



Padilla Bay

National Estuarine Research Reserve

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**SEASONAL CONTROL OF PHYTOPLANKTON GROWTH BY
ANTHROPOGENIC NUTRIENT LOADING IN PADILLA BAY**

NATIONAL ESTUARINE RESERVE

Gisele Muller-Parker

and

Emily R. Peele

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Nutrient Loading in Padilla Bay National Estuarine Research Reserve**

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Table of Contents

Abstract	1
Introduction	2
Methods	3
Water Quality and Environmental Conditions	3
Nutrient Enrichment Bioassays	6
Primary Productivity	7
Phytoplankton Growth	9
Statistical Analyses	10
Results	11
Salinity, Temperature and Inorganic Nutrients	11
Phytoplankton Biomass	12
Phytoplankton Productivity	13
Nutrient Enrichment Bioassays	14
Chl <i>a</i> concentrations	14
Primary productivity	15
Phytoplankton growth	16
Seasonal patterns in phytoplankton growth and relative availability of N and P	18
Phytoplankton biomass yields	19
Discussion	20
Conclusions	23
Acknowledgements	24
Literature Cited	25
List of Tables	28
List of Figures	29
Tables 1-10	35-44
Figures 1-60	45-104

ABSTRACT

The effects of nutrient (N,P) availability on phytoplankton growth, abundance and productivity were assessed seasonally from stations representing a freshwater slough, mid-bay, and outside Padilla Bay, Wa. in 1994 and 1995. We used a growth bioassay method with surface water samples containing phytoplankton (<153 μm) incubated under natural light with three different inorganic nutrient additions (nitrogen, phosphate, nitrogen plus phosphate, and an ambient control). Under ambient nutrient levels, summer phytoplankton biomass approached 8 $\mu\text{g chlorophyll } a \cdot \text{L}^{-1}$ and growth rate was 1.3 d^{-1} ; during winter phytoplankton biomass averaged 0.5 $\mu\text{g chlorophyll } a \cdot \text{L}^{-1}$ and growth rate was 0.5 d^{-1} . Significant increases in phytoplankton growth and biomass occurred only in response to nitrogen enrichment, and only during spring and summer seasons. Phytoplankton from the mid-bay station showed the greatest response to nitrogen additions. Phosphorus stimulated the growth rate and final biomass yield of phytoplankton from the slough station once, in February 1995. Nutrients had no effect on phytoplankton growth and biomass yields during fall and winter. Light is likely to limit phytoplankton growth during these seasons.

Key words: nutrient enrichment, nitrogen and phosphorus, primary production, Padilla Bay, National Estuarine Reserve Reserve, phytoplankton growth

INTRODUCTION

Padilla Bay National Estuarine Research Reserve is an important habitat for indigenous and migratory organisms. It contains over 10,300 acres of intertidal and subtidal mudflats including the largest contiguous seagrass meadows in Washington State (Bulthuis, 1991). The Padilla-Bayview watershed land use is primarily agricultural and rural and encompasses an area of 20,800 acres. Non-point sources include nutrient (nitrogen and phosphorus) inputs from agricultural practices, eroded soils as a result of forest removal, and anthropogenic control of freshwater inputs to the estuary (via tide gates) at the outflows of the major sloughs. Residential, recreational and industrial uses also contribute to non-point pollution. These influences may act to increase or decrease phytoplankton abundance on a temporal or seasonal basis, depending on precipitation and insolation. Freshwater inflows to Padilla Bay are low in summer and early fall.

The potential for anthropogenic alteration of this estuary is high, since it is subject to increased coastal development. Previous studies have suggested that nutrient limitation, particularly by nitrogen, is likely to occur in shallow coastal areas of the Puget Sound region during the summer months (Thom and Albright, 1990). For Padilla Bay, Bernhard and Peele (1997) showed that additions of NH_4^+ to surface water samples during the summer of 1992 resulted in dramatic increases in algal biomass. However, the effects of nutrient addition on primary production rates and phytoplankton growth rates were not measured in their study. We examined the effects of nutrient enrichments on phytoplankton growth and production in this estuary at different times of the year to determine if there is a seasonal component to eutrophication, and whether N or P is the limiting nutrient during those periods.

Controls on phytoplankton growth are important to monitor, since phytoplankton abundance affects water quality in several important ways. Increased algal biomass in the water column decreases light availability to benthic seagrass, leads to oxygen depletion at night, and increased retention of nutrients in the Bay. The seagrass community is at greatest risk from eutrophication, as documented for Chesapeake Bay (D'Elia, 1987). High plankton biomass leads to a general deterioration of habitat for benthic estuarine organisms. This process has been well documented for several tributaries of the Chesapeake Bay, where increased nutrient input has resulted in a shift from benthic to water column production and a decline in water quality (D'Elia, 1987). There is also direct evidence for decline of seagrass by the addition of water-column nitrate (Burkholder *et al.*, 1992).

Our project also addressed the need for measurements of dissolved inorganic nutrient distributions in Padilla Bay, particularly with respect to proximity to land and freshwater inputs. Our project extends the water quality monitoring base provided by a 1985-1986 study by Cassidy and McKeen (1986) by providing data for three stations during 1994 and 1995. We also present the first data for seasonal trends in primary productivity, chlorophyll biomass, and phytoplankton growth for this National Estuarine Research Reserve.

METHODS

Water Quality and Environmental Conditions

We conducted surveys of physical and chemical water quality parameters at three stations in Padilla Bay on 14 dates during 1994 and 1995 (Figure 1, Table 2). Station 1 was located next to the point of discharge from Joe Leary Slough (48°31.25' N, 122°29.05' W). Station 2

(48°31.14' N, 122°30.16' W; P.B. 05, Cassidy and McKeen, 1986), a mid-bay site, drains a relatively large area of Padilla Bay and is in the vicinity of extensive seagrass meadows. Station 3 (48°31.86' N, 122°33.12' W) was located north of Hat Island in Guemes Channel, adjacent to Padilla Bay. We tried to sample all stations as close to the start of the ebb tide as possible to obtain representative water samples.

Surface water temperature, salinity, dissolved oxygen, and pH were measured with a Surveyor 3 Hydrolab[®] (Table 1). The Hydrolab[®] sensors were calibrated immediately prior to each sampling date. The specific conductance electrode was calibrated with a 0.5 M KCl solution, and salinity was calculated internally and directly from specific conductance values. The dissolved oxygen (DO) sensor was calibrated to atmospheric conditions. The calibration value was calculated from a solubility table and the ambient temperature and barometric pressure. The pH sensor was calibrated with pH 7 and pH 10 buffers. Irradiance was measured as photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$) using two LiCor[®] quantum sensors: a cosine quantum sensor for surface irradiance and a 4π quantum sensor for underwater irradiance. At each station, vertical water column profiles of temperature, salinity, dissolved oxygen, and light were obtained from measurements taken at 0.5 m depth intervals. Data from the vertical profiles of irradiance, dissolved oxygen, pH, temperature and salinity are not presented in this report (available by request from the P.I.s).

Surface water samples were collected in an acid-washed bucket and poured gently through 153 μm mesh to remove large zooplankton. The filtered water was stored in acid-washed 20-L carboys. Three discrete surface water samples were collected from each of the 3 stations to provide replicates for statistical analyses. A Van Dorn collection bottle was used to collect near bottom (0.25 m from bottom) water samples. Each water sample was analyzed for

dissolved inorganic nutrients, *in vivo* chlorophyll *a* fluorescence, chlorophyll *a* concentration, and primary productivity (surface samples only).

Subsamples for *in vivo* chlorophyll *a* fluorescence were collected in 20-ml acid-washed glass vials. The samples were stored on ice in the dark for 2-4 h until fluorescence was measured using a Turner digital fluorometer equipped with an extended range photomultiplier. Samples (40-60 ml) for chlorophyll *a* and phaeopigment analysis were filtered onto Poretics[®] GF-75 filters (0.7 μm nominal pore size). Filters were stored on ice in the dark for 2-4 h during transport to the laboratory. Chlorophyll was extracted from the filters in 10 or 20 ml 90% acetone (Parsons *et al.*, 1984). The pigment was extracted overnight in the dark at -20°C . The acetone extracts were mixed, the filters were removed, and the extracts were centrifuged at 3,800 rpm for 5 min. Fluorescence of the samples before and after acidification with HCl were used to determine the concentrations of chlorophyll *a* and phaeopigments in the acetone extracts according to equations in Parsons *et al.* (1984).

Samples for nutrient analyses were filtered through Poretics[®] GF-75 filters (0.7 μm nominal pore size) in the field. Filtered samples were stored in acid-washed polyethylene bottles on ice in the dark for 2-4 h during transport to the laboratory. Samples were stored at -20°C for less than one month. Nitrate ($\text{NO}_3^- + \text{NO}_2^-$) concentrations were determined using an Alpkem[®] rapid flow autoanalyzer. The Alpkem method (A303-S170) is a modification of the standard cadmium reduction method (Parsons *et al.*, 1984). Nitrate standards ranged from 1.0 to 50.0 μM NO_3^- . Ammonium (NH_4^+) concentrations were determined using the alternative phenolhypochlorite method (Parsons *et al.*, 1984). Ammonium standards ranged from 0.75 – 24.0 μM NH_4^+ . Concentrations of soluble phosphate (PO_4^{3-}) were determined by the ascorbic acid colorimetric method (Parsons *et al.*, 1984). Phosphate standards ranged from 0.12 – 4.8 μM

PO_4^{3-} . Nutrient concentrations are reported as $\mu\text{M NH}_4^+$, $\mu\text{M} [\text{NO}_3^- + \text{NO}_2^-]$, and $\mu\text{M PO}_4^{3-}$, and are within the detection limits for the colorimetric methods.

Nutrient Enrichment Bioassays

The three separate surface water samples from each station for the productivity and growth bioassays were covered with screening to shield the plankton from direct light en route to Cap Sante marina, Anacortes. At the marina, we subdivided each water sample as follows, with a total of 9 water samples (three water samples from each of the three stations) processed in the same manner on the first day of the experiment. A two-liter bottle was also filled for subsequent determination of the primary productivity of each water sample (field productivities).

Water from each carboy was dispensed after gentle mixing into four 4-liter polycarbonate bottles, designated (C, control, no nutrients; N, nitrogen-enriched; P, phosphorus-enriched; and NP, nitrogen and phosphorus-enriched). Each acid-washed polycarbonate bottle was rinsed at least twice with small volumes of the water sample prior to filling to the 4-liter mark. When three complete series of bottles (representing the three independent water samples from a given station) were filled, the bottles designated for nutrient additions were spiked with 4 ml of a concentrated nutrient stock solution to provide calculated additions of N ($15 \mu\text{M NH}_4^+$ and $15 \mu\text{M NO}_3^-$) or PO_4^{3-} ($2 \mu\text{M}$), or with a combination of N and PO_4^{3-} (same concentrations as single nutrient additions). After gentle mixing, each polycarbonate bottle was sub-sampled for determination of initial *in vivo* fluorescence (whole water sample), and inorganic nutrients (filtered on site, as described in field sampling methods). The bottles, equipped with clips, were then suspended at 0.5 m depth on the floating arrays deployed in the marina. After initial

deployment, we returned to the lab to conduct the primary productivity experiments with the water samples from each station (field productivities).

Primary Productivity

The primary productivity of each water sample was assessed using standard ^{14}C incorporation methods (Parsons *et al.*, 1984). Equal volumes (100 or 200 ml) of each water sample (taken from the 2-liter bottle filled at the marina) were dispensed into a set of clear and dark glass screw-cap bottles (pre-rinsed with the sample) at Shannon Point Marine Center. Dark bottles were wrapped with electrical tape to exclude light. A dark bottle incubation was run for each light bottle to correct for heterotrophic fixation of ^{14}C . Known amounts of radioactive carbonate, $^{14}\text{CO}_3^{2-}$, were added to achieve final concentrations of $1 \text{ uCi } 100 \text{ ml}^{-1}$. This usually consisted of dispensing 50 μl of a stock ($20 \text{ uCi } \text{ml}^{-1}$) into each bottle, noting the time of addition to each. After the isotope was added, 100 μl from each bottle was removed for determination of the total activity in each bottle. Five ml of Ecolume (ICN) scintillation fluid was added to total activity samples, and these were immediately counted in a Packard TR 1900 scintillation counter using the DPM mode. The primary productivity bottles were then capped and incubated in a sea table in flow-through seawater to maintain ambient seawater temperature. Photosynthetically-saturating irradiance of $437 \pm 11 \text{ } \mu\text{mol}/\text{m}^2/\text{sec}$ was provided to the clear bottles by a bank of eight cool-white fluorescent lamps suspended above the flow-through seawater table. The bottles floated in loose racks in the seatables, and were gently rotated several times during the incubations to mix the plankton samples. Light and dark bottles were incubated in the seatable for 1 hour. Incubations were terminated by filtering the entire contents of each bottle under gentle vacuum ($< 10 \text{ psi}$) through a 47 mm GF/F filter held in a filtration manifold, recording the

time of filtration as the end of the incubation period. The isotope lab was darkened during these steps to minimize plankton fixation of ^{14}C during the filtration procedure. The filter towers and filters were rinsed with filtered seawater, then the filters were transferred to labeled glass petri dishes and were placed in a desiccator containing a shallow layer of concentrated hydrochloric acid. The desiccator was located in a radioisotope fume hood. The desiccator vent was opened, and the filter samples exposed to the acid fumes to remove unincorporated inorganic ^{14}C as CO_2 for several hours. The filters in the petri dishes were then removed from the desiccator and allowed to air-dry in the fume hood for a day or two. When dry, the filters were placed into labeled 20-ml glass scintillation vials and 20 ml of Ecolume (ICN) scintillation fluid added to each. Vials were counted in the DPM mode in the scintillation counter, after a 48-hour equilibration period.

Rates of carbon fixation of plankton in light and dark bottles were calculated from DPM fixed and the inorganic C content of filtered seawater at each temperature. The amount of C fixed in the dark (representing heterotrophic fixation and/or incomplete evolution of inorganic ^{14}C) was subtracted from the amount of C fixed in the light. Carbon content of the seawater at each temperature was determined from total alkalinity (Parsons *et al.*, 1984) measurements. Primary productivity (mg C fixed per h) is expressed on a volume basis (per m^3) and on a chlorophyll *a* basis (per mg Chl *a*). These rates were used to compare the productivity of surface waters from the three stations on a seasonal basis. They were also used as a basis of comparison for the productivity responses of the water samples exposed to the nutrient treatments on the floating arrays in the marina.

The primary productivity of the four nutrient treatment samples (marina incubations) was also measured when the *in vivo* chlorophyll *a* fluorescence values of the incubation bottles

suspended on the array indicated that exponential growth was taking place in one or more of the nutrient treatments (see next section). We chose this time point to indicate the maximal productivity response of the plankton to the nutrient treatments. The actual length of time from the initial enclosure of samples to the determination of productivity rates varied with season. We measured the primary productivity of water samples two days after the start of the experiment during the early summer through early fall months, and after 5 days for the winter experiments. The same procedure was followed as described above, except that we staggered incubations in groups of 12 bottles at a time (4 runs, 48 ^{14}C incubations total), and only ran dark bottles for one water sample from each station. We used the dark value from water sample #1 from each station to correct for dark fixation by the three water samples exposed to the same nutrient treatment from each station. We also took samples for chlorophyll and nutrient analysis at the same time, to assess the amount of nutrients remaining in solution and the algal biomass for standardization of productivity rates. We extended the water sample incubations on the arrays for several days to a week after the primary productivity measurements to determine the continued biomass and growth response of the plankton for interpretation of the productivity rates. A stabilization or severe decline in *in vivo* fluorescence after the primary productivity experiments would indicate senescence or nutrient limitation of the plankton. We also determined final extracted chlorophyll concentrations and nutrients remaining in the seawater at the end of each experiment.

Phytoplankton Growth

Growth of phytoplankton in each nutrient enrichment bioassay treatment was determined from rates of change in *in vivo* chlorophyll *a* concentrations. Each bottle on the marina arrays was sampled daily (sometimes every other day, depending on weather conditions) by removing

small subsamples and taking *in vivo* fluorescence readings of these subsamples. A jonboat was used to row out to the arrays, and samples were removed by pulling each bottle into the boat, gently shaking it, and pouring a 10 to 20-ml sample into a glass vial. The time of sampling was noted. After removing subsamples from each bottle, the vials were taken to the lab for measurement of *in vivo* fluorescence as described above. The *in vivo* fluorescence values from each bottle were plotted against time and specific growth rates calculated from linear regressions of the natural log of fluorescence versus time. Subsamples were taken every 24 to 48 hours until the experiment was terminated (up to 12 days).

Statistical Analyses

The chlorophyll *a* concentrations and rates of primary production of samples collected from the three stations were each compared using one-way ANOVA, with station as the Type I model variable. For the nutrient enrichment bioassays, chlorophyll *a* concentrations, rates of primary production, and growth rates of samples in the four treatments for each station were compared using one-way ANOVA, with nutrient treatment (N, NP, P, C) as the Type I model variable. In these comparisons, the data for each station were analyzed separately.

All data subjected to statistical analysis were checked for homogeneous variances using Bartlett's test of equal variances. Log or square root transformations were done if needed to meet the assumption of homogeneous variances. A p-value of ≤ 0.05 for the F-test was used to assign significance. For results of ANOVAs that indicated a significant difference between stations or nutrient treatments, a comparison of means test, Tukey's Honestly Significant Difference Test ($p \leq 0.05$), was used to compare the means of the different groups within each data set. The statistical program Statistix[®] for Windows was used for the analyses.

RESULTS

Salinity, Temperature, and Inorganic Nutrients

Temperature and salinity of surface water samples collected from the three stations in Padilla Bay (Figure 1) during 1994 and 1995 are presented in Table 2. For both salinity and temperature, the extreme values were obtained at Station 1. Salinity at this station, located closest to shore and influenced by freshwater input from Joe Leary Slough, ranged from 24 in December 1995 to 29 during the fall of 1995, averaging 27.8 (± 1.5 S.D., $n = 13$). Salinity of surface waters at Station 2 (mid-bay) averaged 28.8 (± 1.3 S.D., $n = 13$), and the salinity at Station 3 averaged 29.5 (± 1.1 S.D., $n = 13$). There was a significant difference in salinity between the three stations (ANOVA, $P = 0.007$). Stations 1 and 2 grouped together had a significantly lower average salinity than did the group of Stations 2 and 3. Temperature, which ranged seasonally from a low of 4.1°C to a high of 20°C at Station 1, did not differ significantly among the three stations (ANOVA, $P > 0.05$) and averaged 12.8°C (± 4.2 S.D., $n = 39$) overall.

There are significant seasonal and spatial variations in nutrient concentrations in surface waters of Padilla Bay. In situ concentrations of dissolved inorganic nitrogen ($\text{DIN} = \text{NH}_4^+ + \text{NO}_2^- + \text{NO}_3^-$) were low in summer and early fall, <10 μM at the two shallow sites in Padilla Bay (Stations 1 and 2), and approximately 15 μM at a deeper more saline site outside the Reserve area (Station 3). DIN increased during winter months, primarily due to increases in nitrate concentrations ($\text{NO}_2^- + \text{NO}_3^-$) at all three stations (Figure 2). The peak nitrate concentrations during winter were highest at Station 1, decreasing progressively with distance from shore. Overall, Station 1 showed the greatest range in nutrient concentrations. For example, both the

highest nitrate levels encountered (150 μM ; December 1995) and the lowest (0.8 μM ; July 1994) were obtained at Station 1. In general, summer nitrate concentrations were lower and winter nitrate concentrations higher in Padilla Bay (Stations 1 and 2) than at Station 3 (Figure 2). Ammonium concentrations at the three stations were variable throughout the year and did not exhibit the pronounced seasonality of nitrate concentrations (Figure 3). Ammonium was highest at Station 1, and never exceeded 5 μM at Stations 2 and 3. Ammonium concentrations at Stations 1 and 2 were greater than or equal to NO_3^- concentrations during summer, while the DIN pool at Station 3 was comprised largely of oxidized N species, NO_3^- and NO_2^- . During winter months, nitrate levels were at least one order of magnitude greater than ammonium levels at all three stations.

Soluble reactive phosphate (SRP) concentrations were less variable than DIN and were less than 5 μM at all stations (Figure 4). There was no consistent pattern in SRP concentrations relative to station location. SRP tended to be higher in the winter (2–3 μM) than during summer months. There was a pronounced drop in phosphate during July 1995, when concentrations ranged from 0.05 μM at Station 1 to 0.03 μM at Stations 2 and 3 (Figure 4).

Phytoplankton Biomass

Phytoplankton biomass was low ($< 10 \mu\text{g chlorophyll } a \text{ L}^{-1}$) and seasonally variable in surface waters of Padilla Bay (Figure 5). Highest chlorophyll *a* concentrations were obtained during July through September, and lowest concentrations occurred in the months of December and February. The strongest seasonal pattern occurred at Station 3, which had the least variable chl *a* concentrations. Although winter concentrations were uniformly lowest at Station 3, summer phytoplankton biomass at this station approached phytoplankton concentrations within

Padilla Bay. Chl *a* concentrations were variable for the two Padilla Bay stations, but were generally higher at Station 1 (Figure 5). Table 3 contains the results of statistical comparisons of Chl *a* concentrations at the three stations for each sampling date. These results confirm that chl *a* concentrations tended to be highest at Station 1. Station 3 had significantly higher chl *a* concentrations than the other stations during August through October 1994, and in May 1995. There was no significant difference in chl *a* concentrations at the three stations on only two sampling dates, in July 1994 and in September 1995 (Table 3).

Phaeophytin pigment is degraded chlorophyll, which results from the grazing activities of zooplankton and natural phytoplankton senescence. Phaeophytin concentrations within Padilla Bay were very similar to chl *a* concentrations (Figure 6), tracking these closely during all seasons. At Station 3, chl *a* concentrations were higher than phaeophytin during all months except June and July 1994. During the large phytoplankton peak sustained at Station 3 during August through October 1994, phaeophytin represented only 50% to 25% of chl *a* biomass (Figures 5 and 6).

Phytoplankton Productivity

Primary productivity of surface waters from Stations 1 through 3 varied seasonally for rates expressed both on the basis of volume (Figure 7) and chl *a* concentration (Figure 8). Volume-specific productivity was often higher at Station 1 than at Stations 2 and 3 (Figure 7; Table 3), although it was higher at Station 3 in October 1994 and May 1995 (Table 3). During the winter, there was no significant difference in productivity at all three stations (December and February sampling dates). Productivity rates normalized to chl *a* correct for differences in phytoplankton biomass at the three stations. Productivity at Station 3 increased with this

correction, resulting in a shift from highest productivity at Station 1 to highest at Station 3 during spring and summer (Table 3). Station 3 exhibited pronounced seasonality in rates (Figure 8).

Nutrient Enrichment Bioassays

The seasonal growth response of phytoplankton to nutrient enrichment was evaluated by comparing chl *a* biomass and primary productivity after two to five days of nutrient enrichment, and by comparing growth rates of the phytoplankton populations during the initial enrichment period.

Chlorophyll *a* concentrations

Figures 9 through 20 show the chl *a* concentrations in water samples from the three stations. Table 4 shows that significant increases in phytoplankton biomass occurred only in response to nitrogen enrichment, and only during the spring and summer months. Nitrogen had no effect on phytoplankton biomass during winter. In general, phosphorus addition had no effect on chl *a* biomass relative to control unenriched samples. Differences in results for water collected from the three stations are described below.

There was no significant effect of nitrogen or phosphorus addition on chl *a* biomass in water collected from Station 1 in eight of the twelve experiments (Table 4). During April through July 1995, and again in September 1995, nitrogen caused a significant increase in phytoplankton biomass at Station 1 (Figures 15-17, 18; Table 4). In contrast, the phytoplankton in water from Station 2 responded to nitrogen addition during both summers (Figures 9-11 and 15-17; Table 4), resulting in a significant increase in chl *a* concentration in six of the twelve experiments. Plankton in water from Station 3 responded to nitrogen enrichment in seven of the

twelve experiments; during July 1994 the enrichment with both nitrogen and phosphorus resulted in a significantly higher chl *a* biomass than with nitrogen addition alone (Figure 10; Table 4). This was the only time where phosphorus addition (in combination with nitrogen) enhanced chl *a* biomass.

Primary productivity

Figures 21 through 32 show the primary productivity of phytoplankton in each experiment, normalized to volume (Figs. 21a–32a) and to chl *a* concentration (Figs. 21b–32b). As expected from the chl *a* results, only nitrogen caused a significant increase in primary productivity expressed on a volume basis during seasons other than winter (Table 5). In April 1995, the addition of both nitrogen and phosphorus resulted in almost a doubling of volume-specific production than with nitrogen alone for samples from all three stations (Figure 27, Table 5). For Station 1, half of the experiments showed no effect of nutrient addition on volume-specific primary production. Nutrients had no effect on productivity of samples from Station 2 in five experiments, while nitrogen enhanced the volume-specific productivity of water from Station 2 in all but two experiments (Table 5). Overall, the volume-specific primary productivity in the experimental treatments varied seasonally as shown for field-collected samples (Figure 7), and for most months there was little difference among the rates for the three stations.

Rates normalized to chl *a* remove the effect of differences in phytoplankton biomass in the different nutrient treatments on primary productivity. For Station 1, chl *a*-specific rates showed treatment effects during April through September, 1995 (Figures 27-31, Table 6). These treatment effects for Station 1 differed from those obtained for chl *a* concentrations and for volume-specific primary productivity; the addition of **both** nitrogen and phosphorus resulted in

the highest chl *a*-specific primary production rates in three of these experiments (April, July, and September; Figure 27, 29, 31), while the **control** treatments had the highest primary productivity rates in May 1995 (Figure 28). For Station 2, only half of the experiments resulted in significant differences among treatments for rates normalized to chl *a* (Table 6), and the results were variable as for Station 1. Five of the six experiments showed significant enhancement by nitrogen and phosphorus, with the April and September 1995 experiments indicating that this enhancement was due to the addition of phosphorus alone (Figures 27 and 31, Table 6). Phosphorus also stimulated chl *a*-specific primary production rates in December 1995 (Figure 32). Phosphorus alone significantly enhanced the chl *a*-specific productivity rates of samples from Station 3 during July and August 1995 (Figures 29 and 30, Table 6), while nitrogen was responsible for the significant increases in productivity observed during August 1994 (Figure 23), April 1995 (Figure 27), and September 1995 (Figure 31).

Phytoplankton growth

All phytoplankton in the experimental treatments increased in biomass during the incubation period. In most cases, an initial exponential increase in relative fluorescence of chlorophyll *a* lasted several days, followed by a prolonged stationary phase. The exponential phase was used to assess growth responses of the plankton to the nutrient treatments, while the stationary phase was used to assess biomass yield. Figures 33 through 44 show the growth curves obtained for plankton from each Station subjected to the four treatments. The specific growth rates derived from these curves are shown in Figures 45 through 59 and in Table 7. Table 8 shows the range of hours used to derive the specific growth rates for each experiment. During the spring, summer, and fall, exponential growth during the first 50 hours was used to

calculate growth rates; during winter, rates were calculated after 90 hours and up to 300 hours after the start of the experiment. The results show that nutrient addition affected the growth rate of only one-third of all phytoplankton samples incubated over the two year period. Where a significant increase in growth rate was observed, nitrogen was responsible for the stimulation. Phosphorus only stimulated the growth of phytoplankton once, in February 1995 for Station 1 phytoplankton only. The results for each station are described below.

For Station 1, growth rates of phytoplankton were not affected by nutrient treatments in seven of the eleven experiments reported (Table 9; data for December 1994 are not included). In February 1995, phosphorus addition caused a significant increase (about 1.4 times greater) in phytoplankton growth rate (Figure 49, Table 10). During April 1995, phosphorus caused a significantly reduced growth rate for Station 1 phytoplankton; growth rates with phosphorus were about one-third of control rates. During May and September 1995, the addition of nitrogen stimulated phytoplankton growth rate (Figure 51 and 54, Table 10).

Phytoplankton from Station 2 showed the greatest growth response to nutrient additions, with half of the experiments yielding significant effects (Table 9). Growth rates were significantly higher in response to nitrogen addition in five experiments during the spring and summer months (Figures 45, 47, 51, 52, and 53). In June and August 1994, the specific growth rate was almost doubled by the addition of nitrogen. The only other Station 2 experiment to show a different nutrient growth response occurred in April 1995, where a significant decrease in growth rate with phosphorus addition was obtained (Figure 50, Tables 9 and 10). This result was also found for Station 1 phytoplankton during April 1995.

For Station 3 phytoplankton, only two experiments (April and July 1995) yielded significant growth responses to the nutrient treatments (Table 9). In both experiments, the addition of nitrogen stimulated growth (Figures 50 and 52, Table 10).

Seasonal patterns in phytoplankton growth and relative availability of N and P

Figures 56 through 59 show the seasonal patterns in specific growth rates obtained under the different treatments. Figure 56 (control treatment) can be used to demonstrate seasonal variation in growth of phytoplankton from each station under natural nutrient conditions, and to compare plankton growth rates for each station. During summer, specific growth rates generally peak at about 1.3 d^{-1} , with a maximum growth rate of 1.5 d^{-1} obtained in August 1995 for Station 3 phytoplankton. During the winter and fall seasons, rates are generally three times lower, around 0.5 d^{-1} . Phytoplankton growth was generally higher in the spring than in fall. Comparing the three stations, growth rates are generally higher for phytoplankton from Stations 2 and 3 than for Station 1 phytoplankton (Figure 56).

Figure 57 shows the seasonal pattern in specific growth rates obtained with nitrogen addition. The seasonal patterns are unchanged; the only effect of nitrogen is an increase in specific growth rates during the summer of 1995 for phytoplankton from Stations 1 and 2 (Figures 56 and 57). During July 1995, specific growth rates for phytoplankton from Station 1 increased from 1.15 d^{-1} to 1.44 d^{-1} with nitrogen addition, and those of phytoplankton from Station 2 increased from 1.38 d^{-1} to 1.91 d^{-1} (Table 7). The specific growth rates shown for the phosphorus treatments (Figure 58) are essentially the same as the control treatment rates (Figure 56), indicating that phosphorus has little effect on specific growth rate. This result is supported by the rates obtained with the combined nitrogen and phosphorus treatment (Figure 59), which

yielded the same rates as the nitrogen treatments alone. The results show that nitrogen stimulated phytoplankton growth, primarily during the summer months.

The seasonal pattern in the relative availability of nitrogen to phosphorus (DIN:DIP) at the field stations (Figure 60) indicates the potential for nitrogen limitation of phytoplankton in Padilla Bay in summer. At Station 3, the DIN:DIP ratio indicates that nitrogen was more available, since the Redfield ratio of 16 was attained (Figure 60). The DIN:DIP ratios also changed seasonally. The DIN:DIP ratios indicate that phosphorus was limiting with respect to nitrogen during the spring, fall and winter months.

Phytoplankton biomass yields

Nutrient loading can result in different phytoplankton biomass accumulations, regardless of growth rate. Examination of the final relative fluorescence levels reached in the different nutrient treatments in Figures 33 through 44 shows that nitrogen caused a large increase in the standing stock of phytoplankton at all three stations during June 1994 (Figure 33), July 1994 (Figure 34), August 1994 (Figure 35), April 1995 (Figure 39), May 1995 (Figure 40; Stations 1 and 2 only), July 1995 (Figure 41), and September 1995 (Figure 43). There was no effect of nutrient treatments on final biomass yields during October 1995 (Figure 36), December 1994 (Figure 37), February 1995 (Figure 38; Stations 2 and 3 only), August 1995 (Figure 42), and December 1995 (Figure 44). The only deviation from this pattern occurred in February 1995, when phosphorus increased the final yield of phytoplankton from Station 1 alone (Figure 38).

DISCUSSION

Padilla Bay is a neutral embayment characterized by low freshwater input. The primary sources of freshwater are gated agricultural sloughs such as Joe Leary Slough. While the seasonal changes in freshwater input and nutrient loading in Padilla Bay are small relative to the seasonal changes in river-dominated systems, ambient nitrate and soluble reactive phosphate (SRP) concentrations in surface waters of the bay varied significantly on a seasonal scale. Nitrate and SRP were higher in the winter than during summer. Non-point source inputs to Padilla Bay are linked to seasonal changes in rainfall, seasonally varying volume of water in the sloughs, and seasonal pulses of freshwater from the Fraser River (80 km to the north) and the Skagit River (15 km to the south). Seasonal agricultural and logging activity in the Padilla watershed are more intermittent and are difficult to measure.

The seasonal patterns of dissolved inorganic nitrogen (DIN) concentrations and phytoplankton biomass argue for nitrogen limitation during summer in Padilla Bay. The seasonal distribution of DIN was negatively correlated with that of chlorophyll *a* concentration. This relationship suggests that summer phytoplankton populations in Padilla Bay are potentially limited by nitrogen availability. DIN:PO₄³⁻ ratios were less than 16:1 at stations 1 and 2 during summer 1994 and late summer 1995. The 16:1 ratio is based on the N:P atomic ratio found in a typical algal cell. Ratios below 16:1 indicated the potential for nitrogen limitation during summer. One must be cautious, however, when inferring nutrient limitation from nutrient concentrations and nutrient ratios. Nutrient concentrations may not always accurately represent what is biologically available to the phytoplankton. Additionally, the pool size of the nutrient and its turnover rate must be considered.

The results from the short-term bioassays support the hypothesis that nitrogen potentially limits phytoplankton biomass and production in Padilla Bay during late spring and summer when DIN concentrations are low. Significant increases in phytoplankton biomass and primary productivity occurred only in response to nitrogen enrichment during summer. Specific growth rates of phytoplankton at stations 1 and 2 in Padilla Bay during summer 1994 and summer 1995 were often stimulated by nitrogen additions. These results are consistent with the results of other studies in Puget Sound, Padilla Bay, and the Strait of Georgia (Harrison *et al.*, 1994). The other studies were limited in scope; they do, however, support our contention that nitrogen is the nutrient in greatest demand during summer. For example, Thom *et al.* (1988) suggested that nitrogen limitation is likely to occur in shallow nearshore systems in Puget Sound region during the summer when light is not limiting. Reports from the Puget Sound Water Quality Authority monitoring program (1994) suggest that nitrate can be depleted in the surface waters of poorly flushed bays and inlets in south Puget Sound. Phytoplankton growth in such areas is nitrogen limited for large parts of the summer. Bernhard and Peele (1997) also concluded that the production of phytoplankton biomass in Padilla Bay is nitrogen limited during the summer months.

Although our data show significant changes in phytoplankton biomass and productivity with nitrogen enrichment, we did not address seasonal changes in species composition. It is possible that the magnitude of the response to nitrogen enrichment relative to the control treatment reflects a shift in species dominance

Phytoplankton growth in Padilla Bay during winter appears to be regulated by factors other than inorganic nutrient supply. These factors include temperature, irradiance, and grazing. In a separate study, Brainard (1996) reported that the growth rates of phytoplankton in north

Puget Sound were equivalent to the maximum temperature-limited rates (μ_{\max}) at 6° C. Her results suggest that temperature does not limit phytoplankton productivity during winter. Irradiance is likely to control phytoplankton growth in Padilla Bay during winter. The daily integrated irradiance is six times less during January ($7 \text{ mol}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$) than during July ($45 \text{ mol}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$) (Padilla Bay NERR data). Our study does not address the removal of phytoplankton biomass through grazers $> 153 \text{ }\mu\text{m}$. However, grazing by microplankton less than $200 \text{ }\mu\text{m}$ can be significant during winter. Brainard (1996) compared seasonal phytoplankton growth and grazing by microzooplankton ($<200 \text{ }\mu\text{m}$) at our Station 3 during 1994 and 1995. She found that microzooplankton grazing rates were equivalent to phytoplankton growth rates during winter, while grazing rates were 50% of phytoplankton growth rates during spring, and 70% of growth rates during summer (Brainard, 1996). Her results show that tight coupling between phytoplankton growth and microzooplankton grazing occurs during seasons when phytoplankton growth rates are highest. It would be interesting to examine the relationship between growth and grazing rates under nitrogen-enriched conditions. The accumulation of phytoplankton biomass we observed with nitrogen enrichment during the summer months suggests that these processes are no longer coupled, and that growth greatly exceeds grazing under these conditions.

Seasonal changes in abiotic environmental factors may have contributed to the seasonal variability in nutrient limitation. For example, changes in temperature and light affect the uptake kinetics and growth rates of phytoplankton. Particular algal species can also shift in dominance from one season to another depending on a number of factors including environmental parameters such as temperature and light.

Conclusions

The results of this study show that summer phytoplankton growth in Padilla Bay is often regulated by nutrient (nitrogen) availability. This type of regulation is typical for estuaries where production is directly responsive to changing terrestrial nutrient inputs. It is also typical for estuaries where NO_3^- is the major form of available nitrogen.

Our results confirm that controlling nitrogen inputs may be essential to managing the primary productivity in Padilla Bay during summer. Padilla Bay is a shallow seagrass-dominated estuary with over 3200 ha of intertidal and subtidal mudflats. Nitrogen limitation of eelgrass and photosynthetic epiphytes in Padilla Bay has already been demonstrated in late spring and summer (Williams and Ruckelshaus, 1993), and our study confirms that nitrogen availability can limit phytoplankton production. Continued non-point source nutrient loading is likely to result in more phytoplankton growth in the water column. Some studies suggest, however, that the benthic macroalgae in shallow bays and lagoons can outcompete phytoplankton for available nitrogen and depress phytoplankton populations (Fong *et al.* 1993; Lee and Olsen 1985). Macroalgal biomass is already increasing in Padilla Bay (Bulthuis 1995). The increase in macroalgae may, therefore, profoundly affect the availability of nitrogen for all other autotrophic components in Padilla Bay and exacerbate the potential for nutrient limitation of phytoplankton biomass.

No standards have been set in Washington State for nutrients as potential contributors to algal blooms and eutrophication. Average DIN and phosphorus concentrations in the agricultural sloughs draining the Padilla Bay/Bay View watershed are, however, higher than the average nutrient concentrations for comparable watersheds in the western United States with 50-75% of land use in agriculture (Omernik 1977). The nutrient concentrations in the sloughs are

above the concentrations at which eutrophication problems are likely to exist (Bulthuis 1993). It is the nutrient in least supply that must be controlled, since reducing the supply of that nutrient may effectively control eutrophication. Based on our results, managers are advised to implement practices to limit the supply of anthropogenic nitrogen sources during summer, a critical time period for enhanced phytoplankton growth. For instance, application of fertilizers and output of freshwater from controlled diked sloughs could be restricted during this time period.

Documentation of the response of phytoplankton to watershed inputs is relevant to the management needs of the Padilla Bay Reserve and other sites that are influenced primarily by non-point nutrient inputs. This would include other embayments in the greater Puget Sound area as well as other NERRS estuaries. The nutrient-addition growth experiments provide a useful approach for testing the potential anthropogenic alteration of this estuary.

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List of Tables

- Table 1. Summary of analytical methods.
- Table 2. Sampling dates and physical characteristics of surface water samples at Stations 1, 2, and 3.
- Table 3. Results of statistical analyses of Stations 1, 2, and 3 field chlorophyll *a* concentrations and primary productivity rates.
- Table 4. Results of statistical analyses of chlorophyll *a* concentrations in water from each station after nutrient additions.
- Table 5. Results of statistical analyses of primary productivity rates normalized to volume (m³) in water from each station after nutrient additions.
- Table 6. Results of statistical analyses of primary productivity rates normalized to chlorophyll *a* in water from each station after nutrient additions.
- Table 7. Specific rate (d⁻¹, mean ± S.D., *n* = 3) in incubation bottles containing water from the three stations after nutrient additions. Growth rates were calculated from changes in *in vivo* chlorophyll *a* fluorescence during the exponential growth phase of the phytoplankton cultures.
- Table 8. Range of hours of *in situ* incubation and number of observations included in calculations of specific growth rates from *in vivo* fluorescence measurements for each month that experimental incubations were performed. Growth during this period was exponential or nearly exponential. The 0 h time point is the beginning of every incubation period at the experiment starting dates shown. The same range of hours was used for all 3 stations, except where indicated otherwise.
- Table 9. P values for ANOVA with replication (*n* = 3) of specific growth rates, comparing the 4 experimental groups: Control, +N, +P, and +NP. The ANOVA was performed on the specific growth rates determined for each experimental bottle during the exponential phase of growth. NS = non-significant at alpha = 0.05.
- Table 10. Results of two-sample *t*-tests for comparison of specific growth rate treatment means (*n* = 3). *T*-tests were performed for comparisons of the Control with +N, +P, and +NP. Tests were performed only for treatment groups for which ANOVA indicated a significant effect of nutrient addition (see Table). Blank cells indicate no significant effect of nutrient addition on growth rate. *P<0.05; **P<0.01; ***P<0.001.

List of Figures

- Figure 1. Map of Padilla Bay National Estuarine Research Reserve showing locations of Stations 1, 2, and 3.
- Figure 2. Nitrate concentrations in surface water samples from the three stations collected from June 1994 to December 1995.
- Figure 3. Ammonium concentrations in surface water samples from the three stations collected from June 1994 to December 1995.
- Figure 4. Phosphate concentrations in surface water samples from the three stations collected from June 1994 to December 1995.
- Figure 5. Chlorophyll *a* concentrations in surface water samples from the three stations collected from June 1994 to December 1995.
- Figure 6. Phaeophytin concentrations in surface water samples from the three stations collected from June 1994 to December 1995.
- Figure 7. Primary productivity of surface water samples from the three stations from June 1994 to December 1995. Samples were incubated at an irradiance of $437 \pm 11 \mu\text{mol m}^{-2} \text{s}^{-1}$; rates are expressed on a volumetric basis.
- Figure 8. Primary productivity of surface water samples from the three stations from June 1994 to December 1995. Samples were incubated at an irradiance of $437 \pm 11 \mu\text{mol m}^{-2} \text{s}^{-1}$; rates are expressed on a chlorophyll *a* basis.
- Figures 9-20. Chlorophyll *a* concentrations in surface waters on collection date ('field') and in experimental treatments after addition of nutrients (N = $\text{NO}_3^- + \text{NH}_4^+$; P = PO_4^{3-} ; NP = both nutrients; C = control, no nutrients added). Top panel = Station 1; middle panel = Station 2; bottom panel = Station 3. Dates in the upper right hand corner indicate the collection date for field samples (first date) and the date when bottles in treatments were sampled for chlorophyll *a* concentrations (second date). Statistical results for field comparisons are shown in Table 3; for treatment effects in Tables 4 and 5. Shared letters above the bars indicate chl *a* concentrations that did not differ among designated treatments. Absence of letters above a set of treatments indicates there is no significant difference among chl *a* concentrations for the different nutrient treatments. Error bars represent +1 S.D.; $n = 3$.
- Figure 9. Chlorophyll *a* concentrations in June 1994. Field samples were collected on June 28; treatment effects were measured two days later on June 30.

- Figure 10. Chlorophyll *a* concentrations in July 1994. Field samples were collected on July 25; treatment effects were measured two days later on July 27.
- Figure 11. Chlorophyll *a* concentrations in August 1994. Field samples were collected on August 22; treatment effects were measured two days later on August 24.
- Figure 12. Chlorophyll *a* concentrations in October 1994. Field samples were collected on October 25; treatment effects were measured four days later on October 29.
- Figure 13. Chlorophyll *a* concentrations in December 1994. Field samples were collected on December 7; treatment effects were measured four days later on December 11.
- Figure 14. Chlorophyll *a* concentrations in February 1995. Field samples were collected on February 4; treatment effects were measured five days later on February 9.
- Figure 15. Chlorophyll *a* concentrations in April 1995. Field samples were collected on April 15; treatment effects were measured five days later on April 20.
- Figure 16. Chlorophyll *a* concentrations in May 1995. Field samples were collected on May 20; treatment effects were measured three days later on May 23.
- Figure 17. Chlorophyll *a* concentrations in July 1995. Field samples were collected on July 5; treatment effects were measured two days later on July 7.
- Figure 18. Chlorophyll *a* concentrations in August 1995. Field samples were collected on August 15; treatment effects were measured three days later on August 18.
- Figure 19. Chlorophyll *a* concentrations in September 1995. Field samples were collected on September 14; treatment effects were measured two days later on September 16.
- Figure 20. Chlorophyll *a* concentrations in December 1995. Field samples were collected on December 2; treatment effects were measured five days later on December 7.

Figures 21-32. Primary productivity in surface waters on collection date ('Field') and in experimental treatments after addition of nutrients (N = $\text{NO}_3^- + \text{NH}_4^+$; P = PO_4^{3-} ; NP = both nutrients; C = control, no nutrients added). All samples were incubated at an irradiance of $437 \pm 11 \mu\text{mol m}^{-2} \text{s}^{-1}$. Top panel = Station 1; middle panel = Station 2; bottom panel = Station 3. Dates in the upper right hand corner indicate the collection date for field samples (first date) and the date when bottles in treatments were sampled for primary productivity measurements (second date). Statistical results for field comparisons are shown in Table 3; for treatment effects in Tables 4 and 5. Shared letters above the bars indicate primary production rates that did not differ among designated treatments. Absence of letters above a set of treatments indicates there is no significant difference among rates for the different nutrient treatments. Error bars represent ± 1 S. D.; n = 3. Rates are expressed on the basis of volume (A) and chlorophyll *a* concentration (B).

Figure 21. Primary production in June 1994. Field samples were collected on June 28; treatment effects were measured two days later on June 30.
A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.

Figure 22. Primary production in July 1994. Field samples were collected on July 25; treatment effects were measured two days later on July 27.
A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.

Figure 23. Primary production in August 1994. Field samples were collected on August 22; treatment effects were measured two days later on August 24.
A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.

Figure 24. Primary production in October 1994. Field samples were collected on October 25; treatment effects were measured four days later on October 29.
A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.

Figure 25. Primary production in December 1994. Field samples were collected on December 7; treatment effects were measured four days later on December 11.
A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.

Figure 26. Primary production in February 1995. Field samples were collected on February 4; treatment effects were measured five days later on February 9.
A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.

Figure 27. Primary production in April 1995. Field samples were collected on April 15; treatment effects were measured five days later on April 20.
A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.

Figure 28. Primary production in May 1995. Field samples were collected on May 20; treatment effects were measured three days later on May 23.
A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.

- Figure 29. Primary production in July 1995. Field samples were collected on July 5; treatment effects were measured two days later on July 7.
A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.
- Figure 30. Primary production in August 1995. Field samples were collected on August 15; treatment effects were measured three days later on August 18.
A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.
- Figure 31. Primary production in September 1995. Field samples were collected on September 14; treatment effects were measured two days later on September 16. A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.
- Figure 32. Primary production in December 1995. Field samples were collected on December 2; treatment effects were measured five days later on December 7.
A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.
- Figures 33-44. Relative fluorescence of phytoplankton from Station 1 (top), Station 2 (middle), and Station 3 (bottom) in experimental treatments. Mean values are shown ($n = 3$) for samples taken during the entire experiment. Specific growth rates were calculated from the linear portion of each curve for individual water samples.
- Figure 33. Relative fluorescence of phytoplankton collected June 28, 1994 in experimental treatments.
- Figure 34. Relative fluorescence of phytoplankton collected July 25, 1994 in experimental treatments.
- Figure 35. Relative fluorescence of phytoplankton collected August 22, 1994 in experimental treatments.
- Figure 36. Relative fluorescence of phytoplankton collected October 25, 1994 in experimental treatments.
- Figure 37. Relative fluorescence of phytoplankton collected December 7, 1994 in experimental treatments.
- Figure 38. Relative fluorescence of phytoplankton collected February 4, 1995 in experimental treatments.
- Figure 39. Relative fluorescence of phytoplankton collected April 15, 1995 in experimental treatments.
- Figure 40. Relative fluorescence of phytoplankton collected May 20, 1995 in experimental treatments.

Figure 41. Relative fluorescence of phytoplankton collected July 5, 1995 in experimental treatments.

Figure 42. Relative fluorescence of phytoplankton collected August 15, 1995 in experimental treatments.

Figure 43. Relative fluorescence of phytoplankton collected September 14, 1995 in experimental treatments.

Figure 44. Relative fluorescence of phytoplankton collected December 2, 1995 in experimental treatments.

Figures 45-55. Specific growth rates (d^{-1}) of phytoplankton from Station 1 (top), Station 2 (middle), and Station 3 (bottom) in experimental treatments. Mean values are shown (\pm S.D., $n = 3$) for each treatment. The dates in the upper right hand corner are the time interval used for the growth rate calculations.

Figure 45. Specific growth rate of phytoplankton collected June 28, 1994 in experimental treatments.

Figure 46. Specific growth rate of phytoplankton collected July 25, 1994 in experimental treatments.

Figure 47. Specific growth rate of phytoplankton collected August 22, 1994 in experimental treatments.

Figure 48. Specific growth rate of phytoplankton collected October 25, 1994 in experimental treatments.

Figure 49. Specific growth rate of phytoplankton collected February 4, 1995 in experimental treatments.

Figure 50. Specific growth rate of phytoplankton collected April 15, 1995 in experimental treatments.

Figure 51. Specific growth rate of phytoplankton collected May 20, 1995 in experimental treatments.

Figure 52. Specific growth rate of phytoplankton collected July 5, 1995 in experimental treatments.

Figure 53. Specific growth rate of phytoplankton collected August 15, 1995 in experimental treatments.

Figure 54. Specific growth rate of phytoplankton collected September 14, 1995 in experimental treatments.

Figure 55. Specific growth rate of phytoplankton collected December 2, 1995 in experimental treatments.

Figures 56-59. Specific growth rates for phytoplankton in each experimental treatment (C, N, P, NP) during the project. Data for December 1994 are not included. Mean specific growth rates for phytoplankton from Station 1 (top), Station 2 (middle), and Station 3 (bottom) are presented (\pm S.D., $n = 3$).

Figure 56. Specific growth rates of phytoplankton in control treatment (no nutrients added).

Figure 57. Specific growth rates of phytoplankton in nitrogen treatment.

Figure 58. Specific growth rates of phytoplankton in phosphorus treatment.

Figure 59. Specific growth rates of phytoplankton in nitrogen and phosphorus treatment.

Figure 60. Dissolved inorganic nitrogen to phosphate ratios (DIN:DIP) in surface waters from the three stations collected from June 1994 to December 1995. The dashed line represents the Redfield ratio of 16.

Table 1. Summary of analytical methods.

Variable	Method
Temperature	Hydrolab [®] thermistor
Salinity	Hydrolab [®] specific conductance nickel electrodes
Dissolved oxygen	Hydrolab [®] standard membrane DO sensor
pH	Hydrolab [®] pH glass electrode
NH ₄ ⁺	Alternative phenolhypochlorite colorimetric method
NO ₃ ⁻ + NO ₂ ⁻	Modified cadmium reduction and colorimetric method
PO ₄ ³⁻	Ascorbic acid colorimetric method
Chlorophyll <i>a</i>	Fluorometric analysis of acetone extracts; <i>in vivo</i> chlorophyll <i>a</i> fluorescence
Primary productivity	¹⁴ CO ₃ ⁻ incorporation

Table 2. Sampling dates and physical characteristics of surface water samples at Stations 1, 2, and 3. The average salinity of all water samples was 28.7 (± 1.4 S.D.) and the average temperature was 12.8°C (± 4.2 S.D.).

Date	Time	Station	Salinity ppt	Temp (°C)
06/28/94	10:00	1	26.5	18.8
	11:05	2	28.3	18.3
	13:00	3	28.8	13.4
07/25/94	8:55	1	27.4	20.3
	9:30	2	28.2	18.2
	11:30	3	28.8	13.7
08/22/94	6:45	1	27.9	18.7
	7:25	2	29.6	16.7
	9:30	3	30.2	12.5
09/27/94	11:10	1	28.6	15.7
	12:03	2	29.5	14.8
	12:41	3	29.9	13.5
10/25/94	9:40	1	28.7	8.8
	10:25	2	30.2	10.3
	12:15	3	30.9	10.3
12/07/94	9:06	1	29.3	4.1
	9:57	2	30.7	6.5
	11:54	3	31.1	7.8
02/04/95	8:30	1	26.9	7.8
	9:18	2	26.1	7.7
	11:50	3	29.0	7.9
04/15/95	16:00	1	26.7	13.6
	15:00	2	27.9	11.0
	12:42	3	27.8	9.3
05/20/95	8:58	1	29.0	13.7
	9:38	2	28.7	13.6
	11:48	3	29.5	12.1
07/05/95	12:35	1	27.6	23.0
	10:20	2	28.7	15.7
	9:05	3	27.6	14.2
08/15/95	9:45	1	29.1	14.9
	10:28	2	29.5	12.7
	12:25	3	29.8	12.4
09/14/95	9:30	1	29.2	17.0
	10:08	2	30.1	14.2
	12:20	3	30.5	13.1
12/02/95	12:34	1	24.2	8.0
	10:02	2	27.0	8.4
	9:06	3	29.2	8.9

Table 3. Results of statistical analyses of Stations 1, 2, and 3 field chlorophyll *a* concentrations ([chl *a*]) and primary productivity rates expressed on the basis of chlorophyll *a* (Prod [chl *a*]) and volume (Prod [m³]). NS = P>0.05.

Date	[chl <i>a</i>]		Prod [chl <i>a</i>]		Prod [m ³]	
	Stations	Statistics	Stations	Statistics	Stations	Statistics
1994						
June 28	2>1,3	(P=0.007)	3>1,2	(P=0.004)	2>3>1	(P<0.001)
July 25	1,2,3	NS	1,2>1,3	(P=0.03)	1>2>3	(P<0.001)
Aug 22	3>1>2	(P<0.001)	1,3>2	(P<0.001)	1>2,3	(P<0.001)
Sept 27	3>1>2	(P<0.001)	1>2>3	(P<0.001)	1>2,3	(P<0.001)
Oct 25	3>2>1	(P<0.001)	1>2,3	(P=0.004)	3>1,2	(P=0.04)
Dec 7	1>2,3	(P<0.001)	1,2,3	NS	1,2,3	NS
1995						
Feb 4	1,2>3	(P<0.001)	3>1,2	(P=0.009)	1,2,3	NS
Apr 15	1>2,3	(P<0.001)	3>1,2	(P=0.008)	1>2,3	(P<0.001)
May 20	3>1,2	(P=0.003)	1,2,3	NS	3>1,2	(P<0.001)
Jul y 5	1,2>2,3	(P=0.048)	3>1,2	(P=0.003)	1,3>2	(P=0.001)
Aug 15	2,3>1	(P=0.001)	2,3>1	(P=0.03)	2,3>1	(P=0.02)
Sept 14	1,2,3	NS	1,3>2	(P=0.01)	1,3>1,2	(P=0.02)
Dec 2	1>2,3	(P=0.006)	2,3>1,2	(P=0.04)	1,2,3	NS

Table 4. Results of statistical analyses of chlorophyll *a* concentrations in water from each station after experimental nutrient additions. Samples were collected two to five days after the start of treatments (see Figures 9-20 for details; N = NO₃⁻ + NH₄⁺; P = PO₄³⁻; NP = both nutrients; C = control). NS = P>0.05.

Date	Station 1		Station 2		Station 3	
	Nutrients	Statistics	Nutrients	Statistics	Nutrients	Statistics
June 30, 1994	C,P,N,NP	NS	NP,N>N,P>C,P	(P=0.003)	C,P,N,NP	NS
July 27, 1994	C,P,N,NP	NS	NP,N>C,P	(P<0.001)	NP>N>P>C	(P<0.001)
August 24, 1994	C,P,N,NP	NS	NP,N>C,P	(P<0.001)	NP,N,C>NP,C,P	(P<0.04)
October 29, 1994	C,P,N,NP	NS	C,P,N,NP	NS	C,P,N,NP	NS
December 11, 1994	C,P,N,NP	NS	CP,N,NP	NS	C,P,N,NP	NS
February 9, 1995	C,P,N,NP	NS	C,P,N,NP	NS	C,P,N,NP	NS
April 20, 1995	NP,N>C,P	(P<0.001)	NP,N>C,P	(P<0.001)	NP,N>P>C	(P<0.001)
May 23, 1995	NP,N>C,P	(P<0.001)	NP,N>C,P	(P<0.001)	NP>N>C,P	(P<0.001)
July 7, 1995	N>NP,C,P	(P<0.001)	NP,N>C,P	(P<0.001)	NP,N>C>P	(P<0.001)
August 18, 1995	C,P,N,NP	NS	C,P,N,NP	NS	NP,N>NP,C>P	(P<0.001)
September 16, 1995	NP,N,C>NP,C,P	(P=0.02)	C,P,N,NP	NS	NP,N>NP,C,P	(P=0.009)
December 7, 1995	C,P,N,NP	NS	C,P,N,NP	NS	C,P,N,NP	NS

Table 5. Results of statistical analyses of primary productivity rates normalized to volume (m^3) in water from each station after nutrient additions. Samples were collected two to five days after the start of treatments (see Figures 9-20 for details; $N = NO_3^- + NH_4^+$; $P = PO_4^{3-}$; NP = both nutrients; C = control). NS = $P > 0.05$.

Date	Station 1		Station 2		Station 3	
	Nutrients	Statistics	Nutrients	Statistics	Nutrients	Statistics
June 30, 1994	NP,N>C,P	($P=0.03$)	N,P,N>C,P	($P<0.001$)	NP,N>C,P	($P=0.005$)
July 27, 1994	NP,N>C,P	($P<0.001$)	NP,N>C,P	($P<0.001$)	C,N,NP,P	NS
August 24, 1994	C,N,NP,P	NS	NP,N>NP,C>C,P	($P=0.003$)	C,N,NP,P	NS
October 29, 1994	C,N,NP,P	NS	C,N,NP,P	NS	C,N,NP,P	NS
December 11, 1994	C,N,NP,P	NS	C,N,NP,P	NS	NP,N,P>NP,C	($P=0.008$)
February 9, 1995	C,N,NP,P	NS	N>NP,P,C	($P=0.04$)	NP,N>C,P	($P=0.003$)
April 20, 1995	NP>N>C,P	($P<0.001$)	NP>N>C,P	($P<0.001$)	NP>N>C,P	($P<0.001$)
May 23, 1995	NP,N>C,P	($P<0.001$)	NP,N>C,P	($P<0.001$)	NP,N>C,P	($P<0.001$)
July 7, 1995	NP,N>N,C,P	($P=0.006$)	NP,N>C,P	($P<0.001$)	NP>N>C,P	($P<0.001$)
August 18, 1995	C,N,NP,P	NS	NP,N,P>C	($P<0.001$)	NP,N>P>C	($P<0.001$)
September 16, 1995	NP,N>C,P	($P=0.005$)	NP>N,C,P	($P<0.001$)	C,N,NP,P	NS
December 7, 1995	C,N,NP,P	NS	NP,N,P>C	($P<0.001$)	C,N,NP,P	NS

Table 6. Results of statistical analyses of primary productivity rates normalized to chlorophyll *a* in water from each station after nutrient additions. Samples were collected two to five days after the start of treatments (see Figures 9-20 for details; N = NO₃⁻ + NH₄⁺; P = PO₄³⁻; NP = both nutrients; C = control). NS = P>0.05.

Date	Station 1		Station 2		Station 3	
	Nutrients	Statistics	Nutrients	Statistics	Nutrients	Statistics
June 30, 1994	C,P,N,NP	NS	C,P,N,NP	NS	C,P,N,NP	NS
July 27, 1994	C,P,N,NP	NS	C,N>NP,N>NP,P	(P=0.003)	C,P,N,NP	NS
August 24, 1994	C,P,N,NP	NS	C,P,N,NP	NS	NP,N >NP,N,C	(P=0.04)
October 29, 1994	C,P,N,NP	NS	C,P,N,NP	NS	C,P,N,NP	NS
December 11, 1994	C,P,N,NP	NS	C,P,N,NP	NS	C,P,N,NP	NS
February 9, 1995	C,P,N,NP	NS	C,P,N,NP	NS	C,P,N,NP	NS
April 20, 1995	NP>N,C,P	(P<0.001)	NP>N,C,P	(P<0.001)	NP,N>P>C	(P<0.001)
May 23, 1995	C>NP,N,P	(P=0.04)	C,P,N,NP	NS	C,P,N,NP	NS
July 7, 1995	NP>P>C,N	(P<0.001)	NP,N>N,P>P,C	(P=0.002)	P>NP>N,C	(P<0.001)
August 18, 1995	NP,N,P>C,N	(P=0.01)	NP,N,P>C,N,P	(P=0.02)	P>NP,N>C	(P<0.001)
September 16, 1995	NP>N,P>C	(P=0.001)	NP,P>C,N	(P=0.001)	NP,P>C,N	(P=0.03)
December 7, 1995	C,P,N,NP	NS	NP,N,P>C	(P=0.004)	C,P,N,NP	NS

Table 7. Specific growth rate (d^{-1} , mean and S.D., $n=3$) in incubation bottles containing water from the three stations after nutrient treatments. Growth rates were calculated from changes in relative fluorescence during the exponential growth phase of the cultures.

<u>Station 1</u>	Jun-94	Jul-94	Aug-94	Oct-94	Feb-95	Apr-95	May-95	Jul-95	Aug-95	Sep-95	Dec-95
<u>mean</u>											
C	0.747	1.162	0.334	0.331	0.680	0.397	0.915	1.146	0.878	0.752	0.192
N	0.876	1.076	0.367	0.341	0.695	0.553	1.232	1.445	1.273	0.991	0.175
P	0.788	1.149	0.313	0.354	1.000	0.144	0.962	1.150	1.645	0.786	0.162
NP	0.873	1.222	0.290	0.379	0.965	0.406	1.231	1.435	1.126	0.915	0.168
<u>SD</u>											
C	0.084	0.089	0.123	0.033	0.050	0.128	0.045	0.278	0.223	0.068	0.087
N	0.080	0.198	0.007	0.028	0.056	0.176	0.053	0.102	0.192	0.033	0.113
P	0.004	0.032	0.057	0.026	0.045	0.034	0.007	0.284	0.743	0.092	0.092
NP	0.113	0.139	0.047	0.039	0.038	0.017	0.034	0.281	0.576	0.118	0.077
<u>Station 2</u>											
<u>mean</u>											
C	0.605	1.368	0.497	0.539	0.914	0.759	0.931	1.382	1.017	0.958	0.330
N	1.040	1.500	0.712	0.610	0.877	0.772	1.138	1.907	1.454	0.822	0.357
P	0.489	1.156	0.248	0.577	0.941	0.612	0.958	1.299	1.123	0.971	0.343
NP	0.988	1.512	0.744	0.586	0.912	0.731	1.124	1.814	1.444	1.012	0.364
<u>SD</u>											
C	0.228	0.304	0.065	0.065	0.033	0.053	0.094	0.102	0.186	0.169	0.050
N	0.015	0.117	0.058	0.049	0.070	0.042	0.061	0.032	0.068	0.248	0.067
P	0.042	0.309	0.211	0.019	0.022	0.069	0.028	0.087	0.103	0.190	0.023
NP	0.059	0.199	0.030	0.051	0.033	0.032	0.054	0.124	0.173	0.181	0.098
<u>Station 3</u>											
<u>mean</u>											
C	0.916	1.231	0.660	0.576	0.897	0.890	1.284	1.130	1.491	0.831	0.350
N	0.916	1.258	0.606	0.605	0.908	0.991	1.352	1.284	1.569	0.923	0.419
P	0.781	1.303	0.626	0.556	0.862	0.951	1.296	1.297	1.414	0.852	0.399
NP	1.106	1.355	0.640	0.644	0.865	1.058	1.485	1.479	1.568	1.062	0.417
<u>SD</u>											
C	0.292	0.092	0.111	0.106	0.062	0.042	0.051	0.024	0.248	0.137	0.068
N	0.143	0.054	0.067	0.018	0.073	0.037	0.085	0.086	0.084	0.144	0.027
P	0.067	0.214	0.035	0.037	0.027	0.040	0.019	0.125	0.086	0.084	0.007
NP	0.083	0.034	0.068	0.021	0.069	0.028	0.129	0.164	0.128	0.153	0.011

note: Data from Dec 1994 not included

Table 8. Range of hours of *in situ* incubation and number of observations included in calculations of specific growth rates from *in vivo* fluorescence measurements for each month that experimental incubations were performed. Growth during this period was exponential or nearly exponential. The 0 h time point is the beginning of every incubation period at the experiment starting dates shown. The same range of hours was used for all 3 stations, except where indicated otherwise.

<u>Starting Date</u>	<u>Range of Hours</u>	<u>Number of Observations</u>
May 1, 1994	0-52	6
June 28, 1994	19-48	4
July 25, 1994	19-47	3
August 22, 1994	21-48	4
October 25, 1995	0-167	7
February 4, 1995	97-175	5
April 15, 1995	0-69	3
May 20, 1995	0-50	4
July 5, 1995 (Station 1)	27-51	3
July 5, 1995 (Stations 2,3)	0-27	3
August 15, 1995	45-74	4
September 14, 1995	0-48	5
December 2, 1995	118-311	5

Table 9. P values for ANOVA with replication (n=3) of specific growth rates, comparing the 4 experimental groups: Control, +N, +P, and +NP. The ANOVA was performed on the specific growth rates determined for each experimental bottle during the exponential phase of growth. NS = non-significant at alpha = 0.05.

<u>Starting Date</u>	<u>Station 1</u>	<u>Station 2</u>	<u>Station 3</u>
June 28, 1994	NS	P=0.0010	NS
July 25, 1994	NS	NS	NS
August 22, 1994	NS	P=0.0025	NS
October 25, 1995	NS	NS	NS
February 4, 1995	P=0.0000	NS	NS
April 15, 1995	P=0.0120	P=0.0177	P=0.0034
May 20, 1995	P=0.0000	P=0.0068	NS
July 5, 1995	NS	P=0.0001	P=0.0333
August 15, 1995	NS	P=0.0104	NS
September 14, 1995	P=0.0261	NS	NS
December 2, 1995	NS	NS	NS

Table 10. Results of two-sample *t*-tests for comparison of specific growth rate treatment means (n=3). *t*- tests were performed for comparisons of the Control with +N, +P, and +NP. Test were performed only for treatment groups for which ANOVA indicated a significant effect of of nutrient addition (see Table 9). Blank cells indicate no significant effect of nutrient addition on growth rate. * P<0.05; ** P<0.01; *** P<0.001.

<u>Starting Date</u>	<u>Station 1</u>			<u>Station 2</u>			<u>Station 3</u>		
	<u>+N</u>	<u>+P</u>	<u>+NP</u>	<u>+N</u>	<u>+P</u>	<u>+NP</u>	<u>+N</u>	<u>+P</u>	<u>+NP</u>
May 1, 1994							*		
June 28, 1994				*		*			
July 25, 1994									
August 22, 1994				**		**			
October 25, 1995									
December 7, 1994									
February 4, 1995		***	***						
April 15, 1995		*			*		*		**
May 20, 1995	***		***	*		*			
July 5, 1995				***		**	*		*
August 15, 1995				*		*			
September 14, 1995	**								
December 2, 1995									
# of Significant Months	2	2	2	5	1	5	3	0	2

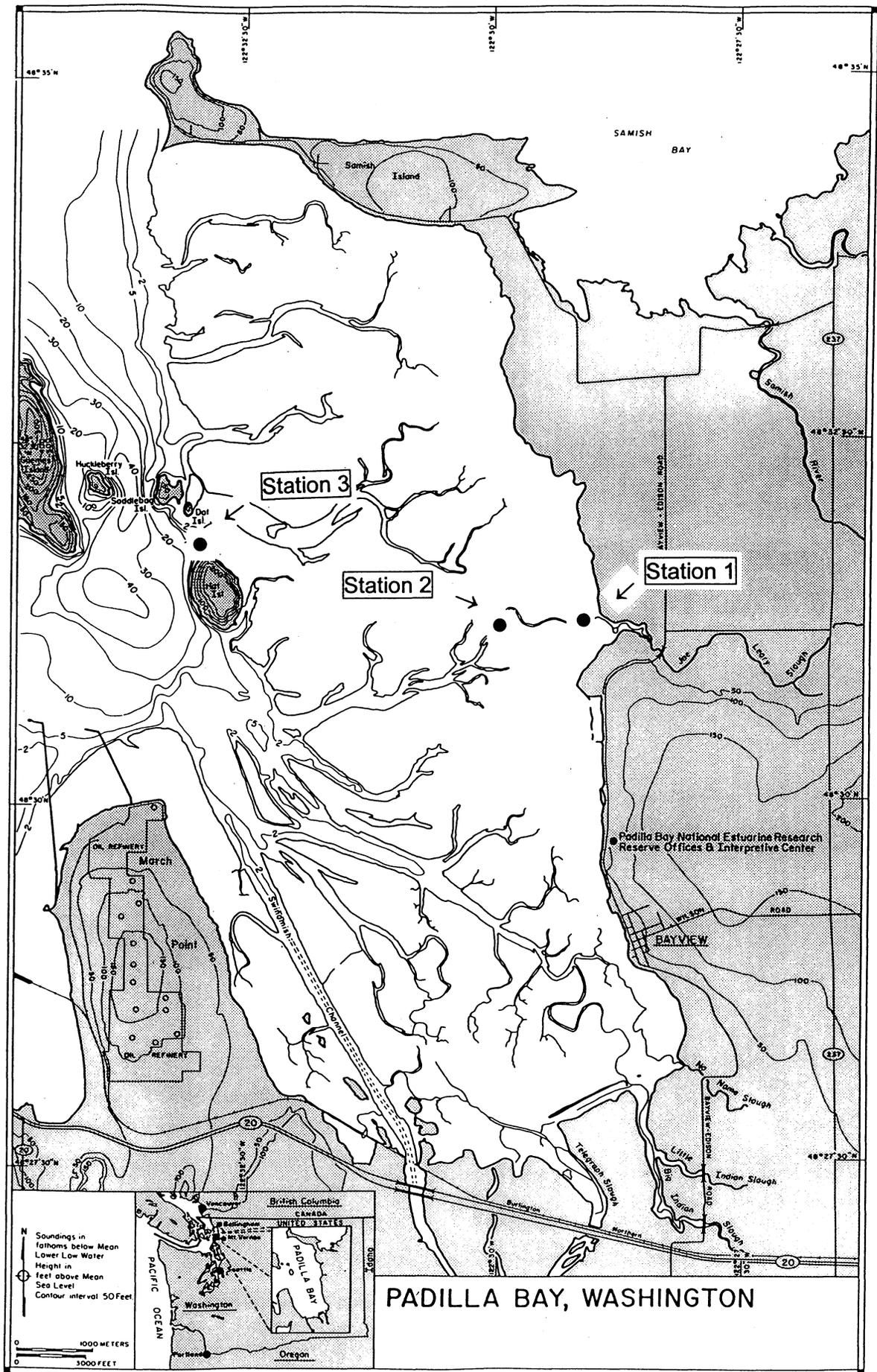


Figure 1. Map of Padilla Bay National Estuarine Research Reserve showing locations of three sampling stations.

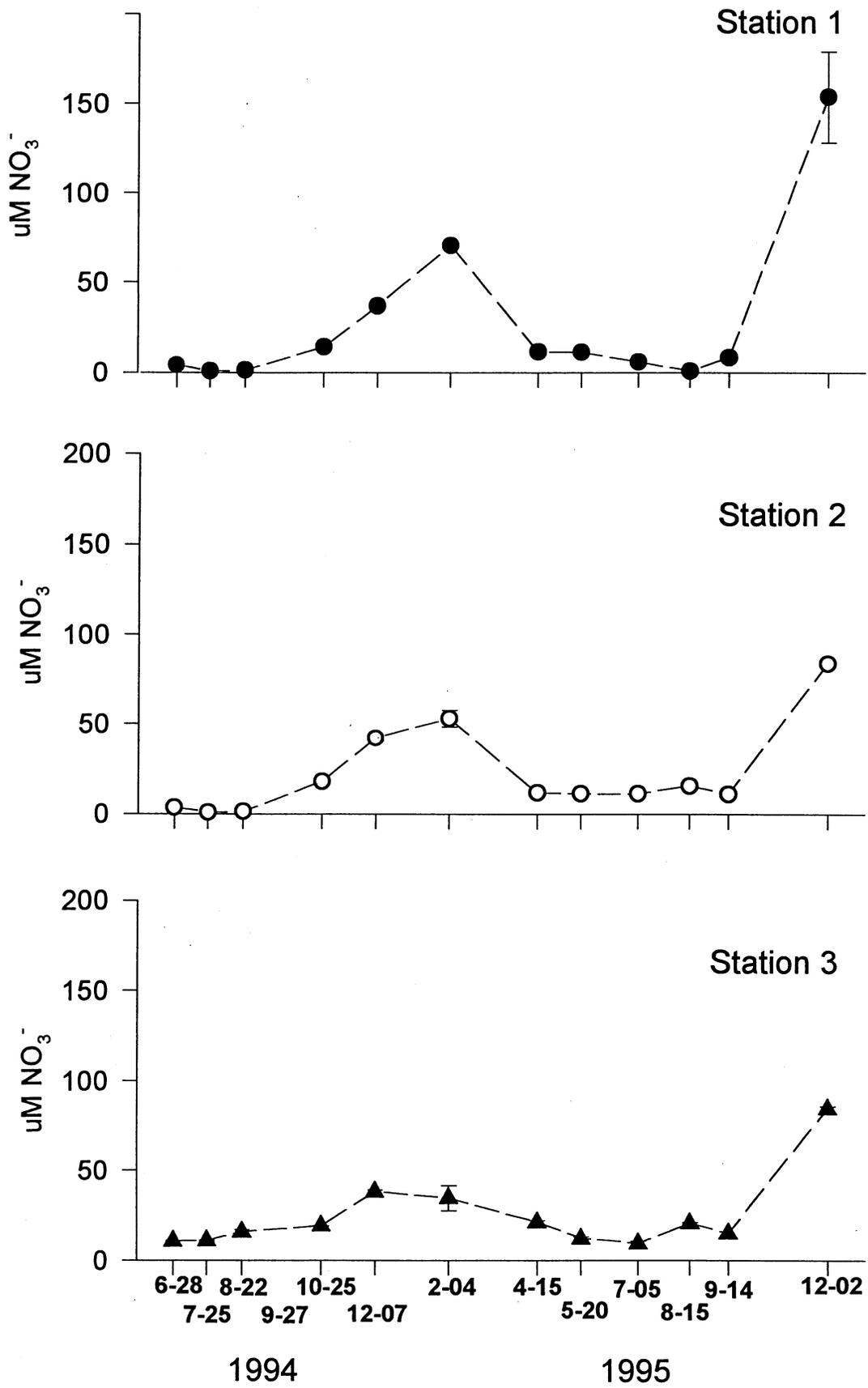


Figure 2. Nitrate concentrations in surface water samples from the three stations collected from June 1994 to December 1995.

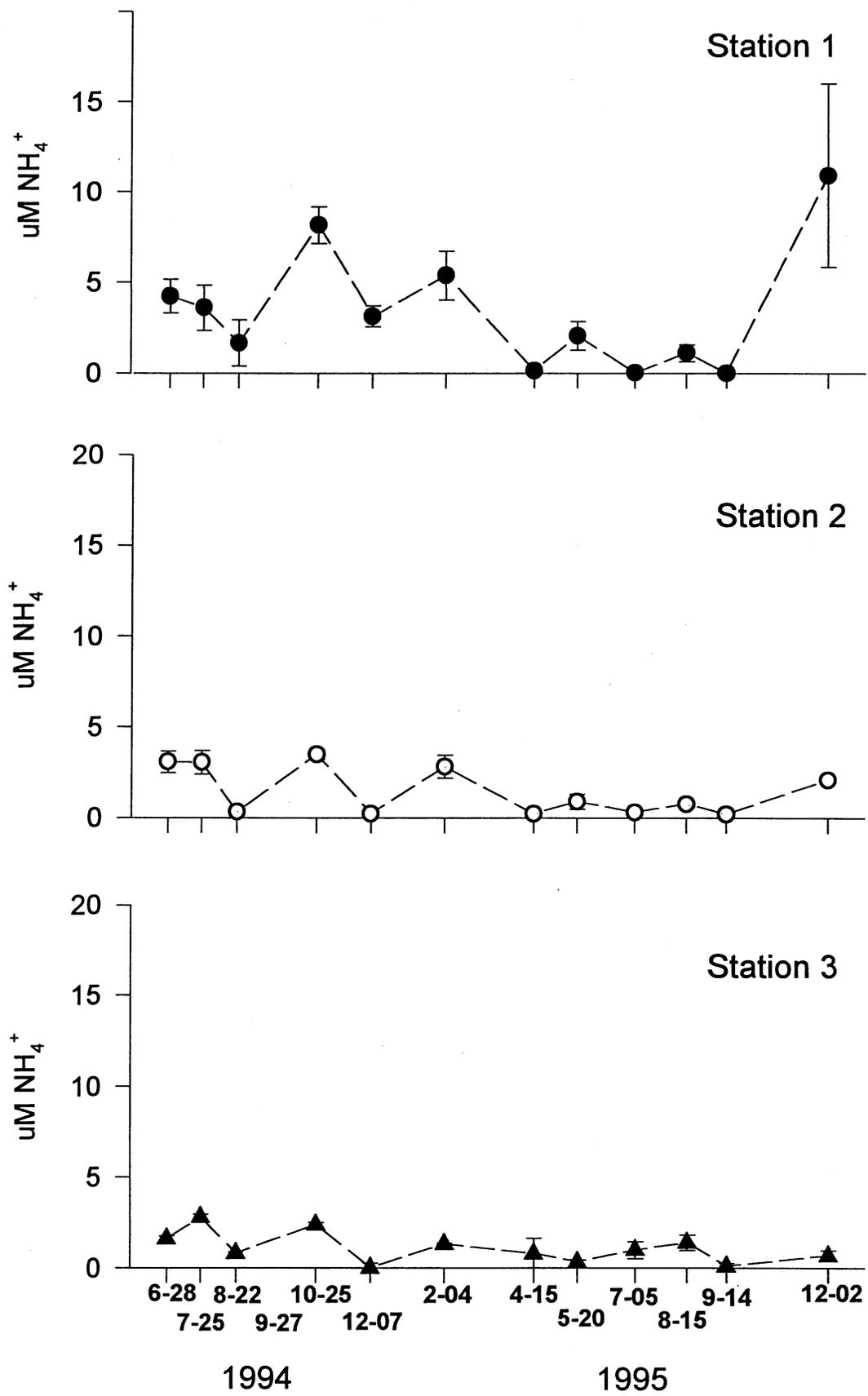


Figure 3. Ammonium concentrations in surface water samples from the three stations collected from June 1994 to December 1995.

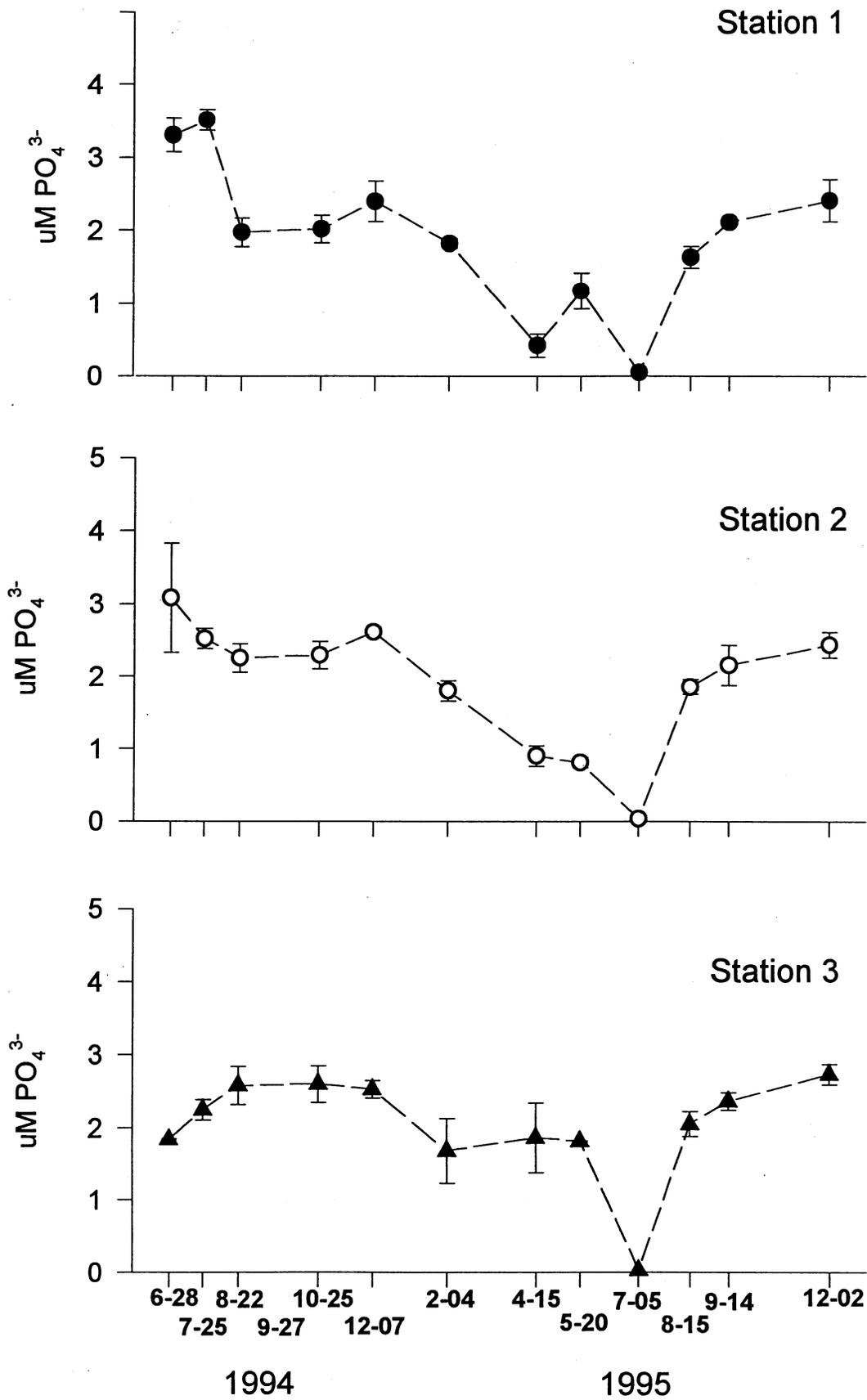


Figure 4. Phosphate concentrations in surface water samples from the three stations collected from June 1994 to December 1995.

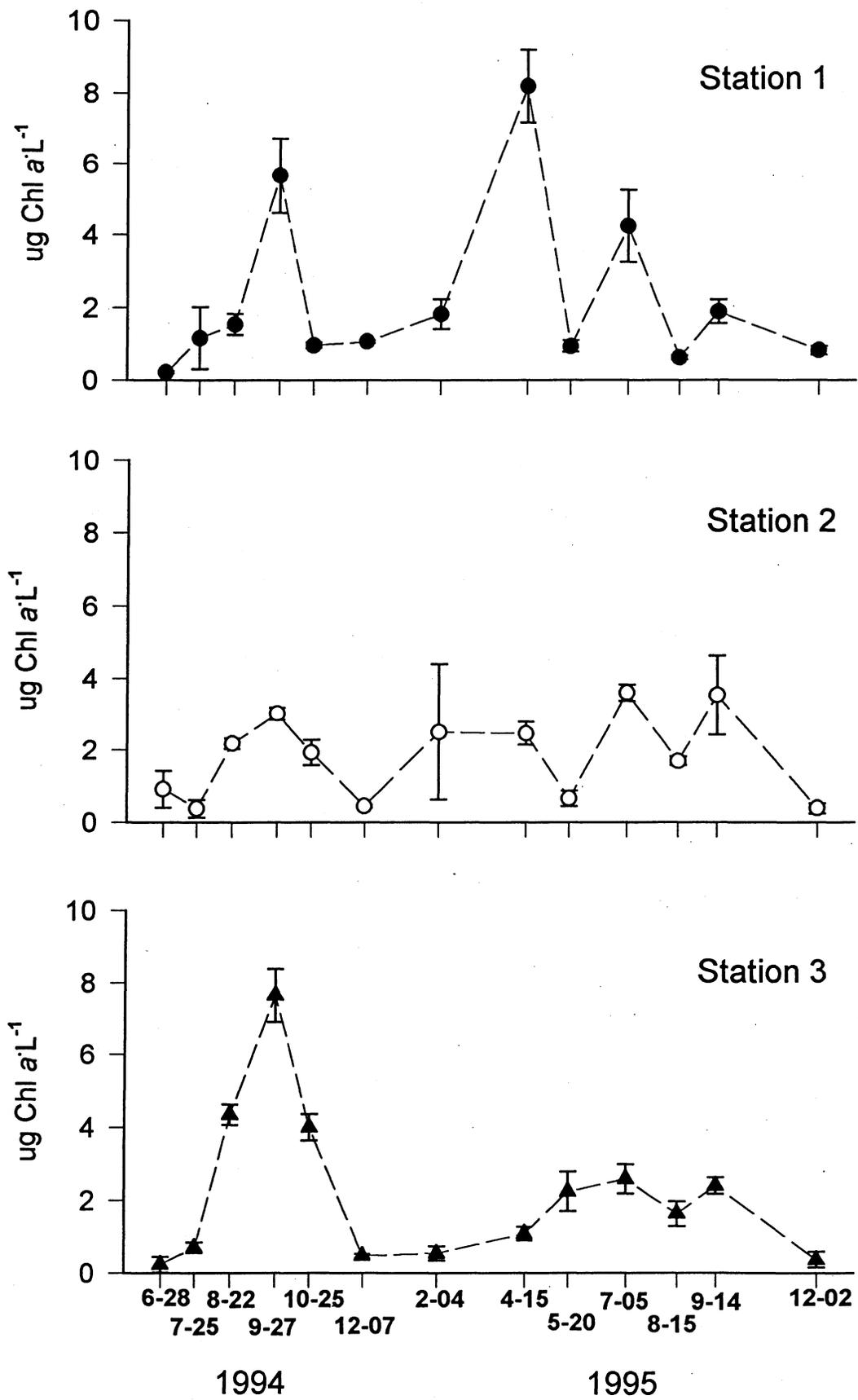


Figure 5. Chlorophyll *a* concentrations in surface water samples from the three stations collected from June 1994 to December 1995.

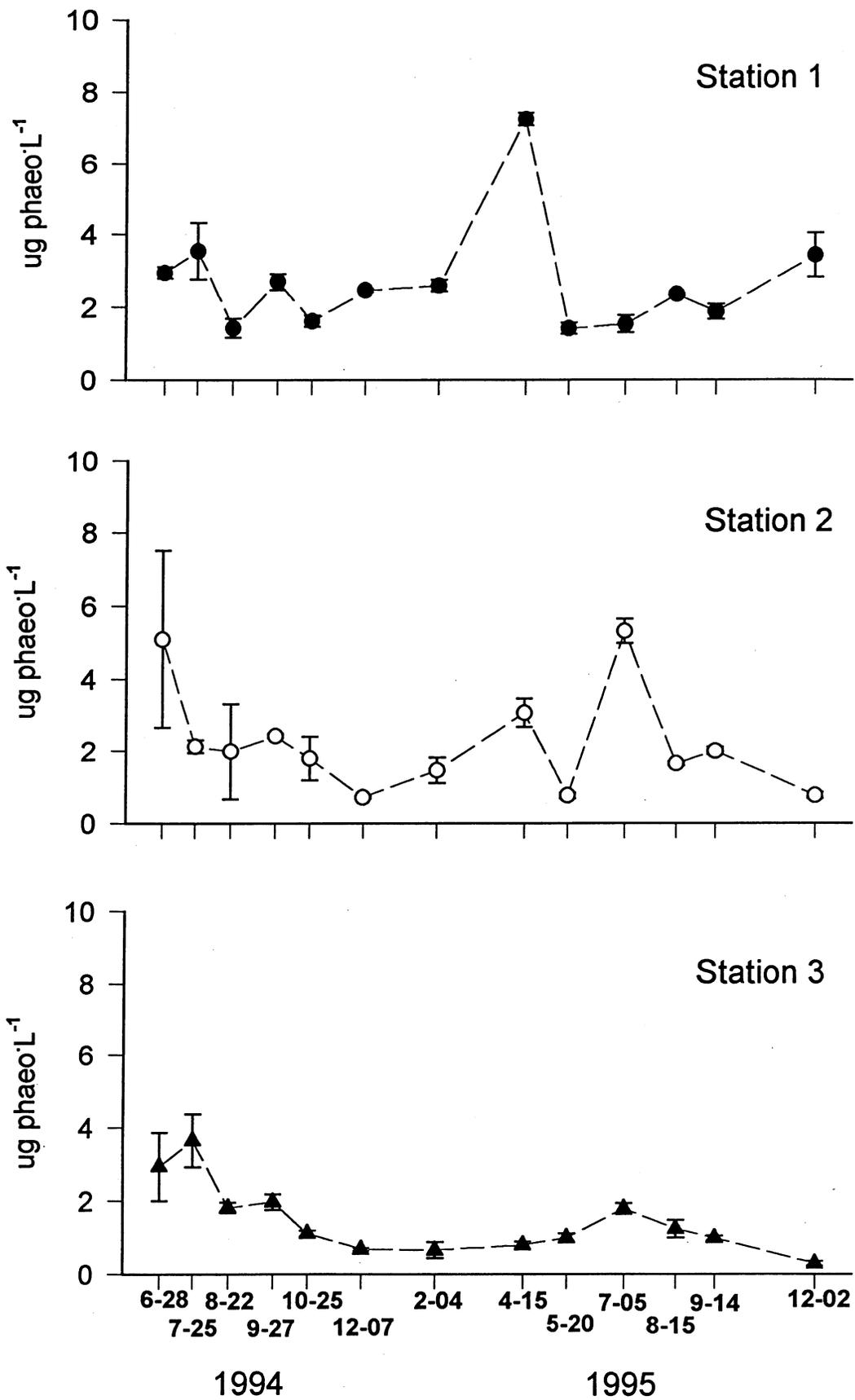


Figure 6. Phaeophytin concentrations in surface water samples from the three stations collected from June 1994 to December 1995.

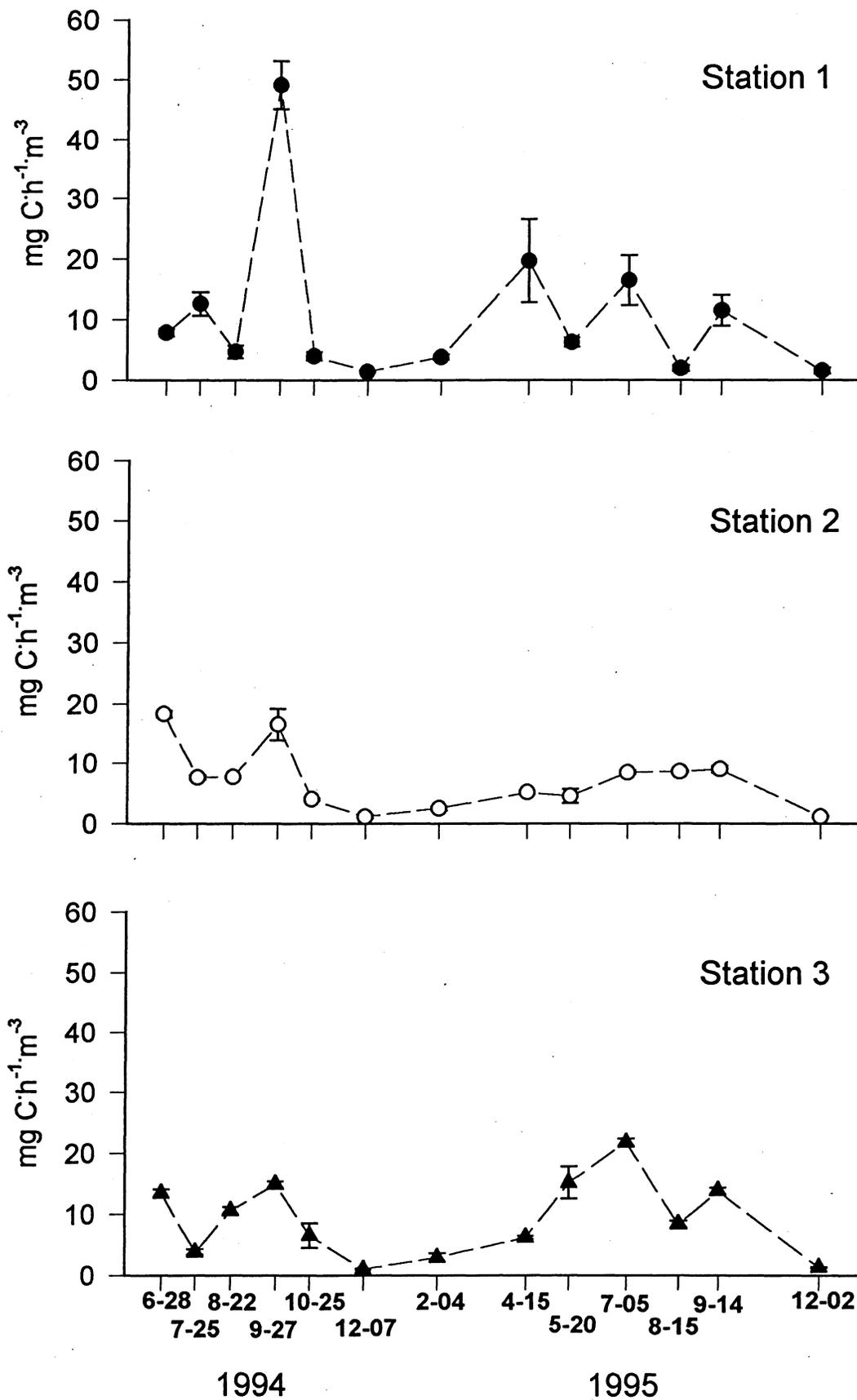


Figure 7. Primary productivity of surface water samples from the three stations from June 1994 to December 1995. Samples were incubated at an irradiance of $437 \pm 11 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; rates are expressed on a volumetric basis.

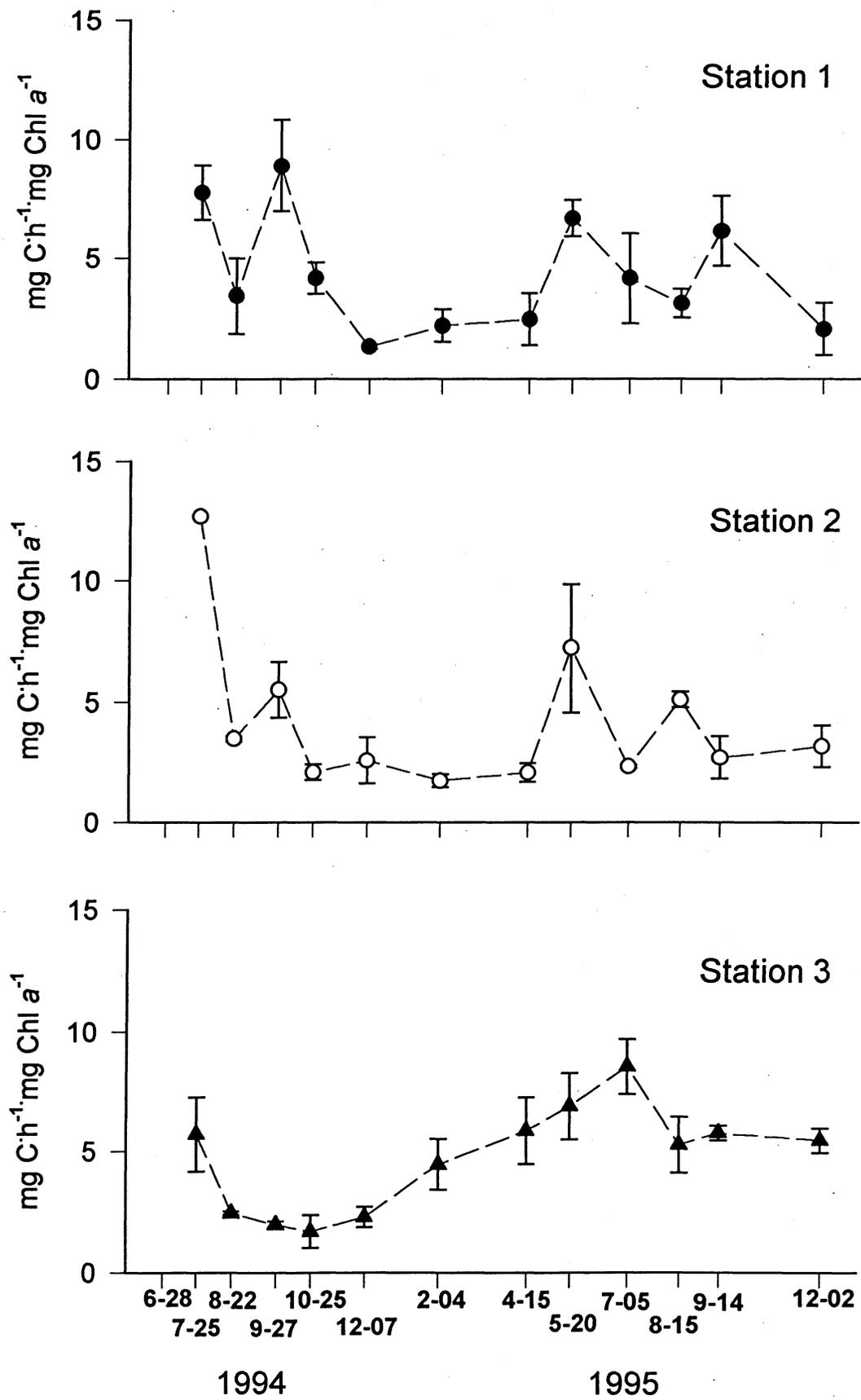


Figure 8. Primary productivity of surface water samples from the three stations from June 1994 to December 1995. Samples were incubated at an irradiance of $437 \pm 11 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; rates are expressed on a chlorophyll *a* basis.

June 28, 30 1994

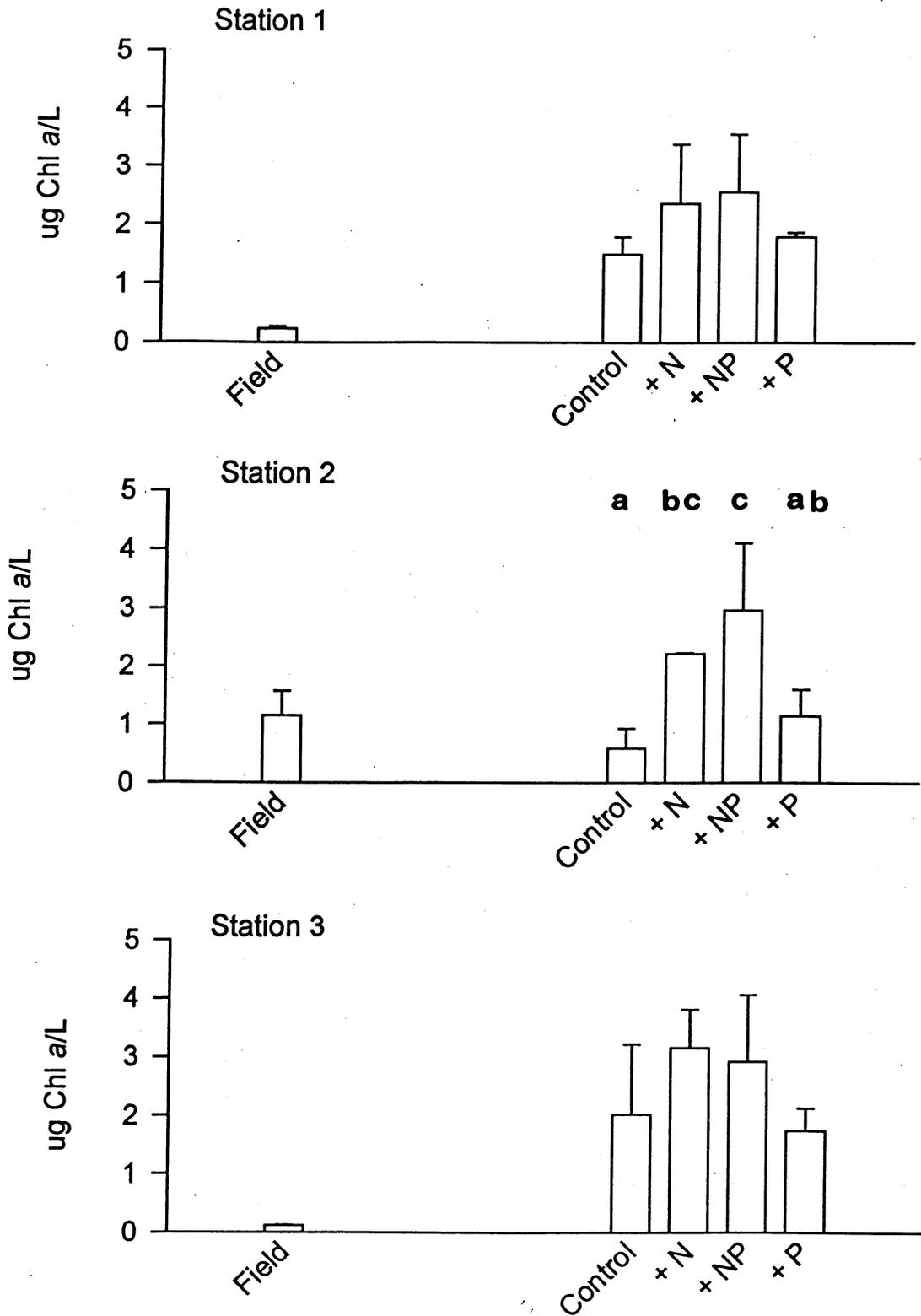


Figure 9. Chlorophyll *a* concentrations in June 1994. Field samples were collected on June 28; treatment effects were measured two days later on June 30.

July 25, 27 1994

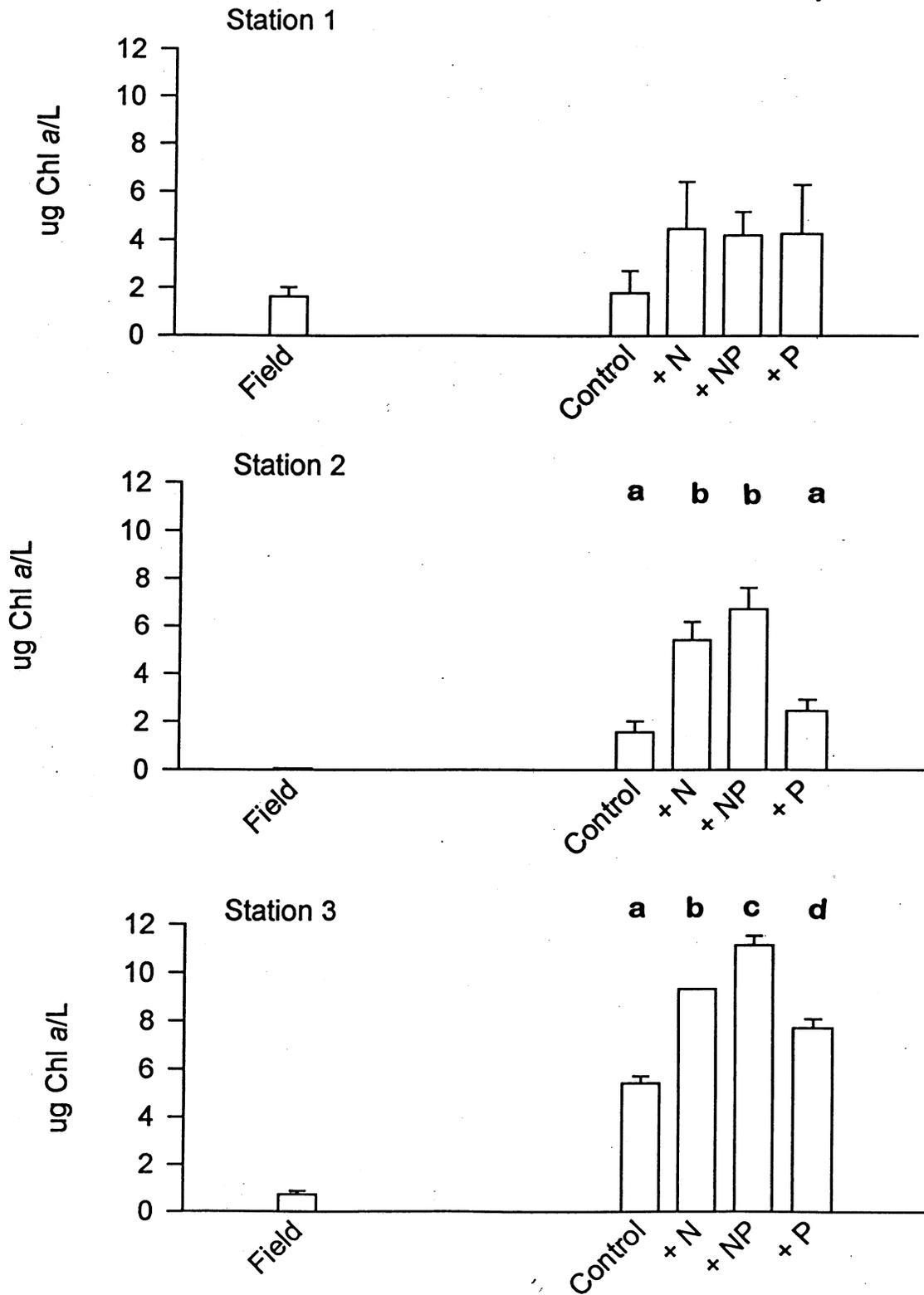


Figure 10. Chlorophyll *a* concentrations in July 1994. Field samples were collected on July 25; treatment effects were measured two days later on July 27.

August 22, 24 1994

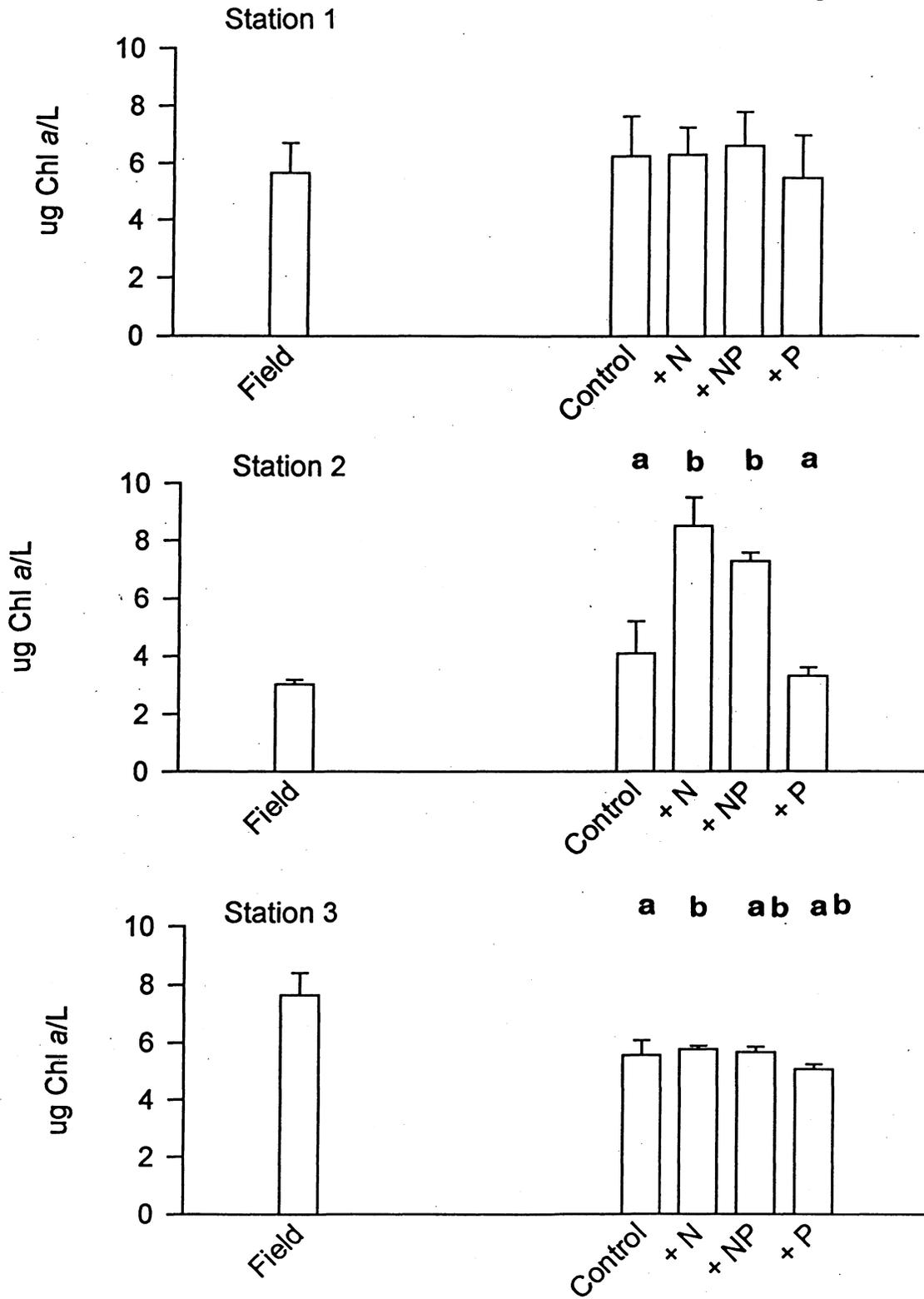


Figure 11. Chlorophyll *a* concentrations in August 1994. Field samples were collected on August 22; treatment effects were measured two days later on August 24.

October 25, 29 1994

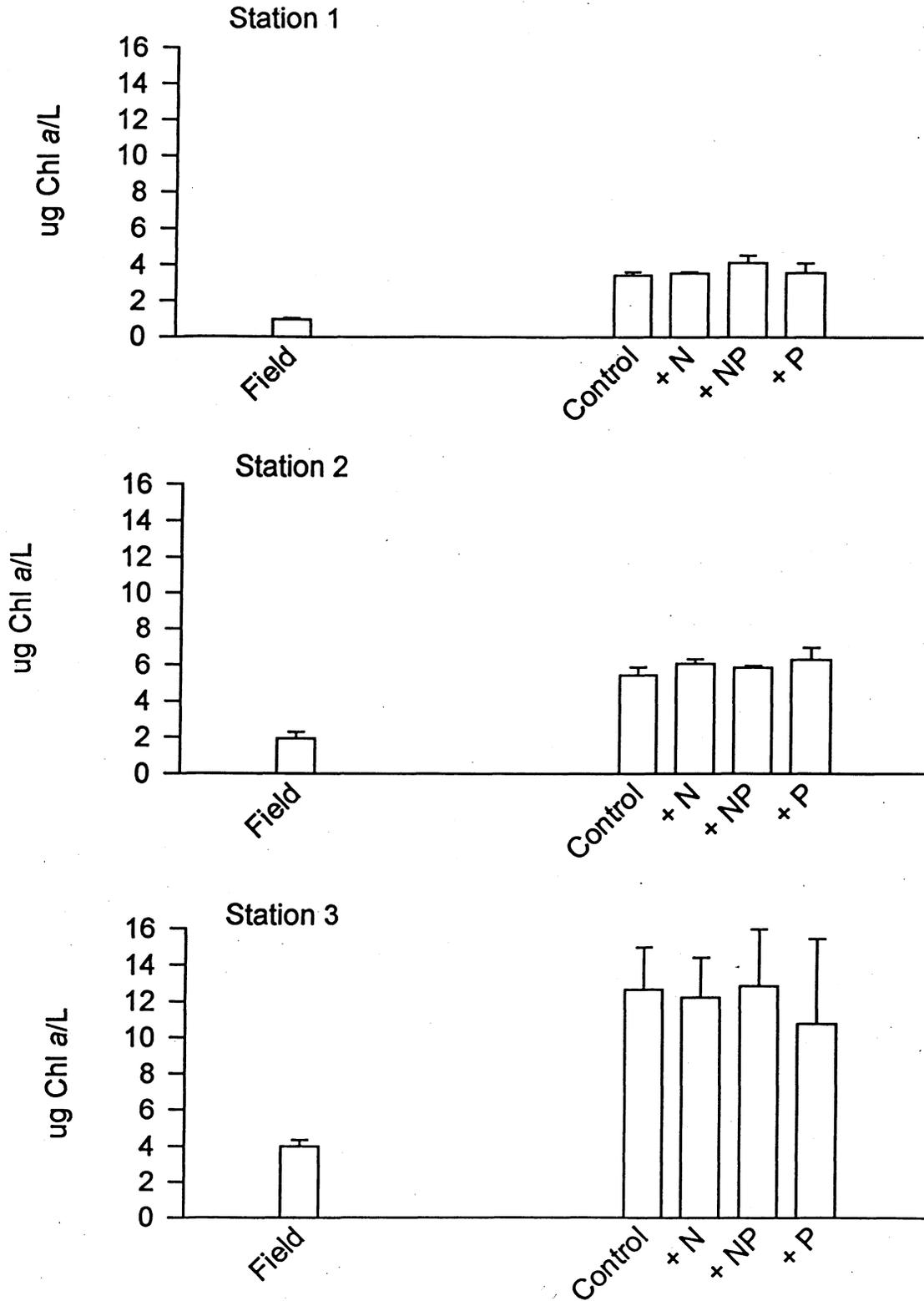


Figure 12. Chlorophyll *a* concentrations in October 1994. Field samples were collected on October 25; treatment effects were measured four days later on October 29.

December 7, 11 1994

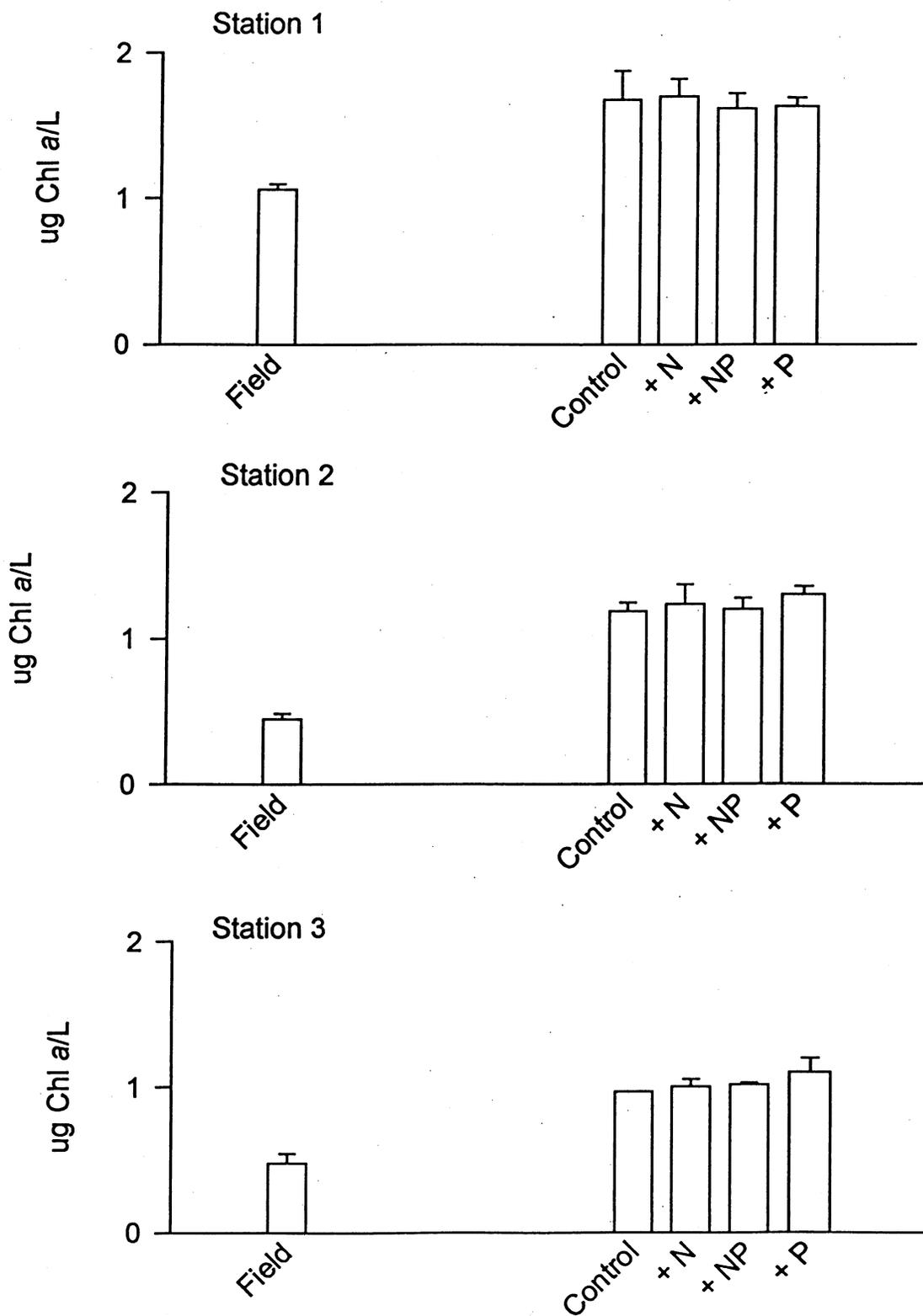


Figure 13. Chlorophyll *a* concentrations in December 1994. Field samples were collected on December 7; treatment effects were measured four days later on December 11.

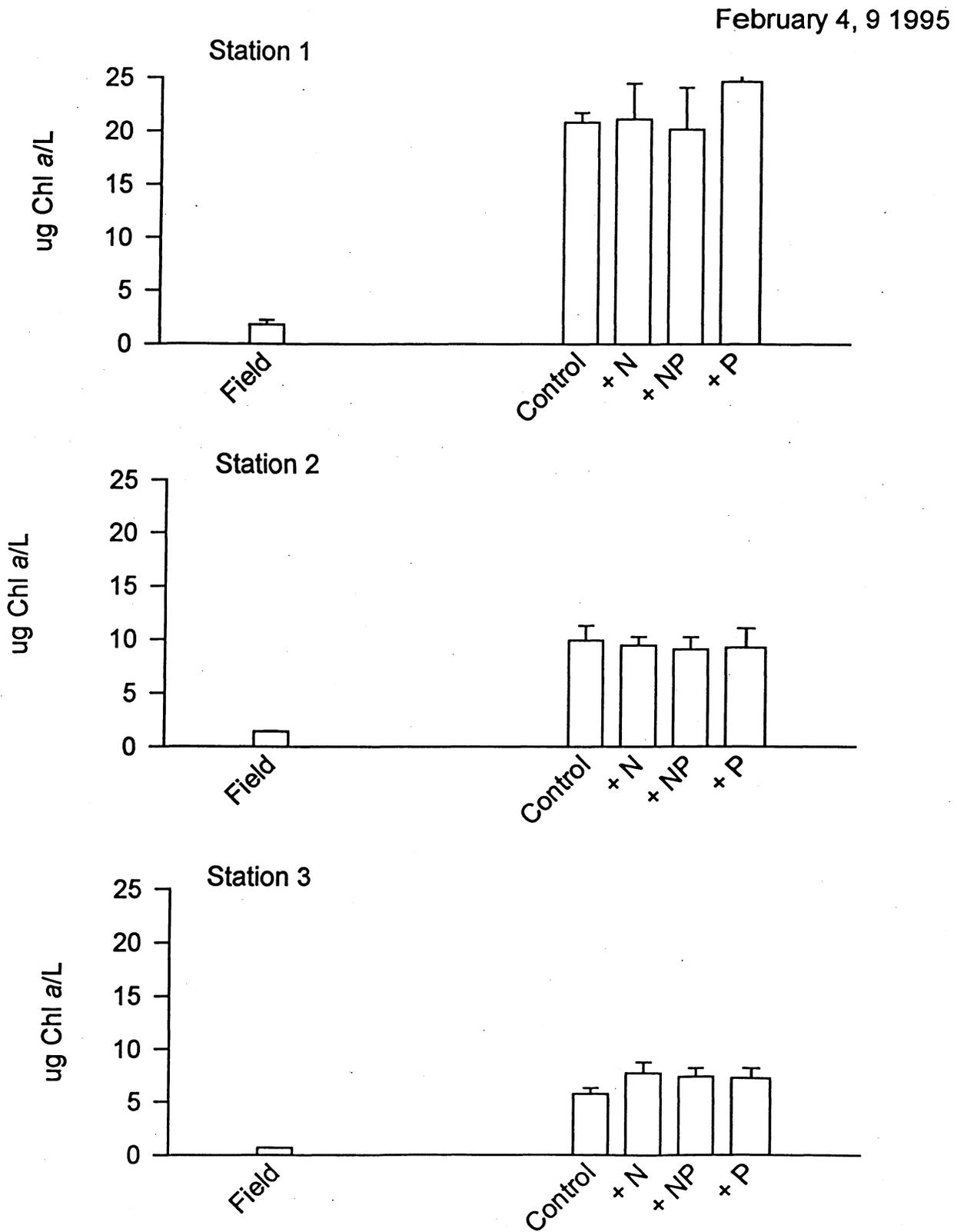


Figure 14. Chlorophyll *a* concentrations in February 1995. Field samples were collected on February 4; treatment effects were measured five days later on February 9.

April 15, 20 1995

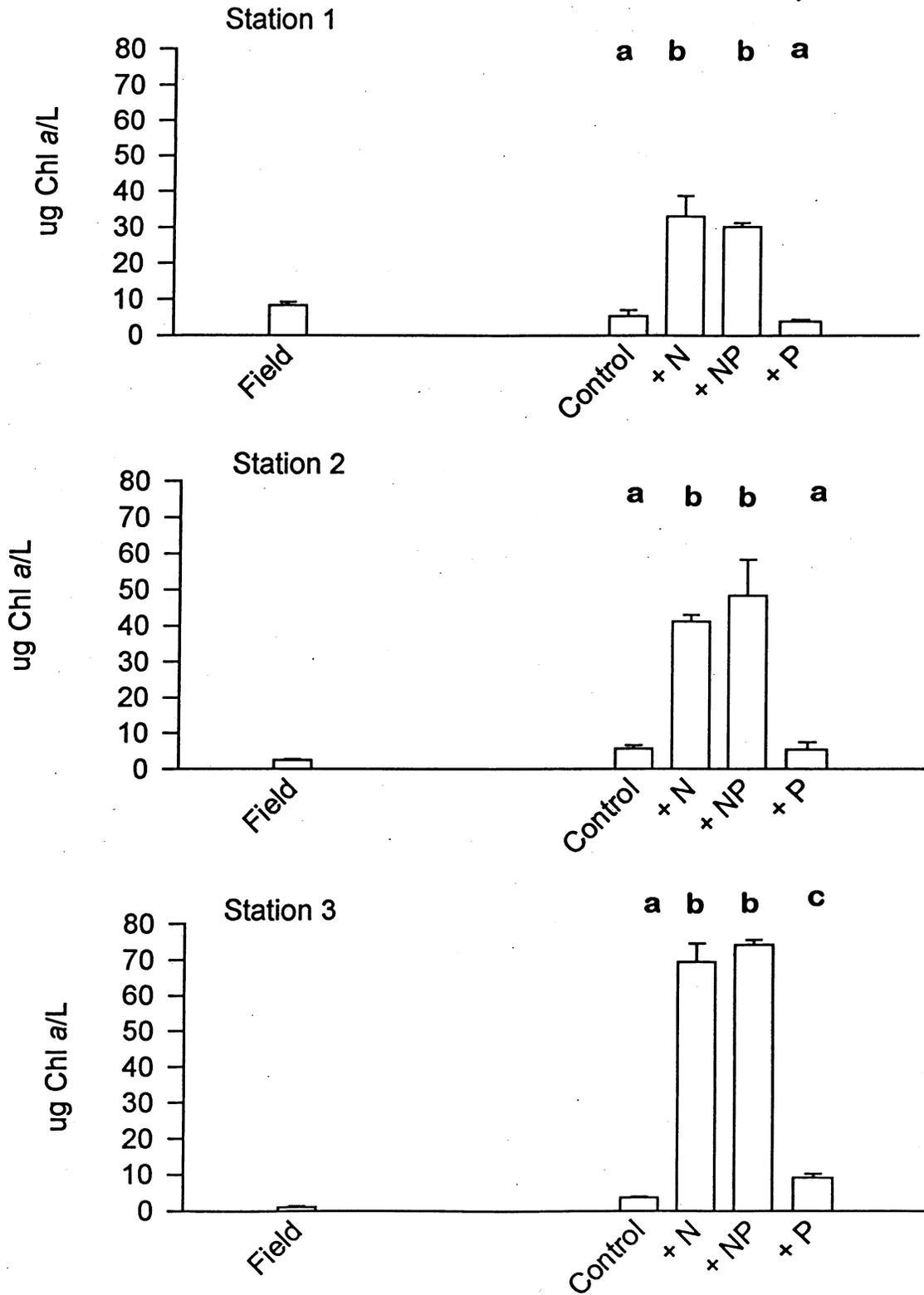


Figure 15. Chlorophyll *a* concentrations in April 1995. Field samples were collected on April 15; treatment effects were measured five days later on April 20.

May 20, 23 1995

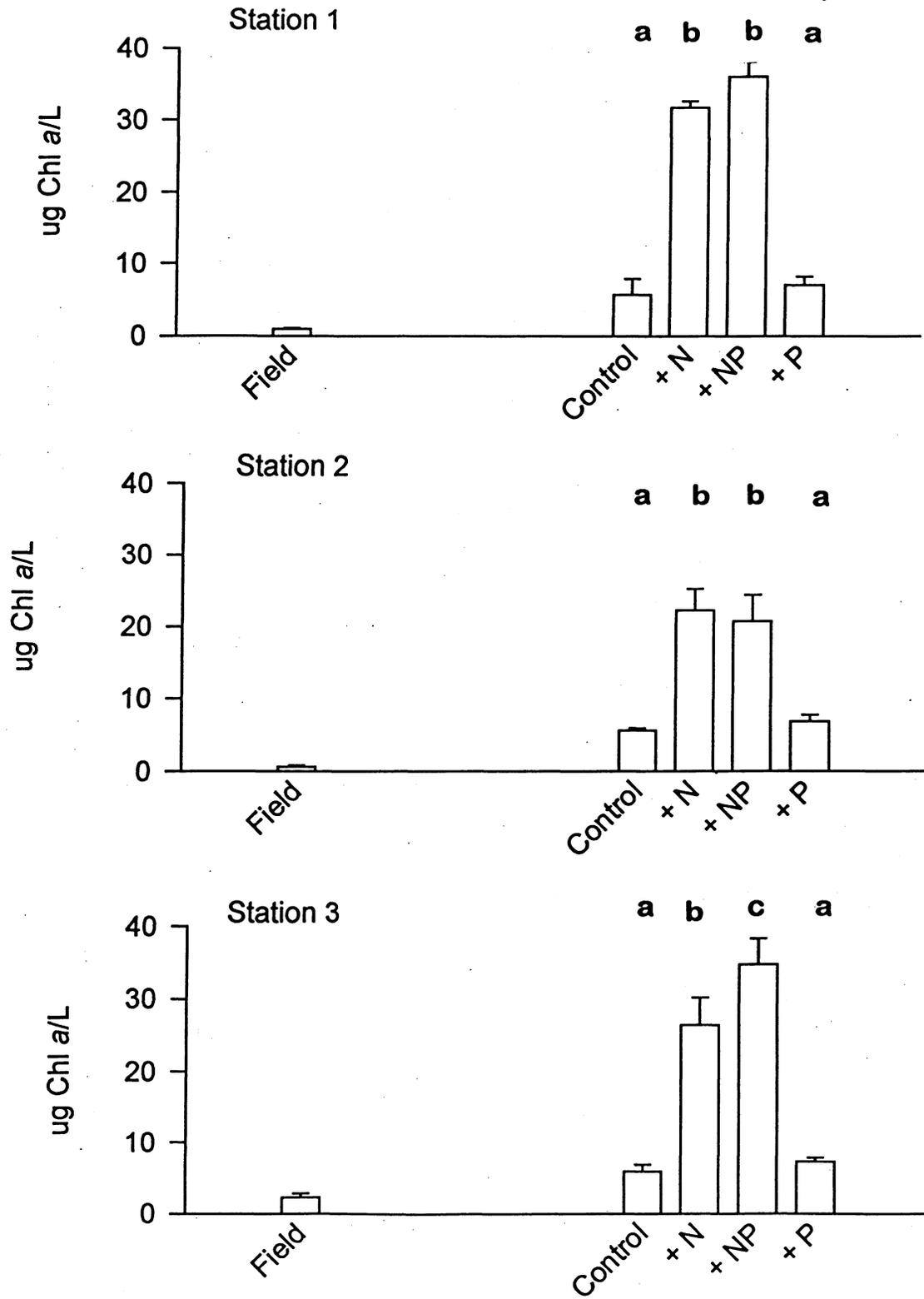


Figure 16. Chlorophyll *a* concentrations in May 1995. Field samples were collected on May 20; treatment effects were measured three days later on May 23.

July 5, 7 1995

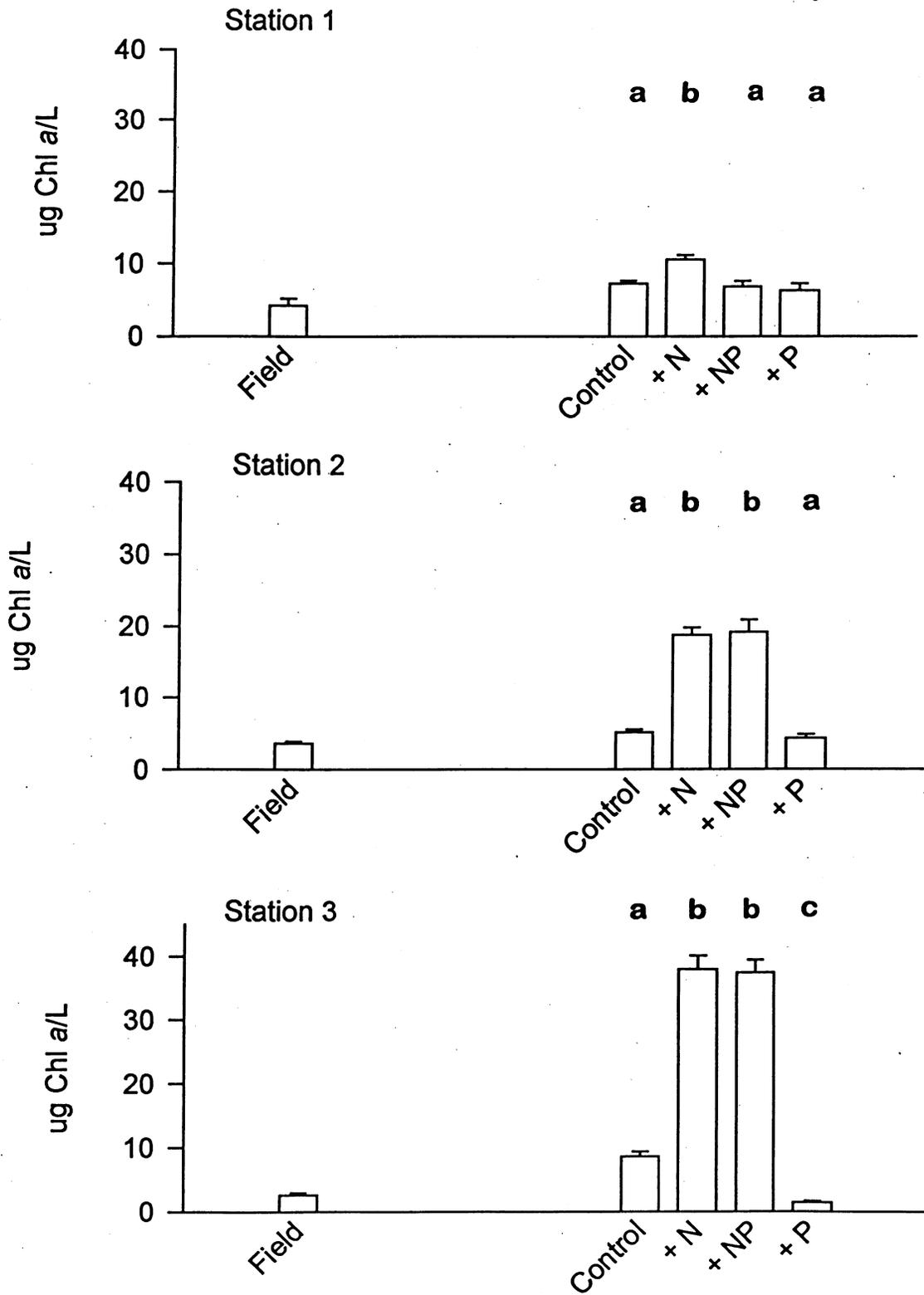


Figure 17. Chlorophyll *a* concentrations in July 1995. Field samples were collected on July 5; treatment effects were measured two days later on July 7.

August 15, 18 1995

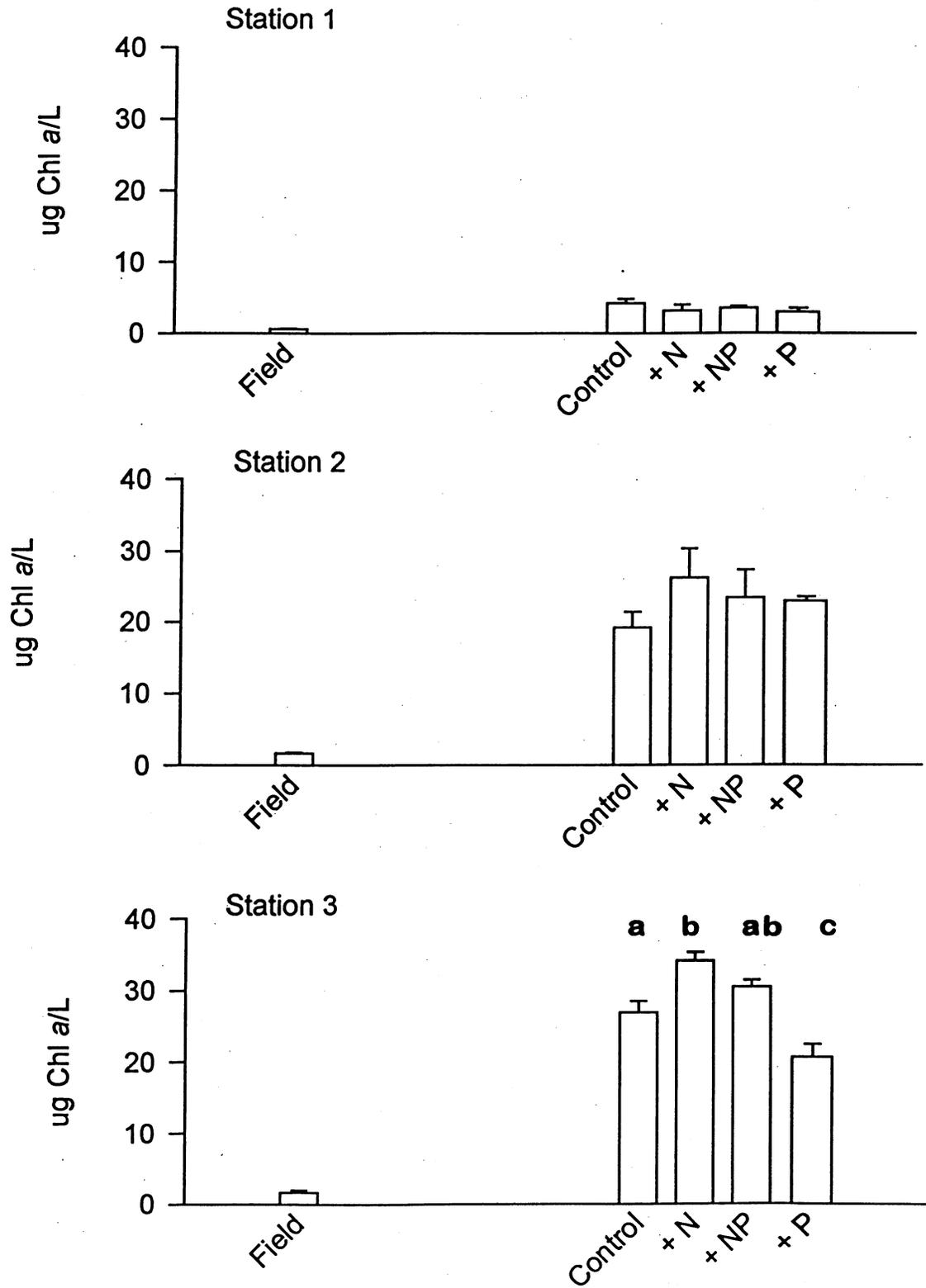


Figure 18. Chlorophyll *a* concentrations in August 1995. Field samples were collected on August 15; treatment effects were measured three days later on August 18.

September 14, 16 1995

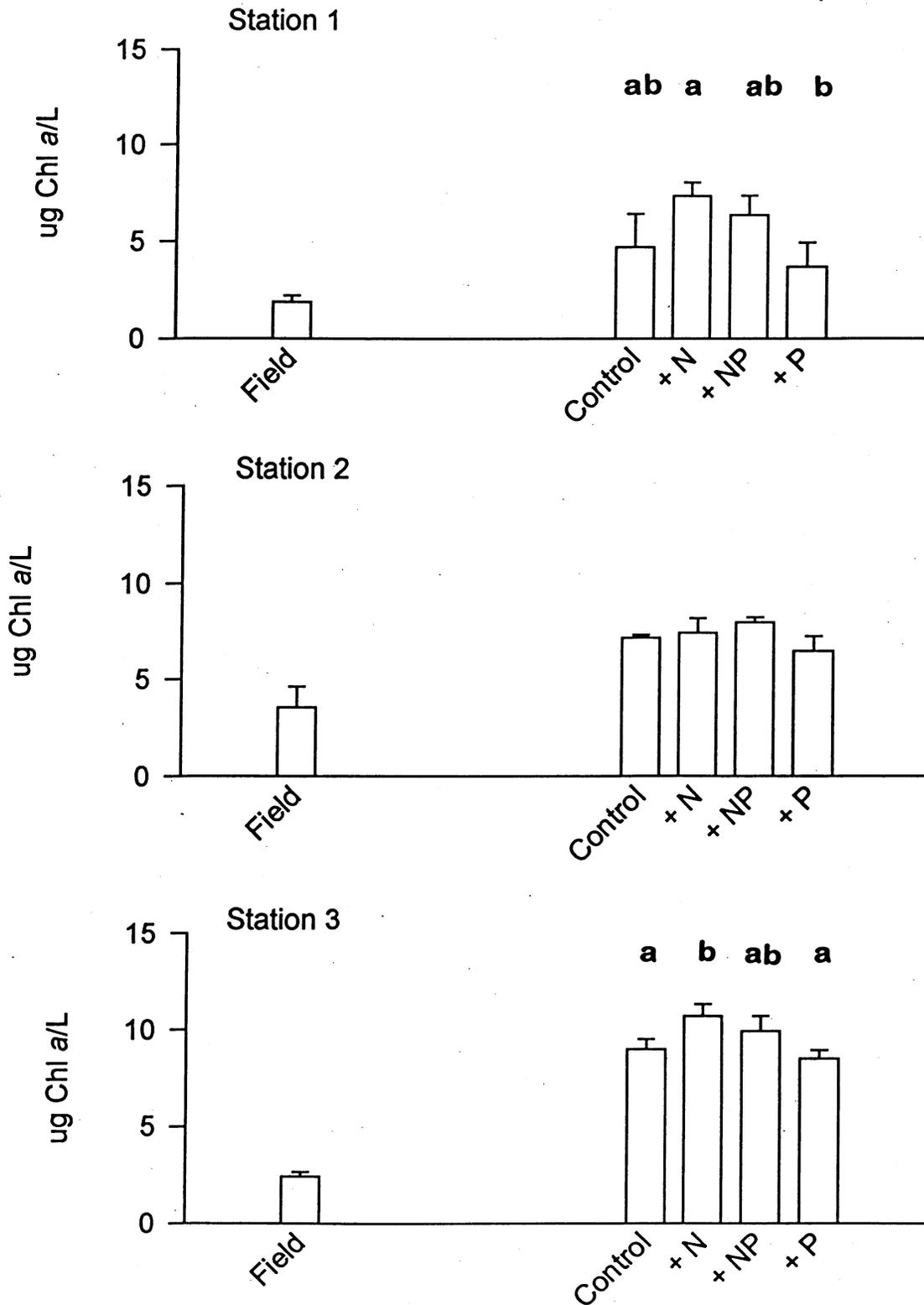


Figure 19. Chlorophyll *a* concentrations in September 1995. Field samples were collected on September 14; treatment effects were measured two days later on September 16.

December 2, 7 1995

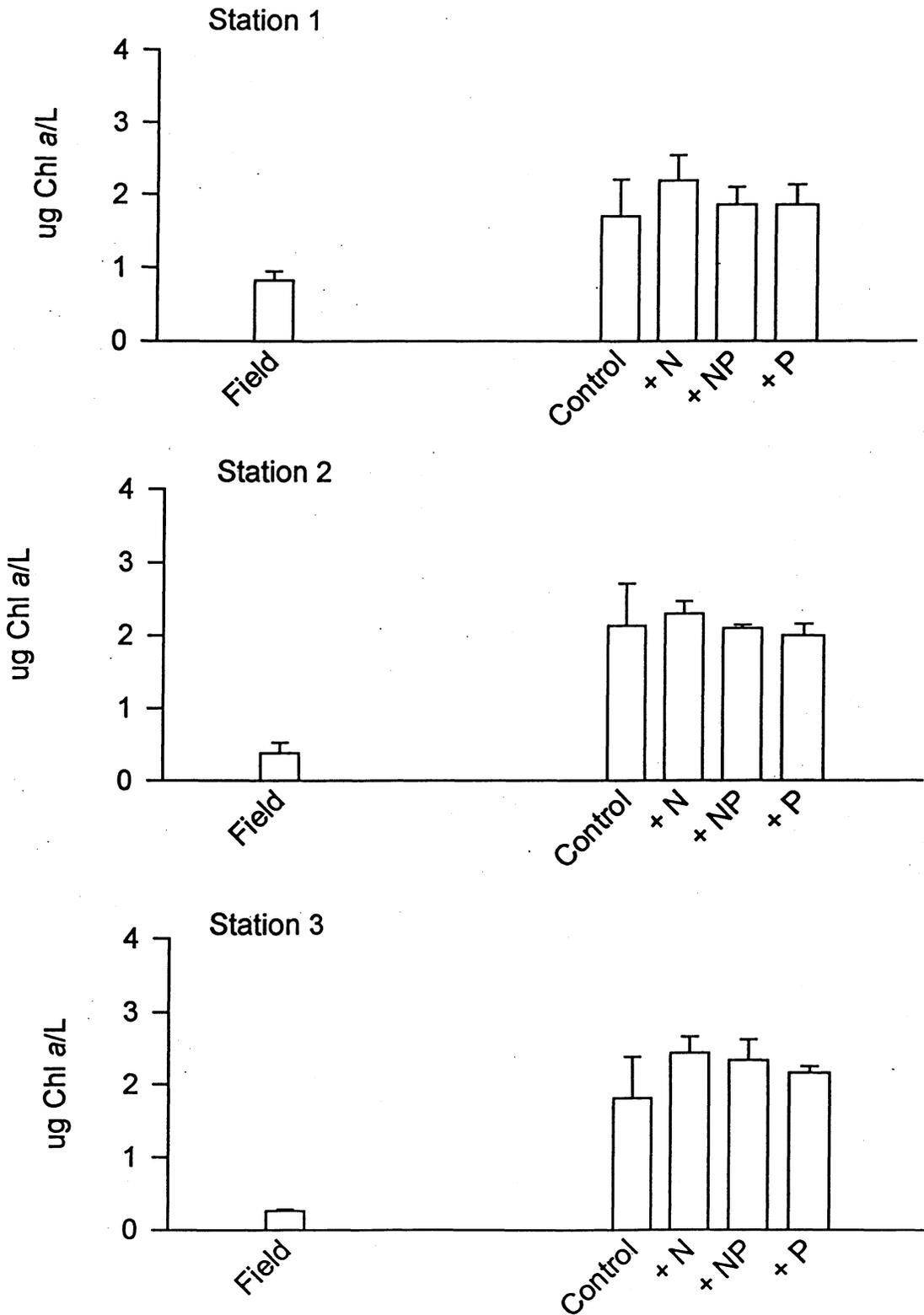


Figure 20. Chlorophyll *a* concentrations in December 1995. Field samples were collected on December 2; treatment effects were measured five days later on December 7.

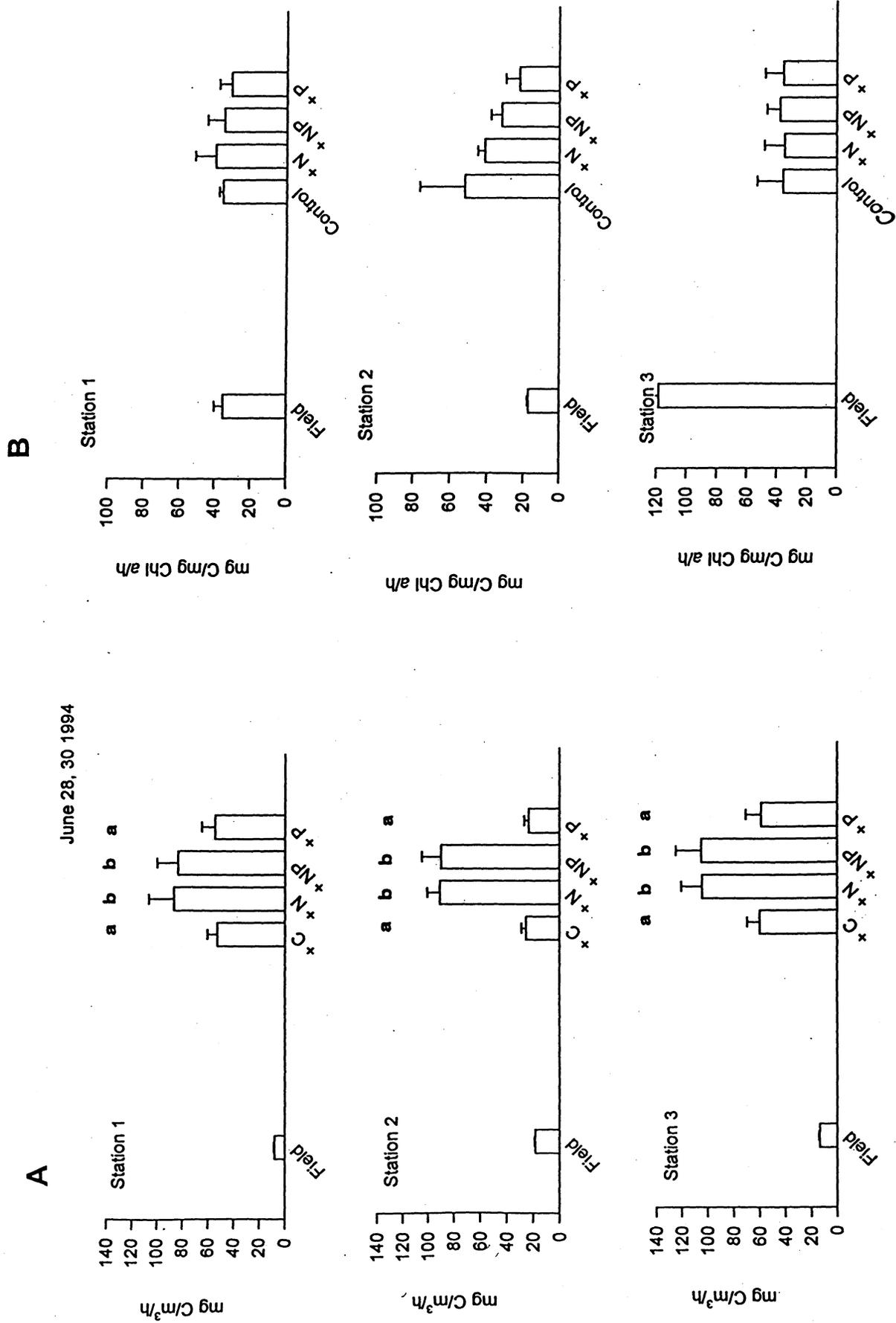


Figure 21. Primary production in June 1994. Field samples were collected on June 28; treatment effects were measured two days later on June 30.
 A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.

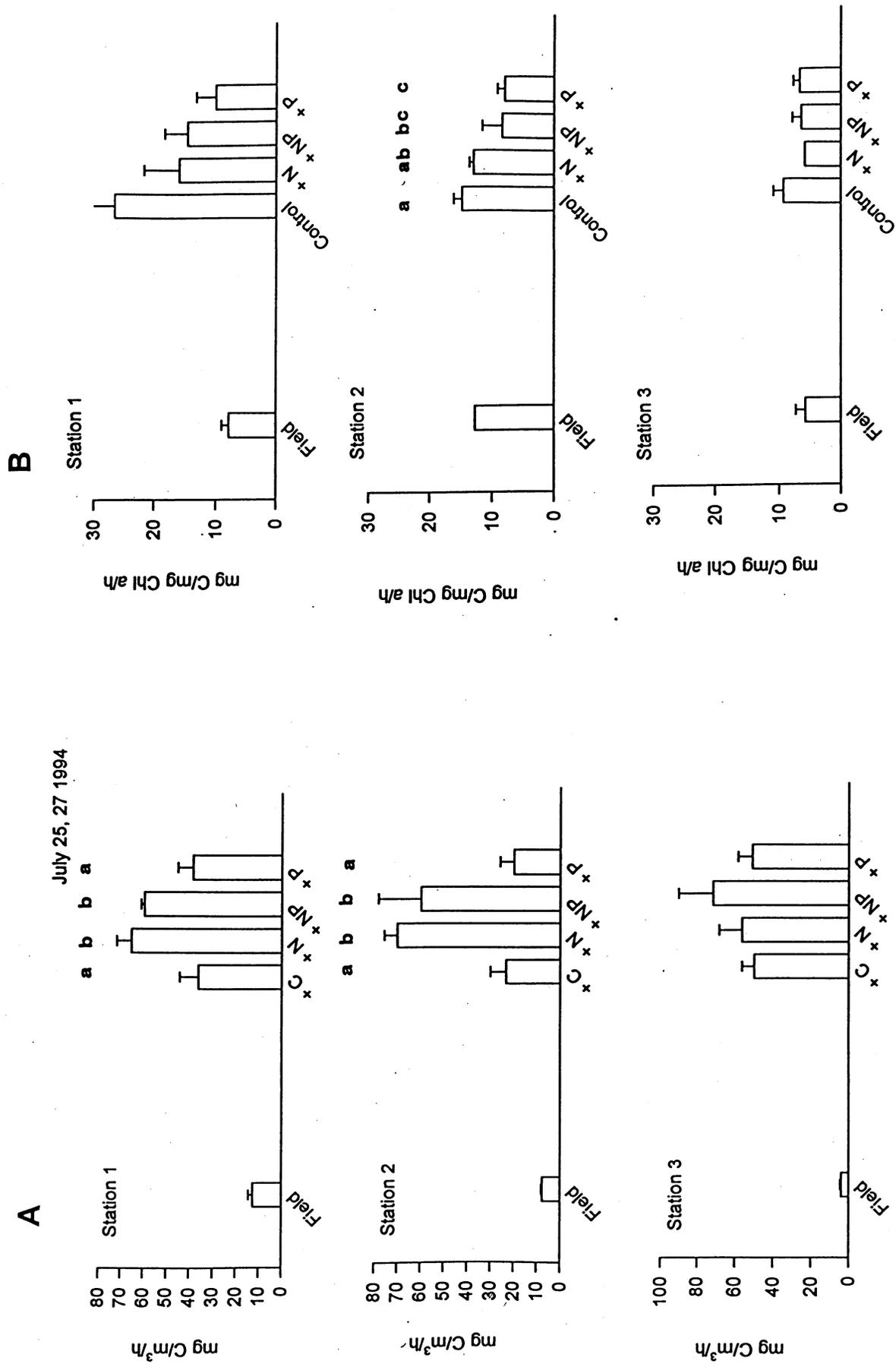
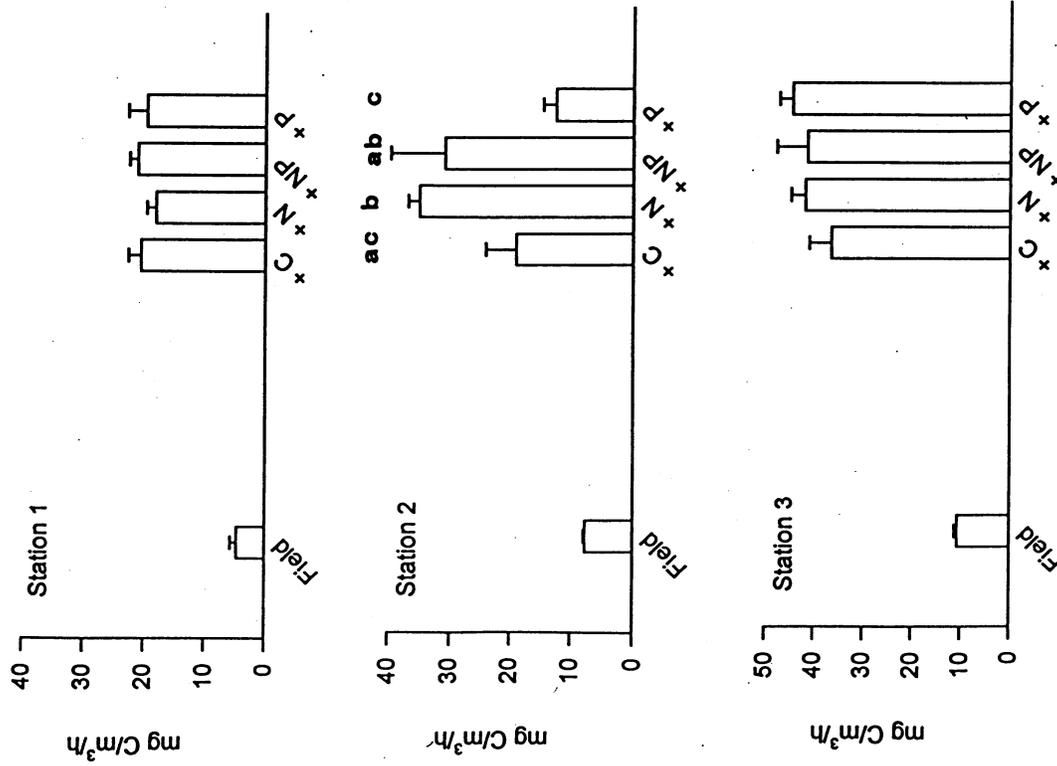


Figure 22. Primary production in July 1994. Field samples were collected on July 25; treatment effects were measured two days later on July 27. A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.

A

August 22, 24 1994



B

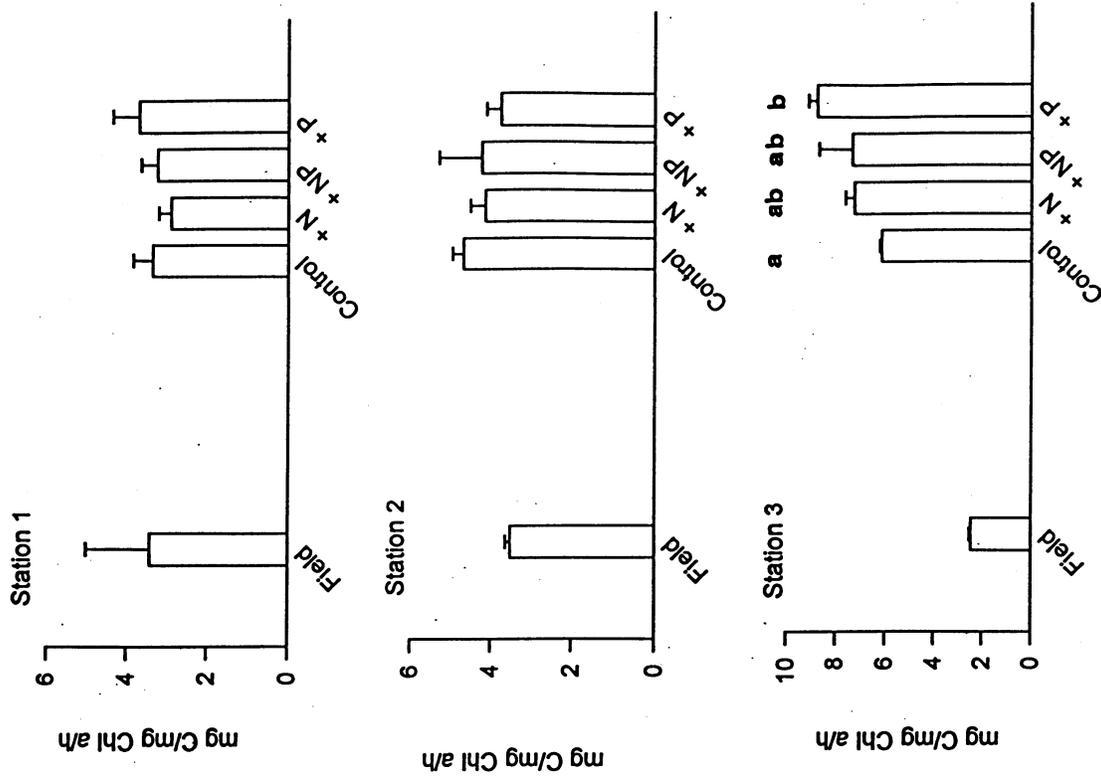


Figure 23. Primary production in August 1994. Field samples were collected on August 22; treatment effects were measured two days later on August 24.
A) rates expressed on a volume basis; B) rates expressed on a chl α basis.

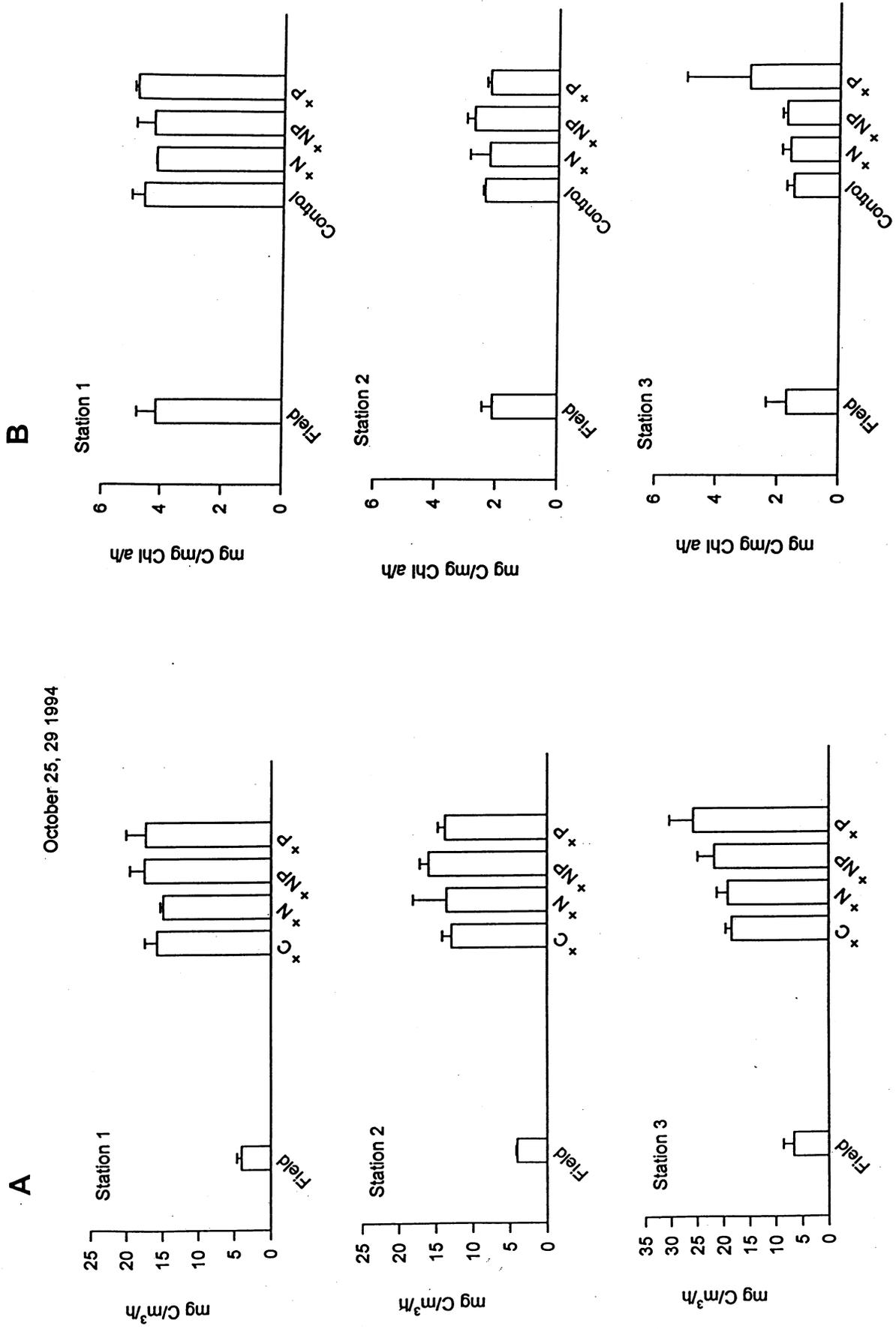


Figure 24. Primary production in October 1994. Field samples were collected on October 25; treatment effects were measured four days later on October 29. A) rates expressed on a volume basis; B) rates expressed on a chl α basis.

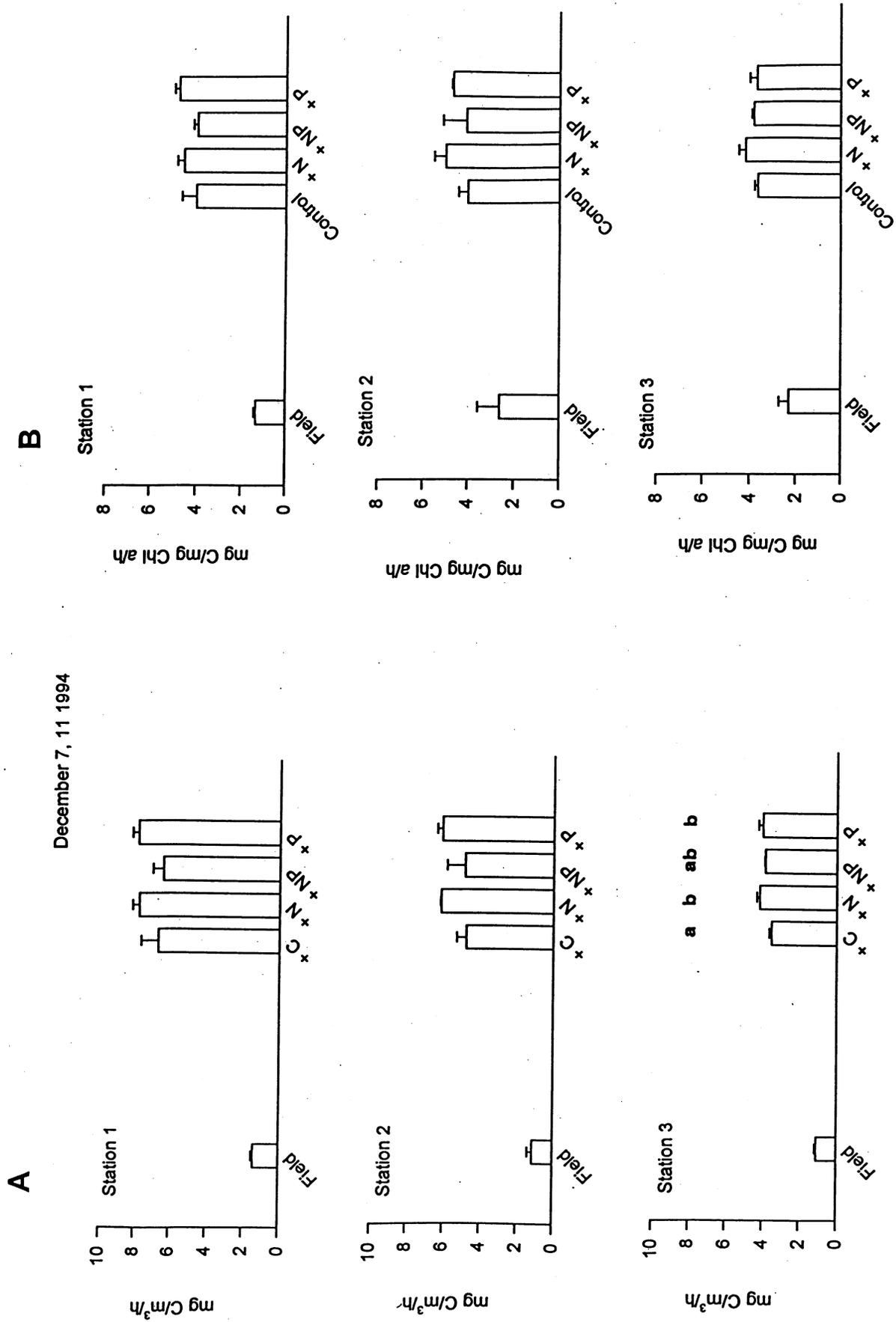


Figure 25. Primary production in December 1994. Field samples were collected on December 7; treatment effects were measured four days later on December 11. A) rates expressed on a volume basis; B) rates expressed on a chl α basis.

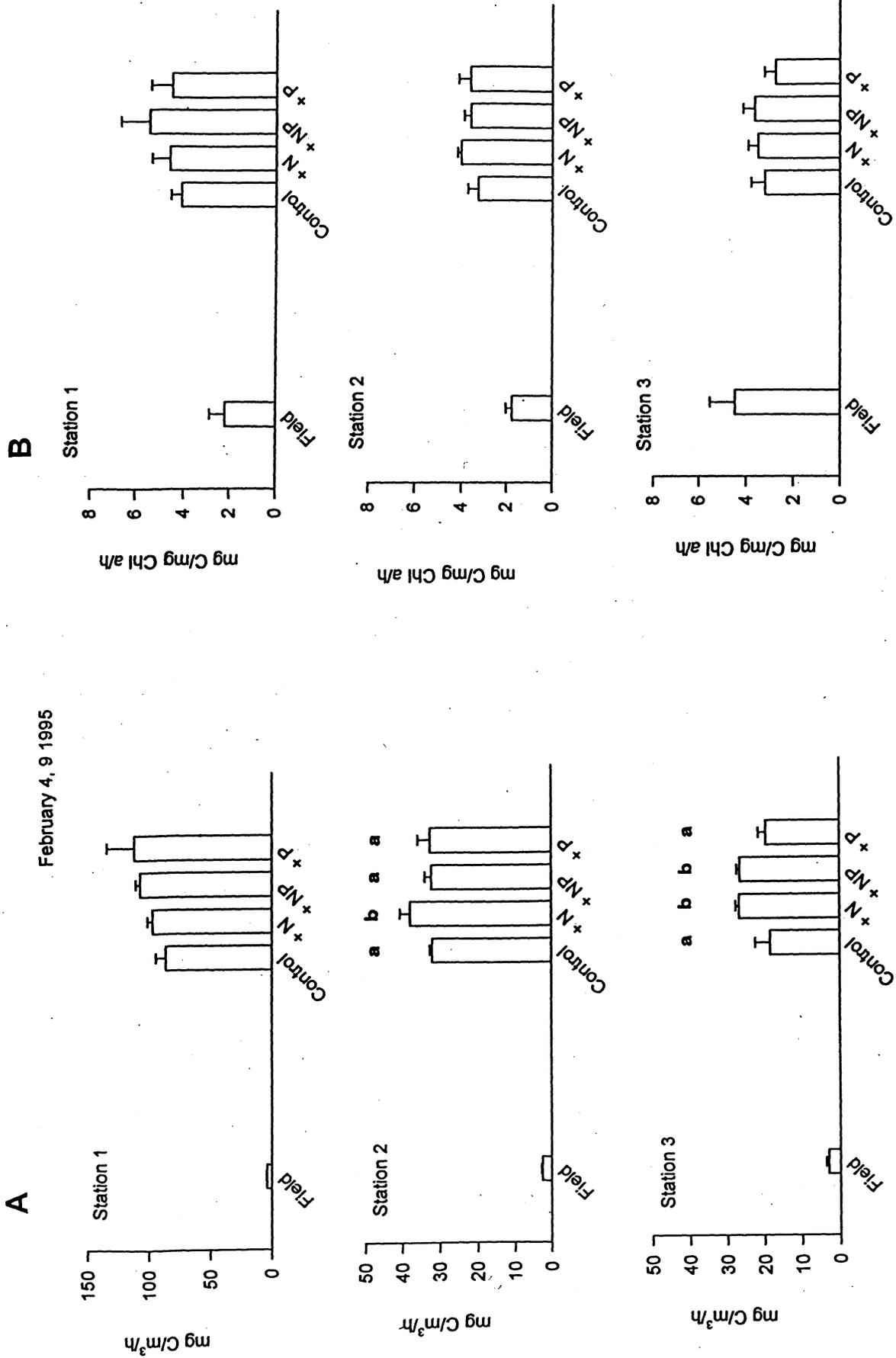


Figure 26. Primary production in February 1995. Field samples were collected on February 4; treatment effects were measured five days later on February 9. A) rates expressed on a volume basis; B) rates expressed on a chl α basis.

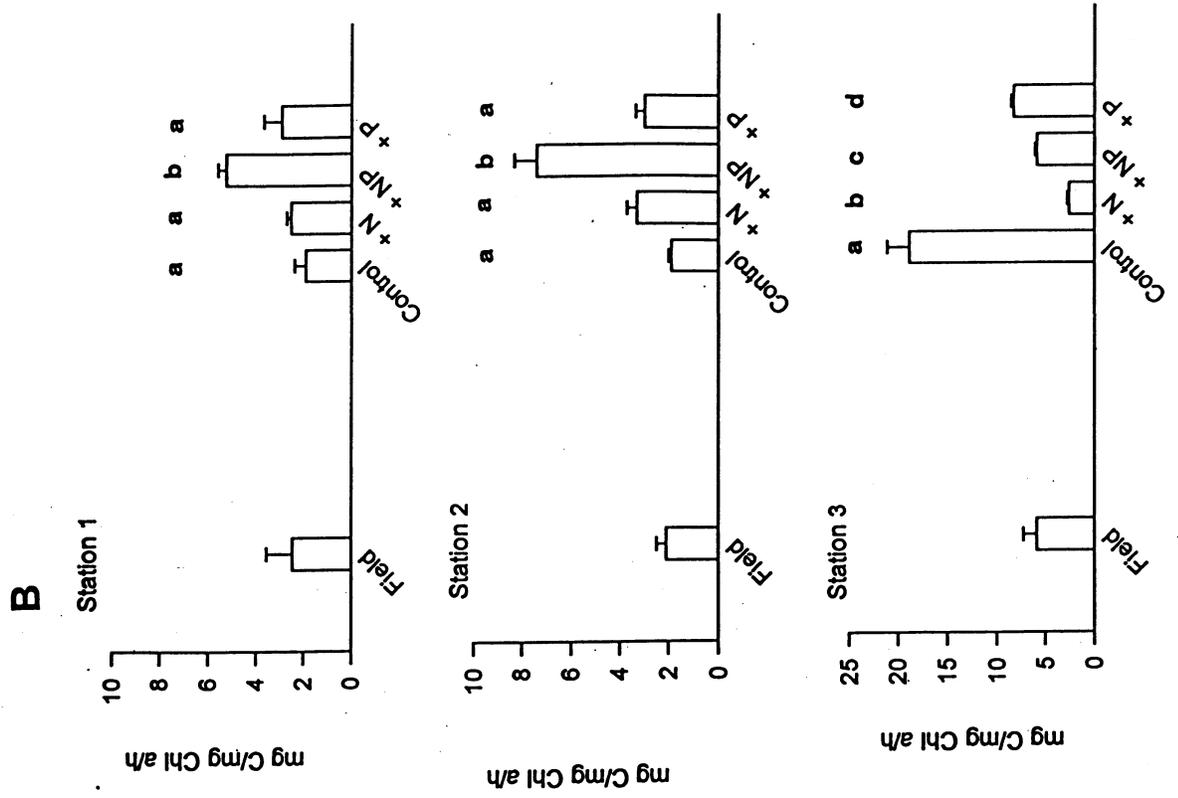
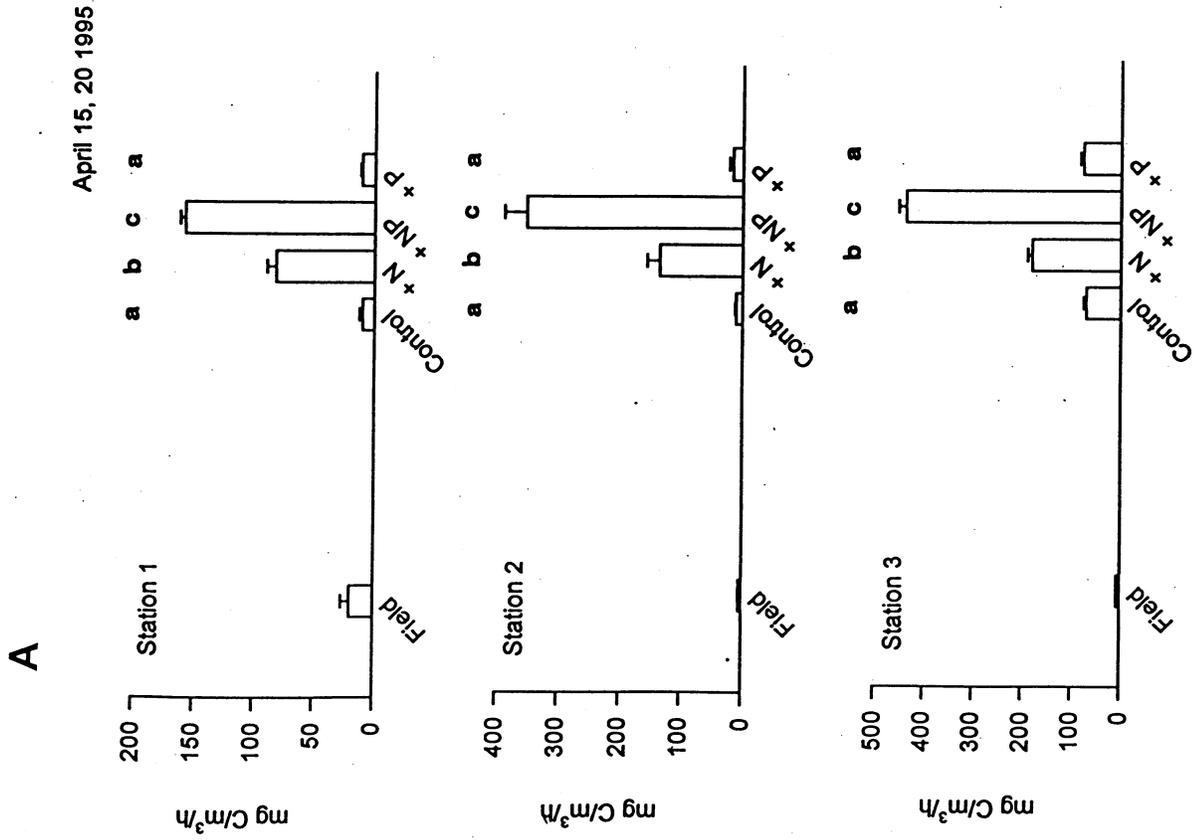


Figure 27. Primary production in April 1995. Field samples were collected on April 15; treatment effects were measured five days later on April 20. A) rates expressed on a volume basis; B) rates expressed on a chl α basis.

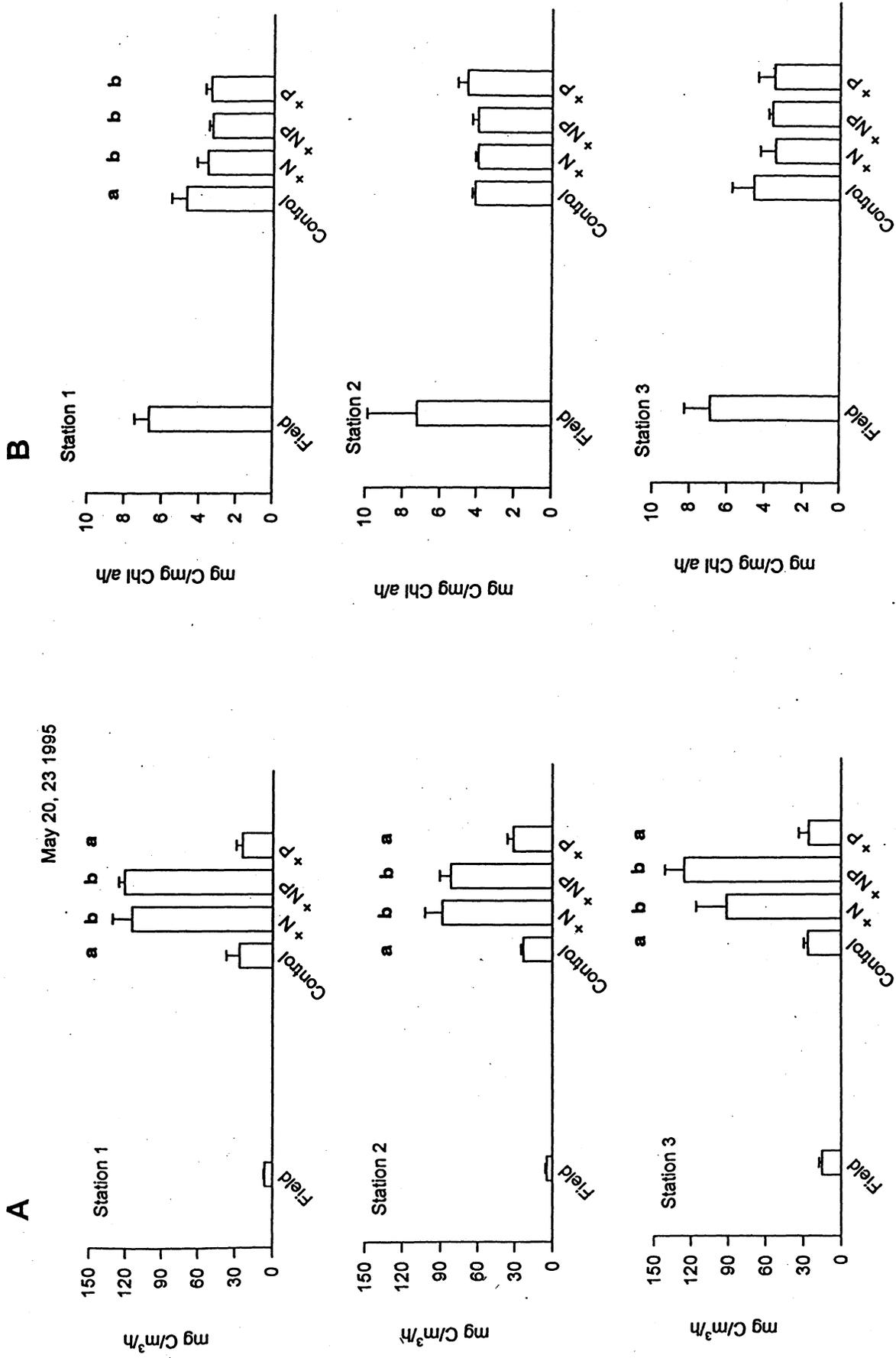


Figure 28. Primary production in May 1995. Field samples were collected on May 20; treatment effects were measured three days later on May 23.
 A) rates expressed on a volume basis; B) rates expressed on a chl *a* basis.

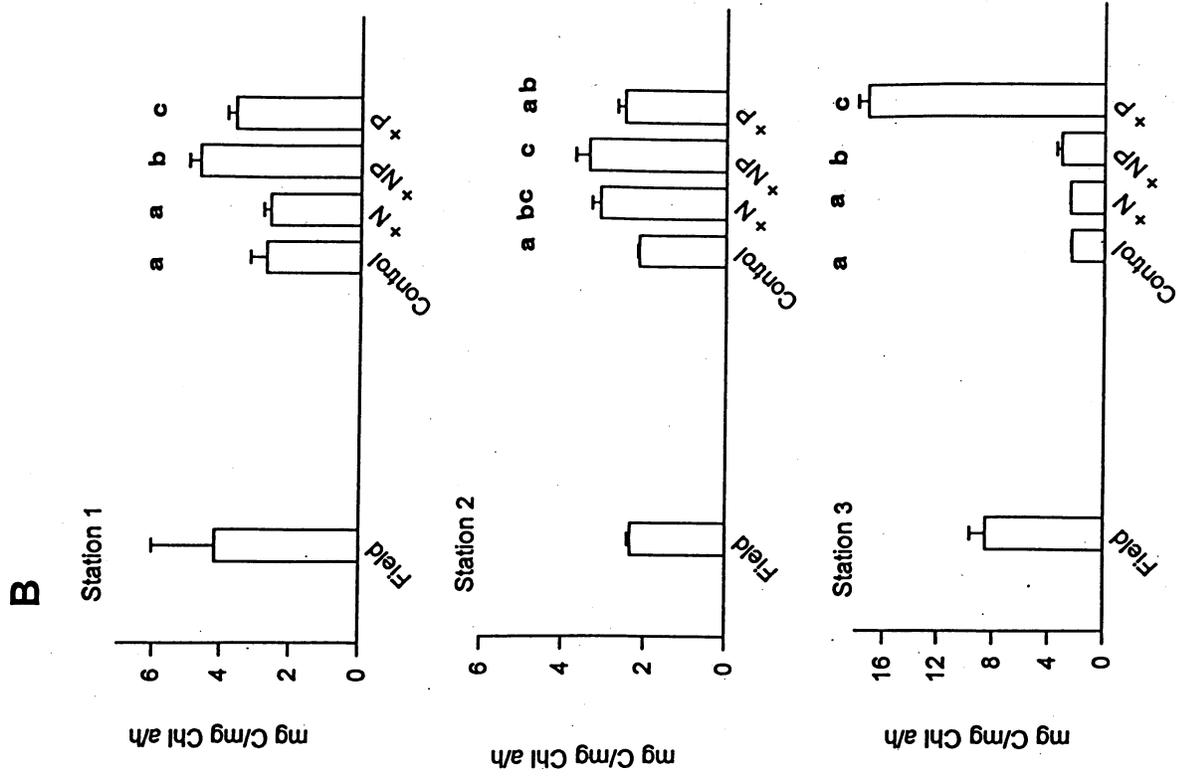
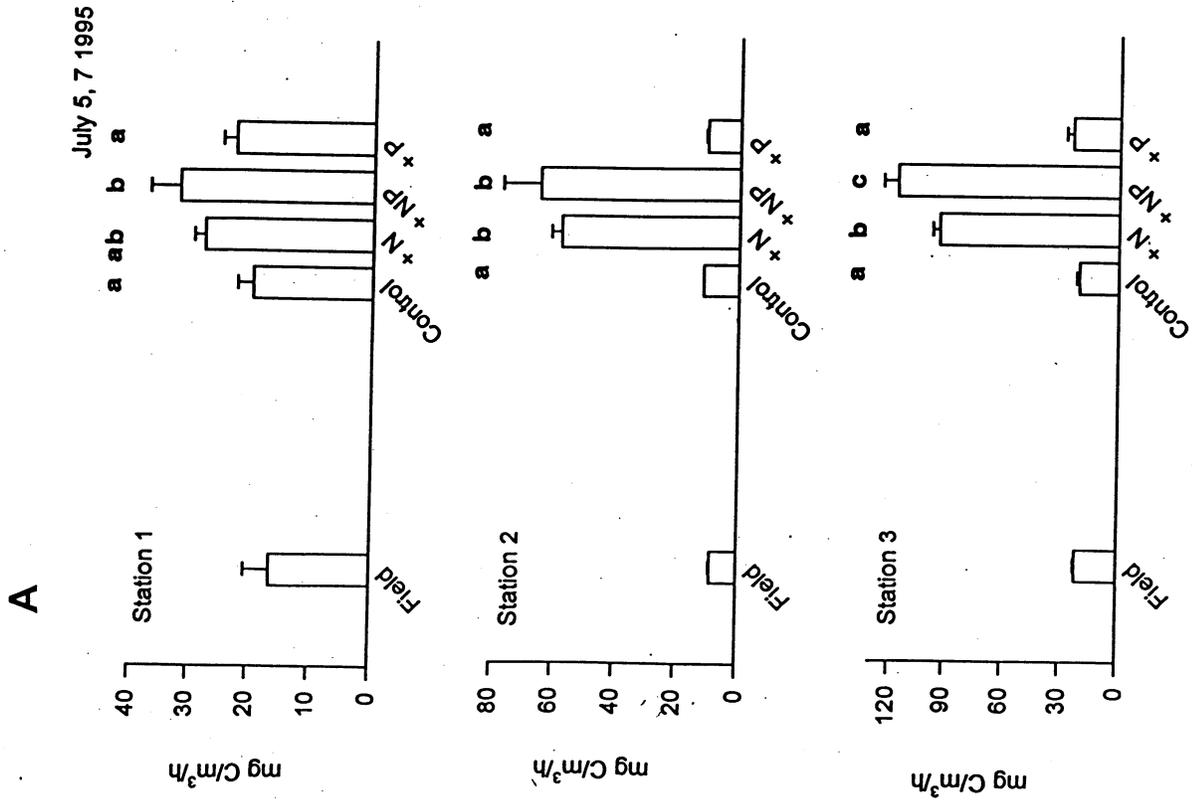


Figure 29. Primary production in July 1995. Field samples were collected on July 5; treatment effects were measured two days later on July 7.
 A) rates expressed on a volume basis; B) rates expressed on a chl α basis.

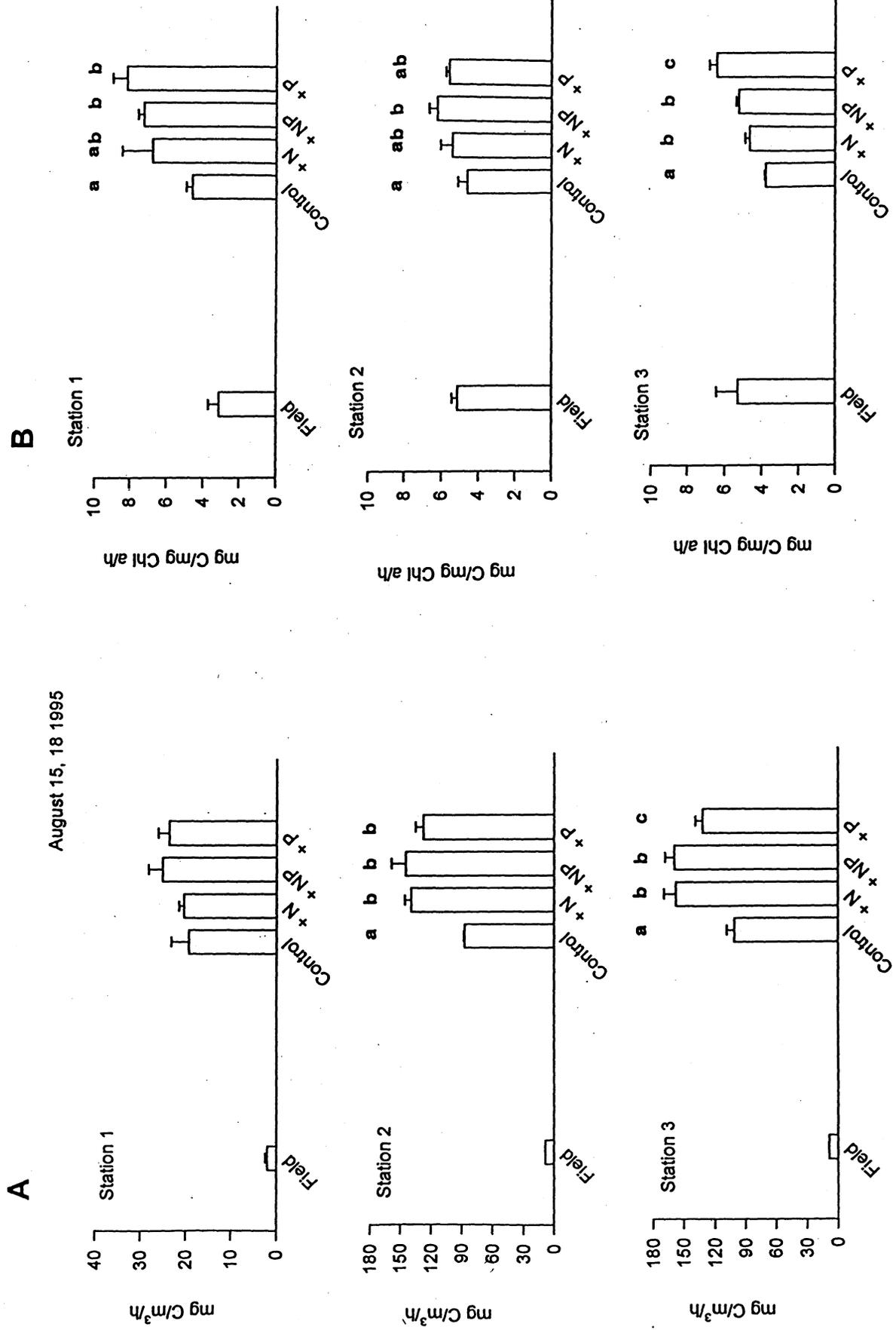


Figure 30. Primary production in August 1995. Field samples were collected on August 15; treatment effects were measured three days later on August 18. A) rates expressed on a volume basis; B) rates expressed on a chl α basis.

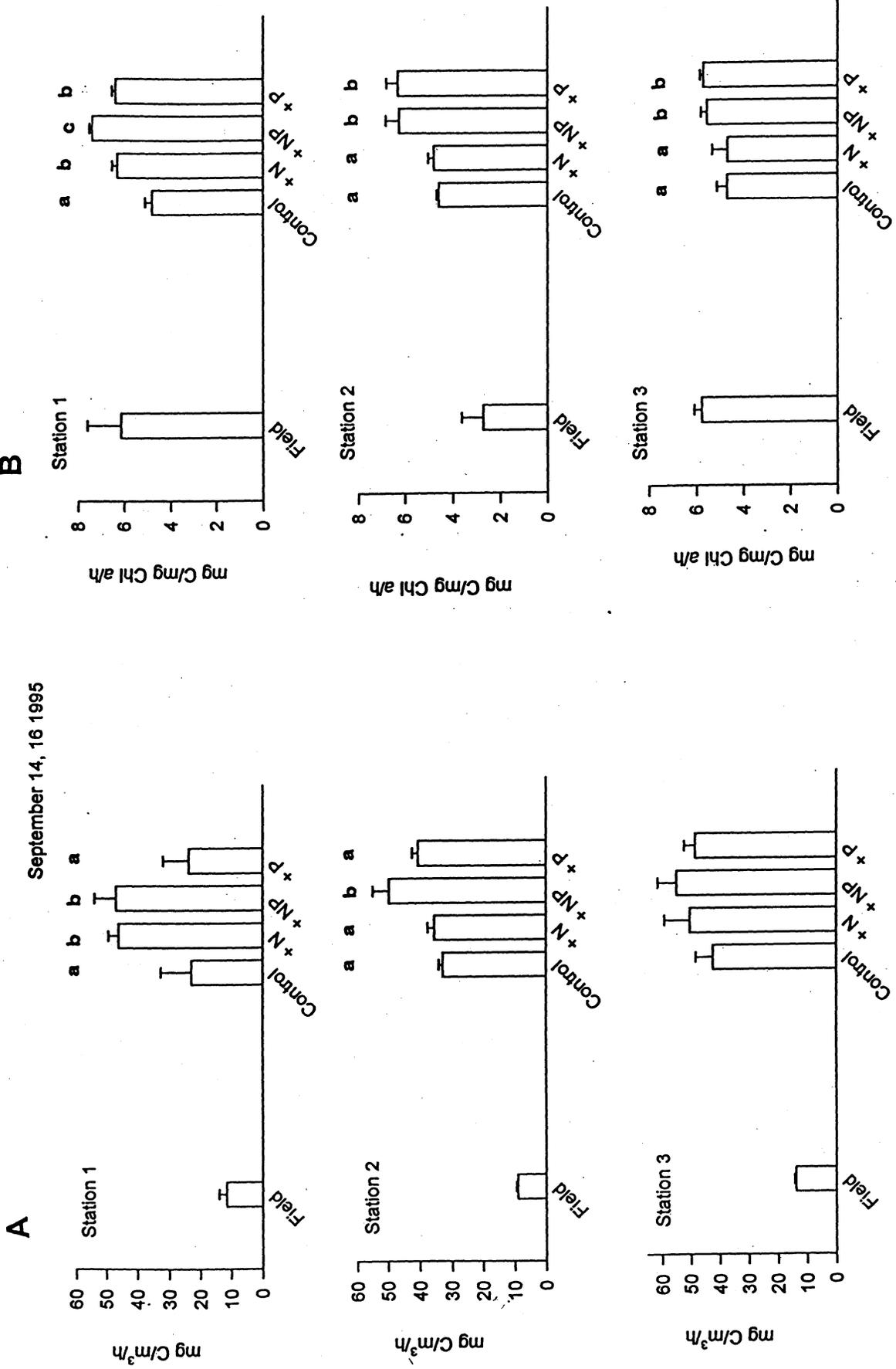
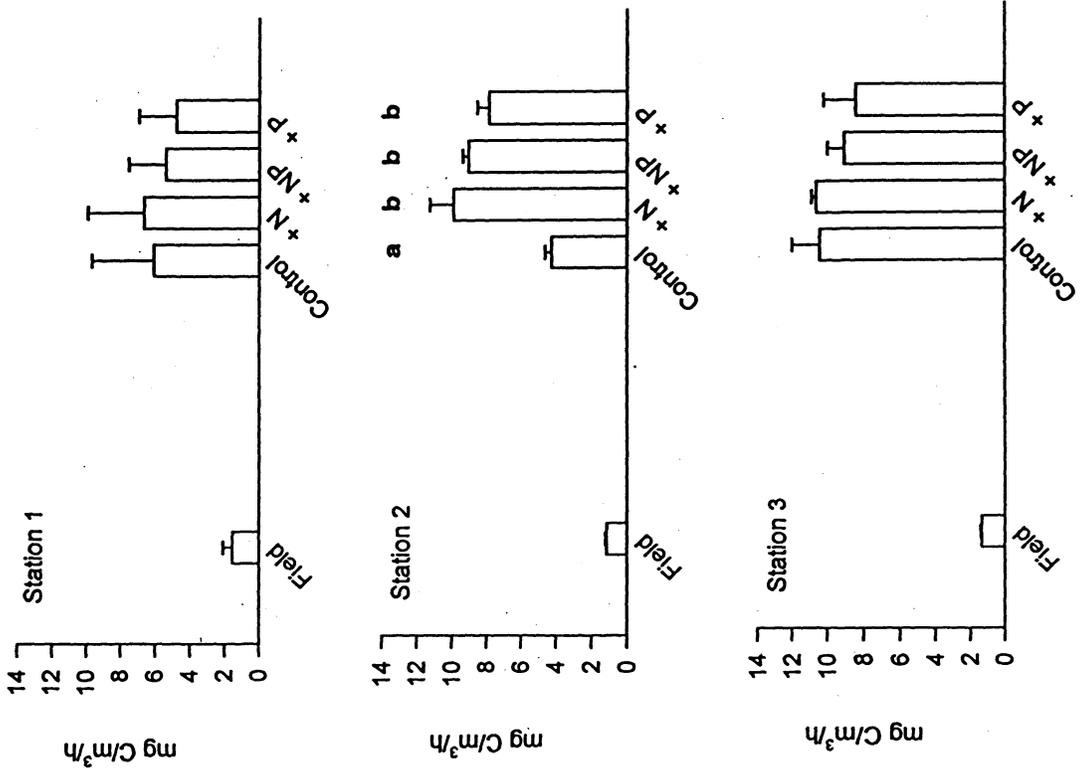


Figure 31. Primary production in September 1995. Field samples were collected on September 14; treatment effects were measured two days later on September 16. A) rates expressed on a volume basis; B) rates expressed on a chl α basis.

December 2, 7 1995

A



B

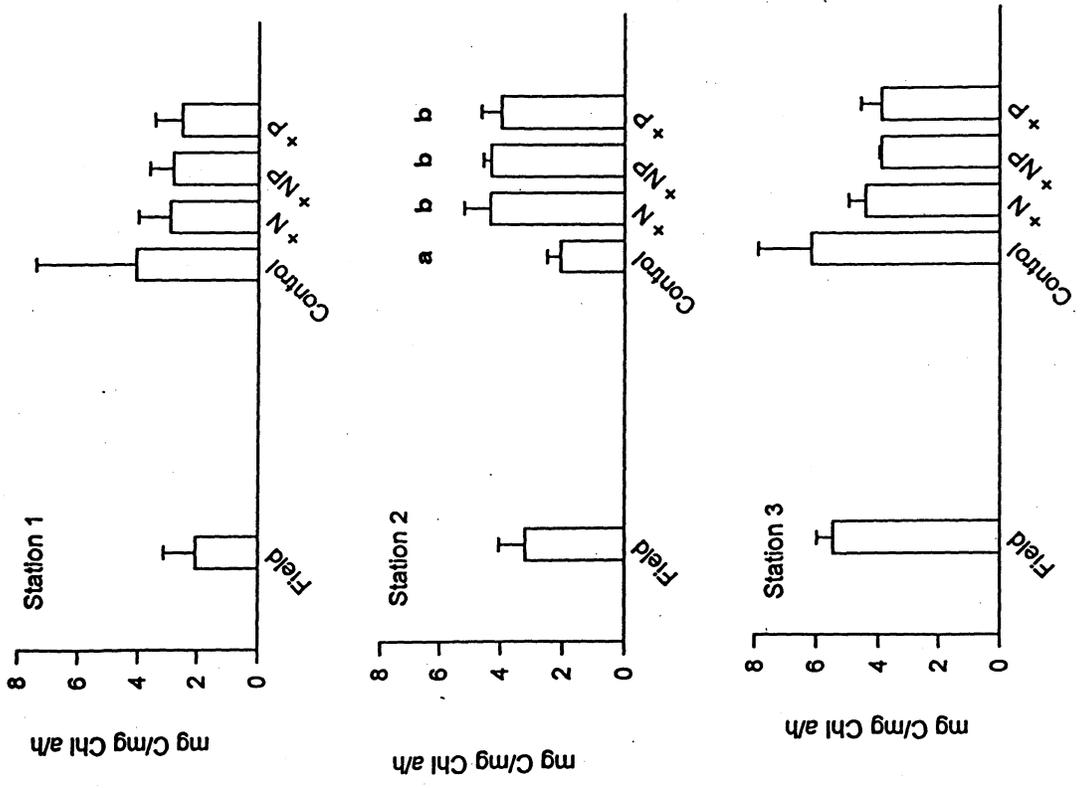


Figure 32. Primary production in December 1995. Field samples were collected on December 2; treatment effects were measured five days later on December 7. A) rates expressed on a volume basis; B) rates expressed on a chl α basis.

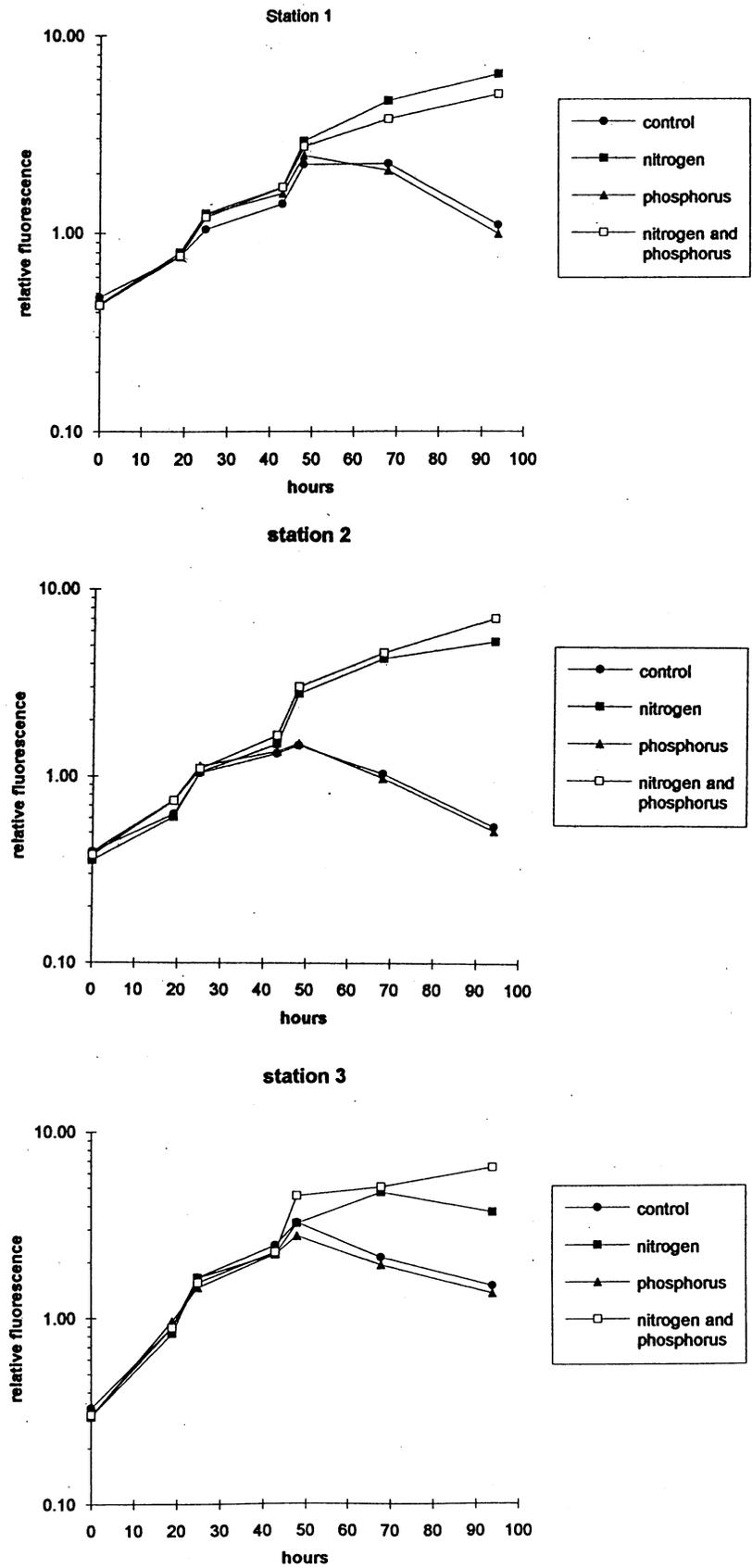
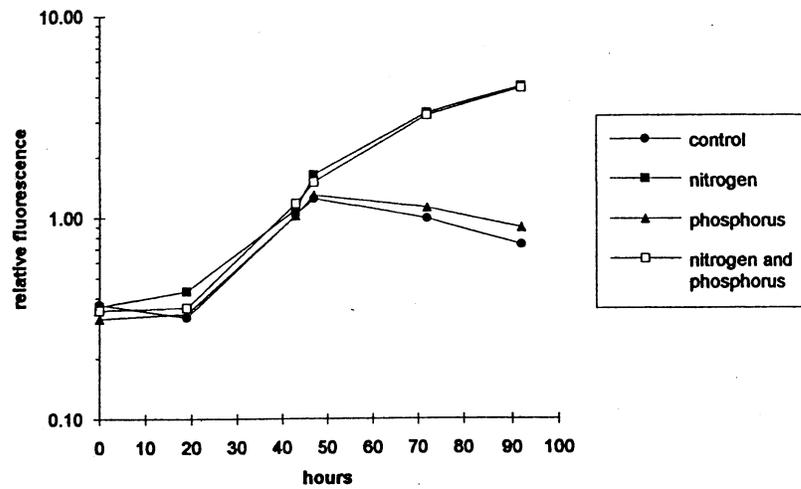
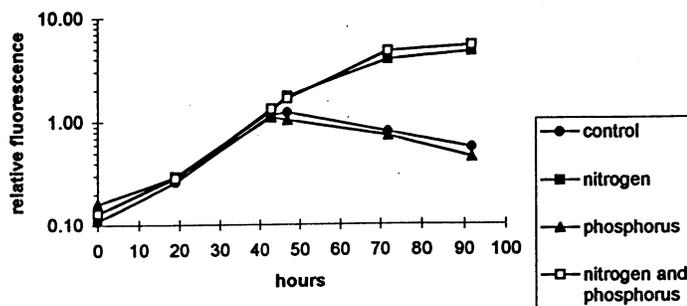


Figure 33. Relative fluorescence of phytoplankton collected June 28, 1994 in experimental treatments.

July, Station 1
treatment means



station 2



station 3

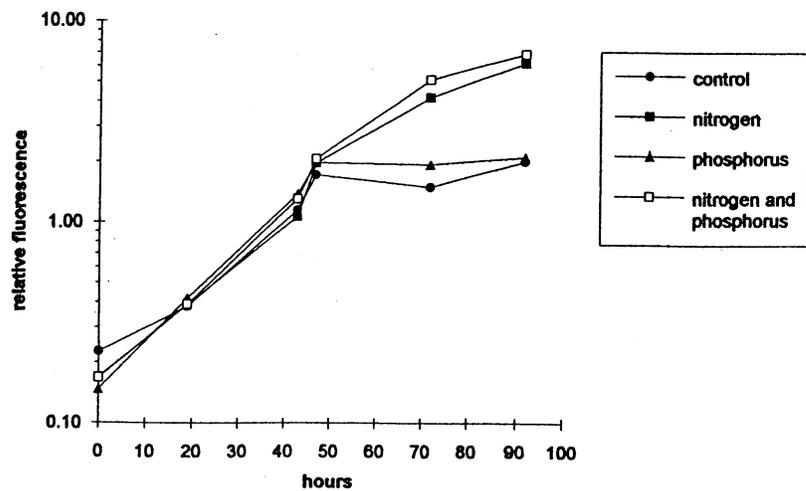
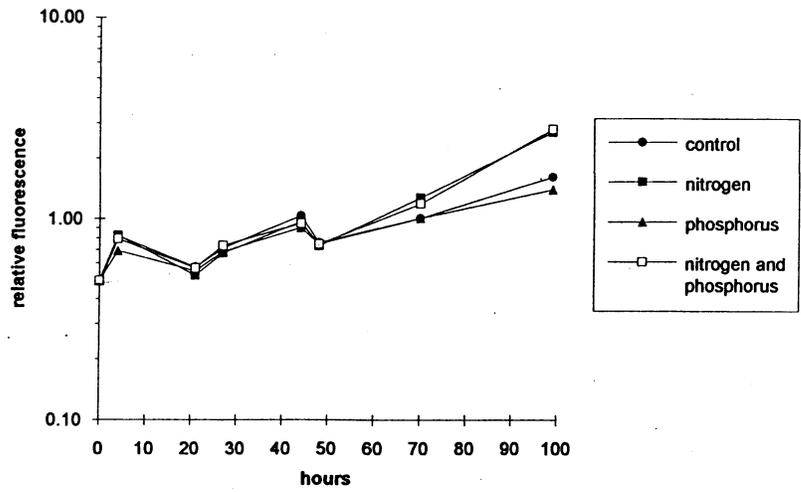
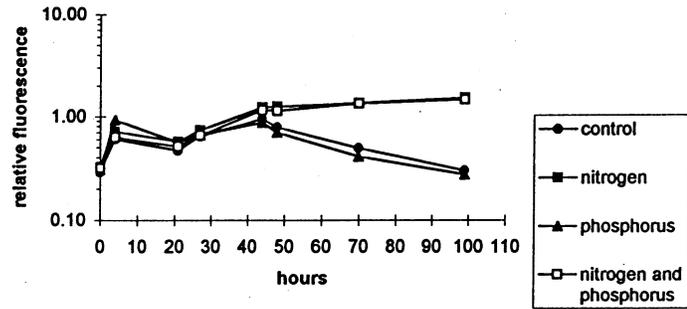


Figure 34. Relative fluorescence of phytoplankton collected July 25, 1994 in experimental treatments.

August, Station 1
treatment means



station 2



treatment means
station 3

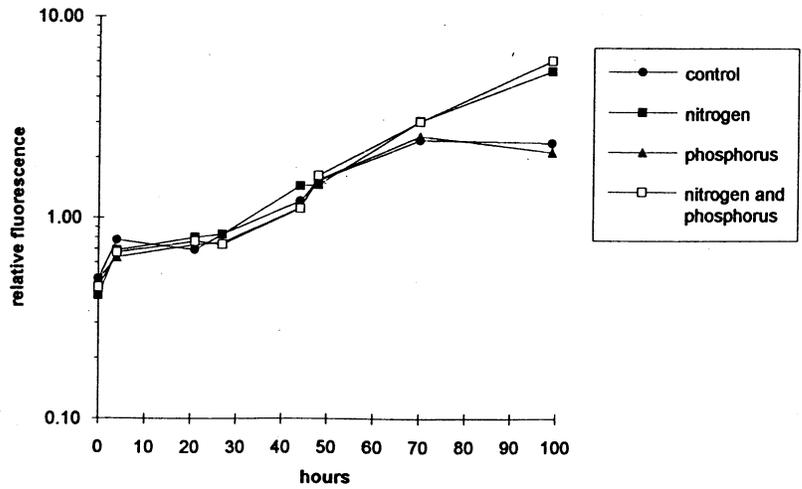


Figure 35. Relative fluorescence of phytoplankton collected August 22, 1994 in experimental treatments.

Oct 1994

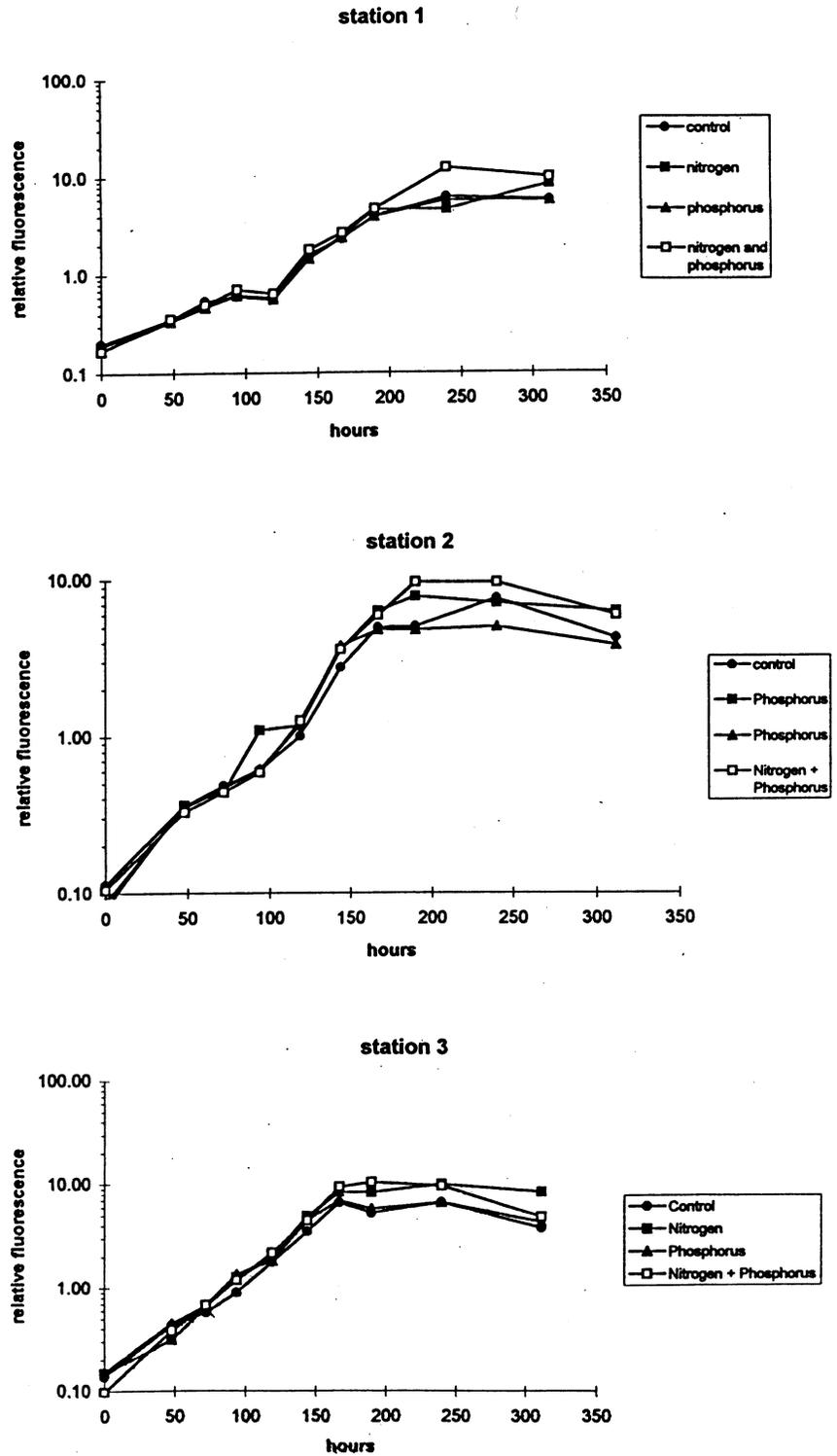


Figure 36. Relative fluorescence of phytoplankton collected October 25, 1994 in experimental treatments.

December 1994

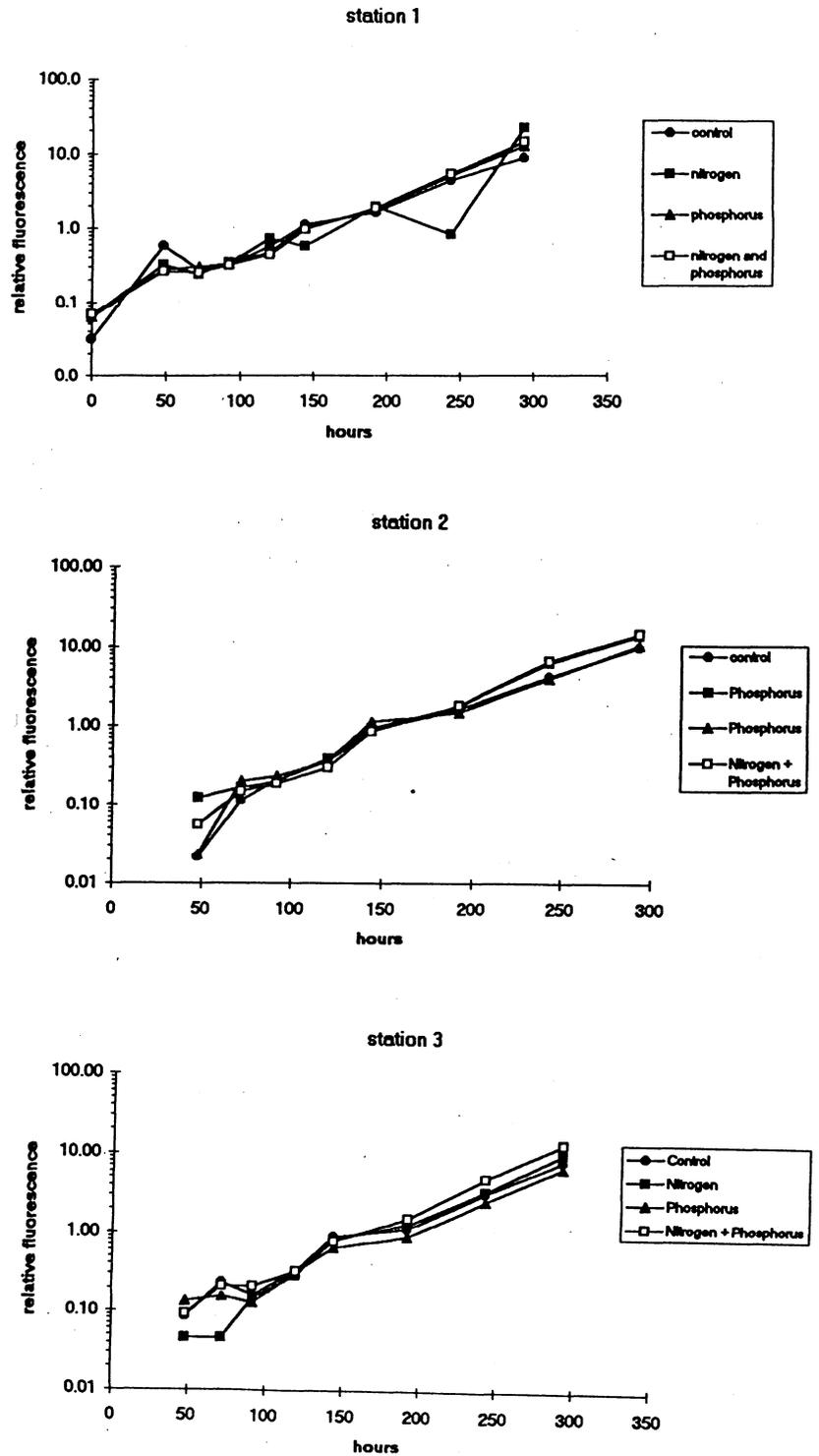


Figure 37. Relative fluorescence of phytoplankton collected December 7, 1994 in experimental treatments.

Feb 15

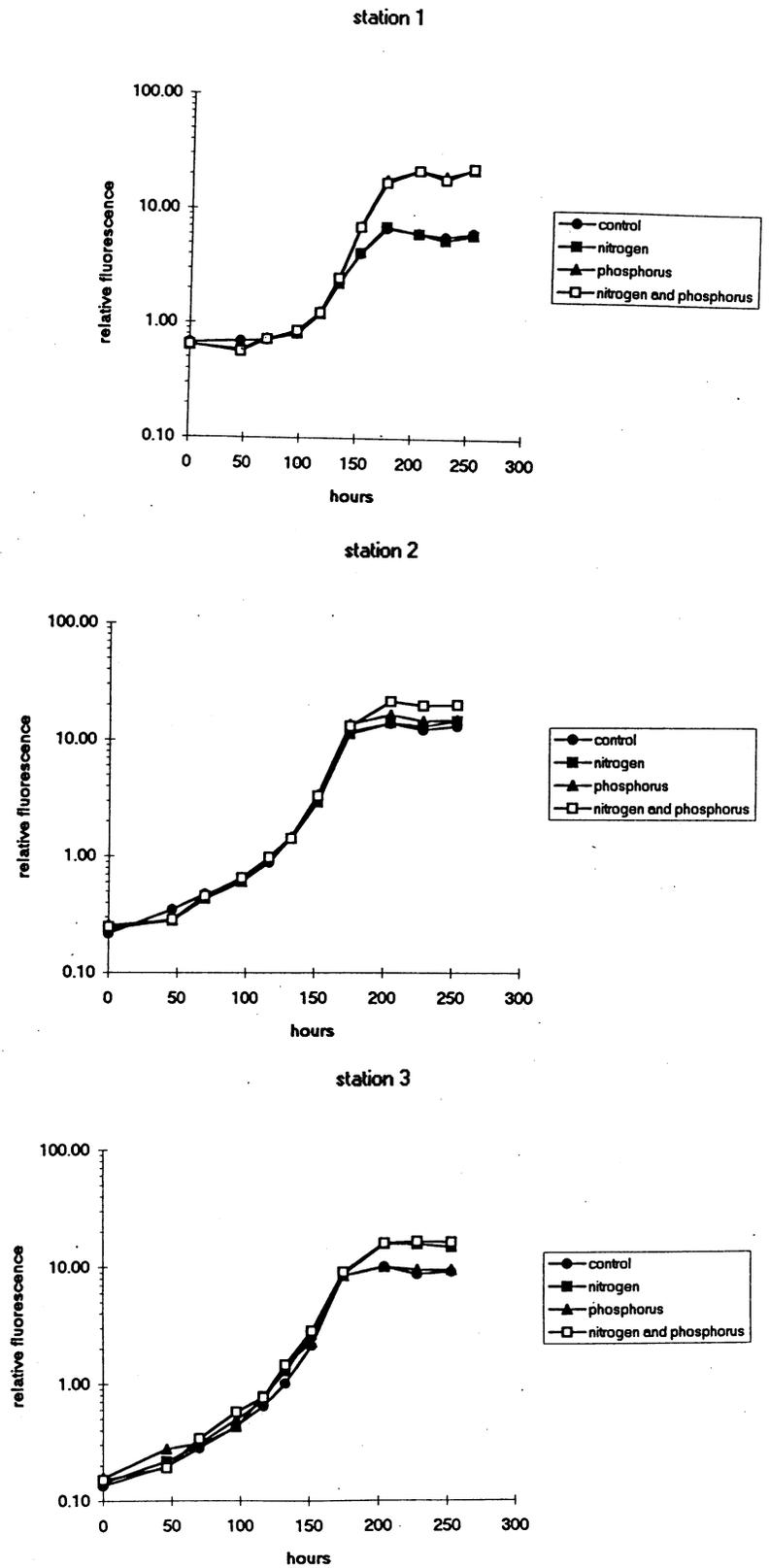


Figure 38. Relative fluorescence of phytoplankton collected February 4, 1995 in experimental treatments.

April 95

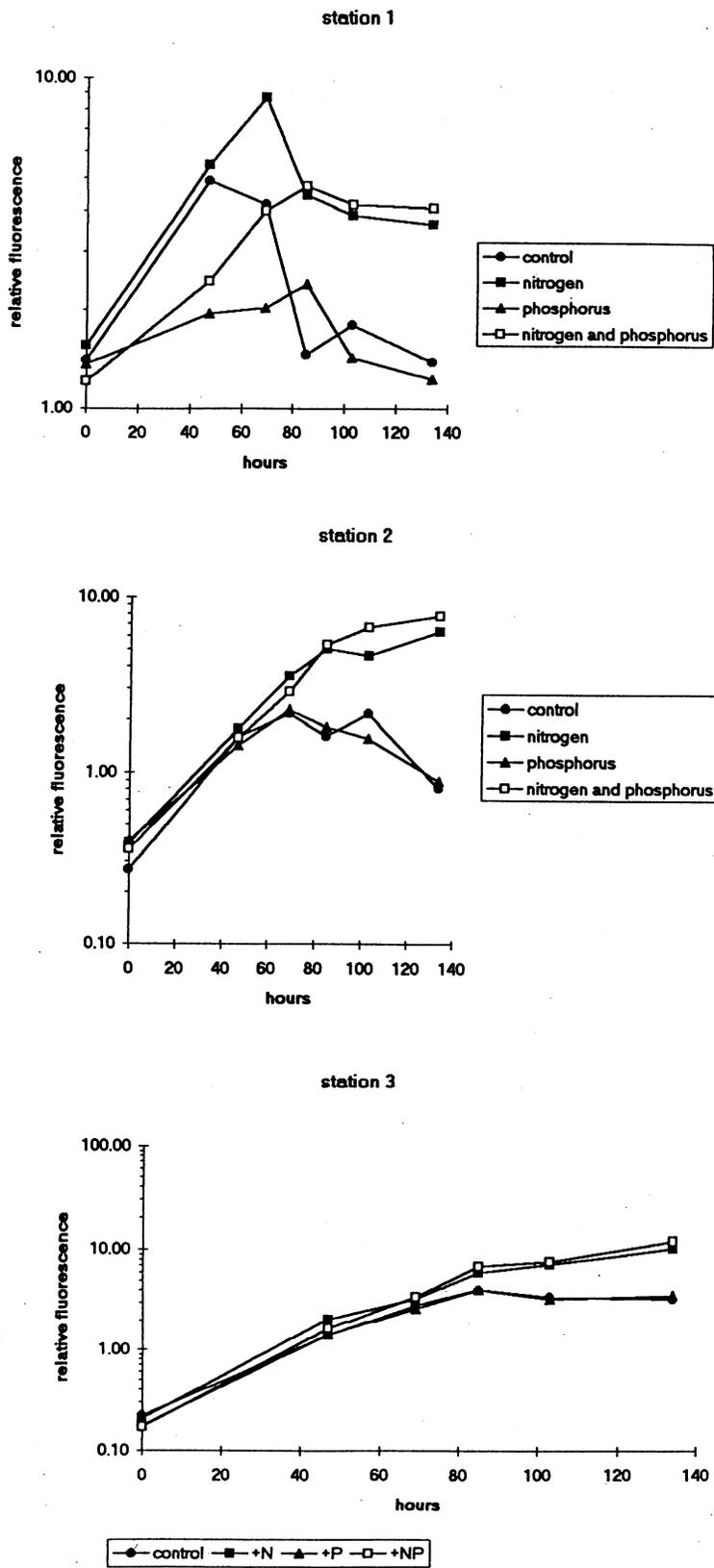


Figure 39. Relative fluorescence of phytoplankton collected April 15, 1995 in experimental treatments.

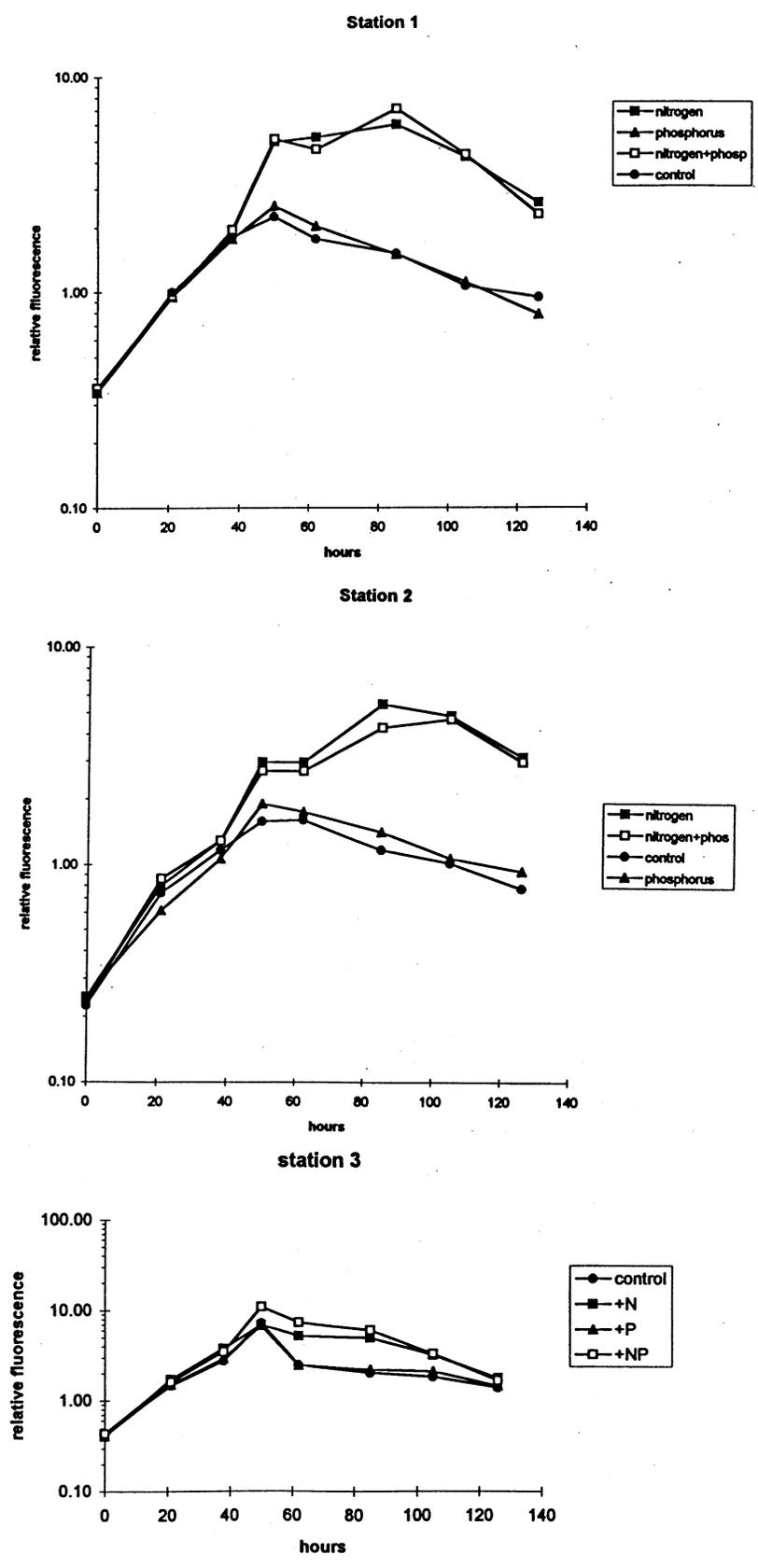


Figure 40. Relative fluorescence of phytoplankton collected May 20, 1995 in experimental treatments.

July 95

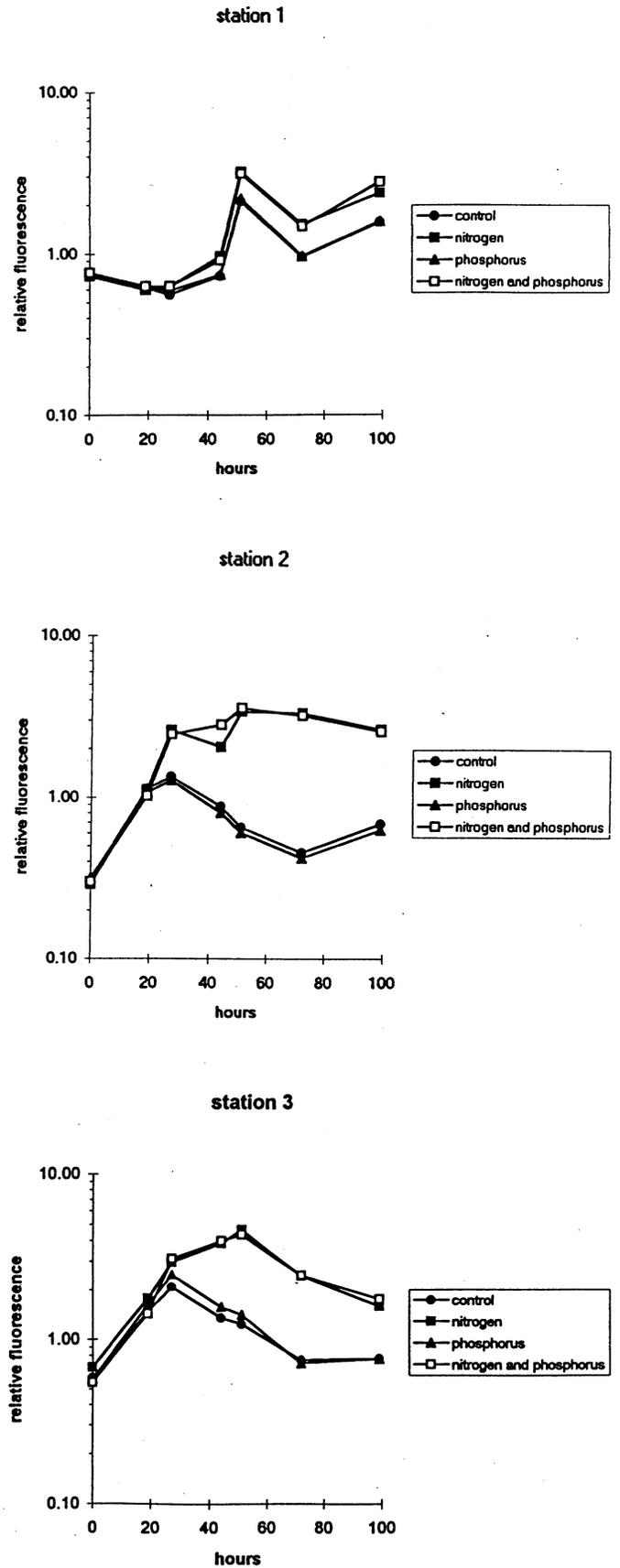


Figure 41. Relative fluorescence of phytoplankton collected July 5, 1995 in experimental treatments.

Aug 95

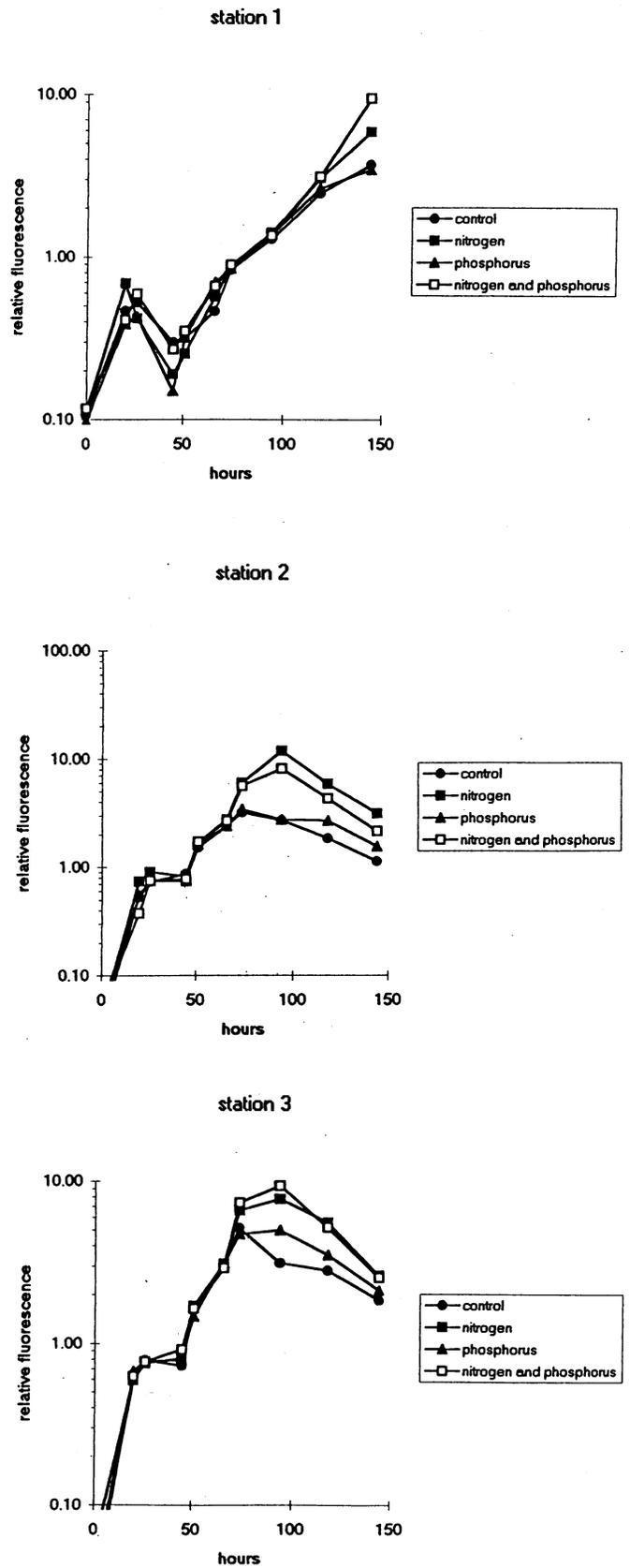


Figure 42. Relative fluorescence of phytoplankton collected August 15, 1995 in experimental treatments.

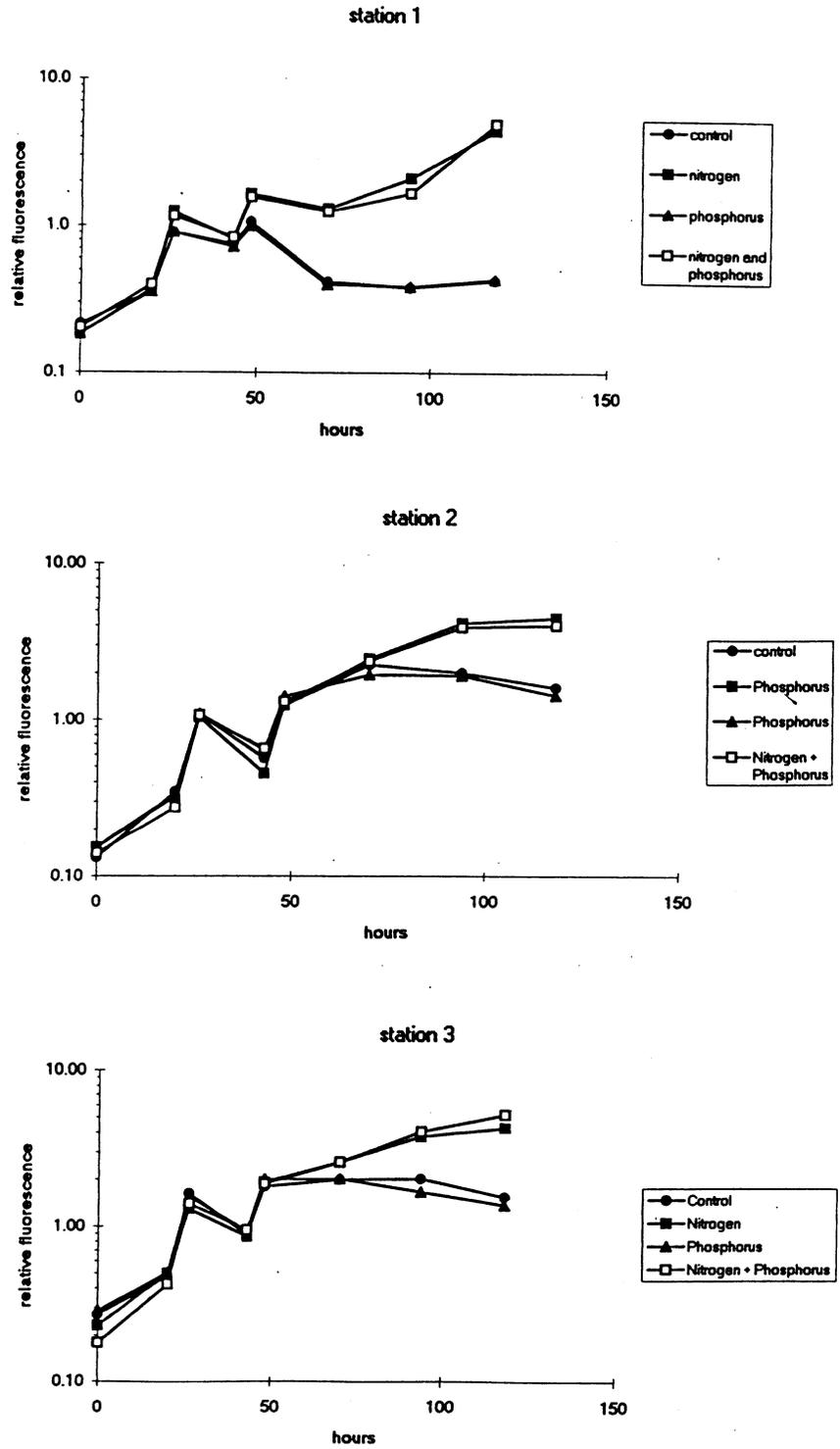


Figure 43. Relative fluorescence of phytoplankton collected September 14, 1995 in experimental treatments.

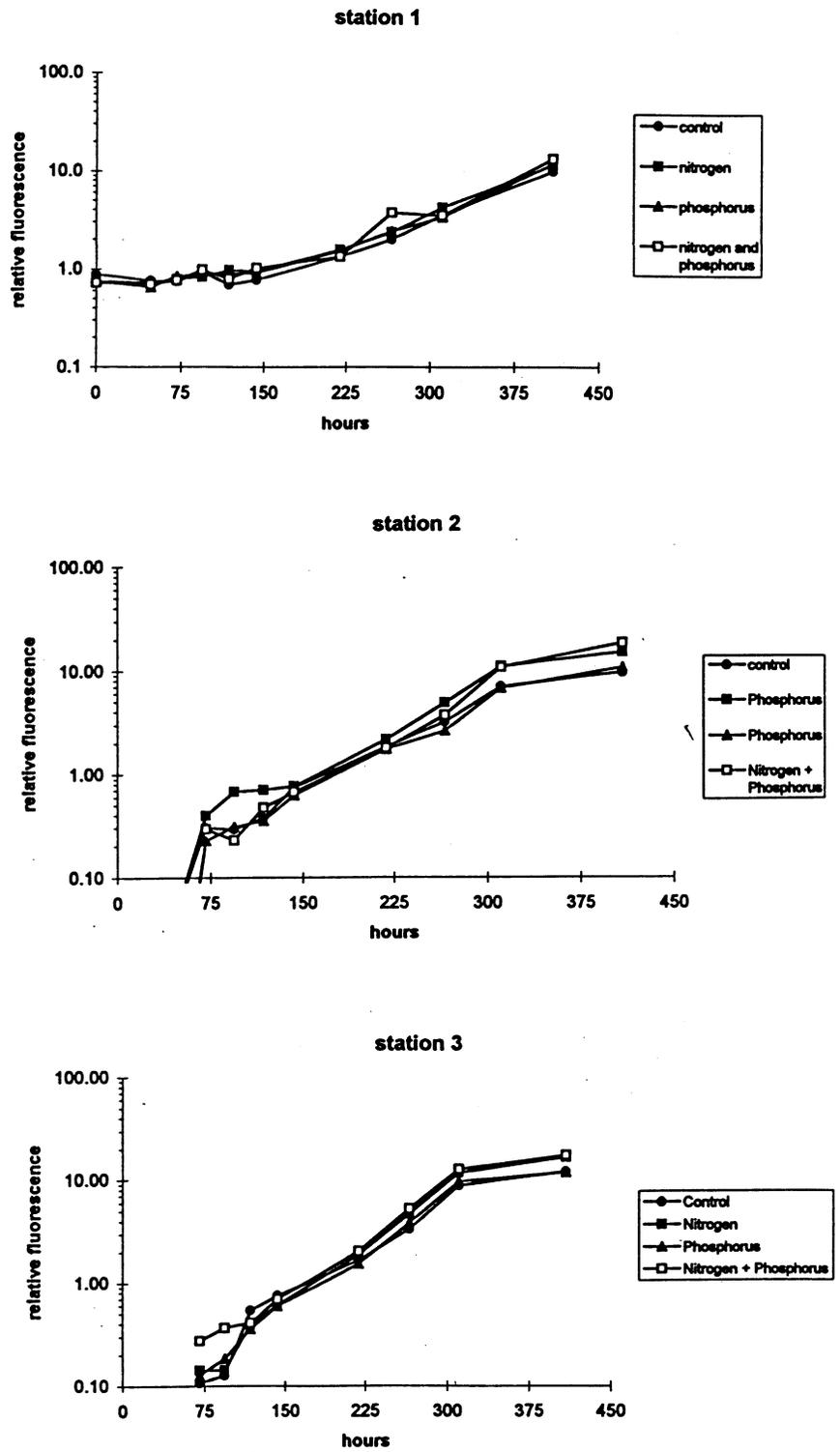


Figure 44. Relative fluorescence of phytoplankton collected December 2, 1995 in experimental treatments.

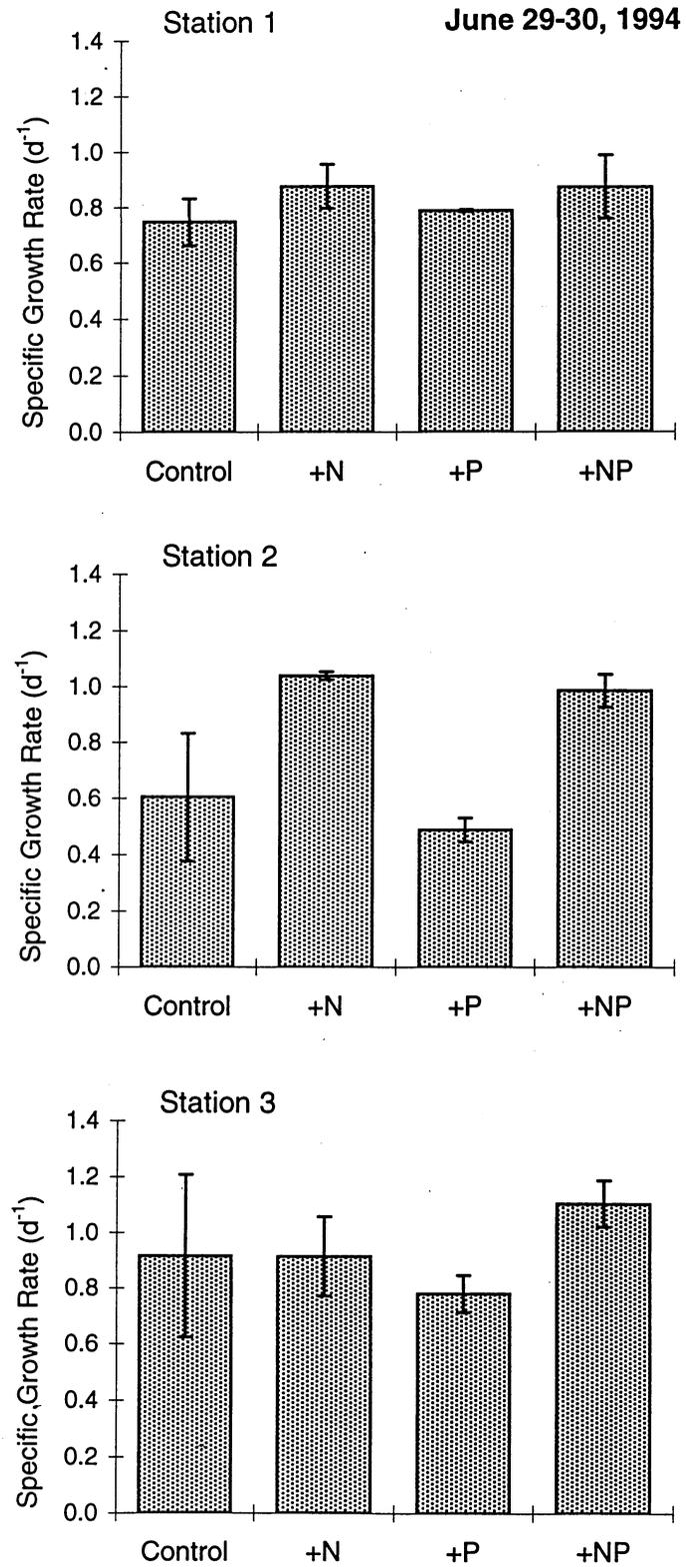


Figure 45. Specific growth rate of phytoplankton collected June 28, 1994 in experimental treatments.

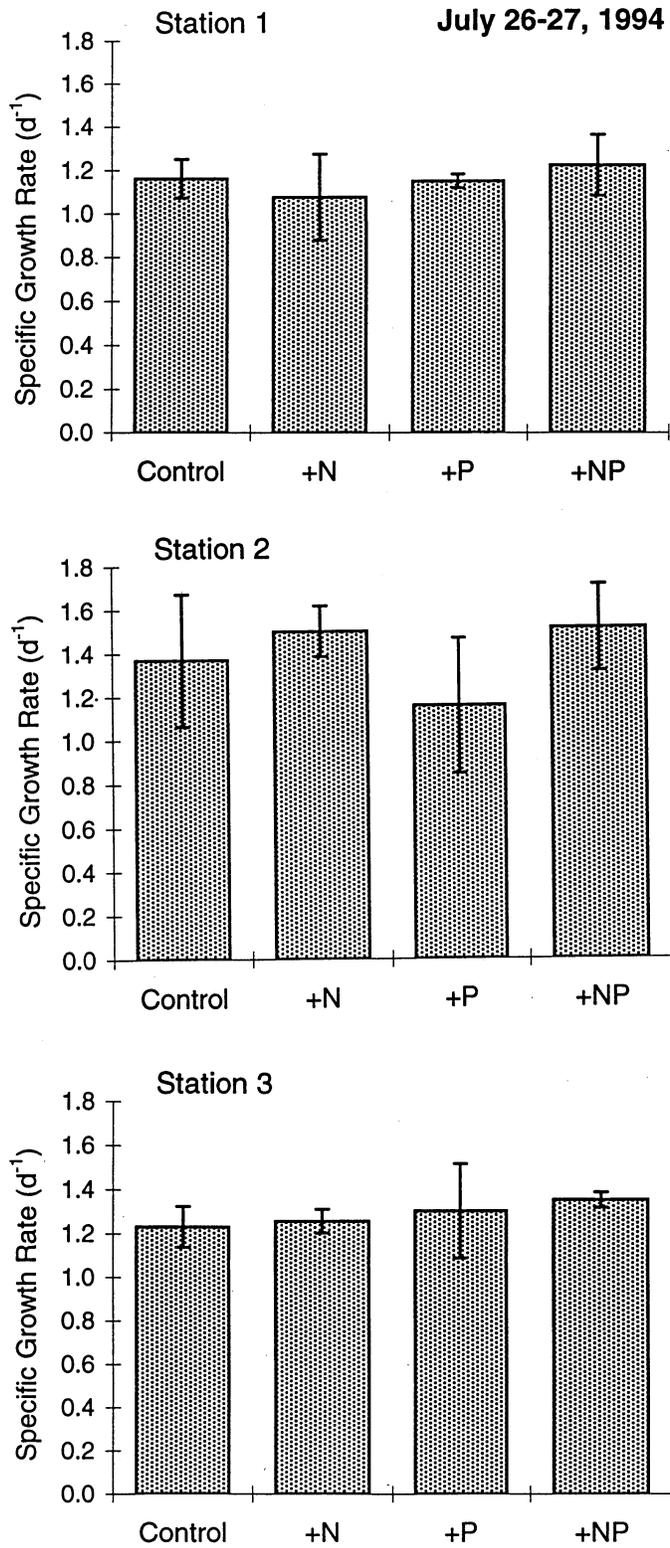


Figure 46. Specific growth rate of phytoplankton collected July 25, 1994 in experimental treatments.

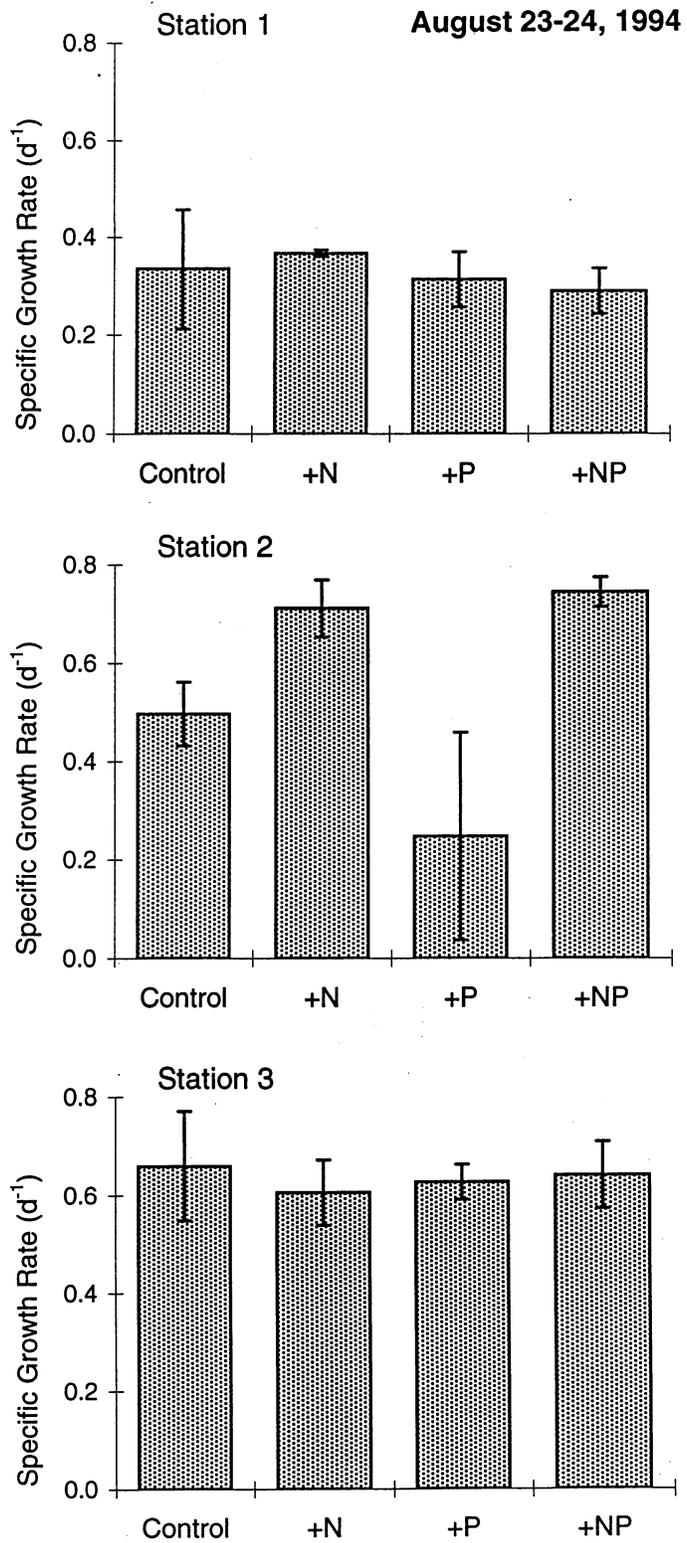


Figure 47. Specific growth rate of phytoplankton collected August 22, 1994 in experimental treatments.

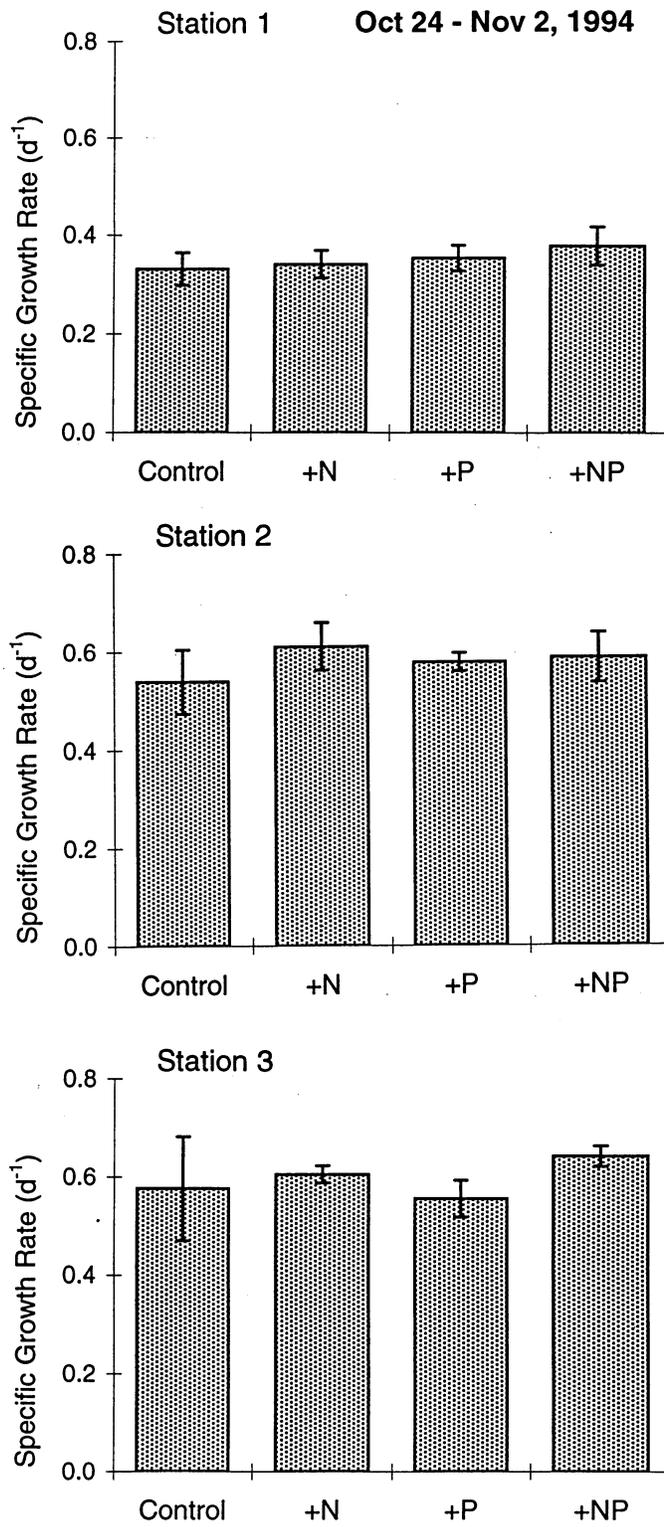


Figure 48. Specific growth rate of phytoplankton collected October 25, 1994 in experimental treatments.

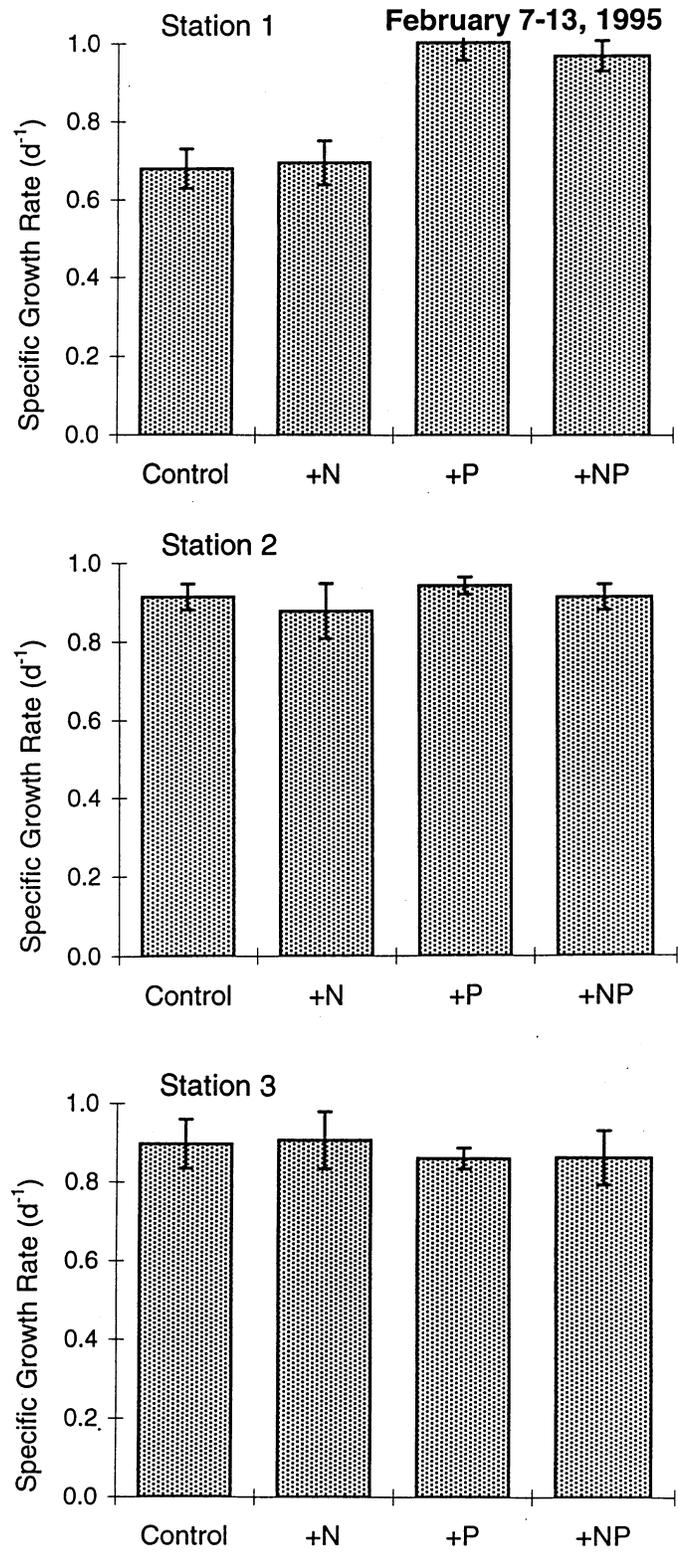


Figure 49. Specific growth rate of phytoplankton collected February 4, 1995 in experimental treatments.

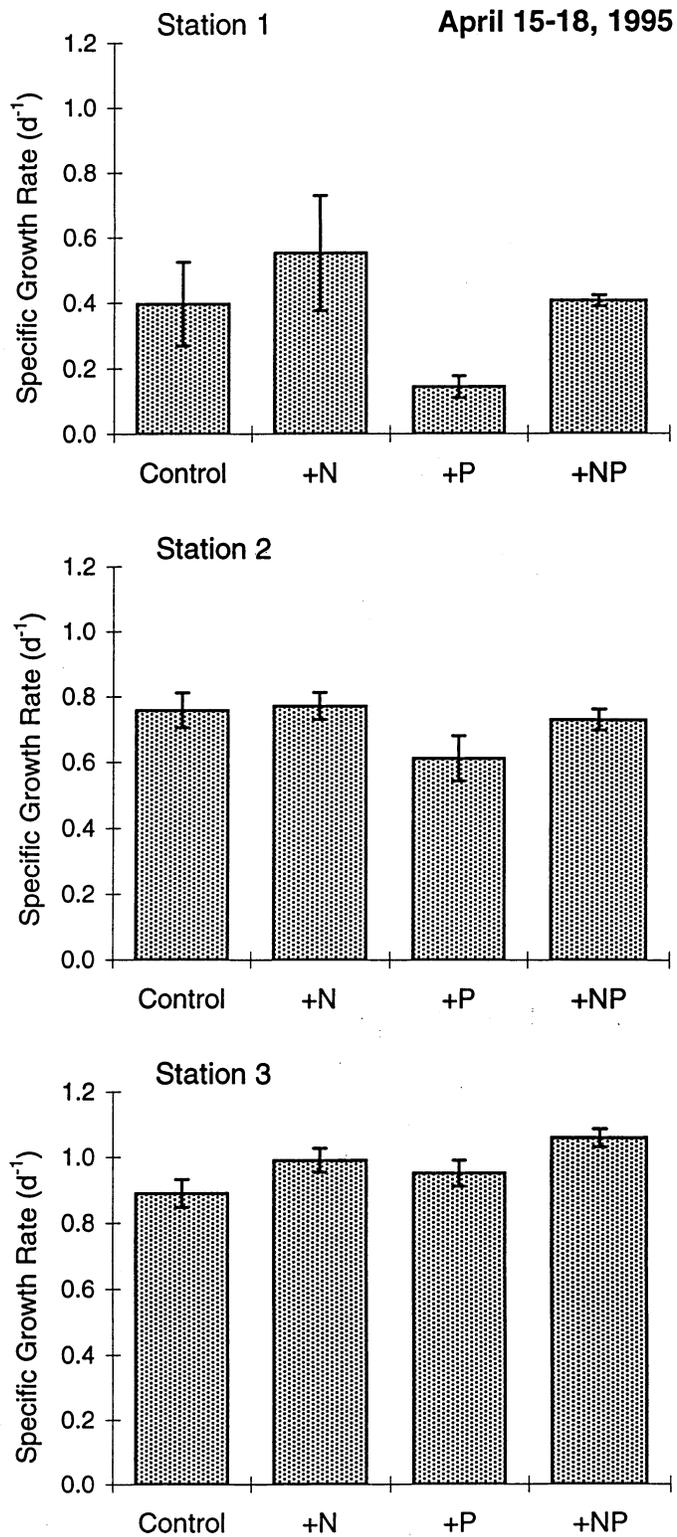


Figure 50. Specific growth rate of phytoplankton collected April 15, 1995 in experimental treatments.

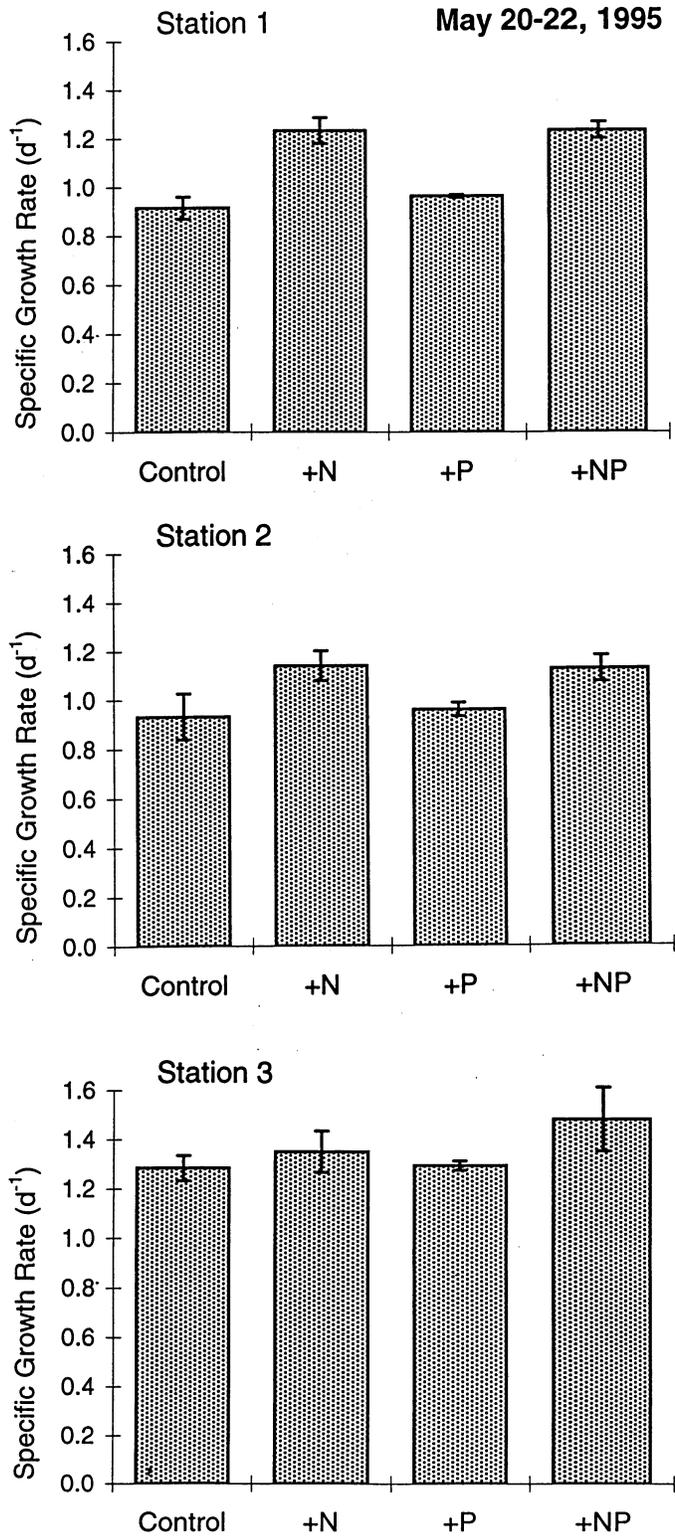


Figure 51. Specific growth rate of phytoplankton collected May 20, 1995 in experimental treatments.

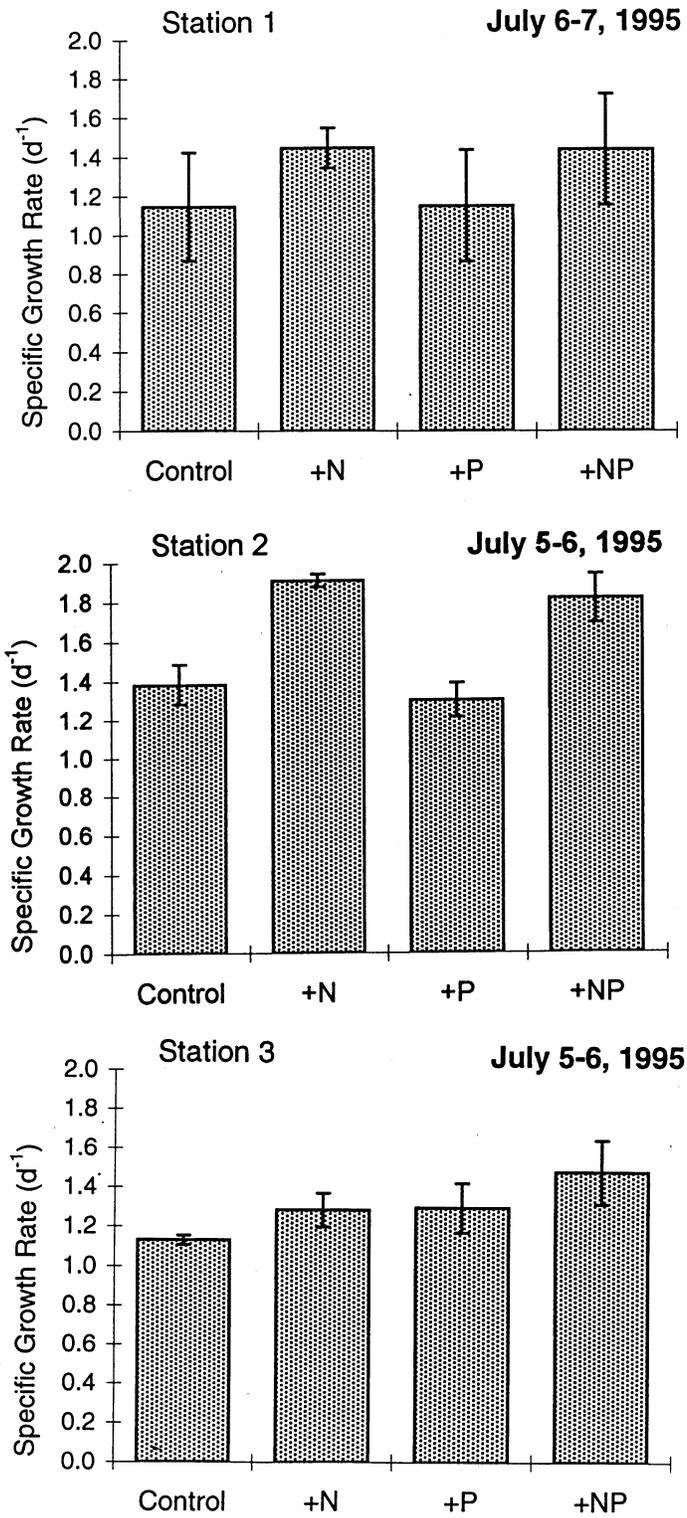


Figure 52. Specific growth rate of phytoplankton collected July 5, 1995 in experimental treatments.

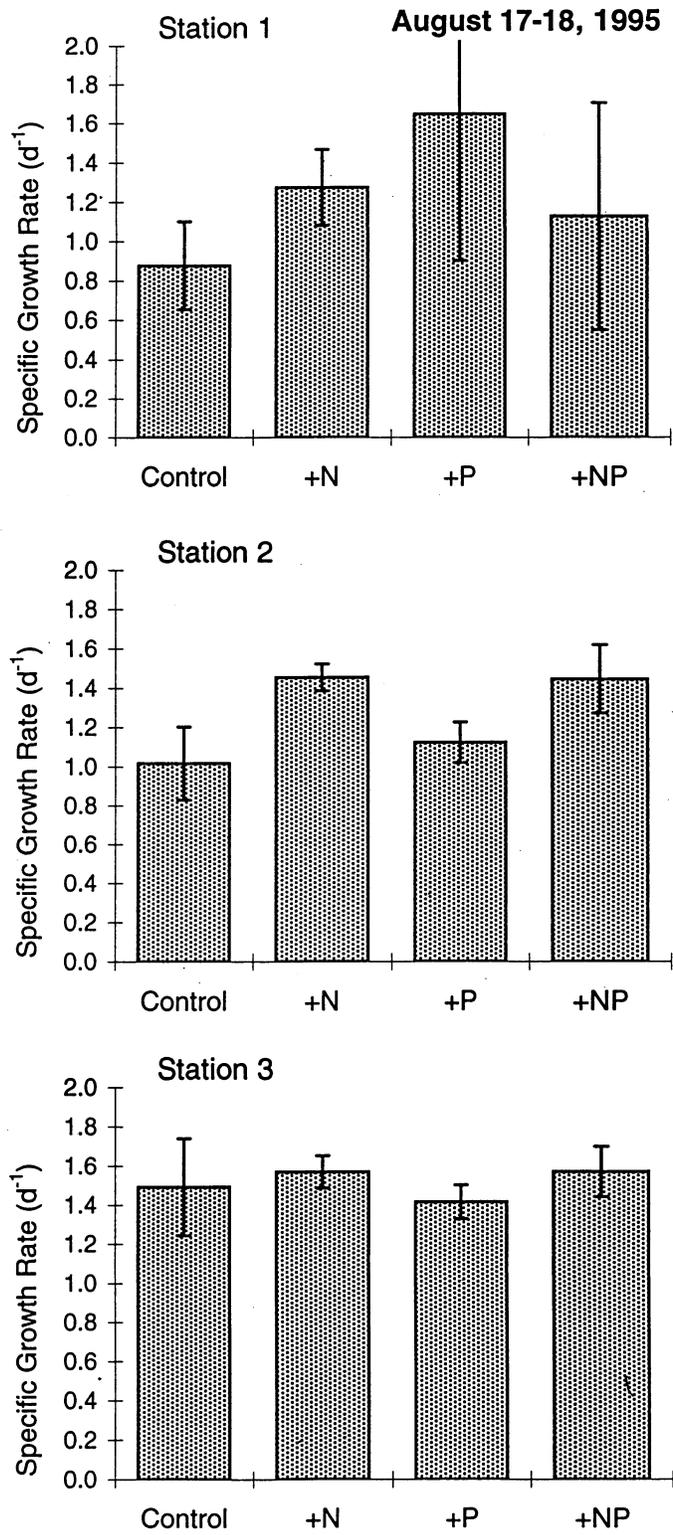


Figure 53. Specific growth rate of phytoplankton collected August 15, 1995 in experimental treatments.

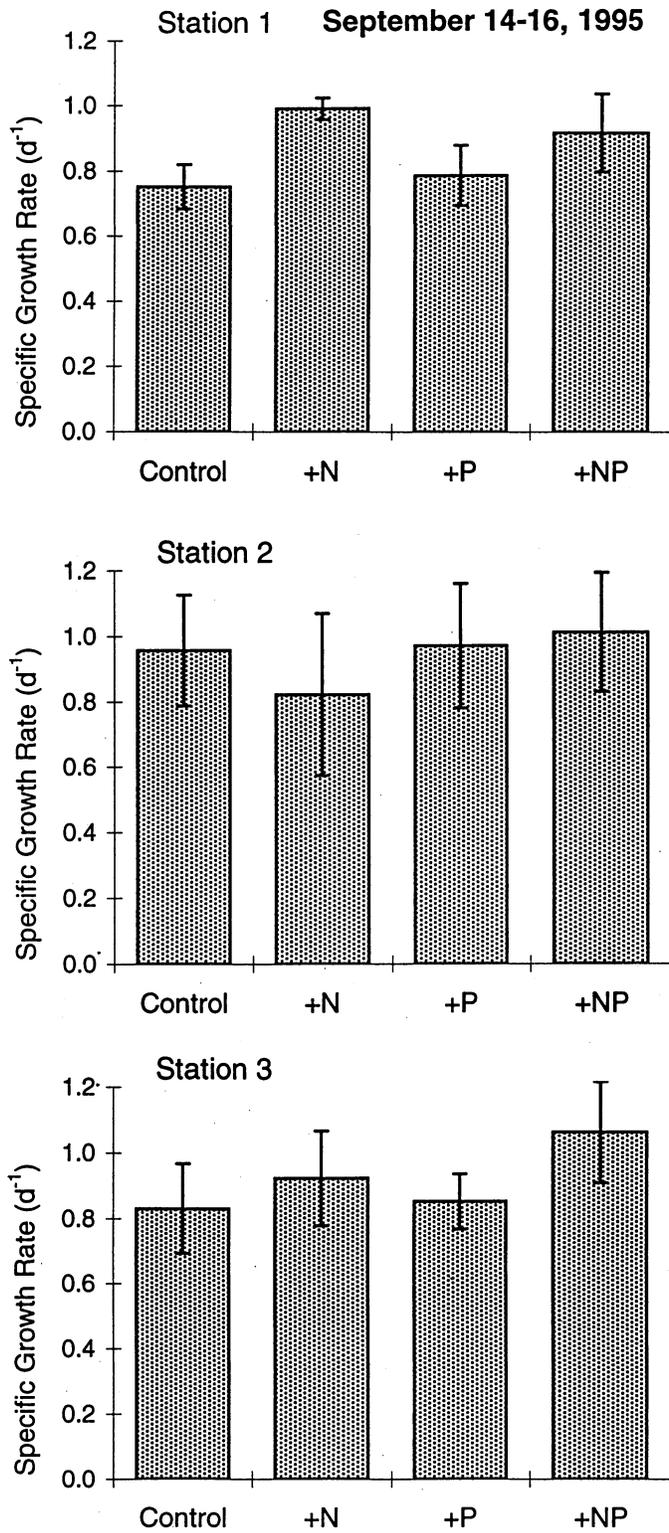


Figure 54. Specific growth rate of phytoplankton collected September 14, 1995 in experimental treatments.

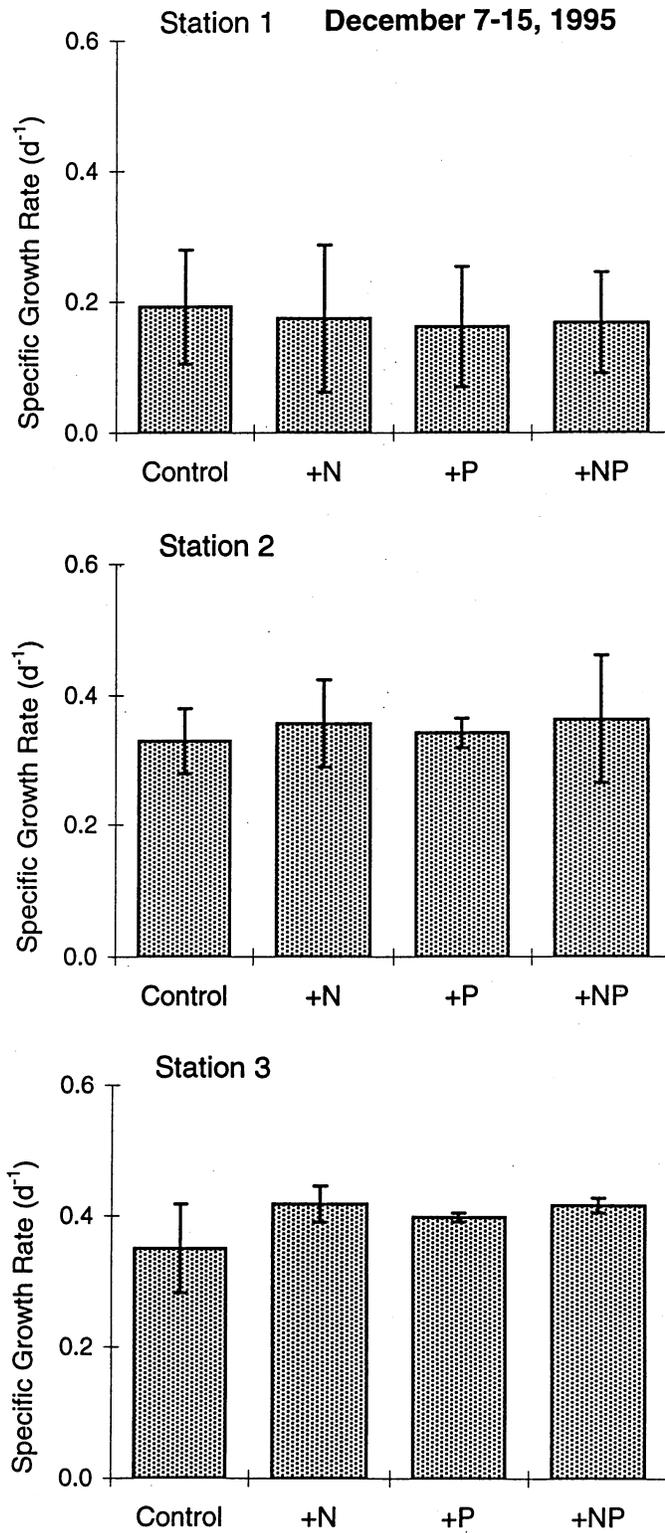


Figure 55. Specific growth rate of phytoplankton collected December 2, 1995 in experimental treatments.

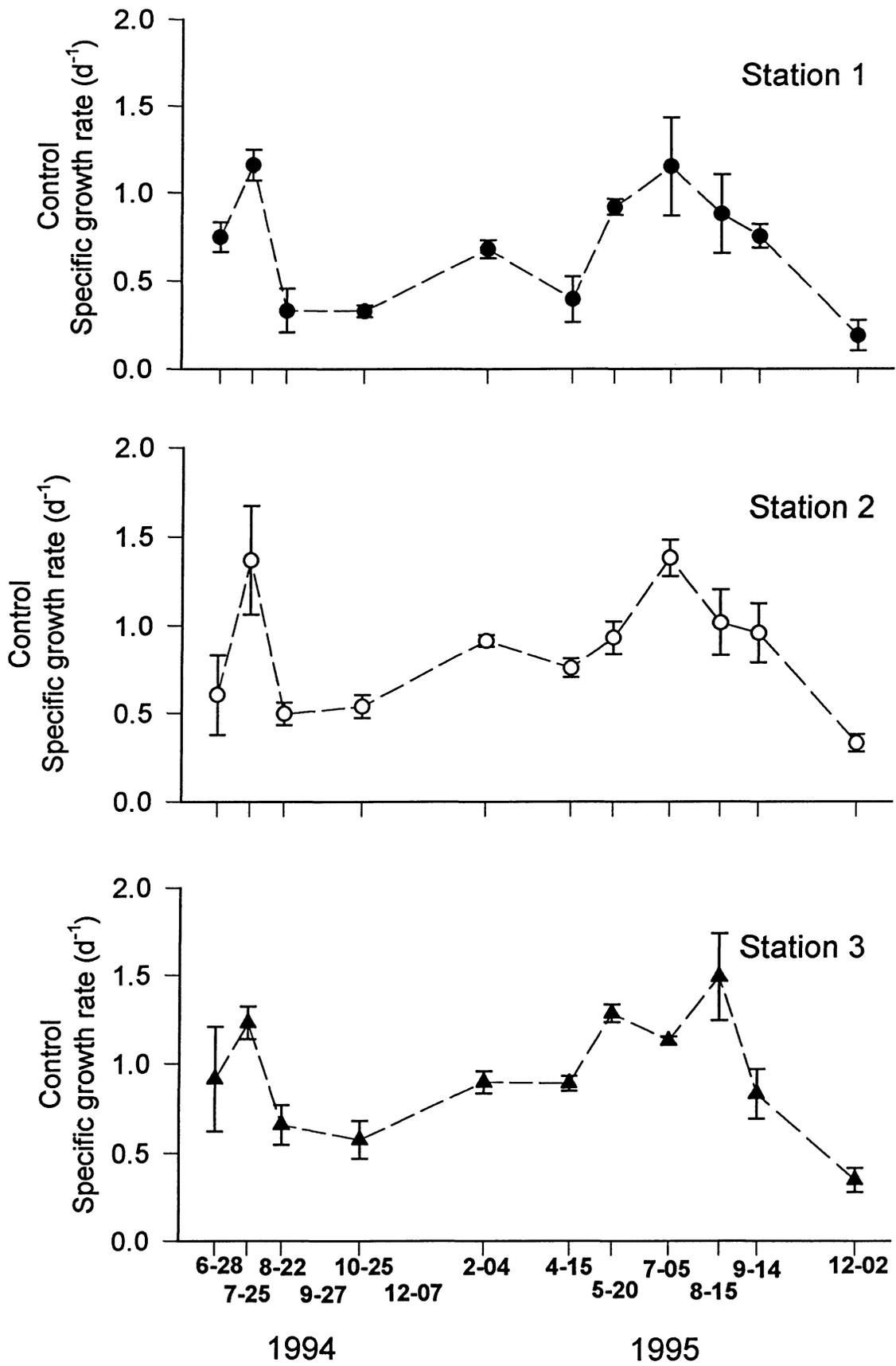


Figure 56. Specific growth rates of phytoplankton in control treatment (no nutrients added).

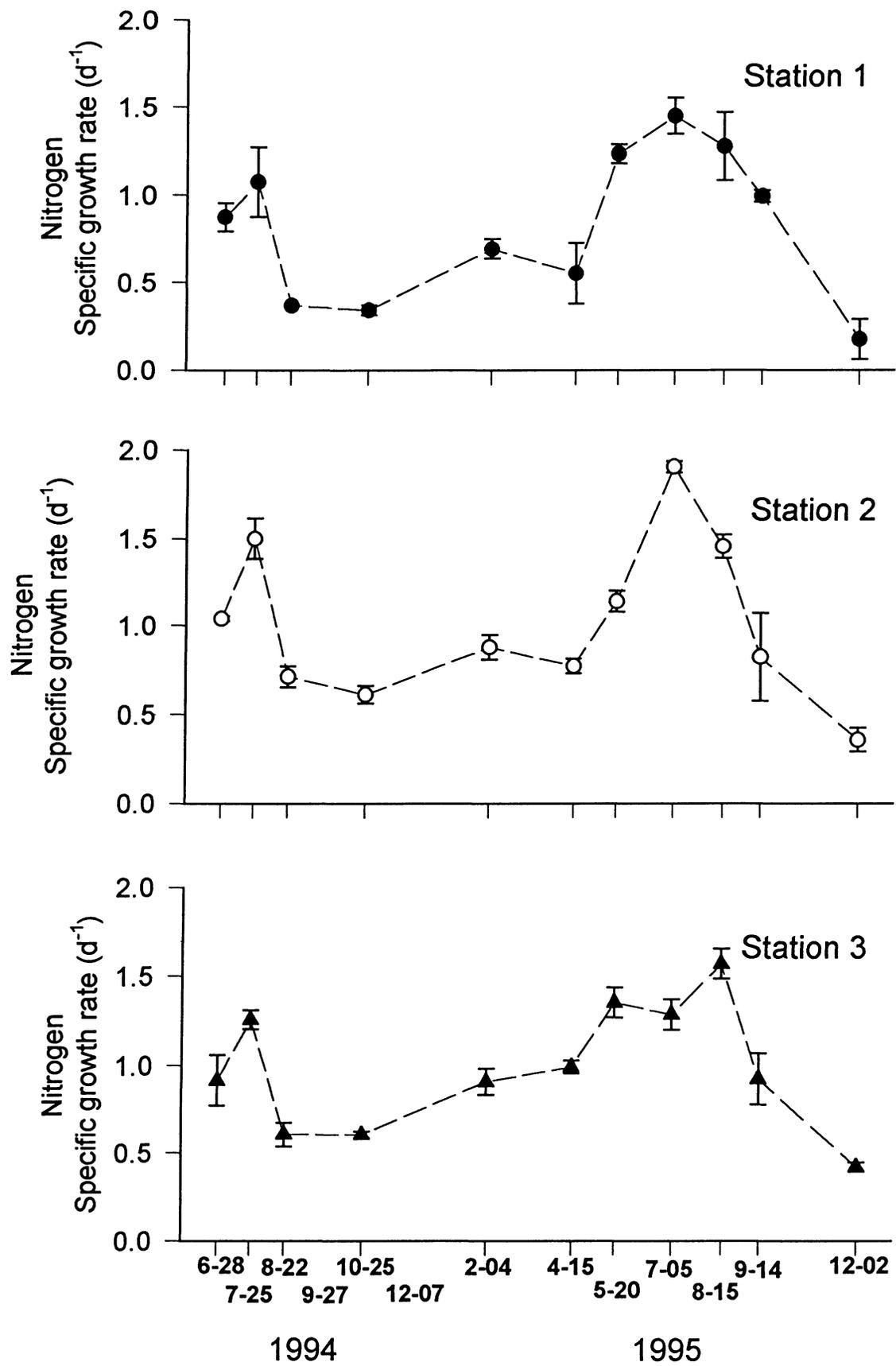


Figure 57. Specific growth rates of phytoplankton in nitrogen treatment.

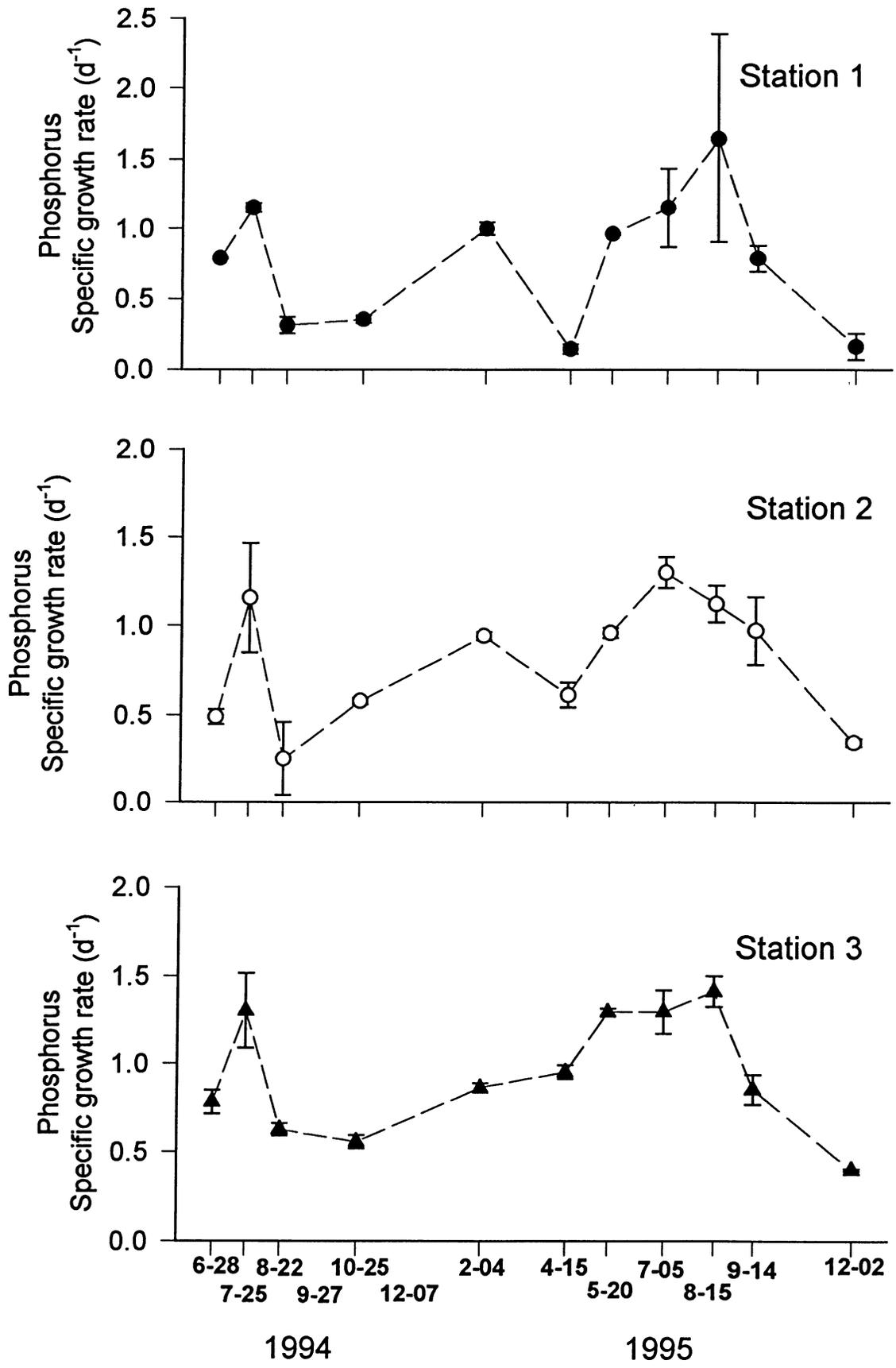


Figure 58. Specific growth rates of phytoplankton in phosphorus treatment.

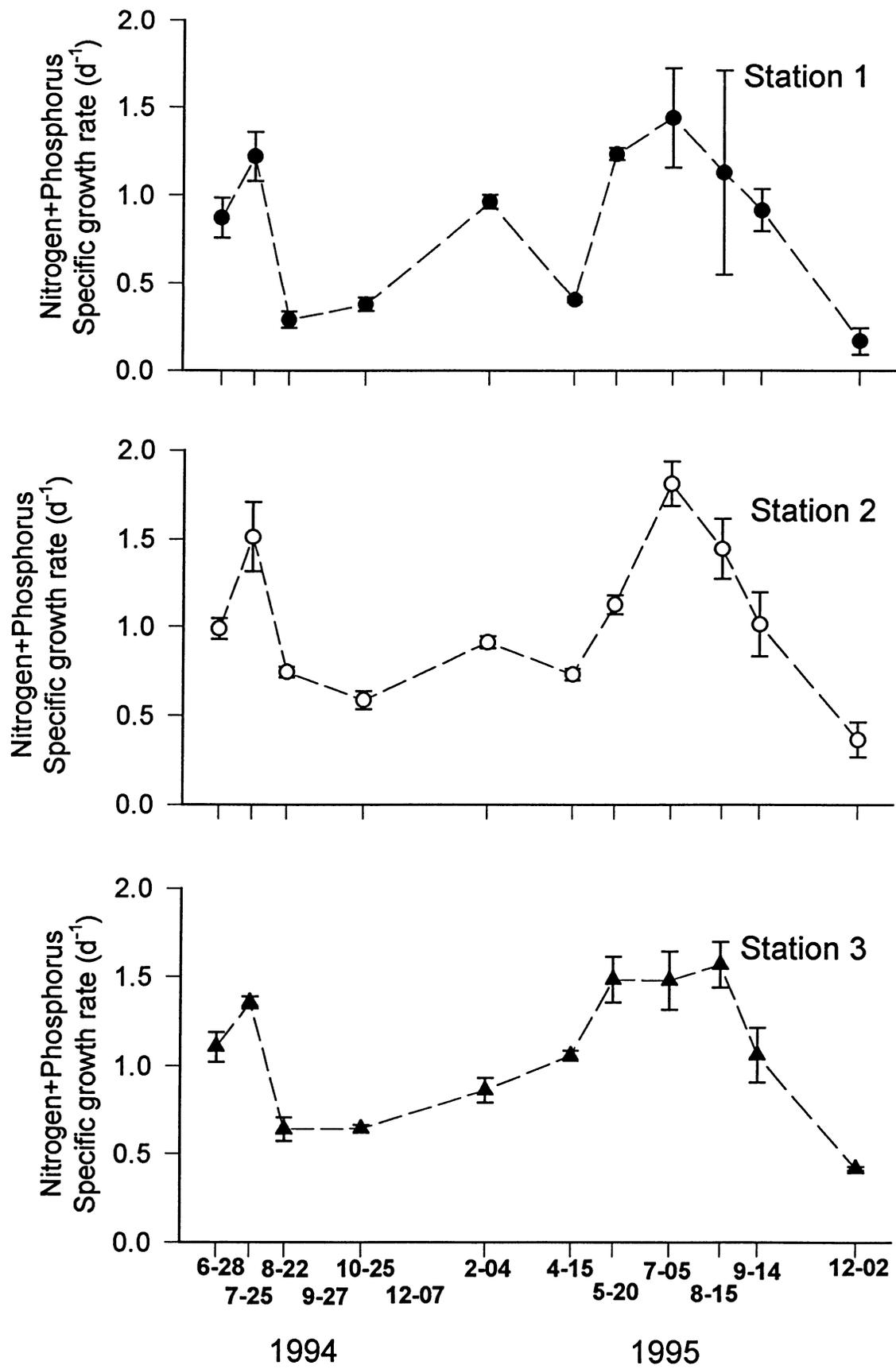


Figure 59. Specific growth rates of phytoplankton in nitrogen and phosphorus treatment.

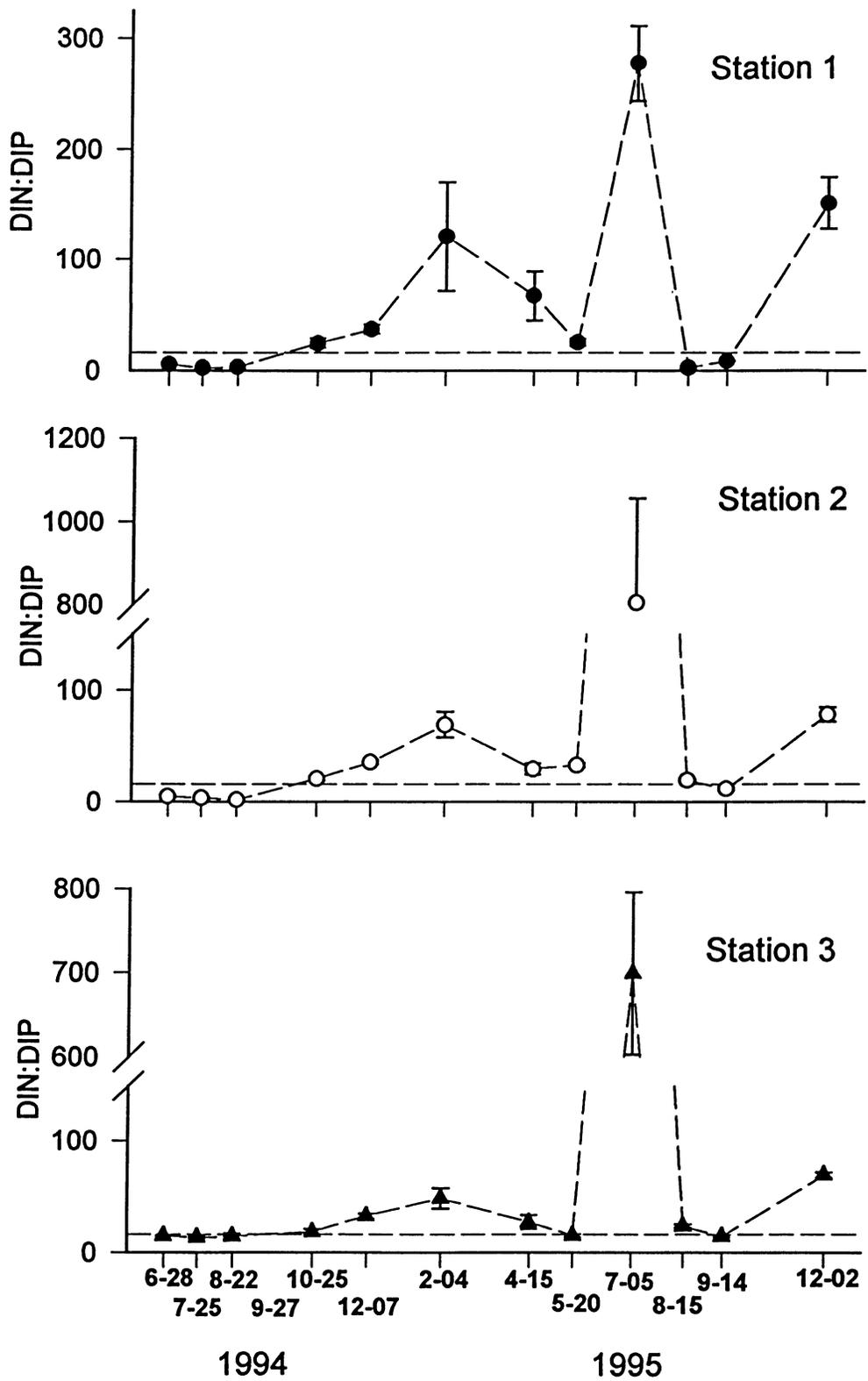


Figure 60. Dissolved inorganic nitrogen to phosphate ratios (DIN:DIP) in surface waters from the three stations collected from June 1994 to December 1995. The dashed line represents the Redfield ratio of 16.

