FINAL REPORT

NUTRIENT REMOVAL BIOASSAY METHODS FOR ASSESSMENT OF THE EFFECTS OF DECREASED NUTRIENT LOADING ON PHYTOPLANKTON COMMUNITIES IN AQUATIC ECOSYSTEMS

Project Report for Research Supported by the Soap and Detergent Association

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Walter K. Dodds
Division of Biology
Kansas State University
Manhattan, KS 66502

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Introduction and Summary

This project was funded to explore techniques for directly estimating phytoplankton response to lowered nutrient loading. Techniques previously used by lake managers have assumed that P limits productivity and utilize regression relationships between P loading and chlorophyll *a* to estimate the effects of lowered nutrient loading. Assuming P is the only limiting nutrient in freshwaters may be misleading; P often limits productivity, but not always. Furthermore, in cases of severe P contamination, internal cycling of P will make efforts to control P loading meaningless. Nutrient addition bioassays can be used to determine what, if any, nutrient limits productivity in a system. They can not be used to quantitatively estimate the effects of lowering nutrients on lakes and are not useful when nutrient levels are extremely high. The research reported here addresses this problem by evaluating techniques to directly test the effects of lowering nutrients in a lake.

Chapter 1 reports a preliminary assessment of the nutrient removal bioassay technique conducted in a hyper-eutrophic farm pond. In this experiment N appeared to be the limiting nutrient until comparisons were made between the treatment carboys and the lake. There was a strong container effect causing a sharp drop in productivity and chlorophyll *a* except when N was added. Addition of N kept levels of productivity up to those seen in the pond. This suggested that cutting the pond water off from internal nutrient loading from the sediments caused a drop in productivity, and that in this system, efforts to control external loading would have little effect without control of internal loading. Nutrient dilution and removal treatments had minor effects on productivity and biomass when compared to control treatments, further enforcing the idea that control of external loading would have minor effects in this system.

Chapter 2 reports on experiments on phosphorus cycling in Milford Reservoir. As part of the main thrust of the proposed research, 1500 l mesocosms were erected and treated with N and P additions. Milford Reservoir is co-limited by N and P; N addition forced P limitation and P addition forced N limitation. These ^{32}P experiments yielded results of interest to investigators of P cycling. Results include: 1) Michaelis-Menten uptake curves may be inadequate to describe maximum uptake rates of phosphate, 2) Chemically determined phosphate overestimates biologically available phosphate more severely as P limitation increases, and 3) Phosphatase activity may not be a good indicator of the severity of P limitation.

Chapter 3 describes a theoretical exploration of why dissolved inorganic nutrient levels resist perturbation in the nutrient addition and dilution

experiments conducted in this study. The main result of general interest from this theoretical treatment of dilution experiments is that inorganic nutrient concentrations do not describe systems well, the total nutrients in a lakewater sample can be almost halved over a week, and there will be little change in the levels of dissolved inorganic nutrients.

Chapter 4 describes the field tests of the bioassays in Milford Reservoir, and describes the largest part of the funded research. Both 10 L carboys and 1500 L mesocosms were used in these experiments. Milford reservoir was limited by both N and P, a result that supports earlier work (Dodds and Priscu) on Flathead Lake. In Milford Reservoir, nutrient removal techniques were tested in conjunction with sewage additions. Algae bloomed when sewage was added, but not when the sewage was first treated with alum to remove P. Removal of ammonium with zeolite had little effect because the bulk of the inorganic nitrogen in the sewage was in the form of nitrate. Dilution of the carboys with 15% per day 1 μm filtered lake water or purified water had small effects on productivity and chlorophyll *a* over a period of 4 days. Dilution with purified water did however decrease the relative abundance of diatoms, suggesting that nutrient control may effect phytoplankton community structure. Zooplankton manipulations in the mesocosms showed little effect of grazing on phytoplankton biomass or productivity, and suggest this is a system controlled by nutrients from the "bottom up".

Each of these chapters will be or is submitted to a peer-reviewed journal for publication. Some of the results presented here were also reported in professional meetings; abstracts are provided in the Appendix.

Acknowledgements

I thank D. Gudder for technical assistance throughout this project. C. Randel, E. Strauss, and C. Edler helped with the work described in Chapter 1. E. Strauss and R. Lehman helped with laboratory and field work described in Chapter 2. R. Lehman, E. Strauss and C. Edler assisted with experiments reported in Chapter 4. Richard Sedlak facilitated this research with his easy-going approach to administering the grant and his tolerance for the quirks of the Office of Research at my home institution.

Chapter 1

PRELIMINARY FIELD ASSESSMENT OF THE EFFECTS OF NUTRIENT REMOVAL ON PHYTOPLANKTON PRODUCTIVITY AND BIOMASS

SUMMARY

Typical nutrient addition bioassays can not be used to quantify the effects of nutrient removal. Nutrient dilution or selective removal of phosphate or ammonium in 10 L containers was employed to assess phytoplankton response to lowered levels of inorganic nutrients in a eutrophic pond. The nutrient removals were compared to controls and nutrient additions. Ammonium addition, with or without phosphate addition, stimulated algal biomass and productivity compared to controls within several days. Phosphate addition alone had no effect. Serial dilution (10% per day over 5 days for a total dilution of 41%) did not lower biomass or productivity compared to controls. A significant ($P < 0.05$) decrease in productivity, relative to the pond, was seen in all mesocosms except in those with ammonium enrichments. The data suggest that nutrient removal in this pond would not lower productivity, presumably because loading of nutrients from groundwater or sediments is sufficient for algal growth. *In situ* experiments may thus help determine the effects of nutrient removal before implementation of costly nutrient control measures.

INTRODUCTION

A frequently used approach for controlling eutrophication in lakes is to calculate the effects of reduced phosphorus loading using a series of loading equations (e.g. Ryding and Rast 1989). In the presence of data confirming phosphorus limitation this is a good approach. However, it has become increasingly evident that factors other than, or in addition to phosphorus can control productivity in many lakes (Carpenter and Kitchell 1988; Suttle and Harrison 1988; Dodds et al. 1989; Dodds and Priscu 1990; Elser et al. 1990). In the absence of data, it may be unwise to assume that controlling phosphorus loading will alleviate algal bloom problems.

Nutrient enrichment bioassays have been used by a number of investigators to determine nutrient deficiencies (e.g. Hecky and Kilham 1988; Dodds et al. 1989; Elser et al. 1990). This approach is useful, except in situations with extremely high nutrient loads (no apparent deficiency) or where it is necessary to assess the quantitative effects of nutrient removals. In many eutrophic systems, a nutrient removal approach may prove more fruitful.

Paerl and Bowles (1987) introduced the concept of nutrient dilution bioassays to assess effects of lowered nutrient loading in the Neuse River, North Carolina. They successfully applied a technique of dilution with reverse osmosis water and re-fertilization with a major ion solution and specific

nutrients to quantitatively assess the effects of nutrient removals. Their approach is promising and merits more intensive study on its applicability to other systems.

In addition to total nutrient dilution, there are also chemical treatments which remove specific nutrients from solution. Alum addition has been used for phosphorus removal in lakes (Ryding and Rast 1989) and treated sewage (De Renzo 1978). Zeolite ion sieves can be used to remove ammonium from sewage (De Renzo 1978) and lake waters (Angelo 1989). These techniques of selective nutrient removal are potentially valuable for determining the effects of nutrient removal for *in situ* bioassays.

The purpose of this paper is to explore short-term response of phytoplankton in a eutrophic pond to addition of filtered lake water treated with zeolite or alum, or dilution with purified water. The techniques are evaluated in terms of their potential for generating information on nutrient removal from lakes.

MATERIALS AND METHODS

The experiments were performed at Kimball Pond, Riley County, Kansas. This is a small (surface area = 0.24 ha, mean depth = 1.52 m) eutrophic (Secchi depth 10 cm, chlorophyll *a* $\approx$ 0.8 mg L^{-1}) cattle pond on the Kansas State University campus. During the study, the pond was dominated by chlorophytes; within 1 month after the study a large bloom of *Anabaena* dominated. Pond water was collected for chemical treatments on 28 April 1991 and the experiment was initiated on 29 April 1991. Ten-L translucent polyethylene carboys were used for experimental treatments and were incubated in the pond. Each carboy was rinsed with 0.1 M HCl, rinsed 6 times with reverse osmosis water and then flushed with pond water immediately before use.

Triplicate carboys were used for each treatment. Treatments to pond water filled carboys were: control (no additions), NH_4Cl, KH_2PO_4, NH_4Cl + KH_2PO_4, + 1 μm filtered pond water, + 1 μm filtered pond water treated with zeolite, + 1μm filtered pond water treated with alum, and + reverse osmosis water. Ammonium was initially added at a concentration of 16 μM; two days later carboys with ammonium addition alone were fertilized with an addition of 160 μM. Ammonium to 160 μM was added to the N+P treatments three days after the experiment began. Phosphate was initially increased by 1 μM and then two days after initiation of the experiment the concentration was increased by 20 μM in both +P and N+P carboys.

Filtered pond water or reverse osmosis water were used to dilute the contents of all dilution treatments by 10% per day. Pond water for nutrient

dilution treatments and controls was filtered through a 1 μm filter 6 h prior to beginning the *in situ* incubations. XLH Bag Filters (Parker Hannifin Corporation, Lebanon IN) were used to filter rapidly large volumes of pond water with high loads of suspended solids. Removal characteristics are presented in Table 1. These bags meet United States FDA regulations for food grade materials and the pond water filtrate from them did not appear to harm algae. The bags are constructed for use in filter housings under vacuum, but only gravity flow and no housing were used for these experiments. The bags were ideal for filtration of large volumes of water; Kimball Pond water filtration rates were 120 L h^{-1} per bag.

Zeolite (Linde Ionsiv W-85, UOP Molecular Sieves, Moorestown, NJ) was used to extract ammonium from pond water. Water was filtered through a 1 μm XLH filter bag and zeolite was added at 3 g L^{-1}, mixed well, settled for 30 min and filtered through a clean 1 μm bag filter to remove zeolite. This treatment resulted in 72% removal of NH_4^+ from the pond water and minimal changes in pH, conductivity, soluble reactive phosphorus (SRP) and nitrate (Table 2).

Alum was used to remove SRP from solution. Alum was added to 1 μm filtered lake water at 1.5 g L^{-1}, pH was lowered to 6.0 with 1M HCl to optimize phosphate precipitation (De Renzo 1978), the precipitate was filtered out with a 1 μm bag filter, and the pH raised to its original level with 1 M NaOH. This treatment lowered SRP by 27%, increased conductivity by 92% and had little effect on NH_4^+, NO_3^- and final pH.

Carboys were filled with water collected from the surface (top 0.25 m) of the pond. Water was collected in 20 L buckets and mixed in a 200 L trash container lined with a polyethylene bag before being dispersed into individual carboys. The large mixing container was used to increase homogeneity in the individual carboys. The trash container was lined with a pre-rinsed plastic bag because new trash containers can contain chemicals that could inhibit or kill phytoplankton.

After filling and chemical treatment, the carboys were attached every 46 cm to a wood rack and weighted with 50 g weights. A small amount of air was left in each carboy so it would float just below the surface. In more oligotrophic systems, less air and a longer tie cord should be used to allow the carboy to sink to a depth where photoinhibition is not problematic.

One liter of water was removed from each carboy daily at 1000 h for analysis. One liter of filtered, treated pond water or reverse osmosis water was added back to each nutrient removal carboy at this time. Samples were returned to the laboratory, and within one hour aliquots were removed for photosynthesis measurements. Aliquots were placed in two, 60 mL borosilicate

BOD bottles from each carboy; one bottle was placed in the dark and one under cool white fluorescent lights (150 μmol quanta m^{-2} s^{-1}, equivalent to light at about 0.1 m in the pond under full sunlight) for 2 h at 25 °C. Oxygen was measured by titration using the azide modification of the iodometric method of APHA (1989). Reduced amounts of the manganous sulfate solution (0.2 mL), the alkali-iodide-azide solution (0.2 mL), and concentrated H_2SO_4 (0.4 mL) were used to accommodate the 60 mL sample sizes; these reagents were prepared at standard concentrations. The sodium thiosulfate titrant was diluted from 25 μM to 6.25 μM to attain adequate precision when titrating the smaller samples.

Additional aliquots were removed for water chemistry analysis. Samples for ammonium, nitrate, and soluble reactive phosphorus (SRP) were filtered through Whatman GF/C filters. Ammonium and SRP were analyzed immediately by the phenol hypochlorite (Solorzano 1969) and the acid molybdate (Strickland and Parsons 1972) methods respectively. Nitrate samples were frozen for later analysis by the cadmium reduction method (Eppley 1978). Ten mL of sample were filtered onto Whatman GF/C filters and frozen for later analysis of chlorophyll *a*. Chl *a* was analyzed spectrophotometrically by a bi-chromatic method with correction for phaeophytin (APHA 1989).

RESULTS

Following nutrient addition, both ammonium (Fig. 1A) and SRP (Fig. 2A) rapidly disappeared from solution. Ammonium (Fig. 1A, B) and SRP concentrations (Fig. 2A, B) in controls also decreased significantly during the experiment (linear regression $P < 0.025$ for ammonium, $P < 0.005$ for SRP). The slope of the decline in ammonium and SRP in the reverse osmosis diluted treatments was not significantly different from the control carboys ($P > 0.05$). The largest SRP decline occurred when ammonium was added concurrently (Fig. 2A), a significantly greater decline than in the control carboys ($P < 0.005$). There were no trends in nitrate concentration with any treatment and levels remained at ca. 2μM (data not shown).

Photosynthesis was stimulated by ammonium addition in the presence or absence of phosphate after 5 days (Fig. 3A) relative to controls ($P < 0.05$, Analysis of Variance, means compared by Tukey's studentized range test) and only when ammonium was added did photosynthetic rate approximate that of the pond after 5 days. The N+P treatment did not respond as rapidly as when N was added, perhaps because the large ammonium addition occurred 1 day later in the N+P than in the N treatments (Fig. 1). Nutrient dilution caused an initial decrease in photosynthetic rates but this was not significant by day 5

(Fig. 3B). The decrease was probably related to dilution of phytoplankton biomass over the course of the experiment (Fig. 4). Chl *a* increased significantly over controls after 5 d in the ammonium treatments ($P < 0.05$, Analysis of Variance, means compared by Tukey's studentized range test). There were no significant differences in chl *a* level after day 5 among the various nutrient dilution treatments and control.

DISCUSSION

That SRP decreased the most rapidly with ammonium addition suggests nitrogen deficiency. With the relaxation of nitrogen deficiency in the carboys when ammonium was added, cells were able to use more phosphate, resulting in an increased rate of lowering of SRP. That N additions only allowed chlorophyll to attain levels found outside of containers suggests nitrogen deficiency in this system is secondary. Possibly, light limited production in the natural pond.

There were small (not significant) differences in productivity between the controls, filtered lake water, reverse osmosis water, zeolite, and alum treatments. This suggests that removal of dissolved nutrients (reverse osmosis, alum and zeolite) had little effect. Even though alum or zeolite may have removed ions other than phosphate and ammonium respectively, there was still little effect on photosynthesis.

Nitrogen loading was probably sufficient for phytoplankton nitrogen requirements in this pond at the time of the study. Ammonium additions only allowed photosynthesis to remain equal to rates in the pond and ammonium concentrations progressively decreased in the enclosures. Since there were no streams flowing into the pond at the time of the experiment and little precipitation, it is possible that the ammonium was supplied by internal loading from the sediments or groundwater. It is likely that little surplus nitrogen was stored in phytoplankton cells because biomass began to decline soon after they were placed in the containers.

The lowering of algal productivity caused by isolating the phytoplankton in containers (and presumably from sediment nutrient regeneration) was a much stronger effect than that caused by nutrient dilution. There was little effect of nutrient dilution (compare filtered lake water to reverse osmosis dilutions), even when 40% of the nutrients were diluted out with reverse osmosis water. This suggests by the criteria of Paerl and Bowles (1987) that this pond was in an extremely hypereutrophic condition.

Nitrogen deficiency and concomitant internal loading of ammonium from sediments supplying nutrients to the phytoplankton would have several

implications for a manager trying to control algal blooms in this pond: 1) control of external nutrient loading may have little effect on the algal population during a single growing season (assuming it is valid to extrapolate from the 5 d experiments) and, 2) control of internal phosphorus loading would probably be ineffective unless levels were so drastically reduced that P limitation was forced and internal loading was stopped.

This study serves as an example of employing nutrient removal and enrichment bioassays to assess controlling nutrient loading in natural eutrophic waters. The techniques presented here utilize standard water quality procedures (inorganic N and P assays, O_2 determinations, chl *a* determination) and are relatively inexpensive. Although the *in situ* bioassay technique requires hours of work, it furnishes an idea of the response of the phytoplankton community to changes in nutrient regime and is probably much more reliable than short-term physiological methods (Hecky and Kilham 1988; Dodds and Priscu 1990; Dodds and Priscu 1991). Given the relative ease of performing bioassays such as those reported here, it may be useful to employ similar techniques (nutrient additions and nutrient removal with dilution by filtered lake water and reverse osmosis water) in other lakes where data on nutrient limitation are unavailable, before initiating costly nutrient control programs. Further studies on the utility of such bioassays will help determine their usefulness.

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Table 1. Characteristics of Kimball Pond water treatments with filter bags under different chemical treatments. 2X = filtered 2 times through the 1 μm filter. Other treatments were first filtered, then treated with zeolite or alum, and filtered again. Values are the mean of 3 replicates, 1 standard deviation in parenthesis. Bacteria were counted with a viable direct count technique (Paul 1982).

FACTOR	POND	2x	ZEOLITE	ALUM
Chl *a* (mg L^{-1})	4.54(1.62)	0.31(0.20)	0.27(0.00)	0.09(0.15)
Bacteria (x10^6 mL^{-1})	1.52(0.43)	0.31(0.14)	0.31(0.12)	0.29(0.11)
Total N (μM)	284(59)	128(9)	118(14)	129(12)
Total P (μM)	47.8(1.5)	25.0(2.4)	24.5(1.4)	21.4(1.3)

Table 2. Chemical effects of alum and zeolite treatment on Kimball Pond water. See text for treatment methods. Values represent the mean of 3 determinations. Standard deviations are given in parentheses.

FACTOR	2x	ZEOLITE	ALUM
NH_4^+ (μM)	5.17(0.23)	1.43(0.06)	5.33(0.26)
SRP (μM)	24.9(0.6)	26.6(0.5)	18.2(0.6)
NO_3^- (μM)	2.02(0.01)	2.38(0.15)	2.08(0.03)
pH	7.73	7.73	7.65
Conductivity (mS)	0.24(0.01)	0.26(0.01)	0.46(0.01)

Figure 1. Ammonium concentrations in nutrient enrichment (A) and removal bioassays (B) with water from Kimball pond. Error bars = 1 std. dev. +N = +160 μM NH_4^+Cl, +P = 10 μM KH_2PO_4, Filt lake = 10% dilution with filtered lake water per day. Alum and zeo represent alum and zeolite treated lake water dilution, and RO = reverse osmosis water at 10% per day.

Figure 2. Soluble reactive phosphorus in nutrient enrichment (A) and removal (B) bioassays with water from Kimball pond. Error bars = 1 std. dev. See Fig. 1 legend for abbreviations.

Figure 3. Photosynthetic O_2 production (mg 0_2 L^{-1} h^{-1}) in nutrient enrichment (A) and removal (B) bioassays. Error bars = 1 std. dev. See Fig. 1 legend for abbreviations.

Figure 4. Chl *a* concentration in nutrient enrichment and removal bioassays at 1 (A) and 5 (B) days after treatment initiation. Error bars = 1 std dev. See Fig. 1 legend for abbreviations.

Figure 1

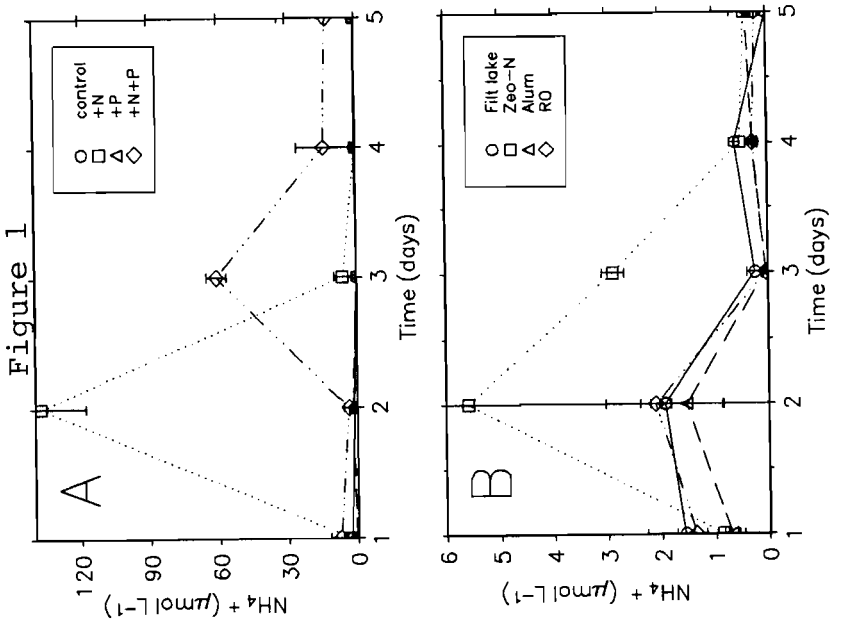
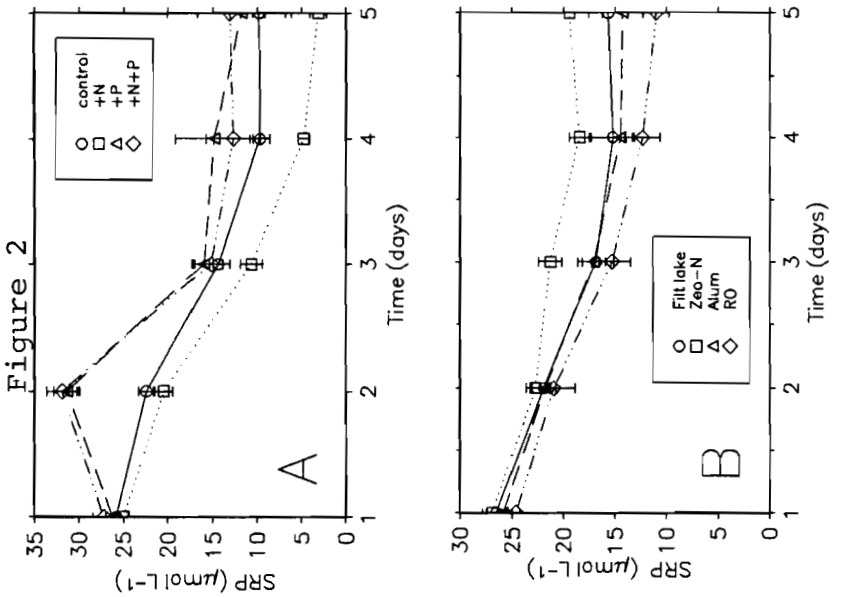


Figure 2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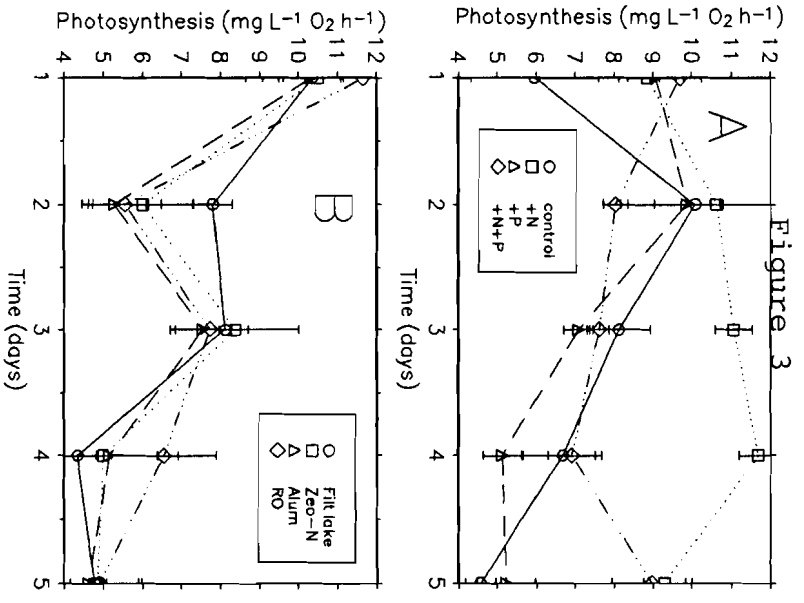
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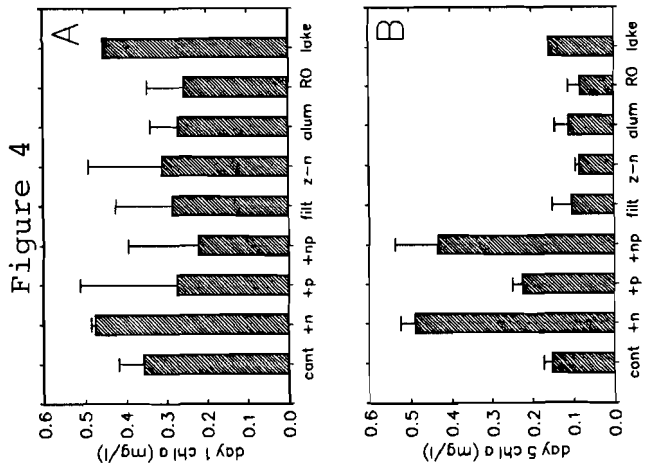
Figure 3

Figure 4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Chapter 2

PHOSPHATE AVAILABILITY, UPTAKE AND REGENERATION IN NITROGEN AND PHOSPHORUS DEPLETE MESOCOSMS

ABSTRACT

Mesocosms (1570 L) in a eutrophic reservoir were treated with NH_4Cl, KH_2PO_4 or nothing (control) and sampled after 8 days for $^{32}PO_4^{3-}$ and phosphatase experiments. Bioassays suggested P deficiency in an ammonium and control mesocosm and no P deficiency with phosphate additions. Biologically available P (BAP) and the ratio of BAP to soluble reactive P decreased as P deficiency increased. Uptake as a function of phosphate concentration exhibited log-log relationships in control and N treatments and was independent of phosphate concentration with P treatment. Particle associated phosphatase activity was greatest in the 0.2-3 μm size fraction in all treatments. Results indicate: 1) Calculating uptake with soluble reactive phosphorus concentrations may overestimate true uptake, with more error as phosphorus deficiency increases, 2) Michaelis-Menten uptake models may be inadequate to describe the relationships between phosphate concentration and uptake by mixed assemblages in some systems, and 3) Organisms < 3 μm may dominate nutrient cycling, independent of deficiency.

INTRODUCTION

Phosphorus is an extremely important element controlling trophic status in lakes (Schindler 1977). P and N dynamics may be coupled in oligotrophic lakes (Dodds and Priscu 1990a), but the relationships between the degree of P deficiency and P dynamics are not well delineated. The recent verification of balances between N and P deficiency in the phytoplankton communities of many natural lakes (Suttle and Harrison 1988*a*, Dodds et al. 1989, Elser et al. 1990) also implies linked P and N dynamics.

There are several conceptual issues regarding P dynamics that have been investigated over the years: 1) To what degree does chemical measurement of soluble reactive phosphorus (SRP) overestimate actual concentrations and prevent accurate estimation of ambient phosphate uptake rates with radio-labeled phosphate? 2) Can Michaelis-Menten uptake be employed to accurately model phosphate uptake as a function of concentrations? and 3) Do picoplankton (bacteria-sized organisms) exhibit primary control of phosphate dynamics (uptake and regeneration) in natural waters?

Since early reports by Rigler (1966) it has become clear that the levels of inorganic P available to microorganisms (biologically available P, BAP) can be overestimated by chemical methods of determination (eg. Peters 1977, Lean and White 1983, Tarapchak and Herche 1986, Bentzen and Taylor 1991, Dodds et al. 1991*a*). However, there has not been a methodical determination of how

the overestimation of BAP may vary as a function of P deficiency.

Uptake of phosphate by mixed planktonic assemblages as modeled by Michaelis-Menten kinetics can be used to estimate phosphate uptake as a function of concentration (eg. Bentzen and Taylor 1991). There has been some criticism of this approach because mixed assemblages do not necessarily exhibit Michaelis-Menten response to increased phosphate concentrations (Tarapchak and Herche 1986). It is possible that the utility of Michaelis-Menten models is influenced by the degree of P deficiency.

Bacteria sized organisms dominate phosphate flux to various degrees in different systems. (Currie and Kalff 1984*a*, *b*) suggest that bacteria can be superior competitors for inorganic phosphorus. Tarapchak and Moll (1990) suggest that, in large lakes, bacteria rely primarily upon organic sources of phosphorus whereas algae rely upon phosphate. Regeneration of phosphate has been shown to be dominated by organisms less than 3 μm in diameter in tropical and temperate lakes (Fisher et al. 1988*a*, Dodds et al. 1991*a*) but not in a marine study (Harrison 1983). The relative importance of bacteria sized plankton as competitors for and regenerators of phosphate may be related to P deficiency.

The purpose of the research reported here was to test if P availability, uptake and regeneration were influenced by the degree of P deficiency in a natural phytoplankton community. To assess this, mesocosms (1560 L) were repeatedly dosed with phosphate, ammonium, or left untreated for 8 days to force varied levels of P deficiency. Samples were then removed and ^{32}P and phosphatase experiments performed on the various planktonic assemblages.

MATERIALS AND METHODS

Research was conducted at Milford Lake, a large (km^2), mesotrophic ($\approx$ 30 μg chlorophyll *a* L^{-1}) reservoir in central Kansas, USA. Mesocosms (1560 L, 1 m diameter, open bottom inserted into the sediments) were erected on 5 August 1991, and repeatedly (5, 8 and 12 August) amended with NH_4Cl to 160 μM, or KH_2PO_4 to 10 μM. Samples were collected from NH_4^+, PO_4^{3-} and control mesocosms on 8 August for primary productivity and chlorophyll measurements. Samples were also returned to the laboratory on 12 August and stored at lake temperature (27 °C) in the dark overnight for ^{32}P and phosphatase experiments.

Photosynthetic oxygen production was estimated with a light dark bottle method (as in Chapter 1). Samples for chlorophyll *a* analysis were collected on Whatman GF/F glass fiber filters or on Poretics membrane filters for size fractionation. Phaeophytin corrected chlorophyll *a* was analyzed by a

bichromatic spectrophotometric method for high levels, and fluorometrically for low levels (APHA 1989). Soluble reactive phosphorus was estimated on Whatman GF/F filtered samples with the colorometric molybdo-phosphate method (APHA 1989).

In all radioisotope experiments, 611 Bq mL^{-1} carrier free $^{32}[P]$-H_3PO_4 were added to 10 mL of lake water. Incubations were terminated after 3 mins (except time course) by filtration onto Milipore type HA (0.45 μm retention) filters followed by washing with 1 Ml of 0.2 μm filtered lake water. A time course of uptake using water from the control mesocosm revealed linear uptake for the first 8 min (data not shown), indicating a 3 min incubation time was adequate. Samples of filtrate were taken before filter washing for regeneration experiments. Filters and filtrate were counted on a scintillation spectrometer after addition of 10 mL of Scintiverse BD fluor (Fisher Scientific). Corrections were made for isotope disintegration, quenching and label adsorbed onto the filters.

Biologically available P was estimated using a technique similar to that described by Peters (1977). Organisms in water from the control mesocosm were used to gauge relative phosphate uptake rates. Five mL of lake water and 5 mL of Type I water from a Barnstead four cartridge E-Pure system were added to each incubation vial. To establish a standard curve, PO_4^{3-} was added to final concentrations of 0, 0.05, 0.1, 0.5, 1, 5, 10, or 100 μM in triplicate vials, and ^{32}P uptake was determined. Uptake of ^{32}P was determined in samples containing 5 mL of 0.2 μm filtered lakewater from one of the three mesocosms and 5 mL of lake water. Rates of uptake associated with samples from each mesocosm were compared to those measured for the standard curve to estimate BAP concentration.

Size fractionated, particle-associated acid and alkaline phosphatase activities were estimated by spectrophotometrically measuring the rate of p-nitrophenyl phosphate hydrolysis (Berman et al. 1990). Bacteria were counted using Hoechstt dye and epifluorescent microscopy (Paul 1982).

It should be noted that the experimental set up is pseudo-replicated. Therefore, conventional statistics can not be applied for comparisons between treatments. Standard deviations are reported for subsamples within each treatment. General trends are noted, but results from statistical tests are not presented.

RESULTS

Photosynthesis and chlorophyll *a* were stimulated by addition of N and P together, but not with either alone (Fig. 1). This indicates that both N and P

limited biomass and productivity of the plankton in the control mesocosm and that addition of N forced P limitation and addition of P forced N limitation. Characteristics of the communities in each mesocosm following size fractionation are presented in Table 1. Between 18 and 32% of the chlorophyll was able to pass through a 3 μm filter, and many of the bacteria were either associated with large particles or could pass the 3 μm filter.

The amount of BAP in the control and + N mesocosms was less than in the type I water. This is known because the amount of label incorporated per time was greater with these two lake water additions than with the addition of type I water. Since the assemblages were identical in these incubations, the specific activity of label must have been lower with type I water (Fig. 2). These data show that levels of BAP were as follows: + P > type I water > control > + N.

Because there was a significant amount of BAP in the type I water, the standard curve had to be scaled by this unknown amount. The maximum amount present was estimated by a modification of Rigler's technique (Bentzen and Taylor 1991). Using this estimate, the relationship between label incorporation and BAP concentration was approximately linear when expressed on a log-log scale. Extrapolating from this curve (assuming linearity to lower concentrations) yields estimates of BAP in the control and + N treatments. Although these estimates still give maximum levels, the relationship between absolute levels (+ P > control > + N) still holds.

It is possible to estimate the ratio of SRP to BAP given the data shown in Fig. 2. This ratio decreases with increasing P deficiency (Table 2), implying that with increased phosphate demand, phosphate is utilized preferentially over organic chemically reactive phosphorus compounds.

The relationship between phosphate concentration and uptake rate did not exhibit saturation even when 0.5 mM phosphate was added in control and + N mesocosms. In the + P mesocosm, there was no relationship between added phosphate and uptake rate, suggesting that uptake was saturated (Fig. 3). When the relationship between concentration and uptake in the control and + N samples was fitted with a Michaelis-Menten model with a non-linear technique (Conway et al. 1970), extremely high values for the maximum uptake rates and the half saturation constants were obtained. When viewing the data plotted on a linear scale, the vast majority of points at low (ecologically relevant) concentrations are not discernable (Fig. 3A). When the data are plotted on a log-log scale of uptake versus concentration (Fig. 3B) there is a linear relationship and values at low concentrations can be seen.

Regeneration was calculated according to equation 4 presented by Laws (1984). This equation assumes that phosphate concentration does not change

over the course of the incubation. Levels of estimated BAP from the control, + N and + P mesocosms were used in these calculations. When the rates associated with each size fractionated sample were used to calculate size fraction specific rates of regeneration, negative values were obtained (Table 3). Negative values suggest either the amount of label in solution increased (impossible) or that it was incorrect to assume that BAP was the same in all different size fractions from a single mesocosm. Consequently, it may be impossible to measure relative size fraction specific regeneration rates without independent determinations of BAP for each size fraction. However, significant label dilution was observed in the < 3 μm size fraction.

Membrane bound phosphatase activity was also used as a gauge of size fraction specific capacity for phosphate regeneration (Fig. 4). These data show that the largest portion of acid and alkaline phosphatase activity occurred in the < 3 μm fractions. The highest overall rates were seen in control samples, with rates in + N and + P treatments approximately equal. This suggests that membrane bound phosphatase and phosphorus deficiency are not closely correlated in this system.

DISCUSSION

That algal productivity in Milford Lake is controlled by both N and P simultaneously (Fig. 1) may be considered unusual. However, recent reports suggest that N and P control of productivity is more ubiquitous than previously thought (Suttle and Harrison 1988*b*, Elser et al. 1990, Dodds and Priscu 1990*a*, *b*). The property of algal community co-limitation can be explained by the fact that planktonic communities contain species that have different abilities to compete for nutrients, and that nutrients are not heterogeneous with time or space in many lakes (Dodds et al. 1989). In this study, balance between N and P deficiency was a useful property of the Milford Lake plankton because addition of P increased N deficiency and addition of N increased P deficiency.

As P deficiency increased, the ratio of BAP:SRP dropped. With heavy phosphate addition in the + P treatments, about 80% of the SRP was biologically available. When N was added, a maximum of 3% of the SRP was biologically available. The relationship between P deficiency and BAP overestimation by SRP values suggests that in cases where it may be the most desirable to estimate uptake velocity (i.e. phosphorus deficient lakes where phosphorus is the controlling element), it is most misleading to use SRP values to calculate that uptake. In this study, ambient uptake rates estimated with SRP rather than BAP would be overestimated by 29, 5, and 1.1 times in +N, control and +P mesocosms respectively.

Phosphorus deficient planktonic communities are apparently extremely efficient at stripping phosphate from water. Thus, the absolute uptake rate of phosphate may never be measured for extremely phosphorus deficient waters. To use bioassay techniques as an estimate of BAP, it is necessary to use water with significantly lower levels of BAP than the water being tested. Commercial water purification systems seem unable to lower phosphate to extremely low levels (T. Fisher, University of Maryland, Horn Point Laboratory, Cambridge, MD 21613, pers. comm., Fig. 2). If the water to be tested contains P deficient plankton, water with significantly less BAP may be impossible to obtain. Rigler's (1966) technique remains a viable alternative, but it can only measure maximum levels of BAP.

Michaelis-Menten models of substrate uptake kinetics seemed to fail in this system, although they appear to be the preferred technique for modelling phosphate uptake (eg. Bentzen and Taylor 1991, Dodds et al. 1991*a*, Suttle and Harrison 1988*b*). Previous investigators have explored the possibility that Michaelis-Menten kinetics underestimate uptake at low concentrations (Tarapchak and Herche 1986). In this study, the lack of fit appears at the high end of the relationship; there did not appear to be a level of phosphate that saturated uptake. A similar lack of saturation at high phosphate concentrations has been reported by others (Suttle and Harrison 1988*b*, Bentzen and Taylor 1991, Dodds, unpubl. data).

That phosphorus uptake continued to increase with phosphate levels up to 0.5 mM in the control and +N samples suggests that Michaelis-Menten uptake kinetics do not apply to the mixed assemblages in this study. The luxury consumption of phosphate by phytoplankton may be related to their ability to efficiently consume very high levels of phosphate. In the +P treatment, when cells were presumably saturated with respect to luxury phosphorus consumption, the uptake rate was lower than it was for the other two treatments at the highest concentrations of added phosphate.

Size fractionation of plankton prior to measurement of uptake and regeneration yielded spurious results. Other investigators have used this approach to measure size fraction specific regeneration and did not report negative values (Dodds et al. 1991*a*, Harrison 1983). Some other studies have shown higher rates of apparent regeneration in filtered subsamples than in whole water (Fisher et al. 1988*b*). It is probable that the size fractionation process causes changes in ambient BAP levels. Such changes could occur very rapidly. Previous ^{32}P uptake studies have shown that presence of *Daphnia* in small volume incubations can lower apparent levels of BAP in minutes (Dodds et al. 1991*b*). In this study, ^{32}P turnover times in the control, +N and +P mesocosm samples were 77, 55 and 64 mins respectively, suggesting significant

changes could occur in pool sizes with as little as 15 min between size fractionation and termination of the ^{32}P regeneration determination. If, for example, fractionation causes a decrease in BAP by removing heterotrophic regenerators, the specific activity of the label will be higher and the apparent label dilution rate (and estimated regeneration) will increase. Although the actual rate of regeneration in this hypothetical case would actually be lower than in the whole sample, assuming no change in BAP upon filtration would cause an overestimation of the calculated regeneration rate. The only way to circumvent this problem is to size fractionate, allow the phosphate pool to come to equilibrium and then measure BAP.

The only reliable estimates for regeneration here are for the whole water samples. The rates of regeneration in whole water samples were approximately equal in the N and control mesocosms, and much higher with P addition. Higher rates of regeneration in the P mesocosms may relate to the fact that heterotrophic organisms (i.e. bacteria) increase regeneration when phosphate demand decreases. Even though regeneration rates were higher in the P mesocosms, ^{32}P turnover times in samples from all three treatments were similar. Both pool size and regeneration rates were maximal with P fertilization.

Membrane bound phosphatase activity offers an alternative method to measure regeneration of phosphate via organo-phosphorus compounds. In this study the < 3 μm size fraction was responsible for the majority of the phosphatase activity in all treatments. Unfortunately, this method does not allow for estimation of phosphorus regeneration caused by heterotrophic excretion or leakage. However, it does suggest that bacteria are very important in cleaving organo-phosphorus compounds. Although it is impossible to estimate the exact importance of the < 3 μm fraction from ^{32}P regeneration experiments, the fact that this size fraction exhibited significant isotope dilution is consistent with the high phosphatase activities associated with small size fractions.

It is surprising that increasing the degree of P deficiency had little effect upon the phosphatase activity. This assay is often used as an indicator of phosphorus deficiency (e.g. Vincent 1981). Longer term exposures to phosphorus deplete conditions may be necessary to cause relative decreases in community phosphatase activity. The data presented here suggest phosphatase activity may be a poor indicator of P deficiency. Turnover times of ^{32}P have also been suggested as an indicator of P deficiency (Vincent 1981). The turnover times in this study did not show a relationship to the degree of P deficiency.

It appears that there are some links between N deficiency and P dynamics

in Milford Lake. Some parameters (alkaline phosphatase activity, ^{32}P turnover times) were not affected by relative degrees of P or N deficiency. Other parameters (level of BAP, BAP:SRP ratio, substrate dependent uptake kinetics, ambient uptake and regeneration rates) were influenced by N deficiency. It is obvious that assuming only one nutrient controls system properties (i.e. extending Leibig's Law of the minimum as a theoretical base to explain planktonic nutrient dynamics) is an inappropriate approach. The complex interactions between P and N documented here probably extend to interactions among other nutrients as well.

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Table 1. Chlorophyll and bacterial numbers in size fractionated samples from N, P and control mesocosms. ± 1 standard deviation.

TREATMENT	CHLOROPHYLL *a* ($\mu g\ L^{-1}$)	BACTERIA (# per mL x 10^6)
control		
whole	14.9	7.1 ± 0.30
< 100 μm	10.8	2.75 ± 0.63
< 8 μm	10.16	3.04 ± 0.67
< 3 μm	4.81	1.74 ± 0.81
+ N		
whole	12.3	8.77 ± 3.4
< 100 μm	4.3	4.94 ± 3.21
< 8 μm	3.5	4.75 ± 0.80
< 3 μm	2.6	5.70 ± 0.60
+ P		
whole	18.4	6.40 ± 1.74
< 100 μm	17.3	4.35 ± 1.50
< 8 μm	11.6	7.11 ± 0.47
< 3 μm	3.3	9.51 ±3.13

Table 2. Soluble reactive phosphorus (SRP) and biologically available P (BAP) in control, + N and + P mesocosms. ± std dev.

TREATMENT	SRP (μ)	BAP (μM)	BAP:SRP
Control	0.085±0.001	0.018±0.001	0.21
+ N	0.147±0.108	0.005±0.001	0.03
+ P	42.2±0.6	37.5±6.3	0.89

Table 3. Overall and size-fraction specific regeneration rates for size fractionated samples in control, + P and + N mesocosm samples.

TREATMENT	CALCULATED REGENERATION (nmol L^{-1} min)	SIZE FRACTION SPECIFIC REGENERATION
control		
whole	0.247 ± 0.069	-0.227
< 100 μm	0.494 ± 0.068	0.134
< 8 μm	0.335 ± 0.114	0.081
< 3 μm	0.254 ± 0.113	0.254
+ N		
whole	0.081 ± ?	-0.013
< 100 μm	0.094 ± 0.022	-0.018
< 8 μm	0.112 ± 0.027	0.047
< 3 μm	0.065 ± 0.024	0.065
+ P		
whole	637 ± 205	-295
< 100 μm	932 ± 171	320
< 8 μm	612 ± 115	62
< 3 μm	550 ± 593	550

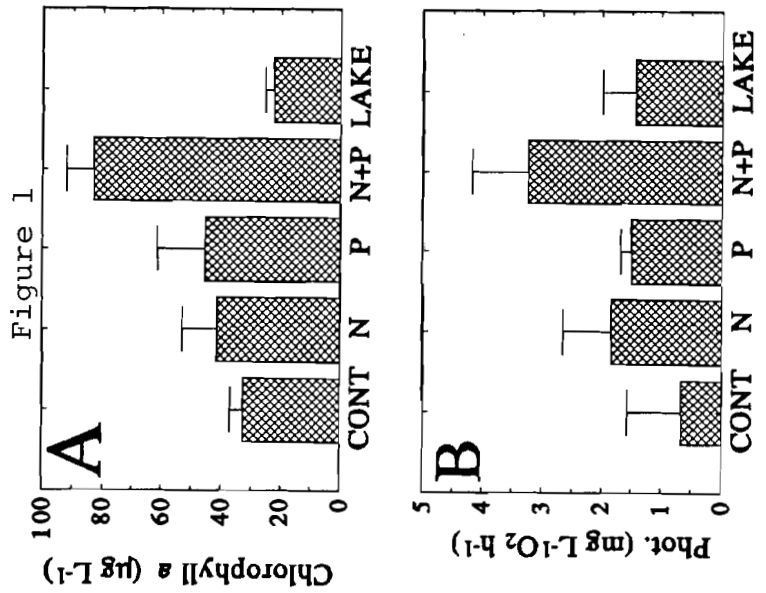
Figure 1. Chlorophyll *a* (A) and O_2 production (B) in control, N, P, N + P mesocosms and in Milford Lake three days after nutrient addition to the mesocosms. Error bars = 1 std dev.

Figure 2. Incorporation of ^{32}P as a function of added concentration using Milford Lake water and type I de-ionized water and label incorporation with addition of 0.2 μm filtered water from control, N and P mesocosms. Line was fit with linear regression.

Figure 3. Relationships between ambient PO_4^{3-} concentration (amount added + estimated biologically available) and uptake plotted as a log-log (A) and a standard (B) relationship. Legend for B is seen in A.

Figure 4. Particle associated alkaline and acid phosphatase activity for 4 size classes of water from control, N and P mesocosms. Absolute rates are given adjacent to the associated section.

Figure 1


A
Chlorophyll a (µg L-1)
100
80
60
40
20
0
CONT
N
P
N+P
LAKE
B
Phot. (mg L-1O2 h-1)
5
4
3
2
1
0
CONT
N
P
N+P
LAKE

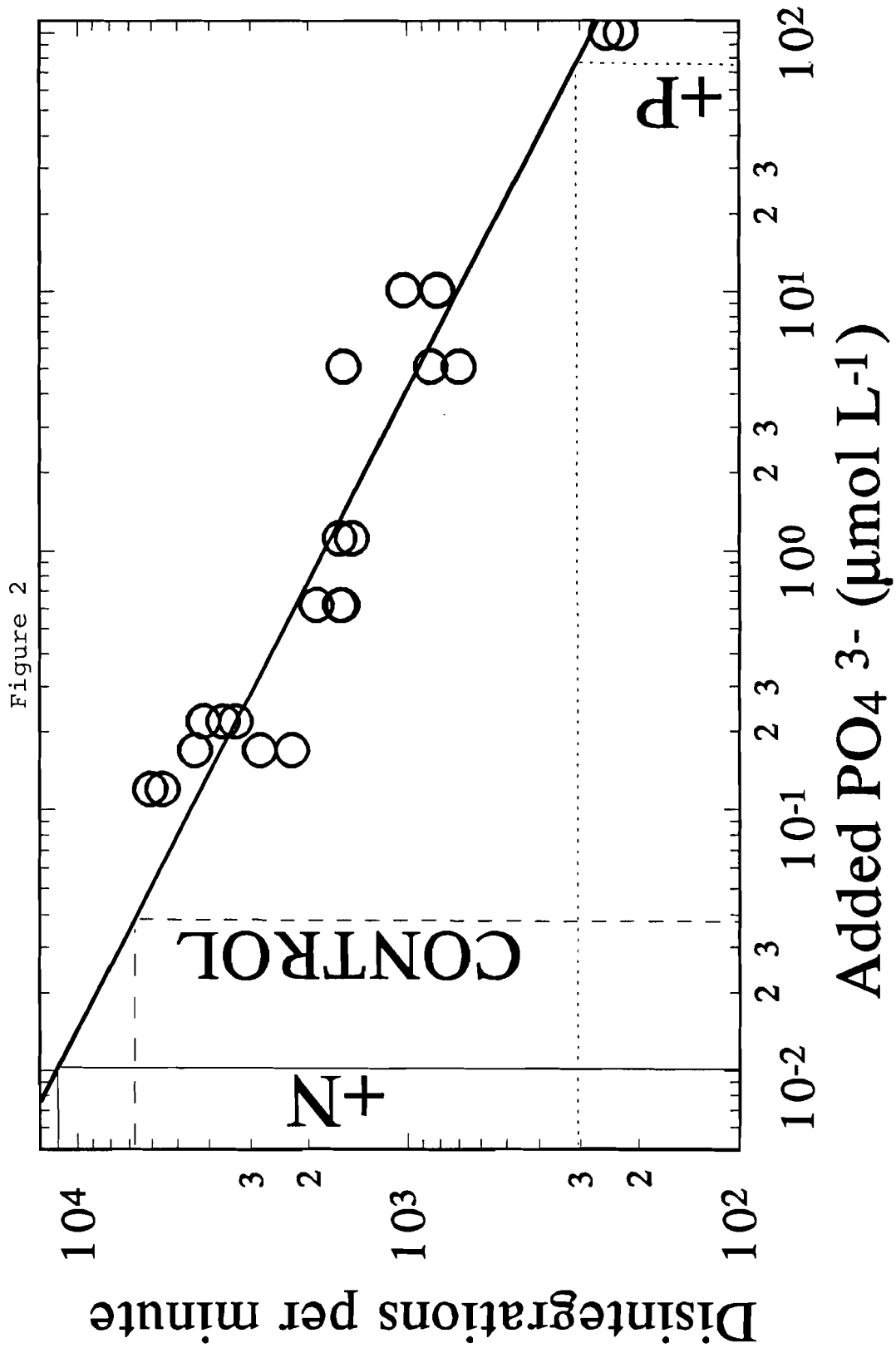

Disintegrations per minute
10⁴
3
2
10³
3
2
10²
+N
CONTROL
+P
10⁻²
2 3
10⁻¹
2 3
10⁰
2 3
10¹
2 3
10²
Added PO4 3- (µmol L-1)

Figure 2

Figure 3

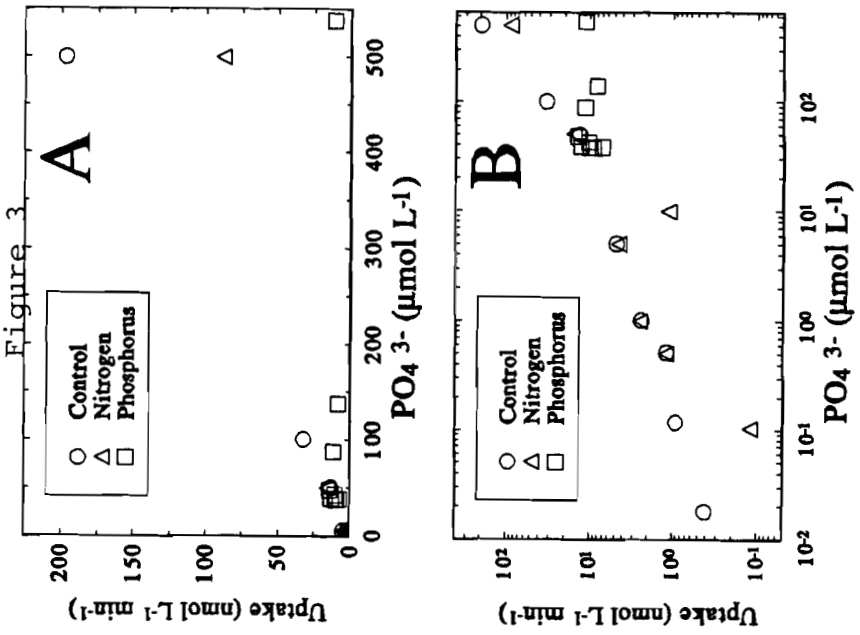
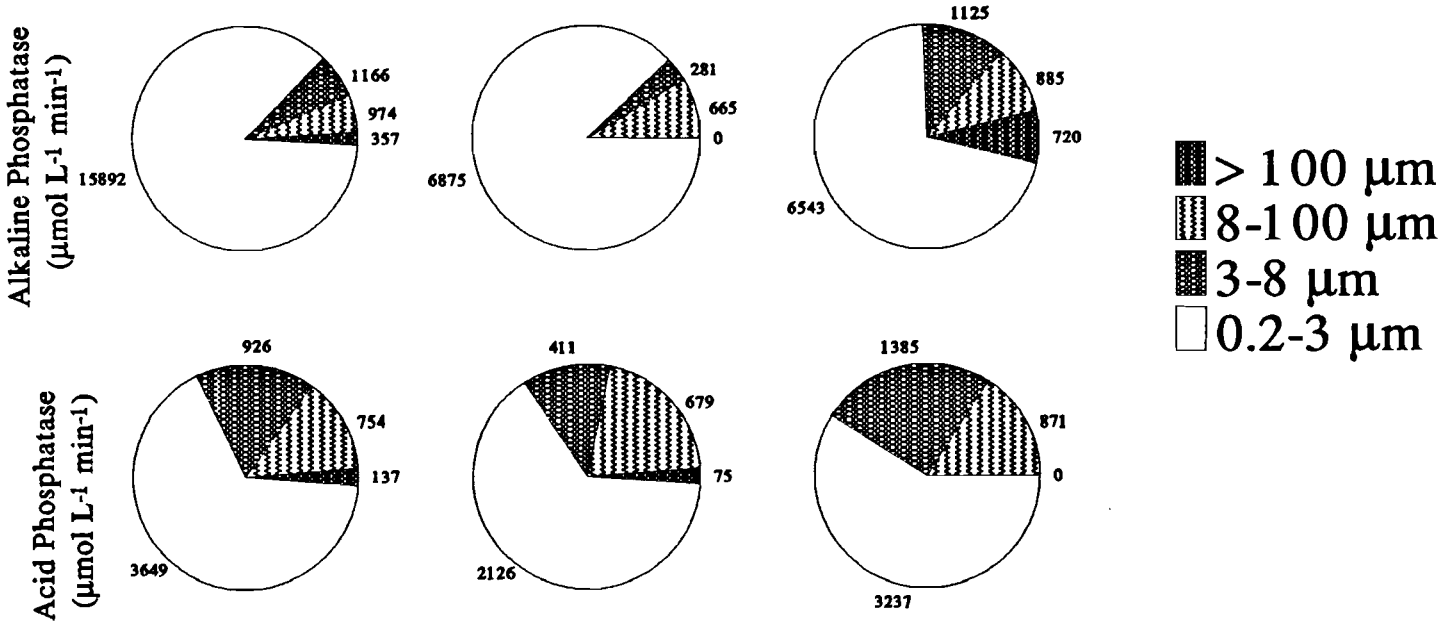

A
B
Control
Nitrogen
Phosphorus
Uptake (nmol L-1 min-1)
PO4 3- (µmol L-1)
0
50
100
150
200
0
100
200
300
400
500
10-2
10-1
100
101
102

Figure 4


Alkaline Phosphatase
(µmol L-1 min-1)
1166
974
357
15892
281
665
0
6875
1125
885
720
6543
Acid Phosphatase
(µmol L-1 min-1)
926
754
137
3649
411
679
75
2126
1385
871
0
3237
> 100 µm
8-100 µm
3-8 µm
0.2-3 µm

Chapter 3

WHAT CONTROLS LEVELS OF DISSOLVED INORGANIC NUTRIENTS IN SURFACE WATERS?

INTRODUCTION

Information on inorganic nutrient levels may be useless without knowledge of the dynamics of nutrient pools. Nutrient concentrations can be controlled by factors including: 1) loading (eg. upwelling, atmospheric input, inflow), 2) losses (eg. sedimentation, transport from the system, efflux of gasses, insect emergence), 3) biotic uptake and adsorption to particles, and 4) regeneration or remineralization either from heterotrophic use of dissolved organic forms or by predation and grazing. Although the maximum level for inorganic nutrients is ultimately controlled by external loading to surface waters, dissolved inorganic nutrient pools can completely turn over in a matter of minutes (Lean and White 1983). Therefore, short-term control over dissolved nutrients is often related to biological uptake and remineralization (e.g. Harrison 1978; Fisher et al. 1988; Glibert et al. 1988; Dodds et al. 1991).

Seasonally, dissolved nutrient concentrations in the euphotic zone of lakes, oceans or streams can vary over several orders of magnitude. Over days, however, dissolved nutrient concentrations often remain fairly constant. This observation, among others, has led investigators to hypothesize that surface plankton communities occur in a well mixed steady-state (Dodds et al. 1989). Temporal and spatial small-scale nutrient patchiness may violate this assumption of equilibrium and at small scales nutrient concentrations may fluctuate wildly (McCarthy and Goldman 1979; Shanks and Trent 1979; Lehman and Scavia 1982). Nevertheless, at the spatial and temporal scales of observation commonly employed by oceanographers and limnologists (i.e. samples about 1 L, filtered and mixed before analysis), levels of dissolved nutrients often show little day to day variation. Given 1) the perturbations associated with small scale nutrient patches and large scale mixing events, 2) the large proportions of nutrients that are often tied up in particulate pools, and 3) the fact that dissolved inorganic nutrient pools can turn over rapidly, one might ask: Why don't dissolved nutrient concentrations fluctuate wildly over a few days or minutes? The answer to this question in many systems is probably related to interactions between biotic uptake and regeneration.

MODEL AND METHODS

I have constructed a model of short-term biological control over dissolved nutrient concentrations which assumes no external nutrient sources or sinks and increasing uptake with substrate concentration. The model assumes small changes in physiological parameters defining substrate dependent uptake kinetics and regeneration (changes from growth, alteration of community structure etc.

are not considered) and thus only applies to short-term (mins to days) variations in substrate concentrations.

Nutrient uptake is modeled by Michaelis-Menten kinetics, a common approach (eg. Glibert and McCarthy 1984; Suttle and Harrison 1988; Bentzen and Taylor 1991). Even if the Michaelis-Menten formalism does not hold because of myriad enzyme systems represented in natural assemblages (Lean and White 1983; Li 1983; Tarapchak and Herche 1986), Michaelis-Menten kinetics approximates the positive relationship between substrate concentration and uptake velocity.

The relationship between dS/dt and substrate concentration [S] in my model is:

$$\frac{dS}{dt} = R - \frac{\rho_{max}[S]}{K_s + [S]} \qquad (1)$$

where ρ_{max} = maximum uptake rate (units in [S] · time^{-1}), K_s is the half saturation constant for uptake, and R = regeneration rate (units in [S] · time^{-1}). The model is represented graphically in Fig. 1A. If $R < \rho_{max}$, then we can solve for [S] at steady state where dS/dt = 0:

$$[S] = \frac{R\,K_s}{\rho_{max} - R} \qquad (2)$$

This solution for Equation 2 occurs where the curve represented as the sum of the dS/dt values for regeneration and uptake crosses 0; this curve is positive at low [S] and negative at high [S]. If the nutrient concentration is perturbed away from the steady state (where the summed line crosses zero), it will tend to return toward that point. When [S] is increased above the steady state value, uptake will exceed regeneration and cause [S] to return to the steady state value. When [S] is below the steady state value, regeneration exceeds uptake. The steady state exists as long as regeneration does not exceed the maximum uptake rate for the nutrient. An alternative version of the model where regeneration is positively related to [S] and exceeds maximum uptake above some critical value of [S] is represented in Fig. 1B. In this case there is still an equilibrium point at low [S].

The model assumes Michaelis-Menten uptake and constant regeneration rates for purposes of analysis, but similar dynamics occur given several basic criteria: 1) when [S] = 0, uptake < R and R > 0, 2) uptake increases with [S] (the exact form of the function is not important), 3) R < the maximum value for uptake over the range of [S] considered, and 4) R is not a decreasing

function of [S]. These generalizations make the model more robust and the basic feature of a steady-state value of [S] remains.

An interesting feature of this model (Fig. 1A) is that dilution of the entire assemblage with low nutrient water has a small effect on the equilibrium nutrient concentration. For example, with 50% dilution there is a 50% decrease in ρ_{max} because half of the organisms are diluted out. R will also be diluted by about 50%; remineralization may decrease slightly more than 50% because food density (eg. prey for grazers (Anderson et al. 1991), dissolved organics for bacteria) will decrease. However, K_s does not change, and the point where the sum of the dS/dt values crosses zero does not change appreciably. This can be validated by inspecting Eq 2. Therefore, even though [S] is diluted by 50% it should still return to approximately the previous equilibrium [S].

Data from field nutrient dilution experiments substantiate model predictions. These experiments were conducted in 10 L polyethylene containers (80% T) in July 1991 using natural phytoplankton assemblages from mesotrophic (summer epilimnetic chlorophyll $a \approx 20$ μg L^{-1}) Milford Lake, KS. The containers were filled with water from 2 m depth with a displacement sampler (Dodds and Priscu 1988). Triplicate treatments included control, 10 μM PO_4^{3-} + 160 μM NH_4^+ added at day 0, 1 μm filtered secondary sewage effluent added at day 0, 20% dilution per day with 1 μm filtered lake water, or 20% dilution per day with reverse osmosis water. A 20% dilution per day with reverse osmosis water results in a 67% dilution of total nutrients after 5 days. Containers were incubated in the lake at 1.5 m and samples were collected daily, filtered through Whatman GF/F filters and soluble reactive phosphorus (SRP) was determined colormetrically (APHA 1989).

RESULTS AND DISCUSSION

Sewage and N+P additions initially increased the size of the SRP pool substantially but SRP approached those of the lake and control containers by day 5 (Fig. 2A). By day 5 there were no significant differences between N+P treatment and lake or control containers, but the SRP in the sewage additions was significantly higher than the control containers ($P < 0.05$, Analysis of Variance, Scheffe's multiple comparison procedure). Biomass in sewage and N+P treatments exceeded that in control and lake treatments (data not shown), so the added phosphate was taken up into biomass.

Even with 67% dilution, the SRP pool did not decrease significantly relative to control over 5 days. This was true whether dilution was accomplished with 1 μm filtered lake water or reverse osmosis water (Fig. 2B).

The control containers exhibited significantly lower levels of SRP than the surrounding lake (*in situ*), the reasons for this effect are not clear, possibly because of grazer removal or adsorption onto container walls. In any case, the SRP results support the idea that after fertilization or dilution perturbations there is a tendency for the SRP pool to return toward a steady state level (i. e. the plankton maintain SRP at an approximate steady-state).

How likely is it that this simple model applies to real systems other than Milford Lake? If uptake and regeneration keep dissolved inorganic nutrient concentrations in steady-state, then ambient uptake and regeneration should approximately balance. This appears to be the case for concurrently measured ammonium and phosphorus uptake (incorporation of label into particulates) and regeneration (isotope dilution) rates from a variety of freshwater and marine systems (Fig. 3). Within a study, uptake and regeneration do not always exactly balance, but across systems there is an approximate balance. Even though log-log plots often give linear relationships, they do not necessarily provide 1:1 relationships. Regression analysis of the data in Figure 3 shows the lines do not depart significantly from a slope of 1. Many of the cases of approximate imbalance of uptake and regeneration may be ascribed to technical problems with measurements (Laws 1984; Glibert et al. 1985).

Another way to verify the model is to use Eq 2 to calculate [S] given measured values for K_s, ρ_{max} and R, and compare the calculated and observed values of [S]. Values for maximum possible biologically available phosphorus from Flathead Lake agree with the calculated [S] within an order of magnitude (Table 1). These values for biologically available phosphorus were determined with Rigler's bioassay, currently the only method available to get estimates of maximum phosphate biologically available concentrations (Dodds et al. 1991). Furthermore, it is encouraging that measured [S] values were reported as maxima, and calculated [S] never exceeded these values.

Similar calculations with ammonium data from Flathead Lake (Dodds et al. 1991) and Lake Fryxell (Priscu et al. 1989) were impossible because in both cases R exceeded ρ_{max}. In these experiments, R was estimated at high ammonium concentrations and the assumption that R is not related to [S] may not have been true; the cases may be similar to that represented in Fig. 1B. Regeneration rates for ammonium measured with low added concentration of ammonium are available from the mouth of Chesapeake Bay (Glibert et al. 1982). These data coupled with roughly contemporaneous uptake kinetic data (Wheeler et al. 1982) allow comparison of observed and calculated values of $[NH_4^+]$. Given the uncertainty involved with analysis of low level ^{15}N addition experiments and determination of K_s, the observed and calculated $[NH_4^+]$ are considered approximately equal (Table 1) lending further support to the model.

The model certainly does not hold in all situations. In systems where catastrophic collapse of phytoplankton communities occurs, very high rates of regeneration may result over hours or days, uptake will drop and [S] increase greatly. In systems where external nutrient input greatly exceeds uptake and regeneration rates (eg. storm flows in streams, fall turnover in lakes, large marine storms, upwelling zones), biotic processes should have little if any effect, and variability in nutrient levels will be forced by abiotic factors.

Increases or decreases in external loading may cause long-term (weeks or months) alteration of nutrient concentrations. Many factors may govern such long-term changes. If there is a time lag between uptake response and nutrient regeneration, regeneration rates will eventually respond to variations in [S]. With a time lag, and with fluctuations in loading that are less than ρ_{max} or R, changes in [S] should occur slowly. Variable grazer abundance and efficiency, rates of algal growth, amount of nutrient deficiency, competition for nutrients between algae and bacteria, and many other factors may also be related to long-term changes in [S]. Over the long-term, fluctuations in loading can result in seston nutrient content changes (Healey 1975; Laws and Redalje 1979) or dissolved nutrient concentrations (Laws and Redalje 1982). My model does not address long-term situations.

Short-term perturbations of nutrient concentrations are common. Dilution with nutrient poor waters, eddy mixing of nutrient rich waters, excreted patches of dissolved nutrients, and depletion zones near actively growing algal colonies or zooplankton (Dodds et al. 1991) may all continuously perturb local concentrations. According to my model the balance between biotic uptake and regeneration and a reserve uptake capacity for nutrients may attenuate such perturbations and maintain relatively constant nutrient concentrations. Because of the balance between uptake and regeneration, the absolute level of dissolved nutrients reveals little. That lakewater can be diluted by 67% over 5 days and still exhibit SRP concentrations similar to those in undiluted treatments underscores the fact that dissolved nutrient concentrations are not indicative of the amount or activity of biota, as has been known for some time (Brylinsky and Mann 1975). Even though dissolved inorganic nutrient concentrations are not related to biomass or activity in many surface waters, biota control nutrient concentrations independently from the rate of external loading.

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Table 1. Observed [S] and values for [S] calculated with Eq. 2 using phosphate data from Flathead Lake (Dodds et al. 1991) and ammonium data for the mouth of the Chesapeake Bay (Glibert et al. 1982; Wheeler et al. 1982).

SITE, MONTH	OBSERVED MAXIMUM [S] (nmol LITER^{-1})	CALCULATED [S] (nmol LITER^{-1})
Flathead		
Jun	0.32	0.08
Jul	0.97	0.14
Sep	0.65	0.09
Nov	0.65	0.09
Feb	0.48	0.44
May	0.32	0.12
Chesapeake		
May-June	314	206

Figure 1. Relationships between uptake and regeneration as a function of nutrient concentration [S], and their combined effects upon substrate rate of change (dS/dt). A) regeneration a constant function of [S], and B) regeneration increasing with [S].

Figure 2. SRP concentrations after enrichment (A) and dilution (B) in Milford Lake water. Lake = *in situ* values, control = carboys with no additions, N + P = 10 μM PO_4^{3-} + 160 μM NH_4^+, 1 μm lake = dilution with lake water filtered through a 1 μm filter, RO = dilution with reverse osmosis water, error bars = 1 std dev. These values are presented in both (A) and (B). Note variations in scale between (A) and (B).

Figure 3. Relationships between independently measured ammonium (A) and phosphate (B) uptake and regeneration rates for a variety of marine and freshwater systems. In all studies uptake was estimated by label incorporation into particulates, regeneration by isotope dilution. Note differences in scale between A and B. Dotted line represents a 1:1 relationship, the solid line represents regression (in A, $r^2 = 0.784$, in B, $r^2 = 0.835$). In A, ○ Flathead Lake (Dodds and Priscu 1990; Dodds et al. 1991), □ Lake Calado, Amazon flood plain (Morrissey and Fisher 1987), ■ marine snow, North Atlantic (Glibert et al. 1988), ▲ Chesapeake Bay (Glibert et al. 1982), ▼ Antarctic lakes (Priscu et al 1989), ▵ Subarctic Pacific (Wheeler et al. 1989), ▽ Chesapeake Bay plume (Glibert et al. 1991). In B, ■ Bedford Basin, Peruvian Upwelling Region and Eastern Tropical Pacific (Harrison 1983), ○ Flathead Lake (Dodds et al. 1991), □ Milford Reservoir (Dodds, unpublished), ▲ Lake Calado, Amazon flood plain (Fisher et al. 1988).

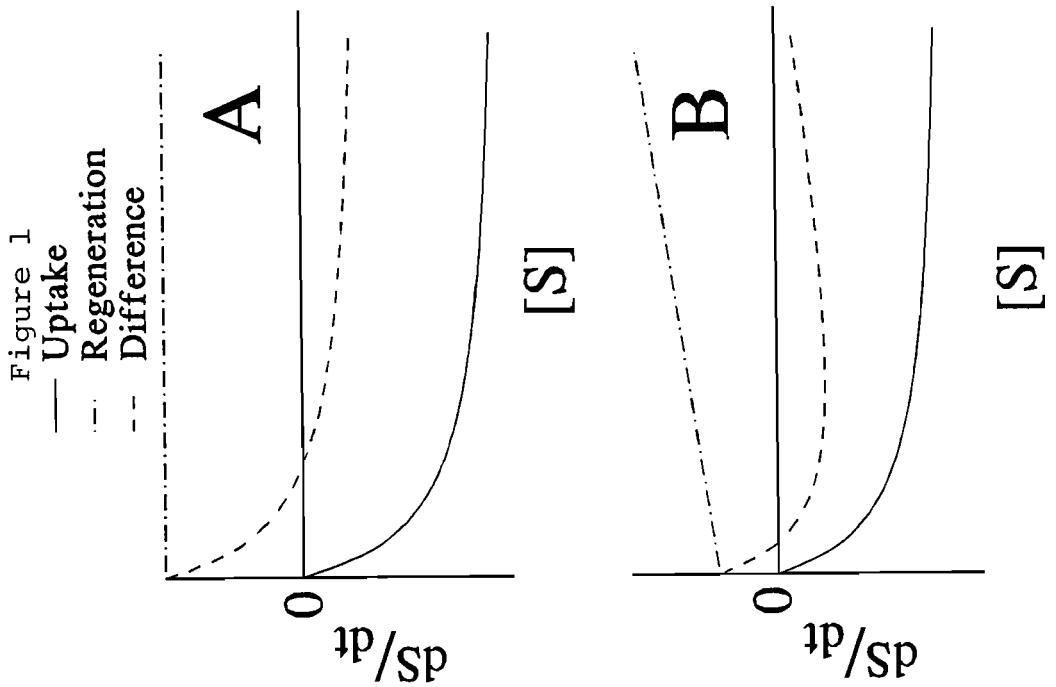


Figure 1
Uptake
Regeneration
Difference
A
B
0
dS/dt
[S]

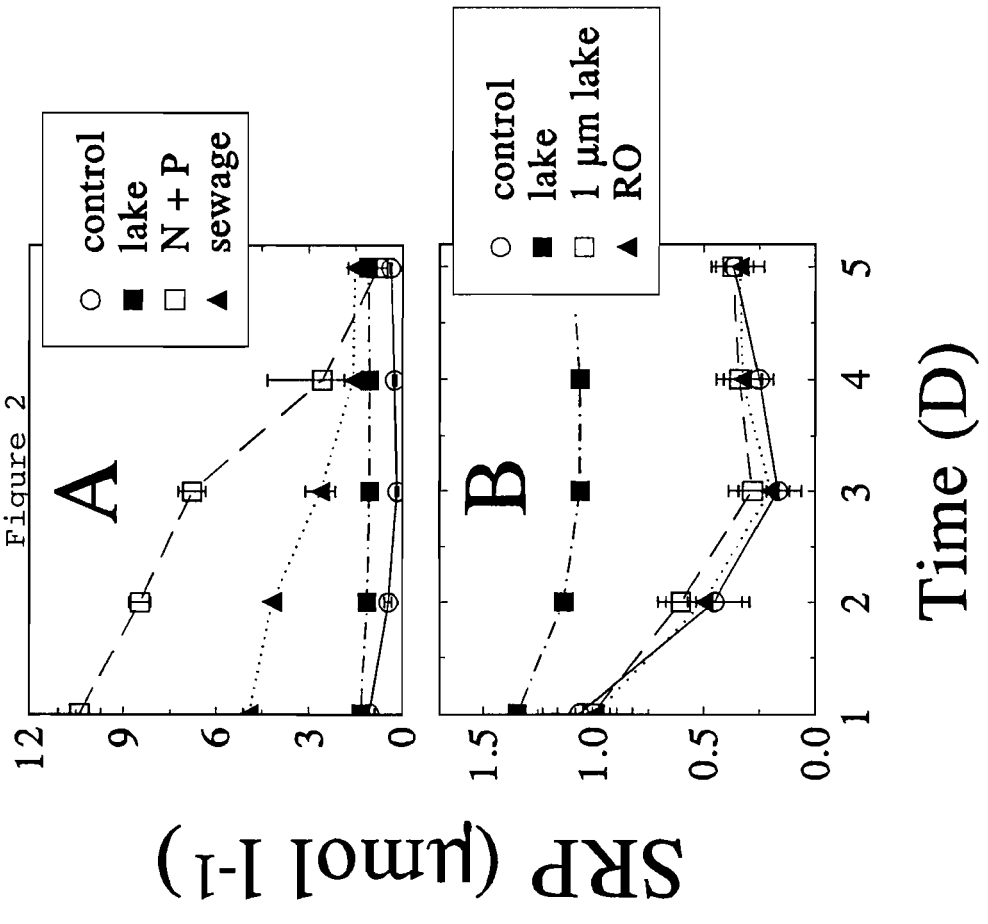
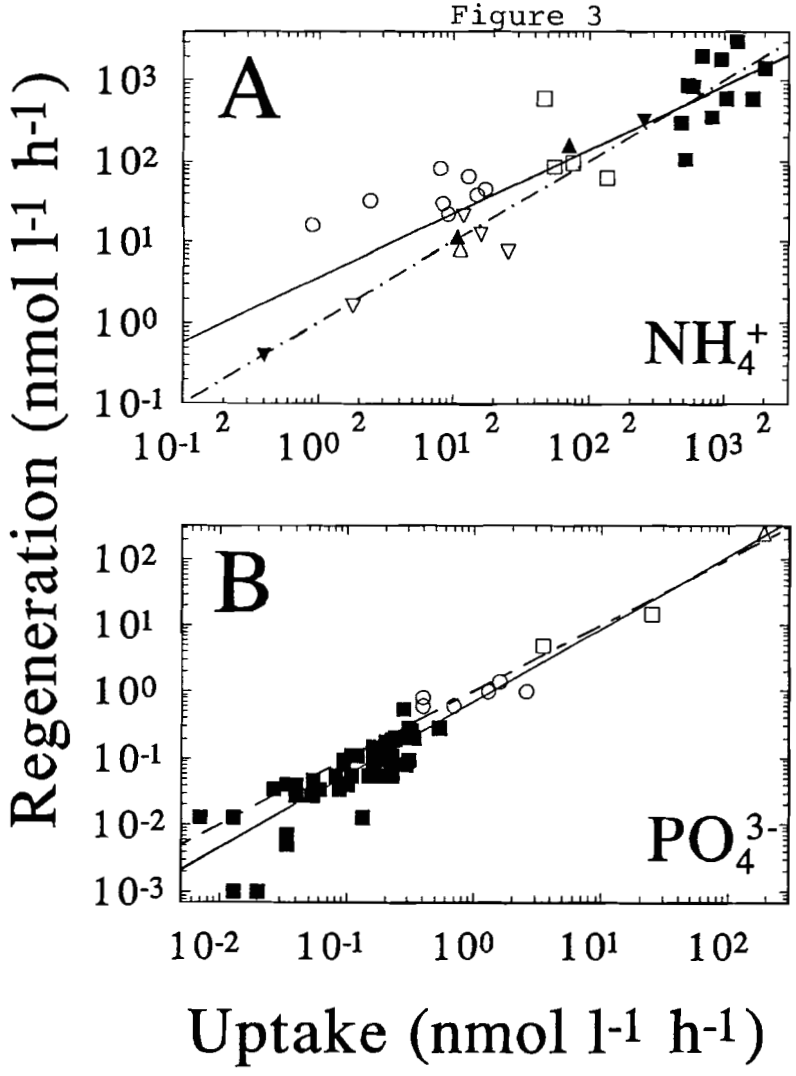


Figure 2
A
B
control
lake
N + P
sewage
1 µm lake
RO
SRP (µmol l-1)
Time (D)
12
9
6
3
0
1.5
1.0
0.5
0.0
1
2
3
4
5

Figure 3


A
B
NH_4^+
PO_4^{3-}
Regeneration (nmol l-1 h-1)
Uptake (nmol l-1 h-1)
10-1 2
100 2
101 2
102 2
103 2
10-2
10-1
100
101
102
10-3

Chapter 4

NUTRIENT DILUTION AND REMOVAL BIOASSAYS TO ESTIMATE PHYTOPLANKTON RESPONSE TO NUTRIENT CONTROL

ABSTRACT

Nutrient addition and dilution bioassays were performed in a large eutrophic reservoir. Experiments were conducted *in situ* in 10 L carboys and 1560 L mesocosms. By day four in carboy experiments, addition of N alone stimulated chlorophyll *a* by 403%, simultaneous N and P addition by 929%, and secondary sewage effluent by 188%. P added alone had little stimulatory effect. Dilution of 15% per day with 1 μm filtered lake water or reverse osmosis water had an insignificant effect on productivity or chlorophyll *a* concentrations compared to controls, but there was a shift from a cyanobacteria to a diatom-dominated phytoplankton assemblage. The results from the mesocosms were consistent with the carboy experiments in addition of N and N + P stimulated chlorophyll *a*, and that dilution with filtered lake water or reverse osmosis water had little effect. Increasing or decreasing zooplankton numbers had only small effects on productivity and chlorophyll *a*. Nutrient addition and dilution bioassays may provide valuable information when evaluating measures to reduce eutrophication in lakes.

INTRODUCTION

Correlations between total phosphorus and chlorophyll in lakes have been used to predict the effects of lowering nutrient loads (Ryding and Rast 1989). The fact that phosphorus is not the only nutrient factor that can limit productivity in lakes (e.g., Dodds et al. 1989, Dodds and Priscu 1990, Elser et al. 1990), and the fact that the correlations can be weakened considerably with increased non-algal turbidity (Jones and Novak 1981, Hoyer and Jones 1983, Jones and Knowlton 1992), suggests the need for system-specific information in addition to using phosphorus loading regression models.

Nutrient enrichment bioassays have been used extensively to determine nutrient limitation (e.g., Hecky and Kilham 1988, Dodds and Priscu 1990, Elser et al. 1990), but such experiments can not provide managers with information regarding the effects of nutrient removals. Paerl and Bowles (1987) developed a technique to dilute nutrients with de-ionized water and then add various amounts of nutrients to estimate the effects of nutrient control on the eutrophic Nuess River. This technique was recently modified by using chemicals to remove ammonium (zeolite) and phosphate (alum) from lake water, and adding the chemically treated water to natural algal assemblages (Dodds and Randal unpublished data). The modified technique had promising results when applied to a hyper-eutrophic farm pond, except that the greatest effect was caused by containing the algae and cutting it off from nutrient

loading from the sediments.

The purpose of this study was to explore and refine nutrient dilution and removal bioassay techniques. The experiments were conducted in microcosms (10 L carboys) and mesocosms (1560 L containers) that included the natural sediment. Nutrient addition experiments were conducted concurrently, and an attempt was made to estimate food web effects (top down control) in the mesocosm experiments.

MATERIALS AND METHODS

Experiments were conducted in Milford Lake (Kansas) during late July and early August 1991. Milford Lake is a large (65.5 km^2), shallow (mean depth 7.8 m), mildly eutrophic (summer epilimnetic chlorophyll *a* $\approx$ 19 $\mu g\ l^{-1}$) reservoir (Environmental Protection Agency 1974). The lake is used for drinking water and recreation, and occasionally is dominated by nuisance blooms of the cyanobacterium *Anabaena* (US Army Corps of Engineers 1983).

Nutrient dilution bioassays were conducted in 10 L polyethylene carboys using water collected from the center of the lake, 1 km offshore from the Rush Creek inlet. Water was collected from the depth of maximum photosynthesis (2 m, unpublished data) with a displacement sampler (Dodds and Priscu 1988), with care taken not to expose the water to full surface irradiance. All treatments (Table 1) were triplicated. Following initial treatment, carboys were incubated at 1.5 m in the lake and sampled daily for photosynthetic O_2 production, chlorophyll *a*, and nutrient concentrations (NH_4^+, PO_4^{3-}, and NO_3^-) as described previously by the methods of APHA (1989). By day 4, one sewage-N and two sewage-P carboys were lost in rough weather.

Mesocosms were located on 5 August 1991 in the bay formed by the inlet of Rush Creek. These mesocosms were 1 m diameter, 2 m deep cylinders, made of semitransparent, layered, reinforced low-density polyethylene sheeting (Canvex II, Raven Industries, Sioux Falls SD), sealed with Griff-tape (Brock White Inc., St. Paul, MN), and held rigid with 1 m diameter hoops of 13 mm PVC pipe at the top and bottom. The mesocosms were placed in 1.6 m of water with bottoms inserted 0.2 m into the sediments, anchored with galvanized wire stakes, and the tops suspended from a floating frame 0.2 m above the water surface.

Mesocosm treatments were not replicated and consisted of: 1) control, 2) 160 μmol $NH_4Cl\ l^{-1}$ final concentration added on days 0 and 3, 3) 10 μmol $NaH_2PO_4\ l^{-1}$ final concentration added on days 0 and 3, 4) 160 μmol NH_4Cl and 10 μmol $NaH_2PO_4\ l^{-1}$ final concentration added on days 0 and 3, 5) 1 μm filtered lake water dilution (150 l per day), 6) reverse osmosis water dilution

(150 l per day), 7) - zooplankton (repeated hauls, 100 $_{\mu}$m net), and 8) + zooplankton (about 2 times natural concentrations). Mesocosms were sampled daily for photosynthesis, chlorophyll *a*, and nutrient concentrations (NH_4^+, PO_4^{3-}, and NO_3^-). Strips of Canvex II were added to the mesocosms at day 0 and removed upon termination of the experiment to analyze for attached chlorophyll *a* at the end of the experiment. Core samples from the sediment were also removed and analyzed for chlorophyll upon termination.

Acetylene reduction (nitrogen fixation) was measured using samples collected from carboys and mesocosms 5 days after initiation of each experiment. An aliquot of 50 ml water from each treatment was added to a 70 ml glass serum vial, and 6 ml acetylene were added through a septum. The vials were incubated at 26 °C (lake temperature) for 6 h under 100 μmol quanta m^{-2} s^{-1} fluorescent light. Incubations were terminated with addition of 5 ml of 10% trichloroacetic acid, and the amount of ethylene in the headspace gas was determined using a Varian 940 GC.

Samples for algal identification were taken from the carboys on day 4. Samples were mounted on filters and counted (Crumpton 1987). Epifluorescence microscopy was used to detect viable algae (containing chlorophyll) and phase transmission was used in identification.

Multiple comparisons of means were done for the carboy experiments using Analysis of Variance followed by Scheffe's pairwise comparison method. Results from the 1560 l enclosure experiments were not analyzed statistically because the experimental design was pseudo-replicated. Triplicate samples were taken from each mesocosm and analyzed, results from these experiments are reported as means of three replicates with associated standard deviation to indicate the amount of variance associated with measurements made within a single treatment.

RESULTS

Carboys

Ammonium added to the +N treatments disappeared less rapidly than in the N+P treatments ($p < 0.05$, day 5 data), suggesting that the amount of phosphorus present controlled the rate of ammonium utilization (Fig. 1A). With sewage addition, ammonium levels were elevated initially, but subsequently returned to levels found in control carboys and lake water. The sewage-N treatments never had significantly more NH_4^+ than control, and the sewage-P treatments had slightly elevated NH_4^+ concentrations initially that fell

to control levels by day 4 (Fig. 1B). There were no significant trends when the amounts of ammonium in the control, lake, filtered lake water dilution, or reverse osmosis dilution were compared (Fig. 1C).

The +N and N+P carboys had significantly elevated levels of nitrate by the end of the experiment (Fig. 2). Although there was significant initial addition of nitrate with the sewage treatments (Table 1), there was only detectable nitrate in the sewage-P treatment at day 5. Nitrate levels in the control carboy were less than those in the lake.

Soluble reactive phosphorus (SRP) disappeared more rapidly in the presence of ammonium than when only phosphate was added ($p < 0.05$, day 5 data). SRP levels in N+P treatments were not significantly different from those in the lake by day 5 (Fig. 3A). This suggests that inorganic nitrogen had to be present for efficient utilization of phosphate.

The sewage-P additions had SRP levels similar to those seen in control carboys and the lake. Sewage-N and sewage treatments both exhibited decreases in SRP throughout the experiment (Fig. 3B). The dilution treatments (filtered lake water and reverse osmosis water) had SRP levels very similar to those in the control carboy (Fig. 3C), and significantly less than in the lake ($p < 0.05$, day 5 data).

Phaeophytin corrected chlorophyll *a* was stimulated slightly with the addition of N, strongly with addition of N+P (Fig. 4A), and early in the experiment by addition of sewage and sewage-N (Fig. 4B), as compared to controls. Chlorophyll *a* did not vary significantly from lake water in sewage-P, filtered lake water dilutions, reverse osmosis dilutions, or controls (Fig. 4B,C). Phytoplankton photosynthetic rates only increased significantly with concurrent N and P addition after 4 days (Fig. 5A,B,C). No other trends were evident. Acetylene reduction data showed no significant differences ($p > 0.05$) between treatments (data not shown).

Algal counts revealed patterns in total biovolume (Fig. 6A) very similar to those observed for chlorophyll *a*, with maximum biovolume with N+P addition (significantly greater than all other treatments except +N) and some stimulation with addition of N alone (N greater than RO dilution $p < 0.05$). The increases in biovolume in the N+P treatment were related to significant increases in the biovolume of green and cyanobacteria. There were no significant trends in diatom biomass.

Mesocosms

Ammonium concentrations in the mesocosms decreased rapidly in the +N and N+P treatments, reaching levels seen in the lake and control treatments by

day 3 (Fig. 7A). At this point ammonium was added again. No clear differences are seen between ammonium concentrations in the filtered lake water, reverse osmosis water, + zooplankton, - zooplankton, and control treatments were observed compared to the values from the lake (Fig. 7B,C).

SRP concentrations dropped rapidly in the +P and N+P treatments (Fig. 8A), analogous to the ammonium concentration drop noted for N addition treatments. On day four there were slight differences in SRP, with reverse osmosis and + zooplankton treatments exhibiting slightly lower SRP concentrations than in the control. The lake had slightly higher SRP concentrations than control by day four (Fig. 8B,C).

Chlorophyll *a* increased markedly in the +N and N+P treatments (Fig. 9A). On day four, there was less chlorophyll in the lake, and the dilution treatments (filtered lake and reverse osmosis) than in the control (Fig. 9B). Both zooplankton treatments exhibited slightly less chlorophyll than the control, but more than in the lake.

By day three, photosynthesis was only stimulated in the N+P treatments, although not strongly. Nitrogenase activity appeared to decrease with nutrient addition, dilution of zooplankton manipulation had little effect (Table 2). The amount of benthic chlorophyll *a* was slightly less with addition of reverse osmosis water, but varied little otherwise. The amount of chlorophyll *a* on the container walls was markedly higher with N and N+P additions, and lower when zooplankton were added. The increase in chlorophyll *a* on the container sides when ammonium was added parallels the increase observed in plankton chlorophyll *a* when ammonium was added. Zooplankton numbers were variable with the lowest recorded values in the -zooplankton treatments, and the highest values with N addition (Table 2).

DISCUSSION

Both nitrogen and phosphorus appeared to limit productivity in the carboy experiments. Ammonium disappeared more rapidly in the presence of phosphate, and phosphate disappeared more rapidly in the presence of ammonium. This suggests that addition of N and P forced P and N limitation respectively. Addition of N increased chlorophyll *a*, but simultaneous N and P addition stimulated both chlorophyll *a* and photosynthesis.

Similar results are common from oligotrophic lakes (Suttle and Harrison 1988, Dodds and Priscu 1990, Elser et al. 1990). The theoretical basis behind such co-limitation (simultaneous N and P limitation) and implications for lake management have been discussed previously (Dodds et al. 1989). Such co-limitation is surprising if Leibig's Law of the Minimum applies to

phytoplankton communities. However, unequal nutrient requirements of algae, heterogeneity of nutrients (regenerated patches and depletion zones near active cells), heterogeneity of algal populations, and selective grazing may all invalidate an equilibrium based approach that predicts only one nutrient can limit phytoplankton communities.

Data from the mesocosms (1560 L enclosures) suggests that nitrogen primarily, and phosphorus secondarily, limited productivity of phytoplankton and periphyton. These experiments differ from the carboy experiments in two key ways: 1) water for the mesocosms was taken from a sheltered bay while water for the carboys was taken from the lake center, and 2) the mesocosms included sediments whereas the carboys did not. The difference in the carboy and mesocosm results may be related to including sediments. The water in the mesocosms was probably influenced by the sediments more heavily before and during the experiment than water in the center of the lake. The sheltered bay is only 4 m deep whereas the lake center is 16 m deep and weakly stratified. The sediments could recycle phosphorus, or remove nitrogen via denitrification, decreasing P limitation and increasing N limitation. The differences between the carboy and mesocosm data are interesting because they imply that nutrient limitation can vary over fairly short distances (ca. 1 km) within a single reservoir.

The results from sewage addition carboys are consistent with those for the inorganic nutrient additions if the fact that the sewage was relatively high in nitrate is considered. In the sewage and sewage-N additions there was stimulation of chlorophyll *a* followed by a drop to initial levels; with sewage-P, there was no stimulation. If both N and P were limiting, one would expect that in sewage with N removed there would be little stimulation of chlorophyll *a*. However, ambient levels of SRP in the sewage and sewage-N treatments increased 4 fold over those in the sewage-P additions. In all treatments, the nitrate in sewage increased the total inorganic nitrogen significantly, and the ammonium concentrations were low at the beginning and only doubled at most. Sewage did not stimulate productivity to the same degree as the N+P additions. This is probably related to the fact that the absolute levels of inorganic nitrogen and SRP were lower with sewage addition.

Nitrate increased in the carboy experiments where ammonium was added which suggests active ammonium oxidization by the planktonic bacterial community. In the sewage additions, the only treatment with significant levels of nitrate remaining at the end of the experiment was the sewage-P treatment, even though this treatment had lower levels of nitrate initially (Table 1). This is consistent with the hypothesis of P limitation in these carboys (although it does not preclude simultaneous N and P limitation). In the case where sewage

without high levels of SRP was added, there was insufficient P available for the plankton to utilize the nitrate. When the SRP remained (in the sewage and sewage-N treatments), it was consumed rapidly (Fig. 3B) along with the nitrate.

There were limited effects of zooplankton addition or removal on chlorophyll *a* although the levels of nitrogenase activity (acetylene reduction) were higher when zooplankton were removed. This suggests that the short-term effects of zooplankton grazing in this system are weak, and that algal biomass in the lake may be most strongly influenced by nutrient supply.

It is surprising that even with 15% dilution per day over four days in the carboy experiments, (52% dilution over all), there was little detectable effect upon the photosynthesis, chlorophyll *a*, NH_4^+, or SRP concentrations. Dilution did, however, cause a minor decrease in total algal biovolume. Investigators of zooplankton grazing have suggested that dilution should increase growth by decreasing grazing (Evans and Paranjape 1992). Theoretical aspects of the lack of response of dissolved inorganic nutrient concentrations have not been addressed. Probably, increased uptake with increasing nutrient concentrations, and weak coupling between nutrient regeneration rates and ambient nutrient concentrations leads to levels of dissolved inorganic nutrients that are resistant to perturbations over short time periods. This means that levels of dissolved inorganic nutrients may be rather poor indicators of the nutrient status of phytoplankton. In spite of this fact, some who manage or study lakes routinely report levels of dissolved nutrients as if they are very important in describing the system. Our dilution results show that inorganic nutrient levels and productivity are not necessarily closely related. The lack of a relationship between inorganic nutrient concentrations and process rates in aquatic systems has been known for some time (Brylinsky and Mann 1975). Furthermore, with nutrient addition, much of the inorganic nutrient was taken up into biomass and levels of SRP and ammonium decreased to their original levels. This happened most rapidly when both ammonium and phosphate were added simultaneously because the phytoplankton community is limited by both nutrients.

Both cyanobacteria and green algae responded positively to N+P addition. Diatoms did not respond to fertilization. It did appear that the proportion of diatoms increased slightly in the RO treatments, but Analysis of Variance of the arc sine transformed proportion revealed no differences at the $p > 0.05$ level. Algal taxonomy did not show significant shifts related to containment.

Bioassays similar to those reported here may represent the best way to determine the effects of nutrient addition and nutrient removal on phytoplankton populations. It has been shown that short term indicators of nutrient deficiency can give ambiguous and contradictory results (Dodds and Priscu 1990, Dodds and Priscu 1991, Hecky and Kilham 1988). There was reasonable agreement

between the mesocosm and the carboy experiments, with the primary differences explained by the inclusion of sediment in the mesocosms, and by the fact that water from a bay was used to fill the mesocosms and water from the center of the lake to fill the carboys.

Techniques such as those presented here may provide useful information for management. Although a significant amount of work is involved in conducting an *in situ* experiment of this scale for one week, none of the procedures presented here require advanced technical capabilities. A version of these bioassays with fewer treatments and less frequent sampling may be as useful. In general, it has been our experience that observation of the carboys will reveal significant differences in chlorophyll and samples do not need to be collected for analysis until this point. Although nutrient levels reveal some information and probably should be measured initially to reveal the appropriate level of addition, nutrient measurements throughout the experiments are probably not necessary. Likewise, phytoplankton community analysis is tedious, requires taxonomic expertise and probably is not necessary to yield results applicable to system management. Recently, a simple version of the bioassays reported here was successfully implemented on another local reservoir by a high school student as a senior research project (Robert Lehmann, personal communication). Therefore, an ecological technician should be able to perform a similar bioassay successfully. Lowering nutrient loading can be quite costly. Using bioassay techniques similar to those presented here may yield important information about a system that the standard loading equations can not, and thus assist in estimating the impact of nutrient reductions.

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Table 1. Nutrient addition and dilution treatments and days treated after experiment initiation in carboy experiments. All treatments were initially triplicated. Day 0 = 29 July 1992.

TREATMENT	DAYS	COMMENTS
Control		No additions
+N	0	Final concentration 160 μM NH_4Cl L
+P	0	Final concentration 10 μM NaH_2PO_4 L
N+P	0	Same concentration as individual additions
Sewage	0	500 ml 2° effluent, Clay Center sewage treatment plant, final concentration= 0.46 μM NH_4^+, 4.03 μM SRP, 27.2 μM NO_3^-
Sewage-N	0	500 ml, treated with zeolite, final concentration= 0.23 μM NH_4^+, 4.46 uM SRP, 26.0 μM NO_3^-
Sewage-P	0	500 ml, treated with alum, final concentration=0.42 μM NH_4^+, 1.04 μM SRP, 17.8 μM NO_3^-
Filt.	0,1,2,3,4	Filtered lake water, 1 μm bag filter (Dodds and Randal 1992), 15% of total volume per day
RO	0,1,2,3,4	Reverse osmosis water, 15% of total volume per day

Table 2. Acetylene reduction, sediment and container wall chlorophyll *a*, and zooplankton numbers from mesocosm experiments. std dev in parentheses, represents mean of 3 determinations. The "other" category of zooplankton includes ostracods and rotifers.

Treatment	Acetylene reduction (μmol L^{-1} h^{-1})	Sediment chl *a* (mg m^{-2})	Container wall chl *a* (mg m^{-2})	Cladoceran (# L^{-1})	Copepod (# L^{-1})	Other (# L^{-1})	Total zooplankton (# L^{-1})
Control	64 (18)	33 (3)	7.9 (6.7)	0	47 (21)	24 (24)	721 (37)
+N	38 (3)	36 (9)	115 (20.9)	108 (48)	115 (71)	78 (29)	3056 (1359)
+P	44 (6)	36 (12)	11.0 (8.4)	10 (10)	108 (16)	14 (12)	1339 (594)
N+P	17 (3)	31 (9)	126 (38.7)	14 (6)	44 (25)	75 (16)	1339 (607)
Filt.	35 (0)	13 (4)	6.2 (6.8)	3 (6)	98 (65)	14 (12)	1168 (560)
RO	83 (16)	34 (6)	7.1 (4.0)	37 (6)	51 (27)	17 (16)	1065 (469)
+Zoo	61 (11)	34 (11)	0 (0.75)	94 (21)	85 (41)	0	1820 (810)
-Zoo	93 (13)	26 (3)	4.9 (3.4)	0	24 (15)	3 (6)	275 (151)

Figure 1. Ammonium concentrations in carboy experiments with nutrient addition (A), sewage addition (B), and nutrient dilution (C). Error bars = 1 std dev.

Figure 2. Nitrate concentrations for day 5 of the carboy experiments. Error bars = 1 std dev.

Figure 3. Soluble reactive phosphorus concentrations in carboy experiments with nutrient addition (A), sewage addition (B), and nutrient dilution (c). Error bars = 1 std dev.

Figure 4. Chlorophyll *a* concentrations in carboy experiments with nutrient addition (A), sewage addition (B), and nutrient dilution (c). Error bars = 1 std dev.

Figure 5. Photosynthetic O_2 production in carboy experiments with nutrient addition (A), sewage addition (B), and nutrient dilution (c). Error bars = 1 std dev.

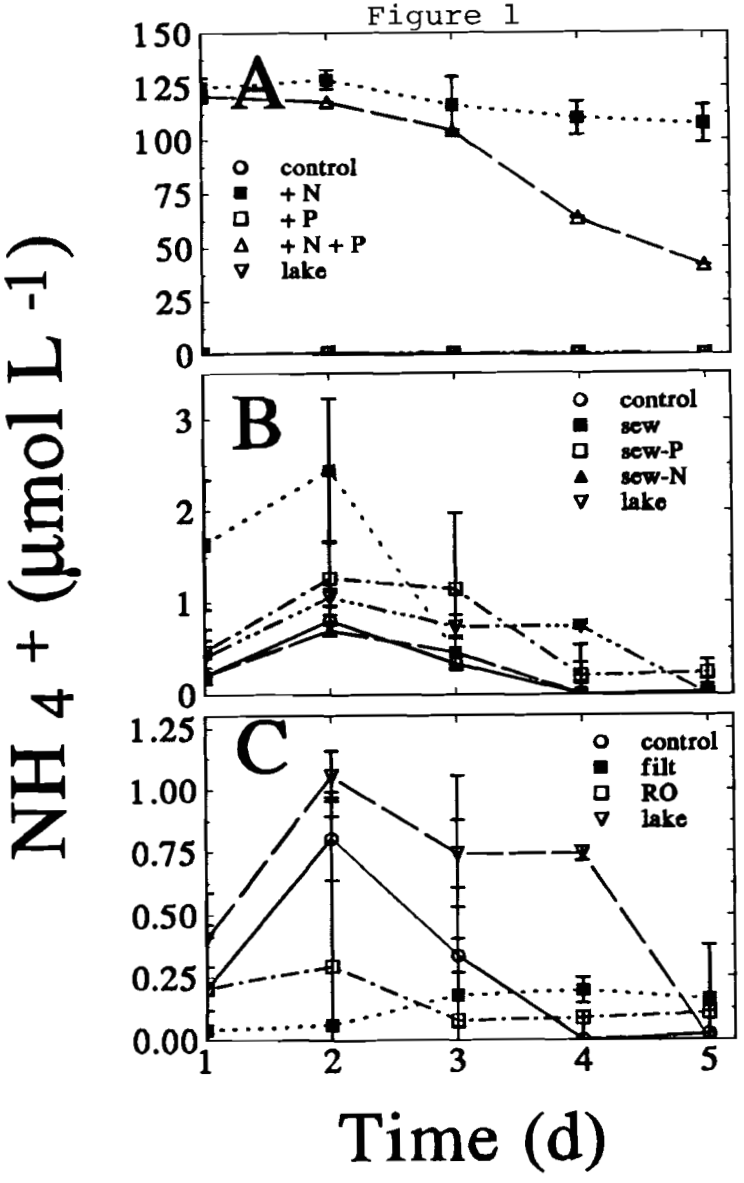
Figure 6. Absolute (A) and relative (B) abundances of phytoplankton after 5 days in the carboy experiments. Error bars in A = 1 std dev and represent the variance in total abundance.

Figure 7. Ammonium concentrations in mesocosm experiments with nutrient addition (A), dilution (B) and zooplankton manipulation (C). Each value = mean of 3 subsamples taken from 1 treatment, error bars = 1 std dev.

Figure 8. Soluble reactive phosphorus concentrations in mesocosm experiments with nutrient addition (A), dilution (B) and zooplankton manipulation (C). Each value = mean of 3 subsamples taken from 1 treatment, error bars = 1 std dev.

Figure 9. Chlorophyll *a* concentrations in mesocosm experiments with nutrient addition (A), dilution (B) and zooplankton manipulation (C). Each value = mean of 3 subsamples taken from 1 treatment, error bars = 1 std dev.

Figure 1


A
control
+N
+P
+N+P
lake
B
control
sew
sew-P
sew-N
lake
C
control
filt
RO
lake
NH 4 + (µmol L -1)
Time (d)

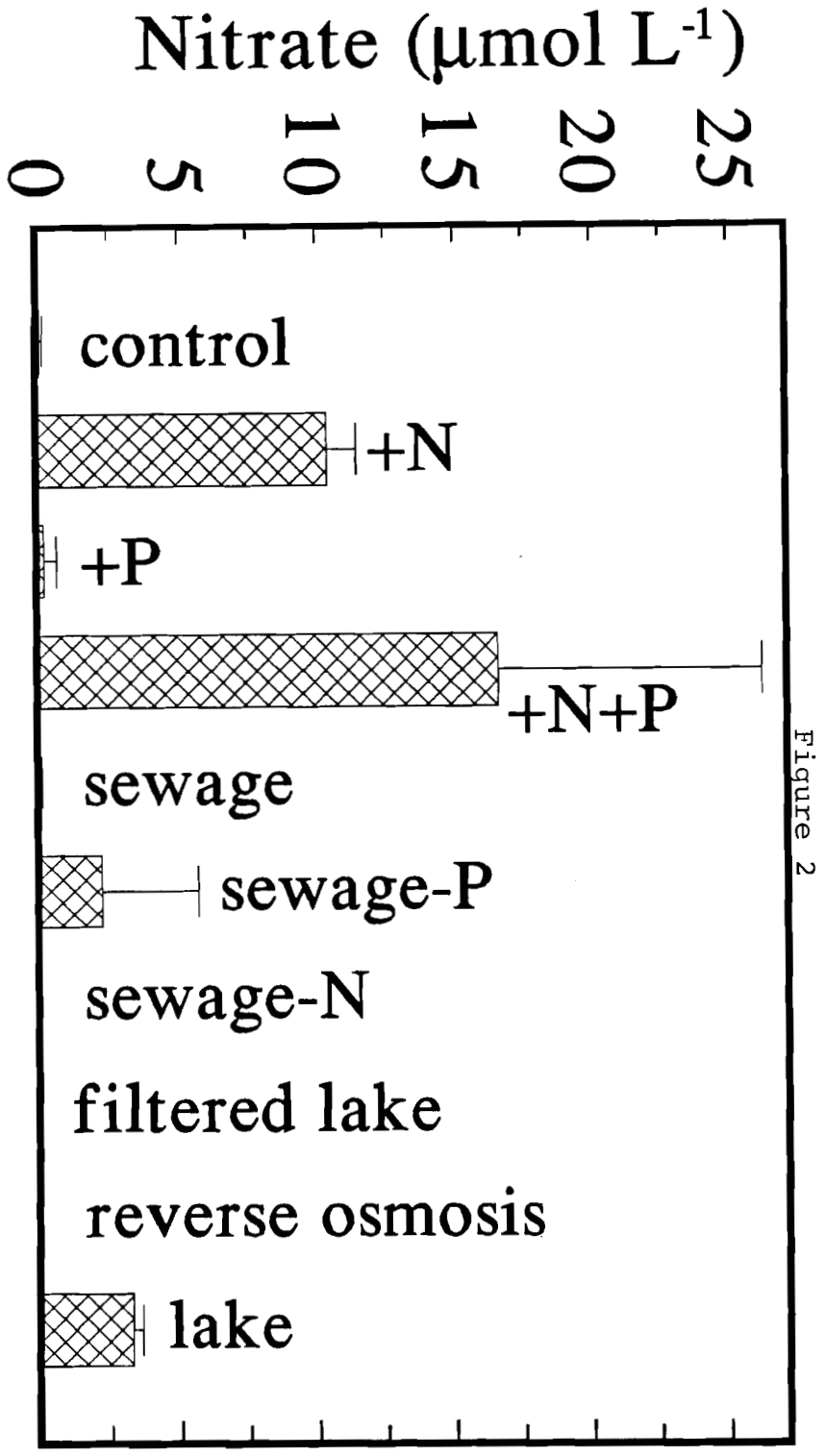
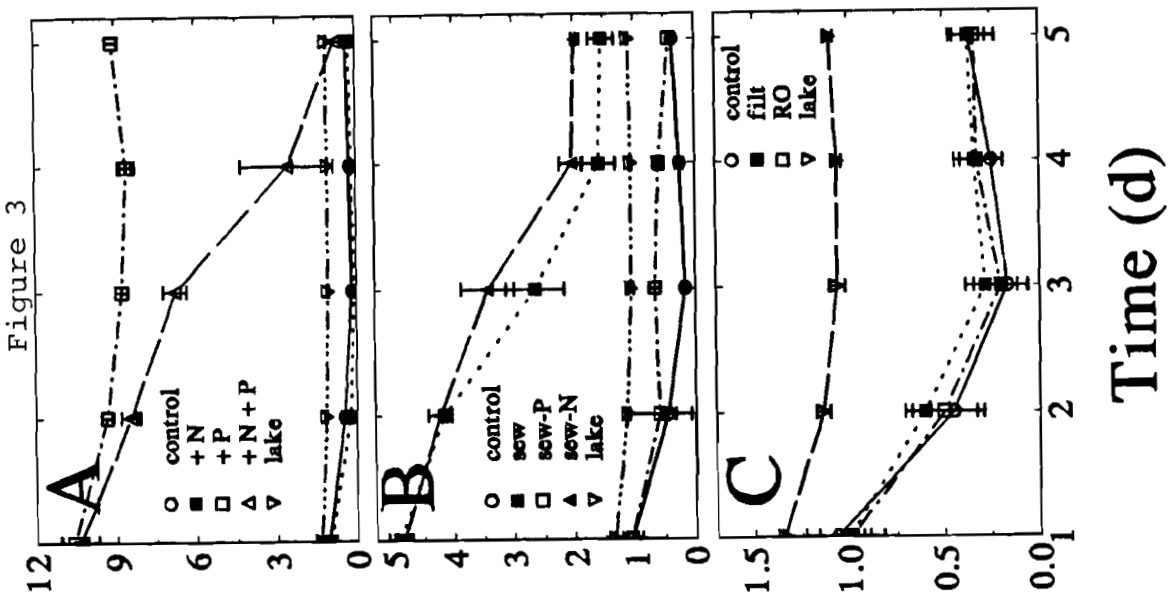

Nitrate (µmol L-1)
0
5
10
15
20
25
control
+N
+P
+N+P
sewage
sewage-P
sewage-N
filtered lake
reverse osmosis
lake

Figure 2

Figure 3


A
control
+N
+P
+N+P
lake
B
control
sew
sew-P
sew-N
lake
C
control
filt
RO
lake
SRP (µmol L -1)
Time (d)

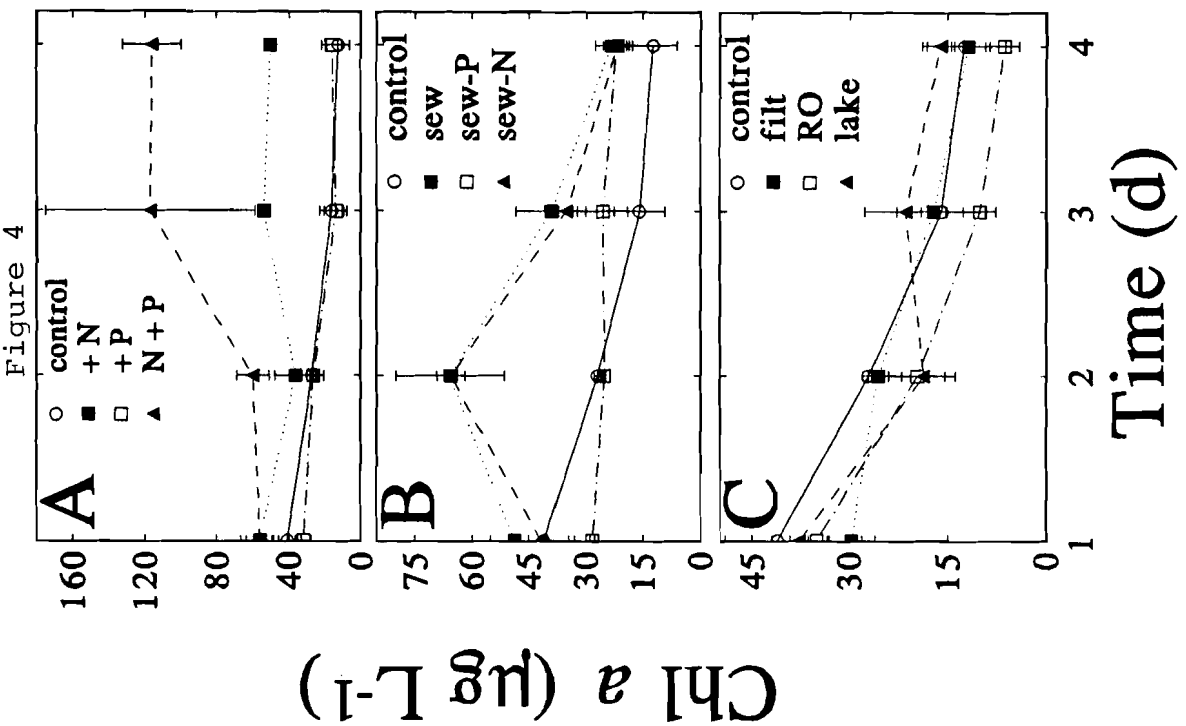


Figure 4
A
control
+N
+P
N+P
B
control
sew
sew-P
sew-N
C
control
filt
RO
lake
Chl a (µg L-1)
Time (d)

Figure 5

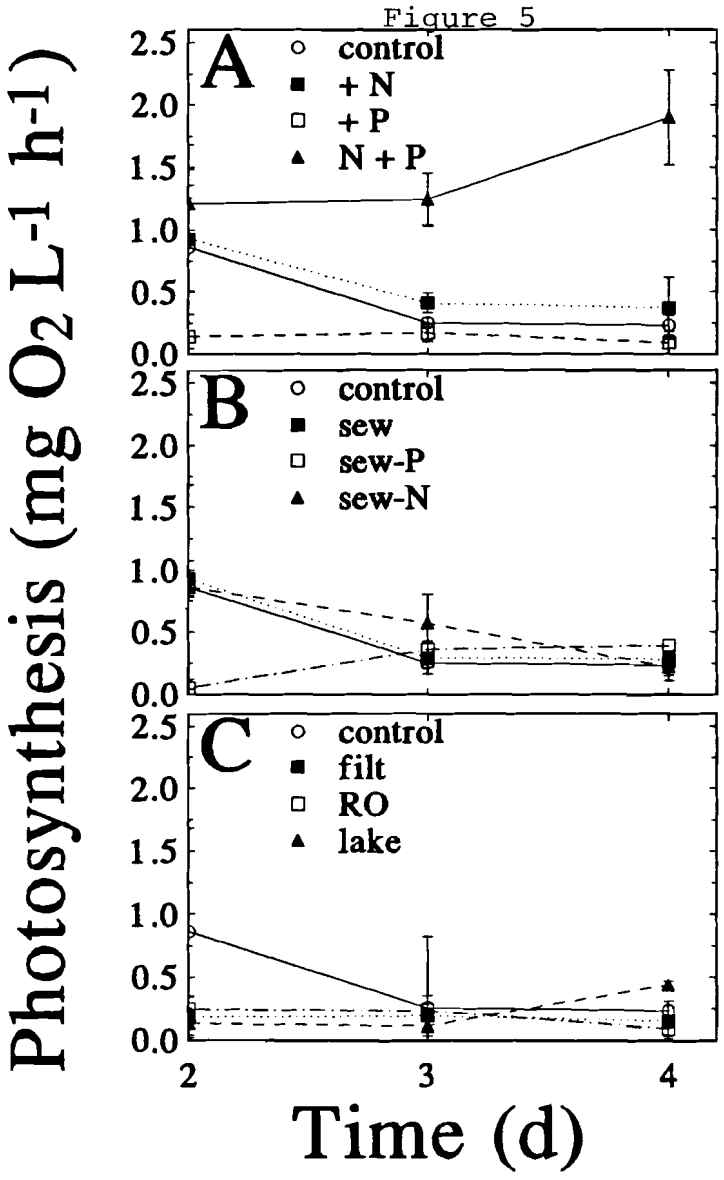
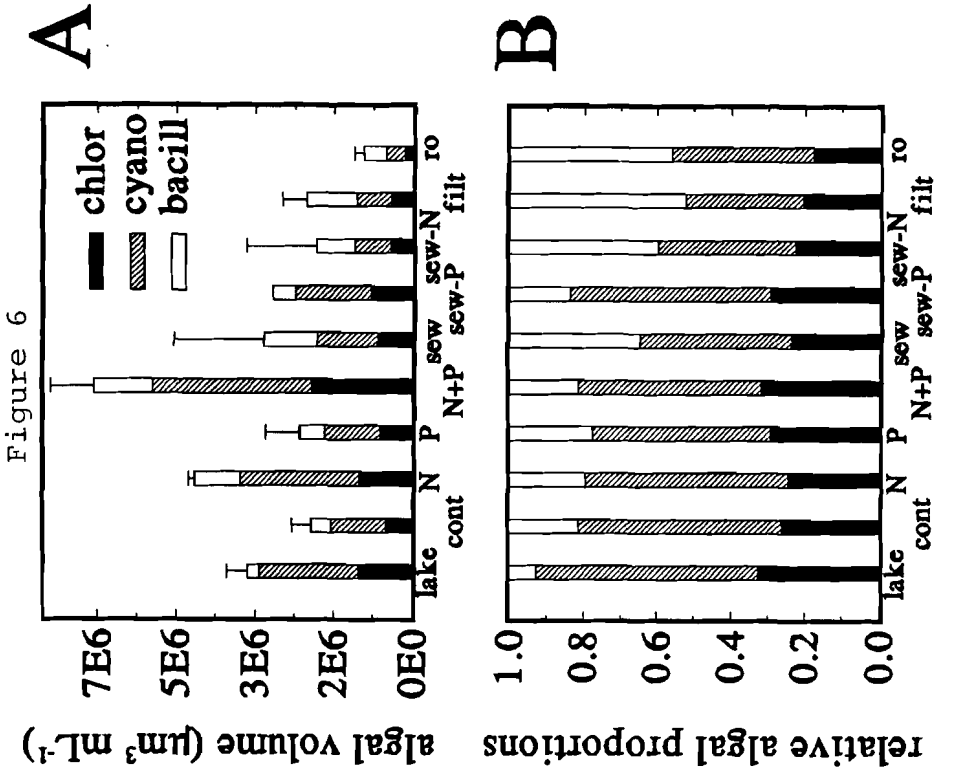

A
control
+ N
+ P
N + P
B
control
sew
sew-P
sew-N
C
control
filt
RO
lake
Photosynthesis (mg O2 L-1 h-1)
Time (d)
0.0
0.5
1.0
1.5
2.0
2.5
2
3
4

Figure 6


A
chlor
cyano
bacill
7E6
5E6
3E6
2E6
0E0
algal volume (µm³ mL⁻¹)
lake
cont
N
P
N+P
sew
sew-P
sew-N
filt
ro
B
1.0
0.8
0.6
0.4
0.2
0.0
relative algal proportions

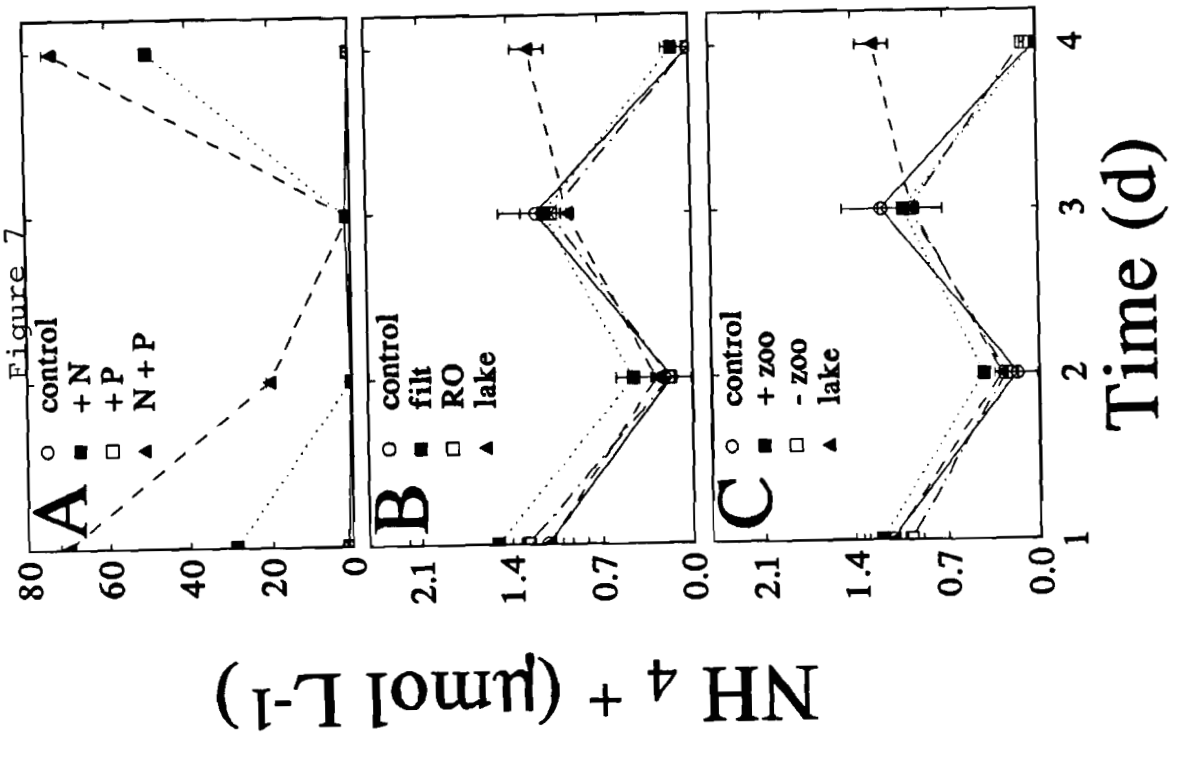

A
control
+ N
+ P
N + P
B
control
filt
RO
lake
C
control
+ zoo
- zoo
lake
80
60
40
20
0
2.1
1.4
0.7
0.0
1
2
3
4
Time (d)
NH 4 + (µmol L-1)

Figure 7

Figure 8

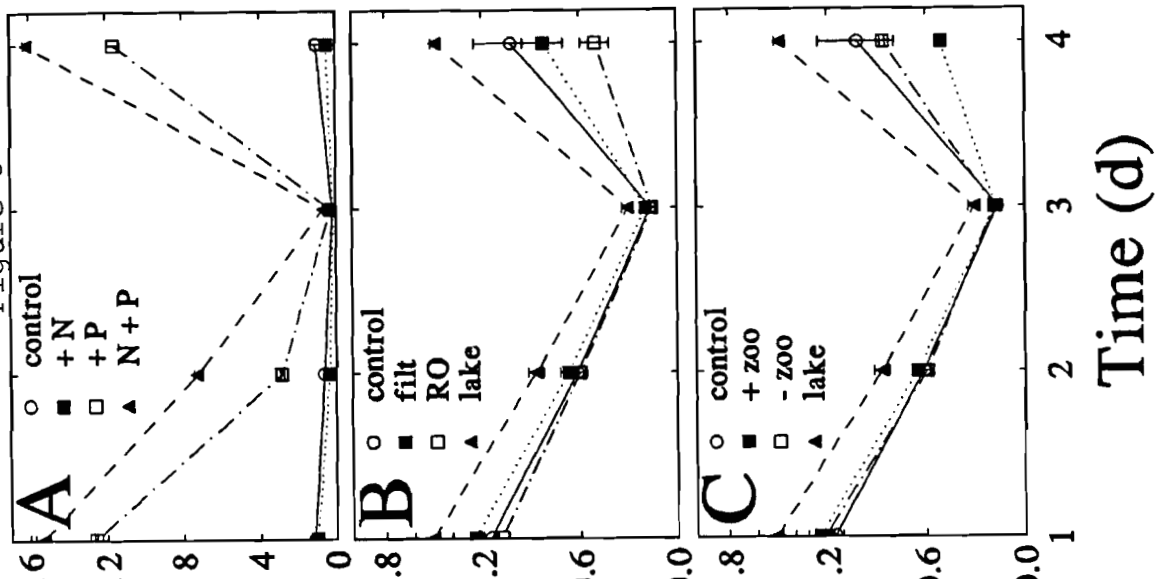
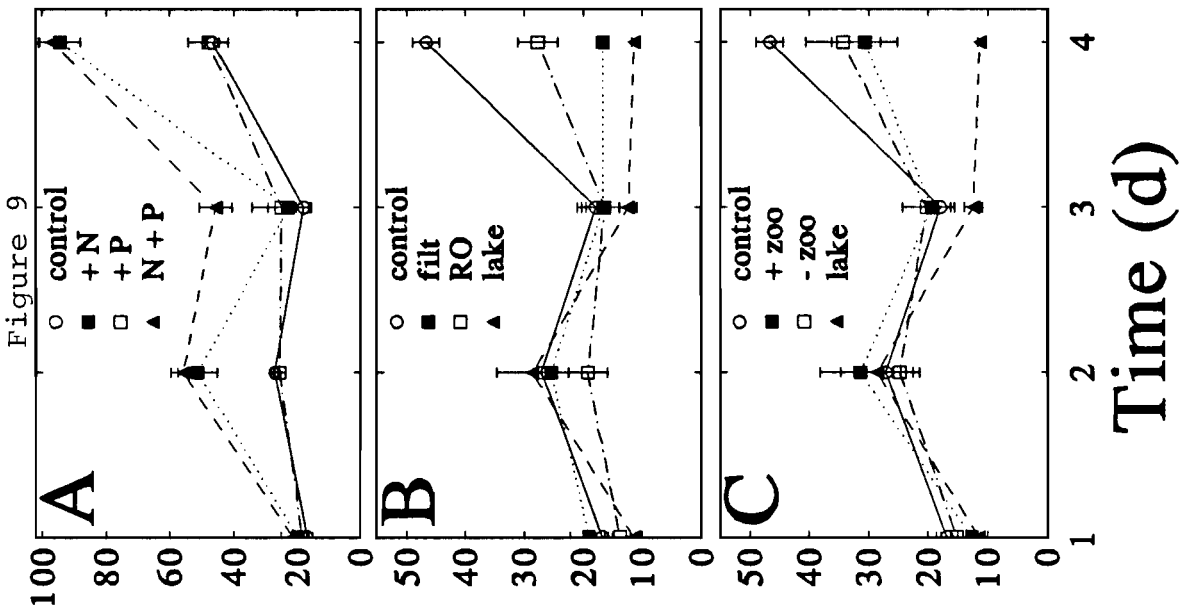

A
control
+N
+P
N+P
B
control
filt
RO
lake
C
control
+ zoo
- zoo
lake
SRP (µmol L -1)
Time (d)

Figure 9


A
control
+ N
+ P
N + P
B
control
filt
RO
lake
C
control
+ zoo
- zoo
lake
Chl a (µg L-1)
Time (d)

APPENDIX

ABSTRACTS OF RESEARCH SUPPORTED BY THE SOAP AND DETERGENT ASSOCIATION PRESENTED AT PROFESSIONAL MEETINGS

Presented in October 1991 to the Great Plains Limnological Society in Omaha, Nebraska and November 1991 to the North American Lake Management Society in Denver, Colorado

FIELD ASSESSMENT OF THE EFFECTS OF NUTRIENT REMOVAL ON PHYTOPLANKTON PRODUCTIVITY AND BIOMASS

Walter K. Dodds and Clay Randel, Division of Biology, Kansas State University, Manhattan, KS 66506

Typical nutrient addition bioassays can not be used to quantify the effects of nutrient removal. Nutrient dilution or selective removal of phosphate or ammonium in 10 L mesocosms was used to assess phytoplankton response to lowered levels of inorganic nutrients in a eutrophic pond. The nutrient removals were compared to controls and nutrient additions. All treatments were triplicated. Ammonium addition, with or without phosphate addition, stimulated algal biomass and productivity compared to controls within several days. Phosphate addition alone had no effect. Serial dilution (10% per day over 5 days for a total dilution of 41%) did not lower biomass or productivity compared to controls. A significant ($P < 0.05$) decrease in productivity relative to the pond, was seen in all mesocosms except in those with ammonium enrichments. The data suggest that nutrient removal in this pond would not lower productivity because nutrients from the sediments continuously supply algal growth needs. *In situ* mesocosm experiments can thus determine the effects of nutrient removal before implementation of costly nutrient control measures.

Presented in February 1992 to the American Society of Limnology and Oceanography at Santa Fe, New Mexico

Walter K. Dodds, Division of Biology, Kansas State University, Manhattan, KS 66506

PHOSPHATE AVAILABILITY, UPTAKE KINETICS AND REGENERATION IN PHOSPHORUS DEPLETE AND REPLETE MESOCOSMS

Mesocosms (1570 L) in a eutrophic reservoir were treated with NH_4Cl, KH_2PO_4 or nothing (control) and sampled after 8 days for $^{32}PO_4^{3-}$ and phosphatase experiments. Bioassays suggested N, N and P and P deficiency in P, control, and N mesocosms respectively. Biologically available P (BAP) and the ratio of BAP to SRP decreased as P deficiency increased. Uptake as a function of $[PO_4^{3-}]$ exhibited log-log relationships in control and N treatments and was independent of $[PO_4^{3-}]$ with P treatment. Particle associated phosphatase activity was greatest in the 0.2-3 μm size fraction in all treatments. Results indicate: 1) Calculating uptake with chemically determined substrate concentrations may overestimate true uptake with more error as substrate deficiency increases, 2) Michaelis-Menton uptake models may be inadequate, and 3) Organisms < 3 μm may dominate nutrient cycling independent of deficiency.

Presented in March 1992 to the Water and the Future of Kansas Conference in Manhattan, Kansas

NUTRIENT POLLUTION AND SURFACE WATER QUALITY

Walter K. Dodds, Division of Biology, Kansas State University, Manhattan, KS 66506

Nutrient enrichment can cause increased growth of algae (eutrophication) in lakes, ponds and streams. This algal growth may impair water quality. In extreme cases, toxic cyanobacterial (blue green algal) blooms can render water unfit for consumption. Algae can also impart undesirable odors or taste to drinking waters. When algae blooms collapse, oxygen depletion events may occur, harming fish populations. Several factors may be involved in determining the future extent of this problem in Kansas.

What Nutrients Limit Algae in Kansas Waters?

The standard belief has been that phosphorus controls algal growth in fresh waters. However, numerous reports have established that nitrogen, nitrogen and phosphorus, other nutrients, or other factors may be important. Bioassays have been performed in several local bodies of water by isolating water in 10 liter carboys and adding different nutrients. In Kimball Pond, a small farm pond heavily influenced by cattle, nitrogen, but not phosphorus stimulated productivity. In Milford Reservoir, both nitrogen and phosphorus were required to stimulate productivity. In Fry's pond, another local farm pond with less nutrient pollution, both nitrogen and phosphorus stimulated productivity. Clearly, phosphorus is not the only important polluting nutrient in all Kansas waters.

Where do Fish and Zooplankton Fit into the Scenario?

Algae are eaten by zooplankton (eg. water fleas), zooplankton by small fish, and small fish by larger fish. It has been demonstrated that in some systems algal biomass can be controlled by zooplankton grazing. How does this apply to Kansas? In Fry's pond, removal of zooplankton by added bluegill (in 1500 liter enclosures) increased algal biomass. Nutrient addition stimulated algae, but a rapid increase in zooplankton rapidly negated this effect. These findings provide evidence that aquatic food chains may well constrain nutrient pollution effects in Kansas.

What Factors Influence Nuisance Cyanobacterial Blooms?

It is clear that high levels of nutrient pollution will stimulate cyanobacterial blooms which at times may be toxic. Control of nitrogen and not phosphorus may stimulate these blooms by causing nitrogen limitation. Furthermore, when blooms occur in low alkalinity waters and float to the surface, they may be limited by atmospheric CO_2. In these situations, the expected doubling in atmospheric CO_2 over the next 65 years may exacerbate the bloom problem.