

## Effects of Zebra Mussels (*Dreissena polymorpha*) on Bacterioplankton: Evidence for Both Size-Selective Consumption and Growth Stimulation

James B. Cotner,<sup>1</sup> Wayne S. Gardner,<sup>2</sup> Jeffrey R. Johnson,<sup>2,3</sup>  
Rosa H. Sada,<sup>1</sup> Joann F. Cavaletto,<sup>2</sup> and Robert T. Heath<sup>4</sup>

<sup>1</sup>Department of Wildlife and Fisheries Sciences  
Texas A&M University  
College Station, Texas 77843-2258

<sup>2</sup>NOAA/Great Lakes Environmental Research Laboratory  
2205 Commonwealth Blvd.  
Ann Arbor, Michigan 48105

<sup>3</sup>Cooperative Institute for Limnology and Ecosystems Research  
University of Michigan  
2200 Bonisteel Blvd.  
Ann Arbor, Michigan 48109

<sup>4</sup>Department of Biological Sciences  
Kent State University  
Kent, Ohio 44242

**ABSTRACT.** Zebra mussels had significant direct and possible indirect effects on heterotrophic bacteria in two contrasting sites in Saginaw Bay. At a eutrophic site in the inner portion of Saginaw Bay, mussels fed directly on bacterial-sized particles and had a negative impact on bacterial abundances. Mussels removed large bacteria ( $> 0.9 \mu\text{m}$ ) more effectively than small bacteria at this site. Individual mussels cleared from 37–89 ml per day. Results using different sizes of fluorescent microspheres suggest that zebra mussels have a lower limit for particle size removal that is less than  $0.4 \mu\text{m}$ . Contradictory to inner bay results, mussels at an outer bay oligotrophic site had a positive impact on heterotrophic bacterial abundance, perhaps as a result of indirect effects, such as nutrient or organic carbon excretion by the mussels. Differences in the impact of mussels on the bacterial communities of the inner bay and outer bay probably result from differences in trophic state and bacterial community structure. A hypothesized smaller size of bacteria at outer bay sites may enable them to escape heavy predation pressure from mussels and the high rates of mussel nutrient excretion may facilitate their growth in these nutrient depleted conditions.

**INDEX WORDS:** Zebra mussel, bacteria, biomass, Lake Huron, particle size.

### INTRODUCTION

Zebra mussels (*Dreissena polymorpha*) were first found in North American waters in 1988 (Hebert *et al.* 1989) and have been reproducing prolifically in many regions of the Laurentian Great Lakes as well as other systems where they have been introduced. In addition to the profound effects these bivalves have had on industries and public water intake systems in the Great Lakes region, they are also having significant impacts on the aquatic foodweb. With particle filtration rates as high as nearly 300 mL

mussel<sup>-1</sup>h<sup>-1</sup> (Kryger and Riisgård 1988), typical rates near 50 mL mussel<sup>-1</sup>h<sup>-1</sup> (Reeders and bij de Vaate 1990) and abundances as high as 350,000 individuals m<sup>-2</sup> in some regions (Griffiths 1993), there have been some major ecological changes. In fact, in the most heavily impacted region, the western basin of Lake Erie, it has been estimated that zebra mussels are presently processing 39–96% of the entire water column daily in various regions (Bunt *et al.* 1993). Over the period of zebra mussel invasion in this region, Secchi disk transparency

has increased by 85% and chlorophyll *a* values have declined by 43% (Leach 1993).

It is clear that zebra mussels are having the most severe impact on those flora and fauna that are in the size ranges that they remove most efficiently. Recent studies indicated that these mussels can filter particles in a size range from 0.7  $\mu\text{m}$  to 450  $\mu\text{m}$  (Jørgensen *et al.* 1984, Sprung and Rose 1988). This broad filtration range would include everything from bacteria to rotifers. Not all particles in this range are retained and ingested with the same efficiency, however, so considerable selectivity may occur. Maximum filtration of particles occurs in the size range from 5–35  $\mu\text{m}$  (Sprung and Rose 1988) and therefore the organisms most severely impacted would be small phytoplankton and protozoans. However, in another study, zebra mussels removed particles as small as 1  $\mu\text{m}$  with a high efficiency, as well (>90%) (Jørgensen *et al.* 1984) and therefore, may have important impacts on heterotrophic bacteria. Few studies, however, have examined the impact of bivalves on heterotrophic bacterial biomass and productivity.

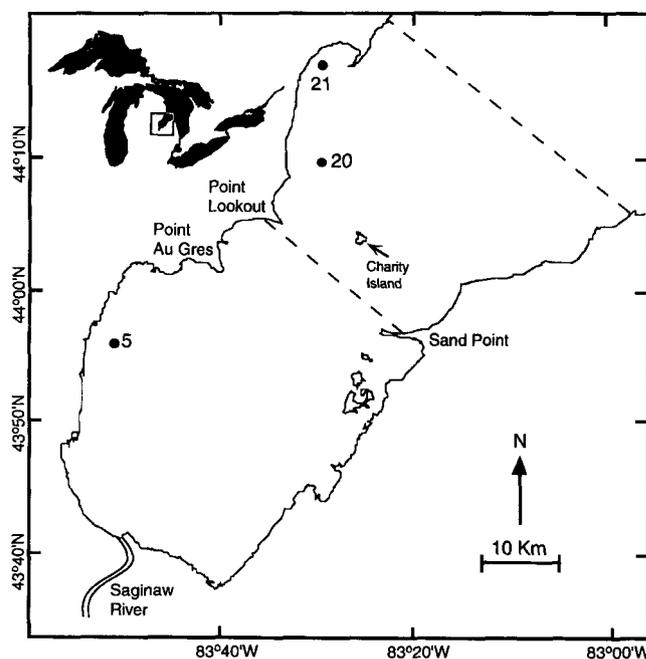
In a study of three marine mussels, *Mytilus*, *Geukensia*, and *Mya*, only *Geukensia* removed a significant quantity of bacteria from the water column (Wright *et al.* 1982). Although the other two species of bivalves removed significant amounts of chlorophyll, there was little change in bacterial abundance in their presence. *Geukensia* was able to filter bacteria at an efficiency nearly half of that for phytoplankton. This species removed some portions of the heterotrophic bacterial community with different efficiencies than others. Particles less than 0.4  $\mu\text{m}$  were only removed at about 30% of the efficiency of particles larger than this, suggesting that large bacteria were being removed more rapidly than small bacteria. Because of differences in particle abundance between phytoplankton and bacteria in the salt marshes where *Geukensia* occurs naturally, it was concluded that bacteria may be the most important food resource for this species in its natural habitat.

The purpose of the present study was to determine the impact of zebra mussels on water-column bacterial abundance. It is the first attempt to quantify the effects of zebra mussels on bacterial dynamics in the Great Lakes. Specifically, we attempted to answer these questions: (1) are zebra mussels capable of removing bacterial-sized particles from the water?, and (2) do zebra mussels impose a significant impact on bacterial-sized particles in regions where they are most abundant?

## MATERIALS AND METHODS

### Study Sites

We conducted experiments at three sites in Saginaw Bay, Lake Huron, and one site in Lake St. Clair. Bottle experiments were performed to examine the impact of zebra mussels on bacterial abundance with water collected at an inner bay site (Station 5; 43°53'43" N, 83°51'38" W) and an outer bay site (Station 20; 44°07'34" N, 83°30'00" W), both located in Saginaw Bay (Fig. 1). These sites were chosen because of the contrasting trophic conditions. Station 5 (hereafter referred to as "inner bay site") is classified as meso- to eutrophic, whereas Station 20 (hereafter referred to as "outer bay site") is classified as oligotrophic. The inner bay site was 3-m deep and had a spring maximum chlorophyll level of 16  $\mu\text{g L}^{-1}$  before zebra mussels became abundant (prior to 1991) and the outer bay site was 16-m deep and has typical chlorophyll values of 1–3  $\mu\text{g L}^{-1}$  (Fahnenstiel *et al.* 1995). A mesocosm experiment was conducted in Tawas Bay, Lake Huron, which is also located in the outer bay near Station 21 (44°15'10" N, 83°30'00" W). This



**FIG. 1.** Locations of sampling sites within Saginaw Bay, Lake Huron. Dashed lines differentiate the inner bay from the outer bay and the outer bay from Lake Huron.

station was comparable in trophic state to Station 20 (oligotrophic). In June 1991 a bottle experiment was also conducted with water collected from a site in the southwestern portion of Lake St. Clair (Nalepa *et al.* 1993). Water samples and mussels were collected from Station 19 (42°22' 30" N, 82°42'30" W) which had a chlorophyll value of 3.7  $\mu\text{g L}^{-1}$  and is 6-m deep. Overall, Lake St. Clair is classified as a mesotrophic lake (Herdendorf *et al.* 1986).

### Bottle Experiments

We examined the effects of mussels on bacterial abundance by incubating lake water with and without mussels present in 4-L bottles. Water was collected from the two Saginaw Bay sites in June, August, and September 1993. Bottles were incubated in waterbath incubators that were designed to maintain *in situ* temperature and light conditions. Water in the incubators was maintained at ambient temperature by circulation through a Neslab Model 110D Digital Refrigerated Bath Circulator equipped with an external temperature control probe suspended in the bath (Gardner *et al.* 1995a). Water was mixed by pumping air bubbles into the bottom of the water bath. Light levels were maintained at approximately 75% of incident solar radiation with a blue polycarbonate filter (Acrylite 625-5). Half of the treatments were covered with aluminum foil to exclude all light.

In Saginaw Bay, water and mussels were collected from the inner bay site and water only was collected from the outer bay site. Mussels used in outer bay water incubations were collected from the inner bay site with an epibenthic sled (Nalepa *et al.* 1995). Fifteen mussels (mean size ca. 10–15 mm length) were removed from their natural substrate by cutting the byssal threads, rinsing with lake water, and removing periphyton before being placed in the 4-L polycarbonate bottle with water collected from either site. Duplicate bottles for each treatment (light/dark, with/without mussels) were placed in the incubators.

Because we found no significant differences in the dark and light treatments with respect to bacterial abundances over the summer (t-test,  $p > 0.05$ ), we pooled the results from both of these treatments and made comparisons only between treatments with and without mussels. Samples were removed with a pipette from the bottles initially and at approximately 48 h for measurements of bacterial abundance. Samples were preserved with formalin

(2% final concentration), stained with acridine orange and counted with a epifluorescence microscope after filtration onto 0.2  $\mu\text{m}$  pore-size filters that had been pre-stained with Irgalan black (Hobbie *et al.* 1977). Duplicate preparations were made for each sample and a minimum of 10 fields or 300 cells was counted.

On 25 June 1991, water was collected from Lake St. Clair (Station 19) to examine the impact of mussels on bacterial abundance and growth rates. Zebra mussels and water were collected, transported to Ann Arbor, where the experiments were set up similar to the Saginaw Bay 4-L bottle experiments except that the incubations were performed in lighted environmental chambers (Percival) at ca. 100  $\mu\text{Einsteins m}^{-2}\text{s}^{-1}$  light levels instead of outdoor incubators with ambient sunlight. Water samples were removed at various time intervals over a 24-h period and bacterial abundance and thymidine incorporation measurements (see below) were performed.

### Fluorescent Microspheres

A similar set of experiments was conducted in summer 1992 to examine zebra mussel removal of fluorescent microspheres that were similar in size to heterotrophic bacteria. Different sizes of microspheres were used to determine whether zebra mussels were filtering bacterial-sized particles. We added polystyrene microspheres of different sizes and corresponding different-colored fluorescent labels (Polysciences, Inc.) at a final concentration of  $2 \times 10^5 \text{ mL}^{-1}$  in 4-liter polycarbonate bottles filled with water collected from the inner Saginaw Bay site. Natural abundances of bacteria at this site vary from  $1\text{--}4 \times 10^6 \text{ mL}^{-1}$  so the microspheres were added at approximately 10% of the ambient community abundance. Bottles were incubated at 100  $\mu\text{Einsteins m}^{-2}\text{s}^{-1}$  light levels and ambient temperature in a Percival Model I30B environmental chamber. Three fluorescently-labeled bead sizes were used: 0.91, 0.36 and 0.22  $\mu\text{m}$  diameters. The different fluorescent labels on the beads enabled experimental treatments to be run in pairs in the same bottles, i.e., a set of mussels with 0.91 and 0.36  $\mu\text{m}$  beads or 0.36 and 0.22  $\mu\text{m}$  beads, etc. Control treatments (no zebra mussels present) were performed and no decrease in bead abundance was observed in any of these treatments. Samples were removed from the bottles at 0.2–10 h intervals over a 24-h period and preserved with formalin. Beads were filtered onto a black polycarbonate filter with a 0.2

$\mu\text{m}$  pore-size and counted with a epifluorescence microscope. We examined the possibility that a significant fraction of the 0.22  $\mu\text{m}$  beads may have passed through the 0.2  $\mu\text{m}$  pore-size filters. A comparison of retention efficiency was made with 0.1 and 0.2  $\mu\text{m}$  pore-size filters and there was no difference between the two sizes. With the 0.2  $\mu\text{m}$  beads, we had a retention efficiency of 92% on 0.2  $\mu\text{m}$  pore-size filters. Furthermore, the mean value for the y-intercept of all the experiments with 0.22  $\mu\text{m}$  beads was  $1.95 \times 10^5 \text{ mL}^{-1}$ , indicating that we were counting nearly all of the beads that were added in the experiment. Counting procedures were the same as those used in counting heterotrophic bacteria (300 beads, a minimum of 10 fields).

The natural log of bead abundance was plotted against time for fitting data into a least-squares regression model. If a significant ( $p < 0.05$ ) regression was obtained, we calculated clearance rates of the mussels as:  $(\text{slope} \times \text{volume of the container}) / (\# \text{ mussels})$ , after Vanderploeg *et al.* (1995).

### Mesocosm Experiments

From 28 August–5 September 1991 we examined the effects of zebra mussels on bacterial processes in some large (1,600 L) mesocosms, anchored at a nearshore site close to Station 21. These enclosures were made of nylon reinforced vinyl, were closed to the sediments at the bottom and had a floating collar at the top to prevent exchange with bay water. Each enclosure was 2-m deep and had a 1-m diameter. Water from the site was pumped into the enclosures until they were full. There were two control enclosures without mussels and two treatment levels of zebra mussels (unreplicated) for a total of four enclosures. One of the zebra mussel treatments contained 892 mussels (4.2 g ash-free dry weight; hereafter, referred to as the low zebra mussel treatment) and another enclosure contained 2,928 mussels (16.1 g ash-free dry weight, hereafter referred to as the high zebra mussel treatment) per enclosure. The mussels were collected from Lake St. Clair (Station 19 in Nalepa *et al.* 1993) and had settled on unionid clam shells. They were suspended at mid-depth in the enclosures by drilling holes in these shells, drawing a line through the holes, and tying both ends to the collar of the enclosure. These abundances are typical of what one might find in western Lake Erie (Heath *et al.* 1995).

Samples were removed daily from a 1-m sampling depth with a Van Dorn water sampler for measurements of bacterial abundance and thymi-

dine incorporation. Abundance measurements were made as in bottle experiments. Radiolabelled thymidine incorporation into DNA was used to estimate the effects of various treatments on bacterial growth rates. Duplicate or triplicate samples were incubated at ambient temperature with 20 nM [methyl- $^3\text{H}$ ]thymidine (Amersham). Nucleic acid and protein fractions were separated with a trichloroacetic acid extraction procedure (Chin-Leo and Benner 1992). Killed blanks were used to correct for abiotic adsorption to filters. Prior to all filtration procedures, filters were soaked in 10 mM cold thymidine to saturate these adsorption sites (Cotner and Gardner 1993).

Bacterial samples from the fourth day of these experiments were examined using scanning electron microscopy. Bacterial samples were preserved with formalin and successively washed with solutions of 40, 60, 80, 90, and 100% ethanol on 0.2  $\mu\text{m}$  polycarbonate filters. Samples were critical-point dried, sputter-coated with a thin gold solution and examined with an Amray 1820I electron microscope at 6,000 $\times$  magnification. Three photomicrographs were taken of randomly selected fields containing 8–20 cells in all treatments. Images were examined using Bioscan Optimas (version 3.01) image analysis software. We measured the axial length and width of all cells in each photomicrograph.

### Statistics

Experimental results of bead and bottle experiments were analyzed using the MGLH module of Systat (version 4.0) for analysis of variance (ANOVA) (Wilkinson 1989). Two-way ANOVA (w/without mussels; month) was performed on bacterial abundance measurements in bottle experiments where zebra mussel presence/absence was manipulated. The  $R \times C$  test for independence was performed on log-transformed bacterial size class data in samples collected in the enclosures after 4 d to determine if there were any differences in the abundance of large ( $>1 \mu\text{m}$ ) bacteria in different treatments (Sokal and Rohlf 1981).

### RESULTS

We examined the impact of mussels on ambient bacterial abundance in the summer of 1993 in bottle experiments with water and mussels collected from inner and outer Saginaw Bay. A 2-way ANOVA was used to examine differences between treatments (with and without mussels added) and

months. There was a significant (ANOVA;  $p < 0.05$ ) impact of mussel treatments on bacterial abundance over the three months that we conducted experiments (Fig. 2a). In all of the treatments where inner Saginaw Bay water was exposed to mussels, the bacterial abundances were lower than those in the control treatments (Fig. 2a). Bacterial abundance was, on average, 41% greater in the control treatments than in the zebra mussel treatments in the inner bay over the entire growing season (range 20–68%). Furthermore, there was much less variance among months in the treatments where mussels were added to the lake water.

Surprisingly, zebra mussels had the opposite effect on bacterial abundance at the outer bay site (Fig. 2b). In the experiments conducted in June, August, and September, adding mussels to the bottles resulted in a net increase in bacterial abundance (ANOVA;  $p < 0.05$ ). On average, the bacterial abundance in the mussel treatments was 50% higher than controls over the growing season (range 28–73%).

To determine the minimum threshold of particle sizes for filtration, we used fluorescent microspheres of various sizes in zebra mussel treatments in experiments with water collected at the inner bay site. Three sizes of beads were used in July and August 1992 (0.22, 0.36, and 0.91  $\mu\text{m}$ ) and two sizes were used in September 1992 (0.22 and 0.91  $\mu\text{m}$ ). In time series measurements, there was a significant decrease in bead abundance with the two largest

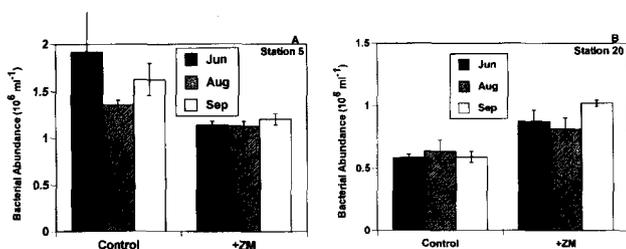
bead sizes at various times. In July, there was not a significant change in the abundance of the 0.22  $\mu\text{m}$  beads but there were significant changes ( $p < 0.05$ ) in both the 0.36 and 0.91  $\mu\text{m}$  beads (Table 1). These results suggest that the lower limit for mussel particle size removal was in the range of 0.22–0.36  $\mu\text{m}$ . In the 0.36 and 0.91  $\mu\text{m}$  bead treatments, the beads were removed at similar rates, suggesting that there was no discrimination of these different particle sizes at this time (Table 1).

In August, the same bead sizes were removed as in July. These mussels filtered the 0.36 and 0.91  $\mu\text{m}$  microspheres at similar rates (43.5 and 36.5  $\text{ml mussel}^{-1}\text{d}^{-1}$  respectively; Table 1). However, the clearance rates of the mussels were only about half as great as those measured in July.

In September, rates were only measured with the smallest and the largest bead sizes. Unlike other months, there was not a significant effect of mussels on bead removal rates at this time (Table 1). Either the mussel filtration rates had decreased relative to rates in the other months or they were not removing bacterial-sized particles at this time.

In all significant treatments, we calculated clearance rates for the bottles (Table 1). These values ranged from 0.5–1.3  $\text{L day}^{-1}$ . These results imply that mussels could have a major impact on bacteria in their local environment, removing as much as 30%  $\text{day}^{-1}$  in the 4-L bottles.

In August–September 1991 we conducted similar experiments in some large (1,600 L) enclosures in Tawas Bay. Mussels were added at abundances similar to what would be found in mesotrophic and eutrophic regions of Saginaw Bay. Over the 6 days that the experiment was conducted, there were great fluctuations in both the bacterial abundance and thymidine incorporation rates of bacteria in the high zebra mussel treatments (Figs. 3 and 4). Bacterial abundance was comparable to control treatments initially but increased significantly on the second day (Fig. 3). On subsequent days bacterial abundance decreased below that of the control and low zebra mussel treatments. Bacterial abundance in the low mussel treatment also increased on the second day and subsequently decreased below the level of the controls but these variations were not statistically different from the controls ( $p < 0.05$ ) (Fig. 3). Bacterial thymidine incorporation showed a similar trend to bacterial abundance in the high zebra mussel treatment (Fig. 4). Growth rates in this treatment were highest on the second day and decreased below the level of the control treatments thereafter.

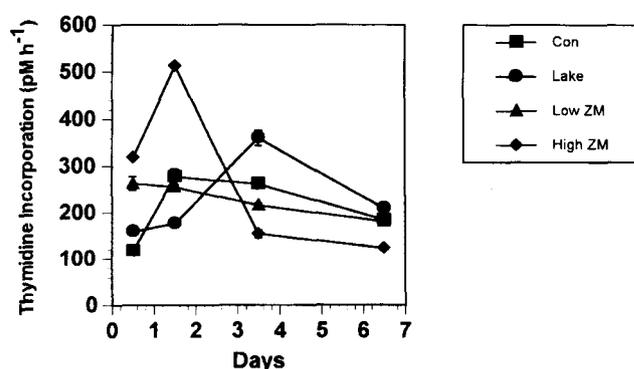
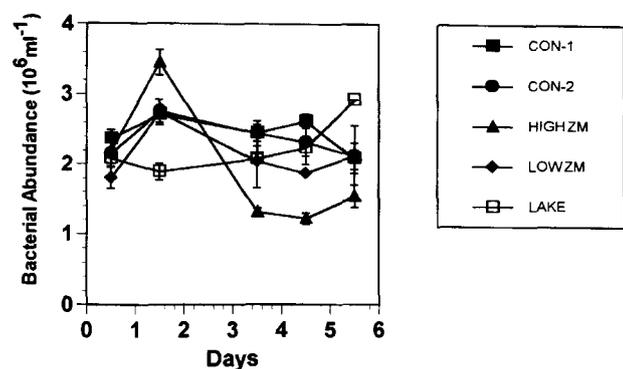


**FIG. 2.** The effect of zebra mussels on bacterial abundances in 4-L bottle experiments at the eutrophic inner bay station (A, Station 5) and the oligotrophic outer bay station (B, Station 20), summer 1993. Error bars (here and in all the figures) represent one standard error of the mean. Control treatments were incubated without mussels present and "ZM" treatments were incubated in the presence of 15 adult mussels.

**TABLE 1.** Clearance rates of zebra mussels feeding on spherical polystyrene beads of various sizes. Beads were inoculated into 4-L polycarbonate containers and abundances were monitored for approximately 24 h. Log bead concentrations were plotted against time and significant regressions were used to estimate clearance rates. Bacterial removal rate is the clearance rate multiplied by the number of mussels in the bottle.

Date	Bead Size ( $\mu\text{M}$ )	Clearance Rate ( $\text{mL mussel}^{-1}\text{day}^{-1}$ )	Bacterial Removal Rate ( $\text{L day}^{-1}$ )	y-Intercept (beads $\times 10^5 \text{ mL}^{-1}$ )
July 92	0.22	n.s.	n.s.	—
	0.36	77.9	1.2	1.1
	0.91	88.6	1.3	0.6
August 92	0.22	n.s.	n.s.	—
	0.36	43.5	0.7	2.9
	0.91	36.5	0.5	1.5
September 92	0.22	n.s.	n.s.	—
	0.91	n.s.	n.s.	—

All rates significant at  $p = 0.05$  unless designated n.s. (not significant).



**FIG. 3.** The effect of zebra mussels on bacterial abundance in mesocosm experiments in the outer bay. Mussels were suspended in 2,000-L enclosures. AODCs were performed at various times during the experiment. There were two control enclosures (CON; no mussels added), one low zebra mussel treatment (Low ZM), one high zebra mussel treatment (High ZM), and the ambient lake water (Lake).

**FIG. 4.** The effect of zebra mussels on thymidine incorporation in mesocosm experiments in the outer bay. Legend shorthand notations are the same as in Figure 3.

This trend was not observed in the low zebra mussel treatment.

To examine the effects of mussels on the size composition of the bacterial community, we measured the longest axial distance of bacterial-sized particles in these mesocosm treatments on the fourth day of the experiment (Fig. 5). There was a decrease in the relative abundance of bacteria

greater than  $1 \mu\text{m}$  in length in the zebra mussel treatments (Fig. 5). In the control treatment, 31% of the bacteria counted were greater than  $1 \mu\text{m}$  in length and zebra mussel treatments had a higher proportion of smaller cells. Only 11% of the bacteria in the high zebra mussel treatment were greater than  $1 \mu\text{m}$  in length whereas 23% were at least this large in the low mussel treatment, suggesting that a

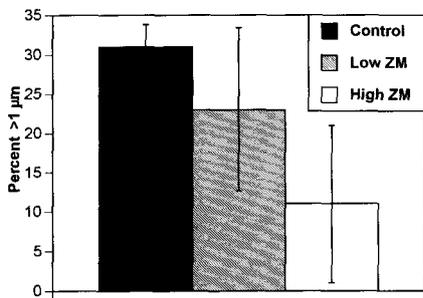


FIG. 5. Percent of bacteria larger than 1  $\mu\text{m}$  in control, low zebra mussel and high zebra mussel mesocosm treatments.

greater abundance of mussels results in a greater decrease in the abundance of large bacteria. However, we were not able to detect significant differences in the various treatments using the  $R \times C$  test of independence, probably because of the few number of cells analyzed. The total number of cells analyzed in control, low and high zebra mussel treatments was only 38, 42, and 12, respectively.

In one set of short-term bottle experiments with water and mussels collected from mesotrophic Lake St. Clair in June of 1991, we examined the dynamics of mussel impact on bacterial abundance and thymidine incorporation simultaneously. Unlike the mesocosm experiments where bacterial abundance and thymidine incorporation rates paralleled each other (Fig. 3), these short-term experiments demonstrated that there was an initial decrease in bacterial thymidine incorporation but no change in bacterial abundance (Fig. 6).

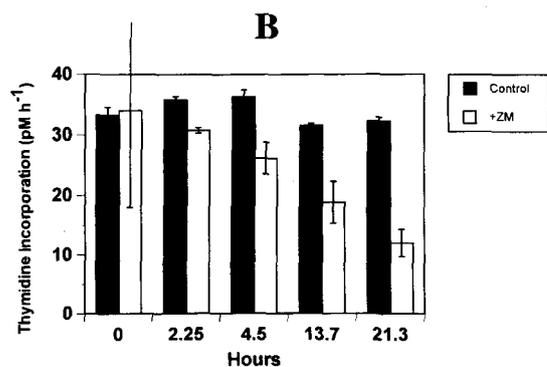
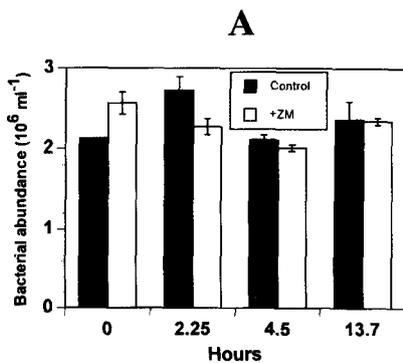


FIG. 6. Effect of mussels on bacterial abundance (A) and thymidine incorporation rates (B) in short-term experiments performed in Lake St. Clair, June 1991.

## DISCUSSION

### Particle Sizes Removed by Zebra Mussels

The maximum clearance rate that we observed with fluorescent polystyrene beads was  $89 \text{ mL mussel}^{-1} \text{ day}^{-1}$  with the  $0.9 \mu\text{m}$  beads in July 1992. The lowest rates observed were about half of this value. There was little effect of the mussels on the smallest particle size used ( $0.22 \mu\text{m}$ ) and few differences were observed between the  $0.9$  and  $0.36 \mu\text{m}$  beads. Measurements of clearance rates of chlorophyll at this same inner bay station on the same dates indicated that phytoplankton were removed at rates of  $240\text{--}720 \text{ mL mussel}^{-1} \text{ day}^{-1}$  (Fanslow *et al.* 1995). These data indicate that the clearance rate for  $1 \mu\text{m}$  diameter particles was 5–37% of the total phytoplankton clearance rate.

Bacteria could be an important supplemental carbon source to mussels in the summer. Given ambient Saginaw Bay summer bacterial abundances at ca.  $1.5 \times 10^6 \text{ mL}^{-1}$ , bacterial carbon content at ca.  $2 \times 10^{-13} \text{ g cell}^{-1}$  (Bratbak 1985) and the measured clearance rates reported here, bacterial consumption could account for about  $2.2 \mu\text{mol C mussel}^{-1} \text{ d}^{-1}$ . Zebra mussels comparable in size to the ones used in the present study (10–15 mm) had respiration rates in Lake St. Clair of  $36 \text{ mg O}_2 \text{ mussel}^{-1} \text{ d}^{-1}$ . This represents a respiratory carbon demand of  $11.3 \mu\text{mol C mussel}^{-1} \text{ d}^{-1}$  assuming a respiratory quotient of 1. Therefore, heterotrophic bacteria could provide about 20% of the mussel's respiratory requirement in summer. However, similar calculations based on phytoplankton chlorophyll concentrations and clearance rates show that phytoplankton can satisfy all of the mussel metabolic requirements in

summer. The relative importance of these two trophic groups to zebra mussel nutrition would also depend on the assimilation efficiency of the carbon biomass from bacteria and algae.

In laboratory experiments with pure cultures, heterotrophic bacteria may not be as nutritious to mussels as small phytoplankton. Zebra mussels were able to grow for 2–3 months on a monoclonal bacterial food, but growth occurred over a longer period when *Chlamydomonas* was used as the sole food (Nichols 1993). Nothing is known about how nutritious a natural bacterial community is to zebra mussels. Perhaps, the diversity of flora and associated biochemical constituents could make them as nutritious as *Chlamydomonas*.

In bivalves the efficiency of bacterial-sized particle removal is species dependent. In a study of three species of marine bivalves (Wright *et al.* 1982), only the ribbed mussel (*Geukensia demissa*) removed bacterial-sized particles. This species removed bacteria from seawater at an efficiency that was 42% of the chlorophyll clearance rate. The greatest efficiency observed was for particles greater than 0.6  $\mu\text{m}$  diameter. However, the blue mussel (*Mytilus edulis*) did not remove a significant amount of bacterial biomass from the water (Wright *et al.* 1982). In another study, the blue mussel removed only about 10% of 1  $\mu\text{m}$  latex beads relative to 6  $\mu\text{m}$  beads (Jørgensen 1975). Both the blue mussel and the American oyster (*Crassostrea virginica*) are most efficient at removing particles in the 3–5  $\mu\text{m}$  range (Møhlenberg and Riisgård 1978).

*Dreissena* and other bivalves with gills having latero-frontal cirri typically can retain particles of at least 1  $\mu\text{m}$  diameter and less (Møhlenberg and Riisgård 1978). At similar filtration rates mussels can remove varying amounts of differing particle sizes by inactivating groups of latero-frontal cirri, shifting their beat trajectory or widening the ostia (Møhlenberg and Riisgård 1978). Zebra mussels are one of the most efficient bivalves in terms of removing small particles, and, as our data indicate, they can remove particles very effectively in the 0.4–1.0  $\mu\text{m}$  range. Other workers have demonstrated that zebra mussels can remove particles effectively down to 0.7  $\mu\text{m}$  diameters (Sprung and Rose 1988). Jørgenson *et al.* (1984) measured a 90% efficiency of zebra mussels feeding on particles 1  $\mu\text{m}$  in diameter and noted that they retained the smallest particles among the bivalves examined.

Our clearance rates measured with 0.9  $\mu\text{m}$  beads were probably underestimated. Although we added beads at a concentration of  $2 \times 10^5$  beads  $\text{mL}^{-1}$ , the

y-intercept with the 0.36 and 0.9  $\mu\text{m}$  beads was less than this value (0.6 and  $1.5 \times 10^5$  beads  $\text{mL}^{-1}$  in July and August, respectively; Table 1). Either the beads were not added at the calculated concentration or the mussels were filtering the beads so rapidly that this intercept was underestimated, i.e., by the time the initial set of samples were taken, a measurable portion of the beads had been removed. In all cases, the intercept for the control treatment was higher than the zebra mussel treatment for the large beads, and the largest bead size always had the smallest intercept, suggesting that clearance rates may have been progressively underestimated with increasing bead size and is consistent with our observations that large beads were removed more effectively than small beads. Furthermore, the intercept deviated most from the calculated concentration in July when filtration rates were most rapid. Alternatively, the larger beads may have settled out of the containers, but the bottles were mixed vigorously prior to removing each sample.

### Impact on the Bacterial Community

What is the potential impact of zebra mussels on heterotrophic bacterial abundance and growth rates in Saginaw Bay? At Station 5 where measurements of clearance rates were estimated in 1992, zebra mussel abundances were estimated with diver-deployed transects to be about 75,000  $\text{m}^{-2}$  (Nalepa *et al.* 1995). Using our mean estimated clearance rate of 62  $\text{mL mussel}^{-1}\text{day}^{-1}$  and assuming that the mussels filtered water from the entire 3 meters of the water column, the entire bacterial biomass would be removed 1.6 times per day. Even if we use our lower estimated clearance rate of 37  $\text{mL mussel}^{-1}\text{day}^{-1}$ , the water column would be cleared of bacteria slightly less than one time per day. Loss rates in the bottle experiments performed at this site (Fig. 2a) indicate an average decrease in abundance of ca. 14% per day over the 2-day experiments.

One might expect, therefore, that bacterial abundance should have decreased in Saginaw Bay with increased zebra mussel abundance. Although our sample size is not very large, we have not detected any long-term changes in mean bacterial abundance at this site in the last three years, suggesting that over long time intervals, bacteria are compensating for losses. Perhaps *Dreissena* does not filter the entire water column effectively and/or losses are being compensated by increased growth rates. In western Lake Erie, the chlorophyll concentration was depleted to  $<1 \mu\text{g L}^{-1}$  above zebra mussel beds,

whereas concentrations higher in the water column were near  $4 \mu\text{g L}^{-1}$  (MacIsaac *et al.* 1992). Although we did not routinely perform thymidine measurements in 1992, a July 1992 estimate of bacterial production, based on thymidine incorporation rates, bacterial abundance, and conversion factors of  $2 \times 10^{18}$  cells produced per mole of thymidine incorporated (Fuhrman and Azam 1982), an average cell volume of  $0.04 \mu\text{m}^3$ , and  $2\text{--}5.6 \times 10^{-13} \text{ g C } \mu\text{m}^{-3}$  (Bratbak 1985, Wetzel and Likens 1991), indicates bacterial community growth rates were ca. 0.8–2.3 per day which is similar to the estimates of bacterial removal rates by mussels and would suggest that bacterial growth rates could compensate for zebra mussel filtering, assuming mussels were the main grazers of bacteria.

Evidence from the mesocosm experiments also suggests that heterotrophic bacterial growth is able to compensate for the increased loss rates, i.e., in addition to the direct negative effect of the mussels, they may also stimulate bacterial growth through indirect effects such as nutrient or organic carbon excretion. In the mesocosm experiments, we observed an increase in bacterial thymidine incorporation 1.5 days after introduction of mussels in the high zebra mussel treatment. Initial increases in bacterial abundance in the presence of mussels also suggest mussels may have stimulated bacterial production. Decreased abundance and thymidine incorporation rates later in the experiment may be the result of increased direct removal of bacteria by mussels. However, these results seem to contradict observations that mussels decreased thymidine incorporation rates in Lake St. Clair in short-term experiments. These different results could arise out of the different time-scales over which the measurements were made, different experimental protocols (enclosures versus bottle experiments) or differences in the bacterial community size composition and mussel filtration.

Although zebra mussels may be removing bacterioplankton, they may also stimulate growth by increasing the turnover of organic matter and inorganic nutrient recycling rates. In these experiments, we also measured significant increases in ammonium concentrations in the presence of zebra mussels (Gardner *et al.* 1995b). In another bottle experiment study, zebra mussels excreted ammonium at rates of  $1\text{--}5 \text{ ng-atom N mg dry weight}^{-1} \text{ hr}^{-1}$  (Quigley *et al.* 1993). There is also evidence that zebra mussels are excreting dissolved inorganic phosphorus compounds, (Heath *et al.* 1995; M. Vanni, personal communication, Miami Univer-

sity). Zebra mussels only assimilate about 50% of the phosphorus that they ingest (Stanczykowska and Planter 1985) and therefore may be an important regenerator of this nutrient. There is increasing evidence that inorganic nutrients, especially phosphorus, can limit bacterioplankton growth in lakes (Toolan *et al.* 1991, Coveney and Wetzel 1992, Morris and Lewis 1992). We have not observed increased dissolved organic carbon or amino acids in the presence of mussels, but this has been observed in other bivalves (Tupas and Koike 1990), suggesting that these components may be released by these organisms. These compounds could stimulate heterotrophic bacterial growth, but may not accumulate because they are rapidly metabolized or incorporated into bacterial biomass.

Our data provide two pieces of evidence supporting the view that mussels are removing primarily the largest bacteria from the community. Experiments with fluorescently labeled beads indicated that mussels removed the  $0.36$  and  $0.9 \mu\text{m}$  beads more effectively than  $0.22 \mu\text{m}$  beads (Table 1). Also, data from the mesocosm experiment (Fig. 5) indicated that only about 30 percent of the bacterial community, on a cell-abundance basis, was greater than  $1 \mu\text{m}$  in diameter in the control treatments, and a smaller percentage (10–23%) was found in zebra mussel treatments.

A likely consequence of removing the largest bacteria is that mussels may have the greatest impact during periods when the bacterial community "blooms." In August 1992, we observed a peak abundance of bacteria of  $4.5 \times 10^6 \text{ cells mL}^{-1}$  but the maximum abundance that we observed in all of 1993 was only  $1.9 \times 10^6 \text{ cells mL}^{-1}$ . Although other factors certainly could explain this change, as the mussels became more established in the bay, they may have been able to harvest the increased bacterial production that occurs in mid-summer. This argument is further supported by evidence that the most rapidly reproducing bacteria are larger than slowly reproducing bacteria (Ammerman *et al.* 1984, Letarte *et al.* 1992).

If bacteria were smaller (and not consumed as efficiently as inner bay bacteria) and more nutrient-limited in the outer bay than the inner bay, it could explain why opposite results were observed in these two locations. Nutrients released by mussels could stimulate growth without leading to an increased grazing rate if the bacterial cells produced were below a threshold size that the mussels filtered. It is obvious, however, that as the community continues to grow in average size toward a threshold that is

effectively removed by zebra mussels, a larger proportion is likely to be grazed, and may contribute to a decline in abundances and growth rates. This contention was supported by data from the mesocosm experiments where, in the high zebra mussel treatment, there was an initial increase in bacterial abundance and growth rate followed by decreased abundance and growth rate (Figs. 3 and 4). In the short-term bottle experiments with outer bay water where there was a significantly positive effect of mussels on bacterial abundance, the experiments may not have been run long enough to observe a subsequent decline in bacterial abundance as mean cell size increased.

It is possible that increased bacterial abundance in the outer bay bottle treatments may have been imported into the bottles with the mussels used in the treatments. Ambient bacterial abundance in the outer bay is only about 30–50% of that in the inner bay so this contamination effect would be more obvious in these treatments. However, time-course measurements of bacterial biomass in mesocosm treatments (Figs. 3 and 4) suggest that bacterial abundance and growth rates only increased 0.5–1.5 days after placement of mussels in the enclosures. These results indicate that the increased abundance was the result of bacterial growth rather than import of bacteria with the mussels.

We believe that the relative importance of direct and indirect impacts may explain the differences observed in the inner bay and outer bay experiments. At the inner bay site, direct effects of the mussels on bacteria through grazing activities is more important, whereas, indirect effects of the mussels on bacteria through nutrient enrichment is more important in the outer bay. In water from the inner bay, there was a decline in bacterial abundance in the zebra mussel treatments relative to the control but in water from the outer bay station, where no zebra mussels occurred naturally (Nalepa *et al.* 1995), there was an increase in bacterial abundance in the zebra mussel treatments relative to the control (Fig. 2). In the inner bay, the indirect effect of the mussel increasing nutrient availability may be of less significance than their direct effects through grazing because of the higher ambient nutrient concentrations and loading rates. The potential impact of the mussels on bacterial productivity is further accentuated by the fact that they are probably grazing the most actively growing, larger cells. A similar impact on rapidly growing, large bacteria has been observed for bacterivorous protists in estuaries (Sherr *et al.* 1992).

Our data from Lake St. Clair also support this observation. These data suggest that mussels may have been removing a disproportionate amount of the bacterial production (thymidine incorporation) but had little impact on bacterial abundance in the short-term (Fig. 6). This result would occur if most of the production was dominated by large cells and these cells were selectively grazed.

However, in the outer bay, the possible increased nutrient regeneration rates that are facilitated by mussels may be the most important factor in this more nutrient-poor habitat. Furthermore, potential differences in the size-distribution of the bacterial community in these two habitats may accentuate these effects. Heterotrophic bacteria growing in oligotrophic conditions are typically smaller than bacteria growing in eutrophic conditions. In nearshore regions of the Georgia continental shelf, 50–80% of bacterial respiration and thymidine incorporation was  $>1 \mu\text{m}$  in diameter, whereas, in offshore regions, 80–99% of these parameters was in particles less than  $1 \mu\text{m}$ . In a survey of eight Danish lakes of various trophic states, bacteria greater than  $1 \mu\text{m}$  in diameter composed 65% of the total bacterial community in eutrophic lakes and only 17% of the total bacterial community in oligotrophic lakes (Letarte *et al.* 1992). Smaller bacteria are less likely to be grazed by protozoans (Andersson *et al.* 1986, Monger and Landry 1992, Sherr *et al.* 1992, Šimek *et al.* 1994) as well as zebra mussels (this study) and therefore can compensate for slower growth rates by decreased predation rates.

It seems clear that mussels have a negative effect on the bacterial communities but may also have a positive indirect effect on bacterial growth rates. The net effect is dependent on the size-structure of the bacterial community and the degree of nutrient limitation. Neither direct nor indirect effects work exclusively in either environment, but rather, direct effects were more important in the inner bay and indirect effects may be more important in the outer bay.

#### ACKNOWLEDGMENTS

This research was supported by the National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory zebra mussel research funds and Sea Grant College Program funding (Award number NA16RG0457). The Biology Department at Eastern Michigan University kindly provided time on the scanning electron microscope. J. Cotner and J. Johnson were partially supported through the Cooperative Institute for

Limnology and Ecosystems Research (CILER) under cooperative agreement from the Environmental Research Laboratory, National Oceanic & Atmospheric Administration, U.S. Department of Commerce. G. Fahnenstiel and R. Heath assisted in the design and implementation of the mesocosm experiment in Saginaw Bay.

## REFERENCES

- Ammerman, J.W., Fuhrman, J.A., Hagström, Å., and Azam, F. 1984. Bacterioplankton growth in seawater: I. Growth kinetics and cellular characteristics in seawater cultures. *Mar. Ecol. Prog. Ser.* 18:31–39.
- Andersson, A., Larsson, U., and Hagström, Å. 1986. Size-selective grazing by a microflagellate on pelagic bacteria. *Mar. Ecol. Prog. Ser.* 33:51–57.
- Bratbak, G. 1985. Bacterial biovolume and biomass estimations. *Appl. Environ. Microbiol.* 49: 1488–1493.
- Bunt, C.M., MacIsaac, H.J., and Sprules, W.G. 1993. Pumping rates and projected filtering impacts of juvenile zebra mussels *Dreissena polymorpha* in western Lake Erie. *Can. J. Fish. Aquat. Sci.* 50: 1017–1022.
- Chin-Leo, G., and Benner, R. 1992. Enhanced bacterioplankton production and respiration at intermediate salinities in the Mississippi River plume. *Mar. Ecol. Prog. Ser.* 87:87–103.
- Cotner, J.B. Jr., and Gardner, W.S. 1993. Heterotrophic bacterial mediation of ammonium and dissolved free amino acid fluxes in the Mississippi River plume. *Mar. Ecol. Prog. Ser.* 93:75–87.
- Coveney, M.F., and Wetzel, R.G. 1992. Effects of nutrients on specific growth rate of bacterioplankton in oligotrophic lake water cultures. *Appl. Environ. Microbiol.* 58:150–156.
- Fahnenstiel, G.L., Bridgeman, T.B., Lang, G.A., McCormick, M.J., and Nalepa, T.F. 1995. Phytoplankton productivity in Saginaw Bay, Lake Huron: effects of zebra mussel (*Dreissena polymorpha*) colonization. *J. Great Lakes Res.* 21:465–475.
- Fanslow, D.L., Nalepa, T.F., and Lang, G.A. 1995. Filtration rates of zebra mussels (*Dreissena polymorpha*) on natural seston from Saginaw Bay, Lake Huron. *J. Great Lakes Res.* 21:489–500.
- Fuhrman, J.A., and Azam, F. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Mar. Biol.* 66:109–120.
- Gardner, W.S., Benner, R., Amon, R.M.W., Cotner, J.B. Jr., Cavaletto, J.F., and Johnson, J.R. 1995a. Effects of high molecular weight dissolved organic matter and light on heterotrophic nitrogen dynamics in the Mississippi River plume. *Mar. Ecol. Prog. Ser.* In press.
- \_\_\_\_\_, Cavaletto, J.F., Johengen, T.H., Johnson, J.R., Heath, R.T., and Cotner, J.B. 1995b. Effects of the zebra mussel (*Dreissena polymorpha*) on community nitrogen dynamics in Saginaw Bay, Lake Huron. *J. Great Lakes Res.* 21:529–544.
- Griffiths, R.W. 1993. Effects of zebra mussels (*Dreissena polymorpha*) on benthic fauna of Lake St. Clair. In *Zebra Mussels: Biology, Impacts, and Control*, ed. T.F. Nalepa, and D.W. Schloesser, pp. 415–437. Boca Raton, FL: Lewis Publishers/CRC Press.
- Heath, R.T., Fahnenstiel, G., Gardner, W.S., Cavaletto, J.F., and Hwang, S.-J. 1995. Ecosystem-level effects of zebra mussels (*Dreissena polymorpha*): an enclosure experiment in Saginaw Bay, Lake Huron. *J. Great Lakes Res.* 21:501–516.
- Hebert, P.D.N., Muncaster, B.W., and Mackie, G.L. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. *Can. J. Fish. Aquat. Sci.* 46:1587–1591.
- Herdendorf, C.E., Raphael, C.N., and Jaworski, E. 1986. *The ecology of Lake St. Clair wetlands: a community profile*. U. S. Fish Wild. Serv. Biol. Rep. 85(7.7).
- Hobbie, J.E., Daley, R.J., and Jasper, S. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33: 1225–1228.
- Jørgensen, C.B. 1975. On gill function in the mussel *Mytilus edulis* L. *Ophelia* 13:187–232.
- \_\_\_\_\_, Kjørboe, T., and Riisgård, H.U. 1984. Ciliary and mucus-net filter feeding, with special reference to fluid mechanical characteristics. *Mar. Ecol. Prog. Ser.* 15:283–292.
- Kryger, J., and Riisgård, H.U. 1988. Filtration rate capacities in 6 species of European freshwater bivalves. *Oecologia* 77:34–38.
- Leach, J.H. 1993. Impacts of the zebra mussel (*Dreissena polymorpha*) on water quality and fish spawning reefs in western Lake Erie. In *Zebra Mussels: Biology, Impacts, and Control*, eds. T.F. Nalepa, and D.W. Schloesser, pp. 381–397. Boca Raton, FL: Lewis Publishers/CRC Press.
- Letarte, Y., Hansen, H.J., Sondergaard, M., and Pinel-Alloul, B. 1992. Production and abundance of different bacterial size-classes: relationships with primary production and chlorophyll concentration. *Arch. Hydrobiol.* 126:15–26.
- MacIsaac, H.J., Sprules, W.G., Johannsson, O.E., and Leach, J.H. 1992. Filtering impacts of larval and sessile zebra mussels (*Dreissena polymorpha*) in western Lake Erie. *Oecologia* 92:30–39.
- Monger, B.C., and Landry, M.R. 1992. Size-selective grazing by heterotrophic nanoflagellates: an analysis using live-stained bacteria and dual-beam flow cytometry. *Arch. Hydrobiol. Beih.* 37:173–185.
- Morris, D.P., and Lewis, W.M. Jr. 1992. Nutrient limitation of bacterioplankton growth in Lake Dillon, Colorado. *Limnol. Oceanogr.* 37:1179–1192.
- Møhlenberg, F., and Riisgård, H.U. 1978. Efficiency of

- particle retention in 13 species of suspension feeding bivalves. *Ophelia* 17:239–246.
- Nalepa, T.F., Cavaletto, J.F., Ford, M., Gordon, W.M., and Wimmer, M. 1993. Seasonal and annual variation in weight and biochemical content of the zebra mussel, *Dreissena polymorpha*, in Lake St. Clair. *J. Great Lakes Res.* 19:541–552.
- \_\_\_\_\_, Wojcik, J.A., Fanslow, D.L., and Lang, G.A. 1995. Initial colonization of the zebra mussel (*Dreissena polymorpha*) in Saginaw Bay, Lake Huron: population recruitment, density, and size structure. *J. Great Lakes Res.* 21:417–434.
- Nichols, S.J. 1993. Maintenance of the zebra mussel (*Dreissena polymorpha*) under laboratory conditions. In *Zebra Mussels: Biology, Impacts and Control*, eds. T.F. Nalepa, and D.W. Schloesser, pp. 733–747. Boca Raton, FL: Lewis Publishers/CRC Press.
- Quigley, M.A., Gardner, W.S., and Gordon, W.M. 1993. Metabolism of the zebra mussel (*Dreissena polymorpha*) in Lake St. Clair of the Great Lakes. In *Zebra Mussels: Biology, Impacts and Control*, eds. T.F. Nalepa, and D.W. Schloesser, pp. 295–306. Boca Raton, FL: Lewis Publishers/CRC Press.
- Reeders, H.H., and bij de Vaate, A. 1990. Zebra mussels (*Dreissena polymorpha*): a new perspective for water quality management. *Hydrobiologia* 200/201: 437–450.
- Sherr, B.F., Sherr, E.B., and McDaniel, J. 1992. Effect of protistan grazing on the frequency of dividing cells in bacterioplankton assemblages. *Appl. Environ. Microbiol.* 58:2381–2385.
- Šimek, K., Vrba, J., and Hartman, P. 1994. Size-selective feeding by *Cyclidium* sp on bacterioplankton and various sizes of cultured bacteria. *FEMS. Microbiol. Ecol.* 14:157–167.
- Sokal, R.R., and Rohlf, F.J. 1981. *Biometry*, 2nd ed. W.H. Freeman.
- Sprung, M., and Rose, U. 1988. Influence of food size and food quantity on the feeding of the mussel *Dreissena polymorpha*. *Oecologia* 77:526–532.
- Stanczykowska, A., and Planter, M. 1985. Factors affecting nutrient budgets in lakes of the R. Jorka watershed (Masurian Lakeland, Poland). X. Role of the mussel *Dreissena polymorpha* (Pall.) in N and P cycles in a lake ecosystem. *Ekol. pol.* 33:345–356.
- Toolan, T., Wehr, J.D., and Findlay, S. 1991. Inorganic phosphorus stimulation of bacterioplankton production in a meso-eutrophic lake. *Appl. Environ. Microbiol.* 57:2074–2078.
- Tupas, L., and Koike, I. 1990. Amino acid and ammonium utilization by heterotrophic marine bacteria grown in enriched seawater. *Limnol. Oceanogr.* 35: 1145–1155.
- Vanderploeg, H.A., Liebig, J.R., and Nalepa, T.F. 1995. From picoplankton to microplankton: temperature driven filtration by the unionid bivalve *Lampsilis radiata siliquoidea* in Lake St. Clair. *Can. J. Fish. Aquat. Sci.* 52:63–74.
- Wetzel, R.G., and Likens, G.E. 1991. *Limnological Analyses*, 2nd ed. Springer.
- Wilkinson, L. 1989. *SYSTAT: The system for statistics*. Systat, Inc.
- Wright, R.T., Coffin, R.B., Ersing, C.P., and Pearson, D. 1982. Field and laboratory measurements of bivalve filtration of natural marine bacterioplankton. *Limnol. Oceanogr.* 27:91–98.

Submitted: 28 October 1994

Accepted: 28 July 1995