observed a one-to-one ratio of DMA to DM′A in normal adults, in agreement with the findings of Kakimoto and Akazawa (*8*) and Carnegie *et al.* (*9*). The ratio of DMA to DM′A changes much more in muscular dystrophies than in liver diseases (*9*). This change, which may relate directly to the etiology of this disease, could, perhaps, be used as a marker for muscular dystrophies and thus provide a means for earlier or preclinical diagnosis of this disease.

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Nitrogenous Nutrition of Marine Phytoplankton in Nutrient-Depleted Waters

Abstract. *Variability in the small-scale temporal and spatial patterns in nitrogenous nutrient supply, coupled with an enhanced uptake capability for nitrogenous nutrients induced by nitrogen limitation, make it possible for phytoplankton to maintain nearly maximum rates of growth at media nutrient concentrations that cannot be quantified with existing analytical techniques.*

Several investigators have described the role that nitrogen plays in regulating the growth of natural populations and laboratory cultures of marine phytoplankton. The rates by which phytoplankton assimilate nitrogen and grow can reasonably be approximated by rectangular hyperbolic functions of either the aqueous (external) nitrogenous nutrient concentration (*1*–*3*) or the cellular nitrogen content (cell quota) (*4*, *5*), or both, depending upon growth conditions. Similar results have been demonstrated for other nutrients under limiting conditions (*6*). Goldman (*7*) recently showed that under steady-state conditions in continuous cultures phytoplankton growth rates and nutrient uptake rates were coupled and could be described simultaneously by external and cellular nutrient concentrations.

In natural marine environments, particularly in impoverished oceanic waters, steady-state conditions for bacterial or phytoplankton nutrition are seldom, if ever, established (*8*), partly because the temporal and spatial patterns in the fluxes of required nutrients into and within the euphotic zone are highly episodic. In several recent studies nitrogenous nutrients in the near-surface waters of the North Atlantic and adjacent seas have been below the limit of detectability, even though considerable photosynthetic activity was noted (*9*, *10*). The absence of measurable concentrations of nitrogenous nutrients in the environment of physiologically active phytoplankton raises the perplexing but crucial question of how nitrogen is made available and utilized by marine phytoplankton in these oceanic waters.

From recent laboratory studies (*11*, *12*) it is evident that, under conditions of nitrogen starvation and during time intervals that are short relative to cell generation periods, the nitrogen uptake rates of marine phytoplankton can exceed the nitrogen uptake rates required for population growth. The major implication of these findings is that a phytoplankton cell need only be exposed to intermittent pulses of nitrogen in order to acquire its daily ration of this nutrient. Such situations conceivably could exist when cells randomly and perhaps frequently pass into microenvironments in which nitrogenous nutrient concentrations are elevated as a result of either metabolic waste excretion by animals or the degradation of organic matter by bacteria. This phenomenon has been addressed conceptually (*13*), but we believe that its significance in the open ocean has not been fully realized because the critical response period of the phytoplankton to nutrient pulses is on the order of minutes rather than tens to hundreds of minutes (*12*). This hypothesis would be consistent with our recent observations that, in oceanic waters where dissolved nitrogenous nutrients were undetectable, rates of primary productivity were measurable.

We explored this question by assessing the short-term nitrogen uptake capabilities of two clones of the marine diatom *Thalassiosira pseudonana* in NH_4^+-limited continuous cultures. Clone 3H was originally isolated from Long Island Sound, and clone 13-1 was from the Sargasso Sea (*14*). Steady-state levels of biomass were first established at different growth rates, and then short-term uptake experiments with added $^{15}NH_4^+$ were carried out immediately, 20 minutes after, and 1 hour after the nutrient supply pump was stopped. Complete details of the continuous culture design and function, culturing protocols, and

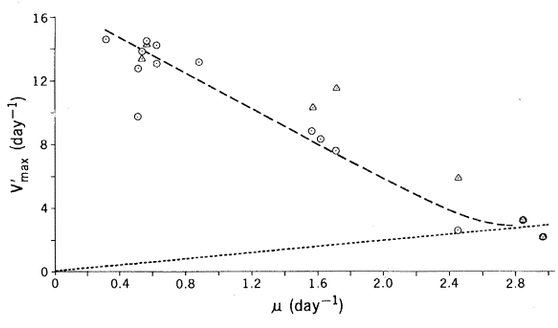

Fig. 1. Maximum rate of NH_4^+ uptake per unit of plant nitrogen (V'_{max}) at specific growth rates (μ) for *Thalassiosira pseudonana* (clone 3H) grown in a NH_4^+-limited chemostat. The circles represent rates determined immediately and 20 minutes after the nutrient supply ceased. The triangles represent rates determined 120 minutes after the nutrient supply ceased. Rates of NH_4^+ uptake adequate to meet the requirements of growth are depicted by the dotted line.

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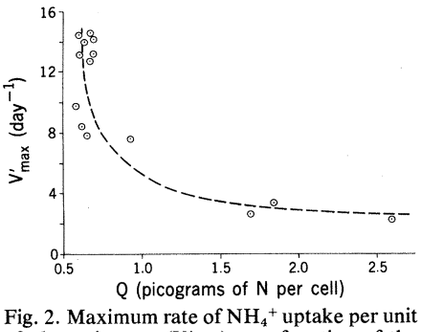

Fig. 2. Maximum rate of NH_4^+ uptake per unit of plant nitrogen (V'_{max}) as a function of the cellular nitrogen content (Q) of *Thalassiosira pseudonana* (clone 3H). Measurements of cellular nitrogen were made at steady-state conditions (*5*).

steady-state measurements are presented elsewhere (*5*).

We carried out the uptake experiments on 50-ml portions of culture exposed to 8- or 16 μg-atom of $^{15}NH_4^+$ per liter during 5-minute incubations at the same temperature (18°C) and light intensity (0.05 to 0.07 langley per minute) used for the continuous cultures (*15*). We terminated the uptake experiments by filtering the samples onto glass-fiber filters which were dried and processed for ^{15}N enrichment by mass spectroscopic analysis (*16*). Dissolved NH_4^+ was measured in the culture media prior to each incubation experiment (*5*).

For the coastal clone, clone 3H, the maximum specific uptake rate of NH_4^+ at a specific growth rate of about 0.5 cellular doubling per day (17 percent of the maximum growth rate μ_{max}) was approximately equivalent to the amount of NH_4^+ that would be needed for 15.5 cellular doublings per day (Fig. 1). Thus under conditions of severe nitrogen starvation (*17*) this alga has the capacity to assimilate NH_4^+ at a rate that is 30 times its steady-state rate of cell replication. Clone 3H needs to be exposed to a saturating NH_4^+ concentration during only about 3.0 to 3.5 percent of a doubling period at this growth rate in order to completely meet its demand for nitrogen during the doubling period (*18*). The enhanced maximum NH_4^+ uptake capacity decreases with increasing growth rate, and at μ_{max} the two must be equal (Fig. 1). We could not discern any measurable change in NH_4^+ uptake capacity when the cultures were deprived of nutrients for 20 minutes prior to the time when the uptake rates were measured. After a delay of 120 minutes, however, additional enhancement of the NH_4^+ uptake capacity was noted at intermediate growth rates (Fig. 1).

At the steady state, the growth rates of clone 3H were described as a function of the nitrogen cell quota, Q (*5*); hence, a more general depiction of enhanced NH_4^+ uptake capability under conditions of nitrogen deprivation can be seen when the maximum NH_4^+ uptake rate is plotted against the steady-state Q (Fig. 2) (*19*). Significant enhancement of the NH_4^+ uptake capacity occurs at low Q values (the region of nitrogen limitation) and decreases to a minimum at the maximum Q value. This upper limit in Q corresponds to the maximum growth rate and represents a region of nonnutrient limitation (*5*). If the rate of population cellular doubling during the 120-minute period of nutrient deprivation prior to NH_4^+ addition was approximately that observed for the steady-state culture at the initiation of the experiment, the corresponding change in Q would be sufficient to account for most of the elevation in the uptake data determined after the 120-minute period (Fig. 1) (*20*). We have defined V'_{max} to be a measure of potential population growth in terms of the nitrogen content of the population. The data in Figs. 1 and 2 can be combined to examine uptake capability per cell as a function of nutrition state, ρ_{max} (*13*). This parameter also increases with increasing nutrient deprivation but not as dramatically as V'_{max}.

Clearly, the enhanced V'_{max} represents uptake that is uncoupled from growth as approximated by the rate of cellular doubling. For a given period of rapid short-term uptake, there must be a coincident and corresponding increase in the amount of nitrogen per cell, and for a steady-state continuous culture population the rate of nitrogenous nutrient uptake must equal the rate of cellular synthesis of organic nitrogen. The rapid increase in the nitrogen per cell arising from a manifestation of V'_{max} in response to a pulse of nutrient results in substantial internal pooling of nutrient. Research is needed in which Q is partitioned into nutrient storage and active metabolic pools.

With the oceanic clone of *T. pseudonana*, clone 13-1, we observed the same enhanced maximum specific NH_4^+ uptake capacity with the increasing severity of nitrogen limitation (Table 1). At 26 percent of the maximum growth rate, the degree of enhancement in V'_{max} was similar to that observed for clone 3H but, when the uptake experiment was delayed for 20 minutes after nutrient delivery ceased, further enhancement in V'_{max} was noted. That only the oceanic clone in our experiments was capable of rapidly adjusting its nutrient uptake capabilities in response to nutrient deprivation is consistent with the hypothesis

Table 1. Ratios of V'_{max} to μ for *Thalassiosira pseudonana* (clone 13-1) determined at three growth rates (μ)(immediately, 20 minutes after, and 120 minutes after nutrient delivery ceased). The maximum growth rate was 1.01 cellular doublings per day.

Time after nutrient delivery (minutes)	μ (doublings per day)		
	0.26	0.60	0.82
0	15.4	4.0	1.1
20	25.7	6.7	1.1
120	33.9	8.2	1.4

that oceanic species, residing in nutrient-impoverished environments, have evolved higher biochemical potential to exploit any quantity of nitrogenous nutrient that might be present (*21*). Such differences in uptake potential may be a major factor determining the geographic distribution patterns for clones of single cosmopolitan species such as *T. pseudonana*, as well as for separate species; however, more research is required to establish firmly the role of clonal variations in the phytoplankton speciation.

Historically, emphasis has been placed on comparing ambient concentrations of nutrients in natural waters with corresponding kinetic constants for nutrient uptake determined in laboratory cultures. These comparisons have served as the basis for estimates of the degree and form of nutrient limitation to be expected in nature. For example, it has been suggested that nitrogen is the limiting nutrient in oceanic waters because both ambient concentrations and phytoplankton kinetic constants for NO_3^- and NH_4^+ uptake have been found to be similar (*22*).

The accuracy of such nutrient data should now be questioned. Through refinements in analytical techniques used at sea, more accurate measurements of nitrogenous nutrients have recently become possible. We now have evidence that NO_3^-, NO_2^-, NH_4^+, and urea concentrations in the upper mixed layer of at least the North Atlantic are frequently less than 0.03 μg-atom/liter, the lower limit of detectability (*9, 10*). Corroborating, albeit indirect, evidence demonstrates a similar absence of nitrogen in the North Pacific (*23*). We believe a major reason for the higher values reported earlier is that the sampling and preservation techniques commonly used aboard ship (in cases where nutrient analyses are delayed until samples reach shore-based laboratories) were inadequate to avoid sample contamination (*9, 23*). For example, when shipboard analyses or biological uptake responses have sug-

gested an absence of measurable nitrogenous nutrient, shore-based analyses of stored portions from the same samples were found to contain measurable concentrations (*9, 23*). Data from a recent study (*24*) on a freshwater green alga, *Selanastrum capricornutum*, grown under nitrogen limitation were related to earlier data for oceanic nutrient concentrations; the conclusion was drawn that nitrogen should not be considered as a primary limiting nutrient in oceanic waters. Based on our comments above, we suspect that the nutrient values used in this comparison contained artifacts (*23*).

In light of our inability to measure nitrogenous nutrients in oceanic surface waters and our experimental evidence for the rapid short-term nitrogen uptake characteristics of marine phytoplankton, we offer a hypothesis on how primary producers obtain their nutrition in oceanic waters that appear to be devoid of nitrogenous nutrient. Bacterial degradation of particulate and dissolved organic nitrogen plus animal excretion products, mainly NH_4^+ and urea, are significant sources of nitrogenous nutrients supporting phytoplankton growth in estuarine, coastal, and oceanic waters (*3, 16, 25*). A single phytoplankton cell with a history of nitrogen deprivation that has the same capacity for enhanced nitrogen uptake displayed by the two clones of *T. pseudonana* in this study could rapidly and efficiently utilize nitrogenous excreta before they are physically dispersed to the surrounding media. Thus the microenvironment surrounding a phytoplankton cell probably determines the magnitude of the cell's supply of nitrogen. Such minute patches, perhaps only nanoliters, with elevated nitrogen content cannot be observed with present-day techniques. The impact of a single zooplankter in supplying nitrogen on this spatial scale is considerable. For example, calculations indicate that herbivorous oceanic copepod such as *Oithona* or *Clausocalanus* can excrete enough NH_4^+ in 5 seconds to increase the ambient concentration of this nutrient by about 5 μg-atom/liter in a volume of water approximating the displacement volume of the animal (*26*).

If our results with *T. pseudonana* typify the nitrogen uptake capabilities of other marine phytoplankton, then conceivably nitrogen that is either excreted by zooplankton or remineralized by bacteria is continually being utilized by adjacent phytoplankton cells at rates that can keep the momentary average concentration for larger water volumes at less than the limits of analytical detection. Extension of these ideas and observations to achieve a more complete understanding of the small-scale temporal and spatial coupling between the nutrient supply and utilization components of near-surface oceanic nitrogen cycling will require a consideration of dispersion processes such as diffusion, shear, and even the physical mixing resulting from phytoplankton and zooplankton motility.

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17. In (*5*) no measurable NH_4^+ was detected in the medium of steady-state continuous cultures at growth rates up to 86 percent of the maximum.
18. In Fig. 1 both V'_{max} and μ are normalized to plant nitrogen in order to permit direct comparison of uptake and growth rates in dimensions of time^{-1}. Eppley and Renger (*11*) grew *T. pseudonana* (clone 13-1) on a light-dark cycle and in a different manner determined maximum uptake rates at three growth rates. Multiplying their hourly uptake value measured at a growth rate of 19 percent μ_{max} by 12 also yields an uptake rate adequate to meet the nitrogen demand for one doubling in 3 percent of the doubling period. The 20-minute experiments of Conway and Harrison (*12*) yielded values of 14 to 25 percent of the doubling period at 20 percent of the maximum growth rate.
19. Data from (*11*) were shown to fall on a curve of similar shape (*13*).
20. At $\mu = 1.6$ cellular doublings per day, Q would be reduced by 14 percent during the 120-minute delay had cellular doubling continued at its steady-state rate, and V'_{max} would have increased by 23 percent (Figs. 1 and 2).
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27. We thank R. R. L. Guillard for providing the culture inocula and J. Nevins for technical assistance. Supported by NSF grants OCE75-14781 and OCE77-26401 and Energy Research and Development Administration contract E(11-1)-2532.

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Cytidylate Cyclase: Possible Artifacts in the Methodology

Cech and Ignarro (*1*) have reported the enzymatic synthesis of cytidine 3′,5′-monophosphate (cyclic CMP) from [α-^{32}P]CTP (cytidine triphosphate) in mouse liver homogenates and other tissues. The method used by the authors to isolate cyclic CMP, however, appears to be unreliable.

Using the assay of cytidylate cyclase in mouse liver as reported by Cech and Ignarro (*1*), we were able to obtain similar values for the formation of apparent cyclic CMP (100 to 120 pmole per milligram of protein per minute) during 1 to 5 minutes of incubation. But the product did not behave as cyclic CMP in various chromatographic systems. On rechromatography on alumina column, more than 99 percent of the apparent ^{32}P-labeled cyclic CMP was retained by the column, while more than 85 percent of authentic [5-^{3}H]cyclic CMP was eluted from the column. This clearly indicated that more than 99 percent of the ^{32}P radioactivity obtained in the assay of Cech and Ignarro (*1*) was not associated with cyclic CMP. Moreover, when the alumina eluate was rechromatographed on a Dowex 50-H$^+$ (0.5 by 4 cm) column (*2*) about 75 to 80 percent of the apparent ^{32}P-labeled cyclic CMP was recovered of which more than 50 percent was eluted in the first 2 ml, and the rest was eluted in the 3- to 10-ml fractions. By contrast more than 85 percent of the recovered authentic ^{3}H-labeled cyclic CMP present

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