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SOME OBSERVATIONS ON MARINE PHYTOPLANKTON KINETICS. 2. THE EFFECT OF NITRATE AND AMMONIUM CONCENTRATIONS ON THE GROWTH AND UPTAKE RATES OF THE NATURAL POPULATION OF UBATUBA REGION, SP (23°S, 045°W)

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Synopsis

The phytoplankton Ks and Vmax as a function of ammonium and nitrate concentrations were determined. The growth rate was estimated from measurements on synthesized chlorophyll-a and the cell number in the culture media. The uptake rate was determined as to the consumption of ammonium and nitrate after the nutrients depletion from culture media.

Descriptors: Phytoplankton, Kinetics, Nitrates, Ammonium, Growth, Chlorophylls, Nutrients (mineral), Limiting factors, Cell counting, Fluorescence, *Phaeodactylum tricornutum*, Ubatuba, SP.
Descritores: Fitoplâncton, Cinética, Nitratos, Amônio, Crescimento, Clorofilas, Nutrientes minerais, Fatores limitantes, Contagem de célula, Fluorescência, *Phaeodactylum tricornutum*, Ubatuba, SP.

Introduction

Marine phytoplankton, as well as all the phototrophic organisms, need light energy, carbon dioxide, water, some metallic ions, inorganic ions and organic compounds to grow. Generally, only nitrogen and phosphorus, and sometimes silicon, occur in concentrations below the optimum for phytoplankton growth. Dugdale & Goering (1967) and Goldman *et al.* (1979) report that nitrogen seems to be the only element limiting the growth of phytoplankton growth in oceans. The predominant forms of available nitrogen in oceans are nitrate and ammonium. Since Dugdale 's (1967) work, the phytoplankton kinetics has been expressed in the Michaelis-Menten equation: $\mu = \frac{Vmax \ vs \ S}{Ks + S}$, where: μ is the specific growth rate constant; Vmax the maximum growth rate constant; S the substract (nutrient) and Ks is the Michaelis-Menten constant. The Michaelis-Menten equation, according to Droop (1974), is based on the following reactions: $E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X \xrightarrow{k_3} p + E$, where:

E is the microorganism; S is the substract (nutrient); P is the product and k_1, k_2 and k_3 are velocity constants. This work aimed to determine Ks and Vmax for ammonium and nitrate growth and uptake rates of phytoplankton natural population of Ubatuba region, and to know the influence of these two nutrients on the phytoplankton percentual composition.

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Material and methods

The sample was collected at sea surface, with a plastic bucket. The cells contained in a 300 ml Erlenmeyer flask were incubated in a BOD type incubador, at 26°C and 5 KLUX given by fluorescent day-light type lamps, continuous regime. Nitrate and ammonium were added to the flasks from 0.0 to 20.0 µg-at/l, a series of 8 concentrations for each nutrient. The standard solution of ammonium was prepared with ammonium chloride and the standard solution of nitrate with potassium nitrate. Chlorophyll-*a* was determined according to Strickland & Parsons (1968). Cell counting were made under a Zeiss inverted microscope, 600 X, with a 2.5 cm^3 chamber. In vivo fluorescence was determined with a Turner fluorometer, Model 111, 30 X sensitivity. Nitrate was determined according to Mullin & Riley (1955) and ammonium by the Solórzano (1969) technique. Phytoplankton carbon was determined by the wet oxidation method, described by Strickland & Parsons (*op. cit.*). Growth and uptake rates were calculated according to Schmidt (1982).

Results and discussion

Figure 1 shows the sampling station. The hydrographic parameters, initial cell number, nitrate, ammonium and chlorophyll-*a* concentrations are listed in Table 1. The relative composition of

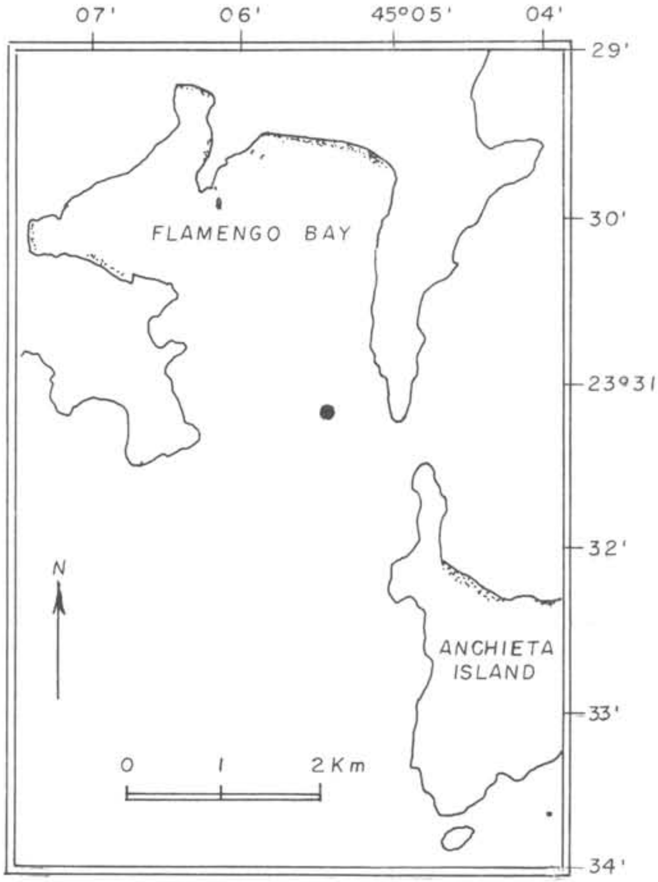

Fig. 1. Localization of the sampling station at Ubatuba region, SP (23°31'S; 45°05'W).

the phytoplankton during the experiment is listed in Table 2. The cells were grouped as phytoflagellates, diatoms, dinoflagellates, *Phaeodactylum tricornutum* (Bohlin) and those with non-conspicuous characteristics were listed as non-identified. A great part of the organisms listed as non-identified are likely to be phytoflagellates that lost their flagelles. In literature they are named "monad" (Hasle, 1978). The initial percentage of the flagellates agrees with Teixeira & Kutner (1962), who observed 97% organisms smaller than 65 μ in the phytoplankton from Cananéia (SP) region. Sassi (1978) recorded at Ubatuba (SP) region 86,24% of chlorophyll-*a* produced by organisms smaller than 20 μ. Teixeira & Tundisi (1967) related that in oligotrophic waters, the flagellates are relatively abundant. The sole difference recorded in Table 2 seems to be the increase, after four days of incubation, of diatom cell number in

Table 1. Hydrographic parameters, initial nitrate, ammonium, cell number and chlorophyll-*a* concentrations

Local Depth (m)	T°C	S°/oo	O_2 ml/l	NO_3 μg-at/l	NH_4 μg-at/l	Cell number/l	Chlorophyll-*a* μg/l
20	26	34.80	5.07	0.90	0.30	1.3×10^5	0.78

Table 2. Percentual composition of the phytoplankton of Ubatuba (SP) region, in culture media of nitrate and ammonium

Culture media	%	Phytoflagellates	Diatoms	Dinoflagellates	Non-identified	*P. tricornutum*	Total phyto cell number (x 10^6)
	Initial	33.57	9.94	18.57	37.90	-	0.13
Nitrate μg-at/l							
0.90	After 4 days of incubation	25.50	59.84	4.67	9.29	0.79	1.00
1.60		13.99	81.08	2.20	2.79	0.25	11.92
2.00		6.86	89.66	1.44	2.02	0.03	13.47
3.00		6.94	65.18	3.32	24.54	0.10	11.46
5.00		6.47	84.30	2.42	7.83	0.14	8.77
11.00		3.33	76.12	3.70	16.81	0.25	10.89
15.00		0.90	94.28	1.44	3.37	0.09	16.63
21.00		3.36	84.38	4.25	8.00	0.12	11.75
Ammonium μg-at/l							
0.30	After 4 days of incubation	5.18	82.65	2.54	9.42	0.18	5.35
1.00		5.80	79.60	2.30	12.23	-	5.36
1.70		2.56	81.34	1.23	14.76	0.09	10.53
2.70		3.83	87.09	0.86	8.16	0.03	13.83
4.30		4.47	75.25	3.68	16.48	0.09	10.10
8.30		4.27	74.41	2.22	19.08	-	20.22
16.30		7.51	60.91	4.92	23.70	2.93	8.52
20.30		2.52	74.98	2.99	19.28	0.21	9.51

both culture media. These results are in accordance with Parsons *et al.* (1978) assumption that diatoms dominate over flagellates under favourable conditions. This advantage could be attributed to a higher rate of cell division or smaller loss of diatom cells. *P. tricornutum* was counted because of its presence in the enriched cultures of water from the region. The chlorophyll-*a*/cell ratio recorded was 0.38pg in ammonium medium and 0.34pg in nitrate. The phytoplankton carbon/chlorophyll-*a* ratio was 48.5. Thomas & Dodson (1972) observed that the phytoplankton carbon/chlorophyll-*a* ratio decreased with the water impoverishment, being approximately 90 for eutrophic and 30 for oligotrophic waters. There was no correlation between cell number and in vivo fluorescence. The correlation value between chlorophyll-*a* and in vivo fluorescence in the ammonium media was 0.77 and 0.56 in the nitrate medium. Figure 2 apparently indicates a better yield of chlorophyll-*a* synthesis in the presence of ammonium. The consumption of ammonium was greater than that of nitrate (Fig. 3). The growth rate of phytoplankton estimated from cell number, has the same hyperbolic shape in both culture media (Figs 4-5). Figures 6-7 show the growth rate, estimated from synthesized chlorophyll-*a*, as a function of nitrate and ammonium concentrations. Figures 6-7 presents only seven points because at the lowest concentration of both nutrients, chlorophyll-*a* yield was < 1.00 µg/l and resulted in a negative rate value. The consumption of nitrate and ammonium was calculated as the difference between their initial and final values in the culture media. The uptake rate, estimated from ammonium and nitrate consumption in culture media, as a function of nitrate and ammonium, is shown in Figures 8-9. Thomas (1970) found a Ks of 1.47 µg-at/l for ammonium and 0.59 µg-at/l for nitrate, estimated from measurements of in vivo fluorescence for the natural phytoplankton from the Tropical Pacific Ocean, showing a better affinity of the phytoplankton for nitrate as nitrogenous nutrient. It seems that the natural phytoplankton of Ubatuba region prefers ammonium as the nitrogenous nutrient (Table 3). McCarthy *et al.* (1977) observed a preference for ammonium in the Chesapeake Bay phytoplankton. They related that water presenting 63% nitrate as nitrogenous nutrient showed 22.1% utilization and that 14.5% ammonium presented 50.9%

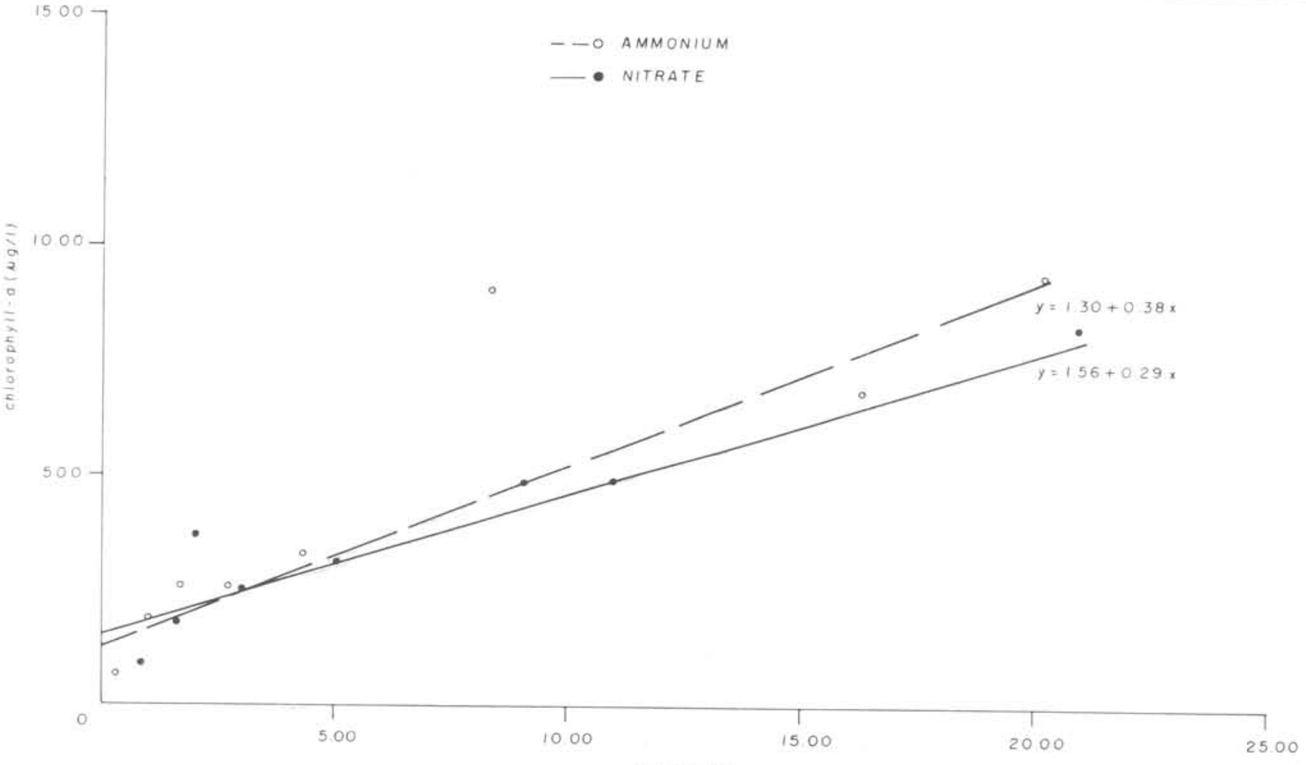

Fig. 2. Chlorophyll-*a* synthesized as a function of nitrate and ammonium concentrations.

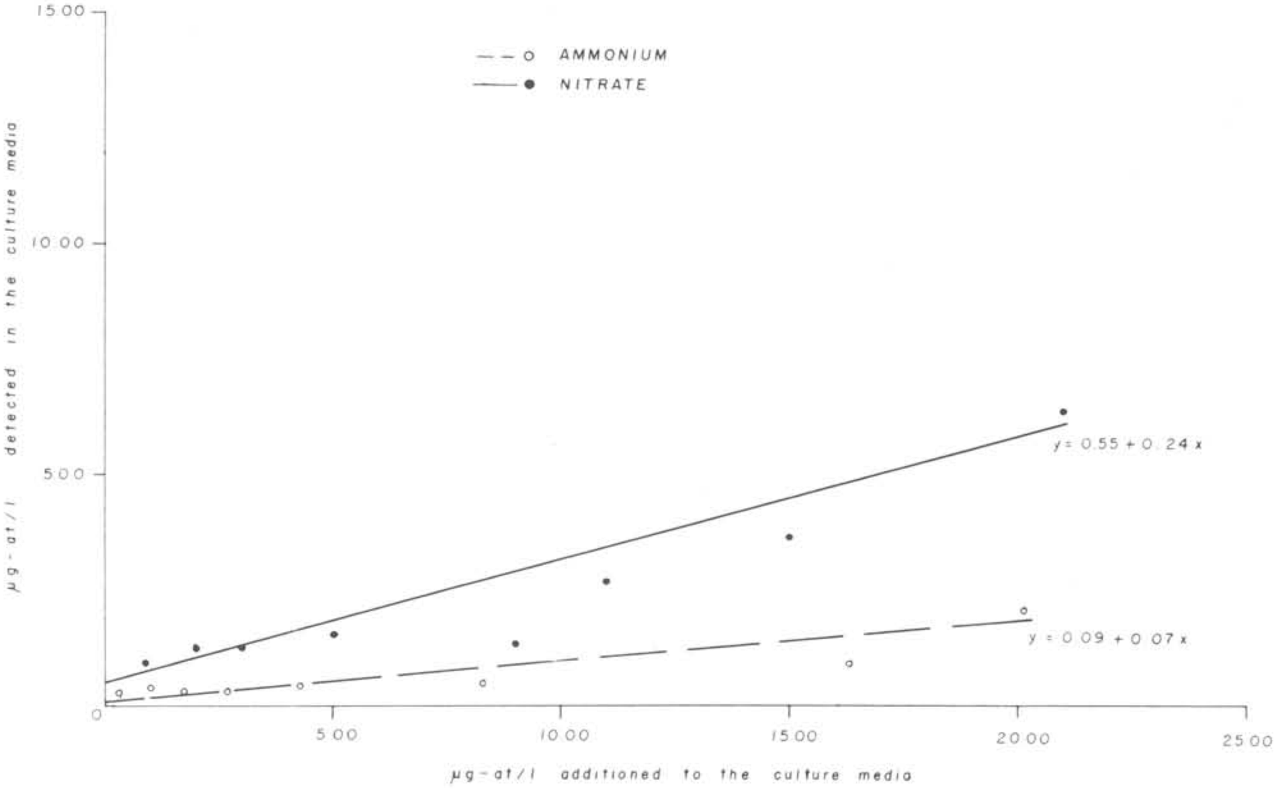

Fig. 3. Nitrate and ammonium consumed as a function of nitrate and ammonium concentrations, measured as its disappearance from culture medium.

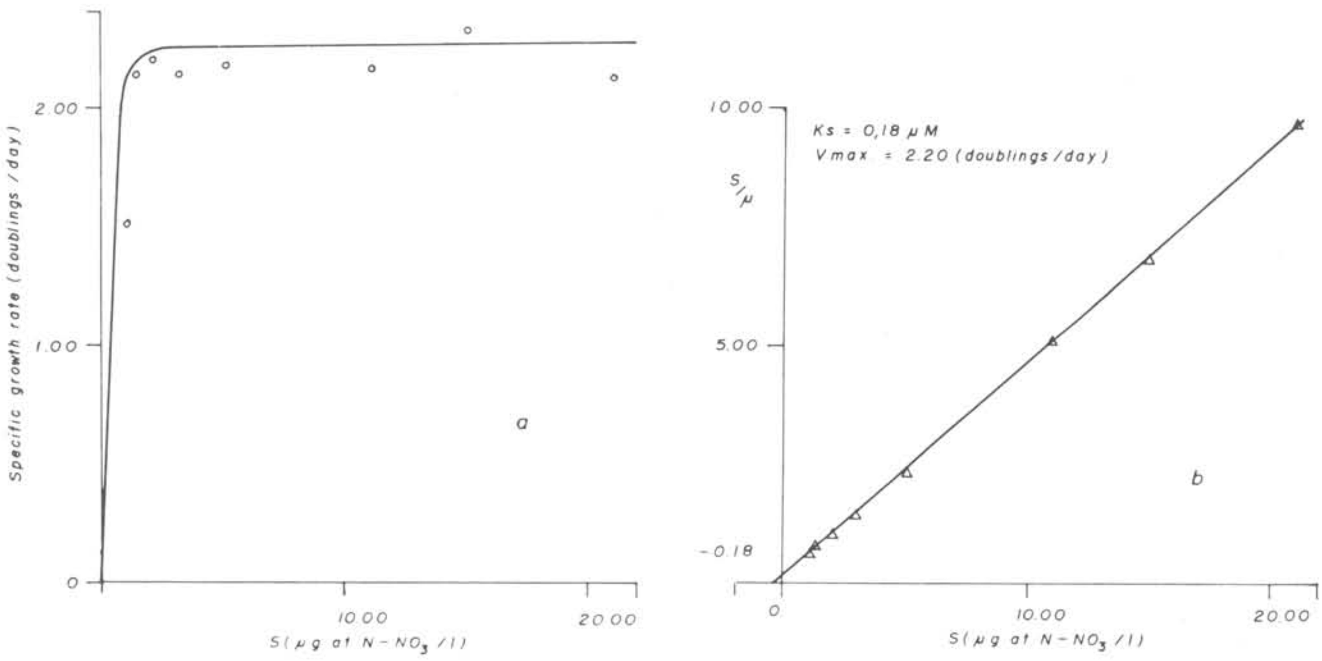

Fig. 4. *a*) Growth rate (doublings/day) of natural phytoplankton, estimated from cell number, as a function of nitrate concentration; *b*) Linearization by plotting S vs S/μ, to give directly Ks and Vmax values.

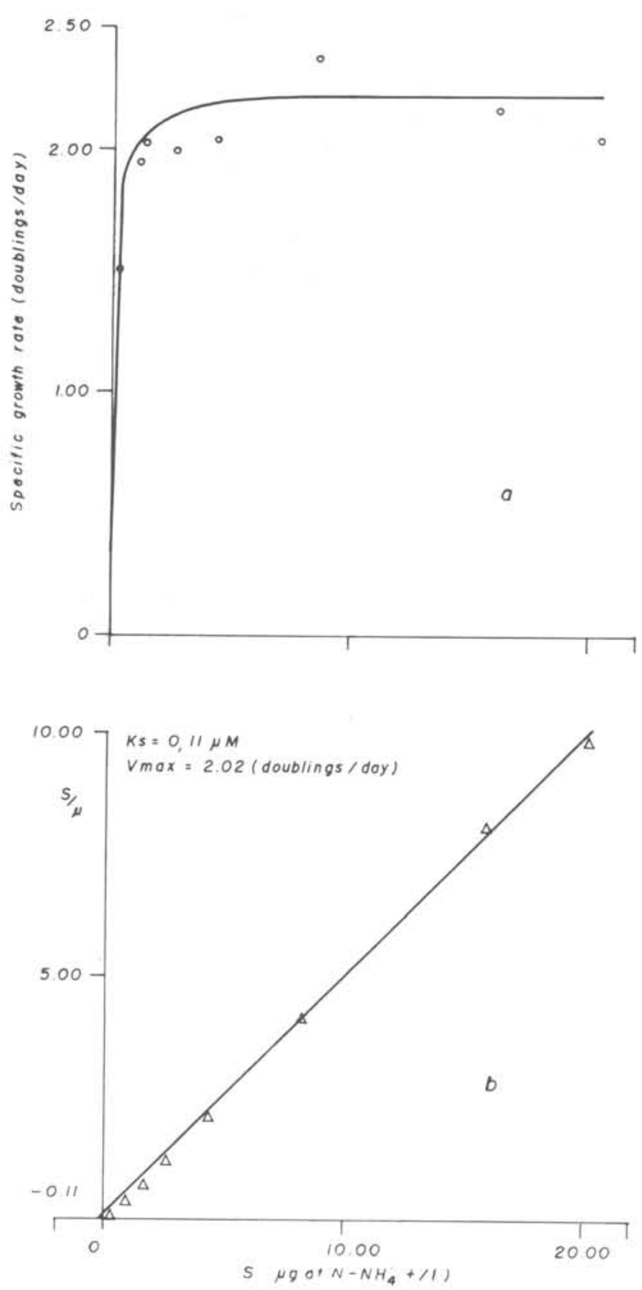

Fig. 5. *a*) Growth rate (doublings/day) of natural phytoplankton, estimated from cell number, as a function of ammonium concentration; *b*) Linearization by plotting S vs S/μ, showing directly Ks and Vmax.

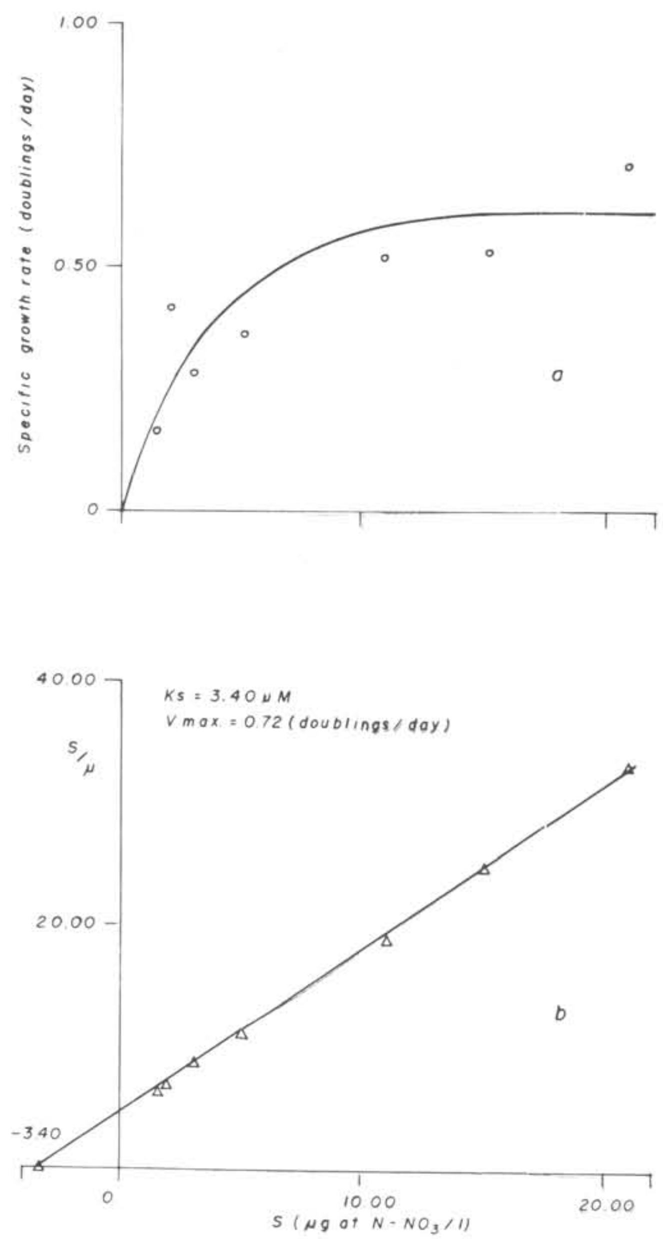

Fig. 6. *a*) Growth rate of natural phytoplankton, estimated from synthesized chlorophyll-*a*, as a function of nitrate concentration; *b*) Linearization by plotting S vs S/μ, to give Ks and Vmax directly.

Table 3. Mean values percentage of utilization of nitrate and ammonium by Ubatuba region phytoplankton; nutrient quantity additioned was considered as 100% values

	Additioned μg-at/l	Detected μg-at/l	% utilized
Ammonium	6.87	0.60	91.12
Nitrate	7.46	2.42	71.71

utilization. Table 3 shows that the percentage of ammonium utilization by the phytoplankton of Ubatuba was greater than that of nitrate. In the present work, the Ks values when estimated as cell number or synthesized chlorophyll-*a* exhibited differences shown in Table 4. These differences probably account for the low chlorophyll-*a* synthesis rate. Also, cell division going on after the nitrogen depletion may have occurred without chlorophyll-*a* synthesis (McAllister *et al.*, 1964). The Ks uptake rate values observed in this work

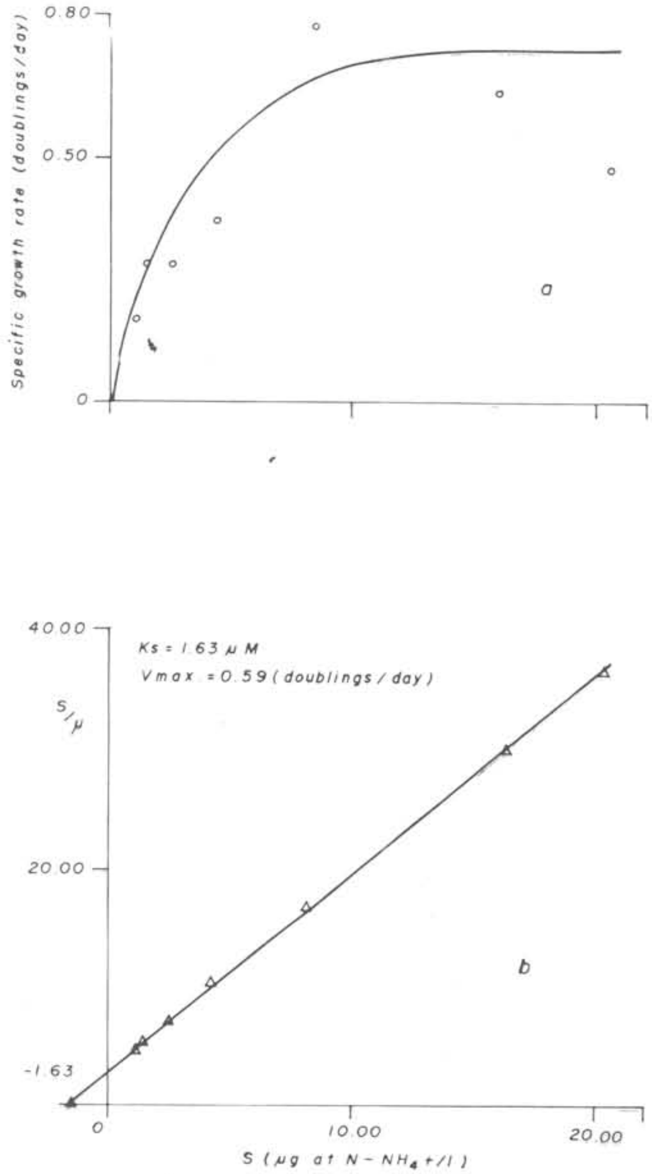

Fig. 7. *a*) Growth rate of natural phytoplankton, estimated from synthesized chlorophyll-*a*, as a function of ammonium concentration; *b*) Linearization by plotting S vs S/µ, to give Ks and Vmax directly.

Table 4. Values of Ks and Vmax estimated from cell number synthesized chlorophyll-*a* in nitrate and ammonium culture media; uptake rate Ks and Vmax in both media; the 95% confidence limits values could be attributed to sample size or experimental variation

	Nitrate		Ammonium	
	Ks µM	Vmax Doublings/day	Ks µM	Vmax Doublings/day
		Growth rate		
Cell number	0.18±0.17	2.20±0.02	0.11±0.45	2.02±2.00
Chlorophyll-*a*	3.40±3.31	0.72±0.48	1.63±5.81	0.59±0.65
		Uptake rate		
Assimilation	3.72±4.32	0.56±0.62	1.68±2.43	1.01±1.12

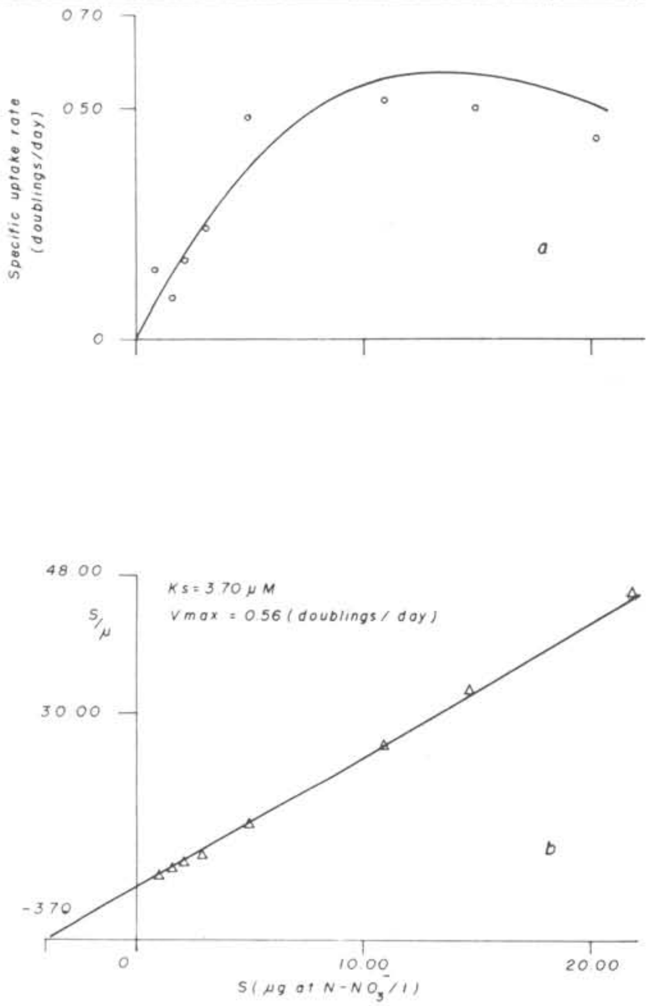

Fig. 8. *a*) Uptake rate of natural phytoplankton, estimated as nitrate consumption in the culture medium, as a function of nitrate concentration; *b*) Linearization by plotting S vs S/µ, showing directly Ks and Vmax.

were higher than those of Ks growth rate (Table 4), the difference being greater with nitrate data. Eppley & Thomas (1969) suggested that Ks uptake rate constants are generally higher than those of Ks growth rate. Some new experiments must be carried on in the same region in order to confirm the results obtained hitherto. The affinity of the natural phytoplankton for ammonium must be confirmed (McCarthy *et al.*, 1977). The Michaelis-Menten constants are important ecological parameters (Thomas & Dodson, 1974) and would been determined for nutrients other than nitrate and ammonium and for other Brazilian regions.

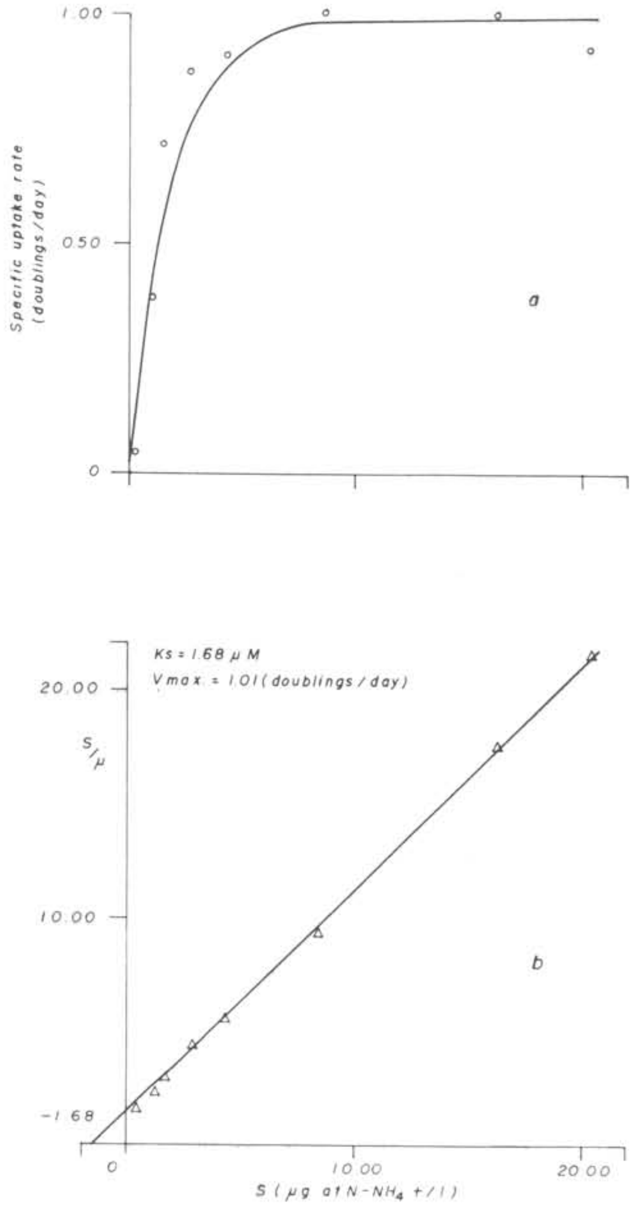

Fig. 9. *a*) Uptake rate of natural phytoplankton, estimated as ammonium consumption in the culture medium, as a function of ammonium concentration; *b*) Linearization by plotting S vs S/μ, showing directly Ks and Vmax.

Resumo

Foram determinadas a Ks e a Vmax do fitoplâncton natural, em função da concentração de amônia e nitrato. A velocidade de crescimento foi determinada como clorofila-*a* sintetizada e número de células no meio de cultura. A velocidade de assimilação foi determinada como o consumo de amônia e nitrato depois do esgotamento desses nutrientes do meio de cultura.

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