

# HALF-SATURATION CONSTANTS FOR UPTAKE OF NITRATE AND AMMONIUM BY MARINE PHYTOPLANKTON<sup>1</sup>

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## ABSTRACT

Uptake rate of nitrate and ammonium was studied as a function of nitrate or ammonium concentration with cultures of 16 species of marine phytoplankton. Half-saturation constants (the concentration supporting an uptake rate one-half the maximum rate) were computed as a measure of the ability of a species to use low levels of nitrate and ammonium. The half-saturation constant ( $K_s$ ) varied approximately in proportion to cell size and inversely with specific growth rate. Small-celled oceanic species, such as *Coccolithus huxleyi*, showed the lowest  $K_s$  values ( $\leq 0.5 \mu\text{M}$ ) and *Gonyaulax polyedra* the highest ( $K_s > 5 \mu\text{M}$ ). The  $K_s$  values are considered to be important characteristics of organisms living in nitrogen-limited environments.

Some apparent success was achieved in predicting the competitive advantage of one species over another by calculating specific growth rates as functions of nitrate and ammonium concentration for species with known growth response to irradiance, temperature, and daylength. For example, at about 20°C and 16 hr daylength *C. huxleyi* should be able to compete successfully with *Skeletonema costatum* when nitrate, ammonium, and irradiance are low. *Skeletonema costatum* should grow faster than *C. huxleyi* only when irradiance and nitrate or ammonium are fairly high. Rock pool forms appear to be relatively unsuccessful in the ocean because of a sluggish response to irradiance and not because of an inability to use low levels of nitrate and ammonium. The success of *G. polyedra*, often the dominant dinoflagellate in local red water blooms, appears to be due to its vertical migration from the sea surface in daylight to nitrate-rich water at 10–15-m depth at night, rather than to any special ability to grow rapidly or use low levels of nitrate and ammonium.

## INTRODUCTION

Uptake rates of nitrate or ammonium by marine phytoplankton give hyperbolas when graphed against the nitrate or ammonium concentration in the environment (Dugdale 1967; Eppley and Coatsworth 1968; MacIsaac and Dugdale 1969). Half-saturation constants (the concentration supporting half the maximum uptake rate) can be calculated for the hyperbolas using the Michaelis-Menten equation:

$$v = V_m S / (K_s + S),$$

where  $v$  is rate and  $S$  is concentration, to allow quantitative comparisons between species. This constant ( $K_s$ ) reflects the relative ability of phytoplankters to use low levels of nutrients and thus may be of ecological significance.

Another characteristic of the uptake hyperbolas, the maximum velocity ( $V_m$ ), is

also of value for ecological interpretations (Dugdale 1967).  $V_m$  is best taken as the maximum specific growth rate of the organism imposed by external conditions, rather than from measured uptake rates uncoupled from growth, and is thus a variable subject to effects of irradiance, daylength, and temperature as well as species-specific properties of the organism.

The half-saturation constant, or  $K_s$ , following the terminology of Epstein and Hagen (1952), varies with temperature (Carter and Lathwell 1967), but we will assume that  $K_s$  is not influenced by irradiance or the other external factors influencing the growth rate of photosynthetic organisms. For example, the same  $K_s$  for nitrate was found for exponentially growing cells and for N-depleted cells with different  $V_m$  values (Eppley and Thomas 1969).

This account provides  $K_s$  values for nitrate and ammonium uptake by several species of marine phytoplankton. These values are then used with existing data on the variation of specific growth rate with

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irradiance to compute the relation between specific growth rate and nitrate or ammonium concentration at a low and at a high irradiance relative to sunlight in the euphotic zone. The calculations are restricted to 20C, 16 hr sunlight per day, and to only four species for which data were available. Finally, environmental conditions suggested by the calculations to be favorable for these species are compared with observed local distributions.

We thank Miss E. Fuglister for computer programming and statistical analysis of the data.

#### METHODS

##### *Uptake measurements*

Cultures were grown in an enriched seawater medium with nitrite as the nitrogen source at 25  $\mu\text{M}$  concentration. Growth of the crops was limited by N-depletion (nitrite no longer detected in the medium) when crop chlorophyll *a* reached about 50  $\mu\text{g/liter}$ . The cultures were then divided into aliquots and enriched with nitrate or ammonium at the beginning of the experiments. The aliquots were incubated for 15–120 min at 18C with fluorescent light at about 7,500 lux (in the same incubator used for culturing), with occasional shaking, until the cells were harvested by filtration. The cell-free filtrates were then analyzed for nitrate and ammonium and uptake was calculated as the difference between the concentration added and that remaining at the end of the experiment. Incubation times were designed to give about 50% uptake of substrate at low substrate concentration or 2  $\mu\text{moles/liter}$  uptake at higher levels. Uptake of ammonium was linear with time, but nitrate uptake showed a lag phase. The lag phase was avoided in these N-depleted cells by a preincubation of 3–4 hr with 1  $\mu\text{M}$  added nitrate; subsequently uptake was linear with time. An earlier value of  $K_s$  for nitrate uptake by *Ditylum brightwellii* (Eppley and Coatsworth 1968) was an overestimate because we were unaware of the lag problem. A revised value is given here.

Nitrate was determined as nitrite, after reduction by passage through a column of

copper-coated cadmium metal. We are indebted to several workers involved in the evolution of this method, namely Morris and Riley, Grasshof, and Wood, Armstrong, and Richards (*see* Strickland and Parsons 1968). We compensated for the slight variation in efficiency of reduction with nitrate concentration in our columns by using nitrate standards at several concentrations. Ammonium was determined by Johnston's (1966) modification of the bispyrazolone method (Procházková 1964).

A linear transform of the Michaelis-Menten equation:

$$S = V_m (S/v) - K_s$$

was used to calculate  $K_s$  and  $V_m$  and their 95% confidence limits by a regression analysis. In this transform,  $K_s$  is given as the Y-intercept and  $V_m$  as the slope of the regression equation of  $S$  on  $(S/v)$ . The  $(S/v)$  vs.  $S$  plot was used in preference to other linear transformations because it gave the best spread of the experimental points. Only 10–14 values were obtained in each experiment (5–7 concns with duplicate determinations), so sample size was small for this kind of statistical analysis and confidence limits are large. In some cases  $v$  vs.  $(v/S)$  transforms of the hyperbolas could be used, but only where the half-saturation constant was large compared to the lowest nitrate or ammonium concentration tested. In these cases, the  $K_s$  and  $V_m$  estimates and their confidence limits were essentially the same as those reported. Lineweaver-Burk  $(1/v)$  vs.  $(1/S)$  plots were not considered suitable for accurate determinations of  $K_s$  and  $V_m$  for the reasons given by Dowd and Riggs (1964). Since at least two replicate experiments were carried out with each organism, we tried combining data from different experiments as a means of increasing sample size. However, the confidence limits were not appreciably reduced, and we consider that most of the variation is experimental.

##### *Experimental organisms*

Since the half-saturation constants of nutrient uptake may be under genetic control and conceivably could be subject to

TABLE 1. *Experimental organisms*

Organism	Clone	Isolator	Location	Date of isolation*
<i>Coccolithus huxleyi</i>	BT-6	R. Guillard	Sargasso Sea	1960
<i>C. huxleyi</i>	F-5	E. Paasche	Oslo Fjord	1959
<i>Chaetoceros gracilis</i>	—	W. Thomas	Costa Rica Dome	1958
<i>Cyclotella nana</i>	13-1	R. Guillard	Sargasso Sea	1958
<i>Skeletonema costatum</i>	Skel.	R. Guillard	Long Island Sound	1956
<i>Leptocylindrus danicus</i>	FCRG 11	J. Jordan	Off La Jolla	1968
<i>Rhizosolenia stolterfothii</i>	FCRG 3	E. Paasche	Off La Jolla	1966
<i>R. robusta</i>	FCRG 13	J. Jordan	Off La Jolla	1968
<i>Ditylum brightwellii</i>	—	T. Smayda	Narragansett Bay (?)	<1963
<i>Coscinodiscus cf. lineatus</i>	FCRG 12	J. Jordan	Off La Jolla	1968
<i>C. wailesii</i>	Cos. R.	R. Holmes	Off La Jolla	1965
<i>Asterionella japonica</i>	—	W. Thomas	San Diego Bay	1963
<i>Gonyaulax polyedra</i>	FCRG 8	J. Jordan	Off La Jolla	1968
<i>Gymnodinium splendens</i>	Gy.	B. Sweeney	Off La Jolla	<1951
<i>Monochrysis lutheri</i>	Mono.	M. Droop	Rock pool	<1953
<i>Isochrysis galbana</i>	Iso.	M. Parke	Rock pool	<1940
<i>Dunaliella tertiolecta</i>	Dun.	B. Foyn	Oslo Fjord	1928

\* The symbol < is used to mean *before*.TABLE 2. *Half-saturation constants for uptake ( $K_s$ ) of nitrate and ammonium by cultured marine phytoplankton at 18C.  $K_s$  units in  $\mu\text{moles/liter}$* 

Organism	Nitrate		Ammonium		Cell diam* ( $\mu$ )
	$K_s$	$\pm 95\%$ conf. limit	$K_s$	$\pm 95\%$ conf. limit	
Oceanic species					
<i>Coccolithus huxleyi</i> BT-6	0.1	0.3†	0.1	0.7	5
<i>C. huxleyi</i> F-5	0.1	1.6	0.2	0.9	5
<i>Chaetoceros gracilis</i>	0.3, 0.1	0.5, 0.2	0.5, 0.3	0.5, 0.3	5
<i>Cyclotella nana</i> 13-1	0.3, 0.7	0.4, 0.5	0.4	0.3	5
Neritic diatoms					
<i>Skeletonema costatum</i> ‡	0.5, 0.4	0.4, 0.1	3.6, 0.8, 0.8	0.8, 0.7, 0.5	8
<i>Leptocylindrus danicus</i>	1.3, 1.2	0.5, 0.1	3.4, 0.9, 0.5	1.4, 0.2, 0.4	21
<i>Rhizosolenia stolterfothii</i>	1.7	0.4	0.5, 0.5	0.9, 0.4	20
<i>R. robusta</i> §	3.5, 2.5	1.0, 1.0	5.6, 9.3	2.0, 1.5	85
<i>Ditylum brightwellii</i>	0.6	1.7	1.1	0.6	30
<i>Coscinodiscus lineatus</i>	2.4, 2.8	0.5, 0.6	2.8, 1.2	2.6, 1.0	50
<i>C. wailesii</i>	2.1, 5.1	0.3, 1.8	4.3, 5.5	5.4, 2.0	210
<i>Asterionella japonica</i>	0.7, 1.3	0.3, 0.5	1.5, 0.6	1.2, 0.8	10
Neritic or littoral flagellates					
<i>Gonyaulax polyedra</i>	8.6, 10.3	—, 2.4	5.7, 5.3	0.6, 1.1	45
<i>Gymnodinium splendens</i>	3.8	0.9	1.1	1.0	47
<i>Monochrysis lutheri</i>	0.6	0.3	0.5	0.4	5
<i>Isochrysis galbana</i>	0.1, 0.1	0.2, 0.2	—	—	5
<i>Dunaliella tertiolecta</i>	1.4	1.1	0.1	0.6	8
Natural marine communities (from MacIsaac and Dugdale 1969)					
Oligotrophic	$\leq 0.2$ (6 expts)		0.1–0.6 (3 expts)		
Eutrophic	$\geq 1.0$ (3 expts)		1.3 (1 expt)		

\* Geometric mean diameter rounded off to the nearest micron.

† This notation means that  $-0.2 < K_s < 0.4$ . Negative  $K_s$  values have no physical interpretations.‡ At 28C,  $K_s$  for nitrate uptake was  $1.0 \pm 0.5$ ; at 8C, it was  $0.0 \pm 0.5$ .

§ An oceanic species according to Cupp (1943).

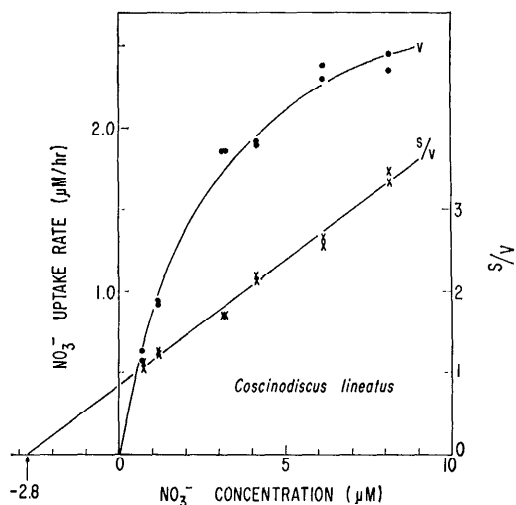


FIG. 1. Nitrate uptake rate ( $v$ ) of *Coscinodiscus lineatus* vs. nitrate concentration ( $S$ ) of the medium (●—●). The half-saturation constant ( $2.8 \mu\text{M}$  in this case) is given as the negative  $S$ -intercept of the linear regression of ( $S/v$ ) vs.  $S$  (x—x). Velocity units are  $\mu\text{moles/liter hr}$  ( $\mu\text{M/hr}$ ).

selection under conditions of laboratory culture, it seems important to identify the organisms as completely as possible and to indicate the approximate date of their original isolation from the sea (Table 1).

#### RESULTS

A list of the half-saturation constants ( $K_s$ ) is provided in Table 2 along with 95% confidence limits. Where only one value is recorded, data for two experiments were combined for the regression analysis. In most cases two values are listed and represent different experiments, usually with the same culture on successive days following nitrite depletion. The two values usually agreed fairly well, but poor agreement was sometimes found, as with *Coscinodiscus wailesii* with nitrate and *Rhizosolenia robusta* with ammonium. These larger organisms were particularly difficult to work with; the cells of *C. wailesii* tended to stick together and to the glass walls of the vessel. With *Skeletonema costatum* and *Leptocylindrus danicus* ammonium uptake experiments, a high  $K_s$  ( $>3.0 \mu\text{M}$ ) was found on the first day and lower values on

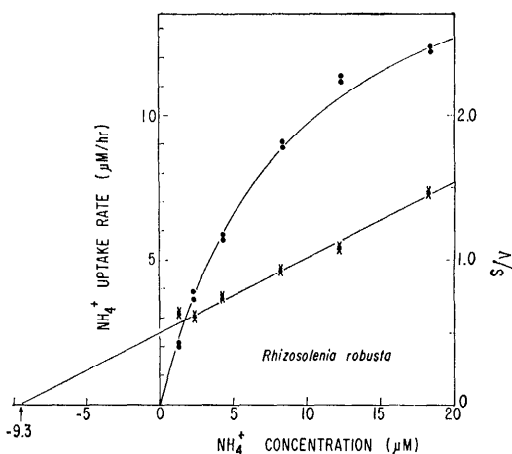


FIG. 2. Ammonium uptake rate ( $v$ ) vs. ammonium concentration ( $S$ ) for *Rhizosolenia robusta* (●—●).  $K_s$  was  $9.3 \mu\text{M}$  from the linear ( $S/v$ ) vs.  $S$  plot (x—x).

the second and third day after nitrite depletion from the growth medium. This was not observed with other organisms and we have no explanation for it.

Graphs of nitrate uptake by *Coscinodiscus lineatus* (Fig. 1) and ammonium uptake by *R. robusta* (Fig. 2) show convincing hyperbolas, as the  $K_s$  was high compared with nutrient concentrations supplied. Data typical of species with low  $K_s$  are shown for *S. costatum* (Fig. 3). Here most of the points approach  $V_m$  with no points at concentrations less than  $K_s$ , due to the limited sensitivity of the analytical methods. MacIsaac and Dugdale (1969) also encountered this problem. In this case the extrapolation of  $K_s$  depends entirely on the assumed linearity of the  $S$  vs. ( $S/v$ ) regression line.

Some trends can be seen in the  $K_s$  data. 1) Organisms with a high  $K_s$  for nitrate uptake usually have a high  $K_s$  for ammonium uptake as well. 2) Large-celled species tend to show high  $K_s$  values. 3) Oceanic species (all small-celled in these experiments, except *R. robusta*) show low  $K_s$  values. 4) Although we lack specific growth rate data for most of these organisms (except when grown in continuous light which does not always permit maximum rates), it appears that fast-growing species tend to have lower  $K_s$  values than slow growers. In

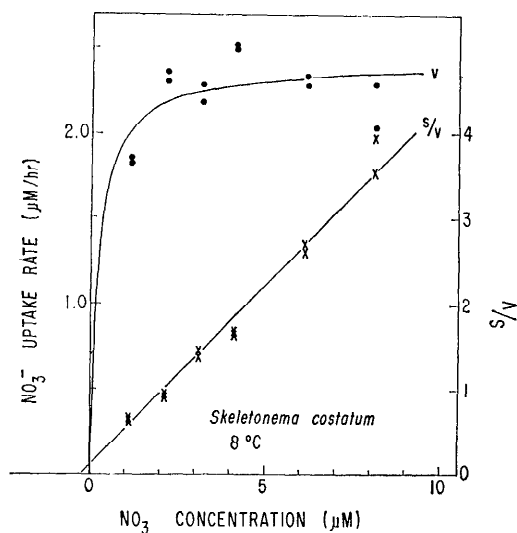


FIG. 3. Nitrate uptake rate ( $v$ ) vs. nitrate concentration ( $S$ ) for *Skeletonema costatum* (●—●). The ( $S/v$ ) vs.  $S$  plot (x—x) indicates a  $K_s$  about  $0.4 \mu\text{M}$  nitrate.

general, phytoplankton growth rates decrease with increasing cell size (Williams 1965; Eppley and Sloan 1966). The rock pool and estuarine species, *Monochrysis lutheri*, *Isochrysis galbana*, and *Dunaliella tertiolecta*, show low  $K_s$  values in spite of their abundance in rock pools which one would expect to be rich in nitrogenous nutrients. Other characteristics possibly limiting their distribution are discussed later.

Uptake rates of cultures grown on nitrite to N-depletion were high compared with growth rates, both for nitrate and ammonium. For example, the specific growth rate during culturing was 0.7–0.9 doublings/day for *R. robusta*, but the corresponding rates calculated from rates of short-term uptake and the nitrogen content of the cells would have been 8 and 13 doublings/day for nitrate and ammonium uptake, respectively. Similarly, the observed specific growth rate of *Gonyaulax polyedra* was 0.4–0.5 doublings/day, although 1.5–4 doublings/day were calculated from uptake rates. Thus these experiments to determine  $K_s$  give no useful information on exponential growth rates of N-sufficient cells but only confirm

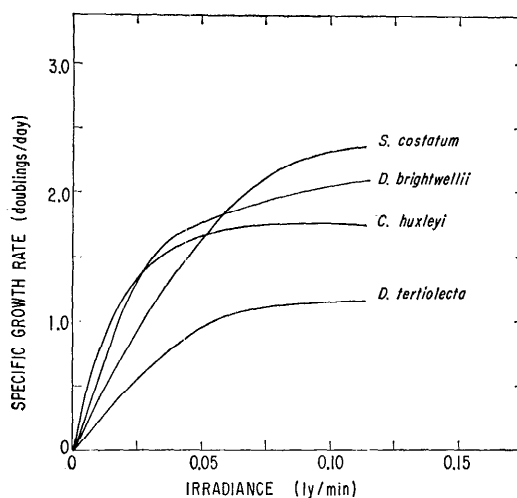


FIG. 4. Specific growth rate as a function of irradiance for *Coccolithus huxleyi* (Paasche 1967), *Skeletonema costatum* (Jitts et al. 1964), *Ditylum brightwellii* (Paasche 1968) and *Dunaliella tertiolecta* (Eppley and Coatsworth 1966). All at 20–21°C. The langley is  $1 \text{ cal/cm}^2$ .

earlier observations that N-deficient cells have high rates of nitrogen uptake (Fitzgerald 1968; Syrett 1962).

#### DISCUSSION

It is difficult to be certain that the  $K_s$  for growth is accurately measured by such short-term uptake experiments. For example, Droop (1968) calculated a 20-fold greater  $K_s$  for short-term uptake of vitamin B<sub>12</sub> by *M. lutheri* than for growth in a chemostat culture; variation of growth rate with the nutrient concentration in the cell, independent of that in the medium, was noted in Droop's vitamin study, in phosphate-limited cultures of *Phaeodactylum tricornutum* (Kuenzler and Ketchum 1962), and in nitrate-limited chemostat cultures of *I. galbana* (Caperon 1968). However,  $K_s$  for uptake in our experiments agreed well with independent measurements of  $K_s$  calculated from exponential growth rates of batch cultures limited by nitrate (Eppley and Thomas 1969) and are consistent with the values observed with 24-hr incubations of samples of natural communities of oceanic and neritic phytoplankton based on uptake of <sup>15</sup>N-labeled nitrate and ammo-

nium (MacIsaac and Dugdale 1969). A more detailed discussion is given elsewhere (Eppley and Thomas 1969).

If these measurements are to be of any ecological value, other than emphasizing the obvious fact that oceanic phytoplankton possess mechanisms for using very low levels of nitrogenous nutrients, they must help explain observed phytoplankton species distribution, for the uptake  $K_s$  values have little meaning for ecology in themselves. Hence we have used the values to calculate specific growth rates of different species in idealized conditions that we think manifest the essential features of natural waters. For this we needed not only the  $K_s$  for nitrogen uptake but measurements of specific growth rate, with adequate nutrients, as influenced by irradiance, daylength, and temperature. Few studies have been extensive enough for such purposes, and none are above criticism. In what follows we have used data for an oceanic (ubiquitous) species, *Coccolithus huxleyi* clone F (Paasche 1967), the coastal diatoms *D. brightwellii* (Paasche 1968) and *S. costatum* (Jitts et al. 1964), and a rock pool form, *D. tertiolecta* (Eppley and Coatsworth 1966). Specific growth rate data are given in the papers cited, or can be calculated from them, as a function of irradiance and temperature with 16-hr light and 8-hr darkness each 24-hr day.

Variation in specific growth rate ( $\mu$ ) with nitrate and ammonium concentration ( $S$ ) was calculated from  $K_s$  values (Table 2) and  $\mu_m$  taken from growth rate vs. irradiance curves (Fig. 4) given in the papers cited above:

$$\mu = \mu_m S / (K_s + S).$$

Results of the calculations are shown in Fig. 5.

At low irradiance (0.025 ly/min), the calculations suggest that *C. huxleyi* is the better competitor and *D. tertiolecta* the poorest at all nitrate concentrations (Fig. 5A). With higher irradiance (Fig. 5B) (0.1 ly/min—about 20% of full sunlight) the situation is complex, with *C. huxleyi* growing fastest with nitrate up to about 0.8  $\mu\text{M}$ ; with higher nitrate, *S. costatum* becomes the better competitor. In fact,

according to these calculations, both neritic diatoms are favored by high irradiance and nitrate. *Dunaliella tertiolecta* generally fares poorly because of its relatively sluggish response to irradiance. Apparently this is not an artifact of poor measurements since Jitts et al. (1964) found a light response curve for *D. tertiolecta* similar to ours. Another rock pool flagellate, *M. lutheri*, with a maximum specific growth rate of 1.2 doublings/day at 20C, has a growth rate vs. irradiance response curve like that for *D. tertiolecta* (Jitts et al. 1964). Thus our comments for *D. tertiolecta* may apply also to *M. lutheri*.

The calculations suggest further that *C. huxleyi* is the best competitor with any ammonium level and low irradiance (Fig. 5C). Because of its low  $K_s$  for ammonium, *D. tertiolecta* would grow faster than the two diatom species at low ammonium concentration ( $<0.5 \mu\text{M}$ ) and low irradiance. At higher irradiance (Fig. 5D), the diatoms would grow faster than *C. huxleyi* only if ammonium is  $>1.5 \mu\text{M}$  for *S. costatum* or  $>3 \mu\text{M}$  for *D. brightwellii*. Such levels are high but are often found in polluted coastal water and in declining phytoplankton blooms. We noted numerous *D. brightwellii* when nitrate was very low in a declining bloom of *Ceratium furca* in November 1967 off Huntington Beach, California.

When irradiance is about 0.05 ly/min and temperature 20C (Fig. 4), *C. huxleyi*, *S. costatum*, and *D. brightwellii* have essentially the same  $\mu_m$ ; only at this irradiance would the  $K_s$  values alone determine the supposed competitive advantage of one species over the others.

According to the laboratory results, *D. tertiolecta* (a rock pool form) would be an effective competitor of diatoms for nitrate or of *C. huxleyi* for ammonium only with bright light, long days (see Eppley and Coatsworth 1966) and appreciable ammonium levels. It would probably be an effective competitor where its broad salinity tolerance (McLachlan 1960; Eppley 1963) would be advantageous. Shallow rock pools probably best meet these criteria. The temperature optimum of this organism (34C, Eppley 1963) is also well suited for such

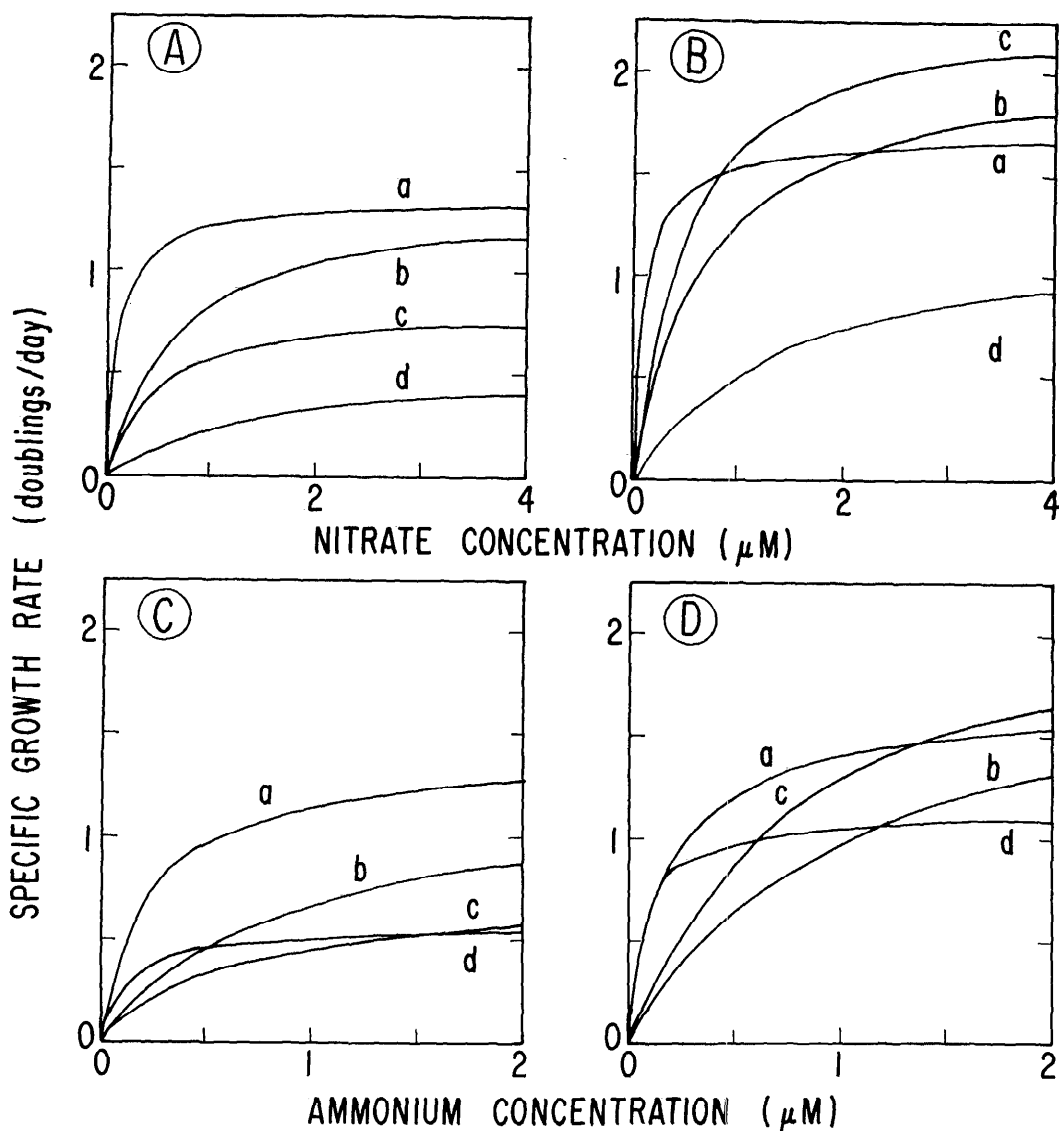


FIG. 5. Calculated specific growth rates vs. nitrate and ammonium concentration at two irradiance levels (approx 1/20, A and C, and 1/5, B and D, of surface sunlight irradiance for wavelengths of 400–700 nm). a = *Coccolithus huxleyi*, b = *Ditylum brightwellii*, c = *Skeletonema costatum*, and d = *Dunaliella tertiolecta*.

habitats. The other species of Fig. 5 would presumably be excluded from rock pools because of low temperature optima and narrow salinity tolerances. Ryther (1954) could account for seasonal changes in the relative abundance of diatoms and unicellular Chlorophyta in Long Island bays

on the basis of differences in their temperature and salinity optima.

*Gonyaulax polyedra*, often the predominant species during local red tides, has a unique strategy that may account for its success despite a low growth rate (about 0.5 doublings/day at 20°C and optimal ir-

radiance, in the laboratory) and very high  $K_s$  values measured for both nitrate and ammonium: its diurnal migration. This species, and several other local dinoflagellate species (Holmes, Williams, and Eppley 1967) swim to the surface in daylight, where nutrients are usually very low, and to a depth of 10–15 m at night (Eppley, Holm-Hansen, and Strickland 1968), where high nitrate levels are found nearshore during upwelling periods. If nitrogen supply limits its growth (Holmes et al. 1967), it would not be a successful competitor unless high nitrate levels were found at shallow depths because of the restricted range of its vertical migration.

The data of Fig. 5 suggest that this kind of analysis can offer a new perspective on the causes of observed phytoplankton distribution and seasonal succession. For example, it can be inferred from Fig. 5 that at nitrate and ammonium concentrations observed off southern California, *C. huxleyi* would predominate over diatoms (at 20°C) unless upwelling increased the nitrate concentration in surface waters (when diatoms would fare well); and this in fact has been observed (Eppley, unpublished). Such inference seems harmless enough since a causal relationship between nitrate, light, and abundance of diatoms off La Jolla was suspected more than 40 years ago (Moberg 1928). But it must be remembered that, in all probability, the measurements in the laboratory were made with different clones than grew in the sea and that *C. huxleyi* is difficult to identify without electron microscopy. Furthermore, these oversimplifications ignore the importance of differential sinking and grazing.

Of course many other species distributions, in addition to *G. polyedra* and *D. tertiolecta*, are not explained by the combination of factors used in Fig. 5. Much work needs to be done in recognizing strains with differing temperature optima (Braarud 1961; Hulburt and Guillard 1968), and day-length effects are probably more general and important than realized (Castenholz 1964; Paasche 1968). The fascinating possibilities remain that exocines (Pratt 1966) and trace metal complexing (Johnston 1964)

may have widespread importance. Physiological characteristics of phytoplankton measurable with existing methods may be just as important to species distribution as the more exotic features remaining for future study.

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