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DISSERTATION

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„Phytoplankton communities and cyanotoxin-production in Kenyan water bodies“

Verfasserin

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Table of Contents

Zusammenfassung

Arbeitsplätze und proteinreiche Nahrung sind in vielen Landstrichen Kenias Mangelware. Diesen Problemen nahm sich das EU-Projekt BOMOSA an, im Rahmen dessen diese Dissertation verfasst wurde. Mit falt- und transportierbaren Netzkäfigen war es das Ziel, Fischzucht auch in ländliche Gegenden zu bringen. In drei klimatisch unterschiedlichen Regionen Kenias – Machakos, Mount Kenia und Viktoria See – wurden an jeweils drei Gewässern (Ngeki, Ngei, Lukenya; Ruthagati, Mailo, Sagana; Harambe, Damside, Bomet) Holzplots gebaut, die Netzkäfige eingesetzt und mit der Buntbarsch-Art *Oreochromis niloticus* besetzt. Die Gewässer unterscheiden sich hinsichtlich maximaler Tiefe, Uferbewuchs und Nutzung durch die Bevölkerung. Teilweise werden sie auch als Wasserstelle für Nutztiere verwendet. Die Probennahmen zu der vorliegenden Arbeit fanden monatlich innerhalb eines Jahres statt (zwei Probennahmen mussten aufgrund von Unruhen nach der Wahl 2007/2008 in Kenia entfallen). Zwei Studien der vorliegenden Arbeit beschäftigten sich mit der Artenzusammensetzung von Phytoplankton, strukturierenden abiotischen Faktoren und der potenziellen Gefährdung der Gewässer durch Microcystin-Produktion. Microcystin ist ein zyklisches Heptapeptid, das von einigen Cyanobakterien-Gattungen synthetisiert werden kann; Hauptproduzent und Namensgeber ist das Cyanobakterium *Microcystis sp.*.

Microcystin steht auch seit Längerem im Verdacht, zum Flamingo-Sterben in den Seen Nakuru und Bogoria im Ostafrikanischen Grabenbruch beizutragen, welches von Zeit zu Zeit auftritt. Manchmal beherbergen diese Seen bis zu 75 Prozent der weltweiten Population des Kleinen Flamingos (*Phoeniconaias minor*) (Vareschi, 1978).

Der dritte Teil der Arbeit befasst sich mit dem plötzlichen Flamingo-Sterben an den Ufern der Sodaseen. Dieser Teil wurde im Rahmen des Projekts "Factors controlling abundance of *Arthrospira fusiformis*" durchgeführt.

Die **erste Studie** beschäftigt sich mit der Phytoplankton-Zusammensetzung und dem Einfluss von abiotischen Faktoren auf Artenmuster der Algen. Auf Basis von Chlorophyll-a- und Total-Phosphor-Daten ergab sich für die Gewässer ein hypertropher Zustand. Einzige Ausnahme war das Reservoir in Sagana, dieses wurde zu Beginn der Probenperiode neu befüllt und zunächst als eutroph eingestuft. Im weiteren Untersuchungsverlauf wechselte aber auch dieses Gewässer zum hyper-eutrophen Zustand. Aufgrund des hohen Nährstoffgehaltes und des Eintrages von Fischausscheidungen und Resten von Fischfutter hatten wir eine Dominanz von Cyanobakterien erwartet, die sich jedoch in den meisten Wasserkörpern nicht einstellte. Ausnahme war der Teich Bomet, der eine hohe Biomasse an *Microcystis* aufwies. Saisonale Veränderungen zeigten sich insbesondere in der Artenzusammensetzung nach der kurzen Trockenzeit im Februar. Anstelle von Cyanobakterien wurden hohe Abundanzen von Chloro-, Crypto, Dino- und Euglenophyten gefunden. Mit einer Hauptkomponentenanalyse – sie erklärt rund 85 % der Datenvarianz – wurden aus den erhobenen abiotischen Faktoren vier Hauptkomponenten (Ions, Location, Zooplankton, Particulate matter) extrahiert. Basierend auf der Algengemeinschaft konnte mit einem multivariaten Analysemodell gezeigt werden, dass sich die unterschiedlichen Algen-Gemeinschaften der einzelnen Gewässer von mit und ohne Zugangsmöglichkeiten für Nutztiere hinsichtlich der Artenzusammensetzung stark unterscheiden. Diese Gewässer zeigen höhere Abundanzen von Cyanobakterien und Chlorophyta.

In der **zweiten Studie** wurden Toxin-Analysen für die neun Teiche durchgeführt. Während Planktonnetzproben mittels HPLC (High Performance Liquid Chromatography) analysiert wurden, mussten für die integrativen Proben aufgrund der geringen Toxinmengen ELISA Kits (Enzyme Linked ImmunoSorbent Assay) eingesetzt werden. Drei der Teiche enthielten hohe Mengen der hochtoxischen Microcystin-Variante Microcystin-LR (Lukenya, Ruthgati, Mailo). Mit einem Diskriminanz-Modell konnten

toxische und nicht-toxische Proben basierend auf Umweltvariablen zu 83 Prozent korrekt zugewiesen werden. Damit kann mittels abiotischer Faktoren, die auch in einfach ausgestatteten Labors analysiert werden können, die Gefahr durch Microcystine abgeschätzt werden.

In der **dritten Studie** wurde untersucht, inwieweit Cyanotoxine für das Flamingo-Sterben in den Seen Nakuru und Bogoria verantwortlich sind. Das Phänomen plötzlich eintretender Massensterben dieser Vögel beschäftigt Wissenschaftler seit Jahrzehnten. Seit Kurzem wird auch Microcystin als potenzieller Grund dafür diskutiert, besonders weil die Hauptnahrungsquelle für den „Kleinen Flamingo" das Cyanobakterium *Arthrospira fusiformis* ist und Einzelnachweise toxischer Stämme vorliegen. *A. fusiformis* dominiert zumeist die Phytoplankton-Gemeinschaft in diesen beiden Seen. Mit HPLC wurden wöchentliche Proben von Juli 2008 bis November 2009 analysiert. Zusätzlich wurden monatliche Stichproben mit Protein-Phosphatase-Inhibitions-Assays und auf polare Toxine analysiert. Während der Dauer des Projekts kam es zu drei Massensterben im Bereich von Lake Bogoria und wir hatten die Möglichkeit, von 20 toten Flamingos Gewebeproben zu entnehmen. Weder in den Planktonproben noch in Proben aus verschiedenen Flamingo-Geweben konnten Cyanotoxine nachgewiesen werden. Für die Flamingo-Sterben während unseres Probenzeitraums können daher Algentoxine ausgeschlossen werden.

Studien an den kenianischen Seen werden seit Jahren durchgeführt, kleine Teiche und Reservoirs waren bisher nur in Einzelfällen Thema von Untersuchungen. Es sind aber gerade diese kleinen Gewässer, die der lokalen Bevölkerung als Wasserquelle dienen. Bei kleineren Gewässern zeigen Regenfälle und Nährstoffeinträge schneller Wirkung, weil die Verweildauer gering ist und Umweltänderungen nicht gut abgepuffert werden können. Die vorliegenden Untersuchungen bereichern die Erforschung von afrikanischen

Gewässern und liefern zudem wichtige Erkenntnisse über Möglichkeiten der Wassernutzung. Studien dieser Art sind enorm wichtig, weil diese kleinen Gewässer in ländlichen Gegenden für Fischzucht verwendet werden und es zum Auftreten von toxischen Cyanobakterien kommen kann. Diese können über die Nahrungskette in den menschlichen Organismus gelangen. Ein regelmäßiges Monitoring ist daher als sinnvoll zu erachten.

Abstract

This thesis was developed within the framework of the EU-project BOMOSA that focused on problems of food supply for local people of rural areas in Kenya; especially there is a shortage of protein-rich diet. The BOMOSA scientists developed a cheap, robust, foldable and transportable net cage system to introduce fish cage farming to rural areas. In three different climate regions in Kenya – Machakos, Mount Kenya and Lake Victoria –wooden plots were established, net cages were installed and stocked with the cichlid fish *Oreochromis niloticus*. In each region three waterbodies were investigated (Ngeki, Ngei, Lukenya; Ruthagati, Mailo Dam, Sagana; Harambe, Damside, Bomet). The waterbodies differed in terms of maximum water depth, shoreline vegetation and utilization by the people. Some of the water-bodies were used for watering cattle. Sampling was carried out monthly over a period of one year. Two studies of the current thesis deal with the phytoplankton community and the factors that influence these communities, as well as microcystin production in these ponds. Microcystin is a cyclic heptapeptide, which is synthetized by some cyanobacterial genera and also a threat to people if ingested via the food chain.

For some time, microcystin is under suspicion to contribute to sudden flamingo mass mortality at saline-alkaline lakes in the East African Rift Valley. From time to time these lakes host up to 75 percent of the world's population of the Lesser Flamingo (*Phaeniconaias minor*) (Vareschi, 1978). The mass mortality threatens the touristic attraction of these regions, what in turn has negative effects on the socio-economy in these regions. The third part of the thesis deals with the potential of microcystin as cause for flamingo mass mortality. This study was carried out in the framework of the FWF-project P-19911 "*Factors controlling abundance of Arthrospira fusiformis*".

The **first study** focuses on phytoplankton communities and the influence of abiotic factors on the species-community-pattern. On basis of data on chlorophyll-a and total phosphorous the

water bodies were classified hyper-eutrophic with the exception of Sagana (eutrophic), which had been refilled at the beginning of the sampling period. In the further course of the study the status switched also to hyper-eutrophic. Due to high nutrient loads and input by fish droppings and fish feed we expected a dominance of Cyanobacteria. For most water-bodies in this study this expectation was however refuted. Only Bomet showed high amounts of *Microcystis* spp. . In the other ponds high abundances of Chloro-, Crypto-, Dino-, and Euglenophytes were found. A principal component analysis explaining about 85 percent of the variance of the data, extracted four principal components. Cattle access turned out to be a key factor for the structure of the algal community; it influences underwater light supply via turbidity and also nutrient supply.

The **second study** focused on cyanotoxin occurrence in these nine ponds. Plankton-net samples were analyzed by HPLC (High Performance Liquid Chromatography). For the depth-integrated water samples more sensitive ELISA kits (**E**nzyme **L**inked **I**mmuno**S**orbent **A**ssay) were used, as toxic content turned out to be too low for HPLC-detection. In three ponds (Lukenya, Ruthagati, Mailo) the highly toxic microcystin variant MC-LR was found by HPLC. With ELISA kits only one pond could be considered toxin-free (Harambe). Some ponds are toxin-free on some occasions. Based on the results of the ELISA measurements two groups were defined: toxin-free and toxin-containing. For discriminating these groups, abiotic factors, bacterial and zooplankton numbers were used. The discriminant model classified 80 percent of the samples correctly with only total phosphorus and nitrite as significant variables. These parameters can easily be obtained by simple means.

In the **third study** potential causes for the flamingo die-offs in the Lakes Nakuru and Bogoria were discussed. Mass mortality of flamingos has been occupying scientists since decades. Recently, also Microcystin came into play as a potential reason for the die-offs, especially

because the main diet of the Lesser flamingo is the cyanobacterium *Athrospira fusiformis,* that is often dominating the phytoplankton community in saline-alkaline lakes. There already exist reports mentioning toxic strains of this taxon. By means of HPLC, weekly samples of Lake Nakuru and Lake Bogoria had been analyzed from July 2008 to November 2009. Additionally, monthly spot samples were analyzed for polar toxins and using PPIA (Protein-Phosphatase-Inhibitions-Assay). During the sampling period three mass-die-offs occurred at Lake Bogoria. We had the opportunity to take samples from 20 dead flamingos. Neither in plankton samples nor in samples from Flamingo tissue cyanotoxins were detected. Factors other than cyanotoxins must be considered for triggering mortalities during our sampling period, such as starvation and/or bird diseases.

Studies on Kenyan lakes are conducted for years, but small ponds had been largely neglected. It is however small reservoirs and ponds that are highly implemented in the daily life of local people, which makes the current study not only interesting for basic research, but also for practical aspects. Small ponds respond much faster to rainfall and nutrient input, because the retention time is low thus buffering against environmental changes minimized. Studies of this kind and monitoring programs are an urgent need for such waterbodies because they are heavily used for various purposes.

Dissertation Outline and Contributors

This dissertation is a compilation of three manuscripts representing the main part of my research:

1) Straubinger-Gansberger N., Kaggwa N. M., Schagerl M. 2014. Phytoplankton patterns along a series of small man-made reservoirs in Kenya. Environmental Monitoring and Assessment, Volume 186, Issue 8, pp 5153-5166. DOI 10.1007/s10661-014-3766-x
2) Straubinger-Gansberger N., Kaggwa N. M., Schagerl M. 2015. Small man-made ponds in Kenya and cyanotoxins – a threat to local people? (submitted)
3) Straubinger-Gansberger N., Gruber M., Kaggwa N. M., Lawton L., Oduor S. O., Schagerl M. 2014. Sudden flamingo deaths in Kenyan Rift Valley lakes. Wildlife Biology. Volume 20, Issue 3, pp 185-187. DOI 10.2981/wlb.00018

The work presented here was conducted within the BOMOSA project (EU-project contract no. 032103), which aimed to establish fish cage farming in the East African countries Kenya, Uganda, and Ethiopia (http://bomosa.oeaw.ac.at). The third part was funded by Austrian Science Fund Project No. P19911 "Factors controlling abundance of *Arthrospira fusiformis*".

	Straubinger-Gansberger N. et al., 2014 a	Straubinger-Gansberger N. et al., submitted	Straubinger-Gansberger N. et al., 2014 b
Experimental design	50%	50%	25%
Experimental work	25%	25%	25%
Laboratory analysis	50%	75%	50%
Data analysis	100%	100%	100%
Writing of manuscript	75%	75%	75%

Introduction

The world's deepest and largest lakes located in the western branch of the Great African Rift Valley (Lake Tanganyika, Lake Malawi, Lake Victoria) have been topic of many studies in the last decades (for a review see Descy and Sarmento (2008)). This study did not focus on these famous lakes but on small, mostly man-made reservoirs in rural areas spread over the country. In contrast to the big lakes, these small water bodies have almost completely been neglected in basic and applied research (Degefu et al., 2011; Kaggwa et al., 2010). Situated in three different areas of Kenya (Machakos, Mount Kenya, Lake Victoria) nine ponds were investigated within the BOMOSA project. The biggest part of the project was carried out in Kenya, while three sites in Ethiopia and two sites in Uganda were examined. The aim of the project was to provide to the people living in rural areas high-quality food at low cost. The locals use the investigated water bodies for different purposes, as watering points for domestic animals, as water supply for the rural community and for washing clothes. Within the BOMOSA project small fish-cage farming was introduced: foldable net cages, that could also be used for temporary water bodies and that are easy to handle, were set in wooden-plots (see Fig. 1).

Fig. 1: Wooden plot that is used for fish cage farming. Foto: Straubinger-Gansberger

Turbidity in the investigated ponds was high and showed fluctuations because of cattle access and the stocking with benthivorous fish. We expected eutrophication processes due to the feed offered to fish and fish excrements (Guo and Li, 2003). Characteristics of hyper-eutrophic ponds such as reduced transparency of the water, high nutrient loads and increased water temperatures often observed in tropical reservoirs, favor development of cyanobacterial dominance (Shapiro, 1990; Smith, 1986; Watson et al., 1997). Such mass occurrence may lead to severe problems, as some genera are known to produce toxins. The most common toxin, which is already well investigated, is the cyclic heptapeptide Microcystin (MC). MC was first isolated from *Microcystis aeruginosa* and thus named after it (Carmichael et al., 1988). In the meanwhile, MCs have also been isolated from many other cyanobacterial genera such as *Anabaena, Planktothrix, Anabaenopsis, Nostoc* and *Phormidium* and the terrestrial genus *Hapalosiphon* (for reviews see Sivonen and Jones (1999) and Codd et al. (2005)). MCs share the common structure cyclo-(D-alanine-L-X-D-erythro-β-methylaspartic acid –L-Z-Adda-D-glutamate-N-methyldehydroalanine). On the positions X and Z bind variable L-amino acids. Until now more than 90 different molecule variants have been identified (del Campo and Ouahid, 2010; Qi et al., 2014; Welker and Von Döhren, 2006)

MCs inhibit the eukaryotic protein phosphatases 1 and 2A (An and Carmichael, 1994; MacKintosh et al., 1990) that are important for the cellular metabolism. Poisoning due to MCs in drinking water and in water for dialysis have been reported worldwide (Azevedo et al., 2002; Carmichael et al., 2001). It is also known that the exposure to even low concentrations of MC in drinking water increases the risk of liver and colorectal cancer significantly (Hernandez et al., 2009).

MCs with their impact on life and health of humans and animals also influence Kenyans in socio-economic ways. The small and shallow lakes in the eastern branch of the Rift Valley, most of them alkaline-saline, like the world-famous Kenyan flamingo lakes Nakuru and Bogoria, are interesting for researchers all over the world because of their very special

environmental conditions, that lead to specialized communities (Ballot et al., 2009; Burian et al., 2012; Descy and Sarmento, 2008; Hubble and Harper, 2002; Lung'Ayia et al., 2000; Schagerl and Oduor, 2008). The water bodies are important touristic attractions in the Rift Valley. They are home to millions of Lesser flamingos *(Phoeniconaias minor)* that mainly feed on the Cyanobacterium *Arthrospira fusiformis*. *A. fusiformis* plays a key role in the food web of these lakes, as it is the dominant primary producer (Oduor and Schagerl, 2007; Vareschi and Jacobs, 1985). Flamingo die-offs have been topic of research for four decades and pose a threat to the economic and conservation status. "Under suspicion" as one cause for these die-offs are also toxic cyanobacteria (Ballot et al., 2004b; Ballot et al., 2009; Ballot et al., 2004; Koenig, 2006; Krienitz et al., 2005; Lugomela et al., 2006). As these die-offs usually occur in remote areas, prompt action is necessary to collect material. Scavengers remove carcasses within a few days. During the sampling period a flamingo die-off could be observed in July 2008 at Lake Bogoria (Krienitz and Kotut, 2010). 2009 two smaller but still noticeable flamingo die-offs occurred in March and August. We were taking weekly samples from July 2008 to November 2009 and screening the lake water for cyanotoxins. Reports on toxin-producing cyanobacteria in the hot springs around Lake Bogoria and in two flamingo carcasses (Krienitz et al., 2003; Metcalf et al., 2013) were basis for our approach.

Paradigm of research and guiding questions

This research focused on MCs in various water bodies located in Kenya, some of them small man-made ponds in Kenya, which were almost neglected before. Which abiotic factors are structuring the algal community? Are seasonal changes (dry and rainy periods) key factors for driving phytoplankton succession? These questions were tried to answer for our studied systems. An important elment was the monitoring of both abiotic and biotic factors, as it is known that cyanobacterial blooms regularly occur in highly eutrophic water-bodies; the reservoirs investigated were classified hyper-eutrophic. MCs accumulate in fish tissue and can

enter the human body via the food chain.

Guiding questions of the thesis:

- How do phytoplankton communities look like in small Kenyan water-bodies?
- Which factors are structuring the community?
- Do seasonal changes in the phytoplankton communities of the nine investigated ponds occur due to dry and rainy season and transition periods?
- Do cyanobacterial blooms occur in these ponds?
- Do toxin-producing cyanobacterial genera occur in the investigated ponds?
- Are the ponds suitable for fish cage farming?
- Do cyanobacterial toxins occur in the lakes Bogoria and Nakuru?
- What does the phytoplankton community look like in the lakes Bogoria and Nakuru?
- Do cyanobacterial toxins play a role in flamingo-die offs at the lakes Bogoria and Kenya?

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Chapter I: Phytoplankton patterns along a series of small man-made reservoirs in Kenya

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Abstract We studied nine small man-made reservoirs located in different climate regions of Kenya to get an insight into the relationship between phytoplankton community structure and its environment. The investigated ponds form three groups of three reservoirs each found in the rural areas of Machakos district, Mount Kenya region, and Lake Victoria area with varied climatic characteristics. The ponds were sampled in monthly intervals between May 2007 and June 2008 for physicochemical variables including water chemistry, phytoplankton community composition, zooplankton abundance, and bacterial numbers. All ponds were classified as hypertrophic. Seasonal changes were reflected in the phytoplankton pattern, as all ponds showed a community shift after the short dry season in February. Due to high nutrient loads and increased turbidity, Cyanobacteria, which were initially thought to be predominating in all investigated water bodies, were found to play only a minor role except for the Bomet reservoir in Lake Victoria region. Instead, Chloro- and Streptophyta, Dinophyta, and Euglenophyta were abundant in the pelagial. A principal component analysis explained around 85 % of the data variance with four principal components (PCs) interpreted as "location", "ions", "zooplankton", and "particulate matter". A clear separation of ponds with and without cattle access based on algal species community data was found indicating the need for a sustainable use and regular monitoring program as the local population is largely dependent on these sensitive small-scale ecosystems.

Keywords Algae · Environment · Fish pond · East Africa

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Introduction

Some of the world's deepest and largest lakes are located in the western branch of the Great African Rift Valley (Tanganyika, Malawi) or between the rift branches (Victoria), and a lot of research has been conducted on these water bodies (for a review, see Descy and Sarmento 2008). Smaller and very shallow lakes, most of them alkaline-saline, are situated in the eastern branch of the Rift Valley and amongst them are the world-famous Kenyan flamingo lakes Nakuru and Bogoria. These lakes have received a lot of attention because of their unique environmental conditions, which in turn lead to specialized communities (Ballot et al. 2009; Descy and Sarmento 2008; Hubble and Harper 2002; Lung'Ayia et al. 2000; Schagerl and Oduor 2008; Burian et al. 2012). In contrast to these lakes, small water

bodies, many of them man-made reservoirs, have almost been completely neglected in both basic and applied researches (Degefu et al. 2011; Kaggwa et al. 2011). Such water bodies are widely spread over the African continent and are commonly used for different purposes, such as water supply to the rural communities, irrigation, and as watering points for domestic animals. Since the main source of water in these reservoirs is rainfall, they are subjected to fast hydrological fluctuations, as well as changes in water quality (Al-Homaidan and Arif 1998) that reflect seasonal patterns (Kaggwa et al. 2011).

The present study focuses on nine small man-made water bodies located in Kenya which differ in morphology and are located in three different climatic zones: Machakos, Lake Victoria, and Mount Kenya (Table 1). The studied ponds are used for fish cage farming and several other purposes such as watering livestock and irrigation. As a result of cattle access and benthivorous fish, turbidity is basically high and largely fluctuating. Due to the stocking of fish, some eutrophication pulse was expected from the introduction of fish feed and a high load of manure (Guo and Li 2003), but fortunately, this has not been observed so far (Degefu et al. 2011; Kaggwa et al. 2011).

Conditions such as low light supply due to decreased transparency, high water temperatures (often observed in tropical reservoirs) and high levels of phosphorus that are characteristics of hyper-eutrophic ponds, favor the development of cyanobacterial dominance (Shapiro 1990a; Smith 1986; Watson et al. 1997). Some cyanobacteria taxa are toxic, and therefore, a health risk for cattle and human beings (Dittmann and Wiegand 2006; Papadimitriou et al. 2010). A regular monitoring program is therefore highly recommended as a risk assessment tool (Borges et al. 2010).

This study aimed to define structuring environmental variables that shape the algal community in these ponds. Both abiotic and biotic parameters were considered in order to identify key variables structuring the algal composition. We focused on major differences between the ponds and also considered algal development over time within the respective ponds. Some authors already focused on succession patterns of phytoplankton in tropical lakes (Hubble and Harper 2002; Melack 1979; Sarmento et al. 2006), but these studies were conducted on larger water bodies such as Lake Naivasha, Lake Nakuru, and Lake Kivu amongst others.

Melack (1979) found for larger water bodies located in the African tropics that seasonal fluctuations of phytoplankton usually correspond well with variations in rainfall. We searched if similar patterns could also be detected in small ponds and reservoirs. We expected a change of the phytoplankton community over time due to autochthonous (e.g., competition, grazing) and allochthonous factors (e.g., weather conditions). In particular, we assumed a shift in phytoplankton composition shortly after the rainy season because water conditions may change due to surface runoffs and increased water exchange. Another aspect structuring the phytoplankton composition could be the geographic region, because the investigated sites receive different temperature regimes and irradiance supply.

Material and methods

Sampling sites

The sampling sites are small man-made reservoirs with an area below 1 ha. They are located in rural areas of

Table 1 Geographic position of all sites including altitude above sea level and information on dry and wet season in the respective area

Region	Dry seasons	Wet seasons	Sites	Geographic coordinates		Altitude [m] a.s.l.
Machakos	Jan/Feb Jun–Oct	Mar–May Nov–Dec	Ngeki	S 01° 30.903′	E 37° 13.041′	1,627
			Ngei	S 01° 31.246′	E 37° 12.079′	1,670
			Lukenya	S 01° 26.958′	E 37° 03.314′	1,630
Mount Kenya	Jan–Mar Jun–Sep	Apr–Jun Oct–Dec	Ruthagati	S 01° 26.387′	E 37° 04.949′	1,759
			Mailo Dam	S 00° 18.398′	E 36° 50.746′	2,159
			Sagana	S 00° 39.834′	E 37° 12.047′	1,210
Lake Victoria	Feb Jun–Oct	Jan Nov–Dec	Damside	S 00° 42.689′	E 35° 02.846′	1,825
			Harambe	S 00° 18.893′	E 34° 53.736′	1,150
			Bomet	S 00° 50.887′	E 35° 18.967′	1,946

three different geographic regions of Kenya (Fig. 1, Table 1); altitudes range from 1,150 to 2,159 m above sea level (ALT). The climate in Kenya is characterized by rainy and dry seasons and transition periods (Table 1).

Water chemistry

In situ measurements of water temperature (TEMP), oxygen saturation, electrical conductivity (COND), and pH were carried out with a WTW multi probe (340i). Water transparency was estimated using a Secchi disc (SEC). Also the mean water depth was calculated (DEPTH). For water chemistry, depth integrated sampling was done ten times between May 2007 and June 2008 by means of a modified Schindler sampler (Uwitec company 5 L, Austria). Two aliquots of 50 mL each were fixed, one with formaldehyde for bacterial counts to a final concentration of 4 % and the other with Lugol's solution (see below) for algal biovolume estimations. Additionally, a net plankton sample was taken (30 μm mesh size) to consider larger and rare taxa. For zooplankton analysis, up to 7 L of pond water were concentrated with a 50 μm net and fixed with formaldehyde to a final concentration of 4 %.

Water samples were transferred in ice boxes to the laboratory at the Sagana Research fish farm and analyzed within 2 days. Nitrate-N (NO_3-N) was analyzed from filtered samples (GF/C filter, Munktell). For total hardness (TH), total alkalinity (ALK), and total phosphorus (TP), raw samples were used. For chlorophyll a (CHL A), a defined volume of the integrated sample was filtered, and the filters frozen over night to assist cell burst. The filters were then homogenized and extracted in 90 % aqueous acetone for 12 h. After centrifugation, the supernatant was measured with a spectrometer at 663 nm. Analyses followed standard procedures (APHA 1995; Legler 1988; Schwörbel 1986; Wetzel and Likens 1991). Trophic levels of ponds were classified following the OECD levels (OECD 1982).

Pelagic community

Heterotrophic bacteria were counted according to Porter and Feig (1980). A defined volume was stained with DAPI (4′,6-Diamidin-2′-phenylindol-dihydrochlorid) for at least 20 min. Then, the samples were filtered on membrane filters (0.2 μm Isopore™ GTBP, Millipore), and the bacteria counted directly with an epifluorescence microscope (Nikon E800).

Phytoplankton was identified to the lowest level possible and quantified from Lugol fixed integrated water samples using an inverted microscope according to Utermöhl (1931). Two diagonals for abundant species and the whole chamber for rare and/or big taxa were counted. In order to estimate the algal biovolume,

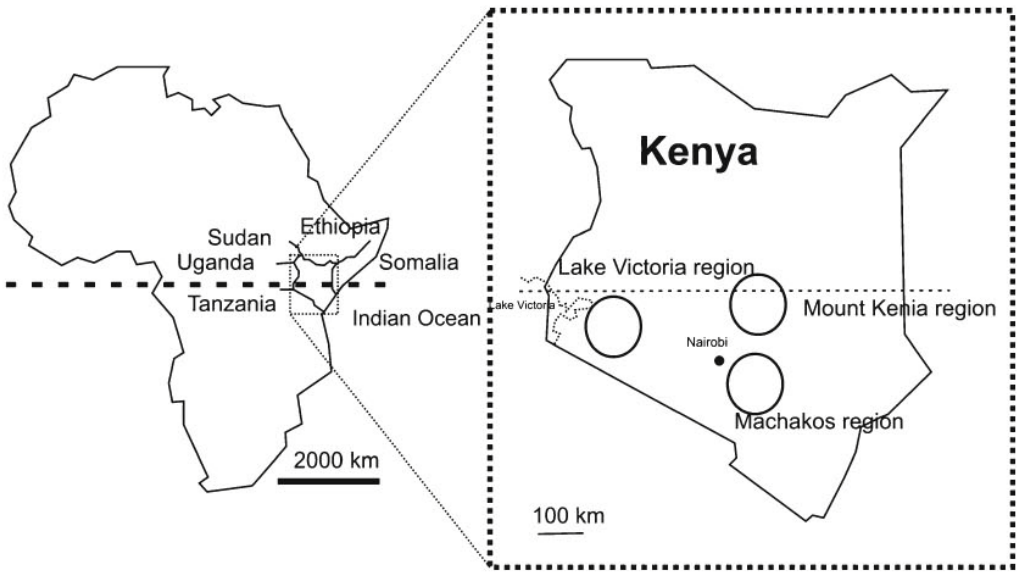

Fig. 1 Map of Africa with focus on Kenya. *Circles* indicate the regions of the investigated ponds; *dotted line* indicates the equator

geometric formulas were used to describe the shape of different algal taxa. A total of 20 measurements per dimension were carried out for abundant species; for very rare ones, literature values were used (Hoehn et al. 1998). Phytoplankton species composition was classified into functional traits (Reynolds et al. 2002; Padisák et al. 2009). For zooplankton quantification, 1 mL aliquots from fixed samples were counted (taxonomic units were adult Copepods (PNAUP), Nauplii (NAUP) larvae, Cladocera, and Rotifers).

Statistical analysis

Data were tested for normal distribution and, if necessary, log+1 transformed for further analysis. Principal component analysis (PCA) was performed to extract a few principal components (PCs) from a number of environmental variables including bacterial numbers and zooplankton counts (criterion of PC extraction was Eigenvalues >1). NO_3-N, Cladocera and rotifer numbers were excluded from PCA because of their low communalities (MacCallum et al. 1999). For applicability of the PCA, variables were checked via Kaiser-Meyer-Olkin (KMO)-measure of sampling adequacy and Bartlett's test statistics.

Based on phytoplankton counts, a nonmetric multidimensional scaling (NMDS) was performed. The analysis was repeated three times with the autopilot function "slow and thorough" (McCune et al. 2002). A minimum of stress was reached with three dimensions and 1,000 runs with 14.165 (p=0.001). Afterwards, the analysis was run six times and the results were compared for consistency of the pattern shown in the graphs. The axes were automatically rotated to orthogonality such that the greatest variation is reflected on the first axis. To find out if the factor scores of the PCA and the NMDS matrix based on phytoplankton data were significantly related to each other, a Mantel test was performed (PCA-ORD uses Pearson correlation McCune et al. 2002). For both matrices, Euclidian distances were used. PCA was carried out using the statistical package SPSS 16; NMDS and the Mantel test were performed in PC-ORD 6.

Results

Environment

Based on high concentrations of CHL A and TP, all ponds were classified as hypertrophic except Sagana which was eutrophic during the first months. However, it had been refilled just before our survey started (Fig. 2). The reservoirs contained soft water except Ngeki which had medium hardness. This was also reflected by the high COND values in this pond with a mean of around 900 μS cm^{-1} (Table 2); lowest COND was found in Sagana (mean 75 μS cm^{-1}). Mean pH was between 6.8 (Damside) and 9.0 (Bomet). PCA revealed four PCs explaining 85 % of the total variation in the data set (Table 3). The first PC showed high loadings with TH, COND, and ALK and was interpreted as "ions". PC "location" was mainly related to ALT, TEMP, and DEPTH. The third PC with loadings on PNAUP and NAUP was labeled "zooplankton". PC "particulate matter" was mainly composed of TP and negatively related to SEC. The factor scores along the PCs "location" and "ions" and the loadings of the variables were used to arrange the samples into four groups (Fig. 3). Group 1 (location>0, ions >0) mainly contained samples from Lukenya, Ruthagati, and some samples of Ngeki, which reflected the variability of the ponds throughout the year. Group 2 (location <0, ions >0) mainly comprised Harambe and some samples of Ngeki. Group 3 (location <0, ions <0) included all sampling occasions in Sagana and one sampling of Damside and Harambe each. Group 4 (location >0, ions <0) included most samplings from Mailo dam, Bomet, and Damside.

Pelagic community

Bacterial densities varied from 4.9×10^5 (Sagana and Damside) to 5.1×10^7 cells mL^{-1} (Ngeki). Highest densities were found in Machakos region, intermediate numbers were observed in the Lake Victoria region and lowest bacterial densities occurred in the Mount Kenya region (Table 2).

Ngeki and Ngei (Machakos) ponds contained high amounts of green algae, Cyanobacteria and diatoms (Fig. 4). Chlorophytes were mainly represented by coccoid taxa like several species of *Scenedesmus* and *Pediastrum* and occurred in all three ponds of this region. Cyanobacteria in Ngei were mostly represented by *Microcystis* and *Merismopedia*, while in Ngeki additionally the diazotrophic genus *Anabaenopsis* showed increased abundances. In May 2008, Ngei experienced a diatom bloom (86 % of total phytoplankton biomass) mainly composed of *Achnanthes* (~96 %) and some Centrales. In contrast to Ngeki and Ngei, Lukenya was

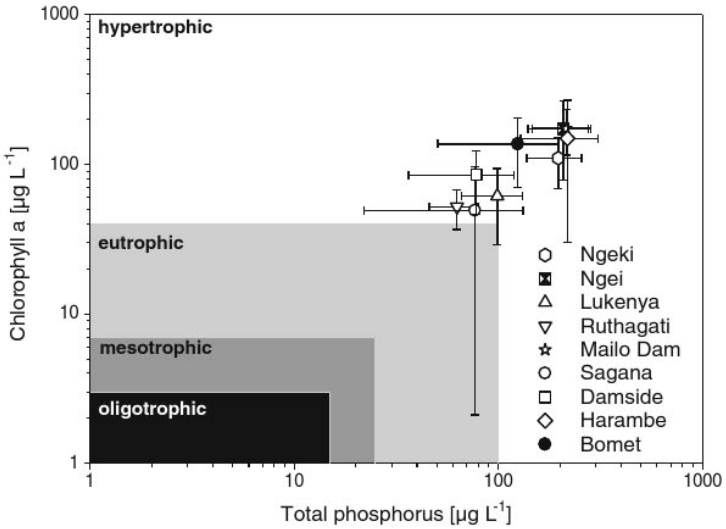

Fig. 2 Trophic status according to total phosphorus and chlorophyll a values with bidirectional error bars (standard deviation (SD)). Different *symbols* indicate different ponds

dominated by Dino- and Euglenophytes throughout the whole sampling period. The main genera were *Peridinium* and an unidentified Raphidophyceae, *Phacus*, and *Trachelomonas*. Machakos region showed the highest phytoplankton biovolume, with a maximum of 200 mm^3 L^{-1} in July in the pond Ngei. The lowest value measured in this region was in Lukenya with around 3 mm^3 L^{-1} in November. However, biovolume varied a lot during the study period. CHL A concentration in the ponds varied between 27.1 µg L^{-1} (Lukenya) in November and 409.6 µg L^{-1} (Ngei) in October. For Lukenya the lowest mean of this region was calculated with 62.4 µg L^{-1} (SD=36.5), the highest for Ngei with 194.6 µg L^{-1} (SD=111.5). In all sites of this region, rotifers were the most abundant group of microzooplankton (Table 2); median of total zooplankton numbers in this region was 1,430 Inds L^{-1}.

Mount Kenya region including Sagana, Ruthagati, and Mailo dam showed high abundances of chlorophytes, diatoms, cryptophytes, and Cyanobacteria (Fig. 4). *Scenedesmus* and *Pediastrum* were the major taxa of green algae; Centrales, and *Achnanthes* built up high densities of Bacillariophyceae in Sagana during 2008. In contrast, *Aulacoseira* was most frequent in Ruthagati. Chrysophyceae were abundant in Mailo Dam (mainly *Chrysococcus*) and Sagana (*Dinobryon* and *Chrysococcus*). Cyanobacteria played only a minor role (*Aphanocapsa, Microcystis*, and *Merismopedia*). The highest biovolume of Mount Kenya region was measured at the Mailo Dam with 167.2 mm^3 L^{-1} in July, while the lowest biovolume with 2.1 mm^3 L^{-1} was in Ruthagati. Mean values of Ruthagati and Sagana were similar with 12.2 and 13.8 mm^3 L^{-1}, respectively. The highest CHL A value measured was 286.7 µg L^{-1} in Mailo Dam in October; the lowest concentration was in Sagana with 5.21 µg L^{-1}. Zooplankton numbers reached an average of about 1,700 Inds L^{-1}; most abundant were rotifers. Mailo Dam had the highest density of all ponds of this region with a maximum count of 2,367 Inds L^{-1}.

The most abundant algal groups occurring in the Lake Victoria region (Fig. 4) were dinophytes, euglenophytes and Cyanobacteria. Damside was mainly populated by an unidentified Raphidophyceae and another species assigned to *Peridinium*. Both forms also showed high amounts in Harambe pond. In addition, euglenophytes (*Euglena, Trachelomonas, Phacus, Strombomonas*) showed high taxa number. Bomet turned out to be the only pond dominated by Cyanobacteria during the whole sampling period. The community mainly consisted of *Microcystis, Planktolyngbya* and *Merismopedia*. Harambe showed the lowest algal biovolume of all ponds with 4.1 mm^3 L^{-1} (average value). Minimum CHL A value measured was 14.2 µg L^{-1} for Bomet, while the highest amount was recorded in Harambe with 465.9 µg L^{-1}. Zooplankton reached a median of around 960 Inds L^{-1} with rotifers being the most abundant group.

Phytoplankton association of Ngeki was stable throughout the whole sampling period and could be assigned to functional group *J*, as different species of *Pediastrum* occurred in high amounts. Codon *J* is

Table 2 Abiotic data, algal biovolume, bacterial numbers, and zooplankton counts (mean, *SD* standard deviation, *Max* maximum, *Min* minimum)

		Ngeki	Ngei	Lukenya	Ruthagati	Mailo Dam	Sagana	Damside	Harambe	Bomet
TP [μg L^{-1}]	Mean	190	196	109	75	227	75	88	214	139
	SD	51	64	47	48	76	56	31	85	78
	Max	274	302	209	196	402	171	142	419	232
	Min	134	130	59	42	154	22	69	121	41
TH [mg $CaCO_3$ L^{-1}]	Mean	84.67	30.63	33.06	32.97	32.39	11.44	10.89	39.00	21.70
	SD	16.81	10.15	4.31	1.64	5.43	0.94	2.57	6.77	2.23
	Max	105.50	45.00	43.95	36.45	43.20	13.00	13.50	54.00	24.75
	Min	60.45	17.50	28.20	31.50	26.50	10.35	6.75	30.00	18.75
NO_3-N [μg L^{-1}]	Mean	169	58	37	29	63	31	140	38	62
	SD	328	39	22	18	23	18	120	11	26
	Max	1,040	140	90	60	90	70	310	60	90
	Min	20	20	10	10	20	10	30	30	30
ALK [mval L^{-1}]	Mean	2.06	1.25	2.12	1.73	1.74	0.59	1.08	2.49	1.54
	SD	0.42	0.36	0.48	0.16	0.33	0.08	0.20	0.56	0.23
	Max	2.63	1.63	3.28	2.03	2.29	0.74	1.37	3.41	1.89
	Min	1.48	0.78	1.60	1.46	1.30	0.49	0.83	1.49	1.21
SEC [m]	Mean	0.24	0.23	0.26	0.46	0.21	0.95	0.40	0.24	0.23
	SD	0.07	0.07	0.07	0.10	0.05	0.70	0.20	0.11	0.07
	Max	0.35	0.35	0.40	0.65	0.30	2.00	0.63	0.50	0.28
	Min	0.14	0.15	0.16	0.35	0.13	0.20	0.18	0.10	0.10
COND [μS cm^{-1}]	Mean	896	185	233	271	211	75	117	252	190
	SD	233	94	42	31	22	29	6	35	44
	Max	1,257	343	287	320	246	150	126	307	244
	Min	590	75	169	237	183	57	112	177	135
pH	Mean	8.00	7.42	7.79	7.53	7.72	7.19	6.82	7.46	8.99
	SD	0.64	0.52	0.43	0.36	0.33	0.26	0.30	0.63	0.46
	Max	8.50	7.92	8.36	7.82	8.00	7.65	7.21	8.10	9.40
	Min	6.98	6.61	6.98	6.69	7.06	6.86	6.31	6.13	8.37
TEMP [°C]	Mean	22.0	21.1	20.5	22.4	19.5	25.4	20.8	24.9	19.3
	SD	1.4	1.2	1.8	1.3	1.0	1.2	1.9	1.3	0.8
	Max	23.8	22.9	23.9	24.5	21.0	27.0	23.0	27.1	20.3
	Min	19.0	19.3	18.3	19.9	17.6	23.5	18.8	22.9	18.3
DEPTH [m]	Mean	2.45	1.76	3.17	2.02	1.76	1.23	3.08	1.91	2.70
	SD	0.51	0.49	0.21	0.15	0.27	0.14	0.14	0.17	0.40
	Max	3.40	2.53	3.49	2.20	2.15	1.41	3.30	2.08	3.40
	Min	1.85	1.23	2.82	1.78	1.22	1.00	2.95	1.52	2.30
Biovolume [mm^3 L^{-1}]	Mean	41.47	35.31	10.59	12.22	55.20	13.81	17.07	4.10	9.27
	SD	32.68	62.82	9.64	11.44	51.98	12.28	8.47	3.57	4.58
	Max	123.88	200.45	27.85	34.09	167.23	44.58	28.53	11.03	18.90
	Min	12.77	5.13	2.55	2.13	13.54	4.71	5.49	0.23	4.26
Bacterial numbers [Cells mL^{-1}]	Mean	1.9E+07	7.1E+06	6.5E+06	5.1E+06	9.3E+06	4.7E+06	5.2E+06	6.6E+06	9.3E+06
	SD	1.8E+07	4.8E+06	4.7E+06	2.5E+06	6.8E+06	3.6E+06	3.1E+06	4.6E+06	1.1E+07
	Max	5.1E+07	1.4E+07	1.6E+07	8.5E+06	2.0E+07	1.2E+07	8.8E+06	1.8E+07	3.0E+07
	Min	4.7E+06	8.0E+05	2.4E+06	4.7E+05	7.6E+05	4.9E+05	4.9E+05	1.5E+06	6.0E+05

Table 2 (continued)

		Ngeki	Ngei	Lukenya	Ruthagati	Mailo Dam	Sagana	Damside	Harambe	Bomet
Post Nauplii [Inds L^{-1}]	Mean	156.1	169.4	117.7	94.8	297.7	104.1	24.8	129.4	130.6
	SD	58.2	76.5	79.8	56.7	206.9	43.1	37.4	121.0	101.6
	Max	233.1	303.5	256.0	205.7	697.0	172.1	90.0	369.3	329.3
	Min	52.0	68.0	30.0	37.0	58.1	31.3	0.0	30.7	45.0
Nauplii [Inds L^{-1}]	Mean	271.5	380.4	315.3	276.8	494.3	368.3	68.7	400.5	308.4
	SD	165.9	157.5	197.2	119.6	376.5	182.2	100.9	256.1	141.5
	Max	582.4	653.3	809.1	552.9	1,237.5	609.0	247.5	940.0	570.0
	Min	11.6	79.3	113.8	129.5	125.8	104.5	11.0	107.3	144.6
Cladocera [Inds L^{-1}]	Mean	1.7	3.9	29.8	90.7	42.3	48.7	2.3	7.2	0.0
	SD	3.4	5.4	61.6	96.4	60.5	24.6	5.0	9.2	0.0
	Max	8.9	13.0	201.5	300.0	187.5	91.5	11.3	24.5	0.0
	Min	0.0	0.0	0.0	7.8	0.0	9.6	0.0	0.0	0.0
Rotifers [Inds L^{-1}]	Mean	905.2	972.4	804.7	1,160.2	2,023.6	831.8	817.1	1,380.5	305.9
	SD	496.8	423.4	548.0	779.2	2,079.1	894.6	684.5	913.4	191.4
	Max	1,741.7	1,600.7	1,691.8	2,881.7	7,275.0	2,515.3	1,530.0	3,666.0	537.5
	Min	57.8	419.3	60.9	364.3	447.1	47.3	110.0	449.2	110.5

typical of shallow mixed and highly nutrient-enriched water bodies. Bomet comprised taxa of group *M* characteristic of eutrophic-hypertrophic small- to medium-sized water bodies with a typical representative *Microcystis*. Ngei started with taxa of *J*, then changed to X_2 (shallow, meso-eutrophic environment) with high amounts of *Cryptomonas* sp., changed back to *J*, and finally reached *MP* (frequently stirred up, meso-eutrophic, turbid shallow lakes) with a phytoplankton community dominated by *Achnanthes* sp. Codon X_2 was also found in Lukenya, Ruthagati, and Damside. Sagana started with *J* followed by X_2; this pattern was repeated, before Sagana shifted to W_2 (meso-eutrophic pond, even temporary shallow lakes) with *Trachelomonas* species as the dominant genus. Also for Mailo dam, a succession of the community could be identified: Mailo started with typical phytoplankton composition for functional group *J* with notable amounts of *Pediastrum* spp. and *Scenedesmus* spp. and then shifted to group *M*. After that, phytoplankton changed to *K* (shallow, nutrient-enriched water bodies) before it switched back to *J*.

Table 3 Varimax rotated factor loadings revealed by the PCA (total variance explained 85.1 %), values >0.7 and <−0.7 are marked in bold for better visibility

	Ions (27.6 %)	Location (20.7 %)	Zooplankton (19.9 %)	Particulate matter (17.0 %)
logCOND	**0.920**	0.127	0.057	0.103
logTH	**0.900**	0.015	0.199	0.277
logALK	**0.863**	0.022	0.002	0.268
logTEMP	−0.005	**−0.928**	−0.023	−0.161
logALT	0.062	**0.909**	0.002	0.092
logDEPTH	0.415	0.517	−0.485	−0.146
logPNAU	0.164	0.086	**0.933**	0.048
logNAU	0.065	−0.069	**0.906**	−0.025
logTP	0.211	0.008	0.101	**0.920**
logSEC	−0.325	−0.289	0.077	**−0.798**

Linking the phytoplankton community to the environment

NMDS based on algal biomass data revealed a distinct pattern with a high phytoplankton community overlap of Ngeki, Ngei, and Mailo dam (Fig. 5); Bomet sampling dates were quite separated. Algal composition of the remaining ponds (Lukenya, Ruthagati, Sagana, Damside, and Harambe) also showed high similarities. Sites with and without cattle access were clearly separated (Fig. 5). Mantel-test statistics revealed a significant negative correlation of both PCs "location" and "zooplankton" with NMDS-axis 1. "Location" was also negatively correlated to axis 2, "zooplankton" showed a

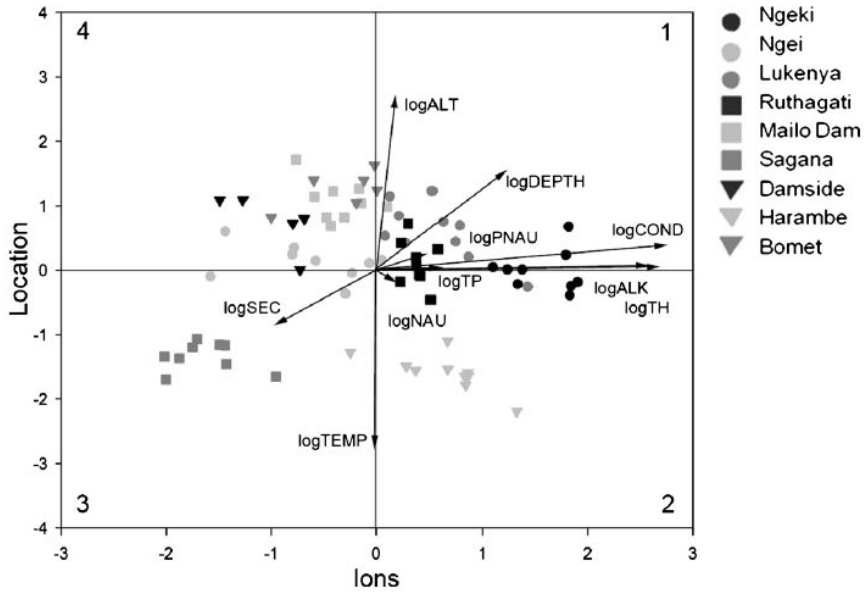

Fig. 3 Biplot obtained by PCA showing the region and the different sites. Group members of a region are labeled with identical symbols, but with different shadings for the sites

slight positive correlation (Fig. 5). The ponds were highly related to PC "zooplankton" (Fig. 6) with the exception of Harambe. PC "particulate matter" was highly linked to sites with cattle access.

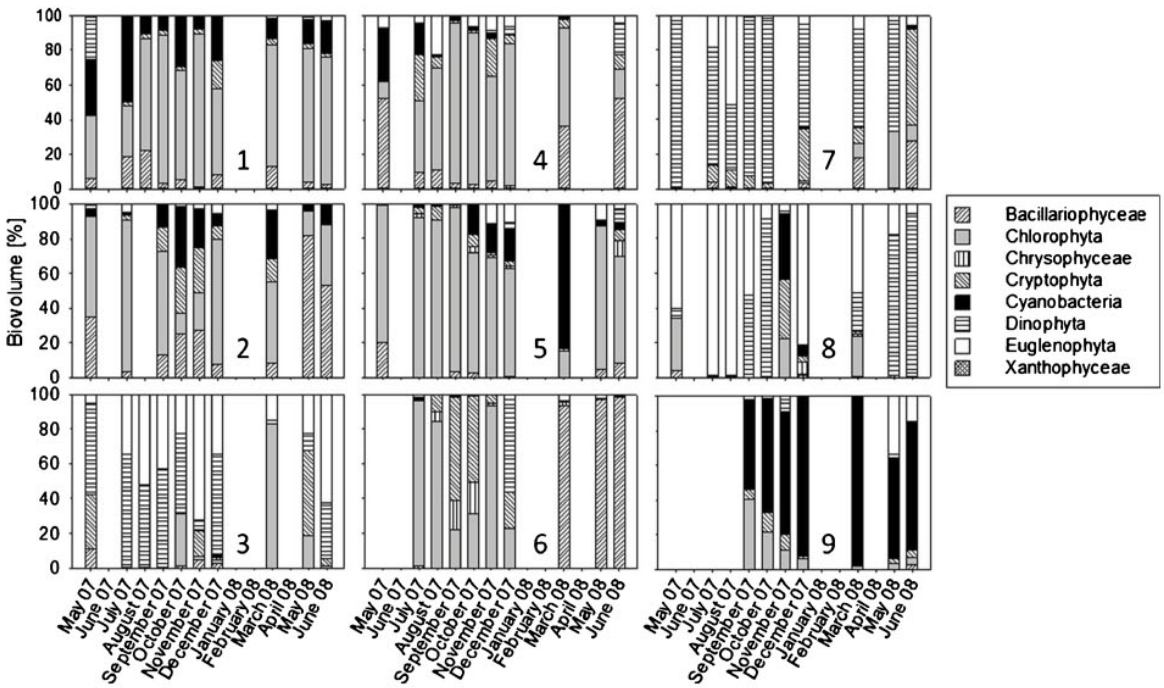

Fig. 4 Percentages of biovolume of different algal classes at BOMOSA sites in the three different regions. Labeled top down for each region: Machakos—*1* Ngeki, *2* Ngei, *3* Lukenya; Mount Kenya—*4* Ruthagati, *5* Mailo Dam, *6* Sagana; Lake Victoria—*7* Damside, *8* Harambe, *9* Bomet

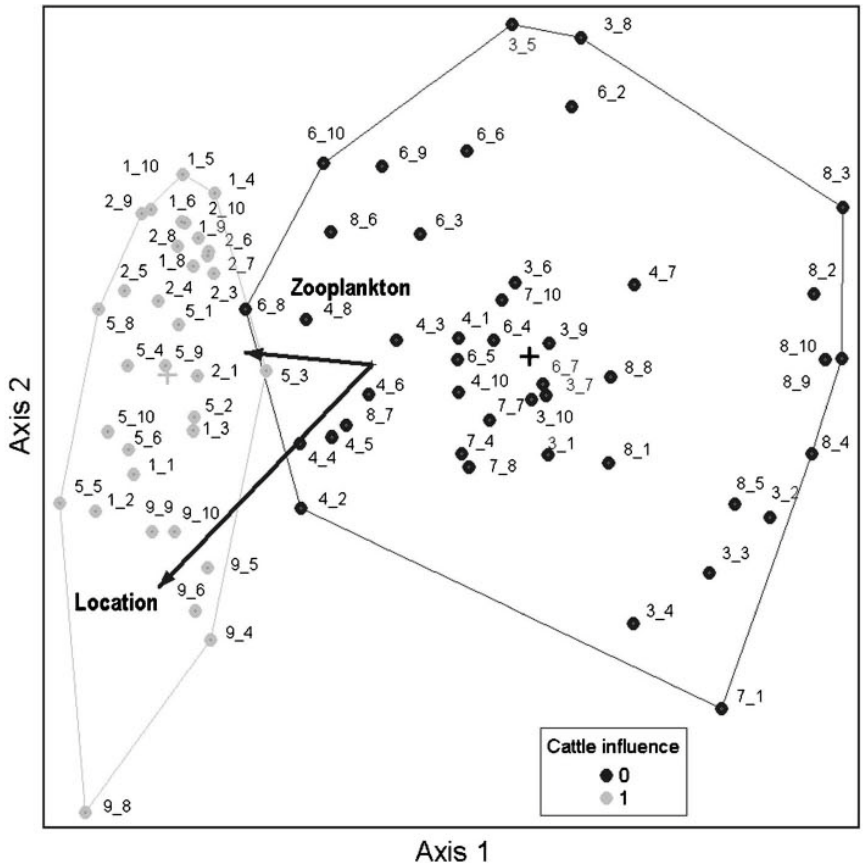

Fig. 5 NMDS plot based on phytoplankton biovolume; the PCs "zooplankton" and "location" obtained from PCA were post hoc projected. Labeling according to site numbers (first number site—*1* Ngeki, *2* Ngei, *3* Lukenya; Mount Kenya—*4* Ruthagati, *5* Mailo Dam, *6* Sagana; Lake Victoria—*7* Damside, *8* Harambe, *9* Bomet; second number sampling date) and sampling date number. Cattle influence is indicated in *grey*; no cattle access is indicated in *black*

Discussion

Environmental factors

Most of the ponds are heavily used for cleaning laundry and also for cattle watering, which is reflected by generally high nutrient loads, e.g., by TP concentrations with means beyond 70 μg L^{-1}. Six ponds (Ngeki, Ngei, Lukenya, Mailo Dam, Harambe, Bomet) had TP means larger than 100 μg L^{-1}, which is in line with other studies on small-sized reservoirs (Degefu et al. 2011; Kaggwa et al. 2011; Ssanyu and Schagerl 2010), thus giving evidence on generally elevated TP concentrations in small-scale water bodies in the tropics and subtropics. Contrarily, Borges et al. (2010) found a TP maximum of 18.6 μg L^{-1} in a big Brazilian tropical reservoir (Rosana reservoir) used for cage fish farming, which clearly points at the higher resistance of large water bodies against pollution compared to smaller ones.

Phytoplankton community structure linked to the environment

Assemblages of green algae and Cyanobacteria have already been described for large East African lakes and were observed especially during periods of thermal stratification (Hecky and Kling 1987). Even if our ponds were shallow, wind exposed, and lacked longer periods of thermal stratification, comparable assemblages were also found in the ponds, but with only minor contribution of Cyanobacteria (exception Bomet, see below). Ex situ experiments with natural freshwater phytoplankton collected from a eutrophic pond showed that both

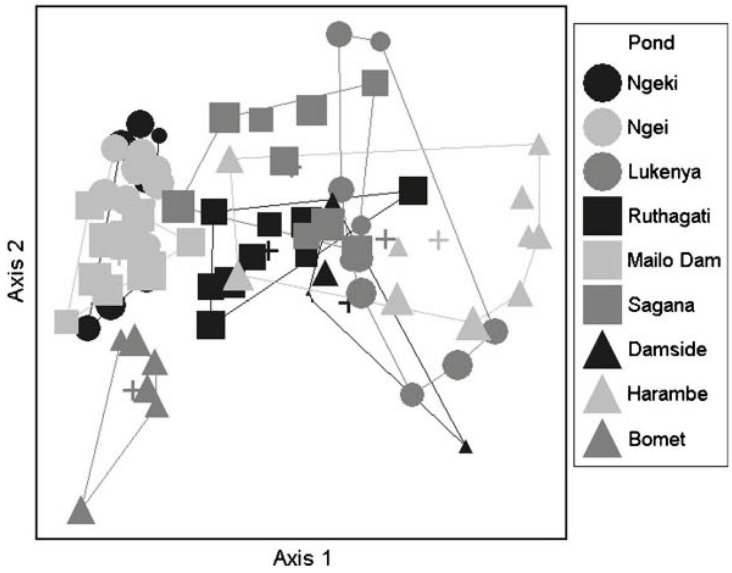

Fig. 6 NMDS plot; the size of the symbols is proportional to the correlation with the PC "zooplankton". Sites of the same region have identical *symbols* but different *colors*

nutrient additions and nutrient plus zooplankton interactions caused a shift in the community (Wang et al. 2010). Cyanobacteria together with chlorophytes turned out to be the most dominant groups similar to long-term succession of phytoplankton genera observed in eutrophic temperate and subtropical ponds and lakes during summer (Chen et al. 2003; DeNoyelles and O'Brien 1978; Schelske and Stoermer 1971).

Except for Bomet located in the Lake Victoria region, we were not able to find dominance of Cyanobacteria, which have been described elsewhere as a typical group of fish ponds (Borges et al. 2010; Lepistö and Rosenström 1998). pH could be one decisive variable for their occurrence, as Bomet showed significantly increased values compared to the other reservoirs (Table 2), and this group is favored by alkaline conditions (Shapiro 1984, 1990b). Another reason for low Cyanobacteria occurrence could be lacking thermal stratification over longer periods, as the presence and dominance of Cyanobacteria has been related to stability of the water column (Calijuri et al. 2002; Komárková et al. 2003; Padisák and Reynolds 1998). Additionally, feeding pressure of Tilapia could be an explanation for low cyanobacterial numbers (Figueredo and Giani 2005), which however remains speculative for our study, because data on fish density were not available. The filtering mechanism of Nile tilapia (*Oreochromis niloticus*) does not prefer any morphological type (filamentous, colony forming) of Cyanobacteria, but the particle size is crucial. Turker et al. (2003) have shown that the number of ingested particles increases with larger particle size. The influence of Tilapia on the phytoplankton community and size structure has also been described by Figueredo and Giani (2005), who found that the biggest changes take place in Cyanobacteria and chlorophytes. Also, the dominance of small rotifers prevailing points at grazing pressure of Tilapia on the plankton community.

Dinophytes were observed in high densities in all regions, especially Lukenya, Damside, and Harambe. Dinophytes like *Peridinium* and *Ceratium* are known to adapt to a wide range of conditions and are therefore called eurytopic (Kangro et al. 2005). Using their phototaxis they are able to move in the water column which is advantageous for nutrient supply (Graham and Wilcox 2000). Reynolds (1996) classified *Ceratium* as a component of late-summer phytoplankton associations in oligo- to eutrophic waters of temperate regions; nevertheless it occurred in notable amounts also in the hypertrophic ponds investigated. *Ceratium* and *Peridinium* were generally absent in ponds with regular cattle access, which points to their vulnerability against high levels of disturbance (Heaney et al. 1988; Reynolds et al. 2002).

PCA based on mainly environmental variables revealed rather spatial patterns than seasonal ones (since sampling dates of the wet and dry seasons were evenly distributed), which indicates larger differences between

the sites than between seasons. Functional phytoplankton traits of some ponds however indicated seasonal patterns. For most of the reservoirs, the phytoplankton community appeared completely different in March 2008, which went along with a change from the hot dry season to the cold rainy season. Such season-driven shifts in the phytoplankton community have already been described for Brazilian reservoirs (Figueredo and Giani 2005). Rainfall has to be considered as one key factor for phytoplankton dynamics in small reservoirs, as in the tropics precipitation seems to be more important than temperature and solar radiation (Figueredo and Giani 2001). In their study of three ponds in the Machakos district (Kenya) with fortnightly sampling intervals, Kaggwa et al. (2011) found highest nutrient concentrations at the onset of the rainy season coinciding with a dominance of Chlorophyta; Cyanobacteria prevailed at the start of thermal stratification and diatoms were abundant in the dry season. Interestingly, even when the geographic distance was low, some of the sites varied a lot in plankton community structure (Fig. 5). Community patterns and their response to environmental changes as observed in the East African Great lakes (Descy and Sarmento 2008) could not be applied to our study sites. Obviously, small man-made water bodies do not have the stability against environmental changes as described for bigger lakes.

Key factors for the community pattern were found to be PCs "location", which was negatively correlated to axis 1 (r=−0.424) and axis 2 (r=−0.432) and to a lesser extent "zooplankton", which was negatively correlated to axis 1 (r=−0.328) and slightly positive to axis 2 (r=0.102). As the impact of "zooplankton" on the structuring of the phytoplankton community is low, we concluded from this result that the algal community is mainly bottom-up controlled. This is in accordance with McQueen et al. (1989, 1986) who suggested that the influence of organisms from higher trophic levels on the lower community is less significant in eutrophic environments. All ponds with cattle access were positively related to the PC "particulate matter", which was explained by nutrient inputs and increased sediment disturbance caused by trampling. The clear differentiation of sites with (Ngeki, Ngei, Mailo Dam, and Bomet) and without cattle access (Lukenya, Ruthagati, Sagana, Harambe, and Damside) was based not only on the lower biomass of phytoplankton occurring in the ponds with cattle exclusion but also on the absence of certain taxa (*Aulacoseira, Tetraedron*, different *Euglena*, *Trachelomonas* and *Phacus* species, *Fragilaria*, *Ceratium* and *Peridinium*) in these ponds. Cyanobacteria (*Microcystis*, *Synechococcus*, *Merismopedia*, *Anabaena*, *Limnothrix*, *Pseudanabaena*) were common taxa in systems with cattle access; nevertheless, they were not able to gain dominance (except for Bomet see above). Also, the chlorophyte *Scenedesmus acuminatus* (Lagerheim) Chodat was found in numerous amounts.

In our study, many taxa indicative of increased nutrient concentrations were observed, some of them characteristic of highly eutrophic water bodies and increased dissolved organic matter (DOM) such as different euglenoids (*Phacus*, *Euglena*, *Lepocinclis*) (Reynolds 1998), as well as different *Microcystis* species (*M. flos-aquae* (Wittrock) Kirchner, *M. aeruginosa* (Kützing) Kützing) (Rawson 1956). Observed chlorophytes such as *Pediastrum* and *Coelastrum* are known to occur mainly in shallow enriched lakes with low light supply (Reynolds et al. 2002).

The diatom species *Aulacoseira granulata* (Ehrenberg) Simonson and *Stephanodiscus* sp. found in the study are sensitive to stratification (Reynolds et al. 2002) and reflect the thermal instability of the investigated systems. These diatoms can be interpreted as r-strategists that are characterized by a high surface area to volume ratio (Reynolds 1997). R-strategists are tolerant to disturbances and can adapt quickly to changes of the environment (Sommer 1981). Even *Cryptomonas* sp. is known as an r-strategist but can also range to c-strategy as described by Calijuri and Dos Santos (2001). Weithoff et al. (2001) characterized c-strategists as phytoplankton with small cell size, high susceptibility to zooplankton grazing, high potential growth rates and low sedimentation losses. After disturbances of the environment, Cryptophyta are often dominating the phytoplankton community, and they have the potential to occur persistently (Barbiero et al. 1999).

Phytoplankton communities were assigned to functional groups in order to find seasonal patterns. For Sagana and Ngei, the same succession of functional traits was observed: samplings of wet seasons were allocated to group *MP* characteristic for shallow water bodies that are frequently stirred up (Padisák et al. 2009); frequent rainfalls lead to instability in the water column. During dry seasons these two ponds were dominated by the functional groups *J* and X_2, which have been described for shallow lakes that are highly enriched with nutrients.

We only found Bomet pond to be dominated by Cyanobacteria during the whole sampling period, which was also clearly separated from the other ponds according to the NMDS model (Fig. 5). The community pattern followed the functional group *M* with high amounts of *Microcystis* sp. Such blooms might become a threat for humans as some Cyanobacteria genera are known to produce toxins. The omnivorous filter feeder Nile tilapia is the most common fish cultivated in the investigated ponds and has already been used for fighting cyanobacterial blooms (Lu et al. 2006). As bioaccumulation of toxins may take place in muscle tissues of fish (Magalhães et al. 2003), there is a serious health risk for fish consumers (Chen et al. 2009). We did not observe high numbers of Cyanobacteria during the sampling period, but shortly after our study was finished, a cyanobacterial bloom was reported from Ruthagati (unpublished observation). This calls for the urgent demand of continuous monitoring of such water bodies, which are strongly linked to the daily needs of local people.

In-depth investigations of algae community–environment relationships are also required in these heavily used, small water bodies—not only for ecological purposes but also for development of future management strategies as the introduction of fish cage farming to different climate areas could ensure a protein-rich diet for the local people.

Acknowledgments The present research was conducted within the BOMOSA project (EU-project contract no. 032103), which aimed to establish fish cage farming in the East African countries Kenya, Uganda, and Ethiopia (http://bomosa.oeaw.ac.at). We are grateful to G. Winkler and D. Liti who coordinated the project and field sampling, respectively.

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Chapter II: Small man-made ponds in Kenya and cyanotoxins – a threat to local people?

Nadja Straubinger-Gansberger, Mary Kaggwa, Michael Schagerl

Small man-made ponds in Kenya and cyanotoxins – a threat to local people?

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Abstract

We studied nine man-made ponds located in different climatic regions of Kenya. The local community uses the water reservoirs for various purposes such as irrigation, fish-cage farming and watering of livestock, which resulted in eutrophication including fast growth of Cyanobacteria, which sometimes are able to produce cyanotoxins threatening human and animal life. We analyzed abiotic variables in monthly intervals over one year, studied the pelagic community and identified microcystins by means of high performance liquid chromatography and with ELISA kits. Three ponds contained high amounts of microcystins with the highly toxic Microcystin-LR identified as the most prominent substance. Cell content of the toxin varied from 7.2 to 686.7 $fg.cell^{-1}$. Based on abiotic and biotic variables, which easily can be obtained even in laboratories with basic equipment, we searched for environmental variables, which are likely to predict the occurrence of cyanotoxins. Although most of the samples did not contain high amounts of visible Cyanobacteria, the cyanotoxin-problem was still evident indicating the urgent need for regular monitoring programs. Our approach can be used as a decision-making tool for/against fish caging, as fish growing in such environments might accumulate toxins in their tissues thus threatening humans via the food chain.

Keywords: Microcystis, fish-cage farming, microcystin

1. Introduction

Cyanobacteria are known to occur worldwide in both aquatic and terrestrial ecosystems (Whitton and Potts, 2000). In lentic freshwater systems, Cyanobacteria are favored by high nutrient loads (Kotak et al., 2000; Kotak et al., 1996), elevated temperatures (Marinho et al., 2002) and slightly alkaline conditions (Summerfield and Sherman, 2008). Some taxa such as *Microcystis* develop gas vesicles (aerotopes) for buoyancy, which enables them to stay in the water column at certain depths (Graham and Wilcox, 2000), which is an efficient strategy especially in turbid water bodies and sometimes resulting in long-lasting scums floating on the surface.

Some cyanobacteria produce toxins, which pose a health risk. The hepatotoxic microcystins (MCs) are the most common toxins produced (Chen et al., 2009; Pflugmacher et al., 1998; Rantala et al., 2006); more than 70 structural variants of MCs have been described so far (Via-Ordorika et al., 2004). Many studies focus on the common MC-LR structural variant carrying leucine (L) and arginine (R) at its variable positions, which is one of the most toxic structural variants with an LD_{50} of 50 µg kg^{-1} as determined by intraperitoneal injection into mice (Carmichael, 1992). The influence on humans has also been documented for several cases: (i) liver cancer caused by exposure to MCs (Fujiki and Suganuma, 1993; Svircev et al., 2009; Ueno et al., 1996), (ii) death of dialysis patients after acute liver failure in two dialysis centers in Brazil (Azevedo et al., 2002) and (iii) identification of MCs in the serum of fishermen at Lake Chaohu in China, for which an average value of 0.39 ng ml^{-1} was measured (Chen et al., 2009). The WHO has determined a maximum tolerable daily intake (TDI) for daily lifetime exposure with 0.04 µg kg^{-1}, which equates 2-3 µg per person.

The East African Great lakes have been extensively studied for MCs occurrence (Ballot et al., 2004; Ballot et al., 2005; Haande et al., 2007; Semyalo et al., 2010), but small man-made reservoirs have been neglected. Our study aimed to bring fish-cage farming into rural areas of

East Africa in order to provide a protein rich diet to the local communities. As most of these water bodies are highly eutrophicated due to human activities (e.g. laundry, watering cattle), the probability for toxic cyanobacterial blooms and therefore the health risk is increased. We aimed to evaluate the risk of toxin production by considering environmental factors, as other studies already described toxicity shifts according to changes under culture conditions (Watanabe and Oishi, 1985) and in nature (Kotak et al., 2000). This study can further be seen as a decision-making tool for or against aquaculture based on variables easily obtained, which is cost effective for local researchers in developing countries.

2. Material and Methods

2.1 Sampling sites

Sampling sites are located in rural areas of three different geographic regions of Kenya (Fig. 1) close to the equator. Altitudes range from 1150 to 2159 m above sea level. The climate in Kenya is characterized by rainy (March – May, short rainy season in November and the biggest part of December) and dry seasons (short dry season from January – February, June – October) and transition periods between the seasons. Detailed information on the sampling sites, chemistry and plankton composition can be obtained from Straubinger-Gansberger et al. (2014). The nine ponds were sampled at approximately monthly intervals (n = 10) between May 2007 and June 2008.

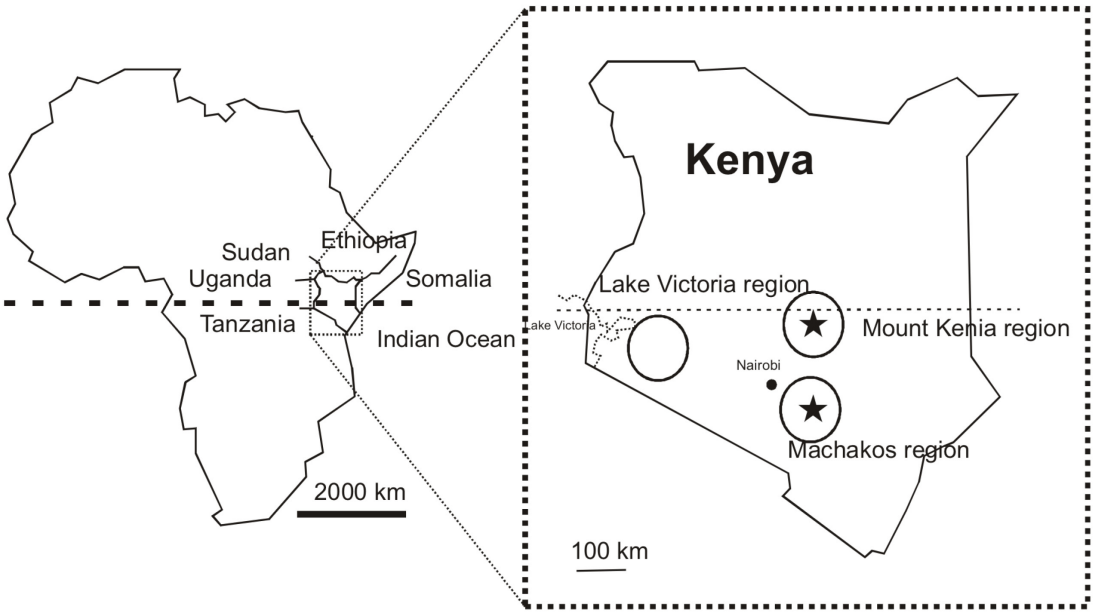

Fig. 1: Kenyan regions, each containing three sampling sites. Stars indicate microcystin occurrence analyzed by means of HPLC (plankton net samples).

2.2 Microcystin analysis

Due to general high turbidity, filtration of higher volumes of depth-integrated water (> 100 ml) was not possible, thus reducing the detectability of MC by High performance liquid chromatography with diode array detection (HPLC-DAD). We therefore took two different

kinds of samples, (1) net hauls for concentrating colonial forms of cyanoprokaryotes (mesh size 30µm) for information on structural variants of MC and (2) depth-integrated samples taken by means of a modified Schindler sampler (Uwitec, Austria). Samples were filtered on glass fiber filters (Munktell MG/C, Sweden) using low vacuum filtration for MC and stored frozen for subsequent MC analysis.

Both (1) and (2) were analyzed by HPLC-DAD (limit of detection = LOD = 1 µg MC-LR equivalents L^{-1}). In order to detect even traces of MCs in (2), an indirect competitive ELISA targeted to the Adda-side chain of the MC molecule was used (LOD 0.1 µg L^{-1}, Abraxis, Warminster, USA). The filters were extracted in aqueous methanol (75%, w/v) following the protocol of Fastner et al. (1998). The clear supernatant was analyzed by HPLC-DAD as described in Lawton et al. (1994). A linear gradient of aqueous acetonitrile containing 0.05% trifluoroacetic acid (TFA) was used at a flow rate of 1 mL min^{-1} increasing from 30% to 70% acetonitrile in 42 min (Lawton et al., 1994). MCs were identified by their retention times and characteristic absorption spectra (Fastner et al., 1999) and quantified at 240 nm using microcystin-LR as external standard (Cyanobiotech, Berlin, Germany). All peaks identified as putative MCs were collected manually and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to identify structural MC-variants by their protonated masses (M+H) as described by Erhard et al. (1997).

For ELISA (Fischer et al., 2001), the extracts used for HPLC were evaporated and re-suspended in 150 µL Millipore water, since ELISA has been shown to be sensitive to methanol (Metcalf and Codd, 2003). Due to the high sensitivity (LOD 0.10 to 5 ng mL^{-1}), extracts were diluted down to 1:10 and 1:100 with Millipore water. The ELISA test was performed in triplicate for each sample according to the manufacturer's instructions using a plate reader (Jupiter, Asys Hitech, Austria).

2.3 Statistical analysis

Two groups, i.e., cyanotoxin-containing and cyanotoxin-free, were predefined based on ELISA results. As the criteria for normal distribution and homogeneity of variances were not met in all cases, non-parametric Mann-Whitney-U tests were used to test all parameters for significant differences. Analyses were carried out using the statistical package SPSS 16.

3. Results

3.1 Trophic status and plankton composition

For details of plankton composition and chemistry, see (Straubinger-Gansberger et al., 2014). Briefly, the water reservoirs turned out to be hypertrophic. Four ponds (Ngeki, Ngei, Mailo Dam, Bomet) allowed cattle access, which resulted in significant differences of the phytoplankton composition, as water transparency was lower in these ponds. Cyanobacteria were only found to be dominant in Bomet reservoir. The other ponds were dominated by Chlorophytes or Dinophytes. Zooplankton was composed of Nauplii, Post Nauplii, Rotifers and Cladocera. The three ponds (Lukenya. Ruthagati, Mailo Dam) that contained toxins in the net samples showed highest amounts of Cladocera. Bacterial densities varied from 4.9×10^5 (Sagana and Damside) to 5.1×10^7 cells mL^{-1} (Ngeki). In the ponds containing higher bacterial numbers Microcystin was measured with ELISA-kits.

The most abundant Cyanobacterium was *Microcystis sp*., but also *Aphanocapsa, Anabaenopsis, Anabaena, Limnothrix, Planktolyngbya, Merismopedia, Phormidium* and *Synechococcus* were observed. *Microcystis* sp. was recognized in every pond at least on a few occasions, *Anabaena* was found in all ponds except Damside and Lukenya. In six ponds (Harambe, Sagana, Ruthagati, Mailo Dam, Ngeki, Ngei) *Anabaenopsis* was observed and in four (Harambe, Ruthagati, Mailo Dam, Ngeki) *Aphanocapsa* occurred.

In total, 76 net samples were analyzed by HPLC and 16 samples were found MC-positive. MCs were detected in three ponds (Lukenya, Mailo Dam, Ruthagati). Four structural variants of MCs could be distinguished (Table 1). The most frequent MC2 variant was assigned to MC-LR (M+H 995) and occurred in all MC-positive samples. MC1 was identified as MC-YR (M+H 1045) and occurred in 44 %; MC3 and MC4 (M+H could not be analyzed due to low amounts) occurred in only 19 % and 13 % of the samples, respectively.

Table 1: At least four different microcystin variants were detected in this study. Retention times in min are given in the first section of the table. Samples analyzed by MALDI-TOF are marked bold. Percentages indicate contribution of the single variants based on the area per sample to total amount.

Sampling date	Site	MC1 (MW 1045.62)	MC2 (MW 995.51)	MC3	MC4	Percentages MC1	MC2	MC3	MC4
7/29/2007	Lukenya		19.805			0	100	0	0
9/3/2007	Lukenya	18.433	19.828			60	40	0	0
10/1/2007	Lukenya	18.306	**19.696**			60	40	0	0
11/24/2007	Lukenya	18.521	19.911			63	37	0	0
12/12/2007	Mailo Dam		**19.458**			0	100	0	0
5/4/2008	Mailo Dam		19.362		23.515	0	77	0	23
6/6/2008	Mailo Dam	**18.074**	**19.46**	22.084		35	50	15	0
5/17/2007	Ruthagati		19.63			0	100	0	0
7/23/2007	Ruthagati	18.269	19.607			36	64	0	0
8/25/2007	Ruthagati	18.333	19.687	22.247	23.671	27	38	18	17
9/26/2007	Ruthagati	18.476	19.825	22.361		42	44	14	0
10/23/2007	Ruthagati		19.919			0	100	0	0
11/21/2007	Ruthagati		19.706			0	100	0	0
12/12/2007	Ruthagati		**19.432**			0	100	0	0
5/4/2008	Ruthagati		19.402			0	100	0	0
6/6/2008	Ruthagati		**19.503**			0	100	0	0

ELISA analyses with its lower detection limits resulted in a much higher MC-positive sample number of 47 (Table 2); only Damside (located in the Lake Victoria region) could be considered as completely toxin-free. The maximal amount of MC measured in integrated water samples by means of ELISA was 614 ng L^{-1}. The overall median was calculated 17 ng L^{-1}.

Table 2: Microcystin detection by means of ELISA versus *Microcystis* cell numbers. Sites are numbered and so are the sampling dates (Machakos – 1 Ngeki, 2 Ngei, 3 Lukenya; Mount Kenya – 4 Ruthagati, 5 Mailo Dam, 6 Sagana; Lake Victoria – 7 Damside, 8 Harambe, 9 Bomet); n.a. = not available, 0.00 = measurement below detection limit of 0.1 ng. mL^{-1}.

Site_Date	MC [$ng.L^{-1}$]	*Microcystis* $cell.L^{-1}$	Site_Date	MC [$ng.L^{-1}$]	*Microcystis* $cell.L^{-1}$	Site_Date	MC [$ng.L^{-1}$]	*Microcystis* $cell.L^{-1}$
1_1	11.72	35,704,259	2_1	44.43	17,792,423	3_1	3.12	0
1_2	47.76	290,888,209	2_2	9.44	104,485,707	3_2	n.a.	0
1_3	n.a.	314,531,832	2_3	n.a.	n.a.	3_3	17.17	86,200
1_4	49.04	75,229,709	2_4	7.33	120,054,077	3_4	14.72	363,200
1_5	16.87	178,670,559	2_5	79.31	232,585,184	3_5	69.71	8,800
1_6	135.96	172,087,960	2_6	218.20	120,759,356	3_6	0.00	0
1_7	76.09	81,028,666	2_7	59.27	129,849,613	3_7	0.00	0
1_8	35.74	39,338,869	2_8	30.07	176,476,359	3_8	n.a.	0
1_9	36.31	150,459,418	2_9	n.a.	1,680,911,381	3_9	n.a.	0
1_10	41.26	421,286,371	2_10	35.39	84,555,058	3_10	0.00	400
4_1	44.69	14,800	5_1	n.a.	0	6_1	n.a.	n.a.
4_2	222.05	24,667	5_2	n.a.	0	6_2	n.a.	0
4_3	455.04	3,462,955	5_3	n.a.	48,000	6_3	n.a.	0
4_4	77.09	60,601,710	5_4	n.a.	96,000	6_4	n.a.	0
4_5	n.a.	42,212,226	5_5	613.26	179,297,473	6_5	n.a.	32,000
4_6	354.93	0	5_6	38.83	245,750,383	6_6	n.a.	0
4_7	0.00	0	5_7	n.a.	245,750,383	6_7	4.06	0
4_8	n.a.	0	5_8	46.71	67,863,467	6_8	17.76	7,451,324
4_9	n.a.	0	5_9	19.13	1,664,000	6_9	5.65	0
4_10	100.91	0	5_10	60.22	65,199,081	6_10	13.97	0
7_1	0.00	0	8_1	0.00	0	9_1	n.a.	n.a.
7_2	0.00	0	8_2	0.00	0	9_2	n.a.	n.a.
7_3	0.00	0	8_3	0.00	0	9_3	n.a.	n.a.
7_4	0.00	0	8_4	3.11	0	9_4	3.20	515,950,422
7_5	n.a.	47,200	8_5	0.00	0	9_5	50.29	161,273,689
7_6	0.00	0	8_6	0.00	2,925,600	9_6	89.21	281,005,090
7_7	0.00	0	8_7	4.24	0	9_7	73.97	3,143,205,096
7_8	0.00	0	8_8	9.17	0	9_8	88.38	1,939,454,889
7_9	0.00	0	8_9	8.01	0	9_9	51.96	282,111,409
7_10	0.00	0	8_10	12.72	0	9_10	80.17	341,041,348

3.2 Abiotic and biotic factors related to MC detection

As *Microcystis* was by far the most abundant cyanobacterial taxon in the samples, we linked *Microcystis* cell abundance to MC occurrence and MC concentrations (ELISA), but no significant spearman rank correlation was found ($r = 0.07$, $p = 0.57$; Fig. 2). Highest *Microcystis* cell numbers were not corresponding with the maximum toxin contents; sometimes, no *Microcystis* cells were observed, but MCs were still detected; also other

cyanobacterial taxa showed no relation with MC occurrence (Table 2). Cellular contents of MCs were very variable (Table 3) and ranged from 7.2 to 686.7 fg cell^{-1}; no seasonal changes due to dry and rainy seasons of cellular MC production could be found.

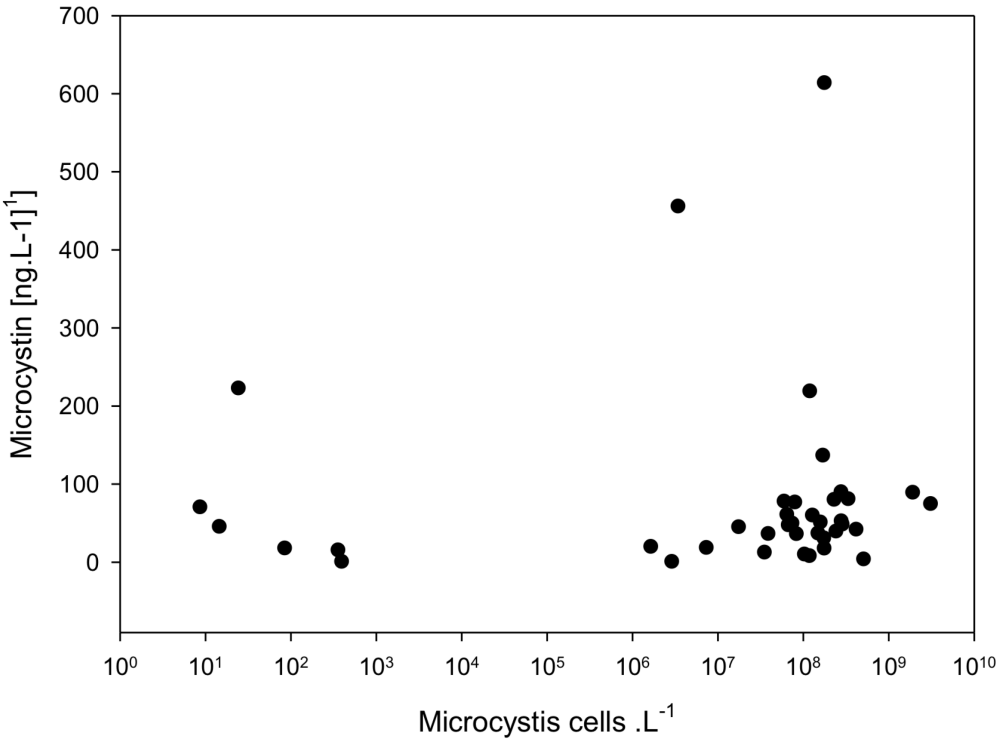

Fig. 2: *Microcystis* cells versus Microcystin concentration obtained by HPLC-DAD showed no significant relationship.

Table 3: Cellular microcystin contents of samples containing microcystin (HPLC-detection).

Site	Date	MC [fg.cell^{-1}]
Lukenya	7/29/2007	79.3
Lukenya	9/3/2007	686.7
Lukenya	10/1/2007	293.4
Lukenya	10/26/2007	87.5
Mailo	12/12/2007	109.4
Mailo	5/4/2008	431.7
Ruthagati	5/15/2007	13.0
Ruthagati	7/23/2007	74.6
Ruthagati	8/25/2007	164.3
Ruthagati	9/26/2007	40.5
Ruthagati	10/23/2007	7.2
Ruthagati	11/21/2007	99.6
Ruthagati	12/12/2007	36.7
Ruthagati	5/4/2008	63.0

Toxin-containing ponds showed significantly lower soluble reactive phosphorus amounts and Nitrite-N amounts (Table 4). The toxin-free ponds were significantly shallower than the ponds that contain toxins. Also total hardness and the Post Nauplii individual numbers differed significantly between groups: Total hardness was higher in the toxin-containing ponds and so were the Post Nauplii numbers.

Table 4: A Mann Whitney U-tests revealed significant differences for toxin free and toxin containing ponds (marked bold, p = 0.05, n = 76).

		Toxin free	Toxin Containing
Soluble Reactive Phosphorus [µg.L^{-1}]	1. Quartile	14.22	5.42
	Median	**23.70**	**11.51**
	2. Quartile	39.28	16.59
Total phosphorus [µg.L^{-1}]	1. Quartile	73.86	76.60
	Median	**111.91**	**153.69**
	2. Quartile	148.96	224.44
Total Hardness [mg $CaCO_3$ L^{-1}]	1. Quartile	12.45	25.05
	Median	**30.00**	**32.55**
	2. Quartile	34.00	41.55
Alkalinity [mval L^{-1}]	1. Quartile	1.16	1.51
	Median	**1.49**	**1.72**
	2. Quartile	1.99	2.06

Chlorophyll a	1. Quartile	76.95	63.89
[µg.L^{-1}]	Median	**87.78**	**95.30**
	2. Quartile	114.11	159.26
Ammonium-N	1. Quartile	35.62	33.33
[µg.L^{-1}]	Median	**83.16**	**48.77**
	2. Quartile	104.89	71.78
Nitrite-N	1. Quartile	3.64	1.44
[µg.L^{-1}]	Median	**7.46**	**2.83**
	2. Quartile	22.78	4.50
Nitrate-N	1. Quartile	0.03	0.03
[mg.L^{-1}]	Median	**0.05**	**0.04**
	2. Quartile	0.07	0.06
Secci-Depth	1. Quartile	0.21	0.20
[m]	Median	**0.28**	**0.25**
	2. Quartile	0.50	0.30
Altitude	1. Quartile	1,150.00	1,627.00
[m a.s.l.]	Median	**1,759.00**	**1,670.00**
	2. Quartile	1,825.00	1,759.00
Conductivity	1. Quartile	120.10	187.28
[µS cm^{-1}]	Median	**182.64**	**245.77**
	2. Quartile	239.93	297.39
Temperature	1. Quartile	19.99	19.76
[°C]	Median	**22.55**	**21.81**
	2. Quartile	23.48	23.12
Water depth	1. Quartile	2.00	1.65
[m]	Median	**2.95**	**2.08**
	2. Quartile	3.05	2.56
Post Nauplii	1. Quartile	18.67	83.26
[Inds L^{-1}]	Median	**40.83**	**136.50**
	2. Quartile	104.63	183.95
Nauplii	1. Quartile	46.67	210.50
[Inds L^{-1}]	Median	**217.00**	**310.50**
	2. Quartile	265.78	450.50
Cladocera	1. Quartile	0.00	0.00
[Inds L^{-1}]	Median	**0.00**	**6.00**
	2. Quartile	11.25	24.13
Rotifer	1. Quartile	449.17	499.75
[Inds L^{-1}]	Median	**962.74**	**994.29**
	2. Quartile	1,437.33	1,375.40
ZOOTotal	1. Quartile	735.00	973.25
[Inds L^{-1}]	Median	**1,502.67**	**1,482.00**
	2. Quartile	1,878.75	2,043.76
Bacteria	1. Quartile	2,841,033.87	4,205,436.28

[Cells mL^{-1}]	Median	**5,144,881.57**	**6,355,441.94**
	2. Quartile	6,785,758.32	11,005,731.13

4. Discussion

Most of the ponds investigated are highly used for cattle watering, domestic purposes, with agriculture being carried out in their close vicinity, which is likely to be the reason for high nutrient loads such as TP concentrations beyond 100 µg L^{-1} (Zhao et al., 2006). Moreover, their shallowness and increased turbidity create ideal conditions for Cyanobacteria (Chen et al., 2003; Scheurs, 1992; Wicks and Thiel, 1990). However, with the exception of Bomet, Cyanobacteria played only a minor role (Straubinger-Gansberger et a, 2014). Instead, chlorophytes, dinophytes and euglenophytes were common in these ponds. Even if Cyanobacteria occurred in low densities, noticeable amounts of MCs were measured in the net samples of Mailo Dam, Lukenya and Ruthagati, which highly calls for considering MCs in monitoring programs of aquaculture. Other studies (Chen et al., 2006; Zhao et al., 2006) on Nile tilapia and Silver carp suggested avoiding fish that feed on toxic Cyanobacteria, in particular when *Microcystis*-blooms occur. Even if no Cyanobacteria were observed in our integrated water samples, *Microcystis* and MCs were detected in net samples due to concentration of the colonies. Every pond contained *Microcystis* at least once throughout the sampling period, which highlights the urgent need of monitoring programs of such water bodies. Rumisha and Nehemia (2013) described that this taxon is the major diet of wild and pond-cultured Nile tilapia in the Lake Victoria basin in Mara, which had already been shown by Figuaredo and Giani (2005). They proved in an experiment that Tilapia feeds selectively on large algae, mainly Cyanobacteria and diatoms.

In some cases, we found MC, but without *Microcystis* being present. Toxin production in absence of *Microcystis* cells was also described by Poste et al. (2013), who explained it with the presence of other cyanobacterial taxa. Other cyanoprokaryotes as toxin producers can be ruled out in our study, because they were only observed in very low abundances. It rather seems that free MC adsorbed to other particles still remained in the water after disintegration

of the MC producers. Bourne et al. (2006) showed that in river water of the Murrumbidgee River (NSW, Australia) 50 % of the total MC-LR - which was the most common toxin in our ponds– still remained in the water column after six days. 20 % of the MC-LR remained after seven days in water from the Las Vegas Bay of Lake Mead (Eleuterio and Batista, 2010). On the other hand, already (Carmichael and Gorham, 1981) proved that some *Microcystis* strains are not able to produce toxins. A linear relationship would implicitly imply the same ratio of toxin- and non-toxin-producing cells in all samples.

MC-LR was most frequent followed by MC-YR; two other structural variants were detected in smaller amounts and therefore could not be identified. Seasonal patterns according to the dry, rainy and transition periods in the tropics as already found in other studies (Kotak et al., 2000; Wicks and Thiel, 1990) could not be observed. Cellular MC concentrations fluctuated strongly throughout the year. Possible explanations are the co-existence of both toxic and non-toxic *Microcystis* strains and also different growth stages of the strains. Lyck (2004) showed that cells in the late stationary phase contain up to twice the amount of MC in comparison to actively growing cells. This hypothesis is strengthened by our finding that the ponds with highest cyanobacterial densities did not necessarily contain MCs (Fig. 2). Bomet was dominated by Cyanobacteria during the whole sampling period, but MCs could be detected only in traces by means of ELISA. Damside was generally toxin-free (tested with both HPLC and ELISA), Harambe showed only low concentrations with ELISA and no toxins with HPLC. While Cyanobacteria densities were generally very low in Damside, Harambe showed an increased contribution of Cyanobacteria (*Anabaena* sp., *Aphanocapsa* sp. and *Limnothrix* sp.) of 40% in December.

Even if MC was only found in traces and always below the limit of the World Health Organization (WHO) guideline for drinking water (1 $\mu g\ L^{-1}$), it still can pose a threat to humans, as it is known to accumulate in fish tissue via the food chain (Magalhães et al., 2003;

Magalhães et al., 2001; Poste et al., 2011). The WHO has set the value of tolerable daily intake for chronic exposure to MC-LR at 0.4µg kg^{-1} body weight (Falconer et al., 1999; WHO, 1998), but no thresholds are given for MC concentration in fish tissue (Poste et al., 2011).

Which environmental factors are responsible for regulating MC production? In general, the *mcy*-genecluster is essential for the expression of MCs. If this sequence is lacking, incomplete or mutated, strains are not able to produce toxins (Kaebernick and Neilan, 2001). Several studies have shown that the amount of MCs produced depends on several factors of the environment such as light intensity (Repka et al., 2004; Sivonen, 1990; Tonk et al., 2005; Utkilen and Gjølme, 1992; Wiedner et al., 2003), temperature (Song et al., 1998; Wicks and Thiel, 1990), pH (Song et al., 1998), nitrogen supply (Repka et al., 2001; Sivonen, 1990; Vezie et al., 2002) and phosphorus availability (Oh et al., 2000; Repka et al., 2004; Repka et al., 2001; Sivonen, 1990; Vezie et al., 2002).

According to the findings of Kotak (2000), who described a strong positive relation between TP and MC-LR concentration in the pelagial of temperate eutrophic lakes, we also found higher TP amounts in toxin-containing ponds, but with the Man-Whitney-U-test we could not prove that there was a significant relationship. NO_3-N, which is commonly the major component of total nitrogen (TN), did also not show a significant relationship. For future research, TN needs to be included, as Kotak (2000) described a strong negative relation of *Microcystis aeruginosa* biomass and the TN/TP ratio. Chen et al. (2011) showed in laboratory experiments that growth rates and maximum cell densities of *Microcystis aeruginosa* showed a significant decline while nitrite concentrations are increasing. Our findings with significantly lower nitrite concentrations in ponds containing toxins point to the findings of Chen et al. (2011), as Microcystin contents are influenced by growth rates.

One attempt to supply the local communities in rural areas with a protein rich diet is aquaculture. Here, small-scale farming in cages might be the method of choice because it only

needs basic equipment (Brummett et al., 2008; Kaggwa et al., 2011). From our results, we conclude that even for small fish-cage farming, a regular monitoring program must be installed as an accompanying measure to ensure that MCs are not posing a threat to health. As direct MCs analysis is expensive and currently not available for routine analysis in developing countries, surrogate variables may be used instead to predict the probability of MCs occurrence. This study provides variables that are easy to obtain and which can also be used as a decision tool. In order to provide healthy food to the people, it should be a matter of course that ponds with toxin production throughout the year are excluded from fish cage programs, even if MC concentrations are below the WHO guideline for drinking water, because fish might accumulate these toxins in tissues over time.

5. Acknowledgements

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Chapter III: Sudden flamingo deaths in Kenyan Rift Valley lakes

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Sudden flamingo deaths in Kenyan Rift Valley lakes

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The East African Rift Valley Lakes Bogoria and Nakuru sometimes host around 75% of the world population of lesser flamingos *Phoeniconaias minor*. In this area, mysterious flamingo die-offs have occupied researchers for four decades. Recently, cyanobacterial toxins came into the fore as a possible explanation for mass mortalities because the main food source of lesser flamingos is the cyanobacterium *Arthrospira fusiformis*. We took weekly samples from July 2008 to November 2009 from Lakes Nakuru and Bogoria and analyzed them by high performance liquid chromatography for microcystins. Monthly, samples were cross-checked using protein phosphatase inhibition assays with lower detection limits and additionally screened for polar toxins. During our study period, three flamingo die-offs occurred at L. Bogoria and we were able to analyze tissues of 20 carcasses collected at the shoreline. No cyanotoxins were detected either in plankton samples or in flamingo tissues. Accordingly, other reasons such as food composition or bird diseases played a key role in the observed flamingo die-offs.

The cyanobacterium *Arthrospira fusiformis* is a well-known dietary supplement because of its high content of essential amino acids and fatty acids, vitamins, minerals and antioxidant functions (Belay et al. 1993, Capelli and Cysewski 2010). In its natural habitat, i.e. tropical alkaline–saline water bodies, *A. fusiformis* commonly plays a key role in the food web as the main photoautotrophic primary producer (Vareschi and Jacobs 1984, Oduor and Schagerl 2007). We focused our research on the Kenyan soda lakes Bogoria and Nakuru, where *A. fusiformis* is the main food source for lesser flamingos *Phoeniconaias minor* (Vareschi 1978).

Die-offs of lesser flamingos at some East African rift valley lakes have attracted researchers interests for four decades (Table 1). They are a threat to the economic and conservation status of the otherwise impoverished regions whose lakes host these birds. Possible causes that have been cited involve habitat degradation (Krienitz and Kotut 2010), infectious diseases (Sileo et al. 1979, Kock et al. 1999) and pollution through heavy metals and pesticides (Greichus et al. 1978, Kairu 1996). Additional suggestions include changes in food quantity and diet quality (Motelin et al. 2000, Ndetei and Muhandiki 2005, Krienitz and Kotut 2010, Kaggwa et al. 2013) and toxic cyanobacteria (Ballot et al. 2004a, b, Krienitz et al. 2005, Koenig 2006, Lugomela et al. 2006).

To date, the ultimate causes for the die-offs are still speculative in most cases, because prompt action is needed to collect material. As this phenomenon usually occurs in remote areas and carcasses are removed by scavengers within a few days, this is rarely possible. In July 2008, a flamingo die-off was observed at L. Bogoria (Krienitz and Kotut 2010) and in 2009, two smaller but noticeable flamingo die-offs (March and August) occurred at the same lake (Fig. 1, Table 1). During our weekly sampling intervals from July 2008 to November 2009, we had the opportunity to screen the lake water for cyanotoxins. This approach was based on reports of toxin-producing cyanobacteria in the hot springs around L. Bogoria and in two flamingo carcasses (Krienitz et al. 2003, Metcalf et al. 2013). Harper et al. (2003), however, argued that those observations were not fully representative of the whole lake as the hot springs represent only a small proportion of the available drinking water. Very recently, Metcalf et al. (2013) detected neurotoxic amino acids in flamingo feathers, which were collected during a sudden mass mortality in 2003, so cyanotoxins must not be precluded as a possible explanation for the recent die-offs.

Methods

The lakes Nakuru and Bogoria sometimes host 75% of the world population of lesser flamingos *Phoeniconaias minor* (Nasirwa 2000, Zaccara et al. 2011), are amongst the Kenyan top tourist attractions and are classified as National Park

Figure 1. Flamingo die-off at the shore of Lake Bogoria in July 2008. Within one week, about 30 000 out of 150 000 flamingos died.

(Nakuru) and Ramsar site (Bogoria). The surface area of L. Nakuru (00°S, 36°E; 1756 m a.s.l.) between 36 and 49 km^2 depends on precipitation, evaporation and human use such as irrigation and varies highly throughout the year. The lake has a maximum depth of about 1 m and is daily mixed. The surface area of L. Bogoria (00°N, 36°E; 990 m a.s.l.) is usually about 34 km^2, but extending during distinct rainy seasons especially in the northern area. With a maximum depth of 10.2 m (Hickley et al. 2003), it is stratified over longer periods (McCall 2010).

We took weekly samples at both lakes. Algal composition and biovolume were determined using lake water samples fixed with formalin to a final concentration of 4%. Taxa were identified down to the lowest possible level and enumerated using an inverted microscope at 100× and 200× magnification (for *Arthrospira* and *Anabaenopsis*) and 400× for other taxa. Taxon specific biovolumes were estimated using geometric formulae of shapes similar to the respective phytoplankton cells. At least thirty cells for each taxon identified were measured to give the average size and biovolume. For conversion of cell volume into biomass, a conversion factor of 1 was used.

A 30-µm-mesh plankton net was used to collect particle sizes that are usually retained by the filter of the lesser flamingo bill and swallowed. Samples were hauled from the shoreline at the central sampling station of the respective lake in weekly intervals. The samples were transported cooled to the Egerton laboratory, immediately filtered through glass-fiber filters and then the filters containing algal biomass were dried and stored frozen until analysis. Prior to analysis, filters were cut into pieces, put in Eppendorf tubes and homogenized with a tissue grinder to assist cell burst and toxin extraction. Extraction was done in 75% (w/v) aqueous methanol. The extracts were concentrated to dryness in a vacuum centrifuge and the residue redissolved in 300 µl of 50% aqueous methanol, vortexed and centrifuged at the highest level for 10 min. Finally, the clear supernatant was analyzed as described in Kurmayer et al. (2003) with high performance liquid chromatography/diode array detection (HPLC-DAD). A gradient of aqueous acetonitrile containing 0.05% triflouroacetic acid (TFA) was used at a flow rate of 1 ml increasing from 30 to 70% acetonitrile in 42 min. MCs with a limit of quantification (LOQ) of 100 ng l^{-1} were identified by their retention times, co-chromatography with authentic standards (Sigma-Aldrich 33578 and CyanoBiotech), and their characteristic absorption spectra. Additionally, 13 dried filters of net samples were analyzed for microcystins in the Aberdeen laboratory for a crosscheck, (MC–LC, –RR, –YR, –LW and –LF; standards available from EnzoLife sciences, Lausen, Switzerland).

To check for traces of MCs, protein phosphatase-inhibitions assays (PPIA) (An and Carmichael 1994) with a detection limit of 10 ng l^{-1} were performed in monthly intervals in 96-well plates; PP1 was used to hydrolyze p-nitrophenol-phosphate. If MC was in the samples, this reaction would have been inhibited and so no color difference would occur with time. The reaction results in a chromogenic product with an absorbance maximum at 405 nm. Plates were then measured by a plate reader.

During periods of die-offs in July 2008, March and August 2009, we additionally analyzed samples for polar toxins, such as cylindrospermopsin, anatoxin-a, anatoxin-a(S), saxitoxin and neosaxitoxin, in the Aberdeen laboratory by means of LC–TOF–MS (Waters Acquity ultra-high performance liquid chromatography coupled to a Xevo quadrupole time of flight mass spectrometer; standards: Cylindrospermopsin EnzoLife Sciences, Phenylalanine Sigma, Anatoxin-a Calbiochem, Saxitoxin and Neosaxitosin NRC Canada). Dried samples (50 mg) were extracted in 1 ml of methanol containing 0.1% TFA for 1 h with intermittent vortexing. An Atlantis hydrophilic interaction (HILIC) column (2.1 mm i.d. ×150 mm long; 5 µm particle size) was used for the separation; mobile phase consisted of acetonitrile (A) and MilliQ containing 0.5 mM formic acid and 0.2 nM ammonium formate (B). Separation was achieved using a gradient increasing from 2% B to 20% B over 7 min, followed by an increase to 70% B from 7 to 10 min at a flow rate of 0.3 ml min^{-1}. Data was acquired in positive ion electrospray scanning from m/z 50 to 600 with a scan time of 2 s and inter-scan delay of 0.1 s. Ion source parameters: capillary and sampling cone were 2.9 V and

Table 1. Reported flamingo mass-mortalities in the Rift Valley Lakes Nakuru and Bogoria from 1991 to 2009.

Year	No. of carcasses	Season	Lake	References
1991	40 000		Bogoria and Nakuru	unpublished data
1993	30 000	Sept–Nov	Bogoria and Nakuru	(Kock et al. 1999, Ndetei and Muhandiki 2005)
1995/1996	50 000		Bogoria and Nakuru	(Motelin et al. 2000, Krienitz et al. 2005)
1999/2000	30 000		Bogoria	(Krienitz et al. 2003)
2006	35 000	July, Aug	Nakuru	(Krienitz and Kotut 2010)
2008	30 000	July	Bogoria	(Krienitz and Kotut 2010), this study
2009	2000	March, Aug	Bogoria	this study

25 V respectively; desolvation temperature 300°C; source temperature 80°C. Cone gas and desolvation gas flows were 50 l h^{-1} and 400 l h^{-1}, respectively. Sodium iodide (2 µg $µl^{-1}$ in 50/50 propan-2-ol/H_2O) was used as the calibrant with leucine-enkephalin (0.5 mg ml^{-1} in 50/50 methanol/Milli-Q) as the lockspray. Instrument control, data acquisition (centroid) and processing were achieved using MassLynx ver. 4.1. LOQ for cylindrospermopsin was 100 ng ml^{-1}, other polar toxins were determined on a qualitative basis due to lack of availability of accurate standards. Phenylalanine is always monitored as this is often misidentified as anatoxin-a.

In addition, material of flamingo carcasses (gizzard, muscle, liver and intestine) from the die-off in August 2009 was analyzed. Tissues were lyophilized, homogenized in a tissue grinder and then extracted according to the aforementioned protocol in 1 ml of methanol containing 0.1% TFA for 1 h with intermittent vortexing. Afterwards the samples were centrifuged at 15 000 g for 10 min. Then the supernatant was removed and used for microcystin and polar toxin analyses.

Results

During the survey, we observed three flamingo die-offs in the Bogoria area. The largest die-off occurred during July 2008, where about 30 000 birds deceased within one week (Fig. 1, Table 1). At this time, the lake hosted about 150 000 flamingos. Two smaller die-offs occurred during 2009 with around 2000 carcasses at the central shoreline area of the lake. During the second die-off in August 2009, we were able to gather different tissues from 20 flamingo carcasses (Table 2) and neither could detect MCs nor polar cyanotoxins.

Net plankton samples were analyzed in weekly intervals for MCs by HPLC and no MCs were detectable. The sensitive PPIA applied in monthly intervals confirmed the HPLC results (Table 2). We also were not able to detect cylindrospermopsin, anatoxin-a, anatoxin-a(S), saxitoxin and neosaxitoxin during the three die-off periods. Summarizing up, in none out of 156 samples analyzed with different methods, we were able to detect any cyanotoxins (plankton n = 136; flamingo-tissues n = 20).

During the study, also the algal composition and biovolume were monitored (Fig. 2). Cyanbacteria turned out to be the dominant group in both lakes. In L. Bogoria, *Arthrospira fusiformis* showed a more or less mono-specific dominance during the whole study period. L. Nakuru showed greater variation in the algal community composition with also green algae and Cryptophyta being abundant. At certain times, *Arthrospira* blooms crashed and were replaced by eukaryotic taxa.

Table 2. Analyzed samples during the investigation. In none of the samples, cyanotoxins were detectable (LOQ HPLC > 100 ng l^{-1}; PPIA > 10 ng l^{-1}; LC/TOFMS > 10 µg l^{-1}).

Samples	HPLC	PPIA	LC/TOFMS
Lakes			
Bogoria central	68	17	13
Nakuru central	68	17	12
Flamingo tissues			
Gizzard	6		6
Muscle	9		9
Liver	2		2
Intestine	3		3

Discussion

Our surprising results contradict other studies (Krienitz et al. 2003, Ballot et al. 2004a, Metcalf et al. 2006, 2013), prompting a search for explanations for this discrepancy: 1) the occurrence of the main toxin producer *Microcystis* in such systems is still disputed. While some scientists proposed a link between *Microcystis* and flamingo die-offs (Ndetei and Muhandiki 2005, Stewart et al. 2008, Githaiga 2003), others challenged the growth of *Microcystis* in such alkaline-saline environments (Kotut and Krienitz 2011). Former *Microcystis* occurrences (Ndetei and Muhandiki 2005) were probably misidentified and may belong to *Anabaenopsis abijatae*, which at a first glance could be mixed up with *Microcystis* colonies. Even molecular techniques could not detect any phylotypes of *Microcystis* (Dadheech et al. 2009, Kotut and Krienitz 2011). 2) *Arthrospira fusiformis* and *A. maxima* are sold as food supplements and considered as non-toxic. Studies on the toxicity of the dominant cyanobacterium *A. fusiformis* revealed that a few clones produce MCs and anatoxin-a, but others not (Ballot et al. 2004a). Mussagy et al. (2006) considered three strains isolated from Mozambique and one from L. Nakuru; none of them produced toxins, nor could the mcyE gene be detected from the analyzed Mozambique clones. At least for our study period, the toxicity of *A. fusiformis* could be excluded as the net samples consisted of mainly cyanobacterial biomass with *A. fusiformis* being the dominant taxon. 3) No cyanotoxins could be detected in flamingo tissues collected during this study, which is in accordance to conclusions of Kock et al. (1999), who examined inner organs of 42 carcasses in 1993 and did not find any typical indications of cyanotoxins poisoning. As cyanotoxins have been detected in flamingo feathers even seven years after collection (Metcalf et al. 2013), we know that flamingos are exposed to these toxicants at least from time to time. It however seems unlikely that the ingested toxins were solely responsible for the die-offs, because unusual mortalities of other bird taxa and mammals frequenting the lakes in high numbers have never been recognized and also scavengers feeding on flamingo carcasses were obviously not affected.

What else could have caused die-offs during our sampling period? Interestingly, this phenomenon has been observed only at Lake Bogoria during our study. Both lakes Nakuru and Bogoria showed varying *A. fusiformis* biomass, which has been identified as driving force for flamingo movements (Kaggwa et al. 2013). The lakes, however, do differ somewhat. While Lake Bogoria was dominated by *A. fusiformis* throughout the sampling period, Lake Nakuru showed pronounced changes in the phytoplankton composition; especially after *Arthrospira* biomass crashes, shifts towards other algae taxa occurred (Kaggwa et al. 2013). The flamingo deaths in March and August 2009 corresponded with the highest bird densities at Lake Bogoria. Kaggwa et al. (2013) proposed that neither food quantity nor food quality in terms of carbohydrates, lipids and crude protein were likely

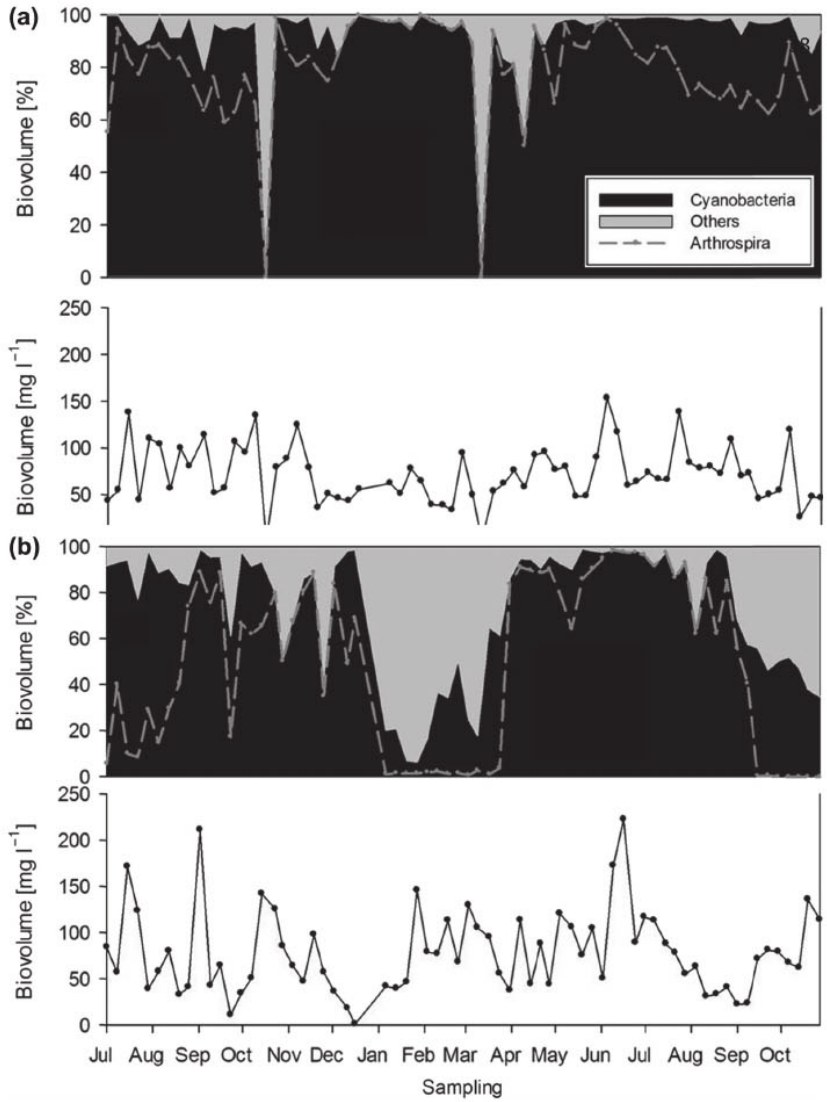

Figure 2. Phytoplankton biovolume during the study showing *Arthrospira* (dashed line), total cyanobacteria (black area) and other algal groups (grey area) in percent (top) and absolute total biovolume (bottom). (2a) Bogoria, (2b) Nakuru; data modified from Kaggwa et al. (2013).

key factors in these die-offs. Food supply has been sufficient and in satisfying quality. At food shortage, flamingos usually start moving to other lakes (Tuite 1979, 2000, Kaggwa et al. 2013) indicating other reasons for die-offs.

Our study suggests excluding cyanobacterial toxins as well as major cause of mass die-off. We used different methods and equipment and never got any positive result. Potential toxin producers such as *Anabaenopsis* sp. were detected, but we did not observe *Microcystis*. Permanent pesticide and heavy metal discharging into the systems may also weaken the birds making them sensitive to infections. For lakes Bogoria, Nakuru and Elementaita, Ochieng et al. (2007) found heavy metal concentrations above the highest desirable level in drinking water (HDL; WHO) and most of the time above the maximum permissible level in drinking water (MPL; WHO). Krienitz and Kotut (2010) proposed that consumed *Anabaenopsis* can clog the filters in the beaks due to its heavy mucilage. Clogging by aggregated *A. fusiformis* has already been described by Vareschi (1978), and we also observed such agglomerations in Bogoria, which could also contribute to a weakening of the flamingos. The current state of knowledge rather suggests that at certain times multiple causes including cyanobacteria aggregations weakens the bird population, making them vulnerable to bird diseases. As only flamingos are affected by this phenomenon, this points towards epizooty as cause of death. In fact, such diseases like avian tuberculosis have already been noted in these ecosystems (Kock et al. 1999).

There is still an urgent need for further comprehensive and systematic investigations, which hopefully will be funded even though flamingo die-offs occur erratically and last only a few days. Key problems seem to be the unpredictability of

flamingo mass mortality and its rapid development. This asks for 'stand by' experts, who must become active within hours to days. Because funding agencies will probably not support such projects based on many coincidences, environmental protection agencies are required. Cooperation between institutions offering state-of-the-art methods and local scientists would achieve the best results. Especially veterinarians should be included in this research topic to gain new insights into behavior of the birds and for diagnosis of avian diseases.

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Conclusions and Recommendations

The present research provides insight into the phytoplankton community of small water bodies in Kenya including possible cyanotoxin production in these ponds. The two Rift valley lakes Bogoria and Nakuru were additionally screened for cyanotoxins. We tried to find out about the risk of intoxication for locals when consuming the fish cultured in fish-cages in small man-made reservoirs. Cyanotoxins are also in the focus as possible cause for mass mortalities of Lesser Flamingo in Lakes Bogoria and Nakuru, both lakes are important tourist attractions of the black continent.

- All nine monitored African ponds turned out to be hyper-eutrophic during the whole sampling period the only exception was Sagana. The pond was filled with water at the beginning of the investigations and showed an eutrophication pulse with time. Changes in the phytoplankton communities of the small ponds followed the change from the hot dry season to the cold rainy season.
- Due to high nutrient loads and conditions that favor the occurrence of Cyanobacteria we assumed this group dominating the phytoplankton community in the investigated ponds. Contrarily to our expectations, mainly genera belong to Chlorophyta, Dinophyta and Euglenophyta occurred in high numbers.
- The phytoplankton community was structured by various factors such as temperature, altitude of the sites, zooplankton. A key factor for the algal composition was accessibility for cattle.
- The investigated ponds were screened for Microcystin, which is produced by some cyanobacterial genera. For depth integrated water samples we used more sensitive ELISA kits and could detect this toxin in most of the samples. The amount was always lower than the suggested WHO-limit for drinking water but Microcystin can accumulate in fish tissue and so it has to be considered too. Via the food chain these highly effective toxins can enter the human body.
- We searched for abiotic factors triggering Microcystin production. The nutrients soluble reactive phosphorus and Nitrite-N as well as total hardness, water depth and the amount of Post Nauplii differ significantly when comparing toxin free and toxin containing ponds.
- For the use of the investigated ponds for fish-cage farming we recommend a regular monitoring including cyanotoxins. Also fish tissue should be analyzed for cyanotoxins. Unfortunately, there is currently no guideline for toxins in fish-tissue

available.

- After our investigation a cyanobacterial bloom was reported from Ruthagati. When such blooms occur, samples should be screened for *Microcystis* cells and analyzed for toxins in any case.
- The lakes Bogoria and Nakuru host the world's largest population of Lesser Flamingos. Mass die-off of these birds threatens the touristic value of these national reserves and Ramsar sites. During our sampling period we found out that cyanobacterial toxins can be excluded from the list of factors that lead to die-off. Although both lakes were dominated by cyanobacterial taxa during the whole sampling period.
- Further investigations on the possible causes for the die-offs have to be carried out, amongst these maybe environmental conditions that weaken the birds and bird diseases such as avian tuberculosis. Also high biomass of *Anabaenopsis/Anabaena,* which sometimes bloom in the saline-alkaline lakes can be a reason for weakening or even die-off, as the colonies are known to clog the gills in the beaks of flamingos.

Curriculum Vitae

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10. 2007 - ongoing:	Doctoral studies in ecology
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N. Straubinger, R. Kurmayer, M. Schagerl. Phytoplanktongemeinschaften und Microcystinproduktion in kenianischen Fischteichen, 12. Tagung der DBG Sektion Phykologie 2008, Wittenberg, Deutschland.

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