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THE EFFECTS OF NITROGEN AND PHOSPHORUS ENRICHMENTS ON PHYTOPLANKTON IN THE REGION OF UBATUBA (LAT. 23°30'S - LONG. 45°06'W), BRAZIL

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Synopsis

Experiments of water enrichments with natural populations of phytoplankton were carried out at the region of Ubatuba (Lat. 23°30'S - Long. 45°06'W) during winter and summer time. Special attention was given to the influence of nitrogen and phosphorus on the standing-stock (chlorophyll-a) and ^{14}C assimilation and dark-fixation in situ conditions. The phytoplankton populations was analysed in terms of its composition and hydrographical parameters were made according to Strickland & Parsons (1968). The enrichments with surface waters reveal that nitrogen may be the major limiting factor for phytoplankton biomass sensu Liebig.

Introduction

The values of primary production of phytoplankton for oceanic or coastal tropical waters are generally low.

Data on the literature report values of 0.05 $mgC.m^{-2}.day^{-1}$ to 0.2 $mgC.m^{-2}.day^{-1}$. This is attributed mainly to the low nutrient concentration, particularly nitrogen (Thomas, 1967; 1970).

Data recorded by Teixeira (1973) and Teixeira & Vieira (1976) for the region of Ubatuba indicated that nitrogen is the main limiting factor throughout the year. Effects of nitrogen on the growth and composition of phytoplankton were studied by Thomas (*op. cit.*), Dunstan & Menzel (1971), Ryther & Dunstan (1971), Vince & Valiella (1973).

In the present paper the authors describe and discuss the results of experiments carried out with natural populations of phytoplankton from the region of Ubatuba. Special attention was given to the effects of enrichment with nitrogen and phosphorus on the standing-stock (total chlorophyll-*a*) and photosynthesis of natural populations of phytoplankton in terms of ^{14}C-counts per minute and some experiments on dark-fixation were performed. The phytoplankton population was analysed superficially only to have a brief idea of its composition.

Material and Methods

Samples were collected at surface in the central region of Ubatuba Bay (Fig. 1), mainly twice a year: winter and summer time.

The water was immediately enriched with nutrients and incubated "in situ" in 10-25 ℓ containers. The experiment lasted from 120 to 192 hours. The concentrations of nitrate and phosphate added were well above the environmental concentrations.

Samples of 400 to 1000 ml were removed daily and the concentration of chlorophyll-*a* was determined according to Strickland & Parsons (1968). Some experiments of relative carbon fixation were carried out "in situ" with the ^{14}C technique according to Steemann-Nielsen (1952).

Samples for analysis of phytoplankton were fixed with lugol and examined under the inverted-microscope according to the technique described by Utermöhl (1931).

The determination of hydrographical parameters, dissolved oxygen, nitrate, phosphate, salinity, was made according to Strickland & Parsons (*op. cit.*). Temperature was determined with reversing thermometer.

Results and Discussion

a) The measurements of standing-stock as chlorophyll-a

The results obtained with the determination of chlorophyll-*a* are shown in

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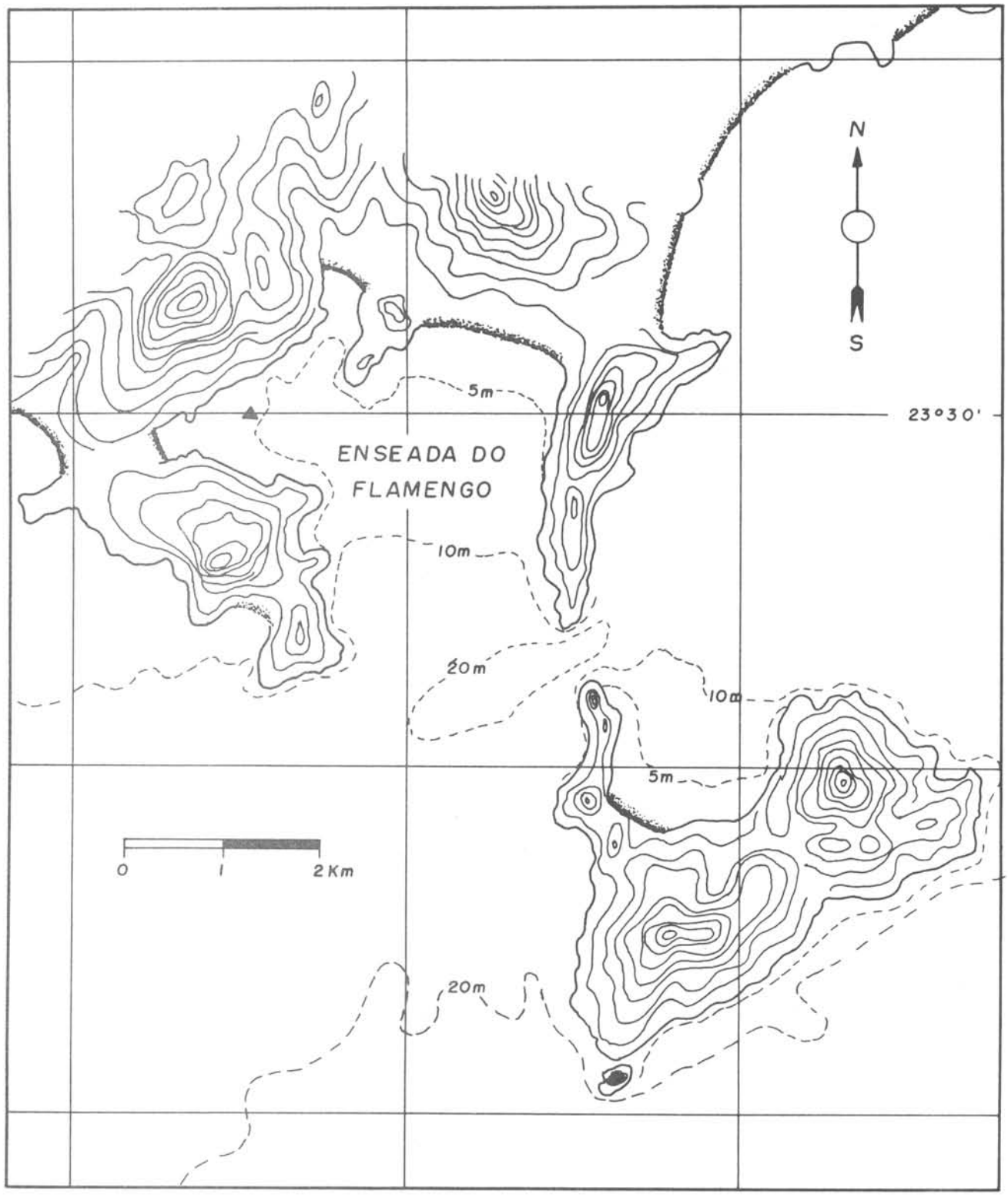

Fig. 1. Map of the region showing the location of environment studied.

Tables I-IX. The original content of nitrogen and phosphorus in the water as well as of the dissolved oxygen and temperature are indicated for each experiment. In general, additions of only nitrogen stimulated the growth of phytoplankton in terms of chlorophyll-*a*. Additions of only phosphorus did not result in differences in the phytoplankton growth. Additions of nitrogen and phosphorus produced an accentuated increase in chlorophyll-*a* synthesis.

Table I - Total chlorophyll-*a* ($mg.m^{-3}$) data from the phytoplankton populations in samples treated with different nutrient additions

DATE: 06/23/1972
$S°/_{oo}$ = 34.95
O_2cc/l = 4.85
T°C = 23.00
NO_3-N = 0.21 µgat/l
PO_4-P = 0.11 µgat/l

TIME (HOURS) \ ADDITION	CONTROL (NO ADDITION)	+ PO_4-P 5.0 µgat/l	+ NO_3-N 50.0 µgat/l	+ PO_4-P + NO_3-N 5.0 µgat/l 50.0 µgat/l
0.0	2.44	-	-	-
24.0	1.72	1.64	3.96	3.76
48.0	1.66	1.42	6.66	5.80
72.0	1.49	1.24	6.91	10.47
96.0	1.28	1.19	5.34	20.04
120.0	1.25	1.08	3.75	23.47
144.0	1.20	1.00	2.40	25.60
168.0	1.17	0.90	2.00	21.30
192.0	1.05	0.90	1.35	18.40

Table II - Total chlorophyll-*a* ($mg.m^{-3}$) data from the phytoplankton populations in samples treated with different nutrient additions

DATE: 12/10/1972
$S°/_{oo}$ = 35.09
O_2cc/l = 5.21
T°C = 25.40
NO_3-N = 0.27 µgat/l
PO_4-P = 0.12 µgat/l

TIME (HOURS) \ ADDITION	CONTROL (NO ADDITION)	+ PO_4-P 5.0 µgat/l	+ NO_3-N 50.0 µgat/l	+ PO_4-P + NO_3-N 5.0 µgat/l 50.0 µgat/l
0.0	2.22	-	-	-
24.0	1.80	1.62	1.95	1.85
48.0	1.84	1.43	2.00	2.10
72.0	1.41	1.18	2.56	2.88
96.0	1.36	1.15	2.80	3.45
120.0	1.34	1.19	2.90	4.68
144.0	1.46	1.22	4.20	5.43
168.0	1.75	1.14	4.00	8.37
192.0	-	1.30	4.25	6.98

Table III - Total chlorophyll-*a* ($mg.m^{-3}$) data from the phytoplankton populations in samples treated with different nutrient additions

DATE: 03/25/1973 NO_3-N = 0.20 µgat/l

$S°/_{oo}$ = 35.02 T°C = 28.35 PO_4-P = 0.08 µgat/l

O_2cc/l = 5.01

TIME (HOURS) \ ADDITION	CONTROL (NO ADDITION)	+ PO_4-P 5.0 µgat/l	+ NO_3-N 50.0 µgat/l	+ PO_4-P + NO_3-N 5.0 µgat/l 50.0 µgat/l
0.0	1.53	-	-	-
24.0	1.02	0.95	4.56	6.73
48.0	0.95	0.80	7.32	8.91
72.0	0.80	0.72	8.41	10.12
96.0	0.78	0.65	6.30	9.35
120.0	0.66	0.70	5.52	8.78
144.0	0.50	0.50	4.31	6.43
168.0	0.58	0.45	4.09	6.00
192.0	0.45	0.30	2.20	5.68

Table IV - The influence of nutrient addition on phytoplankton populations in terms of chlorophyll-*a* ($mg.m^{-3}$)

DATE: 08/16/1973 NO_3-N = 0.12 µgat/l

$S°/_{oo}$ = 34.80 T°C = 23.35 PO_4-P = 0.03 µgat/l

O_2cc/l = 4.65

TIME (HOURS) \ ADDITION	CONTROL (NO ADDITION)	+ PO_4-P 5.0 µgat/l	+ NO_3-N 50.0 µgat/l	+ PO_4-P + NO_3- N 5.0 µgat/l 50.0 µgat/l
0.0	0.97	-	-	-
24.0	0.73	0.68	1.15	2.31
48.0	0.54	0.51	1.87	3.96
72.0	0.51	0.48	1.73	4.08
96.0	0.43	0.48	1.45	3.34
120.0	0.41	0.39	0.98	1.83
144.0	0.40	0.41	0.81	1.05
168.0	0.35	0.30	0.65	0.91
192.0	0.35	0.35	0.50	0.78

Table V - The influence of nutrient addition on phytoplankton populations in terms of chlorophyll-*a* ($mg.m^{-3}$)

DATE: 03/27/1975
S°/oo = 34.90
O_2cc/l = 4.97
T°C = 27.50
NO_3-N = 0.06 µgat/l
PO_4-P = 0.05 µgat/l

TIME (HOURS) \ ADDITION	CONTROL (NO ADDITION)	+ PO_4-P 5.0 µgat/l	+ NO_3-N 50.0 µgat/l	+ PO_4-P + NO_3-N 5.0 µgat/l	50.0 µgat/l
0.0	0.63	-	-	-	
24.0	0.60	0.93	2.92	9.83	
48.0	0.43	0.81	4.01	21.38	
72.0	0.35	0.70	3.43	17.21	
96.0	0.35	0.60	3.30	10.34	
120.0	0.30	0.54	3.05	8.76	

Table VI - Total chlorophyll-*a* ($mg.m^{-3}$) data from the phytoplankton populations in samples treated with different nutrient additions

DATE: 04/08/1975
S°/oo = 34.95
O_2cc/l = 5.02
T°C = 25.0
NO_3-N = 0.13 µgat/l
PO_4-P = 0.07 µgat/l

TIME (HOURS) \ ADDITION	CONTROL (NO ADDITION)	+ PO_4-P 5.0 µgat/l	+ NO_3-N 50.0 µgat/l	+ PO_4-P + NO_3-N 5.0 µgat/l	50.0 µgat/l
0.0	1.03	-	-	-	
24.0	0.97	0.98	4.63	9.75	
48.0	0.53	0.61	2.87	5.43	
72.0	0.51	0.58	1.64	3.43	
96.0	0.51	0.55	1.35	2.57	
120.0	0.47	0.50	1.05	2.02	

Table VII - The influence of nutrient addition on phytoplankton populations in terms of chlorophyll-*a* ($mg.m^{-3}$)

DATE: 05/09/1975 NO_3-N = 0.10 µgat/l

$S^o/_{oo}$ = 34.00 T°C = 23.00 PO_4-P = 0.05 µgat/l

O_2cc/l = 4.84

TIME (HOURS) \ ADDITION	CONTROL (NO ADDITION)	+ PO_4-P 5.0 µgat/l	+ NO_3-N 50.0 µgat/l	+ PO_4-P + NO_3-N 5.0 µgat/l 50.0 µgat/l
0.0	0.84	-	-	-
24.0	0.74	0.87	0.89	0.88
48.0	0.87	1.13	1.63	14.16
72.0	1.05	1.16	2.78	33.41
96.0	1.00	1.22	5.23	27.98
120.0	1.00	1.12	5.37	22.34

Table VIII - The influence of nutrient addition on phytoplankton populations in terms of chlorophyll-*a* ($mg.m^{-3}$)

DATE: 07/02/1975 NO_3-N = 0.09 µgat/l

$S^o/_{oo}$ = 34.75 T°C = 22.0 PO_4-P = 0.03 µgat/l

O_2cc/l = 4.73

TIME (HOURS) \ ADDITION	CONTROL (NO ADDITION)	+ PO_4-P 5.0 µgat/l	+ NO_3-N 50.0 µgat/l	+ PO_4-P + NO_3-N 5.0 µgat/l 50.0 µgat/l
0.0	0.66	-	-	-
24.0	0.72	0.78	1.10	1.32
48.0	0.75	0.81	1.76	1.78
72.0	0.72	0.71	2.57	3.36
96.0	0.65	0.63	1.85	2.03
120.0	0.58	0.55	1.12	1.28

Table IX - The influence of nutrient addition on phytoplankton populations in terms of chlorophyll-*a* ($mg.m^{-3}$)

DATE: 03/15/75 NO_3-N = 0.07 µgat/l

$S°/_{oo}$ = 34.93 T°C = 27.10 PO_4-P = 0.03 µgat/l

O_2cc/l = 4.98

TIME (HOURS) \ ADDITION	CONTROL (NO ADDITION)	+ PO_4-P 5.0 µgat/l	+ NO_3-N 50.0 µgat/l	+ PO_4-P 5.0 µgat/l + NO_3-N 50.0 µgat/l
0.0	1.08	-	-	-
24.0	1.12	0.93	2.44	5.82
48.0	1.41	1.36	2.62	8.45
72.0	0.97	1.15	6.23	15.91
96.0	0.56	0.70	6.55	19.33
120.0	0.96	1.10	6.97	20.99
144.0	1.08	1.15	7.18	23.09
168.0	1.30	1.40	9.41	25.65
192.0	1.10	1.35	8.23	31.63

b) The assimilation of radioactive carbon

These measurements were made only during the winter and summer. The increment in the carbon fixation followed approximately the same pattern as shown in the experiments on standing-stock changes (Fig. 2).

The addition of only phosphate did not produce any significant increase in the carbon fixation, while the addition of nitrogen produced an increment of up to 300%. These experiments also show that phosphate may be sufficient in the environment in relation to the nitrogen concentration. When this nitrogen increases artificially there is possibly an alteration of the ratio N:P, giving a secondary limitation effect due to the depletion of phosphate.

Changes in the dark-fixation of carbon were also determined (Fig. 2). In the samples with the addition of only phosphate, the rate of dark-fixation of ^{14}C was about the same as the control and no significant alteration was found. In the samples where nitrate and phosphate were added, the values were very low, probably due to changes in the specific composition of phytoplankton and high rates of growth, as well as the physiological condition of the "new" population of phytoplankton enriched with nutrient salts.

c) Qualitative study of phytoplankton

While the control samples or the samples enriched with phosphate did not show any change in the specific composition, unless by the death of many cells a drastic change occurred in the samples enriched with nitrogen or both nitrogen and phosphorus. Different associations were found, for example, in the experiments carried out in 05/09/75 and 03/15/75 (Tabs VII and IX). After 24 and 72 hours, there was a bloom composed predominantly of *Chaetoceros* sp probably *simplex*, *Skeletonema costatum*, phytoflagellates, and mainly the diatom *Thalassiosira* sp. This algae is the predominant element in all enriched samples.

In the other experiments, 08/16/73 and 03/27/75 (Tabs IV and V), the specific composition showed a less pronounced change with the absence of *Skeletonema costatum*, *Chaetoceros* sp and the presence of *Nitzchia longissima*.

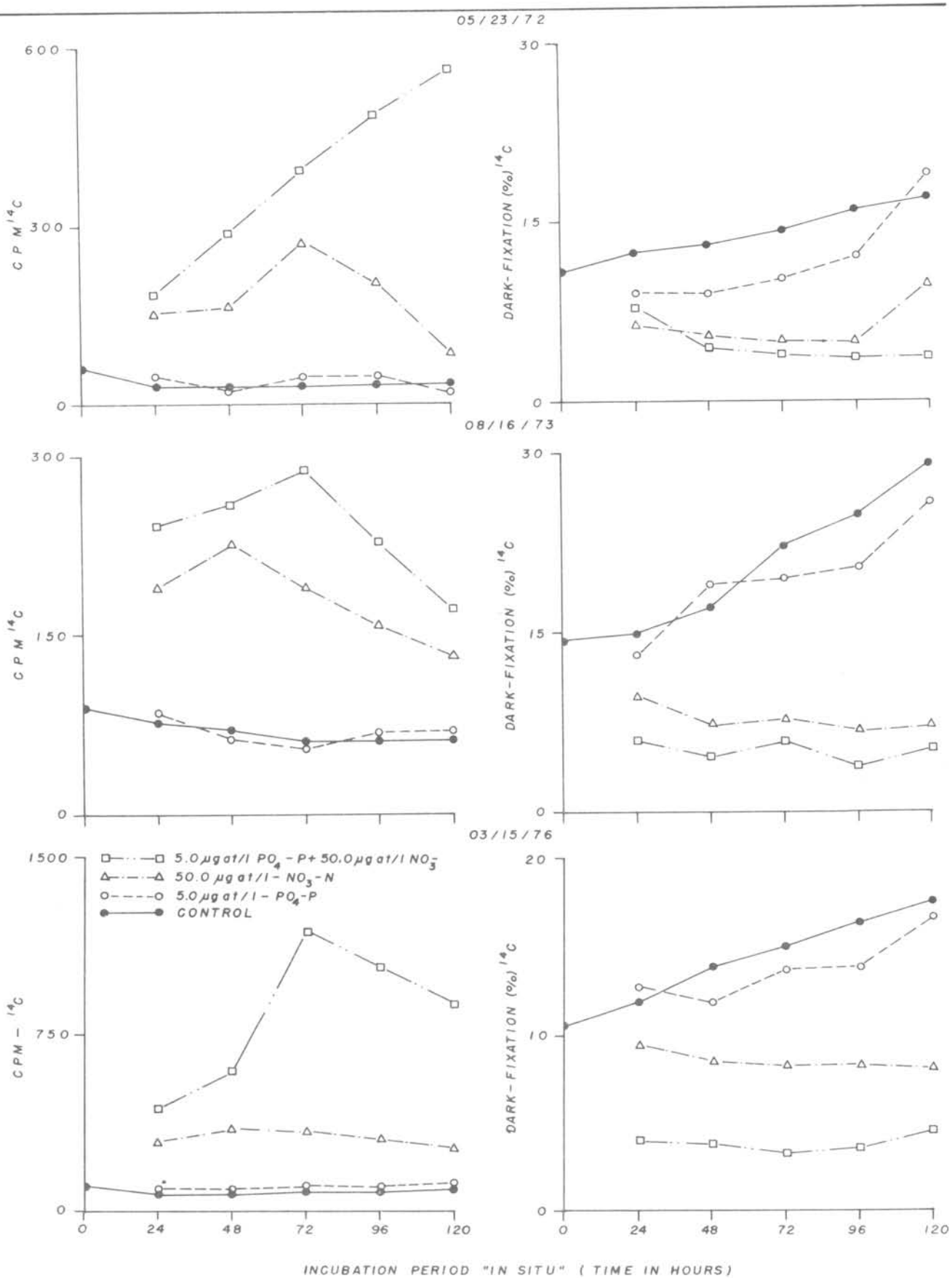

Fig. 2. The effects of enrichment with nitrogen and phosphorus on the natural phytoplankton populations in terms of particulate ^{14}C (CPM) assimilation and dark-fixation (%).

Small phytoflagellates and diatoms, *Phaeodactylum tricornutum* and phytoflagellates were predominant in the other experiments. These small forms probably occur in the natural environment in very low numbers since no record of its presence was made in natural samples. The occurrence of *Phaeodactylum tricornutum* seems to be associated with lower temperature.

Ubatuba Bay shows some characteristics of many coastal waters in tropical or subtropical regions: high light penetration, a thermal stratification during the summer and generally low concentration of nutrients. The results of the enrichment experiments with surface waters only support the claim that nitrogen is the main limiting factor for phytoplankton biomass *sensu* Liebig.

Based on the nutrients determined we verified unfavourable inorganic N:P quotients which seem to be a demonstration of nitrogen limitation in relation to the phytoplankton populations mainly in terms of biomass.

The low nutrient concentration is probably due to the lack of circulation during periods of thermal stratification; this imposes limiting conditions to the phytoplankton community. Therefore this community probably depends mainly on the regeneration of nutrients by zooplankton and decomposition processes above the thermocline. The differences in the composition of the community, when subjected to enrichment, can explain some patterns for seasonal succession of phytoplankton found in coastal waters.

These enrichment experiments carried out "in situ" may be an useful tool to understand the complex dynamics of phytoplankton growth and photosynthesis in these environments where the physical conditions such as the presence of a thermocline may impose limiting factors mainly related to nutrient concentration.

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Resumo

Uma série de experimentos com águas superficiais da região de Ubatuba contendo populações naturais de fitoplâncton, foi utilizada para se avaliar o papel do nitrogênio e do fósforo sobre a síntese da clorofila-*a* e da assimilação do ^{14}C na luz e da fixação do ^{14}C no escuro. A composição do fitoplâncton foi analisada e alguns parâmetros hidrográficos determinados.

Pelos resultados obtidos, concluímos que o nitrogênio é o principal fator limitante, com relação à biomassa do fitoplâncton, segundo "sensu" de Liebig.

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