# NUTRIENT LIMITATION AND TOP-DOWN, BOTTOM-UP CONTROLS ON PHYTOPLANKTON IN MIRROR LAKE

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*Abstract.* Primary production in many freshwater lakes is limited by nutrient availability. The nutrients most commonly limiting are nitrogen or phosphorous, and many lakes are co-limited by both nutrients. Experiments in the 1970's determined that Mirror Lake was co-limited by nitrogen and phosphorous. The purpose of this experiment was to determine whether nutrient limitation in Mirror Lake had changed since that time. In situ nutrient enrichment experiments performed in polyethylene enclosures anchored in the lake were used to determine the limiting nutrients. The results of the experiment showed that the lake was still co-limited by both nitrate and phosphate. Historical lake concentrations and influxes of nutrients into the lake were also examined and supported the findings that nutrient levels in the lake had not changed since the experiments in the 1970's. The experiments in the 1970's also found that after nitrogen and phosphorous limitation was relieved, another factor became limiting. It was suggested that zooplankton grazing exhibiting a top down control may have been responsible for this limitation. This experiment also explored that possibility; however, results were inconclusive because of a simultaneous decline in production in all of the experimental enclosures. The decline in all of the enclosures suggests limitations to the method of experimentation used. Further exploration is necessary in order to discover these limitations.

## INTRODUCTION

Lakes are often classified by the amount of nutrient availability: eutrophic (nutrient-rich), oligotrophic (nutrient poor), or mesotrophic (moderately nutrient rich). A natural succession from oligotrophic to eutrophic occurs in all lakes at varying speeds, but this natural progression can be accelerated by anthropogenic factors, such as runoff from farms or developed areas in a process called cultural eutrophication (Harper 1992). Eutrophication, whether natural or artificial, results in changes in the lake ecosystem. Phytoplankton are the first organisms to be affected, increasing in numbers and biomass as they are no longer limited by nutrients. Because phytoplankton are the base of aquatic foodwebs, changes in their productivity can impact other trophic levels. This increase in primary production can cause an increase in biomass at higher trophic levels by supplying more food for these organisms. As production continues to increase and organisms begin to die and decay, dissolved oxygen is removed from the system through the respiration of decomposers feeding on the increased amount of dead biomass. This creates anoxic conditions and this lack of oxygen can lead to fish kills (Harper 1992).

Elser et al. (1990) found that many freshwater temperate lakes are co-limited by both nitrogen and phosphorous. As inputs of these nutrients are increased by human activities, phytoplankton in lakes are released from their limitation by nitrogen and phosphorus, and two things occur: either eutrophication is accelerated, or another nutrient becomes limiting before eutrophication occurs. If eutrophication occurs, the effects described above may occur. If another nutrient becomes limiting, however, other effects could occur. One possible effect would be a change in the species composition of the phytoplankton community, which could result in changes in the zooplankton community, etc., resulting in a change in the ecology of the lake (Elser et al. 1990).

Human development and activities have been causing the acceleration of eutrophication in many lakes around the world by causing increased influxes of nitrogen and phosphorous. Since the industrial revolution began, chemicals generated by human activities have increased in the atmosphere. With these increases, the amount of these nutrients, especially nitrogen, entering lake systems has also increased, through both wet and dry deposition. Acid deposition resulting from the emissions of $NO_x$ gases from industrial processes adds nitrogen to these water bodies. Land use changes also increase the amount of nutrients entering a lake through runoff. Land clearing for development or agriculture results in leaching of nutrients from the soil, which then runoff into streams, lakes, and rivers, increasing nutrient levels, especially phosphorous. Both nitrogen and phosphorous are also leached into lakes and rivers from fertilizers used for agriculture, gardening, and lawn care (Harper 1992). Another input of phosphorous into rurally developed lakes is possibly from septic tank leakage that diffuses into lake waters (Moore et al 2003).

In order to study the effects and progression of eutrophication, and also to prevent it from occurring, lakes must be monitored. One of the most common ways to do this is to determine the limiting nutrients through enrichment experiments (Harper 1992). Nutrients are purposely added to a system in order to determine which nutrients will cause an increase in phytoplankton production and biomass. It is addition of the limiting nutrients that causes eutrophication. Additions of non-limiting nutrients will not result in increased biomass, so nutrients that cause increased growth should be prevented from entering the system.

Mirror Lake is a small oligotrophic lake in the White Mountains region of New Hampshire (Likens 1985). It has a surface area of approximately 15 ha, and a maximum depth of 10.9 meters. In the 1970's, it was determined that Mirror Lake was limited by both nitrogen and phosphorous (Gerhart 1973). Using polyethylene enclosures and chemostat bioassays, Gerhart found that it was only with the addition of both nitrogen and phosphorous together, that phytoplankton production increased significantly.

Since nitrogen and phosphorous were no longer limiting in the Gerhart (1973) study, phytoplankton productivity should have increased until all of the extra nitrogen and phosphorous was utilized, however this did not occur. Instead, Chlorophyll *a* concentrations, used to measure phytoplankton productivity, reached a plateau. This suggested a new limitation had been reached after the primary limitation of nitrogen and phosphorous had been overcome. Limitation by other nutrients, such as carbon, was explored, but none of the other nutrients tested were found to be limiting. Another explanation for this limitation was that zooplankton grazing was preventing phytoplankton biomass from rising further. Grazing experiments were inconclusive, however (Gerhart 1973).

Land around Mirror Lake and its watershed has been developed since the 1970's. In his thesis Gehart indicated that development may have already been affecting lake productivity. He cited evidence of blooms of filamentous algae in areas where the inlets entered the lake, and an increase in pH, which may have indicated an increase in productivity (Gerhart, 1973). Seepage from septic tanks was suspected to be a significant source of phosphorous and possibly nitrogen in the form of ammonium (Likens 1985).

The purpose of this experiment was to determine whether the lake chemistry had changed since Gerhart's experiment, specifically whether the limiting nutrients had changed, using in situ nutrient enrichment experiments. Another goal of this experiment was to explore the relationship of zooplankton grazing on phytoplankton productivity, in order to determine if grazing was a stronger control on phytoplankton biomass than nutrient limitation.

The results expected from this experiment were that nutrient levels in Mirror Lake had increased since the 1970's, however, the increase would not be large enough to have changed the limiting nutrients (nitrogen and phosphorous) in the lake. Removal of zooplankton was expected to cause an increase in phytoplankton biomass, but was not expected to be a stronger control than nutrient limitation because the lake is oligotrophic. Spencer and Ellis (1998) found that in Flathead Lake, another oligotrophic lake, the bottom-up control of nutrient limitation was stronger than the top-down control of zooplankton grazing on phytoplankton biomass. Phytoplankton populations in oligotrophic lakes may exist at levels below a certain threshold level where zooplankton grazing would significantly impact the phytoplankton population (Spencer and Ellis 1998). Similar findings were observed in an earlier study by Elser and Goldman (1991), in which they found that zooplankton grazing was an important process in mesotrophic lakes, but was not very important in oligotrophic and eutrophic lakes.

In Gerhart's (1973) experiments, chlorophyll *a* concentrations increased from ambient concentrations to approximately 6 $^{\mu g}/_{L,}$ but did not increase any further, which could support Spencer and Ellis's findings of a threshold level. The same plateau Gerhart found in his experiments was expected in this experiment, except for the enclosures where zooplankton were removed. Enclosures where zooplankton were removed would exhibit an increase in chlorophyll concentrations due to the removal of grazing pressure.

## METHODS

In situ nutrient enrichment experiments were conducted using polyethylene enclosures similar to the experiments conducted by Goldman (1962) and Gerhart (1973). Each enclosure had a diameter of approximately 0.6 m and a depth of 4 m, with a volume of approximately 1100 L. Each enclosure consisted of an outer and an inner tube to protect against damage and spillage. Contrary to the study by Gerhart (1973), the enclosures were heat sealed at the bottom, so they were totally enclosed from lake to prevent nutrients from leaking into the lake. The enclosures had a spill guard at the top of the tube, which extended approximately 0.25 m above water level, to protect against spillage caused by wave action. Each enclosure was attached to one of two rafts anchored in the lake at a place where the water depth was approximately 5 m. Four treatments used in this study were: control (C), nitrogen addition (+N), phosphorous addition (+P), and a combined nitrogen and phosphorous addition (+N +P). Each of the two rafts contained a total of four enclosures, one for each of the four treatments used. The location of each treatment was randomly chosen on each raft separately.

Nutrients were added to each of their respective enclosures every morning between the hours of 7am and 10am, before productivity reached its peak. A 4 m length of tube was inserted into the enclosure and nutrients were poured into the tube which was then removed in a quick, but steady motion from the enclosure; distributing the nutrients throughout the water column of the enclosure. Phosphorous was added in the form of $Na_2HPO_4$ in an amount that would yield an addition of a concentration of 35 $^{\mu g}/_{L}$, and nitrogen was added in the form of $NaNO_3$ in an amount that would yield an addition of a concentration of 70 $^{\mu g}/_{L}$.

Just before nutrients were added each day samples were taken from each enclosure at a depth of 1.5 m. Samples were collected using a 1.5 m hose and a battery-powered pump. A sample from each enclosure was pumped out and taken back to the lab where a sub-sample was then filtered and analyzed fluorometrically for chlorophyll *a* concentrations. Samples for determination of nitrogen and phosphorous concentration were also filtered and stored frozen for later analysis. Data was collected for 18 days, beginning July 24, 2005, and ending August 12, 2005. On August 5, 2005 zooplankton were removed from the enclosures on Raft 2 in an effort to discover whether zooplankton grazing was a top-down control on phytoplankton populations. This removal was done by towing a plankton net through each enclosure 15 times.

Historical data of nutrient concentrations in the lake were analyzed to show the history of nitrogen and phosphorous concentrations in the lake. Some of this information was obtained from Likens (1985). This data was then reviewed and average summer concentrations were extracted from each year from 1967 to 1999. Influxes of nitrogen and phosphorous to the lake from streams and precipitation were also examined.

## RESULTS

### *Historical nutrient analysis*

Data for the influx of nitrate and phosphate was analyzed to determine the yearly influx of each nutrient between 1981 and 2000. The average yearly influx of nitrate into Mirror Lake for this time period was approximately 329 kg, with a high of 423 kg in 1990, and a low of 259 kg in 1999. The influxes from the three inlets to the lake

(West, Northwest, and Northeast inlets) were small compared to the total input (Fig. 1). The Northeast inlet contributed the smallest amount of influx to the lake, with a high of 2.82 kg. The West inlet contributed the most nitrate of the three inlets, introducing a larger amount of nitrate in most years compared to the other two inputs. Until 1992, the West inlet averaged an input between 20 and 30 kg, before it declined to an input of less than 10 kg per year. The largest contribution of the total influx came from precipitation. The influx from precipitation was almost as high as that of the total influx, and the total influx mirrored the same trends as precipitation.

The average yearly influx of phosphate into Mirror Lake between 1981 and 2000 was approximately 8.91 kg, with a high of 16.4 kg and a low of 2.01 kg. The Northeast inlet contributed the smallest amount of phosphate, with a high of 0.504 kg. The total influx was influenced almost equally by precipitation and the West and Northwest inlets. Total phosphate influx showed three distinct peaks (Fig. 2). The first peak coincided with a steep increase in influx from the West inlet, and the third peak overlapped a steep increase in the influx of precipitation, while the second peak corresponded with an increase in both the West and Northwest inlets and in precipitation. In 1998, the phosphate inputs from all three inlets decreased to almost zero, and the total influx into the lake was almost exactly the same as the influx from precipitation.

Data collected between 1967 and 1999 was analyzed in order to obtain the history of summer nitrate and phosphate concentrations in Mirror Lake (Fig. 3). Concentrations of nitrate were initially high between 1967 and 1970, then dropped to lower concentrations until rising sharply to its highest concentration of 0.217 $^{mg}/_{L}$ in 1989. The concentration of nitrate then dropped again to less than 0.05 $^{mg}/_{L}$. With the exception of 1971 when concentrations rose to 0.278 $^{mg}/_{L}$, phosphate concentrations remained less than 0.05 $^{mg}/_{L}$.

### *Nutrient limitation experiments*

Chlorophyll *a* concentrations taken from replicate enclosures in both rafts were consistent (Figures 4 and 5). In both rafts the chlorophyll concentrations of the control, +N, and +P enclosures were similar to chlorophyll concentrations measured in open waters of the lake, fluctuating around 1$^{\mu g}/_{L}$. There was some deviation in corresponding enclosures between the two rafts, however, this deviation was small compared to the response observed in the +N+P enclosures. The chlorophyll concentration of +N enclosure on Raft 1, as well as the control and the +P enclsosures on Raft 2, initially rose to concentrations just above 2 $^{\mu g}/_{L}$, but then decreased back to around 1 $^{\mu g}/_{L}$.

The chlorophyll concentrations in the +N +P enclosures on both rafts also exhibited patterns similar to each other, although these patterns differed from the other enclosures and from the lake. Concentrations in both +N +P enclosures rose above the levels of the other enclosures on July 26, and continued to fluctuate between 3.0 and 5.5 $^{\mu g}/_{L}$, until approximately August 7, when chlorophyll concentrations began to decrease to lake levels by the end of the experiment. The concentrations of chlorophyll *a* in the other enclosures also decreased below lake concentrations to concentrations less than 0.5 $^{\mu g}/_{L}$ at about the same time the +N +P enclosures began to decline.

Zooplankton were excluded from Raft 2 on the afternoon of August 5; after morning chlorophyll samples were taken. After that date the chlorophyll concentrations began to decline in both rafts. The +N +P enclosure on Raft 2 reached its peak concentration on the morning that zooplankton were excluded, but then declined steadily until the end of the experiment. Chlorophyll levels in the other three enclosures on Raft 2 also declined during this time. Zooplankton were not excluded from Raft 1, but also had declining chlorophyll concentrations. The +N +P enclosure on Raft 1 did not decline steadily as its counterpart on Raft 2 did, but instead rebounded and increased a couple days after August 5th, before finally declining until the end of the experiment.

## DISCUSSION

Historical data on the influx of nitrate and phosphate was analyzed to help determine whether nutrient levels in the lake had changed. Nitrate and phosphate inputs appear to have declined slightly from 1981 to 2000. However, there is considerable variability in the annual inputs, which suggests this decrease may not be substantial or permanent. The total influx of nitrate into the lake is derived mainly from precipitation to the lake. The increases and decreases in the total influx seem to be due to differences in the amount of nitrate carried in precipitation from year to year. Nitrate inputs from the three inlets contribute only a small amount of nitrate to the total influx. Influences on total phosphate influx were more evenly balanced among the different inputs. Although the northeast inlet contributed little to the total influx, the other two inlets and precipitation accounted for almost equal parts, at least until 1998. Beginning in 1998, influx of phosphate from precipitation was the principal source, and influx from all three inlets was almost zero. The influx of phosphate from precipitation had not increased significantly above past levels, however, so this decrease in total phosphate influx was due to the decreased phosphate levels in the inlets.

The concentrations of nitrate and phosphate in the lake between 1967 and 1999 were also analyzed. This data showed that summer concentrations were fairly constant over the years. There was a large spike in both nutrient concentrations throughout this history, but concentrations again settled to a constant level. Overall, the historical data from influx and lake concentration, seem to indicate that there has not been a significant change in nutrient levels in Mirror Lake, which would be a good indication that the limiting nutrients have not changed either. Since nitrogen and phosphorous were the limiting nutrients in the 1970's, and since the concentration of these nutrients does not seem to have changed since then, it is reasonable that these nutrients are still the limiting nutrients in the lake.

The conclusion that the limiting nutrients in Mirror Lake have not changed is also supported by the experimental data obtained from the enrichment experiments. Only the addition of nitrogen and phosphorous together led to an increase in chlorophyll *a* concentrations above those of lake concentrations. Chlorophyll *a* concentrations in the enclosures treated with nitrogen and phosphorus increased to a high of approximately 5.5 $^{\mu g}/_{L}$ similar to the 6 $^{\mu g}/_{L}$ maximum in Gerhart's (1973) study. Chlorophyll *a* concentrations did not stay at 5.5 $^{\mu g}/_{L,}$ but rather reached a peak at that level and then declined to concentrations observed in the lake. This same pattern was observed in Gerhart's experiment; chlorophyll concentrations increased to a peak around 5.5 to 6.0 $^{\mu g}/_{L,}$ and then declined toward lake concentrations (Gerhart, 1973). In an effort to discover the reason for this decline, Gerhart performed experiments in which other nutrients were also added, but these additions did not result in an increase, so it was determined that perhaps zooplankton grazing was the factor that prevented chlorophyll concentrations from increasing past 6 $^{\mu g}/_{L}$ in the past experiments.

In an attempt to further explore the Gerhart's suggestion of a relationship between zooplankton grazing and chlorophyll concentration, zooplankton were excluded from the enclosures on Raft 2 after the experiment had been running for approximately two weeks. Results were inconclusive, however. Just after the zooplankton were excluded the chlorophyll concentrations in the enclosures began declining. Raft 1, the raft in which zooplankton was still present, began to decline first. Raft 2 reached its peak concentration on the day that exclusion occurred, and then declined to lake levels. There was no evidence that the enclosure zooplankton was excluded from had any increase in chlorophyll concentration over that of the enclosure that had zooplankton still present. In fact, the opposite seems to have occurred. While Raft 2 showed a steady decline, concentrations in Raft 1 actually rebounded after an initial decline before decreasing to lake levels.

The +N +P enclosures were not the only enclosures where concentrations declined. All of the enclosures on both rafts began declining at approximately the same time. This suggests that something may have been depleted from all of the enclosures. Perhaps a common nutrient, other than nitrate or phosphate, was depleted from all of the enclosures. This depletion may have occurred due to the length of the experiment, which was perhaps too long. Lack of mixing with lake water due to the nature of the enclosures may also have been a factor. Because the enclosures were sealed at the bottom, and had an extension high enough above lake level to prevent wave mixture, any nutrients that were depleted would not have been replaced unless they were added artificially into the columns.

## CONCLUSION

Historical data showed that nitrate and phosphate concentrations in Mirror Lake do not seem to have changed. The results of the nutrient enrichment experiments demonstrated that the lake is still limited by nitrogen and phosphorous. Zooplankton removal experiments were inconclusive because chlorophyll *a* concentrations in all of the enclosures began to decline at about the same time as zooplankton removal. This decline suggests that there may be limitations to the enclosure experiments where the water in the enclosures are separated entirely from lake waters so that mixing is not allowed. While the enclosures may keep some of the properties, such as temperature and light exposure the same as lake waters, other properties, such as mixing are lost. Loss of mixing may be responsible for the decline in chlorophyll concentrations. Because of this, in future experiments like this one, it would be beneficial to keep track of other nutrient concentrations throughout the experiment, as well as dissolved oxygen levels and other properties such as pH, in order to determine what is responsible for these declines.

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## LITERATURE CITED

Elser JJ and Goldman CR. 1991. Zooplankton effects on phytoplankton in lakes of contrasting trophic status. Limnol. Oceanogr. 36: 64-90.
Elser JJ, Marzolf ER, and Goldman CR. 1990. Phosphorous 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.
Gerhart DZ. 1973. Nutrient limitation and production of phytoplankton in Mirror Lake, West Thornton, New Hampshire.—Ph.D. thesis, Cornell Univ. 160 p.
Gerhart DZ. 1975. Nutrient limitation in a small oligotrophic lake in New Hampshire. Verh. Internat. Verein. Limnol. 19: 1013-1022.
Goldman CR. 1962. A method of studying nutrient limiting factors in situ in water columns isolated by polyethylene film. Limnol. Oceanogr. 7: 99-101.
Harper D. 1992. Eutrophication of Freshwaters: Principles, problems and restoration. Chapman & Hall, London, Great Britain.
Likens,G.E. 1985. An Ecosystem Approach to Aquatic Ecology: Mirror Lake and Its Environment. Springer-Verlag.
Moore JW, Schindler DE, Scheuerell MD, Smith D, & Frodge J. 2003. Lake Eutrophication at the Urban Fringe, Seattle Region, USA. Ambio 32: 13-18.
Spencer CN and Ellis BK. 1998. Role of nutrients and zooplankton in regulation of phytoplankton in Flathead Lake (Montana, U.S.A.), a large oligotrophic lake. Freshwater Biology 39: 755-763.

# APPENDIX

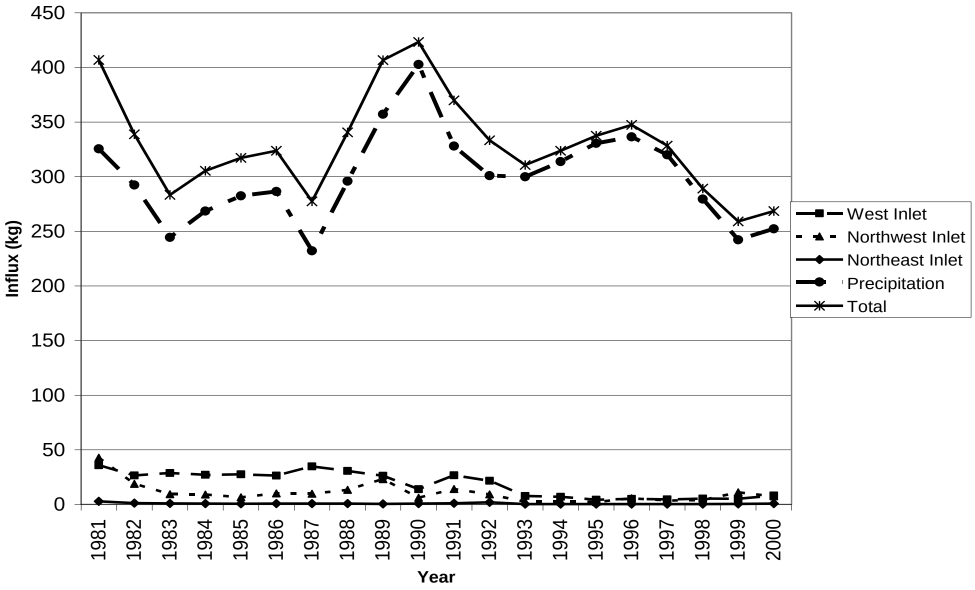

**FIGURE 1**. Inputs of nitrate into Mirror Lake between 1981 and 2000.

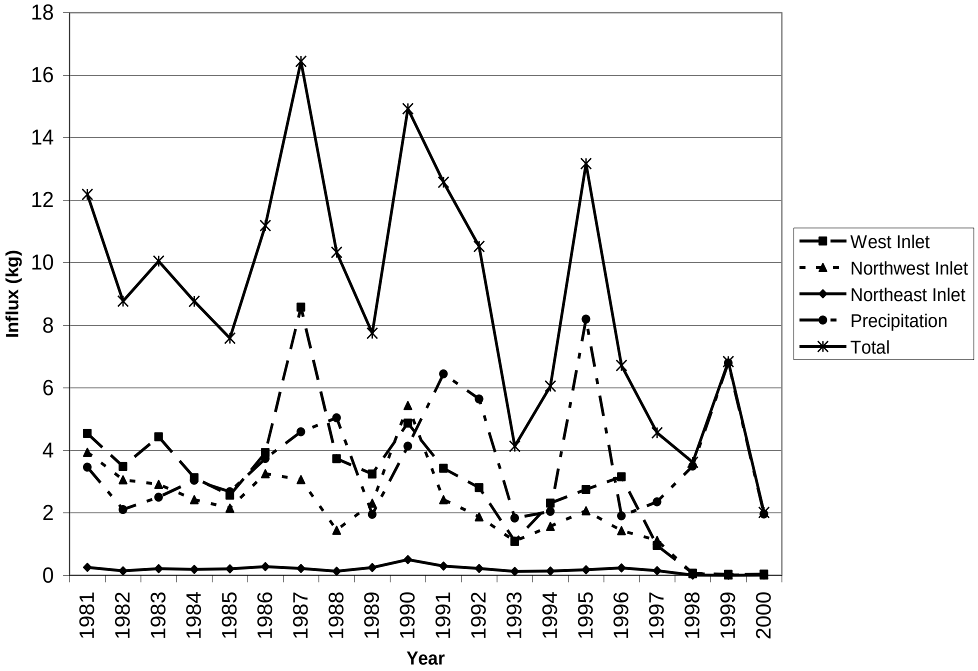

**FIGURE 2.** Inputs of phosphate into Mirror Lake between 1981 and 2000

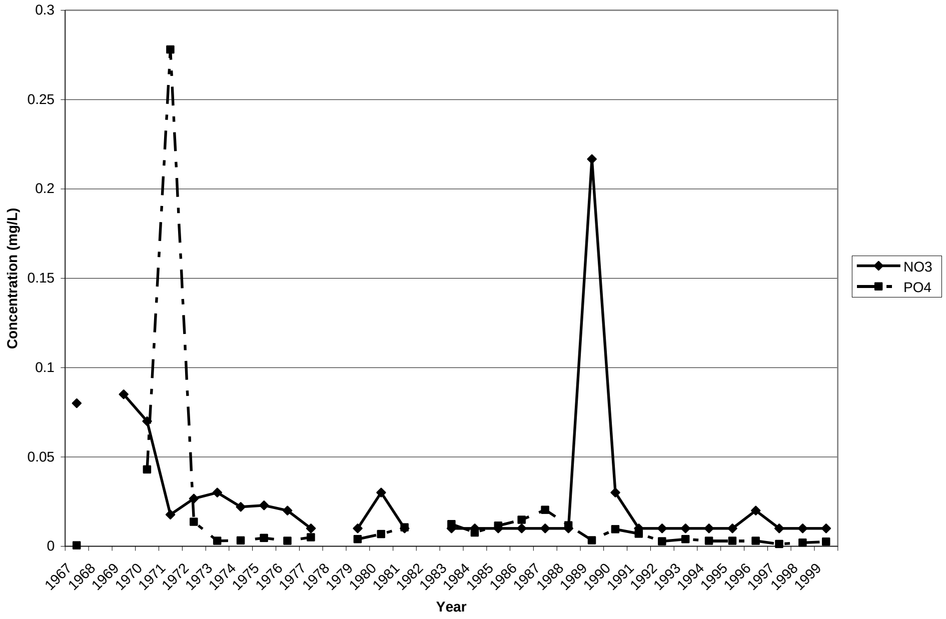

**FIGURE 3.** Average Summer Concentrations of Nitrate and Phosphate in Mirror Lake from 1967 to 1999

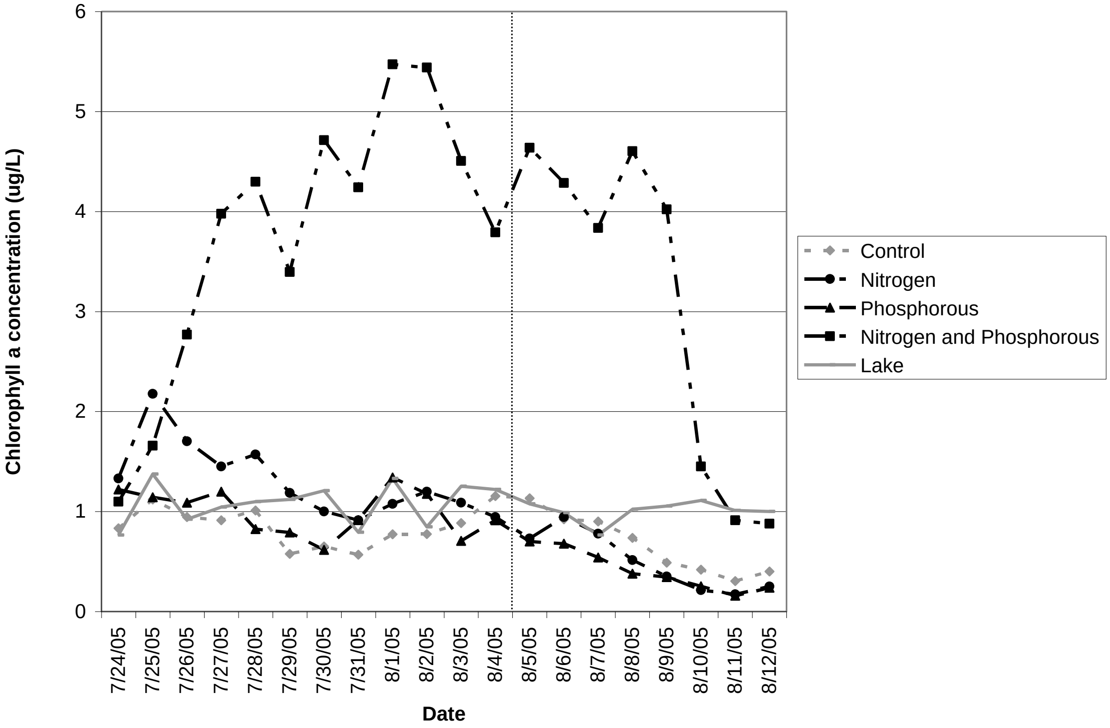

**FIGURE 4.** Chlorophyll *a* concentrations in Raft 1 enclosures. Vertical dotted line marks date of zooplankton exclusion from Raft 2 enclosures.

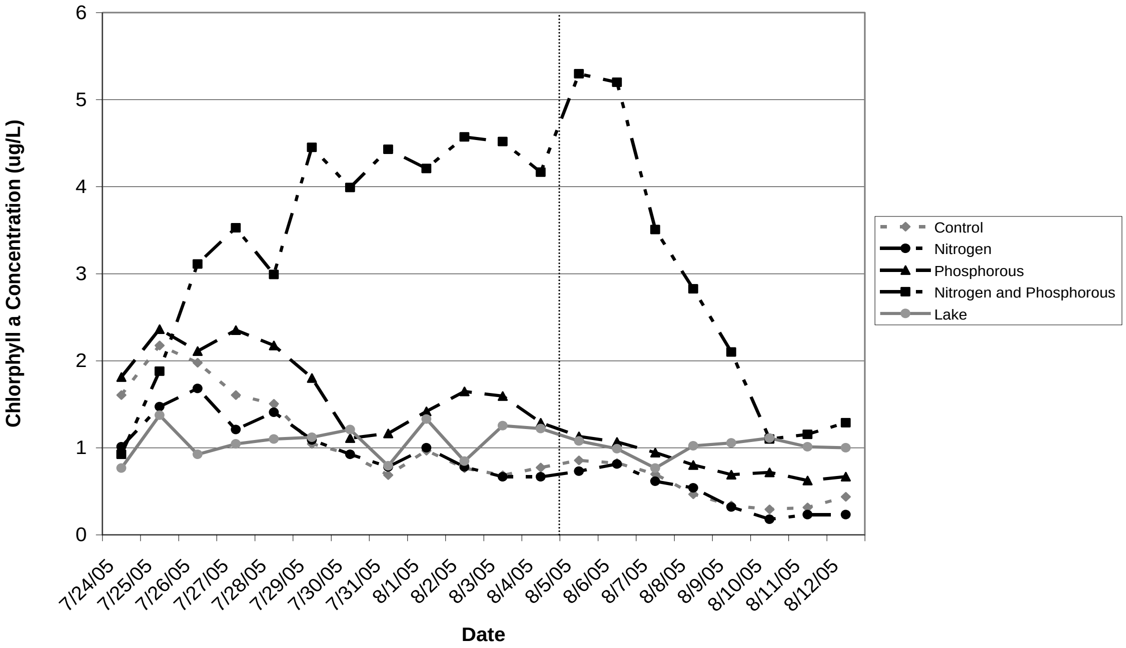

**FIGURE 5.** Chlorophyll a concentrations of Raft 2 enclosures. Vertical dotted line indicates the date zooplankton were excluded from Raft 2 enclosures.