Arch. Hydrobiol.	128	4	467–481	Stuttgart, Oktober 1993

Nutrient dilution and removal bioassays to estimate phytoplankton response to nutrient control

By W. K. Dodds, E. A. Strauss and R. Lehmann[1]

Kansas State University

With 6 figures and 3 tables in the text

Abstract

Nutrient addition and dilution bioassays were performed in a large eutrophic reservoir. Experiments were conducted in situ in 10 L carboys and 1256 L mesocosms. By day 4 in carboy experiments, addition of N alone stimulated chlorophyll-a by 403 %, simultaneous N and P addition by 929 %, and secondary sewage effluent addition by 188 %. P added alone had little stimulatory effect. Dilution of 15 % per day with 1 μm filtered lake water or reverse osmosis water had an insignificant effect on productivity, algal biovolume, and chlorophyll-a concentrations compared to controls. The results from the mesocosms were consistent with the carboy experiments; addition of N and N + P stimulated chlorophyll-a, and 12 % dilution per day with filtered lake water or reverse osmosis water had little effect. Increasing or decreasing zooplankton numbers had only small effects on productivity and chlorophyll-a. Nutrient addition and dilution bioassays may provide valuable information when evaluating measures to reduce eutrophication in lakes.

Introduction

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

Nutrient enrichment bioassays have been used extensively in oligotrophic lakes to determine nutrient limitation (e.g., Hecky & Kilham, 1988; Dodds & Priscu, 1990; Elser et al., 1990), but such experiments can only be used to predict response to nutrient additions. Enrichment experiments may be less valuable in more eutrophic systems when information regarding the effects of

[1] Address of the authors: Division of Biology, Kansas State University, Manhattan, KS USA 66506.

0003-9136/93/0128-0467 $ 3.75

nutrient removals is needed. PAERL & BOWLES (1987) developed a technique to dilute nutrients with de-ionized water and then replenish various nutrients to estimate the effects of nutrient control on the eutrophic Nuese River. This technique was recently modified by using chemicals to remove ammonium (zeolite) and phosphate (alum) from lake water, and adding the chemically treated water to natural algal assemblages (DODDS & RANDEL, 1992). The modified technique had promising results when applied to a hyper-eutrophic farm pond, except for a strong effect from containers isolating algae from sediment nutrient loading.

The purpose of this study was to explore and refine nutrient dilution and removal bioassay techniques and compare them to nutrient addition bioassays. The experiments were conducted in microcosms (10 L carboys) and mesocosms (1256 L containers) that included the natural sediment, and an attempt was made to estimate food web effects (top down control) in the mesocosm experiments.

Materials and methods

Experiments were conducted in Milford Lake (Kansas) during late July and early August 1991. Milford Lake is a large (65.5 km^2, 36.6 km long, max. width 4 km), shallow (mean depth 7.8 m), mildly eutrophic (summer epilimnetic chlorophyll-a in 1974 $\approx$ 19 $\mu g L^{-1}$, drainage basin 64,465 km^2, turbidity 9 nephelometric turbidity units) dimictic prairie reservoir (EMANUEL, 1983; U.S. Environmental Protection Agency, 1974). The lake is used for drinking water and recreation, and occasionally is dominated by nuisance blooms of the cyanobacterium *Anabaena* (EMANUEL, 1983).

Nutrient dilution bioassays were conducted in 10 L polyethylene carboys using water collected from the center of the lake, 3 km upstream from the dam. Water was collected from the depth of maximum photosynthesis (2 m, unpublished data) with a displacement sampler (DODDS & PRISCU, 1988), with care taken not to expose the water to full surface irradiance. All treatments (Table 1) were triplicated.

Fifteen percent dilution was selected for several reasons. 1) Dilution effects at 10 % d^{-1} were difficult to detect in earlier experiments on a nearby eutrophic pond (DODDS & RANDEL, 1992). 2) Very high dilution rates would be unrealistic given the effectiveness of most nutrient management schemes. 3) After five days of dilution at 15 % per day, only 44 % of the original water and biomass should be present in the carboys.

Lake water was filtered by gravity through 1 μm filter bags (DODDS & RANDEL, 1992). Following initial treatment, carboys were incubated at 1.5 m in the lake because the carboys attenuated some light, and this depth of incubation approximated the light at the depth of collection. One liter was removed from each carboy daily for analysis. Oxygen production was determined by the light/dark method, concentrations by Winkler titration (APHA, 1989). A 100 mL subsample was filtered through a Whatman GF/F glass fiber filter (0.7 μm retention). The filter was analyzed for phaeophytin corrected chlorophyll-a by the three wavelength spectrophotometric method for high concentrations or fluoremetrically for lower concentrations (APHA, 1989). The filtrate was analyzed colorimetrically for NH_4^+ (sodium hypochlorite), $NO_3^- + NO_2^-$ (cadmium reduction) and soluble reactive phosphorus (phospho-molybdate, APHA, 1989). By day 4 (the last day), one sewage-N and two sewage-P carboys were lost in rough weather.

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

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

Mesocosms were isolated on 5 August 1991 in the protected bay formed by the inlet of Rush Creek, a small ephemeral creek 3 km up from the dam. These mesocosms were 1 m diameter, 2 m deep cylinders, made of semitransparent, layered, reinforced low-density polyethylene sheeting (Canvex II, Raven Industries, Sioux Falls SD), sealed with Griff-tape (Brock White Inc., St. Paul, MN), and held rigid with 1 m diameter hoops of 13 mm PVC pipe at the top and bottom. The mesocosms were placed in 1.6 m of water with bottoms inserted 0.2 m into the sediments, anchored with galvanized wire stakes, and the tops suspended from a floating frame 0.2 m above the water surface.

Mesocosm treatments were not replicated and consisted of: 1) control, 2) 160 μmol $NH_4Cl\,L^{-1}$ final concentration added on days 0 and 3, 3) 10 μmol $NaH_2PO_4\,L^{-1}$ final concentration added on days 0 and 3, 4) 160 μmol $NH_4Cl\,L^{-1}$ and 10 μmol $NaH_2PO_4\,L^{-1}$ final concentration added on days 0 and 3, 5) 1 μm filtered lake water dilution (150 L per day), 6) reverse osmosis water dilution (150 L per day), 7) −zooplankton (repeated hauls, 100 μm net), and 8) +zooplankton (about 2 times natural concentrations). Mesocosms were sampled daily for photosynthesis, chlorophyll-a, and nutrient concentrations (NH_4^+, PO_4^{3-}, and NO_3^-). Strips of Canvex II were added to the mesocosms at day 0 and removed on day 8 to analyze for attached chlorophyll-a. Core samples from the sediment were also removed and analyzed for chlorophyll upon termination of the experiment.

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

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

Multiple comparisons of means were calculated for the carboy experiments with Analysis of Variance followed by SCHEFFE's pairwise comparison method. Results from the 1256 L enclosure experiments were not analyzed statistically because the experimental design was pseudo-replicated. Triplicate samples were taken from each mesocosm and analyzed, results from these experiments are reported as means of the three samples with standard deviation to indicate the amount of variance associated with measurements made within a single treatment.

Results

Carboys

Ammonium added to the +N treatments disappeared less rapidly than in the N+P treatments ($P < 0.05$, day 5 data), suggesting that the amount of phosphorus present controlled the rate of ammonium utilization per unit volume (Fig. 1 A). With sewage addition, ammonium levels were slightly elevated initially, but subsequently returned to levels found in control carboys and lake water. The sewage-N treatments never had significantly more ammonium than control, and the sewage-P treatments initially had slightly elevated ammonium concentrations that fell to control levels by day 4 (Fig. 1 B). There were no significant trends when the amounts of ammonium in the control, lake, filtered lake water dilution, or reverse osmosis dilution treatments were compared (Fig. 1 C).

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

Table 2. Nitrate concentration and photosynthetic rates for carboys 5 days after initiation of experiments. Mean values are presented, errors one standard deviation, (M) standard deviation missing, replicates lost. BLD = below detection (0.5 μM).

Treatment	NO_3^- (μM)	Photosynthesis (mg O_2 L^{-1} h^{-1})
control	0.05 (0.08)	0.23 (0.08)
+N	10.40 (1.07)	0.37 (0.25)
+P	0.16 (0.47)	0.09 (0.09)
+N+P	16.60 (9.62)	1.90 (0.38)
sewage	BLD	1.90 (0.38)
sewage-P	2.20 (3.45)	0.28 (0.07)
sewage-N	BLD	0.39 (M)
filtered lake	BLD	0.22 (0.11)
reverse osmosis	BLD	0.09 (0.08)
lake	3.26 (0.34)	0.44 (0.03)

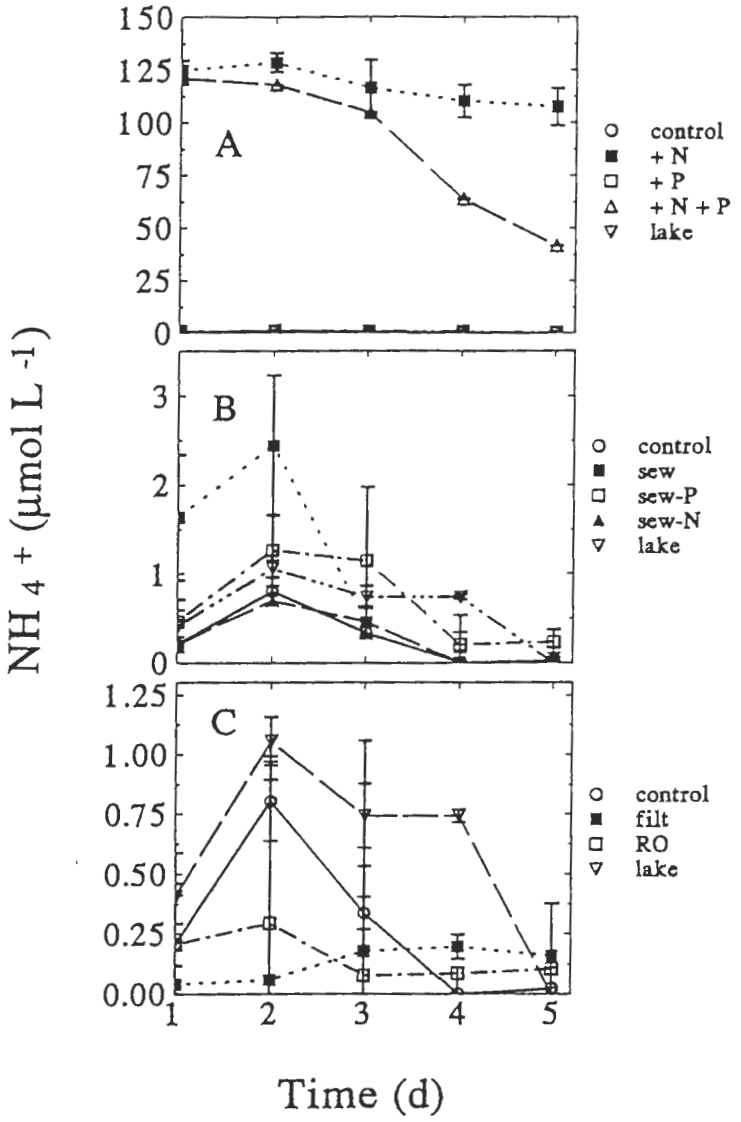

Fig. 1. Ammonium concentrations in carboy experiments with nutrient addition (A), sewage addition (B), and nutrient dilution (C). Error bars = 1 std dev.

Soluble reactive phosphorus (SRP) disappeared more rapidly in the presence of ammonium than when only phosphate was added ($P < 0.05$, day 5 data) suggesting P utilization was controlled by the amount of nitrogen and the disappearance was related to biological utilization, not abiotic absorption. SRP levels in N + P treatments were not significantly different from those in the lake by day 5 (Fig. 2 A). This suggests that inorganic nitrogen had to be present for efficient utilization of phosphate.

The sewage-P addition treatments ended with SRP levels similar to those seen in control carboys and the lake. Sewage-N and sewage treatments both exhibited decreases in SRP throughout the experiment (Fig. 2 B). The dilution

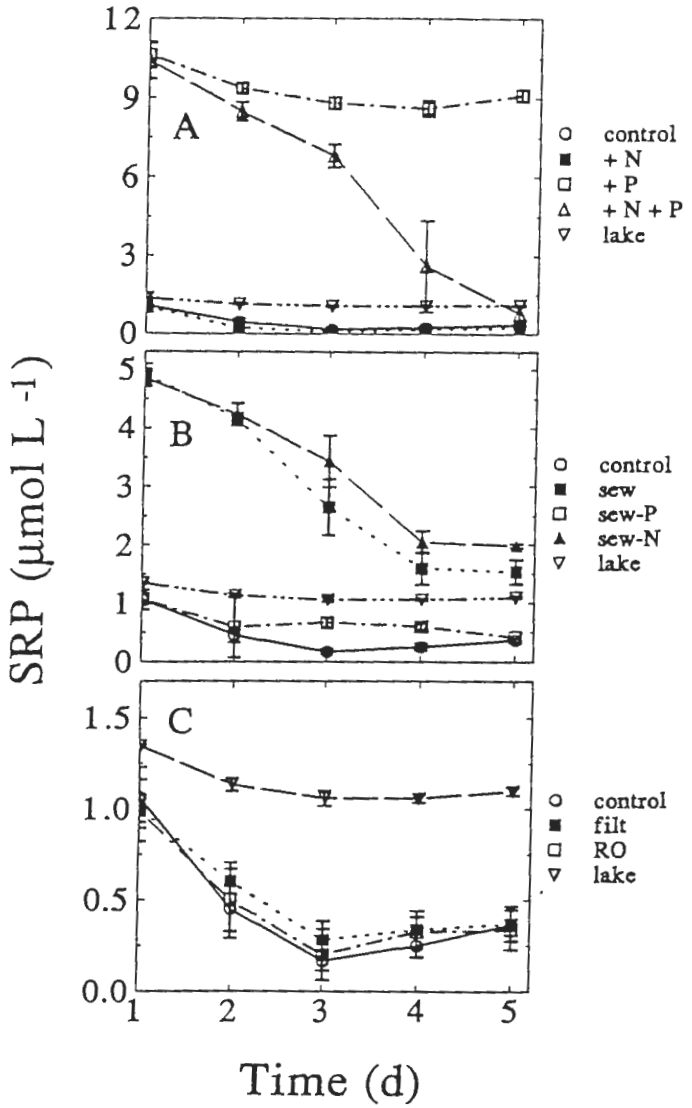

Fig. 2. Soluble reactive phosphorus concentrations in carboy experiments with nutrient addition (A), sewage addition (B), and nutrient dilution (C). Error bars = 1 std dev.

treatments (filtered lake water and reverse osmosis water) had SRP levels very similar to those in the control carboy (Fig. 2 C), and significantly less than in the lake ($P < 0.05$, day 5 data).

Phaeophytin corrected chlorophyll-a was stimulated slightly with the addition of N, strongly with addition of N + P (Fig. 3 A), and early in the experiment by addition of sewage and sewage-N (Fig. 3 B), as compared to controls. Chlorophyll-a did not vary significantly with comparison among lake water, sewage-P, filtered lake water dilutions, reverse osmosis dilutions, or controls (Figs. 3 B, C). Phytoplankton photosynthetic rates only increased significantly over rates in the lake and control mesocosms with concurrent N and P addi-

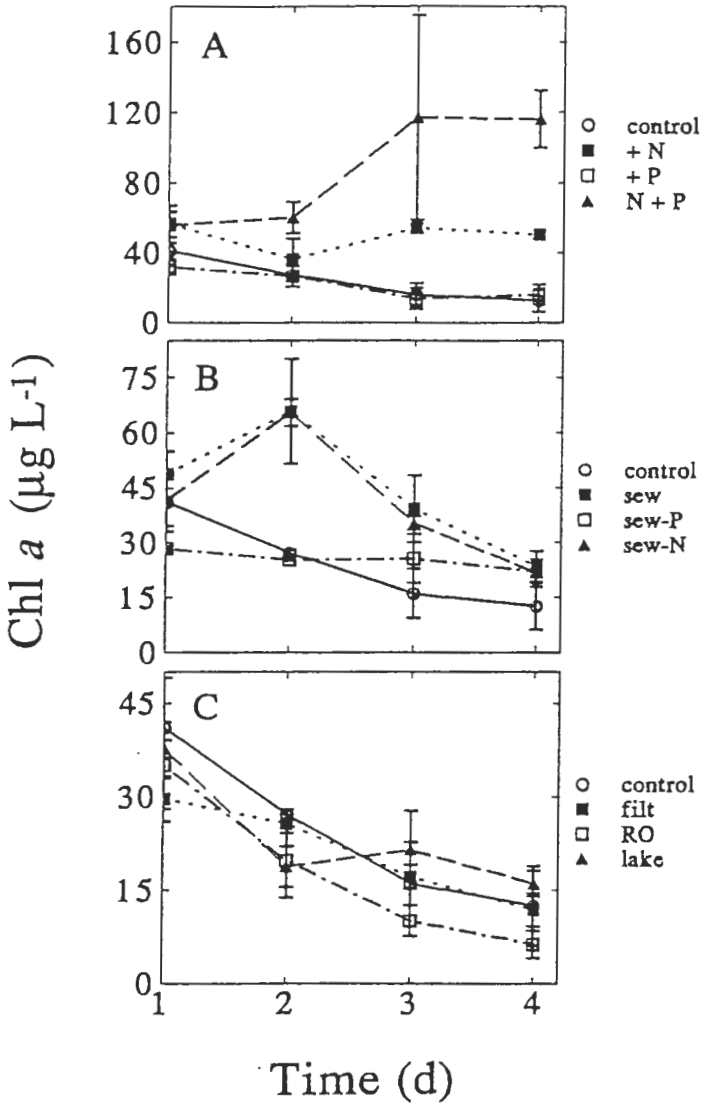

Fig. 3. Chlorophyll-a concentrations in carboy experiments with nutrient addition (A), sewage addition (B), and nutrient dilution (C). Error bars = 1 std dev.

tion after five days (Table 2). No other trends were evident. Acetylene reduction data showed no significant differences ($P > 0.05$) between treatments suggesting P addition alone did not stimulate heterocystous cyanobacteria (data not shown).

Algal counts revealed patterns in total biovolume (Fig. 4) very similar to those observed for chlorophyll-a, with maximum biovolume with N+P addition (significantly greater than all other treatments except +N) and some stimulation with addition of N alone (N greater than RO dilution $P < 0.05$). The increases in biovolume in the N+P treatment were related to significant increases in the biovolume of green algae and cyanobacteria (primarily *Anabeana spiroides*). There were no significant trends in diatom biomass.

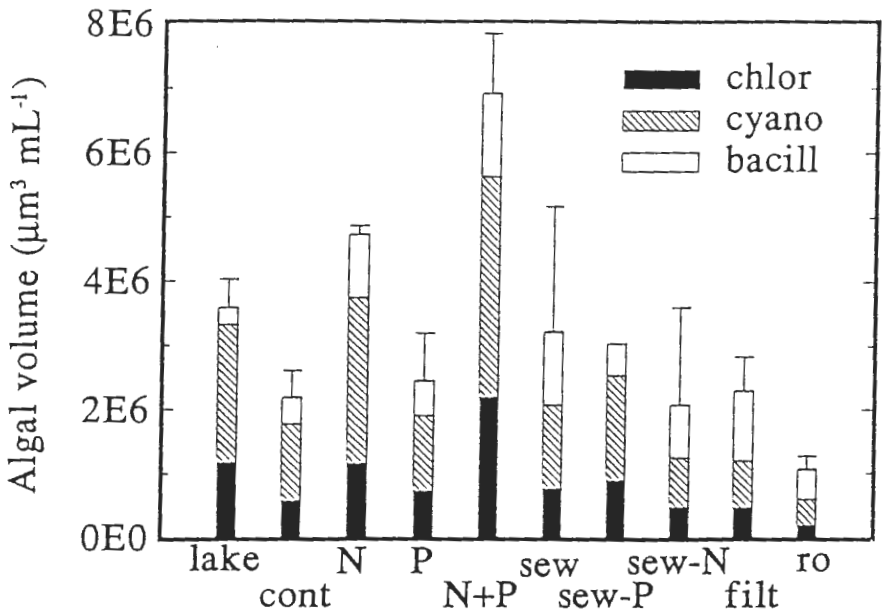

Fig. 4. Absolute abundances of phytoplankton after five days in the carboy experiments. Error bars = 1 std dev and represent the variance in total abundance. chlor = chlorophytes, cyano = cyanobacteria, bacill = bacillariophytes (diatoms).

Mesocosms

Ammonium concentrations in the mesocosms decreased rapidly in the +N and N+P treatments. By day 1, more NH_4^+ was lost in the +N than in the N+P mesocosm. Ammonium reached levels seen in the lake and control treatments by day three (Fig. 5 A). At this point ammonium was added again. No clear differences between ammonium concentrations in the filtered lake water, reverse osmosis water, +zooplankton, −zooplankton, and control treatments were observed compared to the values from the lake (Figs. 5 B, C).

SRP concentrations dropped rapidly in the +P and N+P treatments (Fig. 6 A), analogous to the ammonium concentration drop noted for N addition treatments. On day 4 there were slight differences in SRP, with reverse osmosis and +zooplankton treatments exhibiting slightly lower SRP concentrations than in the control. The lake had slightly higher SRP concentrations than control by day 4 (Figs. 6 B, C).

Chlorophyll-a increased markedly in the +N and N+P treatments (Table 3). On day four, there was less chlorophyll in the lake, and the dilution treatments (filtered lake and reverse osmosis) than in the control (Table 3). Both zooplankton treatments exhibited slightly less chlorophyll than the control, but more than in the lake (Table 3).

By day 3, photosynthesis was only stimulated in the N+P treatments, although not strongly. Nitrogenase activity appeared to decrease with nutrient addition. Dilution or zooplankton manipulation had little effect (Table 3). The amount of benthic chlorophyll-a was less with addition of reverse osmosis

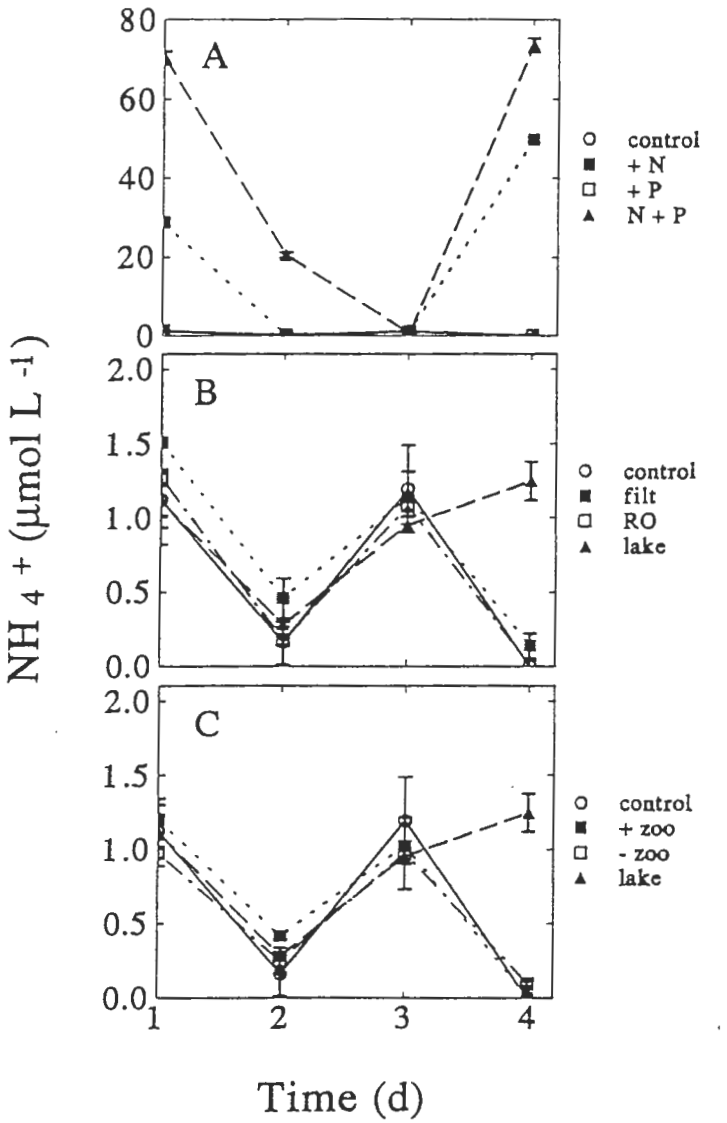

Fig. 5. Ammonium concentrations in mesocosm experiments with nutrient addition (A), dilution (B) and zooplankton manipulation (C). Each value = mean of 3 subsamples taken from 1 treatment, error bars = 1 std dev.

water, but varied little otherwise. The amount of chlorophyll-a on the container walls was markedly higher with N and N + P additions, and lower when zooplankton were added. The increase in chlorophyll-a on the container sides when ammonium was added parallels the increase observed in plankton chlorophyll-a when ammonium was added. Zooplankton numbers were variable with the lowest recorded values in the −zooplankton treatments, and the highest values with N addition (Table 3). The +zooplankton mesocosm ended with approximately equal numbers of copepods and cladocerans, the control and −zooplankton treatments were dominated by copepods.

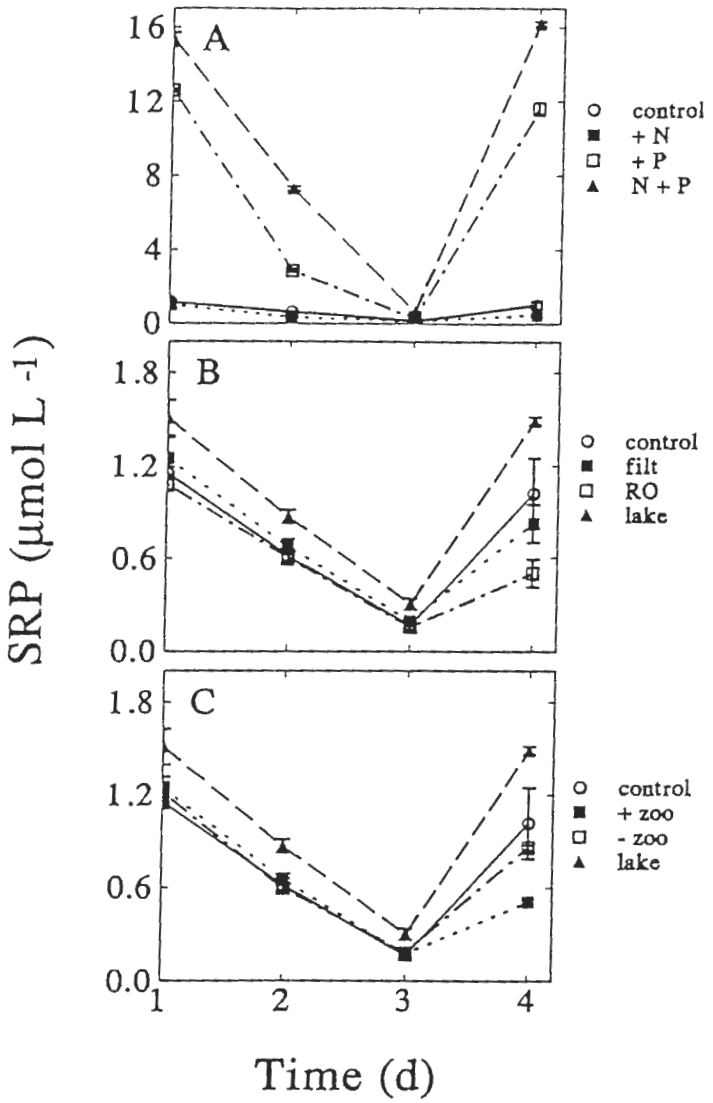

Fig. 6. Soluble reactive phosphorus concentrations in mesocosm experiments with nutrient addition (A), dilution (B) and zooplankton manipulation (C). Each value = mean of 3 subsamples taken from 1 treatment, error bars = 1 std dev.

Discussion

Both nitrogen and phosphorus appeared to limit productivity in the carboy experiments. Ammonium disappeared more rapidly in the presence of phosphate, and phosphate disappeared more rapidly in the presence of ammonium. This is most likely related to the fact that biomass increased the most with concurrent N + P addition. This suggests that addition of N and P forced P and N limitation respectively. Addition of N increased chlorophyll-a, but simultaneous N and P addition stimulated chlorophyll-a, algal biovolume and photosynthesis. These experiments of several days duration did not suggest that the phytoplankton community could make up for N deficiency by increased rates of N_2 fixation. Neither rates of acetylene reduction nor counts of

Table 3. Acetylene reduction, sediment and container wall chlorophyll *a*, and zooplankton numbers from day 8 and planktonic chl-a from day 5 of the mesocosm experiments. std dev in parentheses, represents mean of 3 determinations. The category of zooplankton includes ostracods, cladocerans, copepods and rotifers.

Treatment	Acetylene reduction (μmol L^{-1} h^{-1})	Planktonic chl-a (μg L^{-1})	Sediment chl-a (mg m^{-2})	Container wall chl-a (mg m^{-2})	Total zooplankton (# L^{-1})
Control	64 (18)	46.64 (2.28)	33 (3)	7.9 (6.7)	721 (37)
+N	38 (3)	94.57 (6.47)	36 (9)	115 (20.9)	3056 (1359)
+P	44 (6)	47.83 (6.18)	36 (12)	11.0 (8.4)	1339 (594)
N+P	17 (3)	96.86 (3.83)	31 (9)	126 (38.7)	1339 (607)
Filt.	35 (0)	16.63 (1.07)	13 (4)	6.2 (6.8)	1168 (560)
RO	83 (16)	27.64 (3.32)	34 (6)	7.1 (4.0)	1065 (469)
+Zoo	61 (11)	30.61 (5.47)	34 (11)	0 (0.75)	1820 (810)
−Zoo	93 (13)	34.26 (6.28)	26 (3)	4.9 (3.4)	275 (151)

heterocystous cyanobacteria increased with P addition over a period of four days. It is possible that a longer term experiment would have yielded different results, but container effects become greater over time.

Similar results are common from oligotrophic lakes (Suttle & Harrison, 1988; Dodds & Priscu, 1990; Elser et al., 1990) but fewer experiments have been done on eutrophic lakes. The theoretical basis behind such co-limitation (simultaneous N and P limitation) and implications for lake management have been discussed previously (Dodds et al., 1989). Unequal nutrient requirements of algae, heterogeneity of nutrients (regenerated patches and depletion zones near active cells), heterogeneity of algal populations, and selective grazing may all invalidate an equilibrium based approach that predicts only one nutrient can limit phytoplankton communities.

Data from the mesocosms (1256 L enclosures) suggested that nitrogen primarily, and phosphorus secondarily, limited productivity of phytoplankton and periphyton. These experiments differ from the carboy experiments in two key ways: 1) water for the mesocosms was taken from a sheltered bay while water for the carboys was taken from the lake center, and 2) the mesocosms included sediments and were exposed to the atmosphere whereas the carboys were not. The difference between the carboy and mesocosm results may be related to including sediments. The water in the mesocosms was probably influenced by the sediments more heavily before and during the experiment than water in the center of the lake. The sheltered bay is only 4 m deep whereas the lake center is 16 m deep and weakly stratified. The sediments could recycle phosphorus, or remove nitrogen via denitrification, decreasing P limitation and increasing N limitation.

The results from sewage addition carboys are consistent with those for the inorganic nutrient additions if the fact that the sewage was relatively high in

nitrate is considered. In the sewage and sewage-N additions there was stimulation of chlorophyll-a followed by a drop to initial levels; with sewage-P, there was no stimulation. Ambient levels of SRP in the sewage and sewage-N treatments were four fold higher than those in the sewage-P additions. In all treatments, the nitrate in sewage increased the total inorganic nitrogen significantly, and the ammonium concentrations were low at the beginning and only doubled at most. Sewage did not stimulate productivity to the same degree as the N + P additions. This is probably related to the fact that the absolute levels of inorganic nitrogen and SRP were lower with sewage addition.

Nitrate increased in the carboy experiments where ammonium was added which suggests active ammonium oxidization by the planktonic bacterial community. In the sewage additions, the only treatment with significant levels of nitrate remaining at the end of the experiment was the sewage-P treatment, even though this treatment had lower levels of nitrate initially (Table 1). This is consistent with the hypothesis of P limitation in these carboys (although it does not preclude simultaneous N and P limitation). In the case where sewage without high levels of SRP was added, there was insufficient P available for the plankton to utilize the nitrate. When the SRP remained (in the sewage and sewage-N treatments), it was consumed rapidly (Fig. 2 B) along with the nitrate.

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

It is surprising that even with 15 % dilution per day over four days in the carboy experiments, (56 % dilution over all), there was little detectable effect upon the photosynthesis, NH_4^+, or SRP concentrations. Dilution did, however, cause a minor decrease in total algal biovolume and chlorophyll-a. Investigators of zooplankton grazing have suggested that dilution should increase net algal growth by decreasing grazing (Evans & Daranjape, 1992) so in our experiments, a decrease in available nutrients for phytoplankton growth could possibly have been offset by somewhat lower grazing rates.

Our dilution results show that inorganic nutrient levels and productivity are not necessarily closely related, because even with 52 % overall dilution of total nutrients, there was not a large effect on ammonium or SRP concentrations. The lack of a relationship between inorganic nutrient concentrations and process rates in aquatic systems has been known for some time (Brylinsky & Mann, 1975). Obviously, using dissolved inorganic nutrient concentrations alone to estimate nutrient deficiency in phytoplankton is questionable (Dodds, 1993).

Bioassays similar to those reported here may represent the best way to determine the effects of nutrient addition and nutrient removal on phytoplank-

ton populations. It has been demonstrated that short-term indicators of nutrient deficiency can give ambiguous and contradictory results (HECKY & KILHAM, 1988; DODDS & PRISCU, 1990; DODDS & PRISCU, 1991). In the experiments presented here, there was some agreement (that nitrogen was important and dilution not important) between the mesocosm and the carboy experiments.

The results suggest that during the week long experiments the algae in Milford Reservoir are nutrient limited, because addition of nutrients increased photosynthetic rates and algal biomass. This suggests that further increases in nutrient loading would be detrimental even though chlorophyll-a levels are already about $20\,\mu g\,L^{-1}$. Dilution experiments yielded a small effect, suggesting lack of a short-term response to nutrient control. All these results should be viewed with caution when extrapolating from several days to months. Even though results from bioassays of several days are more relevant to management than those from short-term bioassays (hours), responses over entire seasons could be significantly different than ours.

Techniques such as those presented here may provide useful information on nutrient limitation. This is especially true for lakes where the limiting nutrients have not been established. Although a significant amount of work is involved in conducting an in situ experiment of this scale for one week, none of the procedures presented here requires advanced technical capabilities. A version of these bioassays with fewer treatments and less frequent sampling may be as useful. In general, it has been our experience that observation of the carboys in about three to five days will reveal significant differences in chlorophyll and samples do not need to be collected for analysis until differences are apparent by visual observation. Although nutrient levels reveal some information and probably should be measured initially to reveal the appropriate level of addition, nutrient measurements throughout the experiments are probably not necessary unless mass balance calculations are attempted. Likewise, phytoplankton community analysis is tedious, requires taxonomic expertise and probably is not necessary to yield results applicable to system management. Recently, a simple version of the bioassays reported here was successfully implemented on another local reservoir by a high school student as a senior research project (ROBERT LEHMANN, personal communication). Therefore, an ecological technician should be able to perform a similar bioassay successfully.

Lowering nutrient loading can be quite costly. Using bioassay techniques similar to those presented here may yield important information on nutrient limitation that the standard loading equations can not, and thus assist in estimating the impact of nutrient reductions.

Acknowledgements

We thank A. EICHEM, C. EDLER and D. GUDDER for technical assistance. R. AXLER and two anonymous reviewers provided valuable comments on the manuscript. This re-

search was supported by the Soap and Detergent Association and represents contribution number 93-262-J from the Kansas Agricultural Experiment Station.

References

APHA (1989): Standard methods for the examination of water and waste water. 17th ed. – American Public Health Association, Washington DC.

Brylinsky, M. & Mann, K. H. (1975): The influence of morphometry and of nutrient dynamics on the productivity of lakes. – Limnol. Oceanogr. **20:** 666–667.

Crumpton, W. G. (1987): A simple and reliable method for making permanent mounts of phytoplankton for light and fluorescence microscopy. – Limnol. Oceanogr. **32:** 1154–1159.

Dodds, W. K. (1993): What controls levels of dissolved phosphate and ammonium in surface waters? – Aquat. Sci. **55:** 132–142.

Dodds, W. K., Johnson, K. R. & Priscu, J. C. (1989): Simultaneous nitrogen and phosphorus deficiency in natural phytoplankton assemblages: theory, empirical evidence, and implications for lake management. – Lake Reserv. Manag. **5:** 21–26.

Dodds, W. K. & Priscu, J. C. (1988): An inexpensive device for sampling large volumes of lake water from discrete depths. – Freshwat. Biol. **20:** 113–115.

– – (1990): A comparison of methods for assessment of nutrient deficiency of phytoplankton in a large oligotrophic lake. – Can. J. Fish. Aquat. Sci. **47:** 2328–2338.

– – (1991): Ammonium stimulation of dark carbon fixation as an indicator of nitrogen deficiency in phytoplankton: potential errors caused by ammonium-oxidizing bacteria. – J. Phycol. **27:** 79–82.

Dodds, W. K. & Randel, C. (1992): Field assessment of the effects of nutrient removal on phytoplankton productivity and biomass. – J. Freshwat. Ecol. **7:** 283–292.

Elser, J. J., Marzolf, E. R. & Goldman, C. R. (1990): Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. – Can. J. Fish. Aquat. Sci. **47:** 1468–1477.

Emanuel, D. J. (1983): Supplemental water quality data for Milford Lake 1969–1981. – U.S. Army Corps of Engineers, Kansas City District.

Evans, G. T. & Paranjape, M. A. (1992): Precision of estimates of phytoplankton growth and micro zooplankton grazing when the functional response of grazers may be nonlinear. – Mar. Ecol. Prog. Ser. **80:** 285–290.

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

Hoyer, M. V. & Jones, J. R. (1983): Factors affecting the relation between phosphorus and chlorophyll-a in midwestern reservoirs. – Can. J. Fish. Aquat. Sci. **40:** 192–199.

Jones, J. R. & Knowlton, M. F. (1992): Limnology of Missouri reservoirs: An analysis of regional patterns. – Lake Reserv. Manag. **8:** 17–30.

Jones, J. R. & Novak, J. T. (1981): Limnological characteristics of Lake of the Ozarks, Missouri. – Verh. Int. Verein. Limnol. **21:** 919–925.

Paerl, H. W. & Bowles, N. D. (1987): Dilution bioassays: Their application to assessments of nutrient limitation in hypereutrophic waters. – Hydrobiologia 146: 265–273.

RYDING, S.-O. & RAST, W. (1989): The control of eutrophication of lakes and reservoirs. Man and the biosphere series, Vol. 1. – UNESCO, Paris.

SUTTLE, C. A. & HARRISON, P. J. (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.

U.S.E.P.A. [United States Environmental Protection Agency] (1974): Natural eutrophication survey. A compendium of lake and reservoir data collected by the national eutrophication survey in the central United States. Working Paper # 476. – Government Printing Office 699-440.

Submitted: 22 January 1993; accepted: 18 May 1993.