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Do zebra mussels (dreissena polymorpha) alter the water chemistry in a way that favours Microcystis

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DO ZEBRA MUSSELS (*DREISSENA POLYMORPHA*) ALTER THE WATER

CHEMISTRY IN A WAY THAT FAVOURS *MICROCYSTIS*

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ABSTRACT

Do zebra mussels (*Dreissena polymorpha*) alter the water chemistry in a way that favours *Microcystis*.

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Many factors may contribute to cyanobacterial bloom formation. This study examined possible relationships between the presence of zebra mussels (*Dreissena polymorpha)* and *Microcystis spp.* abundance. Experiments were conducted in twelve microcosms designed to mimic shallow lake ecosystems. Zebra mussels significantly reduced nitrate, dissolved organic nitrogen, and total dissolved nitrogen concentrations, and had no effect on ammonia, phosphate levels, or dissolved organic carbon. Consequently, the N:P ratio was reduced in microcosms with zebra mussels to ~ 6:1, which is below the Redfield ratio of 16:1. Zebra mussels also increased the abundance of *Microcystis* and the *Microcystis* : *Pseudokirchneriella* biovolume. In experiments done without zebra mussels, nutrient ratios were manipulated and low N:P caused a similar increase in *Microcystis* and *Microcystis* : *Pseudokirchneriella* biovolume. The shift in N:P in the presence of zebra mussels was related to higher rates of nitrate flux into sediments and reduced flux of phosphate into sediments. It is this shift in N:P, and possibly some level of selective feeding, that is believed to have driven changes in the relative abundance of *Microcystis*. Finally, in order to compare the experimental results with changes caused by zebra mussel invasion in the natural environment, the data from 15 Wisconsin lakes before and after the zebra mussel invasions were analysed.

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This thesis is dedicated to the memory of my grandfather, Boris Voishel, and my great-

grandfathers, Vladislav Voishel

and Rudolph Tolmats.

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INTRODUCTION

Cyanobacterial blooms

Freshwater cyanobacteria (blue-green algae), may pose a threat to other organisms due to the ability of some cyanobacterial genera, such as *Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Microcystis, Nodularia,* and *Planktothrix* to produce toxins (Carmichael 1997; Codd *et al.* 1999; Hamill 2001). A wide variety of toxins that are produced by these species can affect nervous, hepatic, and dermatologic systems (Fleming and Stephan 2001) and may contribute to human and animal health problems (Katircioglu *et al.* 2004). High phosphate loading can trigger blooms of photosynthetic microbes (including cyanobacteria), decreasing water transparency in lakes (Schindler 1977; Mason 1991; Welch and Lindell 1992). Some other factors contributing more specifically to cyanobacterial blooms include nutrient loading (Klemer 1976; Watson *et al.* 1997), nutrient ratios (N:P, Si:N, Si:P) (Pearsall 1930, 1932; Rhee 1978; Tilman *et al.* 1982; Smith 1983, 1986; Pick and Lean 1987; Stockner and Shortreed 1988; Suttle *et al.* 1991; Bulgakov and Levich 1999), high water temperature (McQueen and Lean 1987; Kong and Gao 2005), high irradiance (Ahn *et al.* 2002), water column stratification (Fogg and Walsby 1971), and high DIC (dissolved inorganic carbon) availability (Klemer *et al.* 1996; Qiu and Gao 2002), which can result in rapid photosynthesis and elevation of pH, a condition favourable for cyanobacteria (Yamamoto and Hiroyuki 2005). Despite the fact that cyanobacteria are a well-studied group (Wetzel 1983), blooms can be difficult to predict as their dominance during bloom formation is a stochastic result of the combination of these factors (Reynolds 1998;

Dokulil and Teubner 2000; Reynolds *et al.* 2000) and combinations of these factors will vary temporally and from system to system.

Macronutrients, such as phosphorus (P), nitrogen (N), and silicon (Si), are known to limit the phytoplankton biomass separately and in relative quantities (Bulgakov and Levich 1999). Interactions of P, N, and Si were discussed in numerous studies that revealed the existence of species-dependent optimal concentration ratios of these macroelements (McQueen and Lean 1987; Bulgakov and Levich 1995, Holm and Armstrong 1981; Tilman 1982; Levich 2000; Rocha *et al.* 2002). Rocha *et al.* (2002) found that the environments with low Si:N and Si:P favoured cyanobacterial dominance, while high Si:N and Si:P favoured increases in diatom abundance (Sommer 1996). Regarding the optimal N:P ratio, data from a study of nutrient enrichment effects showed that cyanobacteria became dominant when this ratio was low (Schindler 1977) which was supported by other authors (Rhee and Gotham, 1980; Levich, 2000). It also should be noted that the limited N supply favours not only N_2-fixing blue-green algae (e.g. *Nostoc spp.*), but also non-fixing genera, such as *Microcystis* (Varis 1992). Schindler (1977) stated that blue-greens dominate in the community at N:P ratios of 2 to 5, while green algae dominate at ratios higher than 29. Fluctuations in N:P determine not only the phytoplanktonic taxomic composition, but also affect the relative biomass of different phytoplanktonic groups (Levich *et al.* 1992) including cyanobacteria.

Zebra mussel invasion

In North America, zebra mussels (*Dreissena polymorpha*) were first discovered in Lake St. Clair in 1988 (Hebert *et al.* 1989), and rapidly spread to other areas of the

Laurentian Great Lakes (Griffiths *et al.* 1991, Dermott *et al.* 1993, Dermott and Munawar 1993, Bailey *et al.* 1999, Dermott *et al.* 2003). This species colonized a large number of North American freshwater systems and by the end of 1993, they became established in all of the Great Lakes, as well as in the waterbodies of 18 American states and at least two Canadian provinces (Moser 2002). Ehrlich (1989) defined successful invaders as species that are able to establish themselves in new areas while others fail to do so. Based on this definition, zebra mussels could definitely be called successful invaders of the Great Lakes region.

Due to their ability to exercise direct effects through their filtering activities and indirect effects that result from the disruption of the regular patterns and processes of the ecosystem, there exists great concern regarding the invasion of zebra mussels. Direct effects include grazing upon algae, thereby depleting a major food source for other aquatic organisms (Caraco *et al.* 1997), mineralization of nutrients (Karatayev *et al.* 2002), increasing the rate of nitrogen and phosphorus recycling (Heath 1993), and alterations to the metabolic structure and function of the benthic microbial community due to the liberation of carbon from *Dreissena* clusters (Lohner *et al.* 2005). Reduction in phytoplankton biomass is one of the most dramatic ecological effects of zebra mussels (Strayer *et al.* 1999; Raikow *et al.* 2004). Wilson (2003), for example, detected a significant negative impact on algal abundance, and stated that zebra mussels in mesocosms were able to decrease the phytoplankton biovolume by 53%. In the Hudson River, zebra mussels decreased the phytoplankton cell densities; however, the proportion of diatoms in this waterbody increased from 14 to 76% (Marshall 1998, Baker *et al.* 1998). In addition to the changes in phytoplankton composition and total phytoplankton

biomass, zebra mussels may also trigger other changes in the aquatic food web. For instance, data collected in Saginaw Bay, Lake Huron (September and October 1994) showed that zebra mussels lowered protozoan numbers by 70-80% and reduced the species richness of the protozoan community by 30-50 % (Lavrentyev *et al.* 1995). Moreover, Wilson (2003) reported an increase in ciliate biovolume of 71%, caused by zebra mussels in *in situ* microcosms. As for the benthic macroinvertebrate communities, Dermott and Geminiuc (2003) detected that benthic fauna in the shallow zone of Lake Ontario (<30 m) had been changed after the arrival of the *Dreissena spp.* These species replaced the filter-feeders *Sphaerium* and *Manayunkia*in in this part of the lake, and increased the benthic biomass by an order of magnitude. Furthermore, with the disappearance of *Diporeia* at depths of less than 70 m, which was associated with the zebra mussel invasion, the benthic community structure became simpler (Dermott and Geminiuc 2003).

Indirect impacts of zebra mussels include bottom-up effects due to alterations in nutrient recycling. For example, the rapid recycling of phosphorus can lead to the alteration from P-limited to P-replete physiological conditions, decreasing the phosphate uptake by phytoplankton at the base of the food web (Heath *et al.* 1995, Heath *et al.* 1996). Alterations to nutrient recycling can thereby alter ecosystem metabolism (Baker *et al.* 1998). Zebra mussels can also hasten the sedimentation processes in freshwater bodies (Wiktor 1963), thereby reducing available carbon for the pelagic food web. Finally, zebra mussels could have ecosystem and food-web consequences not yet explored (Lohner 2005).

Possible causal link between zebra mussel invasion and cyanobacterial blooms

Some blooms of *Microcystis aeroginosa*, one of the most common toxic freshwater cyanobacteria (Oberholster *et al.* 2004), have been observed in North America during the last ten years and are assumed to be connected with the zebra mussel invasions (Sarnelle *et al.* 2005). *Microcystis* blooms that caused taste and odour problems were observed in Saginaw Bay in 1991-1995 following invasions by zebra mussels (Bierman *et al.* 2005). Moreover, Nicholls *et al.* (2002) reported that *Microcystis* was the only taxon to increase (13-fold) after the zebra mussel invasion in the Bay of Quinte (north-eastern Lake Ontario) in the 1990s. Similar recent blooms have been observed in other bodies of waters since the establishment of zebra mussels in those areas. In Lake Erie during 1995, *Microcystis aeroginosa* formed a large bloom that created a surface scum covering much of the western end of the lake (Budd *et al.* 2002). Such blooms had not been observed in Lake Erie since the institution of phosphorus loading reductions in the early 1970s (Makarewicz 1993; Nicholls and Hopkins 1993). During investigations of 39 inland lakes in southern Michigan during 2002-2003, Sarnelle (2005) concluded that *Microcystis aeroginosa* abundance was 3.6 times higher in the invaded lakes than in lakes without zebra mussels. Additional anecdotal evidence of *Microcystis* blooms in Michigan lakes following zebra mussel invasions are cited as personal communications by Vanderploeg *et al.* (2001). The response of phytoplankton to zebra mussels may further depend on the nutrient status of the lake. Raikow *et al.* (2004) found that *Microcystis aeruginosa* blooms in Michigan lakes were associated with zebra mussels where total P concentration of less than 25 µg l^{-1} existed. However, if total P exceeded 25 µg l^{-1}, the occurrence of *Microcystis* blooms was independent of the presence of zebra mussels.

One hypothesis that has been advanced to explain the shifts to cyanobacteria in bodies of water populated by zebra mussels has been selective feeding or particle sorting by zebra mussels (Heath *et al.* 1995). Despite the fact that Wilson (2003) detected an equal decrease in all algal groups by zebra mussels, Baker *et al.* (2000) stated that selective feeding existed, and defined the role of ctenidial or gill morphology as an important factor in particle selection by zebra mussels. Endoscopic observations and video image analysis suggested that preferential feeding took place and that the particle sorting occurred directly in ctenidia (Baker *et al.* 2000), which is a gill found in molluscs that has a central axis with a fringe of filaments on each side. Moreover, Vanderploeg *et al.* (2001) stated that changes in the phytoplankton composition of Saginaw Bay, specifically shifts toward greater abundance of *Microcystis* and other cyanobacteria, mainly were due to the selective rejection of cyanobacteria (*Microcystis aeruginosa*) as seen in the pseudofaeces when mussels were given *Microcystis* in combination with a preferred food source (*Cryptomonas*). However, while zebra mussels appear to be highly capable of sorting particles and prefer the flagellated crytomonad species (Baker *et al.* 2000), *Microcystis aeruginosa* appears to be preferred to a variety of green algae (*Micractinum* sp., *Curcigenia tetrapedia*, and *Scenedesmus quadricauda*) and the diatom *Cyclotella meneghiniana* (Baker *et al.* 1998). Moreover, zebra mussels appear more efficient than native mussels in discriminating between nutritious particles and particles with low nutritional value (Baker and Levinton 2003) and selective feeding on *Microcystis* would be expected to increase as zebra mussel populations displace native populations. Therefore, while selective feeding by zebra mussels may play a role in phytoplankton community shifts toward cyanobacterial dominance in some systems, it

seems that a shift toward less cyanobacterial biomass should be at least as common if selective feeding drives community shifts.

Kaur *et al.* (2005) emphasized that selective filtration could not be discussed as the only factor that affected cyanobacterial abundance, and that the ability of zebra mussels to recycle nutrients (Karatayev *et al.* 2002; Arnott and Vanni 1996; James *et al.* 2001) in the water body should not be overlooked. Johengen (1995) stated that filtering activity has a direct effect in reducing nutrients associated with particles and plankton and advanced this as an explanation of the changes observed in Saginaw Bay. Recently, the reduction of phosphorus and nitrogen turnover times by zebra mussels was demonstrated as zebra mussels mineralize organic nitrogen and phosphorus, excreting some inorganic N and P (Conroy *et al.* 2005). Conroy *et al.* (2005) suggested that nutrient flux from dreissenids modified nitrogen cycles in Lake Erie, thereby facilitating cyanobacterial blooms in this area. They suggested that zebra mussels became a new source of nutrient remineralization in Lake Erie and were more important in nitrogen recycling than in phosphorus recycling. Further, Conroy *et al.* (2005) stated that zebra mussel excretion at low nitrogen to phosphorus ratios could lead to cyanobacterial dominance in Lake Erie, which is consistent with the statement made by Arnott and Vanni (1996). The latter found that zebra mussels excreted nutrients at N:P ratios of less than 20, with smaller mussels excreting even more P relative to N, and suggested nutrient excretion ratios might promote cyanobacterial blooms. Another study on Lake Erie conducted by Howell *et al.* (1996) reported fine particulate matter deposition and total organic carbon content of sediment increased in an eastern Lake Erie site following colonization by zebra mussels. Presumably, deposition of organically-bound nitrogen to

sediments would also increase, intensifying remineralization and N-cycling processes in sediments. Furthermore, Yamamuro and Koike (1998) suggested that the activities of filter-feeders (e.g. bivalves) could enhance bacterial activity and physical transport of ammonium into sediments, and that this might be responsible for changes in dissolved nitrogen concentrations, due to acceleration of coupled nitrification–denitrification processes. The authors explained the high content of DON and ammonium by the influence of macrobenthos in the partitioning of nitrogen species though their motion and excretion. Finally, the enhancement of denitrification in zebra mussel beds was recently demonstrated (Bruesewitz *et al.* 2004).

Denitrification, a microbial reduction of nitrate through various gaseous inorganic forms to N_2, represents the only permanent sink in the nitrogen cycle for aquatic systems; moreover, its enhancement could alter N:P availability in a way that favours growth of cyanobacteria over other phytoplankton. Despite the fact that high water temperature is considered to be one of the main factors that accelerate the denitrification rates (Carrera *et al.* 2003; Inwood 2004), the impact of filter-feeding activities should not be underestimated. The denitrification processes take place mainly in the top few millimetres of the sediment surface of freshwater systems (Venohr *et al.* 2003), and their rates are significantly affected by zebra mussels (Conroy *et al.* 2005; Brusesewitz *et al.* 2004). Moreover, data from a number of experiments with different types of filter- feeders showed the enhancement of denitrification rates associated with the filter-feeding activities. For instance, Mugg *et al.* (2001) found that increased organic sedimentation by filter-feeders, such as oysters, could contribute to increased rates of sediment deposition leading to increased denitrification. Newell and Cornwell (2000)

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also suggested that net rates of nitrogen loss via denitrification could be enhanced in areas with higher levels of benthic-pelagic coupling. Finally, after the comparison of nitrogen pools and transformation rates at a green-lipped mussel (*Perna canaliculus*) farm and a reference site, Kaspar *et al.* (1985) discovered that total denitrification at the mussel farm was 21% higher than in the reference site. The data from that study showed that loss of bound N through mussel harvest and denitrification was 68% higher at the mussel farm.

The present study was conducted to find the possible causal link between the zebra mussel invasions and *Microcystis* blooms in the Great Lakes region. It considers the ability of zebra mussels to change water chemistry in a way that would favour cyanobacterial growth. Three specific hypotheses were tested: 1) zebra mussels would enhance the removal of nitrate from the water column, with little effect on phosphate; 2) enhanced removal of nitrogen would decrease N:P in the water column; 3) this shift in N:P would favour the growth of cyanobacteria over green algae. These hypotheses were tested using freshwater microcosms containing a water column and sediment core. Hypothesis two was also tested by looking at water chemistry data from lakes prior to and following invasion by zebra mussels. The potential role of particle selection on shifts in phytoplankton was also considered.

MATERIALS AND METHODS

Organism collection and maintenance

Zebra mussels (*Dreissena polymorpha*) were collected from a breakwater in the Niagara River during the summer of 2005 (June-August, 2005). Attached mussels were transported to the laboratory where they were carefully detached from rocks by clipping bysal threads. Stock mussels used in the experiment were maintained in a 50-litre aerated tank and were fed with a mixture of *Pseudokirchneriella* spp. and *Microcystis* spp. *Microcystis* were obtained from Carolina Biological supply company (Burlington, NC, USA) and *Pseudokirchneriella* were obtained from Ward's Natural Science supply company (Rochester, NY). These organisms were cultured on a synthetic nutrient medium (Miller *et al.*1978). Large cultures were maintained in 15- litre carboys, and 25% of the volume was replaced with fresh medium daily.

Microcosm description

Twelve microcosms were designed to simulate freshwater ecosystems. Each had a sediment core and an overlying shallow (~2 m) water column. The water column was made of Tygon tubing (4 cm diameter i.d.) and suspended vertically from a steel frame. At the base, the tubing fit a PVC column with three ball valves in series (Figure 1). The top valve was used to isolate the water column. At the collar below this valve, the remainder of the column could be detached when zebra mussels were being added or removed. A nylon mesh screen (1 mm mesh size) above the middle valve supported zebra mussels. A removable sediment core was attached at the collar below the bottom valve.

Water Column

Ball Valve

Collar

Mesh with
Zebra Mussels

Ball Valve

Sampling Port

Ball Valve

Inflow

Sampling Port

Collar

Sediment Column
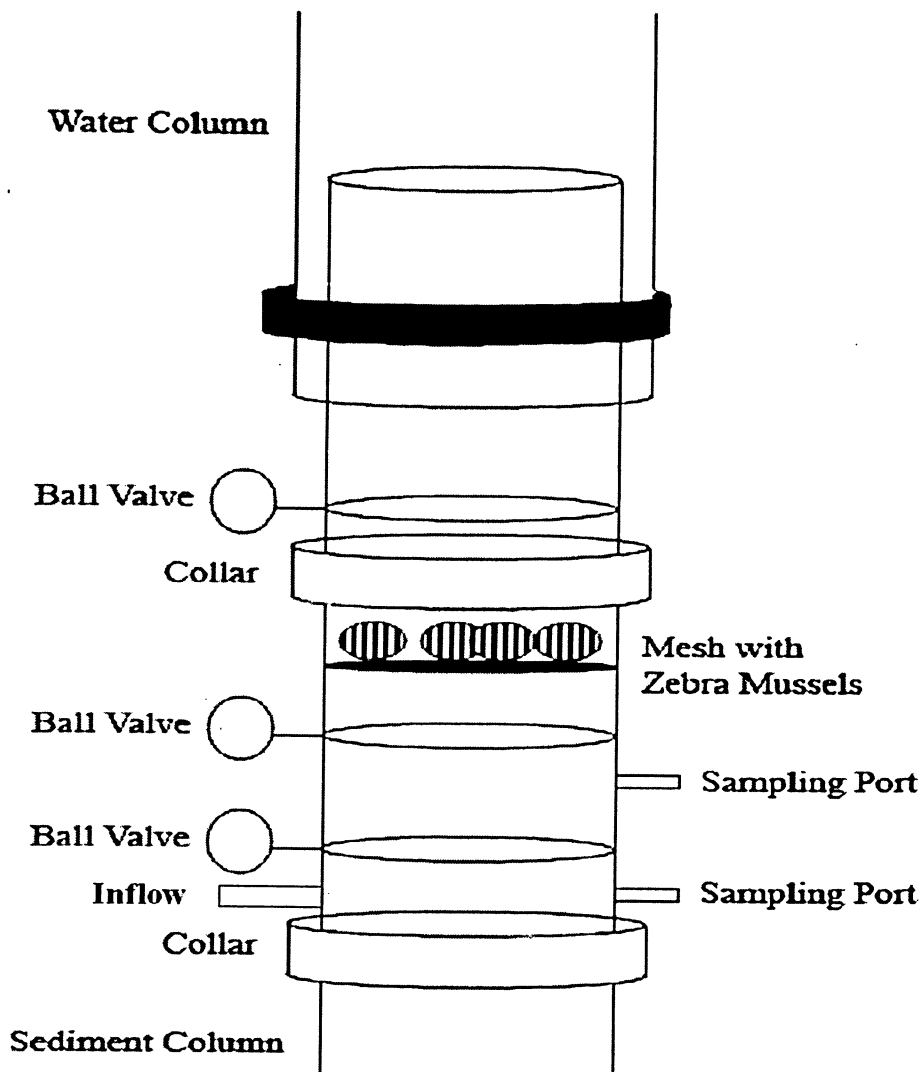
Figure 1. Diagram of microcosms used in experiments

Sediment in these cores was collected from the western end of Lake Ontario, near St. Catharines, ON. No zebra mussels were in the immediate vicinity of collected sediment. With the middle and bottom valves closed, two parcels of water (50 ml volume, each) could be isolated, one in contact with sediments and the other separated from sediments. Ports above and below the bottom valve allowed discrete sampling of these parcels.

Water was pumped into these columns from a 50-litre reservoir containing dechlorinated tap water using a submersible pump capable of generating a 3.7 m hydrostatic head (Little Giant Pump Company, Oklahoma City, OK). Water entered the microcosms through an inlet below the bottom valve. This ensured that water passing over the sediments and through the mesh supporting zebra mussels was oxygenated. At the top of the microcosm, the tubing was connected to a short (15 cm) piece of PVC pipe with an overflow outlet. Water was directed from the outlet through a sand trap to remove any zebra mussel veligers (larval stage) before discharging waste. The microcosms were kept at room temperature with natural, diffuse lighting.

The following are the series of experiments that were conducted to test the aforementioned hypotheses.

A. Effects of zebra mussels on nutrients and photosynthetic microorganisms

Five zebra mussels with an average shell length of 1.8 cm were placed onto the mesh in six of the microcosms (average density ~ 5,000 individuals m^{-2}). Empty mussel shells were added to the six remaining microcosms. The entire water column volume (~1.5 l) was replaced daily by pumping from the reservoir. A mixture of *Pseudokirchneriella* spp. and *Microcystis* spp. was added to the reservoir as a source of

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food for zebra mussels. Inorganic nutrients (KNO_3 and K_2HPO_4) were added to the reservoir with a target concentration of 48 μM N and 3 μM P. Before the water was pumped into the columns, samples from the reservoir were collected for nutrient analyses and cell counts. Samples were also collected from the overflow outlet during pumping and analyzed for nutrient concentrations and cell numbers. Flushing and sampling were performed three times over a five-day period.

B. Effects of nutrients on photosynthetic microorganisms

The second series of experiments were conducted in the same twelve microcosms but without zebra mussels. Instead, N:P (molar ratio) was manipulated based on differences in N:P ratios observed in the zebra mussel and control microcosms from the previous experiment. Water was pumped into the microcosms from two 30-litre reservoirs. Both reservoirs contained aerated and dechlorinated tap water with *Pseudokirchneriella,Microcystis,* and inorganic nutrients. Dissolved inorganic N:P in the first reservoir was adjusted to 6 (target concentrations 18 μM N and 3 μM P), while in the second reservoir N:P was adjusted to 12 (target concentrations 36 μM N, 3 μM P). Each reservoir fed six microcosms. The flushing and sampling procedure was the same as above; however, samples were collected only for cell counts. Flushing and sampling were performed on three consecutive days.

C. Nutrient flux

Experiments were conducted to determine effects of zebra mussels on sediment nutrient flux. Each of six microcosms had five live zebra mussels (mean shell length of

1.5 cm) and the other six microcosms had five empty shells. Aerated, dechlorinated tap water, amended with inorganic nutrients (48 μM N and 3 μM P) and *Pseudokirchneriella* and *Microcystis,* was pumped into each microcosm as above. The water volume of each microcosm was replaced daily. Every week for the duration of the experiment, the top valve of each microcosm was sealed and columns were opened to replace any dead mussels. Organic matter was permitted to accumulate on sediment surfaces for three weeks prior to nutrient flux measurements, which were made on days 21, 28, and 35. Samples were collected from the ports above and below the bottom valve of each microcosm. The bottom and middle valves were then closed to isolate parcels of water for 3 hours. At the end of this incubation, water samples were collected from these ports. Nitrate flux was calculated as the rate of change in nitrate concentration in the parcel of water overlying the sediment, assuming change was linear over this short interval. This flux was corrected by subtracting the rate of change in nitrate concentration in the water column (based on the parcel of water isolated from sediments). Ammonia, phosphate, and organic nitrogen concentrations were similarly calculated.

D. Selective filtration

First experiment on selective filtration

Three zebra mussels were placed in each of five 500-ml beakers, with another five beakers serving as controls (no zebra mussels). Each beaker contained 200 ml of dechlorinated tap water and *Pseudokirchneriella* and *Microcystis. Microcystis* and *Pseudokirchneriella* were counted in a representative sample at the beginning of the experiment, assuming that distribution of cells to all ten beakers was consistent. After a

24-hour period, water samples for cell counts were collected from all the beakers. Pseudofaeces in the bottoms of the beakers with zebra mussels were sampled and the relative proportions of *Microcystis* and *Pseudokirchneriella* were determined.

Second experiment on selective filtration

Five zebra mussels with an average shell length of 1.8 cm were placed in each of four 10-liter tanks; another four tanks without zebra mussels served as controls. Each tank was filled with dechlorinated tap water with an addition of inorganic nutrients at Redfield ratio (48 μM N and 3 μM P) (Appendix 1) and *Pseudokirchneriella* and *Microcystis* cells. Initial *Pseudokirchneriella* and *Microcystis* biovolumes were assumed to be the same in all tanks, all that tanks were seeded with the same volume of cell stock. Samples for cell counts were collected from all of the tanks on days 1, 2, 3, 7, 14, and 35.

E. Effects of zebra mussels on the water chemistry of Wisconsin lakes

This analysis was conducted to detect changes in the water chemistry of small water bodies that could be caused by zebra mussel activities. The Wisconsin University Sea Grant Program website (http://www.seagrant.wisc.edu) maintains a list of Wisconsin (USA) lakes that have been invaded by zebra mussels. The information includes the date zebra mussels were first observed in each of these lakes. Water chemistry data for these lakes were obtained from the United States Environmental Protection Agency's STORET Data Warehouse (www.epa.gov/storet) covering a range of dates from 1970 – 2002. Data were not available for all lakes or for all water chemistry parameters of interest (pH, alkalinity, inorganic nitrogen, organic nitrogen, inorganic phosphorus, organic

phosphorus). A total of 15 Wisconsin lakes with an established date of zebra mussel invasion had water chemistry data prior to and following zebra mussel invasion (Appendix 2). The data were analyzed to determine if there were patterns of change in water chemistry parameters (Appendix 3) for these Wisconsin lakes following the establishment of zebra mussels.

Cell counts

During all of the experiments, cell counts were done using a Sedgewick-Rafter counting cell on the day of sampling. A total of ten 100 x 100 μm grids were counted for each sample. Cell counts were converted to cell biovolumes based on geometric shape (Wetzel and Likens 1991).

Nutrient analyses

Samples to assess nitrate, ortho-phosphate, and dissolved organic carbon (DOC) concentrations were filtered through nylon membrane filters (0.45μm) and frozen until analysis (< 2 weeks). Samples checking for levels of ammonia and organic nitrogen were frozen unfiltered. Nutrient analyses were performed following the methods of the APHA (1998). Nitrate was analyzed using a copperized-cadmium reduction method (4500-NO_3^- E). Organic nitrogen was oxidized to nitrate using the persulfate method (4500-N C) and was then analyzed as nitrate. Phosphate was analyzed using the ascorbic acid method (4500-P). Ammonia was analyzed using the phenate method (4500-NH_3 F). Dissolved organic carbon was analyzed by catalytic oxidation using a Shimadzu TOC-VCSH (Shimadzu Scientific Instruments, Columbia, MD, USA).

Statistical Analysis

Data from the microcosm experiments and the second experiment on selective filtration were analyzed by ANCOVA (analysis of covariance), using mussels (presence or absence of live mussels) as an independent variable and date as a covariate. ANCOVA was used rather than repeated measures ANOVA because the contents of each microcosm, including water and microbial plankton, were replaced daily, and within each treatment, there was no systematic temporal relationship between response variables and the columns from which samples were collected. Single factor ANOVA was used to analyze data from the first experiment on selective filtration conducted in beakers. The water chemistry data from Wisconsin lakes were analyzed using paired samples t tests comparing average water chemistry parameter values (annual and seasonal) during the pre-invasion and post-invasion periods for each lake. All data were analyzed with Systat version 6.0 (SPSS, Inc., Chicago, IL)

RESULTS AND DISCUSSION

A. Effects of zebra mussels on nutrients and photosynthetic microorganisms

After 24 hours and 48 hours, ammonia concentrations were generally higher in microcosms without zebra mussels, although the effect of zebra mussels was not significant (ANCOVA, p = 0.074; Figure 2a). On the third sampling date (day 5), ammonia concentrations were highly variable, and the mean concentrations in microcosms with mussels more than doubled (Appendix 4). This release of ammonia may have been caused by physiological stress as more than one-third of the zebra mussels had died between sampling days 2 and 5. Since data from this third date would strongly influence total nitrogen and N:P measurements, only data from days 1 and 2 were analyzed for nutrient response to zebra mussels.

Nitrate concentrations were reduced in all columns from the inflow concentration of 48 µM. The loss of nitrate was greatest in the microcosms with zebra mussels, as concentrations were approximately three times lower than in the reference microcosms (ANCOVA, p = 0.023; Figure 2b). Similarly, organic nitrogen was much lower in microcosms with live zebra mussels (ANCOVA, p = 0.003; Figure 2c). Ortho-phosphate concentrations were not affected by zebra mussels (ANCOVA, p = 0.491; Figure 2d). Total phosphorus measurements were attempted; however apparent contamination of reagents rendered these data unusable. Therefore N:P was estimated as the sum of ammonia, nitrate, and organic nitrogen levels divided by ortho-phosphate concentrations, recognizing that this underestimated true N:P. In microcosms with zebra mussels, N:P was less than half that in reference microcosms (ANCOVA, p = 0.042) and was well

below the Redfield ratio of 16:1. Dissolved organic carbon showed no response to the presence of zebra mussels (ANCOVA, p = 0.820; Figure 2f).
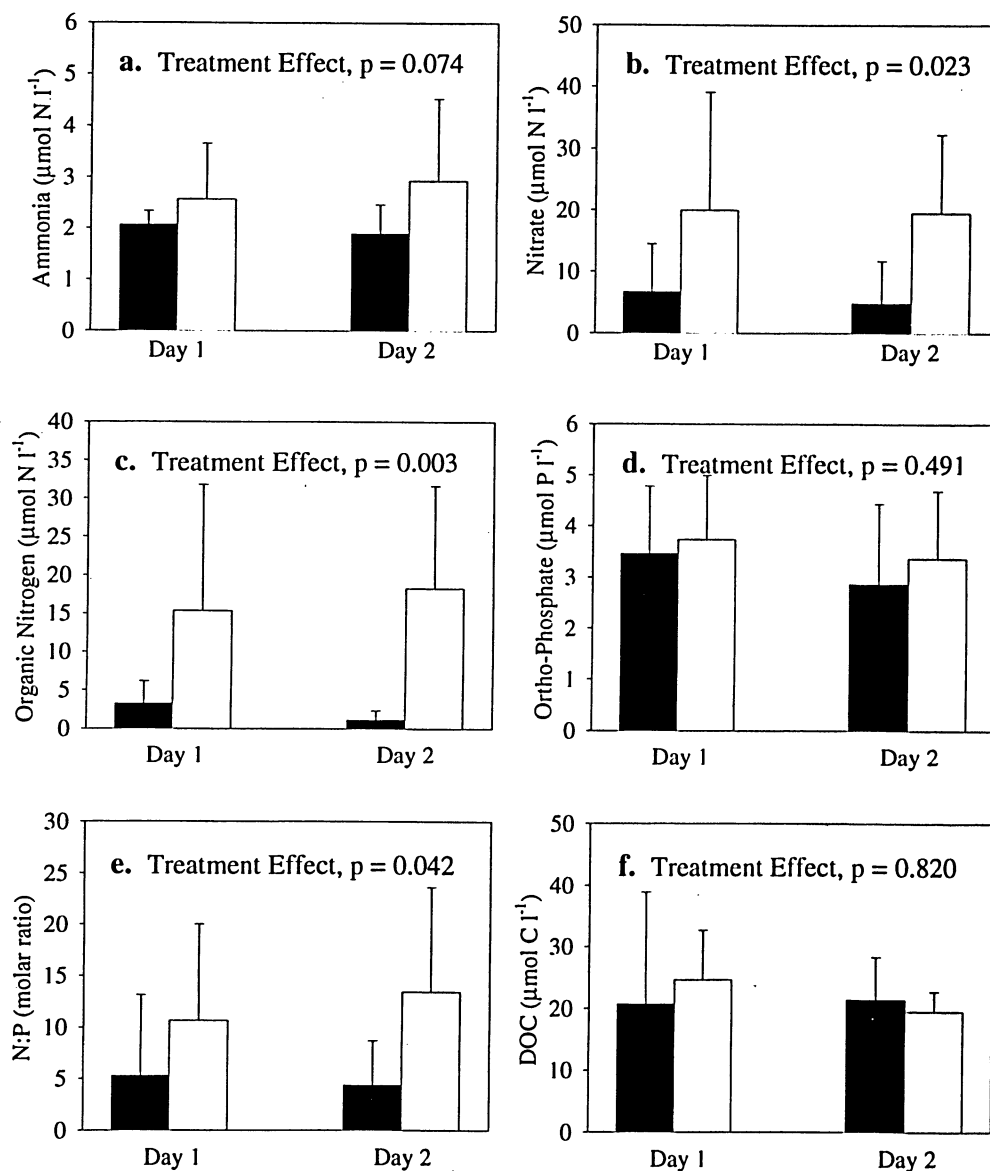
Figure 2. Water column nutrient concentrations. All values mean ± standard deviation. Black bars represent microcosms with zebra mussels, white bars represent microcosms without live mussels.

The presence of zebra mussels resulted in significant changes to the composition of photosynthetic microorganisms. *Pseudokirchneriella* and *Microcystis* biovolumes were similar in inflowing reservoir water and in microcosms without zebra mussels, indicating that cell settling did not significantly alter planktonic abundance. However, in microcosms with zebra mussels, *Pseudokirchneriella* biovolume was greatly reduced, presumably through filtering, while *Microcystis* biovolume increased (ANCOVA, $p < 0.001$ for each organism; Figure 3a and b). Consequently, *Microcystis* : *Pseudokirchneriella* was much higher in microcosms with zebra mussels relative to reference microcosms (ANCOVA, $p < 0.001$; Figure 3c).
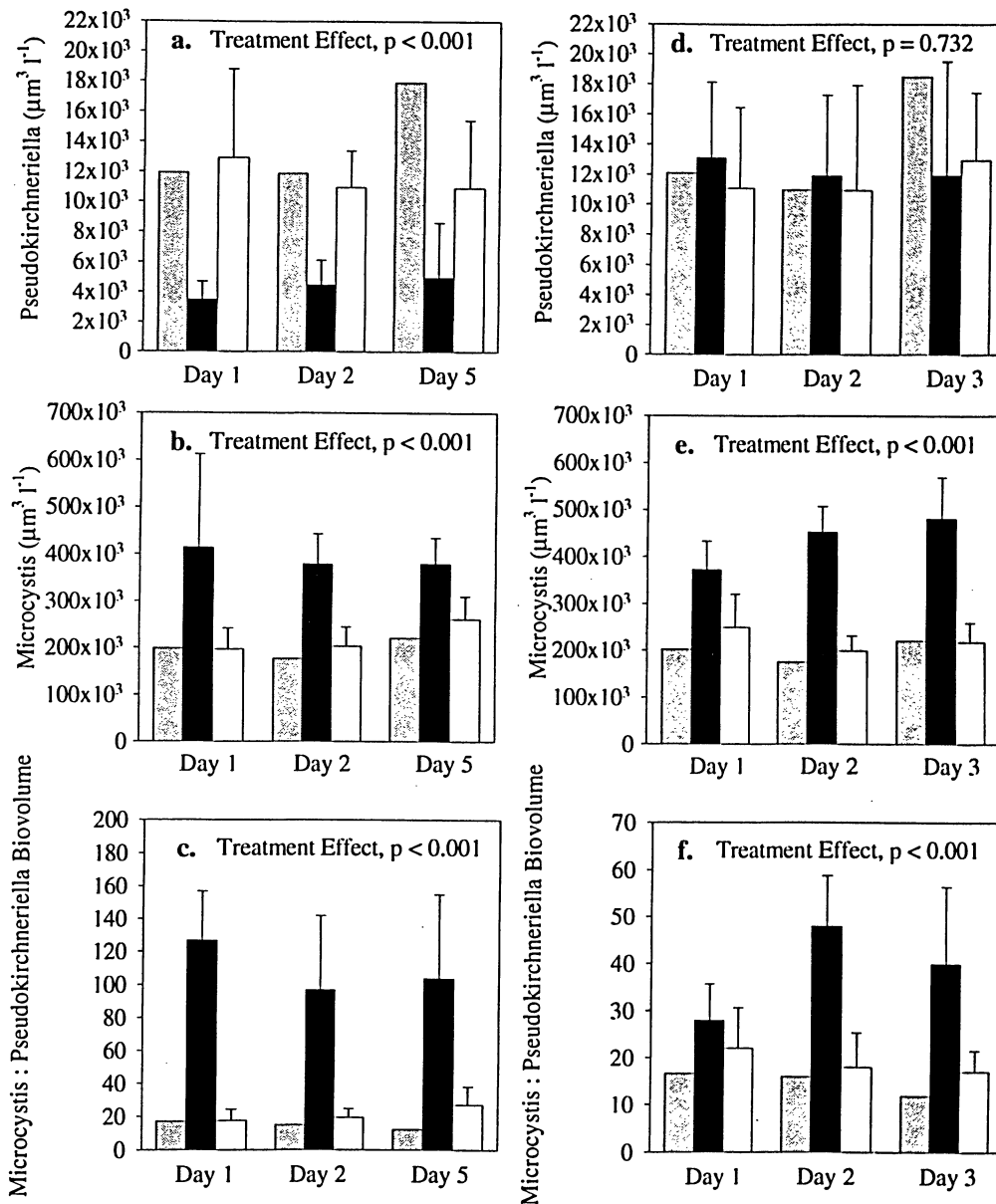
Figure 3. Photosynthetic microbial biovolumes. All values mean ± standard deviation. Panels a – c: grey bars represent initial values (reservoir), black bars represent microcosms with zebra mussels, white bars represent microcosms without live mussels. Panels d – f: grey bars represent initial phytoplankton abundance values (reservoir), black bars represent microcosms with low N:P (6:1), and white bars represent microcosms with high N:P (12:1).

B. Effects of nutrients on photosynthetic microorganisms

Manipulation of N:P had no effect on *Pseudokirchneriella* biovolume, and biovolume was similar in the reservoir and all microcosms, suggesting that cell settling did not reduce abundance of *Pseudokirchneriella* in plankton (ANCOVA, $p = 0.732$; Figure 3d). *Microcystis* biovolume, however, responded positively to low N:P (ANCOVA, $p < 0.001$; Figure 3e). Therefore, a decrease in N:P resulted in higher *Microcystis : Pseudokirchneriella* biovolume in the plankton (ANCOVA, $p < 0.001$; Figure 3f). Nutrient concentrations, in the reservoir and in the water columns after 24 hours, were relatively high and phytoplankton growth should not have been limited in any of the microcosms. However, cyanobacterial growth may respond more strongly to the relative quantities, not the absolute quantities, of nutrients in the water (McQueen and Lean 1987; Levich *et. al.* 1992). The proliferation of *Microcystis* in columns with N:P of 6 is consistent with other studies finding optimal growth of *Microcystis* at low N:P (2 – 10) (Rhee and Gotham 1980; Bulgakov and Levich 1999), or which observed natural blooms of *Microcystis* in water when N:P was less than 10 (Schindler 1977; Smith 1983; Levich *et al.* 1992; Levich 2000).

C: Nutrient flux

In experiments to determine the effects of zebra mussels on nutrient fluxes, nitrate consumption by sediments was significantly higher in microcosms with zebra mussels than in microcosms lacking mussels (ANCOVA, $p = 0.019$; Figure 4a). Microcosms with mussels were consistently a sink for nitrates, whereas microcosms without zebra mussels were, on average, a net source of nitrate. Ammonia flux was generally much

lower than nitrate flux, and the direction of net flux was variable. Although ammonia flux into sediments was high on Day 28 in microcosms with zebra mussels, there was no overall effect of mussels on flux (ANCOVA, p = 0.289; Figure 4b). Sediments were consistently sinks for ortho-phosphate. Flux into sediments was greater in microcosms lacking zebra mussels than in microcosms with mussels (ANCOVA, p = 0.047; Figure 4c). The ratio of dissolved inorganic nitrogen to dissolved inorganic phosphorus (DIN and DIP, respectively) flux into sediments was greater in microcosms with zebra mussels than in those microcosms without mussels (ANCOVA, p = 0.029; Figure 4d). On two of the three sampling dates, DIN:DIP flux in zebra mussel microcosms exceeded the Redfield ratio, indicating these systems were becoming depleted in nitrogen relative to biological demand. In microcosms without zebra mussels, DIN:DIP did not differ from 1, indicating that neither nutrient was becoming less abundant relative to biological demand. Due to the acceleration of sedimentation and denitrification processes, the sediments with zebra mussels were consuming the nutrients at a ratio which was higher than a Redfield ratio (16:1), decreasing the N:P in the water. Nitrogen became a limiting factor and could cause an increase in *Microcystis* biovolume and *Microcystis* dominance.
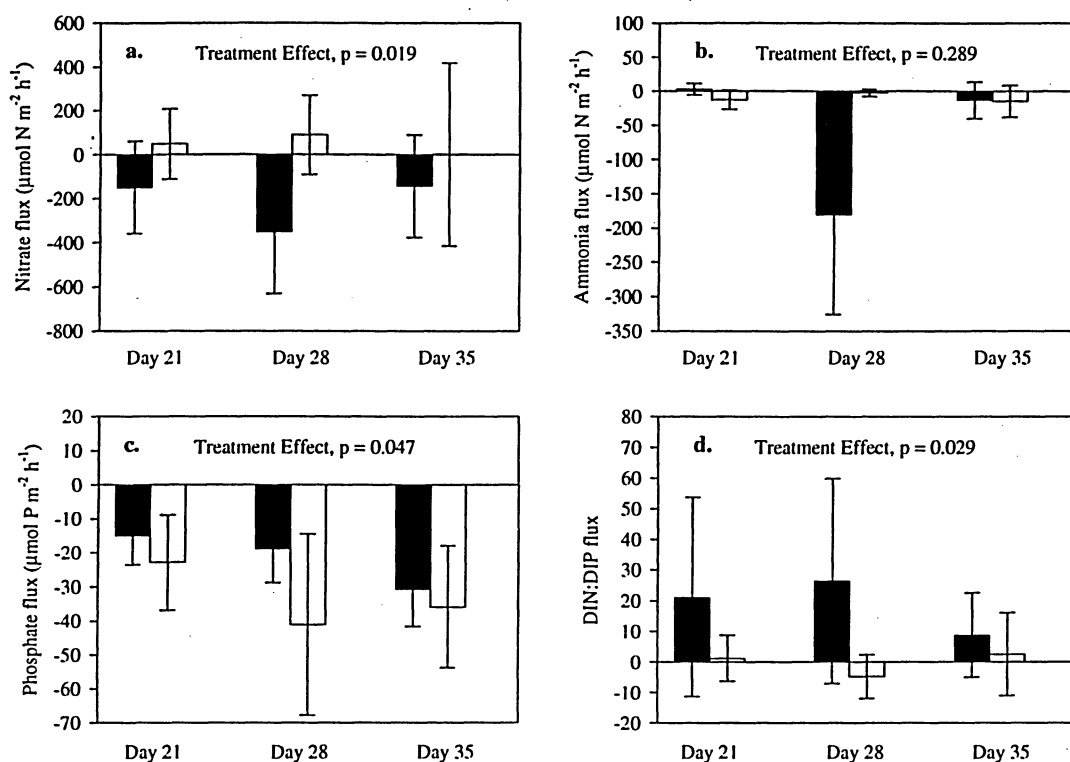
Figure 4. Nutrient fluxes. All values mean ± standard deviation, negative values indicate flux into sediments. Black bars represent microcosms with zebra mussels, white bars represent microcosms without live mussels.

Over all three days, the mean difference in nitrate flux into sediments between reference microcosms and microcosms with zebra mussels was ~ 310 μmol N m^{-2} h^{-1}. This flux was likely a combination of biological uptake and denitrification. As DIN:DIP uptake exceeded the Redfield ratio, a significant portion of this nitrate was likely denitrified, consistent with findings of enhanced denitrification in sediments below zebra mussel beds (Bruesewitz *et al.* 2004). The mean difference in flux would account for a net removal of ~ 3.7 μmol N l^{-1} over a 24-hour period assuming a well-mixed, 2 m water column. Enhanced nitrate flux into sediments (due to enhanced denitrification or

biological uptake) likely contributed to the decrease in nitrate concentration in the water columns observed in the previously described experiment. The shift in N:P in the water column resulting from nutrient flux into sediment in excess of Redfield (and possibly excretion of N:P at ratios lower than Redfield (Arnott and Vanni, 1996) would have created conditions conducive to *Microcystis* growth (low N:P), due to the appearance of nitrogen limiting conditions that favour cyanobacterial dominace.

D. Selective filtration

First experiment on selective filtration

In beakers without live zebra mussels, *Pseudokirchneriella* and *Microcystis* biovolumes in the water column did decrease over 24 hours (Figure 5a, 5b), indicating that cells did settle to the bottom. However, in beakers containing live mussels, both *Pseudokirchneriella* and *Microcystis* biovolumes were greatly reduced after 24 hours (Figure 5a, 5b), and were significantly lower than in reference beakers (ANOVA, $p < 0.001$, for both *Pseudokirchneriella* and *Microcystis*) indicating that zebra mussels were actively removing cells from the water column. However, *Microcystis* : *Pseudokirchneriella* biovolumes in the water column did not change over 24 hours in either beakers with mussels, or those with no live mussels and there was no difference between treatments (Figure 5c). Furthermore, the ratio of *Microcystis* to *Pseudokirchneriella* biovolume in pseudofaeces was not different than the ratio in the water column (ANOVA, $p = 0.919$) for either treatment, suggesting that zebra mussels

were not selectively rejecting either type of microorganism (Figure 5c).
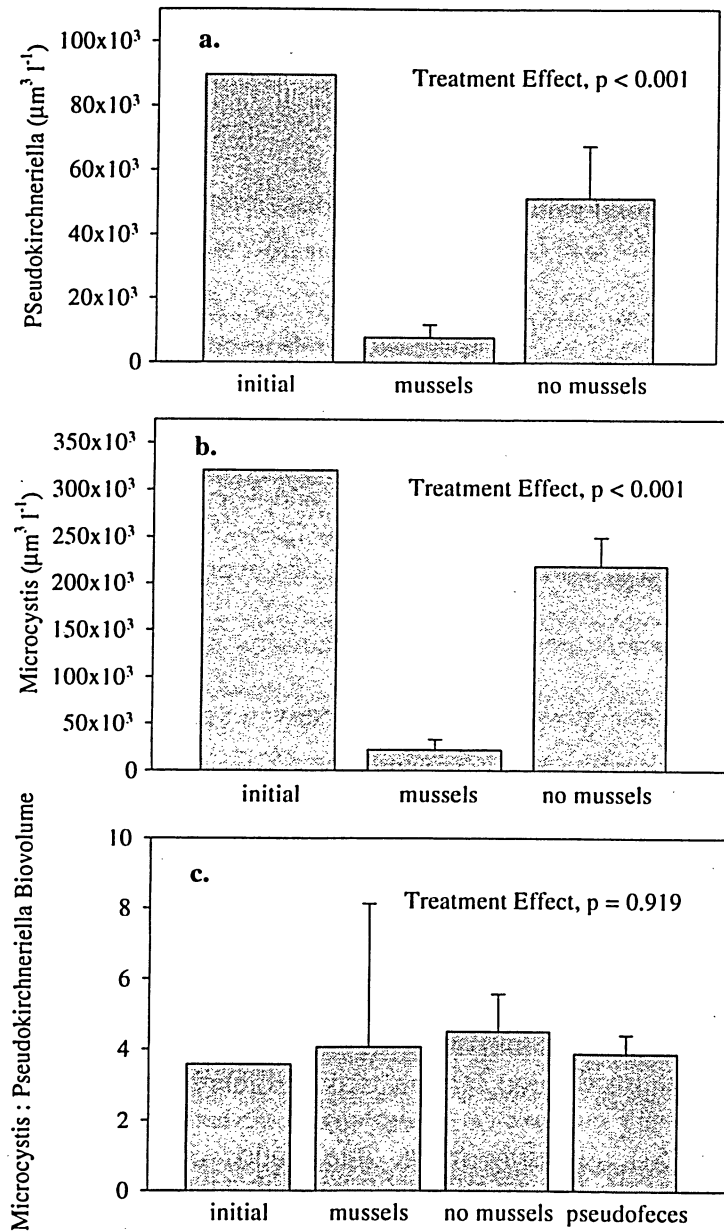
Figure 5. Photosynthetic microbial biovolumes. All values mean ± standard deviation. Panels a & b: water column biovolumes for *Pseudokirchneriella subcapitata* and *Microcystis*. Panel c: *Microcystis* : *Pseudokirchneriella subcapitata* biovolumes in water columns with or without zebra mussels, and in pseudofaeces of mussels.

Second experiment on selective filtration

This experiment showed an increase in *Pseudokirchneriella* biovolume over time in both treatments; nevertheless, this increase was greater in the tanks with zebra mussels than in those without mussels (p = 0.019; Figure 6a). Moreover, *Microcystis* biovolume increased in tanks without zebra mussels and the difference over time in *Microcystis* biovolume was significant (p = 0.003; Figure 6b). In addition, *Microcystis* : *Pseudokirchneriella* biovolume was consequently greater in tanks without zebra mussels (p = 0.010; Figure 6c), which is in contrast to the beaker experiment (first experiment on selective filtration). Unlike the beaker experiment, a decrease in abundance of either microorganism was not observed. Zebra mussels, therefore, did not appear to exert strong grazing pressure on either phytoplankter.

Preferential feeding by zebra mussels is likely to play a key role in some phytoplankton community shifts. The increased abundance of *Microcystis* in Saginaw Bay following zebra mussel establishment may have been the result of selective rejection of *Microcystis* (Vanderploeg *et al.* 2001; Bierman *et al.* 2005). However, the increase in *Microcystis* abundance in the current experiments cannot be explained by preferential feeding on *Pseudokirchneriella*. Rather, *Microcystis* growth responded to favourable changes in elemental ratios (low N:P) resulting from nitrate flux into sediments, presumably related to enhanced denitrification in microcosms with zebra mussels. Changes in water chemistry resulting from intense filter feeding of zebra mussels, and processing of deposited organic matter (faeces and pseudofaeces) is likely to affect nutrient concentrations in lake water, and resulting shifts in elemental ratios may be

another important factor in determining cyanobacterial abundance in lakes following zebra mussel establishment.
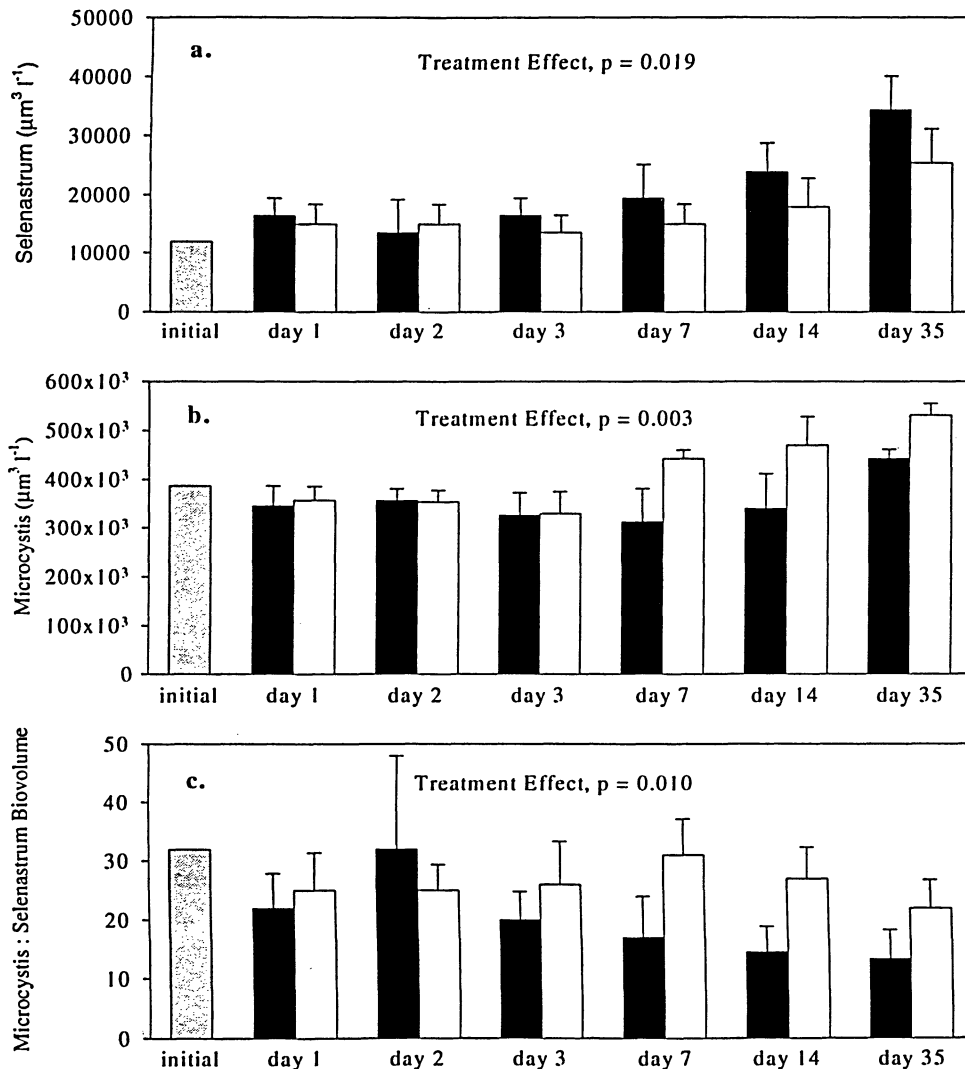
Figure 6. Photosynthetic microbial biovolumes. All values mean ± standard deviation. Panels a & b: water column biovolumes for *Pseudokirchneriella subcapitata* and *Microcystis*. Panel c, *Microcystis* : *Pseudokirchneriella subcapitata* biovolumes in water columns with or without zebra mussels. Grey bars represent initial values (reservoir), black bars represent tanks with zebra mussels, and white bars represent tanks without zebra mussels.

E. Effects of zebra mussels on the water chemistry of Wisconsin lakes

Analysis of data from Wisconsin lakes prior to and following zebra mussel invasions showed a marginally significant decrease in Secchi depth (Appendix 1) following invasion ($p < 0.10$; Figure 7). The effect of zebra mussels on Secchi depth was strongest in spring. Data analysis also suggested an increase in pH over all seasons following invasion by zebra mussels ($p < 0.05$; Figure 8). The effects of zebra mussels on pH were most notable in autumn when pH of invaded lakes was greatest (mean ~8.7). Elevated pH, in combination with decreased Secchi depth, would suggest that primary production in these lakes was greater following the invasion by zebra mussels. However, there was no apparent relationship between invasion of lakes by zebra mussels and chlorophyll a, as chlorophyll a did not increase following the invasion of zebra mussels ($p = 0.766$; Figure 9). This apparent disconnect may be the result of little temporal overlap in collection samples for pH, Secchi depth, and chlorophyll a as not all types of samples were collected on the same dates.

The observed decrease in water clarity in these Wisconsin lakes is surprising, given a number of other studies that discussed zebra mussels as species that could significantly improve water clarity (Horgan and Mills 1999; Aldridge *et al.* 2004). Idrisi *et al.* (2001) found that grazing of phytoplankton by zebra mussels increased water clarity in Oneida Lake, New York resulting in a significant increase in Secchi depth for all seasons following zebra mussel invasion. Elevated pH is commonly associated with

Figure 7. Secchi Depth in Wisconsin lakes prior to and following zebra mussel invasions; y axis represents individual lakes, x axis - difference between the average water chemistry parameter values for pre-invasion and post invasion period for each lake.

Figure 8. pH in Wisconsin lakes prior to and following zebra mussel invasions; y axis represents individual lakes, x axis - difference between the average water chemistry parameter values for pre-invasion and post invasion period for each lake.
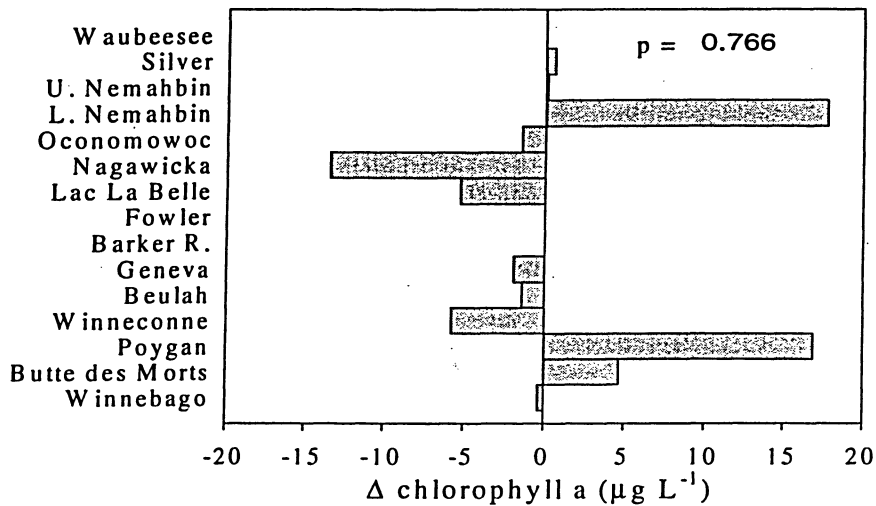
Figure 9. Chlorophyll a in Wisconsin lakes prior to and following zebra mussel invasions; y axis represents individual lakes, x axis - difference between the average water chemistry parameter values for pre-invasion and post invasion period for each lake.

high rates of photosynthesis. Xie *et al.* (2003), for example, found high pH (9-10) to co-occur with *Microcystis* blooms. Secchi depth and pH data from the Wisconsin lakes suggest that primary production and, perhaps, phytoplankton biomass may have increased following invasion.

A possible implication of higher pH following invasion by zebra mussels is formation of conditions conducive to cyanobacterial dominance. This statement could be supported by the study conducted by Korneva (1996) who studied the response of the phytoplankton community to pH, and demonstrated a strong positive correlation between the cyanobacterial abundance and pH. Moreover, Fontes *et al.* (1987) noted that

Anabaena variabilis (a nitrogen-fixing cyanobacterium) had optimal productivity at pH 8.2 -8.4, while at 7.4 -7.8 its productivity was slightly lower.

Based on microcosm experiments, zebra mussels were expected to cause a decrease in N:P in the Wisconsin lakes due to increased export of organic N to sediment and subsequent denitrification, and excretion of N and P at a ratio less than Redfield. The data showed a marginally significant decrease in inorganic phosphorus ($p < 0.10$; Figure 10) and an increase in the percentage of organic phosphorus ($p < 0.01$; Figure 10) following invasion by zebra mussels. Organic nitrogen did decline marginally following zebra mussel invasions ($p < 0.10$; Figure 11). However, there was no effect of zebra mussels on nitrate ($p = 0.826$; Figure 11) or total inorganic nitrogen ($p = 0.131$; Figure 11). Consequently N:P in these lakes did not decrease following zebra mussel invasions as predicted ($p = 0.189$; Figure 12). This apparent lack of response in nitrogen pools and N:P could be related to limited availability of nutrient data for these lakes. Non-point sources of nitrogen (and phosphorus) could also mask any changes in the nutrient concentrations caused by zebra mussel activities.
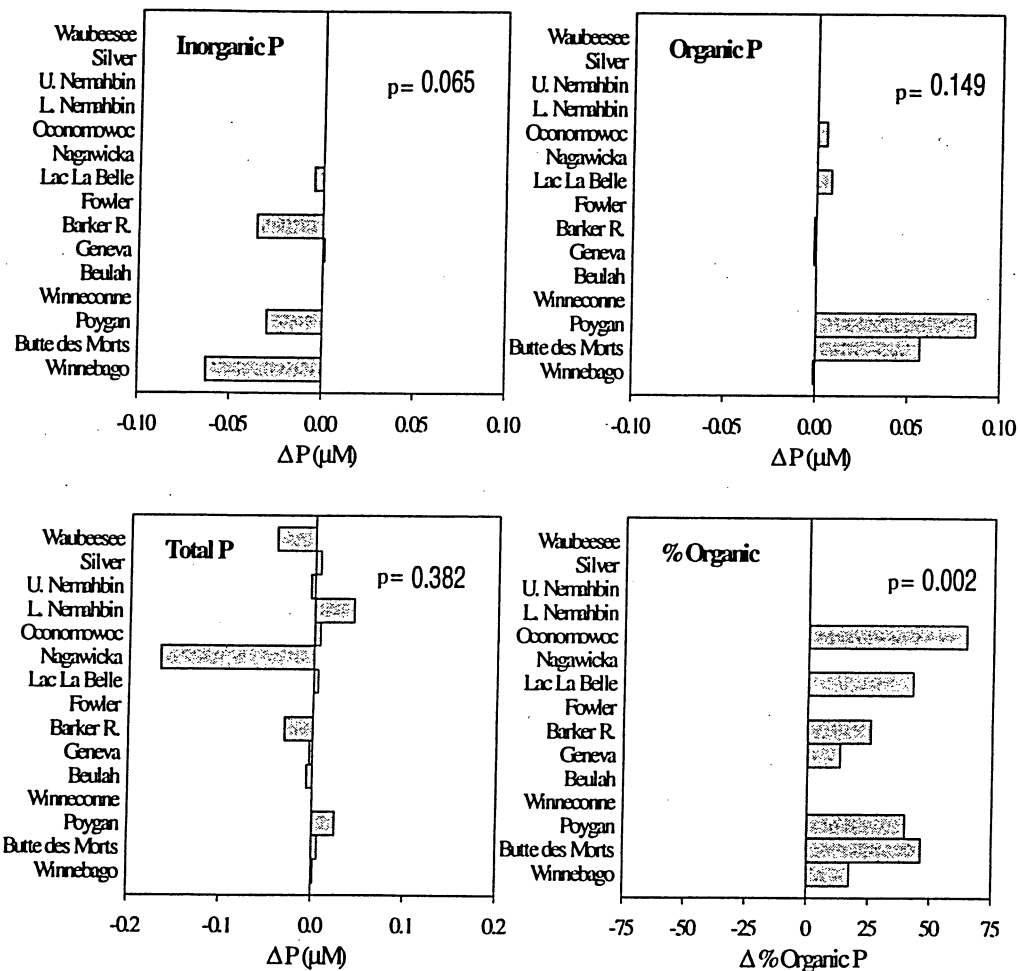
Figure 10. Phosphorus concentrations in Wisconsin prior to and following zebra mussel invasions; y axis represents individual lakes, x axis - difference between the average water chemistry parameter values for pre-invasion and post invasion period for each lake.
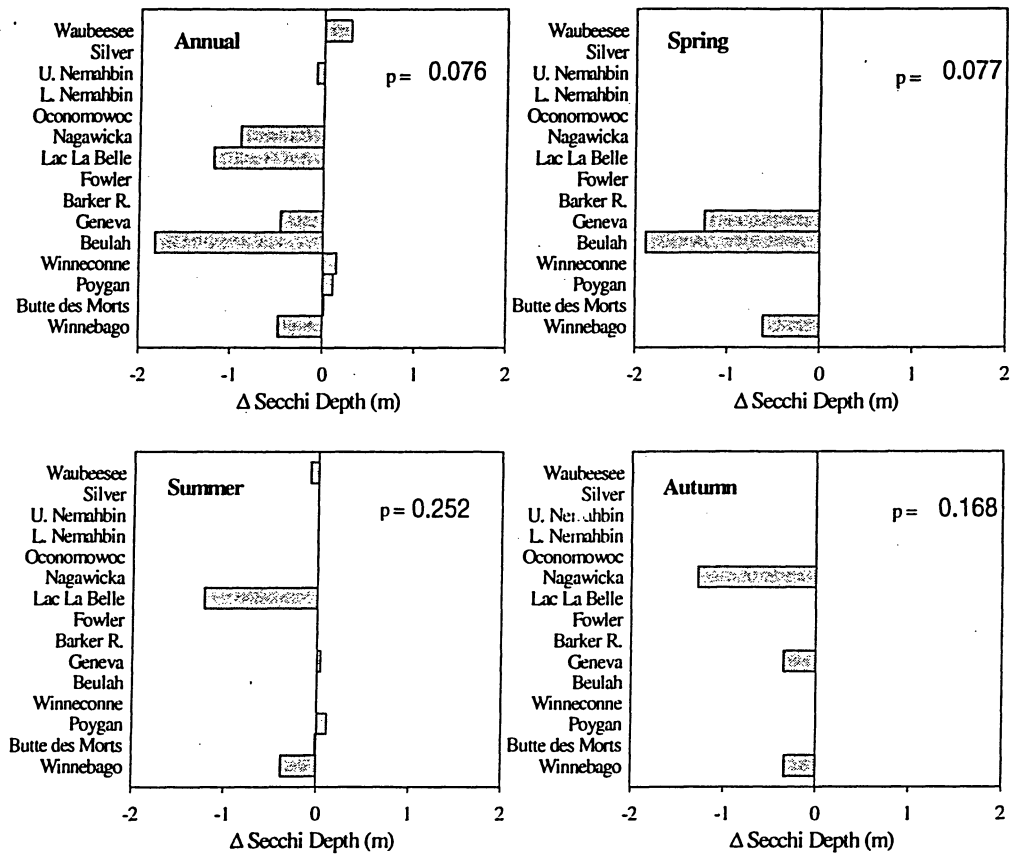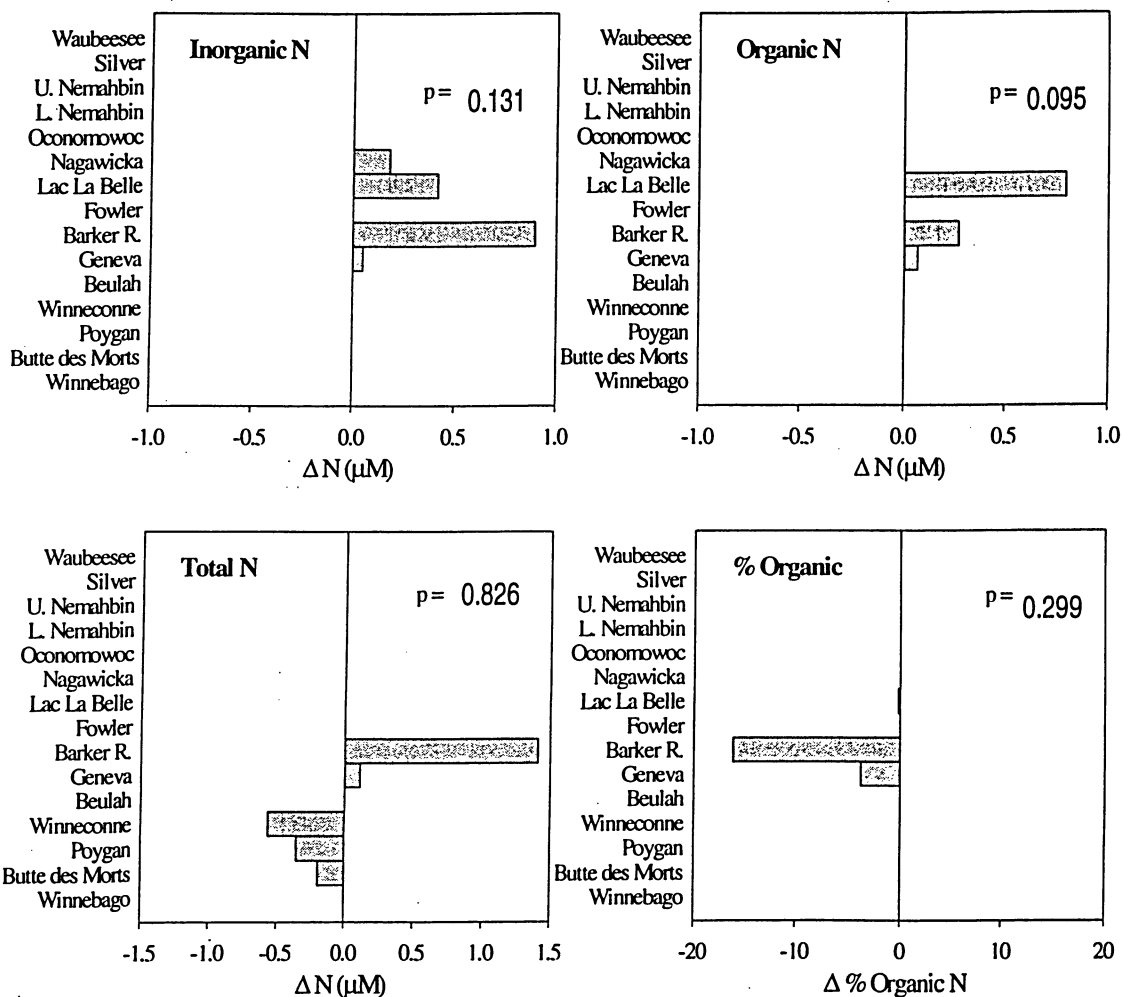
Figure 11. Nitrogen concentrations in Wisconsin lakes prior to and following zebra mussel invasions; y axis represents individual lakes, x axis - difference between the average water chemistry parameter values for pre-invasion and post invasion period for each lake.
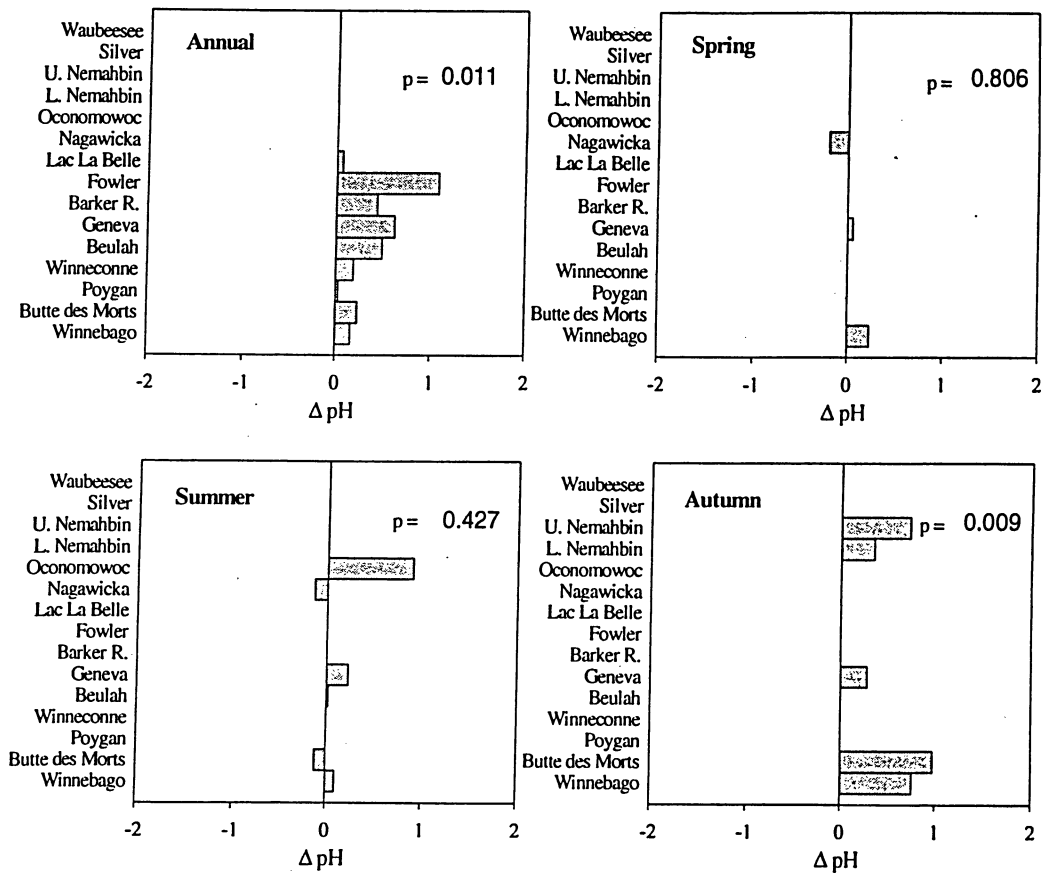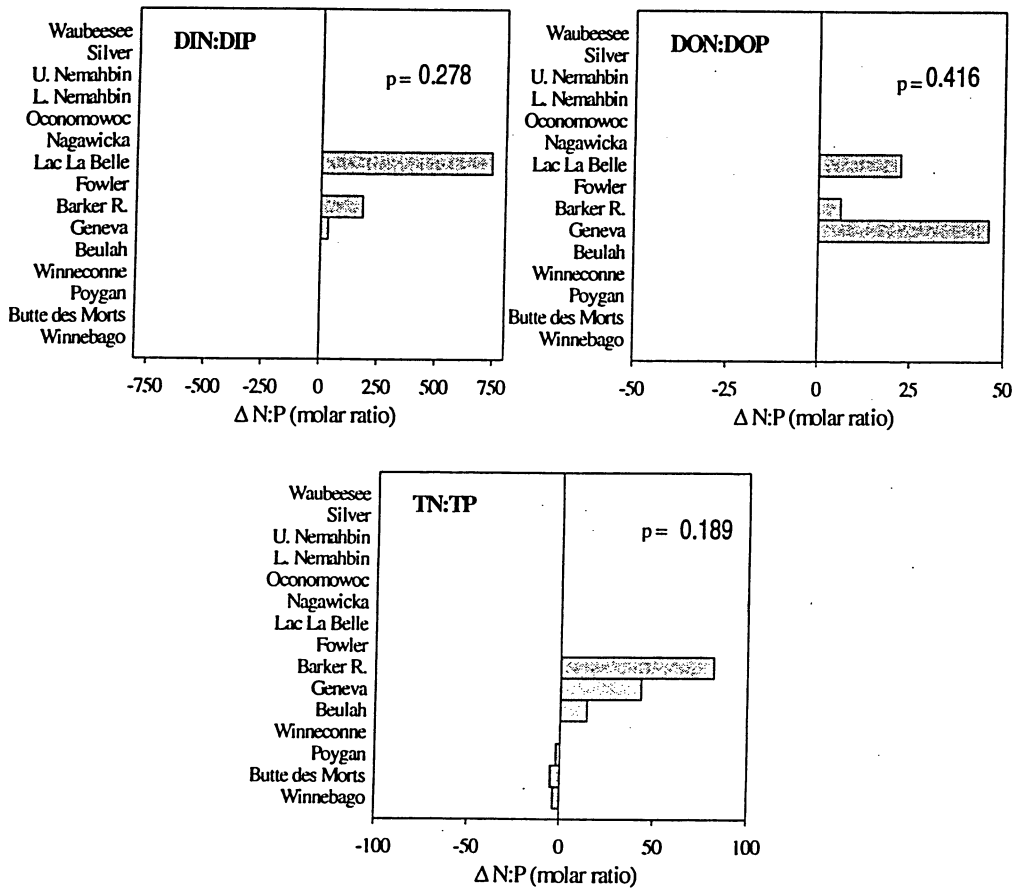
Figure 12. N:P in Wisconsin lakes prior to and following zebra mussel invasions; y axis represents individual lakes, x axis - difference between the average water chemistry parameter values for pre-invasion and post invasion period for each lake.

CONCLUSIONS

In summary, experiments have shown that zebra mussels are affecting nitrogen flux into sediment resulting in a decrease in N:P, which corresponded with the increase in *Microcystis* biovolume, and *Microcystis* dominance. Moreover, selective feeding did not appear to cause the observed shift toward *Microcystis* dominance. While other studies cited in this thesis have explored effects of filter-feeding mussels on nutrients and still other studies have explored nutrient ratio effects on phytoplankton composition, this is the only research the author is aware of that experimentally demonstrates the link between changes in water chemistry (i.e. nutrient ratios) associated with zebra mussels and the increase in cyanobacterial dominance of the experimental system.

The analysis of data from Wisconsin lakes prior to and following zebra mussel invasions suggests that primary production and, perhaps, phytoplankton biomass may have increased following invasions. However, N:P did not decrease following invasions by zebra mussels, and these mussels may not alter water chemistry in a way that would favour dominance by cyanobacteria in these lakes, as was predicted from laboratory experiments. This could be due to the limited availability of nutrient data for Wisconsin lakes, and it does not mean that we will not see the shift in N:P in other lakes. Moreover, one of the main characteristics of the lake that might determine the likelihood of shifts in N:P following zebra mussel invasion is the absence of non-point sources of N and P. In the case with Wisconsin lakes, the presence of these non-point sources in the lakes with available nutrient data could also explain the lack of predicted shift in N:P.

Finally, governments are spending thousands of dollars on the phosphate reduction programmes in order to reduce the cyanobacterial dominance in the freshwater

systems and to prevent human and animal health problems that are associated with the production of cyanobacterial toxins. However, not enough attention is paid to the investigation of direct and indirect effects of zebra mussels and their role in reappearance of cyanobacterial blooms in North America. This study highlighted the importance of the examination of zebra mussel effects on water chemistry, thereby affecting *Microcystis* dominance. The deficiency of water chemistry data from North American lakes with established histories of zebra mussel invasions make it difficult to assess changes in water chemistry parameters that are associated with zebra mussel activities and may lead the improper management of cyanobacterial blooms.

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Appendix 1. Redfeild Ratio and Secchi depth.

Redfield Ratio. The Redfield ratio (Redfield et al., 1963) is a molar ratio, and refers to the stoichiometry of carbon (C), nitrogen (N) and phosphorus (P) in unicellular algae, such as phytoplankton and benthic microalgae. This ratio provides a measure of nutritional status of phytoplankton. It was defined that in conditions when nutrients are not limiting, most phytoplankton has the following ratio of elements:

C:N:P = 106: 16: 1.

Deviations from N:P of 16 in water are commonly taken as indicators of relative N or P limitation.

Secchi Depth. The transparency of the water which depends on absorption due to dissolved substances and scattering by suspended particles could be measured using the Secchi disk. The Secchi disk is an 8-inch disk divided into black and white quadrants that is lowered into the water of a lake until the observer can no longer see it. The depth of disappearance of the disk is called the Secchi Depth.

Appendix 2. Wisconsin lakes invaded by zebra mussels (from the Wisconsin University Sea Grant Program website (http://www.seagrant.wisc.edu))

#	Name of the water body	County	Date of invasion
1.	Silver Lake	Kenosha	1994
2.	Geneva Lake	Walworth	1995
3.	Nagawicka Lake	Waukesha	1998
4.	Upper Nemahbin Lake	Waukesha	1998
5.	Winnebago Lake	Winnebago	1999
6.	Butte des Morts Lake	Winnebago	1999
7.	Winneconne Lake	Winnebago	1999
8.	Bark River	Waukesha	1999
9.	Lac La Belle	Waukesha	1999
10.	Oconomowoc Lake	Waukesha	1999
11.	Lower Nemahbin Lake	Waukesha	1999
12.	Waubeesee Lake	Racine	1999
13.	Poygan Lake	Winnebago	2000
14.	Beulah Lake	Walworth	2000
15.	Fowler Lake	Waukesha	2002

Appendix 3. Lake water chemistry (Wisconsin Lakes) (from the United States

Environmental Protection Agency's STORET Data Warehouse (www.epa.gov/storet))

pH	Pre-invasion (mean)	Post-invasion (mean)
Winnebago Lake	8.465	8.6286
Butte des Morts	8.346	8.58
Beulah Lake	8.3778	8.41
Geneva Lake	8.3481	8.538
Lac La Belle	7.895	8.39
Nagawicka Lake	7.8067	8.43
Oconomowoc Lake	8.0179	8.455
Upper Nemahbin Lake	7.7938	8.875
Lower Nemahbin Lake	7.8142	7.8795
Alkalinity (carbonate as CaCO3 (mg/L))		
Winnebago Lake	157.052	161.375
Poygan Lake	142.17	165
Beulah Lake	219	198
Geneva Lake	207	185.25
Lac La Belle	178.83	209
Nagawicka Lake	241.82	219
Oconomowoc Lake	195.5	215
Upper Nemahbin Lake	216.5	245
Lower Nemahbin Lake	232.5	198
Waubeesee Lake	176	147
Calcium (mg/L)		
Winnebago Lake	36.893	42
Butte des Morts	32	35.333
Poygan Lake	26	34.5
Geneva Lake	42.5	34.333
Lac La Belle	48.5	52
Upper Nemahbin Lake	53.75	43
Lower Nemahbin Lake	43	35.1
Chlorophyll a (ug/L)		
Winnebago Lake	36.176	35.8261
Butte des Morts	34.591	39.28

Poygan Lake	20.607	37.5
Winnebago Lake	38.79	33
Beulah Lake	3.949	2.58
Geneva Lake	4.8939	2.994
Lac La Belle	7.4689	2.2
Nagawicka Lake	16.383	2.9429
Oconomowoc Lake	3.067	1.6627
Upper Nemahbin Lake	2.8237	2.96
Waubeesee Lake	3.8	3.2
Lower Nemahbin Lake	4.242	22
TN (mg/L)		
Winnebago Lake	1.2747	1.2681
Butte des Morts	1.6701	1.4738
Poygan Lake	1.7858	1.432
Winnebago Lake	1.915	1.3503
Geneva Lake	0.4595	0.5719
Bark River	1.2003	2.6072
IN (mg/L)		
Geneva Lake	0.0672	0.1159
Bark River	0.5245	1.42
Lac La Belle	0.1908	0.606
Nagawicka Lake	0.8328	1.0122
ON (mg/L)		
Geneva Lake	0.395	0.4619
Bark River	0.6759	0.946
% ON		
Geneva Lake	85.4	81.6
Bark River	57.1	41
Lac La Belle	52.5	52.3
TP (mg/L)		
Winnebago Lake	0.08624	0.087869
Butte des Morts	0.071	0.0771
Poygan Lake	0.08	0.1045
Beulah Lake	0.0208	0.0141
Geneva Lake	0.018	0.0138
Bark River	0.089	0.058
Lac La Belle	0.0133	0.0179
Nagawicka Lake	0.184	0.01784
Oconomowoc Lake	0.0165	0.0229
Waubeesee Lake	0.0561	0.01367

Silver Lake	0.0201	0.0263
Lower Nemahbin Lake	0.0117	0.055
Upper Nemahbin Lake	0.0186	0.01417
IP (mg/L)		
Winnebago Lake	0.027299	0.008615385
Poygan Lake	0.0327	0.0025
Geneva Lake	0.005	0.006
Bark River	0.054	0.0182
Lac La Belle	0.0073	0.002
OP (mg/L)		
Winnebago Lake	0.05973	0.0597
Butte des Morts	0.0394	0.096
Poygan Lake	0.0479	0.135
Geneva Lake	0.0101	0.0088
Bark River	0.041	0.0398
Lac La Belle	0.006	0.014
Oconomowoc Lake	0.0067	0.012
% OP		
Winnebago Lake	69.4	86.8
Butte des Morts	53.7	100
Poygan Lake	58.4	98.1
Geneva Lake	62.7	76
Bark River	43.4	69.1
Lac La Belle	44.9	87.5
Oconomowoc Lake	36	100
TN:TP		
Winnebago Lake	48.9	45.4
Butte des Morts	46.7	41.7
Poygan Lake	33.6	31.6
Beulah Lake	60.9	75.5
Geneva Lake	80.2	123.6
Bark River	35.4	117.2
IN:IP		
Geneva Lake	31.7	64.4
Bark River	31.5	213.4
Lac La Belle	59.7	800.5
ON:OP		
Geneva Lake	106.9	152.9
Bark River	50.1	56.2

Secchi Depth (m)		
Winnebago Lake	3.00083	1.426
Butte des Morts	1.7786	1.844
Poygan Lake	1.533	1.9
Winneconne Lake	1.3	1.8
Beulah Lake	11.292	5.3
Geneva Lake	13.557	12.044
Lac La Belle	6.7	2.8
Nagawicka Lake	6.325	3.375
Waubeesee Lake	2.7714	2.5
Upper Nemahbin Lake	4.3091	5.3

Appendix 4. Mean ammonia concentrations in microcosms with and without zebra mussels.

	Day 1	Day 2	Day 3
Columns with zebra mussels	2.1	1.9	35.8
Columns without zebra mussels	2.6	2.9	2.1
Reservoir	11.6	17.1	15.0