aboriginal population in Palm Island, Queensland (Australia) was associated with drinking water contaminated with *Cylindrospermopsis raciborskii* and 148 persons were hospitalized (Byth, 1980; Hawkins et al., 1997). In Brazil, at least 55 patients died after receiving a microcystin-containing dialysis medium in a haemodialysis centre (Jochimsen et al., 1998). Chronic exposure to microcystins through drinking water may increase incidents of human liver cancer (Codd et al., 1999; Kuiper-Goodman et al., 1999). In China, higher incidents of liver cancer were observed among the people drinking pond and ditch water than among people using deep well water, and the higher incidences of cancer were thought to be linked to cyanobacterial toxins in the ponds and ditches (Yu, 1995). There is also a risk for exposure to cyanotoxins to humans by consumption of freshwater "seafood" like fish, crayfish, prawns and mussels from water bodies with cyanobacterial blooms (for a review see Ibelings and Chorus, 2007). In addition, field observations of cyanotoxin bioaccumulation in zooplankton (Watanabe et al., 1992; Kotak et al., 1996), macroinvertebrates (Zurawell et al., 1999), and mussels (Falconer et al., 1992; Duy et al., 2000) indicate that cyanobacterial toxins may be transferred in aquatic food webs and thus have effects on higher trophic levels, including exposure to humans.

1.2.7 The possible ecological role of cyanobacterial metabolites

The ecological role of cyanobacterial toxins and oligopeptides remains unclear despite the numerous studies regarding the physiological and ecological effects of the production of the cyanotoxins and other cyanobacterial bioactive oligopeptides. The fact that cyanobacterial

populations comprise both non-producing and producing strains with respect to cyanobacterial toxins and oligopeptides complicates the understanding of the ecological role of these compounds. The toxin production is likely to be related to the physical, chemical, and biotic environment and the competition with other organisms (Paerl and Millie, 1996) and most of the proposed theories regarding the function of the toxin production are related to either grazing protection or to allelopathy (Babica et al., 2006). There is evidence that cyanobacterial toxins have an effect on growth and survival of zooplankton (DeMott and Moxter, 1991; Rohrlack et al., 1999; Rohrlack et al., 2001). Cyanobacterial cells are generally a poor food source for zooplankton and are often selectively avoided (DeMott et al., 1991). *Daphnia* populations are known to decline during cyanobacterial blooms, when alternative food sources for zooplankton have been exhausted (DeMott et al., 1991). Laboratory experiments have shown that intoxication of *Daphnia* upon ingestion of cyanobacterial cells largely is dependent on the microcystin content of the cells (Rohrlack et al., 1999). There is, however, no clear evidence that cyanobacterial toxins have evolved as a response to grazing pressure by zooplankton. The microcystin synthetase genes are found to be ancient and probably predate the metazoan lineage (Rantala et al., 2004). Therefore, the primary role of microcystins may not be a defense mechanism against grazing. As previously mentioned, the toxicity of microcystins to eukaryotic cells is caused by the inhibition of protein phosphatases 1 and 2A, which are important enzymes in intercellular regulatory mechanisms (Honkanen et al., 1990; Dawson, 1998). Also for a number of cyanopeptolins, aeruginosins and microviridins, a protease inhibitory activity has been reported, however, little is known about the function, ecological effects and impact of these bioactive oligopeptides on aquatic biota, animals and humans (Welker and van Döhren, 2006).

The role of cyanobacterial toxins and other cyanobacterial bioactive oligopeptides in allelopathic interactions is unclear, but the discussion regarding allelopathic effects of cyanobacterial metabolites mostly concerns the reduction of photosynthetic activity and growth rates of other planktonic autotrophs and macrophytes (Pflugmacher, 2002; Gross, 2003; Legrand et al., 2003). A recent review by Babica et al. (2006) showed that only a limited number of studies describe effects of microcystins at concentrations that are usually found in the environment and concluded that the ability of microcystins to act as general allelopathic compounds seems unlikely. Microcystins are also thought to have ecophysiological functions, including a role in basic cyanobacterial metabolism, as metal ion chelators (Utkilen and Gjølme, 1995), in signaling and gene regulation (Dittmann et al., 2001;

Rantala et al., 2004), as an infochemical in intraspecific communication (Schatz et al., 2007), in light adaptation processes (Hesse et al., 2001), as an internal storage for N during N deficiency (Kotak at al., 2000), in inhibition of the carbon-concentration enzyme RuBisCo (Jähichen et al., 2001) or as mediators in colony formation (Kehr et al., 2006).

1.3 Cyanobacterial taxonomy

1.3.1 Classification of cyanobacteria

The taxonomic classification of cyanobacteria is quite complex. There are presently two main classification systems available: the botanical classification system (Komárek and Anagnostidis, 1989; 1999; 2005; Anagnostidis and Komárek, 1991) and the bacteriological classification system (Castenholz, 2001). Cyanobacteria were traditionally classified on the basis of their morphology only, according to the International Code of Botanical Nomenclature, ICBN (Greuter et al., 2000). Despite the fact that the cyanobacterial morphology is complex compared to most other prokaryotic microbes, the taxonomy based on morphological characteristics alone does not necessarily result in a phylogenetically reliable taxonomy (Giovanni et al., 1988; Wilmotte, 1994). Moreover, morphological features are also problematic as they may vary considerable in response to different environmental conditions (Wilmotte and Golubic, 1991). The cyanobacteria are also classified according to the International Code of Nomenclature of Prokaryots, ICNP (Oren and Tindall, 2005). The bacteriological classification is today widely based on phenotypic, chemotypic and genotypic characteristics, the so called polyphasic approach, of pure cultures of cyanobacteria. It is a challenge to combine the traditional morphological classification and the classification based on molecular methods, however, effort is made to unify these two systems (Hoffmann, 2005; Oren and Tindall, 2005). In the current botanical classification system, Komárek and Anagnostidis (1989; 1999; 2005) and Anagnostidis and Komárek (1991) have revised the taxonomy of cyanobacteria and also applied phenotypic and genotypic data in this work. The botanical approach distinguishes four orders of cyanobacteria (Komárek and Anagnostidis, 1989; 1999; 2005; Anagnostidis and Komárek, 1991). The bacteriological taxonomic system created for cyanobacteria is divided in five subsections (Rippka et al., 1979; Castenholz, 2001) and they are to a large extent in agreement with the orders in the botanical system (see Table 1). Ideally, taxonomy reflects evolutionary relationships of the classified organisms, and the taxa are monophyletic groups of organisms (e.g. Wilmotte and Golubic, 1991; Wilmotte, 1994). DNA sequences make it possible to infer phylogenies of organisms (e.g.

Moritz and Hillis, 1996) and DNA is not affected by environmental factors in the same manner as many morphological traits are. The 16S rDNA gene is universally present in bacteria and cyanobacteria and is rather conserved. Woese et al. (e.g. Woese et al, 1976; Woese, 1987) established the modern bacterial phylogenetic classification mainly based on the 16S rDNA gene sequence.

Table 1 The different orders of the botanical classification of cyanobacteria and their correspondence to the subsections of the bacterial classification and a short description of main morphological features and the occurrence in the environment.

Botanical classification	Bacteriological classification	Main morphological features, occurrence in the environment and typical species
Order Chroococcales	Subsection I	Unicellular cyanobacteria that reproduce by binary cell division or budding, either single cells or in colonies held together by mucilage or laminated sheaths. Many species are planktonic and contain gas vesicles. They occur in freshwaters as well as marine environments. Typical genera are *Synechocystis* and *Microcystis*
	Subsection II	Some species can sometimes or always reproduce by small spherical cells (baeocytes) which are produced by multiple divisions of the mother cells. They generally grow in aquatic environments attached to substrata. A typical genus is *Pleurocapsa*.
Order Oscillatoriales	Subsection III	Filamentous, mostly uniseriate, cyanobacteria without special cells. The trichomes usually have sheath and many species have gas vesicles. The group is ecologically diverse and they occur in plankton, benthic and periphytic environments in freshwater and in marine environments. Typical genera are *Oscillatoria*, *Planktothrix* and *Spirulina*.
Order Nostocales	Subsection IV	Filamentous, mostly uniseriate, cyanobacteria that may form specialized cells (heterocysts and akinetes), some may form hormogonia (formation of motile trichomes that give rise to young filaments). Some species have gas vesicles. They occur in plankton, benthic and periphytic habitats in freshwater and marine environments and can also be found in terrestrial environments. Typical genera are *Anabaena*, *Aphanizomenon* and *Nodularia*.
Order Stigonematales	Subsection V	Filamentous, usually branched (false or true) multiseriate cyanobacteria that may form specialized cells (heterocysts and akinetes) and some form hormogonia. They occur in aquatic and terrestrial environments but usually not in the plankton. Typical genus is *Fischerella*.

1.3.2 Molecular methods used for inference of cyanobacterial phylogeny

Several molecular methods are used for phylogenetic and taxonomic studies of cyanobacteria (Wilmotte, 1994) including DNA-DNA hybridization (Stam, 1980), fingerprinting based upon PCR with primers from short and long tandemly repeated elements (Rasmussen and Svenning, 1998), classification of clone cultures based upon 16S rDNA sequences (Neilan et al., 1994), and sequencing of other coding and non-coding regions of DNA (Nelissen et al., 1994; Neilan et al., 1995). The widely used 16S rDNA region has been useful in several phylogenetic analyses of cyanobacteria (e.g. Wilmotte and Golubic, 1991; Ben-Porath and Zehr, 1994; Nelissen et al., 1996; Fergusson and Saint, 2000). Phylogenetic investigations

using this region have shown that many unicellular and filamentous non-heterocysteous cyanobacterial genera are probably polyphyletic and do not form natural taxa, whereas heterocysteous strains form a monophyletic group (Giovannoni et al., 1988; Wilmotte, 1994; Castenholz et al., 2001; Rippka et al., 2001; Wilmotte and Herdman, 2001). However, the number of variable positions is low in the 16S rDNA and the gene is unsuitable for studying the relationship at or below the species level (e.g. Neilan et al., 1997a; Rudi et al., 1997).

A number of other coding and non-coding regions of DNA are also used to infer the phylogeny of cyanobacteria. The ITS1 between the 16S and 23S rDNA genes is more variable than the 16S rDNA gene sequence and has been used as a marker for studies of inter- and intraspecific variability in cyanobacteria (Nelissen et al., 1994; Wilmotte, 1994; Neilan et al., 1997b; Otsuka et al., 1999). The ITS1 region of bacteria is variable both in length and the nucleotide sequence (Gürtler and Stanisich, 1996) and multiple copies of the ITS1 may be found within one genome and then usually varies in length and sequence (Iteman et al., 2000). Although the ITS1 region itself is non-coding, it may carry one or two tRNA genes (Iteman et al., 2000). The ITS1 has been investigated in a number of studies focussing on phylogeny and genetic diversity of cyanobacteria (e.g. Nelissen et al., 1994; Neilan et al., 1997b; Iteman et al., 2000; 2002; Laamanen et al., 2001). Phycocyanin (PC) is a phycobiliprotein that is found in cyanobacteria, cryptophytes, and rhodophytes (e.g. Glazer, 1989) and the genes encoding phycocyanin are good targets for studying cyanobacerial inter- and intraspecific variations in cyanobacteria (Neilan et al., 1995). The entire PC operon contains genes coding for two bilin subunits (*cpcB* and *cpcA*) and three linker polypeptides (Belknap and Hazelkorn, 1987), and the intergenic spacer (IGS) between the two bilin subunit genes, designated PC-IGS, which is a potentially highly variable region of DNA. The PC-IGS has been used in a number of studies of phylogenetic relationships and genetic diversity in cyanobacteria (e.g. Neilan et al., 1995; Bolch et al., 1996; Laamanen et al., 2001; Dyble et al., 2002; Rohrlack et al., 2008). Other genetic markers that are commonly used in studies of cyanobacterial phylogeny and genetic diversity are the DNA-dependent RNA polymerase gene *rpoC1* (Fergusson and Saint, 2000; Wilson et al., 2000), the nitrogenase genes *nifH* (Ben-Porath and Zehr, 1994) and the IGS between two adjacent copies of the gene encoding the major structural gas vesicle protein *gvpA* (Barker et al., 1999). Many cyanobacterial species have a world wide distribution (Sivonen and Jones, 1999; Codd et al., 2005a), and the use of genetic tools can lead to a better understanding of the geographical distribution of cyanobacteria.

1.4 Cyanobacteria in Lake Victoria

1.4.1 The Lake Victoria basin

The equatorial Lake Victoria is the second largest freshwater lake in the world by area (68 800 km^2) and the largest tropical lake in the world. It is situated at high altitude (1134 m) on an elevated plateau between the western and eastern part of the African Great rift valley (Fig. 3). The East African Great Lakes are unique natural resources and form a freshwater eco-region with a unique biodiversity (Sturmbauer and Meyer, 1992; Bootsma and Hecky, 2003). Lake Malawi and Lake Tanganyika in the western rift valley are two of the deepest and oldest lakes in the world, whereas Lake Victoria is comparably younger and relatively shallow for its size (max. depth 84). The catchment area of 194 000 km^2 includes large parts of the three riparian countries Kenya, Tanzania and Uganda, and the neighbouring states of Rwanda and Burundi, and has a high and rapidly growing population whose activities influence the lake intensively (Verschuren et al., 2002).

Figure 3 Map of the Lake Victoria region with important cities. Other lakes in the region from where we have isolated cyanobacterial strains (see Table 3): KC=Kazinga Channel, LM=Lake Mburo, MB=Murchison Bay, LB=Lake Baringo, NP=Nakuru final sewage pond, PP=Pilsner Pond, LN=Lake Naivasha, KS=Kenyatta University sewage pond

The Lake Victoria ecosystem has undergone substantial changes over the last five decades, including introduction of exotic species, assumed over-exploitation of fish, severe eutrophication and climate change (Hecky, 1993; Verschuren et al., 2002). The lake originally had an extremely rich fish fauna (Witte and van Oijen, 1990) and the best known ecological change is probably the introduction and successful establishment of Nile perch (*Lates niloticus*) which dramatically altered the indigenous fauna in Lake Victoria (for a review see Goudswaard et al., 2008). The lake supports one of the largest commercial freshwater fisheries in the world (Simonit and Perrings, 2005), but the fisheries are currently considered overexploited largely due to failures in controlling the fishing effort (Simonit and Perrings, 2005). Another introduced species to the Lake Victoria ecosystem is the water hyacinth (*Eichhornia crassipes*), which has caused large scale infiltration of the lake with major negative ecological and economic impacts to the Lake Victoria region (Twongo, 1991; Albright et al., 2004). A considerable pressure to Lake Victoria is eutrophication (Hecky, 1993). Over the last 50 years, substantial increase in agriculture, deforestation, domestic, municipal and industrial effluents, and human encroachment on the shoreline leading to wetland degradation have caused historically high nutrient loadings into the lake (Hecky, 1993; Verschuren et al., 2002). The overall phytoplankton biomass has increased (e.g. Ochumba and Kibaara, 1989; Ochumba, 1990; Mugidde, 1993; Lung'aiya et al., 2000), with a notable decrease in water transparency (Mugidde, 1993). Whereas rates of primary production have accelerated near the lake surface, the rates of decomposition have depleted dissolved oxygen concentrations in the deepest one-third of the water column (Hecky et al., 1994). In near shore areas, episodes of massive fish kills have been reported as a result of oxygen depletion (Ochumba, 1990). A disproportionate increase in phosphorous loading relative to nitrogen and silica loadings to the lake (Lehman and Branstrator 1994; Hecky, 1993; Lindenschmidt et al., 1998) has led to a pronounced shift in the phytoplankton composition, from a historical phytoplankton community dominated by green algae and large diatoms (e.g. Evans, 1962; Talling, 1966) to a present dominance of cyanobacteria (Ochumba and Kibaara, 1989; Lung'ayia, 2000; Kling et al., 2001). The dramatic ecosystem alterations have to a large extent been explained by the large food-web changes caused by "top-down" predation by the introduced Nile perch and the assumed overfishing (Goudswaard et al., 2008). Accordingly, the reduction in indigenous phytoplanktivorous fish has partly been blamed for the increase in algal biomass (Witte et al., 1992). On the other hand, the increased nutrient loading to the lake evidently gives a "bottom-up" effect and influences the phytoplankton productivity (Mugidde, 1993) meaning that the eutrophication also is a serious threat to the Lake Victoria ecosystem.

1.4.2 Phytoplankton community and cyanobacterial abundance in Lake Victoria

Phytoplankton investigations in Lake Victoria have been conducted ever since the late 19th century and the first reported phytoplankton investigation in the lake was accomplished by Schmidle (1902). After that, more than twenty studies on phytoplankton in the lake were published (review by Talling, 1987). Over the last two decades, the taxonomic and floristic observations have mainly appeared incidentally in relation to ecological studies. There are, however, some more recent studies on cyanobacterial population in Lake Victoria (Komàrek and Kling, 1991). Based on all of these investigations, Cocquyt et al. (1993) made a list of a total of 601 taxa of algae (mainly phytoplankton), belonging to 117 genera for the whole of Lake Victoria. Komárek and Kling (1991) emphasize that the extreme variation of populations and taxa complicates the identification of the genera and delimitation of different taxa, and further that Lake Victoria comprises several extremely variable species which are impossible to identify using the available keys and associated literature based on morphology. The phytoplankton community of Lake Victoria before severe antropogenic eutrophication (1960's) was dominated by diatoms, cyanobacteria and chlorophytes (Talling, 1987). The present composition of the phytoplankton community has to a large extent changed and there is proportionally more dominance of cyanobacteria and the diatom *Nitzschia* (Kling et al., 2001). Lake Victoria is differentiated in two main types of environments (Worthington, 1930): the shallow semi-enclosed gulfs and bays that are not deep enough to be persistently stratified, and the open lake waters with stratification and clear seasonality. Consequently, the phytoplankton communities in the inshore and offshore areas are differentiated (Talling, 1987). Cyanobacteria are increasingly predominant in the whole of Lake Victoria, and the diatoms are more abundant in offshore than inshore waters (Hecky, 1993; Lung'ayia et al., 2000; Kling et al., 2001). The offshore waters show seasonality with periods of stratification and periods of full mixing and changes in the mixed layer depth (Talling, 1987; Hecky et al. 1994; Spiegel and Coulter, 1996). Accordingly, diatoms develop during periods of vertical mixing, whereas cyanobacteria generally are more dominant in periods of stratification (Talling, 1987). In inshore shallow waters where cyanobacteria are dominating, the number of cyanobacterial species is found to be more diverse during dry season than in the rainy season (Lung'ayia et al., 2000).

The occurrence of cyanobacterial blooms in Lake Victoria was evident at the beginning of the 20th century (Ostenfeld, 1908) and currently mass populations of cyanobacteria near the shores of Lake Victoria are becoming an increasingly common phenomenon (Ochumba and

Kibaara, 1989; Hecky and Bugenyi, 1992; Hecky, 1993; Gophen et al., 1995; Lung'ayia et al., 2000; Kling et al., 2001; Krienitz et al., 2002). The most commonly reported cyanobacteria are *Anabaena* spp., *Microcystis* spp., *Planktolyngbia* spp., *Anabaenopsis* spp., and *Merismopedia* spp. (e.g. Talling, 1987; Ochumba and Kibaara, 1989; Lung'ayia et al., 2000; Kling et al., 2001; Krienitz et al., 2002). In Lake Victoria, changing nutrient conditions, silicon depletion and nitrogen limitation, light limitation, influence of dry and wet season, and food-web changes have been proposed as main factors regulation the phytoplankton populations (Mugidde, 1993; Lung'ayia et al., 2000; Kling et al., 2001; Gikuma-Njuru, 2005; Silsbe et al., 2006).

1.4.3 Cyanobacterial toxins in Lake Victoria and other East African lakes

Despite the large number of taxonomic and ecological studies in Lake Victoria, there is little knowledge on cyanobacterial toxins. Krienitz et al. (2002) reported an incident of microcystins (<1 μg L^{-1}) during a heavy bloom of *Anabaena flos-aquae* and *Anabaena discoidea* in a near shore area of Nyanza Gulf (Kenya). Sekadende et al. (2005) performed sampling over a four months period in Mwanza Gulf (Tanzania) and found microcystins (about 1 μg L^{-1}) in one sample dominated by the cyanobacterial species *Aphanocapsa* sp., *Anabaena* sp., *Planktolyngbya* spp. and *Microcystis* sp.

As for Lake Victoria, most of the research on cyanobacteria in other water bodies in the East African region has been related to taxonomic and ecological studies. The region comprises the large, deep freshwater lakes of the Western Rift Valley, a number of alkaline lakes and hot springs mainly in the Eastern Rift Valley, in addition to a number of smaller and middle sized freshwater lakes. In recent years, a series of studies of cyanobacteria and cyanobacterial toxins have been conducted in lakes in the Eastern Rift Valley in Kenya (Ballot et al., 2003; 2004; 2005; Krienitz et al., 2003; Mussagy et al., 2006) and Tanzania (Lugomela et al., 2006).

2 Aims of the study

The overall aim of this study was to improve the knowledge of the eutrophication level and the proliferation of toxin-producing cyanobacteria in Murchison Bay, Lake Victoria (Uganda).

This has been accomplished by addressing the following sub-goals:

- Assess the level of eutrophication in Murchison Bay and determine possible mechanisms affecting the eutrophication in the bay

- Investigate the diversity of the phytoplankton community in Murchison Bay, in particular the cyanobacterial population, and assess the influence of environmental factors

- Identify and quantify microcystins in Murchison Bay, identify the microcystin producing species and assess the influence of environmental factors on the microcystin production

- Characterise the morphological, genetical and chemical diversity of strains of *Microcystis aeruginosa* isolated from Murchison Bay and other water bodies in the East African region and infer phylogenetic relationships to strains from other geographic regions.

- Characterise the morphological, genetical and chemical variation of strains of *Cylindrospermopsis raciborskii* isolated from Uganda and Germany and infer phylogenetic relationships to strains from other geographic regions, and assess the distribution patterns of this species

3 Material and methods

3.1 Study area

The field studies were carried out in Murchison Bay in the north western part of Lake Victoria (Fig. 4). The bay is located between 00°04'N, 32°37'E and 00°18'N, 32°38'E and is an extension of Lake Victoria to the north towards Kampala, the capital of Uganda. The shallow embayment is 30 km long and the bottom has a gentle slope from the outlet of the Nakivubo channel to about 11 m depth at the Gaba narrows and to about 12 m at the outer part of the bay. The bay is divided in a semi-enclosed inner part and a wider outer part by narrows about 5 km from the inner shores. Murchison Bay serves as drinking water supply for Kampala and two water works, Gaba I and II, are situated just inside the narrows about 4 km out in the bay.

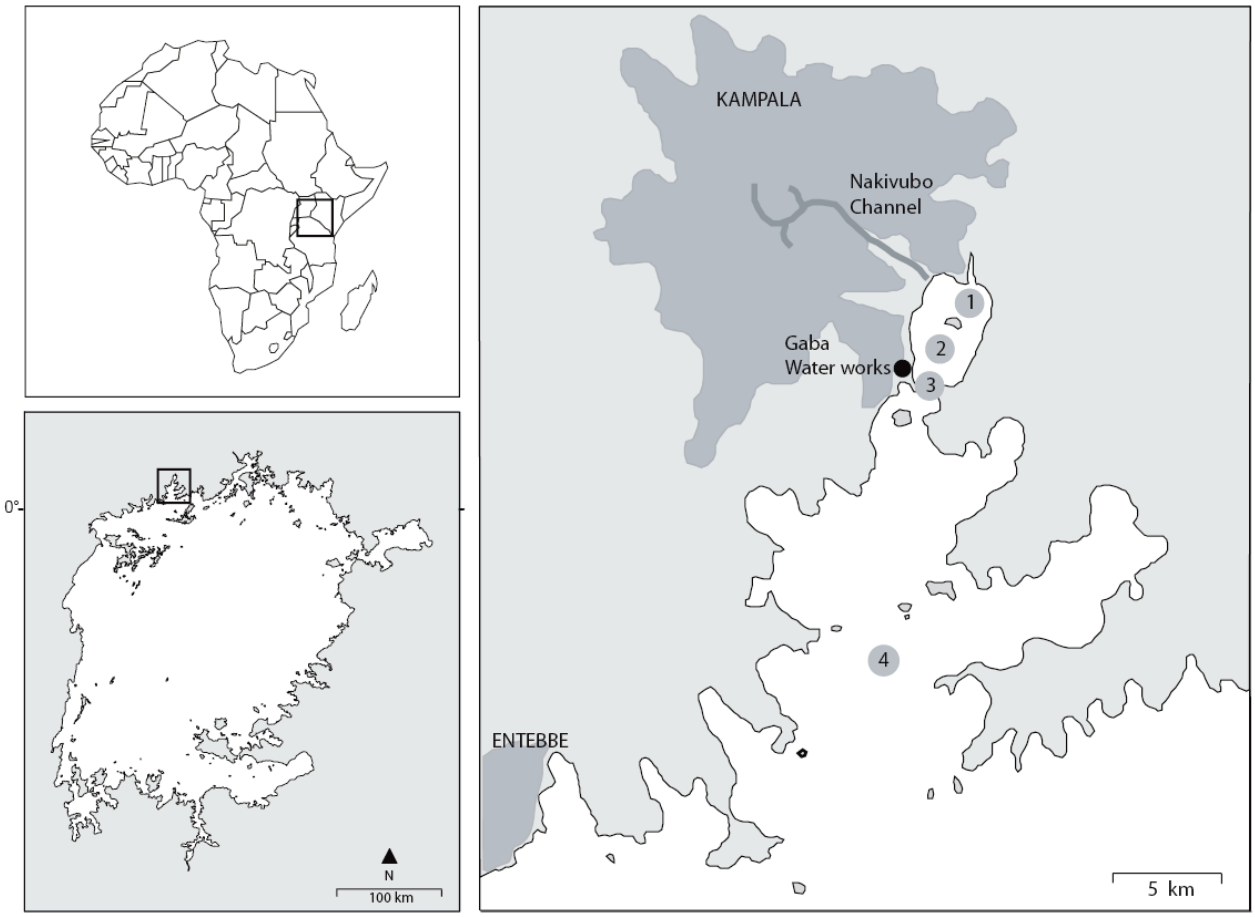

Figure 4 Map of Murchison Bay with the four sampling stations. The map also shows the cities of Kampala and Entebbe, the location of the water works and the Nakivubo Channel. The upper left map shows the African continent and the lower left map shows Lake Victoria and the position of Murchison Bay.

The Inner Murchison Bay (Fig. 5; mean depth 3.2 m) covers an area of about 18 km^2 and has a catchment area of 282 km^2, comprising large parts of the urban areas and settlement of Kampala (>1 million inhabitants), in addition to small scale farmlands and grasslands, and the shoreline is surrounded by large papyrus swamps (Schröder et al., 1998). As a consequence of urban development and expansion, the wetlands in most parts of the city have been drained and turned into agricultural areas or developed for commercial, industrial or residential purposes (Kansiime et al., 2005). The most significant drainage of the catchment area is the Nakivubo channel which runs through Kampala and the wetland areas surrounding the bay, entering the inner part of the bay from the north (Kansiime et al., 2005). Nakivubo channel was constructed some fifty years ago and was designed to carry storm water as fast as possible from the city of Kampala into Murchison Bay. The drainage channels in Kampala were developed to terminate in the Nakivubo channel and sewage was routed through a high level and low level system to Bugolobi sewage treatment works. Kampala is now expanding fast both in population and infrastructure, and unfortunately the sewage systems and the wastewater treatment have not expanded accordingly. In addition, due to old age and blockage of some sewers, part of the raw sewage leaks directly into the Nakivubo channel. Consequently the Nakivubo channel is now receiving a considerable portion of partially treated and untreated wastewater, both of industrial and domestic origin (Schröder et al., 1998).

Figure 5 Murchison Bay at Gaba. The arrow indicates the intake of water to the Gaba II water works.

In the past, the Nakivubo channel ended in the outer part of the wetland areas, allowing the water to be drained in the papyrus swamps before entering the bay. After heavy rain, the flow in the Nakivobo channel increases very fast, and consequently the runoff water flushes into the bay (Schröder et al., 1998; Kansiime et al., 2005). In 2001-2003, however, the channel was enlarged in order to remove storm water more efficiently from the urban areas. It was widened to about 20 m and stretched through the wetland ending only a couple of hundred meters before Murchison Bay. The water in the channel is a mixture of secondary effluents from the Bugolobi sewage treatment works and heavily polluted untreated wastewater from the city. Today, however, the predominant papyrus wetland has been converted to cocoyam fields, and the retention of nutrients and other pollutants is moderate to absent, increasing the concerns for the water quality in the bay (Kansiime et al., 2005).

The Lake Victoria region has an equatorial climate with small seasonal variations in solar radiation. Measurements of global photosynthetic active radiation (PAR) at Makerere University in Kampala, over a 5 year period (2003-2007), show that there is an annual average light intensity of 1500-2500 μmol m^{-2} s^{-1} in the area of Murchison Bay (NUFU project 03/22). The mean annual temperatures in areas close to Murchison Bay range between 21.5 and 22.5 °C. As a rule, there are two rainy seasons, the long rains from March to May with a peak in April and the shorter rains from November to October. There are, however, geographical and annual variations.

3.2 Sampling and cultures

We established four stations in a longitudinal transect from the inner to the outer bay (Fig 4; Table 2). The sampling of Murchison Bay was carried out from November 2000 to March 2004. The sampling frequency was generally every month in the period from November 2000 to March 2003 and every second week in the period from April 2003 to March 2004. All material and methods used in the studies are described in detail in the respective papers **I**-**V**.

Table 2 Geographical position, names and maximum depth of the stations in Murchison Bay

Station nr.	Depth (m)	Name	Geographical position
St. 1	1.5	Mouth of Nakivubo Channel	00°17.076'N, 32° 38.496'E
St. 2	5	South of Namalusu Island	00°15.727'N, 32° 38.749'E
St. 3	11	Gaba Narrows	00°14.480'N, 32° 38.589'E
St. 4	12	South of Makusu Island	00°08.715'N, 32° 37.580'E

We have isolated a number of cyanobacterial strains from different water bodies in East Africa in addition to Murchison Bay. An overview over the strains and their geographical origin is given in Table 3. The strains were used in the studies **IV** and **V**. The geographical locations of the other lakes where strains were isolated from are shown on the map in Fig. 3.

Table 3 Cyanobacterial strains used in this study and their geographical origin. The year of isolation and the person responsible for strains isolation is also given (SH=Sigrid Haande, RS=Randi Skulberg, AB=Andreas Ballot, MB=Martin Beck)

Strain	Geographic origin	Isolation year	Isolated by
Microcystis aeruginosa			
NIVA-CYA 431	Murchison Bay, Uganda	2000	RS
NIVA-CYA 432	Murchison Bay, Uganda	2000	RS
NIVA-CYA 433	Murchison Bay, Uganda	2000	RS
NIVA-CYA 463	Murchison Bay, Uganda	2003	SH
NIVA-CYA 464	Murchison Bay, Uganda	2003	SH
NIVA-CYA 465	Murchison Bay, Uganda	2003	SH
NIVA-CYA 475	Murchison Bay, Uganda	2003	SH
NIVA-CYA 476	Murchison Bay, Uganda	2004	SH
NIVA-CYA 477	Murchison Bay, Uganda	2003	SH
NIVA-CYA 478	Murchison Bay, Uganda	2003	SH
NIVA-CYA 482	Lake Mburo, Uganda	2004	RS
NIVA-CYA 495	Kazinga Channel, Uganda	2004	RS
NIVA-CYA 496	Kazinga Channel, Uganda	2004	RS
NIVA-CYA 497	Kazinga Channel, Uganda	2004	RS
NIVA-CYA 502	Murchison Bay, Uganda	2004	SH
NIVA-CYA 503	Murchison Bay, Uganda	2004	SH
NIVA-CYA 522	Murchison Bay, Uganda	2004	SH
AB2002/21	Nakuru final sewage pond, Kenya	2002	AB
AB2002/22	Kenyatta University sewage pond, Kenya	2002	AB
AB2002/23	Lake Baringo, Kenya	2002	AB
AB2002/24	Pilsner Pond, Kenya	2002	AB
AB2002/40	Lake Naivasha, Kenya	2002	AB
AB2002/52	Lake Baringo, Kenya	2002	AB
AB2002/55	Kenyatta University sewage pond, Kenya	2002	AB
Cylindrospermopsis raciborskii			
NIVA-CYA 506	Kazinga Channel, Uganda	2004	RS
NIVA-CYA 507	Kazinga Channel, Uganda	2004	RS
NIVA-CYA 508	Kazinga Channel, Uganda	2004	RS
NIVA-CYA 509	Kazinga Channel, Uganda	2004	RS
NIVA-CYA 510	Kazinga Channel, Uganda	2004	RS
NIVA-CYA 511	Lake Victoria, Uganda	2004	RS
ZIE05CR	Zierker See, Germany	2005	MB
ZIE11CR	Zierker See, Germany	2005	MB
ZIE13CR	Zierker See, Germany	2005	MB

4 Results and discussion

4.1 Eutrophication in Murchison Bay

During the period of our study from 2000-2004, the Inner Murchison Bay had high average concentrations of total phosphorous (>90 µg L^{-1}), total nitrogen (>1100 µg L^{-1}) and Chl-*a* (> 30 mg L^{-1}) and the phytoplankton community was dominated by a variety of cyanobacterial species (**I**, **II**, **III**). These results correspond to the general view of an ongoing eutrophication in Lake Victoria and several studies from other inshore bays and gulfs like Napoleon Gulf, Pilkington Bay, Nyanza Gulf and Mwanza Gulf of Lake Victoria have reported similar trends of nutrient enrichment (Hecky, 1993; Calamari, 1995; Mugidde, 1993; Lung'ayia et al., 2001; Gikuma-Njuru and Hecky, 2005; Sekandende et al., 2005). One of the most severe problems related to eutrophication in freshwater ecosystems worldwide is the occurrence of cyanobacterial blooms. We found that the concentration of total microcystin in the bay at times exceeded the WHO guide line value of 1 µg L^{-1} MC for drinking water (**III**), and this may present a health risk for the users of the lake water.

Two surveys in the inner part of the Murchison Bay conducted in the 1990s concluded that there had been a major increase in nutrient loading to the bay since the 1960's (Källqvist et al., 1996; Schröder et al., 1998, and references therein). Källqvist et al. (1996) measured concentrations of TP <50 µg L^{-1}, TN <500 µg L^{-1} and Chl-*a* about 20 mg L^{-1} in a surface sample (0-2 m) at a site close to the Gaba water works (close to our St. 2). In a seven months long monitoring survey in 1997, Schröder et al. (1998) found increased concentration of nutrients and Chl-*a* in surface samples at the same location; 82 µg L^{-1} TP, 782 µg L^{-1} TN and 54 mg L^{-1} Chl-*a*, however, emphasizing that the study period was characterized by unusually heavy rainfalls, thus causing unusually high runoff to the bay. Our data showed even higher values of TP and TN, but the Chl-*a* values were lower than found in 1997. In general, there has been an increase in the nutrient loading of Murchison Bay over the last decade (**I**, **II**, **III**), indicating an expanding eutrophication of the bay.

Kampala is the largest city in the Lake Victoria region, and most of the urban settlement lies within the catchment area of Murchison Bay. Most of the waste water from the city is discharged through the enlarged Nakivubo channel, ending in the swamps close to the Inner Murchison Bay. The main pollution sources to Murchison Bay are domestic and industrial waste water, sewage effluents and surface runoff mainly from the urban areas of Kampala

(Schröder et al., 1998; Kansiime et al., 2005). It has been estimated that about 80 % of the nutrients from the Nakivubo channel come from domestic (non-industrial) sources (Schröder et al., 1998). It is likely that the enlargement of Nakivubo channel can lead to even higher nutrient loadings in Murchsion Bay. Our data showed a marked increase in the nutrient concentration in Murchison Bay after September 2003 (**II**, **III**), and this may be an effect of construction work of the Nakivubo channel in the wetland areas close to the bay around this time (Kansiime, pers. com.). Data from sampling in 2005 indicate that there has been a continued increase in the nutrient concentration in Murchison Bay (Ronald Semyalo, unpublished data), however, long term monitoring must be performed to investigate the possible effects of the enlarged channel and thereby also give an even better understanding of the eutrophication process in the bay.

4.2 Surface seiches mediated eutrophication in Murchison Bay

The nutrient enrichment in the Inner Murchison Bay was evident (**I, II, III**), but the data also show that there was a rapid decrease in conductivity and nutrient concentrations from the innermost part of the bay (St. 1) towards St. 4 in the outer part of the bay (**I**). Schröder et al. (1998) found a similar gradient of decreasing nutrient concentrations with increasing distance from the Nakivubo Swamp. Figure 6 is based on the data collected in the period from April 2003- March 2004 and illustrates the gradient of TP and TN from St. 1 to St. 4. At St. 1, the levels of TP and TN were varying from 70-600 µg L^{-1} and 500-5000 µg L^{-1}, respectively, reflecting a highly variable input to the bay. At the other stations in the bay, the nutrient levels were less variable.

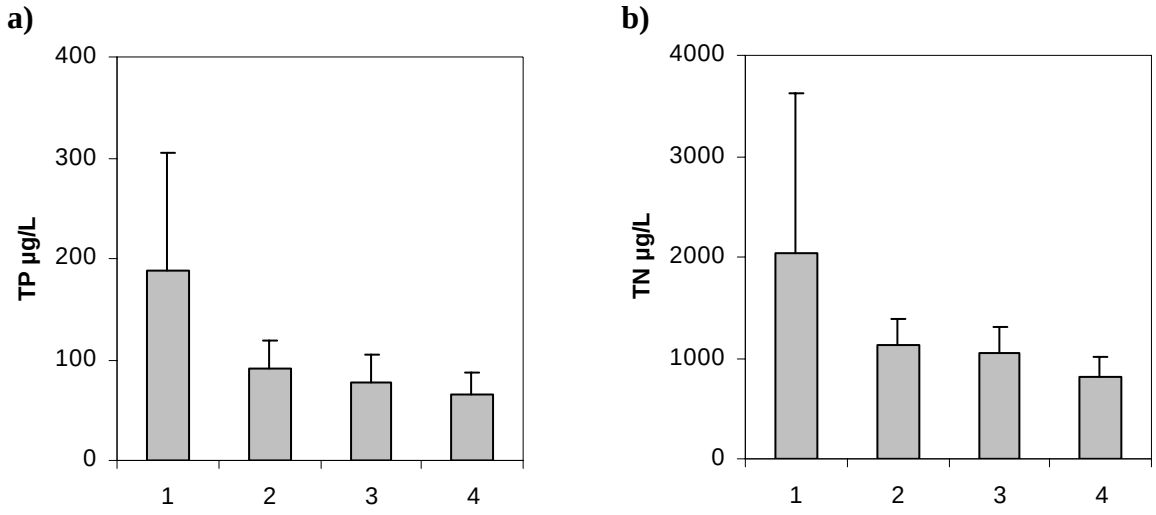

Figure 6 Average concentration and standard deviation (*n*=25) of a) total phosphorous (TP) and b) total nitrogen at the four stations in Murchison Bay in April 2003-March 2004

Despite the excessive amounts of nutrients entering the bay, the nutrient concentration decreased rapidly (**I**) and the phytoplankton biomass (**II**, **III**) was not as high as expected with respect to the nutrient input. Murchison Bay is an open system and is therefore also influenced by water exchange with the main lake. Likely, biotic uptake of nutrients causes some reduction of the nutrient concentrations in the bay, however, Kansiime and Nalubega (1999) pointed out that water exchange between the inner and outer parts of the bay may additionally be responsible for the dilution of pollutants from the Inner Murchison Bay. The water quality and the biological processes in a bay will be influenced both by the water from the watershed and the water exchange with the main lake.

Seiches in great lakes are recognised to play a similar role to that of tides in marine environments in organizing the structure and function of estuaries, embayments and coastal ecosystems (Bedford, 1992; Keough, et al., 1999; Trebitz, 2006). Seiches are standing waves that move water up and down (Ji and Jin, 2006), and in coastal areas, seiche driven water level fluctuations can cause water exchange in shallow embayments (Trebitz, 2006). Seiches are mainly caused by wind and air pressure variation over the lake, but in addition there is a small component of astronomic tide that will modify the seiches (Trebitz, 2006). In the Great Lakes in North America, short-term water level fluctuations have a typical variation from a couple of cm to about 50 cm (Trebitz, 2006) and Mortimer (2004) stated that the Great Lakes are large enough to have substantial and persistent surface seiches. We therefore hypothesized that surface seiches in Lake Victoria might be a main factor in the water exchange in Murchison Bay, and thereby strongly affect the dilution process and water quality in the bay (**I**). Measurements of hourly water level at Entebbe showed a daily variation in water levels between 1.9 and 18.8 cm. Generally, there was one main maximum and one main minimum in water level each day, with additional 4-7 smaller maxima and minima. These water level variations seemed to be both a daily rhythm and more frequent oscillations appearing in 3-5 hour cycles. On average, the total daily water level amplitude was 6.4 cm. We expected these fairly regular water level fluctuations to mainly be caused by surface seiches and thus the seiches caused water transport in and out of the bay. The daily water level fluctuations in Murchison Bay were in the same order of magnitude as found in other large lakes like Lake Okeechobee in Florida (Ji and Jin, 2006), and in the lower end of what has been recorded in the Great Lakes in North America (Trebitz, 2006).

In Murchison Bay, the incoming water from the catchment area had higher conductivity and nutrient concentrations than the water in the bay, and we could therefore calculate an estimated dilution of these parameters in the bay (**I**). Based on the measured water level fluctuation data, we performed simple calculations describing a seiche driven water exchange in Murchison Bay in order to investigate the dilution process in the bay (**I**). The calculations and equations used are described in detail in the materials and methods part of Paper **I**. Briefly, the volume of the daily water exchange between the bay and the lake was calculated as the average daily difference in water volume at maximum and minimum water level. Based on the volume of water in the bay (Schröder et al., 1998), the volume of water exchanged with the main lake (estimated on basis of water level fluctuation data, **I**) and the volume of water coming from the watershed (Schröder et al., 1998), we found a dilution factor of the incoming water of 9.6. The expected conductivity or nutrient concentration after mixing in the bay was calculated for each sampling date on the basis of the concentrations at St. 1 and at St. 4 and the estimated dilution ratio (9.6). A non-linear least square regression model was used to describe the reduction of the various components throughout the bay. The estimated values for each station were compared to the actually measured values of a given parameter at the same stations, and a good agreement between estimated and measured values would indicate that the water exchange was driven by seiches. The results showed a good agreement between estimated and measured conductivity in the bay (**I**). Conductivity is regarded to be biologically neutral, and therefore the accordance in measured and estimated conductivity supported our theory that seiches dominated the water exchange in the bay. The corresponding estimated and measured values of conductivity likely excludes a significant impact of other factors affecting water exchange, like currents driven by local wind or currents caused by inflowing water from the catchment area. TP and particularly PO_4-P tended to be more reduced in the inner part of the bay when compared to the estimated values, and this is most likely due to biological uptake by phytoplankton and water hyacinths, and also sedimentation processes. The measured values of TN did not decrease accordingly to the estimated values, and this might be due to N-fixation. Murchison Bay is dominated by N-fixing cyanobacteria, mostly of the genera *Anabaena* (**II**, **III**), and we found that the phytoplankton community is likely to be N-limited (**II**). N-fixing species of cyanobacteria are abundant both in inshore and offshore areas of Lake Victoria (Kling et al., 2001). Mugidde (2003) found that N-fixation in Napoleon Gulf, an inshore area in the northern part of Lake Victoria, significantly contributed to the overall N budget of this gulf.

The semi-enclosed Inner Murchison Bay has so far been regarded to have quite limited water exchange with the main lake (Schröder et al., 1998), but our results contradicted this view, and showed that there is a significant daily transport of water in and out of the bay (**I**). The water exchange in the bay caused a dilution of nutrients in Murchison Bay and thus mediated the eutrophication process in the bay. There was a rapid transport of nutrients to the open lake and this emphasizes the significance of Murchison Bay as a major contributor to the eutrophication process of Lake Victoria. However, water from the main lake will also be transported into the bay and thereby influence the bay ecosystem. Gikuma-Njuru and Hecky (2005) found similar values of TP, TN and Chl-*a* in Nyanza Gulf in the Kenyan part of the lake as we found in Murchison Bay. However, they found the TP concentrations to be higher in the open lake, than in the gulf, and they claimed that the gulf was a sink for P rather than contributing to the P values in the open lake. Our study has revealed that Murchison Bay has considerable water exchange with the main lake and is probably a more dynamic system than first recognized (**I**).

4.3 Phytoplankton community and cyanobacterial dominance in Murchison Bay

The phytoplankton community in Murchison Bay was studied in the period from April 2003-March 2004 (**II**, **III**). We selected St. 2 as the representative site for the Inner Murchison Bay and St. 4 as a representative for the outer part of the bay (**II**, **III**). Our studies show that the phytoplankton community was dominated by cyanobacteria and diatoms. The cyanobacteria were equally dominant in both parts of the bay, whereas the diatoms were more abundant in the outer part of the bay. Species of Chlorophyceae and Cryptophyceae and different types of picoplankton (cell diameter < 2 μm) were present throughout the year, whereas species of Chrysophyceae, Dinophyceae and Euglenophyceae only were found at some occasions. The average total phytoplankton biovolume was 1.7 ± 1.0 mm^3 L^{-1} (mean value SD, n=24) at St. 2 and 2.6 ± 1.0 mm^3 L^{-1} at St. 4 (**II**, **III**). The composition of phytoplankton in Murchison Bay is in accordance to other studies on phytoplankton in other inshore and coastal areas of Lake Victoria (Ochumba and Kibaara, 1989; Hecky and Bugenyi, 1992; Hecky, 1993; Gophen et al., 1995; Lung'ayia et al., 2000; Kling et al., 2001; Krienitz et al., 2002; Sekandende et al., 2005).

Despite the clear dominance of cyanobacteria in the phytoplankton community, the diatom *Nitzschia acicularis* was the single most abundant species in Murchison Bay and comprised about 28 % of the total biomass at St. 4 (**II**). At St. 2, this species was less dominant, but here

the diatom *Aulacoseira granulata* was the single most abundant species and constituted about 15 % of the total biomass (**II**). This species was nearly absent at St. 4 (**II**). Other studies from inshore and coastal areas of Lake Victoria found a similar distribution pattern of these diatom species (Lung'ayia et al., 2000; Kling et al., 2001). In Murchison Bay, the silicon (Si) concentration was higher in the inner part of the bay and decreased to lower concentrations at St. 4 in the outer part of the bay (**II**), and this can explain the different distribution of the two diatom species. The increased nutrient input to Lake Victoria since the 1960s caused an overall increase in the diatom biomass and as a consequence, the concentration of dissolved Si has been depleted in the lake (Kilham et al., 1986; Hecky, 1993). *Aulacoseira* was earlier the most abundant diatom in the offshore areas of Lake Victoria, but is now excluded by the continuously low Si concentrations (Hecky, 1993; Kling et al., 2001) and can only be abundant in near-shore areas where there is input of Si from the catchment area. The smaller *Nitzschia acicularis* is less Si dependent (Kilham et al., 1986) and is now the most dominant diatom species in the offshore waters of Lake Victoria (Hecky, 1993; Kling et al., 2001). The total biomass was highest in the outer part of the bay, whereas the Chl-*a* concentration was higher in the inner part of the bay, and this is most likely due to the different composition of the phytoplankton community in the different parts of the bay with a very high proportion of the diatom *Nitzschia acicularis* in the outer part of the bay.

More than 20 species of cyanobacteria were found in Murchison Bay (**II**, **III**). Species of the genus *Anabaena* (mainly *Anabaena flos-aquae*) were dominating at both stations in the bay, and constituted about 12 % (St. 2) and 25 % (St. 4) of the total phytoplankton biomass. Other abundant cyanobacterial species at St. 2 were (% of total biomass); *Microcystis wesenbergii* (11.6 %), *Gomphosphaeria aponina* (8.6 %), *Microcystis aeruginosa* (8.5 %), and *Planktolyngbya circumcreta* (6.2 %), and at St. 4; *P. circumcreta* (6.7 %), *M. wesenbergii* (5.8 %), and *G. aponina* (5.5 %) (**II**, **III**). The average total biomass of cyanobacteria was 1.0 ± 0.7 mm^3 L^{-1} (mean value SD, n=24) at St. 2 and 1.6 ± 0.7 mm^3 L^{-1} at St. 4 (**II**, **III**). Our data confirm the general view that *Anabaena* spp. are the most abundant cyanobacterial species in inshore and offshore waters in Lake Victoria (Talling, 1987; Ochumba and Kibaara, 1989; Lung'ayia et al., 2000; Kling et al., 2001). Other commonly reported cyanobacterial species in inshore areas are *Microcystis* sp., *Planktolyngbya* sp., and *Aphanocapsa* sp. (Talling, 1987; Lung'ayia et al., 2000; Kling et al., 2001; Sekandende et al., 2005).

Murchison Bay is situated at the equator where rainy and dry seasons create seasonal variations. The measured water temperatures were high over the whole year (23.3 - 27.8 °C; **II**, **III**), generally higher during the rainy seasons, but all in all comprising a good environment for phytoplankton growth. We did not observe higher biomass of phytoplankton during the rainy seasons (**II**), as found in other studies in inshore areas of Lake Victoria (Lung'ayia et al., 2000). However, the relative proportion of cyanobacteria and diatoms changed from rainy to dry seasons at both stations, with a higher proportion of cyanobacteria in the periods of dry season and a higher proportion of diatoms in the periods of rainy seasons (**II**). Earlier investigations also found a higher proportion of diatoms during rainy seasons in Lake Victoria (Talling, 1987; Lung'ayia et al., 2000; Kling et al., 2001). Cyanobacterial dominance is generally associated with high nutrient concentrations and eutrophication (e.g. Dokulil and Teubner, 2000). As already described, there was a substantial nutrient loading to the Inner Murchison Bay (**I**, **II**, **III**), and in general, the phytoplankton biovolume was comparably low over the whole study period (0.7-4.7 mm^3 L^{-1} at St. 2 and 0.9-5.3 mm^3 L^{-1} at St. 4) (**II**, **III**). In addition, the nutrient concentrations in Murchison Bay clearly increased in September 2003-March 2004 without any corresponding change of the phytoplankton biovolume (**II**, **III**). The average phytoplankton biomasses we found in our investigations (**II**, **III**) did not differ significantly from what was found by Källqvist et al. (1996) in the Inner Murchison Bay, whereas the nutrient loading has increased over the last decade. Taken together, it is likely that there is a limitation of the phytoplankton growth or that loss factors control the phytoplankton biovolume in Murchison Bay. Several factors have been proposed for regulating phytoplankton populations in Lake Victoria: N-limitation which favours N-fixing cyanobacteria (Kling et al., 2001), self shading by high-chlorophyll standing crops (Mugidde, 1993), deep mixing depth in the open lake imposing light limitation (Mugidde, 1993; Kling et al., 2001), light-limitation in inshore areas (Gikuma-Njuru and Hecky, 2005; Silsbe et al., 2006) the influence of dry or wet season (Lung'ayia et al., 2000), and depletion of silica (Verschuren et al., 2002). We estimated the euphotic depth to be 4 m in the outer part of the bay (depth, 12 m) and 3 m in the shallower inner part of the bay (depth 5 m) (**II**, **III**). The regular vertical mixing of the water column in Murchison Bay causes a frequent movement of the phytoplankton well below the euphotic zone, thus leading to possible light limitation in periods of the day, especially in the outer part of the bay. This regular mixing, however, may also counteract the effect of self-shading proposed by Mugidde (1993). Most cyanobacterial species can have maximal growth when PO_4-P concentrations are >10 µg L^{-1}, and in addition, most cyanobacterial species have a capacity to store P (Isvánovics et al.,

2000). The concentrations of free PO_4-P in Murchison Bay were >10 µg L^{-1} at both stations in the whole study period, and it is therefore likely that the phytoplankton growth was not limited by P. Lehman and Branstrator (1994) also concluded that the Lake Victoria phytoplankton rarely, if ever experienced P limitation. N-limitation can be suspected, since there were detected low NO_3-N values (< 10 µg L^{-1}) at both stations at most times of the study period. There was, however, a period of higher detected NO_3-N concentrations at St. 2 in June-July without any detectable effect in the phytoplankton biomass. The composition of the cyanobacterial population was clearly different in the two parts of the bay, with a higher proportion of heterocystous N-fixing species like *Anabaena* sp. in the outer part of the bay, and a higher proportion of non-heterocystous species like *Microcystis* sp. in the inner part of the bay, thus indicating an effect of nitrogen on the cyanobacterial composition.

Murchison Bay is a dynamic system with horizontal and vertical water movements causing continuous water turbulence (**I**, **II**, **III**), and this may strongly affect the development of phytoplankton in Murchison Bay. The continuous mixing of the water column may reduce the capability of buoyancy by cyanobacterial species with gas-vacuoles. In addition, the significant water exchange with the main lake is also likely to cause transport of phytoplankton in the bay. Regular fishing in Murchison Bay revealed an abundance of the phytoplankivorous Nile Tilapia, *Oreochromis niloticus* (Ronald Semyalo, unpublished results). Cyanobacteria account for a significant part of the diet of adult Nile Tilapia (McDonald, 1987) and studies have shown that this species may cause reduction of cyanobacteria in eutrophic water bodies (Miura, 1990; Datta and Jana, 1998). Therefore, grazing by phytoplanktivorous fish may also affect the phytoplankton community in Murchison Bay, but this was not investigated closer in our study.

4.4 Microcystin production in Murchison Bay

Several studies in Lake Victoria, ever since the beginning of the last century, have reported episodes of cyanobacterial blooms (for a review see Talling, 1987), and more recent records have reported increased mass populations of cyanobacteria, especially in near shore areas of the lake (eg. Ochumba and Kibaara, 1989; Hecky and Bugenyi, 1992; Hecky, 1993; Gophen et al., 1995; Lung'ayia et al., 2000; Kling et al., 2001; Krienitz et al., 2002; Sekandende et al., 2005; papers **II**, **III**). There are comparably few reports on the occurrence of cyanotoxins, and to our knowledge, only two studies have investigated the presence of cyanotoxins (microcystins) in Lake Victoria (Krinietz et al., 2002; Sekadende et al., 2005). We therefore

aimed to study the occurrence of microcystins in Murchison Bay by analysing samples collected in the period from April 2003 to March 2004 at St. 2 and St. 4 (**III**). We detected microcystins in all samples at both stations, and there were times when the microcystin concentration exceeded the WHO guide line value of 1 μg L^{-1} MC for drinking water (**III**). We analysed for three different types of microcystins (MC-YR, MC-RR, MC-LR) and found all types at both stations throughout the studied year. Microcystin-RR was the most abundant type of microcystin, followed by MC-YR and –LR.

We found species of *Microcystis*, *Anabaena* and *Anabaenopsis* in the diverse cyanobacterial population in Murchison Bay (**II**, **III**), and these genera are known to contain microcystin producing species (Sivonen and Jones, 1999; Codd et al., 2005a). Based on probability analysis (for details, see Paper **III**), *M. aeruginosa* and *Anabaena* sp. were identified as the possible microcystin producers in Murchison Bay. The t-values, however (**III**), were higher for *M. aeruginosa* than for *Anabaena* sp., indicating that most of the variation in microcystin concentration can be explained by the abundance of *M. aeruginosa*. *M. aeruginosa* is the most commonly reported bloom-forming species in lakes and reservoirs worldwide (e.g. Carmichael, 1996; Sivonen and Jones, 1999) and can produce microcystin in a variety of forms and with varying toxicity (Carmichael et al., 1988; Lee et al., 2000; Sivonen and Jones, 1999). Lanaras and Cook (1994) have reported microcystin in bloom material dominated by *Anabaenopsis millerii* from Lake Porto Lagos, Greece, suggesting that members of the genus *Anabaenopsis* may produce microcystins. Species of *Anabaena* are also known microcystin producers (Sivonen and Jones, 1999; Codd et al., 2005a), but our analyses did not identify *Anabaena* sp. to contribute to the microcystin-production in Murchison Bay. We therefore concluded that *M. aeruginosa* most likely was the main microcystin producer among the species found in Murchison Bay (**III**). Krienitz et al. (2002) studied a dense cyanobacterial bloom dominated by *Anabaena flos-aquae*, *Anabaena discoidea* and *Microcystis aeruginosa* in Nyanza Gulf (Kenya) and found detectable values (< 1μg/L) of microcystin-RR, -LR, -LA and LF. The microcystin concentration was, however, comparably low relative to the biomass of potentially microcystin producing species. Sekadende et al. (2005) performed sampling over a four months period in Mwanza Gulf (Tanzania) and found microcystins (about 1 μg/L) in one sample dominated by the cyanobacterial species *Aphanocapsa* sp., *Anabaena* sp., *Planktolyngbya* spp. and *Microcystis* sp.

In paper **II** we studied the possible effect of environmental factors on the phytoplankton community in Murchison Bay, and in paper **III** we made an attempt to investigate the possible effects of environmental factors on the microcystin production in the bay. Several environmental factors, however, affect both the growth and composition of the cyanobacterial population as well as the production and composition of microcystins (e.g. Kotak, 2000; Oh et al., 2001), hence highlighting the invariable complexity in the relationship between cyanobacteria, microcystin concentration and environmental factors.

We used correlation analyses and found that the most influencing environmental parameters on the microcystin concentration were TP, PO_4-P, TN, Si and euphotic depth (**III**). The influence of light is important for cyanobacterial growth and microcystin production (Utkilen and Gjølme, 1992; Havens et al., 1998; Kaebernick et al., 2000; White et al., 2003; Kurmayer et al., 2003; Wiedner et al., 2003). We found that the phytoplankton in Murchison Bay may be influenced by light limitation (**II**), thus the microcystin production may also be influenced by the light climate in the bay (**III**). Nutrient dynamics can influence the cyanobacterial biomass as well as the microcystin concentration (Kotak et al., 2000; Oh et al., 2001). In this study, TP and TN were positively correlated with microcystin concentration (**III**) which is in accordance to other field studies (e.g Graham et al., 2004; Albay et al., 2005). In Murchison Bay, the highest microcystin concentration was detected at the same time as the highest detected biomass of *M. aeruginosa* and coincided with the highest detected concentrations of TP (140 μg L^{-1}) and the third highest detected concentration of TN (1400 μg L^{-1}) in the studied period. An increase in the microcystin concentration after September 2003 (**III**) coincided with the increase in nutrient concentration in Murchison Bay in September 2003-March 2004 (**II**, **III**). Graham et al. (2004) found highest microcystin production with increasing TP concentrations (maximum at 100-600 μg L^{-1}) and increasing TN concentrations (maximum at 1500-4000 μg L^{-1}). It is therefore most likely that the increase in N availability in Murchison Bay affected the microcystin production. We found that the phytoplankton community in Murchison Bay could be N limited (**II**), and it is therefore likely that an increase in N may influence the microcystin production (**III**).

There was no good correlation between microcystin production and the total biomass of cyanobacteria (**III**), and this probably reflects the diverse cyanobacterial community in Murchison Bay consisting of both microcystin producing and non-producing strains. Studies have shown that a potential microcystin producing species like *M. aeruginosa* can consist of

both producing and non-producing strains (e.g. Shirai et al., 1991; Kurmayer et al., 2004; Paper **IV**). Further, the cellular microcystin concentration is strain dependant and can vary by several orders of magnitude between strains (Chorus, 2001). The microcystin production is therefore dependent on the strain composition and the regulation of microcystin biosynthesis in specific strains under certain environmental conditions. Individual strains also have different environmental optima for growth and microcystin production, and respond differently to changing environmental conditions (Sivonen, 1990; Véize et al., 2002). Taken together, nutrient availability and light climate seemed to be the most probable influencing environmental parameters on the microcystin production in Murchison Bay, however, the microcystin concentrations could not be predicted by a given environmental factor alone, by the biovolume of cyanobacteria or of certain cyanobacterial species (**III**).

4.5 Phylogeny and characterisation of cyanobacterial strains from Murchison Bay and other East-African water bodies

We isolated strains of *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii* from Murchison Bay and other water bodies in the East African region and strains of *C. raciborskii* from Europe (see Table 2 and 3). The strains were morphologically, genetically and chemically characterized in order to obtain knowledge on the diversity and toxicity of these cyanobacterial species from this region (**IV**, **V**). We also analysed the phylogenetic relationship between strains of the same genus from different geographical origins, and assessed the overall genetic variation and distribution patterns of these species (**IV**, **V**).

4.5.1 Microcystis aeruginosa

M. aeruginosa has a cosmopolitan distribution (Komárek and Anagnostidis, 1999) and is one of the most studied species among cyanobacteria. The knowledge on its taxonomy, toxicity and diversity is increasing, however, few studies have included strains from the African continent. We isolated 24 strains of *M. aeruginosa* and found a large degree of morphological, genetical, and chemical diversity (**IV**).

Traditionally, the assessment of diversity within *M. aeruginosa* has focused on morphological variation, such as cell size and colony shape (Watanabe, 1996). However, *M. aeruginosa* is known to comprise large morphological variability, and thus the differentiation within this species is difficult and several morphotypes can occur within the same population (Otsuka et al., 2000). Additionally, the morphology can change in response to environmental factors,

causing problems in the application of morphological criteria in the classification of *M. aeruginosa* (Dor and Hornoff, 1985). Strains of *M. aeruginosa* also tend to change their morphological characteristics when isolated and subjected to different culture conditions (e.g. Krüger et al., 1981; Komárek, 1991; Otsuka et al., 2000). Despite the possible changes of morphological features of isolates during culturing, we distinguished four morphotypes of *M. aeruginosa* among the isolated strains (**IV**). We sequenced the PC-IGS gene region and the ITS1 rDNA region of the isolated strains and found some correspondence between morphotypes and genotypes (**IV**). Several studies with these genetic markers have shown contradictory results; both various genotypes with uniform morphological characteristics (Kato et al., 1991; Nishihara et al., 1997; Otsuka et al., 1999; Bittencourt-Oliveira et al., 2001) and genotypes with poor consistence to morphology (Otsuka et al., 1999; 2000; Tillett et al., 2001; Janse et al., 2004; Yoshida et al., 2005). Consequently, the concordance between morphological variation and genetic variation is often not clear in *M. aeruginosa* populations.

The two genetic markers showed congruent phylogenies and discriminated 10 genotypes among the strains from East Africa (**IV**). We found four different genotypes among the thirteen strains isolated from Murchison Bay, and this is in accordance to previous studies that have found two to six genotypes in a single water body (Kato et al., 1991; Bolch et al., 1997; Bittencourt-Oliveira et al., 2001). It is, however, likely that the genetic diversity in the bay itself is higher due to the possibility that some clones of *M. aeruginosa* have low survival rates when isolated (Wilson et al., 2005). The phylogenetic analyses with the PC-IGS sequences of the East African *M. aeruginosa* strains and sequences from *Microcystis* spp. strains from different geographical regions showed a separation in three clusters (**IV**). Cluster I included most of the strains from Uganda and Kenya, in addition to strains of *M. aeruginosa* from (sub)-tropical regions like Australia and Brazil, with exception of a strain of *M. flos-aquae* from USA. Cluster II comprised one strain from Uganda and one strain from Kenya, in addition to strains of *M. aeruginosa* and *M. flos-aquae* from all parts of the world. On the basis of the sequences included in this study, there seems to be one group (Cluster II) which includes the "cosmopolitan species" suggested for some temperate cyanobacterial species by Komàrek (1985). However, as noted by Bittencourt-Oliveira et al. (2001), the *Microcystis* sp. genotypes most likely represent a series of related populations sharing a common phylogenetic history.

Cyanobacterial populations typically comprise a number of coexisting oligopeptide chemotypes suitable for characterisation of intra-species diversity (Fastner er al., 2001; Welker et al., 2004; Rohrlack et al., 2008). MALDI-TOF-MS analysis of the *M. aeruginosa* strains detected different types of microcystins and an aeruginosin called microcin SF 608, in addition to some unknown aeruginosins and cyanopeptolins (**IV**). Based on oligopeptid composition, the strains in this study could be divided into ten chemotypes, and seven of them were found in Murchison Bay (**IV**). The oligopeptide diversity found among the strains in this study is in good agreement to findings from European lakes (Fastner er al., 2001; Welker et al., 2004, Rohrlack et al., 2008). Two strains from Uganda (Kazinga Channel) and two strains from Kenya (Pilsner Pond; Lake Naivasha) were microcystin producing, but they synthesized different types of microcystins (**IV**). Microcystin desmethyl-YR was produced by all four strains and microcystin-YR by three strains, whereas microcystin-LR only was produced by one strain. *M. aeruginosa* was identified to be the main microcystin producer among the species found in Murchison Bay (**III**), but none of the *M. aeruginosa* strains from Murchison Bay were microcystin producing (**IV**). Hence, the diversity of this species is probably higher in Murchison Bay than detected in this study. The *M. aeruginosa* strains possessed different level of diversity depending on whether genetic, chemical, or morphological methods were used for their characterisation, emphasising the importance of using a polyphasic approach when studying diversity within a cyanobacterial species.

4.5.2 Cylindrospermopsis raciborskii

C. raciborskii was originally described as a tropical cyanobacterium (Geitler and Ruttner, 1936), but it is now increasingly expanding in temperate latitudes, becoming prevalent in temperate freshwater lakes world wide (Krienitz and Hegewald, 1996; Chapman and Schelske, 1997; Padisàk, 1997; Rücker et al., 1997; Druart and Briand, 2002; Wood and Stirling, 2003; Hamilton et al., 2005). Phylogenetic studies concerning *C. raciborskii* have revealed a geographic discrimination of strains (Dyble et al., 2002; Neilan et al., 2003; Gugger et al., 2005b); however, relatively few strains and often many of the same strains have been used in these studies. In our study, we isolated nine new strains of *C. raciborskii* from Uganda and Germany (**V**). The six Ugandan strains were isolated from Murchison Bay and Kazinga channel between Lake Edward and Lake George, a region considered by Padisák (1997) to be the possible origin of the species *C. raciborskii*. The three strains from Germany were isolated from Lake Zierkersee, one of the northernmost lakes where *C. raciborskii* has

been detected in Germany (Stüken et al., 2006). These strains therefore represent the invasive population of *C. raciborskii* found to be spreading in this region of north-eastern Germany.

The strains from the two geographical regions possessed two different morphotypes. The African strains had flexuous trichomes, terminal heterocysts and lacked akinetes (**V**), whereas the European strains had straight trichomes, terminal heterocysts and produced akinetes (**V**). Straight, curved and flexuous morphotypes have been found in most parts of the world (Chapman and Schelske, 1997; Saker et al., 1999; Berger et al., 2006), though only straight morphotypes have been observed in Europe (e.g. Padisák, 1997; Couté et al., 1997; Stüken et al., 2006). In general, akinetes are rarely observed among *C. raciborskii* in tropical areas where the strains can persist in its vegetative form throughout the year (Saker et al., 2003). Strains growing in temperate areas, on the other hand, are more likely to develop akinetes as an adaptation to lower growth temperatures and ability to survive during winter periods.

We analysed the strains chemically (by LC-MS/MS) for cylindrospermopsin production and genetically for the presence of cylindrospermiosin synthetase gene fragments (PKS and PS), but none of the strains were cylindrospermopsin producers (**V**). Cylindrospermopsin production is not found in any other strains of *C. raciborskii* from European water bodies (Fastner et al., 2003; Valério et al., 2005). Cylindrospermopsin has not been detected in strains from African water bodies (Berger et al., 2006), but Mohamed (2007) showed hepatotoxic effects in mouse bioassays. Cylindrospermopsin producing strains of *C. raciborskii* have been found in Australia and Asia (Li et al., 2001b; Hawkins et al., 1997; Saker et al., 1999), Brazilian strains of *C. raciborskii* have been reported to produce paralytic shellfish poisoning (PSP) toxin (Lagos et al., 1999) whereas many of the European strains are found to be hepatotoxic in the mouse bioassay (Bernard et al., 2003; Saker et al., 2003; Fastner et al., 2003).

The strains of *C. raciborskii* were genetically characterised with respect to ITS1-L, PC-IGS, *nifH* and *rpoC1* DNA regions and compared to corresponding sequences of *C. raciborskii* from other geographical regions (**V**). The phylogenetic analyses revealed a clustering of the strains due to geographic origin. The ITS1-L and *nifH* markers separated into American, European and Australian-African groups (**V**), thus congruent to earlier findings with *nifH*, ITS1-L and 16S rDNA markers (Dyble et al., 2002; Neilan et al., 2003 and Gugger et al., 2005b). The PC-IGS and *rpoC1* markers separated into American and

European/Australian/African groups (**V**), and this is in accordance to earlier findings with PG-IGS (Dyble et al., 2002) and *rpoC1* (Gugger et al., 2005b) markers. An analysis of concatenated data (ITS1-L+*nifH*+PC-IGS) supported the division into American, European and African/Australian groups, and even indicated a subdivision into an African and an Australian group (**V**). *C. raciborskii* showed a surprisingly low degree of diversity in comparison to other groups of cyanobacteria. Studies on *M. aeruginosa* (e.g. Bittencourt-Oliveira et al., 2001; Wilson et al., 2005; Paper **IV**) and *Planktothrix* sp. (e.g. Mbedi et al., 2005; Kurmayer and Gumpenberger, 2006) have revealed that even the genetic variation within a population in a single water body is considerable.

Different existing theories have been proposed to explain the phylogeography of *C. raciborskii*; radiation to the other continents from a primary evolutionary centre in Africa (Padisàk, 1997), a more recent radiation to the other continents from a secondary evolutionary centre in Australia (Padisàk, 1997), or a present colonization across the continents from warm refugee areas within the respective continents (Gugger et al., 2005b). The results from our study do not permit any resolution concerning the origin of *C. raciborskii*. The genetic similarity between the sequences from Africa, Australia and Europe was high for all genetic markers used, whereas the American sequences were more diverse both within the strains from this continent and in comparison to the sequences from the other continents (**V**). There are, however, geographical differences within the species *C. raciborskii* and perhaps different mechanisms favour the expansion in temperate regions. In tropical areas, like East African water bodies, *C. raciborskii* can grow all year round (Padisàk, 1997). We found *C. raciborskii* in Murchison Bay throughout the year (**II**, **III**). The species has high temperature optima (> 25 °C) (Padisàk, 1997), and therefore the proliferation in temperate regions has been proposed to be linked to increased water temperatures due to climatic change (Briand et al., 2004; Wiedner et al., 2007). Others have proposed that adapted ecotypes with a wide physiological tolerance may spread in temperate regions (Chonudomkul et al., 2004). Most likely, a combination of coexisting mechanisms enables *C. raciborskii* to proliferate into temperate areas. The spreading of cyanobacteria within and between continents is most likely a dynamic process enabling a continuous changing of the genotype composition of a population.

5 Human health aspects

7th March 2008, New Vision (Ugandan national newspaper) (http://www.newvision.co.ug/PA/9/183/615463)

Lake Victoria Bay turns green

…"A thick layer of algae is floating on Lake Victoria near Gaba Water Works, the source of Kampalas piped water"…

…"The problem is municipal waste and sewerage that flows untreated into the Nakivubo Channel and then into the lake"…

…"People are exposed to health risks since many of them depend on raw water from the lake for cooking, drinking and other domestic uses"…

Murchison Bay serves as a drinking water supply for Kampala and the two water works are situated at the shores of the Inner Murchison Bay (Fig. 3). The microcystin concentrations in Murchison Bay were at times higher than the WHO recommended limit of 1 μg L^{-1} for drinking water (**III**), possessing a health risk for lake water users. The water works use standard water treatment methods in the purification process. Sand filtration and standard coagulation and sedimentation methods will remove many cyanobacterial cells and chlorination can be effective in removing dissolved microcystin if used in sufficient concentrations and contact times (Hitzfield et al., 2000). Water from different steps in the purification process at the water works were analysed, and microcystins were not detected (data not presented). There is however a risk for exposure to microcystins for those using the lake water directly as drinking water. A local household survey in the parishes surrounding the Inner Murchison Bay revealed that 5-50 % of the local people used lake water for drinking water for animals and humans and for other domestic uses (Waiswa Dauda, pers. com.). To minimize harmful effects by using the lake water it is important to filter the water before use, for instance with bank filtration or by using a cloth (dense woven fabric).

There is a need for urgent action in the water management in Murchison Bay in order to protect the unique water resources in this region and to obtain a secure sustainable water resources development. The most important actions are to 1) plan and sustainably manage the land use and the human activities in the wetland areas surrounding Murchison Bay; 2) improve the management of waste disposal; and 3) perform awareness raising among the local population.

6 Conclusions

This study showed that:

- Murchison Bay was strongly eutrophicated with average concentrations of total phosphorous > 90 µg L^{-1} and total nitrogen > 1100 µg L^{-1} in the inner part of the bay (**I**, **II**, **III**).
- There was a rapid decrease in conductivity and nutrient concentrations from the innermost part of the bay to the outer part of the bay, and we found that surface seiches caused water exchange with the main lake and thereby mediated the eutrophication in the Inner Murchison Bay (**I**).
- Murchison Bay is a more dynamic system than first recognized, and the rapid transport of nutrients to the open lake indicates that Murchison Bay contributes to the eutrophication process of Lake Victoria (**I**).
- The phytoplankton community was dominated by a variety of cyanobacterial species and diatoms. The phytoplankton community, especially in the outer part of the bay, may be influenced by light limitation, and low NO_3-N concentrations in the bay may also indicate a possible N-limitation, thus favouring growth of N-fixing cyanobacteria. The proportion of N-fixing species like *Anabaena* sp. was higher in the outer part of the bay whereas species like *Microcystis* sp. were more abundant in the inner part of the bay (**II, III**).
- There were microcystins (MC-RR, -LR, -YR) in Murchison Bay, on average 1.1 µg L^{-1} in the inner part of the bay and 0.6 µg L^{-1} in the outer part of the bay. Based on probability analysis, *Microcystis aeruginosa* was identified as the main microcystin producer, and the maximum total microcystin concentration of 2.98 µg L^{-1} was detected in the inner part of the bay and coincided with the highest detected biomass of *M. aeruginosa*. Nutrient availability and light climate seemed to be the most probable influencing environmental parameters on the microcystin production in Murchison Bay, but the microcystin concentrations could not be predicted by a given environmental factor alone, by the biovolume of cyanobacteria or of certain cyanobacterial species (**III**).
- 24 strains of *M. aerugionsa* isolated from Murchison Bay and other lakes in the East-African region possessed different levels of diversity depending on characterisation method. Four morphotypes were identified based on the traditional morphological

approach, 10 genotypes by DNA sequence comparison of the PC-IGS and ITS1 rDNA regions, and 10 chemotypes based on MALDI-TOF-MS oligopeptide analysis. The phylogenetic analyses showed that the East-African strains of *M. aeruginosa* were closely related to other strains of *M. aeruginosa* of different geographical regions. Only four of the 24 isolated strains from East Africa were found to produce microcystins, while oligopeptides belonging to the aeruginosin and cyanopeptolin class were detected in most strains (**IV**).

- The phylogenetic analyses of nine strains of *Cylindrospermopsis raciborskii* isolated from Murchison Bay, Kazinga Channel (Uganda) and Zierker See (Germany) compared to other strains of *C. raciborskii* of different continents revealed a clustering of the strains due to geographic origin. The strains from Africa and Europe exhibited two different morphotypes and none of the strains produced cylindrospermopsin. *C. raciborskii* is increasingly spreading in temperate freshwater habitats world wide, and most likely, a combination of coexisting mechanisms enables *C. raciborskii* to proliferate into temperate areas.
- The Inner Murchison Bay serves as a drinking water supply for Kampala, the capital of Uganda, and the concentration of total microcystin in the bay was at times exceeding the WHO guide line value of 1 μg L^{-1} MC for drinking water. There is a risk for exposure to microcystins for those using the lake water directly as drinking water.

This study has gained more knowledge regarding blooms of cyanobacteria in Lake Victoria and can be used as a basis for more research and management in Lake Victoria and other lakes in the region.

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