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thesis entitled

UNDERSTANDING ZEBRA MUSSEL (*DREISSENA POLYMORPHA*) IMPACTS ON AUTOTROPHIC AND HETEROTROPHIC PLANKTON OF INLAND LAKE ECOSYSTEMS

presented by

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has been accepted towards fulfillment
of the requirements for the

M.S. degree in FISHERIES AND WILDLIFE

Major Professor's Signature

May 7, 2004

Date

UNDERSTANDING ZEBRA MUSSEL *(DREISSENA POLYMORPHA)* IMPACTS ON AUTOTROPHIC AND HETEROTROPHIC PLANKTON OF INLAND LAKE ECOSYSTEMS

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

2004

ABSTRACT

UNDERSTANDING ZEBRA MUSSEL IMPACTS ON AUTOTROPHIC AND HETEROTROPHIC PLANKTON OF INLAND LAKE ECOSYSTEMS

By

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Zebra mussels (*Dreissena polymorpha*) invaded the Great Lakes of N. America in the mid-1980's, and have rapidly spread into inland lakes, particularly in Michigan. Most research on the impact of *Dreissena* invasion has been conducted in well-mixed aquatic ecosystems, while little is known about such impacts in thermally-stratified inland lakes. *Dreissena* invasion has recently been associated with an increase in the toxic cyanobacterium (bluegreen), *Microcystis aeruginosa*, in low-nutrient lakes but here again, there is little compelling survey data from inland lakes indicating significantly elevated *M. aeruginosa* biomass in invaded habitats. In addition, no studies have examined whether bluegreen toxins are elevated in *Dreissena*-invaded lakes.

To address these needs, I conducted a large-scale survey of inland lakes in Michigan that contain or lack *D. polymorpha*. The surveyed lakes were otherwise similar in nutrients, morphometry, and location. *Microcystis aeruginosa* biomass was 3.6 times higher and total particulate toxin concentration was 3.3 times higher in invaded lakes. Ciliate biomass was 45% lower and rotifer biomass was 44% lower in invaded lakes. Rotifer richness and diversity were also lower in lakes with *D. polymorpha*. In addition, a shift in the size distribution of ciliates was found between lake categories indicating that zebra mussels may be reducing larger, algivorous ciliates in favor of smaller bacterivorous ciliates. In general, zebra mussel impacts on microzooplankton biomass in the stratified lakes I sampled were weaker than those reported from well-mixed systems.

ACKNOWLEDGEMENTS

I thank my advisor Dr. Orlando Sarnelle and my committee members Dr. Patricia Soranno and Dr. Stephen Hamilton for their assistance during all stages of my research. Special thanks to my graduate student collaborator, Carrie Scheele, for all of her help. Thanks to Joan Rose and Mechelle Woodall for ELISA analysis. Thanks to Alan Tessier and Spencer Hall for their bathymetric map of Warner Lake. Thanks to all of those of helped in the field and laboratory: Chelsea Stephen, Kendra Cheruvelil, Brian Elkington, Katherine Gurin, Scott Kissman, Konrad Kulacki, Drew Kramer, Sherry Martin, Andrea Stoddard, Alan Wilson, and Craig Wynne. Financial support was provided by: Michigan Sea Grant, MSU Research Enhancement Award, MSU Ecology, Evolutionary Biology and Behavior Research Award, Sigma Xi Grant-in-aid of Research, MSU Minority and Women Graduate Assistantship Fellowship, and George H. Lauff Research Award.

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Zebra mussel invasion is associated with increased *Microcystis* biomass and toxin concentrations in low-nutrient lakes

ABSTRACT

Bluegreens typically comprise a minor portion of the phytoplankton community in low-nutrient lakes. However, previous studies have shown a positive effect of exotic zebra mussels (*Dreissena polymorpha*) on the relative dominance (as percentage of total phytoplankton biomass) of *Microcystis aeruginosa*, a bloom-forming, toxin-producing bluegreen, in low-nutrient lakes (total phosphorus 10 - 25 $\mu g\ L^{-1}$). I sampled thirty-nine low-nutrient lakes (average TP ~ 10 $\mu g\ L^{-1}$), 20 with *D. polymorpha* and 19 without, that were otherwise similar in nutrients, morphometry, and location. I report a positive influence of *D. polymorpha* on the biomass of *M. aeruginosa* and on the concentration of microcystin, a toxin produced by *M. aeruginosa* and a few other bluegreen taxa. An increase in *M. aeruginosa* and the toxins produced by it are unexpected and undesirable, particularly in low-nutrient lakes. *Microcystis aeruginosa* colony sizes were similar invaded and uninvaded lakes. Soluble reactive phosphorus concentrations were similar between lake categories, but NH_4^+-N was 1.8 times higher in invaded lakes. Chlorophyll *a* and phytoplankton dry mass were 30 % lower in invaded lakes. Other phytoplankton species influenced by *D. polymorpha* presence in the survey were: *Anabaena* and *Ceratium* (both lower in invaded lakes), and *Cryptomonas* (higher in invaded lakes).

Introduction

Bloom-forming bluegreen algae (species in the genera *Anabaena*, *Aphanizomenon*, *Microcystis,* and *Oscillatoria* in temperate lakes) have long been the focus of intense research and concern because of their unpleasant odor and appearance, and their ability to produce toxins harmful to animals and humans (Chorus and Bartram 1999). In addition, poor nutritional quality of bluegreens, coupled with the toxins they produce, can reduce the growth and survivorship of herbivorous zooplankton (de Bernardi and Giussani 1990, DeMott et al. 1991, DeMott 1999), and so reduce food-chain efficiency. It is widely accepted that the relative dominance of noxious bloom-forming bluegreens increases with nutrient enrichment, and that these taxa usually comprise a small percentage of the phytoplankton community in nutrient poor systems (Trimbee and Prepas 1987, Downing et al. 2001). As a result, lake management practices aimed at improving water quality generally focus on reducing the nutrient loads coming into lakes. However, recent evidence suggests that the invasive zebra mussel (*Dreissena polymorpha*) may alter the well-established positive relationship between nutrients and bluegreen dominance (Vanderploeg et al. 2001, Raikow et al. 2004), which may require re-evaluation of nutrient management strategies in lakes with *D. polymorpha*.

Although changes in phytoplankton community structure are variable following *D. polymorpha* invasion across freshwater systems, one surprising trend is emerging. In shallow, mesotrophic areas of the Great Lakes, *Microcystis aeruginosa* seems to have increased following *D. polymorpha* invasion, despite recent reductions in phosphorus loading (Vanderploeg et al. 2001). Similarly, a survey of low-nutrient (total phosphorus, TP, 10 - 25 $\mu g\ L^{-1}$) inland lakes showed higher relative dominance of *M. aeruginosa* in

lakes invaded by *D. polymorpha*. Such lakes would not be expected to harbor high levels of bloom-forming bluegreens given their relatively low nutrient status. Thus, zebra mussels seem to be affecting the dominance relationships of phytoplankton communities in unexpected ways.

The aforementioned survey found higher *M. aeruginosa* **dominance** in inland lakes invaded by *D. polymorpha*, but no significant influence of invasion on the actual **biomass** of this bluegreen species (Raikow et al. 2004). In addition, no measurements of toxin concentrations were reported in that survey. Thus, the primary focus of my study was to determine whether lakes invaded by *D. polymorpha* have higher *M. aeruginosa* biomass and higher microcystin concentrations. The previous survey indicated that the response of *M. aeruginosa* to invasion was limited to lakes within a relatively narrow range of TP (10 – 25 $\mu g\ L^{-1}$), so I focused on such lakes in my survey.

The mechanism by which *D. polymorpha* promotes *M. aeruginosa* dominance is not fully understood, but one proposed mechanism involves reduced filtering rates of *D. polymorpha* on *M. aeruginosa*. This may result in a selective advantage for the bluegreen over other phytoplankton taxa (Vanderploeg et al. 2001). Reduced filtration rates may be a function of the colonial habit and/or toxicity of *M. aeruginosa* (Vanderploeg et al. 2001). Some strains of *Microcystis aeruginosa* and other bluegreen genera (*Anabaena*, *Oscillatoria*, and *Nostoc*) produce microcystin, a hepatotoxic cyclic peptide toxin (Chorus and Bartram 1999) that might act as an herbivore deterrent. Thus, *D. polymorpha* invasion could result in an increase in per capita microcystin production by these species, via phenotypic or evolutionary responses. Such an effect would have ecological, as well

as public health consequences, given that increased per capita toxicity might further decrease food-chain efficiency.

To address the above issues, I conducted a lake survey of 39 invaded and uninvaded, low-nutrient (total phosphorus < 20 μg/L) inland lakes in Michigan that thermally stratify in the summer. In addition to examining the responses of *M. aeruginosa* biomass and toxin concentration, I also investigated how basic limnological parameters were influenced by *D. polymorpha* in these lakes, to provide some insight into potential mechanisms leading to increased *M. aeruginosa*. Based on previous studies, I expected to see lower chlorophyll *a* (Holland 1993, Nicholls and Hopkins 1993, Fahnenstiel et al. 1995 Caraco et al. 1997, Yu and Culver 2000, Idrisi et al. 2001, Raikow et al. 2004), leading to higher water clarity (Holland 1993, Caraco et al. 1997, Yu and Culver 2000, Idrisi et al. 2001) and dissolved nutrients (Holland et al. 1995, Effler et al. 1996, Caraco et al. 1997, Raikow et al. 2004) in invaded lakes. I also examined the influence of *D. polymorpha* on major solutes (Ca, Mg, Na, K, Cl, SO_4, and Si), which has rarely been attempted (Holland et al. 1995, Johengen et al. 1995).

Methods

Lake Selection

For the survey, I selected low-nutrient lakes (TP < 20 μg L^{-1}) in southern Michigan (Figure 1) with a maximum depth of a least 9 m, such that thermal stratification was likely to be present during the summer. For the purposes of lake selection, the presence/absence of *D. polymorpha* was initially assessed using the list of invaded lakes assembled by the Michigan Sea Grant College Program (www.miseagrant.org). Presence/absence of *D. polymorpha* was verified in each lake by searching for adults in

the field and veligers in zooplankton samples. To ensure that invaded and uninvaded lakes included in the survey were similar in depth, mean depth was determined by digitizing bathymetric lake maps in ArcView GIS 3.2. Based on these criteria, 39 lakes were selected, 20 with *D. polymorpha* and 19 without, for the survey.

Field Sampling

Each lake was visited once, in late summer of 2002 or 2003 (2 August – 4 September 2002, 3 August – 20 August 2003). I took samples from the deepest spot in each lake, as determined by bathymetric maps and a depth finder. Temperature, conductivity, and pH were measured at 1 m intervals with a Hydrolab Surveyor and Datasonde. Photosynthetically available radiation (PAR) was measured at 0.5 m intervals with a LiCor model Li-1000 quantum photometer radiometer equipped with a spherical underwater sensor. Vertical extinction coefficients were calculated as the slope of ln PAR versus depth via linear regression. A depth-integrated water sample was taken through the entire mixed layer (epilimnion) with a flexible tube (5 cm inner diameter) and placed on ice until processing (approximately 6 hours). Subsamples of epilimnetic water were taken for chlorophyll *a*, nutrients, and water chemistry. A 125 ml subsample of epilimnion water was preserved in Lugol's solution for phytoplankton enumeration.

Laboratory Analysis

For chlorophyll *a* analysis, samples were filtered onto a Gelman A/E glass fiber filter and kept frozen. The filter was subsequently extracted with 95% ethanol and chlorophyll *a* quantified using a Turner Model 10-AU fluorometer calibrated with standards (Welschmeyer 1994). Dissolved nutrients were filtered through a Gelman A/E glass fiber filter. NH_4^+-N was measured colorimetrically following an adapted version of

the phenylhypochlorite method (Aminot et al. 1997). Soluble reactive phosphorus (SRP) and total dissolved phosphorus (TDP) were measured colorimetrically following the acid molybdate method (Wetzel and Likens 2001); the TDP colorimetric analysis was preceded by a persulfate digestion to decompose organically bound P (Valderrama 1981). Total phosphorus (TP) was analyzed colorimetrically (Langner and Hendrix 1992) after persulfate oxidation. Upon return to the laboratory, water chemistry samples were refrigerated (alkalinity, conductivity) or filtered through Gelman A/E glass fiber filters and then refrigerated (anions, silica), or acidified with 8 N HNO_3 (cations) until analysis. Conductivity was measured in the laboratory using an Orion model 135 conductivity meter. Ca, Mg, Na, and K were measured by flame atomic absorption spectrometry, and total alkalinity, which generally represents HCO_3 in lake waters, was determined by titration with 0.3 N HCl and calculation of the Gran function (Wetzel and Likens 2001). SO_4 and Cl were measured by membrane-suppressor ion chromatography. Si was measured colorimetrically by the ammonium molybdate method (Wetzel and Likens 2001). DOC was measured with a high-temperature combustion DOC analyzer.

Phytoplankton were generally identified to species and were enumerated using the inverted microscope method (Hasle 1978). Subsamples were settled in tubular chambers (Hydro-Bios), the bottoms of which were divided into inner and outer zones of equal area (Sandgren and Robinson 1984). Within each zone, at least 20 random fields were counted at 100x, 400x, and 1000x. I determined phytoplankton biovolume by measurements of cell dimension at 1000x of at least ten individuals of common species using a digital camera system and image-analysis software. Biovolume was calculated by using a geometric volume equation appropriate for the species shape and converted to dry

biomass assuming a specific gravity of 1 g cm^{-3} and a dry mass to wet mass ratio of 0.10. To estimate *M. aeruginosa* colony size, I searched the entire chamber at 100x and estimated the area of each colony (μm^2) by counting the number of grid squares the colony occupied.

To collect particulate microcystin, a large quantity of integrated epilimnion water (generally $\geq$ 1L) was filtered through a Gelman A/E glass fiber filter and the filter was frozen until analysis. Enzyme-linked immuno sorbent assay (ELISA) (An and Carmichael 1994) was used to obtain particulate microcystin concentrations.

Data and statistical analyses

To examine the influence of *D. polymorpha* on all variables, I used t-tests. However, for a sub-set of the physico-chemical parameters (alkalinity, conductivity, NH_4^+-N, DOC, and pH) only data from 2002 were available.

Since the focus of this study was to investigate the impact of *D. polymorpha* on *M. aeruginosa*, separate analyses were performed for the rest of the phytoplankton community. To examine the impact of *D. polymorpha* on phytoplankton species composition other than *M. aeruginosa*, I used principal components analysis (PCA) on phytoplankton relative biomass (exclusive of *M. aeruginosa*). The objective of the PCA was to reduce the phytoplankton data set to a small number of variables to avoid data mining for significant responses. To reduce zero values, only common taxa were included: *Anabaena*, *Aphanocapsa*, *Ceratium*, *Chroococcus*, *Cryptomonas*, *Chrysochromulina*, *Fragilaria*, *Oocystis*, *Peridinium*, *Rhodomonas*, *Scenedesmus*, *Sphaerocystis*, and an unidentified small flagellate.

PCA was also applied to the major solute data (Ca, Mg, Na, K, Cl, SO_4,and Si) to determine the influence of *D. polymorpha* presence on these variables. For this analysis, only data from 2002 were available. Data were log-transformed when necessary so that assumptions for parametric statistics were met. All analyses were conducted in SYSTAT 9.0.

Results

To rule out the influence of potential confounding factors, I assessed whether invaded and uninvaded lakes were similar in morphometry and nutrients. There were no significant differences in either mean depth or TP between the two groups of lakes (Table 1). I assessed if day of year affected any *M. aeruginosa* variable since bluegreens tend to become more dominant as the summer progresses and found no effect of day of year on any *M. aeruginosa* variable ($P > 0.1$). Average epilimnetic temperatures were similar between years (2002 average = 25.4, 2003 average = 25.2), and for all response variables examined, there were no interactive effects of year sampled ($P > 0.1$).

Microcystis aeruginosa biomass was 3.6 times higher in invaded lakes (Table 2; Figure 2) and the relative biomass of *M. aeruginosa* in lakes with *D. polymorpha* was 5.1 times higher (mean invaded = 14.15%, mean uninvaded = 2.79%, $P = 0.001$).

For the phytoplankton community exclusive of *M. aeruginosa*, PCA reduced the data to two factors that explained 34% of the total variance. PCA factor 1 was significantly higher in invaded lakes (Figure 3), while PCA factor 2 was not different between invaded and uninvaded lakes (Figure 3). Factor 1 PCA scores were negatively correlated with *Anabaena* ($r = -0.63$, $P = 0.009$, t-test) and *Ceratium* ($r = -0.79$, $P = 0.00003$, t-test) and positively correlated with *Cryptomonas* ($r = 0.69$, $P = 0.002$, t-test).

This suggests that *D. polymorpha* had a negative effect on *Anabaena* and *Ceratium*, but a positive effect on *Cryptomonas* (Figure 4).

Microcystin toxin concentrations were 3.3 times higher in invaded lakes (Table 2; Figure 5). However, the total biomass of microcystin-producing genera was not different between lake categories (Table 2). Toxin concentration per unit of microcystin-producing algal biomass was 2.9 times higher in lakes with *D. polymorpha* (Table 2; Figure 6A), but toxin concentration per unit of *Microcystis* biomass was not different between lake categories (Table 2; Figure 6B).

To determine if *D. polymorpha* affected colony sizes of *M. aeruginosa*, analyses were conducted on average colony area and median colony area as viewed under the microscope. Colony sizes were similar in uninvaded and invaded lakes for both average colony area (Figure 7) and median colony area ($P = 0.62$).

Dreissena polymorpha also significantly affected several limnological parameters. Both chlorophyll *a* and phytoplankton dry biomass were 30 % lower in invaded lakes (Table 2; Figure 8). Extinction coefficients were significantly lower in invaded lakes (Table 2). No difference was found for SRP (Table 2) or TDP (mean invaded = 6.02, uninvaded = 6.74, $P = 0.46$) between lake categories, while NH_4^+-N was 1.8 times higher in *D. polymorpha* lakes (Table 2). There was no difference between lake categories for molar ratios of NH_4^+-N: SRP (mean invaded = 14.93, uninvaded = 19.18, $P = 0.31$).

Invaded and uninvaded lakes were similar in alkalinity, conductivity, DOC, and pH (Table 1). Principal components analysis was performed on the major solutes (Ca, Mg, Na, K, Cl, SO_4, and Si) to determine the relationship between *D. polymorpha* presence on these variables. The PCA reduced the data to two factors that explained 81%

of the total variance. PCA factor 1 was significantly higher in invaded lakes (P = 0.04, t-test) and PCA factor 2 was not different between lake categories (P = 0.23, t-test). Factor 1 PCA scores were positively correlated with Na (r = 0.88, P = 0.2, t-test), K (r = 0.85, P = 0.14, t-test), Cl (r = 0.89, P = 0.13, t-test), and SO_4 (r = 0.66, P = 0.0008, t-test), but only SO_4 was significant.

Discussion

There was a strong positive influence of *D. polymorpha* on *M. aeruginosa* biomass in the survey lakes. This result builds upon a previous lake survey that found the relative dominance of *M. aeruginosa* to be higher in invaded lakes (Raikow et al. 2004). The conflicting results between my study and Raikow et al.'s study (2004) may be because they did not sample enough lakes in the 'low' nutrient range, or because they did not control for lake mean depth or thermal stratification. These factors are likely important because some bloom-forming bluegreens, such as *Microcystis,* prefer stable water columns (Dokulil and Teubner 2000). For example, in the well-mixed Hudson River and in enclosures in the Ohio River, bluegreen abundance actually declined and diatoms increased in the presence of *D. polymorpha* (Smith et al. 1998, Jack and Thorp 2000), which suggests mixing regime may play a role in the *D. polymorpha – Microcystis* relationship.

I also documented an increase in microcystin toxin concentrations and toxin per microcystin-producing biomass in *D. polymorpha* lakes, which has not been shown previously. However, it is difficult to determine if higher toxin concentrations are a result of *M. aeruginosa* or *Anabaena*, the only other common microcystin-producing genus in the survey lakes. It may be possible that the increased toxin concentrations in invaded

lakes is simply a consequence of increased *M. aeruginosa* biomass. However, this explanation assumes the toxin concentrations produced by *Anabaena* remain the same in invaded and uninvaded lakes. Thus, the differences in toxin concentrations cannot be fully explained from my results.

The mechanism by which *D. polymorpha* promotes *M. aeruginosa* in low-nutrient lakes is not fully understood, but three mechanisms have been proposed: 1) the colony size of *M. aeruginosa* may be too large for *D. polymorpha* to feed on effectively, 2) toxins produced by *M. aeruginosa* may cause *D. polymorpha* to preferentially feed on non-toxic phytoplankton (Vanderploeg et al. 2001) giving *M. aeruginosa* selective advantage over non-toxic phytoplankton and 3), the low N: P excretion of *D. polymorpha* (Arnott and Vanni 1996) may favor phytoplankton that can take advantage of low N conditions (such as bluegreens).

Although I cannot positively determine which of these mechanisms is operating in inland lakes, my data can provide some general insights. First, the size of *M. aeruginosa* colonies were similar between lake categories and this suggests *D. polymorpha* did not shift the size distribution of colonies to be dominated by larger individuals (Figure 7). The third proposed mechanism may not explain the increased *M. aeruginosa* dominance either. NH_4^+-N: SRP ratios were similar in invaded and uninvaded lakes, suggesting that changes in nutrient availability of N and P may not be promoting *M. aeruginosa* in *D. polymorpha* lakes. A similar lake survey (Raikow et al. 2004) also concluded that the ratio of available nutrients does not appear to explain bluegreen dominance in low-nutrient lakes with zebra mussels. Based on my results, the most plausible explanation for the dominance of *M. aeruginosa* in *D. polymorpha* lakes is explained by the second

mechanism. *Microcystis aeruginosa* toxins may cause *D. polymorpha* to feed on non-toxic phytoplankton because toxin concentrations were higher in invaded lakes. Feeding studies have shown that *D. polymorpha* generally prefer to feed on other, non-toxic phytoplankton over colonial, toxic *M. aeruginosa* (Bastviken et al. 1998, Vanderploeg et al. 2001).

Because microcystin is toxic to animals (Chorus and Bartram 1999), the promotion of the toxin-producing *M. aeruginosa* by *D. polymorpha* could have serious ecological implications. An increase in the actual biomass of *M. aeruginosa* may reduce food-chain efficiency because bluegreens are generally of poor nutritional quality and are not a preferred food source for herbivorous zooplankton (DeMott et al. 1991, DeMott 1999). The impacts of toxic bluegreens on several aquatic organisms have been identified (Fulton and Paerl 1987, Rabergh et al. 1991, Kotak et al. 1996). In particular, toxic bluegreens have been found to inhibit feeding, reproduction and survivorship of *Daphnia* (Fulton and Paerl 1987, de Bernardi and Giussani 1990, DeMott et al. 1991, DeMott 1999). In addition, Lurling (2003) found that as the microcystin concentrations of *M. aeruginosa* increased, *Daphnia* growth rates decreased. Thus, higher toxin concentrations in *D. polymorpha* lakes may seriously harm zooplankton and possibly other aquatic organisms.

I found *Dreissena polymorpha* to also significantly impact the overall phytoplankton community (exclusive of *M. aeruginosa*). *Dreissena polymorpha* appear to be filtering large quantities of phytoplankton out of the water column because both chlorophyll *a* and phytoplankton biomass were significantly lower in invaded lakes. Furthermore, extinction coefficients show that *D. polymorpha* lakes are clearer than lakes

without mussels, apparently directly related to the lower phytoplankton biomass in invaded lakes. The trend of decreased chlorophyll *a* concentration and increased water clarity is commonly observed after *D. polymorpha* invasion in rivers (Effler et al. 1996, Caraco et al. 1997), the Great Lakes (Holland 1993, Nicholls and Hopkins 1993, Fahnenstiel et al. 1995), and inland lakes (Yu and Culver 2000, Idrisi et al. 2001, Raikow et al. 2004).

I also found species-level effects on the phytoplankton community. For example, *Anabaena*, a colonial, filamentous bluegreen and *Ceratium*, a large dinoflagellate, were negatively affected by *D. polymorpha* presence, while *Cryptomonas*, a cryptophyte, was positively affected by *D. polymorpha* presence. Bastviken et al. (1998) found *Anabaena* relative abundance to decrease in the presence of *D. polymorpha* although longer *Anabaena* filaments were not as affected as shorter filaments and Smith et al. (1998) found *Anabaena* to be sensitive to *D. polymorpha* presence in the Hudson River. Thus, my *Anabaena* results are consistent with past studies. The effect of *D. polymorpha* on *Ceratium* has been scarcely investigated although Smith et al. (1998) found *Ceratium* (relative abundance) to be sensitive to *D. polymorpha* in the Hudson River, which is in agreement with my results. However, previous studies show that *Cryptomonas* does not always respond in the same manner to *D. polymorpha* presence. Experimental studies have found cryptophytes (includes species other than *Cryptomonas*) (Bastviken et al. 1998) and *Cryptomonas* (Lavrentyev et al. 1995) to be highly preferred by *D. polymorpha*, although Smith et al. (1998) found *Cryptomonas* (relative abundance) to be indifferent to *D. polymorpha* in the Hudson River and Makarewicz et al. (1999) found no

difference in cryptophyte biomass before and after *D. polymorpha* invasion in Lake Erie. Thus, it is unclear what the general trends associated with *Cryptomonas* are.

I also found that few water chemistry variables differed between invaded and uninvaded lakes. For example, a PCA on all ions (Ca, Mg, Na, K, Cl, Si, and SO_4) revealed that only SO_4 was significantly higher in *D. polymorpha* lakes. The effect of *D. polymorpha* on chemical parameters, such as major solutes, has been poorly investigated. In western Lake Erie, silica and chloride increased after *D. polymorpha* invasion (Holland et al. 1995), and in Saginaw Bay, particulate silica decreased while dissolved silica increased (Jonengen et al. 1995). In my study, only SO_4 was significantly higher in invaded lakes and there is no obvious explanation for this result.

The only nutrient that was influenced by invasion status was NH_4^+-N, which was 1.8 times higher in invaded lakes. My results are only partially consistent with previous studies. Similar to other studies, I found an increase in NH_4^+-N in *D. polymorpha* lakes (Holland et al. 1995, Effler et al. 1996, Heath et al. 1995, Wilson 2003), but no difference in SRP. It is reasonable to expect NH_4^+-N and SRP to increase in the presence of *D. polymorpha* because mussels can excrete dissolved nutrients at high rates (Quigley et al. 1993, Arnott and Vanni 1996). However, changes to SRP concentrations following *D. polymorpha* invasion have been somewhat inconsistent. For example, SRP increased in the Hudson River (Caraco et al. 1997), Seneca River (Effler et al. 1996), and western Lake Erie (Holland et al. 1995), while there was no change in Oneida Lake (Idrisi et al. 2001) and SRP slightly decreased in Saginaw Bay of Lake Huron (Johengen et al. 1995) after *D. polymorpha* invasion. The lack of a *D. polymorpha* effect on SRP in my study may be attributable to the degree of nutrient limitation in the lakes I surveyed. In lakes

where phosphorus strongly limits phytoplankton growth, the demand for SRP may still be high, despite reductions in phytoplankton biomass from *D. polymorpha* filtering. SRP concentrations in the survey lakes were low (~1.6 $\mu g\ L^{-1}$) indicating that any increase in supply induced by *D. polymorpha* may be quickly taken up by phytoplankton, making it difficult to detect an impact on SRP concentration. In systems where *D. polymorpha* invasion positively influenced SRP, concentrations prior to invasion were higher than in my study (Hudson River ~10 $\mu g\ L^{-1}$ and western Lake Erie ~ 6 $\mu g\ L^{-1}$).

There are many health-related and management consequences of low-nutrient lakes with *D. polymorpha* having more toxins than uninvaded lakes. This is especially a concern since the impacted lakes are low-nutrient and therefore considered to be highly desirable for recreational purposes. Microcystins are potentially harmful to humans through consumption and skin exposure and can cause a variety of ailments from rashes to liver damage as well as the promotion of tumors (Chorus and Bartram 1999). The World Health Organization has set drinking water guidelines so that microcystin concentrations should not exceed 1 $\mu g\ L^{-1}$ (WHO 1996). Although none of the survey lakes exceed this guideline (highest concentration = 0.097 $\mu g\ L^{-1}$), the values reported in my study may be underestimated. Samples were collected from the deepest spot of the lake, which was generally in the center of the lake. Bluegreen blooms are often buoyant and tend to blow towards the shoreline and accumulate densely there. For example, Johnston and Jacoby (2003) found microcystin to range from 1.5 – 3.1 $\mu g\ L^{-1}$ throughout most of Lake Sammamish, but near the boat launch the concentration was much higher (43 $\mu g\ L^{-1}$), which they attribute to wind causing accumulations of *M. aeruginosa*. Thus, the values I report are likely at the low end of the range and there is the possibility for

microcystin values in low-nutrient lakes to exceed guidelines set by the World Health Organization. In particular, the toxin concentrations may be higher in locations of high human contact and use, such as along the shoreline and in swimming areas.

Because of the negative consequences that can be associated with microcystin toxins, it should be a high priority to determine how *D. polymorpha* are able to promote *M. aeruginosa* and microcystin in lakes they invade. Although previous feeding studies have attempted to answer if *D. polymorpha* choose other phytoplankton over *M. aeruginosa*, more studies are needed that mimic natural conditions. In particular, studies investigating how a range of colony sizes, rather than unicellular *Microcystis* versus colonial *Microcystis* (Bastviken et al. 1998, Dionisio Pires and Van Donk 2002, Dionisio Pires et al. 2004) or colony sizes split only into two categories (Vanderploeg et al. 2001), are needed. In addition, *D. polymorpha* may be enhancing strains of *M. aeruginosa* that produce high levels of toxins or may be stimulating a phenotypic or evolutionary response by inducing *M. aeruginosa* to increase the amount of toxin produced. Thus, studies aimed at determining if *D. polymorpha* induce greater toxin production by *M. aeruginosa* are also necessary. Many inland North American lakes, particularly those in Michigan and in bordering states, are relatively low in TP and capable of supporting *D. polymorpha* populations (Raikow et al. 2004), and therefore determining how *D. polymorpha* increase *M. aeruginosa* and microcystin is crucial.

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Table 1. Average, range and standard deviation of limnological parameters in survey lakes. P values are for t-tests. For NH_4^+-N, alkalinity, conductivity, DOC, and pH, P values are for t-tests only using 2002 data.

Variable	Uninvaded		Invaded		P values
	Average (range)	SD	Average (range)	SD	
Mean depth (m)	4.93 (2.31 – 8.8)	2.11	6.16 (2.13 – 12.41)	2.8	0.16
Alkalinity ($\mu eq\ L^{-1}$)	2670.43 (1700 – 3579	525.36	2804.83 (2163 - 3402)	342.36	0.2
pH	8.06 (7.66 – 8.89)	0.32	8.08 (7.82 – 8.29)	0.12	0.76
Conductivity ($mg\ L^{-1}$)	329.36 (188 – 483)	92.29	400.67 (270 – 681)	114.86	0.073
DOC ($mg\ L^{-1}$)	8.38 (5.35 – 15.82)	2.95	7.18 (4.76 – 9.3)	1.53	0.23
TP ($\mu g\ L^{-1}$)	10.4 (4.9 – 19.32)	3.83	10.62 (5.32 – 19.25)	3.43	0.73
SRP ($\mu g\ L^{-1}$)	1.63 (0.08 – 3.72)	0.87	1.61 (0 – 6.08)	1.57	0.94
NH_4^+-N ($\mu g\ N\ L^{-1}$)	6.81 (0 – 15.6)	4.98	12.4 (6.5 – 28)	7.04	0.015
Extinction coefficient	-0.275 (- 0.428 – - 0.16)	0.07	-0.212 (- 0.308 – - 0.06)	0.05	0.003
Chlorophyll *a* ($\mu g\ L^{-1}$)	4.59 (2.36 – 9.29)	1.77	3.23 (0.89 – 5.25)	1.1	0.008
Phytoplankton biomass ($\mu g\ L^{-1}$)	50.77 (18.71 – 95.08)	19.44	35.33 (12.88 – 115.8)	22.16	0.006

Table 2. Average, range, and standard deviation of variables related to *M. aeruginosa* and microcystin toxin. P values are for t-tests.

Variable	Uninvaded		Invaded		P values
	Average (range)	SD	Average (range)	SD	
M. aeruginosa biomass ($\mu g\ L^{-1}$)	1.33 (0 – 4.17)	1.34	4.8 (0.11 – 20.5)	5.44	0.01
Microcystin-producing biomass ($\mu g\ L^{-1}$)	5.18 (0 – 13.2)	4.49	5.69 (0.16 – 21.67)	5.52	0.83
Microcystin concentration ($ng\ L^{-1}$)	9.87 (2.14 – 31.38)	8.25	33.06 (1.6 – 96.64)	30.55	0.009
Microcystin per producing biomass ($ng\ \mu g^{-1}$)	2.76 (0 – 11.49)	2.62	7.88 (0.83 – 20.6)	5.39	0.00009
Microcystin per *M. aeruginosa* biomass ($ng\ \mu g^{-1}$)	0.019 (0 – 0.158)	0.035	0.013 (0.004 – 0.05)	0.011	0.5

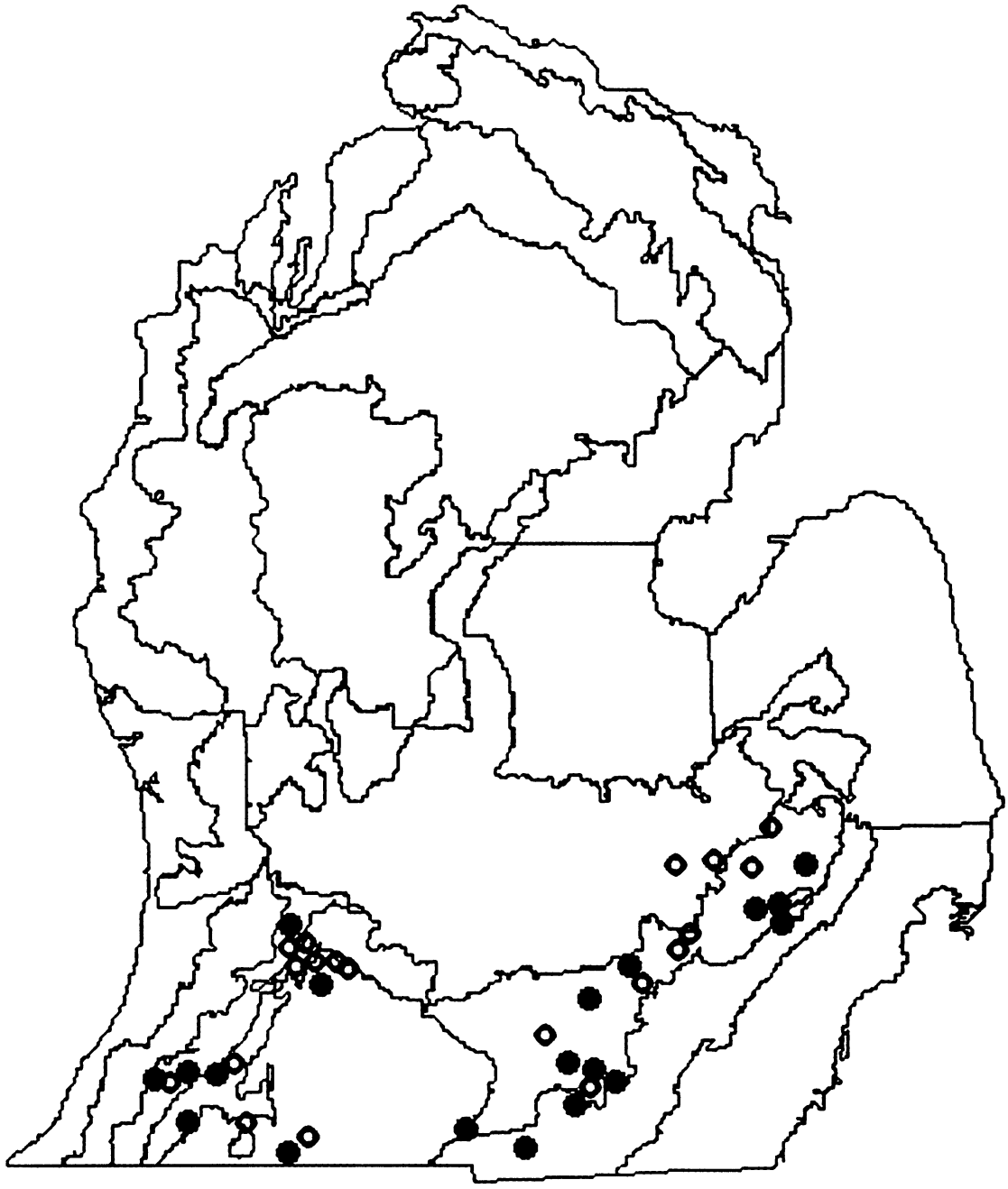

Figure 1. Location of survey lakes in the lower peninsula of Michigan. The state is divided into Albert ecoregion subsections. Closed circles represent invaded lakes and open circles represent uninvaded lakes.

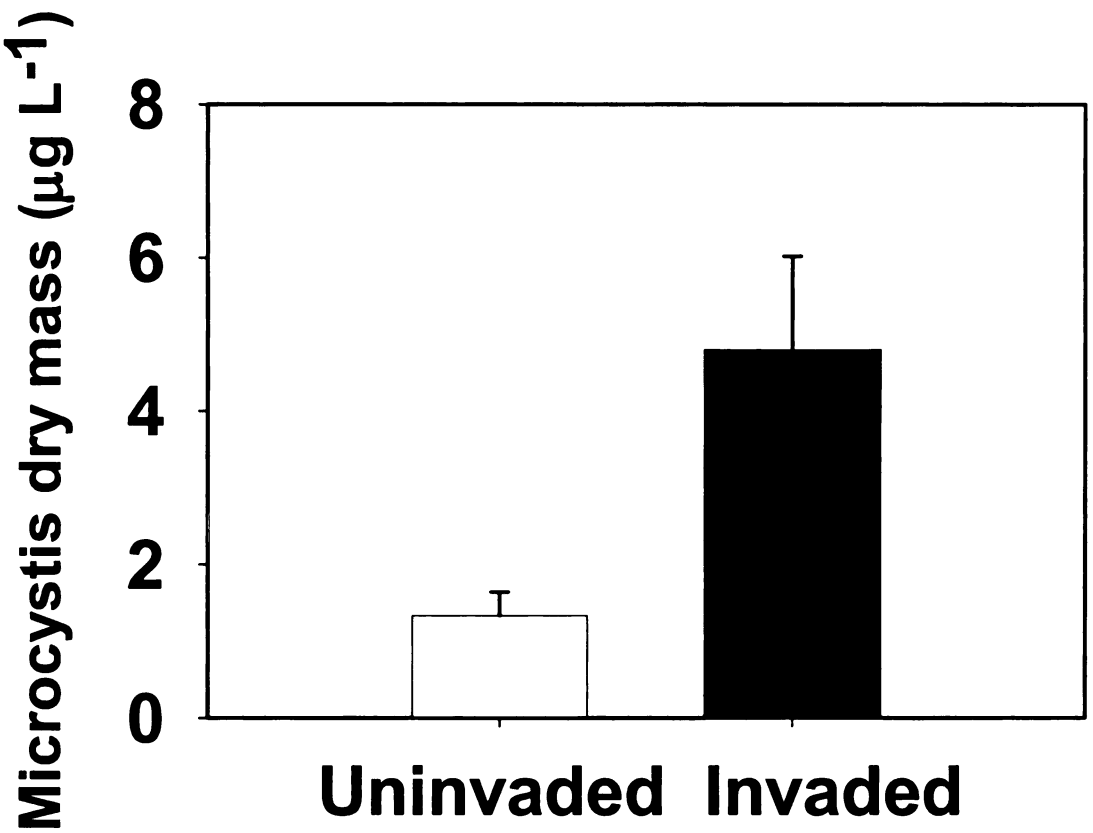

Figure 2. *Microcystis aeruginosa* dry mass in lakes with and without *D. polymorpha* (P = 0.01). P value for a t-test. Bars are SE.

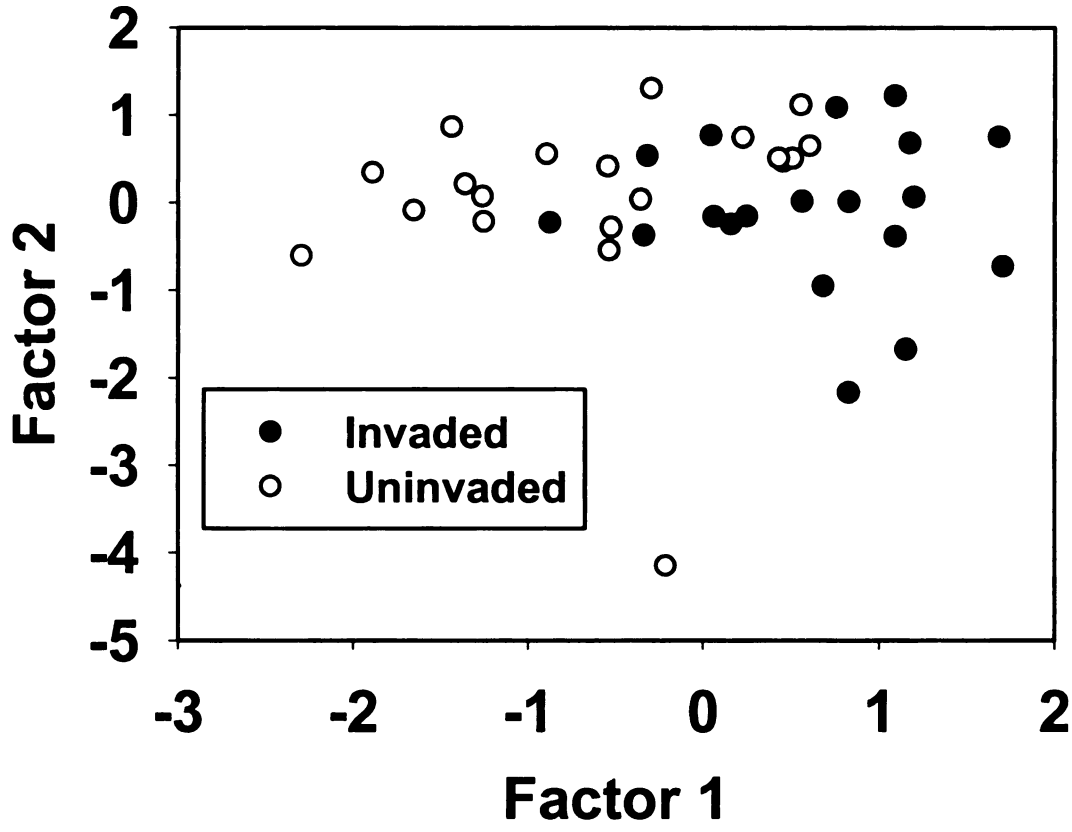

Figure 3. Factor scores from the phytoplankton (exclusive of *M. aeruginosa*) PCA. Factor 1 (P = 0.00002) and Factor 2 (P = 0.65), which indicates a positive effect of *D. polymorpha* on Factor 1. P values are for t-tests.

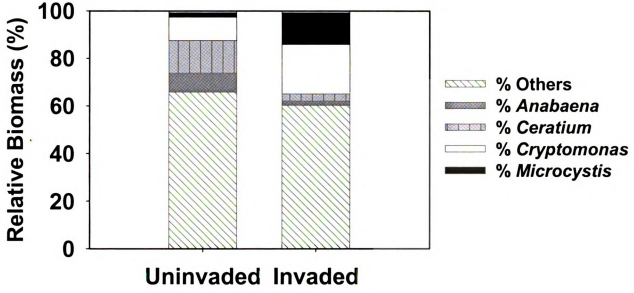

Figure 4. Relative biomass values (%) of the phytoplankton genera affected by *D. polymorpha* presence (*Anabaena*, *Ceratium*, *Cryptomonas*, and *Microcystis*) and the remaining phytoplankton grouped together as "other".

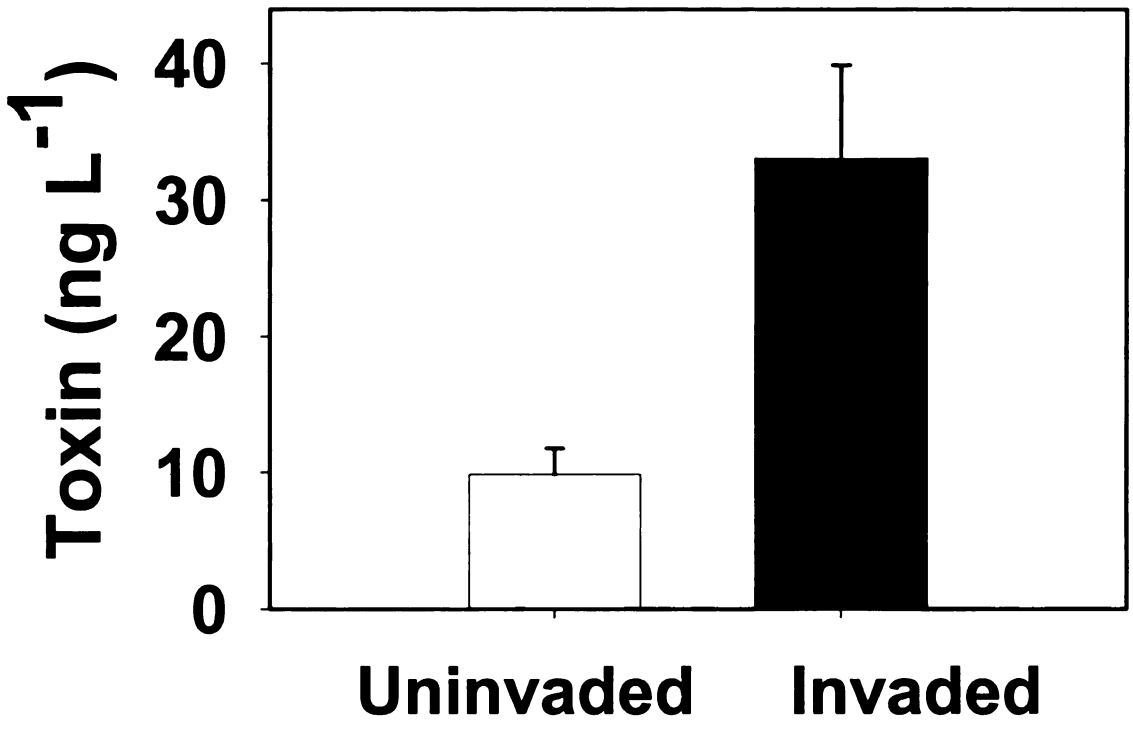

Figure 5. Microcystin concentration in lakes with and without *D. polymorpha* (P = 0.009). P value for a t-test. Bars are SE.

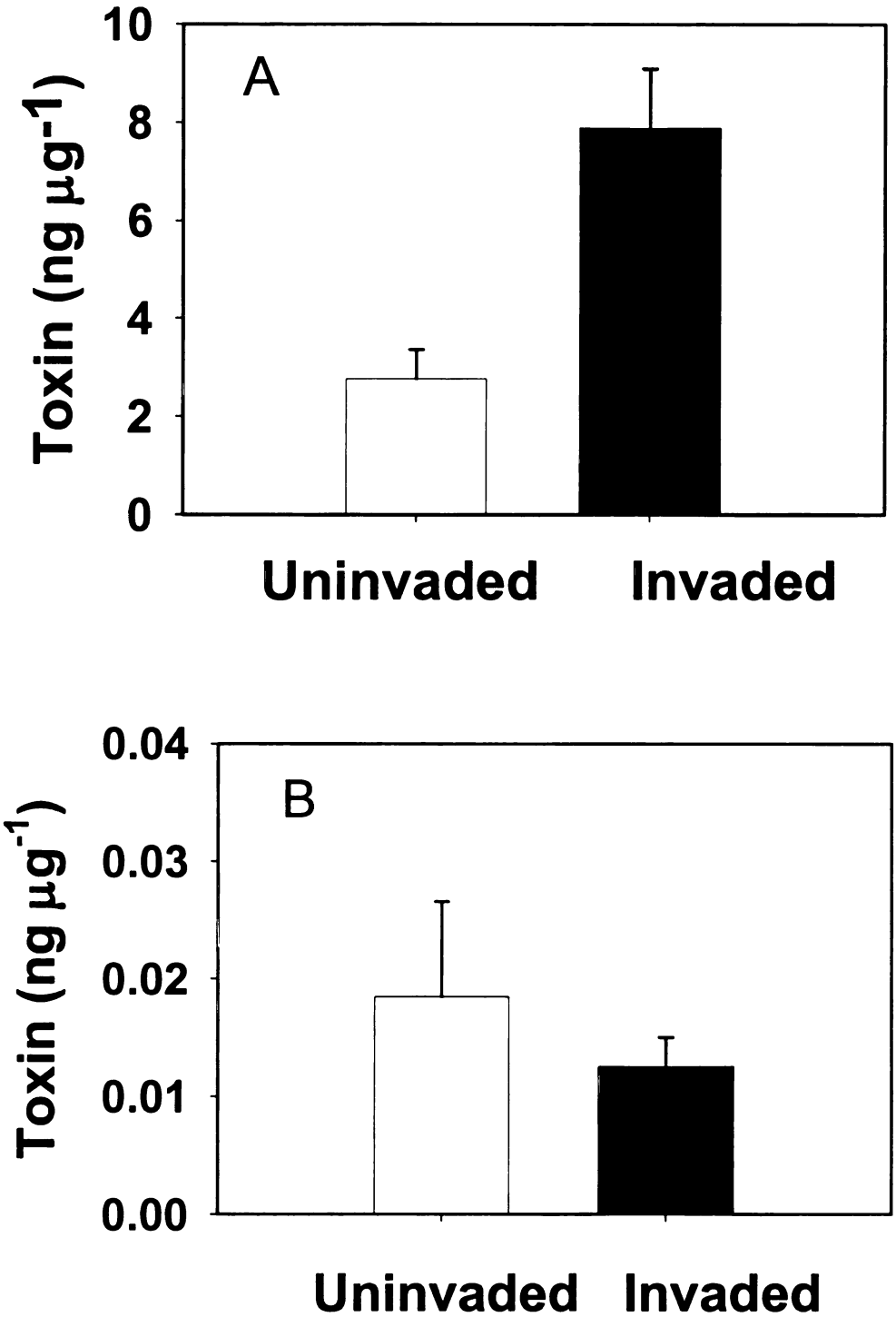

Figure 6. (A) Microcystin concentration per microcystin-producing biomass in lakes with and without *D. polymorpha* (P = 0.0009). Microcystin producing genera are *Anabaena*, *Microcystis*, *Oscillatoria*, and *Nostoc*. (B) Microcystin concentration per *M. aeruginosa* biomass (P = 0.5). P values are for t-tests. Bars are SE.

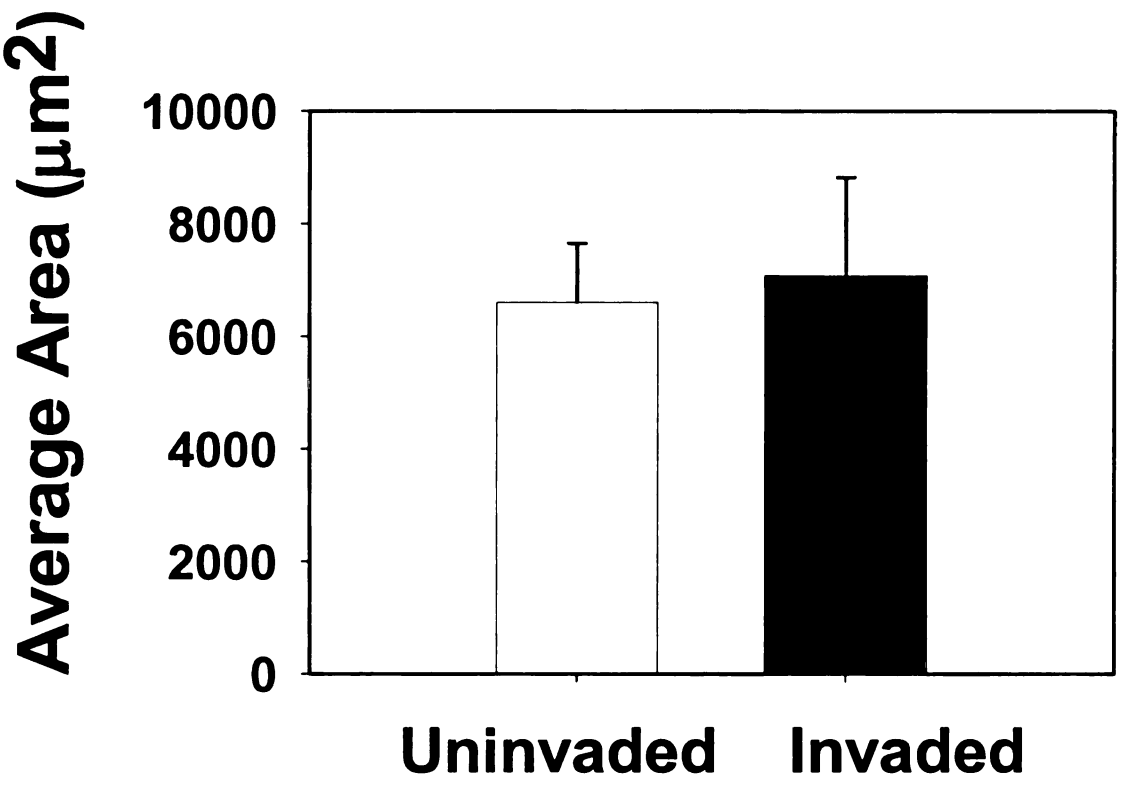

Figure 7. Colony size of *M. aeruginosa* as average area (P = 0.47). P value for a t-test. Bars are SE.

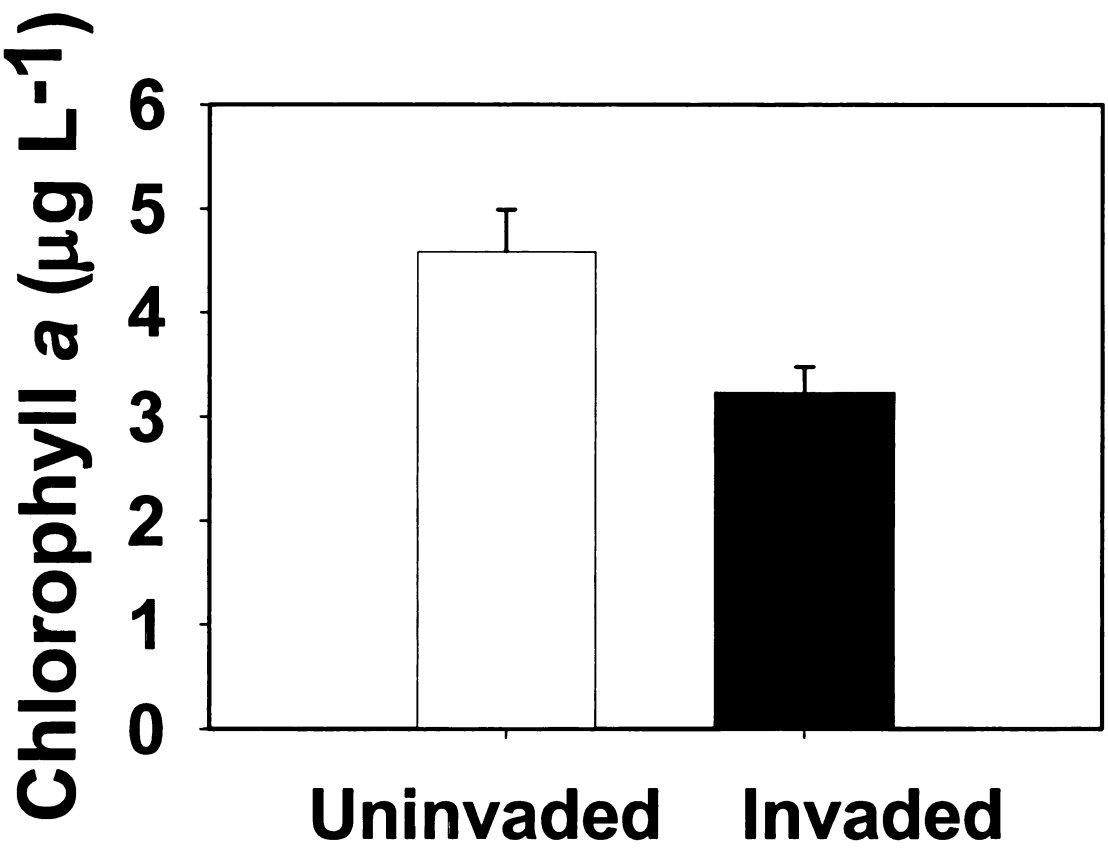

Figure 8. The effect of *D. polymorpha* on chlorophyll *a* (P = 0.008). P value is for a t-test. Bars are SE.

CHAPTER 2

Influence of zebra mussels (*Dreissena polymorpha*) on microzooplankton communities in stratified low-nutrient lakes

ABSTRACT

Although planktonic rotifers and ciliates are an important component of secondary production in lakes, few studies have investigated how zebra mussels (*Dreissena polymorpha*) affect these organisms. Existing studies have largely been conducted in well-mixed systems, yet *D. polymorpha* is invading stratified inland lakes at a rapid rate. It is very likely that the effects of *D. polymorpha* may vary with mixing regime. Thus, I conducted a lake survey to examine how *D. polymorpha* influences the biomass and community structure of rotifers and ciliates in stratified lakes. Forty-six low nutrient lakes were sampled, 24 with *D. polymorpha* and 22 without. The total biomass of rotifers and ciliates were lower in invaded lakes (44% and 45%, respectively) and in both cases, the difference between invaded and uninvaded lakes was highly significant. Thus, I found no evidence that *D. polymorpha* invasion has stronger negative effects on ciliates than rotifers in general. Lakes with zebra mussels also had lower rotifer species richness and diversity. In addition, a shift in the size distribution of ciliates was found between lake categories indicating that zebra mussels may be reducing larger, algivorous ciliates in favor of smaller bacterivorous ciliates. In general, zebra mussel impacts on microzooplankton biomass in the stratified lakes I sampled were weaker than those reported from well-mixed systems.

Introduction

Microzooplankton (heterotrophic flagellates, ciliates and, in freshwaters, rotifers) comprise, at times, a major fraction of total zooplankton biomass in freshwater lakes (Pace and Orcutt 1981, Gates and Lewg 1984). Microzooplankton function as consumers of bacteria and small phytoplankton and as a food resource for larger organisms (e.g. macrozooplankton, larval fish), and thus can link microbes to higher trophic levels (Sherr et al. 1986, Sherr et al. 1987, Sanders et al. 1989, Arndt 1993). Microzooplankton biomass is strongly regulated by predation and resource competition with macrozooplankton (Gilbert 1988, Pace and Funke 1991, Wickham and Gilbert 1991, Jack and Gilbert 1994, Marchessault and Mazumder 1997). Zebra mussels directly consume microzooplankton (MacIsaac et al. 1991, MacIsaac et al. 1995) and act as a resource competitor by reducing phytoplankton abundance (Fahnenstiel et al. 1995, Heath et al. 1995, Caraco et al. 1997, Idrisi et al. 2001). Thus, it is not surprising that invasion of freshwater habitats by the exotic zebra mussel (*Dreissena polymorpha*) has been associated with large declines in microzooplankton abundance (MacIsaac et al. 1995, Pace et al. 1998).

Negative effects of *D. polymorpha* on abundance of ciliates and rotifers have been observed largely in well-mixed systems as before and after studies (MacIsaac et al. 1995, Pace et al. 1998) or experimentally (MacIsaac et al. 1995, Lavrentyev et al. 1995, Jack and Thorp 2000, Thorp and Casper 2002, Wilson 2003). The few studies conducted in stratified systems have only examined macrozooplankton (crustaceans, copepods) (Idrisi et al. 2001) or combined zooplankton (crustacean + rotifer) biomass (Yu and Culver 1999). In thermally stratified systems, contact between the pelagic biota and benthic filter

feeders like *D. polymorpha* should be reduced relative to well-mixed systems (MacIsaac and Sprules 1991, MacIsaac 1996, Noonburg et al. 2003). Thus, we might expect *D. polymorpha* impacts on the plankton to be weaker in stratified systems. The ongoing *Dreissena* invasion of inland lakes in the upper Midwest U.S., especially in Michigan, provided an opportunity to examine this question.

Dreissena polymorpha filtering also has the potential to affect ciliate and rotifer biomass differently. The preferred food size range of zebra mussels (5-45 μm, Ten Winkle and Davids 1982, Sprung and Rose 1988) indicates that they should inflict greater mortality on ciliates than rotifers, since ciliate cell sizes are commonly within the preferred range, while rotifers are generally larger (often > 100 μm). However, the abundance of bacteria, a potential food source for microzooplankton, is typically not affected by *D. polymorpha* presence (Cotner et al. 1995, Findlay et al. 1998) because of the small size of bacteria. Ciliates generally consume bacteria more effectively than rotifers. Thus, the relative magnitude of *Dreissena's* predatory effect may be larger for ciliates, while *Dreissena's* competitive effect may be larger for rotifers (Figure 1). It is not obvious whether mussel invasion will have a greater overall impact on ciliates or rotifers. Previous studies have considered the effects of *D. polymorpha* on ciliates and rotifers separately and have not compared differences in magnitude.

Dreissena polymorpha may also have species-specific effects on rotifers and ciliates. Because *D. polymorpha* are size-selective (Ten Winkle and Davids 1982, Sprung and Rose 1988), they may shift the size distribution of ciliates toward species larger than 45μm, for example. Additionally, few studies have examined the effects of *D. polymorpha* on species diversity in general and those that have were focused mainly on

benthic invertebrates (Stewart and Haynes 1994) and native unionids (Herbert et al. 1991). No studies have assessed whether *D. polymorpha* invasion affects the diversity of pelagic assemblages.

In this paper, I examine the influence of *D. polymorpha* invasion in stratified lakes on two groups of microzooplankton, ciliates and rotifers, via a large-scale lake survey. The main questions I address are: 1) do zebra mussels reduce microzooplankton biomass in stratified inland lakes as much as they do in well-mixed systems?, 2) within microzooplankton, are ciliates or rotifers more negatively affected by zebra mussel presence?, and 3) how do zebra mussels impact rotifer diversity?

Methods

Lake Selection

The survey was restricted to low-nutrient lakes (TP < 21 $\mu g\ L^{-1}$) in southern Michigan (Figure 2) with a maximum depth of a least 9 m, such that thermal stratification was likely to be present during the summer. For the purposes of lake selection, the presence/absence of *D. polymorpha* was initially assessed using the list of invaded lakes assembled by the Michigan Sea Grant College Program (www.miseagrant.org). Presence/absence of *D. polymorpha* was later verified in each lake by searching for adults in the field and for veligers in zooplankton samples. To ensure that invaded and uninvaded lakes included in the survey were similar in depth, mean depth was determined by digitizing bathymetric lake maps in ArcView GIS 3.2. Based on these criteria, 46 lakes (24 with *D. polymorpha* and 22 without) were selected and rotifer biomass was assessed in all lakes, while ciliate biomass was only assessed in 38 randomly chosen lakes within each lake group (20 with *D. polymorpha* and 18 without).

Field Sampling

Each lake was visited once, in late summer of 2002 or 2003 (2 August – 4 September 2002, 29 July – 20 August 2003). Samples were taken from the deepest point in each lake, as determined by bathymetric maps and a depth finder. Temperature was measured at 1 m intervals with a Hydrolab Surveyor and Datasonde. A depth-integrated water sample was taken through the entire mixed layer (epilimnion) with a flexible tube (5 cm inner diameter) and placed on ice until processing (approximately 6 hours). Subsamples of epilimnetic water were taken for TP, chlorophyll *a*, and ciliates (125 mL preserved in Lugol's solution). Rotifers were collected by passing 10 L subsample through a 35-μm mesh screen and rinsing organisms on the screen into sample bottles containing glutaraldehyde (final concentration: 2%).

Laboratory Analysis

TP was analyzed colorimetrically (Langner and Hendrix 1992) after persulfate oxidation. For chlorophyll *a* analysis, samples were filtered onto a Gelman A/E glass fiber filter and kept frozen. The filter was subsequently extracted with 95% ethanol and chlorophyll *a* was quantified using a Turner Model 10-AU fluorometer calibrated with standards (Welschmeyer 1994). Dissolved organic carbon (DOC) was measured with a high-temperature combustion DOC analyzer. Ciliates were identified and enumerated with the inverted microscope technique using a Nikon model TE2000-S inverted microscope. Subsamples (subsample volume 30 – 100 mL) were settled in tubular chambers (Hydro-Bios), the bottoms of which were divided into inner and outer zones of equal area (Sandgren and Robinson 1984). Within each zone, at least 20 random fields were counted at 100x. Ciliate cell volume was determined by measuring at least five

individuals per taxon at 400x using a digital camera and image-analysis software. Biovolume was calculated by using a geometric equation appropriate for the species shape. Biovolume was converted to dry biomass assuming a specific gravity of 1 g cm^{-3} and a dry mass to wet mass ratio of 0.10. Rotifers were identified to species using a Nikon model E600 compound microscope at 100x and a Sedgwick-Rafter counting chamber. For each lake, approximately 400 individuals (average 394, range 341 – 475) were counted in a minimum of 2 subsamples. Dry biomass for each species was estimated from established literature values (Pauli 1989). Rotifer richness was quantified as the total number of species in each lake and diversity was assessed using the Shannon index, (Shannon and Weaver 1949), $H' = - \Sigma(p_i \ln p_i)$, where p_i is the proportion of individuals found in the i^{th} species.

Statistical analyses

There was not an effect of year sampled on any variable ($P > 0.10$), so t-tests were used to determine the influence of *D. polymorpha* on all variables. Rotifer assemblage structure was reduced via principal components analysis (PCA) on the relative biomass of each genus. To reduce zeros in the data set, the genus level was used for the PCA. I restricted the PCA to the following common taxa: *Ascomorpha*, *Colletheca*, *Conochilus*, *Keratella*, *Polyarthra*, *Synchaeta*, and *Trichocerca*. Data were log-transformed as necessary so that assumptions for parametric statistics were met. All analyses were conducted in SYSTAT 9.0.

Results

To rule out the influence of potential confounding factors, I assessed whether invaded and uninvaded lakes were similar in morphometry and nutrients. There were no

significant differences in either mean depth or TP between the two groups of lakes (Table 1). Average epilimnetic temperatures were similar between years (2002 average = 25.4, 2003 average = 25.2), and for all response variables examined, there were no interactive effects of year ($P > 0.1$).

Total ciliate biomass was 45% lower in invaded lakes (Figure 3). Similarly, total rotifer biomass was 44% lower in lakes with *D. polymorpha* (Figure 3). Chlorophyll *a* was 26% lower in invaded lakes (Table 1, Figure 4A) and there was no difference in DOC between lake categories (Figure 4B).

In order to examine if *D. polymorpha* presence affected the size distribution of ciliates, I divided ciliates into two size classes (< 30 μm and > 30 μm). The biomass of ciliates in the < 30 μm range was similar between lake categories, but the biomass of > 30 μm ciliates was significantly lower in invaded lakes (Figure 5B). As a consequence, zebra mussel invasion significantly shifted the ciliate assemblage toward dominance by smaller size category.

Uninvaded lakes had 1.2 times higher rotifer richness (Figure 6A) and 1.2 times higher diversity as measured by the Shannon index (Figure 6B). By using PCA, rotifer relative biomass was reduced to two factors that explained 46% of the total variance. Zebra mussel presence was significantly related to PCA factor 1 (Figure 7), but not to factor 2 (Figure 7). Factor 1 PCA scores were negatively correlated with *Keratella* relative biomass although is was not significant ($r = -0.82$, $P = 0.15$, t-test). Whereas, *D. polymorpha* was positively correlated to *Polyarthra* relative biomass ($r = 0.71$, $P = 0.028$, t-test) suggesting that *D. polymorpha* had a positive influence on *Polyarthra*.

Discussion

My results suggest that *D. polymorpha* has strong negative effects on microzooplankton in stratified inland lakes (Figure 3). However, the magnitude of effect of zebra mussels on microzooplankton appears to be weaker in stratified lakes. In my study, lakes with *D. polymorpha* had 44% lower rotifer biomass and 45% lower ciliate biomass. Experimental studies have shown *D. polymorpha* presence to reduce ciliate biovolume by 77% (Wilson 2003) and protozoan abundance by 70-80% (Lavrentyev et al. 1995). In the Hudson River, total zooplankton biomass declined by 70% (Pace et al. 1998) and mean total zooplankton density (excluding ciliates) was 55-71% lower in Lake Erie (MacIsaac et al. 1995) following *D. polymorpha* invasion, and in both cases, reductions were mainly attributed to negative effects on rotifers. The lesser reduction in my survey compared to Lake Erie and Hudson River may be attributable to mixing regime. The plankton in shallow, well-mixed systems may experience greater *D. polymorpha* impacts than deep, stratified systems (MacIsaac and Sprules 1991, MacIsaac 1996, Noonburg et al. 2003) because pelagic organisms are more likely to come into contact with benthic populations of *D. polymorpha* in systems lacking distinct vertical stratification.

Although it was unclear whether *D. polymorpha* would have a greater impact on rotifers or ciliates, I expected *D. polymorpha* to inflict greater predatory effects on ciliates than rotifers and greater competitive effects on rotifers. However, my results show that the magnitude of effect was remarkably similar (45% versus 44%). Although it seems likely that *D. polymorpha* should filter ciliates more effectively than rotifers because ciliates are generally smaller, my results indicate that *D. polymorpha* may filter

rotifers and ciliates similarly. This is surprising given that feeding studies have shown *D. polymorpha* to ingest some size ranges (5-45 μm) more effectively (Ten Winkle and Davids 1982, Sprung and Rose 1988) and the majority of ciliates in my study were in this range (85% in uninvaded and 98% in invaded, based on the biomass in this size range).

The unexpected similar effect of *D. polymorpha* on ciliates and rotifers may also be explained by bacteria abundance and the feeding behavior of microzooplankton. Typically, *D. polymorpha* does not reduce the total abundance of bacteria in systems they invade (Cotner et al. 1995, Findlay et al. 1998) despite their ability to consume bacteria (Roditi et al. 1996, Frischer et al. 1998). Planktonic ciliates feed primarily on other protozoa and phytoplankton (Fenchel 1987), but they also consume bacteria and sometimes rely on bacteria as a key resource (Christophersen et al. 1990). While rotifers can feed on bacteria with limited ability (Arndt 1993), they generally consume phytoplankton between 4-17 μm because particles larger and smaller are difficult for them to ingest (Bogdan et al. 1980, Bogdan and Gilbert 1984). Thus, in invaded lakes ciliates may be able to take advantage of bacteria more easily than rotifers, allowing ciliates to compensate for predation losses and phytoplankton reductions.

It cannot be determined from my survey results whether indirect (competition) or direct (predation) mechanisms account for the negative impact of *D. polymorpha* on microzooplankton. As mentioned above, it is likely that predation is an important factor. However, phytoplankton biomass (measured by chlorophyll *a*) was significantly lower in invaded lakes, as found in previous studies (Fahnenstiel et al. 1995, Caraco et al. 1997, Idrisi et al. 2001). By consuming phytoplankton, *D. polymorpha* are competing with microzooplankton for resources. Thus, it is reasonable to assume that reductions in

phytoplankton, mediated through *D. polymorpha*, could indirectly affect microzooplankton abundance. However, previous studies conclude that *D. polymorpha* predation may be more important than resource competition in reducing microzooplankton abundance (MacIsaac and Sprules 1991, MacIsaac et al. 1995, Thorp and Casper 2002). In these studies, small-bodied zooplankton were primarily reduced while large-bodied were not, even though both compete for resources with *D. polymorpha*. However, these experiments were either conducted in small containers (MacIsaac et al. 1991, MacIsaac et al. 1995), or short-term (Thorp and Casper 2002). In small-scale experiments, predators and prey may experience greater spatial overlap than in thermally stratified lakes, which may exaggerate the importance of predation over resource competition, particularly on smaller organisms (Sarnelle 1997). Short-term experiments may also overstate the importance of predation on microzooplankton because the effects of resource competition often take longer to observe than those of predation (Sarnelle 1997). Furthermore, it is possible that microzooplankton communities could be regulated by macrozooplankton in the survey lakes. Macrozooplankton are known to prey on ciliates (Pace and Funke 1991, Burns and Gilbert 1993, Jack and Gilbert 1993, Wiackowski et al. 1994) and compete with rotifers for resources (Gilbert 1985). Consequently, macrozooplankton may be an extremely important covariate and the absence of data on their biomass makes it difficult to conclude that *D. polymorpha* is the sole factor controlling microzooplankton in the survey lakes.

Given that zebra mussels exert more grazing pressure on phytoplankton than bacteria, invasion might lead to a shift in ciliate assemblages toward greater dominance by bacterivorous ciliates. One way to examine this is to divide ciliates into size classes,

ciliates < 30 μm can be roughly considered bacterivorous and those > 30 μm algivorous (Fenchel 1987). By dividing ciliates into these classes, I found a shift toward smaller (<30 μm) ciliates in invaded lakes (Figure 5), indicating bacterivorous ciliates may be more prominent in *D. polymorpha* lakes. The size-selective predation hypothesis would expect < 30 μm ciliates to be more negatively affected by *D. polymorpha* than > 30 μm ciliates. It is possible that smaller ciliates are able to compensate for predatory losses better than larger ciliates because smaller ciliates may not be experiencing such severe resource competition with *D. polymorpha*. Because I did not measure bacteria, I cannot determine if bacteria abundance was similar in invaded and uninvaded lakes. However, DOC was similar between lake categories (Figure 4) and since bacteria use DOC (Figure 1), this indicates that bacteria resources may not differ between uninvaded and invaded lakes.

Although evidence suggests that *D. polymorpha* increases benthic invertebrate diversity (Stewart and Haynes 1994) but decreases native unionid diversity (Herbert et al. 1991), no studies have investigated possible diversity changes in pelagic organisms. Both rotifer richness and diversity were lower in invaded lakes. Lower richness might be attributed to *D. polymorpha*'s ability to filter large quantities of material that span a wide size range (Ten Winkel and Davids 1982). A reduction in richness may indicate that *D. polymorpha* strongly preys on and competes with rotifers. Only rotifer species with effective defense mechanisms (e.g. spines, large size, escape mechanisms) may be able to avoid ingestion, so rotifers without such defenses should be severely affected in zebra mussel lakes. For example, faster swimming species may be able to escape *D. polymorpha* filtering currents (MacIsaac and Sprules 1991). The only taxa that differed

between uninvaded and invaded lakes was *Polyarthra* and its relative biomass was significantly higher in invaded lakes. Unlike most rotifers, *Polyarthra* is able to avoid predators (e.g. *Asplanchna*, *Chaoborus*, *Daphnia*) by a jump mechanism (Gilbert and Williamson 1978, Gilbert 1985, Gilbert 1987, Moore and Gilbert 1987). *Polyarthra* may be able to escape *D. polymorpha* through this jump mechanism. However, *Polyarthra* abundance was dramatically reduced by *D. polymorpha* presence in experiments (MacIsaac and Sprules 1991, MacIsaac et al. 1995, Thorp and Casper 2002), the Hudson River (Pace et al. 1998), and Lake Erie (MacIsaac et al. 1995). In these studies, particularly the experiments, *Polyarthra* may come into such close proximity with *D. polymorpha* that its jump mechanism cannot facilitate escape. However, *Polyarthra* is a selective feeder on cryptophyte phytoplankton (Gilbert and Bogdan 1984) and the biomass of the cryptophyte, *Cryptomonas* was found to be higher in invaded lakes (Chapter 1), thus possibly allowing *Polyarthra* to withstand relatively high predation rates. It must also be noted that because I sampled each lake only once per summer, my rotifer diversity results might represent natural community fluctuations since rotifer communities can change rapidly. However, I restricted my study so that lakes were sampled within a few weeks of each other during a stable time of late summer stratification. Controlling for time should help alleviate problems associated with natural fluctuations.

My results show that *D. polymorpha* has the potential to significantly alter the ciliate and rotifer community assemblages. The data also suggest that similar to well-mixed systems, microzooplankton are negatively impacted by *D. polymorpha* in stratified inland lakes. However, the magnitude of effect may be weaker in stratified than in well-

mixed systems. Despite this potential dampening, the considerable reduction of microzooplankton (25 %) in *D. polymorpha* lakes may extend to higher trophic levels. For example, reduced zooplankton biomass may decrease larval or planktivorous fish growth rates. However, if no negative effects of *D. polymorpha* on macrozooplankton are seen, fish may not be affected because an adequate food supply may still be available. Thus, it is important to determine how *D. polymorpha* affect macrozooplankton in stratified systems.

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Table 1. Average, range, and standard deviation of physical and biological parameters in uninvaded and invaded lakes. P values are for t-tests.

Variable	Uninvaded		Invaded		P values
	Average (range)	SD	Average (range)	SD	
Mean depth (m)	5.04 (2.31-8.8)	2.17	6.17 (2.13-12.41)	2.76	0.17
Total phosphorus ($\mu g\ L^{-1}$)	10.75 (4.9-20.76)	4.28	10.53 (5.26-19.25)	3.8	0.94
Chlorophyll *a* ($\mu g\ L^{-1}$)	4.48 (1.91-9.29)	1.82	3.33 (0.89-6.2)	1.19	0.04

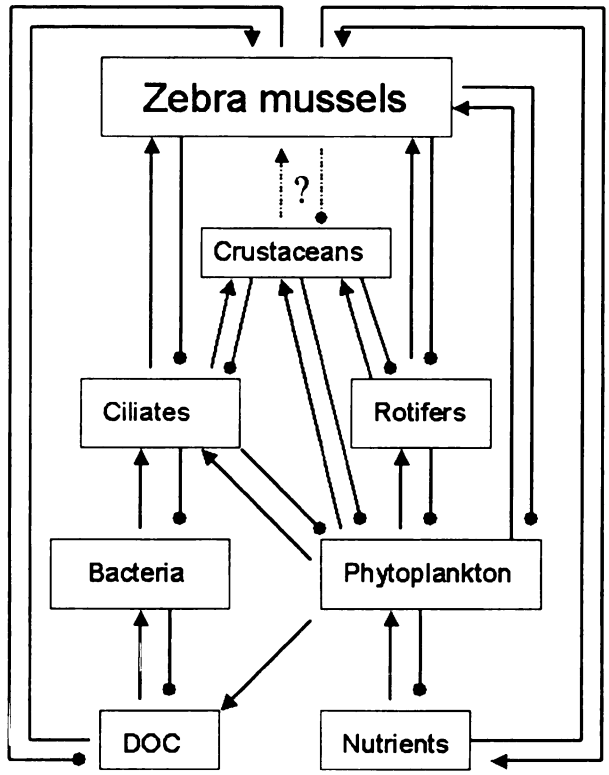

Figure 1. Schematic representation of hypothesized direct and indirect effects of *D. polymorpha* on lower trophic levels of lake ecosystems. Arrows represent a positive effect and circles represent a negative effect.

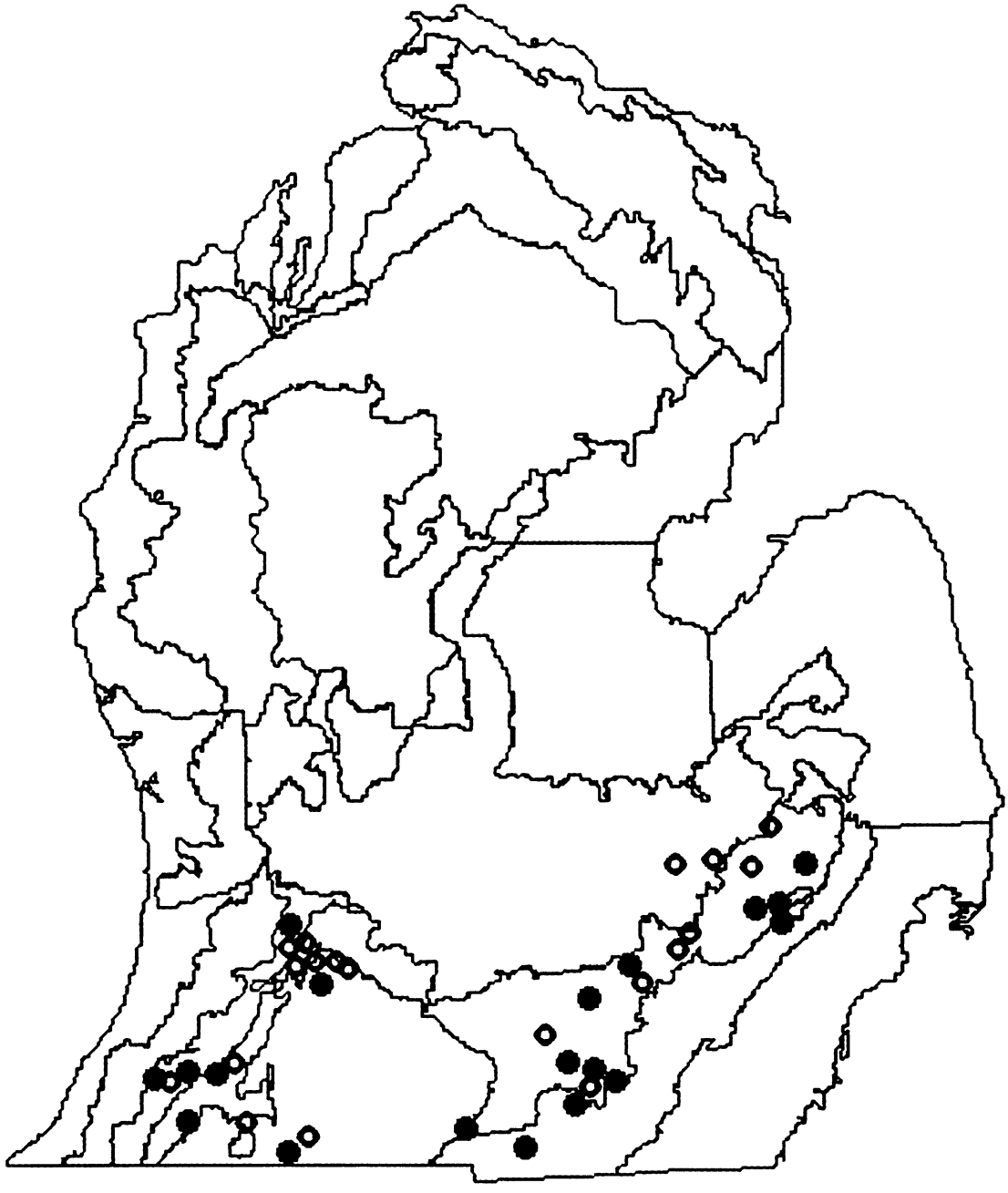

Figure 2. Location of survey lakes in the lower peninsula of Michigan. The state is divided into Albert ecoregion subsections. Closed circles represent invaded lakes and open circles represent uninvaded lakes.

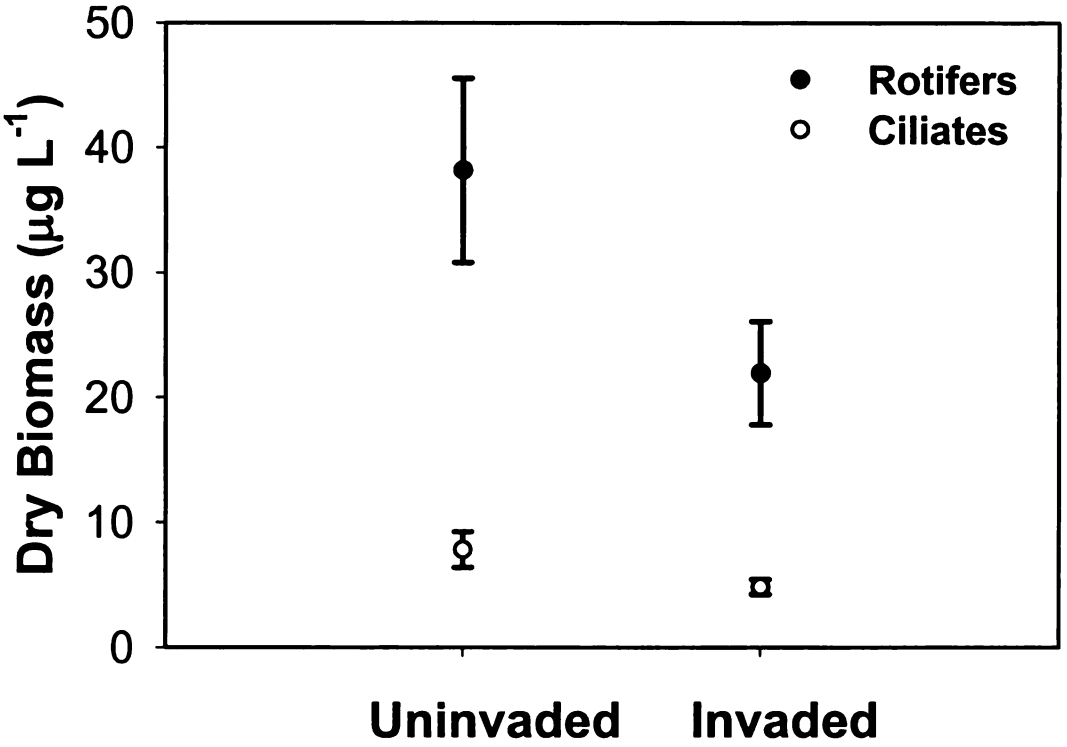

Figure 3. Influence of *D. polymorpha* on ciliate and rotifer dry biomass. Both ciliate and rotifer biomass were significantly lower in *D. polymorpha* lakes (P = 0.009, 0.01, respectively). P values are for t-tests. Bars are SE.

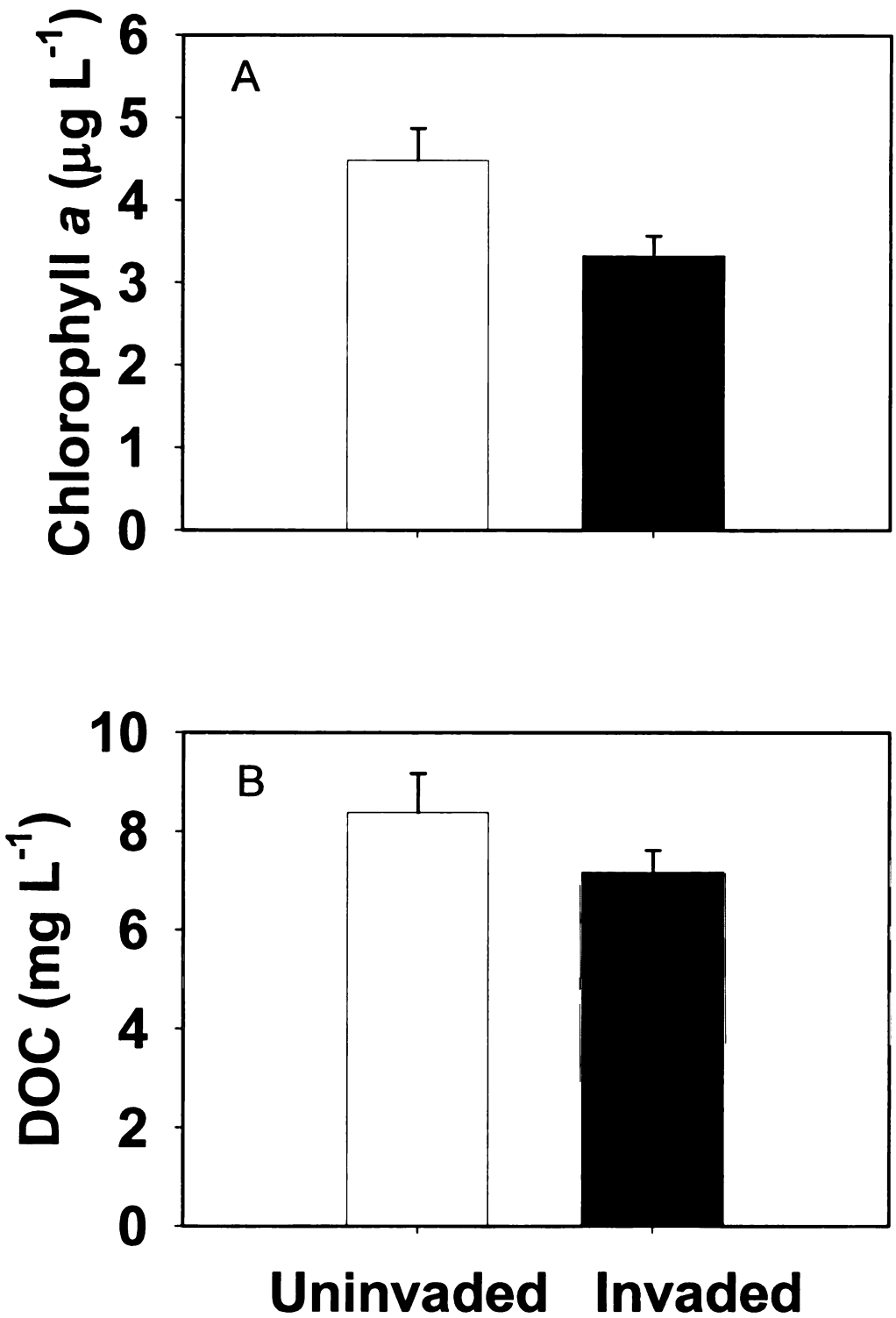

Figure 4. The effect of *D. polymorpha* on (A) chlorophyll *a* (P = 0.02) and (B) DOC (P = 0.23) in the survey lakes. P values are for t-tests. Bars are SE.

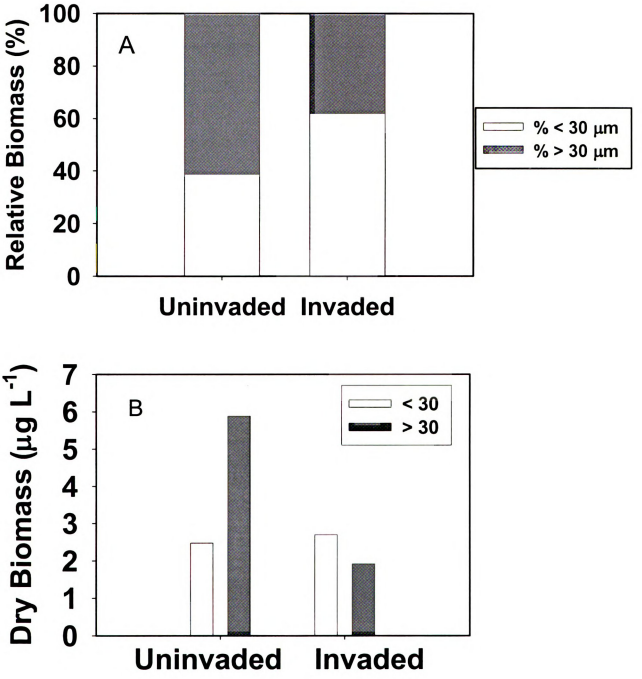

Figure 5. (A) Relative biomass (%) and (B) actual biomass of ciliates below 30 μm in size (bacterivorous) and above 30 μm (algivorous) in the survey lakes. Ciliates above 30 μm were higher in uninvaded lakes for both relative biomass and actual biomass ($P = 0.01$, 0.003, respectively). The actual biomass of ciliates below 30 μm were similar between lake categories ($P = 0.75$). P values are for t-tests.

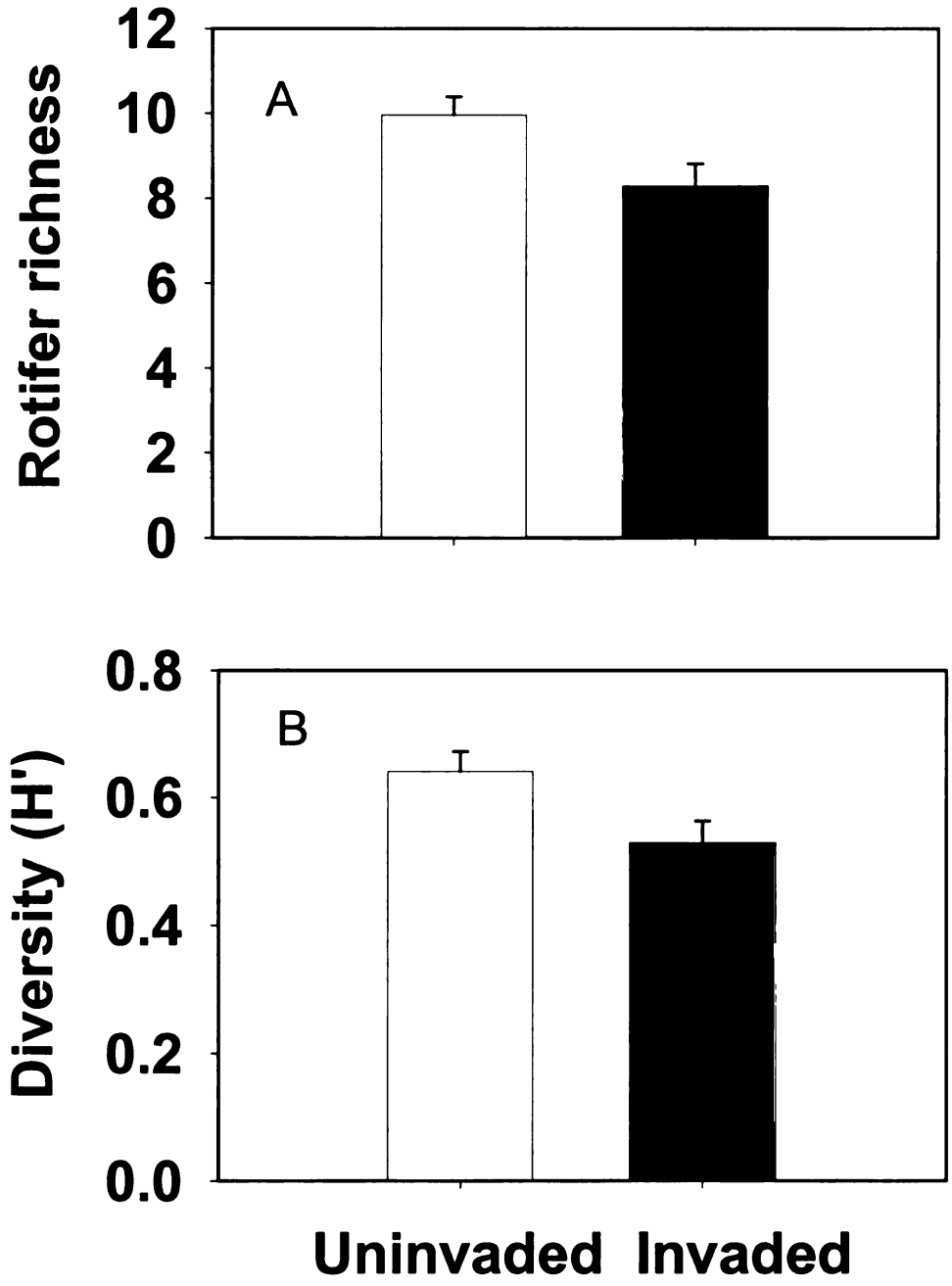

Figure 6. The influence of *D. polymorpha* on (A) rotifer richness (P = 0.01) and (B) diversity (P = 0.02). Both richness and diversity were lower in invaded lakes. P values are for t-tests. Bars are SE.

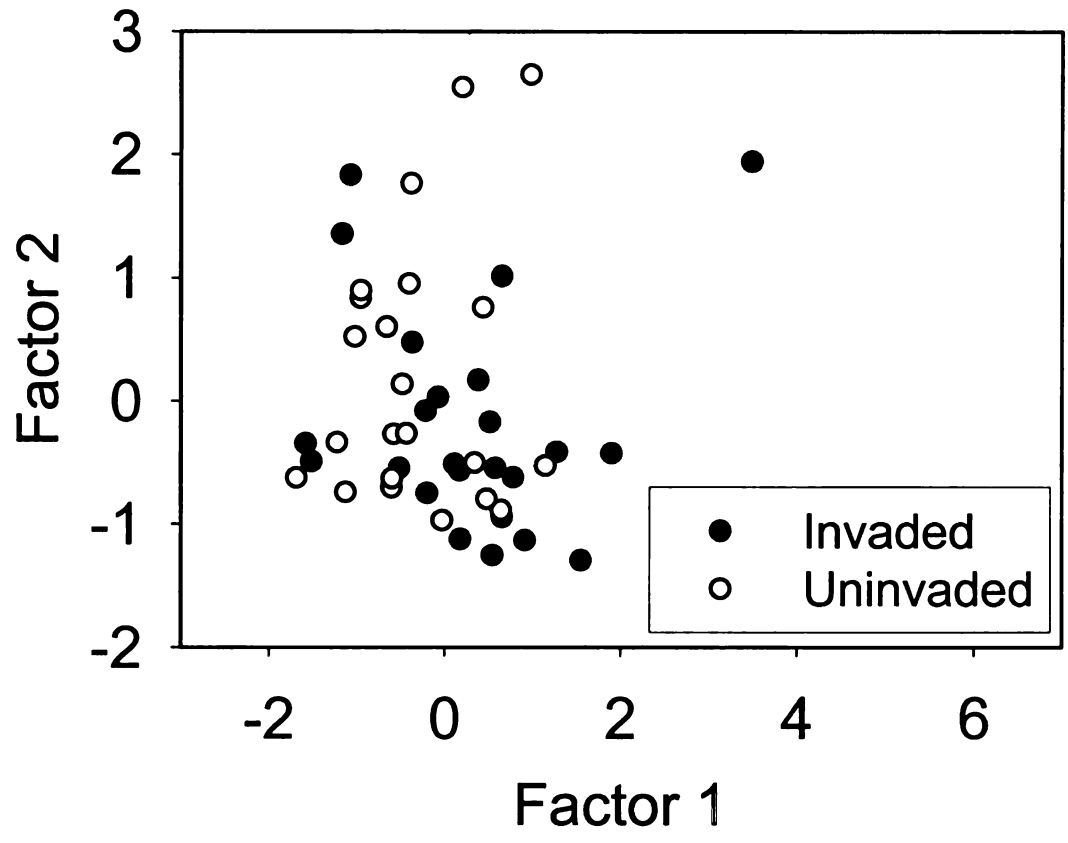

Figure 7. Factor scores from the rotifer PCA. Factor 1 (P = 0.036) and Factor 2 (P = 0.2). P values are for t-tests.

APPENDIX

INDIVIDUAL SURVEY LAKE INFORMATION

Appendix A. Lake name, county, year sampled, mean depth, total phosphorus (TP), and chlorophyll *a* of survey lakes.

Lake	County	Status	Year sampled	Mean depth	TP $\mu g\ L^{-1}$	Chl *a* $\mu g\ L^{-1}$
Banksons	Van Buren	Uninvaded	2003	3.60	14.16	4.29
Big Fish	Lapeer	Uninvaded	2002	5.66	5.52	7.36
Big Portage	Jackson	Invaded	2002	3.16	15.57	3.48
Big Seven	Oakland	Uninvaded	2002	3.07	10.41	5.50
Bird	Hillsdale	Invaded	2003	9.37	9.63	2.98
Bishop	Livingston	Uninvaded	2002	2.34	6.33	3.93
Bristol	Barry	Uninvaded	2003	6.47	11.65	3.06
Cass	Oakland	Invaded	2002	9.17	9.83	2.46
Cedar	Van Buren	Invaded	2003	7.37	9.29	0.89
Cedar Island	Oakland	Invaded	2003	8.68	6.27	2.21
Clark	Jackson	Invaded	2002	3.56	19.25	2.37
Corey	St. Joseph	Uninvaded	2003	7.79	8.62	2.67
Deep	Lenawee	Uninvaded	2003	7.20	10.96	2.82
Devils	Lenawee	Invaded	2003	4.19	11.84	4.29
Diamond	Cass	Invaded	2002	5.08	9.32	5.25
Donnell	Cass	Invaded	2003	8.41	6.94	2.55
Fine	Barry	Uninvaded	2002	3.38	10.68	4.28
Fish	Barry	Uninvaded	2002	8.80	7.78	2.36
Fish	St. Joseph	Uninvaded	2002	8.19	7.00	3.24
Gilkey	Barry	Uninvaded	2002	4.99	4.90	4.20
Gravel	Van Buren	Invaded	2002	5.61	12.29	3.84
Gull	Kalamazoo	Invaded	2002	12.41	5.32	1.95
Gun	Barry	Invaded	2002	2.98	11.38	4.84
Halfmoon	Washtenaw	Invaded	2003	8.50	10.25	2.67
Hemlock	Hillsdale	Invaded	2003	9.91	11.31	4.24
Heron	Oakland	Uninvaded	2002	3.33	11.23	4.23
Klinger	St. Joseph	Invaded	2003	6.43	8.12	3.84
Lake of the Woods	Van Buren	Invaded	2002	4.36	8.30	2.79
Lake Orion	Oakland	Invaded	2002	5.32	15.67	2.49
Lee	Calhoun	Uninvaded	2003	8.18	6.61	1.91
Lobdell	Livingston	Uninvaded	2002	3.87	10.36	6.03
Magician	Cass	Invaded	2002	2.13	12.69	4.02
North	Washtenaw	Uninvaded	2003	3.65	14.50	6.12
Orchard	Oakland	Invaded	2002	7.11	7.38	2.20
Payne	Barry	Invaded	2003	4.91	9.46	3.63

Table 1 (cont'd)

Lake	County	Status	Year sampled	Mean depth	TP $\mu g\ L^{-1}$	Chl *a* $\mu g\ L^{-1}$
Pine	Barry	Uninvaded	2002	3.24	11.01	5.48
Saddle	Van Buren	Uninvaded	2003	2.62	20.76	6.48
Sand	Lenawee	Invaded	2003	3.52	11.67	4.14
Stone	Cass	Uninvaded	2003	5.73	13.16	2.40
Union	Oakland	Invaded	2003	8.92	5.26	2.83
Upper Crooked	Barry	Uninvaded	2002	2.87	19.32	5.13
Vandercook	Jackson	Uninvaded	2002	6.86	15.82	9.29
Vineyard	Jackson	Invaded	2002	4.30	6.94	3.66
Wamplers	Jackson	Invaded	2003	2.66	18.74	6.20
Warner	Barry	Uninvaded	2002	6.75	5.64	3.81
Woodland	Livingston	Uninvaded	2002	2.31	10.12	3.99