YALE
PEABODY
MUSEUM

P.O. BOX 208118 | NEW HAVEN CT 06520-8118 USA | PEABODY.YALE. EDU

JOURNAL OF MARINE RESEARCH

The *Journal of Marine Research,* one of the oldest journals in American marine science, published important peer-reviewed original research on a broad array of topics in physical, biological, and chemical oceanography vital to the academic oceanographic community in the long and rich tradition of the Sears Foundation for Marine Research at Yale University.

An archive of all issues from 1937 to 2021 (Volume 1–79) are available through EliScholar, a digital platform for scholarly publishing provided by Yale University Library at https://elischolar.library.yale.edu/.

Requests for permission to clear rights for use of this content should be directed to the authors, their estates, or other representatives. The *Journal of Marine Research* has no contact information beyond the affiliations listed in the published articles. We ask that you provide attribution to the *Journal of Marine Research*.

Yale University provides access to these materials for educational and research purposes only. Copyright or other proprietary rights to content contained in this document may be held by individuals or entities other than, or in addition to, Yale University. You are solely responsible for determining the ownership of the copyright, and for obtaining permission for your intended use. Yale University makes no warranty that your distribution, reproduction, or other use of these materials will not infringe the rights of third parties.

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.
https://creativecommons.org/licenses/by-nc-sa/4.0/

Journal of Marine Research, 43, 437–464, 1985

Nitrogen storage and use of biochemical indices to assess nitrogen deficiency and growth rate in natural plankton populations

by Q. Dortch,[1,2] J. R. Clayton, Jr.,[3] S. S. Thoresen,[1] J. S. Cleveland,[1] S. L. Bressler,[4] and S. I. Ahmed[1]

ABSTRACT

Various newly developed indicators of N deficiency, physiological state (approximate growth rate), and N source for growth were measured during five cruises to Dabob Bay, Washington from early spring to summer. Although nitrate and ammonium in the surface layer were depleted early in the spring, the plankton populations never became extremely N deficient, as indicated by high intracellular amino acid/protein ratios. However, growth rates, estimated from protein/DNA or RNA/DNA ratios, were usually low unless nitrate concentrations were high or had recently been high, as indicated by large intracellular nitrate pools or high nitrate reductase activities. High growth rates were observed during the spring bloom or as a result of the sporadic supply of nitrate to the euphotic zone, which was inferred from measurements of biochemical indicators on several cruises after the spring bloom. The sporadic supply of nitrate could account for the lack of N deficiency in these populations and mask diel periodicity in N utilization. These results demonstrate that biochemical indicators can be easily measured in the field and that variations in indicators such as intracellular amino acid/protein, protein/DNA, RNA/DNA ratios, NR activities and intracellular nitrate concentrations are an aid in understanding plankton dynamics.

1. Introduction

Many N compounds found in marine algae have a dual role. Besides their primary biochemical function, they can serve as an N reserve. If the external N supply is not adequate, the reserves can be used for growth (Dortch, 1982; Dortch *et al.*, 1984). By this means phytoplankton adapt to the potentially debilitating effect of a variable and sometimes growth-limiting N supply in the ocean (Dortch, 1980, 1982; Dortch *et al.*, 1984). Furthermore, as N reserves are utilized, the chemical composition changes in a manner which is highly dependent on the availability of N. Thus, the concentrations or ratios of some cellular N-containing compounds or the activities of selected N-

1. School of Oceanography, University of Washington, Seattle, Washington, 98195, U.S.A.
2. Present address: Bigelow Laboratory for Ocean Sciences, McKown Point, W. Boothbay Harbor, Maine, 04575, U.S.A.
3. School of Fisheries, University of Washington, Seattle, Washington, 98195, U.S.A.
4. Department of Botany, University of Washington, Seattle, Washington, 98195, U.S.A.

assimilating enzymes can indicate the nutritional status (i.e., N sufficient, deficient, or starved), physiological state (approximate growth rate), and likely N sources for growth (Dortch *et al.*, 1979, 1983, 1984; Dortch 1980, 1982).

Several indices of physiological state or nutritional status have been developed for use in the field (e.g., Morris *et al.*, 1971; Yentsch *et al.*, 1977; Healey, 1979; Healey and Hendzel, 1979; Vincent, 1981a,b; Sakshaug *et al.*, 1983; Goldman and Dennett, 1983; Glibert and McCarthy, 1984), but N-specific biochemical indicators should be particularly useful when the availability of N is likely to limit phytoplankton production. Although there are some earlier measurements of these N specific indicators in natural populations (Conover, 1975b; Collos and Slawyk, 1976; Morris *et al.*, 1983), they have not been used before to explain or predict phytoplankton dynamics. Two exceptions are nitrate reductase activities as an indicator of growth on nitrate (e.g., Eppley *et al.*, 1970; Packard *et al.*, 1971; Harrison, 1973; Blasco and Packard, 1974; Packard and Blasco, 1974; Collos and Slawyk, 1976, 1977; Devol and Dortch, 1981; Blasco and Conway, 1982; Dortch and Maske, 1982; Blasco *et al.*, 1984) and RNA/DNA ratios as an indicator of physiological state (Dortch *et al.*, 1983).

To assess the utility of biochemical indicators in natural populations, a seasonal study was conducted in Dabob Bay, Washington (Fig. 1) between March and July, 1981. Dabob Bay is a fjord-type bay off Hood Canal which has been studied extensively (e.g., Kollmeyer, 1965; Ebbesmeyer, 1973; Christensen, 1974; Shuman, 1978; Copping, 1982; Dortch *et al.*, 1983). Because there is minimal current movement, tidal excursion (100–200 m), or freshwater run-off (Shuman, 1978; Copping, 1982), it is an ideal place to test two specific hypotheses. 1) Above the pycnocline the amount of stored intracellular N should decrease as the phytoplankton progress from N sufficiency to N deficiency over the course of the season. Accordingly, the indicators of N deficiency and physiological state should reflect this transition. Below the nitracline, predictions are more difficult because the effect of low light levels on N storage has not been studied, but the presence of high nitrate concentrations should make plankton populations N sufficient. 2) Diel periodicity should be observed in many aspects of N utilization, particularly when nitrate is the predominant N source or the phytoplankton are N sufficient (e.g., Eppley *et al.*, 1970; Packard *et al.*, 1971; Eppley and Renger, 1974; Bates, 1976; Collos and Slawyk, 1976; Terry, 1982).

On each of five cruises, depth profiles and diel variations were measured (1) in intracellular N compounds, including nitrate (IN NO_3^-), nitrite, ammonium (IN NH_4^+), free amino acids (AA), protein (Pr), RNA, DNA and chlorophyll; (2) in enzymes of N assimilation, including nitrate reductase (NR), glutamate dehydrogenase (GDH), and glutamine synthetase (GS); and (3) in ambient nutrient concentrations (Table 1). Other routine field measurements were made for additional depth profiles and a spatial survey (Table 1). Consequently, both temporal (seasonal, diel, and other) and spatial (horizontal and vertical) variations in indicators could be examined. The results were interpreted by comparison with data obtained from

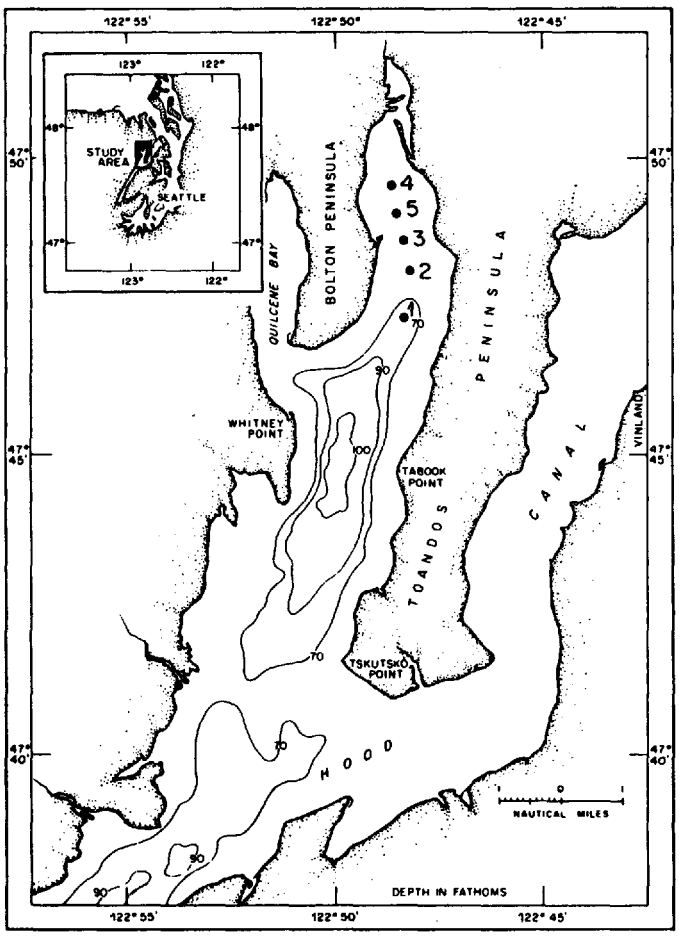

Figure 1. Station locations in Dabob Bay, Washington.

cultures grown under controlled conditions (Dortch, 1982; Dortch *et al.*, 1984). By this means indicators of growth rate/physiological state (RNA/DNA, Pr/DNA), N deficiency (AA/Pr, IN NO_3^-, IN NH_4^+), or use of a particular N compound for growth (IN NO_3^-, IN NH_4^+, NR, NR/GDH, GS, and GDH) can be used to explain phytoplankton dynamics in the field.

2. Materials and methods

a. Field program. Approximately the same procedure was followed at station 5 on each cruise on the R/V *Onar* (Fig. 1; Table 1). First a depth profile of salinity, temperature, chlorophyll and nutrient concentrations ("Routine"; Table 1) was obtained using Scott-Richards bottles; sometimes this same profile was repeated on

Table 1. Cruises to Dabob Bay, Washington in 1981. In the column "Analyses," "All" means that samples were collected for all analyses (except temperature); "Routine" means that, usually, only temperature, salinity, nutrient, and chlorophyll concentrations were measured. Time (da) is calculated from 1 Jan 1981 at 00:00 hr.

Cruise	Date	Time (da)	Cruise plan	Analyses
MP-3	Mar 9–12	68.86–70.77	Diel study (6 times; 5,15 m)	All
			Spatial survey (5 stations; 5,15,25 m)	Routine
			Vertical profile	Routine (3) All (1)
MP-4	Apr 6–9	96.82–98.10	Diel study (5 times; 3,15 m)	All
			Spatial survey (5 stations; 3,15,25 m)	Routine
			Vertical profile	Routine (2)
MP-5	May 4–5	124.85–125.62	Diel study (3 times; 3,20 m)	All
			Vertical profile	Routine (1)
MP-6	May 18–21	138.72–140.67	Diel study (6 times, 6,15 m)	All
			Spatial survey (5 stations; 3,9,25 m)	Routine
			Vertical profile	Routine (2), All (1)
MP-7	Jul 6–9	187.90–189.78	Diel study (6 times; 6,12 m)	All
			Spatial study (5 stations; 3,12,25 m)	Routine
			Vertical profile	Routine (1), All (1)

later days. On 4 of 5 cruises the station was then occupied for longer than 24 hours and samples were collected for all analyses from two depths at 4–6 hr intervals. For the remaining cruise (May 4–5), the sampling period was reduced to 12 hr due to a problem with the research vessel. The two depths were chosen to correspond with an N-depleted surface layer in late spring and a deeper layer with high N concentrations (Christensen, 1974; Shuman, 1978). The primary purpose for occupying this station for longer than 24 hr was to conduct studies of diel periodicity, but the mean values at each depth also show seasonal changes. On 3 cruises, after sampling the two depths for 24 hr or more, water samples from 5 depths above 25 m at station 5 were collected and processed as rapidly as possible in order to obtain more detailed depth profiles of all the same chemical and biological parameters ("All"; Table 1). When samples were

collected for "All" analyses, a large volume (60 ℓ) stainless steel sampler (Young *et al.*, 1969) was used. A reversing thermometer could not be attached to the sampler, so no temperature data are available when it was used. Finally, on 4 cruises a spatial survey of routine measurements was conducted at 3 depths at all 5 stations.

b. Sampling and analyses. Samples were first prefiltered through 333 μm Nitex and then onto combusted (350°C, 24 hr) Whatman 934-AH (nominal pore size 1 μm) glass fiber filters. The vacuum differential for filtration was kept low (<10 cm Hg) because of earlier results showing that cell contents were lost with a greater differential vacuum (Dortch, unpubl.). Depending on the amount of biomass present, between 1 and 3 ℓ was filtered except 50 ml was filtered for nutrient samples. External and internal nutrient samples were frozen at −15°C. From many years of experience we have learned that they can be frozen with no deleterious effects if (1) the sample is filtered through an ammonium-free filter (precombustion removes contaminating ammonium), (2) it is frozen in a bottle which was acid washed, rinsed copiously with distilled, deionized water and stored dry, and (3) the other usual precautions are taken. The filters for total N and C analyses were frozen over desiccant at −15°C. Samples for phytoplankton counts and identification were preserved with Lugol's solution. The filters with samples for enzyme assays and determination of biochemical composition were frozen in liquid N_2 (−196°C). All samples were then analyzed after returning to the laboratory.

Salinity and temperature were determined by the standard methods of Paquette (1958) and Sverdrup *et al.* (1942), respectively. Sigma *t* was calculated by the revised method of Millero and Poisson (1981). Light penetration was estimated with a Secchi disk that was not calibrated, but the data are only used qualitatively.

External nitrate, nitrite, and ammonium concentrations were measured using a Technicon AutoAnalyzer® (Friederich and Whitledge, 1972). Internal nitrate and ammonium were determined with the AutoAnalyzer after extraction with boiling fresh water (Dortch, 1982; method Q-1 in Thoresen *et al.*, 1982). Although internal nitrite was measured in all experiments, it never accumulates, and therefore these data are not reported.

Protein and amino acids were extracted by grinding the filters in cold, 10% TCA. After centrifuging, the pellet was analyzed for protein by the Lowry method (Lowry *et al.*, 1951) and the supernatant for amino acids by the fluorescamine method (Udenfriend *et al.*, 1972), with bovine serum albumin and glutamate, respectively, as the standards, following the procedure of Dortch *et al.* (1984). RNA and DNA were measured using the ethidium bromide method of Prasad *et al.* (1972), as adapted for use with phytoplankton by Dortch *et al.* (1983). Total particulate N (PN) and C (PC) analyses were made with a Perkin Elmer model 240 Elemental Analyzer. Chlorophylls *a*, *b*, and *c* were measured spectrophotometrically according to Jeffrey and Humphrey (1975). Although only chlorophyll *a* concentrations are reported in Table 2, when the chlorophyll-N was calculated for Figure 3, chlorophylls *b* and *c* were included.

Table 2. Seasonal variation in measured concentrations; mean ± 1 SE (number of measurements) of all samples collected during diel study. Ex NO_2^- concentrations <0.04 μM.

Time (da)	Depth (m)	Ex NO_3^- (μM)	Ex NH_4^+ (μM)	PN (μM)	PC (μM)	Chl *a* ($\mu g/l$)	RNA (nM-N)	DNA (nM-N)
69.06–70.66	5	19.7 ± 0.51(6)	0.00 ± 0.000(6)	1.32 ± 0.090(6)	9.0 ± 0.44(6)	1.76 ± 0.139(6)	102 ± 12.5(6)	43 ± 3.1(6)
69.00–70.53	15	23.1 ± 0.36(6)	0.00 ± 0.000(6)	0.87 ± 0.065(6)	6.0 ± 0.44(6)	1.62 ± 0.147(6)	52 ± 8.5(6)	25 ± 4.0(6)
97.14–98.10	3	1.9 ± 0.97(5)	0.09 ± 0.018(5)	6.0 ± 0.48(5)	44 ± 4.9(5)	10.4 ± 0.40(5)	495 ± 28.8(5)	56 ± 4.0(5)
97.06–98.07	15	15.8 ± 1.34(5)	0.17 ± 0.080(5)	5.8 ± 0.80(5)	36 ± 5.5(5)	11.4 ± 1.30(4)	450 ± 59(5)	53 ± 4.4(5)
125.18–125.62	3	0.0 ± 0.00(3)	0.00 ± 0.000(3)	4.6 ± 0.64(3)	43 ± 3.8(3)	3.34 ± 0.237(3)	260 ± 35.0(3)	127 ± 1.6(3)
125.11–125.60	20	22 ± 12.5(3)	4.6 ± 0.96(3)	1.79 (2)	18.4 ± 0.92(3)	3.48 ± 0.254(3)	115 ± 13.8(3)	65 ± 13.9(3)
139.01–140.54	6	0.0 ± 0.00(6)	0.00 ± 0.000(6)	3.51 ± 0.257(6)	25.2 ± 1.52(6)	3.8 ± 1.00(3)	228 ± 8.4(6)	67 ± 6.0(6)
139.01–140.59	15	13.2 ± 0.90(6)	1.42 ± 0.073(6)	2.06 ± 0.249(6)	16.2 ± 1.74(5)	2.66 ± 0.298(6)	121 ± 11.3(5)	56.1 ± 2.96(5)
188.17–189.43	6	0.6 ± 0.56(6)	0.00 ± 0.000(6)	5.3 ± 1.31(6)	30 ± 7.9(6)	4.9 ± 2.39(6)	180 ± 34(6)	53 ± 8.4(6)
188.25–189.56	12	9.5 ± 2.45(6)	0.00 ± 0.000(6)	5.6 ± 1.00(6)	30 ± 6.1(6)	7.58 ± 1.086(6)	240 ± 34(6)	40 ± 8.3(6)

Table 2. (Continued)

Time (da)	Depth (m)	AA (nM-N)	IN NO_3^- (nM-N)	IN NH_4^+ (nM-N)	Protein (μM-N)	NR (nM/hr)	GDH (nM/hr)	GS (nM/hr)
69.06–70.66	5	57 ± 4.6(6)	4.9 ± 1.72(6)	1.0 ± 0.75(6)	0.99 ± 0.045(6)	2.90 ± 0.274(6)	14.9 ± 1.46(6)	ND (6)
69.00–70.53	15	37 ± 3.3(6)	3.2 ± 0.52(6)	0.58 ± 0.351(6)	0.69 ± 0.061(6)	2.8 ± 0.71(6)	11.1 ± 1.29(6)	ND (6)
97.14–98.10	3	390 ± 86(5)	160 ± 31(5)	10.0 ± 2.97(5)	4.3 ± 0.73(5)	46.7 ± 1.26(3)	26.7 ± 2.58(3)	ND (6)
97.06–98.07	15	300 ± 74(5)	249 ± 24.8(5)	7 ± 3.6(5)	3.7 ± 0.34(5)	31 ± 9.2(5)	30 ± 5.0(5)	ND (5)
125.18–125.62	3	190 ± 37(3)	0.0 ± 0.00(3)	0.0 ± 0.00(3)	1.91 (1)	5.6 ± 1.32(3)	78 ± 14.5(3)	ND (3)
125.11–125.60	20	104 ± 9.9(3)	17 ± 4.3(3)	0.0 ± 0.00(3)	1.35 ± 0.156(3)	3.8 ± 1.12(3)	31 ± 4.4(3)	ND (3)
139.01–140.54	6	216 ± 11.8(6)	12 ± 5.5(5)	11.0 ± 2.38(5)	1.99 ± 0.098(6)	2.0 ± 0.81(5)	41 ± 8.5(5)	ND (5)
139.01–140.59	15	109 ± 5.6(6)	14.2 ± 1.40(6)	7.5 ± 1.43(6)	1.08 ± 0.045(6)	2.4 ± 0.49(6)	21.0 ± 2.41(6)	ND (5)
188.17–189.43	6	280.67 ± 86(6)	35 ± 15.2(6)	13 ± 3.8(6)	2.2 ± 0.73(6)	10.1 ± 2.14(6)	20 ± 5.6(6)	11 ± 3.8(6)
188.25–189.56	12	260 ± 40(6)	62 ± 27.9(6)	17 ± 4.1(6)	2.43 ± 0.298(6)	6.8 ± 2.46(6)	32 ± 9.2(6)	16 ± 6.2(6)

Concentrations of all N-containing compounds, except chlorophylls, are expressed as μmol N or nmol N/ℓ of seawater, after conversion from weights as described in Dortch *et al.* (1983, 1984). The use of a single standard amino acid, protein, RNA or DNA to quantify a mixture of amino acids, proteins, RNA's or DNA's introduces some uncertainty (Dortch, 1983; Dortch *et al.*, 1984).

After settling, total cell numbers were counted using an inverted microscope (Utermöhl, 1958) and dominant phytoplankton species were identified. The preservation method, the length of sample storage (up to 6 months), the method of concentrating the cells, and the type of illumination, suggest that very small cells (e.g. Li *et al.*, 1983; Murphy and Haugen, 1985) and fragile flagellate species would not have been counted or identified.

NR activities were measured by the method of Eppley *et al.* (1969) except that the uncentrifuged homogenate was used for the incubations and the homogenate was diluted to minimize depletion of NADH, an essential cofactor. GDH and GS activities were determined according to Ahmed *et al.* (1977) and the biosynthetic assay of Bressler and Ahmed (1984), respectively. Reaction mixtures were incubated for 30 min at 15°C for NR and 25°C for GS and GDH. Rates were corrected to *in situ* temperatures (Packard *et al.*, 1971) using the appropriate activation energies (Eppley *et al.*, 1970; Ahmed *et al.*, 1977; Bressler and Ahmed, 1984). GS activities were below detection limits except on the last cruise when a more sensitive adaptation of the Bressler and Ahmed (1984) method was used (Clayton, 1985). Although an earlier study (Ahmed *et al.*, 1976) indicated that freezing in liquid N_2 preserves enzyme activity, the ability to store the 3 enzymes examined in this study was also tested using laboratory cultures of phytoplankton. For GS, 80% of the initial activity remained after 8 days of storage in liquid N_2 (2 species, 3 replicates each; Bressler, 1981); for NR, 84% remained (4 species, 3 replicates each; Dortch, unpubl.); and for GDH, after 7 or 30 days, 84% remained (3 species, 3 replicates each; Clayton, 1985). Despite slight losses in activity, the difficulty of performing so many enzyme assays on board ship with limited time, space, and personnel, is so great that freezing in liquid N_2 is an acceptable alternative, particularly when the activities are to be used as a general indicator rather than as a measure of V_{max} for that enzyme. Enzyme activities, uncorrected for freezing loss, are expressed in terms of nmol N assimilated or reduced/liter seawater/hr (nM/hr).

3. Results

The profiles of σ_t, nutrients and chlorophyll showed steady progression from plentiful nitrate in the surface layer to N depletion during the first 3 cruises (Fig. 2A–C), and represent pre-phytoplankton bloom, bloom and post-bloom conditions, respectively. On the last two cruises (Fig. 2D,E), the pycnocline and the nitracline were shallower than in the early May cruise, and chlorophyll levels were higher, although occurring in a subsurface maximum. On these two later cruises, less detailed profiles

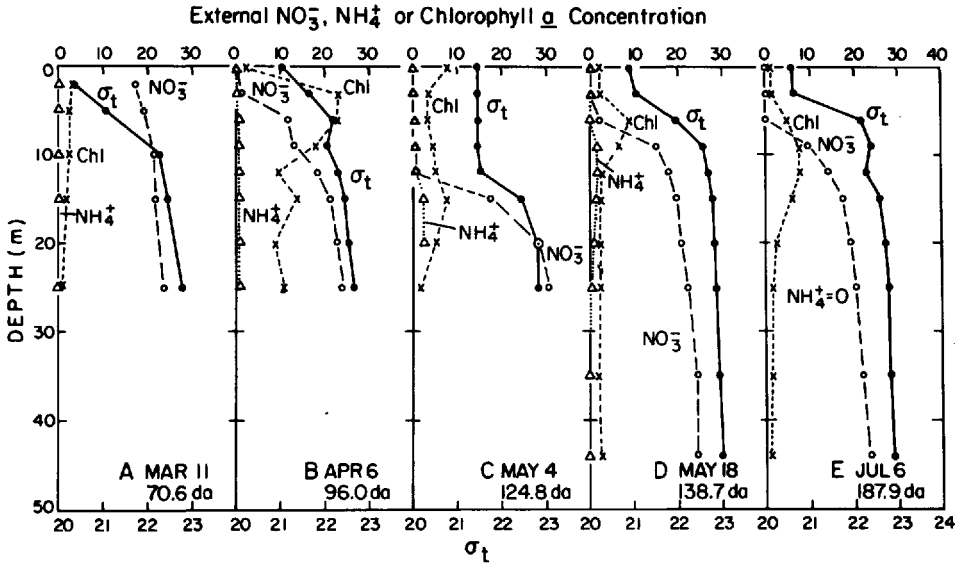

Figure 2. Depth profiles of σ_t, chlorophyll *a* (μg/liter seawater) and external nitrate and ammonium (μM) taken immediately upon arrival at station 5 on each cruise (except MP-3, when it was taken at same time as profile in Fig. 4A).

obtained approximately 2 days later (Fig. 4B,C) show significant changes in chlorophyll, nitrate, and ammonium concentrations. Nitrite concentrations were always <0.05 μM. Ammonium concentrations could be quite high (>2 μM), but the maximum was usually below the nitracline and the chlorophyll maximum, and ammonium was frequently undetectable in the surface layers (Fig. 2). The 1% light depth at 10:00 hr ranged between 9–13 m, which was similar to that obtained in a more extensive early study (8–15 m; Shuman, 1978). On the March cruise, the phytoplankton population was extremely diverse. By April, during the bloom period, it was predominantly a mixture of pennate and centric diatoms, while the post-bloom population in early May was dominated by *Chaetoceros* spp. On the two later cruises, the numbers of dinoflagellates increased, although *Chaetoceros* spp. were still important. However, during all the cruises, one of the most numerous phytoplankters was an unidentified, biflagellate, green monad.

Naturally occurring plankton populations contained at some time all the N compounds which had been measured earlier in laboratory cultures (Table 2). If the concentrations are normalized to PN (Fig. 3), protein was the major component, although it represented only 39% to 79% of PN. Except for unidentified N (discussed in detail later), RNA-N and AA-N were most abundant after protein, whereas, generally concentrations of DNA-N > total chlorophyll-N > IN NH_4^+. In contrast, IN NO_3^- was quite variable. Mean values (Table 2, Fig. 3) are somewhat misleading about the importance of compounds which are subject to rapid changes in concentra-

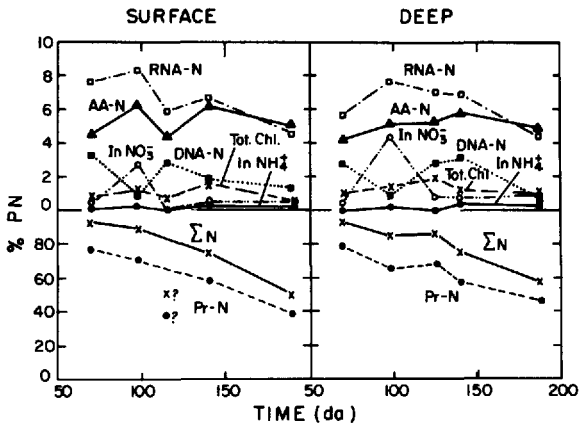

Figure 3. Seasonal variation in mean concentration of intracellular N compounds expressed as a % (by mole N) of the PN. ΣN is the sum of all the measured concentrations. The depths corresponding to Surface and Deep are given in Table 1 or 2.

tion (Dortch, 1982; Dortch *et al.*, 1983, 1984). Maximum observed values, 5.55% PN for IN NO_3^- (MP-4, 15 m, 97.06 da), 0.97% for IN NH_4^+, 13.76% for RNA-N, and 20.98% for AA-N (MP-6, 9 m, 140.61 da) show that all these compounds can at times constitute a significant fraction of cellular N.

Seasonal variations in chemical composition of phytoplankton were quite complex (Fig. 3), but several features are notable. IN NO_3^- was high in April during a phytoplankton bloom when nitrate and light levels were both adequate. Furthermore, large centric diatoms and *Gonyaulax* sp., which may have a greater nitrate storage capacity than other species (Thoresen *et al.*, 1982; Dortch *et al.*, 1984), were numerous. On later cruises, a greater variety of species was present, but *Chaetoceros* spp., which may not store much internal nitrate (Collos, 1982; Dortch, 1982; Dortch *et al.*, 1984), predominated. In contrast, the % AA/PN was relatively constant throughout the season, even when external N in the surface layer was completely depleted. Finally, there was a steady decline in the % protein/PN during the season that was accompanied by an increase in the amount of unidentified N. At the extreme (MP-7, surface layer), almost 50% of the PN was not identifiable, although earlier in the season % ΣN/PN > 90%.

When depth profiles of phytoplankton chemical composition and enzyme activities (Fig. 4) are compared, the seasonal variations are more pronounced. After the surface layer became depleted of N and biomass, a subsurface maximum in chlorophyll, most intracellular N compounds, and enzyme activity formed near the nitracline. The dynamic nature of the population in the subsurface maximum was demonstrated by the intensification of the maximum between July 6 (Fig. 2E) and July 8 (Fig. 4C). Some of the highest intracellular N concentrations and enzyme activities encountered in this study were observed on July 8.

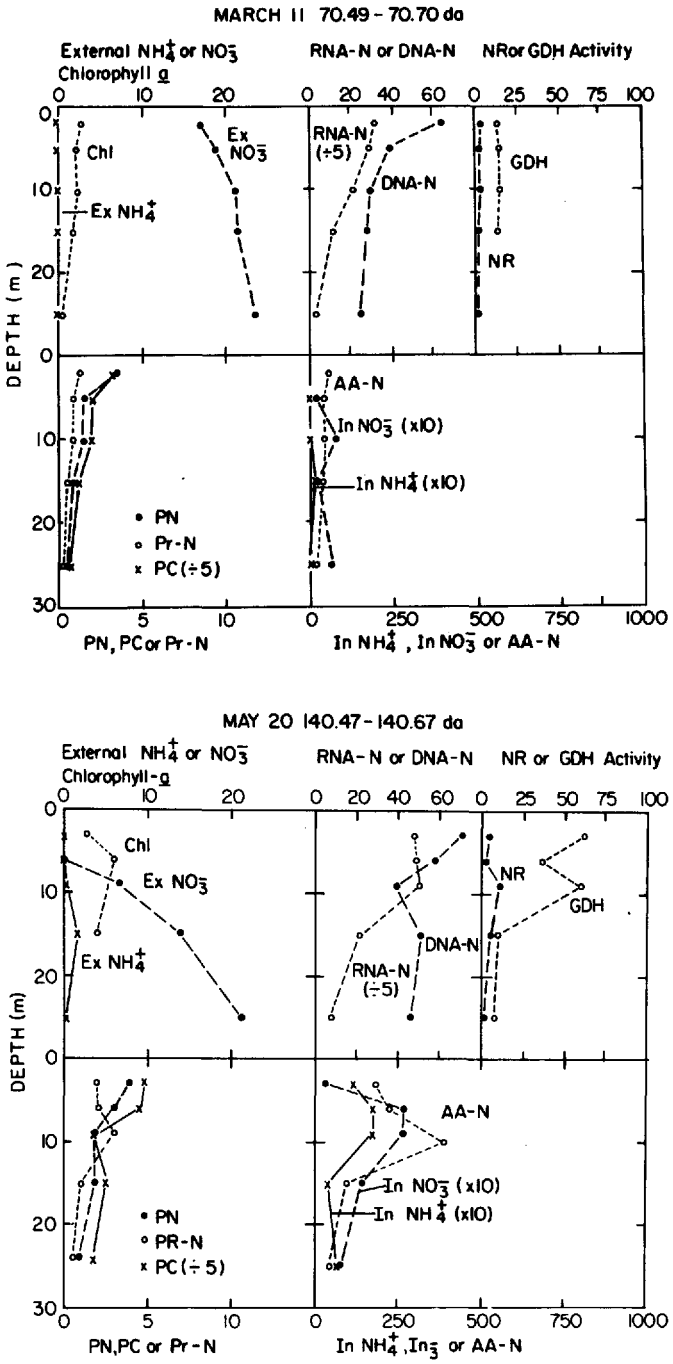

Figure 4. Depth profiles of cellular N compounds (IN NO_3^-, IN NH_4^+, AA-N, RNA-N, DNA-N in nmol N/ℓ seawater; Pr and PN in μmol N/ℓ seawater), chlorophyll *a* (μg/ℓ), external nitrate and ammonium concentrations (μM), PC (μmol C/ℓ seawater), and enzyme activities (nM/hr). Values *xn* are magnified by *n*; values ÷ *n* are reduced by *n* to be on scale.

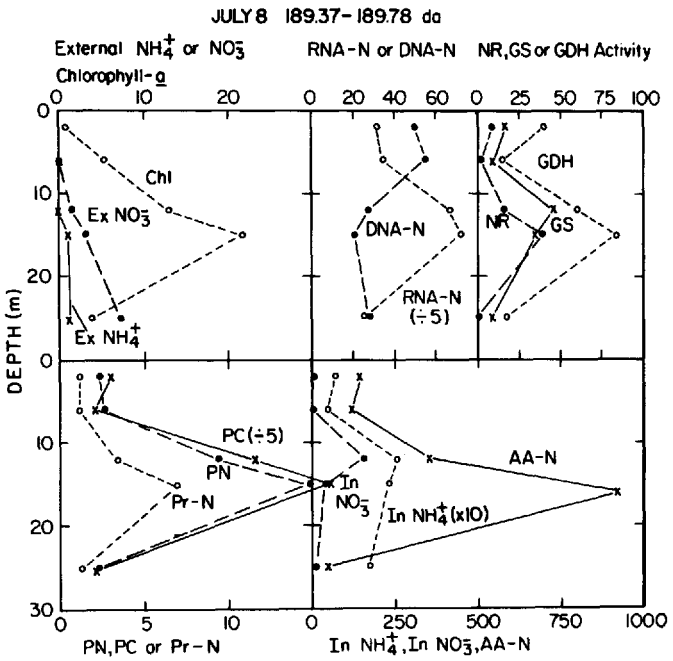

Figure 4. (Continued)

In addition to IN NO_3^-, it is the changes in ratios of compounds and enzyme activities that may be most useful in determining nutritional status and physiological state. Seasonal variation is shown in Figure 5 for the surface and deep layers, and the depth profile from the July cruise (MP-7), showing some of the most extreme values, is given in Figure 6. AA/Pr ratios were lowest early in the spring when light levels were low (Fig. 5), on the May cruise when the nitracline and pycnocline were very deep (Fig. 5), and at the greatest depth sampled in the water column (e.g., Fig. 6). Thus, the AA/Pr appears to be influenced by both light levels and N availability. However, at no time were values as low as those observed in severely N-deficient phytoplankton cultures (AA/Pr <2.5; Dortch *et al.*, 1984) and frequently they exceeded values typical of N-sufficient phytoplankton (AA/Pr >10; Dortch *et al.*, 1984). Similarly, the C/N ratios were quite low for all cruises and depths. Although the phytoplankton were not severely N deficient, high RNA/DNA and Pr/DNA, which indicate high growth rates, were only observed during the bloom ($\mu \simeq 1$ div/da; MP-5) and in the subsurface chlorophyll maximum ($\mu \simeq 3$ to 4 div/da) during the July cruise. The lowest RNA/DNA and Pr/DNA ratios, corresponding to growth rates as low as 0.3 div/da, occurred early in the spring (Fig. 5), after the bloom (Fig. 5, except the last cruise), and at depth in the water column (Fig. 6). Growth rates estimated from Pr/DNA ratios, according to Eq. (2) in Dortch *et al.* (1984), tended to be somewhat lower than those estimated from RNA/DNA ratios, according to Eq. (2) in Dortch *et al.* (1983).

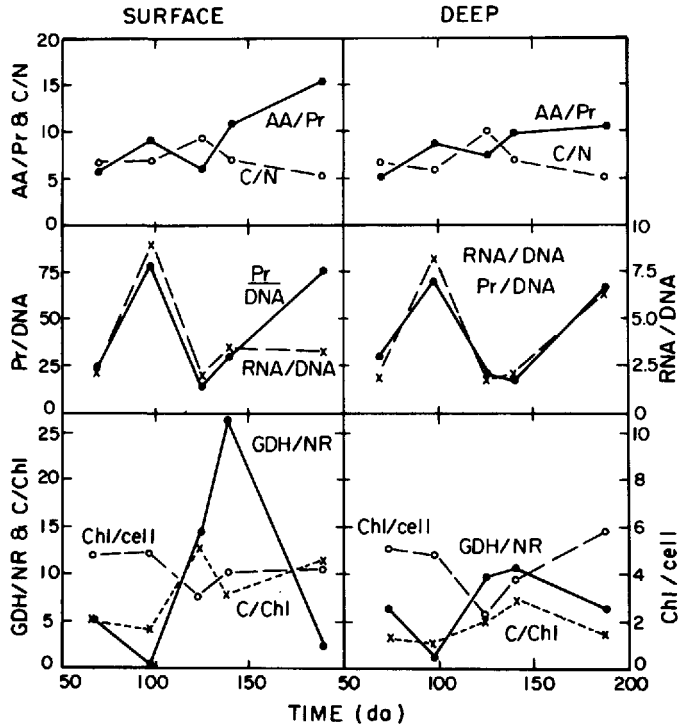

Figure 5. Seasonal variation in mean indices of physiological state and nutritional status. The depths corresponding to Surface and Deep are given in Table 1 or 2. Pr/DNA, RNA/DNA, and GDH/NR on a mole N basis, AA/Pr as % mole N, C/N by mole and C/Chl by mole C/g chlorophyll *a*, and Chl/cell as pg/cell.

Nevertheless, a linear relationship with a high correlation coefficient resulted from the regression of RNA/DNA vs PR/DNA ratios for all five cruises and all depths (Fig. 7). This provides further evidence of the usefulness of Pr/DNA ratios, which are easy to measure, compared with RNA/DNA ratios, which are more difficult to measure. The two points in Figure 7 corresponding to Pr/DNA > 300, but anomalously low RNA/DNA, occurred after the nitrate pulse on the July cruise and in both cases the RNA data are suspect. The C/chlorophyll *a* ratio (note that it is in mol C/g chl *a*) is included because it is a more familiar index. Values were lowest in the early spring and at depth, probably due to low light levels, and highest at the surface when external N concentrations were zero (see Hunter and Laws, 1981; Laws *et al.*, 1983). The highest NR activities were observed when nitrate was available in the water column: during the spring bloom (MP-5; Table 2), in the deep chlorophyll maximum (Fig. 4B,C), and in response to nitrate pulses (Figs. 8 and 9; to be discussed later); GDH activities were more variable, but often tended to be high when NR was low. The role of GDH in ammonium assimilation in general is unclear, but the highest GDH activities appeared

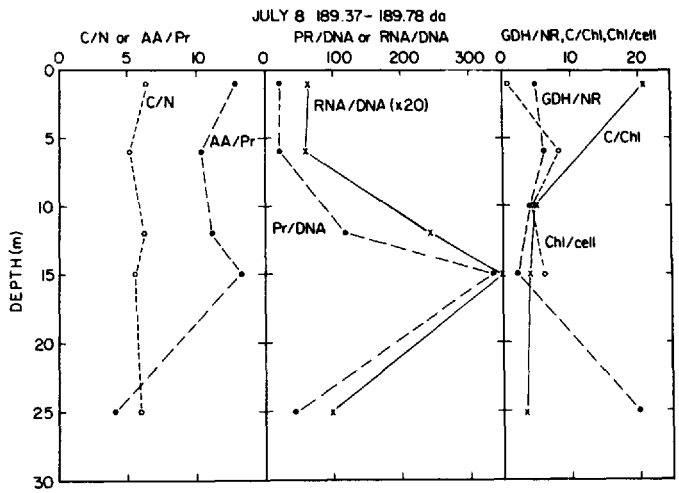

Figure 6. Depth profile of indicators of physiological state and nutritional status on July 8. Units as in Figure 5.

to occur where or when ammonium was probably the major N source, either because of nitrate depletion and the concomitant dependence on regeneration or because light levels were too low for efficient nitrate utilization. Thus, the GDH/NR ratio may be an indicator of the N source being used for growth. The increase in almost all the indicators on the last cruise was due to a nitrate pulse which occurred part way through the diel study (discussed in detail later, see Fig. 9).

Diel studies were conducted on four out of five cruises by sampling at two depths for longer than 24 hr. No diel periodicity was apparent that could be related specifically to light/dark cycles. However, considerable temporal variation did occur in all the

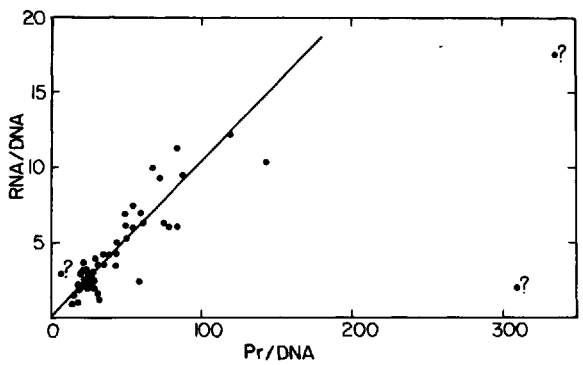

Figure 7. RNA/DNA vs. Pr/DNA (by mole N). Line described by geometric mean linear regression (Laws and Archie, 1981) of all data (except 3 points with ?): slope = 0.1035; y-intercept = 0.0550; $r^2 = 0.8168$; $n = 55$.

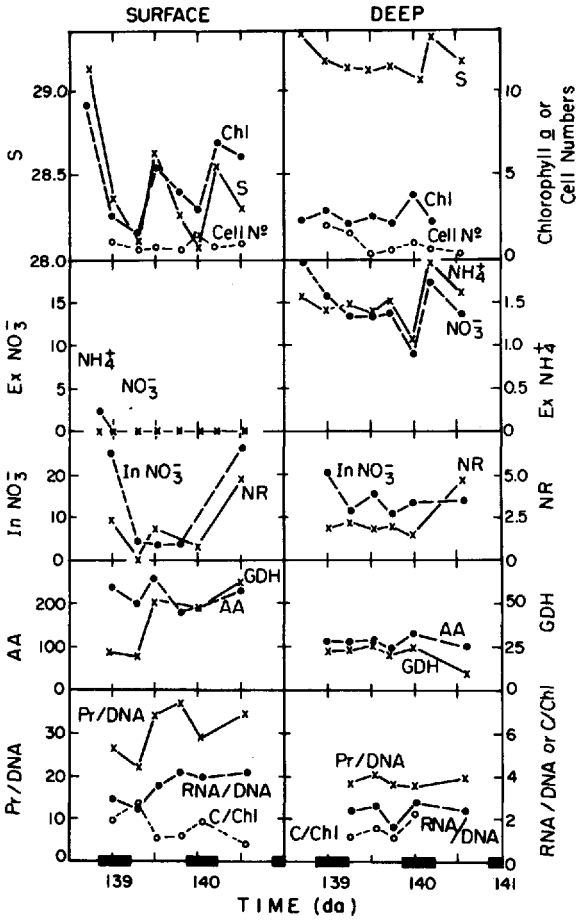

Figure 8. Results of diel study from May 18–20. Salinity expressed as ‰ and cell numbers as cells/ℓ seawater ($\times 10^6$). Other units as in Figure 4 and 5. Surface and Deep as in Table 1 or 2. Bars on abscissa indicate dark period.

measurements, especially in the surface layer on the last two cruises (Figs. 8 and 9). In both cases, the sampling depth was located just above the pycnocline/nitracline. Since it was not possible to put a reversing thermometer on the large volume sampler, σ_t data are not available for the diel studies. There was also a considerable salinity difference across the pycnocline, and, therefore, its variation can be used to indicate movements of the pycnocline.

During the late-May cruise (Fig. 8), salinity and chlorophyll varied in the surface layer with a mean period of approximately 9 hr. Although elevated, external nitrate concentrations occurred only once, some internal N concentrations, enzyme activities, and physiological indicators varied more frequently. For example, the minima and maxima of NR, GDH, AA and Pr/DNA in the surface layer correspond to the same in

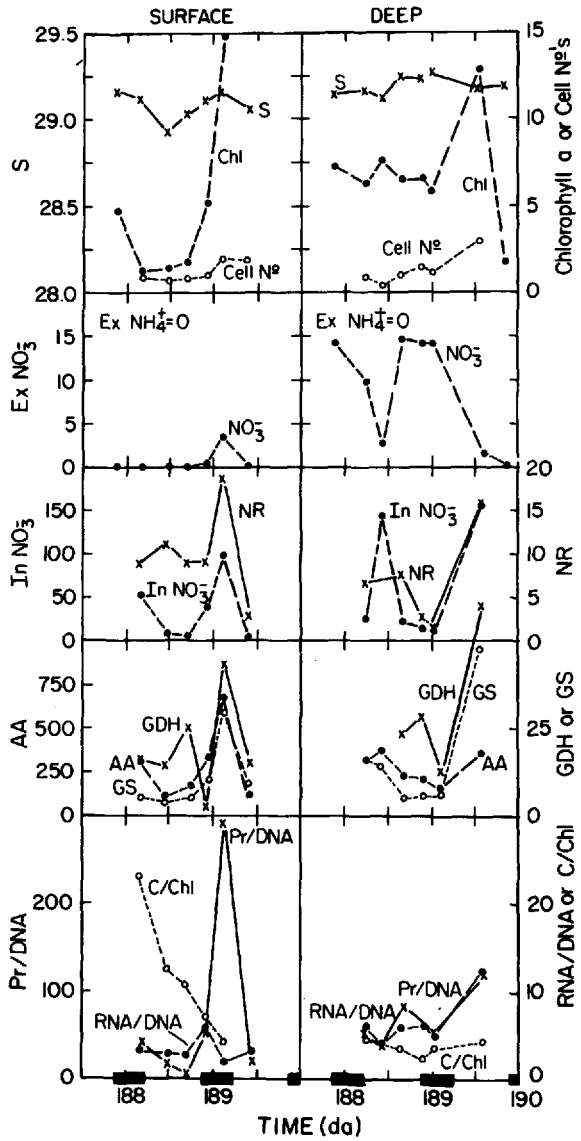

Figure 9. Results of diel study from July 6–9. Units as in Figure 8.

the salinity and chlorophyll distributions. Furthermore, corresponding changes in ambient N concentrations at depth suggested that nitrate, and perhaps ammonium, were available at a shallower depth in the surface layer but may have been rapidly removed by the phytoplankton.

On the July cruise, especially in the surface layer, even more extreme variation in biological parameters was observed (Fig. 9). In this case, the period could not be

determined, although it was clearly longer than on the May cruise (at least 29 hr). Furthermore, the second elevated concentration of nitrate in the surface layer was associated with the development of the large subsurface chlorophyll maximum, the largest intracellular N pools measured on these cruises, and high values of all indicators of physiological state. Since it was calm and overcast throughout, and the water column remained quite stratified, these major fluctuations cannot be attributed to sudden changes in the weather.

The possibility that temporal variations at one station could result from horizontal differences and tidal water movements was examined in a brief spatial survey. Four stations, located north and south of the main station (#5), were occupied within 2 hr and sampled for salinity, temperature, nutrients, and chlorophyll. Coefficients of variation (standard deviation/mean × 100) are compared in Table 3 for the temporal and spatial surveys. From this limited data set, there appeared to be as much horizontal as temporal variation. Furthermore, variations in salinity and σ_t were generally less than for nitrate and chlorophyll.

4. Discussion

a. Occurrence of nitrogen storage. A primary purpose of this study was to assess the importance of N storage in natural plankton populations. In N-sufficient cultures, some phytoplankton species can store enough excess N internally for up to 3 divisions without an external N supply (Dortch, 1982; Dortch *et al.*, 1984). This N is stored in the form of inorganic N, free amino acids, excess RNA, and excess protein (some associated with photosynthetic pigments and some not), which can be diverted to support growth if they are not needed for their primary biochemical function in N-deficient cells. It was hypothesized that if similar storage occurred in the field, it would allow phytoplankton to continue growing when the N supply was sporadic and/or potentially growth limiting.

Measuring concentrations of stored N in natural populations is not as straightforward as in cultures. For compounds such as IN NO_3^-, IN NH_4^+, and AA, which are primarily N storage compounds and are almost completely depleted in N-starved cells, the problem is minimal. But for compounds like Pr and RNA, which have many roles in a cell and are major constituents even in very N-starved organisms, it is much more difficult. In laboratory cultures, this problem is circumvented by calculating concentrations on a cell or cell volume basis. For example, after N is depleted from the medium, cell numbers continue to increase accompanied by a sharp drop in both Pr/cell and Pr/cell volume (Dortch, 1982; Dortch *et al.*, 1984). Obviously, growth is fueled by excess Pr, but there is not necessarily a change in either the ratio of Pr/PN or in the concentration of Pr expressed on a culture volume basis. Since it was not practical to measure total cell volumes on the cruises, Pr and RNA could not be normalized to cell volume. Consequently, it is impossible to determine the number of divisions which could be achieved with stored N for natural populations. However, it is

Table 3. Coefficient of variation (standard deviation/mean × 100) of samples collected at same depth during spatial and temporal surveys. Number in parentheses indicates number of times or locations sampled; blanks indicate no data was collected.

	Spatial survey					Temporal survey			
Cruise	Depth	Coefficient of variation				Depth	Coefficient of variation		
		S	σ_t	NO_3	Chl a		S	NO_3	Chl a
MP-3	5	0.80(5)	0.88(5)	2.24(5)	0.75(5)	5		6.30(6)	19.32(6)
	15	0.28(5)	0.30(5)	1.96(5)	27.53(5)	15		3.85(6)	22.22(6)
	25	0.31(5)	0.32(5)	0.81(5)	43.09(5)				
MP-4	3	0.20(5)	0.19(5)	0.00(5)	38.63(5)	3		114.21(5)	8.54(5)
	15	0.42(5)	0.48(5)	26.63(5)	19.62(5)	15		18.88(5)	22.85(5)
	25	0.14(5)	0.16(5)	1.51(5)	20.98(5)				
MP-5						3		0.00(3)	12.27(3)
						20		28.90(3)	12.64(3)
MP-6	3	0.94(5)	1.62(5)	0.00(5)	62.87(5)	6	0.82(6)	0.00(6)	45.93(6)
	9	0.41(5)	0.47(5)	23.09(5)	62.00(5)	15	0.29(6)	16.70(6)	27.44(6)
	25	0.09(5)	0.08(5)	6.95(5)	16.81(5)				
MP-7	3	0.06(5)	0.42(5)	0.00(5)	14.41(5)	6	0.49(6)	230.51(6)	119.14(5)
	12	0.15(5)	0.34(5)	199.24(5)	69.75(5)	12	0.32(6)	63.41(6)	35.09(6)
	25	0.08(5)	0.08(5)	3.71(5)	32.21(5)				

still possible to look at the patterns of stored N and the various ratios mentioned, and by comparison with appropriate laboratory data, assess the likely importance of N storage.

The seasonal pattern observed in Dabob Bay was quite different from that which was expected. With some variations (IN NO_3^-, Pr, and unaccounted-for-N), the amounts of stored N remained relatively high and constant instead of being high before the bloom and low afterwards. The unexpected low values could have occurred because low light and/or short daylength, typical of early spring, either prevented maximal N storage or altered the pattern of storage. Later in the season, although the surface layer was generally depleted of N, mechanisms for adding N must have been operating so that the phytoplankton never became N deficient (to be discussed later).

Both N uptake and assimilation are highly light dependent (reviewed in Collos and Slawyk, 1980; McCarthy, 1980; Syrett, 1981), but not to the same extent as photosynthesis. Consequently, we had initially expected that when light levels and growth rates were low, cells would be N replete. If not, perhaps more N storage occurs in the form of Pr in low light than under the saturating light intensities used in culture studies. While it is not possible to assess the amount of stored Pr (as opposed to total Pr) in the field, such a scenario would explain the somewhat low AA/Pr ratios and the very high Pr/PN ratios in the early spring. Furthermore, Perry *et al.* (1981) suggest that under low light/high N conditions, phytoplankton synthesize chlorophyll-Pr complexes which can be used for growth if N is in short supply and Morris *et al.* (1974) show that under low light, most ^{14}C that is recently taken up goes into Pr synthesis.

All of the intracellular N compounds which were detectable in cultures were also found in natural populations. This allays the concern that large intracellular pools measured in N-sufficient batch cultures (Dortch, 1982; Dortch *et al.*, 1983, 1984) were due only to the culture conditions and would not be typical of natural populations. Some, like IN NO_3^-, reached high concentrations only after recent exposure to nitrate. IN NH_4^+ on the other hand, was barely detectable throughout the season, although in culture some species can accumulate very high concentrations (Conover, 1975a; Dortch *et al.*, 1984). The fact that it was measurable at all still suggests that the populations were not N deficient. The increasing concentrations of unaccountable-N and decreasing %Pr/PN from spring to summer is somewhat puzzling. Small differences between ΣN and PN would not be surprising because of the approximations required in calculating Pr, AA, RNA, and DNA concentrations (Wheeler *et al.*, 1983; Dortch *et al.*, 1984). However, the large persistent decrease over the course of the season suggests that there may have been some unmeasured compound(s) which accumulated after the bloom rather than unavoidable errors in calculating the concentrations. Several classes of N compounds, lipid-N and chitin, which can account for between 0 and 23%, and 2 and 46% of the cellular N, respectively (Conover, 1975a), were not measured in our study and might account for some of the discrepancy. In cultures, such large quantities of unidentified N are found either in

N-sufficient cells of some species (Dortch *et al.*, 1984) or without regard for nutritional state (Conover, 1975a). Alternatively, the unidentified N could represent detritus which has accumulated after the bloom.

It is clearly evident from the data reported here that naturally occurring plankton populations store N. Whether phytoplankton also use it for growth can only be inferred since at no time were truly N-deficient phytoplankton encountered, even on the third cruise in early May when the nitracline was deepest and coincident with the 1% light depth. However, the fact that phytoplankton in the surface layer were not N-deficient, despite extremely low ambient N concentrations, implies they were obtaining N by some means. Many of the mechanisms which could be postulated to supply adequate N would be sporadic and phytoplankton would have to rely on internal N stores to maintain growth between N pulses.

b. Utility of indicators. One of the major questions facing biological oceanographers interested in phytoplankton dynamics is the determination of algal growth rates and the extent to which they are influenced by N supply in the field. A number of methods, which take advantage of physiological or chemical composition changes as cells become nutrient stressed, have been used to answer this question (e.g., Morris *et al.*, 1971; Yentsch *et al.*, 1977; Healey, 1979; Healey and Hendzel, 1979; Vincent, 1981a,b; Goldman and Dennett, 1983; Sakshaug *et al.*, 1983; Glibert and McCarthy, 1984). The results have been contradictory (Goldman *et al.*, 1979; Eppley, 1981), but the problems may be resolved as the indicators become better defined. Our studies of the biochemistry of N metabolism in phytoplankton has led us to propose indices which can answer the following specific questions about the role of N in phytoplankton dynamics (Dortch *et al.*, 1979, 1983, 1984; Dortch, 1982).

1) What is the degree of N deficiency? The AA/Pr ratio is by far the most effective indicator of N status since N deficient cells rapidly lose their free AA. It can be used in a manner similar to particulate C/N ratios, but it varies over a wider range and is less likely to have a large detrital component. A secondary indicator of N-deficiency is the internal inorganic N concentration. If cells contain any measurable nitrate or ammonium, they are probably N sufficient. However, the absence of inorganic N pools does not necessarily mean the cells are N deficient because some species do not accumulate significant amounts of either.

2) What is the N source used for growth? The presence of large IN NO_3^- and/or high NR indicates that phytoplankton are growing primarily on nitrate. As noted above, not all species can accumulate nitrate and there are a number of phytoplankton which have no measurable NR, even when growing on nitrate (Hersey and Swift, 1976; Dortch, unpubl.), so that their absence does not necessarily mean that nitrate is not important. Similarly, the presence of large ammonium pools is an indicator of growth on ammonium. Activities of ammonium assimilating enzymes may also indicate growth on ammonium, although, obviously, they must also be present when nitrate is

utilized. GDH is thought to be the primary ammonium assimilating enzyme when intracellular ammonium concentrations are high and GS the enzyme when they are low (Bressler, 1981; Syrett, 1981; Bressler and Ahmed, 1984).

3) What is the growth rate or physiological state (approximate growth rate)? Dortch *et al.* (1983) first proposed using RNA/DNA ratios as an indicator of plankton growth rates and obtained very good results (same field data as this study). However, even with improved analytical techniques (Thoresen *et al.*, 1983), it is a time-consuming analysis because RNA and DNA are chemically quite similar. To circumvent this problem, Dortch *et al.* (1984) proposed using the Pr/DNA ratio since (1) it is easier to measure analytically, (2) high RNA content is associated with rapid protein synthesis so the Pr/DNA ratio should substitute for the RNA/DNA ratio, and (3) Pr/DNA was correlated with growth rate in phytoplankton cultures (Dortch *et al.*, 1984). However, because it is not yet certain whether there is a general relationship between growth rate and either the RNA/DNA or Pr/DNA ratio which is applicable for all planktonic marine organisms, these ratios should probably be interpreted as general indicators of physiological state, rather than specific indicators of growth rate.

All of these indicators were helpful in elucidating the phytoplankton dynamics in Dabob Bay. Despite low ambient N concentrations on some cruises, the phytoplankton were not severely N-deficient at any time, as indicated by the generally high AA/Pr ratios and measurable IN NH_4^+ pools. The most nearly N-deficient phytoplankton occurred on the early May cruise (MP-5) and in the upper part of the water column on the last 2 cruises (MP-6 and MP-7). However, high Pr/DNA and RNA/DNA ratios, indicating high growth rates, were only obtained during the bloom (MP-4), in the subsurface chlorophyll maximum on the last 2 cruises, and after an input of nitrate into the surface layer on the last 2 cruises (MP-6 and MP-7). The low ratios on the first cruise may have resulted from low light or short daylength, although "spring" blooms can occur during the winter in Dabob Bay if conditions are suitable (Shuman, 1978). Similarly, low values were often observed at 25 m, presumably also due to low light. The low RNA/DNA and Pr/DNA ratios obtained later near the surface corresponded to the lowest AA/Pr ratios, suggesting that the availability of N was affecting growth rates if not causing severe N deficiency. This supports the hypothesis of Goldman *et al.* (1979) that even when ambient N concentrations are low, phytoplankton are rarely severely N limited, although the μ_{max} of organisms which predominate under those conditions may not be as high as under more favorable conditions. Nitrate was clearly an important N source, as indicated by elevated IN NO_3^- and NR activities at the times that RNA/DNA and Pr/DNA ratios were highest, suggesting that high growth rates were only achieved when nitrate was plentiful. It was surprising that GS was detectable only during the last cruise because other indicators showed that the most N-deficient phytoplankton were present in early May. The GDH/NR ratio was calculated to see if it would be a suitable index of growth on nitrate or ammonium.

While the results presented here suggest that it may be useful for this purpose, Dortch *et al.* (1979) found in culture studies that neither NR nor GDH activities correlated well with assimilation rates. Finally, the failure to observe severe N deficiency and the variations over time in many of the physiological measurements, indicate that Dabob Bay was a much more dynamic environment than originally expected.

A number of factors have been ignored in this analysis. (1) Phytoplankton N uptake also responds to N deficiency. Enhanced ammonium uptake (e.g., McCarthy and Goldman, 1979), decreased nitrate uptake (Dortch *et al.*, 1982), and alterations in the relative uptake of nitrate and ammonium (Dortch and Conway, 1984; Glibert and McCarthy, 1984) could all be used as indicators of N deficiency in addition to those mentioned herein. (2) The effect of variations in light intensity on these indicators is unknown. (3) The presence of bacteria, zooplankton, and detritus in the same samples with phytoplankton may complicate the interpretation of the results (Dortch *et al.*, 1983, 1984). However, the high AA/Pr ratios argue against a sizable detrital protein component. Only living cells contain free amino acids in such high concentrations and the maximum concentrations appear to be similar for phytoplankton, bacteria, and zooplankton (see Dortch *et al.*, 1984). If there had been a sizable contribution from detrital protein, the AA/Pr ratios, which were generally quite high for the particulate matter in Dabob Bay, would have been much lower. Since the factors affecting the proportion of detrital protein would be similar for detrital DNA and RNA, it also seems unlikely that a significant fraction of the DNA and RNA would be nonliving. Whether the presence of bacteria and small zooplankton interferes with the use of these biochemical indicators to explain phytoplankton dynamics depends on the relative biomass of phytoplankton and nonphytoplankton and on the variation of the indicators with environmental conditions in nonphytoplankton organisms. (4) The possibility that some other nutrient is growth limiting, resulting in low growth rates but relatively N-sufficient populations, was not examined. In an earlier study in Dabob Bay, phosphate and silicate concentrations remained nonlimiting although nitrate and ammonium were undetectable in the surface layers (Christensen, 1974). This does not rule out the availability of trace metals or vitamins as a factor.

While it is obvious that more research is required before these indices can be interpreted with complete confidence, the results presented here are promising. No single indicator appears to be adequate by itself because of possible species variations. However, several judiciously chosen indicators can be used to determine the degree of N deficiency, physiological state, and N sources used for growth.

c. Short-term variations. Considerable short-term (>4–6 hr and <30 hr) variation was observed both in the physical and chemical environment but especially in the biological parameters. Such variation was initially expected to be induced by the light/dark cycle, but no clear diel periodicity was apparent. Alternatively, Dabob Bay

may be physically quite dynamic, so that variation in the N supply can account for changes in biology.

Diel periodicity is often observed in many aspects of N utilization, particularly enzyme activities (reviewed in Collos and Slawyk, 1980; McCarthy, 1980; Syrett, 1981). However, the amplitude of the variation is rarely as great for N utilization as it is for C uptake and assimilation, the intensity and timing can vary considerably between species, and the periodicity tends to disappear when cells are N deficient. For example, in three studies where IN NO_3^- was measured over the course of one or more diel cycles, maximum concentrations were observed at night at the highest growth rate only (Malone *et al.*, 1975), sometimes, but not always at night (Picard, 1976), and only during the day (Collos and Slawyk, 1976). Recently, Olson and Chisholm (1983) and Wheeler *et al.* (1983) demonstrated that the timing of N uptake, assimilation, and cell division and their relationship to the light/dark cycle and sporadic input of N can be quite species-dependent. Thus, in a natural population, which consists of a mixture of species, which is dependent on a sporadic supply of N, and which is neither N deficient nor growing extremely rapidly with excess external N, it is not surprising that diel periodicity was not readily apparent.

A more reasonable explanation for the variability in biological parameters, particularly in the surface layer, is that N is re-supplied sporadically. One possible mechanism for this resupply was observed on two cruises. Near the nitracline/pycnocline, a small change in density (indicated by salinity) can mean a significant change in nitrate concentration, which can stimulate nitrate assimilation and phytoplankton growth. Similar changes have been observed in other highly stratified coastal areas as a result of tidally generated internal waves (Herman and Denman, 1979; Cullen *et al.*, 1983). Even if it only causes temporary vertical displacement of the pycnocline, it increases the average light level experienced by phytoplankton populations which may have adequate nitrate but inadequate light. If, at the same time, some of the higher nutrient water is injected into the surface layer, even greater stimulation of phytoplankton growth can occur. Our data were not collected to examine these questions, but it appears that during the July cruise (MP-7; Fig. 9), a combination of both events occurred, stimulating considerable phytoplankton growth at depth. In an earlier study, Ebbesmeyer (1973) documented the formation of discrete, identifiable water parcels in Dabob Bay that might account for both the temporal and horizontal variation that was observed. Furthermore, in attempting to construct an N budget for Dabob Bay, Copping (1982) was unable to balance the loss of N from the euphotic zone by a combination of reasonable diffusion, advection, and excretion rates, particularly during the spring, suggesting that there must be other mechanisms for adding N to the euphotic zone. Finally, in other years large scale mixing events have been observed during the late spring and summer during which the highly stratified, N depleted surface layer is turned over and extremely high nutrient concentrations are found at the surface (Christensen, 1974; Shuman, 1978). Besides the possible injection of

nitrate by these mechanisms, McCarthy and Goldman (1979) hypothesized that ammonium may also be supplied sporadically by zooplankton excretion. Thus, there are a number of ways to explain the short-term variability and the lack of severe N deficiency.

5. Conclusion

Natural plankton populations contain compounds that indicate they are capable of storing excess N. The concentrations and ratios of cellular N compounds and the activities of N assimilating enzymes can be used to determine the degree of N deficiency, physiological state (approximate growth rate), and major N sources for growth. In Dabob Bay, phytoplankton were not severely N deficient, despite generally low ambient concentrations in the surface layer, although growth rates were only high when external nitrate was abundant. Nitrate was supplied sporadically to the euphotic zone with a periodicity ranging from 9 hr to 1 day. This, combined with ammonium regeneration could explain the lack of N deficiency. Furthermore, it suggests that in coastal areas nitrate may be a more important N source in N-depleted waters than previously considered.

Acknowledgments. This research was supported primarily by NSF grants OCE 79-1959-1 and OCE 81-17937-2 awarded to S. I. Ahmed, and in part by NSF grant OCE 82-19746 awarded to Q. Dortch. We would like to thank the crew of the R/V *Onar*, P. Sparks, T. Roberts, M. Stein and A. Ahmed for their help on the cruises, J. Rollins for drafting and P. Boisvert for typing the manuscript. We appreciate the very helpful comments of Dr. Paul J. Harrison and three unknown reviewers. This is contribution no. 1424 from the School of Oceanography, University of Washington, and contribution no. 84006 from Bigelow Laboratory.

REFERENCES

Ahmed, S. I., R. A. Kenner and F. D. King. 1976. Preservation of enzymatic activity in marine plankton by low-temperature freezing. Mar. Chem., *4*, 133–139.

Ahmed, S. I., R. A. Kenner and T. T. Packard. 1977. A comparative study of glutamate dehydrogenase activity in several species of marine phytoplankton. Mar. Biol., *39*, 93–101.

Bates, S. S. 1976. Effects of light and ammonium on nitrate uptake by two species of estuarine phytoplankton. Limnol. Oceanogr., *21*, 212–218.

Blasco, D. and H. L. Conway. 1982. Effect of ammonium on the regulation of nitrate assimilation in natural phytoplankton populations. J. Exp. Mar. Biol. Ecol., *61*, 157–168.

Blasco, D., J. J. MacIsaac, T. T. Packard and R. C. Dugdale. 1984. Relationship between nitrate reductase and nitrate uptake in phytoplankton in the Peru upwelling region. Limnol. Oceanogr., *29*, 275–286.

Blasco, D. and T. T. Packard. 1974. Nitrate reductase measurements in upwelling regions. I. Significance of the distribution off Baja California and Northwest Africa. Tethys, *6*, 239–246.

Bressler, S. L. 1981. Nitrogen assimilation in marine phytoplankton: detection and optimization of glutamine synthetase, a key biosynthetic enzyme. M.S. thesis, University of Washington, 80 pp.

Bressler, S. L. and S. I. Ahmed. 1984. Detection of glutamine synthetase activity in marine phytoplankton: optimization of the biosynthetic assay. Mar. Ecol. Prog. Ser., *14*, 207–217.

Christensen, J. P. 1974. An oxygen budget for the deep waters of Dabob Bay using respiration rates estimated from plankton electron transport activities. M.S. thesis, University of Washington, 237 pp.

Clayton, J. R., Jr. 1985. Nitrogen metabolism as a function of its availability in the marine diatom *Skeletonema costatum*. Ph.D. dissertation, University of Washington (in preparation).

Collos, Y. 1982. Transient situations in nitrate assimilation by marine diatoms. III. Short-term uncoupling of nitrate uptake and reduction. J. Exp. Mar. Biol. Ecol., *62*, 285–295.

Collos, Y. and G. Slawyk. 1976. Significance of cellular nitrate content in natural populations of marine phytoplankton growing in shipboard culture. Mar. Biol., *34*, 27–32.

—— 1977. Nitrate reductase activities as a function of *in situ* nitrate uptake and environmental factors of euphotic zone profiles. J. Exp. Mar. Biol. Ecol., *29*, 119–130.

—— 1980. Nitrogen uptake and assimilation by marine phytoplankton, *in* Primary Productivity in the Sea, P. G. Falkowski, ed., Plenum Press, NY, 195–211.

Conover, S. A. M. 1975a. Partitioning of nitrogen and carbon in cultures of the marine diatom *Thalassiosira fluviatilis* supplied with nitrate, ammonium, or urea. Mar. Biol., *32*, 231–246.

—— 1975b. Nitrogen utilization during spring blooms of marine phytoplankton in Bedford Basin, Nova Scotia, Canada. Mar. Biol., *32*, 247–262.

Copping, A. E. 1982. The distribution and passage of organic matter in the marine food web, using nitrogen as a tracer. Ph.D. dissertation, University of Washington, 155 pp.

Cullen, J. J., E. Stewart, E. Renger, R. W. Eppley and C. D. Winant. 1983. Vertical motion of the thermocline, nitracline, and chlorophyll maximum layers in relation to currents on the Southern California Shelf. J. Mar. Res., *41*, 239–262.

Devol, A. H. and Q. Dortch. 1981. Nitrate reductase activity in Lake Washington plankton. Verh. Internat. Verein. Limnol., *21*, 320–325.

Dortch, Q. 1980. Nitrate and ammonium uptake and assimilation by three marine diatoms. Ph.D. dissertation, University of Washington, 299 pp.

—— 1982. Effect of growth conditions on accumulation of internal pools of nitrate, ammonium, amino acids and protein in three marine diatoms. J. Exp. Mar. Biol. Ecol., *61*, 243–264.

Dortch, Q., S. I. Ahmed and T. T. Packard. 1979. Nitrate reductase and glutamate dehydrogenase activities in *Skeletonema costatum* as measures of nitrogen assimilation rates. J. Plankton Res., *1*, 169–186.

Dortch, Q., J. R. Clayton, Jr., S. S. Thoresen and S. I. Ahmed. 1984. Species differences in accumulation of nitrogen pools in phytoplankton. Mar. Biol., *81*, 237–250.

Dortch, Q., J. R. Clayton, Jr., S. S. Thoresen, S. L. Bressler and S. I. Ahmed. 1982. Response of marine phytoplankton to nitrogen deficiency: decreased nitrate uptake vs. enhanced ammonium uptake. Mar. Biol., *70*, 13–19.

Dortch, Q. and H. L. Conway. 1984. Interactions between nitrate and ammonium uptake: variations with growth rate, nitrogen source and species. Mar. Biol., *79*, 151–164.

Dortch, Q. and H. Maske. 1982. Dark uptake of nitrate and nitrate reductase activity of a red-tide population off Peru. Mar. Ecol. Prog. Ser., *9*, 299–303.

Dortch, Q., T. L. Roberts, J. R. Clayton, Jr. and S. I. Ahmed. 1983. RNA/DNA ratios and DNA concentrations as indicators of growth rate and biomass in planktonic marine organisms. Mar. Ecol. Prog. Ser., *13*, 61–71.

Ebbesmeyer, C. C. 1973. Some observations of medium scale water parcels in a fjord: Dabob Bay, WA. Ph.D. thesis, University of Washington, 213 pp.

Eppley, R. W. 1981. Relations between nutrient assimilation and growth in phytoplankton with

a brief review of estimates of growth rate in the ocean. Can. J. Bull. Fish. Aquat. Sci., *210,* 251–263.

Eppley, R. W., J. L. Coatsworth and L. Solorzano. 1969. Studies of nitrate reductase in marine phytoplankton. Limnol. Oceanogr., *14,* 194–205.

Eppley, R. W., T. T. Packard and J. J. MacIsaac. 1970. Nitrate reductase in Peru Current phytoplankton. Mar. Biol., *6,* 195–199.

Eppley, R. W. and E. H. Renger. 1974. Nitrogen assimilation of an oceanic diatom in nitrogen-limited continuous culture. J. Phycol., *10,* 15–23.

Friederich, G. E. and T. E. Whitledge. 1972. Autoanalyzer procedures for nutrients, *in* Phytoplankton Growth Dynamics, Tech. Ser. 1, Chemostat Methodology and Chemical Analysis, S. P. Pavlou, ed., Spec. Rep. Dept. Oceanogr., University of Washington, *52,* 38–55.

Glibert, P. M. and J. J. McCarthy. 1984. Uptake and assimilation of ammonium and nitrate by phytoplankton: indices of nutritional status for natural assemblages. J. Plankton Res. *6,* 677–697.

Goldman, J. C. and M. R. Dennett. 1983. Effect of nitrogen source on short-term light and dark CO_2 uptake by a marine diatom. Mar. Biol. *76,* 7–15.

Goldman, J. C., J. J. McCarthy and D. G. Peavey. 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. Nature, *279,* 210–215.

Harrison, W. G. 1973. Nitrate reductase activity during a dinoflagellate bloom. Limnol. Oceanogr., *18,* 457–465.

Healey, F. P. 1979. Short-term responses of nutrient-deficient algae to nutrient addition. J. Phycol., *15,* 289–299.

Healey, F. P. and L. L. Hendzel. 1979. Indicators of phosphorus and nitrogen deficiency in five algae in culture. J. Fish. Res. Bd. Can., *36,* 1364–1369.

Herman, A. W. and K. L. Denman. 1979. Intrusions and vertical mixing at the shelf/slope water front south of Nova Scotia. J. Fish. Res. Bd. Can., *36,* 1445–1453.

Hersey, R. L. and E. Swift. 1976. Nitrate reductase activity of *Amphidinium carterae* and *Cachonina niei* (Dinophyceae) in batch cultures: diel periodicity and effects of light intensity and ammonia. J. Phycol., *12,* 29–35.

Hunter, B. L. and E. A. Laws. 1981. ATP and chlorophyll *a* as estimators of phytoplankton carbon biomass. Limnol. Oceanogr., *26,* 944–956.

Jeffrey, S. W. and G. F. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls *a*, *b*, *c*, and c_2 in higher plants, algae, and natural phytoplankton. Biochem. Physiol. Pflanzen, *167,* 191–194.

Kollmeyer, R. C. 1965. Water properties and circulation in Dabob Bay Autumn 1962. M.S. thesis, University of Washington, 111 pp.

Laws, E. A. and J. W. Archie. 1981. Appropriate use of regression analysis in marine biology. Mar. Biol., *65,* 13–16.

Laws, E. A., D. M. Karl, D. G. Redalje, R. S. Jurick and C. D. Winn. 1983. Variability in ratios of phytoplankton carbon and RNA to ATP and chlorophyll *a* in batch and continuous cultures. J. Phycol., *19,* 439–445.

Li, W. K. W., D. V. Subbao Rao, W. G. Harrison, J. C. Smith, J. J. Cullen, B. Irwin and T. Platt. 1983. Autotrophic picoplankton in the tropical ocean. Science, *219,* 292–295.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem., *193,* 265–275.

Malone, T. C., C. Gorside, K. C. Haines and O. A. Roels. 1975. Nitrate uptake and growth of *Chaetoceros* sp. in large outdoor continuous cultures. Limnol. Oceanogr., *20,* 9–19.

McCarthy, J. J. 1980. Nitrogen, *in* The Physiological Ecology of Phytoplankton, I. Morris, ed., University of California Press, Berkeley, 191–233.

McCarthy, J. J. and J. C. Goldman. 1979. Nitrogenous nutrition of marine phytoplankton in nutrient depleted waters. Science, *203,* 670–672.

Millero, F. J. and A. Poisson. 1981. International one-atmosphere equation of state of seawater. Deep-Sea Res., *28A,* 625–629.

Morris, I., H. E. Glover and C. S. Yentsch. 1974. Products of photosynthesis by marine phytoplankton: the effect of environmental factors on the relative rates of protein synthesis. Mar. Biol., *27,* 1–9.

Morris, I., M. J. McCartney and G. A. Robinson. 1983. Studies of a spring phytoplankton bloom in an enclosed experimental ecosystem. I. Biochemical changes in relation to the nutrient chemistry of water. J. Exp. Mar. Biol. Ecol., *70,* 249–262.

Morris, I., C. M. Yentsch and C. S. Yentsch. 1971. Physiological state with respect to N of phytoplankton from low nutrient subtropical water as measured by effect of NH_4^+ on dark CO_2 fixation. Limnol. Oceanogr., *16,* 859–868.

Murphy, L. S. and E. Haugen. 1985. The distribution and abundance of phototrophic ultraplankton in the North Atlantic. Limnol. Oceanogr., *30,* 47–58.

Olson, R. J. and S. W. Chisholm. 1983. Effects of photocycles and periodic ammonium supply on three marine phytoplankton species. I. Cell division patterns. J. Phycol., *19,* 522–528.

Packard, T. T. and D. Blasco. 1974. Nitrate reductase activity in upwelling regions: 2. Ammonia and light dependence. Tethys, *6,* 269–280.

Packard, T. T., D. Blasco, J. J. MacIsaac and R. C. Dugdale. 1971. Variations of nitrate reductase activity in marine phytoplankton. Invest. Pesq., *35,* 209–219.

Packard, T. T., M. L. Healy and F. A. Richards. 1971. Vertical distribution of the activity of the respiratory electron transport system in marine plankton. Limnol. Oceanogr., *16,* 60–70.

Paquette, R. G. 1958. A modification of the Wenner-Smith-Soule salinity bridge for determination of salinity in seawater. University of Washington, Dept. Oceanogr., Ref. 59-14.

Perry, M. J., M. C. Talbot and R. S. Alberte. 1981. Photoadaptation in marine phytoplankton: response of the photosynthetic unit. Mar. Biol., *62,* 91–101.

Picard, G. A. 1976. Effects of light and dark cycles on the relationship between nitrate uptake and cell growth rates of *Chaetoceros* sp. (STX-105) in continuous culture. Ph.D. dissertation, City University of New York, 220 pp.

Prasad, A. S., E. DuMouchelle, D. Donvich and T. Oberleas. 1972. A simple fluorometric method for the determination of RNA and DNA in tissues. J. Lab. Clin. Med., *80,* 598–602.

Sakshaug, E., K. Andresin, S. Nyklestad and Y. Olsen. 1983. Nutrient status of phytoplankton communities in Norwegian waters (marine, brackish, and fresh) as revealed by their chemical composition. J. Plankton Res., *5,* 175–196.

Shuman, F. R. 1978. The fate of phytoplankton chlorophyll in the euphotic zone—Washington coastal waters. Ph.D. dissertation, University of Washington, 238 pp.

Sverdrup, H. U., M. W. Johnson and R. H. Fleming. 1942. The Oceans. Prentice Hall, Englewood Cliffs, 1087 pp.

Syrett, P. J. 1981. Nitrogen metabolism of microalgae. Can. Bull. Fish. Aquat. Sci., *210,* 182–210.

Terry, K. L. 1982. Nitrate uptake and assimilation in *Thalassiosira weissflogii* and *Phaeodactylum tricornutum:* interactions with photosynthesis and with the uptake of other ions. Mar. Biol., *69,* 21–30.

Thoresen, S. S., J. R. Clayton, Jr., Q. Dortch and S. I. Ahmed. 1983. A rapid technique for the determination of RNA and DNA in marine phytoplankton. J. Plankton Res., *5,* 253–261.

Thoresen, S. S., Q. Dortch and S. I. Ahmed. 1982. Comparison of methods for extracting intracellular pools of inorganic nitrogen from marine phytoplankton. J. Plankton Res., *4,* 695–704.

Udenfriend, S., S. Stein, P. Böhler, W. Dairman, W. Leimgruber and M. Weigle. 1972.

Fluorescamine: a reagent for assay of amino acids, peptides, proteins, and primary amines in the picomole range. Science, *178,* 871–873.

Utermöhl, H. 1958. Zur vervollkommung der quantitativen phytoplanktonmethodik. Mitt. Int. Ver. Limnol., No. 9, 38 pp.

Vincent, W. F. 1981a. Rapid physiological assays for nutrient demand by plankton. I. Nitrogen. J. Plankton Res. *3,* 685–697.

—— 1981b. Rapid physiological assays for nutrient demand by plankton. I. Phosphorus. J. Plankton Res., *3,* 699–710.

Wheeler, P. A., R. J. Olson and S. W. Chisholm. 1983. Effects of photocycles and periodic ammonium supply on three marine phytoplankton species. II. Ammonium uptake and assimilation. J. Phycol., *19,* 528–533.

Yentsch, C. M., C. S. Yentsch and L. R. Strube. 1977. Variations in ammonium enhancement as indication of nitrogen deficiency in New England coastal phytoplankton populations. J. Mar. Res., *34,* 537–555.

Young, A. W., R. W. Buddemeier and A. W. Fairhall. 1969. A new 60-liter water sampler built from a beer keg. Limnol. Oceanogr., *14,* 634–637.

Received: *22 May, 1984;* revised: *18 January, 1985.*