

**Figure 1.** Chlorophyll *a* (mean  $\pm$  s.e.) through time in enrichment experiments done with N plus P additions and P additions only for three salinities (0.2‰, 2.3‰, and 5.0‰). Note scale differences on x and y axes. Different letters denote significant differences at the  $P < 0.05$  level using one-way ANOVA and Tukey's honest significant difference test. Water was collected from the northern end of Oyster Pond and mixed with deionized and Vineyard Sound water to produce the salinities. The experiment was done on July 21, 2002.

not statistically significant. The large increase in heterocysts in the 2.3‰ treatment may have influenced the final chlorophyll value by adding N to the water, allowing other species to grow.

The stimulation of phytoplankton growth in the P addition treatment contrasts with the finding of a companion study (5) which found that P additions to undiluted Oyster Pond water

incubated under the same conditions did not significantly increase phytoplankton biomass. Two differences may explain this. The experiment described here ran for twice as long, allowing more time for the typically slow-growing cyanobacteria, present in the pond water at very low abundances, to respond. Further, our P addition treatment had much lower inorganic N (owing to the 10-fold dilution of Oyster Pond water), which also may have provided conditions more favorable for heterocyst development and N fixation, resulting in enough increase in N availability to increase phytoplankton biomass. This apparent difference between the two experiments bears further experimental investigation.

This short-term experiment should be interpreted with caution because over time cyanobacteria might adapt to a change in salinity. Cyanobacteria can grow and fix N up to 32‰ salinity, although they do so more slowly at higher salinities (3). Also, heterocyst abundance in Oyster Pond is low compared to lakes with high rates of N-fixation (6). Thus N-fixing cyanobacteria may not be present in great enough numbers in Oyster Pond at this time of year to alleviate N-limitation. Nonetheless, these experiments suggest that there may be a potential in Oyster Pond for eutrophication in response to both P enrichment alone as well as to N + P enrichment. Thus, managers should consider the sources of and possible controls on both N and P inputs to the pond. Further, it does not appear that manipulating salinity within the range tested here (0.2‰–5‰) will substantially affect phytoplankton growth directly.

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### Nutrient Limitation of Phytoplankton Growth in Vineyard Sound and Oyster Pond, Falmouth, Massachusetts

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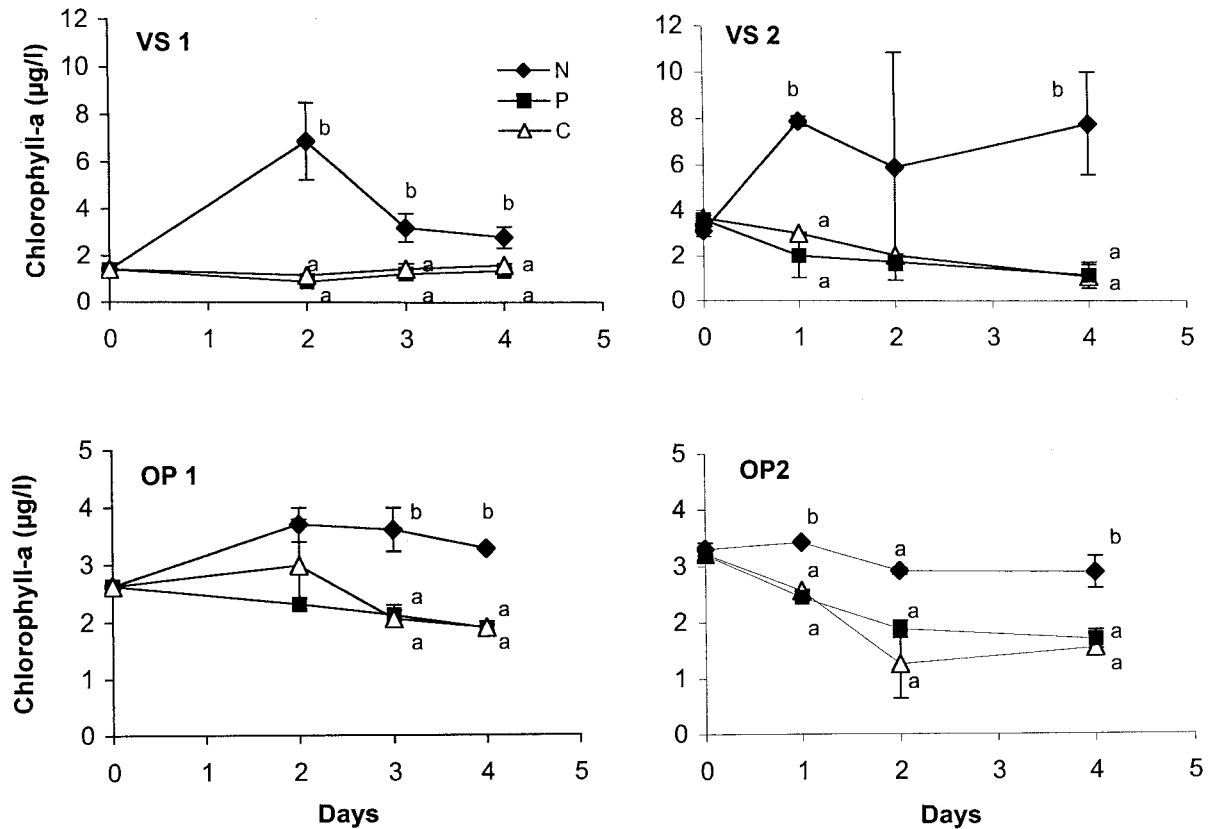
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Phytoplankton growth requires nitrogen (N) and phosphorus (P) in an approximate molar ratio of 16:1 (the Redfield ratio; 1). N or P limitation in an aquatic system is considered to occur when the availability of N relative to P is well below or above this ratio, respectively (2, 3). Past studies have shown that marine systems of moderate to high productivity are typically N limited, while sim-



**Figure 1.** Chlorophyll *a* concentrations (mean  $\pm$  1 s.e.) during experiment 1 and experiment 2 with water collected from Oyster Pond (OP, bottom panels) and Vineyard Sound (VS, top panels). Where standard errors cannot be seen they are smaller than the symbol. Statistical similarities and differences, as denoted by lowercase letters, were determined by a one-way ANOVA followed by Tukey's honest significant difference test ( $P < 0.05$ ).

ilarly productive freshwater systems are most often P limited (2, 3). However, relatively little is known about low-salinity estuaries. The Baltic Sea is perhaps the best-studied estuary of this type; there, productivity has been shown to be limited by P at salinities lower than 3 to 4‰ and by N at higher salinities (4).

Here, we report the results of a comparative set of nutrient limitation experiments in two coastal systems in Falmouth, Massachusetts, of very different salinities: Vineyard Sound and Oyster Pond (32‰ and 2.3‰, respectively). Previous studies have reported N limitation in Vineyard Sound (5, 6) as would be expected for a high-salinity coastal ecosystem (2, 3). In an October 1986 study, phytoplankton in Oyster Pond did not respond to N or P enrichments (5); Boston University Marine Program students obtained the same result from a similar experiment performed on Oyster Pond in October 2001. However, these experiments were not done during the peak growing season. Oyster Pond is currently considered to be mesotrophic to eutrophic (7), and with the watershed nearing buildout, effective management of nutrient inputs may be important in controlling eutrophication and algal blooms of concern.

We conducted two sets of bottle enrichment experiments, from June 30 to July 5, 2002, and from July 22 to July 26, 2002. For both experiments, we sieved water through a 150- $\mu$ m mesh to remove large zooplankton. In the first experiment, 12 replicate, 2-l polycarbonate bottles from each system received enrichments of

$\text{NaNO}_3$  or  $\text{NaH}_2\text{PO}_4$  that increased ambient concentrations of nitrate by about 50  $\mu\text{M}$  (N treatment) or phosphate by about 10  $\mu\text{M}$  (P treatment); 12 control bottles from each system received no nutrient additions (C treatment). As a safeguard against short-term  $\text{CO}_2$  depletion in the bottles, we added  $\text{NaHCO}_3$  (2.0 mM) to the Oyster Pond samples. At the beginning of the experiment, nine bottles were sampled immediately (three each of controls and three each of the  $\text{PO}_4$  and  $\text{NO}_3$  additions) to determine initial chlorophyll *a* concentrations and confirm the effectiveness of the nutrient enrichments. The remaining bottles containing Oyster Pond or Vineyard Sound water were incubated 0.5 m to 1 m below the surface of Oyster Pond on a floating rack, at a light intensity of about 330–560  $\mu\text{E m}^{-2}\text{s}^{-1}$  (peak daylight hours). We collected three replicate bottles of each treatment on days 2, 3 and 4. Subsamples were filtered (GF/F) and chlorophyll *a* concentrations were determined fluorometrically (8).

We started our second set of experiments on July 22, 2002. The nutrient treatments were identical to the first experiment, except a treatment was added for Oyster Pond water in which both  $\text{NO}_3$  and  $\text{PO}_4$  were added to increase ambient concentrations to 50  $\mu\text{M}$  and 3  $\mu\text{M}$ , respectively, to parallel another concurrent set of experiments done in Oyster Pond (7). We repeatedly removed 100-ml samples from each of twelve 2-l bottles over time for chlorophyll analysis, rather than having replicate bottles for each time point.

We incubated the bottles in a growth chamber on a 15:9 h light:dark cycle at a light intensity of 280–350  $\mu\text{E m}^{-2} \text{s}^{-1}$  and a temperature of 24 to 29 °C. All treatments were sampled initially, and on days 1, 2, and 4.

In the first experiment with Vineyard Sound water, chlorophyll *a* concentrations increased in the N-enriched treatment by day 2, and rapidly declined thereafter (Fig. 1); Concentrations were significantly higher than those of the controls and P-enriched treatment. In the second experiment, chlorophyll concentrations in the N-enriched bottles peaked on day 1 and were always significantly higher than the controls. In contrast, P-enriched treatments were never significantly different from the controls in either experiment (Fig. 1). Both experiments indicate that phytoplankton growth in Vineyard Sound was N limited, as previously reported (5, 6).

In the experiments with Oyster Pond water, chlorophyll *a* concentrations in the N-enriched treatment were significantly higher on two out of the three sampling dates for both experiments (Fig. 1). Chlorophyll *a* concentrations in P-enriched bottles did not differ significantly from controls at any time (Fig. 1). In the second experiment when both N and P were added, the response was far greater, with a final chlorophyll *a* concentration of 23.2  $\mu\text{g l}^{-1}$  on day 4 (data not shown). This suggests that P can quickly become limiting if enough N is supplied. The significant response in the N-enriched treatment in both our experiments differs from previous studies in Oyster Pond, which found no nutrient limitation (5), and from studies in low-salinity parts of the Baltic Sea (<3 to 4‰) which concluded that P was limiting (4).

Our results contribute to the large body of experimental evidence that finds N limitation in temperate coastal marine ecosystems of moderately high salinity, such as Vineyard Sound. For low-salinity estuaries, there are fewer studies on nutrient limitation, but our finding of N limitation is unusual. The difference

between earlier studies in Oyster Pond and our study may reflect seasonal changes in nutrient limitation. Nitrogen may be limiting during the summer (our study) while neither N nor P is limiting in mid-fall (previous studies), either because there is less overall demand for nutrient late in the season or because N fixation over the summer and early fall has helped alleviate N limitation. Further research is needed to better understand nutrient limitation in low-salinity ecosystems, and to evaluate the relative importance of the many biogeochemical processes including N fixation that may regulate limitation in these systems. Nonetheless, our study suggests that N availability, rather than P, currently regulates phytoplankton growth in Oyster Pond during the summer.

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## Response of Shrimp Populations to Land-Derived Nitrogen in Waquoit Bay, Massachusetts

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Land-derived nitrogen impacts are a major agent of change affecting estuarine populations. Some changes include macroalgae and phytoplankton blooms, which alter food webs and benthic habitats (1). N loads may influence the abundance, species composition, and growth rates of the shrimp species that are common in estuaries of Cape Cod, such as those in the genera *Palaemonetes* and *Crangon* (2). Estuaries of the Waquoit Bay estuarine system offer the opportunity to examine how shrimp of different species respond to different land-derived N loads, because different sub-estuaries are subject to different land-derived loads. For example, Sage Lot Pond, Quashnet River, and Childs River receive N loads of 15.9, 310.3, and 360 kg N ha<sup>-1</sup> y<sup>-1</sup>, respectively (3). The

estuaries have similar water residence times, about 1–2 days, and range from 0–32 ppt (1).

In this study we assessed the effects of differences in land-derived N loads on shrimp abundance, shrimp species composition, growth rate, and reproduction in estuaries of Waquoit Bay, Massachusetts.

To estimate the abundance and size of shrimp of the different species, we walked a 5-m seine for 10 m in each of five arbitrary locations along the shore, beginning with the most fresh to the most saline of each estuary, during high tide. Shrimp were identified, counted, and measured from the tip of the rostrum to the end of the carapace. To estimate growth rates in *Palaemonetes pugio*, we first identified the modal carapace length of each cohort present, using the software program Mix 3.1.3, and calculated the increment in size per month. In addition, we recorded percent of ovigerous females in each estuary. We used ANOVA to compare species abundance and percent ovigerous females among the

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