



Padilla Bay

National Estuarine Research Reserve

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**NUTRIENT LIMITATION OF PHYTOPLANKTON
IN PADILLA BAY**

Anne Bernhard

June 1993

Publication No. 94-92

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Nutrient Limitation of Phytoplankton
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the Faculty of
Western Washington University

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of the Requirements for the Degree
Master of Science

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Anne E. Bernhard

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Abstract

The effect of nutrient additions on natural phytoplankton assemblages in Padilla Bay was examined from June to October, 1992 by short-term nutrient enrichment bioassays incubated *in situ*. Nitrogen additions ($15 \mu\text{M NH}_4$) significantly ($p < 0.001$) stimulated phytoplankton growth during all six experiments. Nitrate additions ($15 \mu\text{M}$) significantly stimulated phytoplankton growth in October, but not in September. Addition of silicate ($15 \mu\text{M SiO}_2$), phosphate ($1.0 \mu\text{M PO}_4$), or trace metals alone did not stimulate phytoplankton growth. In most experiments, phytoplankton growth was most enhanced by combined additions of nitrogen and phosphorus. In three of the experiments, the response of the nanoplankton to ammonium additions was compared to that of the total phytoplankton. Nanoplankton growth exceeded that of the total phytoplankton during August, but showed no response to ammonium additions in October. Additionally, DIN/ PO_4 ratios were below 16:1 during four out of six experiments, which would indicate the potential for nitrogen limitation. These results indicate that there is the potential for eutrophication in Padilla Bay as a result of nutrient inputs during the summer when light is less likely to be limiting phytoplankton growth.

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Introduction

The rate of phytoplankton growth in marine environments is the result of complex interactions among physical, chemical, and biological factors. Nutrients, light, temperature, grazing, and sinking are some of the major variables controlling phytoplankton growth (Valiela, 1984). This study focuses on the effects of nutrients on phytoplankton growth. According to Riley (1965), phytoplankton biomass is controlled by three major nutrient groups: 1) nitrogen, phosphorus, and silicon, 2) trace metals, and 3) vitamins. Any one of these nutrients may be limiting at a particular time. Studies in coastal areas, however, have shown that *in situ* concentrations of trace metals and vitamins are usually not limiting (Thomas, 1970; Maestrini *et al.*, 1984).

The term nutrient limitation has several different meanings. It may refer to: 1) limitation of growth of the current phytoplankton population, 2) limitation of net ecosystem production, or 3) limitation of the potential rate of net primary production or biomass accumulation (Howarth, 1988; Graneli *et al.*, 1990). It is this last definition that is most often used by aquatic ecologists and is the definition used in this study. A nutrient is considered to be limiting, if when added to a system, there is an increase

in net primary production or biomass accumulation. Consequently, changes in species composition of the phytoplankton community are not important (Howarth, 1988). The effect of nutrient addition on phytoplankton growth can be estimated by determining increases in cell numbers (Ryther and Dunstan, 1971; Vince and Valiela, 1973), chlorophyll *a* concentration (Vince and Valiela, 1973), or the rate of ¹⁴C fixation (Graneli *et al.*, 1990).

Studies of nutrient limitation of phytoplankton growth and identification of the limiting nutrient in marine systems have generated variable results. This variability may be attributed to differences in study sites, methods, duration of the studies, or species-specific responses to nutrient concentrations. Historically, nitrogen has been considered to be the nutrient that limits phytoplankton production in both coastal (Ryther and Dunstan, 1971; Vince and Valiela, 1973) and oceanic systems (Eppley *et al.*, 1973; Goldman, 1976). The case in estuaries, however, is not as clear due primarily to the seasonally varying mixtures of freshwater and seawater. Although some studies have reported nitrogen to be the limiting nutrient, there is evidence that phosphorus (Myers and Iverson, 1981; Smith, 1984) or silicon (Paasche, 1973; Officer and Ryther, 1980) may limit phytoplankton production in estuaries. Other studies have shown phosphorus to have a significant effect on

phytoplankton growth only when supplied in combination with nitrogen (Eppley *et al.*, 1973; Vince and Valiela, 1973; Caraco *et al.*, 1987; Fisher *et al.*, 1992). Results from more recent studies in estuaries provide evidence for a seasonal (D'Elia *et al.*, 1986; Webb *et al.*, 1988; Fisher *et al.*, 1992) or spatial (Caraco *et al.*, 1987; Caraco *et al.*, 1988) shift in the limiting nutrient from nitrogen to phosphorus. This shift may be the result of seasonal changes in freshwater runoff, which may change the nutrient loading ratios (D'Elia *et al.*, 1986; Fisher *et al.*, 1992). Caraco *et al.* (1987) found the shift in the limiting nutrient to be related to differences in salinity, which is also affected by changes in runoff.

Another important variable affecting nutrient limitation of phytoplankton growth is the size class distribution of algal species, which may affect nutrient uptake efficiency. Phytoplankton species range in size from about 2.0 μm to >200 μm (Sieburth *et al.*, 1978). Smaller phytoplankton are presumably more efficient at nutrient uptake because of a greater surface area to volume ratio (Valiela, 1984). Malone (1980a) found that nanoplankton (<20 μm) in the open ocean may account for 90% of the primary production. In coastal areas, however, the contribution of netplankton (>20 μm) to primary production is, at times, greater than the nanoplankton contribution.

The controlling factors of primary production are important when evaluating the potential for eutrophication. Natural eutrophication is a process that occurs over geological time scales. In contrast, anthropogenic eutrophication, caused by nutrient-rich wastewater discharge and agricultural run-off, may occur over a period of years or decades. Eutrophication leads to an increase in biochemical oxygen demand, producing seasonal anoxic conditions in the bottom waters and temporary displacement of aerobic organisms. Although anthropogenic eutrophication is most commonly associated with lakes, eutrophication in estuaries and coastal waters has become a concern within the last few decades (Jaworski, 1981; McErlean and Reed, 1981; Rosenberg, 1985). Eutrophication in estuaries, resulting from nutrient enrichment, has been reported worldwide including Kanehoe Bay in Hawaii (Smith, 1981), Peel-Harvey Estuary in Australia (McComb *et al.*, 1981) Chesapeake Bay (D'Elia, 1987), and the Baltic Sea (Graneli *et al.*, 1990).

Past studies in Puget Sound, however, suggest a different scenario. Primary productivity in Puget Sound is generally considered moderate to high relative to other estuaries (Thom *et al.*, 1988). Studies in southern and central Puget Sound have shown this high productivity is not nutrient limited (Campbell, 1977). According to Winter *et al.* (1975), phytoplankton in the central basin of Puget

Sound are limited primarily by vertical advection and turbulence, sinking of algal cells, and changes in light intensity, but rarely by nutrients. More recently, however, Rensel (1991) reported water quality problems in certain estuarine and innerbay areas of Puget Sound. These problems were associated with nutrient enrichments and phytoplankton blooms which can cause depleted dissolved oxygen levels and massive fish kills. Thom *et al.* (1988) indicate that increases in nutrients from agricultural and sewage discharges cause phytoplankton blooms and eutrophic conditions in localized areas in southern Puget Sound (Figure 1). For example, during the summer of 1985, conditions in Fautleroy Cove, an embayment in central Puget Sound, were indicative of localized eutrophication (Thom *et al.*, 1988). Eutrophication and oxygen depletion were reported in 1985 in Budd Inlet, also in central Puget Sound (Boatman, 1988). This study implicated algal blooms, resulting from nitrogen-rich secondary wastewater treatment discharge, as the cause of the oxygen depletion. Thom and Albright (1990) suggest that shallow, nearshore systems in Puget Sound may be more susceptible to the effects of anthropogenic nutrient loading from May to October when light is not limiting.

The control of eutrophication in Puget Sound will require management strategies for reduction of nutrient inputs (Boatman, 1988; Thom *et al.*, 1988). Before effective

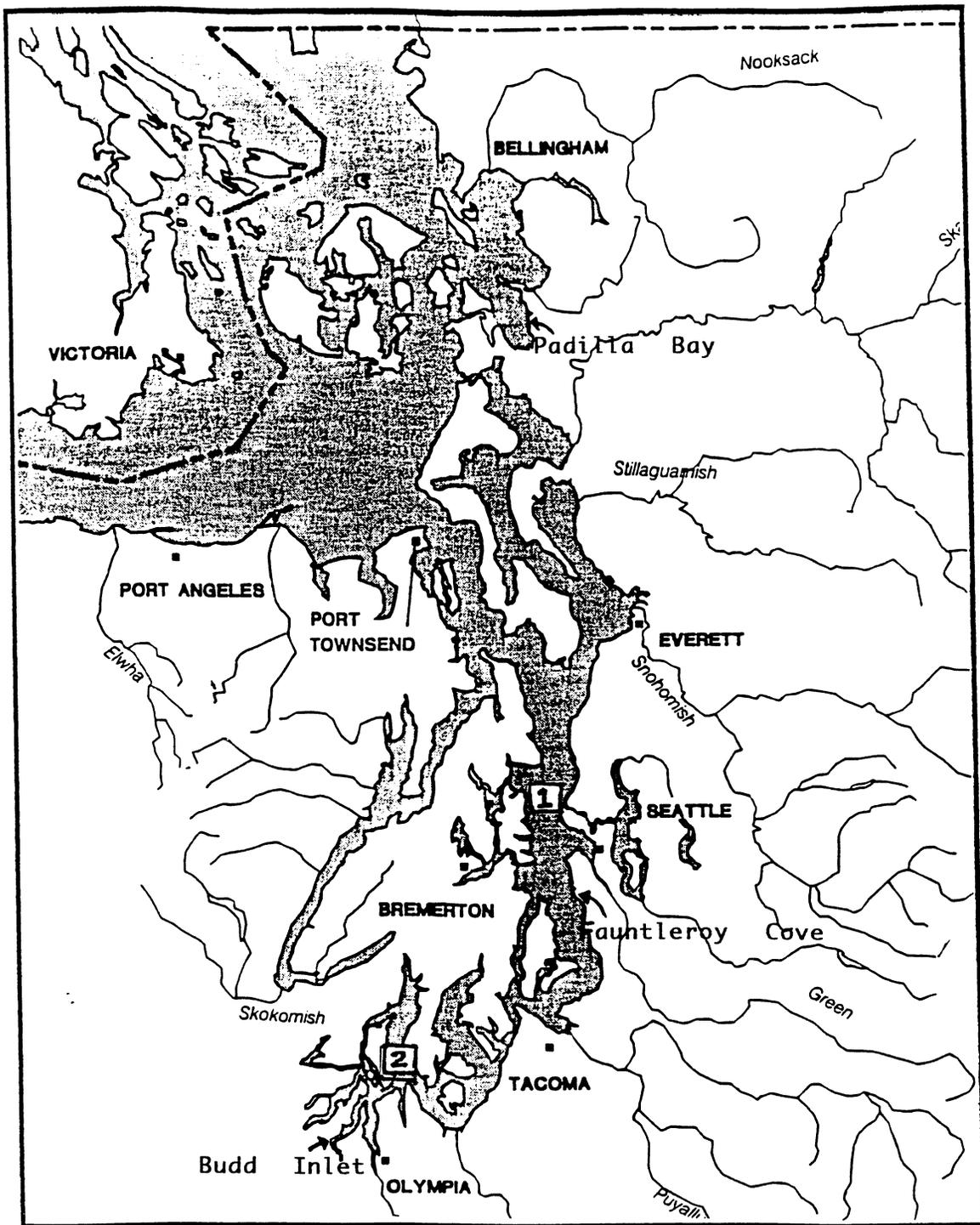


Figure 1. Map of Puget Sound showing location of study site (Padilla Bay) in relation to other study sites in the Sound. Sites 1 and 2 are those studied by Winter *et al.* (1975). Eutrophic conditions have been reported in Budd Inlet (Boatman, 1988) and Fautleroy Cove (Thom *et al.*, 1988).

management practices can be adopted, however, more studies of the effects of nutrient loading are needed for localized embayments (Thom *et al.*, 1988; Rensel, 1991). Dissolved inorganic nutrient concentrations in surface water from a previous 12-month water quality study in Padilla Bay (Cassidy and McKeen, 1986) indicate the potential for nitrogen limitation of phytoplankton growth during most of the year. These results helped to form the hypothesis that phytoplankton in Padilla Bay are nitrogen limited during the summer. The primary objective of this study was to examine the effects of nutrient enrichment on the growth of natural phytoplankton assemblages in Padilla Bay. A secondary objective was to compare the responses of nanoplankton and total phytoplankton to nutrient enrichments.

Methods

Study Site Description

Experiments were conducted at Padilla Bay National Estuarine Research Reserve near Mount Vernon, Washington. All samples were collected from a mid-channel station in Padilla Bay $-122^{\circ} 29' 30''$ W and $48^{\circ} 28' 53''$ N (Figure 2). The depth at the site varied from 3 to 7 m depending on the tide. Padilla Bay is a shallow embayment surrounded by agricultural and industrial land. The seagrass-dominated estuary contains over 10,000 acres of intertidal and subtidal mudflats and the largest contiguous seagrass meadows in Washington state (Bulthuis, 1991). Freshwater inputs come from several agricultural drainage sloughs and the Swinomish Channel which carries water from the Skagit River northward to Padilla Bay.

Water quality

Water quality parameters were measured during each nutrient enrichment experiment from June to October, 1992. Temperature and total irradiance were measured at one meter intervals from surface to bottom at the study site daily during each incubation, except during June when only irradiance was measured. Dissolved oxygen and salinity

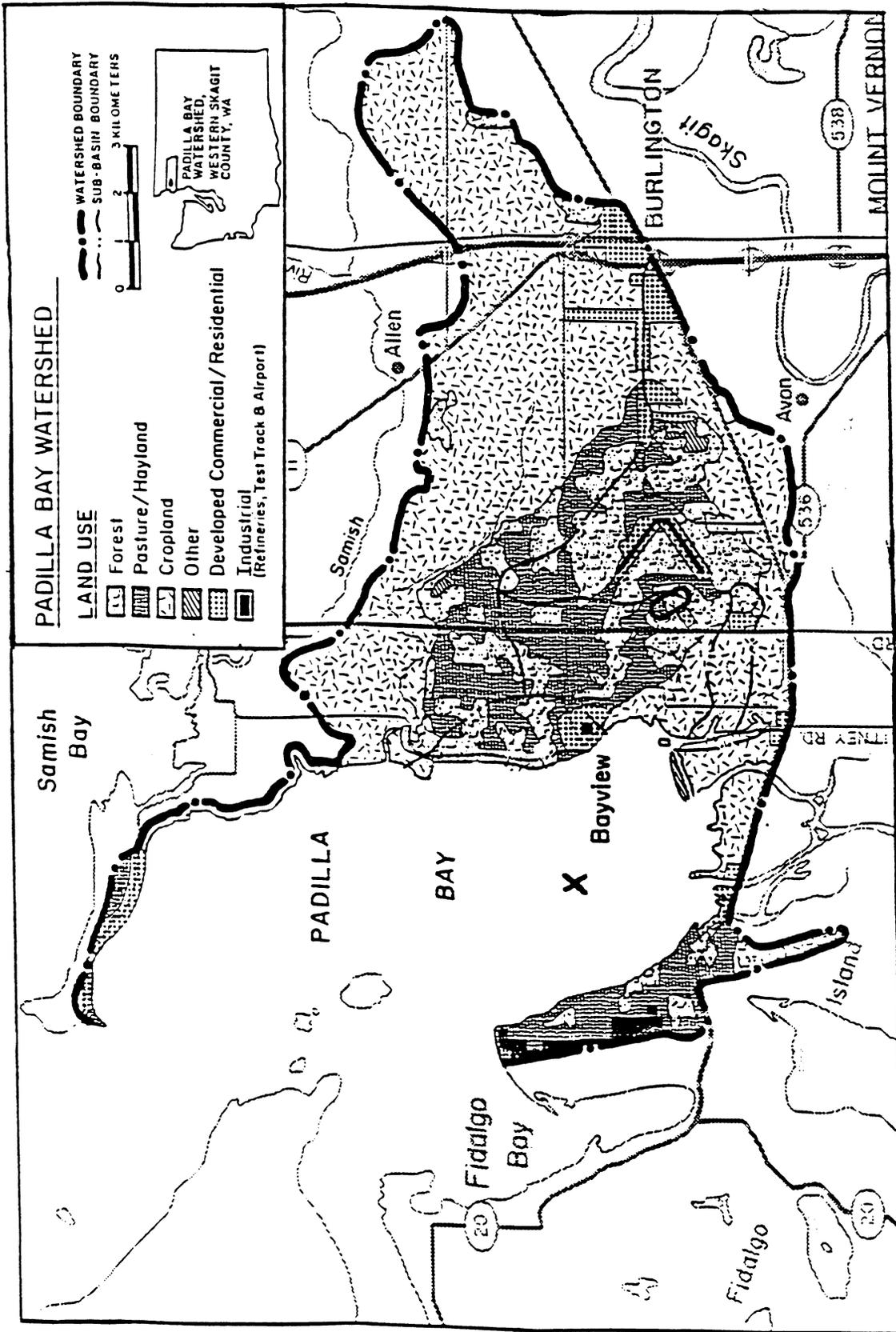


Figure 2. Study site (X) in Padilla Bay and surrounding watershed.

measurements were taken at one meter intervals at the study site on the first day of each incubation and every day during the incubations in late August, September, and October. Temperature and salinity were measured with a YSI model 33 salinity-conductivity-temperature meter. Dissolved oxygen was measured with a YSI model 57 oxygen meter. Total irradiance was determined with two LiCor sensors and a LiCor LI-1000 Data Logger. Above water irradiance was measured with a cosine quantum sensor. A 4π quantum sensor was used for underwater measurements. One 60 second average (above water) and one 10 second average (underwater) irradiance measurements were taken for each depth. During all incubations except August 24-27, measurements were taken at midday. During August 24-27, sampling began in late afternoon. Hourly percent cloud cover data for each incubation was obtained from Port of Skagit Airport, Burlington, Washington.

On the first day of each incubation, surface, mid, and bottom water samples were collected with a 3-liter Van Dorn bottle and analyzed for chlorophyll *a* and dissolved inorganic nutrient concentrations (nitrate [$\text{NO}_3 + \text{NO}_2$], ammonium [NH_4], phosphate [PO_4], and silicate [SiO_2]). Water samples for nutrient analysis were filtered through Whatman GF/F glass fiber filters ($0.7 \mu\text{m}$ nominal pore size) in the field. Samples were stored in acid-washed bottles on ice in the dark

for 2-3 hours during transportation to the lab. Upon return to the lab, samples were frozen at -20°C . Nutrient concentrations were determined using the methods of Parsons *et al.* (1984). Water samples were analyzed within 2 weeks after collection. The following ranges of standards were used: nitrate (6-50 $\mu\text{M-N}$); ammonium (0.25-6.0 $\mu\text{M-N}$); phosphate (0.15-4.8 $\mu\text{M-P}$); silicate (3-100 $\mu\text{M-Si}$). All nutrient concentrations reported are within the detection limits of the methods used.

One liter water samples for chlorophyll analysis were buffered with MgCO_3 , filtered in the laboratory onto Whatman GF/F glass fiber filters, and frozen at -20°C immediately. Within two weeks of collection, filters were homogenized in approximately 15 mL of 90% acetone and extracts stored overnight at 4°C in the dark. Samples were centrifuged for 10 minutes and supernatants were read at 630 nm, 647 nm, 664 nm and 750 nm (for turbidity) in a 1 cm cell with a Hewlett Packard model 8452A diode array spectrophotometer. Chlorophyll *a* concentrations were calculated using the spectrophotometric equation for chlorophyll *a* of Parsons *et al.* (1984). Samples were not acidified.

Nutrient enrichment bioassays

Experiments were conducted from June to October, 1992. Water samples were pumped from 0.5 - 1.0 m below the surface

through a 63 μ m sieve into a 30 gallon container from which 2.2 liter samples were pumped into acid-washed 2.5 liter polycarbonate bottles. The bottles were then treated with the following additions: 1) 15 μ M NH₄, 2) 1.0 μ M PO₄, 3) 15 μ M SiO₂, 4) 15 μ M NH₄ + 1.0 μ M PO₄, 5) a trace metal solution containing: 0.08 μ M zinc, 0.9 μ M manganese, 0.03 μ M molybdenum, 0.05 μ M cobalt, 0.04 μ M copper, 11.7 μ M iron, and 11.7 μ M EDTA (metal concentrations were similar to the concentrations in f/2 marine growth media [McLachlan, 1979]), 6) 15 μ M NH₄ + 1.0 μ M PO₄ + 15 μ M SiO₂ + trace metals and 7) an unenriched control. Table 1 lists the treatments included in each experiment. Four replicates of each treatment were incubated *in situ* from a floating array (Figure 3). Bottles were weighted to hang approximately 0.5 - 1.0 m below the surface. 15 mL subsamples from each bottle were collected daily and brought back to Shannon Point Marine Center where *in vivo* chlorophyll fluorescence was measured using a Turner 112 fluorometer with a CS 5-60 filter for excitation and a CS 2-64 filter for emission (Lorenzen, 1966). Subsamples were kept dark until read. Nutrient enrichment experiments were terminated when a decrease or a slowing of the increase of *in vivo* fluorescence in the control was observed. Experiments ranged from 4 to 13 days. Final chlorophyll *a* concentrations were determined spectrophotometrically as described above

Table 1. Treatments included in each experiment from June to October, 1992. Additions were 15 μ M NH₄ (N), 15 μ M NO₃ (NO₃), 1.0 μ M PO₄ (P), 15 μ M SiO₃ (Si), and trace metals (Tm).

Treatment	Incubation period					
	June 9-14	July 6-10	Aug 3-7	Aug 24-27	Sept 7-14	Oct 7-19
<u>basic expts:</u>						
control	X	X	X	X	X	X
N	X	X	X	X	X	X
P	X	X	X		X	
N + P	X	X	X	X	X	X
Si	X	X	X		X	
Tm	X	X	X			
N+P+Si+Tm	X	X	X ^a			
N+P+Si			X	X	X	
<u>< 20 μm expts:</u>						
control			X	X		X
N			X	X		X
N+P				X		X
N+P+Si				X		
<u>Nitrate expts:</u>						
control					X ^a	X ^a
NH ₄					X ^a	X ^a
NO ₃					X ^a	X ^a

^aindicates the use of one liter instead of 2.5 liter bottles

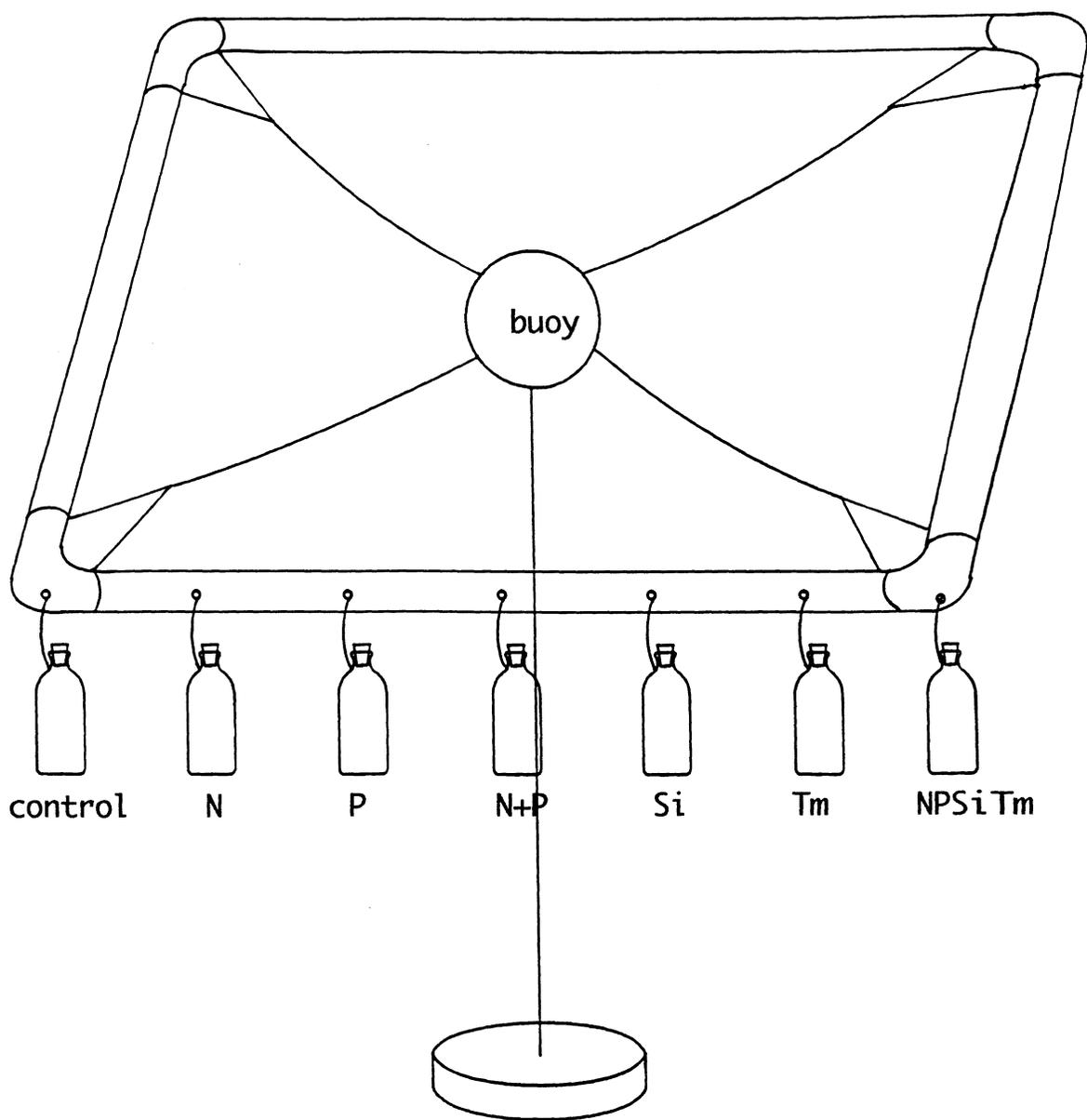


Figure 3. Typical bottle arrangement on the sampling apparatus. Bottles were weighted and hung approximately 0.5 - 1.0 m below the surface. The array was made with 2" PVC pipe drilled for bottle ties and filled with styrofoam for flotation. The apparatus was suspended from a stainless steel cable attached to a 80kg concrete anchor.

except the volume filtered varied from 200 to 800 mL depending on the chlorophyll *a* concentration.

During September and October, 1992 additional treatments were used to compare the differential response of phytoplankton growth to additions of nitrate and ammonium. These experiments included the following treatments: 1) 15 μM NO_3 , 2) 15 μM NH_4 , and 3) a control (Table 1). Samples were treated the same as described above with the following exceptions: 1) samples were placed in 1 liter polycarbonate bottles, and 2) each treatment had three replicates.

Size-Fractionation experiments

In September, 1992, initial and final water samples from the incubation were divided into two subsamples. One sample was passed through a 20 μm Nitex screen to remove netplankton. The other sample was not filtered. Both samples were analyzed for chlorophyll *a* as described above.

In August and October, experiments included a size-fractionation study. Water was collected in the same manner as the nutrient enrichment bioassays as described above. Half of the volume from the 30 gallon container was filtered through a 20 μm Nitex screen to remove the netplankton. Only those nutrients that produced a positive response in the earlier experiments (June and July) were added. These included: 1) 15 μM NH_4 , 2) 15 μM NH_4 + 1.0 μM PO_4 , 3) 15 μM NH_4

+ 1.0 μM PO_4 + 15 μM SiO_2 , and 4) an unenriched control (Table 1).

Data Analysis

The research was designed so that final chlorophyll *a* data could be analyzed by ANOVA, as a systematic block design with the significance level set at 5%. Tukeys Honestly Significant Difference Multiple comparison test was used to determine pairwise differences.

Results

Water quality and environmental conditions

Ambient chlorophyll *a* concentrations in surface waters are shown in Figure 4. Chlorophyll *a* concentrations were below $2 \mu\text{g L}^{-1}$ during all experiments except late August when the chlorophyll *a* concentration was $3.6 \mu\text{g L}^{-1}$.

Dissolved inorganic nutrient concentrations in surface waters at the study site fluctuated throughout the study (Figure 5). Ambient silicate concentrations (SiO_2) decreased by almost 40% from July 6 to August 3. Highest SiO_2 concentrations were measured in July and October (31 and $25 \mu\text{M-Si}$, respectively). During the rest of the months, concentrations were below $25 \mu\text{M-Si}$. Nitrate + nitrite concentrations [$\text{NO}_3 + \text{NO}_2$] were highest in July ($16 \mu\text{M-N}$) and lowest on both sampling dates in August ($8 \mu\text{M-N}$). Ammonium concentrations (NH_4) ranged from $3.2 \mu\text{M-N}$ in early June to less than $0.5 \mu\text{M-N}$ in October. Both [$\text{NO}_3 + \text{NO}_2$] and NH_4 concentrations decreased by more than 50% from July 6 to August 3. Soluble reactive phosphate concentrations (PO_4) showed an inverse relation to NH_4 concentrations. PO_4 concentrations ranged from $1.1 \mu\text{M-P}$ in June to $2.5 \mu\text{M-P}$ in September.

Dissolved inorganic nitrogen ($\text{DIN} = \text{NO}_3 + \text{NO}_2 + \text{NH}_4$) to PO_4 ratios (by atoms) were calculated for surface water at

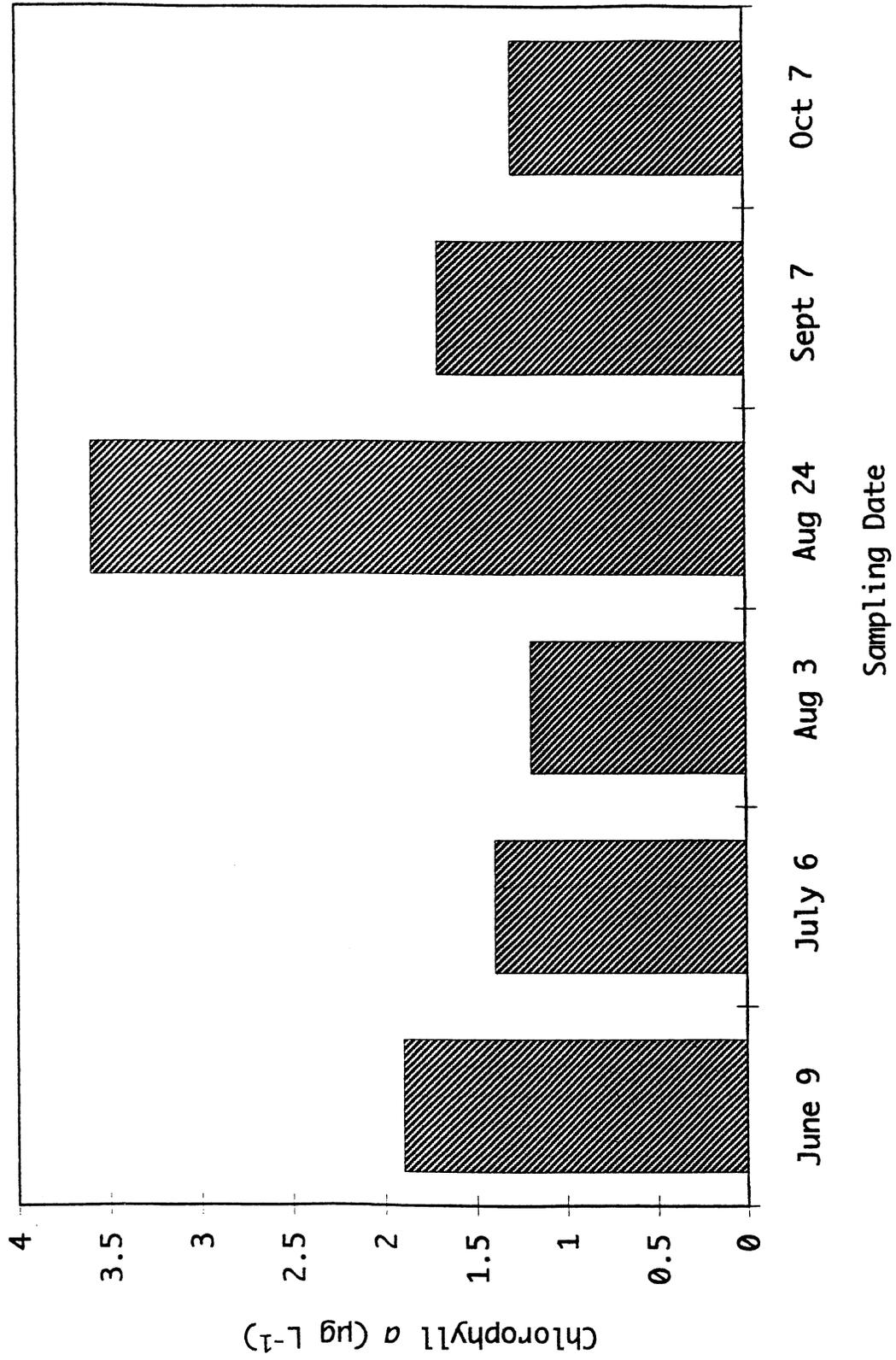


Figure 4. Temporal distribution of ambient chlorophyll *a* concentrations in surface water at the study site in Padilla Bay, 1992.

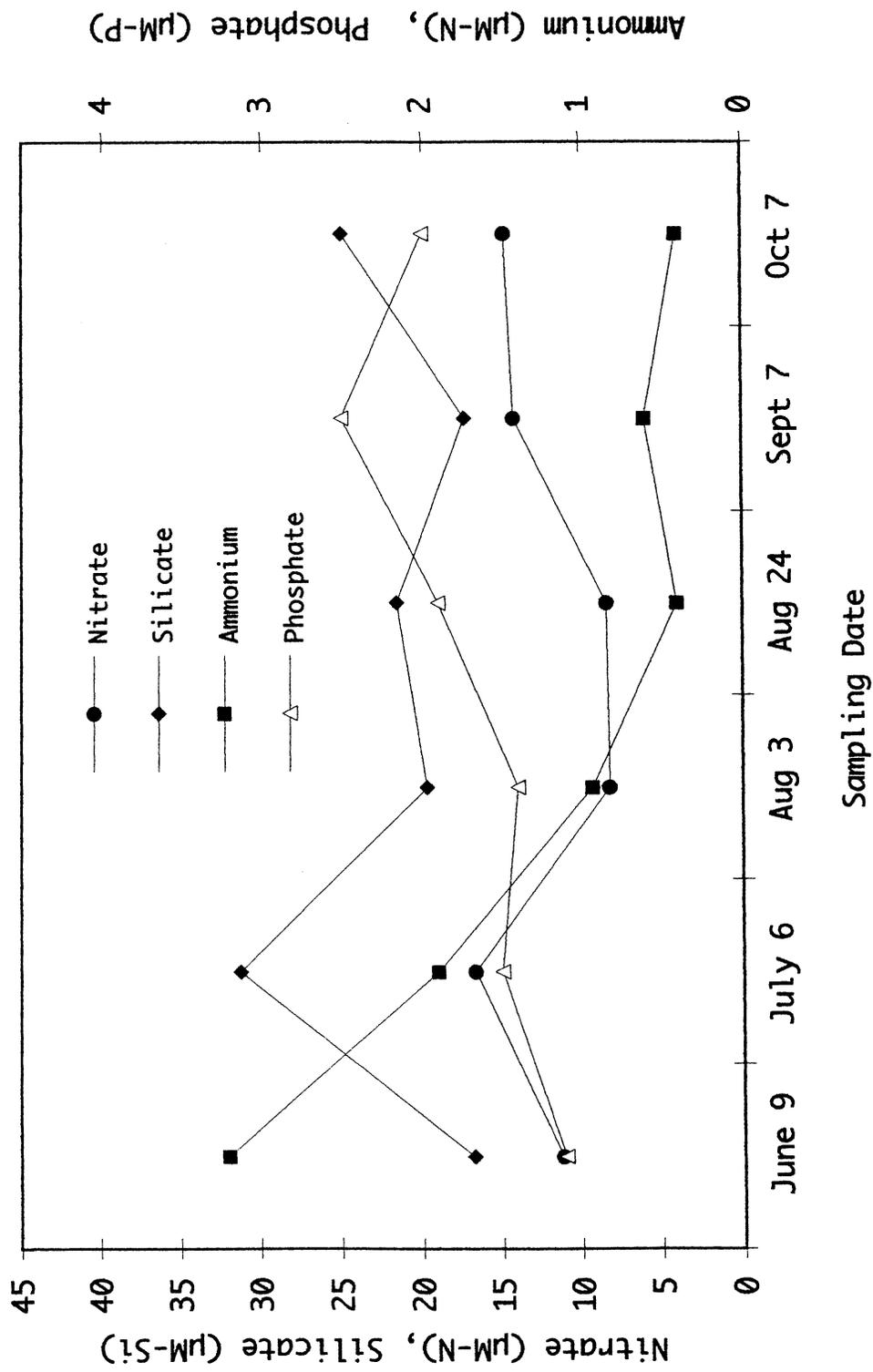


Figure 5. Dissolved inorganic nutrient concentrations of surface water at the study site in Padilla Bay, 1992.

the study site (Figure 6). During June and July, the DIN/PO₄ ratios were between 25:1 and 30:1 which are greater than the Redfield ratio of 16:1. DIN/PO₄ ratios were less than 16:1 for all other sampling dates.

Table 2 shows the ranges of ambient light, temperature, dissolved oxygen, and salinity measurements at 1 meter during each incubation. In general, temperatures showed an inverse relationship to cloud cover and a direct relationship to light intensity. Highest temperatures were recorded during late August (13.2-16.2°C) when cloud cover was the least. The lowest temperatures were recorded during October (9.2-11.6°C). In October, the highest light intensity measurement (600 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was less than half of the highest measurement during any other month. Generally, temperatures showed a significant negative correlation to tidal height (Table A1 in Appendix A). Dissolved oxygen ranged from 6.8 - 11.2 mg L⁻¹, with the highest concentration in early August and the lowest in July and October. Salinities fluctuated within a range of 26.3 - 31.0 psu and did not show any correlation with tides (Table A1 in Appendix A).

Figures 7-12 show depth profiles for all measured parameters (temperature, salinity, dissolved oxygen, dissolved inorganic nutrients, and chlorophyll *a*) on the first day of each incubation. Based on the profiles of salinity and temperature, the water column was isothermal and

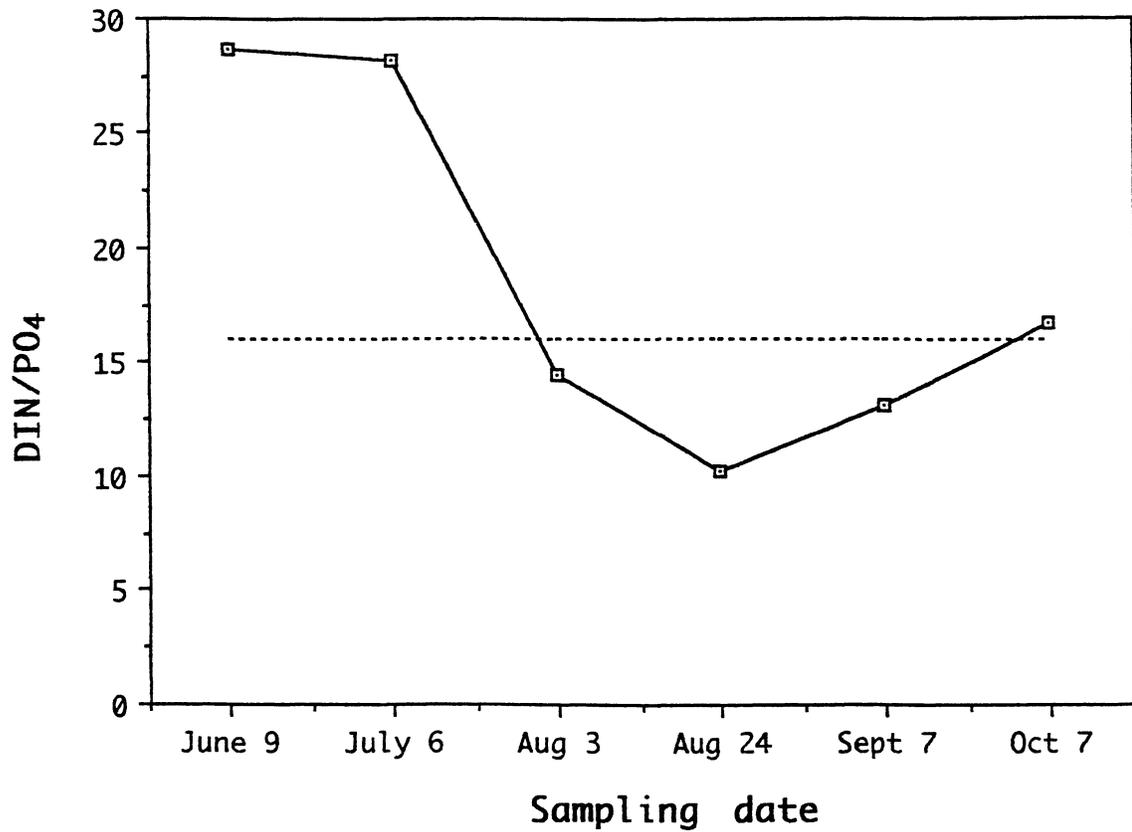


Figure 6. DIN/PO₄ ratios in surface water at the study site in Padilla Bay, 1992. DIN is NO₃ + NO₂ + NH₄. PO₄ is soluble reactive phosphate. The dotted line indicates the Redfield ratio of 16:1.

Table 2. Ranges of temperature, light, dissolved oxygen, and salinity at 1 meter at the study site in Padilla Bay from June to October, 1992. Values represent one measurement taken daily.

Dates	Temperature (°C)	Light $\mu\text{mol m}^{-2} \text{s}^{-1}$	DO (mg L ⁻¹)	Salinity (psu)
June 9-14	11.2*	166-1927	7.6*	29.1*
July 6-10	11.9-15.1	221-1529	6.8*	29.0*
Aug 3-7	11.9-14.5	324-1703	11.2*	28.5*
Aug 24-27	13.2-16.2	41-1683	6.4-9.0	28.2-29.1
Sept 7-14	11.8-14.6	41-1405	7.0-7.6	28.2-31.0
Oct 7-19	9.2-11.6	30-600	6.8-8.1	26.3-30.2

*only one measurement taken on the first day of the incubation

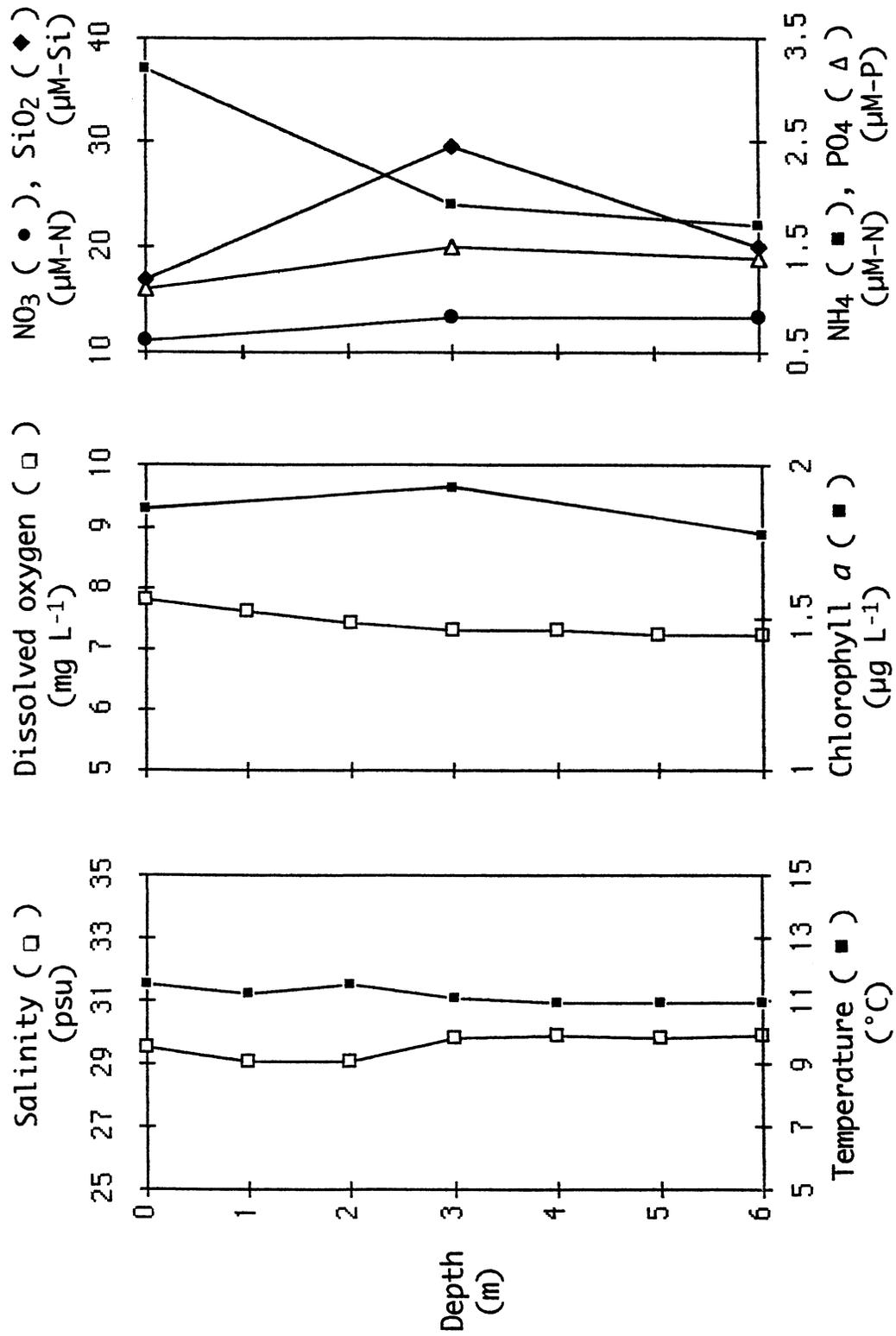


Figure 7. Vertical profiles of certain physical, biological, and chemical properties at the study site in Padilla Bay on June 9, 1992.

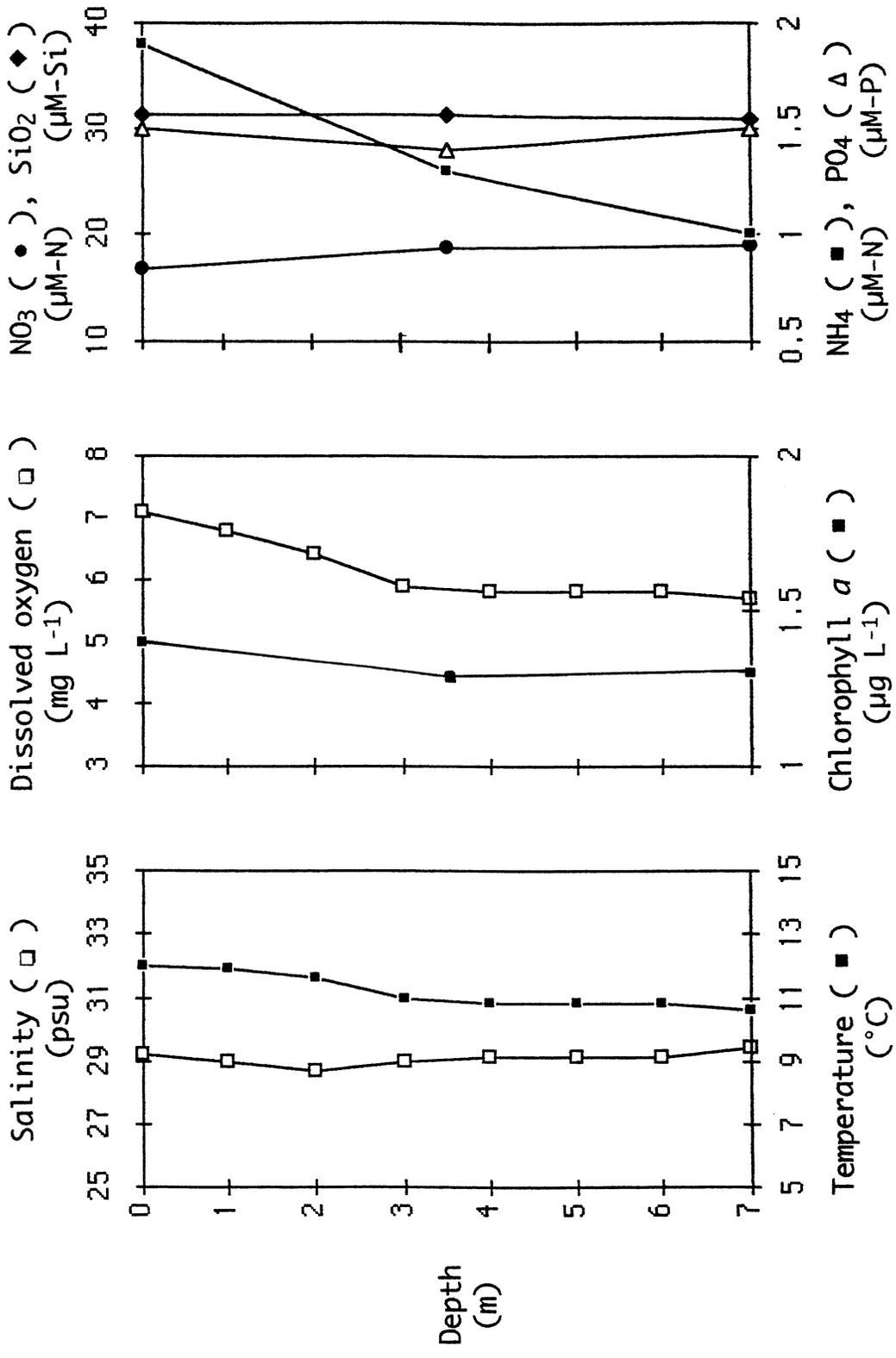


Figure 8. Vertical profiles of certain physical, biological, and chemical properties at the study site in Padilla Bay on July 6, 1992.

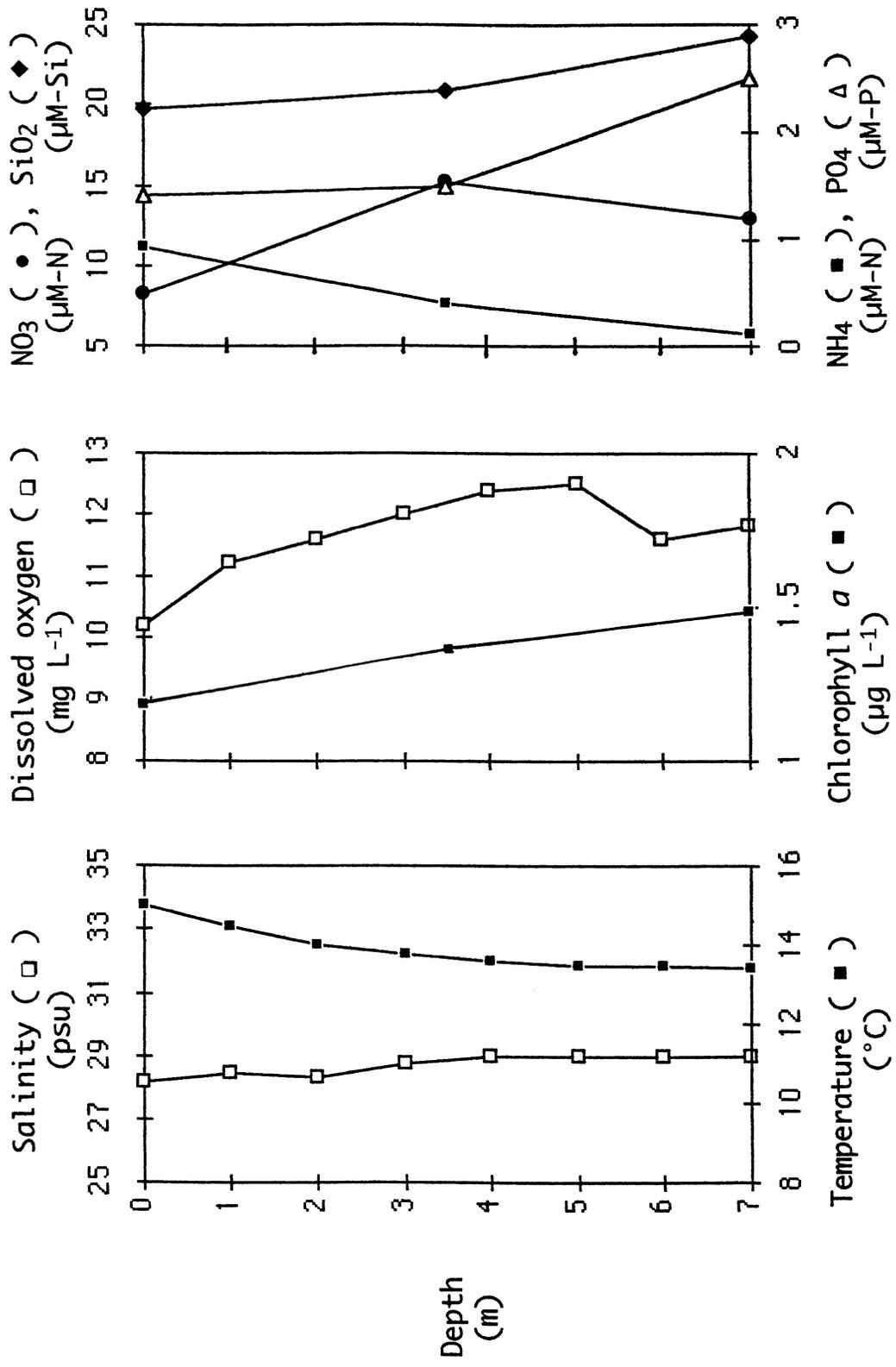


Figure 9. Vertical profiles of certain physical, biological, and chemical properties at the study site in Padilla Bay on August 3, 1992.

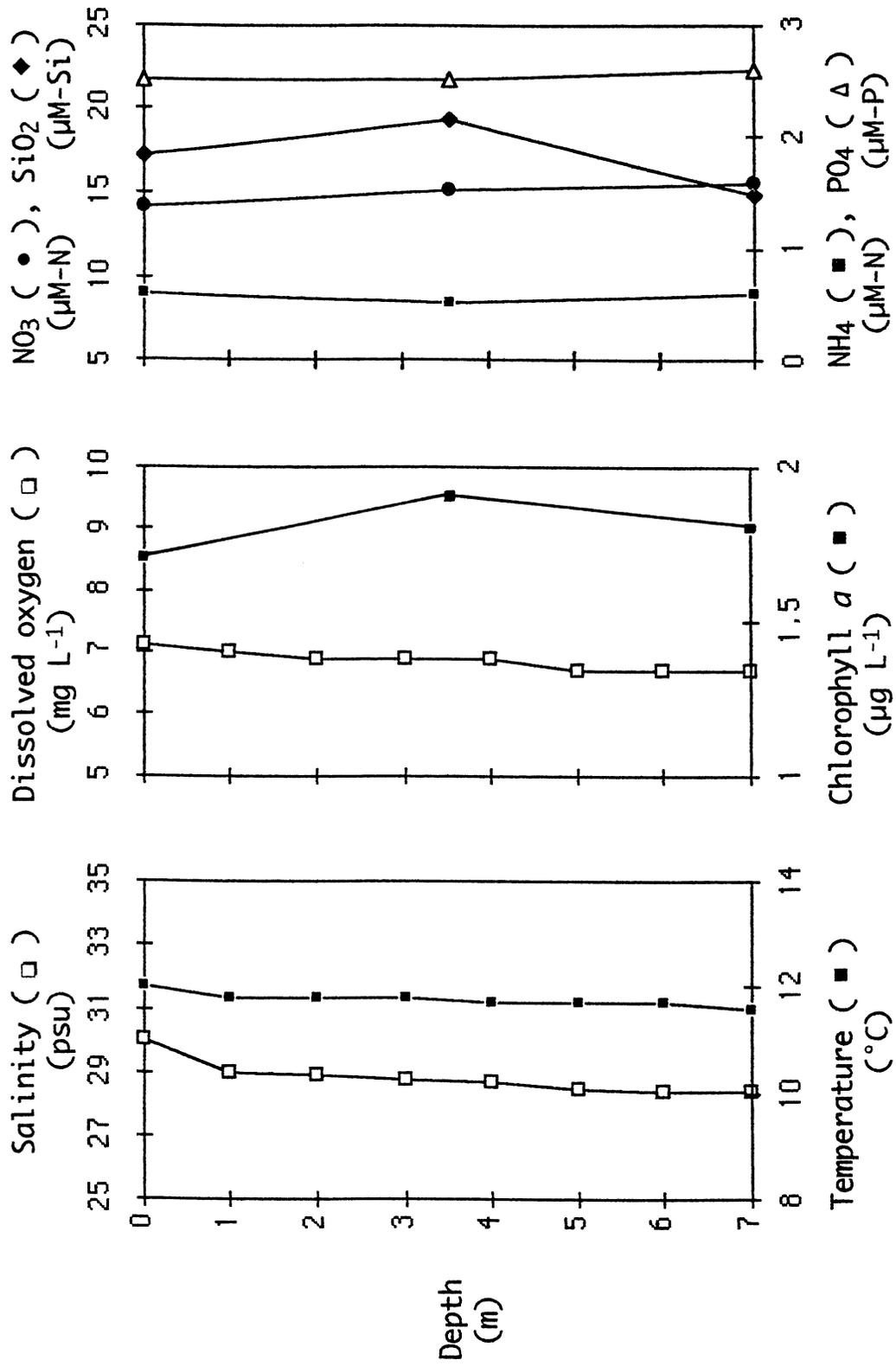


Figure 11. Vertical profiles of certain physical, biological, and chemical properties at the study site in Padilla Bay on September 7, 1992.

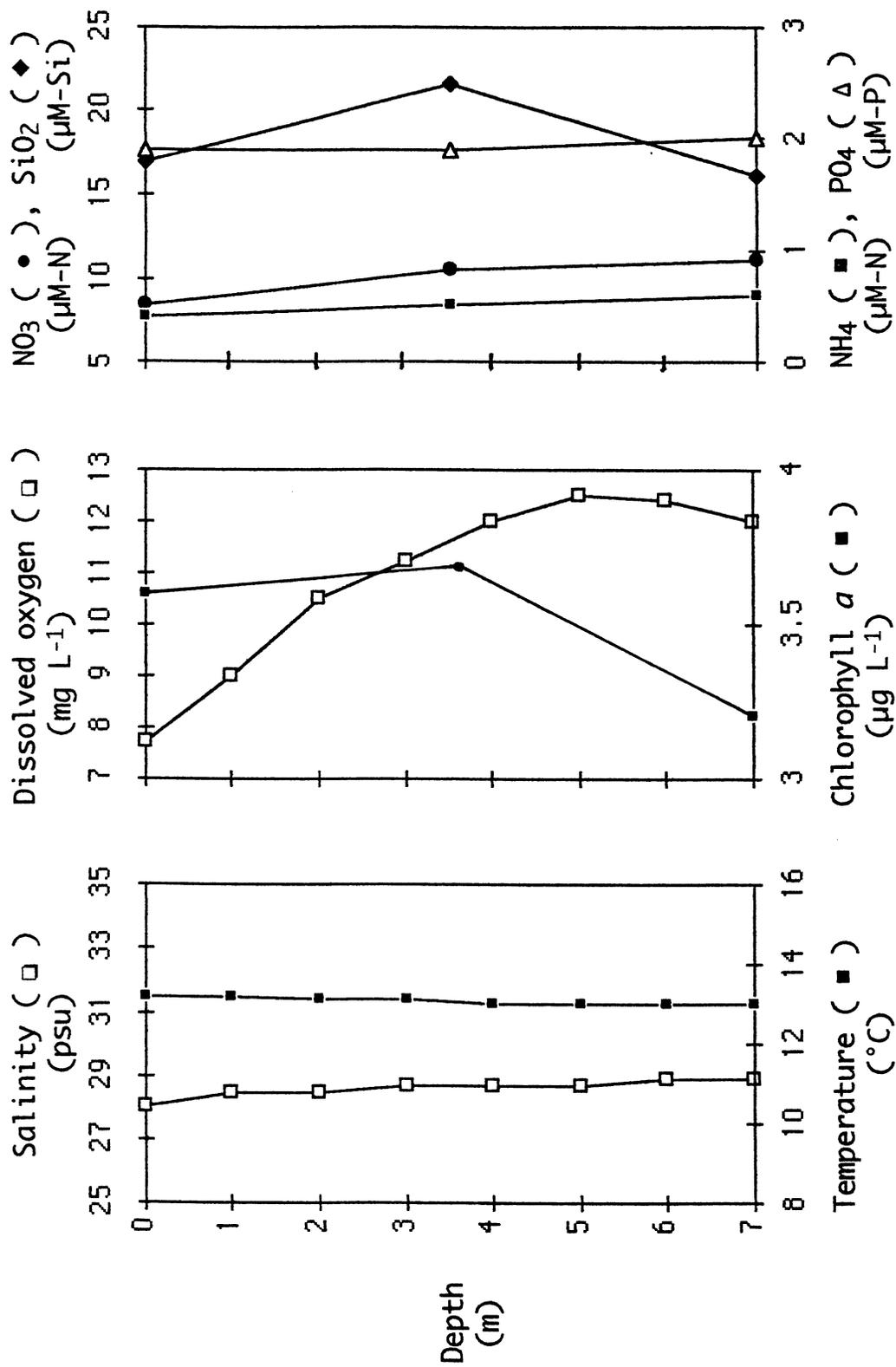


Figure 10. Vertical profiles of certain physical, biological, and chemical properties at the study site in Padilla Bay on August 24, 1992.

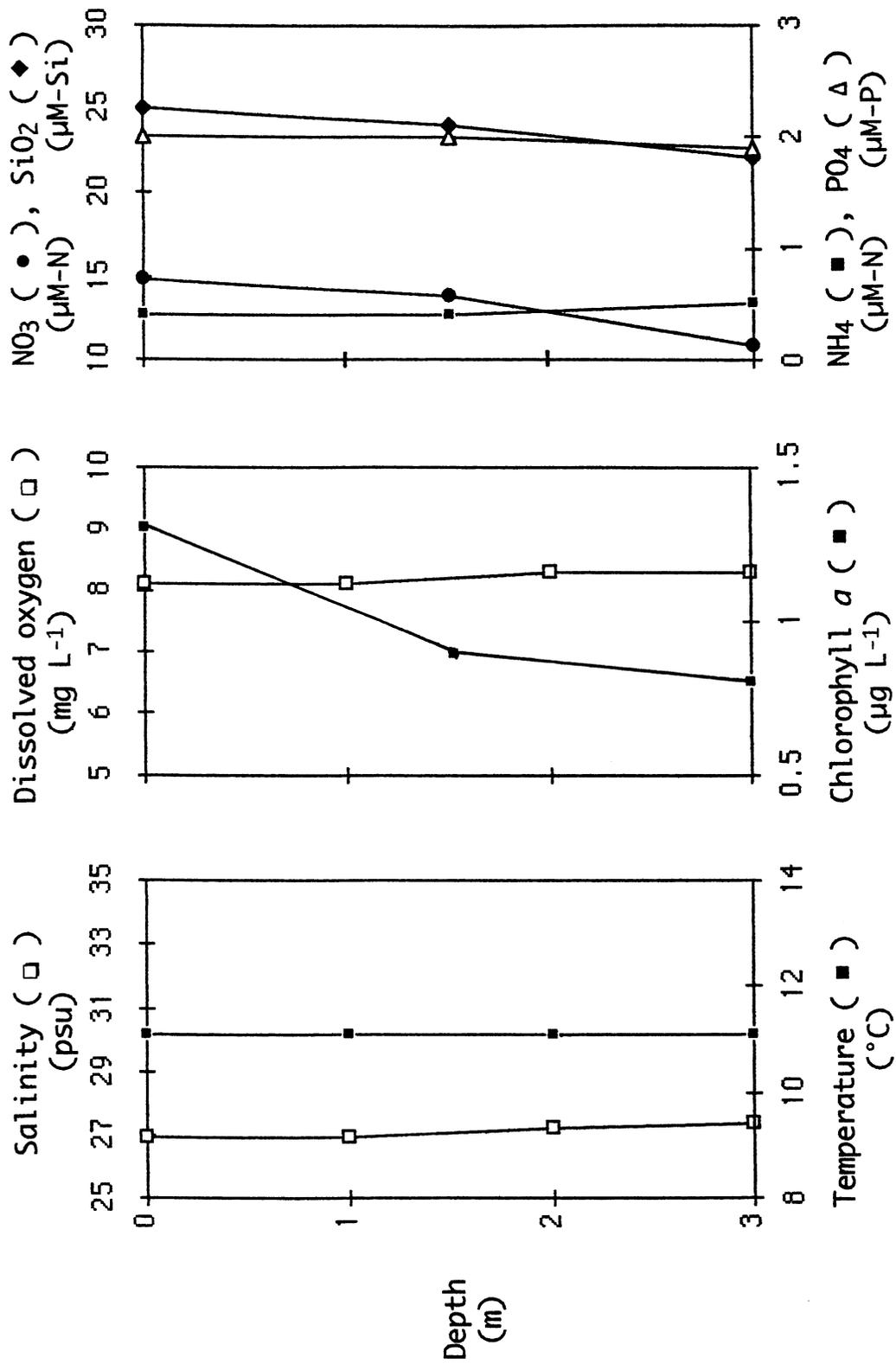


Figure 12. Vertical profiles of certain physical, biological, and chemical properties at the study site in Padilla Bay on October 7, 1992.

isohaline at the time of sampling on all of the dates. Dissolved oxygen concentrations (DO) showed no stratification on June 9, September 7, or October 7. On July 6, however, dissolved oxygen concentrations were highest at the surface, decreased slightly to 3 meters and leveled off. On August 3 and 24, DO concentrations reached levels of supersaturation, with highest concentrations at 5 meters. Chlorophyll *a* concentrations changed less than 20% from surface to bottom on all sampling dates except October 7 (38% change from surface to bottom). On all sampling dates except August 3 and September 7, lowest concentrations of chlorophyll *a* were at the bottom. On August 3 and September 7, chlorophyll *a* concentrations were lowest at the surface.

Vertical profiles of $[\text{NO}_3 + \text{NO}_2]$ and PO_4 concentrations (Figures 7-12) showed no stratification from surface to bottom for all sampling dates except on August 3 when both $[\text{NO}_3 + \text{NO}_2]$ and PO_4 concentrations were approximately 50% higher at the bottom than at the surface. NH_4 concentrations on the first three sampling dates were 2 to 6 times higher at the surface than at the bottom, but showed little change with depth on the last three sampling dates. SiO_2 concentrations were higher at mid depth on June 9, August 24, and September 7. On July 6 and October 7, there was no change in SiO_2 concentrations from surface to bottom, but on August 3, SiO_2 concentrations were higher at the bottom.

Nutrient enrichment bioassays

Results from *in vivo* chlorophyll fluorescence measurements are shown in Figures 13-19. There is a significant correlation ($r^2=0.9$) between *in vivo* chlorophyll fluorescence and extracted chlorophyll *a* concentrations (Figure 20). Accordingly, changes in relative fluorescence can be used to predict changes in actual chlorophyll *a* concentrations during the incubations. In all incubations except August 24, there was a delay or a very slow increase in fluorescence at the beginning of the incubation varying from 1 to 6 days. The longest lag period was in October (Figure 18). During this period, light levels were lower and surface water temperatures were cooler (Table 2). Additionally, ambient NH_4 concentrations were low (Figure 5).

In vivo chlorophyll fluorescence during the August 24-27 incubation differed from the other incubations (Figure 16). There was an immediate increase in fluorescence in all treatments on the first day. However, in the control treatment, this increase was followed by a decrease that continued until the experiment ended. By the last day of the incubation (August 27), fluorescence of the control treatment was less than the initial fluorescence.

Figure 21 shows the final chlorophyll *a* concentrations in each treatment from all six incubations. Using the Tukey

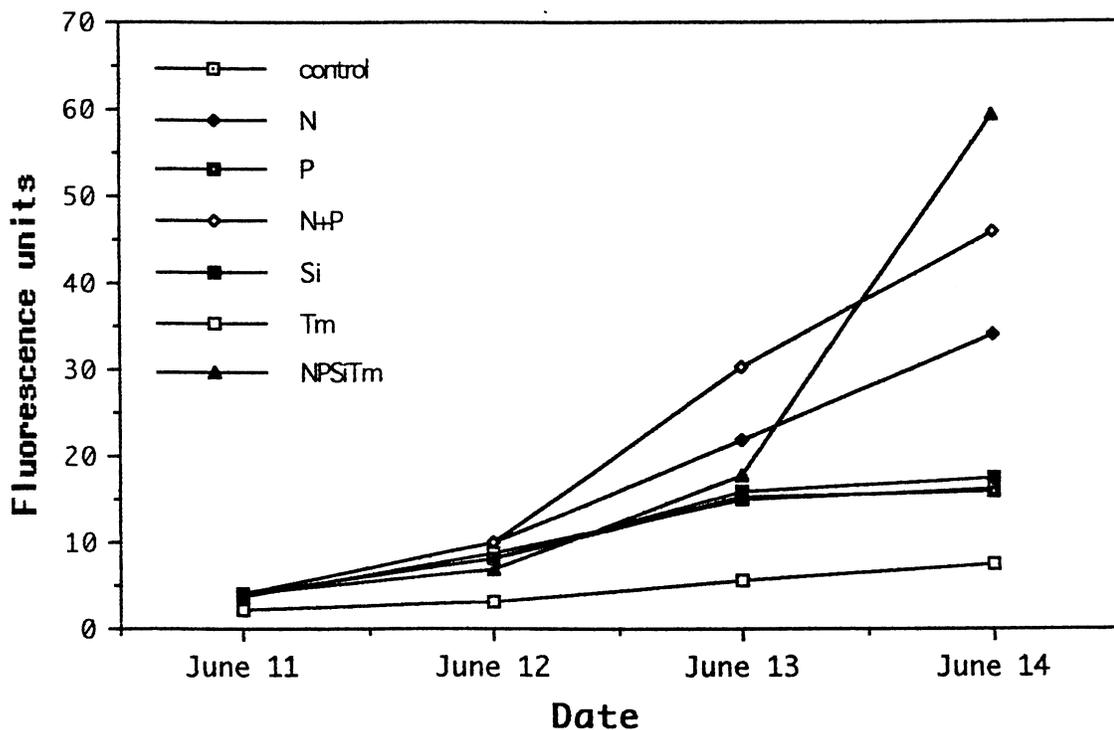


Figure 13. *In vivo* chlorophyll fluorescence during nutrient enrichment bioassays in June, 1992. Additions include 15 μM ammonium (N), 1.0 μM phosphate (P), 15 μM silicate (Si), trace metals (Tm), 15 μM ammonium + 1.0 μM phosphate (N+P), 15 μM ammonium + 1.0 μM phosphate + 15 μM silicate + trace metals (NPSiM), and an unenriched control. Each point represents the mean of four replicates. Coefficients of variation for measurements taken on June 14 are given in Table B1 in Appendix B.

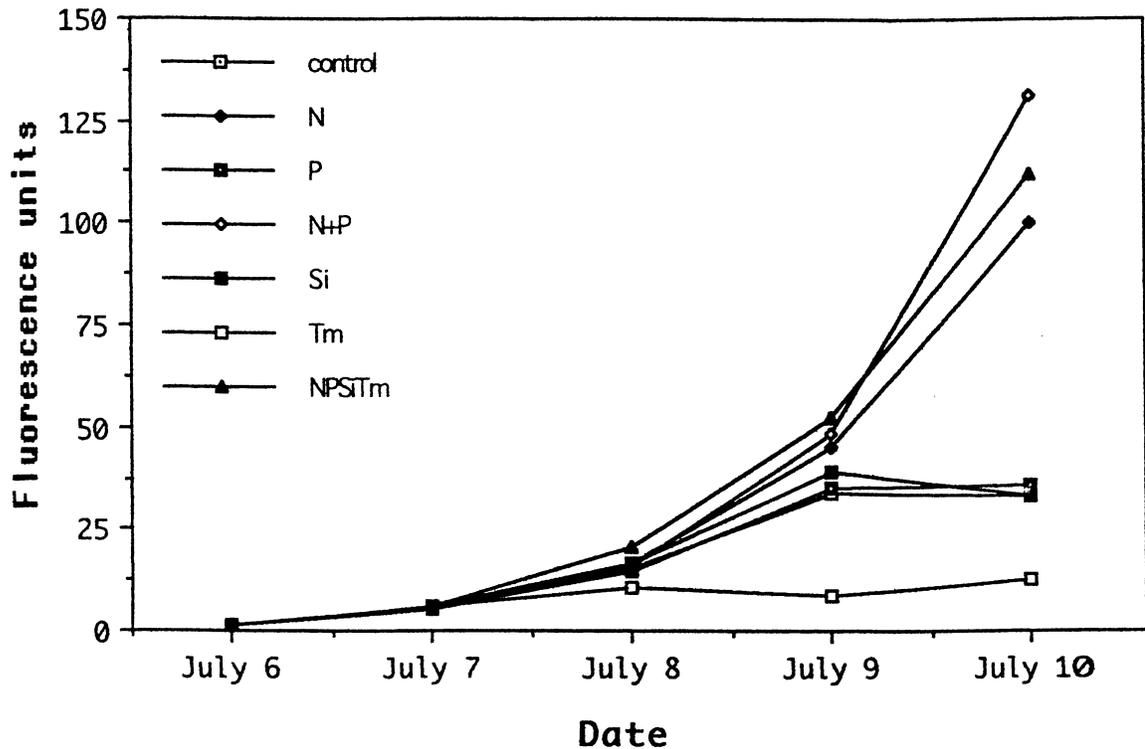


Figure 14. *In vivo* chlorophyll fluorescence during nutrient enrichment bioassays in July, 1992. Additions include 15 μM ammonium (N), 1.0 μM phosphate (P), 15 μM silicate (Si), trace metals (Tm), 15 μM ammonium + 1.0 μM phosphate (N+P), 15 μM ammonium + 1.0 μM phosphate + 15 μM silicate + trace metals (NPSiTm), and an unenriched control. Each point represents the mean of four replicates. Coefficients of variation for measurements taken on July 10 are given in Table B1 in Appendix B.

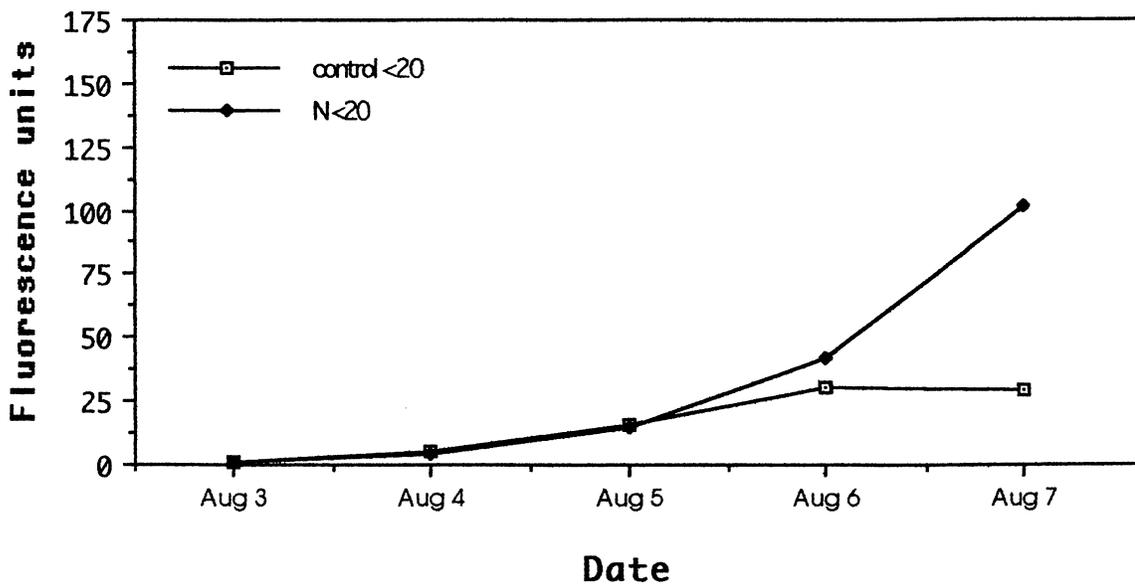
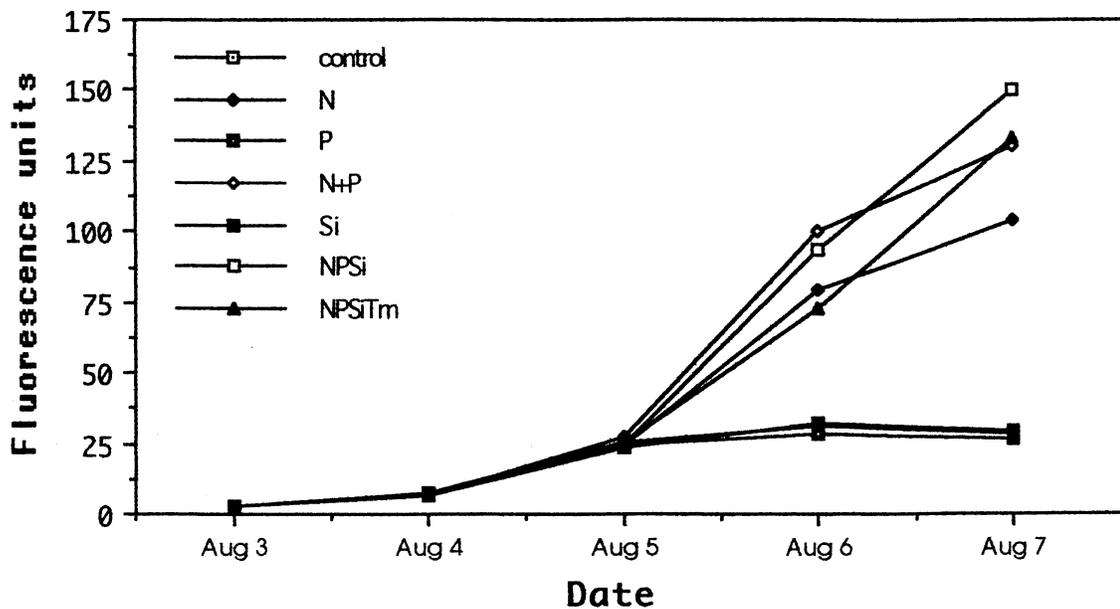


Figure 15. *In vivo* chlorophyll fluorescence during nutrient enrichment bioassays of whole water and < 20 μm fractions August 3-7, 1992. Additions include 15 μM ammonium (N), 1.0 μM phosphate (P), 15 μM silicate (Si), 15 μM ammonium + 1.0 μM phosphate (N+P), 15 μM ammonium + 1.0 μM phosphate + 15 μM silicate (NPSi), 15 μM ammonium + 1.0 μM phosphate + 15 μM silicate + trace metals (NPSiTm), and an unenriched control. Each point represents the mean of four replicates. Coefficients of variation for measurements taken on August 7 are given in Table B1 in Appendix B.

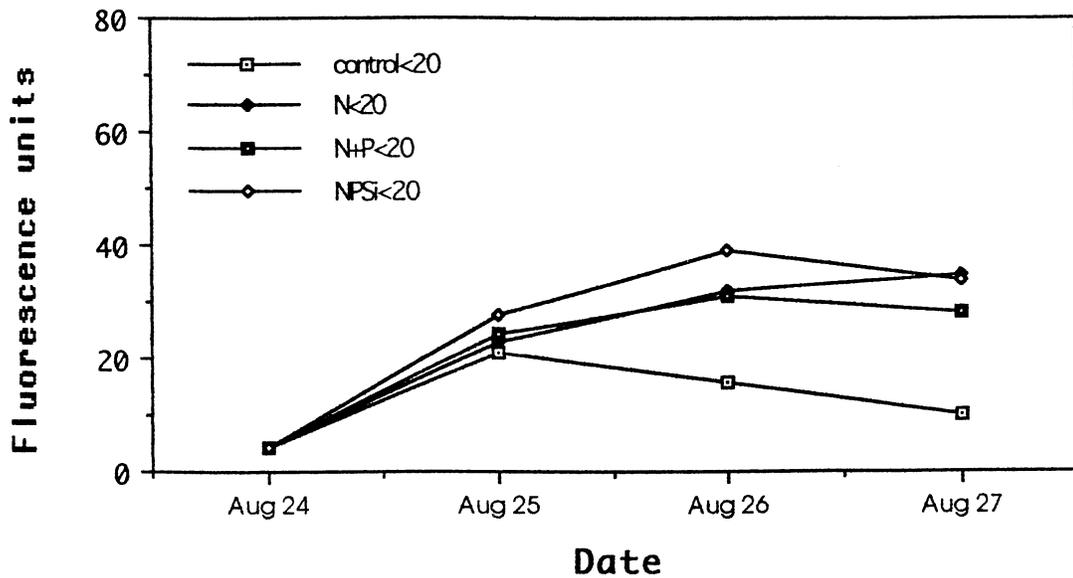
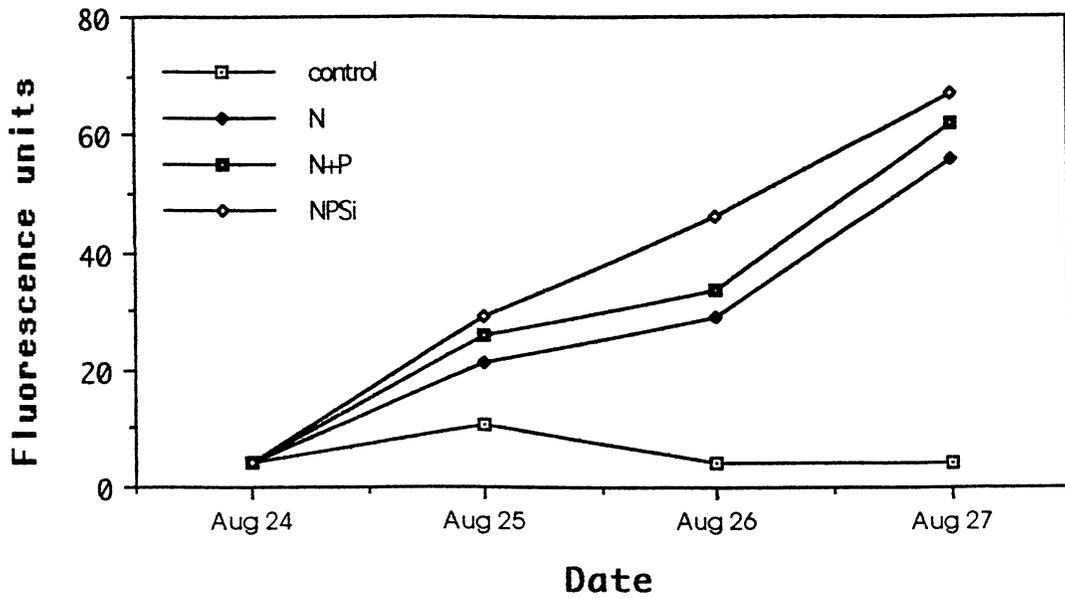


Figure 16. *In vivo* chlorophyll fluorescence during nutrient enrichment bioassays of whole water and < 20 μm fractions August 24-27, 1992. Additions include 15 μM ammonium (N), 15 μM ammonium + 1.0 μM phosphate (N+P), 15 μM ammonium + 1.0 μM phosphate + 15 μM silicate (NPSi), and an unenriched control. Each point represents the mean of four replicates. Coefficients of variation for measurements taken on August 27 are given in Table B1 in Appendix B.

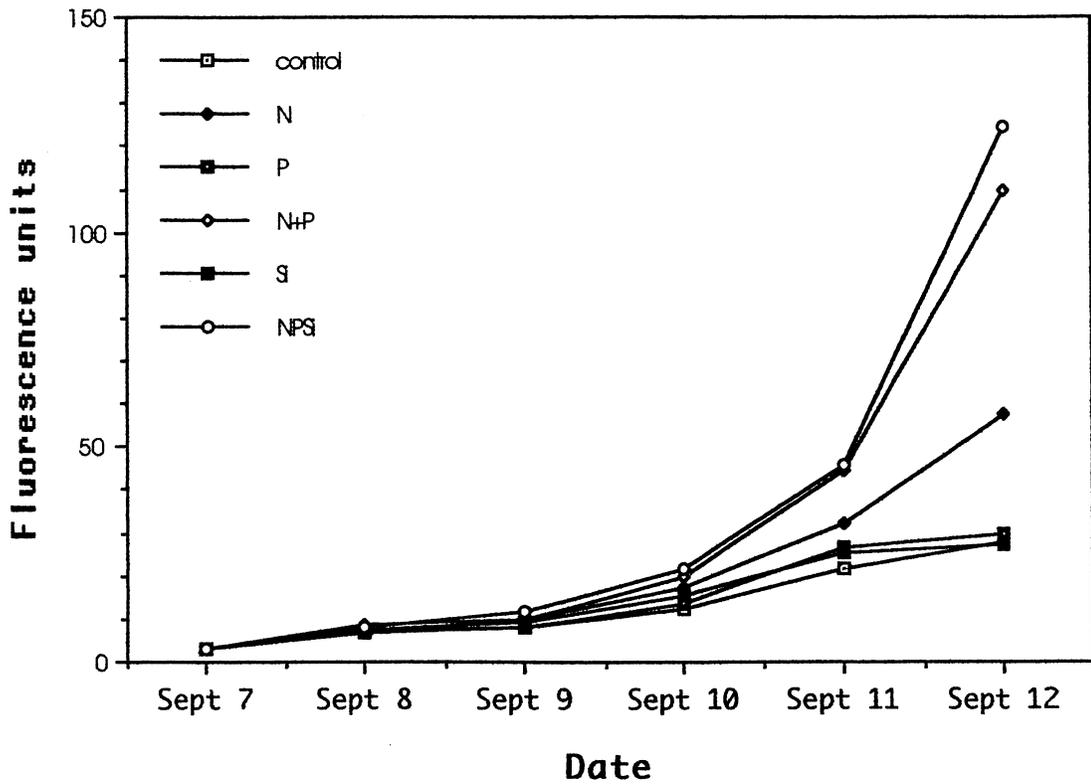


Figure 17. *In vivo* chlorophyll fluorescence during nutrient enrichment bioassays in September, 1992. Additions include 15 μM ammonium (N), 1.0 μM phosphate (P), 15 μM silicate (Si), 15 μM ammonium + 1.0 μM phosphate (N+P), 15 μM ammonium + 1.0 μM phosphate + 15 μM silicate (NPSi), and an unenriched control. Each point represents the mean of four replicates. Coefficients of variation for measurements taken on September 12 are given in Table B1 in Appendix B.

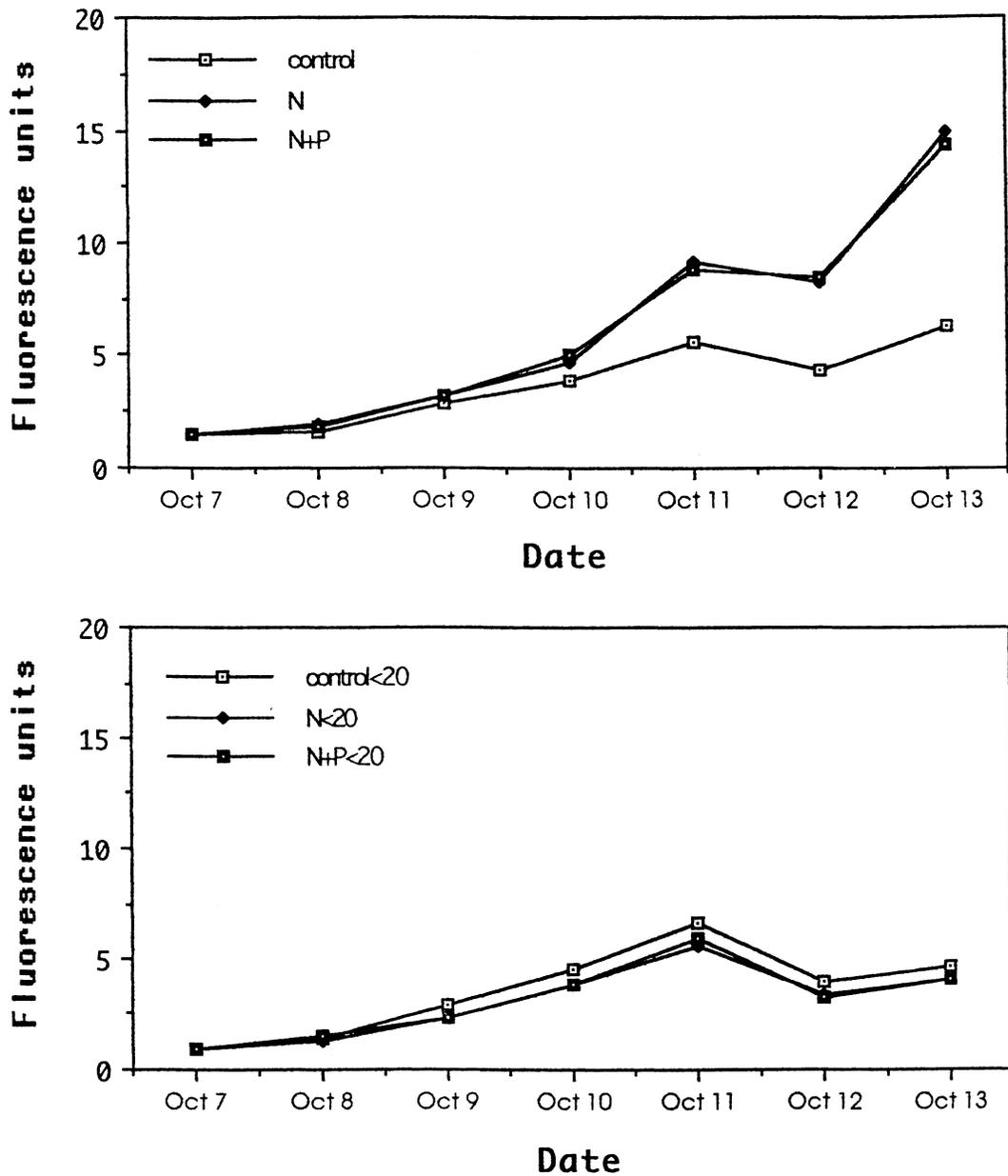


Figure 18. *In vivo* chlorophyll fluorescence during nutrient enrichment bioassays of whole water and < 20 μm fractions in October, 1992. Additions include 15 μM ammonium (N), 15 μM ammonium + 1.0 μM phosphate (N+P), and an unenriched control. Each point represents the mean of four replicates. Coefficients of variation for measurements taken on October 13 are given in Table B1 in Appendix B.

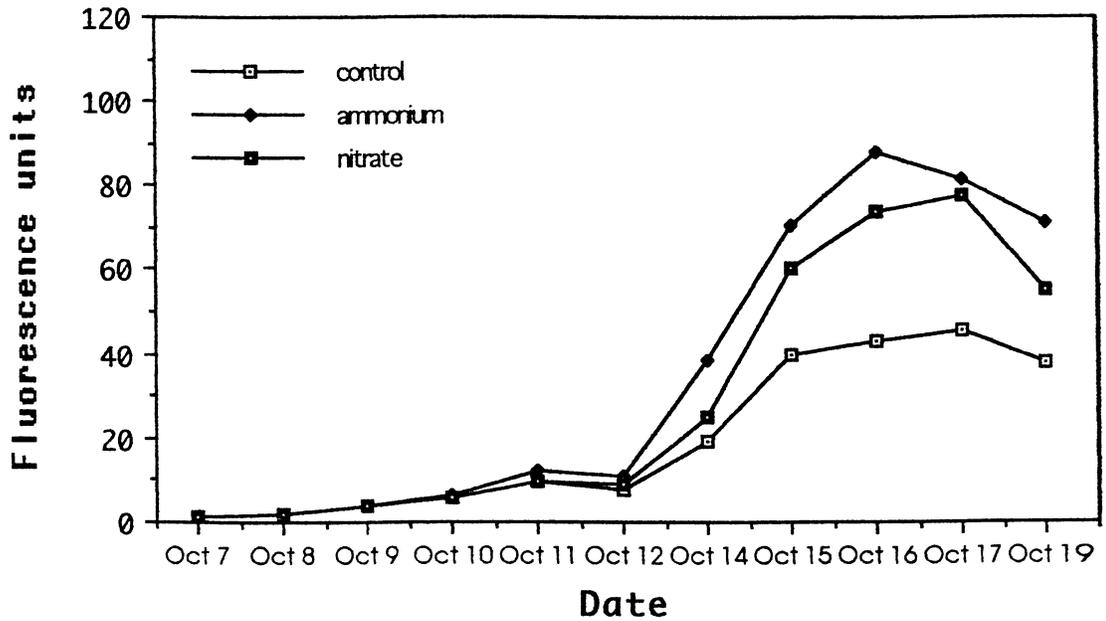
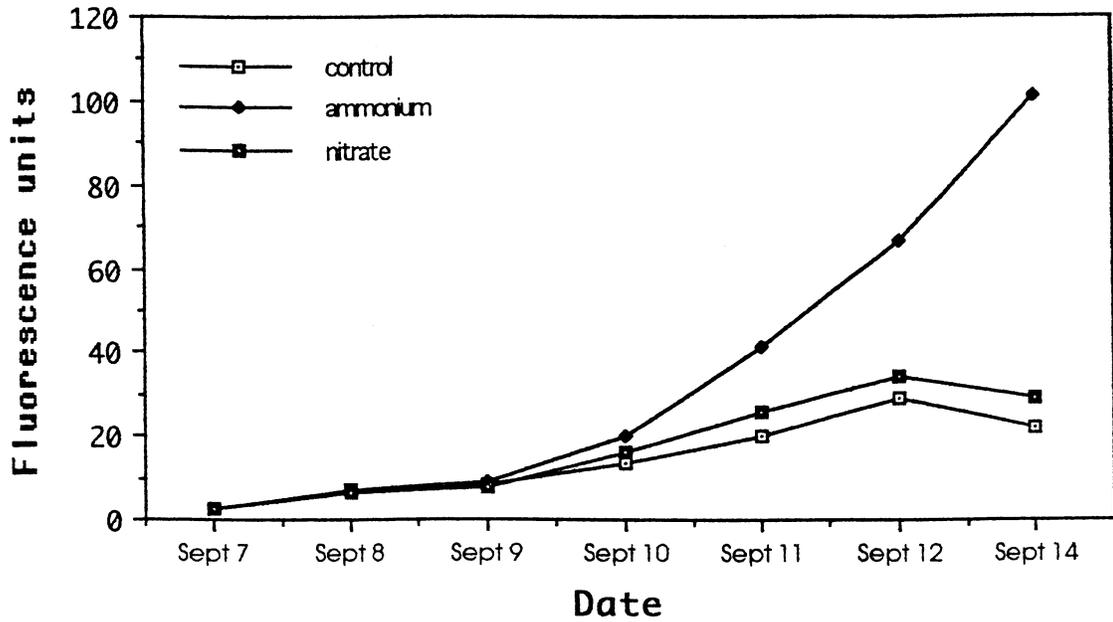


Figure 19. *In vivo* chlorophyll fluorescence during nutrient enrichment bioassays comparing responses of phytoplankton to additions of ammonium versus nitrate in September and October, 1992. Additions include 15 μM ammonium, 15 μM nitrate, and an unenriched control. Each point represents the mean of three replicates. Coefficients of variation for measurements taken on September 14 and October 19 are given in Table B1 in Appendix B.

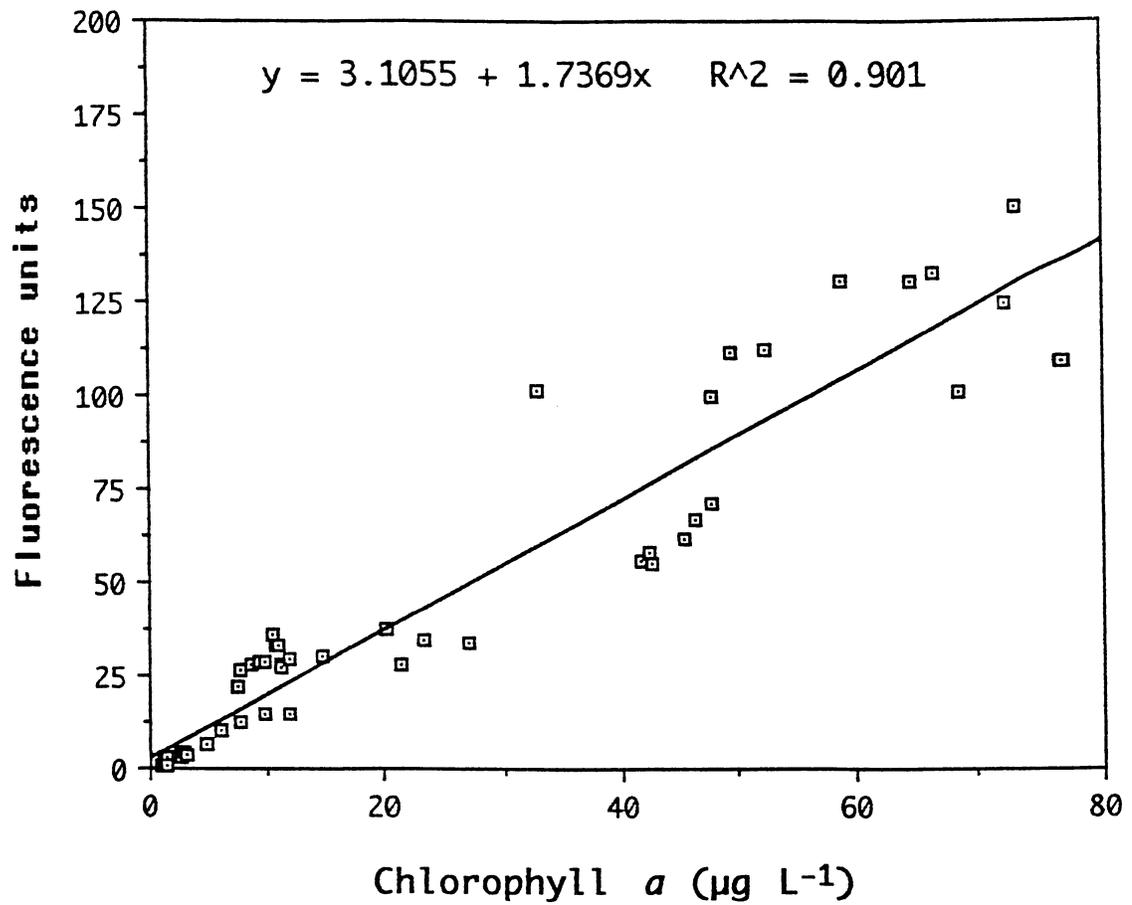


Figure 20. Correlation between initial and final *in vivo* chlorophyll fluorescence and acetone-extracted chlorophyll *a* concentrations from nutrient enrichment bioassays in Padilla Bay from June to October, 1992.

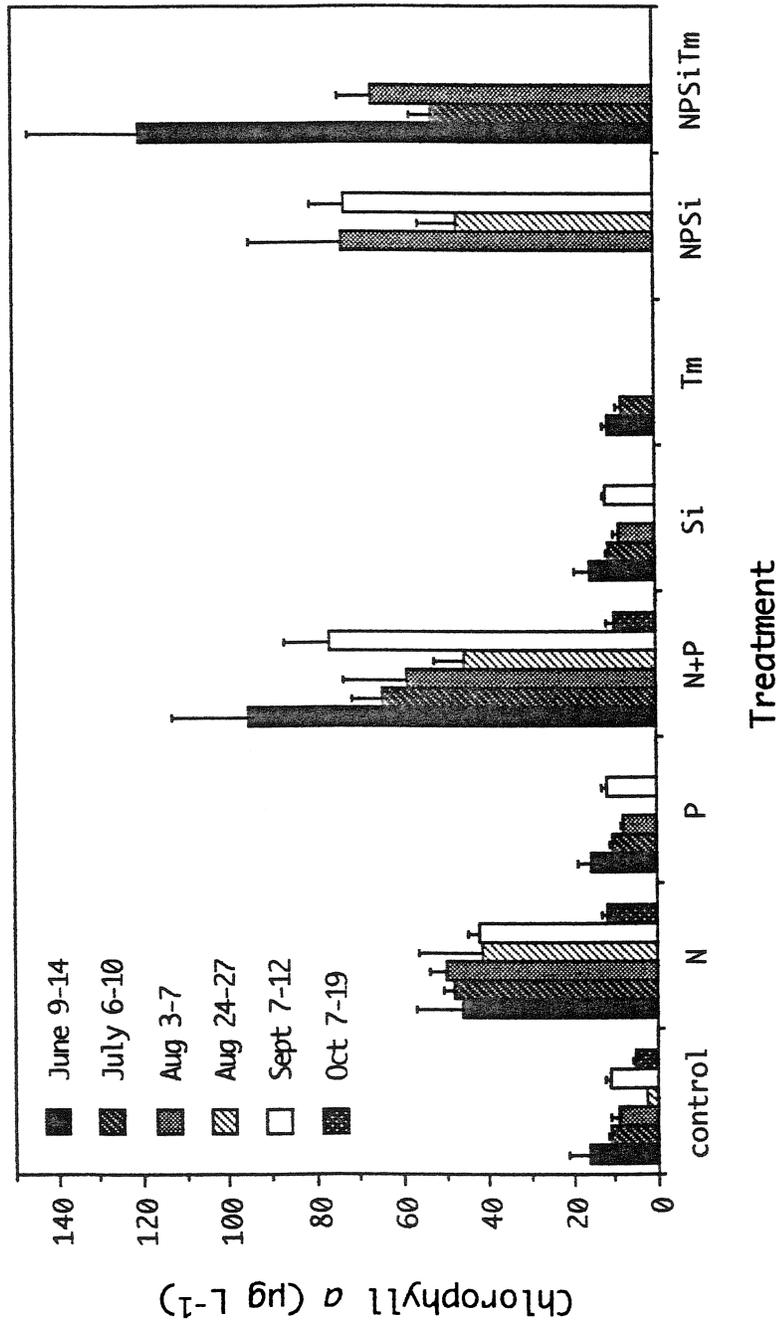


Figure 21. Mean chlorophyll *a* concentrations in surface water samples after nutrient enrichment bioassays during summer and early fall, 1992. Additions include 15 μM ammonium (N), 1.0 μM phosphate (P), 15 μM silicate (Si), trace metals (Tm), 15 μM ammonium + 1.0 μM phosphate (N+P), 15 μM ammonium + 1.0 μM phosphate + 15 μM silicate (NPSi), 15 μM ammonium + 1.0 μM phosphate + 15 μM silicate + trace metals (NPSiTm), and an unenriched control. One standard deviation is shown.

HSD multiple comparisons test, neither silicate nor phosphate additions alone caused significant increases in phytoplankton biomass compared to the control during any of the incubations ($p \geq 0.921$). During both June and July, the addition of trace metals inhibited phytoplankton biomass accumulation. The difference in chlorophyll *a* concentrations between the control and the trace metal treatment was significant during July. Consequently, the trace metal treatment was omitted in the rest of the incubations.

There were significant differences between the control and N treatments in all experiments (Figure 21). P values between treatments are given in Table C1 of Appendix C. Significant differences were also found between the control and all combination treatments (N+P, NPSi, and NPSiTm) in all incubations (Figure 21). The incubation from August 24-27 yielded the greatest difference in chlorophyll *a* concentrations between the control and the N treatments (as much as 18.6 times greater than the control, compared to less than 8 times the control during other incubations). During this incubation, ambient chlorophyll *a* concentration was $>2 \mu\text{g L}^{-1}$ (Figure 4), NH_4 and $[\text{NO}_3 + \text{NO}_2]$ concentrations were low (Figure 5), and the DIN/ PO_4 ratio were less than 16:1 (Figure 6).

In all incubations except October, chlorophyll *a* concentrations were greater in treatments receiving a

combination of N and P than when only N was added (Figure 21). However, significant differences were found only during June, July, and September. In June, the N treatment was significantly different from all combination treatments (N+P and NPSiTm). In July, the N treatment was significantly different from the N+P treatment, but not the NPSiTm treatment. Again, in September, the N treatment was significantly different from all combination treatments (N+P and NPSi).

In the experiments comparing accumulation of phytoplankton biomass in response to the addition of either NO_3 or NH_4 , the NH_4 treatment caused a significant response ($p=0.001$) compared to the control in both September and October (Figure 22). The NO_3 treatment, however, produced a significant response ($p=0.001$) only during October.

Size-fractionation experiments

Results from the size-fractionation experiment in September indicate that nanoplankton account for most of the chlorophyll *a* in the initial water sample (Figure 23). During the incubation, however, the nanoplankton decreased by as much as 80%, depending on the nutrient addition. The decrease was greatest in the combination treatments (N+P and NPSi) and least in the control treatment.

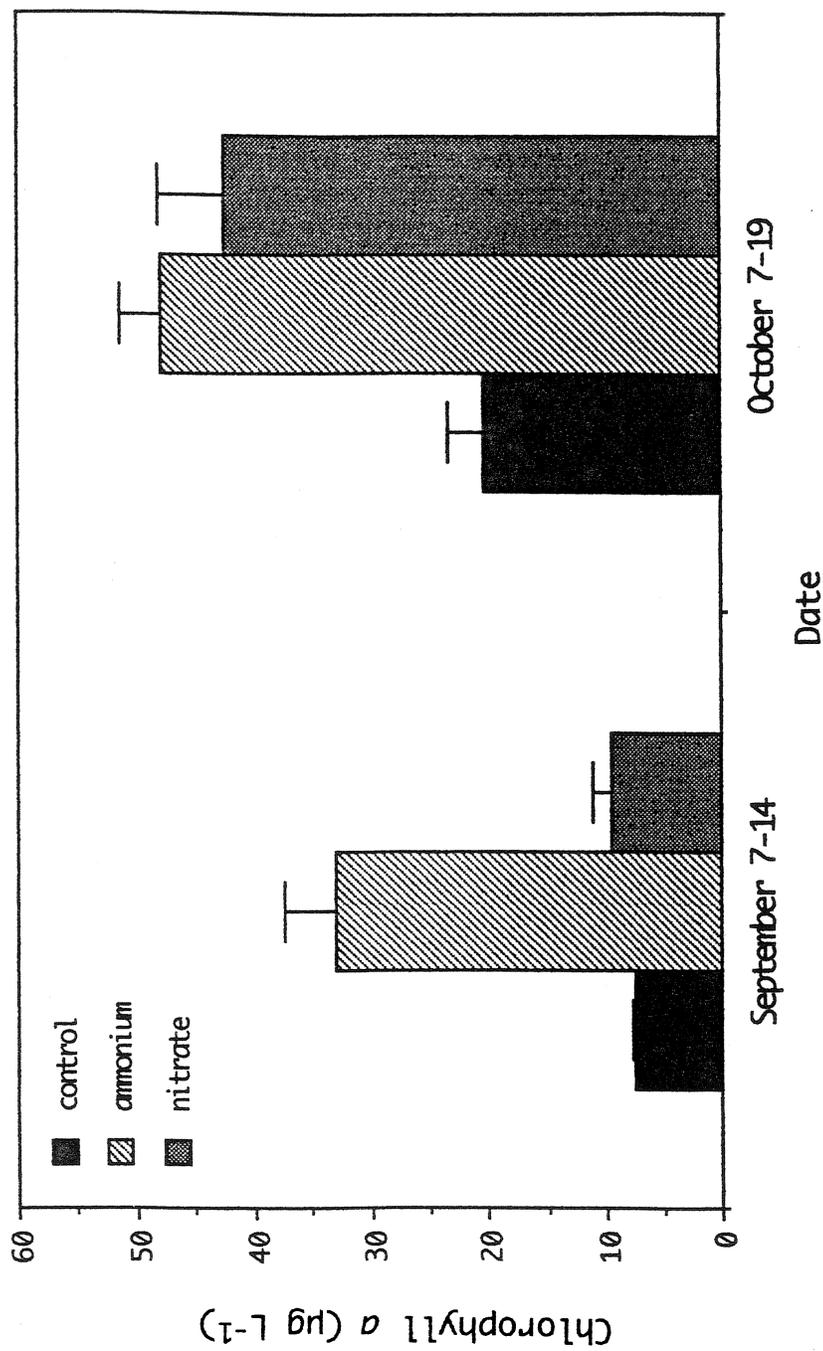


Figure 22. Mean chlorophyll *a* concentrations in surface water samples after nutrient enrichment bioassays during September and October, 1992. Nutrient additions include 15 µM ammonium, 15 µM nitrate, and an unenriched control. One standard deviation is shown.

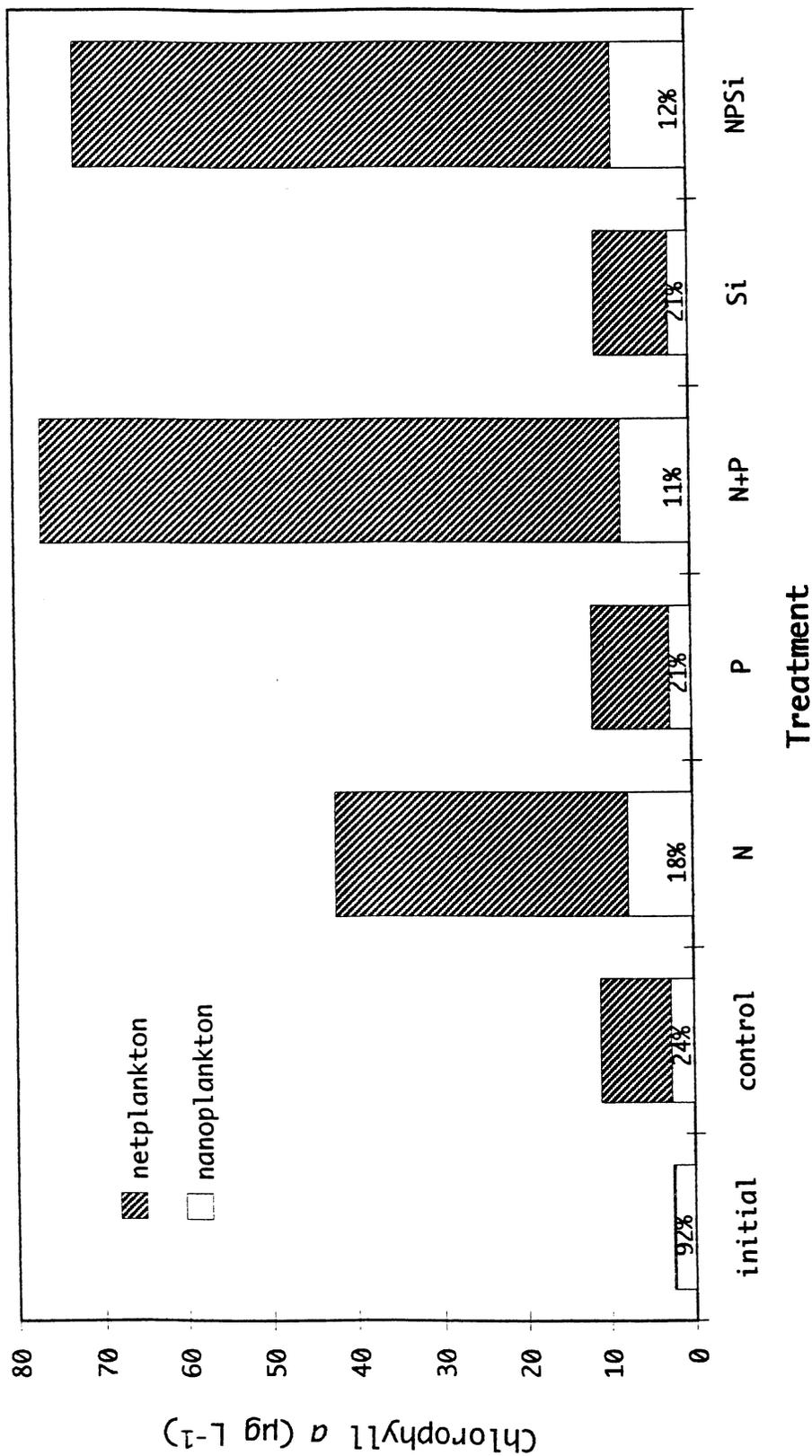


Figure 23. Contributions by netplankton and nanoplankton to total chlorophyll *a* concentrations in initial and final water samples during the incubation from September 7-12, 1992. Additions include 15 µM ammonium (N), 1.0 µM phosphate (P), 15 µM silicate (Si), trace metals (Tm), 15 µM ammonium + 1.0 µM phosphate (N+P), 15 µM ammonium + 1.0 µM phosphate + 15 µM silicate (NPSi), and an unenriched control.

In vivo chlorophyll fluorescence data for size-fractionation incubations are shown in Figures 15, 16, and 18. Again there is a lag period in all incubations except August 24-27. During the August 3-7 incubation, nanoplankton and whole water responded similarly in both the N treatment and the control. During the August 24-27 incubation, fluorescence in all treatments responded similarly until the second day when the fluorescence in both the nanoplankton and whole water controls began to decrease. On the third day, fluorescence in all N-treated nanoplankton samples (N, N+P, NPSi) began to level off or decrease while fluorescence in the N-treated whole water continued to increase. In October, fluorescence in N-treated nanoplankton (N and N+P) paralleled that in the nanoplankton control throughout the incubation. Fluorescence in N-treated whole water was much higher compared to the control, as in the previous incubations. Overall, *in vivo* chlorophyll fluorescence in October was 4-6 times lower than during the other incubations.

Chlorophyll *a* concentrations in all N-treated nanoplankton samples (Figure 24) were significantly greater than nanoplankton controls in both experiments during August, but showed no significant response to any nutrient additions in October. During August 24-27, the response of nanoplankton to N+P addition was significantly different from the response to NPSi additions.

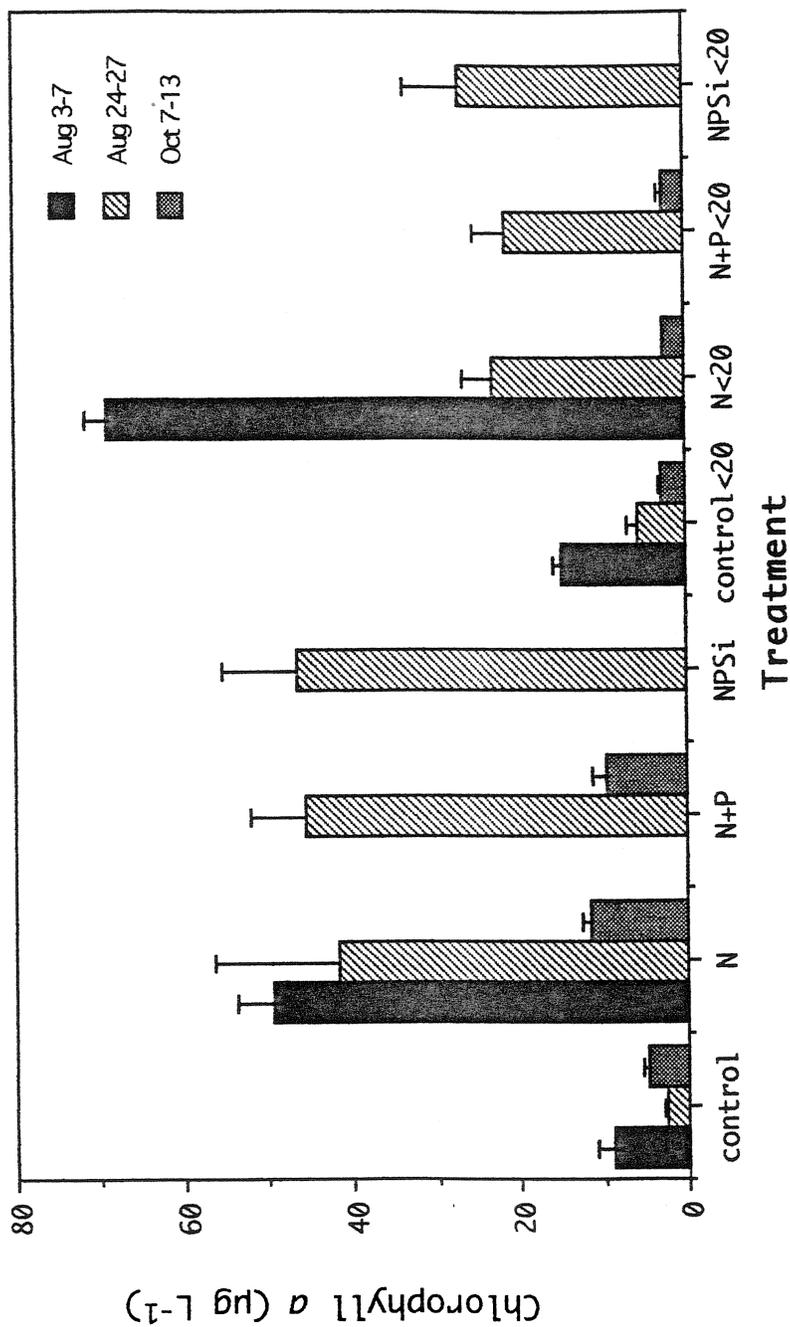


Figure 24. Mean chlorophyll *a* concentrations of surface water samples after nutrient enrichment bioassays comparing the response of nanoplankton (<20 µm) to that of total phytoplankton during August and October, 1992. Additions include 15 µM ammonium (N), 15 µM ammonium + 1.0 µM phosphate (N+P), 15 µM ammonium + 1.0 µM phosphate + 15 µM silicate (NPSi), and an unenriched control for both size fractions. One standard deviation is shown.

In comparing the response of nanoplankton to whole water samples, the nanoplankton control was significantly greater than the whole water control in both the August 3-7 and August 24-27 incubations. Chlorophyll *a* concentrations in the N-treated nanoplankton samples were also significantly greater than those in the N-treated whole water samples during August 3-7.

In the August 24-27 incubation, chlorophyll *a* concentration in the N treatment of whole water was significantly greater than chlorophyll *a* concentrations in all N treatments (N, N+P, and NPSi) of nanoplankton. The chlorophyll *a* concentrations in N+P and NPSi treatments of whole water were both significantly greater than chlorophyll *a* concentrations in N and N+P treatments of nanoplankton, but not significantly different from the NPSi-treated nanoplankton.

In October, chlorophyll *a* concentration in the whole water control was significantly greater than in the nanoplankton control. The chlorophyll *a* concentrations in both N treatments (N and N+P) of whole water were significantly greater than chlorophyll *a* concentrations in the same treatments of nanoplankton.

Discussion

Water quality and environmental conditions

Water quality parameters reported in this study are similar to those reported from other studies in Padilla Bay (Cassidy and McKeen, 1986; Thom, 1990; Williams and Ruckelshaus, 1993; Johnson, in progress) and other areas of Puget Sound (Winter *et al.*, 1975; Thom *et al.*, 1988; Thom and Albright, 1990; Shannon Point Marine Center, unpublished data). Although dissolved inorganic nutrient concentrations in surface waters reported in this study are similar to those reported in other studies, there is more variability. In Padilla Bay, nitrate concentrations ranged from levels below detection limit to 26.9 $\mu\text{M-N}$, most values being less than 10 $\mu\text{M-N}$ (Cassidy and McKeen, 1986; Johnson, in progress). Ammonium and phosphate showed less variability, ranging from 0.7 to 3.7 $\mu\text{M-N}$ and 0.2 to 2.8 $\mu\text{M-P}$. Other studies in Puget Sound (Winter *et al.*, 1975; Thom and Albright, 1990) reported the following ranges in surface waters: nitrate (1 to 24 $\mu\text{M-N}$), ammonium (0.4 to 3.5 $\mu\text{M-N}$), phosphate (1.2 to 2.2 $\mu\text{M-P}$), and silicate (25 to 45 $\mu\text{M-Si}$).

DIN/PO₄ ratios in surface waters reported throughout Puget Sound are generally less than 16:1 (Table 3). A ratio of 16:1 is based on the chemical composition of phytoplankton (Redfield, 1958). Accordingly, ratios less than 16:1

indicate the potential for nitrogen limitation. DIN/PO₄ ratios calculated from previous studies in Padilla Bay are also generally less than 16:1 during the summer and early fall (Table 3). Cassidy and McKeen (1986) found ratios above 16:1 only for brief intervals during January, May, and June, 1985. Johnson (in progress) reported ratios above 16:1 only once during summer, 1991 in Padilla Bay. Based on these data, nitrogen limitation of phytoplankton in Padilla Bay is an expected result. DIN/PO₄ ratios in this study, however, indicate the potential for nitrogen limitation only from August to October. In this case, DIN/PO₄ ratios are not always sufficient to predict nutrient limitation. The pool size of a nutrient and its turnover rate must also be considered (Harris, 1986). Additionally, one must consider analytical limits of detection.

Nutrient enrichment bioassays

The results of these experiments support the hypothesis that nitrogen limits the accumulation of phytoplankton biomass in Padilla Bay during the summer and early fall. Nitrogen limitation of phytoplankton growth, especially during the summer, has been demonstrated by nutrient enrichment studies in other estuarine and coastal areas (Vince and Valiela, 1973; D'Elia *et al.*, 1986; Graneli *et al.*, 1990; Fisher *et al.*, 1992). In Puget Sound, however,

Table 3. Percentage of DIN/P0₄ ratios less than 16:1 found in surface waters at the current study site and other sites in Puget Sound.

Location	% DIN/P0 ₄ <16:1	Reference
Padilla Bay	93 (n=46)	Cassidy & McKeen, 1986
Northern Puget Sound (including Padilla Bay)	73 (n=15)	Johnson, in progress
East Passage of Puget Sound	97 (n>100)	Thom <i>et al.</i> , 1988
Central Puget Sound	89 (n=47)	Thom & Albright, 1990
Padilla Bay	67 (n=6)	current study

Winter *et al.* (1975) suggested that nutrient limitation is a rare occurrence. Although nutrients in the surface may be sufficiently exhausted to limit algal growth, Winter *et al.* (1975) reported that these nutrients will be replenished from nutrient-rich upwellings. Their conclusions were based on data from sites in the southern and central basin of Puget Sound, 90-150 km south of the present study site (Figure 1). Because of differences in study site locations, the conclusions of Winter *et al.* (1975) may not necessarily apply to all areas of Puget Sound.

Although there were significant differences between the controls and nitrogen treatments in all six incubations, chlorophyll *a* concentrations in the same treatment from different dates differ by as much as one order of magnitude. Some of these differences may be the result of differences in water quality and environmental conditions. Unfortunately, the variability in ambient conditions precludes comparison of final chlorophyll *a* concentrations among different incubations. However, the available data suggest some possible relationships between ambient conditions and phytoplankton response. For example, the conditions during August 24-27 (elevated DO, low DIN concentrations, and ambient chlorophyll *a* concentration > 3 $\mu\text{g L}^{-1}$) suggest a possible phytoplankton bloom at the time of sampling. Because of the higher ambient chlorophyll *a* concentrations

during August 24-27, compared to other incubations, the nutrients may have been depleted more rapidly or a build-up of toxic waste products may have occurred. This would result in a decline of the algal population. This decline was evidenced by the decrease in *in vivo* chlorophyll fluorescence data for the control treatment.

Nitrate and ammonium are generally considered to be the most important sources of nitrogen for marine phytoplankton (Dugdale and Goering, 1967). Ammonium was used as the primary nitrogen source in these experiments because it is taken up more readily by phytoplankton than is nitrate (Dugdale and Goering, 1967; McCarthy *et al.*, 1977). However, since nitrate is generally the nitrogen source in greatest supply, it is important to compare accumulation of phytoplankton biomass in response to both forms of nitrogen. The preferential uptake of ammonium is presumably an energy-saving adaptation since nitrate must be reduced (Thompson *et al.*, 1989).

In September, the lack of a response to nitrate addition may have been due to premature termination of the incubation. In order to reduce nitrate to a useable form, the phytoplankton must produce nitrate reductase, a constitutive enzyme. Also, ambient nitrate concentrations were low (<10 μM) prior to the September 7 incubation. Low nitrate concentrations may lead the phytoplankton to use an alternate

nitrogen source (eg. ammonium or urea). If that were the case, the phytoplankton would not produce nitrate reductase, and consequently would not be able to take up the added nitrate immediately (Eppley *et al.*, 1969). In October, the delayed response in both nitrogen treatments was most likely due to less light and cooler temperatures. Despite the delayed response, these results confirm nitrogen limitation of phytoplankton biomass accumulation, regardless of the nitrogen form.

The enhanced response in treatments receiving a combination of N and P are similar to results from other estuarine and coastal studies (Vince and Valiela, 1973; Graneli, 1987; Fisher *et al.*, 1992). This combined effect of N + P suggests secondary phosphorus limitation. Although nitrogen limits biomass yield initially, phosphorus becomes limiting once nitrogen is no longer limiting.

Results indicating that trace metals inhibit the accumulation of phytoplankton biomass are similar to the results from another study in Laholm Bay on the west coast of Sweden (Graneli, 1987). Although inhibition of phytoplankton growth may be an indication that an increase in trace metal concentrations in Padilla Bay might be toxic, it is also possible that inhibition in these experiments was due to the adverse effects of an iron precipitate. During incubations in which trace metals were added, the iron formed a yellow

precipitate in the bottles, despite the addition of EDTA. The possibility that trace metals limit phytoplankton growth in certain areas of the ocean has appeared in recent literature (eg. Morel *et al.*, 1991). However, it is unlikely that trace metals would be limiting in coastal or estuarine systems since concentrations tend to be higher than that required by phytoplankton (Maestrini *et al.*, 1984).

Size-fractionation experiments

The results from the size fractionation experiments provide evidence for nitrogen limitation of nanoplankton populations during 2 out of 3 incubations in August and October. The lack of a response to ammonium additions during October may have been a result of higher ambient nitrate concentrations compared to nitrate concentrations in August (14 μM compared to 8 μM , respectively). Nanoplankton are better suited to take advantage of low nutrient concentrations than are the larger phytoplankton because of a larger surface area to volume ratio (Valiela, 1984). The nitrogen concentration during October, while still low enough to induce nitrogen limitation of the total phytoplankton population, was sufficiently high to satisfy the nitrogen requirements of the nanoplankton. Additionally, the DIN/PO₄ ratio increased from 10.3 in late August to 16.8 in October, which is above the Redfield ratio.

The contribution of different size classes of phytoplankton to primary productivity has been an area of interest recently. Several studies have shown that the smaller size classes (i.e. $< 20 \mu\text{m}$) dominate both biomass and productivity in oceanic systems (Malone, 1980a; Harris, 1986). In coastal and estuarine systems, however, the contribution of nanoplankton to total phytoplankton biomass may fluctuate seasonally (Malone, 1980a). In September, nanoplankton in Padilla Bay account for a sizable portion (over 90%) of the total ambient chlorophyll *a* concentration. This agrees with results from other studies that have shown that nanoplankton may frequently dominate during summer months in some estuarine environments when temperatures are warm and nutrient inputs high (Malone, 1980a; Malone, 1980b). However, at the end of the incubation, nanoplankton chlorophyll *a* concentrations accounted for only 11-24% of the total phytoplankton chlorophyll *a* concentration, depending on the nutrient addition. This reduction in the contribution of the nanoplankton may possibly be explained by the effects of grazing. Initial water samples were filtered through a $63 \mu\text{m}$ sieve. This mesh size excluded larger zooplankton, but allowed smaller zooplankton, especially protozoans, into the samples. The diet of these microzooplankters is probably composed mainly of photosynthetic nanoplankton (Beers and Stewart, 1969; Ryther, 1969; Malone, 1980b). This would

result in disproportionate grazing pressures on the phytoplankton in the bottles.

Although disproportionate grazing pressures may explain some of the differences in the responses between nanoplankton and the total phytoplankton assemblages, these differences may also be due to variations in environmental conditions and growth rates. The contribution of nanoplankton to total phytoplankton biomass may be affected by ambient nutrient concentrations, temperature, and light (Durbin *et al.*, 1975; Probyn, 1985). Nanoplankton have higher intrinsic growth rates (Williams, 1964) and more chlorophyll *a* volume⁻¹ (Malone *et al.*, 1979) than netplankton, given optimal environmental conditions (Durbin *et al.*, 1975). High nanoplankton growth rates are favored by high temperatures and light (Durbin *et al.* 1975; Malone, 1980a; Malone, 1980b). In this study, the higher temperatures and irradiance during August may have led to a phytoplankton population dominated by nanoplankton.

Under optimal combinations of light and temperature, nanoplankton grow more rapidly in response to nutrient enrichments than do netplankton (Malone, 1980b). This may account for the higher percentage of nanoplankton in the nitrogen treatments during September (Figure 23). Conversely, larger phytoplankton, such as diatoms, grow more rapidly than nanoplankton when conditions are favorable for

diatom growth, such as cooler temperatures and higher nitrate concentrations (Durbin *et al.*, 1975; Probyn, 1985). In October, when temperatures were cooler and DIN was increasing, the total phytoplankton control was greater than the nanoplankton control, unlike the previous incubations in August.

Nanoplankton growth may be affected not only by the concentration, but also by the form of nitrogen present. According to some studies, nanoplankton production is dependent primarily on ammonium as the nitrogen source and netplankton production is dependent primarily on nitrate (Malone, 1980a; Probyn, 1985). Therefore, the higher ammonium concentrations during August 3-7 might favor nanoplankton growth and the higher nitrate concentration in October would favor netplankton.

Management considerations

The results from small scale studies should be used with careful consideration when developing management strategies for whole ecosystems. Studies such as this one can only suggest the potential for nutrient limitation. The limitations of such small scale experiments make it difficult to extrapolate the results to whole ecosystems (Hecky and Kilham, 1988). However, whole ecosystem experiments such as

those conducted in lakes are impractical in marine systems and we must use the available data.

A study of the effects of nutrient inputs on primary production has been designated by Padilla Bay National Estuarine Research Reserve and Puget Sound Water Quality Authority as a priority research topic (Bulthuis and Stevens, 1991). The relatively unpolluted waters of Padilla Bay are threatened by the proximity to two oil refineries and the largely agricultural watershed surrounding the bay. Because fertilizers used on these croplands are mostly ammonium-based, there is the potential for an increased nutrient load. Increased nutrient loads have been shown to cause a cascading effect, which eventually leads to eutrophication. Because eutrophication has already been reported in several areas in Puget Sound, it is important to take steps to control eutrophication, especially in areas where the potential has been established.

The results from this study suggest that close monitoring of nitrogen inputs is essential to maintaining the nutrient-phytoplankton biomass accumulation balance. In addition to nitrogen limitation of phytoplankton, Williams and Ruckelshaus (1993) showed evidence of nitrogen limitation of both eelgrasses and epiphytes in late spring and summer in Padilla Bay. The current study was conducted during summer and early fall (June to October) when light and temperature

are less likely to be limiting. Based on Padilla Bay nutrient data from Cassidy and McKeen (1986), there is considerable seasonal variation of nutrients, especially N and P. Because of these fluctuations, there is the possibility of a shift in the limiting nutrient, as has been shown in several other estuaries (D'Elia *et al.*, 1986; Graneli *et al.*, 1990; Fisher *et al.*, 1992). Further nutrient limitation studies should be done to determine the extent of nitrogen limitation, if at all, during winter.

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Appendix A

Table A1. Temperature and salinity at 1 meter in relation to the tides on sampling dates from June to October, 1992. Correlation coefficient (r^2) relating temperature to tidal height is 0.325; correlation coefficient relating salinity to tides is 0.039; correlation coefficient relating salinity to tidal height is 0.034.

Date	Time	Temp. (°C)	Salinity (psu)	Tide	Tidal height (cm)
June 9	1300	11.2	29.1	high slack	174
July 6	1020	11.9	29.0	low slack	-62
July 8	1330	12.4	-	high slack	189
July 9	1000	15.1	-	flood	90
Aug 3	1235	14.5	28.5	flood	70
Aug 4	1230	13.2	-	ebb	150
Aug 5	1100	11.9	-	high slack	192
Aug 24	1400	13.2	28.5	flood	190
Aug 25	1806	14.0	28.6	ebb	220
Aug 26	1740	13.6	29.1	ebb	225
Aug 27	1043	16.2	28.2	flood	-34
Sept 7	1445	11.8	29.0	flood	241
Sept 10	1030	14.1	30.1	low slack	34
Sept 11	1150	14.6	30.5	flood	90
Sept 12	0820	12.8	30.1	ebb	100
Sept 14	1220	11.9	31.0	low slack	101
Oct 7	1600	11.1	26.9	ebb	200
Oct 8	1017	11.6	27.6	flood	110
Oct 9	1500	10.9	28.5	high slack	229
Oct 10	1340	11.0	28.1	flood	180
Oct 11	1600	11.1	26.3	high slack	232
Oct 12	0930	11.2	29.5	ebb	165
Oct 14	1020	10.0	29.0	ebb	200
Oct 15	1345	9.8	28.1	flood	189
Oct 16	1113	9.2	30.2	low slack	229
Oct 17	1230	9.9	30.2	ebb	227

Appendix B

Table B1. Coefficient of variation [(s/X) 100%] of *in vivo* chlorophyll fluorescence taken on the last day of each incubation.

	June	July	Aug	Aug	Sept	Oct
treatment	9-14	6-10	3-7	24-27	7-14	7-19
<u>basic expts:</u>						
control	11.0	4.5	7.3	7.3	11.3	12.7
N	6.7	7.8	3.8	15.4	19.3	12.1
P	8.8	6.5	19.5		2.4	
N + P	29.6	9.1	0.7	27.6	9.0	19.4
Si	10.9	6.3	17.5		9.5	
Tm	7.9	10.2				
N+P+Si+Tm	13.0	2.6	3.8			
N+P+Si			13.7	6.7	12.7	
<u>< 20 µm expts:</u>						
control			6.7	33.3		4.3
N			1.0	24.4		2.4
N+P				20.2		9.8
N+P+Si				24.2		
<u>Nitrate expts:</u>						
control					13.7	22.5
NH ₄					2.6	5.5
NO ₃					5.9	12.9

Appendix C

Table C1. P values based on results from Tukeys HSD multiple comparison test for controls and all ammonium treatments from nutrient enrichment bioassays from June to October, 1992. Values less than 0.05 are considered significant.

Date	Treatment	Control	N	N+P	N+P+Si
June 9-10	control	1.000			
	N	0.001	1.000		
	N+P	0.001	0.001	1.000	
	NPSiTm	0.001	0.001	0.416	
July 6-14	control	1.000			
	N	0.001	1.000		
	N+P	0.001	0.005	1.000	
	NPSiTm	0.001	0.821	0.085	
Aug 3-7	control	1.000			
	N	0.001	1.000		
	N+P	0.001	0.924	1.000	
	NPSi	0.001	0.125	0.618	1.000
	NPSiTm	0.001	0.339	0.919	0.996
Aug 24-27	control	1.000			
	N	0.001	1.000		
	N+P	0.001	0.998	1.000	
	NPSi	0.001	1.000	0.129	
Sept 7-12	control	1.000			
	N	0.001	1.000		
	N+P	0.001	0.001	1.000	
	NPSi	0.001	0.001	0.983	
Oct 7-13	control	1.000			
	N	0.001	1.000		
	N+P	0.001	0.326		

Table C2. P values based on results from Tukeys HSD multiple comparison test for < 20 µm size fractions during August and October, 1992. Values less than 0.05 are considered significant.

Date	Treatment	Control<20	N<20	N+P<20
Aug 3-7	control<20	1.000		
	N<20	0.001	1.000	
Aug 24-27	control<20	1.000		
	N<20	0.001	1.000	
	N+P<20	0.001	0.750	1.000
	NPSi<20	0.001	0.343	0.033
Oct 7-13	control<20	1.000		
	N<20	0.873	1.000	
	N+P<20	0.991	0.994	1.000

Table C3. P values based on results from Tukeys HSD multiple comparison test between < 20 µm size fractions and whole water samples during August and October, 1992. Values less than 0.05 are considered significant.

Date	Treatment	Whole water samples			
		Control	N	N+P	NPSi
Aug 3-7	control<20	0.006	0.001		
	N<20	0.001	0.032		
Aug 24-27	control<20	0.001	0.001	0.001	0.001
	N<20	0.001	0.001	0.001	0.001
	N+P<20	0.001	0.001	0.001	0.001
	NPSi<20	0.001	0.045	0.129	0.101
Oct 7-13	control<20	0.001	0.001	0.001	
	N<20	0.001	0.001	0.001	
	N+P<20	0.001	0.001	0.001	1.000

