NUTRIENT-PHYTOPLANKTON INTERACTIONS IN
FLATHEAD LAKE, MONTANA

A final report on research supported by
the Soap and Detergent Association.

Submitted September 1988

Walter K. Dodds
John C. Priscu

Department of Biology
Montana State University
Bozeman, MT 59717

Table of Contents   Page

Introduction

This report represents, to the best of our knowledge, the most extensive documentation of nutrient dynamics and deficiency in an oligotrophic lake where both nitrogen and phosphorus control primary productivity. Although much of the work originally proposed was to support an ecosystem model developed by Dr. Charles Hall, for Flathead Lake, Montana, the work completed thus far stands on its own, presenting new insights into nutrient dynamics in aquatic systems. Moreover, additional experiments, inspired by the originally proposed research, were conducted. Explanation of these additional experiments is included in the following report since they contribute to the further understanding of nutrient dynamics in aquatic systems and were supported by funding from the Soap and Detergent Association.

Our first development in this project was a water sampler. This was required for collection of large amounts of water necessary for the time-course nutrient bioassays. The design of this sampler is covered in Chapter 1. The manuscript was recently published (Freshwater Biology 20:113-116).

Chapter 2 deals with nutrient deficiency in Flathead Lake. The inclination of lake managers and aquatic scientists in the past has been to assume a priori phosphorus deficiency in fresh waters, even though deficiency of other nutrients has been shown to be important (see introduction to Chapter 3). This is why it was originally

1

proposed to test for phosphorus, as well as other nutrient (primarily nitrogen) deficiencies in Flathead Lake. The results of a series of different bioassays are reported in Chapter 2 and compared to the standard technique of measuring $^{14}CO_2$ uptake after nutrient fertilization of lake water. Interestingly, simultaneous addition of N and P was virtually always required to stimulate primary production. Significant effects of single nutrient additions occurred for N during February only; P enrichment alone never significantly stimulated $^{14}CO_2$ uptake.

Chapter 3 further explores the idea of simultaneous deficiency of N and P. This chapter states the assumptions which lead aquatic scientists and lake managers to believe that phytoplankton communities can be deficient in only one nutrient at a time in lakes and then presents theory and evidence from Flathead Lake and three other western Montana lakes which challenges these assumptions. In addition, the implications of simultaneous deficiency of N and P are discussed with regard to lake management.

Stimulation of dark $^{14}CO_2$ uptake by NH_4^+ was one of the bioassays used to assess N deficiency in this study. Despite the fact that this bioassay is commonly used by limnological and oceanographic investigators, we determined that nitrifying bacteria can confound the results of this bioassay. Chapter 4 presents a series of tests we conducted which challenge past use of this assay. We then propose alternative ways to conduct this the assay correctly.

Chapter 5 describes a project in which a new method for nutrient enrichment of sediments and subsequent determination of photosynthetic O_2 production was tested. This technique, when applied to sediments in Flathead Lake (Yellow Bay), indicated N deficiency. The method was not included in the original proposal, but was later incorporated into our study plan to examine nutrient deficiencies in a different component of the Flathead Lake ecosystem.

Starting with Chapter 6, we address the issues of nutrient fluxes. In this chapter we present seasonal data which demonstrate: 1) how uptake kinetics are dependent upon NH_4^+, NO_3^- and PO_4^{3-} concentrations, 2) how NH_4^+ and PO_4^{3-} regeneration rates change over the course of a year, and 3) how NH_4^+ and PO_4^{3-} uptake and regeneration is partitioned between different size classes of plankton. Regeneration experiments also led to the unexpected discovery that _Daphnia_ can actually decrease PO_4^{3-} uptake by some size classes of phytoplankton (Chapter 7). Seasonal responses of NO_3^-, NH_4^+, PO_4^{3-} and CO_2 uptake to light levels are discussed in Chapter 8. These experiments indicated that CO_2, NH_4^+, and NO_3 uptake was dependent on light, whereas PO_4^{3-} uptake never showed a clear response to variable light. In Chapter 9 the increases in PO_4^{3-} concentrations and fluxes of $^{15}NO_3^-$ and $^{15}NH_4^+$ in 4 day mesocosm experiments are described.

Chapter 10 provides baseline data which is not included in previous chapters, and a summary. Please keep in mind

that individual chapters were written for publication in different peer-reviewed journals. Thus, there is some overlap of contents. Figures and tables are located at the end of each chapter. You will also notice some slight formatting differences between chapters. Again, this is necessary to meet the varying requirements of scientific publications and their constituencies. We hope this does not cause you any inconvenience.

Acknowledgements

Chapter 1

An Inexpensive, Simple Device for Sampling

Large Volumes of Lake Water from

Discreet Depths

5

Certain limnological experiments, such as large scale bioassays and some enclosure (limnocorral) studies require the collection of large volumes of water from discreet depths. Conventional methods to sample large volumes of water (eg. > 10 l) are costly and/or complicated and often require a considerable amount of time and effort. It is possible to sample large volumes of lake water with a device which is inexpensive and can be constructed using common hand tools in less than 2 hours. In addition the materials of construction are usually readily available.

The sampler (Fig. 1) is manually operated and works using water displacement to bring up water from a pre-determined depth. The operator puts the weighted sampling tube into the water and pushes the bottom of the collecting bucket below the surface of the water. The displaced water is replaced by water at the depth of the bottom of the sampling tube. As much volume is discarded as it takes to fill the sampling tube and all subsequent water is collected. The sampling tube is connected to the collection bucket by a flexible hose so the bucket can be emptied easily.

Variable depths can be sampled by varying the length of sampling tube. The sampling tube can be lengthened on board using polyvinyl chloride (PVC) tubing with male/female screw fittings. It is also possible to put several stoppered holes along the length of the sampling tube and remove the

stopper at the specific depth at which collection is desired. Transparent sampling tube can be used to minimize zooplankton avoidance or opaque tubing can be used to minimize light shock to phytoplankton.

A 5 m sampler was constructed for less than $30 (June 1987). Three hundred l of water was collected with this device at a rate of 7.5 l/min. The rate of collection is limited by the rate the bucket refills. Water was collected from 5 m using a 17.5 l Van Dorn bottle and a winch at about the same rate as the displacement sampler.

To test if the displacement sampler is toxic, the rate $^{14}CO_2$ uptake of phytoplankton collected with the displacement sampler was compared to the rate from samples collected with a 2 l Van Dorn bottle. Six separate water samples were collected from 5 m alternating between the displacement sampler and the Van Dorn bottle. $^{14}[C]-NaH_2CO_3$ was added at 0.011 µCi/ml to 100 ml bottles which were incubated **in situ**. After 4 hours, samples were filtered onto Whatman GF/F filters and counted. The Van Dorn samples took up a mean of 975 DPM/ 4 h/ 100 ml and the displacement samples took up 1227 (pooled Student's t test, P < 0.01). This indicated inhibition by the Van Dorn bottle or stimulation by the displacement sampler. Next, a 8 l sample was taken with the Van Dorn Bottle and 1/2 of this sample was poured through the displacement sampler. There was no significant difference in the rates of $^{14}CO_2$ between lake water taken with the Van Dorn bottle and that subsequently poured

7

through the displacement sampler ($P > 0.10$, pooled Student's t test). This indicates that the Van Dorn bottle had inhibitory effects, rather than stimulation of photo-synthesis by the sampler.

Although the sampler is not as versatile as a line operated sampler with a messenger, it allows investigators to take large volumes of water rapidly from a specific depth at very low cost. It may also be less toxic to phytoplankton than collection with a standard Van Dorn bottle.

Figure 1. Displacement Water Sampler. a) bail on bucket. b) black plastic cover to minimize light shock to phytoplankton. c) polyethylene liner (Glad, Sheer Strength plastic bag) which is attached on the outside of the bucket. d) plastic sampling bucket. e) FDA- approved flexible vinyl tubing (1.25 inch O.D.). f) hose clamps. g) poly vinyl chloride (PVC) adapter (1.25 inch OD pipe to male screw fitting). h) 1.25 inch O.D. PVC tubing. i) 2 kg weight to keep sampling tube at depth.

Figure 1
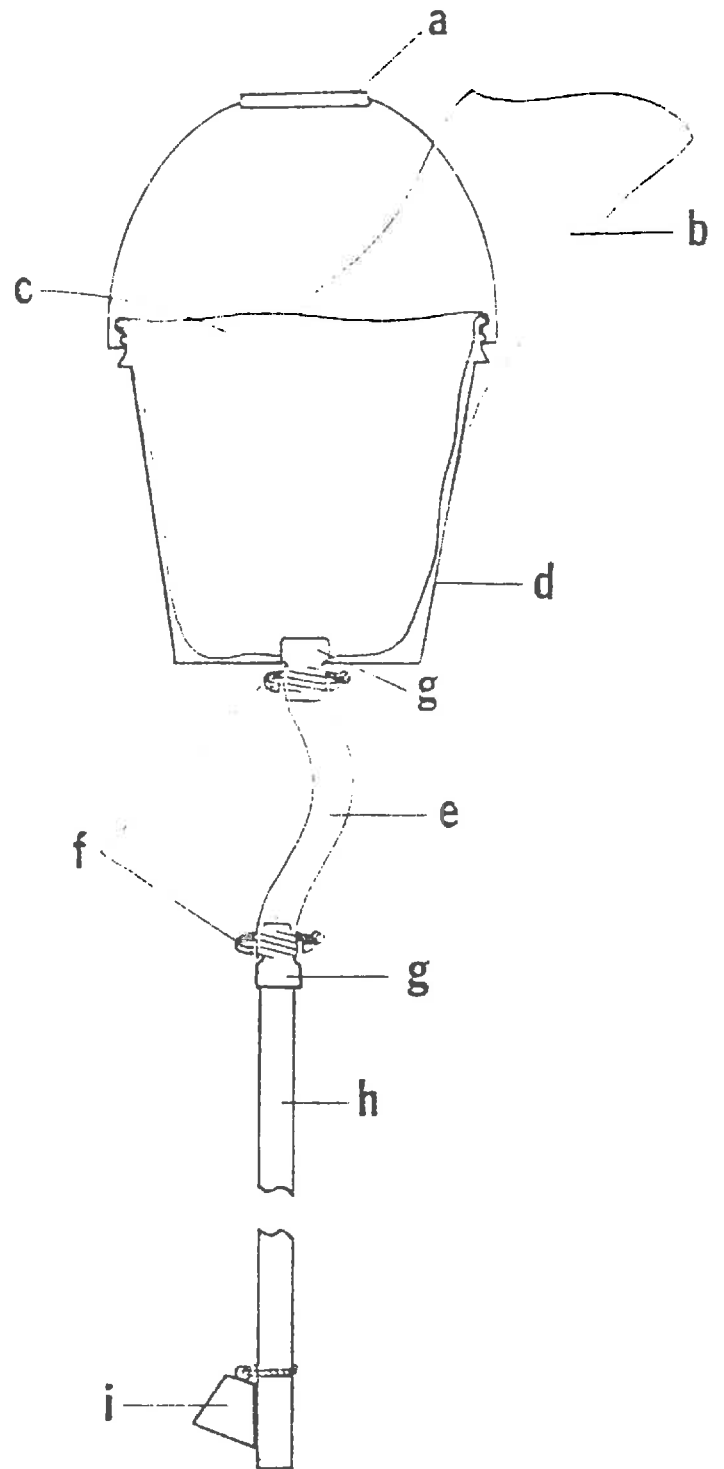
Chapter 2

**A Seasonal Comparison of Methods for Assessment of Nutrient
Deficiency of Phytoplankton in a Large Oligotrophic Lake**

Short-term (hours) physiological and long-term (days) biomass-based bioassays were compared to assess nutrient deficiency in Flathead Lake, Montana over the course of a year. Epilimnetic particulate C:N and C:P ratios were always above Redfield proportions indicating N and P deficiency respectively, whereas N:P ratios were generally above 16:1, indicating P deficiency. Simultaneous additions of NH_4^+ and PO_4^{3-} to 20 l carboys significantly increased chlorophyll a concentrations, $^{14}CO_2$ uptake, and particulate N concentrations after 4.5 days, whereas single additions of N or P had no effect, except during the winter when NH_4^+ alone stimulated $^{14}CO_2$ uptake. Particulate P increased within 0.5 days in the P fertilized treatments, but further increases in particulate P usually occurred only in the presence of N. Stimulation of dark $^{14}CO_2$ incorporation by NH_4^+ enrichment occurred during June and October, indicating N deficiency. At times, stimulation of saturated $^{15}NH_4^+$ uptake by PO_4^{3-} and saturated uptake of $^{32}PO_4^{3-}$ by NH_4^+ occurred, indicating P and N deficiency, respectively. Deficiencies in internal pools of N and P were occasionally indicated by decreased uptake of $^{15}NH_4^+$ after 12 h N exposure and decreased uptake of $^{32}PO_4^{3-}$ after 12 h of P exposure respectively. Of the assays employed, long-term responses (4.5 days) of $^{14}CO_2$ uptake and chlorophyll a concentrations provided the most reliable results. Results from short-term physiological bioassays were not as consistent. In general our results show that natural phytoplankton communities can exhibit N and P

deficiency concurrently.

Determination of nutrient deficiencies by phytoplankton is crucial to understanding how primary productivity is controlled in aquatic systems. Accurate information regarding nutrient deficiency is useful to both lake managers and aquatic ecologists. Therefore, reliable, easily conducted nutrient bioassays must be available. Stimulation of $^{14}CO_2$ uptake by nutrient enrichment has been used both as a short and long-term bioassay indicator of nutrient deficiency. The effectiveness of this technique has been criticized, mainly because short-term nutrient enrichment can lead to a decrease in $^{14}CO_2$ uptake caused by a limiting nutrient (eg. Lean and Pick 1981). Other short-term physiologically based techniques have been employed, but none have been shown to provide incontrovertible results (eg. White et al. 1985; Vincent 1981a,b) and results of such studies are often not compared to long-term, growth based bioassays (Hecky and Kilham 1988).

We applied both short-term physiological bioassays and long-term growth based assays over the annual cycle in a large oligotrophic lake. We attempted to relate short-term physiological response to increased growth associated with N and P enrichment.

Rationale for Bioassays
Biochemical Composition of Suspended Particulate Matter

Molar ratios of N:P in algal cells tend to reflect the N:P supply ratio under which they were grown (Suttle and

Harrison 1988). Therefore, the N and P content of particulate matter may reflect nutrient supply ratios and thus nutrient deficiencies in natural phytoplankton assemblages. Consequently, we collected data on N and P concentrations of particulate matter in the epilimnion throughout the year.

Growth Bioassays

Assays which use long-term growth of natural phytoplankton to gauge the response to nutrient enrichment, directly address how nutrient loading relates to eutrophication. Containment effects (Hecky and Kilham 1988) and spurious short-term effects (Lean and Pick 1981) can complicate the use of $^{14}CO_2$ uptake to gauge nutrient response. For these reasons, we enriched large (20 l) carboys of lake water with nutrients, incubated the carboys in situ to approximate natural conditions, and followed the enrichment response of $^{14}CO_2$ uptake, chl a, particulate N, and particulate P concentrations over several days. We were thus able to discern when algal growth had actually occurred, and stop experiments before populations within the carboys started to collapse. Furthermore, the large volume allowed repeated sampling for all of the parameters we followed.

Dark ^{14}C Uptake

Stimulation of dark $^{14}CO_2$ uptake by NH_4^+ has been proposed by Morris et al. (1971) as a short-term physiological assay to indicate N deficiency. This assay is based upon the observation that NH_4^+ enrichment of N-deficient uni-algal phytoplankton cultures causes an increase in dark $^{14}CO_2$ incorporation. Despite documented uncertainties about using this assay on natural phytoplankton populations (Vincent 1981a), it is often used to indicate N deficiency (ie. Elser et al. 1988, Groeger and Kimmel 1988, White et al. 1985).

Stimulation of V_{max}

It has been shown that PO_4^{3-} addition stimulated saturated uptake of dissolved inorganic N in an oligotrophic lake (Whalen and Alexander 1984) but not in a eutrophic reservoir (Toetz, 1981). Availability of a deficient nutrient can control the rate of incorporation of a nutrient which has a relatively high supply rate. It follows that enrichment by a nutrient which is in short supply can also stimulate uptake of other nutrients. Therefore under P deficiency, N uptake should be stimulated by P fertiliza-tion, and with N deficiency N addition should stimulate P uptake. We exposed aliquots of lake water to NH_4^+ or PO_4^{3-} for 12 h and then measured the uptake of NH_4^+ or PO_4^{3-} respectively. Twelve hour pre-incubation was used to allow

the enriching nutrient time to relieve deficiency of that
nutrient.

Internal Pools

Depletion of intracellular pools of a nutrient has
been shown to indicate deficiency of that nutrient. If
experimental nutrient exposure lowers the maximum rate of
uptake (V_{max}) of a particular nutrient, the following is
implied: (i) internal pools of the nutrient were below
saturation, and (ii) incorporation of the nutrient into
cellular material has limited uptake. Therefore, the
response of $^{15}NH_4^+$ or $^{32}PO_4^{3-}$ uptake to 12 h prior exposure to
NH_4Cl or KH_2PO_4 was used as an assay for N and P deficien-
cies, respectively. This assay approximates the luxury
consumption assays (which examined short-term changes in PN
and PP) used by Vincent (1981a, b) and White et al. (1985)
to estimate nutrient deficiency, except that the problem of
detrital interference is avoided by using isotopic
incorporation.

Materials and Methods
Study Site and Common Methods

Experiments were conducted on Flathead Lake, a 460 km^2,
100 m deep lake in northwestern Montana (Potter and
Stanford, 1975). The lake is oligotrophic with epilimnetic
chl $\underline{a}$ ranging from 0.1 to 1.1 µg l^{-1}, and epilimnetic NH_4^+,
NO_3^- and PO_4^{3-} concentrations usually below 0.93, 0.11 and

0.064 µM, respectively, during summer stratification. Water was collected with a displacement sampler (Dodds and Priscu 1988) from 5 m in the deepest part of the lake (zone of maximum primary productivity (Stanford et al. 1983)).

Phaeophytin corrected chlorophyll retained on Whatman GF/F filters was determined fluorometrically (Strickland and Parsons 1972). NH_4^+, NO_3^- + NO_2^- and soluble reactive phosphorus (SRP) were determined on 10 ml samples by the phenol-hypochlorite (Solorzano 1969), cadmium reduction (Eppley 1978), and phospho-molybdate (Strickland and Parsons 1972) methods respectively. Particulate P was determined by a dry oxidation technique (Solorzano and Sharp 1980) and particulate C and N on a Carlo-Erba 1106 elemental analyzer after filtration onto pre-combusted Whatman GF/F filters.

Growth-Based Bioassays

Water was collected in early evening, mixed in 100 l polyethylene lined containers, and siphoned into twenty liter collapsible polyethylene carboys. Care was taken during filling and subsequent sampling not to expose water to surface light which would cause long-term inhibition of phytoplankton. Immediately after filling, carboys were fertilized with a final enrichment of 0.2 µM KH_2PO_4 (low P), 1.0 µM NH_4Cl (low N), 2 µM KH_2PO_4 (high P), 10 µM NH_4Cl (high N), 10 µM $NaNO_3$, 10 µM $NaNO_3$ + 1 nM Na_2Mo_4, 1/10 strength micronutrient solution (Castenholz 1981) or combinations of the above. Treatments were duplicated in June and August

and triplicated in November, February, and May. Carboys were suspended in the lake to simulate light at the depth of collection. Rate of $^{14}[C]-NaH_2CO_3$ incorporation was determined daily on 100 ml aliquots from each carboy. Isotope was added to a final concentration of 11 nCi ml^{-1} and samples were incubated in situ at 5 m for 4 h during mid-day. Samples were then filtered on Whatman GF/F filters, washed 5 times with 5 ml deionized water, and counted with liquid scintillation spectrometry. Aliquots were also removed daily from the carboys for determination of water chemistry and chlorophyll a concentration.

Dark $^{14}CO_2$ Uptake

Dark $^{14}CO_2$ incorporation was measured on 3 control and 3 samples enriched with 10 µM NH$_4$Cl. $^{14}[C]-NaH_2CO_3$ was added to a final concentration of 0.33 µCi ml^{-1}. Following 8 h of dark incubation at lake temperature, the samples were filtered under reduced light onto Whatman GF/F filters and counted as above.

Stimulation of V_{max}

Experiments to examine stimulation of NH$_4^+$ uptake after 12 h of PO$_4^{3-}$ enrichment were conducted in 6 500 ml bottles of lake water collected in the early evening. Half of these bottles were enriched with 2 um KH$_2$PO$_4$ and all 6 bottles were incubated in the dark at 20^{0}c. After 12 h, the 3

19

unenriched bottles were enriched with 2 um KH_2PO_4, and 10 uM of 99 atom% $^{15}NH_4Cl$ was added to all bottles which were then incubated for 8 h _in situ_ with the isotope. Samples were then filtered on precombusted Whatman GF/F filters, which were analyzed for enrichment by optical emission spectrometry (Timperley and Priscu 1986) following Dumas combustion (Fiedler and Proksch 1975). Particulate N was determined on a subsample of each filter. We did not see significant growth or decrease in SRP levels during the 12 h pre-incubation in this experiment. This design allowed us to examine the effect of P addition on N uptake in samples exposed to P for different amounts of time.

To measure stimulation of PO_4^- uptake by NH_4^+, 8 100 ml bottles of lake water were filled each evening. Four bottles were immediately enriched with 10 uM NH_4Cl and all 8 bottles were incubated for 12 h in the dark at 20^0c. After 12 h, the 4 unenriched bottles were enriched with 10 uM NH_4Cl. All bottles were then enriched with 2 uM KH_2PO_4 and 666 Bq ml^{-1} carrier free $^{32}[P]-H_3PO_4$. Samples were incubated for 1 h _in situ_ and then filtered on Gelman GN-6 filters. Blank filters beneath the sample filters were used to correct for label absorbed on filters. Formalin kills showed significant amounts of abiotic labeling in the particulate fraction. Gelman GN-6 filters showed the same retention of ^{32}P labeled particulates as Whatman GF/F filters (P > 0.10, n=6, _t_ test), but background filters showed much lower counts and less variability within

treatments. Filters were counted by liquid scintillation spectrometry.

Depletion of Intracellular Pools

To test if prior exposure to NH_4^+ lowered uptake of NH_4^+, 6 500 ml bottles of lake water were collected in the early evening. Three of these bottles were enriched with 5 uM NH_4Cl and all bottles were incubated for 12 h at 20^0c in the dark. After 12 h, the 3 unenriched bottles were enriched with 5 μM NH_4Cl and then all bottles were enriched with 5 μM, 99 atom % $^{15}[N]-NH_4Cl$. After 8 h of incubation at 5 m in the lake, the samples were analyzed for ^{15}N enrichment as described above.

To test if pre-exposure to PO_4^{3-} lowered uptake of $^{32}[P]-H_3PO_4$, 8 100 l bottles of lake water were collected during the early evening. Four of these bottles were enriched with 2 μM KH_2PO_4 and all 8 bottles were incubated for 12 h at 20^0c in the dark. After 12 h, the 4 unenriched bottles were enriched with 2 μM KH_2PO_4 and 666 Bq ml^{-1} of carrier free $^{32}[P]-H_3PO_4$ was then added to all samples. After 1 hour of incubation, ^{32}P uptake was measured as discussed above.

Results

Biochemical Composition of Suspended Particulate Matter

Chemical ratios of particulate matter did not conclusively indicate N or P deficiency in Flathead Lake.

The molar C:P ratio was always above the ratio of 106:1 which has been observed for cells growing under nutrient sufficient conditions (Redfield 1958), (Fig. 1) indicating P deficiency. The ratio of C:N was also above the Redfield ratio of 6.6:1, indicating N deficiency (Fig. 1). The ratio of N:P was above the Redfield ratio at all times of the year (except February) (Fig. 1), indicating P deficiency. These molar ratios of particulate matter should be used with caution for inferring algal nutrient deficiencies, because detrital seston (which can vary seasonally) can contribute significantly to the particulate organic matter of lakes (Priscu and Goldman 1983).

Long-Term Growth-Based Bioassays

Simultaneous additions of N and P were required to increase primary productivity in Flathead Lake during most of the year. June time-course experiments showed no affect 4.5 days after N or P addition alone, but simultaneous addition of N and P caused an approximately 5 fold increase in primary productivity (Fig 2B). Approximately 2.5 days of fertilization were required before a measurable response to nutrient enrichment was recorded. June chlorophyll data showed similar trends (Fig. 2A).

Except in February, the largest increase in $^{14}CO_2$ incorporation and chlorophyll a was measured in treatments receiving simultaneous additions of N and P (Fig. 3). At other times of the year, there was slight stimulation by N

or P alone, but the response was never equal to that seen with simultaneous N and P enrichment. Chlorophyll $\underline{a}$ response to nutrient enrichment after 4.5 days usually reflected the ^{14}C response (Fig. 3), but a significant nutrient enhancement was not always present using both indicators, or both $^{14}CO_2$ uptake and chl $\underline{a}$ responded in differently (ie. Fig 4C and E, in the P treatments). Also, after 4.5 days, 2 μM PO_4^{3-} often decreased ^{14}C uptake, chlorophyll $\underline{a}$ concentrations, or both (ie. Fig. 4A, C, D, and E).

In June, when micro-nutrients were added in addition to N and P, there was less of an increase in primary productivity than seen with the simultaneous additions of N and P (Fig. 3A). In August, $NO_3^- + P$ treatments also showed significant increases in $^{14}CO_2$ uptake and chl $\underline{a}$, but they were about 45% less than $NH_4^+ + P$ treatments. Mo added with $NO_3^- + P$ had no additional effect (Fig. 3B). In November, as in August, $NH_4^+ + P$ treatments caused a greater increase than $NO_3^- + P$ treatments (Fig. 3C). Results of addition of KCl in August were not significantly different than control ($P > 0.05$) showing KH_2PO_4 or NH_4Cl additions did not cause inhibition because of added K or Cl (Fig. 3B).

In June, particulate P (PP) concentrations increased significantly within in 0.5 days, in all treatments with P addition, but further increases in PP were only seen in the presence of N (Fig. 4A).

The same PP response to N and P fertilization that was seen in June was present throughout the year (except during

February) (Figs. 5A, B). In cases where PP only increased after 0.5 d in the presence of NH_4^+ (June, August and November) simultaneous N and P deficiency was shown. When PP increased in the presence of PO_4^{3-} alone, it showed P deficiency (Fig. 5B). The 0.5 d surge in P incorporation should be interpreted as a short-term physiological response, and the increase in PP over 4.5 days as a growth response.

In contrast to the PP response to nutrient fertilization, there was never a response of particulate N (PN) to single additions of N or P after 0.5 days (Fig. 4B). There was, however, a significant increase in PN concentration when N and P were added simultaneously in June, August, and November (Fig. 4B, Fig. 7), indicating simultaneous N and P deficiency.

Short-term physiologically-based bioassays

N enrichment significantly stimulated V_{max} for PO_4^{3-} in July only (Table 1). However, the $^{14}CO_2$ uptake bioassays indicated supply of both N and P limited productivity throughout the year suggesting that stimulation of V_{max} of PO_4^{3-} by NH_4^+ is not indicative of nutrient deficiency when compared to long-term growth assays. In contrast, P stimulated V_{max} of $^{15}NH_4^+$, except in May and November (Table 2).

Prior exposure to N decreased N uptake significantly in August only, and rates of N uptake were significantly higher

24

after 12 h prior exposure to N in September and May (Table 3). Prior exposure to P decreased P uptake in February only, the one time of year when the $^{14}CO_2$ bioassays indicated no P deficiency. However, prior exposure to P never actually caused a significant stimulation of P uptake as seen in the N stimulation of N uptake experiments discussed above (Table 4). NH_4^+ stimulated dark $^{14}CO_2$ uptake significantly in June and October only (Table 5). In contrast, carboy experiments showed N to be important at all times of the year.

Discussion

Simultaneous N and P deficiencies

Under simultaneous nutrient deficiency, various components of a phytoplankton assemblage can be deficient in different nutrients both temporally and spatially. This can occur when supply of the deficient nutrients is close to the relative demands of the phytoplankton species present. Spatial and temporal variation in nutrient supplies, coupled with varied competitive abilities of phytoplankton, creates an assemblage which exhibits simultaneous nutrient deficiencies. If the supply of either N or P increases, the assemblage shows signs of deficiency in the other nutrient. Other studies have shown that individual species within a natural phytoplankton assemblage respond differently to available nutrients (Lehman and Sandgren 1985, Tilman 1982).

25

Simultaneous deficiency in N and P is indicated during most of the year in Flathead Lake. A common a priori assumption about freshwaters is that they are deficient in P only, and that they are limited by only one nutrient at any one time (Schindler 1977). However, theory and empirical data indicate that more than one nutrient can control the primary production of a phytoplankton community (Hecky and Kilham 1988). Simultaneous deficiency in P and N has been documented for coastal oligotrophic lakes in British Columbia (Suttle and Harrison, 1988), African Lake George (Viner 1973), several New Zealand Lakes (White et al. 1985) and in Crater Lake and Lake Tahoe (Lane and Goldman 1984). Some Canadian shield lakes exhibit characteristics of mild N deficiency at the same time that they exhibit P deficiency (Healey and Hendzel, 1980). In addition, many systems show temporal transitions between N and P deficiency, eg., several Minnesota lakes (Elser et al. 1988), a tundra pond (Kalff 1971), some deep prairie lakes (Prepas and Trimbee 1988), and a European hyper-eutrophic lake (Zevenboom et al. 1982). Estuarine systems often exhibit gradients between freshwater P deficiency and marine N deficiency (Sakshaug and Olsen 1986). In the transition area (either temporal or spatial) between N and P deficiency, these systems presumably exhibit simultaneous N and P deficiencies.

Comparison of Bioassays

Combined data from all bioassays (Table 6) show that assays indicate N and P deficiency at all times of the year. Of the assays used in our study to assess nutrient deficiency, we believe that the strongest conclusions can be drawn from time-course measurements of $^{14}CO_2$ uptake coupled with measurements of changes in chlorophyll a, PN, and PP concentrations. ^{14}C bioassays can be unreliable over short time periods (Lean and Pick 1981) but our results show that a time-course over several days in large containers (20 l) can overcome the problems associated with short-term incubations.

Two types of problems occurred in our study when using short-term physiologically-based bioassays or biochemical composition of suspended particulate matter: 1) Short-term response does not necessarily reflect the effect of nutrient enrichment on the entire community, and 2) Assays which show only one type of nutrient deficiency will be misleading (ie. N:P ratios) because they do not allow for the possibility of simultaneous nutrient deficiency.

There has been considerable discussion about deviations from Redfield ratios of particulate matter as indicators of nutrient deficiency (Healey and Hendzel 1980; Hecky and Kilham 1988; Sakshaug and Olsen 1986; Suttle and Harrison 1988; White et al. 1985). In Flathead Lake, C:P ratios indicate P deficiency year round, C:N ratios indicate N deficiency all year, and N:P indicate P deficiency except

27

in February. The $^{14}CO_2$ bioassays indicated simultaneous N and P deficiency except in February, when N deficiency appeared to predominate. Therefore, particulate C:N:P ratios support the long-term ^{14}C bioassays in that they imply both N and P deficiency in Flathead Lake.

Luxury consumption of P over a few hours has been used to indicate P deficiency in a number of New Zealand lakes (Vincent 1981a). We saw a large increase in PP concentrations in the carboy experiments after 0.5 days except in February. However, it is hard to be certain if this surge in uptake was caused by bacteria or phytoplankton. Bacteria may out-compete phytoplankton for PO_4^{3-} (Currie and Kalff 1984), and bacteria appear to have a higher requirement for P than phytoplankton (Vadstein et al. 1988). Therefore, this type of bioassay may not always be directly applicable to phytoplankton P deficiency. Similarly, bacteria have been shown to compete with phytoplankton for N (Wheeler and Kirchman 1986), so increases in PN may not be directly related to increased N uptake by phytoplankton. The fact that we never observed an increase in PN after 0.5 d may reflect the slower turnover rate of PN with respect to PP.

Long-term changes (over periods of days) in PP and PN concentrations following enrichment by N and P appeared to be a more reliable indicator of N and P deficiency than following changes over a few hours. These assays are related to the short-term V_{max} experiments. For example, if

28

P stimulates V_{max} of NH_4^+, then P enrichment should result in higher observed PN concentrations over longer periods. A problem with the short-term V_{max} experiments is that longer time periods may be required for physiological response. This is illustrated by Fig. 2 which shows that 2.5 days were required before there was a nutrient-caused increase in $^{14}CO_2$ uptake. Such a delay may explain why P stimulated N uptake (20 h total incubation time), but N stimulated P uptake only once (13 h total incubation time).

Results from turnover experiments, designed to test for depletion of internal nutrient pools did not support the results from other assays. P uptake decreased with prior P exposure once, and N uptake decreased with prior N uptake once. Considering the time and effort involved with ^{32}P and ^{15}N experiments, and the inconsistent nature of the data generated by the turnover experiments, we do not advise using such experiments to determine nutrient deficiency in oligotrophic systems.

We saw mixed results using NH_4^+ stimulation of dark $^{14}CO_2$ uptake as an indicator of N deficiency. It has been suggested that NH_4^+ addition should cause at least a 100% stimulation of dark $^{14}CO_2$ uptake before it conclusively shows N deficiency (White et al. 1985), and that NH_4^+ stimulation of dark $^{14}CO_2$ uptake is not a reliable indicator of combined demand for inorganic N by heterocystous cyanobacteria (Vincent 1981b). Furthermore, dark $^{14}CO_2$ uptake by NH_4^+

oxidizing bacteria can be stimulated by NH_4^+, which may confound results (unpublished data).

In summary, long-term bioassays were more consistent indicators of nutrient deficiency than short-term physiological assays. Photosynthetic $^{14}CO_2$ uptake measured with chl **a**, PP and PN over a period of days gave the most pertinent results because these values directly reflect algal growth. Results from short-term physiological bioassays can be confounded by bacterial metabolism and may reflect short-term (min) variations in physiological state caused by factors other than nutrients (i.e. light, temperature, etc.). Because cyanobacterial picoplankton are often abundant (Stockner 1988), neither size fractionation or antibiotic treatment can be used to successfully remove bacteria from physiological bioassays and have the bioassays remain representative of the entire phytoplankton community. Finally, long-term nutrient responses are usually the most interesting to lake managers, and results from short-term bioassays often cannot be extrapolated over more than one day. Results from the assays we employed in this study suggest both N and P deficiency and that both nutrients are important in regulating algal growth.

Acknowledgements

References

Castenholz, R. W. 1981. Isolation and cultivation of thermophilic cyanobacteria, p. 236-246. IN: M. P. Starr, H. G. Truper, A. Balows and H. G. Schlegel [ed.] The Prokaryotes. Springer Verlag, Berlin.

Currie, D. J., and J. Kalff. 1984. A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus. Limnol. Oceanogr. 29:298-310.

Dodds, W. K., and J. C. Priscu. 1988. An inexpensive device for sampling large volumes of lake water from discrete depths. Freshw. Biol. 20:113-116.

Elser, J. J., M. M. Elser, N. A. MacKay, and S. R. Carpenter. 988. Zooplankton-mediated transitions between N- and P-limited algal growth. Limnol. Oceanogr. 33:1-14.

Eppley, R. 1978. Nitrate uptake, pp. 401-409 in Handbook of phycological methods. Physiological and biochemical methods. J. A. Hellebust and J. S. Craigie (eds.). Cambridge Univ. Press, Cambridge.

Fiedler, R., and G. Proksch. 1975. The determination of nitrogen15 by emission and mass spectrometry in biochemical analysis: a review. Anal. Chim. Acta. 78:1-62.

Groeger, A. W., and B. L. Kimmel. 1988. Photosynthetic carbon metabolism by phytoplankton in a nitrogen-limited reservoir. Can. J. Fish. Aquat. Sci. 45:720-730.

Healey, F. P., and L. L. Hendzel. 1980. Physiological
    indicators of nutrient deficiency in lake
    phytoplankton. Can. J. Fish. Aquat. Sci. 37:442-453.

Hecky, R. E., and P. Kilham. 1988. Nutrient limitation of
    phytoplankton in freshwater and marine environments: A
    review of recent evidence on the effects of enrichment.
    Limnol. Oceanogr. 33(4):796-822.

Kalff, J. 1971. Nutrient limiting factors in an Arctic
    tundra pond. Ecology 52(4):655-659.

Lane, J. L. and C. R. Goldman. 1984. Size-fractionation of
    natural phytoplankton communities in nutrient bioassay
    studies. Hydrobiologia 118:219-223.

Lean, D. R. S. and F. R. Pick. 1981. Photosynthetic response
    of lake plankton to nutrient enrichment: a test for
    nutrient limitation. Limnol. Oceanogr. 26(6):1001-1019.

Lehman, J. T., and C. R. Sandgren. 1985. Species-specific
    rates of growth and grazing loss among freshwater
    algae. Limnol. Oceanogr. 30:34-46.

Morris, I., C. M. Yentsch, and C. S. Yentsch. 1971. The
    physiological state with respect to nitrogen of
    phytoplankton from low nutrient subtropical water as
    measured by the effect of ammonium ion on dark carbon
    dioxide fixation. Limnol. Oceanogr. 16:859-868.

Potter, D. S., and J. A. Stanford. 1975. Influences on the
    plankton communities of oligotrophic Flathead
    Lake. Verh. Internat. Verein. Limnol.
    19:1790-1797.

Prepas, E. E., and A. M. Trimbee. 1988. Evaluation of
    indicators of nitrogen limitation in deep prairie lakes
    with laboratory bioassays and limnocorrals. Hydrobiol.
    159:269-276.

Priscu, J. C. and C. R. Goldman. 1983. Suspensoid
    characteristics in subalpine Castle Lake, California I.
    Chemical composition. Arch. Hydrobiol. 97:373-388.

Redfield, A. C. 1958. The biological control of chemical
    factors in the environment. Amer. Sci. 46:205-221.

Sakshaug, E., and Y. Olsen. 1986. Nutrient status of
    phytoplankton blooms in Norwegian waters and algal
    strategies for nutrient competition. Can. J. Fish.
    Aquat. Sci. 43:389-396.

Schindler, D. W. 1977. Evolution of phosphorus limitation in
    lakes. Science 195:260-262.

Solorzano, L. 1969. Determination of ammonia in natural
    waters by the phenolhypochlorite method. Limnol.
    Oceanogr. 14:799-801.

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

Stanford, J. A., P. J. Stewart, and B. K. Ellis. 1983.
    Limnology of Flathead Lake, Final Report. Flathead
    River Basin Environmental Impact Study. U.S.
    Environmental Protection Agency, Helena, Montana.

Stockner, J. G. 1988. Phototrophic picoplankton: An overview
from marine and freshwater ecosystems. Limnol.
Oceanogr. 33(4):765-775.

Strickland, J. D., and T. R. Parsons. 1972. A practical
handbook of seawater analysis. Bull. Fish. Res. Board
Can. (2nd ed.) 167.

Suttle, C. A., and P. J. Harrison. 1988. Ammonium and
phosphate uptake rates, N:P supply ratios, and evidence
for N and P limitation in some oligotrophic lakes.
Limnol. Oceanogr. 33(2):186-202.

Tilman, D. 1982. Resource competition and community
structure. Princeton Univ. Press, Princeton, N. J.

Timperley, M. H., and J. C. Priscu. 1986. Determination of
nitrogen-15 by optical emission spectrometry using
an atomic absorption-spectrometer. Analyst
111:23-28.

Toetz, D. W. 1981. Effects of pH, phosphate and ammonia on
the rate of nitrate and ammonia uptake by freshwater
phytoplankton. Hydrobiologia 76:23-26.

Vadstein, O., A. Jensen, Y. Olsen, and H. Reinertsen. 1988.
Growth and phosphorus status of limnetic phytoplankton
and bacteria. Limnol. Oceanogr. 33(4):489-503.

Vincent, W. F. 1981a. Rapid physiological assays for
nutrient demand by the plankton I. Nitrogen. J.
Plankton Res. 3:685-697.

35

Vincent, W. F. 1981b. Rapid physiological assays for
   nutrient demand by the phytoplankton II. Phosphorus. J.
   Plankton Res. 3:699-710.

Viner, A. B. 1973. Responses of phytoplankton population to
   nutrient enrichments of ammonia and phosphate, and
   some associated ecological implications. Proc. R.
   Soc. Lond. B. 183:351-370.

Whalen, S. C., and V. Alexander. 1984. Influence of
   temperature and light on rate of inorganic nitrogen
   transport by algae in an arctic lake. Can. J. Fish.
   Aquat. Sci. 41:1310-1318.

Wheeler, P. A. and D. L. Kirchman. 1986. Utilization of
   inorganic and organic nitrogen by bacteria in marine
   systems. Limnol. Oceanogr. 31:998-1009.

White, E., K. Law, G. Payne, and S. Pickmere. 1985. Nutrient
   demand and availability among planktonic
   communities - an attempt to assess nutrient
   limitation to plant growth in 12 central volcanic
   plateau lakes. New Zealand J. Mar. Freshw. Res.
   19:49-62.

Zevenboom, W., A. B. de Vaate, and L. R. Mur. 1982.
   Assessment of factors limiting growth rate of
   Oscillatoria agardhii in hypertrophic Lake Wolderwijd,
   1978, by use of physiological indicators. Limnol.
   Oceanogr. 27:39-52.

Table 1. Seasonal nutrient deficiency of phytoplankton as determined by stimulation of Vmax of PO_4^{3-} by N in Flathead Lake. The + P or + N treatments indicate 12 h prior exposure to the nutrient, the control treatments had the nutrient added with no pre-incubation. Yes = the assay was positive for N deficiency.

date	Uptake rate ($\mu M \ P \ h^{-1}$)	std. dev.	T value	d. f.	P	
11 July 1987						
+ N	0.011	0.0007				
control	0.001	0.0002	19.64	4	< 0.0005	yes
6 August 1987						
+ N	0.115	0.0116				
control	0.170	0.0883	0.87	4	> 0.25	no
21 September 1987						
+ N	0.114	0.0085				
control	0.050	0.0538	1.67	4	> 0.05	no
3 November 1987						
+ N	0.068	0.0085				
control	0.109	0.0451	1.57	6	> 0.05	no
9 February 1988						
+ N	0.034	0.0052				
control	0.037	0.0032	0.88	6	> 0.05	no
6 May 1988						
+ N	0.009	0.0057				
control	0.013	0.0029	1.20	6	> 0.10	no

Table 2. Seasonal nutrient deficiencies of phytoplankton as determined by stimulation of Vmax of NH_4^+ by P in Flathead Lake. The + P or + N treatments indicate 12 h prior exposure to the nutrient, the control treatments had the nutrient added at the beginning of the incubation. Yes = the assays was positive for P deficiency.

date	Uptake rate ($\mu M\ Nh^{-1}$)	std. dev.	T value	d. f.	P	
11 July 1987						
+ P	0.081	0.0235				
control	0.069	0.0053	12.6	4	< 0.0005	yes
6 August 1987						
+ P	0.090	0.0058				
control	0.046	0.0055	7.71	4	< 0.001	yes
21 September 1987						
+ P	0.409	0.1240				
control	0.113	0.0056	3.37	4	< 0.025	yes
3 November 1987						
+ P	0.038	0.0018				
control	0.037	0.0023	0.360	4	< 0.025	no
9 February 1988						
+ P	0.037	0.0050				
control	0.028	0.0022	2.17	4	< 0.05	yes
6 May 1988						
+ P	0.040	0.0014				
control	0.045	0.0037	1.90	4	> 0.25	no

Table 3. Decrease in NH_4^+ incorporation caused by prior exposure to NH_4^+ as an indicator of nutrient deficiency in Flathead Lake. The + P or + N treatments indicate 12 h prior exposure to the nutrient, the control treatments had the nutrient added at the beginning of the incubation. Yes = the assay was positive for N deficiency, see text for details.

date	Uptake rate (μM N h^{-1})	std. dev.	T value	d. f.	P	N deficiency
6 August 1987						
+ N	0.0655	0.0053				
control	0.0822	0.0025	4.08	4	< 0.0025	yes
21 September 1987						
+ N	0.0873	0.0063				
control	0.0615	0.0040	-4.20	4	< 0.01	no
3 November 1987						
+ N	0.0526	0.0263				
control	0.0518	0.0289	-1.20	4	> 0.10	no
9 February 1988						
+ N	0.0245	0.0007				
control	0.0249	0.0008	0.484	4	> 0.25	no
6 May 1988						
+ N	0.0546	0.0082				
control	0.0291	0.0083	-3.095	4	> 0.05	no

Table 4. Decrease in PO_4^{3-} incorporation caused by prior exposure to PO_4^{3-} as an indicator of nutrient deficiency in Flathead Lake. The + P or + N treatments indicate 12 h prior exposure to the nutrient, the control treatments had the nutrient added at the beginning of the incubation. See text for details. Yes = the assay was positive for P deficiency.

date	Uptake rate (μM P h^{-1})	std. dev.	T value	d. f.	P	
6 August 1987						
+ P	0.0779	0.0419				
control	0.0856	0.0541	0.159	4	> 0.25	no
21 September 1987						
+ P	0.0070	0.0009				
control	0.0114	0.0012	2.91	2	> 0.05	no
3 November 1987						
+ P	0.0988	0.0123				
control	0.1162	0.0275	0.299	4	> 0.25	no
9 February 1988						
+ P	0.0307	0.0009				
control	0.0595	0.0138	2.57	6	< 0.05	yes
6 May 1988						
+ P	0.0652	0.0107				
control	0.212	0.1370	1.867	6	> 0.05	no

40

Table 5. Stimulation of dark $^{14}CO_2$ uptake by 10 uM NH_4+ in Flathead Lake. Yes = the assay was positive for N deficiency.

Date	Uptake (DPM l^{-1} h^{-1})	std dev.	% increase	T value	P	
16 June 1987						
control	980	71				
+ NH_4^+	1350	145	37	3.95	< 0.01	yes
15 July 1987						
control	490	54				
+ NH_4^+	424	33	−13	1.709	> 0.10	no
10 August 1987						
control	11200	1400				
+ NH_4^+	9540	904	−15	1.77	> 0.10	no
26 October 1987						
control	4580	4420				
+ NH_4^+	20000	2970	336	5.04	< 0.005	yes
4 February 1988						
control	8390	3370				
+ NH_4^+	11900	2980	42	1.595	> 0.10	no
2 May 1988						
control	13600	1030				
+ NH_4^+	12500	2400	−8	0.676	> 0.25	no

Table 6. Summary of Responses to Bioassays In Flathead Lake from June 1987 - May 1988. N = N deficiency, P = P deficiency, B = simultaneous deficiency of N and P was recorded. NC = the assay showed no clear results.

Assay	June	July	August	November	February	May
Stimulation of $^{14}CO_2$ uptake	B		B	B	N	B
Stimulation of chlorophyll	B		B	B	NC	B
0.5 day partic. P "surge"	P		P	P	NC	P
4.5 day partic. P increase	B		B	B	NC	P
4.5 day partic. N increase	B		B	B	NC	NC
Stimulation of $V_{max,P}$ by N	N	NC	NC	NC	NC	NC
Stimulation of $V_{max,N}$ by P	P	P	P	NC	P	NC
Decrease in $V_{max,P}$ by P	NC	NC	NC	NC	P	NC
Decrease in $V_{max,N}$ by N	N	NC	NC	NC	NC	NC
Dark stimulation of $^{14}CO_2$ uptake by NH_4^+	N	NC	NC	N	NC	NC
Ambient molar ratios						
particulate N:P ratio	P	P	P	P	NC	P
particulate C:N ratio	N	N	N	N	N	N
particulate C:P ratio	P	P	P	P	P	P
# assays showing deficiency						
N	8	5[*]	5	6	2	3
P	8	8*	8	7	3	6

*July data for carboy experiments assumed to be the same as that for June and August.

Fig. 1A-C. Molar ratios of ambient particulate N:P, C:P, and C:N in Flathead Lake. Data are from water collected at 5 m from June 1987 - May 1988. Redfield ratio was below the scale in A and B. Dashed line in C gives Redfield ratio.

Fig. 2A,B. Response of $^{14}CO_2$ uptake and chlorophyll $\underline{a}$ to enrichment of water from Flathead Lake from 11 July 1987. Measurements were made from 10:00 - 14:00 on each day. A) $^{14}CO_2$ incorporation; B) Pheophytin corrected chl $\underline{a}$.

Fig. 3A-E. Change in chlorophyll $\underline{a}$ and $^{14}CO_2$ uptake after 4.5 days compared to unfertilized carboys for 5 times during the year in Flathead Lake. Solid bars represent the % increase over control in $^{14}CO_2$ uptake, crosshatched bars represent % increase in chlorophyll $\underline{a}$. Error bars represent 1 std. dev. Treatments which are significantly different than the control are denoted by * ($P < 0.05$) or ** ($P < 0.01$). Unless otherwise indicated 10 μM N and 2 μM P were added. A) 11 July 1987; low N = 1 μM NH_4^+Cl; high N = 10 μM NH_4Cl; low P = 0.2 μM P; high P = 2 μM P; B) 14 August 1987; C) 6 November 1987; D) 3 February 1988; E) 1 May 1988.

Fig. 4A,B. Time-course of particulate P and N concentrations under different nutrient additions in Flathead Lake water for 11 July 1987.

Fig. 5A,B.   Change in particulate P concentrations in
nutrient enrichment experiments 0.5 days after enrichment
and between 0.5 and 4.5 days after enrichment. N=3 for all
experiments except August when N=2. Significance is
determined with a students' $\underline{t}$ test. *=P<0.05, **=P<0.01. N
additions were 10 µM NH_4Cl and P additions were 2 uM KH_2PO_4;
A) % difference from control 0.5 days after fertilization;
B) % change in particulate P concentrations between 0.5 and
4.5 days after fertilization.

Fig. 6.   Particulate N concentrations 4.5 days after
nutrient enrichment in Flathead Lake. In August, n = 2, for
the rest of the experiments, n = 3. Significance determined
by a pooled $\underline{t}$ test as compared to control values, * = P <
0.05, ** = P , 0.01. N additions were 10 µM NH_4Cl, P
additions were 2 µM KH_2PO_4.

Figure 1

Figure 2

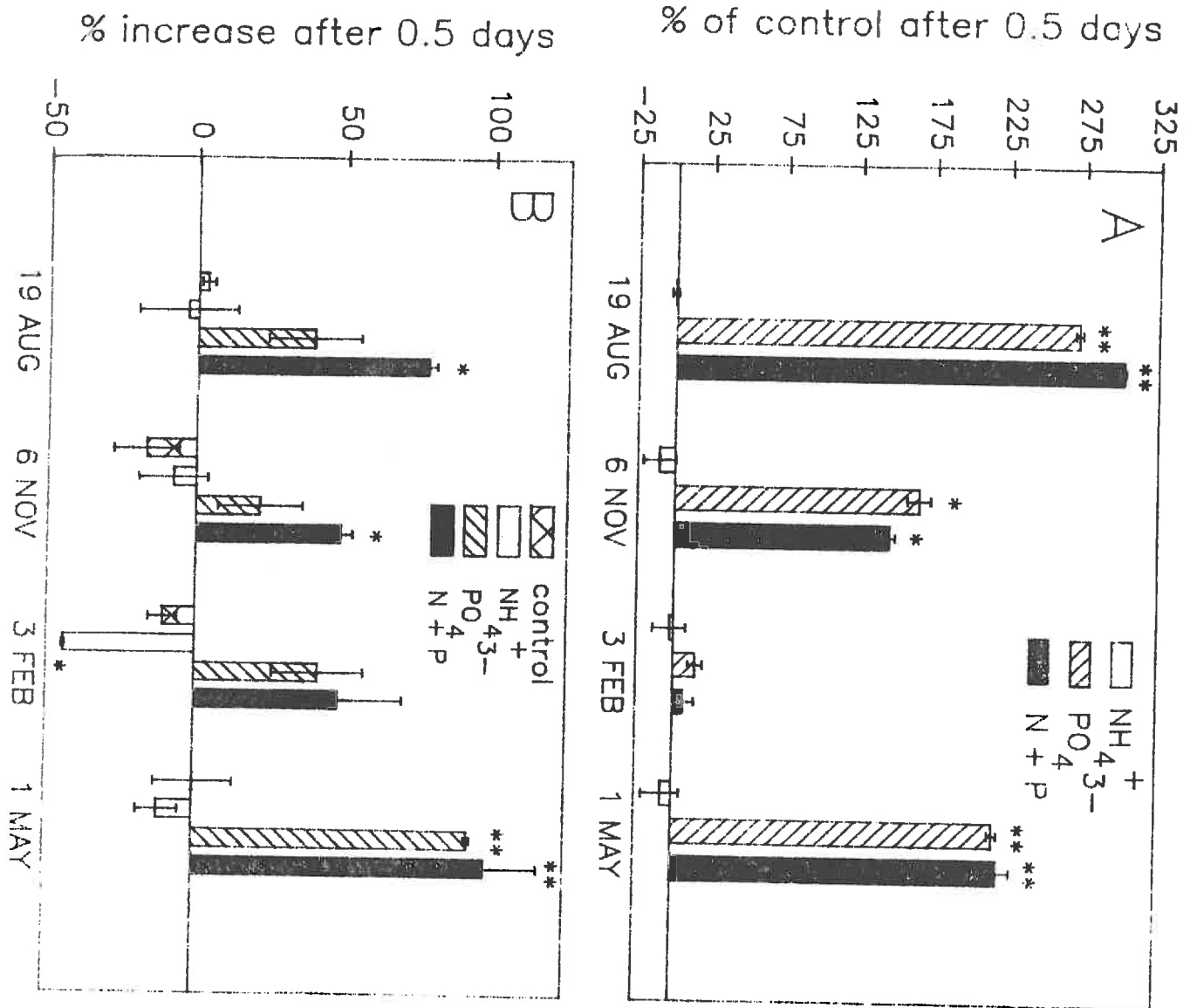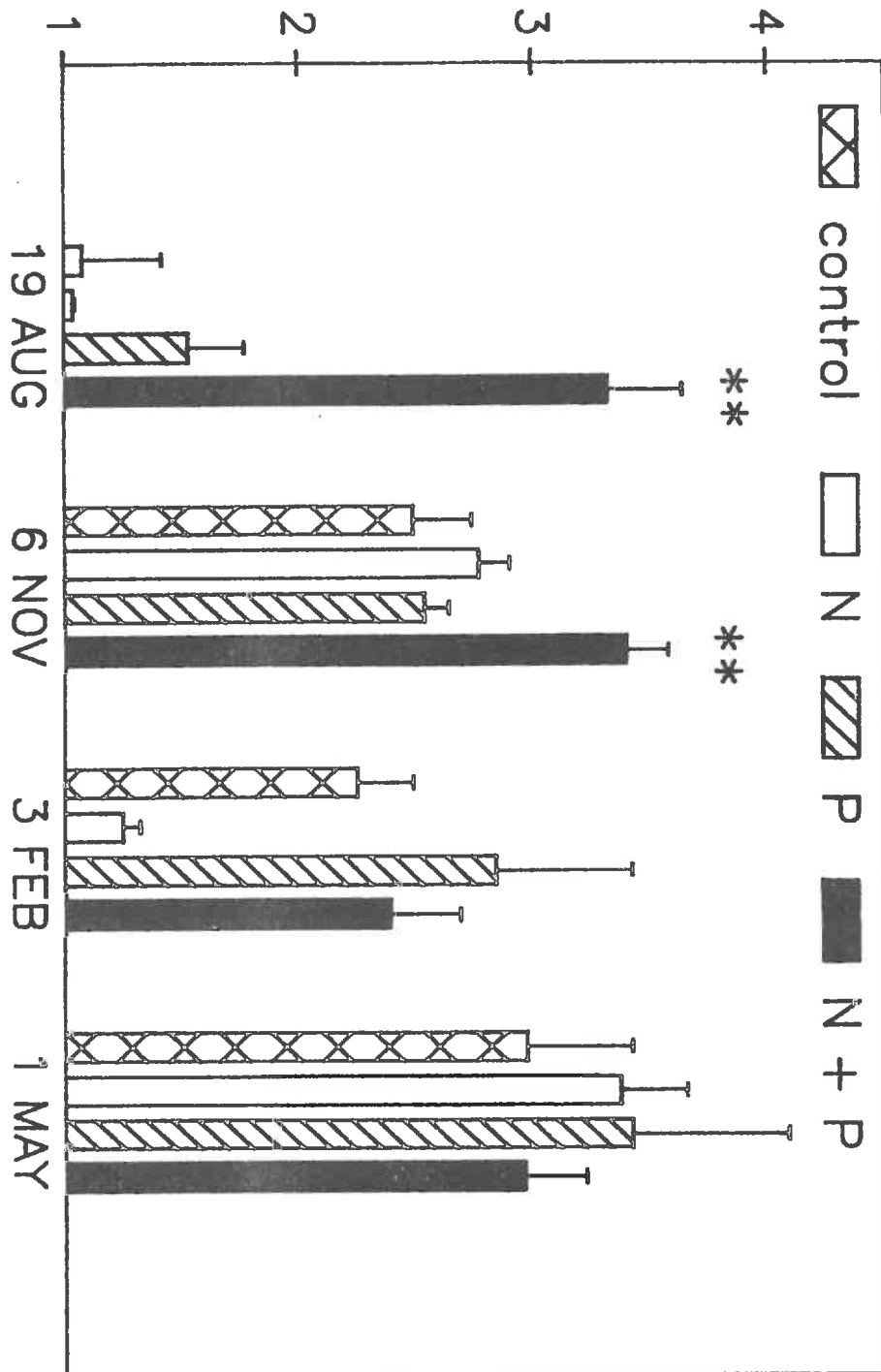# Figure 3

47
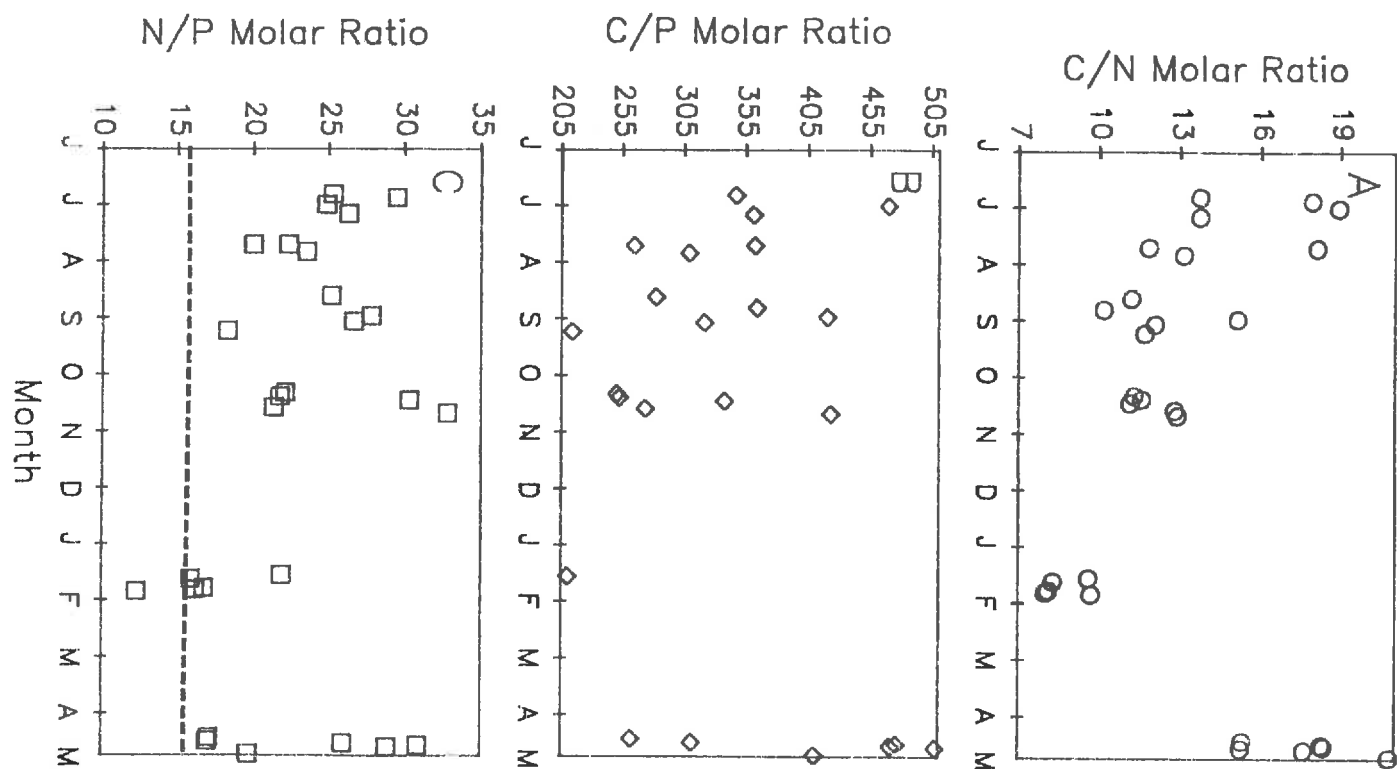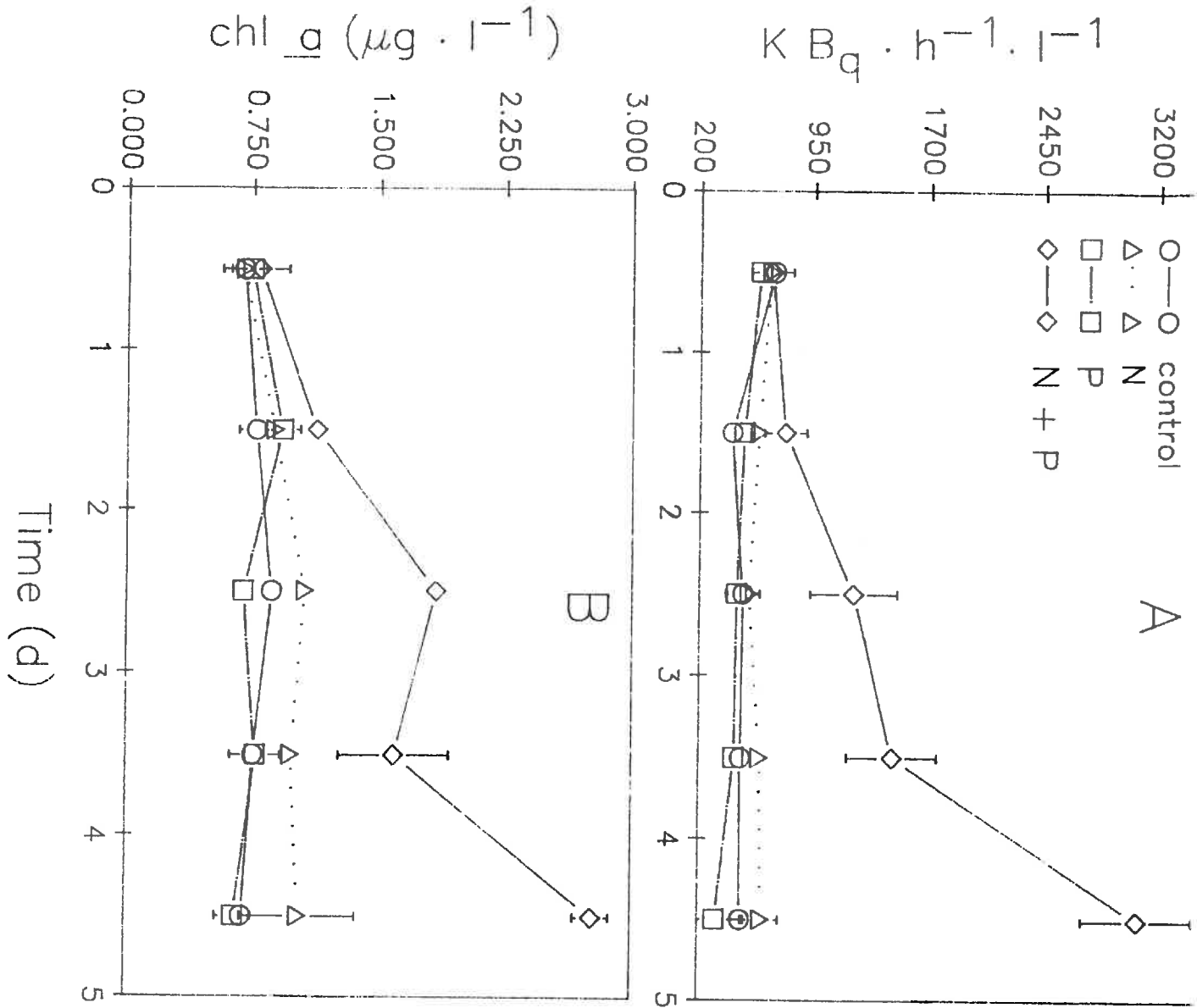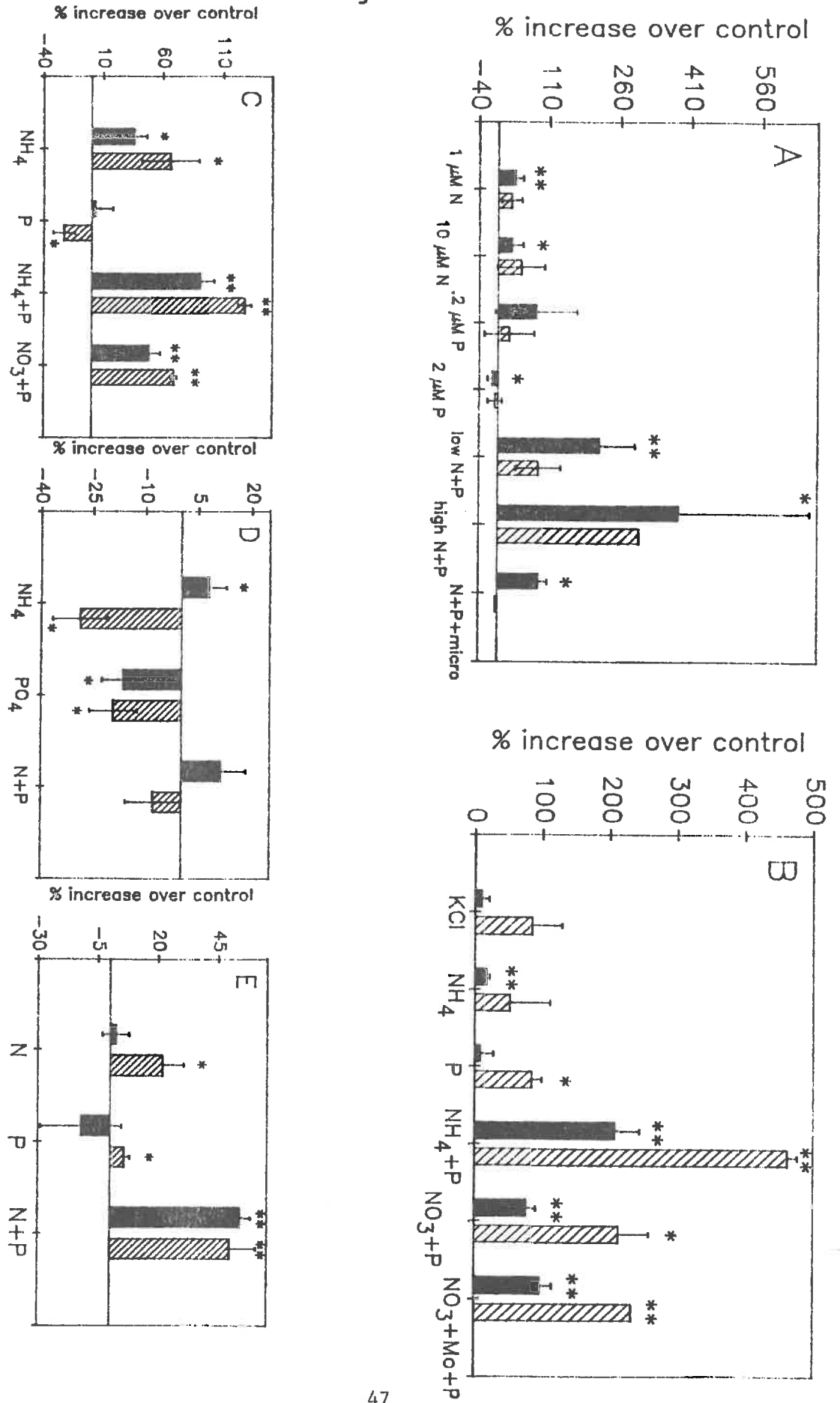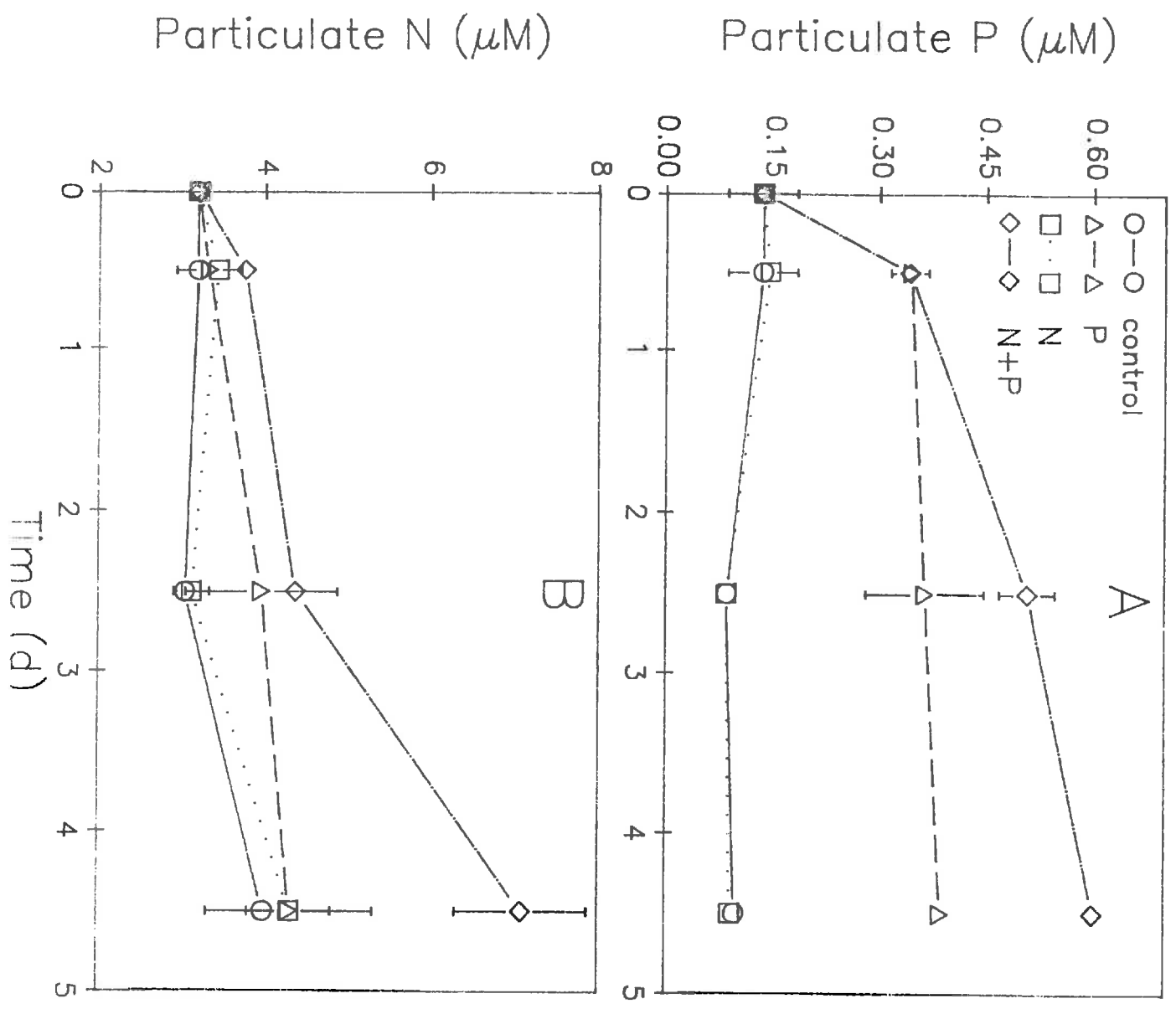
Figure 4

Figure 5

Figure 6

Particulate N (μM)

Chapter 3

Simultaneous Nitrogen and Phosphorus Deficiency in

Natural Phytoplankton Assemblages:

Theory, Empirical Evidence, and

Implications for Lake Management

Abstract

Published measurements have shown that some co-occurring algal species have varied abilities to utilize required nutrients. Therefore, it is theoretically possible that not all species of an algal community are deficient in the same nutrient, challenging the concept that phosphorus is _the_ universal nutrient limiting phytoplankton growth in lakes.

Simultaneous addition of NH_4^+ and PO_4^{3-} was necessary to stimulate community primary productivity (uptake of $^{14}CO_2$) during summer stratification in a number of northern Rocky Mountain lakes and reservoirs; single nutrient additions had no significant effect on primary productivity. However, size-fractionated bioassays showed that additions of N alone stimulated primary productivity of specific size classes of phytoplankton in these systems.

Our data have the following implications for lake management: i) Phosphorus should not be assumed _a priori_ to be the only nutrient which controls primary productivity; ii) Simultaneous enrichment of N and P may be necessary to cause an increase in community primary productivity; iii) Addition of N may cause changes in phytoplankton community structure without changing community primary production.

52

Introduction

The general view of aquatic scientists regarding nutrient deficiency of phytoplankton is based upon Leibig's law, which, as applied in ecology, states that only one factor can limit growth of an organism under steady-state (equilibrium) conditions (Goldman and Horne 1983). The assumptions defining steady-state or equilibrium conditions which underlie the application of this law to phytoplankton communities are: 1) the aquatic environment is unstructured (homogeneous in time and space) and mixing and diffusion make all nutrients equally available to all organisms; 2) Temporal changes in bulk nutrient concentrations are slow relative to the generation times of phytoplankton; 3) All phytoplankters have roughly the same nutrient requirements. For many scientists, this translates into the idea that lake phytoplankton communities can be deficient in only one nutrient at a time.

The competitive exclusion principle states that, at equilibrium, the dominant species will be the one which is the best competitor for the resource in shortest supply (Hardin 1960). This idea was scrutinized by Hutchinson (1961) who noted that the number of phytoplankton species is high despite competitive exclusion, a concept he termed the "paradox of the plankton". Hutchinson felt that this paradox could only be resolved by challenging the basic assumption of equilibrium, or invoking species interactions

53

such as grazing or mutualism. A formal disequilibrium hypothesis was later developed by Richerson et al. (1970).

The above assumptions have also been challenged from other fronts. It has been documented that each phytoplankton species has characteristic requirements and competitive abilities for each nutrient (Tilman et al. 1986). This allows several species to co-exist and a phytoplankton assemblage to be limited contemporaneously by more than one nutrient (Tilman 1982). Such an approach predicts that growth of 2 different species must be limited by 2 different nutrients if the nutrients are homogeneous in space and time.

However, there is indirect evidence that nutrient concentrations are not necessarily homogeneous with space and time. Culture experiments have shown that phytoplankton can benefit from encountering micro-patches of phosphorus regenerated by <u>Daphnia</u> (Lehman and Scavia 1982). Time-course experiments indicate that nutrient deficient marine (Glibert and Goldman 1981) and freshwater phytoplankton (Priscu 1987, Priscu and Priscu 1984) exhibit elevated uptake rates when such pulses are encountered, allowing them to effectively utilize nutrient micro-patches. Also, larger phytoplankton may benefit more from encountering such patches than smaller phytoplankton (Suttle et al. 1988).

Despite the above advances in theory and empirical evidence, many aquatic scientists and management agencies view phosphorus as the only nutrient which controls primary

54

productivity (Schindler 1977) in lakes and attempt to use phosphorus loading data to estimate the trophic status of lakes (Vollenweider 1976). Even if only one nutrient controls productivity, there is no reason to assume that the nutrient is phosphorus. Mild nitrogen deficiency has been observed in central Canadian Lakes (Healy and Hendzel 1980). Stronger N deficiency has been documented for Castle Lake, California (Goldman 1978), Lake Tahoe, California (Goldman 1974), the lower Great Lakes (Murphy 1980), New Zealand Lakes (Priscu and Priscu 1984, White et al. 1985) certain Antarctic lakes (Priscu et al. 1988, Vincent and Vincent 1982), and Lake Titicaca, Peru-Bolivia (Vincent et al. 1984). N deficiency also occurs occasionally in several Minnesota lakes (Elser et al. 1988), in Canadian prairie lakes (Prepas and Trimbee 1988), in an arctic tundra pond (Kalff 1971), and in a eutrophic Scandinavian lake (Zevenboom et al. 1982). Furthermore, micro-nutrients have been shown to control productivity in several lakes (Goldman 1960, Goldman 1971). Therefore, generalizations stating that phosphorus is the primary nutrient controlling primary productivity in freshwater systems (Hecky and Kilham 1988, Schindler 1977, Smith 1984) should be viewed with caution. Indeed, it has been shown that total N used in combination with total P data from a number of geographically distinct lakes can explain a greater degree of the variance in epilimnetic chlorophyll concentrations than regressions using only phosphorus to predict chlorophyll (Smith 1982).

We present further empirical evidence showing that some
nutrient deficiency in natural phytoplankton communities
cannot be explained by phosphorus deficiency alone. We also
show that some components of a phytoplankton community can
respond differently to nutrient enrichment than the entire
community.

Study Sites

Nutrient bioassays were conducted in 4 western Montana
systems; Flathead Lake, Hungry Horse Reservoir and Koocanusa
Reservoir in the Columbia river drainage in northwestern
Montana, and Canyon Ferry Reservoir on the Missouri River.
Flathead Lake is a large (460 km^2), oligotrophic lake (0.1 –
1.1 µg chl $\underline{a}$ l^{-1} in the epilimnion). Hungry Horse Reservoir
(97.2 km^2 full pool, 0.2 – 1.4 µg chl $\underline{a}$ l^{-1} in the
epilimnion) is an oligotrophic reservoir upstream from
Flathead Lake on the south fork of the Flathead River.
Koocanusa (188 km^2, full pool) is an oligotrophic reservoir
(0.7 – 1.9 µg chl $\underline{a}$ l^{-1} in the epilimnion) which stretches
from northwest Montana into southeast British Columbia.
Canyon Ferry is the first major (142 km^2, full pool)
reservoir on the Missouri River. Canyon Ferry is eutrophic
(5 – 200 µg chl $\underline{a}$ l^{-1} in the epilimnion) and dominated by
cyanobacteria during the ice-free seasons.

Materials and Methods

Bioassays in Flathead Lake were started on 19 August 1987 in 20 1 l collapsible polyethylene carboys which were filled using a displacement sampler (Dodds and Priscu 1988) with water collected from 5 m in the deepest portion of the lake. Duplicate carboys were amended with no nutrients (controls), 10 µM NH_4Cl, 2 uM KH_2PO_4, or both, and incubated in situ. Duplicate 100 ml aliquots were taken 0.5, 1.5, 2.5, 3.5, and 4.5 days after fertilization for $^{14}CO_2$ incorporation experiments. The aliquots were inoculated with 0.011 µCi $NaH_2^{14}CO_3$ ml^{-1}, and incubated at 5 m for 4 h. Incubations were stopped by filtration onto 8 µm Nuclepore filters or Whatman GF/F filters (effective retention 0.7 um). Filters were rinsed 3 times with 5 ml of de-ionized water and counted by standard liquid scintillation spectrometry.

Samples from Hungry Horse Reservoir (15 July 1987) and Koocanusa Reservoir (29 August 1987) were collected from 0.5 m, and amended with no nutrients (control), 3.6 µM NH_4Cl, 1.6 µM KH_2PO_4, or both N and P and pre-incubated for 12 h (overnight) at 0 m. Pre-incubation was necessary to avoid spurious fertilization effects upon $^{14}CO_2$ uptake (Lean and Pick 1981). Following pre-incubation, triplicate sub-samples were inoculated with $NaH_2^{14}CO_3$ and incubated in situ. They were then either pre-filtered with 20 µm Nitex mesh and filtered on Whatman GF/C filters (1.2 µm retention) or filtered onto GF/C filters without pre-filtration.

Samples from Canyon Ferry Reservoir were collected from 0.5 m (14 August 1986). Samples were either filtered through 154 µm Nitex netting to remove large filamentous cyanobacteria (<u>Aphanizomenon</u> <u>flos-aquae</u>) or left intact and placed in 1 l polyethylene bottles. Both size fractions were then amended with no nutrients (control), 14.3 µM NH_4Cl, or 0.63 µM KH_2PO_4. Samples were incubated at 200 µE m^{-2} s^{-1} under "cool white" fluorescent lights at the collection temperature for 12 h followed by removal of three aliquots from each treatment for ^{14}C uptake measurement. After 4 h of incubation, samples were filtered on Whatman GF/C filters.

Results

There was no significant response of Flathead Lake phytoplankton ^{14}C uptake to nutrient addition until 2.5 d after fertilization (Fig. 1). At 2.5 d, the productivity of the N and P additions was significantly greater than control ($P < 0.01$, pooled $\underline{t}$ test), and the N and the P additions showed a slight increase over control. After 4.5 days productivity in the N + P addition had increased 60% over control, but the N and the P additions were indistinguishable from the control ($P > 0.10$, pooled $\underline{t}$ test). This suggests that the community, as a whole, is deficient in both N and P simultaneously and that when either nutrient was added alone, it stimulated productivity only until the other nutrient was depleted.

Size fractionation of the same experiment at 4.5 d showed that the less than 8 μm fraction accounted for significantly less of the total primary production in the N and N + P treatments than in the control (Fig. 2). Therefore, even though the total community primary productivity in the control and the N treatments was indistinguishable (Fig. 1), N addition stimulated productivity of the greater than 8 μm fraction.

Data from Koocanusa Reservoir showed that N alone stimulated primary productivity by 22%, that P increased primary productivity by 18%, and N and P added simultaneously stimulated productivity by 40% (Fig. 3). These data are similar to those from Flathead Lake at 2.5 days (Fig. 1), again implying deficiency in more than one nutrient at a time.

Size fractionation data from Hungry Horse Reservoir showed significantly more primary productivity in the less than 20 μm size class of the N treatment than the control treatment (Fig. 4) and the N + P treatment ($p < 0.05$, pooled t test, Fig. 4). However, primary productivity of the entire community was not significantly different from control in any treatment ($p > 0.25$, pooled t test) (Fig. 4). If stimulation of ^{14}C uptake translates into increased growth, this implies a shift in community structure. But rather than N causing an increase in productivity in the larger size classes as in Flathead Lake (Fig. 2), it caused an

increase in productivity of the smaller size classes in
Hungry Horse Reservoir.

In Canyon Ferry Reservoir, the entire community was
stimulated by addition of N or P, but when the large
cyanobacteria were removed, only N alone caused significant
stimulation of productivity (40%) compared to the control
(Fig. 5). With the majority of the large algae removed, it
appears that the community is only deficient in N. This
again illustrates that the short-term community response can
be different than that of individual phytoplankton species.

Discussion

Our data from four topographically and trophically
distinct Montana lakes indicate that N must be considered as
an important nutrient regulating primary production. The
data also document that, in some lakes, simultaneous
community wide N and P deficiency exists. This does not
imply that single species are limited by more than one
nutrient at one time. Rather, the _entire_ community requires
addition of both N and P at the same time, before a
significant stimulation of primary production occurs. Such
simultaneous deficiency has also been shown in coastal lakes
in British Columbia (Suttle and Harrison 1988), at times in
some Michigan lakes (Elser et al. 1988), in some New Zealand
Lakes (White et al. 1985), and in Crater Lake, Oregon and
Lake Tahoe, California (Lane and Goldman 1984). If there is
a close balance in supply rates of N and P relative to

demand, then long-term stimulation of primary productivity by one nutrient is unlikely, since the other nutrient would be rapidly depleted. In systems such as these, where there seems to be a balance between N and P deficiency, a manager who wishes to lower algal production only needs to decrease supply of one of the two nutrients controlling primary production. However this approach could cause changes in community structure such as the eventual stimulation of heterocystous cyanobacteria when N loading is controlled and P loading is not (Tilman et al. 1986). If a manager wants to increase productivity in a system exhibiting simultaneous deficiency in N and P, addition of both N and P in physiologically balanced proportions is required.

We also document that stimulation of specific size fractions by addition of a single nutrient can occur in lakes ranging from ultra-oligotrophic to eutrophic. This was shown previously in ultra-oligotrophic Lake Tahoe and Crater Lake (Lane and Goldman 1984), and in oligotrophic Lake Taupo (New Zealand) (Priscu and Priscu 1984). Furthermore, individual species can respond differently than the entire community to single nutrient or simultaneous nutrient addition (Lehman and Sandgren, 1985). These observations are quite relevant to lake management. In a hypothetical example, if nitrogen additions stimulate the large size classes of algae without increasing total community primary productivity (as in Flathead Lake), and these size fractions are more easily grazed by zooplankton,

fertilization could actually increase production of zooplanktiverous fish without increasing overall levels of primary production in the lake.

It is important that the mechanisms by which nutrients control primary productivity are understood in each system, before manipulation or costly nutrient control measures are implemented. It is not always phosphorus which controls primary productivity, and it is not always a single nutrient which controls community productivity. Moreover, some changes in nutrient loading which do not change the overall primary productivity of the system may change the makeup of the phytoplankton community. Simple experiments, as described above, will provide information allowing sound decisions to be made regarding nutrient deficiencies in specific lakes.

References

Dodds, W. K. and J. C. Priscu. 1988. An inexpensive device
  for sampling large volumes of lake water from discrete
  depths.   Freshwater Biology 20:113-116.

Elser, J. J., M. M. Elser, N. A. MacKay, and S. R.
  Carpenter. 1988. Zooplankton-mediated transitions
  between N- and P-limited algal growth. Limnol.
  Oceanogr. 33:1-14.

Glibert, P. M and J. C. Goldman. 1981. Rapid ammonium uptake
  by four species of marine phytoplankton. Limnol.
  Oceanogr. 27:814-827.

Goldman C. R. 1960. Molybdenum as a factor limiting primary
  productivity in Castle Lake, California. Science
  132:1016-1017.

Goldman, C. R. 1971. The role of minor nutrients in limiting
  the productivity of aquatic ecosystems. pages 21-38, in
  Nutrients and Eutrophication. G. E. Likens ed. American
  Society of Limnology and Oceanography.

Goldman, C. R. 1974. Eutrophication of Lake Tahoe
  emphasizing water quality. Research Report. United
  States E. P. A., Corvallis, Oregon #5501-00996.

Goldman, C. R. 1978. The use of natural phytoplankton
  populations in bioassay. Mitt. Internat. Verein.
  Limnol. 21:364-371.

Goldman, C. R. and A. J. Horne. 1983. Limnology. McGraw-Hill
  Book Company. New York.

63

Hardin, G. 1960. The competitive exclusion principle.
    Science. 131:1292-1297.

Healey, F. P. and L. L. Hendzel. 1980. Physiological
    indicators of nutrient deficiency in lake
    phytoplankton. Can. J. Fish. Aquat. Sci. 37:442-453.

Hecky, R. E. and P. Kilham. 1988. Nutrient limitation of
    phytoplankton in freshwater and marine
    environments: A review of recent evidence on the
    effects of enrichment. Limnol. Oceanogr. 33(4):79-
    6-822.

Hutchinson, G. E. 1961. The paradox of the phytoplankton.
    Am. Nat. 95:137-145.

Kalff, J. 1971. Nutrient limiting factors in an Arctic
    tundra pond. Ecology 52:655-659.

Lane, J. L. and C. R. Goldman. 1984. Size fractionation of
    natural phytoplankton communities in nutrient bioassay
    studies. Hydrobiologia. 118:219-223.

Lean, D. R. S. and F. R. Pick. 1981. Photosynthetic response
    of lake plankton to nutrient enrichment: a test for
    nutrient limitation. Limnol. Oceanogr. 26(6):1001-1019.

Lehman, J. T. and C. R. Sandgren. 1985. Species-specific
    rates of growth and grazing loss among freshwater
    algae. Limnol. Oceanogr. 30:34-46.

Lehman, J. T. and D. Scavia. 1982. Microscale patchiness of
    nutrients in plankton communities. Science
    216:729-730.

Murphy, T. P. 1980. Ammonium and nitrate uptake in the lower
    great lakes. Can. J. Fish. Aquat. Sci. 37:1365-1372.

Prepas, E. E. and A. M. Trimbee. 1988. Evaluation of
    indicators of nitrogen limitation in deep prairie lakes
    with laboratory bioassays and limnocorrals.
    Hydrobiologia 158:269-276.

Priscu, J. C. and L. R. Priscu. 1984. Inorganic nitrogen
    uptake in oligotrophic Lake Taupo, New Zealand. Can. J.
    Fish. Aquat. Sci. 41:1436-1445.

Priscu, J. C. 1987. Time-course of inorganic nitrogen uptake
    and incorporation by natural populations of freshwater
    phytoplankton. Freshw. Biol. 17:331-339.

Priscu, J. C., W. F. Vincent and C. Howard-Williams. 1988.
    Inorganic nitrogen uptake and regeneration in
    perennially ice-covered Lakes Fryxell and Vanda,
    Antarctica. (in press J. Plank. Res.).

Richerson, P., R. Armstrong and C. R. Goldman. 1970.
    Contemporaneous disequilibrium, a new hypothesis
    to explain the "paradox of the plankton". Proc.
    Nat. Acad. Sci. USA. 67:1710-1714.

Smith, S. V. 1982. The nitrogen and phosphorus dependence of
    algal biomass in lakes: An empirical and theoretical
    analysis. Limnol. Oceanogr. 27:1101-1112.

Smith, S. V. 1984. Phosphorus versus nitrogen limitation in
    the marine environment. Limnol. Oceanogr. 29:1149-1160.

Schindler, D. W. 1977. Evolution of phosphorus limitation in
    lakes. Science 21:260-262.

Suttle, C. A. and P. J. Harrison. 1988. Ammonium and
     phosphate uptake rates, N:P supply ratios, and evidence
     for N and P limitation in some oligotrophic lakes.
     Limnol. Oceanogr. 33:186-202.

Suttle, C. A., J. G. Stockner, K. S. Shortreed and P. J.
     Harrison. 1988. Time-courses of size-fractionated
     phosphate uptake: are larger cells better competitors
     for pulses of phosphate than smaller cells? Oecologia
     74:571-576.

Tilman, D. 1982. Resource Competition and Community
     Structure. Princeton University Press, Princeton, NJ.

Tilman, D., R. Kiesling, R. Sterner, S. S. Kilham and F. A.
     Johnson. 1986. Green, blue green, and diatom
     algae: Taxonomic differences in competitive
     ability for phosphorus, silicon, and nitrogen.
     Arch. Hydrobiol. 106:473-485.

Vincent, W. F. and C. L. Vincent. 1982. Response to nutrient
     enrichment by the plankton of coastal lakes and
     the inshore Ross Sea. Polar Biol. 1:159-165.

Vincent, W. F., W. Wurtsbaugh, C. L. Vincent and
     P. J. Richerson. 1984. Seasonal dynamics of nutrient
     limitation in a tropical high altitude lake (Lake
     Titicaca, Peru-Bolivia): Application of physiological
     bioassays. Limnol. Oceanogr. 29:540-552.

Vollenweider, R. A. 1976. Advances in defining critical
loading levels for phosphorus in lake eutrophication.
Mem. Ist. Ital. Idrobiol. 13:87-113.

White, E., K. Law, G. Payne and S. Pickmere. 1985. Nutrient
demand and availability among planktonic communities -
an attempt to assess nutrient limitation to plant
growth in 12 central volcanic plateau lakes. New
Zealand J. Mar. Freshw. Res. 19:49-62.

Zevenboom, W., A. B. de Vaate and L. R. Mur. 1982.
Assessment of factors limiting growth rate of
Oscillatoria agardhii in hypertrophic Lake Wolderwijd,
1978, by use of physiological indicators. Limnol.
Oceanogr. 27:39-52.

Figure 1. Time course of $^{14}CO_2$ uptake after fertilization of unaltered Flathead Lake water. Error bars denote $\pm$ one standard deviation.

Figure 2. Percent of $^{14}CO_2$ uptake by the < 8 um size fraction 4.5 days after fertilization of Flathead Lake water. Error bars denote one standard deviation. Data are from the same experiment shown in Fig. 1. * = significantly lower than control ($P < 0.05$, pooled t test).

Figure 3. Photosynthetic response of Koocanusa Reservoir water to N and P fertilization. Error bars denote 1 standard deviation. * = indicates a significant difference from control at the $P < 0.05$ level, ** = $P < 0.01$ (pooled t test).

Figure 4. Size-selective stimulation of phytoplankton photosynthesis by NH_4^+ and whole community photosynthesis in Hungry Horse Reservoir. Error bars denote 1 standard deviation. ** indicates the N treatment is significantly greater than control ($P < 0.01$). The N treatment was also significantly greater than the N + P treatment ($P < 0.05$).

Figure 5. Size selective response of photosynthesis to single nutrient addition in Canyon Ferry Reservoir. Error bars denote 1 standard deviation. * = significantly greater than control ($P < 0.05$), ** = $P < 0.01$.

Figure 1
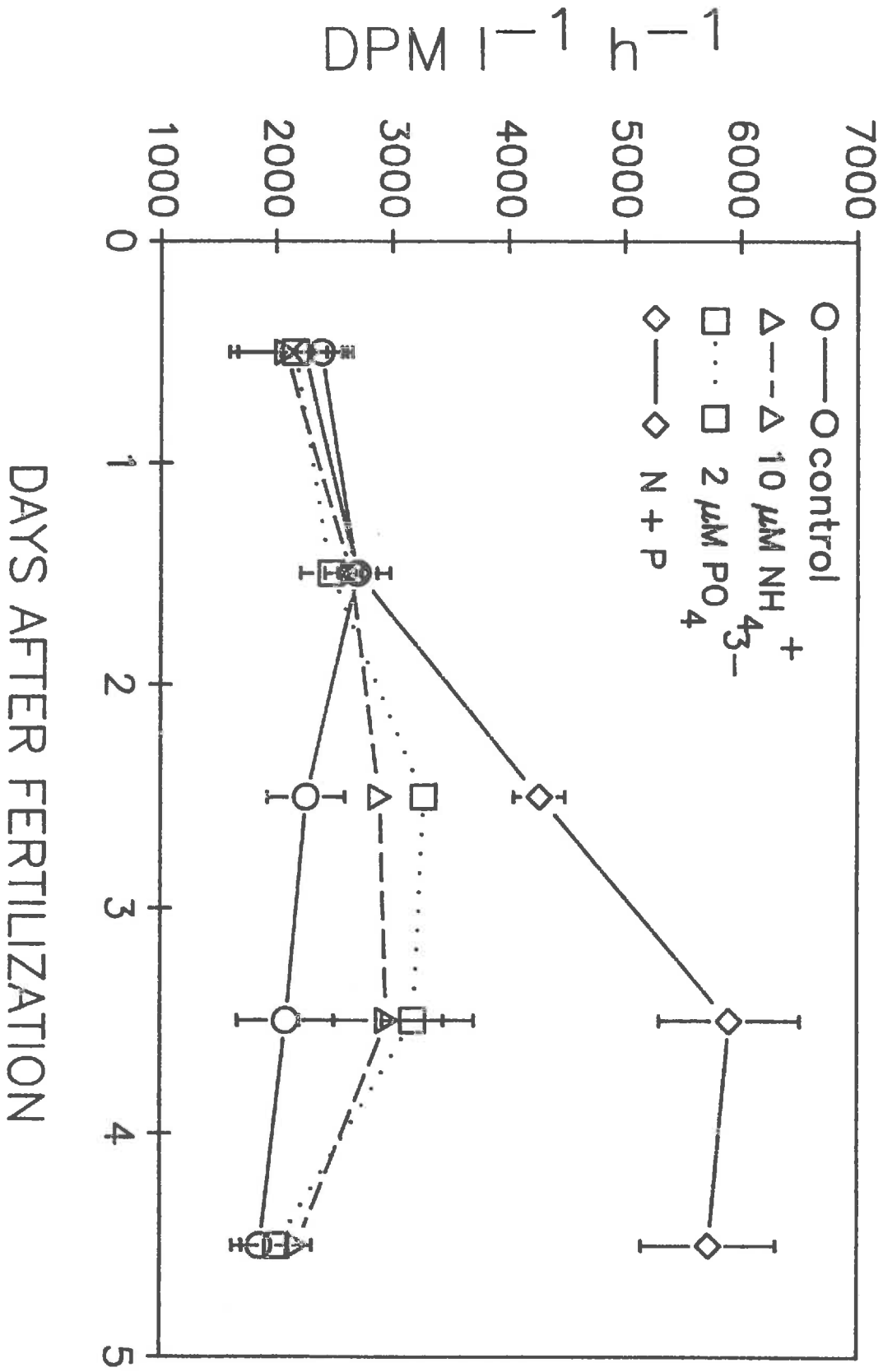
Figure 2

% ^{14}C uptake < 8 μM
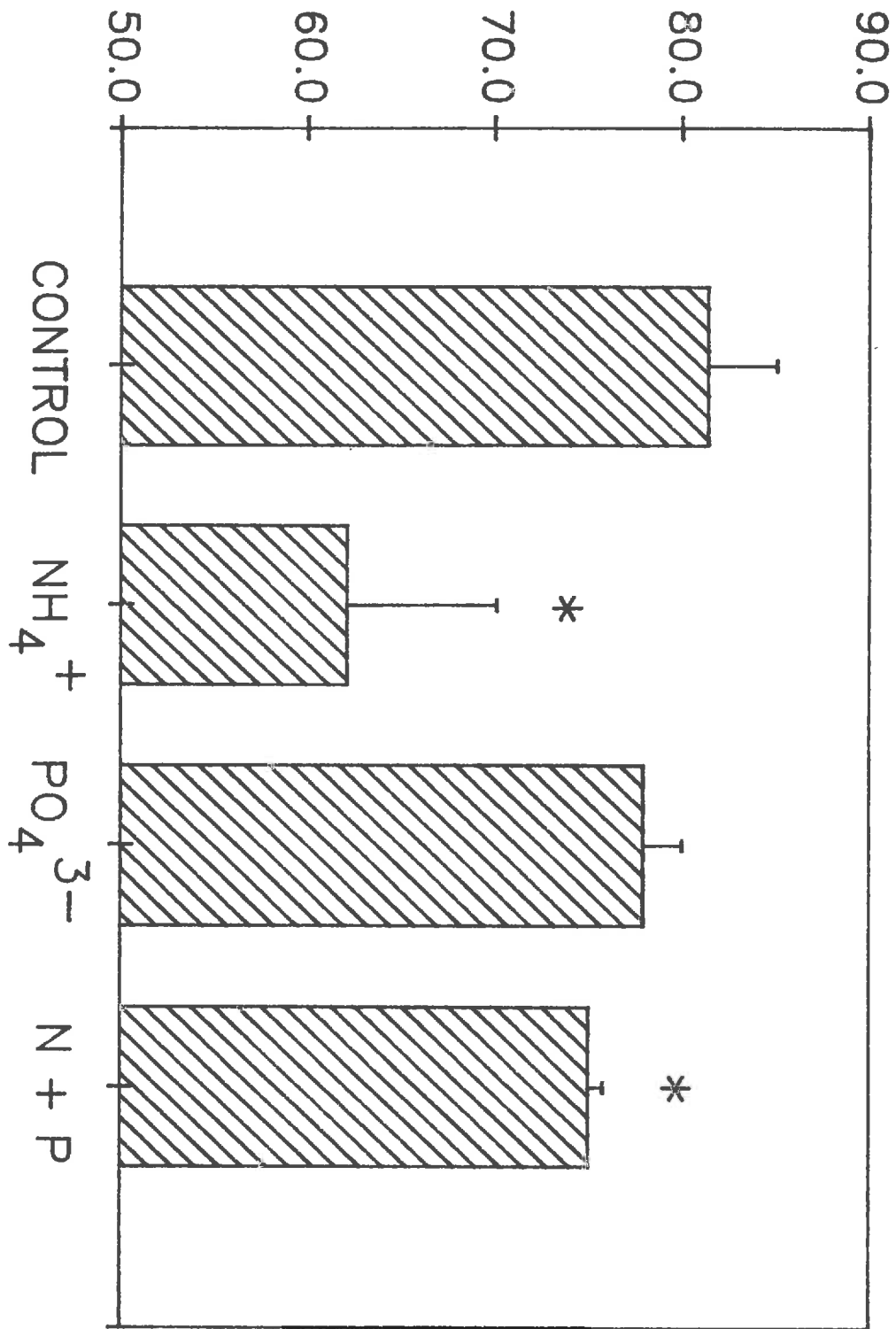
Figure 3

DPM l^{-1} h^{-1}

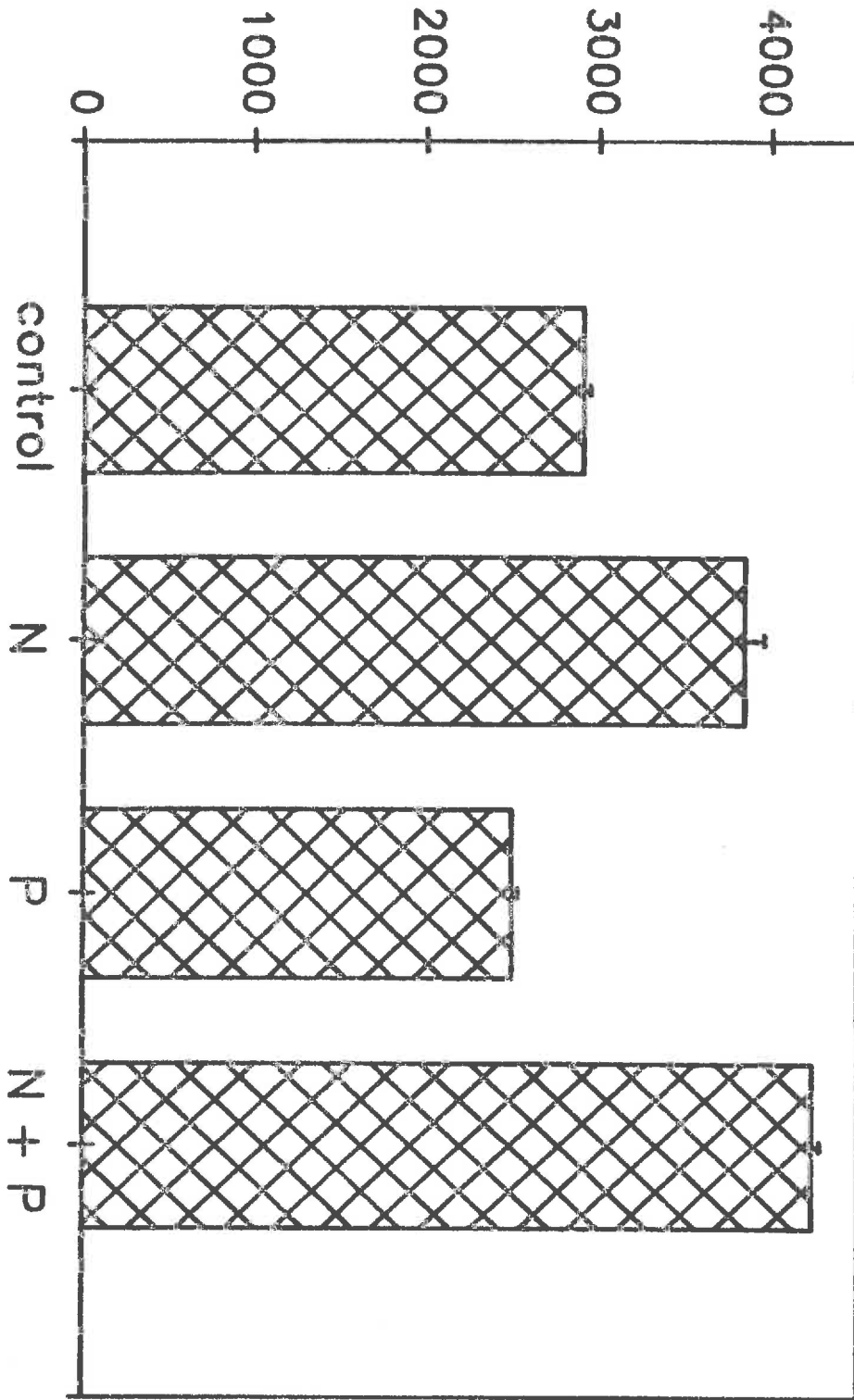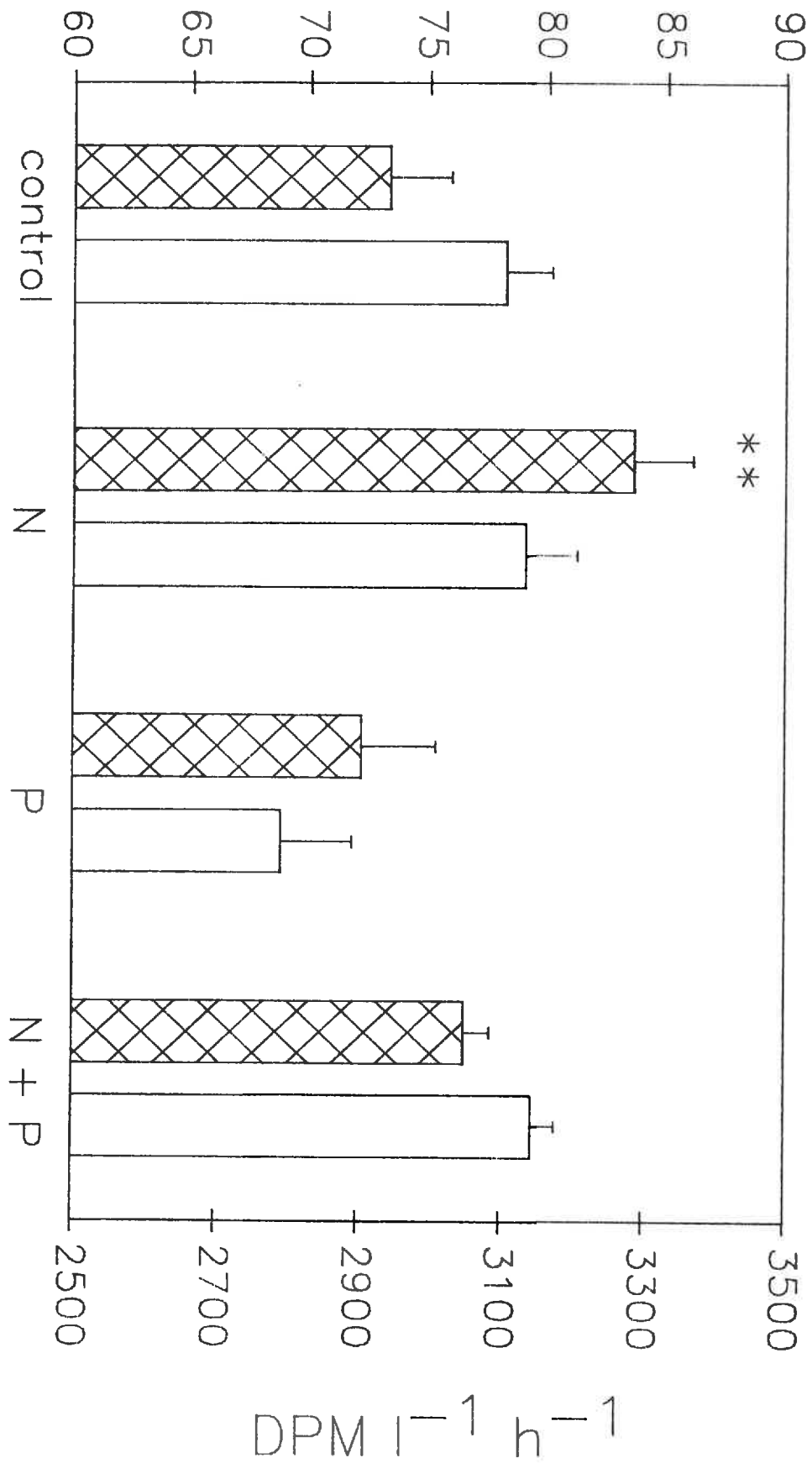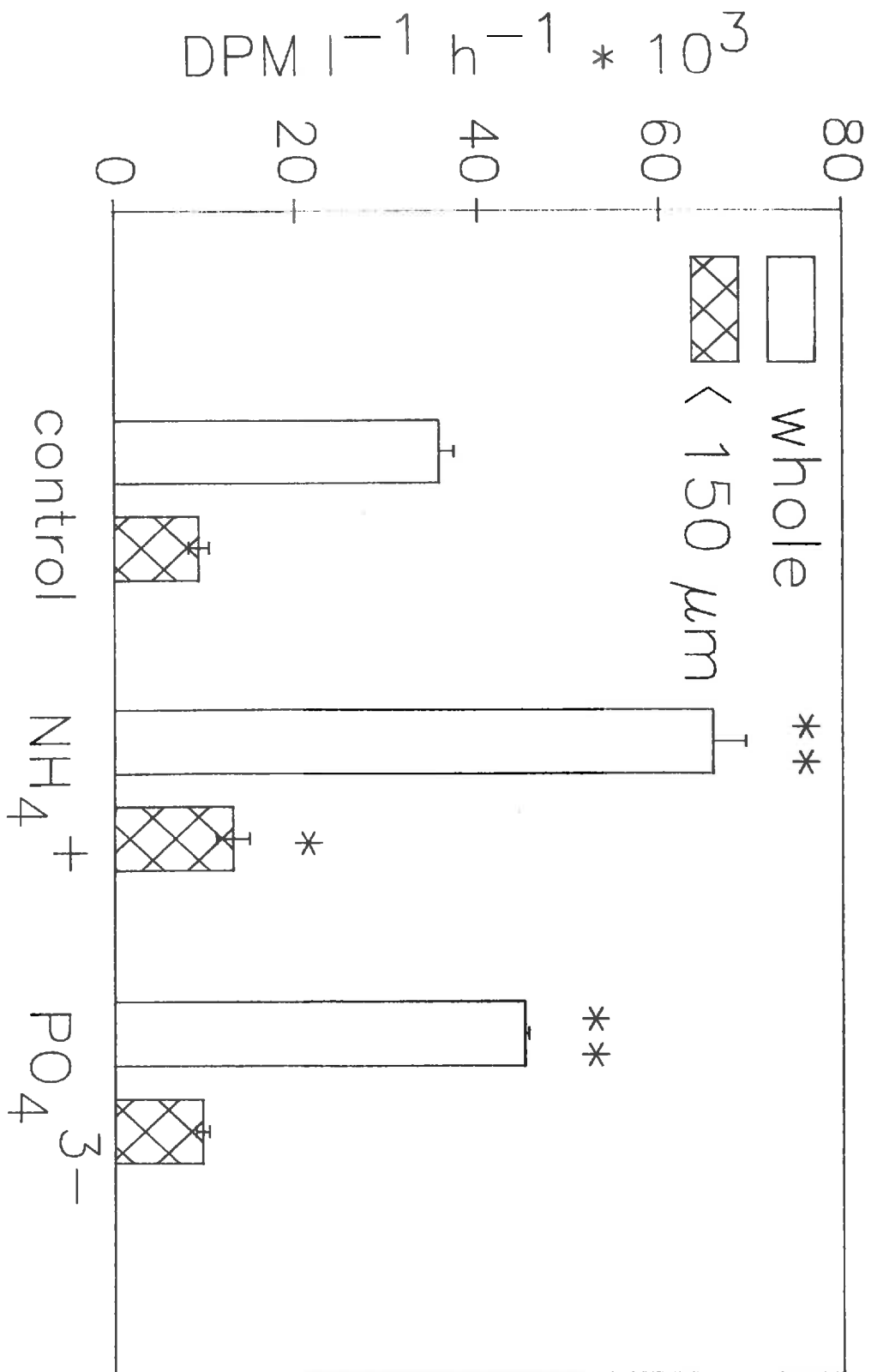**Figure 4**

Figure 5

Chapter 4

Stimulation of Dark Inorganic Carbon Incorporation by
Ammonium as an Indicator of Phytoplankton Nitrogen
Deficiency: Possible Misinterpretation Caused
by Ammonium Oxidizing Bacteria

Abstract

Stimulation of dark uptake of $^{14}CO_2$ by NH_4^+ is often used as an indicator of nitrogen deficiency. Theoretically this assay should measure the influence of NH_4^+ on anapleurotic CO_2 fixation by algae. However, dark CO_2 fixation by NH_4^+ oxidizing bacteria can also be stimulated by NH_4^+ enrichment, which may mask the algal response in natural communities. NH_4^+ enhanced dark $^{14}CO_2$ uptake up to 300% relative to unamended controls in oligotrophic Flathead Lake (Montana, USA), but the effect was not detectable in the presence of nitrapyrin, an inhibitor of NH_4^+ oxidizing bacteria. There was enhancement of dark $^{14}CO_2$ uptake with addition of NH_4^+ in organisms retained on a 2 μm filter, but these organisms were responsible for less than 10% of the community dark $^{14}CO_2$ incorporation. These results imply that treatments to separate the processes of dark $^{14}CO_2$ incorporation by nitrifiers and phytoplankton may improve accuracy when using NH_4^+ stimulation of dark $^{14}CO_2$ incorporation as an indicator of N deficiency in natural algal communities.

Introduction

When first introducing the use of NH_4^+ stimulation of dark $^{14}CO_2$ uptake as an indicator of nitrogen deficiency in natural phytoplankton populations, the authors cautioned the method "is only as good as our knowledge of how the carbon is fixed" (Morris et al., 1971). Dark CO_2 uptake in pure algal cultures most likely involves β-carboxylation activity leading to protein synthesis over long dark periods, although the light history of the alga and length of the dark period can influence the pathway of dark uptake (Mortain-Bertrand et al. 1988). Dark $^{14}CO_2$ stimulation has become one of an array of bioassays used to assess nitrogen limitation in natural phytoplankton communities (eg. Elser et al., 1988; Vincent, 1981; White et al., 1985). It has been observed that NH_4^+ oxidizing bacteria also take up CO_2, that this process may become relatively more important in the dark, and that this uptake can be dependent upon NH_4^+ concentration (Taguchi 1983). However, investigators using NH_4^+ stimulation of dark $^{14}CO_2$ uptake to indicate N deficiency have ignored this problem. We (i) present data on the influence of NH_4^+ oxidizing bacteria on dark uptake of $^{14}CO_2$, and (ii) examine how NH_4^+ oxidizing bacteria may confound results when relying upon the NH_4^+ stimulation of dark $^{14}CO_2$ uptake to indicate N deficiency of phytoplankton in a large oligotrophic lake.

76

Materials and Methods

Water for all experiments was collected from 5 m with a displacement sampler (Dodds and Priscu 1988) from Flathead Lake, a large (460 km^2), oligotrophic (epilimnetic chlorophyll $\underline{a}$ 0.1-1.1 μg l^{-1})) lake in western Montana (USA). Overall nutrient deficiency was determined by measuring $^{14}CO_2$ uptake of 100 ml aliquots from nutrient amended 20 l carboys. Carboys were filled with lake water in the evening, nutrients were added (3 replicates of each: control, 10 μM NH_4Cl, 2 μM KH_2PO_4, and N + P treatments), and carboys were incubated $\underline{in}$ $\underline{situ}$ for 4-5 days. During this time sub-samples were removed and analyzed for photosynthetic $^{14}CO_2$ incorporation which was determined during 4 h mid-day incubations. Samples were filtered onto Whatman GF/F filters and counted with liquid scintillation spectrometry.

Dark $^{14}CO_2$ uptake was measured in 3 lake water samples with a final concentration of 10 μM NH_4Cl and 3 samples with no additions. Nitrapyrin (2-chloro-6-(trichloromethyl) pyridine) was dissolved in 95% ethanol to a final concentration of 6.25 mg l^{-1} and added to one set of the control and NH_4^+Cl amended bottles. This concentration has been shown to effectively inhibit NH_4^+ oxidizers (Jones et al. 1984, Priscu and Downes 1985). $^{14}[C]-NaH_2CO_3$ was added to a final concentration of 0.33 μC ml^{-1}, to all treatments. Bottles were incubated in the dark for 8 hours and filtered with

77

gentle vacuum under reduced light onto Whatman GF/F filters (0.7 µm retention) or 2 µm pore sized Nuclepore filters. ^{14}C incorporation was determined by standard scintillation spectrometry.

To illustrate if ambient NH_4^+ was below saturating levels with respect to dark NH_4^+ uptake, dark $^{15}NH_4^+$ uptake was measured by adding 99 atom % $^{15}NH_4Cl$ to a final concentration of 0.36 or 7.1 µM to lake water samples incubated in 500 ml borosilicate bottles. Incubations were stopped after 3.8 h by filtration through Whatman GF/F filters. Subsamples of the filters were analyzed for particulate N on a Carlo Erba 1106 elemental analyzer; the remainder of the filter was analyzed for ^{15}N enrichment with emission spectrometry (Timperley and Priscu 1986). Loss of $^{15}NH_4^+$ from solution, which was not accounted for by uptake into particulate, was used to indirectly measure NH_4^+ oxidation.

To determine if light uptake could account for all NH_4^+ loss from solution, we measured light uptake and regeneration of NH_4^+ as outlined by Priscu et al. (1988). Total NH_4^+ loss from solution uptake was calculated as decrease in NH_4^+ concentration + regeneration. This was compared to incorporation to particulate matter retained on Whatman GF/F filters. Incorporation was corrected for regeneration.

Results and Discussion

Bioassays based on nutrient stimulation of photosynthetic CO_2 uptake showed that simultaneous addition of N and P were necessary to stimulate primary production (Fig. 1). Similar bioassays yielded the same results in both February and May 1988 when NH_4^+ enriched treatments showed greater productivity than control treatments after 4.5 days (unpublished data). Therefore, NH_4^+ stimulation of dark $^{14}CO_2$ uptake would be expected to show N deficiency, since photosynthetic bioassays suggested N (and P) deficiency.

Dark $^{14}CO_2$ incorporation was enhanced with NH_4^+ on 26 October 1987. The effect was negated by addition of nitrapyrin (Table 1). There was some enhancement of dark $^{14}CO_2$ uptake in nitrapyrin treatments (dissolved in ethanol), compared to unamended lake water, but addition of ethanol to lake water caused a similar enhancement (Table 1).

Nitrapyrin probably did not inhibit β-carboxylation in algae, because dark $^{14}CO_2$ uptake was not lowered by the presence of nitrapyrin relative to unamended lake water in October, February, and May (Table 1). If nitrapyrin inhibited β-carboxylation activity, it would be expected to lower dark $^{14}CO_2$ uptake relative to rates measured in unamended lake water.

β-carboxylation activity in the presence of NH_4^+ in phytoplankton may go undetected against the high background

levels of chemolithotrophic or heterotrophic bacterial $^{14}CO_2$ uptake. Inhibition of NH_4^+ oxidizers also negated any stimulation of dark $^{14}CO_2$ uptake by NH_4^+ on 4 February 1988, but there was significant NH_4^+ stimulation of dark $^{14}CO_2$ uptake by organisms retained on a 2 µm filter (most bacteria presumably passed through a 2 µm filter). This difference was small relative to total uptake, and would not be detectable in the entire community. Chloramphenicol significantly lowered dark $^{14}CO_2$ uptake, demonstrating that procaryotes are responsible for up to 64% of the measured dark $^{14}CO_2$ uptake (Table 1). These results illustrate the importance of dark uptake of $^{14}CO_2$ by bacteria, and results discussed above suggest that some of this dark $^{14}CO_2$ uptake may be accounted for by NH_4^+ oxidizing bacteria.

Despite apparent phytoplankton N deficiency during May (Fig. 1), there was no stimulation of dark $^{14}CO_2$ incorporation by NH_4^+ on 2 May 1988 with or without nitrapyrin (Table 1). There was a 34% stimulation in the > 2 µm fraction, suggestive of NH_4^+ stimulation of β-carboxylation by phytoplankton, but this difference was not statistically significant (P > 0.10).

Independent $^{15}NH_4^+$ uptake data further indicate that NH_4^+ oxidation is important in this system. Total loss from the NH_4^+ pools over time on 29 July 1987 was 32.3 nM N h^{-1}, but incorporation rates into particulate matter were 20.4 nM N h^{-1}. Up to 37% (n = 2, std. dev. = 6.3%) of the loss of NH_4^+ from solution may be accounted for by NH_4^+ oxidation. Other

factors have also been hypothesized to account for the discrepancy between changes in the NH_4^+ pool and incorporation of NH_4^+ into particulates in other systems such as absorption onto abiotic particulates or incubation chamber walls (Laws 1984).

Dark CO_2 uptake, even when inhibitors such as nitrapyrin or size fractionation are used to remove NH_4^+ oxidizers, can only be used as an indication of whether or not the phytoplankton community experiences ambient NH_4^+ concentrations significantly below those which saturate uptake (V_{max}). This is because at NH_4^+ concentrations near V_{max}, further addition of NH_4^+ will not change total N uptake and will not cause an increase in β-carboxylation activity or in dark $^{14}CO_2$ uptake.

There was a significant stimulation of dark $^{14}CO_2$ uptake by NH_4^+ in November, and ambient levels of NH_4^+ were probably below V_{max} with respect to dark uptake at this time. Dark ^{15}N uptake experiments on 26 October 1987 showed that N uptake rates were 2.94 ± 0.382 nM h^{-1} with addition of 0.37 µM $^{15}NH_4^+$, and 3.95 ± 0.839 with addition of 7.0 µM $^{15}NH_4^+$. There was a significant difference between the two treatments, (P < 0.05, pooled $\underline{t}$ test). Therefore ambient substrate concentrations were below saturation with respect to dark $^{15}NH_4^+$ uptake by the entire community.

Measurement of NH_4^+ influences on dark CO_2 incorporation may indirectly indicate phytoplankton N deficiency. The assay may indicate NH_4^+ deficiency in NH_4^+ oxidizing bacteria

which may coincide with NH_4^+ deficiency in phytoplankton (if both types of organisms display similar NH_4^+ uptake kinetics). Stimulation of dark $^{14}CO_2$ incorporation by NH_4^+ does not unequivocally indicate N limitation, because NO_3^- levels may be high enough to support phytoplankton requirements even though NH_4^+ levels are low enough to limit dark CO_2 incorporation by NH_4^+ oxidizing bacteria. We suggest that future use of the dark CO_2 incorporation assay include nitrapyrin or size fraction treatments to control for NH_4^+ oxidation and that assay results be interpreted cautiously.

References

Dodds, W. and J. Priscu. 1988. An inexpensive, simple device for sampling large volumes of water at discreet depths. Freshwater. Biol. 20:113-116.

Elser, J. S., M. M. Elser, N. A. Marky and J. P. Carpenter. 1988. Zooplankton-mediated transitions between N- and P-limited algal growth. Limnol. Oceanogr. 33:1-14.

Jones, R. D., R. Y. Morita and R. P. Griffiths. 1984. Method for estimating in situ chemolithotrophic ammonium oxidation using carbon monoxide oxidation. Mar. Ecol. Prog. Ser. 17:259-269.

Laws, E. 1984. Isotope dilution models and the mystery of the vanishing ^{15}N. Limnol. Oceanogr. 29:379-386.

Mortain-Bertrand, A., C. Decolas-Gros and H. Jupira. 1988. Pathway of dark inorganic carbon fixation in two species of diatoms: influence of light regime and regulator factors on diel variations. J. Plank. Res. 10:199-217.

Morris, I., C. M. Yentsch and C. S. Yentsch. 1971. The physiological state with respect to nitrogen of phytoplankton from low-nutrient subtropical water as measured by the effect of ammonium ion on dark carbon dioxide fixation. Limnol. Oceanogr. 16:859-868.

Priscu, J. C., W. F. Vincent and C. Howard-Williams. 1988. Inorganic nitrogen uptake and regeneration in perennially ice covered Lakes Fryxell and Vanda, Antarctic. (in press J. Plank. Res.)

83

Taguchi, S. 1983. Dark fixation of CO_2 in the subtropical north Pacific Ocean and the Weddell Sea. Bull. Plankt. Soc. Jap. 30:115-124.

Timperley, M. H. and J. C. Priscu. 1986. Determination of nitrogen-15 by optical emission spectrometry using an atomic absorption spectrometer. Analyst 111:23-28.

White, E., K. Law, G. Payne and S. Pickmere. 1985. Nutrient demand and availability among planktonic communities - an attempt to assess nutrient limitation to plant growth in 12 central volcanic plateau lakes. New Zealand J. Mar. Freshwater Res. 19:49-62.

Vincent, W. F. 1981. Rapid physiological assays for nutrient demand by the plankton. I. Nitrogen. J. Plankton Res. 3:685-697.

Table 1.   Stimulation of dark $^{14}CO_2$ uptake by NH_4^+ in three experiments on Flathead Lake water. Values for $^{14}CO_2$ uptake and standard deviation should be multiplied by 10^3. Sample size is in the n column. Comparisons were tested with a pooled $\underline{t}$ test. Values for the test are in the t column and significance level is shown in the P column. *compared to 10 µM NH_4^+ with no other additions.

Treatment	$^{14}CO_2$ Uptake DPM l^{-1} h^{-1}	Standard Deviation	n	$\underline{t}$	P
26 October 1987					
control	4.58	4.42	3		
+ 10 uM NH_4^+	20.0	2.97	3	5.015	P < 0.005
+ nitrapyrin	14.3	1.79	3		
+ NH_4^+ + nitrapyrin	15.5	3.45	3	0.544	P > 0.25
+ 50 ul Ethanol	13.7	4.27	3		
4 February 1988					
control	8.39	3.37	2		
+ 10 uM NH_4^+	11.90	2.98	6	1.410	P > 0.10
+ nitrapyrin	8.39	1.20	3		
+ NH_4^+ + nitrapyrin	9.57	1.32	3	1.146	P > 0.10
+ 50 ul Ethanol	9.00	1.59	3		
> 2 um	0.34	0.018	3		
> 2 um + 10 uM NH_4^+	0.54	0.006	3	18.83	P < 0.0005
+ NH_4^+ + Chloramp	3.05	1.75	3		
				4.44	P < 0.01*

Table 1 continued.

Treatment	$^{14}CO_2$ Uptake DPM l^{-1} h^{-1}	Standard Deviation	n	$\underline{t}$	P
2 May 1988					
control	13.6	1.03	3		
+ 10 uM NH_4^+	12.5	2.44	3	0 .72	P > 0.25
+ nitrapyrin	13.7	0.91	3		
+ NH_4^+ + nitrapyrin	11.7	1.42	3	1.95	P > 0.10
+ 50 ul Ethanol	12.6	3.22	3		
> 2 um	3.17	2.22	3		
> 2 um + 10 uM NH_4^+	4.26	0.67	3	0.81	P > 0.10

Figure 1. Photosynthetic $^{14}CO_2$ uptake time-course after nutrient enrichment in Flathead Lake. Bars give 1 standard deviation. (A) 1 November 1987; (B) 5 May 1988.

Figure 1
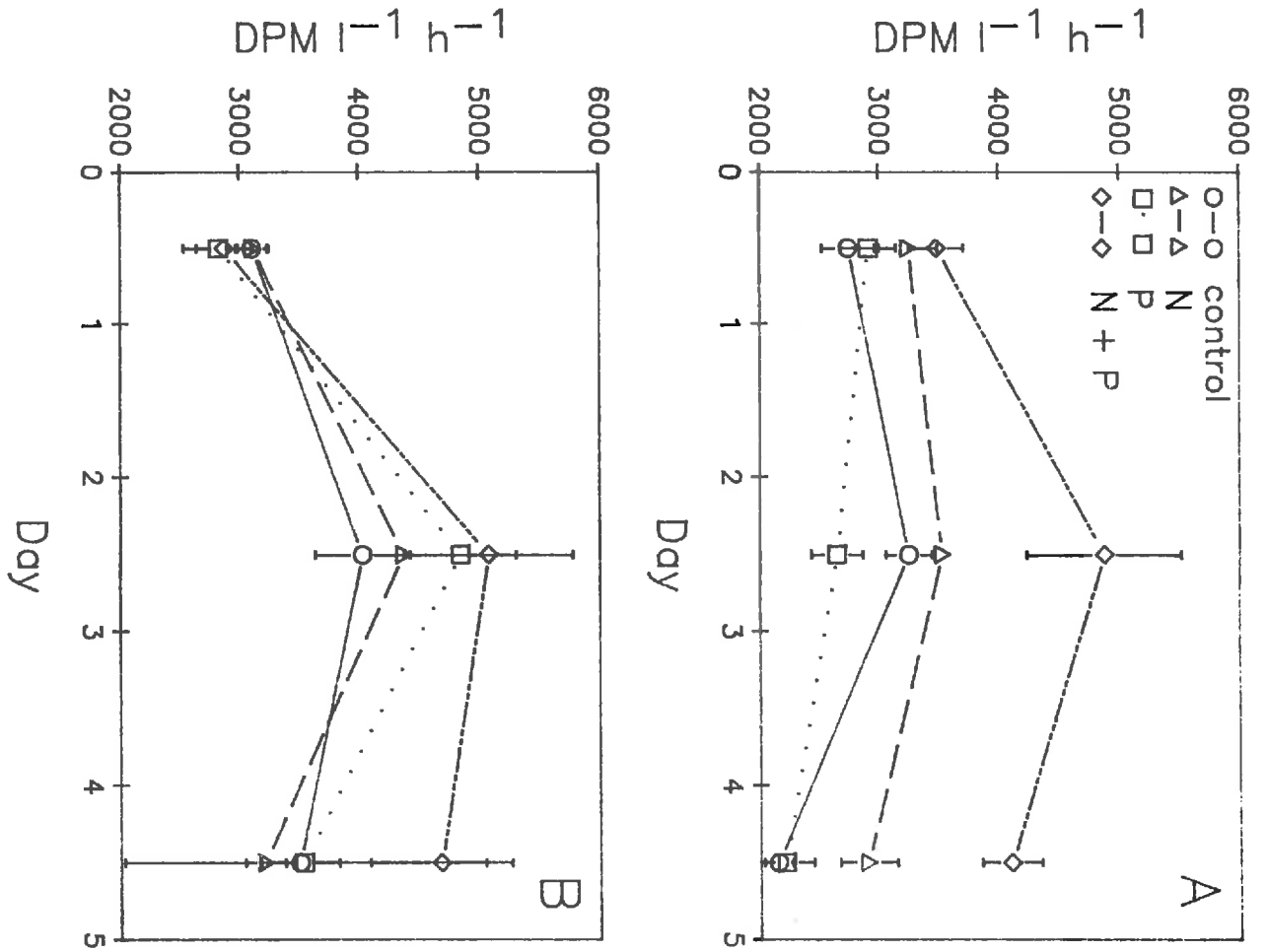
Chapter 5

Development and Application of a Technique for
Estimating Nutrient Deficiency in Soft Sediments

Abstract

A diffusion enrichment technique is presented which allows for chemical enrichment of soft surficial and shallow subsurface sediments and subsequent measurement of O_2 production. The sediment is enriched by inserting a perforated tube containing dialysis tubing which is filled with a nutrient/agar mixture. O_2 production by surficial sediment is measured using an inverted, opaque polyethylene dish over the sediment. The inside of the dish contains a collapsible bag connected to the water outside the dish. When water overlying the sediment is withdrawn from a sampling port, it is displaced with water from outside the dish, and thus water samples are not contaminated by an influx of pore water from below. The technique was tested by enriching near-shore sediments in a large oligotrophic lake with organic N and P. NH_4^+ additions significantly stimulated benthic primary production as measured by O_2 production, whereas enrichment with PO_4^{3-} had no effect.

Introduction

Two methods previously used to expose lentic benthic communities to nutrient enrichment are diffusing artificial substrates (Carrick and Lowe 1988, Fairchild and Everett 1988) and open bottom limnocorrals (mesocosms) secured to the sediments (Seitzinger and Nixon 1985, Stewart et al. 1983). Diffusing substrates are usually clay pots filled with an enriched nutrient solution, sealed and placed on the lake bottom. The pots are then naturally colonized by epilithic algae which are subjected to increased levels of nutrients leaching from the pots. Productivity, biomass, and species composition of the algae coating the pots is then measured. These substrates can be useful for determining the effect of added nutrients on epilithic communities, but may not accurately simulate environmental conditions in soft sediments. For example, soft sediments exhibit small scale vertical heterogeneity in oxygen (Revsbech and Jørgensen, 1986) and light (Jørgensen and Des Marais, 1986) which may not be present on the surfaces of artificial substrates.

Open bottom limnocorrals placed over the sediments may approximate natural situations, but the lack of water replacement can slow the growth of benthic algae over a few weeks (Dodds and Castenholz 1988). In addition, limnocorrals are relatively difficult and costly to construct making numerous replicate treatments burdensome.

We developed a system for measuring the effect of nutrient enrichment on photosynthetic O_2 production in soft sediments which is inexpensive, can be easily replicated, and minimally perturbs the benthos. We present results from a pilot study using our enrichment technique on the sediments of an oligotrophic lake.

Materials and Methods

Diffusion enrichment tubes were constructed using polyvinylchloride encasements plugged on one end (Fig. 1A). The plugged end was cut to form a point for easy insertion into sediments. Holes (0.5 cm diameter) were drilled into the sides of the encasements to allow outward chemical diffusion. Enrichment media was made by adding agar (1.5%, g:g) to an aqueous nutrient solution and then autoclaving. Immediately before solidification, the enrichment media was poured into 1.6 cm diameter dialysis tubing (Spectrapor cellulose 12,000-14,000 molecular weight cut off) which had previously been leached in deionized water for 12 h and tied off at one end. After media addition, the other end of the tubing was tied, and the filled dialysis tube was inserted into the polyvinylchloride encasement which was then plugged on top.

In a pilot test of the enrichment technique in a large oligotrophic lake (Flathead Lake, Montana, USA), we tested the enrichment tubes in near-shore sediments. The sediments were silty and interspersed with rocks (sediment porosity =

0.357 g H_2O/g sediment). Triplicate treatments included controls and tubes enriched with 0.1 M KH_2PO_4 or 0.5 M NH_4Cl. Tubes were inserted in sediments covered by 1.5 m of water on 26 July 1987, and sediment O_2 production and chlorophyll were measured on 6 October 1987 in the sediments adjacent to the enrichment tubes.

Diffusion rates from the enrichment tubes was tested by placing water saturated sediment from Flathead Lake in 20 l open containers. Tubes were enriched with 0.011 M uranin (sodium fluorescein, a biologically conservative chemical), inserted into the sediment, and sediment cores were taken 5 cm from the enrichment tubes at days 1, 3, 7, and 14. The pore water was separated by centrifugation and the concentration of dye was determined spectrophotometrically at 478 nm.

Sedimentary O_2 production was measured with a chamber inserted 1 cm into the sediment (Fig. 1B). The chambers were designed so water withdrawn from the chamber through the sampling port would be replaced with water from above the chamber which flowed into a collapsed, clear, polyethylene bag inside the chamber. This allowed for sampling without drawing pore water up from below and contaminating the sample. The chambers had an average light transmission of 79.2%. The chambers were left in place for 5.6 h after which 30 ml of water was sampled from each chamber by a SCUBA diver. It was necessary to sample the chambers using SCUBA so as not to disturb the sediments.

Oxygen in water from the chambers was measured using a Winkler technique (EPA 1971) modified to 0.1 scale.

Sediment samples were removed for chlorophyll a determination by inserting a 2 m long tube with a cross-sectional area of 9 cm^2, 3 cm into the sediment and using suction to pull the top 1 cm of sediment from the bottom of the tube into a collection container above the surface. This sediment was re-suspended in 1 liter de-ionized H_2O with vigorous stirring. A 10 ml aliquot of the suspension was then filtered onto a Whatman GF/C filter, extracted by grinding in 10 ml 9:1 acetone:water and the extracts were analyzed fluorometrically for chlorophyll a (Strickland and Parsons 1972).

Results and Discussion

Diffusion loss from the fluorescein filled tube was 1.2% d^{-1} (std. dev. = 1.8, n = 16). Variance of diffusion rates in natural sediments was high (CV = 150%), but this may not be extremely important if diffusion supplies nutrients to the area to be sampled so they remain at concentrations at or near V_{max} for the nutrient being tested. Determination of diffusion rates before application in any particular sediment system is necessary so the appropriate sampling time can be determined. If the enrichment tubes lost nutrient at the same rate as the fluorescein, 42% of the original nutrient would have been left after the 73 day incubation of our pilot study in Flathead Lake.

94

O_2 production measurements from our pilot study implied that Flathead Lake sediments are N deficient. NH_4^+ enrichment stimulated O_2 production by 36%, and P enrichment stimulate O_2 production by only 16% indicating N deficiency (Fig. 2B). There were no significant changes in the levels of chlorophyll $\underline{a}$ cm^{-2} in any treatment and levels of variance were high (Fig. 2A).

Given the level of variance within the chlorophyll treatments, 15 replicates of each treatment would have been required to obtain a significant difference between the P and control treatments. The level of replication was, however, sufficient to show a significant response of O_2 production to N enrichment owing to smaller variances in O_2 production within treatments. The discrepancy in the two levels of variance is probably related to the sampling scale. The chlorophyll samples were taken over 9 cm^2, whereas the O_2 production chambers covered and area of 50 cm^2. The larger area sampled by the O_2 production method would average smaller scale inhomogeneities.

We developed a technique to enrich sediments with nutrients and quantify the response of primary producers. In many systems, benthic primary production can be extremely important to the aquatic system, and until now, no simple method existed for replicating multiple chemical perturbations in sediments. Our technique need not necessarily be restricted to nutrient enrichment experiments. It may also be used to gauge diffusion rates

in the sediments using dyes or the environmental impact of other chemicals.

Acknowledgements

References

Carrick, H. J. and R. L. Lowe. 1988. Response of Lake Michigan benthic algae to in situ enrichment with Si, N, and P. Can. J. Fish. Aquat. Sci. 45:271-279.

Dodds, W. K. and R. W. Castenholz. 1988. The biological effects of nitrate fertilization and water replacement in an oligotrophic cold water pond. Hydrobiologia 162:141-146.

Environmental Protection Agency. 1971. Methods for chemical analysis of water and wastes. U. S. Government Printing Office. Number 5501-0067.

Fairchild, G. W. and A. C. Everett. 1988. Effects of nutrient (N, P, C) enrichment upon periphyton standing crop, species composition and primary production in an oligotrophic softwater lake. Freshw. Biol. 19:57-70.

Jørgensen, B. B. and D. S. Des Marais. 1986. A simple fiber-optic microprobe for high resolution light measurements: application in marine sediment. Limnol. Oceanogr. 31:1376-1383.

Revsbech, N. P. and B. B. Jørgensen. 1986. Microelectrodes: their use in microbial ecology, pp. 293-352. IN: Marshall, K. C. (ed.), Advances in Microbial Ecology, volume 9. Plenum Press, New York.

Seitzinger, S. P. and S. W. Nixon. 1985. Eutrophication and
      rate of denitrification and N2O production in coastal
      marine sediments. Limnol. Oceanogr. 30:1332-1339.

Stewart, W. D. P., T. Preston, A. N. Rai and P. Rowell.
      1983. pp. 1-27. IN: Lee, S. A., S. McNeill and I. H.
      Rorison (eds.), Nitrogen as an Ecological Factor.
      Blackwell Scientific Publications, Oxford.

Strickland, J. D. and T. R. Parsons. 1972. A practical
      handbook of seawater analysis. Bull. Fish. Res. Board
      Can. (2nd ed.) 167.

Figure 1. A novel chemical enrichment device and an O_2 incubation chamber for determining nutrient deficiency in soft sediments. A) Cut-away view of a diffusion enrichment tube. Scale bar = 4 cm. a) shows the top plug, b) the enrichment plug c) the polyvinylchloride tube, and d) the bottom plug and end cut for insertion into the sediment. B) Chamber for estimating oxygen production in soft sediments, top and side views. Scale bar = 4 cm. a) shows the main chamber, b) the sampling port, c) the port connecting a bag to the outside, d) a collapsible bag inside, e) a weight collar.

Figure 2. Chlorophyll a concentration and O_2 production of sediment under various nutrient enrichments in Flathead Lake. Error bars = 1 std. dev., n = 3 for all treatments. * = a treatment which is significantly greater than control ($P < 0.05$, pooled t test).

Figure 1
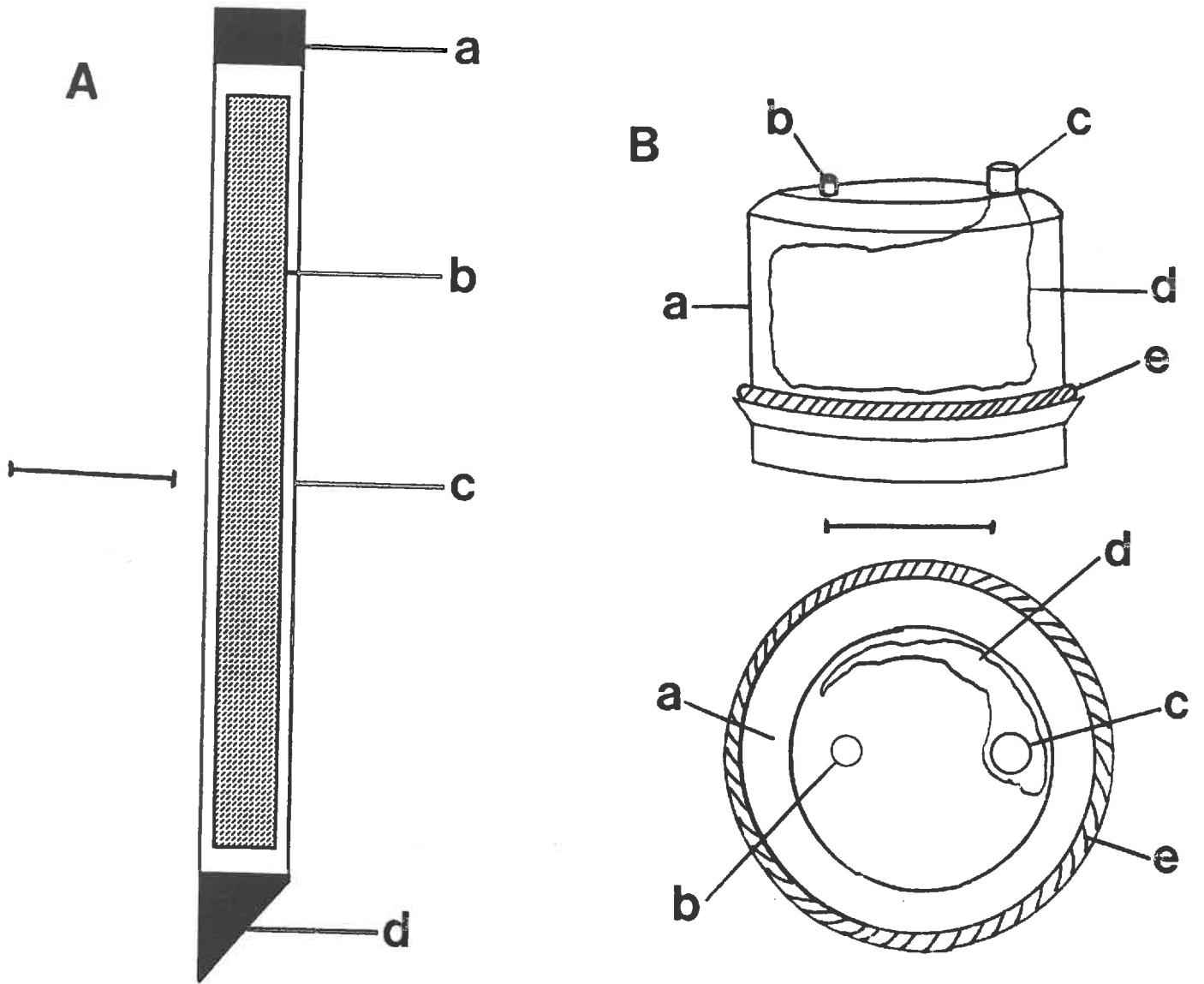
Figure 2
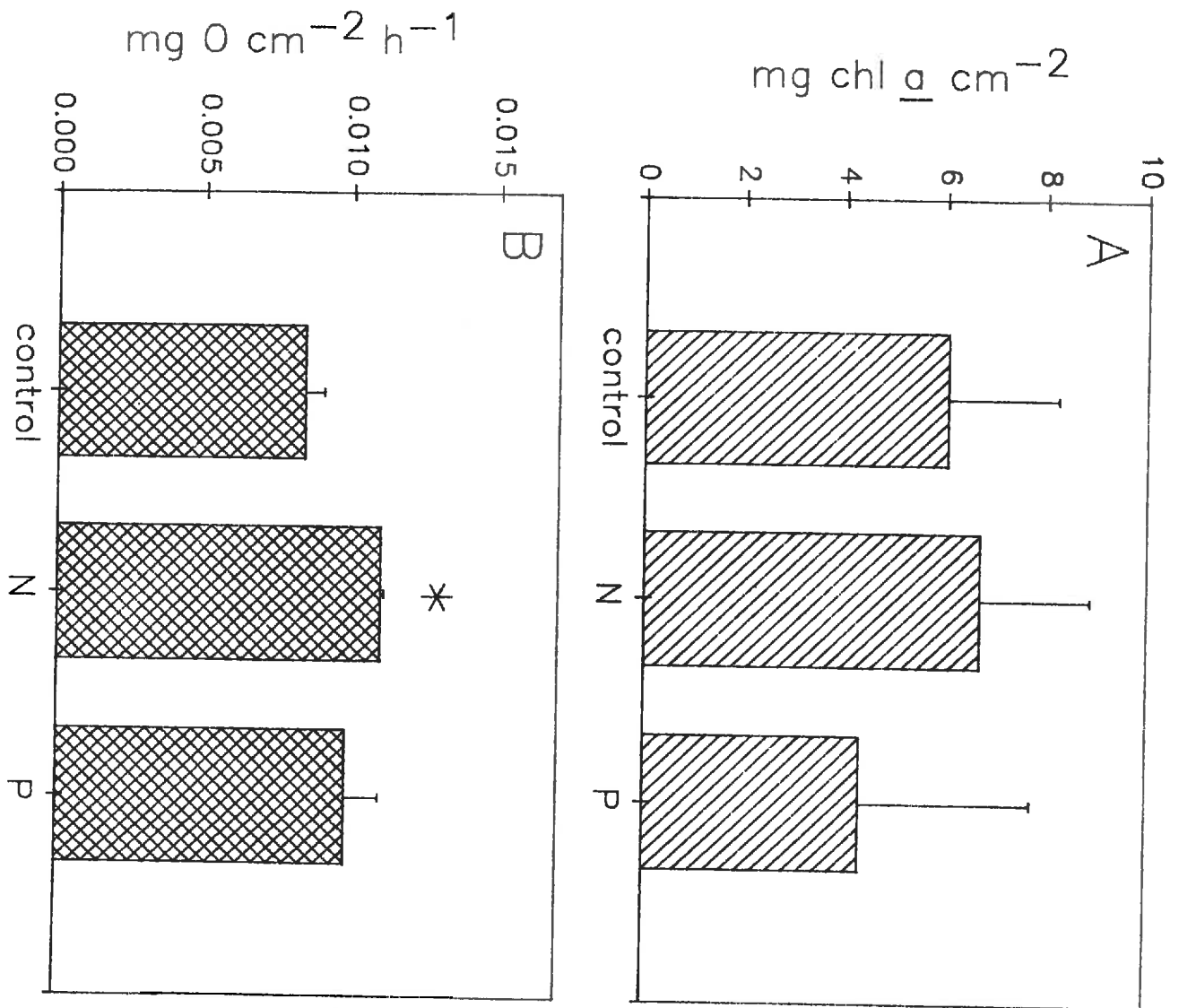
Chapter 6

Temporal Dynamics of Nitrate, Ammonium, and Phosphate
in a Large Oligotrophic Lake: Uptake, Regeneration,
Size-fractionation and Antibiotic Treatment

Abstract

Uptake and regeneration of inorganic N and P in oligotrophic Flathead Lake (Montana) were characterized over a seasonal cycle. N and P uptake and regeneration rates were lowest in the winter. Ambient concentrations of nutrients were usually below K_s for NH_4^+, NO_3^-, and PO_4^{3-}, indicating a potential deficiency of these nutrients. The molar ratios of ambient uptake rates of inorganic N:P ranged from 2.11 to 25.6 with a mean of 8.6; the lowest rates occurred in the winter and spring. Experimental additions of as little as 0.2 µM NH_4^+ reduced $^{15}NO_3^-$ uptake significantly at all times of the year, with the strongest effect occurring in the winter.

Given ambient uptake rates, the relatively low and constant nutrient pools, and low allochthonous nutrient input, nutrient supply via regenerated NH_4^+ and PO_4^{3-} controls the availability of these nutrients to phytoplankton. The molar ratio of regenerated N:P ranged from 22-75 with a mean of 40.8, indicating that N:P regeneration ratio is in excess of the ambient N:P uptake ratio. Organisms in the > 280 µm size fraction were responsible for 0-60% of regenerated NH_4^+ and 0-40% of the regenerated PO_4^{3-}; 40-100% of the NH_4^+ and PO_4^{3-} regeneration occurred in the < 3 µm fraction. The < 3 µm fraction also accounted for 40-100% of NH_4^+ and PO_4^{3-} uptake, except during May, when organisms > 3 µm were responsible for more than 80% of the PO_4^{3-} uptake. Cycloheximide (inhibits eucaryotic protein synthesis)

103

reduced rates of uptake and regeneration of NH_4^+ in whole lake water throughout the year. Chloramphenicol (inhibits procaryotic synthesis) decreased uptake and increased regeneration of NH_4^+. Antibiotic treatments were less useful for explaining PO_4^{3-} dynamics because they did not consistently effect uptake or regeneration. Our data show that microbial nutrition is tightly coupled with regenerative supply rates and that the ambient nutrient pool sizes give limited information about nutrient deficiencies.

Introduction

The uptake of nutrients by micro-organisms is usually dependent upon concentration and can be modeled using Michaelis-Menten kinetics (Button 1985, Dugdale 1967). Michaelis-Menten parameters have been used to examine nutrient deficiencies in marine (Dugdale 1967) and freshwater systems (Fisher et al. 1988, Murphy 1980). However, in most oligotrophic systems, substrate levels are at or near the level of detection. This leads to errors when the Michaelis-Menten formalism is used to estimate ambient uptake rates and makes it difficult to draw meaningful conclusions regarding phytoplankton nutrient deficiencies. Consequently rates of nutrient supply (internal nutrient regeneration) must also be used to determine nutrient deficiencies although few investigators have used this approach.

It has been demonstrated that regenerated N (Harrison 1978, Morrissey and Fisher 1988, Priscu and Priscu 1984) and P (Nalewajko and Lean 1980) can contribute significantly to phytoplankton growth in nutrient deficient systems lacking significant allochthonous inputs. However, concurrent measurements of N and P regeneration in fresh waters (eg. Fisher et al. 1987, Harrison and Harris 1986) are rare, and have not been conducted seasonally.

Recently, there has been an emphasis on the importance of small size fractions (the microbial loop) to P (Currie and Kalff 1984a) and N (Kokkinakis and Wheeler 1988,

Harrison and Wood 1988) cycling, and attempts have been made to separate eukaryotic and prokaryotic components of N dynamics using antibiotic treatments (Wheeler and Kirchman 1987). However, these data are available for few systems and not on a seasonal basis. Our study examines how supply of, and demand for, N and P effect seasonal phytoplankton nutrient deficiency in a large oligotrophic lake. We also employ size fractionation and antibiotic techniques to determine, seasonally, the types of organisms responsible for these fluxes.

Materials and Methods

Common Methods

Samples were taken from Flathead Lake (Montana), a large (460 km^2), oligotrophic (epilimnetic chl $\underline{a}$ conc. 0.1 - 1.1 μg l^{-1}) lake. Water was collected 2 km from shore in the deepest portion of the lake with a displacement sampler (Dodds and Priscu 1988) from 5 m (the depth of maximum primary productivity (Stanford et al. 1983)). For each sample date, NH_4^+, NO_3^-, and soluble reactive phosphorus (SRP) were measured using the phenol-hypochlorite (Solorzano 1969), cadmium reduction (Eppley 1978) and mixed-molybdate (Strickland and Parsons 1972) methods respectively.

N Uptake

Time-course measurements of $^{15}NO_3^-$ and $^{15}NH_4^+$ uptake were made after addition of $^{15}NH_4^+$ or $^{15}NO_3^-$ to a final concentration of 10 μM in a series of 500 ml bottles. These samples were stopped at 0 min with formalin, while all other samples were stopped by filtration onto Whatman GF/F filters. All time-course filters were analyzed for ^{15}N enrichment according to Timperley and Priscu (1986).

$^{15}[N]-NH_4Cl$ or $^{15}[N]-NaNO_3$ was used to measure N incorporation. Incubations were conducted in a series of 500 ml bottles inoculated with $^{15}NH_4^+$ or $^{15}NO_3^-$ to final concentrations ranging from 0.15 to 22 μM. $^{15}NH_4^+$ incorporation experiments were incubated for 4 hours and $^{15}NO_3^-$ for 6 hours at 5 m (the depth of collection). Incubations were stopped by filtration onto precombusted Whatman GF/F glass-fiber filters. C and N concentration of particulates on each filter was measured with a Carlo-Erba 1106 elemental analyzer. The remainder of the filter was analyzed for ^{15}N enrichment by Dumas combustion followed by optical emission spectrometry (Timperley and Priscu 1986). Formalin kills were used to account for label which absorbed to the filters and exchanged abiotically with intracellular pools. Uptake was calculated as in Collos (1987) to avoid errors associated with change in particulate N concentrations during the incubation period. Ammonium uptake was corrected for isotope dilution using the equation

of Laws (1984). When calculating the effect of NH_4^+ on $^{15}NO_3^-$ uptake, the approach of Collos (1987) was used to avoid artifacts from concurrent uptake of unlabeled N. Ambient uptake was calculated by using data on ambient substrate pool sizes and uptake kinetic parameters.

Trichloroacetic acid (TCA) extractions were used to estimate ^{15}N incorporation into TCA precipitable material (protein). Half of each filter used to measure net uptake was vortexed vigorously in a solution of 5% TCA at 0° C and allowed to extract for 5 min. The extracted filter and supernatant were then re-filtered through a Whatman GF/F filter, washed 3 times with ice-cold 5% TCA and dried at 50° C. ^{15}N analysis was conducted on the filters as explained above. The particulate N (PN) content of TCA extracted filters was not significantly different than those which had not been extracted ($P > 0.25$, n=6, pooled $\underline{t}$ test); the particulate N concentration for non-extracted filters was used to calculate incorporation into TCA insoluble material. Michaelis-Menten uptake kinetic curves were fitted with a non-linear curve fitting program (Li 1983).

NH_4^+ Uptake and Regeneration

Regeneration experiments were conducted in 4 l bottles enriched with 20 atom % $^{15}NH_4Cl$ to a final concentration of 5 µM. Aliquots (0.5 l) were removed at 0, 3, 6, 12, 24, and 36 h and filtered through pre-combusted Whatman GF/F filters which were analyzed for ^{15}N enrichment by emission

spectrometry. NH_4^+ was scavenged from solution using
Zeolite ion-sieve (Lipschultz 1984), dried and subsequently
analyzed for ^{15}N enrichment with emission spectroscopy.
Values were corrected for isotopic discrimination by
Zeolite, which was important in this study. Total NH_4^+ was
determined for each aliquot (Solorzano 1969). Regeneration
rates were calculated using a modification of the Blackburn-
Caperon model (Laws 1984). Size fractionated regeneration
was determined by measuring isotope dilution in samples
which passed through either a 280 μm nylon mesh or 3 μm
Nuclepore filters before incubation. The effectiveness of
size fractionation was tested with phase and epi-fluorescent
microscopy. Cycloheximide and chloramphenicol (which
inhibit eukaryotic and prokaryotic protein synthesis,
respectively) were added at 10 mg l^{-1} final concentration 2
h before the experiment started, except in February and May
1988 when a 12 hour pre-incubation was used.

PO_4^{3-} uptake and regeneration

For the $^{32}PO_4^{3-}$ time-course experiment, a 50 ml aliquot
of lake water was amended with $^{32}[P]-H_3PO_4$ to 20,000 DPM ml^{-1}.
Subsamples of 5 ml were removed over time and filtered
through Gelman GN-6 filters which were counted with liquid
scintillation spectrometry.

For uptake kinetic experiments, 4 replicates of 20 ml
samples of lake water were amended with unlabeled KH_2PO_4 to
final concentrations ranging from 0 to 325 nM. Carrier free

$^{32}[P]-H_3PO_4$ was then added to all samples to a final concentration of 20,000 DPM ml^{-1}. Incubations were terminated after 4 min by filtration onto Gelman GN-6 membranes (0.45 μm pore size). Filters were counted with liquid scintillation spectrometry. Kills showed that abiotically labeled particulates were insignificant and stacked double filters were used to account for label absorbed onto filters. Particulate P was measured using dry oxidation (Solorzano and Sharp 1980). Maximum ambient levels of biologically available P were estimated using the method of Rigler (1966).

For size fractionated and antibiotic treated regeneration and uptake experiments, $^{32}PO_4^{3-}$ was added to 20 ml samples of lake water as above, but with no carrier (labeled PO_4^{3-}). After 4 min of incubation, samples were filtered through Gelman GN-6 membranes and filtrates were counted with liquid scintillation spectrometry. Size fractionation was done prior to incubation by filtering through Nuclepore filters for the < 3 μm fraction or by pouring through nylon mesh for the < 280 μm fraction. Cycloheximide and chloramphenicol were added for a 30 min pre-incubation period at 10 mg l^{-1}. Regeneration was calculated using an equation which assumes no measurable change in substrate concentration (Laws 1984).

Results

Time-course of NH_4^+, NO_3^-, and PO_4^{3-} uptake

Time course measurement of $^{15}NH_4^+$ incorporation into particulate matter was essentially linear over the first 4 h (Fig. 1a). When ^{15}N atom % enrichments were converted to PN specific uptake rates, the rates during the first 5 min after receiving the N pulses were 3 times higher than after 15 min (Fig. 1b). This surge was not present with TCA extracted filters. We used 4 h incubations for all other ^{15}N experiments because non-saturating $^{15}NH_4^+$ additions would not show measurable incorporation into particulates with shorter incubations.

Conversely, time-course enrichment of $^{15}NO_3^-$ showed little difference between TCA and non-TCA extracted particulates (Fig. 1c) and surge uptake was not obvious in these incubations (Fig. 1d).

Time-course $^{32}PO_4^{3-}$ incorporation became non-linear within minutes, rather than after hours as seen with N cycling (Fig. 2). The 4 min incubations we used to measure uptake and regeneration in all other ^{32}P experiments were based on the linearity of the uptake curve over approximately the first 10 min.

NO_3^- and NH_4^+ Uptake Kinetics

$^{15}NO_{3-}$ uptake kinetic parameters varied throughout the year (Fig. 3, Table 1). The lowest values for V_{max} occurred in February, coincident with the highest NO_3^- concentrations

(Table 1). Values of K_s were always higher than the ambient concentrations. The uptake kinetics parameters for TCA precipitable material were similar to those for non-TCA extracted samples (Table 1).

$^{15}NH_{4+}$ uptake kinetic experiments showed that V_{max} and ambient uptake rates were lowest in February (Fig. 4, Table 2). Both volume and biomass specific rates of NH_4^+ uptake were lowest during the winter because particulate N was relatively constant over the year (Tables 1 and 2). In addition, the V_{max} and K_s values for TCA precipitable material were always lower than for intact cells, except in May when K_s for whole cells was lower. With the exception of July, ambient NH_4^+ concentration was below K_s, indicating that ambient levels of NH_4^+ are low with respect to phytoplankton uptake capacity. The cases in which the predicted curves did not fit the data at low concentrations well, were also times when the lowest $^{15}NH_{4+}$ addition was above K_s, causing poor resolution on the low part of the kinetic curve.

Values for $^{15}NH_4^+$ uptake measured at the lowest NH_4^+ addition (0.14 µM) were 6-35% higher when corrected for isotope dilution by regeneration (Table 3). Also, computer modeling (Garside and Glibert 1984) showed that the pool size of NH_4^+ did not change considerably over the course of these incubations. Therefore, our uptake kinetics should represent actual physiological characteristics of the cells rather than an artifact of the protocol (Garside 1984).

Relationships between NH_4^+ and NO_3^- Uptake

The ambient uptake of NO_3^- was always at least ten fold lower than that of NH_4^+ (Tables 1 and 2). V_{max} for NH_4^+ was also higher than for NO_3^-. K_s values were lower for NO_3^- than for NH_4^+ which coincided with lower ambient levels of NO_3^- than NH_4^+ (Tables 1 and 2). Even though NH_4^+ demand was higher than NO_3^- demand, the ambient NH_4^+ concentrations were higher than NO_3^-, indicating that the rate of NH_4^+ supply was higher than the rate of NO_3^- supply.

The rate of NO_3^- uptake was lowered by addition of as little as 0.2 µM NH_4Cl at all times of the year (Fig. 5). Addition to a final concentration of 5 µM NH_4Cl lowered NO_3^- uptake by 37.8% in June, 62.7% in July, 80.0% in September, 89.8 % in November, 100% in February, and 94.7% in May. The strongest effect was in February, when ambient NO_3^- concentrations were greatest (Table 1). The ambient NH_4^+ concentrations in these incubations were always less than 1 uM.

PO_4^{3-} Uptake Kinetics

V_{max} for PO_4^{3-} uptake was lowest in February, highest in November and intermediate in July, August, September, and May. Values for K_s varied considerably (Fig. 6, Table. 4). If chemically determined PO_4^{3-} (SRP) was used to calculate uptake, the uptake appeared to increase with decreased

concentration (i.e. an inverse hyperbola occurred).
Therefore, it was necessary to use the biologically
available P (Rigler 1966), which in all cases was much less
than SRP values (Table 3), as has been shown in other
systems (Tarapchak et al. 1982).

Uptake and Regeneration: Size Fractionation and Antibiotic Treatment

The < 3 μm fraction accounted for 40-100% of the N
regeneration and uptake (measured as loss of NH_4^+ from
solution plus regeneration) (Fig. 7). The > 280 μm fraction
was of relatively little importance in uptake or
regeneration of NH_4^+. Cycloheximide generally decreased
uptake and regeneration of NH_4^+ (Fig. 7). Chloramphenicol
also always decreased NH_4^+ uptake, but caused up to a 160%
increase in NH_4^+ regeneration (Fig. 7).

Phosphate uptake and regeneration of whole lake water
was more variable than NH_4^+ over the year (Fig. 8). The
lowest values for PO_4^{3-} uptake and regeneration were recorded
in August, February, and May. PO_4^{3-} dynamics were dominated
by small organisms at all times except May. Excluding May,
the < 3μm fraction accounted for 42-64% of the P uptake
measured in whole lake water, and the < 280 μm fraction
contributed 45-88% of the uptake, suggesting that the < 3 μm
fraction is extremely important in P uptake. The < 3 μm
fraction also accounted for 33-98% of the P regeneration
(Fig. 8). During May, the < 3 μm fraction contributed only

20% of the PO_4^{3-} uptake. Effects of cycloheximide or chloramphenicol on P uptake or regeneration were not consistent (Fig. 8).

We observed that procaryotic primary producers in Flathead Lake (_Aphanothece_ and _Chroococcus_) were able to pass through a 3 μm Nuclepore filter indicating that the < 3 μm fraction still contained algae. The 280 μm mesh filtration removed large zooplankton and detrital particles only; few phytoplankton cells were retained.

Discussion

Nutrient Uptake

We measured surge uptake with NH_4^+, but not with NO_3^-. Similar time-courses on $^{15}NO_3^-$ and $^{15}NH_4^+$ uptake were reported for tropical Lake Calado (Fisher et al. 1988). The surge in NH_4^+ uptake did not, however, occur in TCA precipitable material. Similar surge uptake has been documented for NH_4^+ in freshwater (Priscu and Priscu 1984, Priscu 1987, Suttle and Harrison 1988a). The fact that the surge is not as evident in TCA extracted material implies that ^{15}N is initially incorporated into internal dissolved N pools (Morel 1987, Priscu 1987). Therefore, uptake kinetic curves generated using TCA insoluble incorporation may relate more directly to growth than measurements made on whole cells, although uptake measured after the initial surge may reflect assimilation into the macro-molecular fraction rather than dissolved pools (Wheeler et al. 1982, Priscu 1987).

115

Low V_{max} and ambient uptake during winter is presumably a response to reduced temperature and light, both of which are important factors controlling uptake of NO_3^-, NH_4^+, and PO_4^{3-} (MacIsaac and Dugdale 1972, Nalewajko et al. 1986, Nalewajko and Lee 1983, Priscu 1984, Priscu 1988, Whalen and Alexander 1984).

The molar ratio of NH_4^+ + NO_3^- uptake to PO_4^{3-} uptake at ambient N and P levels was usually lower than the Redfield ratio suggesting N deficiency (Table 5). Ambient substrate concentrations were lower than K_s, and V_{max}/V_{amb} was always greater than 1 for NH_4^+, NO_3^-, and PO_4^{3-} which suggests deficiencies in both N and P for the community as a whole (Glibert and McCarthy 1984). Bioassays conducted during the same period showed that both N and P are important determinants of primary productivity.

N and P Regeneration

N and P supply rates are approximated by NH_4^+ and PO_4^{3-}. Given ambient uptake rates and assuming no regeneration, the PO_4^{3-} pool would be depleted in 15-120 minutes whereas, the ambient NH_4^+ pool would be depleted in about 1 day indicating that these nutrient pools are rapidly replenished internally. Volumetric rates of external loading (from rivers and the atmosphere) are 0.7% of regeneration rates for N and 4% for P (Stanford et al. 1983) and during the

summer the diffusive input of nutrients from the hypolimnion is low. Therefore, regeneration is probably the most important source of nutrients during the period of stratification. In addition, PO_4^{3-} uptake rates were closely coupled to regeneration rates (Fig. 11), and NH_4^+ regeneration exceeded incorporation rates (Fig. 10), indicating that remineralization adequately meets the demands for ambient PO_4^{3-} and NH_4^+ uptake. Ammonium pools remained stable throughout the year (Table 2), but regeneration exceeded uptake. However, because there are losses of NH_4^+ from solution other than uptake into particulates, regeneration must exceed uptake if pools are to remain constant.

Net loss of NH_4^+ from solution exceeding uptake into particulate has been reported in other studies (Laws 1984) and may reflect ammonia oxidation, adsorption of NH_4^+ to containers, or release of ^{15}N as DON. It is also possible that organisms able to pass through a 0.7 μm Whatman GF/F filter take up $^{15}NH_4^+$.

The < 3 μm fraction was relatively important in both uptake and regeneration of NH_4^+ and PO_4^{3-}. Similar results have been obtained by others (Currie and Kalff 1984a,b, Fischer et al. 1987, Suttle and Harrison 1988b), but smaller size fractions were shown to be less important to P regeneration in a marine system (Harrison 1983). The low amount of P uptake in the < 3 μm fraction in May (6.3 %) agrees with ^{33}P autoradiography previously done on Flathead Lake phytoplankton in the spring (Ellis unpublished data).

Interrelationship between uptake and regeneration of N and P

N and P uptake rates cannot be used to accurately estimate the N:P supply ratio. Suttle and Harrison (1988c) used the ratio of $V_{max} PO_4^{3-}/V_{max} NH_4^+$ to estimate N:P supply ratio, and the resultant ratio is used to predict algal nutrient deficiency. If the measured N:P uptake rate does not directly apply to N:P supply to algae, then this technique may not be a rigorous indicator of algal nutrition.

Since N and P regeneration rates are indicative of N and P supply in Flathead Lake, it is possible to test the equation proposed by Suttle and Harrison (1988c) to estimate N:P supply ratios using ratios of maximum uptake rates of NH_4^+ to PO_4^{3-}. Table 5 gives the results of such a calculation. There is some agreement between calculated and observed ratios of N:P supply, but most of the values we obtained fell outside of the 95% confidence interval proposed by Suttle and Harrison (1988c). Hence, their method for calculating N:P supply ratios may not be generally applicable to oligotrophic systems. Most of the N:P supply ratios calculated by the method of Suttle and Harrison (1988c) fall between the 5:1 - 45:1 limits they proposed for simultaneous deficiency of N and P, so their method is consistent with our data in this regard.

The N:P supply ratios are essentially all higher than the N:P ambient uptake ratios (Table 5). The higher supply

ratios result from actual NH_4^+ incorporation rates being less than measured rates of NH_4^+ loss from solution (Fig. 10). If all regenerated PO_4^{3-} is incorporated into particulate matter, but all regenerated NH_4^+ is not, measured N:P supply ratios will be higher than N:P ambient uptake ratios.

The < 3 μm fraction controlled most of the nutrient dynamics and consequently nutrient availability to primary producers. Because the organisms > 3 μm were relatively unimportant in N or P regeneration, zooplankton are probably not important remineralizers of nutrients in this system.

Analysis of N and P uptake and regeneration did not conclusively demonstrate N or P deficiency, rather, the plankton community demonstrated high affinity for both nutrients. Moreover, our data indicate that measurement of quasi-steady-state pools in oligotrophic systems yields little information regarding nutrient deficiencies.

References

Button, D. K. 1985. Kinetics of nutrient-limited transport and microbial growth. Microbiol. Rev. 49:270-297.

Collos, Y. 1987. Calculations of ^{15}N uptake rates by phytoplankton assimilating one or several nitrogen sources. Appl. Radiat. Isot. 38:272-282.

Currie, D. J. and J. Kalff 1984a. The relative importance of bacterioplankton and phytoplankton in phosphorus uptake in freshwater. Limnol. Oceanogr. 29:311-321.

Currie, D. J. and J. Kalff. 1984b. A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus. Limnol. Oceanogr. 29:298-310.

Dodds, W. K. and J. C. Priscu. 1988. An inexpensive device for sampling large volumes of lake water from discrete depths. Freshwat. Biol. 20:113-116.

Dugdale, R. C. 1967. Nutrient limitation in the sea: Dynamics, identification and significance. Limnol. Oceanogr. 12:685-695.

Eppley, R. W. 1978. Nitrate uptake. pp. 401-409 in Handbook of phycological methods. Physiological and biochemical methods. J. A. Hellebust and J. S. Craigie (eds.). Cambridge Univ. Press, Cambridge.

Fisher, T. R., R. D. Doyle and E. R. Peele. 1987. Size-frac-
tionated uptake and regeneration of ammonium and
phosphate in a tropical lake. Verh. Internat. Verein.
Limnol. (in press).

Fisher, T. R., K. M. Morrissey, P. R. Carlson, L. F. Alves
and J. M. Melack. 1988. Nitrate and ammonium uptake by
phytoplankton in an Amazon River floodplain lake. J.
Plank. Res. 10:7-29.

Garside, C. 1984. Apparent ^{15}N uptake kinetics resulting
from remineralization. Limnol. Oceanogr. 29:204-210.

Garside, C. and P. M. Glibert. 1984. Computer modeling of
[15]N uptake and remineralization experiments. Limnol.
Oceanogr. 29:199-204.

Glibert, P. M. and J. J. McCarthy. 1984. Uptake and
assimilation of ammonium and nitrate by phytoplankton:
Indices of nutritional status for natural assemblages.
J. Plankt. Res. 6:677-696.

Harrison, W. G. 1978. Experimental measurements of nitrogen
remineralization in coastal waters. Limnol.
Oceanogr. 23:684-694.

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

Harrison, W. G. and L. R. Harris. 1986. Isotope-dilution and
its effects on measurements of nitrogen and phosphorus
uptake by oceanic microplankton. Mar. Ecol. Prog. Ser.
27:253-261.

Harrison, W. G. and L. J. Wood. 1988. Inorganic nitrogen
    uptake by marine picoplankton: Evidence for size
    partitioning. Limnol. Oceanogr. 33:468-475.

Kokkinakis, S. A. and P. A. Wheeler. 1988. Uptake of
    ammonium and urea in the northeast Pacific: Comparison
    between netplankton and nanoplankton. Mar. Ecol. Prog.
    Ser. 43:113-124.

Laws, E. 1984. Isotope dilution models and the mystery of
    the vanishing ^{15}N. Limnol. Oceanogr. 29:379-386.

Li, W. K. 1983. Consideration of errors in measuring kinetic
    parameters based on Michaelis-Menten formalism in
    microbial ecology. Limnol. Oceanogr. 28:1001-1019.

Lipschultz, F. J. 1984. Environmental factors affecting
    rates of nitrogen cycling. Ph.D. Thesis. Harvard
    University.

MacIsaac, J. S. and R. C. Dugdale. 1972. Interactions of
    light and inorganic nitrogen in controlling nitrogen
    uptake in the sea. Deep Sea Res. 19:209-232.

Morrissey, K. M. and T. R. Fisher. 1988. Regeneration and
    uptake of ammonium by plankton in an Amazon floodplain
    lake. J. Plankt. Res. 10:31-48.

Morel, F. M. 1987. Kinetics of nutrient uptake and growth in
    phytoplankton. J. Phycol. 23:137-150.

Murphy, T. P. 1980. Ammonia and nitrate uptake in the lower
    Great Lakes. Can. J. Fish. Aquat. Sci. 37:1365-1372.

Nalewajko, C. and D. R. S. Lean. 1980. Phosphorus, p 235-258. In: I. Morriss (ed.) Physiological ecology of phytoplankton. Blackwell. Oxford.

Nalewajko, C. and K. Lee. 1983. Light stimulation of phosphate uptake in marine phytoplankton. Mar. Biol. 74:9-15.

Nalewajko, C., B. Paul, K. Lee and H. Shear. 1986. Light history, phosphorus status, and the occurrence of light stimulation or inhibition of phosphate uptake in Lake Superior phytoplankton and bacteria. Can. J. Fish. Aquat. Sci. 43:329-335.

Priscu, J. C. 1984. A comparison of nitrogen and carbon metabolism in the shallow and deep-water phytoplankton populations of a subalpine lake: Response to photosynthetic flux density. J. Plank. Res. 6:733-749.

Priscu, J. C. 1987. Time-course of inorganic nitrogen uptake and incorporation by natural populations of freshwater phytoplankton. Freshw. Biol. 17:331-339.

Priscu, J. C. 1988. Photon dependence of inorganic nitrogen transport by phytoplankton in perennially ice-covered antarctic lakes. Hydrobiologia (in press).

Priscu, J. C. and L. R. Priscu. 1984. Inorganic nitrogen uptake in oligotrophic Lake Taupo, New Zealand. Can. J. Fish. Aquat. Sci. 41:1436-1445.

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

Solorzano, L. 1969. Determination of ammonia in natural
waters by the phenolhypochlorite method. Limnol.
Oceanogr. 14:799-801.

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

Stanford, J., P. J. Stewart and B. K. Ellis. 1983.
Limnology of Flathead Lake. Final Report. Flathead
River Basin Environmental Impact Study. U. S.
Environmental Protection Agency, Helena, Montana.

Strickland, J. H. and T. R. Parsons. 1972. A practical
handbook of seawater analysis. 2nd ed. Bull. Fish. Res.
Bd. Can.

Suttle, C. A. and P. J. Harrison. 1988a. Rapid ammonium
uptake by freshwater phytoplankton. J. Phycol. 24:13-
16.

Suttle, C. A. and P. J. Harrison. 1988b. Ammonium and
phosphate uptake kinetics of size fractionated plankton
from an oligotrophic freshwater lake. J. Plank. Res.
10:133-149.

Suttle, C. A. and P. J. Harrison. 1988c. Ammonium and
phosphate uptake rates, N:P supply ratios, and evidence
for N and P limitation in some oligotrophic lakes.
Limnol. Oceanogr. 33:186-202.

Tarapchak, S. J., S. M. Bigelow and C. Rubitschun. 1982. Overestimation of orthophosphorus concentrations in surface waters of southern lake Michigan: Effects of acid and ammonium molybdate. Can. J. Fish Aquat. Sci. 39:296-304.

Timperley, M. H. and J. C. Priscu. 1986. Determination of nitrogen-15 by emission spectrometry using an atomic absorption spectrometer. Analyst. 111:23-28.

Whalen, S. C. and V. Alexander. 1984. Influence of temperature and light on rate of inorganic nitrogen transport by algae in an arctic lake. Can. J. Fish. Aquat. Sci. 41:1310-1318.

Wheeler, P. A., P. M. Glibert and J. S. McCarthy. 1982. Ammonium uptake and incorporation by Chesapeake Bay phytoplankton: short term uptake kinetics. Limnol. Oceanogr. 27:1113-1128.

Wheeler, P. A. and D. L. Kirchman. 1986. Utilization of inorganic and organic nitrogen by bacteria in marine systems. Limnol. Oceanogr. 31:998-1009.

Table 1. Seasonal nitrate uptake kinetic parameters, ambient NO_3^- levels and ambient uptake rates in Flathead Lake. Amb. up. = uptake rate at ambient concentration. NA = data not available.

Date	Ambient NO_3^- (μM)	Partic. N (μM)	whole cells			TCA extract	
			K_s (μM)	V_{max} (h^{-1})	amb. up. ($\mu M\ h^{-1}$)	K_s (μM)	V_{max} (h^{-1})
22 June 1987	0.005	1.82	0.25	0.000956	0.0034	NA	NA
20 July 1987	0.043	1.97	1.14	0.000699	0.050	NA	NA
8 Sept. 1987	0.078	3.14	0.52	0.000434	0.177	1.19	0.00044
28 Oct. 1987	0.130	1.93	1.92	0.000997	0.120	1.46	0.00052
5 Feb. 1988	0.826	1.53	NA	0.000411	NA	NA	NA
11 May 1988	0.143	2.57	0.25	0.000314	0.060	1.23	0.00033

Table 2. Seasonal NH_4^+ uptake kinetic parameters, ambient uptake rates, and ambient NH_4^+ concentrations in Flathead Lake. Amb. up. = uptake rate at ambient concentration.

Date	Ambient NH_4^+ (μM)	Part. N (μM)	Whole Cells			TCA extract	
			K_s (μM)	V_{max} (h^{-1})	amb. up. (nM h^{-1})	K_s (μM)	V_{max} (h^{-1})
22 June 1987	0.106	2.56	0.51	0.0103	4.56		
30 July 1987	0.795	2.96	0.68	0.0069	11.00		
17 Sept. 1987	0.259	2.15	2.27	0.0114	2.51	0.485	0.00774
6 Nov. 1987	0.486	2.91	8.32	0.0063	8.32	1.01	0.00418
12 Feb. 1988	0.346	2.53	3.67	0.0033	0.73	2.46	0.00127
9 May 1988	0.506	2.79	3.41	0.0048	1.72	5.97	0.00344

Table 3. Regeneration corrected and uncorrected values for NH_4^+ uptake with addition of a final concentration of 0.14 μM $^{15}NH_4^+$.

date	uncorrected value (h^{-1})	corrected value (h^{-1})	%
22 June 1987	0.00461	0.00621	35
	0.00410	0.00547	33
30 July 1987	0.00416	0.00462	11
	0.00400	0.00443	11
17 Sept. 1987	0.00663	0.00795	20
	0.00452	0.00567	25
6 Nov. 1987	0.00267	0.00316	18
12 Feb 1988	0.00048	0.00051	6
9 May 1988	0.00070	0.00082	17
	0.00071	0.00084	18

Table 4. Seasonal PO_4^{3-} uptake kinetic parameters, biologically available P, SRP and ambient uptake of PO_4^{3-} in Flathead Lake. Biologically available P was determined using Rigler's bioassay, SRP gives PO_4^{3-} levels which were determined chemically.

Date	Biologically available P (nM P)	SRP (μM)	Partic P (μM)	K_s (nM)	V_{max} (h^{-1})	ambient up nM h^{-1}
9 July 87	0.323	0.011	0.076	10.2	0.56	1.33
6 Aug 87	0.968	0.021	0.070	113	0.75	0.44
18 Sept 87	0.645	0.011	0.080	128	0.78	0.31
9 Nov 87	0.645	0.016	0.128	89.3	1.74	0.89
11 Feb 88	0.484	0.016	0.128	11.0	0.06	0.32
5 May 88	0.323	0.011	0.109	15.0	0.37	0.84

Table 5. Seasonal N:P uptake and estimated and measured N:P supply ratios in Flathead Lake. [1]Calculated from maximum uptake rates as in Suttle and Harrison (1988b), see text for details.

month	Ambient uptake N:P	N:P Supply Ratio Via Regeneration	Calculated N:P Supply Ratio[1]
June	3.43	27.3	35.2
July	25.6	22.2	34.4
September	8.62	75.5	34.4
November	9.53	29.7	44.8
February	2.30	22.0	27.2
May	2.11	68.1	20.0

130

Fig. 1. Time-course of $^{15}NH_4^+$ and $^{15}NO_3^-$ incorporation into particulate matter at saturating nutrient concentration in Flathead Lake on 8 October 1987. Closed circles represent the uptake into whole cells, open squares the uptake into TCA extracted cells. A) $^{15}NH_4^+$ incorporation into particulates at 10µM NH_4Cl; B) Specific uptake rates of $^{15}NH_4^+$; C) $^{15}NO_3^-$ uptake at 10 µM $^{15}KNO_3$; D) Specific uptake rates of $^{15}NO_3^-$ incorporation.

Fig. 2. Time-course of ^{32}P incorporation (% of total added) into particulate matter in Flathead Lake water.

Fig. 3. Seasonal uptake kinetic curves of NO_3^- in Flathead Lake. Data for whole cells are represented with circles, data for TCA extracted material are represented by closed squares. The curves are drawn for non-TCA extracted cells only. A) 22 June 1987; B) 20 July 1987; C) 8 September 1987; D) 28 October 1987; E) 5 February 1988; F) 11 May 1988.

Fig 4. Seasonal uptake kinetic curves of NH_4^+ in Flathead Lake. Data for whole cells are represented by circles, open squares represent data for TCA extracted cells. The curves are drawn for whole cells only. Graphs represent the following dates: A) 22 June 1987; B) 30 July 1987; C) 17

131

September 1987; D) 6 November 1987; E) 12 February 1988; F) 9 May 1988.

Fig. 5. The effect of added NH_4^+ on $^{15}NO_3^-$ incorporation in Flathead Lake. The x axis represents ambient NH_4^+ + added NH_4^+. The first 2 values which appear on the far left of each graph were measured at ambient NH_4^+ concentrations. A) 22 June 1987; B) 20 July 1987; C) 8 September 1987; D) 28 October 1987; E) 5 February 1988; F) 11 May 1988.

Fig. 6. Seasonal uptake kinetic curves of PO_4^{3-} in Flathead Lake. A) 9 July 1987; B) 6 August 1987; C) 18 September 1987; D) 9 November 1987; E) 11 February 1988; F) 5 May 1988.

Fig. 7. Seasonal effects of size fractionation on uptake and regeneration of NH_4^+ in Flathead Lake. Open bars represent regeneration rates, Solid bars represent the uptake calculated as loss of NH_4^+ from solution + regeneration. Cross hatched bars represent uptake measured into particulates. Error bars show 1 std. dev. Where there are no error bars, only 1 replicate was used. A) 25 June 1987; B) 29 July 1987; C) 14 September 1987; D) 5 November 1987; E) 12 February 1988; F) 8 May 1988.

Fig. 8. Seasonal effects of size fractionation and antibiotic treatment on uptake and regeneration of PO_4^{3-} in

Flathead Lake. Open bars represent regeneration, closed
bars represent uptake onto particulates. Error bars show 1
std. dev. All samples represent 4 replicates, error bars
are not visible where the std. dev. was smaller than the
symbol. A) 9 July 1987; B) 6 August 1987; C) 18 September
1987; D) 9 November 1987; E) 11 February 1988; F) 5 May
1988.

Figure 1
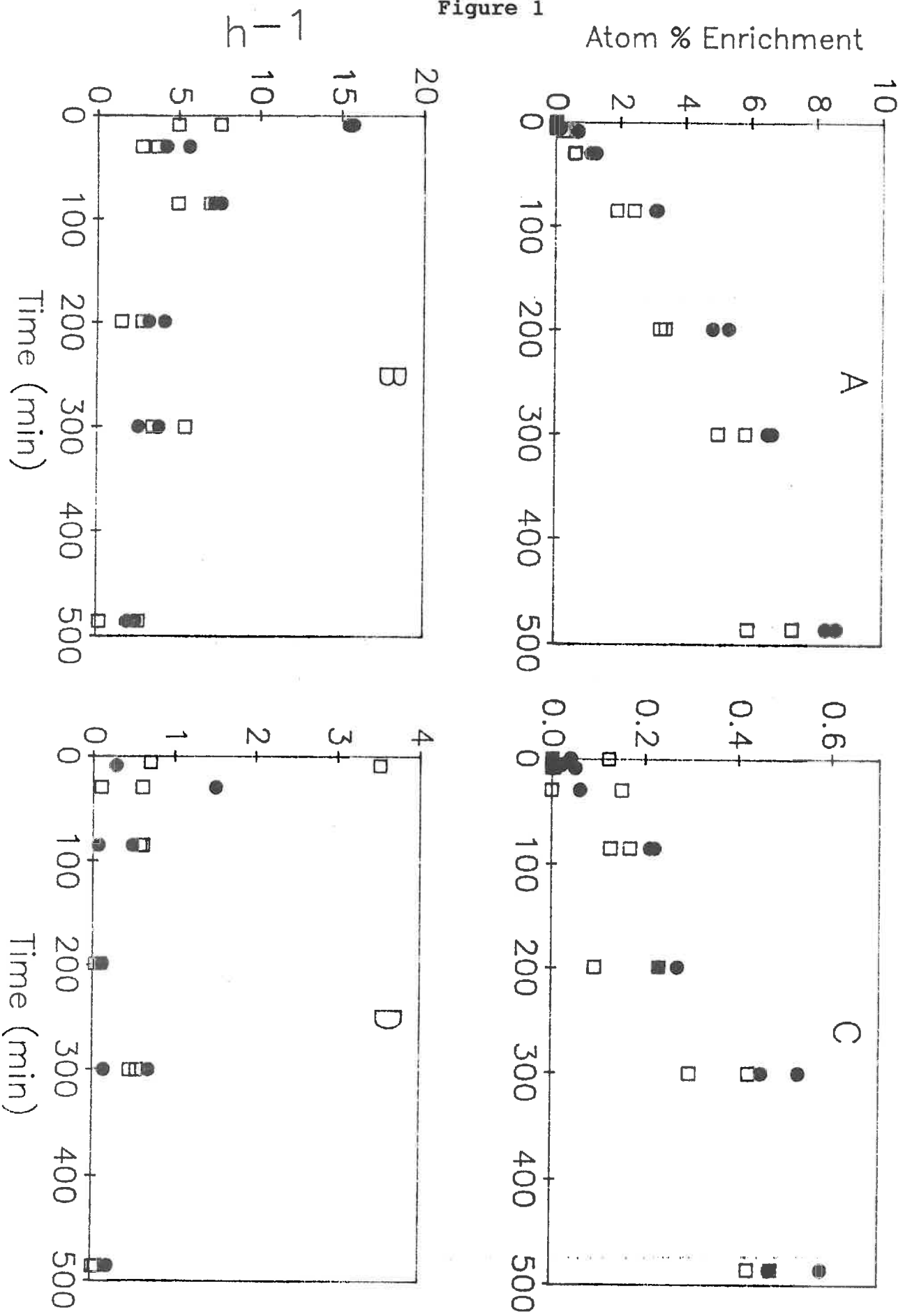
134

Figure 2

135

Figure 3

Figure 3

Figure 4

137

Figure 5

Figure 6

139
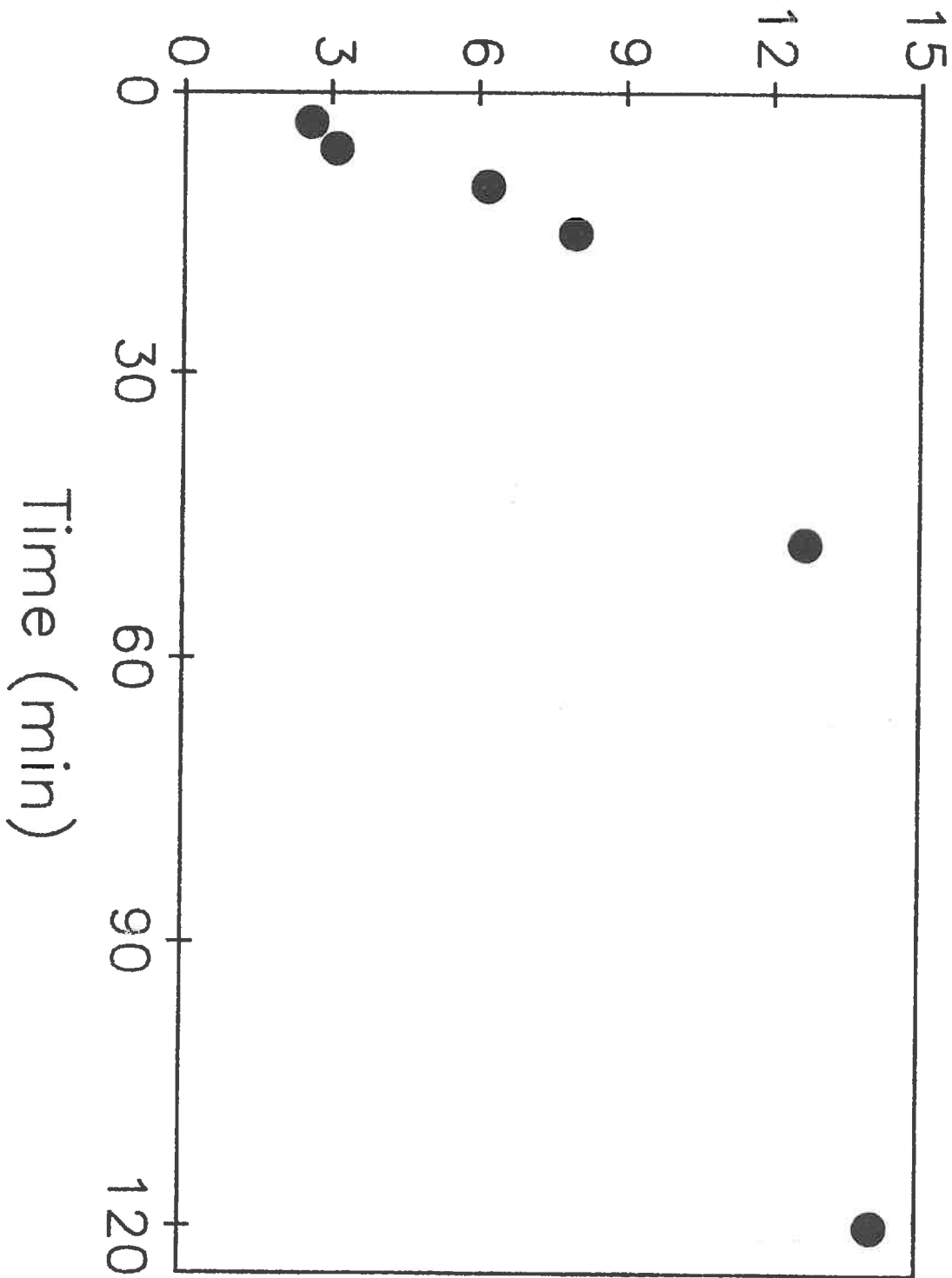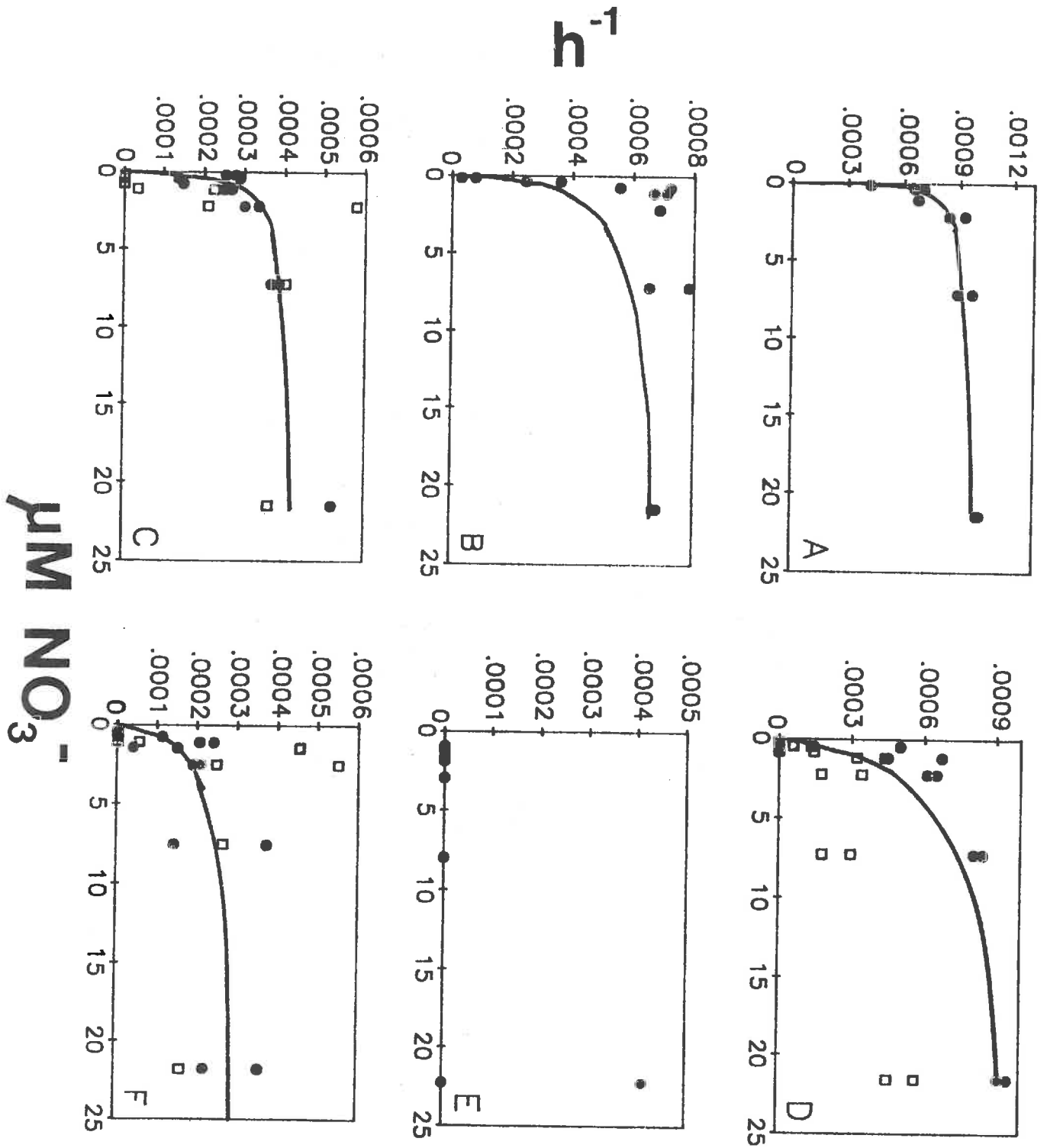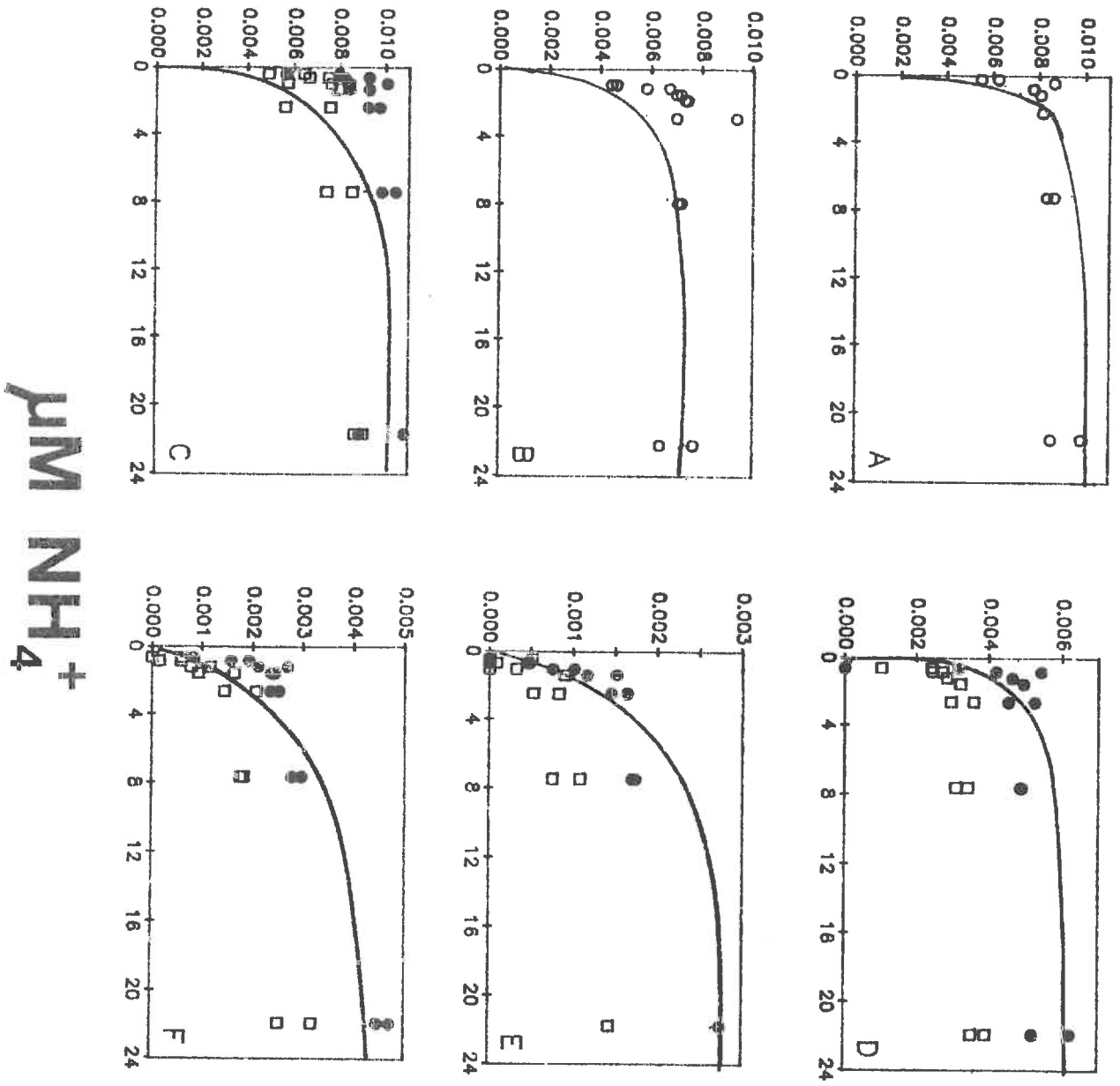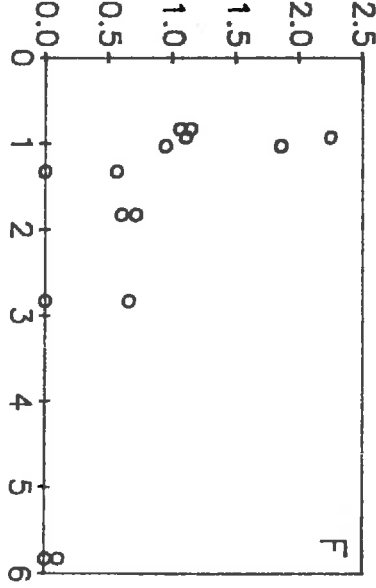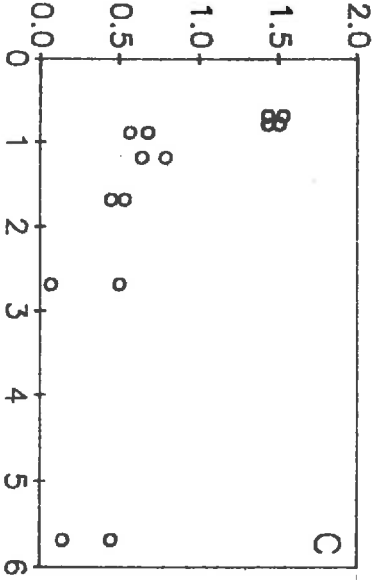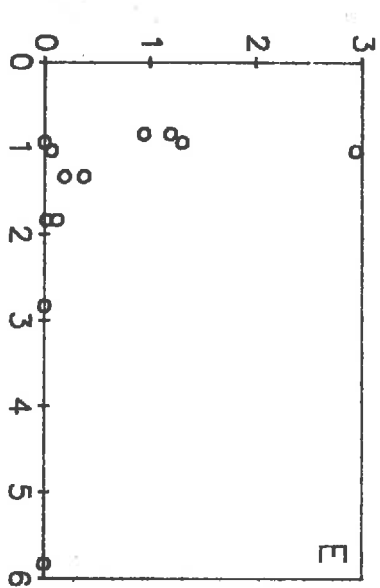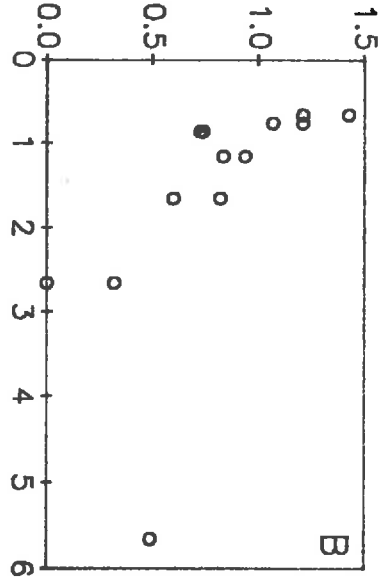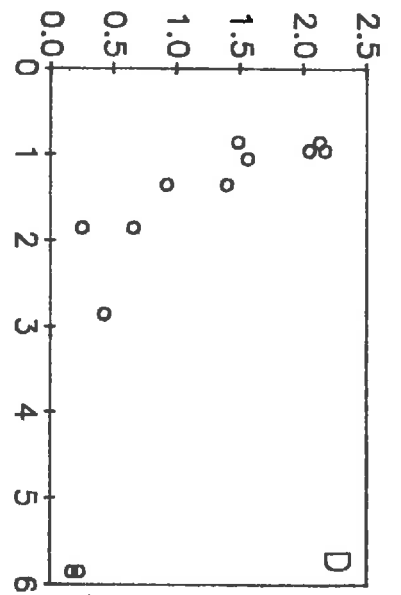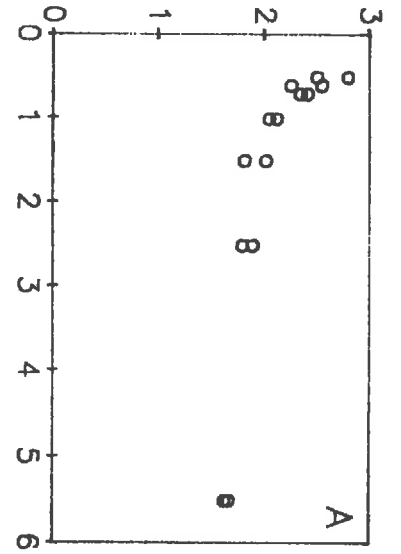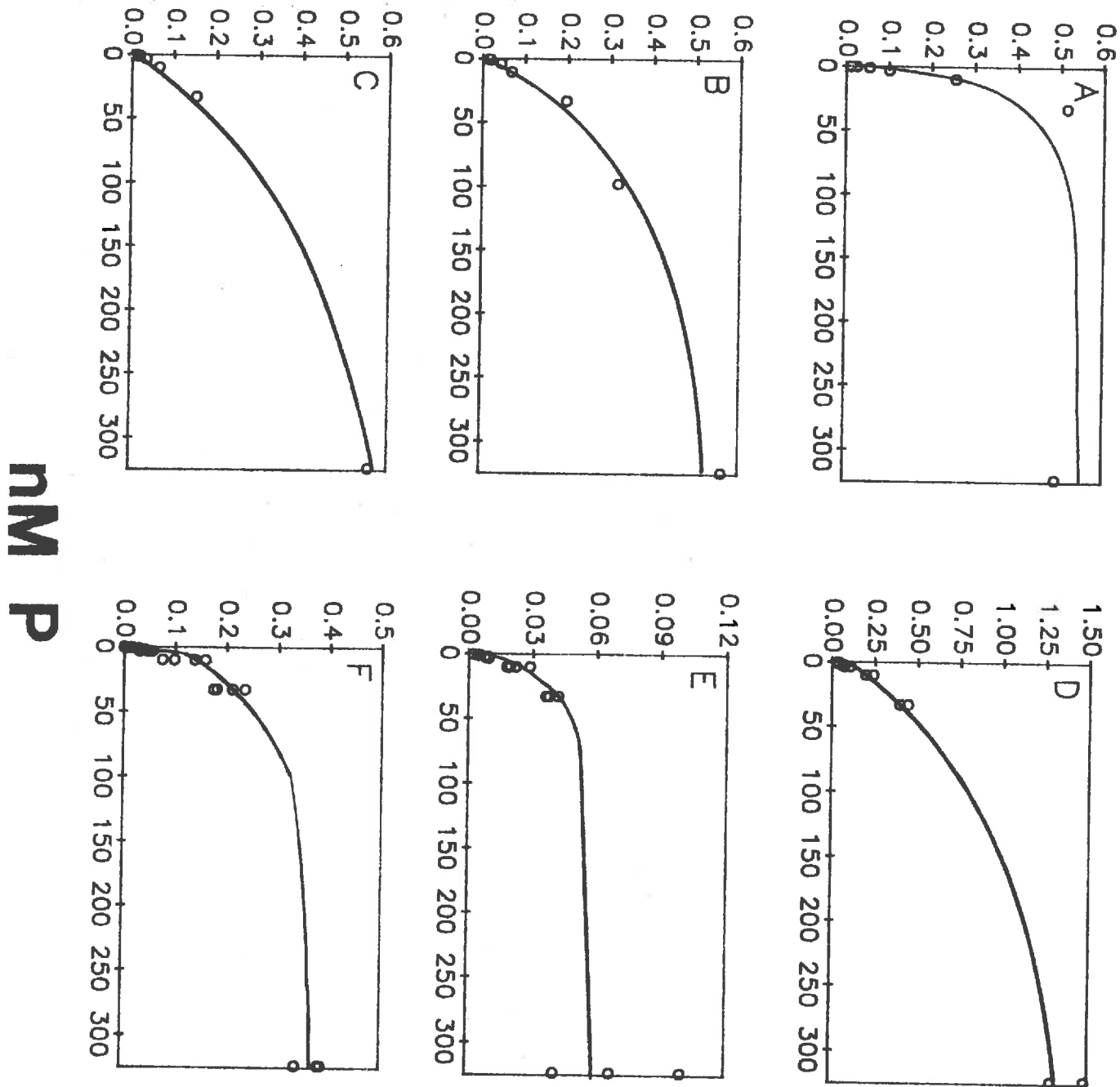
Figure 7

nM NH$_4^+$ h^{-1}
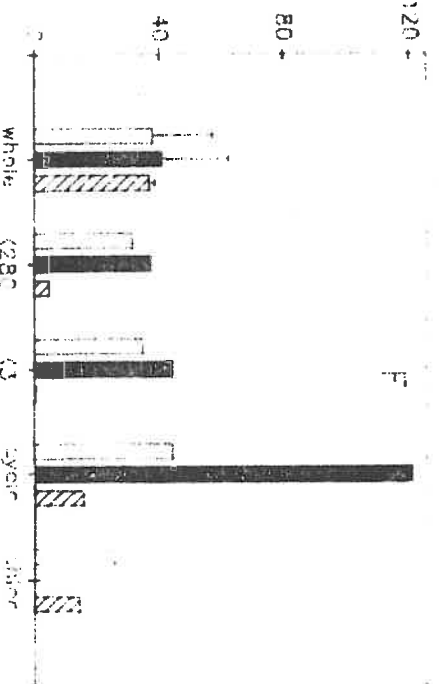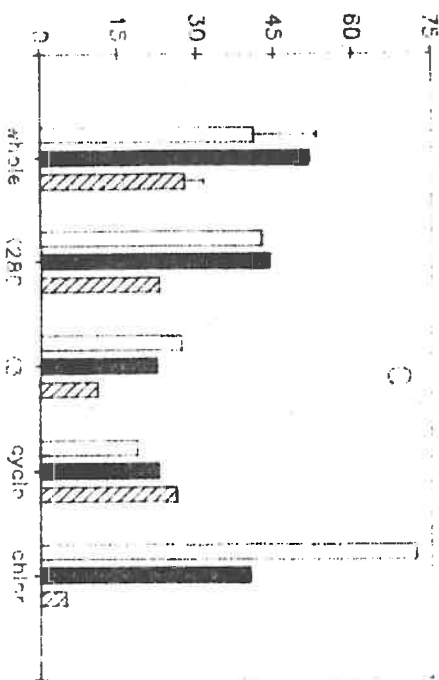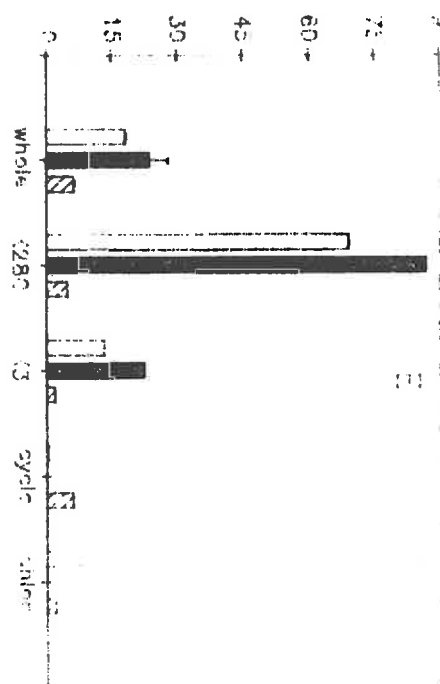
Figure 8
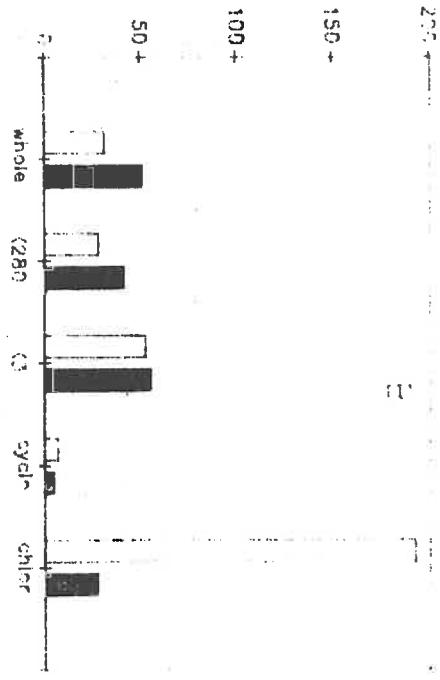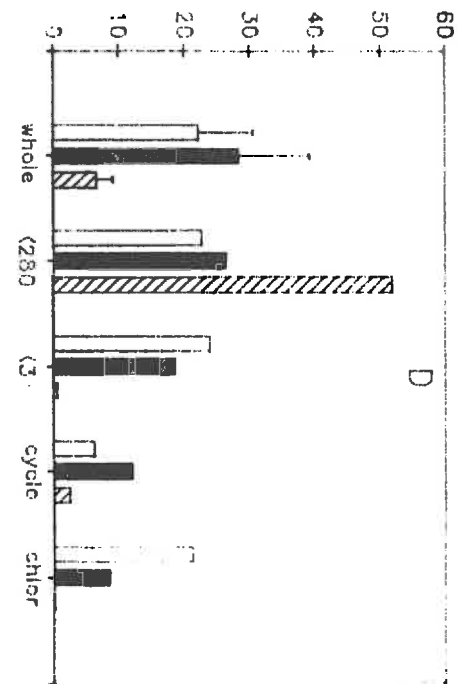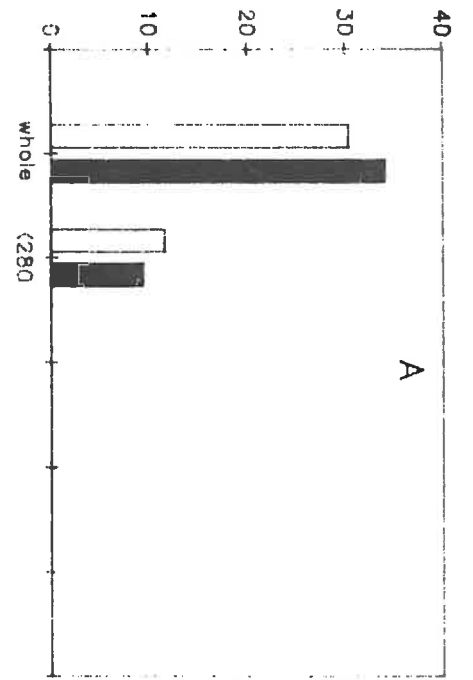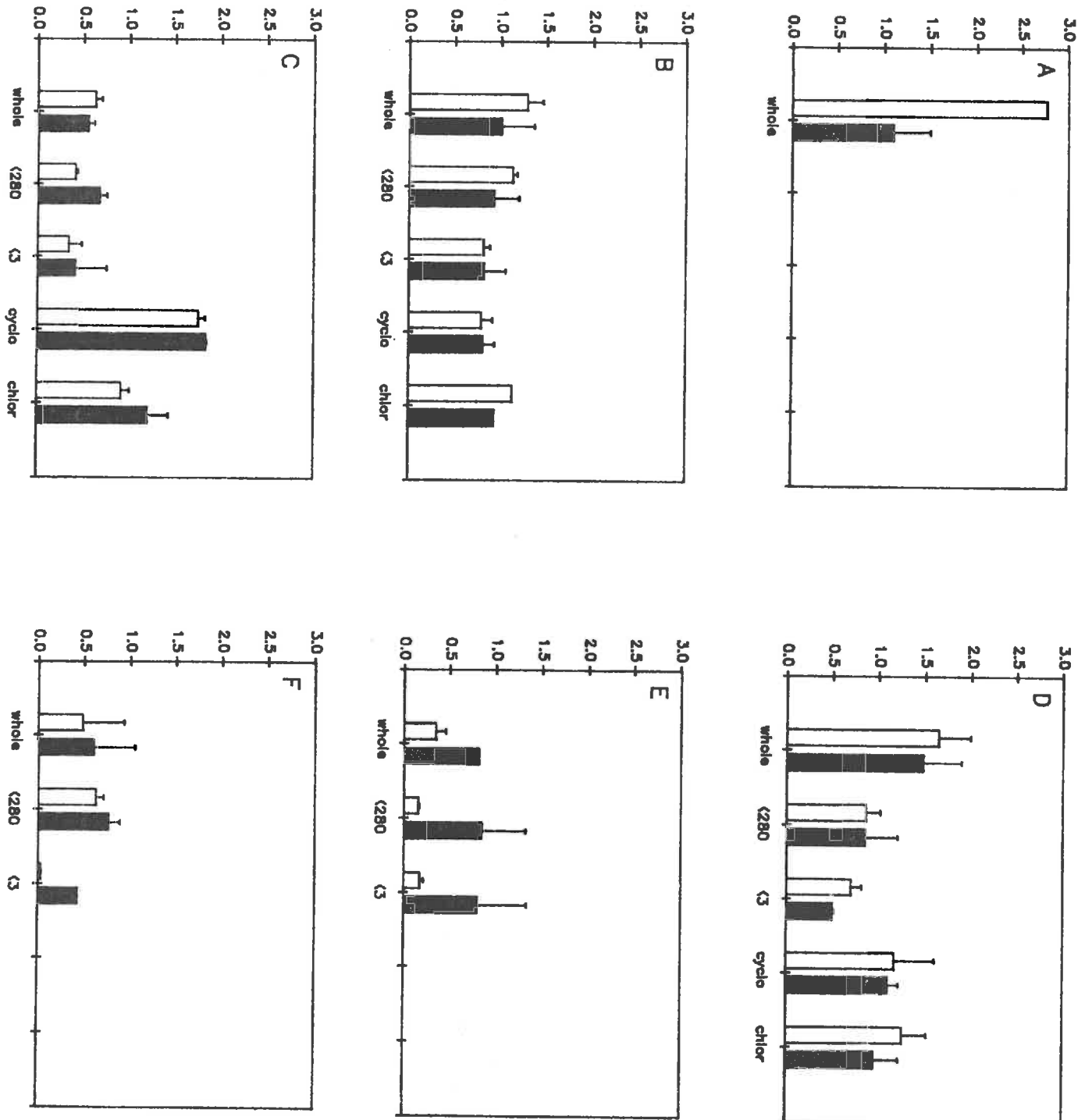
nM P h⁻¹

Chapter 7

Decreased Nutrient Availability to Phytoplankton

Caused by Zooplankton

Abstract

In water collected from an oligotrophic lake, addition of $^{31}PO_4^{3-}$ lowered the uptake rate of $^{32}PO_4^{3-}$ by lowering specific activity. Regeneration of $^{31}PO_4^{3-}$ by <u>Daphnia thorata</u> did not lower the specific activity of $^{32}PO_4^{3-}$ in lake water as expected. In addition, <u>D. thorata</u> appeared to decrease net uptake of PO_4^{3-}. The addition of <u>D. thorata</u> to lake water preferentially increased PO_4^{3-} uptake by bacterio-plankton and algal picoplankton smaller than 1 μm. The mechanism for these responses was not clear, but the data imply that phytoplankton encountering water recently inhabited by zooplankton may experience zones of decreased phosphorus availability rather than regenerated pulses of nutrients.

Introduction

The "indirect" effect of zooplankton on phytoplankton growth has usually been considered synonymous with nutrient regeneration (Sterner 1986). Zooplankton excretion has been hypothesized to increase nutrient availability to nearby phytoplankton by forming ephemeral patches of regenerated nutrient which can sustain primary production at higher levels than would be expected given the ambient bulk nutrient concentration (McCarthy and Goldman 1979). Theoretical arguments have disputed the importance of such patches in nature because the patches may disperse before phytoplankton can use them (Currie 1984, Jackson 1980). Laboratory experiments using quiescent uni-algal cultures have demonstrated that D. thorata can cause ephemeral patches of PO_4^{3-} which stimulate uptake by algae that encounter these patches, prompting speculation that such patches are important in waters with low PO_4^{3-} concentrations (Lehman and Scavia 1982a, b) Furthermore, experiments with natural assemblages in semi-continuous culture showed that short-term nutrient patches can alter algal community structure (Scavia et al. 1984). We measured short-term $^{32}PO_4^{3-}$ uptake by phytoplankton in the presence and absence of D. thorata in 20 ml aliquots of phosphorus deficient natural waters to ascertain if PO_4^{3-} regenerated by D. thorata provides short-term benefits to phytoplankton.

144

Materials and Methods

Water samples were collected at 5 m from Flathead Lake, on 6 August 1987, 18 September 1987 and 9 November 1987 with a displacement sampler (Dodds and Priscu 1988). Flathead lake is a large (460 km^2), oligotrophic lake in western Montana, USA (Potter and Stanford 1975). Carrier free $^{32}[P]$-H_3PO_4 at 333 Bq ml^{-1} was added to 20 ml aliquots in all ^{32}P uptake experiments. The reaction was stopped after 4 min by filtration onto Gelman GN-6 filters (effective retention 0.45 µm). Blank filters placed under the sample filter and formalin killed treatments were used to correct for ^{32}P absorption to the filters. Filters and total ^{32}P in solution were counted by liquid scintillation spectrometry.

Water was collected containing adult D. thorata (unconcentrated). A wide mouth pipette was used to gently isolate D. thorata from lake water samples 30 min before $^{32}PO_4^{3-}$ was added in the August experiments, and 5 min before $^{32}PO_4^{3-}$ was added in the other 2 experiments.

Maximum levels of biologically available PO_4^{3-} were measured using Rigler's bioassay (Rigler 1966). Unlabeled KH_2PO_4 was used as a source of biologically available PO_4^{3-}. Experiments to determine how D. thorata effects were partitioned between different size fractions of the community were done as above except that, after incubation, the communities were filtered first through a 1 µm pore size Nuclepore membrane filter and then on a Gelman GN-6 filter

(effective retention 0.45 um). Both filters were counted by
liquid scintillation spectrometry, and blank filters placed
under the sample filter were used to account for label
absorbed by filters. Chlorophyll was measured fluorometri-
cally on Whatman GF/F filters (effective retention 0.7 μm)
after size fractionation through Nuclepore membrane filters
(Strickland and Parsons 1972).

To determine D. thorata impacts on bacterial and
picoplankton biomass, cell counts were made on 20 ml samples
incubated with or without D. thorata for 45 min. Samples
were stained with DAPI and bacterial counts represent total
DAPI fluorescing cells - auto-fluorescent cells.

Bacterial activity in the presence and absence of D.
thorata was estimated by measuring thymidine incorporation
(Fuhrman and Azam 1982). ^{3}[H]-thymidine was added to a
final concentration of 20 μM thymidine at a final activity
of 0.27 kBq ml^{-1}. The reaction was incubated for 45 min
either with or without D. thorata, and stopped by addition
of 10 ml ice cold trichloroacetic acid (10% w/v in H_2O).
Samples were filtered on 0.45 μm filters (Millipore HA),
rinsed with 20 ml of ice cold 5% trichloracetic acid and
counted by liquid scintillation spectrometry.

To test if regeneration of NH_4^+ by D. thorata could
affect P uptake, samples were inoculated with a final
concentration of 10 μM NH_4Cl at 0 h and 12 h on 9 November
1987. 333 Bq ml^{-1} of ^{32}[P]-H_3PO_4 and 2 μM KH_2PO_4 were added at

146

12 h, and samples were filtered after 40 min. Filters were counted as above.

To determine if dead D. thorata leached a compound which affected $^{32}PO_4^{3-}$ uptake, D. thorata were killed by 30 min exposure to air at 20^0, added to lake water, and $^{32}PO_4^{3-}$ uptake was determined as above. $^{32}PO_4^{3-}$ uptake was also measured in the presence of glucose and stirring to estimate the effects of organic carbon and turbulence caused by D. thorata swimming on PO_4^{3-} uptake.

Results and Discussion

Maximum levels of biologically available P in Flathead Lake were determined to be 0.968 nM, 0.65 nM, and 0.64 nM on 6 August, 18 September and 9 November 1987, respectively. It has been previously shown that chemical determinations of PO_4^{3-} usually give higher values than what is biologically available (Peters 1978, Rigler 1966, Tarapchak et al. 1981).

An increase in $^{31}PO_4^{3-}$ greater than 1 nM over ambient concentration caused a significant decrease in incorporation of $^{32}PO_4^{3-}$ ($P < 0.05$, pooled t test, n = 6, Fig. 1). This decrease in incorporation over time is not caused by satiation of internal pools of PO_4^{3-} because time-course measurement of ^{32}P uptake in whole lake water showed no decrease in uptake over a 15 min incubation (Fig. 2). This trend results from a decrease in specific activity of $^{32}PO_4^{3-}$ in solution. The lowered $^{32}PO_4^{3-}$ uptake occurs even though

147

$^{31}PO_4^{3-}$ uptake increases as substrate concentration increases (ie. Button 1985, Tarapchak and Herche 1986, Rigler 1966).

Ambient PO_4^{3-} concentrations did not change during the course of a 4 min incubation as shown by a time-course of ^{32}P uptake in whole water (Fig. 2). This is because the $^{32}PO_4^{3-}$ uptake appears to be roughly linear for the first 4 minutes and any change in $^{31}PO_4^{3-}$ concentration would cause a change in the uptake rate (Fig. 1). If there was a net increase in unlabeled PO_4^{3-} concentration over the course of the incubation (as would happen with high rates of regeneration), there would have been a net decrease in ^{32}P incorporation over time owing to isotopic dilution.

A decrease in ^{32}P incorporation because of isotope dilution caused by PO_4^{3-} regeneration was expected in the presence of D. thorata, and such a decrease would indicate an increase in net uptake of PO_4^{3-} by phytoplankton. However, in August and September, treatments containing D. thorata showed increased incorporation of $^{32}PO_4^{3-}$ which means there was a decrease total $^{31}PO_4^{3-}$ uptake (Fig. 3). Therefore, levels of biologically available PO_4^{3-} must have been lowered by D. thorata.

On 9 November, D. thorata caused an increase in $^{32}PO_4^{3-}$ uptake in the < 1 μm size fraction and no increase in the > 1 μm size fraction. Whole water ^{32}P incubations with and without D. thorata were size fractionated. In whole lake water without D. thorata, the < 1 μm fraction accounted for 42.2% of the total inorganic P uptake (n = 3, std. dev. =

2.5%), and after 5 minutes of incubation with D. thorata, 61.4% (n = 3, std. dev. = 10.7%) of the uptake was accounted for by the less than 1 μm fraction, significantly more than with no D. thorata addition (P < 0.05, pooled t test, 4 df, t = 2.44). These experiments show that D. thorata caused an overall decrease in PO_4^{3-} uptake by phytoplankton greater than 1 um, since total community uptake was the same with and without D. thorata (Fig. 2 and 3) and uptake by the less than 1 μm fraction increased at the expense of the greater than 1 μm fraction. Most of the uptake in the less than 1 um fraction was probably heterotrophic since more than 95% of the total chlorophyll was retained on a 1 μm filter and only 5-10% of the living cells less than 1 μm contained chlorophyll.

D. thorata did not preferentially increase PO_4^{3-} uptake in the less than 1 μm fraction by increasing bacterial biomass or turbulence. There was no significant increase in bacterioplankton thymidine or biomass caused by D. thorata. Both ^{3}[H]-Thymidine incorporation and bacterial counts were similar in untreated lake water (137 kBq l^{-1} hr^{-1}, n = 3, std. dev. = 18.7; 7.50 x 10^5 bacteria ml^{-1}) and samples exposed to D. thorata for 45 min (132 kBq l^{-1} hr^{-1}, n = 3, std. dev. = 28.0; 7.45 x 10^5 bacteria ml^{-1}). There is no a priori reason to believe that there is a direct relationship between thymidine uptake rates and PO_4^{3-} uptake rates in bacteria. The small amount of turbulence caused by D. thorata was not important because $^{32}PO_4^{3-}$ uptake of a

149

treatment stirred at 300 rpm was not significantly different from that of un-stirred lake water (Table 1).

Excretion of biologically oxidizable organic materials may have caused the stimulation of PO_4^{3-} uptake by heterotrophs in the less than 1 µm fraction because 0.1 µM glucose lowered $^{32}PO_4^{3-}$ incorporation. Killed D. thorata (which may leach PO_4^{3-} or organic material) also decreased measured uptake of $^{32}PO_4^{3-}$ (Table 1). It is possible that D. thorata excretes a dissolved organic compound which chelates and makes PO_4^{3-} unavailable.

Grazing was not responsible for the apparent decrease in PO_4^{3-} uptake by phytoplankton. Time-course measurements showed no decrease in the rate of $^{32}PO_4^{3-}$ incorporation into particulates over a 5 min period with D. thorata present in November (Fig 2). If D. thorata removed a significant portion of the plankton during this time, the rate of $^{32}PO_4^{3-}$ uptake should have decreased over time. Furthermore, net increases in PO_4^{3-} uptake with D. thorata present were observed in August and September. Moreover, D. thorata filtration rates, based on literature estimates, cannot account for filtration of more than 1% of the total volume used in these experiments. If maximum filtration rate of each D. thorata is assumed to be 7.0 ml d^{-1} (Wetzel 1986), during a 30 min incubation in 20 ml, only 0.73% of the volume would be filtered. NH_4^+ excretion may have preferentially stimulated heterotrophic uptake of PO_4^{3-}, partially explaining our observations. However, experiments showed

that there was no community-wide enhancement of uptake of PO_4^{3-} after 12 h exposure to NH_4+. Samples with 12 h prior exposure to N took up 67.6 nM PO_4^{3-} h^{-1} (std. dev. = 8.5, n = 4) and those with no prior exposure took up 109 nM PO_4^{3-} h^{-1} (std. dev. = 45, n = 4). These rates were not significantly different (P > 0.05, pooled t test).

The above experiments were not designed to determine any benefit obtained by phytoplankton ingested and excreted whole (Porter 1976) since they may be exposed to a lower specific activity of $^{32}PO_4^{3-}$ inside D. thorata than in the open water, and we did not include D. thorata on any of our filter counts. Therefore any labeled organisms in the gut of D. thorata were not counted.

Our data show that D. thorata can cause a short-term decrease in PO_4^{3-} availability to phytoplankton, presumably by increasing PO_4^{3-} uptake by heterotrophs. However, D. thorata could have decreased regeneration by other organisms. These results imply that, in some situations, phytoplankton experience patchiness caused by D. thorata not as plumes of increased PO_4^{3-} concentration near D. thorata, but as decreased concentrations of PO_4^{3-} in regions recently inhabited by D. thorata. The data presented indicate that zooplankton-phytoplankton interactions may be even more complex than previously reported.

Acknowledgements

References

Button, D. K. 1985. Kinetics of nutrient limited transport and microbial growth. Microb. Rev. 49:270-297.

Currie, D. J. 1984. Microscale nutrient patches: Do they matter to the phytoplankton? Limnol. Oceanogr. 29:211-214

Dodds, W. K. and J. C. Priscu. 1988. A inexpensive device for sampling large volumes of lake water from discrete depths. Freshw. Biol. 20:113-116.

Fuhrman, J. A. and F. Azam. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol. (Berlin) 66:109-120.

Jackson, G. A. 1980. Phytoplankton growth and zooplankton grazing in oligotrophic oceans. Nature. 284:439-440.

Lehman, J. T. and D. Scavia. 1982a. Microscale nutrient patches produced by zooplankton. Proc. Nat. Acad. Sci USA 79:5001-5005.

Lehman, J. T. and D. Scavia. 1982b. Microscale patchiness of nutrients in plankton communities. Science 216:729j-730.

McCarthy, J. J. and J. C. Goldman. 1979. Nitrogenous nutrition of marine phytoplankton in nutrient-depleted waters. Science 203:670-672.

Peters, R. H. 1978. Concentration and kinetics of phosphorus fractions in water from streams entering Lake Memphremagog. J. Fish. Res. Bd. Can. 35:315-328.

Porter, K. G. 1976. Enhancement of algal growth and
        productivity by grazing zooplankton. Science 192:1332-
        1334.

Potter D. S. and J. A. Stanford. 1975. Influences on the
        plankton communities of oligotrophic Flathead Lake.
        Verh. Internat. Verein. Limnol. 19:1790-1797.

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

Scavia, D., G. L. Fahnenstiel, J. A. Davis, and
        R. G. Kreis, Jr. 1984. Small-scale nutrient
        patchiness: Some consequences and a new encounter
        mechanism. Limnol. Oceanogr. 29:785-793.

Sterner, R. W. 1986. Herbivores' direct and indirect effects
        on algal populations. Science 231:605-607.

Strickland and Parsons. 1972. A Practical Handbook of
        Seawater Analysis. Fish. Res. Bd. Canada 167.

Tarapchak, S. J. and L. R. Herche. 1986. Phosphate uptake by
        microorganisms in lake water: deviations from simple
        Michaelis-Menten kinetics. Can. J. Fish. Aquat. Sci.
        43:319-328.

Tarapchak, S. J., D. R. Slavens, and L. M. Maloney. 1981.
        Abiotic versus biotic uptake of radiophosphorus in lake
        water. Can. J. Fish. Aquat. Sci. 38:889-895.

Wetzel, R. G. 1986. Limnology.

Table 1. Rate of $^{32}PO_4^{3-}$ uptake on 9 November 1987 in Flathead Lake. All treatments n = 4, except lake water where n = 3. Pooled t tests showed the dead D. thorata and glucose treatments were significantly different than lake water (P < 0.025), but the stirred treatment was not (P > 0.15).

Treatment	kBq l^{-1} min^{-1}	s.d.
lake water	0.541	0.139
dead D. thorata	0.312	0.023
0.1 uM glucose	0.356	0.042
stirred	0.373	0.023

Figure 1.   Uptake of $^{32}PO_4^{3-}$ by whole Flathead Lake water
versus total $^{31}PO_4^{3-}$ concentration.   Data for November 9 1987.
Data were similar for the other two sampling dates.   Bars
give plus or minus 1 standard deviation.   The value for 0.64
nM was measured at ambient PO_4^{3-} concentration.

Fig. 2.   Time-course of $^{32}PO_4^{3-}$ uptake in whole Flathead Lake
water on 9 November 1987 in the presence and absence of D.
thorata.

Figure 3. $^{32}PO_4^{3-}$ uptake of Flathead Lake water with and
without D. thorata for three sampling dates.   Values are
means from 3 replicates, the bars give one standard
deviation.   Pooled t tests showed a significant difference
between untreated lake water (control, given by open bars)
and water with D. thorata added (given by solid bars) on 6
August and 18 September ($P < 0.05$) and none on 9 November ($P
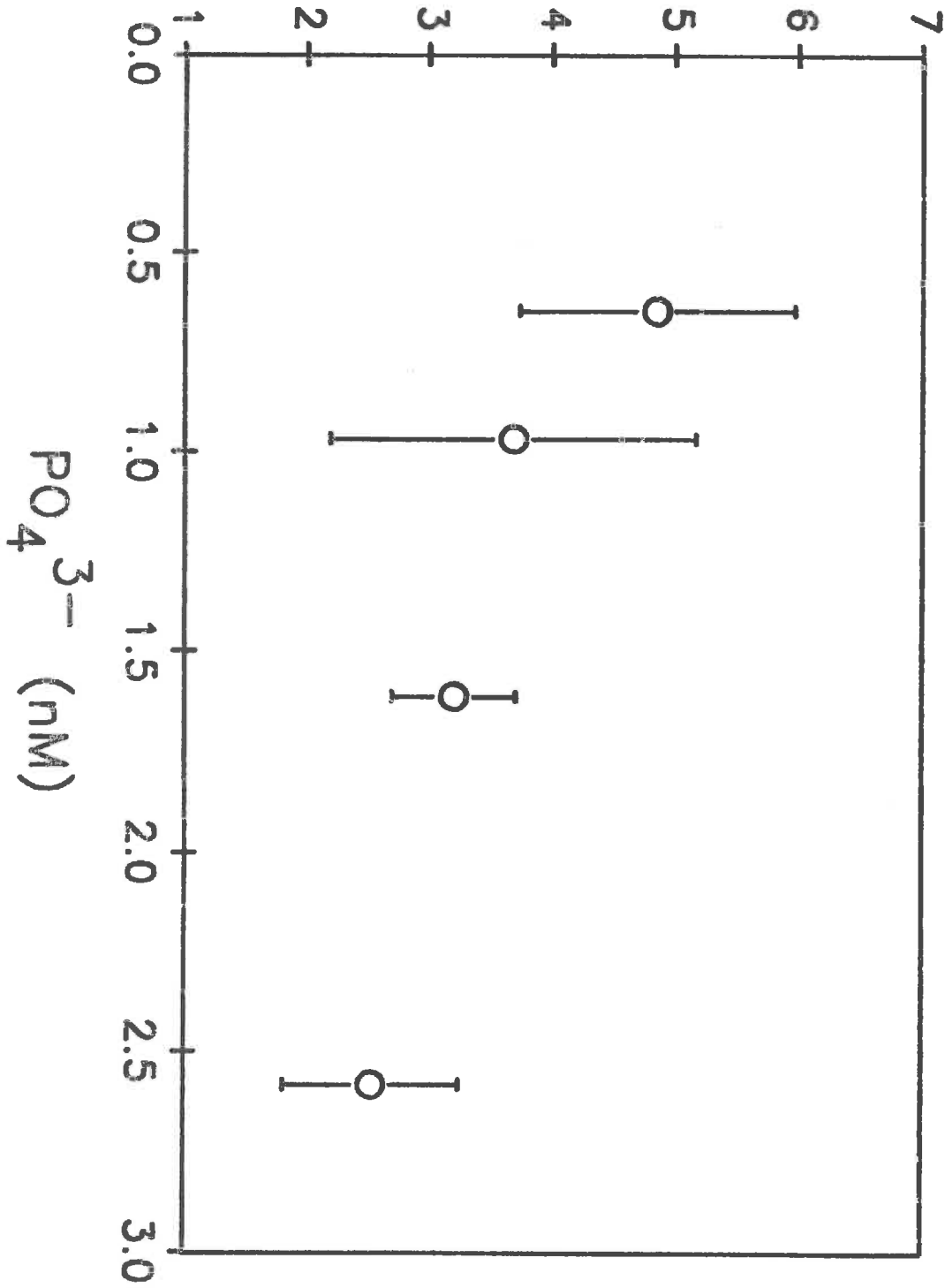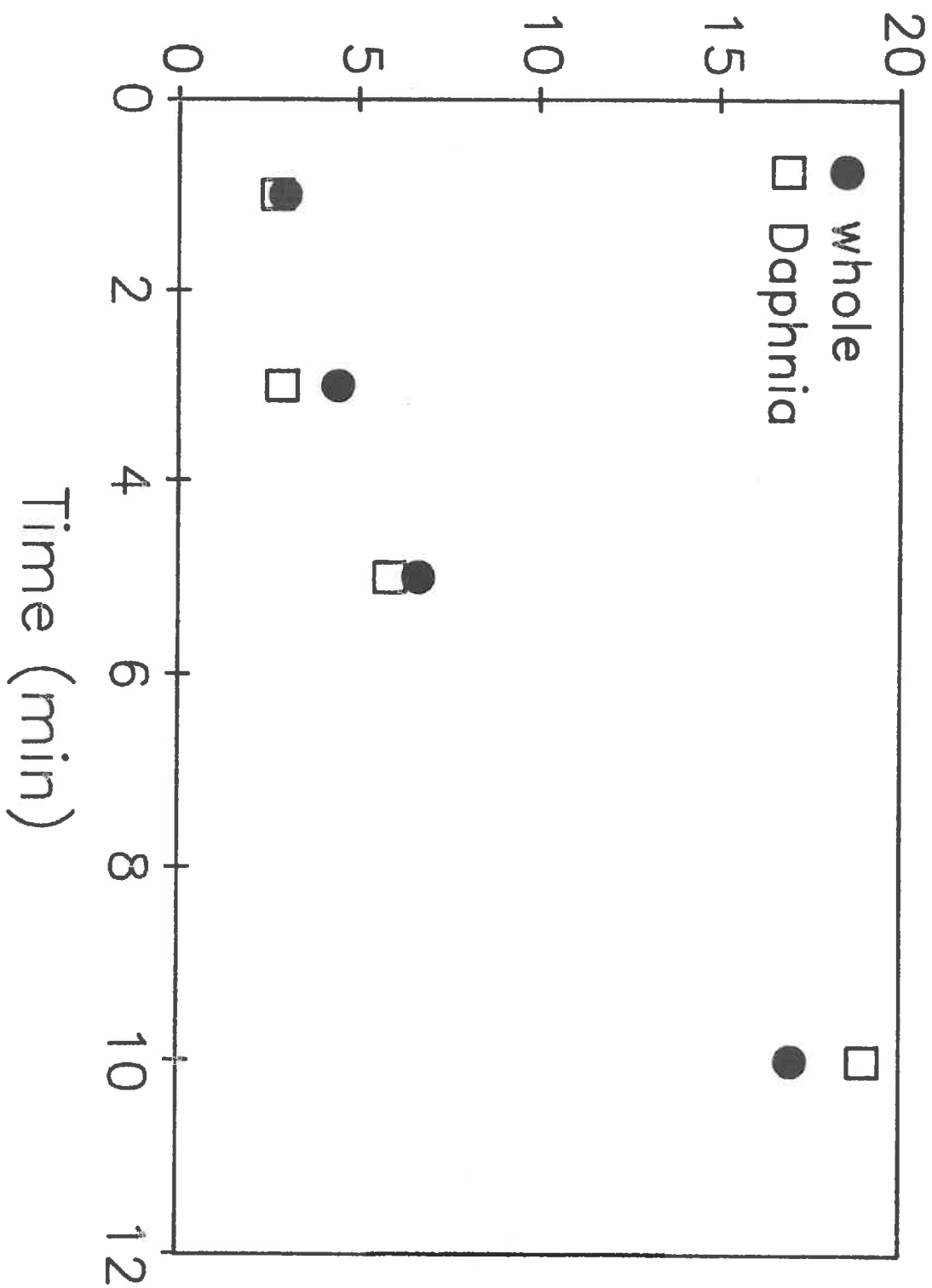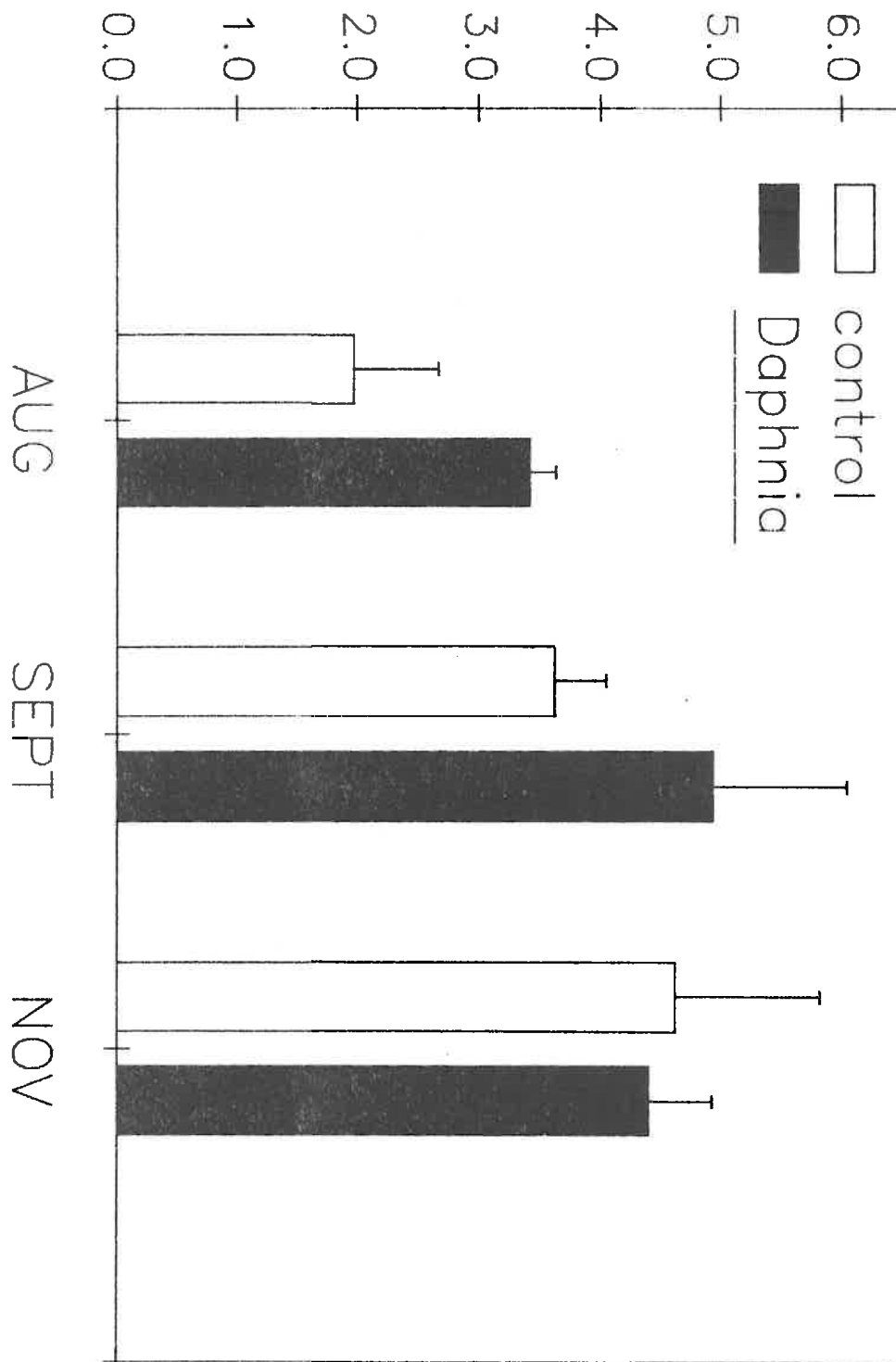> 0.35$).

Figure 1

Figure 2

% ^{32}P on filter

% 32P on filter min^{-1} Figure 3

Chapter 8

Ammonium, Nitrate, Phosphate, and Inorganic Carbon Uptake

in an Oligotrophic Lake: Seasonal Variations in

Light Response Variables

Abstract

The dependence of nutrient saturated uptake of $^{15}NH_4^+$, $^{15}NO_3^-$, $^{32}PO_4^{3-}$, and $^{14}CO_2$ on photosynthetic photon flux density (PPFD) was characterized seasonally in oligotrophic Flathead lake (Montana). PO_4^{3-} uptake was not dependent upon PPFD at any time of the year, while NH_4^+, NO_3^-, and CO_2 uptake were consistently dependent on PPFD. There was usually an inhibition of NH_4^+, NO_3^-, and CO_2 uptake above 40% surface PPFD indicating that maximum rates of uptake occurred at a lower PPFD than measured in the surface water. NH_4^+, NO_3^- and PO_4^{3-} were taken up in the dark at measurable rates most of the year, whereas CO_2 uptake was always negligible in the dark. CO_2 and NO_3^- uptake were more strongly influenced by PPFD than NH_4^+ uptake. The data show that NH_4^+, NO_3^- and CO_2 uptake are dependent upon light regime, and that light may regulate primary productivity in ways other than its direct influence on photosynthesis.

Introduction

Photosynthetic Photon Flux Density (PPFD) is of obvious importance as the controlling factor for photosynthesis. In addition, a relationship between light and uptake of inorganic N (Priscu 1984, Priscu 1988, MacIsaac and Dugdale 1972, Terry 1982, Nelson and Conway 1979, Whalen and Alexander 1984) and P (Nalewajko et al. 1986, Nalewajko and Lee 1983, Young and King 1980) has been shown for aquatic systems.

Investigators have used Michaelis-Menten formalism to describe the uptake of inorganic N (eg. Priscu 1984, MacIsaac and Dugdale 1972, Whalen and Alexander 1984). This approach assumes no dark nutrient uptake, and no photoinhibition of uptake at high light intensities. Platt et al. (1980) described the relationship between photosynthesis and light in an equation which also took into account inhibition of photosynthesis at high light intensities. Priscu (1988) slightly modified this equation by adding a term for dark uptake and showed that when the equation was fit to actual NH_4^+ and NO_3^- uptake data in two Antarctic lakes, it provided an adequate model of the observed trends.

There are few concomitant data available on the seasonal trends of dependence of CO_2, NO_3^-, NH_4^+, or PO_4^{3-} uptake on PPFD in freshwater or marine systems. We present data on the PPFD response of CO_2, NO^{3-}, NH_4^+, and PO_4^{3-} uptake

162

and discuss their relevance to the primary productivity in a large oligotrophic lake.

Materials and Methods

Flathead Lake is a large (460 km^2), oligotrophic (epilimnetic chl $\underline{a}$ 0.1 - 1.1 ug l^{-1}) lake in northwestern Montana (USA). Samples were collected from 5 m (the depth of maximum productivity, Stanford et al. 1983) at a point 2 km off shore in the deepest portion of the lake with a displacement sampler (Dodds and Priscu 1988).

Incubations were conducted in a lake-side incubator maintained at lake temperature using water pumped from the lake. Neutral density filters were used to create a gradient from 0.7-100% of surface PPFD. A LI-COR LI-190 sensor, which continuously integrated surface PPFD over 15 min periods was used to measure PPFD (in $\mu E \cdot m^{-2} \cdot s^{-1}$) over the course of each experiment, except during November when values were obtained from a Weathermeasure pyrheliograph, and transformed to $\mu E \cdot m^{-2} \cdot s^{-1}$ by correlation with data collected with the LI-COR sensor on other dates. Phaeophytin corrected chlorophyll $\underline{a}$ was measured fluorometrically after filtration onto Whatman GF/F filters (Strickland and Parsons 1972).

After collection, samples for nitrogen uptake experiments were pre-filtered through a 280 um mesh to remove large zooplankton and detritus, and aliquots were placed in 500 ml borosilicate bottles. A final

163

concentration of 10μM $^{15}NH_4Cl$ or 10 μM $^{15}KNO_3$ was added to each bottle. This concentration was shown to saturate uptake (unpublished data). Incubations were stopped after 6 h by filtration onto pre-combusted Whatman GF/F (0.7 um effective retention) glass-fiber filters. Dumas combustion followed by optical emission spectrometry was used to analyze the ^{15}N enrichment of particulates (Timperley and Priscu 1986). Concentration of particulate N (PN) was determined on a sub-sample of each filter with a Carlo Erba 1106 elemental analyzer. Ambient levels of NH_4^+ were determined using the phenol-hypochlorite method (Solorzano 1969) and ambient NO_3^- was determined by the cadmium reduction technique (Eppley 1978).

To determine CO_2 uptake, 50 μl of 22.2 μCi ml^{-1}, $^{14}[C]$-NaH_2CO_3 was added to 100 ml of lake water in 125 ml borosilicate bottles. Samples were incubated in the lake-side incubator for 4 h and then filtered onto Whatman GF/F filters. ^{14}C activity was counted using liquid scintillation spectrometry. Alkalinity was determined titrametrically for each water sample (Standard Methods 1975).

P uptake experiments were conducted on 50 ml samples of lake water enriched with KH_2PO_4 to 2 μM (a level which is saturating to uptake, (unpublished data)). Carrier free $^{32}[P]$-H_3PO_4 was then added to 20,000 DPM ml^{-1} and the samples were incubated in the lake-side incubator for 1 h. The reaction was stopped by filtration onto Gelman GN-6 filters

(0.45 um pore size) and filters were counted by liquid scintillation spectrometry.

PPFD dependency of nutrient uptake was parameterized using the model of Platt et al. (1980) as modified by Priscu (1988). Uptake rates were normalized to chlorophyll $\underline{a}$ because it is the primary pigment which absorbs photons for biochemical energy production, and to allow for comparison with previously published literature. A brief summary of the parameters we used is provided in Table 1. Refer to Platt et al. (1980) and Priscu (1988) for derivation and discussion of the parameters.

Results

PO_4^{3-} uptake showed no obvious dependence upon PPFD (Fig. 1) and no attempt was made to model the data. However, there was significant dark uptake of PO_4^{3-} (Fig. 1).

Uptake of $^{14}CO_2$ was strongly dependent upon PPFD throughout the year (Fig. 2, Table 2). Inhibition of photosynthesis at surface PPFD levels occurred at all times of the year. This inhibition occurred at the lowest light levels (I_m was the lowest) in fall, winter, and spring, and at highest light levels during summer stratification. I_m ranged from 15 - 80% of surface PPFD, and was closest to surface PPFD during February. P_{Pm}^B was also the lowest in February.

NO_3^- uptake also exhibited inhibition by high PPFD during most of the year (Fig. 3, Table 2). In addition,

dark uptake was important during September, October and May (Table 2). It was not possible to model NO_3^- uptake for February since NO_m^B was very low and rates of NO_3^- incorporation were below detection at all but the intermediate PPFD (Fig. 3). During the rest of the year, I_m for NO_3^- was 10 - 28% of surface PPFD (Table 2).

$^{15}NH_4^+$ uptake was consistently dependent upon PPFD levels and showed dark uptake at all times of the year (Fig. 4, Table 2). As with NO_3^- and CO_2, the lowest NH_m^B values were during February. I_m was lower than surface PPFD, except during November, when there was minimal dependence of NH_m^B on PPFD (Fig. 4, Table 2).

There was a general relationship between P_m^B and α and P_m^B and β, with large values of P_m^B giving large values of α and β (Fig. 5). This is to be expected since α and β are scaled by P_m^B (Platt et al. 1980). I_k (the slope in Fig. 5a) and I_b (the slope in Fig. 5b) are roughly comparable for all nutrients. This graph also shows that P_m^B for CO_2 is highest for CO_2, intermediate for NH_4^+, and lowest for NO_3^-.

I_k for CO_2 was high during summer stratification, and low during periods of mixing (Fig. 6a). This trend was not present for I_b with regard to CO_2 uptake (Fig. 5b). I_k was usually lower for NO_3^-, than for CO_2 and NH_4^+ (Fig. 6a) and I_b for NO_3^- was always lower than I_b for NH_4^+ and CO_2. Low values for I_k and I_b indicate low light adaptation (Platt et al. 1980). There was a significant positive correlation between I_k and I_b ($P < 0.01$), but the regression only had an

166

r^2 of 0.34; there did not appear to be any nutrient specific trends in I_k versus I_b (Fig. 7).

Discussion

NH_4^+ is energetically preferable to NO_3^- (Reynolds 1984), thus it is not surprising that NO_3^- uptake is more strongly influenced by PPFD (lower values for I_b and I_k) than NH_4^+ uptake. The greater influence of light on NO_3^- than on NH_4^+ uptake has been previously documented for freshwater phytoplankton (Fisher et al. 1988, Priscu 1988, Whalen and Alexander 1984).

PO_4^{3-} uptake was never dependent on PPFD in our study, whereas other authors have shown that light can affect P uptake (Nalewajko et al. 1986). Perhaps bacteria are more important with regard to P than N uptake in Flathead Lake. There has been documentation of the importance of bacteria as competitors for P (Currie and Kalff 1984a,b) and N (Wheeler and Kirchman 1986). If bacteria are more important to P uptake, P uptake would be less dependent on light than NH_4^+, NO_3^- or CO_2, and experiments such as ours would probably not show a dependence of P uptake on PPFD. Also, P uptake was measured using filters with 0.45 μm retention, while N uptake was measured on filters with 0.7 μm retention. The different filters may have emphasized bacterial uptake of P to a greater extent than bacterial uptake of N.

In general, all maximum biomass specific uptake rates were lowest during February. This may be a function of

167

temperature because I_m was not always lowest during winter and low values for PPFD were observed at other times of the year (Table 1). It has previously been shown that temperature has a strong effect on dissolved inorganic N uptake (Priscu et al. 1988, Whalen and Alexander 1984).

I_k values for CO_2 uptake were lowest during periods of deep convective mixing, i.e. November, February, and May. The low values for I_k during periods of mixing allow the cells to utilize the overall lower light levels which they experience at this time. This adaptation may reflect changes in either the photobiology of the cells or community composition. The seasonal trends observed in I_k for CO_2 were not present for NO_3^- and NH_4^+ uptake. Energy for inorganic N uptake may be derived from intermediary metabolism (Priscu 1984). Consequently, the dependence of inorganic N uptake on PPFD may be a function of the overall physiological state of the cell which may not be directly coupled with the ambient light regimes.

Although low values of I_k and I_b are used as indicators of low-light adaptation, low I_k values were not always correlated with low I_b values in our study. Neale (1987) has suggested that ow values of I_b should occur with low values of I_k, because cells which have high photosynthetic efficiency are more susceptible to photoinhibition when increased light causes electron build-up at the photosystem II reaction center (Neale 1987).

Our uptake measurements were made under saturating concentrations of nutrients and the PPFD relationships may not necessarily extrapolate to ambient nutrient concentrations except for $^{14}CO_2$ which was added as a tracer. This problem was discussed with respect to DIN uptake in an arctic lake by Whalen and Alexander (1984).

Dark PO_4^{3-} and NH_4^+ uptake was significant during the incubation periods, whereas dark rates of NO_3^- and CO_2 uptake were much lower. This disparity suggests that nutrient uptake by phytoplankton in this system is dependent upon diel variations in PPFD.

Ambient uptake rates of C, N and P (unpublished data) were scaled with light data to show the diel variations in the nutrient uptake ratio. In August the light C:N:P uptake ratio was 454:6:1, and the dark ratio was 0:3:1. In February the light C:N:P uptake ratio was 478:3:1, and the dark ratio was 0:2:1. At both times, in the light, C was incorporated at a higher rate than N and P, relative to the Redfield ratio (106:16:1), but in the dark, relative rates of C uptake dropped to 0. These data show the importance of considering diel variation of PPFD when examining nutrient dynamics.

Acknowledgements

References

Currie, D. J. and J. Kalff 1984a. The relative importance of bacterioplankton and phytoplankton in phosphorus uptake in freshwater. Limnol. Oceanogr. 29:311-321.

Currie, D. J. and J. Kalff. 1984b. A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus. Limnol. Oceanogr. 29:298-310.

Dodds, W. K. and J. C. Priscu. 1988. An inexpensive device for sampling large volumes of lake water from discrete depths. Freshw. Biol. 20:113-116.

Eppley, R. W. 1978. Nitrate uptake, pp. 401-409 in Handbook of Phycological Methods. Physiological and biochemical methods, J. A. Hellebust and J. S. Craigie eds. Cambridge Univ. Press, Cambridge.

Fisher, T. R., K. M. Morrissey, P. R. Carlson, L. F. Alves, and J. M. Melack. 1988. Nitrate and ammonium uptake by phytoplankton in an Amazon River floodplain lake. J. Plank. Res. 10:7-29.

MacIsaac, J. S. and R. C. Dugdale. 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. Deep Sea Res. 19:209-232.

Nalewajko, C. and K. Lee. 1983. Light stimulation of phosphate uptake in marine phytoplankton. Mar. Biol. 74:9-15.

Nalewajko, C., B. Paul, K. Lee and H. Shear. 1986. Light history, phosphorus status, and the occurrence of light stimulation or inhibition of phosphate uptake in Lake Superior phytoplankton and bacteria. Can. J. Fish. Aquat. Sci. 43:329-335.

Neale, P. J. 1987. Algal photo inhibition and photosynthesis in the aquatic environment. In: D.J. Kyle C. B. Osmond and C. J. Arntzen (eds.) Photoinhibition. Elsevier, Amsterdam pp. 39-65.

Nelson, D. M. and H. L. Conway. 1979. Effects of the light regime on nutrient assimilation by phytoplankton in the Baja California and northwest Africa Upwelling systems. J. Mar. Res. 37:301-318.

Platt, T. C., C. L. Gallegos and W. G. Harrison. 1980. Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J. Mar. Res. 38:687-701.

Priscu, J. C. 1984. A comparison of nitrogen and carbon metabolism in the shallow and deep-water phytoplankton populations of a subalpine lake: response to photosynthetic photon flux density. J. Plank. Res. 6:733-749.

Priscu, J. C. 1988. Photon dependence of inorganic nitrogen transport by phytoplankton in perennially ice-covered antarctic lakes. Hydrobiologia (in press).

Reynolds, C. S. 1984. The Ecology of Freshwater
    Phytoplankton. Cambridge University Press, Cambridge.

Solorzano, L. 1969. Determination of ammonia in natural
    waters by the phenolhypochlorite method. Limnol.
    Oceanogr. 14:799-801.

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

Standard Methods. 1975. 14th ed. American Public Health
    Association, New York. pp. 278-282.

Stanford, J. A., P. J. Stewart and B. K. Ellis. 1983.
    Limnology of Flathead Lake. Final Report, Flathead
    River Basin Environmental Impact Study. US EPA. Helena,
    Montana.

Strickland, J. H. and T. R. Parsons. 1972. A practical
    handbook of seawater analysis. 2nd ed. Bull. Fish.
    Res. Bd. Can.

Terry, K. L. 1982. Nitrate uptake and assimilation in
    Thalassiosira weissfloggi and Phaedoctylum tricornutum:
    Interactions with photosynthesis and with the uptake of
    other ions. Mar. Biol. 69:21-30.

Timperley, M. H. and J. C. Priscu. 1986. Determination of
    nitrogen-15 by emission spectrometry using an atomic
    absorption spectrometer. Analyst. 111:23-28.

Whalen, S. C. and V. Alexander. 1984. Influence of temperature and light on rate of inorganic nitrogen transport by algae in an arctic lake. Can. J. Fish. Aquat. Sci. 41:1310-1318.

Wheeler, P. A. and D. L. Kirchman. 1986. Utilization of inorganic and organic nitrogen by bacteria in marine systems. Limnol. Oceanogr. 31:998-1009.

Young, T. C. and D. L. King. 1980. Interacting limits to algal growth: Light, phosphorus, and carbon dioxide availability. Wat. Res. 14:409-412.

Table 1. Parameters Describing the Dependence of Nutrient Uptake on PPFD. P refers to CO_2 uptake, NH to NH_4^+ uptake, and NO to NO_3^- uptake.

Symbol	Units	Description
P^B, NH^B, NO^B	$nM\ h^{-1}\ (\mu g\ Chl\ 1^{-1})^{-1}$	rate of nutrient uptake normalized to chlorophyll conc.
P_s^B, NH_s^B, NO_s^B	$nM\ h^{-1}\ (\mu g\ Chl\ 1^{-1})\text{-}1$	theoretical maximum uptake in the absence of inhibition
P_m^B, NH_m^B, NO_m^B	$nM\ h^{-1}\ (\mu g\ Chl\ 1^{-1})^{-1}$	maximum chlorophyll specific uptake rate
D^B	$nM\ h^{-1}\ (\mu g\ Chl\ 1^{-1})^{-1}$	dark uptake rate
I	$\mu E\ m^{-2}\ s^{-1}$	PPFD (photosynthetic photon flux density)
α	$(nM\ h^{-1}\ (\mu g\ Chl\ 1^{-1})^{-1})/(\mu E\ m^{-2}\ s^{-1})$	slope in the initial (PPFD limited) portion of the uptake curve
β	$(nM\ h^{-1}\ (\mu g\ Chl\ 1^{-1})^{-1})/(\mu E\ m^{-2}\ s^{-1})$	slope of the inhibition portion of the uptake curve
I_m	$\mu E\ m^{-2}\ s^{-1}$	PPFD at which the maximum uptake occurs
I_k	$\mu E\ m^{-2}\ s^{-1}$	PPFD level where extrapolations of and Pm occur (eg. P_m^B/α)
I_s	$\mu E\ m^{-2}\ s^{-1}$	PPFD level where and extrapolations of P_s and β intersect
Ib	$\mu E\ m^{-2}\ s^{-1}$	an index of photoinhibition, a small I_b is indicative of strong inibition (Platt et al. 1980)

Table 2. Parameters Describing Seasonal Trends in PPFD effects upon $^{14}CO_2$, $^{15}NH_4^+$, and $^{15}NO_3^-$ uptake in Flathead Lake. See Table 1 for identification of parameters. PPFD column gives average value at the water surface over the period of the experiment % column represents $I_m/PPFD*10^2$. NA means the parameter was not applicable or could not be calculated on the basis of the fitted parameters.

CO_2 Uptake

date	P_s^B	α	β	PPFD	P_m^B	I_k	I_s	I_b	I_m	%
6-22-87	1417	9.78	0.484	1173	1161	119	145	2930	443	37.7
7-15-87	1080	2.75	0.873	1597	521	190	393	1238	559	35.0
8-10-87	1327	5.96	0.315	1313	1076	180	223	4219	666	50.7
10-30-87	585	14.85	0.644	821	489	33	39	909	125	15.3
2-4-88	169	2.40	0.120	262	138	58	70	1408	214	81.7
5-2-88	596	8.38	0.218	1476	528	63	71	2732	261	17.7

NO_3^- Uptake

date	NO_s^B	α	β	D^B	PPFD	NO_m^B	I_k	I_s	I_b	I_m	%
6-25-87	4.34	0.265	0.0026	NA	752	4.11	15.5	16.3	1669.2	75.8	10.1
7-20-87	8.00	0.045	0.0085	NA	1590	4.75	106.3	179.2	942.7	328.7	20.7
9-3-87	1703	0.062	5.7000	1.99	1187	6.78	109.3	27467	298.8	297.2	25.0
10-28-87	5.15	0.087	0.0091	1.93	502	3.64	42.0	59.3	567.3	139.9	27.9
2-5-88	NA	NA	NA	NA	378	NA	NA	NA	NA	NA	NA
5-11-88	7.97	0.064	0.0031	1.87	1460	6.56	102.7	124.9	2594.1	384.8	26.3

NH_4^+ Uptake

date	P_s^B	α	β	D^B	PPFD	P_m^B	I_k	I_s	I_b	I_m	%
6-27-87	53.4	0.143	0.0165	18.5	1082	36.9	257.8	373.2	3245.3	847.8	78.3
7-30-87	109.7	0.618	0.0513	35.9	1354	81.9	132.4	177.5	2139.5	455.9	33.7
9-17-87	151.4	0.898	0.1380	46.4	1071	96.3	107.2	168.6	1097.1	339.8	31.7
11-6-87	37.68	0.153	0.0135	22.9	518	27.8	180.9	245.5	2789.2	617.4	119.3
2-12-88	16.9	0.137	0.0627	5.5	727	6.8	49.8	123.4	269.4	142.9	19.7
5-9-88	26.8	0.163	0.0087	15.5	1317	21.7	133.0	164.4	3070.8	489.9	37.2

Figure 1.   Seasonal relationship of PO_4^{3-} uptake to PPFD in Flathead Lake.

Figure 2.   Seasonal relationship of CO_2 uptake to PPFD in Flathead Lake.   See text for a description of the model used to fit the curves to the data.

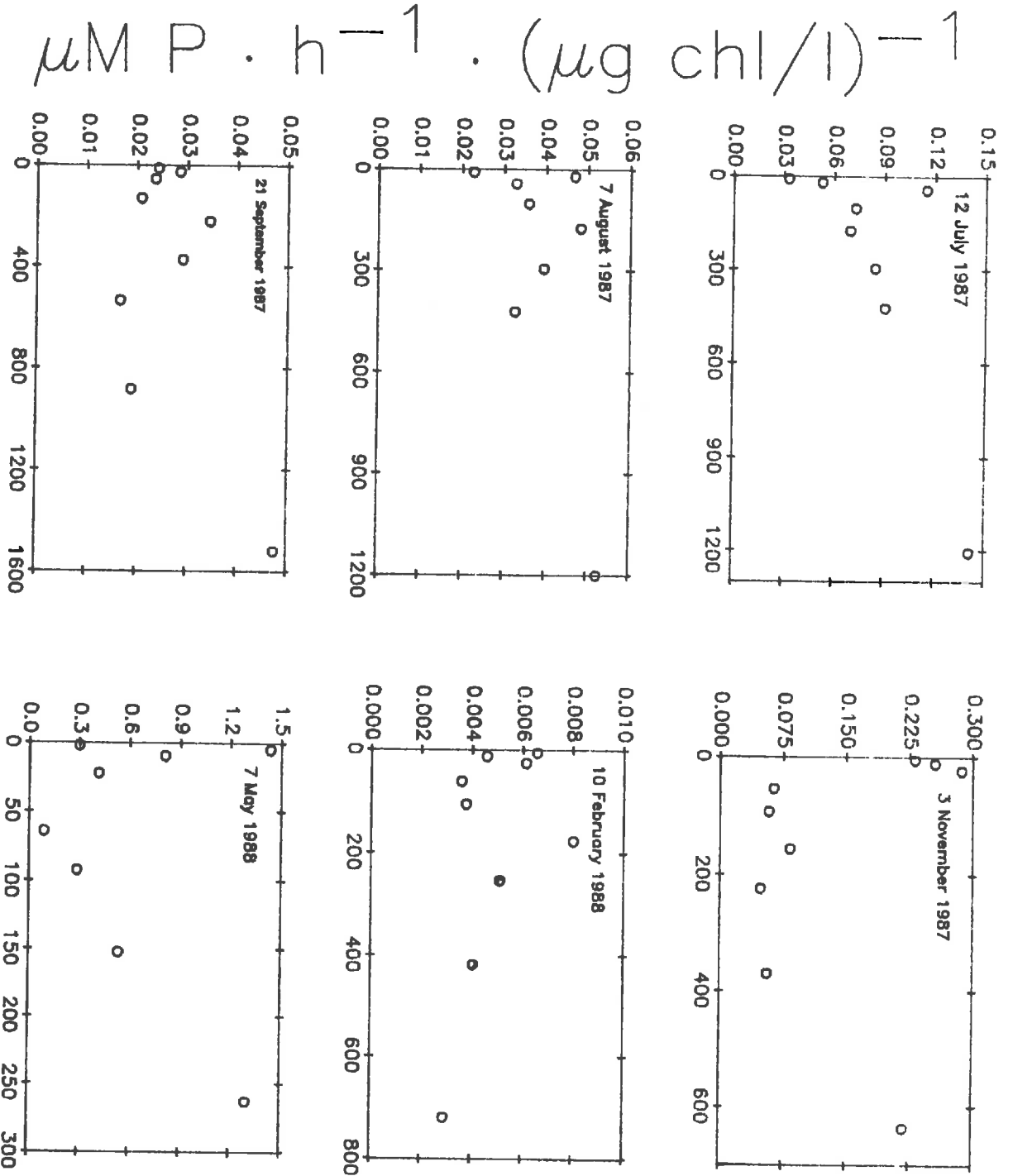Figure 3.   Seasonal relationship of $^{15}NO_3^-$ uptake to PPFD in Flathead Lake.

Figure 4.   Seasonal relationship of $^{15}NH_4^+$ uptake to PPFD in Flathead Lake.

Figure 5.   Relationship between P_m^B and α or β for NH_4^+, NO_3^- and CO_2 uptake in Flathead Lake for all seasonal data.

Figure 6.   Seasonal trends in I_k and I_b for NH_4^+, NO_3^- and CO_2 uptake in Flathead Lake.

Figure 7.   Relationship between I_k and I_b for NH_4^+, NO_3^- and CO_2 uptake.   Symbols are defined in Figure 5a.
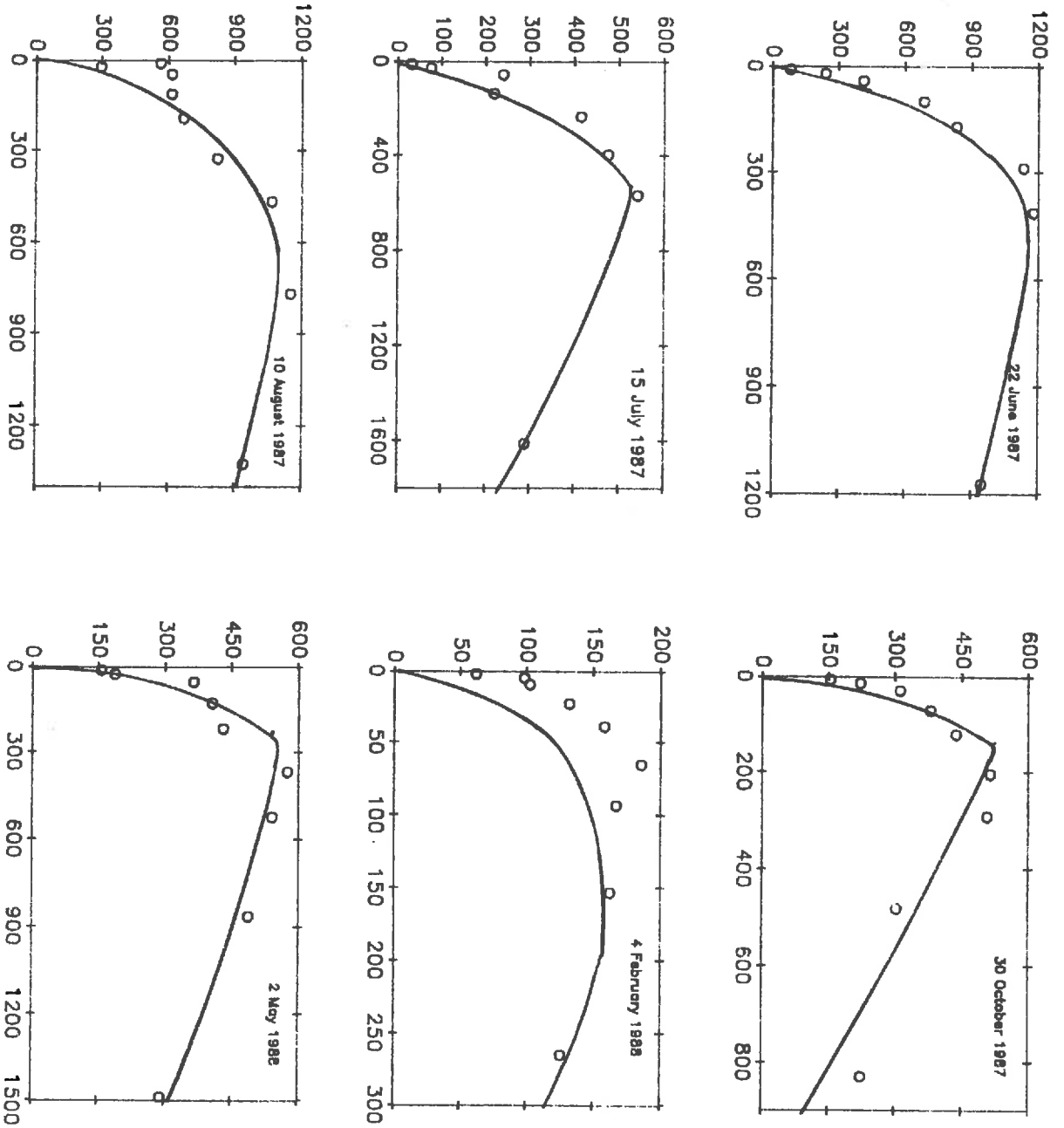
Figure 1

$$\mu M \; P \cdot h^{-1} \cdot (\mu g \; chl/l)^{-1}$$

$$\mu E \cdot m^{-2} \cdot s^{-1}$$

12 July 1987

3 November 1987

7 August 1987

10 February 1988

21 September 1987

7 May 1988

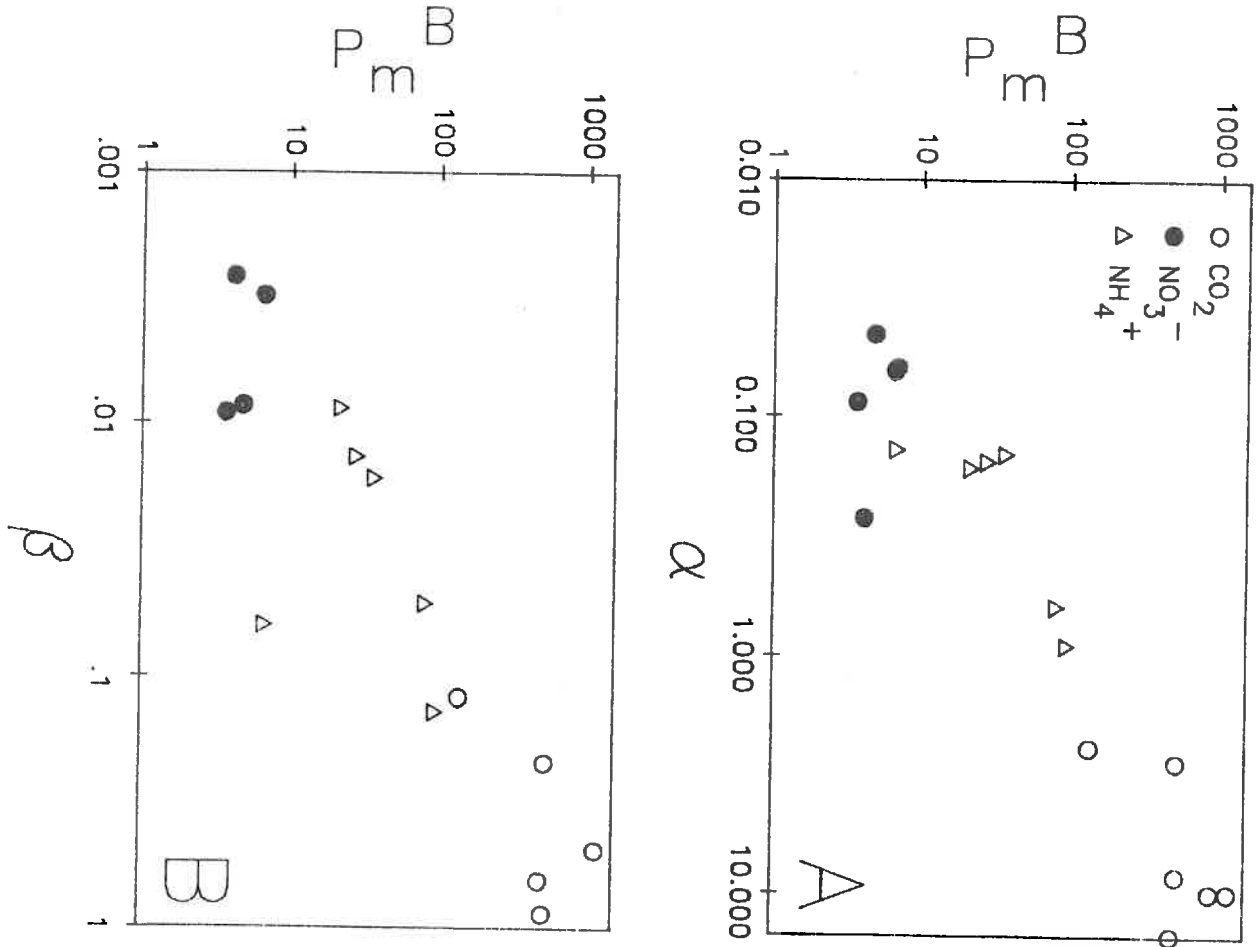Figure 2

179
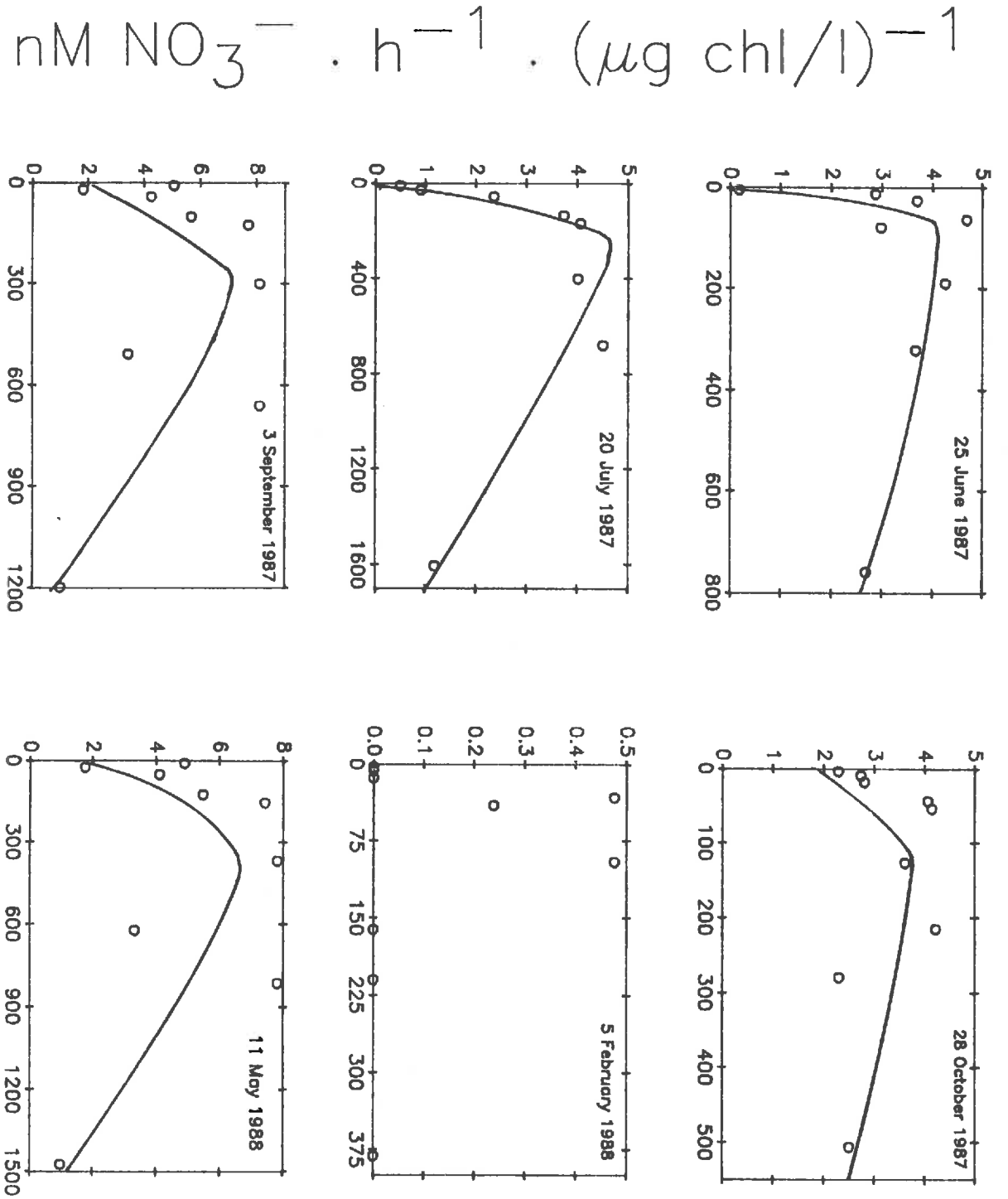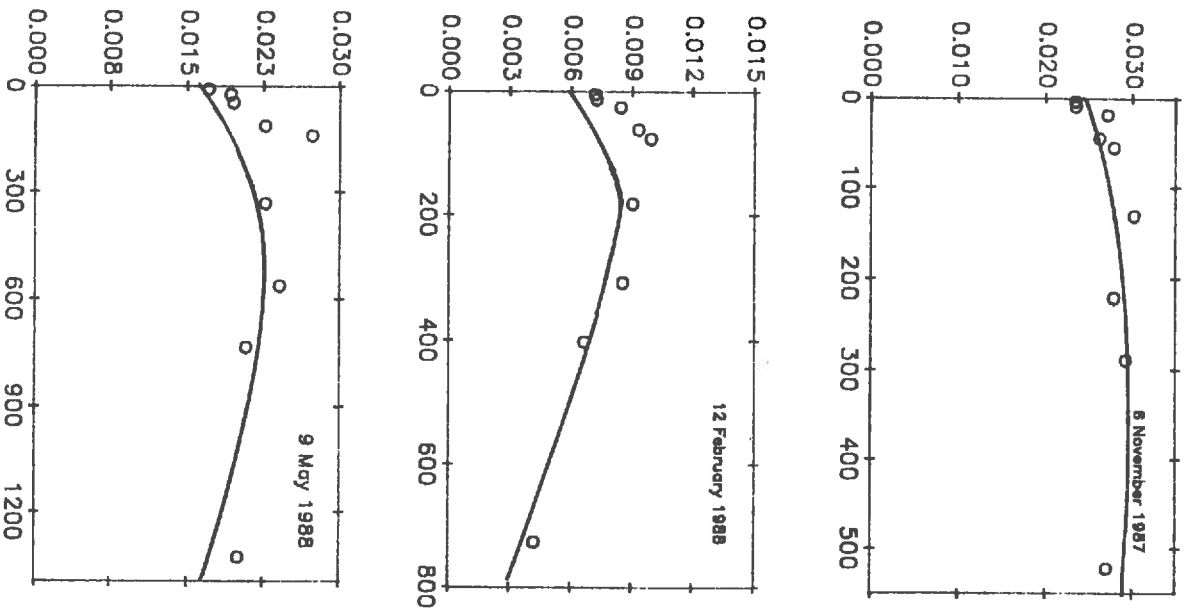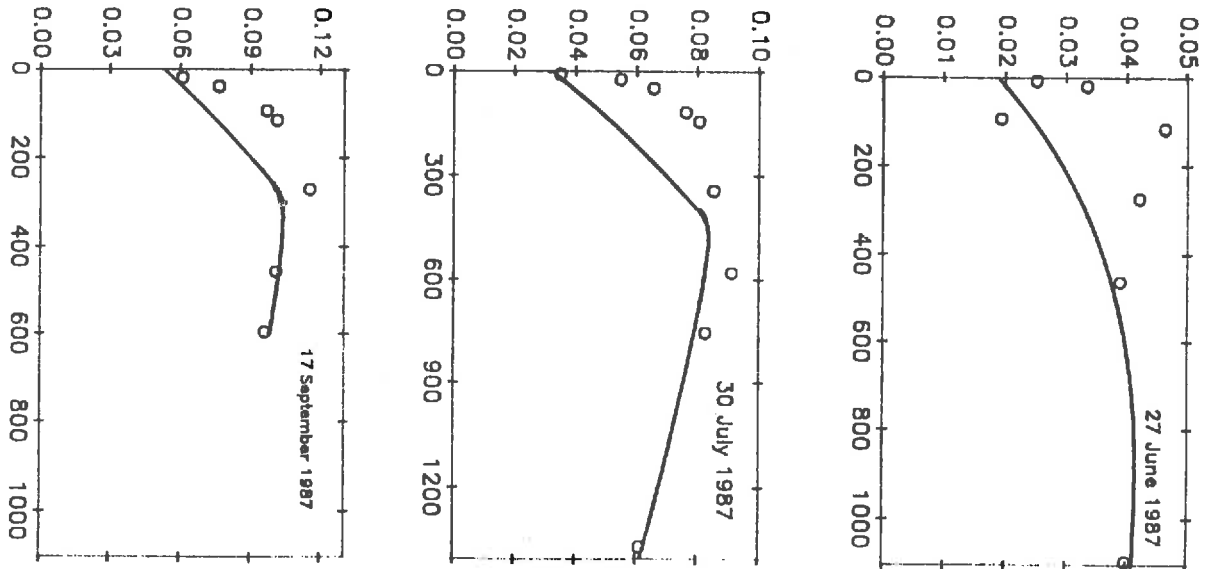
Figure 3

Figure 4
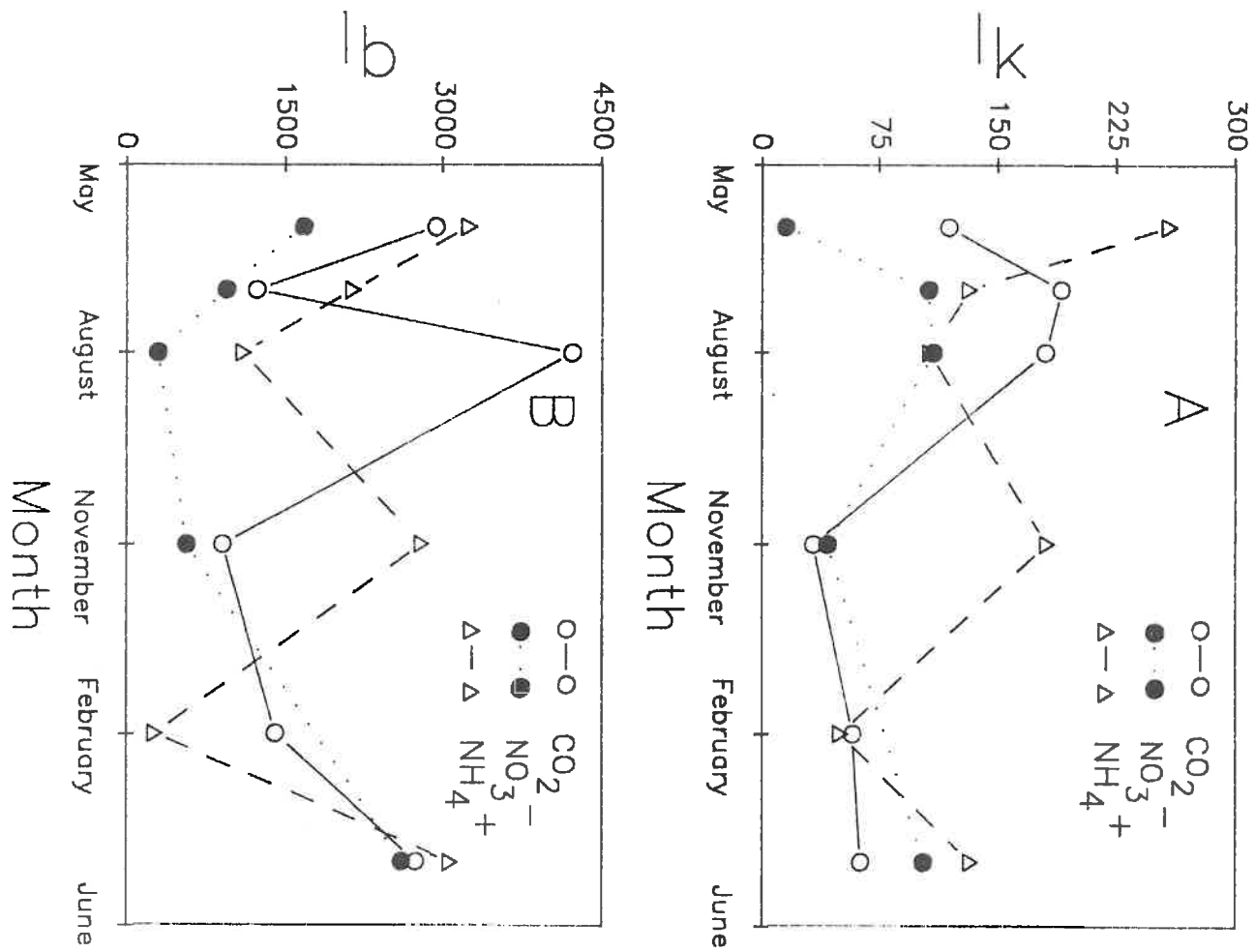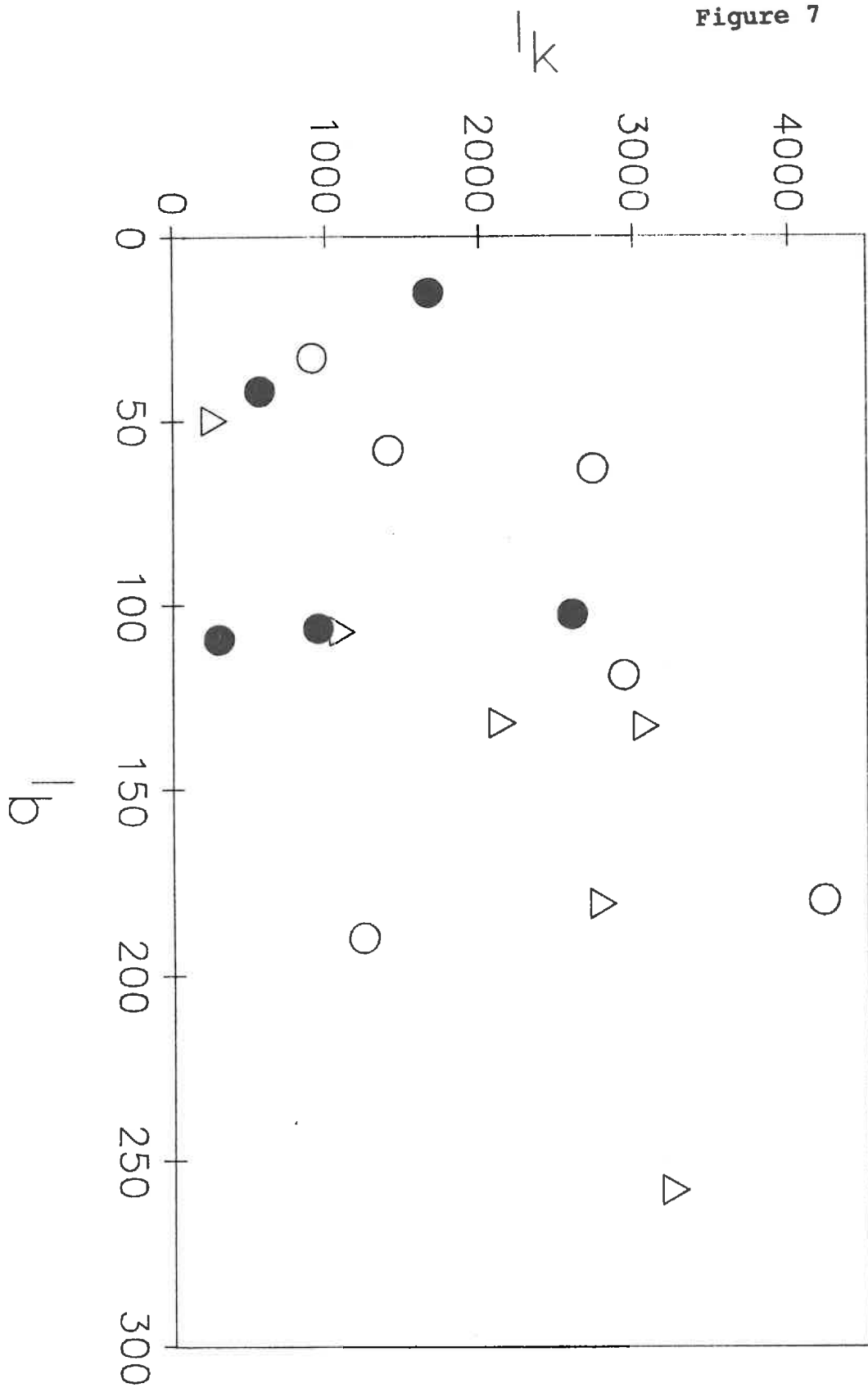
181

Figure 5

Figure 6

Figure 7

Chapter 9

The Influence of Phosphate Enrichment on Ammonium and

Nitrate Flux in an Oligotrophic Lake: Mesocosm Studies

Abstract

The fate of $^{15}NH_4Cl$ or $^{15}NO_3$ was followed in control and PO_4^{3-} enriched mesocosms filled with epilimnetic water from an oligotrophic lake. Incorporation of ^{15}N into phytoplankton and bacteria (particulates between 280 and 0.7 μm size), and crustaceous zooplankton > 80 μm was enhanced by P, but P caused no increase in efficiency of uptake by phytoplankton and bacteria of either $^{15}NO_3^-$ or $^{15}NH_4^+$ (biomass specific rates were not enhanced by P). Isotope dilution occurred in both $^{15}NH_4^+$ and $^{15}NO_3^-$ experiments, showing regeneration of both nutrients. The results illustrate the strong coupling between N dynamics and levels of phosphorus enrichment in this system.

Mesocosms (limnocorrals) have been used extensively to study nutrient limitation since their introduction more than 25 years ago (Goldman 1962). Most of these studies have examined the influence of nutrient additions on phytoplankton production. Few mesocosm experiments have focused on the fluxes of added nutrients through various components of the community. The few that examined these fluxes were conducted in eutrophic or coastal moving systems (Koike et al. 1982; Seitzinger and Nixon 1985; Stewart et al. 1983). We report the results of meso-scale experiments we designed to determine the effect PO_4^{3-} enrichment on both $^{15}NH_4^+$ and $^{15}NO_3^-$ transformations.

Materials and Methods

Experiments took place in Yellow Bay, located on the east side of Flathead Lake, MT. Flathead is a large (460 km^2), oligotrophic (epilimnetic chl a 0.1 - 1.1 μg l^{-1}) lake in the Columbia River drainage. During summer stratification, ambient epilimnetic levels of NH_4^+ range from 0.25 - 0.90 μM, NO_3^- from 0.004- 0.11 μM and soluble reactive phosphorus (SRP) from 0.01 - 0.06 μM.

Mesocosms were constructed of 1 m diameter polyethylene tubes, 2 m deep and sealed at the bottom. The top was open and floated about 20 cm above the water surface. The mesocosms were filled with near-surface water. Three separate experiments were conducted during the summer of 1987. In the first experiment, both mesocosms were amended

with 10 μM, 50 atom %, $^{15}NH_4Cl$ (final concentration) and one was amended with 2.3 μM KH_2PO_4 (final concentration). In the second experiment both mesocosms were amended to 2.3 μM KH_2PO_4 and one was enriched to a final concentration of 10 μm $^{14}NH_4Cl$. In the third experiment, both mesocosms were amended with 50 atom % $^{15}NaNO_3$ to a final concentration of 10 μM and one of the mesocosms had 2.3 μM KH_2PO_4 added. Mesocosms were well mixed daily immediately before sampling, from 0.5 m.

Photosynthesis was measured on 100 ml aliquots of water with $NaH_2^{14}CO_3$ added to a final concentration of 0.011 μC ml^{-1}. The aliquots were incubated at 1 m for 4 h, after which they were filtered on Whatman GF/F glass fiber filters and counted by liquid scintillation spectrometry. NH_4^+, NO_3^-, and soluble reactive phosphorus (SRP) were analyzed by the phenol-hypochlorite (Solorzano 1969), cadmium reduction (Eppley 1978), mixed molybdophosphate (Strickland and Parsons 1972) methods respectively. Dissolved organic N was determined after persulfate digestion (Solorozano and Sharp 1980a). Chlorophyll was determined fluorometrically after filtration onto Whatman GF/F filters (Strickland and Parsons 1972). Particulate N (PN) and particulate P (PP) samples were filtered through a 280 μm mesh to remove large zooplankton and then filtered onto pre-combusted Whatman GF/F filters. PN concentration was determined with a Carlo Erba 1106 elemental analyzer. PP concentration was

determined with a dry oxidation technique (Solorzano and Sharp 1980b).

Incorporation of ^{15}N into particulates was determined on samples which were screened through a 280 μm nylon mesh to remove large zooplankton and then filtered onto Whatman GF/F filters. ^{15}N enrichment was determined by emission spectrometry (Timperley and Priscu 1986). The atom % of ^{15}N in dissolved NH_4^+ was determined by extracting NH_4^+ with zeolite ion sieve (Lipshultz, 1984), drying the zeolite, and measuring enrichment with emission spectrometry. ^{15}N enrichment of zeolite extracts was corrected for isotopic discrimination which was significant in our study system. Enrichment of the NO_3^- and NO_2^- pool was determined by an aniline sulfate extraction technique (McCarthy et al. 1984), followed by Dumas combustion and emission spectrometry. The technique was modified by washing the extract with 0.1% NaOH immediately before the final de-ionized water washes, because excessive amounts of β-naphthol left in the extract can prevent the emission tubes from firing (Joseph Boyer, personal communication). This concentration of NaOH was high enough to remove excess β-naphthol without removing the dye from the extract. The extracts were reduced in volume on a hot plate to 2 ml and the residue was transferred into emission tubes which were dried for 24 h at 50^0 C. Twice the amount of reagents usually used for Dumas combustion (Timperley and Priscu 1986) was added before vacuum sealing

the emission tubes to ensure that Dumas combustion would remove all gases except N_2.

Regeneration was calculated with the equations of Laws (1984). These equations were modified to account for the regeneration of ^{15}N as well as ^{14}N. We assumed that the ratio of $^{15}N:^{14}N$ regenerated was equal to the mean $^{15}N:^{14}N$ ratio of particulates over the previous day.

Large zooplankton were removed at the end of each experiment with repeated hauls of an 80 µm net. Samples were dried, weighed and analyzed for ^{15}N enrichment. Uptake rates of N represent incorporation of N into zooplankton tissue plus the gut contents at the time of collection. The mean atom % enrichment of particulates in each treatment over the 4 days of the experiments was used to estimate the N uptake by zooplankton.

Acetylene reduction was measured according to Flett et al. (1976). Samples were analyzed for ethylene on a Carle AGC series 100 gas chromatograph with a flame ionization detector. Rates of nitrogen fixation were calculated from acetylene reduction rates assuming 3 moles of ethylene produced to 1 mole N fixed (Hardy et al. 1968).

Results

Simultaneous additions of N and P were required in all three experiments to increase primary productivity (Fig. 1) suggesting that both N and P dynamics are important regulators of productivity in this system. There was a

190

significant difference in primary productivity between the $^{15}NH_4^+$ and $^{15}NH_4^+$ + P treatments within two days after fertilization (Fig. 2) illustrating the rapid response of these treatments to simultaneous fertilization. Chlorophyll a was also higher in the N + P than in the N treatments after 4 days regardless of whether N was added as $^{15}NO_3^-$ or $^{15}NH_4^+$ (Fig. 3).

Particulate N concentrations increased more rapidly when both N and P were added as compared to N addition alone (Fig. 4). $^{15}NO_3^-$ and $^{15}NH_4^+$ incorporation by particulates were both higher in the presence of PO_4^{3-} (Fig. 5). However, when rates are expressed as biomass specific uptake (h^{-1}) there are no clear trends in uptake of $^{15}NO_3^-$ or $^{15}NH_4^+$, in the presence or absence of PO_4^{3-} enrichment (Table 1).

Soluble nutrient pools showed less obvious trends with regard to fertilization. SRP levels decreased with time in N + P treatments (Table 1), and there was a concurrent increase in particulate P (Table 1) in these treatments. NO_3^- levels were consistently low in the $^{15}NH_4^+$ and $^{15}NH_4^+$ + PO_4^{3-} experiments, but were variable in both mesocosms containing $^{15}NO_3^-$ (Table 1). Dissolved organic N values dropped in both the $^{15}NO_3^-$ and the $^{15}NO_3^-$ + P treatments (Table 1).

In the $^{15}NO_3^-$ and $^{15}NO_3^-$ + P treatments, a measurable portion of ^{15}N ended up in the NH_4^+ pool (Fig. 6). There was no detectable $^{15}NO_3^-$ in the $^{15}NH_4^+$ and $^{15}NH_4^+$ + P treatments (data not shown). However, the variability was high in the

$^{15}NO_3^-$ extractions, and it was not as easy to discern low levels of $^{15}NO_3^-$ as it was low levels of $^{15}NH_4^+$.

There was dilution of the $^{15}NH_4^+$ by $^{14}NH_4^+$ in the $^{15}NH_4^+$ and $^{15}NH_4^+$ + P treatments and of the $^{15}NO_3^-$ by $^{14}NO_3^-$ in $^{15}NO_3^-$ and the $^{15}NO_3^-$ + P treatments (Fig. 7) implying regeneration of both $^{14}NH_4^+$ and $^{14}NO_3^-$ occurred. Regeneration rates of $^{15}NH_4^+$ dropped throughout the $^{15}NH_4^+$ and $^{15}NH_4^+$ + P treatments (Table 1) and no clear trends were evident. Regeneration rates of $^{15}NO_3^-$ in $^{15}NO_3^-$ and $^{15}NO_3^-$ + P treatments were much more variable (Table 1). The negative value seen for NO_3^- regeneration in Table 1 is an artifact because of the variance in the $^{15}NO_3^-$ data. Therefore, $^{15}NO_3^-$ regeneration data can only be interpreted as showing that regeneration occurs, but that no specific trends in the rates are evident.

Zooplankton (particulates > 80 µm) also showed significantly higher ^{15}N enrichments and higher rates of N incorporation in the presence of both N and P than in the presence of N alone. (Table 2). The higher incorporation of ^{15}N in zooplankton does not necessarily signify higher grazing rates since the particulates the zooplankton were consuming in the N + P treatments had higher enrichments than those in the N alone treatments (Table 1). The zooplankton in NH_4^+ treatments had higher rates of N incorporation than those in the NO_3^- treatments. Also, rates of zooplankton N incorporation were about 100 fold lower than the incorporation into particulate matter. However, these rates of N incorporation may be over estimates because

the zooplankton were not allowed to clear their guts before analysis, and we do not know what portion of the total zooplankton N is represented by algae remaining in their guts.

P enrichment stimulated volumetric acetylene reduction with respect to volumetric rates measured outside the mesocosms (Table 3), but there was not a significant difference between P and N + P volumetric rates. However, biomass specific rate of estimated N_2 fixation in the PO_4^{3-} treatment was significantly higher than in the N + P treatment. This is because there was not an increase in particulate N in the PO_4^{3-} treatment as there was in the N + P treatment, this leads to a higher calculated rate of biomass specific N_2 fixation in the P treatment. The calculated rates of N_2 fixation were close to the maximum uptake rates of $^{15}NO_3^-$ and $^{15}NH_4^+$ into particulates.

Discussion

The Flathead Lake phytoplankton community exhibited simultaneous deficiency in N and P. Simultaneous nutrient deficiency (N and P) has also been reported for oligotrophic coastal lakes in British Columbia (Suttle and Harrison 1988), for some parts of the season in two Michigan lakes (Elser et al. 1988), and in Lake Tahoe and Crater Lake (Lane and Goldman 1984).

In general, our data show a strong impact of P enrichment on N fluxes. Much of this impact is probably

related to the fact that simultaneous N and P enrichment is required to stimulate primary productivity. If P is added along with N, the phytoplankton photosynthesize at a higher rate, produce more chlorophyll and particulate N per unit volume, and utilize nutrients at a higher rate per unit volume. The higher nutrient utilization rates translate up the food chain as well, since the zooplankton in the N + P treatments incorporated N at a greater rate per unit volume than did those when N was added alone.

The data show the importance of N regeneration in this system. The regeneration rates for both NH_4^+ and NO_3^- were in excess of uptake rates, suggesting regeneration rates were sufficient to meet the requirements of the planktonic community. If our estimates of uptake and regeneration accurately reflect conditions in the lake, then a continual accumulation of NO_3^- and NH_4^+ pools in the epilimnion would be expected during summer stratification. Seasonal sampling did not show an accumulation of NO_3^- or NH_4^+ in the epilimnion (unpublished data), which indicates that uptake and regeneration may not be in a steady-state in our mesocosms. Because kinetics experiments (unpublished data) showed that the $^{15}NO_3^-$ and $^{15}NH_4^+$ uptake rates were at Vmax in the meso-cosms, actual uptake rates at ambient concentrations would be considerably lower than measured regeneration rates, leading to an even greater discrepancy between uptake and regeneration rates. The apparent uncoupling between uptake and regeneration may have resulted from using mesocosms with

closed bottoms. In a natural situation, N is lost as PN from the trophogenic zone through sedimentation and river outflow. However, because this loss was eliminated in our closed bottom mesocosms, regeneration rates may appear higher than in the open water.

NH_4^+ regeneration has been previously documented to be an important source of N in certain freshwater systems (eg. Fisher et al. 1987; Fisher et al. 1988; Priscu et al. 1988) and marine systems (eg. Glibert et al. 1982; Harrison 1978). However, data indicative of significant pelagic NO_3^- regeneration is only available for the Cook Strait, New Zealand (Priscu and Downes 1985). Because our mesocosms were open to the atmosphere, the $^{14}NO_3^-$ may have originated from atmospheric deposition. However, data on atmospheric deposition of NH_4^+ and NO_3^- at Yellow Bay for the same time period (R. Steinkraus, R. Metler, personal communication) suggest that only 0.1 % of the NH_4^+ and 3.0 % of the NO_3^- regeneration can be accounted for by atmospheric deposition. This brings into question the general accuracy of $^{15}NO_3^-$ uptake rates measured at low level additions. It has been shown that isotope dilution can cause several-fold underestimation of NH_4^+ uptake rates (Fisher et al. 1988; Glibert et al. 1982), this may also be true for $^{15}NO_3^-$ uptake work.

The fact that $^{15}NH_4^+$ appeared in the $^{15}NO_3^-$ addition experiments shows that the NO_3^- pool is tightly coupled to the NH_4^+ pool. The pathway for this was probably $^{15}NO_3^-$ incorporated into biomass and then regenerated as $^{15}NH_4^+$ by

heterotrophs. Therefore, addition of NO_3^- to the system will rapidly cycle through other parts of the food web such as bacteria and zooplankton, the organisms responsible for regeneration.

The results from our experiments also show the importance of N enrichment to P cycling. Addition of NO_3^- or NH_4^+ stimulated the incorporation of P into particulates (Table 1). Because the system exhibit N and P deficiency, it is very likely that many processes of the P cycle are impacted by the levels of N in the system.

Acetylene reduction was elevated the most dramatically when PO_4^{3-} alone was added to mesocosms. Continued P fertilization in the system may cause in overall increase in N_2 fixing cyanobacterial biomass. Our experiments were not long enough to support this contention directly, however, if N_2 fixation increased several fold, then it would become an important source of new N to the system. In this case, the additional N could act in concert with the added P to increase productivity.

P fertilization increased $^{15}NO_3^-$ and $^{15}NH_4^+$ uptake by phytoplankton, ^{15}N incorporation by zooplankton and acetylene reduction. Because our experiments were conducted under saturating levels of N and P, it is not possible to directly extrapolate our data to the unaltered system. However, we do demonstrate that P can impact N fluxes and this is probably the case in the lake as well as the mesocosms. There appears to be a tight coupling between N

and P dynamics in Flathead Lake, and we expect this is also true in other oligotrophic systems lacking significant inputs of allochthonous nutrients.

Acknowledgements

Elser, J. J., M. M. Elser, N. A. MacKay and S. R. Carpenter.
1988. Zooplankton-mediated transitions between N- and
P-limited algal growth. Limnol. Oceanogr. 33:1-14.

Eppley, R. W. 1978. Nitrate uptake. pp. 401-409
J. A. Hellebust and J. S. Craigie (eds.) Handbook of
Phycological Methods. IN: Physiological and biochemical
methods. Cambridge Univ. Press, Cambridge.

Fisher, T. R., R. D. Doyle and E. R. Peele. 1987. Size-frac-
tionated uptake and regeneration of ammonium and
phosphate in tropical lake. Verh. Verein. Internat.
Limnol. (in press).

Fisher, T. R., K. M. Morrisey, P. R. Carlson, L. F. Alves
and J. M. Melack. 1988. Nitrate and ammonium uptake by
plankton in an Amazon River floodplain lake. S. Plank.
Res. 10:7-29.

Flett, R. J., R. D. Hamilton and N. E. R. Campbell. 1976.
Aquatic acetylene-reduction techniques: solutions to
several problems. Can. J. Microbiol. 22:43-51.

Glibert, P. M., F. Lipschultz, J. J. McCarthy and
M. A. Altabet. 1982. Isotope dilution models of uptake
and remineralization of ammonium by marine plankton.
Limnol. Oceanogr.
27(4):639-650.

Goldman, C. R. 1962. A method of studying nutrient limiting
factors in situ in water columns isolated by
polyethylene film. Limnol. Oceanogr. 7:99-101.

Hardy, R. W., R. D. Holsten, E. K. Jackson, and R. C. Burns. 1968. The acetylene-ethylene assay for N_2 fixation: Laboratory and field evaluation. Pl. Physiol. 43:1185-1207.

Harrison, W. G. 1978. Experimental measurements of nitrogen remineralization in coastal waters. Limnol. Oceanogr. 23:684-694.

Hecky, R. E. and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. Limnol. Oceanogr. 33(4):796-822.

Koike, I., A. Haltori, M. Takaheshi and J. Goering. 1982. The use of enclosed experimental ecosystems to study nitrogen dynamics in coastal waters, pp. 291-303. IN: Grice, G. D. and M. R. Reeve (eds.), Marine Mesocosms. Springer-Verlag, Berlin.

Lane, J. L. and C. R. Goldman. 1984. Size-fractionation of natural phytoplankton communities in nutrient bioassay studies. Hydrobiologia 218-223.

Laws, E. 1984. Isotope dilution models and the mystery of the vanishing ^{15}N. Limnol. Oceanogr. 29:379-396.

Lipschultz, F. J. 1984. Environmental factors affecting rates of nitrogen cycling. Ph.D. Thesis. Harvard University.

McCarthy, J. S., W. Kaplan and S. L. Nevins. 1984.
Chesapeake Bay nutrient and plankton dynamics 2.
Sources and sinks of nitrate. 29:84-98.

Priscu, J. P and M. T. Downes. 1985. Nitrogen uptake,
ammonium oxidation and nitrous oxide (N_2O) levels in
the coastal waters of western Cook Strait, New Zealand.
Est. Coast. and Shelf Sci. 20:529-542.

Sietzinger, S. P. and S. W. Nixon. 1985. Eutrophication and
rate of denitrification and N2O production in coastal
marine sediments. Limnol. Oceanogr. 30:1332-1339.

Solorzano, L. 1969. Determination of ammonia in natural
waters by the phenolhypochlorite method. Limnol.
Oceanogr. 14:799-801.

Solorozano, L and H. Sharp. 1980a. Determination of total
dissolved nitrogen in natural waters. Limnol.
Oceanogr. 25:751-754.

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

Stewart, W. D. P., T. Preston, A. N. Rai and P. Rowell.
1983. pp. 1-27. IN: Lee, S. A., S. McNeill and I. H.
Rorison (eds.), Nitrogen as an Ecological Factor.
Blackwell Scientific Publications, Oxford.

Strickland, J. D. and T. R. Parsons. 1972. A practical
handbook of seawater analysis. Bull. Fish. Res. Board
Can. (2nd ed.) 167.

Suttle, C. A. and P. J. Harrison. 1988. Ammonium and
    phosphate uptake rates, N:P supply ratios, and evidence
    for N and P limitation in some oligotrophic lakes.
    Limnol. Oceanogr. 33(2):186-202.

Timperley, M. H. and J. C. Priscu. 1986. Determination of
    nitrogen-15 by optical emission spectrometry using an
    atomic absorption-spectrometer. Analyst 111:23-28.

Table 1. Daily Data From $^{15}NH_4^+$ and $^{15}NH_4^+ + PO_4^{3-}$, $^{15}NO_3^-$, and $^{15}NO_3^- + PO_4^{3-}$ mesocosms experiments. na = data not available

Treatment date	Dissolved Nutrients (uM)				Particulates		regeneration	uptake
	NH_4^+	SRP	NO_3^-	DON	atom %	µM P	µM h^{-1}	h^{-1}
+ $^{15}NH_4^+$								
7-24-87	12.6	0.042	0.501	5.81	0.72	3.17		
7-25-87	11.7	0.021	0.292	4.34	7.75	na	0.147	0.0096
7-26-87	11.4	0.011	0.300	5.36	5.42	2.47	0.096	-0.0041
7-27-87	10.2	0.011	0.090	4.82	5.15	na	0.077	-0.0006
7-28-87	10.8	0.011	0.122	4.56	16.26	2.57	0.000	0.0256
+ $^{15}NH_4^+ + PO_4^{3-}$								
7-24-7	13.0	1.82	0.154	na	0.72	5.1		
7-25-87	12.3	1.84	0.100	3.66	7.75	na	0.087	0.0104
7-26-87	11.5	1.83	0.154	4.16	9.38	12.6	0.025	0.0026
7-27-87	10.1	1.44	0.100	5.70	17.9	na	0.063	0.0154
7-28-87	7.93	1.49	0.110	na	29.4	17.4	0.043	0.0237
+ $^{15}NO_3^-$								
8-28-87	0.202	0.0214	8.93	6.49	0.17	4.51		
8-29-87	0.288	0.0107	4.65	5.15	2.63	2.67	0.0046	0.015
8-30-87	0.287	0.0107	10.8	7.02	3.68	3.35	0.0073	0.011
8-31-8	0.259	0.0107	10.2	5.95	4.97	3.74	na	
9-1-87	0.372	0.0107	3.67	3.71	5.73	4.10	na	0.020
+ $^{15}NO_3^- + PO_4^{3-}$								
8-28-87	0.316	2.25	4.99	13.46	0.22	11.3		
8-29-87	0.538	1.65	9.35	6.95	2.04	14.6	0.0097	0.0093
8-30-87	0.259	1.51	10.02	8.48	3.29	15.9	0.0111	0.0122
8-31-8	0.401	2.02	10.27	7.10	7.04	15.8	-0.0049	0.0462
9-1-87	0.429	0.84	2.36	3.06	11.13	18.5	0.0041	0.0474

Table 2. ^{15}N enrichment of zooplankton > 80 µm 4 days after fertilization by $^{15}NH_4^+$ or $^{15}NO_3^-$ and P in Flathead Lake. Comparisons are made with a pooled $\underline{t}$ test, * = P < 0.05 ** = P < 0.001. Comparison is made between the NH_4^+ and NH_4^+ + PO_4^{3-} treatments and the NO_3^- and NO_3^- + PO_4^{3-} treatments, n=3 for all.

				N uptake		
Treatment	atom % ^{15}N	std. dev.	t value	nM h^{-1}	std. dev.	t
$^{15}NH_4^+$	5.8	0.44		0.370	0.028	
$^{15}NH_4^+ + PO_4^{3-}$	11.6	1.09	8.532**	0.445	0.042	2.57**
$^{15}NO_3^-$	0.8	0.07		0.067	0.006	
$^{15}NO_3^- + PO_4^{3-}$	2.2	0.14	14.73**	0.195	0.013	15.20**

Table 3. Nitrogen fixation in P and N + P Mesocosms

Treatment	Partic N (uM)	n M N h^{-1}				mole N fixed h^{-1} mole N^{-1}			
		mean	std. dev.	t value	P	mean	std. dev.	t value	P
lake	1.70	9.42	2.14	21.18[1]	<0.005	0.00554	0.0013	20.49[1]	<0.0025
P	1.82	186	11.9	1.72[2]	>0.10	0.102	0.0065	2.11[2]	>0.05
N + P	3.85	124	49.7	3.24[3]	<0.05	0.032	0.109	6.85[3]	<0.025

[1]Comparison between lake and P treatment. [2]Comparison between P and N + P treatments.

[3]Comparison between lake and N + P treatments.

Fig. 1. $^{14}CO_2$ uptake of lake water collected outside and from within two mesocosms for three separate mesocosm experiments in Flathead Lake. Measurements were made 4 days after fertilization. Error bars = 1 std. dev.; * = significantly different from the lake treatment (pooled t test); n = 3 for each bar.

Fig. 2. Time-course of $^{14}CO_2$ uptake in $^{15}NH_4^+$ and $^{15}NH_4^+ + PO_4^{3-}$ mesocosms. Circles represent data from the $^{15}NH_4^+$ treatment, squares represent data from $^{15}NH_4^+ + PO_4^{3-}$ treatment. Error bars = 95% confidence interval (std. dev. were smaller than the symbols). n = 3 for all treatments.

Fig. 3. Chlorophyll $\underline{a}$ concentrations after N or N + P fertilization in mesocosms. Circles show treatments with N alone, open squares N + P treatments. A) $^{15}NH_4^+$ and $^{15}NH_4^+ + PO_4^{3-}$ treatments. B) $^{15}NO_3^-$ and $^{15}NO_3^-$ + PO_4^{3-} treatments.

Fig. 4. Particulate N concentration after N or N + P fertilizations. Circles show treatments with N alone, open squares N + P treatments. A) $^{15}NH_4^+$ and $^{15}NH_4^+ + PO_4^{3-}$ treatments. B) $^{15}NO_3^-$ and $^{15}NO_3^-$ + PO_4^{3-} treatments.

Fig. 5.   Volume specific $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake in N and N + P fertilized mesocosms. A) Open circles represent the $^{15}NH_4^+$ treatment, open squares represent the $^{15}NH_4^+ + PO_4^{3-}$ treatment, B) Open circles represent the $^{15}NO_3^-$ treatments, open squares represent the $^{15}NO_3^- + PO_4^{3-}$ treatment.

Fig. 6. ^{15}N atom % enrichment of the NH_4^+ fraction in the $^{15}NO_3^-$ and $^{15}NO_3^- + PO_4^{3-}$ mesocosms.   Open circles represent the $^{15}NO_3^-$ treatment, open squares represent the $^{15}NO_3^- + PO_4^{3-}$ treatments.

Fig. 7.   Isotopic dilution of $^{15}NH_4^+$ in $^{15}NH_4^+$ and $^{15}NH_4^+ + PO_4^{3-}$ mesocosms, and of $^{15}NO_3^-$ in $^{15}NO_3^-$ and $^{15}NO_3^- + PO_4^{3-}$ mesocosms.   A) Open circles represent atom % $^{15}NH_4^+$ in the $^{15}NH_4^+$ treatment, open squares represent the $^{15}NH_4^+ + PO_4^{3-}$ treatment.   B) Open circles represent the atom % $^{15}NO_3^-$ in the $^{15}NO_3^-$ treatment, open squares represent $^{15}NO_3^- + PO_4^{3-}$ treatments.
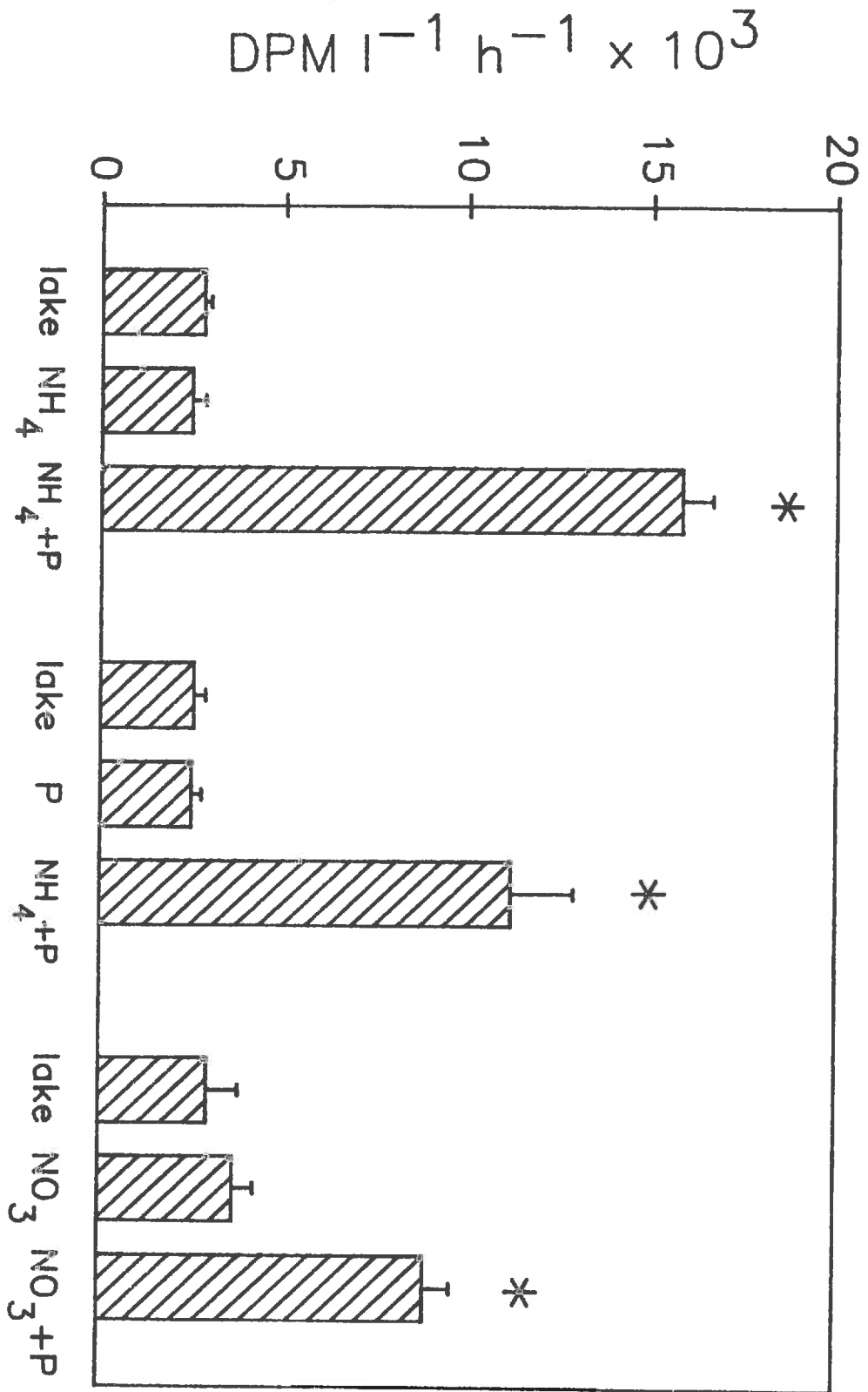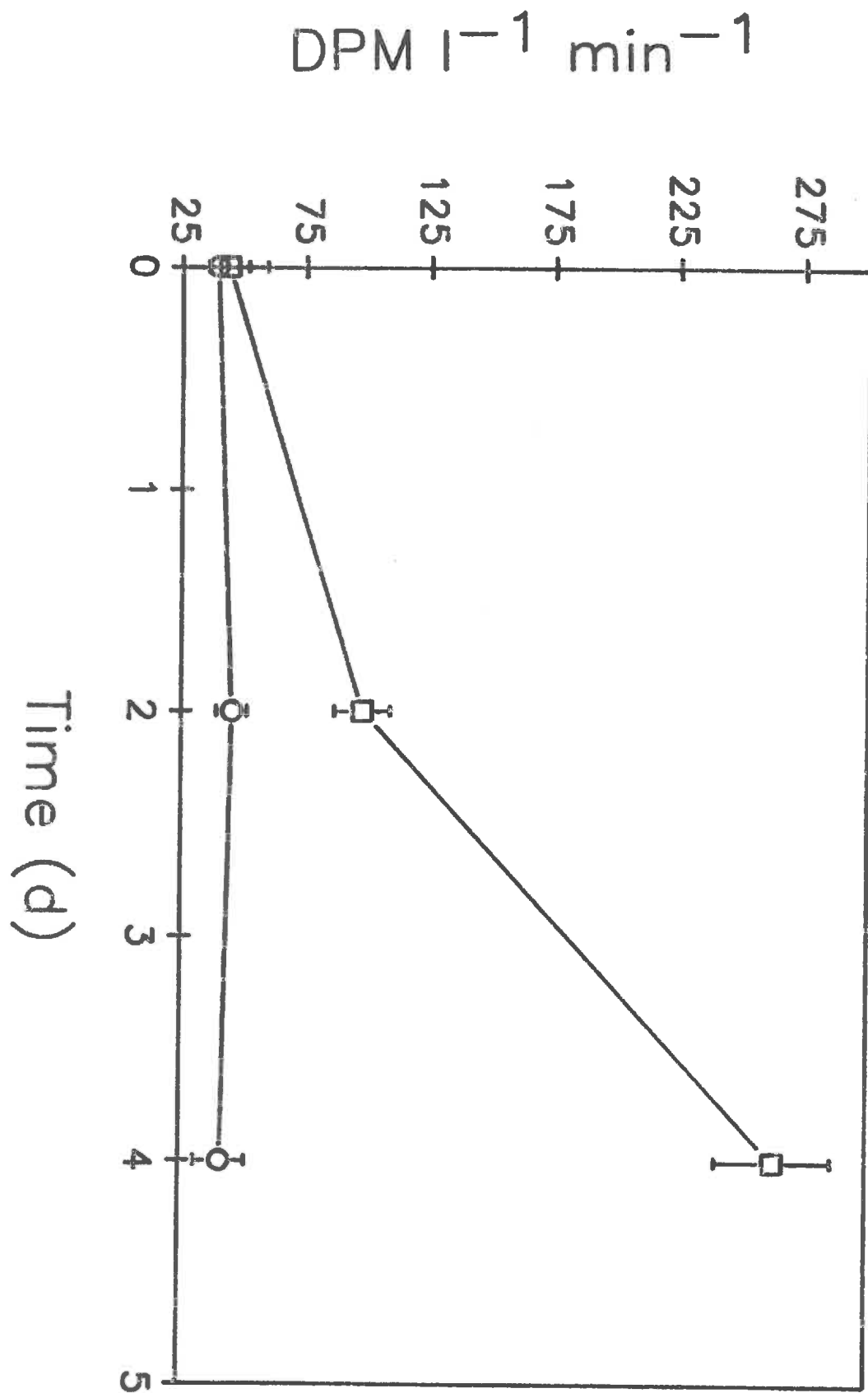
Figure 1
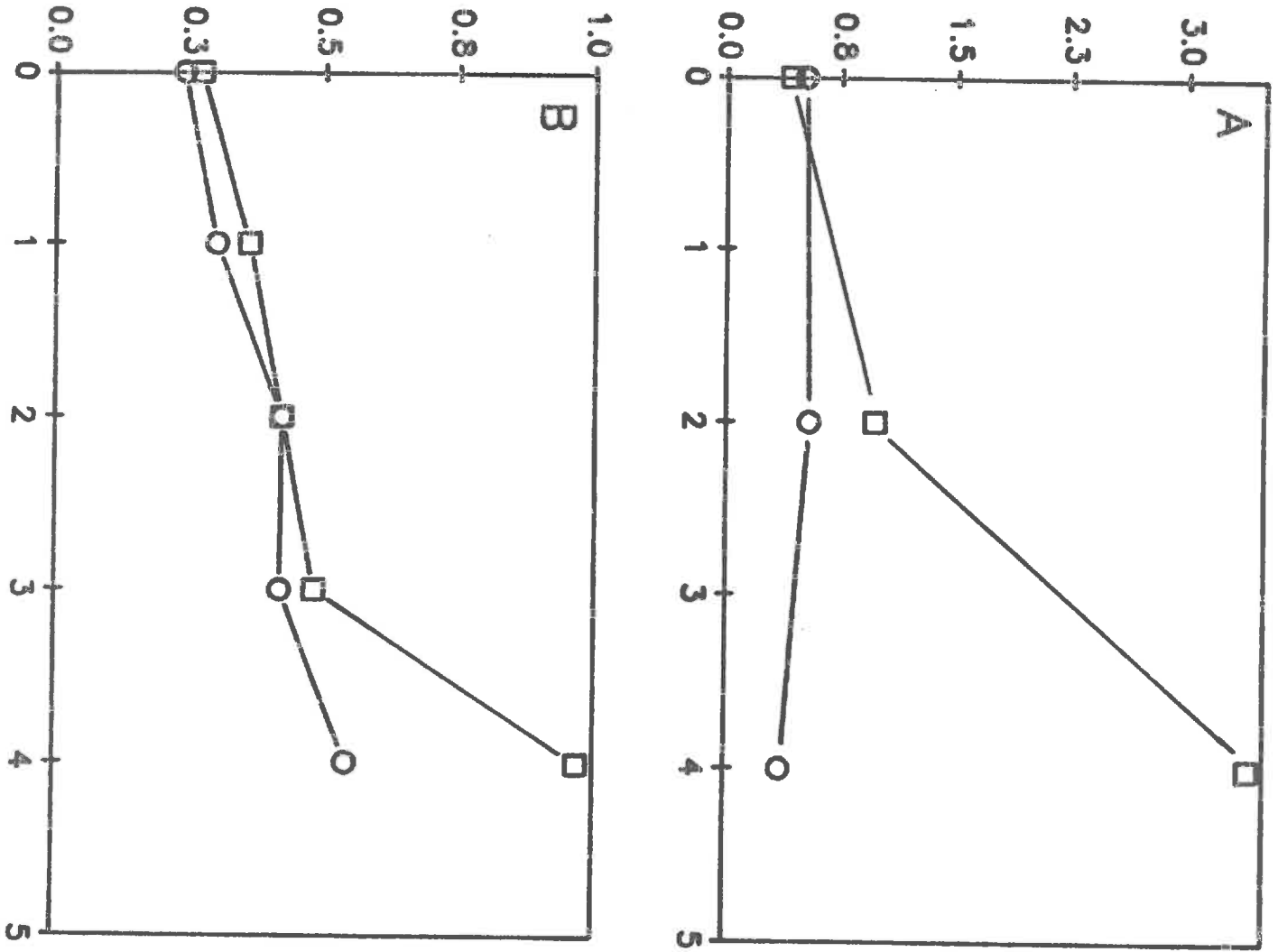
$$DPM\ l^{-1}\ h^{-1} \times 10^3$$

Figure 2

Figure 3

Chl (µg l⁻¹)
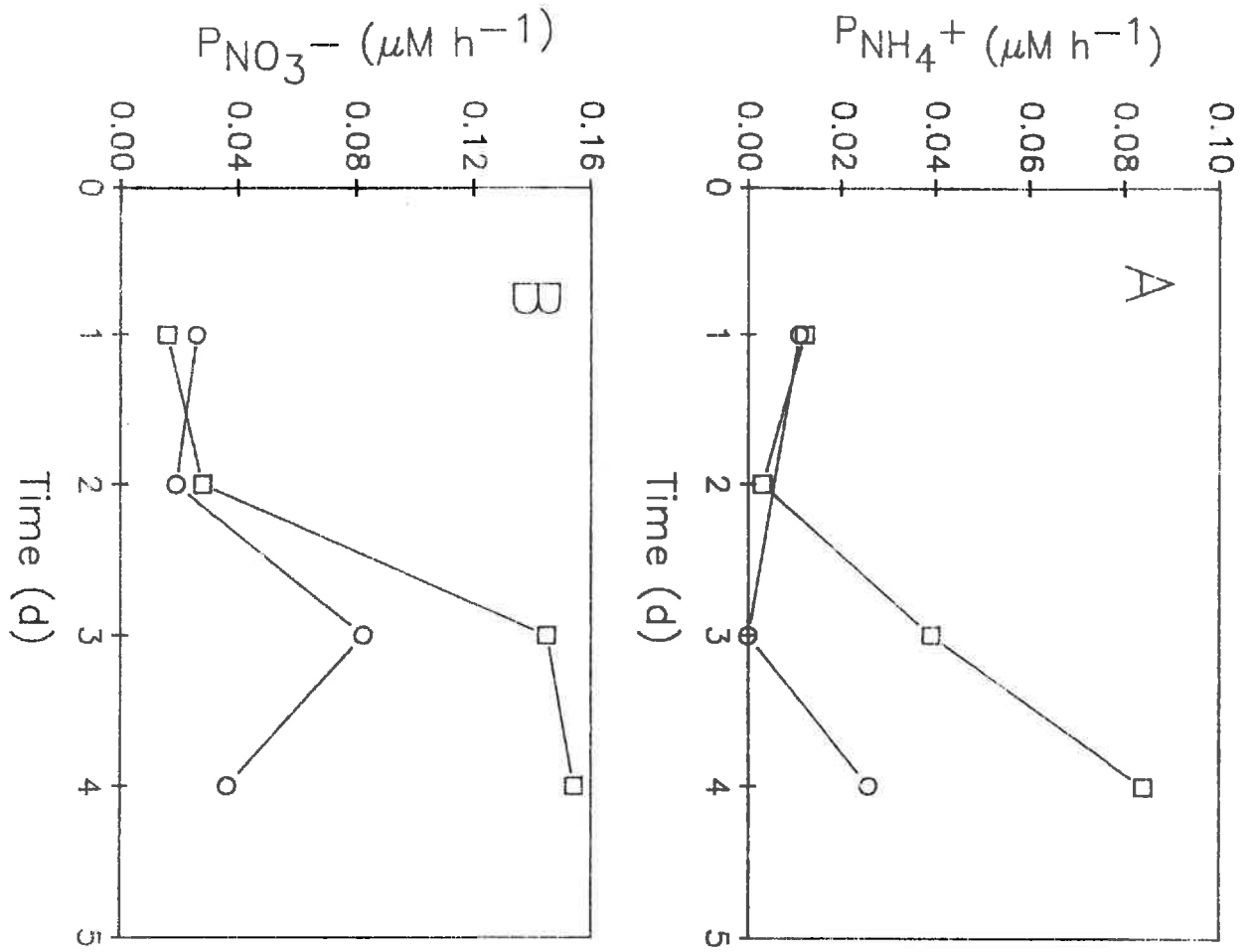
Figure 4

P N(µ M)
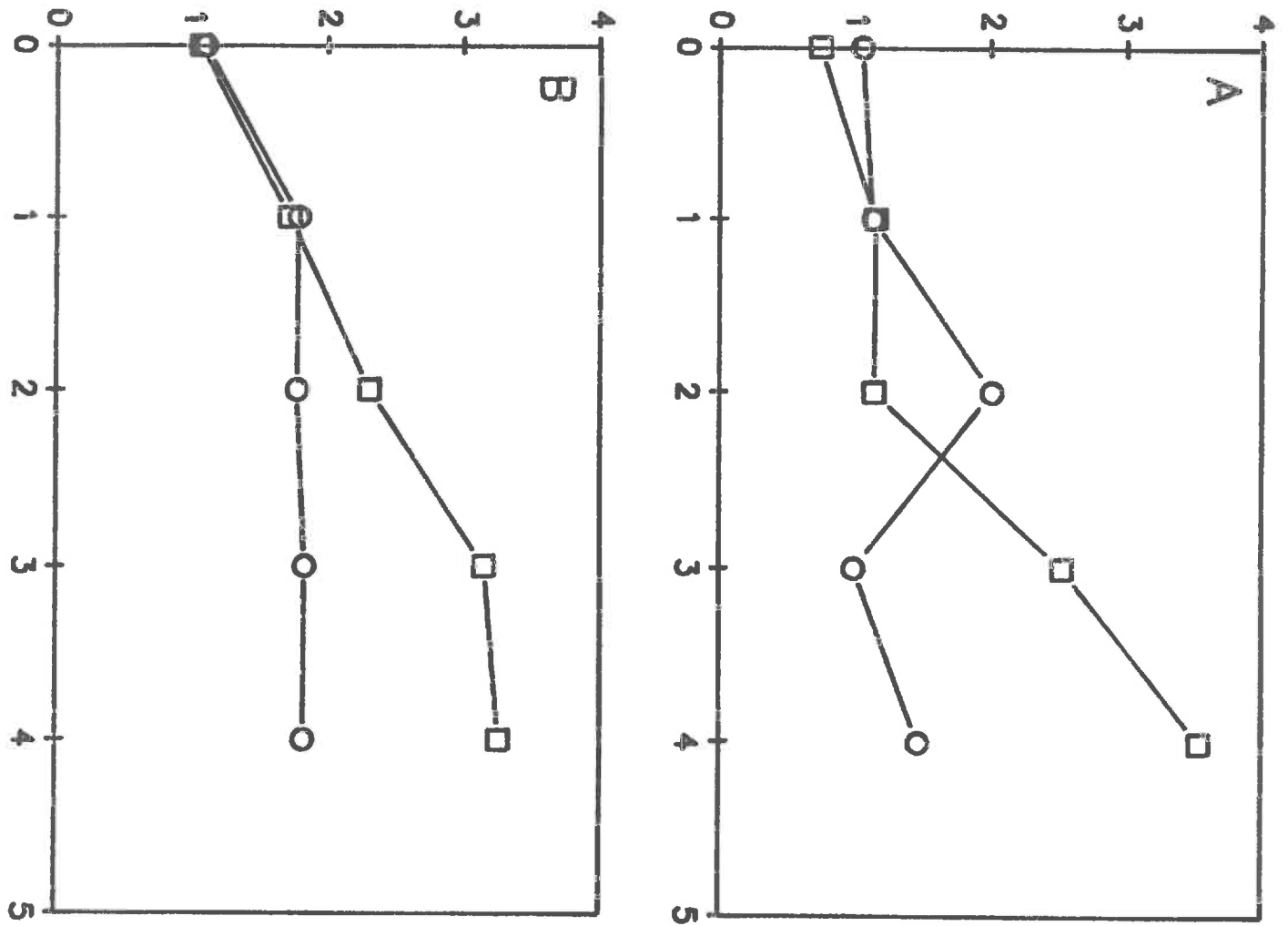
Figure 5

Figure 6

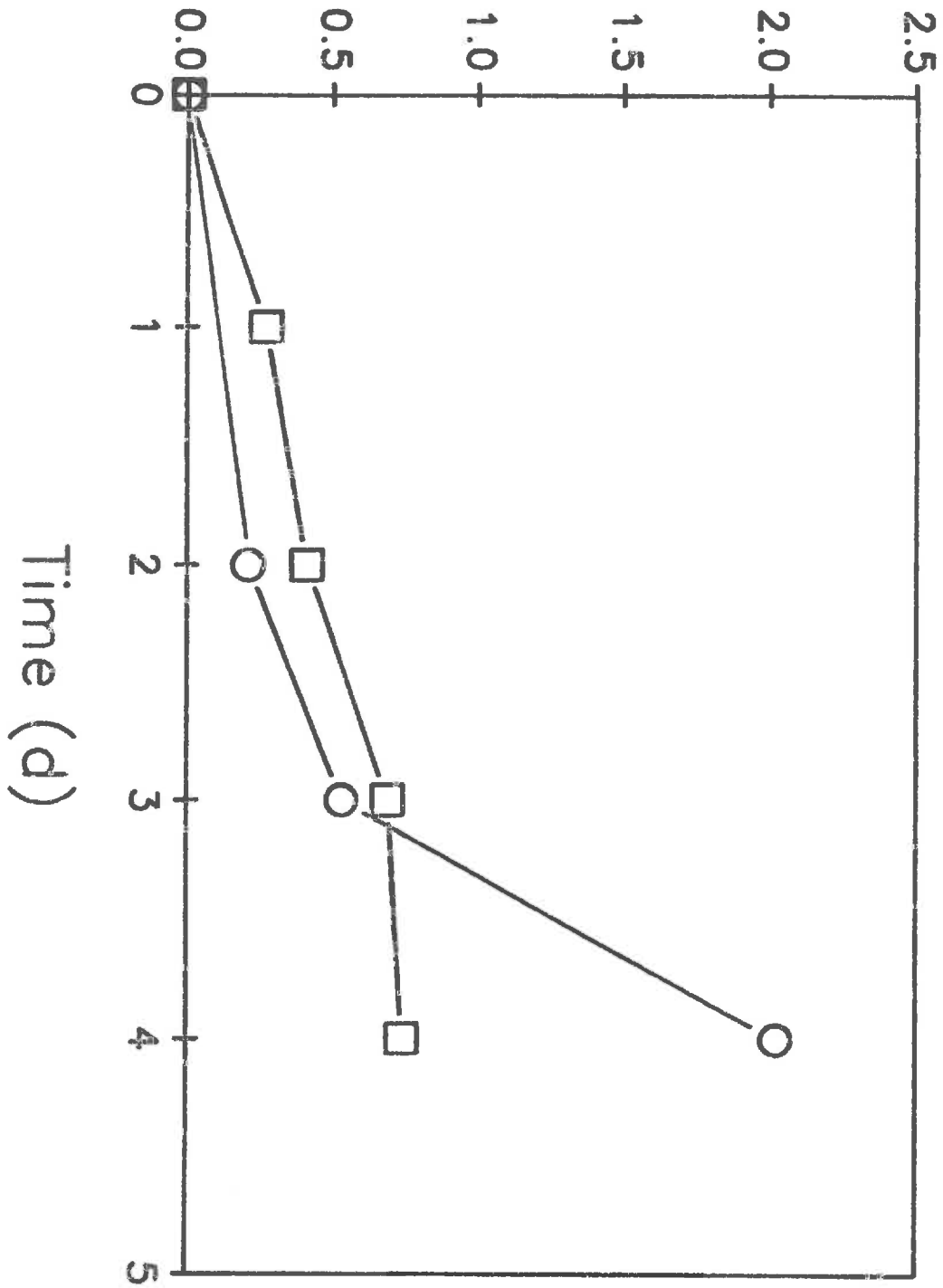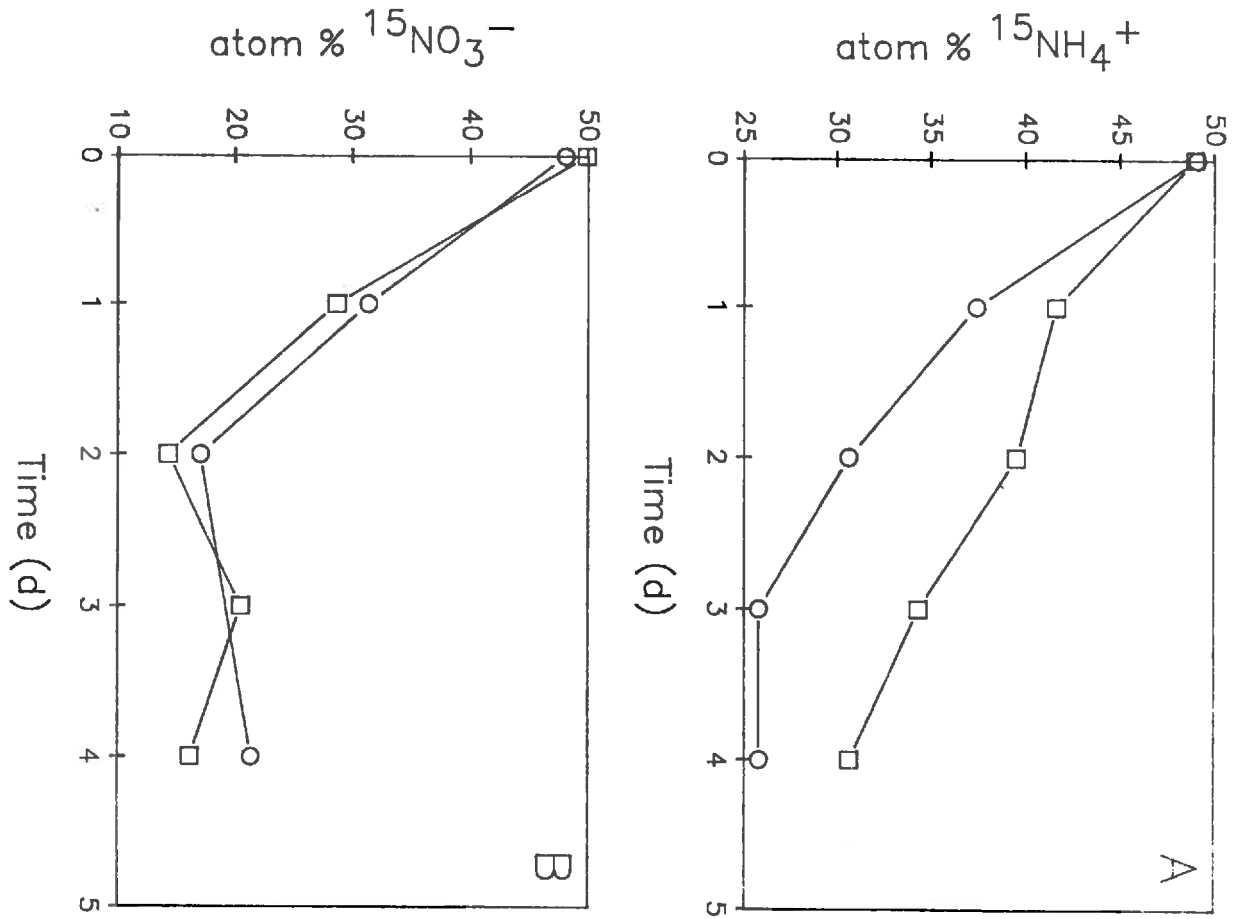^{15}N enrichment

Time (d)

Figure 7

Chapter 10

CONCLUSIONS

The basic goal of our research has been to determine which nutrients control primary productivity, and the dynamics of these nutrients, in Flathead Lake. Because N and P were both shown to control primary productivity, knowledge of the dynamics of both nutrients is important for understanding the relationships between the supply and demand by phytoplankton. Levels of NH_4^+ (Fig. 1) and SRP (Fig. 2) were consistently low throughout the year. In contrast, distinct peaks in NO_3^- occurred in winter and spring (Fig. 3, Table 1). However, our results show that nutrient concentrations alone do not allow for accurate determination of nutrient deficiency. Rather, one must know the rates of internal nutrient supply to define specific deficiencies, particularly in oligotrophic systems like Flathead Lake, where N and P pools are low and in a steady-state. In such systems, an experimental approach, employing nutrient enrichment bioassays and internal regeneration measurements, must be used to delineate the nutrients most important to algal growth. Of the bioassay experiments we used, time-course measurements of $^{14}CO_2$ uptake, chl a, particulate N and particulate P gave the most consistent and, we feel, the most accurate representation of nutrient deficiencies in Flathead Lake. These data are complimented by regeneration experiment results, which show that N:P regeneration rates are only slightly above the Redfield

ratio, indicating a physiological balance in the supply of nutrients.

The main conclusion we draw from our experiments in Flathead Lake is that both N and P are important regulators of primary productivity. The only time a single nutrient addition significantly altered algal growth was in February when N enrichment alone stimulated primary productivity. These results indicate that both N and P must usually be added simultaneously to increase productivity, inferring that decreased input of either nutrient may reduce productivity in the system. However, it was shown that P fertilization increased rates of N flux (Chapter 9), and that specific size classes could be stimulated by N addition alone (Chapter 3). Therefore, control of just one nutrient may have unforeseen consequences, particularly with respect to community structure.

Evidence presented in Chapter 6 shows that N and P uptake and regeneration were approximately balanced in Flathead Lake. Therefore, it is necessary to examine the coupling between uptake and regeneration in order to understand the mechanisms by which phytoplankton growth is influenced by nutrients, and how adding or removing nutrients affects Flathead Lake phytoplankton production.

We found that rates of N and P uptake and regeneration were low during winter, that rates of NO_3^-, NH_4^+ and CO_2 uptake were dependent upon light (but PO_4^{3-} uptake was not), and that organisms able to pass through a 3 μm filter

accounted for the largest portion of N and P uptake and regeneration. Furthermore, it appears that N and P dynamics were highly independent, with increased levels of PO_4^{3-} increasing NH_4^+ and NO_3^- fluxes.

Long-term changes in the planktonic organisms responsible for nutrient regeneration (organisms < 3 μm) may change nutrient dynamics and thus primary productivity. Therefore, "top down" perturbations which change zooplankton biomass (ie. the population increase of _Mysis_ in Flathead Lake), may not affect primary production directly, but may indirectly change the makeup of the microflagellate community which grazes upon bacteria and algal picoplankton < 3 μm in size (the "microbial loop"). Because little is known about the microbial loop in Flathead Lake, and our data show that this part of the community supplies most of the nutrients to phytoplankton, we suggest that further Flathead Lake nutrient studies center on the small organisms (bacteria, algal picoplankton, and microflagellates) of the planktonic community.

Although many questions concerning nutrient dynamics, and how they relate to primary productivity in Flathead Lake, remain unanswered, some important discoveries were made in this study. Methods for examining nutrient deficiencies and dynamics were developed. Using these methods, baseline data on were established which increased understanding of the relationship between nutrients and primary productivity in Flathead Lake. The fact that both N

218

and P dynamics must be considered when discussing nutrient deficiencies in Flathead Lake is perhaps one of the most important aspects of this data set.

Table 1. Physical and biological measurements from 5 m water in Flathead Lake

date	Particulates			Molar ratios of particulates						
	μM N	μM C	μM P	C/N	N/P	C/P	μg/l chl	μM NO_3^-	μM NH_4^+	μM PO_4^{3-}
6-16-87							0.511		0.430	0.0319
6-22-87							0.633		0.514	0.0213
6-27-87							0.205	0.056	0.514	0.0213
7-9-87	1.92	26.3	0.076	13.7	25.3	347	0.205	0.044	0.570	0.0106
7-9-87			0.064					0.044	0.570	0.0106
7-11-87	1.74	31.2	0.059	17.9	29.5	528	0.175	0.090	0.570	0.0319
7-11-87			0.052					0.090	0.570	0.0319
7-15-87	2.06	39.0	0.083	18.9	24.8	469	0.389		0.655	0.0642
7-20-87	1.92	26.3	0.073	13.7	26.3	361	0.511		0.655	0.0642
7-30-87			0.064				0.267	0.044	0.796	0.0106
8-6-87am	2.01	23.8	0.090	11.8	22.3	264	0.206	0.112	0.514	0.0429
8-6-87pm	1.04	18.8	0.052	18.1	20.0	362	0.175	0.078	0.430	0.0213
8-10-87	1.13	14.8	0.048	13.1	23.5	309	0.145	0.033		0.0535
8-19-87			0.048				0.175	0.044		
9-3-87	2.09	23.4	0.083	11.2	25.2	282	0.206	0.090	0.683	0.0213
9-8-87	2.22	22.6	0.062	10.2	35.8	364	0.114	0.078	0.880	
9-14-87	1.78	26.9	0.064	15.1	27.8	420	0.206	0.033	0.457	0.0319
9-17-87	2.13	25.7	0.080	12.1	26.6	321	0.328	0.045	0.259	0.0106
9-18-87	2.43						0.267		0.936	
9-22-87	1.41	16.5	0.077	11.7	18.3	214	0.113	0.078	0.344	0.0106
10-26-87	1.55	17.5	0.070	11.3	22.1	250	0.328	0.055	0.852	0.0213
10-28-87	2.2	25.5	0.101	11.6	21.8	252	0.603	0.100		0.0106
10-30-87	1.910	21.3	0.063	11.1	30.3	338	0.48			0.0213
11-3-87	1.710	21.9	0.080	12.8	21.4	273	0.541		0.457	0.0319
11-5-87			0.072				0.42	0.100	0.907	0.0319
11-6-87	2.43	31.3	0.074	12.9	32.8	423	0.481	0.056	0.486	0.0213
11-9-87			0.071				0.42			
2-3-88	2.19	21.0	0.100	9.6	21.9	210	0.97	3.257	0.826	0.0159
2-5-88	2.05	17.0	0.129	8.3	15.9	132	1.092	3.079		0.0159
2-10-88	2.55	20.7	0.152	8.1	16.8	136	1.092	3.729	1.359	0.0110

Table 1 continued

date	Particulates			Molar ratios of particulates						
	$\mu M\ N$	$\mu M\ C$	$\mu M\ P$	C/N	N/P	C/P	$\mu g/l$ chl	$\mu M\ NO_3^-$	$\mu M\ NH_4^+$	$\mu M\ PO_4^{3-}$
2-11-88	2.07	16.6	0.128	8.0	16.2	129	1.153	2.436	0.240	0.0159
2-12-88	1.67	16.2	0.136	9.7	12.3	119	1.03	2.979	0.346	0.0110
5-2-88	3.24	49.5	0.189	15.3	17.1	262	0.601	0.857	0.880	0.0110
5-4-88	2.3	42.0	0.135	18.3	17.0	311	0.697	0.329	0.720	0.0060
5-5-88	2.83	51.9	0.109	18.3	26.0	476	0.615	1.157	0.560	0.0110
5-6-88	3.12	47.6	0.101	15.2	30.9	471	0.408	1.071	0.560	0.0110
5-7-88	2.48	43.7	0.086	17.6	28.8	508	0.326	0.529	0.506	0.0110
5-11-88	2.09	43.5	0.106	20.8	19.7	410	0.298	0.529	0.826	0.0110

Figure 1. Epilimnetic NH_4^+ concentrations for 1987-1988 in Flathead Lake.

Figure 2. Epilimnetic soluble reactive phosphorus concentrations (SRP) for 1987-1988 in Flathead Lake.

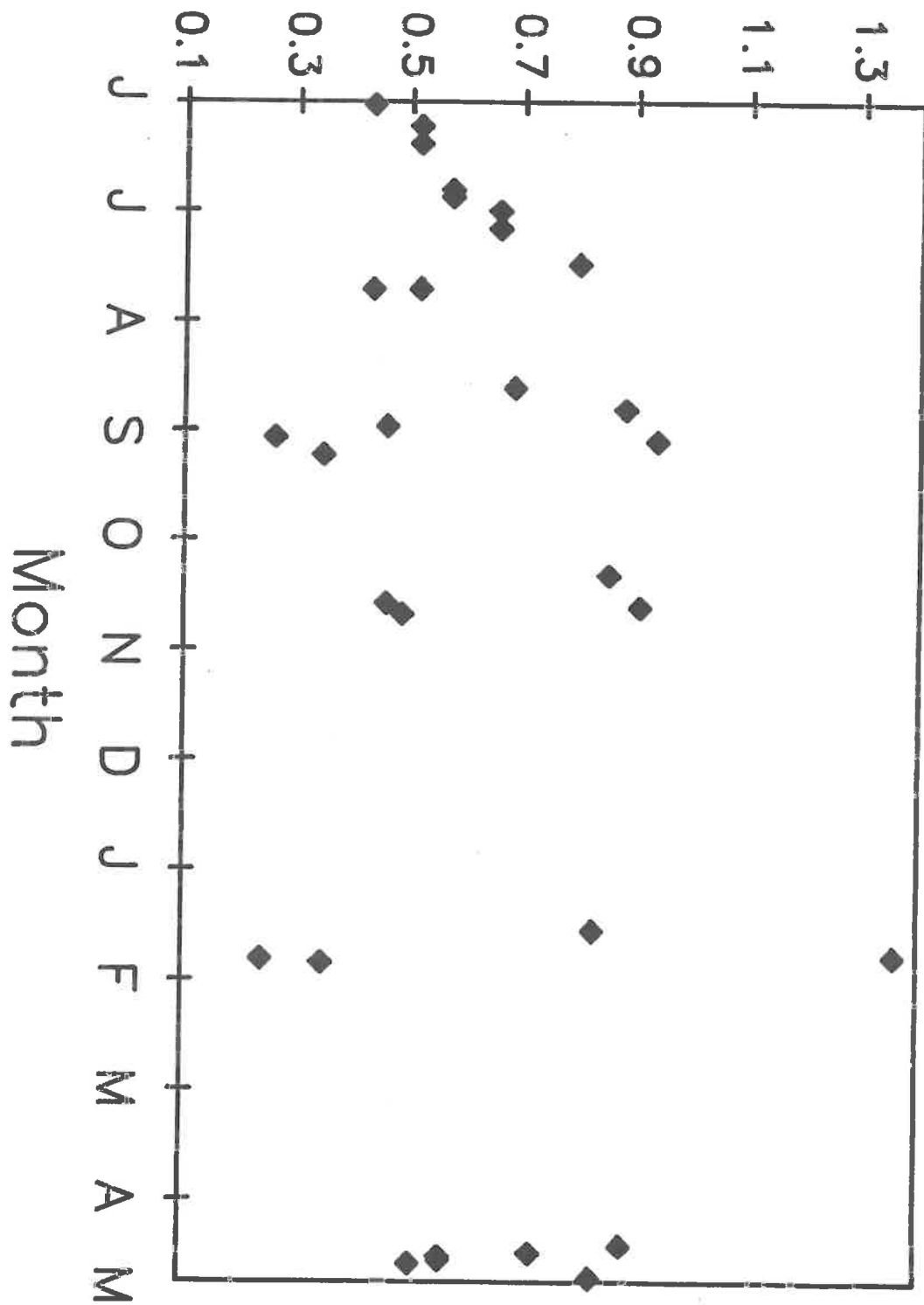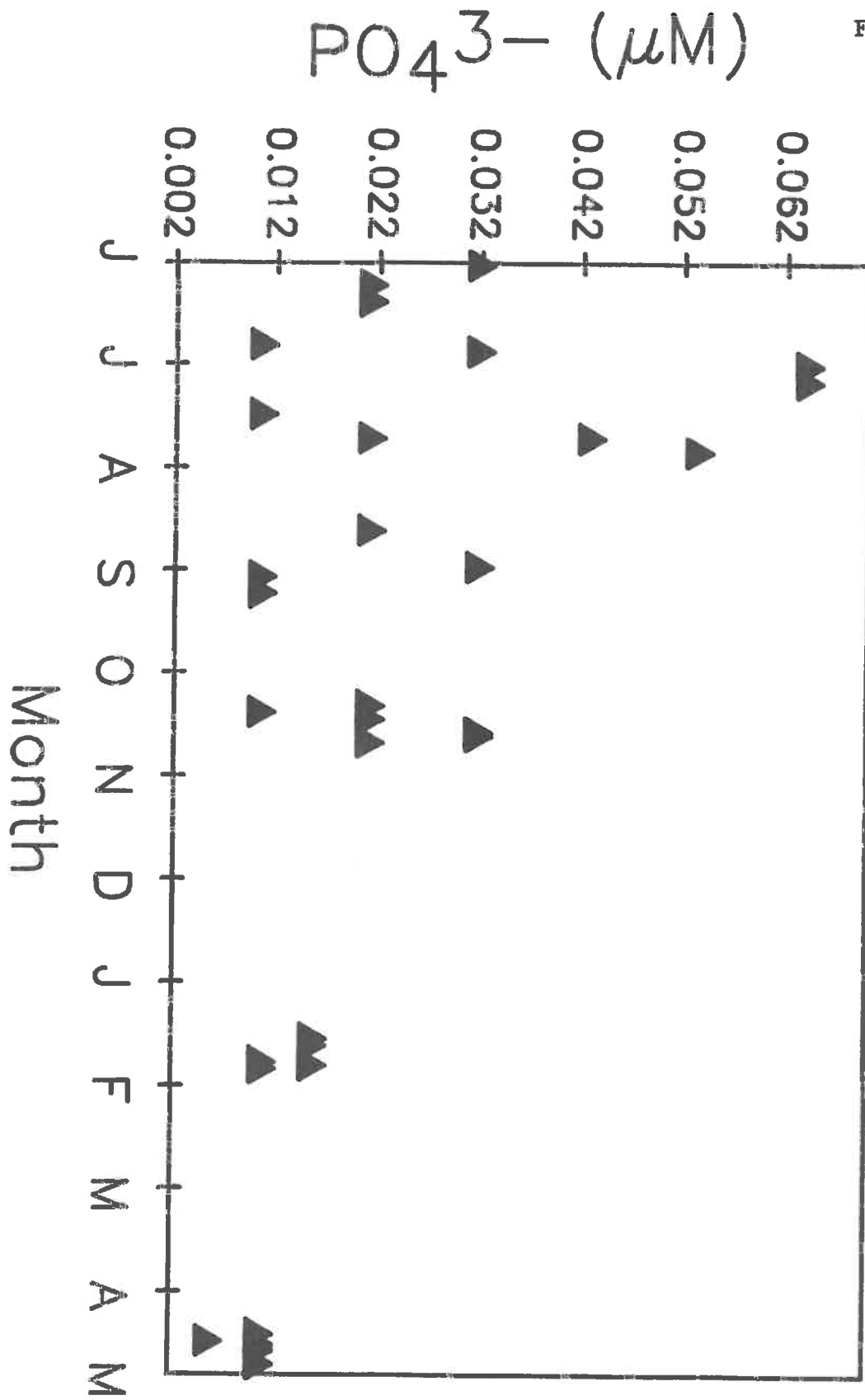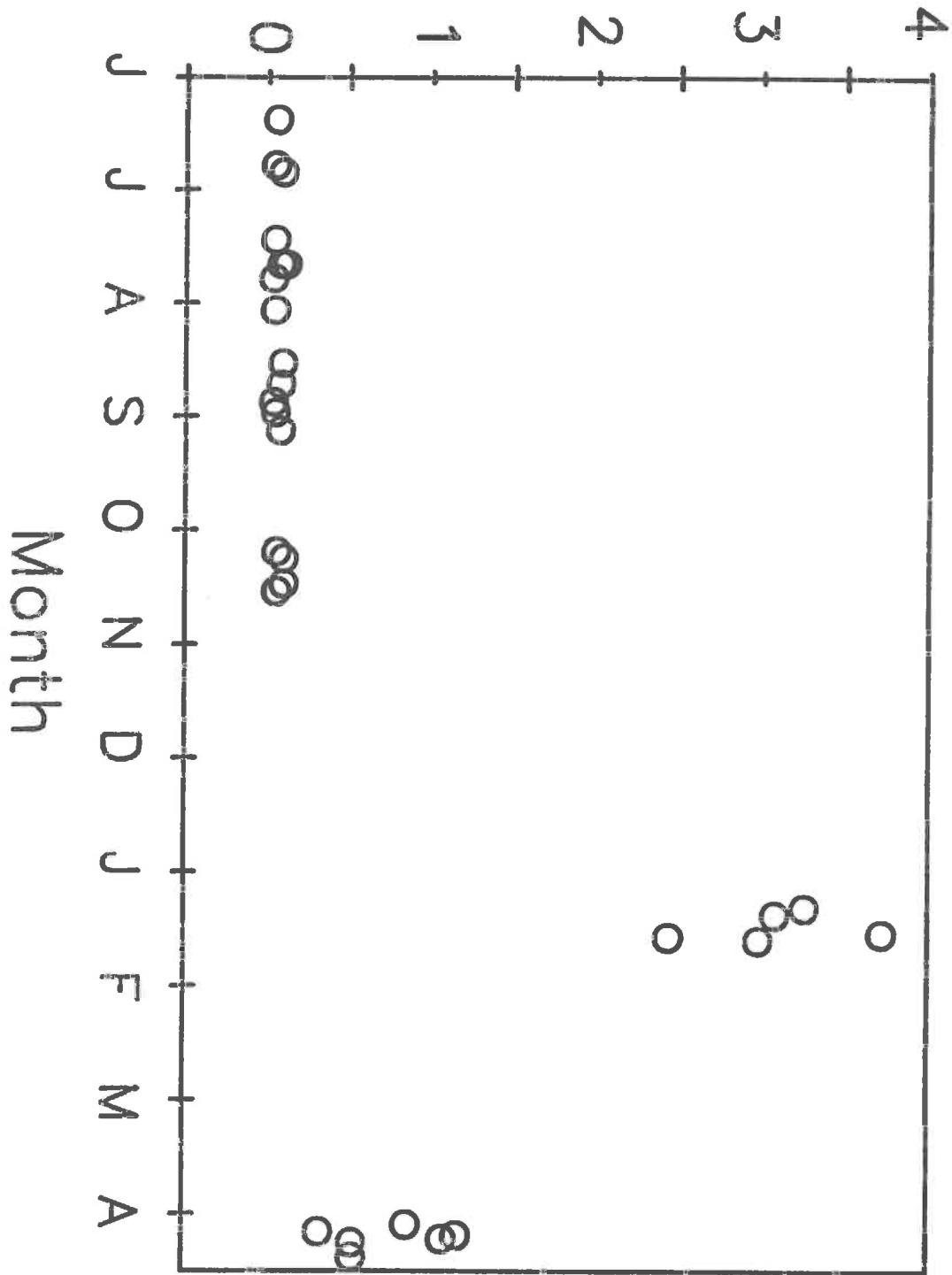Figure 3. Epilimnetic NO_3^- concentrations for 1987-1988 in Flathead Lake.

Figure 1

Figure 2

NO₃⁻ (μM) Figure 3

Appendix I

Attached are abstracts from seminars which were, or will be, presented at the following meetings:

1)   "An Assessment of N or P Limitation in a Large Oligotrophic Lake using Physiological Methods" was presented to the American Society of Limnology and Oceanography in New Orleans in February 1988.

2)   "Pelagic Uptake and Regeneration of Ammonium and Phosphate: Size Fractionation and Antibiotic Treatment" was presented to the American Society of Limnology and Oceanography in Boulder, CO in June 1988.

3)   "Simultaneous Nitrogen and Phosphorus Deficiency in Phytoplankton: Theory, Empirical Evidence and Implications for Lake Management" will be presented to the North American Lake Management Society in St. Louis in November 1988.

4)   "Influence of PO_4^{3-} on $^{15}NH_4^+$ and $^{15}NO_3^-$ Fluxes in an Oligo-trophic Lake: Results from Mesocosm Studies" will be presented as an invited paper to the American Society of Limnology and Oceanography in San Francisco in December 1988.

An Assessment of N or P Limitation in a Large Oligotrophic Lake Using Physiological Methods

Walter K. Dodds, John C. Priscu
Department of Biology, MSU, Bozeman, MT 59717

A variety of nutrient bioassays were conducted during summer stratification on Flathead Lake, Montana, to assess the importance of N and P in controlling phytoplankton productivity. Twenty liter carboys were fertilized with NH_4^+, PO_4^{3-}, NO_3^-, NO_3^- plus PO_4^{3-}, NH_4^+ plus PO_4^{3-}, or NH_4^+ plus PO_4^{3-} plus micronutrients. $^{14}CO_2$ uptake rates, chlorophyll a, and particulate N, P and C increased several fold in N plus P treatments with respect to controls ($P < 0.05$). NO_3^- plus PO_4^{3-} treatments showed less response than NH_4^+ plus PO_4^{3-}, and even less response occurred with addition of NH_4^+ or PO_4^{3-} alone ($P < 0.05$). Addition of micronutrients did not enhance the NH_4^+ plus PO_4^{3-} response. Limnocorrals (1600 liters) gave similar results to those from the 20 liter carboys, indicating that both N and P control primary productivity.

In laboratory experiments, particulate P concentration increased with 12 hours of exposure to PO_4^{3-} ($P < 0.05$), and V_{max} for PO_4^{3-} decreased with 12 hours of exposure to PO_4^{3-} ($P < 0.05$). This implies that internal pools of P were depleted, and P is limiting. However, 12 hours exposure to NH_4^+ increased V_{max} of PO_4^{3-} ($P < 0.0005$), and rate of PO_4^{3-} uptake was greater than the rate of NH_4^+ plus NO_3^- uptake at ambient concentrations, suggesting N deficiency. These physiologically based assays support results from carboy and limnocorral fertilization suggesting that both N and P regulate primary productivity.

1. Ocean Sciences Meeting

2. ASLO

3. (a) Walter Dodds
Dept. of Biol.
MSU
Bozeman, MT 59717

(b) (406) 994-4548

4. B

5. 4845 Nutrients

6. oral presentation

7. material not presented elsewhere

8. $35 enclosed check

9. C

o   (0)

31   38

Walter Dodds

Department of Biology, Montana State University

Bozeman, MT 59717

(406) 994-4548

Dodds, W. K., J. C. Priscu, Dept. of Biology, Montana State University, Bozeman, MT 59717, and B. K. Ellis, Flathead Lake Biological Station, Polson MT 59860. PELAGIC UPTAKE AND REGENERATION OF AMMONIUM AND PHOSPHATE: SIZE FRACTIONATION AND ANTIBIOTIC TREATMENT

$^{15}NH_4^+$ and $^{32}PO_4^{3-}$ were used to measure uptake and regeneration during summer stratification meso-oligotrophic Flathead Lake, Montana. The > 280 μm fraction was relatively unimportant in N or P regeneration, and only accounted for 0 - 20% of N uptake and 13-32% of total P uptake. The < 3 μm fraction accounted for 60-100% of N regeneration, 75-82% of P regeneration, 43-65% of N uptake, and 54-64% of P uptake. Cycloheximide (inhibits eukaryotic protein synthesis) caused a decrease of 56-91% in N uptake and regeneration as compared to whole lake water. Chloramphenicol (inhibits prokaryotic protein synthesis) caused a 1-5 fold increase in regeneration, and a 22-70% decrease in N uptake, suggesting eucaryotes < 280 μm are relatively important to both uptake and regeneration of N. The antibiotics had no consistent effect on P uptake or regeneration. The data show that organisms < 3 μm dominate P and N dynamics in this lake.

Walter Dodds

SIMULTANEOUS NITROGEN AND PHOSPHORUS DEFICIENCY IN PHYTOPLANKTON: THEORY, EMPIRICAL EVIDENCE, AND IMPLICATIONS FOR LAKE MANAGEMENT

Walter K. Dodds, Kirk R. Johnson, and John C. Priscu. Department of Biology, Montana State University, Bozeman, MT 59717

Published measurements have shown that some co-occurring algal species have varied abilities to take up required nutrients. Therefore, it is theoretically possible that not all species of an algal community are deficient in the same nutrient, challenging the concept of phosphorus as the universal limiting nutrient in lakes.

Simultaneous addition of nitrogen and phosphorus was necessary to stimulate total primary productivity (uptake of $^{14}CO_2$) during summer stratification in meso-oligotrophic Flathead Lake and oligotrophic Hungry Horse Reservoir (Montana, USA). Furthermore, these bioassays showed that single nutrient additions stimulated primary production of some size classes of phytoplankton even though the same nutrient did not increase total community productivity.

The above data have the following implications for lake management: i) Phosphorus may not be the only nutrient which controls primary productivity. ii) Simultaneous nutrient enrichment may be necessary to cause an overall increase in primary productivity. iii) Single nutrient additions may cause changes in phytoplankton community structure without changing total primary production.

Influence of PO_4^{3-} on $^{15}NH_4^+$ and $^{15}NO_3^-$ Fluxes in an Oligotrophic Lake: Results from Mesocosm Studies

Walter K. Dodds, John C. Priscu (Department of Biology, Montana State University, Bozeman, MT 59717 (406) 994-2360)

$^{15}NH_4Cl$ or $^{15}NO_3$ were added to control and PO_4^{3-} mesocosms filled with epilimnetic water from oligotrophic Flathead Lake (Montana). $^{14}CO_2$ uptake experiments showed that simultaneous addition of N and P was necessary to increase in primary production. Incorporation of ^{15}N into phytoplankton and bacteria (particulates between 280 and 0.7 µm size), and crustaceous zooplankton was enhanced by P, but P caused no increase in efficiency of uptake by phytoplankton and bacteria of either $^{15}NO_3^-$ or $^{15}NH_4^+$ (biomass specific rates were not enhanced by P).

Isotope dilution occurred in both $^{15}NH_4^+$ and $^{15}NO_3^-$ experiments, implying regeneration of both nutrients. Acetylene reduction of Anabaena, (a minor component of the phytoplankton community) was stimulated by P addition to a greater degree than N + P addition ($P < 0.05$). The results illustrate a strong coupling between N dynamics and levels of phosphorus in this system.