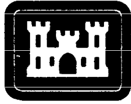

US Army Corps
of Engineers
Waterways Experiment
Station

Zebra Mussel Research Technical Notes

Section 1 — Environmental Testing

Technical Note ZMR-1-35

September 1998

Nutrient Regeneration by Zebra Mussels: Implications for the Phosphorus Budget of Lake Pepin, Upper Mississippi River

Background and purpose

Changes in suspended seston and soluble nutrients were examined as a function of zebra mussel density in flow-through sediment-water microcosms to determine impacts on nutrient recycling. Overall, zebra mussels removed >70 percent of the suspended seston and >80 percent of the total chlorophyll *a* input to the microcosms. Declines in organic nitrogen in microcosms were accompanied by density-dependent increases in nitrate-nitrite nitrogen.

Research described in this technical note suggests that microflora associated with the sediments in the microcosms were responsible for transforming ammonium nitrogen, an excretory product of zebra mussels, into nitrate nitrogen. Declines in total phosphorus in the microcosms also coincided with density-dependent increases in the concentration of soluble reactive phosphorus (SRP) and its rate of regeneration, suggesting enhanced nutrient cycling via excretion. Evidence of measured rates of soluble phosphorus regeneration (up to 2.5 mg m^{-2} day^{-1}) via zebra mussels at densities of only ~1,300 individuals per square meter (ind./m^{-2}) has important implications for the phosphorus economy of Lake Pepin, a natural impoundment of the upper Mississippi River (UMR) that has been recently invaded by zebra mussels.

These studies examined the influence of varying zebra mussel densities on particulate matter and soluble nutrient dynamics in laboratory sediment-water microcosms for application to ongoing investigations of nutrient loadings and water quality in Lake Pepin.

Additional information

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Approach

One of the concerns over the introduction of zebra mussels into aquatic systems within North America is the rapid colonization of these organisms with accompanying changes in water quality and sediment composition. Zebra mussels at

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high densities can graze seston (including phytoplankton) and thus filter water at tremendous rates, resulting in the transfer of suspended particulate matter from the water column to sediments (Reeders and de Vaate 1990). Excretion of feces and pseudofeces on the sediment surface may affect sediment chemistry and nutrient dynamics at the sediment-water interface. Considered collectively, these processes may have important impacts on food web dynamics, contaminant cycling, and water/sediment quality (Holland 1993; Leach 1993; Nicholls and Hopkins 1993; Bruner, Fisher, and Landrum 1994; Effler and Siegfried 1994; Cotner and others 1995; Gardner and others 1995; Heath and others 1995; Holland, Johengen, and Beeton 1995).

While much is known about the impacts of zebra mussel grazing on seston concentrations in aquatic systems (Leach 1993; Nicholls and Hopkins 1993; Fanslow, Napela, and Lang 1995; Holland, Johengen, and Beeton 1995; Madenjian 1995), little information exists regarding the roles of zebra mussels in soluble nutrient regeneration, particularly in rivers.

These processes were evaluated in assessing the nutrient loadings and water quality in Lake Pepin, a eutrophic impoundment on the UMR system that was invaded (~1994) and has been colonized by zebra mussels. The lake, created naturally as a result of river delta formation via the Chippewa River, is large (93.4 km^2), has a mean and maximum depth of 5.4 m and 17 m, respectively, and a water residence time of ~19 days. Its watershed (~122,000 km^2) includes drainage from the Minnesota and St. Croix Rivers.

Methods

Laboratory microcosms consisted of 4-L polycarbonate containers measuring 20 cm in height and 17 cm in diameter. Three replicate control microcosms contained sediment and lake water, while replicate experimental microcosms (three replicates for each experimental treatment) contained sediment, lake water, and zebra mussels. Experimental treatments consisted of four levels of zebra mussel densities ranging from 170 to 1,300 ind./m^2 in the microcosms (Table 1).

Table 1. Zebra Mussel Density Levels in Replicate Microcosms

Density Level (ind./m^2)
170 (3 ind./microcosm)
340 (6 ind./microcosm)
620 (11 ind./microcosm)
1,300 (23 ind./microcosm)

Surface sediment was collected with a ponar sediment sampler from a backwater region of the upper Mississippi River located below Lake Pepin (Finger Lakes region). The sediment was drained of excess water and homogeneously mixed before dispensing ~1.5 L into each microcosm. Water in each microcosm was flushed via a peristaltic pump system at a rate of approximately 2.4 L/day (residence time = 0.6 day) throughout the study with fresh lake water obtained daily from Eau Galle Reservoir, Wisconsin.

At daily intervals, freshly collected lake water was homogeneously mixed, with 3.5-L aliquots dispensed into separate 5-L plastic containers for inflow into each microcosm. The inflow containers were gently aerated to maintain particles in suspension during pumping. Outflow water from each microcosm was collected daily in plastic containers.

To maintain accurate water and material fluxes for each microcosm, inflow and outflow volumes (liters) were measured at daily intervals. The assembled flow-through microcosms were placed in a temperature-controlled water bath (18 °C) where they were gently aerated with air stones to maintain aerobic conditions. The duration of the study was 2 weeks (4-16 October 1995).

The zebra mussels used for the study (mean length = 21 mm ± 0.2 S.E. (standard error); minimum length = 15 mm; maximum length = 30 mm; mean fresh weight = 1.5 g/ind. ± 0.1 S.E.) were collected from the southern basin of Lake Pepin and placed in a constant-temperature (18 °C) water tank (~100 L) for an equilibration period of about 2 weeks prior to initiation of the study. Fresh lake water was constantly circulated though the tank at a rate of ~100 L/day to maintain a food supply for the zebra mussels during the equilibration period.

Every Monday, Wednesday, and Friday during the 2-week period of study, subsamples were collected from microcosm outflows for chemical analyses. In addition, three replicate samples of homogeneously mixed inflow water were collected for chemical analysis. Samples were filtered onto precombusted glass fiber filters (Gelman A/E), dried to a constant weight at 105 °C for suspended seston analysis, and then combusted at 500 °C for 1 hr for particulate organic matter (POM) determination (American Public Health Association, APHA 1992). Samples for total nitrogen and phosphorus were predigested with potassium persulfate according to Ameel, Axler, and Owen (1993) before determining concentrations colorimetrically on an automated system (Lachat Methods 10-107-04-1-A and 10-115-01-1-A; Lachat QuikChem AE System, Zellweger Analytics/Lachat Instruments Division, Milwaukee, WI).

For analysis of soluble constituents, water samples were filtered through a 0.45-µm filter (Gelman Metricel) prior to analysis. Soluble reactive phosphorus (Lachat Method 10-115-01-1-A), nitrate-nitrite nitrogen (Lachat Method 10-107-04-01-A), and ammonium nitrogen (Lachat Method-A) were analyzed colorimetrically using automated procedures (APHA 1992). Total inorganic nitrogen was calculated as the sum of ammonium nitrogen and nitrate-nitrite nitrogen. Organic nitrogen was calculated as the difference between total nitrogen and total inorganic nitrogen.

Samples for the determination of chlorophyll were filtered onto glass fiber filters, macerated with a tissue grinder, and extracted in 90 percent alkaline acetone at 4 °C for a minimum of 2 hr (APHA 1992). Viable chlorophyll *a* and phaeophytin *a* were determined from the clarified extract according to APHA (1992). Total chlorophyll was calculated as the sum of viable chlorophyll *a* and phaeophytin *a*. At the end of the study, zebra mussels from each microcosm were weighed for fresh weight biomass determination.

To determine concentration changes and rates of soluble nutrient regeneration due to zebra mussel activities, mean outflow concentrations obtained from the control microcosms were compared with outflow concentrations obtained from individual zebra mussel density treatments.

It was assumed that outflow concentrations of the control microcosms reflected the difference between inflow concentrations and sedimentation in the inflow containers.

Rates of suspended seston, POM, and total chlorophyll removal via zebra mussels and rates of soluble nitrogen and phosphorus regeneration via zebra mussels were calculated as

$$(C_{exp} - C_{control}) \times Q/A$$

where

C_{exp} = mean daily concentration of the outflow for experimental microcosms

$C_{control}$ = mean daily concentration of the outflow for control microcosms

Q = daily flow rate (L/day)

A = cross-sectional area of the microcosms (0.0176 m^2) or fresh weight biomass of the zebra mussels

Filtration rates (FR) were calculated as the decrease in total chlorophyll *a* according to the equation (Kraak and others 1994)

$$FR = Q/n \times \ln C_i/C_f$$

where

n = number (or biomass) of zebra mussels per microcosm

C_i = mean total chlorophyll *a* concentration in control microcosms

C_f = mean total chlorophyll *a* concentration in experimental microcosms

Results and discussion

Mean concentrations of suspended seston, POM, and total chlorophyll declined significantly in experimental microcosms containing zebra mussels, compared with controls, indicating substantial removal of particles via filtration activities of the zebra mussels (Figures 1a-c). Overall, zebra mussels accounted for removal of >70 percent of the suspended seston and POM and >80 percent of the total chlorophyll input.

Zebra mussel filtration rates were 0.6 to 0.8 L g fresh weight^{-1} day^{-1} (1.0 to 1.5 L ind.$^{-1}$ day^{-1}), indicating that the entire daily water income to the microcosms (that is, ~2.4 L/day) was filtered by zebra mussels, even at the lowest density levels. Rates of removal of suspended particles per gram of zebra mussel biomass decreased as a function of increased density level (Table 2), suggesting that inflow seston concentrations were insufficient in relation to zebra mussel needs at the higher density levels.

Table 2. Mean (±1 S.E.) Rates of Removal of Suspended Seston, Particulate Organic Matter, and Total Chlorophyll in Microcosms as a Function of Zebra Mussel Density[a]

Density (ind./m^2)	Suspended Seston (mg g^{-1} biomass day^{-1})	POM (mg g^{-1} biomass day^{-1})	Total Chlorophyll[b] (µg g^{-1} biomass day^{-1})
170	1.32 (0.19) c	0.99 (0.16) b	15.04 (2.36) b
340	1.18 (0.09) b, c	0.90 (0.08) b	14.31 (0.89) b
620	0.40 (0.04) a, b	0.27 (0.03) a	4.59 (0.52) a
1,300	0.24 (0.03) a	0.18 (0.03) a	2.91 (0.43) a

NOTES:
[a] Rates of removal are corrected for the control rates and normalized with respect to zebra mussel fresh weight biomass. Different letters indicate significant differences at $p < 0.05$, based on Duncan's Multiple Range Analysis (ANOVA; SAS 1988).
[b] Viable chorophyll plus phaeopigments.

Mean concentrations of total and ammonium nitrogen did not differ significantly among any of the microcosms (Figures 1d and 1e). Organic nitrogen (not shown) decreased in experimental microcosms, relative to control microcosms, but there were no trends in concentration as a function of zebra mussel density. Mean nitrate-nitrite nitrogen and the percentage (relative to total nitrogen) of total inorganic nitrogen increased significantly as a function of zebra mussel density (Figures 1f and 1g).

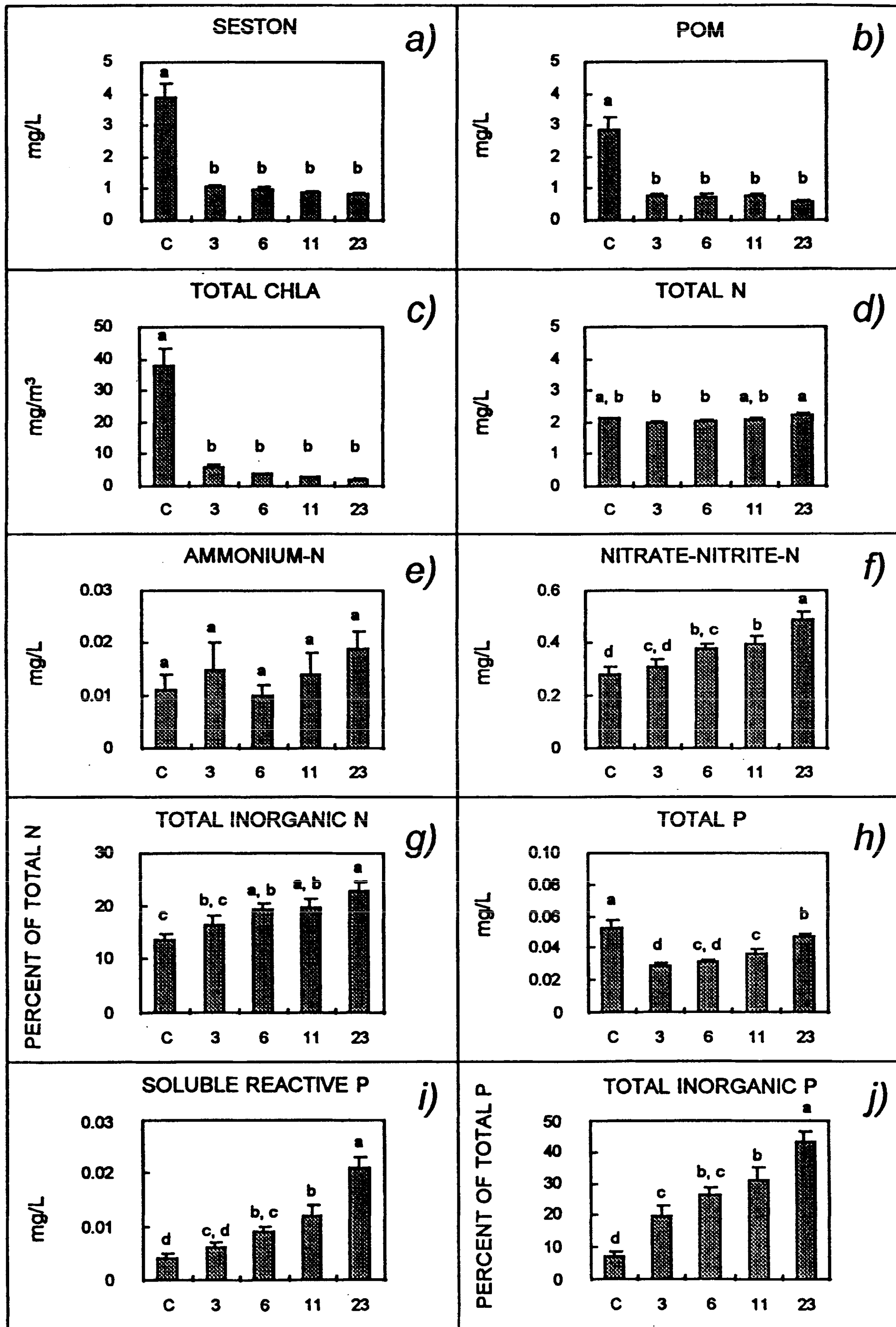

Figure 1. Mean (±1 S.E.) variations as a function of zebra mussel density. For the x-axis, C is the control, and 3, 6, 11, and 23 are the numbers of zebra mussels in each treatment (see Table 1). Different letters above the means represent significant differences at $p < 0.05$ (analysis of variance, ANOVA; Statistical Analysis System, SAS 1988)

Others researchers (Gardner and others 1995, Heath and others 1995) have found that the primary excretory product of zebra mussels is ammonium nitrogen, which contrasts with the observations of this study, perhaps owing to differences in microbial activity. Mean rates of soluble nitrogen regeneration due to zebra mussels tended to increase with increasing zebra mussel density (Table 3). The mean rate of soluble nitrogen regeneration, normalized with respect to zebra mussel biomass (25.5 μg N g^{-1} fresh weight day^{-1}, ±1.8 S.E.), was statistically similar over all density levels.

Declines in total phosphorus concentration (Figure 1h) in all experimental microcosms were associated with marked density-dependent increases in SRP (Figure 1i) relative to control concentrations. At high zebra mussel densities, SRP accounted for >40 percent of the total phosphorus in the outflow (Figure 1j). In contrast, SRP accounted for <10 percent of the total phosphorus in control microcosms (Figure 1j).

Table 3. Mean (±1 S.E.) Areal Rates of Soluble Reactive Phosphorus and Nitrate-Nitrite Nitrogen Regeneration as a Function of Zebra Mussel Density

Density (ind./m^2)	SRP (mg m^{-2} day^{-1})	NO_2NO_3 (mg m^{-2} day^{-1})
170	0.3 (0.2) c	5.3 (1.8) c
340	0.8 (0.2) b, c	15.3 (1.3) b
620	1.0 (0.3) b	16.9 (1.1) b
1,300	2.5 (0.3) a	34.2 (1.0) a

NOTES: Rates of regeneration are corrected for the control rates and normalized with respect to area. Different letters indicate significant differences at $p < 0.05$, based on Duncan's Multiple Range Analysis (ANOVA; SAS 1988).

Mean rates of SRP regeneration increased significantly with increasing zebra mussel density (mean SRP rate, mg m^{-2} day^{-1} = mean zebra mussel density (ind./m^2) × 0.0019; $r^2 = 0.99$), with rates ranging between 0.3 and 2.5 mg m^{-2} day^{-1} (Table 3). When normalized with respect to zebra mussel biomass, rates of SRP regeneration were statistically uniform (mean = 1.4 μg P g^{-1} fresh weight day^{-1}; ±0.2 S.E.). A mean regeneration rate of 0.08 μg P $individual^{-1}$ hr^{-1} (±0.01 S.E.) was estimated, which is similar to the hourly rate of phosphorus regeneration reported by Mellina, Rasmussen, and Mills (1995) for zebra mussels (~20-mm shell length) incubated at 17 °C.

These results support the general findings of others that zebra mussel populations can graze large portions of seston and chlorophyll, thereby markedly altering energy flow and nutrient dynamics in aquatic systems (Leach 1993; Nicholls and Hopkins 1993; Fanslow, Napela, and Lang 1995; Holland, Johengen, and Beeton 1995; Madenjian 1995). While zebra mussels can act as an important sink for nutrients (Stańczykowsla and Lewandowski 1993), the current study results and those of Heath and others (1995) and Mellina, Rasmussen, and Mills (1995) indicate that zebra mussels can also play an important role as a nutrient source by regenerating nutrients back into the water column in soluble forms.

In addition, Holland, Johengen, and Beeton (1995) observed increases in soluble nitrogen and phosphorus forms in the western basin of Lake Erie since invasion by zebra mussels. These investigators attributed the increases to nutrient regeneration via excretion. In our study, in particular, areal rates of soluble phosphorus regeneration measured in laboratory microcosms at modest zebra mussel densities of ~1,300 ind./m^2 were comparable to rates of phosphorus release from profundal sediments measured for eutrophic systems under anoxic conditions (Nürnberg and others 1986).

Enhanced soluble phosphorus regeneration by zebra mussels has important implications for the phosphorus economy of Lake Pepin, since they have recently invaded this system and are currently found at densities of up to 1,700 ind./m^2 in shallow regions of the southern end of the basin (personal communication, M. Davis and R. Hart, Minnesota Department of Natural Resources).

Using the relationship between zebra mussel density and rates of soluble phosphorus regeneration obtained from our laboratory microcosms, a regeneration rate of 3.2 mg m^{-2} day^{-1} is estimated, which is equivalent to estimates of phosphorus release from the sediments of Lake Pepin under oxic conditions during the summer (James, Barko, and Eakin 1995). Although the phosphorus budget of this lake is usually (during normal flow) dominated by external loading from the watershed, internal phosphorus loading via zebra mussels may become increasingly more important under conditions of low flow, particularly as zebra mussels occupy larger areas of this system in future years.

The overall future impacts of zebra mussels on water quality in Lake Pepin or downstream are uncertain at this time. However, marked reductions in chlorophyll and concomitant increases in soluble phosphorus have been reported in shallow-water systems (lakes and rivers) elsewhere at high-population densities of zebra mussels. Examples include Lake Erie (Holland, Johengen, and Beeton 1995), Lake St. Clair (Mellina, Rasmussen, and Mills 1995), and the Seneca River (Effler and others 1996). In contrast, European lakes with natural, stable populations of zebra mussels at lower densities have not exhibited major deviations in chlorophyll:phosphorus relationships (Mellina, Rasmussen, and Mills 1995).

References

Ameel, J. J., Axler, R. P., and Owen, C. J. (1993). "Persulfate digestion for determination of total nitrogen and phosphorus in low nutrient waters," *American Environmental Laboratory* (Oct 1993), 8-10.

American Public Health Association. (1992). *Standard methods for the examination of water and wastewater.* 18th ed., Washington, DC.

Bruner, K. A., Fisher, S. W., and Landrum, P. F. (1994). "The role of the zebra mussel, *Dreissena polymorpha*, in contaminant cycling; II. Zebra mussel contaminant accumulation from algae and suspended particles, and transfer to the benthic invertebrate *Grammarus fasciatus*," *Journal of Great Lakes Research* 20, 735-50.

Cotner, J. B., Gardner, W. S., Johnson, J. R., Sada, R. H., Cavaletto, J. F., and Heath, R. T. (1995). "Effects of zebra mussels (*Dreissena polymorpha*) on bacterioplankton: Evidence for both size-selective consumption and growth stimulation," *Journal of Great Lakes Research* 21, 517-28.

Effler, S. W., and Siegfried, C. (1994). "Zebra mussel (*Dreissena polymorpha*) populations in the Seneca River, New York: Impact on oxygen resources," *Environmental Science and Technology* 28, 2216-21.

Effler, S. W., Brooks, C. M., Whitehead, K., Wagner, B., Doerr, S. M., and Perkins, M. G. (1996). "Impact of zebra mussel invasion on river water quality," *Water and Environmental Research* 205-14.

Fanslow, D. L., Napela, T. F., and Lang, G. A. (1995). "Filtration rates of the zebra mussel (*Dreissena polymorpha*) on natural seston from Saginaw Bay, Lake Huron," *Journal of Great Lakes Research* 21, 489-500.

Gardner, W. S., Cavaletto, J. R., Johengen, T. H., Johnson, J. R., Heath, R. T., and Cotner, J. B. (1995). "Effects of the zebra mussel, *Dreissena polymorpha*, on community nitrogen dynamics in Saginaw Bay, Lake Huron," *Journal of Great Lakes Research* 21, 529-44.

Heath, R. T., Fahnenstiel, G. L., 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," *Journal of Great Lakes Research* 21, 501-16.

Holland, R. E. (1993). "Changes in planktonic diatoms and water transparency in Hatchery Bay, Bass Island Area, western Lake Erie since the establishment of the zebra mussel," *Journal of Great Lakes Research* 19, 617-24.

Holland, R. E., Johengen, T. H., and Beeton, A. M. (1995). "Trends in nutrient concentrations in Hatchery Bay, western Lake Erie, before and after *Dreissena polymorpha*," *Canadian Journal of Fisheries and Aquatic Science* 52, 1202-09.

James, W. F., Barko, J. W., and Eakin, H. L. (1995). "Internal phosphorus loading in Lake Pepin, upper Mississippi River," *Journal of Freshwater Ecology* 10, 269-76.

Kraak, M. H. S., Kuipers, F., Schoon, H., de Groot, C. J., and Admiraal, W. (1994). "The filtration rate of the zebra mussel *Dreissena polymorpha* used for water quality assessment in Dutch rivers," *Hydrobiologia* 294, 13-16.

Leach, J. H. (1993). "Impacts of the zebra mussel (*Dreissena polymorpha*) on water quality and fish spawning reefs in western Lake Erie." *Zebra mussels, biology, impacts, and control.* T. F. Nalepa and D. W. Schloesser, ed., Lewis Publishers, Ann Arbor, MI, 381-98.

Madenjian, C. P. (1995). "Removal of algae by the zebra mussel (*Dreissena polymorpha*) population in western Lake Erie: A bioenergetics approach," *Canadian Journal of Fisheries and Aquatic Science* 52, 381-90.

Mellina, E., Rasmussen, J. B., and Mills, E. L. (1995). "Impact of zebra mussel (*Dreissena polymorpha*) on phosphorus cycling and chlorophyll in lakes," *Canadian Journal of Fisheries and Aquatic Science* 52, 2253-2573.

Nicholls, K. H., and Hopkins, G. J. (1993). "Recent changes in Lake Erie (North Shore) phytoplankton: Cumulative impacts of phosphorus loading reductions and the zebra mussel introduction," *Journal of Great Lakes Research* 19, 637-47.

Nürnberg, G. K., Shaw, M. Dillon, P. J., and McQueen, D. J. (1986). "Internal phosphorus load in an oligotrophic Precambrian shield lake with an anoxic hypolimnion," *Canadian Journal of Fisheries and Aquatic Science* 43, 574-80.

Reeders, H. H., and de Vaate, A. Bij. (1990). "Zebra mussels (*Dreissena polymorpha*): A new perspective for water quality management," *Hydrobiologia* 200/201, 437-50.

SAS (Statistical Analysis System). (1988). "SAS users guide, statistics; Version 6.03 edition." SAS Institute, Cary, NC.

Stańczykowska, A., and Lewandowski, K. (1993). "Effect of filtering activity of *Dreissena polymorpha* (Pall.) on the nutrient budget of the littoral of Lake Mikolajskie," *Hydrobiologia* 251, 73-79.