

**THE EFFECTS OF CYANOBACTERIA ON THE GROWTH,
SURVIVAL, AND BEHAVIOUR OF A TROPICAL FISH (NILE
TILAPIA) AND ZOOPLANKTON (DAPHNIA LUMHOLTZI).**

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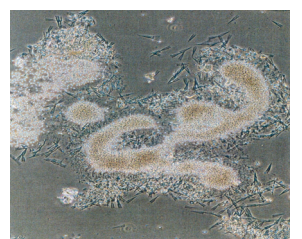
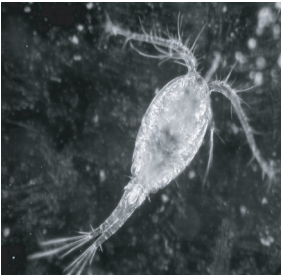
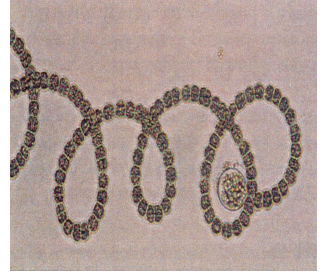
SCIENTIFIC ENVIRONMENT

This study was undertaken at the Department of Biology, University of Bergen, under the main supervision of Prof Petter Larsson, with Dr. Thomas Rohrlack and Associate Professor Y.S. Kizito (RIP) as co-supervisors.

The experimental work in this study was carried out both at the Department of Zoology, Makerere University and the Department of Biology University of Bergen. All the field work was conducted in Uganda in Lake Victoria and Lake Mburo under programmes initiated by the University of Bergen/Makerere University collaboration Programme. Laboratory analysis of field sample material was carried out at the Department of Chemistry Makerere University, the Department of Botany Makerere University and the Norwegian Institute of Water Research (NIVA).

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'Plans fail for lack of counsel, but with many advisers they succeed'. (Prov. 15:22)

ABSTRACT

Recent studies on the ecological effects of cyanobacteria in lakes have focused on the harmful effects of blooms to other aquatic organisms including fish and zooplankton. Much of this work has been conducted on temperate and subtropical species whilst few studies have involved tropical species, despite the almost chronic occurrence of cyanobacteria blooms in eutrophic tropical lakes. The main purpose of this study was to fill some of this knowledge gap on the ecological effects of cyanobacteria on fish and zooplankton in tropical lakes and to discover in what ways tropical species may be better adapted to deal with cyanobacteria than temperate species. The study tested fish (Nile tilapia, *Oreochromis niloticus* L.) and zooplankton growth, survival, and behaviour in relation to different variants (toxic and non toxic) and species of cyanobacteria at varying quantities as well as environmental conditions.

Field studies on the diet and presence of cyanobacteria toxins (microcystins) in the guts and tissues (muscle and liver) of Nile tilapia were carried out in two eutrophic tropical aquatic ecosystems (Lake Mburo and Murchison Bay, Lake Victoria) in Uganda, East Africa. The zooplankton behaviour of diel vertical migration (DVM) was investigated in the Murchison Bay in relation to environmental conditions. Laboratory studies investigated the effect of the cyanotoxins, microcystins, on Nile tilapia and zooplankton (*Daphnia lumholtzi*) growth, survival, and behaviour, using the toxic cyanobacterium *Microcystis aeruginosa* PCC 7806.

Cyanobacteria are the most abundant phytoplankton group in the two study areas contributing to over 60% of the total phytoplankton biovolume. The most dominant cyanobacteria species is *Microcystis sp.* which makes up approximately 70% of the total cyanobacteria biovolume. Low water transparencies were well associated with high levels of chlorophyll *a* in the lakes, suggesting that the abundance of cyanobacteria in these lakes may have a significant effect on the light environment. Water transparency is observed to have a close association with the DVM behaviour of at least one zooplankton group in the Murchison Bay. Phytoplankton especially of the cyanobacteria is an important diet item (> 30%) to the Nile tilapia in both lakes.

Microcystins (RR, LR & YR) were detected in water samples from both study lakes and in the Murchison bay the concentration of microcystins in water was well associated with the microcystins concentration in the gut of Nile tilapia ($p < 0.05$). Although detected, we found no

evidence of microcystin accumulation in the liver or muscle tissue. The ingestion rates and growth rates of juvenile Nile tilapia were not significantly affected by the presence of microcystins in their diets. Likewise growth and survival rates of the tropical cladoceran *Daphnia lumholtzi* in laboratory experiments were not significantly affected by the presence of microcystins in their diets. The cyanobacteria *M. aeruginosa* PCC7806 was a poor source of nutrients for juvenile fish and zooplankton, since it did not seem to stimulate growth both in the wild type and mutant type form.

Though microcystins were present throughout the study period, our findings do not show that they have a strong effect on the diets of Nile tilapia. Nile tilapia in these lakes probably ingest cyanotoxins mostly through the cell-bound fraction, since cyanobacteria (especially *Microcystis* sp.) are an important constituent of their diet. Adult fish may have the ability to efficiently get rid of ingested cyanotoxins, but this may not be so for juvenile tilapia and zooplankton. Small amounts of cyanobacteria may actually stimulate growth in juvenile tilapia and even zooplankton, yet when provided in high proportions or as the sole source of nutrients they hinder growth, survival, and reproduction. Juvenile fish and most especially zooplankton, are unlikely to graze on cyanobacteria either due to their frequent formation of large colonies or because juvenile fish and zooplankton may lower their ingestion rates in the presence of cyanotoxins. Findings in this study do not provide any evidence for tolerance of tropical juvenile fish and zooplankton towards cyanobacteria toxins, yet adult fish show both the ability to utilise cyanobacteria and cope with levels of microcystins registered in the lakes.

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Fig. 2: Chemical structure of microcystin-LR. (From Rinehart et al. 1994).

Fig 3: Map showing the location of Murchison Bay (Lake Victoria) and Lake Mburo with sampling sites for Murchison Bay (black circles). (Drawings in ArcGis: Ingelinn Aarnes)

LIST OF PAPERS

This thesis was based on the papers listed below. These papers are referred to in the text by their roman numerals (I – V).

Paper I

Ronald Semyalo, Juliet K. Nattabi and Petter Larsson. Diel Vertical Migration of zooplankton in a eutrophic bay of Lake Victoria. (*Submitted*).

Paper II

Ronald Semyalo, Thomas Rohrlack, Duncan Kayiira, Yusuf S. Kizito, Stephen Byarujali, Petter Larsson. On the diet of Nile tilapia in two eutrophic tropical lakes containing toxin producing cyanobacteria. (*Submitted*)

Paper III

Semyalo, R., T. Rohrlack, and P. Larsson. (2009). Growth and survival responses of a tropical *Daphnia* (*D. lumholtzi*) to cell bound microcystins. *Journal of Plankton Research*. **31**:827-835

Paper IV

Ronald P. Semyalo, Thomas Rohrlack, Christine Naggawa, George Nyakairu. Microcystin contamination in catches of *Oreochromis niloticus* in Murchison Bay, Lake Victoria and Lake Mburo - Uganda. (*Submitted*).

Paper V

Ronald Semyalo, Knut H. Jensen, Moberg, O. The effects of a toxic cyanobacterium *Microcystis aeruginosa* PCC 7806 on the ingestion and growth of juvenile Nile tilapia *Oreochromis niloticus* (*Manuscript*).

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INTRODUCTION

Cyanobacteria are often the most important group of phytoplankton in eutrophic tropical lakes (Kling et al. 2001). Besides dominating the phytoplankton community composition, they also frequently build up dense populations. High phytoplankton abundance is normally perceived as a precursor to increases in zooplankton abundance, as is the case in several temperate lakes (Talling 2003), especially for the efficient filter feeders the cladocera. Yet high cyanobacteria crops are rarely grazed down (Gliwicz 1990b) and are instead associated with the disappearance or reduction in abundance of efficient grazers (Pattinson et al. 2003). The occurrence of high cyanobacteria abundance in temperate lakes is often seasonal, with a few exceptions of shallow eutrophic lakes where it could last for years (Berger 1989). In some eutrophic tropical lakes, cyanobacteria blooms often last all year round and despite contributing quite significantly to the diets of herbivorous fish (Getachew 1987, Balirwa 1992), fish and zooplankton grazers in these lakes are often unable to graze down populations of cyanobacteria. This has been attributed to a number of factors, for example, formation of 'ungrazable' filaments and colonies Gliwicz (1990b). Cyanobacteria tend to have a negative effect on grazer populations (Landsberg 2002). In this thesis I look at the effects cyanobacteria may have on both tropical fish and zooplankton grazers. In doing so I hope to generate new knowledge in the understanding of why cyanobacteria populations in tropical lakes are rarely grazed down and how tropical grazers may cope with the negative effects associated with cyanobacteria.

The cyanobacteria

Cyanobacteria are gram negative photosynthetic prokaryotes consisting of over 1000 species of unicellular and multicellular micro-organisms belonging to the class Cyanophyceae under the orders *Chroococcales*, *Chamaesiphonales*, *Pleurocapsales*, *Nostocales* (*Oscillatoriaceae*, *Nostocaceae* and *Rivulariaceae*) (Rippka et al. 1979). According to Rippka et al. (1979), cyanobacteria may exist in several forms that may either be unicellular (single or forming colonial aggregates) or filamentous (possessing or lacking heterocysts and akinetes) (Fig. 1). The dominance of cyanobacteria in eutrophic aquatic systems has also been attributed to a variety of factors typical to cyanobacteria including possession of phycobiliproteins (Glazer 1977), production of gas vesicles (Walsby 1994), ability of some species to fix nitrogen (Fay

1992) and the ability to produce allelopathic chemicals that may inhibit growth of algae (Gantar et al. 2008) and macrophytes (Pflugmacher 2002).

Unlike the eukaryotic algae, cyanobacteria lack organelles, but instead have intracellular membranes (thylakoids), which incorporate the photosynthetic pigment (phycobilisomes-phycobiliproteins in a supramolecular structure) within cyanobacterial cells (Fay 1992). Probably the most interesting aspect about cyanobacteria is their ability to harvest light from a wide spectrum and use it to photosynthesise. All cyanobacteria possess the photosynthetic pigment chlorophyll *a* and the light harvesting phycobiliproteins; allophycocyanin B, allophycocyanin, and C-, or R-phycocyanin (others like phycoerythrin and C-phycoerythrin may also be present in some cyanobacteria groups - red algae) that allow them to use light energy outside the chlorophyll maxima in the fixation of carbon dioxide, converting it into organic carbon (Glazer 1977). Light energy may be trapped by using both chlorophyll *a* in photosystem I (PSI) and a series of phycobilosomes (λ 400 – λ_{\max} 671 nm) in photosystem II (PSII) and is used in the production of ATP and NADPH. Cyanobacteria can thus perform better than most algae under low light conditions.

Several cyanobacterial species, especially those possessing heterocysts, are capable of fixing nitrogen using the enzyme nitrogenase (Fay 1992), thus compensating for any shortfalls in aquatic nitrogen that is essential for primary production. In filamentous cyanobacteria, nitrogenase which catalyses the reduction of dinitrogen to ammonia, may be contained in cells known as heterocysts which are formed particularly under conditions of nitrogen limitation, yet non-heterocyst forming cyanobacteria may also fix nitrogen (Fay 1992).

Some cyanobacteria may also possess hollow gas-permeable and water-impermeable protein structures called gas vesicles that provide buoyancy (Walsby 1994). The gas vesicles, which vary in width in different species, regulate buoyancy allowing the cyanobacteria to occupy the most optimum position within the water column.

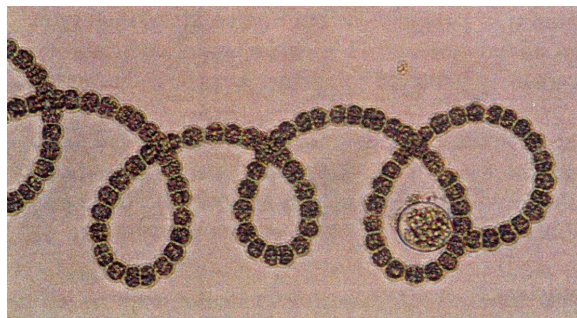
Of current global interest, however, is the ability of some cyanobacteria to form blooms (Skulberg et al. 1984) and produce secondary metabolites (cyanotoxins) (Carmichael 1992) that can be harmful to other tissues, cells, or organisms.

Fig. 1

A)



B)



Harmful algal blooms

Intense proliferation of cyanobacteria or the so-called cyanobacterial blooms are a major symptom of eutrophication. Cyanobacteria will tend to dominate in aquatic ecosystems that have high concentrations of total phosphorus (TP) (Downing et al. 2001, Schindler et al. 2008) or as earlier suggested in environments with low TN (total nitrogen): TP ratios (Smith and Bennett 1999).

In deep eutrophic temperate and subtropical lakes, the summer rise in water temperature, followed by depletion of dissolved inorganic nitrogen and carbon are characteristic of a progression from a clear water phase to cyanobacteria bloom formation which eventually disappears during the cold autumn and winter. In shallow lakes, however, cyanobacteria blooms may persist for years (Havens 2008). In temperate regions cyanobacteria blooms often occur in late summer (Havens et al. 2000, Pattinson et al. 2003), whereas the warmer temperatures, intense solar radiation, and year-round 12-hour days in the tropical regions, often promote all year round cyanobacteria blooms in eutrophic lakes (Ferrão-Filho et al. 2000, Sarma et al. 2005).

Cyanobacterial blooms may have far-reaching ecological effects on aquatic ecosystems (Landsberg 2002). Formation of cyanobacteria blooms typically leads to a reduction of light penetrating through the water column, causing a shading effect. This lowered transparency causes poor growing conditions for epiphytes, phytoplankton, and benthic algae (Scheffer et al. 1993). Increases in pH due to carbon dioxide depletion by cyanobacteria blooms and/or anoxia resulting from a collapsed bloom could lead to massive fish kills (Paerl and Ustach 1982, Ochumba 1990, Vos and Roos 2005). Additionally, when these blooms die off they sink to the bottom, where they decompose causing a depletion of bottom water oxygen or hypoxic conditions (Hecky et al. 1994).

In highly eutrophic lakes, cyanobacteria blooms could provide a rich and abundant source of nutrients for zooplankton and fish. For example, the success of several fish species including roach (*Rutilus rutilus*) (Kamujunke et al. 2002), silver carp (*Hypophthalmichthys molitrix*) (Chen et al. 2006), and Nile tilapia (*Oreochromis niloticus*) (Bwanika et al. 2006) in eutrophic lakes has been attributed to their ability to utilise cyanobacteria. This, however, is not always the case (Bednarska 2006), since blooms are often dominated by colonial and filamentous

forms, which, among the already mentioned undesirable traits, may cause mechanical interference for zooplankton grazers and clogging of gills in fish (Landsberg 2002). A few studies have also found some species of cyanobacteria to be nutritionally deficient to a number of zooplankton species (e.g. Tillmanns et al. 2008). For a number of zooplankton species and for juveniles of most fish species, under certain conditions, some cyanobacteria species can be a poor source of nutrition (Bury et al. 1995, Wilson et al. 2006).

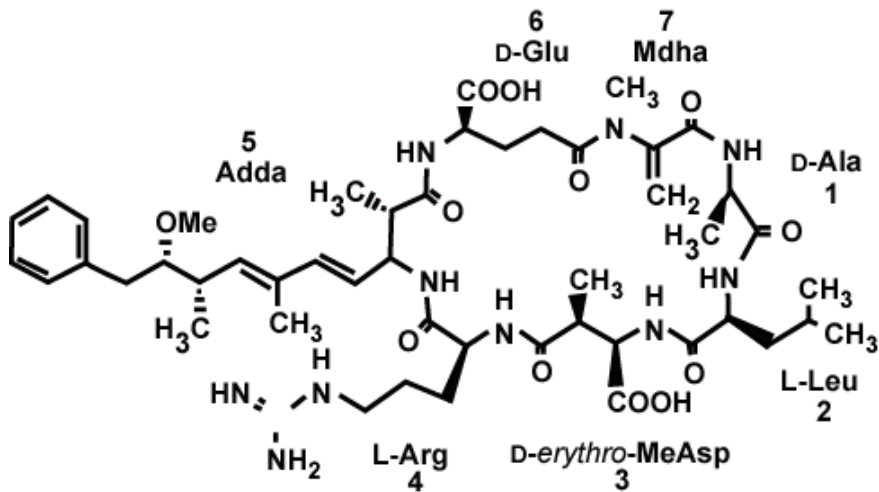
Cyanotoxins

Cyanobacteria produce secondary metabolites the so called cyanotoxins; these may be cytotoxic or biotoxic (hepatotoxins and neurotoxins) (Carmichael 1992). Cyanotoxins may have allelopathic effects on dinoflagellates (Sukenik et al. 2002) green algae, and other cyanobacteria (Singh et al. 2001, Gantar et al. 2008), affecting photosynthesis and growth. Cyanotoxins may also account for the disappearance of submerged macrophytes (Weiss et al. 2000).

The toxicity of cyanobacteria to zooplankton can be attributed not to one but to several toxic compounds including the non-ribosomal oligopeptides that may lower ingestion rates (Rohrlack et al. 1999b), disrupt moulting (Rohrlack et al. 2004b), and lower survival (Demott et al. 1991, Rohrlack et al. 2001). The most commonly assayed of these are the hepatotoxic microcystins (fig. 2) and the neurotoxic anatoxins (Wilson et al. 2006, Tillmanns et al. 2008). Studies examining the effects of these toxic compounds in cyanobacteria on the growth and survival of zooplankton, indicate that though they may have no effect on population growth they greatly affect survival (Wilson et al. 2006).

Whereas fish have been considered quite tolerant to the toxicity of cyanobacteria (Kotak et al. 1996), there are some reports linking toxic cyanobacteria to massive fish kills (Rodger et al. 1994). However, not enough evidence is available to implicate cyanobacteria toxins in occurrences of fish kills and related cyanobacteria blooms either in tropical or temperate regions (Ochumba 1990, Rodger et al. 1994). Fish kills may be a result of other cyanobacteria bloom related circumstances such as oxygen depletion (Hecky et al. 1994). Nonetheless, several laboratory and field experiments have demonstrated that the presence of aqueous and cell-bound cyanotoxins in the diet of the fish is not good for their physiology, morphology, and behaviour (Malbrouck and Kestemont 2006).

Fig. 2



Importance of cyanobacteria to tropical fish and zooplankton

The importance of size, shape, toxicity, and nutritional value of cyanobacteria has been studied on several invertebrates (Burns 1968, Lampert 1987, Gliwicz 1990a, Demott et al. 1991, Rohrlack et al. 2001, Chan et al. 2004, Gustafsson and Hansson 2004, Repka et al. 2004) and fish (Lawton and Codd 1991, Lindholm et al. 1992, Tencalla et al. 1994, Bury et al. 1995, Oberemm et al. 1999, Wiegand et al. 1999, Jacquet et al. 2004, Wang et al. 2005) in temperate regions. These studies leave the impression that cyanobacteria contribute very little to the food chain from primary producers to fish. They are more a hindrance to the development of the biodiversity of secondary producers and may have the ability to reduce being grazed upon; this, however, might be different in tropical waters. Blooms of cyanobacteria often persist for long periods in tropical lakes yet studies on tropical lakes are few and it is difficult to see exactly how the cyanobacteria affect the food web, but some studies (Beveridge et al. 1993, Keshavanath et al. 1994, Matveev et al. 1994, Perschbacher 2003, Sarma et al. 2005) indicate that tropical fish and invertebrates might be better adapted to utilize cyanobacteria than their temperate relatives.

AIMS OF STUDY

The main aim of this study was to investigate the effects of the Cyanobacteria *Microcystis aeruginosa* on the growth, behaviour, and survival of tropical fish and zooplankton. *M. aeruginosa* is a cyanobacterium with the ability of forming blooms, producing toxins, and forming colonies: all these properties have an impact on the behaviour, growth, and survival of fish and zooplankton. The tropical fish *Oreochromis niloticus* (Nile tilapia) and the tropical zooplankton *Daphnia lumholtzi* were chosen as model species and were used in laboratory experiments. In the experiments we investigated the effects of the commonly assayed cyanotoxins microcystins MCYST-LR & (D-Asp³) MCYST-LR from the toxic *M. aeruginosa* strain PCC7806 (wild type) and its microcystin deficient PCC 7806 variant (mutant type), which has been genetically engineered to knock out microcystin synthesis (Dittmann et al. 1997).

This study was achieved by fulfilling the following specific objectives.

- a. Investigating the diel vertical migration behaviour of zooplankton in a eutrophic bay of Lake Victoria.
- b. Investigating the diet composition, availability of food items, and factors affecting the diet of *O. niloticus* in two tropical eutrophic lakes where cyanotoxins are present, Lake Victoria and Lake Mburo, Uganda.
- c. Verifying the presence of microcystins in tissues of fish (Nile tilapia) obtained from fishermen's catches in Murchison Bay, Lake Victoria and Lake Mburo.
- d. Investigating whether cell-bound microcystins have an effect on ingestion rates and growth of juvenile *O. niloticus*.
- e. Investigating the effects of cell-bound microcystins on life history variables of *D. lumholtzi*.

MATERIAL AND METHODS

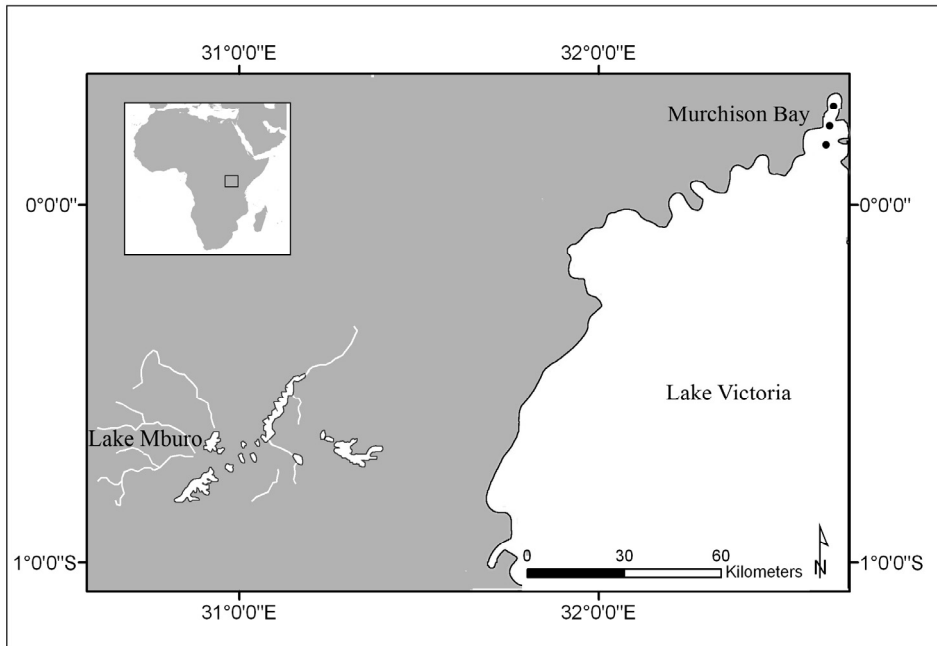
Field studies

These were conducted in two eutrophic tropical lakes, Lake Mburo and Lake Victoria in Uganda (Fig. 3). These lakes were selected because they are both eutrophic and their phytoplankton communities are dominated by bloom-forming cyanobacteria (Kayiira 2007, Haande 2008).

Lake Mburo is located between 00°30' - 00°45'S, and longitude 45°00' - 31°05'E in western Uganda, at an altitude of 1210 m. It is a shallow lake with an average depth of 2 m and a maximum depth of 4 m. It is located within a protected National Park that supports a large wildlife population including at least 1000 hippos that frequently utilise the lake (Wronski 2002). Cyanobacteria are the most dominant phytoplankton group in this lake. Sample collection and physico-chemical measurements were conducted at three sites along a transect across the lake (**IV**).

Murchison Bay is a shallow bay of Lake Victoria located between latitude 00°15'N-00°18'N and longitude 32°33'E-32°41'E, at an altitude of 1135 m with an average depth of 3.2 m. The bay covers an area of about 200 km² and is divided into two parts; the inner bay (18 km²) and the outer bay separated by a narrows. Studies on the phytoplankton community structure of Murchison Bay indicate that cyanobacteria of the genus *Microcystis* (31%) and the genus *Anabaena* (20%) make up over half the cyanobacteria in the inner Murchison Bay (Haande 2008). Sample collection and physico-chemical measurements were conducted at three sites along a transect extending from the inner part of the bay to the outer part of the bay (**IV**).

Fig. 3



Laboratory studies

Laboratory experiments were conducted at the Department of Zoology, Makerere University, Uganda and at the Department of Biology, University of Bergen, Norway. In all our laboratory experiments we used the cyanobacteria *M. aeruginosa* strain PCC 7806 and its microcystin deficient mutant (III, V). In some of the experiments (III, V – experiment 2) the green algae *Scenedesmus* sp. was also used. The cyanobacteria stock cultures were obtained from the Norwegian Institute of Water Research (NIVA). The green algae *Scenedesmus* sp. was obtained from stock cultures maintained at the Department of Biology, University of Bergen.

The tropical zooplankton *Daphnia lumholtzi* was obtained from stock cultures maintained at the Department of Biology, University of Bergen as a single clone. This clone was originally obtained from Lake Victoria and maintained in culture since 2003. For the fish ingestion and growth rate experiments, Nile tilapia *Oreochromis niloticus* fry were obtained from the

Kajjansi fisheries research Institute, Uganda and the Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, respectively.

RESULTS AND DISCUSSION

Cyanobacteria blooms

Conditions in our study lakes were characterised by a high abundance of cyanobacteria and the absence of strong temperature and oxygen gradients (**I, II, IV**). Surface temperatures were high (23 – 30°C), and in Murchison Bay, temperature was mostly uniform throughout the water column during the night and any stratification that had formed during the day was broken down during the evening and night time (**I**). Levels of dissolved oxygen were relatively high in surface waters often exceeding 60% saturation, however, during some of the sampling months, dissolved oxygen levels close to the bottom would drop below 20% saturation (**I, II**). A Wilcoxon rank sum test on seasonal differences in average dissolved oxygen (DO) concentration in Lake Mbuoro showed that DO was higher in dry seasons than wet seasons (**IV**). Water transparency in both study areas was often below 1 m (**I, II, IV**), probably because of the high turbidity due to high quantities of silt and phytoplankton (**II**).

One major difference between lakes in tropics and lakes in temperate regions is the lack of strong seasonal patterns in tropical lakes. Whereas temperate lakes transit from a cold winter season (characterised by short days, low primary and secondary production), to a warm spring period (characterised by increased primary production followed by an increase in secondary production) followed by even warmer summers (characterised by long days, reduction in secondary production and an increase in cyanobacteria dominance), tropical lakes experience conditions of high temperatures and high light intensities all year round. Under such circumstances cyanobacteria blooms tend to persist all year, especially in shallow lakes (Havens 2008). Yet in tropical lakes, even in the presence of year-round blooms that may include toxin producers, several invertebrate and fish species continue to be abundant (**I, II**), with some fish like the Nile tilapia even utilising toxin producing cyanobacteria as a source of nutrition (**II, IV**). Whether or not this can be attributed to tolerance (Gustafsson and Hansson 2004) or toxin avoidance (Keshavanath et al. 1994) is still debatable.

Phytoplankton community

The phytoplankton community of Murchison Bay and Lake Mburo was dominated by cyanobacteria (II). Cyanobacteria accounted for > 90% of the phytoplankton in both lakes (I, II). Previous work on Murchison Bay (Schröder et al. 1998, Haande et al. 2007) and Lake Mburo (Byarujali 1995, Kayiira 2007) has shown that they are both eutrophic with high levels of TP and TN nutrient loading. High levels of phosphorus are a major driving factor for high cyanobacteria abundance (Schindler et al. 2008).

The Lake Mburo catchment consists of a hilly terrain where agriculture and pastrolism are practised (D. Muwhezi *pers comm.*). Lake Mburo is eutrophic mainly as a result of high nutrient loading from surface runoff. Extra nutrient loading is assumed to come from the River Ruizi, which runs from an urban centre (Mbarara town) and partly drains into the wetlands surrounding Lake Mburo. Animal waste from the large population of hippos that live around the lake is also considered an important source of nutrient loading. High total nitrogen and total phosphorus coupled with high turbidity due to silt from surface runoff, provide conditions that favour the growth of cyanobacteria over other phytoplankton (Byarujali 1995). High nutrient conditions, in turn, favour the formation of algal blooms affecting water transparency levels, which were < 1 m (II).

The eutrophication of Murchison Bay is part of a trend of eutrophication that has been documented in Lake Victoria over the last 50 years (Verschuren et al. 2002). This eutrophication has been attributed to several factors including climate change, explosion of the piscivorous Nile tilapia populations, and anthropogenic sources (Ogutuohwayo and Hecky 1991, Verschuren et al. 2002).

The phytoplankton strain responsible for observed microcystins in our study lakes has so far not been identified from isolated strains but it is highly likely that the microcystins come from one or more *Microcystis aeruginosa* strains (Haande et al. 2007). The strain composition of this cyanobacterium varies quite a lot within the lake (Haande et al. 2007). Microcystins were present in both study areas, sometimes exceeding $1.0 \mu\text{g L}^{-1}$ (IV), especially during wet seasons when temperatures were highest. Changes in microcystins concentrations could not be explained by measured environmental variables but may be related to changes in strain composition (Rohrlack et al. 2008).

Cyanobacteria and diet of Nile tilapia

Cyanobacteria are often an important constituent, contributing as much as 50%, of the diet of Nile tilapia in eutrophic tropical lakes (Getachew 1987, Bwanika et al. 2006) and were found to contribute over 30% to the diets of *O. niloticus* in Lake Mburo and Murchison Bay (II).

Colonial and filamentous cyanobacteria were the most abundant forms in the fish diets contributing up to 90%. Colonial and filamentous forms can be ingested more efficiently than single celled forms for filter feeding fish (Turker et al. 2003b).

The Nile tilapia is a generalist filter feeder, with a long gut capable of efficiently ingesting and assimilating carbon from phytoplankton (Moriarty 1973, Moriarty and Moriarty 1973a, 1973b, Moriarty et al. 1973). Its filter feeding mechanism (Sanderson et al. 2001) will often favour the ingestion of the larger colonial and filamentous phytoplankton (Northcott et al. 1991, Turker et al. 2003a), which in Lake Mburo and Murchison Bay were mostly colonies of *Microcystis* spp. (II). As a result, over 80% of ingested phytoplankton in diets of fish from both lakes was *Microcystis* spp. The Nile tilapia is also efficient at filtering small phytoplankton (Robinson et al. 1995), thus several other groups of phytoplankton were also found in its diet (II).

Nile tilapia from our study lakes also ingested large amounts of detritus. Other items in their diets included zooplankton and insects. The contribution of insects to their diets was correlated with water transparency in Murchison Bay, but none of the other food items (phytoplankton, zooplankton, detritus) could be explained by any of the measured environmental variables (II). In our study (II), water transparency was negatively correlated with chlorophyll *a*. Low competition from haplochromines due to the piscivorous Nile perch is believed to be an important factor in allowing Nile tilapia to assume a more omnivorous diet (Bwanika et al. 2006), therefore with improved visibility conditions Nile tilapia may utilise active pursuit to include a variety of animal and plant material in their diet. Populations of Nile tilapia in the lakes studied may flourish largely due to their ability to utilise a broad spectrum of food resources (Bwanika et al. 2006). The Nile tilapia in our study ingested a wide range of food items (II).

Experimental studies on ingestion rates of Nile tilapia have suggested that the presence of toxic cyanobacteria may have a negative effect on its ingestion rates (Keshavanath et al. 1994). We observed low ingestion rates when fish were fed a toxic cyanobacterium (IV), however, we did not observe any effect of the presence of microcystins on these ingestion rates. In the lakes studied, the contribution of cyanobacteria to fish diets also seem to be unaffected by the presence of microcystins in the lake. Since *Microcystis* spp. have been identified as the most likely source of measured microcystins in a number of East African lakes (Haande et al. 2007), the high contribution of these cyanobacteria to Nile tilapia diet (II) may explain the high amount of microcystins observed in its gut ($> 300 \mu\text{g kg}^{-1}$ fw) and its positive correlation with the microcystin concentration in the lake (IV).

Effects of cyanotoxins on fish

Though the presence of toxins has been suggested as an important factor in reducing fish ingestion rates (Keshavanath et al. 1994), presence of microcystins alone in cyanobacteria does not significantly affect the ingestion rate of Nile tilapia (V). Ingestion rates of Nile tilapia on the toxic *M. aeruginosa* strain PCC 7806 were generally low in our study (V), which could be explained by the presence of other substances in the *M. aeruginosa* that were not investigated or it could be attributed to the use of single cell cultures in the ingestion experiments (Wilson et al. 2006). Nonetheless, this study has demonstrated that there can be inhibition effects of cyanobacteria on fish ingestion rates, which may be attributed to factors other than microcystins. Such factors could include extra-cellular properties of toxic cyanobacteria which act as a deterrent to fish ingestion, a so-called bad taste factor (Nizan et al. 1986). Several studies have suggested that the presence of toxic algae can induce stress in fish (Baganz et al. 2004) which in turn would lead to low grazing rates (Beveridge et al. 1993).

In our study we conducted growth experiments on juvenile fish fed with diets containing both toxic cyanobacteria and green algae (V). The presence of high amounts of cyanobacteria (20%) in the diet significantly affected growth ($p < 0.001$) after a two week period, regardless of whether microcystins were present or not ($p > 0.05$). We did not observe a significant effect ($p > 0.05$) on fish growth when diets were supplemented with green algae instead (V). Our findings suggest that when toxic cyanobacteria are present in small amounts in proportion to other food sources, fish growth is not significantly affected. In fact, adding a small amount (2%) of cyanobacteria seems to result in a trend of substantial gains in growth (V), yet when

toxic cyanobacteria amounts are as high as 20% this is no longer beneficial (V). Wild populations of juvenile tilapia (1-5 g) seem not to rely on phytoplankton as much as adult fish (II), probably because they lack the necessary morphological structures to efficiently utilise phytoplankton (Northcott and Beveridge 1988), which could explain the low ingestion rates observed in our experiments (V). Despite our inability to carry out a full study documenting other behavioural responses of Nile tilapia to toxic cyanobacteria, evidence from a single fish pilot experiment utilising four treatments (formulated feed (30% protein), microcystin containing *M. aeruginosa*, microcystin lacking *M. aeruginosa*, green algae *Scenedesmus* sp.) suggests that fish in the cyanobacteria treatments exhibited less motility and an opercula beat frequency as seen in situations of stress (Baganz et al. 2004).

Cyanotoxins in fish

Several laboratory studies have shown that microcystins may accumulate in fish (Malbrouck and Kestemont 2006), yet only a few have shown this to be the case in the field (Magalhaes et al. 2003, Chen et al. 2006), even for Nile tilapia (Mohamed et al. 2003, Mohamed and Hussein 2006). Our study provides crucial evidence for the ingestion and uptake of microcystins by fish in eutrophic lakes (IV).

The levels of microcystins ingested by the fish were closely associated with the level of microcystins in the water (IV) but not to any other measured environmental variables. None of the measured environmental variables had an association with the level of microcystins in fish muscle or liver. There is an almost one hundred fold difference between the levels of microcystins in fish gut and the levels in the muscles (IV) but we found no correlation between the level of microcystins in the fish gut, liver and muscle. While not all ingested microcystins may be assimilated, e.g. due to some cells going through the gut undigested (Lewin et al, 2003), the observed difference in microcystins levels between gut and muscle may be largely due to depuration processes (Sahin et al. 1996, Mohamed and Hussein, 2006). We did not observe any seasonal patterns of microcystin concentrations in fish (IV).

The level of microcystins in fish gut may be closely associated with the proportion of cyanobacteria in their diet. Study II suggests that adult tilapia ingest higher amounts of the cyanobacteria that is believed to be the source of microcystins (Haande et al. 2007) than younger fish. However, we found no relationship between fish size and either the amount of

ingested microcystins or the amount of assimilated microcystins in fish tissue. This may be because our selection of fish for microcystin analysis (fishermen's catch) did not allow us to get a substantial number of younger tilapia (IV). It is also likely that any microcystins taken in by the older fish may be physiologically eliminated (Sahin et al. 1996). The microcystin congeners RR, LR, and YR were observed in fish guts and muscle (IV) and although our laboratory findings, which were limited to juvenile fish, suggest that microcystins, especially LR, are detrimental to the welfare of fish (V), a number of studies have shown that fish (mostly adults) may have a high tolerance to the toxicity of the commonly known cyanotoxin, microcystin-LR (Kotak et al. 1996).

Cyanobacteria and zooplankton behaviour

The presence of cyanobacteria, especially in temperate lakes, often coincides with a reduction in the abundance of zooplankton (Havens 2008), particularly of large filter feeding zooplankton (Pattinson et al. 2003). The efficient filter feeding cladocerans are sometimes unable to graze down high densities of filamentous algae even in the absence of predation (Gliwicz 1990b). Often there will be a shift in the zooplankton community to dominance by small zooplankton (Gliwicz and Lampert 1990). The zooplankton community in our study lake was dominated by juvenile copepods (I) which accounted for over 70% of the entire zooplankton abundance. Though this dominance of small plankton in Lake Victoria has been attributed to a number of factors, including the rise in predation pressure on large herbivores by the planktivorous cyprinid *Rastrineobola argentea* (Gophen et al. 1995) and food limitation due to eutrophication (Mwebaza ndawula 1994, Wanink et al. 2002), very little evidence exists to support these claims. Nonetheless, it is obvious that cyanobacteria dominance has a part to play in the present zooplankton composition and structure. Although cyanobacteria may dominate the phytoplankton community, preferred algal food sources are also abundant enough to provide adequate food resources for zooplankton at all depths in the water column (I, II). Zooplankton were often uniformly distributed throughout the water column due to the absence of temperature and resource (food) gradients (Lampert et al. 2003). The abundance of bloom-forming cyanobacteria may contribute to the observed low levels of water transparency. Diel vertical migration of zooplankton in lakes that lack temperature and food gradients may be associated with water transparency (Gliwicz and Pijanowska 1988), and a correlation between water transparency and amplitude of migration was observed for adult calanoid copepods in Murchison Bay (I). Owing to the generally poor water

transparency conditions and absence of better quality food in surface waters, most zooplankton in our study do not exhibit DVM, despite the high number of zooplankton predators in the lake (Mwebaza-Ndawula 1994, Wanink 1999).

Cyanobacteria are considered poor food for zooplankton (Lampert 1987). Filamentous and colonial natures, production of protease inhibitors, lack of essential fatty acids, and extra-cellular herbivore deterrents may all contribute to making cyanobacteria a poor source of nutrients for zooplankton growth and reproduction (Wilson et al. 2006), but these effects are, species and even strain specific (Tillmanns et al. 2008). Zooplankton that are continually exposed to cyanobacteria, as in eutrophic tropical lakes, may develop some degree of tolerance (Gustafsson and Hansson 2004) especially to the effects of cyanotoxins. However our results indicate that, compared to green algae, cyanobacteria are detrimental to the growth and reproduction of the tropical cladocera *Daphnia lumholtzi* (III). Yet this daphnia is considered to survive well even during cyanobacteria blooms (Pattinson et al. 2003).

Effects of cyanotoxins on zooplankton

Cyanobacteria produce a wide range of bioactive compounds that are toxic to zooplankton (Wiegand and Pflugmacher 2005). Although the commonly assayed cyanotoxins – microcystins – may not have an effect on population growth they have a negative effect on survival (Tillmanns et al. 2008). We observed low survival rates for *D. lumholtzi* when the proportion of cyanobacteria in their diet was more than 0.5 mg DW L⁻¹ both in the presence and absence of microcystins (III). Below this value, it was possible to recognise the effects of microcystins on zooplankton survival (III), as small amounts of cyanobacteria may actually stimulate growth, but above a certain threshold they affect growth negatively (Lampert 1981). The effect of microcystins on survival is therefore also linked to the rate at which they may be ingested (Rohrlack et al. 1999a, Rohrlack et al. 2001). Several other compounds, such as the protease-inhibiting cyanopeptolins and microviridins (Jungmann 1995, Rohrlack et al. 2004a) as well as mucilage polysaccharides that cause mechanical hindrances (Rohrlack et al. 1999b), may also account for observed effects of toxic cyanobacteria on zooplankton survival (III). Toxins in cyanobacteria cells do not seem to have a defence function against zooplankton grazing (Rohrlack and Hyenstrand 2007), yet when ingested will most certainly have a fatal effect on the grazer (III). The presence of toxic cyanobacteria in an aquatic environment may cause a loss in biodiversity by reducing the number of non-tolerant strains. This may be the

reason why some populations of *Daphnia* such as *D. lumholtzi* (Pattinson et al. 2003) and *D. carinata* (Matveev et al. 1994) continue to thrive in lakes with cyanobacteria blooms.

CONCLUSIONS

This study has shown that:

- Cyanobacteria especially of the genus *Microcystis* are an important diet component for adult Nile tilapia in the eutrophic Murchison Bay and Lake Mburo. In addition to phytoplankton, Nile tilapia of all sizes studied consume detritus, zooplankton, insects, and other items (including fish eggs). Juvenile tilapia consume a lower proportion of phytoplankton (cyanobacteria) than adult fish.
- Nile tilapia exhibit generalist feeding habits but may also show a preference for colonies of cyanobacteria. Ingestion of cyanobacteria cells is probably the most important route for intake and accumulation of cyanotoxins in Nile tilapia and probably zooplankton.
- Juvenile fish may not be as efficient at dealing with ingested cyanotoxins as adult fish, nonetheless, when provided in small amounts, cyanobacteria (with or without microcystins) may stimulate growth both in juvenile fish and zooplankton, but when provided as the sole source of nutrients it may hinder growth, survival, and reproduction.
- It is likely that in eutrophic tropical lakes, juvenile fish and zooplankton continue to survive because they are unable to ingest the often too large colonies of toxic cyanobacteria, but feed predominantly on other items like insects and zooplankton (fish) or green algae 'undergrowth' (zooplankton) which may still be abundant.
- The occurrence of microcystins both in the water and fish tissue may be well correlated but shows variation throughout the year that can not be explained by the seasons or measured environmental variables.
- Conditions of water transparency that are likely related to phytoplankton abundance can have an effect on the range of vertical migration for zooplankton.

REFERENCES

- Baganz, G., S. Staaks, Pflugmacher, and C. E. W. Steinberg. 2004. A comparative study on the microcystin induced behavioural changes of two fish species, *Danio rerio* and *Leucaspisus delineatus*. *Environmental Toxicology* **19**:564-570.
- Balirwa, J. S. 1992. The Evolution of the Fishery of *Oreochromis niloticus* (Pisces, Cichlidae) in Lake Victoria. *Hydrobiologia* **232**:85-89.
- Bednarska, A. 2006. Cyanobacteria and their influence on herbivore zooplankton. *Wiadomosci Ekologiczne* **52**:59-87.
- Berger, C. 1989. In situ primary production, biomass and light regime in the Wolderwijd, the most stable *Oscillatoria-agardhii* lake in the Netherlands. *Hydrobiologia* **185**:233-244.
- Beveridge, M. C. M., D. J. Baird, S. M. Rahmatullah, L. A. Lawton, K. A. Beattie, and G. A. Codd. 1993. Grazing Rates on Toxic and Nontoxic Strains of Cyanobacteria by *Hypophthalmichthys molitrix* and *Oreochromis niloticus*. *Journal of Fish Biology* **43**:901-907.
- Burns, C. W. 1968. Relationship between Body Size of Filter-Feeding Cladocera and Maximum Size of Particle Ingested. *Limnology and Oceanography* **13**:675-&.
- Bury, N. R., F. B. Eddy, and G. A. Codd. 1995. The Effects of the Cyanobacterium *Microcystis aeruginosa*, the Cyanobacterial Hepatotoxin Microcystin-Lr, and Ammonia on Growth-Rate and Ionic Regulation of Brown Trout. *Journal of Fish Biology* **46**:1042-1054.
- Bwanika, G. N., L. J. Chapman, Y. Kizito, and J. Balirwa. 2006. Cascading effects of introduced Nile Perch (*Lates niloticus*) on the foraging ecology of Nile tilapia (*Oreochromis niloticus*). *Ecology of Fresh Water Fish* **15**:470-481.
- Byarujali, S. M. 1995. Phytoplankton production in L. Mbuero - Western Uganda. Pages 284-290 Proceedings of the first conference on ecology and sustainable natural resource management for development, Mweya, Queen Elizabeth National Park, Uganda.
- Carmichael, W. W. 1992. Cyanobacteria secondary metabolites - the cyanotoxins. *Journal of Applied Bacteriology* **72**:445-459.
- Chan, F., M. L. Pace, R. W. Howarth, and R. M. Marino. 2004. Bloom formation in heterocystic nitrogen-fixing cyanobacteria: The dependence on colony size and zooplankton grazing. *Limnology and Oceanography* **49**:2171-2178.

- Chen, F. Z. and P. Xie. 2004. The toxicities of single-celled *Microcystis aeruginosa* PCC7820 and liberated *M. aeruginosa* to *Daphnia carinata* in the absence and presence of the green alga *Scenedesmus obliquus*. *Journal of Freshwater Ecology* **19**:539-545.
- Chen, J., P. Xie, D. W. Zhang, Z. X. Ke, and H. Yang. 2006. In situ studies on the bioaccumulation of microcystins in the phytoplanktivorous silver carp (*Hypophthalmichthys molitrix*) stocked in Lake Taihu with dense toxic *Microcystis* blooms. *Aquaculture* **261**:1026-1038.
- Demott, W. R., Q. X. Zhang, and W. W. Carmichael. 1991. Effects of Toxic Cyanobacteria and Purified Toxins on the Survival and Feeding of a Copepod and 3 Species of *Daphnia*. *Limnology and Oceanography* **36**:1346-1357.
- Dittmann, E., A. Neilan, H. Erhard, v. Dören, and T. Börner. 1997. Insertional mutagenesis of a peptide synthetase gene that is responsible for hepatatoxin production in the cyanobacterium *Microcystis aeruginosa* PCC7806. *Molecular Microbiology* **26**:779-787.
- Downing, J. A., S. B. Watson, and E. McCauley. 2001. Predicting Cyanobacteria dominance in lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **58**:1905-1908.
- Fay, P. 1992. Oxygen relations of nitrogen-fixation in cyanobacteria. *Microbiological Reviews* **56**:340-373.
- Ferrão-Filho, A. S., S. Azevedo, and W. R. DeMott. 2000. Effects of toxic and non-toxic cyanobacteria on the life history of tropical and temperate cladocerans. *Freshwater Biology* **45**:1-19.
- Gantar, M., J. P. Berry, S. Thomas, M. L. Wang, R. Perez, and K. S. Rein. 2008. Allelopathic activity among Cyanobacteria and microalgae isolated from Florida freshwater habitats. *FEMS Microbiology Ecology* **64**:55-64.
- Getachew, T. 1987. A study on a herbivorous fish, *Oreochromis niloticus* L., diet and its quality in two Ethiopian Rift valley lakes, Awasa and Zwai. *Journal of Fish Biology* **30**:439-449.
- Glazer, A. N. 1977. Structure and molecular-organization of photosynthetic accessory pigments of cyanobacteria and red algae. *Molecular and Cellular Biochemistry* **18**:125-140.
- Gliwicz, Z. M. 1990a. *Daphnia* Growth at Different Concentrations of Blue-Green Filaments. *Archiv Fur Hydrobiologie* **120**:51-65.
- Gliwicz, Z. M. 1990b. Why do Cladocerans fail to control algal blooms? *Hydrobiologia* **200**:83-97.

- Gliwicz, Z. M. and W. Lampert. 1990. Food Thresholds in *Daphnia* Species in the Absence and Presence of Blue-Green Filaments. *Ecology* **71**:691-702.
- Gliwicz, Z. M. and J. Pijanowska. 1988. Effect of predation and resource distribution on vertical migration of zooplankton. *Bulletin of Marine Science* **43**:695-709.
- Gophen, M., P. B. O. Ochumba, and L. S. Kaufman. 1995. Some aspects of perturbation in the structure and biodiversity of the ecosystem of Lake Victoria (East-Africa). *Aquatic Living Resources* **8**:27-41.
- Gustafsson, S. and L. A. Hansson. 2004. Development of tolerance against toxic cyanobacteria in *Daphnia*. *Aquatic Ecology* **38**:37-44.
- Haande, S. 2008. On the Ecology, Toxicology, and Phylogeny of Cyanobacteria in Murchison Bay of Lake Victoria, Uganda. Ph.D thesis. University of Bergen, Bergen.
- Haande, S., A. Ballot, T. Rohrlack, J. Fastner, C. Wiedner, and B. Edvardsen. 2007. Diversity of *Microcystis aeruginosa* isolates (Chroococcales, Cyanobacteria) from East-African water bodies. *Archiv Microbiol* **188**:15-25.
- Havens, K. E. 2008. Cyanobacteria blooms: effects on aquatic ecosystems. Pages 733-747 in H. H. Kenneth, editor. *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*. Springer, New York.
- Havens, K. E., T. L. East, J. Marcus, P. Essex, B. Bolan, S. Raymond, and J. R. Beaver. 2000. Dynamics of the exotic *Daphnia lumholtzi* and native macro-zooplankton in a sub-tropical chain of lakes in Florida, U.S.A. *Freshwater Biology* **45**:21-32.
- Hecky, R. E., F. W. B. Bugenyi, P. B. O. Ochumba, J. F. Talling, R. Mugidde, M. Gophen, and L. Kaufman. 1994. Deoxygenation of the deep water of Lake Victoria, East Africa. *Limnology and Oceanography* **39**:1476-1481.
- Jacquet, C., V. Thermes, A. de Luze, S. Puiseux-Dao, C. Bernard, J. S. Joly, F. Bourrat, and M. Edery. 2004. Effects of microcystin-LR on development of medaka fish embryos (*Oryzias latipes*). *Toxicol* **43**:141-147.
- Jungmann, D. 1995. Isolation, purification and characterisation of a new *Daphnia* toxic compound from axenic *Microcystis flos-aquea* strain PCC 7806. *Journal of Chemical Ecology* **21**:1665-1676.
- Kamujunke, N., K. Schmidt, S. Pflugmacher, and T. Mehner. 2002. Consumption of cyanobacteria by roach (*Rutilus rutilus*) useful or harmful to the fish? *Freshwater Biology* **47**:243-250.
- Kayiira, D. 2007. Algal community of Lake Mburo and Murchison Bay, Lake Victoria. Masters Thesis. Makerere University, Kampala.

- Keshavanath, P., M. C. M. Beveridge, D. J. Baird, L. A. Lawton, A. Nimmo, and G. A. Codd. 1994. The Functional Grazing Response of a Phytoplanktivorous Fish *Oreochromis niloticus* to Mixtures of Toxic and Nontoxic Strains of the Cyanobacterium *Microcystis aeruginosa*. *Journal of Fish Biology* **45**:123-129.
- Kling, H. J., R. Mugidde, and R. E. Hecky. 2001. Recent changes in the phytoplankton community of Lake Victoria in response to eutrophication. Pages 47-65 in M. Munawar and R. E. Hecky, editors. *The great lakes of the world: food web, health and integrity*. Backhuys Publishers Leiden
- Kotak, B. G., S. Semalulu, D. L. Fritz, E. E. Prepas, S. E. Hrudey, and R. W. Coppock. 1996. Hepatic and renal pathology of intraperitoneally administered microcystin-LR in rainbow trout (*Oncorhynchus mykiss*). *Toxicon* **34**:517-525.
- Lampert, W. 1981. Inhibitory and toxic effects of Blue-Green-Algae on *Daphnia*. *Internationale Revue der Gesamten Hydrobiologie* **66**:285-298.
- Lampert, W. 1987. Laboratory Studies on Zooplankton-Cyanobacteria Interactions. *New Zealand Journal of Marine and Freshwater Research* **21**:483-490.
- Lampert, W., E. McCauley, and B. F. J. Manly. 2003. Trade-offs in the vertical distribution of zooplankton: Ideal free distribution with costs? *Proc. R. Soc. Lond. B* **270**:765-773.
- Landsberg, J. H. 2002. The effects of harmful algal blooms on aquatic organisms. *Reviews in Fisheries Science* **10**:113-390.
- Lawton, L. A. and G. A. Codd. 1991. Cyanobacterial (Blue-Green-Algal) Toxins and Their Significance in Uk and European Waters. *Journal of the Institution of Water and Environmental Management* **5**:460-465.
- Lewin, W. C., N. Kamjunke, and T. Mehner. 2003. Phosphorus uptake by *Microcystis* during passage through fish guts. *Limnology and Oceanography* **48**:2392-2396.
- Lindholm, T., J. E. Eriksson, M. Reinikainen, and J. A. O. Meriluoto. 1992. Ecological Effects of Hepatotoxic Cyanobacteria. *Environmental Toxicology and Water Quality* **7**:87-93.
- Magalhaes, V. F., M. M. Marinho, P. Domingos, A. C. Oliveira, S. M. Costa, L. O. Azevedo, and S. Azevedo. 2003. Microcystins (cyanobacteria hepatotoxins) bioaccumulation in fish and crustaceans from Sepetiba Bay (Brasil, RJ). *Toxicon* **42**:289-295.
- Malbrouck, C. and P. Kestemont. 2006. Effects of microcystins on fish. *Environmental Toxicology and Chemistry* **25**:72-86.

- Matveev, V., L. Matveeva, and G. J. Jones. 1994. Study of the Ability of *Daphnia carinata* King to Control Phytoplankton and Resist Cyanobacterial Toxicity - Implications for Biomanipulation in Australia. *Aust J Mar Freshwat Res* **45**:889-904.
- Mohamed, Z. A., W. W. Carmichael, and A. A. Hussein. 2003. Estimation of microcystins in the freshwater fish *Oreochromis niloticus* in an Egyptian fish farm containing a *Microcystis* bloom. *Environmental Toxicology* **18**:137-141.
- Mohamed, Z. A. and A. A. Hussein. 2006. Depuration of microcystins in tilapia fish exposed to natural populations of toxic cyanobacteria: A laboratory study. *Ecotoxicology and Environmental Safety* **63**:424-429.
- Moriarty, C. M. and D. J. W. Moriarty. 1973a. Quantitative estimation of the daily ingestion of phytoplankton by *Tilapia nilotica* in Lake George, Uganda. *Journal of Zoology* **171**:15-23.
- Moriarty, D. J. W. 1973. The physiology of digestion of blue green algae in the cichlid fish, *Tilapia nilotica*. *Journal of Zoology* **171**:25-39.
- Moriarty, D. J. W., P. E. C. J. Darlington, I. G. Dunn, M. C. Moriarty, and M. P. Tevlin. 1973. Feeding and Grazing in Lake George, Uganda. *Proceedings of Royal Society of London* **184**:299-319.
- Moriarty, D. J. W. and C. M. Moriarty. 1973b. The assimilation of carbon from phytoplankton by two herbivorous fishes: *Tilapia nilotica* and *Haplochromis nigripinnis*. *Journal of Zoology* **171**:41-55.
- Mwebaza ndawula, L. 1994. Changes in Relative Abundance of Zooplankton in Northern Lake Victoria, East-Africa. *Hydrobiologia* **272**:259-264.
- Mwebazandawula, L. 1994. Changes in Relative Abundance of Zooplankton in Northern Lake Victoria, East-Africa. *Hydrobiologia* **272**:259-264.
- Nizan, S., C. Dimentman, and M. Shilo. 1986. Acute toxic effects of the cyanobacterium *Microcystis aeruginosa* on *Daphnia magna*. *Limnology and Oceanography* **31**.
- Northcott, M. E. and M. C. M. Beveridge. 1988. The development and structure of pharyngeal apparatus associated with filter feeding in tilapias (*Oreochromis niloticus*). *Journal of Zoology* **215**:133 -149.
- Northcott, M. E., M. C. M. Beveridge, and L. G. Ross. 1991. A Laboratory Investigation of the Filtration and Ingestion Rates of the Tilapia, *Oreochromis niloticus*, Feeding on 2 Species of Blue-Green-Algae. *Environmental Biology of Fishes* **31**:75-85.

- Oberemm, A., J. Becker, G. A. Codd, and C. Steinberg. 1999. Effects of cyanobacterial toxins and aqueous crude extracts of cyanobacteria on the development of fish and amphibians. *Environmental Toxicology* **14**:77-88.
- Ochumba, P. B. O. 1990. Massive fish kills within the Nyanza Gulf of lake Victoria, Kenya. *Hydrobiologia* . **208**:93–99.
- Ogutuohwayo, R. and R. E. Hecky. 1991. Fish Introductions in Africa and Some of Their Implications. *Canadian Journal of Fisheries and Aquatic Sciences* **48**:8-12.
- Paerl, H. W. and J. F. Ustach. 1982. Blue-green-algal scums - an explanation for their occurrence during fresh-water blooms. *Limnology and Oceanography* **27**:212-217.
- Pattinson, K. R., J. E. Havel, and R. G. Rhodes. 2003. Invasibility of a reservoir to exotic *Daphnia lumholtzi*: experimental assessment of diet selection and life history responses to cyanobacteria. *Freshwater Biology* **48**:233-246.
- Perschbacher, P. W. 2003. Biological control of off-flavor cyanobacteria. *Off-Flavors in Aquaculture* **848**:167-177.
- Pflugmacher, S. 2002. Possible allelopathic effects of cyanotoxins, with reference to microcystin-LR, in aquatic ecosystems. *Environmental Toxicology* **17**:407-413.
- Repka, S., M. Meyerhofer, K. von Brockel, and K. Sivonen. 2004. Associations of cyanobacterial toxin, nodularin, with environmental factors and zooplankton in the Baltic Sea. *Microbial Ecology* **47**:350-358.
- Rinehart, K. L., M. Namikoshi, and B. W. Choi. 1994. Structure and Biosynthesis of Toxins from Blue-Green-Algae (Cyanobacteria). *Journal of Applied Phycology* **6**:159-176.
- Rippka, R., J. Deruelles, J. B. Waterbury, M. Herdman, and R. Y. Stanier. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* **111**:1-61.
- Robinson, R. L., G. F. Turner, A. S. Grimm, and T. J. Pitcher. 1995. An Experimental-Study of Phytoplankton Feeding in 3 Tilapiine Cichlids. *Journal of Fish Biology* **46**:449-456.
- Rodger, H. D., T. Turnbull, C. Edwards, and G. A. Codd. 1994. Cyanobacterial (blue-green-algal) bloom associated pathology in brown trout, *Salmo-trutta* L, in Loch Leven, Scotland. *Journal of Fish Diseases* **17**:177-181.
- Rohrlack, T., K. Christoffersen, P. Hansen, W. Zhang, O. Czarnecki, M. Henning, J. Fastner, M. Erhard, A. B. Neilan, and M. Kaebernick. 2004a. Isolation, Characterization, and Quantitative Analysis of Microviridin J, a New Microcystis Metabolite Toxic to *Daphnia*. *Journal of Chemical Ecology* **29**:1573-1561.

- Rohrlack, T., K. Christoffersen, M. Kaebernick, and B. A. Neilan. 2004b. Cyanobacterial protease inhibitor microviridin J causes a lethal moulting disruption in *Daphnia pulex*. *Applied and Environmental Microbiology* **70**:5047-5050.
- Rohrlack, T., E. Dittmann, T. Borner, and K. Christoffersen. 2001. Effects of cell-bound microcystins on survival and feeding of *Daphnia spp.* *Applied and Environmental Microbiology* **67**:3523-3529.
- Rohrlack, T., B. Edvardsen, R. Skulberg, C. B. Halstvedt, H. C. Utkilen, R. Ptacnik, and O. M. Skulberg. 2008. Oligopeptide chemotypes of the toxic freshwater cyanobacterium *Planktothrix* can form subpopulations with dissimilar ecological traits. *Limnology and Oceanography* **53**:1279-1293.
- Rohrlack, T., M. Henning, and J. G. Kohl. 1999a. Does the toxic effect of *Microcystis aeruginosa* on *Daphnia galeata* depend on microcystin ingestion rate? *Archiv Fur Hydrobiologie* **146**:385-395.
- Rohrlack, T., M. Henning, and J. G. Kohl. 1999b. Mechanisms of the inhibitory effect of the cyanobacterium *Microcystis aeruginosa* on *Daphnia galeata's* ingestion rate. *Journal of Plankton Research* **21**:1489-1500.
- Rohrlack, T. and P. Hyenstrand. 2007. Fate of intracellular microcystins in the cyanobacterium *Microcystis aeruginosa* (Chroococcales, Cyanophyceae). *Phycologia* **46**:277-283.
- Sahin, A., F. G. Tencalla, D. R. Dietrich, and H. Naegeli. 1996. Biliary excretion of biochemically active cyanobacteria (blue-green algae) hepatotoxins in fish. *Toxicology* **106**:123-130.
- Sanderson, S. L., A. Y. Cheer, J. S. Goodrich, J. D. Graziano, and W. T. Callan. 2001. Crossflow filtration in suspension-feeding fishes. *Nature* **412**:439-441.
- Sarma, S. S. S., S. Nandini, and R. D. Gulati. 2005. Life history strategies of cladocerans: comparisons of tropical and temperate taxa. *Hydrobiologia* **542**:315-333.
- Scheffer, M., S. H. Hosper, M. L. Meijer, B. Moss, and E. Jeppesen. 1993. Alternative Equilibria in Shallow Lakes. *Trends in Ecology & Evolution* **8**:275-279.
- Schindler, D. W., R. E. Hecky, D. L. Findlay, M. P. Stainton, B. R. Parker, M. J. Paterson, K. G. Beaty, M. Lyng, and S. E. M. Kasian. 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *Proceedings of the National Academy of Sciences of the United States of America* **105**:11254-11258.

- Schröder, H., J. Dørge, S. C. Nielsen, N. Thygesen, and N. Ipsen. 1998. Kampala water quality monitoring programme - Murchison Bay water quality project., COWI/ VKI.
- Singh, D. P., M. B. Tyagi, A. Kumar, and J. K. Thakur. 2001. Antialgal activity of a hepatotoxin-producing cyanobacterium, *Microcystis aeruginosa*. *World Journal of Microbiology & Biotechnology* **17**:15-22.
- Skulberg, O. M., G. A. Codd, and W. W. Carmichael. 1984. Toxic Blue-Green-Algal Blooms in Europe - a Growing Problem. *Ambio* **13**:244-247.
- Smith, V. H. and S. J. Bennett. 1999. Nitrogen : phosphorus supply ratios and phytoplankton community structure in lakes. *Archiv Fur Hydrobiologie* **146**:37-53.
- Sukenik, A., R. Eshkol, A. Livne, O. Hadas, M. Rom, D. Tchernov, A. Vardi, and A. Kaplan. 2002. Inhibition of growth and photosynthesis of the dinoflagellate *Peridinium gatunense* by *Microcystis* sp (cyanobacteria): A novel allelopathic mechanism. *Limnology and Oceanography* **47**:1656-1663.
- Talling, J. F. 2003. Phytoplankton-zooplankton seasonal timing and the 'clear-water phase' in some English lakes. *Freshwater Biology* **48**:39-52.
- Tencalla, F. G., D. R. Dietrich, and C. Schlatter. 1994. Toxicity of *Microcystis aeruginosa* Peptide Toxin to Yearling Rainbow-Trout (*Oncorhynchus-Mykiss*). *Aquatic Toxicology* **30**:215-224.
- Tillmanns, A. R., W. A. E., F. R. Pick, and O. Sarnelle. 2008. Meta-analysis of cyanobacterial effects on zooplankton population growth rate: species specific responses. *Fundam. Appl. Limnol.* **171**:285-295.
- Turker, H., A. G. Eversole, and D. E. Brune. 2003a. Comparative Nile tilapia and silver carp filtration rates of Partitioned Aquaculture System phytoplankton. *Aquaculture* **220**:449-457.
- Turker, H., A. G. Eversole, and D. E. Brune. 2003b. Filtration of green algae and cyanobacteria by Nile tilapia, *Oreochromis niloticus*, in the Partitioned Aquaculture System. *Aquaculture* **215**:93-101.
- Verschuren, D., T. C. Johnson, H. J. Kling, D. N. Edgington, P. R. Leavitt, E. T. Brown, M. R. Talbot, and R. E. Hecky. 2002. History and timing of human impact on Lake Victoria, East Africa. *Proceedings of the Royal Society of London Series B-Biological Sciences* **269**:289-294.
- Vos, A. T. and J. C. Roos. 2005. Causes and consequences of algal blooms in Loch Logan, an urban impoundment. *Water Sa* **31**:385-392.

- Waldby, M., T. Kroglund, and N. I. f. W. R. (NIVA). 2002. The Baltic Sea - the largest brackish sea in the world.
- Walsby, A. E. 1994. Gas vesicles. *Microbiological Reviews* **58**:94-144.
- Wang, P. J., M. S. Chien, F. J. Wu, H. N. Chou, and S. J. Lee. 2005. Inhibition of embryonic development by microcystin-LR in zebrafish, *Danio rerio*. *Toxicol* **45**:303-308.
- Wanink, J. H. 1999. Prospects for the fishery on the small pelagic *Rastrineobola argentea* in Lake Victoria. *Hydrobiologia* **407**:183-189.
- Wanink, J. H., E. F. B. Katunzi, K. P. C. Goudswaard, F. Witte, and W. L. T. van Densen. 2002. The shift to smaller zooplankton in Lake Victoria cannot be attributed to the 'sardine' *Rastrineobola argentea* (Cyprinidae). *Aquatic Living Resources* **15**:37-43.
- Wehr, D. J. and G. R. Sheath. 2003. Fresh water algae of North America: Ecology and classification. Elsevier science, USA.
- Weiss, J., H. P. Liebert, and W. Braune. 2000. Influence of microcystin-RR on growth and photosynthetic capacity of the duckweed *Lemna minor* L. Pages 100-105. Blackwell Wissenschafts-Verlag GmbH.
- Wiegand, C. and S. Pflugmacher. 2005. Ecotoxicological effects of selected cyanobacterial secondary metabolites a short review. *Toxicology and Applied Pharmacology* **203**:201-218.
- Wiegand, C., S. Pflugmacher, A. Oberemm, N. Meems, K. A. Beattie, C. E. W. Steinberg, and G. A. Codd. 1999. Uptake and effects of microcystin-LR on detoxication enzymes of early life stages of the zebra fish (*Danio rerio*). *Environmental Toxicology* **14**:89-95.
- Wilson, A. E., O. Sarnelle, and A. R. Tillmans. 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: Meta-analyses of laboratory experiments. *Limnology and Oceanography* **51**:1915 - 1924.
- Wronski, T. 2002. Feeding ecology and foraging behaviour of impala *Aepyceros melampus* in Lake Mburo National Park, Uganda. *African Journal of Ecology* **40**:205-211.