# Studies of Phytoplankton Response to Nutrient Enrichment in Cherry Creek Reservoir, Colorado

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## Summary

1. Nutrient limitation of suspended algae (phytoplankton) in Cherry Creek Reservoir, Colorado, was studied on eight dates between May and October, 2003. The study involved collection of data on temperature and oxygen, nutrient concentrations, algal biomass (measured as chlorophyll *a*), and abundances of individual species of algae. Primary production (growth) of phytoplankton also was measured. In addition, nutrient enrichment experiments were performed on samples taken from the lake on each of the sampling dates. The experimental incubations included controls (no addition of nutrients), addition of phosphorus, addition of nitrogen, and addition of a combination of phosphorus and nitrogen; response to enrichment was quantified as change in biomass (chlorophyll *a*).
2. Vertical profiles of temperature and oxygen showed that the lake stratifies only briefly during the growing season. Although phytoplankton within the top meter of the water column grow rapidly for brief intervals (e.g., two hours), algae that are moving freely with the lake water probably experience light deprivation during periods of deep mixing because growth is possible only within the top 1 to 2 m of the water column.
3. Nutrient samples taken from the upper water column during the growing season had very low concentrations of inorganic nitrogen, but relatively high concentrations of total dissolved phosphorus, thus suggesting the possibility of nitrogen deficiency.

4. Enrichment experiments consistently showed that the phytoplankton were nitrogen deficient, i.e., that algal biomass during the growing season of 2003 was determined by nitrogen and not by phosphorus.
5. Estimates of the threshold for phosphorus limitation in phytoplankton biomass indicate that removal of at least half (30 μg/L) of the total phosphorus in the upper water column would have been necessary to induce phosphorus limitation of phytoplankton growth during year 2003. Until this threshold of phosphorus limitation is reached, incremental phosphorus control cannot be expected to suppress phytoplankton biomass in the reservoir.
6. Phytoplankton in Cherry Creek Reservoir during year 2003 were dominated by bluegreen algae, but the nitrogen fixing bluegreen algae were abundant only briefly, probably because these taxa are suppressed by weak light exposure connected with deep mixing, which is commonplace in Cherry Creek Reservoir.

# Introduction

Cherry Creek Reservoir is an especially valuable recreational amenity in Colorado because of its size and its proximity to the Denver metropolitan population. The reservoir, which was built for flood control and is operated by the U.S. Army Corps of Engineers, impounds the waters of Cherry Creek, which drains large areas of undeveloped rangeland as well as small amounts of farmland and several areas that have been intensively developed for residential land use. The point sources of wastewater discharge within the drainage basin are relatively small; they are estimated by CDPHE to account for about 16% of the external load to the reservoir (CDPHE 2001).

Cherry Creek Reservoir develops dense growths of suspended algae (phytoplankton) during the summer months. During the last half of the growing season in some years, mass mortality of fish has occurred. Poor water quality, as shown by low transparency, dense growths of suspended algae, occasional loss of oxygen in deep water, and mass mortality of fish, is considered to be the byproduct of high nutrient concentrations in the lake, which in turn implicates high nutrient supply from the watershed as a cause of the eutrophic status of the lake.

In attempting to protect the public interest in the water quality of Cherry Creek Reservoir, the Colorado Water Quality Control Commission has adopted site-specific standards and has created a special control regulation for Cherry Creek Reservoir (Regulation 72). Originally, a standard was set for the concentrations of phosphorus in the reservoir during the growing season (35 μg/L P, July-September). The current version of the standard for Cherry Creek Reservoir is based on the concentration of

chlorophyll *a* in the upper water column (15 µg/L chlorophyll *a*, July-September). The control regulation is based on the assumption that phosphorus originating from the watershed is responsible for producing dense growths of algae and other associated water-quality problems. In basing the standard on algal biomass (expressed as chlorophyll *a*), the Water Quality Control Commission has avoided defining in exact terms the relationship between phosphorus and chlorophyll, and thus has left the WQCD staff and the Cherry Creek Basin Water Quality Authority to work out methods for suppressing the growth of algae in the lake, presumably by restricting its nutrient supply; emphasis has been on phosphorus.

Control of phosphorus concentrations in the water reaching Cherry Creek Reservoir currently is the objective of the Cherry Creek Basin Water Quality Authority. Reduction of phosphorus loading to lakes in the single most widely used strategy for reducing excessive algal growth and associated water-quality problems (symptoms of eutrophication) throughout the world. The effectiveness of this strategy, however, varies in relation to a number of factors (e.g., relative availability of nitrogen and phosphorus, importance of uncontrollable sources of phosphorus), some of which are unknown for Cherry Creek Reservoir. The study summarized here is intended to provide information on two of these factors: (1) The degree to which the algal community presently is limited by phosphorus, and (2) The degree of reduction in phosphorus concentration that would be required to induce limitation by phosphorus if algal growth is not limited by phosphorus. The present study does not deal with relationships between nutrient loading and nutrient concentrations in the lake, or with the relative contributions of various nutrient sources to total nutrient loading.

## Rationale for the Study

Phytoplankton require approximately 20 elements for the synthesis of protoplasm (Reynolds 1984). Almost all of these elements are present at concentrations exceeding the needs of algal populations, even when algae are abundant. The two exceptions are phosphorus and nitrogen, which commonly are in short supply relative to algal needs. Therefore, phosphorus and nitrogen are designated as key nutrients or limiting nutrients for algal growth (USEPA 1999).

When limiting nutrients are added to waters that have low to moderate abundances of algae or other aquatic plants, the typical response is an increase in the rate of plant growth leading to higher plant biomass toward the end of the growing season. Thus, enrichment with phosphorus and nitrogen produces higher plant biomass in most cases.

Because high nutrient concentrations often lead to high plant biomass, one means of controlling biomass is to reduce nutrient concentrations. Although it would seem advisable to restrict both the nitrogen and phosphorus supplies to a water body in an attempt to control plant growth, the focus of control often falls specifically on phosphorus rather than nitrogen. Phosphorus is easier to intercept than nitrogen. Also, the ability of some kinds of algae (cyanobacteria, also called bluegreen algae) to fix (convert to a useful form) gaseous nitrogen dissolved in water from the atmosphere, thus offsetting shortages of nitrogen and potentially defeating the purpose of any nitrogen control project. The nitrogen fixation process does not always offset nitrogen deficiency, however, as indicated by the fact that approximately half of the lakes in North America

that have been studied for nutrient limitation show nitrogen limitation and the other half show phosphorus limitation (Elser et al. 1990). The organisms that fix nitrogen efficiently are not always able to grow in quantity in a specific lake, and they are unable to fix large enough amounts of nitrogen to offset nitrogen deficiency in some lakes where they do grow. Thus, nitrogen deficiency is possible under many circumstances, as has been shown for Colorado as well as other locations (Morris and Lewis 1988).

If algal growth is limited in a particular lake by nitrogen deficiency, and an attempt is made to control the growth of algae by reducing phosphorus concentrations, the control process will pass through two stages (Figure 1). Because phosphorus is present in excess (as indicated by the fact that nitrogen is limiting), phosphorus first must be reduced from being more abundant than needed by the algae to a point at which it replaces nitrogen as the limiting nutrient. Over the concentration range between the initial conditions prior to control and the onset of control by phosphorus, the reduction in concentrations has no effect because nitrogen is limiting. At some point, progressive reduction of phosphorus concentrations without any change in nitrogen will cause phosphorus to overtake nitrogen as the limiting element. From this point forward, incremental reduction in algal growth and biomass can be expected if incremental reduction can be achieved in concentrations of phosphorus within the lake.

The general presumption has been that incremental reductions in phosphorus concentration in Cherry Creek Reservoir will be accompanied by incremental reductions in algal mass. As shown in Figure 1, however, incremental reductions in algal mass would not be expected if phosphorus is not actually limiting to the growth of algae in Cherry Creek Reservoir under current conditions. This does not necessarily mean that a

phosphorus control strategy is wrong, in that phosphorus restriction at some point could lead to restriction of algal growth. The disjunction between phosphorus concentrations and algal growth when nitrogen is limiting does, however, raise the question as to how much phosphorus would need to be restricted in order to induce a phosphorus limitation that then could be used for the control of algae in the lake, and whether this amount of restriction is feasible or not. Thus, the purpose of the current study is to determine for

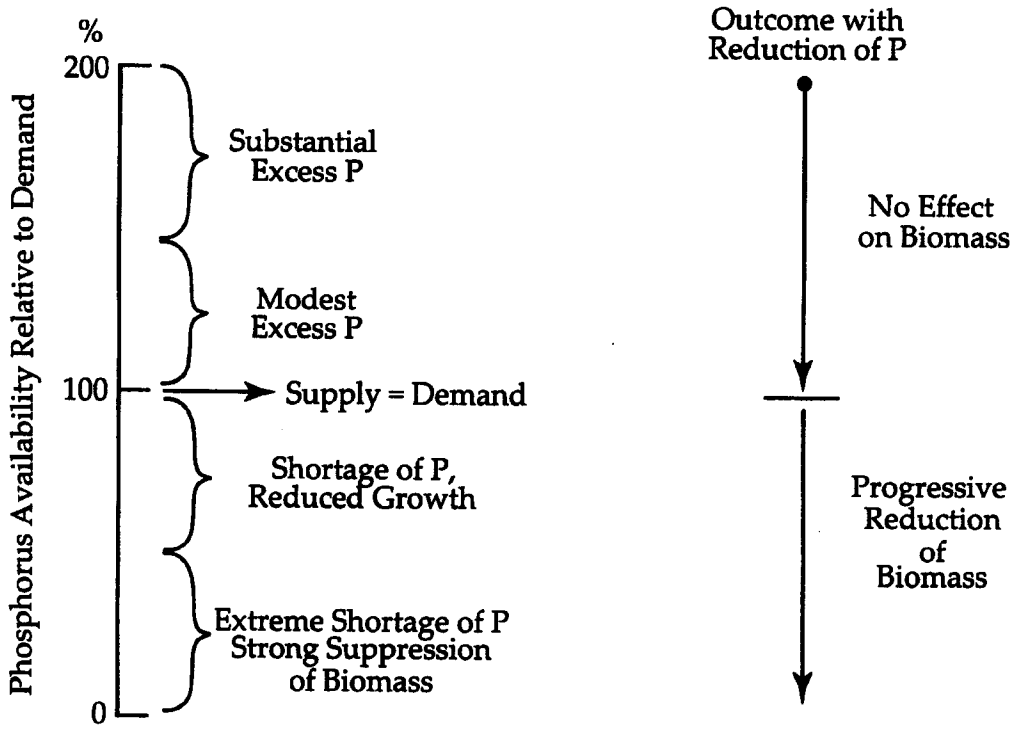


Figure 1. Conceptual basis for control of algal biomass by restriction of phosphorus in a lake that initially is deficient in nitrogen.

various times in the growing season whether or not the algae in Cherry Creek Reservoir are limited by phosphorus or by nitrogen and, if nitrogen is limiting for all or part of the

year, how much phosphorus would need to be removed in order to establish a phosphorus limitation rather than a nitrogen limitation.

There are various methods for determining nutrient limitation of algae in lakes. The simplest of these are bioassays, called nutrient enrichment experiments, based on the addition of increments of phosphorus, nitrogen, or a combination of these to closed containers containing samples of water from a lake, followed by measurement of the amount of growth that occurred in each case. While nutrient enrichment experiments can be difficult to interpret where nutrients are extremely scarce (oligotrophic waters), they often present straightforward results for waters that are relatively rich in nutrients, as is Cherry Creek Reservoir.

For Cherry Creek Reservoir, nutrient enrichment experiments were conducted eight times during the growing season. As is typical for such experiments, the indicator of response to nutrient additions was algal biomass, which was measured as amount of chlorophyll per unit volume of water (μg/L chlorophyll *a*).

Because the value of nutrient enrichment experiments is enhanced by ancillary information relevant to mechanisms that affect growth, the nutrient enrichment studies were accompanied by studies of the conditions in Cherry Creek Reservoir (temperature, stratification, nutrient concentrations, and other related variables) at the time of each of the enrichment studies, and by measurements of primary production (photosynthesis rates as measured by change in oxygen concentrations) as well as quantitative counts of algal taxa and of zooplankton, which consume phytoplankton.

## Methods

Enrichment experiments were conducted once per month during May, June, July, and October and twice per month during August and September. The interval May through October spans the growing season for Cherry Creek Reservoir (i.e., the season for maximum algal growth rates leading to peak algal biomass). The highest amounts of biomass were expected to occur in August and September, at which time nutrient limitation may be most extreme.

At the time when samples were collected for the nutrient enrichment experiments in Cherry Creek Reservoir, vertical profiles of temperature and dissolved oxygen were measured with a field meter (YSI or Hydrolab) at a site approximately 50 m south of the outlet structure over the deepest part of the lake. At the same location, a vertical profile of light intensity was measured with a LiCor quantum sensor. In addition, transparency was determined with a Secchi disk.

On each date when an enrichment experiment was initiated, a sample was taken from the uppermost portion of the water column and preserved with Lugol's solution. Subsequently, phytoplankton in these samples were counted in sedimentation chambers with an inverted microscope, and were assigned during counting to the lowest possible taxonomic unit (typically species). A townet sample was taken for zooplankton (vertical tow, bottom to top); it was preserved and the taxa were subsequently counted.

On the date of each enrichment study, a sample was taken from the upper mixed layer (at a depth of about 1 m) for analysis of nutrients. Three fractions of phosphorus were analyzed (Table 1): soluble reactive phosphorus (SRP), total soluble phosphorus

(TSP), and total particulate phosphorus (TPP). The sum of TSP and TPP is used as the estimate of total phosphorus (TP). The particulate component of phosphorus was separated from the dissolved component by filtration of a measured volume of water through glass fiber paper (pore size approximately 1 μm).

Concentrations of nitrate and ammonium also were analyzed (Table 1); these are the primary nitrogen supplies for phytoplankton. Samples were filtered prior to analysis.

The abundance of algae was estimated on the basis of chlorophyll *a*, which was extracted from the phytoplankton and measured by a standard spectrophotometric procedure (Table 1). Primary production was measured by the oxygen-difference method (Wetzel and Likens 2000) on dates when enrichment experiments were initiated. Water was drawn from the upper part of the mixed layer with a diaphragm pump, which minimizes the risk of damage to algal cells. A 120-L plastic drum was filled with the water, which was kept well mixed before and during the process of filling bottles (300 mL glass) within which the incubations occurred. Five replicate samples of the water were fixed immediately for Winkler analysis of dissolved oxygen (APHA 4500-O C) to

| Variable | Methods | Authority |
|---|---|---|
| Soluble Reactive Phosphorus | Phosphomolybdate/Spectrophotometry | APHA 4500-P E |
| Total Soluble Phosphorus | Acid Digestion/Phosphomolybdate/Spectrophotometry | APHA 4500-P E |
| Total Phosphorus | Pyrolysis/Phosphomolybdate/Spectrophotometry | APHA 4500-P E |
| Nitrate | Ion Chromatography | APHA 4110-B |
| Ammonia | Indophenol Blue/Spectrophotometry | APHA 4500-NH3 F |
| Chlorophyll *a* | Hot Ethanol Extraction/Spectrophotometry | Marker et al. 1980 |
| Secchi Disk Transparency | Secchi Disk | Wetzel and Likens 2000 |
| Algal Species Composition | Inverted Microscope Counts | Wetzel and Likens 2000 |
| Zooplankton Composition | Tow Sample, Microscope Counts | Wetzel and Likens 2000 |
| Dissolved Oxygen Profile | Meter | Wetzel and Likens 2000 |
| Dissolved Oxygen in Bottles | Winkler | APHA 4500-O C |
| Temperature Profile | Meter | Wetzel and Likens 2000 |
| Conductance Profile | Meter | Wetzel and Likens 2000 |
| Light Intensity Profile | Meter | Wetzel and Likens 2000 |

Table 1. Analytical methods used in the Cherry Creek enrichment study.

establish the initial oxygen concentrations. Three replicates were suspended at each of six depths in the water column (0, 0.5, 1, 2, 3, and 5 m). In addition, five replicates were placed in a dark bag suspended at approximately 6 m. All bottles were incubated in Cherry Creek Reservoir for about two hours, after which all samples were fixed for Winkler analysis of oxygen.

During the primary production incubation, light intensity at the lake surface was measured with a LiCor sensor located near the Cherry Creek Reservoir administration building. Average readings of light intensity were taken over 10-minute intervals. Volumetric estimates of production ($mgO^2/m^3/h$) were integrated over depth to give an areal estimate of production per unit time ($mgO_2/m^2/h$).

Water to be used in the enrichment experiments was pumped from the mixed layer of the lake (at a depth of about 1 m) into a 120-gallon plastic drum by use of a diaphragm pump. The water in the drum was used to fill experimental containers, which consisted of 2.5-gallon collapsible, transparent carboys. The carboys were transported immediately in coolers to an experimental pond facility where the water temperature and the amount of light reaching the carboys could be controlled. Four types of treatments were used for the carboys: control (no nutrients added), phosphorus addition, nitrogen addition, phosphorus plus nitrogen addition. Three replicates were done of each of these four treatments, for a total of 12 carboys.

The three carboys to be used for the phosphorus treatment received potassium dihydrogen phosphate in sufficient amounts to achieve an increase of 50 to 70 μg/L P. Nitrogen was added to the nitrogen treatments as ammonium chloride to achieve an

increase of 200 to 250 μg/L N. The carboys used for the phosphorus plus nitrogen treatment received a combination of the additions for the phosphorus and nitrogen treatments.

The temperature of the experimental pond facility was held close to the temperature of the lake; the temperature in the pond cycled on a 24-hour basis in the same manner as the temperatures in the upper water column of the lake. The light regime was adjusted by the use of shade cloth to reduce the amount of light received by the containers to approximately 25% of ambient light, thus representing conditions in the upper mixed layer of Cherry Creek Reservoir between 0.5 and 1.0 m depth (near the depth of maximum photosynthesis). The incubations lasted two or three days. Containers were agitated several times per day to simulate turbulence. One container was examined to verify that no significant algal growth was occurring on the walls of the containers.

At the end of the incubation, aliquots of water were withdrawn from each carboy for analysis of chlorophyll, nutrients, and phytoplankton, by methods identical to those used for the samples taken from the lake (Table 1).

## Results

The surface water of Cherry Creek Reservoir was close to 14°C at the time of the first enrichment study in May, rose to a peak above 25°C in the first part of August, and descended to approximately 13°C by the middle of October, at the time of the last nutrient enrichment experiment (Figure 2). Vertical profiles of temperature show that the

lake was stratified intermittently, but that stratification was repeatedly disrupted by full mixing (Figure 3). The most pronounced episode of stratification was evident in the middle of July, at which time the temperature difference between the upper and lower water column reached approximately 4°C.

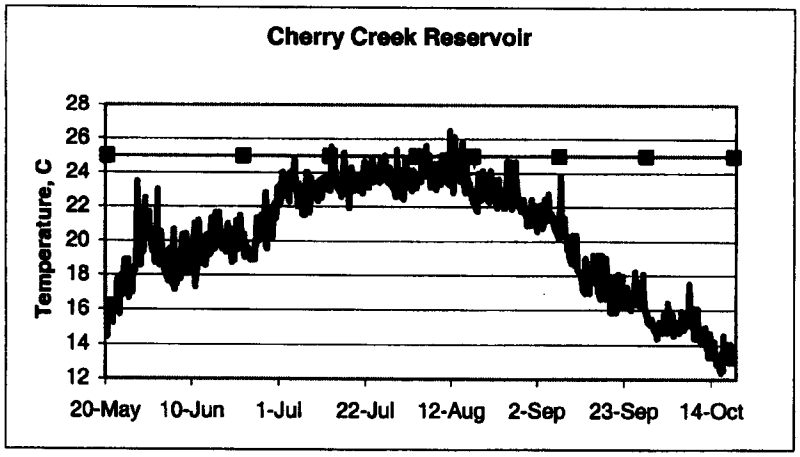


Figure 2. Hourly record of temperature in the upper mixed layer of Cherry Creek Reservoir, 5/21-10/20/03. The solid symbols across the top of the figure indicate the sampling dates.

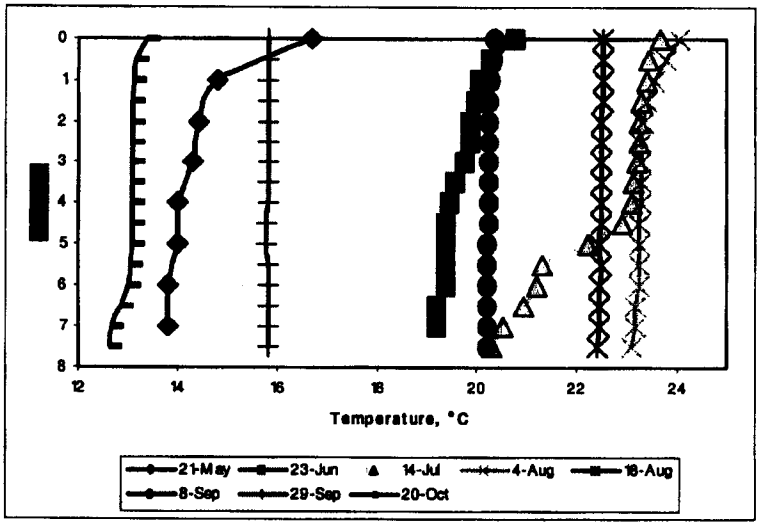


Figure 3. Vertical profiles of temperature in Cherry Creek Reservoir near the outlet structure.

| Sampling Date | Max Depth, ft | Secchi Depth, m | Surface Temperature, °C | Surface Oxygen, mg/L | Surface Oxygen, % saturation | Light Extinction Coefficient, $m^{-1}$ |
|---|---|---|---|---|---|---|
| 5/21 | 7.5 | 1.10 | 13.4 | 9.15 | 116 | 1.43 |
| 6/23 | - | 0.85 | 20.8 | 7.98 | 110 | 1.67 |
| 7/14 | 7.9 | 0.76 | 23.7 | 8.53 | 122 | 1.80* |
| 8/04 | 7.9 | 0.85 | 24.0 | 6.21 | 92 | 1.71 |
| 8/18 | 7.9 | 0.82 | 22.6 | 4.78 | 67 | 1.50 |
| 9/18 | 7.9 | 0.74 | 20.4 | 5.86 | 79 | 2.16 |
| 9/29 | 7.9 | 0.71 | 15.8 | 5.76 | 67 | 1.76 |
| 10/20 | 7.9 | 0.84 | 13.4 | 9.40 | 110 | 1.65 |

*Estimated from Secchi depth.

Table 2. Selected limnological features of Cherry Creek Reservoir on the 8 sampling dates.

Oxygen profiles are good indicators of the recency of complete mixing. A sharp oxycline leading to an anoxic deepwater zone typically indicates that the water within and below the oxycline has not been mixed into the entire water column for at least a few days. As shown by the studies of primary production, dark portions of the water column (i.e., below the depth of 1% surface irradiance) would be expected to lose oxygen at rates between 1 mg/d and 10 mg/d, depending on date. Thus, strong depletion of oxygen in deep water in this case would suggest stabilization of the water column for a day or more, and complete loss of oxygen would suggest stable stratification for two or more days. The most extreme oxycline appeared on 14 July, suggesting multi-day stratification (Figure 4), and another strong oxycline appeared on 20 October but did not reach zero near the bottom, thus suggesting stability lasting at most a few days. In other cases, oxyclines were weaker, which would be suggestive of stabilization lasting a few hours to a day or two. Judging from the vertical profiles of oxygen and discounting a bulge of

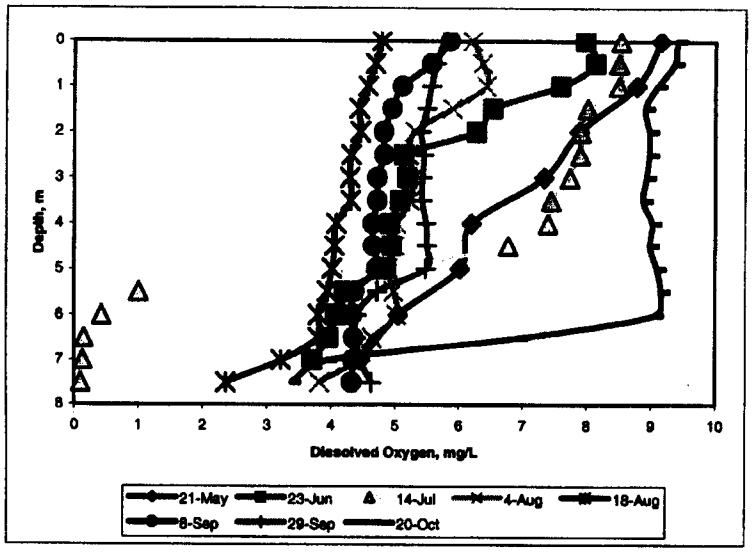


Figure 4. Vertical profiles of dissolved oxygen in Cherry Creek Reservoir near the outlet structure.

oxygen concentrations within the top meter accounted for by temporary daytime stability of the uppermost water column, mixing depth prior to sampling varied between approximately 3 m (23 June) to 7 m (the maximum depth of the reservoir).

Because phytoplankton were abundant in the lake on all sampling dates, saturation or supersaturation of the surface water with oxygen would be expected during the daylight hours. As indicated by the data in Table 2, this was not the case on all dates. The water column, including the surface, was markedly undersaturated during August and September, which suggests failure of the algae within the upper mixed layer to photosynthesize with sufficient vigor to offset nocturnal respiratory losses.

As expected, Cherry Creek Reservoir shows relatively high extinction coefficients for light (Table 2; Figure 5). The reservoir has high phytoplankton biomass, which is the main cause of the removal of light within the water column. A depth of 1% light penetration, which is taken as an approximate index of the thickness of the layer over

which daytime net photosynthesis can be positive, was a little over 3 m at its greatest and close to 2 m at its minimum (Figure 5).

Nitrate was essentially absent from the mixed layer of Cherry Creek Reservoir for the entire growing season (it was below the detection limit, which was approximately 5 μg/L) except on one date (4 August), when the concentration was at 51 μg/L. Ammonium was consistently detectable but at very low concentrations (ca. 10-20 μg/L), except on 4 August, when 32 μg/L of ammonia was present. Thus, the amount of dissolved inorganic nitrogen (DIN) was extremely low throughout the growing season except on 4 August (Table 3; Figure 6).

Total phosphorus in the mixed layer consistently exceeded 50 μg/L and approached 90 μg/L on 4 August. Soluble reactive phosphorus was consistently detectable (> 1 μg/L) and total soluble phosphorus varied between 18 and 30 μg/L. After May, about half of the phosphorus was in the particulate fraction.

The amount of chlorophyll in the upper mixed layer varied within a relatively narrow range (30-60 μg/L), except on the first sampling date, when it was markedly lower (13 μg/L; Figure 7). Algal composition, expressed as cells per unit of water volume, was accounted for primarily by chlorophytes (green algae) in spring (May), and was strongly dominated through the remainder of the growing season by cyanophytes (cyanobacteria or bluegreen algae) (Figure 8). On the basis of biomass rather than cell counts, the bluegreen algae would likely have been dominant as well, but less extremely so because the average cell size for the bluegreens was lower than for any other group.

| | 21-May | 23-Jun | 14-Jul | 4-Aug | 18-Aug | 8-Sep | 29-Sep | 20-Oct |
|---|---|---|---|---|---|---|---|---|
| Total P | | | | | | | | |
| Initial | 49.1 | 72.5 | 63.6 | 85.4 | - | 74.5 | 69.0 | 62.8 |
| Control | 56.4 | 75.6 | 58.0 | 74.4 | 72.0 | 38.9 | 66.3 | 61.0 |
| Add P | 94.3 | 129.5 | 82.6 | 104.0 | 109.1 | 75.2 | 91.4 | 83.5 |
| Add N | 54.5 | 74.6 | 58.8 | 72.9 | 73.1 | 38.9 | 73.0 | 60.9 |
| Add P+N | 85.2 | 111.5 | 83.7 | 103.3 | 112.0 | 53.1 | 84.4 | 85.7 |
| SRP | | | | | | | | |
| Initial | 5.6 | 9.2 | 1.6 | 6.2 | - | 7.5 | 1.8 | 2.1 |
| Control | 1.9 | 2.9 | 1.3 | 3.2 | 2.9 | 1.4 | 2.4 | 1.3 |
| Add P | 18.3 | 36.5 | 1.5 | 16.0 | 31.0 | 27.3 | 4.2 | 3.8 |
| Add N | 2.0 | 1.9 | 1.2 | 3.1 | 2.2 | 1.3 | 3.4 | 1.1 |
| Add P+N | 2.6 | 6.3 | 1.5 | 7.0 | 22.4 | 3.4 | 3.5 | 2.8 |
| TDP | | | | | | | | |
| Initial | 22.8 | 28.4 | 23.0 | 30.0 | - | 24.6 | 18.6 | 18.7 |
| Control | 23.5 | 23.5 | 20.8 | 29.0 | 25.8 | 19.9 | 20.3 | 19.4 |
| Add P | 45.6 | 61.0 | 25.8 | 43.8 | 55.7 | 48.7 | 22.3 | 22.9 |
| Add N | 21.7 | 23.9 | 20.3 | 25.9 | 23.7 | 18.9 | 20.5 | 18.2 |
| Add P+N | 27.5 | 31.2 | 25.9 | 36.0 | 47.5 | 25.5 | 21.3 | 22.9 |
| TPP | | | | | | | | |
| Initial | 26.3 | 44.1 | 40.6 | 55.4 | 48.3 | 49.8 | 50.4 | 44.1 |
| Control | 32.9 | 52.1 | 37.2 | 45.5 | 46.2 | 19.1 | 46.1 | 41.6 |
| Add P | 48.6 | 68.5 | 56.8 | 60.2 | 53.4 | 26.4 | 69.1 | 60.6 |
| Add N | 32.7 | 50.7 | 38.5 | 47.0 | 49.4 | 20.0 | 52.5 | 42.8 |
| Add P+N | 57.7 | 80.3 | 57.7 | 67.2 | 64.5 | 27.6 | 63.1 | 62.8 |
| $NH_4^+$-N | | | | | | | | |
| Initial | 10.0 | 9.0 | 11.4 | 32.3 | 15.4 | 12.7 | 16.2 | 19.4 |
| Control | 6.7 | 5.9 | 9.1 | 12.4 | 13.5 | 7.8 | 9.9 | 6.7 |
| Add P | 7.2 | 6.4 | 9.5 | 13.5 | 11.4 | 6.8 | 10.1 | 6.6 |
| Add N | 10.2 | 12.5 | 8.7 | 15.5 | 30.7 | 7.0 | 9.8 | 10.0 |
| Add P+N | 7.5 | 6.0 | 9.5 | 13.8 | 12.8 | 7.1 | 9.3 | 6.4 |
| $NO_3^-$-N | | | | | | | | |
| Initial | 0.0 | 0.0 | 0.0 | 50.8 | 0.0 | 0.0 | 0.0 | 0.0 |
| Control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Add P | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Add N | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Add P+N | 0.0 | 0.0 | 0.0 | 0.0 | 0.9 | 0.0 | 0.0 | 0.7 |
| DIN | | | | | | | | |
| Initial | 10.0 | 9.0 | 11.4 | 83.1 | 15.4 | 12.7 | 16.2 | 19.4 |
| Control | 6.7 | 5.9 | 9.1 | 12.4 | 13.5 | 7.8 | 9.9 | 6.7 |
| Add P | 7.2 | 6.4 | 9.5 | 13.5 | 11.4 | 6.8 | 10.1 | 6.6 |
| Add N | 10.2 | 12.5 | 8.7 | 15.5 | 30.7 | 7.0 | 9.8 | 10.0 |
| Add P+N | 7.5 | 6.0 | 9.5 | 13.8 | 13.7 | 7.1 | 9.3 | 7.1 |

Table 3. Initial nutrient concentrations and average final concentrations for each of the four treatments in the enrichment study.

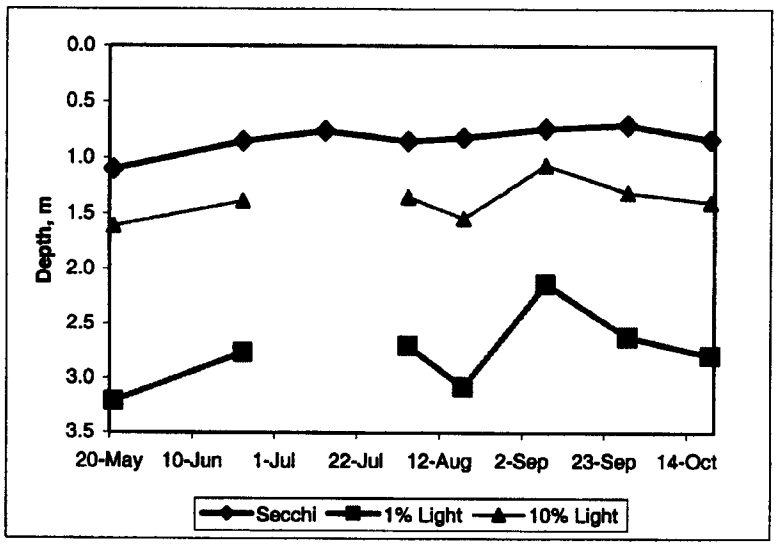


Figure 5. Light attenuation in Cherry Creek Reservoir. Temporal trends are shown for Secchi depth and for the depth of 1% and 10% light as calculated from the extinction coefficient.

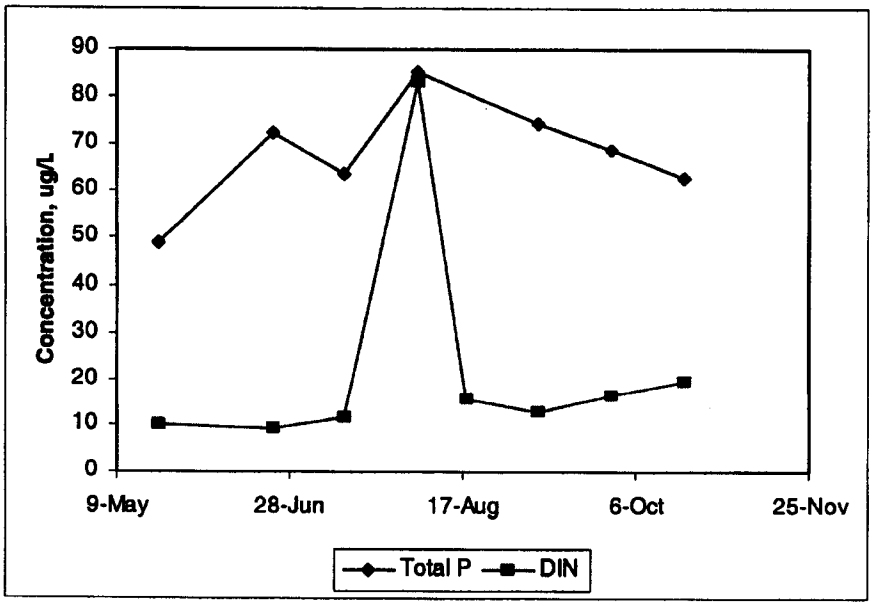


Figure 6. Concentrations of total phosphorus and dissolved inorganic nitrogen (DIN) in the mixed layer of Cherry Creek Reservoir on each sampling date. All DIN was in the form of ammonia, except on 4 August when nitrate was present due to a mixing event.

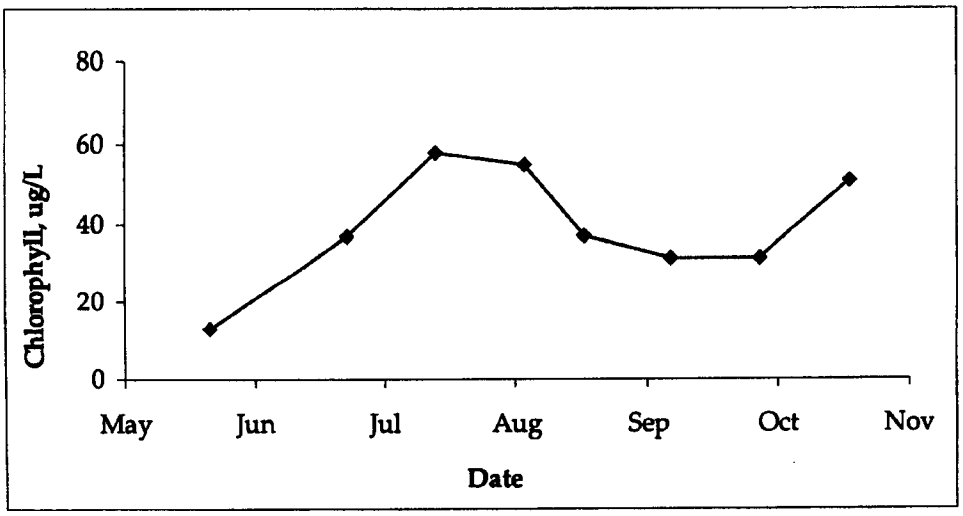


Figure 7. Concentrations of chlorophyll *a* in the upper water column on the 8 sampling dates.

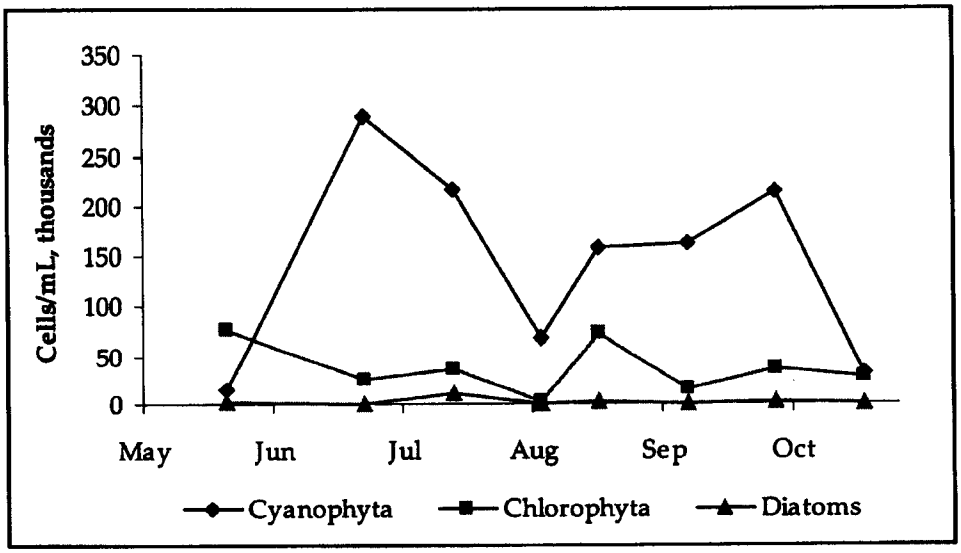


Figure 8. Abundance of the major phytoplankton groups in the mixed layer of Cherry Creek on the 8 sampling dates expressed as cell number.

Among the bluegreens, the dominant genus was *Aphanothece* (Table 4; see Appendix A for more detail), which is a coccoid colonial bluegreen with very small cells (about 1 $\mu m^3$). Cherry Creek Reservoir has had notable pulses of abundance in the nitrogen fixer *Aphanizomenon* in some years (1996) but not others (1997) (Chadwick et al. 1998). *Aphanizomenon* was rare, however, during the summer of 2003. *Anabena* was the most abundant nitrogen fixer, but was rare except for a brief interval near the middle of July. Among the other algae, the dominants belonged to genera that are commonly found in Colorado lakes. Among the green algae (Chlorophyta) a very small solitary coccoid green, *Chlorella minutissima*, was very abundant, as it is in many other Colorado lakes. The chlorophytes were most diverse, as is typically the case in lakes generally.

Table 5 summarizes the concentrations of chlorophyll *a* for the four treatments that were included in the enrichment experiments, as well as the initial conditions at the start of the experiment on each of the eight dates. The response is characterized in the last line of the table as indicating nitrogen limitation, phosphorus limitation, reciprocal limitation by nitrogen and phosphorus, or no limitation. No limitation would be suggested by very similar concentrations of chlorophyll *a* in the enrichments and in the controls; no such results were observed in this study. P limitation would be indicated by a marked response to P but no response to N; N limitation would be indicated by the reverse. Reciprocal limitation (defined by Morris and Lewis 1988) is designated when there is a response to both P and N alone, and typically an even larger response to P plus N. The statistical significance of the interpretations was determined through application of an analysis of variance test (ANOVA) supplemented by an a posteriori grouping procedure, the Bonferroni multiple range test. The results indicate N limitation

| | Cells/mL | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 21-May | 23-Jun | 14-Jul | 4-Aug | 18-Aug | 8-Sep | 29-Sep | 20-Oct* |
| CYANOPHYTA | | | | | | | | |
| *Anabaena perturbata* | | | 62000 | 5045 | 220 | 40 | 20 | |
| *Aphanizomenon issatschenkoi* | | | 300 | 3680 | | | | |
| *Aphanocapsa delicatissima* | | 150 | 7500 | | 101000 | | | |
| *Aphanothece smithii* | 14000 | 290000 | 145000 | 48000 | 20000 | 143000 | 200750 | |
| *Oscillatoria* spp. | 140 | 0 | 2650 | 12240 | 36835 | 20800 | 13920 | |
| BACILLARIOPHYTA | | | | | | | | |
| *Cyclotella* spp. | 2120 | 760 | 1760 | 80 | 1120 | 400 | 1120 | |
| *Fragilaria crotonensis* | | | 8080 | | | | | |
| *Stephanodiscus* spp. | 735 | 120 | 850 | 125 | 90 | 560 | 10 | |
| CRYPTOPHYTA | | | | | | | | |
| *Komma (Chroomonas acuta) caudata* | 7440 | 800 | 1040 | 2240 | 1840 | 800 | 640 | |
| PRASINOPHYTA | | | | | | | | |
| *Tetraselmis cordiformis* | | 2760 | 960 | | 1760 | 2880 | 1440 | |
| CHLOROPHYTA | | | | | | | | |
| *Chlorella minutissima* | 73500 | 1000 | 24250 | 2250 | 66000 | 5500 | 11250 | |
| *Crucigenia tetrapedia* | 1120 | 160 | 1920 | 80 | | 1280 | 80 | |
| *Diplochloris lunata* | 1720 | 2000 | | 480 | 4960 | 6960 | 17600 | |
| *Pseudodictyosphaerium* sp. | | 21000 | 8000 | | 320 | | 2000 | |
| *Scenedesmus* spp. | 760 | 1640 | 1960 | 200 | 1120 | 1440 | 4520 | |
| TOTAL DENSITY (cells/mL) | 101535 | 320390 | 266270 | 74420 | 235265 | 183660 | 253350 | |
| CYANOPHYTA | 14140 | 290150 | 217450 | 68965 | 158055 | 163840 | 214690 | |
| BACILLARIOPHYTA | 2855 | 880 | 10690 | 205 | 1210 | 960 | 1130 | |
| CRYPTOPHYTA | 7440 | 800 | 1040 | 2240 | 1840 | 800 | 640 | |
| PRASINOPHYTA | 0 | 2760 | 960 | 0 | 1760 | 2880 | 1440 | |
| CHLOROPHYTA | 77100 | 25800 | 36130 | 3010 | 72400 | 15180 | 35450 | |

*Sample impaired.

Table 4. Numerical abundances of the most important phytoplankton in Cherry Creek Reservoir, 2003.

| | 21-May | 23-Jun | 14-Jul | 4-Aug | 18-Aug | 8-Sep | 29-Sep | 20-Oct |
|---|---|---|---|---|---|---|---|---|
| Initial | 13.1 | 36.5 | 57.9 | 54.6 | 36.6 | 31.3 | 31.0 | 50.6 |
| Control | 9.7 | 18.2 | 61.6 | 26.0 | 28.7 | 23.4 | 26.8 | 32.5 |
| Add P | 8.0 | 19.3 | 77.3 | 28.9 | 29.1 | 28.9 | 26.8 | 38.6 |
| Add N | 41.2 | 45.6 | 70.9 | 47.4 | 42.9 | 44.8 | 50.7 | 59.0 |
| Add P+N | 35.4 | 46.3 | 93.9 | 52.2 | 42.8 | 40.7 | 53.2 | 64.9 |
| Response | N | N | N+P | N | N | N | N | N |

Table 5. Average chlorophyll concentrations (ug/L) for initial conditions and in experimental treatments at the end of the incubation period for each experiment. The nutrient response on each date is characterized on the basis of statistical analyses described in the text.

on seven out of the eight dates and reciprocal limitation by N and P on one date.

Table 3 shows the nutrient concentrations within each of the treatments at the end of the experiment, as well as the initial conditions. Added N was consistently reduced to very low concentrations by algal uptake, despite the relatively large addition of N (200-250 µg/L).

Primary production, measured as oxygen per unit area per hour or per unit of light, was high and without strong patterns during the growing season (Table 6).

| Item | 5/21 | 6/23 | 7/14 | 8/04 | 8/18 | 9/18 | 9/29 | 10/20 |
|---|---|---|---|---|---|---|---|---|
| Incubation, h | 2:00 | 2:15 | 2:00 | 2:07 | 2:10 | 1:55 | 2:35 | 2:10 |
| GPP, $gO_2$/m2/h (0-5 m) | 0.462 | 1.271 | 1.780 | 1.399 | 0.794 | 0.926 | 0.443 | 0.964 |
| NPP, $gO_2/m^2$/h (0-5 m) | 0.221 | 0.872 | 0.648 | 1.005 | 0.205 | 0.474 | 0.364 | 0.447 |
| Incident light, E/m2/h | 7.1 | 6.2 | 4.8 | 5.6 | 2.6 | 3.8 | 2.8 | 3.4 |
| Comparative yield, $mgO_2$/E | 64.9 | 204.2 | 367.7 | 249.0 | 306.8 | 246.0 | 159.7 | 286.6 |
| Depth of 1% light, m | 3.2 | 2.8 | - | 2.7 | 3.1 | 2.1 | 2.6 | 2.8 |
| Depth of zero NPP (m) | 4 | 3 | 2 | 4 | 2.5 | 2 | 3 | 2.5 |

Table 6. Primary production in Cherry Creek Reservoir. GPP = gross primary production; NPP = net primary production.

The zooplankton counts showed a relatively simple zooplankton community consisting mostly of small-bodied taxa (Appendix B). Zooplankton can contribute to the suppression of algal biomass in lakes when their grazing rates are high relative to algal abundances. For this reason, total zooplankton biomass was calculated as shown in Appendix B. The median zooplankton abundance was close to 600 $mg/m^2$ wet mass (0.6 $g/m^2$). An approximation of the potential effect of this mass of zooplankton on the phytoplankton can be derived from information on consumption rates, which for mixed zooplankton communities often cluster around 50% of body mass per day (Horn 1981). Chlorophyll *a* typically constitutes close to 1% of dry biomass of phytoplankton, or about 0.2% of the wet mass of phytoplankton. Therefore, chlorophyll concentration multiplied by 500 provides a rough approximation of phytoplankton wet mass. Estimates of phytoplankton biomass derived in this way and converted to a per unit area basis for comparison with the zooplankton yield an estimate of approximately 122 $g/m^2$ phytoplankton wet mass. Thus, the estimated consumption rate for the zooplankton community is roughly 50% times the zooplankton mass of 0.6 $g/m^2$ distributed over an algal mass of 122 $g/m^2$, or less than 1% per day. This estimate indicates that there is no reason to suspect significant effects of zooplankton grazing on the biomass of algae in Cherry Creek Reservoir. This situation is in fact typical of eutrophic lakes (Kalff 2001). Even this estimate is conservative insofar as the dominant zooplankton biomass (cyclopoid, apparently *Diacyclops thomasi*) is more likely to feed on protozoans than on phytoplankton (Dobberfuhl et al. 1997).

## Discussion and Interpretation

Many lakes in Colorado develop summer stratification during May caused by selective warming of the upper water column. The density differences inherent in warm water overlying cold water are sufficient to maintain a physical separation of the upper and lower layers (epilimnion, hypolimnion) until cooling in the fall weakens the density difference to such an extent that wind can break down the stratification. Lakes that have low relative depth (are shallow in relation to their size), however, may not sustain seasonal stratification. The shallowest of lakes (e.g., 2 m or less) are likely to mix on a daily basis because of nocturnal cooling. These are called continuous polymictic lakes (Lewis 1983). Lakes that have low relative depth but are deeper than the nocturnal mixing threshold may stratify temporarily for a period of days or even weeks, but do not sustain stratification for the entire season. These are called discontinuous polymictic lakes (Lewis 1983).

Discontinuous polymictic lakes can be identified on the basis of vertical profiles of temperature and oxygen. Such lakes often show temperature differences of several degrees between top and bottom, suggesting the possibility of stratification, and also show an erratic pattern of oxygen concentrations in deep water. When temperature differences are strong enough to keep the entire lake from mixing, oxygen depletion occurs, leading to low oxygen concentrations or complete anoxia near the bottom. The situation is reversed, however, during cool or windy weather when the stratification is lost and the deeper water is reoxygenated.

Cherry Creek Reservoir is a discontinuous polymictic lake. As indicated by the vertical profiles of temperature and dissolved oxygen, the lake shows some symptoms of thermal stratification, but the information on dissolved oxygen in deep water indicates that stratification does not last for long intervals. Thus, the mixing depth for Cherry Creek Reservoir changes substantially and erratically during the growing season.

Mixing events may affect availability of light and nutrients for phytoplankton. On 4 August, for example, a mixing event brought nutrients from an isolated deep layer, which probably was enriched with nutrients because of decomposition, into the surface layer when short-term stratification broke down. Thus, the mixing event had a fertilizing effect. During the mixing event, however, light deprivation could have slowed overall algal growth rates because most of the net production occurs in the top 1-2 m. Light deprivation and nutrient concentrations likely have complementary kinds of effects on the algal community. Light deprivation suppresses average growth rates even when nutrients are abundant, while the concentration of the limiting nutrient sets a cap on maximum biomass when light is sufficient to support growth.

Information on nutrient concentrations in the upper water column over the growing season in a lake can be suggestive of nutrient limitation, although a definitive determination of limitation requires enrichment studies. Because the upper water column of Cherry Creek Reservoir was chronically deficient in dissolved inorganic nitrogen during 2003, nitrogen deficiency might be suspected. Although soluble reactive phosphorus was not abundant, total soluble phosphorus, which is potentially available for uptake by algae (Kalff 2002) was steadily present in amounts that would suggest a sufficiency of phosphorus for phytoplankton.

The enrichment studies provide unambiguous evidence of nitrogen limitation throughout the growing season. The control incubations, to which no nutrients were added, consistently showed a decline in biomass as compared with the initial condition. The suppression of growth in the control incubations is explained by failure of the algae in the enclosures to benefit from nutrient recycling mechanisms that operate in the lake, thus creating greater extremes of nutrient deficiency than would be observed in the lake itself. Reciprocal limitation, which occurred on one date, occurs when the availability of both nitrogen and phosphorus is marginal with respect to the needs of the algae for growth. The exact mechanisms are not well understood, but they may involve one group of species that is more acutely starved for nitrogen and another group that is more acutely starved for phosphorus, in which case there are responses to the addition of either nutrient and especially strong responses to the addition of both nutrients.

Implications of nutrient limitation vary according to the severity of limitation. Under the most severe circumstances, growth may cease, in which case there may be a regression of biomass, as observed in the control incubations. In nature, nutrient limitation typically is less extreme. The critical nutrient (in this case nitrogen) typically is available in small amounts through recycling mechanisms, but not at sufficient concentrations to support biomass accumulation. Thus, biomass often is sustained, but at a level below what would be expected in the absence of nutrient limitation (200-300 μg/L chlorophyll *a* is the approximate biomass limit in the absence of nutrient limitation).

The photosynthesis measurements provide information on the growth rates of the algae in the upper water column. Algal production per unit area for Cherry Creek Reservoir falls within the expected range for eutrophic lakes. Because of high biomass

leading to rapid extinction of light in the water column, however, positive net production is limited to the upper 2 m of the water column (primarily 0-1 m). Under windy conditions, turbulence and wind-generated currents move the algal population across the full range of depths that range from those where the light climate is ideal for photosynthesis (0-1 m) to those where net photosynthesis is negative and algal growth is not possible (2-7 m). When the water column is calm, the portion of the population within the top 2 m has sufficient light to build up biomass, but is unable to surpass specific limits (about 50 μg/L chlorophyll *a*) that are determined by the limiting nutrient (N). An increase in the amount of inorganic nitrogen in the lake would reduce the severity of limitation on biomass accumulation near the surface under calm conditions, thus producing more extreme peaks of algal biomass than are currently observed.

Another viewpoint on the abundance of algae in Cherry Creek Reservoir can be obtained from data on algal abundance in relation to phosphorus concentrations. The Redfield ratio is often used to define the relationships between carbon, phosphorus, and nitrogen in healthy cells that occupy a nutrient scarce environment (Sterner and Elser 2002). The ratio of C:P in phytoplankton cells under these conditions is 106:1 on a molar basis and 41:1 on a mass basis. P starvation begins when P declines to about 0.5% P dry mass (Healey 1982, Nalewajko and Lean 1980), which corresponds to a C:P mass ratio of about 100:1.

The ratio of carbon to chlorophyll *a* for phytoplankton is variable, but clusters around a value of 30 (Saraceni et al. 1978). With this ratio as a basis for estimating carbon in phytoplankton biomass, and with the measurements of particulate phosphorus in the water column, which typically is concentrated in phytoplankton biomass, it is

possible to estimate the C:P ratio for phytoplankton on each of the eight dates in the upper water column of Cherry Creek Reservoir. The results are shown in Table 7. As indicated by the table, the C:P ratios are far less than 100, suggesting that the phytoplankton on all of these dates had internal phosphorus supplies exceeding their immediate phosphorus requirements. This phenomenon, which is designated "luxury

| | 21-May | 23-Jun | 14-Jul | 4-Aug | 18-Aug | 8-Sep | 29-Sep | 20-Oct |
|---|---|---|---|---|---|---|---|---|
| Chl *a*, µg/L | 13.1 | 36.5 | 57.9 | 54.6 | 36.6 | 31.3 | 31.0 | 50.6 |
| Carbon, µg/L | 393 | 1095 | 1737 | 1038 | 1098 | 939 | 930 | 1518 |
| Particulate P, µg/L | 26.3 | 44.1 | 40.6 | 55.4 | 48.3 | 49.8 | 50.4 | 44.1 |
| Mass Ratio, C:P | 15 | 25 | 43 | 30 | 23 | 19 | 18 | 34 |
| Excess P, µg/L | 22 | 33 | 23 | 45 | 37 | 40 | 41 | 29 |
| Total P, µg/L | 49 | 72 | 64 | 85 | - | 74 | 69 | 63 |
| Excess P, % | 45 | 46 | 36 | 53 | - | 54 | 59 | 46 |

Table 7. Estimation of excess P available to phytoplankton in Cherry Creek Reservoir, 2003.

consumption," is characteristic of phytoplankton exposed to external concentrations of phosphorus above their immediate needs (Reynolds 1984). It indicates that phosphorus was not limiting, as also shown by the enrichment studies.

Information in Table 7 can be used in estimating the minimum amount of phosphorus that would need to be removed from the water column in order to induce phosphorus limitation in the phytoplankton. This minimum is the amount of phosphorus tied up in phytoplankton biomass in excess of a C:P mass ratio of 100:1. The estimates shown in Table 7 could be considered conservative insofar as they exclude TDP, some fraction of which would likely be taken up by phytoplankton if the phytoplankton were not limited by nitrogen.

Table 7 indicates that about half of the total P in the upper water column on most dates during 2003 was in excess of the needs of phytoplankton for growth under the nitrogen limiting conditions prevailing in the reservoir. Thus, a reduction of phosphorus concentrations by at least half would be required in order to induce a state of phosphorus limitation in the reservoir. As shown in Figure 1, no beneficial results from phosphorus reduction could be expected until this threshold is approached.

## Conclusions

The phytoplankton biomass of Cherry Creek Reservoir during 2003 was limited by nitrogen deficiency. If nitrogen became more available to the phytoplankton, they would likely respond with an increase in biomass. An increase in nitrogen availability could occur through an increase in source strength within the watershed or through the development of more extensive blooms of nitrogen fixing bluegreen algae (heterocystous cyanobacteria). Conditions in the lake seem generally unfavorable for nitrogen fixing bluegreens at present, probably because of the suppression of these taxa by frequent deep mixing of the reservoir, which is related to the low stability of the reservoir during the summer. Reduction in phosphorus concentrations sufficient to induce phosphorus deficiency in the phytoplankton of year 2003 would involve decreases in upper water column concentrations of at least 50%, or about 30 µg/L.