The impact of a recurrent coastal plume on phosphorus dynamics and production in Lake Michigan

James B. Cotner
Department of Wildlife and Fisheries Sciences
Texas A&M University
College Station, TX 77843-2258
Telephone: 409-845-0169
Fax: 409-845-4096
Internet:j-cotner@tamu.edu

Thomas H. Johengen
NOAA/Great Lakes Environmental Research Laboratory and University of Michigan
2205 Commonwealth Blvd.
Ann Arbor, MI 48105

Noel R. Urban
Department of Civil and Environmental Engineering
Michigan Technological University
Houghton, MI 49931-1295

Duration: 1 Jan 98 - 31 Dec 2002

Amount requested: $849,158

Submitted to:

   NSF/NOAA

Project Summary

The proposed work will examine the influence of a major episodic event on phosphorus (P) availability and dynamics in the southern basin of Lake Michigan. Efforts will focus on a recurrent coastal plume that develops in the spring. Understanding the impact of episodic events on P availability has important implications for ecosystem structure and function because primary productivity in this system is P-limited.

The goal of this project is to determine the importance of the recurrent coastal plume to transport, composition and biological availability of P. To achieve this goal, we will address the following objectives: (1) To determine the potential sources, distributions and fluxes of P in the recurrent coastal plume; (2) To determine the impact of P associated with the recurrent coastal plume on phytoplankton and bacterioplankton production in Lake Michigan; and (3) To determine the impact of biotic utilization and settling on P composition and availability within the plume.

These objectives will be fulfilled by examining the geochemical composition and mineralogy of suspended particles, and the effects of these particles on biological processes. Specifically, we will examine the impact of these suspended particles on microbial (heterotrophic bacteria and phytoplankton) community productivity and nutrient limitation through a combination of bioassays (growth rate measurements, alkaline phosphatase activity), nutrient chemistry and stoichiometry, and P regeneration rates.

The proposed work will address both NSF and NOAA goals. It will contribute to the understanding of the processes controlling transport of a potentially limiting nutrient (P) from nearshore to offshore regions and especially the impact of sediment-water interactions and biological transformations. It will also provide insight into the potential impact of major episodic events that deliver large quantities of non-point source material on nutrient cycling and productivity in the Great Lakes.

Prior NSF support

The first cruise for this project occurred in fall 1996. We have another cruise scheduled for March-April 1997. Initial results from the fall cruise indicated potential N-limitation of *Synechococcus* and other phytoplankton and P-limitation of heterotrophic bacteria. Other observations include the following:

- Dissolved organic phosphate (DOP) was the dominant phosphate pool in the upper water column at the BATS site with concentrations of 100 to 200 nM. Both particulate phosphate and dissolved inorganic phosphate were 10 nM or less, as measured by chemical and kinetic methods.

- Orthophosphate turnover times were a few hours, which is rapid for oligotrophic marine systems.

- Hydrolysis and assimilation of monomeric and polymeric components of DOP were also rapid, though not as rapid as that of orthophosphate.

- Nutrient enrichment experiments suggest that both autotrophic and heterotrophic plankton growth at the BATS site can be phosphate-limited.

A. Publications resulting from this award:

Ammerman, J.W., M.J.D. MacRae, E. Bentzen, E.R. Peele, J.B. Cotner, and W.H. Jeffrey, "Phosphorus cycling at the Bermuda Time-Series Station: An overview". Presented at the AGU/ALSO Ocean Sciences Meeting, February 1994, San Diego.

Cotner, J.B., E.R. Peele, J.W. Ammerman, and E. Bentzen, "Phosphorus-limited plankton growth in the Sargasso Sea and implications for the pelagic food web." Presented at the AGU/ALSO Ocean Sciences Meeting, February 1994, San Diego.

Peele, E.R., J.W. Ammerman, E. Bentzen, and J.B. Cotner, "Microbial hydrolysis and utilization of dissolved organic phosphorus compounds at the Bermuda Time-Series Station." Presented at the AGU/ALSO Ocean Sciences Meeting, February 1994, San Diego.

B. Papers in preparation: Cotner, J.B., J.W. Ammerman, E.R. Peele, and E. Bentzen. Evidence for phosphorus-limited heterotrophic bacterial growth in the Sargasso Sea. (In review: *Aquatic Microbial Ecology).*

Ammerman, J.W. and M.J. Beifuss. Alkaline phosphatase activity: Results of a new assay for field use. (To be submitted to *Applied and Environmental Microbiology).*

Relation of the completed work to the proposed work. The proposed work will examine P cycling in Lake Michigan and is similar in scope to the work that is being performed at the BATS site. An exception, however, is the emphasis on the importance of sediment and physical processes in the current proposal.

This study was designed to determine whether the forms of sulfur in lake sediments provide a useful index of lake conditions. Sediment cores were taken from two, well-characterized Swiss lakes, and successive depth increments were analyzed for approximately ten different forms of sulfur. The lakes represented strongly contrasting conditions, and the recent history of lake conditions was well-known from previous work in each lake. The results indicated that sulfur is a useful marker of lake eutrophication and the oxygen content of bottom waters. The onset and acceleration of eutrophication were evident from the sulfur speciation in both lakes. In the lake

which had been artificially aerated to reverse eutrophication, the onset of aeration was clearly marked by a shift in sulfur speciation. The major advantage of sulfur speciation as a paleolimnological tool is that the indices of lake productivity and bottom water aeration appear to be independent. Hence, it is possible to distinguish between anoxia induced by eutrophication and anoxia induced by climatic factors that diminish lake mixing. This tool should prove useful in studying lake responses to climate change.

The international collaboration initiated by this project was continued for an additional two years with funding from the Swiss government. In addition, collaboration was established with the University of Bayreuth, Germany, as a direct result of this grant. The P.I. supervised one undergraduate research project at the University of Bayreuth and additional exchanges are planned. In addition to the presentations and publications listed below, and additional four collaborative publications are in various stages of completion.

Publications and presentations resulting from this award:

Urban, N.R. 1994. Retention of sulfur in lakes, In: Baker, L.A. (ed.), Environmental chemistry of lakes and reservoirs. Amer. Chem. Soc., Washington, D.C., pp. 323-369.

Urban, N.R., and K. Ernst. 1995. Addition of sulfur to organic matter during early diagenesis of lake sediments: Insights from XPS. Organic Geochem., In press.

Urban, N.R. 1992. Interactions of S, Fe, and Mn in lake sediments, Symposium on microbial catalysis of trace metal geochemistry. Amer. Chem. Soc. Mtg., San Francisco, CA 4/92.

Urban, N.R., S. Peiffer, and B. Wehrli. 1991. Cycling of sulfur at the sediment-water interface of lakes. European Environmental Research Org. Conference on Interfacial phenomena, Davos, Switzerland, 10/91.

Urban, N.R., B. Wehrli, and S. Peiffer. 1991. Mechanisms for recycling of sulfur in lake sediments. 2nd Internat. Symposium on Environmental Geochemistry, Uppsala, Sweden, 9/91

Urban, N.R. 1991. Sulfur retention in lakes, Symposium on Chemistry of Pollutants in Lakes. Amer. Chem. Soc. Mtg., Atlanta, GA, 4/91.

Introduction

Lake Michigan is a phosphorus (P) limited, oligotrophic lake that underwent increased eutrophication from the 1940's to 1975. It is believed that a major factor contributing to eutrophication was increased loading of P associated with point and non-point source pollution (Schelske et al. 1986). Nonetheless, an approximately two-fold decrease in P loading between 1975 and 1990 did not result in a consistent trend of decreased concentrations of either chlorophyll or total phosphorus (Johengen et al. 1994). Johengen et al. concluded that the lack of change may have been caused by a delayed response (ca. 20 years) between loading and planktonic biomass. A key factor in the response time of a lake is the magnitude of the internal cycling of P (i.e., the release of P from the sediments to the water column; Nürnberg 1991). The magnitude and the mechanisms of this P recycling and internal loading in Lake Michigan are not well understood.

Because most P is transferred from terrestrial to aquatic environments in particulate form, soil and shoreline erosion are major processes increasing P availability in aquatic environments (Schelske and Stoermer 1972). Most erosion occurs episodically, therefore, relatively rare events can have an extremely large impact on P loading to aquatic systems. Very little is known regarding the importance of major storms and other episodic events in aquatic ecosystems in general, and especially in large basins such as Lake Michigan. Preliminary measurements during a recurrent coastal plume in April 1996 indicated a total of 1×10^9 g of total particulate P in the plume. This one event represents a P input of 60 mg m^{-2} over the southern basin which is approximately one-fifth the annual flux of P into the sediments (Eadie et al. 1984). Because this lake is P-limited, this input is likely important to "new" production in this system (Caraco et al. 1992). In the proposed

work, we will quantify P-loading associated with episodic events and its relevance to productivity in the Lake Michigan basin.

The classic work of Einsele (1995) and Mortimer (1941) described the factors contributing to high fluxes of inorganic P from sediments to the water column under anaerobic conditions. This model is not appropriate to Lake Michigan sediment P fluxes for several reasons. First, areas of anoxic sediment surfaces are quite restricted in extent. Green Bay, the one area of known anoxia, represents only 0.6% of the total surface area of Lake Michigan. Second, only about 10% of phosphorus in surface sediments of Lake Michigan is bound to Fe and Al oxyhydroxides (Eadie et al. 1984), the important redox species. Third, measured release rates of P from the sediments are much lower than calculated internal recycling rates (Conley et al. 1988). These considerations suggest that either most P is recycled efficiently within the water column before reaching the sediments (Conley et al. 1988), or some other mechanism of P release from the sediments must be operative.

Sediment resuspension is one alternative mechanism of recycling P from the sediments to the water column. Eadie et al. (1984) pointed out more than a decade ago that sediment resuspension loaded the lake with weakly bound phosphorus. More recently, Brooks and Edgington (1994) observed that increases in the mass of phosphorus in the water column of Lake Michigan between spring and early summer are more than 10-fold greater than external, annual P loadings to the lake. These authors speculated that this P input is derived from sedimentary pools and that the equilibrium of phosphate with a solid-phase, calcium phosphate mineral maintained phosphate concentrations at a constant level despite the large uptake of phosphate by phytoplankton. It is not clear, however, that equilibrium with a mineral phase could be maintained in the face of high rates of biological uptake and regeneration.

Nonetheless, the magnitude of phytoplankton growth in the epilimnion may depend on the mass of phosphorus initially present in the epilimnion at the onset of stratification. This inventory of phosphorus would, in turn, depend on the mass of sediment particles resuspended in the water column, the sedimentation rate and the efficiency of regeneration.

Incorporation of P into heterotrophic bacteria may be an important mechanism maintaining P-availability in the water column after resuspension events. Although bacteria can compete with phytoplankton for P (Currie and Kalff 1984a, b; Cotner and Wetzel 1992; Vadstein et al. 1993), and thus, decrease productivity, they are relatively P-rich with internal concentrations double to ten times those of phytoplankton (Bratbak 1985; Vadstein et al. 1993). Phosphate half saturation uptake constants for these organisms have been estimated to be as low as <1 to 20 nM in freshwater lakes (Bentzen et al. 1992; Cotner and Wetzel 1992) and are in the range of measured phosphate values in Lake Michigan (Tarapchak and Rubitschun 1981; Tarapchak et al. 1982). Furthermore, sinking rates of bacteria are low because of their small size. Bacterial growth limitation by P has recently been demonstrated in several freshwater (Toolan et al. 1991; Coveney and Wetzel 1992; Morris and Lewis 1992) and marine ecosystems (Zweifel et al. 1993; Pomeroy et al. 1995; Cotner et al. 1996).

The present study provides an excellent opportunity to determine the role of sediment resuspension in phosphorus cycling in Lake Michigan. The multi-faceted nature of this investigation will allow unambiguous identification and quantification of the sources of P associated with the plume, the rates of P removal from the water column during the migration of the suspended plume, the rates, magnitude and mechanisms for P uptake or release from the plume particles, and the effects of the plume on phytoplankton and bacterioplankton productivity.

The coastal plume

In recent years, it was recognized that a coastal plume of elevated total suspended matter develops in the nearshore region of southern Lake Michigan (see cover proposal; Mortimer 1988; Eadie et al., 1997). This recurrent coastal plume develops during the unstratified period, and its timing corresponds with the disappearance of ice in the southern basin. In winter 1996, it was estimated

that the plume covered an area ca. 10 km wide and 100 km long, at its maximum extent, and persisted for about a month (Eadie et al., EOS, In press). The amount of particulate matter associated with the plume represented ca. 25% of the total annual particulate load to southern Lake Michigan. The origin of this particulate matter is somewhat equivocal, however; stable isotope (δ ^{15}N) analysis and C/N ratios are consistent with the idea that these particles originated as terrigenous material in bluffs along the western shore (B. Eadie, personal communication). An alternative source of the plume particulate matter may be resuspension of particles that were recently eroded from exposed glacial deposits near the coast.

Initial analyses indicated that total particulate P concentrations in the plume were three times open lake concentrations and NaOH extractable (available) P was nearly 10 times higher in the plume than open lake (Fig. 1). Interestingly, the ratio of available to total P was nearly four times greater (52% vs. 14%) for particles in the plume vs. the open lake. In 1996, total particulate P concentrations for plume particles (1.1 mg P g^{-1}) were lower than for the open lake particles (2.9 mg P g^{-1}), however, over 50% of the total particulate P in the plume was characterized as potentially bioavailable compared to 15% in the open lake. Total particulate P concentrations in plume materials were similar to levels measured in trap material near the plume (0.9 mg P g^{-1}) collected from January to March, 1995, and surficial sediments (0.9 mg P g^{-1}; Johengen, unpublished data). The fraction of available:total P for the surficial sediments was 13%, similar to that of open-lake particles (Fig. 2). However, the fraction of available P in the trap material was ca. 32%, indicating that enriched particles were also present in winter-spring 1995. We do not know whether the variation between the 32 and 50% available P represents re-working of the material as it settles or natural annual variation.
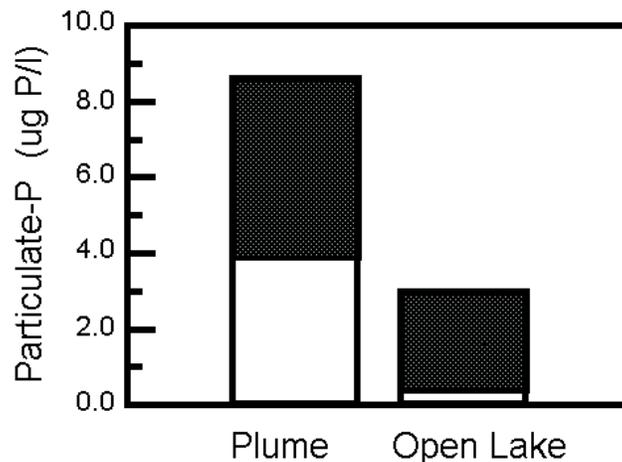

Figure 1. Total and bioavailable (NaOH-extractable; no shading) particulate phosphorus concentrations from samples collected within the turbidity plume and offshore of the plume on 1 April and 10 April 1996 in southern Lake Michigan.

Levels of P on plume particles were slightly higher than P levels in the presumed source material - western coastal bluffs (Fig. 2) which suggests that these storm-induced increases in terrigenous or resuspended particles are scavenging dissolved P from the water column. Nonetheless, our bioavailability measurements suggest that a high proportion of the P on this material is capable of being incorporated by microbial plankton (Fig. 2). These analyses and spatial patterns of nutrients and biomass suggest that the plume physically mixes particles across the coastal margin, and that this physical process is highly interactive with biological processes throughout the plume.
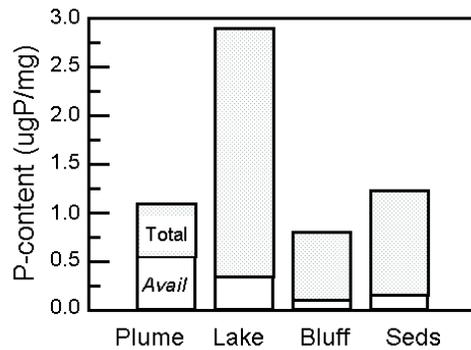
Figure 2. Total and bioavailable phosphorus content of particles on a per mg dry weight basis. Plume and open lake samples collected as in Fig. 1. The bluff sample was collected from a single shoreline site near Milwaukee, WI.

These data have important implications for P and plankton dynamics in the southern basin. If all of the P associated with the plume were deposited in sediments beneath the plume area, it would constitute a flux of nearly 1500 mg P m^{-2}. If it were deposited over the entire southern basin, it would constitute a flux of ca. 60 mg P m^{-2}. This flux combined with the particulate P in the open lake represents a combined flux of 276 mg P m^{-2}, which is comparable to a previous estimate of P sediment fluxes in this region and balances the annual load of P into the basin (Eadie et al. 1984).

Objectives/Hypotheses

The proposed work is one of several projects designed to examine the importance of episodic events on the transport and transformation of biogeochemically active materials across the coastal margin. We believe erosion and resuspension provide particles that sorb P from the water column (Fig. 3). Most of this P is regenerated in the water column but some is transported down plume and offshore in southern Lake Michigan.
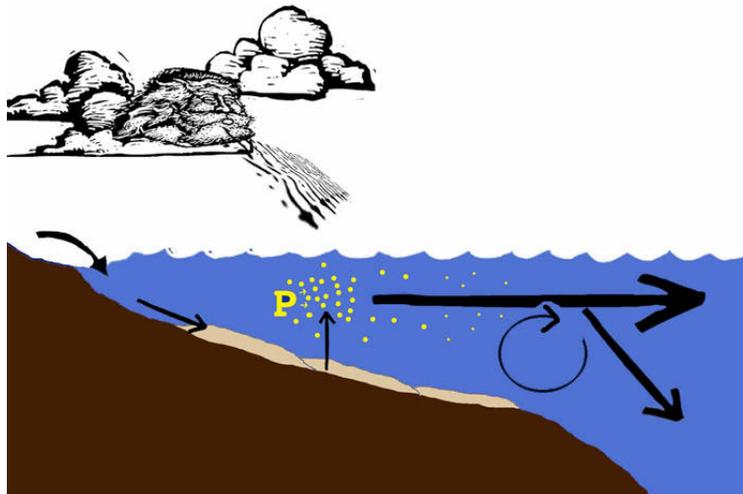

Figure 3. Conceptual diagram showing north wind generating erosion of particulate matter, and transport into transitional deposition regions. During the recurrent coastal plume, particles are resuspended, sorb P from the water column and are transported across the coastal margin. Most of

the P associated with the plume is regenerated but some is transported into permanent depositional regions (downward fluxing arrow).

The general objective of this work is to determine the role of this episodic turbidity plume in the cycling of phosphorus within southern Lake Michigan. In this study we will answer the following questions: (a) Is P from the recurrent coastal plume in southern Lake Michigan an important factor controlling the magnitude and duration of the spring bloom?, i.e., Is annual "new" production tightly coupled to the amount of P mobilized in the plume?, (b) Does P availability change (decrease) spatially and temporally, i.e., as the plume travels from the west shelf to the southern and eastern shelf, as a result of sedimentation and biological utilization; (c) What is the importance of resuspension events for P transport and availability across the coastal margin?

Specific objectives include:

- Determine the quantity and forms (phases) of phosphorus introduced into the water column as a result of sediment resuspension;

- Determine phosphorus losses from the water column as a result of settling of plume particles and plankton;

- Determine the kinetics of abiotic and biotic uptake and release of P from the particles;

- Monitor changes in speciation of P on the particles along the plume trajectory to determine the mechanisms for P uptake and release;

- Determine the impact of P associated with the plume on phytoplankton and bacterioplankton growth and nutrient limitation;

- Determine the importance of P-regeneration relative to sediment resuspension as a source of P for plankton growth.

Hypothesis 1- The recurrent coastal plume and/or resuspension of bottom sediments generates an increased inventory of total P and bioavailable P in the water column. This increased inventory is important to the development of the spring phytoplankton bloom.

This hypothesis will be evaluated by measuring water column depth profiles of dissolved, particulate, total, and potentially bioavailable (NaOH-extractable) P prior to plume development and inside and outside of the plume. In addition, measurements of particle and particulate P settling rates and lifetimes in the water column will be performed.

We will measure dissolved and particulate nutrient concentrations (C, N and P), characterize the phases in which P is bound, characterize the mineralogy as well as the Fe and Al content of suspended particles, determine the kinetics and capacity of the particles for abiotic uptake and release, and measure the effects of suspended particles on biotic P-uptake kinetics inside of and outside of the plume.

The magnitude of P resuspension will be characterized each year of this study, and compared with measures of phytoplankton abundance and productivity (primary productivity group) to determine if resuspension is important to the magnitude and duration of the spring bloom.

Hypothesis 2-Heterotrophic bacteria function as "scavengers" of P in the coastal plume and maintain P availability in the water column.

As P associated with abiotic particles is desorbed, a high proportion is incorporated into bacterioplankton biomass. Because of their high P composition, potential for being grazed by other components of the microbial food web, and low sinking rates, they function to maintain P availability in the euphotic zone. Light availability is likely important to this function. As the light field shifts from low to high availability through the plume, primary productivity increases. In

many freshwater systems, organic carbon is more readily available for bacterioplankton as a result of increased primary productivity and excreted organic carbon (Baines and Pace 1991). When organic carbon is readily available, decomposers incorporate inorganic nutrients into biomass more effectively (DeAngelis 1992). Alternatively, dissolved organic carbon (DOC) loading from eastern rivers flowing into Lake Michigan (Mortimer 1988) may also facilitate bacterial growth (Moll and Brahce 1986; Scavia and Laird 1987) as well as bacterial P incorporation (Cotner and Wetzel 1991a). Consequently, bacterioplankton become more effective competitors for P, decreasing the loss rate from the mixed layer.

To address this hypothesis, we will measure bacterial productivity and biomass, examine the potential for P-limited bacterial growth, and measure P-uptake kinetics and DOC concentrations inside and outside of the plume. We will also directly measure the quantity of P in heterotrophic bacteria by concentrating them from lake water using tangential flow filtration and particulate P measurements.

Hypothesis 3- The inventory of TP and available P within the plume decreases as the plume ages, as a result of biotic incorporation and settling.

In conjunction with the particle flux group (Eadie et al.), samples of suspended and settling (sediment traps and Lagrangian samplers) particles will be collected throughout the lifetime of the plume. These particles will be characterized in terms of size distribution (sequential filters and field flow fractionation for suspended particles), P speciation, and elemental (N, Si, P, Fe, Al, Ca and inorganic and organic C) content. These measurements will enable us to model the residence time of available P within the plume. In addition, we will determine P sedimentation rates associated with plume development to enable dynamic modeling of P availability.

The time of year when the plume is established is likely to impact the relative loss of P through this process. When plume conditions develop in late spring, as was observed by Mortimer (Mortimer 1988), warmer temperatures and higher bacterial productivities (Scavia and Laird 1987) are likely to increase the quantity of P sequestered by these organisms.

Hypothesis 4- The bioavailability of P on the particles is governed by (a) equilibrium with solid Ca-PO$_4$ phases; (b) dissolution kinetics of CaPO$_4$ phases; and/or (c) adsorption/desorption kinetics of PO$_4$ on the Fe and Al oxides of the particles.

Evaluation of this hypothesis will require measurements of ortho-phosphate concentrations, P speciation on the sediment particles, and the kinetics of P uptake and release from the particles. Concentrations of ortho-phosphate will be assayed in selected samples using both the alkaline phosphatase inhibition technique (Pettersson 1979) and Rigler's radiobioassay (Rigler 1966). Because it can be difficult to distinguish P bound to Ca minerals from P bound to Fe and Al oxides with sequential extractions (Hieltjes and Lijklema 1980; Pettersson et al. 1988), element associations will also be examined with electron microprobe analysis (EMPA).

Hypothesis 5- Water column P regeneration is more efficient during development of the recurrent coastal plume, minimizing losses to the sediments at this time.

During the coastal plume development in 1996, there was a shift in the microbial flora from large diatoms to smaller primary and secondary producers (Eadie et al. 1997, submitted). Because of decreased density of smaller, less silicified plankton, settling rates would decrease and a higher proportion of production would be regenerated before it reaches the sediments (Eadie et al., in preparation).

Conley (1988) estimated that sediments provided less than 1% of the annual biological P demand for annual primary productivity. We hypothesize that resuspended sediments from the coastal plume provide an important P source in the late winter/early spring and that biological regeneration plays an important role in minimizing losses of assimilated P to the sediments both during plume development and after the lake stratifies.

Significance

The results of this study have great relevance to the dynamics and productivity of Lake Michigan as well as other large lakes of the world. Because this lake is P-limited, system productivity is closely coupled to the availability of P. The proposed work will examine the importance of physically driven, cross-shelf transport process on phosphorus dynamics in this system. We believe this coastal plume may play an important role in controlling annual ecosystem productivity.

The results of this study have important implications for management of the Laurentian Great Lakes. Data gathered will be used to predict P inventories in Lake Michigan. If P inventories can be related to the duration and extent of the spring bloom or annual productivity, one could develop a predictive model based on P availability. Furthermore, if P availability is related to the duration and extent of the recurrent plume development, it may be possible to estimate P availability as a function of total suspended matter present in the plume each year. If so, satellite imagery could be used to predict productivity in this, and possibly, other systems where physical processes dominate nutrient cycles.

The proposed work has important implications for cross-shelf transport of P, and possibly other potentially limiting nutrients (such as N or Fe), in the Great Lakes as well as marine systems. There is increasing evidence that P can limit microbial productivity in marginal seas (Zweifel et al. 1993; Pomeroy et al. 1995) as well as oceanic gyres (Karl et al. 1995; Cotner et al. 1996), so that the dynamics of P may be relevant to oceanic productivity and carbon dynamics as well.

Relation to long-term goal of the investigators

The proposed work is a collaboration of investigators from three institutions that all have interests in biogeochemical cycles in the Great Lakes.

The goals of the project help fulfill the career goals of each of the P.I.s. Cotner has studied phosphorus and nitrogen cycling in freshwater and marine systems, including the Great Lakes and small kettle lakes in Ohio and Michigan, the nearshore Gulf of Mexico, Florida Bay (with Johengen) and recently in the North Atlantic. All of this work is directed at a long-term goal of understanding the role of heterotrophic bacteria in P and N cycling.

Johengen studies nutrient loading, dynamics and the importance of watershed management practices on these processes. He has significant experience constructing watershed nutrient budgets and understanding the impact of human activities on non-point source nutrient loads.

Urban studies C, N, P and S geochemistry in lakes. He has been involved in several studies on the effects of sediment geochemical processes on nutrient cycles. In addition, he has studied the efficacy of lake restoration projects in eutrophic European lakes, and he participated in a long-term study of sediment - water exchange processes in seepage lakes in Wisconsin.

Experimental Plan

Overview and sample sites

We will conduct cruises in collaboration with the other synoptic components of this group proposal. As part of our proposed program, we will conduct one spring cruise the first year, and three cruises in years 2 and 3. All cruises are planned for ca. two weeks. In the first year, the cruise will be conducted during plume development and in years two and three, cruises will occur before, during and after plume development. Each cruise will consist of a transect survey in the southern basin, intensive sampling at five master stations along the southeast region of the basin, and a 5-7 day Lagrangian experiment that begins in area A offshore of St. Joseph, MI (see cover proposal). These cruises will benefit from a series of five moored arrays that will be deployed in this region of the lake out to a depth of ca. 100m. These meters will make current, particle and temperature measurements prior to and during plume development.

The proposed work complements and contributes to several other proposals in the CoOP program, especially those on phytoplankton dynamics (Fahnenstiel et al.), lower food web dynamics (Vanderploeg et al. and Gardner et. al), biophysical modeling (Cheng et al.), particle fluxes (Eadie et al.), and satellite imagery (Leshkevich et al.). This work will also benefit from an expanded biological monitoring program at NOAA's Great Lakes Environmental Research Laboratory that will enable collection of samples for DOC and bacterial abundance measurements at ca. biweekly intervals from January to May at study site A and a deepwater site near Muskegon. This will facilitate characterization of the southern basin before and after plume development.

The overall strategy is to characterize the particles prior to suspension, throughout plume development, and during deposition. Particles will be characterized in terms of their mineral composition, size distribution, major elemental content, Fe oxide content, and P speciation. The impact of particles on bacterial productivity, bacterial and phytoplankton nutrient limitation, and P regeneration will also be assessed.

Methods

Underway measurements

Continuous measurements of alkaline phosphatase activity, nutrients (PO_4, SiO_2) and various other water quality parameters will be made while the ship is underway. Water will be pumped to a common reservoir and subsequently to a fluorometer (for alkaline phosphatase measurements) or an auto-analyzer (for nutrient measurements). Total suspended matter will be measured using a Seabird® or Hydrolab® CTD unit with attached transmissometer or turbidometer connected in-line to a continuous flow-through delivery system. Ancillary measurements of temperature, pH, dissolved oxygen and conductivity will also be measured. Discrete samples will be measured periodically to calibrate instruments and provide specific particle concentrations at meaningful locations inside and outside of the plume.

Alkaline phosphatase measurements

Alkaline phosphatase is a periplasmic enzyme produced in aquatic microbes that has been used as an indicator of P-limitation in aquatic communities (Healey and Hendzel 1979, 1980; Cembella et al. 1984). It is produced by both phytoplankton (Healey and Hendzel 1979) and bacterioplankton (Cotner and Wetzel 1991b; Chróst 1992) in freshwater ecosystems. We recently used alkaline phosphatase and plankton biomass data to conclude that western Sargasso Sea bacterioplankton, and possibly phytoplankton, are P-limited (Cotner et al. 1996).

Alkaline phosphatase activity is measured fluorometrically with the artificial substrate methyl-umbelliferyl phosphate (MUF-P; Hoppe 1983). MUF-P is added to lake water at saturating concentrations (ca. 10-100 µM), and samples are incubated at ambient temperature for 0.5 - 3 hr prior to measuring fluorescence.

This method has recently been adapted to make continuous underway measurements in the Mississippi River plume (Ammerman and Beifus, in preparation). To adapt the method to continuous flow samples, measurements are made at a constant temperature (25∞C) and incubation time (20 -40 min). We have measured these rates on discrete samples using a Turner 10-AU fluorometer, and propose to adapt this fluorometer to make continuous measurements by adding a smaller flow cell (3 mm), and a low-flow peristaltic pump to deliver lake water to the fluorometer from the reservoir. In addition, we propose to add GPS capability to the fluorometer by feeding data from the fluorometer and GPS directly into a datalogger. Consequently, spatially explicit data will be available from all areas tracked by the ship. These data can be normalized to continuous ambient chlorophyll measurements (made by Fahnenstiel et al. group) and bacterial biomass as an indicator of P-limitation throughout the basin (Cotner et al. 1996). Water samples will also be collected at various depths along transects to obtain a depth profile of enzyme activity. In addition, some of these measurements will be made in both whole lakewater and 0.8 µm pre-filtered

lakewater to separate activity of small microbes (mostly heterotrophic bacteria) from phytoplankton (Cotner and Wetzel 1992).

Nutrient measurements

Dissolved nutrients

Dissolved nutrient concentrations (PO_4^{3-} [soluble reactive P - SRP], NH_4^+, and SiO_2) and the conservative Cl^- ion will be determined using a ship-based auto-analyzer system designed to sample from a continuous water delivery system. Cl^- measurements will be used to distinguish different water masses across the plume. Nutrient analyses will be performed using standard colorimetric techniques (USEPA 1974; American Public Health Association 1992) as detailed by Davis and Simmons (1979).

In addition to continuous underway measurements of surface concentrations, samples will be collected from multiple depths at master stations within and outside of the plume and analyzed. Sampling depths will be chosen based on the depth of the water column and CTD profiles. We will also measure dissolved organic P (DOP) on these samples by determining total dissolved P (0.2

μm pore-size filtered) and subtracting SRP. Although rate constants suggest that DOP is an important source of P to microbes in Lake Michigan (Tarapchak and Moll 1990), virtually no systematic concentration measurements have been made. Typically this pool represents the major fraction of total P in oligotrophic lakes (Wetzel 1983). Observations in Lake Michigan indicate total dissolved P pools are approximately 40% of total P pools (Johengen, unpublished data).

Dissolved organic carbon concentrations (DOC) will be measured by a high-temperature combustion method using a Shimadzu TOC 5000 Pt catalyst analyzer (Sugimura and Suzuki 1988). Water samples are collected, filtered through a combusted GF/F filter into a combusted serum bottle and frozen. Measurements will be corrected for instrument and water blanks (Sharp et al. 1993).

We have recently successfully used these techniques to measure extremely low (60-70 μM C) concentrations at the Bermuda Atlantic Time-Series Station (Cotner, unpublished data).

Particulate nutrients

Particulate phosphorus (PP) will be determined by the combustion method of Solorzano and Sharp (1980) after collection of particulate samples on Whatman GF/F filters. After combustion and acid hydrolysis, the molybdenum blue spectrophotometric method is used for detection (American Public Health Association 1992).

Available phosphorus content will be determined using a 0.1N NaOH extraction procedure (Williams et al. 1980). This chemical extraction procedure compares favorably to estimates of biological available phosphorus determined by algal assay procedures (Williams et al. 1980; Sonzogni et al. 1982; Dorich et al. 1985) and has the advantage of taking only several hours rather than two weeks to complete. The method was reported to yield a coefficient of variation of 7.5 percent for determinations on 5 replicates.

Potentially, bioavailable particulate P will be estimated by mixing particulate matter with 0.1 N NaOH for 17 h at room temperature (Eadie et al. 1984). Filtrates are subsequently analyzed using standard colorimetric techniques. This measurement provides a chemical analogue of biologically available particulate P (Eadie et al. 1984).

CHN analyses will be performed on a Perkin-Elmer Model 2400 elemental analyzer. We will combine these measurements with our measurements of particulate phosphorus concentrations (see above) to determine stoichiometric nutrient ratios. Presumably, the nutrient most likely to be limiting will be deficient relative to nutrient requirements of the plankton (Redfield 1958; Hecky and Kilham 1988).

Kinetics and particle characterization

The kinetics of abiotic P uptake and release will be measured on particles suspended in the plume for varying lengths of time. Sediment sampling prior to resuspension will be done in conjunction with the particle flux group with gravity cores, box cores, and in situ settling chambers. In addition, grab samples from the shoreline bluffs will be collected. This group also plans to use a ROV to collect sediment floc from transient deposition regions, and this material may also be characterized and analyzed kinetically. Suspended particles will be collected and filtered for particle characterizations. Large unfiltered samples will be collected for uptake and desorption experiments; particles will be concentrated as necessary using continuous flow centrifugation. Settling particles will be collected with the Lagrangian samplers and the sediment traps deployed by the particle flux group. Gravity cores will be collected from the eastern depositional basin to characterize the particles that have reached the sediments.

Characterization of the particles is intended to elucidate mechanisms of P binding and to aid in modeling of P dynamics. Mineralogy will be assessed by X-ray diffraction. In conjunction with other groups, the major elemental composition will be determined on an elemental analyzer (C, N, S) and by digestion followed by colorimetric or atomic absorption techniques (total P, Si, Fe, Ca, Al). The Fe oxide content of particles will be measured by extraction after the methods of Canfield (Canfield 1989). The extraction scheme of Hieltjes and Lyklema is particularly suited for determining P speciation in these particles because it can distinguish between P bound to Fe and that bound to Ca (Hieltjes and Lijklema 1980). This fractionation scheme will be applied to size-fractionated particles (settling chambers, differential centrifugation) from the sediments as well as to the suspended particles. A Sedigraph will be used for measuring size distributions of soil samples and surface sediments. Size distributions of suspended solids will be measured with both field flow fractionation and a Coulter Counter.

To assess whether phosphate concentrations in the lake are controlled by equilibrium with mineral phases, careful measurements of the relevant dissolved constituents (orthophosphate , Ca, pH) will be made in filtered plume water. Because molybdate-reactive phosphorus includes more than just orthophosphate (e.g., Hieltjes and Lijklema 1980; Tarapchak and Rubitschun 1981) orthophosphate estimates will be determined in a few samples using both the alkaline phosphatase inhibition technique (Pettersson 1979) and Rigler's radiobioassay (Rigler 1966). Concentrations of dissolved Fe and Al are assumed to be controlled by gibbsite and $Fe(OH)_3$, and equilibrium with potential solid phases (e.g., $AlPO_4$, $Ca_4H(PO_4)_3$, $Ca_{10}(PO_4)_6(OH)_2$) will be assessed using the computer program MINEQL (Westall et al. 1976) with the equilibrium constants of Stumm and Morgan (1981). Particles also will be examined by scanning electron microscopy and electron microprobe analysis to confirm element associations.

Dissolved phosphate concentrations may also be controlled by equilibrium adsorption. Phosphate adsorption isotherms in the plume will be measured after the methods of Detenbeck and Brezonik (Detenbeck and Brezonik 1991) to assess binding constants. Samples will be collected and particles concentrated by filtration, sterilized with chloroform, and rediluted in filter-sterilized (0.2 μm) lake water at ambient pH. The alterations induced by filtration and redilution are not likely to affect the sorption capacity or binding strength although the kinetics may be altered. The variable surface charge model will be applied to calculate the binding constant. This binding constant may then be compared with values in the literature for binding to iron oxides (e.g., Detenbeck and Brezonik 1991).

It also is possible that the kinetics, rather than equilibrium, of adsorption/dissolution and precipitation, control dissolved phosphate concentrations. Kinetics of sorption and desorption will be measured with radiolabelled PO_4^{3-}. Rates of isotope exchange will be assessed by adding carrier-free $^{32}PO_4^{3-}$ to sterilized plume water samples and monitoring activity in the dissolved phase over time. Adsorption rates will be measured by adding ^{32}P with increments of cold phosphate and monitoring the dissolved isotope activity with time. Concentration increments of cold phosphate will be kept in the range of 0.05 to 1 μM.

Desorption rates will be measured after equilibrating a sterilized suspension of particles for one week with 5 µmol L^{-1} PO$_4$$^{3-}$. Radiolabelled PO$_4$$^{3-}$ will be added on the fifth day, and on the seventh day the particles will be filtered from the original lakewater and resuspended in artificial phosphate-free lakewater. Radioactivity and concentrations of SRP will be monitored in the dissolved phase as a function of time. These experiments are not adequate to thoroughly characterize the kinetics (cf. Detenbeck and Brezonik 1991), but should be adequate to determine a rate constant for sorption and desorption. These rates of sorption and desorption may then be compared with rates of alkaline phosphatase activity, other indicators of the rates of biological P uptake, and the residence times of the particles in the water column.

Biotic P uptake

We will assay algal (>0.8 µm) and bacterial (<0.8 µm) PO$_4$$^{3-}$ uptake using radiolabelled phosphate and differential filtration. Tracer levels of ^{33}P-PO$_4$$^{3-}$ will be added to whole lakewater and incubated at ambient temperature for 1-30 min (depending on activity). Killed controls (2% formalin final concentration) will be used to distinguish biotic and abiotic uptake. Incubations will be terminated by filtering through either a 0.2 µm pore-size or 0.8 µm pore-size polycarbonate filter. Filters are dried, and assayed for radioactivity in a scintillation counter. Assuming that most heterotrophic bacteria pass the 0.8 µm pore-size filter, we will use uptake onto the 0.2 µm pore-size filter as an estimate of total phosphate uptake during the incubation and the difference in uptake on the two filter sizes as an estimate of uptake into the heterotrophic bacterial component (Cotner and Wetzel 1992). This assumption will be verified by microscopic examination of the various size-fractions after filtration or flow cytometry (see below).

Alternatively, we will use flow cytometry to fractionate plankton. We are currently running experiments where radioisotopes are added to samples and different planktonic components are separated using flow cytometric sorting and are subsequently examined for radioisotope content. This is a powerful tool in that the specific uptake into different planktonic groups can be measured directly. Li (1994) recently used this method to examine ^{14}C bicarbonate uptake into planktonic autotrophs. We have successfully sorted and detected radioactively labeled bacteria in culture and will test this method on field samples this spring.

P regeneration

An isotope dilution method will also be used to measure P-regeneration in water column samples (Harrison 1983; Hudson and Taylor 1996). In this method, radiolabeled phosphate is added to water samples and incubated at ambient temperature for 20-80 h. Subsequently, cold (unlabeled) phosphate is added to block radiolabeled phosphate and the increase of labeled P in the dissolved pool is monitored and the regeneration rate determined (Hudson and Taylor 1996). Alkaline phosphatase measurements will also provide a relative estimate of P regeneration. MUF-P is an organic P compound, but because it will be added at high concentrations, it provides an estimate of "potential" regenerative capacity from the dissolved organic P pool rather than of ambient activity.

Bacterial production and biomass

Bacterial productivity will be determined by the radiolabeled leucine incorporation method (Kirchman et al. 1985; Viollier et al. 1995). Measurements will be made at a concentration determined empirically to saturate incorporation rates (ca. 10-40 nM). Conversion factors will be estimated empirically in lakewater cultures that have been diluted with filtered (0.2 µm) lakewater. The change in bacterial biomass and incorporation rate with time will be used to estimate the conversion factor.

Bacterial biomass will be estimated using two methods. The first, most developed, method used in our laboratories is epifluorescence microscopic counts of preserved (2% formalin) cells using

acridine orange (Fahnenstiel and Scavia 1987) or DAPI (Porter and Feig 1980) with an image analysis system (OPTIMAS).

Alternatively, we have been developing flow cytometric methods for counting heterotrophic bacteria in our laboratory. We have been using a Becton-Dickinson FACSCalibur flow cytometer with an air-cooled laser and sorting capabilities. Prior to analyses samples will be preserved with paraformaldehyde and frozen in liquid nitrogen (Olson et al. 1993). Sorting may also be used with the ^{33}P-uptake experiments to differentiate uptake by bacteria and phytoplankton. In these experiments, counting is performed with live samples. Similar measurements have recently been made on photosynthetic picoplankton after incorporation on ^{14}C-HCO$_3^-$ (Li 1994).

Bacteria from lake water will be concentrated using tangential flow filtration to measure bacterial P content. We have used this technology effectively in the Mississippi River plume as part of the NECOP program to examine the impact of heterotrophic bacteria on ammonium regeneration rates (Cotner and Gardner 1993). Whole lake water is pre-filtered (<1 μm) to remove non-bacterial particles. The bacteria are then concentrated using a recirculating tangential flow filter (Amicon) with a 0.2 μm pore-size filter. Particles will subsequently be enumerated and particulate P concentrations determined as above. These analyses will be performed at several sites in the plume and outside of the plume on each cruise.

Year 4 and 5

In the last two years of this program, most efforts will be devoted to publishing results from cruises. As part of this effort, we will synthesize our data and put our results in context of the entire Lake Michigan ecosystem. We will obtain data on P pool sizes and, where available, fluxes from other regions of the lake and to determine the relative significance of loading from the recurrent coastal plume to ambient stocks in other regions of the lake.

Schedule

Time	Activity
1998	Pilot cruise activities; development of continuous sampling apparati for nutrient and alkaline phosphatase measurements; particle characterization
1999	Field intensive measurements (Mar-July); Evaluation of results and laboratory analyses (Aug-Dec)
2000	Field intensive measurements (Mar-July); Evaluation of results and laboratory analyses (Aug-Dec); manuscript preparation
2001	Synthesis; interactions with other groups to facilitate model development; further experimentation if necessary; manuscript preparation
2002	Synthesis; interactions with other groups to facilitate model development; further experimentation if necessary; manuscript preparation

Data management

We will work with the physical oceanography group to coordinate data management. They are using the network and communication links already developed for the Great Lakes Forecasting System, and plan to create a distributed environment to provide access to a database of research data and implement analysis tools by which two- and three-dimensional data can be initially and rapidly analyzed. Both numerical model data and field data will be stored in the three-dimensional time varying database. Access will be available by date, type, location, variable type, and will be available on the program file server. The basis for the Lake Michigan Information Data Analysis System (LAMIDAS) is derived from the GLOBEC system for data achieving. A key component of this project is the sharing of the field, retrospective, modeled, and derived data collected by scientific investigators in different fields. The JGOFS (U.S. Joint Global Ocean Flux Study) data

management software is used to serve data and information to the involved scientists. World Wide Web browsers are selected as the standard Graphic User Interface (GUI). Limited two-dimensional plotting, mechanical routines, and filters are also made available for scientists. For more information see cover proposal and Schwab et al. proposal.

References Cited

American Public Health Association. 1992. Standard methods for the examination of water and wastewater, 18th ed. APHA.

Baines, S.B., and M.L. Pace. 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: Patterns across marine and freshwater systems. Limnol. Oceanogr. **36:** 1078-1090.

Bentzen, E., W.D. Taylor, and E.S. Millard. 1992. The importance of dissolved organic phosphorus to phosphorus uptake by limnetic plankton. Limnol. Oceanogr. **37:** 217-231.

Bratbak, G. 1985. Bacterial biovolume and biomass estimations. Appl. Environ. Microbiol. **49:** 1488-1493.

Brooks, A.S., and D.N. Edgington. 1994. Biogeochemical Control of Phosphorus Cycling and Primary Production in Lake Michigan. Limnol. Oceanogr. **39:** 961-968.

Canfield, D.E. 1989. Reactive iron in marine sediments. Geochim. Cosmochim. Acta **53:** 619-632.

Caraco, N.F., J.J. Cole, and G.E. Likens. 1992. New and recycled primary production in an oligotrophic lake: Insights for summer phosphorus dynamics. Limnol. Oceanogr. **37:** 590-602.

Cembella, A.D., N.J. Antia, and P.J. Harrison. 1984. The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae: a multidisciplinary perspective: Part 1. Crit. Rev. Microbiol. **10:** 317-391.

Chróst, R.J. 1992. Significance of bacterial ectoenzymes in aquatic environments. Hydrobiologia **243/244:** 61-70.

Conley, D.J., M.A. Quigley, and C.L. Schelske. 1988. Silica and phosphorus flux from sediments: Importance of internal recycling in Lake Michigan. Can. J. Fisheries. Aquat. Sci. **45:** 1030-1035.

Cotner, J.B.Jr., and W.S. Gardner. 1993. Heterotrophic bacterial mediation of ammonium and dissolved free amino acid fluxes in the Mississippi River plume. Mar. Ecol. Prog. Ser. **93:** 75-87.

Cotner, J.B., and R.G. Wetzel. 1991a. 5'-Nucleotidase activity in a eutrophic lake and an oligotrophic lake. Appl. Environ. Microbiol. **57:** 1306-1312.

Cotner, J.B.Jr., and R.G. Wetzel. 1991b. Bacterial phosphatases from different habitats in a small, hardwater lake, p. 186-204. *In* R.G. Chróst (ed.), Microbial enzymes in aquatic environments, Springer.

---. 1992. Uptake of Dissolved Inorganic and Organic Phosphorus Compounds by Phytoplankton and Bacterioplankton. Limnol. Oceanogr. **37:** 232-243.

Cotner, J.B., J.A. Ammerman, E.R. Peele, and E. Bentzen. 1996. Phosphorus limited bacterioplankton growth in the Sargasso Sea. Aquatic Microbial Ecology.

Coveney, M.F., and R.G. Wetzel. 1992. Effects of nutrients on specific growth rate of bacterioplankton in oligotrophic lake water cultures. Appl. Environ. Microbiol. **58:** 150-156.

Currie, D.J., and J. Kalff. 1984a. Can bacteria outcompete phytoplankton for phosphorus? A chemostat test. Microbial Ecol. **10:** 205-216.

---. 1984b. A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus. Limnol. Oceanogr. **29:** 298-310.

Davis, C., and M. Simmons. Water chemistry and phytoplankton field and laboratory procedures.

DeAngelis, D.L. 1992. Dynamics of nutrient cycling and food webs. Chapman & Hall.

Detenbeck, N.E., and P.L. Brezonik. 1991. Phosphorus sorption by sediments from a soft-water seepage lake. 1. An evaluation of kinetic and equilibrium models. Environ. Sci. Technol. **25:** 395-402.

Dorich, R.A., D.W. Nelson, and L.E. Sommers. 1985. Estimating algal available phosphorus in suspended sediments by chemical extraction. J. Environ. Qual. **14:** 400-405.

Eadie, B.J., R.L. Chambers, W.S. Gardner, and G.L. Bell. 1984. Sediment trap studies in Lake Michigan: resuspension and chemical fluxes in the southern basin. J. Great Lakes Res. **10:** 307-321.

Fahnenstiel, G.L., and D. Scavia. 1987. Dynamics of Lake Michigan phytoplankton: Recent changes in surface and deep communities. Can. J. Fisheries. Aquat. Sci. **44:** 509-514.

Harrison, W.G. 1983. Uptake and recycling of soluble reactive phosphorus by marine microplankton. Mar. Ecol. Prog. Ser. **10:** 127-135.

Healey, F.P., and L.L. Hendzel. 1979. Fluorometric measurement of alkaline phosphatase activity in algae. Freshwater. Biol. **9:** 429-439.

---. 1980. Physiological indicators of nutrient deficiency in lake phytoplankton. Can. J. Fisheries. Aquat. Sci. **37:** 442-453.

Hecky, R.E., and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence. Limnol. Oceanogr. **33:** 796-822.

Hieltjes, A.H.M., and L. Lijklema. 1980. Fractionation of inorganic phosphates in calcareous sediments. J. Environ. Qual. **9:** 405-407.

Hoppe, H.-G. 1983. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Mar. Ecol. Prog. Ser. **11:** 299-308.

Hudson, J.J., and W.D. Taylor. 1996. Measuring regeneration of dissolved phosphorus in planktonic communities. Limnol. Oceanogr. **41:** 1560-1565.

Johengen, T.H., O.E. Johannsson, G.L. Pernie, and E.S. Millard. 1994. Temporal and seasonal trends in nutrient dynamics and biomass measures in Lakes Michigan and Ontario in response to phosphorus control. Can. J. Fisheries. Aquat. Sci. **51:** 2570-2578.

Karl, D.M., R. Leteller, D. Hebel, L. Tupas, J. Dore, J. Christian, and C. Winn. 1995. Ecosystem Changes in the North Pacific Subtropical gyre attributed to the 1991-92 El Niño. Nature **373:** 230-234.

Kirchman, D., E. K'nees, and R. Hodson. 1985. Leucine Incorporation and Its Potential as a Measure of Protein Synthesis by Bacteria in Natural Aquatic Systems. Appl. Environ. Microbiol. **49:** 599-607.

Li, W.K.W. 1994. Primary Production of Prochlorophytes, Cyanobacteria, and Eucaryotic Ultraphytoplankton - Measurements from Flow Cytometric Sorting. Limnol. Oceanogr. **39:** 169-175.

Moll, R., and M. Brahce. 1986. Seasonal and spatial distribution of bacteria, chlorophyll, and nutrients in nearshore Lake Michigan. J. Great Lakes Res. **12:** 52-62.

Morris, D.P., and W.M.J. Lewis. 1992. Nutrient limitation of bacterioplankton growth in Lake Dillon, Colorado. Limnol. Oceanogr. **37:** 1179-1192.

Mortimer, C.H. 1941. The exchange of dissolved substances between mud and water in lakes (Parts I and II). J. Ecol. **29:** 280-329.

Mortimer, C. 1988. Discoveries and testable hypotheses arising from Coastal Zone Color Scanner imagery of southern Lake Michigan. Limnol. Oceanogr. **33:** 203-226.

Nürnberg, G.K. 1991. Phosphorus from internal sources in the Laurentian Great Lakes, and the concept of threshold external load. J. Great Lakes Res. **17:** 132-140.

Olson, R.J., E.R. Zettler, and M.D. DuRand. 1993. Phytoplankton analysis using flow cytometry, p. 175-186. *In* P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.), Handbook of methods in aquatic microbial ecology, Lewis.

Pettersson, K. 1979. Orthophosphate, alkaline phosphatase activity and algal surplus phosphorus in Lake Erken. Acta **505:** 3-15.

Pettersson, K., B. Bostroem, and O.S. Jacobsen. 1988. Phosphorus in sediments - speciation and analysis. Hydrobiologia **170:** 91-101.

Pomeroy, L.R., J.E. Sheldon, W.M. Sheldon, and F. Peters. 1995. Limits to growth and respiration of bacterioplankton in the Gulf of Mexico. Mar. Ecol. Prog. Ser. **117:** 259-268.

Porter, K.G., and Y.S. Feig. 1980. the use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25:** 943-948.

Redfield, A.C. 1958. The biological control of chemical factors in the environment. Amer. Scientist**:** 205-221.

Rigler, F. 1966. Radiobiological analysis of inorganic phosphorus in lakewater. Verh. Internat. Verein. Limnol. **16:** 465-470.

Scavia, D., and G.A. Laird. 1987. Bacterioplankton in Lake Michigan: Dynamics, controls, and significance to carbon flux. Limnol. Oceanogr. **32:** 1017-1033. (in set)

Schelske, C.J., and E.F. Stoermer. 1972. Phosphorus, silica, and eutrophication of Lake Michigan. Limnol. Oceanogr. **Special Symposium Vol. 1:** 157-171.

Schelske, C., E. Stoermer, G. Fahnenstiel, and M. Haibach. 1986. Phosphorus enrichment, silica utilization, and biogeochemical silica depletion in the Great Lakes. Can. J. Fisheries. Aquat. Sci. **43:** 407-415.

Sharp, J.H., R. Benner, L. Bennett, C.A. Carlson, R. Dow, and S.E. Fitzwater. 1993. Re-evaluation of high temperature combustion and chemical oxidation measurements of dissolved organic carbon in seawater. Limnol. Oceanogr. **38:** 1774-1782.

Sharpley, A.N., M.J. Hedley, E. Sibbesen, A. Hilbricht-Ilkowska, W.A. House, and L. Ryszkowski. 1995. Phosphorus transfers from terrestrial to aquatic ecosystems, p. 171-199. *In* H. Tiessen (ed.), Phosphorus in the global environment: Transfers, cycles and management, V. Vol. SCOPE 54, Wiley.

Solorzano, L., and J.H. Sharp. 1980. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. Limnol. Oceanogr. **25:** 754-758.

Sonzogni, W.C., S.C. Chapra, D.E. Armstrong, and T.J. Logan. 1982. Bioavailability of phosphorus inputs to lakes. J. Environ. Qual. **11:** 555-563.

Stumm, W., and J.J. Morgan. 1981. Aquatic chemistry, 2nd ed. Wiley.

Sugimura, Y., and Y. Suzuki. 1988. A high-temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. Mar. Chem. **24:** 105-131.

Tarapchak, S.J., and R. Moll. 1990. Phosphorus sources for phytoplankton and bacteria in Lake Michigan. J. Plankton Res. **12:** 743-758.

Tarapchak, S., and C. Rubitschun. 1981. Comparisons of soluble reactive phosphorus and orthophosphorus concentrations at an offshore station in southern Lake Michigan. J. Great Lakes Res. **7:** 290-298.

Tarapchak, S.J., S.M. Bigelow, and C. Rubitschum. 1982. Overestimation of orthophosphorus concentrations in surface waters of southern Lake Michigan: effects of acid and ammonium molybdate. Can. J. Fisheries. Aquat. Sci. **39:** 296-304.

Toolan, T., J.D. Wehr, and S. Findlay. 1991. Inorganic phosphorus stimulation of bacterioplankton production in a meso-eutrophic lake. Appl. Environ. Microbiol. **57:** 2074-2078.

USEPA. 1974. Methods for chemical analysis of water and wastes, V. 625-/6-74-003a. EPA.

Vadstein, O., Y. Olsen, H. Reinertsen, and A. Jensen. 1993. The Role of Planktonic Bacteria in Phosphorus Cycling in Lakes - Sink and Link. Limnol. Oceanogr. **38:** 1539-1544.

Viollier, E., D. Jezequel, G. Michard, M. Pepe, G. Sarazin, and P. Alberic. 1995. Geochemical study of a crater lake (Pavin Lake France): Trace-element behaviour in the monimolimnion. Chem. Geol. **125:** 61-72.

Westall, J., J.L. Zachary, and F.M. Morel. MINEQL, a computer program for the calculation of chemical equilibrium composition of aqueous systems. (MIT Dept. Civil Engineering Technical Note 18; 91pp).

Wetzel, R.G. 1983. Limnology, 2nd ed. Saunders College Publishing.

Williams, J., H. Shear, and R. Thomas. 1980. Availability to *Scenedesmus quadricauda* of different forms of phosphorus in sedimentary materials from the Great Lakes. Limnol. Oceanogr. **25:** 1-11.

Zweifel, U.L., B. Norrman, and A. Hagstrom. 1993. Consumption of Dissolved Organic Carbon by Marine Bacteria and Demand for Inorganic Nutrients. Mar. Ecol. Prog. Ser. **101:** 23-32.

Budget Justification

Salaries. Cotner will be responsible for coordination of all activities. In addition, he will be responsible for alkaline phosphatase measurements, P regeneration measurements, bacterial productivity, and biomass determinations, DOC and plankton P-uptake experiments. We have asked for 10% time support for Cotner. A post-doctoral position (ca. $25-30 K) has been requested to assist in these measurements. After completion of the field components, the post-doctoral fellow will assist in analyzing data and writing publications.

Travel. We have requested money for travel to Lake Michigan from College Station for the P.I. and the post-doctoral fellow. This money will be used for one trip from College Station to Lake Michigan in the first year and 3 trips in the following two years (2 persons x 3 trips per year). Any money not used for travel to Lake Michigan will be used for travel to professional meetings.

Equipment. We have requested money ($9,000) for a water quality probe to be set-up and used for underway measurements and modifications ($3,000) to a Turner Designs 10-AU fluorometer. The fluorometer will be modified by adding GPS capability and a datalogger. This will enable spatially explicit alkaline phosphatase measurements.

Materials and supplies. We have requested $6,000 for supplies each of the first three years and $3,000 for subsequent years. This money will be used to purchase expendable supplies for alkaline phosphatase, P regeneration measurements, bacterial productivity measurements and uptake experiments, including radioisotopes, scintillation cocktail, filters, and reagent chemicals. This money will also be used to purchase time on the flow cytometer.

Subcontracts. We have requested $79,553 (year 1), $60,308 (year 2), $62,572 (year 3), $63,648 (year 4) and $60,235 for Michigan Technological University (P.I. Noel Urban). Urban will be responsible for particle characterization and abiotic kinetic measurements. In addition, we have requested $59,738 (year 1), $51,156 (year 2), $52,214 (year 3), $49,493 (year 4) and $36,654 (year 5) for CILER/University of Michigan (P.I. Thomas Johengen). Johengen will be responsible for nutrient measurements (both profiles and underway measurements), and bioavailable P measurements.

Other. $900 are requested for publication page charges for the last four years. Funds ($1,200) are requested for the first three years for shipping costs. Fringe benefits are charged as 23% of salaries for P.I.s and 15% of salaries for post-doctoral fellows. Health care costs for post-doctoral fellow assumes coverage of employee and spouse only.

Facilities, equipment and other resources

Laboratory. James Cotner's laboratory is equipped for modern limnological and microbiological studies. His laboratory has a centrifuge (bench models), a laminar flow hood, fume hoods, water baths, pipets, balances, incubators, a spectrophotometer, a gas chromatograph, several filtration manifolds, an autotitrator for Winkler dissolved O_2 or alkalinity determination, several incubators and a total organic carbon analyzer with an autosampler, computers and a epifluorescence microscope and imaging software. His laboratory is fully equipped for field work, including: a Hydrolab CTD water profiler and logger, with probes for dissolved oxygen, pH, turbidity, conductivity and redox; underwater chambers for measuring sediment oxygen demand in the light and the dark, several dissolved oxygen meters and a datalogger; a field fluorometer; and a Licor underwater light meter.

Computer. All P.I.s have access to several PCs (Pentiums) and Macintosh machines. There are also image analysis facilities located in the department's Biosystematics Center.

Major equipment. Also available are a scintillation counter, floor model high-speed centrifuges. In the Department of Wildlife and Fisheries Sciences, we have access to two flow cytometers, numerous computers, centrifuges, autoclaves, and cold rooms.