CRITICAL ESSAY

Master's Program

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THE MARINE PHYTOPLANKTON AND ITS NUTRIENTS:
NITROGEN AND PHOSPHORUS.

* INTRODUCTION *

Growth of phytoplankton can go on reasonably with only very small quantities of nutrients amounting to a few milligrams of phosphorus as phosphate per cubic meter of sea water and usually a somewhat larger amount (about eight times by weight) of nitrate nitrogen. Their remarkable growth as compared with the growth of land plants at such great dilution is partly explained by the microscopic size of the phytoplankton cells, which makes for better diffusion of nutrients as well as a greater surface to volume ratio which promotes absorption. (Raymont, 1963).

In the tropical regions of the ocean, and in most parts of the temperate zones, however, the water above the thermocline contains low levels of dissolved nitrogen and phosphorus compounds, which may become limiting both in space and in time. Different species of phytoplankton have different nutrient requirements. For example, members of Cyanophyeae are able to fix dissolved molecular nitrogen, whereas members of other phyla must satisfy their needs for this element by using dissolved inorganic forms of the element, and the heterotrophs can utilize organic nitrogen compounds. Dissolved inorganic phosphate ion is utilized readily by all species of phytoplankton, yet only some species can also take up phosphorus heterotrophically by using dissolved or particulate organic forms of the element.

Although most species can grow well at high concentrations of phosphorus and nitrogen, (> 20 times the maximum encountered in the oceans), they will not grow when the concentrations of these micronutrients in the medium fall below critical levels. Deficiency of nutrients, especially nitrate, is one of the main factors limiting marine primary production. It has been recently shown that the relationship between phytoplankton growth and these nutrient levels is an important factor in determining species succession, competition, and geographical variations in natural populations.

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* THE DISSOLVED FORMS OF NITROGEN AND PHOSPHORUS *

Although the ocean contains several dissolved forms of nitrogen and phosphorus which may be utilized by the plants, nitrate, ammonium and phosphate are the more important forms.

The following is a discussion of various chemical forms of the two elements.

Nitrate, the most highly oxidized form of nitrogen in ocean, is usually the most abundant source of the element available to phytoplankton. The concentration of the ion varies considerably, both seasonally and geographically. In general, it tends to be low on the surface and rapidly increases to a maximum at intermediate depths of from 500-1000 meters. The surface water is largely depleted of nitrate with the phytoplankton out-burst. The maximum amount of nitrate, usually 50-100 µg-at./l, occurs in winter, due to its accumulation after the phytoplankton outburst comes to an end in late autumn. In upwelling areas, the amount is greatly increased due to turbulence. For example, the winter maximum exceeds 300 µg-at./l and the summer minimum falls only to 200 µg-at./l in the Friday Harbour upwelling area. The depletion of nitrate is not so great in deeper waters; and usually a small amount, e.g. 10 µg-at./l remain even during the summer period. Nitrate is regenerated in deep water mainly from nitrification by bacteria while river effluent contribute a small amount to the ocean.

Ammonium ion is the main inorganic form of fixed nitrogen in the ocean. In general, the upper strata of water (50 meters depth) contains most of the ammonium, though the extreme surface layer may show very little. For example, off the Northwest Pacific coast, the majority of the ammonium occur in the upper 100 meters layer where the average concentration was 20 µg-at./l; in deep layer (1500 meters depth) the average concentration was only 2 µg-at./l. Similar to the seasonal variation of nitrate, the lowest ammonium values occur in spring, and the amount reaches a maximum during autumn/winter. (Raymont, 1963). The maximum concentration presumably arises largely from the bacterial decomposition of the organic material produced over the summer. Although small quantities of ammonium are released during the summer, especially in the upppermost layers with the excretion of the zooplankton, this ammonium is taken up by phytoplankton immediately.

Vaccaro (1963) evaluated the relative importance of ammonium in the Atlantic ocean off New England and demonstrated that when nitrate concentrations were very low in the late summer, ammonium was a major source of available nitrogen for phytoplankton growth. Thomas (1966) studied nitrogenous nutrients in the Northeastern tropical Pacific ocean and showed that the low nitrate concentration (5 µg-at./l) present probably limited the rate of growth of the dinoflagellate *Gymnodinium simplex*. He suggested ammonium should be included with nitrate as a measure of the total nitrogen available to phytoplankton, since the ammonium concentration was greater than that of nitrate in the area. The conclusion was that ammonium may moderate the extreme nitrogen deficiency if nitrate were considered to be the only source of nitrogen. Dugdale and Goering (1967) concluded that the algal cells do depend primarily on ammonium as a source of nitrogen; yet only new sources such as nitrate from deep water or nitrogen fixation will allow increases in population size, because ammonium is the result of short-term regeneration of nitrogen in the same area concerned. These proposals seem quite logical to me and I believe with more research on other parts of the oceans, the conclusion will be widely recognised.

The concentration of nitrite in almost all parts of the ocean in general tends to be low, very much lower than that of nitrate and usually considerably lower than that of ammonium. Thomas (1966) found nitrite concentration was always very low in the northeastern tropical Pacific ocean and concluded that it would not serve as an appreciable source of nitrogen for phytoplankton.

Nitrification, the oxidation of ammonium to nitrite and then nitrate, may be represented by the following equations (Cooper, 1937):

$$NH_4^+ + OH^- + 1.5\ O_2 \rightleftharpoons H^+ + NO_2^- + 2H_2O \qquad \Delta G = -59.4 \text{ Kcal.}$$

and

$$NO_2^- + 0.5\ O_2 \rightleftharpoons NO_3^- \qquad \Delta G = -18.0 \text{ Kcal.}$$

Both reactions are exothermic and require only activation to occur, This process appears to occur largely by a bacterial mechanism and it takes place in the most parts immediately below the photosynthetic zone in the ocean. However, measurements showed that the rate of oxidation was usually too slow to account for the estimated rate of disappearance of ammonium in the ocean.

It is therefore believed that the process must also occur by other mechanism, which remain to be studied. The photochemical oxidation of ammonium to nitrate by solar ultra-violet radiation was reported by Rakestraw and Hollaender (1936). Study on this mechanism had been ignored until 1964, when Hamilton re-evaluated the role of this process in the ocean, but was unable to confirm the result, and thus concluded that the process was not likely to cause significant oxidation of ammonium. Moreover, he observed that photoreduction of nitrate to nitrite took place rather easily, as in contrast to the inability of the photooxidation process. In a discussion that the sea water used in his experiment contained low level of colloidal silica, a catalyst needed for the process, Hamilton believed the catalysis would not exert enough influence on the process. This conclusion does not appear to be a good one to me since it is known that catalysis does in fact influence chemical reaction in great extent. Another problem is that in his experiment, the sample of solution was exposed to sunlight for only 24 hours, which period may be too short for the process to start in the absence of a necessary catalyst. A repeat of the experiment in the presence of a catalyst would be very helpful and probably very interesting.

not clear wonder the alternative experiment

Nitrogen fixation is a source of new nitrogen. Nitrogen-fixing organisms occur in the marine environment. However, the fixation process is endothermic and requires a plentiful supply of organic material as an energy source. It ~~is thus~~ has been suggested therefore that it is unlikely that these organisms in the marine environment fix significant amounts of nitrogen. In contrast, certain blue green algae, e.g. Tricho-

desmium species have been shown to be capable of fixing nitrogen on a large scale in tropical and subtropical waters (Dugdale et al., 1964) even though the concentrations of organic matter are low. It was postulated that these organisms obtained the energy from solar radiation, thus nitrogen fixation occurs only in the light. Dugdale and Goering (1967) emphasized the importance of nitrogen- fixing phytoplankton in the ocean, showing that nitrogen fixation could support the growth of *Trichodesmium* species at rates which are similar to those of species which rely on nitrate. Although there are still many problems as to the significance of nitrification and nitrogen fixation in the ocean, it appears that these two processes have been believed to be the main sources of nitrogen. Rivers also contribute nitrate to the ocean but the amount is relatively small.

waffle

Thus is nitrogen fixation significant or not

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Dissolved inorganic phosphate, mainly orthophosphate, is the most abundant form of phosphorus in the sea. In salinity 3.3% at 20° C, 87% of the orthophosphate is present as HPO_4^{2-}, with 12% as PO_4^{3-} and 1% as $H_2PO_4^-$. Sillen (1961) suggested that the orthophosphate in deep sea water, without any biological influence, might be in equilibrium with hydroxyapatite, a mineral present in marine sediments. The equilibrium reaction is represented by the following equation:

$$Ca_5(PO_4)_3OH(s) + 4H^+ \rightleftharpoons 5Ca^{2+} + 3HPO_4^{2-} + H_2O$$

This idea ~~may~~ suggests that phosphorus in sea water can come from marine sediments at the bottom of the ocean.

Solorzano and Strickland (1968) showed that dissolved organic phosphorus can be oxidized to orthophosphate under the influence of dissolved oxygen and ultra-violet irradiation. This might indicate that the oxidation process could proceed in the euphotic zone of the ocean and would thus act as a regeneration for phosphorus in the ocean.

The phosphorus cycle is probably not a completely closed one. Unlike the nitrogen cycle in which the air-sea interface is involved, the sea-bottom interface is involved in the phosphorus system. As dead organisms sink to the sea floor, much of their phosphorus will be regenerated to the water, mostly by bacterial action. Rock-weathering may contribute new phosphate to the ocean while hydroxyapatite in marine sediments could be changed to orthophosphate if the equilibrium is disturbed physically or biologically.

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So American situation?
Guano depo's &
effect on nutrient rich water

* NUTRIENT ASSIMILATION BY PHYTOPLANKTON *

Early in 1926, H.W. Harvey discovered that the concentrations of nitrate and phosphate salts in the sea water were apparently in a ratio in which they were assimilated by the phytoplankton. Redfield (1934) showed that there actually was an approximately linear correlation between the concentrations of the two salts for sea water from different geographical locations and different depths.

Redfield found that the average nitrogen:phosphorus ratio (by atoms) in a mixed group of phytoplankton and zooplakton was 18.7:1, which is in agreement with the constant ratio of 20:1 for the two dissolved nutrients in sea water. The ratio was later corrected to be 15:1 due to a salt error in the experiment. This ratio of N:P is called the ' assimilation ratio '. Redfield (1934) commented that 'it appears to mean that the relative quantities of nitrate and phosphate occurring in the oceans of the world are just those that are required for the composition of the animals and plants which live in the sea'. (Quoted from Ketchum, 1958).

The ratio describes the biological effects of both assimilation and regeneration of the nutrients. A phenomenon was noted that 'if the N:P ratio in the medium is high, the metabolism of the cells brings the ratio closer to the usual one; if the ratio is low, the metabolism of the cells increases the original departure from the usual ratio'. (Quoted from Ketchum, 1939). The implication was that the relative changes in the concentrations of inorganic nitrogen and phosphorus in sea water resulting from the biological changes should be directly related to the elementary composition of the plankton. There is evidence that phosphorus utilization by phytoplankton continues after the nitrate-nitrogen concentration has fallen below the detectable limit. Stefansson and Richards (1963) observed that, in the euphotic zone of the northeastern Pacific Ocean, nitrate was depleted before the phosphate, and growth continued, however, until the phosphorus had also disappeared, implying that the nutrients were utilized in a N:P ratio higher than it was present in the water. This may represent recycling of nitrate or the addition of traces of nitrogen compounds from other sources.

Corner and Davies (1971) believed that regeneration of nitrogen could account for the continued growth, while the remineralized forms were being used up very rapidly so that inorganic nitrogen concentrations were not detectable. My doubt on this idea is that if the amount of the remineralized form is undetectable, it may not be sufficient for the organisms to have a continued growth. Ketchum et al. (1958) thought that remineralization occurred at depth, and the nitrogen is returned to the euphotic zone by eddy diffusion. Vaccaro (1963) considered the presence of ammonium-nitrogen as the cause for the continued production for the water off the New England coast. He argued that when ammonium-nitrogen concentration was included in the value, the atomic ratio of N:P in the particulate matter suspended in the euphotic zone would be similar to the assimilation ratio. This seems to be a good argument for the case.

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'Apparent oxygen utilization' (AOU) is a concept related to the study of biologically induced changes in nutrient levels. It is believed that a mass of water on the sea surface is in equilibrium with the atmospheric oxygen which has different solubility at different temperature and salinity. The oxygen content of this mass of water should be the same when it sinks below waters of lower density. If the oxygen content changes, it is due to either oxygen used for remineralization of organic matter, or oxygen produced as a by-product during photosynthesis. AOU would be positive for the former case and negative for the latter. It is believed that oxygen, carbon, nitrogen, and phosphorus are assimilated and subsequently regenerated in the same atomic ratios:

$$O:C:N:P = 276:106:16:1$$

If carbon is assumed to be in carbohydrate form and nitrogen in the amino form, the ratios will correspond to the overall reaction of the respiratory and biochemical decomposition of organic matter, (Richards, 1965):

$$\begin{aligned}(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 138O_2 = {} & 106CO_2 + 122H_2O \\ & 16HNO_3 + H_3PO_4\end{aligned}$$

It is assumed that the first step of organic decomposition is the hydrolytic release of NH_4^+ and PO_4^{3-} ions, then the oxidation of CH_2O, represented by the equations:

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 = 106CH_2O + 16NH_3 + H_3PO_4$$
$$106CH_2O + 106O_2 = 106CO_2 + 106H_2O$$
$$16NH_3 + 32O_2 = 16HNO_3 + 16H_2O$$

The complete oxidation of the organic matter associated with one atom of phosphorus would require 276 atoms of oxygen, and would be accompanied by the release of one phosphate ion, 16 nitrate ions, and 106 molecules of carbon dioxide to the water. In the ocean, the intermediate steps are represented by the occurrence of ammonium and nitrite ions.

There are factors causing deviations from the expected oxidative ratio. One is that, in the euphotic zone, oxygen produced by photosynthesis may result in supersaturation of the surface layers, so that some oxygen would be lost to the atmosphere. Another main factor is that the water mass is likely to have contained some nutrients before it sinks, thus the total measured nutrient will then consist of both the 'preformed nutrient' and the 'regenerated nutrient'. The 'preformed nutrient' is that amount of nutrient that has not been derived from the decomposition of organic material but was present in the dissolved inorganic form when the water sank below the surface. Both factors can result in a lower oxidative ratios. The second possibility of deviations was illustrated in Broenkow's report on AOU values and nutrient concentrations in the Costa Rica Dome, a region of upwelling in the eastern tropical Pacific Ocean where deep waters of higher than normal salinities and nutrient levels but lower oxygen concentrations are brought to the surface. Broenkow (1965) found that the N:P value of 16.5:1 was independent of depth, while the AOU:P ratio was -274:1 for water above 120 meters and -114:1 for water below the depth. Since he was able to show that loss of oxygen to the atmosphere was significant only in the upper 20 meter depth, he explained that the AOU:P ratio observed below the 120 meter depth results from a mixture of equatorial subsurface water containing little preformed phosphate and intermediate water containing large amounts of preformed phosphate, rather than froma change in the oxidation ratio. His report shows that the preformed phosphate content increases from 0.65 to 1.25μg-at./l. between the 120 meter depth and the maximum sampling depth,which was 1100 meters below the sea surface.

Broenkow further showed the fact that the ratio N:P is constant over the complete range of observed depths support his idea which stated 'the change in the AOU:P relationship is caused by inequalities in the preformed phosphate distribution'.

Although he gave a remarkable explanation for the abrupt change in the ratio for the water at 120 meter depth, his report did not give data on the ratios for deeper water, which contain higher values of preformed phosphate, and I suspect that there should have an even bigger deviation of the ratio.

Besides deviations of the oxidative ratio, some recent data show doubt on Fleming's original C:N:P ratios of 106:16:1 (proposed by Fleming in 1940) as representive of plankton. Parsons et al. (1961) analysed eleven species of phytoplankton grown in culture and found only two containing N:P ratios near 16:1, while six contained as low as 6:1 ratios. Antia et al. (1963) studied coastal phytoplankton and found C:N:P ratios vary from 85:16.5:1 to 52:13.5:1. An oceanic population analysed gave ratios of 86:17:1 when nitrate was plentiful. The oxidative ratio O:P in this case would be only -240:1. These findings remain to be explained, though many values have been consistent with Fleming's ratios.

Uptake of nitrogenous compounds by phytoplankton has been studied extensively recently. Attention is directed mostly towards inorganic forms of nitrogen since the organic form such as amino acids do not appear to be satisfactory nitrogen sources for phytoplankton growth.

It is believed that before nitrogen is incorporated into the cellular material, nitrate is reduced stepwise within the plant cells through nitrite, hyponitrite, hydroxylamine and ammonium. The reduction process is endothermic, involving a free energy of 77.4 Kcals/mole at 25°C. Cooper (1937) wrote the equation as follows:

$$NO_3^- + H^+ + 2H_2O \rightarrow NH_4^+ + OH^- + 2O_2$$

It can be expected that both ammonium and nitrate are present in culture media, and ammonium is used preferentially by phytoplankton. Fig. 1 illustrates the variation in the concentrations of the two forms of nitrogen during the growth of the dinoflagellate Cachonina niei observed by Strickland et al. (1969). The increase in chlorophyll a resulting from plant growth was accompanied in early stages by a decrease in the ammonium concentration only; and on the sixth day, when the ammonium-nitrogen had been reduced to about 1 μg-at./l., nitrate and ammonium were both utilized.

Using the same cultures, Eppley et al. (1969) found that nitrate assimilation was associated with production of the enzyme nitrate reductase within the cell. The enzyme was absent at the beginning, as the stock cultures were grown on nitrite. On the eighth day, the cells were allowed to migrate to the surface by stopping the mixing; thus it was able to replace most of the original culture medium by sea water of low nitrate content without losing the phytoplankton. An almost complete disappearance of the nitrate reductase from the cells was noted. On the 13th day, nitrate was added to the culture, causing a dramatic rise in enzyme activity.

A suggestion was made that nitrate was consumed later than ammonium because the formation of nitrate reductase was repressed by the levels of ammonium initially present in the culture medium. In the case of the ocean, ammonium concentration is usually so low that repression does not occur. Eppley et al. (1969) showed that synthesis of nitrate reductase occurred during ammonium assimilation in three experiments, with levels of ammonium approximately 0.5, 1.0 and 0.5 μg-at./l., which are probably typical of oceanic water. It thus appears unlikely that ammonium assimilation commonly represses nitrate reductase synthesis in marine phytoplankton. I would believe this repression does occur in the Atlantic Ocean off New England in the late summer, when the ammonium level was higher than that of nitrate, as described by Vaccaro (1963). Eppley et al. (1969) emphasized the importance of the enzyme assay in studying the time course of phytoplankton blooms because it provides a sensitive measure of the initiation and cessation of nitrate assimilation.

Under natural conditions, the simultaneous uptake of ammonium and nitrate may be expected. Dugdale and Goering (1967), using [^{15}N] labelling, followed the assimilation of these nutrients by phytoplankton populations obtained from several sea areas. In all cases, both nutrients were taken up simultaneously and the average of the specific rates of dark and light nitrate uptake, $V\ NO_3^-$ was always lower than the average of the specific rates of ammonium uptake $V\ NH_4^+$. These observations support the idea that ammonium in the ocean is probably a rather important source of nitrogen for phytoplankton. It was also observed that the relative increases in nitrate assimilation occurred during periods with high nitrate concentrations in the euphotic zone, which may be caused by a higher nitrate reductase activity induced by the high nitrate levels.

Nutrient assimilation took place in the dark at a lower rate than in the light. For tropical phytoplankton, the specific rate of ammonium uptake in the dark was only 60% of the light value, and that of nitrate was 30%. Eppley and Coatsworth (1968) showed that light is involved in nitrate reduction within plant cells. Cultures of <u>Ditylum brightwellii</u> containing little nitrate reductase was observed. In the dark, the rate of uptake decreased over a period of two hours and 84% of the accumulated nitrate was recovered from the cells. In the light, the rate remained constant and only 44% could be recovered. No nitrite was detected; thus it was concluded that a light-induced reduction was occurring. This study appears to show that the effect of light on phytoplankton growth is not only related to the rate of photosynthesis in the plant cells but also to the rate of nutrient uptake by the organisms. Meanwhile, it seems to me that this might show that the rate of photosynthesis is directly related to the rate of nutrient uptake.

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* THE HYPERBOLIC RELATIONSHIP *

The absorption of phosphate and nitrate by pure cultures of a marine phytoplankton *Nitzschia closterium* was studied by Ketchum to determine what effect its growth might have upon the ratio of the nitrate and phosphate ions in the water. Ketchum (1939) observed that the rate of absorption of both nitrate and phosphate are dependent upon the nitrate concentrations in the medium with a hyperbolic relationship. That is to say, the nutrient is taken up very fast at low concentration and slowly at high concentration. Recently, it has been shown that this relationship applies to nitrite and nitrate assimilation by *Ditylum brightwellii*, and to nitrate and ammonium by a wide range of species in culture, (Eppley et al., 1969). It has been demonstrated that this applies in the case of uptake of nitrate and ammonium by natural phytoplankton communities.

The relationship is described in an equation:

$$v = \frac{-dS}{dt} = \frac{VmNS}{K_s^u + S}$$

Experimental results are illustrated in Fig. 2 and 3.

In the equation: v = rate of removal of nutrient from the medium.

S = concentration of nutrient.

N = cell population.

t = time.

Vm = the maximal specific uptake rate

$$V_m = v/N \text{ when } S \gg K \text{ .}$$

$$K_s^u = \text{the half-saturation constant.}$$

K_s^u has a value of S for an uptake rate of one half the maximal value. It provides a quantitative measure of the potential of a phytoplankton species to assimilate nutrients, especially at low concentrations.

Using a linear transformation of the equation, we can obtain values of the two constants:

$$S = NVm\ (S/v) - K_s^u.$$

It can be shown in the equation that for a constant N, S/v against S should be linear, the intercept on the abscissa corresponding to $-K_s^u$. Several features were suggested :

(1) a high K_s^u value for nitrate uptake is usually associated with a high value for ammonium uptake.

(2) larger species, which tend to grow more slowly, usually have higher K_s^u values.

(3) oceanic species usually have low K_s^u values, which would be in accord with their need to assimilate nutrients at the low concentrations present away from the land.

The features appear to apply in general, illustrating the useful application of the half-saturation constant. However, I find a contradiction in the data from Eppley et al. (1969). The report showed that the diatom *Rhizosolenia robusta* of 85 μ cell diameter has a K_s^u value of 9.3 for ammonium uptake while the large sized Coscinodiscus wailesii of 210 μ cell diameter has a K_s^u value of 5.5, which is in contradiction to his (2) suggestion. This deviation might have shown that the feature may not hold for very large sized species, while it applies well for the species of cell size from 5 to 50 μ in diameter.

The study for the uptake of phosphorus compounds by phytoplankton is not as detailed. It was noticed that phytoplankton in culture medium where the phosphate concentration is limiting continue to grow for a long time after the nutrient has been stripped from solution. Thus cells are able to accumulate phosphate in excess of their immediate requirements, so that they can utilize the storage at times of nutrient depletion in the external medium.

Unlike the nitrogen uptake, in which organic forms do not play a role, organic phosphate esters may also provide as a source in phosphate uptake, (Chu, 1946). It has been recently found that phytoplankton cells will produce an enzyme alkaline phosphatase under phosphate-deficient conditions, and the enzyme is suppressed when the external phosphate level is adequate for growth. This enzyme is responsible in enabling the assimilation of the phosphate portion of such esters. Glucose-6-phosphate, adenosine monophosphate, and α glycerophosphate, three types of ester, were found to be taken at the same rate by a given phytoplankton species, suggesting that there is a similar uptake mechanism. This ability in utilizing organic phosphate indicates that the recycling rate of phosphate in the euphotic zone would be greatly enhanced since complete remineralization was unnecessary. I would suggest this idea helps explain why phosphorus is less often a limiting factor for phytoplankton growth.

Two interesting demonstrations were made in the early work of Ketchum (1939). One is that the quantitative relationship between the rate of phosphate uptake by phytoplankton and the concentrations of the nutrient in sea water is also hyperbolic in form. The second one shows the phosphate uptake rates are also hyperbolically related to the nitrate concentration in the medium. However, these two relationships have not yet been elaborated, otherwise they might have been of much use in generalizing the hyperbolic relationship.

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Variation in the rate of uptake of nitrate by marine phytoplankton with nitrate concentration follows a hyperbola. Eppley and Thomas (1969) showed that the growth rates of Asterionella japonica and Chaetoceros gracile also vary hyperbolically with the nitrate level in the culture medium. This variation in specific growth rate with nutrient concentration is probably an important characteristic of species living in low nutrient environments, such as phytoplankton in the ocean, and may determine the geographical and seasonal distribution of species. If the specific rates of nutrient uptake V, and of cell growth, μ, are both described to be hyperbolic functions of the nutrient concentration in the medium, i.e.

$$V = \frac{VmS}{K_s^u + 1} \quad \text{and} \quad \mu = \frac{\mu_m S}{K_s^g + 1}$$

Then the relationship between the specific growth rate and the cellular nutrient content depends upon the relative magnitudes of the half-saturation constant for uptake K_s^u and for growth K_s^g. Using the steady state relationship $V = \mu Q$ where Q is the cellular nutrient content, hypothetical curves illustrating this were constructed (Fig. 4). The graph shows that the specific growth rate is independent of the cellular nutrient content only when the half-saturation constants are equal. Usually $K_s^u > K_s^g$, and a hyperbolic relationship occurs. The intercept corresponding to zero growth rate represents the minimum cellular nutrient content which will permit cell division.

Phytoplankton succession was explained by the application of half-saturation constants. Assuming that values of K_s^u are the same as their measured values of K_s^g, they calculated how the specific growth rates of four phytoplankton species would vary with the available nitrate and ammonium concentrations (Fig. 5). For example, the data show that at low nutrient levels, the growth of Coccolithus huxleyi would be favored; while at high levels, the diatoms would predominate. The hypothesis was supported as they actually observed changes in the phytoplankton in agreement off Southern California, where upwelling increases the nitrate levels of the surface waters.

The half-saturation constants attain their important role in explaining species succession and more evidence will be needed to prove of its general use.

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* SUMMARY *

As for the utilization of nitrogenous material by phytoplankton, ammonium and nitrate ions are taken up with comparable rates. Generally speaking, ammonium seems to be the preferred form. Recent work suggests that ammonium is an important nitrogen source serving to maintain the phytoplankton cell in a healthy state and provide much of the nitrogen used in reproduction when nitrate levels are low, whereas nitrate and nitrogen fixation are the most important parameters with respect to nitrogen limitation of primary productivity. It seems that the importance of ammonium for phytoplankton growth is going to be widely recognised, as opposed to the early belief that only nitrate and nitrite are the major sources of nitrogen in the ocean. Phosphorus is less often a limiting nutrient. Unlike the nitrogen cycle, organic phosphorus esters take part in the biocycle, resulting in a higher recycling rate.

The assimilation ratio, originally proposed by Fleming in 1940, has long been recognised. The ratio describes the biological effects of assimilation and regeneration of the nutrients. The fact that marine organisms contain in their protoplasm nitrogen and phosphorus in approximately the same ratio as they are found in the ocean would then be attributed to the adaptive ability of the biological system. The nearly complete assimilation of nitrate from the surface waters suggests that it may be an important nutrient limitation to the growth of phytoplankton. Phophorus is always present in some excess in the ocean, thus it would not be limiting.

Ketchum first found the hyperbolic relationship between the rate of nitrate uptake and the nitrate concentration in the culture medium. Demonstrations have been made to show its wide application to nitrate, nitrite and ammonium uptake by many species of phytoplankton.

In the studies on this hyperbolic relationship, 'the half-saturation constant' is recently introduced, (Eppley and Thomas, 1969), with which explanation of phytoplankton succession could be made. The application of this constant gains its significant advantage, since the measurements of uptake rate are much more easily carried out than growth rate determinations.

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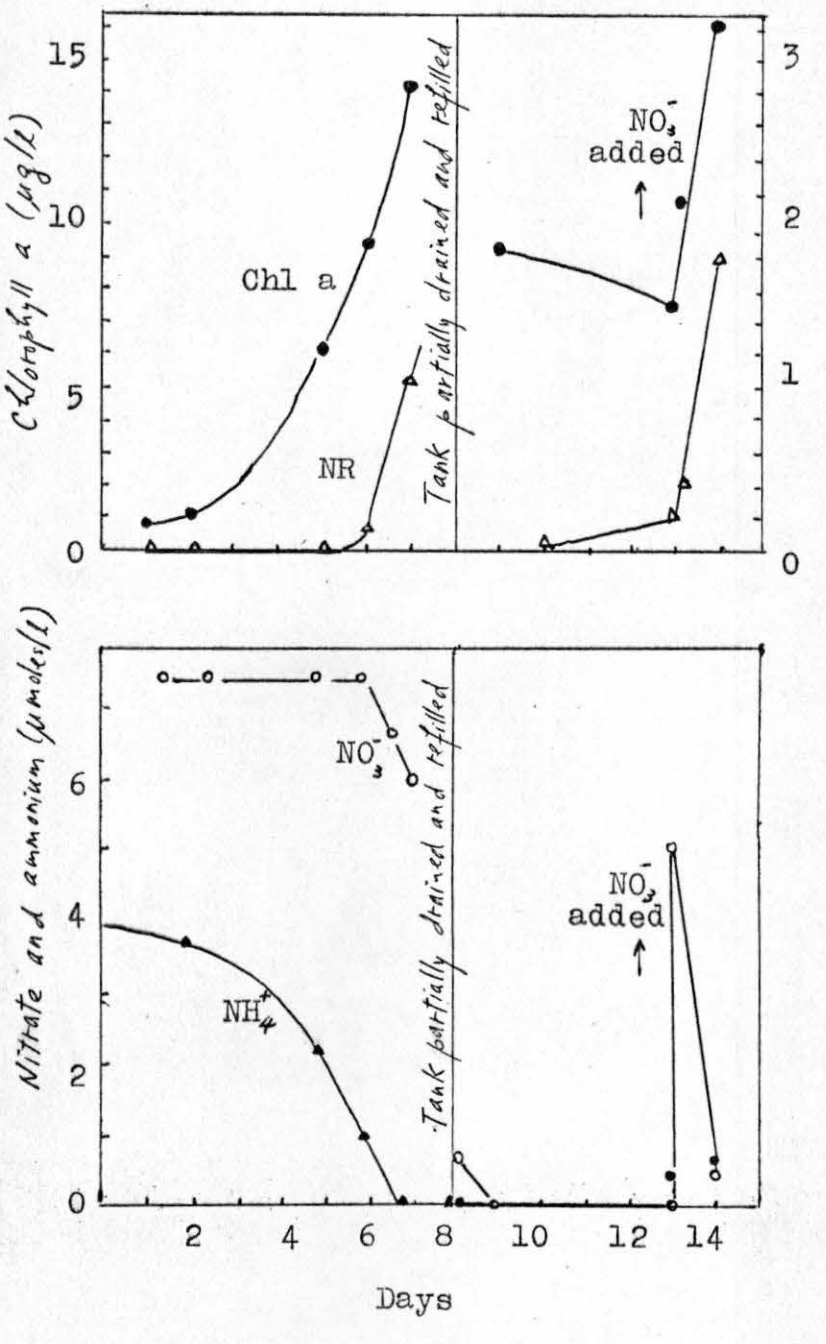
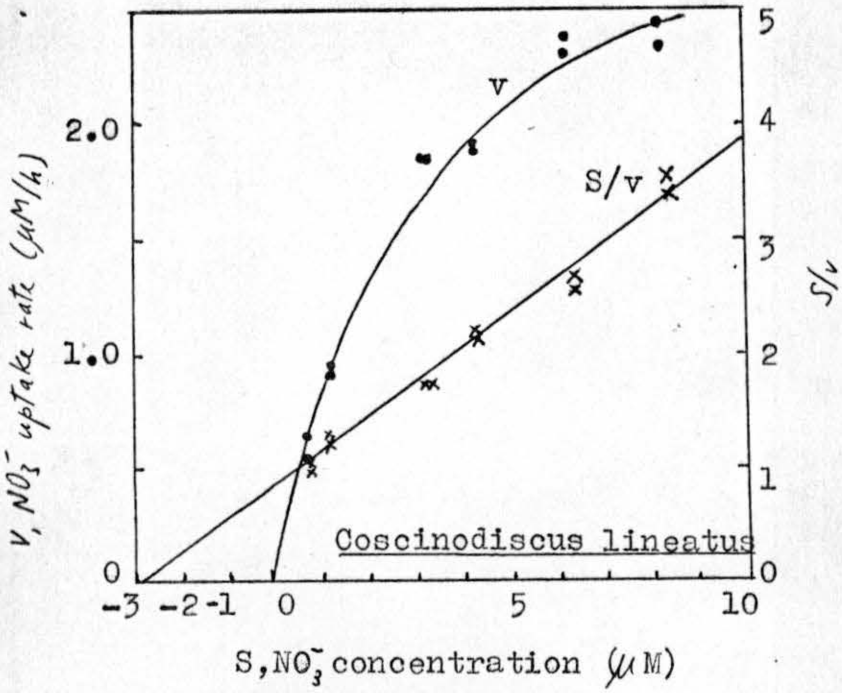


Fig.1. The variation in ammonium and nitrate ion concentrations and the production of chlorophyll a and nitrate reductase (NR) during the growth of *Cachonina niei* in the deep-tank cultures studied by Strickland et al. (1969).

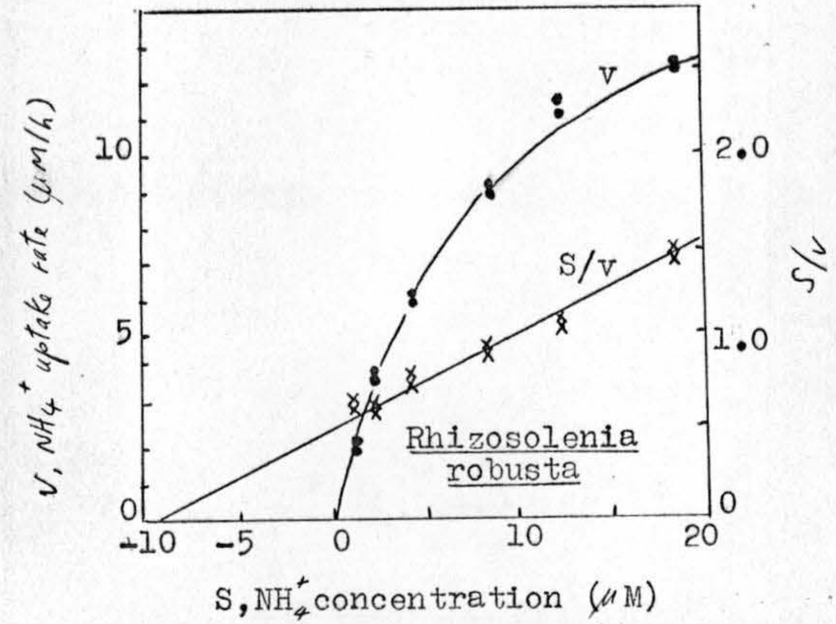
Fig. 2



The rate of nitrate uptake (v) by Coscinodiscus lineatus as a function of the nitrate concentration (S) in the medium.

Ref

Fig. 3



The rate of ammonium uptake (v) by Rhizosolenia robusta as a function of the ammonium concentration (S) in the medium.

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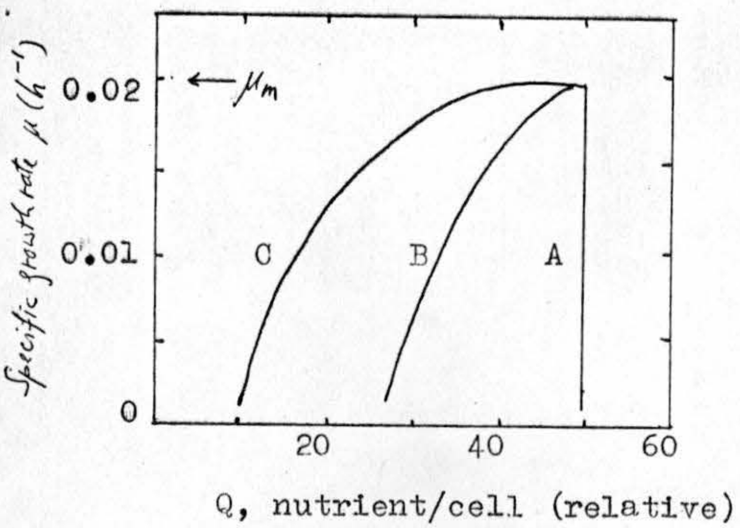

Fig. 4. This illustrates how the relationship between the specific growth rate, μ, and the cellular nutrient content, Q, depends upon the relative magnitudes of the half-saturation constants for nutrient uptake, K_s^u, and for growth, K_s^g. μ was assumed to vary with the nutrient concentration in the medium, S, according to the equation $\mu=\mu_m S/(K_s^g + S)$ where $\mu_m = 0.02\ h^{-1}$ and $K_s^g = 10\ \mu M$. The equation used for the rate of uptake was $V = V_m S/(K_s^u + S)$ with

(A) $V_m = 1\ h^{-1}$ and $K_s^u = 1\ \mu M$,

(B) $V_m = 1\ h^{-1}$ and $K_s^u = 2\ \mu M$,

(C) $V_m = 1\ h^{-1}$ and $K_s^u = 5\ \mu M$,

Only when $K_s^g = K_s^u$ is the value of μ independent of Q; for $K_s^u > K_s^g$, a hyperbolic relationship between μ and Q results.

Ref

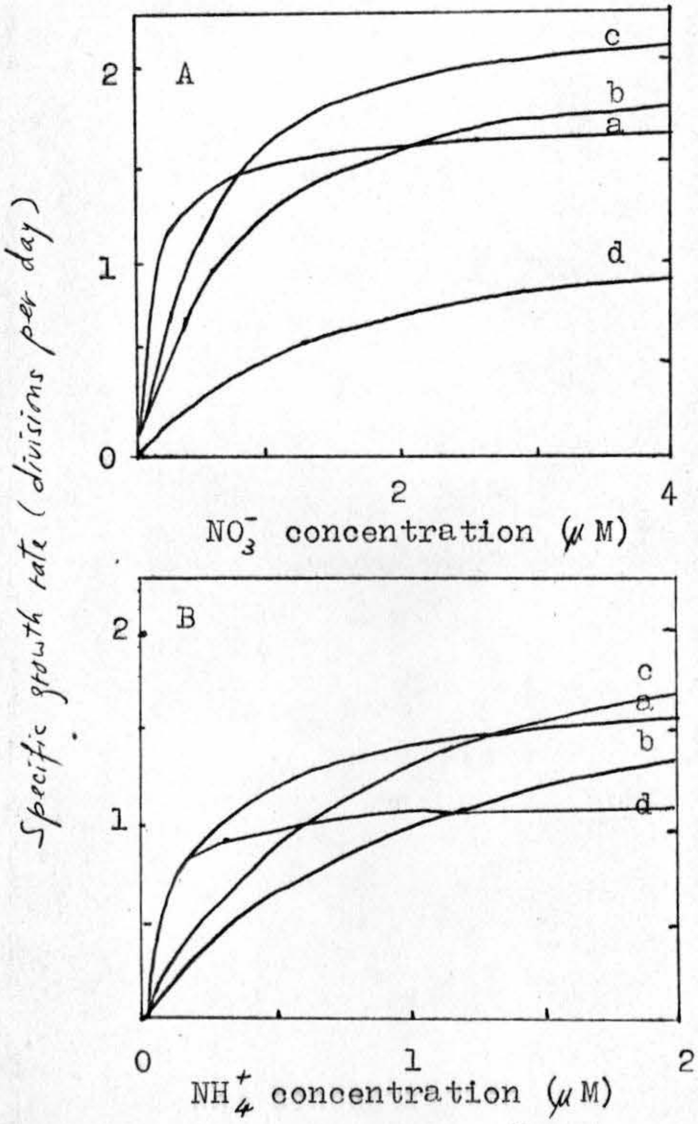

Fig. 5. Calculated specific growth rates of four marine phytoplankton species as a function of (A) nitrate and (B) ammonium concentration.

a - *Cocclithus huxleyi*,
b = *Ditylum brightwellii*,
c = *Skeletonema costatum*
d = *Dunaliella tertiolecta*.

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