

FIELD AND LABORATORY TESTS OF THE HYPOTHESIS THAT ZEBRA MUSSELS RELEASE PHYTOPLANKTON FROM P-LIMITATION

Final Report

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Introductory Rationale

From the beginning of the dreissenid invasion into the Great Lakes, most of the attention has focused at the population level. Most studies have examined either the effects of dreissenids on indigenous species or sought methods for control of the population size and spread of the initial invader, *Dreissena polymorpha*, the zebra mussel. Relatively few studies have focused on ecosystem level effects of zebra mussels, yet it is this level of organization that provides many of the "natural services" of the lake from which the public expects to benefit. Ecosystems are those units of nature that regulate the quantity and efficiency of metabolic energy available for growth of organisms in the lake community. Ecosystems also are those natural units that regulate the availability of critical nutrients that limit the growth and activities of organisms. These two general functions of ecosystems are related: the availability of critical nutrients frequently limits the amount of energy that can enter a lake ecosystem by limiting phytoplankton growth and physiology. Availability of limiting nutrients can even affect the efficiency of trophic transfer as metabolic energy moves from the base of the food web to the highest trophic levels if it influences the rate at which phytoplankton are grazed.

Virtually all of the metabolic energy available for growth and activities of Lake Erie organisms is ultimately from photosynthesis by phytoplankton. Factors that affect the growth of phytoplankton will therefore affect the energy available to other trophic levels, conceivably limiting the yield of fish that can be grown in the lake. There is considerable evidence that growth and activities of phytoplankton communities of the Great Lakes and especially of Lake Erie are generally limited by P-availability (Schelske 1979); accordingly, management strategies to limit growth of noxious phytoplankton have sought to decrease P-loading to the lake (GLWQA, IJC 1987). Nutrient amendment bioassays of phytoplankton in the western basin have shown that the limiting nutrient was seasonally dependent (Hartig and Wallen 1983): silica availability limited phytoplankton growth (largely diatoms) in early spring (April), P-availability limited growth (largely cyanophytes and chlorophytes) in summer (May - September), and trace metals limited growth (largely chlorophytes) in October. In the late 1980's the soluble reactive phosphorus concentrations (SRP, phosphate and possibly other readily available P-compounds) were very low in mid-to-late summer in Lake Erie, which is characteristic but not diagnostic of P-limited lake communities (Charlton, et al. 1993). There was a consistent decrease in phytoplankton biomass from 1958 through 1987, with dramatic decreases in the abundance of nuisance cyanophytes (Makarewicz 1993) that paralleled the decline in P-loading (Nichols and Hopkins 1993). Also, populations of diatoms characteristic of eutrophic conditions declined (e.g. *Stephanodiscus binderanus*, *S. niagarae*, and *S. tenuis*), while

diatom populations characteristic of mesotrophic conditions increased (e.g. *Asterionella formosa*) in relative abundance (Nichols and Hopkins 1993). Phytoplankton communities in Lake Erie in the late 1980's were dominated by "mesotrophic" species, having high affinity for nutrients in low concentrations and departing from rapidly growing "eutrophic" species of the early 1970s (Makarewicz 1993).

Even in the absence of increased P-loading to the lake, P-availability to phytoplankton communities can increase if processes that re-mineralize bound (unavailable) P increase. Many aquatic organisms are involved in re-mineralization as they eat and digest food particles then release inorganic waste products. Mussels are known to be active remineralizers and are especially important when found in large numbers. Adult marine mussels, *Modiolus demissus*, filtered large portions of plankton, releasing about 87 percent as pseudofeces. Of the particulate P ingested by these mussels, 36 percent of P was excreted as dissolved compounds; 92 percent was phosphate, and the remaining material was dissolved organic phosphorus (DOP) of unknown composition (Kuenzler 1961). In Lake St. Clair in 1985, the large unionid, *Lampsilis radiata siliquoidea*, filtered 210 MT of P between May and October, representing 13.5 percent of the total phosphorus load, biodepositing 134 MT of P to sediments, while releasing 28 MT of soluble P to the surrounding waters via excretion (Nalepa et al. 1991). Zebra mussels excrete large amounts of both ammonium and phosphate with an N:P ratio below 20:1, reported to be as low as 5:1 (Arnott and Vanni 1996). If the food ingested is assumed to have C, N and P in Redfield ratios of "universal" organic matter of 106:16:1 (C:N:P, expressed as mole ratios rather than weight ratios), N:P ratios less than 16:1 indicate that N is assimilated in preference to P, and P is preferentially released to the surrounding water, as seems to be the case. Arnott and Vanni (1996) indicated that this may shift the phytoplankton to N-limitation. Conversely, their observations may be interpreted as potentially relieving phytoplankton communities from P-limitation.

Our previous observations indicated that this may well be the case. Previously we have examined ecosystem-level effects of zebra mussels on natural assemblages in experiments conducted in 1600 L mesocosms in the field and in 4 L bottles incubated under ambient light and temperature conditions. We examined the influence of zebra mussels on photosynthetic rates, as well as alterations in phytoplankton community composition and water chemistry and found that P-dynamics at the base of the food web was radically altered (Heath, et al. 1995). In the presence of zebra mussels algal composition changed. Diatom and chlorophyte populations declined most rapidly and cyanophyte populations remained unchanged or increased slightly, but it was unclear whether differences were due to differential grazing by zebra mussels or to differential growth rates of algal taxa (Hwang and Heath 1993). Bacterial populations were grazed but slightly (Hwang and Heath 1993, Cotner, et al. 1995). In communities heavily grazed by zebra mussels SRP and NH_4^+ concentrations in the mesocosms increased, and apparent phosphate uptake and nascent DOP release were slowed to approximately 1% that of controls. Bacterial productivity decreased (Heath, et al. 1995), but algal growth rates increased significantly, from 0.3 day^{-1} to near "theoretical" rates ($1.2 - 1.5 \text{ day}^{-1}$). These observations are consistent with the view that phytoplankton were P-limited in the control communities but were relieved from P-limitation when in the presence of zebra mussels in these field mesocosms.

P-limitation of phytoplankton is a physiological state, an adaptation to limited P resources. This physiological adaptation is not an all-or-nothing response, rather it is a graded set of responses resulting from the physiological needs of the organism and the availability of P in its chemical environment. A wide range of algal species are believed to respond physiologically in similar ways to P- and N-deficiency (Healey and Hendzel 1979). Under P-limitation, taxa of chlorophytes, cyanophytes and cryptophytes increase cell carbon content, alkaline phosphatase activity (APA) and P-debt, while decreasing the ATP, and P-content of the cell and the P-uptake rate per unit chlorophyll. Ratios (APA/ATP) and (P-debt/ATP) are sensitive and reliable physiological indicators of P-limitation in laboratory cultures (Healey and Hendzel 1979, 1984) and natural communities (Healey and Hendzel 1980). Natural communities also undergo certain physiological alterations when growth-limited by P-availability. Additions of phosphate have been reported to depress photosynthesis temporarily. A Phosphorus Deficiency Index (PDI) can be constructed which provides a graded response to P-limitation; the smaller the PDI, the greater the P-limitation (Lean and Pick 1981).

Although P-limitation fundamentally takes place at the cellular level, not all taxa respond equally. Some taxa are competitively favored under some nutrient, temperature and light conditions but competitively disadvantaged under others (Reynolds 1984, Tilman et al. 1986). The different responses of cells of different taxa to the concentration of available-P present *in situ* can result in altered phytoplankton communities. Phytoplankton taxa differ according to their competitive responses to phosphate concentrations (Kilham and Kilham 1980). Some species are competitively advantaged in low phosphate concentrations by having a high affinity for the phosphate ion, but these taxa generally have slowly growing cells, even when the phosphate concentration is relatively high. Such species have been called "oligotrophic" species and are characterized by having high values of μ_{\max} / k_s , where μ_{\max} is the maximum growth rate and k_s is the concentration required for half-maximal velocity of uptake of the P-substrate (the greater affinity for phosphate, the lower the k_s). Other taxa are taxonomically advantaged to grow rapidly when phosphate concentrations increase. These have been called "eutrophic" species, competitively advantaged by having high μ_{\max} and large V_{\max} , the maximum velocity of phosphate uptake, achievable when phosphate is in relatively high concentrations, sufficient to saturate the uptake phosphate transport mechanisms (Sommer 1989).

From these concepts, it is possible to envision that increased remineralization of seston by zebra mussels could lead to a shift from low to high phosphate concentrations that would not only stimulate the growth of P-limited phytoplankton but also shift the community from one dominated by "oligotrophic" species to one dominated by "eutrophic" species (e.g. *Microcystis*). Because of the plausibility of this scenario we have previously hypothesized that the zebra mussel is a "keystone remineralizer" (Heath et al. 1995) capable of re-structuring phytoplankton communities that it invades.

Goals and Objectives

The goal of this study was to examine the view that zebra mussels are capable of relieving Lake Erie phytoplankton communities from P-limitation through increased release of available phosphate as a result of remineralization of grazed seston. We examined this hypothesis in several ways, coupling field observations with laboratory manipulations.

Objective 1) We tested the plausibility of the hypothesis experimentally in 8-liter enclosures in the laboratory by comparing the effects of zebra mussels on natural plankton assemblages with nutrient amendments to those assemblages without zebra mussels (compared against control enclosures amended neither with zebra mussels nor nutrients). We reasoned that the hypothesis would be supported by decline in P-limitation physiological responses in the enclosures containing zebra mussels and nutrients.

Objective 2) We conducted Schelske-style nutrient amendment bioassays, examining growth of natural phytoplankton assemblages to amendments of phosphate, nitrate, or both. We reasoned that the hypothesis would be supported if the communities no longer responded to nutrient amendments of phosphate, whether in combination with nitrate amendments or not.

Objective 3) We examined the physiological behaviors characteristic of P-limitation in natural phytoplankton assemblages. We reasoned that the hypothesis would be supported if the specific activity of phosphatase was significantly decreased, if the P-debt was significantly decreased, if the phosphate uptake time was significantly slowed and if the Phosphorus Deficiency Index was significantly increased.

Results and Findings

General Approach

The purpose of this investigation was to determine the effect that zebra mussels have on P-dynamics and especially on the P-limitation Lake Erie phytoplankton traditionally are recognized to exhibit during the summer growth period. In oceanic-scale freshwater systems such as Lake Erie no single approach is entirely satisfying in addressing questions such as determining ecosystem level effects of an organism or event. In this particular case, the problem is how best to compare the ecosystem with and without zebra mussels. One approach is to examine ecosystem properties before and after the invasion of zebra mussels. But concluding that changes over that interval are due to the presence of zebra mussels assumes that the lake has not changed for any other reason over that interval. Many changes have occurred over the past decade, from 1987 when zebra mussels are thought to have been introduced to Lake Erie until the beginning of the current study; likely many other changes have occurred that have gone unnoticed. In large lake ecosystems which change continually, we felt that this approach would not yield meaningful conclusions.

The most direct and easily interpreted approach is an **experimental approach** under controlled conditions. In such an approach, sub-samples of the lake are placed in enclosures that can be replicated and manipulated, mussels being added to some replicates and not to others. This approach provides a true replicated experiment with experimental treatments running concomitantly alongside controls, where the only difference between experimentals and controls is the single independent variable of interest, in this case the presence of zebra mussels. The chief advantage of this approach is that it likely would provide the clearest results of any possible approach; because the manipulations can be so well controlled, any statistically significant difference between experimental treatments and controls is due to zebra mussels. Another advantage is that it can readily be replicated, allowing for arbitrarily high precision. Finally, that manipulations can be "graded" (i.e. different amounts of zebra mussels can be added to experimental treatments to examine "dose effects" of zebra mussels. The chief disadvantage of controlled, replicated experiments is that the size of the container (e.g. 8 liter carboy) may not be able to hold all of the variables necessary for the effects seen in natural conditions. Further, the investigator can and will only control variables that he or she believes may affect community function. In short, the experimental approach is useful for exploring well known phenomena that can be replicated in small confines, but it has limited usefulness because "it is not natural" and may not contain all variables that influence the behavior of plankton populations.

A means of providing some measure of control under natural conditions is the approach generally called "**comparison of matched pairs**". In this approach what is controlled is the selection of the sites visited in nature, selecting the sites in such a way that the greatest evident difference between one set of sites and another is the variable of interest. That is, sites are investigated in pairs that match well in many regards (weather, depth of lake, configuration of the sediments, etc.) but differ in the variable of interest (e.g. presence of zebra mussels). Its chief advantage is that it provides a stratified approach to investigation of natural phenomena under natural field conditions without manipulation. Its chief disadvantage is that the ideal is never reached. No two sites are really "alike in all regards except for the presence of zebra mussels", and frequently the difference between "similar" sites is not well documented, preventing a thorough analysis of variance that may be able to ferret out unknown variables.

In the study reported here, we combined the power of the experimental approach and the comparison of matched pairs approach. Field studies were conducted with an awareness of the proximity to dense populations of zebra mussels.

Objective 1: We examined the plausibility of the hypothesis experimentally. Approach: The hypothesis states that P-limited phytoplankton communities can be relieved of P-limitation because zebra mussels release large quantities of phosphate to the surrounding waters. If the hypothesis is plausible if addition of phosphate in quantities similar to those released by zebra mussels should have the same effect on the physiology of the phytoplankton as zebra mussels.

Methods: We collected natural plankton assemblages in eight 8-liter carboys. We amended two carboys with 30 zebra mussels apiece (+ZM). Two carboys were amended with 1 mL (1 mM sodium phosphate) per hour for 24 hours (1X +P), and two carboys were amended with 2 mL (1

mM sodium phosphate) per hour. These additions of phosphate were designed to increase the phosphate concentration by 125 nM per hour in the 1X +P carboys, a value similar to that reported by Arnott and Vanni (1996). Two carboys were unamended and used as treatment controls. Carboys were incubated together in the laboratory for 24 hours. Each experiment was conducted in duplicate, and the experiment was repeated on four occasions. This experiment was the topic of a Senior Honors Thesis and conducted by Ms. Jennifer Cline (Cline 2000).

Additionally, we worried that exposing a community that had been exposed to zebra mussels for several years may have selected for algae resistant to grazing by mussels or that may have adapted in some way unclear to us. To make sure that the phytoplankton would be grazed by zebra mussels and would not have adapted to their presence, we used a phytoplankton community from a lake unaffected by zebra mussels: East Twin Lake, near Kent, OH. East Twin Lake has been studied frequently by investigators at Kent State University for over 30 years (Cooke et al. 1978). The phytoplankton community of the lake have frequently been studied as good examples of P-limited communities (e.g. Heath and Cooke 1975, Heath 1986). The proximity of East Twin Lake to Kent State University, provided a ready source of material for these experiments. Waters were sampled from the 1 m stratum over the deepest portion of the lake, a frequently visited reference point. Zebra mussels were collected from the rocks in the shallow portion along the shoreline at Old Woman Creek NERR in Huron, Ohio. They were removed by cutting their byssal threads with a scalpel, then they were placed in 1 liter portions of the experimental water for 20 minutes to clear their guts of foreign material and to acclimate to the new water; this was repeated three times before the mussels were added to carboys of freshly collected lake water. Only mussels in the size range from 1.1 to 1.7 cm were used in these experiments, to control for changes in feeding rate and assimilation efficiency with mussel age.

After 24 hours we measured the amount of chlorophyll in the water (as a measure of zebra mussel grazing) and the SRP concentration. To determine the nutritional status of the phytoplankton regarding P-limitation we measured algal phosphate uptake rate, P-debt, and alkaline phosphatase activity; all measures were scaled for chlorophyll for comparison. P-limited communities were identified as having a rapid phosphate uptake rate (> 0.1 nmol per $\mu\text{g chl a}$), a detectable P-debt (> 0.2 nmol $\mu\text{g chl a}$) and an elevated alkaline phosphatase activity. Reported here are the results from 5 August 1999. Our findings are summarized below.

Table 1. Experimental amendment of water for 1 m, East Twin Lake, 5 Aug 1999. 8-liter carboys were incubated at ambient conditions for 24 hours.

	SRP nM	Chlorophyll <i>a</i> $\mu\text{g} / \text{L}$	PO ₄ -uptake nM / min	P-debt nmol / $\mu\text{g Chl}$	APA Units / Chl
Control	115 \pm 20	5.57 \pm 0.25	0.237 \pm 0.044	0.586 \pm 0.059	0.85
1X +P	250 \pm 30	5.6 \pm 0.30	0.160 \pm 0.030	0.079 \pm 0.029	0.83
2X +P	300 \pm 35	5.8 \pm 0.20	undetec.	0.054 \pm 0.33	0.83
+ 30 ZM	300 \pm 50	0.81 \pm 0.25	0.026 \pm 0.015	0.054 \pm 0.045	0.84

These findings show first of all that the community we examined was moderately but not severely P-limited. The SRP concentration was consistent with P-limited communities, the chlorophyll content of the water used above was that consistent with a mesotrophic lake, and the phosphate uptake rate was in the range typically seen of P-limited communities, and the community showed a significant P-debt. The detected amount of alkaline phosphatase was modest. It is clear from the decrease of chlorophyll *a* that the zebra mussels were capable of grazing heavily on this assemblage in 24 hours: Chlorophyll levels dropped from 5.6 to 0.8 μg per liter in that time. Also note that consistent with our earlier work, SRP was elevated in the carboys containing zebra mussels, consistent with the view that the mussels release large quantities of phosphate into the water and that most but not all of it was assimilated by planktonic organisms. The phosphate uptake rate was greatly slowed and the P-debt was significantly decreased in the presence of the mussels. In bottles unamended with mussels but amended with phosphate additions, we found that the 2X but not the 1X amendments gave results very similar to that in the presence of the mussels. In the 2X amendments the SRP was elevated, the phosphate uptake was slowed to undetectable levels, and the P-debt was not significantly different from that seen in the mussel amendment carboys. Based on these results, we conclude that phosphate amendments similar to that released by zebra mussels alone are able to relieve phytoplankton communities from P-limitation. Our findings also showed that APA did not respond sufficiently rapidly to be a useful indicator of P-limitation in this experiment.

A caveat here is that it was the 2X, rather than the 1X phosphate amendment that achieved results similar to that seen in the presence of zebra mussels. This may indicate that the mussels have other effects on the phytoplankton besides amendments with phosphate, or it may mean that the mussels used in these experiments released more phosphate than reported by Arnott and Vanni. Another caveat is that this was only an experiment; it was not a field observation. The significance of observations in enclosures under controlled conditions is useful for determining the possible effects of certain variables, but they are not able to show that this indeed is what happens in the natural conditions in Lake Erie.

Objective 2: We examined this hypothesis through the use of Schelske-style factorial design nutrient amendment bioassays for – or P-limitation. Approach: Nutrient amendment bioassays, virtually identical to those conducted by Schelske et al. (1978) were conducted at selected sites on integrated samples of the phytoplankton community on selected dates in 1998 and 1999. Statistically significant trends ($p < 0.05$) in dependence of algal production on P amendments were interpreted as evidence of P-limitation. We also examined the growth trends for dependence on N-amendments and for interaction between – and P- amendments. Observation of lack of P-limitation would be consistent with the hypothesis; continued P-limitation of phytoplankton growth would not support the hypothesis.

Methods: We conducted nutrient amendment bioassays for nutrient limitation essentially according to Schelske et al. (1978). Nutrient bioassays were conducted twice per season, once in early to mid- June and again in early - to mid-August. We chose these times because they corresponded to the beginning of the development of the summer bloom (e.g. June) and at the height of the phytoplankton blooms (e.g. August; Hartig and Wallen, 1984) and are times most

likely to exhibit P-limitation. Both shallow and deep sites were examined. Tests on water drawn from sites determined to be proximal to dreissenid beds (i.e. most likely to be greatly affected) and on waters drawn from sites distant from zebra mussel beds (i.e. least likely to be affected) were examined simultaneously.

Water was collected into 8 L carboys; 150 mL portions were distributed into 250 mL flasks and amended essentially according to Schelske et al. (1978); our design differed from theirs by including unamended controls for each element. We amended all flasks with sodium silicate to bring the final concentration to 750 $\mu\text{g Si per L}$. We did this to assure that diatoms would not be silicate limited. Then flasks were amended with N as sodium nitrate or P as dibasic sodium phosphate or both according to the following factorial design. Including the “no amendment” of N (or P) there were three treatments of N and four treatments of P, giving a total of 12 different treatments:

Nutrient Amendment Factorial Design
Schelske-style Experiments

levels:	$\mu\text{g L}^{-1}$
N ($\mu\text{g L}^{-1}$) N-0	0
N-1	120
N-2	320
P ($\mu\text{g L}^{-1}$) P-0	0
P-1	25
P-2	35
P-3	45

All treatments were triplicated (i.e. 36 flasks per site). Flasks were incubated at ambient temperature. Samples were examined every 2 or 3 days using the *in vivo* fluorescence method (Welschmeyer 1994). Aliquots of 5-10mL were removed and their fluorescence detected in a Turner Designs TD-700 fluorometer equipped with a red-sensitive PMT, similar to but more sensitive than that used by Schelske et al. (1978). Samples were read at ambient temperature. Data were analyzed by linear regression and pair-wise ANOVA for significant trends in stimulation of production.

We stratified our investigation using comparison of matched pairs, so that at each time we conducted a study, we compared communities at sites we judged to be relatively less affected by zebra mussels with communities we judged to be relatively more affected by zebra mussels. These judgements were based on proximity to large beds of zebra mussels. Sites nearer to places with dense populations of zebra mussels were compared with sites further away from dense populations of zebra mussels. We attempted to match the sites as closely as possible in every regard except proximity to dense populations of zebra mussels. We examined eight sites in this part of the study:

<u>Relatively more affected by ZM</u>	<u>Relatively less affected by ZM</u>
1. Flat Rock Reef	Between West Sister and Middle Sister Is.
2. Crane Reef	West of North Bass Is.
3. Niagara Reef	Sandusky Sub-basin, north of Huron, OH
4. Middle Is. (west), 10 m, just above dense populations of zebra mussels on the sediment surface	Gull Is. Shoal (water from 1 m. depth, distant from zebra mussels on the bottom (7 m. depth).

Water was collected and amended to provide a factorial amendment schedule, Table 2:

P-0, N-0	P-1, N-0	P-2, N-0	P-3, N-0
P-0, N-1	P-1, N-1	P-2, N-1	P-3, N-1
P-0, N-2	P-1, N-2	P-2, N-2	P-3, N-2

Table 2: Amendment Schedule. Top row (P- amendments without N). Left column (N-amendments without P). Remaining treatments (both N and P).

Each amendment combination was done in triplicate. Flasks were marked and placed in randomized positions in the incubator, incubated at $30 \mu\text{E m}^{-2} \text{sec}^{-1}$ at ambient temperature. The growth of the phytoplankton was followed by detecting the increase in chlorophyll content of the flasks using a non-destructive fluorometric method. The growth of the phytoplankton populations was followed for 21 days. We found that populations consistently reached a maximum growth within that time, often declining before the end of the incubation period. Replicate treatments did not necessarily reach their maximum at the same time. We observed a greater variance in the amount of chlorophyll in replicate flasks at the end of the incubation period than we observed by comparing the maximum chlorophyll detected. For that reason, analyses were done using the maximum amount of chlorophyll observed.

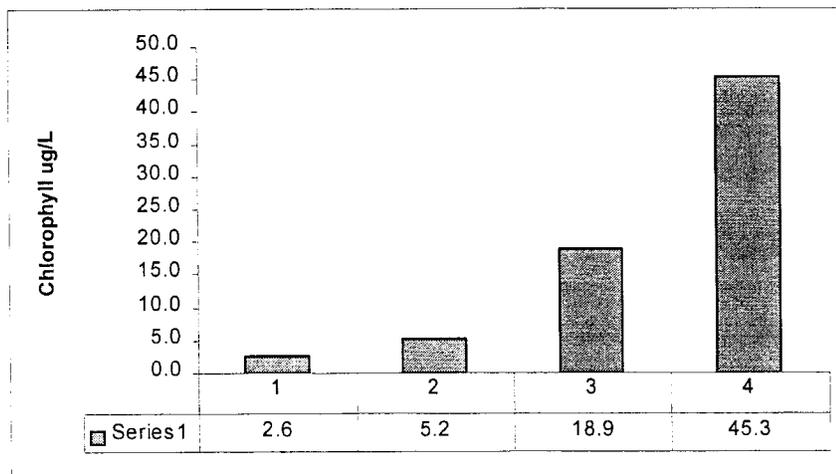
If amendments of P alone stimulated growth significantly (i.e. significant growth along the top row): the community was judged to be "**P-limited**." If amendments of N alone stimulated growth significantly (i.e. significant growth down the left column): the community was judged to be "**N-limited**." If neither P-limitation nor N-limitation was observed, but significant growth was observed in the amendments that involved both N and P, then the community was judged to be "**N and P Co-limited**." Using these criteria, our findings from this set of experiments are summarized in Table 3.

Table 3: Summary of results observed from Schelske-style factorial nutrient amendment growth experiments.

	Low ZM site	High ZM site
July 1997	N and P Co-limitation	no sig. limitation
August 1997	no sig. limitation	N-limitation
July 1998	strong P-limitation N and P Co-limitation	strong P-limitation N and P Co-limitation
August 1998	no sig. limitation	N and P Co-limitation

Our observations indicated that the communities examined in this way generally behaved as if they were not P-limited (i.e. additions of phosphate alone in general did not stimulate phytoplankton growth under the conditions provided). Our most frequent observation was that the communities examined behaved as if they were co-limited by both the availability of N and P (i.e. additions of both N and P stimulated more growth under the conditions provided than either N-amendments alone or P-amendments alone). Only in July 1998 did we observe strong P-limitation: Figure 1. We observed no significant differences between sites near dense populations of zebra mussels and those communities from sites distant from zebra mussel populations.

Figure 1: P-limitation of phytoplankton community: July 1998. Niagara Reef. Chlorophyll produced following amendments of 0, 25, 35, or 45 µg P as phosphate.



Our observations from this set of experiments were inconclusive. On only one date did we find communities that grew significantly when amended with phosphate alone, but communities that we judged to be distant from zebra mussel populations responded the same as communities drawn from a "high zebra mussel density" site on that date (July 1998). Response to P-amendments generally was insignificant or significant only when accompanied with N-

amendments, indicating a N and P co-limitation is most likely. In a sense the clearest finding was that our observations differ from those of Schelske et al. (1978) by being much more equivocal in support of – or P-limitation.

Objective 3: We examined the physiological behaviors characteristic of P-limitation in natural phytoplankton assemblages. Approach: We reasoned that a good way of determining whether algae are P-limited is to examine their biochemical status. When growth-limited by availability of P, algae adapt biochemically in several ways. Generally, these responses are "graded" responses, showing a greater activity when the algae are more severely P-limited. We were especially keen to use responses that are rapid physiological responses, can be measured reliably and are sensitive, graded responses (rather than qualitative "all-or-none" responses).

Many phytoplankton species are known to take up phosphate in excess of their immediate biochemical needs and to store this "excess P" as polyphosphates or as certain organic-P compounds (Healey and Hendzel 1979). Any capability to store excess amounts of phosphate was termed "**Phosphorus Debt**" by Healey and Hendzel (1979). We have developed a radiometric procedure, adding radiolabeled phosphate to unlabeled phosphate, storing the algae being tested overnight in the dark (to prevent photosynthesis and growth), then determining the amount of radioactivity per algal biomass. We have found that phytoplankton in culture with sufficient P, take up no label in this test, while P-limited algae rapidly take up radiolabel, providing a sensitive indicator of P-limitation.

A second physiological indicator we used was the rate of phosphate uptake. We regularly measure the rate of phosphate uptake by algae radiometrically. Spiking water samples with radiolabeled phosphate, we measured the rate of uptake of radioactive P, then multiplied by the dilution factor, and assume that radioactive P is taken up at the same rate as unlabeled P. When phytoplankton are P-limited they rapidly take up phosphate, but when they are in P-replete conditions they take up phosphate very slowly. Our approach has been to scale the P-uptake rate for algal biomass, as a means of comparing one community to another. Because both algae and bacteria take up phosphate quickly, we discriminate between phytoplankton and bacteria by differential filtration. "Algal" uptake is that which does not pass through a filter with pores of 1.0 μm ; "bacterial" uptake is that which passes through a 1.0 μm filter but not through a 0.2 μm filter. These definitions are not perfect, but we have calibrated them in the field and find that less than 5 percent of bacteria adhere to 1.0 μm filters, and less than 1 percent of the phytoplankton pass through the 1.0 μm filters and adhere to 0.2 μm filters in the field sites we visit (the western basin of Lake Erie has low numbers of pico-phytoplankton). We find this acceptable discrimination (Heath 1986).

A third sensitive physiological indicator of P-limitation of phytoplankton we used is the "Phosphorus Deficiency Index" of Lean and Pick (1981). The PDI is based on the observation that both the maximum velocity (V_{max}) of phosphate uptake and the photosynthetic optimum uptake (P_{opt}) of CO_2 are sensitive to P-limitation. When phytoplankton cells are severely P-limited the V_{max} of P-uptake is maximal and the P_{opt} is at a minimum.

The PDI was calculated as:

$$PDI = \frac{P_{opt}}{V_{max}}$$

With all units in moles taken up per liter per hour, the index is unit-less. The greater the degree of P-limitation, the lower the PDI value. Values less than 10 are considered "extremely" P-limited, $10 < PDI < 30$ indicate "moderate" limitation, $30 < PDI < 100$ indicate a "low" degree of P-limitation. PDI greater than 100 are considered to indicate that the phytoplankton are not growth limited by P-availability.

The P_{opt} of C-fixation is determined using a "photosynthetron" - a water jacketed light box that illuminates 18 sub-samples of a water sample under different irradiances (Lewis and Smith 1983). After being spiked with ^{14}C -bicarbonate the samples are incubated for 60 minutes, excess bicarbonate is sparged off and the remaining material is considered photosynthetically fixed C. The sub-samples are irradiated in glass liquid scintillation vials, which are then filled with liquid scintillation cocktail and counted by liquid scintillation. The P_{opt} is determined from the resulting plot of photosynthesis vs. irradiance (P-I curve). The V_{max} of phosphate uptake is determined from a Michaelis-Menten plot of velocity vs. phosphate concentration, following addition of known amounts of phosphate to amend natural water samples. Lean and Pick (1981) showed that the PDI was a sensitive and reliable graded response of plankton communities to P-limitation, and Lean et al. (1987) showed that phytoplankton in Great Lakes communities responded in ways that indicated they were severely P-limited in the summer season, and moderately P-limited or not P-limited at other times of the year.

Some species will produce alkaline phosphatase adaptively, especially when strongly P-limited (Heath and Cooke 1975); others don't seem to respond in this way. We have found that alkaline phosphatase activity (APA) can be a good indicator of P-limitation and a graded response to P-limitation (Heath 1986). Phytoplankton respond slowly to produce APA in response to P-limitation, requiring perhaps as long as a month to achieve full expression of their status (Heath and Cooke 1975). On occasions we found APA to be very low and barely detectable, even when the sensitive methyl-umbelliferyl fluorometric procedure was used.

We first conducted a feasibility study to show that zebra mussels could relieve natural phytoplankton assemblages from Lake Erie from P-limitation in enclosures. Then we examined selected stations in Lake Erie for those same characteristics.

a) Feasibility study: This study was conducted at the F. T. Stone Laboratory on South Bass Island. We collected water from Lake Erie into six 8-liter carboys near the Red Can Buoy in the channel near the lab on 21 August 1998. The carboys were translucent, permitting light with an intensity equivalent to ca. 10% that of the surface illumination, approximating light levels at approximately the secchi depth (1.8 m.). Zebra mussels were collected on rocks near the lab and removed by slicing their byssal threads with a scalpel. The mussels were rubbed by hand to remove aufwuchs. Then they were acclimated to the collected water by placing them in 1 liter quantities of water and allowing them to graze for 20 minutes; this process was repeated three

times. Following cleaning and acclimation to the water to be studied, 15 mussels (*D. polymorpha*) with a shell length of 1.2 to 1.8 cm in length were placed in each of three carboys; the remaining three carboys were used as unamended controls. The carboys were then hung from the dock at the Research Building at about 0.2 m. in sunlight and incubated for 16 hours (4 p.m. until 8 a.m. the following day). The water temperature was $25 \pm 1^\circ\text{C}$. These conditions represented conditions commonly found in Lake Erie in the summer.

After incubation was complete we determined the P-debt, rate of phosphate uptake by algae, and the PDI, all physiological variables we have previously shown to be sensitive to P-limitation. We also determined variables related to water chemistry: Soluble Reactive P (SRP) and chlorophyll a. Each measurement reported is the result of triplicate measures. We found significant differences between carboys amended with zebra mussels and unamended controls in each of the variables examined.

Table 4: Results of Observations of P-limitation Variables in 8-Liter Carboys Containing Lake Erie Natural Assemblages Amended with 15 Washed *D. polymorpha*
 Values represent mean \pm S.E. n = 3

Variable	Unamended Control	Amended with 15 <i>D. polymorpha</i>
SRP (nM)	40 \pm 5	390 \pm 25
Chlorophyll ($\mu\text{g L}^{-1}$)	15.6 \pm 0.8	1.5 \pm 0.3
P-uptake per $\mu\text{g chl a}$	1.8 \pm 0.2	undetectable
P-debt (nmol per $\mu\text{g chl a}$)	13.2 \pm 0.4	undetectable
P Deficiency Index	39 \pm 5	4500 \pm 100

Each of these observations is consistent with the view that zebra mussels rapidly release sufficient quantities of phosphate to relieve Lake Erie phytoplankton populations of P-limitation. The column of observations on the unamended control carboys indicates that **the natural phytoplankton assemblage in the sample examined was moderately P-limited**. The SRP was on the edge of detection by the standard colorimetric procedure read in 1 cm cuvettes at 885 nm (Murphy and Riley 1962). The chlorophyll content was high and typical of late season chlorophyll content of these waters. We noted that a moderate bloom of *Microcystis* spp. was in progress. Rate of phosphate uptake, determined radiometrically and scaled for chlorophyll was typical of phytoplankton assemblages in Lake Erie actively growing under P-limitation. When P-limited, the rate of phosphate uptake is very high, ranging between 1 and 3 nmol P per L per min. P-limitation of this assemblage was especially evident in the final two observations. P-debt was significant, characteristic of assemblages undergoing severe P-limitation. The PDI was characteristic of assemblages under moderate but not severe P-limitation.

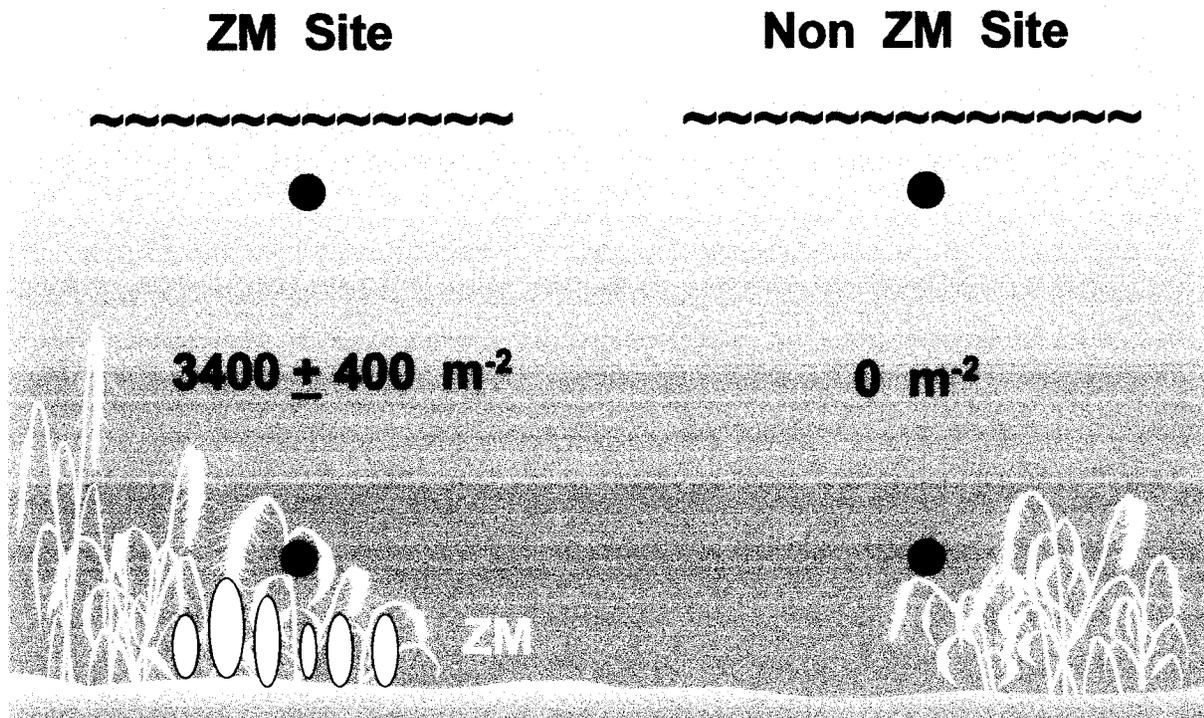
By contrast, the same assemblage exposed to zebra mussels for 16 hours, showed no evidence of P-limitation. **Each of the observations was consistent with the view that zebra mussels heavily grazed this assemblage and relieved the remaining phytoplankton from P-limitation.** The chlorophyll content of the water was about 10% that of the controls, indicating that the assemblage had been heavily grazed by the mussels in the carboys. The SRP in these carboys was high and uncharacteristic of P-limited communities. Under P-limitation phosphate is rapidly taken up and the SRP (an estimate of phosphate and other compounds) remains low, even if phosphate is released by grazing activities. That the SRP attained such high concentrations indicated that planktonic algae and bacteria had greatly slowed in their assimilation of phosphate. As noted above, phytoplankton assimilate phosphate either for immediate use or for storage to be used at a later time. The capacity for storage was measured as P-debt. Phytoplankton exposed to zebra mussels in this experiment showed that assimilation of phosphate had slowed to undetectable levels; no capacity for excess storage was detectable, either. Finally, the PDI was exceedingly high, indicating that the assemblage was not P-limited. Each of these observations was consistent with the view that zebra mussels are capable of releasing substantial quantities of phosphate into the water, relieving those phytoplankton that remain from P-limitation. Although we have reported the results of a replicated experiment designed to focus directly on this issue, our findings here were completely consistent with observations we have made since 1992 on the effects of zebra mussels on plankton assemblages in enclosures such as used here and reported elsewhere.

This experiment shows that zebra mussels are capable of releasing sufficient phosphate that they can relieve natural phytoplankton from P-limitation. It doesn't show whether that happens under the natural conditions regularly found in Lake Erie. Experiments of this sort need to be interpreted strictly, and investigators need to be careful that they do not extend their conclusions beyond reasonable limits. There are many differences between 8 liter carboys and Lake Erie. An obvious difference is that bottles and carboys are closed off from their unaffected surroundings, whereas the water that surrounds zebra mussel populations can be mixed with unaffected water through naturally occurring turbulence. The second part of this objective was designed to answer this question: do zebra mussel populations relieve natural phytoplankton assemblages of P-limitation in nature? This was accomplished by examining various sites in Lake Erie for the same variables and indicators of P-limitation we used above.

b) Field observations to examine the possibility that zebra mussels may relieve natural assemblages of phytoplankton from P-limitation in Lake Erie. We approached this by "two-way comparison of matched pairs". We reasoned that the best match of a site containing dense populations of zebra mussels would a nearby site that didn't contain zebra mussels. Nearby sites would have the similar temperatures and weather conditions but may differ only in the presence of adequate substrate for support of mussels, but even nearby sites can differ in microclimates, so the closer the sites, the better. The closest possible site to a plankton community within 1 meter of dense populations of zebra mussels to use for comparison would be the surface waters at the same site. It could be recognized that the surface waters at a site with dense populations of zebra mussels on the sediment surface may or may not be affected by the presence of the mussels.

To account for the possibility that surface and deep waters are affected by the presence of mussels on the sediments we did two comparisons at once. We compared sites in the western and central basin, confirmed by diver to have dense populations of zebra mussels on the sediments, with sites that did not have zebra mussels present but otherwise had similar biological and chemical characteristics. Simultaneously, we also compared P-limitation characteristics of phytoplankton in surface waters (1 m. depth) with phytoplankton 1 m above the sediment surface. That is, for each set of comparisons, we examined four sets of data, shown graphically in Figure 2.

Figure 2: Graphic representation of two-way comparison of matched pairs. The data shown were taken on 14 July 1998 at a site in Schoolhouse Bay near Middle Bass Island, confirmed to have dense populations of *D. polymorpha*, and from a site near Ballast Island, sampled on the same day and confirmed not to have zebra mussels. Red dots (●) indicate sampling positions.



Samples collected from these four positions were then examined for characteristics diagnostic of P-limited communities. As before, we used specific phosphate uptake rate, P-debt, alkaline phosphatase activity, and Phosphorus Deficiency Index (PDI) to indicate the physiological status of the phytoplankton, and SRP to determine the P-availability at the site. The limnological characteristics of these sites were closely matched in transparency, temperature, pH and conductivity:

	Ballast Island Site	Schoolhouse Bay Site
LAT:	41° 41.15'	41° 41.42'
LON:	82° 47.67'	82° 48.11'
Depth:	8.1 m	5.5 m
Secchi Depth:	3.6 m	3.8 m
Temperature:	25.0	25.3
pH:	8.3	8.0
conductivity (mS/cm):	0.27	0.28

We found that the samples taken within 1 m above dense populations of zebra mussels (Schoolhouse Bay) differed significantly from samples taken at the same time from the 1 m depth at the same site and from samples taken 1 m deep and 1 m from the sediments from the site not colonized with zebra mussels (Ballast Island).

Table 5. Two-way comparison of matched pairs: Comparison of P-limitation characteristics in samples from similar sites, with and without zebra mussels present, and in samples at the top (distant from ZM) and bottom waters (close to ZM). Significant differences shown in **bold red**.

	Ballast Is. (Top)	Ballast Is. (Bottom)	Schoolhouse Bay (Top)	Schoolhouse Bay (Bottom)
Sample Depth	1 m	7.1 m (-1 m)	1 m	4.5 m (-1 m)
Zebra mussels m^{-2} <small>within 1 m of sampled water</small>	0	0	0	3400 ± 400
SRP nM	32 ± 30	88 ± 30	88 ± 30	145 ± 30
Chl <i>a</i> ($\mu g L^{-1}$)	3.0 ± 0.2	4.1 ± 0.2	5.0 ± 0.2	4.8 ± 0.2
P-uptake (nM/min/ μg Chl <i>a</i>)	0.314 ± 0.002	0.515 ± 0.042	0.465 ± 0.002	0.071 ± 0.004
Phosphatase Units $\times 10^{-3} / \mu g$ chl	2.00 ± 0.03	1.13 ± 0.02	1.67 ± 0.01	0.17 ± 0.03
P-debt (nM / nM algae P)	0.34 ± 0.02	0.55 ± 0.06	0.29 ± 0.03	0.07 ± 0.06
P D I (P_{opt} / V_{max})	14	79	80	167

These findings clearly show that phytoplankton in the sample drawn from within 1 m of dense populations of zebra mussels (i.e. the bottom water sample from Schoolhouse Bay) differed significantly from phytoplankton drawn from the bottom waters of a site similar except for the presence of zebra mussels. The phytoplankton physiology drawn from the site proximal to zebra mussels was also significantly different from phytoplankton physiology in the sample drawn from surface waters at the same site. Only in the sample drawn from a position within 1 m of dense populations of zebra mussels was the SRP significantly elevated, the phosphate uptake by algae depressed, the activity of phosphatase (scaled for chlorophyll) was depressed and the Phosphorus Deficiency Index was significantly elevated. We found it interesting to note that the chlorophyll detected in this sample drawn proximal to zebra mussel beds did not differ significantly from the chlorophyll detected elsewhere. The slow P-uptake per unit chlorophyll, the diminished phosphatase activity per unit chlorophyll, and a PDI greater than 100 were all characteristic of communities not limited by P-availability. Those characteristics were seen only in those samples drawn from bottom positions directly over zebra mussels beds.

Samples drawn from the surface of the water column at Schoolhouse Bay did not differ significantly from top or bottom samples from Ballast Island site: the physiological characteristics of these phytoplankton were generally characteristic of moderately P-limited communities. SRP concentrations less than 100 nM, relatively rapid phosphate uptake by phytoplankton per unit chlorophyll, a modest amount of phosphatase per unit chlorophyll, a small but detectable P-debt and a PDI between 30 and 100 are characteristic of weakly P-limited communities (Lean et al. 1987). The PDI in the surface waters at the Ballast Island site indicated that phytoplankton at this site may be severely P-limited.

Tables 6 and 7 show the generality of these findings. **Table 6** follows through on all the observations we made comparing the physiological status of phytoplankton in top and bottom positions of the water column at a given anchor point. We consistently found that phytoplankton assemblages taken from the bottom of the water column were less P-limited than those same assemblages sampled from the top of the water column at the same time. We also consistently found that those assemblages sampled from bottom positions directly over dense populations of zebra mussels were not P-limited. The central basin site was a point directly north of the Huron River about 10 miles from shore. It was sampled late in 1999 and believed to be relatively free of zebra mussels at that time, but that was not confirmed by diver and may have been an inaccurate impression. The results from it are equivocal, showing evidence that the phytoplankton were very weakly P-limited (as determined by the PDI) or not at all (as indicated by the P-uptake rate and the P-debt). At each site, the phytoplankton assemblage in the surface waters was P-limited, although the physiological indicators were weak at the Schoolhouse Bay site.

Table 7 compiles findings of observations taken from the water column at many selected sites in the western basin and the western portion of the central basin. The data are stratified according to whether they were taken from sites with confirmed dense populations of zebra mussels on the bottom. The data are then presented as being consistent with P-limitation or not.

The following criteria were used:

	NO P-limitation
SRP	greater than 100 nM
P-uptake	less than 0.100 nM per hour per $\mu\text{g chl}$.
P-debt	less than 20 nmol per $\mu\text{g chlorophyll}$
PDI	greater than 100
APA	less than 0.01 units of activity

Using these criteria we found that phytoplankton assemblages in Lake Erie distant from zebra mussels were P-limited in general. Samples taken from the top waters in the central basin and in regions between the islands generally showed physiological and biochemical evidence consistent with P-limited phytoplankton. The SRP concentrations at those sites were consistent with recent levels found in the lake (i.e. less than 100 nM = 3 $\mu\text{g P per L}$). P-debt, PDI and APA were consistent with phytoplankton populations that are weakly to moderately P-limited. P-uptake at times was slower than we could account for by P-limitation and may have been slow because growth of algae slows as the season progresses and especially as the temperature rises above 25°C. In those cases where one indicator may have implied P-replete conditions, it was not confirmed by other indicators.

Samples drawn from near the island areas were in relative close proximity to dense populations of zebra mussels and frequently had physiological and biochemical characteristics indicating that they were **not P-limited**. Note that many of these samples were drawn from the middle of the water column rather than immediately above mussel beds. At these sites in relative close proximity to zebra mussels we found that SRP was elevated above historic norms, the P-uptake was slowed, P-debt was minimal, PDI was very high and APA was very low, indicating that these phytoplankton were not P-limited. In all cases reported here, several indicators of P-replete conditions supported the conclusion that these populations were relieved of P-limitation.

Table 6. P-limitation status of top water and bottom water (within 1 m of ZM) at sites confirmed to have dense populations of zebra mussels (a) and sites confirmed not to have many zebra mussels (b). Bottom strata shown in **BOLD**; Indicators of P-replete conditions are shown in **RED**

a) sites with dense populations of zebra mussels on the bottom

	Z m	Z _{sample} m	SRP nM	P-uptake nM/min	P-debt/chl nM	P D I	APA Units	P-lim ?
Niagara Rf. (top)	6.5	1	32	0.005	46	4	0.009	med.
Niagara Rf. (bot)	6.5	6	115	0.009	16	110	0.001	NO
S'lhse. By. (top)	5	1	88	0.465	47	80	0.002	weak
S'lhse. By. (bot)	5	4.5	145	0.071	41	167	< 0.001	NO
Mid. Is. W (top)	10.7	1	117	0.474	49	-	-	med.
Mid. Is. W (bot)	10.7	10	175	0.053	12	-	-	NO
Gull Is. Sh.(top)	4.7	1	146	0.176	24	-	-	weak
Gull Is. Sh. (bot)	4.7	4.5	88	0.022	14	-	-	NO

b) sites with relatively few zebra mussels present on bottom

CB-Huron (Top)	13.5	1	55	0.125	184	19	0.002	med.
CB-Huron (Bot)	13.5	13	110	0.078	8	74	0.003	weak
Ballast Is. (Top)	7.4	1	32	0.314	73	14	0.002	med.
Ballast Is. (Bot)	7.4	7	88	0.515	114	79	0.001	weak

Table 7. Indicators of P-limitation in plankton samples from selected sites in Lake Erie.
 (Z = depth of site, Z_{sam} = sampled depth, other symbols as in Table 6, NO = indication of no P-lim)

a) In close proximity to dense populations of zebra mussels

Site	Z	Z _{sam}	SRP	P-uptake	P-debt	PDI	APA
Hen Island Shoal	9	4	233	NO	NO	--	NO
Red Can @ Stone Lab	6	4	86	NO	NO	--	NO
Flat Rock Reef	6	4	145	P-lim	P-lim	--	P-lim
Crane Reef	7	4	87	NO	NO	--	NO
Middle Island Shoal	6	4	290	P-lim	NO	--	NO
Kelley's Island Bay	7	4	226	NO	NO	--	NO
Niagara Reef (Bot.)	6.5	6	55	NO	NO	110	P-lim
Schoolhouse Bay (Bot.)	5	4.5	145	NO	??	167	NO
Middle Island W (Bot.)	10.7	10	175	NO	NO	--	--
Gull Island Shoal (Bot.)	4.7	4	88	NO	NO	--	--

b) Distant from dense populations of zebra mussels

W. Sis -- Middle Sis. Is.	9	4	145	P-lim	P-lim	--	P-lim
Middle Sister Island	10	4	132	P-lim	P-lim	--	P-lim
W. Sis. Is -- S. Bass Is.	10	4	--	NO	P-lim	--	P-lim
East Sister Is.	11	4	--	NO	P-lim	--	P-lim
W. Sis. Is. -- S. Bass Is.	7	4	34	P-lim	P-lim	--	P-lim
CB -- Huron R.	14	4	10	NO	P-lim	--	P-lim
Niagara Reef (top)	6.5	1	55	NO	??	--	P-lim
Sandusky Sub-basin (top)	14	1	32	P-lim	P-lim	19	P-lim
Schoolhouse Bay (top)	5	1	88	P-lim	??	80	P-lim
Ballast Island (top)	7.5	1	32	P-lim	P-lim	14	P-lim
Middle Island W. (top)	10.7	1	117	P-lim	P-lim	--	--
Gull Island Shoal (top)	4.7	1	146	P-lim	??	--	--

DISCUSSION

In the 1970's there was considerable concern regarding the factors that limit phytoplankton growth. At that time, eutrophication was viewed largely as a problem of excessive growth of phytoplankton caused by excessive nutrients that stimulated more growth than could be grazed; ungrazed phytoplankton eventually died, and the decay processes led to consumption of dissolved oxygen. Oxygen consumption in the deepest parts of the lake in turn led to regions of anoxia that diminished habitat for fish populations, especially those that required deep cold waters in which to thrive. Lake managers sought to control eutrophication by controlling those factors that limited phytoplankton growth. Although a considerable debate raged regarding this view of eutrophication and the identity of the "limiting factor," eventually it became clear that phytoplankton as a whole were growth limited by P-availability. Nutrient amendment bioassays were frequently used because they were seen as objective indicators of phytoplankton growth under controlled conditions. They clearly indicated that when phosphate was added to natural assemblages, growth was stimulated, and P stimulated growth more clearly than addition of any other single element (Schelske et al. 1974). However, they also frequently indicated that searching for "the single factor that limited phytoplankton growth" was simplistic. Frequently growth was stimulated most by the increase in two or more factors simultaneously. In addition to the stimulatory effects of P alone on phytoplankton growth, interactions with nitrogen and trace metals or both were frequently observed (Schelske et al. 1978, Lin and Schelske 1978, and Stoermer et al. 1978). Even so, their consistent finding was that the phytoplankton were P-limited, and that other nutrients often were co-limiting factors. Today we look at the process of eutrophication differently. High nutrient levels stimulate growth of particular, noxious "eutrophic" species that are inefficiently grazed, die and are decomposed at the expense of oxygen in the deep waters. "Eutrophic" algal species grow in preference to efficiently grazed "oligotrophic" species when local nutrient concentrations are high (Sommer 1989).

Although we were using virtually the same factorial nutrient amendment test used by Schelske and others, our findings were generally much more equivocal than those reported by investigators in the 1970's. Although we found that phytoplankton were often P-limited, our observations indicated that they were generally co-limited by N, and on one occasion we observed clear N-limitation without evidence of P-limitation in a sample drawn from an area near dense mussel populations. Using the factorial nutrient amendment bioassays we did not see consistent significant differences between stations near and far from large populations of zebra mussels.

By contrast, physiological indicators of P-limitation that are able to indicate the strength of P-limitation through graded responses, frequently indicated that the phytoplankton of Lake Erie at the sites sampled were P-limited. These physiological characteristics also indicated that the P-limitation was seldom severe and was frequently moderate or low in intensity. Our comparison of sites near and distant from dense populations of zebra mussels consistently showed that those phytoplankton assemblages closest to zebra mussels were most affected by them, as shown by the strength of the physiological response and the corroboration of several physiological indicators simultaneously indicating release from P-limitation. We are unable to state unequivocally that phytoplankton in Lake Erie are less P-limited than similar natural assemblages were 30 years ago. The best indicators able to make such comparisons have been developed over that interval and

were not available at the height of the eutrophic blooms of the late 1960's and the early 1970's. We found that P-debt (developed in 1980 and refined by us in 1998) and the PDI (developed in 1982 and most used in 1987) were most consistent in identifying P-limitation. We found that APA was not a consistently useful indicator of P-limitation.

CONCLUSIONS

From our observations of mussel and nutrient amendments to natural plankton assemblages in 8-liter enclosures we conclude:

- (1) zebra mussels are capable to relieving P-limited phytoplankton from P-limitation**
- (2) amendments of phosphate alone in quantities similar to those released by zebra mussels is sufficient to relieve phytoplankton from P-limitation; we conclude that phosphate alone can account for physiological alteration of phytoplankton under controlled conditions.**

From our field observations we conclude:

- (3) The closer phytoplankton assemblages were to dense populations of zebra mussels, the lower the degree of P-limitation they exhibited in graded physiological responses. In several cases, we observed (P-debt and PDI data) that natural assemblages responded as if they were not P-limited.**
- (4) Phytoplankton as a whole appeared to have a lower degree of P-limitation than reported for Great Lakes phytoplankton 30 years ago (reported by nutrient amendment bottle assays), but exact comparisons were not possible because many of the physiological indicators best suited for comparison of graded responses of P-limitation were unavailable then.**
- (5) Lake Erie phytoplankton were generally observed to be P-limited. Seldom were natural assemblages of phytoplankton in Lake Erie relieved of P-limitation.**

SIGNIFICANCE TO LAKE ERIE MANAGEMENT

This project addressed one of the most important problems facing Ohio and the conduct and management of its coastal programs. Studies in the Great Lakes and Lake Erie in particular have indicated that phytoplankton growth and activities are generally P-limited (Schelske 1979). Predicated on the assumption that phytoplankton growth would remain P-limited, the International Joint Commission developed a management plan and implemented an agreement, the Great Lakes Water Quality Agreement of 1978 (GLWQA), revised and amended in 1987, to control water quality of the Great Lakes by controlling P-loading from the watershed. If these communities are no longer P-limited, current management strategies need to be reassessed and perhaps new ones planned. Our research reported here indicated that zebra mussels *can* release sufficient phosphate to relieve algae from P-limitation (in a bottle) but seldom, if ever, were phytoplankton assemblages relieved from P-limitation under natural conditions. Those phytoplankton apparently most affected by zebra mussels were those closest to the densest mussel populations in the shoaly island areas of the western basin. Phytoplankton physiology and biochemistry related to responses to P-limitation in the upper waters of the central basin appeared to be insignificantly affected by zebra mussels.

Our findings provide evidence that current management strategies are based on assumptions that remain valid: phytoplankton assemblages appear still to be P-limited in all but places very close to zebra mussels both horizontally and vertically in the water column. Control of P-loading to the lake will likely continue to provide an effective constraint to the growth of phytoplankton assemblages because they continue to be P-limited. *But* the presence of zebra mussels in large quantities in selected areas may lead to stimulation of phytoplankton blooms not anticipated from consideration of external P-loading records alone. Zebra mussels are potent remineralizers of P and N. Mussels increase the “internal loading” of P as phosphate and N as ammonium and likely will affect proximate phytoplankton assemblages, causing them to grow faster and to a greater extent than can be expected on the basis of external P-loading. It may be that blooms will occur unexpectedly, especially of those species most likely to grow rapidly in response to high nutrient concentrations. Those species likely to respond to the greatest extent to such nutrient stimuli are “eutrophic” species of noxious algae such as *Microcystis*, conceivably altering the usefulness of the phytoplankton to higher trophic levels and potentially damaging the quality of the water for human drinking and recreational use. Lake Erie managers would be wise to maintain vigilance on the spread of zebra mussels and the possibility of noxious blooms originating from regions most affected by these mollusc invaders.

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