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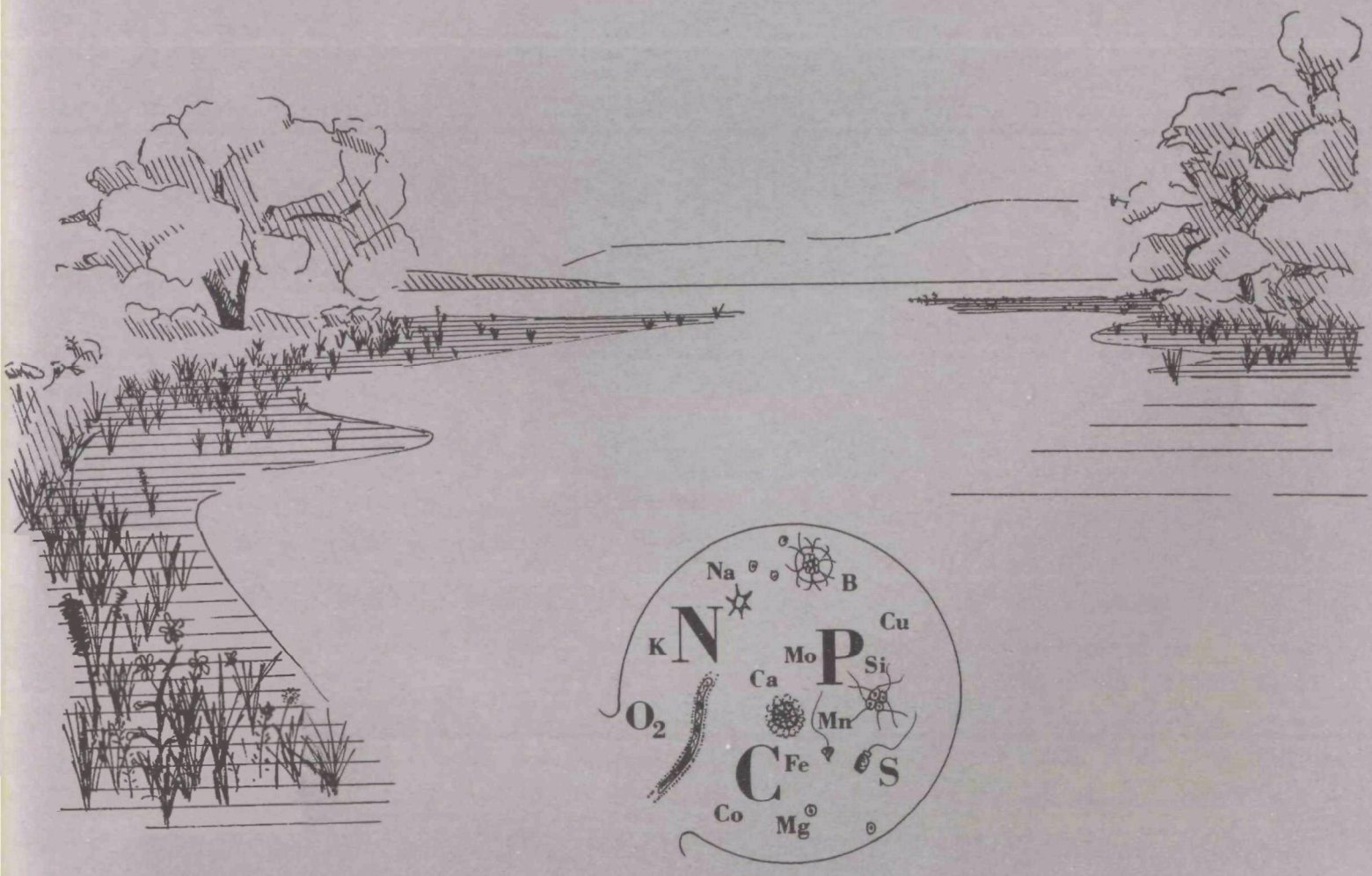
Eutrophication In Coastal Waters: Nitrogen As A Controlling Factor



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EUTROPHICATION IN COASTAL WATERS: NITROGEN AS A CONTROLLING FACTOR

by

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ENVIRONMENTAL PROTECTION AGENCY

Project #16010 EHC

December 1971

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C. 20402 - Price 70 cents

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ABSTRACT

The role of southern California coastal sewage outfalls in the eutrophication of local seawater was investigated. The outfall effluents have a measureable influence on standing stocks of phytoplankton, and on primary production. Two cruises were undertaken, in July, 1970 and June, 1971. Kinetic parameters for the assimilation of ammonium, nitrate and urea were determined at the outfall sites using ^{15}N-labelled substrates. These parameters will be useful for simulation models of phytoplankton growth as influenced by local sewage effluents.

The utilization of various forms of nitrogen by phytoplankton, mechanisms and rates of nitrogen assimilation, and enzymes of nitrogen assimilation were investigated in laboratory cultures. Ammonium and nitrate assimilation were found to vary from day to night as does the capacity for photosynthesis when cultures were grown on light-dark cycles simulating natural illumination.

In fitting data on rates of nitrogen assimilation vs. concentration of nitrogen to the Michaelis-Menten equation, modified to describe nutrient uptake, it was found that the maximum growth rate was a variable while the saturation constant was uniform over a range of dilution rates of N-limited chemostat cultures. The chemical composition of phytoplankton, particularly ratios of carbon/chlorophyll and carbon/nitrogen, varied with dilution rate in reproducible ways. By varying the dilution rate of such cultures one seems to regulate the degree of nitrogen-deficiency of the phytoplankton.

This report was submitted in fulfillment of Project Number 16010 EHC under the sponsorship of the Water Quality Office, Environmental Protection Agency.

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SECTION I

CONCLUSIONS

1. Southern California coastal sewage outfalls have measureable effects on the concentration of nutrients (ammonium) available for phytoplankton growth in the immediate vicinity of the outfalls. Phytoplankton standing stocks are also elevated in these areas.

2. Nitrogen appears to be limiting for the growth of phytoplankton stocks in Southern California coastal waters, and ammonium and urea exceed nitrate in importance as nitrogen sources for phytoplankton growth except during upwelling periods. Increased ambient nitrate concentrations during upwelling permit increases in the phytoplankton standing stocks along the coast to levels observed about the outfalls.

3. Laboratory studies of phytoplankton growth and physiology complement the work at sea by providing data and concepts necessary to an understanding of mechanisms and rates of processes to be expected in nature.

SECTION II

RECOMMENDATIONS

The study of phytoplankton standing stocks and nutrient levels in waters about Southern California sewage outfalls is being continued under the auspices of the Southern California Coastal Water Research Project. The increases in phytoplankton stocks attending sewage inputs are not in themselves detrimental for present uses of coastal waters, although blooms of certain species such as in dinoflagellate red tides may present problems, largely in enclosed embayments. Bottom-living organisms are more readily perturbed by the outfalls, and it is gratifying that studies of these benthic communities are also in progress under SCCWRP.

It is not clear at present why nutrient enrichment of our coastal waters sometimes results in a preponderance of diatoms and at other times of dinoflagellates in the phytoplankton crop. Solution of this problem would be significant for the eventual control of dinoflagellate blooms. Laboratory work on the problem seems in order.

SECTION III

INTRODUCTION

The Marine Food Chain Research Group of the Institute of Marine Resources, University of California, San Diego, is the site of an interdisciplinary program of research into factors which control the food web in the plankton and is concerned not only with the kinetics of phytoplankton growth, but with the more subtle effects that a change in the nature (as well as the biomass) of the flora may have on the whole food chain leading to the production of harvestable marine life. These aspects must be considered, as the undesirable feature of eutrophication is often not so much the high productivity but the production of the wrong organisms at the wrong time for a food web to be established which is favorable to man.

It is impossible to predict the course of eutrophication in the marine environment with any degree of sophistication without an understanding of how the phytoplankton respond to an added load of nitrogen compounds, in particular ammonia and nitrate. These compounds are nearly always "limiting" nutrients in the sea and affect the standing stock and nature of the plant crop (ref. e.g. Strickland, 1965; Ryther and Dunstan, 1971). A shortage of silicate and organic trace factors may influence the nature of the flora but probably has little effect on the total biomass.

The near-shore coastal area of Southern California, where pollution now occurs and will increase in the future, has been surprisingly little studied with respect to phytoplankton ecology, despite the fact that the California Current is one of the most extensively investigated regions with respect to physical oceanography and certain aspects of the zooplankton, in particular fish larvae.

Sverdrup and Allen (1939) and Sargent and Walker (1948) related diatom populations to the large-scale eddies and areas of upwelling off the coast. In addition, there have been several investigations arising from projects to study the disposal of raw and treated sewage (e.g. Stevenson and Grady, 1956, Water Resources Engineers, 1967), but these and numerous published and unpublished floristic studies have produced no basic understanding of the phytoplankton ecology or conclusive evidence for the effect of pollution on the planktonic flora, even adjacent to a sewage outfall. In this respect the area is more difficult to study than an estuary to marshy bay where there is poor circulation. In such contained areas abnormal blooms of organisms are clearly associated with pollution. Such situations have been documented and the general ecology worked out

(e.g. coccolithophores in the Oslo Fjord; Berge, 1962; or green algae in parts of Long Island Sound exposed to the run-off from duck ponds, Ryther, 1954).

Much of the year surface waters off southern California are depleted of plant nutrients, especially nitrogen, with nitrate undetectable at the surface and ammonium concentrations less than 1 μM (cf. Strickland, ed., 1970, and earlier references cited therein). Urea concentrations are likewise less than 1 μM (McCarthy, 1971). Enrichment takes place periodically, especially in spring and summer, when the upwelling of nutrient-rich water markedly increases the concentrations of nitrate, phosphate, and silicate. A detailed study carried out April through September, 1967, off La Jolla, California, provided comparative data on phytoplankton crop size and nutrient concentrations in both quiescent and upwelling periods (Strickland, ed., 1970). The physical oceanography of upwelling has been reviewed recently (Smith, 1968) and the importance of the processes for local phytoplankton production has been recognized for many years (Moberg, 1928). Nutrient enrichment during upwelling tends to increase the size of the phytoplankton crop in local waters. In some cases, especially offshore, diatoms are the principal components of the resulting blooms (Sverdrup and Allen, 1939; Sargent and Walker, 1948) whereas dinoflagellates often form blooms (red tides) within a few miles of shore (Allen, 1946; Holmes *et al.* 1967). At present we cannot predict whether dinoflagellates or diatoms will increase in response to nutrient enrichment nearshore (Strickland, ed., 1970) and further research on the character and mechanisms of species succession is needed.

Enrichment of surface waters also results from sewage disposal off southern California in outfalls serving Ventura, Los Angeles, Orange and San Diego counties. At present we lack sufficient data to compare the nutrient contributions from upwelling and sewage disposal to local surface waters. Very preliminary and approximate estimates suggest that natural upwelling may exceed sewage by an order of magnitude as a source of nitrogen for phytoplankton growth over a year. Fairly accurate estimates of the nitrogen input from sewage can be made, but determining the contribution from upwelling would be very costly of ship time and no doubt variable from year to year.

Upwelling provides nitrogen as nitrate while sewage would be expected to supply ammonium as the principle form of nitrogen. Phytoplankton appear to utilize both forms equally well although their chemical composition, especially C/N and C/chlorophyll *a* ratios, may vary somewhat with the nitrogen source used for growth (Eppley *et al.*, 1971a). Since upwelling is seasonal and intermittent a survey of the region during a quiescent period

(no upwelling) would be expected to show the outfall areas as as points of nutrient-rich water, with high phytoplankton crops, against a low nutrient, low crop background. This was the case, in part, in July 1-15, 1970, for coastal waters between Los Angeles and San Diego: phytoplankton crops were high only at the outfalls but surface nutrient concentrations were low everywhere. In June, 1971, crops were high at all stations and the effects of sewage effluents were less obvious.

Grigg and Kiwala (1970) and Turner, Ebert and Given (1968) provide maps of the White Point and Point Loma outfall areas, respectively, the two outfalls we studied, and report studies on benthic organisms.

Laboratory studies were carried out to aid in the interpretation of the results from cruise work. Intensive studies of diel periodicity in growth (cell division), photosynthetic rate, and nitrogen assimilation were carried out with single phytoplankton species in the laboratory and with cultures of natural phytoplankton aboard ship. Continuous cultures, operated as nitrogen-limited chemostats, were also studied in the laboratory. Such cultures are particularly amenable to studies of nitrogen assimilation rates and the kinetics of assimilation.

Results of the culture studies fall into four categories: (1) periodicity in nitrogen assimilation, and in cell division, chlorophyll synthesis, and photosynthetic carbon assimilation rate; (2) kinetic studies of nitrogen assimilation; (3) the chemical composition of phytoplankton in N-limited chemostat cultures and the influence of rate of nitrogen input on carbon/nitrogen, carbon/chlorophyll, and nitrogen/chlorophyll ratios in the cells; (4) comparison of results of measuring nitrogen assimilation by direct chemical, isotopic ^{15}N, and enzymatic methods.

SECTION IV

METHODS

Nutrient and Particulate Analyses

The determination of chlorophyll *a* by the fluorometric technique (Holm-Hansen *et al*. 1965), nitrate by the cadmium-copper reduction and subsequent determination of nitrite (Wood, Armstrong, and Richards, 1967), and particulate nitrogen by the Kjeldhal method with a ninhydrin finish (Holm-Hansen, 1968) and particulate adenosine triphosphate by the luciferin luciferase method (Holm-Hansen and Booth, 1966) followed the procedures outlined in Strickland and Parsons (1968). Ammonium was determined by the phenolhypochlorite method (Solórzano, 1969) and urea by the urease method (McCarthy, 1970), and both incorporated the modifications of technique, sample preparation, and sample storage reported elsewhere (McCarthy, 1971; McCarthy and Kamykowski, unpublished results). Reactive phosphate and silicate were analyzed according to methods in Strickland and Parsons (1968).

Primary Production

Samples were taken at depths corresponding to sunlight irradiances of 100, 45, 20, 8, 4, and 1% of surface irradiance for measurement of both carbon and nitrogen productivity. (These depths were chosen to match the transmission of neutral density filters in the deck incubators.) The light depths for sampling were based on three times the Secchi disc depth as the 1% light level and the further assumption of a constant attenuation coefficient with depth.

The water samples were passed through 183-μ netting and placed in 300-ml glass-stoppered bottles. Radiocarbonate solution (5 or 20 μCi in one ml) was added with rinsing, the contents of the bottles mixed and the bottles placed in incubators on deck in unobstructed sunlight. The incubators provided cooling water at sea surface temperatures. Samples were incubated for 24 hours. They were then passed through 0.45-μ membrane filters and the filters were dried immediately in a vacuum desiccator over silica gel. Finally, the radiocarbon of the filtered particulate matter was assayed with a scintillation counter and the counts were corrected for counter efficiency, background radiation and coincidence effects. Carbon assimilation was calculated as mg C/m^3/day. These values were integrated over depth to express production as g C/m^2/day.

Phytoplankton Uptake of Nitrogen Using the ^{15}N Isotope Technique

The ^{15}N analyses of phytoplankton nitrogen uptake followed the procedure outlined by Dugdale and Goering (1967). After incubation (24 hours unless noted) with the ^{15}N isotopes [99 at % $K^{15}NO_3$, 95 at % $Cl^{15}NH_4$, and 97 at % $(^{15}NH_2)_2CO$] the particulate material was collected on a Reeve Angel 984H glass fiber filter, desiccated under a partial vacuum over silica gel, and converted to gaseous nitrogen by an automated Dumas method. A Coleman^R nitrogen analyzer was used to combust the particulate sample and filter and to sweep it into a glass vacuum system which trapped the CO_2 and reduced the volume of the gaseous nitrogen sample (Barsdate and Dugdale, 1965). The sample then entered a single beam Nier sector-type mass spectrometer for determination of the $^{15}N/^{14}N$ ratio. The resultant ratio was compared with standards to determine the quantity of ^{15}N isotope incorporated by the algae during the incubation. The precision of the mass spectrometer was examined using 13 replicate samples of low enrichment (0.448 at % ^{15}N); and one SD was 0.0124 at % ^{15}N.

From the mass spectrographic analysis the variable V was determined. $V = \frac{\text{mass of N taken up}}{\text{mass of particulate N X time}}$ and hence has only dimensions of $(\text{time})^{-1}$ and can be considered a specific growth rate in terms of nitrogen (Dugdale and Goering, 1967).

When detrital particulate nitrogen is present in a sample, V will underestimate growth rate since the biologically active particulate nitrogen is diluted by the inert fraction in the mass spectrographic analysis. For laboratory cultures and enriched shipboard cultures, where there is proportionately little particulate detrital nitrogen, the consequences are negligible.

The product of V and the particulate nitrogen concentration for the same sample is an assimilation rate (ρ), which has dimensions of mass/volume·time and units of µmole N/l·hour or µmole N/l·day will be used. Dugdale and Goering (1967) have shown that this parameter is not affected by detrital particulate nitrogen, hence assimilation rates may be compared both within and between samples.

Enzyme Assays

All cell-free extracts for enzyme assay were prepared by filtering cells on a glass fiber filter paper, homogenizing filter and cells in 0.2 M phosphate buffer, pH 7.9, and centrifuging 1-2 min at about 2000 RCF to obtain a clear extract (Eppley, Coatsworth and Solórzano, 1969).

Nitrite reductase (NiR). The enzyme was assayed essentially as described by Joy and Hageman (1966) with reduced (by dithionite) methyl viologen as reductant. The 1.0 ml methyl viologen solution (40 mg/10 ml water), 0.1 ml $NaNO_2$ solution (9 mM in water), and 0.1 ml sodium dithionite solution (75 mM in buffer, freshly prepared). Reaction tubes were fitted with serum stoppers and evacuated through a hypodermic syringe needle plumbed to a vacuum pump. Incubations were carried out at room temperature (20-25 C) usually for 60 min. Activity was calculated as the difference in NO_2^- remaining in control tubes (reaction tubes lacking enzyme, with boiled enzyme, or lacking MV) and that in experimental tubes. Nitrite in the reaction tubes was determined on 1-ml samples (in duplicate or triplicate) after 1/10 or other appropriate dilution, by adding 1 ml sulfanilamide solution and and 1 ml N-(1-naphthyl)-ethylenediamine dihydrochloride solution, and absorbance was read at 543 nm with a spectrophotometer.

Glutamic dehydrogenase (GDH). The ammonium-dependent oxidation of pyridine nucleotide in the presence of α-ketoglutarate was taken as GDH activity. To each reaction tube was added 0.6 ml cell extract in 0.2 M phosphate buffer, pH 7.9, 0.1 ml α-keto-glutarate solution (0.2 M), 0.1 ml $(NH_4)_2\ SO_4$ solution (1.5 m), and 0.2 ml NAD(P)H solution (1 mM). After an appropriate incubation time (usually 10 min) the reaction was stopped by adding 0.3 ml 3 N HCL followed by 1.0 ml 30% NaOH solution. The reaction mixture was then placed in a boiling water bath for 5 min if NADH was included, but not if NADPH was used. Five ml water was then added and the fluorescence of oxidized pyridine nucleotide, formed in the reaction, was determined (Turner and Associates, 1968). Activity was calculated from the difference in fluorescence between tubes with and without added $(NH_4)_2\ SO_4$, i.e. from the ammonium-dependent fluorescence. Standard curves were prepared using NAD(P) dissolved in buffer and treated as the samples.

Viologens were purchased from Mann Research Laboratories, Inc.; pyridine nucleotides from Sigma Chemical Co.

Shipboard Culture Methods

Polyethylene culture vessels on an exposed deck of the ship were filled with 200 l of low-nutrient, near-surface seawater which had been passed through 183-μ nylon mesh to remove the larger zooplankton. The vessels were covered with translucent lids, wrapped with cheesecloth, and cooled by a continuous spray of surface seawater. A full complemement of nutrients in the proportions recommended by Eppley, Holmes, and Strickland (1967), except for nitrogen, were introduced at the initiation. Nitrogen as the limiting nutrient was added as nitrate to one culture,

ammonium to another, urea to the third (hereafter referred to as the nitrate, ammonium, and urea cultures) and a fourth culture received no nitrogen. Additional quantities of nutrients were added when necessary to prevent depletion during the experimental period. The cultures were sampled at 6 hour intervals through 24 hours of the stationary phase of growth in one experiment and at 6 hour intervals through 42 hours of the logarithmic phase of growth in another. Every 6 hours analyses were made for nitrate, ammonium, urea, particulate nitrogen, chlorophyll a, enzyme activities, and photosynthetic rate (samples incubated 1 hr with ^{14}C under artificial light). At the same time each of three 200 ml samples from each culture was enriched with 5.0 µg-at N/l ^{15}N labeled nitrate, ammonium, or urea for determining N-assimilation rates. The bottled samples were suspended in the culture vessels and incubated for one hour.

Laboratory Culture Methods

Organisms cultured were the coccolithophorid, Coccolithus huxleyi, clone BT-6, isolated by Dr. R. R. L. Guillard in 1960 from the Sargasso Sea, two coastal diatoms: Skeletonema costatum, isolated from Long Island Sound in 1956 by Dr. Guillard, and Leptocylindrus danicus, isolated by Mr. J. B. Jordan off Pt. Loma, California, in 1970, and a large-celled dinoflagellate, Gymnodinium splendens, isolated off the Scripps Institution pier by Mr. Jordan in 1969. The latter is often a minor species component in local dinoflagellate blooms.

Enriched sea water media were used both for maintenance of cultures and for the experiments (half-strength IMR medium described by Eppley, Holmes, and Strickland, 1967). Nitrogen levels were reduced for N-limited chemostat experiments and are indicated in the appropriate data tabulations. All experiments were carried out at 18°C. Illumination was provided by tungsten lamps with the light filtered through copper sulfate solutions. Details of the light source are given in Eppley and Coatsworth (1966). Irradiance during the experiments, and daylength are given in the data tables. Dilution rate of the continuous cultures was regulated by a Technicon^{R} peristaltic pump. Cell concentration was measured in the laboratory cultures with a Celloscope^{R} model 101 electronic particle counter.

SECTION V

SHIPBOARD STUDIES OF EUTROPHICATION AROUND SOUTHERN CALIFORNIA COASTAL SEWAGE OUTFALLS, JULY, 1970, AND JUNE, 1971

The White Point (Los Angeles County) and Point Loma (San Diego) coastal sewage outfalls were visited on cruises of the R/V ALPHA HELIX and ALEXANDER AGASSIZ in July, 1970, and June, 1971, respectively (Fig. 1). The outfall diffuser piping lies at approximately 70 meters depth at each site allowing the ship to move directly over the pipes for taking samples. The outfall diffusers are said to be designed for an initial dilution of sewage with sea water of the order 200-fold, hence one would see only small salinity changes resulting from fresh water dilution, even at depth. We have found the best "tracer" for the sewage to be ammonium ion. Ammonia in sewage is probably of the order 1-2 millimolar in concentration and is generally present at <1 micromolar in sea water. The 200-fold dilution would be expected to produce an ammonia concentration 5 to 10 micromolar directly above the outfall, a concentration easily measured with present methods. We have found concentrations up to 30 micromolar at White Pt. (Table 1) but concentrations were usually lower.

The depth of the euphotic zone at the outfalls is roughly 20 m or about 1/4 the water depth (Table 2). Ammonia concentration in the euphotic zone is low, presumably because the abundant phytoplankton rapidly absorb the nutrient in the presence of light. Other nutrient substances measured include phosphate, silicate and nitrate, none of which were different, either elevated or depressed, at the outfalls in comparison with concentrations measured at control stations (Eppley *et al*. 1971b).

Phytoplankton crops were high at the outfalls at each visit. In July, 1970, phytoplankton concentrations were fairly low along the southern California coast and the outfall crops stood out clearly above background levels. In June, 1971, crops were elevated everywhere measurements were made and the outfalls were not conspicuous by their phytoplankton crops (Table 2). Limited areal coverage was achieved in 1971 by recording several depth profiles of chlorophyll fluorescence (Lorenzen, 1966) about the outfalls with a hose, pump and fluorometer (Table 3). However, the above conclusions based upon chlorophyll concentration were supported by other parameters related to the standing stock of phytoplankton: primary productivity, particulate carbon, particulate nitrogen, and adenosine triphosphate (Eppley *et al*. 1971b). The phytoplankton crop off southern California has shown marked temporal fluctuations

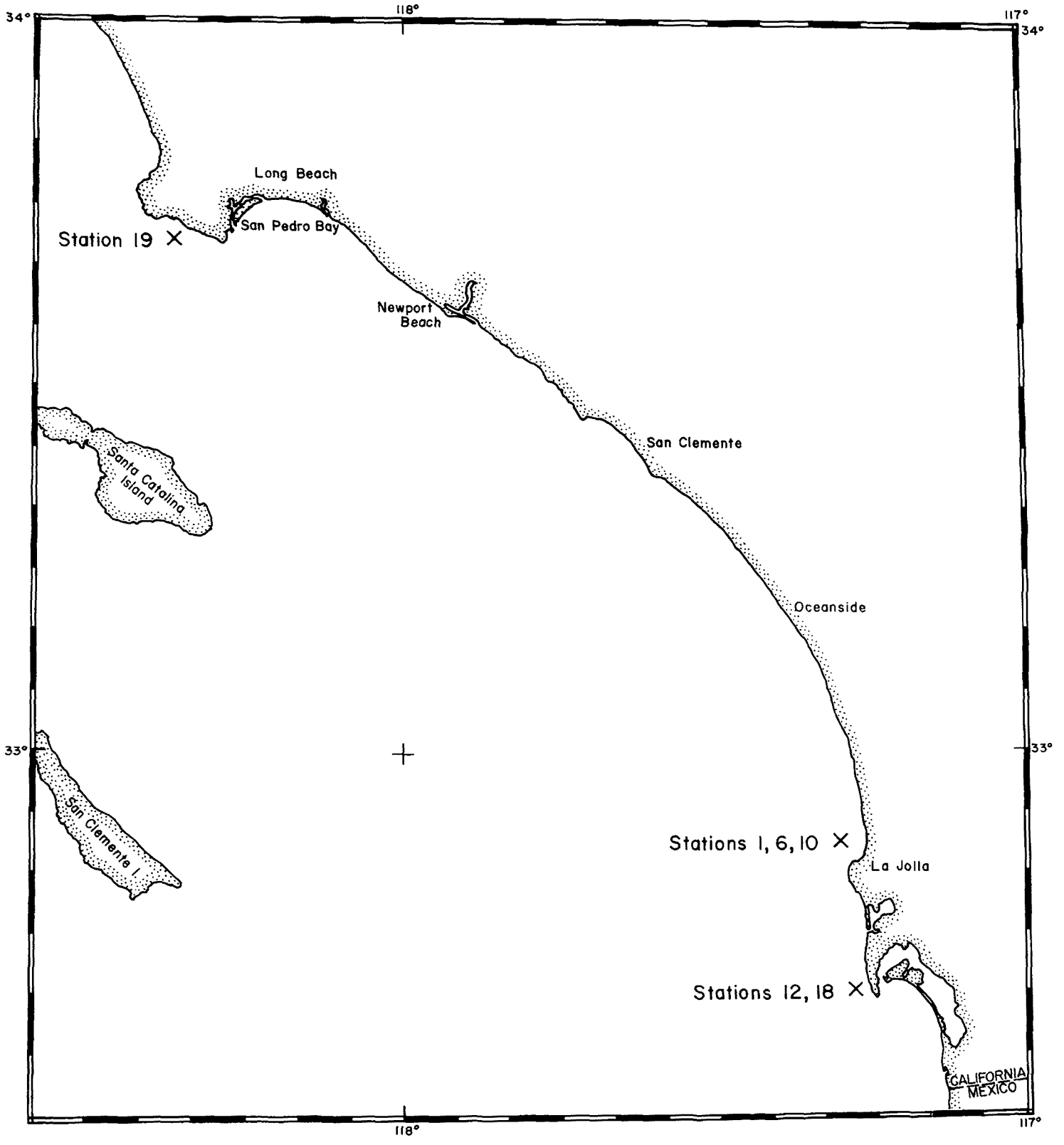

Fig. 1. Map showing the location of the White Point (station number 19) and Point Loma (station numbers 12, 18) sewage outfalls and the control station of La Jolla, California (station numbers 1, 6, 10). The station numbers refer to the 1970 cruise and are not used further in this report.

Table 1. Depth profiles of ammonium concentration (μmoles/liter) off southern California in June, 1971.

Pt. Loma Outfall				White Pt. Outfall		Control Station off La Jolla, Calif.	
5 June 1971		8 June 1971		6 June 1971		7 June, 1971	
Depth m	NH_4^+ μM	Depth m	NH_4^+ μM	Depth m	NH_4^+ μM	Depth m	NH_4^+ μM
5	0.16	0	0.83	0	0.40	0	0.08
15	0.20	5	1.04	5	1.30	5	0.19
25	0.31	10	0.79	10	18.3	10	0.17
40	2.72	25	0.91	25	26.0	25	0.23
45	0.07	45	2.19	35	30.1	35	0.19
50	0.24	60	0.35	48	27.6	50	0.13

Table 2. Photosynthetic carbon assimilation, nitrogen assimilation, and chlorophyll *a* at coastal seawater stations off southern California. Nitrogen assimilation values are the sum of nitrate, ammonium, and urea assimilation. Values were integrated over the depth of the euphotic zone.

Location	Date	Photosynthetic Rate g C/m^2·day	Nitrogen Assimilation m moles N/m^2·day	Chlorophyll *a* mg/m^2	Euphotic Depth m
		July, 1970			
Pt. Loma Outfall	9 July	2.64	ND	163	21
White Pt. Outfall	13 July	1.76	10.0	83	17
Control station off La Jolla	1 July	1.37	10.4	33	33
	3 July	1.10	9.4	18	33
	8 July	0.36	ND	14	46
		June, 1971			
Pt. Loma Outfall	5 June	2.07	14.0	21	20
	8 June	0.96	12.8*	50	23
White Pt. Outfall	6 June	2.54	10.5	66	16
Control station off La Jolla	7 June	2.46	18.5*	53	28

*Based on three depths only, instead of the usual six depths.

NB: Ratios of the rate of carbon assimilation to nitrogen assimilation in these experiments averaged 9.7 ± 2.5 (s.d.) g C/g N.

Table 3. Variation in the chlorophyll *a* content of the upper 50 meters of water about the Pt. Loma and White Pt. outfalls and about a control station off La Jolla, California.

Station	Time	Position N. Latitude	Position W. Longitude	Chlorophyll *a* mg/m^2	
		Point Loma Outfall	5 June, 1971		
1*	0930	32°40.3'	117°17.0'	33	
2	1406	41.3'	17.3'	83	
3	1500	43.5'	17.3'	32	(40 m)
4	1545	46.3'	17.2'	18	(25 m)
5	1648	40.3'	22.8'	115	
6	1828	39.1'	17.2'	36	
7	1912	37.0'	17.2'	50	
		White Point Outfall	6 June, 1971		
8	0800	33°42.2'	118°20.3'	292	
9	0906	45.0'	26.6'	250	
10	0952	43.4'	23.4'	247	
11	1030	42.6'	21.4'	168	(40 m)
12*	1130	42.1'	19.9'	206	(45 m)
13	1500	41.6'	19.1'	201	(40 m)
14	1539	40.8'	16.9'	85	(30 m)
15	1636	40.5'	21.6'	141	(45 m)
		Control Station Off La Jolla, California	7 June, 1971		
17	0850	32°55.9'	117°19.0'	53	
18	0940	54.0'	18.0'	55	
19	1106	49.4'	21.3'	79	
20	1200	50.8'	19.7'	78	
21*	1236	52.2'	18.0'	72	

*Stations for productivity and chemical measurements.

in the past and much of this variation is attributable to intermittent coastal upwelling (Strickland, 1970). Nutrient inputs from upwelling could account for the year to year differences we observed.

Primary production, as nitrogen assimilation, was measured as well as the customary carbon-14 measure of photosynthetic carbon assimilation. Nitrate and ammonium uptake rates were measured with the ^{15}N-labeled compounds following procedures of Dugdale and Goering (1967). Uptake of ^{15}N-urea was also measured (apparently for the first time at sea) with rather surprising results. Urea appeared to provide as much as one-half the nitrogen assimilated by coastal phytoplankton (Table 4). Urea concentrations in seawater are of the same order as those of ammonium (McCarthy, 1971) but were not elevated at the outfall stations even when high ammonia concentrations were noted. The source of urea in seawater is thought to be the excretory metabolism of animals, including zooplankton, bony fishes and sharks (McCarthy, 1971).

The distribution of ammonia and of phytoplankton was patchy about the outfalls. This would be expected if one visualized the effluent leaving the diffusing mechanism of the outfall and maintaining a certain integrity over time and space before intermixing completely with the ambient seawater. Depth profiles in several instances showed an elevated ammonia concentration at a single depth, as if that sample bottle had filled in such a discrete "patch" or "plume" of sewage-seawater mixture (Table 1, see for example, Pt. Loma: depths 40-50 m). Phytoplankton patchiness was evident in continuous depth profiles of chlorophyll fluorescence and in the total quantity of chlorophyll integrated over the upper 50 meters (Table 3). This kind and scale of patchiness in chlorophyll distribution was seen earlier off La Jolla (Strickland, 1970) where its origin was presumably the temporal and spatial irregularity in natural upwelling off headlands and submarine canyons. The apparent patchiness in the properties measured about the outfalls makes one wonder how typical or representative our results may be. Finding high ammonia concentrations may depend largely upon fortune, depending on the ships drift rate over the outfall piping and the geometry of the plumed "high ammonia water." Chlorophyll distribution would be expected to be less patchy since chlorophyll synthesis, like other aspects of plant growth, is not instantaneous but occurs on a time scale of days and mixing could be more extensive on this time scale.

Our chlorophyll profiles, made at intervals about the outfalls, gave no evidence of a discrete phytoplankton bloom developing "downstream" of the outfall, as would be predicted if a steady longshore current were present (Dugdale and Whitledge, 1970).

Table 4. Relative importance of urea as a source of nitrogen for phytoplankton growth in southern California coastal waters.

Light Depth as % of Surface Irradiance	Urea Assimilation as % of Urea + Nitrate + Ammonium Assimilation Mean Values of 1970 and 1971 Pt. Loma	White Pt.	Control Stations off La Jolla, Calif.
87	40	62	41
43	60	47	37
20	30	43	38
8	44	38	49
4	17	36	16
1	3	12	15

However, it is quite possible that such a bloom could occur at those times when a persistent longshore current is present. One wonders if the frequent dinoflagellate blooms observed off Newport, California are caused by longshore transport of sewage nutrients from outfalls "up" the coast toward Los Angeles.

An interesting parameter which can be calculated from the nitrogen assimilation data and the concentrations of nitrogenous substances in the water is the time required for the phytoplankton crop to deplete the available nutrient, assuming no new input. Such turnover times ranged about 2-3 days in July, 1970, and 1-2 days in June, 1971 for the upper ten meters. Values tended to increase with depth either because of decreased crop, decreased rate of uptake per unit crop, or increased ambient nutrient (Table 5). Turnover times as low as those in the upper ten meters must be regarded as unusual for waters with high rates of nutrient input as one would intuitively consider near shore coastal waters to be. They imply rather close coupling between nutrient input rate and phytoplankton growth as in continuous phytoplankton cultures of the chemostat variety. The waters sampled for measurement of nitrogen assimilation fortuitously included a range of nitrate and ammonium concentrations so that the dependence of assimilation rate upon the concentration of these nitrogen compounds could be assessed and the maximum assimilation rate, V_m, and the half-saturation constant, K_s, could be evaluated in the Michaelis-Menten equation:

$$V = V_m S / K_s + S \qquad (1)$$

Values of V_m were 5 to 6 X 10^{-3} day^{-1} for nitrate and ammonium and the corresponding K_s values were 0.3 to 0.5 micromolar (Figs. 2 & 3). The K_s values agree well with earlier measurements with natural eutrophic phytoplankton (MacIsaac and Dugdale, 1968) and with cultures of neritic phytoplankton species (Eppley, Rogers, and McCarthy, 1969; Eppley and Thomas, 1969; Carpenter and Guillard, 1971). The V_m values are typical of oligotrophic waters of the Pacific Ocean (MacIsaac and Dugdale, 1968; Goering, Wallen and Naumann, 1970) rather than eutrophic waters of rich coastal regions. Assimilation of nitrate and urea decline with depth in a way which suggests a dependence upon light intensity (Tables 4 & 5). However, ammonium assimilation showed no light dependence in these ocean profiles (Table 5).

Table 5a. Nitrogen productivity in southern California coastal waters in July, 1970. Assimilation rates of nitrate, ammonium, and urea (μmoles/liter·day) were determined with ^{15}N-labelled substrates. Turnover times (days) were calculated for the nutrients dissolved in the water as (ambient concentration of nutrient + ^{15}N-nutrient added)/assimilation rate.

Depth (m)	Light Level†	Assimilation Rate			Turnover Time		
		NO_3	NH_4	Urea	NO_3	NH_4	Urea
		White Point Outfall, 13 July, 1970					
1	100	0.444	0.125	0.338	2.4	1.9	1.5
3	45	0.526	0.139	0.125	2.0	1.6	1.6
6	20	0.422	0.166	0.154	2.5	1.5	1.6
9	8.1	0.377	0.187	0.074	3.1	1.3	1.3
12	4.9	0.163	0.120	0.091	3.6	1.9	2.8
15	1.4	0.259	0.216	0.122	2.3	1.8	2.5
		Control Station off La Jolla, Calif., 3 July, 1970					
2	100	0.380	0.038	0.115	3.0	2.4	2.8
7	45	0.255	0.079	0.230	1.8	1.9	3.0
13	20	0.144	0.106	0.134	15*	2.5	3.0
16	8	0.029	0.085	0.149	100	2.6	4.0
23	4.9	0.010	0.060	0.017	>100	3.0	5.8
30	1.4	0.010	0.043	0.017	>100	3.8	7.5

*Sudden increase in nitrate turnover time represents increase in nitrate concentration with depth. Concentrations were 0.0, 0.0, 5.50, 7.30, 8.70, and 12.0 μmolar for depths shown. This effect is the rule rather than the exception. Ammonium and urea concentration show little depth variation and are usually <1 μM.

†As % surface irradiance.

Table 5b. Data for June, 1971. Values and units as in Table 5a.

Depth (m)	Light Level†	Assimilation Rate NO_3	Assimilation Rate NH_4	Assimilation Rate Urea	Turnover Time NO_3	Turnover Time NH_4	Turnover Time Urea
		Pt. Loma Outfall, 5 June, 1971					
1	100	0.389	0.158	0.613	1.5	1.8	1.4
3	45	0.140	0.190	0.565	1.4	1.5	1.5
7	20	0.382	0.332	0.303	18	2.1	3.2
11	8.1	0.178	0.209	0.178	67	4.4	7.2
15	4.9	0.171	0.102	0.054	94	6.2	18
20	1.4	0.187	0.266	0.0083	96	23	140
		White Point Outfall, 6 June, 1971					
1	100	0.083	0.180	0.529	1.2	1.3	1.2
3	45	0.076	0.150	0.740	1.3	1.5	1.5
6	20	0.077	0.139	0.389	1.3	1.4	1.3
9	8.1	0.066	0.114	0.305	1.5	1.5	1.4
12	4.9	0.056	0.260	0.290	1.8	1.2	1.3
16	1.4	0.028	0.738	0.038	12	1.8	2.6
		Control Station off La Jolla, Calif., 7 June, 1971					
1	100	0.223	0.173	0.350	1.8	1.9	1.7
10	20	0.272	0.421	0.322	1.5	1.5	1.5
21	4.9	0.169	0.133	0.037	69	3.5	8.0
		Point Loma Outfall, 8 June, 1971					
5	45	0.202	0.130	0.437	1.7	1.8	1.9
15	8.1	0.016	0.136	0.238	1.5	1.7	1.9
25	1.4	0.028	0.290	0.014	43	1.1	1.7

*Nitrate hardly detectable above 16 m.

†As % surface irradiance

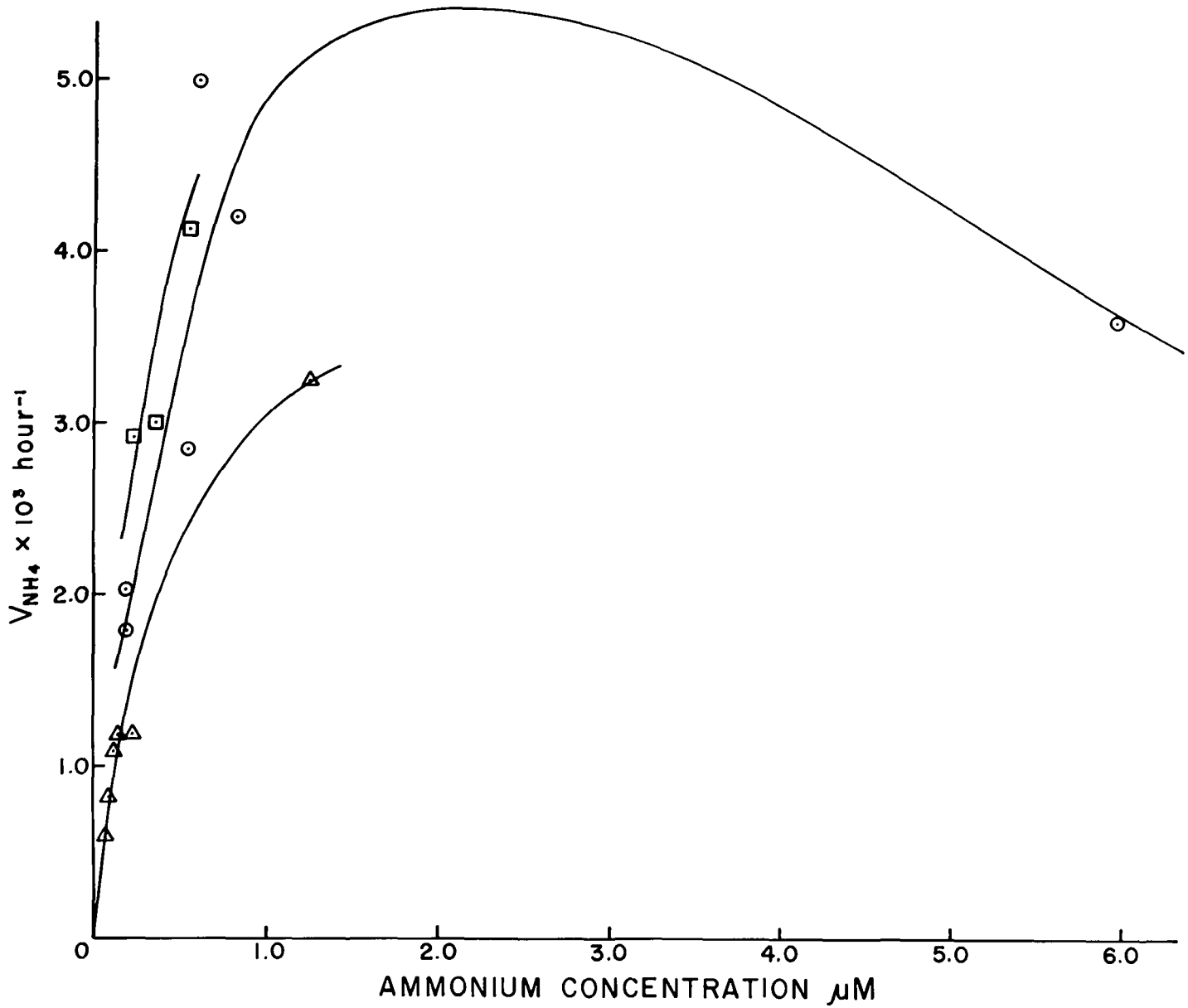

Fig. 2. The rate of ammonium assimilation by natural marine phytoplankton vs. concentration of ammonium in the water. Circles represent measurements at the Pt. Loma outfall, triangles are for White Pt. data and squares are for a control station off La Jolla, California.

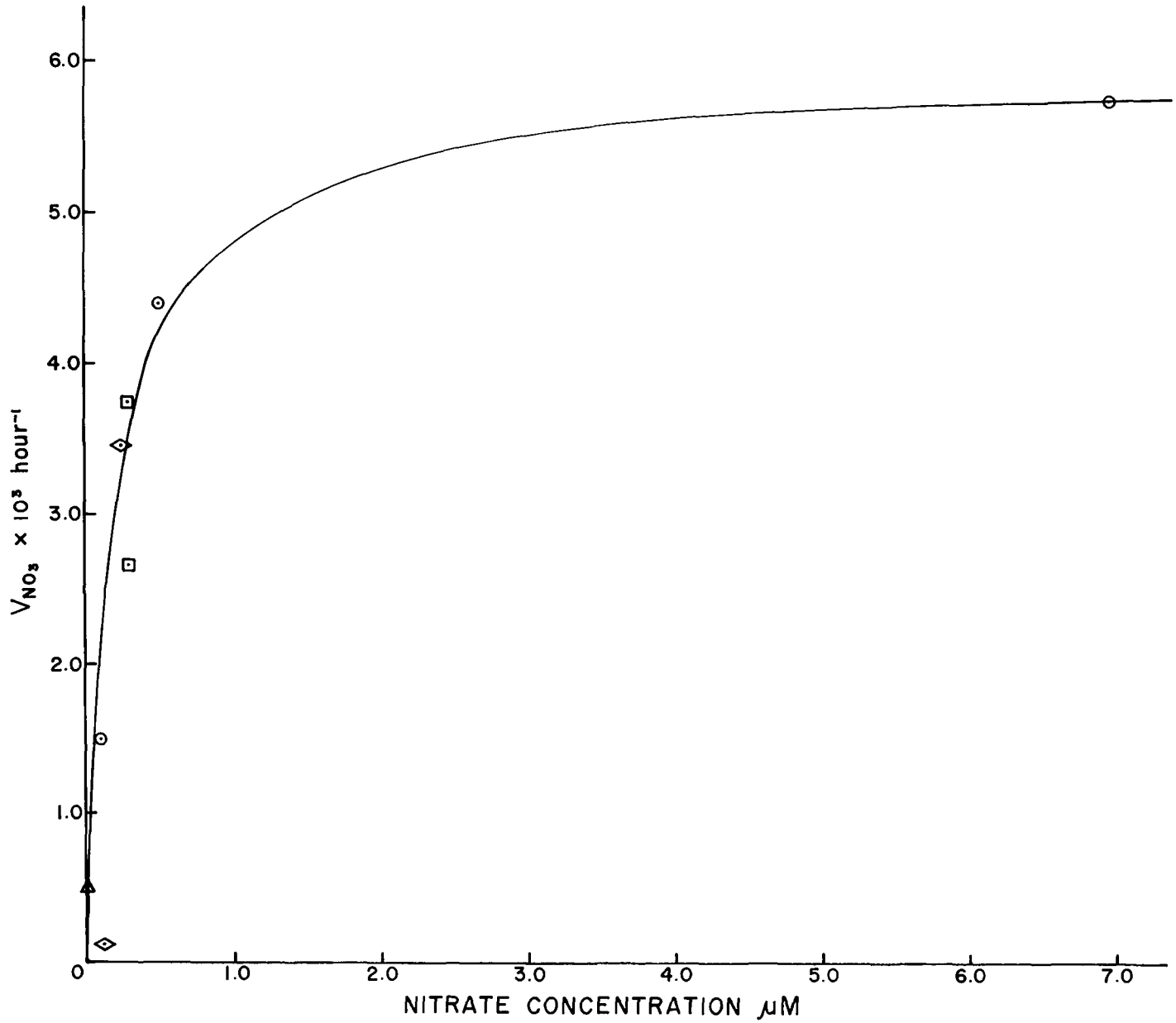

Fig. 3. The rate of nitrate assimilation by natural marine phytoplankton vs. nitrate concentration in the water. Circles: Pt. Loma; triangles: White Pt.; square; off La Jolla, California; diamonds: a second station at Pt. Loma.

SECTION VI

DIEL PERIODICITY IN NITROGEN ASSIMILATION

Three experiments were carried out for the purpose of studying day-night differences in nitrogen assimilation rate of marine phytoplankton: (1) a shipboard batch culture series in which 200 liters of seawater containing its natural phytoplankton flora were enriched with phosphate, silicate, chelated trace metals and vitamins and with either no added nitrogen, with nitrate, with ammonium, or with urea as the nitrogen source for growth. Details of the experimental procedures are described in Eppley *et al*. (1971a). There was essentially no growth in the culture with no added nitrogen as is typical of local waters except during upwelling (Eppley, 1968; W. H. Thomas, unpublished results). In the other cultures chlorophyll *a* synthesis was exponential over time with no evidence of diel periodicity (Fig. 4). The sudden change in slope for the nitrate and ammonium cultures represents depletion of vitamin B_{12}. Cell division was periodic (Fig. 5) and was most rapid in the afternoon and early evening. Nitrate and ammonium assimilation were also periodic (Fig. 6) and photosynthetic capacity showed its usual diel periodicity in the nitrate, ammonium, and urea cultures. Phosphate assimilation rate likewise showed diel periodicity irrespective of the form of nitrogen added (Fig. 7).

In a second experiment *Coccolithus huxleyi*, a small 5 μ diameter phytoplankter of cosmopolitan distribution in the oceans, was studied in a nitrogen-limited chemostat culture in the laboratory. Measurements included cell concentration, nitrate and ammonium in the culture (both forms of nitrogen were offered in the feed medium), cell nitrogen content, photosynthetic rate, and enzymatic activity of nitrate reductase, nitrite reductase, and glutamic dehydrogenase. Further details are given in Eppley, Rogers, McCarthy and Sournia (1971). Results are presented in Table 6 and Figure 8. Diel periodicity was noted in cell division and photosynthetic rate (Table 6) and in the activity of all three enzymes assayed (Fig. 8). However, nitrogen assimilation rates sufficed to maintain low ambient concentrations of nitrate and ammonium in the culture and no periodicity in assimilation rate of nitrogen could be observed.

In the third experiment *Skeletonema costatum*, a common coastal diatom, was studied in an N-limited chemostat culture grown, as was *C. huxleyi*, with illumination provided in light-dark cycles. Cell division and photosynthetic rate again showed diel periodicity (Table 7) and the ambient nitrate and ammonium concentration within the culture fluctuated between day and night

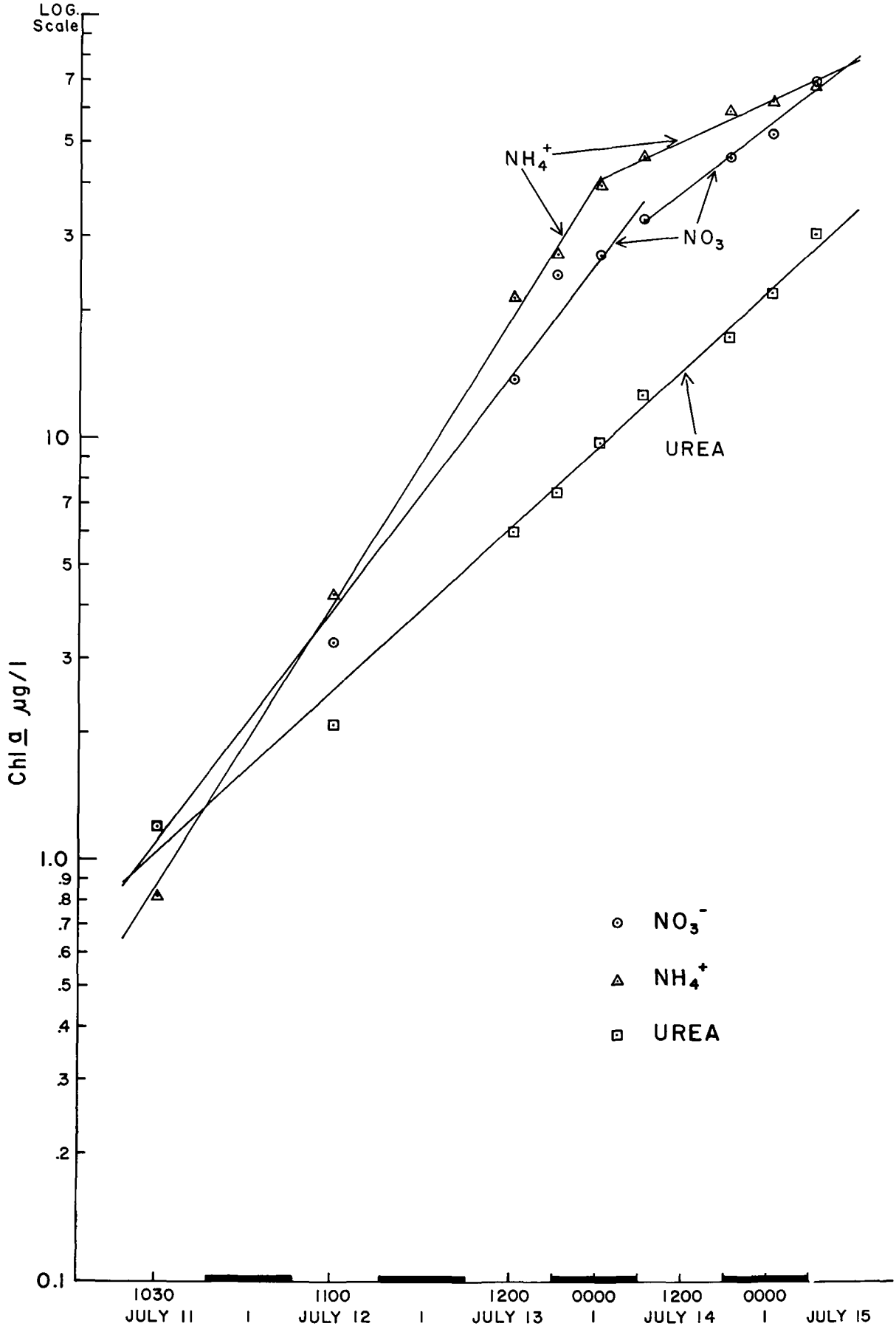

Fig. 4. Chlorophyll a concentration in shipboard cultures of natural marine phytoplankton during growth with nitrate, ammonium, or urea as the nitrogen source. Abrupt changes in slope represent depletion of vitamin B_{12} from the culture media.

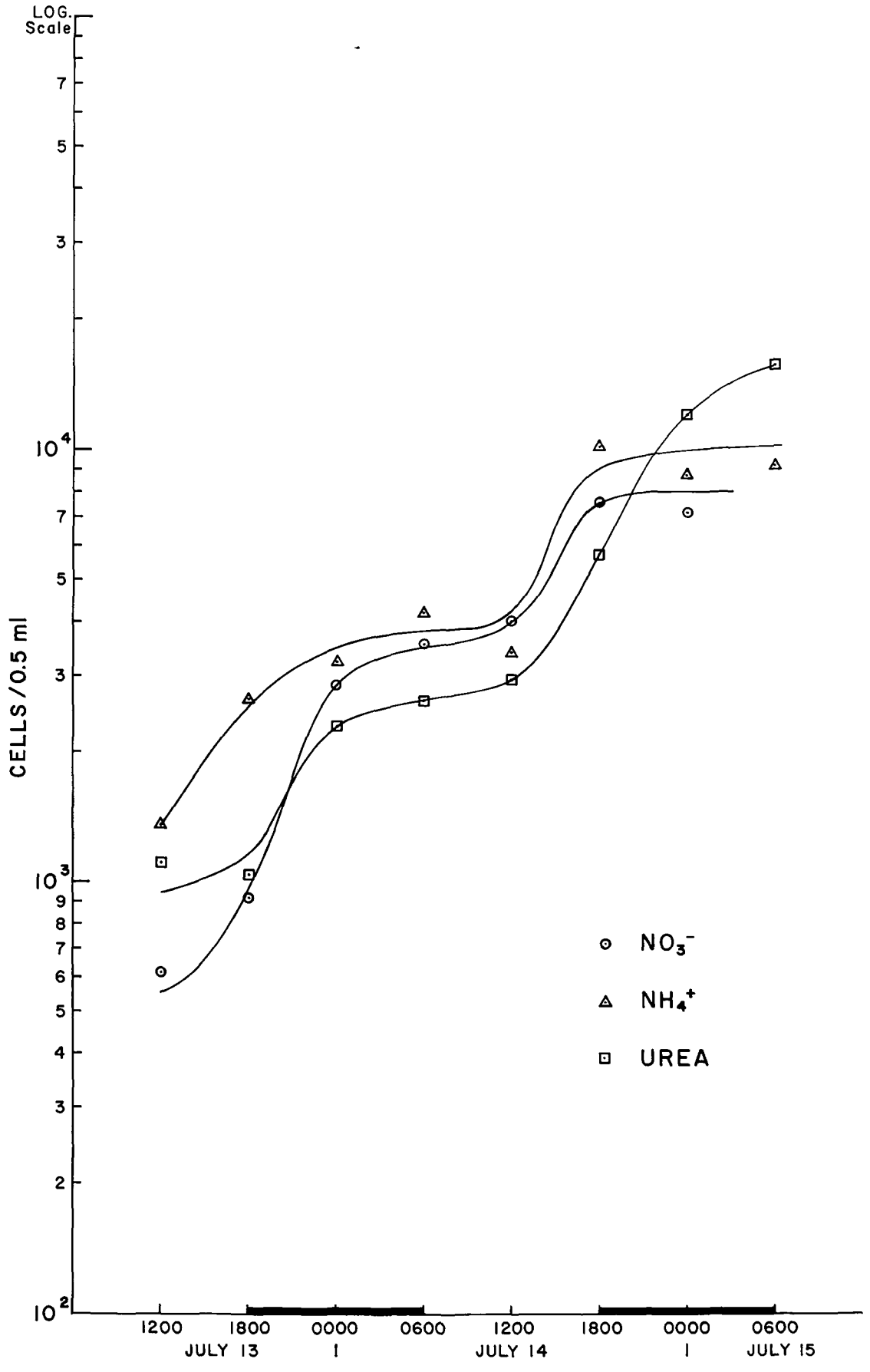

Fig. 5. Concentration of diatom cells in the shipboard cultures. Cell division appears to take place in the late afternoon and early evening.

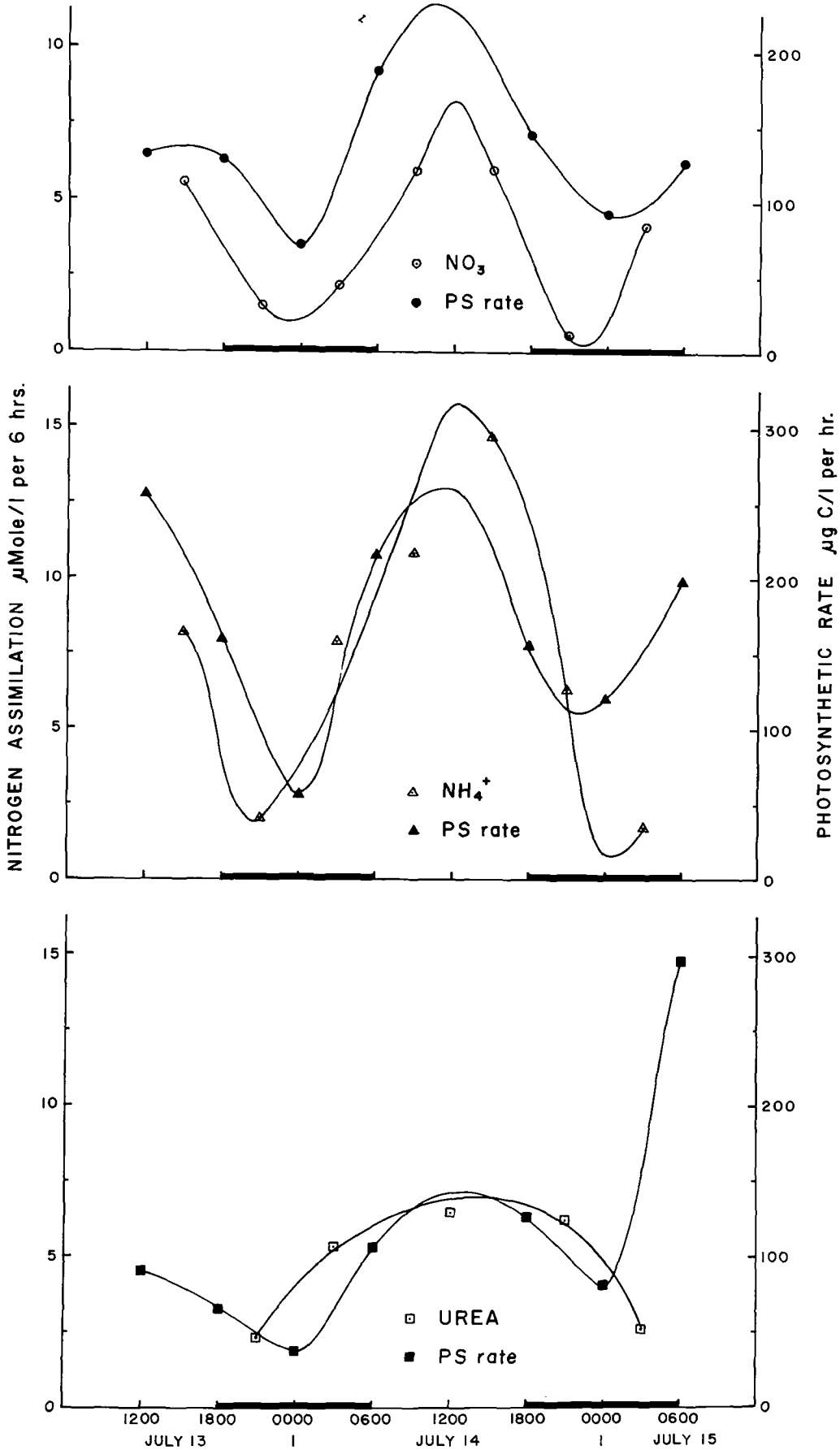

Fig. 6. Diel periodicity in the rate of nitrate (upper), ammonium (middle), and urea assimilation of the shipboard cultures. The capacity for photosynthesis (measured under artificial light) is also plotted (solid symbols).

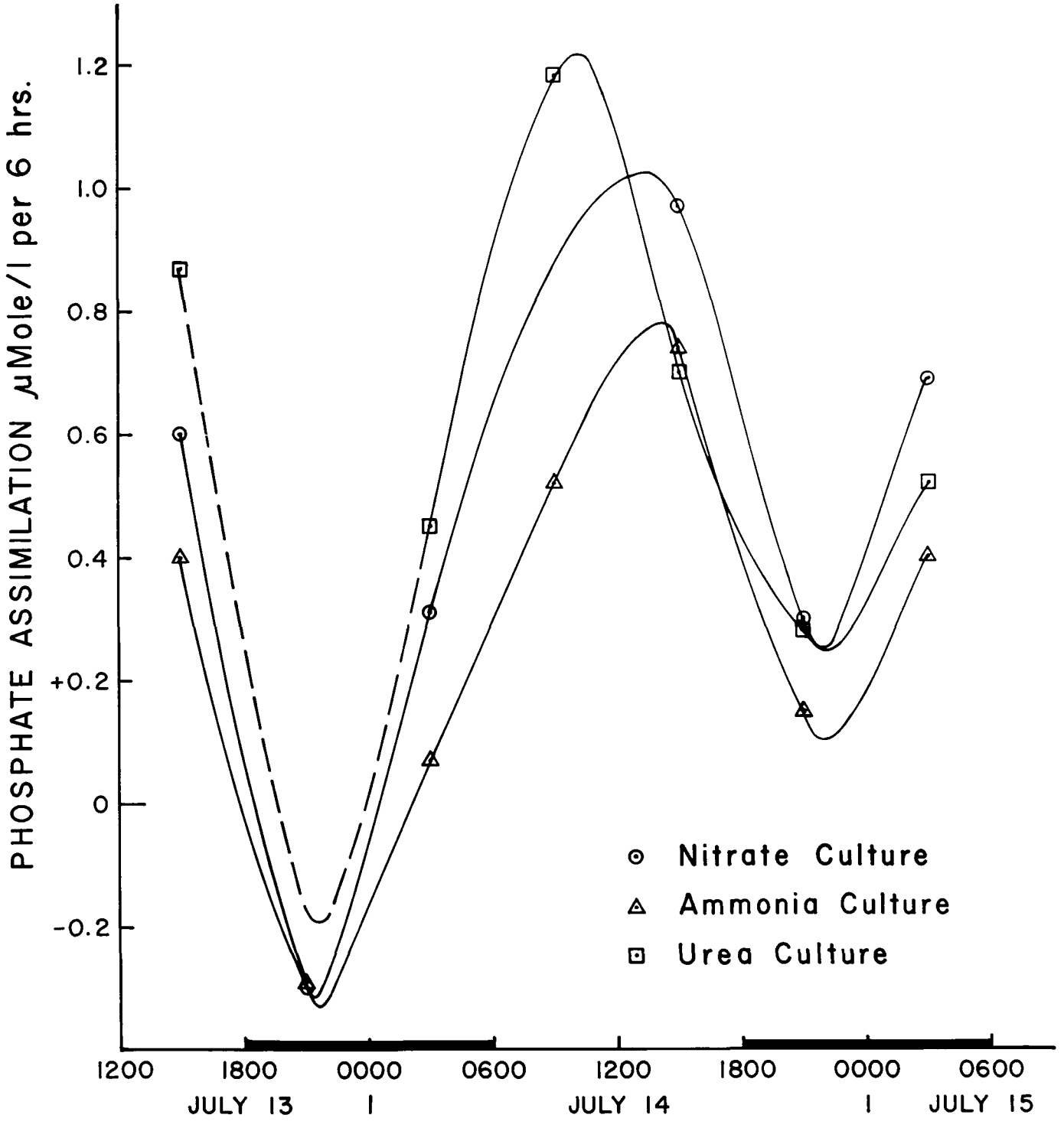

Fig. 7. Diel periodicity in the rate of phosphate assimilation in the shipboard cultures.

Table 6. N-limited chemostat culture of Coccolithus huxleyi, clone BT-6, with 16 hr. light/8 hr. dark illumination cycle: cell concentration, nitrate and ammonium concentration in the culture. Dilution rate was 0.78/day.

Day	Hour		(1) Cell Concentration (millions/l)	(2) (1) Expected From Dilution Rate*	(2) - (1) Cell Concentration Due to Cell Division	NO_3^- µM	NH_4^+ µM	Photosynthetic Rate g C/g Chl. a·hr.	N/cell (picograms)†
16 Feb.	0800	L	920	920*	0	.12	.02	3.4	0.73
	1500	L	780	760	20	.14	.05	6.0	0.86
17 Feb.	0000	D	660	570	90	.19	.09	1.0	1.0
	0400	D	790	500	290	.13	.14	0.38	0.85
	0800	L	920	440/920*	480/0	.13	.08	5.6	0.73
	1500	L	800	760	40	-	0.3	6.1	0.84
18 Feb.	0000	D	630	570	60	-	0.15	1.4	1.1
	0500	D	750	470	280	-	0.1	0.67	0.89
	0930	L	980	420/980	560/0	-	0.02	6.8	0.68
	1500	L	680	840	-160	-	0.25	6.4	0.98
19 Feb.	0000	D	660	630	30	-	0.1	0.45	0.83
	0800	L	900	490	410	-	0.1	2.5	0.74

†A picogram is 10^{-12} grams.

*Assuming no cell division. $N = N_o e^{-0.78t}$ where N_o is taken as the cell concentration at 0800 on Feb. 16 and 17 and 0930 on Feb. 18; t is in days.

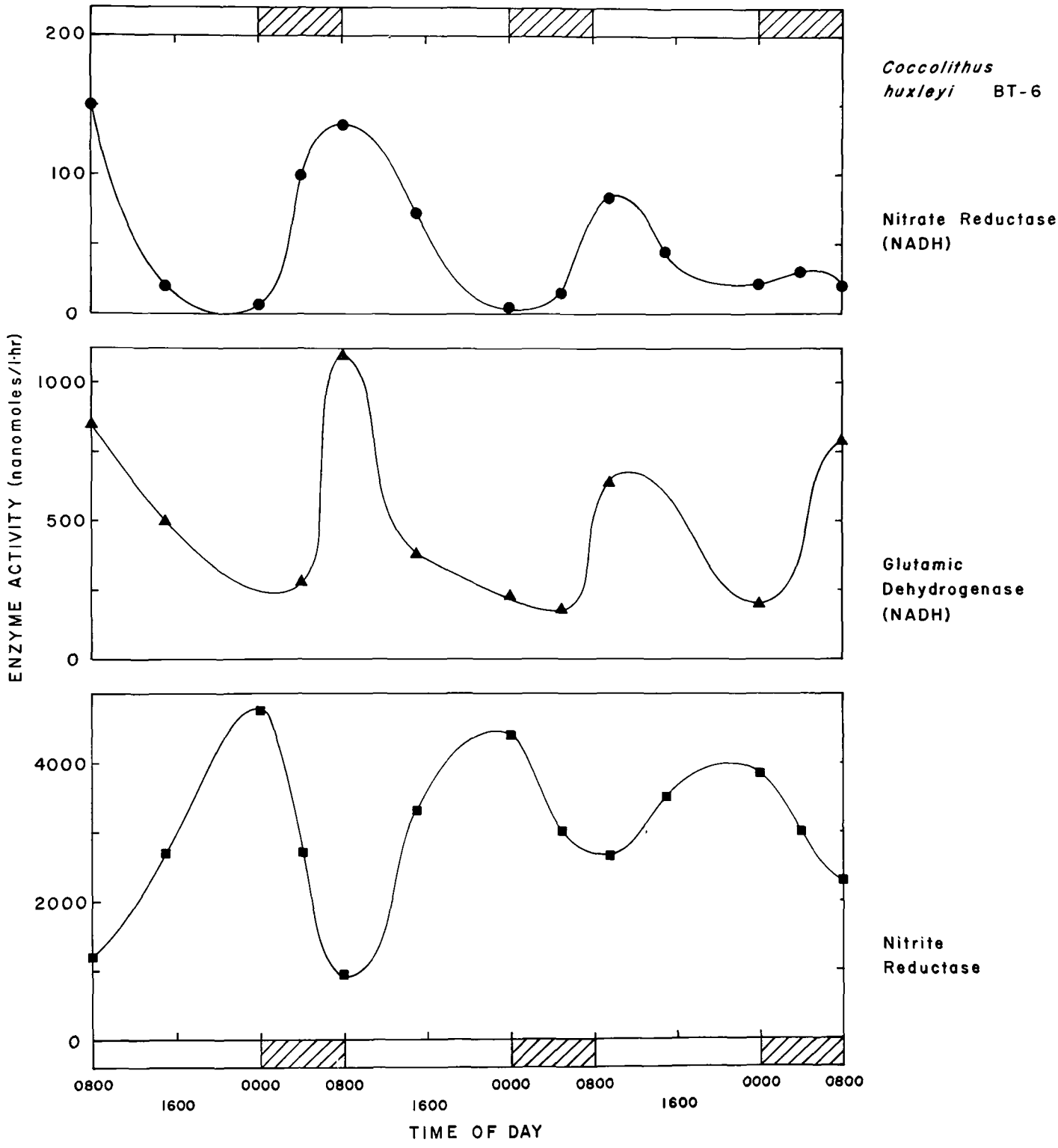

Fig. 8. Diel periodicity in the activity of three enzymes of nitrogen assimilation in an N-limited chemostat culture of Coccolithus huxleyi grown on a light-dark cycle. Upper curve: nitrate reductase; middle: glutamic dehydrogenase measured with NADH; lower: nitrite reductase. Activity of the latter appears to be out of phase with nitrate reductase and glutamic dehydrogenase activity, but in phase with photosynthetic capacity (see Table 6).

Table 7. N-limited chemostat culture of Skeletonema costatum, clone Skel. with 12 hr. light/12 hr. dark illumination cycle: cell concentration, chlorophyll a and N-content of a cell and photosynthetic rate.

Day	Hour	Cell Concentration (10^5/liter)	Cells/chain	Chlorophyll a per cell (picograms)*	Nitrogen/cell (picograms)	Photosynthetic Rate g C/g Chl. a·hr.
13 May	1400 L	455	5.6	3.4	15	3.00
	2000 D	272	3.5	5.7	25	2.25
14 May	0200 D	292	4.8	3.4	20	1.87
	0800 L	342	6.6	2.6	15	3.01
	1400 L	230	5.2	4.8	30	3.65
	2000 D	193	5.4	6.9	36	2.35
15 May	0200 D	157	4.5	5.7	31	2.14
	0800 L	254	7.2	2.5	14	2.72
	1400 L	220	5.0	2.9	17	3.11
	2000 D	167	-	-	-	-

*A picogram is 10^{-12} grams.

such that assimilation rate of these ions could be evaluated (Fig. 9). Rates in the figure are expressed as V with units hr^{-1} and were calculated as the uptake per hour per cell nitrgen content. Units so expressed are equivalent to a specific growth rate and can be compared with the data from ocean profiles based upon the ^{15}N method of measuring nitrogen assimilation. Values of V in the culture experiments are nearly an order of magnitude greater than in the ocean profiles. This may result from the dilution effect of detrital nitrogen on the calculation of V in natural samples, as well as from the higher ambient nutrient concentrations in the cultures and higher specific growth rates.

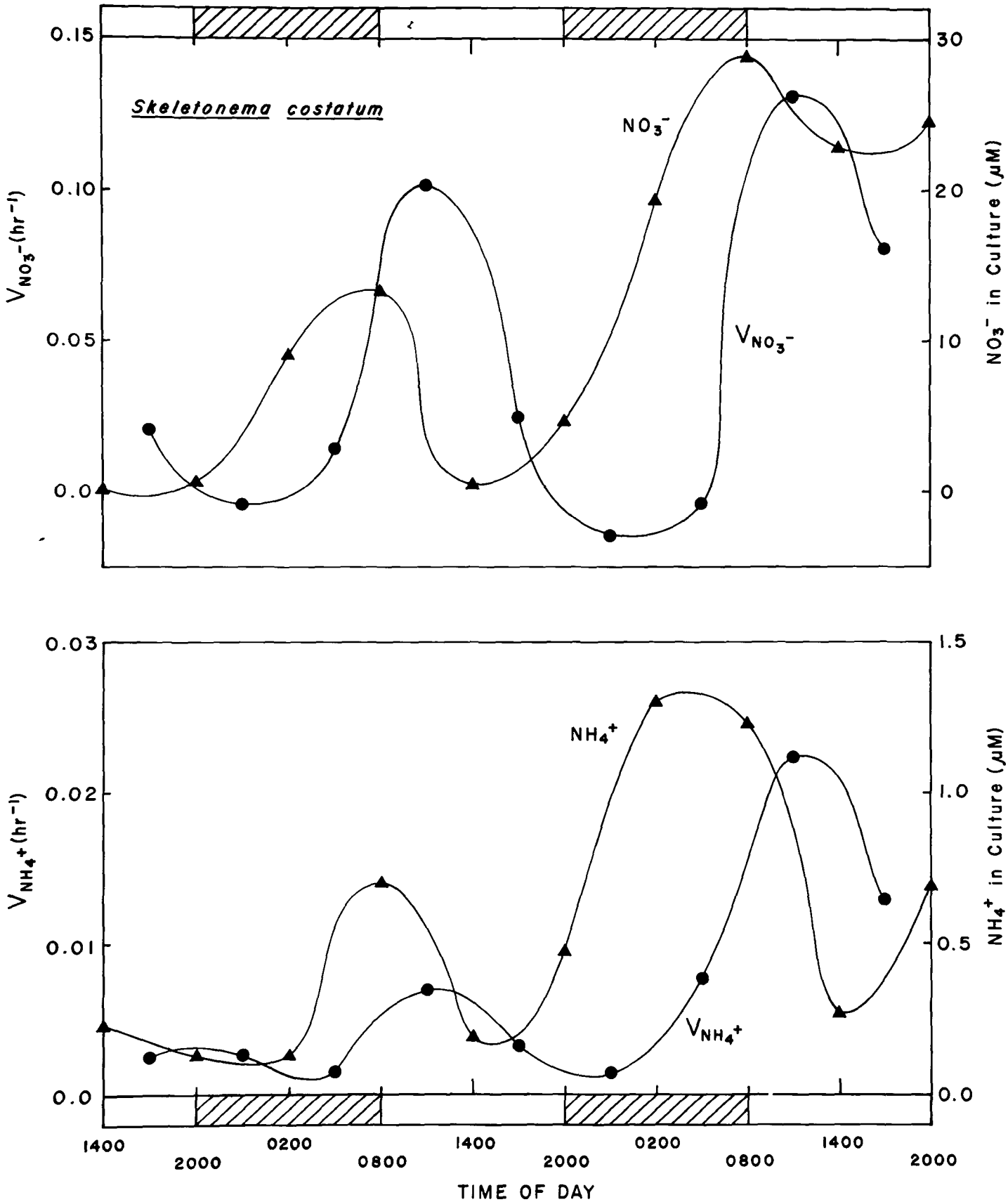

Fig. 9. Diel periodicity in ambient concentration of nitrate and in the velocity of nitrate assimilation (upper curves) and in the concentration of ammonium and rate of ammonium assimilation in an N-limited chemostat culture of Skeletonema costatum grown on light-dark cycles.

SECTION VII

KINETICS OF NITROGEN ASSIMILATION

Coccolithus huxleyi was grown in a nitrogen-limited chemostat culture until a steady-state was reached (constant cell concentration from day to day) at each of two dilution rates. A large volume of the culture was withdrawn and divided into aliquots. To these was added ammonium sulfate solution to achieve a range of ammonium concentrations 0.1 to about 10 μM. The aliquots of cell suspension were incubated 10 to 30 minutes, as appropriate, the cells were removed by filtration and the remaining ammonium measured. Uptake rate was computed from the difference between initial and final concentrations and the half-saturation constant and maximum uptake rate were evaluated using Wilkinson's method (Wilkinson, 1961). The values could be compared with results from a stationary phase batch culture obtained earlier (Eppley, Rogers, and McCarthy, 1969). The half-saturation constant (K_s) was found to be invariant with dilution rate but large differences were noted in the maximum velocity of uptake (V_m) as shown in Table 8. The V_m values increased as the dilution rate decreased, that is, as the cultures became progressively more nitrogen-deficient.

The apparent half-saturation constant (K_s') can also be computed in another way, from the dilution rate and the concentration of ammonium in the culture, if μ_m is evaluated and if data are compiled from several steady state cultures at different dilution rates (cf. Droop, 1970) using the equation

$$\mu = {}^{\mu}{}_m \, S/K_s + S \qquad (1)$$

The value of μ_m is taken as the dilution rate required to washout the culture, S is the ammonium of nitrate concentration in the vessel and μ is the dilution rate. This technique gives apparent K_s' values which decrease systematically with increasing dilution rate (Table 9). Droop (1970) regards the "true" value of K to be that minimal value obtained as μ approaches μ_m and this agrees with our data (Table 10).

A culture of C. huxleyi and S. costatum, together was studied in an attempt to observe species succession resulting from different kinetic parameters of nitrogen uptake between the two species. The cosmopolitan C. huxleyi has a lower K_s for uptake of both ammonium and nitrate than does the neritic diatom, S. costatum. However, the latter has a greater maximum growth rate, μ_m. Hence if one graphs expected growth rate against concentration

Table 8. Kinetics of short-term ammonium uptake by Coccolithus huxleyi, clone BT-6.

Dilution Rate as doublings/day (actual doublings/day of cell-N)	K_s μM ± S.E.	V_{max} as doublings/day of cell-N
0*	0.35 ± 0.10	7.3
0.32	0.28 ± 0.07	4.8
0.63	0.33 ± 0.10	2.4

*N-depleted batch culture in stationary phase of growth.

Table 9. Variation with dilution rate of the apparent half-saturation constant (K_s) for the uptake of nitrate and ammonium by phytoplankton grown in nitrogen-limited chemostat cultures. 18°C. Values in parenthesis are extrapolated to the maximum dilution rate by graphing apparent K_s' vs μ.

Dilution Rate as doublings/day (μ)	Apparent Half-Saturation Constant in moles/liter (K_s)	
	Nitrate	Ammonium
	Gymnodinium splendens	
0.24	0.66	0.62
0.37	0.75	1.0
0.51	2.4	1.2
0.57 = μ_m	72.4	(1.2)
	Leptocylindrus danicus	
0.23	4.9	4.3
0.78	1.8	1.2
1.43	0.57	0.44
1.85	0.62	0.85
2.35 = μ_m	(0.6)	(0.5)
	Coccolithus huxleyi BT-6	
0.32	1.08	0.73
0.56	0.51	0.42
0.76	0.36	0.16
1.08	0.14	0.10
1.41	0.05	0.03
1.7 = μ_m	(0.1)	(<0.1)

Table 10. Comparison of half-saturation values determined by two independent methods. In column (a) are values based on short-term uptake experiments in which uptake was measured vs. concentration with N-depleted batch cultures. In column (b) values are obtained from extrapolating the apparent K_s' to $\mu = \mu_m$, as in Table 9. Values in column (a) from Eppley, Rogers, and McCarthy, 1969.

Species	Half-Saturation Constant, K_s, μmoles/liter			
	For Nitrate		For Ammonium	
	(a)	(b)	(a)	(b)
Coccolithus huxleyi	0.1	0.1	0.1	<0.1
Leptocylindrus danicus	1.2	0.6	0.5	0.5
Gymnodinium splendens	3.8	>2.4	1.1	1.2

of nitrate or ammonium, based on eq (1) and with measured values of K_s and V_m, the curves cross. The coccolithophorid would be expected to grow fastest, i.e. to eventually win the competition, at low nutrient concentrations and low dilution rates while the diatom would do better at higher levels. However, both species maintained themselves at the dilution rates employed although the ratio of their concentrations did follow the expected trend (Table 11). The experiment was not entirely satisfactory for two reasons. At the lowest dilution rate S. costatum lost its buoyancy and appeared to become sticky. Even vigorous aeration did not keep the cells suspended and they tended to settle out and stick to the walls of the vessel. Furthermore C. huxleyi showed a high percentage of motile cells at the low dilution rates and this may represent a significant alteration of its life cycle with possible physiological differences from the usual non-motile cells. The ability to become motile under conditions of nutrient stress has obvious survival value (Munk and Riley, 1952).

Data from N-limited chemostat cultures of Leptocylindrus danicus, a coastal, chain-forming diatom, Gymnodinium splendens, a 50 micrometer diameter dinoflagellate often present as a minor species component of local red water blooms, and C. huxleyi were collected for evaluation of the apparent K_s value for ammonium and nitrate uptake using methods analogous to those of Droop. K_s values obtained earlier from short-term uptake measurements of N-depleted batch cultures were available for comparison. Results were as noted by Droop: i.e. the apparent K_s value obtained as μ approached μ_m agreed with the earlier values reported (Tables 10 & 11).

The implication of these comparisons is that μ_m in eq (1) varies with dilution rate while K_s remains constant. If this is so then this variation in apparent μ_m can be calculated from eq (1) taking a constant K_s (the minimum value as μ approaches μ_m or the value determined in short-term uptake experiments). When this is done for the three species studied the value of μ_m was found to be a linear function of the dilution rate (μ) and had a value about twice the value of μ (Fig. 10). Recent results of Thomas and Dodson (1971) show that the rate of photosynthesis at light saturation varied with dilution rate in N-limited chemostat cultures of marine phytoplankton in the same direction as the variation in μ_m. Since one would expect the two parameters to be closely related Thomas' observations seem to confirm variation in apparent μ_m we calculated.

Table 11. Ratios of Skeletonema costatum cell concentration to that of Coccolithus huxleyi BT-6, when the two phytoplankters were grown together in the same culture, as a function of the dilution rate of the culture. Dilution rate is expressed as doublings/day.

Dilution Rate	Skeletonema/Coccolithus
0.26	No steady state reached*
0.79	2.60
0.84	3.07
1.49	5.97
1.52	7.10

*See text for explanation.

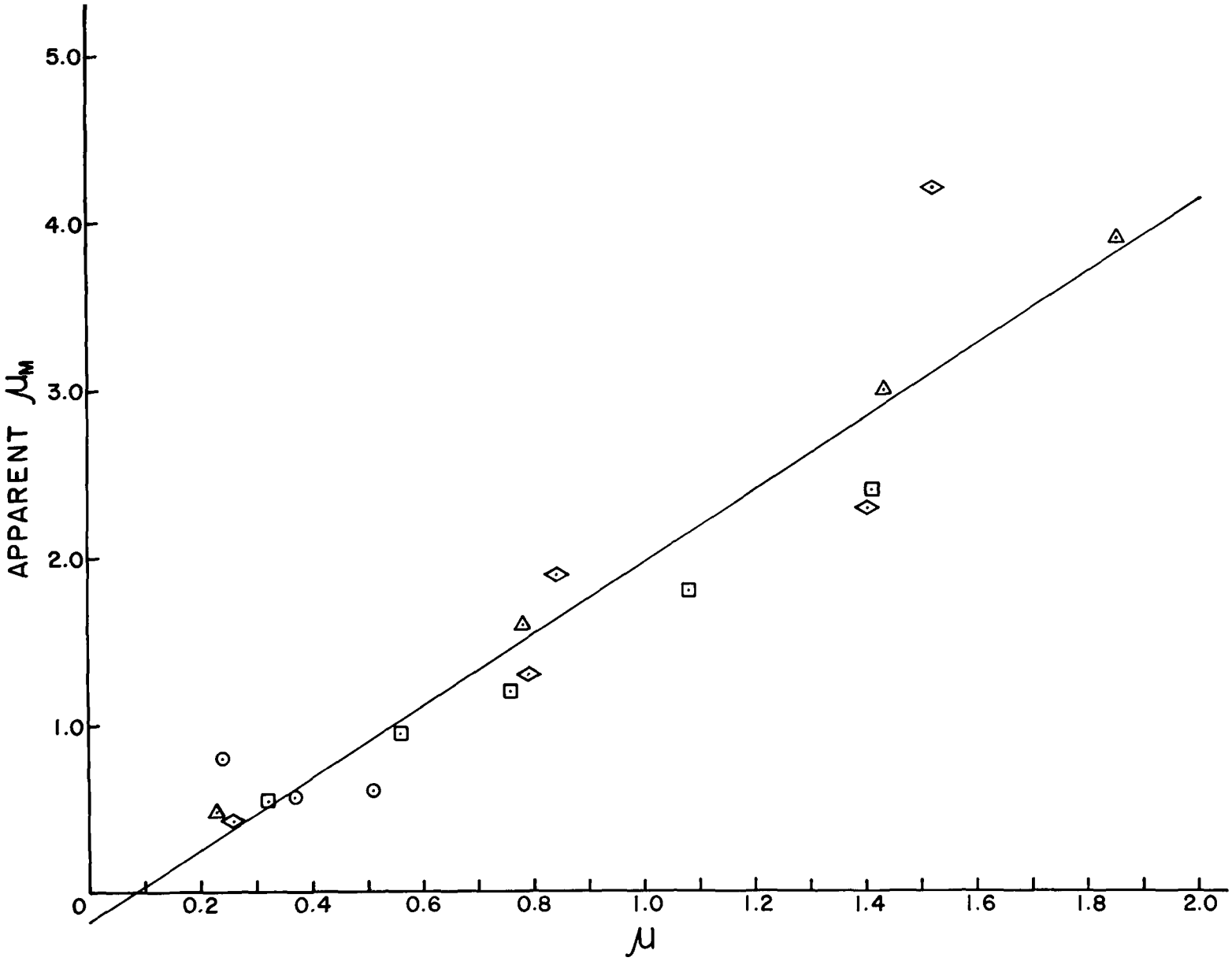

Fig. 10. Variation in the apparent value of μ_m with dilution rate, μ, based on phytoplankton growth in N-limited chemostat cultures. The value of μ_m' (the apparent μ_m) was calculated from equation 1 (see text). Circles: Gymnodinium splendens; triangles: Coccolithus huxleyi; squares: mixed culture of C. huxleyi with Skeletonema costatum; diamonds: Leptocylindrus danicus.

SECTION VIII

INFLUENCE OF THE RATE OF NITROGEN INPUT ON THE CHEMICAL COMPOSITION OF PHYTOPLANKTON

The carbon, nitrogen, and chlorophyll *a* content of the phytoplankton cultures grown as N-limited chemostats were measured at steady-state with several dilution rates. The carbon/nitrogen and carbon/chlorophyll *a* ratios varied systematically with dilution rate as would be expected if the dilution rate of an N-limited cultures were a quantitative measure of the degree of N-deficiency of the cells in the culture (Thomas and Dodson, 1971). Certain similarities between the species can be seen if the composition ratios are plotted not against dilution rate, μ, but against the ratio μ/μ_m as Thomas has done. The C/N ratio then shows little variation among the species (Fig. 11). Species differences are still noted, however, in the C/Chlorophyll *a* ratio (Fig. 12).

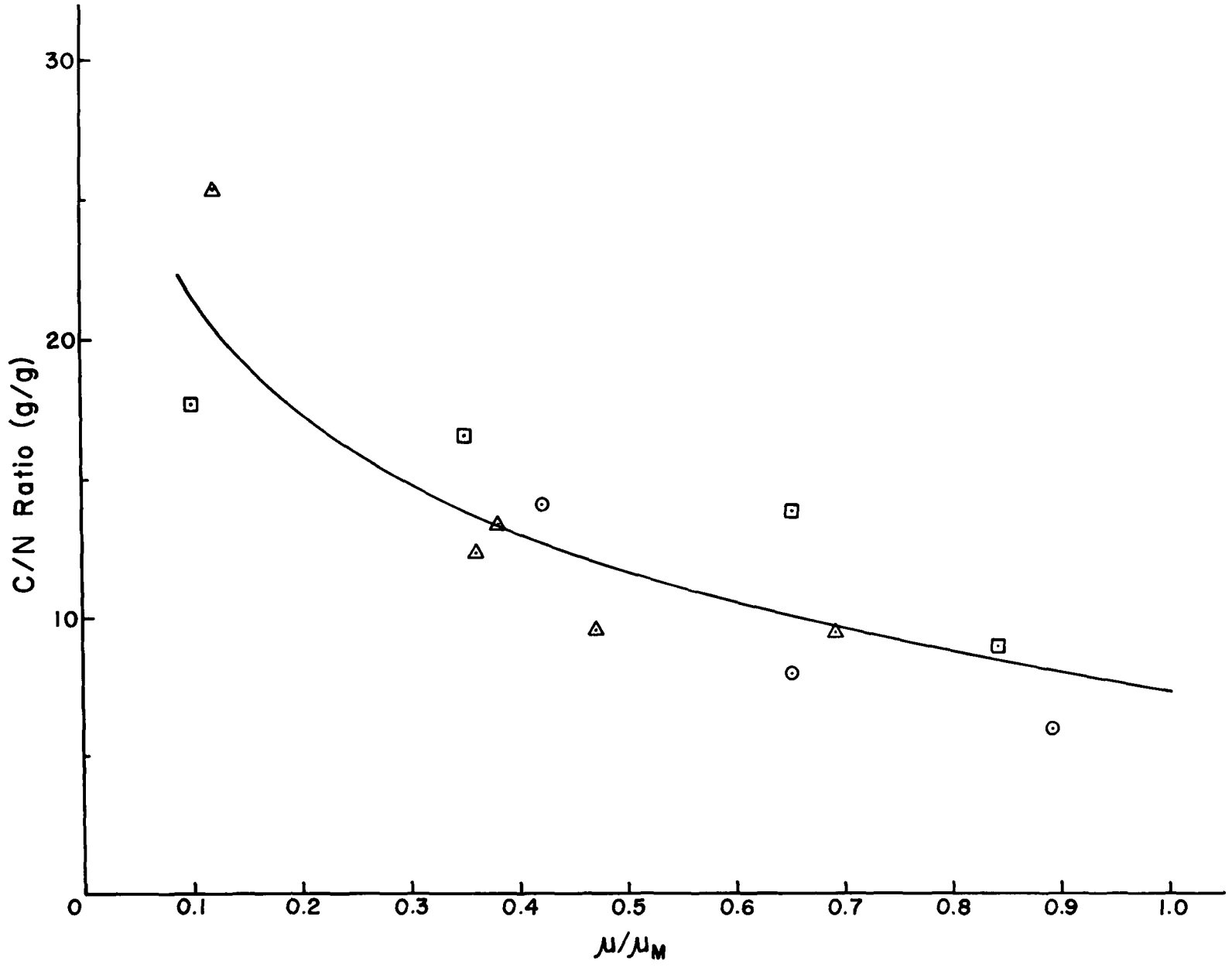

Fig. 11. Ratios of carbon to nitrogen in phytoplankton grown in N-limited chemostat cultures at different dilution rates. The abscissa represents the dilution rate, μ, as a fraction of the dilution rate at washout of the culture, μ_m. Circles: Gymnodinium splendens; triangles: a mixed culture of Coccolithus huxleyi with Skeletonema costatum; squares: Leptocylindrus danicus.

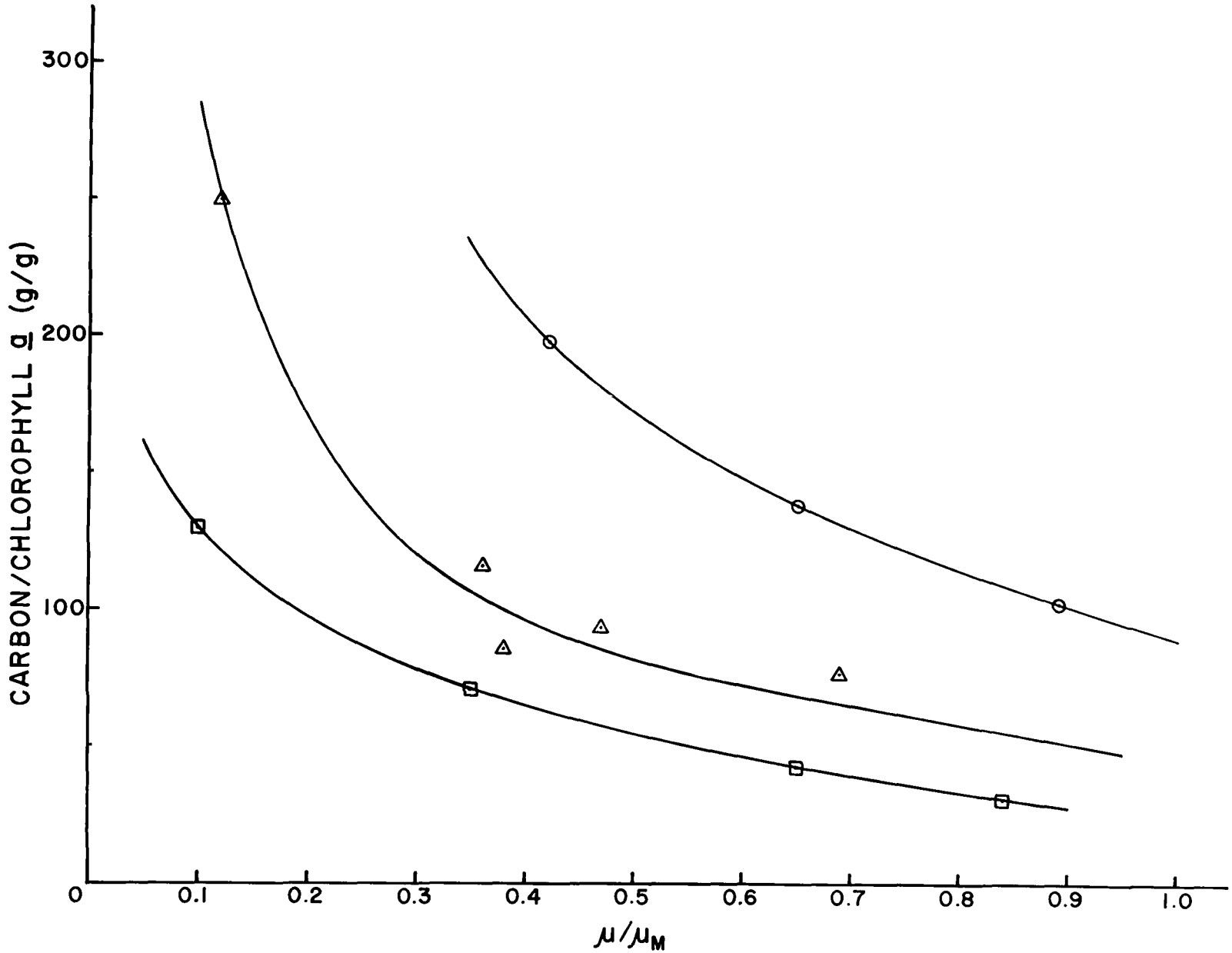

Fig. 12. Ratios of carbon to chlorophyll a of phytoplankton grown in N-limited chemostat cultures. Symbols as in Fig. 10.

SECTION IX

COMPARISON OF METHODS OF MEASURING NITROGEN ASSIMILATION RATE

Direct chemical measurements lack sufficient sensitivity and precision for measuring rates of phytoplankton assimilation of nitrate, ammonium and urea or of the increase of phytoplankton particulate nitrogen in natural seawater samples. Typical rates are of the order nanomoles of N assimilated per liter and hour and depend upon the phytoplankton and substrate concentrations in the water, as well as upon the metabolic rate of the organisms, as influenced by irradiance, temperature, and the species composition of the phytoplankton assemblage.

The introduction of ^{15}N methodology (Dugdale and Goering, 1967) for measuring nitrogen productivity of seawater samples incubated on shipboard provided a significant addition to the available methods for studying phytoplankton growth in the sea. The technique has broad appeal for use in field phytoplankton studies. It allows one to measure nitrogen productivity and to distinguish between the fractions due to different forms of nitrogen utilized (Dugdale and Goering, 1967; Goering, Wallen and Naumann, 1970). Assay of phytoplankton enzymes involved in nitrogen assimilation, such as nitrate reductase, is also feasible at sea (Eppley, Coatsworth and Solórzano, 1969; Eppley, Packard and MacIsaac, 1970) and may indicate qualitatively the utilization of nitrate or its suppression by ammonium.

The following study used shipboard culture experiments to compare the ^{15}N-method and direct chemical analyses of nitrogenous nutrients and particulate nitrogen for estimating the phytoplankton uptake of nitrate, ammonium, and urea. At the same time enzyme assays of nitrite reductase and glutamic dehydrogenase were carried out with the heuristic purpose of assessing their utility in studies of phytoplankton nitrogen assimilation. Moreover, the shipboard cultures provided an opportunity to observe interactions between phytoplankton uptake of nitrate, ammonium, and urea when more than one of these forms of nitrogen was added.

Results of the ^{15}N- and enzyme methods are also compared for several stations off the California coast, between San Diego and Los Angeles, where assessment of phytoplankton uptake of nitrogen by direct chemical measurement was not possible. Other aspects of this work are described in Eppley *et al.* (1971a, b).

Shipboard Cultures

The ^{15}N technique gave good results in comparison with the direct chemical methods of measuring N-assimilation, i.e. loss of nitrate, ammonium, or urea from the culture medium and increase in particulate nitrogen in the cultures (Table 12). The isotope technique overestimated nitrogen utilization over a 36 hour period by 9.2% in the nitrate cultures and by 22.8% in the ammonium culture and underestimated it by 0.5% in the urea culture, when compared to an average value of particulate nitrogen increase and nutrient loss. Furthermore, specific growth rates computed from nitrogen uptake rates compared well with those computed from increases in cell concentration.

Nitrite reductase (NiR) activity would be expected to follow the capacity for nitrate uptake and it showed a fair correspondence to the measured rate of nitrate assimilation (Tables 12, 13). Rates of nitrate uptake capacity and NiR activity were highest in the nitrate culture, intermediate in urea cultures and lowest when ammonium was the nitrogen source. A linear regression of nitrite reductase specific activity vs $^{V}NO_3$ gave

$$NiR/PN = 0.95\ V_{NO_3} - 0.09$$

with 95% confidence limits on the slope of 0.55 with 17 pairs of measurements.

Glutamic dehydrogenase activity would be expected to parallel the capacity for ammonium assimilation but GDH assays proved to be a poor measure of the latter, either when enzymatic activity was measured with NADH, NADPH or as the sum of that with both pyridine nucleotides (Table 13). The ratio of the total GDH activity to the rate of ammonium assimilation measured with ^{15}N in the cultures was 0.73 ± 0.56 (2σ) for samples taken during the day when the two measures would be expected to most closely correspond to one another.

The close relation between nitrite reductase activity and rate of nitrate assimilation noted in the culture experiments was not observed in the natural seawater samples. Nitrite reductase activity was always higher than the rate of NO_3^- assimilation measured with $^{15}NO_3^-$, often by an order of magnitude. Since NiR also occurs in micro-organisms other than phytoplankton, heterotrophic NiR offered a potential explanation. Hence, we compared NiR activity per weight of chlorophyll a (present only in photosynthetic organisms) and per weight of ATP (present in all organisms) in the filtered particulate matter, for both seawater samples and culture samples (containing few heterotrophs) without finding any significant

Table 12. Changes in particulate nitrogen; nitrate, ammonium, and urea concentrations; utilization* of nitrogen; and nitrite reductase activity for each six-hour period by shipboard cultures.

NITRATE CULTURE

Date	Δ Particulate Nitrogen (µg at N/l)	Δ NO_3^- (µg at N/l)	Utilization of Nitrogen (ρNO_3) (µg at N/l)	Nitrite Reductase Activity (µg at N/l)
13 July 1200				
	4.5	7.6	2.6	5.1
13 July 1800				
	5.1	1.5	2.5	5.1
14 July 0000				
	-0.7	2.2	3.2	3.9
14 July 0600				
	13.1	8.0	4.8	4.8
14 July 1200				
	10.4	6.0	4.5	4.8
14 July 1800				
	3.7	0.6	5.7	6.5
15 July 0000				
	0.0	4.2	8.9	6.2
15 July 0600				
Σ 13-15 July 1800 0600	31.6	22.5	29.6	31.3

Table 12, Cont'd.

AMMONIUM CULTURE

Date	Δ Particulate Nitrogen (μg at N/l)	Δ NH_4^+ (μg at N/l)	Utilization of Nitrogen (ρNH_4^+) (μg at N/l)
13 July 1200			
	13.8	8.2	3.5
13 July 1800			
	4.3	2.0	5.8
14 July 0000			
	15.9	7.9	7.9
14 July 0600			
	12.1	10.8	13.1
14 July 1200			
	4.5	14.7	12.4
14 July 1800			
	9.8	6.3	8.3
15 July 0000			
	1.2	1.7	9.0
15 July 0600			
Σ 13-15 July 1800 0600	48.8	43.4	56.5

Table 12, Cont'd.

UREA CULTURE

Date	Δ Particulate Nitrogen (µg at N/l)	Δ Urea (µg at N/l)	Utilization of Nitrogen (ρ_{urea}) (µg at N/l)
13 July 1200	-	-	-
13 July 1800	1.1	2.3	2.9
14 July 0000	1.0	5.3	2.5
14 July 0600	4.7	6.4	7.1
14 July 1800	8.4	6.2	3.2
15 July 0000	2.0	2.6	4.2
15 July 0600			
Σ 13-15 July 1800 0600	17.2	22.8	19.9

*The uptake rate of ^{15}N-labelled nitrate, ammonium, or urea.

Table 13. The mean rate of uptake (V) for nitrate, ammonium, and urea and nitrite reductase (NiR) and glutamic dehydrogenase (GDH) activities per weight of particulate nitrogen in each culture averaged over the duration of the experiment. All units are hrs.$^{-1}$. GDH activity is the sum of that with NADH and NADPH measured separately.

Culture	$V_{NO_3^-}$	$V_{NH_4^+}$	V_{urea}	$\frac{NiR}{PN}$	$\frac{GDH}{PN}$
		Experiment I			
Nitrate	.0236	.0304*	.0087*	.017	.025
Ammonium	.0034*	.0309	.0052*	.004	.025
Urea	.0080*	.0367*	.0237	ND	.022
		Experiment II			
Nitrate	.0278	.0245*	.0105*	.025	ND
Ammonium	.0014*	.0318	.0039*	.008	ND
Urea	.0164*	.0417*	.0418	.012	ND

*Potential uptake. Phytoplankton were exposed to this form of nitrogen only during measurement period.

differences: NiR/Chl. a and NiR/ATP were similar in the nitrate culture and in the seawater samples. Furthermore, for natural seawater samples, a graph of the ratio NiR/ATP vs Chl. a/ATP had a positive slope, with 95% confidence limits not including a slope of zero, and the intercept was not significantly different from zero ($\rho<.05$). This seems to imply that the nitrite reductase activity observed is primarily in chlorophyll-containing cells. Differences between NiR activity and rate of nitrate uptake would be due to low nitrate uptake rates rather than lack of assimilatory enzymes. Nitrite reductase activity was found even in surface sea water samples lacking nitrate and with low rates of nitrate assimilation.

Results with glutamic dehydrogenase assays of seawater samples were even less satisfactory. Control samples, containing a complete reaction mixture except ammonium sulfate, showed considerable oxidation of pyridine nucleotide not seen in work with cultures. This suggested that high levels of ammonium were somehow introduced in the enzyme preparation from the filtered particulate matter and this suggestion was later confirmed by ammonium analysis of homogenated particulate matter from sea water collected at the Scripps Institution Pier. It is apparent that we were, in fact, measuring the rate of oxidation of reduced pyridine nucleotides in the presence of α-ketoglutarate and ammonium and that actual GDH levels could not be calculated for lack of adequate experimental controls, lacking ammonium.

Since even this information may be of value in assessing metabolic activity, the data for culture and sea water samples were recalculated as rate of pyridine nucleotide oxidation. The sum of the activities with both nucleotides was a constant fraction of the ATP content of the samples (Table 14) in both culture and seawater samples over 3 orders of magnitude variation in ATP content. The summed activities (i.e. that with both pyridine nucleotides) were also proportional to the chlorophyll content of the sample, although with greater scatter in the seawater samples. In contrast to results with NiR a graph of the summed activity/weight ATP vs chlorophyll a/ATP had a slope not significantly different from zero ($\rho>0.2$). To the extent that the Chl. a/ATP ratio represents the proportion of phytoplankton in all living organisms in the sample, this result implies that the activities were not restricted to phytoplankton.

Table 14. Ratios of enzymatic activity to chlorophyll a and to adenosine triphosphate (ATP). Units are μmoles/μg·hr. (average ± standard deviation).

	Rate/Chlorophyll a	Rate/ATP
Cultures		
Nitrite reductase*	0.027 ± .019	0.080 ± 0.023
Pyridine nucleotide oxidation	0.12 ± 0.04	0.29 ± 0.07
Seawater Samples		
Nitrite reductase	0.020 ± 0.017	0.065 ± 0.058
Pyridine nucleotide oxidation	0.16 ± 0.10	0.22 ± 0.06

*Nitrate cultures only

SECTION X

DISCUSSION

The timing of the first cruise in July, 1970, was propitious for showing the eutrophication around southern California sewage outfalls where phytoplankton crops and primary production exceed typical levels off this coast and approach values characteristic of upwelling periods.

Concentrations of nutrients were not elevated in samples taken at the outfall stations (except occasional ammonia values) even when bottle casts were taken directly over outfalls. This would seem to imply that the hydraulic diffusers were effective in diluting the wastes prior to emission and that the phytoplankton growth was sufficiently vigorous to keep up with the nutrient input rate.

If a value for inorganic nitrogen (nitrate and ammonium) of 2 mM is taken for sewage (Weibul, 1969), with a 200-fold dilution with sea water during emission, the final nitrogen concentration would be about 10 µM. This concentration of nitrogen would produce a phytoplankton crop equivalent to about 10 µg/l of chlorophyll *a*, using conversion factors found in this laboratory. Maximum observed chlorophyll *a* concentrations were 10 to 17 µg/l at the two outfalls, in reasonable agreement with the hypothesis that the phytoplankton crop was assimilating the inorganic nitrogen of the effluent as fast as it was released. This hypothesis was supported by the 1-3 days turnover times of nitrate, ammonium, and urea in the upper 10 meters observed both in July, 1970 and June, 1971.

Nitrate and phosphate concentrations found in this study were much lower than in eutrophic estuaries of the eastern coast of the United States (Ketchum, 1969; Carpenter, Pritchard and Whaley, 1969; Flemer *et al*. 1970; Ryther and Dunstan, 1971) and fall within the range of values observed elsewhere along the southern California coast (Strickland, ed., 1970). Chlorophyll *a* and phosphate concentrations resemble those from Long Island Sound rather than polluted estuaries (see Fig. 4, in Ketchum, 1969; Flemer *et al*. 1971; Ryther and Dunstan, 1971). The high ammonium levels (up to 30 micromolar) observed below 15 meters in June, 1971, at the White Point outfall constitute an exception to this generalization.

Earlier work has shown that while growth and uptake rates of nitrogen by coastal marine phytoplankton are dependent upon the ambient nutrient concentration, rates are essentially saturated between one and five micromolar concentrations of nitrate, ammonium (MacIsaac and Dugdale, 1968; Eppley, Rogers, and McCarthy, 1969; Eppley and Thomas, 1969) and urea (McCarthy, 1971). Since concentrations were less than one micromolar in the upper 10 to 15 meters the phytoplankton seem to be in a favorable position to adjust rapidly to changes in ambient nutrient levels. That is, their growth and uptake rate is on a steep portion of the rate vs. concentration curve, and any increase in nutrient can be accomodated by increasing uptake rate of each algal cell. If nutrient concentrations were so high as to be rate-saturating then the phytoplankton could respond to increased nutrient concentration only by an increase in crop size, certainly a slower and less sensitive mechanism than that postulated, and one which requires the cooperation of grazing animals as well. (I am indebted to Drs. John Caperon and Alan Cattell, Univ. Hawaii, for this insight). This is perhaps another way of saying that the input of nitrogen at the outfalls, monumental as it is, is not yet great enough to exceed the capacity of the local phytoplankton to assimilate it. However, the elevated ammonium concentrations noted in 1971 at White Point below 15 meters depth seem to suggest that the phytoplankton may not be able to keep up indefinitely. The consequence of such a failure would be multifold: (a) increases in phytoplankton crops about the outfalls; (b) increased concentrations of ammonium at the surface near the outfalls; and (c) an increase in the spatial extent of the outfalls influence; i.e. increased eutrophication of the southern California coastal waters. Perhaps the most dangerous consequence for local food webs would be a shift in the species composition of the phytoplankton flora to minute coccoid green algae as observed in bays receiving duck farms wastes (Ryther, 1954; Hulburt, 1970). Samples taken for determination of phytoplankton species will not be counted by the time this report is due but will be available ultimately.

Some of the results of laboratory experiments suggest other observations which might be made at the outfalls:

(1) The diel periodicity in assimilation rate of nitrate and ammonium suggests that uptake rates might slow greatly at night in natural phytoplankton populations. If so, this would lead to increased ambient ammonia levels at night in the upper few meters where the impact of phytoplankton nutrient assimilation is greatest.

(2) If one assumes that the phytoplankton crop about the outfalls behaves as if in a nitrogen-limited chemostat culture, albeit of unknown volume, some further inferences can be made. From the variation in the ratio of cell carbon to nitrogen (Fig. 10) in the natural phytoplankton one can guess the specific growth rate of the crop as a fraction of its maximum growth rate (i.e. μ/μ_m). In samples where large crops of phytoplankton allow such estimates, relatively free of error due to particulate detritus in the samples, ratios were in the range 7 to 9. This suggests that μ/μ_m is about 0.85. If μ_m were about 0.7 doublings/day, as measured in nutrient-rich waters off Peru (Strickland et al. 1969; Beers et al. 1971) then μ would be about 0.6 doublings/day. Other estimates of μ from rates of nitrogen and carbon assimilation calculated as daily increments of the existing crops (Eppley et al. 1971; McCarthy, 1971) suggest lower values about 0.3 to 0.4 doublings/day, i.e. μ/μ_m 0.4 - 0.6. This discrepancy may be due to differences in irradiance between the cultures and natural situations as irradiance has a marked influence on C/N ratios of cultures.

(3) Perhaps the greater utility of N-limited chemostats as models or natural phytoplankton growth about the outfalls derives from the possibility of mathematical descriptions permitted by chemostat theory.

However, with the present lack of measurements over the seasons it cannot be assumed that our estimates of the phytoplankton crop and growth rate represent a steady-state. Advective processes, variation in the quantity and quality of wastes discharged, and periodic upwelling would complicate mathematical simulation modeling but efforts in this direction would be interesting and useful (Dugdale and Whitledge, 1970).

While our data are too limited for good quality simulation modeling because of infrequent sampling and poor areal coverage certain of the measurements may prove useful, such as the kinetic parameters for nitrate and ammonium uptake. The short turnover times for these nutrients and for urea in the upper few meters imply a close coupling between nitrogen input and assimilation by the phytoplankton. These turnover times are generally less than the doubling times calculated for the phytoplankton. The ambient nitrate and ammonium concentrations measured in the upper 10-15 meters are so low that uptake rate is on the steep portion of uptake rate vs. concentration curves for the crops. These two observations strengthen the conclusion that the phytoplankton crops are able to keep up with the rate of nitrogen input. They furthermore show the power of the ^{15}N methods of measuring nitrogen assimilation in southern California coastal waters in which nitrogen is usually the growth rate-limiting nutrient.

SECTION XI

ACKNOWLEDGMENTS

This work was planned and carried out at the Scripps Institution of Oceanography by members of the Food Chain Research Group, Institute of Marine Resources, University of California at San Diego. Mr. E. H. Renger, Mrs. Gail Hirota, Mrs. Jane Rogers, Mrs. Melita Dee, Dr. James J. McCarthy (then a graduate student); Dr. R. W. Eppley and Dr. O. Holm-Hansen provided experimental or analytical contributions. Dr. Eppley prepared this report.

Shiptime was funded by the National Science Foundation in 1970 via the R/V Alpha Helix Eastern Pacific Program and in 1971 via a grant to the Scripps Institution of Oceanography. The U.S. Atomic Energy Commission, Contract AT(11-1)GEN 10, P.A. 20 shared in the support of the laboratory aspects of this program.

Drs. W. H. Thomas, A. F. Carlucci, P. M. Williams and J. R. Beers provided advice and counsel. The late Dr. J. D. H. Strickland, head of the Food Chain Research Group, helped the work in innumerable ways. We thank Dr. T. Enns for the use of his mass spectrometer for the ^{15}N work.

SECTION XII

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SECTION XIII

PUBLICATIONS

The following research papers have resulted wholly or in part from this research grant. Some of the papers are in press, have been submitted for publication, or are in preparation. Only those actually submitted are listed.

1. Eppley, R.W., and Rogers, J.N., "Inorganic nitrogen assimilation of Ditylum brightwellii, a marine plankton diatom," J. Phycology, 6, pp 344-351 (1970).

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1 Accession Number	2 Subject Field & Group	SELECTED WATER RESOURCES ABSTRACT INPUT TRANSACTION FORM

5 Organization

Institute of Marine Resources, Scripps Institution of Oceanography, University of California, San Diego

6 Title

Eutrophication in Coastal Waters: Nitrogen as a Controlling Factor,

10 Author(s)	11 Date	12 Pages	15 Contract Number
Eppley, Richard W.	Dec., 1971	67	16010 EHC
	16 Project Number: 16010 EHC		21 Note

22 Citation

23 Descriptors (Starred First)

25 Identifiers (Starred First)

*Southern California Coastal Sewage Outfalls, Nitrogen Assimilation in marine phytoplankton.

27 Abstract

The role of southern California coastal sewage outfalls in the eutrophication of local seawater was investigated. The outfall effluents have a measureable influence on standing stocks of phytoplankton, and on primary production. Two cruises were undertaken, in July, 1970, and June, 1971. Kinetic parameters for the assimilation of ammonium, nitrate and urea were determined at the outfall sites using ^{15}N-labelled substrates. These parameters will be useful for simulation models of phytoplankton growth as influenced by local sewage effluents.

The utilization of various forms of nitrogen by phytoplankton, mechanisms and rates of nitrogen assimilation and enzymes of nitrogen assimilation were investigated in laboratory cultures. Ammonium and nitrate assimilation were found to vary from day to night as does the capacity for photosynthesis when cultures were grown on light-dark cycles simulating natural illumination. (Eppley - UCSD).

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WR:102 (REV. OCT. 1968) WRSIC

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