Experimental Evidence for Nutrient Limitation of Phytoplankton Growth in Oyster Pond, Falmouth, Massachusetts

Y. Erlitz
S. Fox
A. Leschen
V. Miller-Sims
Boston University Marine Program
Marine Biological Laboratory
Woods Hole, Massachusetts 02543

Abstract

We conducted an enrichment study to determine nutrient limitation of phytoplankton along the salinity gradient of Oyster Pond, Falmouth, MA. The experimental enrichment increased the supply of nitrate, phosphate, and ammonium in the sample bottles. At salinities up to 7‰ enrichment with nitrate and phosphate increased phytoplankton growth. Nitrogen or phosphorus alone did not increase growth at these salinities. In Vineyard Sound (30 ‰) chlorophyll *a* concentration increased in response to nitrate and ammonium additions suggesting nitrogen limitation. Phytoplankton growth in response to nitrate was two times higher than that of ammonium.

Introduction

In aquatic systems, nitrogen or phosphorus is the most common limiting nutrient since other minerals required for growth may be present in abundance (Ryther and Dunstan 1971; Vince and Valiela 1973; Tomasky et al. 1999). Redfield et al. (1958) proposed that a ratio of nitrogen to phosphorus of 15:1 is required for phytoplankton growth. This ratio can vary considerably; algae exhibit a range of N:P ratios from 5:1 to 15:1 with an average ratio of 10:1 (Ryther and Dunstan 1971). In the surface waters of the sea where photosynthesis rapidly depletes these nutrients, a ratio considerably lower than 15:1 exists, and nitrogen limitation has been observed. Freshwater systems tend to have higher N:P ratios and are limited by phosphorus (Ryther and Dunstan, 1971).

Local studies have demonstrated this difference in nutrient limitation across a salinity gradient. Caraco et al.(1986) and Tomasky et al. (1999) demonstrated that coastal ponds of salinity below 6.5-10‰ in Falmouth were phosphorus limited. A pond with a salinity of 31 ‰ and Vineyard Sound (32‰) were limited by nitrogen. Ponds were both nitrogen and phosphorus limited at intermediate salinities. Since the salinity of Oyster Pond is low (2‰), phytoplankton growth is expected to be limited by phosphorus.

The salinity of Oyster Pond is kept low by means of a weir that controls entry of seawater. Saltwater incursions occasionally occur over the weir with high tides and extreme weather. It is important to know whether these incursions will result in changes in pond salinity, and if so, what implications such changes would have for phytoplankton growth.

Previous attempts to understand nutrient limitation in Oyster Pond have yielded conflicting results. A study conducted in October 2001 found that nutrient enrichments had no effect on phytoplankton growth in Oyster Pond (Doverspike et al. 2001). In contrast, an enrichment study performed in July 2002 found that nitrogen was the limiting nutrient in Oyster

Pond (Weber et al. 2002), despite its expected phosphorus limitation. These conflicting results suggest that further investigation might be of interest.

Nitrogen is present in aquatic systems and is used by phytoplankton in two forms, nitrate and ammonium. Ammonium is thought to be preferentially taken up by phytoplankton, so that other forms of nitrogen are used only when ammonium concentrations are nearly depleted (Valiela 1995). Previous studies in Oyster Pond and other Cape Cod waters (Caraco et al. 1986 and Tomasky et al. 1999) have enriched samples with only the nitrate form of nitrogen, and have not included ammonium enrichments in their experimental design (Weber et al. 2002; Doverspike et al. 2001; Tomasky et. al. 1999). Some of these studies found no nutrient limitation, leading to the question of whether ammonium may be the limiting source of nitrogen for phytoplankton growth.

We performed10-day nutrient enrichment experiments to determine whether nitrogen or phosphorus is currently the limiting nutrient in the Oyster Pond system. In addition to nitrate, phosphate, and nitrate and phosphate (N+P) treatments, we included an ammonium enrichment to determine which form of nitrogen, nitrate or ammonium, is more limiting in this system.

Materials and Methods

To determine whether nitrate, phosphate, or ammonium were limiting growth of phytoplankton in the Oyster Pond system, we enriched bottles of ambient water with nutrients and assessed phytoplankton growth across 10 days by analyzing chlorophyll *a* concentrations. Each experiment consisted of filling 40 2-liter acid-washed polyethylene bottles with ambient water that we filtered through a 200 μm mesh to exclude larger planktonic grazers. To create a nutrient-enriched condition in excess (250 μM) of the ambient, we treated bottles with one of five enrichments: nitrate ($NaNO_3$), phosphate (KH_2PO_4), ammonium (NH_4Cl), nitrate and phosphate, or control (no treatment). We suspended 40 bottles approximately 50 cm below the water surface from PVC rafts anchored at each site. A total of 40 bottles were incubated in situ representing the five treatments, four collection days, and 2 replicates of each. In addition to the bottles incubated in the field, we filled two replicate bottles to determine initial concentrations of nutrients and chlorophyll *a* (Table 1).

To examine whether there was a relationship between salinity and the response of phytoplankton present within the Oyster Pond system, we selected four sites representing salinities between 2 and 30‰ : two in Oyster Pond (2‰), and one each in the lagoon (7‰) and Vineyard Sound (30 ‰) (Fig. 1). The first pond site (Pond 1) was at the northern end of the pond across from the Treetops condominium complex. The second pond site (Pond 2) was at the southern end of the pond approximately 40 m north of the weir, with the lagoon sampling site approximately 20 m south of the weir. Samples from Vineyard Sound were collected adjacent to the mouth of Trunk River. All samples were incubated at the collection site, with exception of the Sound. Vineyard Sound bottles were incubated about 300 m to the west of Trunk River where they would be less likely to be disturbed by bathers or fishermen.

To obtain a description of the time course of phytoplankton growth as a response to the treatments, we collected samples from the field on days 0, 2, 4, 6, and 10, and filtered one liter of each sample through a Whatman 47-mm glass microfiber filter using a vacuum pump. We stored the filter and 250 ml of the filtrate at $-20^{\circ}C$ for chlorophyll and nutrient analyses, respectively. Phosphate and ammonium concentrations in the samples were determined according to Strickland and Parsons (1972), and nitrate concentration according to Jones (1984). Filters were added to 25 ml of cold 90% acetone, and stored for 12 hours at $4^{\circ}C$ to extract the chlorophyll. We centrifuged the samples, and analyzed the supernatants for cholorophyll *a* using spectrophotometric methods and the Lorenzen (1967) equation.

Results and Discussion

Enrichments successfully increased concentrations of nutrients in all bottles (Table 2). Concentrations of phosphate, nitrate, and ammonium in day 4 control samples did not differ between sites. Treatments enriched with phosphate, nitrate, or ammonium created concentrations significantly higher that those of the controls. The N+P treatments also increased nutrient concentrations to levels much higher than those in the controls. All enrichments resulted in concentrations of nutrients that were not likely to be depleted within the time course of this experiment.

Chlorophyll *a* concentrations in water from the four sites showed different patterns in response to nutrient enrichment over the time course (Fig. 2). In Oyster Pond and the lagoon, enrichments with nitrate, phosphate, and ammonium created no significant differences relative to untreated controls. Enrichment with N+P, however, increased chlorophyll *a* to a peak 2-7 times higher than that of the control on day 4. After day 4, the chlorophyll *a* concentrations decreased and returned to control levels by day 10.

These findings suggest that neither nitrogen nor phosphorus independently limited chlorophyll *a* growth in Oyster Pond and the lagoon. Phosphorus limitation would be expected in freshwater ponds, however, our study demonstrates that phytoplankton growth of Oyster Pond can be limited by nitrogen as well. The N+P limitation observed in this study differs from the results of previous studies of the system, which found either no limitation (Doverspike 2001) or nitrogen limitation (Weber et al. 2002). The current N:P ratio in Oyster Pond is 6:1 (Dixon et al. 2002). An unusually high abundance of macrophytes have been observed in the pond and could be contributing to this low ratio. One species of macrophyte, *Ceratophyllum somersum*, is currently holding an estimated 32% of the modeled nitrogen load entering Oyster Pond (Cermak et al. 2002). Future studies should be carried out in this system throughout the year to determine whether nutrient limitation changes over time with other biotic changes in the pond.

In Vineyard Sound, chlorophyll *a* concentrations of phosphorus-enriched bottles mirrored those of the controls, as seen in Oyster Pond and the lagoon (Fig. 2). This site differed from the others, however, in its response to nitrogen enrichment. Both ammonium and nitrate resulted in increased chlorophyll *a* concentrations, peaking on day 2 (14.57 and 24.82 μg/l, respectively), with the effect of nitrate nearly twice that of the ammonium. The N+P enrichment yielded similar results to the other sites, peaking with the greatest increase in chlorophyll concentration on day 4 (38.24 μg/l).

The three enrichments containing nitrogen (nitrate, ammonium, and N+P) increased chlorophyll *a* concentrations supporting the findings of previous studies which have shown nitrogen to be limiting in Vineyard Sound (Caraco et al. 1986; Weber et al. 2002). Unexpectedly, nitrate had a larger effect on phytoplankton growth than ammonium, which is thought to be preferentially absorbed. In the N+P bottles the continued phytoplankton growth after growth in nitrate enrichments has leveled off could be due to secondary limitation by phosphorus.

The similarity in our findings for Oyster Pond (2 ‰ salinity) and the lagoon (7‰) suggests that additions of saltwater to Oyster Pond either by nature (storms) or design (manipulation of the weir) will not change nutrient limitation status under its current dynamic structure. Because we found nitrogen and phosphorus together to be limiting in Oyster Pond, future management of the pond should be concerned with the level of both of these nutrients in order to control algal blooms.

Literature Cited

Caraco, N., Tamse, A., Boutros O., and I. Valiela.1987. Nutrient limitation of phytoplankton growth in brackish coastal ponds. Canadian Journal of Fisheries and Aquatic Sciences 44:473-476.

Jeffrey, S.W. and G.F. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls *a, b,* c_1 and c_2 in higher plants, algae, and natural phytoplankton. Biochemische Physiologie Pflanzen 167:191-194.

Jones, M. N.1984. Nitrate reduction by shaking with cadmium. Water Research 18:643-646.

Klochenko, P.D. and V.A Medved.1993. Transformation of nitrogen fertilizers during the growth of certain green and blue-green algae. Hydrobiological Journal 29: 64-69.

Lorenzen, C. J.1967. Determination of chlorophyll and pheo-pigments: spectophotometric equations. Limnology and Oceanography 12:343-346.

Redfield, A. C.1958. The biological control of chemical factors in the environment. American Scientist 46:205-221.

Ryther, J. H. and W. M. Dunstan.1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science 171:1008-1013.

Strickland, J.D.H. and T.R. Parsons.1972. A Practical Handbook of Seawater Analysis, 2nd ed. Fisheries Research Board of Canada, Ottawa.

Tomasky, G., Barak, J.,Valiela, I., Behr, P., Soucy L. and K. Foreman.1999. Nutrient limitation of phytoplankton growth in Waquoit Bay, Massachusetts, USA: a nutrient enrichment study. Aquatic Ecology 33:147-155.

Valiela, I.1995. Marine Ecological Processes, 2nd ed. Springer-Verlag, New York, New York.

Vince, S. and I. Valiela.1973. The effects of ammonium and phosphate enrichments on chlorophyll *a*, pigment ratio and species composition of phytoplankton of Vineyard Sound. Marine Biology 19:69-73.

Weber, C. F., Barron, S., Marino, R., Howarth R.W., Tomasky G., and E. A. Davidson. 2002. Nutrient limitation of phytoplankton growth in Vineyard Sound and Oyster Pond, Falmouth, Massachusetts. In press.

Sources of Unpublished Materials

Cermack M., Crandall E., Young J. Boston University Marine Program, Marine Biological Laboratroy, Woods Hole, MA 02543.

Dixon, S., Rowan, W., Grant, D., and J. Whitney. Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543.

Doverspike, S., A. Gewirtzman, P. Jekielek, and K. Priesner, Boston University Marine Pogram, Marine Biological Laboratory, Woods Hole, MA 02543.

Good S. Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543

Table 1. Experimental Layout of Nutrient Enrichments

	Collection Day			
Treatment	2	4	6	10
NO_3	2 replicates	2 replicates	2 replicates	2 replicates
NH_4	2 replicates	2 replicates	2 replicates	2 replicates
PO_4	2 replicates	2 replicates	2 replicates	2 replicates
NO_3+PO_4	2 replicates	2 replicates	2 replicates	2 replicates
Control	2 replicates	2 replicates	2 replicates	2 replicates

Table 2. Mean Nutrient Concentrations ± Standard Error of Unenriched Control Samples and Those that Received Enrichments in Each of the Four Sites.

	Phosphate Concentration (μ M)		
Sample Site	Control	PO_4 Enrichment	$NO_3 + PO_4$ Enrichment
Pond 1	0.01 ± 0.05	270 ± 0.0	221 ± 58
Pond 2	0.27 ± 0.23	249 ± 47	195 ± 33
Lagoon	0.10 ± 0.02	249 ± 47	308 ± 21
Vineyard Sound	0.07 ± 0.04	293 ± 40	249 ± 24

	Nitrate Concentration (μ M)		
Sample Site	Control	NO_3 Enrichment	$NO_3 + PO_4$ Enrichment
Pond 1	1.12 ± 1.13	560 ± 54	527 ± 14
Pond 2	2.33 ± 0.20	447 ± 150	545 ± 23
Lagoon	2.36 ± 0.17	531 ± 49	537 ± 35
Vineyard Sound	2.19 ± 0.06	522 ± 44	514 ± 3.0

	Ammonium Concentration (μ M)	
Sample Site	Control	NH_4 Enrichment
Pond 1	0.71 ± 0.14	161 ± 0.0
Pond 2	0.99 ± 0.47	231 ± 2.0
Lagoon	1.83 ± 0.88	221 ± 25
Vineyard Sound	0.32 ± 0.32	227 ± 14

Figure Legends

Fig. 1. Map of Oyster Pond estuary system in Falmouth, MA, on the south shore of Cape Cod. Sample sites are indicated by solid circles.

Fig 2. Time course of mean chlorophyll *a* concentrations (mg m^{-3}) over the ten day incubation period is plotted for the four sites and five treatments. Sites are as follows Pond 1 (P1), Pond 2 (P2), Lagoon (L), Vineyard Sound (VS). The y-axis error bars represent standard error.

Figure 1

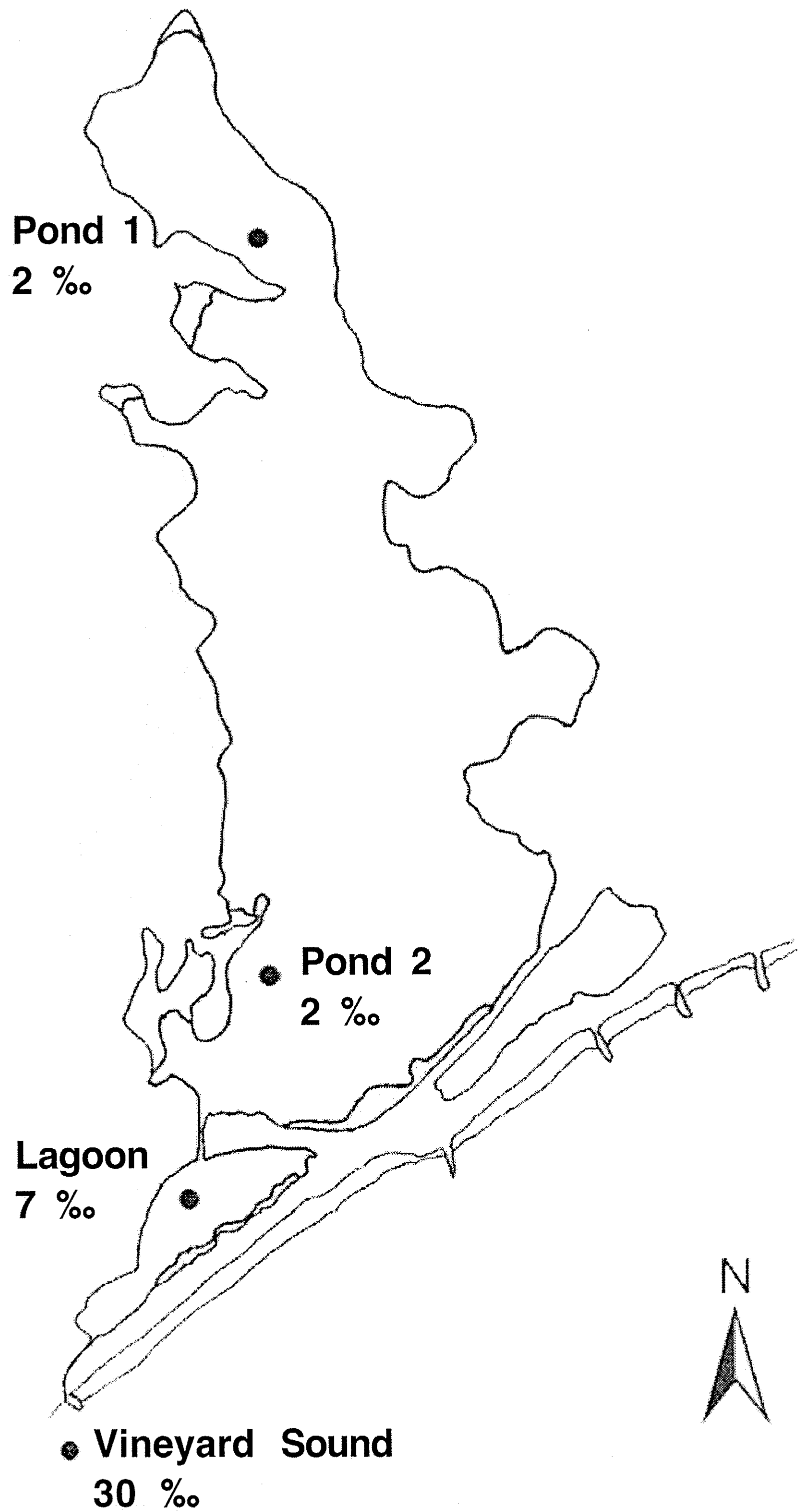
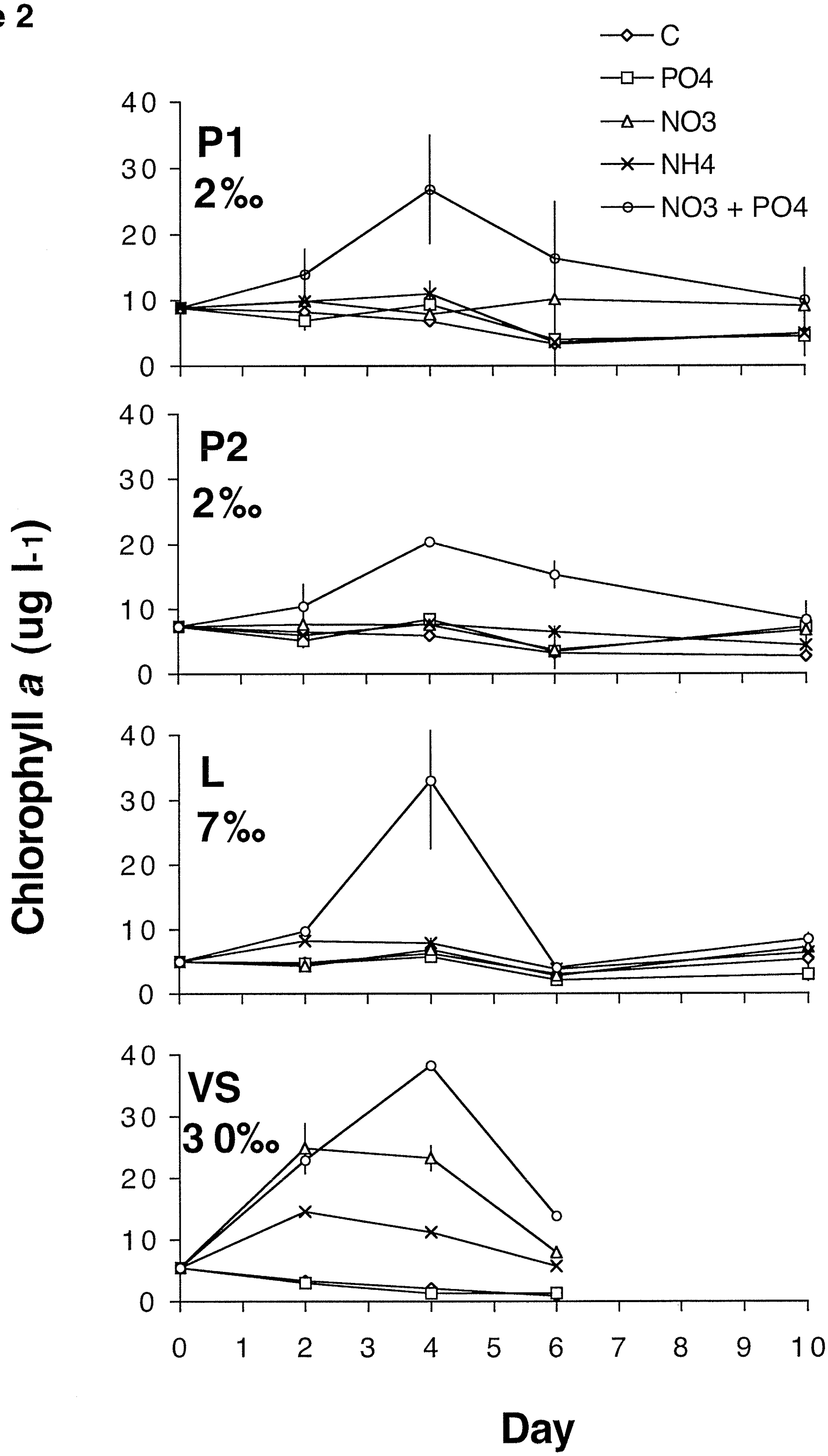


Figure 2



C
PO4
NO3
NH4
NO3 + PO4
P1
2‰
P2
2‰
L
7‰
VS
3 0‰
40
30
20
10
0
Chlorophyll a (ug l-1)
0
1
2
3
4
5
6
7
8
9
10
Day