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John Michael DeManche (Name) for the degree of Master of Science (Degree)

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Title: UREA AND AMMONIA AS REGENERATED NITROGEN NUTRIENTS FOR PHYTOPLANKTON

Abstract approved: Redacted for privacy

Herbert C. Curl, Jr.

A detailed survey of phytoplankton nitrogen nutrients was conducted at Auke Bay, Alaska during a two week period in June, 1972. Spatial and temporal patterns of nitrate, ammonia and urea abundance were observed. Surface concentrations of these nutrients were low and fairly uniform, averaging 0.40 μg atom urea-N liter^{-1}, 0.29 μg atom ammonia-N liter^{-1} and 0.27 μg atom nitrate-N liter^{-1}. The predominant phytoplankton species during this period was Skeletonema costatum, contributing 50% to 80% of the phytoplankton biomass. Laboratory experiments with this strain of S. costatum indicated that uptake rates for urea and nitrate were similar at the low nutrient concentrations observed in Auke Bay. Since average nutrient concentrations remained constant during the period of observation, it was concluded that the upper photic zone was in quasi-steady state, with input of nitrogen nutrients equal to uptake by phytoplankton.

Regenerated urea supplied as much nitrogen for phytoplankton growth as advected nitrate. Based on published values for uptake of urea and ammonia by S. costatum, as well as published values for ammonia and urea excretion by zooplankton, the conclusion was made that ammonia was at least as significant as urea as a nitrogen source. It was estimated that regenerated nitrogen contributed a minimum of 67% of the total nitrogen required by phytoplankton during this period.

Urea and Ammonia as Regenerated Nitrogen Nutrients for Phytoplankton

by

John Michael DeManche

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Redacted for privacy

Dean of Graduate School

Date thesis is presented 27 August 1974

Typed by Marjorie Wolski for John Michael DeManche

To Linda,

whose love, encouragement, and support helped to make this thesis possible.

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I wish to thank Mauri Pelto and the crew of the R/V Murre II for providing logistic support in Auke Bay. I would like to acknowledge the following grants and contracts for their support: Sea Grant Project 061 through NOAA contract no. 2-35187.

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UREA AND AMMONIA AS REGENERATED NITROGEN NUTRIENTS FOR PHYTOPLANKTON

INTRODUCTION

In many marine enviornments the supply of nitrogen is a major factor in the regulation of phytoplankton growth (Dugdale and Goering, 1967; Ryther and Dunstan, 1971; Thomas, 1966, 1969, 1970a, 1970b; Thomas and Owen, 1971). Nitrate has usually been considered the most important form of nitrogen for phytoplankton nutrition. Recently, however, regenerated forms of nitrogen have received increased attention as important sources of phytoplankton nitrogen. Regenerated forms of nitrogen as defined by Dugdale and Goering (1967) are any form of nitrogen other than nitrate or molecular nitrogen. Vaccaro (1963) suggested that in coastal water off New England in summer, ammonia was a more important source of nitrogen than nitrate. Several other forms of regenerated nitrogen have been suggested as being important sources, including urea and amino acids (McCarthy, 1971; North and Stevens, 1968).

Surface concentrations of regenerated nitrogen forms are highly variable depending on location and season, but representative ranges have been reported for ammonia as 0.00 to 2.38 μg atom N liter^{-1}, for urea as 0.00 to 3.46 μg atom N liter^{-1}, and for amino acid as 0.0 to 4.0 μm liter^{-1} (McCarthy and Kamykowski, 1972; Menzel and

Spaeth, 1962; Remsen, 1971; McCarthy, 1970; Bruce, 1969; Coughenower and Curl, 1974). A wealth of information on the growth of laboratory and natural populations of phytoplankton with ammonia has been reported (Eppley and Rogers, 1970; Grant, Madgwick and DalPont, 1967; Corner and Davies, 1971; Eppley et al., 1971; Goering, Dugdale and Menzel, 1964; MacIsaac and Dugdale, 1969; Strickland et al., 1969). At concentrations greater than one μg atom N liter^{-1}, ammonia is a preferred source of nitrogen compared to nitrate or urea (Eppley et al., 1969; McCarthy and Eppley, 1972) and can be used by almost all marine phytoplankton as sole nitrogen source. McCarthy (1971) has studied phytoplankton growth with urea as sole nitrogen source for 35 species. Over half showed at least fair growth, and the capacity for growth varied between genera, within genera, and between clones of the same species. Laboratory kinetics experiments for Skeletonema costatum (Carpenter, Remsen and Watson, 1972) and for various species of diatoms (McCarthy, 1972a) have shown that urea can be taken up by some species of phytoplankton at ecologically significant rates for the concentrations of urea often found in coastal waters. Recently Carpenter, Remsen and Schroeder (1972) have compared the rate of urea decomposition for S. costatum for laboratory and in situ populations, finding the in situ rate to be well over an order of magnitude greater than that for laboratory culture. Guillard (1963), Bruce (1969), and Wheeler et al. (1974) have shown

that some, but not all forms of amino acids could support phytoplankton growth. However, Bruce (1969) and Schell (1974) found that many of the most abundant forms of amino acid in Alaskan waters allowed only poor growth of phytoplankton when compared with nitrate.

Eppley et al. (1971) have shown that natural populations of surface phytoplankton off southern California have similar specific growth rates when enriched separately with high levels of nitrate, ammonia, or urea. McCarthy (1972b) has demonstrated that in natural surface populations of phytoplankton off southern California, regenerated nitrogen (ammonia and urea) provided up to 86% of the daily requirement of nitrogen. Eppley et al. (1973) reported that regenerated nitrogen nutrients were the primary nitrogen source for phytoplankton in the upper 80 meters of the central North Pacific, with urea contributing up to 85% of the total.

Several sources of regenerated nitrogen have been reported. Zooplankton are generally considered to be primary sources for ammonia (Corner and Davies, 1971; Riley, 1963), although in some locations input by excretion from teleost fish can be as large or larger than that from zooplankton (Whitledge and Packard, 1971). Zooplankton may also be major contributions to urea regeneration. Corner and Newell (1967) reported that only 10% of nitrogen excretion by zooplankton was in the form of urea, whereas McCarthy (1971) and Eppley et al. (1973) found that zooplankton excretion in the Pacific

was roughly half ammonia, half urea. Many fish also excrete urea, especially elasmobrachs. Sharks excrete 80 to 90% of their nitrogen waste as urea (Scheer, 1963). Small teleosts also excrete a significant fraction of nitrogen as urea. Peruvian anchovy and jack mackerel excrete 16 to 25% of their nitrogen waste as urea (McCarthy, 1971). Zooplankton have also been shown to excrete amino acids (Webb and Johannes, 1967) but usually only as a small fraction, generally in a ratio of 4:1, ammonia:amino acids. Bruce (1969) has suggested that a major source of amino acid regeneration may be from spillage of phytoplankton cell contents into the water during active zooplankton grazing.

For the past several years a series of intensive oceanographic investigations have been conducted in Auke Bay, Alaska (Bruce, 1969; Curl, Iverson and O'Connors, 1971; Iverson, 1971; Kirk, 1972; Coughenower, 1972; Iverson et al., 1974; Schell, 1974). Auke Bay, a small 11 km^2 embayment located near Juneau, Alaska (Fig. 1), is part of the larger Inside Passage system of southeastern Alaska. Iverson (1971) found that wind mixing in Auke Bay could account for most phytoplankton blooms during periods with strong southeast winds, but could not explain blooms and high standing stocks which sometimes occured in the absence of strong winds. Bruce (1969) and Schell (1974) have investigated the significance of regenerated amino acid nitrogen in Auke Bay and found that amino acids alone were not

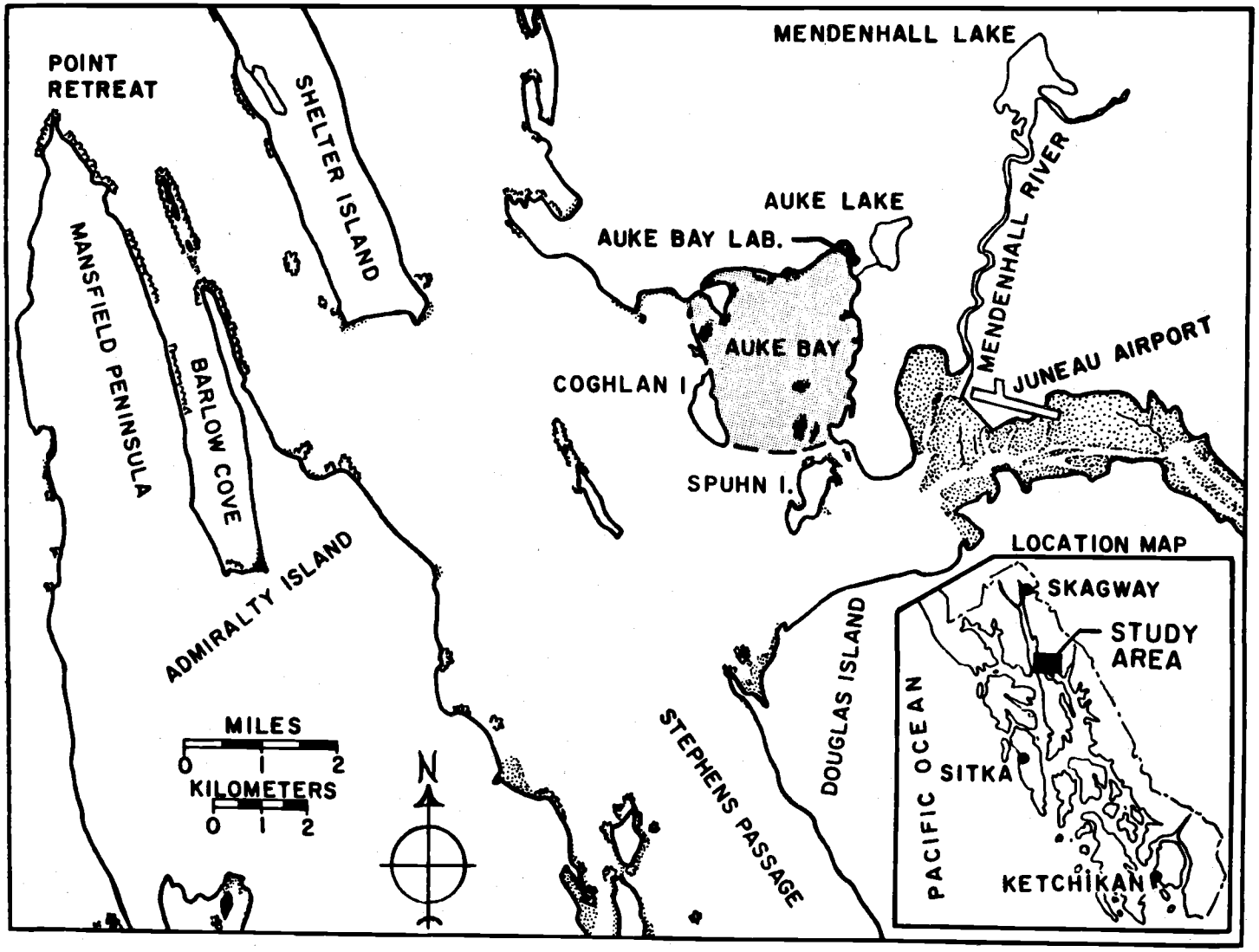

Figure 1. Location of Auke Bay in relation to major straits and passages in southeastern Alaska (from Bruce, 1969).

found in sufficient concentrations to permit phytoplankton growth at the rates observed. Surveys have been made of southeastern Alaskan waters for total available nitrogen, including nitrate, nitrite, ammonia, and organic nitrogen (Coughenower, 1972), but no data were available for the amounts of regenerated nitrogen available for phytoplankton during the summer growing season. Moderate standing stocks of phytoplankton had been observed to continue to grow at rates which could not be accounted for by wind mixing of new nitrate into the photic zone. This led me to suspect that a major fraction of the supply of nitrogen to phytoplankton was being overlooked. This study was undertaken to determine to what extent regenerated forms of nitrogen were contributing to phytoplankton population growth and maintenance. Since the role of urea in these waters had not been previously examined, major emphasis was placed on determining its significance as a regenerated nitrogen form in Auke Bay.

METHODS

Field date were collected during cruise C7306C on R/V Cayuse during June and July, 1973, in Auke Bay, Alaska. Temperature and salinity were measured with a Bissett-Berman Model STD sensor (Becker and Curl, 1971) below ten meters depth and with a hand held YSI salinometer-thermometer at ten meters and above. Most samples for nutrients, chlorophyll, and particulate matter were obtained by means of a pumping system to which the STD sensor was attached. A few samples were collected by means of Van Dorn bottles. Chlorophyll a was determined by the method of Richards as modified by Strickland and Parsons (1972). Pheo-pigments were also measured by the Strickland and Parson procedure. Particulate nitrogen and carbon were determined by filtering samples through Whatman GFA glass fiber filters. The filters were vacuum dried over silica gel and analyzed in a Carlo Erba CHNO Elemental Analyzer. Nutrients were measured with a Tecnicon Auto-Analyzer, usually within one to two hours of collection. Nitrate plus nitrite was determined by the method of Wood, Armstrong, and Richard (1967) as modified by Atlas et al. (1971). The method for ammonia was that of Head (1970). Urea was measured by the method of DeManche, Curl, and Coughenower (1973).

Primary productivity was measured using $H^{14}CO_3^-$ additions to light and dark bottles with incubation in situ. Solar radiation was

measured with a recording pyronometer located at the National Marine Fisheries dock at Auke Bay. Tide was recorded with a tide gauge installed at the National Marine Fisheries Lab. Wind velocities were obtained from the U. S. National Weather Service (Juneau Municipal Airport located three km southeast of Auke Bay).

For laboratory experiments cell numbers and particle size distributions were obtained with a Coulter particle counter, either a model B or model ZBI with a P64 channel analyzer. Samples from cultures for nutrient analysis were removed from the culture vessels by drawing gently through ten μm Nitex netting (Lundy, 1974).

RESULTS

I. Auke Bay

Stations occupied during the cruise are shown in Figure 2. Transects along the line from Station 24 to Station 31 were made on June 24, 27, 29, and July 1. Each survey required approximately six to eight hours to complete. Station 31 was also occupied for two 24 hour periods June 26-27 and 28-29. Station 28 was occupied for 24 hours on June 30-July 1.

Hydrography

Hydrographic conditions on June 24 were fairly uniform throughout the bay (Fig. 3) and remained so during the survey period. The bay was stratified into three layers (Fig. 4). The upper layer, five to seven meters deep, was well mixed. The upper layer was separated from a deep water layer by a pycnocline, two to three meters thick, which was highly stable according to Sverdrup's definition of stability (Sverdrup et al., 1942). The depth of the pycnocline at Station 31 was observed to oscillate with the tide.

Nitrogen Nutrients

The concentrations of nitrate + nitrite, ammonia, and urea were uniformly low throughout the upper layer (Figs. 5, 6, and 7). The

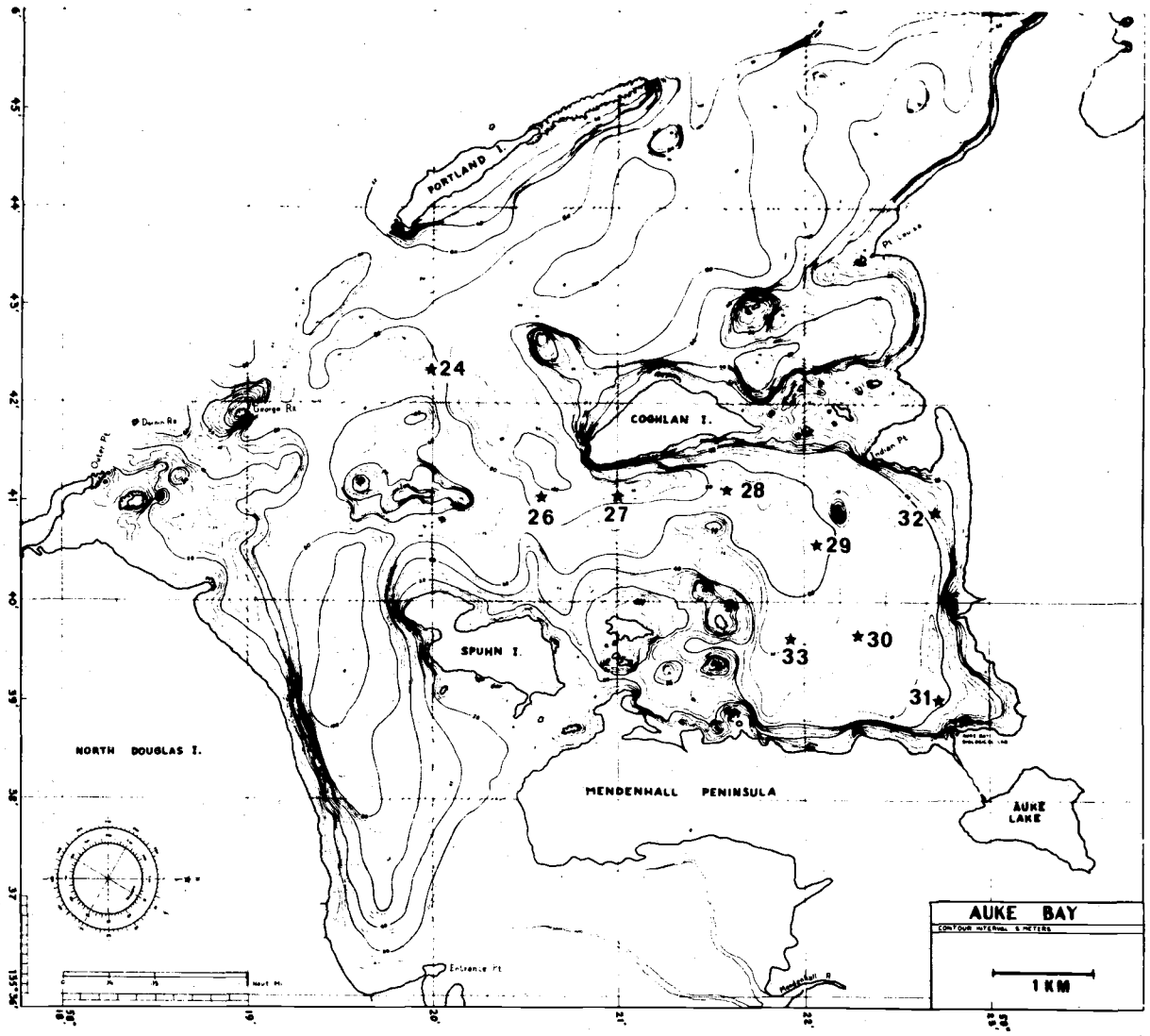

Figure 2. Station locations in Auke Bay

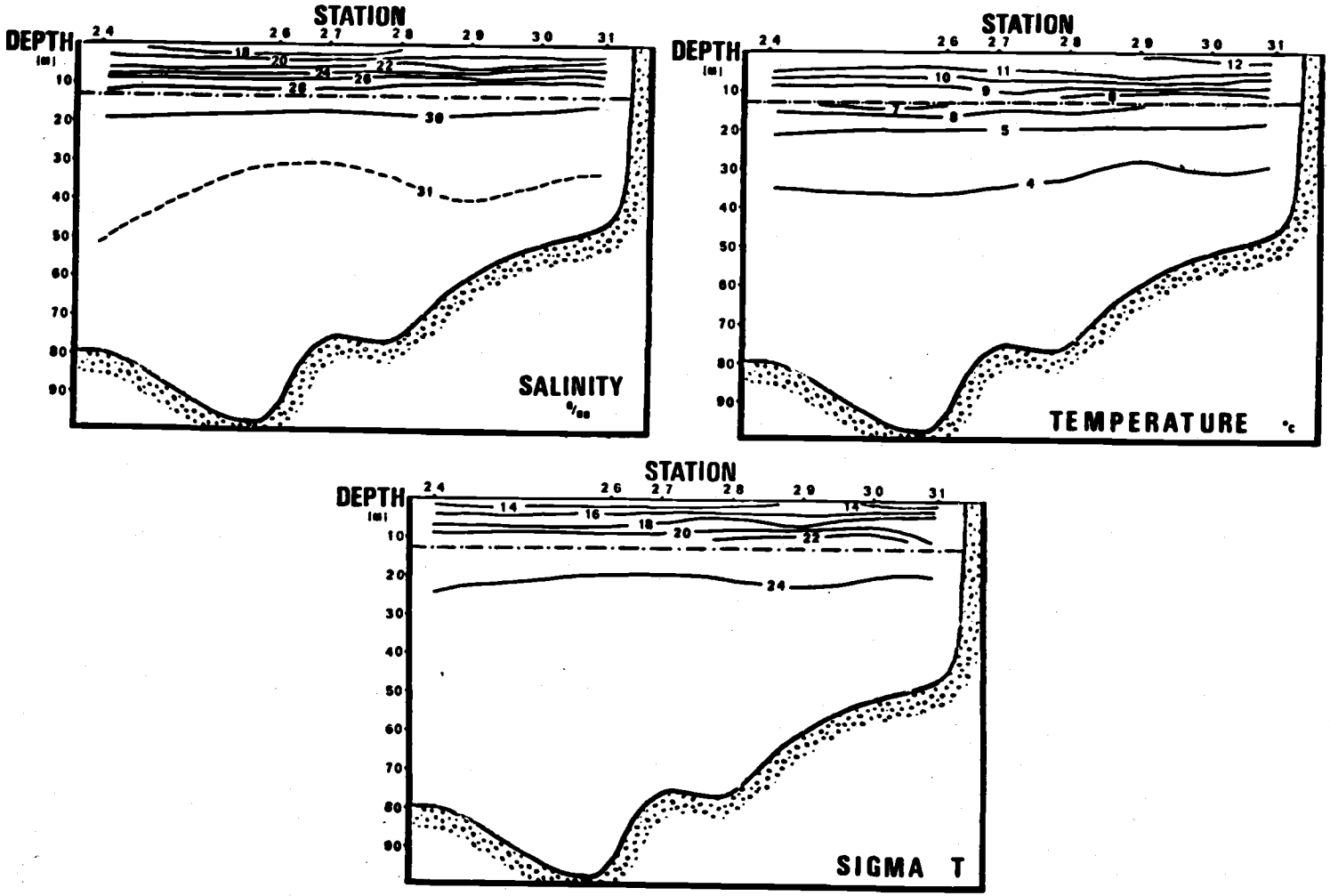

Figure 3. Hydrography of Auke Bay, 24 June.

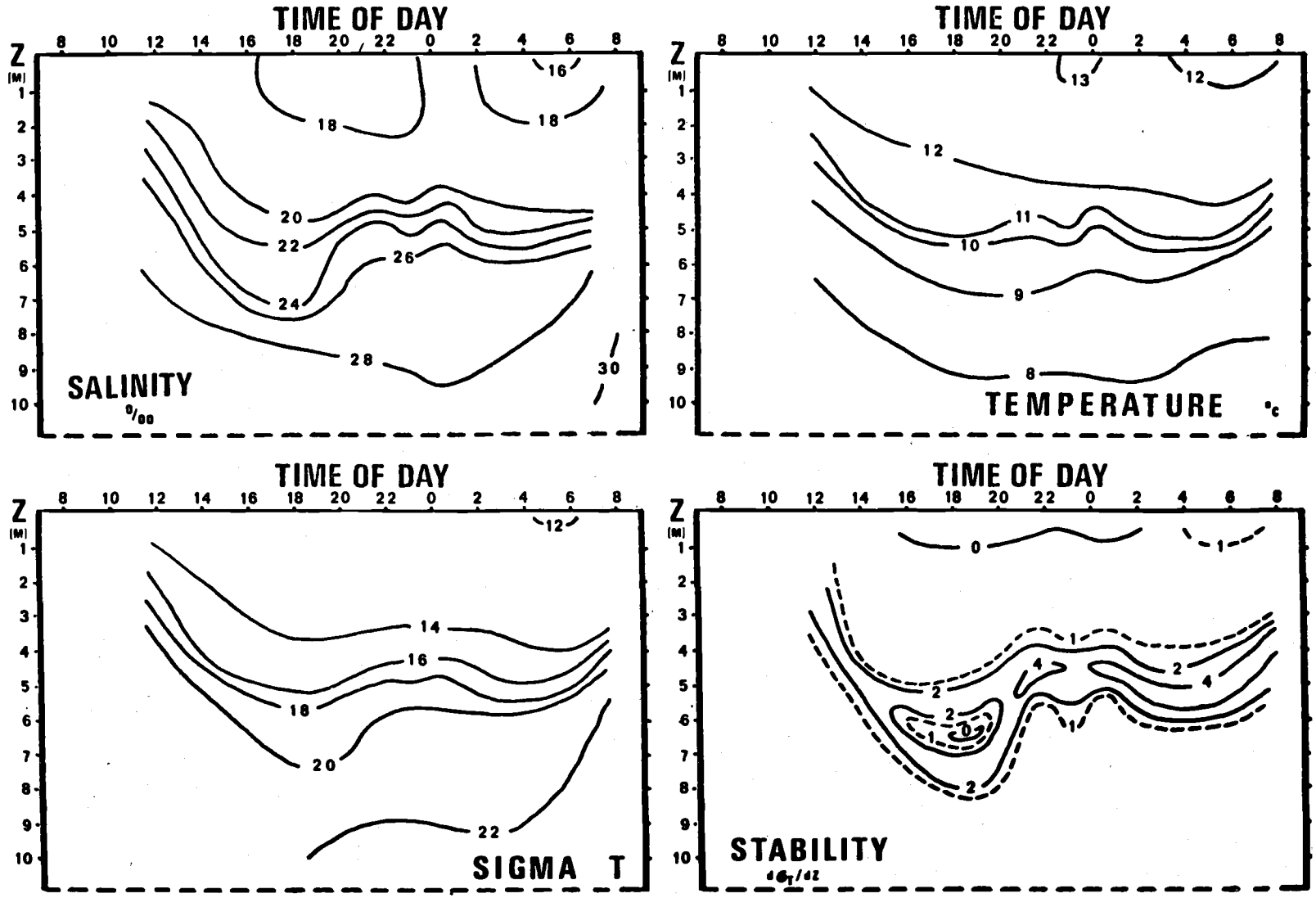

Figure 4. Upper layer hydrography at Station 31, 26-27 June.

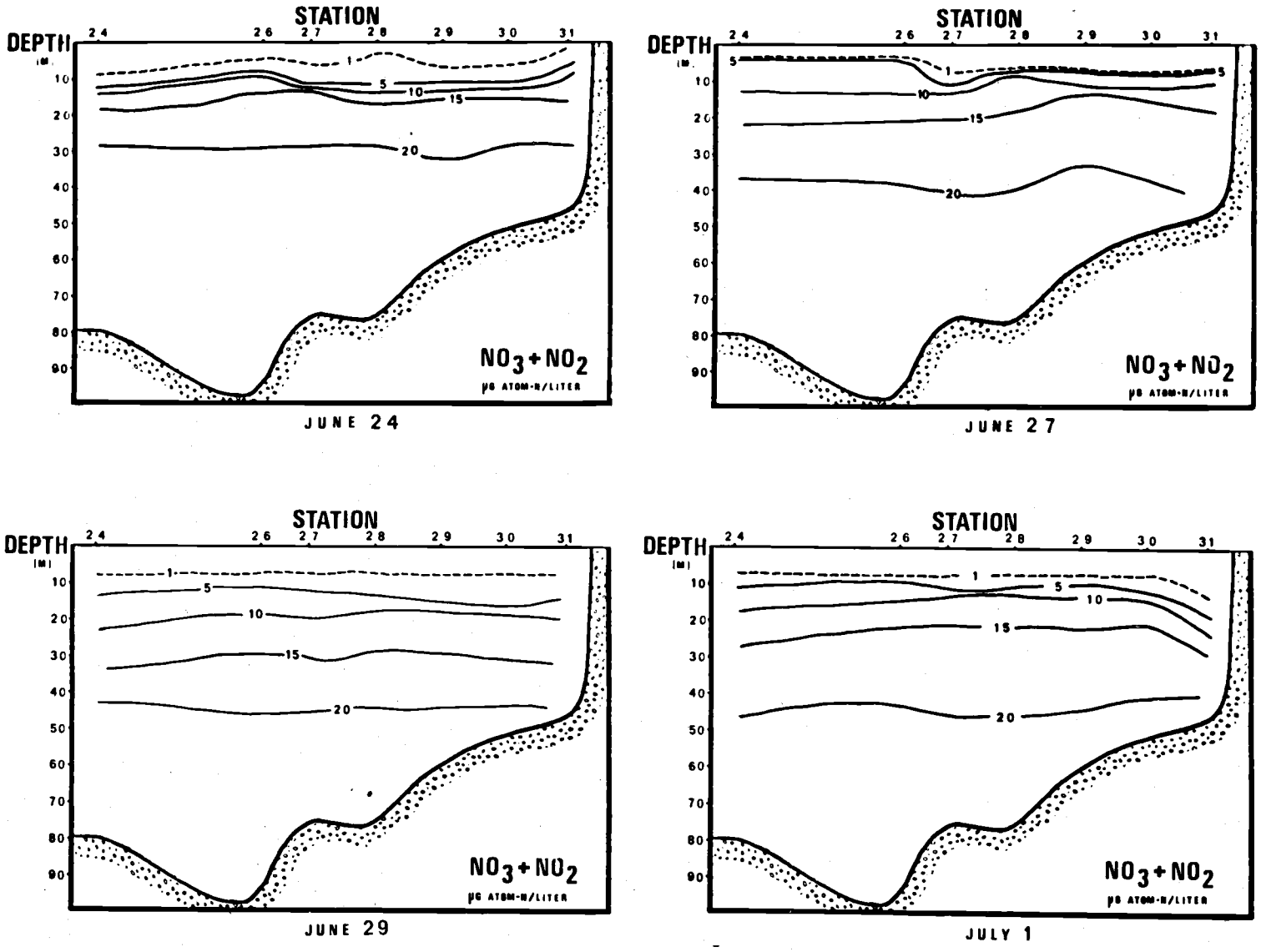

Figure 5. Contours of nitrate + nitrite.

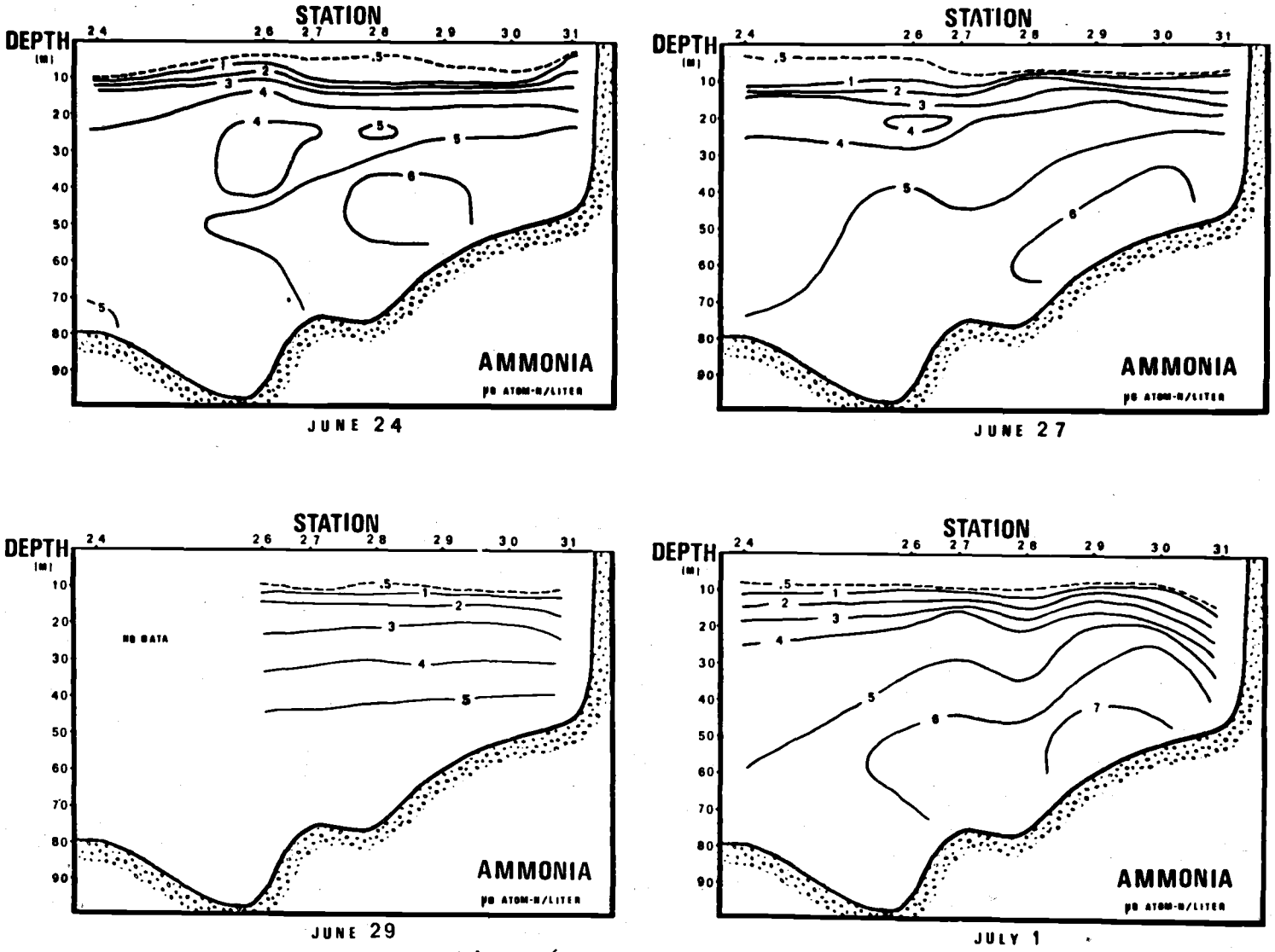

Figure 6. Contours of ammonia.

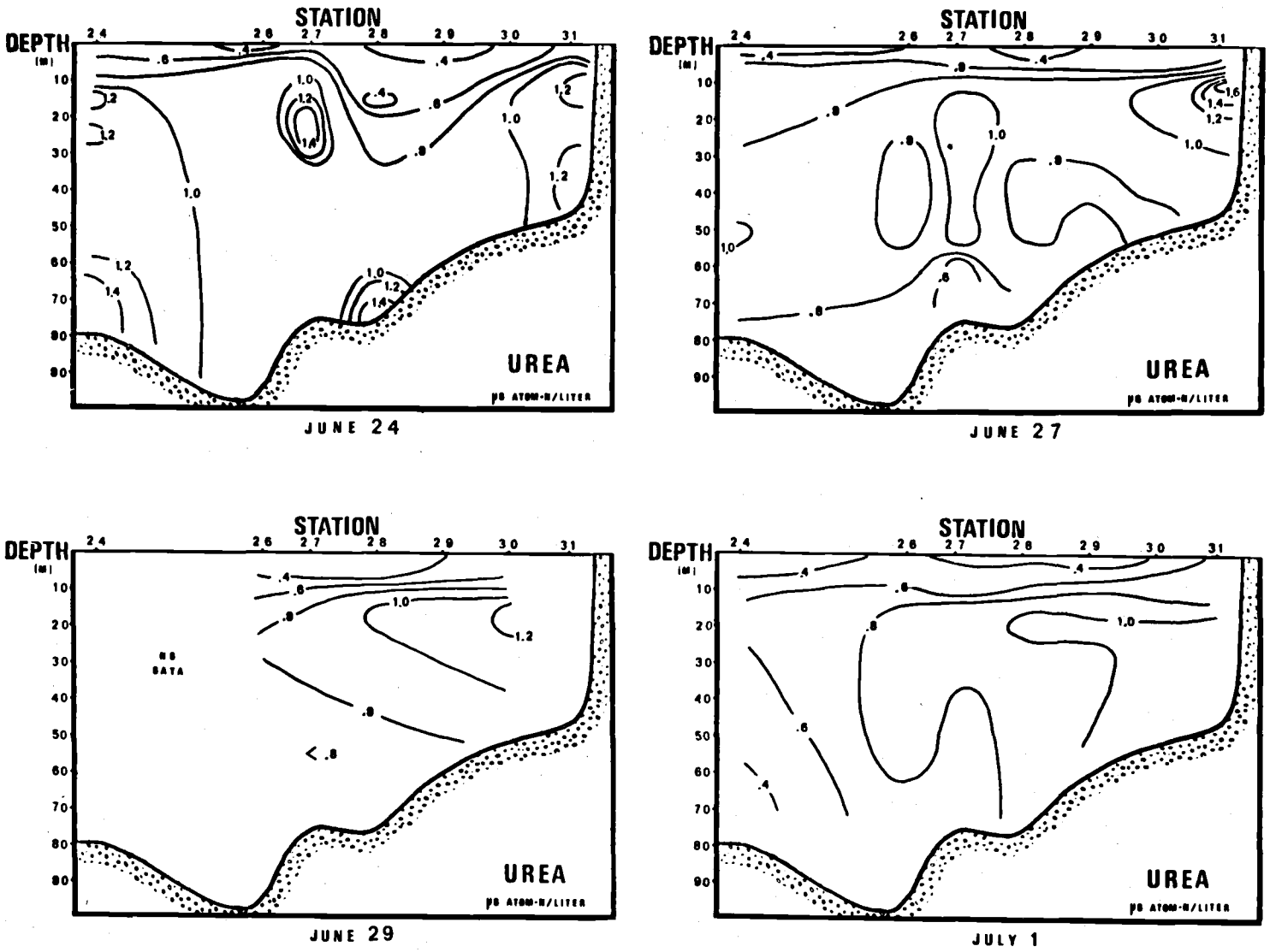

Figure 7. Contours of urea.

mean surface value for nitrate + nitrite was 0.27 μg atom N liter^{-1}, ammonia 0.29 μg atom N liter^{-1}, and urea 0.42 μg atom N liter^{-1}. At most stations urea was usually the predominant form of free, surface nitrogen. Surface values of nitrate + nitrite were often undetectable. The layer of low nitrogen nutrients coincided with the well-mixed surface layer, a sharp gradient in nitrate and ammonia concentration occuring at the pycnocline. At depth urea was the least significant nitrogen form, usually exhibiting a maximum between 1.0 and 1.6 μg atom N liter^{-1} at a depth of 20 meters. A linear regression of surface nitrogen nutrient concentrations against time was not significant at the 0.1 level. At both Stations 28 and 31, surface nitrogen concentrations showed a periodicity that was approximately in phase with tidal oscillations (Figs. 12, 13, and 14).

Phytoplankton Biomass

The phytoplankton biomass, as indicated by both chlorophyll *a* and particulate nitrogen, was high to moderate during the survery period (Figs. 8 and 10). Initially biomass was not horizontally uniform; a bloom preexisted at Stations 24 and 26. This bloom decreased rapidly, and by June 29, the biomass was fairly homogeneous across the bay. The nearshore biomass, as indicated by Station 31, remained relatively constant for each survey, approximately 5 μg atom N liter^{-1} in the upper layer. Ratios of particulate carbon to particulate

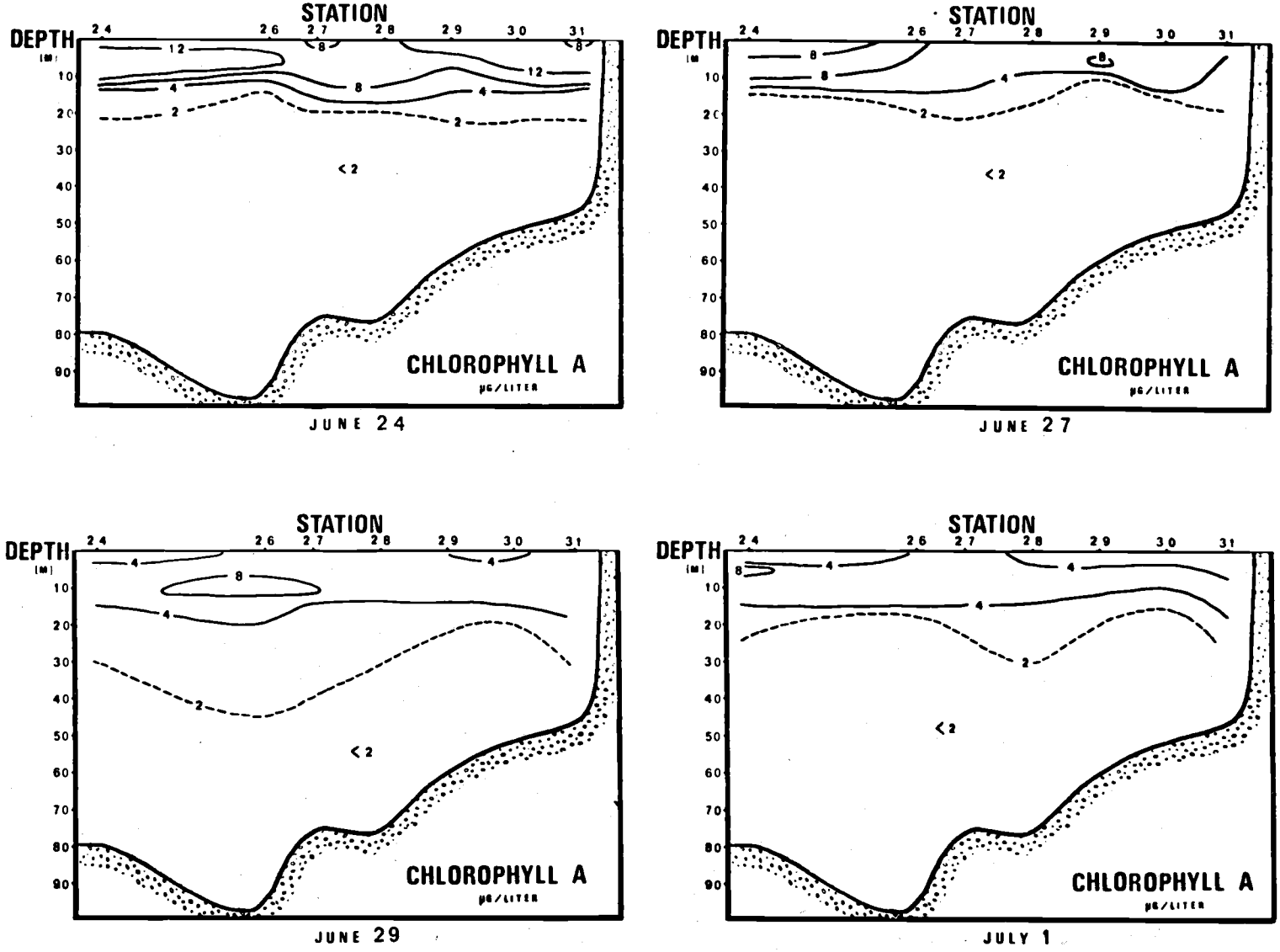

Figure 8. Contours of chlorophyll a.

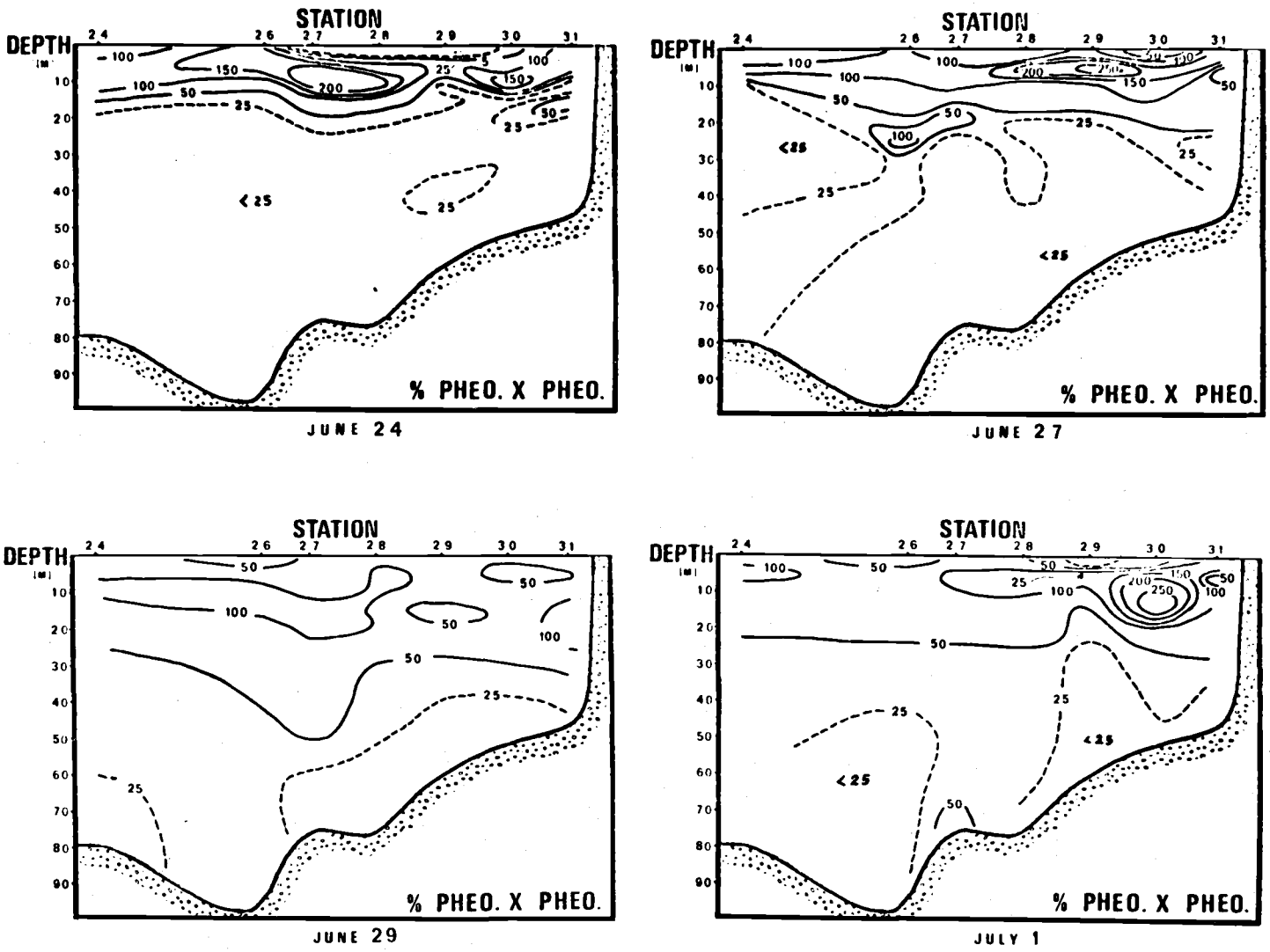

Figure 9. Contours of percentage of pheophytin as fraction of (total chlorophyll a + pheophytin) X pheophytin.

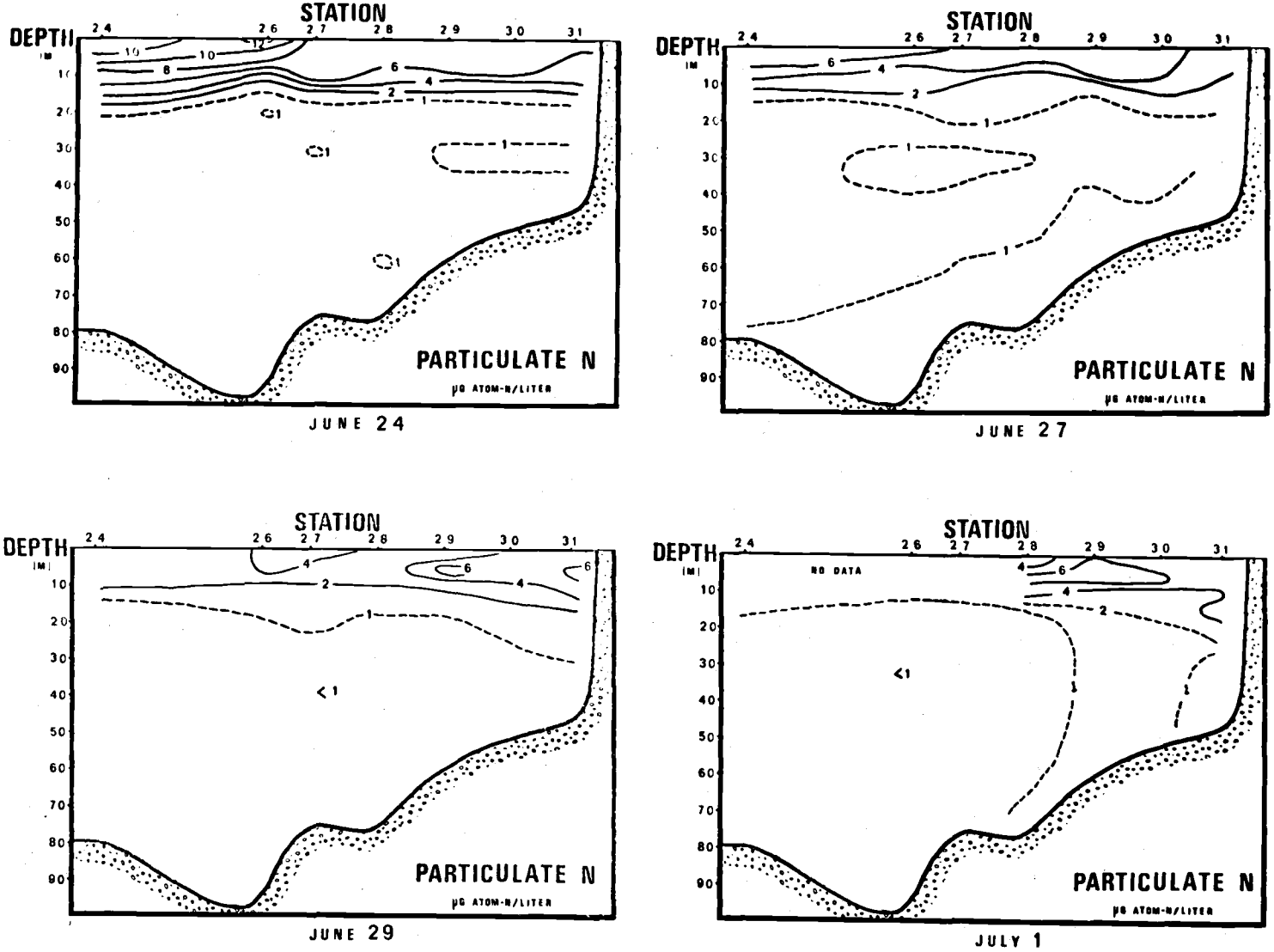

Figure 10. Contours of particulate nitrogen.

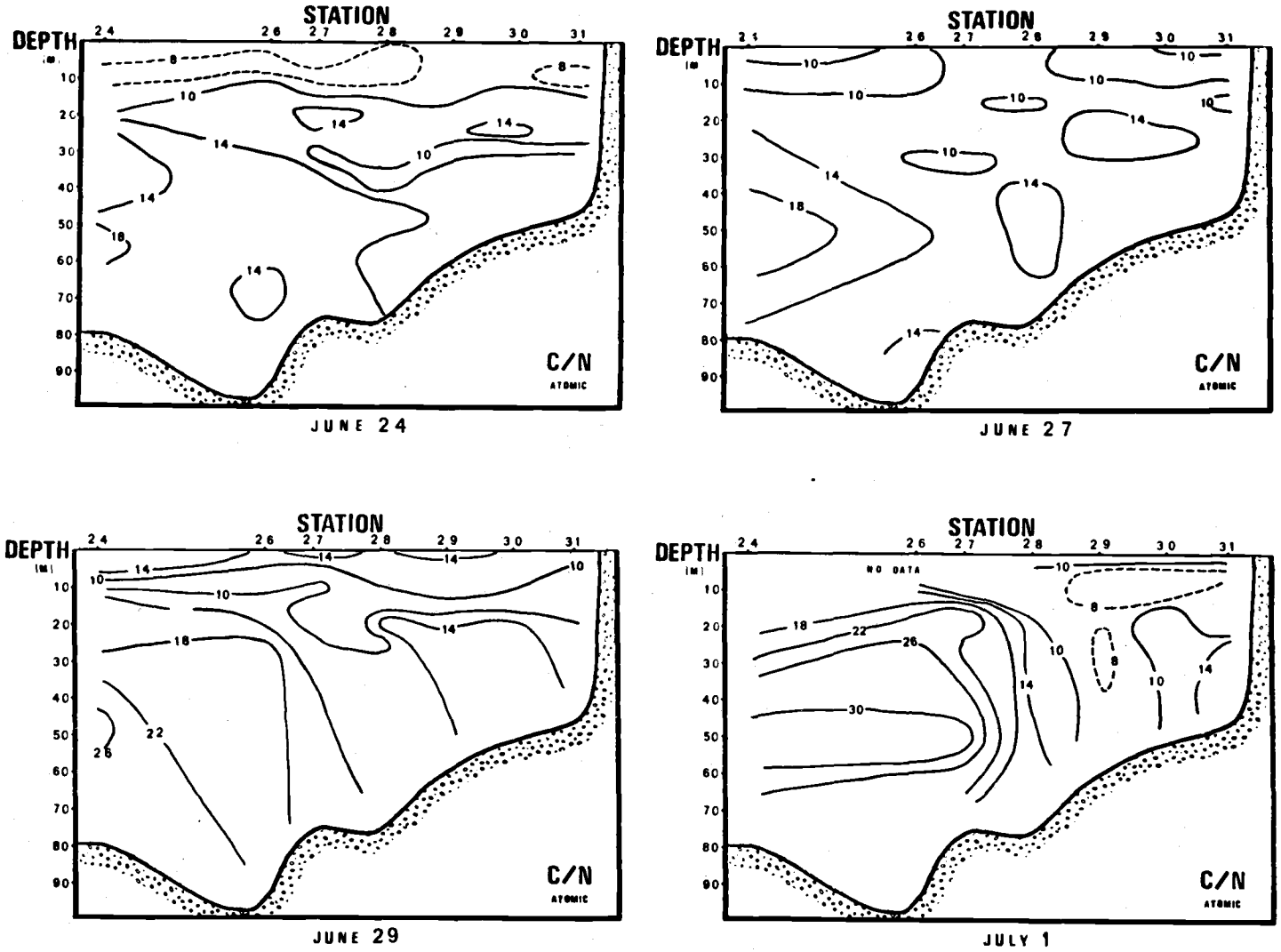

Figure 11. Contours of particulate carbon to particulate nitrogen ratio on an atom to atom basis.

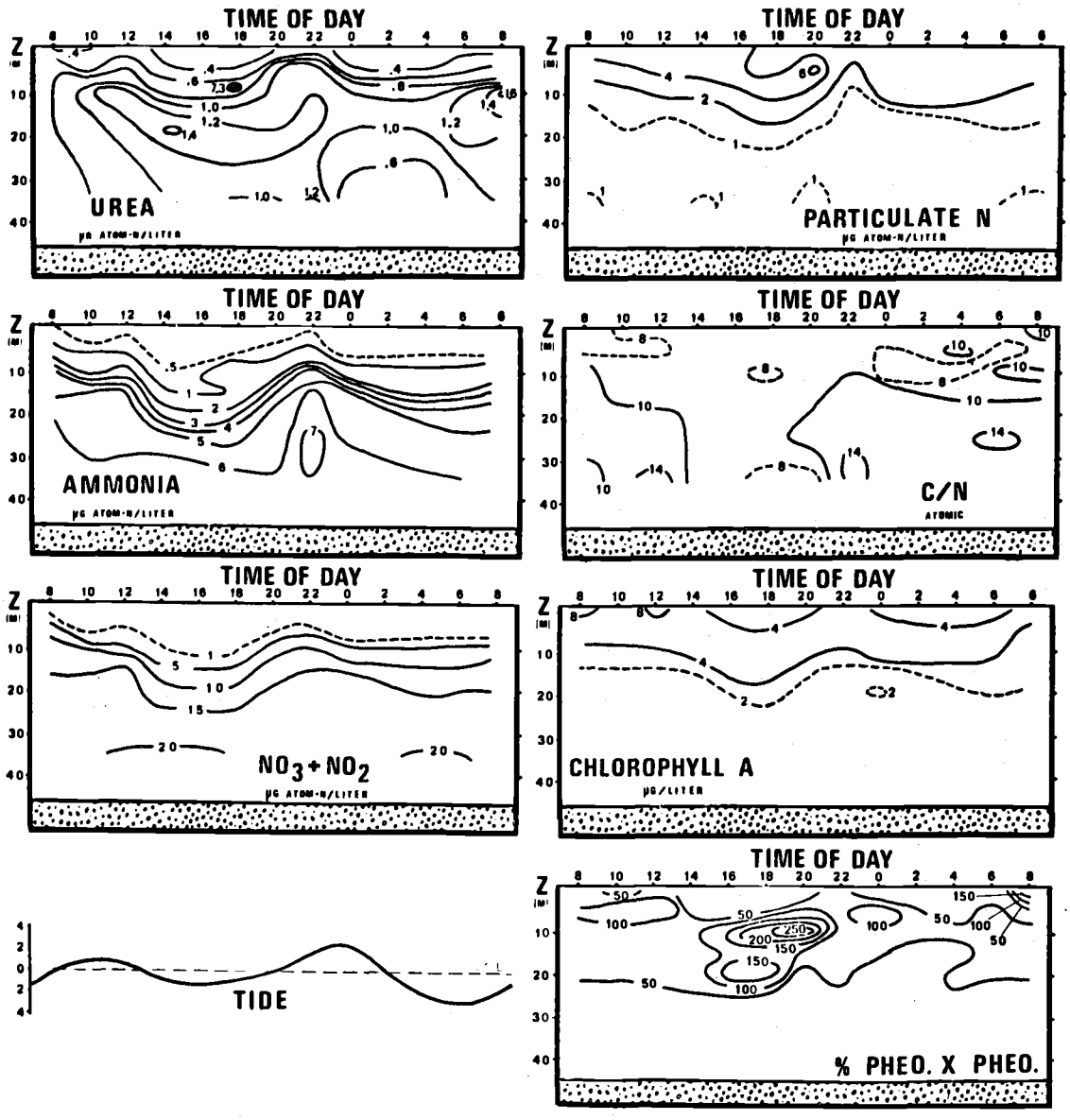

Figure 12. Twenty-four hour survey at Station 31, 26-27 June.

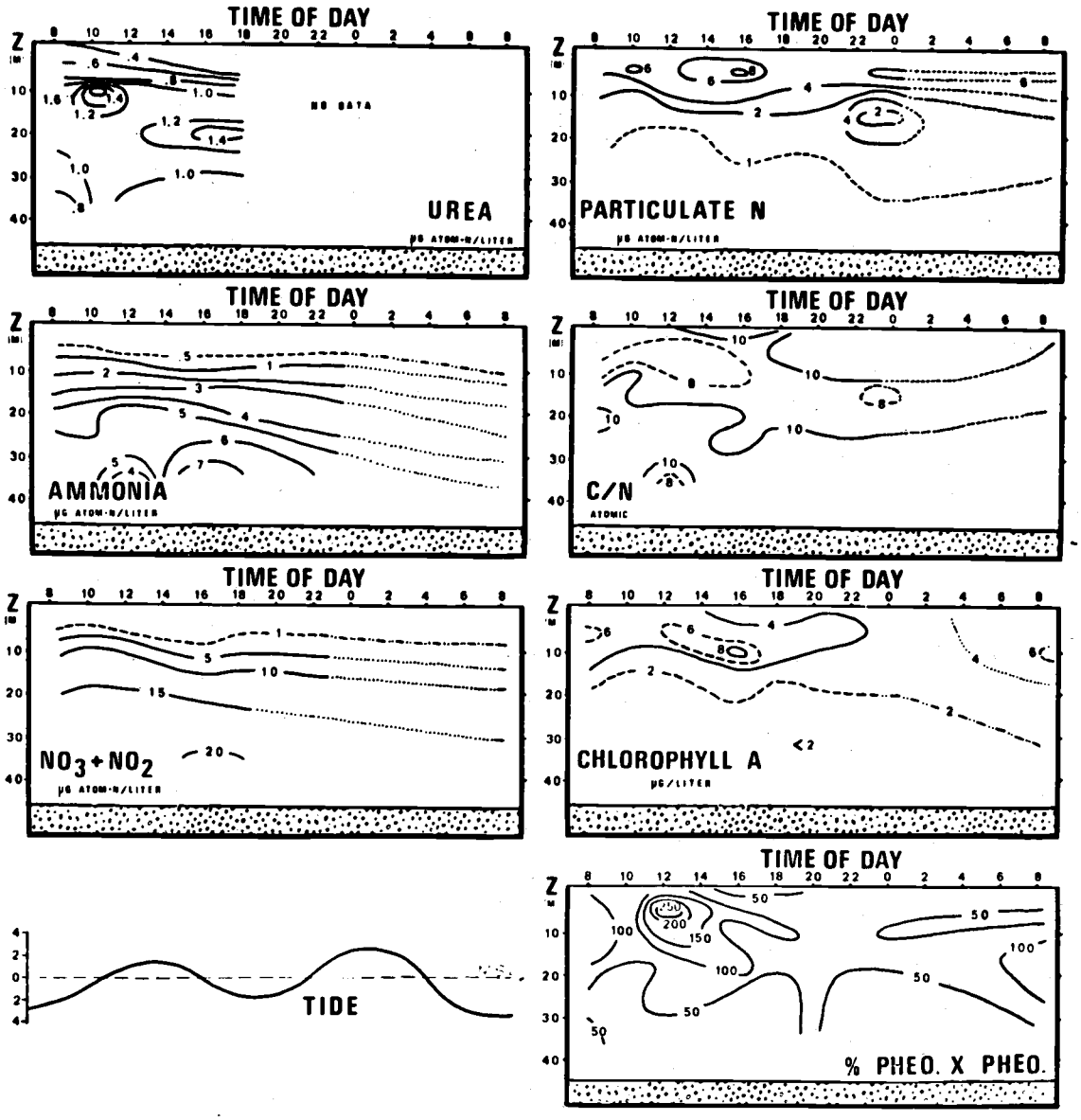

Figure 13. Twenty-four hour survey at Station 31, 28-29 June.

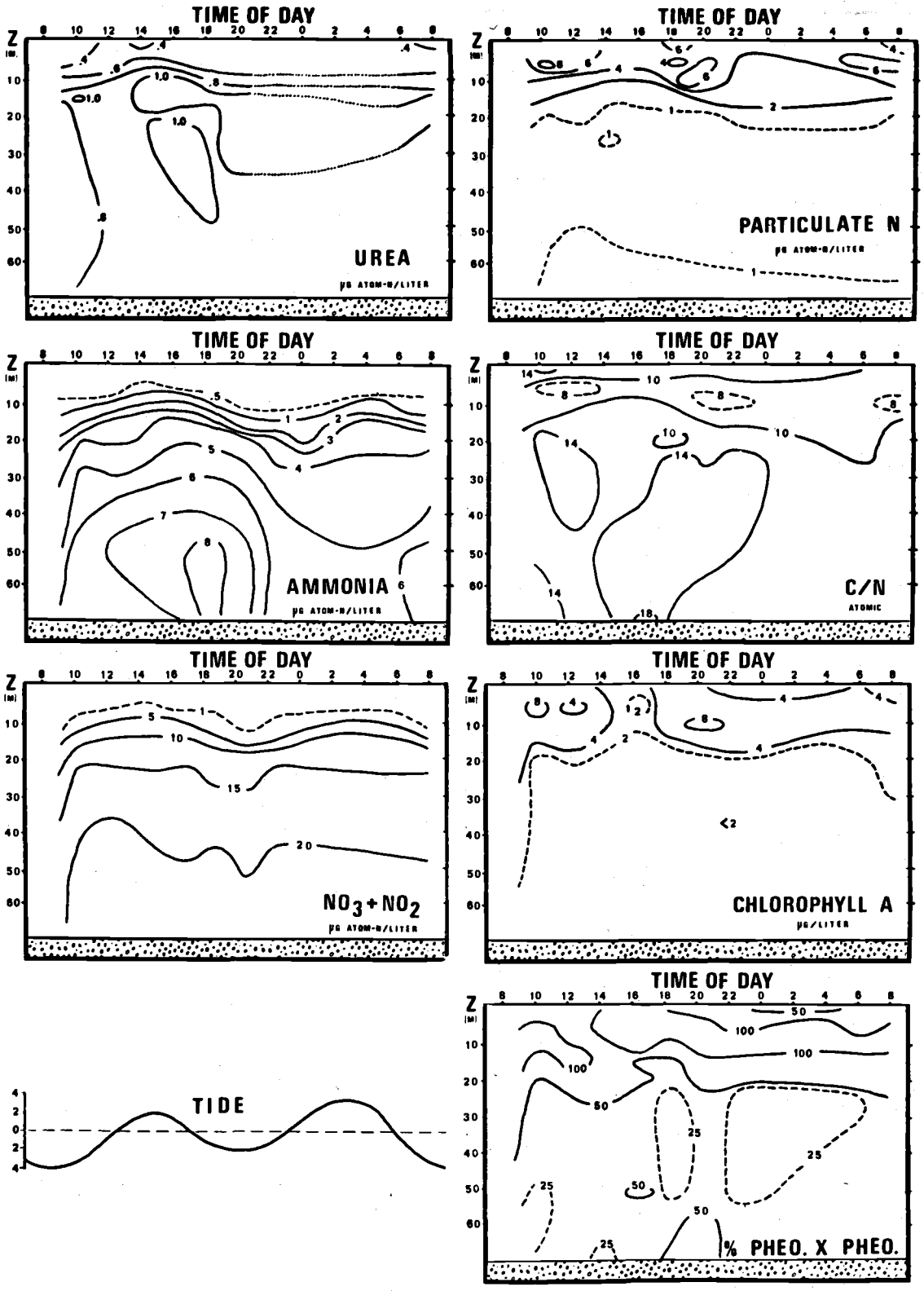

Figure 14. Twenty-four hour survey, Station 28
30 June - 1 July.

nitrogen (C/N) ranged between 7.2 and 16.5 atom/atom at the surface (Fig. 11).

Meteorology

Wind was light throughout the survey period (Figs. 15 and 16). Just prior to the initial survey, wind was blowing strongly from the southeast with gusts to 12 m/sec. During the surveys the wind was from the northeast and averaged only 2.5 m/sec. Low surface light intensities (Fig. 17) were the result of overcast skies. Total solar insolation was recorded for each 24 hour period, but intensities were measured only at the times indicated in Figure 17.

Primary Productivity

Two sets of primary productivity data were obtained by slightly different methods. In each case $H^{14}CO_3^-$ enriched light and dark bottles were incubated in situ in 100 ml bottles for two to three hours. One set was obtained by incubations at 1, 4, 8, and 12 meters depth at mid-day (Table 1). Particulate carbon and nitrogen were measured concurrently for each sample in this set. In the second set (Table 2) incubations were made between one and ten meters depth at one meter intervals. Incubations were spaced so as to cover an entire 24 hour period, but no particulate carbon or nitrogen analyses were made. The mean primary productivity for the upper six meters at Station 31

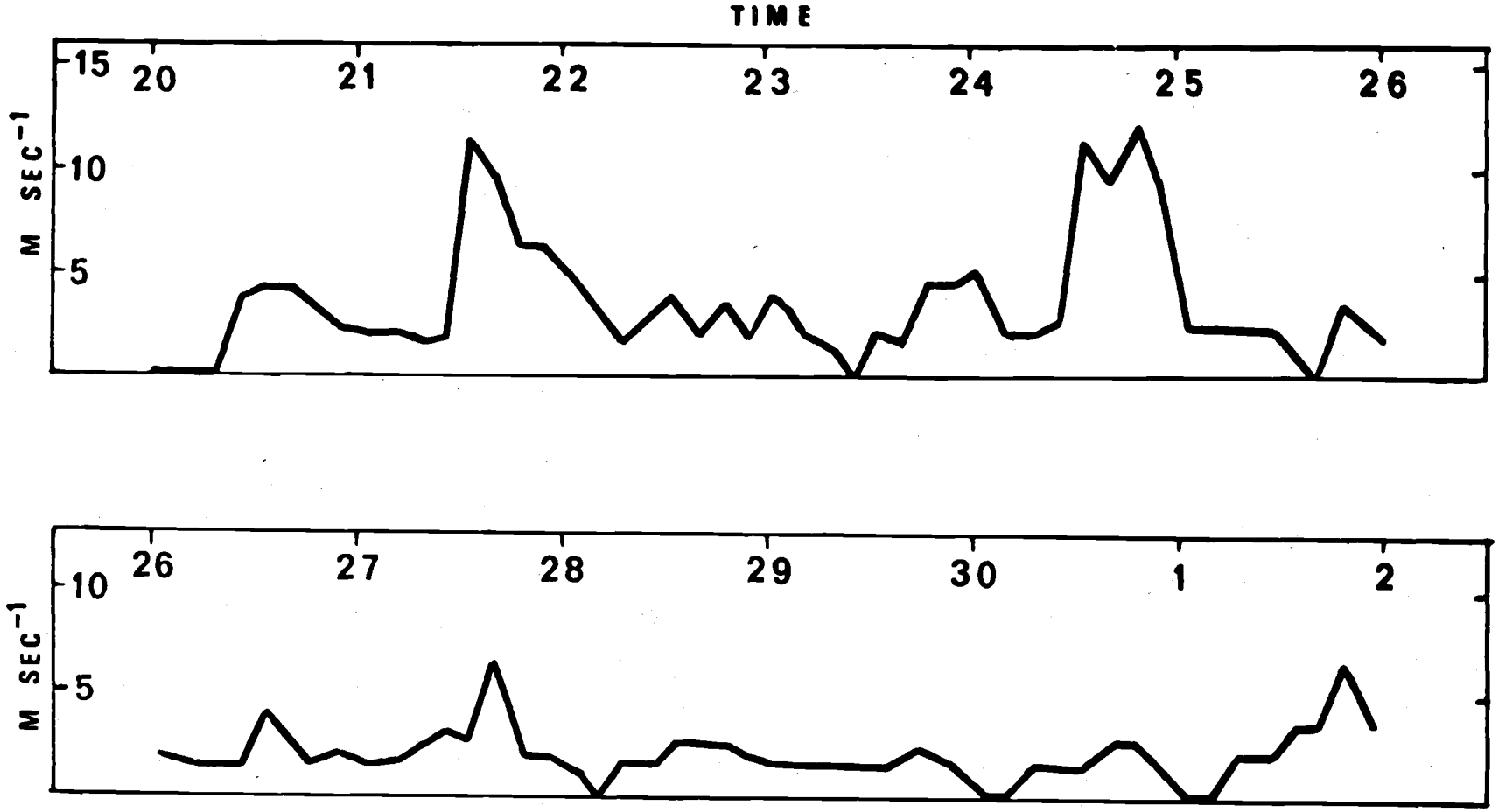

Figure 15. Wind speed, 20 June - 2 July.

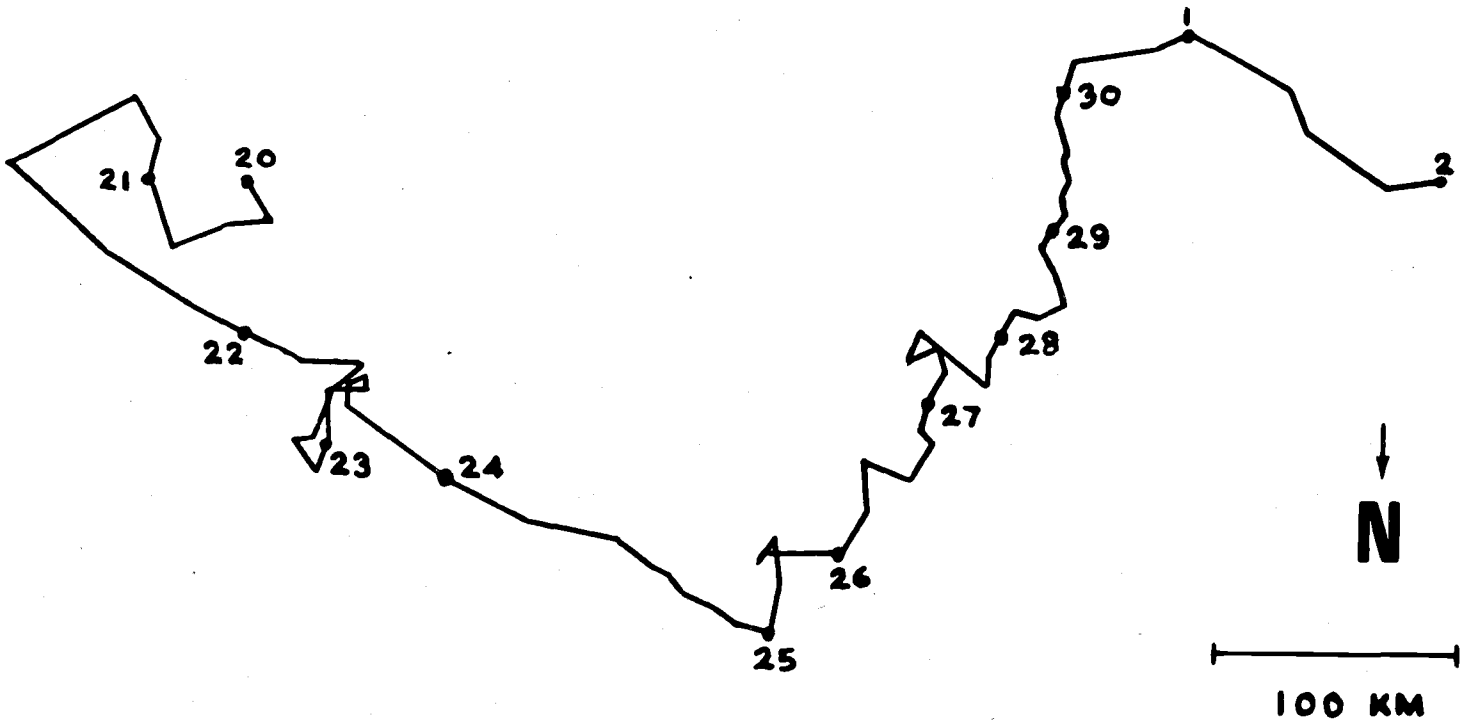

Figure 16. Progressive vector diagram for wind velocity, 20 June - 2 July.

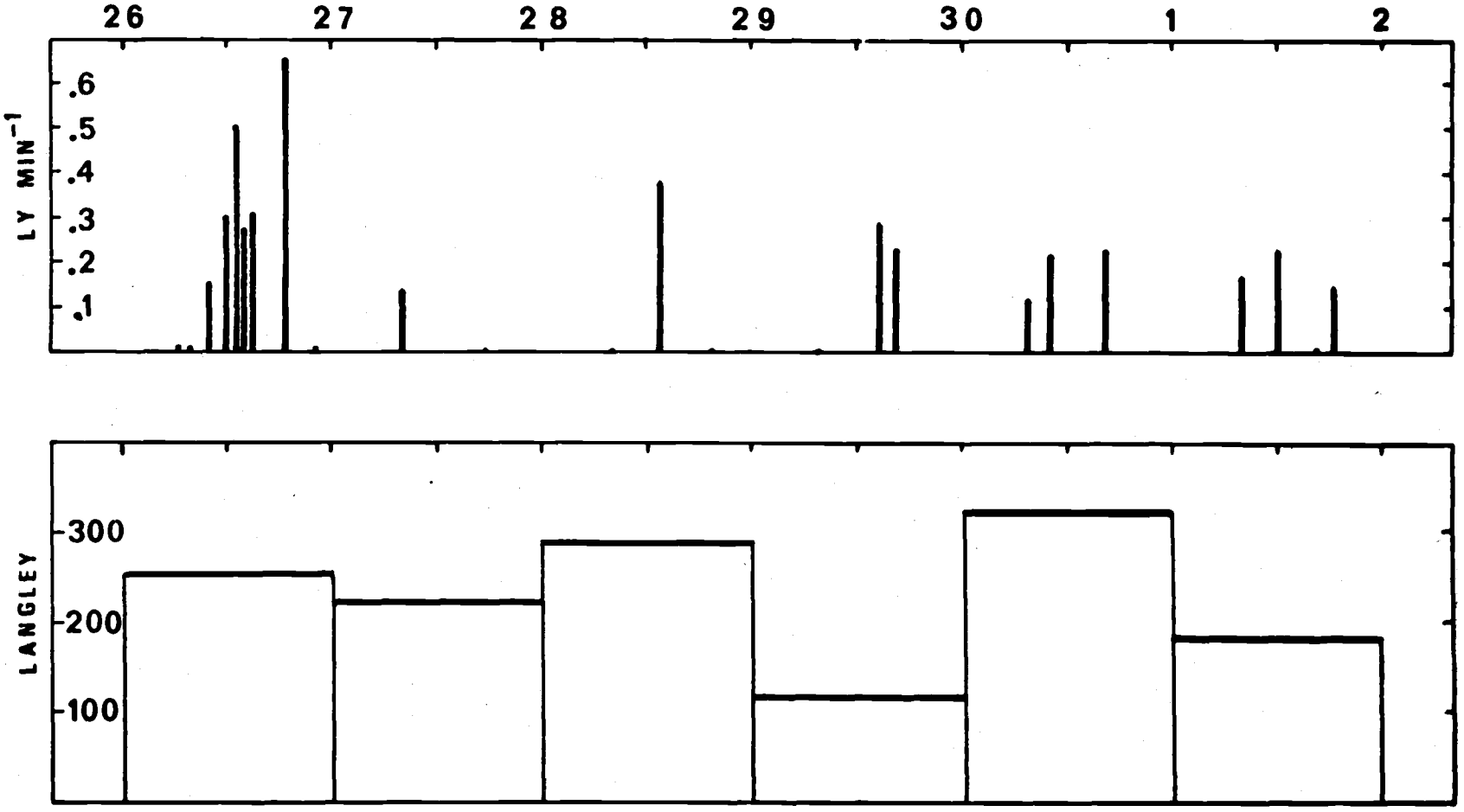

Figure 17. Solar insolation: rates and daily totals.

Table 1. Primary Productivity, Station 31 (I)

Date/Time	Depth m	Productivity mg atom C/ m^3 X hr	Particulate C mg atom C/m^3	Chlorophyll a mg/m^3
26 June	1	5.18	22.26	4.32
1230-1330	4	2.20	18.31	7.60
	8	0.40	23.93	5.84
27 June	1	9.66	47.87	3.74
1000-1130	4	2.54	88.94	3.58
	8	0.88	20.92	7.71
28 June	1	11.28	44.95	10.21
1030-1130	4	3.72	48.49	7.00
	8	0.54	13.43	7.44
29 June	1	15.18	46.62	6.21
1030-1130	4	2.10	57.46	6.81
	8	0.92	37.12	6.39
30 June	1	12.28	40.06	5.81
1010-1130	4	3.62	21.86	7.84
	8	1.26	37.79	5.15
1 July	1	20.66	22.49	6.23
1000-1145	4	3.30	16.94	5.40
	8	0.98	31.26	5.22

Date	Total primary productivity, upper six meters (mg atom C m^{-2} day^{-1})
26 June	13.07
27 June	13.66
28 June	22.07
29 June	14.77
30 June	17.52
1 July	26.79

Table 2. Primary Productivity, Station 31 (II)

Date	Time	Primary productivity (mg C m^{-3} hr^{-1}) at one meter depth intervals							
		1	2	3	4	5	6	7	8
6/26	1020-1220	2.30	1.49	0.89	1.07	0.93	0.94	0.40	0.15
	1520-1745	3.35	1.36	1.27	1.00	0.69	0.86	0.45	0.40
	1930-2130	1.37	0.98	0.86	0.52	0.31	0.68	0.35	0.16
	2330-0200	0.12	0.14	0.13	0.08	0.14	0.14	0.08	0.06
6/27	0445-0645	0.18	0.20	0.14	0.08	0.17	0.12	0.12	0.04
6/28	0930-1130	5.71	3.88	2.49	1.87	1.54	1.04	0.73	0.43
	1330-1530	4.10	3.76	2.40	1.65	1.17	1.04	0.73	0.85
	1730-1930	4.43	3.49	2.79	1.59	0.92	0.62	0.45	0.46
	2130-2345	0.20	0.19	0.19	0.17	0.17	0.13	0.15	0.11
6/29	0145-0400	0.11	0.09	0.09	0.11	0.10	0.46	0.07	0.05
	0530-0730	0.66	0.34	0.19	0.14	0.16	0.13	0.12	0.12
6/30	0700-0900	2.54	1.52	0.97	0.83	0.42	0.27	0.18	0.16
	1005-1250	3.22	3.25	2.77	1.82	1.69	1.38	0.87	0.67
	1400-1600	4.03	3.55	2.92	2.02	1.69	1.13	0.82	0.61
	1700-1900	3.27	3.42	2.44	1.74	1.14	0.90	0.52	0.46
	2000-2200	1.56	1.06	0.61	0.38	0.35	0.28	0.27	0.27
	2300-0110	0.09	0.10	0.13	0.12	0.14	0.13	0.11	0.09
7/1	0200-0400	0.12	0.09	0.10	0.14	0.12	0.08	0.04	0.02
	0500-0700	0.83	0.56	0.41	0.24	0.21	0.14	0.09	0.06
	0800-1000	3.52	2.61	1.60	1.04	0.95	0.68	0.44	0.33

between June 26 and July 1 was 18.0 mg at C m^{-2} day^{-1}.

Nitrogen Uptake Experiment

A 30 liter water sample was collected at Station 28 on June 30 at 0930 from a depth of five meters. The water was mixed well and placed into three 20-liter translucent polyethylene carboys. The carboys were placed back into the water, floating at the surface, for ten hours. At this time the water was again mixed well, and ten liter aliquots were placed back into three separate carboys. No nitrogen addition was made to Carboy I; Carboy II received an addition of 8.8 µg atom urea-N $liter^{-1}$; Carboy III received an addition of 17.2 µg atom NO_3-N $liter^{-1}$. The carboys were placed back into the water at the surface and incubated a total of 24.5 hours. The samples received only ambient light which was attenuated approximately 25% by the containers. Samples were removed periodically for nutrient, chlorophyll, and particulate carbon and nitrogen analyses (Table 3). Skeletonema costatum was the most numerous phytoplankton species present. Initial samples for quantitative microscopic analysis were lost as were the samples for initial particulate carbon and nitrogen. Quantitative microscopic examination of the control (Carboy I) at the end of the incubation showed the following diatoms present in order of abundance: Skeletonema costatum, various species of Chaetoceros, Thalassiosira aestivalis, Thalasionema sp. and a few penates. Cell

Table 3. In Situ Nitrogen Uptake Experiment

Date	Time	Carboy	Nitrate μg at N/l	Ammonia μg at N/l	Urea μg at N/l
6/30	0900	I, II, III	0.00	0.20	0.25
6/30	2030	I	0.20	0.26	0.32
	2330	I	0.00*	0.15*	0.00*
7/1	0900	I	0.20	0.20	0.31
	1300	I	0.04*	0.39*	0.21*
	1830	I	0.20*	0.34*	0.21*
6/30	1800	II	--	--	8.80
	2030	II	0.20	0.26	6.63
	2330	II	0.00*	0.60*	4.10*
7/1	0900	II	0.20	0.30	4.71
	1300	II	0.00*	0.38*	1.98*
	1830	II	0.20*	0.58*	0.47*
6/30	1800	III	17.20	--	--
	2030	III	14.75	0.26	0.32
	2330	III	11.15*	1.05*	0.43*
7/1	0900	III	10.20	0.30	0.33
	1300	III	8.12*	0.28*	0.11*
	1830	III	6.05*	0.50*	0.35*

Total uptake over 24.5 hours:

Urea (II)	8.3 μg atom N liter^{-1}
Nitrate (III)	8.7 μg atom N liter^{-1}

Final biomass 7/1 1830	Part. N μg at N/l	Part. C μg at C/l	C/N	Chlorophyll a μg/l
I	5.04	61.1	12.1	2.63
II	7.01	69.6	9.9	5.51
III	7.54	84.8	11.2	5.07

* Indicates frozen sample.

dimensions were measured optically. On a volume basis, 80% of the phytoplankton consisted of S. costatum.

Excretion Experiment

An experiment was performed to measure the rate of excretion of native zooplankton species. Zooplankton were collected from the upper 15 m at Station 28 using a Clark-Bumpus zooplankton net. The most numerous were a species of Podon and a species of barnicle nauplii. The animals were sorted by hand and placed into beakers containing 200 ml of filtered seawater. No significant amounts of ammonia excretion were observed although urea was released by the Podon over the 12 hour incubation period (Table 4) at a rate of 0.068 μg atom N Podon^{-1} day^{-1}.

Table 4. Zooplankton Excretion Experiment

	Nitrogen Concentration after 12 hours μg atom N liter^{-1}	
	Ammonia	Urea
A. 20 Podon	4.3	3.43
B. 20 Podon	4.8	5.71
C. 25 Barnicle nauplii	4.0	2.47
D. 25 Barnicle nauplii	4.6	2.52
E. Control (filtered seawater)	4.4	2.34

II. Laboratory Nitrogen Uptake Experiments

Several species of phytoplankton were isolated from Auke Bay which could grow well using urea as their sole nitrogen source. These included Skeletonema costatum, Chaetoceros didymus, Chaetoceros septentrionales, and Thalassiosira aestivalis. All isolations and subsequent growth experiments were made in a modified, half-strength Basic Medium as used at Oregon State University for culturing marine phytoplankton (Table 5). Medium was prepared using seawater with less than one μg atom total dissolved nitrogen per liter as measured by nitrate, nitrite, ammonia, urea, and amino acid analyses. All the following experiments were with S. costatum isolated from Auke Bay.

Batch Growth

Small equal inoculations of exponentially growing S. costatum culture with urea as nitrogen source were transferred to three 500 ml flasks, each containing 250 ml of culture medium without additional nitrogen. Nitrogen was added as follows: Flask I (control), no additional nitrogen; Flask II, 50 μg atom urea-N liter^{-1}; Flask III, 50 μg atom NO_3-N liter^{-1}. Cells were incubated under two cool-white fluorescent lamps at 15° C. Cell growth was monitored with a Coulter particle counter (Fig. 18). There was no significant difference between particle size distributions for Flasks II and III, and no

Table 5. Half Strength Basic Medium

Major Ions, Concentration per liter of seawater:*		
$NaH_2PO_4 \cdot H_2O$	5 mg	36.25 µg atom P
Fe sequestrene	0.5 mg	1.16 µg atom Fe
$Na_2SiO_3 \cdot 9H_2O$	15 mg	53.5 µg atom Si
$NaHCO_3$	0.1 mg	added as 10 ml of stock sol.
Thiourea	0.5 mg	
$NaNO_3$	Various amounts added in each experiment. Stock cultures maintained with 50 µg atom urea-N.	
Urea		
Vitamins, Weight per liter of seawater:		
Thiamin HCl	0.1 mg	
Biotin	0.5 µg	
B_{12}	0.5 µg	
Trace Metals, Concentration per liter of seawater:		
$CuSO_4 \cdot 5H_2O$	0.098 mg	0.039 µg atom Cu
$ZnSO_4 \cdot 7H_2O$	0.022 mg	0.00765 µg atom Zn
$CoCl_2 \cdot 6H_2O$	0.010 mg	0.042 µg atom Co
$MnCl_2 \cdot 4H_2O$	0.280 µg	0.91 µg atom Mn
$NaMoO_4 \cdot 2H_2O$	0.0065 µg	0.0265 µg atom Mo

* Millipore filtered, autoclaved.

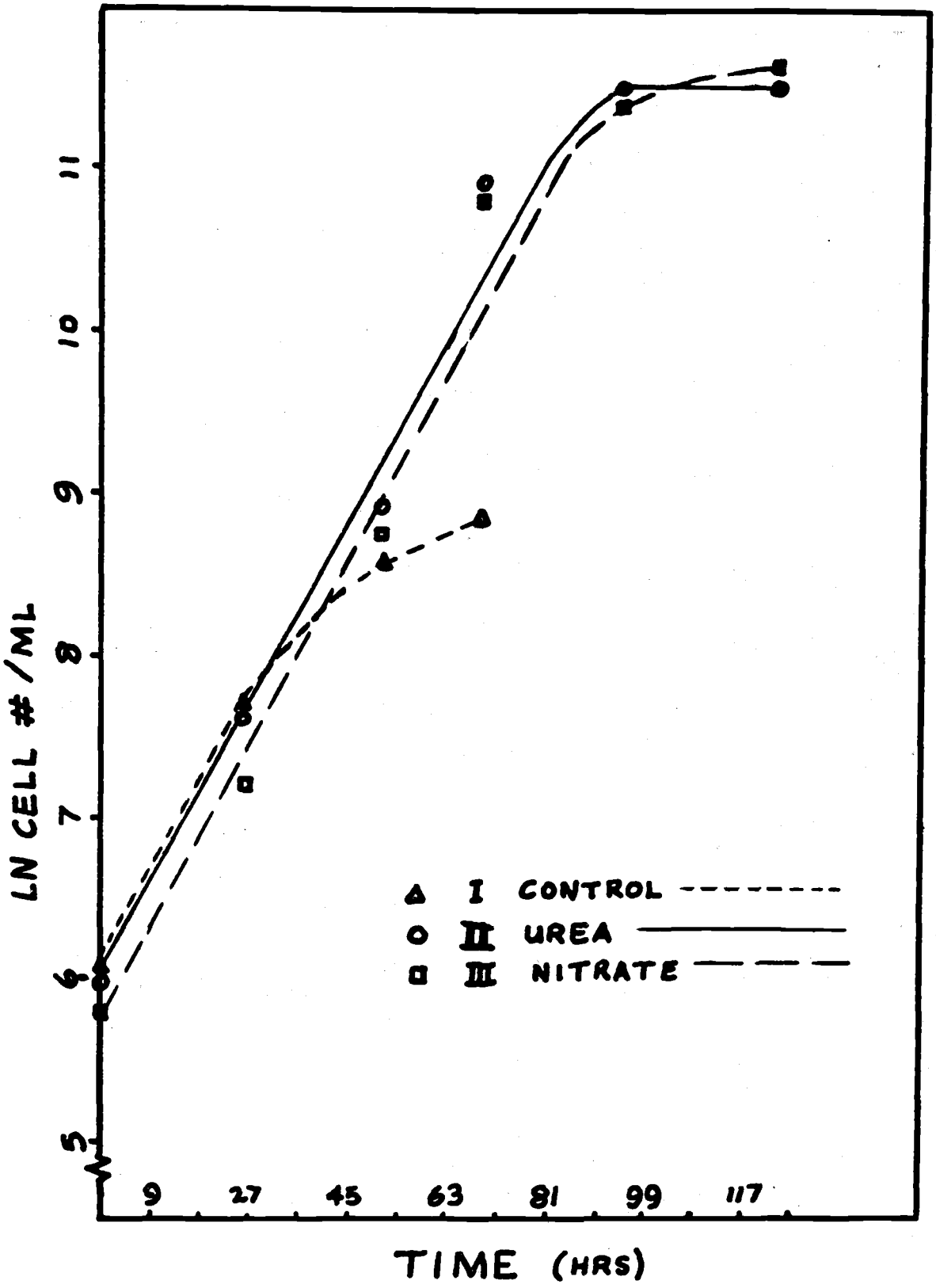

Figure 18. Batch nitrogen uptake.

change in distribution with time. A linear regression of the first 100 hours of incubation against the natural logarithm of cell number per milliliter was used to calculate the rates of growth for S. costatum with urea vs. nitrate. There was no significant difference in the two rates.

Continuous Culture

Since the batch experiment was conducted at high levels of nutrient, an attempt was made to measure uptake rates of nitrate and urea, separately and in combination, by S. costatum at low nitrogen concentrations. A large (ten liter) batch culture of S. costatum with 50 μg atom urea-N liter^{-1} was cultured as previously described. Thiourea was reduced to 1/50th of normal concentration to prevent interference with urea analysis at low concentrations of urea. Growth was monitored by Coulter particle counter, CN analysis, fluorometry, and nitrogen nutrient disappearance. When the urea concentration reached approximately three μg atom urea-N liter^{-1}, 85% of the cells were removed by filtration to slow the rate of urea disappearance. Attempts were made, by close interval monitoring of urea concentrations, to maintain urea levels in the range of 0.5 to 1.0 μg atom urea-N liter^{-1}. Fresh additions of urea were made after each analysis to maintain this level. The culture was maintained in this manner for 40 hours. After the culture had been at a low urea

concentration for 11 hours, a portion was removed, to which nitrate was added. Both nitrate and urea concentrations were held in the low range 0.5 to 1.0 μg atom N liter^{-1} in the same manner as for urea alone. This culture was incubated for 26 hours, during which time the average rate of disappearance of urea was 0.6 μg atom liter^{-1} hr^{-1} and nitrate 0.5 μg atom N liter^{-1} hr^{-1}. Average nitrogen concentration during this time was 0.64 μg atom urea-N liter^{-1} and 0.86 μg atom NO_3-N liter^{-1}. No difference could be detected in the rate of urea uptake for urea alone or when urea was in combination with nitrate.

DISCUSSION

To determine the significance of regenerated nitrogen for phytoplankton in Auke Bay, a combination of field and laboratory investigations were required. It was first necessary to obtain surveys of the various forms of free surface nitrogen and to measure any changes in concentration with time. From such measurements, as well as measurements of phytoplankton productivity, estimates of the flux of nitrogen uptake experiments were required to determine the potential for the natural phytoplankton population to use regenerated nitrogen relative to advected nitrogen. Finally, laboratory studies with the major species of phytoplankton found in Auke Bay were needed to give a quantitative assessment of the ability of Auke Bay phytoplankton to use the various forms of nitrogen observed in the bay at natural nitrogen concentrations.

I. Auke Bay

Wind conditions during the period of investigation were ideal for studying the significance of regenerated nitrogen nutrients. Light, variable, northeast winds fit the pattern described by Kirk (1972) under which little deep water nitrate is advected across the pycnocline. However, the low light levels during this period complicated the picture of nutrient limitation. Although no measurements of

light attenuation with depth were made, the data in Table 2 suggests that only occasionally did ambient light levels approach saturation at the surface.

Surveys of nitrogen nutrients indicate that the entire upper layer was in a "quasi-steady state" with respect to nitrogen. Also, the entire bay was horizontally homogeneous with respect to nitrogen nutrients with only two major exceptions. A urea maximum was observed at 20 meters depth only near shore, especially in the vicinity of Stations 31, 32, and 33, and occasionally at Station 26. It appears that a source for urea exists near shore, almost in a ring around the bay at 20 meters depth. Also there appeared to be a deep (50 m) source of ammonia centered at Station 29. Although these distributions are of interest in understanding the complete nitrogen dynamics of the bay, they did not have any immediate significance in resupplying nutrients to the surface. This is due to the fact that below 10 meters depth, all forms of nitrogen were insignificant compared to nitrate. The 24 hour surveys at a single station gave a better indication of the surface nutrient dynamics. Because of the coincidence of tidal and daylight periods during the surveys, it was difficult to distinguish between tidal advection and diel biological processes. However, the coincidence of almost all parameters for the first 24 hour survey at Station 31 (Fig. 12) with the tidal period strongly suggests that one is observing a spatial gradient of parameters oscillating horizontally

past a fixed observation point. Of special interest in regards to regeneration is the unusually high urea value observed at Station 28 at 10 m on the morning of June 30 (Fig. 14). That this sample was accompanied by elevated ammonia and highly elevated amino acid concentrations reduces the possibility of an error or contaminated sample. The most likely explanation is that a spatially small regeneration or excretion event was observed. The loose coincidence of this event with a large surface pheophytin concentration suggests that active feeding of zooplankton on phytoplankton might have been the source for this event.

The fairly constant, moderate levels of phytoplankton biomass maintained at the surface at Station 31, in spite of the constant low free nitrogen, indicates that resupply of nitrogen to the surface at this location must be occurring at a rate sufficient to maintain the phytoplankton population. This conclusion is supported by the observed C/N ratios in the upper layer (Figs. 11, 12, 13, and 14). Although those at one meter were sometimes fairly high (> 10), in general C/N ratios in the surface layer were in the range of seven to ten, a range indicative of non-nitrogen deficient phytoplankton (Thomas and Dodson, 1972). The phytoplankton population maintained itself against what appears to be moderate zooplankton grazing pressure as demonstrated by the presence of chlorophyll degradation products in the upper waters (Fig. 9).

In past years, at the end of a phytoplankton bloom in Auke Bay, a rapid sinking out of phytoplankton has often been observed. A secondary chlorophyll and pheophytin maximum is frequently observed on the bottom after a bloom. The absence of such an event, as shown by the lack of a secondary or bottom chlorophyll maximum, is further indication that the phytoplankton biomass was in a steady state with respect to supply of nutrients and loss to zooplankton grazing.

Productivity

Several approaches were used to arrive at a total daily productivity per square meter at Station 31. The problem was complicated by the fact that no measurements of light attenuation with depth were made, nor was light intensity always measured at the same time as the ^{14}C incubations were made. Data from Table 1 were used for calculations of productivity because they spanned the entire period of investigation. An estimation of light attenuation with depth was made using a regression of light attenuation as a function of particulate carbon previously reported for Auke Bay (Iverson, 1971). Using this formula, the 1% light level was located approximately eight meters deep. The assumption was made that carbon fixation in the upper layer was directly proportional to total solar insolation during a 24 hour period. This assumption appears to be valid from examination of the decrease in productivity with depth as shown in Table 2, the rate

of production generally decreasing at the same rate as calculated light attenuation. Using these two approximations, total daily productivity was calculated by first obtaining the light specific productivity rate per square meter. Since only three depth intervals were used in the upper eight meters, weighted integration was performed to obtain a total for the upper six meters. Two different weighting schemes, based on different formulas for attenuation of light with depth (Iverson, 1971; Lorenzen, 1972), gave essentially identical results for integrated light specific productivity. This rate was then multiplied by the 24 hour insolation to obtain a total daily productivity. The result obtained was within 10% of that calculated using the more complete productivity data of Table 2 for days when comparisons could be made.

From this estimate of total carbon productivity for the surface layer, an estimate of total nitrogen productivity was made. The assumption was made that nitrogen productivity followed carbon productivity in proportion to the C/N ratio observed in the particulate matter. The slight change in C/N ratio with time was taken into account in the calculation. The result is that an estimated 1.75 mg atom N m^{-2} day^{-1} was required to balance carbon production. This value will be an underestimate in as much as that fraction of non-living detritus inflated the observed C/N ratios. Had resupply not been occurring at a rate similar to uptake, one would have expected

the observed surface N concentrations to have decreased approximately 20% per day. No such change was observed.

Resupply of Nitrogen

The results of both in situ uptake experiments at Auke Bay (Table 3) and laboratory uptake experiments at high and low nitrogen concentrations indicate that the uptake rates for both urea and nitrate were similar for the phytoplankton of Auke Bay at the time of this study. Calculations by Kirk (1972) for wind and hydrographic conditions similar to those observed indicate that a flux of 0.5 mg atom NO_3-N m^{-2} day^{-1} and 0.3 mg atom NH_3-N m^{-2} day^{-1} could be supplied by wind mixing across the pycnocline. If regenerated urea-N were being supplied at the same rate as advected nitrate-N, then approximately 0.5 mg atom N m^{-2} day^{-1} must be supplied by other sources to account for the observed phytoplankton growth. A possible source would be regenerated ammonia. McCarthy (1971) has shown that zooplankton nitrogen excretion of actively feeding zooplankton consists of approximately equal portions of ammonia and urea, and that urea excretion decreases rapidly after feeding ceases. The experiment to measure zooplankton excretion was inconclusive. No ammonia excretion was observed for either of the zooplankton species tested. The high ures excretion observed for the species of Podon could have been the result of stress. The similarity in concentration

of urea and ammonia in the surface layer leads one to suspect that ammonia was being utilized at a rate similar to that for urea, and ammonia is probably supplying the fraction of regenerated nitrogen unaccounted for. This conclusion is supported by observations by McCarthy (personal communication) of nitrogen uptake by phytoplankton in similarly low nitrogen concentrations in Chesapeake Bay and off the coast of southern California (McCarthy, 1972b). McCarthy found that when concentrations of nitrogen nutrients were below one μg atom N liter^{-1}, the three forms, nitrate, ammonia, and urea, were assimilated equally.

Several alternative sources of the urea maximum at 20 m are possible. Zooplankton may be excreting at depth following vertical migration. The extent to which vertical migration of zooplankton occurs in Auke Bay is unknown. A rock outcropping occurs at this depth around Auke Bay (Schell, 1974). A large population of sea urchins is found on this rock bottom, but few sea urchins are found at greater depths (Shabica, personal communication). The primary excretory product of sea urchins is urea (Fechter, 1973). A third possibility is that seepage from spetic tanks associated with dwellings around Auke Bay enters the bay at this depth. However, input of urea from Auke Creek was low, the concentration in the creek being only 0.5 μg atom urea-N liter^{-1}. This creek flows into the bay near Station 31 from Auke Lake. The lack of urea contamination seems to

rule out input into the surface layer from pollution but does not exclude the possibility that pollution enters through ground water.

In Situ Uptake Experiment

Uptake rates for nitrate and urea were similar for the in situ uptake experiment (Table 3). The lack of change in free nitrogen in the control was surprising, but it is doubtful that a change as small as 20% could have been detected. Chlorophyll for the control at the end of the incubation was about half that of either of the enriched samples. This indicates that nitrogen input was limiting growth, at least at the surface. The samples that were frozen were stored for approximately two months before analysis and do not fit with the pattern established by the fresh analyses. Possibly variable amounts of internal nutrient reservoirs were released by rupturing cells upon freezing. Also the length of time between collection and freezing was variable. This conclusion is supported by the transitory increase in ammonia concentration observed in the frozen samples from both the nitrate and urea enriched cultures, reflecting an increase in ammonia as an intermediate within the cells (Lundy, 1974). In spite of the discrepancy between fresh and frozen samples, the rates of uptake calculated using frozen samples is similar to that using fresh samples.

II. Laboratory Nitrogen Uptake Experiments

Results of uptake experiments at high nutrient concentrations establish the potential for a phytoplankton population to utilize urea as sole nitrogen source. By maintaining a population at a low concentration for a long period of time, it was hoped that uptake rates, especially relative uptake rates for urea vs. nitrate, could be more readily extrapolated to the naturally low nitrogen conditions existing in Auke Bay. The management of such a culture proved to be difficult to accomplish. Constant attention to the culture was required in the form of repeated nutrient analyses and nutrient additions, and substances other than nitrogen could have become limiting near the end of the experiment. Measurements of nitrogen uptake by disappearance from the medium at low concentrations were further complicated by the occurrence of internal cell reservoirs of the nutrients being taken up. Reservoirs were previously reported by Lundy (1974) for nitrate and ammonia. Cell reservoirs of urea amounting to as much as 12.4% of the total particulate nitrogen were observed. With particulate nitrogen concentrations much larger than the free nitrogen concentration, accidental rupture of only a small fraction of the cell population would give a significant error in the nutrient analysis. However, the detection of an internal cell urea reservoir is strong evidence that S. costatum actually assimulates urea rather than

utilizing ammonia from external hydrolysis of urea.

SUGGESTIONS FOR FUTURE RESEARCH

1) Use of ^{15}N tracers could go a long way toward giving a definative picture of the nitrogen dynamics of Auke Bay. Although ^{15}N enriched incubations of urea, nitrate, ammonia, and amino acids were made concurrently with this study, suitable techniques for analyzing these samples are not yet available.

2) Measurements of internal cell reservoirs for the various forms of nitrogen using the method of Lundy (1974) could be made fairly easily at sea. Such information would be of little use in assessing the contribution for ammonia, but might indicate the significance of other regenerated forms such as urea or amino acids.

3) Quantitative measurements of nitrogen excretion by zooplankton under natural conditions are needed to determine the role of zooplankton in nitrogen regeneration. However, even if excretion measurements could be made under natural conditions, the problem of obtaining estimates of zooplankton biomass throughout the bay remains a major difficulty in directly estimating regeneration.

4) A more promising line of investigation appears to be a continuation of the laboratory uptake experiments. Techniques need to be developed for more closely simulating natural conditions of low nitrogen concentration in laboratory cultures. Possibly chemostats or diffusion cultures would be more suitable for examining

phytoplankton growth response at low nitrogen concentrations. In combination with systems modeling and computer simulation, a more complete picture of the interactions of phytoplankton, zooplankton, advected nitrogen, and regenerated nitrogen might be obtained.

CONCLUSIONS

1. During the period of investigation, surface water in Auke Bay was in steady state with respect to nitrate, ammonia, and urea.

2. Urea and nitrate both contributed equally to the phytoplankton nitrogen requirements.

3. The contribution of ammonia could not be estimated directly, but was indirectly estimated to be as large as or larger than that of urea or nitrate.

4. The phytoplankton population growth was limited by the rate of flux of nitrogen in the surface layer, although the population was not nitrogen deficient.

5. Regenerated nitrogen contributed at least 67% to the phytoplankton's nitrogen needs.

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