

# Nutrient Addition Experiments in a Nitrogen-Limited High Plains Reservoir Where Nitrogen-Fixing Algae Seldom Bloom

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## ABSTRACT

Cherry Creek Lake in Colorado is a shallow flood control reservoir that exhibits infrequent blooms of nitrogen-fixing cyanobacteria one of which occurred in summer 1992. In nutrient addition experiments run before and during the bloom, added nitrogen significantly stimulated algal growth. The magnitude of the N-effect was much greater in the pre-bloom experiments in which added phosphorus had no effect. During the bloom, added P had small but significant effects on growth indicating concurrent P and N limitation. Monitoring data suggest that phytoplankton is usually N-limited in the lake, but, for unknown reasons, blooms of N-fixing algae are rare.

## INTRODUCTION

The ability of heterocystous cyanobacteria to fix atmospheric nitrogen means that phosphorus, rather than nitrogen, is likely to limit the total algal community biomass in a given freshwater system (Schindler 1977). For this reason, management of eutrophication has usually focused on phosphorus control (Hecky and Kilham 1988). But in some lakes with abundant P supplies, N-fixing cyanobacteria occur rarely and so N usually limits algal biomass (Howarth et al. 1988). This situation is both boon and bane to lake managers. On one hand, the preponderance of N-limitation will result in lower algal biomass, and hence, better water quality than predicted on the basis of phosphorus. Maximum, P-limited biomass will be observed only during rare blooms of N-fixers. On the other hand, the rarity of such blooms creates problems in justifying the expense of P-control measures needed to regulate these infrequent, but objectionable, maximum blooms.

Cherry Creek Lake, near Denver, Colorado, provides an example of this scenario. The lake and its catchment are subject to comprehensive regulations for control of phosphorus inputs. But lake monitoring data suggest that algal biomass is well below the threshold of P-limitation except during blooms of colonial cyanobacteria. In summer 1992, we conducted four *in situ* algal growth experiments to document the nutrient status of the phytoplankton community. This paper presents the results of these experiments together with an overview of nutrient-algal relations in this waterbody.

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## STUDY SITE DESCRIPTION

Cherry Creek Lake is a 45-year-old flood control reservoir about 15 km southeast of Denver, Colorado in Cherry Creek State Park. At normal pool (1692 m MSL) the lake has a surface area of  $\approx 400$  ha with mean and maximum depths of 3.2 m and 7.9 m, respectively. The lake is ice covered from late December through March in most years. In summer the lake shows ephemeral stratification often mixing diurnally; but stratified periods lasting several days are common and usually result in depletion of dissolved oxygen near the lake bottom. Complete anoxia, however, has not been observed.

Inflow to the lake is sporadic. Most of the lake's 995 km<sup>2</sup> catchment is undeveloped pasture land drained by Cherry Creek, an intermittent stream that contributes surface inflow only during floods. As estimated by the Corps of Engineers (C.O.E.), total inflow ranged from 0.002 to 0.046 km<sup>3</sup>/y between 1950 and 1991, averaging 0.009 km<sup>3</sup> or about 73% of the current mean lake volume. Consequently, theoretical water residence times have varied from a few months to several years during the period of record. In intervals between floods, much of the lake's water income is from direct precipitation and inflow from three mostly urban watersheds that comprise the  $\approx 5\%$  of the catchment not drained by Cherry Creek. Inflow from these minor streams is partially intercepted by a complex of natural and constructed wetlands bordering the lake. Evaporation averages about 0.003 km<sup>3</sup>/y and exceeds tributary inflow in about one year in four.

To protect water quality in the lake, the Colorado Water Quality Control Commission adopted an in-lake total phosphorus (TP) standard of 35  $\mu\text{g/L}$  in 1984 based largely on results from a "Clean Lakes Study" in 1982 (Denver Regional Council of Governments 1984). To comply with this standard, stringent measures were adopted in the mid-1980's to regulate phosphorus loading in the Cherry Creek Basin (Cherry Creek Basin Water Quality Authority 1989). Nonetheless, mean TP has exceeded 35  $\mu\text{g/L}$  in every year since 1982. Even so, mean chlorophyll (Chl) concentrations have usually been near or below the target concentration of 15  $\mu\text{g/L}$  upon which the 35  $\mu\text{g/L}$  TP standard was based.

## METHODS AND MATERIALS

In June and August 1992 we conducted *in situ* algal growth experiments (Morris and Lewis 1988, Jones et al. 1989) in which lake water was incubated 3-5 d in 1 liter Cubitainer<sup>®</sup> polyethylene containers suspended at three depths (0.5, 1.0, and 1.5 times the Secchi depth) and subsequently analyzed for chlorophyll content. Six experimental treatments were represented by three replicate containers at each depth. Treatments were: P - unfiltered lakes water spiked with potassium phosphate; N - unfiltered lake water spiked with ammonium-nitrate; NP - unfiltered lake water spiked with both potassium phosphate and ammonium-nitrate; F - lake water filtered through 35  $\mu\text{m}$  Nitex netting; NS - unfiltered lake water spiked with ammonium-nitrate and a suspension of lake bottom sediment; and C - unfiltered lake water with no additions. Table 1 shows initial Chl, TN and TP values for water placed in the containers.

Table 1. Starting conditions in June and August experiments. Data ( $\mu\text{g/L}$ ) are the means of triplicate analyses of subsamples of amended lake water. For the August experiments, the first and second numbers in each column represent the experiments begun on 8 and 12 August, respectively. Also for the August experiments, separate Chl determinations were only made for the F and NS treatments. See text for descriptions of treatments.

TREATMENT	JUNE			AUGUST		
	TP	TN	Chl	TP	TN	Chl
C	43	610	6.8	41 - 43	780 - 650	21.3 - 16.9
P	110	620	7.5	197 - 137	840 - 680	21.3 - 16.9
F	41	590	6.1	33 - 31	670 - 520	7.5 - 6.7
N	45	1340	7.0	43 - 44	1460 - 1210	21.3 - 16.9
NS	67	1170	7.5	92 - 75	1440 - 1280	21.6 - 19.8
NP	108	1340	7.5	190 - 182	1370 - 1210	21.3 - 16.9

In June, two experiments were run concurrently. One lasted four days; the other five days. In August, two three-day experiments were run in succession. After incubation, duplicate 250 mL subsamples were taken from each container and analyzed for total chlorophyll by the ethanol-fluorometric method (Sartory and Grobbelaar 1984, Knowlton 1984). Duplicate or triplicate measurements of TP (Prepas and Rigler 1982), TN (Crumpton et al. 1992), ammonium-N (Stainton et al. 1977), nitrate-nitrite-N (American Public Health Association 1985), and volatile and non-volatile suspended solids (Whatman AH-934 filters, American Public Health Association 1985) were made at the beginning of each experiment along with triplicate measurements of Chl.

Additional measurements of nutrients, Chl and suspended solids were made on surface samples collected at several sites during the June and August visits and monthly from a mid-lake site during May-September in 1992-1994. Historic lake monitoring data bases compiled by C.O.E., the U.S. Geological Survey, and private contractors were also reviewed.

## RESULTS

### Summer Conditions

Phytoplankton counts have been made routinely in Cherry Creek Lake since 1987 and have shown that either *Anabena*, *Aphanizomenon*, or both occurred every summer, usually without forming noticeable blooms. Since 1984, peak summer Chl has been 15-30  $\mu\text{g/L}$  in every year except 1988 and 1992 when large cyanobacteria blooms pushed peak Chl to 55-70  $\mu\text{g/L}$ .

In 1992 Chl increased from 4.1  $\mu\text{g/L}$  on 20 May to 56.8  $\mu\text{g/L}$  on 27 August at the peak of the bloom. During this period TP increased from 34 to 86  $\mu\text{g/L}$  and TN from 630 to 1240  $\mu\text{g/L}$ , mostly due to increased particulate P and N. Dissolved TP and TN remained nearly constant over this period at  $\approx 20 \mu\text{g/L}$  and  $\approx 550 \mu\text{g/L}$ , respectively. Nitrate-N remained below 15  $\mu\text{g/L}$  throughout the summer and ammonium-N remained  $< 10 \mu\text{g/L}$  except in late August and September when concentrations of 40-70  $\mu\text{g/L}$  were measured. The bloom, mostly *Aphanizomenon*, continued from early August through late September when summer sampling was terminated. On 30 September Chl was  $> 35 \mu\text{g/L}$ .

### June Experiments

On 31 May Cherry Creek Lake was nearly homothermal at 17 C with a

Secchi depth of 1.5 m and Chl of about 7  $\mu\text{g/L}$  (Table 1). Experimental containers were filled and suspended at 0.75, 1.5, and 2.25 m, but raised to 0, 0.75, and 1.5 m on 1 June because of increased turbidity (Secchi depth 0.9 m) and heavy overcast following a storm front. Skies were mostly clear after 1 June and the lake stratified with mixing depth reduced to about 2 m by 5 June. Surface temperature increased to 19 C and Secchi depth increased to 1.5 m. One half of the containers were collected on 4 June and the rest on 5 June.

Analysis of variance showed the proportional change in Chl (final/initial) to differ significantly with treatment at all depths (Table 2). Treatments with added N (i.e., N, NS and NP) significantly exceeded those without N (i.e., C, P and F). Added P had no effect alone but significantly increased yield in conjunction with added N. The removal of zooplankton and large algae (F treatment) reduced initial Chl about 1.1  $\mu\text{g/L}$  (Table 1) but had no effect on proportional growth. Addition of N together with lake sediment approximately doubled TP (Table 1), but significantly increased growth relative to N alone in only one of six depth/treatment combinations.

Addition of ammonium-nitrate increased Chl by an average factor of  $\approx 5$  during these experiments, which clearly indicates that ambient P was sufficient to support a substantial increase in algal biomass. Nitrogen may also have been in slight excess. Treatments without added N (i.e., C, P and F) increased as much as 56% at 1.5m. Chl at this depth declined between days four and five, suggesting that Chl may have peaked earlier during the incubation period. Also, Chl in the lake increased about 54% between 31 May and 5 June. Light conditions improved during this period due to increased transparency and reduced mixing depth, so phytoplankton in containers and in the lake may have been somewhat light-limited at the outset. In any case, factors other than N deficiency probably influenced biomass.

#### August Experiments

Qualitative phytoplankton samples collected in June contained few colonial cyanobacteria (three trichomes of Anabena in a total sample volume of 15 mL), but by early August large colonies of heterocystous Aphanizomenon and Anabena dominated the algal community. Between June and August total biomass increased 2-3 times and the proportion of Chl removed by 35  $\mu\text{m}$  netting increased from  $\approx 15\%$  to  $> 60\%$ . Total P remained nearly constant at 40-45  $\mu\text{g/L}$ , but TN increased approximately one third (Table 1).

Results from two three-day experiments run in August suggest that the degree of N-deficiency indicated by the June data was greatly reduced and that both N and P were in relatively short supply. As occurred in June, the August experiments showed that added N significantly enhanced growth at all depths, but the degree of stimulation was only a fraction of that observed in June. Growth in the N treatment in August was never more than 1.6 times that of controls. In June the two treatments differed by an average factor of 4.7. Phosphorus insufficiency may explain this difference. In the first August experiment, added P significantly increased growth at two depths, although the effect was significantly less than N. And lake sediment plus N produced faster growth than N alone at all depths. In August only the NP treatment yielded peak final Chl more than twice the concentration in controls.

Table 2. Results of algal growth experiments expressed as a proportion of initial chl. Each value is the mean for three replicate containers for each treatment/depth. Analysis of variance (ANOVA) results show significant differences ( $p < 0.05$ ) among treatment means at the same depth as determined by Duncan's multiple range test (SAS, 1991).

EXPERIMENT	DEPTH	$\Delta\text{chl (FINAL/INITIAL)}$							ANOVA
		C	P	F	N	NS	NP		
JUNE	0.0	0.83	0.87	0.83	3.62	3.66	5.95	NP > NS = N > P = F = C	
	0.8	0.98	0.87	0.92	4.61	4.60	10.85	NP > N = NS > C = F = P	
	1.5	1.56	1.44	1.51	5.93	6.35	10.99	NP > NS = N > C = F = P	
2	0.0	0.85	0.87	0.77	3.83	4.08	10.88	NP > NS > N > P = C = F	
	0.8	0.82	0.75	0.76	4.50	4.55	11.12	NP > NS = N > C = F = P	
	1.5	1.16	1.18	0.98	6.14	6.16	15.16	NP > NS = N > P = C = F	
AUGUST 1	0.6	1.03	1.21	1.30	1.53	1.82	3.09	NP > NS > N > F = P > C	
	1.2	1.09	1.33	1.62	1.69	2.11	2.58	NP > NS > N = F > P > C	
	1.8	1.33	1.43	1.83	1.88	2.33	2.35	NP = NS > N = F > P = C	
2	0.9	1.17	1.02	1.26	1.70	1.89	2.82	NP > NS = N > F = C, F > P, C = P	
	1.9	1.44	1.32	1.78	1.94	1.85	2.21	NP > N = NS = F > C = P	
	2.7	1.61	1.64	1.75	1.76	1.46	1.88	NP = N = F, NP > P = C, N = F = P N = F = P, N = F > C > NS	

In August, removal of zooplankton and algae  $> 35 \mu\text{m}$  increased proportional growth compared to controls. This effect was probably due to removal of larger, slower growing algae. After the second August experiment we pooled unused water from the NP treatment (0.9 and 1.9 m depths) and measured Chl retained by  $35 \mu\text{m}$  netting and in the filtrate. These results suggest that growth in the  $< 35 \mu\text{m}$  fraction was about 1.5 times that of larger algae. Differential growth among different varieties of phytoplankton may also explain why both N and P could separately stimulate growth in the first August experiment. In a mixed community, different species need not be limited by the same factor at any given time (Suttle and Harrison 1988, Dodds et al. 1989, Elser et al. 1990).

## DISCUSSION

Results of these experiments suggest that P supplies in Cherry Creek Lake can support a larger biomass of phytoplankton than is usually observed. Chl in N-spiked containers increased as much as six fold without additional P. The substantial effect of added N in our June experiments and the success of N-fixing algae during the summer bloom indicate that lack of sufficient N may usually limit phytoplankton biomass in the lake. Such circumstances indicate a strong potential for dominance of the algal community by species equipped for N-fixation and yet such dominance is seldom achieved. Large cyanobacteria blooms have occurred in only two of last eleven years and a thorough review of available monitoring data revealed no strong indication of why this was so.

Weather, inflow, water temperature, stratification, water levels, and other physical factors (Reynolds and Walsby 1975) did not consistently differ among years with and without blooms. Concentrations of total nutrients were elevated during blooms but the best data available, those from 1992, suggest that higher nutrients may be more a result of blooms, rather than a cause. In summer 1992, concentrations of particulate P and N were correlated very strongly with Chl ( $r > 0.9$ ,  $n = 16$  observations) and time series showed no suggestion that increased Chl was preceded by increased total nutrients or accompanied by losses of dissolved N or P. It comes as no surprise that particulate N should increase during a bloom of N-fixing algae, but what is the source of the additional P? Our current hypothesis is that the extra phosphorus associated with blooms is derived from bottom sediments, probably from luxury uptake in uplake portions of the reservoir, and that these nutrients are subsequently circulated with the algae to the main body of the lake. Over half of Cherry Creek Lake is  $< 3$  m deep. About a third of the lake is  $< 1.2$  m deep. Lake bottom sediments contain about 1 mg P per dry weight g of sediment. In 1992 we found consistently higher concentrations of both TP and Chl at sampling sites  $< 2$  m deep. And one of our four experiments showed a significant positive effect of sediments on algal growth. It is possible that the phosphorus from these littoral sediments fuels cyanobacteria blooms (Osgood 1988).

But why do blooms not occur every year? Does the available sediment P require "re-charging" before another bloom can occur? Are cyanobacterial usually limited by some micronutrient, pathogens, or unmeasured physical

constraint (Paerl 1988)? Recent research (e.g. Morris and Lewis 1988, Elser et al., 1990) has shown N-limitation to be more common than would be expected if N-fixation was readily able to supply the needs of the algal community (Schindler 1977). Thus the situation in Cherry Creek Lake is probably not uncommon. There may be many lakes in which P rarely limits biomass and for which the efficacy of P control is uncertain. For these waterbodies the key to successful lake management may lie in discovering the factor or factors that limit the growth of N-fixing algae in P-rich environments (Paerl 1988, Howrath et al. 1988).

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