Lake and Reservoir Management 23:161-168, 2007

Microcystin distribution in physical size class separations of natural plankton communities

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Abstract

Graham, J.L. and J.R. Jones. 2007. Microcystin distribution in physical size class separations of natural plankton communities. Lake and Reserv. Manage. 23:161-168.

Phytoplankton communities in 30 northern Missouri and Iowa lakes were physically separated into 5 size classes (>100 µm, 53-100 µm, 35-53 µm, 10-35 µm, 1-10 µm) during 15-21 August 2004 to determine the distribution of microcystin (MC) in size fractionated lake samples and assess how net collections influence estimates of MC concentration. MC was detected in whole water (total) from 83% of lakes sampled, and total MC values ranged from 0.1-7.0 µg/L (mean = 0.8 µg/L). On average, MC in the >100 µm size class comprised ~40% of total MC, while other individual size classes contributed 9-20% to total MC. MC values decreased with size class and were significantly greater in the >100 µm size class (mean = 0.5 µg/L) than the 35-53 µm (mean = 0.1 µg/L), 10-35 µm (mean = 0.0 µg/L), and 1-10 µm (mean = 0.0 µg/L) size classes ($p < 0.01$). MC values in nets with 100-µm, 53-µm, 35-µm, and 10-µm mesh were cumulatively summed to simulate the potential bias of measuring MC with various size plankton nets. On average, a 100-µm net underestimated total MC by 51%, compared to 37% for a 53-µm net, 28% for a 35-µm net, and 17% for a 10-µm net. While plankton nets consistently underestimated total MC, concentration of algae with net sieves allowed detection of MC at low levels ($\leq$0.01 µg/L); 93% of lakes had detectable levels of MC in concentrated samples. Thus, small mesh plankton nets are an option for documenting MC occurrence, but whole water samples should be collected to characterize total MC concentrations.

Key words: cyanobacteria, cyanotoxin, microcystin, sampling methods, size fractions

Cyanobacteria produce a chemically and bioactively diverse group of toxins that target fundamental cellular processes, thereby affecting a wide range of organisms, including humans. Concern for public health and environmental consequences has prompted cyanotoxin research worldwide. Hepatotoxic microcystins are the most common class of cyanotoxins and the specific focus of many studies. Over 80 microcystin variants (herein referred to collectively as microcystin, MC) have been isolated with the -LR, -LA, and -YR variants being most toxic (Watanabe *et al.* 1996, Chorus 2001). Microcystin is produced by strains in at least 13 cyanobacterial genera ranging in size from <1 to >100 µm (Carmichael 1997, Chorus and Bartram 1999). Toxic incidents involving MC, however, are frequently associated with large (>64 µm) bloom forming genera such as *Anabaena* and *Microcystis* (Chorus and Bartram 1999) so risk of exposure is associated with abundance of large genera. Not well understood is the contribution of smaller forms such as *Anabaenopsis* and *Synechococcus* (Bláha and Maršálek 1999, Domingos *et al.* 1999, Oudra *et al.* 2002) to environmental MC concentrations.

Together, particulate and dissolved MC constitute total MC (MC_T) concentrations. MC is maintained intracellularly as particulate MC (MC_P), and the MC_P compartment typically composes the majority of total MC (MC_T). Upon cell lysis MC is released, and dissolved MC (MC_D) often occurs in the water column during cyanobacterial bloom senescence (Bláha and Maršálek 1999, Chorus and Bartram 1999, Chorus 2001). Because MC_P is the predominant compartment, cyanobacteria are often concentrated for MC analysis using plankton nets with mesh sizes between 10 and 100 µm (Kotak *et al.* 1995, Vézie *et al.* 1998, Wiedner *et al.* 2002, Baldia *et al.* 2003, Graham *et al.* 2004), but there are no consistent guidelines for selecting mesh size. Plankton nets generally concentrate larger cyanobacteria while excluding smaller size

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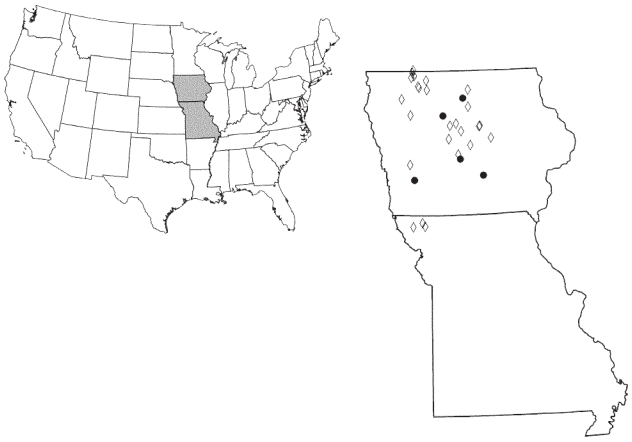

Figure 1.-Location of northwestern Missouri and Iowa lakes included in the study. Open diamonds indicate lakes where microcystin (MC) was detected in whole water samples. Closed circles indicate lakes where MC was not detected in whole water samples.

classes, but large forms may also pass through nets (Allen 1919, Johnstone *et al.* 1924, Hardy 1956); therefore, net collected MC measurements are influenced by mesh size, and potentially are underestimated.

Cultured cyanobacteria of all sizes are known to produce MC (Bláha and Maršálek 1999, Domingos *et al.* 1999, Chorus 2001, Oudra *et al.* 2002), but in natural assemblages the distribution of MC among cyanobacterial size classes has not been well described. Understanding the contribution of various cyanobacterial size classes to environmental MC concentrations is critical to development of effective methods to monitor and assess environmental health risks. To address this question the cyanobacterial communities in 30 northern Missouri and Iowa, U.S.A., lakes were separated into 5 discrete size classes to: (1) determine the distribution of MC in size fractionated lake samples and (2) assess how plankton nets influence estimates of MC concentration.

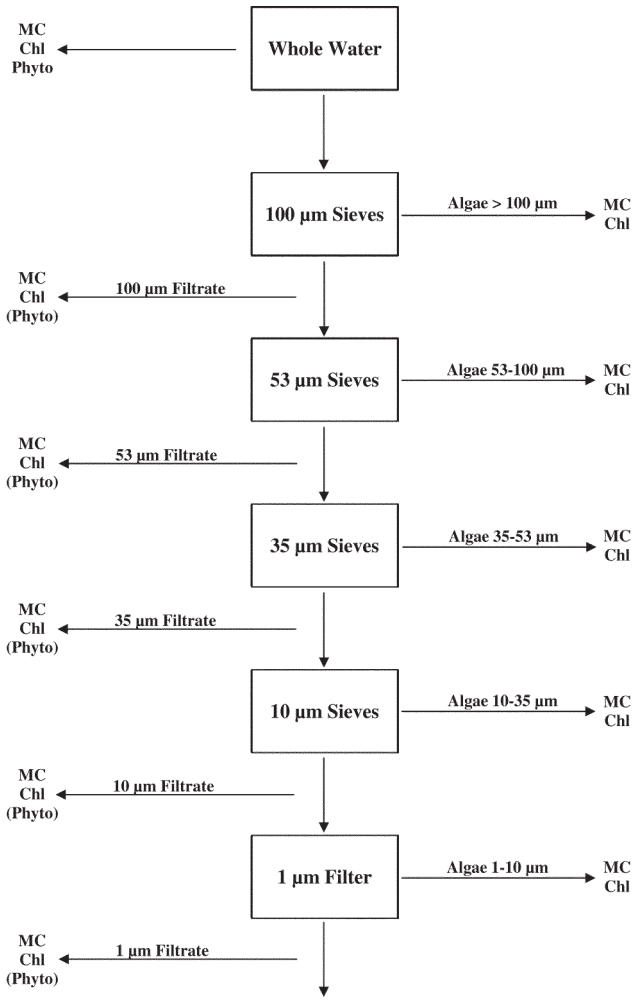

Figure 2.-Method used to separate cyanobacteria into discrete size classes and the microcystin (MC), chlorophyll (Chl), and phytoplankton (Phyto) samples collected. Phytoplankton samples were only collected after each filtration step at 6 lakes. Each size class was filtered separately. For each size class >1 µm 4 sieves of the same mesh size were stacked in series. Samples were collected from both filtrate and algal material concentrated on the sieves.

Methods

Sample collection

Thirty lakes and reservoirs in northern Missouri and Iowa, selected to represent the range of limnological conditions encountered in the region (Graham *et al.* 2004), were sampled during 15-21 August 2004 (Fig. 1). Temperature, dissolved oxygen profiles and Secchi transparency were measured at open pelagic locations on each lake. Water samples, integrated from the surface to Secchi depth, were analyzed for total phosphorus, total nitrogen, and volatile and nonvolatile suspended solids after Graham *et al.* (2004).

The phytoplankton community was separated in the field by sequentially filtering 6 L of the integrated sample through 100-µm, 53-µm, 35-µm, and 10-µm nitex mesh sieves and a 1-µm glass fiber filter (Fig. 2). Pilot studies indicated some large cyanobacteria passed through the sieves; this finding is consistent with the observation that it is difficult to predict what size organisms will be retained with a particular mesh size (Johnstone *et al.* 1924, Hardy 1956, De Bernardi 1984). To enhance separation, 4 same-size sieves were stacked in series for each filtration step. Microcystin, chlorophyll (Chl), and phytoplankton samples were collected from whole water prior to each filtration (n = 5). Following filtration both MC and Chl samples were collected from filtrate and from algal

material concentrated on the sieves. The volume of water passed through each consecutive sieve decreased by ~1 L as a result of sample collection (Fig. 2). Concentrated algal material was rinsed from the sieves using tap water. The volume filtered and final volume of the concentrated sample were recorded and used to calculate actual MC and Chl values from the concentrated algal material. Microcystin was not detected in tap water used for the rinse step. At 6 randomly selected lakes, phytoplankton samples were collected from the filtrate after each step in the sequence.

Microcystin samples underwent 3 freeze-thaw cycles to lyse cyanobacterial cells and release cell-bound MC. Samples were subsequently filtered through 0.45-µm membrane filters to remove particulates (Gjølme and Utkilen 1994, Chorus and Bartram 1999, Johnston and Jacoby 2003). Envirogard® ELISA kits (detection limit 0.1 µg/L; includes -LR, -YR, -RR, and nodularin) were used to measure MC. Total microcystin was measured on whole water. Microcystin in the 1-µm filtrate (Fig. 2) was considered dissolved (Chorus and Bartram 1999). Particulate microcystin was calculated by taking the difference between MC_T and MC_D.

Chlorophyll was collected on 1.0-µm glass fiber filters, extracted in heated ethanol, and analyzed fluorometrically (Knowlton 1984, Sartory and Grobbelar 1986). Phytoplankton samples were preserved with gluteraldehyde and sent to PhycoTech (St. Joseph, Mich.) for identification and biovolume analysis. In addition, the average greatest axial linear dimension (GALD, the length of the longest axis of a cell, filament, or colony) was estimated for all species.

Microcystin distribution in size class separations

Sequential separation of the cyanobacterial community created 5 size classes: >100 µm, 53-100 µm, 35-53 µm, 10-35 µm, and 1-10 µm (Fig. 2). Microcystin and Chl in each size class were measured directly on algal material concentrated by the sieves (sieve MC) and indirectly by calculating the difference between filtrate (or whole water) values before and after each step in the filtration sequence (filtrate MC). Agreement between sieve and filtrate measures of MC and Chl in each size class was expected.

Simulated sampling with plankton nets

To simulate potential MC values in net collections with 100-µm, 53-µm, 35-µm, and 10-µm mesh, filtrate estimates of MC in the larger size classes were cumulatively summed. For example, sampling with a 53-µm net (MC > 53 µm) was simulated by summing filtrate estimates of MC in the >100 µm and 53-100 µm size classes. Simulated MC values were compared with MC_T values to determine % underestimation. Only lakes with detectable MC_T were included in this component of the analyses (n = 25).

Statistical analyses

Relations between MC, Chl, and cyanobacterial biovolume were developed using Spearman-Rank correlation analysis (significance set at $p < 0.05$). Significant differences in MC among size classes were determined using one-way ANOVA and Tukey's pairwise comparisons ($p < 0.05$). The relation between whole water and simulated plankton net estimates of MC was determined using linear regression analysis, and significant differences between estimates were determined using Wilcoxon pairwise comparisons ($p < 0.05$). Data were $\log_{10}$ transformed when the assumptions of normality and heteroscedasticity were not met.

Results

Descriptive limnology

Lake conditions were encountered wherein total phosphorus (28-521 µg/L), total nitrogen (700-12750 µg/L), and chlorophyll (8-294 µg/L) values spanned 2-3 orders of magnitude (Table 1) across the eu-hypereutrophic range of lakes/reservoirs in this mid-continent region (Graham *et al.* 2004). Cyanobacteria dominated the phytoplankton biovolume in 70% of lakes, and all lakes had at least one genera known to contain MC producing strains, with *Microcystis* (present in 80% of lakes), *Oscillatoria* (77%), *Synechococcus* (77%), and *Anabaena* (70%) most common. Although ubiquitous, potential MC producers dominated cyanobacterial biovolume in only 37% of lakes. Several genera with potential to produce toxins other than MC, including *Aphanizomenon* (33% of lakes) and *Cylindrospermopsis* (10%) also dominated cyanobacterial biovolume.

Microcystin was detected in whole water from 83% of lakes sampled (25 of 30; Fig. 1) and MC_T ranged from 0.0-7.0 µg/L (median: 0.4 µg/L), MC_P from 0.0-6.8 µg/L (median: 0.3 µg/L), and MC_D from 0.0-0.4 µg/L (median: 0.0 µg/L; Table 1). Among lakes with detectable MC (n = 25), 52% had only MC_P and 4% only MC_D. The remaining 44% had both MC_P and MC_D, with MC_P comprising 57-97% of MC_T. Thus, MC_P was the predominant compartment.

Microcystis was present in 96% of lakes with detectable MC (n = 25) and absent from lakes without (n = 5), which is consistent with the role of *Microcystis* as a common MC producer. Total microcystin was most strongly correlated with *Microcystis* biovolume ($r_s = 0.84$, $p < 0.01$, n = 30) and correlated with total cyanobacterial biovolume ($r_s = 0.75$, $p < 0.01$), Chl ($r_s = 0.67$, $p < 0.01$) and *Oscillatoria* biovolume ($r_s = 0.40$, $p = 0.03$).

Table 1.-General limnological conditions in lakes sampled during 15-21 August 2004. All n = 30. Total microcystin (MC_T) was measured on whole water and dissolved MC (MC_D) on 1-µm filtrate. Particulate MC (MC_P) was calculated as the difference between MC_T and MC_D.

Variable	Mean	Median	Range
Z_{mean} (m)	2.6	3.0	0.9-11.6
Area (ha)	710	153	6-7689
Secchi (m)	0.9	0.8	0.2-2.8
Total Phosphorus (TP, µg/L)	106	76	28-521
Total Nitrogen (TN, µg/L)	3430	2340	700-12570
TN:TP	54	25	5-282
Chlorophyll (µg/L)	69	52	8-294
Nonvolatile Suspended Solids (mg/L)	9.1	4.5	0.4-55.5
Volatile Suspended Solids (mg/L)	12.6	10.0	0.1-38.0
Cyanobacterial Biovolume (μm^3/mL)	1.4×10^7	9.9×10^6	6.5×10^4-7.6×10^7
Total microcystin (µg/L)	0.8	0.4	0.0-7.0
Dissolved microcystin (µg/L)	0.1	0.0	0.0-0.4
Particulate microcystin (µg/L)	0.8	0.3	0.0-6.8

Table 2.-Microcystin (MC) in discrete size classes based on indirect (filtrate) estimates calculated by differences between each successive filtration and direct (sieve) measurements in algal material collected on sieves (See Methods, Fig. 2). Means ±1 SE and ranges. All n = 30. For filtrate and sieve measures, significant differences among size classes, indicated by different letters, were determined using one-way ANOVA and Tukey's pairwise comparisons.

	Filtrate MC (µg/L)			Sieve MC (µg/L)		
Size Class	Mean	Range	Lakes with Detectable MC	Mean	Range	Lakes with Detectable Chl
> 100 µm	0.5±0.22[a]	0.0-6.4	22	0.3±0.10[a]	0.0-2.3	27
53-100 µm	0.2±0.04[a, b]	0.0-0.8	19	0.1±0.03[a, b]	0.0-0.7	26
35-53 µm	0.1±0.01[b]	0.0-0.2	17	0.0±0.01[b]	0.0-0.2	20
10-35 µm	0.0±0.01[b]	0.0-0.4	14	0.0±0.01[b]	0.0-0.2	12
1-10 µm	0.0±0.02[b]	0.0-0.3	11	0.0±0.01[b]	0.0-0.1	21
	F=3.48, p<0.01			**F=6.18, p<0.01**		

Sieve and filtrate estimates of microcystin in size classes

Direct measurement of MC in the algal material collected on sieves improved the rate of MC detection in individual size classes relative to indirect estimates based on differences between successive filtrations (see Methods, Fig. 2). In algal material MC was detected in 71% of size-class samples (106 of 150) and 97% of sampled lakes (29 of 30). In contrast, in differences between successive filtrations, MC was only detected in 55% of size class samples (83 of 150) and 83% of sampled lakes (Table 2). Concentrating algal material on the sieves likely facilitated MC detection in direct measurements. Extrapolated environmental MC values were always an order of magnitude less than the ELISA detection limit of 0.1 µg/L when MC was detected in sieve samples but not in filtrate. Thus, environmental MC values were low enough in some samples that MC was not detectable without the concentration step.

Despite increased detection of MC, sieve estimates of MC were generally smaller than filtrate estimates (Table 2). When MC was detected in both sieve and filtrate measurements, 80% (60 of 75) of sieve estimates were less than filtrate estimates, and the overall difference was significant ($Z = 5.96$, $p < 0.01$, $n = 75$). Algal material continued to pass through sieves after the separation step was complete but before algal samples were collected for analysis; these

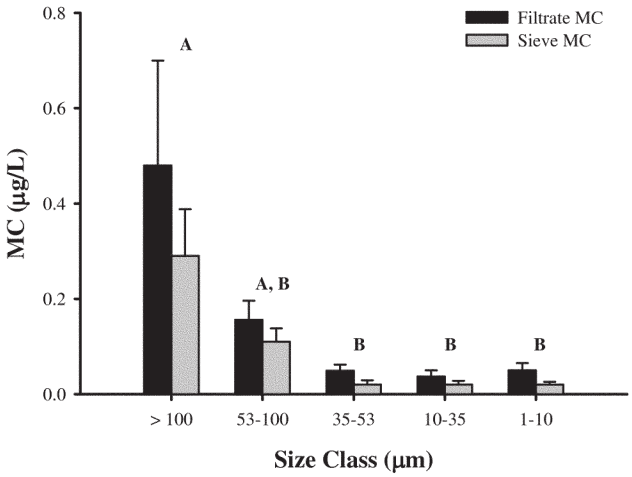

Figure 3.-The distribution of microcystin (MC) among size classes. Values based on indirect (filtrate) estimates of MC based on differences between successive filtrations and direct (sieve) measurements in algal material collected on sieves (see Methods, Fig. 2). Mean ±1 SE, all n = 30. Significant differences among size classes, indicated by different letters, were determined using one-way ANOVA, and were the same for both filtrate ($F = 3.48$, $p < 0.01$) and sieve ($F = 6.18$, $p < 0.01$) estimates.

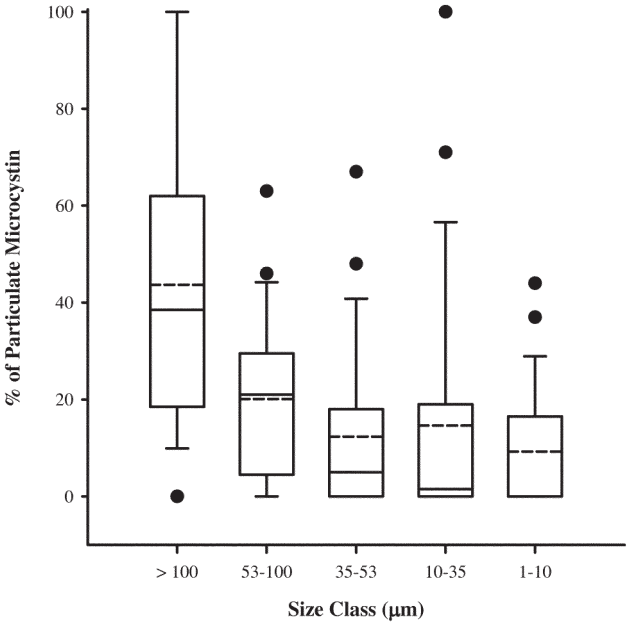

Figure 4.-The contribution of each size class to particulate microcystin (MC_P) in individual lakes (all n = 24). Solid lines represent median values and dashed lines represent mean values, the box represents 25th and 75th percentiles, and whiskers represent 10th and 90th percentiles. Closed circles represent points that fall outside the 10th and 90th percentiles. Figure based on indirect (filtrate) measures of MC (see Methods, Fig. 2).

losses likely account for differences between sieve and filtrate estimates of MC. Based on differences in Chl between sieve and filtrate measurements, an average of 34-62% (medians 18-60%) of algal material was lost through the sieves after each separation step. This substantial loss of algal material from net sieves indicates environmental MC values are likely underestimated by field net collections of algae.

Microcystin distribution in size class separations

Regardless of differences in detection and concentration, sieve and filtrate measurements show similar patterns in MC distribution among size classes (Fig. 3; Table 2). Microcystin was most abundant in the >100 µm size class and decreased significantly among progressively smaller size classes (Fig. 3; Table 2). Mean MC decreased by 60-70% between the >100 µm and 53-100 µm size classes and 50-100% between the 53-100 and 35-53 µm size classes. Microcystin was similar among the 35-53 µm, 10-35 µm, and 1-10 µm size classes (Fig. 3; Table 2). While MC values were greatest in the >100 µm size class, on average this fraction only comprised ~40% of MC_P, with other individual size classes contributing 9-20% to MC_P (Fig. 4).

Size separation of algae by net sieves was not discrete, and large algae passed through the small sieves during the separation process. Based on the 6 lakes where cyanobacteria were enumerated and GALD was measured in each filtrate fraction, ≥70% of cyanobacteria were retained by sieves <GALD (data not shown). Most large cyanobacteria that passed through small sieves were filamentous forms, particularly *Oscillatoria*, which have both long and short axes. Pass through resulted in cyanobacteria with GALDs >100 µm present in all filtrate fractions. Therefore, MC distribution in the various size classes may be attributed to either MC production by smaller cyanobacteria and/or by larger cells that passed through during the separation process.

Simulated sampling with plankton nets

Whole water (MC_T) and simulated plankton net estimates of MC were linearly associated ($r^2 = 0.77$, $p < 0.01$, $n = 100$) but the net approach significantly underestimated MC_T (pairwise comparisons, all $p < 0.01$; Fig. 5A). Use of 100-µm, 53-µm, and 35-µm sieves underestimated MC_T in 80-88% of lakes with detectable MC (n = 25), and the 10-µm net underestimated MC_T in 68%. On average, simulated use of a 100-µm plankton net underestimated MC_T by 51% compared to 37% using a 53-µm net, 28% using a 35-µm net, and 17% using a 10-µm net (Fig. 5B). Thus, as expected, underestimation decreased with decreasing net size. The 10-µm net estimates of environmental MC concentrations represented ~80% of whole water values and were a substantial improvement

over 100-µm net estimates, which were only ~50% of whole water estimates.

Discussion

Lakes selected to determine MC distribution among physical size class separations reflected regional diversity in lake fertility, cyanobacterial community structure, and occurrence of toxin producing strains. Despite this variability, there was a distinct overall pattern in MC distribution among size classes. Microcystin abundance was greatest in the >100-µm collection and decreased systematically with smaller size classes (Table 2; Fig. 3). Similarly, 2 studies on hypereutrophic German lakes physically separated communities dominated by *Microcystis* into size classes ranging from 33 to 340 µm; both studies found MC concentrations were greatest in the >100 µm size classes, and MC content decreased with decrease in size class (Jungmann *et al.* 1996, Kurmayer *et al.* 2003). Laboratory studies assessing MC production by cyanobacterial strains also indicate larger (>64 µm) colonial or filamentous forms have a greater MC content than picocyanobacterial (<2 µm; size classes as defined in Callieria and Stockner 2002) genera (Bláha and Maršálek 1999, Domingos *et al.* 1999, Chorus 2001, Oudra *et al.* 2002). For example, Oudra *et al.* (2002) found on a µg/g basis *Microcystis* had MC concentrations an order of magnitude greater than the picocyanobacterial genera *Synechocystis* and *Anabaenopsis*. Therefore, the presence and abundance of potential MC-producing cyanobacteria >64-100 µm may be a good indicator of MC occurrence.

While MC abundance was greatest in the >100 µm size class, collectively the smaller size classes contributed ≥60% of MC_P in most lakes (Fig. 4). Simulated sampling with net sieves demonstrated plankton net sampling potentially excludes portions of the MC-producing community resulting in underestimates of MC_T (Fig. 5). Underestimates were minimized using 10-µm nets, as recommended by Chorus and Bartram (1999), but on average, even the 10-µm net underestimated environmental MC concentrations by ~20% in this assessment (Fig. 5).

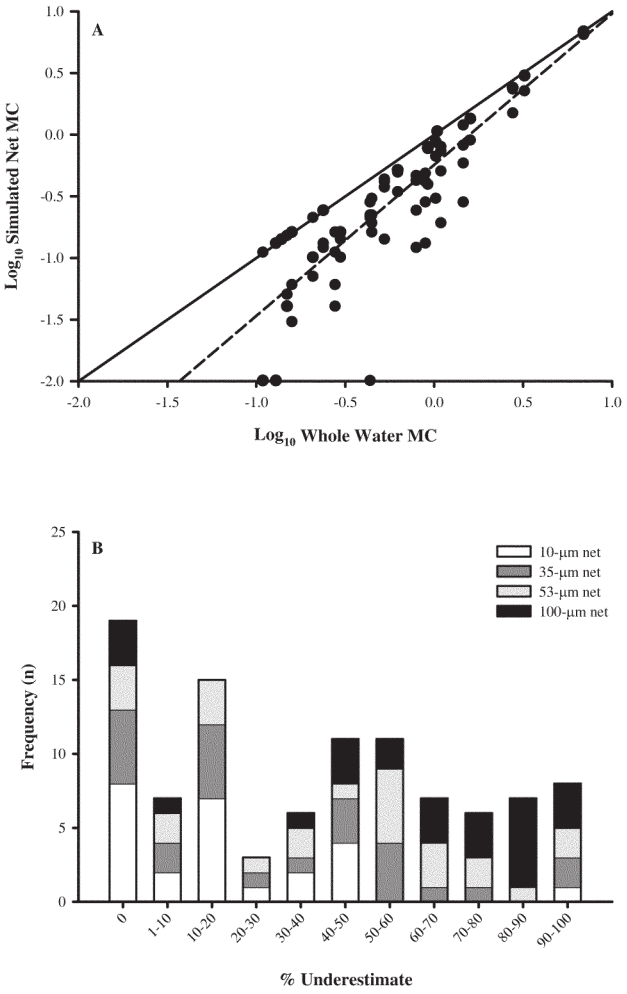

Figure 5.-Underestimation of whole water microcystin (MC_T) by simulated sampling with plankton nets. (A) Relation between MC_T and simulated plankton net estimates of MC ($r^2 = 0.77$, $p < 0.01$); based on lakes with detectable MC_T (n = 25), all simulated estimates were pooled (n = 100). The solid line represents a 1:1 relation and the dashed line represents the regression relation. (B) Frequency distribution of the % underestimation of MC_T by simulated plankton net sampling. For each net size (100, 53, 35, and 10 µm) all n = 25. Total n = 100.

Underestimates of MC calculated from simulated sampling with plankton nets are likely conservative. While simulations excluded smaller size classes and dissolved fractions, they did not account for the concentration and loss of algal material that typically occurs when sampling with plankton nets. Theoretically, algae larger than the mesh size are retained and concentrated, while smaller forms pass through. The passage of larger forms is well-known, although rarely quantified. Losses are influenced by phytoplankton community composition, mesh quality and size, speed of sampling, volume sampled, and net clogging (Allen 1919, Johnstone *et al.* 1924, Hardy 1956, De Bernardi 1984, Chorus and Bartram 1999). In this study pass through was minimized during size class separation by stacking 4 same-size sieves in series and gently filtering the sample. But, substantial losses still occurred as demonstrated by the smaller MC values in algal material collected on sieves (Fig. 3; Table 2). Losses would likely be greater when collecting algal material with a single plankton net tow.

Due to the potential loss of algal material with plankton net collections, whole water samples that incorporate both the particulate and dissolved microcystin compartments are required to characterize MC_T concentrations. However, an advantage of plankton net collection is the concentration of algal material, and therefore MC, to levels greater than

encountered in the environment, thereby increasing the probability of detection with commercial ELISA kits (minimum detection limit ~0.1 µg/L). In the current study, MC was detected ~15% more frequently in concentrated algal material than in whole water samples. By comparison, during 2000-2001 regional surveys Graham *et al.* (2004) found that 98% of algal samples concentrated with a 64-µm plankton net had detectable levels of MC. Small-mesh plankton nets may therefore afford an advantage when documenting MC occurrence.

Pass through likely influenced MC distribution among size classes. Colonies and filaments >100 µm were present in all size classes in the 6 phytoplankton samples that were quantified, indicating larger forms may comprise a greater proportion of MC_T than indicated by size class separation. Larger colonies and filaments also may have broken up into smaller forms during the separation process, thereby contributing to MC content of smaller size classes. Large forms may be the dominant contributor to MC_T, but cyanobacteria of all size classes have been documented to produce MC in the laboratory (Bláha and Maršálek 1999, Domingos *et al.* 1999, Oudra *et al.* 2002) and likely contribute to environmental MC concentrations. Although separations were not discrete, results suggest smaller size classes contribute to MC_T. For example, not all lakes had detectable MC in the >100 µm size class, and 6 lakes had >50% of MC_P in the 35-53-µm size class or smaller.

Accurate estimates of environmental MC concentrations are essential to understanding the ecology of toxin production and minimization of human health risks. Quantifying and comparing the relative benefits of sampling methods is critical to making informed decisions about which methods best meet research and monitoring goals. Consistent guidelines for collection of MC samples are not presently available, resulting in a range of techniques being used, including sampling with plankton nets of all sizes (Kotak *et al.* 1995, Vézie *et al.* 1998, Wiedner *et al.* 2002, Baldia *et al.* 2003, Graham *et al.* 2004), small pore filters (Gjølme and Utkilen 1994, Lawton *et al.* 1994, Chorus and Bartram 1999, Carmichael 2001), and whole water samples (Chorus and Bartram 1999, Hirooka *et al.* 1999, Johnston and Jacoby 2003). This study demonstrates MC abundance is greatest in cyanobacteria >100 µm, but smaller size-classes also contribute to environmental MC concentrations. Thus, monitoring for large cyanobacteria is likely a good indicator of MC occurrence, but sampling with plankton nets excludes portions of the MC-producing community and underestimates MC_T concentrations.

Acknowledgments

The authors thank A. St. Amand for sharing her ideas on separating phytoplankton into size classes and assistance with pilot studies, and C. Reynolds for sharing his thoughts on collecting algal samples with plankton nets. The authors also thank C. Pollard for field and laboratory assistance. Funding for this project was provided by the U.S. Environmental Protection Agency. This study has not been subjected to EPA administrative review and may not reflect the view of the agency; no official endorsement should be inferred. Funding and support were also provided by the Department of Fisheries and Wildlife Sciences at the University of Missouri.

References

Allen, E.J. 1919. A contribution to the quantitative study of plankton. J. Mar. Bio. Assoc. UK 12:1-8.

Baldia, S.F., M.C.G. Conaco, T. Nishijima, S. Imanishi and K.-I. Harada. 2003. Microcystin production during algal bloom occurrence in Laguna de Bay, the Philippines. Fish. Sci. 69:110-116.

Bláha, L. and B. Maršálek. 1999. Microcystin production and toxicity of picocyanobacteria as a risk factor for drinking water treatment plants. Algol. Stud. 92:95-108.

Callieri, C. and J.G. Stockner. 2002. Freshwater autotrophic picoplankton: a review. J. Limnol. 61:1-14.

Carmichael, W.W. 1997. The cyanotoxins. Adv. Bot. Res. 27:211-256.

Carmichael, W.W. 2001. Assessment of blue-green algal toxins in raw and finished drinking water. AWWA Research Foundation and American Water Works Association.

Chorus, I. and J. Bartram (eds.). 1999. Toxic Cyanobacteria in Water. WHO, E & FN Spon.

Chorus, I. (ed.). 2001. Cyanotoxins: occurrence, causes, consequences. Springer.

De Bernardi, R. 1984. Methods for the estimation of zooplankton abundance. P. 59-85. *In* J.A. Downing and F.H. Rigler (eds.). A manual on methods for the assessment of secondary productivity in freshwaters. Blackwell Scientific.

Domingos, P., T.K. Rubim, R. Molica, S.M.F.O. Azevedo and W.W. Carmichael. 1999. First report of microcystin production by picoplanktonic cyanobacteria isolated from a northeastern Brazilian drinking water supply. Environ. Toxicol. 14:31-35.

Gjølme, N. and H. Utkilen. 1994. A simple and rapid method for extraction of toxic peptides from cyanobacteria. P. 168-171. *In* G.A. Codd, T.M. Jefferies, C.W. Keevil and E. Potter (eds.). Detection Methods for Cyanobacterial Toxins. The Royal Society of Chemistry.

Graham, J.L., J.R. Jones, S.B. Jones, J.A. Downing and T.E. Clevenger. 2004. Environmental factors influencing microcystin distribution and concentration in the Midwestern United States. Water Res. 38:4395-4404.

Hardy, A.C. 1956. The Open Sea Its Natural History: The World of Plankton. Houghton Mifflin Company.

Hirooka, E.Y., M.H.P. Pinotti, T. Tsutsumi, F. Yoshida and Y. Ueno. 1999. Survey of microcystins in water between 1995 and 1996 in Parana, Brazil using ELISA. Nat. Toxins 7:103-109.

Johnston, B.R. and J.M. Jacoby. 2003. Cyanobacterial toxicity and migration in a mesotrophic lake in Western Washington, USA. Hydrobiologia 495:79-91.

Johnstone, J., A. Scott and H.C. Chadwick. 1924. The Marine Plankton. The University Press of Liverpool Limited.

Jungmann, D., K.-U. Ludwichowski, V. Faltin and J. Benndorf. 1996. A field study to investigate environmental factors that could effect microcystin synthesis of a *Microcystis* population in the Bautzen reservoir. Int. Rev. Hydrobiol. 81:493-501.

Knowlton, M.F. 1984. Flow-through microcuvette for fluorometric determination of chlorophyll. Water Resour. Bull. 20:1198-1205.

Kotak, B.G., A.K.-Y. Lam and E.E. Prepas. 1995. Variability of the hepatotoxin microcystin-LR in hypereutrophic drinking waters. J. Phycol. 31:248-263.

Kurmayer, R., G. Christiansen and I. Chorus. 2003. The abundance of microcystin-producing genotypes correlates positively with colony size in *Microcystis* sp. and determines its microcystin net production in Lake Wannsee. Appl. Environ. Microbiol. 69:787-795.

Lawton, L.A., C. Edwards and G.A. Codd. 1994. Extraction and high-performance liquid chromatographic method for the determination of microcystins in raw and treated waters. Analyst 119:1525-1530.

Oudra, B., M. Loudiki, V.M. Vasconcelos, B. Sabour, B. Sbiyyaa, Kh. Oufdou and N. Mezrioui. 2002. Detection and quantification of microcystins from cyanobacteria strains isolated from reservoirs and ponds in Morocco. Environ. Toxicol. 17:32-39.

Sartory, D.P. and J.U. Grobbelar. 1986. Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. Hydrobiologia 114:117-187.

Vézie, C., L. Brient, K. Sivonen, G. Bertru, J.-C. Lefeuvre and M. Salkinoja-Salonen. 1998. Variation of microcystin content of cyanobacterial blooms and isolated strains in Lake Grand-Lieu (France). Microb. Ecol. 35:126-135.

Watanabe, M.F., K.I. Harada, W.W. Carmichael and H. Fujiki (eds). 1996. Toxic Microcystis. CRC Press.

Wiedner, C., B. Nixdorf, R. Heinze, B. Wirsing, U. Neumann and J. Weckesser. 2002. Regulation of cyanobacteria and microcystin dynamics in polymictic shallow lakes. Arch. Hydrobiol. 155:383-400.