Diatom biochemistry with respect to nutrient concentrations and the quality of light

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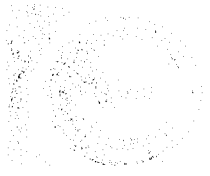


CONTENTS

Abstract

Experiments were conducted to determine whether a relationship exists between the composition and metabolism of phytoplankton cells, and the chemistry of their environment.

The study was designed to simulate in the laboratory natural conditions as occur in upwelling zones of the sea.

Two diatom species *Thalassiosira pseudonana* and *Skeletonema costatum* were cultured in nutrient enriched and nutrient deficient seawater medium to create conditions comparable to newly upwelled water and 'aged' water respectively. Such conditions occur in upwelling areas of the world's oceans where oil rich deposits of possibly planktonic origin have been discovered. Differences in the biochemical constituents of the algae cultured in these specific conditions have been examined with a view to establishing background information of relevance to oil generation.

The major findings of the study included:

1 Increased carbohydrate and lipids, storage materials, relative to protein and amino acids, as nutrient concentrations decreased.

2 Silicon uptake diminished in phosphate-deficient cultures.

3 Mucilage material built up around stationary phase cells.

4 The frustules of silicon limited *Skeletonema* cells showed structural abnormalities. This was not the case in *Thalassiosira* cells.

5 The rate of photosynthesis decreased in nutrient deficient cultures, and there was a reduction in the number of chromatophores per cell.

CHAPTER 1

INTRODUCTION

It has been estimated that 2 x 10^{13} Kg carbon is fixed each year by the various groups of marine phytoplankton species, about 40% of the total net primary production of all plants on earth (Strickland, et al 1972). Diatoms predominate in productive areas of the ocean, such as upwelling regions and continental shelves, and contribute 20-25% of the world's net primary production. Since this class of phytoplankton was recognised as being important primary producers in marine ecosystems, many aspects of their biology have been researched.

Early workers favoured ultrastructure research, and with the aid of the electron microscope diatom shells were examined (Muller & Pasewaldt 1942; Gerloff & Golz 1944).

Even before this diatoms played a key role in the origins of mariculture and sea farming. Allen and Nelson (1910) cultured several diatom species and used them successfully to rear mollusc, copepod and echinoderm larvae. Mariculture is today an expanding industry, and diatom species, particularly the centric diatoms *Skeletonema costatum* and *Chaetoceros calcitrans*, have proved to be the best food for larval growth (Walne 1970) of echinoderms, molluscs and copepods.

Research has included some diatom cytology (Mazia 1961, Robards 1970), and the significance of diatom species as indicators of water quality and pollution, a rather controversial study (Patrick et al 1954, Fjerdingstadt 1964, Chelnoky 1968). Silicon metabolism has been investigated in diatoms (Davis et al 1973, Reuter 1983), and this group was also specially favoured for early permeability studies (Hofler 1940).

Detailed plankton field studies made on numerous oceanographic cruises increased the knowledge of diatom ecology and distribution throughout the oceans. (Hensen 1887, Cleve 1900, Gran 1908). More recently planktological and ecological work conducted on cruises considered physiological aspects. (Boyer 1926, Hendey 1937, Cupp 1943, Chelnoky 1968). This trend has continued with an increasing number of

projects relating diatom physiology with environmental factors. Pugh (1975) investigated variations in the biochemical composition of the diatom *Coscinodiscus eccentricus* in relation to water salinity and culture age. Fawley (1984) documented the effects of temperature on the growth of *Phaeodactylum tricornutum* a subject also covered in a paper by H.R. Jitts et al (1964).

Areas of the world's oceans where upwelling occurs have attracted a great deal of attention in recent years. These upwelling zones are extremely productive biologically, and valuable commercial fishing grounds, waters off South West Africa and Peru are typical of such regions.

Dense phytoplankton blooms predominantly of diatoms, develop in the upwelling water providing surplus food for large shoals of fish. (Smayda 1963, 1966, Strickland et al 1969, Belyaeva 1972, Reyssac 1973).

Interest in the plankton blooms not only arises from the key role they play in the food chains of pelagic and demersal fish, but also in the contribution made by the diatom cells to underlying sediments. As blooms die down with the onset of nutrient depletion in surface water, the cells sink rapidly down to the sea bed. Current research is investigating theories which suggest polymerisation/association reactions occur which transfer the organic remains of plankton blooms into the sediments, resulting in organic rich deposits, possibly pre-requisites for subsequent oil reservoirs (Morris, 1977, Cronin, 1982).

Barlow (1981) completed a series of field projects in Southern Benguela upwelling system, and successfully related biochemical parameters of phytoplankton blooms (in which diatoms were the dominant species), with the water temperature, and the concentration of major nutrients in the water. However, field studies to establish a relationship between cell biochemistry and water chemistry, particularly the association of these factors in upwelling areas, are not easily conducted. To be in an area at the exact time a bloom develops involves a lot of good luck, and to use up valuable ship time waiting for blooms to occur in an area can become prohibitively expensive.

It has proved more feasible to carry out investigations in controlled field ecosystem enclosures or in the laboratory. A large volume plastic sphere has been used in a lake near Nanaimo, British Columbia, Canada (Antia et al 1963) for a series of experiments, and large ecosystem bags in Loch Ewe N.W. Scotland, have provided discrete water columns in which detailed productivity studies have been conducted (Davies et al 1980, Morris et al 1983). Data from the Loch Ewe experiments indicate that significant changes occur in the cell biochemistry of phytoplankton species, which are related to changes in the nutrient chemistry of the water. To evaluate individually the effect of various growth parameters on phytoplankton cell composition, field work had to be extended to carefully controlled laboratory experiments, and this was the objective of the present project.

It had been intended that experiments would include the effects of nutrient limitation, and light quality on diatom biochemistry and growth. Nutrient limitation has received considerable attention for several years. (Morris et al 1971, Eppley et al 1977, Pingree et al 1977, McCarthy & Goldman 1979, Goldman et al 1979, Fogg 1982). These workers have considered the open ocean situation where a position of equilibrium is established between available nutrients, primary and secondary production and bacterial reactions. Nutrients regenerated in the euphotic zone are assimilated throughout the water column at approximately the same rate. (Fogg 1982).

This balance is disturbed in areas of upwelling, with the intrusion of extremely rich waters into relatively nutrient deficient surface water. Generally the nutrients are rapidly depleted by the phytoplankton blooms which develop. The blooms collapse and the cells sink to become incorporated into the sediments eventually giving rise to oil deposits.

The laboratory experiments of this project are intended to simulate the extreme conditions experienced in upwelling areas. Frequent sampling throughout the induced bloom period and biochemical analyses of the plankton, together with the determination of the chemistry of the water, it was hoped would enable a relationship to be established between the parameters.

Skeletonema costatum and *Thalassiosira pseudonana* were the two diatom species used for the study owing to their dominance in most phytoplankton communities in coastal waters and also in upwelling zones. The two diatoms are members of the class Bacillariophyceae, and the family Bacillariaceae. They have siliceous tests, and in common with all diatoms, when cultured they require extra silicate in the nutrient media. Shell structure in the two species is very different. *Thalassiosira* have circular disc shaped plates which fit together like a pill box (Plate I, IV & V, see Result Chapter). The surface of the frustules is patterned with a siliceous lattice (Plate VI, Result Chapter). The cells grow in chains, filaments of mucilage passing through pores between adjacent cells holding them firmly together (Plate V, Result Chapter). *Skeletonema* is cylindrical with siliceous projection arising from the end discs (Plate VII-X, Result Chapter).

A more detailed description of the shell structure of the diatoms is given in the Electron microscope section of the Results Chapter.

Recently attention has turned towards the response of plankton to light quantity (intensity) and quality (wavelength). (Soeder & Stengel 1974, Voskresenskaya 1972, Ballantine et al 1979, Wallen and Geen 1971).

Both parameters are important to diatoms due to the differential absorption of light of various wavelengths with depth in the marine environment.

Wallen and Geen (1971) considered the effect of light quality on *Thalassiosira pseudonana*. They found that blue or green light favoured protein synthesis at the expense of carbohydrate production, compared to white light of the same intensity. If this reaction occurs in the oceans, phytoplankton composition will vary with depth. This study also revealed that on a per cell basis, chlorophyll and protein amounts were higher in blue, and lower in green light than in white light of equal intensity.

Light intensity has been shown to determine chlorophyll amounts per cell, and the size of the chloroplasts. (Strickland 1965). High light intensities appear to lower the chlorophyll content per cell, and reduce the size of chloroplasts, but increase the rate of photosynthesis.

Experiments to investigate the effects of light on plankton growth had been planned, but problems arose with the equipment for the experiments (Result Chapter). This is an area of research in which further work would be merited.

CHAPTER 2

METHODS

I Culturing Techniques

a) Master Cultures of *Skeletonema costatum* and *Thalassiosira pseudonana* were maintained in 100 ml Erlenmeyer flasks, each containing 50 ml of Guillards' "f/2" enriched seawater culture medium (Guillard and Ryther 1962, Appendix 1), which had been sterilized by autoclaving. The flasks were kept at 20°C in continuous white light provided by fluorescent tubes with an emission intensity of 2000 $\mu w\ cm^{-2}$. To ensure a rigorous stock, 1 ml subsamples were withdrawn from each flask every 10 days, and reinoculated into fresh media.

b) Experimental Cultures. Experiments were carried out in 10 litre pyrex vessels, which had been acid washed to prevent contamination by bacteria and micro organisms. Each flask was equipped with a rubber bung through which passed air inlet and outlet tubes, and a tube to withdraw samples for analysis. Guillards' "f/2" medium was used (Appendix 1); the anion and cation concentrations were altered to meet the conditions required for each experiment (Appendix 2). Six litres of medium were autoclaved at 15 lbs^{-2} for 15 minutes, cooled, and the pH adjusted to between 7.9-8.5. An airstream was bubbled through the medium to keep the algal cells in suspension, and to provide the carbon dioxide needed for cell growth. The temperature was kept constant at 20°C for all the experiments. Continuous white light produced by fluorescent tubes at an intensity, measured externally to the flasks, of 4000 $\mu w\ cm^{-2}$ was used for all nutrient experiments. The light source was altered for all the light quality experiments, using translucent squares of coloured acrylic (3.2 mm thick, ICI Perspex, red 400). The intensity of the red light, as with all light measurements, was determined using an irradiation lightmeter, and was found to be 1250$\mu w\ cm^{-2}$. Three flasks were set up for each experiment, two experimental and one as a control. Into each was inoculated 10 ml of exponentially growing cells from master cultures.

From the start of all the experiments regular cell counts were made using a haemocytometer. The cells were fixed by addition of 2-3

drops of Lugol's Iodine stain, then at least 4 counts were made of each sample.

At fixed intervals, samples were drawn off and centrifuged. The supernatant was removed and frozen for analysis of the nutrient content. The cell residue provided the material for biochemical analysis.

II Chemical Analyses

A) Nutrient Analyses were effected on the supernatant. The major anions required for algal growth were determined as follows:

1) Phosphate PO4

The method used was that described in Department of the Environment guide (Phosphorus in Waters, Effluents and Sewage 1980). The spectrophotometric method is derived from that of Murphy and Riley (1962).

In essence the method involves the reaction of phosphate and ammonium molybdate in acid solution to give phosphomolybdic acid, which can be reduced by a number of reagents to molybdenum blue. Ascorbic acid is utilised in this method as the reducing agent. An antimony salt is added to accelerate the formation of the molybdenum blue complex, the concentration of which is read spectrophotometrically. All spectrophotometric measurements in the project were made using a Pye Unicam SP6-350 Visible Spectrophotometer.

Reagents:

Mixed Reagent. The solutions must be added in the given order, mixed well, and used within 24 hours. The quantities given are suitable for 50 determinations.

250 ml	14% v/v	sulphuric acid
75 ml	4% m/v	ammonium molybdate
150 ml	1.76% m/v	ascorbic acid
25 ml	0.28% m/v	antimony potassium tartrate

Procedure. The frozen samples were thawed, and 20 ml aliquots pipetted into 50 ml acid-washed volumetric flasks. Each sample was replicated 3 times. 8 ml of mixed reagent was added, and the volume made up to 50 ml with distilled water. All samples were well mixed and left to stand for 10 minutes. The optical density of the solutions was then measured in the spectrophotometer at a wavelength of 882 nm, in a 1 cm disposable cuvette against a reagent blank.

A calibration curve was drawn (Appendix 3), using a standard phosphate solution diluted to cover the range 1-15 µg phosphate/50 mls. The standard error of the curve was ±.005.

2) Silicate SiO_4

The silicate available to growing plant cells, reactive silicate, was determined following the 1-amino-2-napthol-4-sulphuric acid (ANSA) reagent reduction method given in a publication by the Department of the Environment, Silicon in Waters and Effluents (1980). In the reaction silicate combines with ammonium molybdate in acid conditions to form yellow molybdosilicic acid. This is reduced with ANSA to yield a molybdenum blue complex, which is measured spectrophotometrically at a wavelength of 810 nm. The corresponding phosphate complex is destroyed using tartaric acid prior to reduction.

Reagents:

a) Ammonium Molybdate - sulphuric acid Reagent

The method involved dissolving 89 g ammonium molybdate in 800 ml distilled water. Into a beaker immersed in cold water 63 ml of concentrated sulphuric acid was slowly added to 100 ml distilled water, and mixed well. The acid solution was cooled, and added to the molybdate solution. The solution was made up to 1 litre with distilled water, and, when stored in a polythene bottle, remains stable for 6 months.

b) 28% m/v Tartaric acid Solution

When this solution is stored in a polythene container it remains stable for 6 months.

c) 0.2% m/v ANSA Solution

Into 10 ml distilled water 2.4 g sodium sulphate was dissolved, and after adding 0.2 g ANSA, the solution was mixed well, and diluted to about 90 ml with distilled water.

Finally 14 g potassium metabisulphate was added, stirred well to dissolve, and the volume of the solution was made up to 100 ml. The reagent must be stored in the dark, and prepared freshly each week.

Procedure. After thawing the samples, 5 ml of each was measured into a clean plastic 100 ml bottle, and 40 ml of distilled water was added. The solutions were mixed well, and 1.25 ml of the ammonium molybdate-sulphuric acid reagent was added to each sample. The samples were left to stand for 10 minutes, then 1.25 ml tartaric acid was mixed into each, and after a further 5 minutes 1 ml ANSA reagent was added to the samples. After mixing well, the samples were allowed to stand for 40 minutes, and finally the extinction of the resultant blue molybdenum complex was read at a wavelength of 810 nm in the spectrophotometer in 1 cm cuvettes against a reagent blank. Three replicates of each sample were made and measured.

The calibration curve (Appendix 4) in the range of 100-500 µg SiO_4/litre was prepared using dilutions of a standard silicate solution. The standard error of the curve was found to be ±.01.

All containers used in the experiment were polythene or plastic as silicate diffusing out of the walls of glass vessels would potentially produce significant contamination.

3) Nitrate NO_3

The method according to Strickland and Parsons (1972) was found to be the most suitable for the high concentrations of nitrate present in the culture medium. Nitrate is reduced almost quantitatively to nitrite when passed through a column containing cadmium filings loosely coated with metallic copper. The nitrite is then diazotized with sulphanilamide and

coupled with N-(1-napthyl)-ethylenediamine to form a pink azo dye, the extinction of which is measured spectrophotometrically. A correction is made for any initial nitrite present.

Reagents:

a) 1% m/v acidified sulphanilamide

Into about 300 ml distilled water, 5 g of sulphanilamide was dissolved, and 50 ml of concentrated hydrochloric acid was carefully added. The solution was mixed well and diluted to 500 ml with distilled water. It remains stable for several months.

b) 0.1% m/v N-1-napthylethylene diamine dihydrochloride

Procedure. The samples had to be diluted to lower the nitrate concentration to within the range of the method, 0-400 µg atoms NO_3-N/litre. After dilution 100 ml of the sample was passed through the copper-cadmium column at a fixed rate of 100 ml per 8-10 minutes. The first 40 ml through the column activates the filings; this volume was discarded. The next 50 ml was collected for measurement in an acid-cleaned 50 ml volumetric flask, and 1 ml of sulphanilamide solution was added immediately and mixed well. After 5 minutes, 1 ml of napthylethylenediamine solution was pipetted into the sample, mixed well and left to react for 10 minutes to allow the development of the pink colour of the azo dye, the optical density of which was then measured in the spectrophotometer at a wavelength of 543 nm in 1 cm cuvettes against a reagent blank.

A standard nitrate solution was used to construct a calibration curve in the range of 0-400µg atoms NO_3-N/litre (Appendix 5), which had a standard error of ±.05.

The accuracy of this method was affected by the high nutrient content of the culture medium. The high dilution factor needed to lower the concentrations to within the measurable range of the technique potentially introduced contaminations which were a source of errors. Reagent blanks were run in an attempt to minimise the error arising from the distilled water used for dilution, but the method was not perfected to produce reliable measurements in the experiments (See Results Chapter).

B) Biochemical Analyses of the Cell Residue.

The components analysed were of major importance in the main metabolic pathways which occur in the plankton cells, and in all living forms. They include carbohydrates, proteins, lipids, free amino-acids. Chlorophyll was also determined.

1) Carbohydrate

The cell carbohydrate was analysed according to the method of Dubois et al (1956).

Simple sugars, oligosaccharides, polysaccharides, and their derivatives condense giving an orange-yellow colour when treated with phenol, and concentrated sulphuric acid. This reaction is used for the quantitative colorimetric determination of carbohydrate, most simple sugars showing absorption peaks between 480-490 nm. For sugar mixtures the wavelength used is 490 nm. The reaction is sensitive, and the colour stable for a few hours, making it particularly useful for the quantitative estimation of carbohydrates in phytoplankton.

Reagents:

a) 5% Phenol solution
b) Concentrated sulphuric acid.

Procedure

A known volume of medium was centrifuged, the residual cell plug was homogenised with 3 ml distilled water. 1 ml of homogenate was pipetted into a boiling tube, 1 ml phenol, and 5 ml concentrated H_2SO_4 was added rapidly, and the contents of the tube gently mixed. The tube was covered with foil and left to stand for 30 minutes. The extinction of the solution was then measured in the spectrophotometer at a wavelength of 490 nm on 1 cm disposable cuvettes against a reagent blank. Three replicates were made on each sample.

A standard glucose solution was used to prepare a standard curve in the range 10-100 µg carbohydrate/ml, with a standard error of ±.001. (Appendix 6).

At this point it should be noted that since the results were plotted in terms of per 10^6 cells, there was no necessity to ascertain the packed cell volume of the plug.

2) Protein

Several methods were investigated. Most were insufficiently sensitive to detect the extremely small quantities of protein found in the algae. The most satisfactory results were obtained following the method of Lowry *et al* (1951) using the Folin-Phenol Reagent.

Colour production occurs in two distinct steps. The protein is first reacted with copper in an alkaline solution. The copper-treated protein then reduces the phosphomolybdic-phosphotungstic reagent (Folin-Phenol reagent), causing it to change colour from yellow to blue. The intensity of the blue solution is measured spectrophotometrically in 1 cm cuvettes at a wavelength of 750 nm.

Reagents:

a) Reagent A — 2% w/v Na_2CO_3 in a 0.1 N Sodium hydroxide solution (NaOH)

b) Reagent B_1 — 5% w/v $CuSO_4.5H_2O$

Reagent B_2 — 10% w/v potassium sodium tartrate

Reagent B — This reagent was made up by mixing 1 volume of B_1 solution and 1 volume of B_2 solution into 8 volumes of distilled water. The reagent had to be used within 24 hours.

c) Reagent C — This was a mixture of 50 volumes of Reagent A and one volume of Reagent B. This reagent was also only stable for 24 hours.

d) Reagent D — The Folin Ciocalteu Phenol Reagent.

e) Reagent E — Into 2 volumes of distilled water was mixed 1 volume Reagent D. The reagent had to be discarded after 24 hours.

Procedure:

The cell plug obtained after centrifuging a known volume of the medium was homogenised with 3 ml distilled water.

Into a boiling tube 1 ml of the homogenate was pipetted, and an equal volume of hot 6% w/v trichloroacetic acid (TCA) solution added to extract soluble non protein material. The sample was centrifuged, and the supernatant discarded.

To the cell residue 5 ml Reagent C (warmed to about 55°C) was added, and after 10 minutes 0.5 ml of Reagent E was added and mixed well. The sample was left to stand for 30 minutes, centrifuged, and the optical density of the supernatant was measured in the spectrophotometer using 1 cm cuvettes, at a wavelength of 750 nm, against a reagent blank.

Each sample was replicated three times.

A standard curve was prepared using an appropriately diluted standard bovine albumin solution in range of 0-100 µg protein/ml (Appendix 7) with a standard error of ±.01.

Turbidity occurring in the solutions interfered with the spectrophotometer readings. This was prevented by thoroughly centrifuging each sample. A further disadvantage with the method was the length of time needed for each sample, but quicker methods, such as the Biuret Method described by Raymont et al (1964), proved to be inadequate when measuring such low protein concentrations.

Minor modifications, as suggested by Price (1965), were made to the reagent formulae.

3) Lipid

The gravimetric technique of Folch, Lees and Sloane-Stanley (1957) was used. The simplicity and accuracy of the method made it preferable to more complicated alternative procedures such as the colorimetric method given by Strickland and Parsons (1972) which is an adaptation of the technique outlined by Mukerjee (1956), or the procedure involving the separation of lipids by thin layer chromatography, (Amenta 1964). It has the additional advantage of also being the first stage in the free amino acid analysis, enabling the first stage of the two analyses to be combined.

Reagents:

a) 2:1 chloroform-methanol solution
b) 0.05N KCl solution.

Procedure:

A known volume of culture medium was centrifuged, and the cell residue retained. In triplicate, each sample was homogenised with 12 ml 2:1 chloroform-methanol solution. (The volume of reagent required for the extraction is twenty time the wet weight of the cell plug).

To the homogenate was added 1 ml of 0.05N KCl solution. The solutions were mixed well, and centrifuged to separate the mixture into two phases.

The upper phase, water-methanol, contained free amino acids, the lower organic phase contained lipids dissolved in chloroform. (Bound lipids were precipitated out of the upper phase by the addition of KCl). The upper phase was carefully pipetted off, and stored in a deep freeze for later amino acid analysis. The lower phase was drawn off, care being taken not to disturb the central protein layer. The organic extract was

transferred to weighed foil cups, and evaporated to dryness in a desiccator through which was passed a nitrogen stream to prevent oxidation of the lipids. The foil containers were than re-weighed.

4) Free Amino Acids

A modification of the ninhydrin method (Troll and Cannan 1953) was used to determine total free amino acids.

Amino acids react with ninhydrin in reducing conditions producing a deep purple solution, the colour intensity is then measured with a spectrophotometer. The method has the advantage over other techniques (eg: that of Moore & Stein, 1948, and Snow & Williams, 1971) in that all amino acids give approximately the same amount of colour.

Reagents:

a) Ninhydrin Reagent. The reagent was made up by mixing the following compounds in the given order. The resulting solution was made up to 1 litre with distilled water, and was stable for several months.

10 gm ninhydrin
150 gm sodium acetate
400 ml absolute alcohol
100 ml glacial acetic acid.

b) Reducing Agent. This was a 0.001% potassium cyanide solution.

Procedure

Into a boiling tube was pipetted 0.5 ml water-methanol extract (The upper phase solutions retained from the lipid extraction). Each sample was done in triplicate. The boiling tubes were then placed into a water bath in a fume cupboard. The water bath was kept between 80-86°C. It was necessary to keep the apparatus in a fume cupboard on account of the poisonous fumes which were given off during the experiment. To the contents of the tube was added 0.5 ml cyanide solution, and 4 ml ninhydrin

reagent. After mixing all the solutions well, the tubes were covered with aluminium foil, and left to react in the water bath for 10 minutes. This enabled the full development of the deep purple colour, which was then read spectrophotometrically at a wavelength of 570 nm, in 1 cm cuvettes.

A glycine standard solution was used to prepare a standard curve between 0.1–0.5 µmols free amino acid/0.5 ml which had a standard error of ±0.001 (Appendix 8).

5) Chlorophyll

Chlorophyll was measured by the trichromatic method of Strickland and Parsons (1972). Plant chlorophylls are of three principal types; chlorophyll a, b, and c. Chlorophyll c principally occurs in diatoms, and can be adequately extracted according to the following procedure.

Reagent:

a) 90% acetone solution

Procedure

A cell residue obtained after centrifuging a known volume of medium, was transferred into a homogenising tube, and homogenised with 10 ml 90% acetone for 5 minutes.

The homogenate was pipetted into a centrifuge tube, which had been wrapped in foil to exclude light from the extract. The sample was left overnight in a refri gerator, then centrifuged for 10 minutes. The acetone supernatant was decanted into a 4 cm glass cuvette, and the extinction of the solution measured in a spectrophotometer at the following wavelengths: 630, 647, 664, and 750 nm.

A matched glass cuvette containing 90% acetone was used to zero the spectrophotometer.

The readings at 750 nm were used to correct for turbidity, they were subtracted from all the other measurements.

The chlorophylls were calculated from the equations:-

$$\text{Chlorophyll a } \mu g/l = [11.85_{E664} - 1.54_{E647} - 0.08_{E630}] \times \frac{v}{VP}$$

$$\text{Chlorophyll b } \mu g/l = [-5.4_{E664} + 21.03_{E647} - 2.66_{E630}] \times \frac{v}{VP}$$

$$\text{Chlorophyll c } \mu g/l = [-1.6_{E664} - 7.6_{E647} + 24.52_{E630}] \times \frac{v}{VP}$$

E_{664}, E_{647}, E_{630} are corrected extinctions at the various wavelengths.

v is the volume of acetone in ml

V is the volume of sample in litres

P is the path lenth of the cuvette in cm.

The equations are updated from Strickland and Parsons (1972), as given in a paper by Jeffrey and Humphrey (1975).

A computer program was obtained which incorporated the equations, and enabled the results to be quickly and easily determined.

III Sample Preparation for the Scanning Electron Microscope

In order to obtain information on the morphology of the diatoms, the cells were studied using a Jeol JSM-P15 Scanning Electron Microscope. Photographs were taken of the cells using Ilford FP4 Safety Film.

Procedure

From an exponentially growing master culture 10 ml of algae were centrifuged, and the supernatent discarded, leaving a plug of cells in the tube. To the plug was added about 10 ml M.HCl; the sample was mixed well and then centrifuged. The supernatant was discarded and 10 ml 5% gluteraldehyde solution shaken up with the cell residue. Prior to the critical point drying procedure the cells were dried by mixing them with alcohol solutions. The samples were put through a series of 50%, 70%, 90% and 100% alcohol. At each stage the alcohol was centrifuged off and the cell plug retained. Once the cells had been washed with absolute alcohol

they were ready for the critical point drying, a final fixing process which is carried out using liquid CO_2. Finally the fixed cells were coated with gold and palladium in an SEM-PREP sputter coater so they could be observed in the Scanning Electron Microscope.

Exponential cells were used for all electron microscopy work, older algae in the stationary phase were unsuitable on account of large quantities of mucous and organic debris adhering to the silica tests. This material was resistant to cleaning even with concentrated acids. (see Results and Discussion Chapters).

IV LIGHT MICROSCOPE STUDY on cell features

While making the regular cell counts throughout the experiments, the morphology and cytological features were noted, and comparisons were made between nutrient limited diatoms, and non limited algae.

The appearance of the cells was also observed to change at the different stages in the life cycle, making it easy to distinguish between exponentially growing cultures, and sessile stationary phase cells.

A Patholux light microscope was used for the study, with a maximum magnification of x47, which was adequate for observing major cellular components; the cell walls and silicate test, and the larger cytoplasmic structures within the cell such as the chloroplasts, nuclear material and storage products.

A drop of algal culture was placed on a haemocytometer for the routine cell count. While the count was being made, details of the cell morphology were noted and recorded.

RESULTS

I Nutrient Experiments

a) Non-limited Skeletonema (Figure 1 and 2)

Three 10 litre flasks, two experimental and one control, were set up containing 6 litres of Guillards' "f/2" media (Appendix 1). The vessels were maintained in continuous white light, the intensity of which measured externally to the flask, was 4000$\mu W/cm^2$. The contents of each flask were continually stirred using an air stream, and the temperature was kept constant at 20°C.

Each flask was inoculated with 10 ml of exponentially growing plankton culture, and sampling was carried out according to the procedure set out in the chapter on methods.

All the major nutrient elements, phosphorus, nitrogen and silicon were non limited. This was shown in the case of phosphorus by the phosphate curve in Figure 2.

As the chlorophyll per cell increased, protein and free amino acid on a cellular basis decreased relative to the cell lipid and carbohydrate content (Figures 1 and 2)

This seems to indicate a build up of storage materials within the cells to carry the diatoms through the stationary phase induced by low nutrient levels. The building materials, protein and amino acids, contained inside the cells, were used up during the production of the storage products, the carbohydrates and lipids.

b) Phosphate-limited Skeletonema (Figures 3 and 4)

Silicate and nitrate were not limiting throughout the experiment. Phosphate became limiting by day 4 (Figure 4).

Again the chlorophyll peak occurred on day 6, but the chlorophyll levels were lower than in the non limited experiment; maximum levels being 75µg/litre compared to 288µg/litre.

The internal cell reservoirs of protein and amino acid decreased on a relative basis to the increasing cell reserves of lipid and carbohydrate, but the extent of the changes were less marked than those occurring in non-limited conditions. This response to reduced nutrient levels has been reported by several workers notably Healey (1973), Strickland (1965), Barlow (1982), Morris et al (1983).

The relative importance of lipid in the cell biochemistry was greater in stationary phase cells in phosphate limited conditions compared to stationary phase cells in non-limited conditions. Lipid accounts for 8.1% of stationary phase cells in phosphate limited cells compared to 7.2% of cells in the stationary phase in the non-limited cultures.

A large build up of lipid relative to other major cell components, has been noted by a number of workers, who have reported that this was particularly evident in silicate-limited cultures. (Healey 1973, Strickland 1965, Werner 1966, Coombs et al 1967 a).

Phosphate and silicate uptake appear to be interdependent (Table 2). On day 4, all phosphate had been used up by the plankton. Thereafter the silicate concentration remained high with only small decreases occurring until day 7. This effect suggests that in the absence of adequate phosphate levels, silicate uptake declines, but this response has not been reported by any other worker.

In relative terms lipid production was increased at the expense of protein and amino acid levels. Carbohydrates were manufactured within the cells at an increased rate towards the completion of the growth cycle, carbohydrate being of major importance as storage material in cells entering unfavourable environmental conditions.

Figure 12 compares cell numbers and cell chlorophyll in non-limited, and phosphate-limited cultures. Cell counts were similar in both experiments, but in the nutrient non-limited experiment there was more chlorophyll/cell than in the phosphate-limited culture. (See Section

in this chapter on light microscopic study). Chlorophyll production under nutrient limitation appears to be reduced, an observation also noted by other researchers. (Healey 1973; Strickland 1965, Holmes 1966).

c) Silicate limited Skeletonema and Thalassiosira (Figures 5-8)

The two diatom species reacted in a similar way to silicate limitation.

Silicate levels were no longer detectable in the media by day 4 in both experiments (Figure 5 and 7). Chlorophyll measurements were not available for the *Skeletonema* culture, cell counts were used as an indication of cell mass. Cell numbers followed the same pattern with both diatoms, but the growth rate of *Thalassiosira* was greater then Skeletonema (Table 6 and Graph 4).

Protein and carbohydrate decreased in both diatoms at the onset of the stationary phase as silicate became limiting, then increased rapidly as the cells began to produce storage material to maintain them for the duration of stress conditions induced by low nutrient concentrations (Fig. 6 and 8).

Protein showed a decrease, followed by a relatively small increase. Protein is not used as storage material so is not manufactured by the cells in unfavourable conditions. Carbohydrates and lipids are produced at the expense of the cell building material, the protein and amino acids.

Both *Thalassiosira* and *Skeletonema* combined their free amino acids to make protein. Free amino acid levels inversely reflected protein concentrations, the latter increasing at the expense of the former. (Figures 6 and 8).

The lipid scale in the *Thalassiosira* graph (Fig. 7) is double that in the *Skeletonema* graph (Fig. 5). Lipid concentrations in both diatoms increase during the stationary phase, lipid accumulating as storage for the cells. The use of lipid in particular as storage material in assisting cells to survive stress conditions has been observed in other experiments. (Werner 1966, Coombs et al 1967).

Again the connection between phosphate and silicate uptake was demonstrated. (Table 6). When silicate became limiting, phosphate uptake was inhibited.

Comparing cell chlorophyll and cell numbers (Fig. 13), the cell chlorophyll in silicate limitation decreased steadily. It would appear silicate plays a role in chlorophyll production. (Werner & Pirson 1967, Werner 1969, Coombs et al 1967, Healey et al 1967).

d) Nitrate limited Skeletonema

Accurate nitrate determinations were not obtained, the nitrate limited experiment was abandoned.

The concentration of nitrate in "f/2" media was too high for most commonly used nitrate analyses. Large dilution factors were needed to lower the nitrate levels to within the working range of the most suitable method of analysis, the copper-cadmium reduction column (Strickland and Parsons 1972). This severely affected the accuracy of the method unless carried out with extreme care. Excellent results had been achieved with standard nitrate solutions, but no results had been obtained using "f/2" media.

The production of carbohydrate and lipid storage material by senescent cells, at the expense of further protein synthesis in nutrient limited and unlimited cultures was shown by plotting total culture protein, carbohydrate and lipid against time (Fig 9-11). In exponentially growing cells protein increased in both nutrient unlimited cells (Culture I, Fig 9), and phosphate limited cells (Culture II, Fig 9), but the increase was smaller in Culture II. For Culture I in the presence of adequate nutrients, protein production levelled off after day 6.

In Culture II, the absence of phosphate resulted in the cells becoming senescent by day 4, the protein synthesis decreasing steadily after this time. (Fig 9).

Carbohydrate and lipid production in Culture I and II increased sharply during exponential growth of the cells, (Fig 10 and 11). In Culture I the build up of these storage materials levelled off as the cells entered the senescent phase of their life cycle, after day 6. But in Culture II carbohydrate and lipid production continued after the cells became senescent (after day 4). Between day 4 and 5 carbohydrate and lipid was synthesised at the expense of protein production; the storage materials carried on being built up in the senescent cells, although protein production had slowed down, and protein levels had begun to fall (Figs 9, 10, and 11). Further accumulation of carbohydrate and lipid after day 6 was prevented by the lack of nutrients now found in both cultures, but especially phosphate in culture II (Table 8).

II Growth Rates and Growth Curves

All experimental data has been tabulated (Table 1-6). Sampling in these experiments commenced at least 48 hours after inoculation. The growth curves (Graphs 1-4) drawn from these data show exponential growth occurs in the first 80 hours after inoculation. This indicates for most of the experiments sampling has been carried out on late exponential-early stationary phase cultures. To include early exponential phase when the cell division rate is at a maximum, the experiments must be reorganized, and sampling times altered.

An experiment was conducted to determine more appropriate sampling times.

Skeletonema was grown in phosphate deficient media in continuous white light at a temperature of 20°C. Exponentially growing cells were used for inoculation, cell counts were made every 8 hours. Chlorophyll samples were made every 24 hours.

Samples for nutrient determinations were also taken once a day. The resultant growth curve plotted from the data collected is given in Graph 1.

All growth rates were calculated applying the equation according to Guillard (1973).

$K = [3.322/t_2-t_1]. \log N_2/N_1$

t_1 = initial time of sampling

t_2 = Sample time after time t_1

N_2 = Cell count at t_2

N_1 = Cell count at t_1

Growth rates do not appear to be significantly altered by nutrient limitation. Cell yield was reduced in nutrient limited cultures, as shown by curves II and III in Graph 1.

The growth curves of the phosphate limited cultures (curves II and III) levelled off between 40-50 hours after inoculation as the diatom cells entered the stationary phase. In non-limited cultures the growth curve does not level out until between 70-80 hours.

Cell yield in the nutrient limited experiments (II and III), was between 20 - 27 x 10^4 cells/ml, compared to the higher yield obtained in the non limited control culture (I) of 188 x 10^4 cells/ml (Table A).

III Light Experiments

No results were obtained owing to problems arising with the apparatus for the experiment. The experimental light box constructed to conduct the light experiments used a 450W xenon lamp which emitted high levels of ozone. A smaller box was built housing fluorescent tube lights. The wavelength of the lights was altered using the coloured perspex squares from the original box, but the intensity of the light was now found to be too low to support healthy growth in algal cultures.

The diatom cultures did not grow, and the experiments had to be discontinued.

IV Electron Microscopy

The cleaned shells of *Thalassiosira* and *Skeletonema* were examined by electron microscopy to determine the effect of nutrient limitation, in particular silicate limitation, on the external morphology of the cells' test.

The frustules of *Thalassiosira* showed no obvious differences when the cells were cultured without silicate. It is possible that this diatom has a relatively low silicate requirement, but there has been no research to prove this, and the reason for the negative reaction of the test to silicate deficiency remains unclear. More prolonged silicate deprivation may be needed before the shell features show signs of alteration.

Several interesting structures were observed, which will now be described. In the centre of the silicate test there is a large raised circular opening, and along the edge of the plates are regularly spaced pores. (Plates I, II and III).

Thalassiosira occurs in chains (Plate IV), cohesion is probably achieved by filamentous binding material passing between adjacent cells through matched pores (Plate V).

Plate VI shows a silicate plate of *Thalassiosira* stripped of all cellular material by prolonged treatment with concentrated sulphuric acid to show the lattice pattern. The lattice also appeared to be unaffected by silicate starvation.

By contrast with the situation in *Thalassiosira* there was some suggestion that silicate limitation affects the structure of the test of *Skeletonema*.

Non nutrient-limited *Skeletonema* cells have a central rod structure circled by 10-12 silicate projections arising out of the end plates of the silicate tests (Plate VII).

Cells which had been cultured in silicate deficient media had only 6-8 projections (Plate VIII), and these appeared abnormal. The tips of the protrusions were flat and "spade-like" in appearance (Plate IX). Occasionally two were observed in close proximity instead of the usual regular spacing between each structure. (Plate X).

These aberrant forms of test were observed in six samples, but to draw any firm conclusions that these abnormalities occurred as a direct consequence of silicate starvation, a more quantitative assessment would have to be conducted.

Skeletonema like *Thalassiosira* grows naturally in chains. The silicate projections link to hold the cells together. When less silicate is available it appears the number of these silicate structures is reduced, but the adhering properties of each is improved by the flattening of the ends of each protrusion. This does not seem to be completely effective, as cohesion between cells in silicate deficient cultures is lowered, and chain lengths are shorter (see light microscope section).

V Light Microscopy

Non-limited Skeletonema

Skeletonema cells were studied using a light microscope at a magnification of X47, at the same time as the cell counts were made during the experiments. All the features visible at this magnification were reported, and these observations are now given in detail.

Exponentially growing non-nutrient-limited *Skeletonema* cells are typically oblong in shape, with well defined outlines bounded by the silicate test enclosing the cell walls. Cytoplasmic material within the cells fill the central vacuole, the nuclear matter and chloroplasts are clearly visible.

The cell size varies, since rapid division occurs at this stage and each cell is at a different division phase. Chains of up to a dozen cells are commonly observed.

After the rapid division phase, the cells enter the stationary stage, and become obscured by copious extracellular mucilage.

Detailed descriptions of the diatom cells in this phase are impossible, large clusters of more than a hundred cells aggregate together surrounded by the organic material.

Phosphate-Limited Skeletonema

In phosphate-limited cultures, several changes were observed in the appearance of the cells. The general shape was long and thin, with indistinct silicate tests and cell walls. They most frequently occurred in pairs of curved cells forming "sickle" shaped chains. The maximum number of diatoms seen in a chain was only four compared to twelve cells per chain in non-limited cultures.

Internally the diatoms appeared to be empty, the cytoplasm and chloroplasts apparently forming a thin layer around the cell walls instead of occupying the central vacuole. (See Discussion).

When the cells entered the stationary phase of their life cycle they did clump together in the same way as in the nutrient non-limited cultures, but less extracellular material resulted in smaller aggregates of twenty or thirty cells.

Silicate-limited Skeletonema

The changes occurring in silicate deprived cultures were similar to those in phosphate limited cells.

The cell shape was elongated, and the majority formed the two cell sickle shaped chain. Again no more than four cells were observed in a chain. An explanation for such short chains can be found by referring to the Electron Microscope section, in which it is reported that in silicate deficient cells, silicate processes which normally link the cells together in chains were reduced in number. Although this was partially compensated for by the flattening of the ends of the protrusions, the cohesive properties of the structures were not so efficient, resulting in fewer cells linking together to form chains. At this point it must be said that

although no electron microscopy was carried out on phosphate limited cells, it is possible that the reduced chain lengths in these cells is also due to a reduction in the number of siliceous processes forming the link between the cells in a chain, due to the apparent interelationship between silicate and phosphate metabolism (see Discussion).

The silicate test was a thin line indicating a considerable reduction in frustule thickness, which was also suggested by comparisons made between the dry weight of non-nutrient-limited, and silicate-limited cultures.

An equal volume of each culture was centrifuged and the cell plug obtained was ashed in a muffle furnace. The dry weight of the nutrient non-limited algae was 1.7 mg/ml compared to 1.3 mg/ml for the silicate deficient cells. (The dry weight was taken as the weight which remained constant for at least 12 hours).

Other changes were observed in silicate and phosphate limited cultures. Large solitary square shaped cells with fragile tests were seen, particularly in "ageing" cultures entering the stationary phase. The cytoplasmic material had again shrunk against the cell walls, but the central vacuole was filled with green spheres of various sizes.

These bodies have been observed by several workers in other diatom species. (Crawford 1973, Dawson 1973 a, Stoermer & Pankratz 1964, Heath & Darley 1972, Lewin et al 1966).

They were particularly abundant in late exponential/early stationary cells, and have been analysed as lipid or oil droplets which occur most commonly in silicate starved diatoms (Lewin et al 1966). These droplets provide further evidence for reports that silicate deficiency results in a rapid acceleration of lipid production, compared to phosphate and nitrate limitation which initially result in increased carbohydrate synthesis, which is later followed by an increased lipid production. (Werner 1966).

Nutrient-limited Thalassiosira

The appearance of *Thalassiosira* did not change a great deal when the cells were cultured under nutrient limitation.

The tests and cell walls were very slightly thinner, and the chains did not consist of more than four cells compared to the eight cell chains of nutrient non-limited cells. Silicate limitation did result in the production of oil droplets, and cytoplasmic matter was seen lining the cell walls rather than filling the central vacuole in nutrient limited conditions, but none of the features were as extensive as the similar reactions in nutrient deficient *Skeletonema* cells. This is surprising since biochemical responses of *Thalassiosira* nutrient-limited cells are typical of other nutrient deficient diatoms. The explanation for the negative response of morphological features to nutrient limitation is not clear; possibly *Thalassiosira* can take up lower concentrations of silicate than *Skeletonema*, and remain unaffected by silicate starvation for longer (see electron microscopy section). More work is required before the reason can be discovered.

Discussion

The problems of investigating individual parameters with any accuracy in a sequential manner in the open ocean situation has limited the number of studies of this kind.

Barlow (1982) has conducted a series of experiments in the Southern Benguela upwelling system in which he determined nutrient concentrations in the water, and analysed the biochemistry of the phytoplankton cells occurring in the water. Diatoms dominated the plankton samples throughout the experiments, and it was concluded that a relationship existed between the biochemical and environmental variables. The protein/carbohydrate ratios were measured and found to be high (>1) in freshly upwelled water with adequate nutrients, but low (<1) in "aged" upwelled water containing low levels of nutrients. With sufficient nutrients available, protein was produced, carbohydrate synthesis proceeding relatively slowly. As nutrient levels decrease, carbohydrate was synthesized as storage material at an enhanced rate, at the expense of other cell constituents. These results were in agreement with those of Myklestad *et al* (1972) who also measured the protein/carbohydrate ratio in the diatom *Chaetoceros affinis* var. willer (Gran) Hustedt.

Myklestad conducted his experiments in controlled laboratory conditions, and found that the protein/carbohydrate ratio rapidly decreased as the diatom growth cycle proceeded. He cultured diatoms in media of varying nutrient concentrations, and concluded that cells grown in high nutrient concentrations have high protein/carbohydrate ratios compared to cells cultured in low nutrient levels. He noted considerable quantities of extracellular mucilage, a polysaccharide, in particular when the algae were grown in a medium with a high proportion of nitrate to phosphate.

"Controlled field conditions" have been achieved by using enclosed ecosystem bags (Morris et al 1983), and large volume plastic spheres. (Antia et al 1963).

The ecosystem bags located in Loch Ewe, Scotland have been used by several workers (Davies et al 1980, Morris et al 1983). Morris et al (1983) noticed a large rise in the carbohydrate content of the cells, as phosphorus and nitrogen became depleted in the water, the relative levels of lipid, protein and free amino acid dropped. On addition of nitrate, protein and lipid levels increased with only a relatively small change in the carbohydrate contents. This suggests the importance of carbohydrate as a storage product produced in unfavourable conditions at the expense of other cell constituents. Included in this study was the assessment of bacterial growth, to determine the significance of microbiol constituents and their possible interference in the data.

Bacterial mass and activity did not appear to increase until well after the completion of the bloom period. Several workers have observed the production of antibiotic compounds by actively growing phytoplankton cells which could provide the explanation for the inhibition of bacteria by exponentially growing phytoplankton cultures. (Al-Ogilvy and Knight Jones 1977, Ohta and Takagi 1977, Gauthier et al 1978).

With these previous studies in mind, the results of the present project are considered. In all the experiments, as the nutrients became depleted, carbohydrate levels increase considerably, at the expense of the protein and amino acid concentrations, which is in agreement with Barlow (1982) Myklestad et al (1972) and Morris et al (1983).

Lipid concentrations did not respond in exactly the same way as in the previous projects, increasing to a greater extent and more rapidly than had been previously noted. A possible explanation for this can be found in the paper by Barlow (1982). He states that nitrate in particular has a marked effect on the biochemical composition of phytoplankton. He quotes nitrate levels of between 2-15 µg atoms NO_3-N.l^{-1} in freshly upwelled water, and less than 2 µg atoms NO_3-N.l^{-1} in aged upwelled water. The nitrate content of "f/2" media used in the culture of diatoms for the experiments of the present project was 885 µg atoms NO_3-N.l^{-1}. The reason for such high nitrate levels is not clear, but nitrate was not limiting throughout the experiments, and lipid synthesis continued uninterrupted until the bloom conditions collapsed. In nitrate limitation, lipid production shows an initial decrease, which is later followed by a stimulation in lipid production (Werner 1966, Morris et al 1983).

Large quantities of extracellular mucilage surrounding stationary phase cells, a feature observed by Myklestad et al (1972), was also noticed in the present project. On completion of the growth cycle the diatom cells fall out of suspension despite the continuous stirring effect of the aeration flow. Aggregated cells settle on the bottom of the flask making cell counts virtually impossible since individual frustules were enclosed by the adhering material. As reported in Chapter II, this extracellular debris also presented a real problem in the cleaning and processing of the diatoms for examination by the electron microscope. Analysis of the material in two species of *Thalassiosira* has revealed long filaments of almost pure chitin (Falk et al 1966, Blackwell et al 1967), which provides a clue to the resistant nature of the macromolecular mass enclosing old diatom cells.

Most nitrate analyses determine concentrations at the levels which occur in ocean conditions. The extremely high nitrate levels of the "f/2" medium were outside this range, making it necessary for large dilutions to lower the concentration to within a measurable quantity. Such large scale dilution inevitably introduced errors.

Extreme care had to be exercised throughout the analysis, and a sufficiently accurate procedure was still being perfected at the conclusion of the project, and no satisfactory nitrate determinations were made on the "f/2" media. Phosphate and silicate did not present any such problem, and both nutrients were accurately measured.

Nitrogen deficiency has been extensively studied, because this element rather than phosphorus is usually the limiting nutrient in the sea (Strickland 1965). Phosphorus deficiency is less well known. Holmes (1966) and Werner (1966) in noting the response of diatoms to phosphorus limitation reported similar, but less intense, reactions as those occurring in nitrogen limitation.

When silicate concentrations become limiting in nature, it appears that cell composition is affected indirectly, with a cessation of growth late in the life cycle, just prior to mitosis or during cell wall formation. (Darley & Volcani 1969). Direct affects arising from silicon

deficiency have been demonstrated in laboratory experiments. (Werner & Pirson 1967), in particular the curtailment of protein and chlorophyll synthesis, followed by the inhibition of R.N.A. and carotenoid production.

In this project a reduced uptake of phosphorus from the media by the diatoms was particularly noted in silicon limited experiments. The absence of silicon in some way prevented phosphorus metabolism. In phosphorus deficient cultures, silicon uptake slowed down considerably. It appears the two elements are interdependent during their uptake, a deficiency in either one resulting in the prevention of the uptake of the other.

The problem of creating "natural" conditions in a laboratory was frequently noticed throughout this work, particularly in the case of the nitrogen in the media. The excess nitrogen resulting in continued lipid production rather than the great increase in carbohydrate storage (Werner 1966, Coombs et al 1967). In this case experiments should have been conducted to determine a balanced nutrient content bearing a greater resemblance to conditions in the wild.

Photosynthetic pigments are sensitive to changing environmental parameters, and are frequently used to detect such changes.

Nutrient deficiency, notably nitrogen, results in a decrease in photosynthetic pigments. (Healey 1973, Strickland 1965, Holmes 1966, Werner & Pirson 1967, Werner 1969, Coombs et al 1967, Healey et al 1967).

In a study by Holmes (1966) in which cytological manifestations of nutrient deprived marine centric diatoms were observed using light microscopy, it was noticed that there was a reduction in chlorophyll a synthesis, but also a concomitant decrease in the number of chromatophores per cell. This effect was especially apparent in nitrogen starved cultures of *Coscinodiscus wailesii*, *Ditylum brightwellii*, *Rhizosolenia setigera f. semispina*, and *Stephanopyxis turris*. The reduction in chromatophore numbers was probably the result of the cessation of division by the chromatophores when chlorophyll a production stopped owing to the absence of adequate nitrate.

(1966)

Holmes, was uncertain exactly what happened in *Skeletonema costatum*. *Skeletonema* cells have only one chromatophore per cell which makes this diatom more difficult to study with respect to photosynthetic pigments. Nitrate deficient *Skeletonema* cells contain very weakly pigmented chromatophores, making it hard to detect their presence with a light microscope. Since in the other diatom species the cessation of chromatophore division seems to be the reason for the reduced chromatophore number per cell, this gives rise to the question whether or not all nutrient deficient *Skeletonema* cells do in fact contain chromatophores, since the parental cells originally only have one chromatophore.

This observation was particularly relevant in this present work, because it was noticed that in nutrient starved cells the chromatophores were extremely indistinct (Light microscope section, Results chapter). It had been thought that the chloroplast retracted away from their normal central position within the cell vacuole, to line the inside of the cell walls. In light of Holmes' work another suggestion could be put forward. Possibly these nutrient deficient cells contain no chromatophores at all, because the single chromatophore per parental cell fails to divide once chlorophyll a production ceases. Electron microscope observations would be necessary to determine the existence of a chromatophore in newly divided nutrient deficient *Skeletonema* cells.

Another observation made by Holmes in his study was proportionately more large diameter cells in phosphate deficient cultures. He suggested that low phosphate levels might induce auxospore formation. In this present study these large diameter cells were noticed in nutrient deficient cultures. (Light microscope section, Results Chapter). Confirmation of the validity of the suggestion that these enlarged cells are more commonly found in cultures grown without sufficient nutrients can be made by statistically analysing a large number of counts of these cells in nutrient limited cultures.

In observations made in this work, it was noted that sickle shaped cell chains were also found in cultures in which low nutrient levels were experienced. In order to support the claim that this phenomenon occurs because of inadequate nutrition again a statistical study should be made,

and counts carried out on nutrient deficient cultures, and control nutrient non-limited cultures.

The effect of light quantity and quality on the pigments has also been studied. (Strickland 1965, Wallen & Geen 1971). Strickland (1965) reported lower chlorophyll concentrations per cell and higher maximum photosynthetic rates in cells grown at higher light intensities.

In the project of Wallen & Geen (1971), it was observed that on a per cell basis the amount of chlorophyll was higher in blue and lower in green light than in white light of the same intensity. This was also true of protein, RNA and DNA levels. (See later in this section, and in the Introduction for more of this study).

There are a number of directions in which the study could be extended. A complete detailed analysis of the lipid, free amino acid, and protein components at each stage in the life cycle of the algae could reveal changes occurring in each fraction as a result of nutrient limitation, light quality and quantity, and provide a more complex picture of the sequence of events which ultimately result in the formation of oil deposits in sediments underlying highly productive waters, especially upwelling areas.

In the enclosed ecosystem bags in Lock Ewe, Morris et al (1983) noted a shift in lipid metabolism from mainly non polar lipids (tri glycerides) at the onset of the growth cycle, to mainly polar lipids (phospho-lipids and sterols) as the bloom proceeded.

Wallen & Geen (1971) in a series of field experiments conducted at Saanich Inlet, British Columbia, Canada, reported a depth-dependent shift in the relative activity of some amino acids, particularly aspartate and glutamate, increased with depth. They also observed the relative concentration of carbohydrates, principally glucose and sugar phosphate, was higher in surface rather than deep water phytoplankton. These depth dependent changes associated with the shift from white to blue-green light were comprable to observations in culture studies (Wallen & Geen 1971 a), and further light quality work could reveal more interesting features in this area of research.

Results obtained from studies done so far, indicate a dynamic system which constantly changes in response to environmental parameters. It appears there is a relationship between nutrient concentrations, light quality and quantity, the age of the plankton cells, and the biochemistry of the algal cells. The reactions vary in extent and direction, making it necessary for more detailed investigations to allow complete understanding of every process occurring. The microscopic size of the diatoms, the low concentrations of some of the photosynthate components make analysis difficult to undertake with sufficient care and precision to obtain accurate results.

In many projects axenic (bacteria-free) unialgal cultures have been used, to ensure the isolation of the reactions of only this species. In this project unialgal cultures were used, but it was considered unecessarily time consuming to maintain axenic cultures when they apparently have an insignificant effect on the results. (Morris et al 1983, Gauthier et al 1978, Onta & Takagi 1977, Al Ogilvy & Knight Jones 1977).

Results from the project were of a very preliminary nature requiring a good deal more work. But arising from the data it appears again possible to conclude a relationship exists between the cell biochemistry and changes occurring in the environment; in nutrient concentrations, and light quality and quantity.

Future research could prove conclusively that given certain conditions, phytoplankton blooms occur producing areas of high productivity in surface waters, and ultimately result in the formation of oil deposits in the organic rich underlying sediments.

Summary

Organic materials are contributed to the sediment's underlying blooms, and there is current interest in the possibility that blooms in upwelling areas have been significant in the generation of oil reservoirs.

The present work has been directed at examining the interaction of nutrient level and culture age on the relative concentrations of lipid, protein and carbohydrate in the diatoms *Skeletonema costatum* and *Thalassiosira pseudonana*. In addition studies of the structure of the frustules of both species have been made in relation to nutrient availability.

The principal findings include:-

1 Increase in carbohydrate levels relative to protein and amino acids as nutrient concentrations decrease.

2 Lipid concentrations tended to increase to a greater extent and more rapidly than has been found in previous studies. The effect is correlated with high nitrate levels in the medium when other nutrients are limiting.

3 Large quantities of mucilage tend to accumulate around stationary phase cells.

4 Diminished uptake of silicon was noted in phosphorus-limited cultures.

5 Structural changes in the frustules are observed in *Skeletonema costatum* cultured in silicon limited conditions. No obvious changes are noted in *Thalassiosira*.

6 Chromatophore numbers and the rates of photosynthesis are decreased in nutrient deficient cultures.

7 These findings are discussed.

TABLE 1

NON LIMITING SKELETONEMA

	CARBO $\mu g/l \times 10^6$	PROTEIN $\mu g/l \times 10^6$	LIPID $\mu g/l \times 10^6$	FREE AA NANOMOLES 1×10^6	CHLORO $\mu g/l$	PO4 $\mu g/l$	Si04 $\mu g/l$	CELL 1×10^4 NO	$K = (3.322/t_2 - t_1) \log N_2/N_1$
T_0									
T_3	13.3	260	6.5	12.5	73	585	400	40	3.4
T_4	10.2	130	7.5	3.7	246	215	336	145	1.4
T_5	15.5	214	13.0	7.5	357	5	290	115	-.4
T_6	28	357	15.5	10.1	307	10	212	90	-.3
T_0									
T_3	10.2	520	2	7.3	10.1	840	385	10	2.5
T_4	7.4	1000	7.5		80.4	620	340	53	2.7
	4.1	40	17.5	7.9	103.9	475	283	107	1.8
T_5	7.4	40	2.2		163.4	315	140	132	1.0
	8.6	40	3.9	3.8	233.8	145	117	152	.4
T_6	22.6	80	9.2	10	288.9	50	60	130	-.18
T_7	32.1	1000	11.1	17	197.7	15	0	90	-.5
T_0									
T_2	3.07	270	4.7	.4	4.6	665	420	19	2.3
T_3	8.6	60	.1	.4	20.5	515	320	66	1.8
T_4	9.9	10	.4	.1	81.2	60	34	206	1.6
	17.1	12	2.9	.1	73.2	15	12	172	-.7
T_5			.9			0	2		
	26.6	11		.07	69.5			209	.4
T_6	23	6	.1	48.9	48.9	0	10	188	-.14
T_7	17.8	14	3.9	.05	36.9	0	17	177	-0.9
T_0									
T_3	8.5	56	2.5	.9	71.7	665	290	40	1.1
T_4	11.7	30	6.4	.4	481	265	167	125	1.6
	10.2	39	11.5	.7	356	240	128	95	-1.1
T_5	37.2	78	23.9	1.5	379	95	112	46	-1.5
T_6	89.4	310	53.3	6.1	280	45	176	15	-1.6

NON LIMITING SKELETONEMA

	CARBO µg/1x10^6	PROTEIN µg/1x10^6	LIPID µg/1x10^6	FREE AA NANOMOLES 1x10^6	CHLORO µg/1	PO4 µg/1	Si04 µg/1	CELL$_4$NO 1x10^4	K=(3.322/t_2-t_1) log N_2/N_1
T_3						595	420		
T_4	6.6	144		3.0	50	475	384	10	
	7.7	70.5	1.4	2.0	151	415	434	34	3.5
T_5	8.6	24.4	3.4	1.4	349	220	290	86	4.5
	12.8	20.6	7.6	1.2	426	60	232	91	0.2
T_6	21.9	53.6	20.4	1.8	340	15	220	49	-1.3
T_0						770	250		
T_3	3.8			2.0	11.1	385	232	11	
T_4	5.3	11.1	10	2.0	227	295	112	67	2.5
T_5	5.5	12.3	10	0.4	478	75	52	152	1.2
T_6	8.2	27.5	10	0.5	586	30	33	136	-0.15
T_0						2000	190		
T_3	4.8	---	3.2	2.0		2000	290	17	
T_4	6.4	2.5	2.8	0.8		1250	249	87	2.4
T_5	8	3.4	7.5	0.3		650	228	158	0.7
T_6	7.4	3.1	6.8	0.5		475	280	202	0.3
T_1									
T_2								1.48	
T_3	6.8	450	65	85	84	655	440	20	1.9
T_4	7.1	163	74	34	487	190	337	139	2.2
T_5	10.1	116	130	21	348	40	274	167	0.26
T_6	22.6	278	104	70	---	0	172	124	-0.43

TABLE 2

PHOSPHATE LIMITED SKELETONEMA

	CARBO µg/1x10^6	PROTEIN µg/1x10^6	LIPID µg/1x10^6	FREE AA NANOMOLES 1X10^6	CHLORO µg/1	PO4 µg/1	Si04 µg/1	CELL NO 1x10^4	K	HOURS
T_0										
T_3	17	360	3.8	1.3	18	180	440	13	2.3	62
T_4	6.5	50	1.7	4.3	74	0	328	116	3.6	83
	7.7	50	0.7	5.5	74	0	145	134	0.3	96
T_5	6.1	35	3.4	4.2	79	0	234	145	0.3	103
	12.7	40	7.3	7.1	84	0	202	109	-0.9	113
T_6	9.5	60	8.2	12	60	0	202	97	-0.13	142
T_7	8.2	80	8.8	15	23	0	223	79	-0.2	167
T_3	7.2	39	0.8	0.9	81	300	222	36		66
T_4	10.3	20	6.0	0.4	392	5	111	99	1.4	90
	9.7	56	-----		365	0	112	74	-1.2	98
T_5	13	58	16	9.6	338	0	112	56	-0.6	114
T_6	23.9	105	179	24	286	0	120	39	-0.5	138
T_0						335	305			
T_3	9.8			4.4		160	231	8		70
T_4	4.6	5.7	6.7	0.8	449	0	93	130	3.8	95
T_5	7.3	18.4	9	0.4	425	0	80	122	-0.09	118
T_6	6.4	12.6	14	0.7	420	0	71	119	-0.03	143
T_0									0.21x 10^4/ml	
T_3	8	210	3.7	7.5	20	80	350	24	2.6	62
T_4	5	60	0.8	2.9	83.8	0	257	124	2.7	83
	5	0.6	1.1	4.0	91.9		228	172	0.26	96
T_5	6	20	3.1	3.9	95.6	0	139	159	-0.03	103
	13	40	8.2	4.3	100.7		194	146	-0.05	113
T_6	15	70	7.9	11	75	0	202	101	-0.64	142
T_7	14	80	8.2	13	49		223	85	-0.26	167

PHOSPHATE LIMITED SKELETONEMA

	CARBO $\mu g/1x10^6$	PROTEIN $\mu g/1x10^6$	LIPID $\mu g/1x10^6$	FREE AA NANOMOLES 1x106	CHLORO µg/l	PO4 µg/l	Si04 µg/l	$CELL_4NO$ 1x10	K	HOURS
T_3	10.6		----	2.7	11.8	115	212	11	2.7	70
T_4	8.7	9.4	9	1.8	259	0	200	79		95
T_5	16.9	17.6	13	0.7	277	0	200	115	0.5	118
T_6	9.5	19.4	18	1	278	0	78	77	-0.5	143
T_3	9	54	0.4	0.52	75	250	280	41	2.7	66
T_4	13.9	48	12.3	0.16	549	15	157	65	0.5	98
	16.1	52	16.6							
T_5	38.8	130	10.8	0.23	386	0	178	23	-2.2	114
T_6	26	228	9.5	0.234	183	0	207	21	-0.13	138

TABLE 3

NON LIMITING SKELETONEMA

	CARBO µg/1x10^6	PROTEIN$_6$ µg/1x10^6	LIPID µg/1x10^6	FREE AA NANOMOLES 1x10^6	CHLORO µg/l	PO4 µg/l	Si04 µg/l	CELL COUNT 1x10^4	K	
1. LOG PHASE	13.3	260	6.5	12.5	73	585	400	40	3.4	48 hrs
STATIONARY	28	357	15.5	10.1	307	10	212	90	-0.3	120 hrs
2. LOG PHASE	10.2	520	2	7.3	10.1	840	385	10	2.5	62 hrs
STATIONARY	32.1	1000	11.1	17	197.7	15	0	90	-0.5	167 hrs
3. LOG PHASE	3.07	270	4.7	0.4	4.6	665	420	19	2.3	43 hrs
STATIONARY	17.8	14	3.9	0.05	36.9	0	17	177	-0.9	159 hrs
4. LOG PHASE	8.5	56	2.5	0.9	71.7	665	290	40	1.1	66 hrs
STATIONARY	89.4	310	53.3	6.1	280	45	176	15	-1.6	138 hrs
5. LOG PHASE	6.6			3.0	50	475	384	10		90 hrs
STATIONARY	21.9	53.6	20.4	1.8	340	15	220	49	-1.3	144 hrs
6. LOG PHASE	3.8			2	11.1	385	232	11		70 hrs
STATIONARY	8.2	27.5		0.5	586	30	33	136	-0.15	143 hrs
7. LOG PHASE	4.8		3.2	2		2000	290	17		66 hrs
STATIONARY	7.4	3.1	6.8	0.5		475	280	202	0.03	140 hrs
8. LOG PHASE	6.8	450	65	8.5	84	665	440	20	1.9	48 hrs
STATIONARY	22.6	278	104	7.0		0	172	124	-0.43	

TAKING THE FIRST IV EXPERIMENTS n=4

TABLE 4

NON LIMITING SKELETONEMA

	CARBO µg/1x10^6	PROTEIN µg/1x10^6	LIPID µg/1x10^6	FREE AA NANOMOLES 1x10^6	CHLORO µg/1	PO4 µg/1	SiO4 µg/1	CELL COUNT 1X10^4	K	
1. LATE LOG PHASE	8	210	3.7	7.5	20	80	350	24	2.6	62 hrs
STATIONARY	15.8	70	7.9	11	75	0	202	101	-0.64	142 hrs
2. LATE LOG PHASE	9	54	0.4	0.52	75	250	280	41	2.7	66 hrs
STATIONARY	26	228	9.5	0.23	183	0	207	21	-0.13	138 hrs
3. LATE LOG PHASE	10.6			2.7	11.8	115	212	11	2.7	70 hrs
STATIONARY	9.5	19.4	18	1	278	0	78	77	-0.5	143 hrs
4. LOG PHASE	17	360	3.8	1.3	18	180	440	13	2.3	62 hrs
STATIONARY	9.5	60	8.2	12	60	0	202	97	-0.13	142 hrs
5. LOG PHASE	7.2	39	0.8	0.9	81	300	222	36		66 hrs
STATIONARY	23.9	105	17.9	24	286	---	120	39	-0.5	138 hrs
6. LOG PHASE	9.8			4.4		160	231	8		70 hrs
STATIONARY	6.4	12.6	14	0.7	420	0	71	119	-0.3	143 hrs

TABLE 5

UNLIMITED SKELETONEMA

	LOGARITHMIC				STATIONARY			
	n	$\bar{x}$	$\pm$SD	RANGE	n	$\bar{x}$	$\pm$SD	RANGE
CARBOHYDRATE µg/1x10^6	4	8.7	3.7	3.07-13.3	4	41.8	27.9	17.8-89.4
PROTEIN µg/1x10^6	3	195	98	56-270	3	227	151.8	14-357
LIPID µg/1x10^6	3	3.7	1.7	2-6.5	3	26.6	18.9	11.1-53.3
FREE AA NM/1x10^6	4	1.9	0.7	0.9-3	4	2.2	2.2	0.5-6.1
CHLORO	4	39.8	32.5	4.1-73	4	205	105	36-307
PO4	4	499	242	585-840	4	17.5		0-45
SiO4	4	373	49	290-420	4	101	93	0-212
CELL COUNT	4	27 54 hrs	13 9	10-40 43-66	4	93 146 hrs	57 18	15-177 120-167
K	4	2.3	0.8	1.1-3.4	4	-0.6	-0.5	-0.9--1.6
PHOSPHATE LIMITED SKELETONEMA								
LOG								
CARBOHYDRATE	2	12.5	4.5	8-17	2	12.7	3.2	9.5-15.8
PROTEIN	2	285	75	210-360	2	65	5.0	60-70
LIPID	2	3.8	0.1	3.7-3.8	2	8.1	0.2	7.9-8.2
FREE AA	2	4.4	3.1	1.3-7.5	2	11.5	0.5	11-12
CHLORO	2	19	1.0	18-20	2	67.5	7.5	60-75
PO4	2	130	50	80-180	2	0	0	0
SiO4	2	395	45	350-440	2	202	0	202
CELL COUNT	2	18.5	5.5	13-24	2	99	2	97-101
K	2	2.5	0.2	2.3-2.6	2			

TABLE 6

SILICA LIMITED SKELETONEMA

	CARBO µg/10^6	PROTEIN µg/10^6	LIPID µg/10^6	FREE AA NANOMOLES/ 10^6	CHLORO µg/l	P04 µg/l	Si04 µg/l	CELL NO $1x10^4$	K	HOURS t
T_0						1500	148			
T_3	6.5	1.8	3.6	1.3		1000	83	73		66
T_4	6.1	2.7	5.9	0.5		590	0	140	0.9	89
T_5	5.4	2.7	4.8	0.3		590	0	207	0.5	116
T_6	8.8	4.4	6.8	0.5		185	0	214	0.04	140

SILICA LIMITED THALASSIOSIRA

T_0						1500	210			
T_1	4.7	0.68	15	1.8	78	1950	192	11		24
T_2	3.4	2.03	6.9	0.92	1262	1990	35	96	3.1	48
T_3	9.8	4.3	11.2	0.46	2580	190	0	173	0.9	69
T_4	6.3	3.2	15.6	0.49	3795	495	0	204	0.2	92
T_5	6.2	3.2	14.1	0.2	4448	230	0	293	0.4	121

Si04 LIMITED SKELETONEMA

EARLY STATIONARY	6.5	1.8	3.6	1.3		1000	83	73		66
STATIONARY	8.8	4.4	6.8	0.5		185	0	214	0.04	140

Si04 LIMITED THALASSIOSIRA

EARLY STATIONARY	3.4	2.03	6.9	0.92	1262	1990	35	96	3.1	48
STATIONARY	6.2	3.2	14.1	0.2	4448	230	0	293	0.4	121

TABLE 7

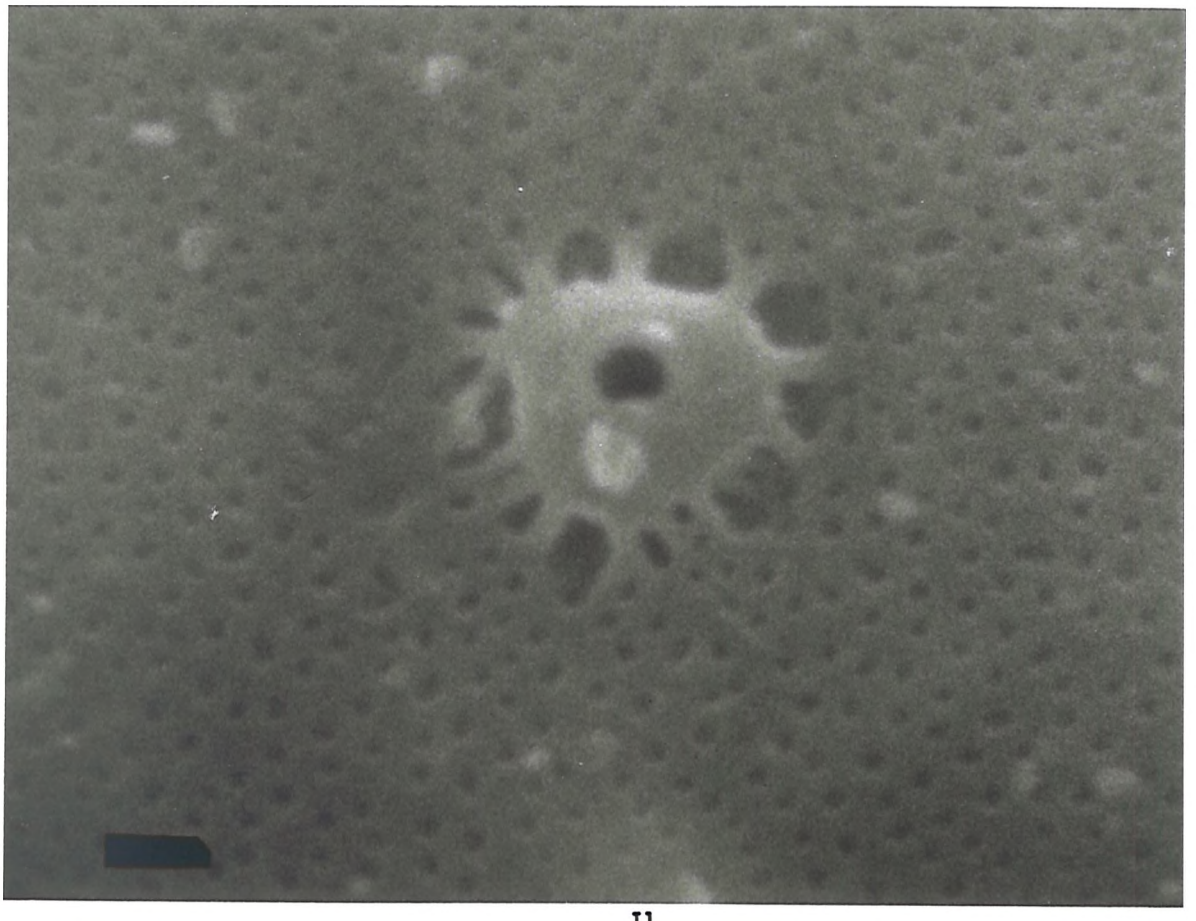
	I			II			III		
	CELL COUNT $1x10^4$	CHLORO µg/l	K	HRS	CELL COUNT $1x10^4$	CHLORO µg/l	K	HRS	CELL COUNT $1x10^4$	CHLORO µg/l	K	HRS
T_0	0.1				0.1				0.1			
T_1	0.36		0.1	7	0.51		0.1	7	0.16		0.4	7
T_2	0.66		0.13	15	0.9		0.6	15	0.38		0.9	15
T_3	1.58		0.1	24	1.93		0.9	24	1.26		1.4	24
T_4	2.87		0.16	32	3.15		0.5	32	2.12		0.5	32
T_5	7.29		0.14	40	7.89		1.0	40	5.49		1.0	40
T_6	16.06		0.56	48	11.9		0.4	48	9.3		0.5	48
T_7	26.66		1.03	56	22.16		0.6	56	13.57		0.4	56
T_8	67.7		0.6	64	26.16		0.18	64	15.4		0.14	64
T_9	116.8		0.2	72	24.3		-0.8	72	17.3		0.12	72
T_{10}	140.5		0.16	80	27.1		0.1	80	15.0		-0.15	80
T_{11}	163		0.16	88	25.4		-0.7	88	20.9		0.3	88
T_{12}	188.8		-0.09	96	25.6		0.008	96	17.2		-0.2	96
T_{13}	173.1		-0.4	104	32.0		0.2	104	18.3		0.06	104
T_{14}	166		-0.05	112	22.8		-0.3	112	20		0.09	112
T_{15}	161			124	21		-0.1	124	22.8		0.14	124

$K = (3.322/t_2-t_1) . \log N_2/N_1$

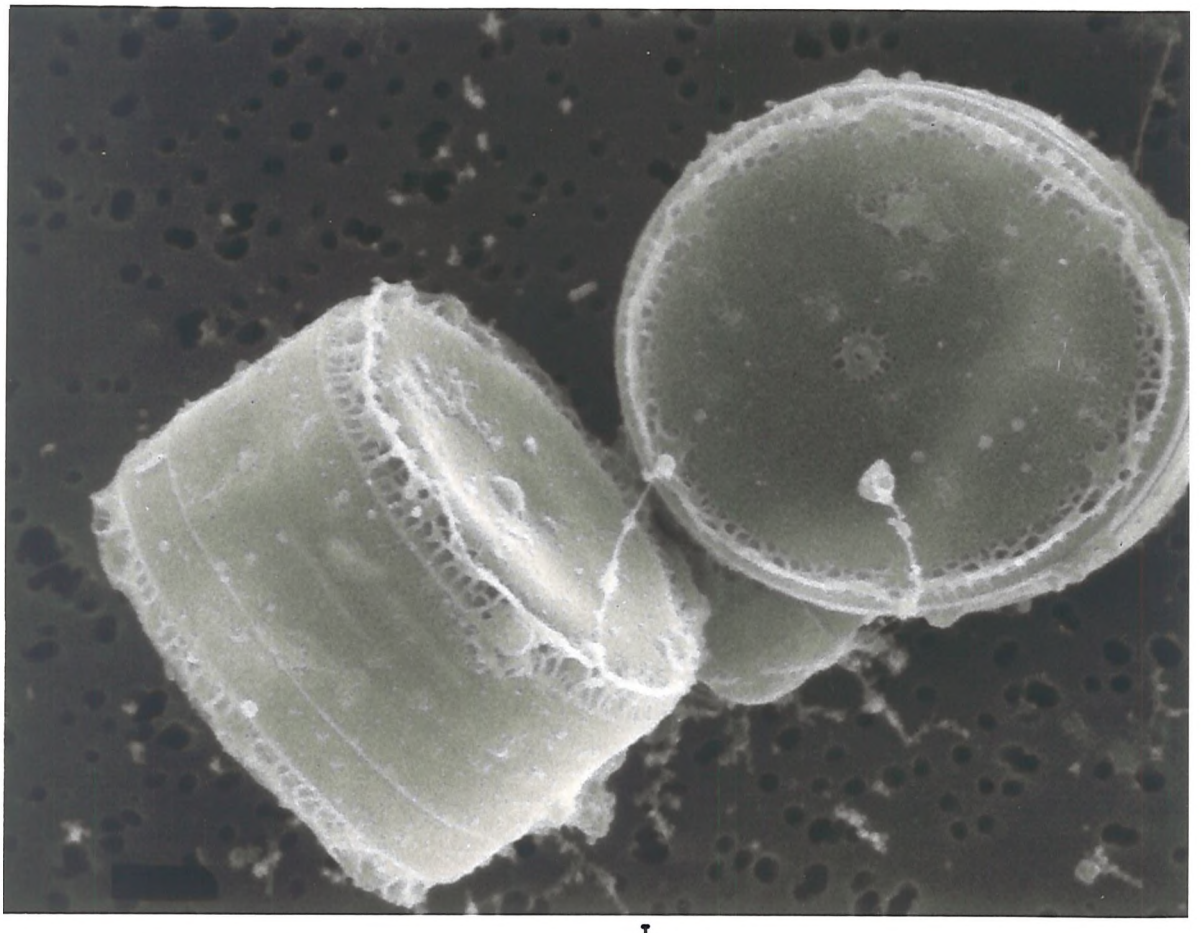
TABLE 8

TIME IN DAYS	PHOSPHATE µg/l		SILICATE µg/l		TOTAL PROTEIN MG/ML		TOTAL CARBOHYDRATE µg/ml		TOTAL LIPID µg/ml	
	I	II	I	II	I	II	I	II	I	II
3	840	80	385	350	0.052	0.051	1.0	1.9	0.2	0.9
4	620	0	340	257	0.055	0.075	3.9	6.2	4.0	2.0
5	145	0	117	150	0.062	0.07	13.1	19	6.0	8.0
6	50	0	60	141	0.11	0.07	29	15	12	8
7	15	0	0	139	0.11	0.06	29	12	10	7

LIST OF PLATES

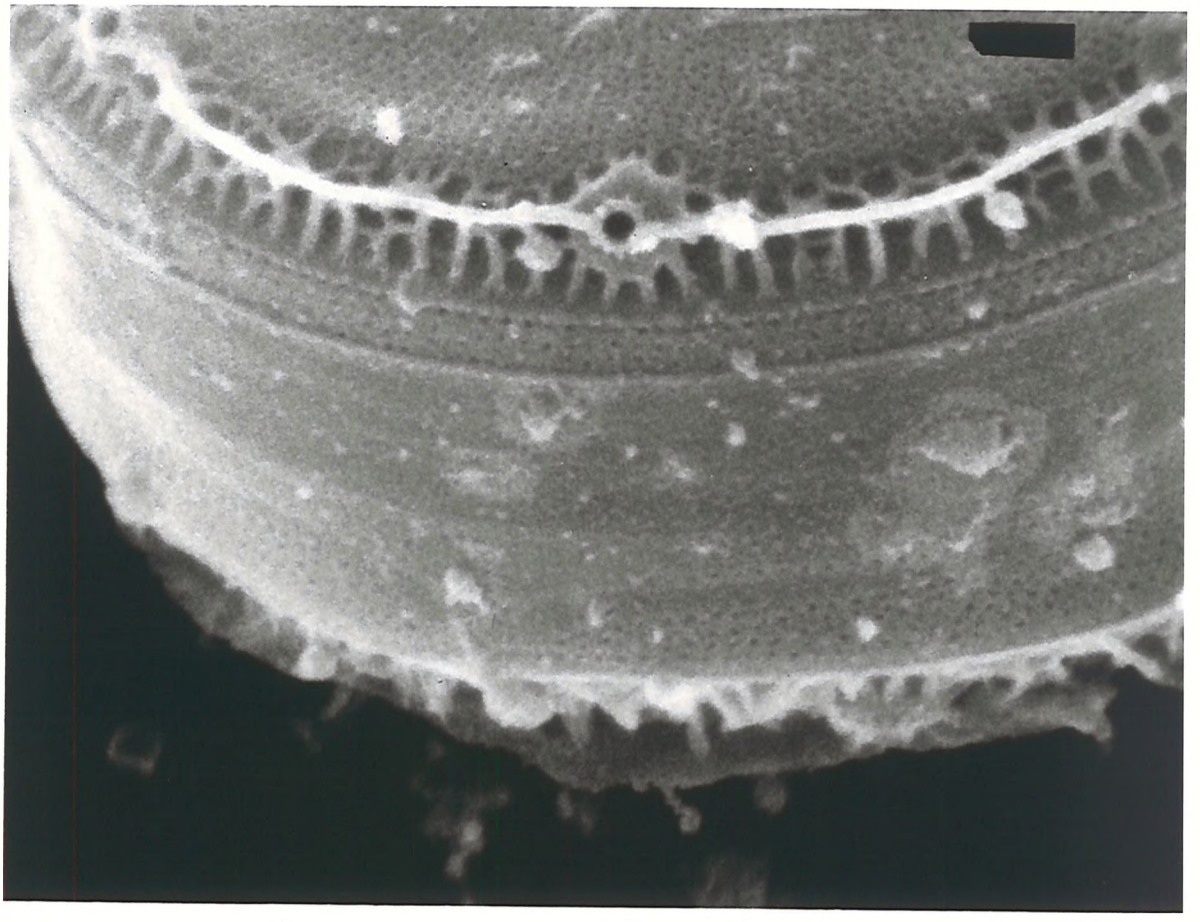


II



I

III



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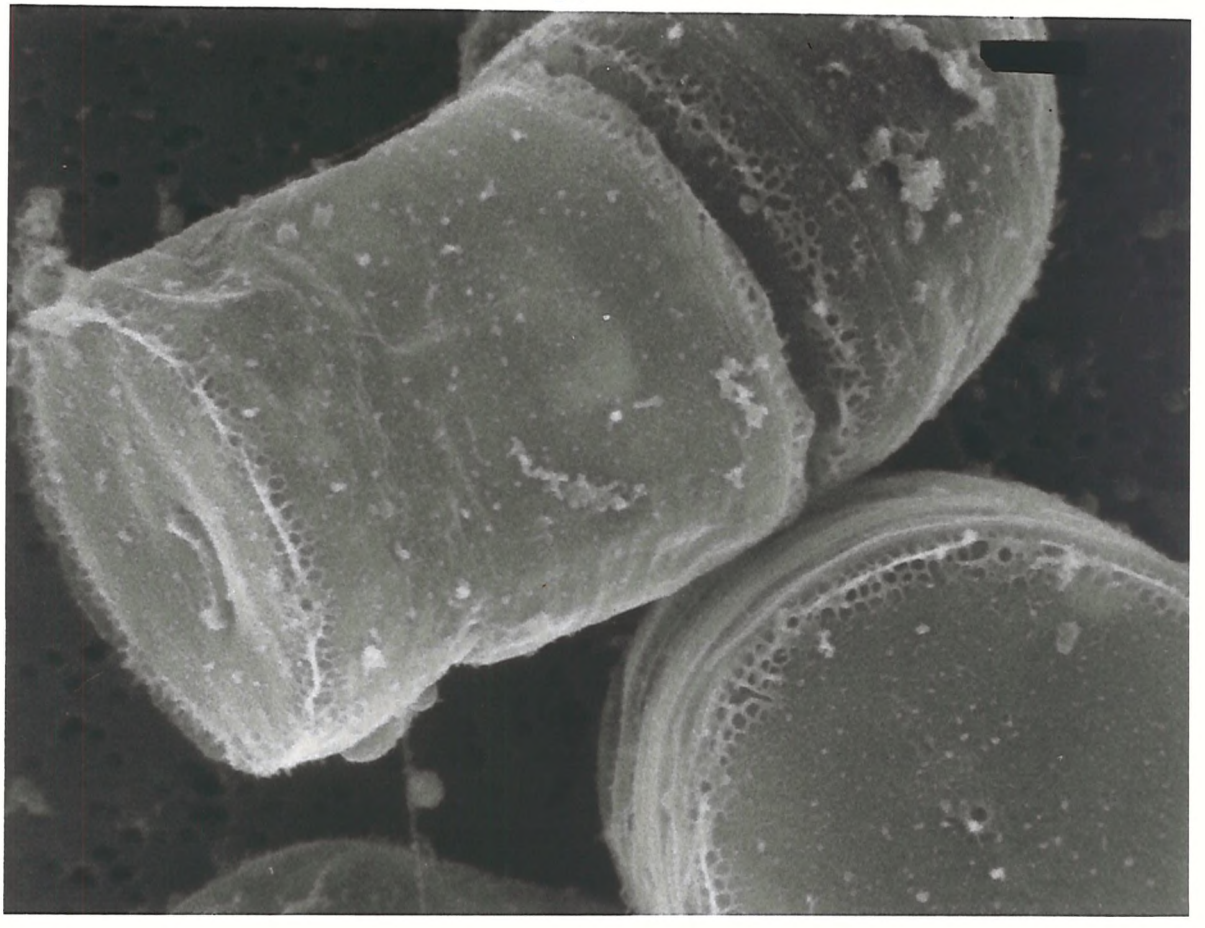

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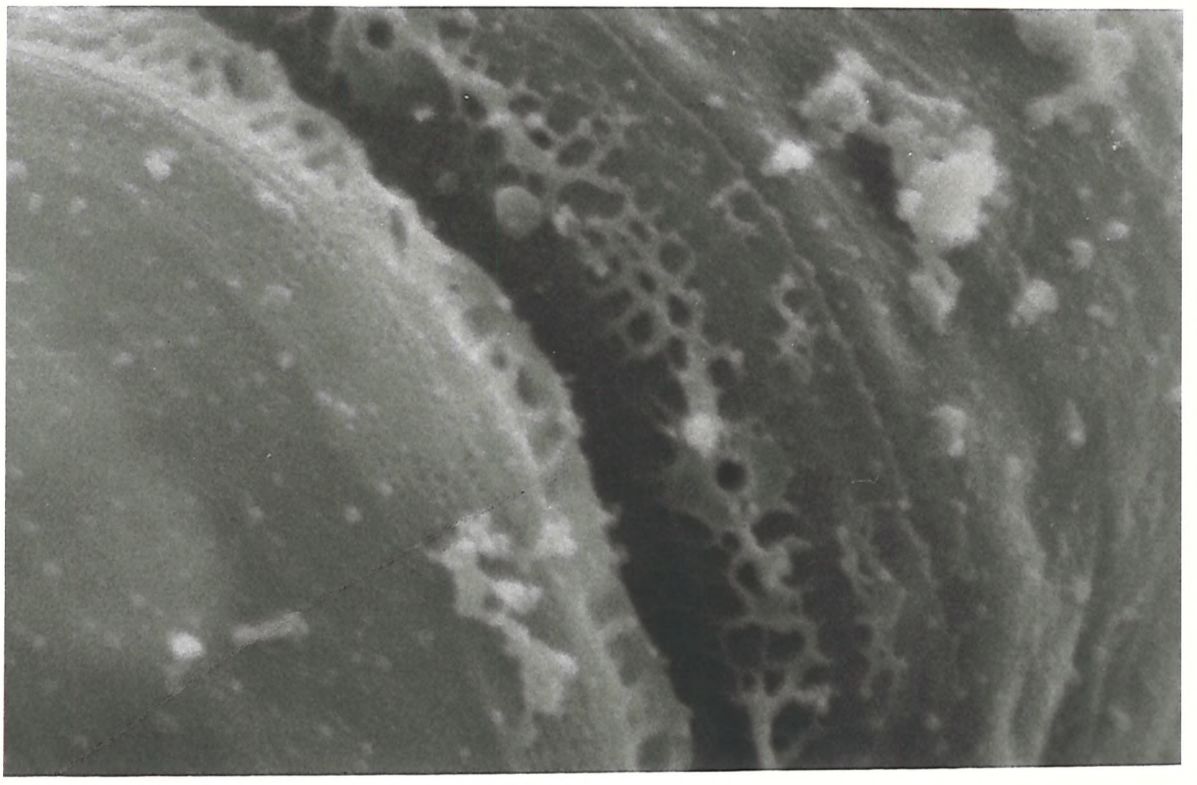

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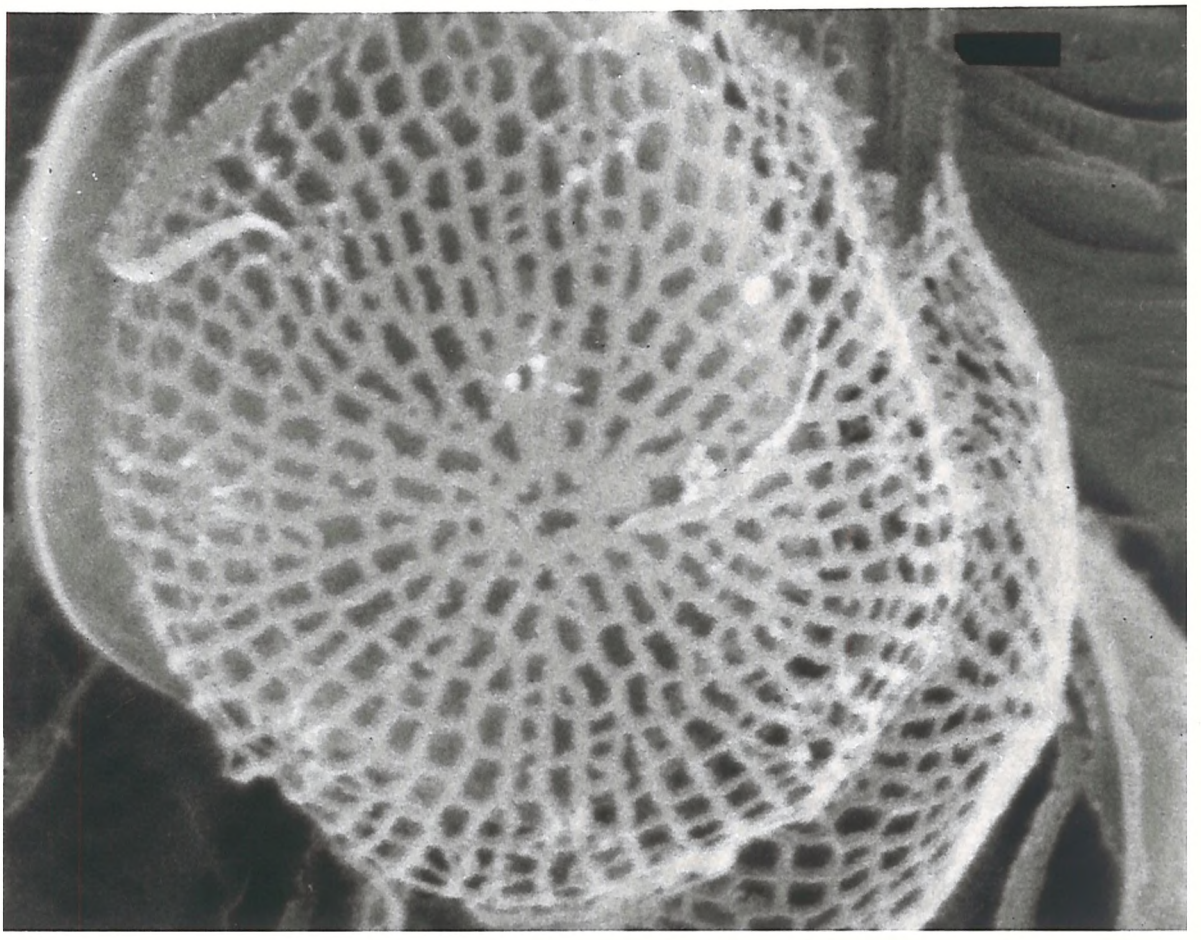

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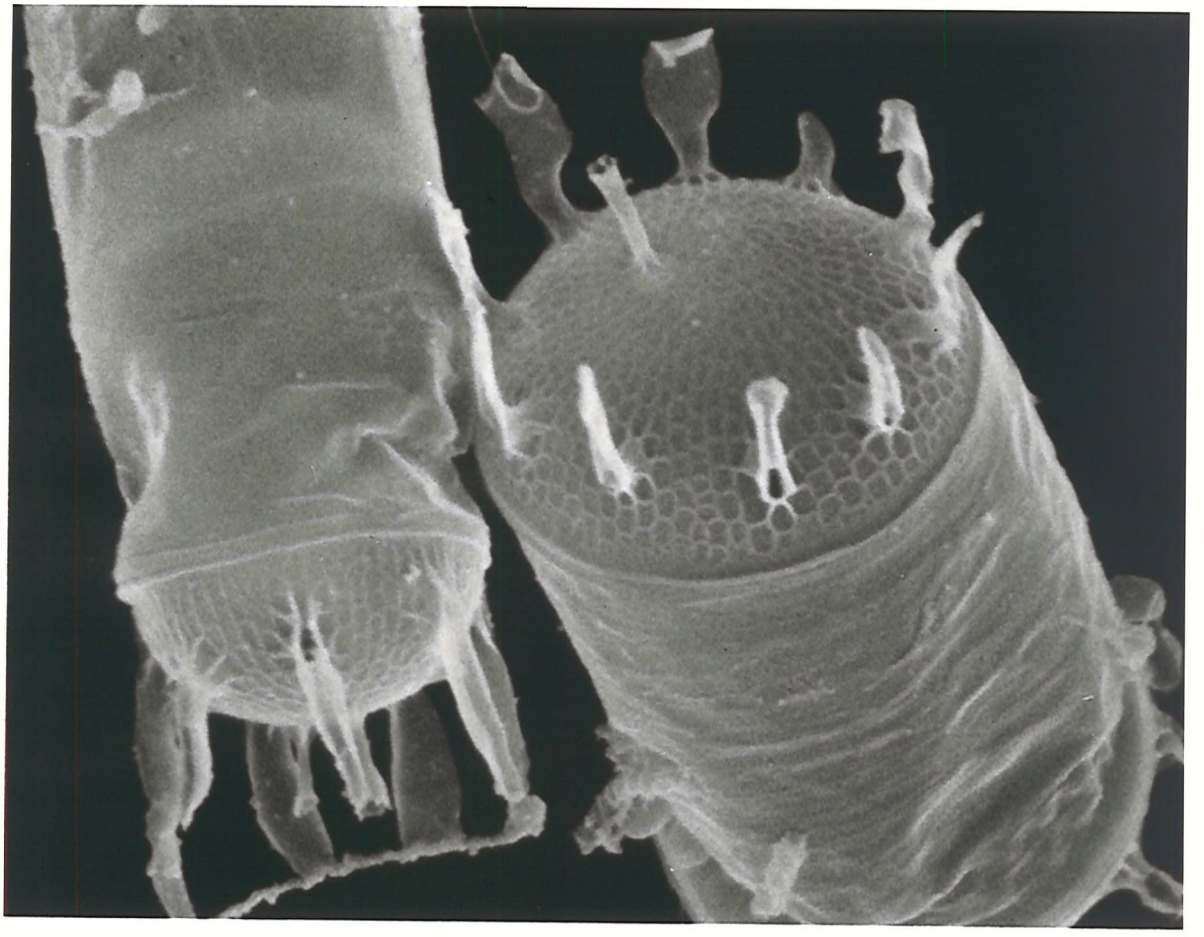
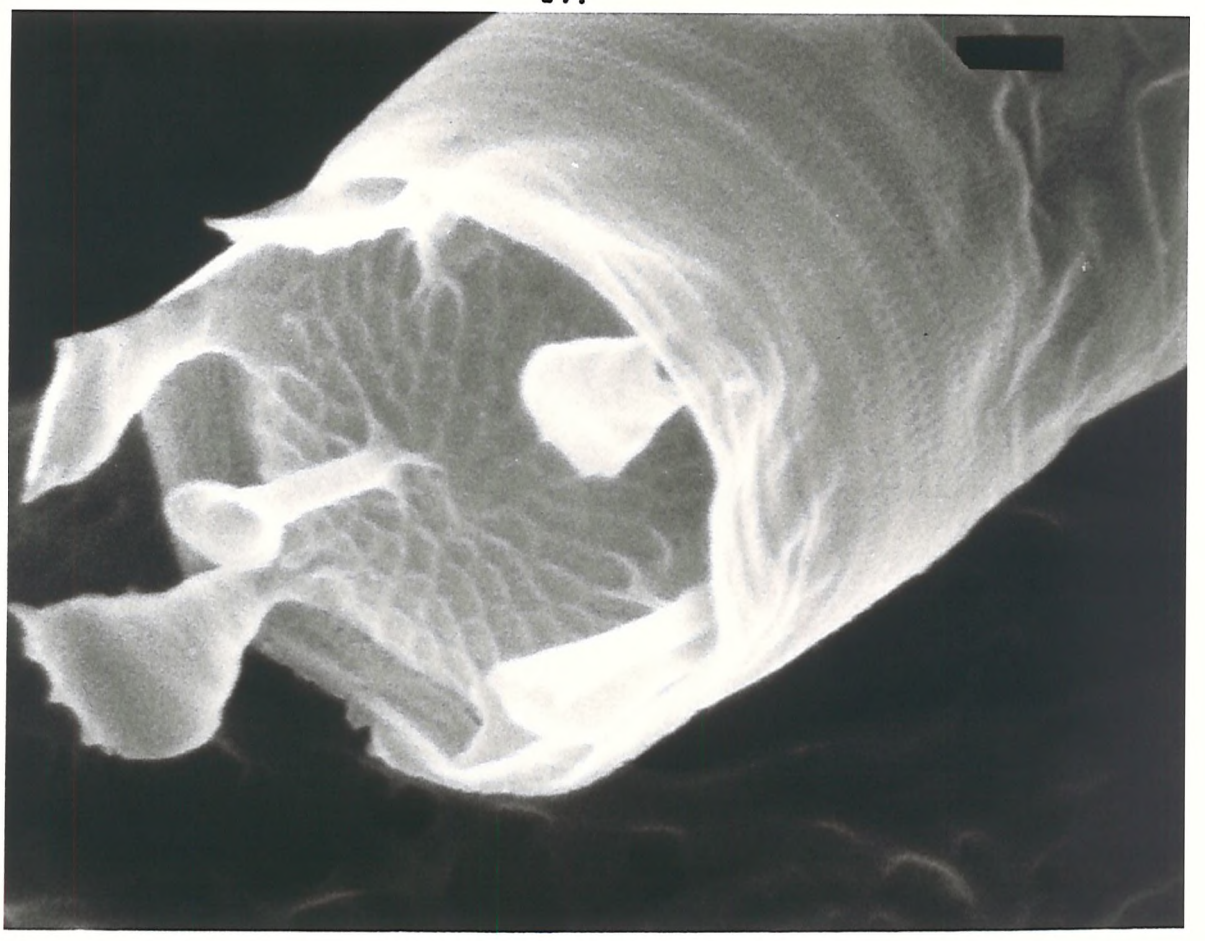


VIII

IX



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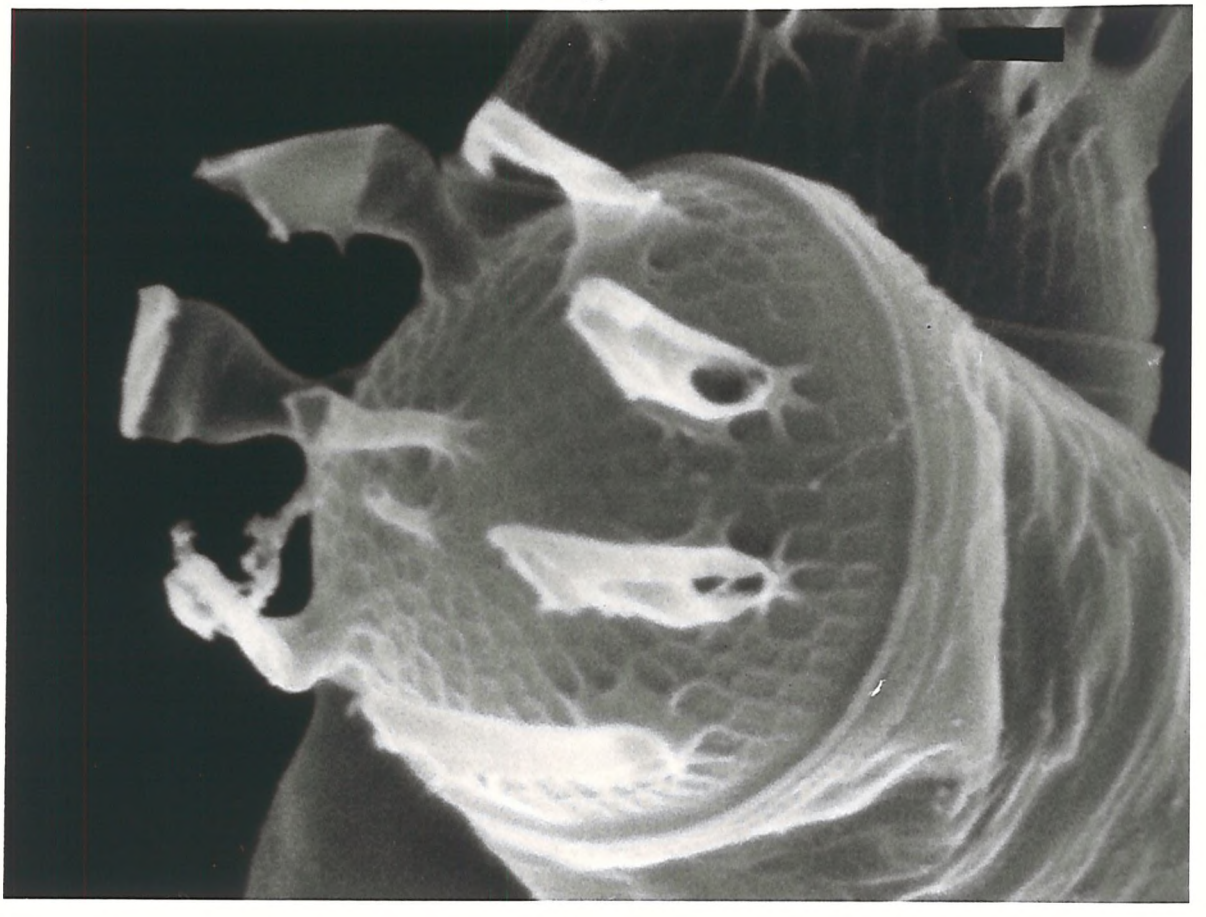


LEGENDS FOR FIGURES AND GRAPHS

FIGURES

1 Changes with time in the carbohydrate, protein and amino acid contents in non-nutrient limited *Skeletonema*.

2 Changes with time in the lipid and chlorophyll contents in nutrient-non-limited *Skeletonema*, including the changing phosphate concentration of the water.

3 Changes with time in the carbohydrate, protein and amino acid contents in phosphate-limited *Skeletonema*.

4 Changes with time in the lipid and chlorophyll contents in phosphate-limited *Skeletonema*, including the changing phosphate concentration of the water.

5 Changes with time in the lipid content in silicate-limited *Skeletonema*, plotted with the changing silicate concentration of the water, and the changing cell count.

6 Changes with time in the carbohydrate, protein and amino acid contents in silicate-limited *Skeletonema*.

7 Changes with time in the lipid content of silicate-limited *Thalassiosira*, plotted with the changing silicate concentration of the water, and the changing cell count.

8 Changes with time in the carbohydrate, protein and amino acid contents in silicate-limited *Thalassiosira*.

9 Total culture protein of nutrient non-limited and phosphate limited *Skeletonema* cultures, plotted against time.

10 Total culture carbohydrate of nutrient non-limited and phosphate limited *Skeletonema* cultures, plotted against time.

11 Total culture lipid of nutrient non-limited and phosphate-limited *Skeletonema* cultures, plotted against time.

12 A comparison between the changes with time in the chlorophyll content per cell in nutrient non-limited *Skeletonema* cultures, and phosphate-limited *Skeletonema* cultures.

13 The changes with time in the chlorophyll per cell in silicate limited *Thalassiosira*.

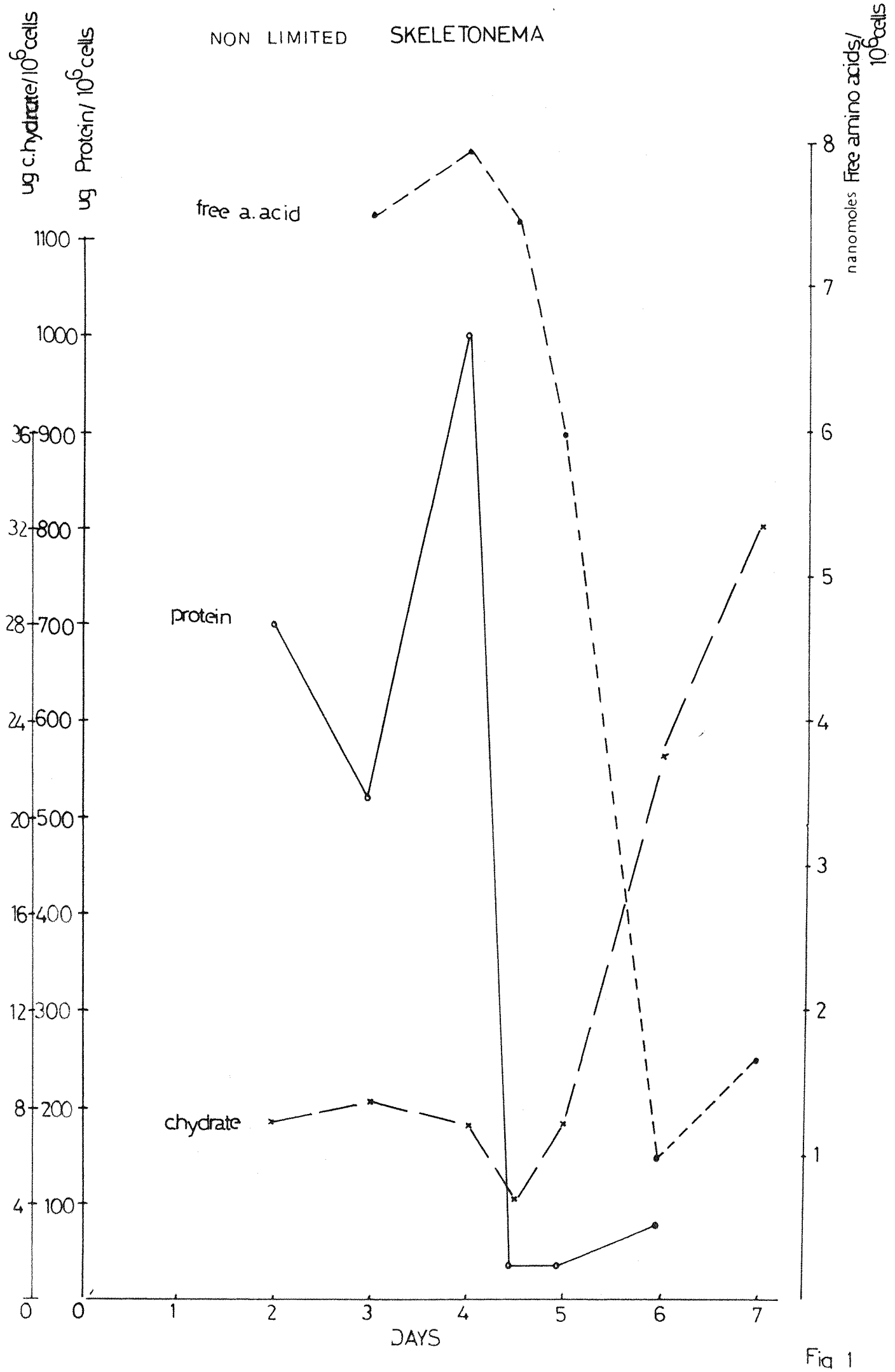

NON LIMITED SKELETONEMA
ug c.hydrate/10^6 cells
ug Protein/ 10^6 cells
nanomoles Free amino acids/ 10^6 cells
free a. acid
protein
chydrate
1100
1000
900
800
700
600
500
400
300
200
100
36
32
28
24
20
16
12
8
4
0
8
7
6
5
4
3
2
1
0
1
2
3
4
5
6
7
DAYS

Fig 1

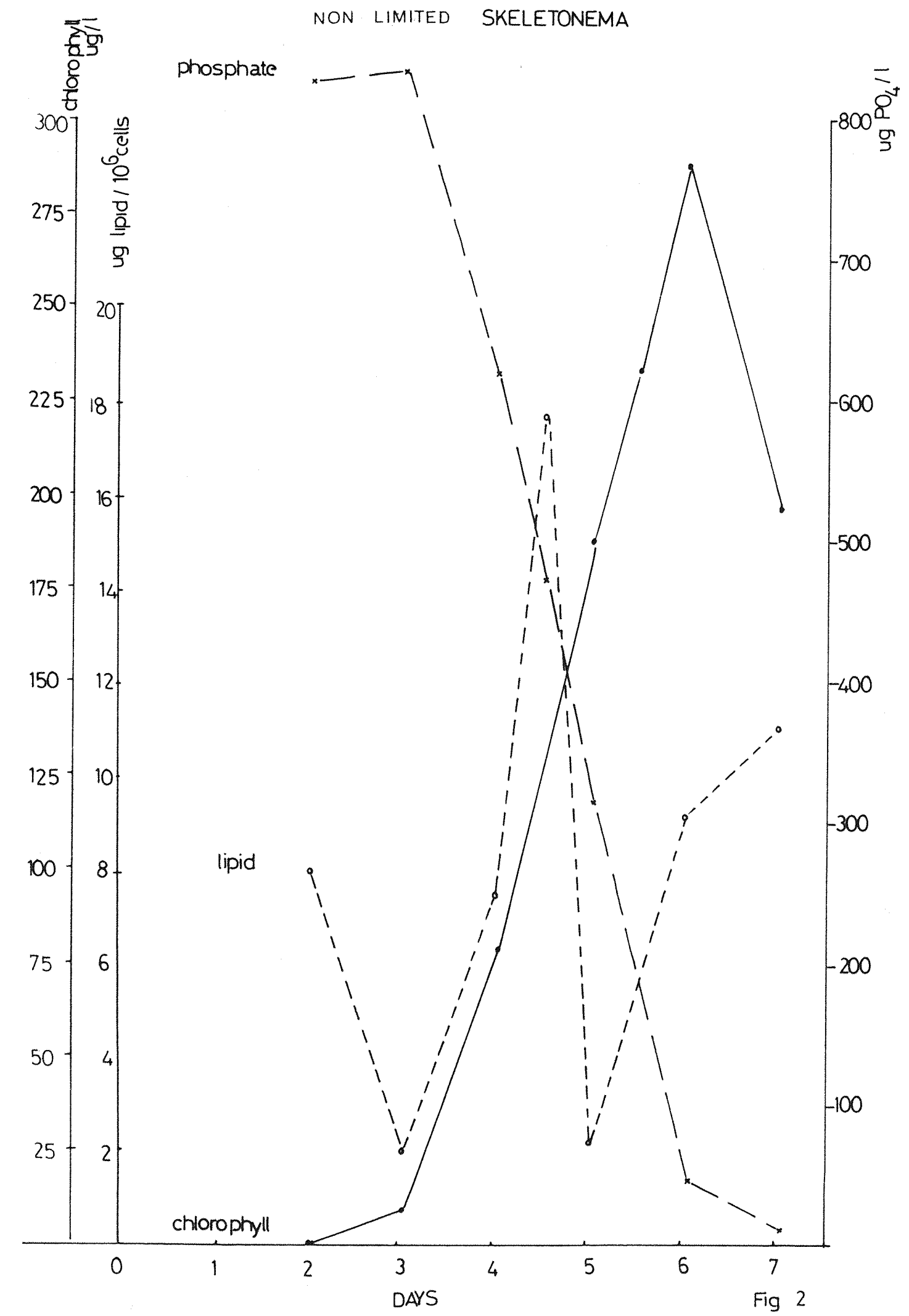

NON LIMITED SKELETONEMA
chlorophyll ug/l
ug lipid / 10^6 cells
ug PO_4 / l
phosphate
lipid
chlorophyll
300
275
250
225
200
175
150
125
100
75
50
25
20
18
16
14
12
10
8
6
4
2
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
DAYS

Fig 2

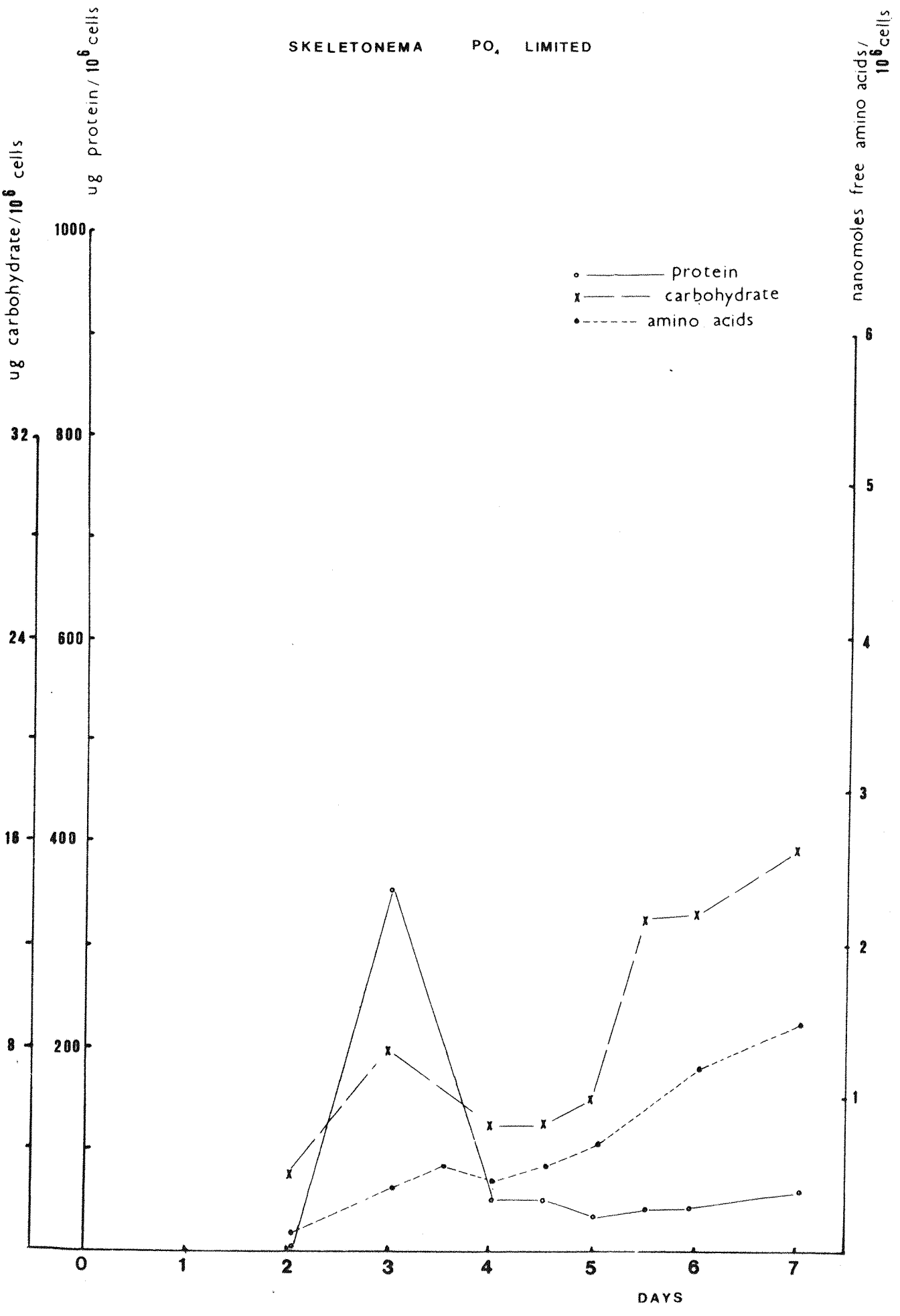

SKELETONEMA PO4 LIMITED
ug protein / 10^6 cells
ug carbohydrate / 10^6 cells
nanomoles free amino acids / 10^6 cells
protein
carbohydrate
amino acids
1000
800
600
400
200
32
24
16
8
6
5
4
3
2
1
0
1
2
3
4
5
6
7
DAYS

FIG 3

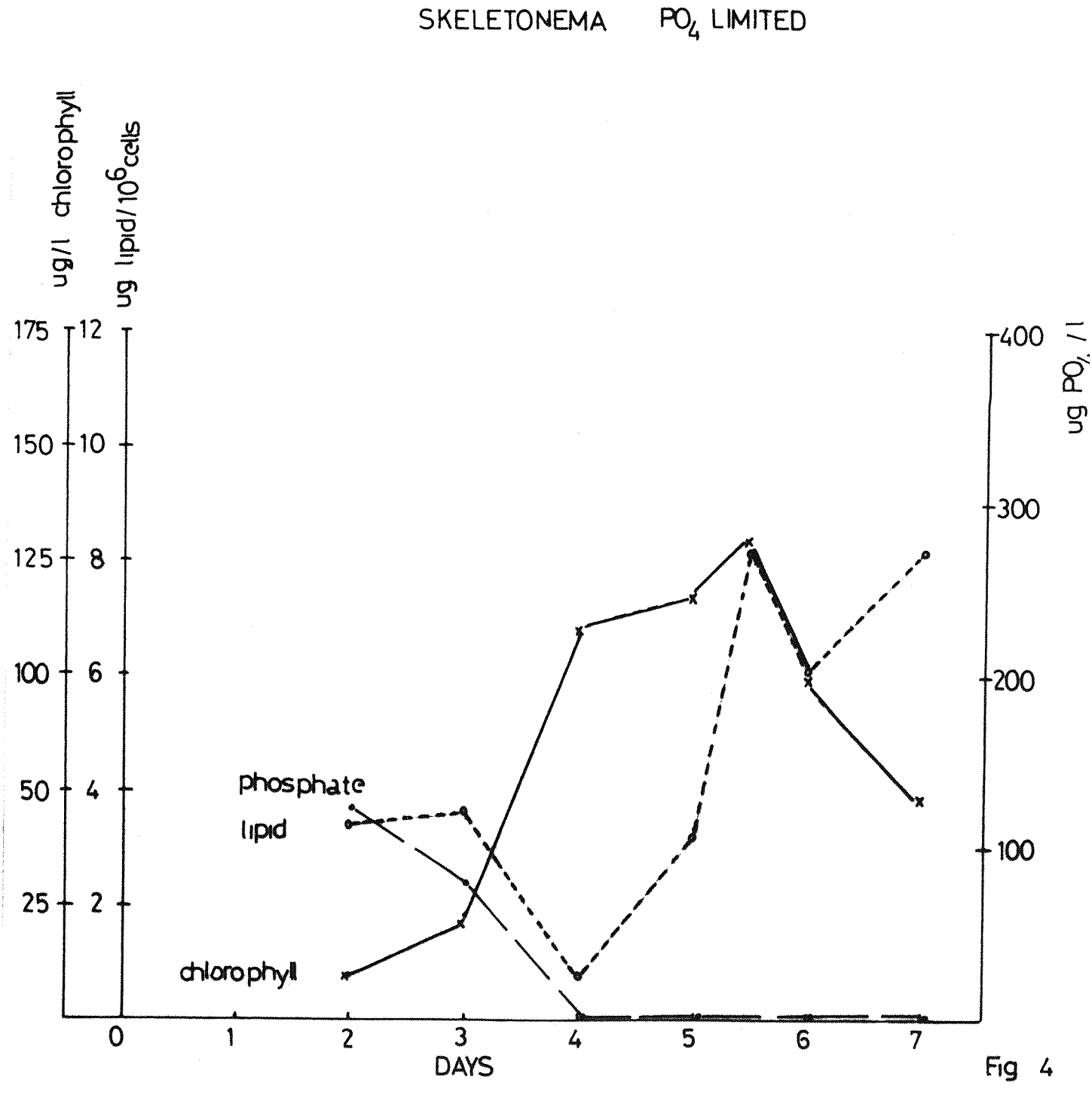

SKELETONEMA PO4 LIMITED
ug/l chlorophyll
ug lipid/10^6cells
ug PO4 / l
175
150
125
100
50
25
12
10
8
6
4
2
400
300
200
100
phosphate
lipid
chlorophyll
0
1
2
3
4
5
6
7
DAYS

Fig 4

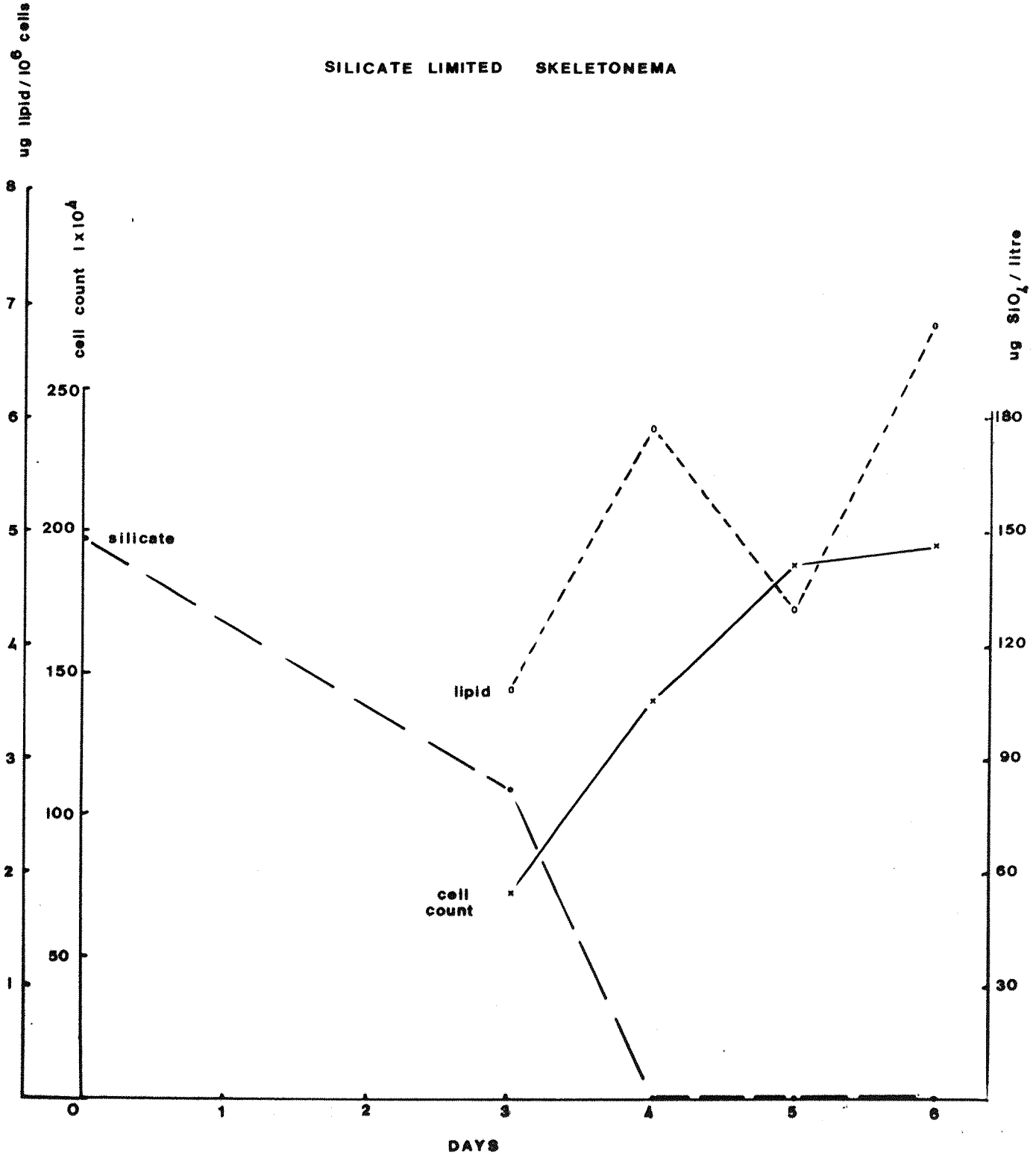

SILICATE LIMITED SKELETONEMA
ug lipid / 10^6 cells
cell count 1 x 10^4
ug SiO2 / litre
silicate
lipid
cell count
DAYS
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
50
100
150
200
250
30
60
90
120
150
180

FIG 5

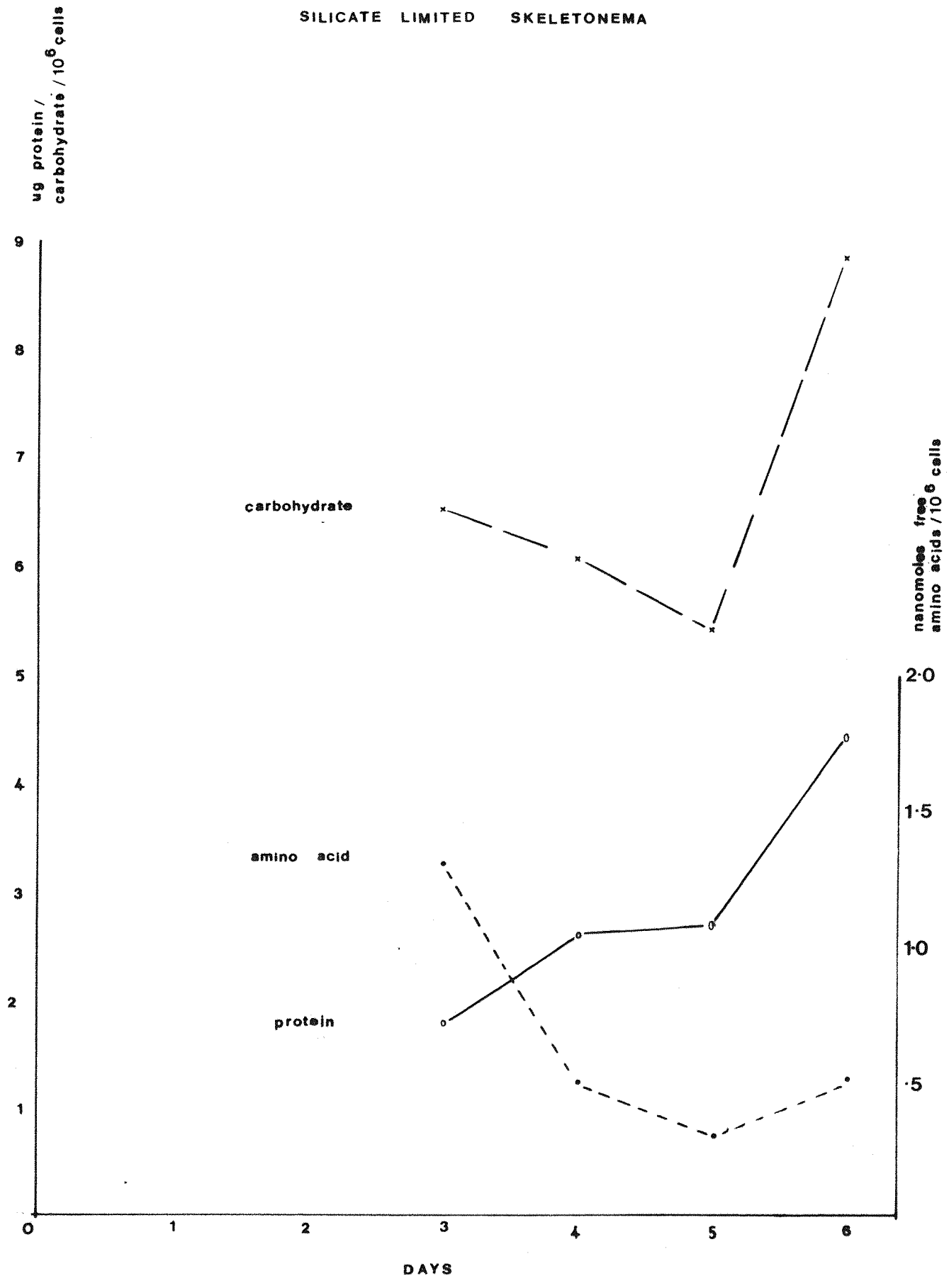

SILICATE LIMITED SKELETONEMA
ug protein / carbohydrate / 10^6 cells
nanomoles free amino acids / 10^6 cells
carbohydrate
amino acid
protein
DAYS
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
9
·5
1·0
1·5
2·0

FIG 6

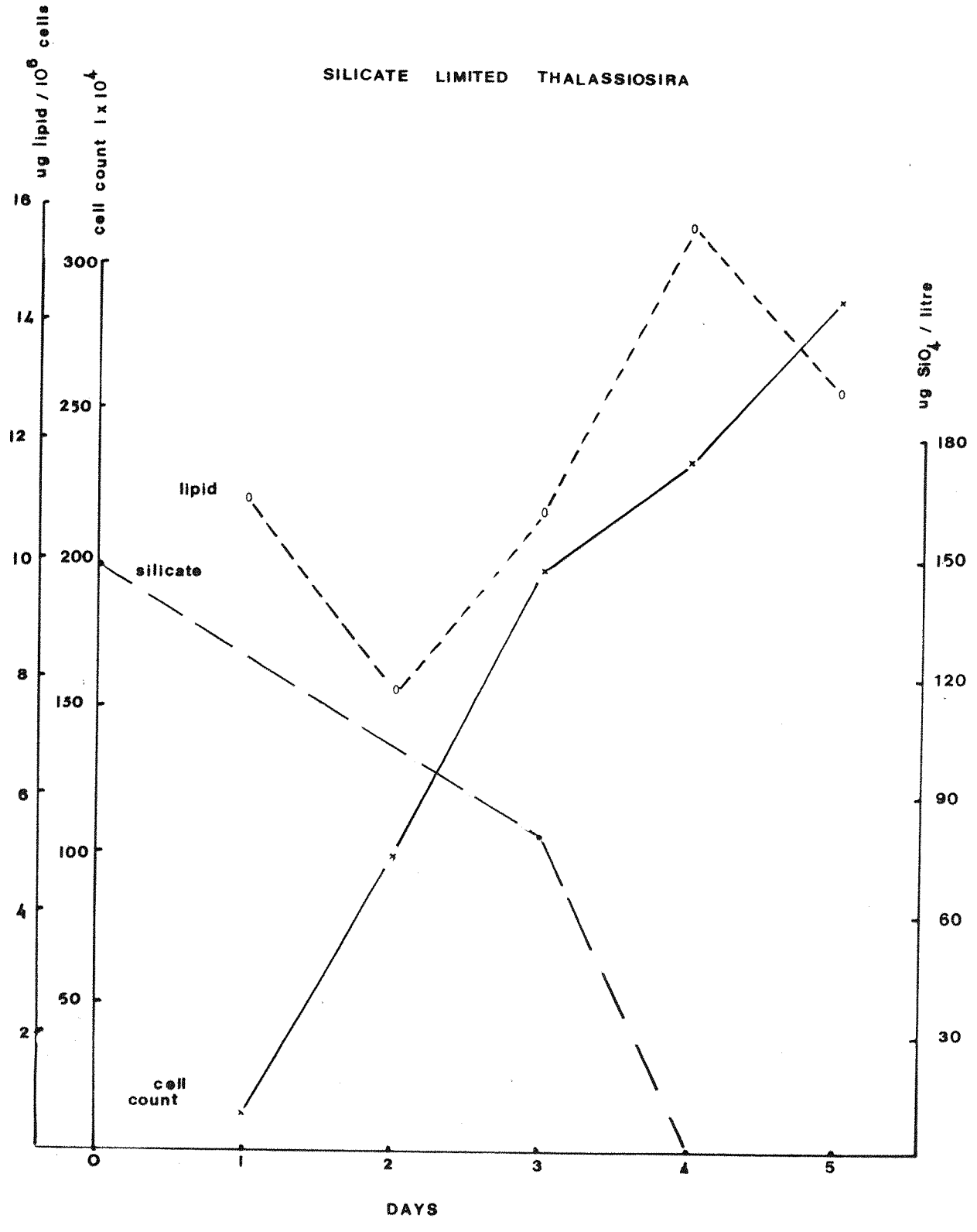

SILICATE LIMITED THALASSIOSIRA
ug lipid / 10^6 cells
cell count I x 10^4
ug SiO_4 / litre
lipid
silicate
cell count
DAYS
0
1
2
3
4
5
16
14
12
10
8
6
4
2
300
250
200
150
100
50
180
150
120
90
60
30

FIG 7

SILICATE LIMITED THALASSIOSIRA

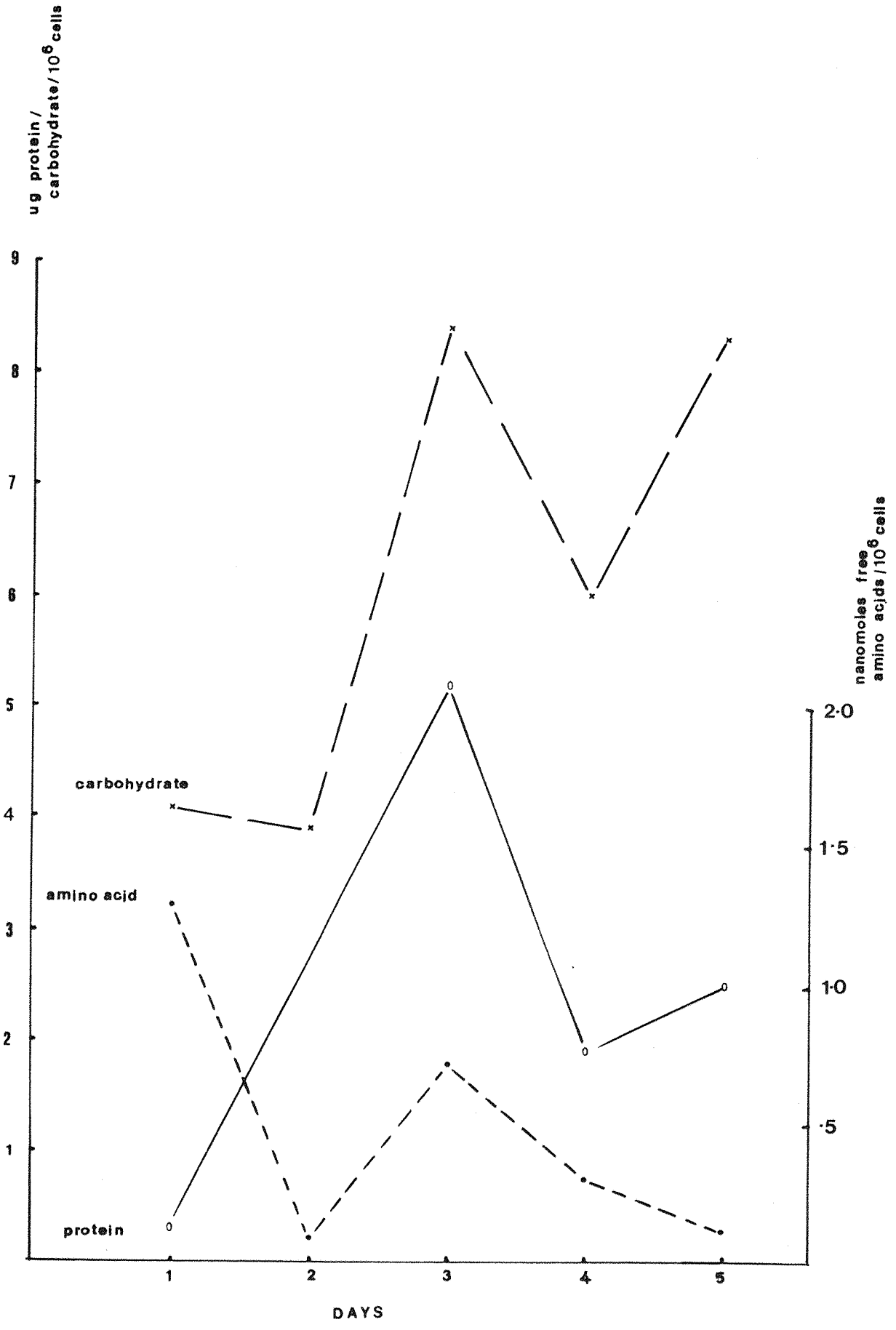


FIG 8

TOTAL CULTURE PROTEIN / TIME (SKELETONEMA)

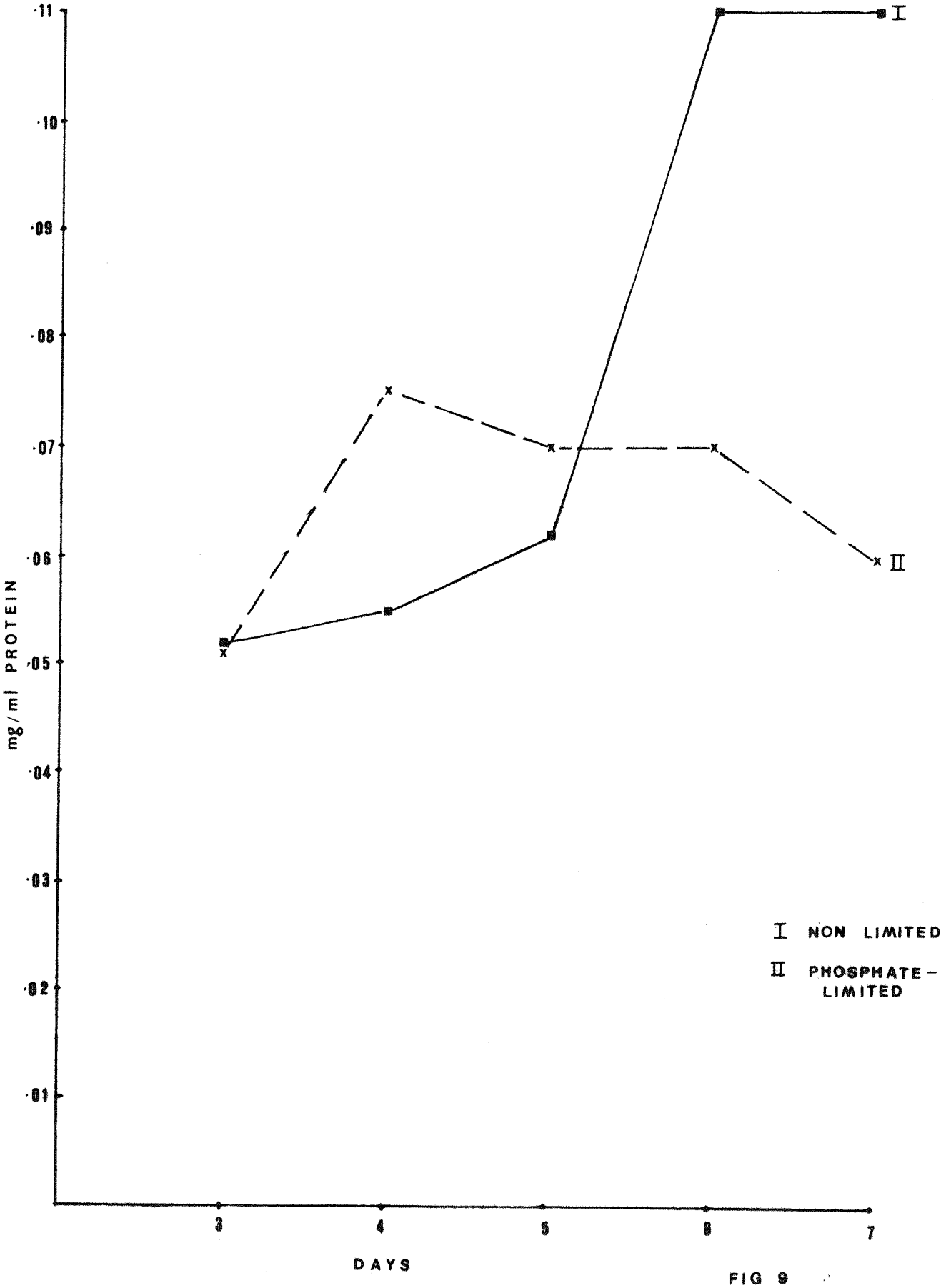


FIG 9

TOTAL CARBOHYDRATE / TIME (SKELETONEMA)

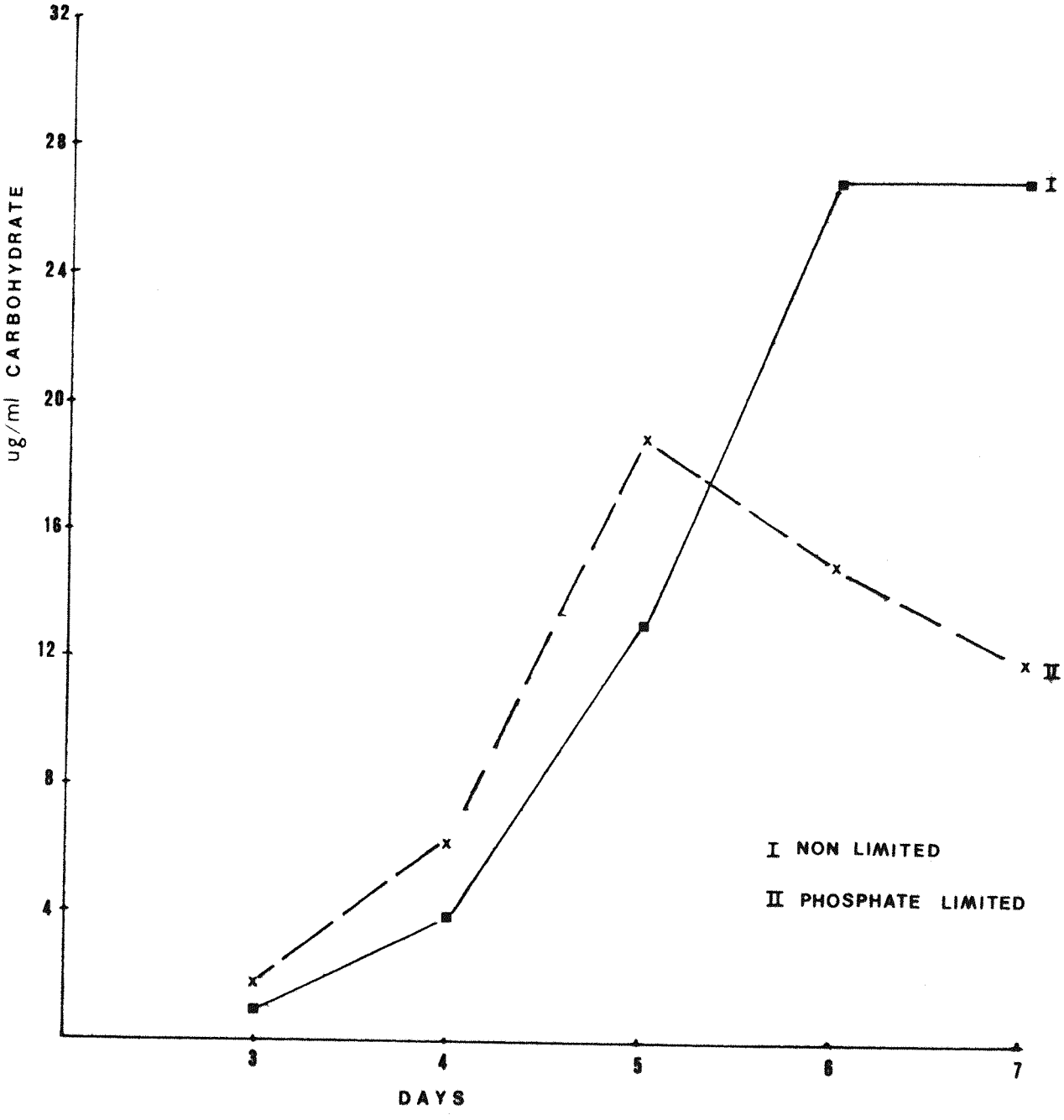


FIG 10

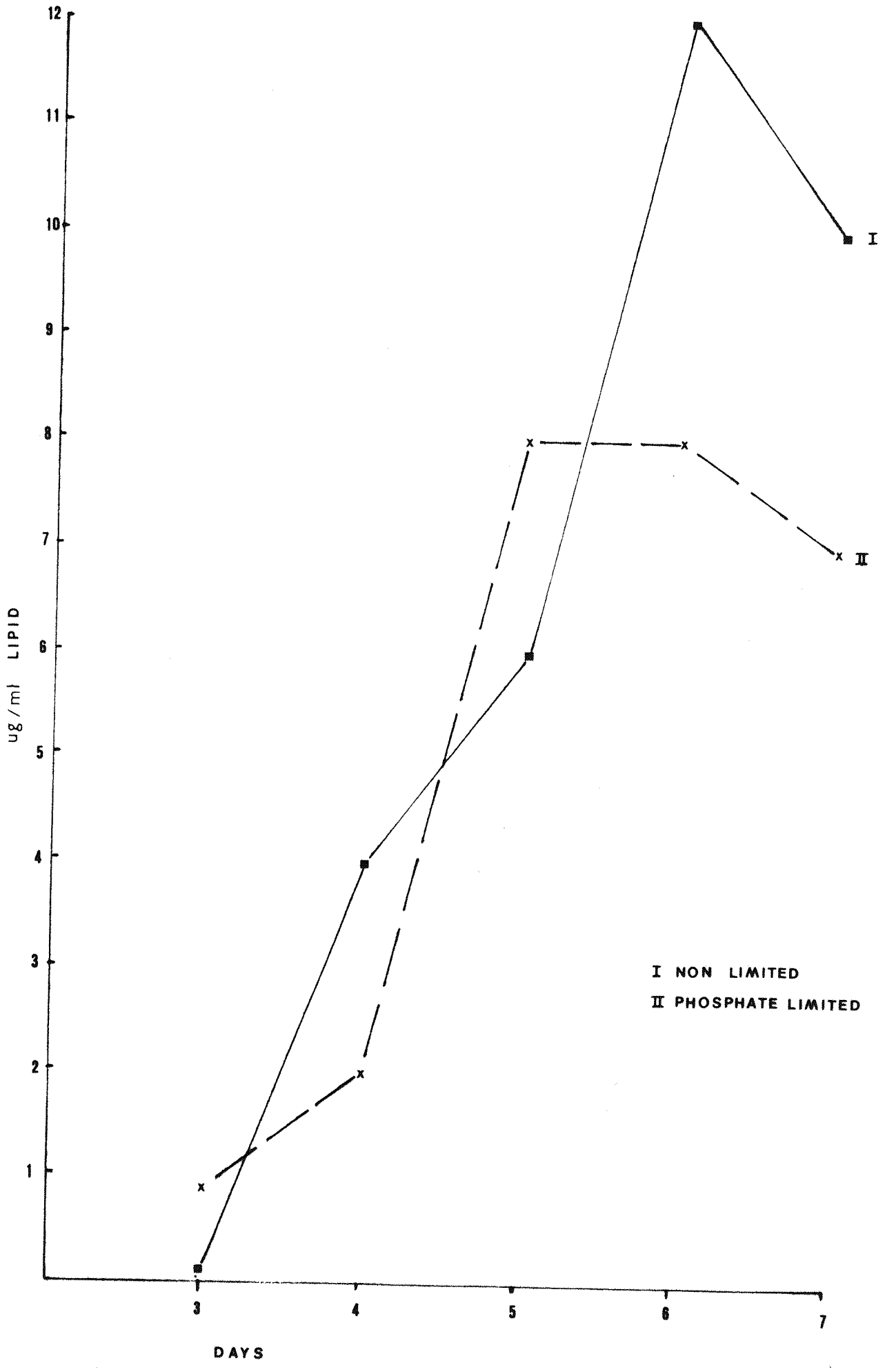

TOTAL LIPID / TIME (SKELETONEMA)
12
11
10
9
8
7
6
5
4
3
2
1
ug/ml LIPID
3
4
5
6
7
DAYS
I
II
I NON LIMITED
II PHOSPHATE LIMITED

FIG 11

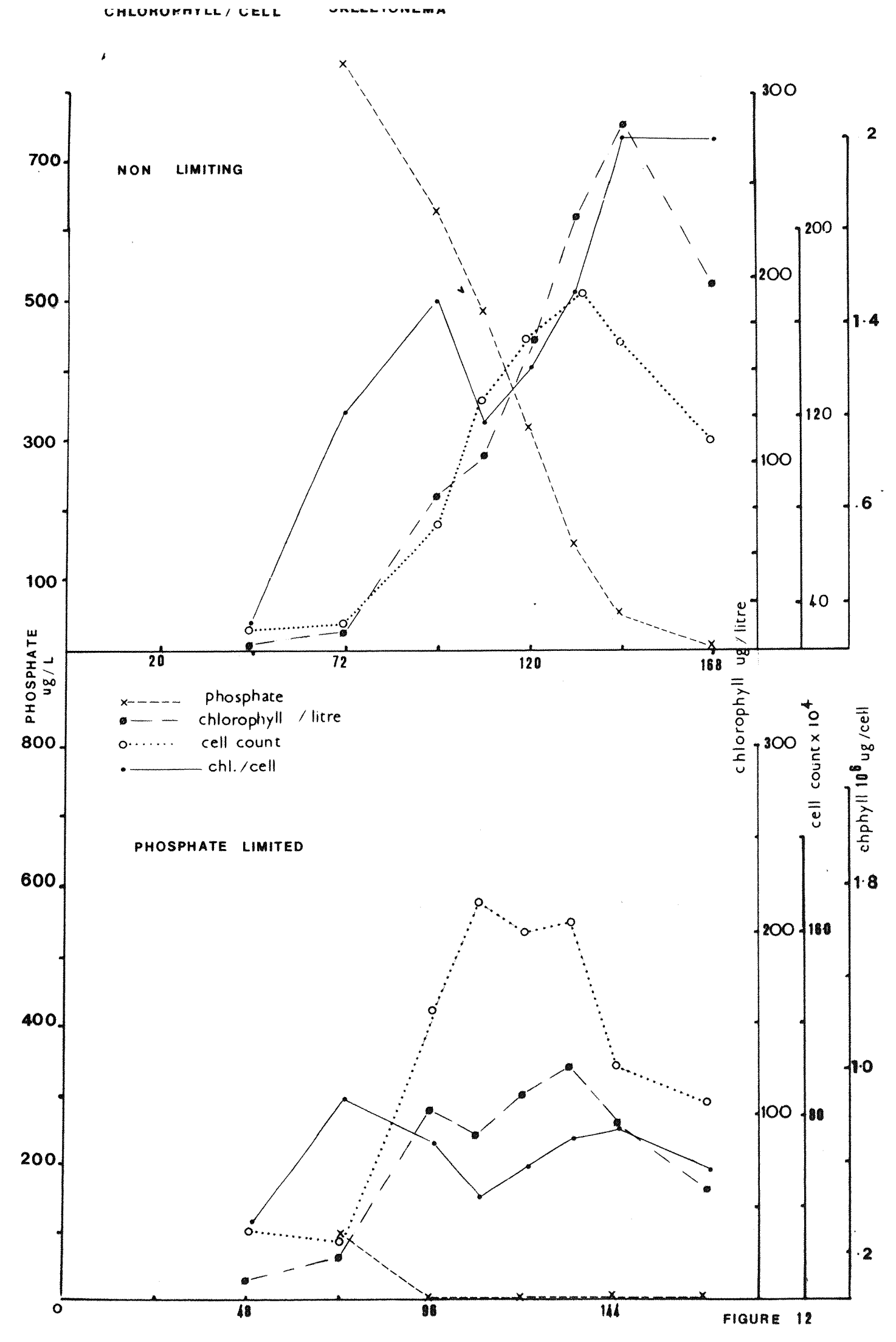

CHLOROPHYLL / CELL
NON LIMITING
PHOSPHATE ug/L
chlorophyll ug / litre
cell count x 10⁴
chphyll 10⁶ ug/cell
phosphate
chlorophyll / litre
cell count
chl. / cell
PHOSPHATE LIMITED
700
500
300
100
800
600
400
200
0
20
72
120
168
48
96
144
300
200
100
2
1·4
·6
120
40
1·8
1·0
·2

FIGURE 12

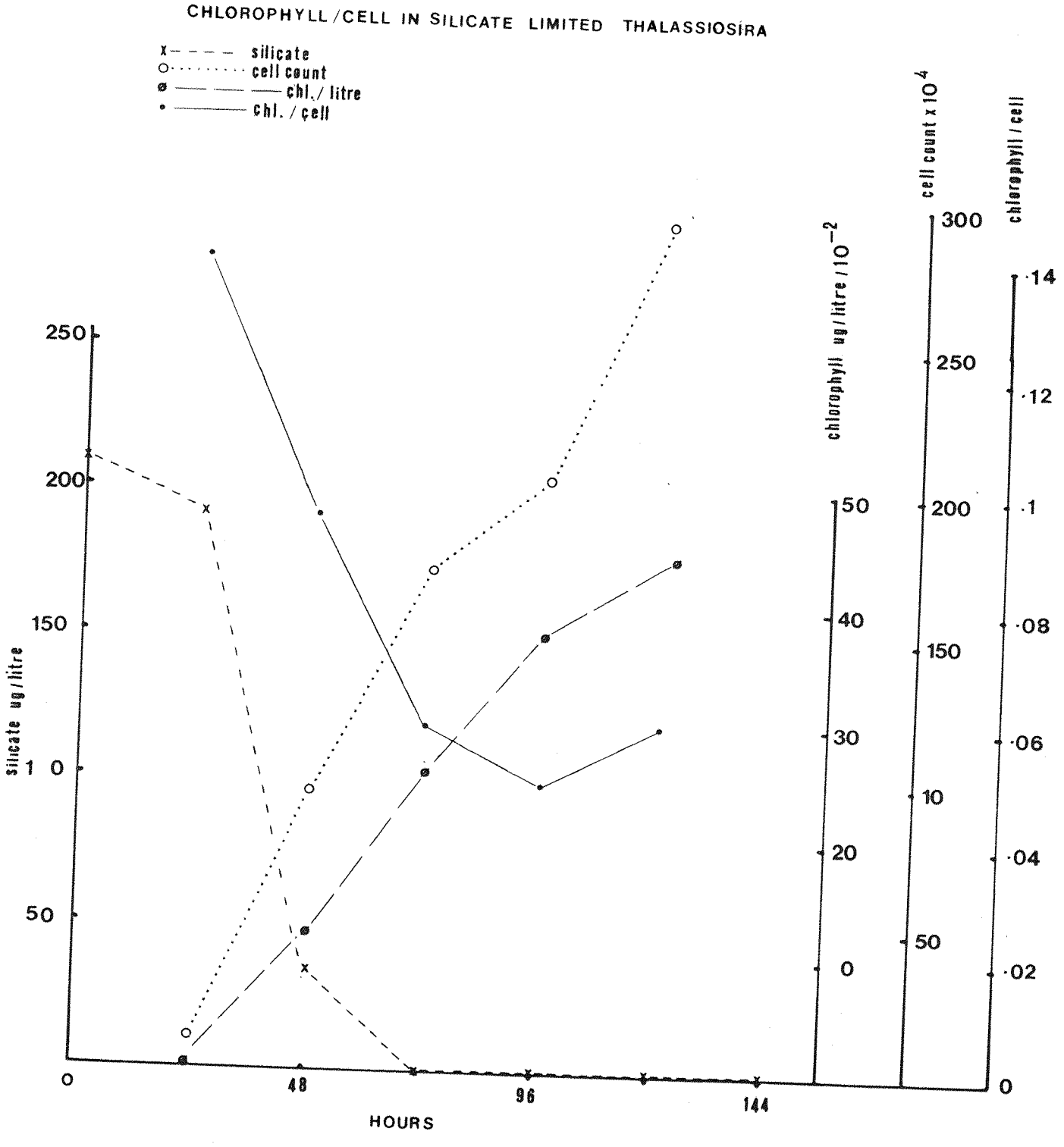
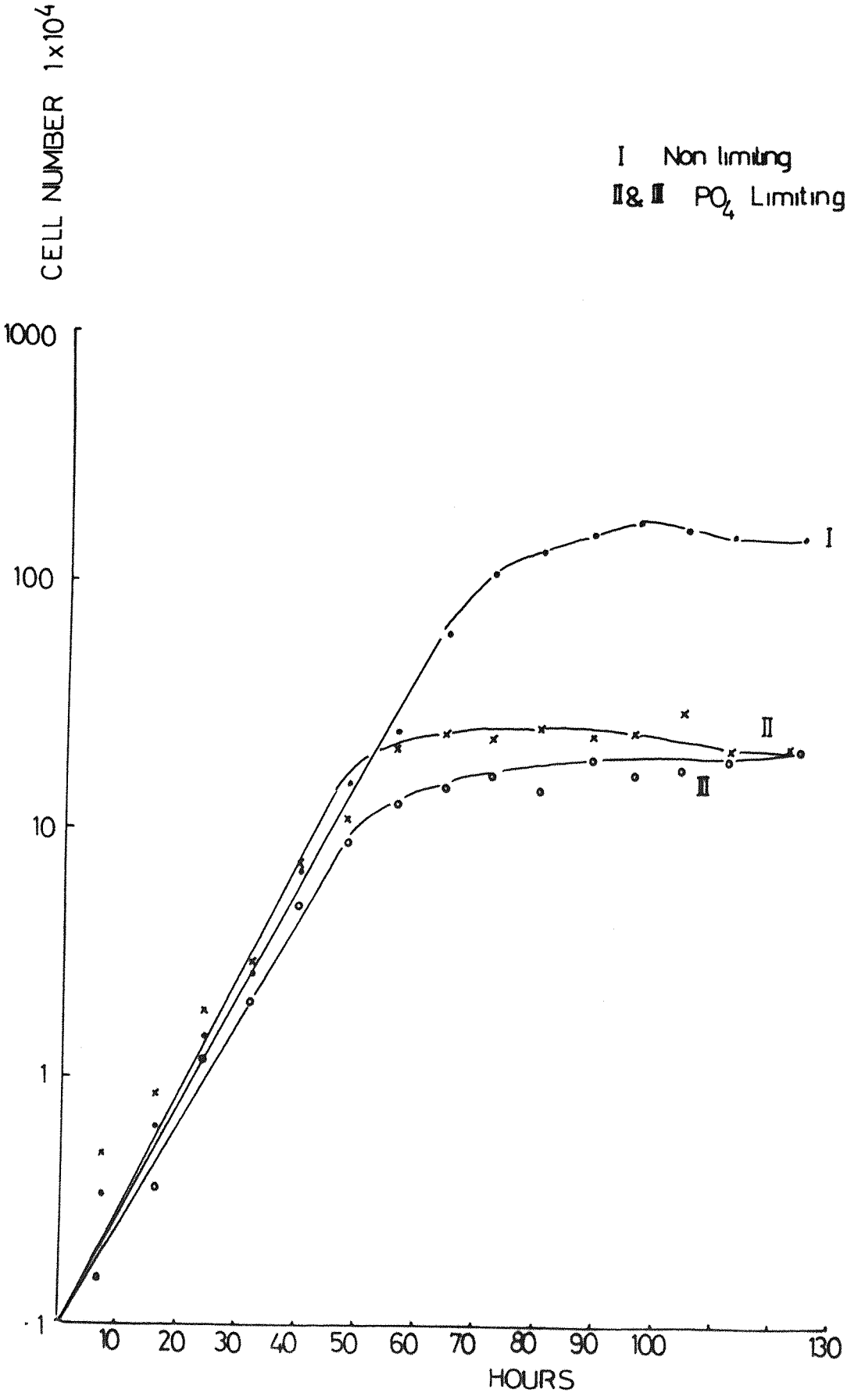

CHLOROPHYLL/CELL IN SILICATE LIMITED THALASSIOSIRA
silicate
cell count
chl./ litre
chl. / cell
Silicate ug/litre
250
200
150
100
50
0
chlorophyll ug/litre/10⁻²
150
40
30
20
0
cell count x 10⁴
300
250
200
150
10
50
chlorophyll / cell
·14
·12
·1
·08
·06
·04
·02
0
0
48
96
144
HOURS

FIGURE 13

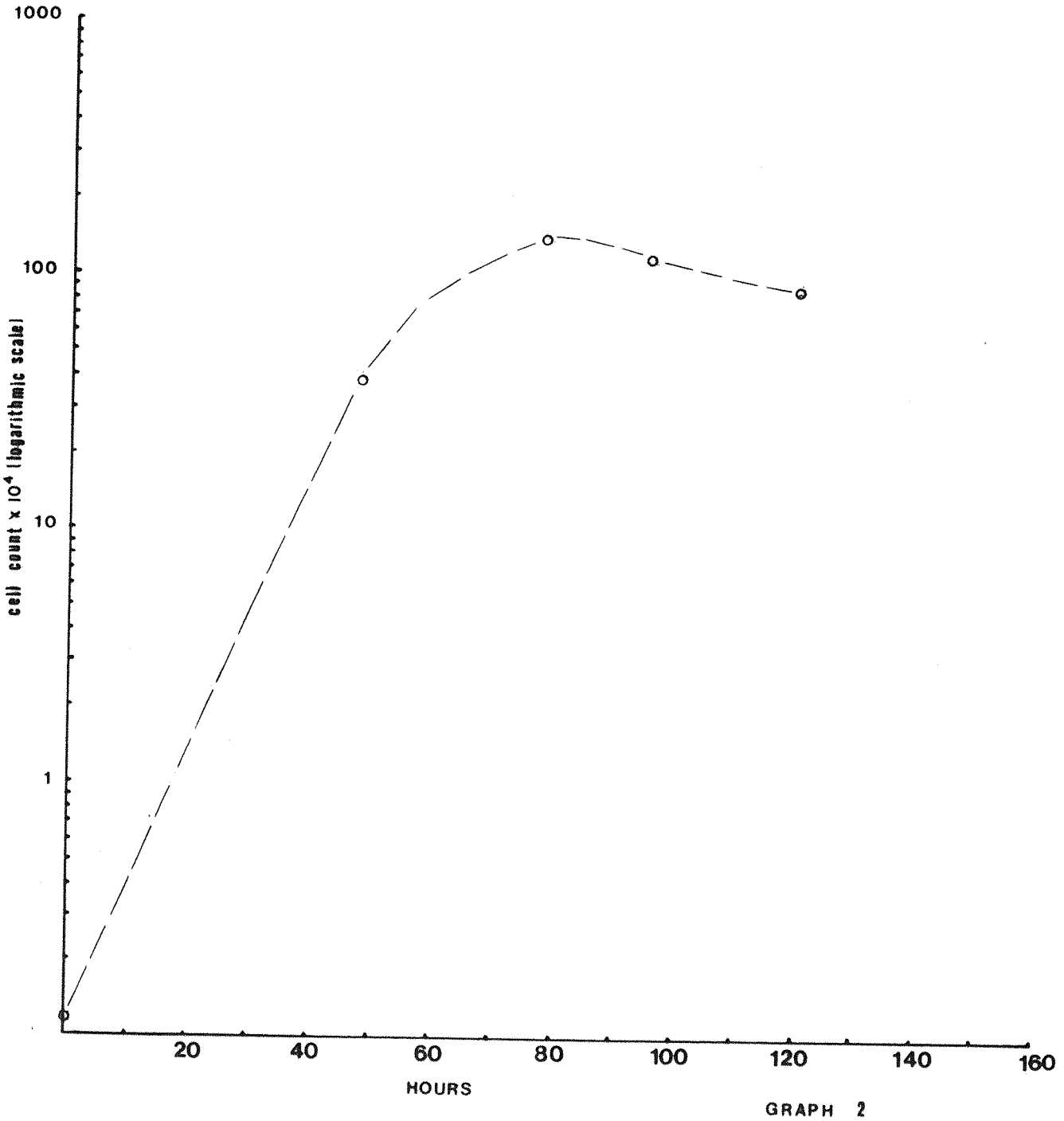
GRAPHS

SKELETONEMA GROWTH CURVES



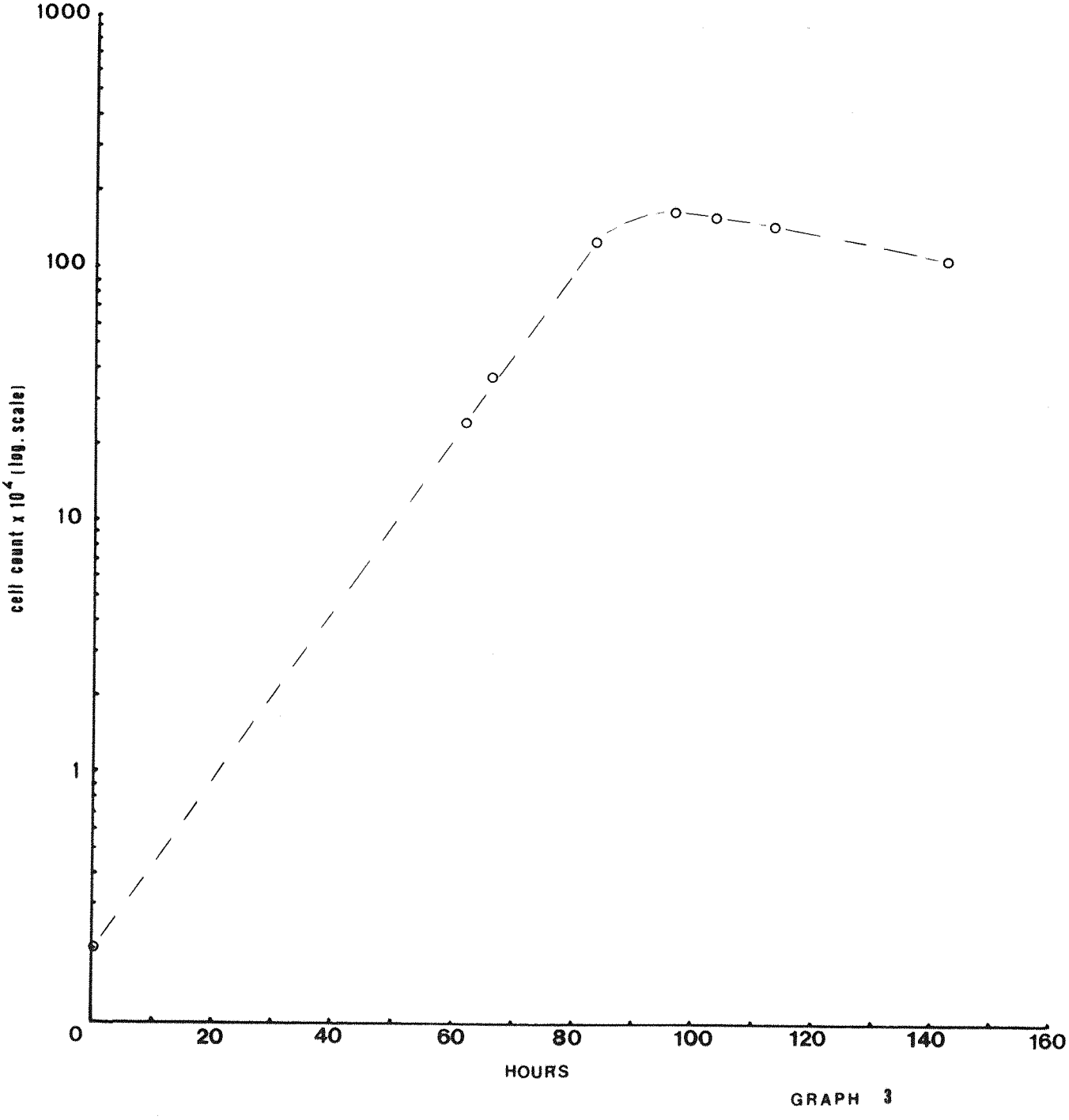
GRAPH 1

GROWTH CURVE NON LIMITED SKELETONEMA



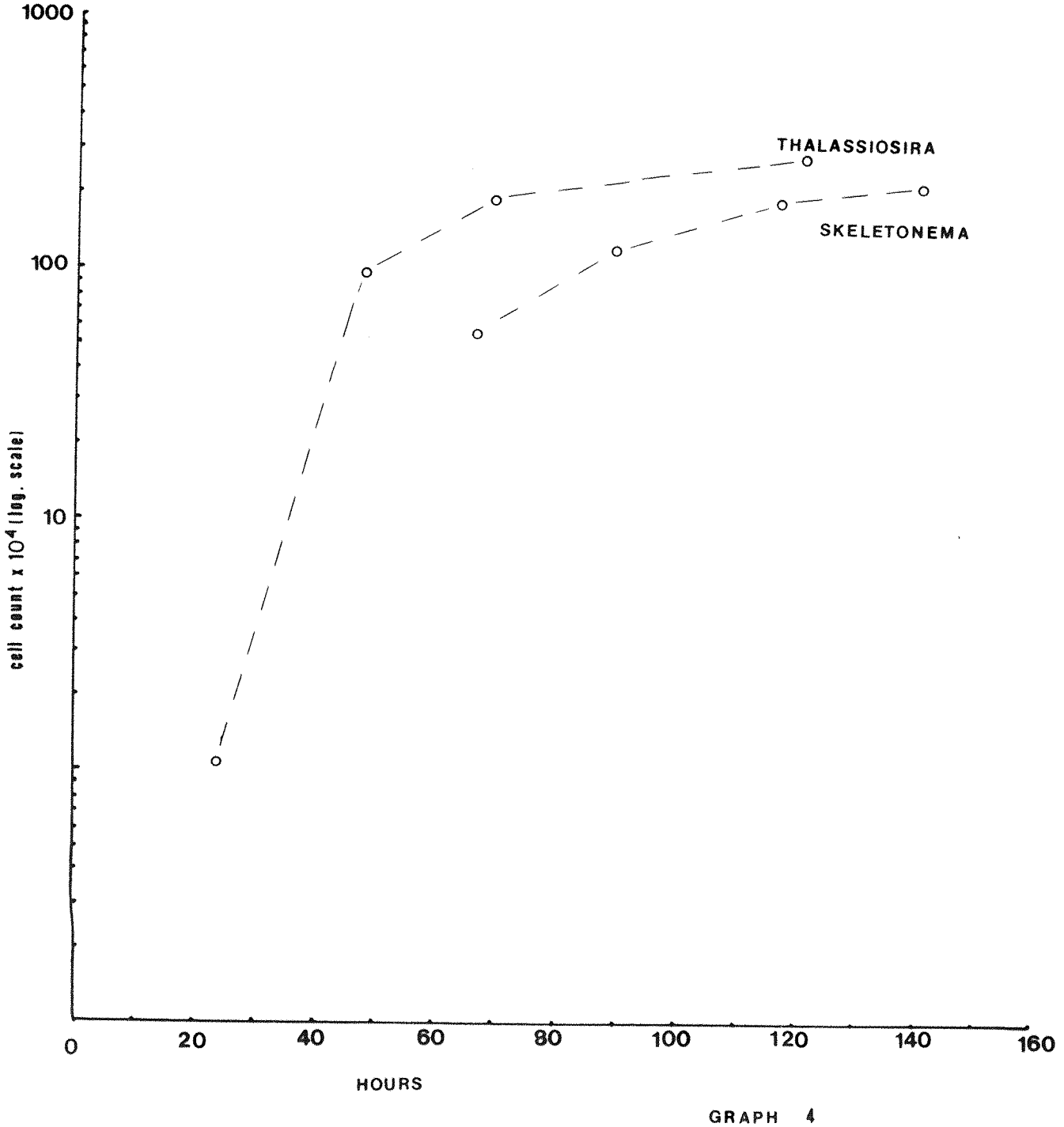
GRAPH 2

GROWTH CURVE PHOSPHATE LIMITED SKELETONEMA



GRAPH 3

GROWTH CURVES SILICATE LIMITED SKELETONEMA AND THALASSIOSIRA



GRAPH 4

Appendix 1

Guillard's Enriched Seawater media - "f/2" Formula. (Guillard and Ryther 1962).

Composition (per litre of filtered seawater).

a) Major Nutrients

$NaNO_3$	75 mg	(883µM)
$NaH_2PO_4.H_2O$	5 mg	(36.3µM)
$Na_2SiO_3.9H_2O$	15-30 mg	(54-107µM)

b) Trace Metals

$Na_2.EDTA$	4.36 mg	(11.7µM)
$FeCl_3.6H_2O$	3.15 mg	(11.7µM)
$CuSO_4.5H_2O$	0.01 mg	(0.04µM)
$ZnSO_4.7H_2O$	0.022 mg	(0.08µM)
$CoCl_2.6H_2O$	0.01 mg	(0.05µM)
$MnCl_2.4H_2O$	0.18 mg	(0.09µM)
$Na_2MoO_4.2H_2O$	0.006 mg	(0.03µM)

c) Vitamins

Thiamin HCl	0.1 mg
Biotin	0.5 µg
B_{12}	0.5 µg

Preparation of Stock Solutions

n% (W/V) means n grams brought to a volume of 100 ml with distilled water.

a) Major Nutrients

Material	Stock Solution, (% W/V)
$NaNO_3$	7.5
$NaH_2PO_4.H_2O$	0.5
$Na_2SiO_3.9H_2O$	3.0

b) Trace Elements

Salt	Primary Stock, (%W/V)
$CuSO_4.5H_2O$	0.98
$ZnSO_4.7H_2O$	2.2
$CoCl_2.6H_2O$	1.0
$MnCl_2.4H_2O$	18.0
$Na_2MoO_4.2H_2O$	0.63

Working Stock Solution

Into 900 ml distilled water was dissolved 3.15 gm ferric chloride ($FeCl_3.6H_2O$) and 4.36 gm di sodium E.D.T.A. To this was added 1 ml of each of the trace metal primary stock solutions, and the volume was made up to 1 litre with distilled water.

The pH of the solution was adjusted to about 4.5.

c) Vitamin Primary Stock Solutions

Biotin — Into 10 ml distilled water was dissolved 1 mg crystalline biotin.

B_{12} — Into 10 ml distilled water was dissolved 10 mg crystalline B_{12}.

Thiamine — No primary stock solution is needed.

Vitamin Working Solution

Into 100 ml distilled water 1.0 ml biotin primary stock and 0.1 ml B_{12} primary stock solution were added. Into this solution was dissolved 20 mg thiamine HCl.

The working stock solution can be frozen in 5 ml aliquots until required.

Preparation of Enriched Seawater Medium

The freshly collected seawater of salinity 33‰ was filtered through a glass fibre filter, porosity >1μ. To each litre of seawater was then added:

1 ml nitrate stock solution
1 ml trace metal working stock solution
0.5 ml vitamin working stock solution

The water was autoclaved at 15lbs/in^2 for 15 minutes in acid-cleaned pyrex flasks of required volume. To this cooled solution was added:

1 ml per litre silicate stock solution
1 ml per litre phosphate stock solution

The silicate and phosphate stock solutions were autoclaved separately in heat resistant bottles because it was found that if these elements were mixed with the medium before it was autoclaved, they would precipitate out of solution during the autoclaving. Only by adding them to the cooled medium could this be prevented.

After all the solutions were mixed well together the pH of the medium was adjusted to between pH 8.5-8.8, and then continuously bubbled with air for about 24 hours prior to inoculation with the algal cultures.

Appendix 2

The formulae of the nutrient-limited medium; adjusted "f/2" medium concentrations.

Phosphate-limited Medium

The same nitrate and silicate content as in "f/2" medium were used, but 1/10 the phosphate was added.

Per litre of seawater this meant 0.1 ml phosphate stock solution was added instead of 1 ml.

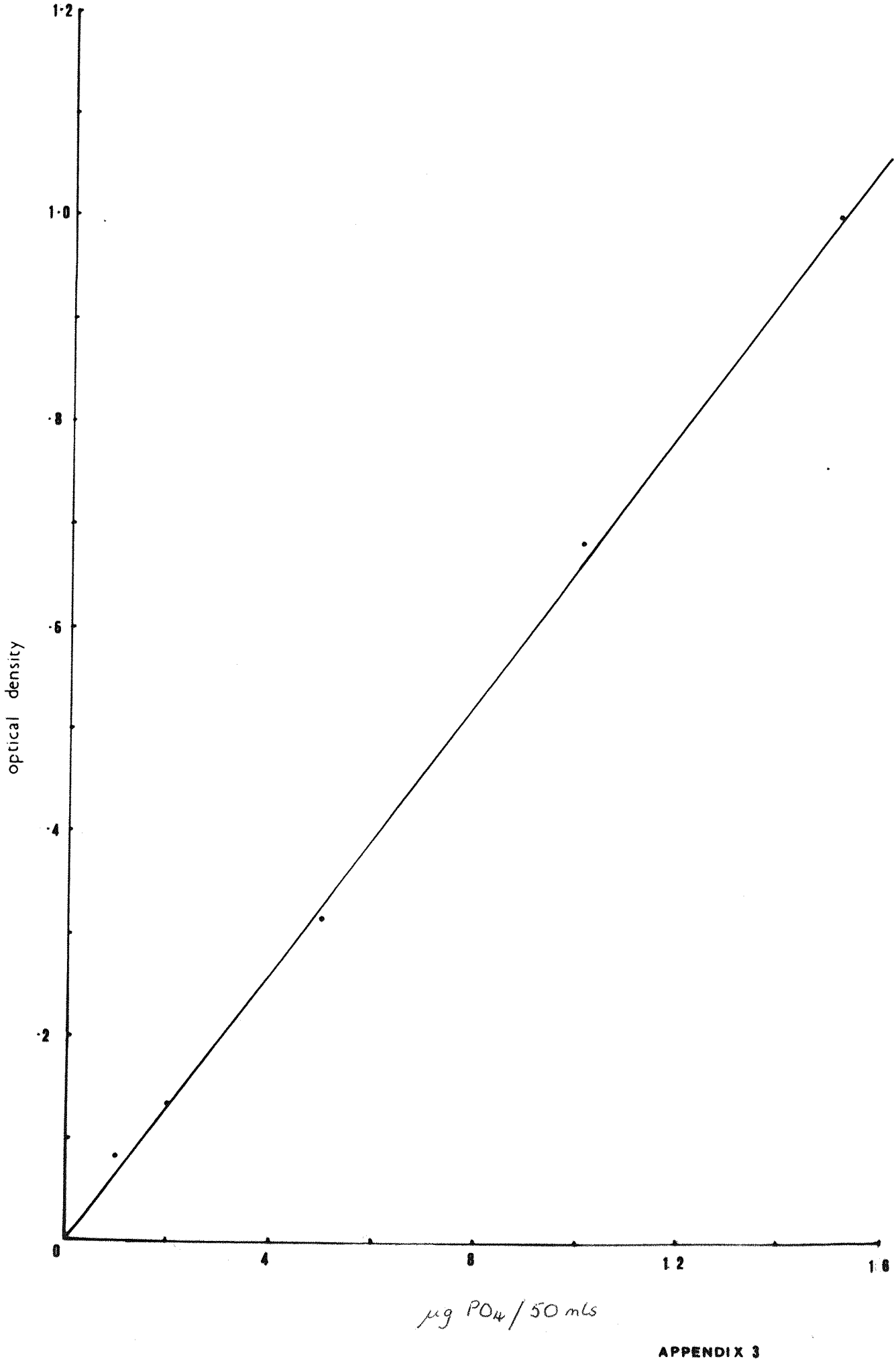
Silicate-limited Medium

The same nitrate and phosphate content as in "f/2" medium were used, but only 1/10 the silicate was added.

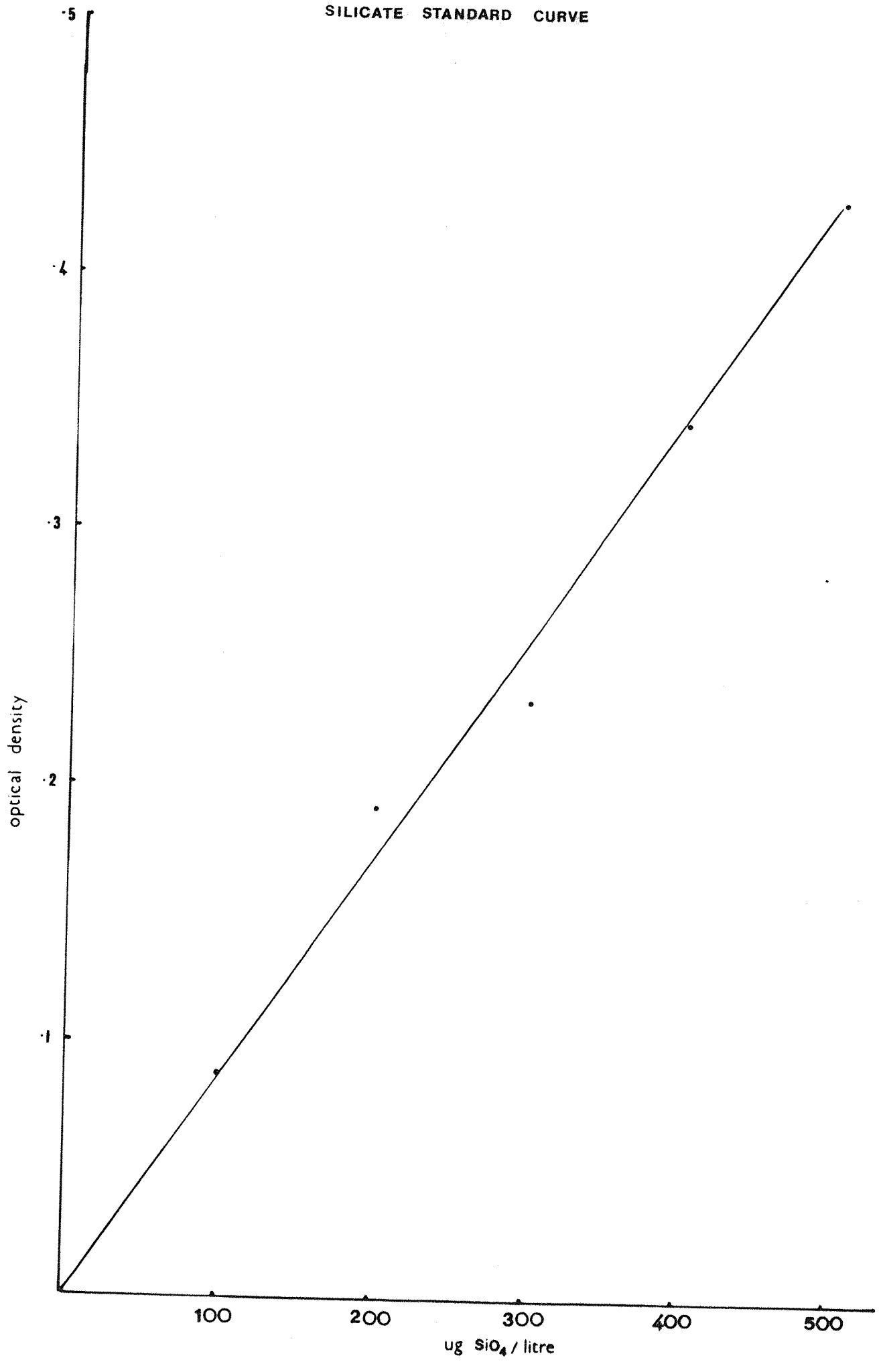
Per litre of seawater this meant 0.1 ml silicate stock solution was added instead of 1 ml.

	PO_4 LIMITED	SiO_4 LIMITED
Nitrate	f/2	f/2
Phosphate	f/10	f/2
Silicate	f/2	f/10

PHOSPHATE STANDARD CURVE

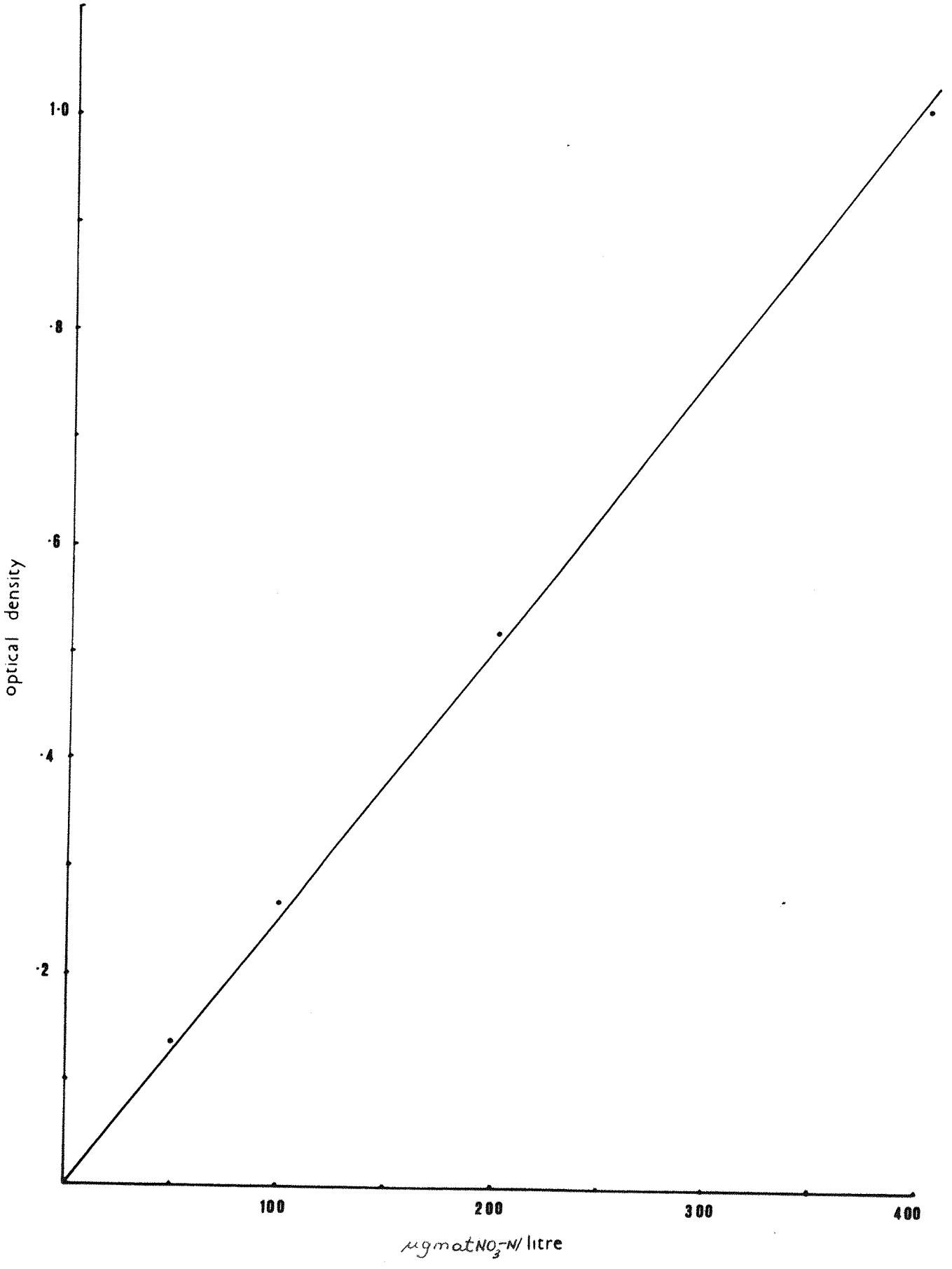


APPENDIX 3


SILICATE STANDARD CURVE
·5
·4
·3
·2
·1
optical density
100
200
300
400
500
ug SiO_4 / litre

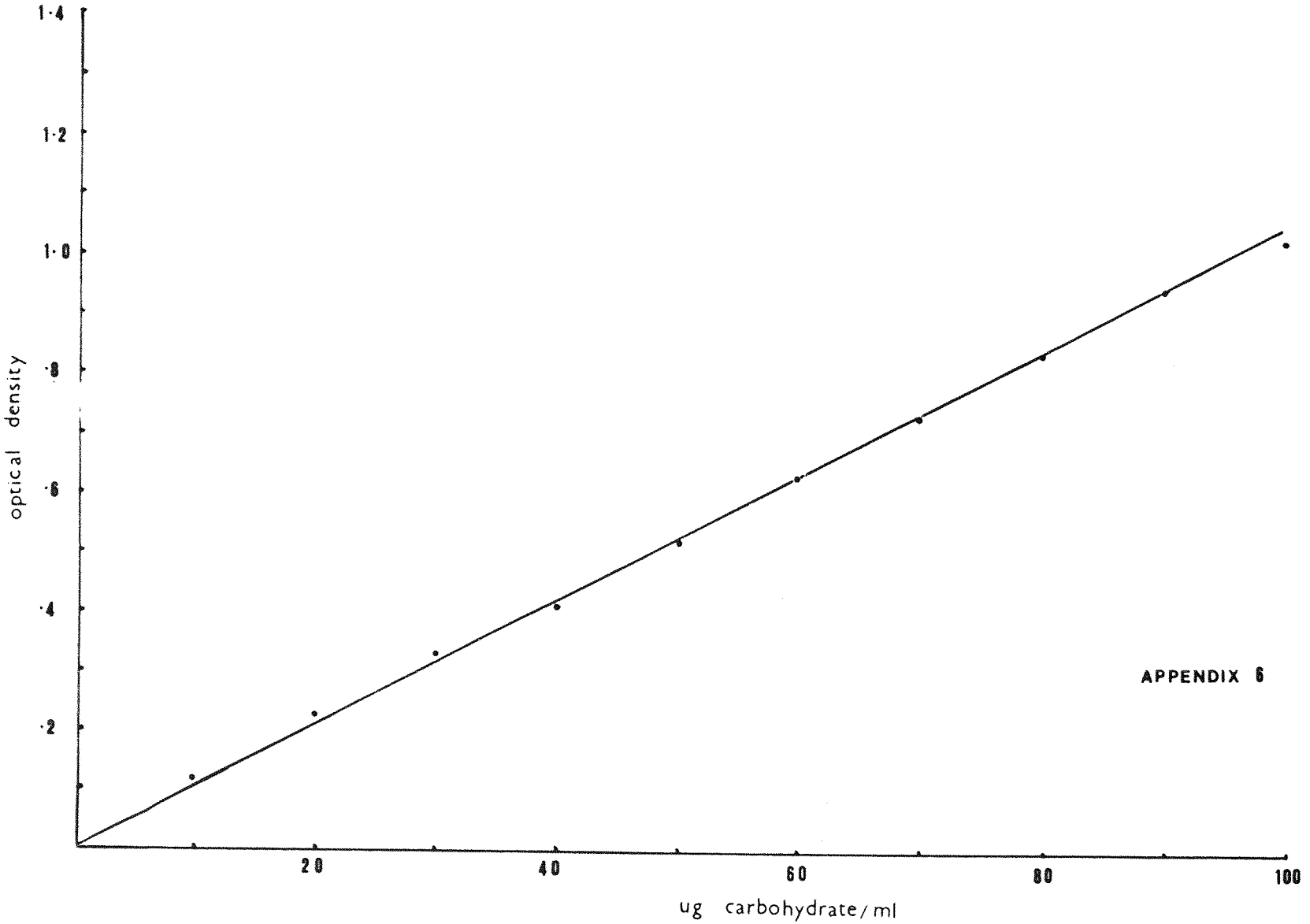
APPENDIX 4

NITRATE STANDARD CURVE

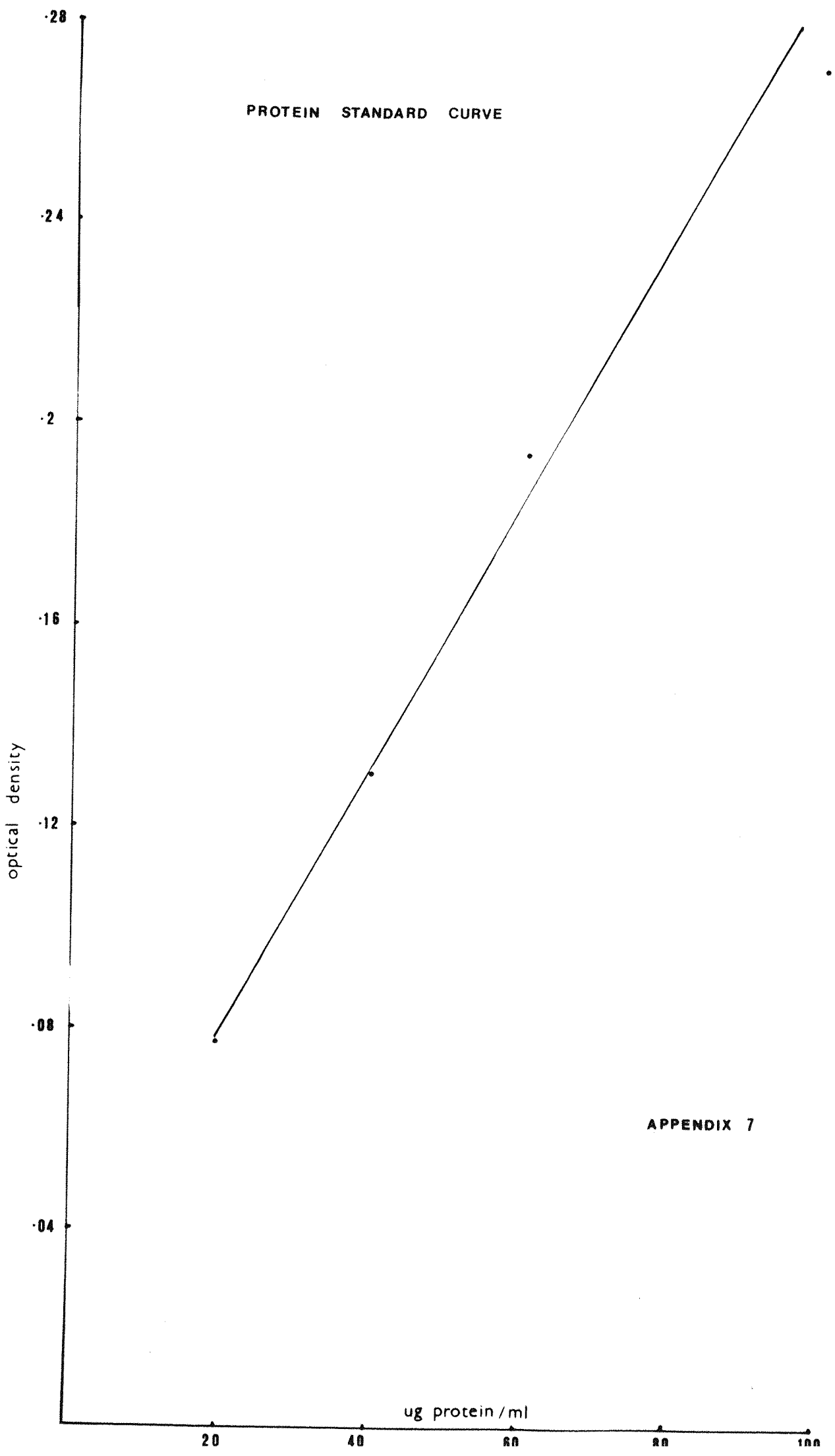


APPENDIX 5

CARBOHYDRATE STANDARD CURVE

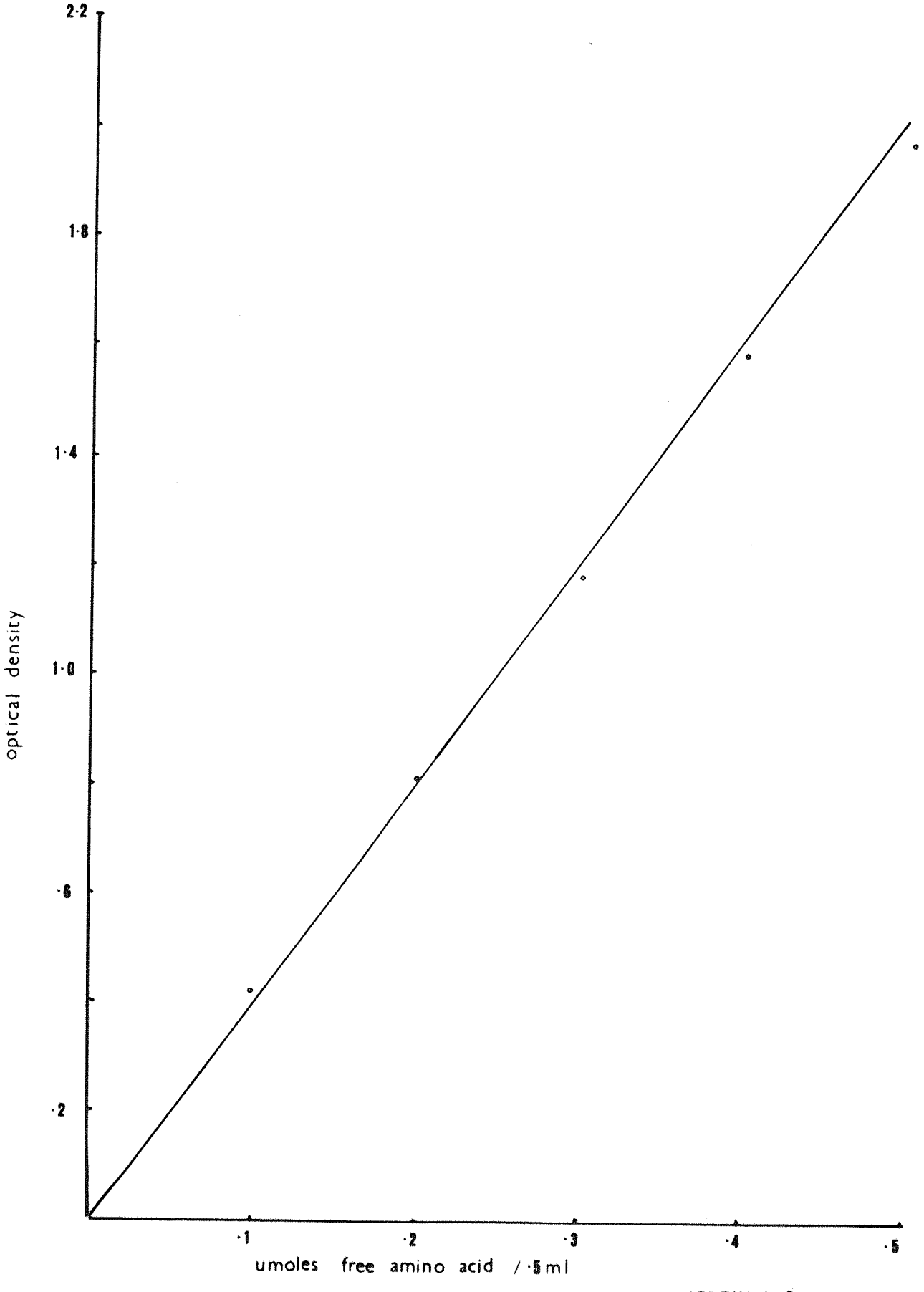


APPENDIX 6


PROTEIN STANDARD CURVE
·28
·24
·2
·16
·12
·08
·04
optical density
ug protein / ml
20
40
60
80
100

APPENDIX 7

FREE AMINO ACID STANDARD CURVE



APPENDIX 8

References

Ackman, R.G., P.M.Jangaard, R.J.Hoyle and H.Brockerhoff (1964). J. Fish. Res. Bd. Canada 21 , 747-756. Origin of marine fatty acids 1. Analyses of the fatty acids produced by *Skeletonema costatum*.

Allen, E.J. and E.W.Nelson (1910). On the artificial culture of marine plankton organisms. J. Mar. Biol. Assoc. U.K. 8 421-74.

Al-Ogilvy, S.M. and E.W.Knight-Jones (1977). Anti-fouling role of antibiotics produced by marine algae and bryozoans. Nature, Lond. 265 728-729.

Amenta, J.S. (1964). A rapid chemical method for the quantification of lipids separated by thin layer chromatography. J. Lipid. Res. 5 270-2 .

Antia, N.J., C.D.McAllister, T.R.Parsons, K.Stephens and J.D.Strickland (1963). Further measurements of primary production using a large volume plastic sphere. Lim. Ocy. 8 166-183.

Ballantine, J., A.Lavis and R.J.Morris (1979). Sterols of the phytoplankton - effects of illumination and growth stage. Phytochemistry 18 1459-1466.

Barlow, R.G. (1980). The biochemical composition of phytoplankton in an upwelling region off South Africa. J. Exp. Mar. Biol. Ecol. 45 83-93.

Barlow, R.G. (1982). Phytoplankton ecology in the Southern Benguela current I. Biochemical composition. J. Exp. Mar. Biol. Ecol 63 209-227.

Barlow, R.G. (1982). Phytoplankton ecology in the Southern Benguela current II. Carbon Assimilation Patterns. J. Exp. Mar. Biol. Ecol. 63 229-237.

Barlow, R.G. (1982). Phytoplankton ecology in the Southern Benguela current III. Dynamics of a bloom. J. Exp. Mar. Biol. Ecol. 63 239-248.

Barlow, R.G. and M.J.Swart (1981). Analysis of protein and carbohydrate in particulate matter. Fish. Bull. S.Afr. 15 89-93.

Belyaeva, T.V. (1972). Distributions of large diatoms in the south eastern Pacific (in Russian, English summary).Okeanologiya 12 475-84. Engl. trans. (1972) Oceanology 12 400-7.

Blackwell, J., K.D.Parker and K.M.Rudall (1967). Chitin fibres of the diatoms *Thalassiosira fluviatilis* and *Cyclotella cryptica*. J. molec. Biol. 28 383-5.

Boyer, C.S. (1926-27). Synopsis of North American Diatomaceae. Part I. *Coscinodiscatae, Rhizosoleniatae, Biddulphiatae, Fragillariatae*. Part II. *Naviculatae, Surirellatae*. Proc. Acad. nat. Sci. Philad. 78 suppl. 3-288 and 79 suppl. 229-583.

Caperon, J. and J.Meyer (1972). Nitrogen limited growth of marine phytoplankton 1. Changes in population characteristics with steady state growth rate. Deep Sea Res. 19 601-618.

Caperon, J. and J.Meyer (1972). Nitrogen limited growth of marine phytoplankton. Deep Sea Res. vol.19 601-618.

Chau, Y.K., L.Chuecas and J.P.Riley (1967). The component combined amino acids of some marine phytoplankton species. J. Mar. Biol. Ass. U.K. 47 543-554.

Cholnoky, B.J. (1968). Die Okologie der DiatomEen in Binnengewässem. J.Cramer, Lehre p.699.

Cleve, P.T. (1900). The plankton of the North Sea, the English Channel and the Skagerak in 1898. Konigl. Svensk. Vet. Stockh. Ak. Handl. 32 No.8 53.

Coombs, J., W.M.Darley, O.Holm-Hansen and B.E.Volcani (1967a). Studies on the biochemistry and fine structure of silica shell formation in diatoms. Chemical composition of *Navicula pelliculosa* during silicon starvation synchrony. Pl. Physiol. 42 1601-6.

Crawford, R.M. (1973). The protoplasmic ultrastructure of the vegetative cell of *Melosira varians* C.A.Agardh. J. Phy col. 9 50-61.

Cronin, J.R. and R.J.Morris (1982). The occurrence of high molecular weight humic material in recent organic-rich sediment from the Nambian shelf. Estuar. Coast. Shelf Sci., vol.15 17-27.

Cupp, E. (1943). Marine plankton diatoms of the west coast of North America. Univ. Calif. Press. Berkeley and London 237 p.

Darley, W.M. and B.E.Volcani (1969). A silicon requirement for deoxyribo nucleic acid synthesis in the diatom *Cylindrotheca fusiformis*, Reimann and Lewin. Expl. Cell. Res. 58 334-42.

Davis, C.O., P.Harrison and R.Dugdale (1973). Continuous culture of marine diatoms under Si limitation. J. Phycol., 9 175-180.

Davis, C.O., J.T.Hollibaugh, D.L.R.Seibert, W.H.Thomas and P.J.Harrison (1980). Formation of spores by *Leptocylindrus denicus* (Bacillariophyceae) in a controlled experimental ecosystem. J. Phycol. 16 296-302.

Dawson, P.A. (1973c). Observations on the structure of some forms of *Gomphonema parvulum* Kütz. II The internal organization. J. Phycol. 9 165-75.

Dept. of Environment H.M.S.O. (Pub.) (1980). Reports of the Standing Committee of Analysis. Phosphorus in waters, effluents and sewages.

Dept. of Environment H.M.S.O. (Pub.) (1981). Reports of the Standing Committee of Analysis. Oxidized nitrogen in waters.

Dept. of Environment H.M.S.O. (Pub.) (1980). Reports of the Standing Committee of Analysis. Silicon in waters and effluents.

Ditullio, G.R. and E.A.Laws (1983). Estimates of phytoplankton N uptake based on $^{14}CO_2$ incorporation into protein. Limnol. Oceanogr. 28 177-185.

Droop, M.R. (1968). Vitamin B_{12} and marine ecology. IV The kinetics and uptake, growth and inhabitation in *Monochrysis lutheri*. J. Mar. Biol. Ass. 48 689-733.

Dubois, M., K.A.Gilles, J.K.Hamilton, P.A.Rebers and F.Smith (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28 350-356.

Eppley, R.W., J.H.Sharp, E.H.Renger, M.J.Perry and W.G.Harrison (1977). Nitrogen assimilation by phytoplankton and other micro organisms in the surface waters of the central North Pacific Ocean. Mar. Biol. 39 111-120.

Falk, M., D.G.Smith, J.Maclachlan and A.C.McInnes (1966). Studies on chitan (β-(1→4)- linked 2 aceta-mido-2-deoxy-D-glucan) fibres of the diatom *Thalassiosira fluviatilis* Hustedt. II. Proton magnetic resonance, infrared and X-ray studies. Can. J. Chem. 44 2268-81.

Fawley, M.W. (1984). Effect of light intensity and temperature interactions on growth characteristics of *Phaeodactylum tricornutum* (Bacillariophyceae). J. Phycol. 20 67-72.

Fjerdingstadt, E. (1964). Pollution of streams estimated by benthal phyco microorganisms. 1. A saprobic system based on communities of organisms and ecological factors. Int. Revue ges. Hydrobiol. 49 63-131.

Fogg, G.E. (1982). Nitrogen cycling in sea waters. Phil. Trans. R. Soc. Lond. B296 511-520.

Folch, J., M.Lees and G.H.Sloanestanley (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226 497-509.

Gauthier, M.J., P.Bernard and M.Aubert (1978). Production d'un antibiotique lipidique photosensible par la diatomée marine Chaetoceros lauderi (Ralfs). Ann. Microbiol. 129B 63-70.

Gerloff, J. and E.Gölz (1944). Uber den Feinbau der Kieselschalen bei einigen Zentrischen Diatomeen. Hedwigia 81 283-97.

Goldman, J.C., J.J.McCarthy and D.G.Peavey (1979). Growth rate influence on the chemical composition of phytoplankton in oceanic waters. Nature (London). 279 210-215.

Gran, H.H. (1908). Diatomeen. Lipsuis and Tischler, Kiel and Leipzig. In Nordisches Plankton-Botanischer Teil (ed. K.Brandt and C.Apstein). pp. XIX 1-155.

Guillard, R.R.L. (1973). Division rates. In: Handbook of Phycological Methods - Culture methods and Growth Measurements (ed. J.R.Stein) p.289-311. Cambridge Univ. Press, Cambridge.

Guillard, R.R.L. (1975). Culture of Phytoplankton for feeding Marine Invertebrates. In: Culture of Marine Invertebrate Animals. Ed. W.L.Smith and M.H.Chanley. Plenum Publishing Corporation.

Guillard, R.R.L. and J.H.Ryther (1962). Studies on marine planktonic diatoms. 1. Cyclotella nana Hustedt and Detonula confervacae (Cleve). Gran. Can. J. Microbiol. 8 229-239.

Hauschild, A.H.W., C.D.Nelson and G.Krotkov (1962). The effect of light quality on the products of photosynthesis in green and blue-green algae, and in photosynthetic bacteria. Canadian Journal of Botany. 40 1619-1629.

Healey, F.P., J.Coombs and B.E.Volcani (1967). Changes in pigment content of the diatom Navicula pelliculosa (Bréb) Hilse in Silicon starvation synchrony. Arch. Microbiol. 59 131-42.

Healey, F.P. (1973). The inorganic nutrition of algae from an ecological viewpoint. CRC Critical Rev. Microbiol. 3 69-113.

Heath, I.B. and W.M.Darley (1972). Observations on the ultrastructure of the male gametes of *Biddulphia levis* Ehr. J. Phycol. 8 51-9.

Hellebust, J.A. (1974). In: *Algal Physiology and Biochemistry* (ed. W.D.P.Stewart). p.838-63 Extracellular products. Blackwell Scientific Publications (Oxford).

Hendey, N.I. (1937). The plankton diatoms of the southern seas. 'Discovery' Rep. 16 151-364.

Hensen, V. (1887). Uber die Bestimmung des Planktons oder des im Meere treibenden Materials an Pflanzen und Thieren. Deutsch. Meere Ber. 5. Bericht der Kommission zur wissenschafltichen Untersuchung der deutschen Meere bei Kiel. J.Phycol. 2 136-40 (für die Jahre 1882-1886) 1-8, 1-XIX.

Hobson, L. and R.Pariser (1971). The effect of inorganic nitrogen on macromolecular synthesis by *Thalassiosira fluviatilis* Hustedt and *Cyclotella nana* Hustedt grown in batch culture. J. Exp. Mar. Biol. Ecol. 6 71-78.

Höfler (1940). Aus der Protoplasmatik der Diatomeen. Ber. dt. bot. Ges. 58 97-120.

Holmes, R.W. (1966). Light microscope observations on cytological manifestations of nitrate, phosphate and silicate deficiency in four marine centric diatoms. J.Phycol. 2 136-140.

Jeffrey, S.W. and G.F.Humphrey (1975). New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanzen (BPP) Bd. 167 S. 191-194.

Jitts, H.R., C.D.McAllister, K.Stephens and J.D.H.Strickland (1964). The cell division rates of come marine phytoplankters as a function of light and temperature. J. Fish. Res. Bd. Canada 21 139-157.

Kates, M. and B.E.Volcani (1966). Lipid components of diatoms. Biochim. Biophys. Acta. 116 264-278.

Kuhl, A (1968). Phosphate metabolism of green algae. In. *Algae, Man and the Environment*. ed. D.F.Jackson. p.37-52. Syracuse University Press, Syracuse.

Lewin, J.C., B.E.Reimann, W.F.Busby and B.E.Volcani (1966). Silica shell formation in synchronously dividing diatoms. p.169-88. In. *Cell Synchrony* (eds. I.L.Cameron and G.M.Padilla). Academic Press Inc., N.Y.

Lowry, O.H., N.J.Rosebrough, A.Lewis Farr and R.Randall (1951). Protein measurement with the Folin Phenol Reagent. J. Biol. Chem. 193 256.

Maske, H. (1982). Cultures of *Skeletonema costatum* in steady and transitional state: experimental results and model simulations. J. Mar. Biol. Ass. UK. 62 919-945.

Mazia, D. (1961). Mitosis and the physiology of cell division. In. *The Cell* (ed. J.Bratchet and A.E.Mirsky) Vol. III, p. 77-412. Academic Press, New York and London.

McCarthy, J.J. and J.C.Goldman (1979). Nitrogenous nutrition of marine phytoplankton in nutrient-depleted waters. Science 203 670-672.

*

Morris, I., C.M.Yentsch and C.S.Yentsch (1971). The physiological state with respect to nitrogen of phytoplankton from low nutrient subtropical water as measured by the effect of ammonium ion on dark carbon dioxide fixation. Limnol. Oceanogr. 16 859-868.

Morris, I., H.E.Glover and C.S.Yentsch (1974). Products of Photosynthesis by marine phytoplankton: the effect of Environmental Factors on the relative rates of protein synthesis. Mar. Biol. 27 1-9.

Morris, R.J., M.J.McCartney and G.A.Robinson (1983). Studies of a spring phytoplankton bloom in an enclosed experimental ecosystem. 1. Biochemical changes in relation to the nutrient chemistry of water. J. Exp. Mar. Biol. Ecol. 70 249-262.

Morris, R.J. and S.E.Calvert (1977). Geochemical studies of organic-rich sediments from the Namibian Shelf. 1. The organic fractions. In: A voyage of discovery: George Deacon 70th anniversary volume, edited by M.Angel, Pergamon Press, Oxford pp. 647-665.

Mukerjee (1956). Analyt. Chem. 28 870. USE OF IONIC DYES IN THE ANALYSIS OF IONIC SURFACTANTS AND OTHER IONIC ORGANIC COMPOUNDS

Müller, H.O. and C.W.Pasewaldt (1942). Der Feinbau der Testdiatomee Pleurosigma ungulatum W.Sm. nach Beobachtungen und stereoskopischen Aufnahmen im Übermikroskop. Naturwissenschaften 30 55-60.

Murphy and Riley (1962). Analyt. Chem. Acta. 27 p.31. A MODIFIED SINGLE SOLUTION METHOD FOR THE DETERMINATION OF PHOSPHATE IN NATURAL WATERS

Myklestad, S. (1974). Production of carbohydrates by marine planktonic diatoms. 1. Comparison of nine different species in culture. J. Exp. Mar. Biol. Ecol. 15 261-274.

Myklestad, S. and A. Haug (1972). Production of carbohydrates by the marine diatom *Chaetoceros affinis var. willei* (Gran) Hustedt. 1. Effect of concentration of nutrients in the culture medium. J. Exp. Mar. Biol. Ecol. 9 125-136.

Myklestadt, S. (1977). Production of Carbohydrates by marine planktonic diatoms: influence of the N/P ratio in the growth medium on the assimilation ratio, growth rate, and production of cellular and extracellular carbohydrates by *Chaetoceros affinis var. willei* (Gran) Hustedt and *Skeletonema Costatum* (Grev.) Cleve. J. Exp. Mar. Biol. Ecol. 29 161-179.

Ohta, K. and M.Takagi (1977). Antimicrobial compounds of the marine red algae *Marginisporum aberrans*. Phytochemistry 16 1085-1086.

Patrick, R., M.Hohn and J.H.Wallace (1954). A new method for determining the pattern of the diatom flora. Net. Natn. Acad. Nat. Sci. Philad. 259 1-12.

Pingree, R.D., L.Maddock and E.T.Butler (1977). The influence of biological activity and physical stability in determining the chemical distribution of inorganic phosphate, silicate and nitrate. J. Mar. Biol. Assoc. U.K. 57 1065-1073.

Price, C.A. (1965). A membrane method for determination of total protein in dilute algal suspension. Analyt. Biochem. 12 213-218.

Pugh, P.R. (1975). Variations in the biochemical composition of the diatom *Coscinodiscus eccentricus* with culture age and salinity. Mar. Biol. 33 195-205.

Raymont, J.E.G., J.Austin and E.Linford (1964). Biochemical studies on marine zooplankton. 1. The biochemical composition of *Neomysis integer*. J. Cons. perm. int. Explor, Mer., 28 354-363.

Reuter, J.J.Jr. (1983). Effect of copper on growth, silicic acid uptake and soluble pools of silicic acid in the marine diatom *Thalassiosira weisflogii* (Bacillariophyceae). J. Phycol. 19 101-104.

Reyssac, J. (1973). Diatomées et dinoflagellés au laige de Walvis Bay (Sud-Ouest africain). Bull. Inst. fondam. Afrique noire, Ser. A. 35 273-97.

Robards, A.W. (1970). Electron microscopy and plant ultrastructure. pp. 297. McGraw-Hill, Maidenhead.

Smayda, T.J. (1963). A quantitative analysis of the phytoplankton of the Gulf of Panama. I. Results of the regional phytoplankton surveys during July and November 1957 and March 1958. Bull. inter.-Am. trop. Tuna. Commn. 7 193-253.

Smayda, T.J. (1966). A quantitative analysis of the phytoplankton of the Gulf of Panama. III. General ecological conditions and the phytoplankton dynamics at 80° 45'N, 79° 23'W from November 1954-May 1957. Bull. inter.-Am. trop. Tuna. Commn. 11 355-612.

Soeder, C.J. and E.Stengel (1974). Physico-chemical factors affecting metabolism and growth rate. In: *Algal Physiology and Biochemistry* (ed. W.D.P.Stewart) p. 714-40. Blackwell Scientific Publications, Oxford.

Stein, J.R. ed. (1973). *Handbook of Phycological Methods*, (Culture methods and growth measurements p.448). Cambridge Univ. Press, Cambridge.

Strickland, J.D.H. and T.R.Parsons (1972). Determination of Reactive Nitrate. In: *A Practical Handbook of Seawater Analysis* Bulletin 167 2nd edition p.77. Fisheries Res. Board of Canada.

Strickland, J.D.H. (1965). Production of organic matter in the primary stages of the marine food chain. In: *Chemical Oceanography* col. I. (eds. J.P.Riley and G.Skirrow). pp. 478-610. Academic Press, New York.

Strickland, J.D.H., R.W.Eppley and B.Rojas de Mendiola (1969). Phytoplankton populations, nutrients and photosynthesis in Peruvian coastal waters. Bol. Inst. Mar. Peru 2 4-45.

Strickland, J.D. (1972). Research on the marine planktonic food web at the institute of marine resources: a review of the past seven years of work. Oceanogr. Mar. Biol. Ann. Rev. (ed. H.Barnes) 10 349-414.

Stoermer, E.F. and H.S.Pankratz (1964). Fine structure of the diatom *Amphipleura pellucida* I Wall Structure. Am. J. Bot. 51(9) 986-90.

Troll, W. and R.K.Cannon (1953). A modified ninhydrin method for the analysis of amino and imino acids. J. Biol. Chem. 200 803-811.

Voskresenskaya, N.P. (1972). Blue light and carbon metabolism. Ann. Rev. Pl. Physiol. 23 219-34.

Wallen, D.G. and G.H.Geen (1971). Light quality in relation to growth, photosynthetic rates and carbon metabolism in two species of marine plankton algae. Mar. Biol. 10 34-43.

Wallen, D.G. and G.H.Geen (1971). The nature of the photosynthate in natural phytoplankton populations in relation to light quality. Mar. Biol. 10 157-168.

Walne, P.R. (1970). Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera Ostrea, Crassostrea, Mercenaria and Mytilus. Fish. Invest. Ser. 2 26 1-62.

Werner (1966). Die Kieselsäure im Stoffwechsel von *Cyclotella cryptica* Reimann, Lewin and Guillard. Arch. Mikrobiol. 55 278-308.

Werner, D. and A.Pirson (1967). Uber reversible Speicherung von Kieselsäure in *Cyclotella cryptica*. Arch. Mikrobiol. 57 43-50.

Werner, D. (1969). Beiräge zur Physiologie und Biochemie der Kieselsäure. Ber. dt. Bot. Ges. 81 425-9.

Werner, D. ed. (1977). Botanical Monographs Volume 13. *The Biology of Diatoms*. Pub. Blackwell Scientific Publications.

*Moore, S. and W.H.Stein (1948). Photometric ninhydrin method for use in the chromatography of amino acids. J. Biol. Chem., 176 367-388.

#Snow, N.B. and P.J.LeB Williams (1971). A simple method to determine the O:N ratio of small marine animals. J. Mar. Biol. Ass. U.K., 51, 105-109.