# Invasive zebra mussels (*Dreissena polymorpha*) increase cyanobacterial toxin concentrations in low-nutrient lakes

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**Abstract:** We investigated whether concentrations of the cyanobacterial toxin microcystin were positively associated with *Dreissena polymorpha* invasion by conducting surveys of 39 inland lakes in southern Michigan with low to moderate total phosphorus concentrations ($\leq$20 µg·L$^{-1}$). Lakes with *D. polymorpha* had 3.3 times higher microcystin concentrations and 3.6 times higher biomass of *Microcystis aeruginosa* (a major producer of microcystin) than comparable lakes without *D. polymorpha*. In contrast, the biomass of *Anabaena* spp. (another potential producer of microcystin) was 4.6 times higher in lakes without *D. polymorpha*. We also conducted a large-scale enclosure manipulation of *D. polymorpha* density in Gull Lake, a low-nutrient lake containing *D. polymorpha*. The experiment revealed a positive effect of *D. polymorpha* on microcystin concentrations and *M. aeruginosa* biomass. The congruence between survey and experimental results provides strong evidence that *D. polymorpha* invasion causes an increase in toxin concentrations in lakes with low to moderate nutrients. An increase in *M. aeruginosa* biomass may negatively impact food webs and public health because microcystins are known to be toxic to aquatic and terrestrial organisms.

**Résumé :** Nous avons recherché s'il existe une association positive entre les concentrations de microcystine, une toxine des cyanobactéries, et l'invasion de *Dreissena polymorpha*, en faisant un inventaire de 39 lacs intérieurs du sud du Michigan qui ont des concentrations faibles à modérées de phosphore total ($\leq$20 µg·L$^{-1}$). Les lacs à *D. polymorpha* ont des concentrations de microcystine 3,3 fois plus importantes et des biomasses 3,6 fois plus grandes de *Microcystis aeruginosa* (un producteur important de microcystine) que des lacs semblables sans *D. polymorpha*. En revanche, la biomasse d'*Anabaena* spp. (un autre producteur potentiel de microcystine) est 4,6 fois plus élevée dans les lacs sans *D. polymorpha*. Nous avons aussi fait une manipulation en enclos à grande échelle de la densité de *D. polymorpha* au lac Gull, un lac avec peu de nutriments qui contient *D. polymorpha*. L'expérience indique un effet positif de *D. polymorpha* sur les concentrations de microcystine et la biomasse de *M. aeruginosa*. L'accord entre l'inventaire et les résultats expérimentaux est une forte indication que l'invasion de *D. polymorpha* cause une augmentation des concentrations de toxines dans les lacs de niveaux faibles à modérés de nutriments. Une augmentation de la biomasse de *M. aeruginosa* peut avoir un impact négatif sur les réseaux alimentaires et la santé publique puisque les microcystines sont connues pour être toxiques pour les organismes aquatiques et terrestres.

[Traduit par la Rédaction]

## Introduction

Filamentous and colonial cyanobacteria are the most important taxa causing harmful phytoplankton blooms in lakes, rivers, and low-salinity estuaries (Reynolds and Walsby 1975; Paerl 1988). It is well established that nutrient enrichment leads to an increase in the fraction of total phytoplankton biomass composed of cyanobacteria (Downing et al. 2001) and an increase in the incidence of harmful blooms (Paerl 1988). However, recent discoveries about the effects of invasion by dreissenid mussels (*Dreissena polymorpha* and *Dreissena bugensis*) on bloom-forming cyanobacteria suggest the need for a re-evaluation of the role of nutrient enrichment as the overarching driver of harmful phytoplankton blooms in freshwater systems (Raikow et al. 2004).

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A recent survey of inland lakes in Michigan revealed a strong positive association between *D. polymorpha* invasion and the relative abundance of the potentially harmful cyanobacterium *Microcystis aeruginosa* (Raikow et al. 2004). Surprisingly, the positive influence of invasion on *M. aeruginosa* was restricted to lakes with low to moderate total phosphorus (TP) concentrations (10–25 µg TP·L$^{-1}$). These observations echo reports from the Laurentian Great Lakes indicating an increase in *M. aeruginosa* biomass after dreissenid establishment in near-shore habitats with moderate nutrient levels (Vanderploeg et al. 2001; Nicholls et al. 2002). Similarly, a large-scale field experiment in a low-nutrient lake has recently confirmed that *D. polymorpha* can have a positive effect on the biomass of *M. aeruginosa* (Sarnelle et al. 2005). It is unclear the mechanism by which *D. polymorpha* promotes *M. aeruginosa* in lakes with low to moderate TP concentrations, while not affecting *M. aeruginosa* in higher-nutrient lakes (>25 µg TP·L$^{-1}$; Raikow et al. 2004). *Dreissena polymorpha* grazing may be low in stratified, high-nutrient lakes because of anoxia (Ramcharan et al. 1992), whereas grazing is likely higher in well-mixed systems (e.g., Hudson River, western basin of Lake Erie) and stratified, low- to moderate-nutrient lakes.

The aforementioned observations are cause for concern, as an increase in the biomass of *M. aeruginosa* may result in an increase in the concentration of cyanobacterial toxins. *Microcystis aeruginosa*, as well as several other genera (*Anabaena*, *Nostoc*, and *Oscillatoria*), can produce secondary metabolites known as microcystins that inhibit the activity of phosphatase enzymes (Carmichael 1994). Microcystin presence in surface waters represents a public health threat to humans (Ueno et al. 1996; Kuiper-Goodman et al. 1999) and may also deter zooplankton grazing thereby affecting herbivore control of harmful phytoplankton and the efficiency of planktonic food chains (Fulton and Paerl 1987).

In this paper, we extend recent findings by focusing on four questions. We address the first three questions with a survey of 39 lakes in southern Michigan with low to moderate total phosphorus concentrations (≤20 µg TP·L$^{-1}$). Such lakes appear to be at greatest risk for water quality degradation upon invasion (Raikow et al. 2004). First, we ask if there is a positive association between *D. polymorpha* invasion and the biomass of *M. aeruginosa* in low-nutrient lakes. The aforementioned survey (Raikow et al. 2004) only reported a significant association between invasion status and the fraction of total phytoplankton biomass comprised by *M. aeruginosa*. Given that total phytoplankton biomass was also lower in invaded lakes, that study was unable to demonstrate statistically that the biomass of *M. aeruginosa* was actually higher in invaded lakes. Second, we ask if there is a positive association between *D. polymorpha* invasion and the concentration of microcystins. It is unclear whether an increase in *M. aeruginosa* typically leads to an increase in microcystins because some naturally occurring strains of *M. aeruginosa* are incapable of producing microcystin (Wilson et al. 2005) and other taxa can produce these toxins and may react differently to invasion.

The third question concerns the possibility that selective grazing by *D. polymorpha* might favor strains of cyanobacteria that produce high amounts of toxin. Intracellular toxins such as microcystins might act as chemical deterrents to grazing (Lampert 1987; Vanderploeg et al. 2001). Thus, *D. polymorpha* may selectively graze on cyanobacterial strains that produce no or low amounts of toxins, which in turn may benefit strains that produce higher amounts of toxin. Thus, we might expect cyanobacteria to have higher biomass-specific microcystin levels in lakes with *D. polymorpha* relative to uninvaded lakes.

The last question addresses cause and effect: is *D. polymorpha* invasion the cause of increased toxin concentrations in lakes with low to moderate TP concentrations? We address this question with an enclosure experiment conducted in a low-nutrient lake that contains *D. polymorpha*. We previously reported on the positive effect of *D. polymorpha* on *M. aeruginosa* biomass in this experiment (Sarnelle et al. 2005) and so here we focus on the response of toxin concentrations.

## Materials and methods

### Lake survey

Lakes were selected for the survey based on maximum depth (≥9 m) and expected summer TP concentration (≤20 µg TP·L$^{-1}$, based on existing data in the USEPA's STORET database and our own surveys). We were interested in examining lakes that would be likely to thermally stratify in the summer, hence the depth restriction, because most previous work on *D. polymorpha* invasion has been conducted in well-mixed systems (MacIsaac et al. 1995; Caraco et al. 1997; Vanderploeg et al. 2001). Thermally stratified lakes may show weaker impacts of *D. polymorpha* because benthic filter feeders like *D. polymorpha* should have less contact with the phytoplankton relative to well-mixed lakes. For the purposes of lake selection, the presence or absence of *D. polymorpha* was initially assessed using a list of invaded lakes assembled by the Michigan Sea Grant College Program (www.miseagrant.umich.edu/ais/lakes.html). Presence–absence was also verified in each lake by searching for adults in the field and for larvae in plankton samples during routine plankton counting. In the field, we examined substrates in the littoral zone in multiple locations of the lake for approximately 1 h. To ensure that lakes with and without *D. polymorpha* were similar in depth, mean depth was determined by digitizing bathymetric lake maps in a geographic information system. Based on the above criteria, 39 lakes were selected: 20 with *D. polymorpha* and 19 without. All survey lakes were located in the southern portion of the Lower Peninsula area of Michigan. Of the 39 lakes in the survey, 16 lakes (seven lakes without and nine with *D. polymorpha*) were also sampled in a 1998 and 1999 lake survey conducted by Raikow et al. (2004).

Each lake was visited once in late summer of 2002 and 2003 (2 August – 4 September 2002, 3–20 August 2003). Samples were taken from the deepest location in each lake, as determined by bathymetric maps and an echosounder. Depth profiles of temperature were measured with a Hydrolab Surveyor 4a equipped with a Datasonde 4a (Hach Environmental, Loveland, California) to determine the depth of the mixed layer (epilimnion). A depth-integrated water sample was then taken through the entire mixed layer with a flexible tube (5 cm inner diameter, 10 m length). Two to four casts of depth-integrated water were pooled, and

subsamples were taken to determine phytoplankton composition and chlorophyll *a*, TP, and particulate microcystin concentrations. Phytoplankton samples were preserved immediately in Lugol's solution (Hasle 1978). Water for chlorophyll *a*, TP, and particulate microcystin testing was placed in a cooler with ice until processed (~6 h). For chlorophyll *a* and particulate microcystin, samples were filtered onto Pall A/E glass fiber filters and kept frozen until analysis.

## Enclosure experiment

The enclosure experiment was conducted in July 2001 in Gull Lake (surface area, 822 ha; mean depth, 12 m; maximum depth, 31 m), a hardwater lake in southwestern Michigan. Gull Lake had exhibited signs of incipient cultural eutrophication by the 1970s, but this was ameliorated via installation of a residential sewer system in 1984 (Moss 1972; Tessier and Lauff 1992). Currently, summer TP content in the mixed layer of the lake is ~4–12 µg TP·L$^{-1}$. *Dreissena polymorpha* were first detected in the lake in 1994, and *D. polymorpha* biomass (dry tissue mass) within the 0–10 m depth stratum in Gull Lake was estimated in 1999 as 6 g·m$^{-2}$ (standard error (SE), 1 g·m$^{-2}$; *n* = 16) (Wilson and Sarnelle 2002). Experimental methods have been described in detail previously (Sarnelle et al. 2005) and are briefly outlined here.

Twelve tubular enclosures (diameter, 2.5 m; depth, 10 m; volume, 50 000 L) constructed of clear polyethylene were suspended from a floating platform at a 15 m deep nearshore site in Gull Lake. Enclosures were open to the atmosphere at the top and sealed from the sediments at the bottom. *Dreissena polymorpha* were collected in mid-June from the littoral zone of Gull Lake by scuba divers, separated from the substrate by cutting their byssal threads, and allowed to attach to polyvinyl chloride (PVC) plates (27 cm wide × 36 cm long × 0.5 cm thick) in a lakeside laboratory for 2–3 weeks before being stocked into the enclosures. Average mussel length, measured along the longest axis of the shell, was 20 mm (range 17–22 mm), which corresponds to an individual dry tissue mass of ~0.02 g (Wilson and Sarnelle 2002). Substrates with mussels were incubated in shallow basins that were continuously supplied with epilimnetic water pumped from Gull Lake.

To initiate the experiment, 12 substrates with attached mussels were hung in a single vertical series (from 0.5–7 m) down the center of each enclosure on July 5. We chose 7 m as the vertical extent of the substrates to simulate mussel grazing within the mixed layer of Gull Lake. Enclosures assigned to zero-mussel treatments received 12 identically treated substrata lacking *D. polymorpha*. A gradient of mussel biomass density (0–4 g·m$^{-2}$, one enclosure per density) was employed to enable examination of nonlinearities in herbivore effects (Sarnelle 2003). The experiment was designed to be analyzed by regression, with mussel density as a continuous rather than a discrete factor. A regression design without replication is statistically valid but potentially more informative than the standard analysis of variance (ANOVA) design (Cottingham et al. 2005).

Enclosures were fertilized weekly with phosphorus starting on day 3. Phosphorus was added throughout the water column as a concentrated solution of reagent-grade

NaH$_2$PO$_4$ (282 mg P·L$^{-1}$) on the day after each weekly sampling (P was added on days 3, 6, and 13). The objective of fertilization was to maintain P levels near 10 µg P·L$^{-1}$, within the range of total phosphorus typically observed in Gull Lake; without fertilization TP concentrations in the enclosures tended to diminish slowly over time (O. Sarnelle, unpublished data).

The enclosure experiment lasted 19 days. Samples for phytoplankton species composition and particulate microcystin were collected on day 19 using the same methods as described for the lake survey except that filters for microcystin were dried and stored in a desiccator until analysis.

## Sample analysis

Chlorophyll *a* was extracted from filters with 90% ethanol and quantified using a fluorometer (model 10-AU; Turner Designs, Sunnyvale, California) (Welschmeyer 1994) that was calibrated against a commercial chlorophyll *a* standard (Sigma-Aldrich Corporation, St. Louis, Misssouri; made from *Anacystis*). Total phosphorus was analyzed colorimetrically (Langner and Hendrix 1982) after persulfate oxidation in an autoclave. Phytoplankton were identified to species (in most cases) and enumerated via the inverted microscope technique (Hasle 1978).

The biovolume (µm$^3$·L$^{-1}$) of *M. aeruginosa* was determined by first examining the entire settling chamber at 100× and measuring the area of every colony in grid units of an ocular micrometer. Total area of colonies in the chamber (*x*) was converted to number of cells in the chamber (*y*) using an empirical relationship, $\log y = 2.14 + 0.91 \log x$ ($R_2 = 0.87$, *n* = 10). The relationship was derived from a series of samples in which total area was recorded, colonies were disaggregated by treatment with NaOH (Reynolds and Jaworski 1978), and individual cells were counted and measured at 1000×. Biovolume was calculated by multiplying cell density by mean cell volume. For species other than *M. aeruginosa*, biovolume was determined from measurements of cell dimensions of at least 10 individuals of common species in each sample at 1000× using a digital camera system and image-analysis software. Biovolume was converted to dry biomass, assuming a specific gravity of 1 g·cm$^{-3}$ and a dry mass to wet mass ratio of 0.10.

Microcystins were extracted from filters with 75% aqueous methanol. Each filter was extracted three times in fresh solvent to increase yield (Oudra et al. 2002), and the three extracts were pooled for analysis. Extracts were analyzed for microcystins (variants LR, LA, RR, and YR) via enzyme-linked immunosorbent assay (ELISA; An and Carmichael 1994) using a commercially available kit and following manufacturer's instructions (Envirologix, Inc., Portland, Maine). Standard curves were established using known concentrations of microcystins followed by colorimetric analysis of the sample extracts at 450 and 620 nm on a Labsystems Multiskan RC plate reader (Thermo Labsystems, Helsinki, Finland). Extract concentrations were calculated using Genesis Lite software (Thermo Labsystems, Helsinki, Finland). We chose to measure microcystins via ELISA because of the method's relatively high sensitivity (Chorus and Bartram 1999), low cost, and broad reactivity with respect to the many varieties of microcystin that occur in nature (Fischer et

**Table 1.** Mean, standard deviation (SD), and range of limnological characteristics for the survey lakes.

| Variable | D. polymorpha absent | | | D. polymorpha present | | | P value |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Range | Mean | SD | Range | |
| Mean depth (m) | 4.9 | 2.1 | 2.3–8.8 | 6.2 | 2.8 | 2.1–12.4 | 0.161 |
| TP ($\mu g \cdot L^{-1}$) | 10.4 | 3.8 | 4.9–19.3 | 10.6 | 3.4 | 5.3–19.3 | 0.728 |
| Chlorophyll *a* ($\mu g \cdot L^{-1}$) | 4.6 | 1.8 | 2.4–9.3 | 3.2 | 1.1 | 0.9–5.3 | 0.008 |
| Phytoplankton biomass ($\mu g \cdot L^{-1}$) | 50.8 | 19.4 | 18.7–95.1 | 35.3 | 22.2 | 12.9–115.8 | 0.006 |

**Note:** Probability values refer to the results of *t* tests of differences in the means between the two classes of lakes. For all variables, *n* = 19 in lakes without *D. polymorpha* and *n* = 20 in lakes with *D. polymorpha*.

al. 2001). Benchmark chemical techniques (high performance liquid chromatography – mass spectrometry) may miss microcystin varieties for which no standards exist (Fischer et al. 2001) and have poorer sensitivity than ELISA (Chorus and Bartram 1999). Methodological comparisons have shown good agreement between benchmark techniques and ELISA for quantifying microcystins in lake samples (Fischer et al. 2001; Fastner et al. 2002; Mountfort et al. 2005). Given that the toxicity of microcystin varieties to warm-blooded animals varies (Mountfort et al. 2005), we make no claim that our estimates of total microcystin concentrations show a one-to-one correspondence to toxicity in humans.

### Statistical analysis

For the lake survey, we used linear regression using day after 1 July as a continuous independent variable to test for the influence of sampling date on all response variables. We also used Student's *t* tests to examine the influence of sampling year (2002 versus 2003) on all response variables. For all lake survey response variables, we used Student's *t* tests to test the effect of presence or absence of *D. polymorpha*. All 39 lakes were used in every *t* test, thus the degrees of freedom for these analyses (df = 37) were the same. When variances were significantly unequal between lake types at *P* < 0.05 (Bartlett's test), data were log-transformed before *t* testing. Log transformation was always effective in eliminating significant heteroscedasticity. To examine the influence of *D. polymorpha* on the biomass of phytoplankton taxa other than *M. aeruginosa* in the lake survey, we reduced the number of response variables by combining species into genera and applying principal components analysis (PCA) to the covariance matrix of genera abundances. To limit the number of zero values in the PCA data set, only commonly occurring genera (found in >60% of lakes) were included: *Anabaena*, *Aphanocapsa*, *Ceratium*, *Chroococcus*, *Cryptomonas*, *Chrysochromulina*, *Fragilaria*, *Oocystis*, *Peridinium*, *Rhodomonas*, *Scenedesmus*, *Sphaerocystis*, and an unidentified small flagellate (~6 µm length). For the lake survey, we used analysis of covariance (ANCOVA) to test for the influence of *D. polymorpha* status on the elevation or slope of the relationship between *M. aeruginosa* biomass and particulate microcystin concentrations. For the enclosure experiment, we examined the effect of manipulated *D. polymorpha* density on microcystin concentrations after accounting for the influence of TP via multiple linear regression with TP and mussel density as independent variables. Levels of significance were set

at $\alpha < 0.05$. All statistical analyses were conducted in SYSTAT (version 9; SPSS Inc., Chicago, Illinois).
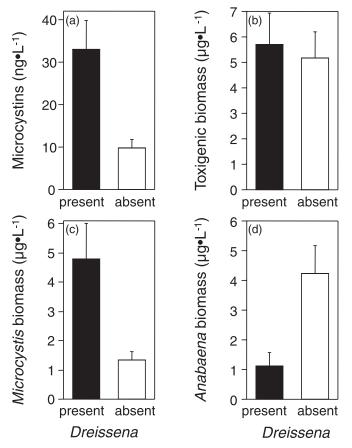
## Results

### Lake survey

Lakes with and without *D. polymorpha* were not significantly different in mean depth or TP concentration ($t = -1.43$, $P > 0.10$, and $t = -0.35$, $P > 0.10$, respectively; Table 1). There was also no influence of sampling date (linear regression and ANCOVA using day after 1 July as a continuous independent variable, $P > 0.10$) or sampling year (*t* test, $P > 0.10$) on any of the response variables. As expected, both chlorophyll *a* and total phytoplankton biomass were lower in lakes with *D. polymorpha* ($t = 2.813$, $P < 0.008$, and $t = 2.911$, $P < 0.006$, respectively; Table 1), although the magnitude of these differences was relatively modest (~30% reduction). Total biomass of cyanobacteria was similar between lakes with and without *D. polymorpha* ($t = 1.703$, $P = 0.097$). In contrast, concentrations of microcystins were 3.3 times higher in *D. polymorpha* invaded lakes ($t = -2.768$, $P < 0.01$; Fig. 1). Microcystins were elevated in invaded lakes despite the fact that there was no difference between lake types in the total biomass of potentially toxigenic taxa (*Anabaena*, *Microcystis*, *Nostoc*, and *Oscillatoria*) ($t = -0.217$, $P > 0.50$; Fig. 1). However, the mean biomass of *M. aeruginosa* was 3.6 times higher in invaded lakes ($t = -2.725$, $P < 0.01$; Fig. 1). There was a strong linear relationship between *M. aeruginosa* biomass and particulate microcystin concentrations across all lakes ($R^2 = 0.77$, $P < 0.0001$; Fig. 2), but no significant influence of *D. polymorpha* status on the elevation or slope of this relationship (ANCOVA, $F_{[1,36]} = 2.388$, $P > 0.10$). There was no relationship between microcystin concentrations and the biomass of *Anabaena*, the only other potentially toxigenic taxon that was common in the lakes ($R^2 = 0.04$, $P > 0.20$).
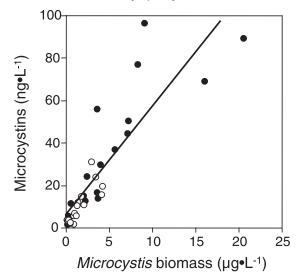
For the phytoplankton community exclusive of *M. aeruginosa*, PCA accounted for 45% of the total variance with two factors. The two factors were rotated to maximize loadings of individual genera on one or the other factor. After rotation, factor 1 accounted for 28% of total variation, with strongest loadings from *Ceratium*, *Anabaena*, *Cryptomonas*, *Fragilaria*, and *Peridinium*. Factor 2 accounted for 17% of total variation, with strongest loadings from *Chrysochromulina*, *Scenedesmus*, unidentified small flagellate, and *Chroococcus*. Scores of factor 1 were significantly higher in lakes with *D. polymorpha* ($t = -5.058$, $P < 0.001$), indicating that *D. polymorpha* status had a significant influence on

**Fig. 1.** Influence of *Dreissena* presence (solid bars) or absence (open bars) on (*a*) toxin (microcystins) concentrations (*P* < 0.01), (*b*) total biomass of microcystin-producing genera (toxigenic biomass = *Microcystis* + *Anabaena* + *Oscillatoria*, *P* > 0.5), (*c*) *Microcystis aeruginosa* biomass (*P* < 0.01), and (*d*) *Anabaena* biomass (*P* < 0.001) for the study lakes. *P* values are for Student's *t* tests of differences between lake types; *n* = 19 in lakes without *D. polymorpha* and *n* = 20 in lakes with *D. polymorpha*. Values are means + 1 standard error.



**Fig. 2.** Relationship between toxin (microcystin) concentrations and *Microcystis aeruginosa* biomass in the survey lakes with (solid circles) and without *Dreissena* (open circles); $y = 5.87 + 5.11x$, $R^2 = 0.77$, $P < 0.0001$; $n = 19$ in lakes without *D. polymorpha* and $n = 20$ in lakes with *D. polymorpha*.
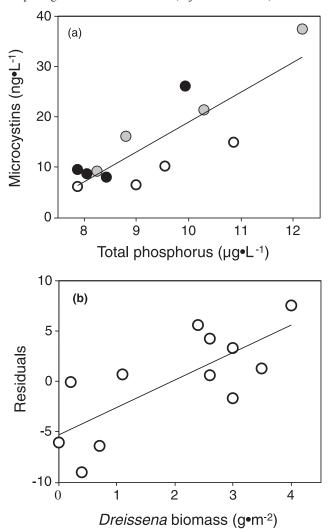


*Anabaena* and *M. aeruginosa*, both responded in opposite directions to *D. polymorpha* invasion (Fig. 1).

**Enclosure experiment**

Periodic P additions to the enclosures maintained TP levels near the target of 10 µg TP·L$^{-1}$, but there was unintended variation in TP across the enclosures (Fig. 3) that had a strong influence on both toxin concentrations and *M. aeruginosa* biomass. There was no relationship between TP and mussel density (linear regression, $n = 12$, $P > 0.95$), so we treated TP as an additional, uncontrolled independent variable (Sarnelle et al. 2005). We chose to use TP data from the final sampling date (day 19) for this analysis because we had toxin data for the final date only. TP concentrations were highly correlated between days 19 and 12 ($R^2 = 0.84$, $P < 0.0001$), and toxin concentrations on day 19 were significantly correlated with TP averaged over days 12 and 19 ($R^2 = 0.65$, $P < 0.01$), but the latter relationship was not as strong as for TP on day 19 alone (adjusted $R^2 = 0.85$; Fig. 3). Thus, using TP data from day 19 enabled us to account for more variation in toxin concentrations when examining the effects of mussel density.

We examined mussel effects after accounting for the influence of TP via multiple linear regression with TP and mussel density as independent variables. We plotted residuals from regressions against TP (Fig. 3) to provide a visual illustration of mussel effects that were analyzed via multiple regression. The latter revealed a significant positive effect of *D. polymorpha* density on microcystin concentrations (Fig. 3). There was a similar positive effect of *D. polymorpha* density on the biomass of *M. aeruginosa* (after adjusting for the influence of TP), but no other phytoplankton taxon was significantly affected by *D. polymorpha* in the experiment (Sarnelle et al. 2005). *Microcystis aeruginosa* was the only potentially toxigenic taxon that was common during the experiment (Sarnelle et

phytoplankton taxa other than *M. aeruginosa*. In contrast, scores of factor 2 were not influenced by *D. polymorpha* invasion status (*t* = 0.831, *P* > 0.41). Factor 1 scores were negatively correlated with the biomass of *Ceratium* (*r* = –0.87, *P* < 0.00001) and *Anabaena* (*r* = –0.43, *P* < 0.006) and positively correlated with the biomass of *Cryptomonas* (*r* = 0.46, *P* < 0.004). Based on these results, we examined the influence of *D. polymorpha* invasion on the biomass of each of these genera using Student's *t* tests. These tests revealed that in invaded lakes, *Ceratium* (*t* = 5.128, *P* < 0.001) and *Anabaena* (*t* = 3.675, *P* < 0.001) were significantly less abundant, and *Cryptomonas* (*t* = –2.047, *P* < 0.05) was significantly more abundant. *Anabaena* was the only other potentially toxigenic taxon besides *M. aeruginosa* that was common in the lakes. *Oscillatoria* was found in only one of the 19 *Dreissena*-absent lakes and in 3 of the 20 *Dreissena*-present lakes. In one lake with *Dreissena*, *Oscillatoria* comprised 20% of the toxigenic biomass. In all other lakes, *Oscillatoria* made up less than 6.1% of the toxigenic biomass. The two common potentially toxigenic taxa,

**Fig. 3.** (*a*) Effects of total phosphorus (TP, *n* = 12, *P* < 0.0001) and (*b*) *Dreissena* density (*n* = 12, *P* < 0.008) on toxin (microcystins) concentrations on day 19 of the Gull Lake enclosure experiment. Symbols in (*a*) reflect *Dreissena* density in each enclosure (open, <1 g·m$^{-2}$; shaded, 1–2.6 g·m$^{-2}$; solid, 3–4 g·m$^{-2}$). The influence of TP on toxin concentrations was removed via linear regression, and residuals from the regression were plotted against *Dreissena* density in (*b*). *P* values are for Student's *t* tests of the significance of each predictor variable in a multiple regression. Probability for the analysis of variance (ANOVA) *F* test of the overall significance of the multiple regression was *P* < 0.0001 (adjusted $R^2$ = 0.85).



**Fig. 4.** Relationship between toxin (microcystin) concentrations and *Microcystis aeruginosa* biomass on day 19 of the enclosure experiment; *y* = –1.23 + 5.35*x*, $R^2$ = 0.90, *n* = 12, *P* < 0.0001.
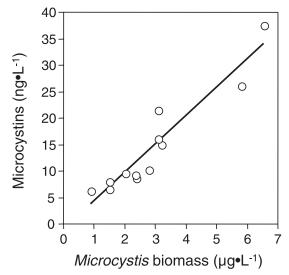


al. 2005), and not surprisingly, there was a strong positive relationship between microcystin concentrations and *M. aeruginosa* biomass across the enclosures (Fig. 4).

## Discussion

We found a strong positive influence of *D. polymorpha* invasion on *M. aeruginosa* biomass, confirming reports from the Great Lakes that invasion of habitats with low to moderate nutrient levels leads to increases in the biomass of this harmful phytoplankton species (Vanderploeg et al. 2001; Nicholls et al. 2002). The greater *M. aeruginosa* biomass in

invaded lakes also builds upon the previous survey of Raikow et al. (2004), who reported a positive *D. polymorpha* influence on the relative abundance of this species. More importantly from the perspective of public health, we found a concomitant elevation in microcystin concentrations in invaded lakes, despite the fact that the combined biomass of all taxa that can potentially produce these toxins was not significantly influenced by invasion. The latter result was a consequence of the opposite responses of the two common toxigenic taxa (*M. aeruginosa* and *Anabaena* spp.) to *D. polymorpha* invasion. The results of the enclosure experiment indicate that the correspondence between *D. polymorpha* invasion and increased *M. aeruginosa* biomass and toxin concentrations in the lake survey likely reflect causality. The range of toxin concentrations in the experiment was within the range of concentrations in the lake survey. This suggests that Gull Lake, the site of the enclosure experiment, was representative of the lakes in the survey with respect to toxin concentrations.

The positive influence of *D. polymorpha* invasion on microcystin concentrations, in the absence of any influence on the combined biomass of taxa known to be capable of producing these toxins (*M. aeruginosa* and *Anabaena* spp.), suggests two alternative hypotheses about the contribution of each taxon to the overall concentration of microcystins and the potential influence of *D. polymorpha* grazing on biomass-specific (per capita) toxin levels. If we assume that *Anabaena*, or at least the genotypes of *Anabaena* that were common in the lakes we surveyed, produce negligible amounts of toxin, the similar responses of microcystin concentrations and *M. aeruginosa* biomass in the lake survey suggest that per capita production of the toxin by *M. aeruginosa* was not influenced by *D. polymorpha* invasion. In support of this hypothesis, the best predictor of microcystin concentrations across all lakes was *M. aeruginosa* biomass and there was no significant influence of invasion status on this relationship. Further, there was no relationship between microcystin and *Anabaena* bio-

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mass ($y = -1.44x + 25.06$, $R^2 = 0.04$, $P = 0.24$). Alternatively, if we assume that both *M. aeruginosa* and *Anabaena* produce substantial amounts of microcystins in these lakes, the elevation in microcystin levels in the absence of an increase in combined toxigenic biomass would suggest higher per capita production of toxins. As evidence against this latter hypothesis, inclusion of *Anabaena* biomass substantially degrades the fit of the relationship between microcystin concentration and combined toxigenic biomass across lakes ($R^2$ declines to 0.41). In addition, the slope of the relationship between microcystin concentration and *M. aeruginosa* biomass in the lake survey (5.11) was similar to the slope for the enclosure experiment (5.35) in which *M. aeruginosa* was the only toxigenic species. The similarity of slopes suggests that *M. aeruginosa* was the only significant producer of microcystins in the lake survey and, by implication, suggests that *D. polymorpha* invasion did not promote higher per capita production of microcystins. Thus, our results provide no clear support for the hypothesis that microcystins act to reduce *D. polymorpha* grazing on *M. aeruginosa*, despite previous laboratory studies suggesting that toxic *M. aeruginosa* reduce clearance rates and survival of *D. polymorpha* (Vanderploeg et al. 2001; Dionisio Pires et al. 2003). However, it must be noted that the large number of chemical variants within this group of toxins (Chorus and Bartram 1999) leaves open the question of whether a certain form of microcystin might have deterrent properties. Further, we do not have measurements of other cyanotoxins, such as neurotoxins, that *Anabaena* is capable of producing. The decline of *Anabaena* biomass in invaded lakes may be associated with a decrease in neurotoxins, which may lessen the overall impact of *Dreissena* on toxins.

An increase in *M. aeruginosa* biomass and microcystin concentration in response to *D. polymorpha* invasion has implications for both the ecology and recreational values of lakes with low to moderate nutrient levels. Increased *M. aeruginosa* may reduce food-chain efficiency because this species is of poor nutritional quality for herbivorous zooplankton, inhibiting feeding, survivorship, and growth (DeMott et al. 1991; DeMott 1999; Lurling 2003). Increased microcystin concentrations may also seriously impact lakes in terms of both drinking water and recreational use. Cyanobacterial toxins are potentially harmful to humans via consumption and skin exposure (e.g., rashes, gastrointestinal illness) (Chorus and Bartram 1999). Drinking water guidelines set by the World Health Organization for total microcystin concentrations (particulate plus dissolved microcystin) are set at less than 1 $\mu g \cdot L^{-1}$ (World Health Organization 1996). Although microcystin concentrations did not approach this guideline in any of the survey lakes (highest particulate microcystin concentration = 0.097 $\mu g \cdot L^{-1}$), levels higher than 1 $\mu g \cdot L^{-1}$ have been reported from invaded habitats in the Great Lakes (Brittain et al. 2000; Vanderploeg et al. 2001). We measured microcystins in particulate matter only, but laboratory studies indicate that in healthy cultures, microcystins occur predominantly within the cells (Rapala et al. 1997). However, our microcystin values may grossly underestimate potential human exposure because samples were collected from the entire mixed layer and far from shore. Cyanobacteria blooms are often buoyant and tend to blow towards the shoreline, where they may create dense surface accumulations. For example, Johnston and Jacoby (2003) found late-summer total microcystin to range from 0.19 to 3.8 $\mu g \cdot L^{-1}$ throughout most of Lake Sammamish (Washington), but near the boat launch, the concentration was much higher (43 $\mu g \cdot L^{-1}$), which was attributed to the wind causing accumulations of buoyant *M. aeruginosa*. Thus, the values we report are potentially much lower than those found in locations of high human and terrestrial animal use along the shoreline.

Management solutions to high levels of cyanobacterial toxins have focused almost exclusively on reductions of phosphorus loading, which has been justified given the strong positive relationship between the abundance of toxigenic phytoplankton species and phosphorus concentrations (Paerl 1988). However, mounting evidence that *D. polymorpha* invasion negates the latter relationship (Raikow et al. 2004) and leads to unexpected increases in microcystins suggests that the exclusive management focus on nutrients may need to be broadened to take into account *Dreissena* invasion. As first suggested by Raikow et al. (2004), the responses of cyanobacteria to *Dreissena* invasion appear to be very different in habitats with low and high nutrient levels, with the unfortunate consequence that lakes of generally higher quality as a resource (i.e., lakes with low to moderate nutrient levels) are the most negatively impacted.

## Acknowledgements

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