Chapter 3

Nitrogen and phosphorus as limiting factors for phytoplankton in the Oyster Pond estuary

SARAH DOVERSPIKE, ARON GEWIRTZMAN, PHOEBE JEKIELEK,
AND KAROLINA PRIESNER

Boston University Marine Program, Marine biological Laboratory,
Woods Hole, MA 02543

Abstract

We conducted an enrichment study to determine nutrient limitation of phytoplankton along the salinity gradient of Oyster Pond, Falmouth, Massachusetts. The experimental enrichment succeeded in increasing supply of nitrate and phosphate to the selected samples. Freshwater sites showed little response to nutrient enrichments, suggesting either that nutrients were already in excess or that there was insufficient time for algal growth. Chlorophyll *a* concentrations increased only in the Vineyard Sound water where nitrate was added to the bottles, suggesting nitrogen limitation in these coastal waters. Cyanobacteria were most common in freshwater sites and least abundant in coastal waters, dinoflagellates showed the opposite trend, and diatoms showed no apparent preference for salinity.

Introduction

Either nitrogen or phosphorus usually limits phytoplankton production in fresh and coastal systems (Ryther and Dunstan 1971, Vince and Valiela 1973, Tomasky 1999). In both fresh and coastal waters, phosphate is by far the dominant form of phosphorus while nitrogen exists in many forms such as nitrate, nitrite, and ammonium. Ammonium is preferentially taken up by phytoplankton so that other forms of nitrogen are used only when ammonium concentrations are nearly depleted (Valiela 1995). In freshwater systems, phytoplankton growth is generally limited by phosphorus availability (Schindler 1977), while coastal systems show nitrogen limitation (Ryther and Dunstan 1971).

Redfield (1958) suggested that primary producers require nitrogen (N) and phosphorus (P) in a ratio of approximately 16:1. Ryther and Dunstan (1971) suggested that this ratio is not rigid and can vary slightly depending on an individual organism's needs and the ratio present in the water. Generally, freshwater organisms require an atom ratio of available N:P greater than 15:1, while saltwater organisms tend to have N:P requirements lower than this Redfield ratio. The higher N:P ratio in freshwaters suggests for phosphorus limitation, while the low N:P ratio in saltwater indicates nitrogen limitation.

Generally, estuaries have a range in salinity between 0 and 30 ppt as well as a shift from P to N limitation of phytoplankton growth (Caraco 1987, Tomasky 1999). Caraco et al. (1987) suggested that in Cape Cod ponds and estuaries the phosphorus supply controls phytoplankton production at low salinities while nitrogen limits production where salinities were greater than 10 ppt. Oyster Pond in Cape Cod, MA offers an opportunity to test these suppositions because the surface of the pond is mainly freshwater while the neighboring surface waters in Vineyard Sound are saline.

To determine whether N or P limited the accumulation of phytoplankton biomass in the fresh to saltwater gradient of Oyster Pond, we experimentally enriched bottles containing fresh and salty water with nitrogen and phosphorus and measured ensuing changes in chlorophyll *a* concentration. Some bottles were enriched with both N and P (NP) to assess the possible response to both nutrients. In addition to a simple increase in phytoplankton biomass, the taxonomic composition of the phytoplankton within the container could change in response to the enrichments. We monitored the change in taxonomic composition in two different ways, noting trends along the salinity gradient and in response to enrichments.

Materials and Methods

To obtain water varying from fresh to salty, we collected samples from two locations within Oyster Pond and one each from the adjacent lagoon and Vineyard Sound (Fig. 1). Salinity at each site was measured with a refractometer. Water samples for nutrient and phytoplankton concentrations were taken from the upper 50 cm of the water and filtered through a 250-_m sieve to remove large zooplankton. Sixteen samples were taken from each location and stored in acid-washed two-liter polyethylene bottles. Two replicate bottles were subjected to each treatment.

The experimental treatments were as follows: control bottles (C) received no additional nutrients; nitrate-enriched bottles (N) received $NaNO_3$ to increase nitrate concentration at least 50 times above that of the ambient; phosphate-enriched bottles (P) received KH_2PO_4 to raise phosphate concentration at least 50 times above the ambient; nitrate and phosphate-enriched bottles (NP) received both enrichments. All sample bottles were incubated in the southern end of the pond at a depth between 0.5-1 m. Bottles from each site and nutrient treatment were collected at day 0 (initial), day 1 (24 hour incubation), day 4 (96 hour incubation), and day 6 (144 hour incubation).

To assess whether the experimental enrichment worked, we measured initial concentrations of phosphate, nitrate, and ammonium in the sample bottles. To prepare samples for nutrient analysis, 500 ml of water was filtered through a Whitman filter and the filtrate was preserved with reagent quality HCl and stored in the dark at $4^{o}C$. All nutrients were measured spectrophotometrically, using procedures described in Murphy and Riley (1962), Strickland and Parsons (1972), and Jones (1984).

Chlorophyll *a* concentrations were measured by filtering 1 L of water through a Whitman filter. Filters were placed in centrifuge tubes filled with 25 ml of cold 90% acetone and stored in the dark at $4^{o}C$ for 12 hours. The acetone containing chlorophyll extract was analyzed after centrifugation using spectrophotometric methods at 665 nm and 750 nm. Absorbances were converted to concentrations using the Lorenzen (1967) equation.

To obtain a measure of relative changes in taxon abundance occurring under each treatment and along the fresh-to-salt water gradient, we preserved 250 ml of unfiltered water in polyethylene bottles containing Lugol's solution. Bottles were stored in the dark at 4°C until analysis. Samples were centrifuged to obtain a pellet, which was then placed on a slide and analyzed microscopically. We assigned values of 0 (absent), 1 (rare), 2 (common), and 3 (abundant) to samples as a qualitative assessment of the relative abundance of cyanobacteria, diatoms, dinoflagellates, rotifers, and copepod nauplii in each sample.

Results and discussion

Nutrients

The experimental enrichment succeeded in increasing supply of the desired nutrients. All enriched bottles showed concentrations of the added nutrient(s) about 50 times higher than ambient concentrations (Fig. 2, Fig. 3), indicating that enriched bottles contained nutrient concentrations at a level unlikely to be completely depleted within the duration of our study.

Ambient phosphate concentrations increased slightly with an increase of salinity, and there was a minor decrease in phosphate concentration by day 6. Because there was no substantial decrease in phosphate in any bottles and no increase in chlorophyll *a* in P-enriched bottles, we suggest that phosphorus might not be limiting growth of phytoplankton. Even though relatively low concentrations were found in the water, phosphate is recycled, and so it might not be limiting in supply (Valiela 1995).

Ambient nitrate concentrations were higher in fresher water (Fig. 3). Nitrate-enriched bottles showed an increase in nitrate concentration by the end of day 6, while bottles not enriched with nitrate showed no trend. While the phytoplankton initially utilized some of the nitrate in the N-enriched bottles, the overall increase in nitrate suggests that some other processes may be involved. First, the high nutrient turnover rate exhibited by phytoplankton may keep the nitrate concentrations from decreasing. Secondly, nitrogen fixers such as cyanobacteria contribute additional inorganic nitrogen to the water column, which may be responsible for the unexpected rise in nitrate concentration. In the case of the Vineyard Sound N-enriched bottles, nitrate concentrations did not decrease even when chlorophyll *a* concentrations increased substantially.

Although no bottles were enriched with ammonium, there was an unexplained increase in concentration in nitrate-enriched bottles (Fig. 4) with no discernible pattern within or among sites over the six-day incubation period. We cannot explain this, but rapid turnover of nitrate and ammonium was also reported by Klochenko and Medved (1993) and Triska (1993).

Chlorophyll a

Initial concentrations of chlorophyll *a* were relatively similar for all sites (Fig. 5). Concentrations were low, as found by Foreman et al. (submitted) who reported low chlorophyll concentrations in October (Fig. 6). This suggests that chlorophyll *a* in Oyster Pond is as low or lower than the chlorophyll *a* concentration found in the nearly pristine estuary, Sage Lot Pond. Phytoplankton in freshwater and saltwater responded differently to treatments. Freshwater sites showed little response to nutrient enrichments, suggesting either that nutrients were already in excess or that there was insufficient time for the algae to grow. Chlorophyll *a* concentrations increased clearly only in the Vineyard Sound water where we added nitrate to the bottles. Chlorophyll growth in nitrogen-enriched bottles indicates that Vineyard Sound is nitrogen limited, as found by Vince and Valiela (1973). Since N and NP bottles showed the same chlorophyll concentrations, we conclude that phosphorous is not secondarily limiting in Vineyard Sound.

Relative abundance of phytoplankton

We analyzed at the relative abundance of major taxa by microscopically observing major groups of phytoplankton: cyanobacteria, diatoms, dinoflagellates, rotifers, and copepod nauplii (Table 1). The relative abundance of these taxa, as measured by our qualitative index, was not altered by the enrichment treatments (Fig. 7). There were no evident differences in abundance assessed with N or P enrichments, suggesting insufficient time for algal growth or adequate ambient nutrient concentrations.

The relative abundance of the major taxa did differ among sites, probably due to the differences in salinity (Fig. 8). The differences can be highlighted by comparing the salinity extremes, NOP and VS, in Fig. 8. While cyanobacteria were abundant in the freshwater, they were rare to absent in salty water. Cyanobacteria often bloom in eutrophic water, so we may suppose that further nutrient enrichment of Oyster Pond might foster greater blooms. Diatoms were abundant in all sites, but dinoflagellates were common only in salty water. Rotifers, a freshwater organism, were absent in salty water.

Acknowledgments

We would especially like to thank Gabrielle Tomasky for her guidance and allowing us to use her incubation rack. We also would to thank Ivan Valiela, Marci Cole, and Joanna York for their guidance and support throughout our study.

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Table 1: Relativeabumdance of species composition for the four treatments [control (C), nitrate (N), phosphate (P), and nitrate plus phosphate (NP)] in each site [North Oyster Pond (NOP), South Oyster Pond (SOP), Lagoon (L), and Vineyard Sound (VS)].
Scale is as follows: abundant (3), common (2), rare (1), and absent (0).

	NOP			
	C	N	P	NP
Cyanobacteria	2.5 ± 0.5	2.5 ± 0.5	3.0 ± 0.0	3.0 ± 0.0
Diatoms	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	3.0 ± 0.0
Dinoflagellates	0.5 ± 0.5	0.5 ± 0.5	1.0 ± 0.0	1.0 ± 1.0
Rotifers	0.0 ± 0.0	0.5 ± 0.5	1.5 ± 0.5	0.5 ± 0.5
Other grazers	1.5 ± 0.5	2.5 ± 0.5	2.0 ± 0.0	1.0 ± 0.0
	SOP			
	C	N	P	NP
Cyanobacteria	2.5 ± 0.5	3.0 ± 0.0	2.5 ± 0.5	3.0 ± 0.0
Diatoms	2.3 ± 0.3	1.5 ± 0.5	2.0 ± 1.0	3.0 ± 0.0
Dinoflagellates	0.0 ± 0.0	1.5 ± 0.5	1.5 ± 0.5	1.0 ± 1.0
Rotifers	0.0 ± 0.0	2.5 ± 0.5	1.0 ± 1.0	2.0 ± 1.0
Other grazers	0.5 ± 0.5	2.5 ± 0.5	1.0 ± 0.0	2.5 ± 0.5
	L			
	C	N	P	NP
Cyanobacteria	2.0 ± 1.0	2.0 ± 1.0	2.0 ± 1.0	1.5 ± 0.5
Diatoms	3.0 ± 0.0	3.0 ± 0.0	2.5 ± 0.5	3.0 ± 0.0
Dinoflagellates	2.0 ± 0.0	2.0 ± 0.0	1.5 ± 0.5	2.0 ± 0.0
Rotifers	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Other grazers	1.5 ± 0.5	1.0 ± 0.0	1.5 ± 0.5	1.5 ± 0.5
	VS			
	C	N	P	NP
Cyanobacteria	1.0 ± 0.0	0.5 ± 0.5	0.8 ± 0.8	0.8 ± 0.8
Diatoms	2.8 ± 0.3	3.0 ± 0.0	2.5 ± 0.5	3.0 ± 0.0
Dinoflagellates	2.0 ± 1.0	2.5 ± 0.5	1.5 ± 0.5	0.5 ± 0.5
Rotifers	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Other grazers	1.5 ± 0.5	1.5 ± 0.5	1.0 ± 0.0	2.0 ± 0.0

Figure Legends

Fig. 1. Map of the Oyster Pond estuary system of Falmouth, MA, on the south shore of Cape Cod. Sample sites are indicated by abbreviation as follows: North Oyster Pond (NOP), South Oyster Pond (SOP), Lagoon (L) and Vineyard Sound (VS).

Fig. 2. Mean (± standard error) phosphate concentrations (_M) during the incubation period for the four sites and nutrient treatments: controls (C), nitrate-bottles (N), phosphate-bottles (P), and nitrogen and phosphate-bottles (NP). Sites are: North Oyster Pond (NOP), South Oyster Pond (SOP), Lagoon (L) and Vineyard Sound (VS).

Fig. 3. Mean (± standard error) nitrate concentrations (_M) during the incubation period for the four sites and nutrient treatments: controls (C), nitrate-bottles (N), phosphate-bottles (P), and nitrogen and phosphate-bottles (NP). Sites are: North Oyster Pond (NOP), South Oyster Pond (SOP), Lagoon (L) and Vineyard Sound (VS).

Fig. 4. Mean (± standard error) ammonium concentrations (_M) during the incubation period for the four sites and nutrient treatments: controls (C), nitrate-bottles (N), phosphate-bottles (P), and nitrogen and phosphate-bottles (NP). Sites are: North Oyster Pond (NOP), South Oyster Pond (SOP), Lagoon (L) and Vineyard Sound (VS).

Fig. 5. Time course of mean Chlorophyll *a* concentration (mg m^{-3}) over the six-day incubation period is plotted for the four sites and treatments. Sites are as follows: North

Oyster Pond (NOP), South Oyster Pond (SOP), Lagoon (L) and Vineyard Sound (VS). The y-axis error bars represent standard error.

Fig. 6. Mean seasonal chlorophyll concentrations for Sage Lot Pond (also found in coastal Massachusetts) adapted from Forman et al. (submitted). Initial chlorophyll *a* concentrations from our study are averaged over the four sites and plotted in relation to seasonal variations.

Fig. 7. Relative abundance of phytoplankton averaged over collection days 4 and 6. Species composition compared by treatment [Control (C), Nitrate (N), Phosphate (P), Nitrate and Phosphate (NP)] to determine effect of enrichment.

Fig. 8. Relative abundance of phytoplankton averaged over collection days 4 and 6. Species composition compared by site [North Oyster Pond (NOP), South Oyster Pond (SOP), Lagoon (L) and Vineyard Sound (VS)] to determine effect of salinity gradient.

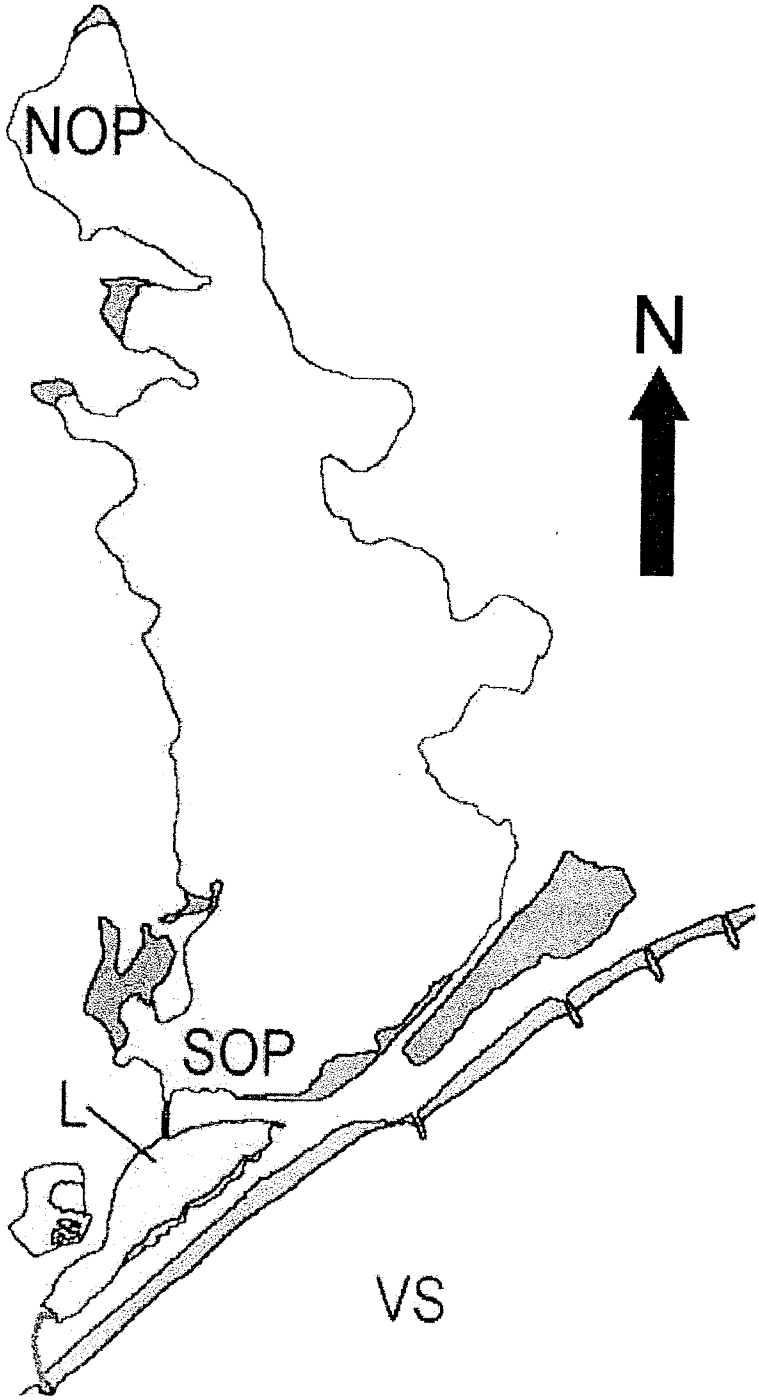
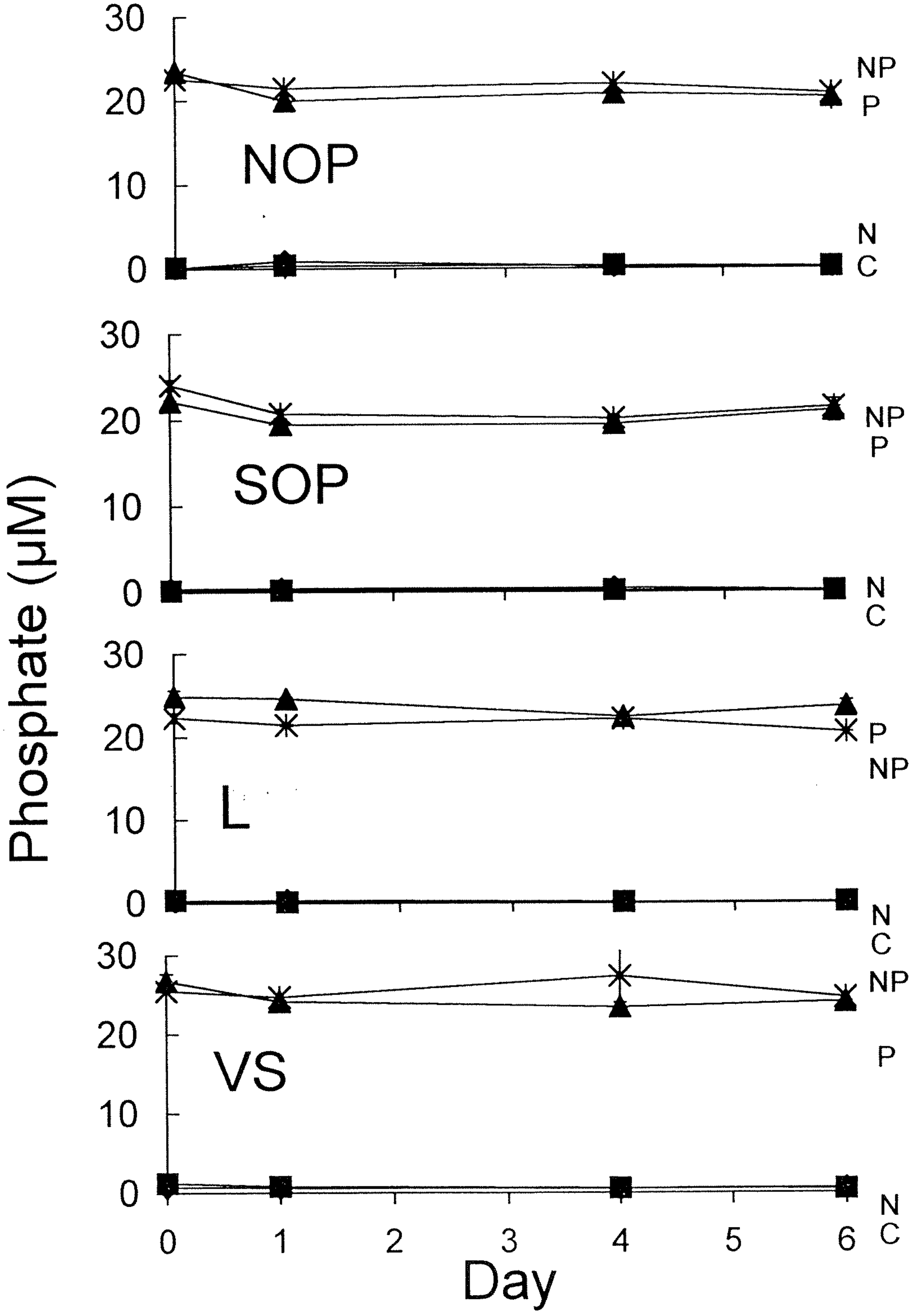


Figure 1



Phosphate (µM)
30
20
10
0
NOP
NP
P
N
C
SOP
NP
P
N
C
L
P
NP
N
C
VS
NP
P
N
C
0
1
2
3
4
5
6
Day

Figure 2

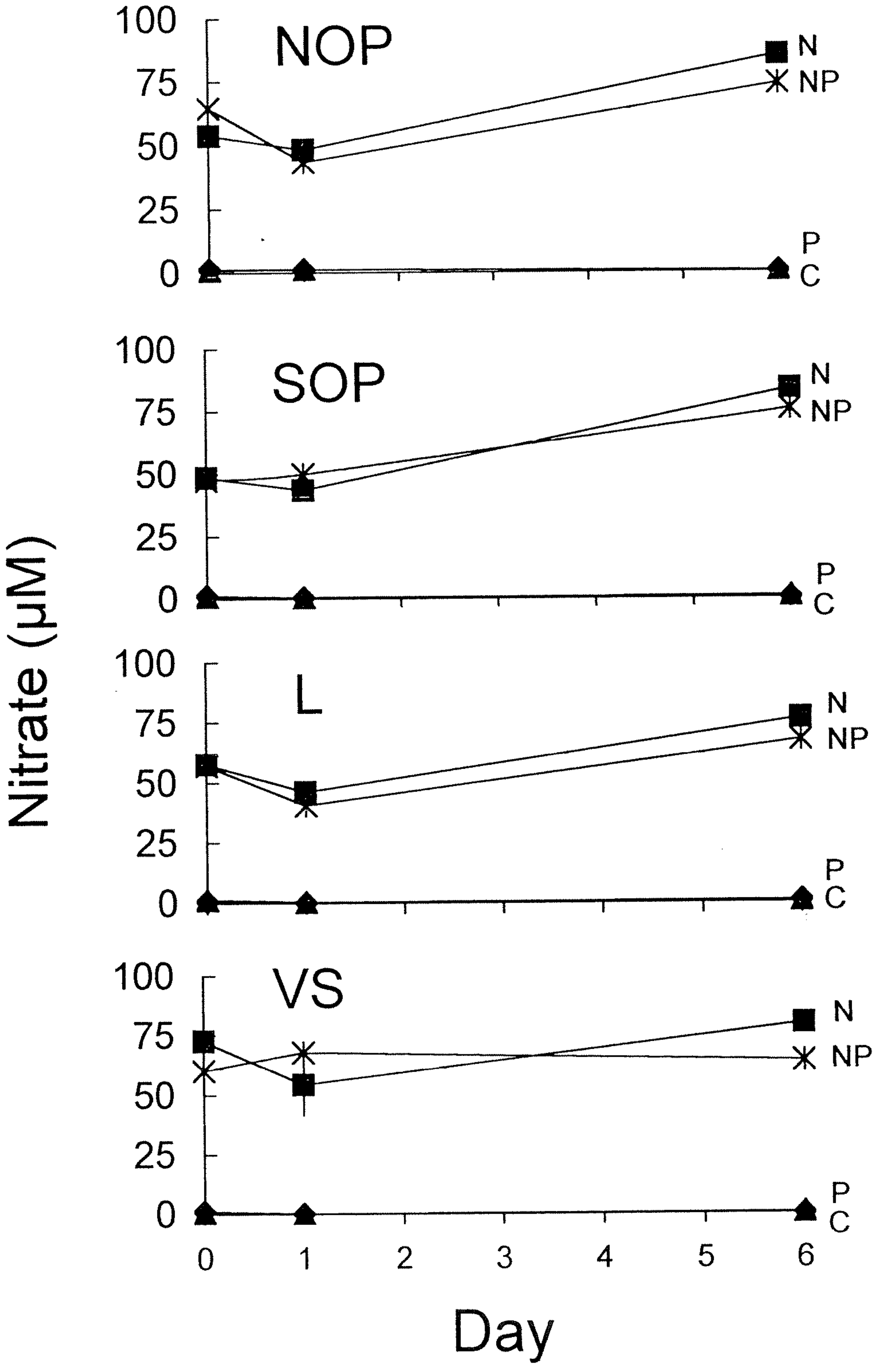


Figure 3

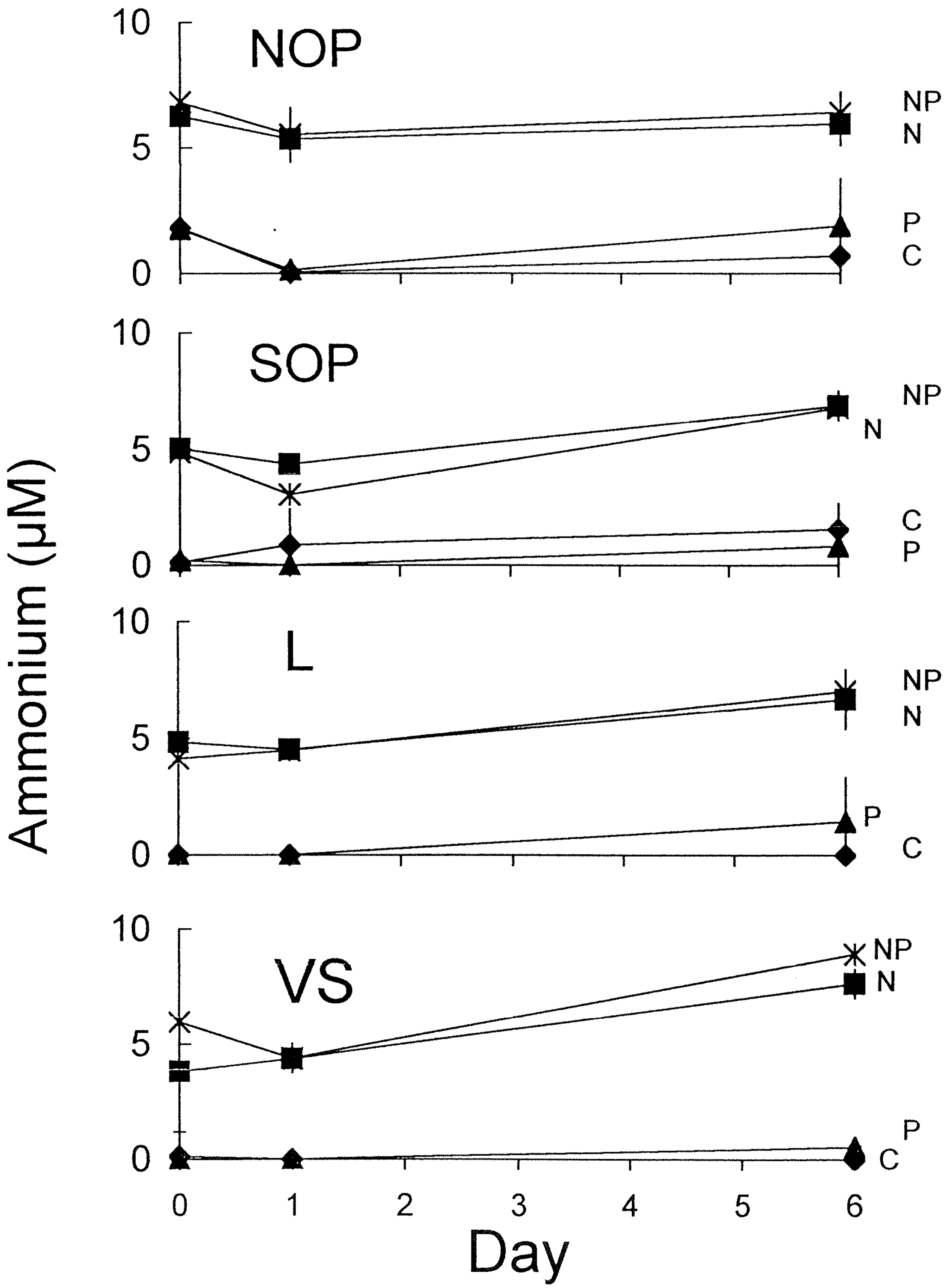


Figure 4

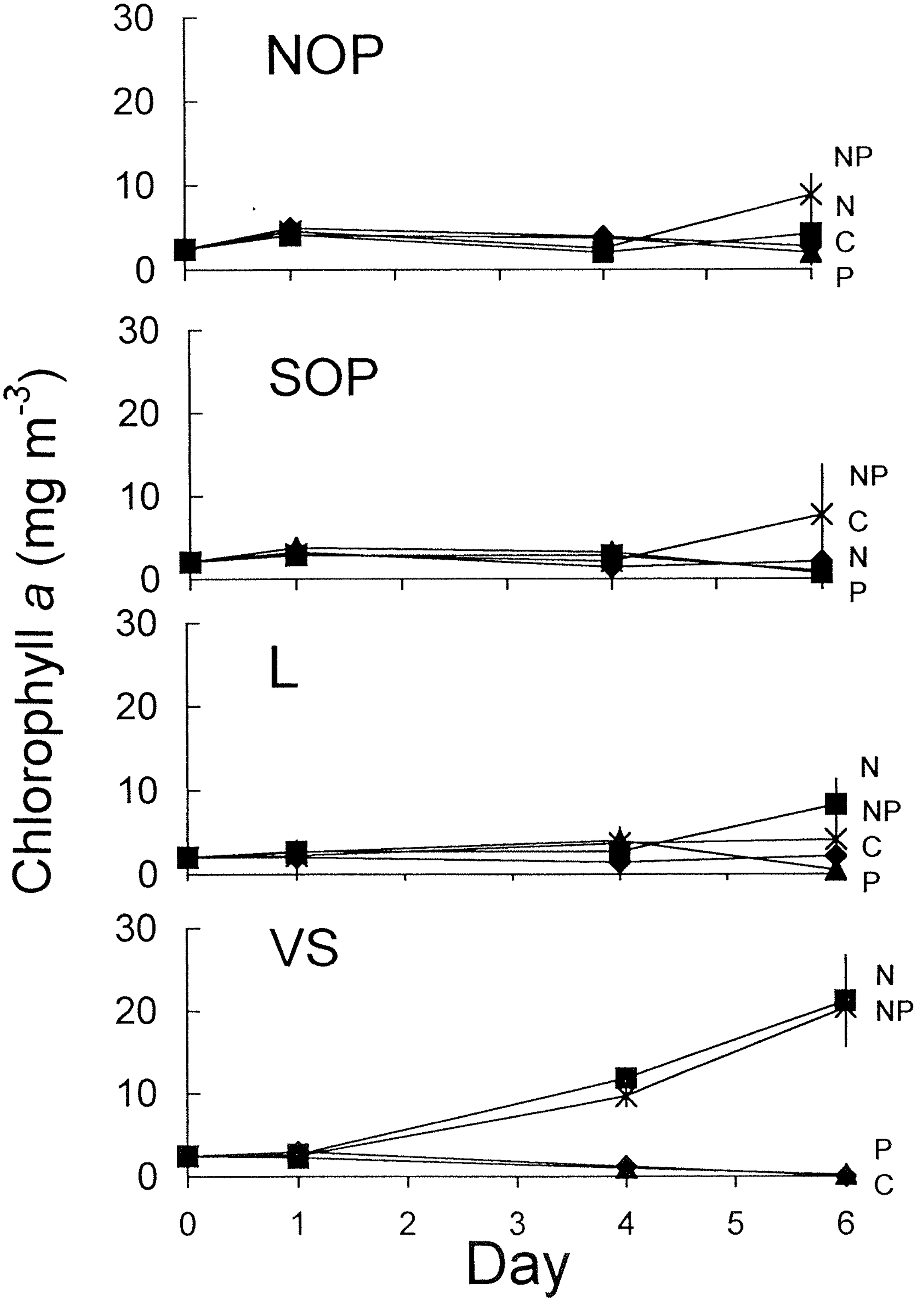


Figure 5

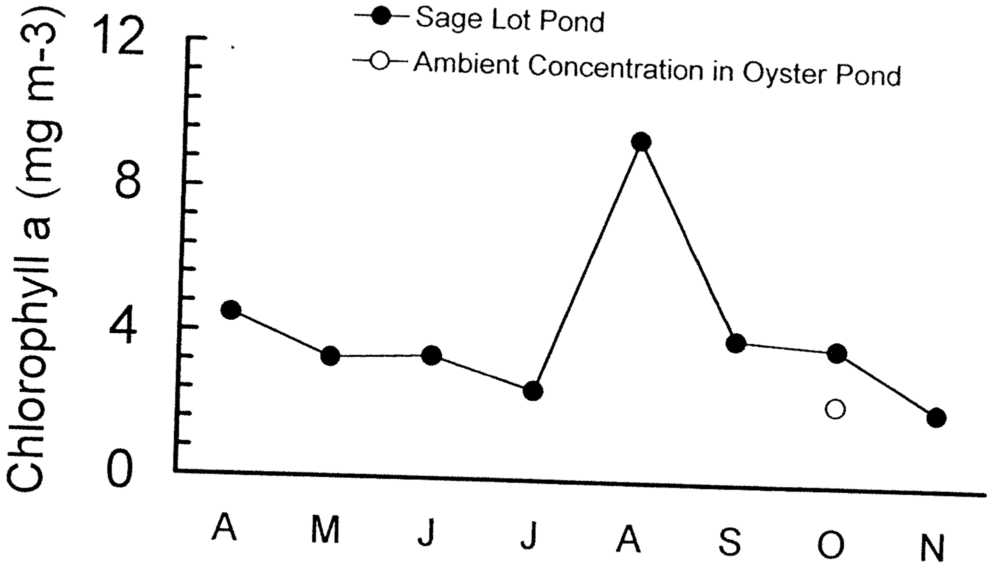

Chlorophyll a (mg m-3)
12
8
4
0
A
M
J
J
A
S
O
N
Sage Lot Pond
Ambient Concentration in Oyster Pond

Figure 6

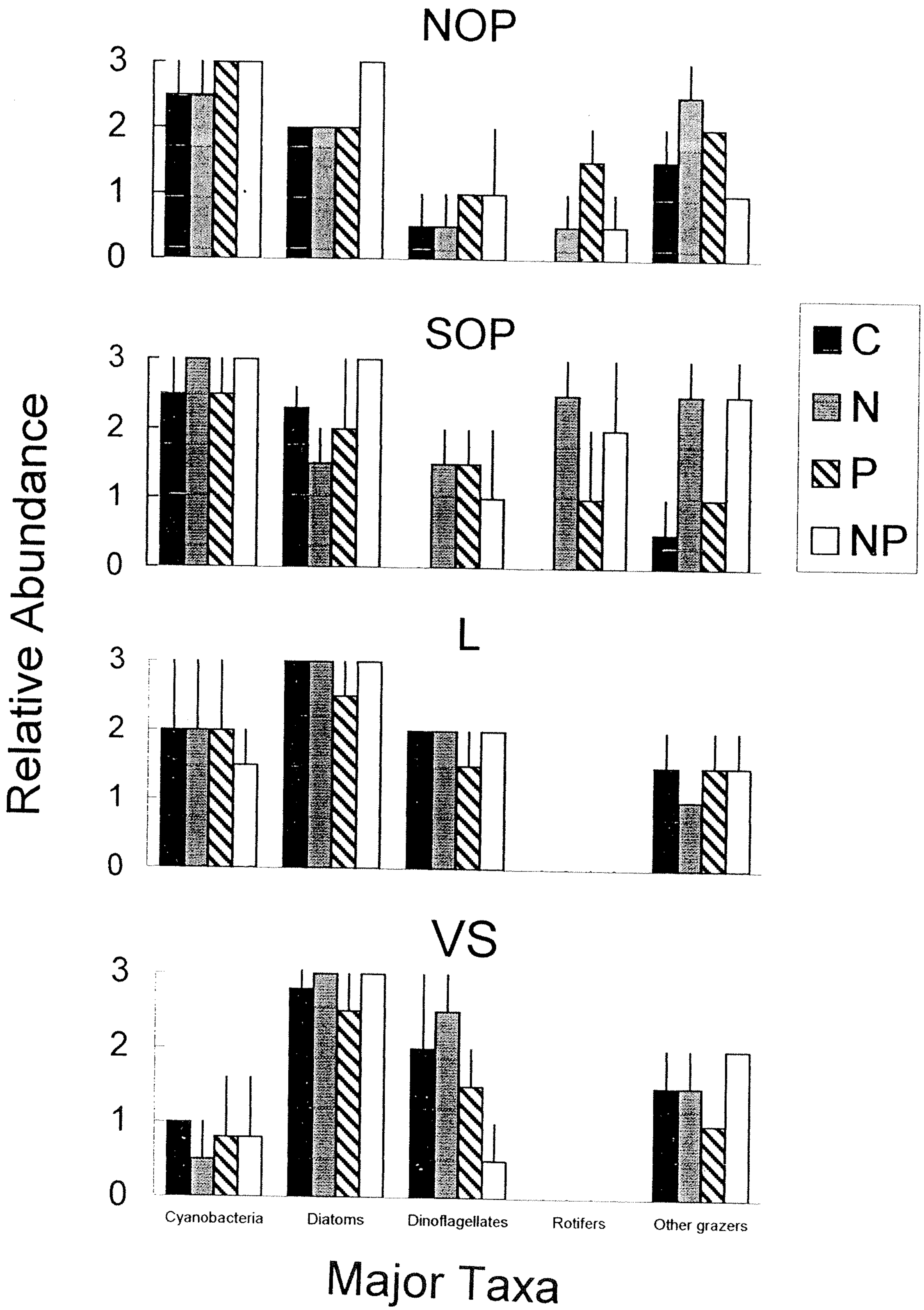
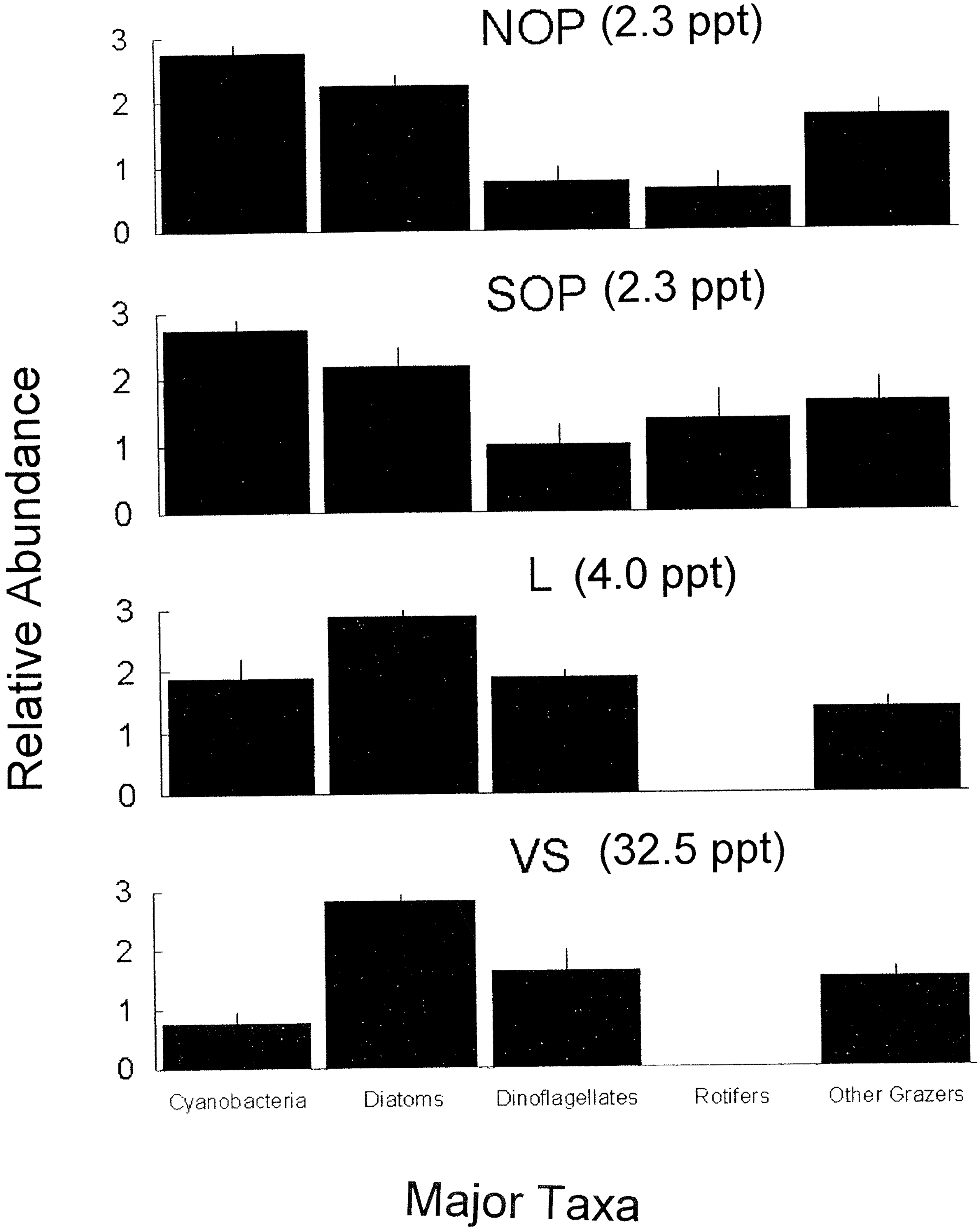

NOP
SOP
L
VS
3
2
1
0
Relative Abundance
Cyanobacteria
Diatoms
Dinoflagellates
Rotifers
Other grazers
Major Taxa
C
N
P
NP

Figure 7



NOP (2.3 ppt)
SOP (2.3 ppt)
L (4.0 ppt)
VS (32.5 ppt)
Relative Abundance
0
1
2
3
Cyanobacteria
Diatoms
Dinoflagellates
Rotifers
Other Grazers
Major Taxa

Figure 8

Chapter 4

Responses of submerged and emergent macrophytes to varying rates of wastewater influx in Oyster Pond

SARA GRADY

Boston University Marine Program, Marine biological Laboratory,
Woods Hole, MA 02543

ELIZABETI MUTO

Instituto Oceanografico, Universidade de Sao Paulo, Sae Paulo, Brazil

Abstract

We used mapping of vegetation coverage, percent nitrogen in vegetation tissue, and stable nitrogen isotope analysis of vegetation tissue to evaluate the response of submerged and emergent aquatic vegetation in Oyster Pond, Falmouth, Massachusetts to nutrient loading from wastewater influx. Tissue nitrogen and $\delta^{15}N$ were greatest in the recharge area with the highest nutrient load. Lemna minor and Najas gracillema were abundant in that region and absent to occasional in other recharge areas, suggesting a unique response to nitrogen loading. These results confirm that vegetation chemistry along with mapping is a good evaluative tool for examining nitrogen loading and suggest a possible management response is needed to the wastewater influx.

Introduction

In recent decades Oyster Pond has become more eutrophic (Howes and Goehringer, 2000). Citizens, scientists and the local government have three primary concerns with regard to Oyster Pond. The first concern is salinity. During summer, a stratification of the water column results from the sinking of dense, high-salinity Vineyard Sound water and high influx of lighter freshwater to the surface. This prevents vertical mixing, which promotes depletion of oxygen over the bottom, particularly in the basins. During fall and winter the declining surface water temperatures and the onset of higher winds results in a deeper mixed layer except in the southern deep basin where the salinity gradient remains strong (Emery, 1997).

The second concern is nutrient influx into the pond. Although it is difficult to determine the threshold for nutrient inputs before the system stabilizes, Oyster Pond seems to have a low tolerance for N loading due to its potential for water column stratification and long residence time (Emery, 1997). 86% of the total influx of fresh water into the pond comes from groundwater. (Emery, 1997) The main sources of N inputs to Oyster Pond via the watershed are on-site disposal of wastewater (63%), direct precipitation (16%) and lawn fertilizer (10%) (Emery, 1997).

The third source of concern is vegetation. Citizens have observed shifts in aquatic vegetation throughout the years, with populations increasing to nuisance levels at times. It is possible that these blooms are due to increased nitrogen loads. Vegetation tissue chemistry could be used to indicate the location and relative concentration of wastewater influx as well as overall reaction to increased nitrogen loading. Natural abundances of the ^{15}N stable isotope have been used widely for studies on nitrogen cycling in organisms and ecosystems (Robinson, 2001). McClelland and Valiela (1998) demonstrated a tight coupling between nitrogen contribution to coastal watersheds and nitrogen use by primary producers in Waquoit Bay by comparing the _^{15}N of producers and dissolved inorganic nitrogen in groundwater.

The goals of this study were to evaluate the effect of nutrient loading on pond vegetation and also to use vegetation as an indicator for the influx of nutrients from various sources within the watershed of Oyster Pond. We approached this using three methods: mapping of vegetation cover, and analysis of elemental nitrogen and stable nitrogen isotopes in tissues.

Materials and Methods

Vegetation mapping

We divided a map of Oyster Pond into 38 sections, which were enlarged and used as templates for sketching vegetation cover in the field. The observations were carried out on a rowboat and all patches of submerged aquatic vegetation visible as well as any emergent aquatic species present within 2-3 meters of the shoreline were sketched. Where more than one species was present in a given area, the fraction of each was visually estimated to the nearest third or fourth. For our analyses, these maps were converted into percent cover within the total area mapped within each recharge area. This differs from the standard definition of percent cover, as plant presence was patchy within each recharge area, not uniformly distributed in varying quantities. Emergent vegetation was not quantified by percent cover but rather by abundance. We ranked each species within each recharge area on a scale of 0 to 3, with 0 being absent, 1 being rare, 2 being common, and 3 being abundant. We had to use this ranking system because it was difficult to estimate percent cover accurately from within the pond.

Tissue chemistry analysis

Vegetation samples for chemistry analysis were taken in nine sites along the shoreline of Oyster Pond and one site along the shore of the lagoon (Fig. 1). Plants were deliberately sampled in the shallows and on the shoreline at each site and placed in plastic bags. The species were separated and identified at the laboratory, washed with distilled water, dried in a drying oven at 60°C, then ground to a fine powder using a combination of a mortar and pestle and a Wiley Mill. The powder was weighed out into samples of 5 mg and analyzed in a Perkin-Elmer 2400C Elemental Analyzer according to standard procedure for estimation of carbon and nitrogen content. The same amount of this powder was used for stable nitrogen isotope analysis. The powder was weighed, packed (5mg), and sent to the University of California at Davis Stable Isotope Facility for analysis.

Data analysis

Vegetation features (% cover, C/N, %N, $\delta^{15}N$) were analyzed in relation to total nitrogen load, wastewater nitrogen load, water salinity and distance from a set point at the south part of the pond (see Fig. 1). Distances were measured starting from a point at the central southern end of the pond and wrapping around the shoreline in a clockwise direction. Data on nitrogen load was taken from Al-Qatami et al. (unpublished data) and salinity data was taken from Defilippi et al. (unpub. data) (Table 1). In order to verify significant correlation between two variables we used regression analysis. Only significant results are represented by trendlines.

Results

Vegetation mapping

A total of twenty-nine species of aquatic plants was identified on the studied area. Eight species were submerged vegetation, one species (Lemna minor) was floating and ten species were emergent vegetation (Table 2). The percent cover of each species varied between recharge areas. With regard to submerged vegetation, Eliocharis and Potamogeton were the dominant species in recharge areas 2,4, and 5 but had low percent cover at areas 1 and 3. Both of these species plus Ruppia were the dominant species at area 6 while Lemna had the greatest percent cover at area 3. Recharge area 1 differed from others because there were seven species present with Eliocharis, Elatine and Rupia representing 70% of the area cover (Fig. 2). With regard to emergent vegetation, recharge area 6 differed from others with Phragmites as the dominant species and Iva, Spartina and Rosa as common species. None of the six emergent species occurred at area 4 and only one rare species occurred at area 2. Typha was the dominant species at recharge area 1 (Fig. 3).

In order to understand the differential pattern of distribution and dominance of submerged and floating species a regression analysis was performed using data on % cover vs. nitrogen load. Lemna minor and Najas gracillema both increased in abundance in areas with greater nitrogen loading (Fig. 4). The remaining species did not show a significant response to nitrogen loading. Salinity was found not to be a confounding factor; the regression of this variable versus nitrogen load was not significant (F=2.72, p=0.14). However, we observed that

Ruppia maritima, a common estuarine species (Fassett, 1957) had a much higher cover in area 6 where salinity was highest (7.4 ppt). The occurrence and abundance of emergent plants appears to have been influenced more by salinity than nitrogen due to presence of some species in limited regions (Fig. 3). T. angustifolia and C. stipata occurred only in areas with low salinity (2.3 to 2.5 ppt), while I. frutescens, S. alterniflora, and R. rugosa occurred only in the area with the highest salinity (7.4 ppt.) Phragmites australis can survive in wide ranges of salinity and occurred in both high and low salinity areas. The only invasive species that were present were Phragmites australis and a small amount of Lythrum salicaria in recharge area 5.

Tissue chemistry Analysis

Percent nitrogen and $\delta^{15}N$ of submerged and emergent aquatic vegetation tissue showed increasing values with distance reaching a peak in recharge area 3 then decreased with distance away from this area (Table 1; Figs. 5,6). The lowest values of both %N and $\delta^{15}N$ occurred in emergent vegetation. Tissue $\delta^{15}N$ increased significantly with increasing N load ($F = 8.71$, $p < .05$), but tissue percent nitrogen, while still increasing with N load, did not do so significantly (Figs 7,8). In order to confirm that the $\delta^{15}N$ signal was primarily from wastewater, we also compared nitrogen load from wastewater with $\delta^{15}N$ of the tissues, which was also significant ($F = 13.32$) (Fig. 9). The relationship between carbon and nitrogen percentage ratios and the nitrogen load in each recharge area was not significant.

Discussion

The data seem to suggest that nitrogen is having an effect on vegetation in Oyster Pond. According to Cloern (2001), nutrient enrichment causes the dominant species to shift from perennials to ephemeral species, because ephemeral species are better adapted to high-nutrient environments while perennials are best adapted to low nutrients. Most of species observed in Oyster Pond are perennial except Elatine americana, Lemna minor, and Vaucheria. This may explain the response of Lemna in recharge area three, although the strong response of Lemna minor and Najas gracillema may also be due to a fertilization effect, which would increase the coverage of a species that is already present. At the moment we do not know of any physiological similarities between these two plants that would cause them to have such a dramatic response.

The determination of emergent vegetation patterns by salinity and not nutrients is most likely a result of the ecology of the plants observed. Phragmites australis, Iva frutescens, Spartina alterniflora, and Rosa rugosa are all salt tolerant, while Typha angustifolia and Carex stipata prefer fresh water (Stuckey and Gould, 2000). The presence of Phragmites australis and Lythrum salicaria, which are invasive species, is primarily limited to the southern end of the pond where nutrient levels are low, so it is unlikely that their presence is an effect of nitrogen.

The maximum levels of percent nitrogen in tissues and $\delta^{15}N$ in recharge area 3 confirm that the vegetation is not only showing higher nitrogen levels in the presence of high N loads but is also assimilating a strong wastewater signal. The increase in tissue $\delta^{15}N$ with increasing wastewater nitrogen also agrees with the results of McClelland and Valiela (1997) as well as Cole (pers. comm.). Recharge area 3 also has the highest density of houses as compared to the other recharge areas, which has implications for future building as well as current land management. The relationship between land use, nutrient loading, and vegetation in Oyster Pond is evident- greater land use leads to a high wastewater influx which changes not only the tissue chemistry but also the distribution and abundance of some species.

It is important to understand the ways that vegetation reacts to nitrogen loading, because this knowledge can be used to address various topics. Vegetation can be used to assess eutrophication in a water body. It is also useful in understanding food webs. Since nutrients are at the bottom of the food web, they can have pervasive, "bottom-up" effects. If we understand how primary producers react to higher nitrogen loads, we may be able to predict the responses of the higher trophic levels to nutrient enrichment.

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Heba Al-Qatami et al, Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts, 02543

Marci Cole, Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts, 02543

Julie-Marie Defilippi, Boston University Marine Program, Marine Biological Laboratory, WoodsHole, Massachusetts, 02543

Table 1: Total nitrogen load, wastewater nitrogen, salinity and distance of sampling sites in Oyster Pond (MA). *Source: Al-Qatami et al.

Recharge area	Sites	Total N load (kg yr-1)*	Wastewater N (kg yr-1)*	Salinity (ppt)*	Distance (m)
1	8	135	94.91	2.3	281
	9				856
2	1	147	101.14	2.4	1280
	2				2047
3	3	385	277.2	2.3	2149
	4				2341
4	5	71	38.84	2.3	2697
5	6	141	112.94	2.5	3272
	7				3621
6	10	55	94.91	7.4	4114

Table 2: Percentage of carbon (C), nitrogen (N), C/N ratio and δ^{15}N per site, watershed and N load (kg yr-1).

Submerged Aquatic Vegetation	**% C**	**% N**	**C / N**	**d^{15}N**	**Site**	**Watershed**	**N load**
Eleocharis	38.6	3.0	12.8	6.7	4	3	385
	33.2	2.4	14.1	6.5	6	5	141
	39.7	3.0	13.4	4.8	7	5	141
	37.7	2.6	14.4		8	1	135
	26.5	1.9	14.0	9.1	9	1	135
Elatine americana	37.1	2.6	14.3	7.5	9	1	135
Lemna minor	38.4	2.0	19.0	6.0	1	2	147
	39.8	2.7	14.6	7.9	3	3	385
Myriophillum	38.3	3.0	13.0	5.2	1	2	147
	40.7	2.1	19.3	6.8	2	3	385
	36.9	3.7	10.0	7.3	4	3	385
	39.5	3.2	12.4	2.1	7	5	141
	37.7	3.0	12.5	3.7	8	1	135
Najas gracillema	37.1	2.8	13.4	7.0	2	3	385
	36.4	2.4	15.4	5.7	5	4	71
Potamageton	37.2	2.0	18.7	8.0	4	3	385
	32.7	2.7	12.2	5.2	5	4	71
	40.1	3.3	12.2	6.6	6	5	141
	40.0	2.4	17.0	3.6	7	5	141
	39.6	2.8	14.1		8	1	135
Vaucheria	34.1	2.9	11.7	10.3	4	3	385
Emergent Aquatic Vegetation							
Carex	38.2	2.2	17.5	7.2	3	3	385
	41.1	1.4	29.6	4.6	7	5	141
Ammophila	44.5	1.2	38.0	-0.1	10	6	55
Iva	50.4	1.8	28.5	-2.7	10	6	55
	49.0	1.5	32.4	2.2	3	3	385
Spartina patens	45.4	0.8	60.6	-3.4	10	6	55
Typha	43.9	1.0	45.2	3.4	7	5	141

Figure Legends

Fig. 1: Oyster Pond and its watershed with six recharge areas. Numbers in the pond represent sampling sites for vegetation tissue chemistry analysis. The X at the southern end of the pond is the starting point (zero) for measuring distance around the pond.

Fig. 2: Percent cover of submerged aquatic vegetation in each recharge area.

Fig. 3: Abundance of emergent aquatic vegetation in each recharge area.

Fig 4: Lemna minor and Najas gracillema increased with nitrogen load.

Fig. 5: Percent nitrogen in tissue for submerged and emergent aquatic vegetation versus distance from X (see Fig. 1)

Fig. 6: $\delta^{15}N$ in tissue for submerged and emergent aquatic versus distance from X (see Fig. 1)

Fig. 7: Percent nitrogen in tissue for submerged and emergent aquatic vegetation versus N load.

Fig 8: $\delta^{15}N$ in tissue for submerged and emergent aquatic vegetation versus N load. ** $p < .05$

Fig 9: $\delta^{15}N$ in tissue for submerged and emergent aquatic vegetation versus wastewater N load. ** $p < .05$

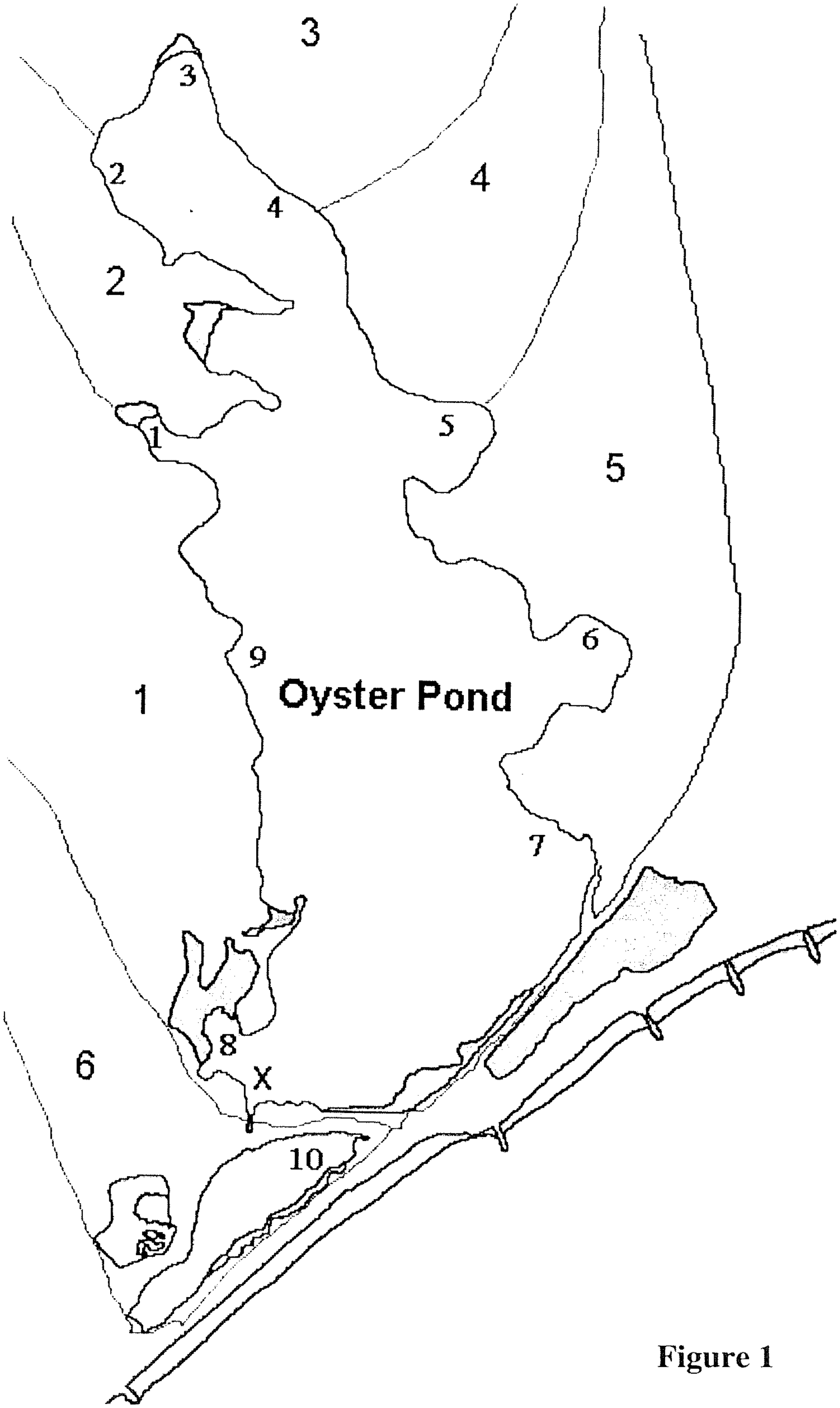


Figure 1

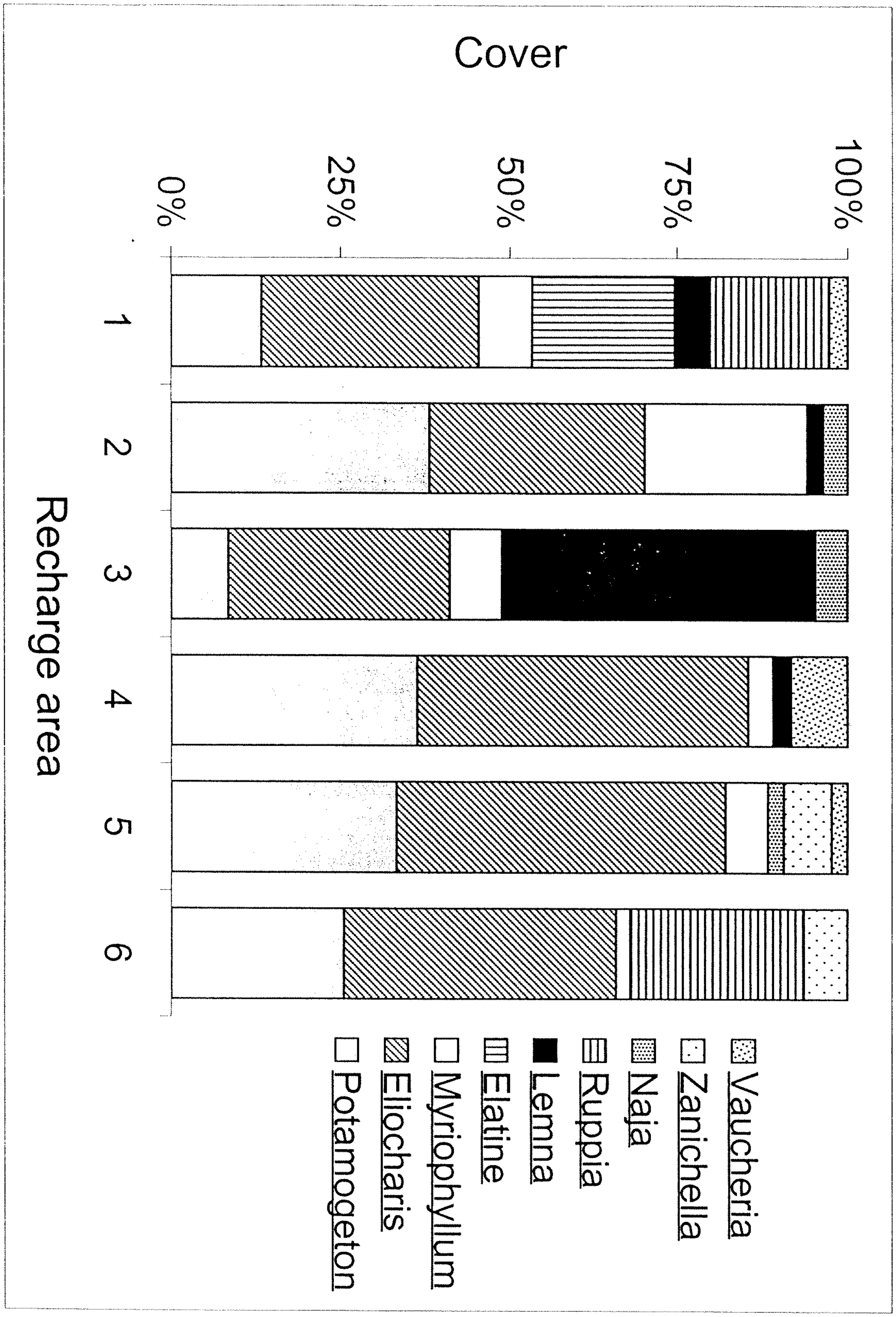
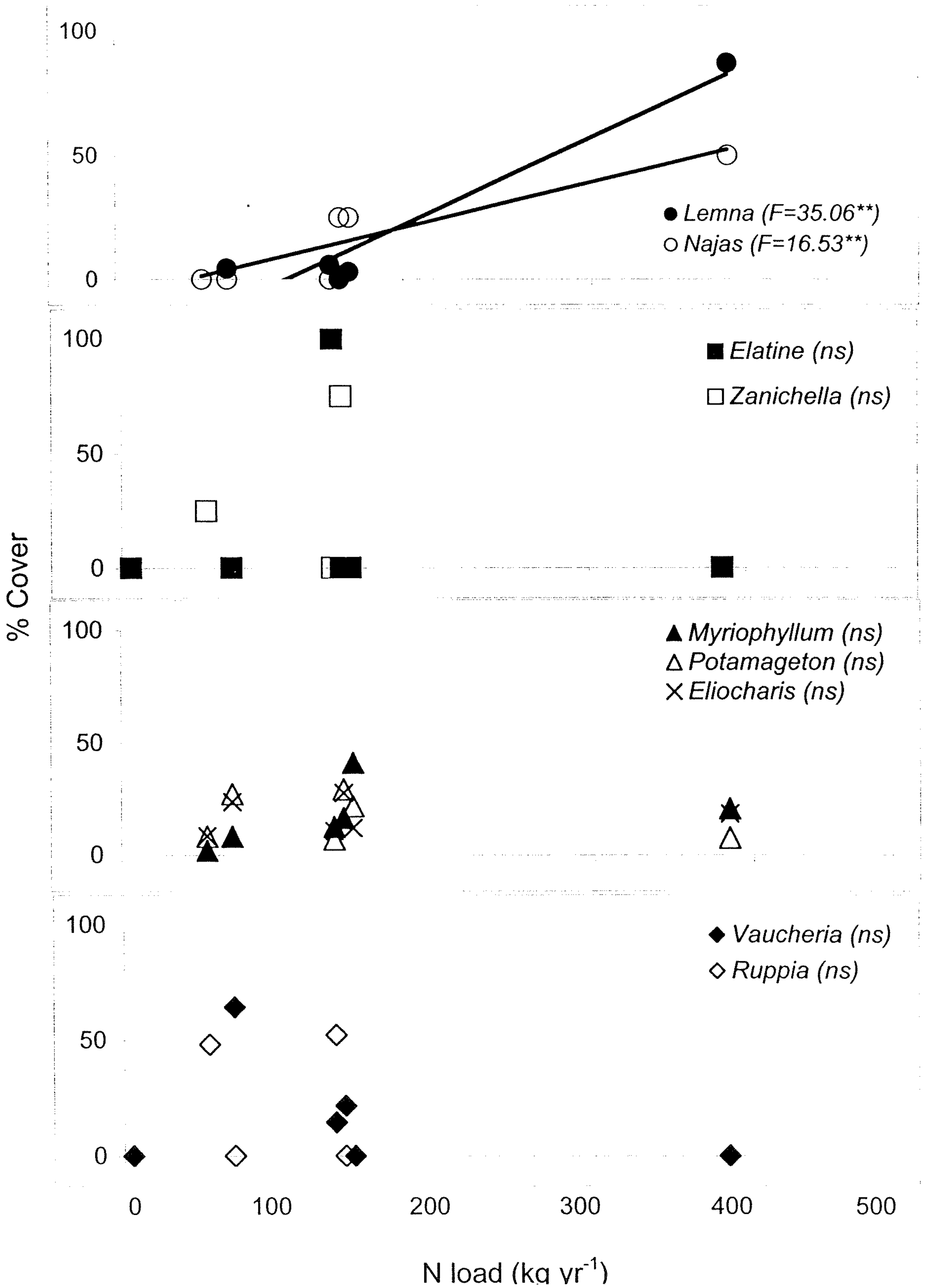

Cover
100%
75%
50%
25%
0%
1
2
3
4
5
6
Recharge area
Vaucheria
Zanichella
Naja
Ruppia
Lemna
Elatine
Myriophyllum
Eliocharis
Potamogeton

Figure 2

Recharge area	Typha angustifolia	Carex stipata	Phragmites australis	Iva frutescens	Spartina alterniflora	Rosa rugosa
1	■	░	▒	□	□	□
2	▒	□	□	□	□	□
3	▒	□	□	□	□	□
4	□	□	□	□	□	□
5	▒	░	░	□	□	□
6	□	□	■	░	░	░

□ absent ▒ rare ░ common ■ abundant

Figure 3



100
50
0
● Lemna (F=35.06**)
○ Najas (F=16.53**)
100
50
0
■ Elatine (ns)
□ Zanichella (ns)
% Cover
100
50
0
▲ Myriophyllum (ns)
△ Potamageton (ns)
× Eliocharis (ns)
100
50
0
◆ Vaucheria (ns)
◇ Ruppia (ns)
0
100
200
300
400
500
N load (kg yr-1)

Figure 4

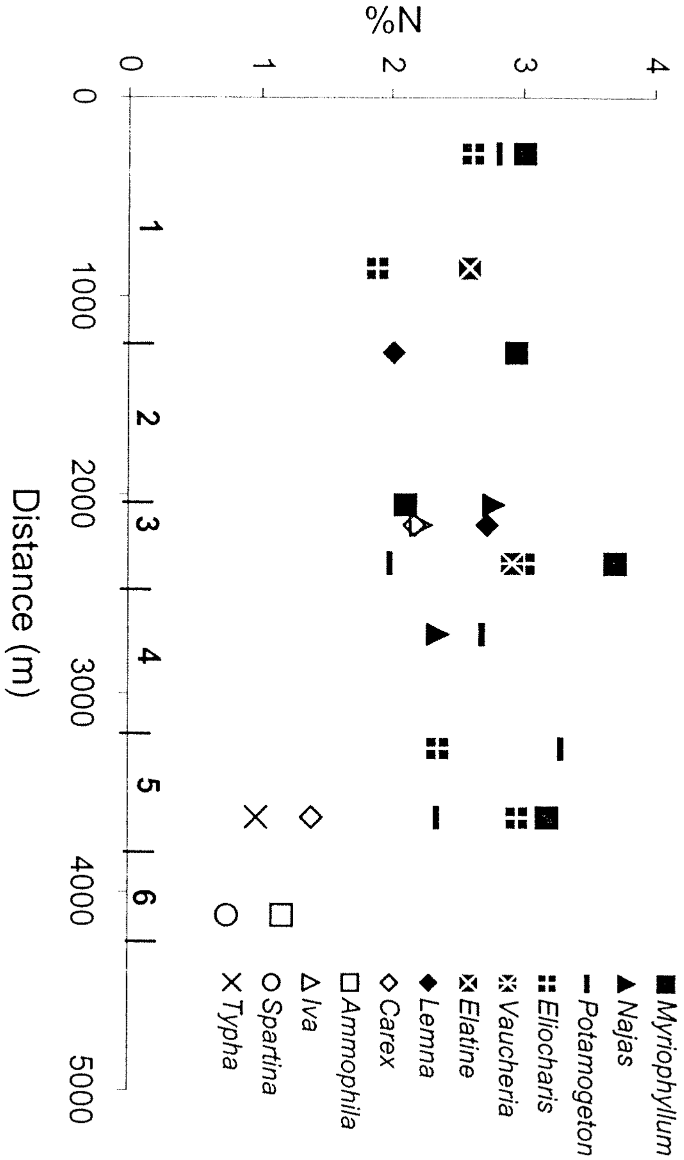

%N
0
1
2
3
4
Distance (m)
0
1000
2000
3000
4000
5000
1
2
3
4
5
6
■ Myriophyllum
▶ Najas
ǀ Potamogeton
∷ Eliocharis
⊠ Vaucheria
⊠ Elatine
◆ Lemna
◇ Carex
□ Ammophila
△ Iva
○ Spartina
× Typha

Figure 5

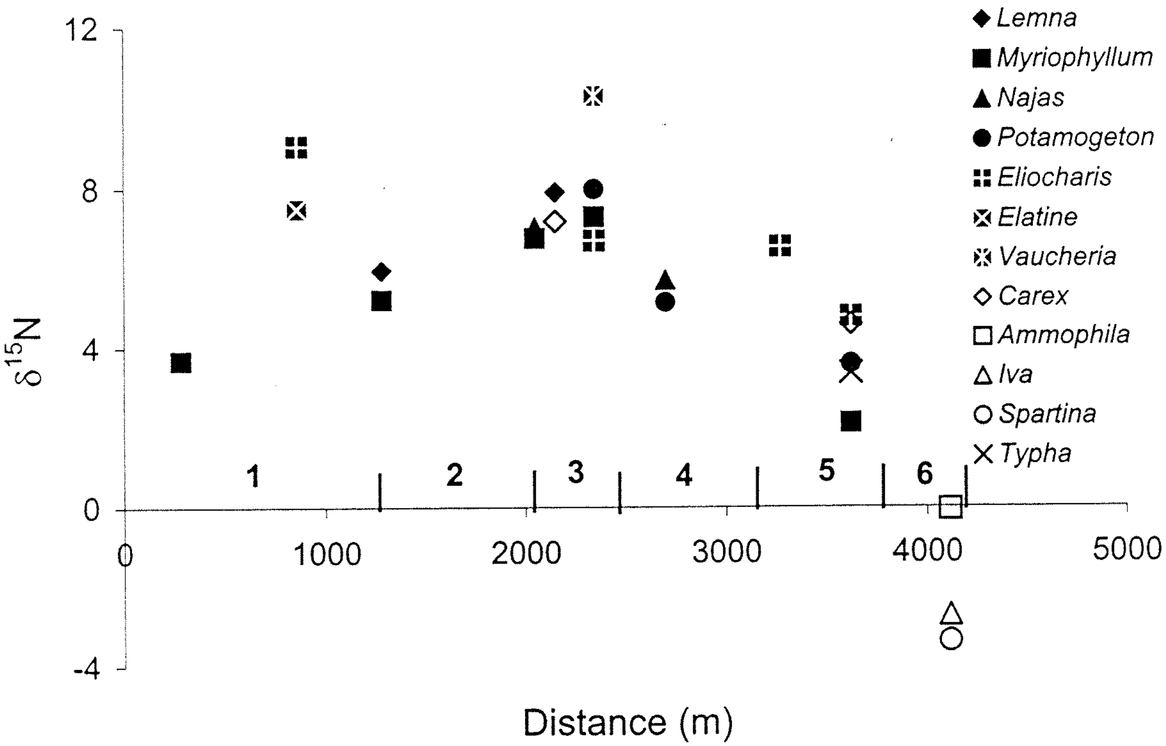

12
8
4
0
-4
$\delta^{15}N$
0
1000
2000
3000
4000
5000
Distance (m)
1
2
3
4
5
6
◆ Lemna
■ Myriophyllum
▲ Najas
● Potamogeton
∷ Eliocharis
⊠ Elatine
⊠ Vaucheria
◇ Carex
□ Ammophila
△ Iva
○ Spartina
× Typha

Figure 6

Figure 7

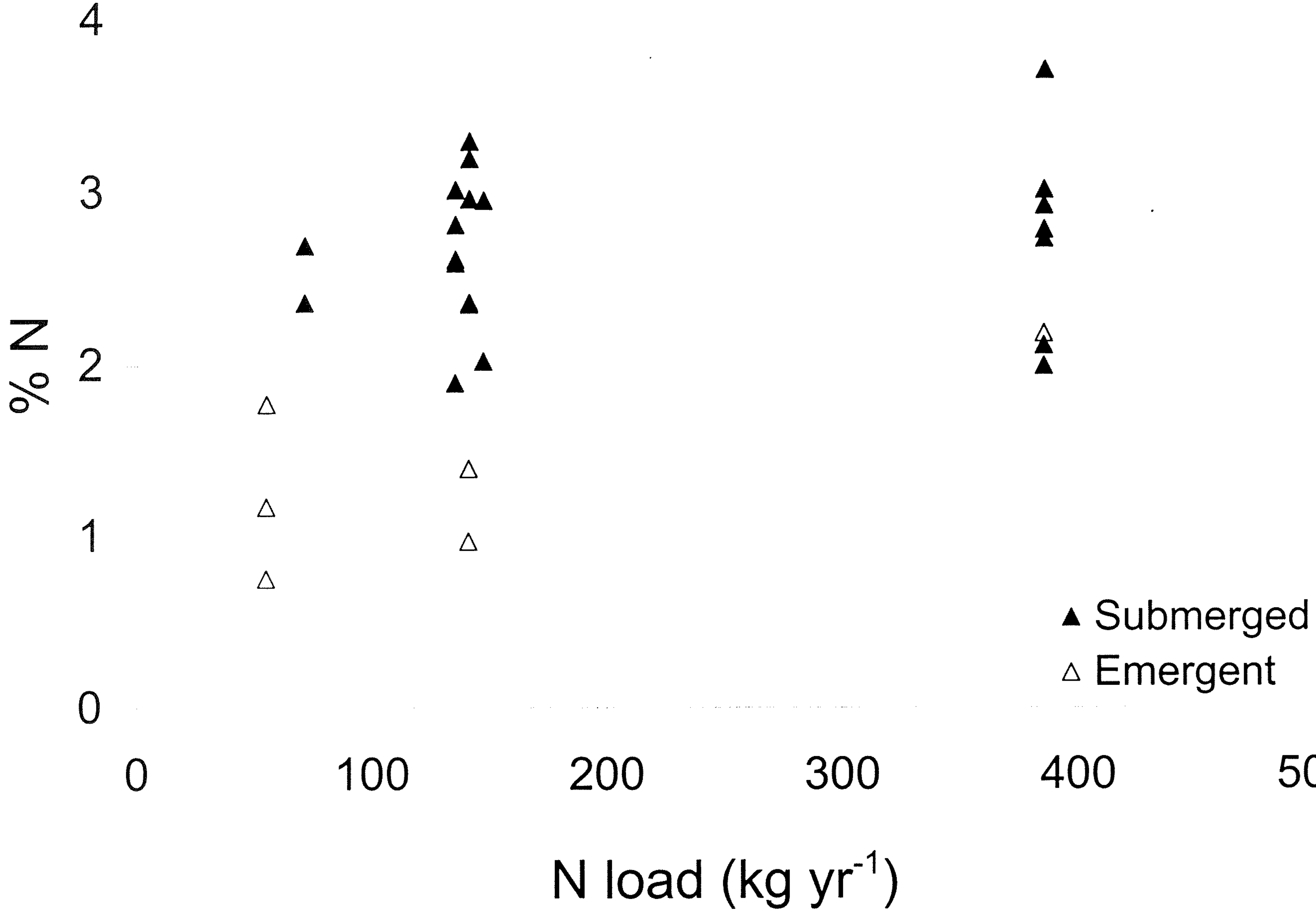


Figure 8

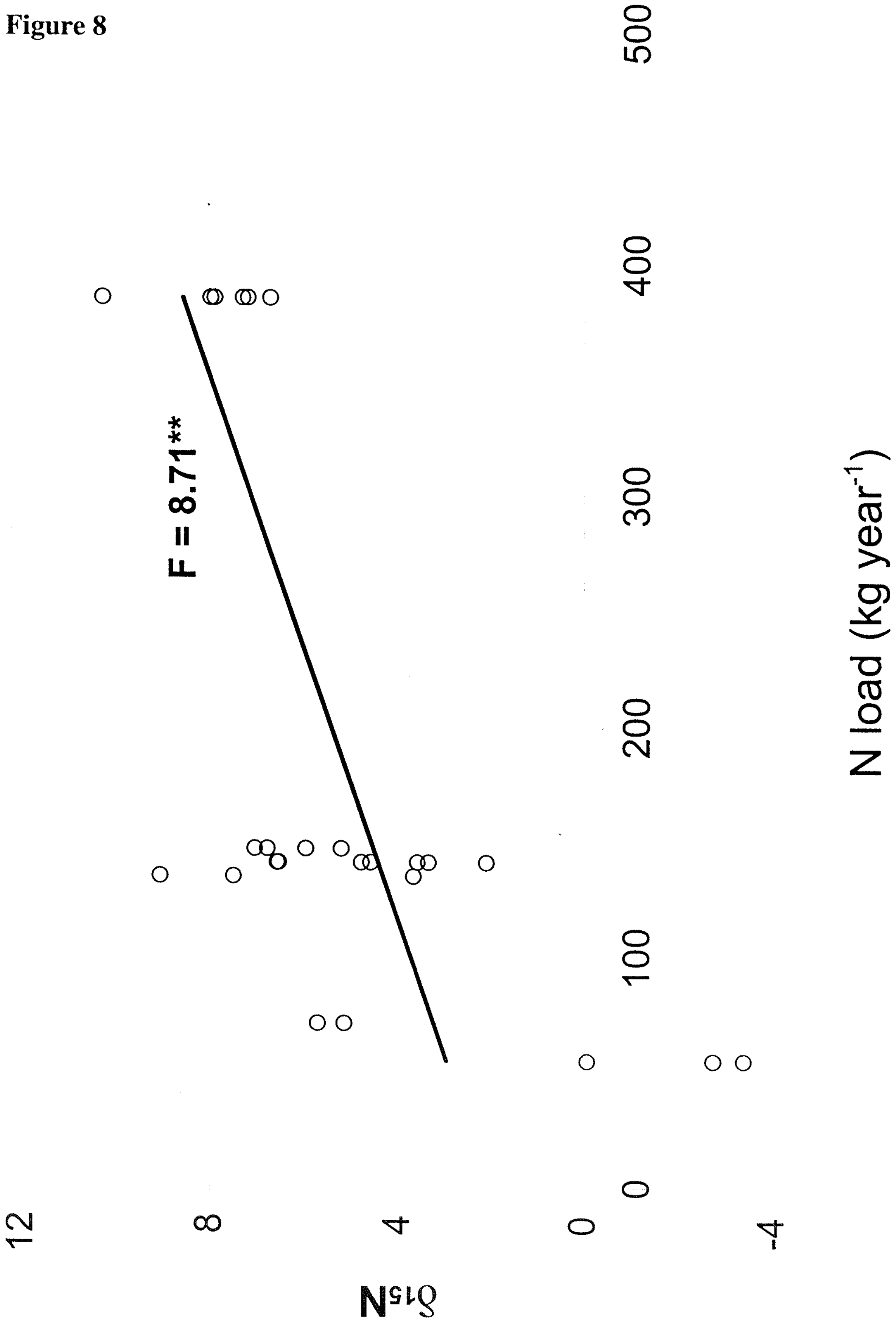


Figure 9