Beyond Total Microcystins

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The importance of examining different microcystin variants

Cyanobacteria and their toxins

Nutrient over-enrichment and cyanobacterial blooms impair freshwater lakes around the world, with consequences on recreational activities, property values, and water supplies. The proliferation of algal blooms is problematic given that cyanobacteria can produce dermato-, hepato- and neurotoxic compounds associated with a range of health risks (from muscle pain, gastrointestinal symptoms, and skin irritation to neurodegenerative diseases, cancer, and death). Among these compounds, the hepatotoxic microcystins are the most commonly reported in freshwater blooms (Chorus and Bartram 1999). In their recent review, Harke et al. (2016) found microcystins in 79 out of 108 countries and territories where blooms were documented; this is a much higher count than the 30 countries reported in the mid-2000s (Zurawell et al., 2005).

Figure 1. Plankton community with potential toxin producing taxa Microcystis *and* Dolichospermum.

A closer look at microcystins

Microcystins are only produced by cyanobacterial strains that have the genes for their synthesis. Microcystins thus tend to be associated with specific cyanobacteria (e.g., *Planktothrix agardhii Dolichospermum*, *Aphanizomenon, Microcystis aeruginosa*; Chorus and Bartram 1999; Zurawell et al. 2005; Figure 1), which carry the gene. Microcystins are a class of small peptides with variability at two positions on the generic molecule, which results in different variants (Chorus and Bartram 1999; Figure 2). Microcystins are harmful to humans and animals because once ingested they can inhibit enzyme activity in the liver, which can lead to cell damage, pooling of blood, and death from hemorrhagic shock.

Variants of microcystin

Although there are over 100 known variants of microcystins, there remains a great deal of variation in the way microcystins are analyzed, quantified, and reported in the literature and by monitoring agencies. In Canada, total microcystin concentrations are more commonly reported (based on ELISA or protein phosphatase inhibition bioassays), and drinking water and recreational guidelines tend to be based on the concentration of the microcystin-LR variant alone (assumed to be the most widely distributed variant of microcystins). This differs from the approach in the U.S., where drinking advisories are for total microcystins, reported as microcystin-LR equivalents when analysed by ELISA. The focus on total microcystins or microcystin-LR could be an issue given that the occurrence of different variants is quite variable across lakes, and there may be considerable differences in their persistence, capacity for bioaccumulation, and toxicological properties.

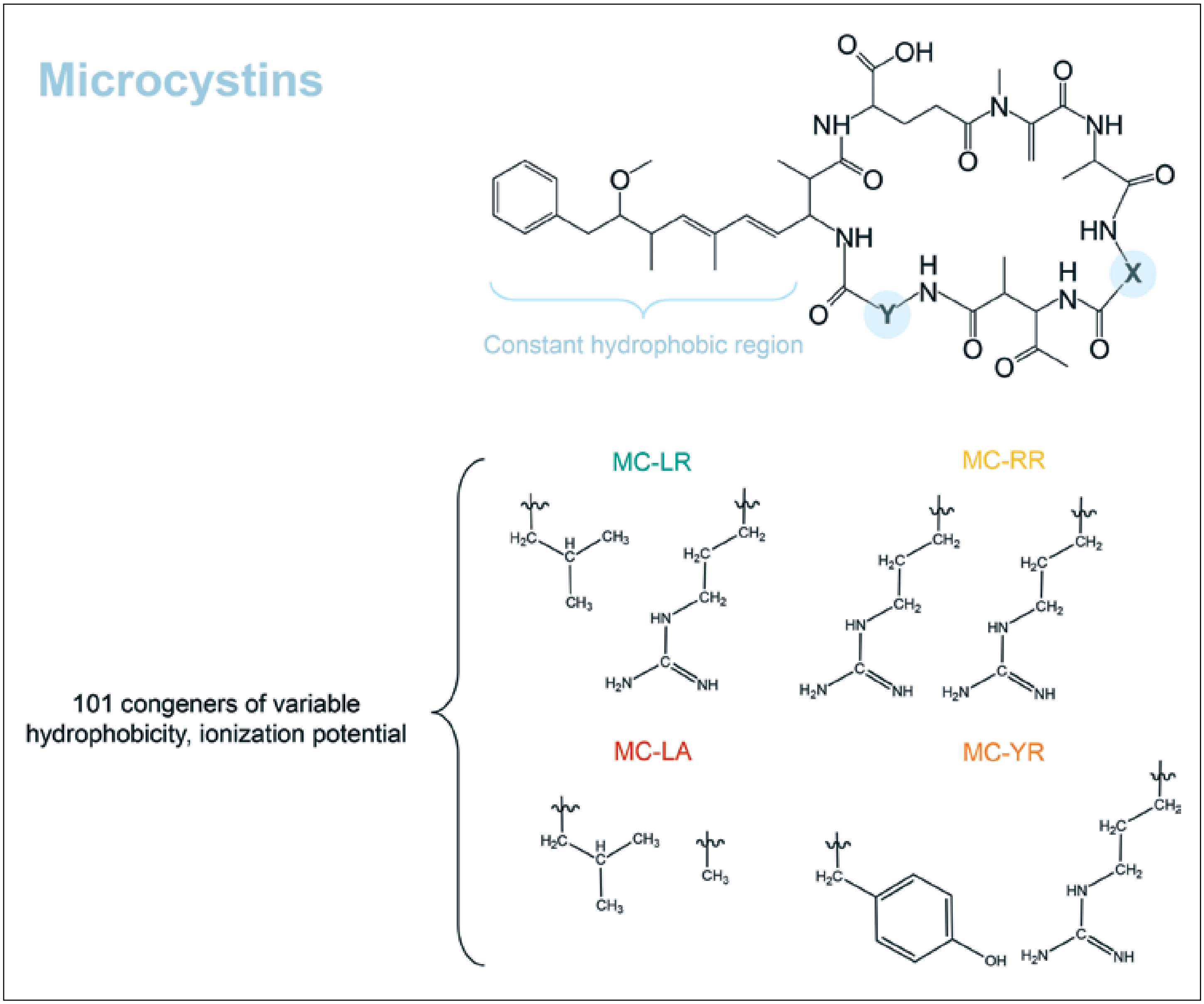

Figure 2. Structure of the peptide hepatotoxin, microcystin.

Microcystin-LA, for instance, may be just as or more persistent and toxic than microcystin-LR, with greater consequences for bioaccumulation (Chorus and Bartram 1999; Zurawell et al. 2005). In Ontario and Quebec, microcystin-LA is encountered in lakes affected by cyanobacterial blooms. In one small Ontario lake, significant levels of microcystin-LA toxin remained long after the spring blooms were visible, and toxin concentrations remained above the Health Canada provisional drinking water guideline (1.5 µg L^{-1}) for the remainder of the summer (Zastepa et al. 2014). The persistence of microcystin-LA is troubling given that the exposure to microcystin-LA from a recurrent *Microcystis* blooms in an upstream lake (~8.5 km inland) was recently found responsible for the intoxication and death of 21 sea otters along the shore of Monterey Bay in central California (Miller et al. 2010). Because sea otters and humans utilize the same coastal habitat and share the same marine foods, these findings have important implications for human health as well. Together, these case studies show that it is important to consider more than just total microcystin analyses, or simply the more commonly studied microcystin-LR variant. Interestingly, microcystin-LA is not reported in most European studies, perhaps due to differences in standards available for microcystin variant analysis.

Predicting the occurrence of MC variants in freshwaters

Although the factors that regulate the composition of different microcystin variants in lakes is largely unknown, the variability in the occurrence of these variants among regions or studies may be due, in part, to ecosystem-specific factors such as lake trophic status. For instance, some experimental work suggests that changes in light and nutrient availability may influence the quantity and composition of microcystin variants (Van de Waal et al. 2009). In addition to these lake-specific effects, the bias in the literature may be due to which cyanotoxin and/or variant are specifically analyzed. A consequence of this amongstudy variability is the difficulty in linking cyanobacterial toxins exposure to human health. To tease apart these different factors and biases (lake trophic status versus analytical methods), we have begun a large-scale meta-analysis quantifying the distribution of microcystin variants and occurrence in lakes around the world.

Using ongoing monitoring of raw water intake data from the Ontario Ministry of the Environment and Climate Change (OMECC; 2007 – 2015), combined with a synthesis of the published literature, we examined the role of lake trophic state in regulating the relative abundance of different microcystin variants. Although we detected (> 0.05 µg/L) microcystins across a range of trophic states (oligo- to hypereutrophic) in the Great Lakes and Lake of the Woods region, the effect of nutrients on each variant was quite variable throughout the OMECC monitoring program. The more commonly studied microcystin-LR tended to increase in a nonlinear fashion with nutrient concentrations, with declining concentrations at the highest nutrient concentrations in a pattern similar to that described for total microcystins in central U.S. lakes (Graham et al. 2004). In contrast, microcystin-LA was most common in nutrient poor sites and sampling dates.

By aggregating the data at the level of major water source, we found that the dominance of each microcystin variant was lake specific: Lake Ontario tended to be dominated by microcystin-LA, whereas lakes Erie and St-Clair were dominated by microcystin-LR. In addition to this spatial heterogeneity, we also noted an important change in dominance over time, where microcystin-LA increased sharply 2013 onwards. Collectively, even at this relatively small geographical scale, there was notable heterogeneity through space and time with respect to which microcystin dominated in freshwater lakes. The focus on solely total microcystins or microcystin-LR may be missing an important part of the story, with potential consequences on the perceived degree of animal or even human intoxication.

At a global scale, our synthesis of studies reporting microcystin variant data showed that few lakes in Europe appear to contain microcystin-LA although it has been reported from North and South America, New Zealand, and other lakes (Figure 3). In contrast, microcystin-LR was detected in Europe and many other regions. The lack of microcystin-LA in the more productive European lakes could be driven by different analytical approaches, making it difficult to quantify whether microcystin-LA is truly increasingly more rapidly in some regions (e.g., North America) relative to others. There is recent paleolimnological evidence, however, that -LA has existed in a eutrophic North American lake since before European settlement and that the variant is indeed on the rise over the last 200 years or so (Zastepa et al. 2016).

In summary, observations from central Canadian lakes with detectable microcystins suggest that microcystin-LA is commonly encountered and appears to be associated with lower nutrient concentrations, whereas this variant appears less common or absent from regions with relatively higher nutrient concentrations than central Canada (e.g., China, The Netherlands). In contrast, microcystin-LR is more prominent in nutrient enriched lakes. More variant analyses and consistent reporting (both as concentration and content) are required to validate these trends in microcystin composition.

Next steps

An important shortcoming to providing generalizable trends in microcystin composition across the globe is the reliable reporting of different microcystin variants, as well as the use of comparable laboratory techniques. Going forward, standardized methods and analytical standards will need to be used across studies and regions in order to provide accurate benchmarks in the face of ongoing global change.

Acknowledgements

The present research was funded by an NSERC CREATE grant, awarded to the Algal Bloom Assessment through Science, Technology and Education (ABATE) program. ABATE is a collaborative training team of academics, government, and industry from Canada, U.S., Europe, Asia, and Africa. The team's objective is to help management harmful algal blooms by improving our understanding of the species responsible for toxic blooms, and the environmental factors (e.g., climate and land us changes) that shift a population towards toxic strain dominance. The program offers interactive lectures and webinars as well as field schools to provide a first-hand experience of the harmful effects of algal blooms on communities. Academic exchanges, government and industry internships, and workshops on translating scientific findings into policies and practices are also a key component of the ABATE program.

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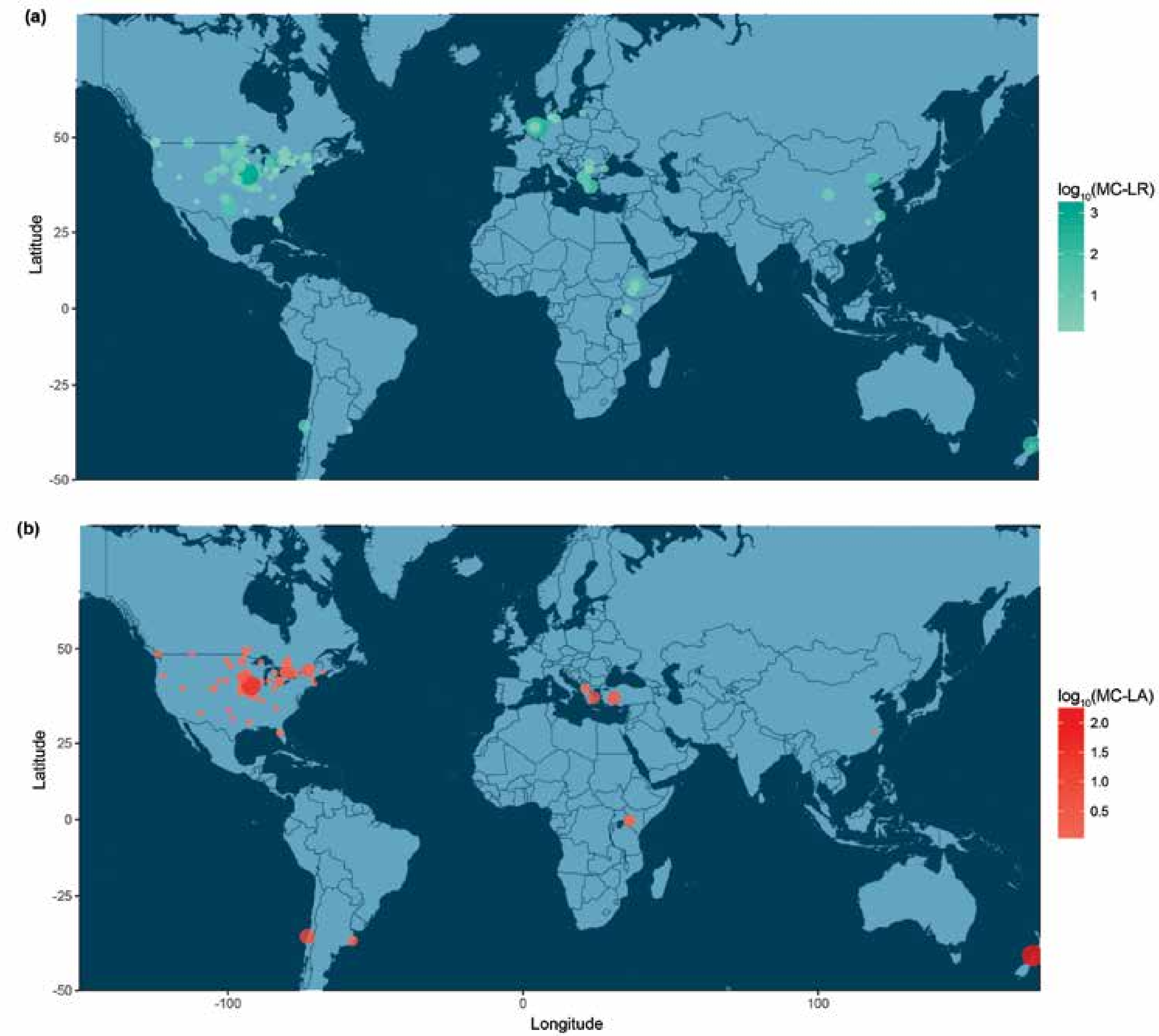
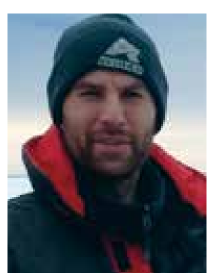


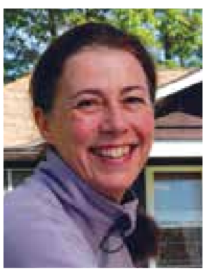
Figure 3. *Map of* ***(a)*** *microcystin-LR (MC-LR) and* ***(b)*** *microcystin-LA (MC-LA) mean concentration (log-transformed) of sites synthesized from the peer-reviewed literature. Data from Australia not currently shown on map.*

Dr. Zofia Taranu is a postdoctoral fellow in the biology department at the University of Ottawa, Ottawa, Canada. Her research focuses on understanding how anthropogenic activities (e.g., land use development, excess nutrient loading) and climate warming interact across different spatial and temporal scales to increasingly impair aquatic ecosystems (i.e., eutrophication and occurrence of symptomatic cyanobacterial blooms). Through quantitative syntheses, her work has advanced our understanding of the effect of Global Change on harmful algal blooms in freshwater lakes over the last 200 years or so.



Dr. Arthur Zastepa is a research scientist at the Canada Centre for Inland Waters at Environment and Climate Change Canada. He is actively involved in collaborative work with toxigenic and harmful algal blooms and source-water impairment in systems across Canada including in the Lake of the Woods, Lake Winnipeg, Lake Erie, and Lake Ontario. His research examines the factors regulating the abundance and diversity of microbes, their chemical ecology, and the fate and consequences of toxins produced in these systems. Dr. Zastepa has developed expertise in the application of bioanalytical technologies and paleolimnological tools to aquatic ecosystem research and has led the design and execution of large-scale field studies and surveys.

Dr. Irena Creed is professor and Canada Research Chair at Western University in the department of biology, with cross-appointments to geography and earth sciences. She investigates links between hydrological and biogeochemical processes and ecological outcomes under present and future climate scenarios, including the interactions between terrestrial and aquatic ecosystems and the formation of potentially harmful algal blooms. She tracks the movement and fate of nutrients within and through watersheds, which are released to the atmosphere (generating greenhouse gases) and aquatic systems (affecting productivity and diversity), work that is relevant to integrative disciplines like ecosystem health and ecosystem services. Her research has been used by governments to support policies and management measures to reduce risk to water supplies.



Dr. Frances Pick is chair of the biology department and professor of biology and environmental sciences at the University of Ottawa, Ottawa, Canada. Her research focuses on the ecology of freshwater ecosystems (lakes, rivers, wetlands) and in particular the structure and function of plant and algal/cyanobacterial communities. With chemists, she has worked on developing analytical methods for various hepatotoxins and neurotoxins produced by cyanobacteria. With molecular biologists, her lab is applying molecular tools for in situ detection of cyanobacterial genes (including microcystin genes) in both present day and ancient eDNA preserved in sediments. She has been a NALMS member for over 20 years and co-chaired the symposium in Toronto, Canada.

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NALMS Seeking New LakeLine Editor

After 18 years at the helm as editor of LakeLine, Bill Jones has decided to fully enact his retirement, and hand over the reins to new blood. As a result, the North American Lake Management Society (NALMS) seeks an editor for its LakeLine magazine which is a quarterly publication that informs and educates readers about current issues regarding lakes and lake management. Issues are theme-based with all of the articles addressing a specific topic. Most NALMS members primarily receive the magazine in print.

The ideal candidate must possess:

1. A bachelor's degree or higher in the water resources field.
2. Working knowledge of NALMS and its history.
3. Excellent verbal and written communication skills.
4. Superior English writing and editing skills, and demonstrated experience.
5. Experience with M.S. Word and Excel, and with Adobe Photoshop or similar publishing software.
6. Ability to work independently, budget time, and to meet deadlines.
7. Ability to oversee/work with production editor to approve/guide layout and presentation.

Specific duties of the Editor include, but are not limited to the following:

1. Identify issue themes independently or after consultation with NALMS members, the NALMS Board, and/or Publications Committee.
2. Solicit 6-7 articles from potential authors for each issue.
3. Article submission dates are Jan. 1 (spring issue), April 1 (summer), July 1 (fall), and Oct 1 (winter) to allow publication and delivery of each issue via postal mail in March-April (spring issue), June-July (summer issue), September-October (fall issue), and December-January (winter issue),
4. Edit each article; send edits back to authors until each is in final form. Use the AP Style Manual and editing guidelines prepared by past LakeLine editors. This stage is the last opportunity for authors to modify their article.
5. Prepare a one-page (~700 words) "From the Editor" column that introduces the issue.
6. Compile all articles, figures, columns, and announcements. Text should be in Word, images and figures in *.tif, *.jpg, or PDF files. Submit complete electronic package to the Production Editor for layout.
7. Work closely with Production Editor to correct any errors; print and proof laid-out issue; return marked-up proof to Production Editor. This can be scanned and returned electronically.
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11. Work with the NALMS Membership and Marketing Director to identify, recruit/obtain, and retain advertisers.

Interested applicants should send a cover letter detailing their experience and how they meet each of the qualifications to: Frank Wilhelm, President, North American Lake Management Society, fwilhelm@nalms.org<mailto:fwilhelm@nalms.org by August 15th, 2017.