JOURNAL OF PLANKTON RESEARCH | VOLUME 25 | NUMBER 8 | PAGES 905–915 | 2003

Effects of zebra mussels on phytoplankton and ciliates: a field mesocosm experiment

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Many observational studies in North American lakes have documented decreases in phytoplankton abundance after the invasion of the zebra mussel (Dreissena polymorpha). However, few field experiments have examined in detail the effect of zebra mussels on phytoplankton abundance and species composition over an extended period. Replicated in situ mesocosms were used to evaluate the impact of natural densities of zebra mussels on phytoplankton and ciliate biovolume, and algal species composition over a 5-week period in a habitat that lacked extant mussel populations. Mussel biomass used in the experiment was determined using a regression model based on a data analysis that predicts zebra mussel biomass from total phosphorus concentration. Within 1 week, zebra mussels decreased phytoplankton biovolume by 53% and ciliate biovolume by 71%. The effect of zebra mussels on ciliate biovolume was sustained throughout the study. However, the effect of zebra mussels on phytoplankton abundance gradually waned over the remaining 4 weeks of the experiment, such that the declining effect of zebra mussels could not be explained by a shift towards less edible and/or faster growing algal species. The mussels' declining condition could help to explain the effect observed over the course of the experiment.

INTRODUCTION

Since their introduction into North America in the mid-1980s, zebra mussels (*Dreissena polymorpha*) have been implicated in significant ecosystem-level changes, including a shift in energy flow from the pelagic to the benthic zone (Johengen *et al.*, 1995; Arnott and Vanni, 1996; Lavrentyev *et al.*, 2000), declines in microzooplankton and phytoplankton biomass (Holland, 1993; MacIsaac *et al.*, 1995; Bastviken *et al.*, 1998), and shifts in phytoplankton species composition from communities dominated by palatable species, like diatoms, to inedible blue-greens (Lowe and Pillsbury, 1995) and vice versa (Reeders and de Vaate, 1990; Caraco *et al.*, 1997; Yu and Culver, 2000).

Several manipulative studies have been performed to assess the effects of zebra mussels on phytoplankton and ciliates (Heath *et al.*, 1995; Lavrentyev *et al.*, 1995; Roditi *et al.*, 1996; Jack and Thorp, 2000). Of these, laboratory experiments have documented zebra mussel feeding preferences for particular groups of protozoans and algae (Lavrentyev *et al.*, 1995; Bastviken *et al.*, 1998), as well as decoupling of a well-established total phosphorus–chlorophyll relationship (Mellina *et al.*, 1995). Although short-term laboratory studies can be useful for examining small-scale effects on individual species and populations, they are inappropriate for studying community processes such as shifts in species composition. For questions of this type, larger-scale experiments or observational studies are needed. Of those experiments aimed at examining the effects of zebra mussels on phytoplankton communities in the field, only three were large-scale field experiments (Reeders and de Vaate, 1990; Heath *et al.*, 1995; Jack and Thorp, 2000). The first of these was unreplicated and the other two studies each lasted less than a week. Because phytoplankton and ciliates can take several weeks to establish a new community equilibrium after a disturbance (Sarnelle, 1993, 1997), studies aimed at examining the effect of a newly introduced grazer on these guilds should allow enough time for populations to attain such an equilibrium. Otherwise, results from relatively brief studies may lead to wrong conclusions. In this paper, the effect of zebra mussels on the phytoplankton and ciliate

community is studied with a 5-week replicated field mesocosm experiment. Specifically, the following questions are addressed:

Do zebra mussels decrease phytoplankton abundance, and if so, over what time scale?
Do zebra mussels cause a shift in phytoplankton species composition from palatable to unpalatable species?
How does the effect of zebra mussels on ciliates compare with their effects on phytoplankton?

METHOD

Experimental set-up

This experiment was performed in an experimental pond (30 m diameter by 1.8 m depth) at the Kellogg Biological Station (Michigan State University, Hickory Corners, MI) that was almost completely surrounded by cattails (*Typha* sp.). The pond contained abundant macrophytes, sunfish (*Lepomis* spp.) and macroinvertebrates, but no zebra mussels.

Two treatments with four replicates each were used in the experiment: zebra mussels present and zebra mussels absent (control). The enclosures were constructed out of clear polyethylene (1.13 m diameter by 1.5 m depth), sealed at the bottom, and supported by floating frames. A fine-mesh insect screen covered the enclosures to prevent pond organisms from entering them.

The enclosures were filled on June 15, 1999 with pond water pumped through a 149 μm mesh net to remove all macrozooplankton. Zooplankton were then collected from the pond with a 102 μm mesh net and stocked at natural abundances into each enclosure on two occasions (June 22 and July 9). The second stocking was performed because zooplankton densities were lower in both sets of enclosures relative to the pond early in the study. Despite the additional stocking, zooplankton densities were higher in the pond than the treatments throughout the experiment.

To prevent large zooplankton from dominating in the enclosures, one juvenile bluegill (*Lepomis macrochirus*; 40 ± 1 mm total length) was added to each enclosure, which was below the natural density of bluegill for local lakes (Mittelbach, 1988). The fish were seined from a local lake on May 27 and held in aquaria until they were transferred to the enclosures on June 18. The enclosures were checked daily for fish mortality. Dead fish were removed and immediately replaced with a similar-sized fish. A total of five fish were replaced during the course of the experiment, but fish mortality did not have a significant impact on any measured responses (ANOVA; $P > 0.35$).

To estimate a reasonable stocking density of zebra mussels for the experiment (≈1 g m^{-2}), an equation that predicts zebra mussel dry tissue biomass (g m^{-2}) from total phosphorus (TP) concentration (μg l^{-1}) was used

$$\text{Dry tissue biomass} = -10.8 + 11.0 \times \log_{10} \text{TP}$$

[$R^2 = 0.19$, $P < 0.04$, $n = 24$; (Wilson and Sarnelle, 2002)]. On May 20, zebra mussels were collected from Gull Lake and excessive detritus was gently scraped from each mussel with a coarse scouring pad. Next, all mussels between 10 and 20 mm were placed into a flow-through tank (2.5 × 0.3 × 0.3 m) where they were allowed to attach to one of eight substrates made from PVC pipe (0.1 m diameter, 0.5 m length and 0.05 m thickness) cut lengthwise to create symmetrical pipe halves. Fresh water was pumped directly from Gull Lake (19 l min^{-1}) and filtered for large debris with a 1 mm mesh net before entering the tank. Settled detritus and faeces were siphoned from the tank twice daily.

To determine the relative condition of the mussels before and after the experiment, length–weight relationships were derived from randomly chosen mussels. Before the experiment, 50 randomly chosen fresh mussels (mean length = 13.9 mm, range 6.3–24.4 mm) were collected from the flow-through tank and measured with callipers to the nearest 0.1 mm. The soft body tissue was removed, dried at 55°C for 22 h, and weighed to the nearest ±0.01 mg. Post-experimental mussels were removed from the experimental substrates at the conclusion of the study and frozen. Twenty-five post-experimental mussels from each enclosure (for all post-experimental mussels: mean length = 13.7 mm, range 9.0–21.0 mm) were later thawed and a length–weight relationship for post-experimental mussels was derived. Because dry weight can be lost through freezing (J. Chiotti, A. E. Wilson and T. Toda, unpublished data), a correction factor was used to adjust the dry weight of frozen samples. To determine the correction factor, additional mussels were collected from Gull Lake and the length–weight protocol was repeated using a set of fresh mussels versus a matched set that had been frozen. The correction factor derived from a linear regression of frozen dry tissue mass on fresh dry tissue mass was:

$$\text{Frozen (g)} = 1.3406 \times \text{fresh (g)} - 0.0003$$
$$(R^2 = 0.997,\ P < 0.001)$$

On Day 1 (June 21), one substrate was hung vertically in the middle of each of the eight enclosures. Before deployment, zebra mussels were removed from four of the eight mussel substrates and these substrates were placed into the control enclosures. Mussels remaining on the four treatment substrates were counted [162 ± 10 mussels per

substrate (mean ± standard error)] and measured prior to deployment [12.2 ± 2.7 mm length (mean ± standard error)], and the average initial dry tissue biomass used in each enclosure was 1.2 ± 0.1 g (dry tissue, mean ± standard error), which is within the 95% confidence intervals predicted for the TP concentration of the pond (13 μg l^{-1}). Zebra mussels were monitored at each sampling date for mortality.

Sampling and laboratory analyses

The enclosures and the pond were sampled from a small boat on June 20 (Day 0), June 21 (Day 1), and at 7-day intervals for the next 5 weeks (n = 37 days).

A YSI multisensor (model 600XL) was used to measure temperature (°C), pH and dissolved oxygen (mg l^{-1}) at three depths (surface, 0.5 m and 1.0 m) in each enclosure and the pond. Readings were averaged over all depths for all analyses.

A rigid plastic tube was used to take integrated water samples (≈2 l per tube) from the enclosures and the pond. The contents of two tubes were placed into 10 l plastic containers and stored in the dark on ice.

After thoroughly mixing each sample, water was filtered through a Gelman A/E filter for particulate phosphorus analysis (PP) and dried until further analysis. Filtrate was collected for analyses of total dissolved phosphorus (TDP) and ammonium (NH_4^+). PP and TDP were determined spectrophotometrically (Lambda 20; Perkin Elmer) after potassium persulphate digestion (Menzel and Corwin, 1965). NH_4^+ was measured by indophenol blue colorimetry (Wetzel and Likens, 1991). TP was calculated as the sum of PP and TDP. Water was filtered for chlorophyll *a* analysis (μg l^{-1}) and measured fluorometrically (Turner Designs 10A) after dark extraction in 95% ethanol for 30 h (Jespersen and Christoffersen, 1987).

Samples from each enclosure and the pond were collected on Days 8, 22 and 36, and preserved in 1% Lugol's solution for the determination of algal and ciliate abundance and phytoplankton species composition (Wetzel and Likens, 1991). Depending on the chlorophyll concentration, 10–100 ml aliquots from each enclosure were settled in Utermöhl settling chambers. Each chamber was divided into circular inner and outer halves and an equal number of visual fields was counted in each half (Sandgren and Robinson, 1984). At least 15 fields per chamber half were counted for the most abundant species and at most 100 fields from each half were counted for most species. Phytoplankton were identified and enumerated to genus or species with an inverted microscope at ×400 and ×1000.

Phytoplankton were grouped into six categories according to morphological and functional characteristics, as well as abundance. These groups consisted of small greens <10 μm (*Elakatothrix* spp., *Nannochloris* spp., *Oocystis* spp.), dinoflagellates (*Ceratium* spp., *Peridinium* spp.), cryptomonads (*Cryptomonas erosa*, *Cryptomonas pusilla*, *Rhodomonas* spp.), miscellaneous flagellates <10 μm (*Dinobryon* spp., *Trachlemonas* spp.), colonials (*Uroglenopsis* spp.) and others [desmids (*Closterium* spp., *Cosmarium* spp., *Staurastrum* spp.), diatoms (*Nitzschia* spp., *Synedra* spp.) and filamentous greens]. For each sample, 10 randomly selected individuals of each common species were measured with a micrometer, and these measurements were used to calculate phytoplankton biovolume (μm^3 ml^{-1}). Average phytoplankton biovolume per cell for each algal species did not vary among treatments or dates, so a single average (across treatments and dates) cell volume was calculated for each species.

Ciliates were counted and measured in the same manner as the phytoplankton, although ciliates were not identified into specific categories. Unlike phytoplankton biovolume, average ciliate biovolume differed significantly ($P < 0.05$) between treatments, so separate average cell volumes for ciliates were used from zebra mussel enclosures, control enclosures and the pond for each sampling date. Ciliate and phytoplankton biovolumes were converted to dry biomass (μg l^{-1}) assuming a specific gravity of 1 and a dry mass to wet mass ratio of 0.10.

Macrozooplankton were sampled by pouring the contents of seven integrated tubes (≈14 l) through a 102 μm mesh net, and were preserved in 95% ethanol (Campbell and Chow-Fraser, 1995). For most dates, the entire sample was counted and measured. Otherwise, the entire sample was poured into a beaker and at least five 2 ml subsamples were taken from the total sample with a Henson–Stempel pipette. At least 50 individuals of each species were measured from each subsample. Zooplankton subsamples were counted with a Ward zooplankton counting wheel at magnifications between ×20 and ×80. Cladoceran taxa included *Bosmina* spp., *Ceriodaphnia* spp., *Chydorus* spp., *Daphnia retrocurva* and *Diaphanosoma* spp. The copepod taxa measured were calanoid juveniles, cyclopoid juveniles, *Diacyclops* spp., *Diaptomus*, *Mesocyclops* spp., *Tropocyclops* spp. and nauplii. Dry biomass (μg l^{-1}) of each species was estimated from measured lengths using regressions derived by Culver *et al.* (Culver *et al.*, 1985).

Data analyses

ANOVA was used to assess treatment effects for most parameters on Days 0, 1, 8 and 36 and on time-averaged data (Days 8–36). If no statistically significant treatment effects were detected for time-averaged data ($P > 0.05$), repeated-measures ANOVA was performed on all data from Day 8 to Day 36 to determine whether any time × treatment interactions were present. None was found, so results from the repeated-measures ANOVA are not

shown. Effect sizes were calculated from treatment means using the following formula:

$$100 \times [(\text{zebra mussel} - \text{control})/\text{control}]$$

Linear regression was used to determine zebra mussel dry body weight (g) from length (mm), and analysis of covariance was used to compare length–weight regressions for mussels analysed at the beginning of the experiment and for zebra mussels in each of the four treatment enclosures at the end of the study. To determine if TP:chlorophyll decoupling had occurred on Day 8, a two-sample *t*-test assuming unknown variance (Welch test) was used. The observed chlorophyll concentrations for the two treatments and the pond were compared with the predicted chlorophyll concentrations from the TP:chlorophyll regression provided in Dillon and Rigler (Dillon and Rigler, 1974). Log transformations were applied to data if they were skewed or their variances were heterogeneous. Arcsine transformations were applied to relative algal biomass estimates. All statistical analyses were performed with SYSTAT 9.0 (SPSS, 1998). The rejection criterion was set at $\alpha < 0.05$.

RESULTS

Physical and chemical parameters

Although no measured parameters differed significantly between treatments on Day 0 (the day before mussels were added), three variables (temperature, TDP and TP) were statistically different 4 h after the addition of the zebra mussels on Day 1 (Table I).

Temperature varied from 22 to 27°C for all enclosures for all days, and the average temperature throughout the experiment was ~25.7°C (Table I). The pH in the

Table I: Treatment and pond averages (Days 8–36), ranges (Days 1–36), P-values and treatment effects {100 × [(zebra mussel – control)/control]} for chemical, physical and biological parameters

Variables	Treatment averages (range: Days 1–36)			Significance of treatment effects (zebra mussels versus controls; *P*-values)				
	Pond	Control	ZM+	5-week average	Day 0	Day 1	Day 8	Day 36
Temperature (°C)	25.86 (22.04–27.21)	25.73 (22.06–26.95)	25.71 (22.31–26.95)	0.32 (–0.08%)	0.05 (–0.76%)	0.01 (1.13%)	0.68 (0.00%)	0.69 (0.00%)
pH	7.35 (7.19–7.44)	7.67 (7.38–7.77)	7.66 (7.36–7.81)	0.83 (–0.13%)	0.93 (0.03%)	0.17 (–0.27%)	0.52 (0.00%)	0.59 (0.55%)
Dissolved oxygen (mg l^{-1})	3.63 (3.07–4.61)	5.57 (4.91–6.93)	5.52 (4.59–7.11)	0.81 (–0.90%)	0.41 (–1.45%)	0.80 (0.66%)	0.10 (–6.52%)	0.63 (2.63%)
Particulate phosphorus (μg l^{-1})	7.55 (2.43–14.61)	2.84 (2.36–3.47)	2.13 (1.60–3.40)	0.01 (–25.12%)	0.15 (–9.22%)	0.22 (–6.82%)	0.02 (–42.77%)	0.21 (–17.23%)
Total dissolved phosphorus (μg l^{-1})	5.52 (3.01–8.26)	3.50 (1.74–7.61)	3.48 (2.11–4.82)	0.84 (–0.63%)	0.12 (17.63%)	0.05 (–41.24%)	0.11 (52.34%)	0.51 (20.87%)
Total phosphorus (μg l^{-1})	13.07 (5.44–19.26)	6.34 (4.10–10.80)	5.60 (4.06–7.45)	0.27 (–11.60%)	0.21 (11.05%)	0.03 (–31.07%)	0.75 (7.72%)	0.84 (–1.05%)
Ammonium (μg l^{-1})	7.89 (4.78–16.71)	9.26 (7.50–11.43)	10.79 (6.95–14.49)	0.02 (16.52%)	0.55 (17.43%)	0.18 (–18.44%)	0.53 (11.78%)	0.99 (–9.51%)
Chlorophyll (μg l^{-1})	2.67 (2.35–2.98)	1.11 (0.76–1.40)	0.59 (0.22–1.07)	0.00 (–46.85%)	0.10 (–15.30%)	0.26 (–16.60%)	0.00 (–71.43%)	0.57 (–23.23%)
Phytoplankton dry biomass (μg l^{-1})	35.30	15.17	9.87	0.03 (–34.91%)	NA	NA	0.00 (–53.11%)	0.69 (–24.33%)
Ciliate dry biomass (μg l^{-1})	2.40	4.23	0.95	0.00 (–77.50%)	NA	NA	0.01 (–70.55%)	0.02 (–80.53%)
Zooplankton dry biomass (μg l^{-1})	6.95	1.48	0.74	0.05 (–50.13%)	NA	NA	0.59 (–81.43%)	0.35 (–63.32%)

On July 19, pH was not recorded because of a malfunction with the electrode.
ZM+, zebra mussel addition; Control, lack of zebra mussels; NA, not available.

enclosures ranged from 7.4 to 7.8 and was similar between treatments (Table I). Similarly, dissolved oxygen concentrations in the enclosures were comparable and averaged ~6 mg l^{-1} in the enclosures (Table I).

TDP tended to decline over the course of the experiment and averaged 3.5 μg l^{-1} for all enclosures (Table I); mean PP and NH_4^+ differed significantly between treatments (P = 0.01 and P = 0.02, respectively, Table I). Particulate phosphorus averaged <3 μg l^{-1} in the enclosures and was consistently higher in the control enclosures. A similar trend was observed for TP (treatments averaged <6.5 μg l^{-1}). As early as Day 8, NH_4^+ was higher in the zebra mussel enclosures, and this effect was maintained throughout the remainder of the experiment (except for Day 36). Ammonium ranged from 7.0 to 14.5 μg l^{-1} in zebra mussel enclosures and from 7.5 to 11.4 μg l^{-1} in the control enclosures.

Biological parameters

Zebra mussels had a significant but ephemeral effect on phytoplankton abundance. Chlorophyll concentrations differed significantly between treatments (5 week average, P = 0.001; Table I) and averaged 1.1 μg Chl *a* l^{-1} in the control enclosures and 0.6 μg Chl *a* l^{-1} in the zebra mussel enclosures. Although a large negative effect on chlorophyll concentration was observed early in the experiment in the zebra mussel enclosures (71.4% less chlorophyll in the zebra mussel enclosures when compared with the control enclosures; Day 8), chlorophyll in the zebra mussel enclosures consistently increased throughout the remainder of the study to eventually reach a concentration no different from that in the control enclosures on Day 0 (P = 0.57; Figure 1a). Additionally, calculation of the zebra mussel effect showed a dramatic decrease soon after mussel introduction (Figure 1b). However, this effect waned over time. The response of total phytoplankton biomass (from microscope counts) paralleled that observed for chlorophyll concentration (Table I).

The Dillon–Rigler TP:chlorophyll equation [from (Dillon and Rigler, 1974)] predicted the chlorophyll concentrations observed in the control enclosures and the pond; however, the observed chlorophyll concentration for the zebra mussel enclosures on Day 8 was significantly lower than predicted (P < 0.0001; Figure 1c). Thus, the negative effect of zebra mussels on phytoplankton biomass does not appear to have been driven by the lower TP concentration observed in the control enclosures.

The effect of zebra mussels on phytoplankton species composition was modest. Two algal groups were significantly lower in the zebra mussel enclosures (cryptomonads, P = 0.003; and small greens, P = 0.01; Table I). When examining phytoplankton species composition on individual days, all groups except colonials were significantly lower in the zebra mussel enclosures when compared with the control enclosures on Day 8 (Table II). However, the effect of zebra mussels on the phytoplankton community composition was not maintained after Day 8. By Day 22,

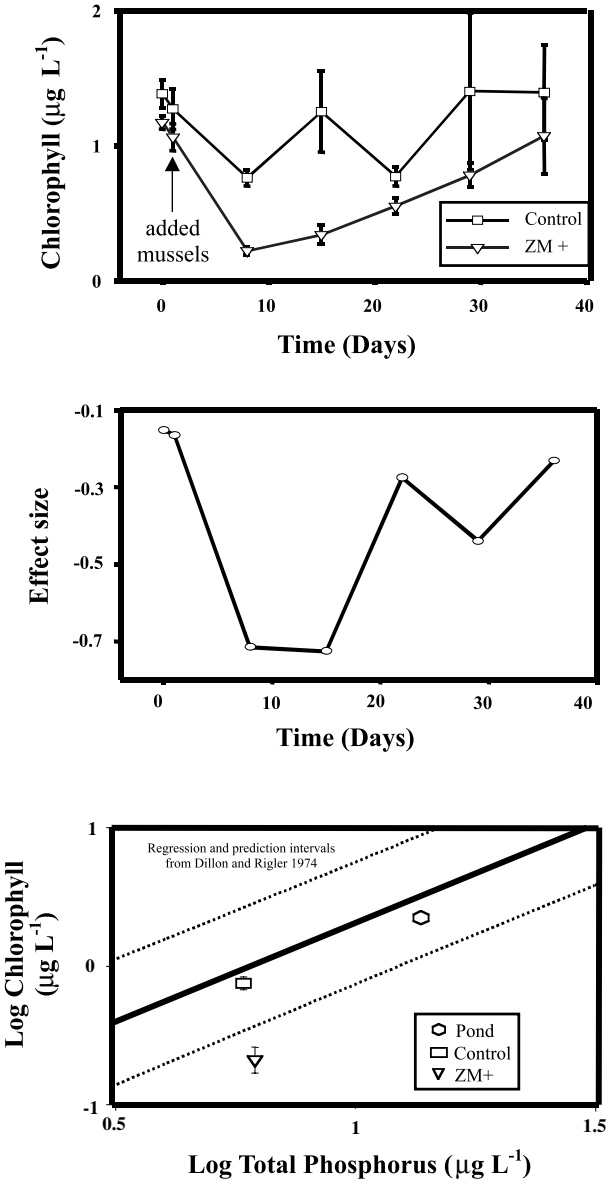

Fig. 1. (Top) Chlorophyll concentration in the control and zebra mussel enclosures (daily treatment averages ± 1 standard error). ZM+ = zebra mussel addition. Control = lack of zebra mussels. (Middle) Measured effect size {100 × [(zebra mussel – control)/control]} of zebra mussels on phytoplankton abundance (measured as chlorophyll *a*) over the 37 days of the experiment. (Bottom) Observed chlorophyll *a* concentrations for Day 8 plotted in relation to the Dillon–Rigler TP–chlorophyll relationship (log Chl *a* = 1.449 × logTP – 1.136). The 95% prediction intervals for Dillon–Rigler are provided. Error bars represent ±1 standard error.

only one group differed significantly between treatments (small greens), and by the end of the study, no groups differed (Table II). The relative phytoplankton biomass data suggest that the mussels were predominantly non-selective in their filtering of the phytoplankton throughout the entire study, with no groups being statistically different on Days 8 and 36, and only one algal group being statistically different on Day 22 (small greens, $P = 0.01$; Figure 2). In addition, time-averaged data showed that only one algal group's relative biomass was significantly different between treatments (5 week average, miscel-laneous flagellates, $P = 0.03$).

Ciliate numbers declined significantly throughout the entire study in the zebra mussel enclosures. Ciliate biomass in the zebra mussel enclosures averaged 77.5% lower than the control enclosures throughout the entire study ($P < 0.0001$; Table I). Ciliate biomass in the zebra mussel enclosures was decreased by 70.6% by Day 8 and

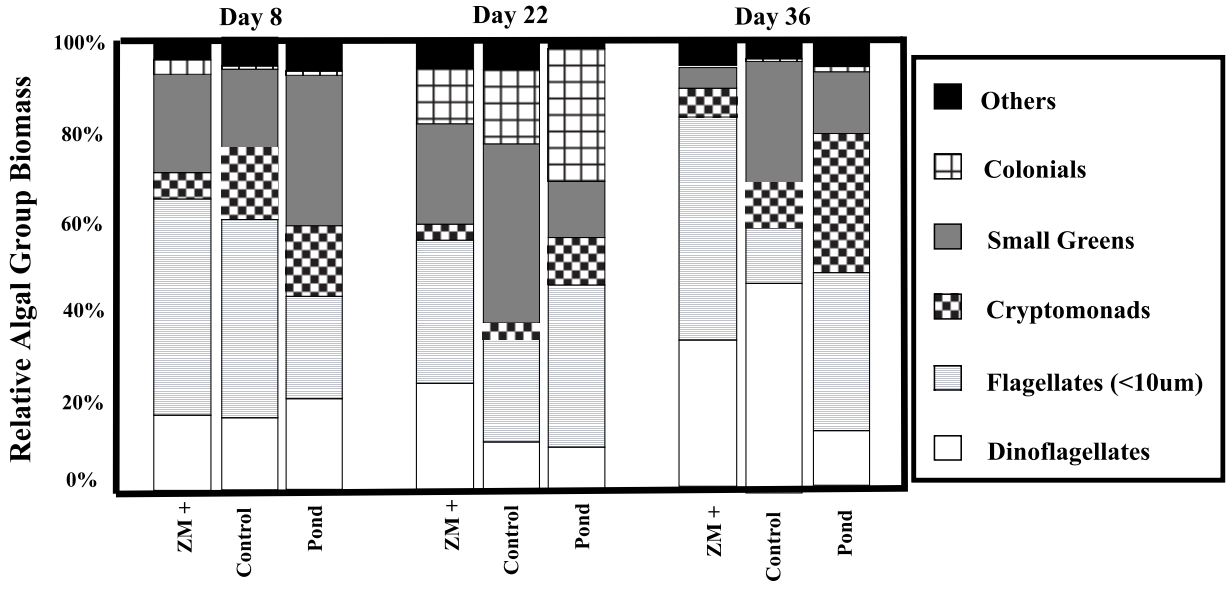

Fig. 2. Relative algal group dry biomass (%) for zebra mussel enclosures, control enclosures and the pond for Day 8, Day 22 and Day 36. ZM+ = zebra mussel addition. Control = lack of zebra mussels.

Table II: Absolute observed treatment differences (ZM+ versus control) in algal groups throughout the study

Algal group	Averages[a]		Day 8		Day 22		Day 36	
	diff.	*P*	diff.	*P*	diff.	*P*	diff.	*P*
Dinoflagellates	–38%	0.44	–51%	0.05	47%	0.24	–46%	0.61
Miscellaneous flagellates (<10 μm)	17%	0.69	–49%	0.00	–2%	0.81	204%	0.20
Cryptomonads	–65%	0.00	–83%	0.00	–33%	0.68	–51%	0.10
Small greens (<10 μm)	–68%	0.01	–41%	0.02	–61%	0.00	–87%	0.08
Colonial	–37%	0.24	133%	0.54	–47%	0.16	–18%	0.54
Other	–32%	0.18	–64%	0.00	–34%	0.32	1%	0.91
Total	–35%	0.03	–53%	0.00	–30%	0.02	–24%	0.69

All groups are measured as total algal dry biomass (μg l^{-1}) and 'Other' denotes desmids, diatoms and filamentous algae.
[a]Average of Days 8, 22 and 36.

was maintained at these lowered levels (or greater) until the end of the study (Figure 3a).

Bosmina spp. and *Diaphanosoma* spp. accounted for >99% of all cladocerans, and calanoid and cyclopoid juveniles accounted for >73% of all copepods for both treatment types. Cladocerans were almost three times less abundant than copepods in the zebra mussel enclosures, but both groups were equally abundant in the control enclosures. Zebra mussels had a significant effect on total zooplankton biomass averaged over all dates ($P = 0.048$; Table I). Zebra mussels significantly decreased total cladocerans throughout the study (time-averaged, $P = 0.035$), but had no effect on copepods.

The first sign of zebra mussel mortality was observed on July 12 (Day 22). Dead mussels were observed throughout the experiment; however, the average total mortality observed in the zebra mussel enclosures over the entire experiment accounted for only 7.6 ± 3.6% (mean ± standard error) of the total density. The mussels that survived were shown to have lost 56% of their initial weight by the completion of the study (Figure 3b). No differences were observed for post-experimental weights between replicates of the zebra mussel treatment (ANCOVA, $P = 0.71$), but there was a highly significant difference between length–weight relationships for pre-experimental mussels [log dry tissue mass (g) = 2.5429 × log length (mm) – 4.9396, $R^2 = 0.93$, $n = 50$] and post-experimental mussels [log dry tissue mass (g) = 2.1455 × log length (mm) – 4.8135, $R^2 = 0.76$, $n = 100$) (Tukey–Kramer test of slopes, $P < 0.0001$), after correcting for the weight lost because of freezing.

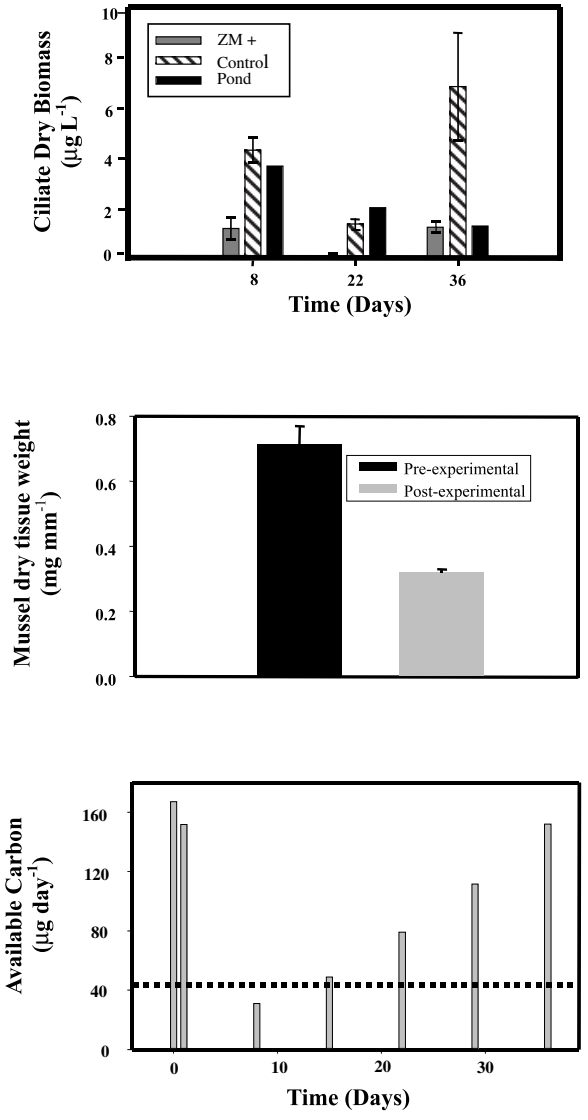

Fig. 3. (Top) Mean ciliate dry biomass (µg l^{-1}, ± 1 standard error) for zebra mussel and control enclosures and pond for Days 8, 22 and 36. ZM+ = zebra mussel addition. Control = lack of zebra mussels. (Middle) Dry tissue biomass (mg mm^{-1}) for average mussel length (12 mm) for pre- and post-experimental zebra mussels. A correction factor for the effect of freezing as a preservative technique was used to determine the post-experimental mussel weights. Error bars represent 1 standard error. (Bottom) Average available carbon µg day^{-1} (as on figure) in the zebra mussel enclosures. The horizontal dotted line indicates the minimum amount of carbon necessary for routine maintenance without growth based on mussels fed *Nitzschia* [from (Walz, 1978)].

Pond conditions

Control enclosures were similar to the pond for several parameters (i.e. temperature, pH and NH_4^+), but chlorophyll, PP, TDP and TP in the control enclosures were almost half those in the pond throughout the entire experiment (5-week treatment averages; Table I). Only on Day 8 were algal assemblages in the control enclosures similar to those observed in the pond (Figure 2). Ciliate abundance in the control enclosures was similar to the pond until Day 22, but by Day 36 the control enclosures had four times greater ciliate biomass than the zebra mussel enclosures and the pond (Table I). In addition, after two zooplankton inoculations, total zooplankton biomass measured in the enclosures averaged 6.2 times lower than that observed in the pond (Table I). Finally, the zooplankton assemblage in the control enclosures was very similar to that observed in the pond.

DISCUSSION

As expected, zebra mussels had a significant negative impact on algal abundance early in the experiment. By the end of the first week, the mussels had decreased algal biomass and chlorophyll concentrations by 53 and 71%, respectively. Similar but less steep declines in chlorophyll occurred in the control enclosures shortly after zooplankton were inoculated in all enclosures on Days 2 and 19. Although zebra mussels removed a majority of the algae

from the enclosures early in the study, their effect on algal abundance was not maintained throughout the experiment. After Day 8, chlorophyll concentrations started and then continued to increase until the completion of the experiment, at which time concentrations were similar to those in control enclosures. The inability of the mussels to maintain the phytoplankton at a low level could potentially be as a result of an algal species shift to inedible species, altered algal size structure, nutrient enrichment via zebra mussel mortality and/or the declining health of the mussels.

Although the zebra mussels significantly decreased most algal groups (except colonials) by Day 8, a shift in phytoplankton species composition was not observed because abundances of all algal groups were similar between treatments by Day 36. Additionally, 'inedible' algal species, such as colonial blue-greens, were not detected, thus it is unreasonable to conclude that a shift to less palatable species occurred. It is also not likely that the size-structure of the algal assemblages affected mussel grazing because all algal species were well within the size range that mussels have been shown to consume [range 1–150 μm; (Ten Winkel and Davids, 1982; Sprung and Rose, 1988; Horgan and Mills, 1997)] and all observed algal groups have been shown to be grazed by mussels in other studies (Heath *et al.*, 1995; Lavrentyev *et al.*, 1995; Bastviken *et al.*, 1998; Smith *et al.*, 1998).

The deteriorating health of the mussels could also help to explain the lack of effect on algal concentration observed later in this experiment. Several studies have examined the role of physical and chemical parameters in regulating zebra mussels in lakes (Ramcharan *et al.*, 1992; Ludyanskiy *et al.*, 1993; Mackie and Schloesser, 1996; Karatayev *et al.*, 1998). Specifically, calcium provides the material for mussel shell construction and mussels require at least 20 mg Ca^{2+} l^{-1} to establish populations (Ludyanskiy *et al.*, 1993). The pond used in this study had calcium concentrations >50 mg Ca^{2+} l^{-1} (S. Hamilton and D. Raikow, personal communication), thus calcium limitation probably did not affect mussel growth and maintenance. Ramcharan *et al.* demonstrated that mussels are sensitive to pH and found mussels to be absent from lakes with pH <7.3 (Ramcharan *et al.*, 1992). The pH in the enclosures ranged from 7.4 to 7.8 therefore it is not likely that pH significantly affected zebra mussel health.

Most temperate species are adapted to seasonally changing thermal environments; however, extremely high (or low) temperatures can be lethal. Karatayev *et al.* suggest that zebra mussels thrive in temperatures <27°C and have difficulty surviving in temperatures >32°C (Karatayev *et al.*, 1998). Although the enclosures reached 27°C, the average measured temperature experienced by the mussels throughout the entire study was 25°C. Thus, it seems unlikely that temperature by itself affected mussel health and grazing; however, higher temperatures could have made routine physiological maintenance more difficult because of higher food requirements at higher temperatures (Walz, 1978; Aldridge *et al.*, 1995; Fanslow *et al.*, 1995; Horgan and Mills, 1997).

An individual zebra mussel's growth rate is dependent on body size and temperature, among other factors (Walz, 1978). Walz indicates that higher temperatures severely restrict zebra mussel growth rates because of a greater demand for food and that larger mussels require less food per gram of mussel than smaller mussels (Walz, 1978; James *et al.*, 2000). Comparisons of length–weight relationships performed on the mussels before and after the study show that the mussels lost 56% of their weight during the experiment (Figure 3b). Average available daily rations were calculated by converting measured chlorophyll concentrations into carbon (μg) [carbon:chlorophyll = 67:1; (Riemann *et al.*, 1989)] and then calculating filtration rates based on the size distribution and density of mussels used in the study (Kryger and Riisgård, 1988). The average daily ration on Day 8 of the experiment was 31 μg C day^{-1} in the mussel enclosures. Walz used *Nitzschia*, a high-quality laboratory cultured diatom, to show that a 5 mg (dry tissue mass) mussel requires 42.5 μg C day^{-1} for routine maintenance at 20°C (Walz, 1978). This is a very conservative estimate given the 5°C temperature difference between this study (Table I) and that of Walz (Walz, 1978). Additionally, the average mussel size used by Walz was slightly larger than the average mussel used in this study (3.7 mg dry tissue mass), and smaller mussels typically require more food per unit body weight than larger mussels (Walz, 1978). Finally, given the lower quality pond water, which contained a considerable amount of detritus, the zebra mussels would have needed to filter more enclosure water when compared with a similar amount of cultured *Nitzschia* medium to acquire the carbon needed for basic maintenance. Calculations indicated that food abundance in the zebra mussel enclosures was below that required for basic maintenance on Day 8 (Figure 3c). Thus, it is reasonable to conclude that the mussels lost weight as a result of insufficient food availability soon after they were added to the enclosures, and consequently were stressed to the point where they were unable to control algal abundance later in the study. Presumably, if algal abundances were greater (i.e. above the minimum maintenance concentration) throughout the experiment, or the added mussel biomass was lower, the zebra mussels may have exerted a sustained control over the phytoplankton.

In addition, although the TP concentration of the pond (13 μg l^{-1}) was used to predict a reasonable stocking

density of zebra mussels for the enclosures, the enclosures were half as productive as the pond (Table I). Consequently, an overestimated amount of mussels was inoculated into each enclosure. With this in mind, careful consideration of the mussels' food availability must be accounted for when designing field experiments with zebra mussels in enclosed, previously mussel-free systems.

Zebra mussels immediately decreased and maintained low levels of ciliates throughout the experiment (Figure 3a), while phytoplankton abundance continued to increase after Day 8 (Figure 1a). This result is not surprising given that phytoplankton were capable of acquiring available nutrients created via mussel excretion and mortality, while ciliates competed with and were preyed upon by zebra mussels. Others have shown similar impacts of zebra mussels on protozoan abundances. For example, Lavrentyev *et al.* conducted laboratory experiments where they showed that zebra mussels decreased protozoan abundance by >70%, while the mussels had less of an impact on phytoplankton abundance (≈45% decline) (Lavrentyev *et al.*, 1995). In addition, competition between zebra mussels and ciliates for small edible algae, like small greens, could help explain the observed effects on ciliate and algal abundances. A positive correlation between ciliates and small green algae for the enclosures (Figure 4) suggests that although the zebra mussels caused a decrease in all algal groups equally, the large absolute reduction of small green algae (68%; Table II) could have helped to keep ciliate numbers low. In addition, the dramatic difference in ciliate abundance between the control enclosures and the pond and the zebra mussel enclosures at the end of the study (Figure 3a) could be attributed to the loss of zooplankton in the mesocosms during the experiment. Zebra mussels significantly decreased zooplankton biomass (Table I); however, cladocerans were more negatively affected than copepods. Although the mechanism that zebra mussels used to control zooplankton is unknown, indirect exploitative competition or direct predation on zooplankton could have been responsible for the decline in zooplankton biomass throughout the experiment. In general, large-bodied cladocerans tend to be considered the dominant planktonic grazers in most lakes (Porter, 1977); thus, decreases in their total abundance could affect benthic–pelagic linkages within aquatic food webs. Also, zooplankton and ciliates compete for algae, and it is likely that the ciliates experienced less exploitative competition in the control enclosures; however, ciliate abundances were controlled by either the zebra mussels in the zebra mussel enclosures or the natural zooplankton community in the pond. This study shows that zebra mussels are extremely effective at controlling ciliates; however, the importance of competition and predation in zebra mussels' ability to control micro- and macrozooplankton is currently unresolved. Thus, further research directed at understanding the competitive and predative interactions among protozoans, zooplankton and zebra mussels will aid in developing more complete and accurate food-web models.

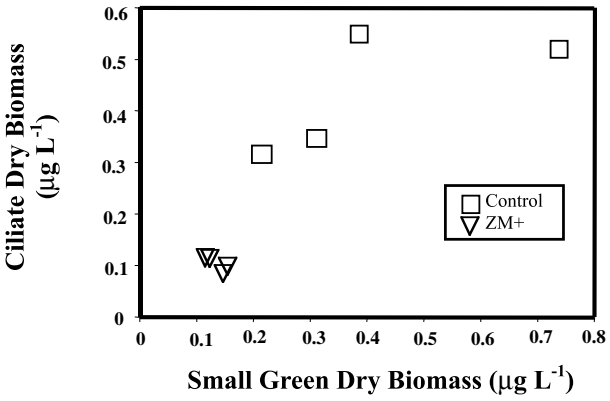

Fig. 4. Time-averaged (Days 8, 22 and 36) data on small green algal group dry biomass (μg l^{-1}) versus ciliate dry biomass (μg l^{-1}) for the four replicates of both treatments. ZM+ = zebra mussel addition. Control = lack of zebra mussels.

Given that many earlier experimental studies involving zebra mussels used excessively high numbers of them, presumably to see a grazing effect, the current study clearly shows how critical the determination of a natural density of zebra mussels can be to the outcome of the study. Also, this is first study to compare length–weight relationships to monitor the health of zebra mussels during an experiment. Without this type of data, conclusions based on the health of the mussels would only be conjecture. Thus, future experiments using zebra mussels should: (i) take precautions not to overstock their zebra mussel treatments by either taking benthic samples to determine a biomass estimate for water bodies where mussels have already invaded or by measuring the TP concentration of the experimental units for mussel-less systems and then calculating dry tissue biomass; (ii) monitor TP levels in enclosures related to the natural system; and (iii) monitor the health of the mussels throughout the experiment.

In conclusion, zebra mussels have been shown to decrease quickly the biomass of algae and ciliates soon after entering a new water body. However, their impact on phytoplankton biomass was shown to diminish within 2 weeks of their introduction. The deteriorating health of the mussels has been proposed to help explain this effect. Future work incorporating a long-term, large-scale, controlled field experiment aimed at examining the

gradual development of a zebra mussel founder population and its effects on the community will elucidate the important complex interactions between zebra mussels and other food-web components.

ACKNOWLEDGEMENTS

I sincerely thank O. Sarnelle, S. Hamilton, G. Mittelbach and P. Soranno for their helpful comments and criticisms throughout the entire project; N. Dorn and R. Valley for their suggestions on the experimental design; D. Raikow and E. Cebelak for their help building the enclosures; M. Machavaram for sharing his knowledge about chemistry; W. Wilson for helping process samples; K. Rogers for helping identify zooplankton; J. Bence and M. Rutter for their statistical expertise; N. Consolatti and the rest of the KBS staff for their kindness and support; K. Hoffman and S. Hanson for technical advice; and C. Steiner for use of his stapler. Additionally, I would like to thank two anonymous reviewers for their helpful suggestions that improved the manuscript. This project was funded by a George H. Lauff Scholarship and a National Science Foundation Research Training Grant DIR-9602252RTG. This is contribution no. 992 of the Kellogg Biological Station.

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Received on December 18, 2001; accepted on April 10, 2003