Variations in ammonium enhancement, an indication of nitrogen deficiency in New England coastal phytoplankton populations

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ABSTRACT

Phytoplankton populations in the coastal waters of the Gulf of Maine have been tested for cellular nitrogen deficiency by adding ammonium to the dark bottle in a conventional ^{14}C experiment. In phytoplankton cultures it has been shown that under conditions of nitrogen limitation to growth, accelerated dark fixation after ammonium addition signals the onset of cellular nitrogen deficiency. The field studies show that ammonium enhanced carbon fixation is correlated with bloom growths of phytoplankton. Most enhancement occurs with terminal chlorophyll concentrations indicating that these blooms are limited by nitrogen.

It is proposed that in these coastal waters the seasonal sequence is regulated by two sets of kinetics. One is referred to as "basal" where population size is controlled by the recycling of ammonium. The other is referred to as "bloom"; these populations arise by the introduction of nitrate into the euphotic zone by vertical mixing.

1. Introduction

Historically, there have been fundamental problems in identifying nutrient limitation in the sea. Two important papers of the sixties discuss the dynamics, identification and significance of *nitrogen limitation* in the sea (Dugdale, 1967) and the uptake of new and regenerated forms of nitrogen by phytoplankton (Dugdale and Goering, 1967). Much of the evidence for these works was from nitrogen-15 data. Of note is the terminology. *Nitrogen limitation* refers to limitation of increases of the phytoplankton population by nitrogen.

In this paper we deal with *nitrogen deficiency* of natural populations. That is, measurement of that physiological state within a phytoplankton cell when metabolism is shifted due to depletion of external as well as internal nitrogen pools. When a sufficient number of cells of a natural population exhibit the same response, the technique shows a rather dramatic response.

In other papers we have attempted to establish a technique that would signal *nitrogen deficiency* in natural phytoplankton (Morris *et al.*, 1971a & b). We call the technique ammonium enhancement. The technique was based on the experiments

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by Syrett (1962) who observed that in nitrogen-starved algae an accelerated rate of carbon fixation occurred in darkness when nitrogen was given in the form of ammonium.

According to Syrett's review article of 1962, based principally on work with cultures of *Chlorella*, the nitrogen content of the cells falls from 8-10% of the dry weight to about 2% upon nitrogen deficiency, and the chief products of photosynthesis change from protein to carbohydrate and eventually to lipid. When nitrogen is added, assimilation by nitrogen-starved cells is accompanied by exaggerated carbon dioxide fixation in darkness, which is not apparent in the light. This is particularly marked when ammonium-nitrogen is assimilated and is presumably a consequence of active amino acid synthesis.

The method developed for use in natural populations adds a known amount of ammonium and carbon dioxide as carbon-14 tracer to a population in a dark bottle. The rate of uptake of the tracer is compared to that by the identical population in a dark bottle without ammonium. The usefulness of the technique for marine algae was demonstrated by Morris *et al.* (1971b), using growing cultures, for large populations in a polluted estuary and oligotrophic tropical populations. The tests carried out in the oligotrophic open ocean suggest that these populations were closely in balance with their nitrogen supply. We have reasoned that tests of the "enhancement method" should be performed in oceanographic areas where primary productivity was more active. By this we mean in areas where large blooms of algae occur seasonally which is the case for most temperate waters such as those off New England.

To sound a negative note, we were apprehensive about conducting these experiments in coastal waters. This was largely because of the variability which could be induced by local change (tidal currents, etc). We reasoned that this could be minimized by intense data gathering during the periods of the blooms, as well as by keeping the experimental time periods short. During January of 1970 sampling at a single station was started in Ipswich Bay at the southern end of the Gulf of Maine (Figure 1). Specifically, our research questions are:

(1) Does the addition of ammonium stimulate the dark fixation of these populations? If so, when?

(2) If significant enhancement is observed, does it correlate with other environmental physiological parameters such as the assimilation number and/or the level of available nitrogen?

To introduce the reader unfamiliar with the process of ammonium enhancement of dark fixation of carbon, we will first present data from batch culture experiments where enhancement is measured under widely different growth rates. Then, because of the close relationship between seasonal succession and the nitrogen experiments, a brief description of the physical and chemical parameters associated with seasonal primary production will be given. This description will emphasize the factors asso-

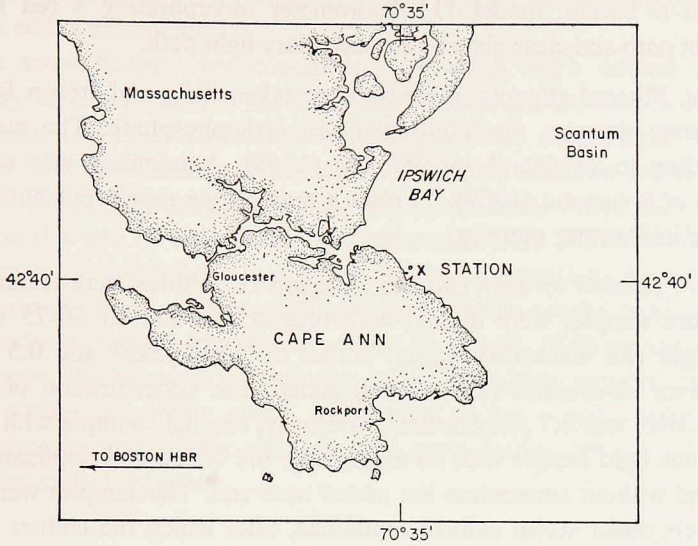

Figure 1. The Ipswich Bay region of the Gulf of Maine.

ciated with appearance of the blooms of phytoplankton during the season. It is the results of the ammonium enhancement experiments on natural populations during these periods that will be elaborated and discussed.

2. Methods and materials, batch culture experiments

a. Cultures. Unialgal cultures of the marine phytoplankton species *Dunaliella tertiolecta* Butcher, and *Phaeodactylum tricornutum* Bohlin, were maintained in the F1 medium (Guillard and Ryther, 1962) at 20°C under continuous, approximately .05 ly/min., fluorescent 12L/12D illumination. Subcultures of the algae were grown in three liters of modified F1 medium, containing no NH_4Cl, and only 10% of the full strength concentration of KNO_3. The depletion of much of the nitrogen source in the medium provokes nitrogen deficiency prior to nutrient deficiency in the cultures. The cultures were stirred and equipped with a sampling siphon to permit convenient and asceptic sampling at various intervals.

b. Chlorophyll and phaeophytin. One ml. of the cultures was taken twice daily and filtered onto 0.45 μ glass fiber filters. Acetone extracts were prepared according to Yentsch and Menzel (1963). The extracts and acidified extracts were read in a Turner fluorometer model 111. In the field, 25 ml. of sample was filtered and frozen prior to analysis.

c. Light scattering for measurement of cell concentration. Undiluted culture samples were placed in 5 ml. square cuvettes twice daily for determination of light

scattering in a Turner, model 111 fluorometer incorporating a red filter in the primary light path and clear glass in the secondary light path.

d. Nutrients. Filtered aliquots of media were taken daily and frozen for measurement of nitrate-nitrogen, nitrite-nitrogen and orthophosphate. The methods used were according to Strickland and Parsons (1965). Ammonium was measured by the method of Solorzano (1969). In such a manner we monitored nutrients in the medium and in seawater samples.

e. Carbon-14 fixation by algal cultures. Aliquots of cultures were taken daily. Five ml. of culture samples were diluted with filtered seawater to 50-75 ml. in glass stoppered light and blackened reagent bottles or Whirl-paks® and 0.5 ml. of carbon-14 sodium bicarbonate (25 μCi/ml) added. The concentration of ammonium ion, when added, was 6.7 μmoles/liter. Routinely, one light sample with ammonium ion added, one light sample with no ammonium ion added and duplicate dark samples with and without ammonium ion added were run. The samples were incubated for four hours under stated culture conditions, after which the culture suspensions were filtered through HA 0.45 μ Millipore membrane filters. The filters were dried and radioactivity counted using a standard end-window Picker counter with an efficiency of approximately 12.4 percent.

3. Methods and materials, field observations

Carbon-14 fixation by natural populations; measurement of nitrogen deficiency by ammonium enhancement. The routine experimental procedure is to incubate four dark bottles, two with ammonium ion added and two without. The bottles, either darkened glass reagent bottles or Whirl-paks placed in black plastic, contained 100 ml samples of natural population. One ml. of carbon-14 sodium bicarbonate (10 μCi/ml) was added to each sample, and an addition of 6.7 μmoles/l of NH_4Cl (where ammonium addition is indicated), were placed in the samples just prior to the incubation period of four to five hours. After incubating the plankton, the suspensions were filtered onto HA 0.45 μ 25 mm Millipore filters. The filters were dried and the radioactivity was counted using a standard end-window Picker counter; or a glass fiber filter and a scintillant (6g butyl PBD in 750 ml toluene + 250 ml methanol) were used in a Nuclear Chicago scintillation counter. Ratio comparisons of dark samples with and without added ammonium indicated ammonium enhancement. The arbitrary level of 2.0 has been selected to indicate cellular physiological nitrogen deficiency.

In both experiments dark fixation rate without added ammonium roughly corresponded to the size of culture biomass. However, with ammonium, the dark fixation rate increased dramatically between days 3-4, this increase followed the change in cell number. By the time the experiments were in stationary phase (6-7

days) the dark fixation rate with ammonium was at least two times greater than the rate without added ammonium.

Although these findings are consistent with other batch culture experiments performed by us (see Morris, *et al.*, 1971 a & b) there are features in these experiments which bear on the interpretation of field data which need further examination. We feel that there must exist a predictable relationship between cell nitrogen content or some component thereof and ammonium enhanced rates. Indirect evidence that supports this can be seen in the *Phaeodactylum* and *Dunaliella* experiments: the increases in photosynthetic rate, chlorophyll concentration, were proportional to the decrease in nitrate concentration, and considerable uptake of nitrate occurred before the cultures moved into the log phase of growth. With nitrate as the only nitrogen source, during rapid growth the cells must share nitrogen taken by the population during the prelog phase. With most of the nitrate depleted and cell division continuing, the cellular nitrogen level must decline, which would correlate with enhanced carbon fixation.

4. Results: nitrogen limited cultures

In both cultures, rapid disappearance of nitrate-nitrogen from the media began on the second day of the experiments (Figures 2 and 3). This was accompanied by increases in photosynthesis, chlorophyll concentration and cell number (measured as light scattering) in that order. For some reason, cell number reached the log phase later in *Dunaliella tertiolecta* than in *Phaeodactylum tricornutum*: for both, the stationary phase for photosynthesis and chlorophyll occurred on day five. The delay in *Dunaliella* is consistent with a major decline in normalized nitrate prior to increase in cell number and indicates that the internal pool of N must increase sizeably prior to division. This is not so extreme in *Phaeodactylum*. Cell number reached a maximum on day seven in *Phaeodactylum* and on day nine in *Dunaliella*. We believe these sequences of batch culture to be similar to those associated with coastal phytoplankton blooms.

5. General description of seasonal change of water mass characteristics of chlorophyll and nitrogen in Ipswich Bay—site of our field experiments

The shallow waters of this bay, ten meters maximum (Fig. 1), undergo extremes in both temperature and salinity throughout the year (Fig. 4). The hydroclimate is dominated by local heating and the discharge of freshwater from marshes and rivers of the region. In winter, the surface waters are near freezing but most of the bay is ice-free. Nearshore ice, especially in areas of freshwater entry, is common. During the winter months the water column is isothermal; the strong, northerly winds keep the water column stirred.

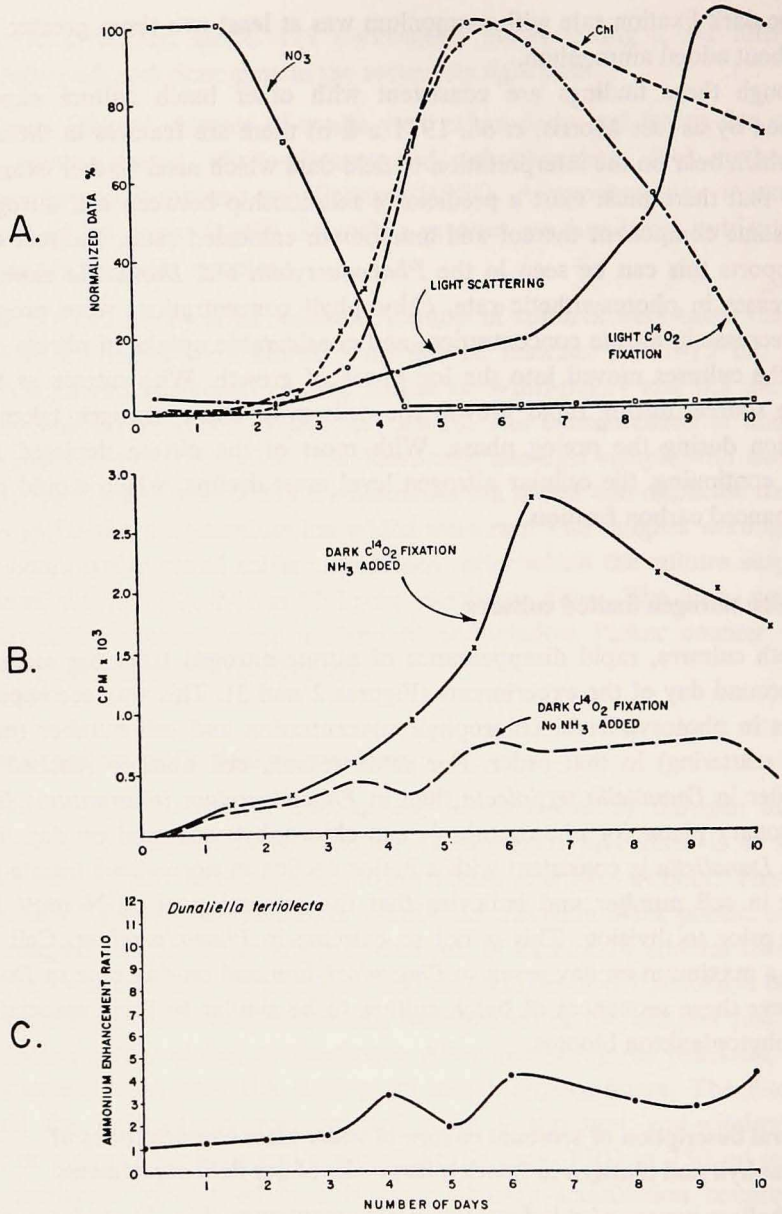

Figure 2. Culture data from *Dunaliella tertiolecta* grown in F1 medium with 1/10th the nitrogen: a) normalized data for nitrate, chlorophyll, carbon-14 fixation and light scattering; b) dark carbon-14 fixation with and without added ammonium ion; c) enhancement ratio.

The first bloom of phytoplankton (Fig. 5) appears in early April and is associated with increasing solar radiation, water temperature, and low salinity due to the spring thaw run-off. The combined effects of increased surface temperature and low

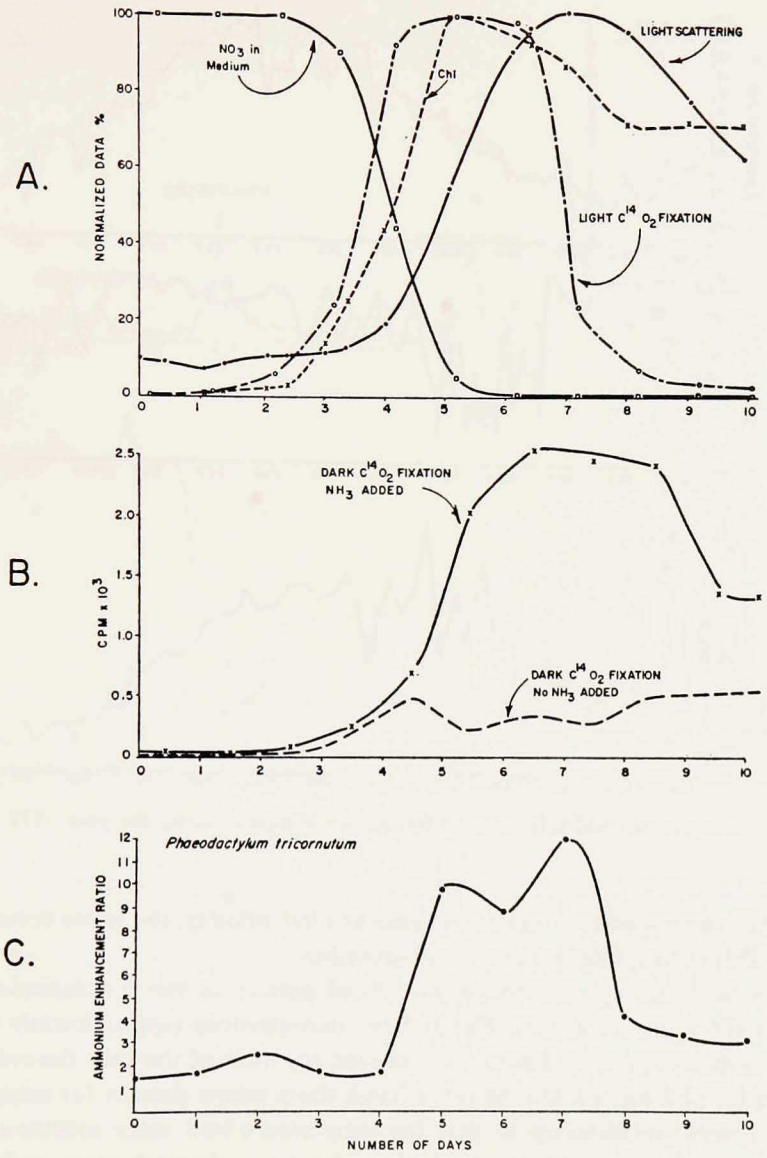

Figure 3. Culture data for *Phaeodactylum tricornutum* grown in F1 medium with 1/10th the nitrogen: a) normalized data for nitrate, chlorophyll, carbon-14 fixation and light scattering; b) dark carbon-14 fixation with and without added ammonium ion; c) enhancement ratio.

salinity tend to reduce vertical mixing. Throughout the spring and into summer the surface temperatures rise rapidly, reaching a maximum in late summer. At this point the thermocline in the water column is fully developed. In autumn, due to a lower-

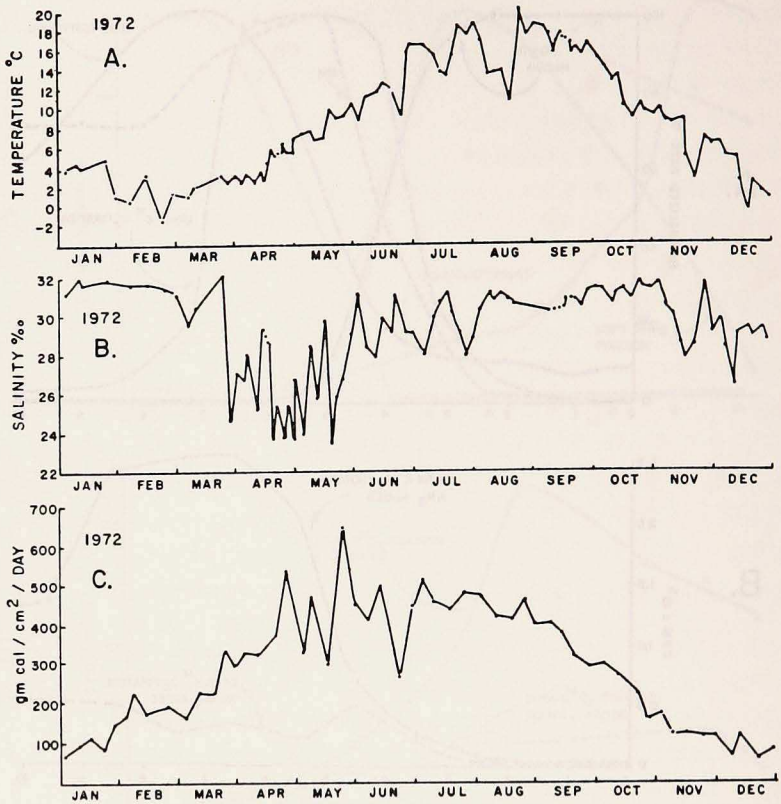

Figure 4. Temperature and salinity data for surface samples during the year 1972 in Ipswich Bay.

ing of surface temperature and an increase in wind velocity, the water column begins to mix. This is complete by the end of November.

Seasonally there appears to be no defined pattern in the concentration of ammonium in the surface waters. The highest concentrations (approximately 5 μg at *l*) appear in mid-summer and autumn, however, for most of the year the values hover between 1 and 2 μg at *l*. On the other hand, the seasonal pattern for nitrate bears a general *inverse* relationship to that for temperature and solar radiation. The sequence is as follows: with the beginning of water column heating and increased photosynthesis in the spring, the nitrate levels drop rapidly, reaching the lowest levels in late spring. For short periods during summer, some relatively high concentrations (approximately 4.0 μg at *l*) do appear, and are associated with decreases in surface temperature. We believe these short-term increases in nutrient-rich water to be due to vertical mixing. With the destruction of the seasonal thermocline, the nitrate is cycled into the surface waters. See Figure 5.

With the exception of the *Gonyaulax* bloom which exceeded 30 mg/m^3, none of

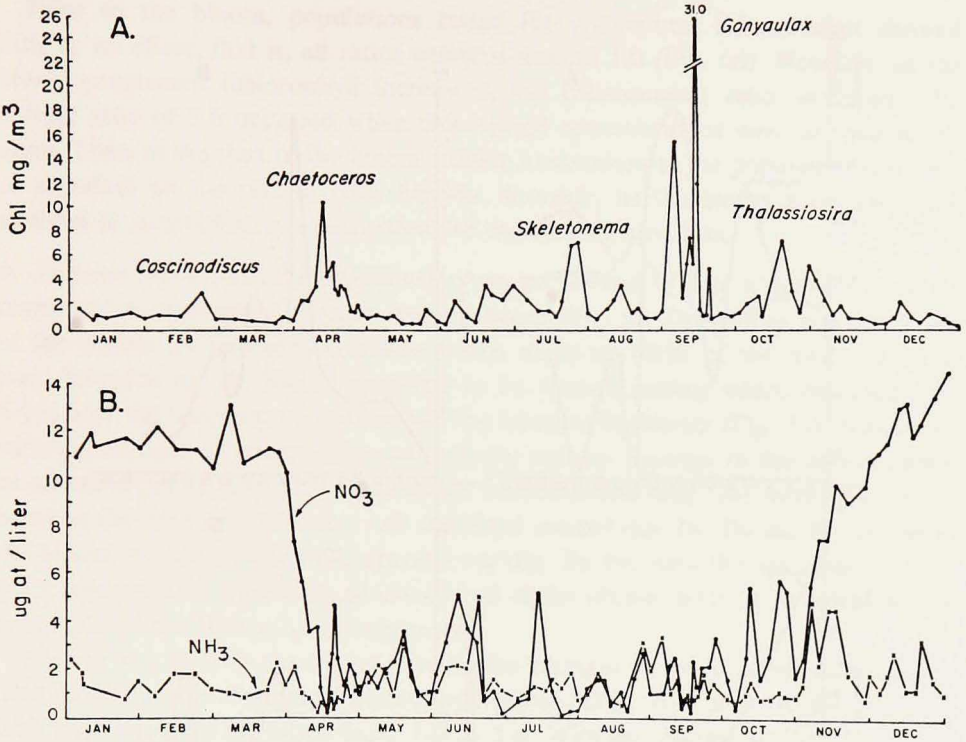

Figure 5. Chlorophyll and nitrate data for surface samples during the year 1972 in Ipswich Bay.

the other blooms exceeded 12 mg/m³ chlorophyll. One could assign a base level (nonbloom condition) for chlorophyll of about 1.0 mg/m³ for the season. We believe that our data show that throughout the growing season, a number of blooms appear and these are of different sizes and dominated by different species. The point we wish to make is that the algae that do appear in different concentrations throughout the season do so at the expense of the level of nitrate in the water and that the nitrate supply is primarily under the control of vertical mixing. With this in mind, we can proceed to examine the results of the ammonium enhancement experiments during three principal bloom periods: (1) spring—*Chaetoceros,* (2) summer—*Skeletonema,* and (3) autumn—*Gonyaulax.* These results will be the testimony in the justification of nitrate as the substance controlling the growth of blooms.

6. Dynamics of three seasonal blooms

a. Spring bloom. The first bloom in the season appeared during the month of April. Floristically the bloom was composed of at least three species of the diatom *Chaetoceros* which made up over 93 percent of the total cell count. At the time of the

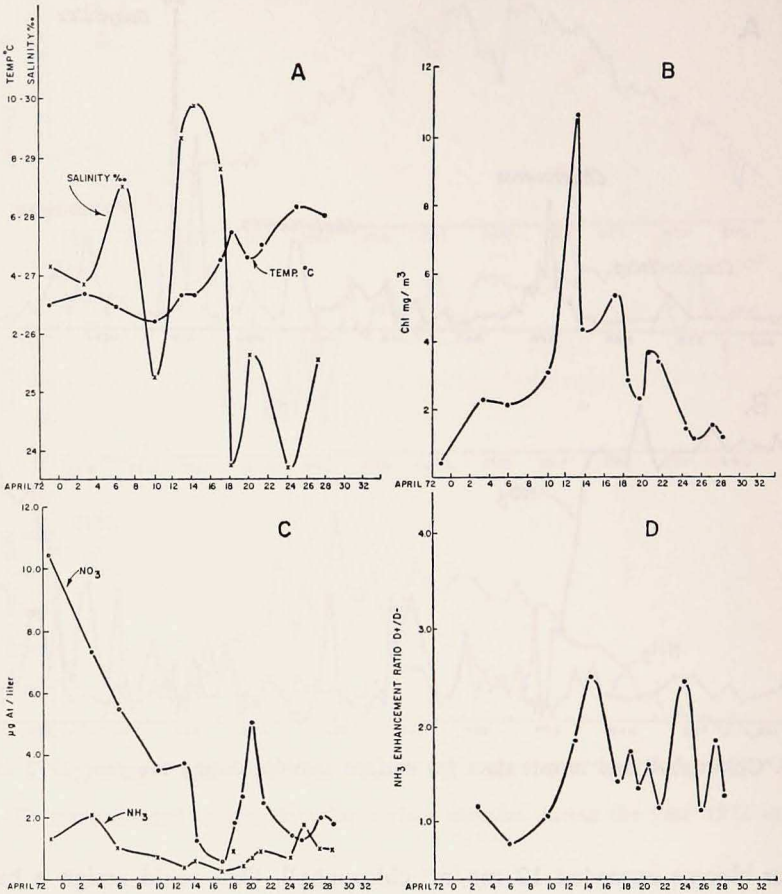

Figure 6. Data from the spring bloom of *Chaetoceros* in 1972: A) temperature and salinity; B) chlorophyll; C) nitrate; D) ammonium enhancement.

bloom, the water mass was changing from winter to summer conditions (Fig. 4) with temperature increasing and the water freshening due to the spring run-off. The burst of phytoplankton growth (see chlorophyll, Fig. 6b) was reflected in a dramatic decline in the nitrate-nitrogen (Fig. 6c). Actually, the disappearance of nitrate commenced before any sizable increase in chlorophyll; the lowest nitrate values coincided with the height of the maximum chlorophyll. A less dramatic decline of ammonium was reflected by the bloom; at the height of the bloom (maximum chlorophyll) nitrate and ammonium concentration were about equal. Note that around the middle of the month, the concentration of nitrogen, mostly nitrate, abruptly increased (Fig. 6c). This coincides with a decline in salinity (Fig. 6a) which suggests that the source of this nitrogen was freshwater runoff.

Prior to the bloom, populations tested for ammonium enhancement showed little or no effect; that is, all ratios centered around 1.0 (Fig. 6d). However, as the bloom progressed (chlorophyll increased), the enhancement ratio increased. The highest ratio of 2.6 occurred when chlorophyll concentrations were at their maximum. Thus, at the start of the bloom, adding ammonium to the population had little or no effect on the rate of dark fixation, however, as the bloom progressed, the addition of ammonium more than doubled the dark fixation rate.

b. Summer bloom. This bloom occurred toward the end of July when surface water temperatures were near their seasonal maximum (Fig. 5). This bloom was composed of the diatom *Skeletonema costatum* which made up 95% of the total cell numbers. Stimulus for the bloom appeared to be vertical mixing which occurred July 6-15; note the temperature decline and the increase in salinity (Fig. 7a). Associated with the changes in temperature and salinity was an increase in the concentration of nitrate-nitrogen (Fig. 7c). Chlorophyll concentration (Fig. 7b) increased rapidly between the 16th and 20th day and stabilized around day 28. During the course of the bloom, nitrate-nitrogen disappeared rapidly. By the time the maximum chlorophyll concentration appeared, practically all of the nitrate, added by vertical mixing at the start of the bloom, had disappeared.

As was the case in the spring bloom, the changes in ammonium enhancement ratio paralleled the changes in chlorophyll content (Fig. 7d). Between the 20th and 24th day, the ratio increased from 1.0 to 2.6. With the decline of the bloom, the enhancement ratio decreased to about 1.0.

c. Autumn bloom. This bloom appeared as nearly a unialgal population of the motile toxic dinoflagellate *Gonyaulax tamarensis.* Because of public health implications, there has been a great deal of speculation as to the factors which caused this bloom. The causes of the bloom are in many respects similar to those of the preceding summer bloom. For example, surface water temperatures dropped between the 6th and 10th day of the month (Fig. 4). Associated with this drop in temperature was a gradual lowering of surface salinity (Fig. 8a). This was due to the heavy rainfall from Hurricane Carrie which passed through this region on September 3, 1972. Also, at the start of the month, the immediate prehistory of the bloom, the concentrations of ammonium were about two to three times higher than the nitrate concentrations (Fig. 8c), but the situation quickly reversed with the temperature drop. Nitrate appeared with the intersection of cooler waters (Fig. 8a). The bloom (chlorophyll increase) appeared to start around the 10th day of the month (Fig. 8b). Increases in chlorophyll were accompanied by a marked decrease in both nitrate and ammonium. At the height of the bloom, on the 14th day, nitrate was almost exhausted and only a residual amount of ammonium remained.

The ammonium enhancement (Fig. 8d), at the start of this month, measured around 1.0. At the peak of the bloom (Fig. 8b), enhancement was 3.7 which was

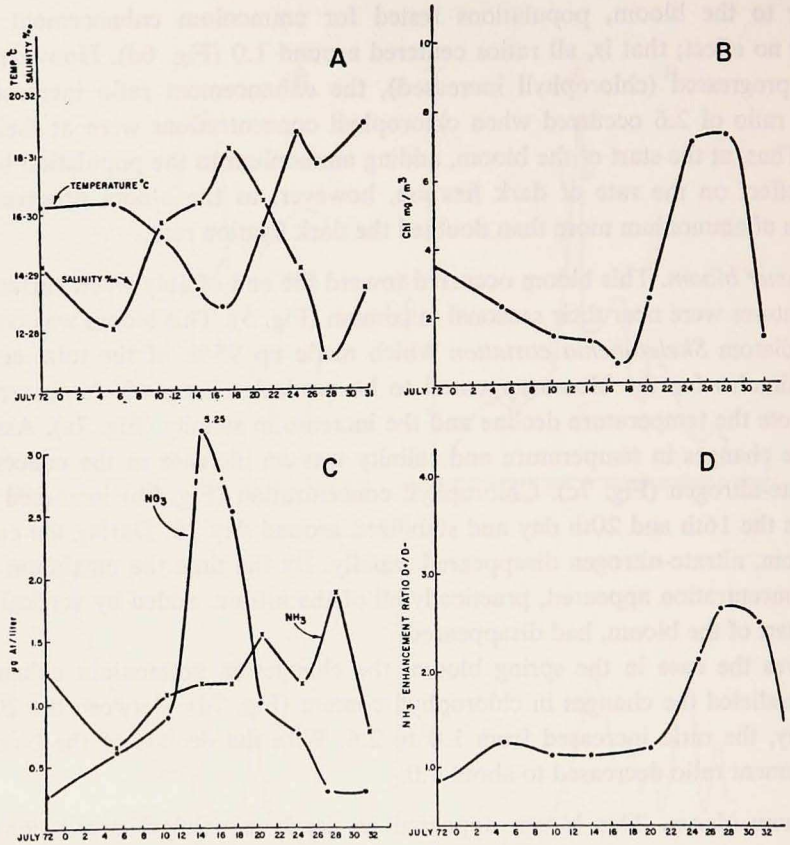

Figure 7. Data from the summer bloom of *Skeletonema* in 1972: A) temperature and salinity; B) chlorophyll; C) nitrate; D) ammonium enhancement.

the highest recorded for natural populations for the year. The explosive nature of this bloom hampered adequate sampling; our schedule simply could not keep up with the population physiology. Several days after the bloom, both the chlorophyll and enhancement ratios returned to "normal levels."

d. Photosynthetic efficiency during the course of the blooms. One of the most common measurements in studies of phytoplankton populations is the assimilation number. Since this is a ratio of the maximum rate of photosynthetic carbon fixation to chlorophyll content, it is a measurement of photosynthetic efficiency. A number of workers have attempted to utilize this ratio as an indicator of nutritional deficiency, but it is affected by a number of widely different parameters (see Yentsch and Lee, 1966 and Eppley, 1972). Therefore, the level of photosynthetic efficiency has not been especially useful in relating the nutritional stress of populations. However, given that other things are equal and that our assessment of the enhancement ratio

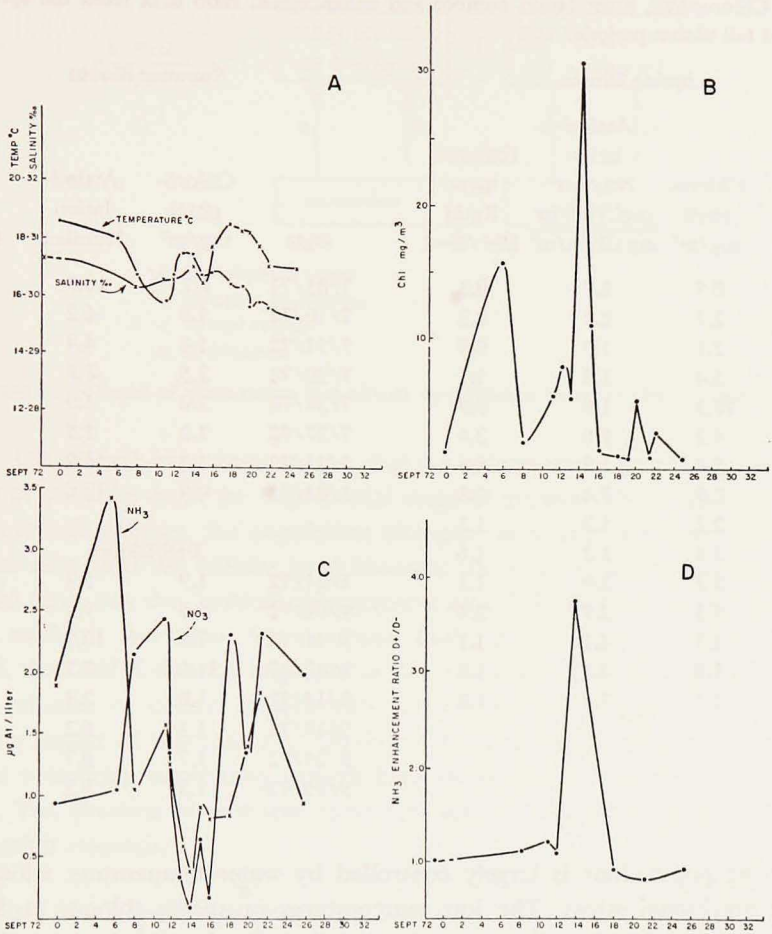

Figure 8. Data from the fall bloom of *Gonyaulax* in 1972: A) temperature and salinity; B) chlorophyll; C) nitrate; D) ammonium enhancement.

as a specific indicator of cellular nitrogen deficiency is correct, one would expect to observe that low assimilation ratios (low photosynthetic efficiency) would correspond to high ammonium enhancement ratios.

With the exception of the spring bloom, this is exactly what was observed. Please see Table 1. For example, the assimilation numbers for the summer *Skeletonema* and fall *Gonyaulax* blooms were the inverse of the enhancement ratios; the lowest assimilation numbers observed coincided with the maximum chlorophyll concentrations. The major discrepancy in the above proposed relationship between assimilation numbers and enhancement ratios concerns the data for the spring bloom (Table 1). We would argue that photosynthetic efficiency (assimilation number) in

Table 1. Chlorophyll, assimilation number and enhancement ratio data from the spring, summer and fall bloom periods.

Spring Bloom				Summer Bloom			
Date	Chlorophyll mg/m³	Assimilation Number mgC/m²/hr / mg Chl α/m³	Enhancement Ratio ($D+/D-$)	Date	Chlorophyll mg/m³	Assimilation Number	Enhancement Ratio ($D+/D-$)
3/30/72	0.5	2.7	0.3	7/05/72	2.2	10.9	1.2
4/03/72	2.1	2.1	1.2	7/10/72	1.6	6.2	1.2
4/06/72	2.1	1.7	0.8	7/14/72	1.4	4.4	1.2
4/10/72	3.4	1.3	1.1	7/20/72	2.5	8.8	1.2
4/13/72	10.3	1.0	1.9	7/24/72	7.0	5.9	2.2
4/14/72	4.2	5.6	2.4	7/27/72	7.2	5.5	2.6
4/17/72	5.4	1.7	1.4	7/31/72	1.5	15.2	2.4
4/18/72	2.9	3.4	1.8	8/03/72	0.8	12.6	1.0
4/19/72	2.2	1.2	1.3				
4/20/72	3.4	3.2	1.6	Fall Bloom			
4/21/72	3.2	2.9	1.1	8/21/72	1.9	5.8	0.9
4/24/72	1.3	2.7	2.4	9/08/72	5.6	9.1	1.1
4/25/72	1.3	4.1	1.1	9/11/72	7.5	10.3	1.2
4/27/72	1.8	4.5	1.8	9/12/72	31.5	7.7	1.1
4/28/72	1.1	7.4	1.3	9/14/72	1.0	2.3	3.8
				9/18/72	1.1	6.2	0.9
				9/21/72	1.7	9.7	0.8
				9/25/72	1.3	9.2	0.9

these spring populations is largely controlled by water temperature which would override nutritional stress. The low temperatures in spring (bloom at 5°C) are known to reduce the rate of photosynthetic carbon fixation (Yentsch *et al.*, 1974). During the period of the spring bloom, the increase in water temperature was paralleled by an increase in the assimilation numbers.

7. Evidence for cellular nitrogen deficiency in natural populations

The most significant finding in this research is that high ratios of ammonium enhancement are associated with blooming conditions and low or no enhancement associated with periods between blooms. In all cases the ammonium enhancement ratio increased as the bloom progressed with the highest ratios occurring on or near the peak concentration of population chlorophyll. The incremental increases in population size appear to be at the expense of the nitrate-nitrogen generally supplied in the bloom pre-history. The blooms appear to be analogous to the culture experiments described earlier in this paper: i.e. highest enhancement ratios occurring during peak concentrations of chlorophyll correspond to those observed in

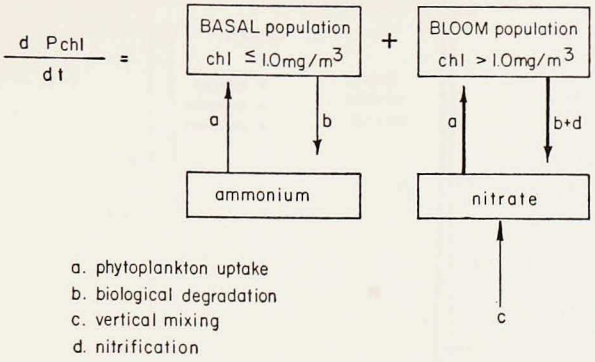

Figure 9. Scheme of ammonium and nitrate as related to basal or bloom conditions.

cultures. The culture experiments show that the enhancement ratio becomes greater than 1.0 as the cells enter the exponential stage of growth. This suggests that with increases in cell number, the population nitrogen (as a fixed amount) is shared by individual cells until the cellular level becomes too low to sustain further division. We would term this the "critical enhancement ratio." Unfortunately we cannot accurately establish this ratio. We do know that when a ratio of 2.0 or larger is observed, then cell division is impaired as the stationary phase of growth is entered. But this reliance on culture experiments to interpret population kinetics is a disappointing aspect of this research. Obviously the enhanced dark fixation rates are telling us something concerning growth kinetics as they relate to the level of cell nitrogen. The question of how well these kinetics relate to other kinetic parameters awaits further research.

8. Basal and bloom periods of growth

We have stressed that the appearance of nitrogen deficiency (as signaled by the ammonium enhancement of carbon fixation) occurs when populations are rapidly dividing in so-called bloom conditions. No less important is the observation that between blooms—the basal growth periods when populations are small—there is no evidence that cells of these populations are nitrogen deficient. This suggests that the seasonal pattern is controlled by two nitrogen-growth related kinetics. Figure 9 is a general outline of the kinetics of the two systems. In the basal populations the important factor is that the uptake of ammonium is balanced by the rate of biochemical degradation which produces ammonium, as has been suggested previously by Dugdale (1967) and Dugdale and Goering (1967). Thus, the stock of ammonium and population organic material fluctuates very little. Nitrogen deficiencies do not occur in the basal populations since the appearance of the new cell material is immediately used in the production of ammonium. The population moves to bloom conditions by receiving added nitrogen as nitrate (Fig. 10).

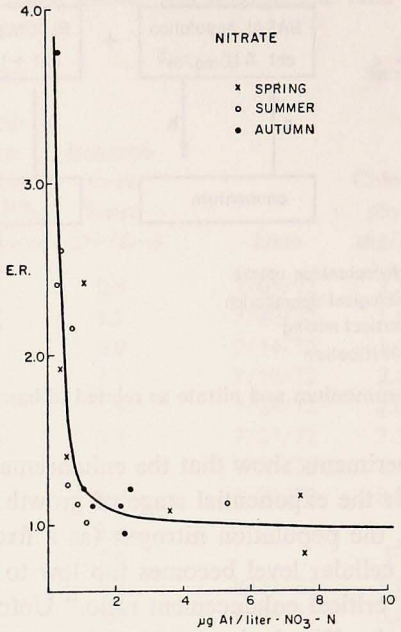

Figure 10. Ammonium enhancement ratio as related to nitrate concentration in the water at the time of experiment.

The nitrification step is comparatively slow. Thus, populations are produced whose size has a demand capable of outstripping the nitrogen supply. In terms of the dual growth model, the source of the added nitrate would be below the euphotic zone. Thus vertical mixing becomes an important factor in fostering bloom status, but since vertical mixing is limited, the source is limited. The nitrate addition by mixing provides the cells with a high growth potential for a brief period. The accelerated cell division produces cells which must share the limited nitrate-nitrogen ration. With each cell division the cellular nitrogen decreases, which eventually throttles cell division.

It is the nitrate that causes a deficiency problem for the algae. This ration appears discontinuous within the time frame of the bloom. We ask to what level the external nitrogen falls before signs of deficiency appear. Figure 10 is a plot of data points for the enhancement ratio and nitrate concentration at the time of the experiment. Values for the so-called basal population cluster around 1.0 and the enhancement ratio begins to increase when the nitrate concentrations are less than 1.0 μg at *l*. Of note is that this break point coincides with K_t of 1 μg at *l* for nitrate in euphotic waters (MacIsaac and Dugdale, 1969).

High enhancement ratios (greater than 2.0) are associated with nitrate concentrations of around 0.5 μg at *l*. A similar plot for the sum of nitrate and ammonium (Fig. 11) shows that enhancement ratios increase when the level reached is 1 to 2

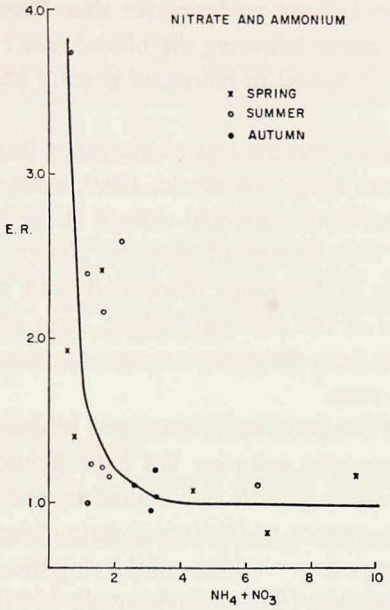

Figure 11. Ammonium enhancement ratio as related to nitrate and ammonium concentration in the water at time of experiment.

μg at l of nitrogen. These limiting estimates (we term them "departure values") are very close to the nitrogen limiting kinetics of Chesapeake Bay phytoplankton (McCarthy *et al.*, 1975). Using N_{15} methods McCarthy clearly established that ammonium was the preferred nitrogen source and when in excess of 1.5 μg at l, nitrogen was not a limiting nutrient.

Both the McCarthy data set and ours suggest the now common hyperbolic substrate-growth relationship. To our knowledge, the departure point has no present kinetic terminology. It is, however, analogous to the I_k value derived from photosynthetic light curves. We would emphasize that the departure point for nitrogen is the point where cells in the population are first experiencing nitrogen stress. Thus the so-called limitation is one of "incipient deficiency."

The basal-bloom dual growth model has certain social-economic analogies—specifically boom or bust economics: as remarkable as the growth of a bloom is its rapid decline. Grazing and/or sinking are the most likely causes. A number of workers have considered the dual role of grazing factor (Eppley, 1972; McCarthy *et al.*, 1975) and biochemical degradation, but it is a difficult role to assess quantitatively. Obviously, during bloom periods, phytoplankton growth has exceeded that removed by the degradation processes; however at the apex of the bloom, the enhancement ratio data suggest that all cell division is at best retarded and hence grazing pressure could be a dominant factor in the control of population size. In

our data set there is some indirect evidence for this. Pronounced increases in the ammonium concentration occur following the bloom (see Figs. 7,8) which could be degradation, or could be attributed to increased grazing and the release of nitrogen by animals.

Cellular nutrient deficiency and the loss of buoyancy have been well documented for cells sinking in cultures (Yentsch & Steele, 1960; Smayda, 1970; Eppley, 1972) and implicated as the cause in the seasonal change in the vertical profile of chlorophyll. Similarly it could well be argued that the abrupt decline of these coastal blooms is due to the loss of buoyancy associated with nitrogen deficiency. This would mean that in terms of the nitrogen budget, most of the particulate nitrogen in the bloom would be lost from the surface waters to undergo decomposition somewhere below the euphotic zone.

The other analogy that exists between basal and bloom growth concerns growth kinetics in batch and chemostat cultures. We have likened the bloom periods to batch culture conditions where growth was limited by the nitrogen in the medium. Basal periods fit more the pattern of the steady state chemostat culture. This latter analogy has found wide appeal to workers considering the dynamics of oligotrophic oceans (Eppley, 1972; Hulburt, 1970; Morris *et al.*, 1971b). It seems worth mentioning that the only difference in oligotrophic ocean situations and those of the coastal waters are the perturbations in nutrient supply brought about by vertical mixing. In the coastal waters of New England the spring bloom sets the stage for the chemostatic nature of the basal growth system. That is, batch culture growth moves into the continuous system of the chemostat. Populations would stay in that growth phase if it were not for the rather unpredictable events of vertical mixing in summer and the break-down of the thermocline in the autumn.

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REFERENCES

Dugdale, R. C. 1967. Nutrient limitation in the sea: dynamics, identification, and significance. Limnol. Oceanogr., *12*, 685–695.

Dugdale, R. C., and J. Goering. 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. Oceanogr., *12*, 196–206.

Eppley, R. W. 1972. Temperature and phytoplankton growth in the sea. Fish. Bull., *70*, 1063–1085.

Guillard, R. R. L., and J. H. Ryther. 1962. Studies on marine planktonic diatoms I. Can. J. Microbiol., *8*, 229–239.

Hulbert, E. M. 1970. Competition for nutrients by marine phytoplankton in oceanic, coastal and estuarine regions. Ecology, *51,* 475–484.

MacIsaac, J. J. and R. C. Dugdale. 1969. The kinetics of nitrate and ammonia uptake by natural populations of marine phytoplankton. Deep-Sea Res., *16,* 45–57.

McCarthy, J. W., R. Taylor, and J. L. Taft. 1975. The dynamics of nitrogen and phosphorus cycling in the open waters of Chesapeake Bay, *in* Marine Chemistry in the Coastal Environment. ACS Symposium Series 18, Thomas C. Church, ed., Washington, D.C., 664–681.

Morris, I., C. S. Yentsch, and C. M. Yentsch. 1971a. Relationship between light carbon dioxide fixation and dark carbon dioxide fixation by marine algae. Limnol. Oceanogr., *16,* 854–858.

—— 1971b. The physiological state with respect to nitrogen of phytoplankton from low-nutrient subtropical water as measured by the effect of ammonium ion on dark carbon dioxide fixation, Limnol. Oceanogr., *16,* 859–868.

Smayda, T. J. 1970. Suspension and sinking of phytoplankton in the sea. Oceanogr. Mar. Biol. A. Rev., *8,* 353–414.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr., *14,* 799–801.

Strickland, J. and T. Parsons. 1965. A manual of seawater analysis. Fish. Res. Bd. Can. Bull. 125.

Syrett, P. J. 1962. Nitrogen assimilation, *in* Physiology and Biochemistry of Algae, R. A. Lewin, ed., New York, Academic Press, 171–188.

Vaccaro, R. F. 1963. Available nitrogen and phosphorus and biochemical cycle in the Atlantic off New England. J. Mar. Res., *21,* 284–301.

Yentsch, C. S., and R. W. Lee. 1966. A study of photosynthetic light reactions and a new interpretation of sun and shade phytoplankton. J. Mar. Res., *24,* 319–337.

Yentsch, C. S., and D. W. Menzel. 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep-Sea Res., *10,* 221–231.

Yentsch, C. S., and J. H. Steele. 1960. The vertical distribution of chlorophyll. J. Mar. Biol. Assoc. U. K., *39,* 217–226.

Yentsch, C. S., C. M. Yentsch, L. R. Strube, and I. Morris. 1974. The influence of temperature on the efficiency of photosynthesis in natural populations of marine phytoplankton, *in* Thermal Ecology, AEC Symposium Series, J. W. Gibbons and R. R. Sharitz, eds., Oak Ridge, Tenn., 508–517.

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