PURPOSE: This technical note examines phosphorus excretion by zebra mussels as a function of zebra mussel density and food availability using laboratory microcosms.

BACKGROUND: In addition to their ability to filter and remove large quantities of seston from the water column, zebra mussels (*Dreissena polymorpha*) play a very important role in accelerating the remineralization of filtered organic matter into soluble forms that can be recycled back to the pelagic food web. Nutrient remineralization may occur directly via excretion of soluble nutrients (Heath et al. 1995) and indirectly via decomposition of feces and pseudofeces (Klerks, Fraleigh, and Lawniczak 1996). Thus, while filtering activities can be beneficial in clarifying the water column (Reeders and de Vaate 1990) and improving light transparency for littoral flora (Skubinna, Coon, and Batterson 1995), enhanced soluble nutrient remineralization by zebra mussels may be detrimental in promoting a shift in phytoplankton dominance to noxious blue-green algae (Arnot and Vanni 1996). Thus, it is important to identify and quantify nutrient remineralization mechanisms to forecast changes in ecosystem structure due to zebra mussels.

Recent studies have estimated excretion of soluble phosphorus (P) by zebra mussels based on laboratory experiments (Mellina, Rasmussen, and Mills 1995; Arnot and Vanni 1996; James, Barko, and Eakin 1997) and mass balance approaches (Effler et al. 1997). These studies indicate that zebra mussels are capable of markedly impacting the P economy of aquatic systems. In addition to excretion, zebra mussels may also recycle P back to the water column via tissue emaciation during temporary periods of growth stress such as seasonal declines in food availability and type of food. This potential avenue of P recycling has received minor attention, yet it may constitute an important flux of P to aquatic systems invaded by zebra mussels. In addition to food availability, other factors that may induce lack of growth and tissue emaciation include occurrences of turbidity, which decrease the filtering efficiency of zebra mussels (Madon et al. 1998), and changes in temperature and salinity (Spidle, Mills, and May 1995). Using laboratory microcosms, the hypothesis that decreases in food availability can lead to enhanced P recycling by zebra mussels due to growth stress and tissue emaciation was tested. To test this hypothesis, zebra mussels over a range of densities were subjected to a constant food resource supply.

METHODS: Flow-through laboratory microcosms (4-L polycarbonate containers), as described in James, Barko, and Eakin (1997), were used to examine P recycling by zebra mussels over a range of densities. Three replicate control microcosms contained lake water, while replicate experimental microcosms (three replicates for each experimental treatment) contained lake water and zebra mussels. Experimental treatments consisted of four discrete levels of zebra mussel densities (Table 1).

Using freshly collected lake water obtained daily from Eau Galle Reservoir, Wisconsin, a residence time of ~ 0.6 day (i.e., flow rate of 2.4 L/day) was maintained for each microcosm using a peristaltic
pump system. Each evening, fresh 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 were measured in liters at daily intervals. The assembled flow-through microcosms were placed in a temperature-controlled water bath (18 °C) where they were gently aerated using air stones to maintain aerobic conditions. The duration of the study was 2 weeks (14-28 August 1996).

The zebra mussels used for the study (mean length = 22 mm ± 0.2 standard error (S.E.); minimum length = 9 mm; maximum length = 33 mm) were collected from Lake Pepin, Upper Mississippi River, 2 weeks prior to initiation of the study. Shortly after collection, they were gently removed from substrate by cutting byssal threads with a razor blade and placed in a constant-temperature (18 °C) water tank (~ 100 L). The zebra mussels were maintained with fresh lake water (collected daily) that was constantly circulated through the tank at a rate of ~ 100 L/day. The lake water had a total chlorophyll (viable chlorophyll a = 10 µg/L, phaeophytin a = 6 µg/L) content of ~16 µg/L. The shell length of each zebra mussel was determined to the nearest 1 mm prior to incubation in microcosms.

Six times during the 2-week study, subsamples were collected from microcosm outflows for chemical analyses. Samples for total P were predigested with potassium persulfate according to Ameel, Axler, and Owen (1993) before determining concentrations colorimetrically on an automated system (Lachat Method 10-115-01-1-A, Lachat QuikChem AE System, Zellweger Analytics/Lachat Instruments Division, Milwaukee, WI). For analysis of soluble reactive P (SRP), water samples were filtered through a 0.45-µm filter (Gelman Metricel) prior to analysis. SRP (Lachat Method 10-115-01-1-A) was analyzed colorimetrically using automated procedures (American Public Health Association (APHA) 1992). Samples for the determination of chlorophyll were filtered onto glass fiber filters (Gelman A/E), macerated with a tissue grinder, and extracted in 90 percent alkaline acetone at 4 °C for a minimum of 2 hours (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.

<table>
<thead>
<tr>
<th>Density, individuals m⁻²</th>
<th>Δmg Fresh Weight/Individual</th>
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<tbody>
<tr>
<td>600</td>
<td>-8.4 (49.0)</td>
</tr>
<tr>
<td>1,300</td>
<td>-4.1 (11.6)</td>
</tr>
<tr>
<td>2,600</td>
<td>-30.2 (5.1)*</td>
</tr>
<tr>
<td>5,200</td>
<td>-62.1 (12.4)*</td>
</tr>
</tbody>
</table>

1 Losses expressed on a per-individual basis (asterisks indicate significant (p < 0.05) losses in fresh weight mass (paired comparisons t-test; SAS 1994))
To determine concentration changes and rates of P recycling 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 total chlorophyll removed by zebra mussels and rates of P recycling via zebra mussels were calculated as

\[(C_{\text{exp}} - C_{\text{control}}) \times Q/A\]  

where

- \(C_{\text{exp}}\) = mean daily concentration of the outflow for experimental microcosms
- \(C_{\text{control}}\) = mean daily concentration of the outflow for control microcosms
- \(Q\) = daily flow rate (L/day)
- \(A\) = either the cross-sectional area of the microcosms (0.0176 m\(^2\)) or the tissue dry mass of the zebra mussels

Immediately before and after the study, the fresh mass (i.e., moist shell and tissue mass) of the entire zebra mussel population contained within each experimental microcosm was determined.

A relationship between shell length and tissue dry mass was determined using additional zebra mussels collected from Lake Pepin. Soft tissue dissected from each zebra mussel was dried at 40 °C to a constant mass for dry mass determination. A relationship between shell length and tissue dry mass described by the equation

\[\text{tissue dry mass, g} = 1 \times 10^{-5} L^{2.8458}\]  

where \(L\) = length, mm, was used to estimate soft tissue dry mass for zebra mussels used in the experimental microcosms.

**RESULTS AND DISCUSSION:** Mean concentrations of chlorophyll in the outflow from the control microcosm available for zebra mussel filtration were low over time, ranging between 3.4 and 15 µg/L (Figure 1a), due to low concentrations in the source water during the study period. In experimental microcosms, mean chlorophyll concentrations were significantly lower (i.e., ≤ 1 µg/L) than control concentrations at all zebra mussel density levels, indicating substantial removal of chlorophyll inputs as a result of zebra mussel filtering activities. While areal rates of chlorophyll filtration were more similar over all density levels (Figure 1b), the rate normalized with respect to tissue biomass declined significantly as a function of increasing density (Figure 1c), indicating less food resource per unit biomass as density levels increased.

Changes in fresh mass of zebra mussels over the 2-week period were insignificant for the 600-individuals/m\(^2\) and the 1,300-individuals/m\(^2\) density levels (Table 1). High variance associated with the 600-individuals/m\(^2\) density level was due to the occurrence of fresh mass gains in two of the microcosms and a net loss of fresh mass in the other microcosm. Significant declines in zebra mussel
bio mass were observed for the 2,600-individuals/m² and the 5,200-individuals/m² density levels, indicating that at higher density levels zebra mussels were losing biomass via emaciation as a result of starvation.

At densities exceeding 1,300 individuals/m², mean total P concentrations in the outflow of experimental microcosms exceeded those from the control, particularly toward the end of the study, indicating net export of total P (Figure 2a). Most of the P exported at density levels >600 individuals/m² was in the form of SRP (Figure 2b). Since the zebra mussels were living and actively filtering throughout the study period, these results suggested that they were losing P from tissue mass as soluble forms at higher densities. As a result of emaciation, areal rates of net total P export increased with increasing density above 600 individuals/m² (Figure 3a). Similarly, areal rates of net SRP export were elevated at densities > 600 individuals/m² (Figure 3b). These patterns coincided with a significantly greater recycling of P per unit tissue biomass at the higher densities (Figures 3c and 3d).

Total P concentrations were not significantly different between control and experimental microcosms at the density level of 600 individuals/m² (Figure 2a), indicating no net export of total P. Thus, zebra mussels did not lose tissue P, and it was likely that food resource was not limiting growth or maintenance of biomass at this lower density level. Although no total P export was observed, some conversion of total P to SRP was observed relative to control microcosms at the 600-individuals/m² density level. Zebra mussels excreted back into the water column as SRP a portion (Figure 3b) of the particulate P filtered. Others (Heath et al. 1995; Mellina, Rasmussen, and Mills 1995; Arnott and Vanni 1996; Effler et al. 1997, James, Barko, and Eakin 1997) have documented soluble P excretion by zebra mussels.

Overall, even at the greatest density levels, these biomass-specific rates of P remineralization (maximum = 74 µg g⁻¹ day⁻¹) were low compared with P rates determined for zebra mussels by Arnott and Vanni (1996). However, these rates fell within the lower range of biomass-specific P rates estimated.
for zebra mussels in the Seneca River between July and September 1994 (min ≈ -47 µg g⁻¹ day⁻¹, max ≈ 519 µg g⁻¹ day⁻¹, mean ≈ 135 µg g⁻¹ day⁻¹; Effler et al. 1997). Lower rates of P remineralization by zebra mussels in the present study may be attributed to lower food supply availability. In addition, rates of P remineralization were determined over a 24-hr time scale versus about a 6-hr time scale used by Arnott and Vanni (1996). Since zebra mussels may not feed (or excrete) for several hours a day due to shell closure (Walz 1978), the 24-hr time scale probably overlapped periods when zebra mussel shells were closed, as well as when they were open and actively feeding. Thus, rates observed for the present study reflect an average daily (i.e., encompassing the diel cycle of active and resting periods) remineralization rate versus an hourly rate that includes only periods of active feeding and excretion as determined by Arnott and Vanni (1996).

The results of this study suggest the occurrence of another mechanism of phosphorus remineralization by zebra mussels, namely, P loss associated with emaciation during periods of temporary starvation. Since zebra mussel populations can be subjected to a variety of factors that may affect their condition over both spatial and temporal time scales, P recycling by zebra mussels may vary accordingly. During periods that are environmentally unfavorable for zebra mussel growth (e.g., limited food resource, poor food quality, or unfavorable temperature regime), temporary emaciation may result in enhanced P recycling (primarily in soluble forms).

Variations in P recycling by zebra mussel populations can have an important impact on phosphorus dynamics in lakes, reservoirs, and rivers. In particular, enhanced P recycling via emaciation may be a dominant P flux by zebra mussels as populations reach carrying capacity in aquatic systems. During periods that are favorable for growth, zebra mussels can accumulate considerable P in their tissue (Stańczyowska and Lewandowski 1993) that can be later transferred back to the water column during emaciation.

Figure 2. Mean (n = 3) and S.E. (vertical bars) for (a) total phosphorus (P) concentration, and (b) soluble reactive P concentration as a function of time for different zebra mussel densities. Different letters above the means represent significant differences at p < 0.05 based on Duncan’s Multiple Range Analysis (ANOVA; SAS 1994)
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REFERENCES


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