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Complex interactions between the zebra mussel, *Dreissena polymorpha*, and the harmful phytoplankter, *Microcystis aeruginosa*

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Abstract

We report a reversal in the sign of the herbivore–phytoplankton interaction between the zebra mussel (*Dreissena polymorpha*) and *Microcystis aeruginosa,* a harmful planktonic cyanobacterium. A pair of large-scale manipulations of mussel density in the same lake in consecutive years showed that when phosphorus concentrations were very low (mean total phosphorus [TP] $\sim$ 3 μg L^{-1}), the effect of *Dreissena* on the biomass of *M. aeruginosa* was monotonically negative across the full range of sustainable mussel densities. When the enclosures were fertilized with phosphorus (mean TP $\sim$ 9 μg L^{-1}), there was a monotonically positive effect of *Dreissena* on *M. aeruginosa* across the same mussel gradient. These contrasting results indicate that *D. polymorpha* feeds on *M. aeruginosa,* as shown in previous laboratory feeding experiments, but that the positive effects of *D. polymorpha* on *M. aeruginosa* can be larger than the negative effects of consumption. A sign reversal in the interaction between these two species is congruent with highly variable patterns in the response of *M. aeruginosa* to *D. polymorpha* invasion across lake and river systems in North America.

A well-established paradigm in limnology holds that the taxonomic composition of summer phytoplankton assemblages shifts with phosphorus enrichment toward greater dominance by cyanobacteria (Smith 1986; Trimbee and Prepas 1987; Watson et al. 1997). It is also widely accepted that harmful species of cyanobacteria (temperate species within the genera *Anabaena, Aphanizomenon, Microcystis,* and *Oscillatoria*) are more likely to attain bloom densities in lakes and rivers that are nutrient rich (Paerl 1988). Given this background, recent reports of increases in harmful cyanobacteria (specifically, the toxin-producing colonial species *Microcystis aeruginosa*) in coastal areas of the Great Lakes (Vanderploeg et al. 2001; Nicholls et al. 2002) are surprising, given the success of phosphorus abatement programs in these habitats (Bierman et al. 1984; Bertram 1993; Vanderploeg et al. 2001; Nicholls et al. 2002). Increases in *M. aeruginosa* are coincident with invasion of the Great Lakes by the zebra mussel (*Dreissena polymorpha*), a filter-feeding bivalve (Vanderploeg et al. 2002). A recent survey of inland lakes (Raikow et al. 2004) shows a strong positive association between *Dreissena* invasion and the relative abundance of *M. aeruginosa,* but only in lakes with relatively low nutrients (total phosphorus [TP] = 10–25 μg L^{-1}). That study also reported no relationship between TP and cyanobacterial dominance in invaded lakes, in contrast to the positive re-

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lationship observed in lakes that lack the invader (Trimbee and Prepas 1987; Watson et al. 1997; Downing et al. 2001). Given the radical implications of these recent observations with respect to the general understanding of the drivers of harmful cyanobacteria, there is a critical need for experimental field tests of the hypothesis that zebra mussels have positive effects on the biomass of *M. aeruginosa.*

Despite significant interest in the consequences of *Dreissena* invasion in North America (Nalepa and Fahnenstiel 1995; MacIsaac 1996; Caraco et al. 1997), there is a relative paucity of reports regarding field manipulations of *Dreissena* density (Heath et al. 1995; Thayer et al. 1997; Stewart et al. 1999; Jack and Thorp 2000; Wilson 2003). None of these field experiments provide direct information about the response of *M. aeruginosa* to manipulations of *Dreissena.* Observations of phytoplankton species composition before and after zebra mussel invasion suggest that the *Dreissena–M. aeruginosa* interaction may be complex, in the sense that *Dreissena* may have opposite effects in different habitats. For example, the biomass of *M. aeruginosa* in the Bay of Quinte in Lake Ontario increased dramatically after *Dreissena* invasion (Nicholls et al. 2002), whereas the biomass of *M. aeruginosa* in the Hudson River declined dramatically after invasion (Smith et al. 1998). The results of laboratory feeding experiments are similarly conflicting, with mussels having relatively high and low selectivities for different clones of *M. aeruginosa* within and across studies (Vanderploeg et al. 2001). *M. aeruginosa* forms colonies that vary greatly in size, and some strains produce intracellular toxins that may act as herbivore deterrents, so vulnerability to herbivory may be highly variable within this species (Vanderploeg et al. 2001). In this paper, we demonstrate both negative and positive effects of *Dreissena* on the biomass of *M. aeruginosa* in a pair of large-scale experiments conducted in the same lake in consecutive years. Logistical, legal, and ethical constraints limited us to conducting mussel-removal manipulations in an already-invaded lake, rather than mussel-addition manipulations in an uninvaded lake. Consequently, we assumed at the outset that the effects of mussel invasion on *M. aeruginosa* could be reversed (by reducing mussel density) within the restricted time frame of our enclosure experiments.

Methods

The enclosure experiments were conducted in Gull Lake (surface area = 8.2 km^2, mean depth = 12 m, maximum depth = 31 m), a hardwater lake in southwestern Michigan. Gull Lake underwent incipient cultural eutrophication during the 1960s–1970s, but this was ameliorated via the installation of a domestic sewer system in 1984 (Moss 1972; Tessier and Lauff 1992). Currently, the lake is low in phosphorus (summer TP in the mixed layer $\sim$ 8–12 $\mu g\ L^{-1}$). Zebra mussels were first detected in the lake in 1994, and observational data indicate an increase in the relative abundance of *M. aeruginosa* in the lake subsequent to invasion (Fig. 1). Zebra mussel biomass (as dry tissue mass) within the 0–10-m-depth stratum in Gull Lake was estimated in 1999 as 6 g m^{-2} (SE = 1 g m^{-2}, n = 16) (Wilson and Sarnelle 2002).

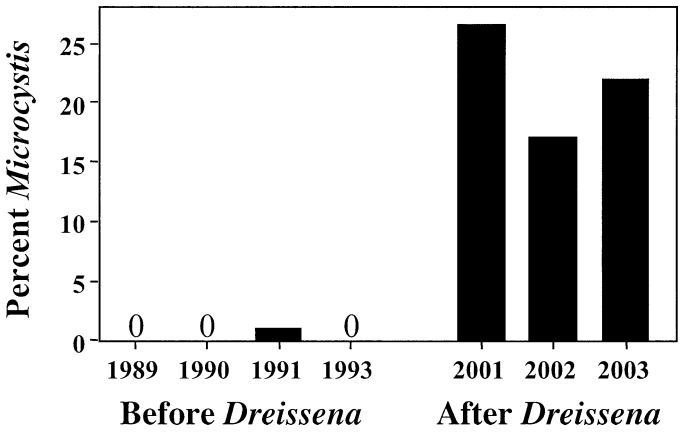

Fig. 1. Relative abundance (as percentage of total phytoplankton biomass) of *Microcystis aeruginosa* in Gull Lake before and after zebra mussel invasion. Zebra mussels were first observed in Gull Lake in 1994. For each year, one to three samples collected during the first half of August were analyzed.

The basic design of the enclosure experiments was similar between years. Large tubular enclosures (diameter = 2.5 m, depth = 10 m, volume = 50,000 liters) 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. Concrete weights were attached to the bottom of each enclosure to provide downward tension and thereby maintain the shape of the enclosure. Enclosures were installed during the first week of July in each year.

Zebra mussels were collected in June from the littoral zone of Gull Lake by scuba divers, separated from the substrate by cutting their byssal threads, and then allowed to attach to plastic substrates (polyvinyl chloride plates, 27 cm wide $\times$ 36 cm long $\times$ 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, which corresponds to an individual dry tissue mass of ca. 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 each enclosure experiment, substrates with attached mussels were hung in a single vertical series (from 0.5 to 7.0 m) down the center of each enclosure. We chose 7 m as the vertical extent of the substrates to simulate mussel grazing within the proximity of the 7-m mixed layer of Gull Lake. Enclosures assigned to zero-mussel treatments received identically treated substrates lacking zebra mussels.

A gradient of mussel biomass density (0–4 g m^{-2}) was employed in each experiment to enable an examination of nonlinearities in herbivore effects (Sarnelle 2003). Our initial intention in 2000 was to include mussel treatments that were above average natural density in Gull Lake. However, we observed high mussel mortality in the three enclosures stocked at or above average natural density (6, 8, 10 g m^{-2}). Consequently, we limited all analyses to the nine enclosures stocked below natural density in 2000. We used stocked biomass as the index of mussel treatment density in 2000.

To verify that enclosure mussels were healthy and actively feeding in the nine enclosures used in 2000, we conducted a laboratory feeding trial with 20 mussels taken from each enclosure on day 13 of the experiment. Mussels for the feeding trial had attached to smaller substrates during the preexperiment incubation period in June. These substrates with mussels (and control substrates lacking mussels) were transferred to the laboratory and incubated in 1-liter glass beakers filled with lake water that was passed through a 1-μm filter and to which a laboratory-cultured alga, *Ankistrodesmus falcatus*, was then added to achieve a high density ($\sim$35 μg L^{-1}) of chlorophyll *a* (Chl *a*). Mussels were allowed to feed for 1 h, after which filtering rates were assessed by comparing initial and final concentrations of Chl *a* in the beakers. The mean filtering rate in the feeding trial was 7.1 L ind^{-1} d^{-1} (SE = 0.3 L ind^{-1} d^{-1}) and was independent of stocked density in the enclosures ($p > 0.30$). This filtering rate is similar to laboratory-based measurements for healthy 20-mm mussels (6.2 L ind^{-1} d^{-1}) (Kryger and Riisgård 1988), indicating that enclosure mussels were actively feeding in 2000.

In 2001, we monitored mussel mortality weekly by videotaping four substrates in each of the 12 enclosures with an underwater camera. The resolution of the camera was sufficient to enable the characterization of mussels as live or dead. Videotapes were examined independently by two observers whose characterizations agreed closely (linear regression of numbers of live mussels counted per substrate for the two observers: $y = 0.96x + 0.14$, $r^2 = 0.99$). These analyses revealed that mortality was low during the first 3 weeks (average percentage of mortality from initial stocking to day 18 = 9.2%, SE = 1.2%; the mortality was not density dependent, $p > 0.50$) and was largely the result of mussels falling off the substrates during or shortly after initial stocking. Mortality increased after 3 weeks, so we restricted data analyses to the first 3 weeks for both experiments. We used average video-determined biomass during the first 3 weeks as the index of mussel treatment density in 2001. There was a strong correlation between stocked density and video-determined density in 2001 ($r = 0.98$), indicating that the treatment gradient was maintained during the first 3 weeks of the experiments. We assumed that mussel mortality was similarly low during the first 3 weeks of the 2000 experiment, since (1) mussel collection and stocking protocols were identical between years, and (2) mussels were actively feeding on day 13, but we do not have mortality data for 2000 to make what may well be small corrections to the treatment-density index in 2000. However, it is highly unlikely that average mussel density during the first 3 weeks differed by more than $\sim$10% between the two experiments.

Enclosures were not fertilized in 2000. In 2001, enclosures were fertilized weekly with phosphorus, starting on day 3. The objective of fertilization was to maintain phosphorus levels near 10 μg L^{-1}, which would bring experimental conditions into the range of TP typically observed in Gull Lake and the aforementioned survey of inland lakes with and without zebra mussels (Raikow et al. 2004). Phosphorus was added as a concentrated solution of reagent-grade NaH_2PO_4 (282 mg phosphorus L^{-1}) on the day after each weekly sampling. Equal volumes were injected through tubing at five depths distributed evenly throughout the enclosures.

Temperature profiles were measured weekly with a Hydrolab Surveyor and Datasonde 4a. Water clarity was measured with a 20-cm Secchi disk. Enclosures were sampled for nutrients and phytoplanktonic Chl *a* immediately before stocking mussels (day 0) in both years and on days 5, 9, 14, and 21 of the 2000 experiment and days 5, 12, and 19 of the 2001 experiment. Samples for phytoplankton species composition analysis were collected on day 0 (both years), days 5 and 21 in 2000, and day 19 in 2001. Depth-integrated nutrient and phytoplankton samples were taken from the entire water column of each enclosure with a 10-m-long tube sampler. Two casts from the sampler were pooled and well mixed, after which subsamples were taken for phytoplankton species counts and TP (unfiltered water), Chl *a* (retained on Gelman A/E filters), and dissolved nutrients (soluble reactive phosphorus [SRP] and NH_4^+, measured in the filtrate passing an A/E filter). Subsamples for phytoplankton were immediately preserved in Lugol solution (Hasle 1978). All filtrations were performed in the lakeside laboratory within 1–2 h after water samples were collected, and water was kept in a cooler during this time. Nutrient samples and filters for chlorophyll were either analyzed promptly (SRP, NH_4^+) or frozen immediately after processing.

Chl *a* was extracted from filters in 90% ethanol. Samples were kept cold and dark during overnight extraction. Chl *a* in extracts was measured via fluorometry (Welschmeyer 1994), and concentrations were calculated from fluorometric readings using a calibration equation developed from the analysis of a commercial Chl *a* standard. Dissolved nutrients (SRP, NH_4^+) were analyzed within 1–3 d of collection using standard colorimetric techniques and long-pathlength spectrophotometry (Murphy and Riley 1962; Solórzano 1969). TP was analyzed using standard colorimetric methods following persulfate digestion. Phytoplankton species biomass was determined via the inverted microscope technique, with counts made at several magnifications ($\times$100–$\times$1,000) to accurately assess the density of both large and small species. Cell volumes (exclusive of spines, horns, and sheaths) were calculated from cell dimensions estimated using images captured with a digital camera at $\times$1,000 and analyzed with image-analysis software. Phytoplankton volumes were converted to dry biomass assuming a specific gravity of 1 g cm^{-3} and a dry mass : wet mass ratio of 0.10. We also estimated a relative index of colony size for *M. aeruginosa* on day 0 of each experiment, as well as on day 19 of the 2001 experiment, by measuring the area (μm^2) of individual colonies at $\times$100. We were unable to assess the colony size for day 21 of the 2000 experiment because the colony matrix disintegrated after long-term (>2 yr) sample storage.

Mussel effects on phytoplankton species abundances were statistically analyzed in two steps to avoid data mining for statistical significance. Our primary intent was to determine the effects on *M. aeruginosa* biomass, so this effect was examined first via linear regression, with mussel treatment density as the independent variable. To examine whether mussels affected any other components of the phytoplankton community in 2000, we next subjected biomass data for the common phytoplankton taxa (excluding *M. aeruginosa*) to

Table 1. Means and ranges of response variables in each enclosure experiment. Data are reported for day 21 of the experiment in 2000 and day 19 of the experiment in 2001. Phytoplankton and *Microcystis* data are given as dry biomass.

Experiment	TP (μg L^{-1})	SRP (μg L^{-1})	NH_4^+-N (μg L^{-1})	Chlorophyll (μg L^{-1})	Phytoplankton (μg L^{-1})	*Microcystis aeruginosa* (μg L^{-1})
2000						
Mean	2.7	0.59	16	0.7	8	0.6
Range	1.8–5.7	0.47–0.65	13–20	0.4–0.9	4–12	0.2–2.1
2001						
Mean	9.3	1.38	9	5.1	19	3.0
Range	7.9–12.2	1.25–1.60	4–15	3.3–8.3	11–32	0.9–6.6

principal components analysis (PCA). Our objective was to reduce the species-biomass data to one or two response variables for regression against mussel density. In 2001, there were only three common taxa besides *M. aeruginosa*, so PCA was unnecessary. Data were log transformed as needed to conform to the assumptions of parametric statistics.

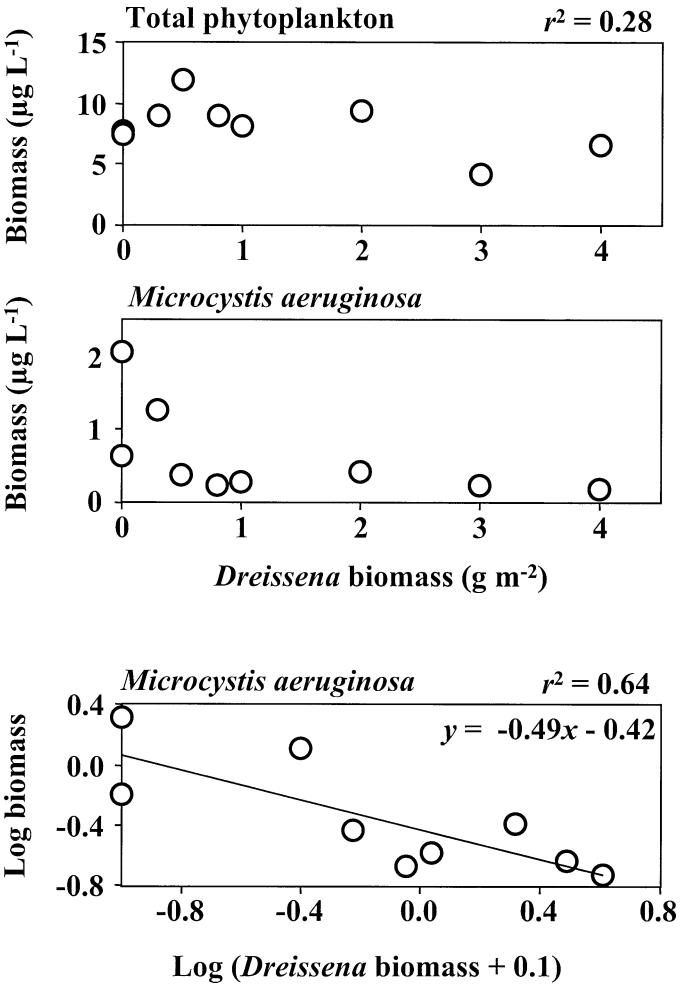

Fig. 2. Effects of zebra mussel (*Dreissena*) density on the biomass of total phytoplankton (upper panel, not significant at $p > 0.14$) and *Microcystis aeruginosa* (middle and lower panels) on day 21 of the 2000 enclosure experiment. The lower panel presents the same data as the middle panel but on log-log axes (*Dreissena* effect was significant for log-transformed data at $p < 0.01$). Probabilities (p values) are for ANOVA F-tests from linear regression analyses (*see* Table 2).

Results

There were no relationships between the intended mussel treatment density and the total phytoplankton biomass, the *M. aeruginosa* biomass, the TP, the SRP, or the NH_4^+ on day 0 in either the 2000 or 2001 enclosures (linear regressions, $p > 0.12$). Thus, there were no preexisting relationships with respect to mussel treatment in the experiments. In addition, there were no mussel effects on any response variable, including the abundances of individual phytoplankton species, on day 5 of the 2000 enclosure experiment (linear regressions, $p > 0.30$). Mixed-layer (0–7 m) water temperatures in the enclosures were similar between years, ranging from 23°C to 24°C in 2000 (mean = 23.7°C) and from 22°C to 26°C in 2001 (mean = 24.0°C). The thermocline extended from 7 to 10 m in both years.

Results of enclosure experiment in 2000—Average phosphorus levels were low in the 2000 enclosures (Table 1), relative to typical summer TP concentrations in the lake (~8–12 μg L^{-1}). Although TP appeared quite variable (range = 1.8–5.7 μg L^{-1} across the nine enclosures), this wide range was a consequence of a single anomalously high measurement of TP. Without the latter value, the TP range was relatively narrow (1.8–2.9 μg L^{-1}). There was no correlation between TP and any phytoplankton response variable.

Zebra mussels had no effect on total phytoplankton biomass, measured either as Chl *a* or via microscope counts (Fig. 2; Table 2). In contrast, zebra mussels had a negative effect on *M. aeruginosa* biomass (Fig. 2; Table 2). *M. aeruginosa* biomass decreased as mussel density increased from 0 to 1 g m^{-2} but did not appreciably decrease further as mussel density increased from 1 to 4 g m^{-2} (Fig. 2). We judged the effect to be nonlinear on the basis of the shape of the response (Fig. 2) and because the log-log regression was highly significant (Table 2), whereas the linear regression was not ($p > 0.10$). Zebra mussels also had a negative effect on the relative dominance (as percentage of total phytoplankton biomass) of *M. aeruginosa* ($p < 0.04$).

PCA of phytoplankton species biomass data (exclusive of *M. aeruginosa*) accounted for 80% of the total variance when two orthogonal factors were analyzed. Zebra mussel

Table 2. Statistical results for the enclosure experiment conducted in 2000. ANOVA statistics for linear regression analyses are reported as proportions of variance (Prop.) explained (r^2) and p values of F-tests. Statistically significant results ($p \leq 0.05$) are underlined.

Response variable	Independent variable (*Dreissena* biomass)	
	Prop. explained	p value
Chlorophyll	0.05	0.56
Phytoplankton biomass	0.28	0.14
Microcystis aeruginosa biomass*	<u>0.64</u>	<u>0.01</u>
SRP	0.02	0.69
NH_4^+-N	<u>0.70</u>	<u>0.01</u>

* Both variables log transformed.

density had a significant negative effect on scores of PCA factor 2 (linear regression, $p < 0.04$) but not of PCA factor 1 ($p > 0.40$). PCA factor 2 scores were positively correlated with the biomass of *Oocystis* sp. ($r = 0.69$, $p = 0.04$), suggesting that zebra mussels had a negative effect on this phytoplankter. The latter was confirmed by regression of *Oocystis sp.* biomass against mussel density ($r^2 = 0.58$, $p < 0.02$). However, the *Oocystis* sp. was only a minor species, comprising 0.3–6.0% of the total phytoplankton biomass across the enclosures. No other common phytoplankton taxon (Table 3) was affected by mussels in 2000 ($p > 0.11$).

SRP concentrations declined during the experiment (mean SRP on day 0 = 1.25 μg L^{-1}, SE = 0.31, mean SRP on day 21 = 0.59 μg L^{-1}, SE = 0.03), but they were not affected by mussels (Fig. 3; Table 2). In contrast, NH_4^+ concentrations increased over time (mean NH_4^+ on day 0 = 8 μg L^{-1}, SE = 1, mean NH_4^+ on day 21 = 16 μg L^{-1}, SE = 1), and there was a positive effect of mussels on NH_4^+ by the end of the experiment (Fig. 3; Table 2).

Results of enclosure experiment in 2001—Fertilization of the enclosures in 2001 maintained TP levels near the target of 10 μg L^{-1} (Table 1), but there was a large unintended variation in TP across the enclosures (Fig. 4). There was no relationship between TP and mussel density (linear regression, $p > 0.95$), so we treated TP as an additional independent variable. Variation in TP was positively associated with variation in the biomass of both total phytoplankton and *M. aeruginosa* across the enclosures (Fig. 4; Table 4), so we

Table 3. Common phytoplankton taxa and their average biomass (dry mass) across the enclosures on day 21 of the 2000 experiment and day 19 of the 2001 experiment. ND, not detected.

Taxon	Average biomass (μg L^{-1})	
	2000	2001
Aphanocapsa pulchra	0.7	ND
Chlorella sp.	ND	1.1
Chroococcus sp.	ND	1.1
Cyclotella atomus	0.5	ND
Cyclotella comta	0.2	<0.1
Microcystis aeruginosa	0.6	3.0
Microspora sp.	0.2	ND
Ochromonas sp.	3.3	9.8
Oocystis sp.	0.2	<0.1
Peridinium spp.	0.9	0.3

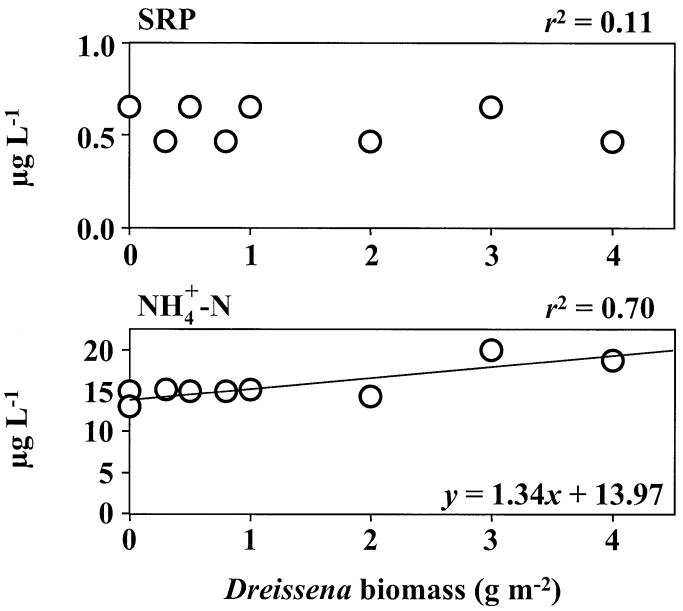

Fig. 3. Effects of zebra mussel (*Dreissena*) density on soluble reactive phosphorus (SRP; upper panel, not significant at $p > 0.69$) and NH_4^+-N (lower panel, significant at $p < 0.01$) on day 21 of the 2000 enclosure experiment. Probabilities (p values) are for ANOVA F-tests from linear regression analyses (*see* Table 2).

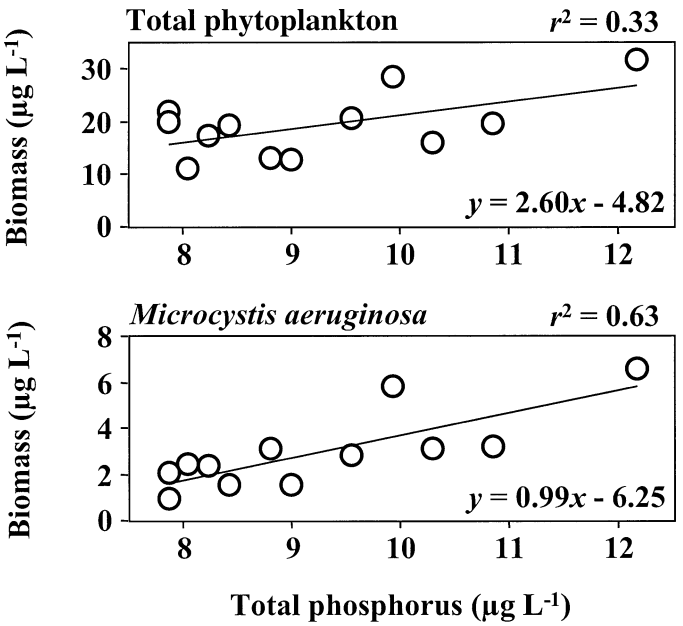

Fig. 4. Relationships between the biomass of total phytoplankton (upper panel, significant at $p < 0.05$) and *Microcystis aeruginosa* (lower panel, significant at $p < 0.01$) versus total phosphorus concentration on day 19 of the 2001 enclosure experiment. Probabilities (p values) are for ANOVA F-tests from linear regression analyses (*see* Table 4).

Table 4. Statistical results for the enclosure experiment conducted in 2001. ANOVA statistics from stepwise linear regression analyses are reported as proportions of variance (Prop.) explained and *p* values of *t*-tests for the effect of each independent variable. Statistically significant results ($p \leq 0.05$) are underlined.

	Independent variable			
	Total phosphorus		*Dreissena* biomass	
Response variable	Prop. explained	*p* value	Prop. explained	*p* value
Chlorophyll	<u>0.49</u>	<u>0.01</u>	0.01	0.65
Phytoplankton biomass	<u>0.33</u>	<u>0.05</u>	0.03	0.57
Microcystis aeruginosa biomass	<u>0.63</u>	<u>0.01</u>	<u>0.17</u>	<u>0.02</u>
SRP	0.15	0.22	<u>0.32</u>	<u>0.05</u>
NH_4^+-N	0.26	0.09	0.04	0.50
Microcystis colony size (area)*	<u>0.26</u>	<u>0.05</u>	<u>0.26</u>	<u>0.05</u>

* Response variable log transformed.

examined mussel effects after removing the influence of TP via stepwise regression. We plotted residuals from regressions against TP (Figs. 5–7) to provide a visual illustration of the mussel effects that were analyzed via stepwise regression. These analyses revealed that there was no effect caused by the mussels on the total phytoplankton biomass (Fig. 5; Table 4), as seen in 2000. In contrast, there was a significant positive effect of mussels on the biomass of *M. aeruginosa* (Fig. 5; Table 4). The relative strengths of the effects of TP and mussel biomass on *M. aeruginosa* can be assessed by comparing the slopes of the relationships in Figs. 4, 5 (Osenberg et al. 1997), since the units of the ordinates are the same. This comparison shows that the TP effect (0.99) was about twice as large as the mussel effect (0.50). No other phytoplankton taxon was affected by mussels ($p > 0.30$), although the biomass of one other species (*Chlorella* sp.) was positively associated with TP ($p < 0.01$). We also did not find that the mussels had any effect on the total phytoplankton biomass exclusive of *M. aeruginosa.*

Also in direct contrast to 2000, we observed a significant positive effect on SRP that was caused by the mussels, but there was no effect on NH_4^+, in 2001 (Fig. 6; Table 4). As expected, mean SRP was higher in 2001 (Table 1), and SRP increased over time (mean SRP on day 0 = 0.67 μg L^{-1}, SE = 0.03, mean SRP on day 19 = 1.38 μg L^{-1}, SE = 0.04), presumably as a result of fertilization. In contrast, NH_4^+ decreased sharply over time (mean NH_4^+ on day 0 = 34 μg L^{-1}, SE = 3, mean NH_4^+ on day 19 = 9 μg L^{-1}, SE = 1).

The colony size of *M. aeruginosa* at the start of each experiment (day 0) was similar between the 2 yr (mean ± SE: 12,682 ± 2,299 μm^2 in 2000; 10,177 ± 964 μm^2 in 2001, which was not statistically different at $p > 0.30$, *t*-test). In 2001, mean colony size across all enclosures decreased to an average of 4,450 μm^2 (SE = 364 μm^2) by day 19. There were significant positive effects of both TP and mussel density on colony size on day 19 in 2001 (Fig. 7; Table 4).

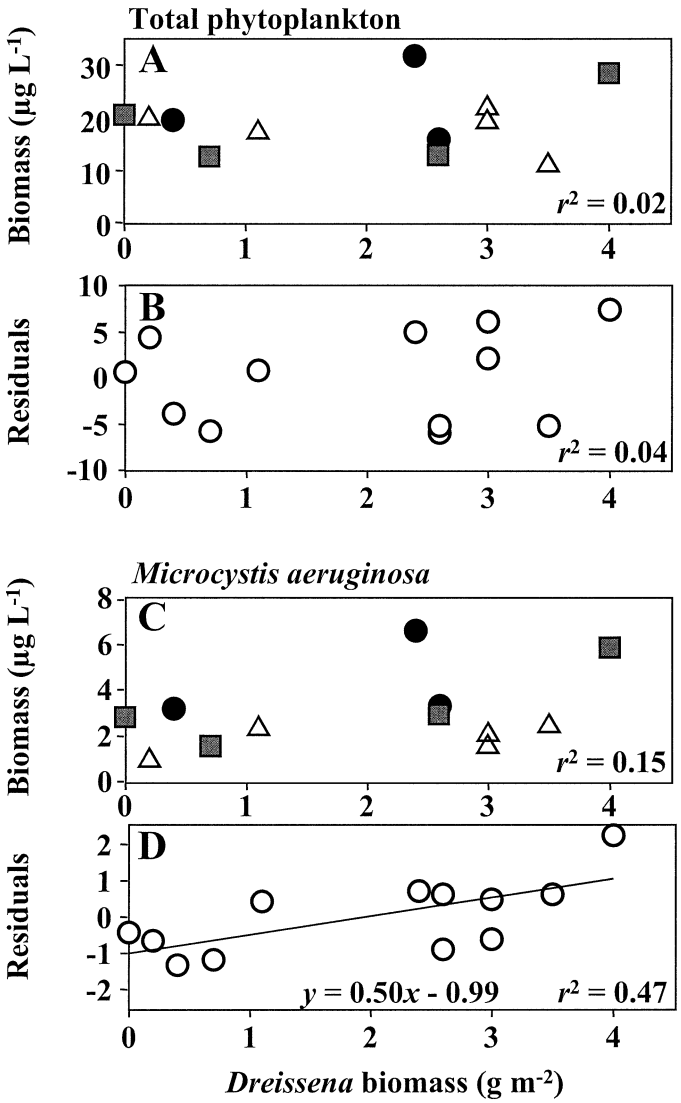

Fig. 5. Effects of zebra mussel (*Dreissena*) density on the biomass of phytoplankton (panels A and B, not significant at $p > 0.57$) and *Microcystis aeruginosa* (panels C and D, significant at $p < 0.02$) on day 19 of the 2001 enclosure experiment. Data points in panels A and C are coded according to total phosphorus concentration: white triangles = low (8.1–8.5 μg L^{-1}), gray squares = medium (8.6–10 μg L^{-1}), and black circles = high (>10 μg L^{-1}). Residuals from linear regressions of each response variable against the total phosphorus concentration (Fig. 4) are plotted in panels B and D. Probabilities (*p* values) are for *t*-tests of the significance of the slope for the effect of *Dreissena* from stepwise linear regressions using total phosphorus and *Dreissena* density as independent variables (*see* Table 4).

Discussion

The effect of *D. polymorpha* on *M. aeruginosa* biomass was negative in 2000 (Fig. 2) and positive in 2001 (Fig. 5) across the same experimental gradient (0–4 g m^{-2}) of mussel density. Taken together, the enclosure results indicate that (1) *D. polymorpha* preys significantly on *M. aeruginosa*, as shown in previous laboratory feeding studies (Baker et al. 1998; Bastviken et al. 1998), and (2) the indirect positive

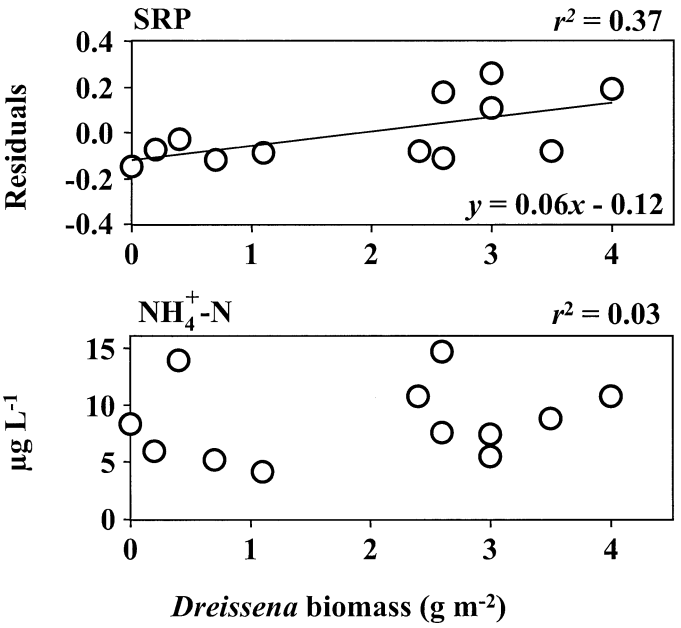

Fig. 6. Effects of zebra mussel (*Dreissena*) density on soluble reactive phosphorus (SRP; upper panel, significant at $p < 0.05$) and NH_4^+-N (lower panel, not significant at $p > 0.50$) on day 19 of the 2001 enclosure experiment. Residuals from a linear regression of SRP against total phosphorus concentration (Fig. 3) are plotted in the upper panel. The probability (p value) is for a t-test of the significance of the slope for the effect of *Dreissena* from a stepwise linear regression using total phosphorus and *Dreissena* density as independent variables (*see* Table 4).

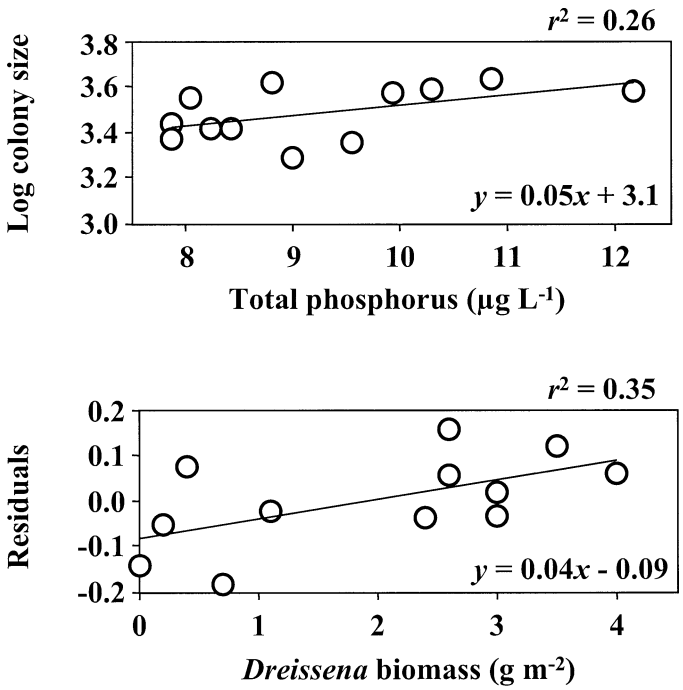

Fig. 7. Upper panel: relationship between *Microcystis aeruginosa* colony size and total phosphorus concentration on day 19 of the 2001 enclosure experiment (significant at $p < 0.05$). Lower panel: effect of zebra mussel (*Dreissena*) density on *M. aeruginosa* colony size (significant at $p < 0.05$) in the 2001 enclosure experiment. Residuals from a linear regression of *M. aeruginosa* colony size against total phosphorus concentration (upper panel) are plotted in the lower panel. The probability (p value) is for a t-test of the significance of the slope for the effect of *Dreissena* from a stepwise linear regression using total phosphorus and *Dreissena* density as independent variables (*see* Table 4).

effects of *D. polymorpha* on *M. aeruginosa* outweigh the negative effects of consumption under certain conditions (Fig. 5). Given that consumers are typically expected to reduce the abundance of their prey, positive effects warrant scrutiny with respect to potential experimental artifacts. An important potential artifact is density-dependent nutrient release from dead or dying mussels (Threlkeld 1988). We monitored mussel mortality weekly in 2001 and found it to be low and not related to mussel density. This observation provides direct evidence against this mechanism of phytoplankton growth stimulation. In addition, data from monitoring programs suggest that positive effects of mussel invasion on *M. aeruginosa* abundance are common in lakes (Vanderploeg et al. 2001; Nicholls et al. 2002; Raikow et al. 2004), so our 2001 results are congruent with a large fraction of the available observational evidence, including observations in Gull Lake before and after *Dreissena* invasion (Fig. 1). Monitoring data, however, also suggest that positive and negative effects are occurring in different ecosystems (Smith et al. 1998). Our experimental results suggest that both types of effects can occur in the same ecosystem.

The negative effect of *D. polymorpha* on *M. aeruginosa* in 2000 was somewhat unexpected given that observational data tend to favor the hypothesis that mussel effects are positive in many lakes. However, observational data are limited to lakes with summer epilimnetic TP concentrations above ca. 10 μg L^{-1} (Raikow et al. 2004), a level that is substantially higher than TP concentrations in the 2000 enclosures (Table 1). Thus, our experimental results in 2000 have no strict analog among the available observational data. Zebra mussels have invaded lakes with TP concentrations <10 μg L^{-1} (Wilson and Sarnelle 2002), but no before-after phytoplankton composition data have been reported from such lakes.

Our primary objective was to provide experimental evidence with respect to the magnitude and direction of *D. polymorpha*'s net effect on *M. aeruginosa*, in light of existing circumstantial evidence from lake surveys (Raikow et al. 2004). Given that we unexpectedly observed both positive and negative effects in our experiments, it seems appropriate to consider briefly some possible explanations for the reversal of effect, as a guide for future mechanistic investigations. We restrict this consideration to simple mechanisms that our data can address and emphasize that more complex mechanisms involving other species of phytoplankton and/or grazers certainly may play a role. We also do not claim that fertilization of the enclosures with phosphorus in 2001 caused the reversal, because there could have been many other environmental differences between years that we did not measure.

Variation in the magnitude of either or both of the negative (via consumption) and positive (via altered nutrient or light availability) effects of *D. polymorpha* on *M. aerugi-*

nosa may have contributed to the reversal of effect that we observed. With respect to consumption, it is possible that the vulnerability of *M. aeruginosa* to *D. polymorpha* grazing varied between the experiments. Grazing vulnerability appears to be a function of *M. aeruginosa* colony size and chemical deterrents (Vanderploeg et al. 2001). We found no difference in colony size between the experiments at the start, suggesting that ambient lake conditions that determine colony size were not appreciably different between years. However, we did find a positive effect for both TP and *D. polymorpha* on colony size by the end of the 2001 experiment (Fig. 7). These results suggest that phosphorus enrichment increases *M. aeruginosa* colony size and that *D. polymorpha* grazing is less effective against larger colonies (since colony size shifted upward as mussel density increased). Laboratory feeding studies have also suggested that large colonies are less vulnerable to mussel grazing (Vanderploeg et al. 2001). Thus, greater phosphorus availability in 2001 (Table 1) may have reduced the vulnerability of *M. aeruginosa* to *D. polymorpha* grazing and contributed to the overall positive effect we observed.

Alternatively, there may have been differences in the magnitude of stimulation that *D. polymorpha* had on the growth of *M. aeruginosa* between the experiments. The mussels had no effect on water clarity in either experiment (as Secchi depth, $p > 0.25$), so we focused our attention on nutrients. We found that the mussels had a positive effect on NH_4^+ but no effect on SRP in 2000 (Fig. 3), when TP levels were very low. The opposite result (positive effect on SRP, no effect on NH_4^+, Fig. 6) was obtained in 2001 when TP levels were higher. Taken together, these results are at least consistent with comparisons of phosphorus excretion by mussels across habitats with varying levels of phosphorus loading (Vanderploeg et al. 2002). In the latter review, it was suggested that zebra mussels excrete less phosphate in habitats with lower TP concentrations, presumably as an adaptive response to phosphorus deficiency (Sterner 1990). If mussels enhanced the supply of available phosphorus to *M. aeruginosa* to a greater degree in 2001 than in 2000, this could explain why the overall effect on *M. aeruginosa* was positive in 2001 but negative in 2000. Results from the 2001 experiment (Fig. 4) and laboratory studies with pure cultures (Holm and Armstrong 1981; Olsen et al. 1989; Fujimoto et al. 1997) also suggest that the growth rate of *M. aeruginosa* is likely to be strongly limited by phosphorus supply. Further experiments are needed to unravel these mechanisms.

It was surprising that *Dreissena* had no effect on total phytoplankton biomass, given previous field studies (Fahnenstiel et al. 1995; Heath et al. 1995; Caraco et al. 1997; Jack and Thorp 2000; Idrisi et al. 2001; Wilson 2003; Raikow et al. 2004), but we note that most of the above studies reported mussel effects on phytoplankton assemblages that were relatively naive with respect to mussel grazing. Further, phytoplankton data from the Bay of Quinte (Lake Ontario) show no sustained decrease in total phytoplankton biomass subsequent to mussel invasion, while the biomass of *M. aeruginosa* was increasing dramatically (Nicholls et al. 2002). Thus, there is precedent for a lack of mussel impact on total biomass. The phytoplankton in Gull Lake were exposed to mussel grazing for 6 yr prior to our first enclosure experiment, which may have fostered the development of an assemblage of relatively grazing-resistant forms, including *M. aeruginosa*. As a consequence, we might expect such a phytoplankton assemblage to be less responsive as a whole to changes in mussel-grazing pressure relative to a naive assemblage (Wellborn and Robinson 1991). In addition, TP concentrations in both enclosure experiments were low, and herbivore effects on total phytoplankton biomass are expected to be weak at low TP (Sarnelle 1992).

Despite the lack of mussel impact on total phytoplankton biomass, we demonstrated a positive effect of *Dreissena* on *M. aeruginosa* that is consistent with comparative studies in both the Great Lakes (Nicholls et al. 2002) and inland lakes (Raikow et al. 2004), including the lake in which the experiments were conducted (Fig. 1). This further supports the conclusion that *Dreissena* invasion is the primary cause of recent increases in *M. aeruginosa* in North American lakes with low-to-moderate nutrient levels. Given that we experimentally reduced mussel densities in an already-invaded lake, the responses of *M. aeruginosa* further suggest that *Dreissena*'s effects are reversible in invaded lakes.

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