UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

DISTRIBUTIONS AND BIOACCUMULATION OF MICROCYSTINS

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

In partial fulfillment of the requirements for the

Degree of

MASTER OF SCIENCE

By

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Norman, Oklahoma
2022

DISTRIBUTIONS AND BIOACCUMULATION OF MICROCYSTINS

A THESIS APPROVED FOR THE
DEPARTMENT OF BIOLOGY

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Acknowledgements

There are so many people to thank. First, my advisor, David Hambright. He as always pushed me to do the best I could possibly do, and given me all the help and support I needed to accomplish it all. The summer job that I took as an eager and inexperienced undergraduate fundamentally changed the course of my life, and I couldn't be happier with where I have ended up. Dave, thank you for everything.

Second, every member of the Plankton Ecology and Limnology Laboratory, current and past. You have welcomed me, supported me, helped me, and been there with me through it all, from kicking goose poop off the docks at Thunderbird to admiring awesome plankton on the microscope. I couldn't have asked for a better group of colleagues and friends.

Next, my dad, Charles Glidewell, and my sister, Gabrielle Glidewell. It's been a long, hard road. I never could have made it without you. The love, support, and encouragement of my family has meant the world to me. A thousand thanks to all of my friends, who stood by me even when I needed someone to feed my dogs unexpectedly on the Fourth of July because, surprise, I wasn't going to be leaving the lab until after midnight. You all kept me sane through this whole process.

Finally, my thanks to Cleo, Phaedra, Felix, Molly, Pretzel, and Poppy, the goodest of dogs. I could handle any stress thrown my way because of you.

Funding came from the University of Oklahoma Department of Biology Adams Summer Scholarships, the Hill Fund, the University of Oklahoma Graduate Student Senate Research Grants, Sigma Xi Grants in Aid of Research, and the National Science Foundation.

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Abstract

Microcystins are hepatotoxins and tumor promotors, with various deleterious effects on plants as well. There are 270+ known congeners of the toxin microcystin, structural variants of the molecule. These hepatotoxins are produced by cyanobacteria, and are some of the most common toxins produced by cyanobacterial harmful algal blooms, or cyanoHABs.

Cyanobacterial harmful algal blooms are increasing in frequency and intensity worldwide. They are already a global threat to human health and ecosystems, occurring on every continent, and occurring from Antarctica to the arctic circle. They occur in freshwater, brackish water, and marine waters, and their toxins have been carried to terrestrial ecosystems as well. However, due to the number of congeners and the difficulties of monitoring their presence, more research is needed on their effects on ecosystems, their global distribution, and whether they can bioaccumulate or biomagnify in food webs.

In Chapter 1, I review the literature on microcystins in food webs. I compare the presence of microcystins in different trophic levels, habitat types, and taxonomic groups. I conclude that microcystin intoxication is widespread. I also conclude that more research is needed on microcystins outside of freshwater ecosystems and on how microcystins spread through food webs. In Chapter 2, I review the literature on the geographic distribution of microcystin reports. I conclude geographic disparities exist in the sampling and reporting of microcystins, and that these disparities have implications for global public health. In Chapter 3, I report a laboratory experiment which I ran to determine if microcystins can bioaccumulate or biomagnify in invertebrates. I fed microcystin-producing cyanobacteria to phytoplanktivorous zooplankton, and

then fed those zooplankton to zooplanktivorous predators. I conclude that microcystins can be transferred between invertebrates, and that while microcystins likely do bioaccumulate they likely do not biomagnify.

Chapter 1 – A review on the distribution of microcystins across ecosystems, food webs, and organisms

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Abstract

Microcystins are a common cyanobacterial toxin and a global environmental concern. Despite their prevalence in the physical environment, less is known about their persistence in tissues and across trophic levels in food webs. This review synthesizes data from 99 publications found on Web of Science to examine the prevalence of microcystin intoxication across taxonomic groups, habitats, trophic levels, and tissue types. Microcystins were found in terrestrial habitats, throughout the water column in aquatic habitats, across trophic levels, and across tissue types within organisms. Opportunities exist for more studies involving multiple trophic levels—especially within a single site or ecosystem—and for research examining species outside freshwater aquatic ecosystems.

Introduction

Microcystins are hepatotoxins, tumor promoters, and some of the most common toxins produced by cyanobacteria. These toxins are a widespread environmental concern, having been found on every continent, including Antarctica (Rastogi et al., 2014). Microcystins are also persistent in aquatic environments due to their chemical stability. Microcystins are resistant to degradation by heat, sunlight, hydrolysis, and oxidation, but are eventually degraded by microorganisms (Rastogi et al., 2014). Though they are usually associated with the cyanobacterial genus *Microcystis*, microcystins are also produced by many other genera such as *Planktothrix* (Laub et al., 2002; Barco et al., 2004), *Nostoc* (Jungblut et al., 2006), and *Woronichinia* (Bober et al., 2011).

While microcystins are broadly distributed and prevalent in the physical environment, there is still uncertainty concerning their spread through food webs. Microcystins can accumulate in tissue by covalently bonding with protein phosphatases (Rastogi et al., 2014). Observations of microcystin accumulation in tissue have led to the hypothesis that these toxins may be transferred across trophic links in a food web. Reports in the literature have both supported and contradicted the hypothesis that microcystins biomagnify across trophic levels, with some providing evidence of biomagnification (Rohrlack et al., 2009), some showing microcystin concentrations decreasing as trophic levels increase (Papadimitriou et al., 2012), and some showing microcystin remaining at similar concentrations across trophic levels (Rezaitabar et al., 2017). A decrease in microcystin concentration as trophic levels increase could indicate microcystins being depurated or otherwise removed from food webs, while an increase in concentration or a consistent concentration across trophic levels could indicate microcystins remaining in tissues.

Bioaccumulation and magnification of microcystins has the potential to pose a major problem for ecosystems, by exposing organisms to microcystins which otherwise would not be directly impacted by harmful algal blooms. However, the difficulty of detecting microcystins in tissues has complicated research into this subject. Two common methods used to detect microcystin are liquid chromatography-mass spectrometry and enzyme-linked immunosorbent assays (ELISA). Liquid chromatography-mass spectrometry is expensive and requires specialized equipment and training. ELISA kits can suffer interference from the tissue and other complex matrices. Additionally, the 270+ known variants of microcystin complicate the detection of all microcystins in a sample with a single test, though the Adda ELISA and MMPB liquid chromatography-mass spectrometry method—both of which rely on a fragment of the molecule common to most microcystins—may make this more manageable.

This paper reviews the current literature on microcystins found in non-cyanobacterial organisms to show how extensively non-cyanobacterial organisms are intoxicated, to demonstrate the prevalence of microcystins across aquatic food webs and aquatic habitats, to draw attention to the occurrence of microcystins in non-aquatic environments, and to highlight the research questions that remain.

Methods

Literature Search

Papers were collected as part of a larger dataset using R 3.5.1 (R Core Team, 2018). The wosr package (v0.3.0; Baker, 2018) was run on February 2, 2020 to search Web of Science. The search terms used were "microcystin", and the 279 congener names listed in Table 1 of Bouaïcha et al. (2019). This resulted in an initial list of 5,646 papers that could potentially be used in this review.

Papers were selected for this review if they reported original research of observational field studies in which microcystins were found in non-cyanobacterial tissue (N = 116). To focus the review on microcystins naturally present in food webs, papers were excluded if the authors manipulated field conditions (N = 4) or did not report microcystin measurements (N = 4). Reviews, meta-analyses, or agency-compiled datasets were also excluded to prevent data duplication (N = 1). Finally, any paper in which the target organism was not identifiable (N = 8) was excluded. This current analysis is based on the remaining 99 publications.

Collecting Data

A data point was defined as one report of a target species per paper. If a target species was reported multiple times in the same paper, such as in multiple months, years, or lakes, the target species was still only counted once per paper. Using this unit of data removed the possibility of pseudoreplication in the analysis and produced the most conservative results by including the minimum number of data points from each paper. Additionally, this method prevented studies with more samples from overwhelming studies with fewer samples. For each data point, the scientific name of the target organism was recorded. The target organism's habitat, trophic level,

taxonomic group, economic significance, and the specific tissues found to contain microcystin were also recorded for each data point. Scientific names were checked for current taxonomic validity as of June 2021. MolluscaBase.org (MolluscaBase eds., 2021) was used to determine the validity of mollusk names, FishBase.org (Froese and Pauly, 2021) to determine the validity of fish names, WorldFloraOnline.org (WFO, 2021) to determine plant taxonomic names, and ITIS.gov (ITIS, 2021) to find the currently accepted scientific names of any other organisms.

Habitats were defined broadly as freshwater, marine, brackish water, and terrestrial. Aquatic habitats were further subdivided into littoral, pelagic, and benthic to examine the distribution of microcystins throughout the water body. Habitat details were recorded from the papers when possible, or from other sources when necessary. FishBase.se provided any missing habitat details for fish species. For the purposes of this review, fish species listed as demersal on FishBase.se were counted as pelagic, and benthopelagic species as benthic.

For trophic levels, target organisms were categorized as primary, secondary, or tertiary consumers. Primary consumers were defined as herbivores, phytoplanktivores, and detritivores. Omnivores and zooplanktivores were considered secondary consumers. Tertiary consumers were defined as carnivores, insectivores, and piscivores. While these broad categories do not describe a species' absolute position within its local food web, they do provide a rough estimate of how many trophic steps a species is from deliberately consuming cyanobacteria in its diet. Therefore, these categories should allow for broad comparisons of microcystin concentrations across papers and ecosystems.

Taxonomic groups were used to demonstrate the spread of microcystin intoxication across the kingdoms Animalia and Plantae. Vertebrates were grouped at the class level, invertebrates were grouped at the phylum level, and all plants were grouped together. These categories allow for a quick overview of the broad range of organisms contaminated by microcystins.

Information on the economic significance of organisms was recorded from the papers when possible, and from other sources if necessary. This information was collected to denote the potentially increased risk factors associated with microcystin intoxication in these species, as species of economic significance are often widely consumed by humans. Fish were considered to have economic significance if the paper mentioned it, or if the FishBase.org entry listed any commercial fishery. If no information could be found describing a species' economic significance, it was considered not to have any for the purposes of this review.

Microcystin Concentrations

Microcystin concentration data were used from any organism for which the concentrations of microcystins were reported in the liver, hepatopancreas, or muscle tissue. Muscle tissue was chosen because it is the tissue most often consumed, and could therefore be said to have the greatest importance to consumers, particularly humans. It was also the most commonly examined tissue. Hepatic tissue—from the liver or hepatopancreas—was selected because microcystins are hepatotoxins known to target hepatic tissue, and because hepatic tissue was the second most commonly examined tissue in this dataset.

Forty-four of the 99 papers reported microcystin concentrations in the selected tissues, resulting in 182 microcystin concentration data points. Only one concentration value was included per selected tissue per target species per paper. If raw concentration data from several samples were reported, the mean was calculated and used for this review. If several means were reported, the median value of the means was used as a conservative estimate. If there were two median values, the lower value was used. If a single mean was reported, it was used. WolframAlpha (Wolfram|Alpha, 2021) was used to convert all reported concentrations to µg microcystin per g tissue dry weight. For conversion from wet to dry weight, hepatic tissue and muscle tissue were assumed to have 65% moisture content (Adrian and Stevens, 1979; Wimmer et al., 1985; Khodabux et al, 2007; Arai et al., 2016;).

Statistical Analysis

Variations in microcystin concentrations were analyzed with respect to tissue type and trophic level. The data were log transformed due to the range of concentrations. Then the data were checked for normality and homogeneity of variance using Shapiro-Wilk tests and Bartlett tests to determine if the assumptions of a two-way ANOVA were met. The data were normally distributed. Some groups did not have equal variance, so a permutational ANOVA and a two-way ANOVA were performed. The results of the two tests were identical, so the results of the two-way ANOVA are presented for clarity. The two-way ANOVA was performed on the consumers, comparing the effects of target tissue, trophic level, and their interaction on the concentration of microcystins. Tukey's post-hoc tests were run if the two-way ANOVA produced significant results. Variations in the distribution of microcystins between primary consumers, secondary, and tertiary consumers in benthic, littoral, and pelagic habitats were

analyzed with chi-square goodness-of-fit tests. Significant ANOVA results were followed by post hoc tests to determine which consumers had significantly greater or fewer reports of microcystin than would be expected of an equal distribution.

Results

The extent of microcystin intoxication in non-cyanobacterial organisms

Microcystins were reported in 95 species of vertebrates, 58 species of invertebrates, and 28 species of plants (Table 1), including economically significance species (Supplemental Tables S1-S4). Interestingly, no papers reported detecting microcystins in fungi. Within the phylum Arthropoda, two species of arachnids, 13 species of crustaceans, and eight species of insects were found to be intoxicated with microcystins. Ray-finned fish, perhaps unsurprisingly, were the organisms most commonly found to be intoxicated with microcystins (Supplemental Table S2). These intoxicated fish included both farmed fish (Greer et al., 2017) and fish with significant fisheries (Nyakairu et al., 2010). Microcystin contamination was not restricted to the liver in fish, but was also found in the muscle tissue and throughout various other organs. This was also true of the intoxicated mollusks (Table 2).

Microcystin distribution in food webs

Microcystin intoxication was found across all trophic levels from primary to tertiary consumers. Significant differences were found between trophic levels and between tissue types (Figure 1). Microcystin concentrations were significantly higher in primary consumers than secondary

consumers, and in primary consumers than tertiary consumers (Tukey's post-hoc test, $p < 0.01$). No difference was found between secondary consumers and tertiary consumers. Hepatic tissue had significantly higher concentrations of microcystins than muscle tissue across all trophic levels (Tukey's, $p<0.01$). No significant interaction was found between the effects of trophic level and tissue type on microcystin concentration (Two-way ANOVA, $F_{2,176} = 0.787$, $p = 0.457$).

Microcystin distribution in aquatic habitats

In aquatic systems, microcystins have spread beyond the freshwater habitats commonly attributed to cyanobacterial harmful algal blooms (Figure 2). The toxins have also been detected in organisms from marine and brackish ecosystems. Marine habitats had the fewest papers reporting the detection of microcystins in organisms (N = 3). Of these, one study traced the origin of the microcystins to freshwater algae blooms that ran downriver and contaminated coastal habitats, intoxicating southern sea otters, likely through the biomagnified microcystins in the shellfish they consumed (Miller et al., 2010). Another paper rejected a freshwater origin and provided evidence that the microcystins accumulating in the mussels in the Amvrakikos Gulf came from marine cyanobacteria (Vareli et al., 2012), while the third paper could not determine the source of microcystins found in dolphin livers (Brown et al., 2018). In fresh water and brackish water, microcystins were detected throughout the water body in benthic, littoral, and pelagic habitats. It is more difficult to draw conclusions on the distribution of microcystins in marine waters from only three papers, which all detected microcystins in coastal organisms (Figure 2).

A pattern emerges in the distribution of reports of microcystin in benthic, littoral, and pelagic habitats (Figure 3). In benthic habitats the number of reports of microcystins were not significantly different across trophic levels ($\chi^2 = 3.59$, df = 2, $p = 0.1657$). However, the number of reports of microcystins were not equally distributed across littoral ($\chi^2 = 27.3$, df = 2, $p < 0.001$) or pelagic ($\chi^2 = 20.275$, df = 2, $p < 0.001$) habitats. The number of reports in littoral habitats was significantly higher in primary consumers. In pelagic habitats the number of reports increased with trophic level from primary consumers to tertiary consumers.

Terrestrial Reports of Microcystins

Terrestrial habitats were not sub-divided into specific sections for this review (Figure 2), though the presence of microcystins in terrestrial ecosystems at all is notable. As mentioned, microcystins are not restricted to aquatic environments (Figure 2). Indeed, terrestrial organisms can be exposed to microcystins through their food, not only through their drinking water. This has been documented in spiders (Takashi et al., 2014), little brown bats (Woller-Skar et al., 2015), warblers (Moy et al., 2016), and possibly in humans (Li et al., 2011). Terrestrial livestock such as pigs and cattle were found to contain microcystins (Manubolu et al, 2014; Classen et al., 2016), as were terrestrial crops (Xiang et al., 2019). Cattle were affected by microcystin-contaminated drinking water in their pastures (Manubolu et al., 2014), while pigs were affected by water piped into their nursery facility (Classen et al., 2016). Microcystins reached terrestrial crops through contaminated irrigation water (Xiang et al., 2019).

Discussion

Papers reported microcystin intoxication in several plants and animals, though not in fungi. Whether this lack of papers is a result of fungi not absorbing microcystins, fungi efficiently depurating microcystins, or because this is a question of little interest that has not been investigated, this review cannot say. Given that the symbiotic cyanobacteria in lichens can and do produce microcystins (Kaasalainen et al., 2012), it seems unlikely that fungi are simply never exposed to these toxins. Similarly, it was unsurprising that fish were the organisms most commonly found to be intoxicated with microcystins. Though whether this is a result of sampling intensity, again, this review cannot say.

The effects of cyanobacterial harmful algal blooms were not confined to freshwater—or even aquatic—ecosystems. Breinlinger et al. (2021) demonstrated the movement of aetokthonotoxin, another cyanobacterial toxin, to bald eagles at concentrations high enough to cause neurological disease, while twenty-three terrestrial species were reported with microcystin intoxication in this review (Supplemental Table S8). Within aquatic ecosystems, microcystins were spread throughout the water column, and the prevalence of microcystin contamination appeared to follow different patterns within different habitats. If the results of the chi-square test presented in Figure 3 are not caused by differences in sampling intensity, this implies different distributions of microcystins in different aquatic habitats, with microcystins being more likely to spread to tertiary consumers in pelagic habitats than littoral or benthic habitats. This could explain why some studies have supported the hypothesis of biomagnification, while others have refuted it. Additionally, the prevalence of microcystins in littoral primary consumers could represent a pathway for microcystin contamination in terrestrial organisms, as littoral habitats are nearest to

terrestrial environments and primary consumers are the animals most likely to be consumed. Therefore, more research needs to be done on non-freshwater ecosystems adjacent to harmful algal blooms to determine the scope of microcystin contamination and its effects on those systems. Coastal marine organisms in particular require closer study, as there were the fewest papers examining marine species and cyanobacterial toxins are spreading to coastal marine ecosystems (Preece, 2017).

When interpreting the trophic level results from Figure 1, certain factors must be considered. These data are not taken from the same food webs. Except in a few instances, these tertiary consumers are not eating these secondary consumers, which are not eating these primary consumers. The data were taken from independent studies conducted across the world, over 30 years, in different seasons, with varying background levels of microcystin, and different environmental conditions. That said, some conclusions can still be drawn. Microcystin concentrations being highest in primary consumers does not support the hypothesis that microcystins biomagnify. However, the lack of a significant difference between secondary consumers and tertiary consumers implies that microcystins are not necessarily being entirely depurated or otherwise removed from food webs. It appears that organisms receiving primary exposure to cyanobacteria have the highest microcystin concentrations, but that organisms receiving secondary exposure have similar concentrations regardless of their trophic level.

Additional factors must be considered when interpreting the tissue level results from Figure 1. The muscle and hepatic values were sometimes taken from the same organism—or pooled

sample of organisms—but more often were not. The two values were only counted separately for this analysis, not for the counts of microcystin-contaminated species. Again, these data were taken from independent studies conducted across a broad range of locations and times. But again, some broad conclusions can still be drawn. Table 2 demonstrates that microcystins accumulate throughout the tissues of fish and mollusks. Figure 1 additionally shows that microcystins accumulate both in hepatic tissue and muscle tissue, though on average, concentrations were highest in hepatic tissue across trophic levels.

Conclusions

The current literature suggests that microcystin intoxication is widespread in non-cyanobacterial organisms. It occurs across taxonomic groups, habitat types, and trophic levels. Within organisms, it occurs across tissues, not being confined to the hepatic tissues known to be targeted by these toxins. These factors should be accounted for when examining animals and plants for microcystin contamination. Given that cyanobacterial harmful algal blooms are a growing, global problem, more studies should be done involving multiple trophic levels—especially within a single site—and examining species outside freshwater aquatic ecosystems. Clearly, several opportunities exist to increase understanding of the spread of microcystins through food webs.

Acknowledgments

Many thanks are due to the entire Plankton Ecology and Limnology Laboratory for all of their discussion and advice, and particularly to Dr. Jessica Beyer for her continuous feedback,

critique, and assistance. I would also like to thank Dr. Claire Curry of the University of Oklahoma Libraries for all her help with Excel and R. Funding for this project came from the University of Oklahoma Department of Biology Adams Summer Scholarships and the National Science Foundation (DEB 1831061).

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Figures

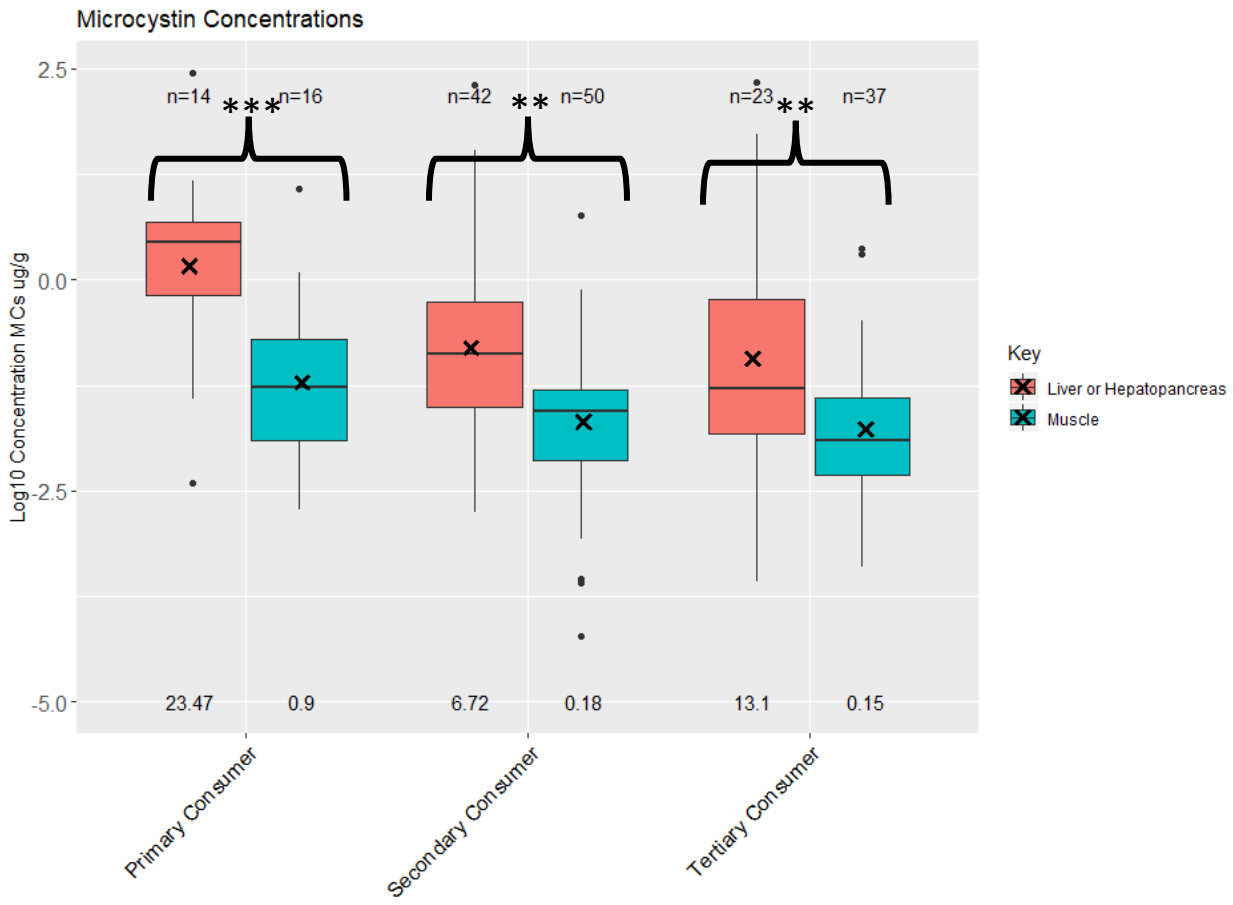

Figure 1: Log_{10} concentrations of microcystins in the hepatic tissue and muscle tissue of consumers across trophic levels. Data were taken from 44 papers. Asterisks indicate statistically significant differences between trophic levels using a Two-way ANOVA ($F_{2,176}$=7.442, $p<0.01$) and Tukey's post-hoc test ($p<0.01$). Numbers below the boxes indicate the un-transformed means. Hepatic tissue had significantly higher concentrations of microcystin than muscle tissue across all trophic levels ($F_{1,176}$=36.687, $p<0.01$). There was no significant interaction between tissue type and trophic level ($F_{2,176}$=0.787, $p=0.457$).

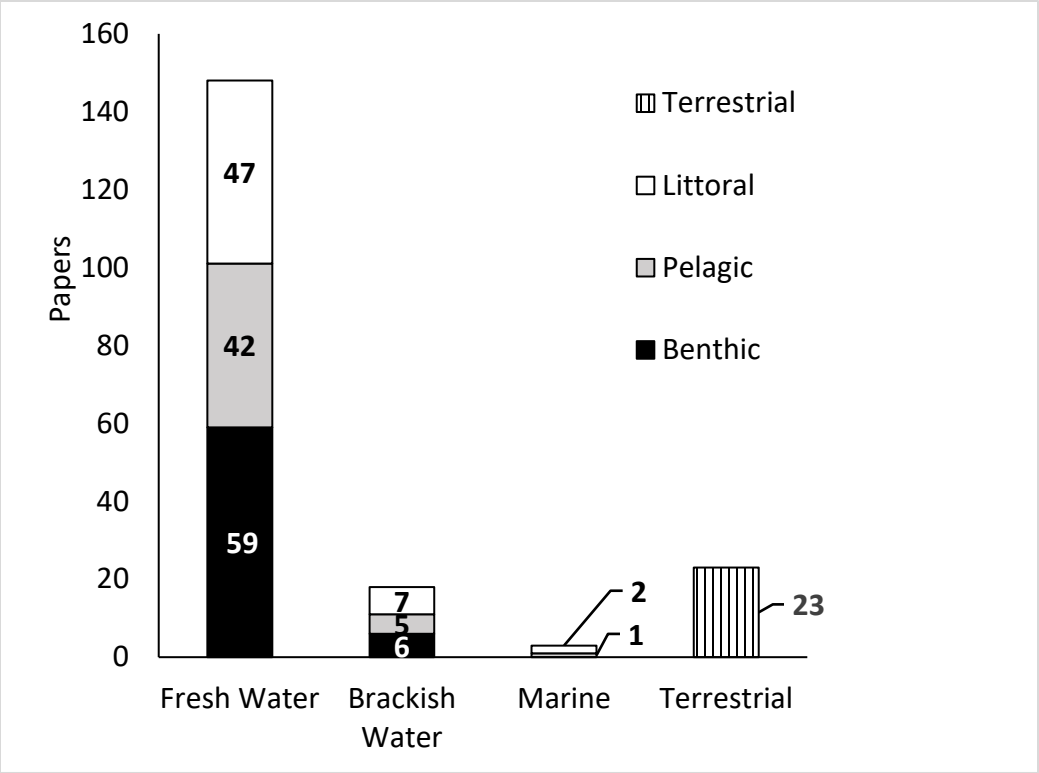

Figure 2: The numbers indicate the number of papers that reported organisms with microcystins in their tissues in each of the habitat types. Aquatic habitats are subdivided into benthic, littoral, and pelagic zones. The details of which organisms were reported from each environment are listed in the supplemental tables S5, S6, S7, and S8.

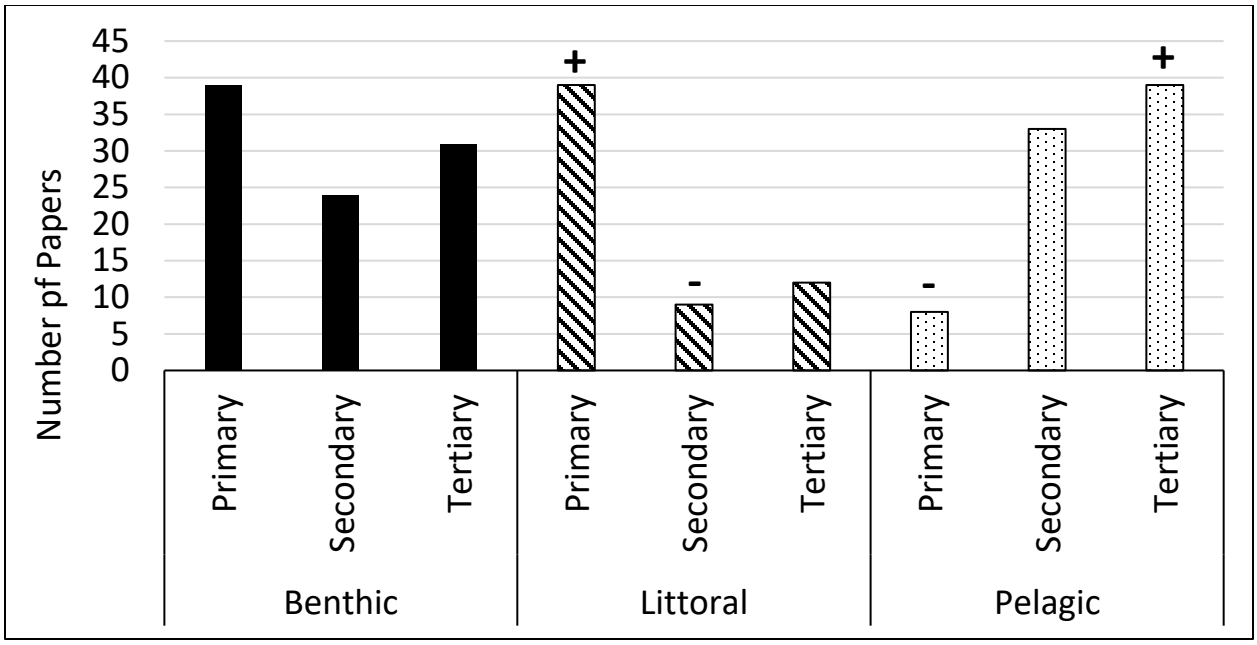

Figure 3: Reports of microcystins in aquatic organisms by habitat subtype. A chi-square goodness-of-fit test found that in benthic organisms, the distribution of reports was not significantly different across consumer levels (χ^2=3.59, df=2, p=0.1657). The same test found that reports were not equally distributed in littoral (χ^2=27.3, df=2, p<0.001) and pelagic (χ^2=20.275, df=2, p<0.001) habitats. The symbol + indicates a category with significantly more observed reports than expected in an even distribution, according to a post hoc test. The symbol – indicates significantly fewer reports than expected.

Tables

Table 1: The numbers indicate the number of species in each category reported to contain microcystins. Microcystin intoxication is broadly distributed across taxonomic groups. Note, unlike the other figures, the numbers in this table refer to the number of species in each category, not the number of papers that reported detecting microcystins in those species.

Vertebrates – 95 species	**Invertebrates – 58 species**	**Plants – 28 species**
Amphibians – 1	Annelids – 1	
Birds – 4	Arthropods – 23	
Fish, lung – 1	Mollusks – 34	
Fish, ray-finned – 77		
Mammals – 7		
Reptiles – 5		

Table 2: The number of papers that reported the detection of microcystins in all fish and mollusks. The portions most often consumed by humans (muscle in fish and foot in mollusks) have been found to contain microcystin.

Fish	**Mollusks**
Bile – 4	Foot – 11
Blood – 10	Gill – 6
Brain – 3	Gonad – 2
Gallbladder – 3	Hepatopancreas – 15
Gill – 3	Intestine – 7
Gonad – 5	Mantle – 1
Heart – 4	Muscle – 1
Intestine – 37	Remainder – 4
Kidney – 20	Stomach – 1
Liver – 91	Viscera – 7
Muscle – 117	
Spleen – 3	

Supplemental Material

Supplemental Table S1: The numbers indicate the number of papers that reported microcystins in each species. Asterisks indicate agricultural or economic significance.

Vertebrates – 173 papers		
Amphibians – 1	Fish, ray-finned – 148	Reptiles – 5
***Pelophylax epeiroticus – 1*	See Ray-Finned Fish, Table S2	**Crocodylus niloticus – 1*
		Emys orbicularis – 1
Birds – 6	Mammals – 12	*Mauremys leprosa – 1*
**Anas platyrhynchos – 2*	**Bos taurus – 1*	**Pelodiscus sinensis – 1*
Nycticorax nycticorax – 1	*Canis familiaris – 2*	*Trachemys scripta – 1*
Phoeniconaias minor – 2	*Enhydra lutris – 1*	
Protonotaria citrea – 1	**Homo sapiens – 4*	
	Myotis lucifugus – 2	
Fish, lung – 1	**Sus scrofa – 1*	
**Protopterus aethiopicus – 1*	*Tursiops truncates – 1*	

Supplemental Table S2: The numbers indicated the number of papers that reported microcystins in each species. Asterisks indicate agricultural or economic significance.

Ray-Finned Fish – 148 papers		
**Alosa pseudoharengus – 1*	**Esox lucius – 2*	**Odontesthes bonariensis – 2*
Ambloplites rupestris – 1	*Gymnocephalus cernua – 1*	**Oncorhynchus mykiss – 1*
**Ameiurus nebulosus – 2*	*Haplochromis squamipinnis – 1*	**Oreochromis esculentus – 1*
**Anguilla anguilla – 2*	**Hemibarbus maculatus – 1*	*Oreochromis leucostictus – 1*
**Aplodinotus grunniens – 1*	**Hemiculter leucisculus – 1*	**Oreochromis mossambicus – 2*
Astatoreochromis alluaudi – 1	**Hoplias malabaricus – 1*	**Oreochromis niloticus – 5*
**Atherina boyeri – 1*	**Hypophthalmichthys molitrix – 13*	**Oreochromis variabilis – 1*
**Bagrus docmac – 1*	**Hypophthalmichthys nobilis – 4*	**Osmerus eperlanus – 1*
**Brevoortia tyrannus – 1*	**Ictalurus furcatus – 1*	**Parabramis pekinensis – 2*
**Brycinus sadleri – 1*	*Labeo rosae – 1*	**Paralichthys olivaceus – 1*
**Carassius auratus – 8*	*Labeobarbus bynni – 1*	**Perca flavescens – 3*
**Carassius gibelio – 2*	**Lates niloticus – 3*	**Perca fluviatilis – 3*
Catostomus macrocheilus – 1	*Lepomis gibbosus – 2*	**Plagiognathops microlepis – 1*
**Chanodichthys erythropterus – 4*	**Lepomis macrochirus – 1*	**Plagioscion squamosissimus – 1*
Chetia flaviventris – 1	*Leporinus friderici – 1*	*Poecilia reticulata – 1*
Cichla monoculus – 1	**Leuciscus aspius – 1*	**Pomoxis nigromaculatus – 2*
**Clarias gariepinus – 3*	*Menidia audens – 1*	**Prochilodus brevis – 1*
**Coilia nasus – 4*	**Micropterus dolomieu – 1*	*Ptychocheilus oregonensis – 1*
**Coptodon zillii – 1*	*Micropterus salmoides – 2*	**Rastrineobola argentea – 1*
**Coregonus clupeaformis – 2*	**Mormyrus kannume – 1*	**Salanx prognathous – 1*
**Coregonus lavaretus – 2*	**Morone americana – 1*	**Sander lucioperca – 2*
**Ctenopharyngodon idella – 2*	*Morone chrysops – 1*	**Sander vitreus – 1*
**Cyprinus carpio – 17*	*Morone saxatilis – 1*	**Tachysurus fulvidraco – 2*
**Dorosoma cepedianum – 1*	**Mugil cephalus – 1*	**Tilapia rendalli – 1*
**Dorosoma petenense – 1*	**Neogobius melanostomus – 1*	**Tinca tinca – 1*
Enteromius neumayeri – 1	*Neosalanx taihuensis – 1*	

Supplemental Table S3: The numbers indicate the number of papers that reported microcystins in each species. Asterisks indicate agricultural or economic significance.

Invertebrates – 81 papers		
Annelids – 2	*Tetragnathidae sp. – 1*	*Nodularia douglasiae – 1*
Limnodrilus hoffmeisteri – 2	*Trichoptera sp. – 1*	*Physella acuta – 3*
	Zooplankton sp. – 3	*Physella gyrina – 1*
Arthropods – 31		*Pisidium sp. – 1*
Amphipod sp. – 1	Mollusk – 48	*Planorbella trivolvis – 1*
**Astacus astacus – 1*	*Ampullaceana balthica – 2*	*Planorbis planorbis – 2*
Atyaephyra desmarestii – 1	*Ancylus fluviatilis – 1*	*Potamopyrgus antipodarum – 1*
**Callinectes sapidus – 2*	*Anodonta cygnea – 1*	*Radix auricularia – 2*
Chironimidae sp. – 1	*Aplexa hypnorum – 1*	**Rangia cuneata – 1*
Chironomus sp. – 2	*Batillaria cumingi – 1*	**Ruditapes decussatus – 1*
Daphnia galeata – 1	*Bithynia tentaculata – 1*	*Sinanodonta woodiana – 2*
**Eriocheir sinensis – 1*	**Corbicula fluminea – 1*	**Sinotaia aeruginosa – 5*
**Exopalaemon modestus – 1*	**Cristaria plicata – 3*	*Sinotaia quadrata – 1*
Hexagenia limbata – 1	*Dreissena polymorpha – 2*	*Sphaerium corneum – 1*
Hexagenia sp. – 1	*Dreissena rostriformis – 1*	*Viviparus contectus – 1*
Macrobrachium amazonicum – 1	*Echyridella menziesii – 1*	
**Macrobrachium nipponense – 4*	*Hippeutis complanatus – 1*	
**Metapenaeus joyneri – 1*	**Hyriopsis cumingii – 1*	
Microchironomus tabarui – 1	*Lamprotula leaii – 1*	
Pantala flavescens – 1	*Lanceolaria lanceolata – 1*	
**Portunus trituberculatus – 1*	*Lymnaea stagnalis – 1*	
**Procambarus clarkia – 2*	**Magallana gigas – 1*	
Tanypus chinensis – 1	*Mytilus galloprovincialis – 1*	
Tetragnatha praedonia – 1	**Mytilus trossulus – 2*	

Supplemental Table S4: numbers indicate the number of papers that reported microcystins in each species. Asterisks indicate agricultural or economic significance.

Plants – 33 papers		
**Allium tuberosum – 1*	**Eruca vesicaria – 1*	*Phragmites australis – 1*
Alternanthera philoxeroides – 1	*Hydrilla verticillate – 1*	*Potamogeton maackianus – 1*
**Amaranthus hybridus – 1*	**Ipomoea aquatica – 1*	**Raphanus raphanistrum – 1*
**Anethum graveolens – 1*	**Lactuca sativa – 2*	**Solanum lycopersicum – 1*
**Apium graveolens – 1*	*Myriophyllum spicatum – 1*	**Solanum melongena – 1*
**Brassica oleracea – 2*	*Nymphaea elegans – 1*	**Trapa natans – 1*
**Brassica rapa – 1*	**Oryza sativa – 2*	*Typha latifolia – 1*
**Capsicum annuum – 2*	*Persicaria glabra – 1*	*Typha sp. – 1*
**Daucus carota – 1*	**Petroselinum crispum – 1*	
Eichhornic crassipes – 2	**Phaseolus vulgaris – 1*	

Supplemental Table S5: The number of papers that found microcystins in organisms living in brackish water.

Brackish Water Habitat Details	Organism	Number of Papers
Benthic	*Metapenaeus joyneri*	1
Benthic	*Portunus trituberculatus*	1
Benthic	*Paralichthys olivaceus*	1
Benthic	*Batillaria cumingi*	1
Benthic	*Dreissena polymorpha*	2
Benthic	*Mytilus trossulus*	2
Littoral	*Anas platyrhynchos*	1
Littoral	*Microchironomus tabarui*	1
Littoral	*Pantala flavescens*	1
Littoral	*Magallana gigas*	1
Littoral	*Amphipod sp.*	1
Littoral	*Daphnia galeata*	1
Littoral	*Zooplankton sp.*	1
Pelagic	*Gymnocephalus cernua*	1
Pelagic	*Menidia audens*	1
Pelagic	*Morone saxatilis*	1
Pelagic	*Mugil cephalus*	1
Pelagic	*Osmerus eperlanus*	1

Supplemental Table S6: The number of papers that found microcystins in organisms living in marine waters.

Marine Habitat Details	Organism	Number of Papers
Littoral	*Enhydra lutris*	1
Littoral	*Mytilus galloprovincialis*	1
Pelagic	*Tursiops truncatus*	1

Supplemental Table S7: The number of papers that found microcystins in organisms living in fresh water.

Fresh Water Habitat Details	Organism	Number of Papers
Benthic	*Limnodrilus hoffmeisteri*	2
Benthic	*Callinectes sapidus*	1
Benthic	*Eriocheir sinensis*	1
Benthic	*Exopalaemon modestus*	1
Benthic	*Macrobrachium amazonicum*	1
Benthic	*Macrobrachium nipponense*	4
Benthic	*Procambarus clarkii*	2
Benthic	*Anguilla anguilla*	2
Benthic	*Bagrus docmac*	1
Benthic	*Carassius gibelio*	2
Benthic	*Chanodichthys erythropterus*	3
Benthic	*Clarias gariepinus*	3
Benthic	*Coptodon zillii*	1
Benthic	*Coregonus clupeaformis*	1
Benthic	*Coregonus lavaretus*	2
Benthic	*Ctenopharyngodon idella*	2
Benthic	*Dorosoma cepedianum*	1
Benthic	*Enteromius neumayeri*	1
Benthic	*Haplochromis squamipinnis*	1
Benthic	*Hemibarbus maculatus*	1
Benthic	*Hemiculter leucisculus*	1
Benthic	*Ictalurus furcatus*	1
Benthic	*Labeobarbus bynni*	1
Benthic	*Lepomis gibbosus*	1
Benthic	*Lepomis macrochirus*	1
Benthic	*Micropterus dolomieu*	1
Benthic	*Morone americana*	1
Benthic	*Morone chrysops*	1
Benthic	*Neogobius melanostomus*	1
Benthic	*Odontesthes bonariensis*	1
Benthic	*Oreochromis esculentus*	1
Benthic	*Oreochromis leucostictus*	1

Benthic	*Oreochromis mossambicus*	1
Benthic	*Oreochromis niloticus*	5
Benthic	*Oreochromis variabilis*	1
Benthic	*Perca fluviatilis*	3
Benthic	*Plagiognathops microlepis*	1
Benthic	*Poecilia reticulata*	1
Benthic	*Pomoxis nigromaculatus*	1
Benthic	*Prochilodus brevis*	1
Benthic	*Sander vitreus*	1
Benthic	*Tachysurus fulvidraco*	1
Benthic	*Tinca tinca*	1
Benthic	*Chironomus sp.*	2
Benthic	*Hexagenia sp.*	1
Benthic	*Tanypus chinensis*	1
Benthic	*Ampullaceana balthica*	2
Benthic	*Ancylus fluviatilis*	1
Benthic	*Bithynia tentaculata*	1
Benthic	*Corbicula fluminea*	1
Benthic	*Echyridella menziesii*	1
Benthic	*Hippeutis complanatus*	1
Benthic	*Lanceolaria lanceolata*	1
Benthic	*Physella acuta*	2
Benthic	*Planorbis planorbis*	2
Benthic	*Radix auricularia*	2
Benthic	*Rangia cuneata*	1
Benthic	*Ruditapes decussatus*	1
Benthic	*Sinotaia aeruginosa*	5
Littoral	*Pelophylax epeiroticus*	1
Littoral	*Anas platyrhynchos*	1
Littoral	*Nycticorax nycticorax*	1
Littoral	*Phoeniconaias minor*	2
Littoral	*Astacus astacus*	1
Littoral	*Atyaephyra desmarestii*	1
Littoral	*Callinectes sapidus*	1
Littoral	*Astatoreochromis alluaudi*	1
Littoral	*Hypophthalmichthys molitrix*	13
Littoral	*Hypophthalmichthys nobilis*	4
Littoral	*Chironimidae sp.*	1
Littoral	*Hexagenia limbata*	1
Littoral	*Trichoptera sp.*	1

Littoral	*Anodonta cygnea*	1
Littoral	*Aplexa hypnorum*	1
Littoral	*Cristaria plicata*	3
Littoral	*Dreissena rostriformis*	1
Littoral	*Hyriopsis cumingii*	1
Littoral	*Lamprotula leaii*	1
Littoral	*Lymnaea stagnalis*	1
Littoral	*Nodularia douglasiae*	1
Littoral	*Physella acuta*	1
Littoral	*Physella gyrina*	1
Littoral	*Pisidium sp.*	1
Littoral	*Planorbella trivolvis*	1
Littoral	*Potamopyrgus antipodarum*	1
Littoral	*Sinanodonta woodiana*	2
Littoral	*Sinotaia quadrata*	1
Littoral	*Sphaerium corneum*	1
Littoral	*Viviparus contectus*	1
Littoral	*Alternanthera philoxeroides*	1
Littoral	*Eichhornia crassipes*	2
Littoral	*Hydrilla verticillata*	1
Littoral	*Ipomoea aquatica*	1
Littoral	*Myriophyllum spicatum*	1
Littoral	*Oryza sativa*	2
Littoral	*Persicaria glabra*	1
Littoral	*Phragmites australis*	1
Littoral	*Potamogeton maackianus*	1
Littoral	*Trapa natans*	1
Littoral	*Typha sp.*	1
Littoral	*Crocodylus niloticus*	1
Littoral	*Emys orbicularis*	1
Littoral	*Mauremys leprosa*	1
Littoral	*Pelodiscus sinensis*	1
Littoral	*Trachemys scripta*	1
Littoral	*Zooplankton sp.*	2
Pelagic	*Alosa pseudoharengus*	1
Pelagic	*Ambloplites rupestris*	1
Pelagic	*Ameiurus nebulosus*	2
Pelagic	*Aplodinotus grunniens*	1
Pelagic	*Atherina boyeri*	1
Pelagic	*Brevoortia tyrannus*	1

Pelagic	*Brycinus sadleri*	1
Pelagic	*Carassius auratus*	8
Pelagic	*Catostomus macrocheilus*	1
Pelagic	*Chanodichthys erythropterus*	1
Pelagic	*Chetia flaviventris*	1
Pelagic	*Cichla monoculus*	1
Pelagic	*Coilia nasus*	4
Pelagic	*Coregonus clupeaformis*	1
Pelagic	*Cyprinus carpio*	17
Pelagic	*Dorosoma petenense*	1
Pelagic	*Esox lucius*	2
Pelagic	*Hoplias malabaricus*	1
Pelagic	*Labeo rosae*	1
Pelagic	*Lates niloticus*	3
Pelagic	*Lepomis gibbosus*	1
Pelagic	*Leporinus friderici*	1
Pelagic	*Leuciscus aspius*	1
Pelagic	*Micropterus salmoides*	2
Pelagic	*Mormyrus kannume*	1
Pelagic	*Neosalanx taihuensis*	1
Pelagic	*Odontesthes bonariensis*	1
Pelagic	*Oncorhynchus mykiss*	1
Pelagic	*Oreochromis mossambicus*	1
Pelagic	*Parabramis pekinensis*	2
Pelagic	*Perca flavescens*	3
Pelagic	*Plagioscion squamosissimus*	1
Pelagic	*Pomoxis nigromaculatus*	1
Pelagic	*Protopterus aethiopicus*	1
Pelagic	*Ptychocheilus oregonensis*	1
Pelagic	*Rastrineobola argentea*	1
Pelagic	*Salanx prognathus*	1
Pelagic	*Sander lucioperca*	2
Pelagic	*Tachysurus fulvidraco*	1
Pelagic	*Tilapia rendalli*	1
Pelagic	*Nymphaea elegans*	1
Pelagic	*Typha latifolia*	1

Supplemental Table S8: The number of papers that reported microcystins in terrestrial organisms.

Terrestrial Organism	**Number of Papers**
Tetragnatha praedonia	1
Tetragnathidae sp.	1
Protonotaria citrea	1
Bos taurus	1
Canis familiaris	2
Homo sapiens	4
Myotis lucifugus	2
Sus scrofa	1
Allium tuberosum	1
Amaranthus hybridus	1
Anethum graveolens	1
Apium graveolens	1
Brassica oleracea	2
Brassica rapa	1
Capsicum annuum	2
Daucus carota	1
Eruca vesicaria	1
Lactuca sativa	2
Petroselinum crispum	1
Phaseolus vulgaris	1
Raphanus raphanistrum	1
Solanum lycopersicum	1
Solanum melongena	1

Chapter 2 – A review of the geographic distribution of the reports of microcystin congeners

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Abstract

Microcystin, a common toxin produced by cyanobacterial harmful algal blooms, is a growing threat to ecosystems and public health. However, there is a geographic disparity in the distribution of microcystin reports. This review synthesizes data from 398 publications found on Web of Science to examine the distribution of published microcystin reports and cyanobacterial harmful algae reports. Areas with no published reports of microcystin often matched areas with insufficient data on public water resources as determined by the World Health Organization and United Nations Children's Fund. This lack of data demonstrates the need for basic research on the presence of microcystin in these regions.

Introduction

Cyanobacterial harmful algal blooms (cyanoHABs) are a growing threat to the safety of drinking water and aquatic ecosystems, due to their increasing frequency and intensity worldwide (Huisman et al., 2018). Microcystins—the most common toxins produced by cyanobacterial harmful algal blooms—have acute effects and chronic, sublethal effects on humans, which

promote tumors and cause liver damage. Therefore, it is necessary to monitor cyanoHABs in general, and microcystins in particular, to protect public health.

However, monitoring microcystins requires time and resources, both of which are often limited. These toxins can be present in water, tissues (Chapter 1), and soil (Zastepa et al., 2015). They have been found in freshwater, brackish, coastal marine, and even terrestrial environments (Chapter 1). Over three percent of the Earth's continental surface area is covered by water, and there are over 300 million natural lakes around the globe (Downing et al., 2006), without even considering reservoirs. These water bodies represent an enormous potential area for microcystin contamination, and would require extensive resources to monitor. Data from the World Health Organization and the United Nation's Children's Fund (2017) suggest that there is a geographic disparity in where data on water quality is currently available, perhaps correlated with a geographic disparity in the availability of resources necessary for water quality monitoring.

The nature of microcystins also presents inherent challenges to monitoring. There are over 270 congeners—variations of the molecule with similar structures—of microcystin (Bouaïcha et al. 2019). Certain tests detect only a small subset of the congeners, often focusing on microcystin-LR (MC-LR) (Zeck et al., 2001). Some tests also are limited in their ability to detect microcystins in matrices other than water (Preece et al., 2015). Tests that can detect a broad range of microcystin congeners across simple and complex matrices, such as the 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) liquid chromatography-mass spectrometry method, require expensive equipment and specially trained personnel. Even quantifying total

microcystins can obscure important information. Different congeners have different toxicities (Sivonen and Jones, 1999), and cyanobacterial toxins may even exhibit synergistic toxicity (Leão et al., 2010), in which multiple toxins present together are more toxic than an equivalent amount of a single toxin.

In this review, I found that geographic disparities appeared in a large set of papers reporting the presence of microcystins, which have implications for public health. To guard public health, especially in the developing world, resources must be used efficiently. Agencies and decision makers should be aware of areas of undersampling and areas where microcystins have been detected previously. Researchers and reviewers need to be aware of the gaps in the current literature. Therefore, this review will examine the geographic distribution of published microcystin reports, and determine areas of monitoring that need to be improved to guard human health.

Methods

Literature search

I collected papers using R 3.5.1 (R Core Team, 2018), by running the wosr package (v0.3.0; Baker, 2018) on February 2, 2020 to search Web of Science. I used the search terms "microcystin", and the 279 congener names listed in Table 1 of Bouaïcha et al. (2019). This search returned a list of 5,646 titles and abstracts. I used the list of titles and abstracts to find papers that appeared to be field studies that detected microcystin, which gave me 1,006 potential papers to examine for this review.

I attempted to download these papers (N = 1,006) in order to read them to determine if they had useful data for this review. I immediately had to exclude 27 papers because I was unable to obtain them, either through an online database or through the University of Oklahoma's Interlibrary Loan service. I had to exclude 13 papers for being written in a language I could not read. I then excluded an additional paper because the digital record included only the abstract of a conference presentation, and one paper for being a preprint not yet subject to peer review. To prevent data duplication, I also excluded eight papers for being reviews and 34 papers for using agency-collected datasets that were available to the public and therefore used in multiple papers. To further reduce this still large dataset of 922 papers, I chose only to include papers that presented their data as figures or tables. Therefore, I excluded 98 papers because the papers did not include their information on the occurrence of microcystins in a graph and/or a table.

The purpose of this review is to provide geographic and temporal information on reports of microcystins in ecosystems. Therefore, I included only papers that reported the month and year in which a sample was collected, and the location in which a sample was collected—a given lake, estuary, location within a river, or coastal bay, for example. Because the microcystins present can change with the environmental conditions (Rapala et al., 1997), I only included papers that reported microcystins collected from field samples from field conditions which had not been experimentally manipulated. I excluded two papers because the papers did not report the methods used to detect the microcystins, and one paper because the methods were reported so poorly it raised questions about the validity of the data. I excluded five papers because the microcystins were found in sediment cores, which made it impossible to determine the year and

month they had been deposited. I excluded one paper for reporting microcystins from historical specimens in museums, which lacked information on when and where they had initially been collected. I excluded 209 papers for not providing the location or the time in which their samples were collected with enough accuracy—either the year and month for the time the sample was collected, or the specific location with the level of detail described above. I excluded 55 papers in which the researchers manipulated field conditions, either by manipulating nutrient concentrations, introducing animals, or introducing cyanobacteria. Finally, I excluded 153 papers which reported microcystin measurements from cyanobacterial cultures.

After excluding ineligible papers, I was left with 398 papers. In 68 of the 398 papers, only some of the microcystin data reported in the paper was eligible for this review, either because only some of the samples reported in the paper were collected from field studies while others were collected from lab cultures, because the field conditions from some samples were manipulated while others were not, or because the month, year, and location in which the samples were collected were not reported for all samples. For these 68 papers, I included the data that met the requirements for the review and excluded the data that did not meet the requirements.

Collecting Data

I recorded data on which congeners were reported, which cyanobacterial genera were present, the month and year in which the samples were collected, and the locations from which the samples were collected. To determine the location, I recorded the GPS coordinates to the second decimal if they were reported in the paper. If the sampling coordinates were not reported, I used

Google Maps to find the location from which the microcystin sample was taken. If images marking the sampling sites were provided in the paper, I compared these with Google Maps to obtain the most reasonable GPS coordinates. When images were not provided, I used descriptions in the papers' methods sections to obtain approximations of the GPS coordinates from which the samples were taken. If the paper provided no information on the exact location from which the samples were taken, I selected GPS coordinates near the middle of the water body or other sampling area. I was forced to exclude an additional 16 papers because I was not able to locate GPS coordinates for the samples during this process. After excluding those final papers, I analyzed the data from 382 papers.

Analyzing Data

In total, I gathered 14,108 reports of microcystin from 382 papers. I mapped the microcystin data using the R package maps (v3.3.0; Becker et al., 2018). I checked the cyanobacterial genera for currency and accuracy using AlgaeBase.org in March 2021 (Guiry and Guiry, 2021).

Results

Microcystins are a global problem, with reports on every continent from the Arctic to the Antarctic. However, certain areas of high human population density have few to no reports of microcystins in the literature. These areas include, but are not limited to, the west coast of Africa, India, and Indonesia (Figure 1).

The maps demonstrate that the three most commonly reported congeners—LR, RR, and YR—all have the same global distributions (Figures 2-4). These distributions also match the global distribution of all microcystin congeners (Figure 1).

Patterns also emerge in the temporal distribution of microcystin reports. In the northern hemisphere, the number of reports and the number of papers is reduced in winter. However, the distribution of the remaining reports is the same as the distribution of reports in the other seasons. In the southern hemisphere, the number of reports and the number of papers is not reduced in winter, and the distribution of the reports remains the same as in the other seasons as well. Additionally, the northern hemisphere has many more papers and reports than the southern hemisphere. (Figure 5).

Finally, *Microcystis* is the most commonly reported genus of cyanobacteria, and MC-LR is the most commonly reported congener of microcystin. However, other genera of cyanobacteria do occur with MC-LR, and other congeners of microcystin do occur with *Microcystis* (Table 1).

Discussion

Given the global distribution of microcystins presented in Figures 1-4, it seems unlikely that reports of microcystins in areas of high human population density are absent because no microcystins are present. It appears more likely that these reports are absent because the basic monitoring and reporting of microcystins is not being done in these geographic areas. Indeed,

many of these areas overlap with areas of insufficient data on safely managed drinking water reported by WHO and UNICEF (2017). Basic survey research still needs to be published, or conducted, in these underserved regions. These studies are critical for filling these gaps in the literature.

Further examining the distribution of microcystin congeners (Figures 2-4, Table 1, Supplemental Table S1, and Supplemental Table S2) makes it clear that sampling for MC-LR alone is inadequate. In Figures 2-4, the other two most commonly reported congeners, MC-RR and MC-YR, have the same global distribution as MC-LR. Clearly, the other congeners are just as broadly distributed. In Table 1 and the supplemental tables S1 and s2, it is apparent that many other congeners are commonly present within a wide variety of cyanobacterial harmful algal blooms. Therefore, to best account for all microcystins present, Adda or MMPB methods should be used to sample for microcystins, instead of MC-LR-specific methods.

The temporal patterns in the distribution of microcystin reports and the number of papers reporting microcystin also reveal gaps in the current literature. The decreased number of microcystin reports in the northern hemisphere in winter could indicate that microcystins occur less frequently in winter, or that microcystins are less monitored in winter and therefore less reported. The fact that the number of microcystin reports in the southern hemisphere is not drastically lower in winter, and that the number of papers reporting those microcystins is also not drastically lower in winter, implies that the reduced reports in the northern hemisphere might be the result of less monitoring, not less prevalence of microcystin.

There are several potential explanations for why microcystins might persist in winter or other times when cyanobacterial blooms are not present. Microcystins are highly stable in the physical environment, resisting degradation by heat, sunlight, hydrolysis, and oxidation (Rastogi et al., 2014). The half-lives of microcystins are longer in deeper water, up to 120 days per meter of depth (Welker and Steinberg, 2000), and the toxins have been found in sediment cores and pore waters from surface sediments to sediments deposited over 100 years ago (Zastepa et al., 2015). They have also been found in shoreline sediments along water bodies contaminated with cyanobacteria (Preece et al., 2021). Additionally, microcystins can be absorbed into the tissues of other organisms and persist in the food web, including terrestrial, agriculturally significant species, which implies humans could be consuming microcystins through unanticipated routes (Chapter 1). Li et al. (2011) determined that the children in their study were consuming higher doses of microcystin through food collected from lakes than the World Health Organization's tolerable daily intake, and that they had elevated liver enzymes. Microcystins have also been reported in terrestrial crops grown in China (Xiang et al., 2019), Nigeria (Chia et al., 2019), Saudi Arabia (Mohamed and Al Shehri, 2009), and Guatemala (Romero-Oliva et al., 2014). Therefore, this chronic, sublethal exposure to microcystins through terrestrial food could be a more widespread problem than is currently realized.

As a side note, the literature could benefit from more consistent reporting of GPS coordinates for sample sites. I had to exclude 16 otherwise usable papers because the descriptions in the methods were inadequate to locate the sample sites, despite extensive effort. Of the 133papers published after 2010 and included in this review, 84 papers reported GPS coordinates and 49 did not.

Conclusions

There is geographic disparity in microcystin monitoring. Given the toxic effects of microcystins and their global distribution, more basic research is needed on where microcystins are occurring, which congeners are present, and how humans are being exposed to them—particularly in areas of the world where this information is lacking. Monitoring should not be stopped in winter until it is determined that microcystins are no longer present, even if cyanobacterial blooms have dissipated. Additionally, when field reports are made of microcystins in the literature, care should be taken to ensure that the sites are easily locatable for the sake of replicability and future research.

Acknowledgments

Again, my thanks to the Plankton Ecology and Limnology Laboratory for all of their discussion and advice, and particularly to Dr. Jessica Beyer and Katherine Cook for their suggestions and feedback. Thanks also to Dr. Claire Curry of the University of Oklahoma Libraries for all her help with Excel and R. Funding for this project came from the University of Oklahoma Department of Biology Adams Summer Scholarships and the National Science Foundation (DEB 1831061).

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Figures

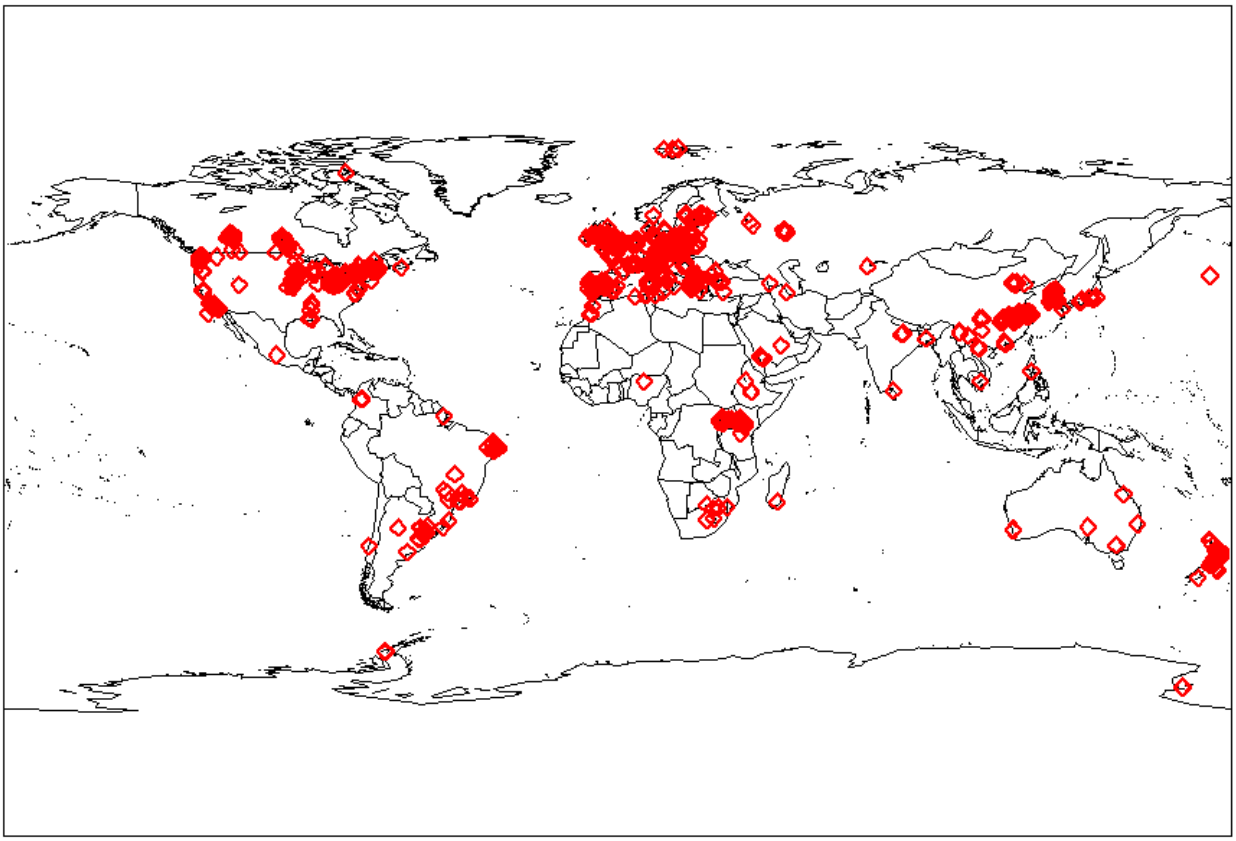


Figure 1: All reports of microcystin (N = 14,108).

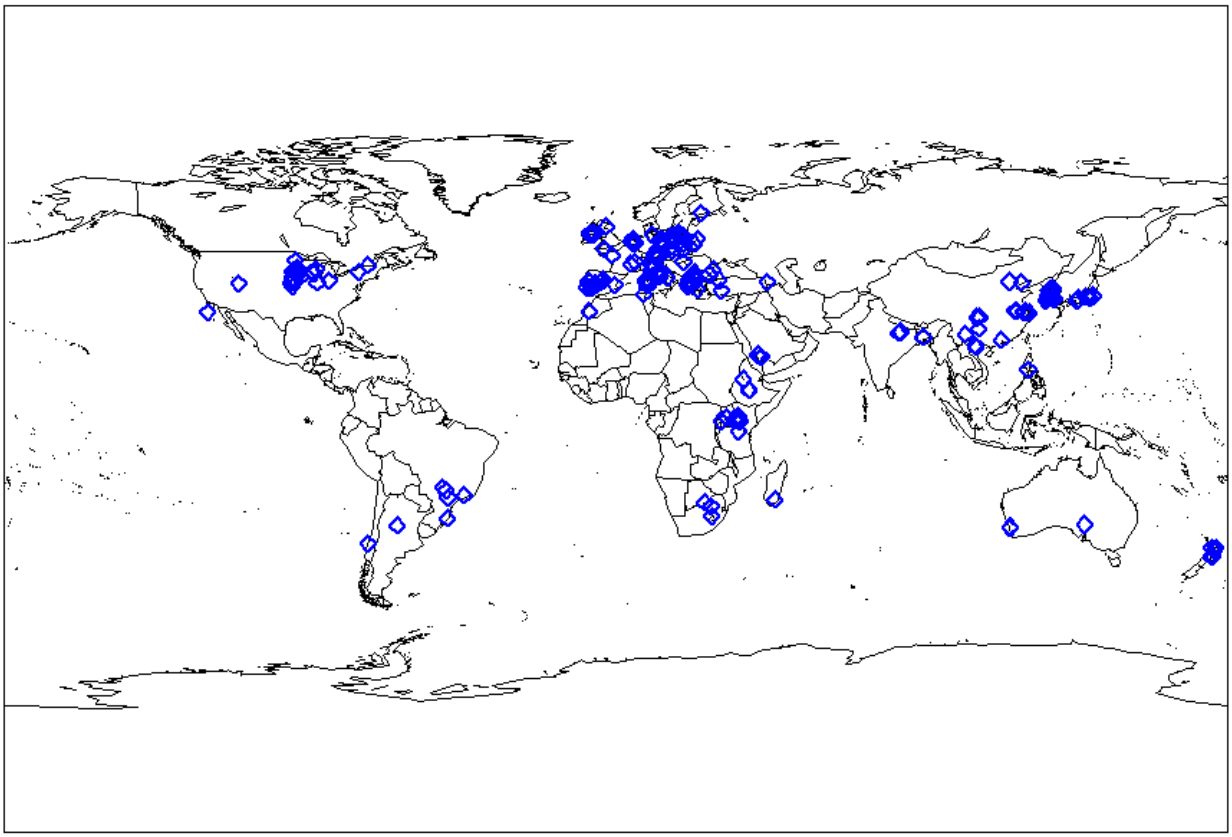


Figure 2: Reports of MC-RR (N = 2,195).

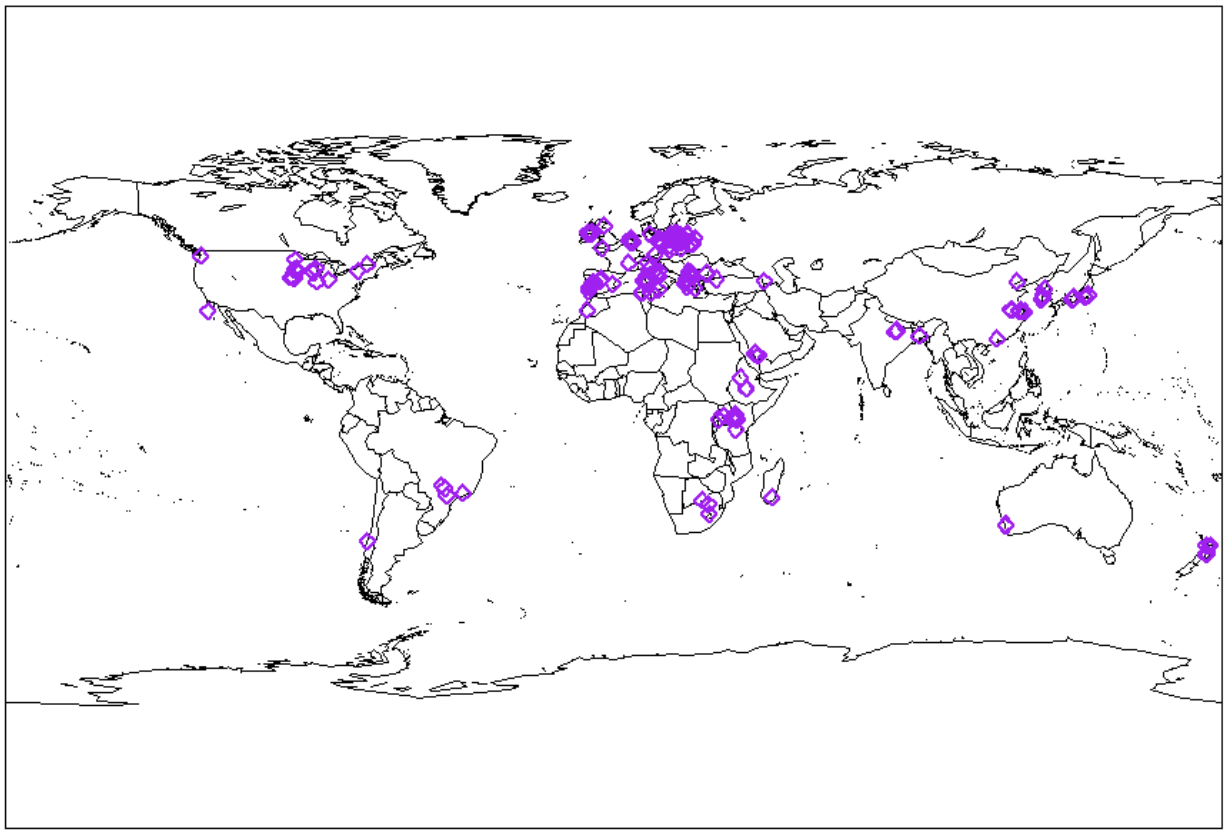


Figure 3: Reports of MC-YR (N = 1,254).

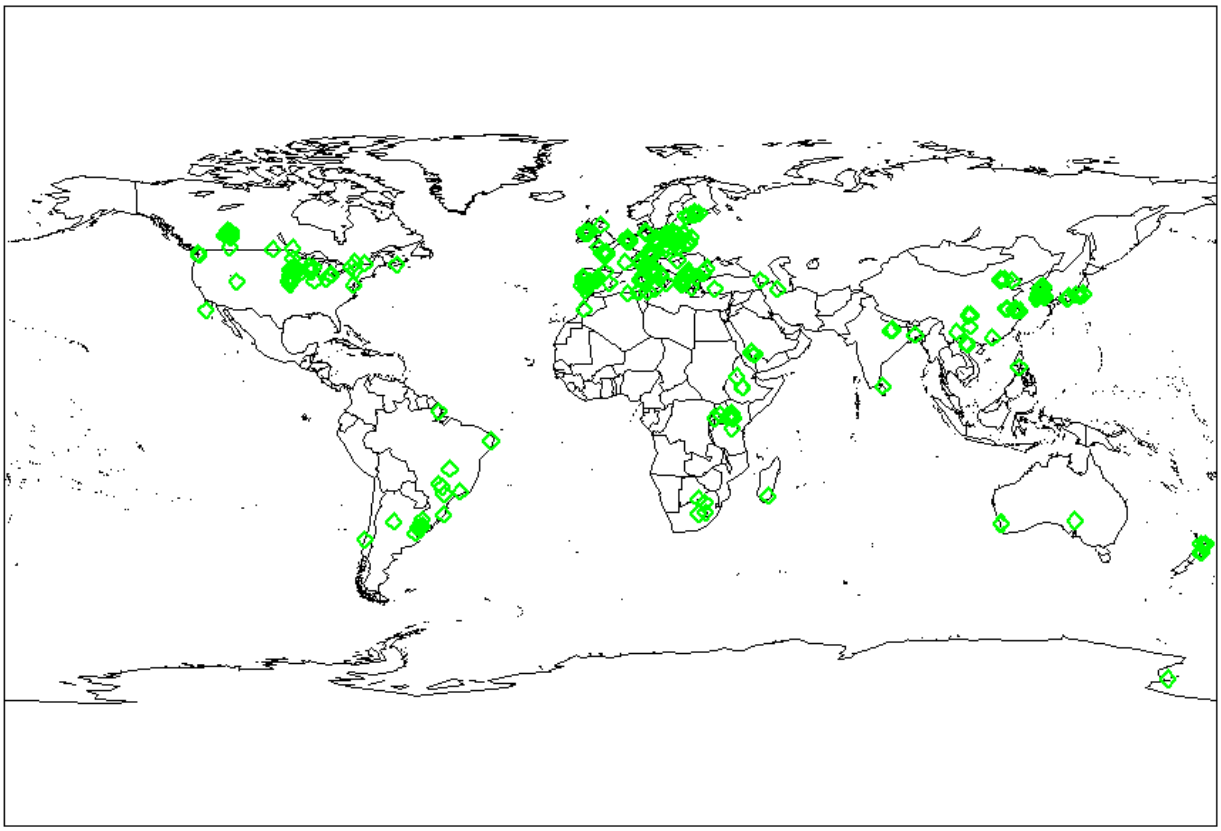
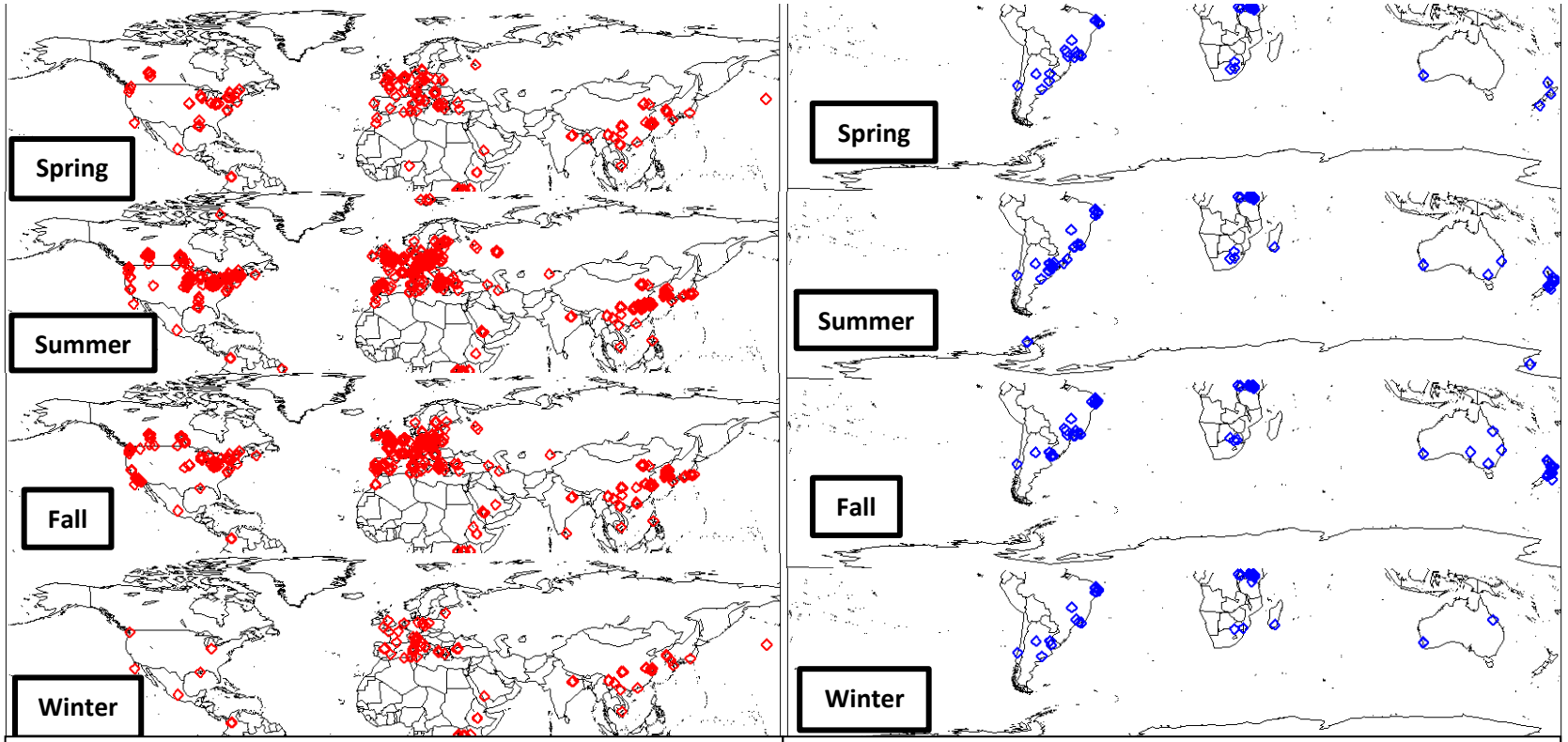


Figure 4: Reports of MC-LR (N = 2,468).



Spring: n = 1,666; papers = 141 Summer: n = 5,227; papers = 269 Fall: n = 4,200; papers = 255 Winter: n = 1,193; papers = 94	Spring: n = 350; papers = 33 Summer: n = 509; papers = 45 Fall: n = 641; papers = 45 Winter: n = 277; papers = 30

Figure 5: Reports of microcystins in the northern and southern hemispheres across the four seasons.

Tables

Table 1: The 10 most commonly reported cyanobacterial genera and the 10 most commonly reported microcystin congeners. Congeners are listed from most common to least common, left to right. Algae are listed from most common to least common, top to bottom. Note that these are reports of the algae genus being present and the congener being present. The congener was not necessarily detected inside the algal cells, and there was often more than one genus of algae present. Full tables with all the algae genera and congeners in alphabetical order are in the supplemental materials, one ordered first by algae and then by congener, the other ordered first by congener and then by algae.

	MC-LR	MC-RR	MC-YR	MC-LA	[D-Asp3]MC-RR	MC-LF	[Dha7]MC-LR	MC-WR	[D-Asp3]MC-LR	MC-LY
Microcystis	1,056	955	574	133	9	16	36	41	17	19
Planktothrix	113	199	139	19	85	18	-	-	22	10
Dolichospermum	248	219	147	47	4	14	14	17	5	10
Aphanizomenon	163	67	51	25	6	3	-	-	1	1
Oscillatoria	97	77	74	17	-	8	-	-	-	-
Pseudanabaena	21	25	18	12	-	4	-	1	1	1
Raphidiopsis	32	24	19	8	3	-	1	1	-	1
Limnothrix	10	6	5	-	-	-	-	-	-	-
Anabaena	39	35	32	10	-	4	-	-	-	-
Phormidium	21	26	18	13	-	10	-	-	-	-

Supplemental Material

Supplemental Table S1: All reports of microcystins and cyanobacteria, listed alphabetically by cyanobacterial genus. Counts represent the number of times a certain congener was reported when a certain cyanobacteria was present. More than one genus of cyanobacteria could be present when the congener was detected, and the congener was not necessarily detected within the cyanobacterial cell.

Algae Genus	**Congener**	**Count**
Alkalinema	MC-LR	5
Anabaena	MC-LA	10
Anabaena	MC-LF	4
Anabaena	MC-LR	39
Anabaena	MC-LW	9
Anabaena	MC-RR	35
Anabaena	MC-YR	32
Anabaena	Unnamed Congener	11
Anabaenopsis	MC-LA	2
Anabaenopsis	MC-LF	3
Anabaenopsis	MC-LR	28
Anabaenopsis	MC-LW	1
Anabaenopsis	MC-RR	14
Anabaenopsis	MC-YR	4
Anabaenopsis	Unnamed Congener	43
Anagnostidinema	Unnamed Congener	1
Aphanizomenon	[Asp3,ADMAdda5,Dhb7]MC-RR	1
Aphanizomenon	[Asp3]MC-HarR	3
Aphanizomenon	[D-Asp3]MC-HtyR	2
Aphanizomenon	[D-Asp3]MC-LR	1
Aphanizomenon	[D-Asp3]MC-RR	6
Aphanizomenon	[D-Asp3]MC-RY	1
Aphanizomenon	[Dha7]MC-RR	1
Aphanizomenon	[DMAdda5]MC-YR	1
Aphanizomenon	[Ser7]MC-RR	1
Aphanizomenon	MC-LA	25

Aphanizomenon	MC-LF	3
Aphanizomenon	MC-LR	163
Aphanizomenon	MC-LW	11
Aphanizomenon	MC-LY	1
Aphanizomenon	MC-RR	67
Aphanizomenon	MC-VR	1
Aphanizomenon	MC-YM	1
Aphanizomenon	MC-YR	51
Aphanizomenon	Unnamed Congener	417
Aphanocapsa	[D-Asp3]MC-LR	2
Aphanocapsa	MC-LA	7
Aphanocapsa	MC-LR	17
Aphanocapsa	MC-RR	21
Aphanocapsa	MC-YR	12
Aphanocapsa	Unnamed Congener	38
Aphanothece	MC-LR	14
Aphanothece	MC-LW	3
Aphanothece	MC-RR	8
Aphanothece	MC-YR	4
Aphanothece	Unnamed Congener	7
Arthrospira	Unnamed Congener	12
Calothrix	Unnamed Congener	2
Cephalothrix	MC-LR	1
Chroococcus	[Asp3,ADMAdda5,Dhb7]MC-RR	1
Chroococcus	[D-Asp3]MC-LR	1
Chroococcus	MC-LA	7
Chroococcus	MC-LR	24
Chroococcus	MC-LW	1
Chroococcus	MC-RR	24
Chroococcus	MC-YR	17
Chroococcus	Unnamed Congener	42
Chrysosporum	MC-LR	2
Chrysosporum	MC-RR	2
Chrysosporum	MC-YR	2
Chrysosporum	Unnamed Congener	2
Coelomoron	MC-LA	6
Coelomoron	MC-LR	6
Coelomoron	MC-RR	6
Coelomoron	MC-YR	2
Coelosphaeriopsis	Unnamed Congener	9

Coelosphaerium	MC-LR	3
Coelosphaerium	MC-RR	3
Coelosphaerium	MC-YR	3
Coelosphaerium	Unnamed Congener	19
Cuspidothrix	[Asp3,DMAdda5]MC-HarW	1
Cuspidothrix	[D-Asp3]MC-LR	3
Cuspidothrix	[D-Asp3]MC-RR	1
Cuspidothrix	MC-(H4)YR	1
Cuspidothrix	MC-AR	1
Cuspidothrix	MC-HilR	2
Cuspidothrix	MC-LA	1
Cuspidothrix	MC-LF	2
Cuspidothrix	MC-LR	7
Cuspidothrix	MC-LW	3
Cuspidothrix	MC-LY	2
Cuspidothrix	MC-RR	10
Cuspidothrix	MC-WR	2
Cuspidothrix	MC-YR	6
Cuspidothrix	Unnamed Congener	38
Cyanocatena	[D-Asp3]MC-LR	1
Cyanocatena	MC-HilR	1
Cyanocatena	MC-LA	4
Cyanocatena	MC-LF	1
Cyanocatena	MC-LR	6
Cyanocatena	MC-LW	1
Cyanocatena	MC-LY	1
Cyanocatena	MC-RR	6
Cyanocatena	MC-WR	1
Cyanocatena	MC-YR	3
Cyanocatena	Unnamed Congener	2
Cyanodictyon	MC-LR	1
Cyanodictyon	MC-RR	1
Cyanonephron	MC-LR	1
Cyanonephron	MC-RR	1
Cylindrospermopsis	Unnamed Congener	54
Cylindrospermum	Unnamed Congener	3
Dactylococcopsis	MC-LR	4
Dactylococcopsis	MC-RR	4
Dactylococcopsis	MC-YR	3
Dolichospermum	[Asp3,DMAdda5]MC-HarW	2
Dolichospermum	[D-Asp3]MC-LR	5

Dolichospermum	[D-Asp3]MC-RR	4
Dolichospermum	[D-Asp3]MC-RY	1
Dolichospermum	[Dha7]MC-LR	14
Dolichospermum	[Dha7]MC-RR	4
Dolichospermum	[Ser7]MC-RR	1
Dolichospermum	MC-AR	2
Dolichospermum	MC-FA	14
Dolichospermum	MC-FAbu	2
Dolichospermum	MC-FR	18
Dolichospermum	MC-HilR	3
Dolichospermum	MC-HtyR	2
Dolichospermum	MC-LA	47
Dolichospermum	MC-LAbu	6
Dolichospermum	MC-LF	14
Dolichospermum	MC-LR	248
Dolichospermum	MC-LW	18
Dolichospermum	MC-LY	10
Dolichospermum	MC-RA	8
Dolichospermum	MC-RAbu	6
Dolichospermum	MC-RR	219
Dolichospermum	MC-VR	1
Dolichospermum	MC-WA	12
Dolichospermum	MC-WAbu	1
Dolichospermum	MC-WR	17
Dolichospermum	MC-YR	147
Dolichospermum	Unnamed Congener	580
Epigloeosphaera	Unnamed Congener	1
Geitlerinema	Unnamed Congener	13
Gloeocapsa	Unnamed Congener	4
Gloeothece	MC-LR	5
Gloeothece	MC-RR	4
Gloeothece	MC-WR	1
Gloeothece	Unnamed Congener	12
Gloeotrichia	MC-LA	1
Gloeotrichia	MC-LR	9
Gloeotrichia	MC-RR	8
Gloeotrichia	MC-YR	6
Gloeotrichia	Unnamed Congener	54
Gomphosphaeria	MC-LF	5
Gomphosphaeria	MC-LR	25
Gomphosphaeria	MC-LY	5

Gomphosphaeria	MC-RR	6
Gomphosphaeria	MC-YR	8
Gomphosphaeria	Unnamed Congener	51
Heteroleibleinia	Unnamed Congener	2
Jaaginema	MC-RR	1
Kamptonema	MC-LA	16
Kamptonema	MC-LF	6
Kamptonema	MC-LR	1
Kamptonema	MC-LW	12
Kamptonema	MC-RR	8
Lemmermaniella	MC-LR	1
Lemmermaniella	MC-RR	1
Leptolyngbya	[Asp3,ADMAdda5,Dhb7]MC-RR	1
Leptolyngbya	MC-LR	3
Leptolyngbya	Unnamed Congener	7
Limnolyngbya	[D-Asp3]MC-LR	1
Limnolyngbya	MC-HilR	2
Limnolyngbya	MC-LA	1
Limnolyngbya	MC-LF	1
Limnolyngbya	MC-LR	5
Limnolyngbya	MC-LW	1
Limnolyngbya	MC-LY	1
Limnolyngbya	MC-RR	6
Limnolyngbya	MC-WR	1
Limnolyngbya	MC-YR	4
Limnolyngbya	Unnamed Congener	8
Limnospira	MC-LA	2
Limnospira	MC-LF	5
Limnospira	MC-LR	11
Limnospira	MC-RR	19
Limnospira	MC-YR	3
Limnothrix	[D-Asp3]MC-HtyR	1
Limnothrix	[DMAdda5]MC-YR	1
Limnothrix	MC-HilR	1
Limnothrix	MC-LR	10
Limnothrix	MC-RR	6
Limnothrix	MC-YR	5
Limnothrix	Unnamed Congener	123
Lyngbya	MC-LA	3
Lyngbya	MC-LR	13

Lyngbya	MC-RR	15
Lyngbya	MC-YR	12
Lyngbya	Unnamed Congener	56
Merismopedia	MC-HilR	1
Merismopedia	MC-LR	11
Merismopedia	MC-RR	8
Merismopedia	MC-YR	6
Merismopedia	Unnamed Congener	13
Microcrocis	MC-LR	1
Microcrocis	MC-RR	1
Microcrocis	MC-YR	1
Microcystis	[(6Z)-Adda5]MC-LR	3
Microcystis	[ADMAdda5]MC-LHar	1
Microcystis	[ADMAdda5]MC-LR	1
Microcystis	[Asp3,DMAdda5]MC-HarW	1
Microcystis	[Asp3,DMAdda5]MC-LR	1
Microcystis	[Asp3]MC-HarAba	1
Microcystis	[Asp3]MC-HarR	3
Microcystis	[Asp3]MC-LR	2
Microcystis	[Asp3]MC-LY	1
Microcystis	[Asp3]MC-Raba	1
Microcystis	[Asp3]MC-RR	2
Microcystis	[D-Asp3,ADMAdda5]MC-LR	1
Microcystis	[D-Asp3,Dha7]MC-RR	1
Microcystis	[D-Asp3]MC-HtyR	2
Microcystis	[D-Asp3]MC-LR	17
Microcystis	[D-Asp3]MC-RR	9
Microcystis	[D-Asp3]MC-YR	1
Microcystis	[D-Glu(OMe)6]MC-LR	1
Microcystis	[D-Leu1]MC-LR	2
Microcystis	[Dha7]MC-LR	36
Microcystis	[Dha7]MC-RR	18
Microcystis	[Dha7]MC-YR	5
Microcystis	[DMAdda5]MC-LR	3
Microcystis	[DMAdda5]MC-YR	1
Microcystis	[Glu(Ome)6]MC-FR	1
Microcystis	[Glu(Ome)6]MC-LR	1
Microcystis	[Mser7]MC-LR	3
Microcystis	MC-(H4)YR	13
Microcystis	MC-AHar	13
Microcystis	MC-AR	22

Microcystis	MC-FA	14
Microcystis	MC-FAbu	2
Microcystis	MC-FR	28
Microcystis	MC-HilR	6
Microcystis	MC-Hty(OMe)R	1
Microcystis	MC-HtyR	2
Microcystis	MC-LA	133
Microcystis	MC-LAbu	6
Microcystis	MC-LF	16
Microcystis	MC-LR	1056
Microcystis	MC-LW	23
Microcystis	MC-LY	19
Microcystis	MC-M(O)R	1
Microcystis	MC-RA	8
Microcystis	MC-RAbu	6
Microcystis	MC-RR	955
Microcystis	MC-RY	1
Microcystis	MC-WA	12
Microcystis	MC-WAbu	1
Microcystis	MC-WR	41
Microcystis	MC-YA	1
Microcystis	MC-YM	2
Microcystis	MC-YR	574
Microcystis	Unnamed Congener	2146
Nodularia	MC-HtyR	1
Nodularia	MC-LR	6
Nodularia	MC-RR	1
Nodularia	MC-YR	1
Nodularia	Unnamed Congener	6
Nostoc	[Asp3,ADMAdda5,Dhb7]MC-LR	1
Nostoc	[D-Asp3]MC-LR	1
Nostoc	MC-LA	1
Nostoc	MC-LR	9
Nostoc	MC-RR	2
Nostoc	Unnamed Congener	7
Oscillatoria	MC-LA	17
Oscillatoria	MC-LF	8
Oscillatoria	MC-LR	97
Oscillatoria	MC-LW	12
Oscillatoria	MC-RR	77

Oscillatoria	MC-YR	74
Oscillatoria	Unnamed Congener	71
Pannus	MC-LR	1
Pannus	MC-LW	1
Pannus	MC-RR	1
Phormidium	MC-LA	13
Phormidium	MC-LF	10
Phormidium	MC-LR	21
Phormidium	MC-LW	9
Phormidium	MC-RR	26
Phormidium	MC-YR	18
Phormidium	Unnamed Congener	42
Planktolyngbya	[D-Asp3]MC-LR	1
Planktolyngbya	[D-Asp3]MC-RR	1
Planktolyngbya	MC-HilR	2
Planktolyngbya	MC-LA	1
Planktolyngbya	MC-LF	1
Planktolyngbya	MC-LR	6
Planktolyngbya	MC-LW	2
Planktolyngbya	MC-LY	1
Planktolyngbya	MC-RR	7
Planktolyngbya	MC-WR	1
Planktolyngbya	MC-YR	4
Planktolyngbya	Unnamed Congener	42
Planktothrix	[Asp3,DMAdda5]MC-HarW	2
Planktothrix	[Asp3]MC-HarR	3
Planktothrix	[D-Asp3,(Z)-Dhb7]MC-HtyR	1
Planktothrix	[D-Asp3,(Z)-Dhb7]MC-LR	1
Planktothrix	[D-Asp3,ADMAdda5,Dhb7]MC-HtyR	1
Planktothrix	[D-Asp3,D-Glu(OMe)6]MC-RR	1
Planktothrix	[D-Asp3,Dha7]MC-LR	1
Planktothrix	[D-Asp3,Dhb7]MC-RR	7
Planktothrix	[D-Asp3]MC-HtyR	5
Planktothrix	[D-Asp3]MC-LR	22
Planktothrix	[D-Asp3]MC-RR	85
Planktothrix	[D-Asp3]MC-RY	1
Planktothrix	[D-Asp3]MC-YR	6
Planktothrix	[D-Glu(OC3H6O)6]MC-LR	6
Planktothrix	[Dha7]MC-RR	2

Planktothrix	[DMAdda5, Glu(Ome)6,Dhb7]MC-YR	1
Planktothrix	[DMAdda5]MC-YR	1
Planktothrix	[seco][D-Asp3]MC-RR	1
Planktothrix	MC-AR	1
Planktothrix	MC-LA	19
Planktothrix	MC-LF	18
Planktothrix	MC-LR	113
Planktothrix	MC-LW	19
Planktothrix	MC-LY	10
Planktothrix	MC-RR	199
Planktothrix	MC-VR	1
Planktothrix	MC-YA	1
Planktothrix	MC-YM	1
Planktothrix	MC-YR	139
Planktothrix	Unnamed Congener	1088
Pseudanabaena	[D-Asp3]MC-HtyR	1
Pseudanabaena	[D-Asp3]MC-LR	1
Pseudanabaena	[DMAdda5]MC-YR	1
Pseudanabaena	MC-HilR	2
Pseudanabaena	MC-LA	12
Pseudanabaena	MC-LF	4
Pseudanabaena	MC-LR	21
Pseudanabaena	MC-LW	14
Pseudanabaena	MC-LY	1
Pseudanabaena	MC-RR	25
Pseudanabaena	MC-WR	1
Pseudanabaena	MC-YR	18
Pseudanabaena	Unnamed Congener	154
Radiocystis	MC-LR	1
Radiocystis	MC-RR	1
Radiocystis	Unnamed Congener	2
Raphidiopsis	[Asp3,DMAdda5]MC-LR	1
Raphidiopsis	[Asp3]MC-HarR	2
Raphidiopsis	[Asp3]MC-LY	1
Raphidiopsis	[D-Asp3,ADMAdda5,Dhb7]MC-HtyR	1
Raphidiopsis	[D-Asp3]MC-HtyR	2
Raphidiopsis	[D-Asp3]MC-RR	3
Raphidiopsis	[Dha7]MC-LR	1
Raphidiopsis	[Dha7]MC-RR	1

Raphidiopsis	[DMAdda5]MC-LR	1
Raphidiopsis	[DMAdda5]MC-YR	1
Raphidiopsis	MC-HilR	1
Raphidiopsis	MC-LA	8
Raphidiopsis	MC-LR	32
Raphidiopsis	MC-LW	1
Raphidiopsis	MC-LY	1
Raphidiopsis	MC-RR	24
Raphidiopsis	MC-RY	1
Raphidiopsis	MC-WR	1
Raphidiopsis	MC-YR	19
Raphidiopsis	Unnamed Congener	106
Rivularia	Unnamed Congener	3
Romeria	MC-HilR	1
Romeria	MC-LR	2
Romeria	MC-LW	1
Romeria	MC-RR	2
Romeria	MC-YR	1
Romeria	Unnamed Congener	1
Snowella	[D-Asp3]MC-LR	1
Snowella	MC-LR	2
Snowella	MC-LW	1
Snowella	MC-RR	3
Snowella	MC-YR	1
Snowella	Unnamed Congener	3
Sphaerocavum	MC-LR	1
Sphaerocavum	MC-RR	1
Sphaerospermopsis	[Asp3,DMAdda5]MC-LR	1
Sphaerospermopsis	[Asp3]MC-LY	1
Sphaerospermopsis	[Dha7]MC-LR	1
Sphaerospermopsis	[DMAdda5]MC-LR	1
Sphaerospermopsis	MC-LA	1
Sphaerospermopsis	MC-LR	2
Sphaerospermopsis	MC-LY	1
Sphaerospermopsis	MC-RR	2
Sphaerospermopsis	MC-RY	1
Sphaerospermopsis	MC-WR	1
Sphaerospermopsis	MC-YR	1
Sphaerospermopsis	Unnamed Congener	11
Spirulina	MC-LF	3
Spirulina	MC-LR	5

Spirulina	MC-RR	5
Spirulina	MC-YR	5
Spirulina	Unnamed Congener	4
Synechococcus	MC-LA	17
Synechococcus	MC-LF	12
Synechococcus	MC-LR	10
Synechococcus	MC-LW	12
Synechococcus	MC-RR	22
Synechococcus	MC-YR	7
Synechococcus	Unnamed Congener	7
Synechocystis	MC-LA	6
Synechocystis	MC-LF	2
Synechocystis	MC-LR	1
Synechocystis	MC-LW	6
Synechocystis	MC-RR	7
Trichodesmium	Unnamed Congener	5
Woronichinia	[D-Asp3]MC-LR	1
Woronichinia	[D-Asp3]MC-RR	2
Woronichinia	MC-(H4)YR	1
Woronichinia	MC-LR	12
Woronichinia	MC-RR	11
Woronichinia	MC-WR	2
Woronichinia	MC-YR	5
Woronichinia	Unnamed Congener	60
NA	[(6Z)-Adda5]MC-LR	3
NA	[ADMAdda5]MC-LHar	1
NA	[ADMAdda5]MC-LR	1
NA	[Asp3,ADMAdda5,Dhb7]MC-LR	5
NA	[Asp3,ADMAdda5,Dhb7]MC-RR	4
NA	[Asp3,ADMAdda5,Thr7]MC-LR	1
NA	[Asp3,Dha7]MC-RR	3
NA	[Asp3,Dhb7]MC-AhaR	1
NA	[Asp3,Dhb7]MC-HtyR	2
NA	[Asp3,Dhb7]MC-LR	2
NA	[Asp3,Dhb7]MC-LY	1
NA	[Asp3,Dhb7]MC-RR	2
NA	[Asp3,Dhb7]MC-RY	2
NA	[Asp3,DMAdda5,Dhb7]MC-LR	2

NA	[Asp3,DMAdda5]MC-HarW	2
NA	[Asp3,DMAdda5]MC-LR	1
NA	[Asp3,Ser7]MC-RR	1
NA	[Asp3]MC-HarAba	1
NA	[Asp3]MC-HarR	3
NA	[Asp3]MC-LR	2
NA	[Asp3]MC-LY	2
NA	[Asp3]MC-Raba	1
NA	[Asp3]MC-RR	4
NA	[Asp3]MC-RY	2
NA	[D-Asp3,(E)-Dhb7]MC-RR	4
NA	[D-Asp3,(Z)-Dhb7]MC-HtyR	1
NA	[D-Asp3,(Z)-Dhb7]MC-LR	1
NA	[D-Asp3,ADMAdda5,Dhb7]MC-HtyR	1
NA	[D-Asp3,ADMAdda5]MC-LR	1
NA	[D-Asp3,D-Glu(OMe)6]MC-RR	1
NA	[D-Asp3,Dha7]MC-LR	2
NA	[D-Asp3,Dha7]MC-RR	4
NA	[D-Asp3,Dha7]MC-YR	1
NA	[D-Asp3,Dhb7]MC-RR	7
NA	[D-Asp3]MC-HtyR	5
NA	[D-Asp3]MC-LF	1
NA	[D-Asp3]MC-LR	49
NA	[D-Asp3]MC-LW	1
NA	[D-Asp3]MC-RR	102
NA	[D-Asp3]MC-RY	2
NA	[D-Asp3]MC-YR	7
NA	[D-Glu(OC3H6O)6]MC-LR	6
NA	[D-Glu(OMe)6]MC-LR	1
NA	[D-Leu1,Glu(OMe)6]MC-LR	1
NA	[D-Leu1]MC-LR	13
NA	[Dha7]MC-LR	56
NA	[Dha7]MC-RR	19
NA	[Dha7]MC-YR	5
NA	[DMAdda5, Glu(Ome)6,Dhb7]MC-YR	1
NA	[DMAdda5]MC-LR	3
NA	[DMAdda5]MC-YR	1
NA	[Glu(Ome)6]MC-FR	1

NA	[Glu(Ome)6]MC-LR	1
NA	[Mser7]MC-LR	3
NA	[seco][D-Asp3]MC-RR	1
NA	[Ser7]MC-RR	1
NA	MC-(H4)YR	22
NA	MC-AHar	13
NA	MC-AR	25
NA	MC-FA	14
NA	MC-FAbu	2
NA	MC-FR	29
NA	MC-HilR	15
NA	MC-Hty(OMe)R	1
NA	MC-HtyR	11
NA	MC-LA	287
NA	MC-LAbu	6
NA	MC-LF	59
NA	MC-LR	2468
NA	MC-LR Cys conjugate	24
NA	MC-LR GSH conjugate	27
NA	MC-LW	44
NA	MC-LY	48
NA	MC-M(O)R	1
NA	MC-RA	8
NA	MC-RAbu	6
NA	MC-RR	2195
NA	MC-RR Cys conjugate	22
NA	MC-RR GSH conjugate	3
NA	MC-RY	1
NA	MC-VR	2
NA	MC-WA	12
NA	MC-WAbu	1
NA	MC-WR	51
NA	MC-YA	2
NA	MC-YM	2
NA	MC-YR	1254
NA	Unnamed Congener	7101

Supplemental Table S2: All reports of microcystins and cyanobacteria, listed alphabetically by congener. Counts represent the number of times a certain congener was reported when a certain cyanobacteria was present. More than one genus of cyanobacteria could be present when the congener was detected, and the congener was not necessarily detected within the cyanobacterial cell.

Congener	**Algae Genus**	**Count**
[(6Z)-Adda5]MC-LR	*Microcystis*	3
[(6Z)-Adda5]MC-LR	NA	3
[ADMAdda5]MC-LHar	*Microcystis*	1
[ADMAdda5]MC-LHar	NA	1
[ADMAdda5]MC-LR	*Microcystis*	1
[ADMAdda5]MC-LR	NA	1
[Asp3,ADMAdda5,Dhb7]MC-LR	*Nostoc*	1
[Asp3,ADMAdda5,Dhb7]MC-LR	NA	5
[Asp3,ADMAdda5,Dhb7]MC-RR	*Aphanizomenon*	1
[Asp3,ADMAdda5,Dhb7]MC-RR	*Chroococcus*	1
[Asp3,ADMAdda5,Dhb7]MC-RR	*Leptolyngbya*	1
[Asp3,ADMAdda5,Dhb7]MC-RR	NA	4
[Asp3,ADMAdda5,Thr7]MC-LR	NA	1
[Asp3,Dha7]MC-RR	NA	3
[Asp3,Dhb7]MC-AhaR	NA	1
[Asp3,Dhb7]MC-HtyR	NA	2
[Asp3,Dhb7]MC-LR	NA	2
[Asp3,Dhb7]MC-LY	NA	1
[Asp3,Dhb7]MC-RR	NA	2
[Asp3,Dhb7]MC-RY	NA	2
[Asp3,DMAdda5,Dhb7]MC-LR	NA	2
[Asp3,DMAdda5]MC-HarW	*Cuspidothrix*	1

[Asp3,DMAdda5]MC-HarW	*Dolichospermum*	2
[Asp3,DMAdda5]MC-HarW	*Microcystis*	1
[Asp3,DMAdda5]MC-HarW	*Planktothrix*	2
[Asp3,DMAdda5]MC-HarW	NA	2
[Asp3,DMAdda5]MC-LR	*Microcystis*	1
[Asp3,DMAdda5]MC-LR	*Raphidiopsis*	1
[Asp3,DMAdda5]MC-LR	*Sphaerospermopsis*	1
[Asp3,DMAdda5]MC-LR	NA	1
[Asp3,Ser7]MC-RR	NA	1
[Asp3]MC-HarAba	*Microcystis*	1
[Asp3]MC-HarAba	NA	1
[Asp3]MC-HarR	*Aphanizomenon*	3
[Asp3]MC-HarR	*Microcystis*	3
[Asp3]MC-HarR	*Planktothrix*	3
[Asp3]MC-HarR	*Raphidiopsis*	2
[Asp3]MC-HarR	NA	3
[Asp3]MC-LR	*Microcystis*	2
[Asp3]MC-LR	NA	2
[Asp3]MC-LY	*Microcystis*	1
[Asp3]MC-LY	*Raphidiopsis*	1
[Asp3]MC-LY	*Sphaerospermopsis*	1
[Asp3]MC-LY	NA	2
[Asp3]MC-Raba	*Microcystis*	1
[Asp3]MC-Raba	NA	1
[Asp3]MC-RR	*Microcystis*	2
[Asp3]MC-RR	NA	4
[Asp3]MC-RY	NA	2
[D-Asp3,(E)-Dhb7]MC-RR	NA	4
[D-Asp3,(Z)-Dhb7]MC-HtyR	*Planktothrix*	1
[D-Asp3,(Z)-Dhb7]MC-HtyR	NA	1
[D-Asp3,(Z)-Dhb7]MC-LR	*Planktothrix*	1
[D-Asp3,(Z)-Dhb7]MC-LR	NA	1
[D-Asp3,ADMAdda5,Dhb7]MC-HtyR	*Planktothrix*	1
[D-Asp3,ADMAdda5,Dhb7]MC-HtyR	*Raphidiopsis*	1
[D-Asp3,ADMAdda5,Dhb7]MC-HtyR	NA	1
[D-Asp3,ADMAdda5]MC-LR	*Microcystis*	1

[D-Asp3,ADMAdda5]MC-LR	NA	1
[D-Asp3,D-Glu(OMe)6]MC-RR	*Planktothrix*	1
[D-Asp3,D-Glu(OMe)6]MC-RR	NA	1
[D-Asp3,Dha7]MC-LR	*Planktothrix*	1
[D-Asp3,Dha7]MC-LR	NA	2
[D-Asp3,Dha7]MC-RR	*Microcystis*	1
[D-Asp3,Dha7]MC-RR	NA	4
[D-Asp3,Dha7]MC-YR	NA	1
[D-Asp3,Dhb7]MC-RR	*Planktothrix*	7
[D-Asp3,Dhb7]MC-RR	NA	7
[D-Asp3]MC-HtyR	*Aphanizomenon*	2
[D-Asp3]MC-HtyR	*Limnothrix*	1
[D-Asp3]MC-HtyR	*Microcystis*	2
[D-Asp3]MC-HtyR	*Planktothrix*	5
[D-Asp3]MC-HtyR	*Pseudanabaena*	1
[D-Asp3]MC-HtyR	*Raphidiopsis*	2
[D-Asp3]MC-HtyR	NA	5
[D-Asp3]MC-LF	NA	1
[D-Asp3]MC-LR	*Aphanizomenon*	1
[D-Asp3]MC-LR	*Aphanocapsa*	2
[D-Asp3]MC-LR	*Chroococcus*	1
[D-Asp3]MC-LR	*Cuspidothrix*	3
[D-Asp3]MC-LR	*Cyanocatena*	1
[D-Asp3]MC-LR	*Dolichospermum*	5
[D-Asp3]MC-LR	*Limnolyngbya*	1
[D-Asp3]MC-LR	*Microcystis*	17
[D-Asp3]MC-LR	*Nostoc*	1
[D-Asp3]MC-LR	*Planktolyngbya*	1
[D-Asp3]MC-LR	*Planktothrix*	22
[D-Asp3]MC-LR	*Pseudanabaena*	1
[D-Asp3]MC-LR	*Snowella*	1
[D-Asp3]MC-LR	*Woronichinia*	1
[D-Asp3]MC-LR	NA	49
[D-Asp3]MC-LW	NA	1
[D-Asp3]MC-RR	*Aphanizomenon*	6
[D-Asp3]MC-RR	*Cuspidothrix*	1
[D-Asp3]MC-RR	*Dolichospermum*	4
[D-Asp3]MC-RR	*Microcystis*	9
[D-Asp3]MC-RR	*Planktolyngbya*	1
[D-Asp3]MC-RR	*Planktothrix*	85

[D-Asp3]MC-RR	*Raphidiopsis*	3
[D-Asp3]MC-RR	*Woronichinia*	2
[D-Asp3]MC-RR	NA	102
[D-Asp3]MC-RY	*Aphanizomenon*	1
[D-Asp3]MC-RY	*Dolichospermum*	1
[D-Asp3]MC-RY	*Planktothrix*	1
[D-Asp3]MC-RY	NA	2
[D-Asp3]MC-YR	*Microcystis*	1
[D-Asp3]MC-YR	*Planktothrix*	6
[D-Asp3]MC-YR	NA	7
[D-Glu(OC3H6O)6]MC-LR	*Planktothrix*	6
[D-Glu(OC3H6O)6]MC-LR	NA	6
[D-Glu(OMe)6]MC-LR	*Microcystis*	1
[D-Glu(OMe)6]MC-LR	NA	1
[D-Leu1,Glu(OMe)6]MC-LR	NA	1
[D-Leu1]MC-LR	*Microcystis*	2
[D-Leu1]MC-LR	NA	13
[Dha7]MC-LR	*Dolichospermum*	14
[Dha7]MC-LR	*Microcystis*	36
[Dha7]MC-LR	*Raphidiopsis*	1
[Dha7]MC-LR	*Sphaerospermopsis*	1
[Dha7]MC-LR	NA	56
[Dha7]MC-RR	*Aphanizomenon*	1
[Dha7]MC-RR	*Dolichospermum*	4
[Dha7]MC-RR	*Microcystis*	18
[Dha7]MC-RR	*Planktothrix*	2
[Dha7]MC-RR	*Raphidiopsis*	1
[Dha7]MC-RR	NA	19
[Dha7]MC-YR	*Microcystis*	5
[Dha7]MC-YR	NA	5
[DMAdda5, Glu(Ome)6,Dhb7]MC-YR	*Planktothrix*	1
[DMAdda5, Glu(Ome)6,Dhb7]MC-YR	NA	1
[DMAdda5]MC-LR	*Microcystis*	3
[DMAdda5]MC-LR	*Raphidiopsis*	1
[DMAdda5]MC-LR	*Sphaerospermopsis*	1
[DMAdda5]MC-LR	NA	3
[DMAdda5]MC-YR	*Aphanizomenon*	1
[DMAdda5]MC-YR	*Limnothrix*	1
[DMAdda5]MC-YR	*Microcystis*	1
[DMAdda5]MC-YR	*Planktothrix*	1

[DMAdda5]MC-YR	*Pseudanabaena*	1
[DMAdda5]MC-YR	*Raphidiopsis*	1
[DMAdda5]MC-YR	NA	1
[Glu(Ome)6]MC-FR	*Microcystis*	1
[Glu(Ome)6]MC-FR	NA	1
[Glu(Ome)6]MC-LR	*Microcystis*	1
[Glu(Ome)6]MC-LR	NA	1
[Mser7]MC-LR	*Microcystis*	3
[Mser7]MC-LR	NA	3
[seco][D-Asp3]MC-RR	*Planktothrix*	1
[seco][D-Asp3]MC-RR	NA	1
[Ser7]MC-RR	*Aphanizomenon*	1
[Ser7]MC-RR	*Dolichospermum*	1
[Ser7]MC-RR	NA	1
MC-(H4)YR	*Cuspidothrix*	1
MC-(H4)YR	*Microcystis*	13
MC-(H4)YR	*Woronichinia*	1
MC-(H4)YR	NA	22
MC-AHar	*Microcystis*	13
MC-AHar	NA	13
MC-AR	*Cuspidothrix*	1
MC-AR	*Dolichospermum*	2
MC-AR	*Microcystis*	22
MC-AR	*Planktothrix*	1
MC-AR	NA	25
MC-FA	*Dolichospermum*	14
MC-FA	*Microcystis*	14
MC-FA	NA	14
MC-FAbu	*Dolichospermum*	2
MC-FAbu	*Microcystis*	2
MC-FAbu	NA	2
MC-FR	*Dolichospermum*	18
MC-FR	*Microcystis*	28
MC-FR	NA	29
MC-HilR	*Cuspidothrix*	2
MC-HilR	*Cyanocatena*	1
MC-HilR	*Dolichospermum*	3
MC-HilR	*Limnolyngbya*	2
MC-HilR	*Limnothrix*	1
MC-HilR	*Merismopedia*	1
MC-HilR	*Microcystis*	6

MC-HilR	*Planktolyngbya*	2
MC-HilR	*Pseudanabaena*	2
MC-HilR	*Raphidiopsis*	1
MC-HilR	*Romeria*	1
MC-HilR	NA	15
MC-Hty(OMe)R	*Microcystis*	1
MC-Hty(OMe)R	NA	1
MC-HtyR	*Dolichospermum*	2
MC-HtyR	*Microcystis*	2
MC-HtyR	*Nodularia*	1
MC-HtyR	NA	11
MC-LA	*Anabaena*	10
MC-LA	*Anabaenopsis*	2
MC-LA	*Aphanizomenon*	25
MC-LA	*Aphanocapsa*	7
MC-LA	*Chroococcus*	7
MC-LA	*Coelomoron*	6
MC-LA	*Cuspidothrix*	1
MC-LA	*Cyanocatena*	4
MC-LA	*Dolichospermum*	47
MC-LA	*Gloeotrichia*	1
MC-LA	*Kamptonema*	16
MC-LA	*Limnolyngbya*	1
MC-LA	*Limnospira*	2
MC-LA	*Lyngbya*	3
MC-LA	*Microcystis*	133
MC-LA	*Nostoc*	1
MC-LA	*Oscillatoria*	17
MC-LA	*Phormidium*	13
MC-LA	*Planktolyngbya*	1
MC-LA	*Planktothrix*	19
MC-LA	*Pseudanabaena*	12
MC-LA	*Raphidiopsis*	8
MC-LA	*Sphaerospermopsis*	1
MC-LA	*Synechococcus*	17
MC-LA	*Synechocystis*	6
MC-LA	NA	287
MC-LAbu	*Dolichospermum*	6
MC-LAbu	*Microcystis*	6
MC-LAbu	NA	6
MC-LF	*Anabaena*	4

MC-LF	*Anabaenopsis*	3
MC-LF	*Aphanizomenon*	3
MC-LF	*Cuspidothrix*	2
MC-LF	*Cyanocatena*	1
MC-LF	*Dolichospermum*	14
MC-LF	*Gomphosphaeria*	5
MC-LF	*Kamptonema*	6
MC-LF	*Limnolyngbya*	1
MC-LF	*Limnospira*	5
MC-LF	*Microcystis*	16
MC-LF	*Oscillatoria*	8
MC-LF	*Phormidium*	10
MC-LF	*Planktolyngbya*	1
MC-LF	*Planktothrix*	18
MC-LF	*Pseudanabaena*	4
MC-LF	*Spirulina*	3
MC-LF	*Synechococcus*	12
MC-LF	*Synechocystis*	2
MC-LF	NA	59
MC-LR	*Alkalinema*	5
MC-LR	*Anabaena*	39
MC-LR	*Anabaenopsis*	28
MC-LR	*Aphanizomenon*	163
MC-LR	*Aphanocapsa*	17
MC-LR	*Aphanothece*	14
MC-LR	*Cephalothrix*	1
MC-LR	*Chroococcus*	24
MC-LR	*Chrysosporum*	2
MC-LR	*Coelomoron*	6
MC-LR	*Coelosphaerium*	3
MC-LR	*Cuspidothrix*	7
MC-LR	*Cyanocatena*	6
MC-LR	*Cyanodictyon*	1
MC-LR	*Cyanonephron*	1
MC-LR	*Dactylococcopsis*	4
MC-LR	*Dolichospermum*	248
MC-LR	*Gloeothece*	5
MC-LR	*Gloeotrichia*	9
MC-LR	*Gomphosphaeria*	25
MC-LR	*Kamptonema*	1
MC-LR	*Lemmermaniella*	1

MC-LR	*Leptolyngbya*	3
MC-LR	*Limnolyngbya*	5
MC-LR	*Limnospira*	11
MC-LR	*Limnothrix*	10
MC-LR	*Lyngbya*	13
MC-LR	*Merismopedia*	11
MC-LR	*Microcrocis*	1
MC-LR	*Microcystis*	1056
MC-LR	*Nodularia*	6
MC-LR	*Nostoc*	9
MC-LR	*Oscillatoria*	97
MC-LR	*Pannus*	1
MC-LR	*Phormidium*	21
MC-LR	*Planktolyngbya*	6
MC-LR	*Planktothrix*	113
MC-LR	*Pseudanabaena*	21
MC-LR	*Radiocystis*	1
MC-LR	*Raphidiopsis*	32
MC-LR	*Romeria*	2
MC-LR	*Snowella*	2
MC-LR	*Sphaerocavum*	1
MC-LR	*Sphaerospermopsis*	2
MC-LR	*Spirulina*	5
MC-LR	*Synechococcus*	10
MC-LR	*Synechocystis*	1
MC-LR	*Woronichinia*	12
MC-LR	NA	2468
MC-LR Cys conjugate	NA	24
MC-LR GSH conjugate	NA	27
MC-LW	*Anabaena*	9
MC-LW	*Anabaenopsis*	1
MC-LW	*Aphanizomenon*	11
MC-LW	*Aphanothece*	3
MC-LW	*Chroococcus*	1
MC-LW	*Cuspidothrix*	3
MC-LW	*Cyanocatena*	1
MC-LW	*Dolichospermum*	18
MC-LW	*Kamptonema*	12
MC-LW	*Limnolyngbya*	1
MC-LW	*Microcystis*	23
MC-LW	*Oscillatoria*	12

MC-LW	*Pannus*	1
MC-LW	*Phormidium*	9
MC-LW	*Planktolyngbya*	2
MC-LW	*Planktothrix*	19
MC-LW	*Pseudanabaena*	14
MC-LW	*Raphidiopsis*	1
MC-LW	*Romeria*	1
MC-LW	*Snowella*	1
MC-LW	*Synechococcus*	12
MC-LW	*Synechocystis*	6
MC-LW	NA	44
MC-LY	*Aphanizomenon*	1
MC-LY	*Cuspidothrix*	2
MC-LY	*Cyanocatena*	1
MC-LY	*Dolichospermum*	10
MC-LY	*Gomphosphaeria*	5
MC-LY	*Limnolyngbya*	1
MC-LY	*Microcystis*	19
MC-LY	*Planktolyngbya*	1
MC-LY	*Planktothrix*	10
MC-LY	*Pseudanabaena*	1
MC-LY	*Raphidiopsis*	1
MC-LY	*Sphaerospermopsis*	1
MC-LY	NA	48
MC-M(O)R	*Microcystis*	1
MC-M(O)R	NA	1
MC-RA	*Dolichospermum*	8
MC-RA	*Microcystis*	8
MC-RA	NA	8
MC-RAbu	*Dolichospermum*	6
MC-RAbu	*Microcystis*	6
MC-RAbu	NA	6
MC-RR	*Anabaena*	35
MC-RR	*Anabaenopsis*	14
MC-RR	*Aphanizomenon*	67
MC-RR	*Aphanocapsa*	21
MC-RR	*Aphanothece*	8
MC-RR	*Chroococcus*	24
MC-RR	*Chrysosporum*	2
MC-RR	*Coelomoron*	6
MC-RR	*Coelosphaerium*	3

MC-RR	*Cuspidothrix*	10
MC-RR	*Cyanocatena*	6
MC-RR	*Cyanodictyon*	1
MC-RR	*Cyanonephron*	1
MC-RR	*Dactylococcopsis*	4
MC-RR	*Dolichospermum*	219
MC-RR	*Gloeothece*	4
MC-RR	*Gloeotrichia*	8
MC-RR	*Gomphosphaeria*	6
MC-RR	*Jaaginema*	1
MC-RR	*Kamptonema*	8
MC-RR	*Lemmermaniella*	1
MC-RR	*Limnolyngbya*	6
MC-RR	*Limnospira*	19
MC-RR	*Limnothrix*	6
MC-RR	*Lyngbya*	15
MC-RR	*Merismopedia*	8
MC-RR	*Microcrocis*	1
MC-RR	*Microcystis*	955
MC-RR	*Nodularia*	1
MC-RR	*Nostoc*	2
MC-RR	*Oscillatoria*	77
MC-RR	*Pannus*	1
MC-RR	*Phormidium*	26
MC-RR	*Planktolyngbya*	7
MC-RR	*Planktothrix*	199
MC-RR	*Pseudanabaena*	25
MC-RR	*Radiocystis*	1
MC-RR	*Raphidiopsis*	24
MC-RR	*Romeria*	2
MC-RR	*Snowella*	3
MC-RR	*Sphaerocavum*	1
MC-RR	*Sphaerospermopsis*	2
MC-RR	*Spirulina*	5
MC-RR	*Synechococcus*	22
MC-RR	*Synechocystis*	7
MC-RR	*Woronichinia*	11
MC-RR	NA	2195
MC-RR Cys conjugate	NA	22
MC-RR GSH conjugate	NA	3
MC-RY	*Microcystis*	1

MC-RY	*Raphidiopsis*	1
MC-RY	*Sphaerospermopsis*	1
MC-RY	NA	1
MC-VR	*Aphanizomenon*	1
MC-VR	*Dolichospermum*	1
MC-VR	*Planktothrix*	1
MC-VR	NA	2
MC-WA	*Dolichospermum*	12
MC-WA	*Microcystis*	12
MC-WA	NA	12
MC-WAbu	*Dolichospermum*	1
MC-WAbu	*Microcystis*	1
MC-WAbu	NA	1
MC-WR	*Cuspidothrix*	2
MC-WR	*Cyanocatena*	1
MC-WR	*Dolichospermum*	17
MC-WR	*Gloeothece*	1
MC-WR	*Limnolyngbya*	1
MC-WR	*Microcystis*	41
MC-WR	*Planktolyngbya*	1
MC-WR	*Pseudanabaena*	1
MC-WR	*Raphidiopsis*	1
MC-WR	*Sphaerospermopsis*	1
MC-WR	*Woronichinia*	2
MC-WR	NA	51
MC-YA	*Microcystis*	1
MC-YA	*Planktothrix*	1
MC-YA	NA	2
MC-YM	*Aphanizomenon*	1
MC-YM	*Microcystis*	2
MC-YM	*Planktothrix*	1
MC-YM	NA	2
MC-YR	*Anabaena*	32
MC-YR	*Anabaenopsis*	4
MC-YR	*Aphanizomenon*	51
MC-YR	*Aphanocapsa*	12
MC-YR	*Aphanothece*	4
MC-YR	*Chroococcus*	17
MC-YR	*Chrysosporum*	2
MC-YR	*Coelomoron*	2
MC-YR	*Coelosphaerium*	3

MC-YR	*Cuspidothrix*	6
MC-YR	*Cyanocatena*	3
MC-YR	*Dactylococcopsis*	3
MC-YR	*Dolichospermum*	147
MC-YR	*Gloeotrichia*	6
MC-YR	*Gomphosphaeria*	8
MC-YR	*Limnolyngbya*	4
MC-YR	*Limnospira*	3
MC-YR	*Limnothrix*	5
MC-YR	*Lyngbya*	12
MC-YR	*Merismopedia*	6
MC-YR	*Microcrocis*	1
MC-YR	*Microcystis*	574
MC-YR	*Nodularia*	1
MC-YR	*Oscillatoria*	74
MC-YR	*Phormidium*	18
MC-YR	*Planktolyngbya*	4
MC-YR	*Planktothrix*	139
MC-YR	*Pseudanabaena*	18
MC-YR	*Raphidiopsis*	19
MC-YR	*Romeria*	1
MC-YR	*Snowella*	1
MC-YR	*Sphaerospermopsis*	1
MC-YR	*Spirulina*	5
MC-YR	*Synechococcus*	7
MC-YR	*Woronichinia*	5
MC-YR	NA	1254
Unnamed Congener	*Anabaena*	11
Unnamed Congener	*Anabaenopsis*	43
Unnamed Congener	*Anagnostidinema*	1
Unnamed Congener	*Aphanizomenon*	417
Unnamed Congener	*Aphanocapsa*	38
Unnamed Congener	*Aphanothece*	7
Unnamed Congener	*Arthrospira*	12
Unnamed Congener	*Calothrix*	2
Unnamed Congener	*Chroococcus*	42
Unnamed Congener	*Chrysosporum*	2
Unnamed Congener	*Coelosphaeriopsis*	9
Unnamed Congener	*Coelosphaerium*	19
Unnamed Congener	*Cuspidothrix*	38
Unnamed Congener	*Cyanocatena*	2

Unnamed Congener	*Cylindrospermopsis*	54
Unnamed Congener	*Cylindrospermum*	3
Unnamed Congener	*Dolichospermum*	580
Unnamed Congener	*Epigloeosphaera*	1
Unnamed Congener	*Geitlerinema*	13
Unnamed Congener	*Gloeocapsa*	4
Unnamed Congener	*Gloeothece*	12
Unnamed Congener	*Gloeotrichia*	54
Unnamed Congener	*Gomphosphaeria*	51
Unnamed Congener	*Heteroleibleinia*	2
Unnamed Congener	*Leptolyngbya*	7
Unnamed Congener	*Limnolyngbya*	8
Unnamed Congener	*Limnothrix*	123
Unnamed Congener	*Lyngbya*	56
Unnamed Congener	*Merismopedia*	13
Unnamed Congener	*Microcystis*	2146
Unnamed Congener	*Nodularia*	6
Unnamed Congener	*Nostoc*	7
Unnamed Congener	*Oscillatoria*	71
Unnamed Congener	*Phormidium*	42
Unnamed Congener	*Planktolyngbya*	42
Unnamed Congener	*Planktothrix*	1088
Unnamed Congener	*Pseudanabaena*	154
Unnamed Congener	*Radiocystis*	2
Unnamed Congener	*Raphidiopsis*	106
Unnamed Congener	*Rivularia*	3
Unnamed Congener	*Romeria*	1
Unnamed Congener	*Snowella*	3
Unnamed Congener	*Sphaerospermopsis*	11
Unnamed Congener	*Spirulina*	4
Unnamed Congener	*Synechococcus*	7
Unnamed Congener	*Trichodesmium*	5
Unnamed Congener	*Woronichinia*	60
Unnamed Congener	NA	7101

Chapter 3 – Bioaccumulation and trophic transfer of microcystin between invertebrates

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Abstract

Microcystins are common hepatotoxins produced by cyanobacteria. As reported in Chapter 1, microcystins have been detected in a wide range of organisms. However, research examining whether microcystins bioaccumulate or biomagnify in ecosystems has generated contradictory results. This experiment sought to determine if microcystins bioaccumulate, biomagnify, or exhibit trophic transfer between invertebrates. The common aquatic crustacean grazer *Daphnia pulex* was fed microcystin-producing cyanobacteria, and then fed to predatory aquatic larvae of the damselfly *Enallagma* sp. The results support the hypothesis that microcystins bioaccumulate and can be transferred trophically, but do not support the hypothesis that microcystins biomagnify.

Introduction

Bioaccumulation, as used here, is the process of biological uptake and short- or long-term storage of a contaminant within an organism's body tissues. Biomagnification is observed when that contaminant becomes more concentrated in successive trophic levels in a food web.

Generally, lipid-soluble compounds—such as mercury (Morel et al., 1998) and DDT (Lushchak et al., 2018)—biomagnify, reaching deleterious concentrations in apex predators (Schaefer et al., 2011; Ames, 1966). Other chemical types also are known to bioaccumulate in consumers. Microcystins are hepatotoxins and tumor promotors that covalently bind with protein phosphatases in animals. Microcystins are some of the most common toxins produced by cyanobacterial harmful algal blooms. Cyanobacterial harmful algal blooms are a threat to human and ecosystem health, occur globally (Chapter 2), and are increasing in frequency and intensity (Huisman et al., 2018).

Despite the fact that microcystins can bind to tissue, there is contradictory evidence on whether or not microcystins bioaccumulate or biomagnify (Chapter 1). Additionally, many studies that measure the bioaccumulation of microcystins do so in vertebrates, generally ray-finned fish (Chapter 1). Invertebrates are common grazers of cyanobacteria, one of the primary routes of carbon transfer in aquatic ecosystems (Hambright et al., 2007), and a possible route of transfer of microcystins to terrestrial food webs (Moy et al., 2016). *Daphnia*, a common invertebrate grazer of cyanobacteria in aquatic ecosystems (Nizan et al., 1986), could have the ability to bioaccumulate microcystins by concentrating cyanobacteria in their guts. By consuming cyanobacteria, they increase the density of algal cells in a small area relative to the water around them. Additionally, *Daphnia* with full guts are more conspicuous to visual predators than *Daphnia* with clear guts (Zaret, 1972), making it even more likely for visual predators to receive a high dose of algal toxins.

In this study, phytoplanktivorous zooplankton were fed microcystin-producing *Microcystis.* These organisms were then rinsed and fed to predatory zooplankton. This tests three hypotheses. First, microcystins can be transferred to aquatic predators through their diet, without direct exposure to cyanobacteria. Second, sequestered microcystin transferred from prey's tissues will sequester in a predator's tissue. Third, microcystin will bioaccumulate or biomagnify in invertebrates.

Methods

Organisms Used

Daphnia pulex, originally collected from the University of Oklahoma's Aquatic Research Facility in 2014, were used as the phytoplanktivorous zooplankton. *Daphnia pulex* were selected because they are common grazers of cyanobacteria (Fey et al., 2010), and are commonly consumed by predatory zooplankton (Hunt and Swift, 2010). The *D. pulex* were maintained in the Plankton Ecology and Limnology Laboratory in COMBO (Kilham et al. 1998) at 20°C and a 12-hour light cycle. Only adult *D. pulex* of similar size, approximately 1.7 mm in body length, were selected for the experiment.

Enallagma sp. damselfly larvae purchased from Carolina Biological were used as the predatory zooplankton. The larval *Enallagma* sp. were selected because damselflies readily consume *Daphnia* (Hunt and Swift, 2010). They are also visual predators, potentially making them susceptible to the bioaccumulation of microcystins through consuming visually conspicuous *Daphnia* with cyanobacteria-filled guts. Additionally, these aquatic larvae metamorphose into

terrestrial damselflies, making them a possible route for transferring microcystin out of freshwater ecosystems. The *Enallagma* sp. were placed in COMBO and maintained at 20°C and a 12-hour light cycle for 24-48 hours before the experiment. All *D. pulex* and *Enallagma* sp. were starved for 24 hours before the experiment.

Microcystis aeruginosa (UTEX LB2385) was used as the microcystin-producing cyanobacteria. This strain has consistently produced microcystins as previously verified by ELISA tests, and is reported to produce microcystins (Bateman et al., 1995). *Microcystis* was added to the toxic trials at 0.5 mg carbon/liter to provide grazing conditions just above the incipient limiting concentration (Burns and Rigler, 1967) for the *D. pulex* and sublethal toxin concentrations, as demonstrated in a previous pilot experiment. The nontoxic trials used the green algae *Scenedesmus acutus* (UTEX 72) at 0.5 mg carbon/liter to be comparable to the toxic trials. Both algae were grown in COMBO at 25°C with a 12-hour light cycle.

Experimental Treatments

Each treatment was made from a combination of three options. First, *D. pulex* were fed either toxic or nontoxic algae. Second, *D. pulex* either had guts full of *Microcystis* or were allowed to clear their guts of *Microcystis*. And third, *Enallagma* sp. either had guts full of *Daphnia* or were allowed to clear their guts of *Daphnia*. The experimental design was not full factorial with all possible combinations of these options, as not all combinations were necessary to test the three hypotheses and limiting samples reduced the cost of microcystin analysis. The experimental treatments used are listed in Table 1.

Feeding toxic cyanobacteria to *D. pulex* exposes them to microcystins. Feeding nontoxic green algae provides a negative control to test if the LC-MS method is detecting false positives due to matrix interference. Feeding the intoxicated *D. pulex* to *Enallagma* sp., which have not themselves been exposed to *Microcystis,* tests the hypothesis that microcystins can be transferred to aquatic predators through their diet without direct exposure to cyanobacteria. Comparing the microcystin levels in *D. pulex* with guts full of cyanobacteria and guts cleared of cyanobacteria allows a comparison of how much microcystin is present in total from the cyanobacteria in the *D. pulex*'s digestive tract and tissues, and how much microcystin remains in the *D. pulex* once the cyanobacteria have been excreted. Comparing the microcystin levels in *Enallagma* sp. fed *D. pulex* with full guts to the microcystin levels in *Enallagma* sp. fed *D. pulex* with clear guts allows a comparison of how much microcystin was transferred from the prey organisms' tissues and how much microcystin was transferred from the cyanobacteria in the prey organisms' guts. Measuring microcystin in *Enallagma* sp. with full guts and comparing it to the microcystin in *Enallagma* sp. with clear guts allows a comparison of how much microcystin remains in the guts of the predators, and how much microcystin sequesters in their tissues once the gut contents have been excreted. Finally, measuring the microcystin in prey and predator will test the hypothesis that microcystins can bioaccumulate or biomagnify in invertebrates.

Experimental Setup

Experiments were conducted in wells in six-well plates filled with 10 ml COMBO. Twenty-four hours before the experiment, *D. pulex* and *Enallagma* sp. were placed in their respective wells.

Ten *D. pulex* were placed in each well for trial one, and five *D. pulex* were placed in each well for trials 2-5. The number of *D. pulex* used in later trials was reduced due to the fact that no *Enallagma* sp. consumed all 10 *D. pulex* during trial one, and the number of adult *D. pulex* available was the limiting factor that determined how many trials could be run simultaneously.

Algae densities were measured using a fluorometer at the start of the experiment. The appropriate amount of COMBO was removed from each well and replaced with the necessary amount of algae culture to reach a density of 0.5 mg C/L. Toxic wells received *Microcystis aeruginosa.* Nontoxic wells and clearing wells received *Scenedesmus acutus*. Wells of pure COMBO which were used to rinse the *D. pulex* between experimental steps, and the wells containing *Enallagma* sp. did not receive any algae.

Feeding Experiment

Five total replicates of the feeding experiment were conducted across three nonconsecutive dates, due to the limited number of comparably sized adult *D. pulex* available. Trial one was conducted on the first day. Trial two and trial three were conducted on the second day. Trial four and trial five were conducted on the third day. Immediately before the feeding experiments, one milliliter of the concentrated *Microcystis* culture and one milliliter of the concentrated *Scenedesmus* culture were each pipetted into separate sample vials and frozen in a -80°C freezer for later analysis of their microcystin content by LC-MS.

D. pulex were allowed to graze on the algae for four hours. These *D. pulex* were either sampled so microcystins could be measured in the first trophic level, or fed to *Enallagma* sp. so microcystins could be transferred to the second trophic level. By feeding both sets of *D. pulex* the same concentration of algae from the same cultures for the same time, the *D. pulex* that were sampled presumably had similar concentrations of microcystin as the *D. pulex* that were fed to the *Enallagma* sp. After grazing, *D. pulex* meant to be sampled with full guts were placed using a plastic transfer pipette into a sample vial using the minimum amount of culture necessary to transfer the *D. pulex.* The sample vial was then filled to 1 mL with fresh COMBO and frozen at -80°C. The *D. pulex* that would be fed with full guts to *Enallagma* sp. were transferred to a rinse well and then to an *Enallagma* sp. well. *D. pulex* that would be sampled with clear guts or fed with clear guts to *Enallagma* sp. were placed in clearing wells and allowed to clear their guts for 2 hours as they fed on the nontoxic *Scenedesmus*. After clearing their guts, these *D. pulex* were either placed in a sample vial with 1 ml fresh COMBO and then frozen, or placed in an *Enallagma* sp. well.

The *Enallagma* sp. that would be sampled with full guts were observed for 2-3 hours until they consumed the *D. pulex*, and then placed in a sample vial with 1 ml of fresh COMBO and frozen at -80°C. The lack of any algae present in the *Enallagma* sp. well should have slowed the rate at which the *D. pulex* cleared their guts, but the differences in the length of the *Enallagma sp*.'s feeding time could have influenced the amount of *Microcystis* present in the *D. pulex*'s guts. The *Enallagma* sp. that would be sampled with clear guts were given an additional 12 hours to clear their guts, and then placed in a sample vial with 1 ml of fresh COMBO and frozen at -80°C.

All samples were stored at -80°C until they could be shipped to GreenWater Laboratories (Palatka, Florida, United States) for extraction and analysis. The samples were shipped overnight on ice in an insulated cooler. The samples were oxidized, extracted, and analyzed for total Adda microcystins/nodularins using the MMPB liquid chromatography-mass spectrometry method similar to Foss and Aubel (2015).

Statistical Analysis

Values less than the limit of detection were treated as 0.13 ng microcystins/mL, 65% of the limit of detection, in the toxic treatments (Palarea-Albaladejo and Martín-Fernández, 2013). Values less than the limit of detection were treated as 0 ng microcystins in the nontoxic treatments, as the green algae *Scenedesmus* cannot produce microcystin.

Toxin concentrations were first converted to ng microcystin/mg dry weight of organism. For *D. pulex*, an average weight was used based on length measurements of 10 similarly sized adult *D. pulex*, like those used in the experiment. The length-weight regression used was

$$W = 0.0116L^{2.67}$$

where W is the dry weight of the *D. pulex* in mg and L is the body length in mm (Burns, 1969). For *Enallagma* sp., an average length was determined from three larvae, and the length-weight regression used was

$$W = 0.0078L^{2.792}$$

where W is the dry weight of the *Enallagma* sp. in mg and L is the total length in mm (Benke et al., 1999).

The data were not normally distributed, so a non-parametric Kruskal-Wallis test was used to check for significant differences in the concentration of microcystin among treatments.

Due to a wide range of initial microcystin concentrations among the replicates, the percent of the total possible microcystin transferred to each trophic level was also calculated. First, the ng microcystin/ml was calculated for each well, using the formula

$$X = \frac{a \times b}{c}$$

where X was the final concentration in ng microcystin/ml, a was the reported value of ng microcystin/ml of concentrated culture, b was the ml of concentrated culture added to the well, and c was the total ml of liquid in the well. Second, the fraction of the total possible microcystin that could have been transferred to the *D. pulex* was calculated using the formula

$$Y = \frac{e}{f}$$

where Y was the fraction of microcystin transferred from the *Microcystis* to the *D. pulex*, e was the observed microcystins reported in the *D. pulex*, and f was the total microcystins from the *Microcystis* in the well with the *D. pulex*. Third, the fraction of microcystin transferred from the *D. pulex* to the *Enallagma* sp. was calculated using the formula

$$Z = \frac{g}{h \times i}$$

where Z was the fraction of microcystin transferred from the *D. pulex* to the *Enallagma* sp., g was the observed microcystins reported in the *Enallagma* sp., h was the number of *D. pulex* eaten by the *Enallagma* sp., and i was the observed microcystins per *D. pulex*.

Using 0.13 ng microcystin/ml as a replacement for values below the limit of detection caused one outlier, in which the Predator Clear Gut Grazer Clear Gut treatment of trial one showed a 333% transfer of microcystin. This outlier was excluded from further analysis.

Results

No nontoxic trial had detectable levels of microcystin. Microcystin was present in each toxic treatment, varied considerably among replicate trials, and was transferred through two trophic levels.

D. pulex, the grazers, had higher concentrations of microcystin per mg dry weight than did *Enallagma* sp., the predators (Figure 1). No significant differences in microcystin concentration were found between the grazer treatments (χ^2=7.418, df=4, p=0.115). No significant differences were found between the predator treatments (χ^2=7.258, df=4, p=0.122).

The predators with full guts which consumed grazers with full guts had nearly complete trophic transfer of microcystins. Even the predators with clear guts which consumed grazers with clear

guts showed trophic transfer of microcystins. The highest values of toxin accumulation and transfer occurred in treatments with cyanobacteria-filled guts (Figure 2).

Discussion

The hypothesis that microcystin can be transferred to invertebrates that were never directly exposed to cyanobacteria was supported. Microcystin clearly transferred from grazers to predators in each of the predator trials. In one trial, over 99% of the available microcystin (× ng in the initial algae) was recovered from a *Enallagma* sp. with a full gut that had consumed *D. pulex* with full guts. The grazers were rinsed in algae-free COMBO and transferred with fresh pipettes to the predator's well, so no cyanobacteria or microcystin (or only negligible cyanobacteria or microcystin) could be present in predator's well. In this trial, none of the microcystin values were below the limit of detection so the result cannot be caused by mathematical artefacts, as in the case of the 333% transfer datum that was excluded.

The hypothesis that microcystin sequestered in a prey organism's tissue could be transferred to a predator's tissue was supported. The *Enallagma* sp. with clear guts that had been fed *D. pulex* with clear guts had detectable microcystin. While the digestive tracts of the *Enallagma* sp. were not clearly visible and there was not a strong color difference between *D. pulex* guts full of cyanobacteria and *D. pulex* guts cleared by ingesting *Scenedesmus*, the *D. pulex* in clear gut trials were given ample time to clear their guts (Murtaugh, 1985) and the *Enallagma* sp. in clear gut trials left visible fecal material in the wells. Thus, the organisms in clear-gut trials likely did have clear guts at the time of sampling. This implies detected microcystin was likely sequestered in

the organisms' tissue, which further implies that microcystin that has been sequestered in tissue once is still capable of bonding to another organism's tissue.

It is possible that the period of starvation before the feeding experiment limited the *D. pulex* and *Enallagma* sp.'s ability to depurate microcystin, and little research is available on the subject. However, a recent study by Castro et al. (2019) found that starvation had no effect on the rates at which *Daphnia magna* depurated four of five chlorinated parafins, another family of toxins with the potential to bioaccumulate and biomagnify.

Because microcystins are water soluble (Rivasseau et al., 1998) and organisms such as *Daphnia* do have the ability to depurate them (Castro et al. 2019), they are less likely to biomagnify than lipid-soluble toxins such as mercury and DDT. Generally, lipid-soluble toxins are sequestered into the fat-storage structures of organisms, where they remain due to the organisms' inability to detoxify the compounds. This allows the toxins to increase in concentration with trophic level. However, β-methylamino-L-alanine (BMAA) is a water-soluble cyanobacterial toxin that does biomagnify. This amino acid was found in the symbiotic cyanobacteria that lived in the roots of the cycad *Cycas micronesica,* increasing in concentration in the cycad's seeds, and increasing again in the tissue of the flying foxes *Pteropus mariannus*, that consumed the seeds (Cox et al., 2003). The data here do not support the hypothesis that microcystin biomagnifies in invertebrates.

Microplastics, another aquatic contaminant of growing concern, do bioaccumulate within trophic levels (Miller et al., 2020). This process is likely similar to the bioaccumulation of cyanobacterial cells concentrated in the guts of grazers seen in this experiment. Predators—including humans (Chapter 1)—could potentially receive a high dose of microcystin from prey which had recently consumed cyanobacteria.

The bioaccumulation and trophic transfer of microcystin to the *Enallagma* sp. demonstrates that damselflies are a potential route of transfer of microcystin from aquatic ecosystems to terrestrial ecosystems, perhaps contributing to some of the microcystin contamination seen in terrestrial food webs in Chapter 1. While the larvae are aquatic, adult damselflies emerge from aquatic habitats after metamorphosis and become terrestrial, where they are preyed upon by several predators such as warblers (Bibby and Green, 1983) and spiders (Rehfeldt, 1992). The trophic transfer of microcystins from aquatic to terrestrial ecosystems has similarly been seen in mayflies, another insect with an aquatic larval stage and a terrestrial adult. Microcystins which bioaccumulated in mayfly larvae were trophically transferred to spiders, and then to warblers (Moy et al., 2016). The contaminant methylmercury has also been observed transferring trophically from aquatic to terrestrial ecosystems along the same route, from emergent aquatic insects to spiders to songbirds (Gann et al., 2015), implying this is a common route of trophic transfer for multiple environmental contaminants. This evidence strongly indicates that cyanobacterial toxins in general, and microcystins in particular, are likely to bioaccumulate in aquatic larvae such as damselflies and trophically transfer through them to terrestrial ecosystems.

In summary, microcystins were clearly passed to organisms that had no direct exposure to cyanobacteria, and microcystin sequestered in prey organisms were sequestered in the tissue of their predators. The results support trophic transfer of microcystin between invertebrates, and bioaccumulation of microcystin, if not biomagnification. The bioaccumulation of microcystin in damselflies could present a route of trophic transfer from aquatic to terrestrial ecosystems.

Acknowledgments

Thank you to the whole Plankton Ecology and Limnology Laboratory for their help, and particularly to Claire Grimmett, Jessica Beyer, and Eve Minkin, for caring for the hungry little *Daphnia* when I could not. Funding for this project came from the University of Oklahoma Department of Biology Adams Summer Scholarships, the Hill Fund, the University of Oklahoma Graduate Student Senate Research Grants, Sigma Xi Grants in Aid of Research, and the National Science Foundation (DEB 1831061).

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Figures

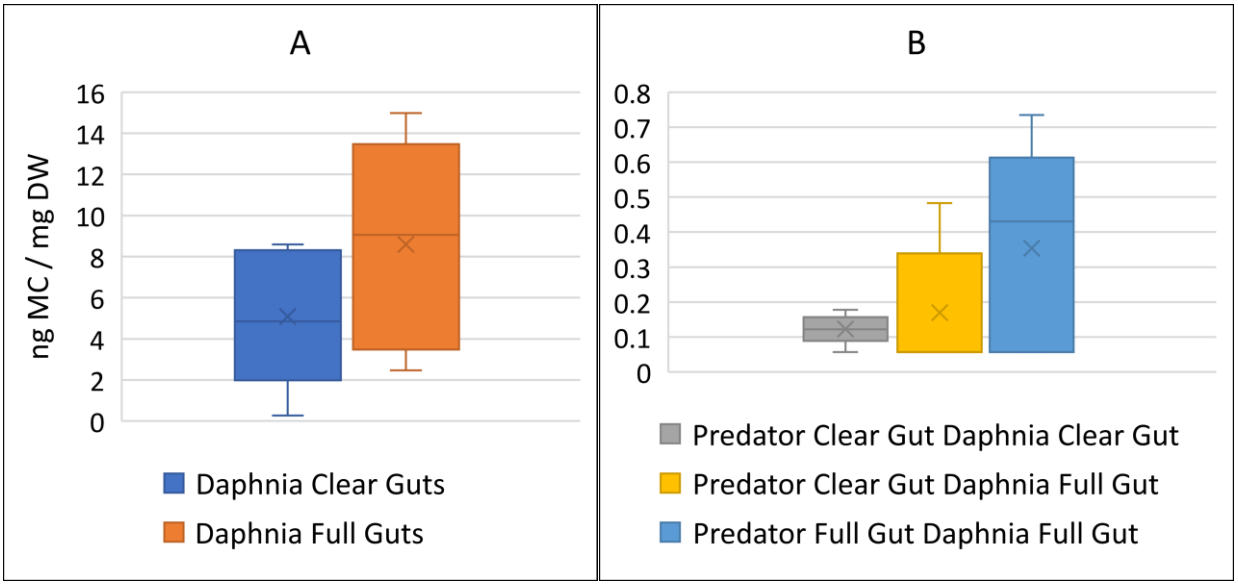

Figure 1: The amount of microcystin present at each trophic level. Panel A represents Daphnia, trophic level 1. Panel B represents damselfly larvae, trophic level 2. Boxes represent interquartile range of microcystin concentrations, and bars represent minimum and maximum values. Horizontal lines across boxes indicate medians. Means are shown by the x within each box. All nontoxic controls were below the limit of detection. No significant differences were detected between Daphnia treatments (Panel A: χ^2=7.418, df=4, p=0.115), and no significant differences were detected between damselfly treatments (Panel B: χ^2=7.258, df=4, p=0.122).

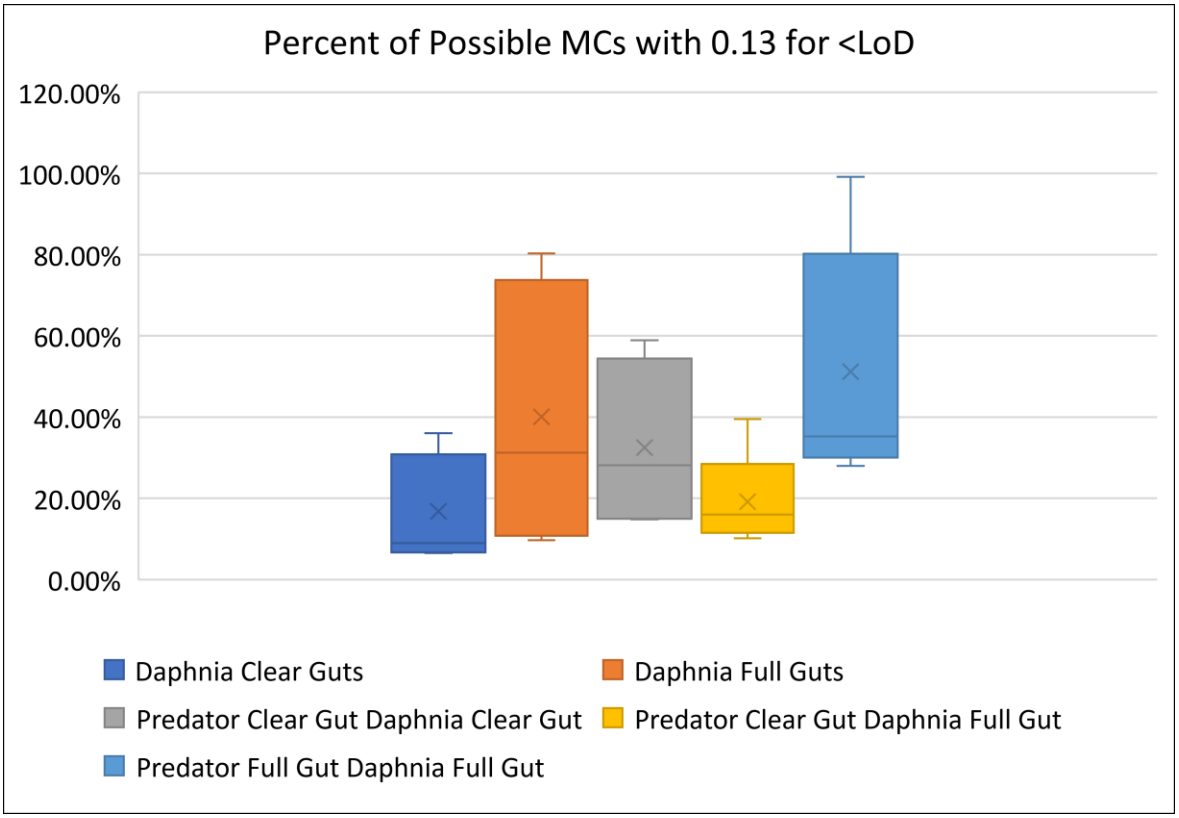

Figure 2: The percent of available microcystin transferred to each treatment. The values take into account the concentration of microcystin in the initial experimental well, and the number of Daphnia eaten by the predatory damselflies. One outlier was excluded: The datum for Predator Clear Gut Grazer Clear Gut on trial one had a value of 333% due to replacing values below the limit of detection with 65% of the limit of detection. All nontoxic controls were below the limit of detection.

Tables

Table 1: The treatments conducted in the experiment. Samples of algae contained 1 ml of the concentrated algae culture. Samples of zooplankton contained 1 ml of clean COMBO and the zooplankton.

Algae	***Daphnia pulex***	***Enallagma* sp.**
Toxic	-	-
Toxic	Clear guts	-
Toxic	Full guts	-
Toxic	Clear guts	Clear guts
Toxic	Full guts	Clear guts
Toxic	Full guts	Full guts
Nontoxic	-	-
Nontoxic	Clear guts	-
Nontoxic	Full guts	-
Nontoxic	Full guts	Clear guts