

Microcystin concentrations in Nile tilapia (*Oreochromis niloticus*) caught from Murchison Bay, Lake Victoria and Lake Mburo: Uganda

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Abstract Catches of the economically important Nile tilapia (*Oreochromis niloticus* L.) from two eutrophic tropical lakes in Uganda, Lake Mburo and Murchison Bay, Lake Victoria, were examined to determine the presence of microcystins (MCs) in gut, liver and muscle of the fish. Analysis for MCs (RR, LR and YR) in both fish and water samples was by liquid chromatography coupled with mass spectroscopy (LC-MS) method. Physico-chemical parameters were also measured to establish the status of both lakes. MC-RR was the most prominent MC detected in Lake Mburo and Murchison Bay samples, there was no evidence of significant seasonal variation in

the concentration of MCs in fish tissue. MCs were detected in all water samples from both study lakes. The mean concentration of MCs in water was found higher in dry times for Lake Mburo ($P < 0.05$) and higher in wet times for Murchison Bay ($P < 0.05$). MC concentrations in the fish guts were positively related with MC concentrations in water samples from Murchison bay ($P < 0.05$), no such correlation was found in Lake Mburo. In eutrophic tropical lakes, fish seem to have a high tolerance to the toxicity of cyanotoxins including MCs. However, there is a possibility of accumulating these toxins in their tissue with the threat of transferring them higher up in the food chain. Due to a low sample size and short sampling period, the results can only serve to highlight the potential risk of MC accumulation in Nile tilapia in these lakes. Further studies are needed for the purpose of risk assessment.

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Introduction

Formation of cyanobacteria blooms is symptomatic of eutrophication in productive lakes. Besides forming colonies that may be unpalatable to fish and zooplankton, cyanobacteria are capable of producing cyanotoxins which are toxic to fish (Malbrouck & Kestemont,

2006), terrestrial animals and humans. The most frequently occurring of these cyanotoxins are the monocyclic heptapeptides-microcystins (MCs) (Malbrouck & Kestemont, 2006) produced by cyanobacteria like *Microcystis*, *Anabaena*, *Oscillatoria* and *Nostoc* (Dawson, 1998). The occurrence of MCs in these cyanobacteria is strain specific (Welker & von Döhren, 2006). Strains producing MCs possess genes for their synthesis (Zurawell et al., 2005; Welker & von Döhren, 2006; Kurmayer & Christiansen, 2009). Microcystins are mainly cell bound but may be released in high concentrations during cell lysis (Chorus & Bartram, 1999). MC biosynthesis and their extracellular concentration may also be enhanced under high light/red light conditions (Wiegand & Pflugmacher, 2005) and higher temperatures (Zurawell et al., 2005). Due to public health concerns, the effects of MCs have been more extensively investigated in mammals than in fish (Zurawell et al., 2005), as the reported toxicity of MCs is much higher in mammals such as mice ($LD_{50} \sim 50 \mu\text{g kg}^{-1}$) than in fish ($LD_{50} > 500 \mu\text{g kg}^{-1}$) (Kotak et al., 1996).

There are over 60 known microcystins (Sivonen & Jones, 1999) which differ mainly in the two L-amino acids and methylation on methylaspartic acid and methyldehydroalanine. Of these, microcystin-LR is the most common having the L-amino acids leucine (L) and arginine (R) (Dawson, 1998). Microcystins are manufactured through a non-ribosomal thio-template mechanism (Arment & Carmichael, 1996). In aquatic ecosystems, microcystins may have severe effects on all levels of aquatic flora and fauna (Zurawell et al., 2005) and could also accumulate within the food web (Laurén-Määttä et al., 1995).

Several freshwater fish are capable of ingesting and assimilating nutrients from toxin producing cyanobacteria (Moriarty et al., 1973; Kamujunke et al., 2002; Turker et al., 2003; Bwanika et al., 2004). In some cases, such as the Nile tilapia, cyanobacteria make up a large proportion of the diet (Getachew, 1987; McDonald, 1987). Fish may, therefore, be exposed to MCs through grazing on toxic cells (Tencalla et al., 1994). Once consumed, MCs act primarily as a hepatotoxin, causing hepatic haemorrhage through inhibition of protein phosphatases 1 and 2A (Kotak et al., 1996). Despite isolated reports implicating toxicity of MCs as responsible for massive fish deaths (Rodger et al., 1994), it is considered likely that fish would continue ingesting

cyanobacteria even in the presence of MCs, which eventually may accumulate in fish tissue (Magalhaes et al., 2003; Mohamed et al., 2003; Zurawell et al., 2005; Zhao et al., 2006). It has also been suggested that fish are able to effectively deal with MCs physiologically through biliary excretion (Sahin et al., 1996) and behaviourally by lowering ingestion rates in the presence of toxic algae (Keshavanath et al., 1994). The ability of fish to consume large amounts of cyanobacteria is sometimes also seen as an advantage in the control of cyanobacterial blooms (Datta & Jana, 1998). Nonetheless, substantial amounts of ingested MCs may accumulate in the fish liver and muscle (Tencalla & Dietrich, 1997; Mohamed et al., 2003; Zhao et al., 2006), and could easily be transferred higher up in the food web.

Studies on the tropical fresh water lakes Victoria and Mburo have shown that their trophic states are eutrophic and hypertrophic, respectively, with a high biomass of potential toxin-forming cyanobacteria (Byarujali, 1995; Verschuren et al., 2002; Okello et al., 2009). Analyses in the bays and gulfs of Lake Victoria (Krienitz et al., 2002; Sekadende et al., 2005; Haande et al., 2007) have revealed the presence of the MCs LR, RR and YR that are most likely produced by *Microcystis* (Haande et al. 2007; Okello et al., 2009). Although levels observed in these studies were not considered to pose any risk for human consumption (Sekadende et al., 2005), there may be potential long-term implications. One way of ascertaining the potential risk of MCs is to investigate accumulation at different levels in the food web.

The generalist filter feeding Nile tilapia is a very important economic fish in Lake Victoria (Ogutu-Ohwayo, 1990; Njiru et al., 2007) and Lake Mburo (Bwanika et al., 2004; Nagayi-Yawe et al., 2006). Nile tilapia, consumes large amounts of the cyanobacteria *Microcystis* and *Anabaena* in its diet (Bali-rwa, 1992). In the present study, the concentrations of microcystins in gut, muscle and to a lesser extent liver tissue, were monitored monthly over a 1-year period. The main aim of our study was to verify the presence of microcystins in Nile tilapia gut, muscle and liver tissue and to investigate whether these concentrations could be explained by the following environmental variables; microcystin concentrations in the water, surface chlorophyll *a* levels, water transparency, water temperature and dissolved oxygen.

Materials and methods

Study area

Our study was carried out in two Ugandan freshwater bodies; Lake Mburo and Murchison Bay, Lake Victoria.

Lake Mburo is located within the protected Lake Mburo National Park in the south-west of Uganda which lies between 00°30′–00°45′S, and 45°00′–31°05′E at an average altitude of 1,210 m (Wronski, 2002). Lake Mburo has a maximum depth of 4 m, an average depth of 2 m and a surface area of approximately 13 km². Fishing and tourism are the main economic activities in and around Lake Mburo. Major fisheries include the haplochromines, Nile tilapia and Graham's tilapia (*Oreochromis esculentus*).

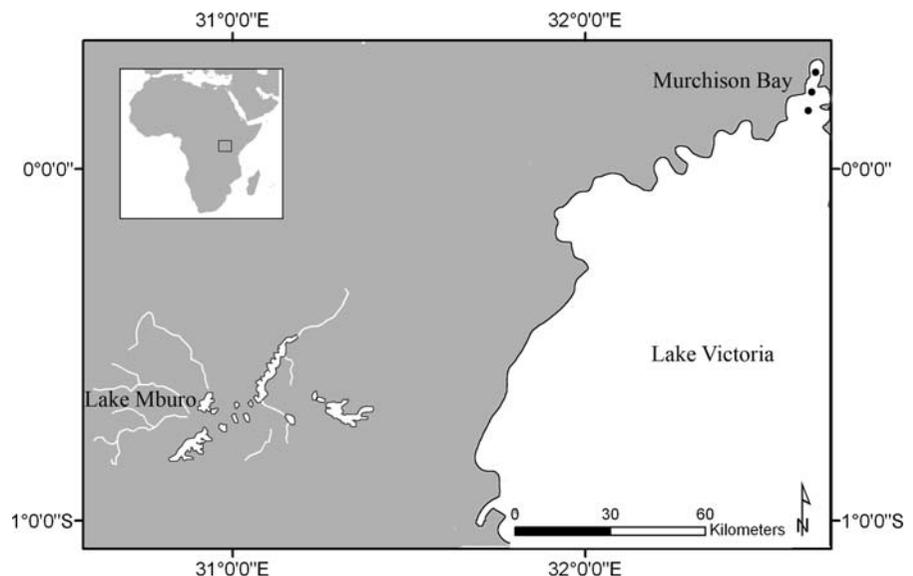
Murchison Bay is the north-eastern part of Lake Victoria. It is only 8 km east of Kampala, the capital city of Uganda, and lies between latitude 00°15′N–00°18′N and longitude 32°33′E–32°41′E at an altitude of 1,135 m. Fishing and water transport are the main economic activities in this bay. The main fisheries include Nile tilapia, Nile perch (*Lates niloticus*) and the Silver cyprinid (*Rastrineobola argentea*).

Environmental variables

In each study area, environmental variables were measured along a transect at three different locations

(Fig. 1). We measured water temperature and dissolved oxygen, using a hand-held dissolved oxygen meter (YSI 85, Yellow Spring Instruments) at 1 m depth intervals from the surface to the bottom. Water transparency was measured using a black and white Secchi disc ($\varnothing = 20$ cm). We filtered 100 ml of surface water using Whatman GF/C filters that were wrapped in aluminium foil and transported in an ice box to the laboratory for later chlorophyll analysis. Chlorophyll *a* was extracted using methanol and measurements made using a spectrophotometer (Hach DR 4000, Hach Co. USA), following the method by Lorenzen (1967). From an integrated water sample taken at each study site using a 5-l water column sampler (modified ramberg type), by sampling at every metre, 200 ml were placed into an acid washed bottle and 2 ml 4 M H₂SO₄ added to it. This was done for measurements of total phosphorus (TP), total nitrogen (TN) and for dissolved nutrients (orthophosphate (PO₄-P) and nitrate (NO₃-N)). In the laboratory nutrients (TP, TN, PO₄-P and NO₃-N) were determined using a spectrophotometer (Hach DR 4000, Hach Co., USA). Integrated water samples (100 cm³) were filtered through a GFC filter for measurement of particle bound microcystin concentration at each site. Filters were wrapped in aluminium foil and placed in an ice box, then transported to the laboratory and stored in a freezer. In the laboratory, filters were placed into an extraction vessel and the extraction agent (75% methanol and 25% distilled water) added

Fig. 1 Map of Uganda showing Murchison Bay Lake Victoria and Lake Mburo with some of the sampling sites (black circles—Murchison bay only. For Lake Mburo three sites were chosen along a transect covering the entire lake)



and shaken vigorously at room temperature for 30 min. The resulting extract was then decanted into an evaporation flask. The extracts were centrifuged at $500\times g$ to remove any particles. The extract was then kept at -18°C prior to analysis. Analysis of all samples for microcystins was done using liquid chromatography coupled with mass spectroscopy. Microcystins LR, YR and RR in phytoplankton extracts were quantified in positive ESI mode and single-ion monitoring using the ions 995.5, 1045.5 $[\text{M} + \text{H}]^{+}$ and 519.8 $[\text{M} + 2\text{H}]^{2+}$.

For both the study lakes, there is a short dry season (January–February) followed by a short wet season (March–May), a long dry season (June–August) and then a long wet season (September–December) every year. However, the main environmental variable is the amount of rainfall rather than the seasons per se.

Fish samples

Nile tilapia is a non-endemic species introduced into these lakes more than 50 years ago for fisheries purposes (Ogutu-Ohwayo, 1990; Balirwa, 1992; Bwanika et al., 2006). Nile tilapia samples were randomly obtained from fishermen's catches each month over a 1-year period. Three randomly selected fish were obtained from the fisherman's first catch of the day and transported in an insulated ice box to the laboratory within 12 h for analysis. In the laboratory, the fish were weighed (g) and measured (total length—cm), then dissected. The whole gut (including stomach and intestines with food content), the liver and 5 g of fish muscle were retained for microcystin analysis.

Lyophilised liver, gut, and muscle (5 g) samples were separately homogenised in a blender and extracted three times with a suitable volume of 75% methanol and 25% water to cover the samples. For each extraction step, the sample was sonicated for 30 min and all extracts of a sample pooled. The methanol extract was washed three times with an equal volume of n-hexane and the organic layer discarded after each wash. The washed extract was centrifuged for 5 min; the upper layer was transferred into a rotary evaporation flask (50 ml) and evaporated to dryness. The dry methanol extract was re-dissolved in 50% methanol and 50% water (2 ml), and then stored at -4°C prior to analysis.

Samples were then analysed using liquid chromatography coupled with mass spectroscopy for MCs content. The extracts were centrifuged at $12,000\times g$ for 10 min and transferred into 1.5 ml HPLC auto sampler vials. The instrumental setup included an ACQUITY UPLC system equipped with a Waters Atlantis C18 column (2.1×200 mm, particle size $5 \mu\text{m}$) set to deliver a gradient from 80% solvent A (water, 0.1% acetic acid; solvent B: acetonitrile, 0.1% acetic acid) to 40% solvent A within 10 min at a flow rate of 0.25 ml min^{-1} . The UPLC system was directly connected to a Quattro Premier XE MS/MS detector. Fish samples were analysed in multiple reaction mode using the 995.5, 1045.5 $[\text{M} + \text{H}]^{+}$ and 519.8 $[\text{M} + 2\text{H}]^{2+}$ as parent ions and the typical 135 Da Adda fragment as daughter ion. Mass spectroscopy settings were optimised with commercial standards for the three toxins (Sigma, Germany), which also served to calibrate the system.

Statistics

We ran a multiple regression analysis on the concentrations of microcystins in fish gut, muscle and liver tissue, using environmental variables as explanatory variables. Pearson's moment correlation was used to analyse correlations between environmental variables. A Wilcoxon rank sum test with continuity correction was used to test for seasonal differences in measured environmental variables and response variables. All statistics were done using R (version 2.8.1) (R Development Core Team, 2008).

Results

Environmental variables

In Lake Mburo, water temperature varied by up to 2°C during the time of the study. The higher temperatures ($>25^{\circ}\text{C}$) were recorded, during February (dry), May and October (wet) and lower temperatures ($<24^{\circ}\text{C}$) were recorded in January and July (dry). Differences of less than 1°C between the different depths on days of sampling suggested a well mixed water environment throughout the year. Mean seasonal water temperatures in Murchison Bay varied between 25 and 30°C . The lowest mean temperatures were recorded

in June–August (dry, 25.33°C) and highest in September–December (wet, 30.03°C). The water column in the Bay was more-or-less fully mixed throughout the year.

Mean percent saturation of dissolved oxygen (%DO) in Lake Mburo ranged from 59 to 103% at all depths. Only in December did the percent saturation drop below 60%. Mean dissolved oxygen concentration was higher during dry times than wet times in Lake Mburo ($W = 234$, $P < 0.01$). In Murchison Bay, mean levels of dissolved oxygen varied from 58% saturation in July to 78% saturation in February. For most of the year, DO saturation was above 60% at all depths and there was no significant difference between seasons ($P > 0.05$). Only in June–August did saturation drop below 20% at <1 m depth and near anoxic conditions (<2 mg l⁻¹) were recorded 1 m from the bottom of the water column.

The water transparency in both Lake Mburo and Murchison Bay was <1 m in all sampling months. In Lake Mburo, the mean water transparency was <0.5 m, in all sampling months, whereas in Murchison Bay the mean water transparency ranged from 0.3 to 0.9 m. We did not observe any seasonal differences in water transparency in either lake.

Both the highest and lowest mean surface chlorophyll *a* values were found during wet times in Lake Mburo (Fig. 2: 98.2 and 39.8 μg l⁻¹) and during dry times in Murchison Bay (Fig. 2: 37.47 and 28.5 μg l⁻¹). Although there were monthly differences in surface chlorophyll *a* values there were no significant seasonal differences of average chlorophyll *a* values in either Lake Mburo ($W = 54$, $P = 1$) or Murchison Bay ($W = 96$, $P = 0.3507$).

Measurements of nutrients in Murchison Bay and Lake Mburo (Table 1) show that mean total phosphorus (TP) values in both lakes were higher during the dry times (213.6 and 142.1 μg l⁻¹, respectively) than during the wet times (211.5, 141.8, respectively) but not significantly so ($W = 564$, P value = 0.5345; $W = 250.5$, P value = 0.6856, respectively). Mean P-PO₄ values in Murchison Bay were higher during the dry times (66.7 μg l⁻¹) than during the wet times (66.3 μg l⁻¹) these differences were, however, not significant ($W = 523.5$, P value = 0.927; $W = 253$, P value = 0.7252, respectively).

Mean values of total nitrogen (TN) were found to be higher during the wet times (450.8 and 495.2 μg l⁻¹, respectively) than during the dry times

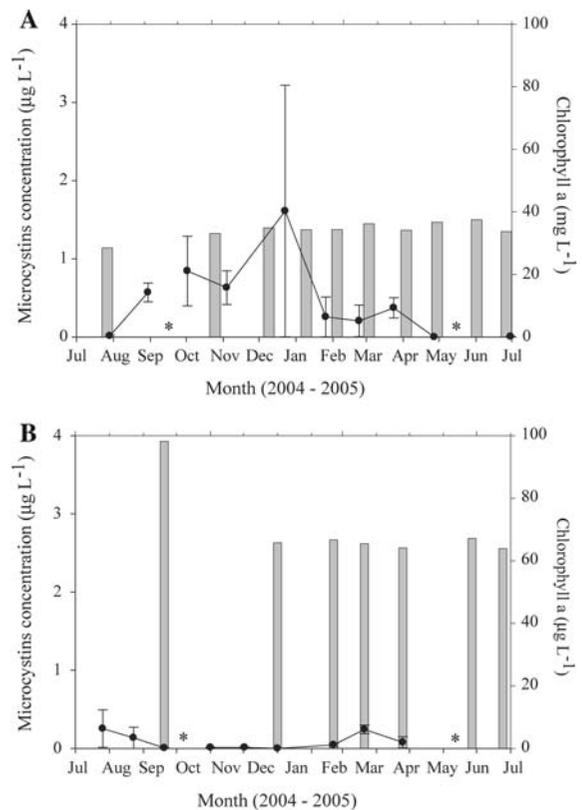


Fig. 2 Mean surface chlorophyll *a* concentration (μg l⁻¹, bars) and mean microcystin concentration (μg l⁻¹, closed circles ± SE) in water samples collected from Murchison Bay (A) and Lake Mburo (B) from July 2004 to July 2005 (* missing values)

(449.1 and 488.7 μg l⁻¹, respectively), however, we did not observe any significant seasonal differences in both Murchison Bay and Lake Mburo significant ($W = 487$, P value = 0.7094; $W = 246$, P value = 0.6167, respectively). Mean values of N-NO₃ in Murchison Bay and Lake Mburo were also higher during the wet times (16.8 and 29.7 μg l⁻¹, respectively) than during the dry times (15.9 and 27.8 μg l⁻¹, respectively). However, we did not find any significant seasonal differences ($W = 444.5$, P value = 0.3531; $W = 231.5$, P value = 0.4183, respectively).

Microcystins were detected in both lakes during wet and dry times (Fig. 2). In Lake Mburo, the highest mean level was recorded during the second dry season (0.26 μg l⁻¹) and the lowest mean level during the first dry season (0.006 μg l⁻¹), MC concentrations in the water were higher during dry times than during wet times ($W = 135$, $P < 0.05$). In Murchison Bay, the highest mean levels were recorded

Table 1 Mean values (\pm SD) of nutrients measured monthly from July 2004 to July 2005 in Murchison Bay and Lake Mburo

Variables	Jan–Feb (dry)	Mar–May (wet)	Jun–Aug (dry)	Sep–Dec (wet)
Murchison Bay (Lake Victoria)				
TP	209.8 \pm 5.9	202 \pm 7.2	215.5 \pm 7.8	216 \pm 9.3
PO ₄	62.7 \pm 2	69.9 \pm 2	68.8 \pm 3.5	64.7 \pm 5.5
TN	408.3 \pm 15.9			
NO ₃	14.1 \pm 0.3	17.3 \pm 0.4	16.8 \pm 1.2	16.51 \pm 2.6
Lake Mburo				
TP	142.5 \pm 8.7	136.2 \pm 8.1	142 \pm 3.8	143.2 \pm 3.3
PO ₄	32.3 \pm 4.3	28.1 \pm 0.9	29.4 \pm 2.3	31.2 \pm 3
TN	585.6 \pm 10.4	419.2 \pm 20	440.2 \pm 12	513 \pm 85.3
NO ₃	30.9 \pm 1.7	23.3 \pm 1.4	26.1 \pm 1.4	32.3 \pm 3

during the second wet season ($0.7 \mu\text{g l}^{-1}$) and the lowest values during the first wet season ($0.2 \mu\text{g l}^{-1}$), MC concentrations in the water were higher in wet times than in dry times ($W = 57$, $P < 0.05$).

Microcystins in gut

We found samples of Nile tilapia gut that contained high levels of MCs from both Lake Mburo and Murchison Bay (Fig. 3) with concentrations of >300 and $>390 \mu\text{g kg}^{-1}$ fw, respectively. MCs were found in the gut of all samples that could be successfully analysed (Fig. 3). The microcystin congeners RR, YR and LR were found in almost all of the analysed gut samples. In Murchison Bay, the average MC concentration in water was positively associated with MC concentration in the gut ($F = 6.0363$, $DF = 23$, $P = 0.02$). No other significant correlate was found between gut MC concentration and any other measured environmental variable. In Lake Mburo, we only found a non-significant trend towards increasing gut MC concentration with increasing water MC concentration ($F = 0.8$, $P = 0.38$) but no significant correlates were found for any of the other measured environmental variables. In Murchison Bay and Lake Mburo, MC levels were not significantly different during the wet times and dry times ($W = 80.5$, $P = 0.1481$ & $W = 135$, $P = 0.3466$, respectively).

Microcystins in muscle

MCs were present in samples of muscle tissue obtained from Lake Mburo at $<6 \mu\text{g kg}^{-1}$ fw (with the exception of two tissue samples from February

2005, which recorded values of 121.6 & $15.7 \mu\text{g kg}^{-1}$) and from Murchison Bay at $<5 \mu\text{g kg}^{-1}$ fw (Fig. 4). Levels of MCs in samples collected during November and December 2004 and July 2005 in Lake Mburo were beyond detection. The MC congener RR was the most prominent microcystin in Nile tilapia muscle tissue samples, accounting for $>70\%$ of detected microcystins in Lake Mburo and 52% in Murchison Bay. Microcystin congener YR was next prominent accounting for $>40\%$ in Murchison Bay and 16% in Lake Mburo, while microcystin congener LR was the least prominent accounting for $<20\%$ of detected MCs in both Lake Mburo and Murchison Bay. None of the measured environmental variables were significantly associated with the concentration of MCs in muscle tissue samples in either lake. There were no seasonal differences in microcystin concentrations in muscle tissue samples obtained from either lake.

Microcystins in liver

Measurements of MCs in the liver were infrequent due to the loss of samples especially from Lake Mburo (the acquisition of dry ice for sample storage was problematic due to frequent electrical power outages at the supplier). The results obtained showed that MC concentrations in fish livers from Murchison Bay (min = 0.00, mean = 7.68, max = 87.89, $n = 21$), did not differ significantly between seasons ($W = 94.5$, $P = 0.1519$). RR was present in all analysed liver samples making up 79.3% of the detected MCs, followed by YR in 50% of samples and 18% of the detected MCs. LR was in 60% of the samples and

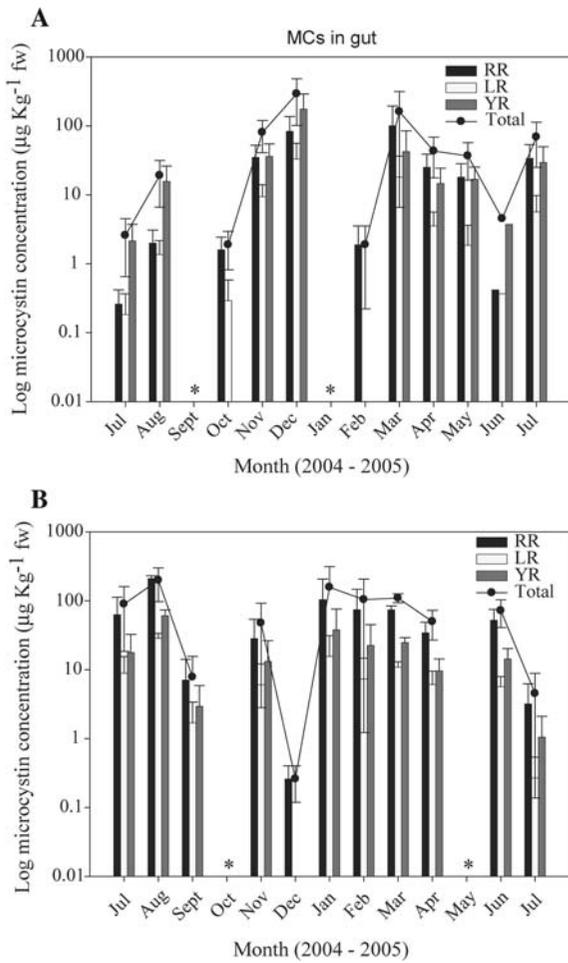


Fig. 3 Microcystins [RR, LR and YR— $\mu\text{g kg}^{-1}$ fw (fresh weight), +S.E; Total MC concentration ($\mu\text{g kg}^{-1}$ fw \pm S.E, closed circles)] extracted from gut samples ($n = 3$) of Nile tilapia in Murchison Bay (A) and Lake Mburo (B) from July 2004 to July 2005 (* missing values)

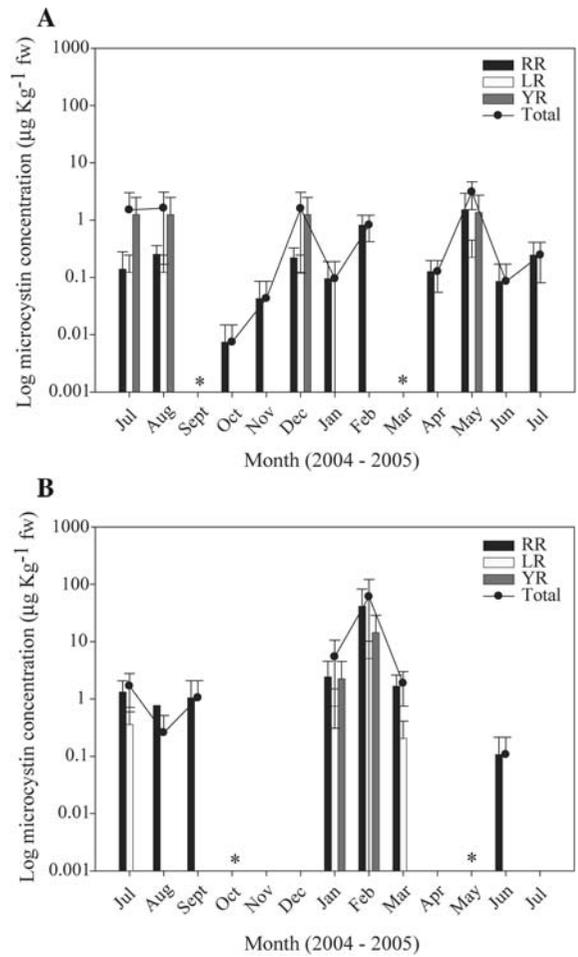


Fig. 4 Microcystins [RR, LR and YR— $\mu\text{g kg}^{-1}$ fw (fresh weight), +S.E; Total MC concentration ($\mu\text{g kg}^{-1}$ fw \pm S.E, closed circles)] extracted from muscle tissue samples (5 g, $n = 3$) of Nile tilapia in Murchison Bay (A) and Lake Mburo (B) from July 2004 to July 2005 (* missing values)

made up 2.5% of the detected MCs. None of the measured environmental variables were a good predictor of amount of MCs in fish liver in Murchison Bay. Not enough material was available from Lake Mburo for seasonal comparisons (min = 2.50, mean = 23.06, max = 56.10, $n = 3$), and only RR and LR were detected in the samples analysed making up 86.5 and 13.5% of the detected MCs, respectively.

Fish physiology

In Lake Mburo, the length (Min = 20.0, Mean = 24.0, Max = 30.4, $n = 33$) and weight (Min.

158.3, Mean = 283.4, Max = 640.0, $n = 33$) of Nile tilapia did not differ between seasons ($P > 0.05$), whereas in Murchison Bay we found that the length (Min = 19.0, Mean = 27.87, Max = 38.0, $n = 36$) and weight (Min = 43.96, Mean = 458.4, Max = 987.3, $n = 36$) of fish obtained during wet times were significantly higher than during dry times ($P < 0.05$). In Murchison Bay, fish weight showed a negative correlation with MC concentration in water ($r = -0.36$, $P = 0.05$). None of the other measured environmental variables were significantly correlated with the weight and length of fish in either lake.

Discussion

Our study shows that Nile tilapia from Lake Mburo and Murchison Bay ingests MCs during all months of the year. MCs were also detected in most of the analysed muscle and liver tissue samples, indicating that they are being assimilated by the fish. The level of MCs in the water in Murchison Bay was positively associated with the amount of MCs in the fish guts ($P < 0.05$), whereas this was not the case for Lake Mburo ($P > 0.05$). In Murchison Bay, the concentration of microcystins in the water was higher during wet times than dry times ($P < 0.01$), whereas MC concentrations were higher during dry times ($P < 0.01$) in Lake Mburo. None of the other measured environmental variables were a good predictor of levels of MCs in gut, muscle or liver tissue. Neither did we observe any seasonal differences in MC concentrations in gut and tissue samples from either lake.

Both the Murchison bay and Lake Mburo are eutrophic with a high level of nutrient loading (Kayiira, 2007; Haande, 2008; Okello et al., 2009, Table 1) with cyanobacteria contributing as much as 90% of the phytoplankton community in both Lake Mburo and Murchison Bay (Kayiira, 2007; Haande, 2008). Some studies have shown that production of oligopeptides like MCs can be positively associated with cyanobacteria biomass (Halstvedt et al., 2008), however, in the present study we do not observe any correlation between either chlorophyll *a* or measured nutrients and MC concentration in water. MC concentrations were more-or-less comparable in both lakes ($P > 0.05$). The cyanobacteria *Microcystis* spp., which may constitute >30% of the phytoplankton community in Murchison Bay and Lake Mburo (Kayiira, 2007; Haande, 2008), are the most likely source of MCs detected in Murchison Bay (Haande et al., 2007) and probably Lake Mburo as well. Studies on tilapia diets in Ugandan lakes have shown that cyanobacteria, especially of the genus *Microcystis* and *Anabaena* can constitute >50% of ingested phytoplankton (Bwanika et al., 2004; Nagayi-Yawe et al., 2006; Semyalo, 2009). An increase in MCs producing cyanobacteria in lakes may result in higher ingestion of microcystins by the filter feeding Nile tilapia, but, transfer or accumulation of microcystins in muscles may vary, depending on the MC variant and study area (Deblois et al., 2008). In the present study, we did not find any correlation between the

concentration of microcystins in fish (gut and muscles) and level of chlorophyll *a* in either lake.

Cell-bound MCs are more of a concern than free MCs because the main uptake route of MCs in fish is through the ingestion of MCs containing cells (Tencalla et al., 1994). Exposure of fish to MCs has been shown to affect osmo-regulation (Gaete et al., 1994; Wiegand & Pflugmacher, 2005), increase liver enzyme activities in the serum and heart rate (Gupta & Guha, 2006), modify behaviour (Baganz et al., 2004), and exert histopathological effects in the liver, intestine, kidneys, heart, spleen or gills (Zurawell et al., 2005). In the present study, none of these effects were investigated. Likewise, our investigations did not find any relationships between the fish size (length and weight) and the concentration of MCs in the gut, liver or muscles. Our study revealed that the levels of MCs in the muscle tissue were ~1% of that measured in the guts of fish from Murchison Bay and up to 30% in Lake Mburo fish. The accumulation of microcystins in muscle has been shown to vary regardless of concentrations measured in water (Mohamed et al., 2003; Deblois et al., 2008), due to depuration processes (Mohamed & Hussein, 2006). Levels of MCs were highest in the gut followed by liver and then the muscle tissue. This indicates that although ingested MCs (as shown in the gut) may finally accumulate in muscles, these levels are significantly lower than the amount ingested. Therefore, it is possible that either ingested MCs containing cells pass through the gut undamaged (Lewin et al., 2003) or that fish are able to get rid of assimilated MCs physiologically through processes like bile excretion (Sahin et al., 1996). The latter is very likely the case for Nile tilapia (Mohamed & Hussein, 2006), some studies, however, shown that microcystins mostly accumulate in the Liver of the Nile tilapia (Zhao et al., 2006).

Cyanobacteria can pass through fish gut undigested (Lewin et al., 2003). However, Nile tilapia possesses a long gut that may allow the effective digestion of cyanobacterial cells (Moriarty, 1973). This will likely result in the release of cell-bound microcystins which could easily be assimilated. Once taken in MCs tend to accumulate quickly in liver cells and muscle tissue (Magalhaes et al., 2003; Wiegand & Pflugmacher, 2005; Deblois et al., 2008).

For human risk assessment, the Total Daily Intake (TDI) for microcystin-LR is 0.04 µg/kg BW per day (Carmichael, 2001). In areas prone to algal blooms

and microcystin production this recommendation can only be enforced if there is a continuous monitoring programme.

Despite reports of the presence of microcystin-producing algae in the Lake Victoria (Ochumba & Kibaara, 1989) and algal bloom related fish kills (Ogutu-Ohwayo, 1990), only a few studies have investigated microcystin production and its potential for production (Krienitz et al., 2002; Sekadende et al., 2005; Haande et al., 2007). To our knowledge, there are no published studies on bioaccumulation in zooplankton, macrobenthos or fish either in Lake Victoria or Lake Mburo. Due to our low sample size and short sampling period, our results can only serve to highlight the potential risk of microcystin bioaccumulation in Nile tilapia in these lakes. Further studies are needed for the purpose of risk assessment.

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