Can Eutrophication Influence Nitrogen vs. Phosphorus Limitation?

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Abstract

As suburban sprawl spreads across Cape Cod, nitrogen and phosphorus, originating largely from septic systems and fertilizers, have caused significant eutrophication in freshwater lakes and ponds. The purpose of my study is to determine if anthropogenic nutrient loading can influence whether the primary production in freshwater ponds is limited nitrogen or phosphorus. I sampled three highly nutrient loaded ponds and three pristine ponds, and incubated each for 14 days under four different treatments: nitrogen enrichment, phosphorus enrichment, enrichment with both, and control. I measured chlorophyll growth, the uptake of inorganic nutrients, and final organic carbon, nitrogen, and phosphorus concentrations of biomass grown in each incubation. For all ponds, incubations enriched with both nitrogen and phosphorus grew the highest concentration of phytoplankton, but eutrophic ponds grew a mean phytoplankton concentration five times greater than the oligotrophic. Growth in eutrophic ponds reacted more strongly to the nitrogen enrichment than the phosphorus, while the oligotrophic ponds did not respond to nitrogen at all. Oligotrophic ponds did respond noticeably to the phosphorus enrichment, indicating a phosphorus limitation.

Key words and phrases: eutrophication, nutrient limitation, phytoplankton

Introduction

Cape Cod's network of freshwater lakes and ponds holds high aesthetic, cultural, and ecological value from the perspective of preservation (Smith et al, 2006). As suburban sprawl spreads across Cape Cod, nitrogen and phosphorus, originating largely from septic systems and fertilizers, have caused significant eutrophication in freshwater lakes and ponds. Subsequent additional net primary production in these systems can lead to loss of species diversity, alterations to natural trophic structures, and hypoxia or anoxia (Valiela, 1995). Understanding the effects of anthropogenic nutrient loading to aquatic ecosystems is important for future ecosystem diversity and stability. If human populations and industrial activity continue to grow, the health of freshwater bodies will be diminished, and potential recovery from eutrophication will unfortunately become very costly and politically difficult (Whittaker, 1975).

The purpose of my study is to determine if the consequences of anthropogenic nutrient loading can influence whether freshwater ponds are limited by nitrogen or phosphorus. Although both nitrogen and phosphorus are known to limit the primary production in freshwater bodies, the conditions under which each nutrient becomes limiting remains unclear (Elser et al, 1990). All recent short term enrichment studies in freshwater demonstrate the highest growth response occurring with the addition of both nitrogen and phosphorus, indicating a possible co-limitation. (Elser et al, 1990, Schindler, 1988). Although the conventional view is that phosphorus is more limiting, a great deal of evidence supporting both nutrient's role in limitation exists. More replication and statistical analysis under a variety of environmental conditions would greatly enhance our understanding of how nitrogen and phosphorus act as limiting nutrients on primary production in freshwater (Elser et al, 1990).

I sampled six similarly sized ponds, grouped into two categories of eutrophication: pristine and highly affected. The conditions of land use in each pond's watershed influence its category. On Cape Cod, high densities of residential housing, with associated septic systems and fertilizers, can load watersheds and groundwater with nitrates and phosphates, which are eventually deposited into aquatic systems. By choosing three oligotrophic ponds, and three highly eutrophic, I seek to establish a difference in how limited each trophic class is by either N or P.

I have conducted a short-term enrichment incubation in which I fertilized the pond samples in different bottles with either potassium nitrate, potassium phosphate, both, or neither (control). I used the increase of phytoplankton growth following nutrient addition and a 14 day incubation to designate whether nitrogen or phosphorus limits the primary productivity in each pond. In addition, I recorded the uptake of inorganic nutrients, such as nitrate, ammonium, and phosphate, and measured final total change in particulate carbon, nitrogen and phosphorus content.

Methods

I chose the six ponds based on size similarity (5-30 acres) and nutrient load. On Monday, November 12, I sampled the ponds, filling 20L carboys from six inches below the surface around the middle of the pond. Until the incubation began on Wednesday, Nov. 14, the samples were kept refrigerated. The following morning I filtered the samples through a 250 μm mesh to remove existing zooplankton.

From each pond's carboy I filled eight clear, plastic two-liter soda bottles. Two of the bottles became controls, and received no nutrient addition. Two were enriched with 1 ml of 400,000 μM potassium nitrate (for a final concentration of 200 μM), two with 1 ml of 36,000 μM potassium phosphate (for a final concentration of 18 μM), and two were enriched with both N and P.

Incubation took place in the MBL growth chamber for 14 days. In the chamber the incubations received 14 hours a day of light, which peaked at approximately 1200 PAR. The temperature in the chamber was a constant 18 degrees C. To prevent the settling of organic matter and inorganic nutrients, I maintained constant circulation of the water in the bottles by incubating on top of a shaker table (Figure 15).

Phytoplankton growth was measured in two ways. Spectrophotometry (Lorenzen, 1967) involved filtering a known volume of the sample through 47 μm GF-F filters, extracting the filters with 35 ml 90% acetone, and analyzing with a spectrophotometer. *In vivo* fluorescence proved to be a more efficient method for the measure of phytoplankton growth, in that I did not have to remove any sample from the incubation. I drew approximately 5 ml form the incubation, analyzed with a fluorometer, and poured the sample back.

Ammonium and phosphate concentrations were determined by filtering the sample through a 0.7 μm GF-F filter, and then analyzed with a Shimadzu 1601 spectrophotometer. Nitrate samples were similarly filtered, and analyzed with a Lachat Flow Injection Analyzer.

Organic carbon and nitrogen concentrations were measured by filtering a known volume of sample through a 25 μm GF-F filter, and analyzing the filter with a CHN machine. Particulate organic phosphorus was measured using the same method as PO_4, except I filtered a sample through 25 μm GF-F, and then extracted the filter with 10 ml 1 normal HCL.

On the first day of incubation, I measured initial chlorophyll concentration in each pond using both the fluorometer and the spectrophotometer method.   I also filtered for initial organic carbon, nitrogen, and phosphorus composition of the phytoplankton. On the final day on incubation, I filtered for a chlorophyll concentration of each incubation bottle, using both the fluorometer and spectrophotometer method. I also filtered for final organic carbon, nitrogen, and phosphate composition of the growth. I measured nutrient concentrations (NH_4, NO_3, PO_4) four times through the course of incubation, on days 0, 2, 4, and 12.

Results

Initial measurements of dissolved inorganic nitrogen (NO_3 and NH_4) revealed asimilar trend to the one predicted by the Cape Cod Commission Water Resources Office in 2003 (Figure 1). The three eutrophic ponds, Emery, Aunt Betty's, and Cedar Lake, showed DIN concentrations of approximately three times as large as the oligotrophic ponds, Pine, Slough, and Mary Dunn (Figure 2). I observed a similar trend in phosphate concentrations, although Aunt Betty's PO_4 concentration was noticeably lower than the other two eutrophic ponds (Figure 3). Eutrophic ponds averaged an almost 20% lower N:P ratio than the oligotrophic (Figure 4).

DIN concentrations in N and N+P enriched incubation averaged over 200 μMolar above concentrations in P enriched and control incubations. In all ponds, incubations enriched with both N and P showed the largest decrease in DIN concentration through the course of the incubation (Figure 5). Incubations enriched with P took up more DIN than control incubations.

Phosphate concentrations showed a similar scale to the DIN, with incubations enriched with phosphate or N+P having almost 20 μMolar most PO_4 than the N enriched and controls. Incubations enriched with both N and P showed the largest uptake of PO_4 through the course of the incubation (Figure 6).

Flourometry measured approximately 15% of the total phytoplankton community growing in all incubations. To correct for this I scaled the flourometer readings up to those of the spectrophotometry using a regression equation (Figure 7). All ponds showed the highest final concentration of phytoplankton in incubations enriched with both nutrients. However, the phytoplankton in eutrophic ponds reached mean concentrations of approximately five times that of the oligotrophic ponds. In most ponds, incubations enriched with P showed slightly more growth than both N enriched and control (Figure 8).

Spectrophotometry revealed that the N+P incubations for all six ponds were significantly greater than the N or P enrichment alone (F Value= 181.14, P= <.0001). Again, the eutrophic ponds showed approximately five time as much chlorophyll growth as the oligotrophic, which proves to be significantly greater (F Value= 103.55, P= <.0001).

In all three cases, eutrophic ponds also showed a noticeable growth response to the N enrichment, and variable responses to P enrichment (Figure 9). In fact, incubations enriched with P for Aunt Betty's Pond actually decreased. Oligotrophic ponds did not respond to the N enrichment, and decreased in all cases (Figure 10). They did, however, respond noticeably and uniformly to the P enrichment.

The carbon to nitrogen ratio in all six ponds approached the Redfield value of 6.625 (Figure 11). While the incubations enriched with N showed a higher ratio, they still grew phytoplankton more rich in organic nitrogen than the phosphorus and control incubations.

All incubations enriched with N or N+P showed a lower C : N ratio, regardless of pond (Figure 12). Similarly, all incubations enriched with P or N+P showed a lower C : P ratio (Figure 13).

Discussion

The concentrations of DIN throughout the incubation decreased because the primary production in the incubation incorporated available inorganic N into biomass. Because the largest phytoplankton growth for both eutrophic and oligotrophic ponds occurred in the N+P incubation, the concentrations of DIN decreased the most in those bottles (Figure 5). Incubations enriched with just phosphorus showed a larger decrease in DIN than control bottles, because the additional P sparked more growth, which in turn incorporated more DIN. When more DIN was available for primary production, as with the N and N+P enrichments, the DIN concentrations decreased more than when it was not enriched, indicating that more DIN was incorporated into biomass. This interpretation is reinforced by the organic C : N ratios of incubations enriched with either N or N+P. All of these bottles incorporated almost one mol of N for every 6.625 mol of carbon, which is the Redfield ratio. When the DIN was less available in the incubation, the ratio almost doubled.

Similar patterns occurred regarding phosphate concentrations. Growth in N+P incubations drew more inorganic phosphate out of the water than just P alone. This is likely due to the overall difference in phytoplankton growth in these two different enrichments; N+P incubations grew almost six times as much phytoplankton than with P alone. Incubations enriched with N drew more phosphate out of the water than control, because more total growth occurred in N incubations. Final organic C:P ratios revealed that when it was available, as with P and N+P incubations, more P was incorporated into biomass than when it was not available.

Several important differences can be observed between the growth in eutrophic ponds and oligotrophic ponds. Most importantly, eutrophic ponds supported almost five times as much phytoplankton growth than oligotrophic. Secondly, eutrophic incubations enriched with N responded noticeably while the P had little effect. This refutes the accepted wisdom that P is the primary limiting nutrient, and N is the secondary limiting nutrient (Elser et al, 1990). Thirdly, oligotrophic ponds responded noticeably to P enrichment, but not at all to N. In all ponds, the significantly greater response to both nutrients indicates that the interplay between N and P has a larger influence on primary production than either nutrient alone, which is a result often found in these types of short term enrichment studies (Elser et al, 1990, Smith et al, 2005).

These patterns imply that eutrophic ponds can withstand massive nitrogen loads, of which it is safe to assume they receive naturally at certain times of year (Elser et al, 1990). This is a potential result of a temporary nitrogen limitation on primary production, possibly due to a high load of phosphorus from local anthropogenic sources. Oligotrophic ponds demonstrated a P limitation, which supported the idea that pristine temperate ponds are typically phosphorus limited (Elser et al, 1990).

Another explanation could be that to this pattern could be that eutrophication has caused a trophic structure shift in the freshwater, and the communities of phytoplankton differ from oligotrophic.

Seasonal variation can influence the availability of both N and P (Elser et al, 1990, Smith et al, 2005). This seasonal variation is certainly of ecological significance to primary production that occurs in Cape Cods ponds. My study did not concern seasonal variation, and similar studies at other time of year could elucidate limitation patterns.

Initial inorganic N:P ratios were different between eutrophic and oligotrophic ponds, with eutrophic ponds having more P to N, and oligotrophic ponds having more N to P. Because I observed strong P limitation in oligotrophic ponds, and possible N limitation in eutrophic ponds, this N:P ratio could have been used to predict the limitation. Future studies may find that initial nutrient ratios can be a reliable precursor to which nutrient is in higher demand at that particular time of year.

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Figures

Figure 1: Characteristics of the Six Ponds, Cape Cod Commission Water Resources Office, Final Report, May 2003

Eutrophic	Town	Acres	Depth (m)	Chlorophyll (μg/liter)	Total Nitrogen (mg/liter)	Total Phosphorus (μg/liter)
Emery Pond	Chatham	14.1	21	21.89	1.43	54.8
Aunt Betty's Pond	Barnstable	7.1	15	86.83	0.73	24.78
Cedar Lake	Falmouth	20.7	10	7.77	0.58	24.78
Mary Dunn Pond	Barnstable	18	9	1.14	0.38	11.46
Pine Pond	Brewster	23.3	-	0.65	0.12	4.65
Slough Pond	Brewster	31.6	20	0.33	0.11	4.65

Oligotrophic

Figure 2: Initial Dissolved Inorganic Nitrogen Concentrations

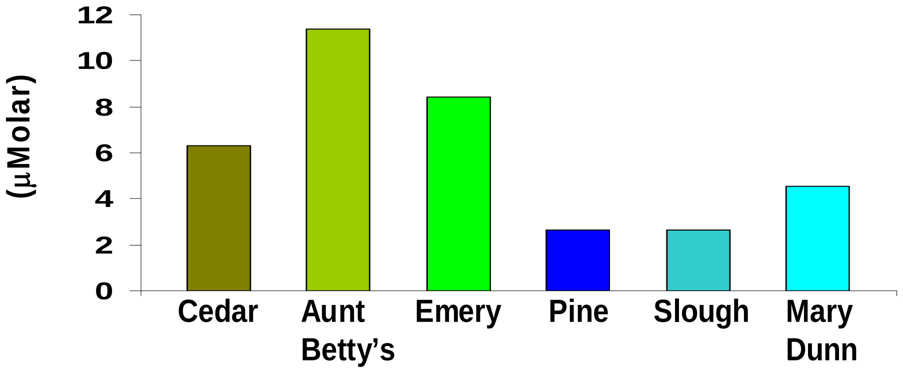


Figure 3: Initial Phosphate Concentrations

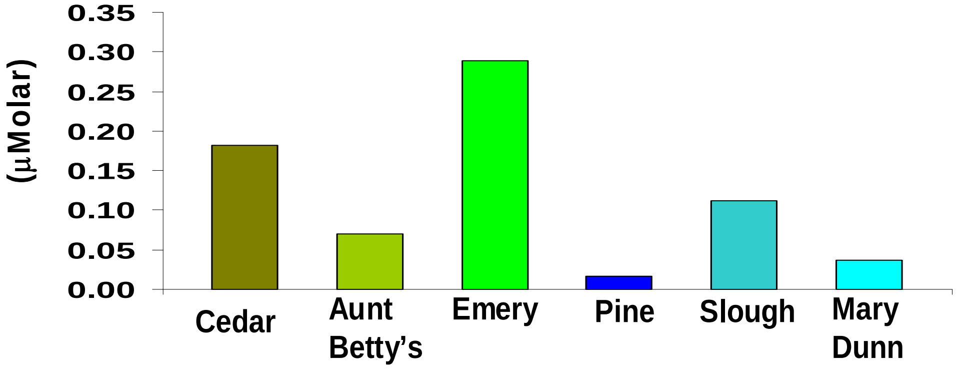


Figure 4: Mean DIN : Phosphate Ratios

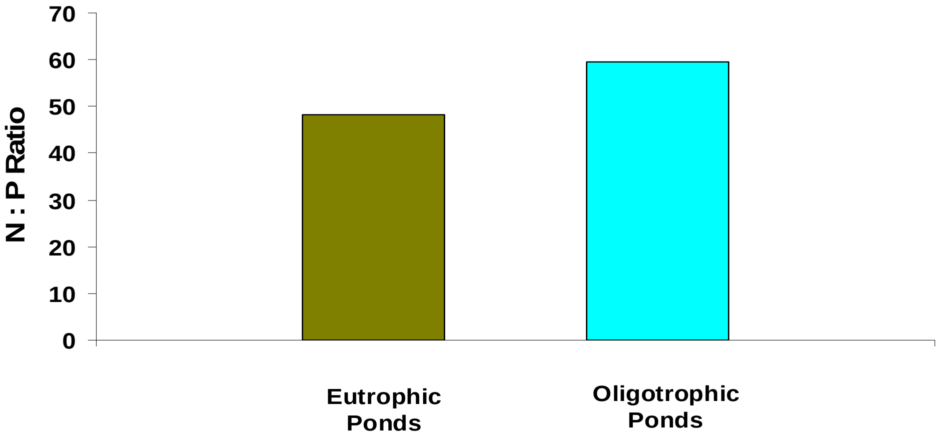


Figure 5: Dissolved Inorganic Nitrogen Concentrations During Incubation

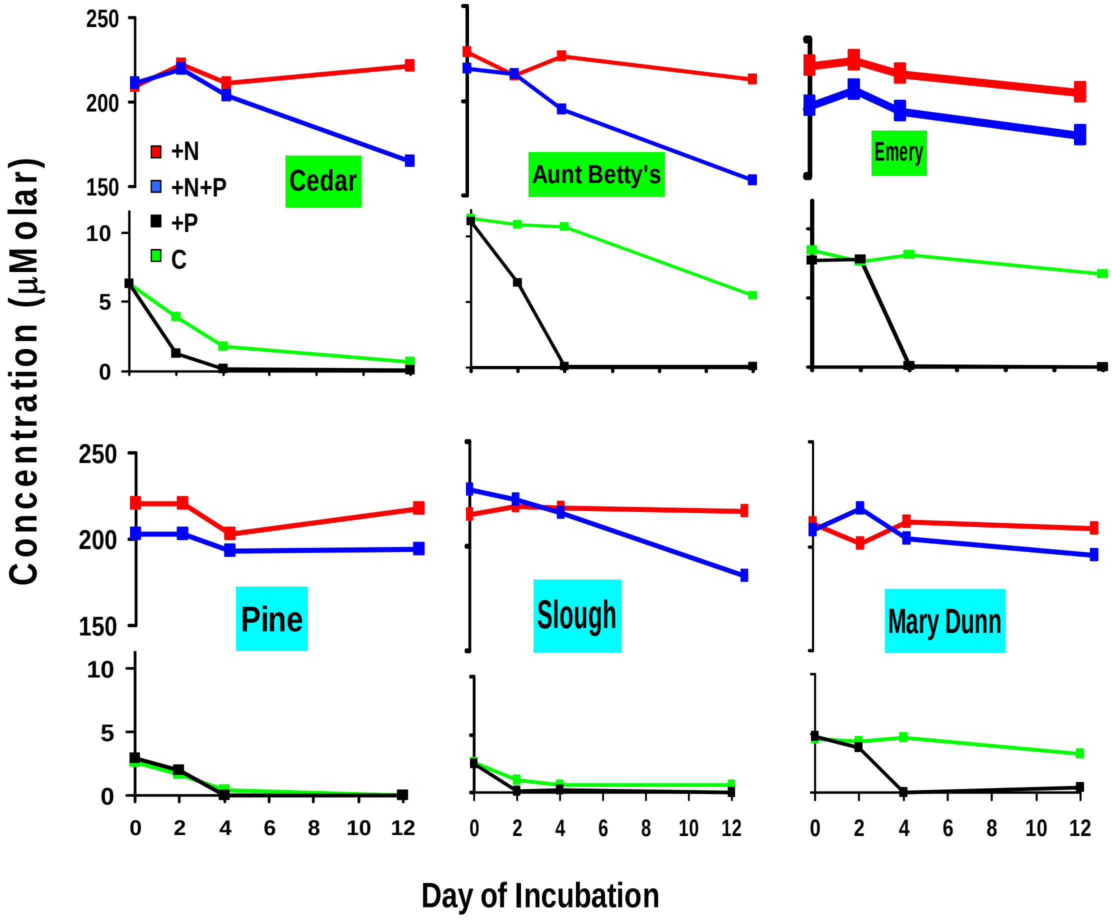


Figure 6: Phosphate Concentrations During Incubation

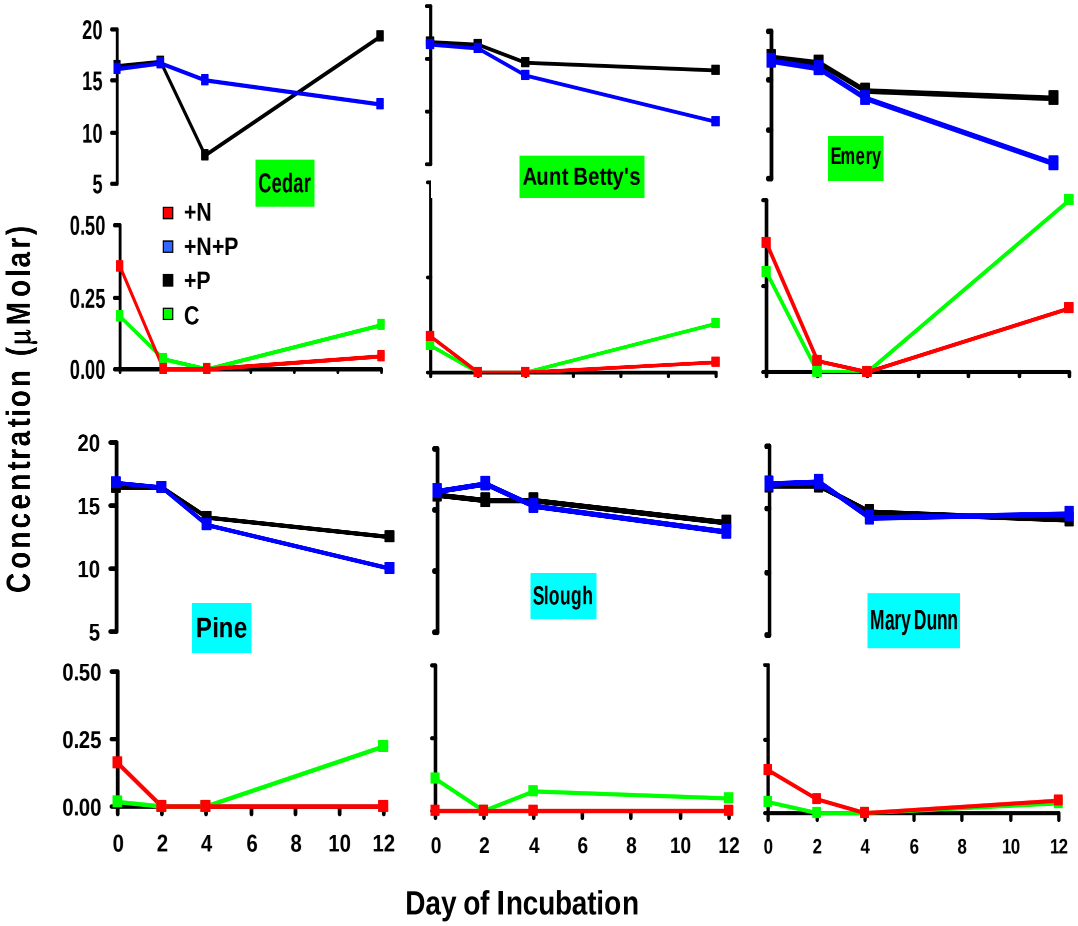


Figure 7: Comparison of Two Measures of Chlorophyll

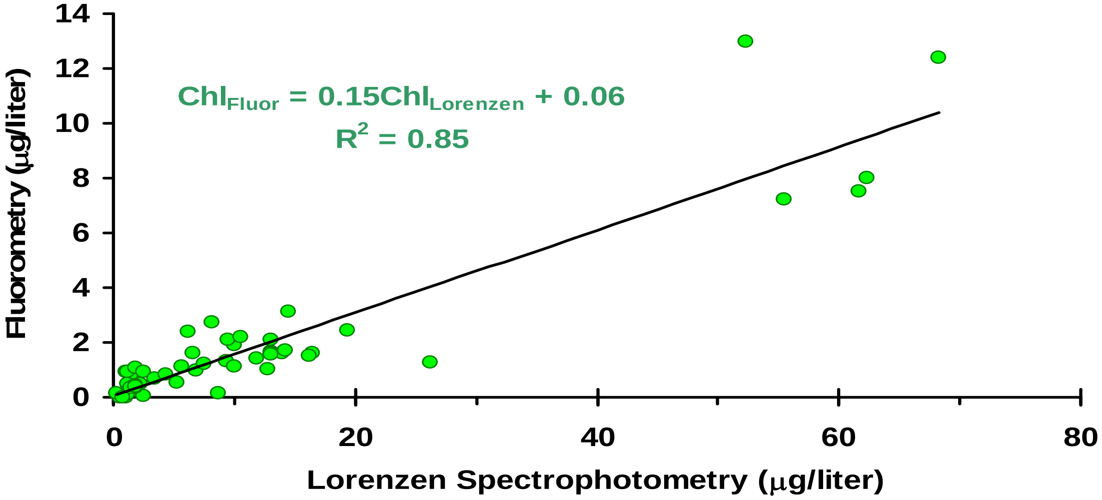


Figure 8: Chlorophyll Concentrations During Incubation, Derived from Fluorometry

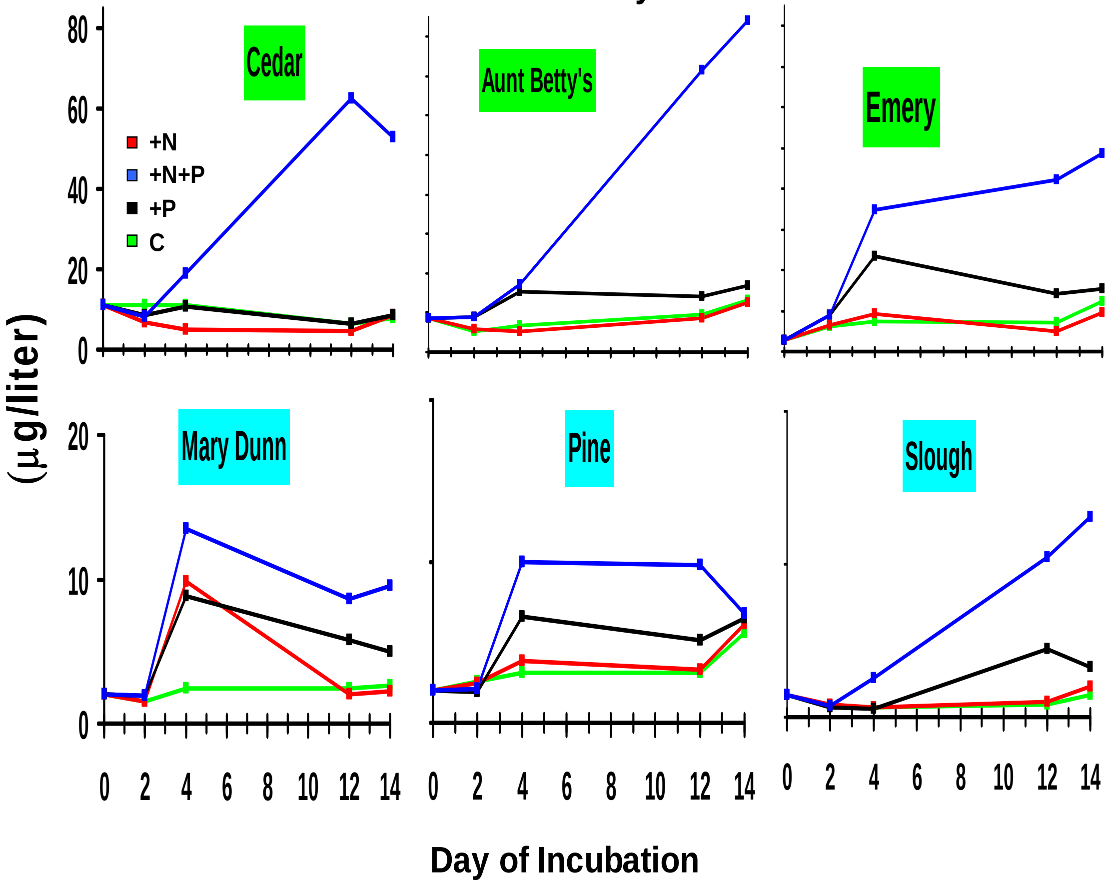


Figure 9: Response of Chlorophyll Concentration Following Nutrient Additions in Eutrophic Ponds

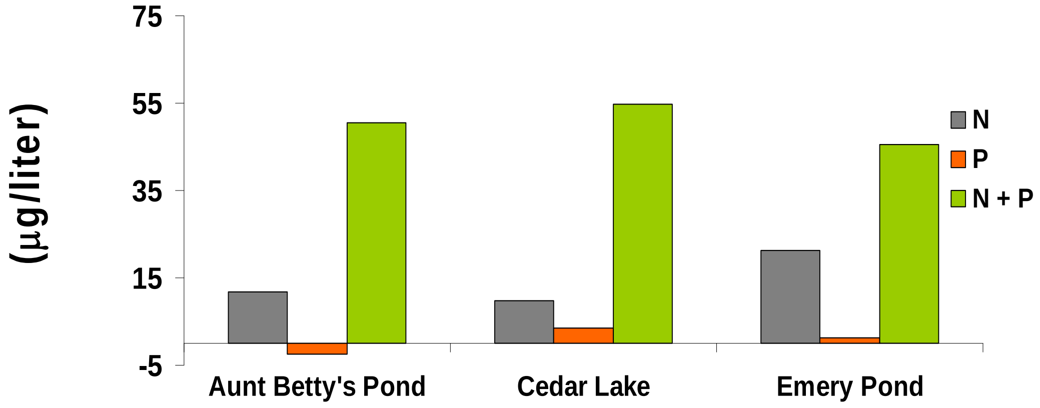
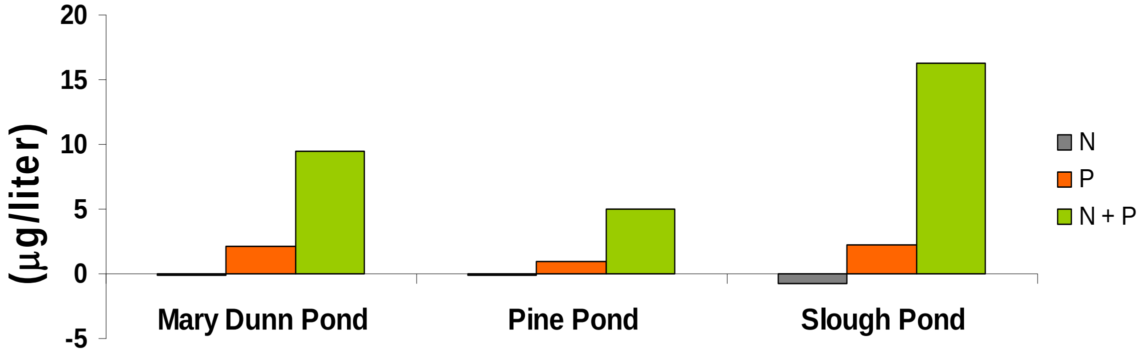


Figure 10: Response of Chlorophyll Concentration Following Nutrient Additions in Oligotrophic Ponds



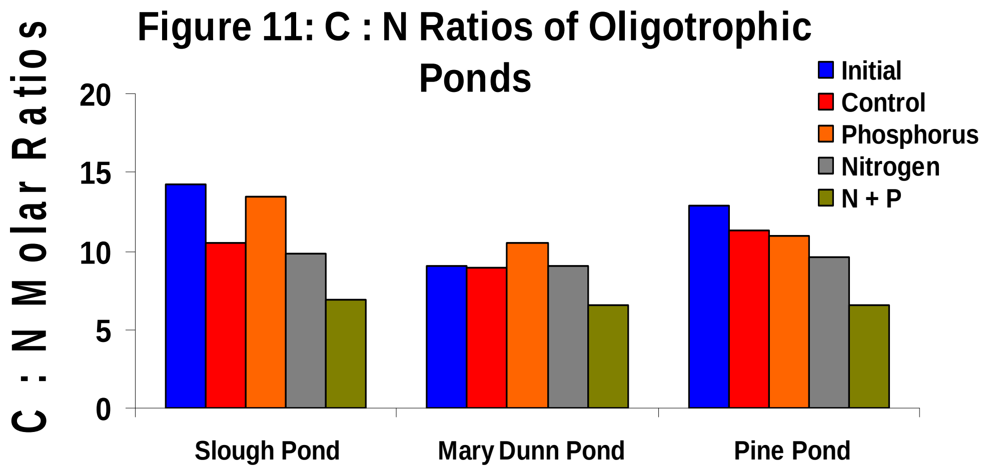


Figure 11: C : N Ratios of Oligotrophic Ponds

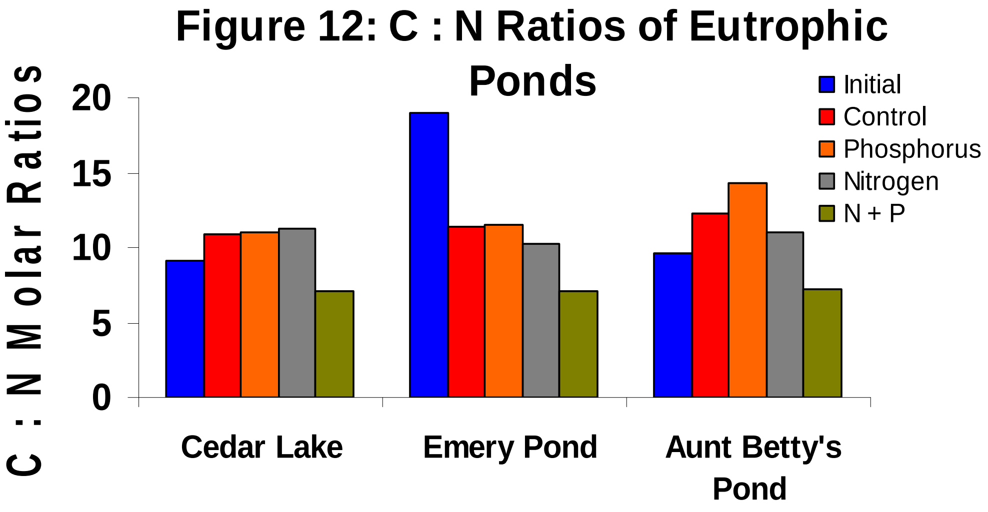


Figure 12: C : N Ratios of Eutrophic Ponds

Figure 13: C : N Ratios of Nitrogen Treatments and Non-Nitrogen Treatments

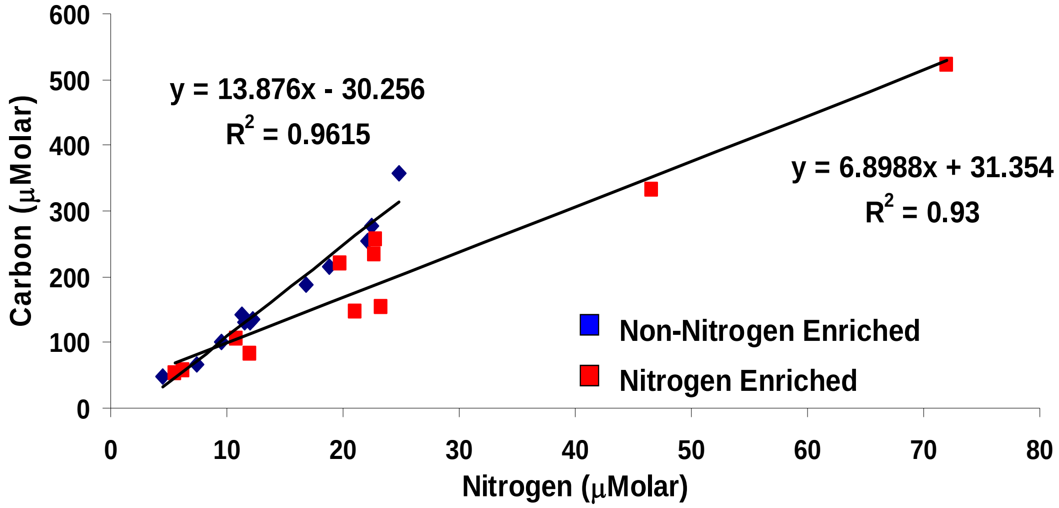


Figure 14: C : P Ratios of Phosphorus Treatments and Non-Phosphorus Treatments

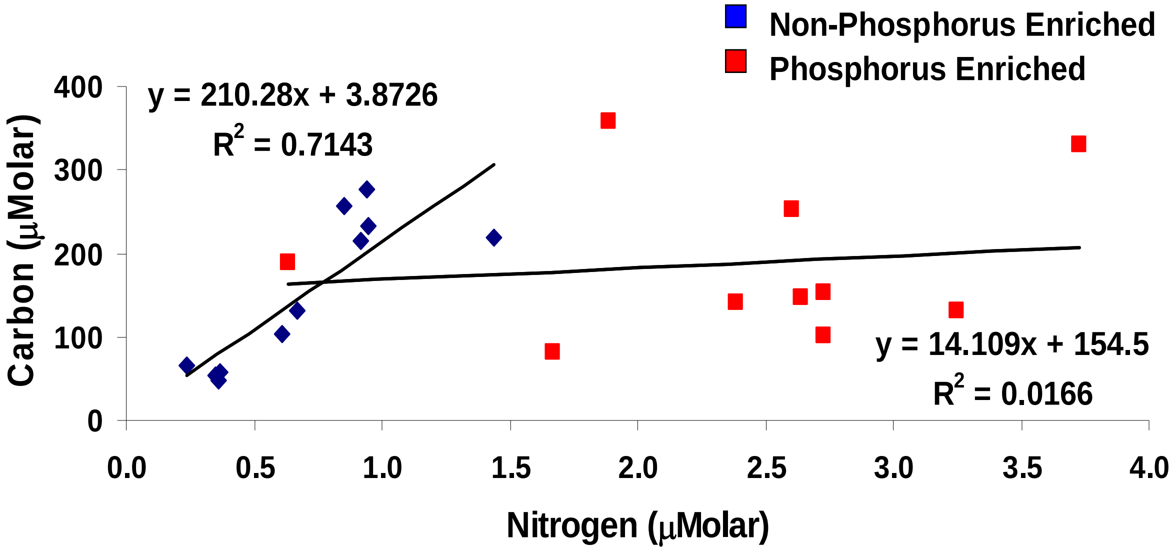


Figure 15: The Growth Chamber Assembly

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