Microcystins in planktonic and benthic food web components from Greenlandic lakes

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Abstract. There is increasing global concern regarding the social, economic, human health, and environmental health implications of cyanotoxins. However, much of what we know about cyanotoxins comes from studies of temperate or tropical systems with conspicuous surface blooms of cyanobacteria. We measured the concentrations of microcystins (MCs), potent cyanotoxins produced by many cyanobacterial taxa, within lake food webs in southwestern Greenland. We detected MCs in six taxonomic groups of organisms and found that median MC concentrations in large (>50 µm) phytoplankton were an order of magnitude higher than benthic cyanobacteria (genus Nostoc) and two orders of magnitude higher than benthic grazers and consumers (snails, dytiscid larvae, and chironomid larvae). Microcystin concentrations generally decreased with increasing trophic position, suggesting that biomagnification does not occur in these lakes. We conclude that MCs are prevalent in multiple components of these Arctic aquatic food webs and that both benthic and pelagic taxa may be sources of MCs.

Key words: aquatic; Arctic; cyanobacteria; cyanotoxins; food webs.

INTRODUCTION

Cyanotoxins are of increasing concern worldwide due to their harmful effects on freshwater resources for drinking, sanitation, and recreational activities (Paerl and Paul 2012). In addition to their adverse health effects on humans and domesticated animals, many of these toxins are also of ecological concern due to their potential to impact aquatic biota and influence ecosystem processes (Ferrão-Filho and Kozlowsky-Suzuki 2011). Organisms encounter cyanotoxins directly by absorbing dissolved cyanotoxins or indirectly by consuming toxic cyanobacterial cells or consuming organisms containing cyanotoxins (Buratti et al. 2017). The ecological effects of cyanotoxins include allelopathic effects on competing phytoplankton (Babica et al. 2006) and behavioral changes in aquatic animals (Ferrão-Filho and Kozlowsky-Suzuki 2011). Additionally, variants of the widespread cyanotoxin microcystin (MC) are known to form complexes with metal ions such as copper, zinc, lead, and iron (Humble et al. 1997, Yan et al. 2000, Saito et al. 2008, Klein-teich et al. 2013), potentially disrupting nutrient cycling in the ecosystem.

Although the presence and abundance of cyanotoxins have been documented in a diverse range of freshwater systems and organisms (Buratti et al. 2017), significant gaps in our understanding...
remain. For example, our knowledge about cyanotoxins in lake food webs comes primarily from studies in temperate and tropical regions. We know much less about cyanotoxins in Arctic ecosystems, including the concentrations at which they naturally occur in Arctic waters and food webs.

Microcystins are a diverse group of cyclic polypeptides that inhibit protein phosphatases in eukaryotes and induce tumor growth and liver hemorrhaging in vertebrates (Sivonen and Jones 1999). We focus here on MCs because they are among the most widely produced and most frequently detected cyanotoxins worldwide, as well as the focus for ongoing regulatory efforts (Salmaso et al. 2017:201). A handful of studies have reported detectable MCs in Antarctic microbial mats (Hitzfeld et al. 2000, Jungblut et al. 2006, Wood et al. 2008, Kleinteich et al. 2014, Puddick et al. 2015), but reports of MCs and other cyanotoxins in Arctic environments are scarce (Kleinteich et al. 2012, 2013, 2018, Chrapusta et al. 2015, Trout-Haney et al. 2016, 2021). Improving our understanding of the occurrence of cyanotoxins in Arctic ecosystems is especially important because cyanobacteria tend to be highly successful in high latitude systems (Vincent and Hobbie 2000), and, as reported in the most recent Intergovernmental Panel on Climate Change (IPCC) assessment, Arctic regions are experiencing the fastest and most pronounced impacts of climate change, including both warming temperatures and increased precipitation (Pachauri and Meyer 2014). Given that warm temperatures and high nutrient loads are associated with an increased risk of cyanotoxin production (Paerl and Paul 2012, Walls et al. 2018), Arctic lakes may become increasingly suitable for cyanobacterial growth and cyanotoxin production in the future (Przytulska et al. 2017).

Further, the accumulation of cyanotoxins in aquatic organisms remains poorly understood, including whether toxins biomagnify (higher toxin concentration in a consumer relative to its food source), biodilute (lower toxin concentration in a consumer relative to its food source), or bioconcentrate (higher toxin concentration in an organism relative to the surrounding water) in a given system (Pham and Utsumi 2018). While cyanotoxins have been shown to transfer to a wide variety of aquatic consumers across systems (Appendix S1: Table S1), the factors influencing whether toxins biomagnify or biodilute in a given system (e.g., length of exposure, assimilation rates, temperature) remain unresolved (Kozlowsky-Suzuki et al. 2012). To begin addressing these knowledge gaps, we tested for the presence and trophic transfer potential of MCs within pelagic and benthic producer and consumer taxa collected from Arctic lakes in southwestern Greenland.

**MATERIALS AND METHODS**

**Study site and focal organisms**

In the last week of July 2015, we collected aquatic organisms from eight lakes in Kangerlussuaq, Greenland (67°01' N, 50°41' W), located along a stretch of tundra between Søndre Stromfjord and the Greenland Ice Sheet (Fig. 1). Ice-out in this region varies across years, but most lakes are ice-free by mid-June and reach maximum surface water temperatures in late July–early August (Brodersen and Anderson 2000). We focused on six taxonomic groups found in these systems. These included two potential toxin-producing groups: the phytoplankton (i.e., phytoplankton >50 µm in size, including large pelagic cyanobacteria, if present; all eight lakes, dominant planktonic cyanobacterial genera in our samples were *Chroococcus*, *Oscillatoria*, *Eucapsis*, and *Oocystis*) and benthic colonial cyanobacteria of the genus *Nostoc* (found in six of the eight lakes). Zooplankton (seven lakes) include primary consumers grazing mostly on phytoplankton, although certain species can consume microbes, detritus, or other zooplankton (Marion et al. 2016). Snails (*Lymnaea*; seven lakes) represented the primary benthic grazers, consuming epiphytic algae growing on aquatic macrophytes and (likely) the surface of *Nostoc* colonies. Chironomid larvae (all lakes) represent a range in feeding strategies from primary consumers to specialized sediment feeders and predators (Reuss et al. 2014). Lastly, dytiscid larvae (predaceous diving beetle, *Colymbetes dolabratus* Paykull, all lakes) are the most abundant top predator, opportunistically preying on invertebrates such as zooplankton, larval insects, and gastropods (L. E. Culler, personal communication).
Sample collection and processing

We collected phytoplankton and zooplankton using 1–2 tows of a 50-µm Wisconsin net to obtain a target wet biomass of 0.001–1.0 g plankton; additional tows were preserved in Lugol’s iodine for identification of the captured taxa. We separated phytoplankton from zooplankton using a customized plankton separation device or Pocket ZAPP (Nancy Leland, North Andover, Massachusetts, USA) that uses zooplankton photo-avoidance behavior to separate them from phytoplankton. We collected separated fractions onto 20-µm mesh, desiccated the samples, and stored them at −20°C. Before toxin extraction, we checked each sample for incomplete separation using a Leica MZ-12 dissecting microscope (Leica Microsystems Ltd, Heerbrugg, Switzerland) and hand-picked samples until only the desired fraction was present. We macerated, weighed, and reconstituted the picked sample with a known volume of distilled water (between 1 and 1.5 mL, depending on the volume of the sample). Microcystin extraction and analysis proceeded as described in Extraction and ELISA for biological samples.

We used D-nets to collect Nostoc, snails, chironomid larvae, and dytiscid larvae from three locations around the shoreline of each lake, when they were present. We separated and identified organisms by hand, placed them into sterile Whirl-Pak bags (Nasco, Fort Atkinson, Wisconsin, USA), and stored frozen (−20°C) until laboratory analyses. We sampled each site until we obtained at least three individuals of each taxon to ensure sufficient wet mass for toxin analyses (0.05–2.0 g, based on a pilot study in 2014). The total contents of each bag (i.e., multiple individuals) represented a lake replicate. We rinsed organisms thoroughly with distilled water and blotted them dry with clean Kimwipes (Kimberly-Clark Professional) prior to obtaining wet weights. In preparation for extraction, we desiccated, froze (−80°C), and thoroughly macerated each sample using a combination of glass rods,
mortar, and pestle, and dissecting blades before reconstituting the entire sample with a known volume of distilled water.

We tested for contamination by integrating negative control (NC) samples (distilled water) into the field sampling and laboratory techniques. This entailed bringing acid-rinsed bottles and sterile Whirl-Paks filled with distilled water into the field, opening, closing, and handling them as we would to collect water and biological samples. We treated NC samples to triplicate cycles of freeze-thaw, sonication, and ELISA using the protocol described for all other samples.

Because we did not flush organisms’ gut contents prior to toxin extraction, our measurements represent both the toxin bound in tissues and any toxin contained in the gut. Thus, our MC measurements reflect the amount of toxin that would be consumed by a predator, but may not necessarily reveal how much MC is accumulated in tissue.

Extraction and ELISA for biological samples

To release free and peptide-bound MCs in reconstituted samples, we used triplicate freeze-thaw cycles (−80°C), interspersed with a sonic water bath incubation (10 min intervals) and 5–10 s vortex, following existing protocols (Banack et al. 2015). We then centrifuged samples at 12,000 rpm (9660 × g) for 2 min and used the supernatant for analysis. Duplicates of each post-extraction homogenate were analyzed, with further replication for the subset of samples included in quality control (QC) analyses.

We detected MCs using an enzyme-linked immunosorbent assay (ELISA, EP 022 HS, High Sensitivity QuantiPlate Kit for Microcystins, Envirologix, Portland, Maine, USA). This method does not distinguish between MC variants, and as such, we use the abbreviation MC to refer to multiple possible MC variants (including but not limited to MC-LR, MC-LA, MC-RR, MC-YR), as well as the structurally similar nodularin toxin also detected with this kit. When extracted samples were below the kit’s detection limit (0.071 ng/mL), we transferred a known volume of sample fluid to borosilicate serum bottles, refreeze the fluid, and lyophilized in a freeze-dry system (Labconco, Kansas City, Missouri, USA) under vacuum (~30 × 10^3 mbar) at −50°C for 18–24 h to dryness. We then rehydrated samples to achieve a 10-fold concentration and bring them into the range of sensitivity of the ELISA.

Data analyses

To obtain comparable units across all taxonomic groups after MC analysis, we converted wet weights (ww) to dry weights (dw) for the non-planktonic taxa (wet weights shown in Appendix S1: Fig. S1). Based on the literature, we estimated dry weight as 12% of wet weight for chironomid larvae (Landahl and Nagell 1978), 27% for dytiscid larvae (Sage 1982), and 9% for snails (Van Aardt 1967). To determine the relationship between dry and wet weight (dw and ww, respectively) for Nostoc, we dried 20 randomly selected colonies weighing between 0.3 and 21 g ww (covering the size range of Nostoc in this study) for 36 h at 105°C. We estimated Nostoc dry weights as a log-linear regression of wet weight (Appendix S1: Fig. S2).

To evaluate assay precision (QC analyses), we haphazardly selected from among our biological samples and replicated subsamples within ELISA plates (intra-assay variation) and across plates run on different days (inter-assay variation). Inter-assay samples were replicates from the supernatant of a given sample, when samples were initially being processed. We calculated intra-assay variation as the percent coefficient of variation (% CV), the standard deviation (SD) of duplicate sample concentrations within a plate divided by the mean concentration of those duplicates × 100. We determined inter-assay variation by calculating the % CV across plate-specific sample means.

We used R 3.6.0 (R Core Team 2019) for all analyses and figures. Statistical tests for differences in MC concentrations among taxonomic groups and lakes were complicated by the unbalanced design—not all taxonomic groups were present in all lakes—as well as a strong right-skew for some taxonomic groups. To accommodate these issues, we took two approaches to data analysis. First, we ran a two-way ANOVA to test for the effects of taxonomic group, lake, and their interaction on log_{10}-transformed MC concentrations in the subset of three lakes for which all taxa were present. Given the results of that analysis, we opted to use one-way ANOVAs to compare log_{10}-transformed MC concentrations.
across taxa for each lake. We also used the nonparametric Wilcoxon rank-sum test to compare median MCs in snails from lakes with and without *Nostoc*.

**RESULTS**

Where possible, we noted the identity of the dominant species within our taxonomic groups. The dominant planktonic cyanobacterial genera in our study lakes were *Chroococcus*, *Oscillatoria*, *Eucapsis*, and *Oocystis*, while the dominant benthic cyanobacteria were *Nostoc pruniforme* and *N. zetterstedtii*. The dominant zooplankton taxa were *Daphnia middendorfiana*, *D. pulex*, *Eurycercus* sp., *Leptodiaptomus minutus*, *Keratella* spp., and *Asplanchna* spp. We did not attempt to identify the chironomid larvae.

We detected MCs in all six taxonomic groups tested, with wide variations within and among taxa as well as among lakes (Fig. 2). Across all lakes, median MC concentrations varied by two orders of magnitude across taxonomic groups, ranging from a high of 745 ng/g dry weight (dw) in phytoplankton to lows of 3 ng/g dw in dytiscid larvae and snails (Fig. 2). Although phytoplankton contained the highest median MC concentration, concentrations varied considerably across lakes, ranging from 141 ± 50 ng/g dw (mean ± 1 SD) in LWL to 3140 ± 539 ng/g dw in LCL (Table 1). Median phytoplankton MC concentrations were an order of magnitude higher than *Nostoc* (36 ng/g dw) and two orders of magnitude higher than the benthic grazers and consumers (snails, dytiscid larvae, and chironomid larvae). Importantly, there were also notable outliers, such as high MCs in snails, chironomid larvae, and dytiscid larvae from LWL and WL relative to other lakes (solid red and orange points, respectively, in Fig. 2).

When we analyzed the MC data statistically, there was a significant interaction between taxon and lake for the subset of three lakes in which all taxa were represented ($F_{17,61} = 17.16, P < 0.001$), suggesting that comparisons across taxonomic groups needed to be considered lake by lake (or...
vice versa, that lakes be compared taxon by taxon). When lakes were analyzed separately, seven of the eight lakes had mean MC concentrations that varied significantly by taxonomic group (Appendix S1: Table S2). In all of those seven lakes, phytoplankton had the highest mean MC concentrations; in six of the seven lakes, zooplankton had the second highest mean MC concentrations and dytiscid larvae had the lowest mean MCs (Appendix S1: Fig. S3). Finally, we did not detect a difference in MCs in snails from lakes with vs. without Nostoc (Wilcoxon rank-sum test, \( W = 26, P = 0.47 \)).

Negative control samples (\( N = 6 \)) were all below the 0.071 ng/mL detection limit of the ELISA, even after 10-fold concentration (\( N = 3 \)). In QC samples, the intra-assay CV was 20.4% (\( N = 4 \) ELISA plates), while the inter-assay CV was 28% (\( N = 6 \) samples run on \( \geq 2 \) ELISA plates), indicating that within-plate measurements were generally more precise than across plate measurements.

**DISCUSSION**

This study is among the first to report detectable levels of MCs within multiple food web components from small Arctic lakes. We detected MCs in all six taxonomic groups tested and found that MCs varied across both taxonomic groups and lakes with nearly all consumers having lower MCs than their food sources (Fig. 2; Appendix S2: Fig. S2). Microcystin concentrations from organisms in Kangerlussuaq lakes fell within the lower end of the range of previously reported MC concentrations (Buratti et al. 2017, Appendix S1: Table S1), potentially influenced by the shorter growing season, which might limit toxin production (Billam et al. 2006, Reza Sham-sollahi et al. 2018, Miller et al. 2019). Taken together, our results demonstrate that MCs are found in both benthic and pelagic components of Kangerlussuaq lake food webs that MCs are highly variable in taxa among lakes and suggest that MCs are transferring, but not biomagnifying, in these Arctic lake food webs.

**Microcysts in planktonic food web components**

Consistent with the expectation of toxin production by pelagic cyanobacteria, the largest tissue-bound concentrations of MCs were generally in large phytoplankton (>50 μm) and their zooplankton consumers (Fig. 2). However, these MC concentrations are several orders of magnitude lower than MC concentrations in cyanobacteria in temperate and tropical freshwater bodies.

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**Table 1. Microcystins (MC) concentrations in whole lake water and six taxonomic groups from eight study lakes in Kangerlussuaq, Greenland, in 2015.**

| Lake | Whole lake water (ng/L) | Phytoplankton (ng/g dw) | Nostoc (ng/g dw) | Zooplankton (ng/g dw) | Snails (ng/g dw) | Chironomid larvae (ng/g dw) | Dytiscid larvae (ng/g dw) |
|------|------------------------|------------------------|------------------|----------------------|------------------|--------------------------|--------------------------|
| BCL  | 63                     | 966 ± 341              | 14 ± 11          | 145 ± 33             | NA               | 24 ± 14                  | 2 ± 2                    |
| N    | 3                      | 6                      | 6                | 3                    | 3                | 3                        | 3                        |
| BSL  | 65                     | 263 ± 59               | 98 ± 68          | 235 ± 56             | 1 ± 1            | 16 ± 10                  | 1 ± 0                    |
| N    | 3                      | 3                      | 6                | 3                    | 3                | 3                        | 3                        |
| HL   | 73                     | 1756 ± 1               | 14 ± 3           | 84 ± 51              | 16 ± 40          | 6 ± 1                    | 3 ± 3                    |
| N    | 3                      | 3                      | 3                | 3                    | 8                | 3                        | 3                        |
| LCL  | 91                     | 3140 ± 539             | NA               | 227 ± 284            | 3 ± 3            | 15 ± 5                   | 3 ± 2                    |
| N    | 3                      | 3                      | 3                | 3                    | 6                | 3                        | 3                        |
| LSL  | 93                     | 249 ± 39               | 112 ± 79         | NA                   | 11 ± 14          | 12 ± 3                   | 3 ± 1                    |
| N    | 3                      | 4                      | 7                | 14                   | 4                | 3                        | 3                        |
| LWL  | 86                     | 141 ± 50               | NA               | 86 ± 16              | 61 ± 58          | 360 ± 330                | 27 ± 23                  |
| N    | 3                      | 3                      | 3                | 3                    | 4                | 3                        | 3                        |
| STL  | 78                     | 1017 ± 229             | 51 ± 19          | 362 ± 284            | NA               | 18 ± 13                  | 1 ± 0                    |
| N    | 3                      | 3                      | 8                | 4                    | 3                | 3                        | 3                        |
| WL   | 81                     | 586 ± 152              | 16 ± 6           | 125 ± 35             | 3 ± 3            | 69 ± 54                  | 9 ± 4                    |
| N    | 3                      | 7                      | 3                | 16                   | 3                | 3                        | 3                        |

**Notes:** Microcysts in taxa are shown as the mean dry weight (dw) concentration ±1 standard deviation together with sample size (\( N \)). Whole lake water MC represent a single integrated water sample from the upper 1 m of each lake. NA denotes organisms that were not present in a lake during the sampling period or for which the collected biomass was insufficient for toxin analyses. Data on whole lake water were previously published in Trout-Haney et al. (2016).
For example, freshwater cyanobacterial samples have been reported to contain 320,000 ng/g dw MC in oligo-mesotrophic lakes in Turkey (Gurbuz et al. 2016), 56,000 ng/g dw MC in a meso-eutrophic Italian lake (Manganelli et al. 2016), between 7 and 3912 ng/g dw MC in a eutrophic lake in the Netherlands (Ibelings et al. 2005), and up to $8.7 \times 10^5$ ng/g dw in hypereutrophic Lake Taihu in China (Hu et al. 2016). Our study lakes did not experience visible cyanobacterial surface blooms during the summer months, and cyanotoxin production is not limited to bloom-forming taxa or to the presence of surface blooms (Quiblier et al. 2013, Jakubowska and Szeląg-Wasilewska 2015). Given that the phytoplankton fraction studied here does not include MCs bound up in nano- and picocyanobacteria, which can constitute an important part of the producer community in Arctic lakes (Vincent and Hobbie 2000), these MC concentrations may underestimate the total MCs found in planktonic primary producers. Picocyanobacteria would have been included in our previously reported whole lake water measurements (Trout-Haney et al. 2016), albeit at low densities since the samples were not filtered to concentrate phytoplankton cells. Additionally, although we collected samples in late July when these lakes typically experience highest surface water temperatures, these temperatures are variable and we do not know whether pelagic cyanobacterial MCs would be slightly higher in August.

Microcystin concentrations were also variable within zooplankton, which may have been due to the variation in phytoplankton MCs, as well as in differences in zooplankton composition, selective feeding on certain taxa, or food sources not measured in this study. For example, many zooplankton are omnivores who also feed on the microbial food web (Wolkovich et al. 2014): Copepods feed on protozoans and *Daphnia* graze on both bacteria and protozoans (Face and Vaque 1994). In shallow Arctic lakes, zooplankton can also utilize non-phytoplankton resources such as organic matter (Rautio et al. 2011). However, *Daphnia* tend to be less efficient when grazing on bacteria (Porter et al. 1983), and microbial or benthic food sources alone generally represent a poor diet for most zooplankton (Martin-Creuzburg et al. 2011). Accounting for contributions from the benthos and microbial loop is an important future step in accurately quantifying the trophic transfer of cyanotoxins to zooplankton, particularly in Arctic ecosystems where photosynthetic picocyanobacteria and benthic cyanobacteria can be abundant.

**Benthic cyanobacteria as sources of microcystins**

This study presents the first documented case of MCs in benthic consumers from Greenland lakes and one of few to report cyanotoxins within natural colonies of *N. pruniforme* and *N. zetterstedtii* (Trout-Haney et al. 2021). Benthic resources such as mats have been shown to serve as important subsidies for pelagic consumers in Arctic and sub-Arctic ponds (Rautio and Vincent 2006, Cazzanelli et al. 2012, Mariash et al. 2014). Benthic cyanobacteria are also increasingly recognized for their role as sources of cyanotoxins in freshwater lotic systems (Fetscher et al. 2015, Bouma-Gregson et al. 2018). Our study suggests that benthic species may also be important sources of cyanotoxins in lakes and ponds, particularly in systems where benthic producers dominate, such as polar regions. In a separate study (Trout-Haney et al. 2021), we evaluated the extent to which benthic *Nostoc* release toxins into the lake water (i.e., actively or passively through the colony sheath) or have toxins transferred through grazing.

In these Greenlandic lakes, snails are commonly found on *Nostoc* colonies, presumably grazing epiphytic matter from colony sheaths and potentially ingesting MCs from trace amounts of *Nostoc* tissue. Although we did not detect a difference in MCs in snails from lakes with vs. without *Nostoc*, the large range in snail MCs could be influenced by lake-specific differences in *Nostoc* such as colony size, abundance, and spatial distribution, or differences in snail species, age, and food preferences. Notably, chironomid larvae also showed high variability in MC concentrations across lakes, suggesting that the chironomid category likely comprises diverse species with a range of feeding strategies (Reuss et al. 2014). If chironomid larvae do utilize diverse food sources, biomagnification within this group might be more sensitive to lake-specific factors such as community composition. Importantly, these larvae are sometimes found in or on *Nostoc* colonies, suggesting that incidental consumption of *Nostoc* tissue or epiphytes is possible.

Finally, the concentrations of MC per gram dry weight in benthic organisms include some error
introduced by the wet to dry weight conversions. However, examining MC concentrations per gram wet weight of these organisms (Appendix S1: Fig. S1) demonstrates that Nostoc remain the highest in MCs relative to the other benthic organisms measured, the relative concentrations of MCs among taxa only change slightly (i.e., median concentration of MCs per gram wet weight in snails is slightly higher than in chironomids and dytiscids) and that MCs in chironomids, snails, and dytiscids are still highly variable across lakes.

**Likely prevalence of biodilution in aquatic taxa**

While cyanotoxin concentrations varied considerably both within and among trophic levels, there was little evidence for biomagnification of MCs: Concentrations either stayed the same across trophic levels (e.g., phytoplankton to zooplankton) or decreased with trophic level (Fig. 2). These patterns suggest that MCs are transferring, but not biomagnifying, in Kangerlussuaq lake food webs. Exploratory calculations of potential biomagnification vs. biodilution across our food web components, given observed MC concentrations, further suggest that most consumers likely exhibited biodilution of MC regardless of dietary scenario (Appendix S2: Fig. S2).

In comparing planktonic to benthic consumers, zooplankton contained an order of magnitude more MCs than chironomids and two orders of magnitude more MC than snails (Fig. 2)—likely because zooplankton and snails differ in feeding rates, assimilation efficiencies, and, perhaps most importantly, graze on spatially distinct resource pools (i.e., pelagic vs. benthic). Similarly, in a meta-analysis of food web MC concentrations from 42 studies, Kozlowsky-Suzuki et al. (2012) found that zooplankton MC concentrations were more variable than those in decapods, mollusks, fish, turtles, and birds. This finding highlights the importance of taking into account not only broad trophic categories, but also species-level feeding strategies and diet in determining the routes of toxin transfer, in future studies.

**Conclusions**

Lake-dwelling cyanobacteria and their toxins are an increasingly serious and global threat to the health of aquatic food webs and the safe use of freshwater by humans. Very little is currently known about the presence and trophic transfer of cyanotoxins in high latitude lakes, yet cyanobacteria are common in Arctic environments as they are among the relatively few organisms capable of tolerating the harsh environmental conditions. Our results demonstrate that MCs can be present in multiple taxonomic groups within Greenlandic lake food webs and that both benthic and pelagic cyanobacteria could be important sources of toxins into the aquatic ecosystem. Results further suggest MCs generally did not biomagnify and that biodilution of MCs may be the dominant process in these lake food webs. Given that many cyanobacterial species are capable of producing more than one type of MC, as well as other cyanotoxins (Buratti et al. 2017), investigating whether additional cyanotoxin classes also occur in these environments and within the aquatic food web represent important next steps. Future studies should also employ more selective detection methods, such as liquid chromatography-tandem mass spectrometry, to verify ELISA results and quantify separately the various isomers of MC, especially in picocyanobacteria and the larger cyanobacterial taxa studied here. This work may be particularly important in polar ecosystems, especially given that previous studies have detected unique MC variants in these environments (Jungblut et al. 2006, Kleinteich et al. 2013, Puddick et al. 2015, Kleinteich et al. 2018) and polar regions are experiencing rapid and ongoing climate-associated changes that may favor increased cyanobacterial growth.

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**Literature Cited**

Babica, P., L. Blaha, and B. Marsalek. 2006. Exploring the natural role of microcystins – a review of effects on photoautotrophic organisms. Journal of Phycology 42:9–20.

Banack, S., T. Caller, P. Henegan, J. Haney, A. Murby, J. Metcalf, J. Powell, P. Cox, and E. Stommel. 2015. Detection of cyanotoxins, β-N-methylamino-L-alanine and microcystins, from a lake surrounded by cases of amyotrophic lateral sclerosis. Toxins 7:322–336.

Billam, M., L. Tang, Q. Cai, S. Mukhi, H. Guan, P. Wang, Z. Wang, C. W. Theodorakis, R. J. Kendall, and J.-S. Wang. 2006. Seasonal variations in the concentration of microcystin-LR in two lakes in western Texas, USA. Environmental Toxicology and Chemistry 25:349–355.

Bouma-Gregson, K., R. M. Kudela, and M. E. Power. 2018. Widespread anatoxin-a detection in benthic cyanobacterial mats throughout a river network. PLOS ONE 13:1–21.

Brodersen, K., and N. Anderson. 2000. Subfossil insect remains (Chironomidae) and lake-water temperature inference in the Sisimiut-Kangerlussuaq region, southern West Greenland. Geology of Greenland Survey Bulletin 186:78–82.

Buratti, F. M., M. Manganelli, S. Vichi, M. Stefanelli, S. Scardala, E. Testai, and E. Funari. 2017. Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. Archives of Toxicology 91:1049–1130.

Cazzanelli, M., L. Forström, M. Rautio, A. Michelsen, and K. S. Christoffersen. 2012. Benthic resources are the key to *Daphnia middendorffiana* survival in a high arctic pond. Freshwater Biology 57:541–551.

Chrapusta, E., M. Węgrzyn, K. Zabaglo, A. Kaminski, M. Adamski, P. Wietrzyk, and J. Bialczyk. 2015. Microcystins and anatoxin-a in Arctic biocrust cyanobacterial communities. Toxicon 101:35–40.

Ferrão-Filho, A. S., and B. Kozlowsky-Suzuki. 2011. Cyanotoxins: bioaccumulation and effects on aquatic animals. Marine Drugs 9:2729–2772.

Fetscher, A. E., M. D. A. Howard, R. Stancheva, R. M. Kudela, E. D. Stein, M. A. Sutula, L. B. Busse, and R. G. Sheath. 2015. Wadeable streams as widespread sources of benthic cyanotoxins in California, USA. Harmful Algae 49:105–116.

Gurbuz, F., O. Y. Uzunmehmetoğlu, Ö. Diler, J. S. Metcalf, and G. A. Codde. 2016. Occurrence of microcystins in water, bloom, sediment and fish from a public water supply. Science of the Total Environment 562:860–868.

Hitzfeld, B. C., C. S. Lampert, N. Spaeth, D. Mountfort, H. Kaspar, and D. R. Dietrich. 2000. Toxin production in cyanobacterial mats from ponds on the McMurdo ice shelf, Antarctica. Toxicon 38:1731–1748.

Hu, L., K. Shan, L. Lin, W. Shen, L. Huang, N. Gan, and L. Song. 2016. Multi-year assessment of toxic genotypes and microcystin concentration in northern Lake Taihu, China. Toxins 8:23.

Humble, A. V., G. M. Gadd, and G. A. Codde. 1997. Binding of copper and zinc to three cyanobacterial microcystins quantified by differential pulse polarography. Water Research 31:1679–1686.

Ibelings, B. W., K. Bruning, J. de Jonge, K. Wolfstein, L. M. D. Pires, J. Postma, and T. Burger. 2005. Distribution of microcystins in a lake foodweb: no evidence for biomagnification. Microbial Ecology 49:487–500.

Jakubowska, N., and E. Szeląg-Wasielewska. 2015. Toxic picoplanktonic cyanobacteria—Review. Marine Drugs 13:1497–1518.

Jungblut, A.-D., S. J. Hoeger, D. Mountfort, B. C. Hitzfeld, D. R. Dietrich, and B. A. Neilan. 2006. Characterization of microcystin production in an Antarctic cyanobacterial mat community. Toxicon 47:271–278.

Kleinteich, J., F. Hildebrand, S. A. Wood, S. Cirés, R. Agha, A. Quesada, D. Pearce, P. Convey, F. C. Kupper, and D. R. Dietrich. 2014. Diversity of toxin and non-toxin containing cyanobacterial mats of meltwater ponds on the Antarctic Peninsula: a pyrosequencing approach. Antarctic Science 26:521–532.

Kleinteich, J., J. Puddick, S. A. Wood, F. Hildebrand, D. H. IV Laughinghouse, A. D. Pearce, R. D. Dietrich, and A. Wilmotte. 2018. Toxic cyanobacteria in Svalbard: chemical diversity of microcystins detected using a liquid chromatography mass spectrometry precursor ion screening method. Toxins 10:147.

Kleinteich, J., S. A. Wood, F. C. Kupper, A. Camacho, A. Quesada, T. Frickey, and D. R. Dietrich. 2012. Temperature-related changes in polar cyanobacterial mat diversity and toxin production. Nature Climate Change 2:356–360.

Kleinteich, J., S. A. Wood, J. Puddick, D. Schleheck, F. C. Kupper, and D. Dietrich. 2013. Potent toxins in Arctic environments – Presence of saxitoxins and an unusual microcystin variant in Arctic freshwater ecosystems. Chemico-Biological Interactions 206:423–431.
Kozlowsky-Suzuki, B., A. E. Wilson, and A. S. Ferrão-Filho. 2012. Biomagnification or biodilution of microcystins in aquatic foodwebs? Meta-analyses of laboratory and field studies. Harmful Algae 18:47–55.

Landahl, C. C., and B. Nagell. 1978. Influence of the season and of preservation methods on wet- and dry weights of larvae of *Chironomus plumosus* L. Internationale Revue der gesamten Hydrobiologie und Hydrographie 63:405–410.

Manganelli, M., M. Stefanelli, S. Vichi, P. Andreani, G. Nascetti, F. Scialanca, S. Scardala, E. Testai, and E. Funari. 2016. Cyanobacteria biennial dynamic in a volcanic mesotrophic lake in central Italy: strategies to prevent dangerous human exposures to cyanotoxins. Toxicon 115:28–40.

Mariash, H. L., S. P. Devlin, L. Forström, R. I. Jones, and M. Rautio. 2014. Benthic mats offer a potential subsidy to pelagic consumers in tundra pond food webs. Limnology and Oceanography 59:733–744.

Marion, A., S. Plourde, and P. Sirois. 2016. Mortality and recruitment in two copepod populations in a subarctic oligotrophic reservoir and the influence of environmental forcing. Journal of Plankton Research 38:915–930.

Martin-Creuzeburg, D., B. Beck, and H. M. Freese. 2011. Food quality of heterotrophic bacteria for *Daphnia magna*: evidence for a limitation by sterols. FEMS Microbiology Ecology 76:592–601.

Miller, T. R., S. L. Bartlett, C. A. Weirich, and J. Hernandez. 2019. Automated subdaily sampling of cyanobacterial toxins on a buoy reveals new temporal patterns in toxin dynamics. Environmental Science & Technology 53:5661–5670.

Pace, M. L., and D. Vaqué. 1994. The importance of *Daphnia* in determining mortality rates of protozoans and rotifers in lakes. Limnology and Oceanography 39:985–996.

Pachauri, R. K., and L. A. Meyer, editors. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland.

Paerl, H. W., and V. J. Paul. 2012. Climate change: links to global expansion of harmful cyanobacteria. Water Research 46:1349–1363.

Pham, T.-L., and M. Utsumi. 2018. An overview of the accumulation of microcystins in aquatic ecosystems. Journal of Environmental Management 213:520–529.

Porter, K. G., Y. S. Feig, and E. F. Vetter. 1983. Morphology, flow regimes, and filtering rates of *Daphnia, Ceriodaphnia*, and *Bosmina* fed natural bacteria. Oecologia 58:156–163.

Przytulska, A., M. Bartosiewicz, and W. F. Vincent. 2017. Increased risk of cyanobacterial blooms in northern high-latitude lakes through climate warming and phosphorus enrichment. Freshwater Biology 62:1986–1996.

Puddick, J., M. Prinsep, S. Wood, S. Cary, D. Hamilton, and P. Holland. 2015. Further characterization of Glycine-containing microcystins from the McMurdo Dry Valleys of Antarctica. Toxins 7:493–515.

Quiblier, C., S. A. Wood, I. Echenique-Subiabre, M. Heath, A. Villeneuve, and J.-F. Humbert. 2013. A review of current knowledge on toxic benthic freshwater cyanobacteria – Ecology, toxin production and risk management. Water Research 47:5464–5479.

R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rautio, M., H. Mariash, and L. Forströ. 2011. Seasonal shifts between autochthonous and allochthonous carbon contributions to zooplankton diets in a subarctic lake. Limnology and Oceanography 56:1513–1524.

Rautio, M., and W. F. Vincent. 2006. Benthic and pelagic food resources for zooplankton in shallow high-latitude lakes and ponds. Freshwater Biology 51:1038–1052.

Reuss, N. S., L. Hamerlik, G. Velle, A. Michelsen, O. Pedersen, and K. P. Brodersen. 2014. Microhabitat influence on chironomid community structure and stable isotope signatures in west Greenland lakes. Hydrobiologia 730:59–77.

Reza Shamsollahi, H., M. Alimohammadi, R. Nabizadeh, S. Nazmara, and A. Mahvi. 2018. Monitoring of microcystin–LR concentration in water reservoir. Desalination and Water Treatment 126:345–349.

Sage, R. D. 1982. Wet and dry-weight estimates of insects and spiders based on length. American Midland Naturalist 108:407–411.

Saito, K., Y. Sei, S. Miki, and K. Yamaguchi. 2008. Detection of microcystin–metal complexes by using cryospray ionization-Fourier transform ion cyclotron resonance mass spectrometry. Toxicon 51:1496–1498.

Salmaso, N., et al. 2017. Basic guide to detection and monitoring of potentially toxic cyanobacteria. Pages 46–69 in J. Meriluoto, L. Spoof, and G. A. Codd, editors. Handbook of cyanobacterial monitoring and cyanotoxin analysis. John Wiley & Sons, Hoboken, New Jersey, USA.

Sivonen, K., and G. Jones. 1999. Chapter 3: cyanobacterial toxins. Pages 55–124 in I. Chorus and J. Bartram, editors. Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. E. & F.N. Spon, London, UK and New York, New York, USA.
Trout-Haney, J. V., A. L. Ritger, and K. L. Cottingham. 2021. Benthic cyanobacteria of the genus Nostoc are a source of microcysts in Greenlandic lakes and ponds. Freshwater Biology 66:266–277.

Trout-Haney, J. V., Z. Wood, and K. Cottingham. 2016. Presence of the cyanotoxin microcystin in Arctic lakes of southwestern Greenland. Toxins 8:256.

Van Aardt, W. J. 1967. Quantitative aspects of the water balance in Lymnaea stagnalis (L.). Netherlands Journal of Zoology 18:253–312.

Vincent, W. F., and J. E. Hobbie. 2000. Ecology of Arctic Lakes. Pages 197–232 in M. Nuttall and T. Callaghan, editors. The Arctic: environment, people, policy. Harwood Academic, Amsterdam, The Netherlands.

Walls, J. T., K. H. Wyatt, J. C. Doll, E. M. Rubenstein, and A. R. Rober. 2018. Hot and toxic: Temperature regulates microcystin release from cyanobacteria. Science of the Total Environment 610–611:786–795.

Wolkovich, E. M., S. Allesina, K. L. Cottingham, J. C. Moore, S. A. Sandin, and C. de Mazancourt. 2014. Linking the green and brown worlds: the prevalence and effect of multichannel feeding in food webs. Ecology 95:3376–3386.

Wood, S. A., D. Mountfort, A. I. Selwood, P. T. Holland, J. Puddick, and S. C. Cary. 2008. Widespread distribution and identification of eight novel microcystins in Antarctic cyanobacterial mats. Applied and Environmental Microbiology 74:7243–7251.

Yan, F., M. Ozsoz, and O. A. Sadik. 2000. Electrochemical and conformational studies of microcystin–LR. Analytica Chimica Acta 409:247–255.

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