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The kinetics of nitrate and ammonia uptake by natural populations of marine phytoplankton*

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Abstract—The response of natural marine populations of phytoplankton to nitrate and ammonia concentrations has been investigated using nitrogen-15 tracer techniques. Experiments made in the Bering Sea, the waters of southeastern Alaska, and the northeastern tropical Pacific suggest that the uptake of both compounds follows the Michaelis–Menten expression for enzyme kinetics. A hyperbola, therefore, describes the relationship between the concentration of nitrate or ammonia and the uptake of that nutrient. Such a hyperbola can be obtained easily in experiments with ammonia and, under suitable conditions, with nitrate.

The value of K_s, the nutrient concentration at which the maximum uptake rate is reduced by one-half, appears to be related to the nutrient and productivity regime of the region inhabited by the population. In the tropical oligotrophic area investigated, $K_s(NO_3) < 0{\cdot}2$ μg-atom/l., while in corresponding eutrophic regions, $K_s(NO_3) > 1{\cdot}0$ μg-atom/l. These values suggest that the phytoplankton populations of oligotrophic regions are adapted to the low ambient nutrient concentrations and are able to take up nutrients at a higher rate under these conditions than would phytoplankton possessing characteristic of eutrophic conditions and showing a higher value of K_s.

INTRODUCTION

The CONTROL of phytoplankton cellular growth rate by limiting nutrient concentrations is a critical element in the regulation of aquatic productivity. However, the exact nature of the relationship between specific nutrient concentrations and instantaneous growth rates has remained elusive. The successful application of Michaelis–Menten kinetics to problems of bacterial growth in chemostats (Monod, 1942) led Dugdale (1967) to apply this expression to Harvey's (1963, p. 96) data for phosphate uptake by algae, and to suggest that this expression eventually would be shown to hold for the uptake of nutrients by marine phytoplankton populations. Parsons and Strickland (1962) and Vaccaro and Jannasch (1966) previously had shown Michaelis–Menten kinetics to be applicable in some cases to natural populations of marine heterotrophs. Wright and Hobbie (1965), working with glucose and acetate in freshwater, obtained comparable results for bacteria but interpreted their results for algae to mean that these kinetics did not apply. The study of nitrate and ammonia uptake reported here was undertaken as a step in resolving this confusion regarding the applicability of the Michaelis–Menten expression to natural populations of marine phytoplankton.

METHODS

The results discussed below were selected from experiments conducted in Alaskan coastal waters and the northeastern tropical Pacific Ocean (Fig. 1). The Alaska

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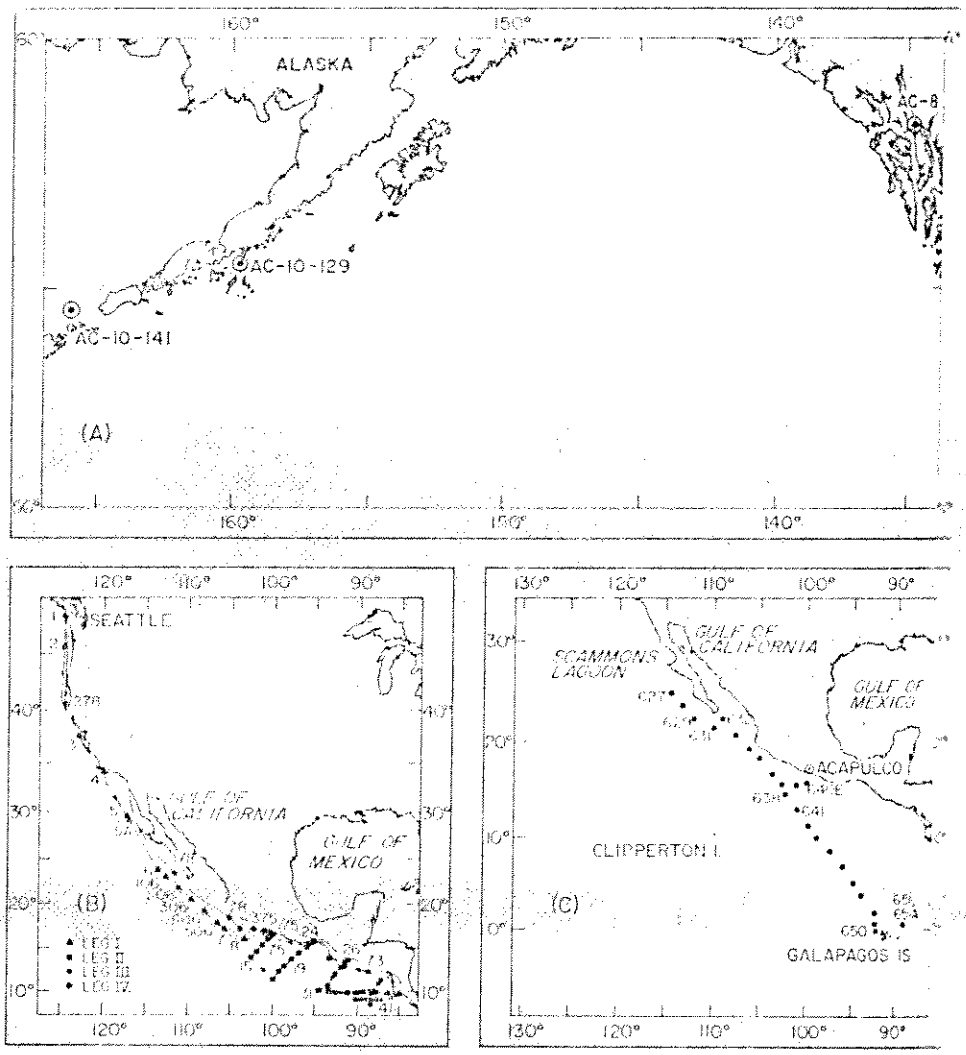

Fig. 1. Station locations from (A) *Acona* cruises 8 and 10, (B) *Thompson* cruise 26, and (C) *Te Vega* cruise 13.

experiments are from Stephen's Passage near Juneau, on the University of Alaska R.V. *Acona* cruise 8 in April 1965, and near the tip of the Alaska Peninsula on *Acona* cruise 10 in July 1965. The tropical measurements were made in February 1967 on Stanford University's R.V. *Te Vega* cruise 13, and in January and February 1968 on the University of Washington's R.V. *Thomas G. Thompson* cruise 26.

The basic experimental design involves exposing a population to several concentrations of nitrogen-15-labeled nitrate or ammonia, incubating for a period of time and analyzing by mass spectrometry for enrichment in the particulate nitrogen pool (NEESS, DUGDALE, DUGDALE and GOERING, 1962; DUGDALE and GOERING, 1967). Seawater was collected with large-volume nontoxic samplers and transferred to 4-liter

Pyrex serum bottles. Four to six bottles were prepared, each enriched with a different concentration of labeled nitrate or ammonia. These bottles usually were incubated for 6–24 hr in seawater-cooled incubators exposed to full sunlight. Neutral-density 50% and 25%-transmission nickel screens* were used to reduce the natural light intensity in certain experiments, and artificial lighting was used in the experiments on *Acona* 8. After incubation the bottle contents were filtered onto glass fiber filters (Hurlbut 984H ultrafilters) which were dried and held for mass spectrometry. The mass spectrometry for the Alaska and *Te Vega* 13 experiments was done at the Institute of Marine Science, University of Alaska, with a Bendix 17–210 time-of-flight instrument, and the *Thompson* 26 experiments were processed at sea with an Associated Electrical Industries MS-10 machine (180° analyzer, 5-cm radius). In all cases the samples were converted to gas by the automated Dumas procedure described by Barsdate and Dugdale (1965).

The limitations of the nitrogen-15 method are common to all tracer work to some degree, but it is well to review them specifically as much of the work discussed here involved conditions leading to minimal sensitivity of the method. The requirements for optimal mass spectrometry include the following: (1) The nitrogen compound initially should be enriched with the labeled form by at least 10%. (2) At the end of incubation the particulate nitrogen (PN) fraction of the sample should be enriched by at least 1% nitrogen-15. (3) The particulate sample size should contain at least 2 μg-atoms nitrogen to overcome background in the converting system. The first condition precludes measurements at natural nutrient concentrations, a significant problem when these concentrations are low. Ordinarily the latter two conditions may be met, respectively, by extending the incubation period sufficiently and by increasing the sample volume when practical. However, in measuring uptake rates related to specific nutrient concentrations it is important that the incubation period be kept minimal to avoid significant depletion of the nutrient. In the work reported here, small populations generally were encountered in areas with low nutrient concentrations, and it is a consequence of the tracer-technique restrictions that rates at these stations were the most difficult to measure.

The initial concentrations of nitrite and nitrate or ammonia and particulate nitrogen had to be determined for each experiment. Nitrite was measured in all cases by the method of Shinn (1941); cadmium-reduction columns were used to determine nitrate, cadmium–mercury amalgam (Grasshoff, 1964) in Alaska, and cadmium–copper on *Te Vega* 13 and *Thompson* 26 (Wood, Armstrong and Richards, 1967). Ammonia was measured by the Procházková (1964) extraction method. Particulate nitrogen was determined with a Coleman nitrogen analyzer. In the tropics, duplicate nitrate and triplicate ammonia samples were processed.

The Michaelis–Menten kinetic expression may be found in most biochemistry texts (e.g., White, Handler, Smith and Stetten, pp. 234–238, 1959):

$$V = \frac{V_{max} S}{K_m + S} \tag{1}$$

where V = velocity of uptake of substrate (in this paper, units of nitrogen taken up per unit time per unit PN, or time^{-1}); V_{max} = maximal velocity of uptake; S = concentration of substrate, or nutrient; and K_m = Michaelis–Menten constant and is

*Perforated Products, Inc., No. 15Gi, 50%; No. 40/10P, 25%.

that substrate concentration at which $V = V_{max}/2$. Rather than using the designation K_m, K_t or "transport constant" (Wright and Hobbie, 1966) is used to emphasize that a mathematical and not necessarily biochemical equivalence to Michaelis-Menten kinetics is being considered here. Using a computer program supplied by Dr. John Caperon, V_{max} and K_t were calculated directly from a hyperbola fitted to the data points by a least squares analysis. In this program, the assumption is made that zero uptake occurs at zero concentration. All calculations for the isotope work on *Thompson* 26 and all of the hyperbola calculations and plots presented here were performed on the *Thompson*'s shipboard IBM 1130 computer and Calcomp 30-in. plotter.

The estimate of V, velocity of uptake, is influenced by nitrogen-containing detritus in the manner discussed by Dugdale and Goering (1967). Briefly, detritus of this nature dilutes the phytoplankton nitrogen, thereby reducing the estimate of the nitrogen-15/nitrogen-14 ratio and of V. In an experiment using the same sample of seawater, all values of V will be reduced in the same proportion and V_{max} will be underestimated to the same degree. The value of K_t, however, is unaffected as it depends only upon the ratios of V and V_{max} whether they are apparent or real. This can be seen by rearranging equation (1):

$$\frac{V}{V_{max}} = \frac{S}{K_t + S} \tag{2}$$

Let

$$V_{(apparent)} = C \cdot V$$

$$V_{max\,(apparent)} = C \cdot V_{max}$$

where C is a dilution factor due to detritus; then

$$\frac{C \cdot V_{(apparent)}}{C \cdot V_{max\,(apparent)}} = \frac{S}{K_t + S}$$

$$\frac{V_{(apparent)}}{V_{max\,(apparent)}} = \frac{V}{V_{max}} = \frac{S}{K_t + S}. \tag{3}$$

In spite of the straightforward experimental method, it was not possible to obtain consistent results at low nutrient concentration until *Thompson* 26 and even this might not have been possible without shipboard mass spectrometry and the opportunity to adjust experiments with the previous results in mind. On the *Thompson*, a 2- to 3-day lag between experiment and result was still the best that could be managed. Stations 6–15 (Fig. 1b) were located in an unproductive region, characterized by a very deep euphotic zone with the 1% light level near 70 m and with extremely low concentrations of the major nutrients. Surface nitrate values of less than 0.05 μg-atom NO_3–N/l. were observed. Considerable patchiness of biological and physical parameters was encountered to the south, but generally speaking more productive waters began at Sta. 16 and continued through the last nitrogen-15 experiments at Sta. 38. The water was productive with the 1% light level occurring as shallow as 20 m and with nutrient-enriched water close to the surface.

The water used on *Thompson* 26 normally was taken from the 25% or 50% light depth, since a suppression of uptake of nitrogen compounds sometimes occurs in open ocean surface water (R. C. Dugdale, unpublished data), similar to the suppression known for carbon fixation. In areas of strong upwelling and high productivity,

suppression is not observed and thus shallower depths were selected near the Costa Rica Dome, Stas. 36 and 38, to avoid the nutrient-laden water close beneath. There was some question as to the best light intensity to use in incubating subsurface water. While it was necessary that light not be limiting in these experiments, there is evidence that surface-light intensities may suppress nitrogen uptake by deeper water in the open ocean (R. C. DUGDALE, unpublished data). Neutral-density screens passing 50% transmission were thus used through Sta. 15. However, an experiment at Sta. 16 in which 25% light depth water was incubated with 25%, 50% and 100% light indicated no significant differences in uptake with the different light intensities (Fig. 2), and it was assumed that full light could be used safely in the productive waters encountered for the remainder of the cruise.

The reliability of the nitrogen-15 method under conditions of a large and active particulate nitrogen pool is demonstrated by the experiment at Sta. 16. The lack of

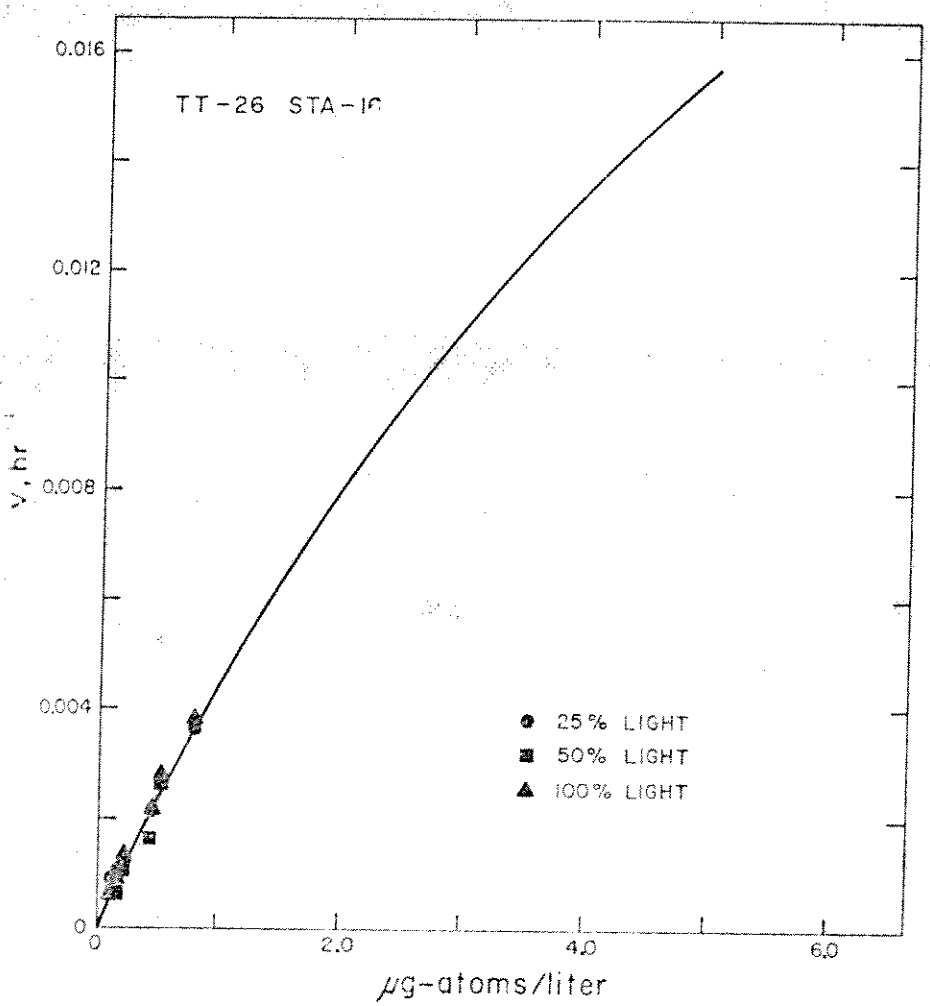

Fig. 2. V_{NO_3} as a function of nitrate concentration, incubated at three light levels.

significant differences among the responses to the three light intensities results in essentially triplicate determinations of uptake rate at each enrichment concentration, and their agreement is good.

RESULTS

The results of two nitrate-uptake experiments from *Thompson* 26 are plotted in Fig. 3. Station 7 is an example from the oligotrophic region while Sta. 38 is from the eutrophic area associated with the Costa Rica Dome. In both cases a hyperbola described by the Michaelis–Menten equation agrees very well with the data points. The differences between the two curves appear to be associated with the regional differences in overall productivity.

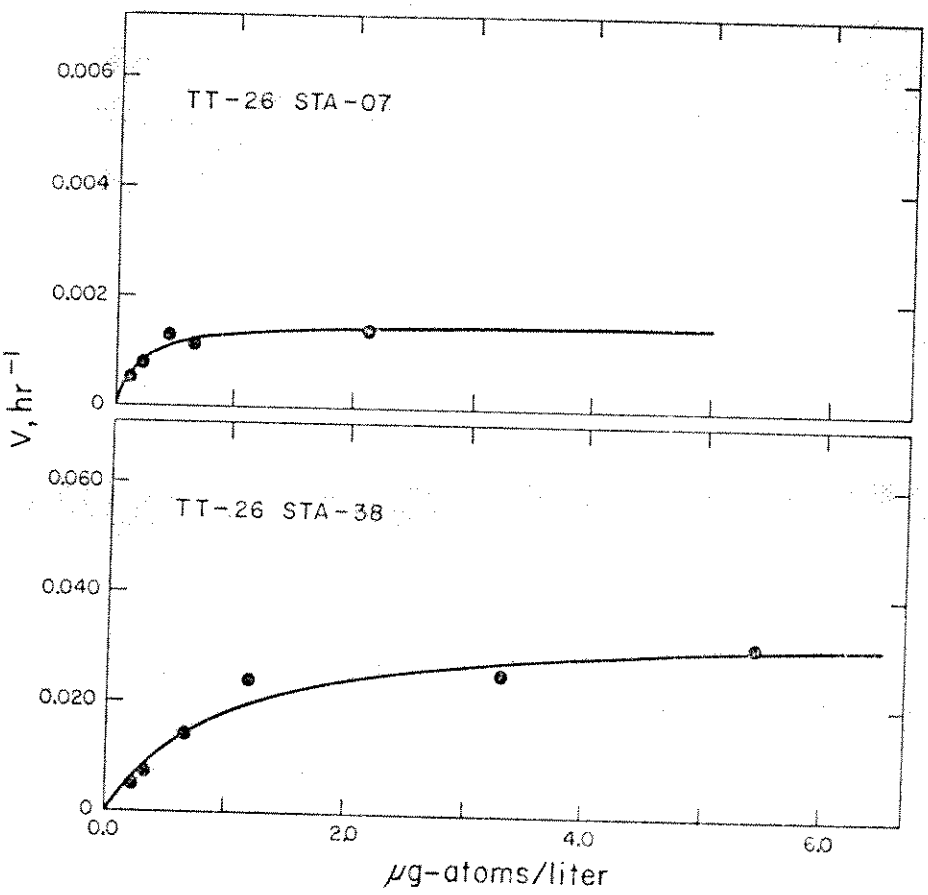

Fig. 3. Representative response curves for V_{NO_3} as a function of nitrate concentration, showing the characteristics of Michaelis–Menten kinetics.

The results obtained at Sta. 7 are exceptionally good when compared with other measurements in the unproductive areas encountered on both *Thompson* 26 and *Te Vega* 13. The combination of small populations and low uptake rates at the oligotrophic stations produced isotope enrichments that approached the limit of

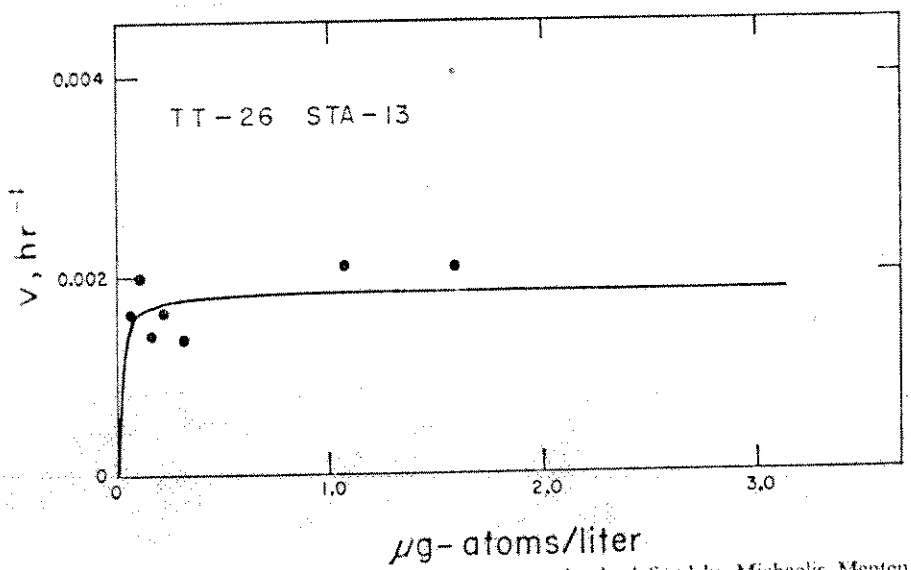

Fig. 4. V_{NO_3} as a function of nitrate concentration, not clearly defined by Michaelis–Menten kinetics.

mass spectrometer sensitivity. The data from *Thompson* 26 Sta. 13 (Fig. 4) illustrate the inconclusive results frequently obtained under these conditions. But if it may be assumed that there is only one uptake mechanism involved at all concentrations of the nutrient, and that zero uptake occurs at least at zero nutrient, then even with these data a curvilinear relation between concentration and uptake must occur. The value for V_{max} at Sta. 13 is indicated by several points, but it is difficult to determine a value for K_t. The statistically fitted hyperbola in Fig. 4 indeed may define the true K_t, but that value, 0·01 μg-atom NO_3 –N/L., is extremely low. Although there is an order of magnitude difference between the K_t for Sta. 7, 0·21 μg-atom NO_3 –N/L., and that for the Sta. 13, even small errors in the isotope-ratio measurements would account for this difference.

DUGDALE (1967) has discussed another condition that would lead to the underestimation of K_t. If, at some point below V_{max}, the uptake of the nutrient under investigation becomes limited by the concentration of another nutrient, i.e., becomes "other limited," only the portion of the curve at the very low end will be described by Michaelis–Menten kinetics. This condition is illustrated in Fig. 5. It is not always possible to distinguish between poor data and poor hyperbola fit, and if a K_t is calculated from an unrecognized other-limited curve, it will be underestimated.

The contrasting environment of the more productive regions alleviates the problems associated with mass spectrometry, but introduces other complications. *Thompson* 26 Sta. 38 (Fig. 3) represented ideal experimental conditions with its large and healthy phytoplankton population and low ambient nitrate concentration. The same situation existed at Sta. 16 (Fig. 2), but the enrichment concentrations were not made high enough to detect the V_{max} plateau. More frequently, areas supporting dense phytoplankton populations have high nitrate levels. Under these conditions there is no way to measure uptake at the low nitrate concentrations where uptake rate dependence is apt to occur. Experiment 1 on *Acoma* 8 is shown in Fig. 6 to illustrate the

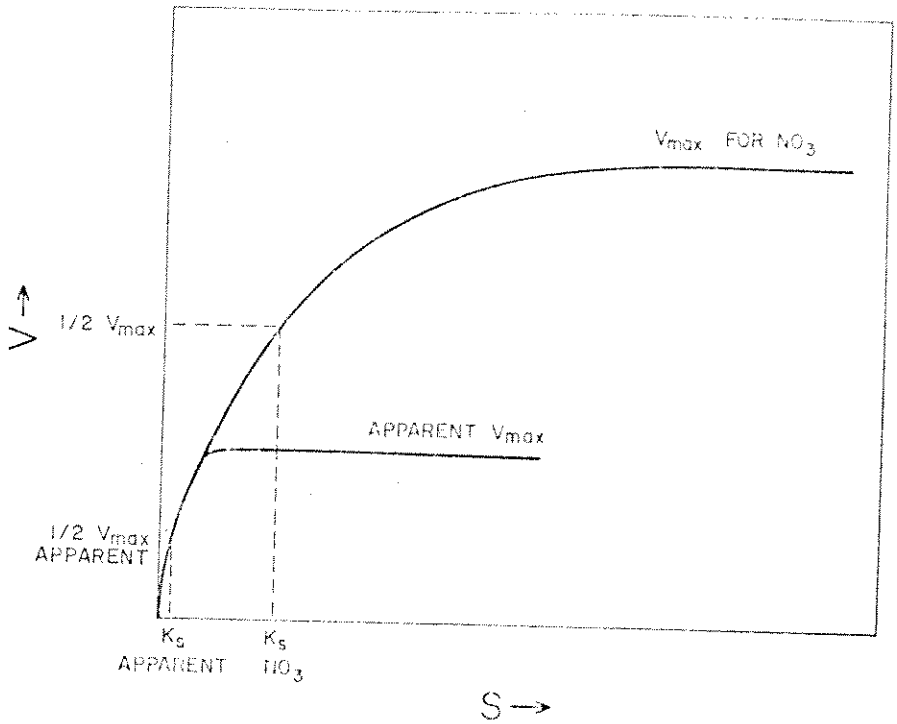

Fig. 5. Portrayal of limitation of V_{NO_3} by something other than nitrate concentration.

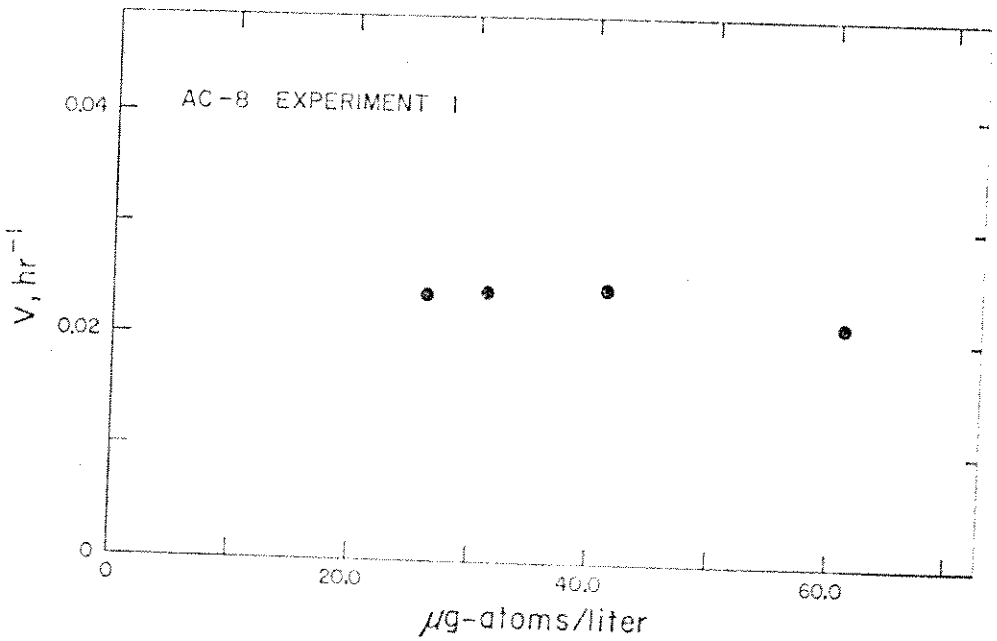

Fig. 6. V_{NO_3} as a function of nitrate concentration under conditions of high ambient nitrate levels.

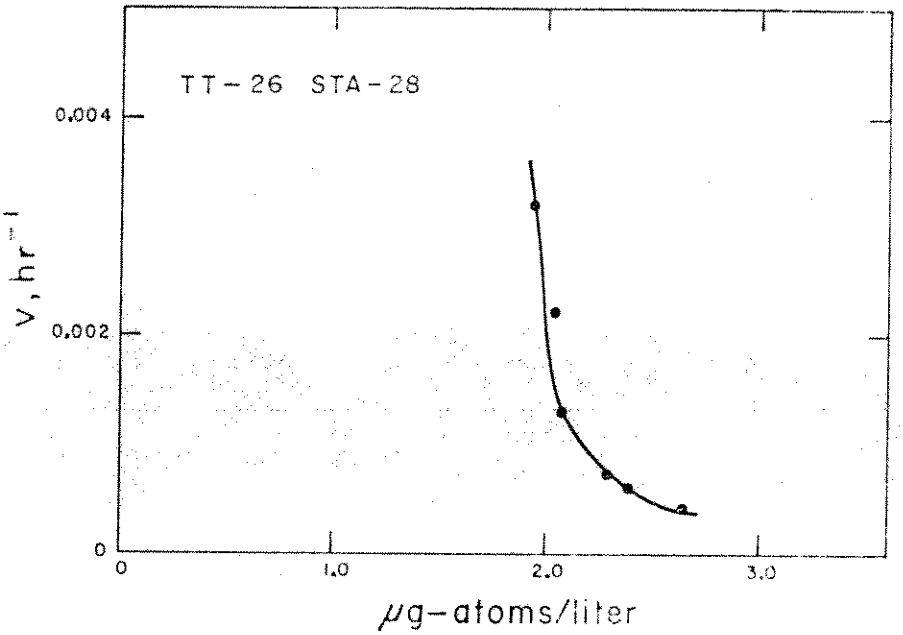

Fig. 7. Inhibition of V_{NO_3} with increasing nitrate concentration.

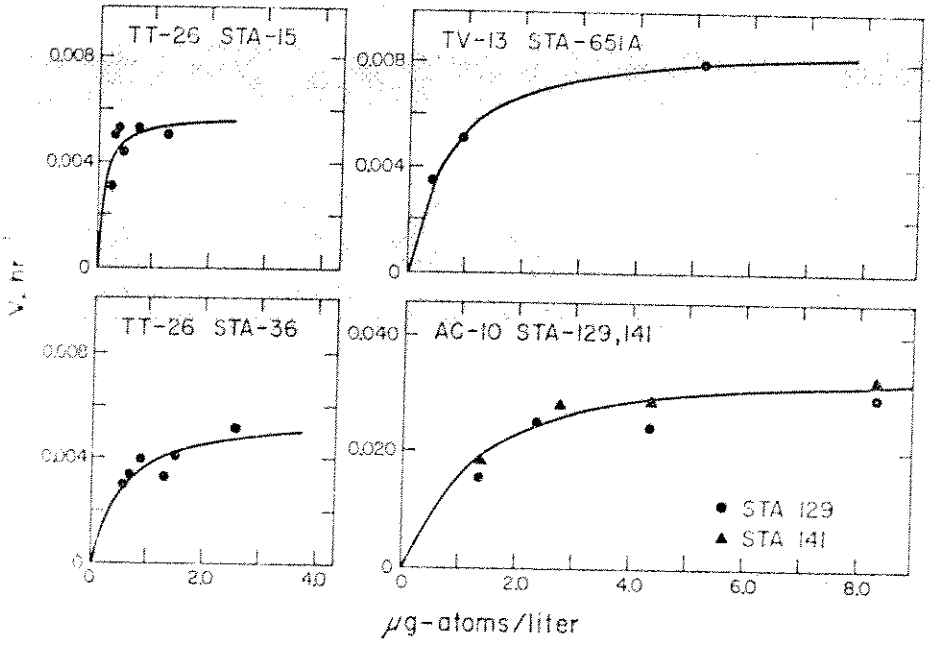

Fig. 8. Representative curves for V_{NH_3} as a function of ammonia concentration, showing the characteristics of Michaelis–Menten kinetics.

results from a nutrient-rich area. While V_{max} is well established in this case, no hyperbola can be fitted to the points and K_t cannot be calculated.

There have been several instances in which, rather than a positive or neutral influence of nitrate concentration on uptake rate, an actual suppression has been observed. An example of this type of response is *Thompson* 26 Sta. 28 shown in Fig. 7. Similar results were obtained in the Peru Current (R. C. Dugdale, unpublished observations). Common to both of these locations were ammonia concentrations of around 1 μg-atom NH_4^+–N/l., considerably above those of adjacent areas. In some preliminary work, H. L. Conway and B. M. Gallis (personal communication) have demonstrated an inhibiting effect of high levels of ammonia on nitrate uptake, dependent to some degree on the concentrations of both compounds. Although the critical range for the onset of this inhibition was 1·5–2·0 μg-atoms NH_4^+–N/l. in their experiments, ammonia control of the nitrate uptake must be considered as a likely explanation of the results at *Thompson* 26 Sta. 28 and the Peru Current station.

Examples of the ammonia-uptake curves are shown in Fig. 8. Variations similar to those of the nitrate hyperbolae (Fig. 3) appear in the ammonia curves and again a correlation of this characteristic with regional productivity is suggested.

Table 1. *Summary of field data, experimental conditions, and results for the nitrate-uptake experiments.*

Cruise	*Station*	*Initial* NO_3–N (μg atoms/l.)	PN–N (μg atoms/l.)	*Depth* (% *light*)	*Incubation* (% *light*)	V_{max} (hr^{-1})*	K_t (μg atom/l.)	*Incubation period* (hr)	
Thompson 26	6	0·03	0·87	25	50	0·0026	0·04	24	Oligotrophic
	7	0·02	0·87	25	50	0·0016	0·21	24	
	8	0·07	0·37	25	50	0·0010	0·01‡	24	
	13	0·02	0·82	25	50	0·0019	0·01‡	22	
Te Vega 13	642†	0	0·89	50	50			6	
	645†	3·41	0·36	50	50	0·0010	0·03‡	6	
	647	0	0·53	50	50	0·0074	0·14	6	
Thompson 26	16	0·02	4·73	25	25 50 100		at least 0·7	16	Eutrophic
	38	0·12	4·32	100	100	0·0361	0·98	6	
Acona 8	Exp. 1	21·10	3·69	100	artificial	0·0210		6	
	Exp. 2	7·20	5·77	100	artificial	0·0163	4·21	6	

*The hourly uptake velocity is based on the total incubation period and no attempt has been made to correct for the effect of reduced uptake during darkness in the longer experiments.
†Calculated together.
‡May be underestimated.

In Table 1, a summary of the field data, experimental conditions, and V_{max} and K_t where calculated is presented for all the nitrate experiments. According to the characteristics of the area where each occurred, the stations have been designated oligotrophic or eutrophic, and similarly designated stations have been grouped together. Table 2 contains the experimental ammonia data in similar form.

Table 2. Summary of field data, experimental conditions, and results for the ammonia-uptake experiments.

Cruise	Station	Initial NH_4^+–N (μg atoms/l.)	PN–N (μg atoms/l.)	Depth (% light)	Incubation (% light)	V_{max} (hr^{-1})*	k_t (μg atoms/l.)	Incubation period (hr)	
Thompson 26	15	0·20	0·56	25	50	0·0058	0·10	25	Oligotrophic
	36	0·46	1·85	50	100	0·0057	0·55	24	
Te Vega 13	651–a	0·18	0·77	10	50	0·0088	0·62	6	
Acona 10	129†	0·30		100	100			6	Eutrophic
	141†	0·33		100	100	0·0362	1·30	6	

*The hourly uptake velocity is based on the total incubation period and no attempt has been made to correct for the effect of reduced uptake during darkness in the longer experiments.
†Calculated together.

DISCUSSION

The data given above offer convincing evidence that the uptake of ammonia and nitrate by natural marine phytoplankton populations is dependent on the concentrations of these elements at their lower natural abundances. The possibility that the independence of uptake rate at higher concentrations is actually a repression of uptake imposed by some parameter other than nitrate or ammonia concentration has been examined in terms of light and other nutrients. It was established earlier in the paper that nonlimiting lighting was used throughout, with the possible exception of the artificially illuminated nitrate experiments in Alaska. The problem of other nutrients is not so easily resolved, as it is difficult to generalize about the supply of elements available in all situations; but the uptake results from pure culture experiments involving a highly enriched medium describe a hyperbola apparently related only to nitrate or ammonia concentration (J. J. MacIsaac, unpublished observations). In another experiment, the 24-hr rate of nitrate uptake at a nutrient-rich station

Table 3. Effect of other nutrients on the uptake of NO_3^-–N *at* Thompson 26, *Sta.* 30, 8 m *water. Basic addition of* 0·78 μg atom NO_3^-–^{15}N *made to each experiment.*

Nutrient	Approximate addition (μg atoms/l.)	Ambient concentration (μg atoms/l.)	V (hr^{-1})
NO_3^-–N (control)	0·78	0·06	0·0082
SiO_4 Si	4·5	0·50*	0·0070
PO_4^{3-}–P	1·0	0·16*	0·0082
Vitamins	μg/l.		
Thiamine	31		0·0084
B_{12}	0·006		
Biotin	0·31		
Trace metals (without Fe)†			0·0088
All above enrichments plus Fe‡	(as above)		0·0085

*Values taken from hydrocast chemistry at 10 m.
†Approximately 0·25 ml/l. of the following trace metal solution made up in 1 liter: 0·28 g H_3BO_3, 0·18 g $MnCl_2 4H_2O$, 0·18 g Na tartrate, 3·93 mg $CuSO_4 \cdot 5H_2O$, 2·08 mg $ZnCl_2 \cdot 2H_2O$, 6·4 mg $CoCl_2 \cdot 6H_2O$, 2·52 mg $Na_2MoO_4 \cdot 2H_2O$.
‡0·15 mg $FeSO_4 \cdot 7H_2O$ added per liter of sample.

(*Thompson* 26, Sta. 30) was not clearly influenced by additional enrichment with various nutrients. The data from this experiment are shown in Table 3. These results from culture and nature do not preclude the suppression of uptake by other limitations under different conditions or at the stations discussed here, but they do indicate that the processes of nitrate and ammonia uptake can be regulated solely by concentrations of these compounds.

The characterization of uptake by a low K_t would be of competitive advantage to species found in nutrient-poor waters, and especially for nitrate (Table 1) such a situation is found. The associated measurements of V_{max} are also low. The eutrophic stations are all characterized by higher K_t and V_{max} values. The role of detritus in leading to underestimation of V should be kept in mind.

It is interesting to note that the K_t values for nitrate measured in laboratory cultures correspond to those given here for the eutrophic regions. For example, in preliminary experiments using nitrogen-15, a K_t for uptake by *Cyclotella nana* of about 1·8 μg-atoms NO_3^-–N/l. and a V_{max} of approximately 0·02 hr^{-1} have been determined, and a K_t for uptake of 2·64 μg-atoms NO_3^-–N/l. can be calculated from Ketchum's (1939) data with *Phaedactylum tricornutum*. In a chemostat study of *Isochrysis galbana*, Caperon (1965) measured K_t values for growth of 2·13 and 2·83 μg-atoms NO_3^-–N/l. and respective V_{max} values of 0·053 and 0·026 hr^{-1}. The diverse sources of these data preclude more than their casual comparison, but the general agreement between uptake and growth K_t and V_{max} values is encouraging. Under low productivity conditions, uptake rates may be determined with far greater sensitivity than growth rates in terms of cell numbers, and it would be advantageous to establish the relation between these two rates.

As can be seen from Table 2, the ammonia data also indicate a distribution of K_t correlated with regional productivity. *Thompson* 26 Sta. 36, in spite of its location in the Costa Rica Dome, actually had the appearance of an unproductive situation, judging from some nitrate-uptake data associated with other work, and it is no doubt the depth of the sample at *Te Vega* 13 Sta. 651-a that makes it appear unproductive in an otherwise productive area. The absolute variability of the ammonia uptake values is not so great as for nitrate uptake and may be related to the relative constancy of ammonia concentrations in the euphotic zone over most of the oceans.

According to the theory of nutrient limitation advanced by Dugdale (1967), the data here may be interpreted to indicate the presence or absence of limitation of phytoplankton uptake or growth rates by the ambient nitrate or ammonia levels. At stations where uptake rate determined by the nutrient level in the water is a point along the plateau of the hyperbola, that nutrient may be assumed nonlimiting. Such a condition is illustrated clearly by the nitrate experiment from *Acoma* 8 shown in Fig. 6. When the ambient nutrient level determines a rate found along the slope of the hyperbola, that concentration may be considered limiting to uptake of that nutrient by the phytoplankton. The ammonia-uptake experiments all indicate this situation, and limitation by nitrate is suggested at *Te Vega* 13 Sta. 647, and at *Thompson* 26 Stas. 7, 16, and 38. At those *Te Vega* 13 and *Thompson* 26 oligotrophic stations where the uptake rates of nitrate are low and uncertain, the presence of limitation or nonlimitation cannot be evaluated.

There can be little argument that the direct uptake measurement of a nutrient under consideration is a powerful tool in studies of nutrient limitation, and where

techniques have been developed for such direct study they should be applied. It also appears that by approaching nutrient limitation from a consideration of uptake or growth rates, rather than of yields, insight into the nature of limitation may be obtained as well as evidence simply of its presence or absence. The combining of tracer technique sensitivity with a kinetic study has permitted a direct approach to problems of nitrogen utilization in natural phytoplankton populations, unique in being applicable to naturally occurring low nutrient levels.

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