Spatial isolation favours the divergence in microcystin net production by *Microcystis* in Ugandan freshwater lakes

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**Abstract**

It is generally agreed that the hepatotoxic microcystins (MCs) are the most abundant toxins produced by cyanobacteria in freshwater. In various freshwater lakes in East Africa MC-producing *Microcystis* has been reported to dominate the phytoplankton, however the regulation of MC production is poorly understood. From May 2007 to April 2008 the *Microcystis* abundance, the absolute and relative abundance of the \textit{mcyB} genotype indicative of MC production and the MC concentrations were recorded monthly in five freshwater lakes in Uganda: (1) in a crater lake (Lake Saka), (2) in three shallow lakes (Lake Mburo, George, Edward), (3) in Lake Victoria (Murchison Bay, Napoleon Gulf). During the whole study period *Microcystis* was abundant or dominated the phytoplankton. In all samples \textit{mcyB}-containing cells of *Microcystis* were found and on average comprised 20±2\% (SE) of the total population. The proportion of the \textit{mcyB} genotype differed significantly between the sampling sites, and while the highest \textit{mcyB} proportions were recorded in Lake Saka (37±3\%), the lowest proportion was recorded in Lake George (1.4±0.2\%). Consequently *Microcystis* from Lake George had the lowest MC cell quotas (0.03–1.24 fg cell\(^{-1}\)) and resulted in the lowest MC concentrations (0–0.5 m\(\mu\)g L\(^{-1}\)) while *Microcystis* from Lake Saka consistently showed maximum MC cell quotas (14–144 fg cell\(^{-1}\)) and the highest MC concentrations (0.5–10.2 m\(\mu\)g L\(^{-1}\)). Over the whole study period the average MC content per *Microcystis* cell depended linearly on the proportion of the \textit{mcyB} genotype of *Microcystis*. It is concluded that *Microcystis* populations differ consistently and independently of the season in \textit{mcyB} genotype proportion between lakes resulting in population-specific differences in the average MC content per cell.

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1. Introduction

During the last decades cyanobacteria in freshwater have been of interest due to their ability to produce various hepatotoxic and neurotoxic substances. It is generally agreed that the hepatotoxic microcystins (MCs) are the most abundant toxins produced by cyanobacteria in freshwater (WHO, 2006; Erdner et al., 2008; Hudnell, 2008). MCs are cyclic...
heptapeptides that share the common structure cyclo\((d-Ala^{1}-X^{2}-d-MeAsp^{3}-Z^{6}-Adda^{9}-d-Glu^{10}-Mdha^{13})\), where \(X\) and \(Z\) are variable \(\alpha\)-amino acids (e.g. MC-LR refers to leucine and arginine in the variable positions of this peptide), \(d-MeAsp\) is \(d\)-erythro-\(\beta\)-iso-methyl-aspartic acid, Adda is \(2S, 3S, 8S, 9S\)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldec-4,6-dienoic acid, and Mdha is \(N\)-methyl-dehydroalanine. Structural variation has been reported most frequently in positions 2, 4, and 7 of the MC molecule resulting in over 80 structural variants that have been characterized from field samples or isolated strains (Krüger et al., 2009). In a recent paper, we could show that cyanobacteria contribute significantly to the phytoplankton of freshwater lakes in Uganda while other algal groups like diatoms, green algae, and cryptomonads are of a relatively minor importance (Okello et al., 2009). We further concluded that in Uganda the genus Microcystis is the only MC-producing genus which isfavoured under more shallow, eutrophic conditions which is in correspondence to the general theory on how physical factors govern phytoplankton associations (Reynolds et al., 2002). In this earlier study we did not monitor Microcystis populations as well as the MC net production of the phytoplankton community during different seasons. This is of relevance as it is known that beside the absolute population abundance it is the proportion of MC-producing genotypes (those containing the mcy gene cluster encoding MC synthesis) vs. non-MC-producing genotypes (those lacking the mcy gene cluster) that has a decisive influence on MC net production (Sivonen and Jones, 1999; Kurmayer and Christiansen, 2009). So far research was unable to document an adaptive value of MC production, thus making it difficult to identify proximate factors triggering MC synthesis (Kaebernick and Neilan, 2001; Schatz et al., 2007). Although the transcription rate of the mcy gene cluster is increased from low to high irradiance conditions it is generally agreed that MC synthesis is constitutive (Kaebernick and Neilan, 2001). In the course of seasonal studies several researchers reported the occurrence of seasonal shifts in the proportion of MC-producing vs. non-MC-producing genotypes in dependence on various abiotic or biotic environmental factors (Briand et al., 2008; Hottol et al., 2008). In East Africa usually dry seasons with precipitation minima and wet seasons with maxima of precipitation have been correlated with changes in phytoplankton composition (Talling, 1986). During the rainy season (from March to March and August–November), the phytoplankton in shallow lakes will be affected directly by a reduced water temperature (2.5 °C in Kasene and 3 °C in Kampala), reduced light availability in the water column as well as increased terrestrial run-off. Only in deep lakes such as the main basin of Lake Victoria, the mixing regime will change, as a higher stability of the water column has been described during the dry season (Talling, 1986). These physical changes in the water column have a significant effect on phytoplankton community composition (Talling, 1987). Generally in shallow lakes less seasonality in phytoplankton composition is observed when compared with the main basin of Lake Victoria.

In contrast to the seasonal influence we recently emphasized that it is rather the spatial isolation of populations than the seasonal influence of biotic or abiotic factors that leads to differences in the population structure of MC-producing genotypes (Kurmayer and Gumpenberger, 2006). We further concluded that the structural variation within the position 2 of the MC molecule is selectively neutral (Kurmayer and Gumpenberger, 2006) implying that genetic drift resulting from geographic isolation has the potential to lead to new MC variants that appear unique and dominant in particular waterbodies, i.e. \([Asp^{3}, Dhb^{7}]\)-MC-HtyY and \([Asp^{3}, Dhb^{7}]\)-MC-HtyHty (Christiansen et al., 2008a). For lakes located in the Alps of Austria, Germany and Switzerland we hypothesized that populations diverge in their mcy genotype composition at least during consecutive years (Ostermaier and Kurmayer, 2009). However, we also observed that the genetic population structure changed between years, for example due to the extinction of the population during winter and a subsequent re-immigration of new genotypes (Kurmayer and Gumpenberger, 2006).

In this study we aimed to investigate the seasonal development of Microcystis and potential MC-producing genotypes and the resulting MC concentrations in five freshwater lakes in Uganda. Microcystis has been reported to dominate in shallow eutrophic lakes such as Lake George at least for decades (Ganf, 1974). While the shallow lakes close to the Ruwenzori mountain (L. George, L. Edward, L. Mburo) are naturally eutrophic, the genus Microcystis also has become abundant in bays of Lake Victoria as a result of human induced eutrophication (Hecky, 1993; Mugidde, 1993; Verschuren et al., 2002). It is hypothesized that if spatial isolation leads to genetic divergence in MC production then the variation in mcy genotype proportion between sites should significantly exceed the variation in mcy genotype proportion that is observed within sites during the season. Vice versa if spatial isolation is of minor importance, the seasonal variation in mcy genotype proportion as caused by unknown biotic and abiotic factors that is observed within sites should significantly exceed the site variation.

2. Materials and methods

2.1. Description of the study sites

From five freshwater lakes in Uganda six sampling sites were chosen (Fig. 1): (1) A site in the center of Lake Saka (NO ’41.670\(^{0}\), E30’14.667\(^{0}\)), mean depth of 3.6 m. Lake Saka is a small crater lake (1.4 km\(^{2}\)) located at an altitude of 1520 m.a.s.l. The other sampling sites included the shallow eutrophic lakes (2) Lake George, (3) Lake Edward, (4) Lake Mbuuro as well as Lake Victoria, (5) Murchison Bay near Kampala, and (6) Napoleon Gulf near Jinja, which were described previously (Okello et al., 2009).

2.2. Field sampling and analysis of Microcystis abundance

Depth-integrated water samples and plankton net samples (30 µm mesh size) were taken monthly from May 2007 until April 2008 as described (Okello et al., 2009). For DNA and MC analysis aliquots (250–2400 ml) were filtered onto GF/C filters (2 47 mm, Whatman, Kent, Great Britain) and the filters were dried at 50 °C. Microcystis were counted by the inverted
microscope technique from Lugol fixed samples following standard techniques (Wetzel and Likens, 2000). The genus Microcystis was discriminated from other cyanobacteria according to Komárek and Anagnostidis (1999). Following the taxonomic revision for the genus Microcystis Küting ex Lemmerman 1907 (Otsuka et al., 2001) all morphospecies were considered morphological varieties of individuals of Microcystis aeruginosa comb. nov. Küting 1833. For each sample 400 specimens of Microcystis and other dominant phytoplankton genera were counted at 400-fold magnification. Only the data on Microcystis cell numbers and Microcystis biovolume are reported here while the phytoplankton community composition will be described in another publication. The average biovolume of a Microcystis cell was 76 ± 12 μm³ (1SE).

2.3. Quantification of the microcystin genotype

To estimate the abundance and the proportion of the MC-producing genotype, DNA was extracted from aliquots of the samples analyzed for Microcystis cell numbers and for MC as described previously (Kurmayer et al., 2003). The absolute and relative cell numbers of Microcystis and the Microcystis genotype containing the mcyB gene that is indicative of MC production were determined by means of quantitative real-time PCR as described (Kurmayer and Kutzenberger, 2003) and the same primers and probes have been used. To quantify the total population of Microcystis the linear regression was $y = -3.4552x + 36.229$ ($n = 6$, $R^2 = 0.998$), where $y$ was the cycle threshold ($C_t$ value) at the set fluorescence threshold level obtained for the mcyB gene and $x$ was the amount of starting DNA aliquots used for PC (given as log10 cell number equivalents of Microcystis strain HUB524). To quantify the Microcystis cells containing the mcyB gene only the linear regression curve was $y = -3.9759x + 40.712$ ($n = 6$, $R^2 = 0.994$), where $y$ was the $C_t$ value at the set fluorescence threshold level obtained for the mcyB gene and $x$ was the amount of starting DNA aliquots used for PC (given as log10 cell number equivalents of Microcystis strain HUB524). The relative abundance of the mcyB genotype of Microcystis was determined by dividing the cell numbers of the mcyB genotype through the cell numbers of the total population (as estimated from the PC genotype). All measurements were done in triplicate using an Eppendorf mastercycler ep realplex system (Eppendorf, Vienna). Both gene regions that were amplified by PCR have been shown to be specific for Microcystis in the presence of other MC-producing cyanobacteria such as Anabaena or Planktothrix (Kurmayer and Kutzenberger, 2003).

In order to include the whole study period all depth-integrated samples from all six sampling sites ($n = 72$) as well as plankton net samples from Lake George ($n = 4$) and Lake Mbuuro ($n = 4$) were analyzed.

2.4. Microcystin analysis

Phytoplankton collected on filter was extracted in aqueous methanol as described (Okello et al., 2009). The clear supernatants of extracts were analyzed by high performance liquid chromatography-diode array detection (HPLC-DAD) as described using a HP1100 Chemstation (Lawton et al., 1994; Kurmayer et al., 2003). MCs were quantified at 240 nm and the concentration of all MC variants was determined as concentration equivalents of [MeAsp, Mdha]-MC-LR (Cyanobiotec GmbH, Berlin, Germany). The concentration of MC-LR was calculated from the regression curve $y = 1885.3x - 6.8775$, ($R^2 = 0.99$), where $y$ was the absorption (mAU) recorded at 240 nm and $x$ was the μg of MC-LR injected.

HPLC fractions identified as MC were collected manually and analyzed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), (PerSepBioSystems, Framingham MS, USA) as described (Erhard et al., 1997). The constitution of the new MC variant [NMeSer7]-MC-YR was assigned by ESI-MS and ESI-MS² experiments that were performed on a Q-TOF Ultima mass spectrometer (Waters, Milford, MA.) equipped with a nanospray source and operated in the positive ionization mode under the control of MassLynx 4.1.

2.5. Statistical analysis

The linear regression curves were fitted using the least square approximation and the associated statistical tests of Sigma Plot 2000 (V 6.10). The data were log10 transformed in order to achieve normal distribution and constant variances. The linear regressions between the total Microcystis cell number (as estimated from the microscope) and the abundance of the PC genotype and the mcyB genotype were compared in slope and intercept using a general factorial model of analysis of variance (ANOVA). The data were modeled as $y = \mu + \pi x + \epsilon$, where $y$ is the measured abundance of the PC genotype or the mcyB genotype, $\mu$ is the overall mean level, $\pi$ is the effect of the PC or the mcyB genotype, $x$ is the effect of the cell number as determined from the microscope as a covariate, and $\epsilon$ is the
random sites. To compare the mcyB proportions between sampling sites one-way ANOVA was used.

Multiple linear regression analysis was used to test the relationship between MC concentration (in µg MC·ml⁻¹) as dependent variable and the influence of Microcystis cell numbers as determined by the microscope or real-time PCR via PC and mcyB as independent variables. A forward stepwise analysis was employed selecting for the independent variable for inclusion that makes the most significant unique contribution to the prediction of the data. Calculations were performed using SPSS 15.0 for Windows and the F value to enter the respective model was set default (p < 0.05).

3. Results

3.1. Microcystis abundance

Microcystis appeared throughout the sampling period in all the lakes. Only on 30 September 2007 Microcystis abundance was below the detection limit in the depth-integrated sample obtained from Lake Victoria (Napoleon Gulf). During the study period Microcystis cell numbers varied between 10⁴–10⁶ (Lake Saka), 10⁵–10⁶ (L. George), 10⁴–10⁵ (L. Edward), 10⁴–10⁶ (L. Mburo), 10⁴–10⁵ (L. Victoria, Murchison bay), and 10⁴–10⁶ cells ml⁻¹ (L. Victoria, Napoleon Gulf). We recorded the highest Microcystis biovolume in samples from Lake George (273 mm³·l⁻¹) and the lowest (0.3 mm³·l⁻¹) in samples from Lake Victoria (Napoleon Gulf). On average Microcystis constituted between 18 and 46% of the total phytoplankton biovolume (Table 1). This implied that the phytoplankton at all the sampling sites had the potential for MC production.

3.2. Quantification of the microcystin genotype

In order to test the hypothesis that populations of Microcystis differ in the proportion of the mcyB genotype, the absolute abundance of both the total population as well as the subpopulation containing the mcyB gene were determined by means of real-time PCR. Overall, both the microscope as well as real-time PCR showed congruent results in estimating the total Microcystis population number that on average differed by one order of magnitude in lakes George and Edward, and less than an order of magnitude in the other lakes (Fig. 2). Consequently over the study period a linear relationship between cell numbers estimated via the microscope and real-time PCR of the PC genotype was found (Fig. 3A): The regression curve was $y = 0.595x + 1.661$ (n = 80, R² = 0.62), where y is the log₁₀ cell number as determined by the real-time PCR (PC) and x is the log₁₀ cell number as counted in the microscope.

In all samples mcyB-containing cells of Microcystis were found and on average comprised 19.9 ± 1.8 (SE) % of the total population (min = 0.5, max = 66.6%). In contrast to PC a relatively weak relationship between cell numbers as counted in the microscope and cell numbers of the mcyB genotype was found (Fig. 3B): $y = 0.322x + 2.1935$ (n = 80, R² = 0.13) and the two regression curves were not parallel (ANOVA, p < 0.001). Particularly the measurements obtained from Lake Saka and Lake George showed a much wider scatter when compared with the measurements on the PC genotype. The proportion of the mcyB genotype differed significantly between the sampling sites (Fig. 3C): While the highest mcyB proportions were recorded in Lake Saka (min – mean = max, 24.4 – 37.0 = 3.3 – 66.6%) and in Napoleon Gulf (12.9 – 31.6 ± 5.8 – 59.3%), the by far lowest proportion was recorded in Lake George (0.49 – 1.4 ± 0.2 – 3.6%). At the other three sites, Lake Edward (7.5 – 14.9 ± 2.8 – 36.7%), Lake Mburo (6.1 – 14.0 ± 2.2 – 32.3%), and Murchison Bay (3.3 – 16.4 ± 3.1 – 39.2%) an intermediate proportion was recorded. It is concluded that the significant differences in the proportion of the mcyB genotype between the lakes resulted in a poor correlation between microscopically determined cell numbers and the cell numbers of the mcyB genotype.

### Table 1 – Cell numbers (min – mean ± SE – max, cells ml⁻¹), biovolume (mm³·l⁻¹) and proportion (%) of Microcystis (in total phytoplankton) in depth-integrated samples from the six sampling sites from May 2007 to April 2008 (n = 12).

<table>
<thead>
<tr>
<th>Cell numbers</th>
<th>Biovolume</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Saka</td>
<td>10⁴–10⁶</td>
<td>3.3 ± 10⁴</td>
</tr>
<tr>
<td>Lake George</td>
<td>10⁴–10⁶</td>
<td>3.3 ± 10⁴</td>
</tr>
<tr>
<td>Lake Edward</td>
<td>10⁴–10⁶</td>
<td>3.3 ± 10⁴</td>
</tr>
<tr>
<td>Lake Mburo</td>
<td>10³–10⁵</td>
<td>3.3 ± 10⁵</td>
</tr>
<tr>
<td>Murchison Bay</td>
<td>10³–10⁵</td>
<td>3.3 ± 10⁵</td>
</tr>
<tr>
<td>Napoleon Gulf</td>
<td>10³–10⁴</td>
<td>3.3 ± 10⁴</td>
</tr>
</tbody>
</table>

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The sampling sites differed significantly in the relative abundance of all the MC variants (Chi square test, p < 0.01). For example MC-RR and [Asp\(^3\)]-MC-RR were most frequent in lakes Saka, Mburo and Edward and in Murchison Bay. In contrast MC-RY and [Asp\(^3\)]-RY were dominant at all sites except in Napoleon Gulf (Table 2). Surprisingly in the samples from Napoleon Gulf the new [NMeSer\(^7\)]-MC-YR variant occurred most frequently. Taking all sampling sites together MC-RY was most abundant, followed by MC-RR and MC-YR. In contrast MC-LR only occurred in 11% of all the samples.

3.3.2. Concentration of microcystins
The contribution of each MC variant to the total MC concentration (calculated as MC-LR equivalents) closely matched the frequency of occurrence. For example, MC-RR contributed on average ≥50% to the total MC in lakes Saka and Mburo. MC-RY contributed >50% to the total MC in lakes George and Edward and in Murchison Bay. All MC detected in Napoleon Gulf was dominated by the [NMeSer\(^7\)]-MC-YR variant (Table 2). The phytoplankton further differed significantly in the concentration of MC in total (p < 0.0001, ANOVA). On average, the MC concentrations were 28-fold higher in Lake Saka (4.7 ± 0.9 \(\mu\)gL\(^{-1}\)) when compared with the average (0.2 ± 0.1 \(\mu\)gL\(^{-1}\)) MC concentration measured in Lake George (Fig. 4). Samples from Lake Saka had the maximum MC concentration (10 \(\mu\)gL\(^{-1}\)) in July 2007. The minimum concentrations (0.02 \(\mu\)gL\(^{-1}\)) were recorded in Lake George in May 07, June 07, January 08 and April 08. At the sampling sites in the other three lakes intermediate MC concentrations (0.1–2.5 \(\mu\)gL\(^{-1}\)) were recorded. Within the lakes, the total MC concentration varied seasonally from 12-fold to 30-fold.

3.4. Genotype determined microcystin production
3.4.1. Relation of the PC and mcyB genotypes to microcystin concentrations
Both PC and mcyB genotype cell numbers were highly significantly related to MC concentrations: \(y = 1.33 \times 10^{-8} + 0.000195 (R^2 = 0.28)\) for the PC genotype and \(y = 6.16 \times 10^{-8} + 0.000385 (R^2 = 0.62)\) for the mcyB genotype. With the exception of Lake George the cells of the mcyB genotype showed an increase of the
average MC content by a factor of 2.9–7.8 when compared with the MC content of the PC genotype (Fig. 5A). Corresponding to the lowest mcyB genotype proportion Microcystis from Lake George consistently showed the lowest MC cell quotas (0.03–1.24 fg cell−1) while Microcystis from Lake Saka showed maximum MC cell contents (14–144 fg cell−1). While the between site variation was found reduced in plankton net samples the ranking of sampling sites by their average MC contents per Microcystis cell was not affected (data not shown). It is concluded that at all sites MC production was related to the occurrence of Microcystis as enumerated in the microscope while between sites the populations differ consistently and independently of the season in their average MC content per cell.

3.4.3. Relation of the mcyB genotype proportion to the microcystin content

Over the study period the proportion of the mcyB genotype was linearly related to the average cellular MC content per cell: y = 1.2884x − 0.7835 (n = 77, R² = 0.58), where x is the log₁₀ proportion of the mcyB genotype and y is the log₁₀ MC content in fg MC cell⁻¹ (Fig. 5C). In order to explain MC concentrations the forward multiple regression analysis revealed a significant inclusion of the mcyB genotype abundance as the first and most significant predictor variable (R² = 0.68) and subsequently the microscopically determined Microcystis cell numbers as the second predictor variable (R² = 0.73): y = 0.862x + 0.256z − 7.805 (R² = 0.73, n = 77), where x is the log₁₀ abundance of the mcyB genotype (cells ml⁻¹), z is the log₁₀ cell number (ml⁻¹) determined in the microscope and y is the log₁₀ MC concentration (µg ml⁻¹). It is concluded that the Microcystis populations differ genetically in the mcyB proportion which can indeed explain the variation in the average MC content of Microcystis cells observed among the lakes during the study period.

4. Discussion

4.1. Correlation of Microcystis cell numbers with microcystin net production

For all lakes the abundance of Microcystis cells was significantly positively related to MC production. In contrast MC production was negatively related to the abundance of Planktothrix in Lake Saka and to the abundance of Anabaena in Lake Victoria in Napoleon Gulf and Murchison Bay. Significant relationships between the total MC concentration and Anabaena cell numbers were observed for the sites in lakes Mbuuro, Murchison Bay and Napoleon Gulf (data not shown). However, as we were unable to detect genes involved in MC production of any other taxa than Microcystis in the same habitats (Okello et al., 2009), we consider this relationship as due to the co-occurrence of these taxa and Microcystis (Okello et al., 2009). In addition nine strains of Planktothrix sp. were isolated from Lake Saka in April 2008 and analyzed for MC production. None of the strains were found to contain MCs and/or the mcyE/ mcyB gene part of the mcy gene cluster (Rainer Kurmayer, unpublished results).
Table 2 – Relative frequency of occurrence (%) and proportion (mean ± SE) in HPLC chromatograms of each microcystin variant in the depth-integrated and the plankton net samples at the six sampling sites from May 2007 to April 2008 (n = 24). For each site the most abundant MC variant is marked in Bold.

<table>
<thead>
<tr>
<th>M + H⁺ Structure variant</th>
<th>MC1</th>
<th>MC2</th>
<th>MC3</th>
<th>MC4</th>
<th>MC5</th>
<th>MC6</th>
<th>MC7</th>
<th>MC8</th>
<th>MC9</th>
<th>MC10</th>
<th>MC11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>1024</td>
<td>1038</td>
<td>1063</td>
<td>1031</td>
<td>1045</td>
<td>995</td>
<td>1013</td>
<td>1031</td>
<td>1045</td>
<td>1024</td>
<td>1031</td>
</tr>
<tr>
<td>[Asp³]MC-RR</td>
<td>14–14.5</td>
<td>15.1–15.7</td>
<td>16.0–16.4</td>
<td>17.0–17.7</td>
<td>18.0–18.9</td>
<td>19.0–19.9</td>
<td>20.9</td>
<td>21.0–21.8</td>
<td>23.0–23.9</td>
<td>24.0–25.0</td>
<td>27.8–27.9</td>
</tr>
<tr>
<td>[NMeSer⁷]-MC-YR</td>
<td>17</td>
<td>67</td>
<td>63</td>
<td>0</td>
<td>67</td>
<td>83</td>
<td>17</td>
<td>0</td>
<td>17</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>[Asp³]MC-YR</td>
<td>0.8 ± 0.6</td>
<td>58.7 ± 4.1</td>
<td>3.2 ± 2</td>
<td>0.4 ± 0.2</td>
<td>17.3 ± 3.5</td>
<td>4.4 ± 1.3</td>
<td>8.0 ± 2.8</td>
<td>7.0 ± 1.3</td>
<td>0.1 ± 0.07</td>
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<tr>
<td>MC-LR</td>
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<td>0.3 ± 0.1</td>
<td>1.8 ± 0.5</td>
<td>0.03 ± 0.0</td>
<td>5.9 ± 4.3</td>
<td>81.1 ± 4.5</td>
<td>1.0 ± 0.2</td>
<td>0.3 ± 0.1</td>
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<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>25</td>
<td>67</td>
<td>0</td>
<td>38</td>
<td>46</td>
<td>4</td>
<td>0</td>
<td>13</td>
<td>96</td>
<td>50</td>
<td>33</td>
</tr>
<tr>
<td>Lake Edward</td>
<td>0.6 ± 0.2</td>
<td>8.3 ± 1.7</td>
<td>0.3 ± 0.1</td>
<td>1.8 ± 0.5</td>
<td>0.03 ± 0.0</td>
<td>5.9 ± 4.3</td>
<td>81.1 ± 4.5</td>
<td>1.0 ± 0.2</td>
<td>0.3 ± 0.1</td>
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<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>0.6 ± 0.2</td>
<td>8.3 ± 1.7</td>
<td>0.3 ± 0.1</td>
<td>1.8 ± 0.5</td>
<td>0.03 ± 0.0</td>
<td>5.9 ± 4.3</td>
<td>81.1 ± 4.5</td>
<td>1.0 ± 0.2</td>
<td>0.3 ± 0.1</td>
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<td></td>
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<tr>
<td>Proportion</td>
<td>58</td>
<td>96</td>
<td>0</td>
<td>54</td>
<td>92</td>
<td>58</td>
<td>67</td>
<td>79</td>
<td>100</td>
<td>17</td>
<td>54</td>
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<tr>
<td>Lake Mburo</td>
<td>2.6 ± 0.9</td>
<td>46.9 ± 2.8</td>
<td>0.8 ± 0.2</td>
<td>12.9 ± 1.5</td>
<td>1.6 ± 0.4</td>
<td>1.8 ± 0.4</td>
<td>2.2 ± 0.5</td>
<td>27.7 ± 3.5</td>
<td>2.6 ± 0.4</td>
<td>0.4 ± 0.1</td>
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<tr>
<td>Frequency</td>
<td>0.6 ± 0.2</td>
<td>8.3 ± 1.7</td>
<td>0.3 ± 0.1</td>
<td>1.8 ± 0.5</td>
<td>0.03 ± 0.0</td>
<td>5.9 ± 4.3</td>
<td>81.1 ± 4.5</td>
<td>1.0 ± 0.2</td>
<td>0.3 ± 0.1</td>
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<tr>
<td>Proportion</td>
<td>58</td>
<td>96</td>
<td>0</td>
<td>54</td>
<td>92</td>
<td>58</td>
<td>67</td>
<td>79</td>
<td>100</td>
<td>17</td>
<td>54</td>
</tr>
<tr>
<td>Murchison Bay</td>
<td>2.6 ± 0.9</td>
<td>46.9 ± 2.8</td>
<td>0.8 ± 0.2</td>
<td>12.9 ± 1.5</td>
<td>1.6 ± 0.4</td>
<td>1.8 ± 0.4</td>
<td>2.2 ± 0.5</td>
<td>27.7 ± 3.5</td>
<td>2.6 ± 0.4</td>
<td>0.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>0.6 ± 0.2</td>
<td>8.3 ± 1.7</td>
<td>0.3 ± 0.1</td>
<td>1.8 ± 0.5</td>
<td>0.03 ± 0.0</td>
<td>5.9 ± 4.3</td>
<td>81.1 ± 4.5</td>
<td>1.0 ± 0.2</td>
<td>0.3 ± 0.1</td>
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<tr>
<td>Proportion</td>
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<td>0</td>
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<td>58</td>
<td>67</td>
<td>79</td>
<td>100</td>
<td>17</td>
<td>54</td>
</tr>
<tr>
<td>Napoleon Gulf</td>
<td>2.6 ± 0.9</td>
<td>46.9 ± 2.8</td>
<td>0.8 ± 0.2</td>
<td>12.9 ± 1.5</td>
<td>1.6 ± 0.4</td>
<td>1.8 ± 0.4</td>
<td>2.2 ± 0.5</td>
<td>27.7 ± 3.5</td>
<td>2.6 ± 0.4</td>
<td>0.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td>0.6 ± 0.2</td>
<td>8.3 ± 1.7</td>
<td>0.3 ± 0.1</td>
<td>1.8 ± 0.5</td>
<td>0.03 ± 0.0</td>
<td>5.9 ± 4.3</td>
<td>81.1 ± 4.5</td>
<td>1.0 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion</td>
<td>58</td>
<td>96</td>
<td>0</td>
<td>54</td>
<td>92</td>
<td>58</td>
<td>67</td>
<td>79</td>
<td>100</td>
<td>17</td>
<td>54</td>
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<tr>
<td>Total</td>
<td>17</td>
<td>58</td>
<td>22</td>
<td>19</td>
<td>41</td>
<td>35</td>
<td>11</td>
<td>33</td>
<td>77</td>
<td>20</td>
<td>15</td>
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<tr>
<td>Frequency</td>
<td>0.7 ± 0.2</td>
<td>23.0 ± 2.1</td>
<td>15.5 ± 2.8</td>
<td>0.3 ± 0.1</td>
<td>6.6 ± 0.9</td>
<td>3.3 ± 0.6</td>
<td>0.3 ± 0.09</td>
<td>4.8 ± 1.2</td>
<td>40.5 ± 3.2</td>
<td>2.6 ± 0.5</td>
<td>0.1 ± 0.02</td>
</tr>
</tbody>
</table>
Microcystis probably constitutes the most widely distributed MC-producing organism on earth. MC-producing strains have been isolated from all continents (Sivonen and Börner, 2008). A phylogenetic analysis of 164 Microcystis strains revealed the occurrence of two phylogenetic clades only that contained the mcy gene cluster, while the other four clades did not (Tanabe et al., 2007). The same authors concluded that those phylogenetic clades either containing or lacking the mcy gene cluster constitute cryptic ecotypes that are adapted to various environmental conditions. Notably these MC-producing ecotypes seem to have a wide geographic distribution, as typically MC-producing genotypes occur in waters inhabiting Microcystis (Kurmayer and Christiansen, 2009). In contrast Anabaena – although occurring frequently worldwide – shows a more restricted pattern of MC production as so far only MC-producing strains from Europe, North America and North Africa have been reported (Sivonen and Jones, 1999). When compared with Microcystis the genus Anabaena shows a much wider genetic variation, for example the taxonomic discrimination of the two morphologically distinct genera Anabaena and Aphanizomenon cannot be confirmed by 16S rDNA sequence analysis (Gugger et al., 2002). Recently, MC-producing Anabaena occurring even in brackish water in the Gulf of Finland in the Baltic Sea has been reported (Halinen et al., 2007) implying that the genus Anabaena is composed of MC-producing ecotypes showing resistance to increased salinity (5.03–6.67 practical salinity units). Consequently, although Microcystis cell numbers as determined in the microscope typically correlate with MC production, Anabaena cells cannot be used to infer MC concentrations in water.

4.2. Differences in microcystin net production between sites

The average MC cell quotas of Microcystis differed significantly between populations (Fig. 5A, B). Environmental conditions such as light availability and nitrogen availability have been shown to increase MC production in Microcystis. For example Wiedner et al. (2003) reported a linear increase in MC content per cell of Microcystis strain PCC7806 from 40 to 80 fg cell\(^{-1}\) under light conditions from 10 to 100 \(\text{m} \text{mol m}^{-2} \text{s}^{-1}\). Long et al. (2001) observed a variation in MC content per cell of Microcystis strain MASH 01-A19 from 0.052 to 0.116 fmol cell\(^{-1}\) under nitrogen limiting and nitrogen-replete conditions. Typically, environmental factors have been shown to modulate MC production per cell up to 5-fold, while larger variation (up to 30-fold) at 30 °C vs. 12.5 °C has been reported in exceptional cases only (Sivonen and Jones, 1999). In this study the average MC contents differed between Microcystis populations by 16–150-fold in integrated samples and 2.5–23-fold in plankton net samples. This range of variation substantially exceeds the variation observed for single strains under variable environmental conditions in the laboratory. Consequently it is more likely that genetic differences between populations such as...
the variable proportion of the mcyB genotype contributed to the variation in MC content that is observed. Indeed by applying real-time PCR to estimate the proportion of the mcyB genotype in the individual Microcystis populations it could be shown that the average proportion of the mcyB genotype was significantly related to the average MC content per cell (Fig. 5C). As suggested by one reviewer it might be that the inclusion of an irradiance in a less densely populated water column) cannot be excluded, but are of minor importance.

4.3. Differences in microcystin genotype proportion between sites

We have shown previously that populations of cyanobacteria in lakes may diverge in mcy genotype composition even if they are located only a few kilometres apart due to spatial isolation (Kurmayer and Gumpenberger, 2006). While this geographical isolation may result in the evolution of MC structural variants that appear to be unique and dominant (Christiansen et al., 2008a), this study is the first that demonstrates, that in consequence MC net production may differ quantitatively between sites as well. The structural analysis of protein phosphatase 1 – MC complexes did not provide evidence that the most variable amino acid residues at positions 2 and 4 of the MC molecule are of functional consequence (Bagu et al., 1997; Maynes et al., 2005). In contrast a quantitative change in MC production might be of a selective consequence. For example it has been shown that dissolved MC affects the growth of several submersed and emersed macrophytes negatively (Wiegand and Pflugmacher, 2005) and allelopathic effects on other phytoplankton and zooplankton species have been repeatedly suggested (Gross, 2003; Leflaive and Ten-Hage, 2007; Martins and Vasconcelos, 2009). However, it has also been shown that herbivorous organisms may develop behavioural or physiological resistance to MC production (Kurmayer and Jüttner, 1999). Following the concept of co-evolutionary interactions between herbivores and plants producing defensive compounds (Futuyama, 1983; Jongsm and Bolier, 1997) one might speculate that particularly in those Microcystis populations showing lowest mcyB proportion the allelopathic role of MC is increasingly replaced by bioactive compounds other than MCs (Welker and von Döhren, 2006). If this conclusion is true then one might expect that MC production is becoming selectively neutral to individual Microcystis colonies. While it is likely that only strong selective pressure led to the evolution of the mcy gene cluster in cyanobacteria it is known that the mcy gene cluster

### Table 3 – Linear regression curves on the dependence of microcystin concentrations on Microcystis cell numbers as determined in the microscope in the depth-integrated and the plankton net samples at the six sampling sites from May 2007 to April 2008.

<table>
<thead>
<tr>
<th>Sample size</th>
<th>R²</th>
<th>Linear regression curve³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Saka</td>
<td>24</td>
<td>0.97 y = 3.18 × 10⁻⁴x + 0.00654</td>
</tr>
<tr>
<td>Lake George</td>
<td>24</td>
<td>0.81 y = 1.3 × 10⁻³x – 0.000787</td>
</tr>
<tr>
<td>Lake Edward</td>
<td>24</td>
<td>0.66 y = 2.38 × 10⁻³x + 0.000475</td>
</tr>
<tr>
<td>Lake Mburo</td>
<td>24</td>
<td>0.94 y = 2.44 × 10⁻³x – 0.000919</td>
</tr>
<tr>
<td>Lake Victoria (Murchison Bay)</td>
<td>24</td>
<td>0.87 y = 2.74 × 10⁻³x + 0.000599</td>
</tr>
<tr>
<td>Lake Victoria (Napoleon Gulf)</td>
<td>24</td>
<td>0.87 y = 6.33 × 10⁻²x + 0.000128</td>
</tr>
<tr>
<td>Total</td>
<td>144</td>
<td>0.40 y = 1.41 × 10⁻²x – 0.00245</td>
</tr>
</tbody>
</table>

³ y is the microcystin concentration (µg MC ml⁻¹) and x is the Microcystis cell concentration (cells ml⁻¹).
probably evolved about two billion years ago (Rantala et al., 2004). According to this hypothesis the majority of the modern cyanobacterial lineages had lost the mcy gene cluster during their evolution. Unexpectedly within species such as Planktothrix the loss of the mcy gene cluster in strains happened on a much shorter time scale in evolution, yet has been found to be a rather rare event that happened a few million of years ago (Christiansen et al., 2008b). It was further concluded that in the meantime both the genotype retaining the mcy gene cluster and the genotype that lost the mcy gene cluster diverged and adapted to various other environmental conditions. Consequently it is impossible to compare costs and benefits of MC production between strains unless these strains have been genetically characterized in total (by comparative genome analysis) in order to elucidate potential hidden ecophysiological differences. It is likely that the Microcystis populations investigated in this study also diverged in other phenotypic characters not directly linked to MC production.

The results are of relevance with regard to the question of whether biogeography can influence toxin production in cyanobacteria. For example, in this study MC-LR that is most frequently found in populations of Microcystis in Europe (Via-Ordorika et al., 2004) could only be rarely detected in Ugandan freshwater lakes. MC-LR is known to have a ten-fold higher toxicity to vertebrates when compared with MC-RR and therefore those Ugandan water samples also should be less toxic to livestock and humans when compared with European habitats. In summary, the seasonal variation in average mcy proportion within each of the sites could not outweigh the between site variation in mcy genotype proportion, thus leading to a rather stable divergence in MC production of Microcystis between the spatially isolated populations. This lake-specific divergence might lead to a divergence in MC production on a wider geographic scale affecting MC production both qualitatively and quantitatively resulting in a so far unrecognised bio geographic pattern.

5. Conclusions

The finding that Microcystis is a consistent MC producer has important implications for water monitoring. By counting Microcystis cells under the microscope, Microcystis cell numbers can be used as a proxy to predict MC concentrations in surface water. Since for a specific sampling site a relatively minor variation in the average MC content both during dry and rainy seasons has been found, worst case MC concentrations could be calculated from cell numbers using the maxima of cellular MC quotas as reported for each sampling site. The microscopical approach is considered feasible as the microscopic enumeration technique is well established and the maintenance of technically sophisticated equipment is avoided. However, quantifying the mcyB genotype directly could make more accurate predictions of MC concentrations. In contrast the influence of the transcriptional rate of the mcyB gene on the observed variation in MC net production between sites is considered of minor importance.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.watres.2010.02.018.

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