EXPLORING THE POSSIBILITY OF EUTROPHICATION IN MIRROR LAKE

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Abstract. In 2005, algal production was found to be limited by both nitrogen and phosphorus in the small, oligotrophic Mirror Lake of West Thornton, NH. In this study, in situ nutrient enrichment experiments were used to explore how increasing nitrogen and phosphorus additions would affect the productivity of the lake and at what concentrations might eutrophication occur. Nitrogen and phosphorus were added to 16 mesocosms in a range from 0 to 70 PO_4 μg L^{-1} d^{-1} (the ratio of N: P constant at 4.4). The experiment ran for 11 days, and the systems were replenished with nutrients every other day. Surrogates for primary productivity used in this study included chlorophyll *a* (Chl. *a*) concentrations and oxygen production in the mesocosms. It was found that all levels of nutrient additions caused an increase in both Chl. *a* and the concentration of dissolved oxygen. The fourth highest addition rate, 26.3 PO_4 μg L^{-1} d^{-1} and 52.5 NO_3 μg L^{-1} d^{-1} increased the final P level to 0.025 P- PO_4 (mg L^{-1}) and the final N content to 0.45 mg N-NO_3 (mg L^{-1}). While these levels are considered eutrophic, even the highest addition rate, 70 PO_4 μg L^{-1} d^{-1} and 140 NO_3 μg L^{-1} d^{-1}, had Chl. *a* concentrations indicative of only slight eutrophication. This study highly suggests that another factor may be limiting productivity in Mirror Lake.

INTRODUCTION

Eutrophication is defined as the over-enrichment of a water body by an increase in nitrogen and phosphorus loading, leading to excessive algal and macrophyte growth. While eutrophic systems are highly productive, these elevated rates of productivity can lead to the degradation of water quality, secondary declines in dissolved oxygen content, toxic algal blooms, loss in macrophyte diversity, and fish kills.

Eutrophication is a naturally occurring process; however, the increase of human activity in an environment often leads to the acceleration of productivity in a water system. Use of fertilizers, land clearing, and improper sewage disposal, are but a few of the activities associated with rising nutrient levels in water bodies (Harper 1992) In addition, atmospheric depositions from industries and fossil fuel burning contribute a great amount of nitrogen to aquatic ecosystems (Carpenter et. al 1998). Further studies have shown that in areas where atmospheric depositions are high, phosphorus often becomes the limiting nutrient (Bergstrom and Jansson 2006, Bergstrom et. al 2005). However, co-limitation of both nitrogen and phosphorus in lakes is not uncommon (Elser et al. 1990, Maberly et al. 2002).

Mirror Lake, located in West Thornton, NH, is classified as a small oligotrophic lake, meaning it is nutrient poor. As Mirror Lake is part of the Hubbard Brook Ecosystem Study, long-term ecological research project, a large amount of data have been collected regarding the quality of the lake since the late 1960's (Likens 1985). In the early 1970's, Gerhart (1973) determined that both nitrogen and phosphorus were limiting algal growth in Mirror Lake. Since that time, there has been continued human activity in the Mirror Lake watershed. Roads have been built, houses have been both constructed and demolished, and septic tanks continue to leach P and NH_4^+ into the subsoil (Likens 1985). This runoff, in addition to mounting atmospheric NO_x depositions (Likens 2004) and overall increased human use of the lake, has concerned many people about the current trophic status of Mirror Lake. However, recent studies investigating possible biogeochemical changes showed the lake continues to be limited by both nitrogen and phosphorus (Bukaveckas and Shaw 1998, Bouchard 2005).

Nevertheless, it remains unknown by what concentration of nitrogen and phosphorus the phytoplankton of Mirror Lake are limited. Will adding low levels of nitrogen and phosphorus to Mirror Lake cause an increase in phytoplankton productivity or is cause for the concern of eutrophication a long way off? If eutrophication is of immediate concern, even adding small amounts of N and P to the lake will cause an increase in algal productivity. However, if the lake were still severely limited by both N and P, it would take a great amount of added nutrients to cause increased algal productivity.

Based on the work by Gerhart (1973), there is some indication that something else may also be limiting algal growth. Therefore, this study is additionally interested in seeing at what level of nutrient additions these other factors may become limiting. Using an in-situ nutrient enrichment experiment, measuring Chl. *a* and oxygen levels to estimate productivity, this study aimed to analyze what amount of nitrogen and phosphorus were necessary to trigger surplus algal growth in Mirror Lake, and if there existed a certain level above which algal growth was suppressed.

METHODS

Sixteen thin polyethylene enclosures were constructed for the in-situ nutrient enrichment experiment. Each tube was built with both an outer and inner layer to protect against any possible damage or leaks. Tubes extended 0.5 m above the waterline to prevent spills from waves. Each tube was approximately 0.6 m in diameter and 4-m long, capable of holding a volume of approximately 1100 L. Unlike the experiments conducted on Mirror Lake by Gerhart (1973), the present experiments were completely enclosed. After construction, the tubes were mounted on floating rafts, and anchored in Mirror Lake at a location with an approximate water depth of 5 m.

Mirror Lake has a maximum depth of 11 m and a mean depth of 5.75 m. During mid-summer, the time when these experiments took place, the upper mixed layer or epilimnion, usually extends about 4-m below the surface. However, light transmission extends several meters deeper in this clear water lake (Likens 1985). Therefore, this experiment most represents conditions that are likely to occur in the epilimnion and does not consider production in the metalimnion. The tubes were filled with lake water collected with a motorized pump. The intake hose was manually lowered and raised throughout the top 4 m of the lake to get a variable mixture of water from the epilimnion.

In a previous research conducted by Bouchard (2005), it was shown that phosphorus and nitrogen, added in the form of Na_2HPO_4 and $NaNO_3$ in an amount that would yield a final concentration of 35 PO_4 $\mu g\ L^{-1}\ d^{-1}$ and 70 NO_3 $\mu g\ L^{-1}\ d^{-1}$ caused a significant increase of algal growth. To observe what would happen above and below this level, a range of eight different loading rates of nitrogen and phosphorus were added to the 16 mesocosms in the forms of $NaNO_3$ and Na_2HPO_4. Each loading level was replicated twice. To follow closely Gerhart's (1973) methods, the molar ratio of N: P was kept constant at 4.4. The loading rates for the eight treatment levels are listed in Table 1.

The experiment ran for 11 days, from July 18th to July 29th, 2006. Dissolved oxygen readings were estimated with a dissolved oxygen meter on the 18th and 29th of July in each tube and in the lake at approximately the same time in the afternoon. Dissolved oxygen measurements were also recorded before sunrise, after noon, and after sunset on July 19, 24, and 28th at depths of 0, 2, and 4 meters to look for overall changes in productivity. Every other day before noon, nutrients were added to keep the concentrations from depleting. The nutrients were distributed evenly using the "garden hose" technique outlined by Goldman (1962).

Before loading the nutrients, water samples were drawn from both the lake and all the enclosures from a 1.5-m depth using a hose and a battery-powered peristaltic pump. The samples were kept in a dark box to stop any further photosynthesis until processed in the lab. One hundred mL of water was filtered through Whatman GF/A filters. The filters were frozen for at least 24 hours and measured fluorometrically (Marker 1980). Approximately 50 mL of filtrate was collected in acid-washed plastic bottles and frozen for further analysis of nitrate and phosphate. The $P\text{-}PO_4$ concentrations were determined using a Shimadzu 1601 spectrophotometer. Phosphate was measured using the ascorbic acid method (APHA 1992). Nitrate was measured using the second-derivative spectroscopy method (Crumpton et al. 1992). For both methods a spectroscopic cell length of 10 mm was used.

RESULTS

Concentrations of both PO_4 and NO_3 increased steadily in nutrient enriched mesocosms over the eleven days (Figure 1 and Figure 2). Final concentrations of nutrients increased with the loading rate. No limitations of either PO_4 and NO_3 were observed. The fourth highest addition rate, 26.3 PO_4 $\mu g\ L^{-1}\ d^{-1}$ and 52.5 NO_3 $\mu g\ L^{-1}\ d^{-1}$, resulted in raising the final PO_4 and NO_3 concentrations to 0.025 mg P-PO_4 L^{-1} and 0.45 mg N-NO_3 L^{-1}. In the mesocosms with the highest nutrient load, final P- PO_4 and N- NO_3 concentrations were 0.72 mg P-PO_4 L^{-1} and 1.5 mg N-NO_3 L^{-1}.

The Chl. *a* levels steadily increased in the enclosures with added nutrients (Figure 3). After eleven days of nutrient loading, the average Chl. *a* concentration in the mesocosms with the highest nutrient load was 6.5 ug/L. At the end of the experiment, the Chl. *a* concentrations of the mesocosms had not yet reached a plateau.

All enclosures with added nutrients exhibited a positive change in dissolved oxygen percent saturation over the eleven days of the experiment. The absolute change in percent saturation increased roughly linearly at the lowest loading rates up to a loading rate of 8.75 $\mu g\ PO_4\ L^{-1}\ d^{-1}$ (Figure 4). The absolute change in percent saturation of oxygen appears to plateau in mesocosms above 8.75 $\mu g\ PO_4 L^{-1}\ d^{-1}$. Despite a further increase in loading of almost 10X above 8.75 $\mu g\ PO_4\ L^{-1}\ d^{-1}$, changes in the percent saturation of oxygen was nearly constant at 11.75. The control enclosures, with no added nutrients, showed lower concentrations of dissolved oxygen and Chl. *a* than in the lake itself.

Algal response to the various nutrient loads was measured by the difference in the Chl. *a* levels between July 19th and the 29th. When compared to the final measured concentration of N and P, Chl. *a* increased linearly at low levels of nutrient loading but quickly came to a plateau (Figure 5 and 6). At final concentrations above about 0.1 mg NO_3 L^{-1} and 0.1 mg PO_4 L^{-1} the algal response was relatively flat, suggesting that other factors beside NO_3 and PO_4 could be limiting the productivity of algae at higher levels of NO_3 and PO_4 loading.

DISCUSSION

Lake trophic levels can be defined by an equation incorporating the total nitrogen (TN) content, total phosphorus (TP) content, Chl. *a* levels, and Secchi depth (Burns et al. 2000). Burns et al. (2000) defines a eutrophic lake as having a concentration of TP around 20-50 mg/m^3, TN concentrations around 300-500 mg/m^3, and Chl. *a* levels around 5-15 mg/m^3 with Secchi readings of 1-3 m.

At the end of this experiment, the concentrations of N-NO_3 and P-PO_4 in mesocosms with nutrient loads of 26.3 $\mu g\ PO_4\ L^{-1}\ d^{-1}$ and 52.5 $\mu g\ NO_3\ L^{-1}\ d^{-1}$ and above indicate eutrophic systems. The final N-NO_3 and P-PO_4 concentrations of the mesocosms with the highest loads could almost be classified as hypertrophic lakes, but Chl. *a* concentrations in the tubes with the highest nutrient loads were only slightly indicative of eutrophication, with an average concentration of 6.5 ug/L. Sechhi depths were not recorded for this project so a full comparison with Burns et al. (2000) is not possible. However, the relatively small concentrations of Chl. *a* suggest that even the mesocosms with the highest N-NO_3 and P-PO_4 concentrations may still be oligotrophic, in the sense that they are relatively unproductive.

The lack of response in Chl. *a* concentrations in these mesocosms suggests that maybe another nutrient other than N or P must be limiting phytoplankton productivity in Mirror Lake. Silica and iron are among some of the other nutrients that have been found to limit algal growth in other water bodies (Chang and Rossman 1987, North et. al 2007). Future studies could be performed to see what micronutrients are limiting productivity of Mirror Lake.

It is also possible that additional algal response could have been observed but the experiment was not extended long enough. The ratio of N:P in this experiment was 4.4:1, which was much lower than the 17:1 ratio shown to be optimal for algal growth (Hillebrand and Sommer 1999). The low N:P ratios may have favored nitrogen fixing algae, such as cyanobacteria, as has been noted before in eutrophic systems in similar conditions (Ferber et. al 2001). However, the short duration of the mesocosms study may not have allowed adequate time for the cyanobacterial community to become established. However, the nitrogen concentrations in the mesocosms did not decline, suggesting N was not in danger of becoming limiting. Therefore it seems unlikely that the lack of response in Chl. *a* could have been solely attributed to the time lag in establishing new algal communities.

Had pronounced changes in the algal community occurred, it is possible that all trophic levels of the lake would have been affected. A change in the algal community may mean the increase or species shift of zooplankton. It is also possible that the opposite may have occurred. Large zooplankton, especially *Daphnia*, have been shown to suppress microzooplankton in lakes fertilized with both N and P (Pace et. al 1998). It may be that zooplankton may also suppress the growth of algae by grazing. However, recent work on Mirror Lake suggests that this may not be the case (Bouchard, pers. comm.) For that reason, studies which observe species composition of both algae and zooplankton at differing N and P loading are highly encouraged.

This study suggests that eutrophication of Mirror Lake may not immediately occur even with large changes of N and P in the water chemistry as even under high levels of N and P, algal growth in the mesocosms were not substantial. However, it is hard to say what would happen if nutrient additions of even the smallest rates used in this experiment occurred in the entire lake. As of yet there have been no studies performed to determine how nutrient additions would affect other organisms of Mirror Lake, especially macrophytes, or how changes in plant growth might alter the biogeochemistry of the water. Therefore, I suggest that Mirror Lake continue to be monitored for any increases in N or P, and subsequent studies performed to identify the other factors that are limiting algal production in Mirror Lake.

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LITERATURE CITED

American Public Health Association (APHA). 1992. Standard methods for the examination of water and wastewater. American Public Health Association, Washington D.C.

Bergstrom, A.K., and M. Jansson. 2006. Atmospheric nitrogen deposition has caused nitrogen enrichment and eutrophication of lakes in the northern hemisphere. Global Change Biology. 12(4): 635-643.

Bergstrom, A.K., P. Blomqvist, and M. Jansson. 2005. Effects of atmospheric nitrogen deposition on nutrient limitation and phytoplankton biomass in unproductive Swedish lakes. Limnol. Oceanogra.. 50(3) 987-994.

Bouchard, K. 2005. Nutrient limitation and top-down, bottom-up controls on phytoplankton in Mirror Lake. Institute of Ecosystems Studies. Undergraduate Research Report.

Bukaveckas, P. and W. Shaw. 1998. Phytoplankton response to nutrient and grazer manipulations among northeastern lakes of varying pH. Can. J. Fish. Aquat. Sci. 55: 985-966.

Burns, N.B., G.E. Bowman. 2000. Protocol for Monitoring Trophic Levels of New Zealand Lakes and Reservoirs. Prepared for Ministry of the Environment by Lakes Consulting, March.

Carpenter, S.R., N.F. Caraco, and V.H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications. 8: 559-568.

Chang, W.B, and R. Rossmann. 1987. Changes in the abundance of blue-green algae related to nutrient loadings In the nearshore of Lake Michigan. Hydrobiologia. (157)3: 271-278.

Crumpton, W.G., T.M. Isenhart, and P.D. Mitchell. 1992. Nitrate and organic N analyses with second-derivative spectroscopy. Limnol. Ocean. 37: 907-913.

Elser, J.J., E.R. Marzolf, and C.R. Goldman. 1990. Phosphorus and nitrogen limitation of phytoplankton growth I n the freshwaters of North America: a review and critique of experimental enrichments. Can. J. Fish. Aquat. Sci. 47:1468-1477.

Ferber, L.R., S. N. Levine, A. Lini, and G. P. Livingston. 2004. Do cyanobacteria dominate in eutrophic lakes because they fix atmospheric nitrogen? Freshwater Biology. 49 (6): 690–708.

Gerhart, D.Z. 1973. Nutrient limitation and production of phytoplankton in Mirror Lake, West Thornton, New Hampshire.—Ph.D. thesis, Cornell Univ. 160.

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

Harper, D. 1992. Eutrophication of Freshwaters: Principles, problems and restoration. Chapman & Hall, London, Great Britain.

Hillebrand H, and U. Sommer. 1999. The nutrient stoichiometry of benthic microalgal growth: Redfield proportions are optimal. Limno. Oceanogr. 44: 440-446.

Likens,G.E. 1985. An Ecosystem Approach to Aquatic Ecology: Mirror Lake and Its Environment. Springer-Verlag.

Likens, G. E. 2004. Some perspectives on long-term biogeochemical research from the Hubbard Brook Ecosystem Study. Ecology 85(9):2355-2362.

Maberly, S.C., L. King, M.M. Dent, R.I. Jones, and C.E. Gibson. 2002. Nutrient limitation of phytoplankton and periphyton growth in upland lakes. Freshwater Biology. 47(11): 2136-2152.

Marker, A. F. H., C. A. Crowther, and R. J. M. Gunn. 1980. Methanol and acetone as solvents for estimating chlorophyll and pheopigments by spectrophotometry. Ergebnisse der Limnologie 14:52-69

North, R.L., S.J. Guildford, and R.E.H. Smith. 2007. Evidence for phosphorus, nitrogen, and iron co limitation of phytoplankton. Limnol. Oceanogr. 52(1): 315-328.

Pace, M.L., J.J. Cole, and S.R. Carpenter. 1998. Trophic cascades and compensation: differential responses of microzooplankton in whole lake experiments. Ecology. 79: 138-152.

APPENDIX

TABLE 1. The loading levels of the 16 mesocosms used in this experiment.

Mesocosms	**PO_4 μg L^{-1} d^{-1}**	**NO_3 μg L^{-1} d^{-1}**
1, 9	70	140
2, 10	52.5	105
3, 11	35	70
4, 12	26.3	52.5
5, 13	17.5	35
6, 14	8.75	17.5
7, 15	4.37	8.75
8, 16	0	0

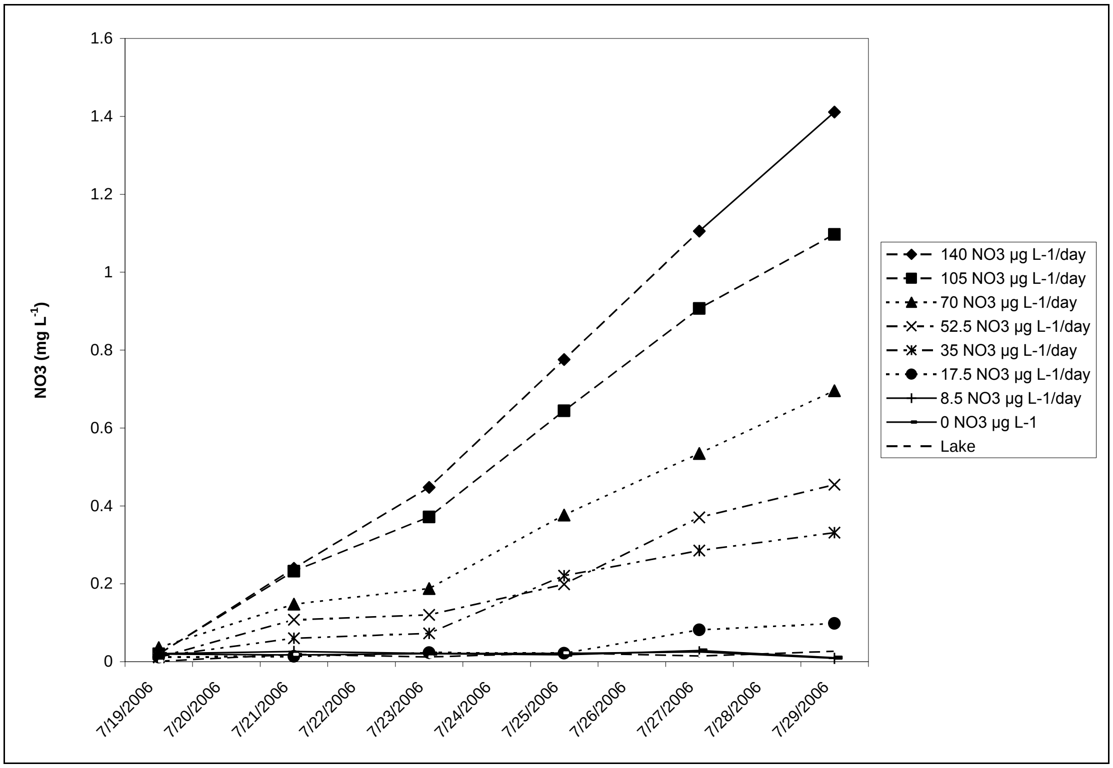

FIGURE 1. The time series of NO3 concentrations over eleven days. Replicate mesocosms were averaged.

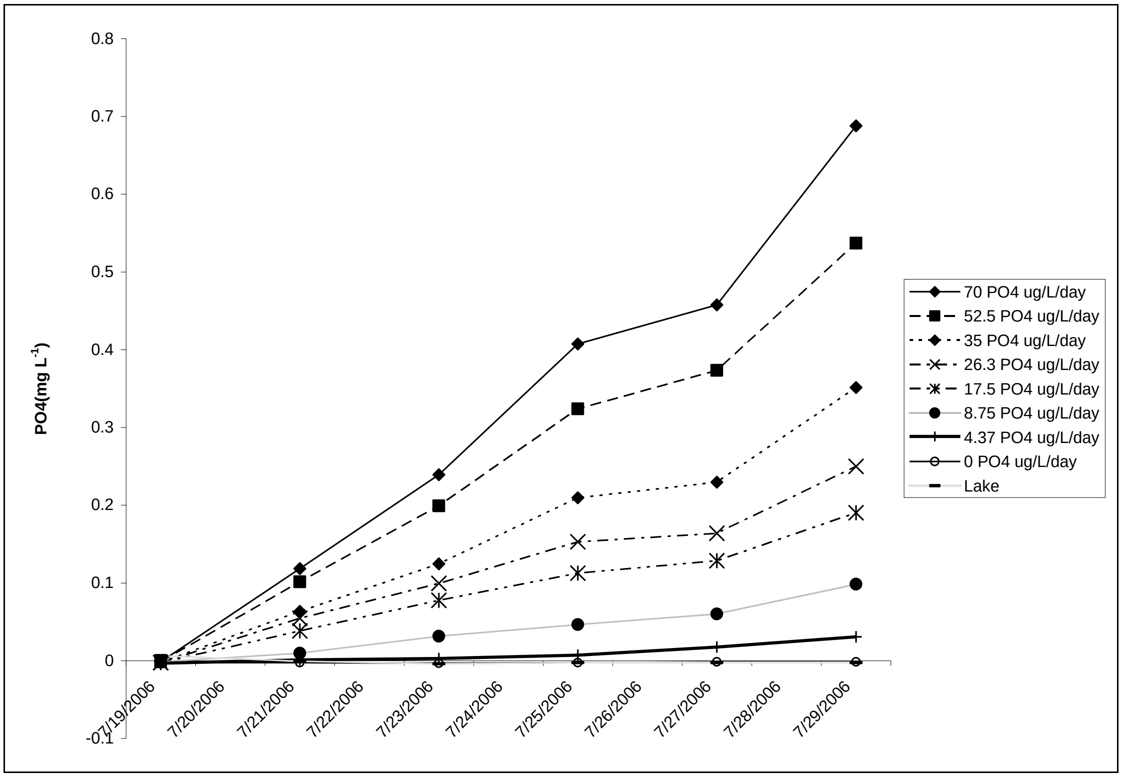

FIGURE 2. The time series for PO4 concentrations over eleven days. Replicate mesocosms were averaged.

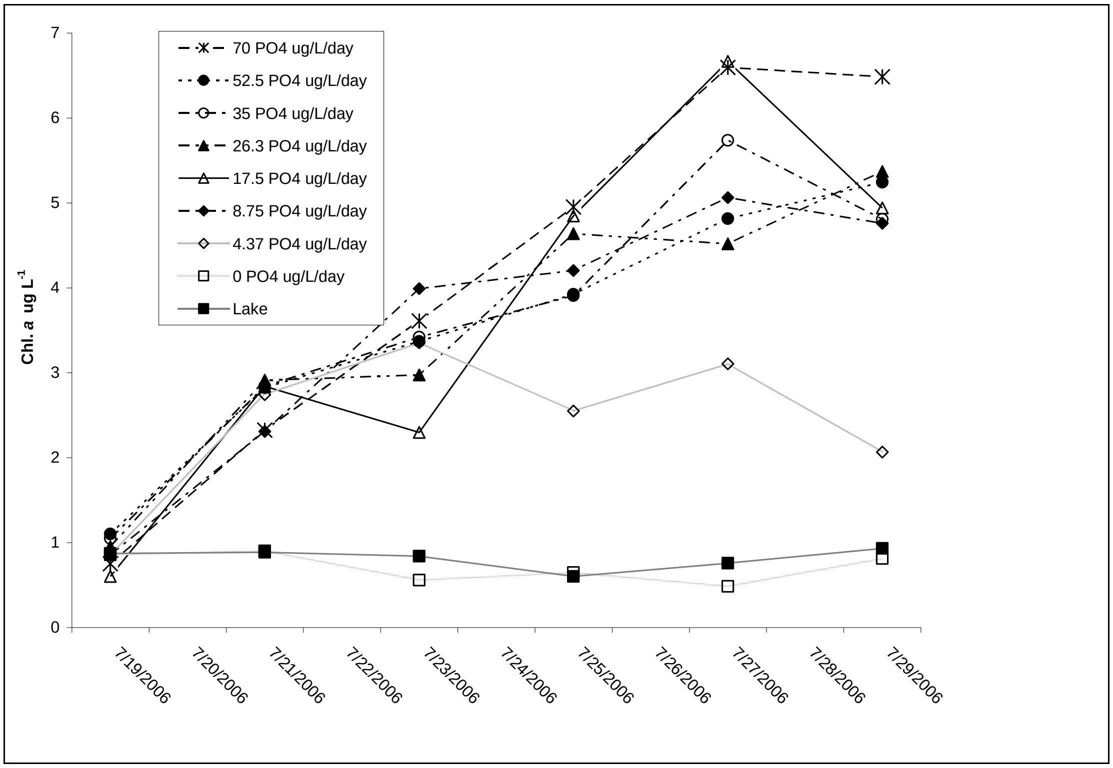

FIGURE 3. Time series of Chl. *a* concentrations over the eleven day experiment. Replicate mesocosms were averaged.

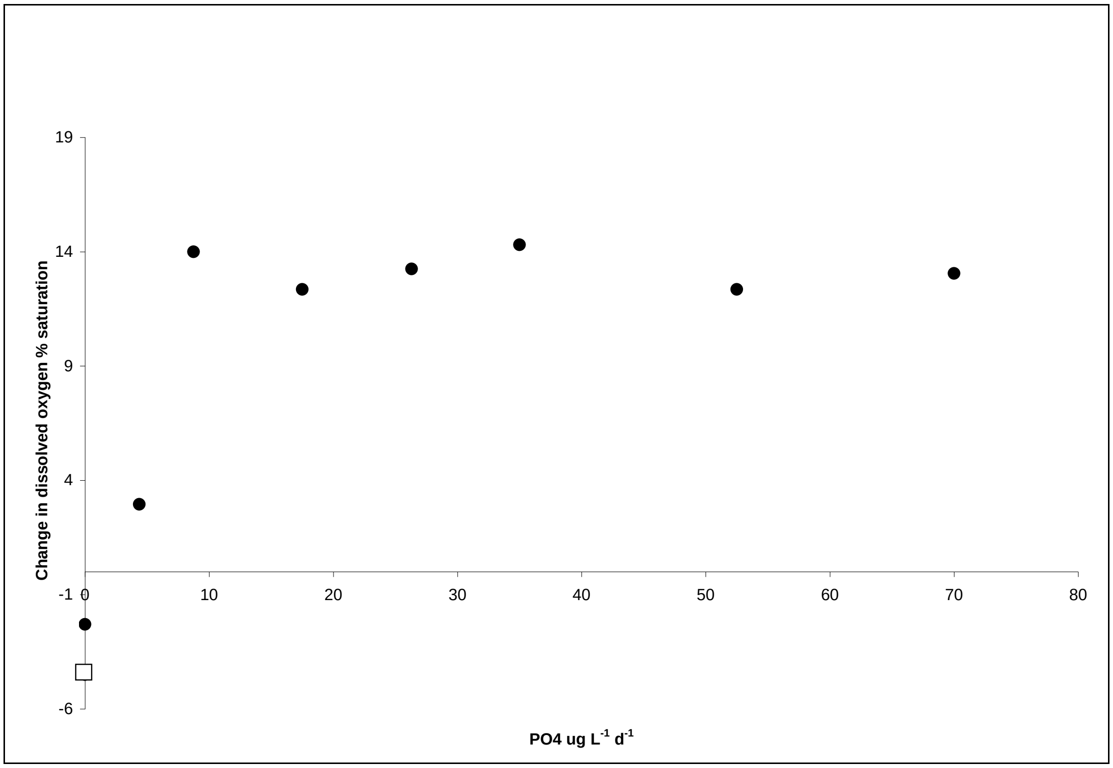

FIGURE 4. The response of oxygen to nutrient loading rates measured as the absolute change in the percent saturation of dissolved oxygen from July 19th to July 28th. Each point represents the average value of the replicate mesocosms. The open square represents the response measured in the lake.

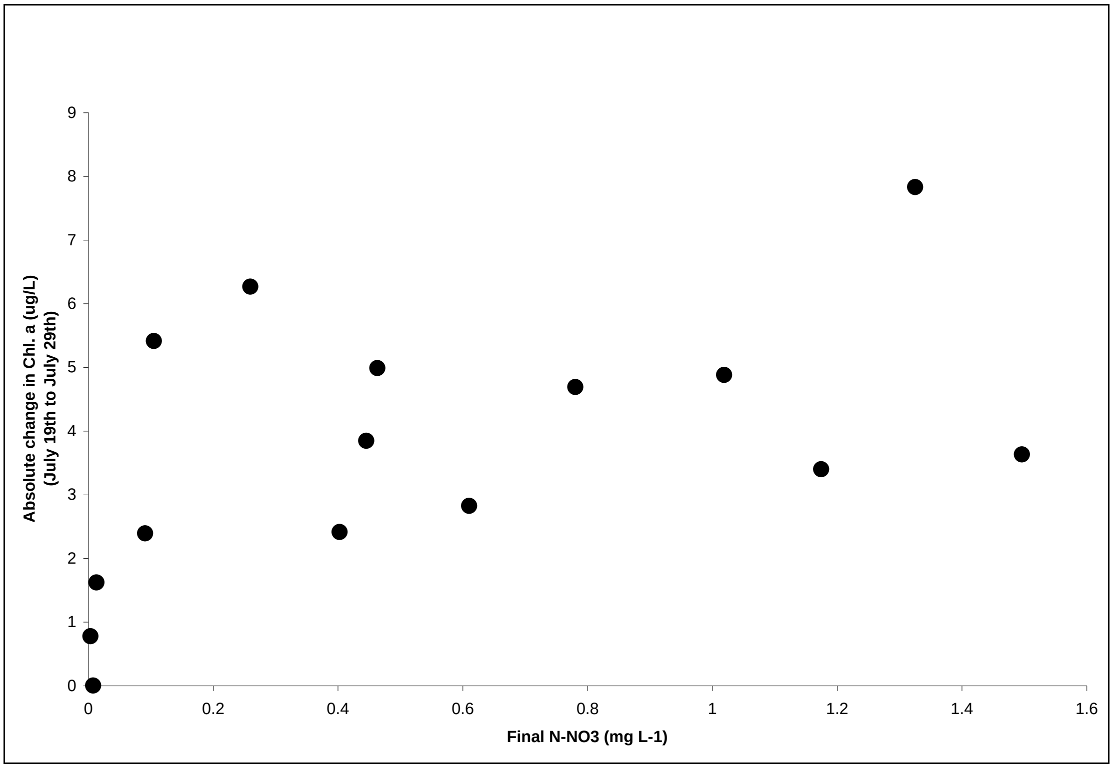

FIGURE 5. Absolute change in algal productivity as measured by Chl a. levels on July 19th and July 28th, in response to the final N-NO_3 concentrations in individual enclosures.

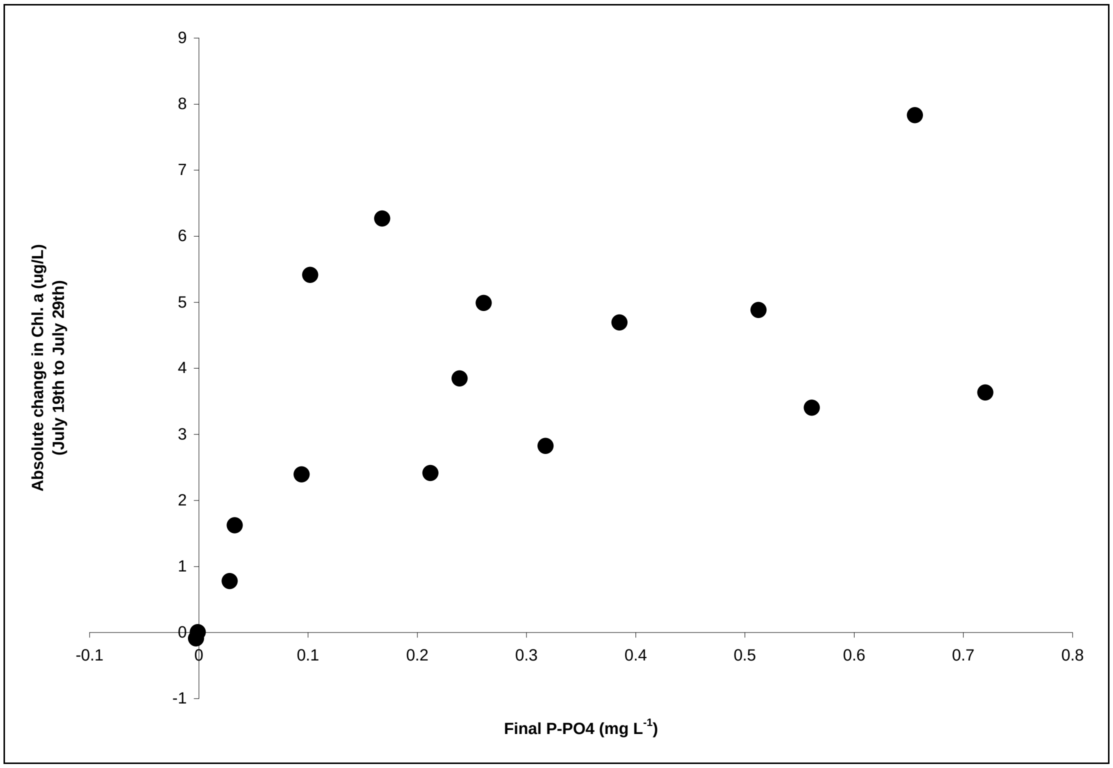

FIGURE 6. Absolute change in algal productivity as measured by Chl a. levels on July 19th and July 28th, in response to the final P-PO_4 concentrations in individual enclosures.