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Title: RAPID EQUILIBRIUM RESPONSE OF A MARINE DIATOM TO EXTERNAL AND INTERNAL NUTRIENT CONCENTRATIONS

Abstract approved: Redacted for privacy
Herbert C. Curl, Jr.

Batch cultures of Skeletonema costatum were grown under continual light saturation, with nitrogen nutrients in limiting concentrations. Population parameters measured included ^{14}C uptake, chlorophyll a, particulate carbon and nitrogen, and ammonia, nitrate, and nitrite concentrations.

A method was developed for measuring cellular nutrient reservoirs in laboratory cultures of marine phytoplankton. The method seems compatible to field measurements of cellular reservoirs in natural plankton communities. Significant cellular nutrient reservoirs of nitrate and ammonia were measured in all three experiments, and the ratio of mg/l of cellular nutrient reservoir to mg/l of particulate nitrogen ranged from zero to 1.0.

Cellular nitrite reservoirs ranging from 0-1μM were observed during nitrite assimilation. A practical method of measuring nitrate

reservoirs was developed.

From the raw data, parameters calculated were nutrient specific uptake rates, particulate nitrogen specific growth rates, relative cellular reservoir size, percent soluble organic nitrogen, photosynthetic assimilation ratios, chlorophyll *a* / carbon ratios, and nitrogen to carbon ratios.

Specific uptake rates were found to be highly variable, and at times were ca. 20 times the highest measured population growth rate. It was concluded that specific uptake rates showed poor correlation to specific growth rates. Calculated relative cellular nutrient reservoir sizes do not correspond directly to trends in specific growth rates, although it was noted that large relative cellular nutrient reservoir sizes preceded the initial large increases in particulate nitrogen specific growth rates.

Photosynthetic assimilation ratios and percent soluble organic nitrogen correlate with trends in population growth rate very well. Changes in N/C ratios, and chlorophyll *a* / carbon ratios were also found to be related to changes in population growth rates. The N/C values ranged from 0.04 to 0.16, and the corresponding C/N ratios from 15 to 6.2. Induction of the chlorophyll *a* synthesis pathway by internal ammonia concentrations is discussed.

Four basic models of phytoplankton growth dynamics are discussed, and the inadequacy of each model, in light of past and

present experimental evidence is pointed out.

A new conceptual model is presented and discussed, which appears to be biologically sound and compatible with experimental evidence. The most important aspect of the model is the induction of assimilatory enzyme systems by internal nutrient concentrations.

Rapid Equilibrium Response of a Marine Diatom to External and Internal Nutrient Concentrations

by

Daniel Wayne Lundy

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RAPID EQUILIBRIUM RESPONSE OF A MARINE DIATOM TO EXTERNAL AND INTERNAL NUTRIENT CONCENTRATIONS

INTRODUCTION

Since the concept of single nutrient limitation was presented by Liebig (1840) and Blackman (1955), there has been considerable effort to understand the growth responses of marine phytoplankton in terms of a single limiting nutrient. Early work by Monod (1942) demonstrated that Michaelis-Menten enzyme kinetics could be successfully extended to describe the growth kinetics of bacteria limited by a single substrate. The assumption that similar processes would apply to phytoplankton, prompted the theoretical papers by Caperon (1967), and Dugdale (1968).

The simplest form of the logistic equation presented by Caperon contains a maximum specific growth constant that is derived expressly from enzyme kinetic theory, and the equation itself relates growth rate to limiting nutrient concentration by Michaelis-Menten kinetics.

Dugdale's discussion of nutrient limitation in the sea involves the concept of nutrient flux through the system, which ultimately controls the productivity in the euphotic zone. In his simplified model, phytoplankton growth is related to Michaelis-Menten uptake kinetics of the limiting nutrient.

The theoretical papers by Caperon and Dugdale have had a

definite effect on the type of research effort in the area of phytoplankton ecology. Half saturation constants (K_s) have been determined for a variety of marine phytoplankton, and nutrient uptake kinetics have been successfully described by hyperbolic Michaelis-Menten type functions (MacIsaac and Dugdale, 1969; Eppley and Coatsworth, 1968; Droop, 1968; and Eppley and Thomas, 1969). However, serious problems have been encountered when nutrient uptake has been related to growth rate in batch and continuous culture.

In batch cultures, rarely can the measured uptake rates be correlated with specific growth rates. Rather, uptake rates can be as much as twenty times higher than the highest growth rate ever measured (Eppley and Thomas, 1969) and with low initial nutrient concentration, the nutrient can be completely depleted before growth is observed (Caperon and Meyer, 1972a). In addition, Ketchum (1939) observed growth well after phytoplankton had been removed from nutrient medium into nutrient depleted medium. The growth rates of phytoplankton in continuous culture have not been successfully predicted by nutrient concentrations in culture vessels, because the measured nutrient concentrations are usually very low, and do not appear to increase with increasing steady state growth rate (Caperon and Meyer, 1972a; Donaghay, 1973; Williams, 1971).

The varied results in batch and continuous cultures of phytoplankton mentioned above may all be explained by the existence of

two phenomena: the ability of phytoplankton to store nutrients internally, and the ability to take nutrients up at very high rates. As early as 1939, Ketchum explained his experimental results by postulating a nutrient reservior, yet it has been only recently that serious attempts have been made at measuring and explaining the significance of nutrient reservoirs in marine phytoplankton. The concept of nutrient reservoirs has been discussed in a number of recent papers, each of which will be mentioned briefly.

Caperon and Meyer (1972a) found excellent correlation between variations in "internal nutritional state" and growth rate at steady state. Internal reservoirs of ammonia and nitrate were never measured directly, but N/C ratios and chlorophyll a/carbon ratios were said to be indicators of the size of the internal reservoirs of these nutrients. Growth rate was related to population nutritional state by the equation:

$$\mu = \mu_m (q - q_o) / K + (q - q_o),$$

where μ_m, q, q_o, and K are equal to maximum specific growth rate, N/C ratio, N/C ratio at zero growth, and half saturation constant, respectively. A similar expression related chlorophyll a/ carbon ratios to steady state growth rates. From the nutrient data in these experiments, the authors state that "there is no evident relationship between nutrient concentration in the growth chamber and steady

state growth for any of the populations studied."

In the second paper, Caperon and Meyer (1972b), the authors demonstrated a definite relationship between steady state growth, and uptake rates in separate two-hour uptake experiments. Higher uptake rates were associated with cells grown at higher dilution rates. The authors then insisted that there must be a relationship between nutrient concentration in the chemostat vessel, and growth rate, and attempted to predict nutrient levels at steady state on the basis of four equations.

In his treatment of internal nutrient reservoirs, Williams (1971) does not distinguish between reserviors of enzymes, nutrients, or amino acid pools, etc., but lumps them all into a "synthetic portions" of the cell. He does not measure the synthetic portion directly, but makes the assumption that chlorophyll is a good representative of the synthetic fraction of the cell, because of the observed linear increase in chlorophyll with increasing steady state growth rates. Using an analog computer, and the set of assumptions developed, the author produces some interesting simulations of batch and continuous culture conditions. However, because he offers no nutrient data for batch cultures, it is not possible to make an adequate comparison between the response to nutrients of actual batch culture experiments and the simulation batch culture.

Williams (1971) does give information about nutrient conditions

in his continuous cultures; there are discrepancies between actual chemostat values and predicted values from the model simulation. Figure 23 in the Williams' paper shows a predicted linear increase in nutrient concentration in the growth chamber, with increasing growth rate. In reality, however, Williams observed nitrate levels "so low that it was impossible to obtain good quantitative estimates." All of the measurements were at the lower sensitivity of the analytical technique.

Grenney, Bella, and Curl (1973, a and b) have developed a mathematical model of phytoplankton growth dynamics assuming Michaelis-Menten saturation kinetics and three compartment cellular reservoirs of nitrogen. The compartments are separated into an inorganic nutrient reservoir and a reservoir of organic nitrogen intermediate molecules, such as amino acids, both of which can vary in relative size with changes in growth rate. The third compartment is cellular protein, which is defined in the model as constant per cell volume, at all growth rates. It is assumed that all flow rates in and out of the various compartments, and relative concentrations of nitrogen in each compartment are controlled by saturation kinetics. Computer "muscle" is utilized to solve for the relative concentrations of nitrogen in each compartment and the rate constants between compartments. The chemostat equilibrium data of Caperon (1968) was simulated by computer, following computer estimation of model

parameters. The simulated equilibria conditions show rather good fit to Caperon's cell number data, and predict interesting changes in internal nutrient reservoirs and organic nitrogen reservoirs with increasing growth rate. It should be noted that the model is consistent with actual observations, in that it does not require increasing nutrient concentrations in the culture vessel with increasing steady state growth rate.

Model simulations of batch cultures are also made. The model predicts an initial lag in growth, and achievement of maximum growth rate at approximately two days, when depleted cells are added to a nitrate rich environment. The transient growth of batch cultures is controlled by rapid changes in composition of the two variable compartment reservoirs.

It seems apparent that the obvious next step in the investigation of the importance of cellular reservoirs in phytoplankton is the actual measurement of these reservoirs in laboratory cultures. The work presented here involves the measurement of cellular reservoirs of ammonia, nitrate, and nitrite in a marine phytoplankton grown in batch cultures. These measurements, along with various measures of "physiological state" will be analyzed, and incorporated into a biologically probable model.

METHODS AND MATERIALS

Skeletonema costatum, the diatom used in this work, was isolated as a clone from the estuarine waters of Auke Bay, Alaska in 1971, and subsequently carried as a unialgal stock culture in the laboratory. The diatom was not axenically cultured. The culture medium used was half strength Basic Medium, used for culturing marine algae at Oregon State University School of Oceanography.

Table 1. Half Strength Basic Medium

Major Ions, Concentration per 1000 ml of Sea water:*		
$NaH_2PO_4 \cdot H_2O$	5 mg	36.25 μg-at. P
Fe sequestrene	0.5 mg	1.16 μg-at. Fe
$Na_2SiO_3 \cdot 9H_2O$	15-30 mg	53.5-107 μg-at. Si
$NaHCO_3$	0.1 mg	added as 10 ml of stock sol.
Thiourea	0.5 mg	
$NaNO_3$	Different amounts added in each experiment. (See experiment descriptions).	
NH_4Cl		
Vitamins, Weight per 1000 ml of Sea water		
Thiamin HCl	0.1 mg	
Biotin	0.5 μg	
B_{12}	0.5 μg	

Table 1, continued:

Trace Metals, Concentration per 1000 ml of Sea Water

$CuSO_4 \cdot 5H_2O$	0.098 mg	0.039 μg-at. Cu
$ZNSO_4 \cdot 7H_2O$	0.022 mg	0.00765 μg-at. Zn
$CoCl_2 \cdot 6H_2O$	0.010 mg	0.042 μg-at. Co
$MnCl_2 \cdot 4H_2O$	0.280 μg	0.91 μg-at. Mn
$NaMoO_4 \cdot 2H_2O$	0.0065 μg	0.0265 μg-at. Mo

* Millipore[R] filtered, autoclaved.

Light Saturation Determination

Determination of photosynthetic light saturation for the diatom was done as follows. A two liter culture was allowed to reach moderate cell density, under non-saturating light intensity. While still growing vigorously, the culture was divided into 50 ml aliquots in 125 ml flasks, and the flasks placed in the dark for 24 hours. At the end of the dark period, two flasks were selected randomly, innoculated with 0.5 μCi of carbon-14 bicarbonate, and placed in a light bath at the lowest light intensity (see Table 2). The light bath (Figure 1) was used for illumination in all experiments.

After a two-hour incubation, the contents of the flasks were

Table 2. Light Intensities Achievable with Combinations of Fluorescent and Incandescent Lamps.

Lamps	Intensity (mw / cm^2)	Langlies/ minute x 10^3	Langlies/ 24 hours
1 cool white Sylvania $Circline_R$ light ring	0.37	5.2	7.7
2 cool white Sylvania $Circline_R$ light rings	0.74	10.4	15.4
3 cool white Sylvania $Circline_R$ light rings	1.11	15.6	23.1
4 cool white Sylvania $Circline_R$ light rings	1.48	20.8	30.8
5 cool white Sylvania $Circline_R$ light rings	1.85	26.0	38.5
5 cool white Sylvania $Circline_R$ light rings 1 filtered,* Sylvania $Cool\text{-}lux_R$ flood lamp	3.60	49.8	72.2
5 cool white Sylvania $Circline_R$ light rings 2 filtered, Sylvania $Cool\text{-}lux_R$ flood lamps	5.4	73.6	105.9

---

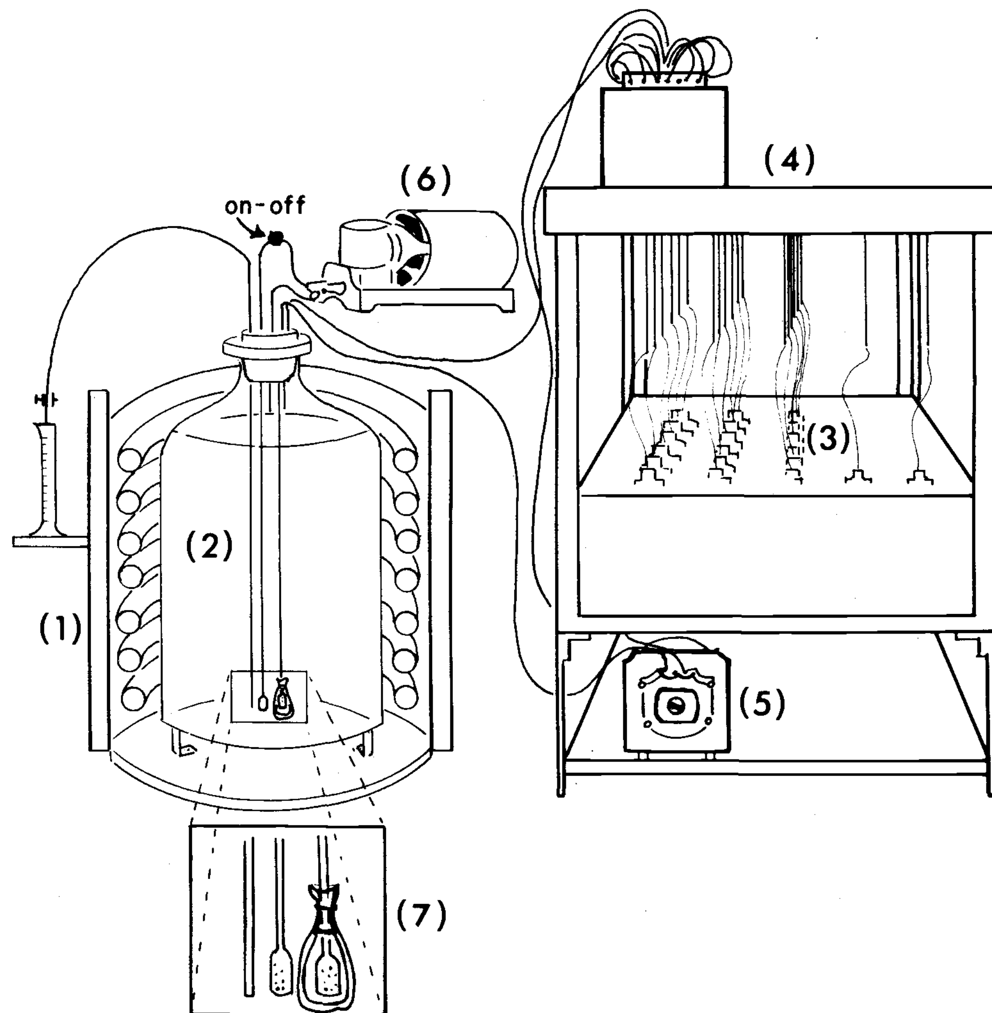
* Corning infra-red absorbing glass filter number 4600

Revised, from Marmelstein, 1970.

Figure 1. Schematic representation of culture and sampling apparatus.

LEGEND

1) Cut-away of light bath, with seven light rings.
2) 20 liter sample vessel.
3) Sample bottles, packed in dry ice.
4) 20 sample automatic sampler.
5) Cole-Parmer[R] peristaltic pump.
6) Sealed diaphram closed circuit pump.
7) Left to right: culture sampling tube, gas dispersion tube, and gas dispersion tube with double pouches of 10µ Nitex[R] netting.
8) Graduate cylinder, for sample volume measurement.



immediately filtered through 0.8 μ Millipore[R] HA membrane filters. The filter pads were placed in scintillation vials with 10 ml of Aquasol[R] scintillation fluor. Two more flasks in the dark were selected randomly, innoculated with ^{14}C, and placed in the light bath for two hours at the next higher light intensity (two circuline lamps), and again filtered onto Millipore[R] filters at the end of the incubation. This procedure was repeated for each light intensity available in the light bath apparatus. The labeled filter pads were counted on a Packard-Tri-Carb[R] scintillation counter, and relative activity in cpm plotted against predetermined light intensities for the light bath (Marmelstein, 1970).

Culturing and Sampling Apparatus

Figure 1 shows a schematic drawing of the culturing and sampling apparatus used in all experiments. The entire apparatus was located in a constant environment room at 12°C. The culture medium was maintained at ambient temperature, ± 1°C, by an overhead forced air duct from the cooling unit, and a blower directed at the base of the culture.

Special precautions were taken to avoid loss of carbon-14 activity in the culture medium. These included a closed circuit bubbling system, and maintainance of positive pressure above the culture medium, when sampling. All Tygon[R] and glass tube

connections were sealed with Dow Corning[R] silicone rubber sealer, to avoid gas leaks.

Illumination in the light bath was by 40 watt Sylvania[R] cool white circuline lamps (seven total). Although two overhead filtered incandescent lamps were used in the light saturation experiments, they were not used in experiments thereafter, because light saturation was accomplished for the diatom with the light rings alone.

An automatic sampler was used to sample from the culture on a 30-minute cycle. The cycle started with five minutes of sampling, followed by 25 minutes of sample bypass through the sampler and back into the culture vessel. "Dead space" in the sample tubing between samples was avoided in this manner.

Sampling and Sample Analysis Procedure

Cell-free medium for nutrient analysis was obtained by removing the medium slowly through a fine pore gas dispersion tube, surrounded by two pouches of 10μ Nitex[R] netting, and into the automatic sampler. A Cole-Parmer[R] peristaltic pump maintained slow constant flow. During the five minute sampling period of each 30-minute cycle, the cell-free culture medium was sampled into 50 ml polethylene bottles packed in dry ice, which froze each sample immediately. This sampling technique was designed to remove cell-free culture medium automatically without causing cell breakage or leakage; i.e.,

to minimize the release of any nutrients taken up by the diatoms, back into the culture medium. A combination of slow flow rate, large netting area through which the culture medium was drawn, and mixing of the culture medium by bubbling, produced very little accumulation of cells on the netting and little or no cell leakage into the culture medium sampled.

Samples to be ultrasonicated and analyzed for nutrients were removed from the culture, placed in 50 ml polyethylene bottles and immediately frozen in dry ice. The samples were thawed, immediately before ultrasonication with a Biosonik III[R], at an intensity of 105 watts/cm^2 for 30 seconds. After ultrasonication, the samples were again immediately frozen in dry ice until nutrient analysis by the Autoanalyzer[R] was performed. Duplicate samples were taken in experiment I and III, and single samples in experiment II.

The cell-free culture medium samples, and ultrasonicated samples were analyzed for nitrate, ammonia, and nitrite with a Technicon Autoanalyzer[R]. The nitrate-nitrite method used was that of Wood, Armstrong and Richard (1967), with some minor modifications by Atlas *et al.* (1971). If significant nitrite was present in samples, the concentrations were subtracted from the calculated nitrate values. Sample-to-sample precision is reported as ± 2% (2σ). The automated ammonia method is that of Head (1970). Sample-to-sample precision for the method is reported to be ± 3.32%

(2σ), although this is under optimum machine operation.

Three replicate chlorophyll samples of 50 ml each were taken and filtered onto Millipore[R] HA membrane filters. Chlorophyll a determination was done by a modified Strictland and Parsons (1972) method (Glooshenko et al., 1972). Calculations were performed according to the equations of Strictland and Parsons (1972) for total chlorophyll a.

Three replicate particulate carbon and nitrogen samples were taken by very gently filtering 50 ml culture through a Whatman GFC filter cut to a 13 millimeter diameter to fit a Swinney adaptor. The filter pads were placed in individual vials and frozen until analysis by a Carlo Erba Elemental Analyzer[R] model 1100. Immediately before analysis, samples were dried in a 60°C oven, for three to four hours. The filtrate of these samples was saved, and placed in 50 ml polyethylene bottles capped tightly and frozen for later analysis of nitrate, ammonia, and nitrite nutrients.

Carbon-14 sampling was done in triplicate and involved removing 50 ml of culture from the culture vessel, and filtering it under vacuum immediately through a 2.5 cm 0.8 μ Millipore[R] HA membrane filter. The labeled filter pads were immediately placed in 10 ml of Aquasol[R] LSC cocktail in scintillation vials. The vials were capped tightly and stored at room temperature until analysis of activity. Aquasol[R] breaks up and partially dissolves membrane filters within

48 hours. Phytoplankton pigments are also apparently extracted and bleached, since the samples become colorless within three to five days. Sample vials were shaken vigorously at weekly intervals. Samples were counted on a Packard Tri-Carb^{R} model 3375 liquid scintillation spectrometer. Because of the apparent homogeneous suspension of labeled sample and filter pad, the counting efficiency was determined by automatic external standardization (AES) and by the channels ratio method. Net counts per minute were corrected to disintegrations per minute using an efficiency "curve" based on both channels ratio, and AES. Disintegrations per minute were converted to mg of carbon according to the method of Strictland and Parsons (1972) using the ratio of activity taken up to the total activity present, and the CO_2 alkalinity of the culture.

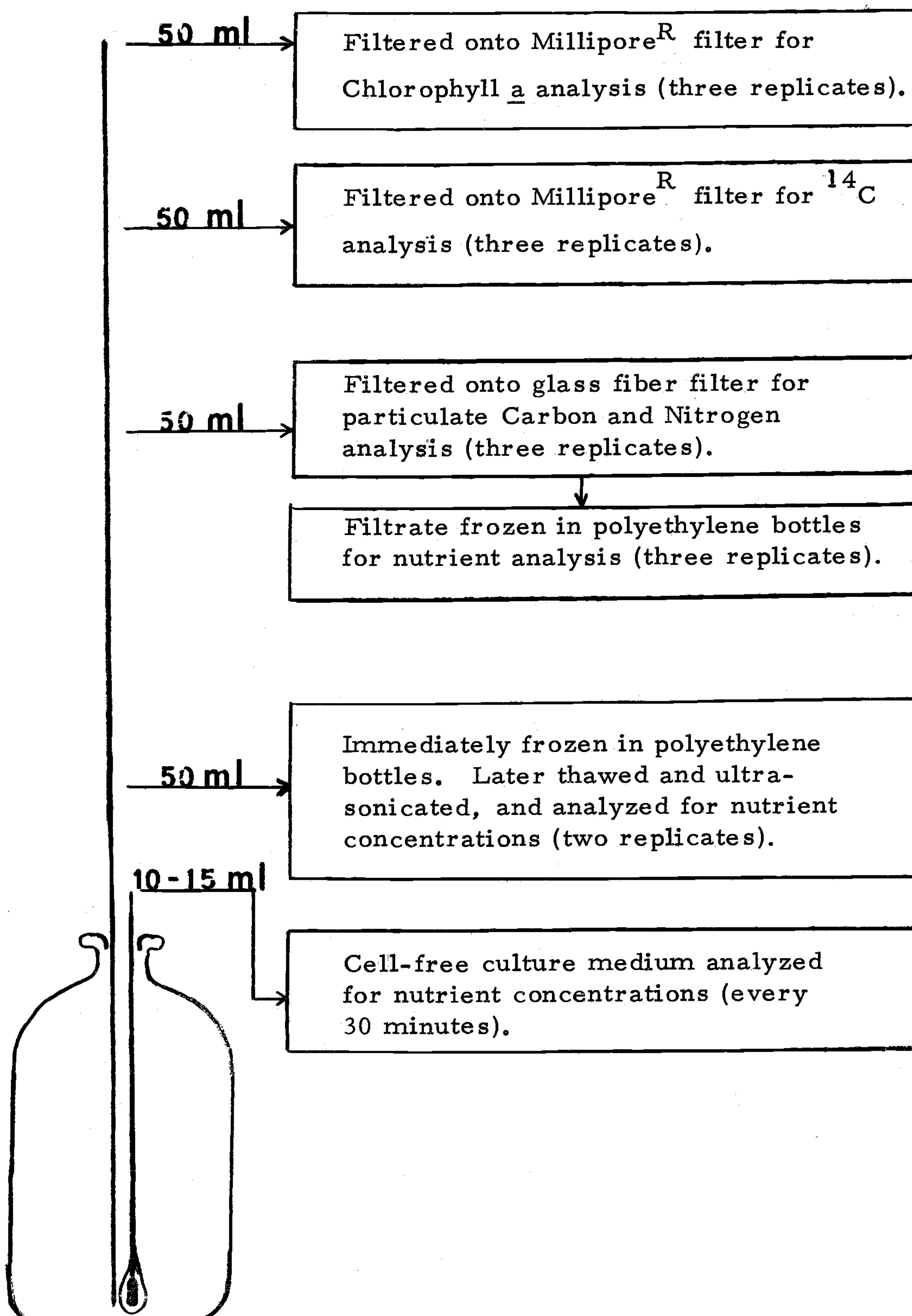
A schematic of the sampling and analytical procedure appears in Figure 2.

Brief Description of Each Experiment

Experiment I: 100 ml of dense, nutrient-depleted culture of *S. costatum* was inoculated into 20 liters of sterile enriched sea water. Initial nitrogen concentrations were 12.25 μM ammonia and 32.4 μM nitrate.

Experiment II: 10 liters of enriched sea water was inoculated

Figure 2. Block diagram of sampling and analytical procedure.


50 ml
Filtered onto Millipore R filter for Chlorophyll a analysis (three replicates).
50 ml
Filtered onto Millipore R filter for 14C analysis (three replicates).
50 ml
Filtered onto glass fiber filter for particulate Carbon and Nitrogen analysis (three replicates).
Filtrate frozen in polyethylene bottles for nutrient analysis (three replicates).
50 ml
Immediately frozen in polyethylene bottles. Later thawed and ultra-sonicated, and analyzed for nutrient concentrations (two replicates).
10-15 ml
Cell-free culture medium analyzed for nutrient concentrations (every 30 minutes).

with *S. costatum*, and the culture allowed to grow four days, until nutrients were depleted. To the culture vessel was then added 10 more liters of enriched sea water, with an initial concentration of nitrogen nutrients of 6.8 μM ammonia 11.7 μM nitrate. Sampling was started immediately after the culture volume was brought up to 20 liters. During the first 40 minutes of sampling, the lighting was reduced to approximately 42% of saturation, after which it was maintained at saturation until shortly after nitrate was depleted from the medium.

Experiment II was designed expressly for accurate measurement of nitrate and ammonia reservoir concentrations and uptake rates. This was the reasoning behind the large initial biomass, and the close interval sampling at the beginning of the experiment. The low light intensity period was an attempt to slow the growth rate of the population slightly, and allow cellular reservoir filling to occur more rapidly.

Experiment II: One hundred ml of a dense nutrient-depleted culture of *S. costatum* was inoculated into 20 liters of sterile enriched sea water. Initial nitrogen concentrations were 8 μM ammonia and 55 μM nitrate. After all nutrients had been depleted, enough nitrate was added to the culture vessel to produce approximately

8 μM concentration. Uptake of this addition was monitored by close interval sampling.

The procedure followed in experiment III was designed to produce results similar to experiment I, except that the role of ammonia was to be reduced, in hopes of measuring a nitrate reservoir.

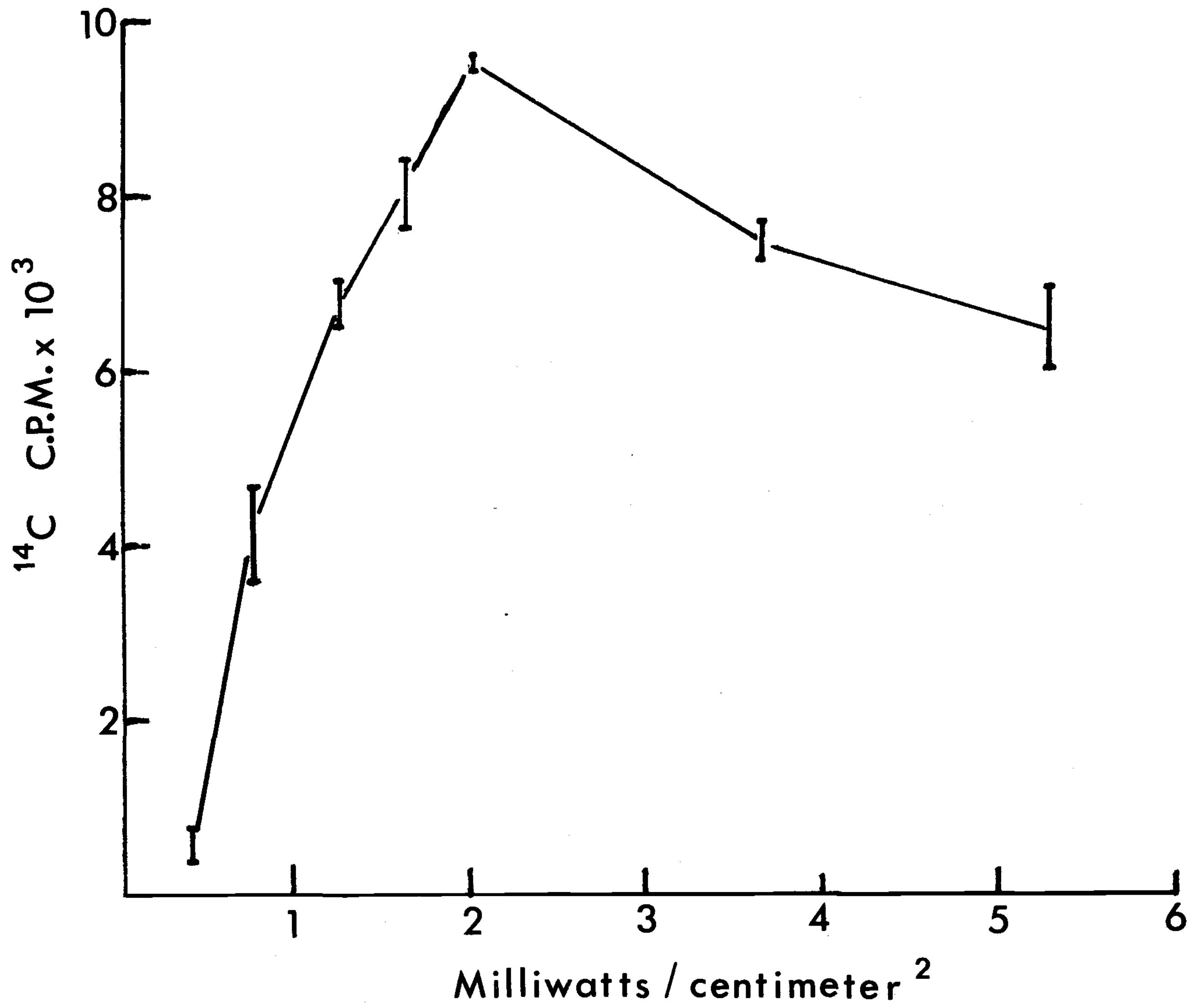
The sea water used in experiments I and II was collected in early January at the mouth of Yaquina Bay, Oregon (salinity 30 ‰). This water was stored approximately one month, before use in experiments I and II. The sea water used in experiment III was collected in April, at the mouth of Yaquina Bay, Oregon (salinity 31‰). There was a phytoplankton bloom in the bay at the time of collection. Also, because time did not allow, this sea water was not "aged," but used immediately in experiment III.

RESULTS

Although maximum ^{14}C uptake occurred with five light rings, a total of seven light rings were used in experiments I, II, and III. This was to insure light saturation throughout the duration of the experiments, as the cultures changed from low cell density to maximum cell density. Light saturation for our laboratory culture occurred at about 2 mw/cm^2 intensity (Figure 3).

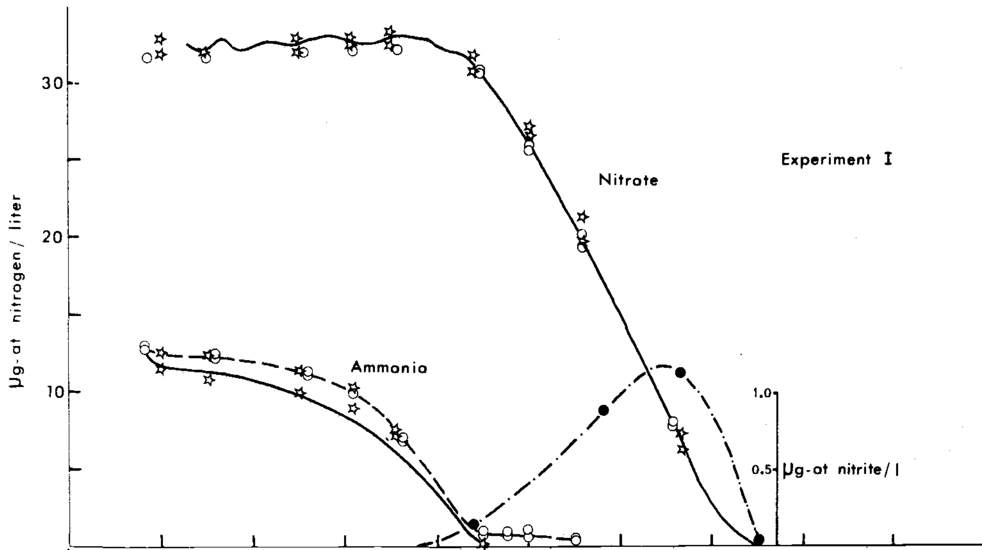
In general, the uptake profiles (Figure 4) resemble data from Caperon and Meyer (1972a) with ammonia effectively inhibiting the

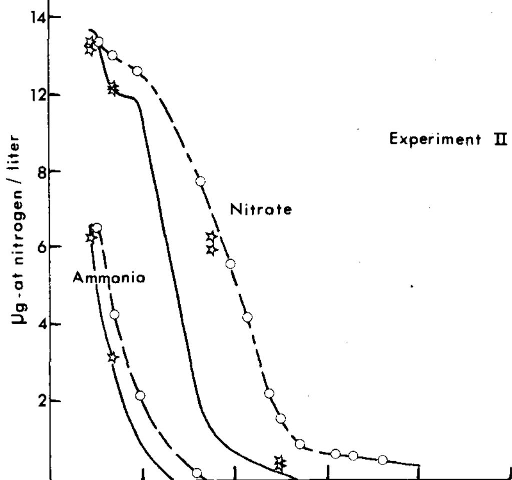
Figure 3. Light saturation plot for *Skeletonema costatum*. Saturation occurs at an intensity of about 2.0 milliwatts/ centimeter^2.

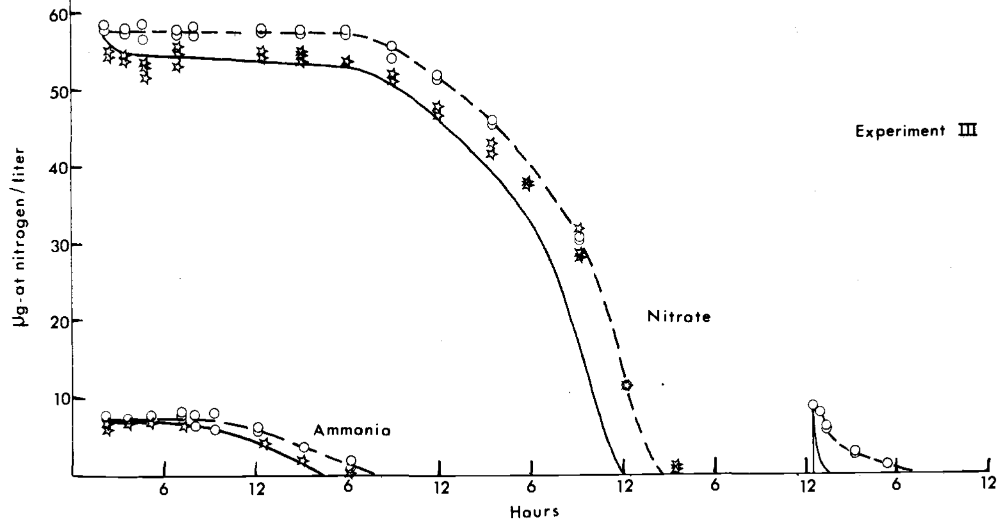


10
8
6
4
2
^{14}C C.P.M. x 10^3
1
2
3
4
5
6
Milliwatts / centimeter ^{2}

Figure 4. Nutrient data from experiments I, II, and III vs. time in hours. Dashed lines with open circles are ultrasonicated values; solid lines are cell-free culture medium values; and solid stars represent glass fiber filtrate values. Nitrite values are shown for experiment I on a separate axis.


Experiment I
Nitrate
Ammonia
μg-at nitrogen / liter
μg-at nitrite / l
30
20
10
1.0
0.5


Experiment II
Nitrate
Ammonia
μg-at nitrogen / liter
14
12
8
6
4
2


Experiment III
Nitrate
Ammonia
μg-at nitrogen / liter
Hours
60
50
40
30
20
10
6
12
6
12
6
12
6
12
6
12

uptake of nitrate. It appears that in all three experiments, nitrate is taken up and assimilated when ammonia concentrations go below about 2 μM in the environment. Caperon and Meyer (1972a) report gradual uptake of nitrate when ammonia is present, similar to the gradual slope in the culture medium concentrations in experiment III (Figure 4). Essentially no assimilation of nitrate took place during this time; the concentration of nitrate from ultrasonication samples has remained constant. Although nitrite was not found in the cell-free culture medium samples, it was observed in low concentrations in ultrasonicated samples (Figure 4). Nitrite is measurable in higher plants, when nitrate is assimilated (Evans and Nason, 1953). Nitrite values are shown for experiment I only.

Extremely rapid nutrient uptake was observed when nutrients became available to depleted phytoplankton populations (Figure 5). This very rapid uptake of nutrients by deficient phytoplankton is thought to be filling of an intracellular nutrient reservoir.

The "raw" data from the three experiments were plotted on a time sequence, such that comparisons of the different measurements could be made (Figures 6, 7, and 8). Calculations performed on the raw data included nitrogen specific uptake rates, and nitrogen specific nutrient assimilation rates. The nitrogen specific nutrient assimilation rates were calculated as the disappearance of nutrients from ultrasonicated samples. Since the amount of particulate nitrogen

Figure 5. Nitrate concentrations taken from experiments II and III. Dashed lines with open circles are ultrasonicated values; and solid lines are cell-free culture medium values.

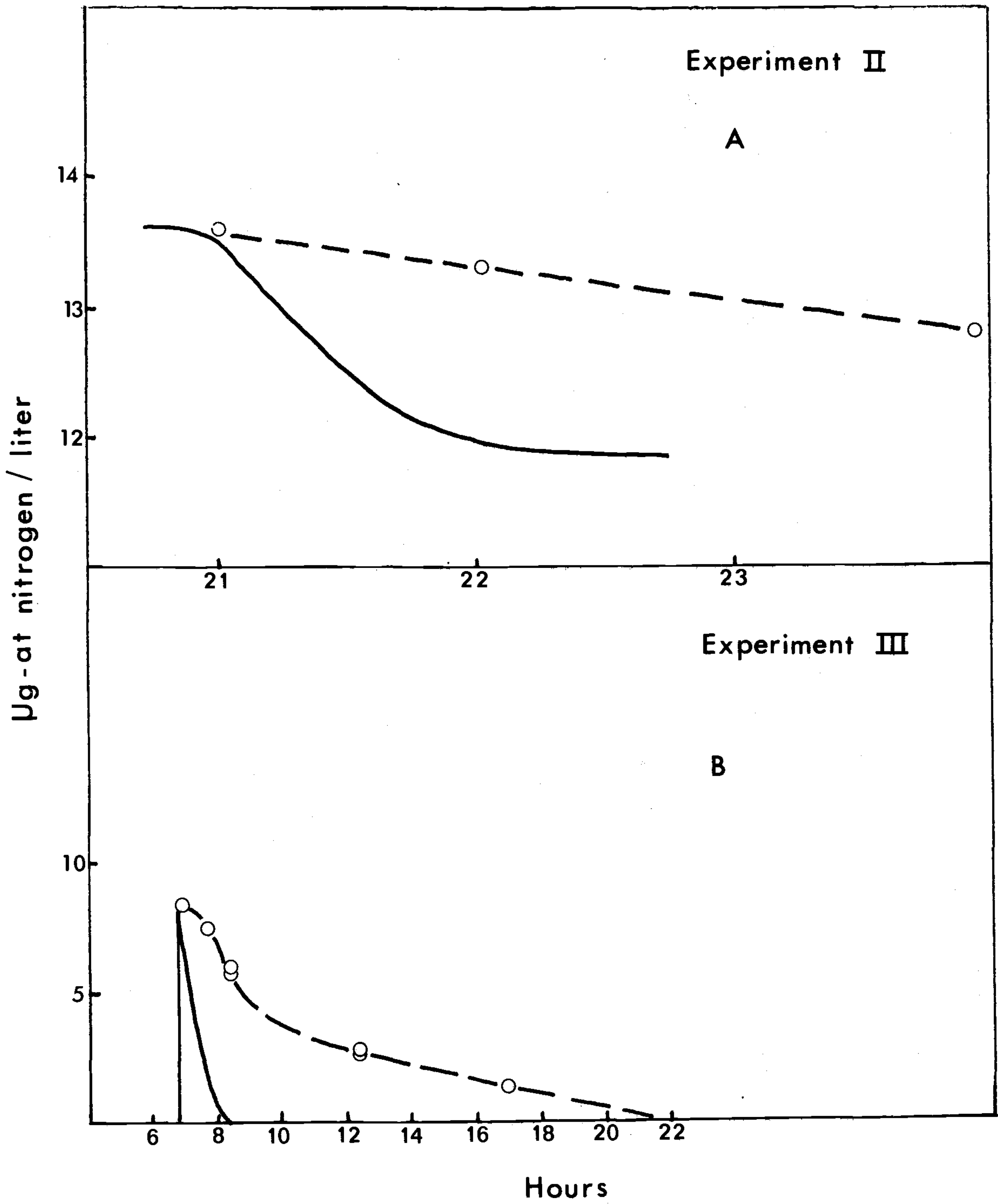

Experiment II
A
14
13
12
21
22
23
Experiment III
B
10
5
6
8
10
12
14
16
18
20
22
Hours
µg-at nitrogen / liter

Figure 6. Raw data from experiment I vs. time in hours.

A. nutrient concentrations (μg-at/liter)
B. chlorophyll *a* (μg/liter)
C. carbon and nitrogen (μg/50 ml)
D. carbon-14 (CPM)

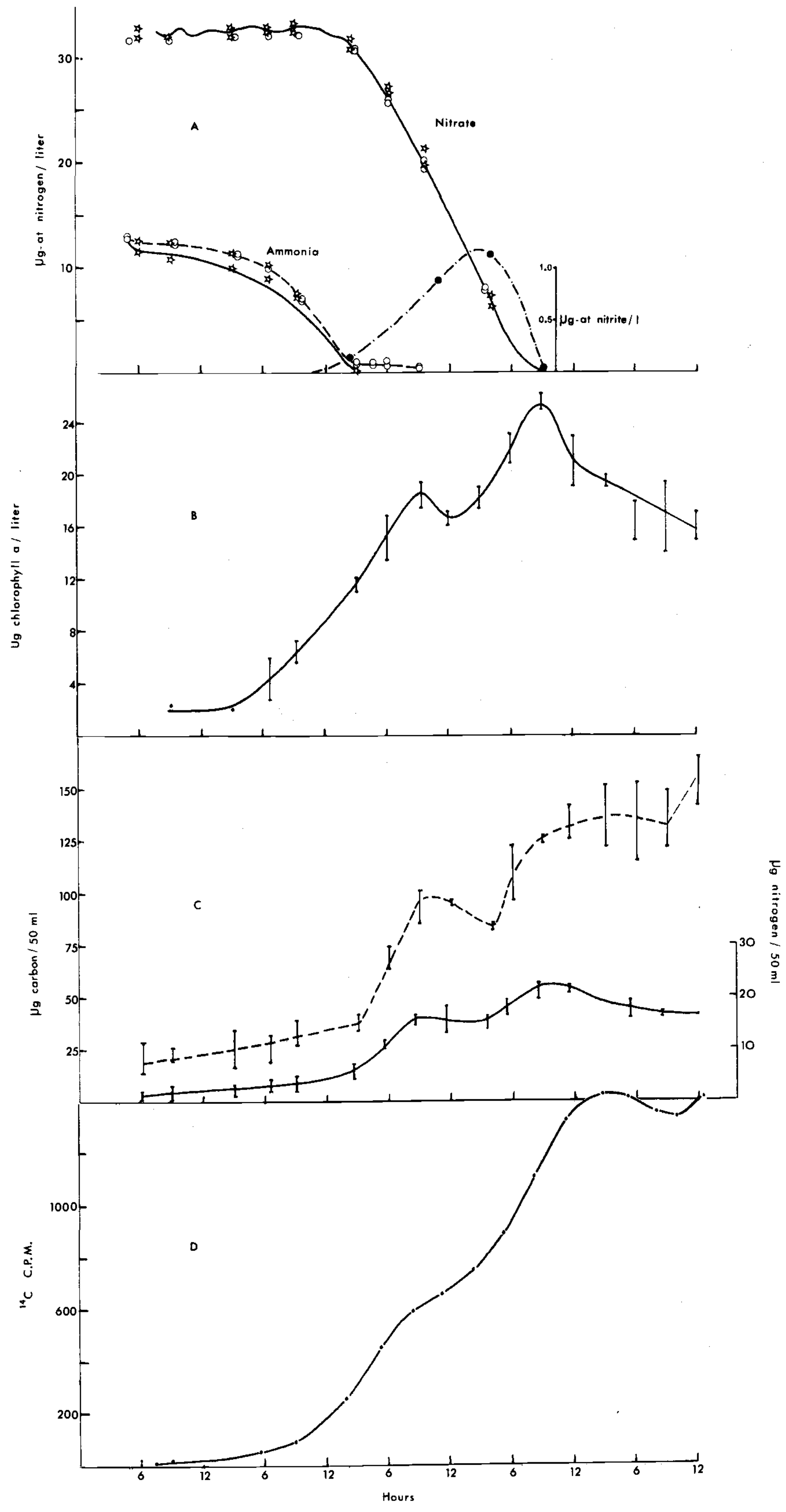

A
Nitrate
Ammonia
µg-at nitrogen/liter
µg-at nitrite/l
B
µg chlorophyll a/liter
C
µg carbon/50 ml
µg nitrogen/50 ml
D
^{14}C C.P.M.
Hours

Figure 7. Raw data from experiment II vs. time in hours.

A. nutrient concentrations (μg-at/liter)
B. chlorophyll a (μg/liter)
C. carbon and nitrogen (μg/50 ml)
D. carbon-14 activity (CPM)

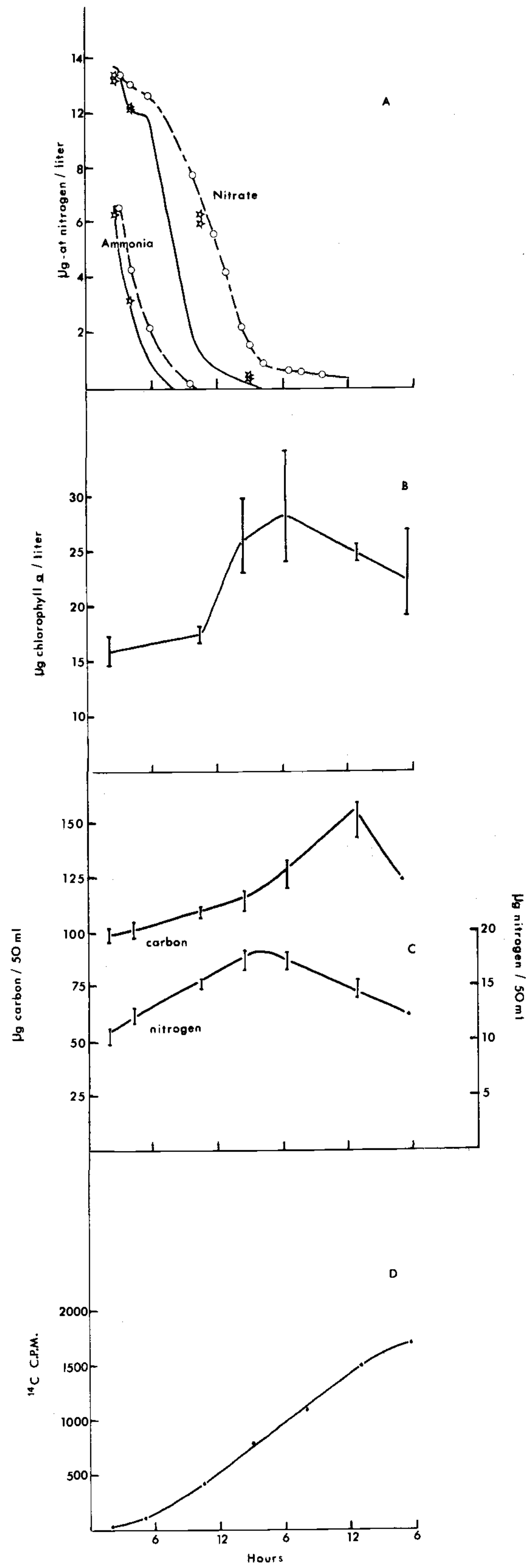
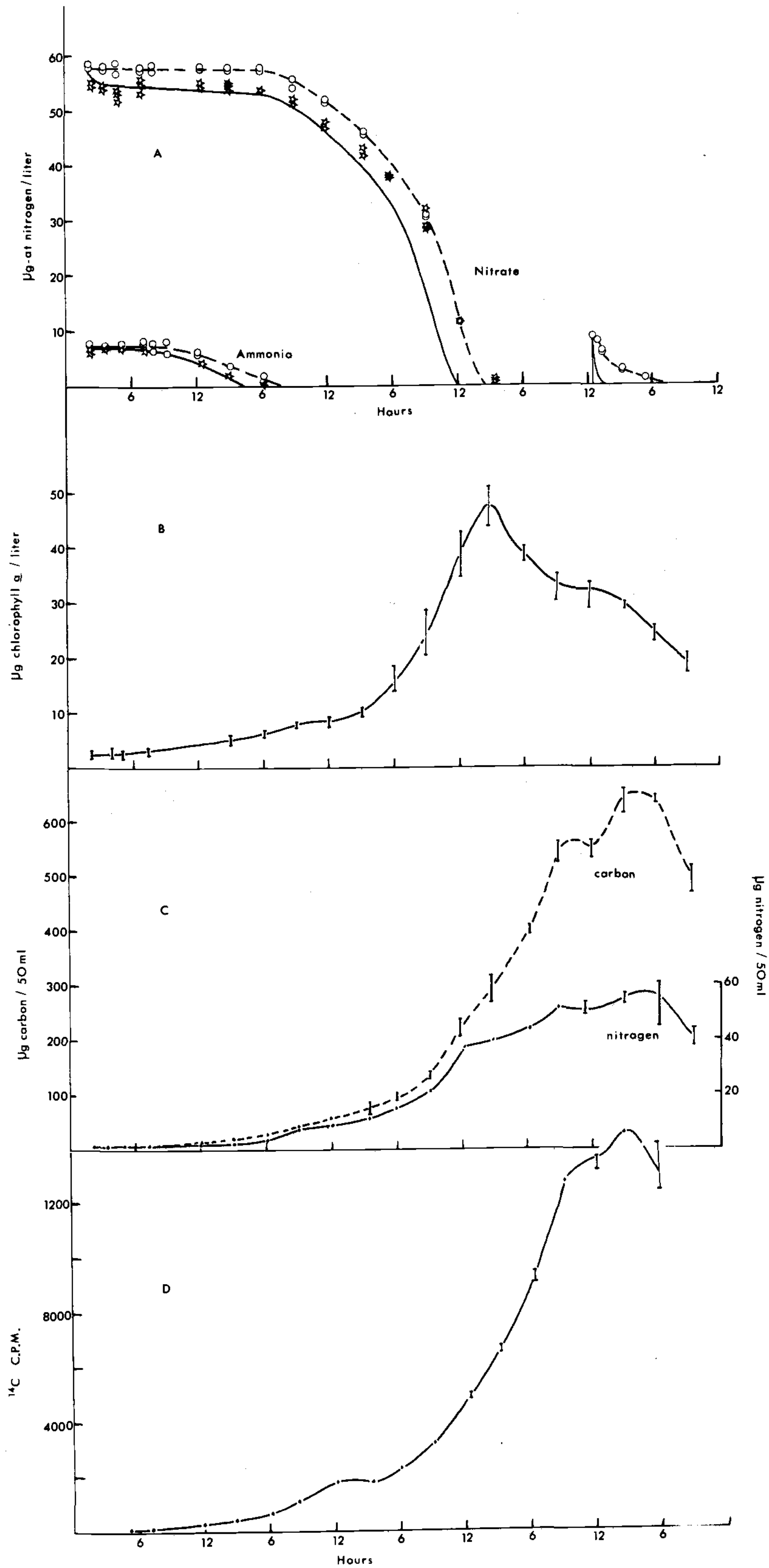

A
µg-at nitrogen / liter
14
12
8
6
4
2
Nitrate
Ammonia
B
µg chlorophyll a / liter
30
25
20
15
10
C
µg carbon / 50 ml
150
125
100
75
50
25
carbon
nitrogen
µg nitrogen / 50 ml
20
15
10
5
D
¹⁴C C.P.M.
2000
1500
1000
500
6
12
6
12
6
Hours

Figure 8. Raw data from experiment III vs. time in hours.

A. nutrient concentrations (μg-at/liter)
B. chlorophyll a (μg/liter)
C. carbon and nitrogen (μg/50 ml)
D. carbon-14 activity (CPM)



μg-at nitrogen / liter
A
Nitrate
Ammonia
Hours
μg chlorophyll a / liter
B
μg carbon / 50 ml
C
carbon
nitrogen
μg nitrogen / 50 ml
^{14}C C.P.M.
D
Hours

was used in the calculations, uptake rates and nitrogen specific nutrient assimilation rates were calculated at each CHN sample time, according to the formula:

$$\frac{\text{(change in nutrient)-}\mu\text{g}}{\text{(amount of particulate N present)-}\mu\text{g (time)-hours}},$$

which reduces to hour^{-1}.

Determination of population specific growth rate* was done by applying a least square fit to the particulate nitrogen data using a natural log transform of the exponential function ab^x. Because growth rates were not constant over the duration of any of the experiments, the average rate of three sequential data points were used. These calculations were made for all successive combinations of sequential data points over the period of particulate nitrogen increase in each experiment.

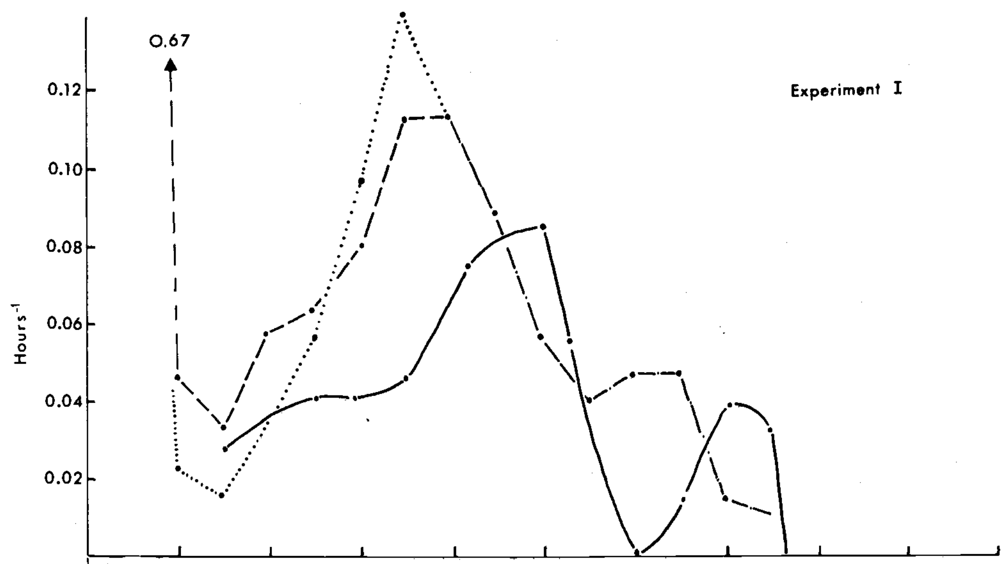
Comparison of assimilation rates and uptake rates with particulate nitrogen specific growth rates reveals no obvious relationship (Figure 9). The most obvious characteristic of the specific uptake rates is that they are transient and highly variable.

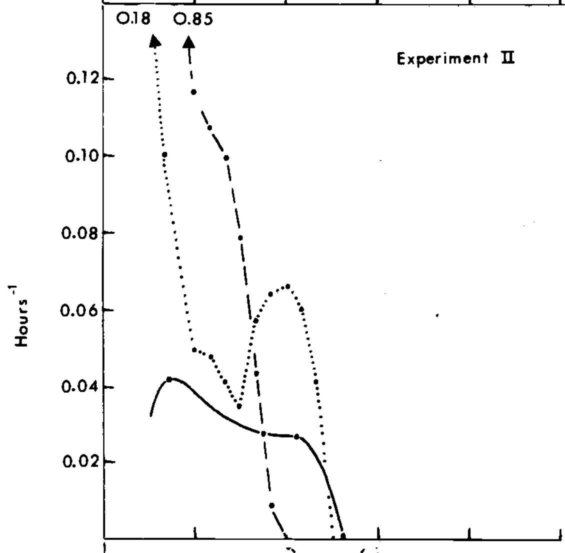
Nitrogen to carbon ratios (N/C) were calculated as an atom-to-atom ratio of the particulate carbon and nitrogen data. Chlorophyll a to carbon ratios were computed as µg

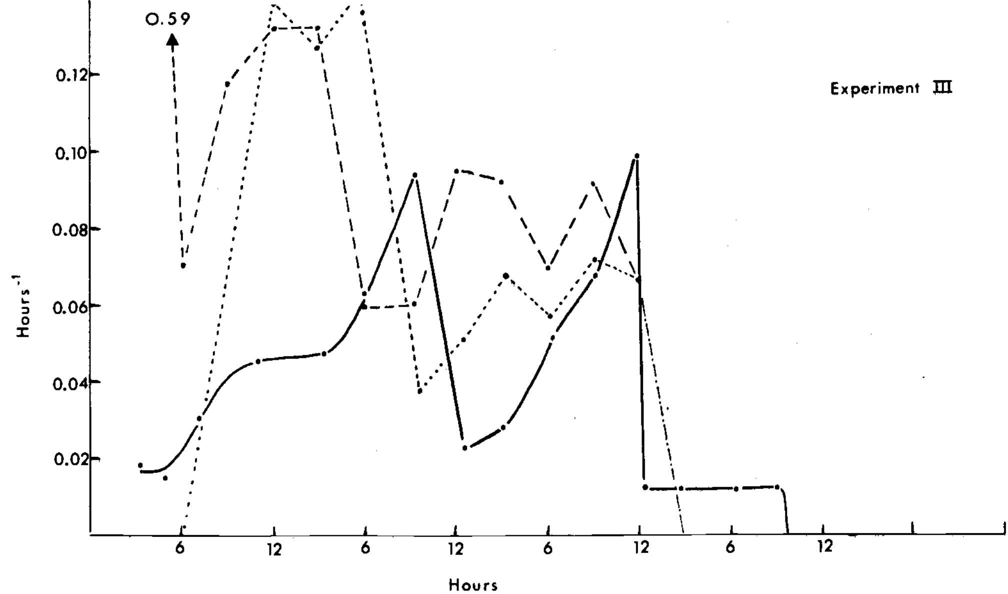
---

* The terms particulate nitrogen specific growth rate and population specific growth rate are used interchangeably.

Figure 9. Particulate nitrogen specific growth rates (solid lines), specific nutrient uptake rates (dashed lines), and specific nutrient assimilation rates (dotted lines) vs. time in hours. All rates are expressed as $hours^{-1}$.


0.67
Experiment I
0.12
0.10
0.08
0.06
0.04
0.02
Hours⁻¹


0.18
0.85
Experiment II
0.12
0.10
0.08
0.06
0.04
0.02
Hours⁻¹

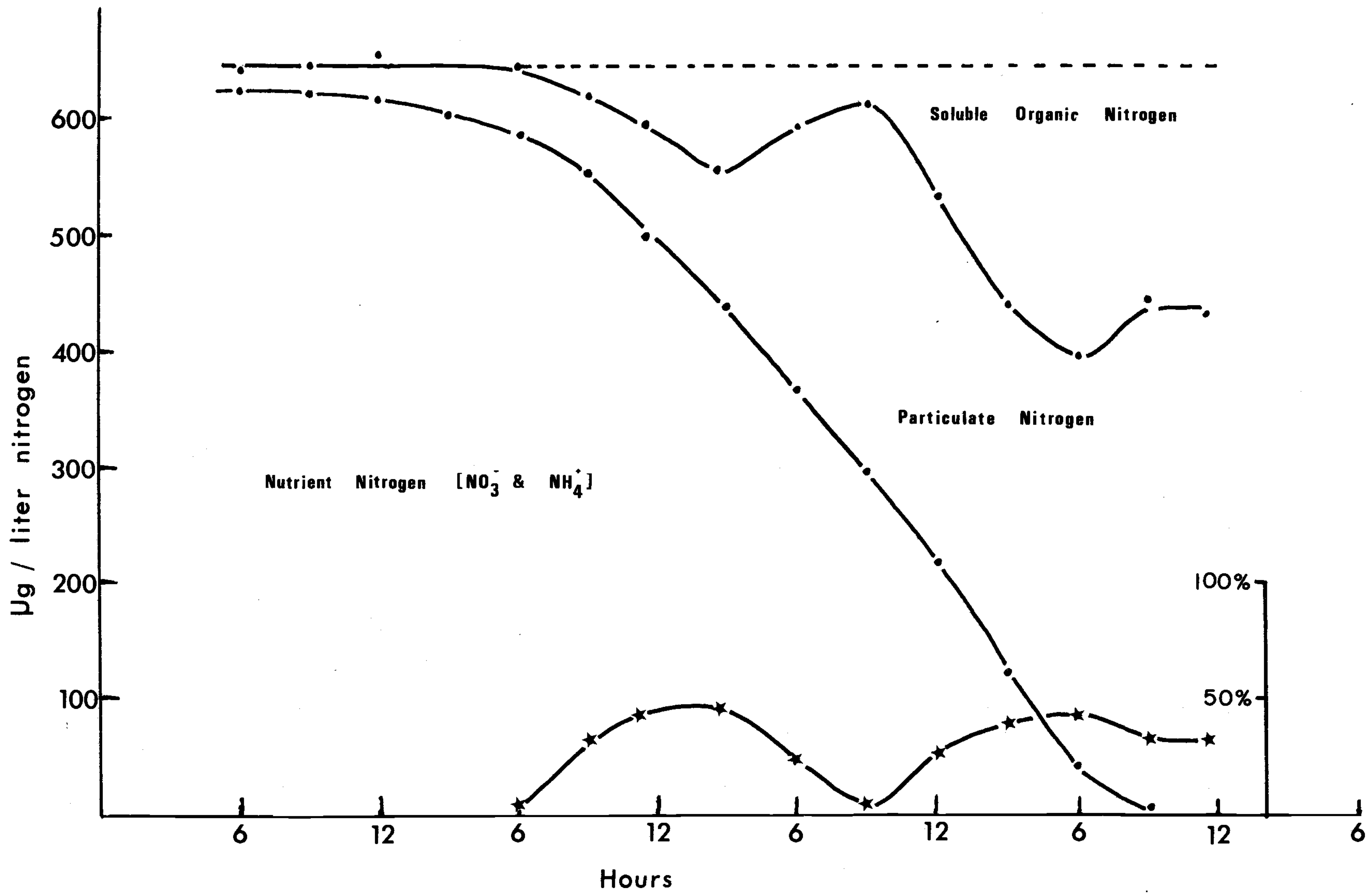

0.59
Experiment III
0.12
0.10
0.08
0.06
0.04
0.02
Hours⁻¹
6
12
6
12
6
12
6
12
Hours

chlorophyll <u>a</u> / μg-at. of carbon from the particulate carbon data. The photosynthetic assimilation ratios (mg carbon / mg chl <u>a</u>-hr.) were calculated from ^{14}C data, and the chlorophyll data. Since the cultures were at light saturation, this data conforms to the definition of Curl and Small (1965). A ratio of mg of nutrient nitrogen in the cellular reservoir to the mg of particulate nitrogen present was also calculated. Since this ratio places the cellular inorganic nutrient reservoir in perspective with the amount of particulate nitrogen it will be termed <u>relative reservoir size</u>.

When the nutrient concentrations in the CHN filtrate samples are converted to μg of nitrogen and added to the μg of particulate nitrogen present, they should total 100% of the initial nitrogen added as nutrients. However, if organic nitrogen was excreted, or if the filtration process caused loss of soluble organic nitrogen from the cells, then the total of filtrate and particulate nitrogen would be less than 100% yield.

I attempted to account for all of the nitrogen in the system through the duration of experiment I (Figure 10). The portion labeled soluble organic nitrogen is not measured, but derived by difference. Since this is a closed system batch culture, loss of nitrogen from the system is expected to be negligible, and estimation of total yield by this method should be reasonable. This nitrogen is labeled soluble organic nitrogen because it was not retained on the glass fiber filter,

Figure 10. Nitrogen budget for experiment I. Percent soluble organic nitrogen (stars and solid line) is shown with separate axis.


Soluble Organic Nitrogen
Particulate Nitrogen
Nutrient Nitrogen [NO_3^- & NH_4^+]
μg / liter nitrogen
600
500
400
300
200
100
100%
50%
6
12
6
12
6
12
6
12
6
Hours

and it is nitrogen of a form different from nutrients; undoubtedly organic. The percent soluble organic nitrogen, determined by this difference method, ranged from zero to 50% (Figure 10).

Because of their similarity in initial conditions, the results of experiments I and III are presented together. The results of experiment II are presented separately, because of the unique initial conditions, and results.

In both experiments (I and III), the populations very rapidly accumulated a substantial internal pool of nitrogen nutrients and maintained that relative portion until the population growth rate began to increase rapidly (Figures 11 and 12). In experiment I, the cellular reservoir is composed entirely of ammonia, and the ratio of mg / l of cellular nutrient reservoir to mg / l of particulate nitrogen (relative reservoir size) is maintained initially at 0.5. The reservoir in experiment III is composed largely of nitrate, with some ammonia, and the relative reservoir size ratio is maintained initially at approximately 1.0. It is not clear why a nitrate reservoir was not found in experiment I, since substantial nitrate reservoirs were measured in experiments II and III.

Trends in population growth rates are followed rather well by trends in photosynthetic assimilation ratios, and soluble organic nitrogen (Figures 13 and 14). On close examination, it can be seen that trends in soluble organic nitrogen directly precede the

Figure 11. Comparison of relative reservoir size to particulate nitrogen specific growth rates from experiment I (time sequence).

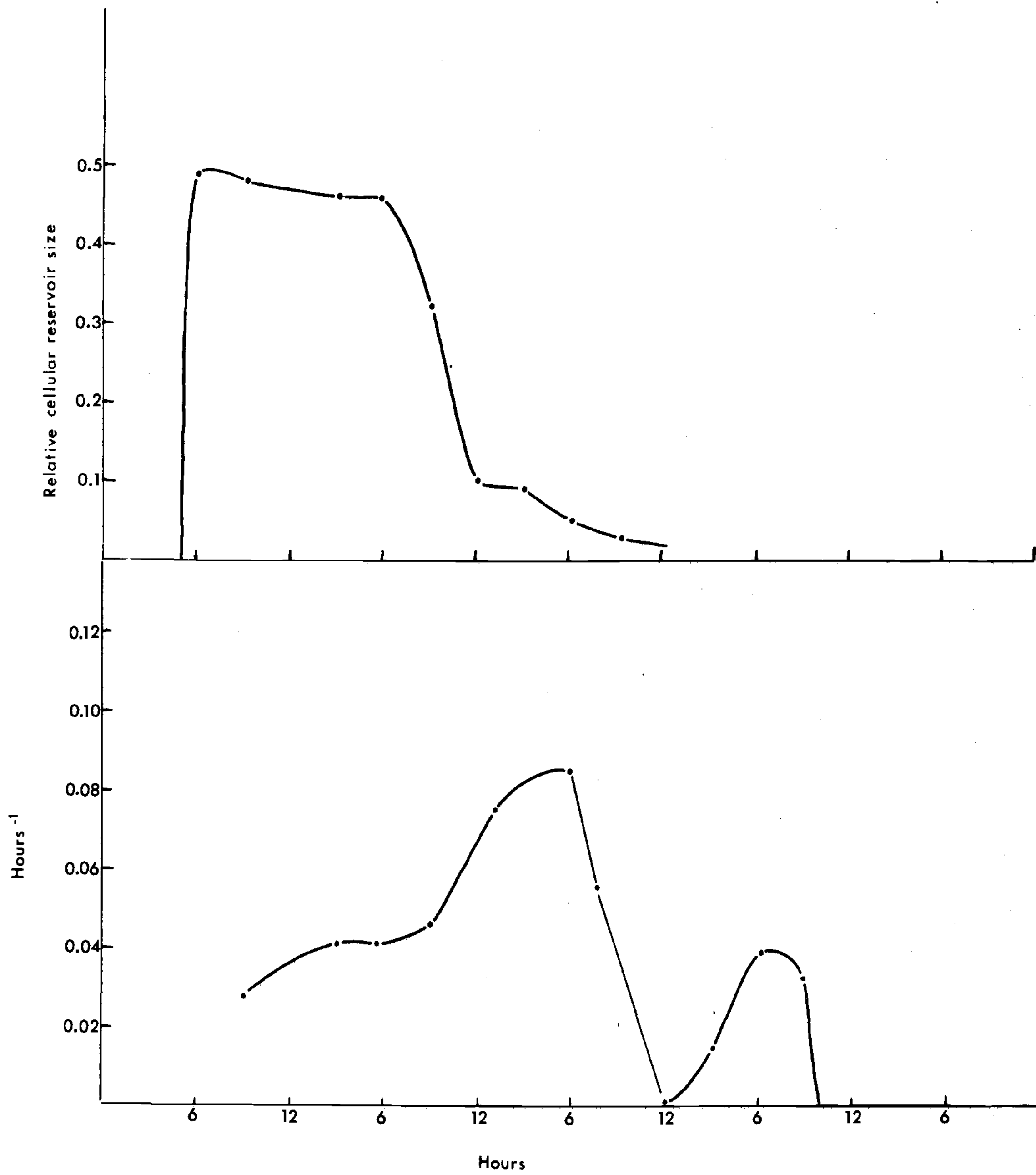

Relative cellular reservoir size
0.5
0.4
0.3
0.2
0.1
Hours -1
0.12
0.10
0.08
0.06
0.04
0.02
6
12
6
12
6
12
6
12
6
Hours

Figure 12. Comparison of relative reservoir size to particulate nitrogen specific growth rates from experiment III (time sequence).

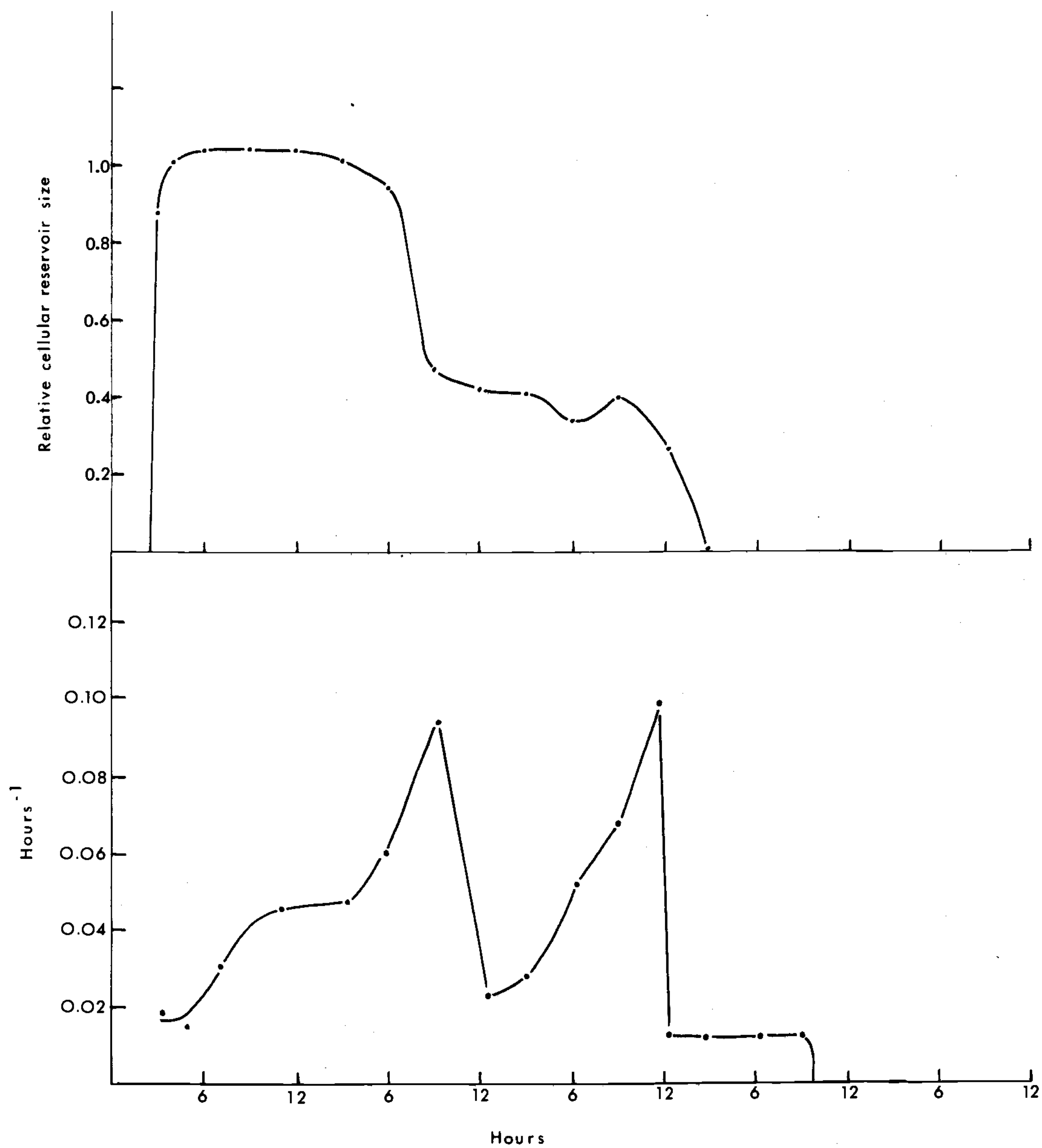

Relative cellular reservoir size
1.0
0.8
0.6
0.4
0.2
0.12
0.10
0.08
0.06
0.04
0.02
Hours -1
6
12
6
12
6
12
6
12
6
12
Hours

Figure 13. Comparison of photosynthetic assimilation ratios and percent soluble organic nitrogen with particulate nitrogen specific growth rates in experiment I (time sequence).

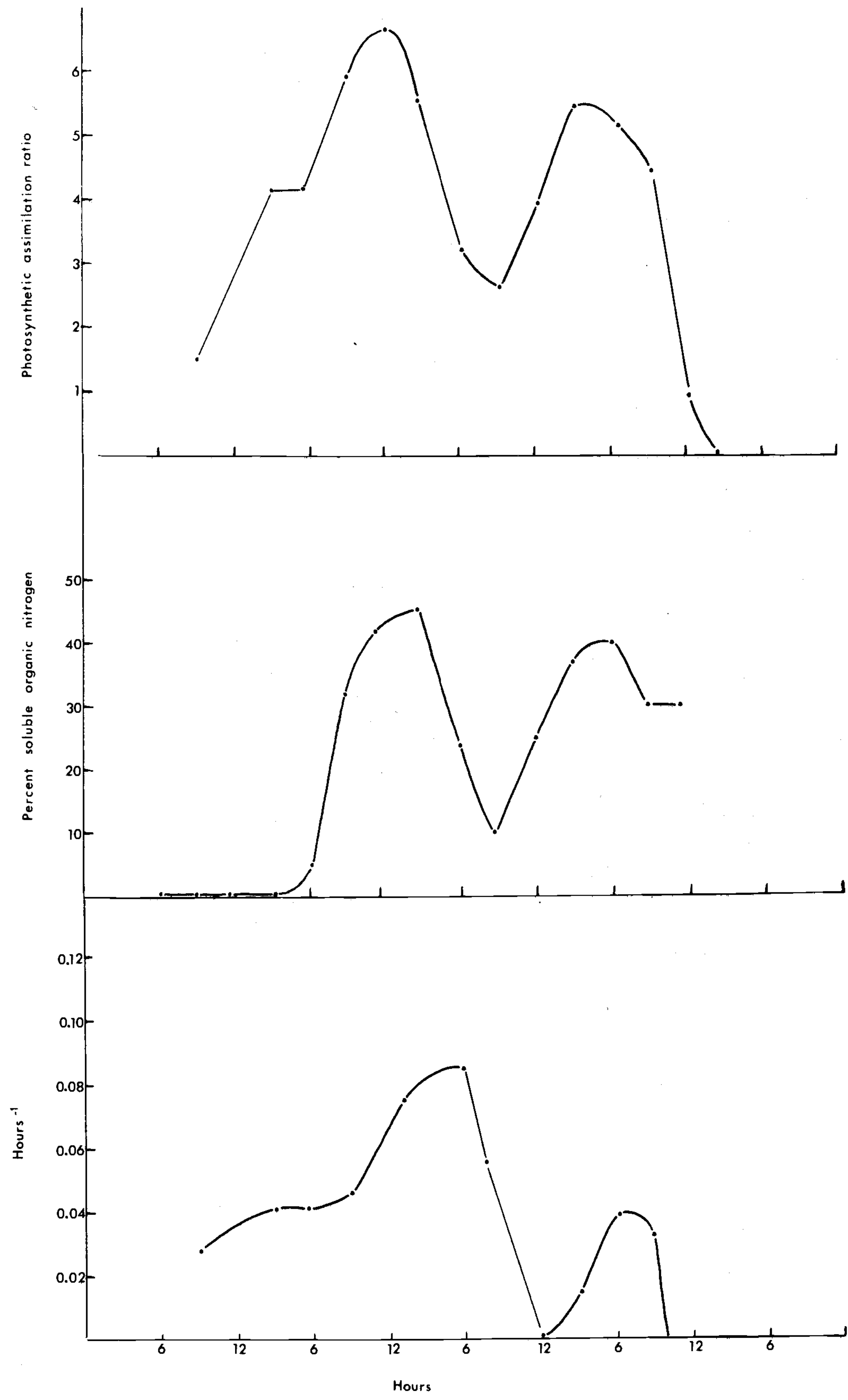
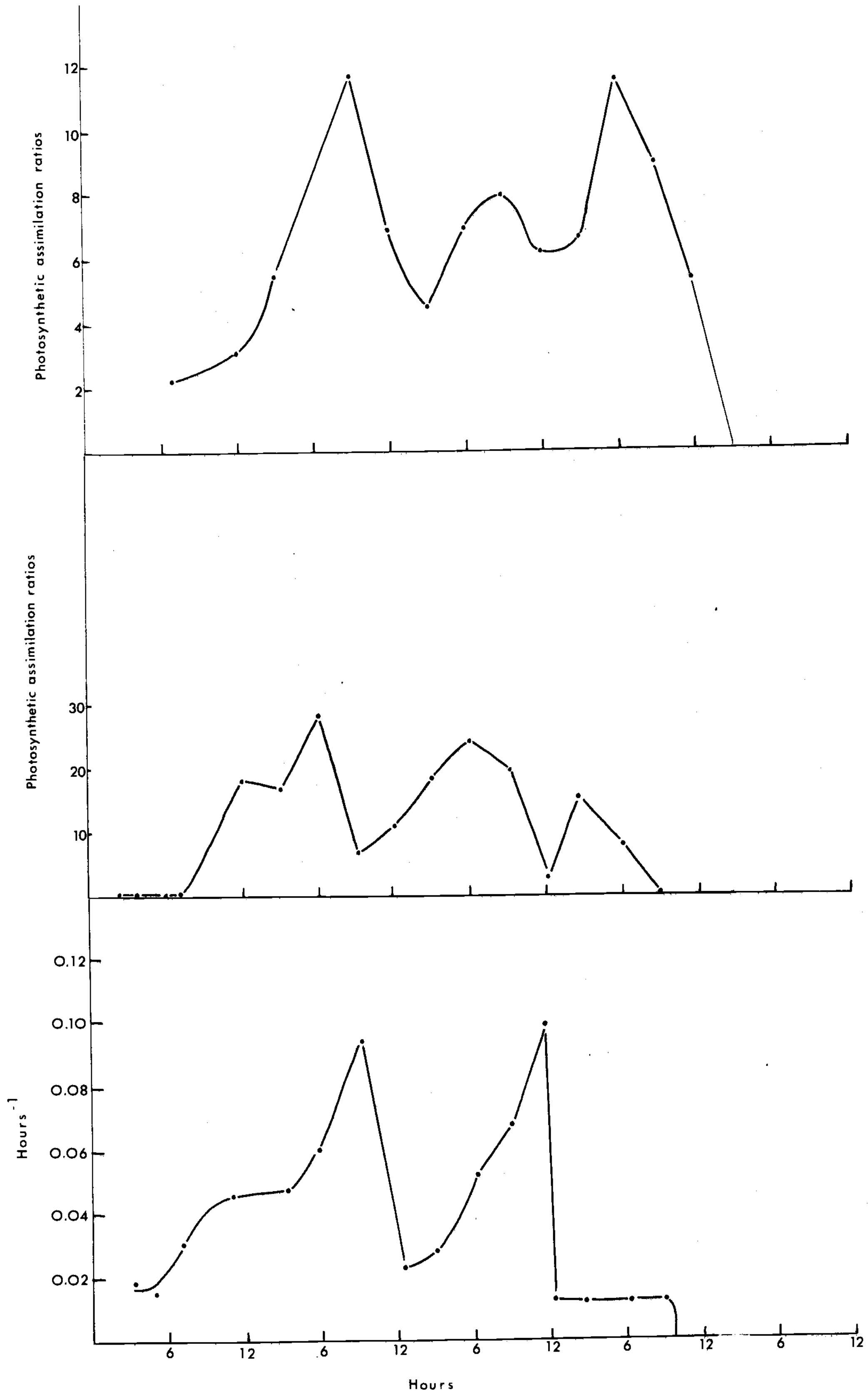

Photosynthetic assimilation ratio
6
5
4
3
2
1
Percent soluble organic nitrogen
50
40
30
20
10
Hours -1
0.12
0.10
0.08
0.06
0.04
0.02
6
12
6
12
6
12
6
12
6
Hours

Figure 14. Comparison of photosynthetic assimilation ratios and percent soluble organic nitrogen with particulate nitrogen specific growth rates in experiment III (time sequence).



Photosynthetic assimilation ratios
12
10
8
6
4
2
Photosynthetic assimilation ratios
30
20
10
Hours -1
0.12
0.10
0.08
0.06
0.04
0.02
6
12
6
12
6
12
6
12
6
12
Hours

corresponding trends in population growth rates by approximately six hours. Photosynthetic assimilation ratios show excellent correlation to growth rates, with the exception of the end of experiment III. Here, the continued uptake of ^{14}C, and decreases in chlorophyll a produce an upward surge in the ratio, while the population growth rate remains constant.

Although N/C ratios do not follow the same up and down trends of population growth rate, they do respond to changes in growth rate (Figures 15 and 16). In both experiments I and III the population growth rate stabilizes at slightly more than 0.04 hr^{-1}, followed by a rapid increase in growth rate in both experiments. N/C ratios are rather constant, or increasing slowly during the first part of both experiments, but show marked increases during the rapid increases in population growth rate. N/C ratios can be seen to level out during the drops in growth rate, and then show increases again as the growth rate increases. When growth completely ceases, the N/C ratios drop rapidly to lower levels, and stabilize.

The chlorophyll a to carbon ratios appear to be responsive to changes in growth rate, although there appears to be some difference between the two experiments.

Figure 17 shows the time sequence plots of the various "physiological state parameters," compared to growth rates, in experiment II. The percent soluble organic nitrogen was not

Figure 15. Comparison of N/C ratios and chlorophyll a/ carbon ratios with particulate nitrogen specific growth rates in experiment I (time sequence).

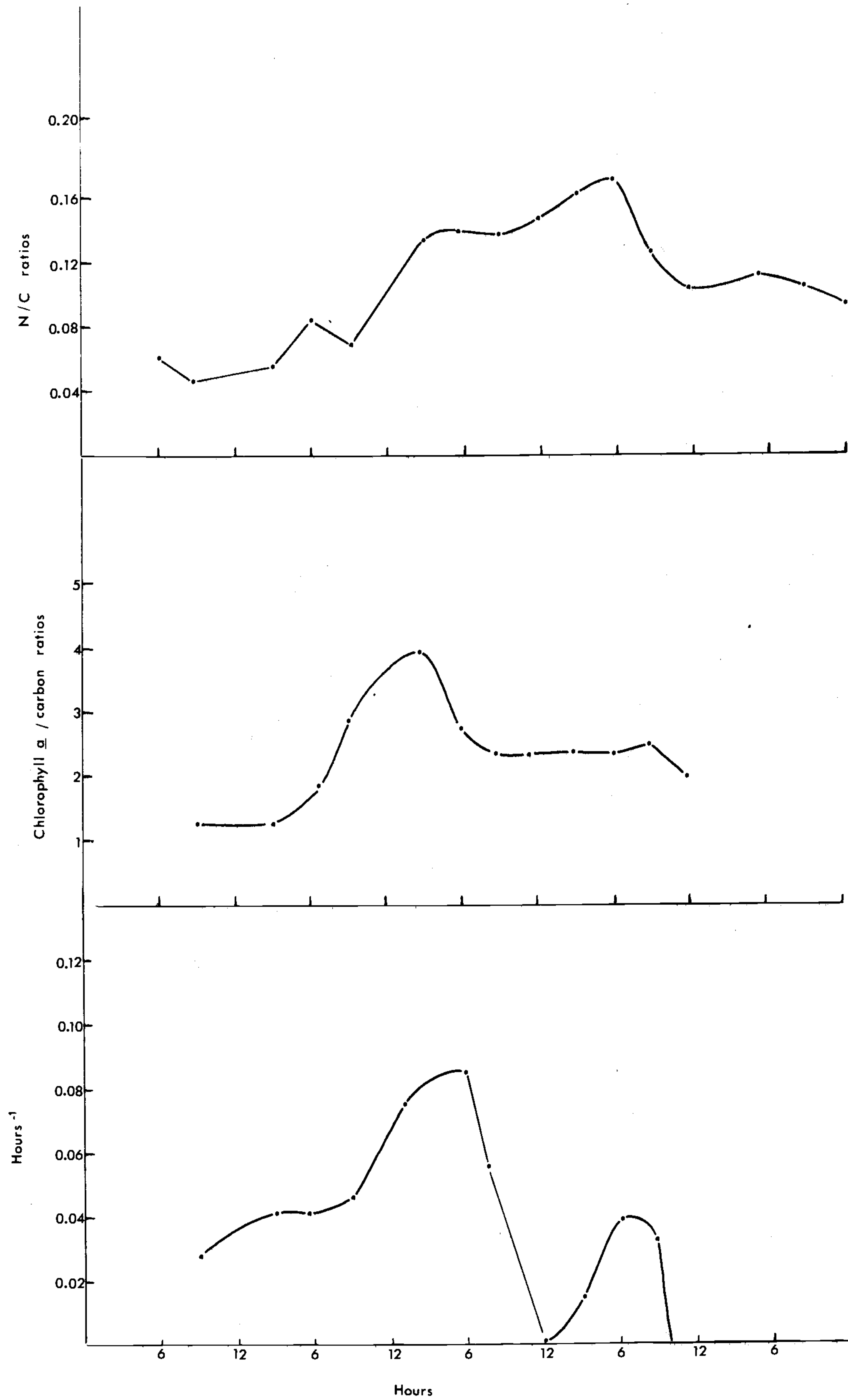
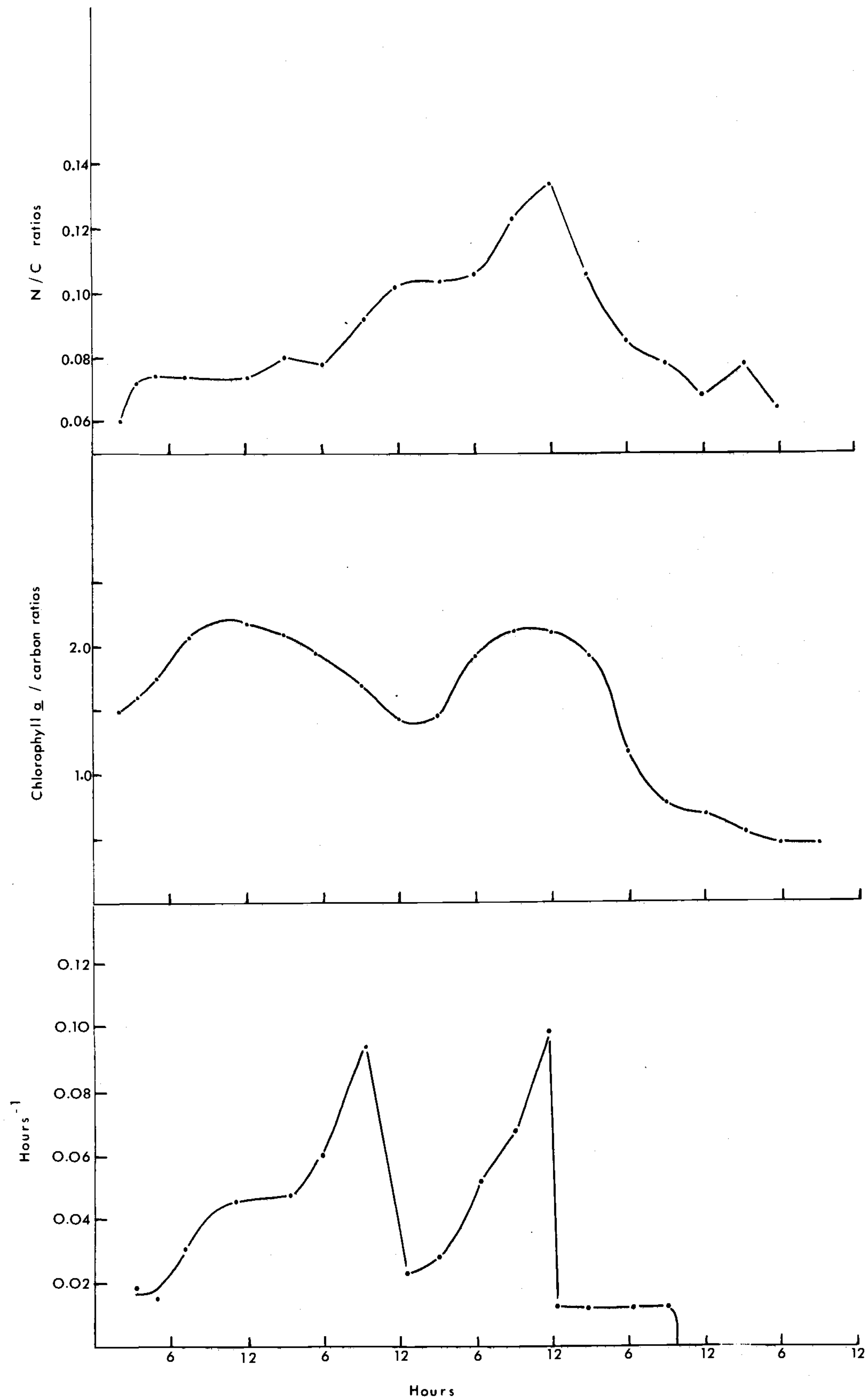
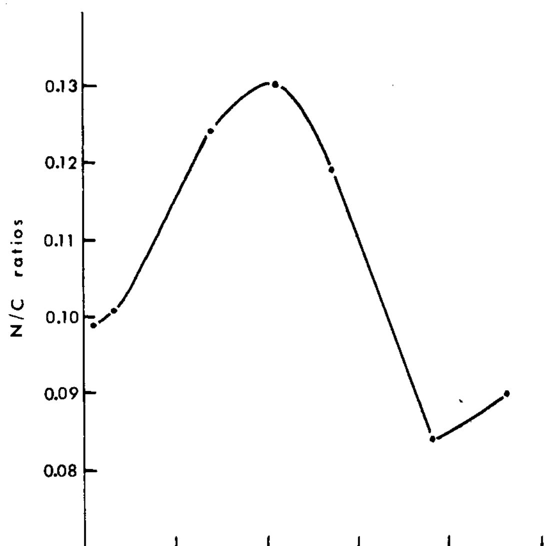

0.20
0.16
0.12
0.08
0.04
N/C ratios
5
4
3
2
1
Chlorophyll a / carbon ratios
0.12
0.10
0.08
0.06
0.04
0.02
Hours -1
6
12
6
12
6
12
6
12
6
Hours

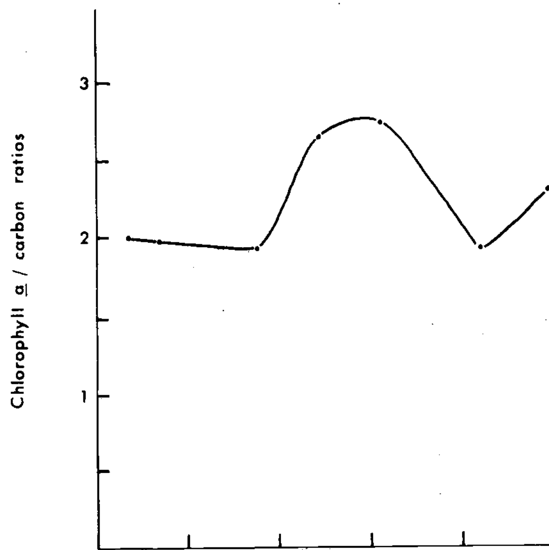
Figure 16. Comparison of N/C ratios and chlorophyll a/carbon ratios with particulate nitrogen specific growth rates in experiment III (time sequence).

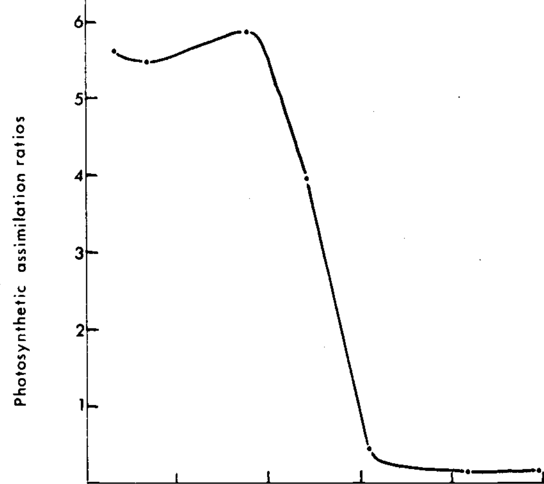


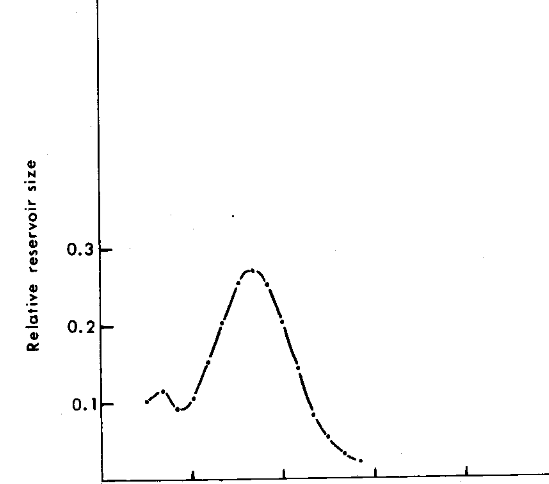
N / C ratios
0.14
0.12
0.10
0.08
0.06
Chlorophyll a / carbon ratios
2.0
1.0
Hours -1
0.12
0.10
0.08
0.06
0.04
0.02
6
12
6
12
6
12
6
12
6
12
Hours

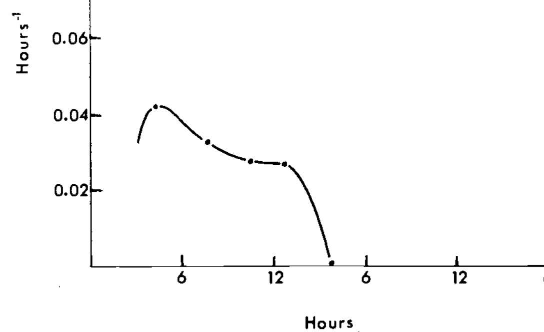
Figure 17. Comparison of N/C ratios, photosynthetic assimilation ratios, chlorophyll a / carbon ratios, and relative reservoir size with particulate nitrogen specific growth rates in experiment II (time sequence).

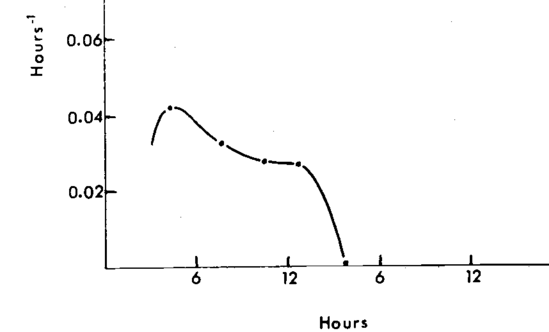

0.13
0.12
0.11
0.10
0.09
0.08
N/C ratios


3
2
1
Chlorophyll a / carbon ratios


6
5
4
3
2
1
Photosynthetic assimilation ratios


0.3
0.2
0.1
Relative reservoir size


Hours-1
0.06
0.04
0.02
6
12
6
12
6
Hours


Hours-1
0.06
0.04
0.02
6
12
6
12
6
Hours

calculated for this experiment. The growth rate in this experiment was fairly constant during nitrogen assimilation. Although the growth rate did not change appreciably over the short duration of the experiment, increases in N/C ratios and chlorophyll a / carbon ratios indicate the population was "primed" to undergo an increased growth rate. In this experiment, however, nutrients were depleted before growth rate increases could occur.

DISCUSSION

Reservoir Measuring Methodology

Because the phytoplankton in the samples to be ultrasonicated were not instantly killed, it is likely that some nitrogen assimilation took place in the samples between the time of sampling, and freezing in dry ice (which was about 10-20 minutes). For this reason, measured nutrient reservoirs measured should be considered a minimum, especially when they were measured during rapid nitrogen assimilation. This may be a partial explanation of the lack of measurable nitrate reservoir in experiment I. This problem could be solved by immediately ultrasonicating samples, and then freezing them.

The procedure used in this work should be readily adaptable to measurement of nutrient reservoirs in phytoplankton in the field. Bottle or bucket samples of phytoplankton could be treated essentially

the same as in these experiments, by removing cell-free sea water very slowly through 10μ $Nitex_R$ pouches, and removing whole samples to be ultrasonicated.

Nutrient Uptake

Droop (1968) observed very rapid uptake of vitamin B_{12}, when it was added to deficient cells. This uptake, which he called adsorbtion, was much faster than steady state uptake. Eppley and Thomas (1969) observed that uptake of nitrate by deficient phytoplankton was 20 times faster than the highest growth rate measured.

Reservoir filling occurs when nutrient becomes available to living phytoplankton with depleted internal reservoirs. This rapid uptake decreases markedly when the reservoir is "filled" (Figure 4A). In experiment III (Figure 4B) there was insufficient nutrient to fill the reservoir of the rather large biomass present, and the rapid uptake proceeded until the nutrient was completely depleted from the medium. It was found that both of these uptake rates were slightly greater than 20 times the maximum growth rate measured in the three experiments.

The rate of disappearance of nutrients in the ultrasonicated samples can be considered the rate of assimilation of these nutrients. As can be seen in Figures 4 and 9, the rate of nutrient uptake into the cell can be much different than the rate of nutrient assimilation.

However, when the cellular reservoir size is measurable zero, or when the relative cellular reservoir size remains constant, then the rate of nutrient assimilation can be considered equal to the rate of nutrient uptake.

From this information, it is possible to label three distinct functions that govern measured uptake rates:

Reservoir Filling Uptake: Occurs when internal nutrient reservoirs are not full, and assimilation of nutrients is negligible.

Assimilatory Uptake: Occurs when the cellular nutrient reservoir is measurable zero, or not changing in size over the time period involved.

Combination Uptake: This uptake rate is a function of both reservoir filling and assimilation of nutrients. In the strictest sense, uptake rates that are a function of both reservoir filling and rate of assimilation are probably most common.

The values of nutrient concentrations in the CHN filtrate samples are represented by ☆'s in Figure 3. In most instances, these values correspond closely to the concentrations in the culture medium, represented by solid lines. However, when a substantial nutrient reservoir is present, the values lie between the culture medium concentration and the ultrasonicated sample concentrations. This indicates at least some of the internal nutrient reservoir is lost due to the filtration process. Although it was mentioned in the

methods section, I will again state that precautions were taken to use very little vacuum during filtration, to insure minimum loss by this method. It is apparent however, that even gentle filtration onto glass fiber filters can cause significant loss of internal nutrient reservoirs.

The data from these experiments confirm the observations of Eppley and Thomas (1969) that specific uptake rates are highly variable and are not correlated with measurable growth rates in any direct manner, and that specific uptake rates of nutrients by deficient phytoplankton can be 20 times the highest measured growth rate. At least a partial explanation of the high variability in measured specific uptake rates is that there appears to be two distinct processes that govern uptake. Reservoir filling uptake which is extremely rapid, can be distinguished from assimilatory uptake, which occurs by definition, when the nutrient reservoir is either measurable zero, or constant, over the time period measuring uptake. When these two processes are working simultaneously, a highly variable combination uptake rate occurs.

Slow input of nutrients into the euphotic zone, by advection across the pycnocline, or regeneration by zooplankton excretion, may not lead to measurable nutrient in the water. This is because of the ability of phytoplankton to remove nitrogen nutrients from the water at very rapid rates, even at low concentrations (Figure 5). This ability of phytoplankton to rapidly remove nutrient input from

the water may explain reported lack of measurable nutrients in marine waters even when blooms are underway (Bruce, 1961).

Caperon and Meyer (1973b) reported good correlation between measured uptake rates in two hour uptake experiments with phytoplankton populations taken directly from chemostats at various equilibrium growth rates. Their success in these experiments can be related to several factors. The phytoplankton populations used had consistent prehistory treatment, because they were taken directly from steady state growth in a chemostat. Thus, all of the physiological parameters were adjusted to growth at the particular dilution rate of the chemostat. The uptake experiments lasted only two hours. This length of time and the presence of high nutrient concentrations, allowed for reservoir filling and establishment of a stable reservoir. The reservoir filling event can stabilize in under 20 minutes, with the remaining nutrient uptake essentially a function of the chemostat determined prehistory assimilation rate (Grenney, Bella, and Curl, 1973a and b) of the population. The two hour time was also short enough that inducement of increases in growth rate due to the high nutrient concentration would be insignificant.

In natural phytoplankton populations or in batch cultures of phytoplankton, the prehistory, physiological state, and fullness of nutrient pools are transient and usually unknown. Since all of these factors have an effect on nutrient uptake, the resulting measured

specific uptake rates can vary greatly from measured specific growth rate.

Unique Aspects of Experiment III

Although experiment III was quite similar in many ways to experiment I, at least part of experiment III is different from both experiments I and II. The particulate nitrogen in experiments I and II did not increase after nitrate was depleted from the culture medium. In experiment III, however, particulate nitrogen increased considerably after the initial nitrate was depleted from the culture medium. Also, in experiment III, the yield of particulate nitrogen was greater than the amount of nitrogen added as nutrients, while in experiments I and II the yield of particulate nitrogen was not more than 70 percent of the total nitrogen added as nutrients.

Because of these observations it must be concluded that a significant amount of nitrogen, other than ammonia, nitrate, or nitrite, was present in the sea water, used as culture medium. This nitrogen was probably organic in nature, and it was not utilized preferentially over nitrate or ammonia.

Because of the method of calculating percent soluble organic nitrogen, the values in experiment III were not affected by the unknown nitrogen <u>during</u> ammonia and nitrate assimilation. While the unknown nitrogen was being assimilated (after nitrate was depleted),

the calculated values of percent soluble organic nitrogen become less meaningful.

This problem can be solved in the future by using sea water of known low nitrogen content, or by doing experiments with artificial sea water.

Measurement of Nitrite

Significant levels of nitrite were found only in ultrasonicated samples, and these concentrations were always quite low. As might be expected (Lui and Roels, 1972; and Roeloffs, 1971), nitrite levels became measurable only during nitrate assimilation, since no nitrite was added to the culture medium. During assimilation, nitrate is reduced to ammonia, with nitrite as an intermediate product of the enzyme catylized process.

Because of the success in measuring intracellular concentrations of nitrite during nitrate assimilation, using ultrasonication of the cells, it seems that this procedure might be a useful indicator of nitrate assimilation. This procedure, which is decidely easier than measuring nitrate reductase activity (Roeloffs, 1971), could be used at sea to estimate the magnitude of nitrate assimilation, as opposed to assimilation of regenerated forms of nitrogen (ammonia, amino

acids, urea, etc.).

Correlation of Particulate Nitrogen Specific Growth Rate to Measured Parameters

Although measured specific uptake rates and specific assimilation rates were at times similar to population specific growth rates, they were found to be highly variable, and in general did not show any positive relationship to trends in population growth rates.

In experiments I and III (Figures 11 and 12), the calculated relative cellular reservoir sizes were maintained at high levels until the population growth rate began to increase rapidly, at which time the reservoir sizes decreased rapidly to lower levels. It appears then, that although the relative cellular reservoir size is not directly related to changes in growth rates, maintainance of a large cellular reservoir may have been important in stimulating or preparing for the initial large increases in population growth rate in both experiments.

The parameters; photosynthetic assimilation ratio, and percent soluble organic nitrogen appear to be excellently correlated to population growth rate. Both parameters show fluctuations that relate to trends in the population growth rates rather well. Although they do not undergo the same fluctuations as population growth rates, N/C ratios and chlorophyll / carbon ratios also appear to be related to

population growth rate.

Further Discussion of Measured Physiological Parameters

The rather excellent correlation between photosynthetic assimilation ratios and nitrogen specific growth rate is understandably because of the similarity of the two parameters. The nitrogen specific growth rate is calculated as a specific growth rate. Photosynthetic assimilation ratios are calculated as a change in carbon uptake per unit time, divided by the amount of chlorophyll *a* present. Because chlorophyll *a* can be considered a biomass indicator, the calculated ratio is actually a quasi specific growth, undergoing the same fluctuations as the nitrogen specific growth rate.

It also seems reasonable that the percent soluble organic nitrogen, "measured" as a loss due to glass fiber filtration, should undergo fluctuations similar to those of the population growth rates. Since amino acids and peptides are direct precursors to cellular protein, the levels of these soluble nitrogen molecules should be closely related to fluctuations in the nitrogen specific growth rate of the population. It is also highly likely that at least some of the soluble organic nitrogen fraction is composed of soluble nucleic acids, especially t-RNA and m-RNA. Levels of soluble RNA should also be indicative of changes in population growth rate (Herbert, 1961).

Caperon and Meyer (1972a) found excellent correlation between steady state growth rates and chlorophyll *a* / carbon ratios. In a less quantitative sense, Williams (1971) showed that chlorophyll *a* increased linearly in his chemostat vessel with increasing steady state growth rates. In batch culture, chlorophyll *a* to carbon ratios appear to be responding to changes in population growth rate also.

Evidence that chlorophyll *a* synthesis is tied closely with nitrogen assimilation, can be seen in both experiments I and III. As can be seen in these experiments (Figures 6 and 8), an interesting event occurs after ammonia is depleted from the culture medium. In both experiments, and especially noticeable in experiment I, chlorophyll *a* momentarily stops its exponential increase, and then resumes its increase in an exponential fashion. The plots of particulate carbon and nitrogen, and ^{14}C also show lags during this period (Figures 6 and 8). This event, which notably, is not detected in experiment II, is believed to be the result of transfer from dependence on nitrate and ammonia for assimilation, to total dependence on nitrate. The change-over produces momentary decreases in chlorophyll *a*, and affects ^{14}C uptake, and particulate nitrogen, and carbon increases. The data from these experiments indicate that ammonia may induce chlorophyll *a* synthesis, while nitrate apparently is not directly capable of induction of the chlorophyll *a* synthesis pathway.

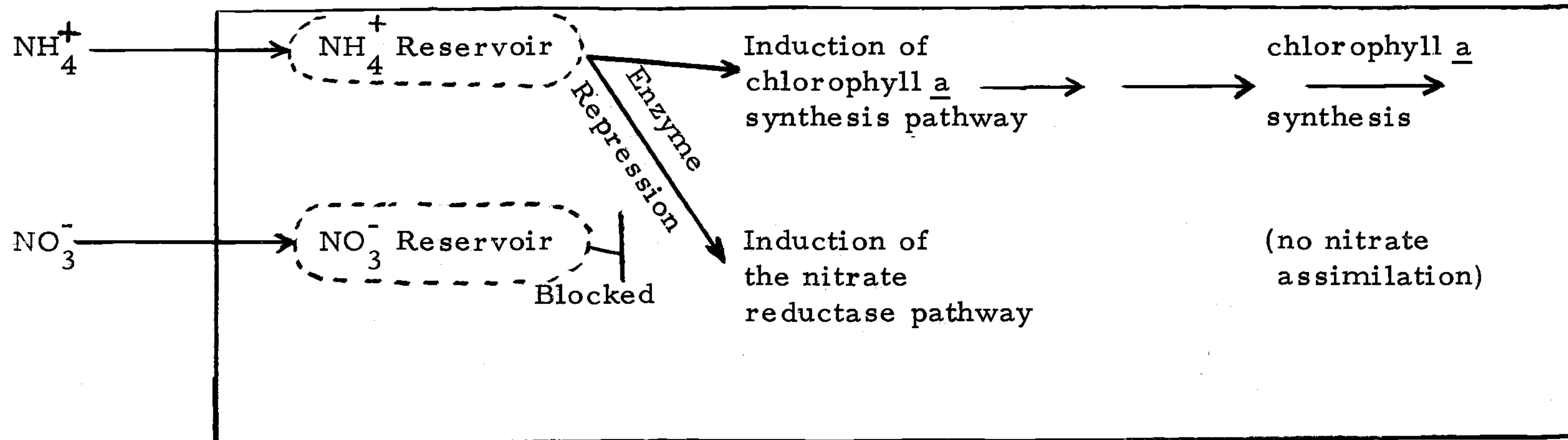
A schematic representative of the possible relationship between chlorophyll _a_ synthesis and the assimilation of either ammonia (case A) or nitrate (case B) is shown in Figure 18. During the direct assimilation of ammonia, chlorophyll synthesis is induced by the presence of an ammonia reservoir, and nitrate assimilation is blocked by repression of the enzyme pathway nitrate reductase (Roeloffs, 1971). During the change-over to assimilation of nitrate, a lag in nitrogen assimilation occurs while the induction of the nitrate reductase enzyme system takes place. Because ammonia, the end product of the nitrate reductase pathway, momentarily lags in production, induction of chlorophyll _a_ synthesis is also momentarily reduced. The terminology and general principles of enzyme control mechanisms were taken from Lehninger (1970).

This type of response to a change in nutrient substrate is termed diauxic growth, and was first demonstrated in the bacterium _Bacillis subtilis_ (in Thimann, 1963). When the bacterium is grown in equal concentrations of fructose and arabinose, it assimilates fructose first. After fructose is depleted, the cells go into a short term stationary phase, as the enzymes capable of assimilating arabinose are induced.

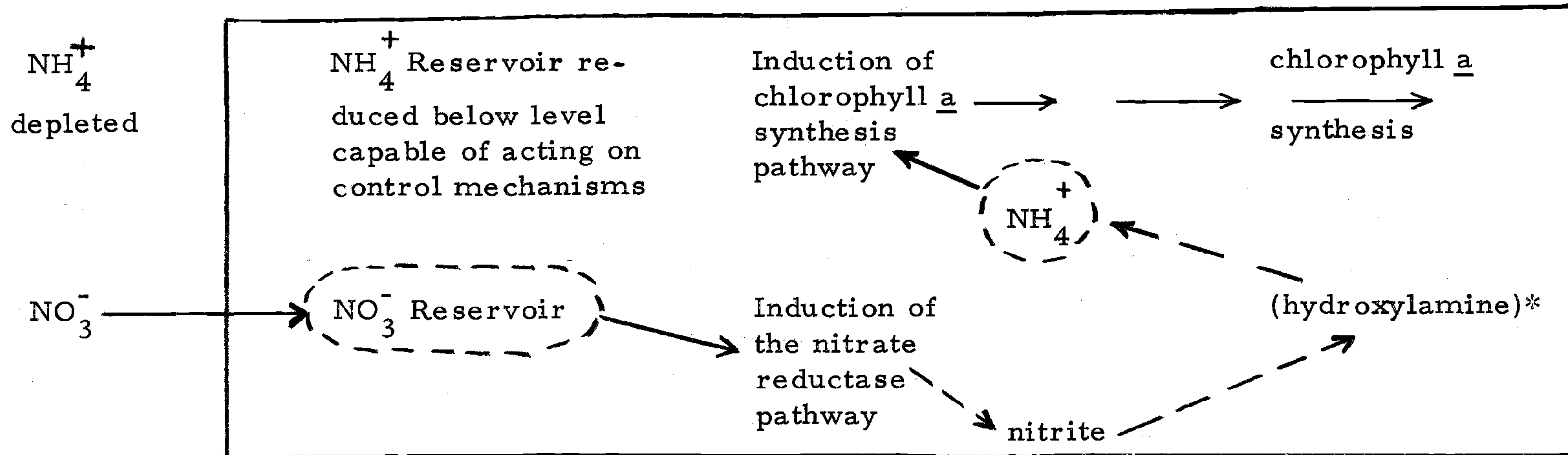
Chlorophyll _a_ can be considered as an indicator of the labile, inducible _synthetic machinery_ (term taken from Williams, 1971) of a phytoplankton population. It's concentration shows almost

Figure 18. Schematic showing the induction of the chlorophyll *a* synthesis pathway in two different cases.

Case A



Case B



*Not found thus far.

instantaneous response to changes in the nitrogen assimilation of the population. Chlorophyll a to carbon ratios should and do respond in a similar manner with population growth rate.

Caperon and Meyer (1972a and b) suggest that changes in the N/C ratio of phytoplankton grown at different steady state growth rates, are due to changes in the nutrient reservoir sizes of the population. Evidence from these experiments tends to indicate other factors must be as much, or more, important in determining changes in N/C ratios. N/C ratios measured in all three experiments did not undergo similar fluctuations with measured nutrient reservoir sizes. It was also noted that when large reservoirs were present, much of the reservoir was lost during glass fiber filtration. It was also shown that N/C ratios showed none of the downward fluctuations in population growth rate that occurred in experiments I and III, except when nitrogen assimilation finally ceased. For these reasons, it appears that the N/C ratio is indicative of the overall nitrogen sufficiency of the population, not merely the level of internal nitrogen nutrients. Changes in N/C ratios may be due to changes in cellular structural protein, nucleic acid levels, enzyme levels, nutrient reservoir levels, protein intermediates (amino acids, peptides), and so on.

Because many factors seem to contribute to changes in N/C ratios, they respond sluggishly. Rapid fluctuations in the population

growth rate (measured as the particulate nitrogen specific growth rate) have little effect on N/C ratios. This sluggish response makes N/C ratios a good estimator of the prehistory of phytoplankton populations.

Although it is apparent that the various measured physiological parameters are well correlated with population growth rates, it remains for their relationship to nutrients to be discussed.

Discussion of Models

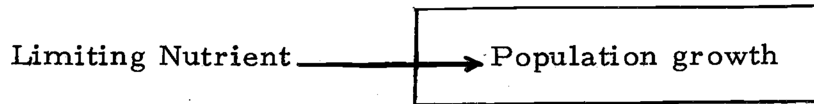
Figure 19 shows schematic representation of the four basic models discussed in the Introduction. The simplest model was that presented in the early papers by Dugdale (1968) and Caperon (1967). Population growth was assumed to be controlled by the Michaelis-Menten kinetics of nutrient uptake. Postulated reasons for the poor relationship between population growth rate, and uptake rates have included the presence of nutrient reservoirs in phytoplankton.

In Caperon and Meyer's two papers (1972a and b), they include the nutrient reservoir concept in their mathematical model. They claim that N/C ratios and chlorophyll a / carbon ratios are indicative of the nutrient reservoir size of the population at different steady state growth rates. In addition, external nutrient concentrations are predicted to increase, with increasing steady state growth rates, with the rates between compartments controlled by Michaelis-Menten

Figure 19. Schematic representation of the four basic models of phytoplankton growth dynamics.

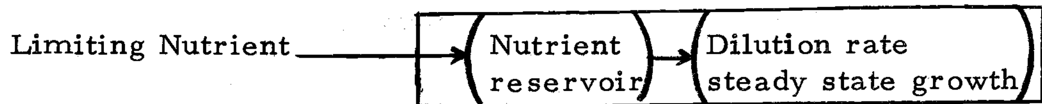
FOUR BASIC MODELS

Caperon's (1968) and Dugdale's (1968) original papers:



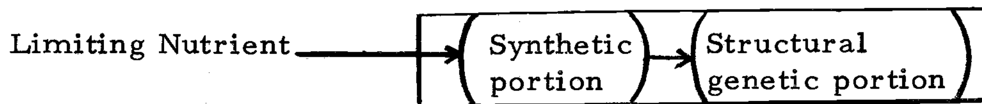
Michaelis-Menten kinetics

Caperon and Meyer (1972a and b):



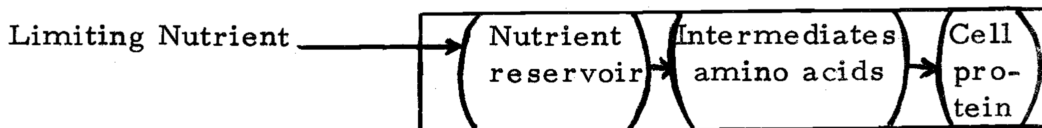
Michaelis-Menten kinetics

Williams (1971):



Bimolecular rate control

Grenney, Bella, Curl (1973a and b):



Michaelis-Menten kinetics

kinetics. The nutrient data of Caperon and Meyer refute the model requirement for external nutrient concentration to increase proportionally with increasing steady state growth rate. Their actual measurements of external nutrient concentrations appear to be quite low in general, and show no significant trend with growth rates. Also, data from our work indicates that N/C ratios and chlorophyll a / carbon ratios should be considered separately from the relative nutrient reservoir size.

Williams (1971) model incorporates a reservoir compartment that is labeled as the synthetic portion of the population. Lumped into this compartment are enzyme levels and the nutrient reservoir. Chlorophyll levels are said to be an indication of the size of the synthetic portion. This model also predicts increasing external nutrient concentrations with increasing steady state growth rates, because of the rate relationships assumed between compartments in the model. Again, actual measurements of nutrient concentrations showed no trend with increasing dilution rates. As has been sufficiently demonstrated, chlorophyll levels can be related to population growth rates, because its synthesis appears to be closely regulated by internal ammonia concentration. However, the data from the experiments presented here indicates there is no reason to lump the nutrient reservoir with the "synthetic machinery."

The three compartment model presented by Grenney, Bella

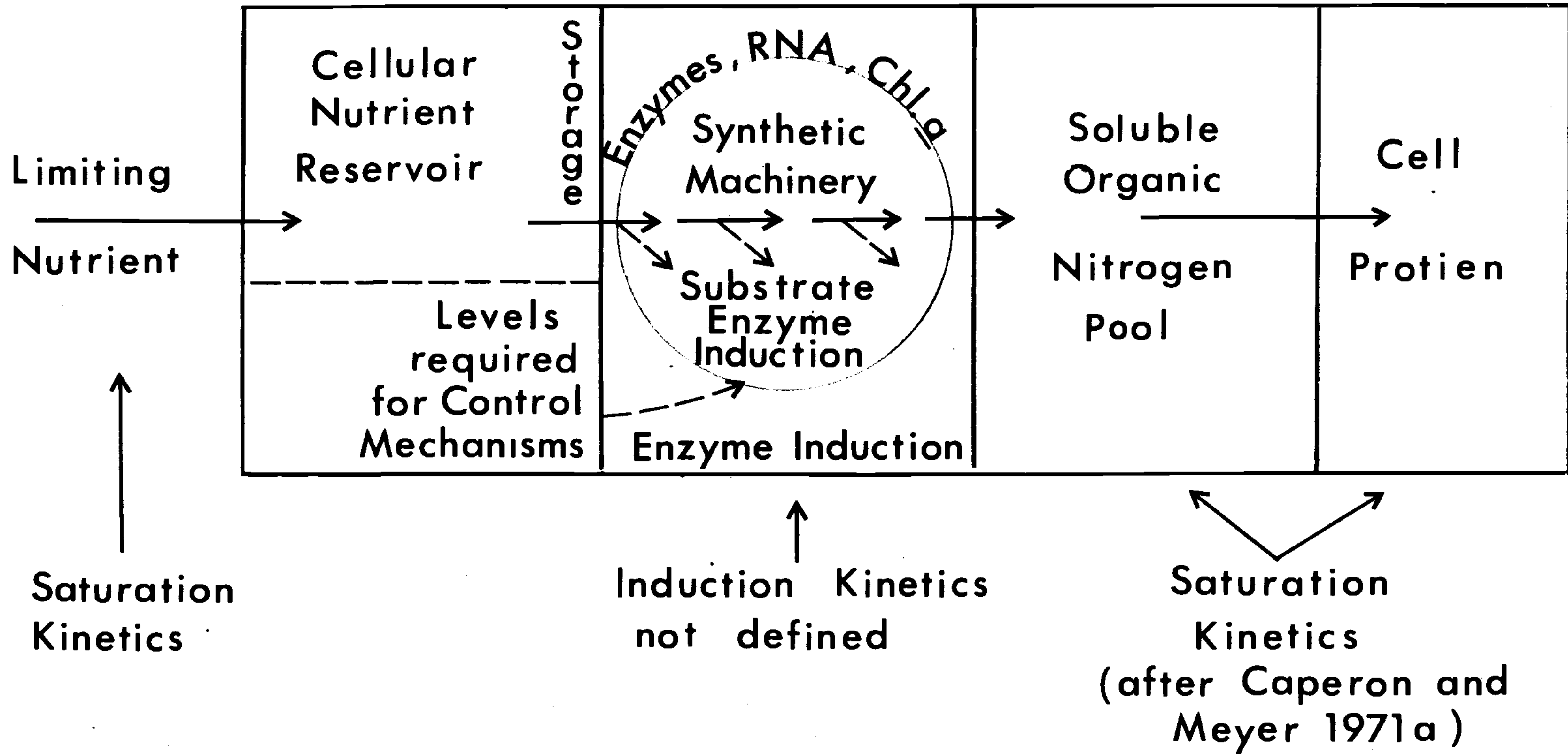
and Curl (1973a and b) assumes that rates between all compartments in the model are controlled by Michaelis-Menten kinetics. The middle compartment, which is called intermediate molecules is analogous to the percent soluble organic nitrogen parameter measured in the experiments presented here. It has been shown that this parameter is exceptionally well correlated with trends in growth rates. However, the nutrient reservoir data from the present experiments indicates that the rates between the first two compartments are not described by simple Michaelis-Menten kinetics. Fluctuations in the relative reservoir data appear to produce no predictable reaction in the percent soluble organic nitrogen measurements.

Because none of the presented models appear to adequately describe phytoplankton growth dynamics, in light of the data presented in these and other experiments, an attempt will be made to present a model consistent with available data, and also biologically feasible.

The model in Figure 20 is not presented as a definitive mathematical model, but rather is a suggested conceptual model. Although mechanisms controlling rate processes are suggested, they should be considered loosely. The one aspect of this model that makes it entirely unique is the introduction of rate control via enzyme induction and repression, initiated by intracellular nutrient concentrations. The induction process, which is not mathematically defined here, has the affect of essentially decoupling nutrient uptake from the

Figure 20. A new conceptual model of phytoplankton growth dynamics.

A Conceptual Model



population growth rates. The enzyme induction process initiated by nutrient reservoirs, is not restricted only to closely interrelated nitrogen metabolism, but must also indirectly affect the entire synthetic machinery of the population related to growth and replication.

Some evidence for a model of the type presented is:

1) Measured uptake rates historically have shown a lack of relationship with phytoplankton growth rates (Eppley and Coatsworth, 1968). The data presented here confirms this.

2) In general, the measured relative reservoir sizes showed a lack of any correlation to measured growth rates. However, an initial large nutrient reservoir was maintained before large increases in growth rates occurred. This initial reservoir may be important in enzyme induction.

3) Chlorophyll *a* / carbon and N/C ratios both respond in a positive manner with growth rate changes. The enzyme pathway responsible for chlorophyll synthesis seems to be inducible by intracellular ammonia concentrations. Both chlorophyll *a* / carbon and N/C ratios can be considered as indicative of the relative levels of synthetic machinery in the population.

4) Finally, it seems highly unlikely that micro-organisms associated with dynamic fluctuations of nutrients in their environment would constantly maintain maximum levels of synthetic machinery, since enzyme systems are energetically costly to maintain. Rather,

these organisms have undoubtedly developed sophisticated control mechanisms involving the induction and repression of enzyme synthesis, and the inhibition of enzyme activity, by various nutrients and assimilatory by-products.

There are two factors that cannot be over-emphasized, when considering the conceptual model presented here. Nutrient cellular reservoirs appear to serve two functions within the phytoplankton cell. The ability to pool substantial amounts of nitrogen nutrients functions as a means of storing nutrients, and of concentrating nutrients for later use. The cellular reservoir also may be important in the induction of assimilatory enzyme systems. The levels of cellular nutrient reservoir required for enzyme induction may be much smaller than the total storage capacity of the cell, however. Evidence for this can be seen in Figures 10 and 11, which shows the relative cellular reservoir decreasing in size sharply when the particulate nitrogen specific growth rate begins rapid increase. The decrease in the relative cellular reservoir size is possibly due to the reduction in the size of the nutrient reservoir that can be maintained at the higher growth rates. However, this reduction in relative cellular reservoir size does not produce a resultant decrease in the growth rate, indicating the levels required for induction of the synthetic machinery of the cell are still being maintained.

The model in no way is intended to imply a stoichiometric

relationship between the rate of removal of nutrients from the cellular reservoir (due to the first step in assimilation), and the rate of formation of the soluble organic nitrogen pool. It is much too simplistic to assume that nutrient assimilation is directly coupled to the rate of formation of the cellular amino acid pool. The soluble organic nitrogen pool is probably made up largely of amino acids and peptides, which are synthesized via a wide variety of enzyme catalyzed reactions. In addition, many of the enzyme systems involved must be induced by their specific substrates. For this reason, the appearance of increases in soluble organic nitrogen (Figures 13 and 14) should be more closely coupled to increased levels of the overall cellular synthetic machinery (enzymes and soluble RNA) than to variations in the size of the cellular nutrient reservoir.

The model suggests that the rates of formation of the soluble organic nitrogen pool, and the rate of formation of cellular protein may be described by saturation kinetics. A possible form of the relationship is the adaptation of the Michaelis-Menten equation used by Caperon and Meyer (1971a and b). However, it should be realized that the Michaelis-Menten equation has no significance in terms of actual mechanisms involved, it is merely a convenient equation that is widely recognized, and which may be useful in approximating rate relationships.

A model is of little practical value, if its parameters are not measurable. All of the parameters suggested in the conceptual model are either directly measurable, or estimatable with current methodology. Nutrient concentrations in the environment, and nutrient reservoirs can be measured in the laboratory and at sea by the methods described in this work. Currently the synthetic machinery levels are estimated by chlorophyll a / carbon ratios, and N/C ratios. It is conceivable that other measurable parameters may be found which are more closely related to nitrogen assimilation. Measurement of the levels of soluble RNA may be a good estimator of the synthetic machinery of the cell. The soluble organic nitrogen pool may be measured directly in glass fiber filtrate samples, or estimated by difference (as in these experiments), when a nitrogen budget can be performed. It is also possible that this fraction could be measured in ultrasonicated samples, as levels of primary amino groups.

Cellular protein is measured as particulate nitrogen by CHN analysis, a well-known procedure. From particulate nitrogen increases, the population specific growth rates can be calculated. Population cell volume increases can also be used to calculate specific growth rates, although there at times may be some uncoupling between volume change and particulate nitrogen changes. Photosynthetic assimilation ratios are a quasi specific growth rate

measurement. For this reason, this parameter may be used to estimate trends in specific growth rates calculated from particulate nitrogen data.

Although it is well established that nutrient uptake is described by saturation kinetics (either Michaelis-Menten, or Langmier adsorbtion isotherm), other rate kinetics must be tested. There is some evidence from chemostat studies by Caperon and Meyer (1972a) that the function

$$\mu = \frac{\mu m \cdot (q - q_o)}{K \quad (q - q_o)}$$

may adequately describe rates between enzyme levels and intermediate molecules, and between intermediate molecules and cell protein. Although no mathematical relationship is offered to describe enzyme induction processes, the function must include a time factor, and a factor involving the concentration of inducing nutrient.

The work presented here is definitely limited because of the lack of mathematical treatment involved. Work is currently underway to compare model responses to the data in these experiments, by computer simulation. Response of the three compartment model of Grenney, Bella, and Curl to these data will be compared to the response of the model presented in this manuscript. Since measured parameters are available to estimate all compartments in both models, the resulting comparison should be definitive.

It appears obvious that further research is warranted using improvements of the techniques described here. Laboratory research with other species of marine phytoplankton is necessary, to determine the extent of variation between species. At-sea measurements of the parameters described here, should be done as soon as possible.

SUMMARY

1) A method has been developed for measurement of nutrient reservoirs in laboratory cultures of marine phytoplankton. Applicability of the method to at sea measurements is discussed. It appears there should be no problem in using this methodology at sea.

2) Significant nutrient reservoirs were measured in all three experiments. The maximum ratio of cellular nitrate reservoir to particulate nitrogen was 1.0. It is feasible that the population particulate nitrogen could completely double, utilizing only stored nitrate.

3) Variations in nutrient uptake rates were discussed, and three uptake functions identified: reservoir filling uptake, assimilatory uptake, and combined uptake. It is felt that a combined uptake function probably occurs most often. Previous observation that maximum uptake rates (reservoir filling uptake) can be at least 20 times the maximum growth rate observed, was confirmed by the data.

4) Nitrite was observed in ultrasonicated samples during

nitrate assimilation in all experiments. The usefulness of this measurement was discussed, in terms of identifying the importance of nitrate assimilation in natural populations.

5) Specific uptake rates and relative reservoir size plots were compared on a time sequence with calculated particulate nitrogen specific growth rates. Both of these parameters show lack of relationship to specific growth rates. It was noted, however, that large relative reservoir sizes were maintained prior to the initial rapid increases in particulate nitrogen specific growth rates.

6) From the raw data, four parameters, called in general, "physiological state parameters," were calculated. They were: photosynthetic assimilation ratios, percent soluble organic nitrogen, chlorophyll a / carbon ratios, and N/C ratios. These parameters were compared to particulate nitrogen specific growth rates. It was found that both photosynthetic assimilation ratios and percent soluble organic nitrogen showed excellent correlation to measured specific growth rates. It was suggested that the percent soluble organic nitrogen trends directly preceded growth rate trends by about six hours. Although their responses were different, it was concluded that both N/C ratios and chlorophyll a / carbon ratios were related to changes in particulate nitrogen specific growth rates.

7) The nature of each physiological state parameter was discussed, and reasons presented for the type of response that each

showed. Because the photosynthetic assimilation ratio is a quasi specific growth rate measurement, it should be expected to undergo similar fluctuations with specific growth rates. Likewise, since the percent soluble organic nitrogen is undoubtedly composed largely of cellular protein precursors (amino acids and short peptides), it is also expected that this parameter should be closely related to changes in particulate nitrogen specific growth rates. It is also reasonable that trends in this parameter directly precede trends in specific growth rates, because of precursor nature of the parameter.

Previous experimenters have shown a positive relationship between chlorophyll a levels, and population specific growth rates. The chlorophyll a / carbon data from the experiments presented here seem to substantiate the relationship. Evidence is presented for the induction of the chlorophyll a synthesis pathway by internal ammonia concentrations. This process explains the lags in measured parameters in experiments I and III, during the change-over from ammonia assimilation to nitrate assimilation. Because of its sensitivity to changes in nitrogen assimilations, chlorophyll a is considered indicative of the labile, inducible synthetic machinery of the phytoplankton population.

Evidence is presented that refutes the assumption by Caperon and Meyer that changes in N/C ratios are due primarily to changes in nutrient reservoir sizes. It is more likely that N/C ratios are

affected by changes in a number of cellular parameters. The fact that many parameters appear to contribute to changes in N/C ratio explains their rather sluggish response. This response, however, makes N/C ratios a good indicator of the prehistory of the population.

8) In an attempt to relate the particulate nitrogen specific growth rates to nutrient data, the four basic models presented are discussed, in turn. None of the presently existing models appear to be compatible with experimental data, past and present.

9) A new conceptual model is presented that appears to be biologically sound, and compatible with observations. The most important aspect of the model, in terms of control functions, is the concept of enzyme induction by internal nutrient concentrations. The process of enzyme induction has the effect of essentially uncoupling nutrient uptake from phytoplankton growth rates. Some evidence supporting the enzyme induction portion of the model is given.

10) The estimation of each parameter in the model is discussed, and it appears that available methodology can adequately estimate the model parameters. Current attempts at a mathematical treatment, by computer simulation, are discussed.

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