# EFFECTS OF LIGHT AND DARK CYCLES ON THE RELATIONSHIP BETWEEN NITRATE UPTAKE AND CELL GROWTH RATES OF CHAETOCEROS SP. (STX-105) IN CONTINUOUS CULTURE.

by

GASTON A. PICARD

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The City University of New York

Abstract

EFFECTS OF LIGHT AND DARK CYCLES ON THE RELATIONSHIP BETWEEN NITRATE UPTAKE AND CELL GROWTH RATES OF *CHAETOCEROS* SP. (STX-105) IN CONTINUOUS CULTURE.

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Gaston A. Picard

Adviser: Professor Oswald A. Roels

The diatom, *Chaetoceros* sp. (STX-105) was grown in a continuous culture at five different turnover rates with nitrate as the limiting nutrient. Light was supplied for 12 continuous hours each day. The effects of the light-dark cycle on cellular particulate organic carbon (POC), particulate nitrogen (PN), chlorophyll *a*, nitrate, free amino acids, protein and carbohydrate, and also the rates of photosynthesis and NADH-dependent nitrate reductase, were studied. Uptake kinetics constants, $K_s^u$ (half saturation constant) and Vm (maximum velocity), were studied by using two techniques not previously compared.

The most stable population and cellular characteristic is particulate nitrogen whereas, the most active cellular

pools were nitrate and carbohydrate. As a general rule larger diel oscillations were observed with increasing growth rate. Protein specific nitrate reductase activity was shown to be corrolated with the intracellular nitrate. Measured intracellular nitrate was shown to be a small portion of the hyperbolically increasing nitrogen yield coefficient. Cellular protein decreased by as much as 45% as the growth rate increased, while nitrate uptake efficiencies were always higher than 96%. It is suggested that the cells growth rate is limited by the rate of nitrate supply at the lower turnover rates whereas protein synthesis is the rate limiting step in the process of nitrate assimilation for higher turnover rates. Exception of carbon appears to occur at low growth rate while, intracellular recycling may be significant at higher turnover rates.

Determination of the nitrate uptake kinetics parameter $K_s^u$ by the short-term uptake technique indicates that the constancy of $K_s^u$ with increasing growth rates (Caperon and Meyer 1972) is questionable.

The results of this study suggest that the growth rate of Chaetoceros sp. (STX-105) in continuous nitrogen-limited culture is neither limited by the rate of nitrate uptake nor by the rate of the enzyme nitrate reductase at any growth rate.

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# 1 INTRODUCTION

Inorganic nitrogen has been shown to be an important regulatory element for the growth of phytoplankton in the sea (Thomas 1969, 1970); it is also well established that the supply of nutrient-rich deep water to the euphotic zone, contributes to the primary productivity in the world oceans (Hargraves et al., 1970). Nitrate is the main source of inorganic nitrogen in deep sea water for phytoplankton; ammonia is a remote second in abundance.

Nutrient-rich, deep water is used in our laboratory for the continuous growth of phytoplankton in the operation of an artificial upwelling system at St-Croix, U. S. Virgin Islands (Othmer and Roels 1973). The resulting crop is fed to shellfish populations. The nitrogen budget of the whole mariculture system is of major importance. The present study of nitrate assimilation by a marine diatom Chaetoceros sp. (STX-105), was undertaken with the general approach of simulating, as much as possible, the existing abiotic conditions of the St-Croix system, such as temperature, medium and continuous culture system. The rationale was that the experimental artificial upwelling system for phytoplankton mass culture could aided by a better knowledge of the

nitrogen metabolism of an indivilual phytoplankter under controlled conditions in the laboratory. A primary requirement of this approach was the study of the theoretical aspects of the nitrogen flow through phytoplankton.

It is known that nitrogen limitation may reduce the nutritional value of the phytoplankton crop in batch culture (Spoehr and Milner 1948) and may affect the growth and cellular composition of unicellular algae in continuous culture (Richardson et al., 1969). By limiting the concentration of one nutrient in the medium of a continuous culture, cells can be grown at a constant degree of limitation. It is well known that nutrient uptake and growth do not occur simultaneously. Thus, at low initial nutrient concentrations, the nutrient is nearly exhausted from batch culture by the time growth rates can be determined (Lui and Roels 1972). Phytoplankton growth does not depend on current environmental nutrient concentrations, but rather upon the population's nutrient history: Caperon and Meyer (1972) have shown that preconditioning of the population affects the population size and uptake kinetics of phytoplankton, and that the past nutrient history of the cells can be controlled best in continuous culture.

The complexity of nitrogen assimilation by phytoplankton is reflected in the literature: Caperon 1968, Eppley and Coatsworth 1968, Eppley and Thomas 1969, Eppley et al. 1969a,b Eppley and Rogers 1970, Eppley et al. 1970a, 1971, Hobson and Pariser 1971, Caperon and Meyer 1972, Lui and Roels 1972,

Eppley and Renger 1974. Other important cellular processes closely related to nitrogen assimilation, have been investigated: photosynthesis (Morris 1971, Hellebust and Terborgh 1967, Paasche 1971, Griffiths 1973), cellular composition (Parsons et al., 1961, Jeffries 1968, Haug and Myklestad 1973) and the effect of light and dark cycles on growth (Eppley and Coatsworth 1966, Eppley et al., 1967, Paasche 1967). Unfortunalely, because of the use of different organisms, growth media, light regimes and methods of culture, these results cannot be compared easily.

It was the purpose of this study to further the understanding of nitrate uptake and assimilation and their relationship to growth using the marine diatom Chaetoceros sp. (STX-105). This study employed frequent measurements of nitrate assimilation, media and cellular properties of Chaetoceros sp. nitrogen-limited continuous cultures at various growth rates with a 12 hour light period each day.

## 1.1 Scientific background

### 1.1.1 Uptake kinetics

Nitrate uptake by marine phytoplankton has been shown to vary hyperbolically with external nitrate concentration (Eppley and Coatsworth 1968, Eppley et al., 1969b, MacIsaac and Dugdale 1969, Carpenter and Guillard 1970). The hyperbolic curves can be described by an equation relating the rate of removal of the nutrient from the medium (v) to its concentrations (S) and having the form:

$$v = Vm\ S\ /\ K_s^u + S \qquad \text{Eq. 1}$$

where Vm is the maximal specific uptake rate and $K_s^u$, the half saturation constant. This equation has the mathematical form of the Michaelis-Menten equation used in enzyme kinetics.

Eppley et al. (1969b) have observed that larger species, which tend to grow more slowly, have in general higher $K_s^u$ values whereas oceanic species usually have low $K_s^u$ values.

Carpenter and Guillard (1970) have reported intraspecific differences in nitrate half saturation constants for oceanic ($K_s^u$= 0.38 µg-at $NO_3^-$-N/l) and coastal ($K_s^u$= 1.0 µg-at $NO_3^-$-N/l) clones of Cyclotella nana (Thalassiosira pseudonana). However, Caperon and Meyer (1972b) did not detect any significant variations in the $K_s^u$ values of four different species, Monochrysis lutheri, Dunaliella tertiolecta, Coccochloris stagnina and Cyclotella nana, in nitrate uptake experiments. Furthermore, they showed in their continuous culture experiments that there is a linear relationship between the maximum uptake rate (Vm) and different preconditioning steady-state growth rates. Previous data on the uptake kinetics parameters Vm and $K_s^u$ had been obtained from batch culture experiments (MacIsaac and Dugdale 1969, Droop 1968, Eppley and Thomas 1969, Eppley et al., 1969b, Carpenter and Guillard 1970) where the nutrient concentrations in the growth chamber varied. In reviewing the earlier work, Caperon and Meyer (1972b) state: "It does not seem possible to obtain populations representing a variety of known preconditioned states without continuous culture techniques."

The successful determination of uptake kinetic parameters ($K_s^u$ and Vm) by the batch culture method (Eppley et al., 1969b, Carpenter and Guillard 1970) requires a linear relationship between uptake rate and time. This criterion must be fulfilled for each organism tested. Failure to obtain this relationship results in questionable values.

When cells are grown in a continuous culture at steady-state, the uptake rate, v, is proportional to the growth rate, u. The proportionality constant is the variable yield coefficient, q (Droop 1968).

$$v = q\ u$$

Caperon (1968) and Droop (1968) showed that the yield coefficient, q (the amount of limiting nutrient per unit population), is related to the steady-state growth rate by a Michaelis-Menten type hyperbola. However, Caperon (1968) and Caperon and Meyer (1972) assumed that $q_o$ represents a fixed minimal amount of organic nitrogen needed for a living cell. By extrapolation to zero growth rate, they determined a $q_o$ value which they believed to be a cellular constant independant of growth rate. The idea of an intracellular nitrate pool is well established, but it is questionable that changes in q result only from changes in intracellular nitrate, and that the minimal amount of organic nitrogen needed for a living cell is a constant for different steady-state growth rates (Grenney et al., 1973). Therefore, the relationship between cellular organic nitrogen and growth rate requires further study.

1.1.2 Growth kinetics

The growth rate (u) of marine phytoplankton can be expressed by an equation analogous to the one used in uptake kinetics

$$u = \frac{Um.S}{K_s^G + S} \qquad \text{Eq. 2}$$

The maximum growth rate is Um and the half saturation constant for growth is $K_s^G$ (Eppley and Thomas 1969). The agreement between $K_s^G$ and $K_s^u$ values is surprising (Eppley and Thomas 1969), in contrast to the finding of Droop (1968), who found a 20-fold greater $K_s^u$ for short-term uptake of vitamin $B_{12}$ by Monochrysis lutheri, than $K_s^G$ for growth, in a chemostat culture. Caperon and Meyer (1972b) also found a ten-fold higher $K_s^u$ for nitrate than $K_s^G$ in nitrate-limited chemostat cultures.

The maximum of nutrient uptake (Vm) has been shown to be much higher than the maximum specific growth rate (Um). Eppley and Thomas (1969) suggest that this might be due to an increase in the number of nitrate absorption sites in the N-depleted cells, but one of his experimental cultures (Asterionella japonica) was in the exponential phase of growth and those cells were not nitrogen-limeted. Caperon and Meyer (1972b) define Vm as a composite parameter, one of the components being the number of uptake sites per unit population. They showed that Vm increases linearly as the rate of nitrate supply increases. The number of uptake sites could be a consequence of either the rate of supply or the cellular concentration of nitrate.

Caperon (1968) observed a hyperbolic relationship between steady-state growth rate and the calculated nutrient concentration in the cell (yield coefficient), in a nitrate-limited chemostat culture of Isochrysis galbana. Droop (1968) also observed the same relationship with his vitamin $B_{12}$ study, the equation being:

$$u = Um\ (q-q_o)\ /q \qquad \text{Eq. 3}$$

The best population measure in terms of yield coefficient (q) suggested by Caperon and Meyer (1972b) is the ratio of nitrogen to carbon (N/C). Droop (1968) and Caperon and Meyer (1972) used vitamin $B_{12}$ and nitrate-limited continuous cultures, with continuous light. All possible variables were under control. Even in such conditions, the amount of date available and applicable to Eq. 3 is very limited.

Malone et al. (1975) used a nitrate-limited continuous culture of a phytoplankter (Chaetoceros sp., STX-105) in large outdoor cultures, at St-Croix, Virgin Islands. Turnover rates of 50, 100, 150 and 200% per day were used. Nutrients ($NO_3^-$, $NO_2^-$, $NH_4^+$ and $S_iO_3^{--}$) and population sizes (chlorophyll a, cell counts, particulate organic nitrogen and carbon) were monitored on a diurnal basis. The situation is more complex than previous similar work since many natural variables such as light, temperature and contamination were not controlled. However, this is probably a better approximation of the real world. One interesting feature of their finding is that the yield coefficient (N/C averaged for 24-hour periods increases over a very narrow range (0.075-

0.119) as the turnover rate was increased from 50% to 200% per day. This may imply that under those conditions $K_s^u$ could be used for calculation of $K_s^G$ (Eppley and Thomas 1969). Only at the 200% turnover rate did they observe significant nitrate in the medium; simultaneously, a small increase in nitrate per cell in the late dark and early light period was observed.

Most of the work on nitrogen uptake and growth kinetics does not examine the important assimilatory enzymes, nitrate reductase (NR), nitrite reductase (NiR) and glutamate dehydrogenase (GDH), but only measures both ends of the process; that is, rates of uptake or dissappearance of the nutrients from the medium and resulting growth rates often are not N specific for the organism. Eppley et al. (1971) studied these enzyme activities as a function of the light and dark cycles, in a nitrogen-limited culture of Coccolithis huxleyi and Skeletonema costatum, but only for one growth rate (1.1 div/day) and both, nitrate and ammonium, were supplied to the culture at the same time. A more complete picture of the nitrate flow through the cells is obtained when rates of individual enzyme activities, such as NR and GDH, are measured as well as the nutrient uptake and growth rates. The activity of the enzyme nitrate reductase in phytoplankton is a good indicator of nitrate utilization. Eppley et al. (1971) have measured NR activity in a continuous culture system but only at one growth rate; in the present study it was decided to investigate the relationship of the NR activity at different growth rates. During the course of this work,

Eppley and Renger (1974) published the results of a similar study in which they found (contrary to their expectation) a decreasing NR activity with increasing growth rate of Thalassiosira pseudonana.

1.1.3 Growth with light-dark cycles.

Eppley et al. (1971) observed diel periodicity of the three enzymes, nitrate reductase (NR), glutamate dehydrogenase (GDH) and nitrite reductase (NiR) in a chemostat culture of Coccolithus huxleyi. The activities of NR and GDH showed a maximum at the beginning of the light period; the minimum was at the onset of the dark period. The diel periodicity of NR was also noted in the Peru current (Eppley et al., 1970) where the maximum activity was reached at noon, indicating a different phasing when compared with the Coccolithus huxleyi study mentioned above. Packard (1973) showed that there is a hyperbolic relationship between light intensity and NR activity of natural phytoplankton population and that saturation occurred at low light intensities.

The rate of nutrient uptake over light-dark cycles seems to be species specific. Eppley et al. (1971) found that Skeletonema costatum culture showed diel changes in ammonium and nitrate concentration. His Coccolithus huxleyi experiment did not show this effect and Malone et al. (1975) observed periodicity only at higher growth rates using Chaetoceros sp. (STX-105). Eppley et al. (1970) noted a diel variation of nutrient uptake rate in natural populations of the Peru Current.

Pigment synthesis also displays diel periodicity in most organisms. Paasche (1968) found that synthesis of chlorophyll *a* occurs within 10 hours after the onset of the light period for a synchronous culture of *Ditylum brightwellii* regardless of the length of the photoperiod. Thus, this could occur in the dark period (Eppley et al., 1967). On the other hand, Paasche (1967) showed that the rate of chlorophyll *a* synthesis is more or less continuous in *Coccolithus huxleyi*. Synthesis of chlorophyll *a* in *Dunaliella tertiolecta* occurs primarily in the light period (Eppley et al., 1966).

The time course of cell division and the extent of synchrony in cell division seem to vary among the species. Eppley and Coatsworth (1966) noted that *Dunaliella tertiolecta* cells divide mainly in the dark period of the cycle, but as the photoperiod increases, more cells divide near the end of the light period, while cells of *Coccolithus huxleyi* divide invariably in the dark period. Successive increases in photoperiod produces a corresponding shift of cell division to the dark period (Paasche 1967). Cells of *Ditylum brightwellii* divide in the late light period and the early dark period (Eppley et al., 1967).

Another important physiological process, which varies on a diel basis, is the photosynthetic capacity. Eppley and Coatsworth (1966) observed the highest rate at midday, followed by an "afternoon depression". Eppley et al. (1967) present a similar picture, but the depression came with the onset of the dark period. Eppley et al. (1971) found that *Skeletonema costatum* and *Coccolithus huxleyi* had their

maximum photosynthetic capacity per unit of chlorophyll a at midday, in a continuous culture system.

It should be kept in mind that all the observations mentioned above, on pigment synthesis, cell division and photosynthetic capacity, were obtained with semicontinuous cultures, where rich medium f, or f/4, (Guillard and Ryther 1962) was used. This daily dilution by itself could probably have induced some of the observations. The diel periodicity can also be affected by the dilution rate of a continuous culture (Malone et al., 1975), who observed a stronger periodicity of population characteristics at turnover rates corresponding to 100% $day^{-1}$ and especially at 200% $day^{-1}$.

1.1.4 Growth in deep-sea water

Studies on phytoplankton growth in artificially upwelled deep water show that natural populations of marine phytoplankton do not grow, or will grow only poorly, in deep water (Menzel et al., 1963). They demonstrated that deep water (700 m) from the Sargasso Sea contains sufficient nitrate and phosphate, but inadequate iron, to stimulate plant production to an extent comparable to the effect produced by enriching surface water with nitrate, phosphate and iron. The enriched surface sample was completely dominated by Chaetoceros simplex, while in the $Fe^{+3}$ supplemented deep water the dominant species was Nitzschia closterium. Further study of phytoplankton growth with upwelled water off the coast of Peru (Barber et al., 1971) showed that enrichment with $Fe^{+3}$ and trace metals (TM) or chelator (EDTA)

increased the specific growth rate with unconditioned sea water (lack of biogenic organic ligands or natural chelators) but not with conditioned water.

Recent work by Haines, Roels and Farmer (1974) has demonstrated that certain species of phytoplankton grow very well in unsupplemented deep sea water, e. g., Chaetoceros curvisetus (STX-167) with generation time of 1.2 division per day in large (2,000 liters) outdoor continuous cultures. Malone et al., (1975) have successfully grown the diatom Chaetoceros sp. (STX-105), at four dilution rates (50, 100, 150 and 200% turnover per day) in large outdoor continuous cultures using deep sea water (870 m) pumped off the north shore of St-Croix and enriched with EDTA, iron and vitamin $B_{12}$. The population in the reactors reached $2 \times 10^8$ cells per liter at steady-state. As a general rule, the literature is sparse as far as phytoplankton micronutrient requirements are concerned and the work of Barber et al. (1971) is an indication that more research is needed in this field.

## 1.2 Objectives

The objectives of this investigation were to determine the relationship between the growth rate and the processes of nitrate uptake, nitrate assimilation and photosynthesis during N-limited continuous growth of Chaetoceros sp. (STX-105) in deep sea water and to evaluate the effects of growth rates on nutrients in the growth medium, nitrate uptake kinetics, cellular characteristics, NADH dependent nitrate reductase and photosynthetic rate.

In specific terms the objectives were, during continuous N-limited growth:

a- To compare the parameters of nitrate uptake kinetics, namely, the maximal specific uptake rate (Vm) and the half saturation constant for uptake ($K_s^u$) as determined by short-term and discrete uptake techniques.

b- To test the hypothesis that the cellular organic nitrogen ($q_o$) is constant with different growth rates.

c- To find the dilution rate for maximum nitrate utilization (nitrate transformed into organic cellular nitrogen).

d- To determine the effect of the dilution rate on the phasing of nitrate uptake and enzymic nitrate reduction.

e- To describe the relationship between dilution rate and nitrate reductase activity.

f- To determine the relationship between photosynthetic rate and dilution rate.

# 2 MATERIALS AND METHODS

## 2.1 Materials

The marine diatom, Chaetoceros sp. (STX-105), was used in this experiment. The organism is only presently identified as Chaetoceros sp. (STX-105) but is similar to Chaetoceros simplex (K. C. Haines, personal communication). The cultures were axenic. Sea water for the preparation of the medium for the culture of Chaetoceros, was pumped from 870 m depth off the North Coast of St-Croix (Othmer and Roels 1973). The water was stored at $4^{o}C$ for 2-4 months, in 55 gallon, opaque polyethylene containers prior to use. To insure an axenic and homogeneous culture, the deep sea water (DSW) was filtered to remove any particulate matter and after addition of the nutrients, the medium was steam-sterilized. Ferric ion was sterilized separately and added aseptically. The concentration of major nutrients in deep sea water (DSW) and the nutrient concentration used for the enrichment are given in table 1. This specific nutrient supplementation provided a medium in which the limiting nutrient was nitrogen.

The continuous culture apparatus consisted of a 10 liter carboy closed with an air tight cover having four ports (Fig. 1). Fresh medium was introduced to the culture vessel by a peristaltic Sage pump (# 375A), which delivered a constant flow at a preselected rate. Aeration and sampling

was achieved by means of a two-way stopcock. The air supplied for mixing was initially bubbled through a 0.1 M solution of $ZnCl_2$ to remove traces of ammonia, then through distilled water and finally through sterile cotton. A constant 8 liter volume of culture was maintained by means of an overflow. The culture vessel was kept at a constant temperature by submergence in a plexiglass container in which water continually circulated at 26 $\pm$ 1°C. Light was supplied by cool white fluorescent tubes at an intensity of 0.1 ly/min, for a period of 12 hours (0900-2100) per 24 hours.

## 2.2 Experimental design

The growth chamber was inoculated with an axenic culture of Chaetoceros sp. (STX-105), in the exponential growth phase. The growth medium of the inoculum was the same as described in table 1. The system was run initially as a batch culture for 3-4 days. The pump was then turned on to the chosen flow rate. A period of 4-5 days was necessary to obtain a steady-state culture, which was defined as repetitive nearly identical cycles of cell densities as a function of time (Jannash 1974).

Based on the work of Malone et al., 1975, the following dilution rates were selected:

30% turnover/day = 100 ml/hr flow.

60% turnover/day = 200 ml/hr flow.

87% turnover/day = 290 ml/hr flow.

105% turnover/day = 350 ml/hr flow.

116% turnover/day = 386 ml/hr flow.

The same inoculum was used for the three chemostat runs (30, 60 and 87%) which lasted for 20, 15 and 15 days, respectively. Sterility tests were conducted at the beginning and the end of the third dilution rate (87%) and were negative. The test consisted of adding one drop of the medium, sampled aseptically, to sterile broth medium. In addition no wall effects of bacterial contamination were seen for the entire period of the experiment.

Samples were collected at 0900, 1300, 1700, 2100, 0100 and 0500 for the 30% turnover rate. The sampling time for the 60, 87,105 and 116% turnover rates was alternated every day between 0900, 1300, 1700, 2100, 0100, 0500 and 0700, 1100, 1500, 1900, 2300 and 0300 hr to cover the 24 hours cycle at intervals of 2 hr. However, some measurements such as pH, cell count, protein, chlorophyll a and nutrients were made every two hours.

## 2.3 Analytical procedures

### 2.3.1 Cell counts

Cells were counted with a Speirs-Levy counting chamber. Each determination represented an average number of 3700 cells per count (range of 3400-4000). The counting error was $\pm$ 2% ($\pm$ 1 SD).

### 2.3.2 Chlorophyll a

Chlorophyll a was measured in duplicate within one hour after sampling in 90% acetone extract by fluorometry (Strickland and Parsons 1972). The standard curve had a

correlation coefficient (r) of 0.9999 and the standard error was ± 0.27 ug chlorophyll a (± 1 SD).

2.3.3 Protein

Cellular protein was determined by the Lowry method (Lowry 1951). Fifty ml of culture were contrifuged and the pellet was resuspended in distilled water to a volume of one mililiter. The samples were immediately frozen until analysis. The standard curve for the protein determination had a correlation coefficient (r) of 0.9988 and the standard error was ± 1.9 µg Prot/l (± 1 SD).

2.3.4 Dissolved nutrients

Nitrate, nitrite, silicate and ammonium were measured by the methods of Strickland and Parsons (1972). The incoming medium and the growth chamber were sampled at intervals of 24 and 2 hours respectively. The samples were filtered through glass fiber filters (0.3 µ) and the filtrate was stored in polyethylene bottles in the freezer until analysis.

2.3.5 Primary productivity

The carbon-14 tracer solution used for the determination of the primary productivity was made according to the methods of Strickland and Parsons (1972). The radioactive stock solution was purchased from International Chemical and Nuclear Corporation. Ampoules having an activity of 5 µ Curie were prepared and the variability of radioactivity between these ampoules was checked on 15 ampoules, giving a mean count of 3369000 CPM ± 43000 (± 1 SD) or an error of ± 1.27%.

The assay was made with bottles having a capacity of sixty-five ml. Each measure was made by adding one ampoule (5 $\mu$ Curie) to a sixty ml sample of the culture. Night samples were manipulated in darkness. The bottles (light and dark) were incubated for thirty (30) minutes in the same water bath as for the culture vessel. Linearity of carbon-14 fixation as a function of time was tested and resulted in a linear relationship up to two hours. The cells used for carbon-14 uptake as a function of time were taken from the continuous culture at the 30% dilution rate and the experiment was made between 1900 and 2100 hr. Sample bottles (light and dark) were then filtered on to a 0.45 $\mu$ pore size Millipore filter. Any residual inorganic carbon-14 on the membrane was removed by holding the membrane for twenty seconds over hydrochloric acid fumes.

The organically bound carbon-14 was then counted without drying by addition of 15 ml of scintillation fluor to the wet membrane (Anderson and Zeutchel 1970). The fluor was a mixture of a toluene solution (7.8 ml) containing 5 gm/l of PPO and 0.5 gm/l of dimethyl POPOP with 2.2 ml of Bio-Solv solubilizer formula BBS-3 (Available from Beckman Instrument Inc.).

The unit $\mu g\ C.hr^{-1}l^{-1}$ was obtained by:

$$\mu gC.\ hr^{-1}l^{-1} = \frac{LB - DB}{\text{Total Activity}} \times MgC\ (1000)\ t^{-1}\ \left(\frac{100}{99}\right)\ 1.06$$

Where:

L.B. = Count per minute (CPM) of the light bottle.

D.B. = Count per minute (CPM) of the dark bottle.

Total Activity = CPM of the 5 $\mu$ Curie ampoule.

MgC. = Total carbon dioxide per liter in the medium. This value was obtained from the determination of the alkalinity of the medium according to the method of Strickland and Parsons (1972).

1000 = Transformation of mg to µg.

t = is equal to the incubation time in hours.

$\frac{100}{99}$ = Counting efficiency, as determined empirically from a quenching curve.

1.06 = Isotopic discrimination factor.

The 0900 sample was incubated in the light, while the 2100 sample, was incubated in the dark.

The experimental procedure for the photosynthetic capacity was the same as mentioned above except that the samples were incubated in a "light-box" illuminated by 4 fluorescent tubes covered with aluminium foil at a light intensity of approximately 95% of the chemostat light. This measurement allowed an estimate of the photosynthetic capacity at different intervals during the dark cycle.

The rate of photosynthesis per unit chlorophyll (P/chl) was calculated from the hourly rate of carbon-14 uptake (Mean of the 24 hour cycle) per unit chlorophyll a in the culture.

### 2.3.6 Total organic nitrogen and carbon

The total particulate nitrogen (PN) and carbon (POC) were analysed by combustion (model 185, CHN Analyzer, Hewlett-Packard Corp.). Samples of 200 ml were collected on a glass fiber filter paper (Gelman 0.3 µ), previously incinerated at 450°C for one hour.

2.3.7 Enzyme assay

The activity of NADH-dependent nitrate reductase (NR) was determined by the method of Eppley et al. (1969a). The standards used for the NR standard curve received the same treatments as the samples, except that nitrate was substituted for nitrite; the standard curve had a correlation coefficient (r) of 0.9986 with a standard error of 0.19 $\mu$ mole of nitrite formed per liter per hour. The sample volume was always 350 ml of culture. Care was taken to ensure that sampling and analysis was made in practically complete darkness, during the dark period of the diurnal cycle. The 0900 hr sample was incubated in the dark, while the 2100 hr was incubated in the light.

2.3.8 Intracellular nitrate

Cellular nitrate was measured according to the technique of Eppley and Coatsworth (1968). Samples of 350 ml were collected for each analysis and the final neutralized extract was diluted to a volume of 15 ml. Then the sample was quickly frozen in a polyethylene bottle, until analysis.

2.3.9 Cellular free amino acids

Based on the work of Jeffries (1968), the cellular free amino acids were extracted with 95% ethanol. Samples of one liter were filtered onto a glass fiber paper (Gelman 0.3 $\mu$) which was ground with a teflon homogenizer (Type A) in the presence of 5 ml of 95% ethanol. Exactly thirty-five strokes were used for each sample to standardize the procedure.

The disintegrated filter was recovered completely by washing of the mortar and pestle with 95% ethanol and filtered through another glass fiber filter, with a maximum vacuum of five pounds per square inches. The final filtrate was then evaporated by heating in a nitrogen atmosphere. Further desiccation was accomplished by storage over silica gel in a desiccator for a period of 48 hours. The dried samples were then analysed with a Durrum Analyzer, Model D-500 at the AAA laboratory, Seattle, Washington.

2.3.10 Carbohydrate

Total cell carbohydrate was measured by the sulphuric acid-indole technique of Dische and Popper as reported by Colowich and Kaplan in Methods in Enzymology (1957). Ten ml of culture was collected for each determination and centrifuged down to 0.1 ml at 2000 g for eight minutes. The pellet was then resuspended to a final volume of one ml with distilled water, and stored frozen until analysis. The standard curve had a correlation coefficient (r) of 0.9972 with a standard error of $\pm$ 1.79 µg sugar/ml ($\pm$ 1 SD).

2.3.11 Nitrate uptake kinetics

For each particular dilution rate at steady-state (1400 hr), the rate of nitrate uptake was determined by three techniques: (1) The uptake rate of nitrate was determined at steady-state, from the nitrate removal from the medium by the population. (2) At 1400 hr, the pump was stopped and nitrate was added to a concentration of approximately 8 µg-at/l. The nutrient depletion was measured every five minutes

(Caperon and Meyer 1972b). (3) Prior to the nitrate spike of the preceding technique, a sample from the chemostat was collected and the uptake rate of nitrate was determined by the method of Carpenter and Guillard (1970), i.e., short-term uptake experiments in which the cells were incubated for 1 to 15 minutes in presence of different concentrations of nitrate. The constants of nitrate uptake kinetics determined by the technique of Caperon and Meyer (1972b), i.e., discrete uptake experiments were compared with the results of the technique of Carpenter and Guillard (1970), i.e., short-term uptake experiments.

To estimate the effects on the uptake kinetics of cells which were fed with nitrate (8 µg-at/l) and cells which were starved for 2 hrs, short-term uptake experiments were done and "$K_s$" and "Vm" were determined following these two treatments. We used three different types of cell populations designated as fresh, fed and starved, defined as follows:

Fresh: The pump of the continuous culture system was stopped and the cells were used within 10 minutes.

Fed: Fresh cells were exposed to a concentration of approximately 8 µg-at $NO_3^-$-N/l and one hour later, these cells were used to do the uptake experiment.

Starved: Fresh cells were utilized two hours after the pump was stopped.

# 3 RESULTS

Raw data are presented in the Appendix . In this section we refer to tables and figures derived from those data.

The results presented in this section represent data obtained from continuous cultures of the oceanic diatom Chaetoceros sp. (STX-105) at five different dilution rates, 30, 60, 87, 105 and 116% turnover per day. The experiments were started only when steady-state growth, as defined above, prevailed. Usually 3-5 days after the beginning of each run, repetitive cycles of 24 hours were observed for a few parameters such as, cell number, chlorophyll a, pH. The cultures were considered in steady-state of growth when the time function of the cycles became repetitive (Jannash, 1974). Sustained steady-state was not achieved at the highest dilution rate (116%) and the results of this run were edited to exclude the data obtained when the population collapsed.

Since the culture did not maintain itself at steady-state at 116%, another dilution rate was tried (105%) to assess the maximum growth rate (u max) under the existing conditions. Unfortunately, only one day was monitored due to air pump failure. Thus, the data for this dilution rate represent a single 24 hour cycle. In spite of this, the data obtained are consistent with what would be expected at

that dilution rate when compared with data obtained at other dilution rates.

3.1 Nutrients:

The mean nutrient concentrations of the medium entering and leaving the culture vessel for each dilution rate are shown in table 2. The incoming nitrate and nitrite concentions did not change whereas the ammonia concentration increased approximately three fold when compared with table 1. The mean values of ammonia plus amino acids (detection of the amino group) are slighly higher than ammonia values, as expected, however, individual values were variable. Finally, the incoming medium contained twice as much silicate (Table 2) as the deep sea water (Table 1), possibly due to leaching from the glass carboy during sterilization.

Nitrate concentrations of the outgoing medium (Table 2) are consistently low for all dilution rates except for the 60% turnover rate and to a certain extent, 87%. The reason for the residual nitrate concentration (0.96 ± 0.87 µg-at) may have been a slow air flow to the culture vessel, resulting in an uneven distribution of obvious repetitive pattern of the temporal nitrate concentration. The aeration flow was increased for the 87% run, but probably not enough; further increase of the aeration flow for the 105% and 116% turnover rates revealed practically no residual nitrate in the culture vessel.

For all five dilution rates tested, nitrite concentrations in the outgoing medium and in the incoming medium were low (Table 2) and no diel variations was observed.

The analysis of the nutrient samples for ammonia and ammonia plus amino acids in the culture vassel are shown in table 2. Except for the 87% turnover rate, ammonia was taken up by the algae. These values were irregular without any trend with time.

Finally, table 2 shows the mean silicate concentrations of the outgoing medium for each turnover rate. These data suggest that the uptake of silicate, by the cells increased as the turnover rate increased. However, the 116% turnover rate did not follow this trend, since the residual concentrations was higher than the 60, 87 and 105% dilutions rates.

## 3.2 Population size

### 3.2.1 Cell number

The diel periodicity of cell density was examined by plotting the mean cell number at each time of the day for each turnover rate (Fig. 2). Each point represents the mean of n values, where n was the number of days for which each turnover rate was maintained. The 30% turnover rate was the most stable whereas 60% showed an overall decrease during the photoperiod and increase in the dark period. A similar pattern was observed for the 87% and 105% turnover rates where the increase in cell number was shifted earlier into the dark period (2100 hr) and cell number peaked at 0300 hr when a gradual washout began and the cells population

recovered only at 1100 hr. The highest turnover rate tested (116%) showed (Fig. 2) a different pattern from the 60%, 87% and 105% dilution rates in which there was no apparent increase in cell number during the dark period. Cell numbers increased between 0900 hr and 1500 hr in the first half of the light period. This was the only turnover rate with consistently lower cell numbers. In general, the range was greater in the region where cell division occurs, indicating that this cellular process did not occur at exactly the same time for each day.

3.2.2 Cellular protein

The mean values of cellular protein per liter of culture at each sampling time during the 24 hour cycle are reported in figure 3 and were taken from the number of 24 hour cycles indicated on the figure. Except for the 30% turnover rate, which showed no significant diel variation, there was a decrease in protein nitrogen during the dark period. There was an increase during the light period which, at the 87% and 105% turnover rates began before the onset of the light period. As the turnover rate increased the phasing of the diel variations changed, in the sense that from 2100 hr the decrease is more and more accentuted, resulting in a progressively more pronounced increase during the early light period, except for 116%, which showed the larger increase at 1900 hr. It should be pointed out that the 30% turnover rate is very stable with variations between 215 and 230 µg protein-N/l (Fig. 3). However, as the dilution rate

increased, the maximum value of 230 µg protein-N/l is not surpassed while the minimum reached for each run is continuously decreasing to a minimum value of 26 µg protein-N/l at 0700 hr for the 116% turnover rate. One interesting feature which should be noted is that during the dark period the decrease in protein content (Fig. 3) of the culture corresponds to an increase in cell number (Fig. 2). The spread of the values (Fig. 3) for the 116% turnover rate is partially attributed to the fact that steady-state could not be maintained, since the high values were mainly from the first 24 hour cycle whereas the lower values from the last cycle.

3.2.3 Chlorophyll *a*

The mean concentrations of chlorophyll *a* in the culture for each sampling time, over the 24 hour cycle for each dilution rate are shown in figure 4. The number of cycles averaged is given in the figure. The diel periodicity of chlorophyll *a* was erregular which is reflected in the relatively large spread of the data. However, with the exception of the 30% turnover rate, a trend seemed to exist in the synthesis of chlorophyll *a* for the diatom *Chaetoceros* sp. (STX-105): chlorophyll *a* increased during the second half of the photoperiod between 1500 and 1900 hr and during the second half of the dark period, starting at 0300 hr up to 0900 hr, except for the 105% turnover rate where there is a rapid decrease between 0300 hr and 0900 hr. As the dilution rate increased, both the amplitude of the diel variations and the absolute values increased.

The concentration of chlorophyll a generally reached its highest values at the beginning and toward the end of the photoperiod and had lower values during the middle of the photoperiod and the dark period.

3.2.4 Particulate organic nitrogen

The diel variations of the mean particulate organic nitrogen for each sampling time over the 24 hour cycle are shown in figure 5 with the range of the values and the number of cycles averaged. The increase in particulate nitrogen (PN) began at the start of the photoperiod and reached a high value at approximately 2100 hr. Except for the 105% turnover rate where the value at 1900 hr is only a single value and may be an experimental error and 116% where two 24 hour cycle were averaged and the 1300 hr mean is lower than the 0900 value. The decrease of PN at each dilution rate started as soon as the light was turned off (Fig. 5). However, there was short period of increasing PN during the dark period, for example at 0500 hr, 2300 and 0500 hr and 0100 hr for the 60%, 87% and 116% turnover rates.

It is interesting to compare the particulate nitrogen concentration (Fig. 5) with the protein data (Fig. 3); as the turnover rate increased the protein content decreased regularly in the dark period while the particulate organic nitrogen showed only a slight decrease. This is an indication that, as the turnover rate increased, the inorganic nitrogen was still taken up (Table 2) but was used differen-

tly at different times during the diel cycle. Thus, as the turnover rate increased, the synthesis of protein took place more and more during the photoperiod while nitrogen must have been used for other cellular needs during the dark period.

3.2.5 Particulate organic carbon

The mean amount of bound carbon produced by the Chaetoceros population at each sampling is shown in figure 6 with indication for the range of each value and the number of cycles used to obtain these mean values. Examination of figure 6 reveals that as the turnover rate increased, the maximum amplitude of the diel variation was reached at the 87% turnover rate. The differences between the lowest and highest values for each turnover rate were 140, 245, 281, 250 and 290 µg-at POC for the 30, 60, 87, 105 and 116% turnover rates respectively. This phenomenon is consistent with the primary productivity data from carbon-14 fixation (section 3.4).

Diel periodicity (Fig. 6) is closely related to the light regime in that POC increased during the photoperiod and continuously decreased in the dark period. For each turnover rate the maximum was reached at the end of the photoperiod.

3.2.6 Carbohydrate

The mean values of total carbohydrate per liter of culture at each sampling time are shown in figure 7 for the 24 hour cycle at each turnover rate. The mean values were taken from different numbers of 24 hour cycle, which are

indicated on the figure. As for the other population size measures, the 30% turnover rate was the most stable. The greatest amplitude, in terms of the absolute quantity of carbohydrate, was observed from the 60% dilution rate. The three other dilution rates, 87%, 105% and 116%, are similar in shape and intermediate in amplitude. The carbohydrate increased during the photoperiod and decreased during the dark period, except for the 105% turnover rate at 1900 hr and 0100 hr; these last two values might be due to experimental error since only one 24 hour cycle is reported (Fig. 7). The trend of these curves is similar to those of the particulate organic carbon data (Fig. 6). The lowest values were always at 0900 hr (at the end of the dark period) and the highest carbohydrate content at 2100 hr (the end of the light period).

3.2.7 Free amino acids

The free amino acid pool was determined for each dilution rate except for 116%, where steady-state could not be maintained. All samples were taken at 1100 hr. As the turnover rate increased from 30% to 60%, 87% and 105%, the number of amino acids detected and measured was 4, 5, 8 and 13 respectively. This increase is attributed to changes in growth rate of the organism. Of all the amino acids, glutamate always had the highest concentration, reflecting the main patways of inorganic nitrogen into the amino acid metabolism via amination of alpha-ketoglutarate in the presence of the

enzyme glutamate dehydrogenase. In general, there was a moderate increase in the concentrations of amino acids as the dilution rate increases up to 87%, but there was a large increases in the free amino acid pool from 87% to 105%. For example, glutamic acid concentrations were 3.87, 12.12, 25.39 and 236 nanomoles for the 30, 60, 87 and 105% turnover rates respectively.

It should be noted from table 3 that the total amount of free amino acids (μg) in the extract represents a small percentage of the total protein (μg) before extraction. Even at the highest dilution rate, the free amino acid pool represents only 0.92% of the total protein. This percentage is probably underestimated because the protein concentration in table 3 is calculated from the total nitrogen number assuming a factor of 6.25 for the conversion.

Table 4 shows that glutamic acid represents a large proportion of the total free amino acid pool (41%, 66%, 59% and 52% for the 30%, 60% 87% and 105% turnover rates, respectively. Among those amino acids which have increasing relative abundance (Table 4) as the dilution rate increased are aspartic acid (6%, 9% and 10% for the 60%, 87% and 105% turnover rates, respectively) and lysine, phenylalanine, proline, tyrosine and valine which appeared only at the 105% dilution rate. Glutamic acid increased from the 30% to 60% but decreased for the 87% and 105% turnover rates. Isoleucine, leucine and threonine follow the same pattern as glutamate (Table 4), in the sense that they increased from the 30% and 60% turnover rates, where they were not detected, to the

87% dilution rate and decreased slightly at the 105% turnover rate. Of the amino acids which show a decline as the turnover rate increased are serine (31%, 16% and 10% for the 30%, 60%, 87% turnover rates), glycine (12%, 4%, 3% and 2% for the 30%, 60%, 87% and 105% turnover rates) and finally alanine which represented 14% of the total free amino acid pool for the slowest dilution rate, decreased to 8% for the 60% dilution rate and levels off for the 87% and 105% turnover rates.

It can be seen from table 4, that the major changes in the relative abundance of individual amino acid, occur between the 30% and 60% turnover rate. This is principally a result of a three fold increase in glutamic acid content.

3.2.8 Intracellular nitrate

Since algae have the ability to store nitrate, the diel variation of intracellular nitrate ($NO_3^-$) at each turnover rate was measured (Fig. 8). The cells from the 30% turnover rate had the lowest intracellular nitrate concentration; the highest amount detected was 0.11 µg-at at 0900 hr. The 60% turnover rate had slightly higher intracellular nitrate (varies between 0.09 and 0.14 µg-at $NO_3^-$/l) than 30% and was more irregular in the sense that cellular nitrate peaked at 1900 and 0300 hr. The highest concentration of cellular $NO_3^-$ for the 87% turnover rate was found at the beginning of the light period at 1100 hr (note the change of scale in the figure), the main increase occurred between 0700 and 1100 hr. The next turnover rate, 105%, also has a high value of 0.33 µg-at $NO_3^-$ during the day time (1100 hr), but the major

difference from the three preceding turnover rates (30%, 60% and 87%) was the relatively high concentration of intracellular nitrate at 2300 hr, which had declined rapidly by 0300 hr. Finally the highest dilution rate tested (116) was similar to 105% except that the day peak shifted to 1500 hr, and the maximum at 2300 hr decreased more slowly than the one at 105%.

Except for the 87% dilution rate, the highest concentrations of intracellular nitrate were found at the end or during the dark period. The diel variation of cellular nitrate increased as the dilution rate increased from 30% to 105% and decreased slightly for 116%. The amplitude of the diel variations were 0.037, 0.067, 0.195, 0.853 and 0.781 µg-at $NO_3^-$ for the 30%, 60%, 87%, 105% and 116% turnover rates respectively. It should be kept in mind that the cell density decreased for the 116% turnover rate (Fig. 2).

3.2.9 Population size as a function of the dilution rates.

Figures 9 and 10 show the mean population size of the Chaetoceros culture at each dilution rate. The mean cell number (Fig. 9) increased from the 30% dilution rate ($9.6 \times 10^8$ cells/l) to the 60% dilution rate ($9.9 \times 10^8$ cells/l) and decreased for the 87%, 105% and 116% turnover rates. There was a substantial decrease at 116%, ($7.7 \times 10^8$ cells/l), at which dilution rate, steady-state could not be maintained. Except for the 116% turnover rate, the mean cell numbers varies within a small range (i.e., $9.2 \times 10^8$ cells/l) Another indicator of population size which showed little variation with dilution rate was particulate nitrogen.

which varied between 28 and 33 µg-at PN/l; the lowest value was observed for the 30% dilution rate, and the highest for the 105% turnover rate. Since particulate nitrogen is often used to calculate the protein content of cells, it is interesting to note the tremendous difference between protein determined by the Lowry technique and total particulate nitrogen (Fig. 9). This decrease in protein content is well supported by the accumulation of the building blocks of protein in the cells as the turnover rate increased; table 3 shows that many free amino acids are below the detection level at the lowest turnover rate (30%) indicating that these materials are rapidly incorporated into protein. However, as the turnover rate increased, the free amino acid pool also increased, and the major increment was at 105% turnover rate.

The mean carbohydrate content in the culture shows a decrease as the turnover rate increased (Fig. 9), and the most abrupt decrease is from 60% to 87% turnover rates. Figure 10 shows that the mean particulate organic carbon decreased as the dilution rate increased. The chlorophyll *a* increased as the dilution rate increased especially from 60 to 87%. Figure 10 shows the mean population size in terms of intracellular nitrate. The main feature is a three fold increase in the latter as the turnover rate changes from 87% to 105% with nearly constant values on either side of this transition.

3.3 Nitrate reductase activity

The rate of reduction of the intracellular nitrate ($NO_3^-$) to nitrite ($NO_2^-$) was measured by the determination of the NADH-dependent nitrate reductase activity. The results shown in figure 11 represent the mean enzymatic activities (per unit volume, per hour) at each sampling time during the 24 hour cycle. The mean for each dilution rate was obtained from the number of cycles indicated on the figure. The lowest enzyme activities were observed at the 30% turnover rate and showed a light decrease during the photoperiod and an increase between 0100 hr and 0500 hr. The first four hours of the light period at the higher dilution rates were characterized by a rapid increase of nitrate reductase activity (Fig. 11), except for the marked increase between 1700 hr and 1900 hr at the 60% dilution rate.

The decrease in nitrate reductase activities did not occur at the same time of the day for each turnover rate. For example, the decrease for the 30% dilution rate was during the first half of the light period, whereas, there were three time intervals during which the NR activity decreased at the 60% dilution rate, i. e., 1300 hr-1700 hr, 1900 hr-2300 hr and 0500 hr-0700 hr. However, a single decrease of the NR activity occurred during the first half of the dark period at the 87% dilution rate. The 105% turnover rate was characterized by a continuous decrease (Fig. 11) between 1300 hr and 0500 hr in the following dark period. The main decline in NR activity for the 116% dilution rate occurred mainly during the dark period between 2300 hr and 0500 hr.

It is interesting to compare the NR activity (Fig. 11) to the cellular $NO_3^-$ concentrations (Fig. 8). For the 30% dilution rate, the cellular $NO_3^-$ is highest at 0900 hr and correspond to a high NR activity but the increasing NR activity between 0300 hr and 0500 hr is not accompanied by higher cellular $NO_3^-$. The 60% turnover rate is particularly interesting since cellular nitrate peaked twice during the 24 hour cycle at an interval of 8 hours (Fig. 8) whereas the nitrate reductase activity had two major increments with an interval of approximately 8-10 hours (Fig. 11). The cellular nitrate for the 87% turnover rate increase during the dark period mainly from 2300 hr to 0300 hr and 0700 hr to 1100 hr (Fig. 8) while the nitrate reductase activity increased and maintained high rates during the photoperiod. The 105% turnover rate was characterized by one peak of cellular $NO_3^-$ at 2300 hr which could correspond to the single, rapid increase of NR activity in the following early light period. Finally, the cellular $NO_3^-$ for the 116% turnover rate increased also at 2300 hr like 105%, but decreased less rapidly, and could be related to the two successive increases of NR activity (1100 hr - 1300 hr and 1700 hr - 2300 hr).

At all dilution rates there is a general trend of increased protein per unit volume (Fig. 3) when nitrate reductase activity per liter increased (Fig. 11). Exception to this pattern can be seen at the 60% dilution rate at 1700 hr; at the 116% turnover rate where the maximum protein per liter preceded the nitrate reductase activity maximum at 2300 hr, by two hours.

## 3.4 Photosynthesis

Primary productivity and photosynthetic capacity were measured by carbon-14 fixation. The rates are expressed in $\mu g\ C\ l^{-1} hr^{-1}$. Figure 12 shows the effects of the light and dark cycles on the rate of carbon fixation by the diatom Chaetoceros sp. (STX-105), when the culture was preconditioned at a 30% turnover per day. The curves of primary productivity and photosynthetic capacity (Fig. 12) are close to each other during the photoperiod, indicating similar light intensities since all other experimental conditions were identical.

The primary productivity was pratically nil in the culture during the dark period (Fig. 12) whereas the results for the photoperiod represents a bell shape that is an increase during the first half of the light period up to a maximum and a decrease during the late light period. It should be mentioned that maximum rate reached is maintained only for a short period, probably within one or two hours, our sampling interval was too long for a more precise definition of this particular feature. The reproducibility of the data was good from day to day (Fig. 12).

Rates of carbon-14 fixation for the determiantion of the photosynthetic capacity of the Chaetoceros population is shown in figure 12. The diel periodicity was strong, the maximum rate being reached during the late light period, while the dark minimum was approximately at 0300 hr. It can be seen that before the onset of the light period, the cells

capacity to fixed carbon increased until a maximum during the photoperiod and that this ability to fix carbon decreased before the onset of the dark period. Since the 2100 hr sample was incubated in darkness for the primary productivity data, the results of the photosynthetic capacity measurement gives a good approximation of the rate of carbon fixation in the culture at that particulate time (2100 hr) or prior to the dark period.

Figures 13, 14, 15 and 16 show the results of the carbon-14 experiments when the culture was preconditioned at turnover rates of 60%, 87%, 105% and 116% respectively. At the 30% turnover rate the highest rate measured was 275 $\mu g\ C\ l^{-1}hr^{-1}$, and a plateau of approximately 500 $\mu g\ C\ l^{-1}hr^{-1}$ was reached for 87%, 105% and 116% turnover rates for the maximum rate of carbon fixation for both, the primary productivity and photosynthetic capacity. It is interesting to note that as the dilution rate increased the daylight photosynthetic values reached a plateau but the night values did not. The minimum values measured for each turnover rate were 35, 100, 135, 200 and 230 $\mu g\ C\ l^{-1}hr^{-1}$.

Another interesting feature noticed in figures 12 through 14 is the shifting of the maximum rate of photosynthesis toward the beginning of the light period as the turnover rates increased. The maximum rate for the 30% run (Fig. 12) is reached at approximately 1700 hr which is eight hours after the start of the photoperiod, whereas for the 60% turnover rate the maximum was about at 1500 hr (with a marked increase in the 1100 hr samples) and for the 87, 105 and 116% turnover

rates the maximum is reached at 1100 hr, which is only two hours after the onset of the light period. One can see that as the dilution rate increased the 0900 hr sample gave much higher values (Figs 12 and 15).

## 3.5 Uptake kinetics

The results of the determination of the uptake kinetics parameters, Vm and $K_s^u$, by the technique of short-term uptake and discrete uptake are shown in table 5. The values for the 116% (3) and 60% (2) by the short-term uptake technique are omitted because the cells at the lower nitrate concentration were incubated too long. Regression analysis on the linear transform $S = S/v$. $Vm - K_s^u$ was used for the determination of Vm and $K_s^u$.

As the turnover rate increased from 30 to 87% (Table 5), for the short-term uptake technique, there was a slight increase of Vm for the fresh population and approximately the same value for the fed population, the starved population ($.0168 \pm .0002$) shows a decrease for Vm as the turnover rate increased to 60% ($.0159 \pm .0003$) and an increase ($.0196 \pm .0053$) for the 87% turnover rate, however the error on the last value does not allow this increase to be significant. By comparison to the fresh population, the maximum rate (Vm) for the fed population were higher and those of the starved cells were lower. The half saturation constant, $K_s^u$, as determined by the short-term uptake technique, were similar (Table 5) for the three populations (fresh, fed, starved) at the 30% turnover rate. This similarity was also observed for the 87% run,

but, the values are about twice those at the 30% turnover rate, if we do not consider the large error of the fed population (87%).

Except at the 116% turnover rate, where Vm = 0.0456, the maximum rate of nitrate uptake by the discrete uptake technique was 0.0196, 0.0197 and 0.0195, essentially equal values (Table 5). These numbers were obtained by taking the maximum negative slope of the culves representing the decreasing nitrate concentration as a function of time. However, the determination of $K_s^u$ for the 30 and 60% turnover rates gave zero because each of these two depletion curves were straight lines. The values of $K_s^u$ for the turnover rate 87% and 116% per day were taken as the substrate concentration where the uptake rate (v) was half of the maximum rate (Vm). Comparison of short-term and discrete techniques (Table 5) shows that the values of Vm are in the same order of magnitude for the three lower dilution rates whereas the $K_s^u$ values are not comparable.

# 4 DISCUSSION

## 4.1 Population characteristics

Caperon and Meyer (1972b) attempted to establish the relationship between population characteristics and phytoplankters growth rate, to provide the best estimator of population size from an ecological standpoint. Their study was conducted under continuous light conditions, whereas in the present study a more natural light-dark regime of 12 hrs light/12 hrs dark was used.

It can be seen from the results presented above that the various population sizes measured in this work showed varying degrees of both absolute and relative diel periodicity at each turnover rate and that there were also variations of the mean population size between turnover rates (Figs 9 and 10). These variations resulted in part, but to a generally minor extent, from experimental error, to some extent from a lack of reproducibility of the daily cycles, and to a large extent to the diel periodicity of the population characteristic in question. Statistically, there are too few daily cycles of measurements to allow an accurate estimate of the contribution of the day to day variations to the total variance, but the magnitude can be seen to be generally

less than the variation due to the diel amplitude of the population characteristic.

Table 6 is a summary of the coefficients of variation of the population characteristics in the culture experiments reported above. As already stated the major component of variance of population characteristics at a particular growth rate derives mainly from the diel periodicity (see for example figures 5 - 8). However, for some population characteristics, the lack of reproducibility of the diel cycle may account for as much as the diel variation alone (see for example figure 4); this is also true for the slowest dilution rate where the diel periodicity was smaller as compared to higher turnover rates. The grand mean for all turnover rates (Table 6) reflects the additional variance due to changes in the population characteristics as a function of growth rate.

It can be seen from table 6 that particulate nitrogen is the most stable population size measured; the coefficients of variation at each turnover rate do not exceed 6% (except for the 116% turnover rate, where steady-state could not be maintained) indicating a relatively small diel variation and the variance of the population particulate nitrogen for all turnover rates is also small (6%). The second most stable population characteristic is cell number with a 10% coefficient of variation for all turnover rates; the major difference with particulate nitrogen is that the 30% had a much smaller diel variation with a 2% coefficient of variation. Particulate carbon is the third most stable population

characteristic with a coefficient of variation of 17% for all turnover rates, but for the first time the coefficient of variation for the 87%, 105% and 116% (reflecting the diel variation) exceedes the grand mean for all turnover rates. It can be seen from figure 10 that the absolute value decreased as the turnover increased while the deviation from the mean (± 1 SD) increased. The fourth most stable population characteristic is chlorophyll a which showed a higher degree of variation (22%) between different growth rates, than the degree of variation due to diel periodicity, day to day reproducibility, for each growth rate. The decrease in the coefficient of variation from 7% to 5% as the turnover rate increased from 60% to 87% is due to the increase of chlorophyll a per liter since the deviation (± 1 SD) from the mean chlorophyll a concentration at 60% and 87% is approximately the same (Fig. 9). Protein is the fifth most stable population characteristic with a coefficient of variation of 30% for all turnover rates. It can be seen from figure 9 that the 30% turnover rate had a low coefficient of variation (10%) because of the relatively small deviation (± 1 SD) from the high mean protein concentration (222 µg Protein/l) whereas for the 116% turnover rate the standard deviation was greater and the mean protein concentration decreased to 114 µg Protein/l, giving a high coefficient of variation (48%). The last two population characteristics which exhibited the highest degree of instability are carbohydrate and cellular nitrate, with coefficients of variation of 62% and 63%, respectively. The main difference between those two popula-

tion characteristics was, that the absolute amount of carbohydrate per liter decreased while the intracellular nitrate increased, as the turnover rate increased (Figs 9 and 10).

The relationship of population characteristics ratios such as chlorophyll *a*/C or N/C vs growth rate is often exploited to determine if they are related by a linear or hyperbolic function (Malone et al. (1975); Caperon and Meyer (1972b); Thomas and Dodson (1972)). In the present study the chlorophyll *a*/C vs dilution rate relationship (Fig. 17) is similar to the results of Caperon and Meyer (1972b) with respect to the slope of the curve whereas they differ from Eppley and Renger (1974) who have obtained a less steep curve. On the other hand, the curve in figure 17 might not be appropriate since a straight line could also be traced between those points and then the value of chlorophyll *a*/C ratio at zero turnover rate would be similar to those of Caperon and Meyer (1972b), Eppley and Renger (1974) and Thomas and Dodson (1972). In spite of having a limited number of points in figure 17, it seems that there is a breaking point in the curve with dilution rates higher than 60%. This could be interpreted as such in view of the work of Malone et al. (1975) who mentioned that at low dilution rates the system is nitrate limited and becomes light limited at high turnover rates.

Another important cellular characteristic ratio is N/C as a function of growth rate. There is excellent agreement between the present results and those of Caperon and Meyer

(1972b), and of Eppley and Renger (1974). Present results agree with the N/C ratio reported by Malone et al. (1975) for the same organism grown in large outdoor cultures. Ratios from this study (Fig. 18) are similar to their lower values, since they operated with higher turnover rates; the ratio of N/C are comparable but it should be noticed that their mean cell number was approximately three times lower than in this study whereas the nitrogen and carbon content of their cells was approximately three times higher. This difference could be explained by the fact that their cultures were exposed to higher light intensities, natural tropical sunlight, and this would imply a lower concentration of chlorophyll a per liter of culture and per cell. However, their chlorophyll a concentration per liter of culture is about twice as high as in the present study and ten times higher when expressed on a cell basis (Table 7). Since the same quantity of nitrate (31.5 µg-at) was removed from the medium by Chaetoceros sp. (STX-105) in the study of Malone et al. (1975) and in the present work, for comparable turnover rates it is probable that the cells adjusted to the different light intensities by increasing their content of POC, PN and chlorophyll a per cell. However, Maddux and Jones (1969) observed an increase in growth rate for the organisms Nitzschia closterium and Tetraselmis sp. as the light intensity increased for identical quantity of nutrient ($NO_3^-$) to start with.

The pattern of the diel cycle of specific rate processes (such as NADH-dependent NR activity) depends greatly on the population characteristic upon which the specific

rate is expressed. Figure 11 shows the diel periodicity of the NR activity expressed per unit volume per hour, as described previously. The interesting point is that diel pattern is similar and damped if NR activity is expressed on the basis of the most stable population characteristic, particulate nitrogen (Fig. 19). If the nitrate reductase activity is expressed on the basis of population characteristics which have increasing coefficients of variation but are still relatively small (Table 6), for example cell numbers and chlorophyll a (Figs 20 and 21), the diel pattern is still similar to the one of NR activity expressed on the basis of PN, but the diel oscillations are increasing. However, if we look at the diel periodicity of nitrate reductase activity expressed per protein (Fig. 22) the diel pattern is opposite to the other specific nitrate reductase activities, except for the 30% turnover rate where the population characteristic protein had a relatively low coefficient of variation of 10% (Table 6). The diel pattern of NR activity expressed on a protein basis for the 60% turnover rate is not completely opposite to the other specific NR activities (Figs 11, 19, 20 and 21) during the photoperiod but it is during the dark period. As the coefficient of variation of the population protein increase, i.e., 87%, 105% turnover rates (Table 6) the diel pattern of NR expressed per protein (Fig. 22) is completely opposite to those of NR activities on the basis of PN, cell numbers and chlorophyll a; the same inversion is observed for the 116% dilution rate except between 1100 hr and 1700 hr, where the usual

increase in protein for other dilution rates (Fig. 3) did not occur. It should be remembered that steady-state could not be maintained for this particular growth rate.

Specific NR activity is most often expressed on the basis of the population characteristic, protein. Expressed on the basis of Lowry protein the NR activity decreased at the onset of the photoperiod and increased as soon as the dark period began, except for 30% and to a minor extent 60% dilution rate. This diel pattern of the specific NR activity (Fig. 22) is in good agreement with the diel pattern of intracellular nitrate (Fig. 8), which is known to induce the protein specific nitrate reductase activity (Lui and Roels 1972). At the 30% dilution rate (Fig. 8), the cellular nitrate peaked at 0900 hr and corresponded with the highest NR activity (Fig. 22) for that particular turnover rate. For the 60% dilution rate it can be seen from figure 8 that the first intracellular nitrate peak (1900 hr) matches with the NR activity increase at 1900 hr (Fig. 22) while the second accumulation of cellular nitrate between 2300 hr and 0300 hr (Fig. 8) gave a broad increase of NR activity which also started at 2300 hr (Fig. 22); figure 8 shows a third increase of intracellular nitrate between 0700 and 1100 hr, which would explain the persistant high NR activity between 0700 hr and 1100 hr (Fig. 8) after which time it decreased until to 1700 hr. The intracellular nitrate concentrations for the 87% dilution rate (Fig. 8) were consistently higher than for 60% (except at 0300 hr) and gave higher NR activity (Fig. 22). (Note the change of scale in

figure 8 for the 87%, 105% and 116% dilution rates). The increase in cellular nitrate between 0700 hr and 1100 hr, at the 87% turnover rate (Fig. 8) corresponded to the increase in NR activity at 0900 hr (Fig. 22). The decrease of cellular nitrate is slow during the photoperiod and the concentrations are relatively higher when compared with the 60% dilution rate, resulting in slightly increasing NR activity during the photoperiod for the 87% dilution rate; there was a decrease of NR activity between 1100 hr and 1700 hr for the 60% turnover rate. Finally the increase of cellular nitrate between 2300 hr and 0300 hr for the 87% dilution rate (Fig. 8) was probably responsible for the increased NR activity between 0100 and 0500 hr (Fig. 22). Probably the most convincing dilution rates, showing the close association between intracellular nitrate and the protein specific nitrate reductase activity are 105% and 116%. Figure 8 shows that there was a tremendous increase in cellular nitrate between 1900 hr and 2300 hr for the 105% dilution rate, which corresponded with a rapid increase of NR activity between 2100 hr and 0100 hr. This first large peak of cellular nitrate decreased quite rapidly (between 2300 hr and 0300 hr) but a second increase between 0700 hr and 1100 hr probably accounted for the large increase of NR activity at 0900 hr (Fig. 22). The 116% turnover rate also showed a large increase of cellular nitrate between 1900 hr and 2300 hr (Fig. 8) which corresponded with a rapid increase of NR activity starting a 2100 hr (Fig. 22); it should be noted that the cellular nitrate decreased at a slower rate than at the 105% dilution

rate (see 0300 hr for both dilution rates), and it can be seen from figure 22 that the NR activity for the 105% turnover rate did not increase as rapidly as at 116%, for the time interval between 0100 hr and 0500 hr. Another interesting feature for the 116% dilution rate, is that there was no second increase of cellular nitrate (Fig. 8) between 0700 hr and 1100 hr (as was the case for 105%) and it can be seen that there was a rapid decrease of NR activity between 0500 hr and 1100 hr.

The phasing between cellular nitrate accumulation and the protein specific nitrate reductase activity seems to exist at all growth rates and the phase lag would be in the region of one to two hours for Chaetoceros sp. (STX-105), under the present conditions of growth.

Except for the 30% turnover rate which is characterized by a great stability in every respect, it can be seen that the population characteristic, cell number, increased during the dark period, for the 60%, 87% and 105% dilution rates whereas washout seems to be imminent at the 116% turnover rate (Fig. 2) and the cell numbers was only just maintained during the dark period. Also during the dark period, the population characteristic, protein, decreased as a general rule (Fig. 3) and the protein specific nitrate reductase activity increased (Fig. 22). This implies that the enzyme NADH-dependent nitrate reductase represents a small proportion of the total protein and it can rapidly reduce the intracellular nitrate. For example, at the 116% dilution

rate the protein decreased continuously during the dark period (Fig. 3) and reached a low concentration of 45 ug Protein-N/l at 0500 hr, whereas the protein specific activity is high (Fig. 22) for the same time. Another implication is that the enzyme, nitrate reductase, must have a high turnover rate to account for the quick increase and decrease in activity mentioned above. Zielke and Filner (1971) have demonstrated that there is a continuous turnover of NR enzyme in cultured tobacco cells.

Observation of the diel periodicity of NR activity expressed on the basis of PN and chlorophyll a, in the Peru current by Eppley et al. (1970), gave comparable diel variations to those in figures 19 and 21 where a maximum occurs during the photoperiod (the exact time depending on the dilution rate) and a minimum is obtained during the late dark period. However, a slow growth rate such as the 30% dilution rate practically does not exhibit any diel periodicity. A similar finding has been reported by Packard and Blasco (1974) who showed that there was no diel variation of NR activity of phytoplankters in the Genyaulax polyedra populations off Baja California.

Packard (1973) has shown the light dependence of nitrate reductase in marine phytoplankton and Hodler et al.(1972) using synchronous cultures of Chlorella pyrenoidosa, showed that the onset of the photoperiod results on a sharp increase in the specific activity of NR expressed on the basis of Lowry protein. The results of this study shows that there is a decreasing protein specific NR activity for the

Chaetoceros cells as soon as the photoperiod begins (Fig.22) and increases when the dark period begins except for slower growth rate such as the 30% dilution rate and 60% to a certain extent (Fig. 22). The amplitude of this diel variation increased as the turnover rate increased up to the 105% dilution rate and levels off. Thus nitrate reductase activity per ug protein does not respond positively to day/light. In an attempt to investigate the effect of light exposure on NR activity of the Chaetoceros population, samples were taken from the continuous culture during the dark period and were incubated for increasing lengths of light exposure (up to two hours). The results showed that the protein specific nitrate reductase activity did not increase but diminished slightly as the period of exposure increased.

The fact that, on a diel basis NR activity does not respond positively to day/light when expressed on a protein basis but that it does when expressed on the basis of PN, chlorophyll a and cell numbers (For the 87%, 105% and 116% turnover rates) can be explained by the magnitude of the diel variation of the population characteristic (Table 6) used to express the specific NR activity. It can be seen from figure 3 that the protein increased and reached a maximum during the photoperiod, and it had a coefficient of variation 4 and 5 fold higher than the coefficients of the population characteristics PN, chlorophyll a and cell numbers (Table 6) for the three higher dilution rates. It is for this reason that the protein specific NR activity

was lower during the photoperiod.

This last paragraph might suggest that the rate of intracellular nitrate reductase as measured by NR activity would not directly depends on light energy, on a diel basis, for each dilution rate, except 30% where this does not show up, because of its stability. Then, this brings up the question: What is the relationship between growth rate and NR activity when expressed on the basis of different population characteristics? Obviously the growth of Chaetoceros sp. (STX-105) is dependent on light energy, but changing the dilution rate of the continuous culture (growth rate) automatically changes the length of light exposure for one generation time. Thus as the growth rate increases, the cells are exposed to less light energy for each division. Figure 23 shows the mean NR activity expressed on the basis of the most stable population characteristic, PN, for each turnover rate. It can be seen that the NR activity increases hyperbolically with growth rate and that a plateau is quickly reached above the 60% dilution rate. Table 7 shows the specific NR activities expressed on the basis of decreasing population characteristics stability. It should be noted that the deviation from the mean ($\pm$ 1 SD) is mainly due to the diel variation of the population characteristic. The main feature that comes out from table 7 is that the specific nitrate reductase activity increases rapidly as the turnover rate increases from 30% to 60% dilution rates and then a plateau is reached, except when it is expressed per chlorophyll a. Eppley and Renger (1974) found that the

mean NR activity (expressed on the basis of cell numbers) was inversely related to the dilution rate. However, they expected the opposite based on previous work (Eppley et al., 1969a) and Eppley et al., 1970). Results obtained in this work show that there was an increase especially from 30 to 60% turnover rates (Table 7) where a plateau is reached. The further increase at the 116% turnover rate is due to a decrease in cell numbers (Fig. 9). The NR specific activity on a basis of Lowry protein (Table 9) increases up to 105% turnover rate and levels off. We have already seen that the protein content at the 105% and 116% turnover rates are about the same (Fig. 9). The specific NR activity based on POC (Table 9) constantly increases and this is not surprising, since the POC concentration is a function of the dilution rate (Fig. 9).

Figure 23 shows that, as the turnover rate increases, a plateau is reached for NR activity in the region of the 60% turnover rate indicating a saturation level for the enzyme nitrate reductase when expressed on the basis of the most stable population characteristic, PN. Saturation implies that the amount of enzyme is limited. Since the ultimate limitation is light energy, the specific NR activity in terms of ug of chlorophyll a should indicate where the energy limitation begins its effect as the turnover rate increases. It can be seen from table 7 that the highest mean value of chlorophyll a specific NR activity is reached at the 60% dilution rate and decreases for higher dilution

rates. In other words these values represent the nitrate assimilation efficiency with increasing growth rate (T. T. Packard, personal communication). Another way to show that energy limitation in the culture was introduced in the region of the 60% turnover rate is the relationship between the population characteristic chlorophyll a and the number of hours of light exposure that the Chaetoceros cells had, for a generation time at each dilution rate. Figure 24 shows that there was practically no increase in chlorophyll a per liter of culture as the dilution rate increases from 30% to 60%; however, for higher growth rate, there is an increasing demand of energy.

The activity of the NADH-dependent nitrate reductase (NR) of Chaetoceros sp. (STX-105) in our work was up to two orders of magnitude higher than previously reported data in the literature (Table 9). For example the NR activities in synchronous culture of Chlorella pyrenoidosa (Hodler et al., 1972), in higher plants (Schrader et al., 1968) and of Thalassiosira pseudonana grown in continuous culture with light and dark cycles (Eppley and Renger 1974) are in the same order of magnitude as for the NR activities of Chaetoceros sp. (STX-105). However, increasing discrepencies are found (Table 9) with the values of LeClair and Grant (1972), Eppley et al. (1969a), Eppley et al. (1970) and Rigano (1971). It is interesting to see that the results of the present study agree with other laboratory cultures (Eppley and Renger 1974) who used the oceanic diatom

Thalassiosira pseudonana while large differences are observed with field data (Eppley et al., 1970); this could be due to different size classes of phytoplankton. Malone (1971) showed that nannoplankton were responsible for 80% of the phytoplankton productivity, off the California coast, while Eppley et al. (1970) used net plankton for the nitrate reductase assay. Thus, it is probable that an important fraction of the phytoplankton was excluded from the enzyme assay. Another reason would be the species-specific response in terms of NR activity, especially when different inorganic nitrogen sources are used in the culture medium or are present in the field.

## 4.2 Cellular characteristics

If the growth rate has a physiological basis its magnitude should be reflected in cell composition. A summary, of the cellular characteristics for each turnover rate is given in table 9. It can be seen that the amount of chlorophyll a per cell does not increase significantly as the turnover rate increases from 30% to 60%, suggesting that energy supply via photosynthesis does not limit growth at these growth rates. This is reflected in the relatively high concentrations of cellular characteristics for the 60% dilution rate as compared with higher turnover rates. For example, carbohydrate represents one important form of cellular energy reserve and table 9 shows that there was not a major decline from 30% to 60% dilution rates. However, as the turnover rate is increased by a factor of 2 from 60%

to 116% the concentration of chlorophyll a per cell also increased by a factor of 2 (0.27 ± 0.03 to 0.55 ± 11 µg Chl a/$10^7$ cells) as shown in table 9. This cellular demand for light energy was reflected in the large decrease of the cellular energy reserve (carbohydrate) as the turnover rate increases from 60% up to 116%. Eppley and Renger (1974) found that chlorophyll a increased by more than 500% for Thalassiosira pseudonana when they increased the turnover rate from 33% to 100%. Thomas and Dodson (1972) found that the chlorophyll a content of the diatom Chaetoceros gracilis doubles as the growth rate increases from 0.57 division/day to approximately 3.0 division/day; however, they used continuous light exposure for their culture, which precludes direct comparison with the present results.

The rate of photosynthesis per unit chlorophyll a however reaches a maximum at the 60% dilution rate as shown in table 10. Thomas and Dodson found slightly increasing assimilation ratios for Chaetoceros gracilis and Eppley and Renger (1974) found a similar steady increase for Thalassiosira pseudonana with increasing dilution rates. Our results show that the rate of photosynthesis per unit chlorophyll a for the Chaetoceros sp. (STX-105) increases up to the 60% dilution rate (Table 10), after which it consistently decreases. These rates were obtained with the ambient light intensity for the culture and no attemp was made to determine if the rates of carbon fixation were light saturated. The pattern followed by the photosynthetic rate per unit chloro-

phyll a as a function of the turnover rate (Table 10) is another indication that light-limitation is introduced between the 60% and 87% turnover rates.

Another interesting cellular characteristic is the intracellular nitrate concentration which does not change significantly between the 30% and 60% turnover rates but increases for higher dilution rates by a factor of 5 (116% turnover rate). This phenomenon has been demonstrated (indirectly or directly) for many organisms for different limiting nutrients, for example, with nitrate for Isochrysis galbana (Caperon 1968) with vitamin $B_{12}$ for Monochrysis lutheri (Droop 1968), with phosphorus for Cyclotella nana (Fuhs 1969), with nitrate for Monochrysis lutheri and Cyclotella nana (Caperon and Meyer 1972), with iron for Monochrysis lutheri (Droop 1973a) and with silicon for Thalassiosira pseudonana (Paasche 1973), and with nitrate for Chaetoceros sp. (STX-105) by Malone et al. (1975). Compared with the continuous culture study of the same organism (Malone et al., 1975) the intracellular concentrations of the present study are approximately 3 to 4 times smaller. This could be due to differences in light intensities as mentioned previously. It should be pointed out that Malone et al. (1975) used an aqueous rather than an acid extraction for the measurements of the cellular nitrate. However, both of us proceeded by a direct measure of the nitrate in the extract, which can be termed "measured" cellular nitrate; whereas Caperon (1968) obtained the concentration of intracellular nitrate by an indirect method, which

can be termed "calculated" cellular nitrate.

The "calculated" intracellular nitrate content is obtained by the relationship between the yield coefficient, $q_i$, and dilution rate. ($q_i$ = PNi/ni where PNi and ni are the particulate nitrogen and the cell density at the $1^{th}$ steady-state dilution rate). This relationship is shown in figure 25. By extrapolation to zero dilution rate (Fig. 25) a "$q_o$" value of 0.0290 µ µg-at PN/cell is obtained. Values of 0.0293 and 0.0328 µ µg-at PN/cell were obtained for Isochrysis galbana (Caperon 1968). By subtraction of the "supposed" fixed value (Caperon 1968) of "$q_o$" from the yield coefficients "$q_i$" then, the nitrate reservoir of the cells or the "calculated" cellular nitrate can be estimated for each dilution rate (Caperon 1968) and the results are shown in figure 26. These values of "calculated" cellular nitrate are in good agreement with the data of Caperon (1968).

If the "measured" intracellular nitrate concentration is plotted as a function of dilution rates (Fig. 27), another hyperbolic curve is obtained but, there are two main distinctions. The shape of the hyperbola is much steeper in the case of the "measured" cellular nitrate, indicating that the saturation is quickly reached and, second, the absolute quantity of cellular nitrate is much smaller if measured rather than calculated. Table 11 shows clearly the differences between calculated and measured cellular nitrate and it seems that most of the calculated intracellular nitrate is not nitrate.

Table 9 shows that particulate nitrogen per cell increased as the turnover rate increased while the protein per cell decreased. Table 12 shows the relative amounts of nitrogen in protein, free amino acids, cellular nitrate and chlorophyll *a*, as a percentage of the total nitrogen, for each turnover rate. Although it is true that intracellular nitrate increased with increasing growth rate, it cannot explained the increasing total nitrogen per cell as the dilution rate increased, neither free amino acids and chlorophyll *a* increases can account for this. It is believed that the protein concentrations determined by the Lowry method (Table 6) are underestimated, in view of recent results of Dorsey et al. (1976) who found that prolonged heating is required to obtain quantitative extraction of protein from phytoplankton cells by the Lowry technique. Table 12 clearly shows that the intracellular pool of nitrate cannot, unlike other nutrients, be calculated from the yield coefficient. As the growth rate increases, the increasing yield coefficient "$q_i$" is mainly in chemical forms other than $NO_3^-$. This is not surprising in view of the multiple metabolic pathways open to $NO_3^-$ metabolism versus the very limited chemical transformation of other nutrients such as phosphate, vitamin $B_{12}$ and silicate. Table 11 shows that, except for the 30% turnover rate, less than 5% of the increase of the nitrogen yield coefficient is due to intracellular nitrate estimated by direct measurement.

Since the particulate nitrogen per cell increases as

the turnover rate increases (Table 9) then, this raises the question of what form(s) of nitrogen is increasing in each cell as the growth rate increases? One of the most important groups of nitrogen containing compounds that should be considered is the protein; however, the results (Table 9) shows that the amount of protein per cell (as measured by the Lowry technique) decreased as the growth rate increased, down to an "apparent" minimal value of 0.09 µg-at Prot-N/$10^7$ cells. Effectively, the percentage of particulate nitrogen present as protein is 57, 42, 38, 24 and 21% for the 30%, 60%, 87%, 105% and 116% turnover rates respectively (Table 12) and follows the model of Grenney et al. (1973). The protein concentration may be underestimated, as mentioned earlier, however, the decreasing trend of protein content per cell as the dilution rate increased (Table 9), exclude this form of nitrogen to account for the increasing yield coefficient "$q_i$". Since particulate nitrogen is often used to calculate the cellular protein content, for example by Hobson and Parizer (1971) it is interesting to note the tremendous difference between protein determined by the Lowry technique and total particulate nitrogen (Table 12). The decrease in protein per cell is consistent with the accumulation of the building blocks of protein in the cells as the turnover rate increased (Table 3); several free amino acids were below the detection level at the lowest turnover rate (30%) and the total free amino acid pool was smallest which is consistent with a high degree of nitrogen limitation. In

other words, the limited supply of inorganic nitrogen as nitrate ions is readily transformed into cellular protein at low turnover rates. Lui and Roels (1972) have demonstrated for the organism Biddulphia aurita that there is a negligible level of amide nitrogen in cells grown in a medium with nitrate as the nitrogen source; this is also true for the diatom Chaetoceros sp. (STX-105) since glutamine and asparagine, which would represent most of the amide nitrogen are absent (Table 3).

The accumulation of free amino acids in the cells, as the dilution rate increases (Table 3) indicates a rate limiting step at the protein synthesis level. However, the increment of free amino acids is not sufficient (Table 12) to justify the increasing yield coefficient, $q_i$, (Fig. 25) as the dilution rate increases. Therefore, it may be suggested that the continuous decrease in protein/cell, as the dilution rate increases, triggers cellular mechanisms for more protein synthesis. At this point, more nitrogen would be channeled to nucleic acid synthesis (Kolata 1974) since we know that the ribosomes represent the "work bench" for protein synthesis, this should imply a larger amount of nitrogen in the form of ribosomal nucleic acid (r-RNA). This hypothesis could be supported firstly by the fact this phenomenon has been observed with yeast ( Mc Murrough 1967 ) and secondly by the results of Eppley and Renger 1974, who showed that as the growth rate increased the GDH activities calculated as specific rates ($day^{-1}$), passed from 8 to 6, finally to 4

times the actual growth rate. This is an indication that, as the growth rate increases, less intracellular ammonia is used for amination of alpha ketoglutarate, and more could be used to form carbamyl phosphate, which is a precursor of nucleic acids.

Knowing that the measured cellular nitrate represents a small portion of the calculated cellular nitrate for all dilution rates studied (Table 11), then the remaining fraction (approximately 95%) of this calculated cellular $NO_3^-$ is the varying $q_o$, which would be organic nitrogen. The variation of "$q_o$" with increasing dilution rates would follow the curve in figure 26; the absolute quantity represented by "$q_o$" for each dilution rate being the calculated cellular nitrate minus the measured cellular nitrate. Thus, $q_o$, increases hyperbolically with increasing dilution rates and should not be considered as a constant value for the calculation of intracellular nitrate pools of cells preconditioned at different growth rates, contrary to Caperon (1968).

A possible reason to explain the decrease of protein per cell as the turnover rate increases (Table 9) would be that the cell volume decreases as the dilution rate increases, however, this would obviously not explain the rising concentrations of particulate nitrogen per cell (Table 9). In the present study the size of the Chaetoceros cells was measured on electron micrographs and no significant variations were observed. On the other hand, frequent cell counts with the light microscope revealed a decrease in cell size

as the growth rates increased especially during the late dark period. More work needs to be done on the exact determination of cell size as a function of different growth rates at steady-state.

Table 9 shows that the particulate organic carbon (POC) of the Chaetoceros cells decreased as the dilution rates increased, except for the highest dilution rate where steady-state could not be maintained. The results agree with those of Malone et al. (1975) who have grown the same organism Chaetoceros sp. (STX-105) in large outdoor continuous cultures, and those of Caperon and Meyer (1972) for Coccochloris stagnina, Cyclotella nana, Monochrysis lutheri and Dunaliella tertiolecta. In this respect, it is surprising to see that the cellular carbon content of Thalassiosira pseudonana increased as the dilution rate increased (Eppley and Renger 1974).

The carbohydrate content of Chaetoceros cells also decreased as the dilution rate increased (Table 9). Also as the dilution rate increases, the carbon content of each cell becomes smaller and this can be explained by the fact that the ultime source of energy for these cells (12 hours of light/day) was constant and thus each cell had less light exposure as the dilution rate increased, i.e., inversely proportional to the residence time in the chemostat. This phenomenon of less light exposure is compensated for by an increase of chlorophyll a per cell (Table 9). Figure 28 shows that there is an inverse linear relationship between chlorophyll a and particulate organic carbon, which reflects

the dependence of the Chaetoceros cells on light energy for the production of organic cellular matter, under the present conditions of growth.

Table 9 shows the difference between the rate of primary productivity and photosynthetic capacity as the turnover rate increased. The values are similar for the slowest dilution rate but the difference between the two sets of data increases as the turnover rate increased. The faster increase of the photosynthetic capacity is due to the increasing dark minimum rate of the photosynthetic capacity. Table 13 shows this difference expressed as a percentage. It can be seen that primary productivity reached a plateau between the 60% and 87% dilution rates, whereas the photosynthetic capacity increased up to the 105% turnover rate and leveled off. Primary production by phytoplankton is most frequently measured by photosynthetic oxygen evolution or by carbon-14 uptake. Comparisons of the different techniques to estimate primary production were reported by many workers, i.e., Antia et al. (1963), Eppley and Sloan (1965), McAllister et al. (1964). Many carbon-14 measurements were done at sea, or in laboratory cultures, where the past history of the cells was unknown. The importance of knowing this history is evident from the finding of Griffiths (1973) who observed that the photosynthetic capacity per cell does not remain constant during the exponential phase of growth. From the primary productivity data it can be concluded that the uptake of carbon-14 during the dark period is negligible.

In this respect, our results disagree with those of Morris et al. (1971a) who found that the dark uptake is significant, i.e., 5400 CPM for Dunaliella tertiolecta; our dark fixation counts were always lower (200-600 CPM). The uptake of carbon-14 during the photoperiod reached a maximum value for each turnover rate and this maximum was shifted towards the beginning of the light period as the turnover rate increased.

The cellular POC (Fig. 6) and cellular carbohydrate content (Fig. 7) showed a decrease from the 30% to the 60% turnover rate at 0900 hr. This was associated with a shift of the maximum carbon-14 uptake during the day from 1700 hr to 1500 hr (Figs 12 and 13). A similar change was observed between the 60% and 87% turnover rates. For all turnover rates the POC and carbohydrate cellular content reached their minimal values at the end of the dark period. However, except for the 116% turnover rate, the absolute quantity of carbon per cell decreased as the dilution rate increased (Table 9) indicating that these cells became relatively depleted in carbon, this is reflected in higher rates of carbon-14 uptake during the early light period. Surprisingly, there was no shifting of the minimum photosynthetic capacity during the dark period as the turnover rate increased, the minimum was always close to 0300 hr (Figs 12 to 16). However, the dark minimum of the photosynthetic capacity (Figs 12 to 16) shows a gradual increase as the dilution rate increased. In fact the minimum values are approximately 35, 100, 135, 200 and 230 $\mu g\ C.l^{-1}hr^{-1}$. for the 30%, 60%, 87%,

105% and 116% turnover rates, respectively. This increase is correlated to the POC value at 0900 hr (Fig. 30) but not with the mean of the 24 hour period.

Thus, even if the dark uptake is negligible for the culture, the physiological state of the cells is different during the dark period for increasing turnover rates. Cells are better prepared for high photosynthetic rate as the photoperiod began, at high turnover rates. The continuous increase of the dark minimum of photosynthetic capacity as the dilution rate increased could be explained by the fact that the carbon-14 uptake system goes from a high activity during the photoperiod to a lower one during the dark period; the "inducer" of the system increases with increasing dilution rate and remains in the cells during the dark period, resulting in higher values for the photosynthetic capacity during the dark priod. Another possible explanation of the continuous increase of the dark minimum of the photosynthetic capacity as the dilution rate increased, could be the decreasing length of light exposure per generation time as the dilution rate increased. This is endorsed by the finding of Griffiths (1973) who showed that light exposure had a deleterious effect on the photosynthetic capacity of Phaeodactylum tricornutum, whereas, dark incubation had a benificial effect. A similar phenomenon occurred in the present study, since the cells were exposed to 40, 20, 13.8 11.5 and 10.2 hours of light per generation time for the 30% 60%, 87%, 105% and 116% dilution rates, respectively. Thus, the cells grown at higher dilution rate would have higher

photosynthetic capacity.

The relative degree of variation of the cellular characteristics at each dilution rate and between them is shown in table 14. It can be seen that the 30% turnover rate had the smallest variation. This is primarily due to the relatively high energy (light energy) available to the cells for each generation time; since the growth rate was low then it obviously implies a low metabolic rate. This is shown in table 9 where the rates of photosynthesis and nitrate reductase are the lowest at 30% turnover rate. The biggest oscillations were always observed at the highest or close to the highest turnover rate (Table 14).

Particulate nitrogen per cell was the most stable for each turnover rate, reflecting its small diel periodicity. However, the coefficient of variation for all dilution rates is slightly higher (15%) than for the particulate carbon (12%), but the larger diel variation of particulate carbon at any particular dilution rate is reflected by higher coefficient of variation (Table 14). The chlorophyll *a* and protein per cell are the next two most stable cellular characteristics. It is interesting to note that the coefficients of variation of chlorophyll *a*, for each dilution rate, are relatively small, but larger for all dilution rates whereas the converse is true for protein. The greatest coefficients of variation for all cellular characteristics were for carbohydrate and intracellular nitrate. This can in part be accounted for by the high activity of these

cellular pools, that is, they are built and consumed rapidly on a diel basis. The high coefficient of variation for all turnover rates is a consequence of the larger variation of the mean value for each turnover rate. The photosynthetic rate per cell and nitrate reductase were quite stable at each dilution rate.

## 4.3 Relationship between nitrate uptake, growth rate and photosynthesis.

For each dilution rate, the residual nitrate concentration in the culture vessel was low (Table 2) and did not show any consistent diel periodicity. This is in agreement with the results of other workers using continuous unialgal cultures with light and dark cycles. Malone et al. (1975) had complete removal of nitrate from large outdoor continuous culture of *Chaetoceros* sp. (STX-105), except at their highest dilution rate, Eppley et al. (1971) and Eppley and Renger (1974) also had similar results with the organisms *Coccolithus huxleyi* and *Thalassiosira pseudonana* respectively. This is consistent with the finding of Caperon and Meyer (1972a), that growth rate cannot be directly related to the residual nitrate concentration in the growth chamber. However, other species of phytoplankton, such as *Skeletonema costatum*, showed diel variation in nutrient uptake (Eppley et al., 1971); this diel periodicity was also observed for natural populations in the Peru current (Eppley et al., 1970).

In the present study, even at the highest dilution rate

(116%), the residual nitrate concentration was low (0.13 ± 0.26 µg-at, see table 2) and increased only when the population collapsed. This indicates that the rate of nitrate uptake was not a limiting step in the process of nitrogen incorporation into cellular protoplasm. Another implication is that the energy required for uptake (Falkowski 1974, 1975) is not only supplied directly from photosynthesis (i.e., ATP produced by photophosphorilation) but also from other photosynthates, at least during the dark period since the uptake rate was constant during steady-state over the diel cycle.

Caperon and Meyer (1972b) showed that there was an increase in Vm for a corresponding increase of the growth rate. The results of uptake experiments in the present study for the fresh population (other populations i.e., fed and starved are not comparable) agree with this finding when Vm is determined by the short-term uptake technique (Table 5). Although there are only two points, they represent a large difference in growth rate (30% and 87% turnover rates). The explanation of Caperon and Meyer (1972b) for their data was that the number of uptake sites per cell increased as the supply of nitrate increased. Since those uptake sites responsible for nitrate uptake are protein (Falkowski 1974), it would imply that as the dilution rate increases, the decrease in protein content per cell predicted by Grenney et al. (1973) and observed in the present study (Table 9) is probably not affecting the protein needed for nitrate

uptake and hence the number of uptake sites. In other words the protein constituting the uptake sites are still synthesized by the cells and are operating at high dilution rate (near washout) at the expense of other cellular protein which would decrease (Table 9).

The preceding discussion of Vm does not explain how the cells can have a higher Vm as the dilution rate increases. Whether it is by increasing the number of uptake sites or by another cellular mechanism which would act as a supplement when needed is unknow. However, Caperon and Meyer (1972b) stated: "The kinetics of nutrient uptake are well described by a Michaelis-Menten type hyperbola with two constants, $K_s^u$ and Vm. Under these conditions $K_s^u$ is the ratio of two rates constants in the uptake process. Thus significant variation in $K_s^u$ indicates that a different uptake mechanims is operating." The results of the present study for the determination of $K_s^u$ of nitrate uptake for the fresh population (the fed and starved populations are not comparable) by the short-term uptake technique (Table 5) are consistent with the constancy of $K_s^u$ at different growth rates, since $K_s^u$ was $0.26 \pm 0.07$ for the 30% turnover rate and $0.84 \pm .17$ for the 87% turnover rate. This difference is substantial when compared with the values reported in the literature (Eppley et al., 1969b). The implication of such findings would be that the mechanism of nitrate uptake changes according to the degree of nitrogen limitation, (that is, as a function of the dilution rate). Thus, cellular physiological changes

would result from different states of preconditioning of cells and would influence both Vm and $K_s^u$. Another important implication is that, for each different preconditioning of the same organism, a different $K_s^u$ would be obtained, which would be incompatible with the classification of organisms according to their $K_s^u$ value for uptake of nitrate (Eppley et al., 1969b). Droop (1973a) has mentioned that, depending on the treatment given to the cells, they could be in a transient state (indeterminate nutritional status) resulting in questionable measurements. However, Caperon and Meyer (1972b) and Eppley and Renger (1974) did not find significantly different $K_s^u$ for nitrate uptake as the dilution rate increased. More work should be done to elucidate this problem. Attempts to determine the nitrate uptake kinetics constants $K_s^u$ and Vm by two different techniques (Table 5) with the same population "fresh" suggest that the discrete nitrate uptake technique used by Caperon and Meyer (1972) gives systematic errors. Indeed, it seems that the $K_s^u$ cannot be measured accurately by this technique (J. J. MacIsaac, personal communication).

The efficiencies of nitrate uptake and assimilation are shown in table 15. The 96% and 98% nitrate uptake efficiencies observed for the 60% and 87% turnover rates compared to better than 99% at the other turnover rates was probably due to incomplete mixing of the culture vessel in these experiments, since increased aeration resulted in complete nitrate removal at even higher turnover rates. The measured intra-

cellular nitrate was small for each dilution rate (Table 15) and varied from 0.26% to 0.98% of the nitrate taken up by the cells. The increasing intracellular nitrate pool, as the dilution rate increased, was shown to be a small portion of the total cellular nitrogen (Table 12). However, we know that the cellular nitrate was in phase with the protein specific nitrate reductase, with a phase lag of one to two hours. This implies that the intracellular nitrate was reduced to nitrite by the enzyme NADH-dependent nitrate reductase.

Table 16 shows that the rate of nitrate uptake (v) in the culture at steady-state, the maximum rate of nitrate uptake (Vm) and the rate of nitrate assimilation as determined by the NR activity for each dilution rate: v was calculated from the data in table 2, the NR rates were taken from table 7 and Vm values are those of table 5 for fresh cultures as determined by the short-term uptake technique.

From the results in table 16, it is obvious that the rate of nitrate uptake (v) at steady-state is not a limiting step, since it never reaches more than 9% of the maximum rate of nitrate uptake (Vm). The ratio v/NR shown in table 16 shows that the rate of nitrate reduction by the enzyme NR is sufficiently high to account for all the nitrate ions which are taken up by the cells for all growth rates. This is in contradiction with the results of Eppley et al. (1969a) and Eppley et al. (1970), who reported that nitrate reduction by NR accounted for 25% and 15% of the nitrate

taken up by the cells (v). However, neither of their populations were preconditioned at steady-state. Since Vm is 10 and 50 times greater than v (Table 10) depending on the growth rate, it is possible that the rates of nitrate uptake (v) of their cultures was close to Vm. This would in turn give a smaller v/NR ratio. As seen in table 16, the values of NR/Vm in the present study are comparable to the values found in their work.

It would have been interesting to verify the fact that NR/Vm reached a plateau or a maximum value of approximately 30% (Table 16). This could be done by determining the rate of $NO_3^-$ uotake by the cell with a high $NO_3^-$ concentration in the medium (e.g. 100 µg-at $NO_3^-$). It is expected that after a given period of time the rate of $NO_3^-$ uptake will decrease below the maximum rate (Vm) because the intracellular $NO_3^-$ pool will be full and the NR activity will then control $NO_3^-$ uptake at a rate of 30% of the maximum rate of nitrate uptake Vm.

Examination of table 16 shows that nitrate reductase activity increased by a factor of 2.4 (0.0027 at 30% and 0.0065 at 116% turnover rates) while the growth rate increased by approximately a factor of 4. The increment of NR activity was due to the synthesis of additional NR enzyme per cell, since all the in vitro enzyme assays were nitrate saturated. The difference (4-2.4=1.6) is probably due to the fact that the NR enzyme (in vivo) were not substrate saturated at low dilution rate but they were at higher

dilution rates. This is supported by the marked increase of cellular nitrate for the 105% and 116% turnover rates (Table 9).

Thus far, neither nitrate uptake nor nitrate reductase rates appears to be rate limiting step in the flow of inorganic nitrate thru the Chaetoceros cells. However, the high proportion of glutamic acid of the free amino acid pool and its increasing absolute quantity as the grwoth rate increased, suggest that the reduced nitrate is incorporated into the amino acid metabolism by amination of alpha-ketoglutarate by the enzyme glutamate dehydrogenase. The large increase in the free amino acid pool as the growth rate increased (table 9) especially from the 87% to 105% turnover rates, indicates that protein synthesis is the rate limiting step at high growth rate. It seems that cells ability to capture energy (light energy) is not the limiting factor since the chlorophyll a/cell doubled as the turnover rate increased from 60% to 116% (Table 9). However, we know that the photosynthetic rate per unit chlorophyll a did not double but, decreased as the turnover rate increased from 60% to 120% (Table 10). This could be a consequence of a decreasing pool of enzyme (Protein, see table 9) responsible for the production of photosynthates or a lack of further photosynthetic energy. Accordingly, the limited amount of protein synthesized could be limited either, by ATP (from photophosphorylation) or by intrinsic rate limitation of this cellular process. It is not possible, from the experimental evidence, to determine weather the limitation of cells growth

rate is caused by the limited availability of light energy or by the rate at which protein can be synthesized.

Table 9 shows that the quantity of protein per cell, decreased to an apparent minimum value as the growth rate increased near washout (105%). The highest growth rate (116% turnover rate) resulted in cells still having the minimum amount of protein per cell in order to survive or perform a normal cell division cycle. However, they had to produce this minimum amount of protein per cell in a shorter time and it appears that they simply could not, since steady-state growth could not be maintained.

Assimilation efficiencies based on protein and particulate nitrogen (mean concentration divided by incoming nitrate concentration) are shown in table 15. As the growth rate increases, less nitrate is transformed into protein. However, consideration of assimilation efficiency in terms of nitrate transformed into particulate organic nitrogen gives much higher values (Table 15). Therefore, it is important to define specifically the nature of the form of nitrogen used for assimilation efficiency calculations. This is even more important when considering the eventual use of the produced cells in a natural or artificial food web, since cells having similar assimilation efficiencies (based on PN) but different protein contents may have greatly different nutritional value to higher trophic levels. For example, it can be seen from table 15, that cells preconditioned at 30% and 116% turnover rates gives assimilation efficiencies,

based on protein, of 50.4% and 23.1% respectively. This decreasing trend is supported by the accumulation of free amino acids in the cells as the turnover rate increased (Table 12).

Table 15 shows that for the slowest turnover rate (30%) some nitrogen compounds must be excreted to the medium since the assimilation based on PN was the lowest (90.2%) while the nitrate uptake efficiency was 99.2%. The second lowest assimilation efficiency based on PN was obtained at the 116% dilution rate, which agrees with the pattern observed by Malone et al. (1975) who also found maximum assimilation (production) efficiency at intermediate dilution rates. The low efficiency of nitrate assimilation based on PN for the lowest dilution rate could be explained by excretion of nitrogen containing compounds since the metabolic rate of those cells was low. These cells were not as healthy as fast growing cells and probably lysed more easily, accounting for some of the nitrogen which is not recovered. This would imply that carbon containing compounds may also be lost or excreted. It is interesting to compare the daily carbon fixed as estimated by the carbon-14 technique, using short time incubation with the daily POC production measured on discrete samples, using the combustion technique. Table 17 shows that a significant percentage of the fixed carbon at the slowest dilution rate is not recovered in the form of particulate organic carbon. As the dilution rate increased this percentage decreased. This may indicate that POC production has

reached a maximum value, suggesting either that energy limitation has been reached or that intracellular carbon recycling has become a significant supplementary mechanism of carbon supply, resulting in a smaller rate of carbon-14 uptake.

Eppley and Sloan (1965) have concluded that the carbon-14 technique gives the best estimate of net production, defined as increase in phytoplankton cell carbon. However, the present work includes variable growth rates which were absent in their comparative study. Thus, it is possible that the decreasing excess of carbon-14 taken up versus the PCC production, as the dilution rate increased, (Fig. 30) could be due to different metabolic rates.

The difference between carbon fixation and production (Table 17) should be near zero at the 105% turnover rate (Fig. 30); it should be the approximate maximum turnover rate at which the steady-state continuous culture can be maintained, under the prevailing experimental conditions. The plot of the percentage of excess carbon-14 taken up (Table 17) against the number of hours of light exposure corresponding to a generation time for each dilution rate (Fig. 30) gives 0% carbon excess at 11.4 hours which corresponds to a turnover rate per day of 105%; from figure 30, one can see that the experimental percentage obtained for this particular turnover rate deviates from the curve. Unfortunately, this turnover rate could not be maintained because of mechanical failure as mentioned earlier. This

resulted in fewer data for this particular dilution rate as shown in figure 6 for the mean POC and figure 15 for the carbon-14 uptake rate.

On the other hand, excretion could also explain in part, the excess of carbon-14 taken up as shown in table 17 and figure 30. Figure 18 shows that the C:N ratio decreased from 19, 15, 13, 12 and 11 as the turnover rate increased from 30%, 60%, 87%, 105% and 116% respectively. The 30% dilution rate had the highest C:N ratio and also the lowest nitrate assimilation efficiency based on PN (Table 15). This indicates that excretion probably took place at the lower growth rate. Hellebust (1965) has demonstrated that Chaetoceros simplex, excreted between 17 and 25% of its organic carbon when grown in batch culture. Analogy with the behavior plants (Black 1973) suggest that the major excreted compound is glycollate.

Light mediated carbon utilization in a nitrogen-limited continuous culture of Chaetoceros sp. (STX-105), cannot be divorced from nitrogen utilization. It has been shown that chlorophyll a reflects the cellular energy demand. Then the rates of nitrate reductase activity and photosynthesis per unit chlorophyll a represents key points in the nitrogen and carbon metabolisms of the cells. Figure 31 shows that there is a striking similarity between those two curves and they have the same trend as the nitrate assimilation efficiency based on PN (Table 15). Such indexes of N and C assimilation both indicates that the 30% turnover rate is the least effi-

cient. These cells had high concentrations of cellular components (Fig. 9) with a low metabolic rate giving unhealthy cells. The low assimilation efficiency is probably accounted for by a combination of factors, among other, cell lysis and excretion. The maximum efficiencies of N and C assimilation (Fig. 31) are in the vicinity of the 60% dilution rate, which was also the most efficient on a PN basis (Table 15). It was shown that the cellular characteristics of that particular growth rate were still relatively high (compared to the 30% turnover rate, see fig. 9) and that cellular processes such as NR and photosynthesis, almost reached a plateau (Fig. 9). This indicated that these cellular processes were now operating at higher velocities without severe depletion of the cellular characteristics components. Finally, as the turnover rates increased to 87%, 105% and 116%, the efficiencies also decreased (Fig. 31), similarly to table 15. Protein synthesis was shown to be the rate limiting step and the lower efficiency can be accounted for by the intracellular accumulation of nitrate, free amino acids and presumably carbohydrate precursor compounds.

It seems that overall, the nitrogen metabolism governs the cells and that any drop in photosynthesis as the growth rate increases may be due to the rate limiting step of protein synthesis in Chaetoceros sp. (STX-105), when grown in a nitrate limited continuous culture.

# 5 CONCLUSIONS

From the results of this study on the diatom Chaetoceros sp. (STX-105) under the present conditions of growth, it may be concluded that the most stable population and cellular characteristics is particulate nitrogen. The most active cellular pools were nitrate and carbohydrate. As a general rule larger diel oscillations were observed with increasing growth rate. The effects of increasing growth rate on the mean cellular characteristics resulted in an increased cellular chlorophyll a, nitrate and particulate nitrogen and a decreased cellular protein, carbon and carbohydrate. The diel periodicity of NADH-dependent nitrate reductase (NR) activity was found to vary systematically depending on the specific activity used. Protein specific NR activity was shown to be corrolated with the intracellular nitrate. As the growth rate increased the NR activity increased hyperbolically and reaches a plateau when it is expressed on the basis of PN, cell number and protein. Chlorophyll a specific NR activity was found to be good index of nitrate assimilation efficiency.

Measured intracellular nitrate was shown to be a small portion (approximately 5%) of the hyperbolically increasing nitrogen yield coefficient "$q_i$" as the dilution rate increased. Based on this finding , $q_o$, is not a constant amount

of organic nitrogen per cell for all growth rates as indicated by Caperon's observations (1968).

Cellular protein decreased by as much as 45% as the dilution rate increased from 30% to 116%, while nitrate uptake efficiencies were always higher than 96%. Consequently the slowest dilution rate gave the best cellular protein production per mole of nitrate taken up by the cells. It is suggested that protein synthesis is a rate limiting step in the process of nitrate assimilation for higher dilution rates; intracellular accumulation of nitrate and free amino acids supports this hypothesis.

Comparison of daily carbon-14 fixation and particulate organic carbon production suggests that excretion might occur at lower dilution rates, while intracellular recycling of carbon may be significant at higher dilution rates. The carbon-14 fixation during the photoperiod was shown to reach a maximum at 87% turnover rate per day for both the primary productivity and the photosynthetic capacity. The dark uptake of carbon-14 was negligible in the cultures while the photosynthetic capacity increased continuously. Therefore, as the dilution rate increased the ability of the cells to fix carbon-14 increased. It is concluded that the increasing dark minimum uptake is related to physiological changes of the cells as the dilution rate increases; the cellular concentration of particulate organic carbon at 0900 hr was inversely related to the minimum dark uptake, as the dilution rate increase. This interpretation is

supported by the fact that the daily maximum of carbon-14 fixation shifted toward the beginning of the light period as the growth rate increased.

The determination of the nitrate uptake kinetics parameter $K_s^u$ by the short-term uptake technique indicates that the constancy of $K_s^u$ with increasing growth rates (Caperon and Meyer, 1972) is questionable. Comparison of the short-term and discrete uptake techniques for the determination of Vm and $K_s^u$, revealed systematic differences. From the results of the present study it seems that the short-term uptake technique is more reliable than the discrete uptake technique. More reseach is needed to elucidate phytoplankton nitrate uptake kinetics.

From the results of this study, it appears that the growth rate of Chaetoceros sp. (STX-105) in continuous nitrogen-limited culture is neither limited by the rate of nitrate uptake nor by the rate of the enzyme nitrate reductase at any growth rate. Growth is probably limited by the rate of nitrate supply at lower dilution rates whereas protein synthesis would be rate limiting process at higher dilution rates, where the "enzymic machinery" is saturated.

Table 1: Nutrient concentration and enrichment concentration of deep-sea water (DSW) from St-Croix.

| Nutrients | DSW Concentration | Enrichment Concentration |
|---|---|---|
| $NO_3^-$ | 31.5 µg-at ± 0.5 | 0 |
| $NO_2^-$ | 0.14 µg-at ± 0.05 | 0 |
| $NH_4^+$ | 0.9 µg-at ± 0.12 | 0 |
| $PO_4^{-3}$ | 1.95 µg-at ± 0.05 | 0 |
| $S_iO_4^{-3}$ | 22.5 µg-at ± 0.27 | 0 |
| $Fe^{+3}$ | * | 1 uM |
| EDTA (or natural chelator in DSW) | * | 1 uM |
| $B_{12}$ | * | $1.8 \times 10^{-10}$ M |
| Trace Metal Mixture: | | |
| $Cu^{+2}$ | * | $7.86 \times 10^{-10}$ M |
| $Zn^{+2}$ | * | $1.53 \times 10^{-9}$ M |
| $Co^{+2}$ | * | $8.50 \times 10^{-10}$ M |
| $Mn^{+2}$ | * | $1.83 \times 10^{-8}$ M |
| $Mo^{+6}$ | * | $5.20 \times 10^{-10}$ M |

*: not measured

Table 2: Mean (± 1 SD) nutrient concentration (µg-at) of the incoming(IN) and outgoing (OUT) medium of the culture vessel for each turnover rate.

| Nutrients | | Turnover rate, day $^{-1}$ (%) | | | | |
|---|---|---|---|---|---|---|
| | | 30 | 60 | 87 | 105 | 116 |
| IN | $NO_3^-$ | 31.5±0.5 | 31.5±0.5 | 31.5±0.5 | 31.5±0.5 | 31.5±0.5 |
| | $NO_2^-$ | 0.39±0.05 | 0.45±0.05 | 0.34±0.05 | 0.26±0.05 | 0.26±0.05 |
| | $NH_4^+$ | 3.17±0.12 | 2.96±0.12 | 2.89±0.12 | 2.88±0.12 | 2.88±0.12 |
| | $NH_4^+$+A.Acids | 4.06±0.12 | 4.31±0.12 | 4.25±0.12 | 4.04±0.12 | 4.04±0.12 |
| | $S_iO_2^{--}$ | 45.00±0.65 | 45.00±0.65 | 45.00±0.65 | 45.00±0.65 | 45.00±0.65 |
| OUT | $NO_3^-$ | 0.25±0.22 | 0.96±0.87 | 0.38±0.32 | 0.18±0.33 | 0.13±0.26 |
| | $NO_2^-$ | 0.16±0.05 | 0.19±0.05 | 0.22±0.05 | 0.26±0.05 | 0.13±0.05 |
| | $NH_4^+$ | 1.73±0.90 | 1.72±1.01 | 2.99±1.08 | 0.97±0.19 | 1.56±0.34 |
| | $NH_4^+$+A.Acids | 3.50±1.12 | 3.00±1.17 | 4.45±1.38 | 2.43±0.26 | 1.77±0.42 |
| | $S_iO_2^{--}$ | 6.78±1.74 | 3.66±1.77 | 2.48±1.58 | 2.27±1.21 | 5.05±4.98 |

Table 3: Free amino acids of Chaetoceros sp. (STX-105). Samples were taken at 1100 hr.

| Turnover rate, day$^{-1}$ | 30% | | 60% | | 87% | | 105% | |
|---|---|---|---|---|---|---|---|---|
| Amino acids | Nanomoles | ug | Nanomoles | ug | Nanomoles | ug | Nanomoles | ug |
| Alanine | 2.19 | .195 | 2.58 | .230 | 5.70 | .508 | 58 | 5.2 |
| Aspartic acid | - | - | 1.22 | .162 | 4.50 | .599 | 50 | 6.7 |
| Glutamic acid | 3.87 | .569 | 12.12 | 1.783 | 25.39 | 3.735 | 236 | 34.7 |
| Glycine | 2.20 | .165 | 1.54 | .116 | 2.42 | .182 | 19 | 1.4 |
| Isoleucine | - | - | - | - | 1.26 | .165 | 8 | 1.1 |
| Leucine | - | - | - | - | 2.04 | .268 | 10 | 1.3 |
| Lysine | - | - | - | - | - | - | 6 | .9 |
| Phenylalanine | - | - | - | - | - | - | 9 | 1.5 |
| Proline | - | - | - | - | - | - | 14 | 1.6 |
| Serine | 4.07 | .428 | 4.02 | .423 | 5.85 | .615 | 78 | 8.2 |
| Threonine | - | - | - | - | 2.18 | .260 | 12 | 1.4 |
| Tyrosine | - | - | - | - | - | - | 5 | .9 |
| Valine | - | - | - | - | - | - | 11 | 1.3 |
| Total free amino acids in extract | - | 1.36 | - | 2.71 | - | 6.33 | - | 66.2 |
| µg N/l before extraction | 415 | | 435 | | 413 | | 450 | |
| µg Protein/l before extraction assuming 6.25% N | 6592 | | 6960 | | 6608 | | 7200 | |
| Total free amino acids in extract as a % of total protein (w/w) in the cell. | .021 | | .039 | | .096 | | .920 | |

Table 4: Percentage of the each free amino acid of the total free amino acids in extract.

| Turnover rate, $day^{-1}$ (%) | Turnover rate, day (%) | | | |
|---|---|---|---|---|
| | 30 | 60 | 87 | 116 |
| Alanine | 14 | 8 | 8 | 8 |
| Aspartic acid | - | 6 | 9 | 10 |
| Glutamic acid | 42 | 66 | 59 | 52 |
| Glycine | 12 | 4 | 3 | 2 |
| Isoleucine | - | - | 3 | 2 |
| Leucine | - | - | 4 | 2 |
| Lysine | - | - | - | 1 |
| Phenylalanine | - | - | - | 2 |
| Proline | - | - | - | 2 |
| Serine | 31 | 16 | 10 | 12 |
| Threonine | - | - | 4 | 2 |
| Tyrosine | - | - | - | 1 |
| Valine | - | - | - | 2 |
| | 98% | 100% | 100% | 98% |

Table 5: Nitrate uptake kinetics parameters Vm and $K_s^u$ (± 1 SD) by the techniques of short-term uptake and discrete uptake.

| Technique | Turnover rate, $day^{-1}$ (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 30 | | 60 | | 87 | | 116 | |
| | Vm | $K_s^u$ | Vm | $K_s^u$ | Vm | $K_s^u$ | Vm | $K_s^u$ |
| Short-term uptake | | | | | | | | |
| FRESH | .0197±.0003 | .26±.07 | .0191* | - | .0235±.0018 | .84±.17 | .0225* | - |
| FED | .0239±.0001 | .41±.02 | - | - | .0243±.0001 | .66±.01 | - | - |
| STARVED | .0168±.0002 | .37±.04 | .0159±.0003 | .28±.06 | .0196±.0053 | .86±.65 | - | - |
| Discrete uptake | | | | | | | | |
| FRESH | .0196 | 0 | .0197 | 0 | .0195 | .65 | .0456 | 2.78 |

Vm: µg-at $NO^{-1}.hr^{-1}.10^6$ $cells^{-1}$.

* : Approximates rates observed in experiments judged inadequate for $K_s^u$ evaluation.

Table 6: Coefficient of variation of the population characteristics of *Chaetoceros* sp. (STX-105).

| | Turnover rate, $day^{-1}$ (%) | | | | | |
|---|---|---|---|---|---|---|
| | 30 | 60 | 87 | 105 | 116 | Grand mean for all turnover rates |
| Particulate nitrogen/l | 6 | 6 | 6 | 5 | 9 | 6 |
| Cells/l | 2 | 7 | 8 | 8 | 9 | 10 |
| Particulate carbon/l | 11 | 15 | 19 | 27 | 28 | 17 |
| Chlorophyll *a*/l | 4 | 7 | 5 | 8 | 11 | 22 |
| Protein-N/l | 10 | 23 | 36 | 36 | 48 | 30 |
| Carbohydrate/l | 17 | 38 | 67 | 112 | 85 | 62 |
| Cellular $NO_3^-$/l | 13 | 20 | 58 | 106 | 94 | 63 |

Table 7: Mean nitrate reductase activity for each turnover rate (± 1 SD)

| Nitrate reductase activity | Turnover rate, $day^{-1}$. (%) | | | | |
|---|---|---|---|---|---|
| | 30 | 60 | 87 | 105 | 116 |
| a | .089 ±.017 | .137 ±.019 | .147 ±.025 | .141 ±.017 | .158 ±.020 |
| b | .27 ±.04 | .46 ±.08 | .49 ±.11 | .53 ±.09 | .65 ±.08 |
| c | .0048±.0011 | .0092±.0016 | .0112±.0020 | .0125±.0027 | .0132±.0023 |
| d | .104 ±.019 | .165 ±.024 | .130 ±.023 | .126 ±.020 | .123 ±.016 |
| e | .0115±.0019 | .0237±.0037 | .0309±.007 | .049 ±.017 | .047 ±.015 |

a= μ moles $NO_2^-$ formed/ (μg-at Particulate nitrogen) (hour).
b= μ moles $NO_2^-$ formed/ ($10^8$cells) (hour).
c= μ moles $NO_2^-$ formed/ (μg-at Particulate carbon) (hour).
d= μ moles $NO_2^-$ formed/ (μg Chlorophyll a) (hour).
e= μ moles $NO_2^-$ formed/ (μg Protein-N) (hour).

Table 8: Comparison of nitrate reductase activities of the diatom Chaetoceros sp. with reported values in the literature.

| Authors | Source | Literature | | Chaetoceros sp. |
|---|---|---|---|---|
| Hodler et al. (1972) | Chlorella pyrenoidosa | 0-0.005 | $\mu$ moles $NO_2^-$ formed. $hr.^{-1}.\mu g^{-1}$ Prot. | 0.010-0.075 |
| Schrader et al. (1968) | Higher plants | 0-0.018 | $\mu$ moles $NO_2^-$ formed. $hr^{-1}.\mu g.^{-1}$ Prot. | 0.010-0.075 |
| Rigano (1971) | Cyanidium caldarium | 0-0.0001 | $\mu$ moles $NO_2^-$ formed. $hr^{-1}.\mu g^{-1}$ Prot. | 0.010-0.075 |
| LeClair and Grant (1972) | Dunaliella tertiolecta | 0.001 | $\mu$ moles $NO_2^-$ formed. $hr^{-1}$. $\mu g^{-1}$ Prot. | 0.010-0.075 |
| Eppley et al. (1970) | Peru current | .0002-0.002 | $\mu$ moles $NO_2^-$ formed. $hr^{-1}$. $\mu$ moles $PN^{-1}$ | 0.070-0.080 |
| Eppley et al. (1970) | Peru current | .0005-0.002 | $\mu$ moles $NO_2^-$ formed. $hr^{-1}$. $\mu g^{-1}$ chl a. | 0.080-0.180 |
| Eppley et al. (1969) | Dytylum brightwellii | 0-0.003 | $\mu$ moles $NO_2^-$ formed. $hr^{-1}$. $\mu g^{-1}$ chl a. | 0.080-0.180 |
| Eppley and Renger (1974) | Thalassiosira pseudonana | .20-0.40 | $\mu$ moles $NO_2^-$ formed. $hr^{-1}.10^{8^{-1}}$ cells | 0.30 -0.70 |

Table 9: Mean cellular characteristics of the Chaetoceros sp. (STX-105) population for each turnover rate. (± 1 SD).

| | Turnover rate, $day^{-1}$ (%) | | | | |
|---|---|---|---|---|---|
| | 30 | 60 | 87 | 105 | 116 |
| µg Chl a/$10^7$ cells | 0.26±.02 | 0.27±.03 | 0.37±.05 | 0.43±.07 | 0.55±.11 |
| µg-at $NO_3^-$/$10^{10}$ cells[1] | 0.80±.12 | 0.83±.22 | 1.24±.81 | 3.41±3.45 | 4.17±4.79 |
| µg-at Free A.Acids/$10^{10}$cells[1,2] | 0.13 | 0.22 | 0.50 | 5.74 | - |
| µg-at Prot.-N/$10^7$ cells | 0.17±.02 | 0.14±.04 | 0.12±.05 | 0.09±.04 | 0.09±.05 |
| µg-at PN/$10^7$ cells | 0.30±.02 | 0.33±.04 | 0.32±.04 | 0.37±.05 | 0.43±.07 |
| µg-at POC/$10^7$ cells | 5.67±.70 | 5.09±.1.09 | 4.33±1.16 | 4.32±.1.52 | 4.97±1.82 |
| µg Carbohydrate/$10^7$ cells | 89.6 ±16.5 | 78.9 ±35.0 | 32.0 ±23.6 | 28.1 ±26.7 | 25.8 ±24.2 |
| Primary productivity: µg-at C.24 $hr^{-1}$/$10^7$cells | 2.29±.08 | 3.63±.28 | 4.12±.37 | 4.35±.41 | 5.38±.54 |
| Photosynthetic capacity: µg-at C.24 $hr^{-1}$/$10^7$cells | 2.66±.10 | 4.82±.38 | 5.77±.52 | 7.04±.66 | 8.83±.89 |
| Nitrate reductase: µg-at $NO_2^-$ formed per hour/ $10^7$cells | 0.036±.008 | 0.061±.013 | 0.065±.018 | 0.070±.015 | 0.089±.017 |

1 Note that these two quantities are calculated per $10^{10}$ cells.
2 Only a single determination was made at each turnover rate.

Table 10: Photosynthetic rate per unit chlorophyll *a* of the *Chaetoceros* population for each turnover rate.

| | Turnover rate, $day^{-1}$, (%) | | | | |
|---|---|---|---|---|---|
| | 30 | 60 | 87 | 105 | 116 |
| $\mu g\ C.hr^{-1}.\mu g\ chl\ a^{-1}$. | 4.39 | 6.72 | 5.57 | 5.05 | 4.89 |

Table 11. Comparison of the calculated and the measured intracellular nitrate.

| Intracellular nitrate | Turnover rate, $day^{-1}$. (%) | | | | |
|---|---|---|---|---|---|
| | 30 | 60 | 87 | 105 | 116 |
| Measured, µµg-at/cell. : | 0.00008 | 0.000083 | 1.000124 | 0.000341 | 0.000417 |
| Calculated, µµg-at/cell. : | 0.001 | 0.004 | 0.003 | 0.008 | 0.014 |
| Measured / Calculated : | 0.08 | 0.021 | 0.041 | 0.043 | 0.030 |

Table 12: Cellular characteristics nitrogen per cell (µg) of *Chaetoceros* sp. (STX-105) expressed as a percentage of total nitrogen per cell.

| | Turnover rate, $day^{-1}$ (%) | | | | |
|---|---|---|---|---|---|
| | 30 | 60 | 87 | 105 | 116 |
| Protein | 57 | 42 | 38 | 24 | 21 |
| Free amino acids | .05 | .06 | .16 | 1.54 | - |
| Cellular nitrate | .26 | .26 | .38 | .93 | .96 |
| Chlorophyll *a* | .38 | .37 | .51 | .52 | .58 |
| | 57.69 | 42.69 | 39.05 | 26.99 | 22.54 |

Table 13: Primary productivity and photosynthetic capacity expressed as $\mu g\ C.l^{-1}.24\ hrs^{-1}$.

| | Turnover rate, $day^{-1}$.(%) | | | | |
|---|---|---|---|---|---|
| | 30 | 60 | 87 | 105 | 116 |
| Primary Productivity: | 2624 | 4332 | 4847 | 4747 | 4905 |
| % | 53 | 88 | 99 | 97 | 100* |
| Photosynthetic capacity: | 3053 | 5746 | 6786 | 7685 | 8051 |
| % | 38 | 71 | 84 | 95 | 100* |

*: Assuming the 116% turnover rate as 100% production.

Table 14: Coefficient of variation of the cellular characteristics of *Chaetoceros* sp. (STX-105)

| | Turnover rate, $day^{-1}$ (%) | | | | | |
|---|---|---|---|---|---|---|
| | 30 | 60 | 87 | 105 | 116 | Grand mean for all turnover rates |
| µg-at PN/cell | 7 | 12 | 12 | 14 | 16 | 15 |
| µg-at POC/cell | 12 | 21 | 27 | 35 | 37 | 12 |
| µg Chl *a*/cell | 8 | 11 | 14 | 16 | 20 | 32 |
| µg-at Prot.-N/cell | 12 | 28 | 42 | 44 | 56 | 28 |
| µg Carbohydrate/cell | 18 | 44 | 74 | 95 | 94 | 61 |
| µg-at $NO_3^-$/cell | 15 | 26 | 65 | 101 | 115 | 76 |
| Primary productivity: µg-at C.24 $hr^{-1}$/cell | 3 | 8 | 9 | 9 | 10 | 29 |
| Photosynthetic capacity: µg-at C.24 $hr^{-1}$/cell | 4 | 8 | 9 | 9 | 10 | 40 |
| Nitrate reductase: µg-at $NO_2^-$ formed per hr/cell | 22 | 21 | 28 | 21 | 19 | 30 |

Table 15 Efficiencies of nitrate uptake and assimilation of the Chaetoceros sp. (STX-105) population, for each turnover rate.

| Turnover rate $day^{-1}$ (%) | Uptake efficiency (%) | Intracellular nitrate (%) | Assimilation efficiency based on: Protein (%) | * (%) | PN (%) | * (%) |
|---|---|---|---|---|---|---|
| 30 | 99.2 | 0.26 | 50.4 | 49.6 | 91.7 | 90.2 |
| 60 | 96.4 | 0.30 | 43.7 | 43.1 | 105.8 | 104.3 |
| 87 | 98.8 | 0.38 | 37.1 | 37.7 | 100.6 | 102.1 |
| 105 | 99.4 | 0.92 | 27.1 | 25.9 | 106.7 | 102.2 |
| 116 | 99.1 | 0.98 | 23.1 | 21.6 | 103.2 | 96.7 |

*: corrected for ammonia, nitrite and free amino acids.

Table 16: Effect of the dilution rate on: the rate of nitrate uptake at steady-state (v) - the maximum uptake rate (Vm) and the rate of nitrate reduction (NR) as measured by the NR activity.

| | Turnover rate, $day^{-1}$.(%) | | | | |
|---|---|---|---|---|---|
| | 30 | 60 | 87 | 105 | 116 |
| v: µg-at $NO_3^-.hr^{-1}.10^6$cells. | 0.00041 | 0.00079 | 0.00117 | 0.00151 | 0.00200 |
| NR: µg-at $NO_2^-$.formed. $hr^{-1}.10^6cells^{-1}$. | 0.0027 | 0.0046 | 0.0049 | 0.0053 | 0.0065 |
| Vm: µg-at $NO_3^-.hr^{-1}.10^6$cells. | 0.0197 | 0.0191* | 0.0235 | - | 0.0225* |
| $\frac{v}{NR}$ x 100 | 15.2 | 17.2 | 23.9 | 28.5 | 30.8 |
| $\frac{v}{Vm}$ x 100 | 2.1 | 4.1 | 5.0 | - | 8.9 |
| $\frac{NR}{Vm}$ x 100 | 13.7 | 24.1 | 20.8 | - | 28.9 |

*: Approximates rates observed in experiments judged inadequate for $K_s^u$ evaluation.

Table 17: Carbon balance of the Chaetoceros sp. (STX-105) population for each turnover rate.

| | Turnover rate, $day^{-1}$ (%) | | | | |
|---|---|---|---|---|---|
| | 30 | 60 | 87 | 105 | 116 |
| Mean POC $\mu gC.\ l^{-1}.24hrs^{-1}$. | 6500 | 6070 | 5090 | 4765 | 4540 |
| (a): POC production " | 1950 | 3642 | 4428 | 5003 | 5266 |
| (b): C-14 uptake " | 2624 | 4332 | 4846 | 4747 | 4905 |
| (b)-(a) " | 674 | 690 | 418 | (-256)≠ | (-361)≠ |
| % of the excess taken up | 25.7 | 15.9 | 8.6 | (-5.4)* | (-7.9)* |

≠: Shortage of carbon uptake.
*: Deficit

Figure 1: Schematic description of the continuous culture apparatus.

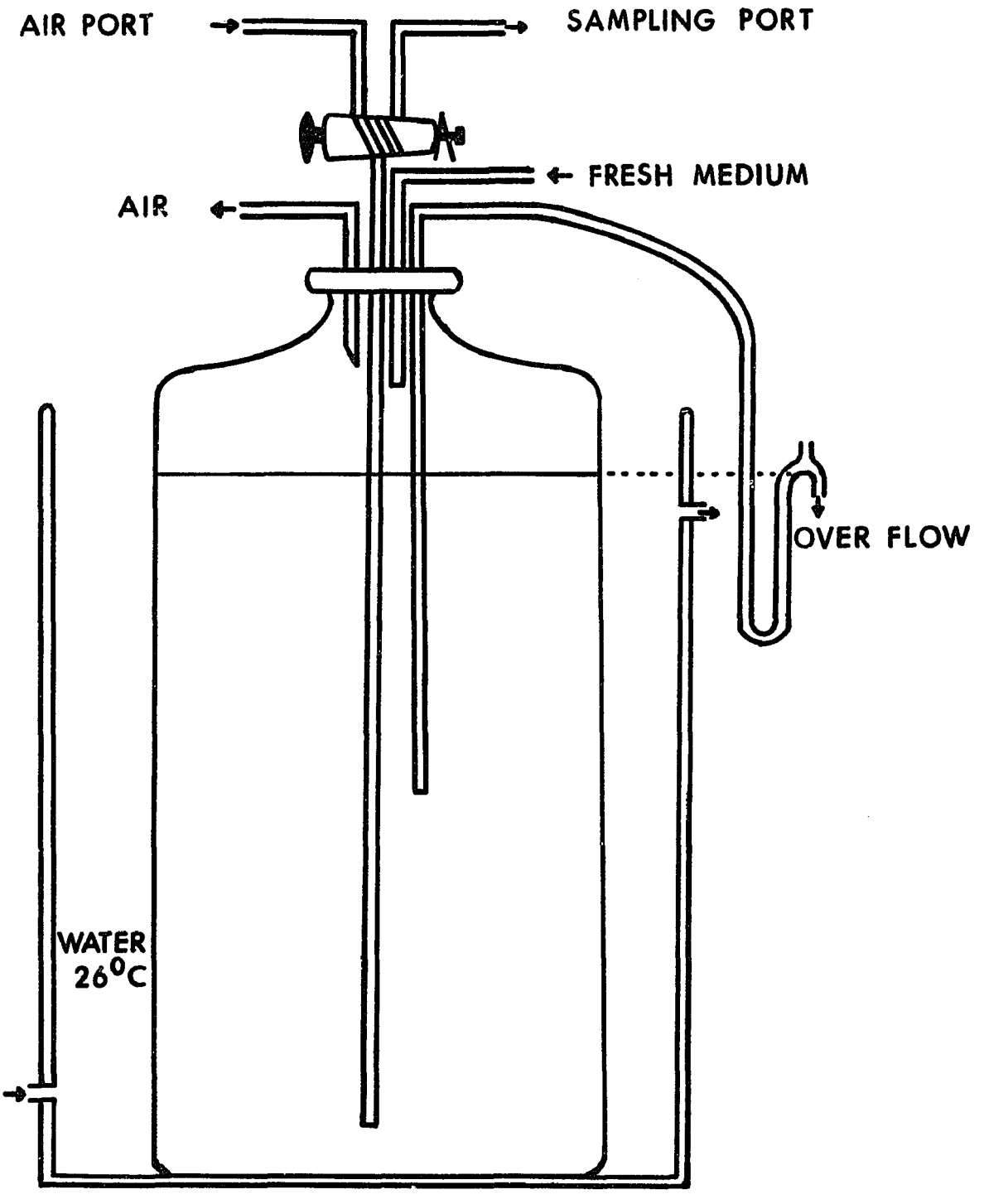

AIR PORT
SAMPLING PORT
FRESH MEDIUM
AIR
OVER FLOW
WATER
26°C

Figure 2: Diel variations of the mean cell density at each dilution rate. Bars indicates the range.

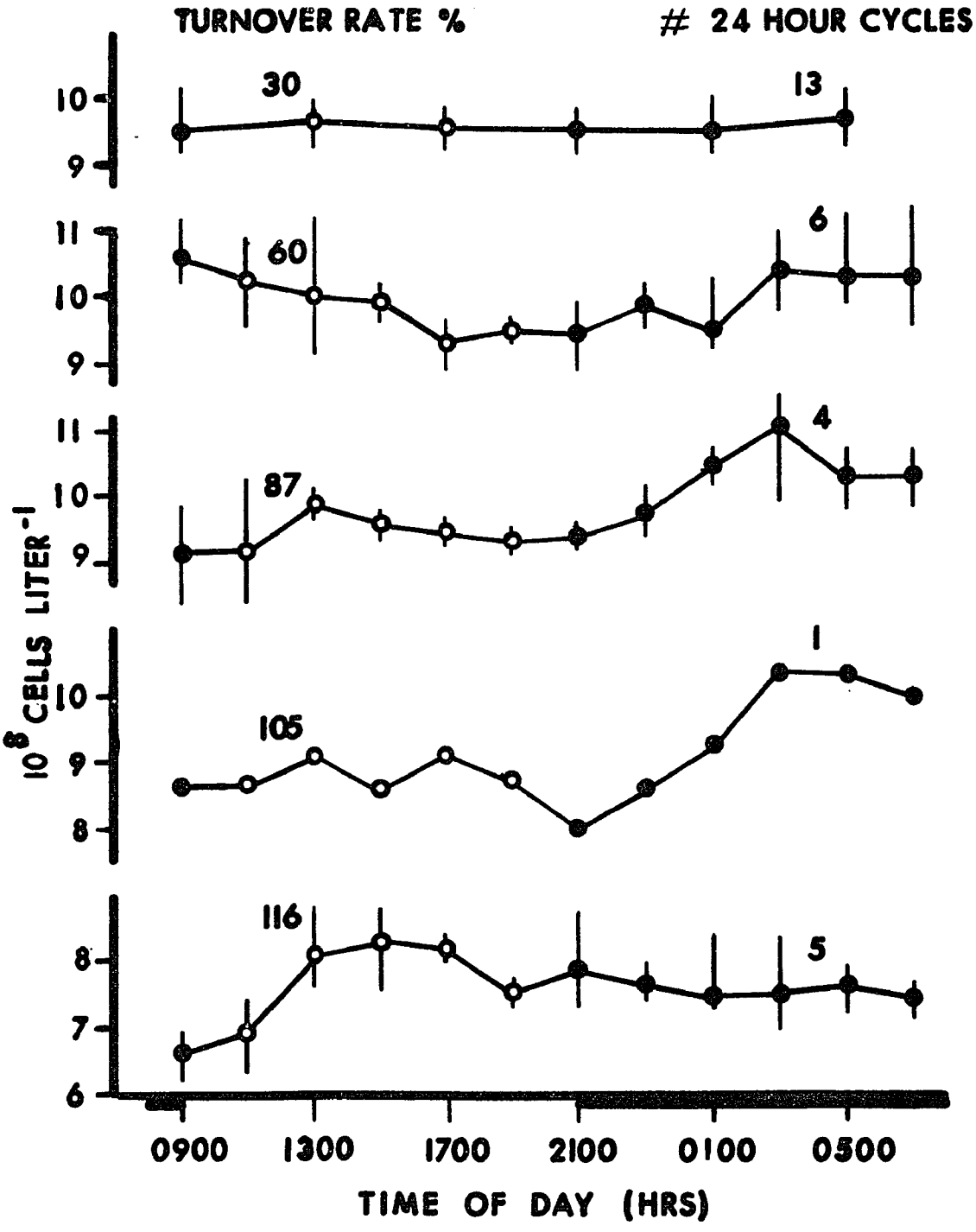

TURNOVER RATE %
# 24 HOUR CYCLES
30
13
60
6
87
4
105
1
116
5
10
9
11
10
9
11
10
9
10
9
8
8
7
6
$10^8$ CELLS LITER$^{-1}$
0900
1300
1700
2100
0100
0500
TIME OF DAY (HRS)

Figure 3: Diel variations of the mean cellular protein at each dilution rate. Bars indicates the range.

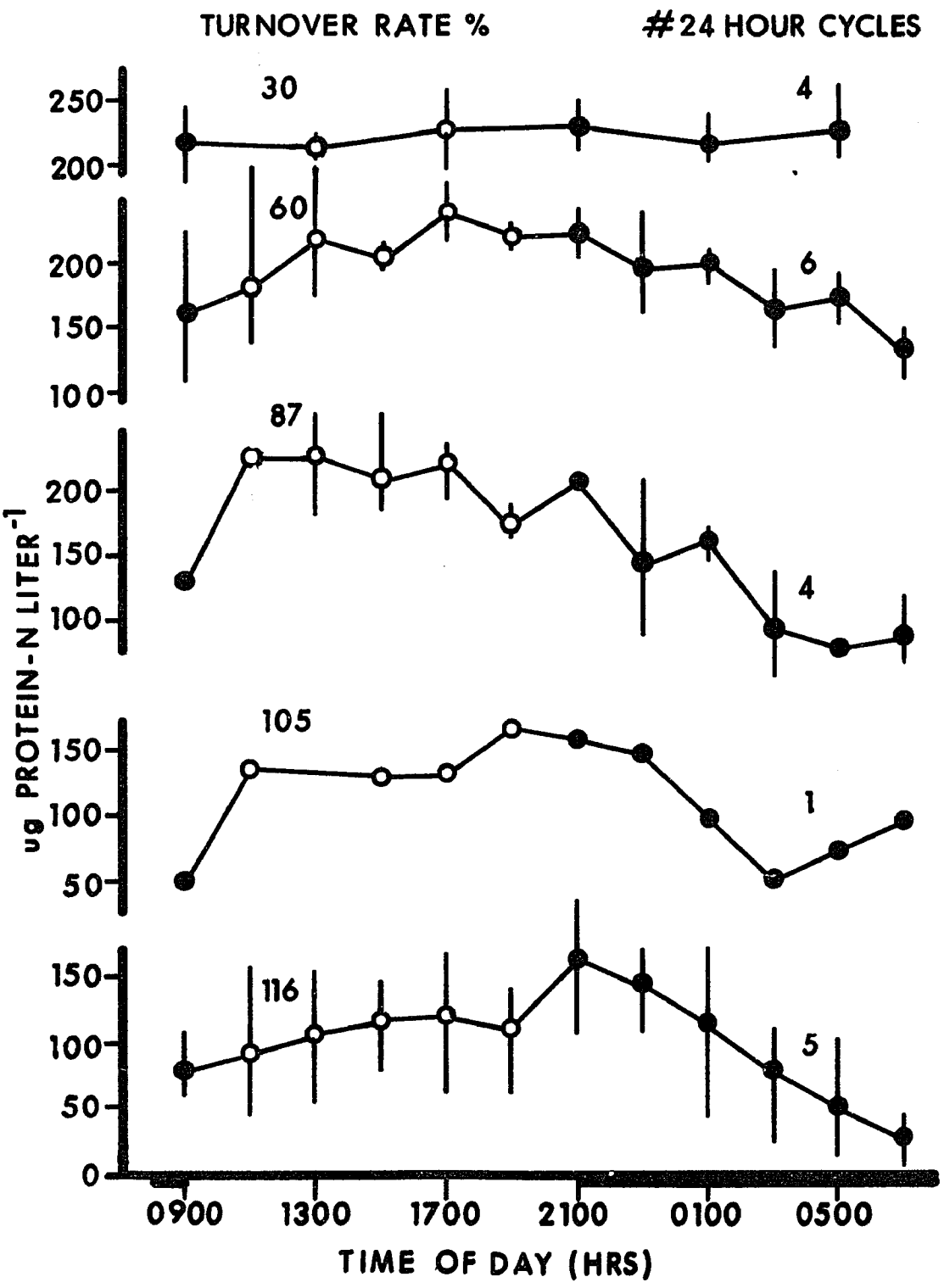

TURNOVER RATE %
#24 HOUR CYCLES
30
4
60
6
87
4
105
1
116
5
250
200
150
100
50
0
ug PROTEIN-N LITER$^{-1}$
0900
1300
1700
2100
0100
0500
TIME OF DAY (HRS)

Figure 4: Diel variations of the mean concentrations of chlorophyll a at each dilution rate. Bars indicates the range.

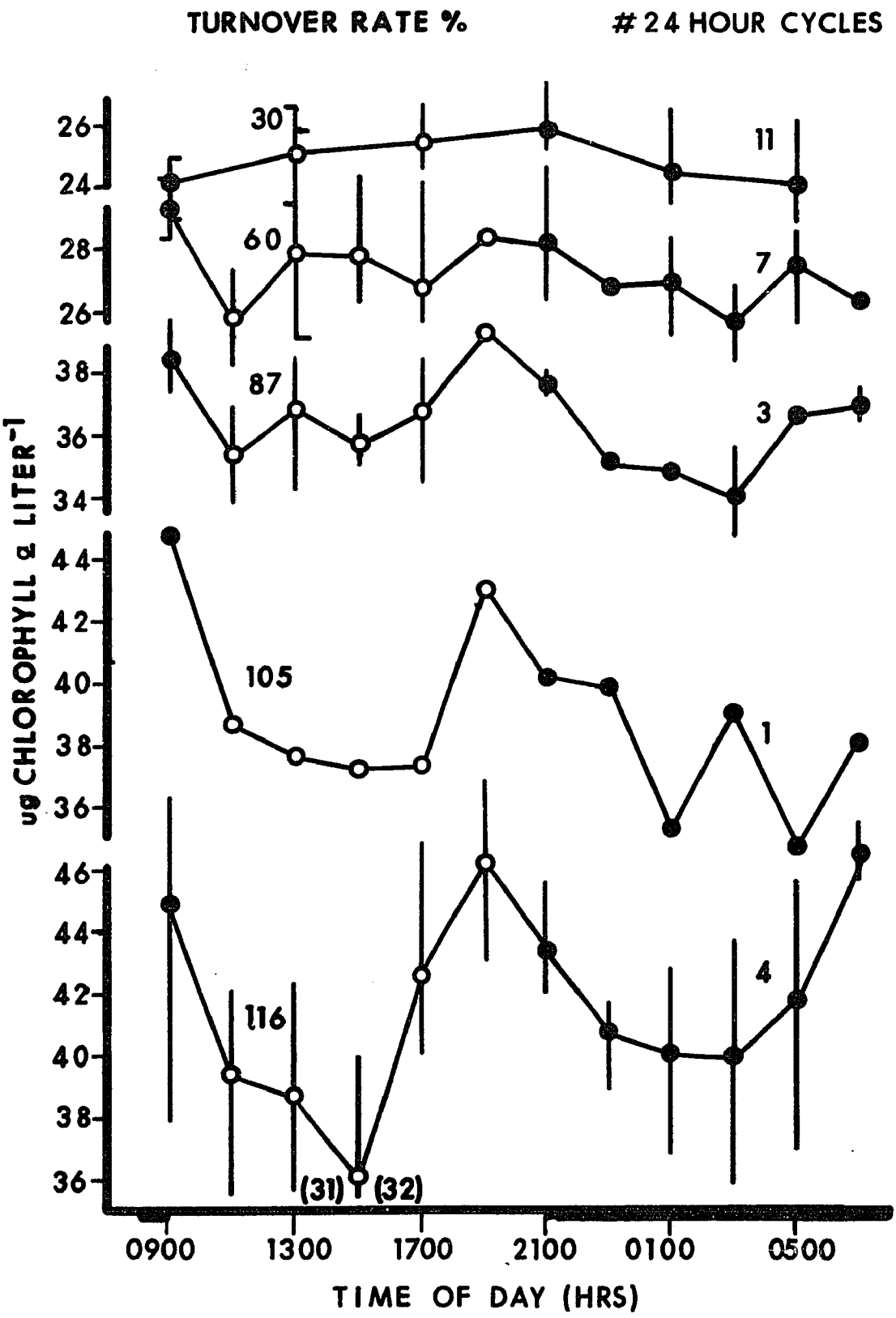

TURNOVER RATE %
# 24 HOUR CYCLES
30
11
60
7
87
3
105
1
116
4
(31)
(32)
26
24
28
26
38
36
34
44
42
40
38
36
46
44
42
40
38
36
ug CHLOROPHYLL a LITER⁻¹
0900
1300
1700
2100
0100
0500
TIME OF DAY (HRS)

Figure 5: Diel variations of the mean particulate organic nitrogen (PN) at each dilution rate. Bars indicates the range.

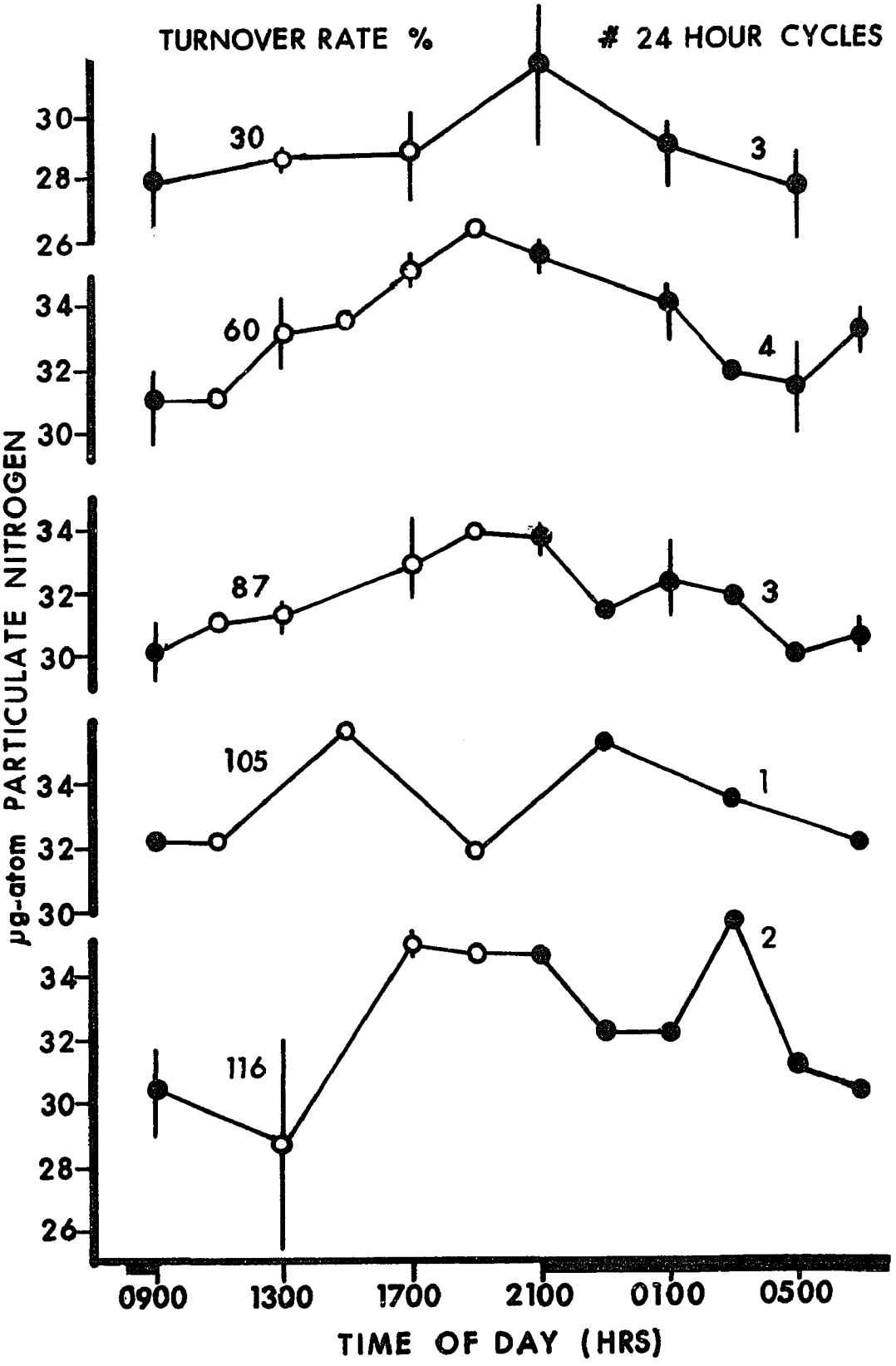

TURNOVER RATE %
# 24 HOUR CYCLES
30
3
60
4
87
3
105
1
116
2
µg-atom PARTICULATE NITROGEN
TIME OF DAY (HRS)
0900
1300
1700
2100
0100
0500

Figure 6: Diel variations of the mean particulate organic carbon (POC) at each dilution rate. Bars indicates the range.

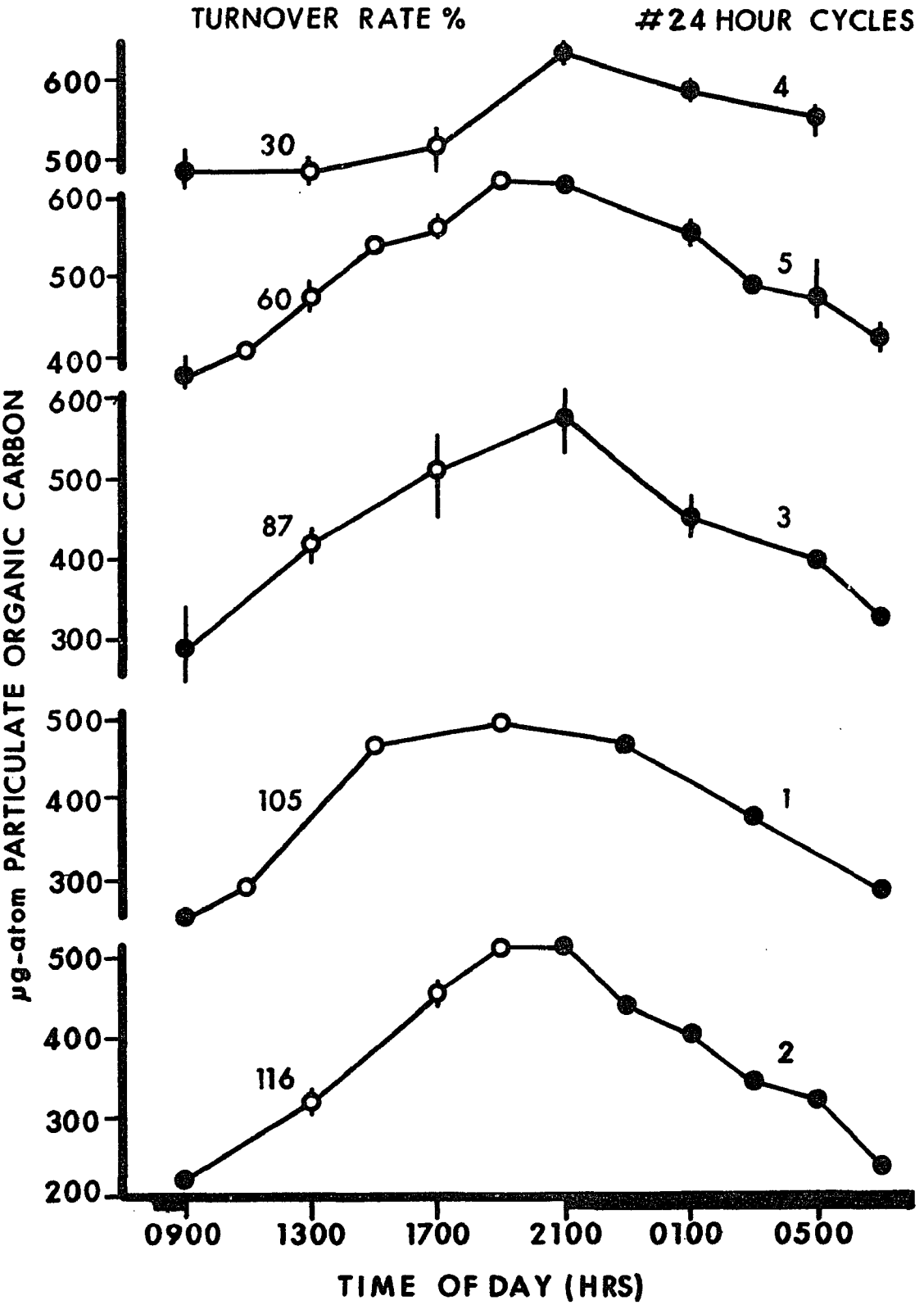

TURNOVER RATE %
#24 HOUR CYCLES
30
4
60
5
87
3
105
1
116
2
600
500
400
300
200
µg-atom PARTICULATE ORGANIC CARBON
0900
1300
1700
2100
0100
0500
TIME OF DAY (HRS)

Figure 7: Diel variations of the mean concentrations of carbohydrate at each dilution rate. Bars indicates the range.

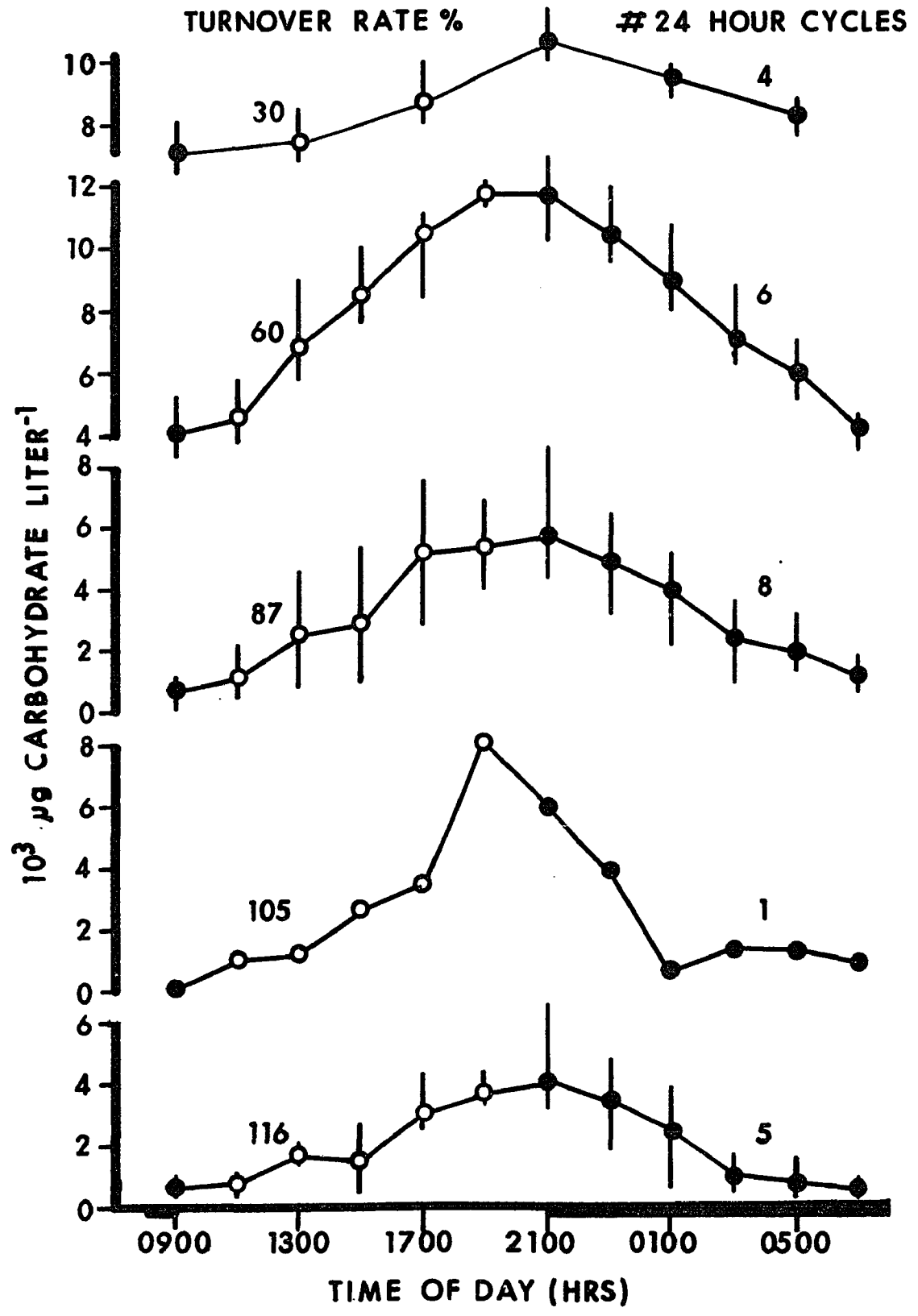

TURNOVER RATE %
# 24 HOUR CYCLES
30
4
60
6
87
8
105
1
116
5
$10^3$ µg CARBOHYDRATE LITER$^{-1}$
0900
1300
1700
2100
0100
0500
TIME OF DAY (HRS)

Figure 8: Diel variations of the cellular nitrate concentration at each dilution rate.

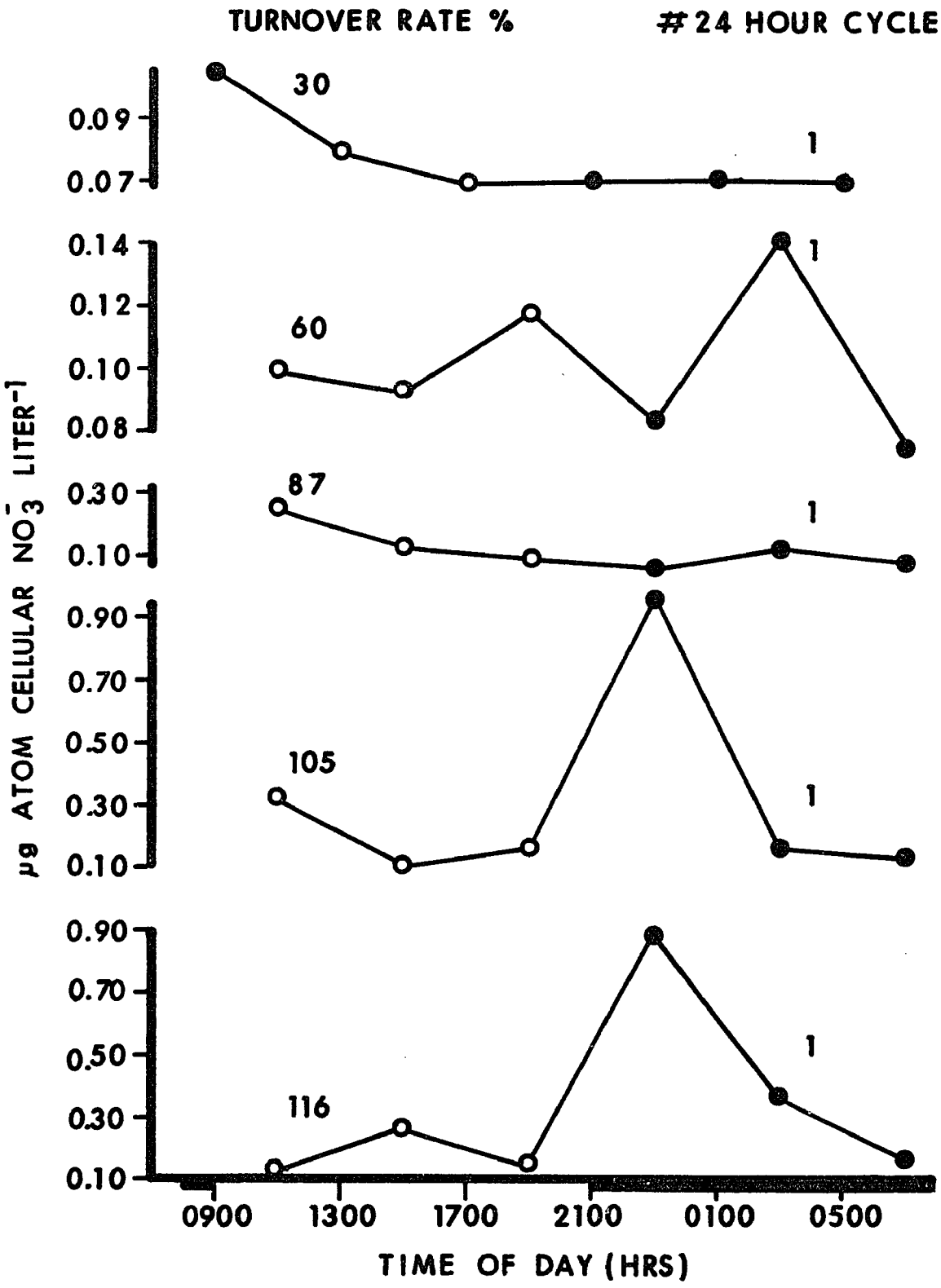

TURNOVER RATE %
#24 HOUR CYCLE
30
1
60
1
87
1
105
1
116
1
0.09
0.07
0.14
0.12
0.10
0.08
0.30
0.10
0.90
0.70
0.50
0.30
0.10
0.90
0.70
0.50
0.30
0.10
μg ATOM CELLULAR $NO_3^-$ LITER$^{-1}$
0900
1300
1700
2100
0100
0500
TIME OF DAY (HRS)

Figure 9: Mean population size (± 1 SD) for each dilution rate: $10^8$ cells liter$^{-1}$ (o), µg protein-N liter$^{-1}$ (△), $10^{-3}$ µg carbohydrate liter$^{-1}$ (□), and µg-atom PN liter$^{-1}$ (◇).

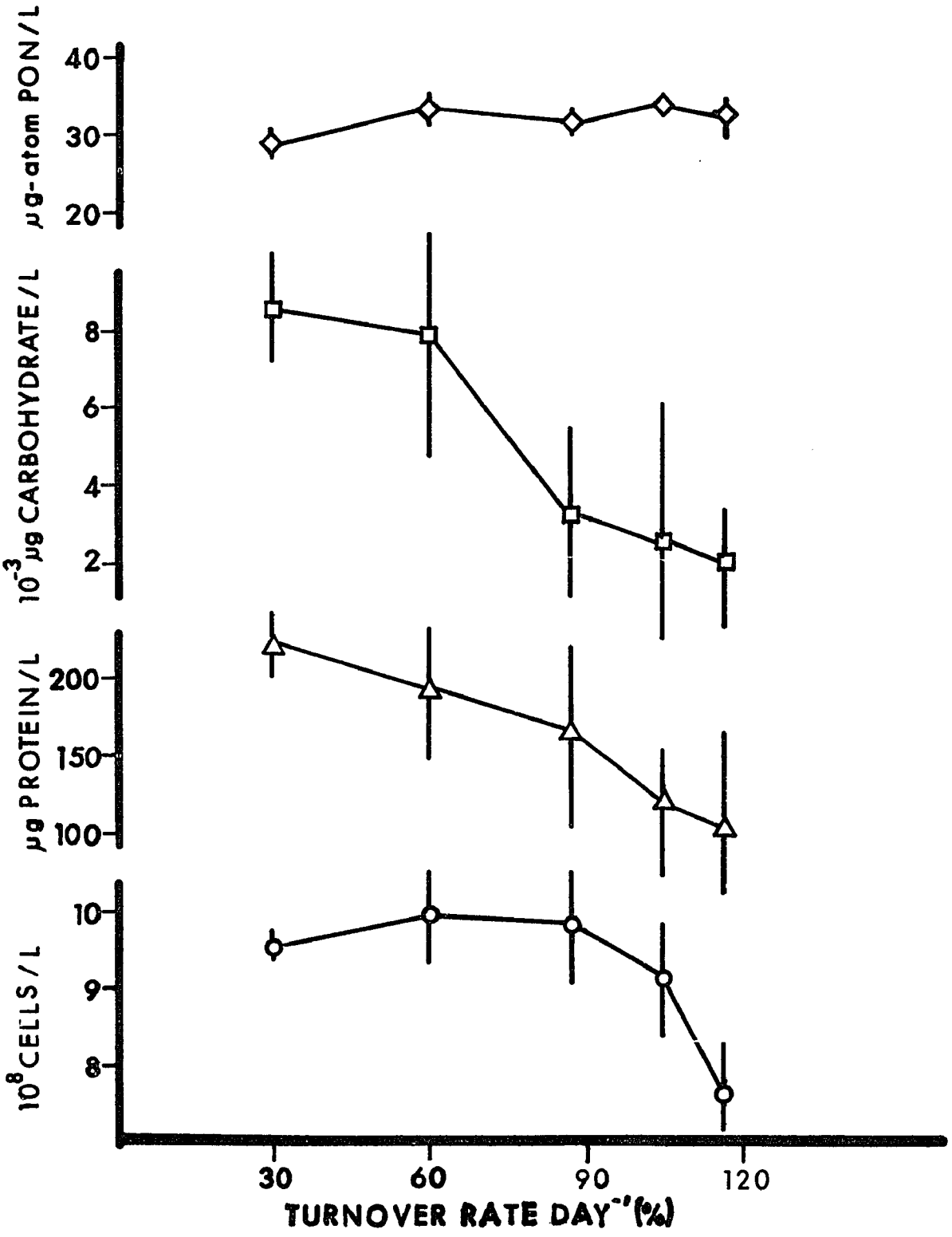

µg-atom PON/L
40
30
20
$10^{-3}$ µg CARBOHYDRATE/L
8
6
4
2
µg PROTEIN/L
200
150
100
$10^{8}$ CELLS/L
10
9
8
30
60
90
120
TURNOVER RATE DAY$^{-1}$(%)

Figure 10: Mean population size ($\pm$ 1 SD) for each dilution rate: µg-atom cellular $NO_3^-$ liter$^{-1}$ (O), µg chlorophyll *a* liter$^{-1}$ (△), and µg-atom POC liter$^{-1}$ (□).

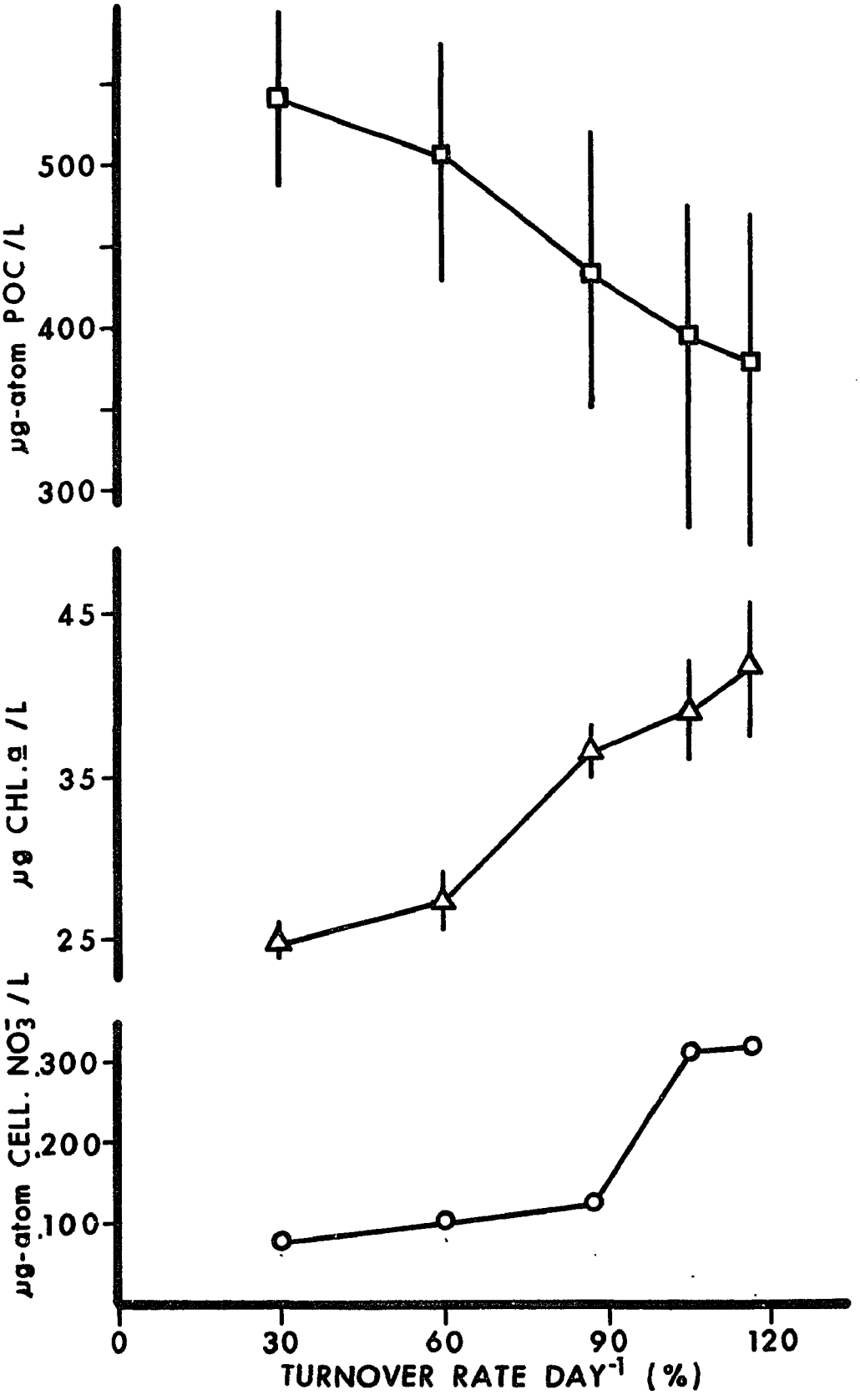

µg-atom POC /L
500
400
300
µg CHL.a /L
45
35
25
µg-atom CELL. NO3 /L
.300
.200
.100
0
30
60
90
120
TURNOVER RATE DAY-1 (%)

Figure 11: Diel variations of the mean NADH nitrate reductase activity as $\mu$ moles $NO_2^-$ formed/ (liter) (hour), at each dilution rate. Bars indicates the range.

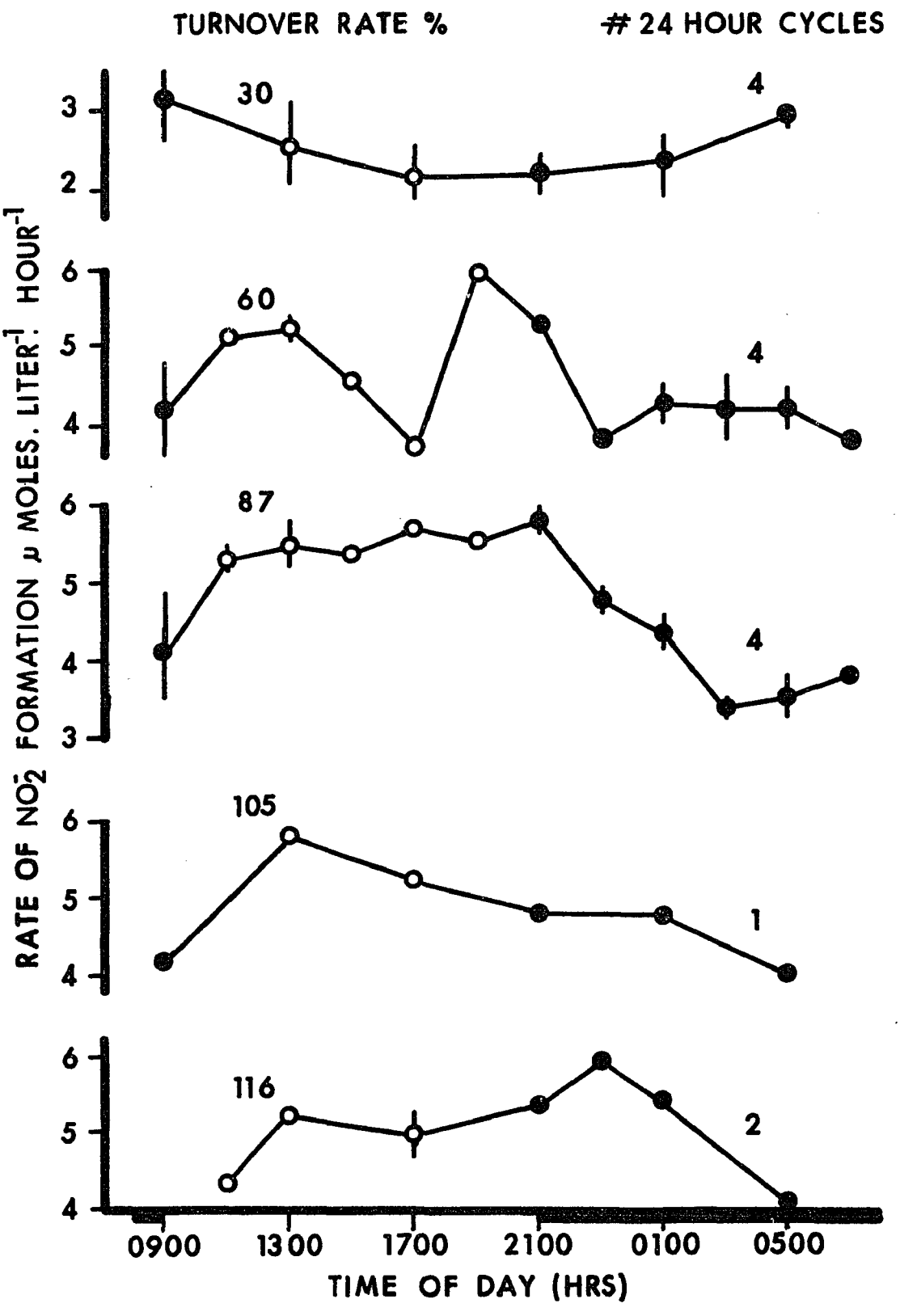

TURNOVER RATE %
# 24 HOUR CYCLES
30
4
60
4
87
4
105
1
116
2
RATE OF NO2 FORMATION μ MOLES. LITER-1. HOUR-1
0900
1300
1700
2100
0100
0500
TIME OF DAY (HRS)

Figure 12: Carbon fixation as a function of the light and dark cycle for the 30% turnover rate.

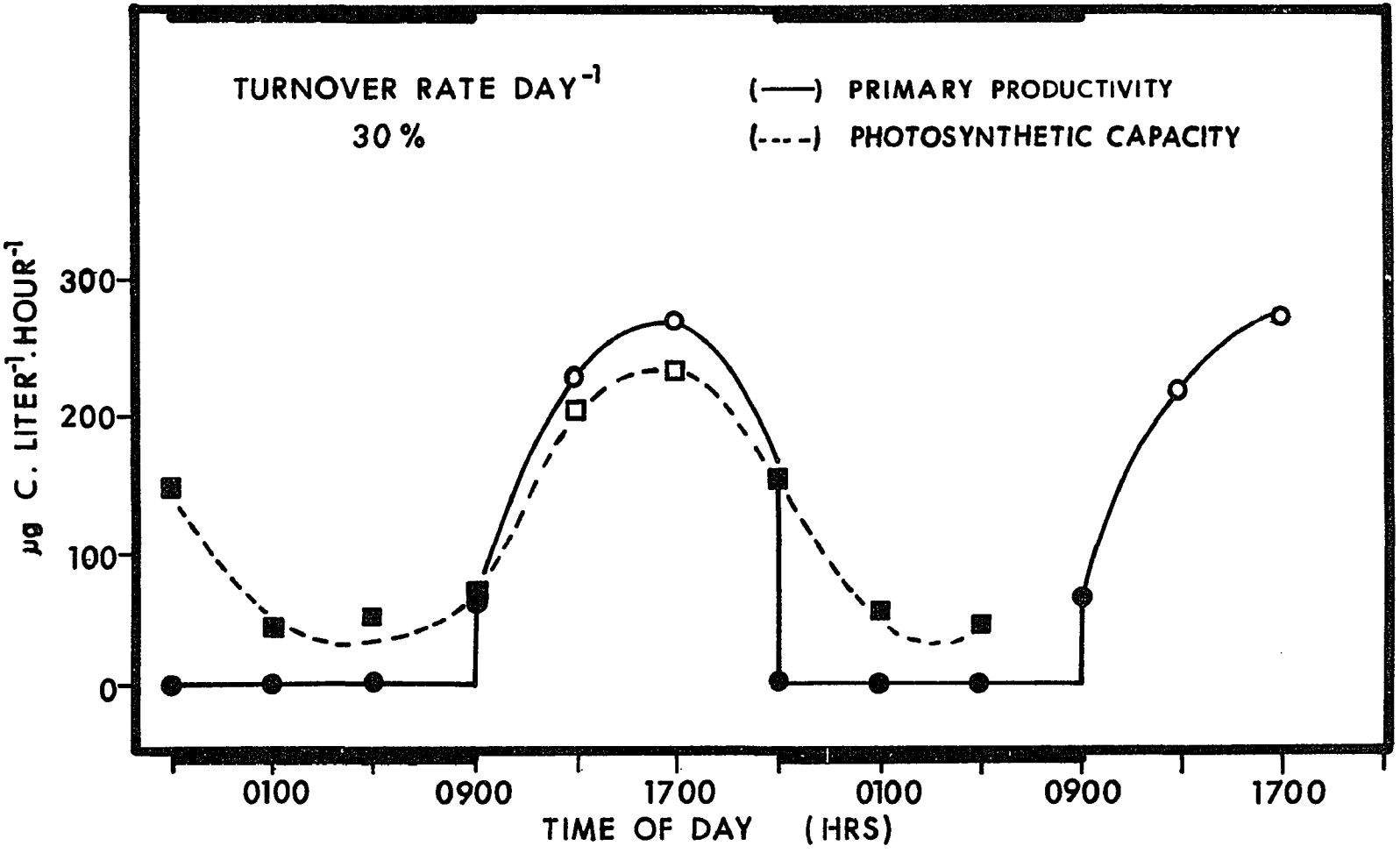

TURNOVER RATE DAY$^{-1}$
30 %
(——) PRIMARY PRODUCTIVITY
(- - -) PHOTOSYNTHETIC CAPACITY
µg C. LITER$^{-1}$.HOUR$^{-1}$
300
200
100
0
0100
0900
1700
0100
0900
1700
TIME OF DAY (HRS)

Figure 13: Carbon fixation as a function of the light and dark cycle for the 60% turnover rate.

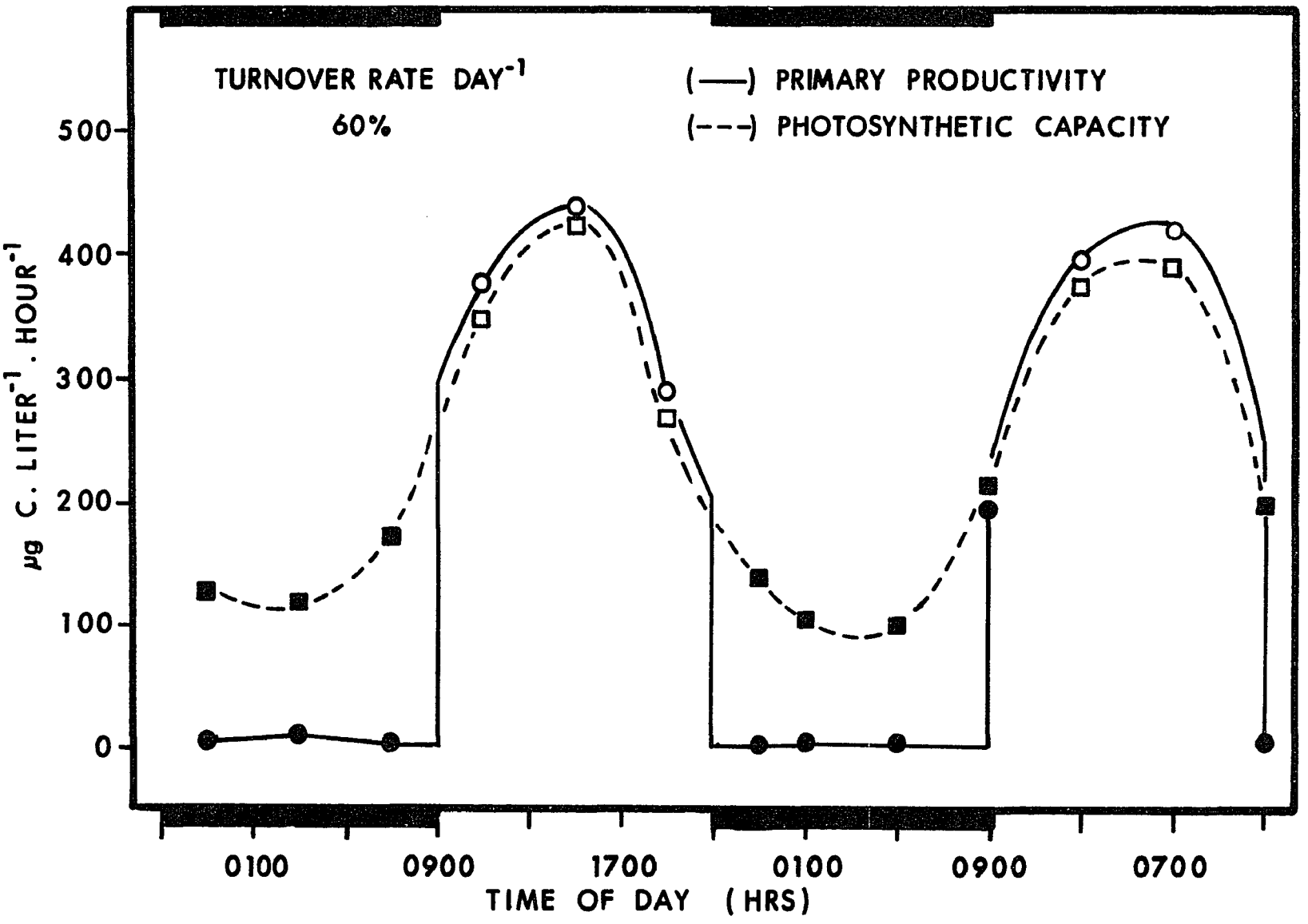

TURNOVER RATE DAY$^{-1}$
60%
(——) PRIMARY PRODUCTIVITY
(---) PHOTOSYNTHETIC CAPACITY
500
400
300
200
100
0
µg C. LITER$^{-1}$. HOUR$^{-1}$
0100
0900
1700
0100
0900
0700
TIME OF DAY (HRS)

Figure 14: Carbon fixation as a function of the light and dark cycle for the 87% turnover rate.

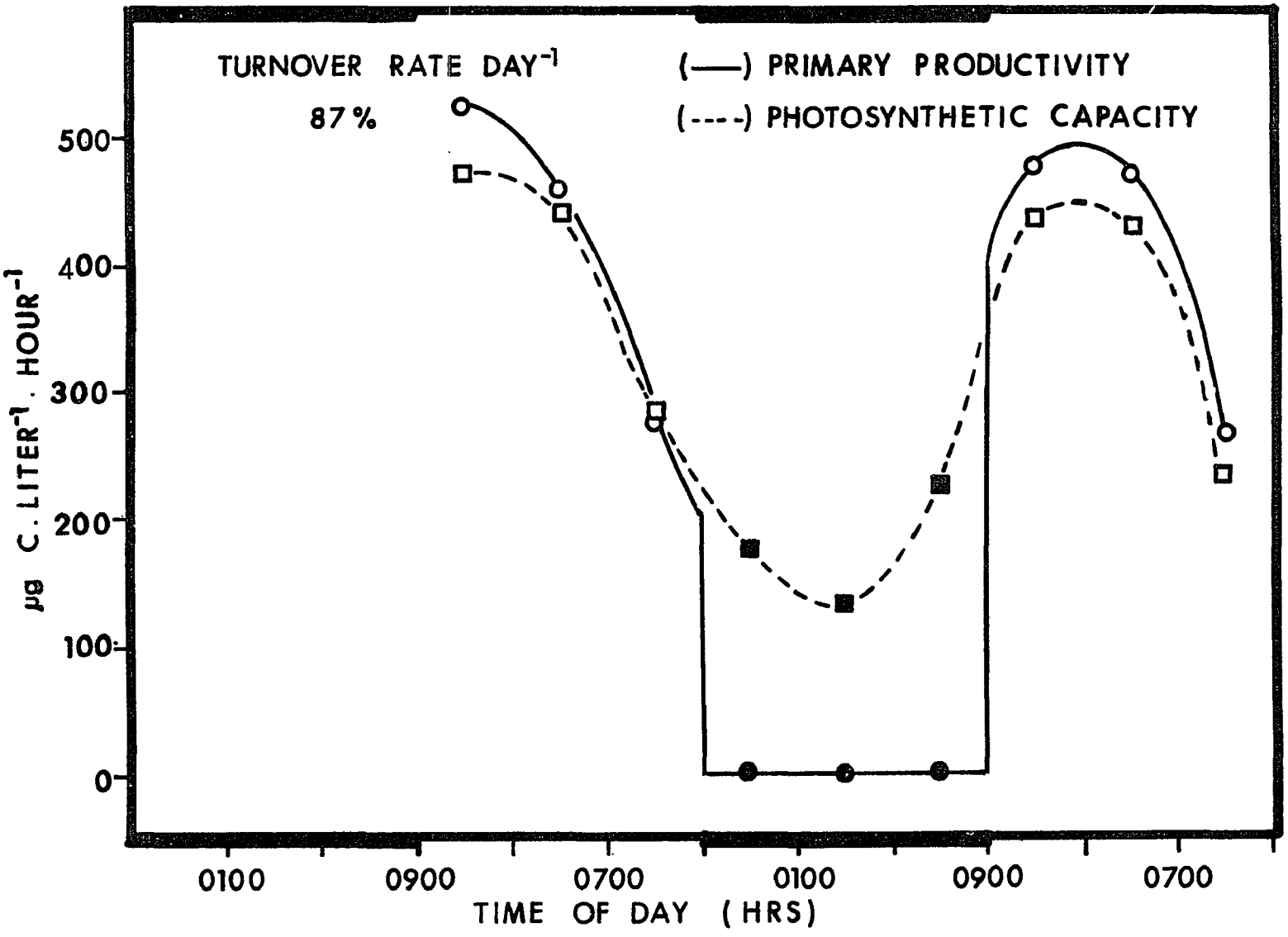

TURNOVER RATE DAY$^{-1}$
87 %
(——) PRIMARY PRODUCTIVITY
(----) PHOTOSYNTHETIC CAPACITY
500
400
300
200
100
0
µg C. LITER$^{-1}$. HOUR$^{-1}$
0100
0900
0700
0100
0900
0700
TIME OF DAY (HRS)

Figure 15: Carbon fixation as a function of the light and dark cycle for the 105% turnover rate.

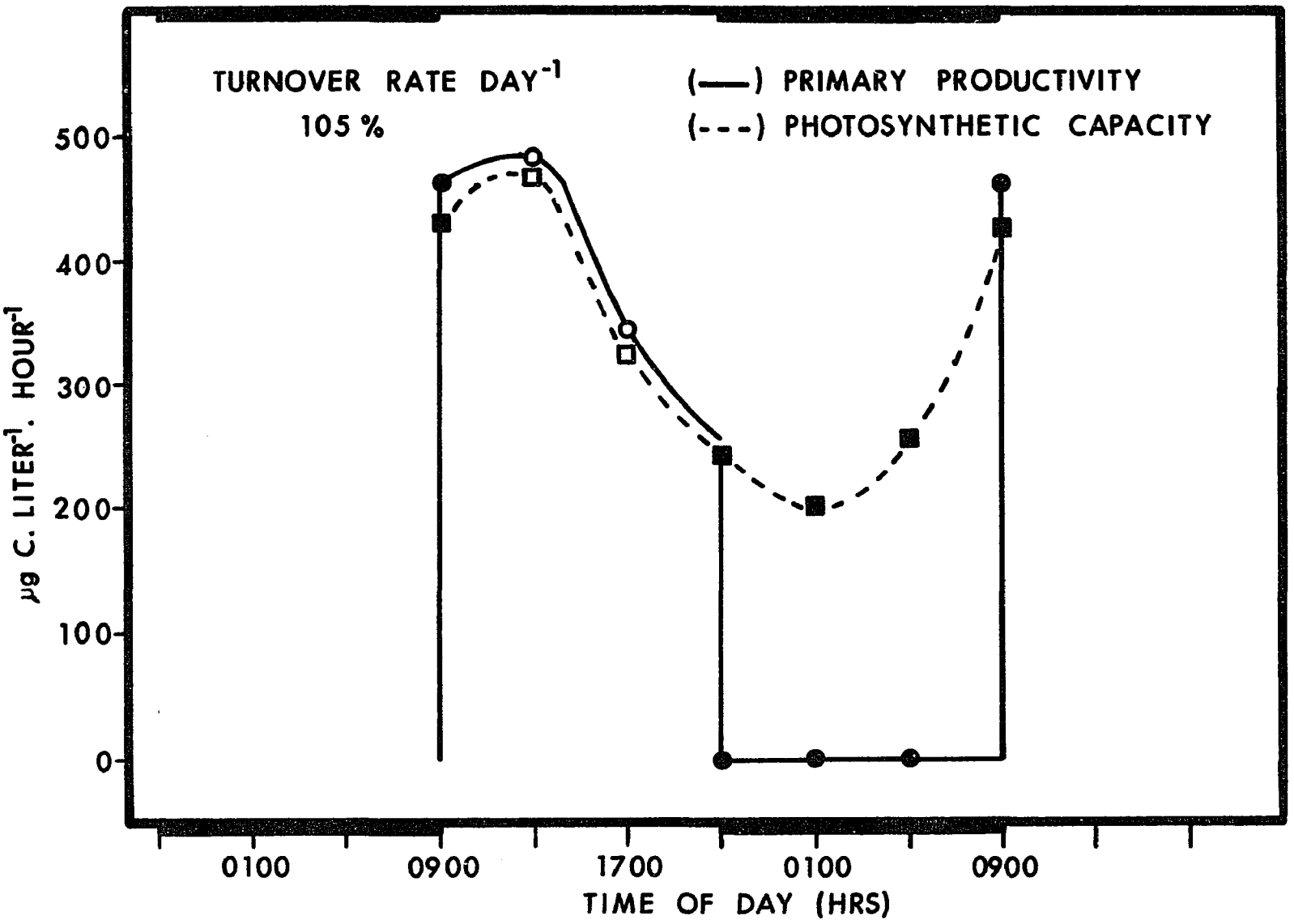

TURNOVER RATE DAY-1
105 %
(——) PRIMARY PRODUCTIVITY
(- - -) PHOTOSYNTHETIC CAPACITY
500
400
300
200
100
0
µg C. LITER-1. HOUR-1
0100
0900
1700
0100
0900
TIME OF DAY (HRS)

Figure 16: Carbon fixation as a function of the light and dark cycle for the 116% turnover rate.

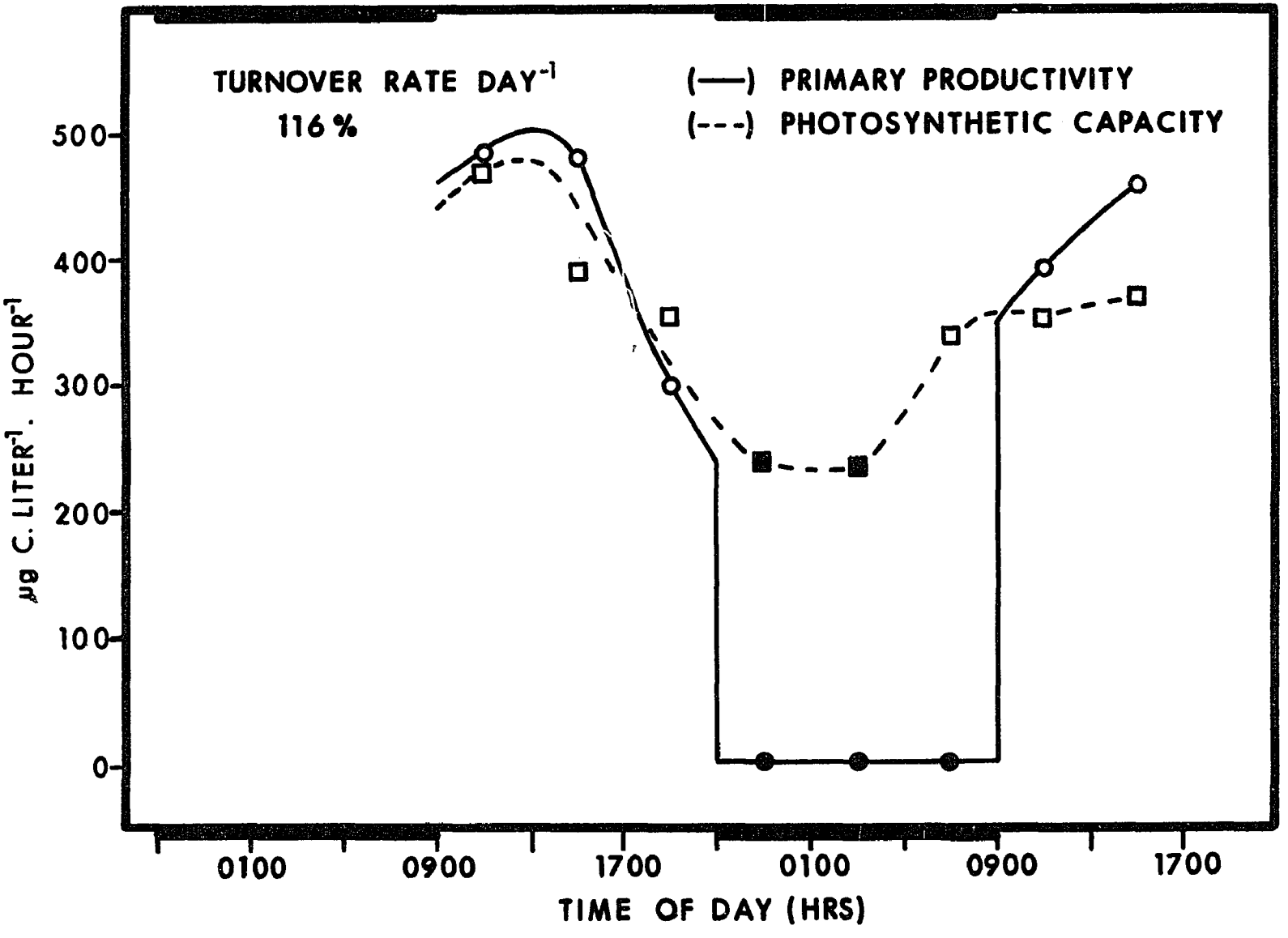

TURNOVER RATE DAY-1
116 %
(——) PRIMARY PRODUCTIVITY
(---) PHOTOSYNTHETIC CAPACITY
500
400
300
200
100
0
µg C. LITER-1. HOUR-1
0100
0900
1700
0100
0900
1700
TIME OF DAY (HRS)

Figure 17: Relationship between the chlorophyll a: carbon ratio and turnover rate per day (%).

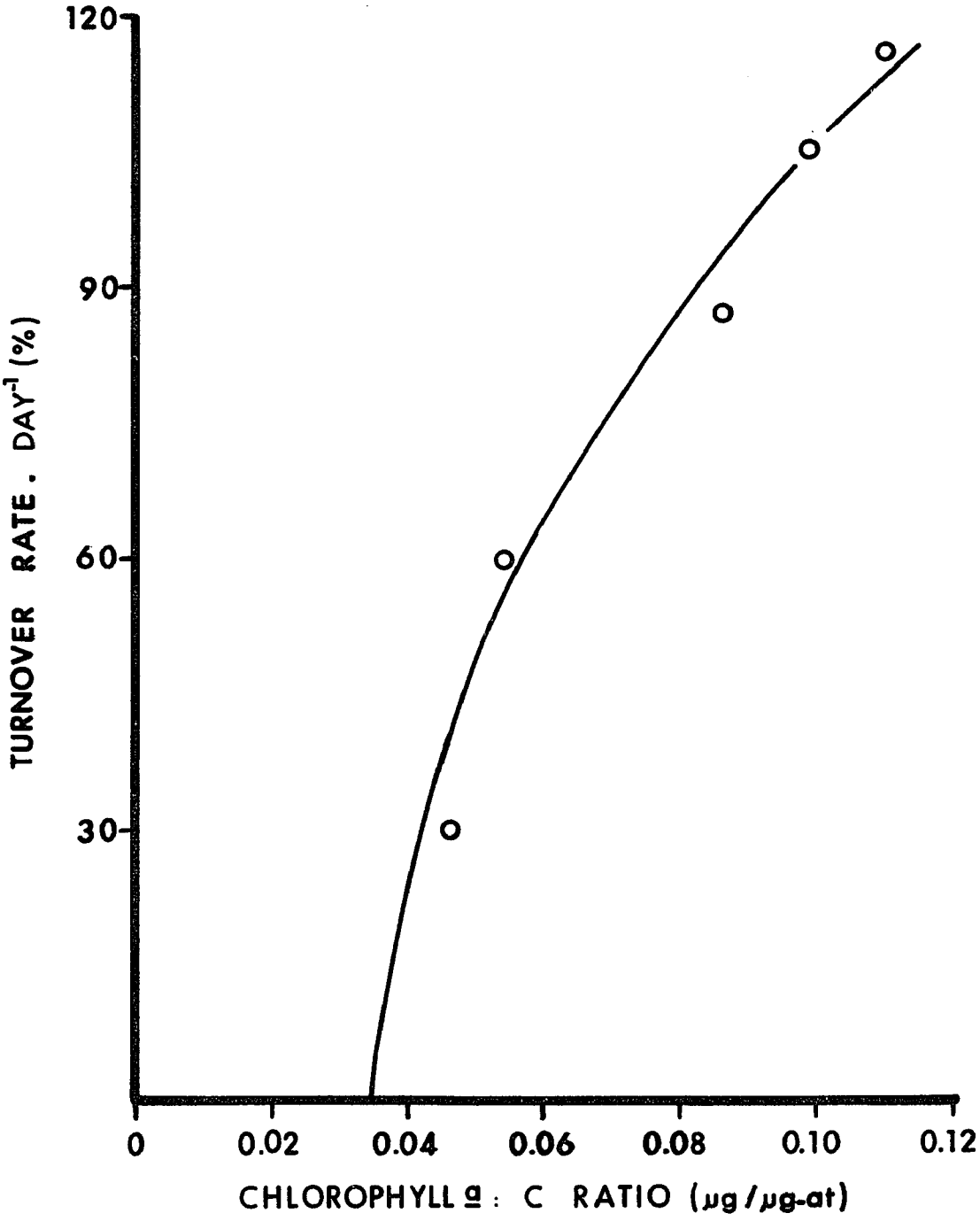

120
90
60
30
TURNOVER RATE. DAY$^{-1}$ (%)
0
0.02
0.04
0.06
0.08
0.10
0.12
CHLOROPHYLL a : C RATIO (µg / µg-at)

Figure 18: Relationship between the nitrogen: carbon ratio and turnover rate per day (%).

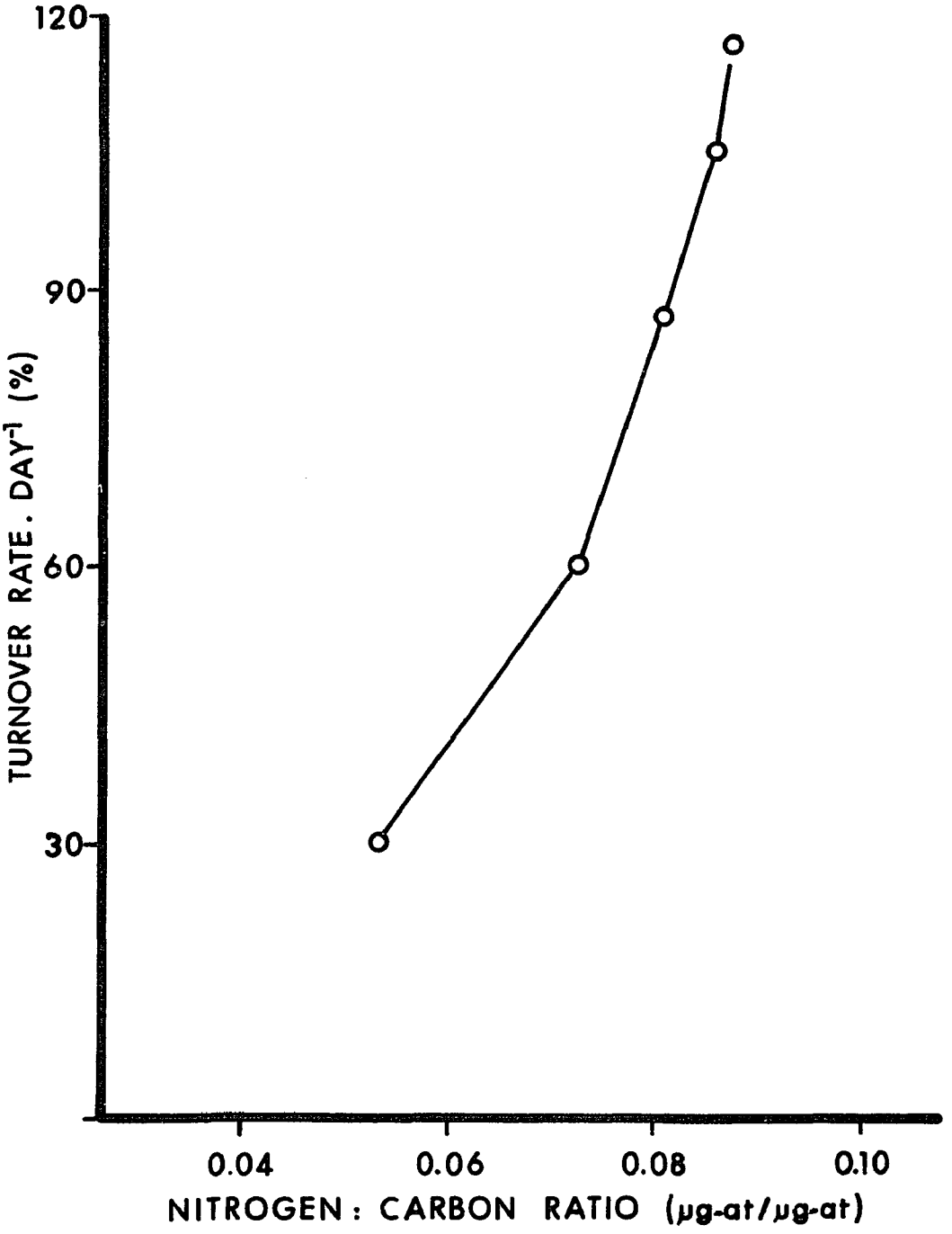

120
90
60
30
TURNOVER RATE. DAY$^{-1}$ (%)
0.04
0.06
0.08
0.10
NITROGEN : CARBON RATIO (µg-at/µg-at)

Figure 19: Diel variations of the mean NADH nitrate reductase activity as μ moles $NO_2^-$ formed/ (μg-at PN) (hour), at each dilution rate.

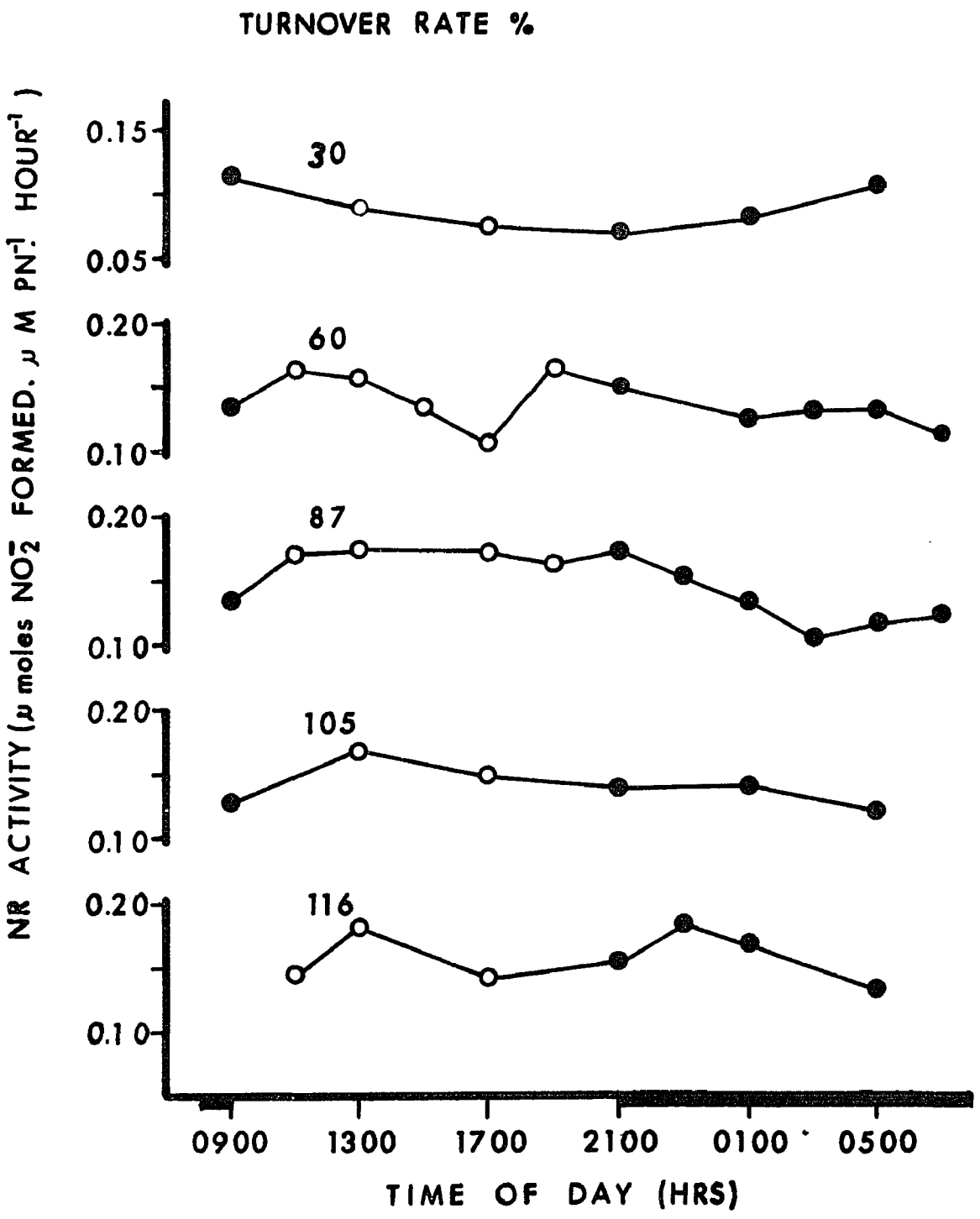

TURNOVER RATE %
NR ACTIVITY (μ moles $NO_2^-$ FORMED. μ M $PN^{-1}$ $HOUR^{-1}$)
0.15
0.05
30
0.20
0.10
60
0.20
0.10
87
0.20
0.10
105
0.20
0.10
116
0900
1300
1700
2100
0100
0500
TIME OF DAY (HRS)

Figure 20: Diel variations of the mean NADH nitrate reductase activity as $\mu$ moles $NO_2^-$ formed/ ($10^8$ cells) (hour), at each dilution rate.

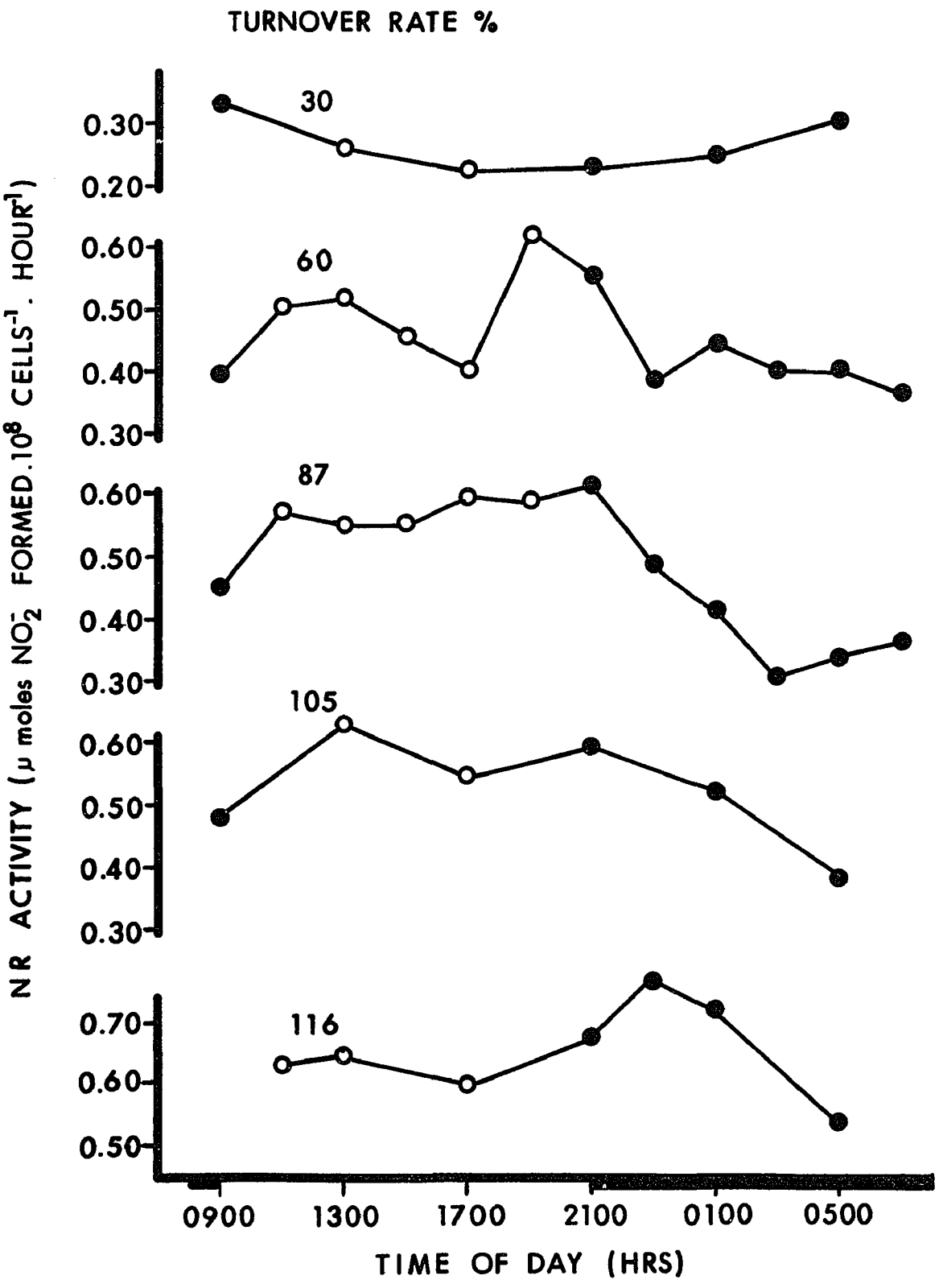

TURNOVER RATE %
30
60
87
105
116
0.30
0.20
0.60
0.50
0.40
0.30
0.60
0.50
0.40
0.30
0.60
0.50
0.40
0.30
0.70
0.60
0.50
NR ACTIVITY (μ moles NO2- FORMED.10^8 CELLS^-1. HOUR^-1)
0900
1300
1700
2100
0100
0500
TIME OF DAY (HRS)

Figure 21: Diel variations of the mean NADH nitrate reductase activity as $\mu$ moles $NO_2^-$ formed/ ($\mu$g chlorophyll *a*) (hour), at each dilution rate.

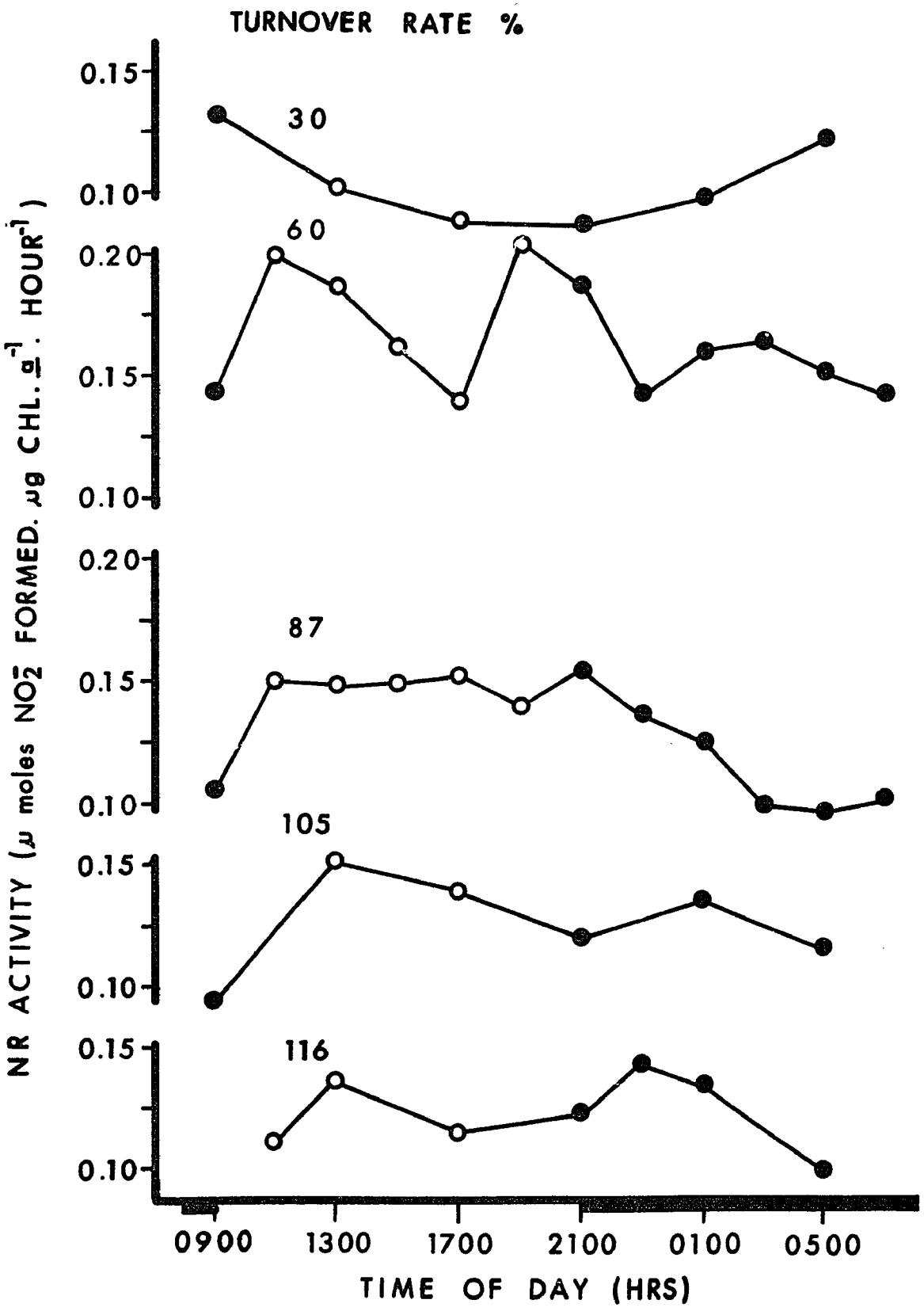

TURNOVER RATE %
30
60
87
105
116
0.15
0.10
0.20
0.15
0.10
0.20
0.15
0.10
0.15
0.10
0.15
0.10
NR ACTIVITY (μ moles $NO_2^-$ FORMED. μg CHL. $g^{-1}$. $HOUR^{-1}$)
0900
1300
1700
2100
0100
0500
TIME OF DAY (HRS)

Figure 22: Diel variations of the mean NADH nitrate reductase activity, as $\mu$ moles $NO_2^-$ formed/ ($\mu$g protein) (hour), at each dilution rate.

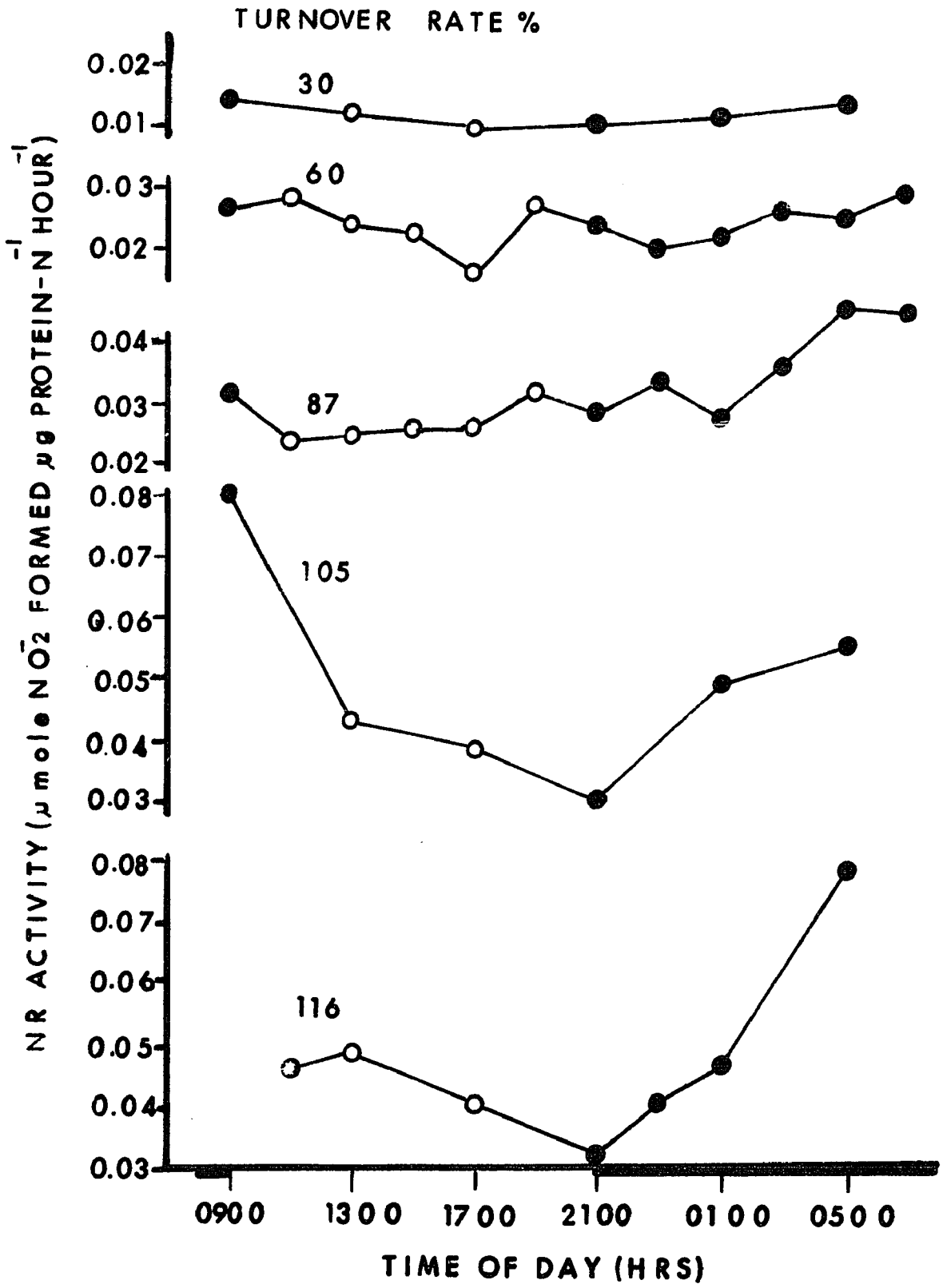

TURNOVER RATE %
30
60
87
105
116
0.02
0.01
0.03
0.02
0.04
0.03
0.02
0.08
0.07
0.06
0.05
0.04
0.03
0.08
0.07
0.06
0.05
0.04
0.03
NR ACTIVITY (μmol• NO2- FORMED μg PROTEIN-N-1 HOUR-1)
0900
1300
1700
2100
0100
0500
TIME OF DAY (HRS)

Figure 23: Relationship between the mean NADH nitrate reductase activity and the turnover rate per day (%).

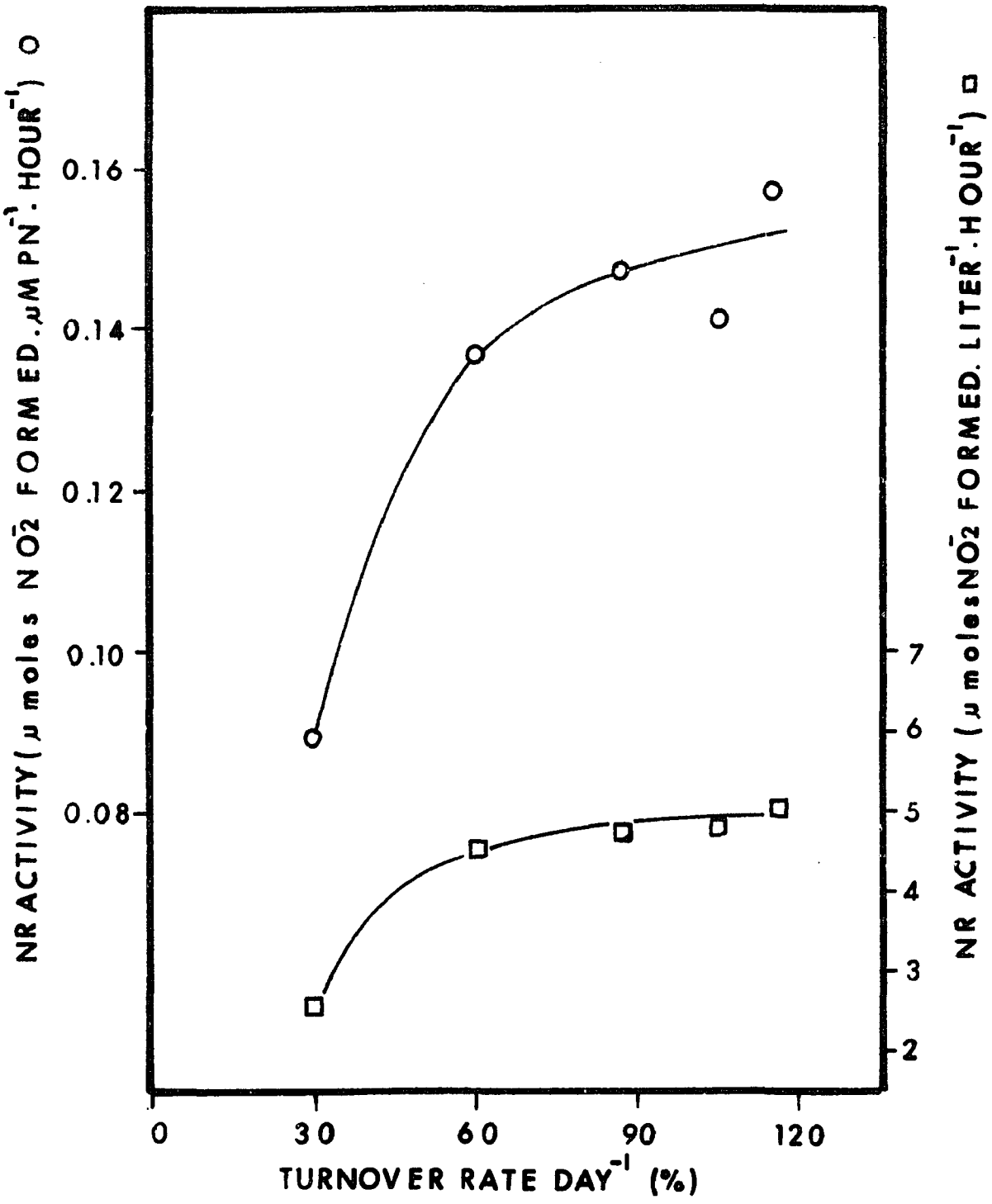

NR ACTIVITY ( μ moles $NO_2^-$ FORMED. μM $PN^{-1}$. $HOUR^{-1}$) ○
NR ACTIVITY ( μ moles $NO_2^-$ FORMED. $LITER^{-1}$. $HOUR^{-1}$) □
0.16
0.14
0.12
0.10
0.08
7
6
5
4
3
2
0
30
60
90
120
TURNOVER RATE $DAY^{-1}$ (%)

Figure 24: Relationship between the chlorophyll *a* concentration in the culture and the hours of light exposure per generation time for each turnover rate.

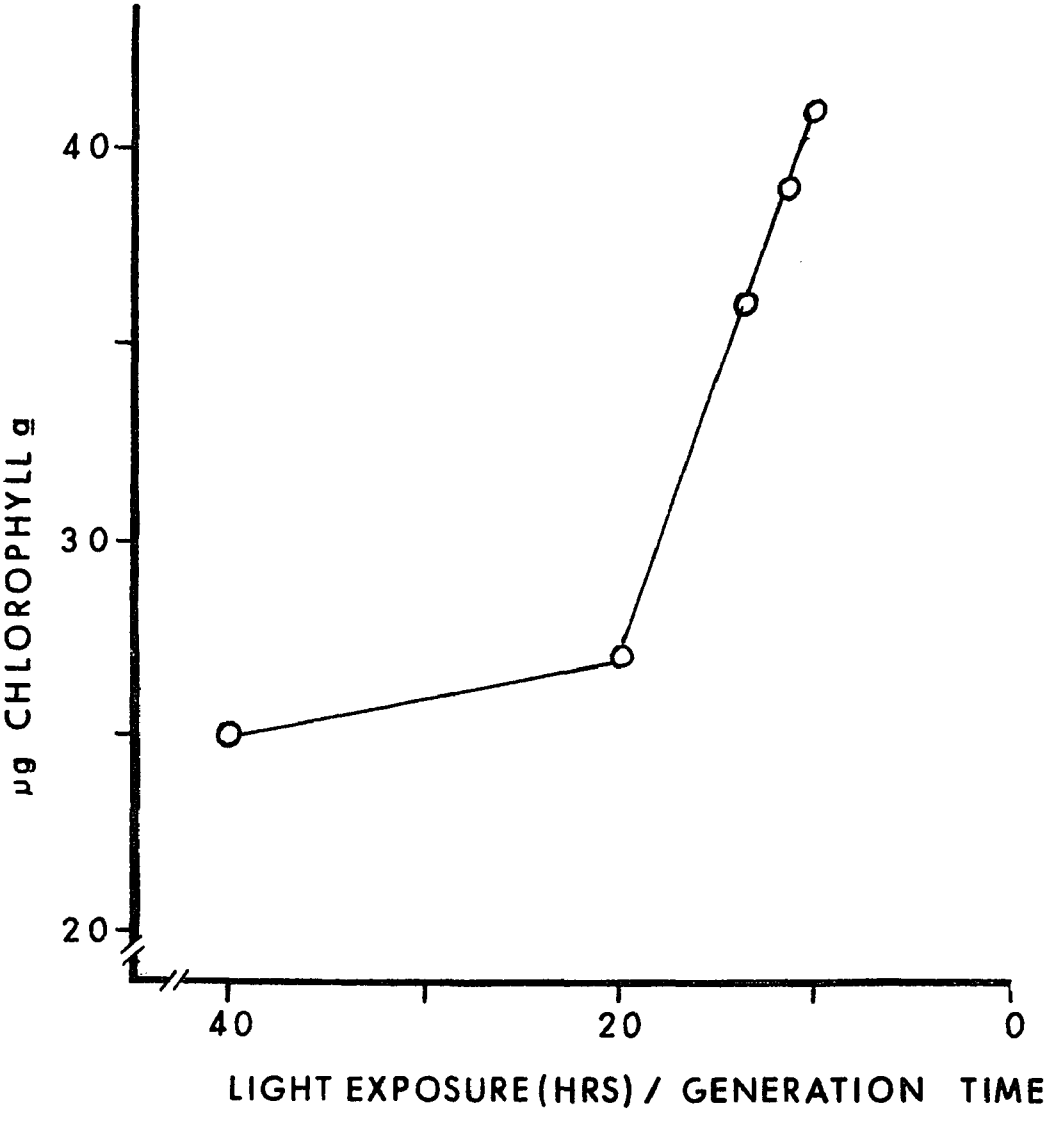

40
30
20
µg CHLOROPHYLL a
40
20
0
LIGHT EXPOSURE (HRS) / GENERATION TIME

Figure 25: Relationship between the population particulate nitrogen per cell or yield coefficient "$q_i$" and turnover rate per day (%)

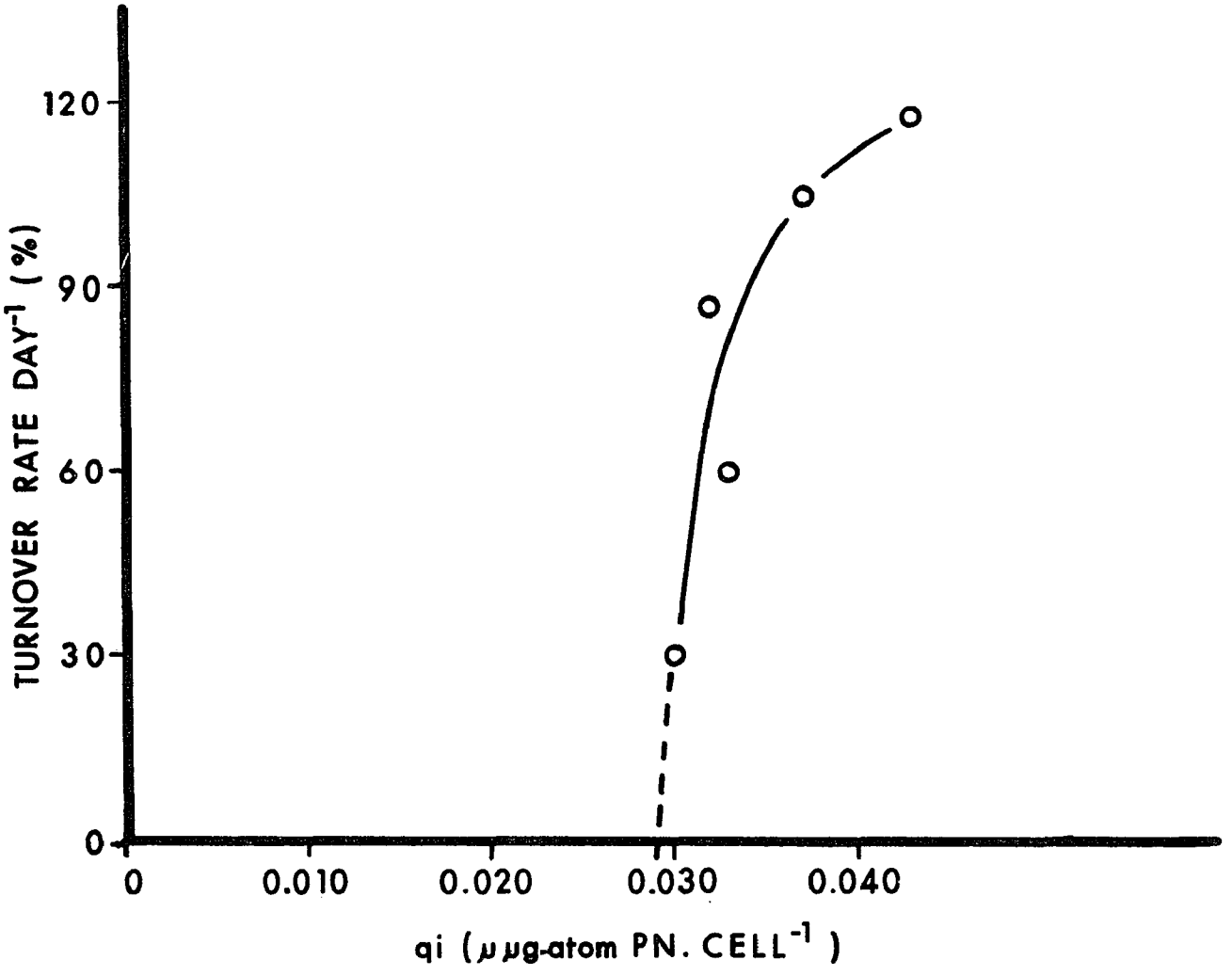

120
90
60
30
0
TURNOVER RATE DAY$^{-1}$ (%)
0
0.010
0.020
0.030
0.040
qi (μμg-atom PN. CELL$^{-1}$ )

Figure 26: Relationship between the calculated intracellular nitrate "q" and turnover rate per day (%).

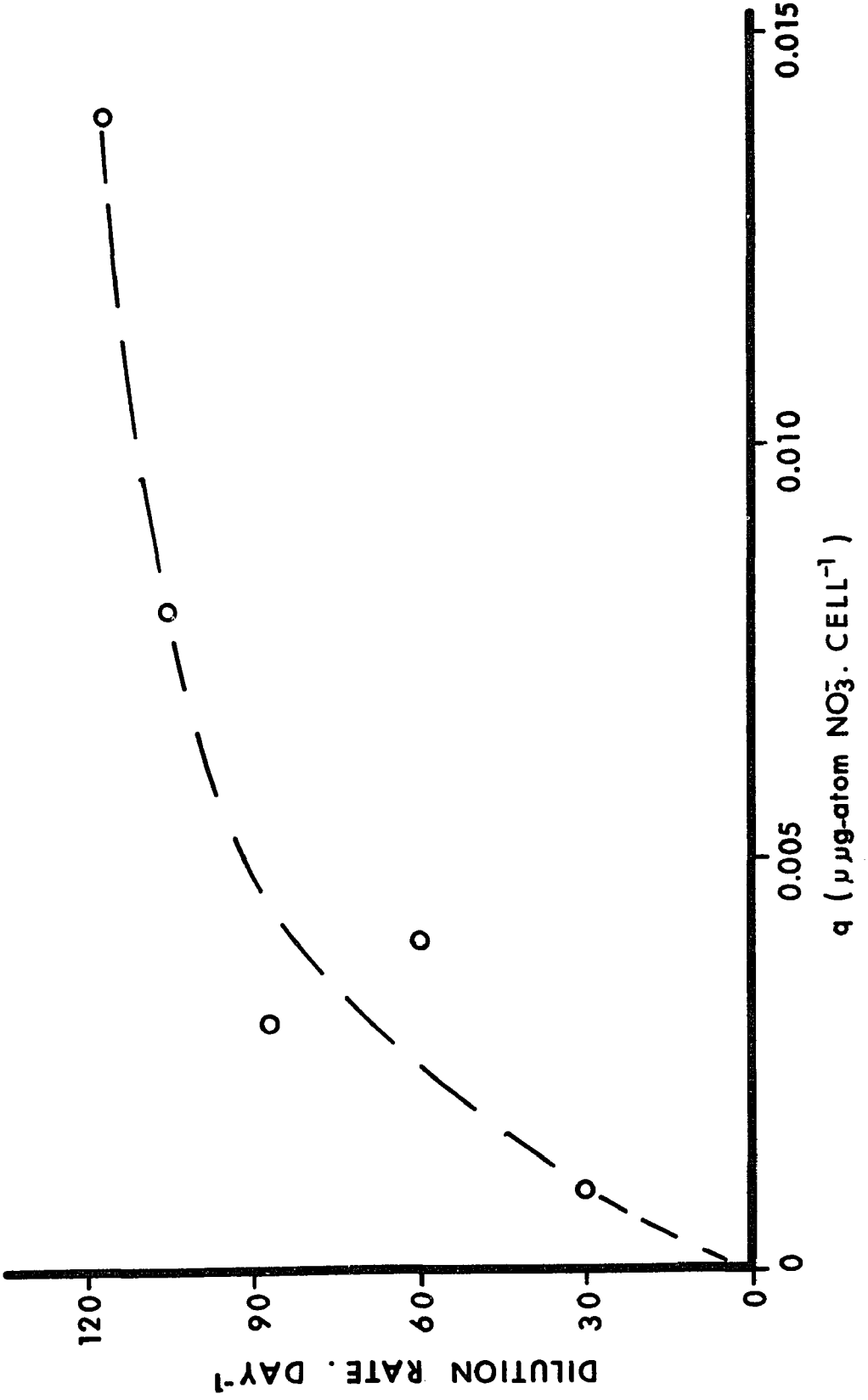

DILUTION RATE . DAY-1
120
90
60
30
0
0
0.005
0.010
0.015
q ( μ μg-atom NO3. CELL-1 )

Figure 27: Relationship between the measured intracellular nitrate and turnover rate per day (%).

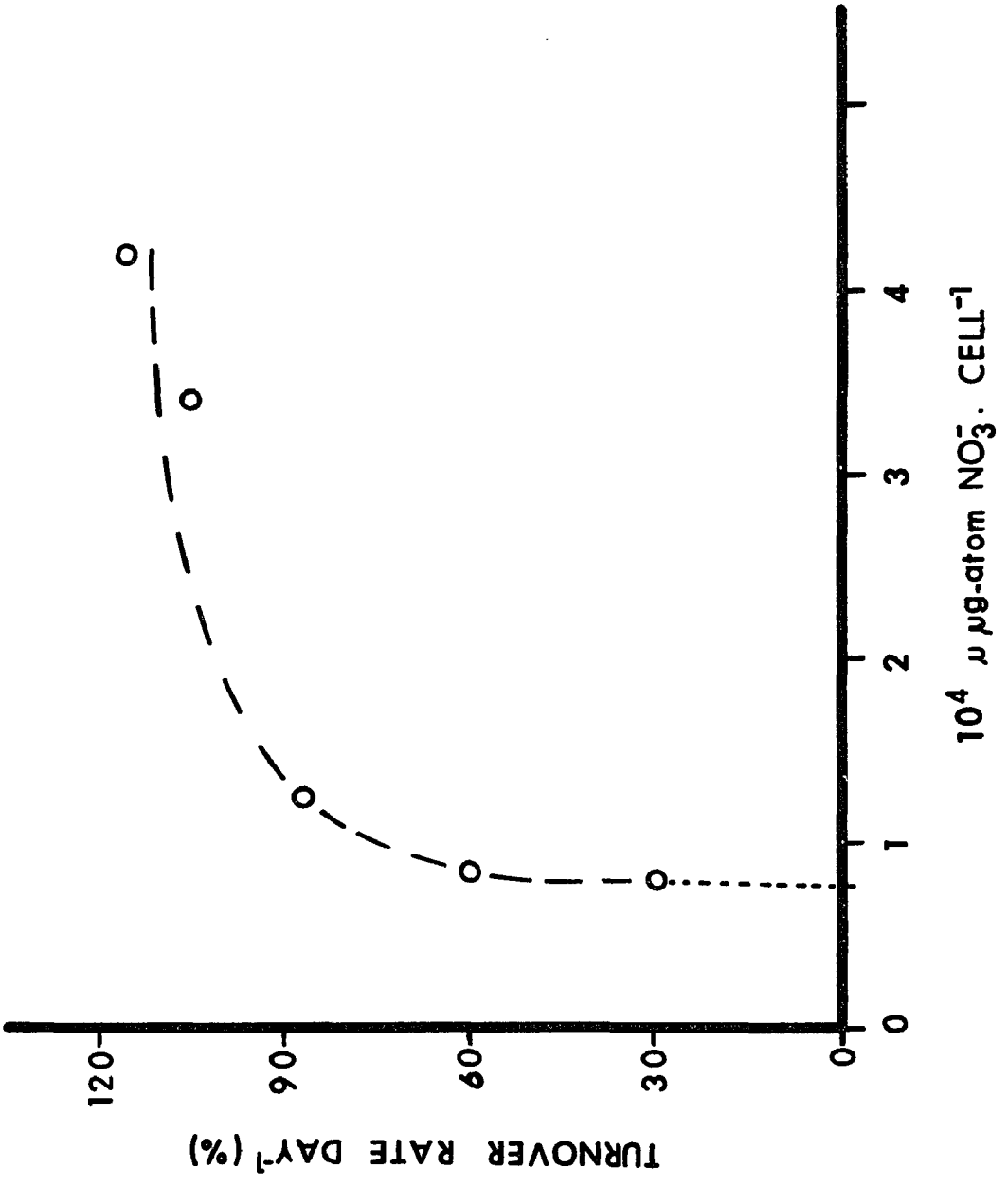

TURNOVER RATE DAY$^{-1}$ (%)
120
90
60
30
0
0
1
2
3
4
$10^4$ μ μg-atom $NO_3^-$. CELL$^{-1}$

Figure 28: Relationship between chlorophyll *a* and particulate organic carbon for each turnover rate per day (%).

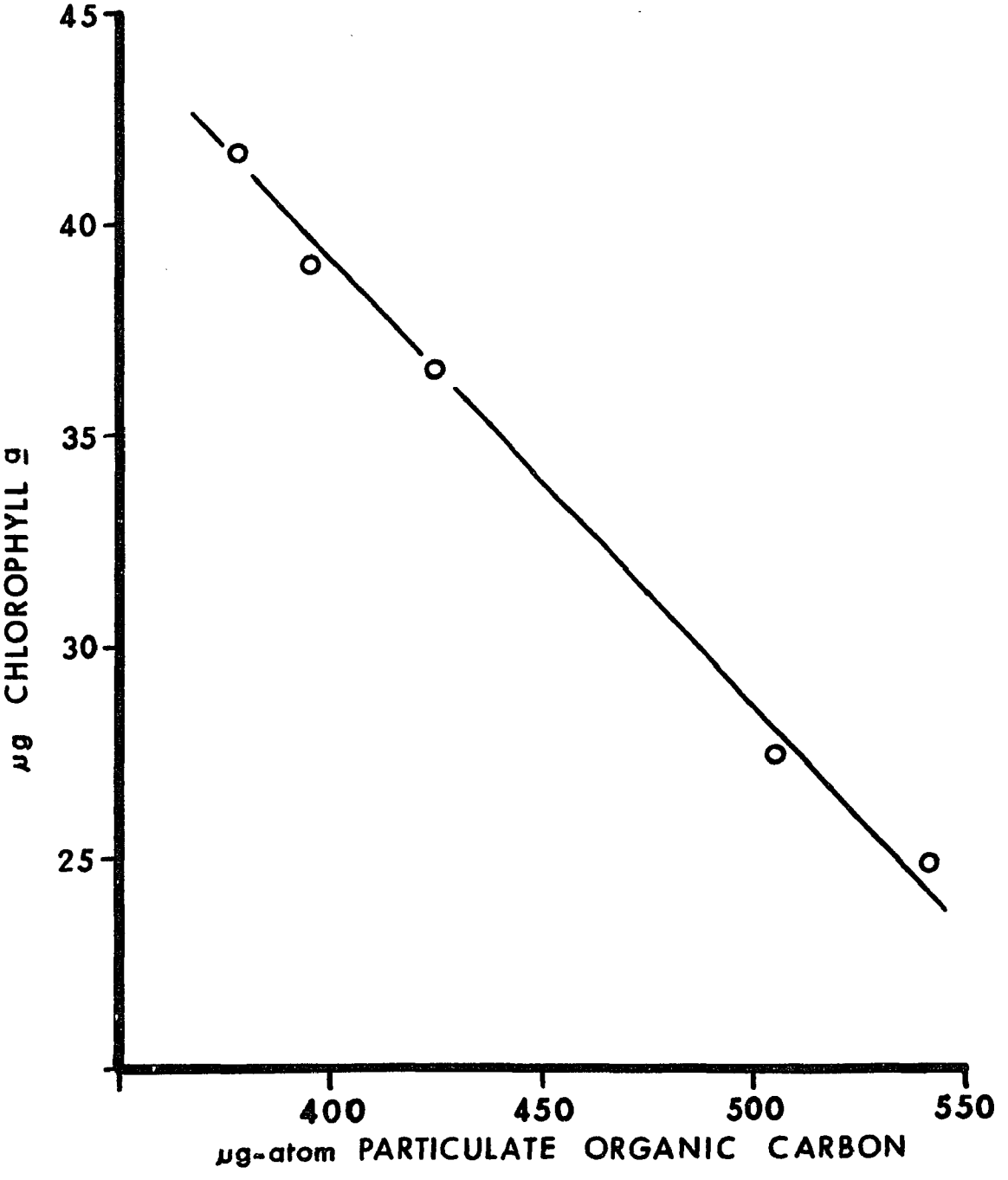

45
40
35
30
25
µg CHLOROPHYLL a
400
450
500
550
µg-atom PARTICULATE ORGANIC CARBON

Figure 29: Relationship between particulate organic carbon (POC) at 0900 hour and the minimum value of photosynthetic capacity for each turnover rate per day (%).

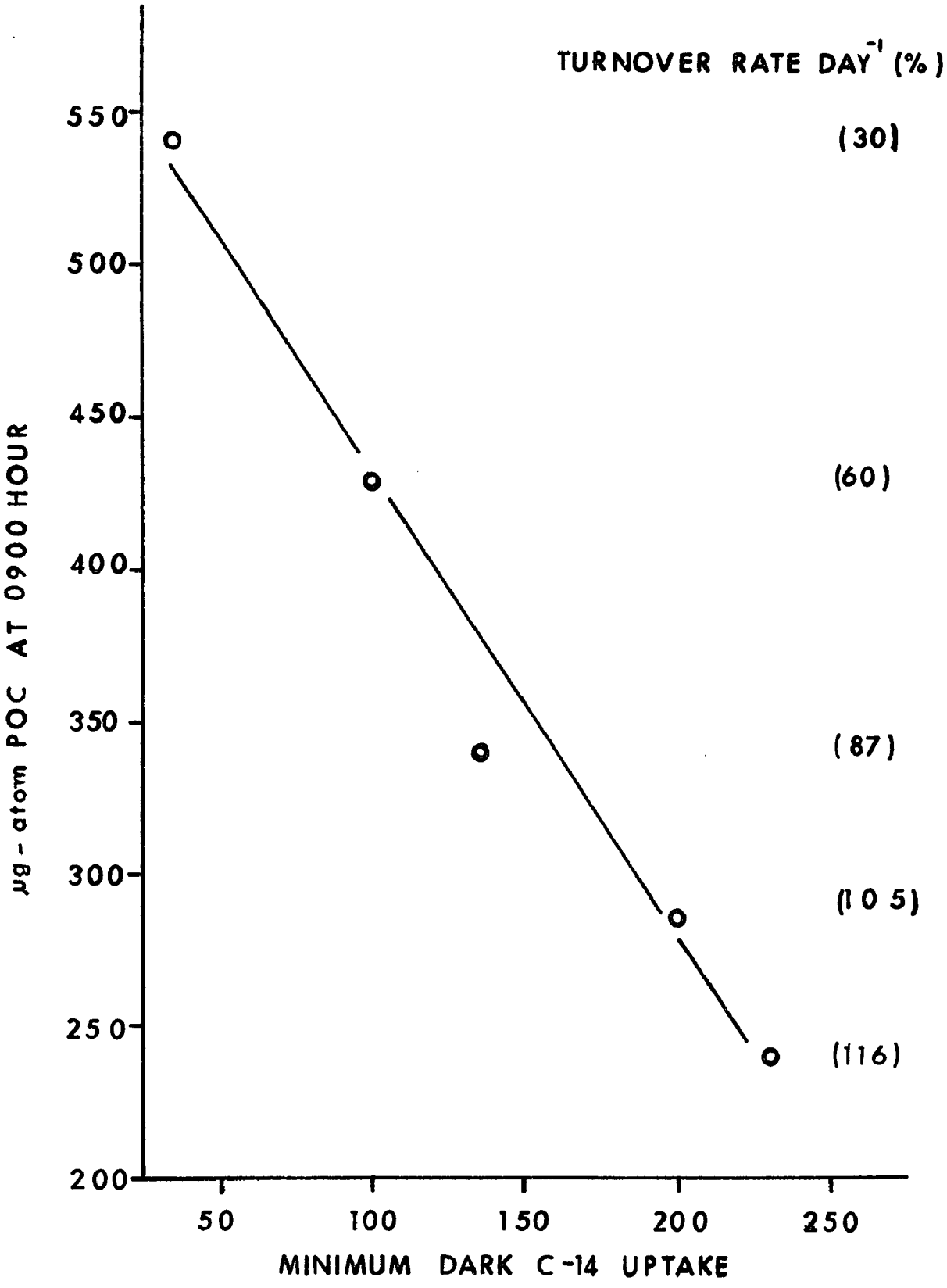

TURNOVER RATE DAY$^{-1}$ (%)
(30)
(60)
(87)
(105)
(116)
550
500
450
400
350
300
250
200
µg - atom POC AT 0900 HOUR
50
100
150
200
250
MINIMUM DARK C-14 UPTAKE

Figure 30: Relationship between hours of light exposure and the percentage of carbon-14 uptake over PCC production, at each dilution rate.

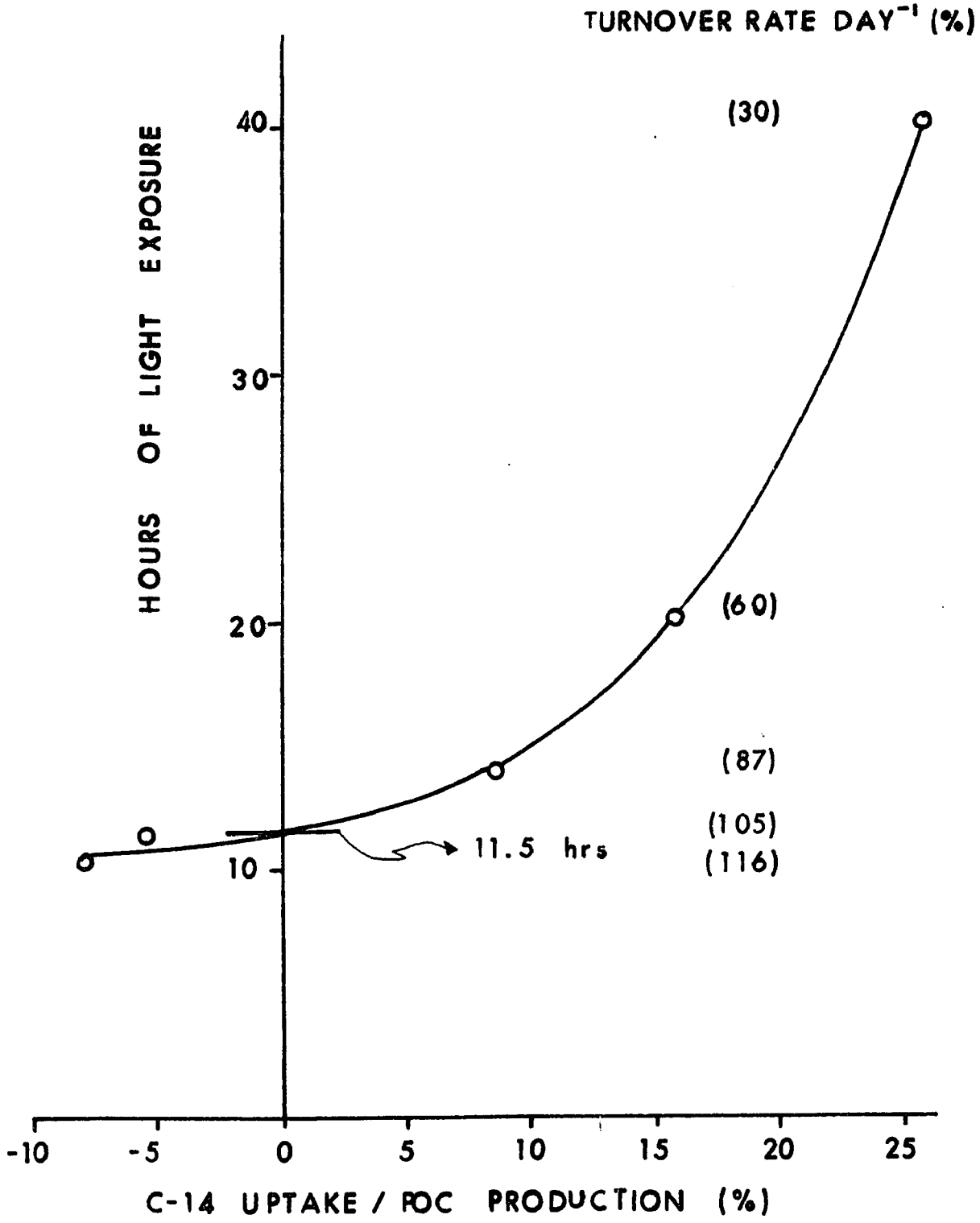
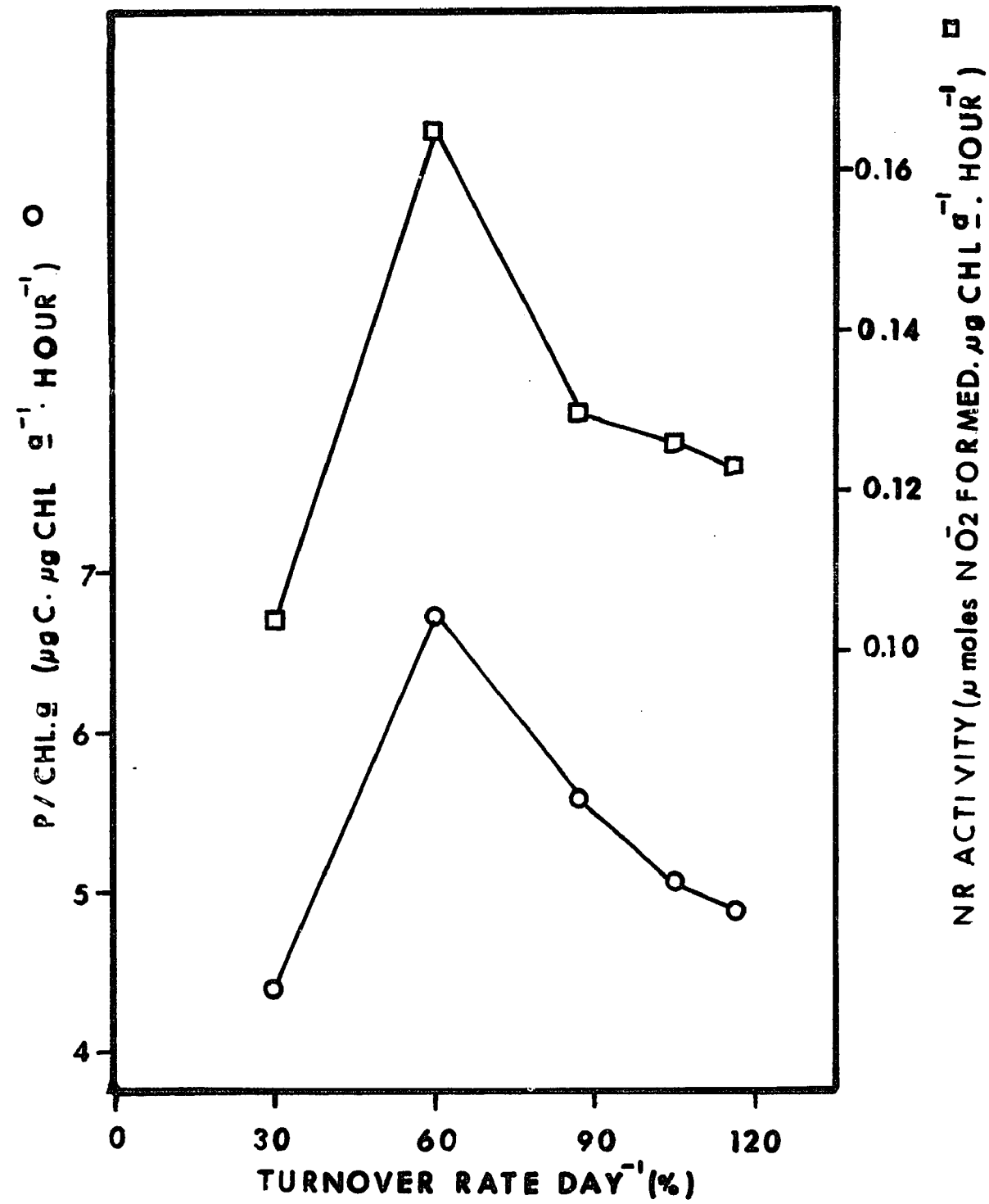

TURNOVER RATE DAY$^{-1}$ (%)
HOURS OF LIGHT EXPOSURE
40
30
20
10
0
(30)
(60)
(87)
(105)
(116)
11.5 hrs
-10
-5
0
5
10
15
20
25
C-14 UPTAKE / POC PRODUCTION (%)

Figure 31: Photosynthetic rate per unit chlorophyll a (P/chl a) (ug C. ug chlorophyll a/hour) and NADH nitrate reductase activity u moles $NO_2^-$ formed/ug chlorophyll a/hour) at each turnover rate per day (%).


P/CHL.a (μgC. μg CHL a⁻¹. HOUR⁻¹) O
NR ACTIVITY (μ moles NO2 FORMED. μg CHL a⁻¹. HOUR⁻¹)
7
6
5
4
0.16
0.14
0.12
0.10
0
30
60
90
120
TURNOVER RATE DAY⁻¹(%)

# 6 APPENDIX

In this section is provided figures reporting raw data.

Figure 1: Temporal variations in the concentration of nitrate ($NO_3^-$) in the growth chamber for each turnover rate.

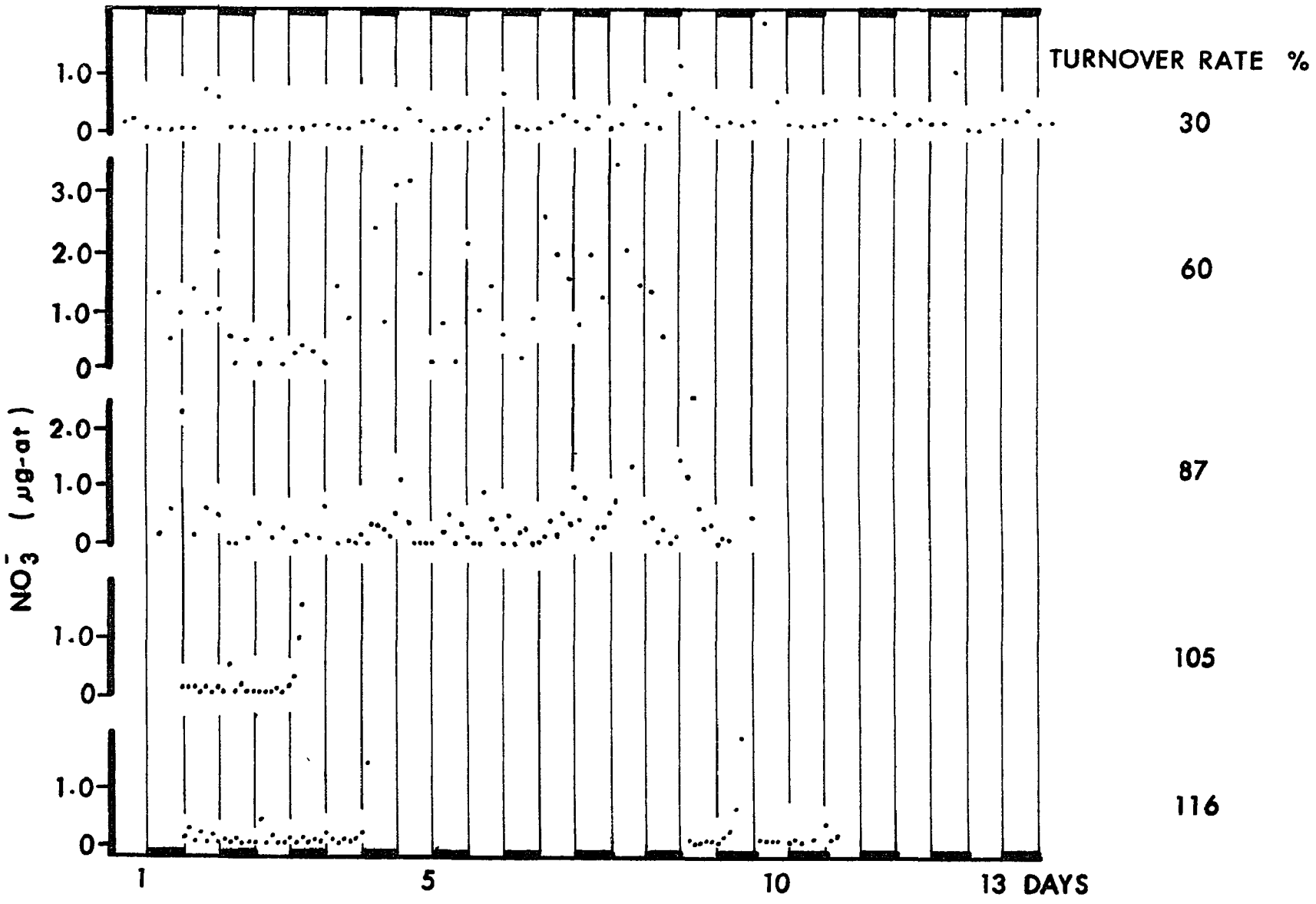

TURNOVER RATE %
30
60
87
105
116
1.0
0
3.0
2.0
1.0
0
2.0
1.0
0
1.0
0
1.0
0
$NO_3^-$ (µg-at)
1
5
10
13 DAYS

Figure 2: Temporal variations in the concentration of silicate ($Si0_2$--) in the growth chamber for each turnover rate.

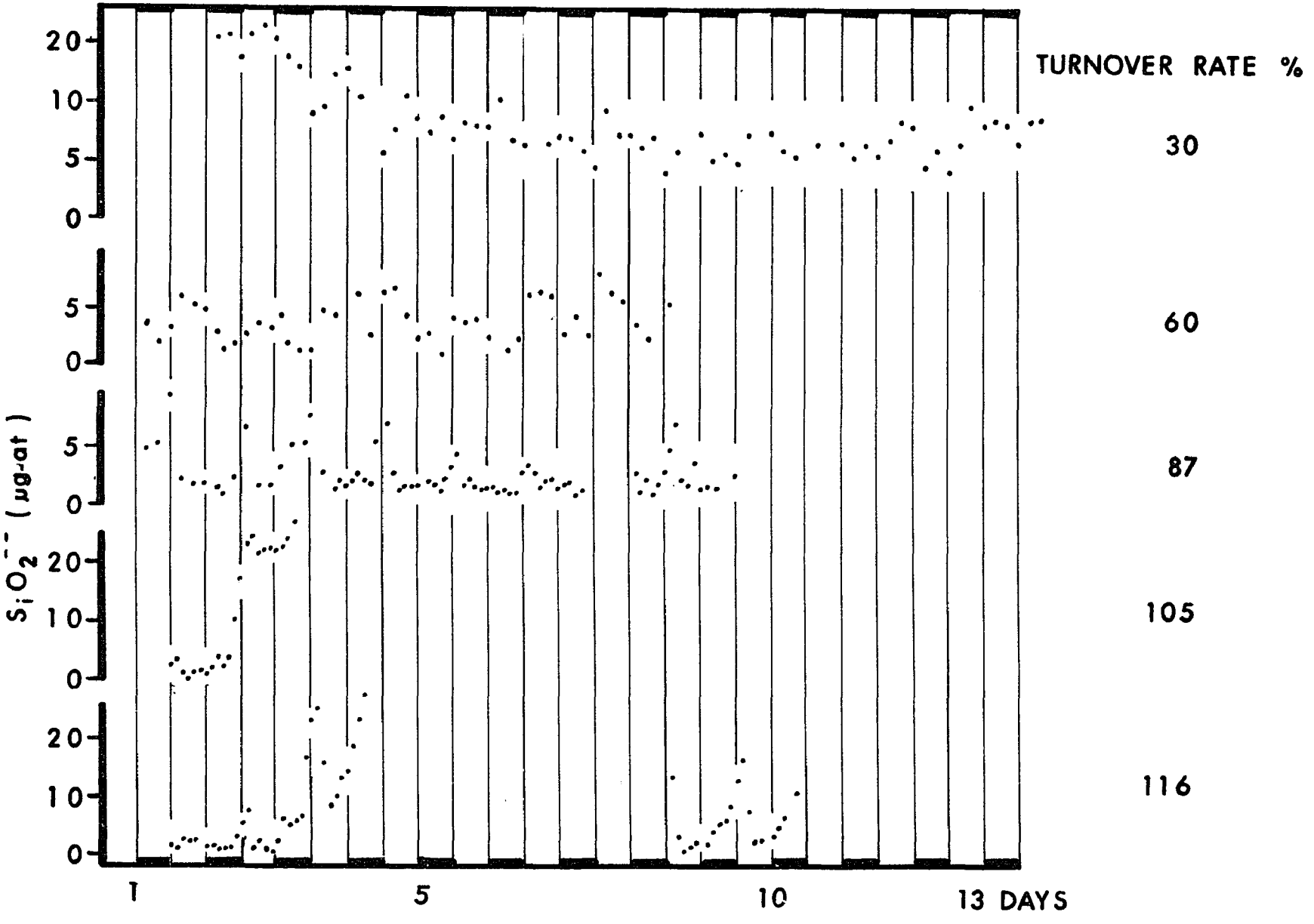

TURNOVER RATE %
30
60
87
105
116
$Si(OH)_2^{--}$ (µg-at)
20
10
5
0
1
5
10
13 DAYS

Figure 3: Temporal variations of cell density in the growth chamber for each turnover rate.

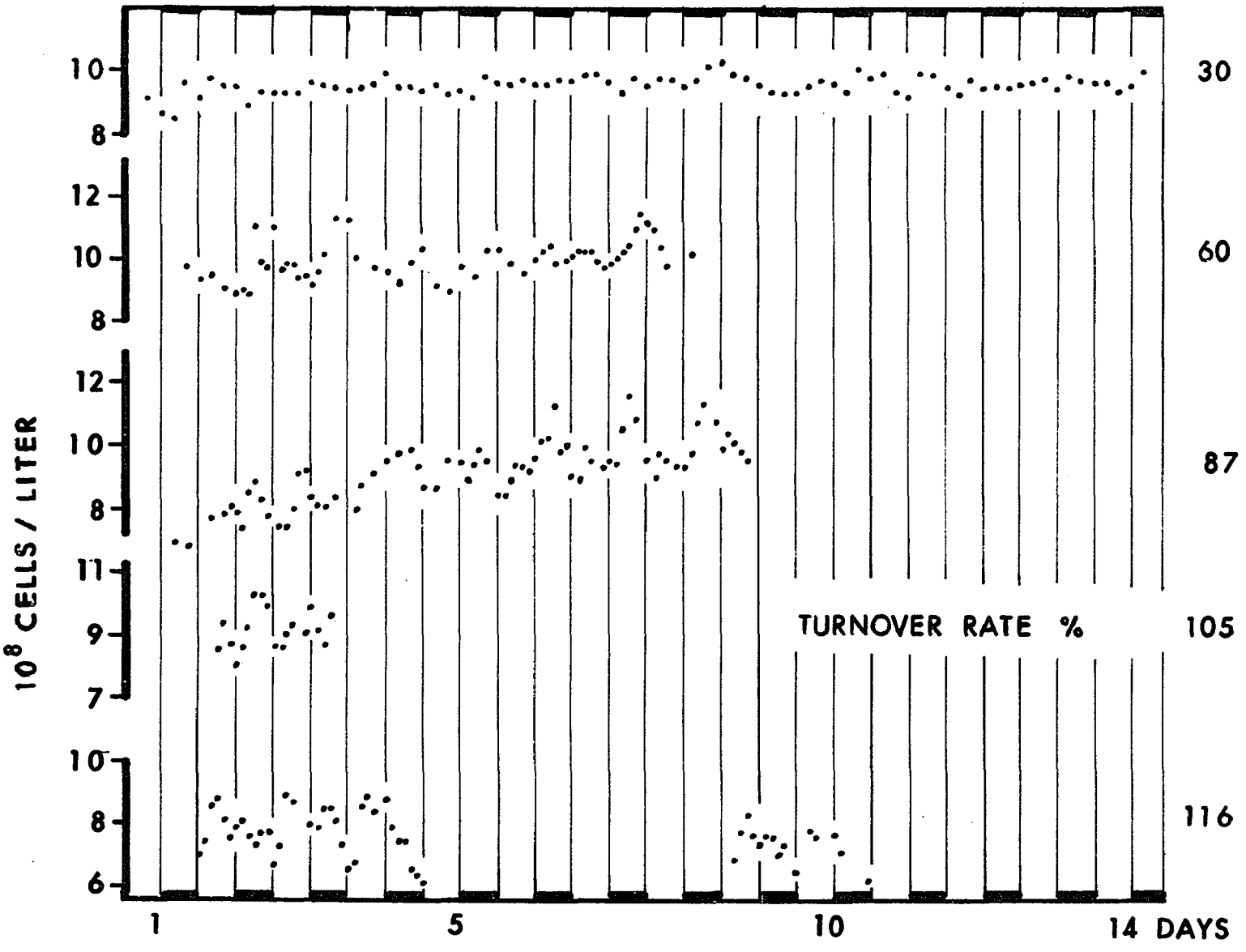

10
8
12
10
8
12
10
8
11
9
7
10
8
6
$10^8$ CELLS / LITER
30
60
87
105
116
TURNOVER RATE %
1
5
10
14 DAYS

Figure 4: Temporal variations in the concentration of protein in the growth chamber for each turnover rate.

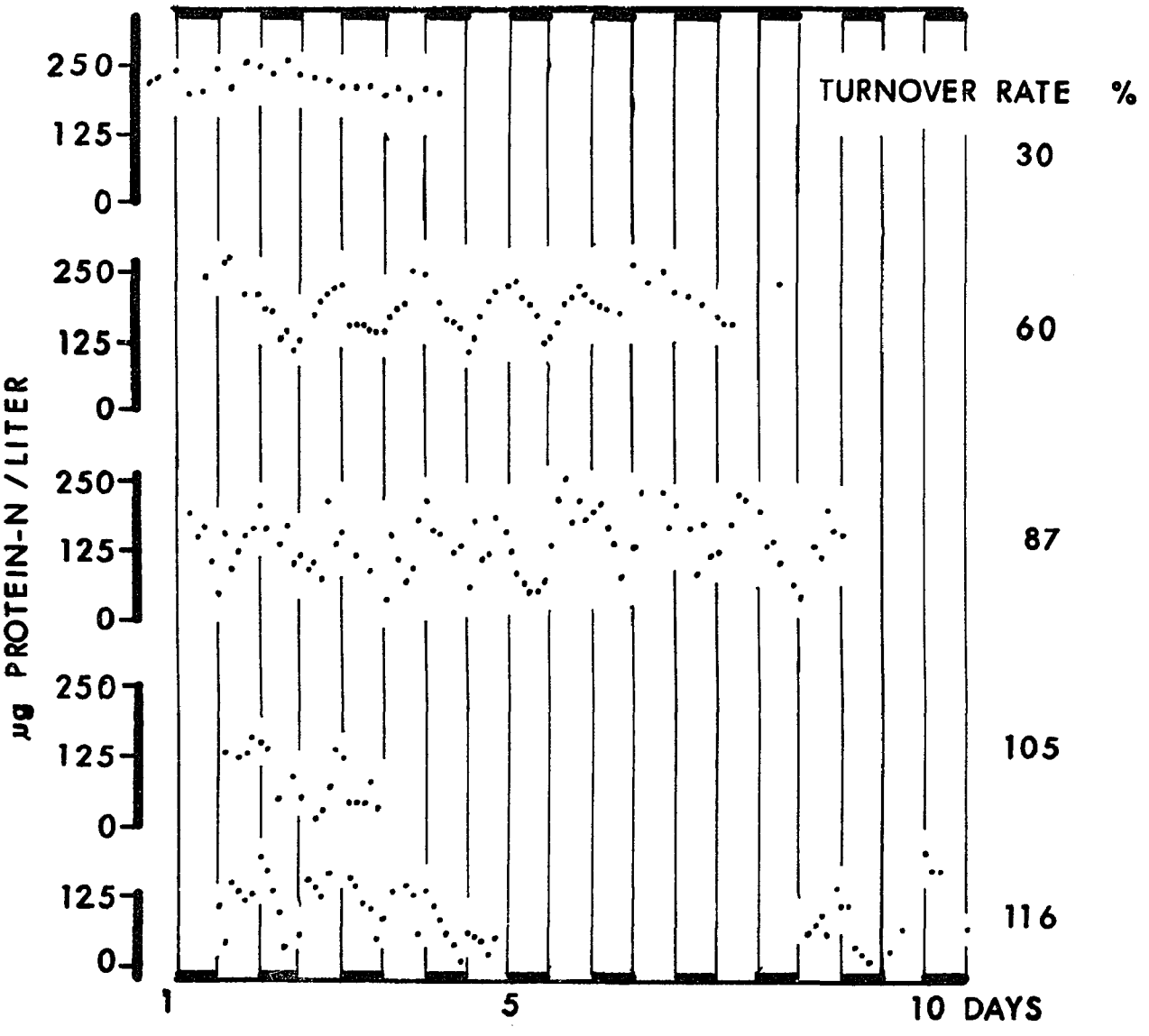

TURNOVER RATE %
30
60
87
105
116
250
125
0
µg PROTEIN-N / LITER
1
5
10 DAYS

Figure 5: Temporal variations in the concentration of chlorophyll *a* in the growth chamber for each turnover rate.

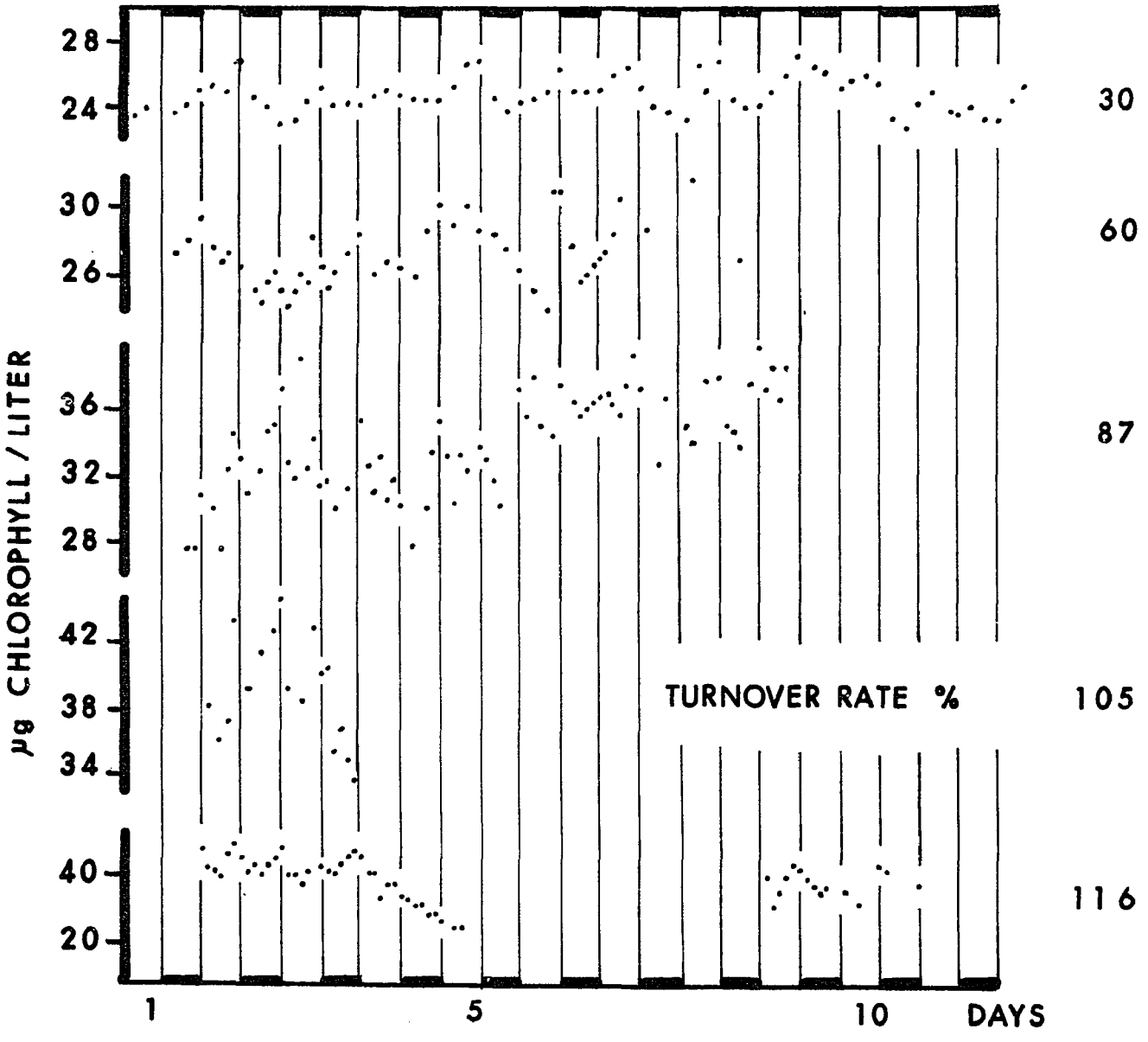

28
24
30
26
36
32
28
42
38
34
40
20
µg CHLOROPHYLL / LITER
1
5
10
DAYS
TURNOVER RATE %
30
60
87
105
116

Figure 6: Temporal variations in the concentration of particulate organic nitrogen (PN) in the growth chamber for each turnover rate.

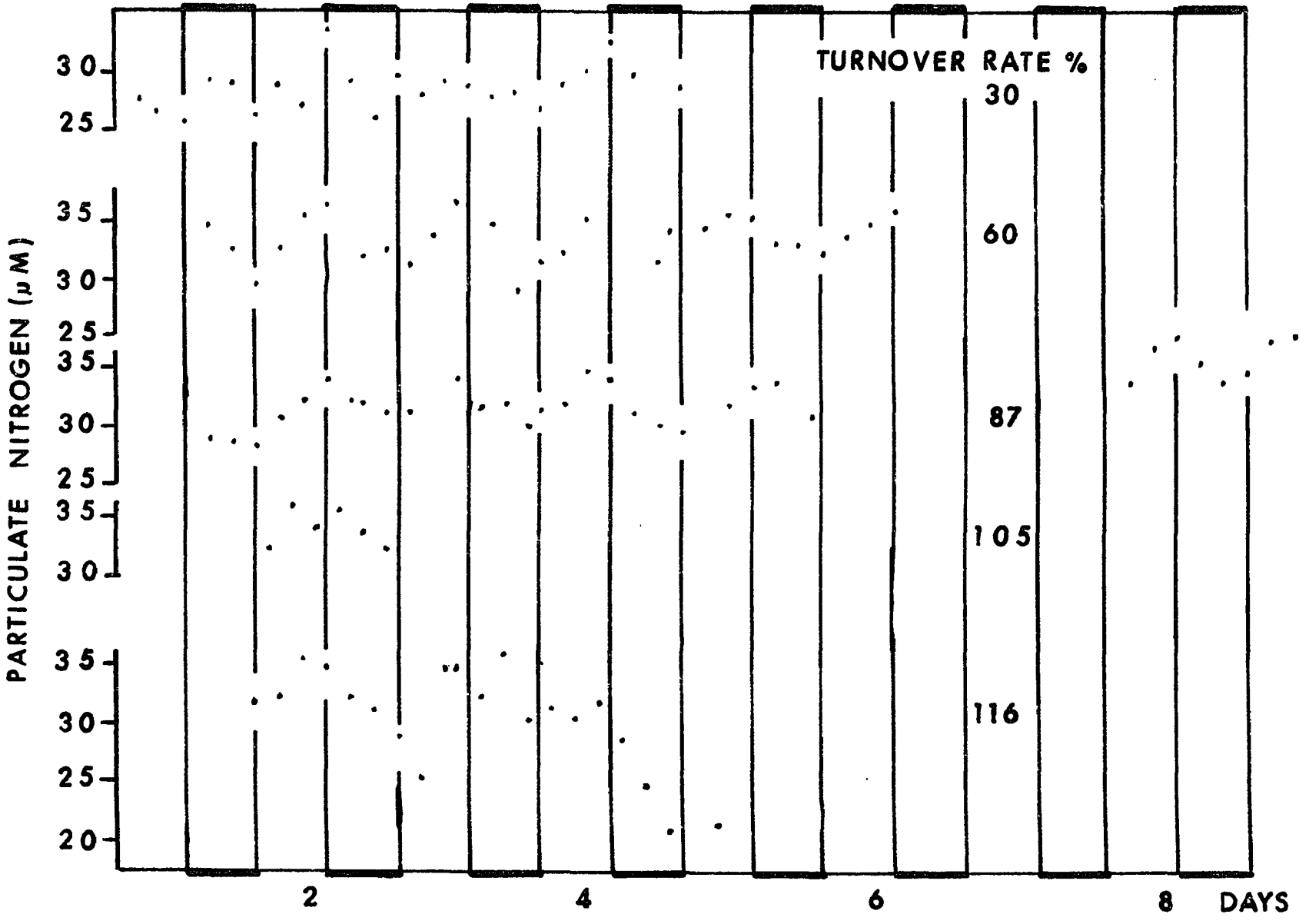

TURNOVER RATE %
30
60
87
105
116
PARTICULATE NITROGEN (μM)
30
25
35
30
25
35
30
25
35
30
25
20
2
4
6
8
DAYS

Figure 7: Temporal variations in the concentration of the particulate organic carbon (POC) in the growth chamber for each turnover rate.

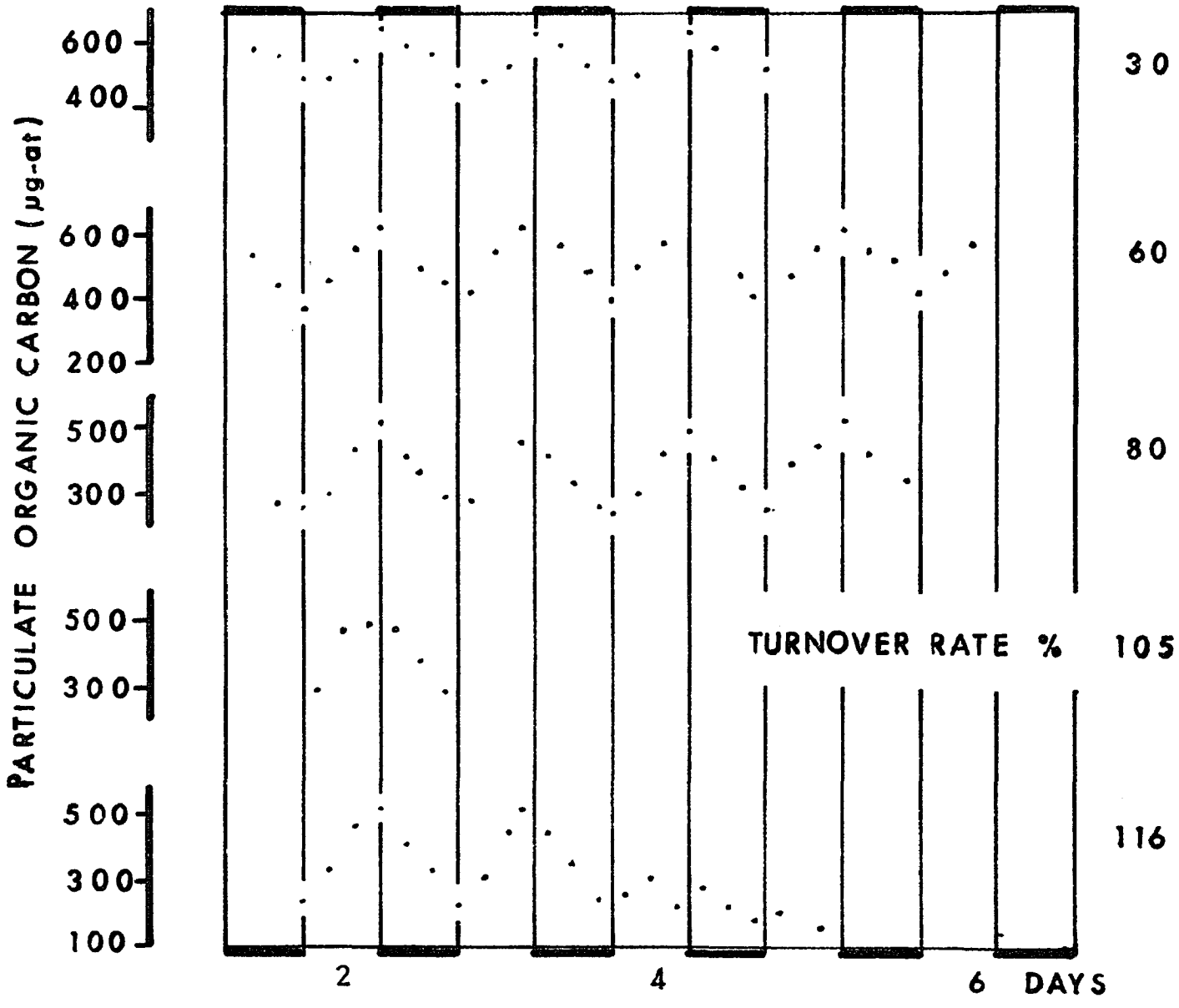

PARTICULATE ORGANIC CARBON (µg-at)
600
400
600
400
200
500
300
500
300
500
300
100
30
60
80
TURNOVER RATE %
105
116
2
4
6
DAYS

Figure 8: Temporal variations in the concentration of carbohydrate in the growth chamber for each dilution rate.

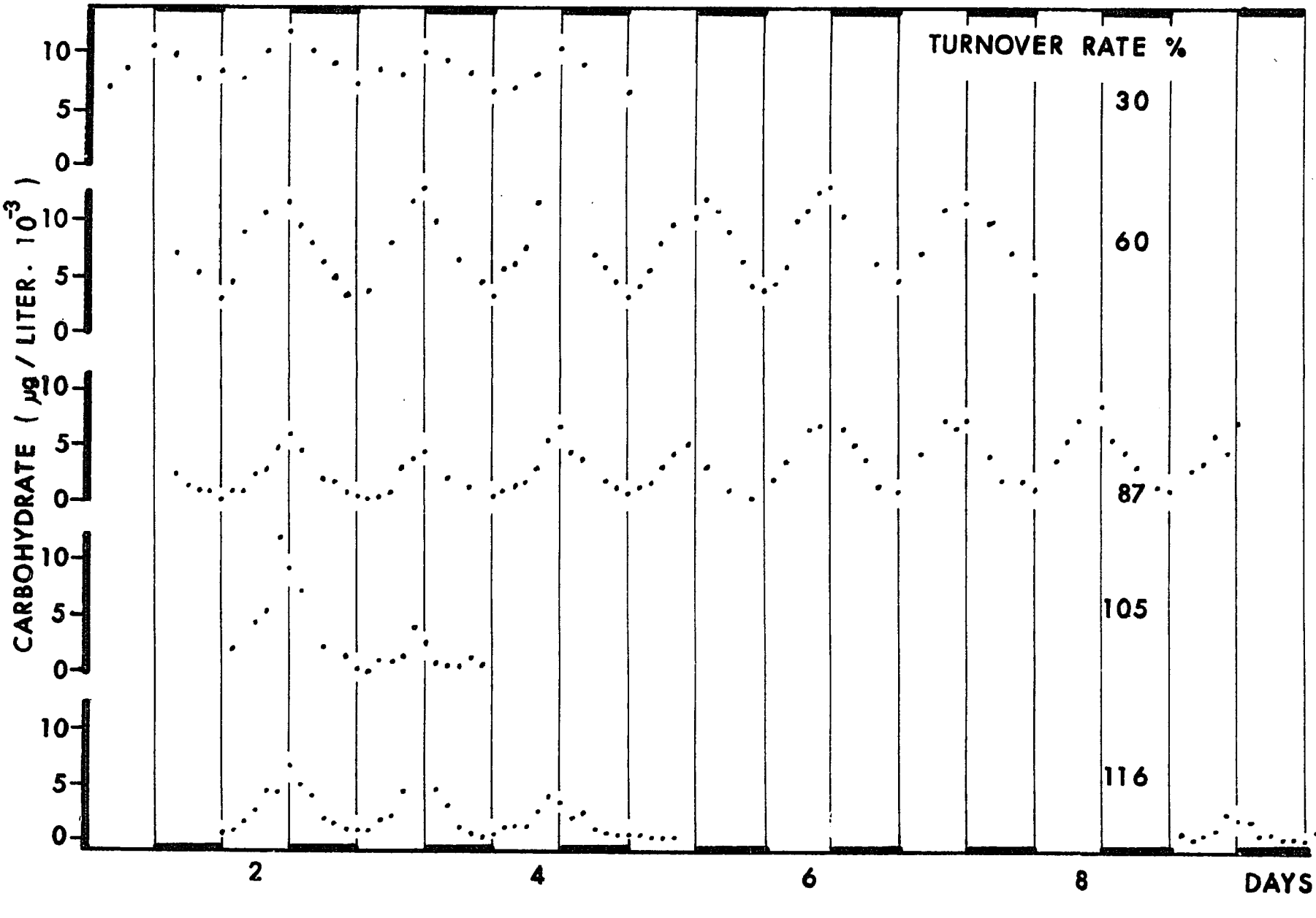

TURNOVER RATE %
30
60
87
105
116
CARBOHYDRATE ( µg / LITER . 10-3 )
10
5
0
2
4
6
8
DAYS

Figure 9: Temporal variations of the NADH nitrate reductase activity in the growth chamber for each turnover rate as u moles $NO_2^-$ formed/liter/hour.

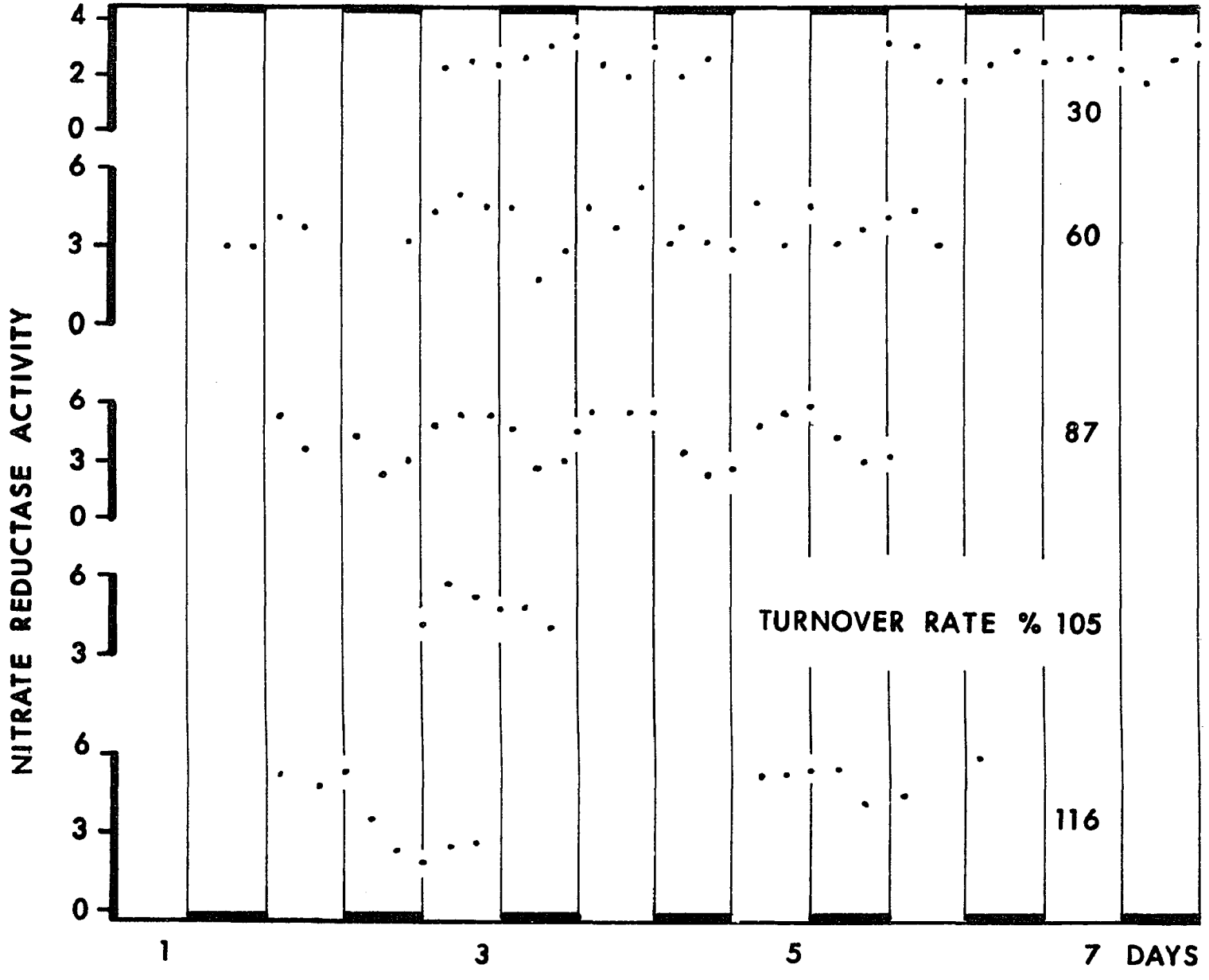

NITRATE REDUCTASE ACTIVITY
4
2
0
6
3
0
6
3
0
6
3
0
30
60
87
TURNOVER RATE % 105
116
1
3
5
7 DAYS

Figure 10: Nitrate uptake rate (v) as a function of nitrate concentration (S) for the "FRESH" population of Chaetoceros sp. (STX-105) preconditioned at the 30% turnover rate. $K_s^u$ and Vm were obtained from the regression analysis of the linear transform of S vs S/v. Each point is the mean value of duplicate determinations for the corresponding nitrate concentration.

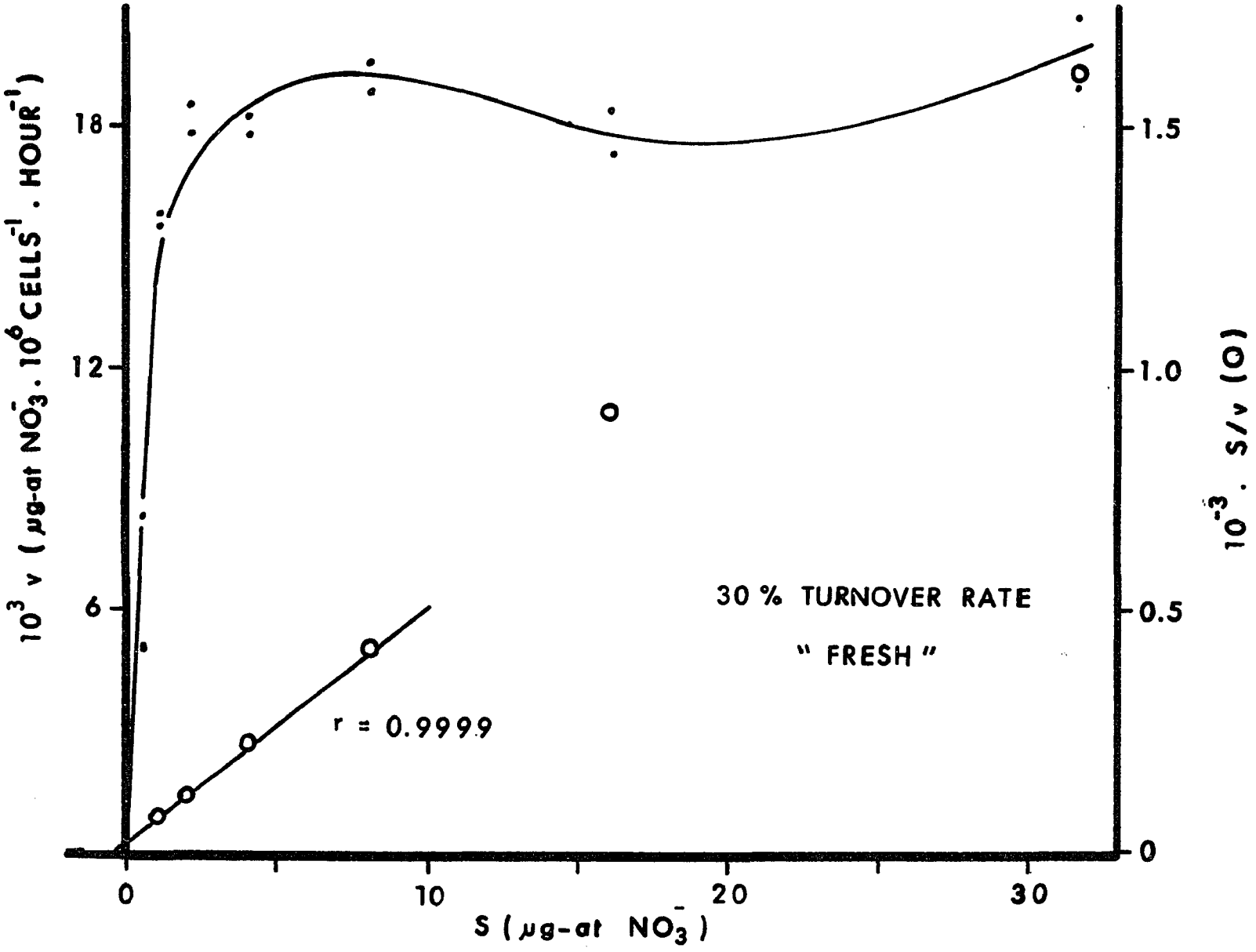

$10^3$ v ( µg-at $NO_3^-$ . $10^6$ CELLS$^{-1}$ . HOUR$^{-1}$ )
18
12
6
0
10
20
30
S ( µg-at $NO_3^-$ )
1.5
1.0
0.5
0
$10^{-3}$ . S/v (O)
30 % TURNOVER RATE
" FRESH "
r = 0.9999

Figure 11: Nitrate uptake rate (v) as a function of nitrate concentration (S) for the "FED" population of Chaetoceros sp. (STX-105) preconditioned at the 30% turnover rate. $K_s^u$ and Vm were obtained from the regression analysis of the linear transform of S vs S/v. Each point is the mean value of duplicate determinations for the corresponding nitrate concentration.

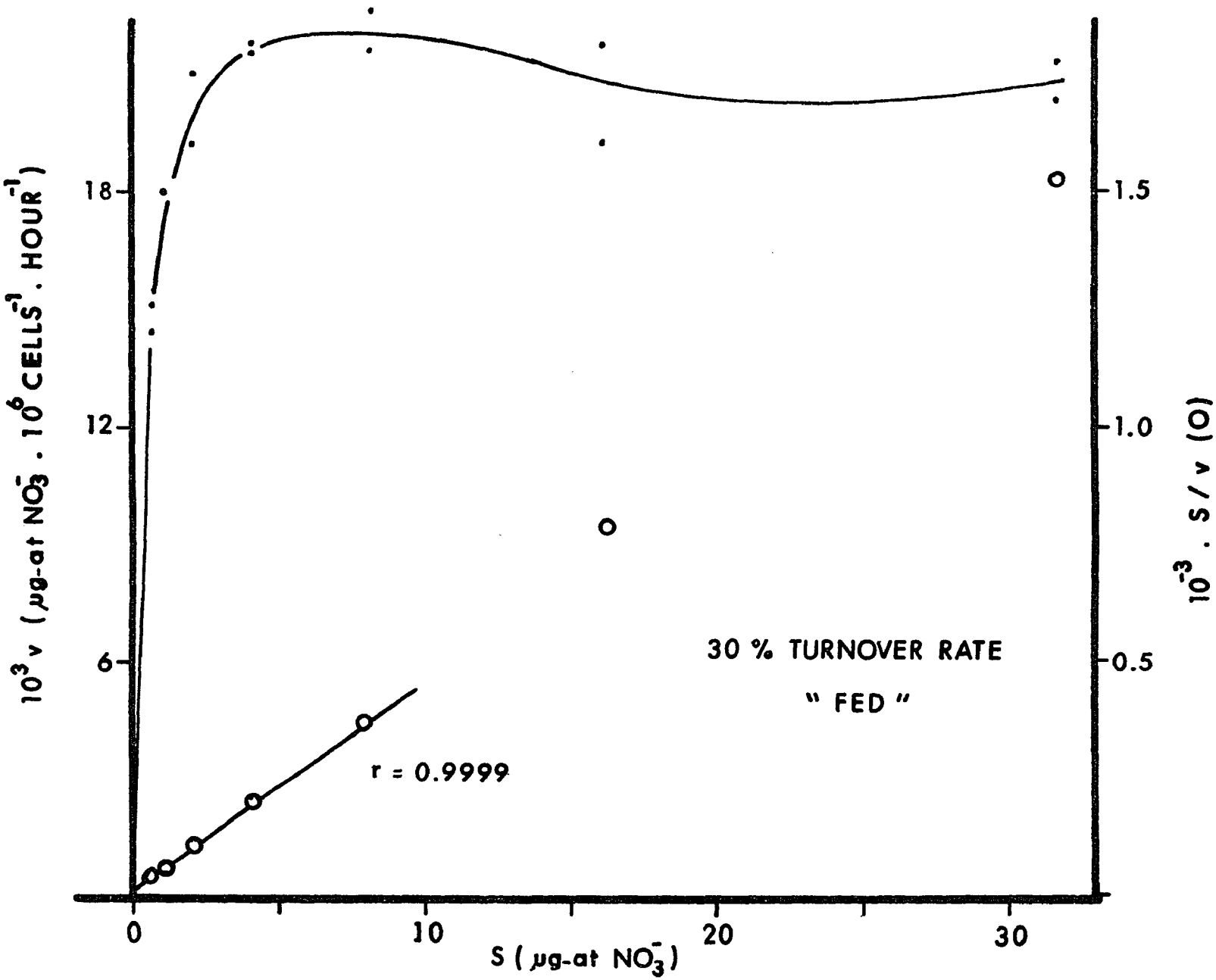

$10^3$ v ( µg-at $NO_3^-$ . $10^6$ CELLS$^{-1}$. HOUR$^{-1}$)
18
12
6
0
10
20
30
S ( µg-at $NO_3^-$)
1.5
1.0
0.5
$10^{-3}$ . S / v (O)
30 % TURNOVER RATE
" FED "
r = 0.9999

Figure 12: Nitrate uptake rate (v) as a function of nitrate concentration (S) for the "STARVED" population of Chaetoceros sp. (STX-105) preconditioned at the 30% turnover rate. $K_s^u$ and Vm were obtained from the regression analysis of the linear transform of S vs S/v. Each point is the mean value of duplicate determinations for the corresponding nitrate concentration.

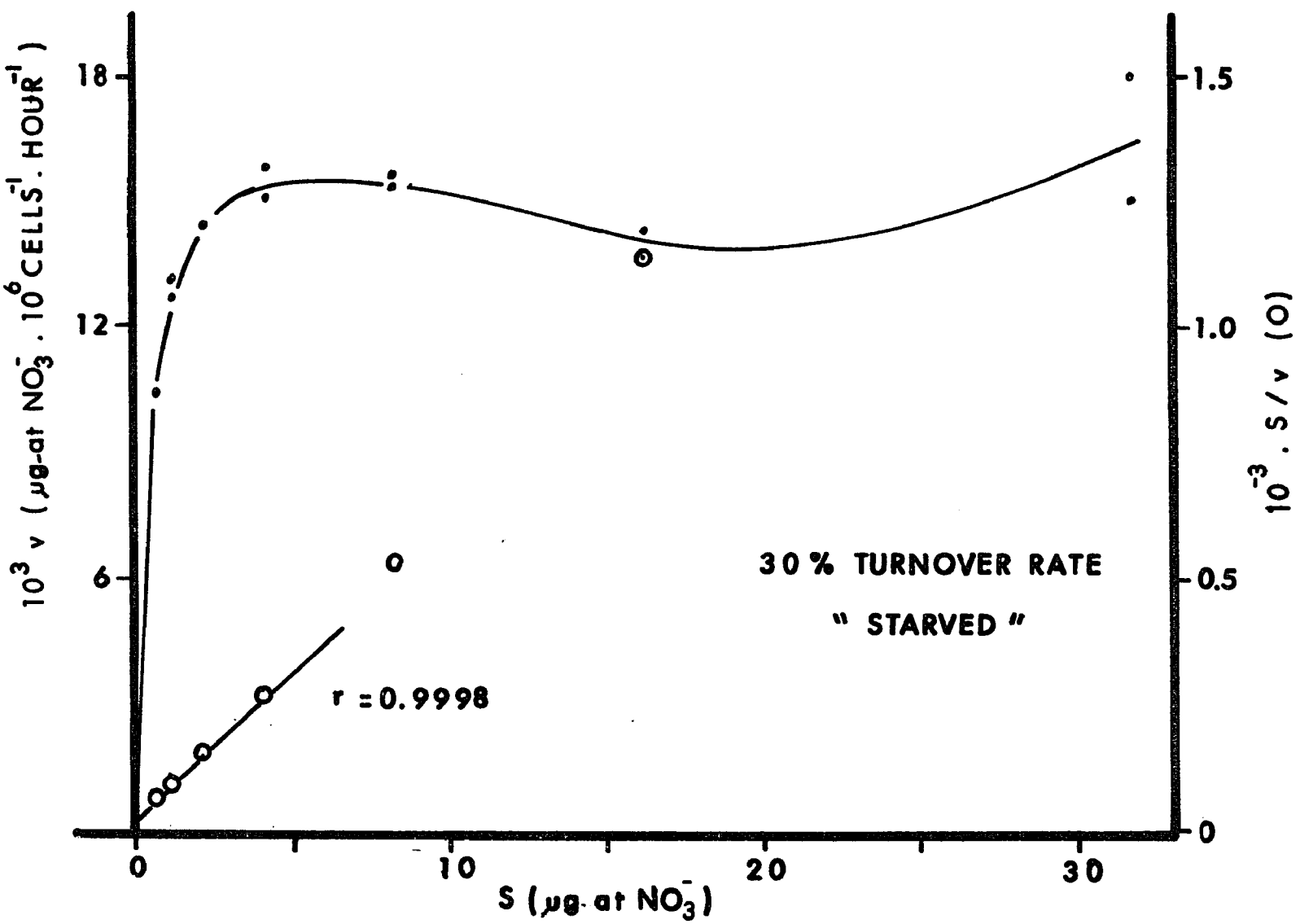

18
12
6
0
10
20
30
S (µg-at $NO_3^-$)
$10^3$ v (µg-at $NO_3^-$ . $10^6$ CELLS$^{-1}$. HOUR$^{-1}$)
1.5
1.0
0.5
0
$10^{-3}$ . S / v (O)
30 % TURNOVER RATE
" STARVED "
r = 0.9998

Figure 13: Nitrate uptake rate (v) as a function of nitrate concentration (S) for the "FRESH" population of Chaetoceros sp. (STX-105) preconditioned at the 60% turnover rate. $K_s^u$ and Vm were obtained from the regression analysis of the linear transform of S vs S/v. Each point is the mean value of duplicate determinations for the corresponding nitrate concentration.

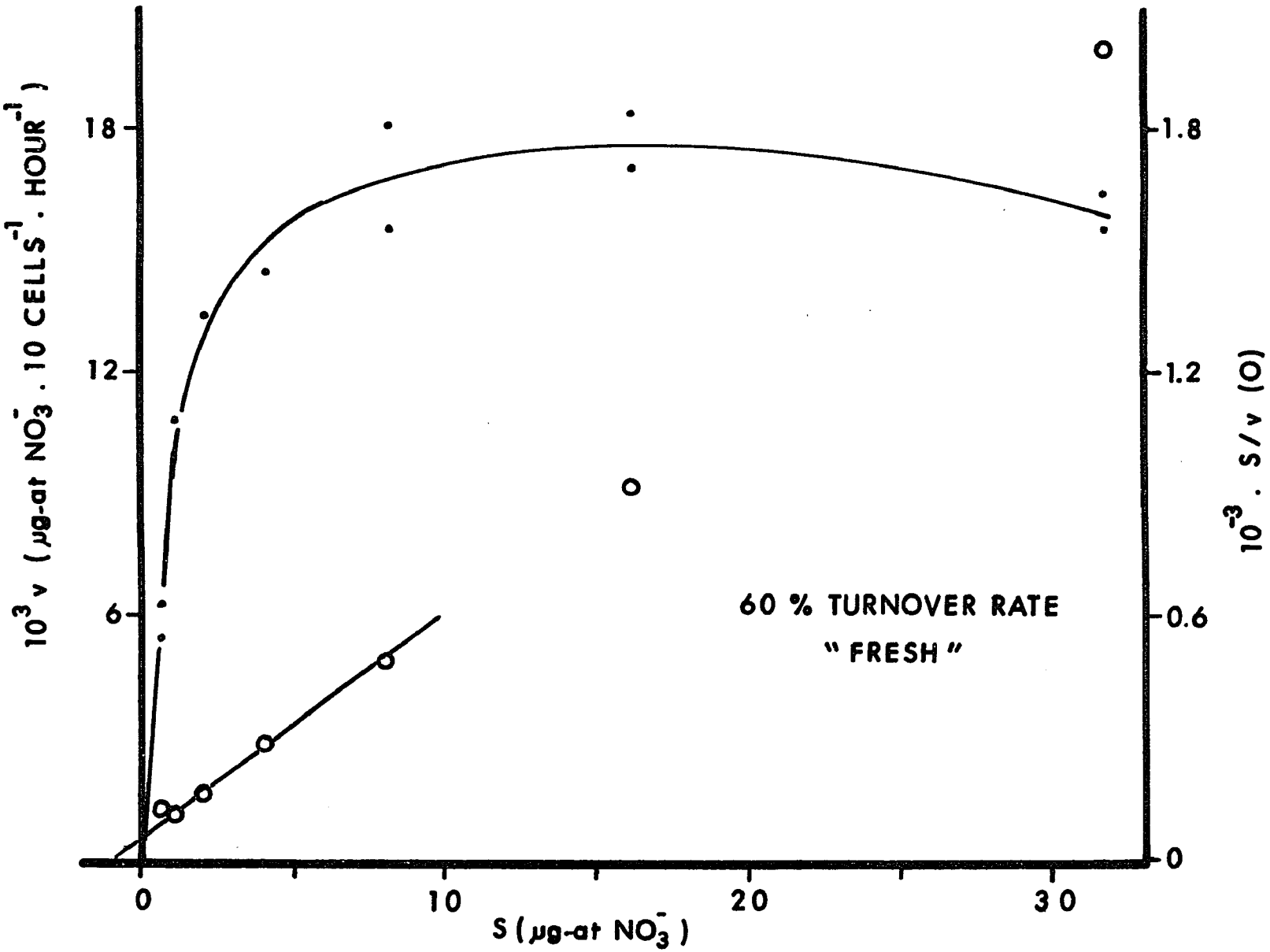

$10^3$ v ( µg-at $NO_3^-$ . 10 CELLS$^{-1}$ . HOUR$^{-1}$ )
18
12
6
0
10
20
30
S ( µg-at $NO_3^-$ )
1.8
1.2
0.6
0
$10^{-3}$ . S / v (O)
60 % TURNOVER RATE
" FRESH "

Figure 14: Nitrate uptake rate (v) as a function of nitrate concentration (S) for the "FED" population of Chaetoceros sp. (STX-105) preconditioned at the 60% turnover rate. $K_s^u$ and Vm were obtained from the regression analysis of the linear transform S vs S/v. Each point is the mean value of duplicate determinations for the corresponding nitrate concentration.

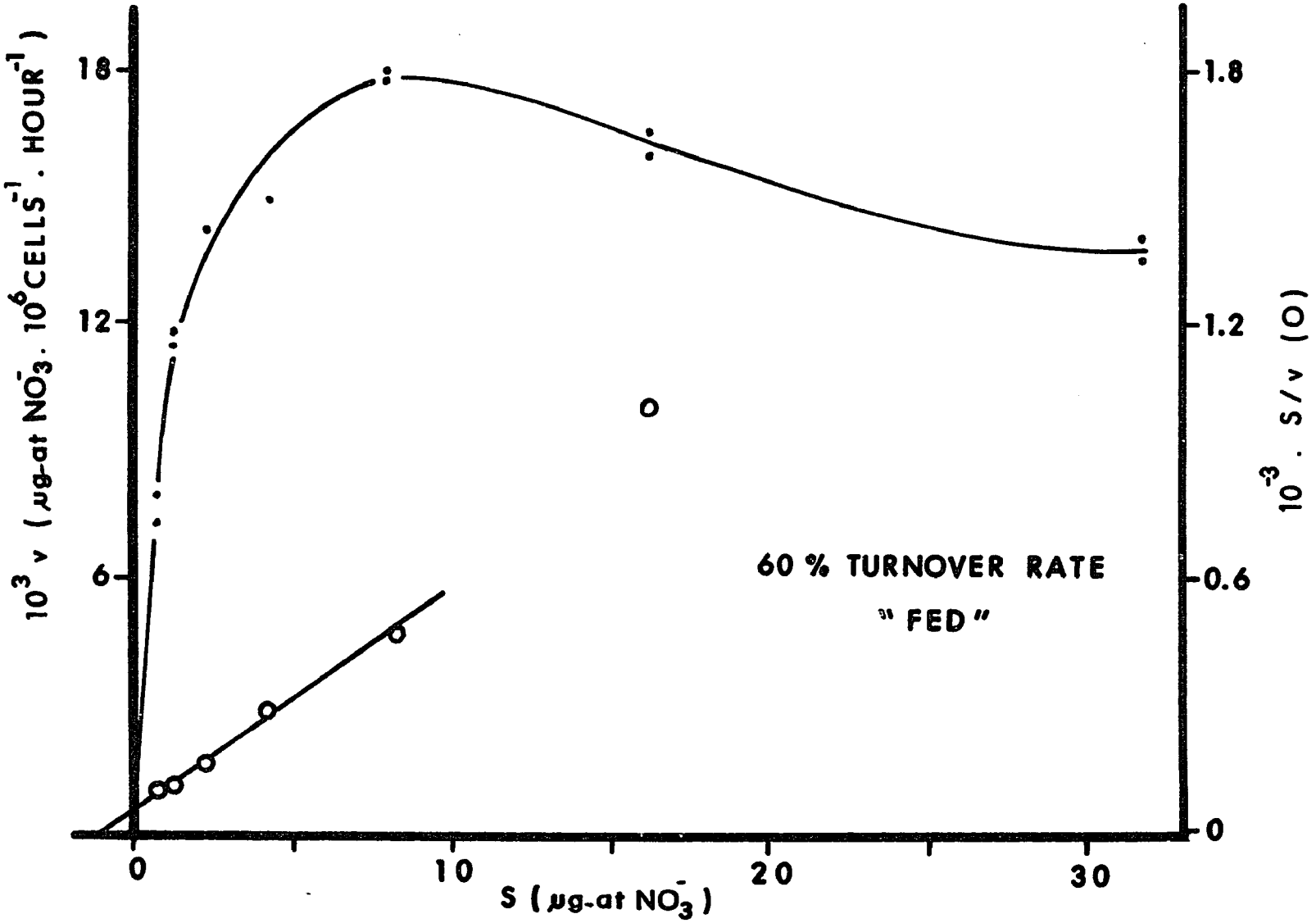

18
12
6
0
10
20
30
S (µg-at NO3-)
10^3 v (µg-at NO3-. 10^6CELLS^-1. HOUR^-1)
1.8
1.2
0.6
0
10^-3 . S/v (O)
60 % TURNOVER RATE
" FED "

Figure 15: Nitrate uptake rate (v) as a function of nitrate concentration (S) for the "STARVED" population of Chaetoceros sp. (STX-105) preconditioned at the 60% turnover rate. $K_s^u$ and Vm were obtained from the regression analysis of the linear transform of S vs S/v. Each point is the mean value of duplicate determinations for the corresponding nitrate concentration.

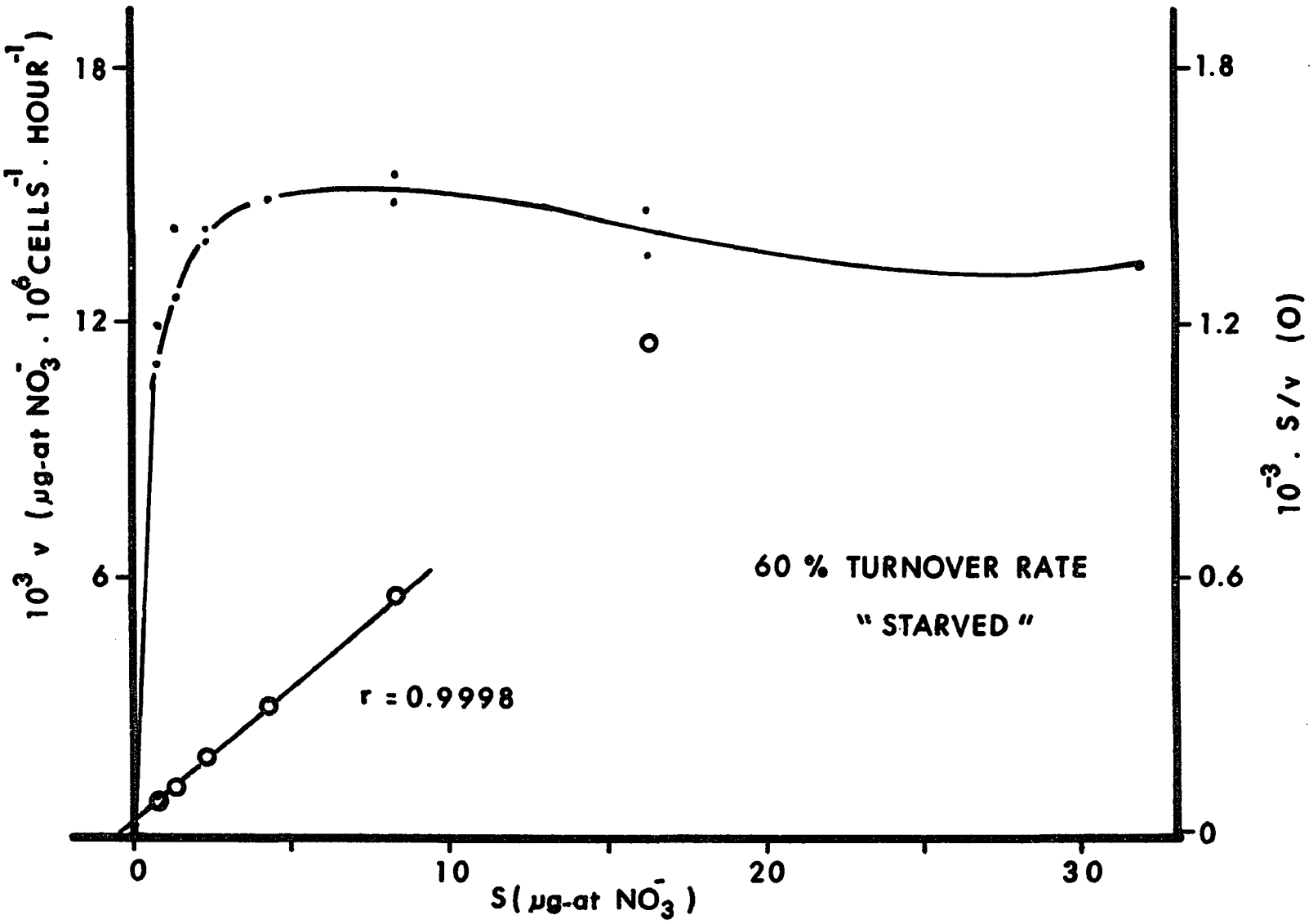

18
12
6
0
10
20
30
S ( µg-at NO3- )
10^3 v ( µg-at NO3- . 10^6 CELLS^-1 . HOUR^-1 )
1.8
1.2
0.6
0
10^-3 . S / v (O)
60 % TURNOVER RATE
" STARVED "
r = 0.9998

Figure 16: Nitrate uptake rate (v) as a function of nitrate concentration (S) for the "FRESH" population of Chaetoceros sp. (STX-105) preconditioned at the 87% turnover rate. $K_s^u$ and Vm were obtained from the regression analysis of the linear transform S vs S/v. Each point is the mean value of duplicate determinations for the corresponding nitrate concentration.

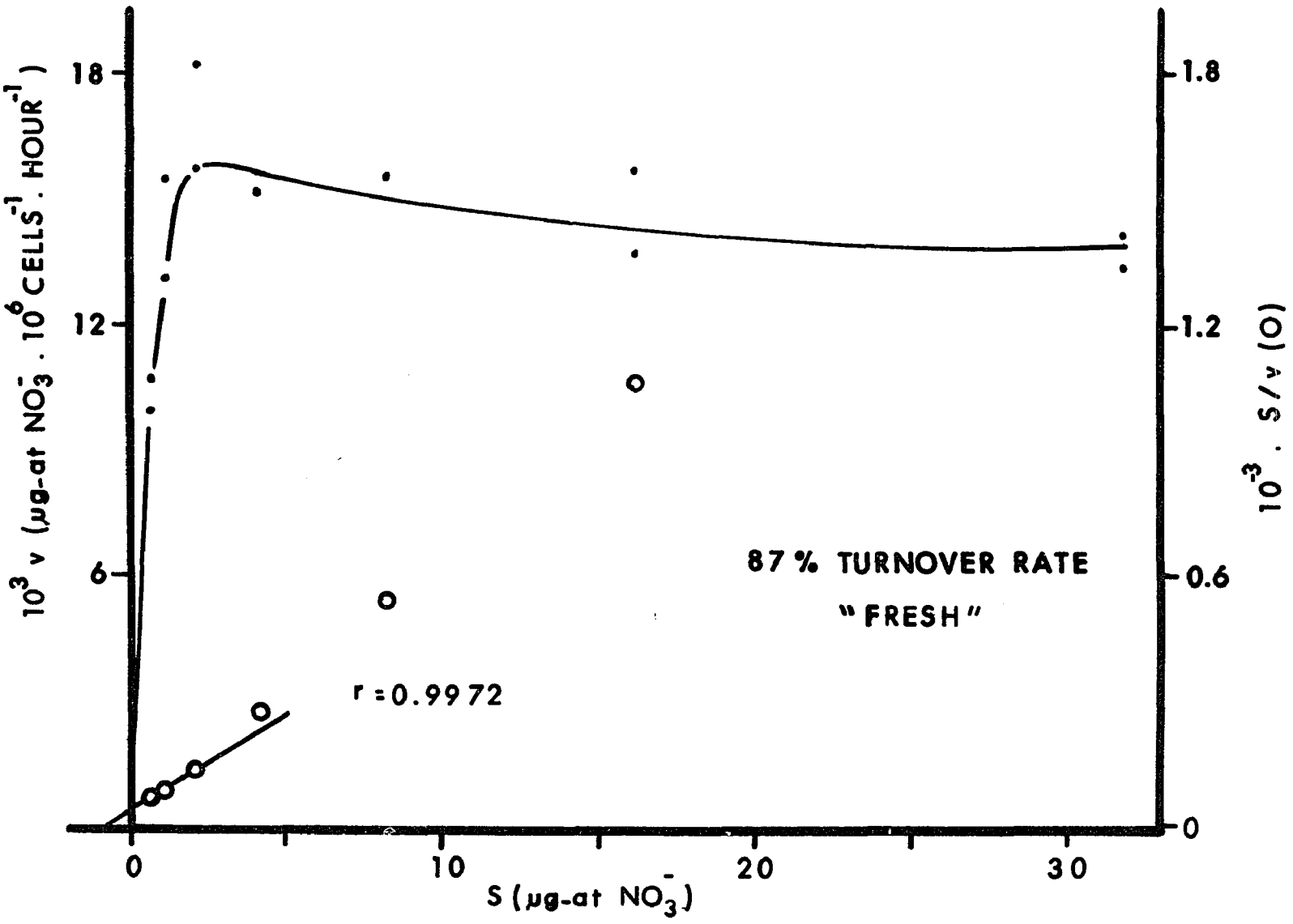

18
12
6
0
10
20
30
S (µg-at $NO_3^-$)
$10^3$ v (µg-at $NO_3^-$ . $10^6$ CELLS$^{-1}$ . HOUR$^{-1}$)
1.8
1.2
0.6
0
$10^{-3}$ . S / v (O)
87 % TURNOVER RATE
"FRESH"
r = 0.9972

Figure 17: Nitrate uptake rate (v) as a function of nitrate concentration (S) for the "FED" population of Chaetoceros sp. (STX-105) preconditioned at the 87% turnover rate. $K_s^u$ and Vm were obtained from the regression analysis of the linear transform S vs S/v. Each point is the mean value of duplicate determinations for the corresponding nitrate concentration.

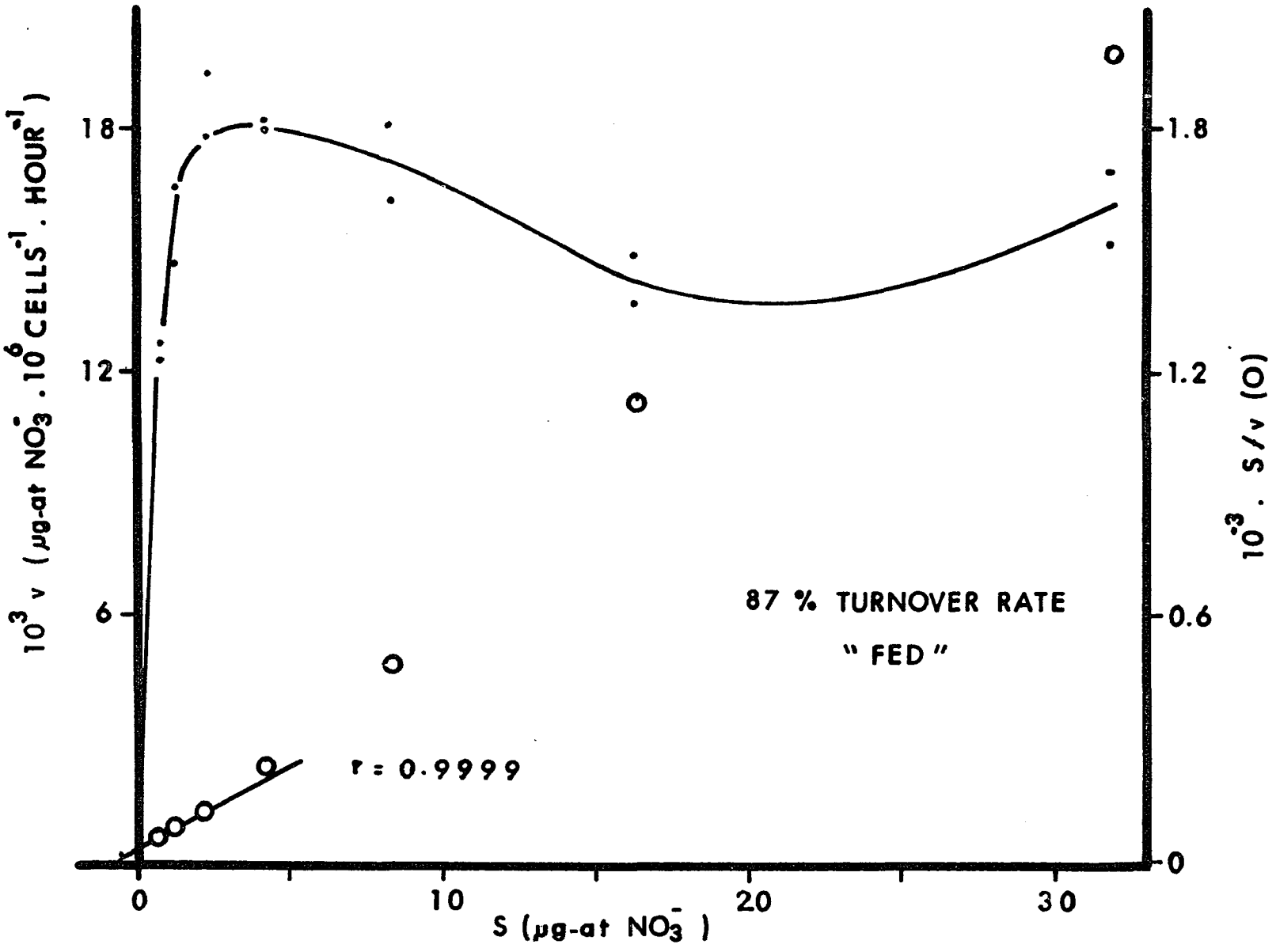

$10^3$ v ($\mu$g-at $NO_3^-$ . $10^6$ CELLS$^{-1}$ . HOUR$^{-1}$)
18
12
6
0
1.8
1.2
0.6
0
$10^{-3}$ . S / v (O)
87 % TURNOVER RATE
" FED "
r = 0.9999
0
10
20
30
S ($\mu$g-at $NO_3^-$ )

Figure 18: Nitrate uptake rate (v) as a function of nitrate concentration (S) for the "STARVED" population of Chaetoceros sp. (STX-105) preconditioned at the 87% turnover rate. $K_s^u$ and Vm were obtained from the regression analysis of the linear transform S vs S/v. Each point is the mean value of duplicate determinations for the corresponding nitrate concentration.

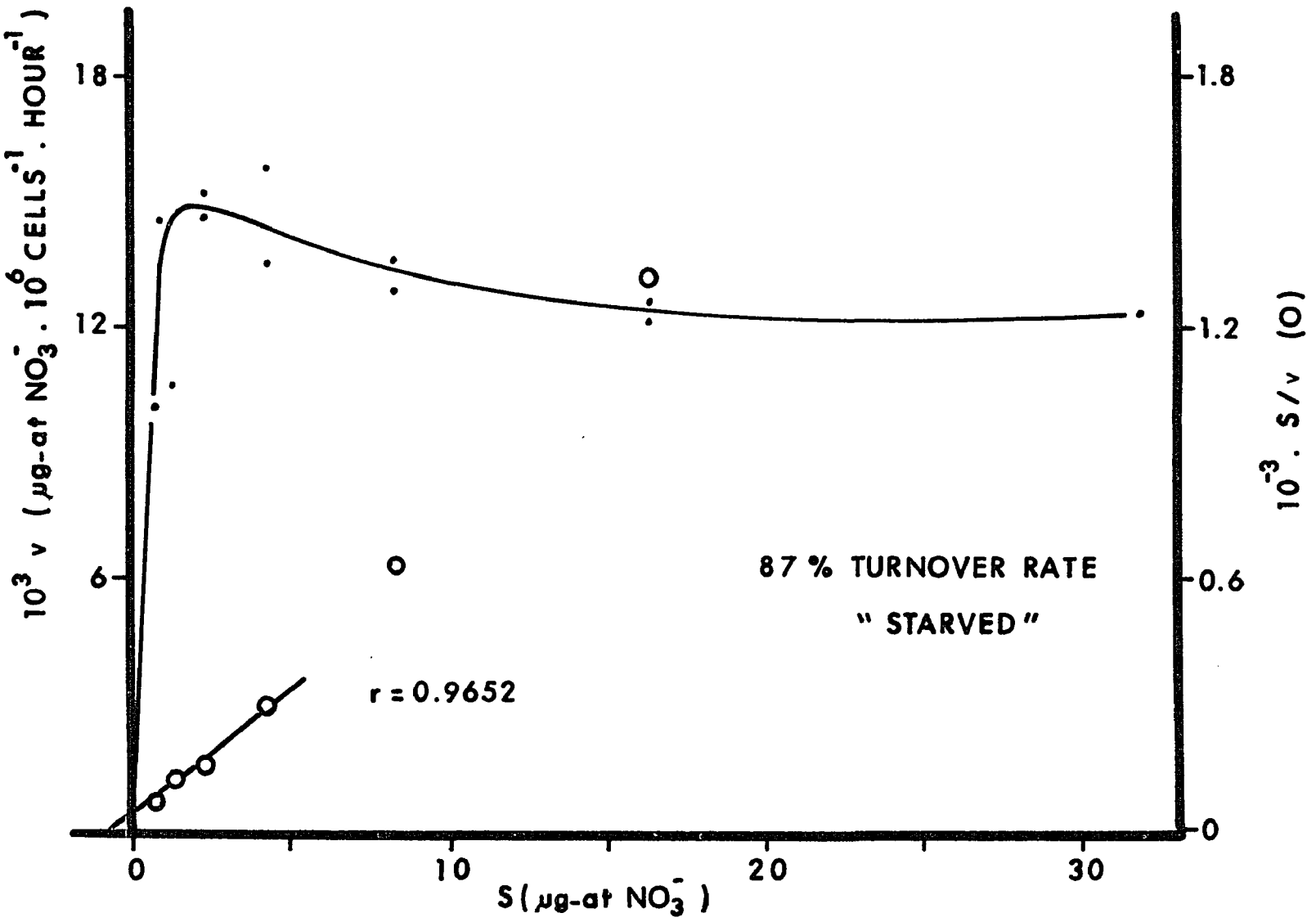

$10^3$ v ( $\mu$g-at $NO_3^-$ . $10^6$ CELLS$^{-1}$ . HOUR$^{-1}$ )
18
12
6
0
10
20
30
S ( $\mu$g-at $NO_3^-$ )
1.8
1.2
0.6
0
$10^{-3}$ . S / v (O)
87 % TURNOVER RATE
" STARVED "
r = 0.9652

Figure 19: Nitrate uptake rate (v) as a function of nitrate concentration (S) for the "FRESH" population of Chaetoceros sp. (STX-105) preconditioned at the 116% turnover rate. $K_s^u$ and Vm were obtained from the regression analysis of the linear transform of S vs S/v. Each point is the mean value of duplicate determinations for the corresponding nitrate concentration.

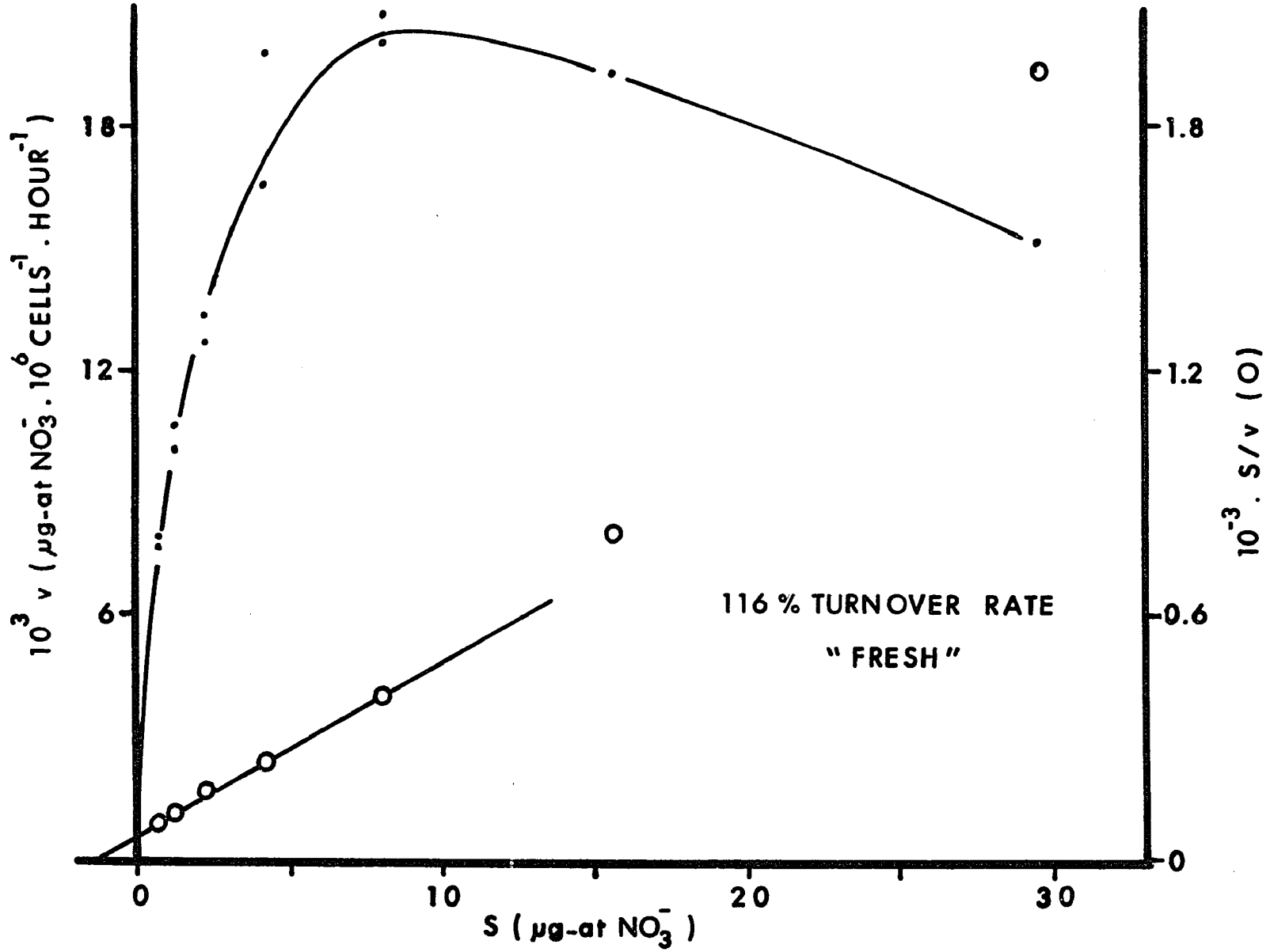

18
12
6
0
10
20
30
S ( µg-at $NO_3^-$ )
$10^3$ v ( µg-at $NO_3^-$ . $10^6$ CELLS$^{-1}$ . HOUR$^{-1}$ )
1.8
1.2
0.6
0
$10^{-3}$ . S / v (O)
116 % TURNOVER RATE
" FRESH "

Figure 20: Nitrate uptake rate (v) as a function of nitrate concentration (S) for the "FED" population of Chaetoceros sp. (STX-105) preconditioned at the 116% turnover rate. $K_s^u$ and Vm were obtained from the regression analysis of the linear transform of S vs S/v. Each point is the mean value of duplicate determinations for the corresponding nitrate concentration.

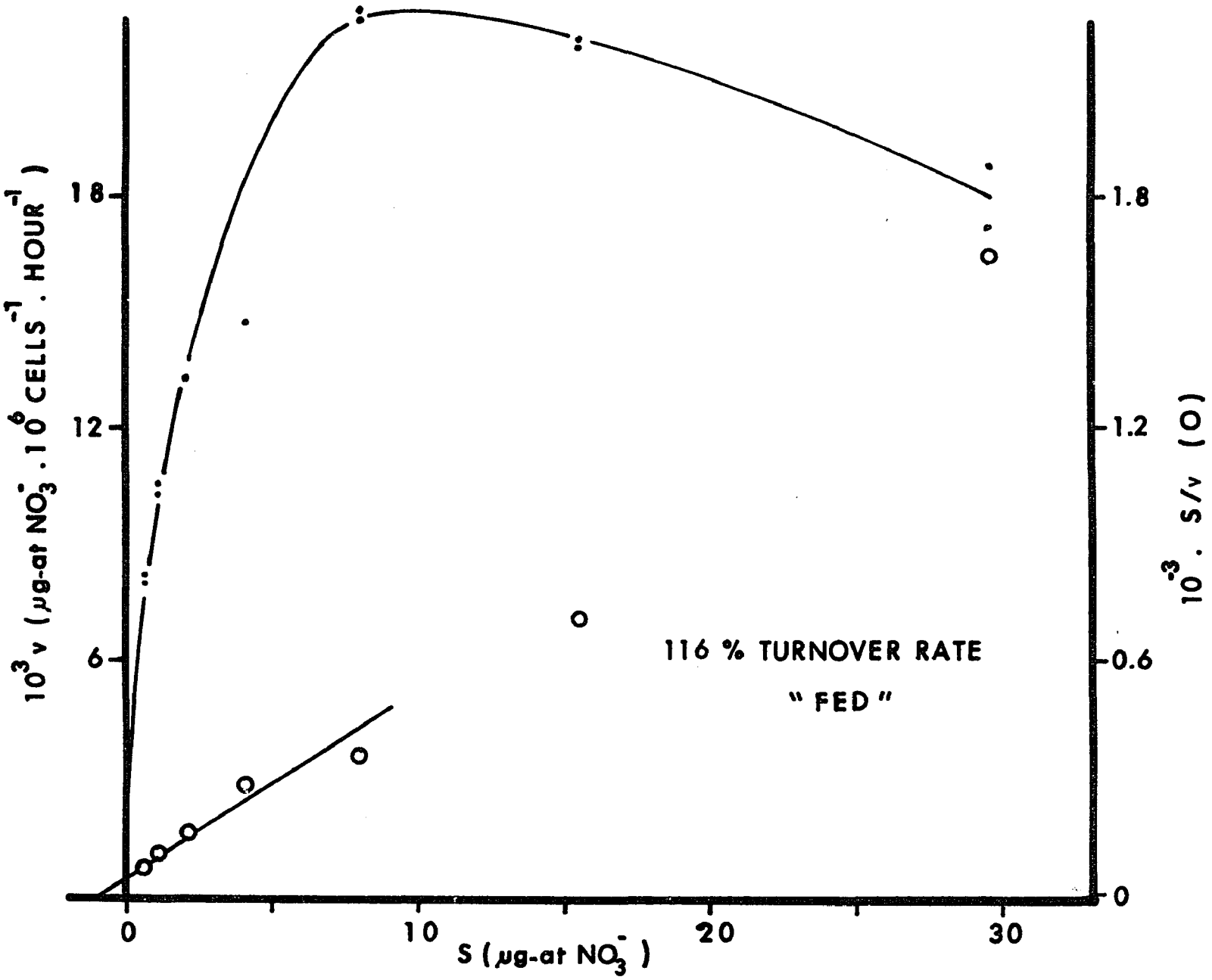

$10^3$ v ($\mu$g-at $NO_3^-$ . $10^6$ CELLS$^{-1}$ . HOUR$^{-1}$)
18
12
6
0
10
20
30
S ($\mu$g-at $NO_3^-$)
1.8
1.2
0.6
0
$10^{-3}$ . S/v (O)
116 % TURNOVER RATE
" FED "

Figure 21: Nitrate uptake rate (v) as a function of nitrate concentration (S) for the "STARVED" population of Chaetoceros sp. (STX-105) preconditioned at the 116% turnover rate. $K_s^u$ and Vm were obtained from the regression analysis of the linear transform S vs S/v. Each point is the mean value of duplicate determinations for the corresponding nitrate concentration.

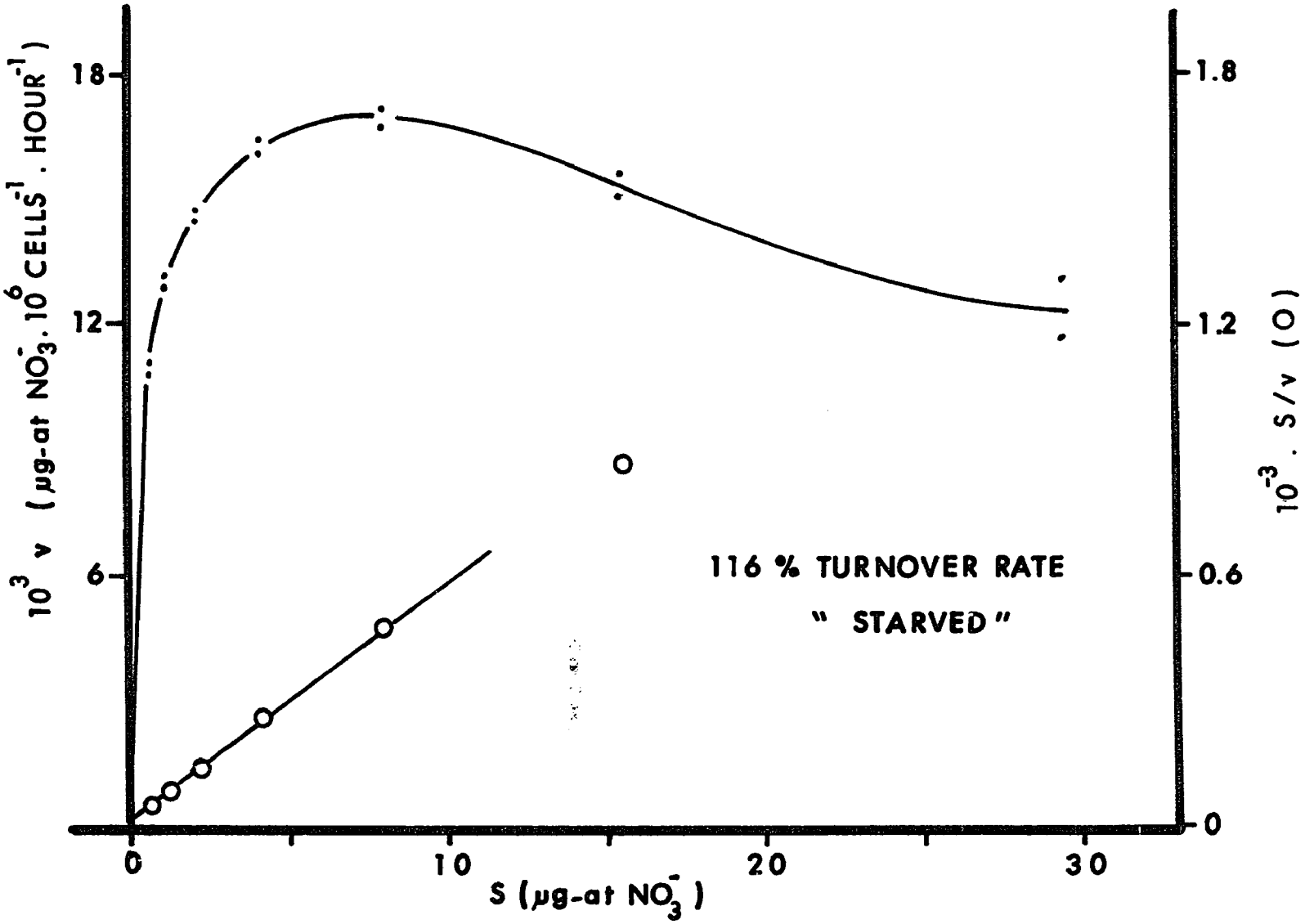

18
12
6
0
10
20
30
S (µg-at NO3-)
10^3 v (µg-at NO3-. 10^6 CELLS^-1 . HOUR^-1)
1.8
1.2
0.6
0
10^-3 . S/v (O)
116 % TURNOVER RATE
" STARVED "

Figure 22: Nitrate concentration as a function of time in the growth chamber during a nitrate uptake experiment with a Chaetoceros population preconditioned at the 30% turnover rate.

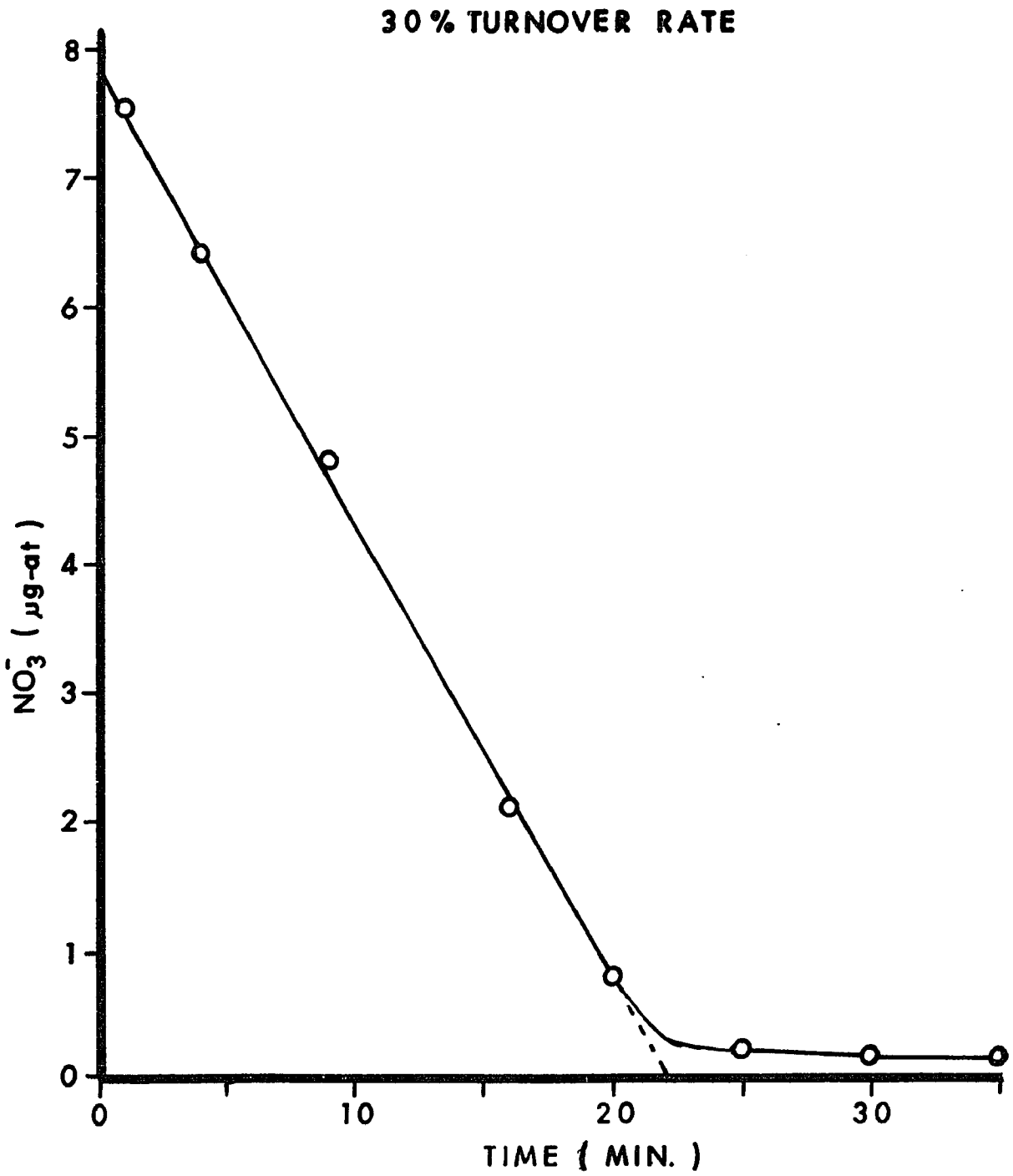

30% TURNOVER RATE
8
7
6
5
4
3
2
1
0
$NO_3^-$ (µg-at)
0
10
20
30
TIME ( MIN. )

Figure 23: Nitrate concentration as a function of time in the growth chamber during a nitrate uptake experiment with a Chaetoceros population preconditioned at the 60% turnover rate.

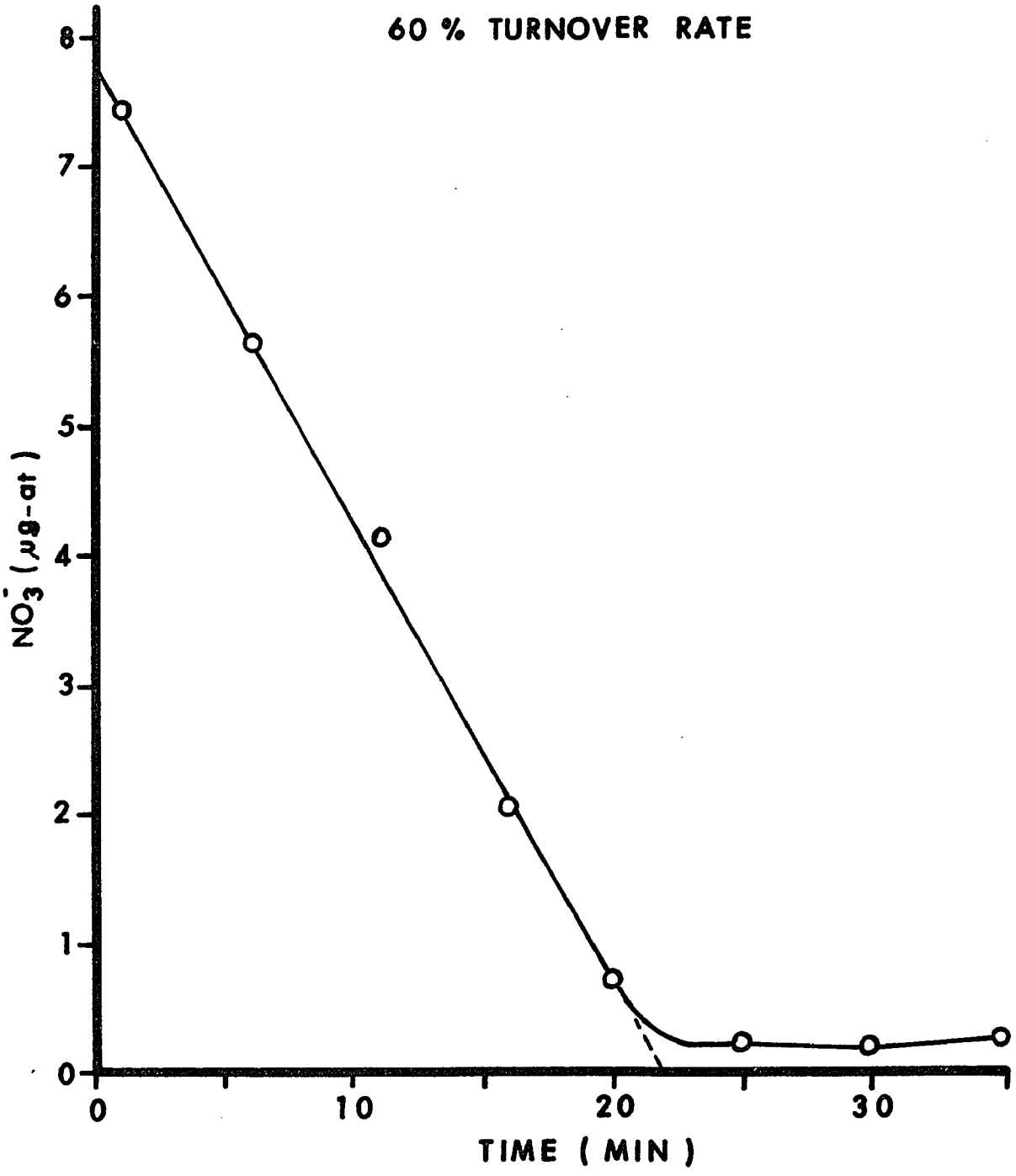

60 % TURNOVER RATE
$NO_3^-$ (µg-at)
0
1
2
3
4
5
6
7
8
0
10
20
30
TIME ( MIN )

Figure 24: Nitrate concentration as a function of time in the growth chamber during a nitrate uptake experiment with a Chaetoceros population preconditioned at the 87% turnover rate.

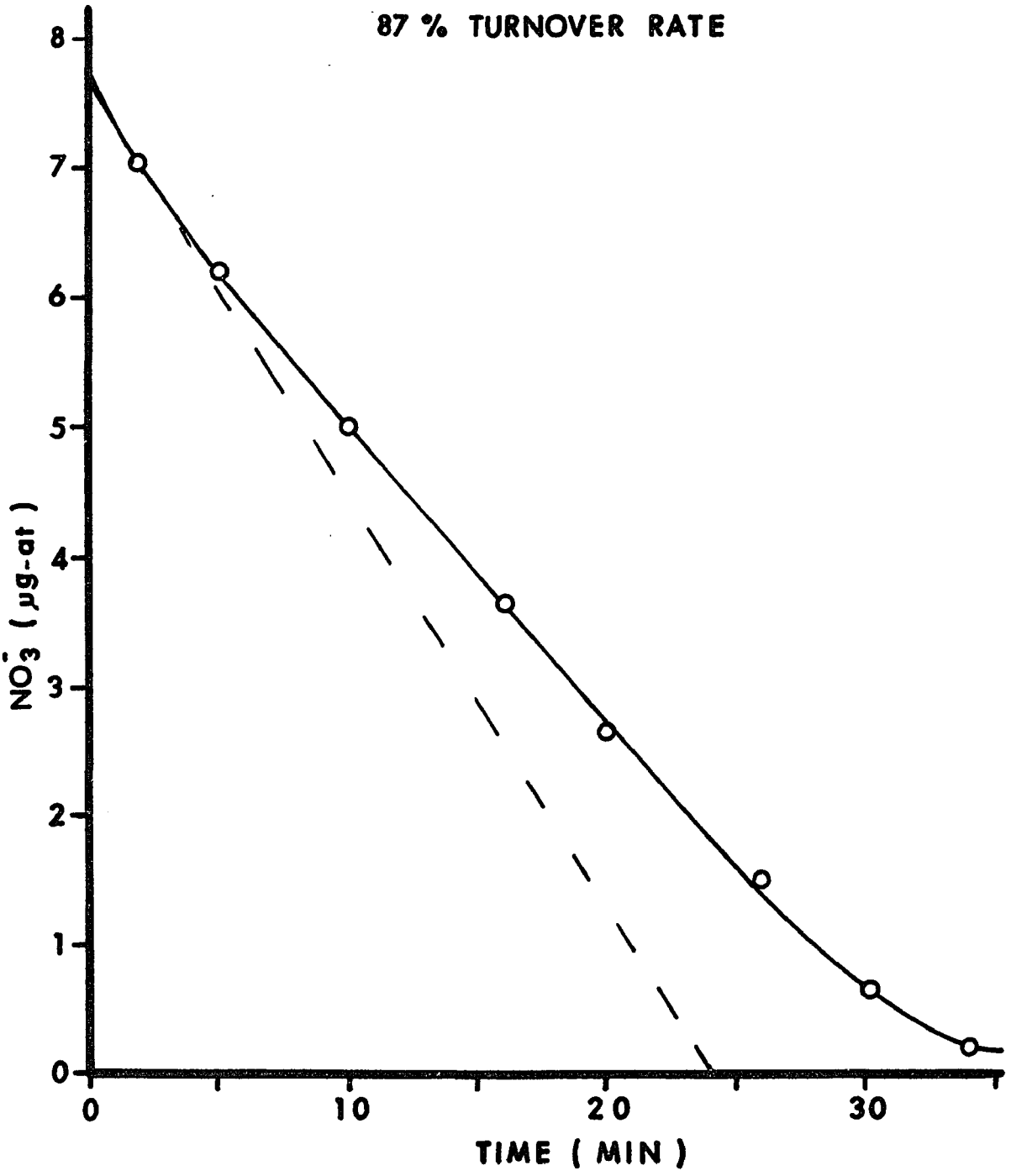
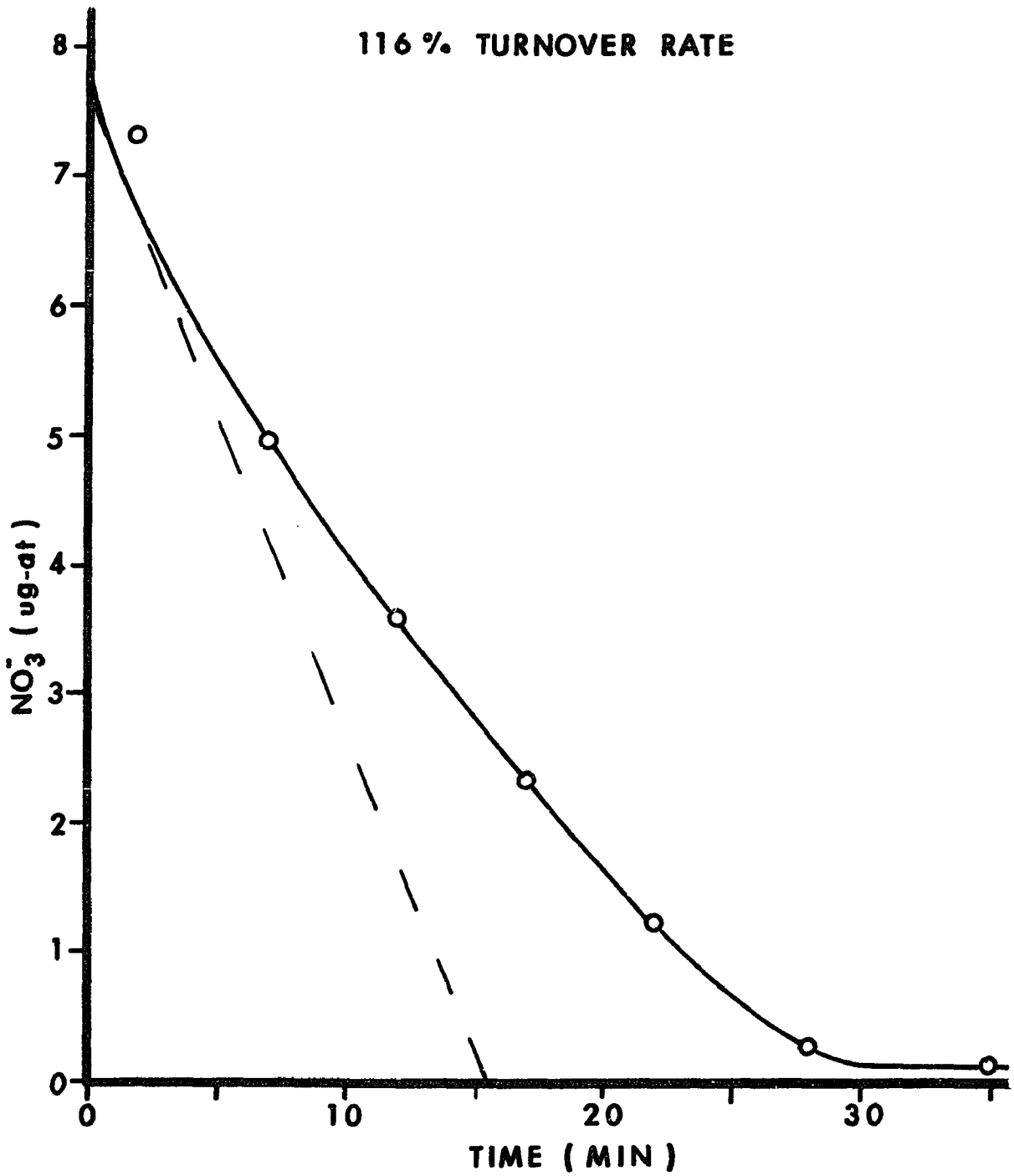

87 % TURNOVER RATE
8
7
6
5
4
3
2
1
0
$NO_3^-$ ( µg-at )
0
10
20
30
TIME ( MIN )

Figure 25: Nitrate concentration as a function of time in the growth chamber during a nitrate uptake experiment with a Chaetoceros population preconditioned at the 116% turnover rate.


116 % TURNOVER RATE
8
7
6
5
4
3
2
1
0
$NO_3^-$ ( ug-at )
0
10
20
30
TIME ( MIN )

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