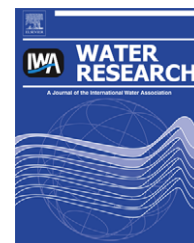


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Citizen monitoring: Testing hypotheses about the interactive influences of eutrophication and mussel invasion on a cyanobacterial toxin in lakes

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ABSTRACT

An existing volunteer monitoring network in the state of Michigan was exploited to conduct a statewide survey of the cyanobacterial toxin, microcystin, and to test hypotheses about the interactive influences of eutrophication and dreissenid mussel invasion. A total of 77 lakes were sampled by citizen volunteers for microcystin, total phosphorus (TP) and chlorophyll *a*. Microcystin was measured in depth-integrated samples collected from the euphotic zone as well as in surface-water samples collected along the shoreline. Average microcystin in samples collected by volunteers was not different from samples collected side-by-side by professionals. Euphotic-zone microcystin was positively related to TP in lakes without dreissenids (uninvaded) but not in lakes with dreissenids (invaded). Regression-tree analysis indicated that euphotic-zone microcystin was eight times higher in the presence of dreissenids for lakes with TP between 5 and 10 $\mu\text{g L}^{-1}$. In contrast, euphotic-zone microcystin was almost identical in invaded and uninvaded lakes with TP between 10 and 26 $\mu\text{g L}^{-1}$. Across all lakes, microcystin concentrations at the surface were on average more than double, and in some cases an order-of-magnitude greater than, concentrations in the euphotic-zone. Given these results, it seems prudent to include dreissenid invasion status in forecasting models for microcystin, and to include shoreline sampling in monitoring programs aimed at assessing recreational exposure to cyanobacterial toxins.

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1. Introduction

Cyanobacteria are a major source of water-quality problems, in part because many species produce toxins that contaminate drinking water and degrade the recreational value of freshwaters. Given the importance of cyanobacteria to water quality, major research efforts have been directed at predicting the influence of environmental factors on the biomass of

bloom-forming nuisance cyanobacteria (hereafter referred to as cyanobacteria). Most notably, this body of work has shown that the biomass of cyanobacteria increases faster with nutrient enrichment than that of phytoplankton biomass in general, such that the percentage of total phytoplankton biomass comprised by cyanobacteria can reach 100% during the summer in lakes in which total phosphorus concentrations exceed $\sim 100 \mu\text{g L}^{-1}$ (Downing et al., 2001; Kalff, 2002;

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Trimbee and Prepas, 1987). More recently, it has been shown that at extremely high enrichment levels (TP > ~500 $\mu\text{g L}^{-1}$), dominance by cyanobacteria may decline again with further enrichment (Jensen et al., 1994).

Recent advances in analytical methodology have enabled more widespread monitoring of particular cyanobacterial toxins, most notably the class of peptides known as microcystins. Microcystins are intracellular compounds produced by several cyanobacterial taxa (most notably *Microcystis aeruginosa*) that act as potent liver toxins when ingested by terrestrial animals and humans (Chorus and Bartram, 1999). Given the relative ease of microcystin measurement, studies have accumulated documenting the distribution and abundance of microcystins in a variety of habitats and geographical regions (Jacoby and Kann, 2007; Kotak and Zurawell, 2007). Despite this increased attention, research into the influence of environmental factors on microcystin concentrations across natural systems remains relatively underdeveloped (Graham et al., 2004). A few studies have correlated microcystin concentrations with environmental factors across lakes, with the general consensus that concentrations increase with nutrient enrichment in all but the most enriched lakes (Giani et al., 2005; Graham et al., 2004; Kotak et al., 2000). This conclusion is not surprising given the existing base of knowledge about the effects of eutrophication on cyanobacteria in general (Kalff, 2002).

The most comprehensive studies of environmental influences on microcystin concentrations have relied on sampling methods that involve concentrating the toxin by collecting particles from lake water using sieves, with the mesh sizes employed being as large as 64 μm (Giani et al., 2005; Graham et al., 2004; Kotak et al., 2000). Concentrating samples via sieving enables detection of very low environmental concentrations, but at the potential cost of underestimating concentrations since microcystin may be associated with particles that pass through the sieve. We have found microcystin concentrations as high as 5 $\mu\text{g L}^{-1}$ in the filtrate from a 35 μm mesh sieve when *Microcystis aeruginosa*, a colony-forming cyanobacterium, was dominant (unpublished data). A recent study estimated that sieving with 53 μm mesh resulted in an average 37% underestimation of total microcystin concentrations in lakes (Graham and Jones, 2007). From the perspective of protecting public health, underestimating toxin levels when they are high would seem to be more of an issue for a monitoring program than not detecting toxin when levels are very low. Thus, we decided to depart from common practice in our survey by measuring microcystin concentrations in unaltered water samples.

In an era of restricted budgets for environmental monitoring, some agencies have begun to rely on citizen-volunteer networks to provide data on large numbers of habitats, including lakes (Bruhn and Soranno, 2005). These networks enlist interested citizens as field workers who collect samples for the analysis of relatively simple parameters such as total phosphorus (TP) and chlorophyll *a*. Studies have shown that sampling by citizens can provide high-quality data for such parameters when the laboratory analyses are performed by professionals (Canfield et al., 2002; Obrecht et al., 1998). We took advantage of an existing citizen-monitoring network in the state of Michigan (the Cooperative Lakes Monitoring Program, CLMP) to conduct a state-wide survey of microcystin

concentrations in 2006. The CLMP has been in existence since 1974 and the current program includes monitoring of more than 100 lakes for water clarity (Secchi Disk depth), TP and chlorophyll *a*. By coordinating with the CLMP, we were able to collect microcystin data from 77 lakes (a total of 378 samples) on a small budget, along with parallel data on TP and chlorophyll *a*. In this paper, we present analyses aimed at validating microcystin data from samples collected by citizen-volunteers, followed by use of that data to examine a set of hypotheses about the interacting influences of total phosphorus and dreissenid-mussel invasion on microcystin concentrations.

The ongoing invasion of North American lakes by dreissenid mussels (*Dreissena polymorpha*, the zebra mussel and *Dreissena bugensis*, the quagga mussel) has been implicated as the cause of a widely-observed increase in a particular species of toxigenic cyanobacteria, *Microcystis aeruginosa* (Knoll et al., 2008; Raikow et al., 2004; Sarnelle et al., 2005; Vanderploeg et al., 2001). Curiously, promotion of *M. aeruginosa* by dreissenids appears to be limited to lakes with low-moderate TP concentrations (Knoll et al., 2008; Raikow et al., 2004; Sarnelle et al., 2005). Thus, there is concern that lakes not normally at risk for high concentrations of cyanobacterial toxins may become so after dreissenid invasion. One survey of lakes in Michigan has also suggested that dreissenid invasion alters the well-established positive influence of TP on cyanobacterial dominance (Raikow et al., 2004).

Although previous studies have investigated the interactive influences of eutrophication and dreissenid invasion on the dominance of cyanobacteria (Raikow et al., 2004), and the separate influences of eutrophication (Giani et al., 2005; Graham et al., 2004; Kotak et al., 2000) and dreissenid invasion (Knoll et al., 2008) on microcystin concentrations, no studies have examined the interactive influences of eutrophication and dreissenid invasion on microcystin concentrations. Based on existing studies, we constructed testable hypotheses about how microcystin should respond to the interacting influences of TP and dreissenid invasion. First, we expected that microcystin concentrations would increase with TP, given that our survey was limited to lakes with TP levels below 200 $\mu\text{g L}^{-1}$ (Giani et al., 2005; Graham et al., 2004; Kotak et al., 2000). However, based on the response of cyanobacteria to dreissenid invasion and TP in a previous survey (Raikow et al., 2004), we also expected that microcystin would increase with TP at a faster rate in uninvaded lakes than in invaded lakes. Finally, we expected that microcystin concentrations would be elevated in invaded lakes, but only if those lakes have relatively low nutrients (Knoll et al., 2008; Raikow et al., 2004).

In addition to examining the influences of dreissenid invasion and TP, we also examined how microcystin levels measured at the surface near the shore compare with concentrations in offshore samples collected from the entire euphotic zone. Surface sampling near the shore targets toxin levels at the point of contact with swimmers and terrestrial animals, whereas depth-integrated sampling through the mixed layer represents the typical method used by limnologists to assess phytoplankton species composition (Sarnelle, 1993), a major driver of variation in microcystin concentrations across lakes (Kotak and Zurawell, 2007). Given that cyanobacteria can regulate their buoyancy and form scums at the surface in calm weather (Reynolds, 1984), we hypothesized that

microcystin concentrations should be generally higher at the surface than in depth-integrated samples from the mixed layer. We are not aware of any broad-scale systematic comparison of this kind for cyanobacterial toxins (but see Johnston and Jacoby, 2003 for an example within one lake).

2. Materials and methods

2.1. Sample collection and processing

Samples were collected by citizen-volunteers in conjunction with the CLMP, which is administered by the Michigan Department of Environmental Quality (MDEQ) in partnership with Michigan Lake and Stream Associations, Inc., Michigan State University (MSU) and the Huron River Watershed Council under the Michigan Clean Water Corps (MiCorps) program. A one-day training session was held in April of 2006 to instruct volunteers in sample collection/processing and to distribute sampling containers, shipping supplies and instruction manuals. Each of 77 lakes (Supplementary Table 1, Fig. 1) was sampled on a single occasion within the period 25 August–29 September, 2006. Water samples from the euphotic zone (defined as 2X the Secchi Depth, (Koenings and Edmundson, 1991)) were collected from a single site over the deepest basin of the lake with a depth-integrating sampler. Samples for TP analysis were taken at the same location but collected by submerging a 250 ml polyethylene sample bottle ~0.3 m below the surface. Surface-water samples were collected from four sites along the north, south, east and west shorelines. Surface water was collected by orienting the sample bottle such that the bottle opening was partially submerged and air could escape the bottle without bubbling during filling. Volunteers were instructed to take shoreline samples where water depth was ~0.6 m and to avoid disturbing the sediment in the vicinity of the sample-collection site. Samples destined for microcystin

and chlorophyll *a* analysis were stored in brown 250 ml polyethylene bottles in the field.

Volunteers were instructed to store samples on ice in the field and process (chlorophyll *a*) and freeze samples at their home immediately after completion of field collection. For chlorophyll *a*, volunteers filtered 50 ml of lake water through a 0.45 μm membrane filter (Millipore, MF) using a syringe-filter holder assembly. For microcystin, one subsample from each of the five 250 ml bottles was dispensed into a matching set of five 60 ml polyethylene bottles. All samples were kept frozen until transported or shipped to the laboratory for analysis. Volunteers transported euphotic-zone samples for chlorophyll *a* and TP to a designated MDEQ district office. Only samples that were frozen upon delivery at the MDEQ office were accepted for analysis. These samples were then kept frozen by MDEQ until analyzed. Chlorophyll *a* from shoreline samples and all microcystin samples were shipped to MSU within 8 days after collection in styrofoam-insulated boxes via Express Mail (U. S. Postal Service). Upon arrival at MSU, samples were transferred immediately to a lab freezer and kept frozen until analyzed. Notes were also recorded about shipping and arrival date and condition of samples. In most cases, water samples were still frozen upon arrival at MSU.

Volunteers were asked to note presence/absence of dreissenid mussels in their lake on a data sheet. Generally speaking, CLMP participants are experienced in the detection of dreissenid presence through participation in other state monitoring programs. We checked volunteer information, where possible, against a database of dreissenid monitoring maintained by Michigan Sea Grant (www.miseagrant.umich.edu/ais/lakes.html). A total of 33 lakes in our survey were listed in Sea Grant's database. Zebra mussel characterizations by citizen-volunteers matched the database in 30 of 33 cases (91% accuracy), indicating that volunteer characterizations were generally reliable. Of the 3 mismatches, one lake was listed as uninvaded by volunteers but as recently invaded (in

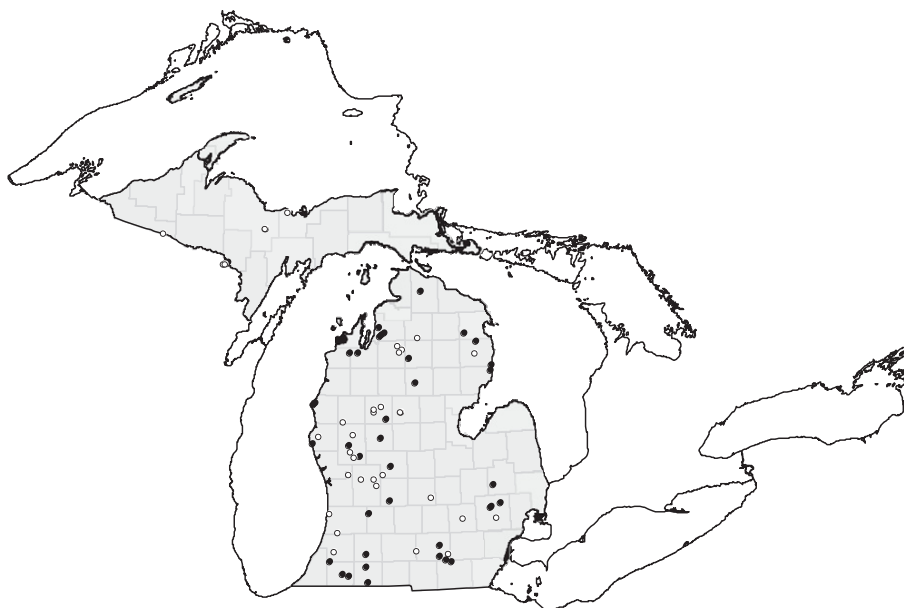


Fig. 1 – Map of Michigan showing surveyed lakes. Solid symbols- invaded by dreissenid mussels, open symbols- not invaded by dreissenid mussels.

2005) by Sea Grant (Pickerel Lake, Kalkaska County). This lake was scored as invaded in our analyses. Two lakes (Gillets Lake, Jackson County and Hubbard Lake, Alcona County) were listed as invaded by volunteers but uninvaded by Sea Grant. However, Sea Grant has no record of invasion monitoring for these two lakes since 1997. We assumed that they were invaded at some point between 1997 and 2006. For lakes that were not listed in the Sea Grant database, we assumed that volunteer characterizations were correct. No attempt to distinguish between *D. polymorpha* and *D. bugensis* was made.

2.2. Sample analysis

Chlorophyll *a* from the euphotic zone and TP were analyzed by MDEQ. Shoreline chlorophyll *a* and all microcystin analyses were conducted by MSU. Total phosphorus in whole-water samples was measured via automated sulfuric acid-mercuric oxide digestion followed by standard colorimetry for phosphate (U. S. Environmental Protection Agency, 1983). For three lakes, no summer TP data for 2006 was available, so we estimated TP from mean TP in 2004 and 2005 and an empirical relationship between averaged 2004–2005 TP and 2006 TP for all CLMP lakes ($y = 2.0 + 0.82x$, $R^2 = 0.63$, $n = 170$, $p < 0.0001$). Chlorophyll *a* in euphotic-zone samples was measured at MDEQ via extraction of filters in 90% acetone followed by fluorometric measurement of extracted chlorophyll *a* (APHA, 1998). Chlorophyll *a* in shoreline samples was measured at MSU via extraction of filters in 95% ethanol followed by fluorometric measurement of extracted chlorophyll *a* (Welschmeyer, 1994). We found generally good correspondence in chlorophyll *a* for parallel samples analyzed by MDEQ versus MSU (unpublished data). Microcystin was measured on whole-water samples via Enzyme-Linked ImmunoSorbent Assay (ELISA) using a commercial kit (Enviroligix, Inc.) according to manufacturer's instructions for high sensitivity. The detection limit for microcystin in lake water using our ELISA protocol was $\sim 0.02 \mu\text{g L}^{-1}$. When microcystin concentration was above $1 \mu\text{g L}^{-1}$, the sample was diluted with deionized water and re-analyzed.

2.3. Methodological assessments

Data quality for microcystin was assessed via comparisons of data from samples collected by volunteers versus MSU personnel. Samples were either collected, processed and shipped to MSU by volunteers or collected by MSU personnel along side volunteers and brought back to the laboratory for processing on the day of collection. This comparison was conducted for 10 lakes, with five microcystin samples per lake. The MDEQ annually conducts side-by-side sampling with volunteers and performs data assessments for TP and chlorophyll *a* in samples collected by volunteers as part of the CLMP Quality Assurance Project Plan (Bednarz, 2007). These assessments have shown that data from volunteer-collected samples are highly comparable to data from MDEQ-collected samples (Bednarz and Wandell, unpublished data).

We also examined the influence of three procedures on whole-water microcystin concentrations using water samples collected from a eutrophic pond with high microcystin concentrations located on the MSU campus. The procedures

investigated were: storage time in insulated shipping containers, heating of samples to release toxin from cells, and extraction in methanol of dried samples. Given that microcystin is a relatively stable, intracellular toxin (Chorus and Bartram, 1999), we expected that incomplete release of toxin from cells would be the most likely source of bias in our data. A standard protocol for microcystin measurement in water samples calls for freezing and thawing samples 2–3 times before analysis (Graham and Jones, 2007; Harada et al., 1999), but we are aware of no published examination of the efficacy of this technique. Preliminary tests indicated that this technique was not significantly superior to our standard protocol of one freeze-thaw cycle.

To examine the effects of storage in insulated shipping containers, we collected water from the MSU pond in mid-summer and dispensed replicate aliquots into 35 60-ml sample bottles. The shipping containers and sample bottles used for this test were identical to those used by volunteers during the survey. Each of six shipping containers was packed the same as by volunteers: five frozen sample bottles at the bottom, two frozen “blue ice” surrounding the samples, and styrofoam peanuts on top of the ice packs such that the container was completely full. A small temperature recorder (Hobo Model U10, Onset Computer Corporation) was placed next to the samples within each container. Containers were placed in a room without air conditioning to simulate summer transport conditions. Containers were opened after 1, 2, 3, 4, 7 and 8 days and bottles were removed and immediately frozen. Results were compared to bottles filled and frozen on the day of collection (day 0 samples).

To examine the effect of heating on microcystin concentrations, we selected 42 volunteer-collected samples of widely-varying toxin concentrations and simultaneously analyzed replicate aliquots for microcystin with our standard protocol (direct analysis of untreated water) versus immersing the samples in a boiling water bath for 30 min before analysis (Metcalf and Codd, 2000). To examine the effect of methanol extraction on microcystin concentrations, we selected 12 volunteer-collected samples and simultaneously analyzed replicate aliquots for microcystin with our standard protocol versus evaporating 25 ml of the sample to dryness and then extracting the residue in 75% methanol.

2.4. Data analysis

Relationships among TP, chlorophyll *a* and microcystin were examined via linear regression after data were normalized by log transformation. The interactive influences of log TP and dreissenid invasion on log microcystin were tested statistically with a least-squares general linear model having invasion status as a categorical variable and log TP as a covariate. To examine the hypothesis that dreissenid invasion only increases microcystin concentrations in low-nutrient lakes, we ran regression-tree analysis (Systat 9.0) with log microcystin as the dependent variable and invasion status and log TP as independent variables. Regression trees subdivide a data set into increasingly homogenous subsets that maximize the reduction in error produced by the partitioning (De'ath and Fabricius, 2000; Knapp and Sarnelle, 2008). This procedure was used as an objective means of defining the “low nutrient”

category of lakes. We estimated error reduction via least squares and used a criterion of 5% for the minimum improvement in the proportion of variance explained to terminate splitting. When the regression tree split the data into subsets based on invasion status, we tested the significance of the difference in microcystin between lake subsets with a t-test.

3. Results

3.1. Methodological assessments

Microcystin data from samples collected and handled by volunteers were highly comparable to data from samples collected and handled by MSU personnel (means not significantly different by paired t-test, $p > 0.80$, $n = 50$), and data from volunteer-collected samples were not more variable (Fig. 2). Our test of the effect of storage in shipping containers showed no significant breakdown of microcystin for the first 2 days (Tukey-Kramer HSD multiple-comparison test, $p > 0.05$), after which toxin concentrations began to decrease (Fig. 3). Temperatures in the shipping containers remained below 5 °C for the first 24 hours and below 20 °C for the first ~40 hours. Shipping records indicated that none of the samples shipped by volunteers spent more than 2 days in transit and most of the bottles were frozen upon delivery, so it is likely that our method of shipping samples did not result in significant losses of microcystin.

Heating samples before analysis resulted in significantly higher microcystin concentrations relative to our standard protocol (Fig. 4, paired t-test, $p < 0.05$). We were not able to heat samples routinely, so we corrected all our microcystin concentrations as follows to account for underestimation stemming from not heating. A linear regression fitted to the relationship between heated and not-heated samples yielded an intercept that was not significantly different from zero ($p > 0.50$), so we fitted a regression with zero intercept to the

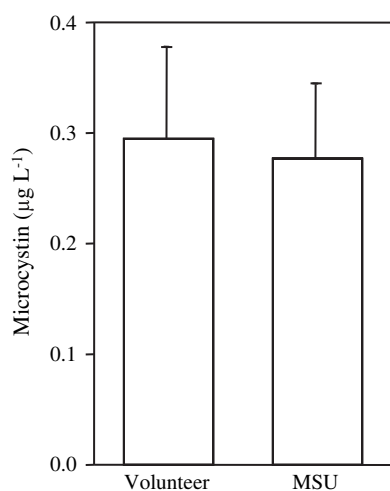


Fig. 2 – Mean microcystin concentration (+ 1 SE) for samples collected by citizen volunteers versus Michigan State University personnel. Means were not significantly different ($p > 0.80$, paired t-test, $n = 50$).

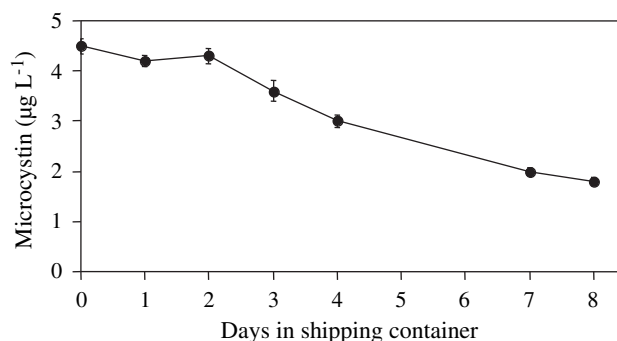


Fig. 3 – Effects of storage time in shipping containers on microcystin concentrations (means ± 1 SE). There was no effect of time from day 0 to day 2 (Tukey-Kramer HSD multiple-comparison test, $p > 0.05$).

data to estimate a factor to account for the underestimation of toxin levels in samples that were not heated ($y = 1.24x$, $R^2 = 0.90$, $n = 42$, $p < 0.0001$). All microcystin concentrations were multiplied by 1.24 to account for this underestimation. In the methanol-extraction test, we found no significant difference in mean concentration for the two methods (mean ± SE for standard protocol, $0.44 \pm 0.24 \mu\text{g L}^{-1}$; for methanol extraction, $0.46 \mu\text{g L}^{-1} \pm 0.28$, paired t-test, $p > 0.70$).

3.2. Interactive influences of TP and dreissenids on microcystin

There was a significant positive influence of TP on euphotic-zone chlorophyll *a* ($\log \text{chlorophyll} = -0.58 + 1.07 \log \text{TP}$, $R^2 = 0.43$, $n = 66$, $p < 0.0001$), which suggests that phosphorus generally limits phytoplankton growth in these lakes, and thus that we should expect TP to influence phytoplankton species composition as well (Watson et al., 1997). In contrast, we found no influence of dreissenid invasion on euphotic-zone chlorophyll *a* nor on the TP-chlorophyll relationship (ANOVA *F*-tests, $p > 0.15$). Thus, we could not detect any

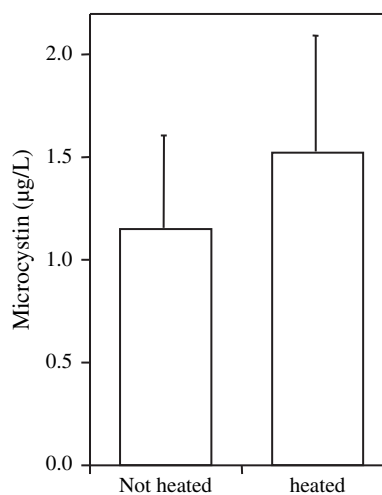


Fig. 4 – Effects of heat treatment (30 min in a boiling water bath) on microcystin concentrations (means ± 1 SE). Means were significantly different at $p < 0.05$ (paired t-test, $n = 42$).

Table 1 – Summary of total phosphorus, chlorophyll *a* and microcystin concentrations ($\mu\text{g L}^{-1}$) for lakes with and without dreissenid mussels.

	Without dreissenids			With dreissenids		
	<i>n</i>	mean	range	<i>n</i>	mean	range
Total phosphorus	33	18	6–103	44	11	1–36
Microcystin, Euphotic zone	32	0.17	0.03–0.95	43	0.34	0.02–8.37
Microcystin, Shoreline	33	0.34	0.03–1.88	44	0.83	0.02–23.6
chlorophyll <i>a</i> , Euphotic zone	29	6.5	1.4–21.1	37	4.8	0.5–31.0
Chlorophyll <i>a</i> , Shoreline	30	16.9	2.1–77.4	42	9.3	0.5–142

Shoreline concentrations represent averages of four locations within each lake.

influence of dreissenid invasion on total phytoplankton biomass, although mean biomass in invaded lakes was somewhat lower (Table 1). The latter may have been a consequence of lower average TP in invaded lakes (ANOVA *F*-tests, $p < 0.01$). Lower average TP in invaded lakes makes the following results for microcystin all the more striking.

Microcystin concentrations from depth-integrated euphotic-zone samples ranged up to $8 \mu\text{g L}^{-1}$ (Table 1), although only 6 of 75 lakes had concentrations above $0.5 \mu\text{g L}^{-1}$. Shoreline concentrations in individual samples ranged up to $46 \mu\text{g L}^{-1}$, but this was also an unusual occurrence. Notably, the two lakes with the highest shoreline concentrations (maxima of 46 and $9 \mu\text{g L}^{-1}$ for individual samples) were both dreissenid-invaded lakes with relatively low TP (9 and $14 \mu\text{g L}^{-1}$ respectively). Within these two lakes, spatial variation in microcystin along the shore was very high, ranging from 0.3 to $9 \mu\text{g L}^{-1}$ and 1 to $46 \mu\text{g L}^{-1}$. We found no statistical differences among microcystin concentrations sampled from the north, south, east and west shores across lakes, so we averaged across the four shoreline sites for all subsequent analyses. There were no significant influences of lake latitude (mean = 43.8°N , median = 43.8°N , range: 41.8 – 46.5°N) or maximum depth (mean 16 m, median = 14 m, range: 2 – 87 m) on microcystin concentrations (ANOVA *F*-tests, $p > 0.15$) so these variables were not included in the analyses that follow.

As expected, microcystin concentrations in both the euphotic zone and along the shoreline were positively influenced by TP (ANOVA *F*-test, $p < 0.0001$), but this influence appeared to differ for lakes with and without dreissenids (Fig. 5). The results of a general linear model suggested that the relationship between log euphotic-zone microcystin and log TP might be different for lakes with and without dreissenids ($p = 0.084$ for the TP \times dreissenid interaction term). Given this suggestive result and our a priori hypothesis based on existing literature, we examined the influence of TP on microcystin separately for each category of lakes. These analyses revealed that the influence of TP on euphotic-zone microcystin was stronger in uninvaded lakes (log-log slope = 0.98 , SE = 0.24 , $R^2 = 0.37$, $p < 0.0002$) than in invaded lakes (log-log slope = 0.35 , SE = 0.23 , $R^2 = 0.05$, $p > 0.10$). A similar result was obtained for shoreline microcystin versus TP although unexplained error was higher (uninvaded lakes: log-log slope = 1.12 , SE = 0.33 , $R^2 = 0.27$, $p < 0.002$, invaded lakes: log-log slope = 0.44 , SE = 0.27 , $R^2 = 0.06$, $p > 0.10$). These results were not driven by the fact that maximum TP for uninvaded lakes was almost three times higher than maximum TP for invaded lakes (Table 1, Fig. 5). Excluding the

lake with the highest TP ($103 \mu\text{g L}^{-1}$) had only a minor effect on the relationship between microcystin and TP in uninvaded lakes (euphotic-zone microcystin: log-log slope = 0.89 , SE = 0.31 , $R^2 = 0.22$, $p < 0.009$, shoreline microcystin: log-log slope = 1.26 , SE = 0.44 , $R^2 = 0.21$, $p < 0.008$). Thus, even when

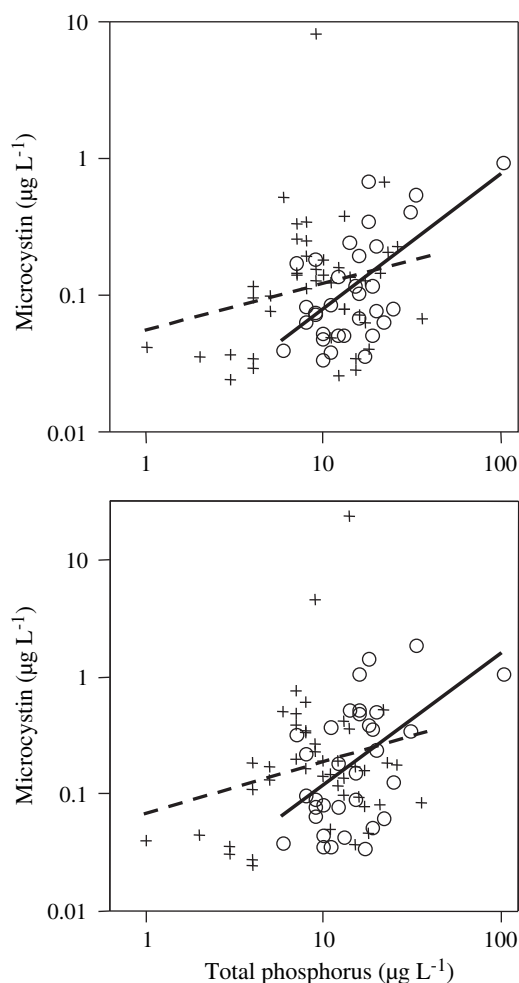


Fig. 5 – Relationships between TP and microcystin in the euphotic zone (top panel) and at the surface (bottom panel). Open circles, solid lines- uninvaded lakes; crosses, dotted lines- invaded lakes. Only regression lines for uninvaded lakes were significant (top panel: log microcystin = $-2.11 + 0.98 \log \text{TP}$, $n = 32$, $r^2 = 0.37$, $p < 0.0002$; bottom panel: log microcystin = $-2.07 + 1.12 \log \text{TP}$, $n = 33$, $r^2 = 0.27$, $p < 0.002$).

maximum TP was equalized between the two lake categories, the influence of TP on microcystin was stronger, and only statistically significant, for lakes without dreissenid mussels.

In contrast to the positive influence of TP on euphotic-zone microcystin, there was no relationship between chlorophyll *a* and microcystin for depth-integrated samples from the euphotic-zone, regardless of invasion status (Fig. 6). Thus, euphotic-zone chlorophyll *a* was not a useful predictor of euphotic-zone toxin levels. In contrast, shoreline microcystin was significantly related to shoreline chlorophyll *a* and the elevation of the relationship was significantly higher in dreissenid-invaded lakes (Fig. 6).

A regression tree split the euphotic-zone microcystin data four times, revealing several interesting patterns (Fig. 7). The first split separated 8 lakes (all invaded) with the lowest TP ($<5 \mu\text{g L}^{-1}$) and very low microcystin levels (untransformed mean = $0.05 \mu\text{g L}^{-1}$) from the remainder of the data set. The second split separated out 5 lakes (2 invaded) with the highest TP ($\geq 26 \mu\text{g L}^{-1}$) and a high average microcystin (untransformed mean = $0.32 \mu\text{g L}^{-1}$). The third split divided the remaining 62 lakes based on a TP criterion of $10 \mu\text{g L}^{-1}$, with the 21 lower-TP lakes (TP $< 10 \mu\text{g L}^{-1}$) being further subdivided into invaded (untransformed mean microcystin = $0.80 \mu\text{g L}^{-1}$) and uninvaded (untransformed mean microcystin = $0.10 \mu\text{g L}^{-1}$). The difference in microcystin concentrations between these last two subsets was significant at $p < 0.02$ (Fig. 8, *t*-test on log-transformed data, $n = 21$). In contrast, microcystin concentrations were not statistically different between invaded (untransformed mean = $0.15 \mu\text{g L}^{-1}$) and uninvaded (untransformed mean = $0.19 \mu\text{g L}^{-1}$) lakes with TP $\geq 10 \mu\text{g L}^{-1}$ (but $< 26 \mu\text{g L}^{-1}$), despite a larger sample size (Fig. 8, *t*-test, $p > 0.65$, $n = 46$). Thus, the regression tree identified an influence of invasion on microcystin concentrations, but only for lakes with TP $< 10 \mu\text{g L}^{-1}$. The lack of uninvaded lakes with TP $< 5 \mu\text{g L}^{-1}$ in the data set made it impossible to be more specific about a lower bound for the positive dreissenid influence.

3.3. Comparison of microcystin concentrations at the shoreline versus in the euphotic zone

As expected, microcystin concentrations were higher at the surface near shore than in depth-integrated samples taken from the euphotic zone (Fig. 9, mean shoreline: $0.62 \mu\text{g L}^{-1}$, mean euphotic zone: $0.27 \mu\text{g L}^{-1}$, paired *t*-test on log-transformed data: $p < 0.0001$). In a few cases, surface concentrations were an order of magnitude higher than contemporaneous levels in the euphotic zone (Fig. 9). Invasion status had no effect on the relationship between shoreline and euphotic-zone microcystin levels (Fig. 9, ANOVA *F*-tests, $p > 0.50$).

4. Discussion

The effects of dreissenid grazing on the biomass of *Microcystis*, a major producer of microcystin, appear to be maximally complex in that all possible outcomes have been documented either in before/after invasion studies or in field experiments (Sarnelle et al., 2005). For example, invasion in the Hudson River was followed by a dramatic decrease in *Microcystis* (Smith et al., 1998), whereas invasion in the Bay of Quinte

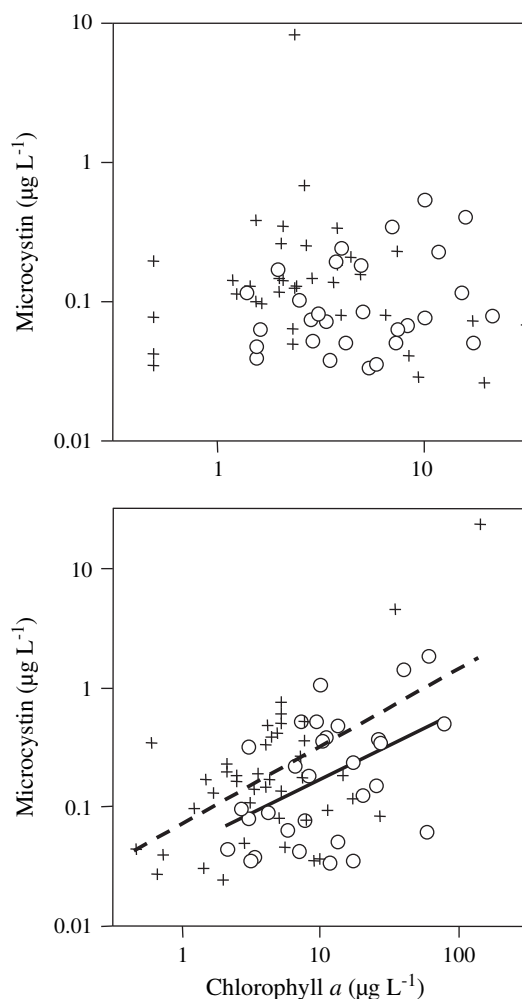


Fig. 6 – Relationships between chlorophyll *a* and microcystin in the euphotic zone (top panel) and at the surface (bottom panel). Open circles, solid lines- uninvaded lakes; crosses, dotted lines- invaded lakes. For surface microcystin, regression slopes were not significantly different between uninvaded and invaded and the influences of chlorophyll *a* ($p < 0.0001$) and invasion status ($p < 0.05$) were both significant (ANCOVA, *F*-tests).

(Lake Ontario) and Gull Lake (southwestern Michigan) was followed by a dramatic increase (Nicholls et al., 2002; Sarnelle et al., 2005). Both positive and negative effects of *Dreissena* on *Microcystis* have also been documented in separate field experiments in Gull Lake (Sarnelle et al., 2005), but the exact mechanisms driving these variable effects are not yet understood. Selective mussel grazing (i. e., selective avoidance of *Microcystis*, Vanderploeg et al., 2001) is not a sufficient explanation, since mussels can sometimes have large negative impacts on *Microcystis* (Smith et al., 1998; Sarnelle et al., 2005). In further contrast, dreissenid invasion of Oneida Lake was accompanied by no significant change in the relative abundance of cyanobacteria, despite a significant decrease in total phytoplankton biomass during summer (Idrisi et al., 2001). Similarly, previous surveys of Michigan lakes found no difference in the abundance of *Microcystis* in lakes with

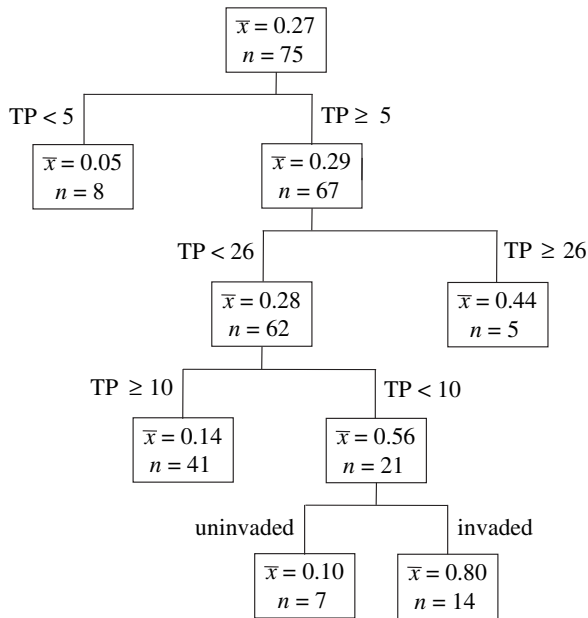


Fig. 7 – Regression tree of log euphotic-zone microcystin versus log TP ($\mu\text{g L}^{-1}$) and invasion status. \bar{x} - untransformed mean microcystin, n - number of lakes.

TP > 25 $\mu\text{g L}^{-1}$, yet a large difference in lakes with TP < 25 $\mu\text{g L}^{-1}$ (Knoll et al., 2008; Raikow et al., 2004). These studies provided the basis for two of the hypotheses we sought to test in this survey.

We hypothesized that microcystin would increase faster with increasing TP in lakes without dreissenids than in lakes with dreissenids, given that invasion seems to promote Microcystis in low-nutrient lakes only (Knoll et al., 2008; Raikow et al., 2004). In support of this hypothesis, we found that the slope of log microcystin versus log TP was almost three times higher in uninvaded than invaded lakes (Fig. 5), although the difference in slope between lake types was not statistically significant at $p < 0.05$. We also found that log TP was a significant predictor of log microcystin in uninvaded lakes ($p < 0.002$) but not in invaded lakes ($p > 0.10$) despite

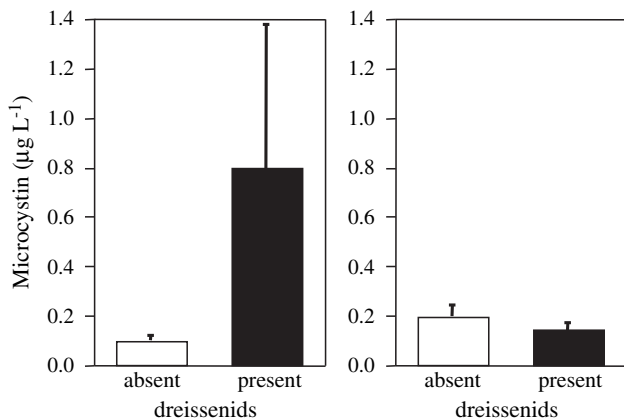


Fig. 8 – Influence of dreissenid mussels on mean euphotic-zone microcystin concentration (+ 1 SE) for lakes with TP between 5 and 10 $\mu\text{g L}^{-1}$ (left panel) and lakes with TP between 10 and 26 $\mu\text{g L}^{-1}$ (right panel).

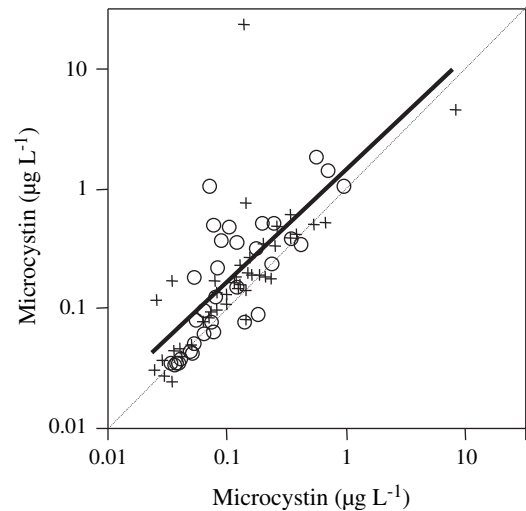


Fig. 9 – Relationship between microcystin in the euphotic zone (x-axis) and microcystin at the surface (y-axis). Open circles- uninvaded lakes; crosses- invaded lakes. One-to-one (dotted) line is depicted for reference. There was no influence of dreissenids on the relationship (log microcystin = 0.12 + 0.93 log microcystin, $n = 75$, $r^2 = 0.56$, $p < 0.0001$).

a larger sample size for invaded lakes (Table 1). This result was not an artifact of a higher maximum TP in uninvaded lakes, since equalizing maximum TP (by excluding one high-TP uninvaded lake) did not materially affect the relationship for uninvaded lakes. After excluding the uninvaded lake with the highest TP, the range of TP was actually greater in invaded lakes, yet no influence of TP was found. Thus, we suggest that the response of microcystin to eutrophication is weaker in lakes with dreissenids. This conclusion is reminiscent of an earlier survey of phytoplankton species composition which indicated that the response of cyanobacteria to eutrophication was different in lakes with and without dreissenids (Raikow et al., 2004). The latter study found the expected positive influence of TP on cyanobacterial dominance in uninvaded lakes (Kalf, 2002) but not in invaded lakes.

We also hypothesized that microcystin would be elevated in invaded lakes with low nutrients based on a previous survey of low-nutrient lakes (Knoll et al., 2008), which could help to explain the lack of positive influence of TP across all invaded lakes. To examine this hypothesis, we ran a regression-tree analysis on euphotic-zone microcystin versus TP and dreissenid presence to objectively categorize lakes with respect to TP. The analysis split 21 lakes with relatively low TP (between 5 and 10 $\mu\text{g L}^{-1}$) into invaded and uninvaded subcategories based on microcystin concentration, with invaded lakes having 8 times higher toxin levels (Fig. 7), a difference that was statistically significant. In stark contrast, at moderate TP levels (between 10 and 26 $\mu\text{g L}^{-1}$), microcystin was almost identical in invaded (0.15 $\mu\text{g L}^{-1}$) and uninvaded (0.19 $\mu\text{g L}^{-1}$) lakes. Notably however, the threshold TP level identified by the regression tree in this survey (10 $\mu\text{g L}^{-1}$) was very different from the level of 25 $\mu\text{g L}^{-1}$ used in previous surveys (Knoll et al., 2008; Raikow et al., 2004). This discrepancy may be in part a function of different methods of measuring TP in the various surveys (for example, TP samples

from the entire mixed layer were taken in earlier surveys but only from the surface in this survey). We suggest that further study is required to more clearly identify levels at which dreissenid effects on microcystin shift from positive to neutral.

Historically, research aimed at predicting water-quality problems stemming from freshwater cyanobacteria has focused on the role of nutrient loading and in particular phosphorus, because of the powerful influence of phosphorus on the success of these phytoplankton (Kalf, 2002). This perspective has informed recent attempts to predict microcystin concentrations across lakes (Giani et al., 2005; Graham et al., 2004; Kotak et al., 2000). We suggest that this perspective be broadened to account for the effects of dreissenid invasion on *Microcystis* and microcystin, especially in light of the observation that cyanobacteria in lakes with *Dreissena* appear to respond differently to nutrient enrichment than in lakes lacking *Dreissena* (Raikow et al., 2004). In our survey, euphotic-zone microcystin concentrations in invaded lakes with TP between 5 and 10 $\mu\text{g L}^{-1}$ were about double that in uninvaded lakes with TP $\geq 26 \mu\text{g L}^{-1}$. From the perspective of public health, the two lakes with the highest shoreline concentrations of microcystin were dreissenid lakes with low TP ($<15 \mu\text{g L}^{-1}$). Given that microcystin appears to be the most common cyanobacterial toxin encountered in temperate freshwaters (Boyer, 2007; Chorus and Bartram, 1999), monitoring programs for toxins should take into account the role of dreissenid invasion in elevating microcystin concentrations in low-nutrient lakes. Dreissenid invasion is limited to waters with sufficient alkalinity, so this accounting can be ignored in lakes with pH or calcium concentrations below established thresholds (Ramcharan et al., 1992).

We monitored microcystin concentrations at the surface near shore since this is the most likely point of contact for recreational users of lakes (none of the lakes in the survey provide drinking water). We predicted that shoreline concentrations would be higher than concentrations in depth-integrated samples from the euphotic zone because of the buoyancy-regulating abilities of *Microcystis* and other cyanobacterial taxa potentially capable of producing microcystin. In addition, zebra mussel impacts on *Microcystis* are likely to be strongest in the littoral zone because contact between the phytoplankton and benthic suspension feeders is greatest in shallow water. Our data clearly support this hypothesis (Fig. 9), with average shoreline concentration ($0.62 \mu\text{g L}^{-1}$) being more than double the average for euphotic-zone samples ($0.27 \mu\text{g L}^{-1}$). In two cases, average shoreline levels were an order of magnitude higher than euphotic-zone levels (Fig. 9), suggesting that measuring the latter may not be the most appropriate sampling strategy for assessing risks from recreational exposure.

The close correspondence in microcystin levels between samples collected by volunteers and those collected by professionals (Fig. 2), the efficacy of the method we employed for shipping samples, and our ability to use the data to test hypotheses with high statistical power all suggest that sample collection by citizen volunteers is a valid, valuable and cost-effective approach for monitoring microcystin in freshwaters. Given that microcystin was positively related to chlorophyll *a* in surface-water samples (Fig. 6), monitoring costs might be reduced by routinely determining chlorophyll *a* at the surface, followed by microcystin analysis on selected samples with high chlorophyll *a*.

The primary limitation of our survey was that we were only able to collect and analyze samples from a single date in each lake. Given that interannual and seasonal variation in lake wide cyanobacterial abundance, and day-to-day variation in scum formation, are likely very high, we suggest that future monitoring included multiple samplings in each lake. A second limitation stems from the fact that we had no control over the lakes selected for sampling. This resulted in there being an unintended difference in TP between invaded and uninvaded lakes (Table 1). To account for this potential source of bias, we used regression-tree analysis to compare microcystin in invaded and uninvaded lakes having the same TP (Fig. 7). Given that TP had an overall positive influence on microcystin, lower TP in invaded lakes was a conservative source of bias with respect to the general conclusion that dreissenid invasion leads to increased microcystin.

5. Conclusions

1. The positive influence of eutrophication (as indexed by TP) on microcystin concentrations appears to be stronger in lakes that lack dreissenid mussels (uninvaded) relative to lakes in which dreissenids have become established (invaded). A positive relationship between log TP and log microcystin was found for uninvaded lakes but not invaded lakes.
2. For lakes with TP between 5 and 10 $\mu\text{g L}^{-1}$, euphotic-zone microcystin concentrations were on average eight times higher in invaded lakes than uninvaded lakes. The highest levels of microcystin in near shore surface waters were found in invaded lakes with low TP.
3. Across all 75 lakes, microcystin concentrations in surface-water samples were on average more than double concentrations in euphotic-zone samples.
4. Sample collection by citizen volunteers is an effective method for monitoring microcystin.

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Appendix. Supplementary information

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.watres.2009.09.014.

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