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EFFECT OF LIGHT INTENSITY ON BIOMASS CHARACTERISTICS OF A DIATOM GROWING IN OUTDOOR CONTINUOUS CULTURE

by

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ABSTRACT

The marine diatom, Chaetoceros curvisetus Cleve, was grown in outdoor continuous culture to assess the relationships between growth, nutrient uptake, and photosynthesis under different light regimes and to help optimize a mariculture operation. Experiments were conducted at four different growth rates and five different light intensities using Antarctic Intermediate water as culture medium and tropical sunlight as light source. Samples were taken for cell number, particulate nitrogen and organic carbon, chlorophyll, and inorganic nutrients (nitrate, nitrite, ammonia, phosphate, and silicate) at sunrise and sunset. Analyses were performed on data from cultures that had attained steady state. Three categories of limitation were identified: nitrogen-limited, light-limited, and intermediate cultures. Growth rate was under experimental control and the processes of uptake and photosynthesis were adapted to maintain that growth rate. Adaptations by light-limited cultures were increased chlorophyll per cell and decreased total biomass so that photosynthesis per cell was maintained at a rate equal to that of cells growing at the same rate at higher light intensities. Nitrogen-limited cultures assimilated less protein and needed less chlorophyll per cell than light-limited cultures to maintain the balance between uptake, photosynthesis, and growth. The light-dark cycle affected the coupling between

uptake of carbon and the assimilation of photosynthetic products into protein. The findings showed that growth rate could be independent of light intensity throughout most of the euphotic zone, depending on rates of nutrient supply, mixing, and removal.

ACKNOWLEDGMENTS

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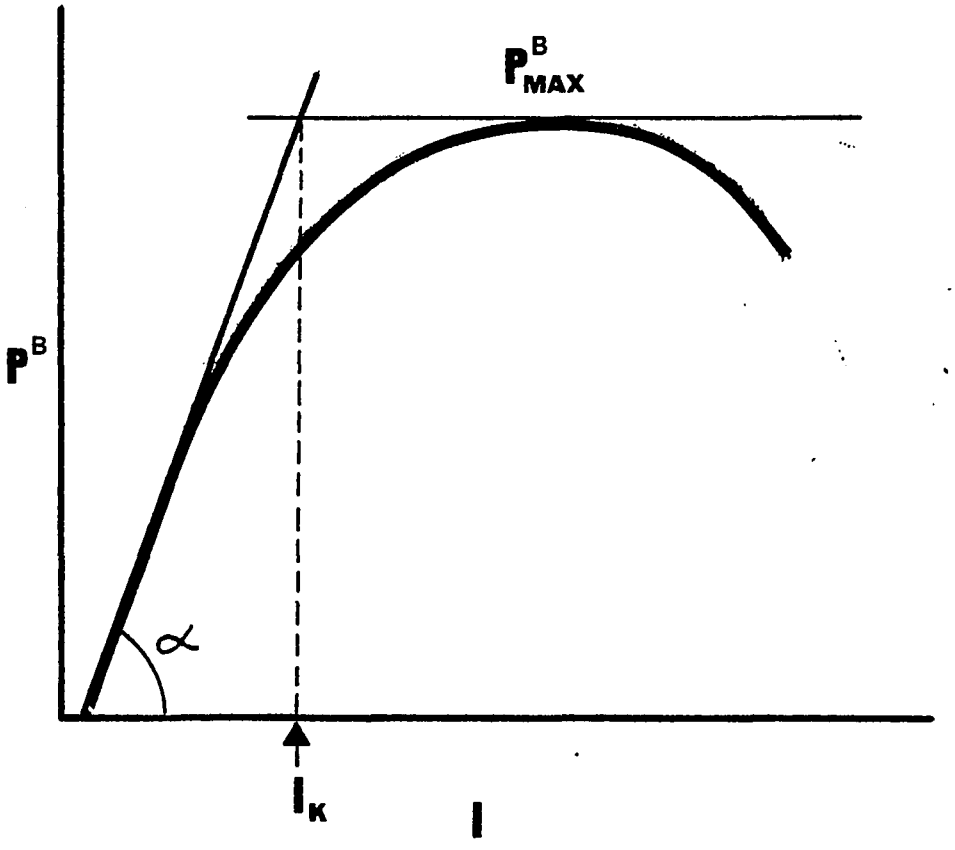
INTRODUCTION

Growth of marine autotrophic phytoplankton is a consequence of photosynthesis, nutrient uptake, and other metabolic processes. These processes depend on environmental factors such as available light, concentration and rate of supply of nutrients, and ambient temperature. The relationships between environmental factors and photosynthesis or nutrient uptake have been clearly defined (for a review, see Parsons and Takahashi 1973). Less clearly understood are the relationships between photosynthesis and growth, between nutrient uptake and growth, and between photosynthesis and nutrient uptake. A better understanding of these relationships will help evaluate the environmental regulation of phytoplankton growth in the sea.

The relationship between photosynthesis and growth is seen in the similarity of response of the two processes to an increase in light intensity. The response is described by a generalized photosynthesis-light (P-I) curve (Fig. 1). The proportional increase in photosynthesis with an increase in light and the leveling off of the curve at a sufficiently strong light intensity were first noted in 1883 by J. Reinke. In 1905, F. F. Blackman interpreted the shape of the curve as evidence of a two-step mechanism in photosynthesis: a photochemical step associated with the initial linear portion

Figure 1. A generalized photosynthesis-light (P-I) curve where P^B = rate of photosynthesis per unit biomass; P^B_{max} = rate P^B attains at saturating light intensity; α = the slope of the initial portion of the curve; I = light intensity; and I_k = the light intensity where a line having the initial slope intersects a line representing maximum rate of photosynthesis.


P^B_{MAX}
P^B
α
I_K
I

and a "dark" step, now known to consist of many enzymatic reactions, associated with the saturation portion of the curve (Rabinowitch and Govindjee 1969). At the end of the saturating portion a further increase in light intensity results in a decline in photosynthesis.

Meyers (1966) measured net photosynthesis and growth rate in batch culture and found that two different curves were obtained when the rates were plotted in the same units as a function of light intensity. The maximum rate was much higher with the photosynthesis measurements than with the growth measurements. The differences were at least partly due to differences in the method of measurement. Growth rate was calculated on different cultures, each grown at a different light intensity. Photosynthesis was measured on cells grown at a single light intensity and removed to new light intensities for the photosynthesis measurements. Nevertheless, Meyers argued (1966) that the differences in the curves could not arise without large variation in the composition and physiology of the algal cells -- variation easily brought about by light intensity. To understand this variation and how it affects the relationship between photosynthesis and growth, it is necessary to have a population that has a known environmental history.

In practice, growth rate is often estimated from the

rate of photosynthesis (Eppley 1972; Parsons and Takahashi 1973). Net photosynthesis (ΔC) is usually assumed to be approximated by ^{14}C uptake measurements (Eppley 1972). If the initial amount of population carbon (C_o) is known, growth rate in doublings per day can be calculated by the equation:

$$\mu = \frac{1}{\Delta t} \log_2 \left(\frac{C_o + \Delta C}{C_o} \right) \quad (1)$$

where Δt is the length of the incubation period. Since population carbon is difficult to evaluate at sea, Eppley (1972) showed how growth rate could be calculated using an estimated population carbon:chlorophyll (C:chl) ratio and the equation:

$$\mu = \frac{1}{\Delta t} \log_2 \left(\frac{C{:}chl + \Delta C/chl}{C{:}chl} \right) \quad (2)$$

where $\Delta C/chl$ is the net rate of photosynthesis per unit chlorophyll.

Evidence does not yet exist that such calculations of growth rate from net photosynthesis measurements are accurate because the quantitative relationship between growth and photosynthesis is not known. Photosynthesis is a rapid process and can vary over time scales that are short relative to doubling times and photosynthetic and growth rates

are often saturated at quite different levels of intensity (Hellebust 1970). The problem is compounded when photosynthesis is measured over time intervals that are short compared to doubling times.

Just as photosynthesis and growth show similar, if not identical, responses to light intensity, so nutrient uptake and growth show similar responses to increases in nutrient concentration. Both uptake and growth have demonstrated hyperbolic curves with respect to nutrient substrate concentration that can be described by a Michaelis-Menton-type equation:

$$\mu = \mu_m \left(\frac{S}{K_s + S}\right) \tag{3}$$

where μ = specific growth rate, μ_m = maximum specific growth rate, $\underline{S}$ = substrate concentration, and $\underline{K_s}$ = the substrate concentration when $\mu = 1/2\ \mu_m$. The uptake equation is identical, with $\underline{v}$ representing uptake velocity rather than u for growth rate. However, some studies have shown no correlation between growth rate and substrate concentration (Caperon 1968; Droop 1968, 1970; Caperon and Meyer 1972a). Instead, growth rate was shown to be proportional to nutrient uptake. The proportionality constant, $\underline{q}$, was identified as a "yield coefficient" or the "cell quota" since it was equated to the concentration of limiting nutrient within the cell. When

q was determined under various conditions, it was found to be related to growth rate by a Michaelis-Menton hyperbola (Caperon 1968; Caperon and Meyer 1972a, b; Thomas and Dodson 1972):

$$\mu = \mu_m \left(1 - \frac{K_q}{q}\right) \tag{4}$$

where $K_q = q$ at $1/2\ \mu_m$.

Some insight into the relationship between nitrate uptake and photosynthesis has been demonstrated by Falkowski and Stone (1975). These authors found that carbon fixation by Skeletonema costatum was temporarily suppressed after enrichment with nitrate or ammonia. They attributed the effect to competition between inorganic carbon and nitrogen for adenosine triphosphate (ATP). After six to eight hours the chlorophyll a content of the cells increased and the effect was reduced. Another insight is provided by the similarity in diel cycles of photosynthesis and nitrogen uptake (Eppley et al. 1971), and by the lack of such periodicity in uptake of other nutrients. Thus, nutrient uptake may be in phase or out of phase with photosynthesis but quantitative relationships have not yet been established.

To investigate these relationships it would be best to have a population with a known environmental history upon which all measurements could be made. The original findings

of photosynthesis-light and nutrient uptake-substrate concentration relationships were based on cells growing in batch culture. In these systems the conditions around each cell constantly change (Venrick et al. 1977) and there is no way of knowing how these changes affect the relationships being studied. The environmental history is not known. However, a continuous culture maintains a constant environment for the cells and biomass characteristics of the cells reflect metabolic adjustments to that environment.

A continuous culture is diluted with fresh medium and maintained at a constant volume. There are two basic types of steady state, continuous cultures. In a turbidostat, a monitoring device records cell concentration or turbidity, and fresh medium is supplied whenever the turbidity exceeds a preset value. Light intensity thus determines growth rate. In a chemostat, fresh medium containing a growth-limiting nutrient is pumped through the culture at a constant rate, and nutrient supply determines growth rate (Eppley and Strickland 1968). The two systems are mathematically equivalent (Herbert et al. 1956). In both cases the rate at which the medium is added to the culture (the dilution rate) is exactly equal to the growth rate of the cells in the culture. In the turbidostat, biomass is preset and the growth rate is determined by the light intensity.

In the chemostat, dilution rate is preset. Since the medium contains a growth-limiting substance, the cells will be able to grow only as quickly as the substance is added. Thus, growth rate is preset with a chemostat and the biomass is determined by the concentration of the limiting nutrient.

Maddux and Jones (1964) used a turbidostat to maintain a constant biomass while examining the effects of nutrient concentration, temperature, and light intensity on growth rate. They found that nutrient concentrations had profound effects on the growth rate responses to temperature and light. At high nutrient concentrations, growth rate was hyperbolic with respect to light intensity. At low nutrient concentrations maximum growth rate was diminished and was inhibited at high light intensities. Temperature optima increased when nutrient concentration was increased. They concluded that the relationship between a single environmental factor and the growth rate of a population in the oceans is likely to be influenced by other factors.

Most of the chemostat experiments reported in the literature have been designed to study nutrient uptake kinetics and have revealed information on the relationship between uptake and growth (e.g. Droop 1968; Thomas and Dodson 1972; Caperon and Meyer 1972 a, b; Eppley and Renger

1974). The initial establishment of a quantitative expression of this relationship was possible because the chemostat provided a population with a known environmental history upon which both uptake and growth could be measured. Heretofore, chemostats have not been used to establish similar expressions for the relationship between photosynthesis and growth or between photosynthesis and uptake. These relationships have been studied in batch culture or in bottles incubated at sea with the attendant difficulties already discussed.

There is another class of continuous cultures that cannot be called either chemostat nor turbidostat. These cultures run under light-dark cycles either in the laboratory (Eppley et al. 1967, 1971; Paasche 1967, 1973) or outdoors (Burlew 1961; Dunstan and Menzel 1971; Goldman et al. 1973; Malone et al. 1975). These cultures are usually run as if they were chemostats, that is, a dilution rate is preset and the medium is constantly provided at that rate. For a true chemostat, however, all environmental conditions, e.g., temperature, light, incoming medium, must be constant.

Outdoor continuous cultures were first used to provide reliable sources of protein as possible food (Burlew 1961) and to use the waste nutrients found in sewage (Dunstan and Menzel 1971; Goldman et al. 1973). More recently these

cultures have been used to study cellular and nutrient uptake characteristics of the algae at different growth rates under more natural conditions than can be created in the laboratory. These cultures contain no detritus so that all measures of biomass are measures of phytoplankton biomass. Malone et al. (1975) demonstrated the effect of growth rate on synchrony of nitrate uptake and net cellular organic nitrogen production in light-dark cycles. The study also contributed to optimization of phytoplankton production in an artificial upwelling mariculture system.

In the present study outdoor continuous cultures were used to examine the effects of light intensity on photosynthesis and nutrient uptake at different growth rates and under different light intensities as well as to provide further information for the optimization of the mariculture system. The aim was to establish steady states so the effects of dilution rate and light intensity could be evaluated in terms of changes in biomass and biomass characteristics.

Approach

Steady state is the condition in which the population size of cultured cells and their chemical composition do not change in time. The biomass characteristics reflect metabolic adjustments made to the environmental conditions of

the experiment. In cultures grown in light-dark cycles steady state has a different meaning, and rate processes may change throughout a 24-hour cycle. However, from day to day the processes are repeatable. Cell numbers averaged for the 24-hour period are constant from day to day. The steady state exists on a different time scale than for a chemostat.

This definition of steady state enables one to simplify analysis of the system by using 24-hour averaged values to calculate rate processes. Thus, dilution rate can be used in any calculation involving growth rate. Carbon and nitrogen assimilation rates can be calculated by multiplying dilution rate by particulate carbon and nitrogen values, since these products had to be formed at the same rate as dilution rate if their weight were constant with time. Finally, the ratio of carbon to nitrogen assimilation rates has to be the same as the final carbon to nitrogen ratio in the cells as long as the cultures are in steady state.

The experiments in this study were designed to study the effects of five different light intensities on the biomass and biomass characteristics of populations growing at four different growth rates. This matrix of data allowed interpretation of the photosynthesis-growth relationship, the nutrient uptake-growth relationship, and the photosynthesis-

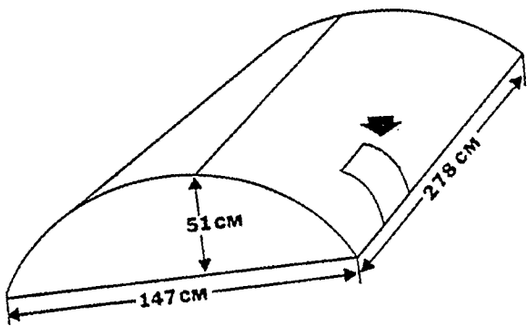
nutrient uptake relationship in terms of biomass characteristics at different steady state growth rates.

MATERIALS AND METHODS

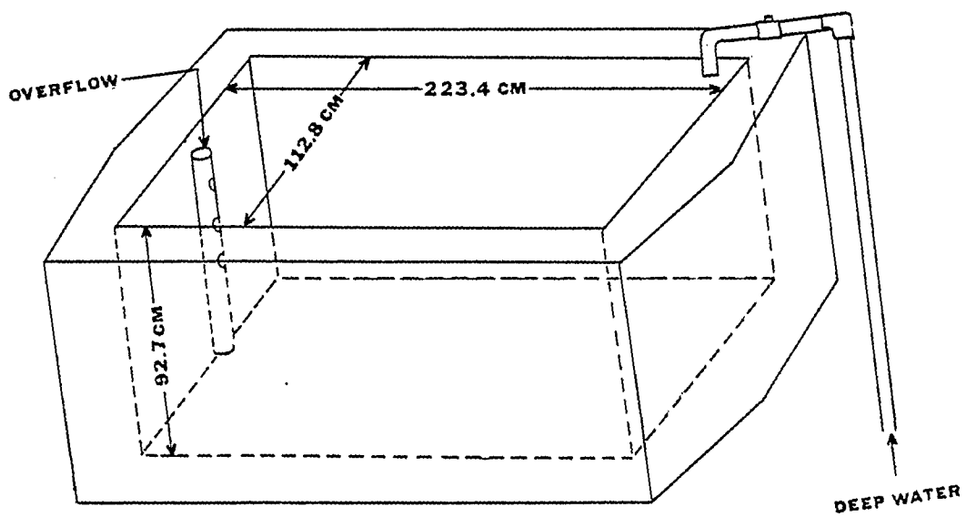
Four experiments were conducted, each at one dilution rate (0.25, 0.70, 0.95, 1.20 dilutions day^{-1}). Each experiment consisted of five cultures running simultaneously under screens of different density (Fig. 2a). Light intensities of 100 (no screen), 46, 30, 20, or 3 per cent of incident light reached the surface of the cultures. The culture vessels were 2000-liter reactors, 83 cm deep with a surface area of 2.4 m^2 (Fig. 2b). The culture medium was Antarctic Intermediate Water ("deep water") pumped continuously from 870 m off the north shore of St. Croix (Roels 1973). Deep water had an average salinity of 34.90°/$_{oo}$ and the following average nutrient concentrations (µg-at l^{-1}): nitrate, 31.25; nitrite, 0.15; ammonia, 0.41; phosphate, 1.97; and silicate, 23.4. Cultures were mixed by aeration from the bottom of the reactors.

The culture organism was Chaetoceros curvisetus Cleve (STX-167), which had been isolated from deep water by K. C. Haines. Stock cultures were routinely maintained in f/4 medium (Guillard and Ryther 1962) in 20 mm X 150 mm Pyrex test tubes at 20 - 25°C under alternating illumination regimes of five days at 4000 lux and two weeks at 400 lux between transfers to new medium. Inocula for the experiments were transferred from stock cultures to f/2 medium in

Figure 2. Screen and culture vessel for outdoor continuous cultures. A. Screen of black plastic mesh used for reducing intensity of sunlight hitting the surface of the cultures. Porthole (arrow) allowed sampling to be done with minimum leakage of ambient light onto the culture surface. B. Culture vessel (reactor).



A



B

125 ml flasks and incubated for two days under continuous illumination. These cultures were transferred to 500 ml aerated flasks and then to aerated carboys with incubation for two days in each vessel. The carboys were used to inoculate 760 liter polytanks containing deep water enriched to f/4 nutrient concentrations and kept under reduced sunlight on the beach. Reactors were inoculated with 20 liters of culture from the polytanks.

The reactors were filled to half-volume on day 0 of an experiment and inoculated at 1600 hours. Cell counts of the inoculum for each experiment were (in 10^8 cells per liter): 0.25 day^{-1}, 2.33; 0.70 day^{-1}, 5.24; 0.95 day^{-1}, 1.78; and 1.20 day^{-1}, 2.47. At sunrise on day 1 the first sample was taken and deep water was turned on at the rate required for the experiment. When the reactors were full and continuous flow had been initiated, shades were applied.

Samples were taken every day at sunrise and sunset for cell density, chlorophyll *a*, particulate nitrogen (PN) and organic carbon (POC), and the following dissolved nutrients in the medium: nitrate, nitrite, ammonia, phosphate, and silicate.

Cells were preserved with Lugol's solution and counted in a Speirs-Levy hemocytometer. Chlorophyll *a* was determined by fluorometry (Strickland and Parsons 1968). Particulate

nitrogen and organic carbon samples were filtered onto pre-heated glass fiber filters and were analyzed by combustion and gas chromatography using a CHN analyzer (Hewlett-Packard Model 185). Nutrient concentrations were determined on samples that had been filtered through glass fiber filters and frozen. Standard Technicon Auto Analyzer manifolds and procedures were used.

Temperature and light attenuation were recorded at each sampling time. Incident light was measured with a mechanical pyrheliograph. Total radiation for the day was calculated by cutting along the traced outline on the paper with a razor, weighing the area under the curve, and comparing the weight with the weight of a calibrated area.

Photosynthesis measurements using ^{14}C-labeled bicarbonate were done near the end of each experiment following the method of Doty and Oguri (1958). Aliquots from each reactor were first diluted with the percentage of deep water that would enter the culture vessel during the time of the ^{14}C incubation. Diluted samples were then distributed to duplicate light and dark bottles and inoculated with 5 uCi of ^{14}C. For the experiment at 0.25 day^{-1} bottles were covered with screening identical to the screening over the reactor and incubated in a long tray open to sunlight and cooled with running deep water. Incubations were done in

the early morning and at noon for two hours each. For the remaining experiments, bottles were suspended 15 cm from the surface of each culture and incubations were done in the early morning, at noon, and in the late afternoon for two hours each.

In addition, light curves for each culture were run for dilution rates of 0.25 and 1.20 day^{-1}. Incubations were done for two hours in the early afternoon.

At the end of each steady state period deep water was shut off at sunrise, and the cultures were enriched with nutrients to bring the concentrations to deep water levels. Aliquots were taken at intervals for the next eight hours (0.25 day^{-1}) or the next 24 hours (all other dilution rates) for batch nutrient-uptake studies.

Specific growth rate (μ) for continuous cultures was calculated by the equation:

$$\mu = \frac{(\ln N_1 - \ln N_o)}{(t_1 - t_o)} + D \qquad (5)$$

where N_o is the population in cell numbers or biomass concentration at time t_o; N_1 is the population at t_1; and D is the dilution rate. When the difference between t_1 and t_o was 24 hours the term $(\ln N_1 - \ln N_o)/(t_1 - t_o)$ approached 0 as the cultures approached steady state.

Nutrient uptake (v) was calculated by the equation:

$$v = \frac{D(C_i - C_o)}{B} \qquad (6)$$

where C_i is incoming nutrient concentration (deep water) and C_o is the outgoing concentration (assumed to be identical to the concentration in the culture medium); B is biomass as expressed in cell numbers.

RESULTS AND DISCUSSION

Most cultures grew rapidly after inoculation and then approached a growth rate approximating dilution rate (steady state) by the fourth day. The experiment conducted at a growth rate of 1.2 day^{-1} deviated from this pattern. These cultures started off slowly, then grew faster than the dilution rate, and did not reach steady state until day 5.

The cultures did not remain unialgal throughout the experiments. Other diatoms, unidentified flagellates, protozoa, amoeba, and bacteria appeared at one time or another in most cultures. However, Chaetoceros curvisetus always accounted for more than 97% of the total cell volume of the cultures, and cell numbers presented here are for C. curvisetus only.

The following tests were used to evaluate the amount of day-to-day variability: (1) $R_x = \bar{x}_0/\bar{x}_1$, where $\bar{x}_0$ is the mean of the first two cell counts and $\bar{x}_1$ the mean of the last two cell counts of the presumed steady state period (Williams 1971) and (2) analysis of variance (ANOVA) of biomass and rate values.

The variability within each culture is apparent with test (1) (Table 1). The difference between growth rate and dilution rate was less than 20% in 16 of the 20 cultures. It was more than 25% in two cultures and between 20% and 25% in

Table 1. R_x values as criteria for steady state for outdoor continuous cultures of Chaetoceros curvisetus.

Dilution Rate (day^{-1})	% Light Intensity	R_x	Percent Difference between μ and D
0.25	100	1.5	<20
	46	0.8	< 20
	30	0.9	< 5
	20	1.4	< 15
	3	2.2	< 35
0.70	100	1.0	< 2
	46	0.7	< 20
	30	0.9	< 10
	20	1.0	< 2
	3	1.3	< 15
0.95	100	1.0	0
	46	1.5	< 20
	30	1.7	< 25
	20	1.8	< 25
	3	2.6	< 40
1.20	100	0.8	<15
	46	0.9	<10
	30	1.0	< 2
	20	1.1	<10
	3	1.6	<20

another two cultures.

Analysis of variance demonstrated that variance in a characteristic examined was significantly related to dilution rate (Table 2) while the variance between days ("replicates") was not significant. It was concluded that while analysis of variance showed no significant variation between days of steady state, the condition of steady state was poorly approximated at the lowest light intensity of cultures growing at 0.25 day^{-1} and 0.95 day^{-1}.

Environmental Variables

Incident light intensity varied from day to day by as much as 41 per cent about the mean during the steady state period of a single experiment (Table 3). The mean incident light intensity varied between experiments by 20 per cent (SD = 67 ly day^{-1}).

Temperature variation was greatest at 100% incident light within each experiment (Table 3). Cultures were coolest at sunrise and warmest at sunset. Temperature variation between experiments was 7 per cent (SD = 1.73°C).

Nitrate and silicate concentrations in the medium generally increased with an increase in growth rate and with a decrease in light intensity (Table 4). Nitrate was removed to the limit of detection (0.40 µg-at NO_3 per liter) at every sampling time during steady state in seven cultures. No

Table 2A. Analysis of variance for biomass. T = time of day (either sunrise or sunset), D = dilution rate, I = light intensity. All sources tested over error term. Angular transformation performed on the ratio, C:chl.

Source of Variation	Degrees of Freedom	Mean Squares				
		Cell No.	Particulate Organic Carbon	Particulate Nitrogen	Chlorophyll	C:chl
T	1	30.87**	1.191**	.000025	23.77	.00138**
D	3	104.18**	7.895**	.06995**	2302.80**	.02513**
I	4	149.52**	7.807**	.13375**	901.80**	.00624**
T,D	3	5.51**	0.874**	.00836**	140.80**	.00074*
T,I	4	2.05	0.146	.00059	44.73	.00021
D,I	12	4.75**	0.544**	.01674**	613.00**	.00505**
T,D,I	12	2.55*	0.077	.00125	44.10	.00015
Error term	80	1.25	0.071	.00111	31.22	.00020

*Significant at the 0.05 level of probability.

**Significant at the 0.01 level of probability.

Table 2B. Analysis of variance for rates. T = time of day (either sunrise or sunset), D = dilution rate, I = light intensity. All sources tested over error term.

Source of Variation	Degrees of Freedom	Mean Squares: Nitrate Uptake per Liter	Nitrate Uptake per Cell	Carbon Production per Liter	Carbon Production per Cell	Carbon Production per Chlorophyll
T	1	105.54**	2.553	19.080**	2,083,568**	16,787
D	3	936.73**	274.793**	5.778**	929,370**	47,416**
I	4	1228.98**	44.148*	6.705**	14,642	17,315*
T,D	3	35.89**	3.846	6.026**	542,837**	60,857**
T,I	4	9.83*	8.513	0.794	31,827	2,751
D,I	12	169.74**	13.029	0.634	28,560	24,037**
T,D,I	12	7.41*	22.658	0.661	70,851**	4,109
Error term	80	3.64	17.129	0.490	28,030	5,129

*Significant at the 0.05 level of probability.

**Significant at the 0.01 level of probability.

Table 3. Environmental factors during the steady state period.

Dilution Rate (day^{-1})	Incident light (%)	Time of Year	Temperature (°C)		Light ($ly\ day^{-1}$)	
			Mean	Range	Mean	Range
0.25	100	June	27.3	25.5-29.8	331.0	259-415
	46		27.0	25.5-29.5	153.0	119-170
	30		27.0	25.5-29.5	99.4	78-125
	20		26.8	25.5-29.2	66.2	52-83
	3		26.7	25.5-29.0	9.9	8-12
0.70	100	December	24.5	22.0-26.5	274.0	223-334
	46		24.1	22.0-26.5	126.0	103-154
	30		23.8	22.0-25.0	82.2	67-100
	20		23.7	22.0-25.0	54.8	45-67
	3		23.7	22.0-25.0	8.2	7-10
0.95	100	January	23.4	22.0-24.6	297.0	253-335
	46		22.9	22.0-23.5	137.0	116-154
	30		22.9	22.0-23.5	89.1	76-100
	20		22.8	22.0-23.2	59.4	51-67
	3		22.7	22.0-23.0	8.9	8-10
1.20	100	September	26.0	24.4-28.2	427.0	226-547
	46		25.4	24.4-26.8	196.0	104-252
	30		25.2	24.4-26.3	128.0	68-164
	20		25.1	24.4-26.0	85.4	45-109
	3		24.9	24.4-25.8	12.8	7-16

Table 4. Residual nutrient concentrations (µg-at l^{-1}) in culture medium during steady state.

Dilution Rate (day^{-1})	Incident Light (%)	Nitrate plus Nitrite	Ammonia	Phosphate	Silicate	Ratios of Uptake N:P	Ratios of Uptake N:Si
0.25	100	<0.4	-*	0.40	1.51	19	1.5
	46	<0.4	-	0.39	1.35	19	1.5
	30	<0.4	-	0.40	1.43	19	1.5
	20	<0.4	-	0.42	1.62	19	1.5
	3	3.61	-	0.48	2.57	17	1.4
Deep Water		30.91	-	2.05	22.43	15	1.4
0.70	100	<0.4	0.30	0.54	2.37	20	1.3
	46	<0.4	0.32	0.53	2.09	20	1.3
	30	0.6	0.32	0.55	2.08	20	1.3
	20	1.47	0.32	0.60	3.83	20	1.4
	3	16.60	0.37	0.98	14.29	14	1.4
Deep Water		31.20	0.34	2.09	25.67	15	1.2
0.95	100		0.33	0.48	2.00	24	1.4
	46	1.83	0.34	0.50	4.80	22	1.5
	30	3.38	0.35	0.50	4.30	21	1.4
	20	14.58	0.36	0.57	12.00	14	1.4
	3	26.72	0.42	1.55	19.00	19	1.0
Deep Water		32.42	-	1.86	24.60	17	1.3
1.20	100	3.99	0.60	0.67	6.70	22	1.8
	46	4.39	0.53	0.64	6.30	21	1.8
	30	8.78	0.47	0.79	7.37	20	1.6
	20	19.91	0.58	1.32	13.98	19	1.5
	3	28.43	0.60	1.75	22.12	19	-
Deep Water		30.46	0.48	1.86	21.00	16	1.4

*No analysis

temporal variation could be observed and nitrate uptake was directly proportional to growth rate and independent of light intensity. These cultures were therefore identified as "nitrogen-limited" cultures.

Rates of removal of nitrate with respect to phosphate increased slightly with increasing growth rate and decreased with decreasing light intensity (Table 4). Uptake ratios for N:Si averaged 1.4 $\pm$ 0.2 and showed no obvious trend. These findings suggested that while cultures were becoming less severely limited by nitrate supply as growth rate increased, neither phosphate nor silicate acted as limiting nutrient under these experimental conditions.

Diel variations in nitrate and silicate were seen in cultures not identified as nitrogen-limited, with low concentrations of nutrient at the end of the light period and high concentrations at the end of the dark period. Phosphate varied slightly and without respect to day-night cycles. These findings are consistent with the study of Malone et al. (1975) who found nitrate uptake was continuous and not related to day-night cycles at slow growth rates whereas it was cyclic at higher growth rates.

There was more ammonia present in the cultures at a dilution rate of 1.2 day^{-1} than was coming into the cultures with deep water (Table 4). No explanation for this

phenomenon could be found.

Biomass Characteristics

Total biomass. Variations in cell number, POC, PN and chlorophyll were significantly related to light intensity (Table 2). Cell number, POC, and PN increased with light intensity up to 20 to 30% light (Fig. 3). Chlorophyll decreased with increasing light intensity at the lowest growth rate but increased with an increase in light up to 20 or 30% at higher growth rates. Cell number, POC, and PN tended to level off at light intensities higher than 20 or 30% while chlorophyll tended to decrease at higher intensities. A sequence of cultures was considered to be light-limited when biomass decreased with a decrease in light and the standard deviations about each mean did not overlap. Nitrogen-limited cultures have already been identified. Remaining cultures were called "intermediate" cultures. The three types of cultures are identified in Table 5.

Total biomass also varied with growth rate (Table 2). Three patterns of change were detected for cell number, POC, and PN (Fig. 4): (1) At 46% and 100% incident light, biomass increased with an initial increase in growth rate and decreased with further increase in growth rate. (2) At 20% and 30% incident light, biomass decreased slowly, then more rapidly, with an increase in growth rate. (3) At 3% light

Table 5. Separation of cultures into three groups of populations, based on responses to light intensity and presence of nitrate in the medium. N = nitrogen-limited cultures; L = light-limited cultures; I = intermediate cultures.

		Dilution Rate (day^{-1})			
		0.25	0.70	0.95	1.20
Light Intensity (%)	100	N	N	N	I
	46	N	N	I	I
	30	N	I	I	L
	20	N	I	L	L
	3	I	L	L	L

Figure 3. Four measures of biomass as functions of incident light intensity at each growth rate. Vertical bars represent one standard deviation about the mean. Horizontal lines in the upper portion of the figure represent the value of particulate nitrogen that would be attained if all the nitrate in the incoming medium were converted to PN. □ = particulate organic carbon (POC) in mg per liter. ■ = particulate nitrogen (PN) in mg per liter. ○ = cell number as 10^7 Chaetoceros cells per liter. ● = chlorophyll a (CHL) in ug per liter.

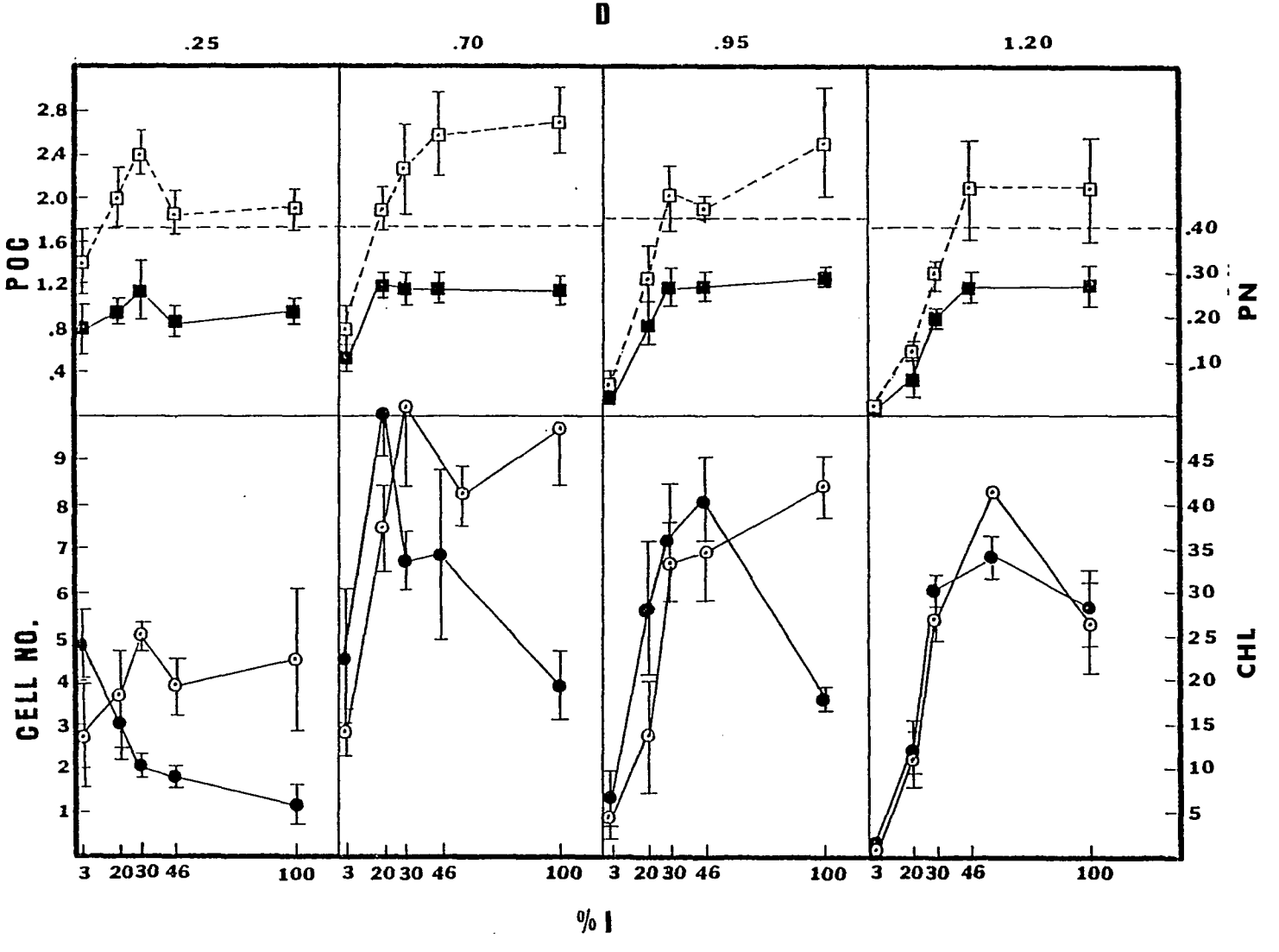
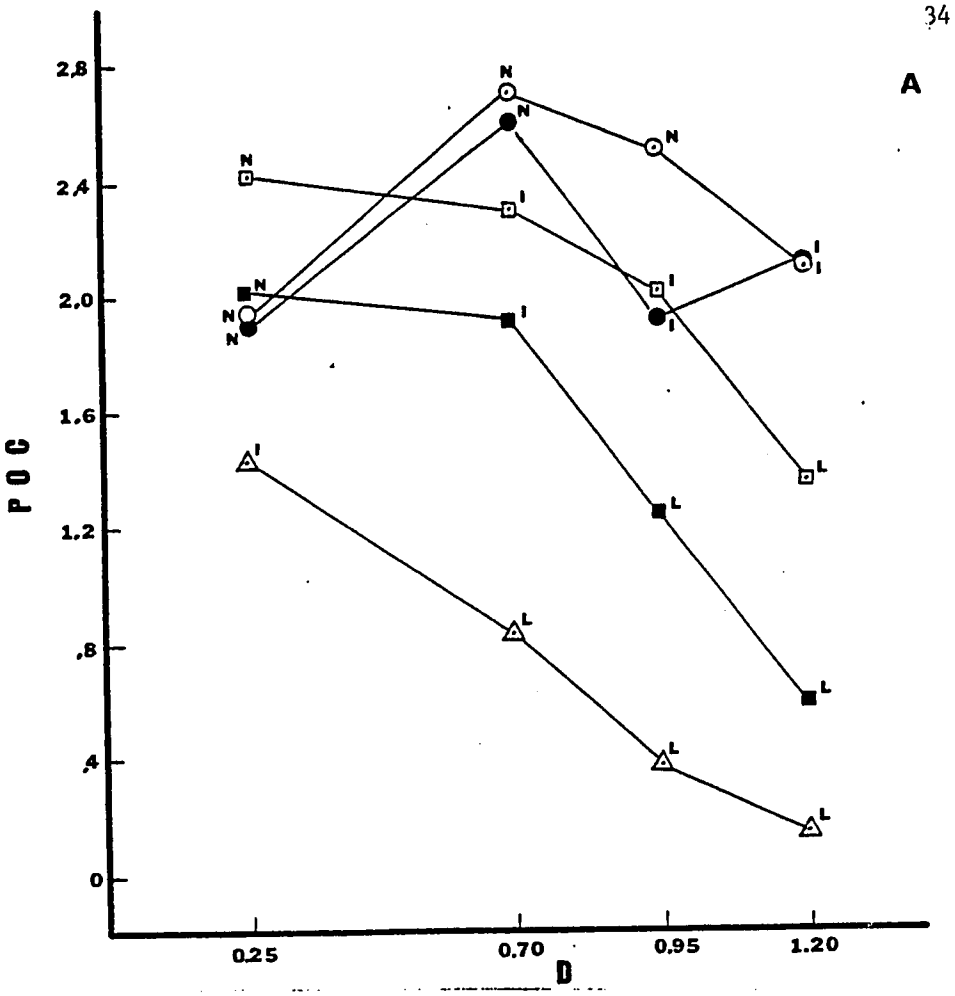
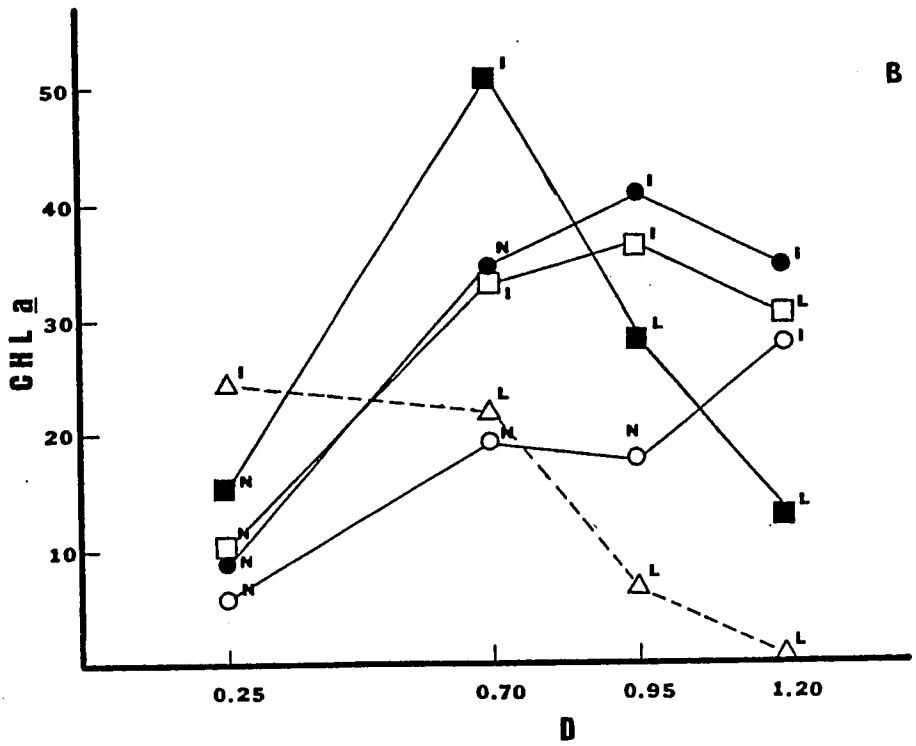

D
.25
.70
.95
1.20
POC
PN
CELL NO.
CHL
%I
3
20
30
46
100

Figure 4. Two measures of biomass as functions of dilution rate (D). A. Particulate organic carbon in mg l^{-1} (POC). B. Chlorophyll *a* in µg l^{-1} (CHL). L = light-limited cultures; N = nutrient-limited cultures; I = intermediate cultures. ○ = 100% light; ● = 46% light; □ = 30% light; ■ = 20% light; △ = 3% light.


A
POC
2,8
2,4
2,0
1,6
1,2
,8
,4
0
0.25
0.70
0.95
1.20
D


B
CHL a
50
40
30
20
10
0.25
0.70
0.95
1.20
D

biomass decreased linearly with an increase in growth rate. These patterns were not observed with chlorophyll, which tended to increase with growth rate at higher light intensities and to decrease with growth rate at lower light intensities (Fig. 4).

These patterns of change were associated with different limiting conditions of the cultures. In pattern (1) the initial increase in biomass with increase in growth rate was associated with a change from nitrogen-limited to intermediate culture conditions. Thus, the limitation of nitrate uptake by rate of nitrate supply was accompanied by less production of carbon and cells relative to production in other cultures. In patterns (2) and (3) the rapid decrease in biomass with increase in growth rate was associated with a change from intermediate to light-limited cultures. Thus, the limitation of carbon production by light intensity was accompanied by less production of nitrogen and cells relative to production in other cultures.

These findings show the interdependence of nitrate uptake and photosynthesis under widely different conditions that resulted in a significant interaction term (D,I) of the ANOVA for measures of biomass (Table 2).

Photosynthesis and growth rate. Photosynthetic carbon production, as the product of growth rate and POC per liter,

increased with light intensity to saturation at 30 to 46% light. Carbon production was much lower, and increased more slowly with an increase in light intensity, at 0.25 day^{-1} than at higher growth rates (Fig. 5a).

Photosynthesis per cell, as the product of growth rate and carbon per cell, increased with increasing growth rate and was not significantly affected by light intensity ($p > 0.10$; Fig. 5b). Consequently, carbon and cell number at steady state were significantly correlated for the entire set of data ($p < 0.001$) and the slope of the least-squares fit to the data was 0.22 mg C per 10^7 cells (Fig. 6a). At sunset the correlation was significant for each category of limitation (Table 5) and the correlation coefficients were not significantly different (Table 6). However, at sunrise the correlation between carbon and cell number for the nitrogen-limited cultures was not significant, and the correlation coefficients for light-limited and nitrogen-limited cultures were significantly different. These differences were accompanied by a high intercept and a slope near zero for the least-squares data fit for nitrogen-limited cultures. Carbon metabolism in these cultures was affected by light history and greater excretion or respiration apparently occurred at night in cultures growing at high light than at low light intensities.

Chlorophyll-specific photosynthesis, as the product

Figure 5. A. Photosynthetic carbon production per volume of culture (D x POC l^{-1}, in g POC day^{-1}) as a function of incident light intensity. B. Photosynthesis per cell (D x POC $cell^{-1}$, in g POC 10^{-7} cells day^{-1}) as a function of incident light intensity. C. Chlorophyll-specific photosynthesis (D x C:chl, in mg POC mg chl^{-1} day^{-1}) as a function of incident light intensity. The equation for the straight line is $y = 23.3 + 0.71x$ ($r = 0.916$; $p < 0.001$) for nitrogen-limited and intermediate cultures. Dashed line represents the mean of light-limited cultures. ○ = 0.25 day^{-1}; ● = 0.70 day^{-1}; □ = 0.95 day^{-1}; and ■ = 1.20 day^{-1} growth rates.

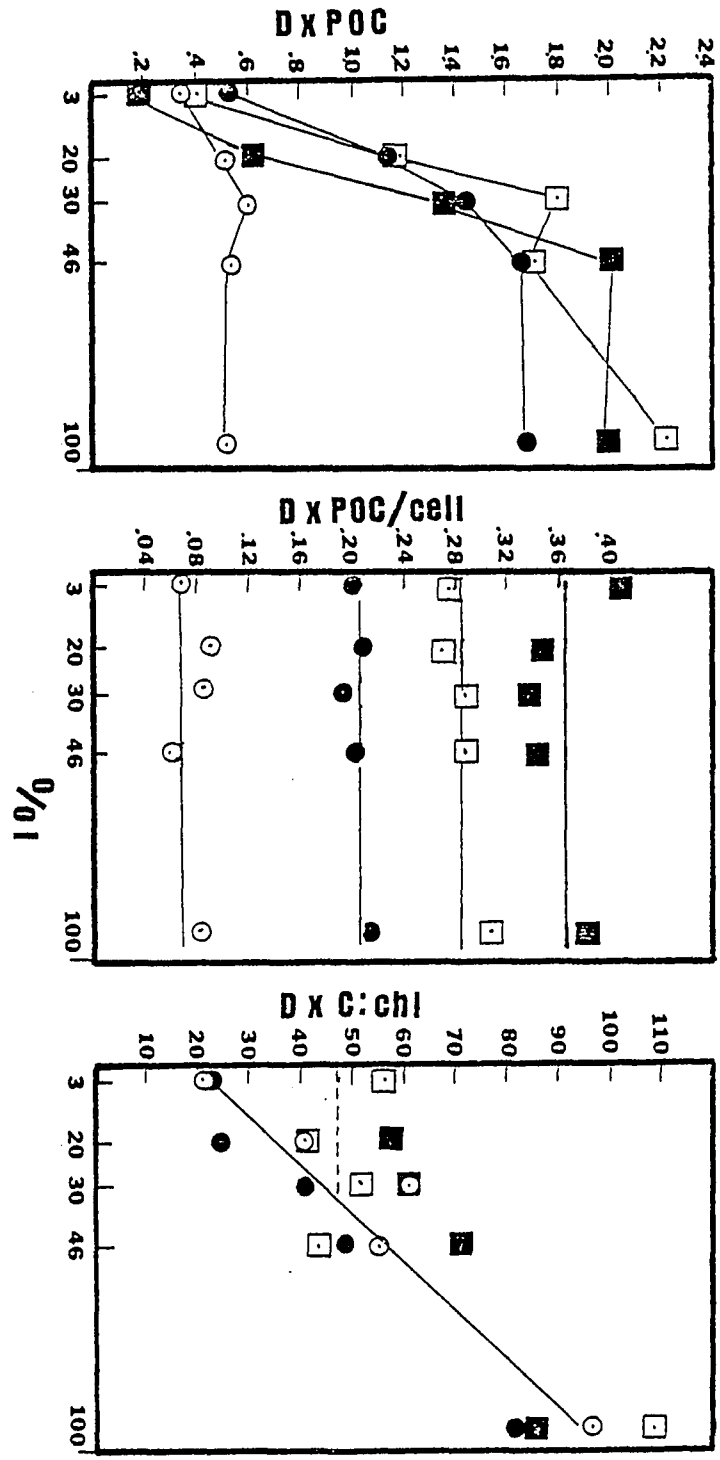
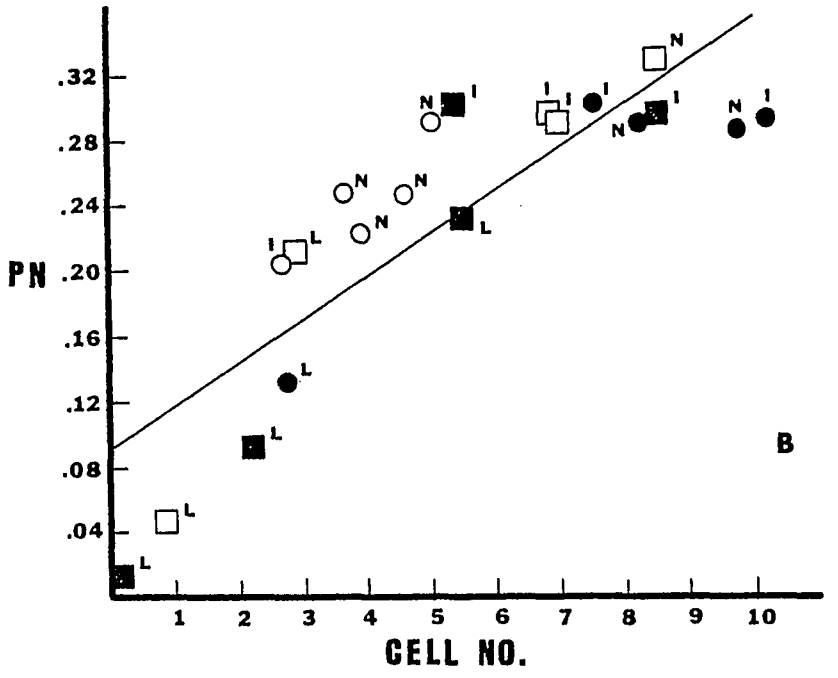

D x POC
D x POC/cell
D x C:chl
% I
3
20
30
46
100

Figure 6. Correlations of carbon and nitrogen to cell number during steady state. A. Particulate organic carbon (POC) in mg l^{-1} and cell number in 10^7 Chaetoceros cells l^{-1} ($y = 0.57 + 0.22x$; $r = 0.849$). B. Particulate nitrogen (PN) in mg l^{-1} and cell number in 10^7 Chaetoceros cells l^{-1} ($y = 0.09 + 0.03x$; $r = 0.840$). Values are means of sunset samples. Line through data represents a least-squares fit. L = light-limited cultures; N = nitrogen-limited cultures; I = intermediate cultures. ○ = 0.25 day^{-1}; ● = 0.70 day^{-1}; □ = 0.95 day^{-1}; and ■ = 1.20 day^{-1} growth rates.


PN
.32
.28
.24
.20
.16
.12
.08
.04
1 2 3 4 5 6 7 8 9 10
CELL NO.
B

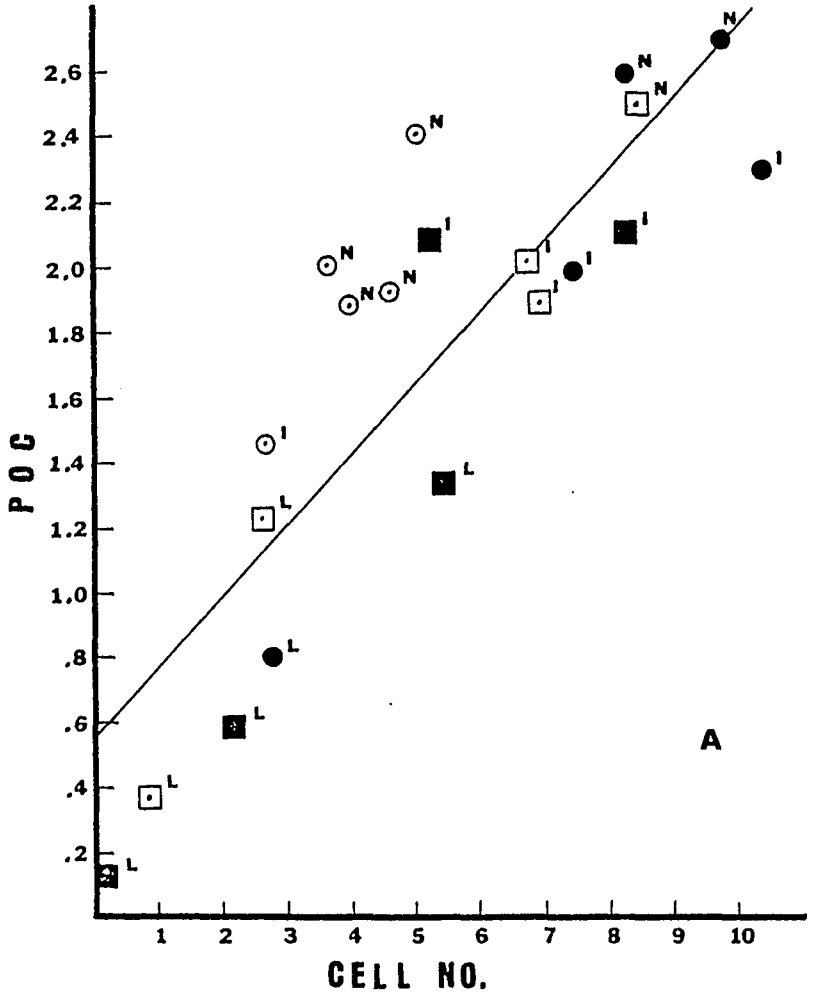

POC
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
1 2 3 4 5 6 7 8 9 10
CELL NO.
A

Table 6. Correlations and least-square fit of line for cellular characteristics of nutrient-limited (N), light-limited (L), and intermediate (I) cultures and for the total matrix of conditions; t-test for difference between populations.

Characteristic	Population	Sunset Values				
		a	b	r	$\bar{y}/\bar{x}$	t test
Carbon (y) and cell no. (x)	N	1.52	0.12	0.910**	0.367	(N-I) ns
	I	1.31	0.10	0.855**	0.288	(N-L) ns
	L	0.18	0.24	0.908**	0.314	(I-L) ns
	Total	0.57	0.22	0.849*	0.324	
Nitrogen (y) and cell no. (x)	N	0.21	0.01	0.768*	0.044	(N-I) ns
	I	0.21	0.01	0.707*	0.041	(N-L) ns
	L	0.02	0.04	0.898**	0.050	(I-L) ns
	Total	0.09	0.03	0.840**	0.044	
Nitrogen (y) and carbon (x)	N	0.07	0.09	0.858**	0.120	(N-I) ns
	I	0.06	0.11	0.840**	0.144	(N-L) **
	L	-0.01	0.18	0.998**	0.161	(I-L) **
	Total	0.03	0.12	0.945**	0.135	
		Sunrise Values				
Carbon (y) and cell no. (x)	N	2.21	-0.01	-0.216^{ns}	0.361	(N-I) ns
	I	1.00	0.11	0.800**	0.326	(N-L) **
	L	0.08	0.33	0.972**	0.379	(I-L) ns
Nitrogen (y) and cell no. (x)	N	0.26	0.01	0.589^{ns}	0.050	(N-I) ns
	I	0.19	0.01	0.896**	0.055	(N-L) **
	L	0.01	0.06	0.991**	0.066	(I-L) ns
Nitrogen (y) and carbon (x)	N	0.36	-0.03	-0.091^{ns}	0.137	(N-I) ns
	I	0.10	0.10	0.848**	0.168	(N-L) **
	L	-0.01	0.19	0.990**	0.175	(I-L) ns

^{ns}Not significant

*Significant at the 0.05 level of probability.

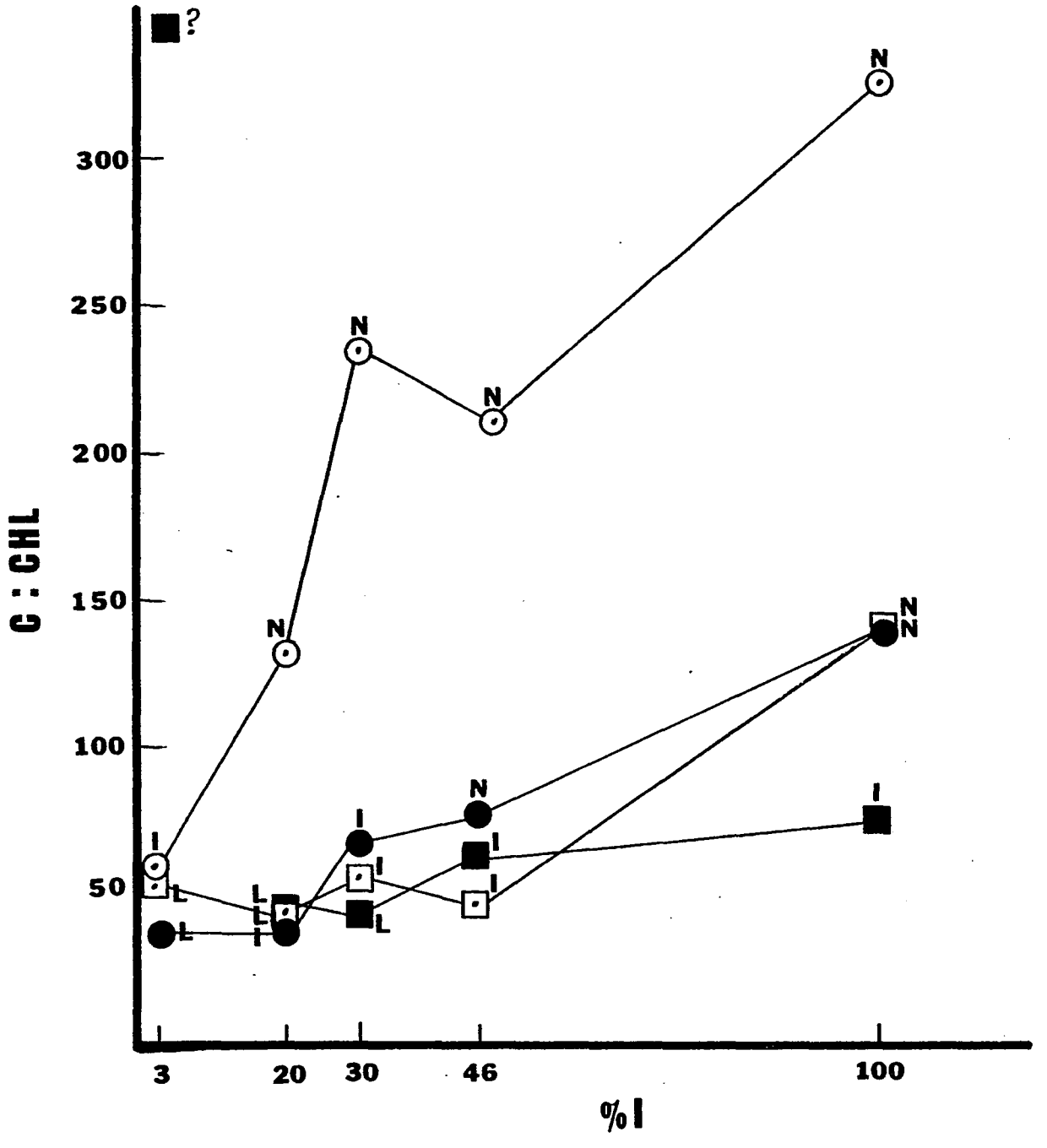
**Significant at the 0.01 level of probability.

of growth rate and the carbon:chlorophyll ratio, increased as light intensity increased without reaching saturation in nitrogen-limited and intermediate cultures (Fig. 5c). It was not significantly related to light intensity in light-limited cultures ($r = 0.157$; $p > 0.10$). An extremely high value (320 mg C mg chl^{-1} day^{-1}) for the culture growing at 3% light intensity, 1.2 day^{-1}, was not included in the figure. Any error in the extremely low biomass determinations of this culture was magnified by the calculation and the validity of the result was questionable.

Because culture conditions influenced chlorophyll-specific photosynthesis, carbon and chlorophyll were not correlated significantly for the entire set of data ($r = 0.570$; $p > 0.10$). Nor were they correlated significantly for nitrogen-limited ($r = 0.411$; $p > 0.10$) or intermediate cultures ($r = 0.222$; $p > 0.10$) where C:chl increased with an increase in light intensity (Fig. 7). However, the correlation was significant for light-limited cultures ($r = 0.983$; $p < 0.001$) and gave a slope of 39.1 C:chl for the least-squares fit to the data. Light intensity did not affect the C:chl ratio in these cultures (Fig. 7).

The effect of light intensity on chlorophyll-specific photosynthesis but not on photosynthesis per cell suggests that chlorophyll per cell was adjusted to accommodate a certain rate of photosynthesis for culture conditions, and chlorophyll

Figure 7. The carbon:chlorophyll (C:chl) ratio as a function of light intensity (I) at different growth rates. L = light-limited cultures; N = nitrogen-limited cultures; I = intermediate cultures. ○ = 0.25 day^{-1}; ● = 0.70 day^{-1}; □ = 0.95 day^{-1}; ■ = 1.20 day^{-1} growth rates.


■?
300
250
200
150
100
50
C : CHL
3
20
30
46
100
%I
N
I
L

per cell did in fact increase with a decrease in light intensity in nitrogen-limited and intermediate, but not light-limited cultures (Fig. 8).

^{14}C incubations. The ^{14}C incubations showed differences due to time of day of the incubation, growth rate, and light intensity (Fig. 9a-d). The late afternoon incubations gave the lowest values, morning incubations the highest, with noon usually in between. Carbon production for the day of incubation was calculated as the product of carbon (sunset value) and growth rate divided by 12 because daylength ranged from 11.5 to 12.0 hours among the experiments. This steady state value is included in the figure for comparison with ^{14}C results.

In general ^{14}C uptake results were lower than would be expected from steady state production values (Fig. 9). Even when sunrise or 24-hour averaged POC values were used to calculate production, most ^{14}C incubations underestimated steady state production. One factor possibly affecting the ^{14}C results was the dilution of the samples with the amount of deep water the cells would have been exposed to during the incubation period if they had remained in the reactor. The exposure would have been gradual and continuous in the reactor, however, whereas the exposure was in effect a spike in the bottles. Falkowski and Stone (1975) have shown initial

Figure 8. Chlorophyll per cell (µg chl *a* 10^{-7} cells) as a function of light intensity (I) at different growth rates. L = light-limited cultures; N = nitrogen-limited cultures; I = intermediate cultures. ○ = 0.25 day^{-1}; ● = 0.70 day^{-1}; □ = 0.95 day^{-1}; ■ = 1.20 day^{-1}.

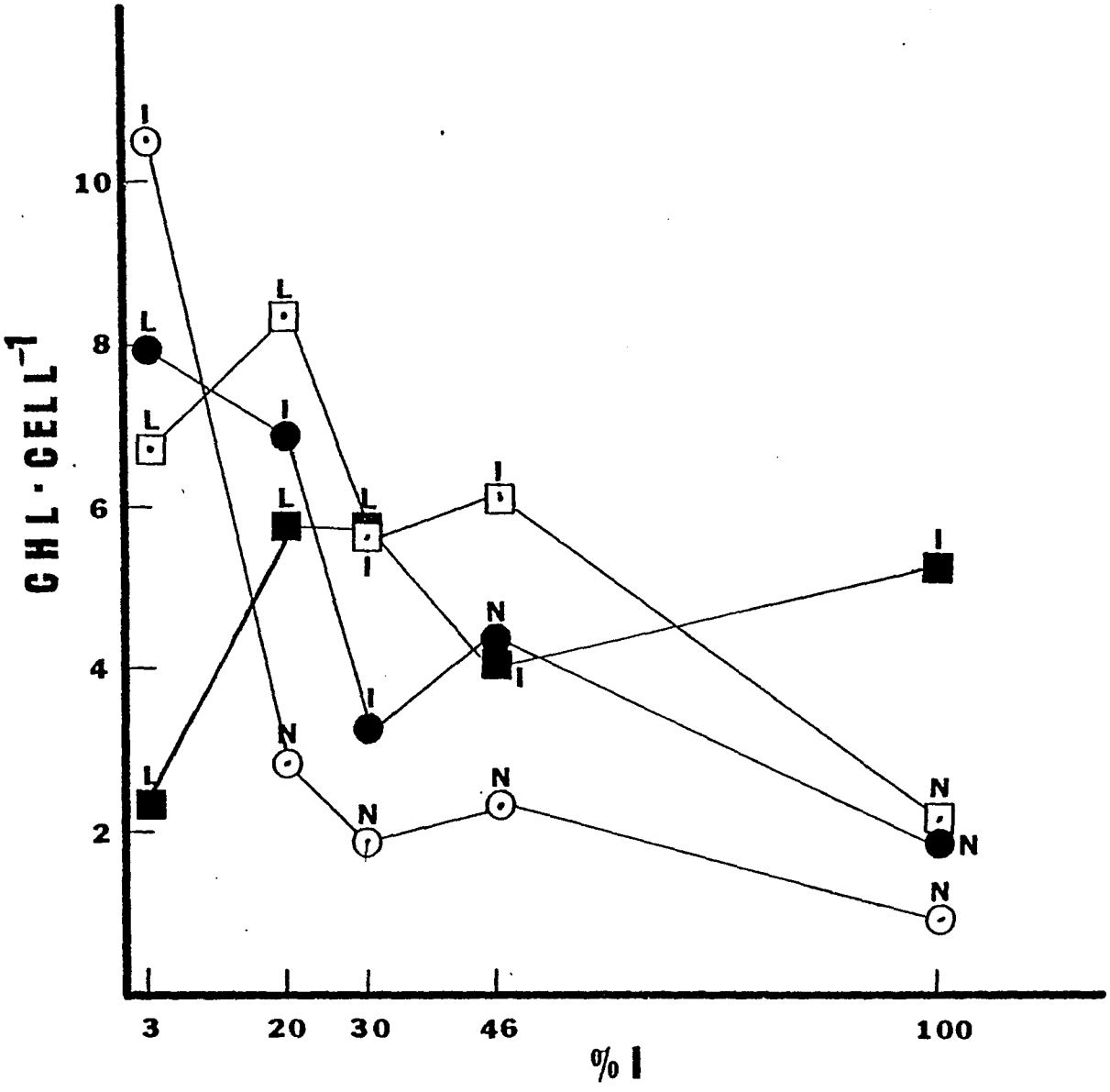
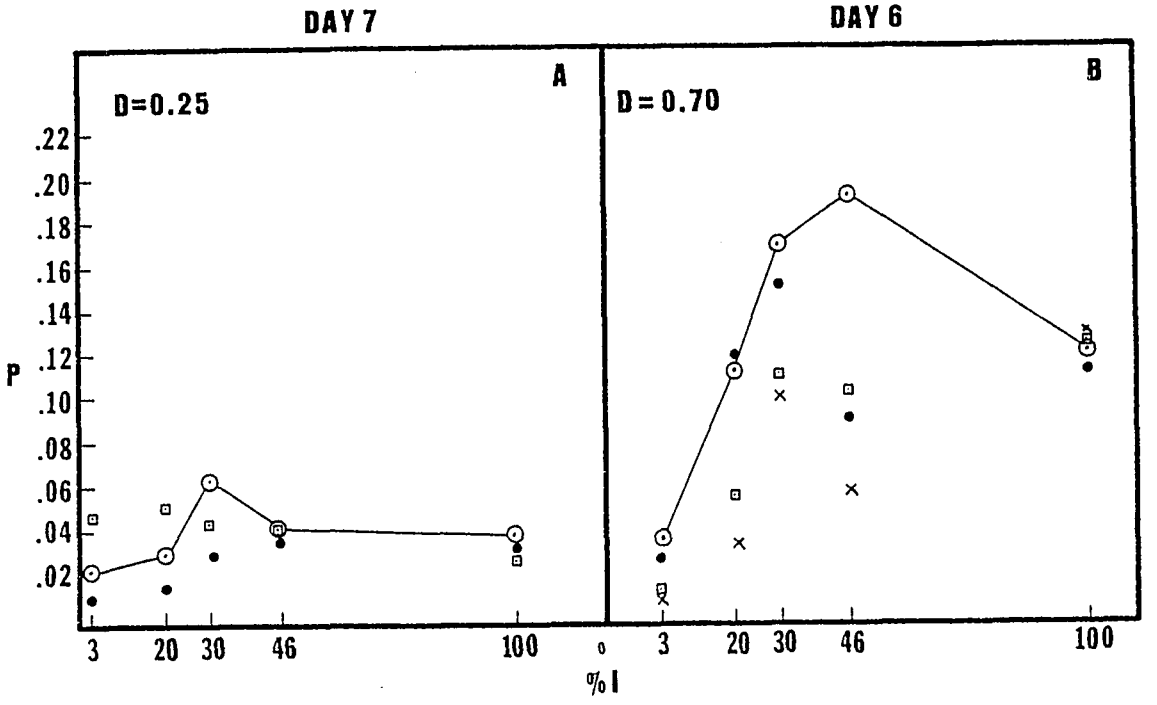
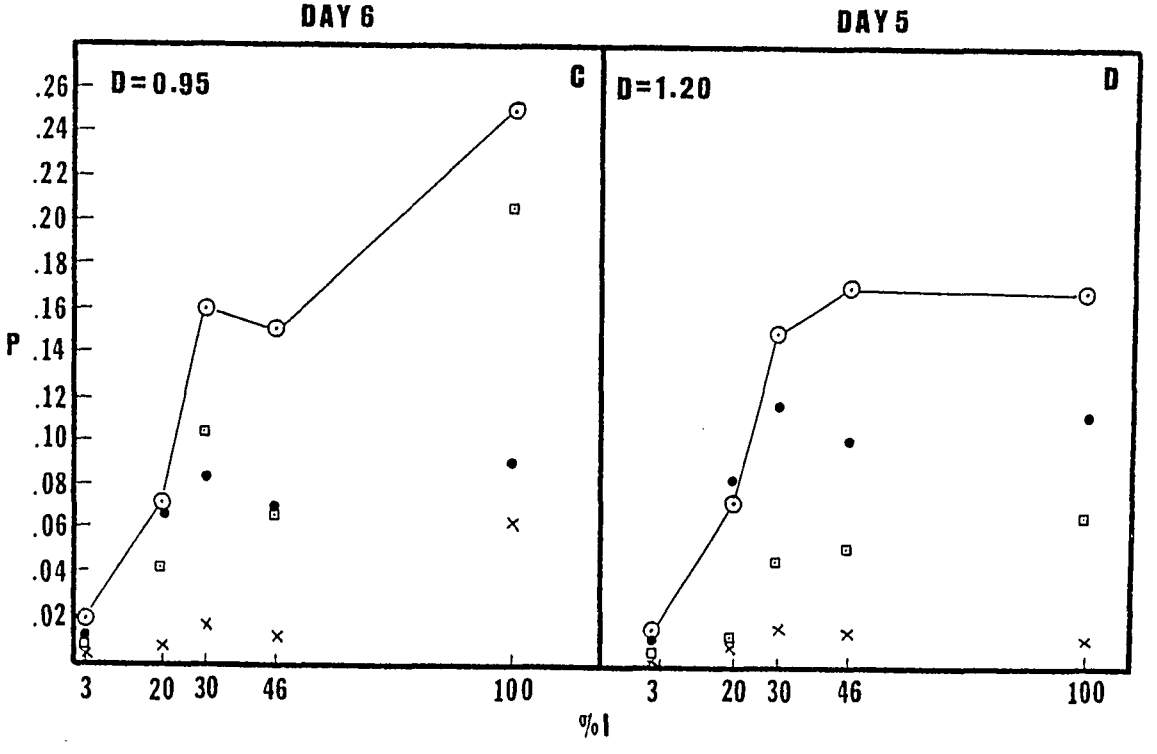

CHL·CELL⁻¹
10
8
6
4
2
3
20
30
46
100
% I

Figure 9. Particulate carbon production (g POC day^{-1}) as estimated by ^{14}C uptake experiments compared with estimations from POC values in steady state culture on the day of the ^{14}C experiment. Estimates shown as a function of light intensity for each growth rate. ● = a.m. incubations; □ = noon incubations; X = p.m. incubations, O = steady state POC values.


DAY 7
DAY 6
A
B
D=0.25
D=0.70
P
.22
.20
.18
.16
.14
.12
.10
.08
.06
.04
.02
3
20
30
46
100
%I


DAY 6
DAY 5
C
D
D=0.95
D=1.20
P
.26
.24
.22
.20
.18
.16
.14
.12
.10
.08
.06
.04
.02
3
20
30
46
100
%I

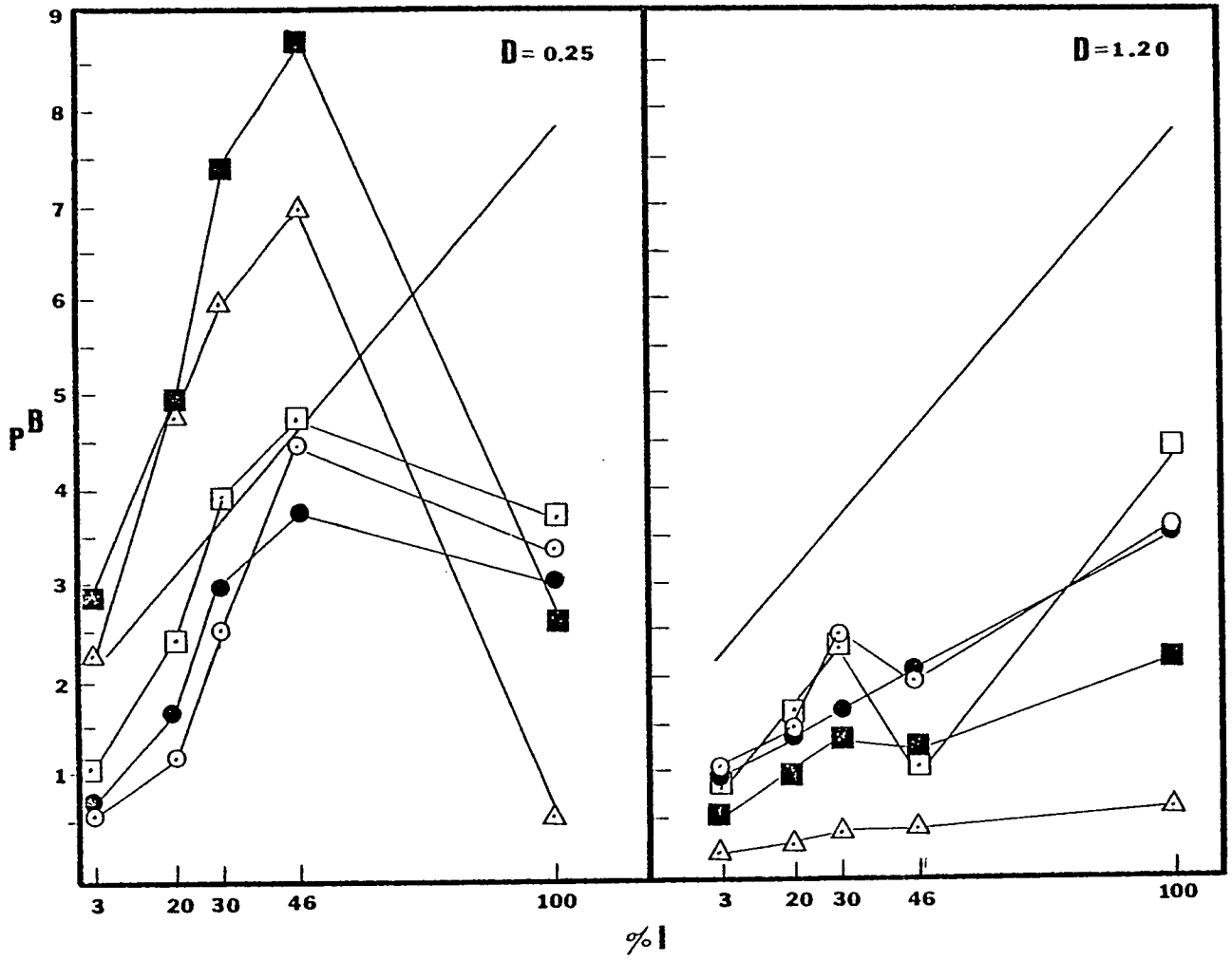
inhibition of photosynthesis when nitrate depleted cells are enriched with nitrate. The ^{14}C samples that most underestimated steady state photosynthesis were those that had the most amount of deep water added before incubation.

Another factor affecting the ^{14}C results was the bottle itself. The larger surface-to-volume ratio in the bottles than in the 2000-liter culture vessel may have caused higher excretion or respiration rates or both in the bottles. The apparent photosynthetic rates would therefore have been lower by ^{14}C estimations than from steady state estimations. Whatever factors were at work, it is clear that in these experiments ^{14}C incubations were measuring somewhat different processes than were measured by the POC values in steady state.

Light curves for each culture were done at growth rates of 0.25 and 1.20 day^{-1}. A greater rate of chlorophyll-specific photosynthesis (P^B) occurred in the ^{14}C incubations for a growth rate of 0.25 than for 1.20 day^{-1}, and the rate for 1.20 day^{-1} was considerably less than steady state P^B (Fig. 10).

Inhibition was seen at 100% light in all incubations from cultures growing at 0.25 day^{-1} but not at 1.20 day^{-1}. The inhibition was greatest for cultures that had been growing at 3 and 20% of incident light. At 0.25 day^{-1} and in the light intensity range of 3 to 30%, P^B was related to light

Figure 10. Light curves for cultures growing at each light intensity and two growth rates. Straight lines represent the regression of chlorophyll-specific carbon production (mg POC mg chl^{-1} day^{-1}) on light intensity from the steady state cultures. Each curve represents the chlorophyll-specific photosynthetic response of a culture growing in steady state at one light intensity. ○ = 100%; ● = 46%; □ = 30%; ■ = 20%; △ = 3% of incident light.


D = 0.25
D = 1.20
P^B
9
8
7
6
5
4
3
2
1
3
20
30
46
100
%I

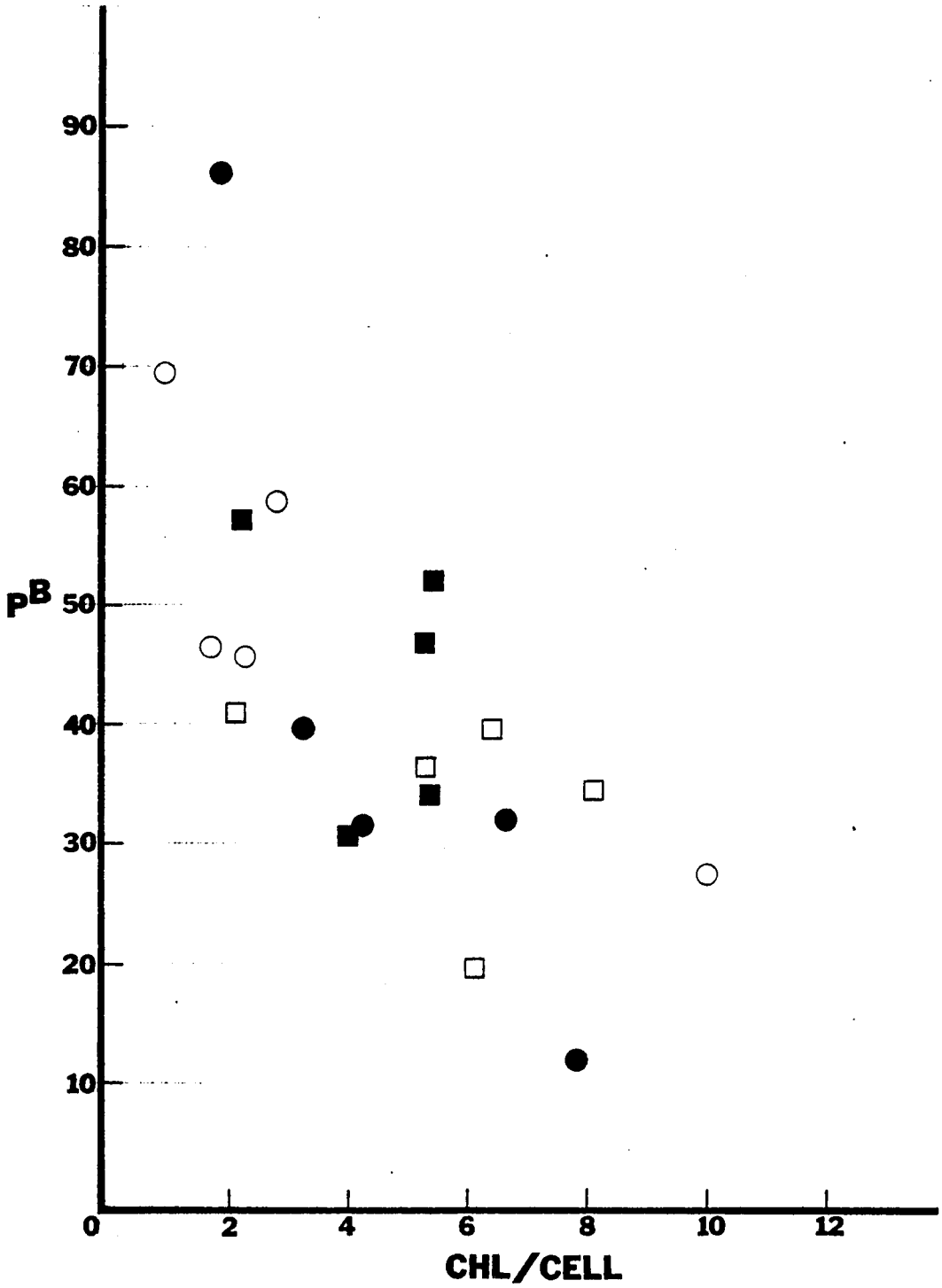
history of the culture and was higher for cultures growing at low light than for cultures growing at high light. This relationship was not seen at 1.20 day^{-1}.

The maximum P^B for each ^{14}C uptake incubation was inversely related to the chlorophyll per cell concentration (Fig. 11). Since the chlorophyll concentration per cell varied both with growth rate and light intensity (Fig. 8), this relationship between P^B and chlorophyll per cell probably reflected self-shading rather than factors external to the cell (Platt et al. 1977).

Saturation was not reached in steady state P^B but was seen in the ^{14}C incubations for 0.25 day^{-1}, again demonstrating that different processes were being measured by the incubations and the C:chl values in steady state.

Nitrogen assimilation, nitrogen uptake, and growth rate. The relationship between nitrogen assimilation and growth varied with light in the same way that the relationship between net photosynthesis and growth varied, as demonstrated in the similarity between PN and POC concentrations in the cultures (Fig. 3). The rate of nitrogen assimilation also followed the pattern of photosynthesis and was considerably less at the lowest growth rate (0.072 mg PN day^{-1}) than at the higher growth rates (0.325 mg PN day^{-1}).

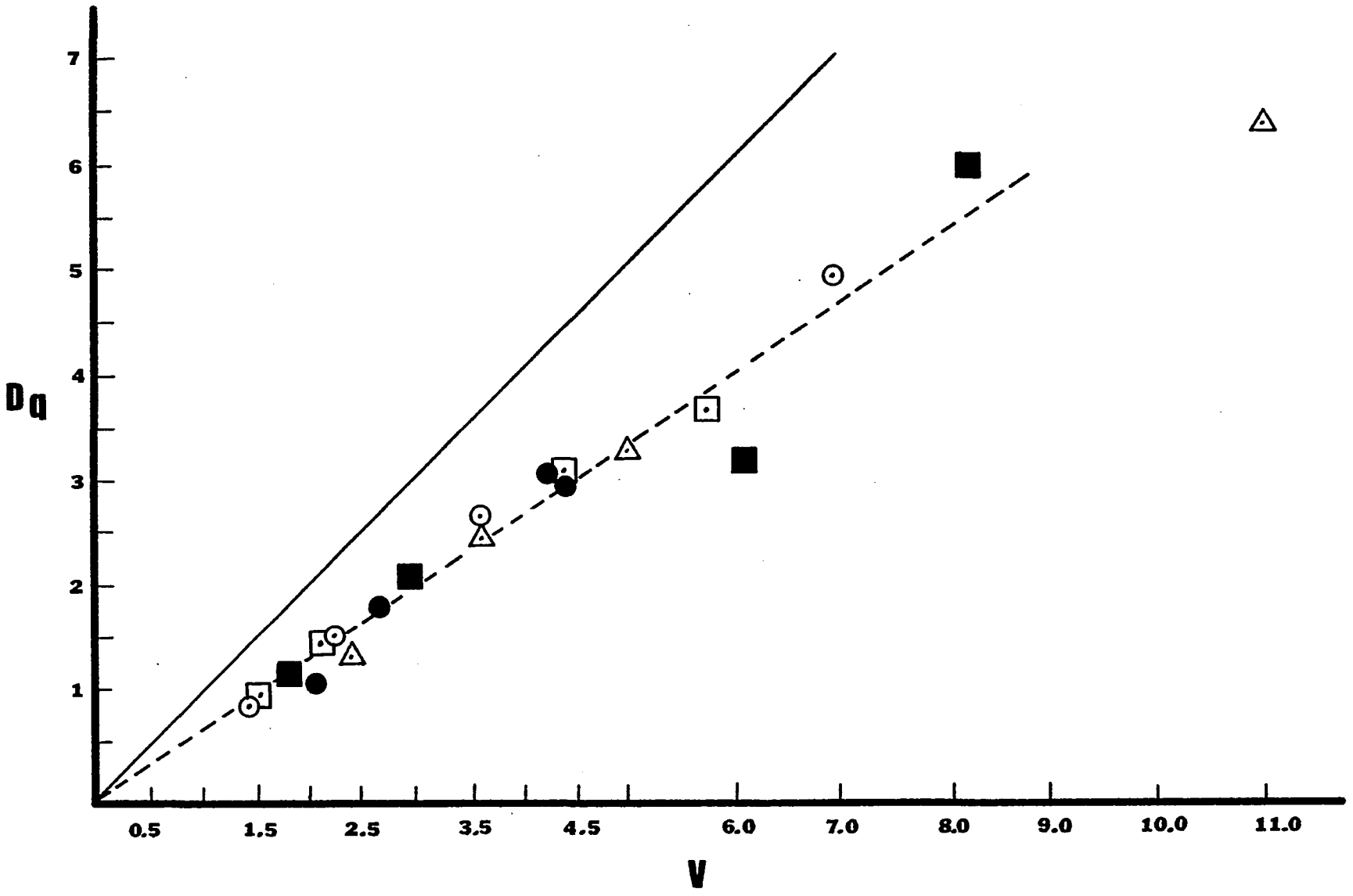
Figure 11. P^B as a function of chlorophyll per cell. P^B = mg C mg chl^{-1} day^{-1} using maximum value attained during incubations done in the morning, noon, and afternoon. CHL/CELL = µg chlorophyll a 10^{-7} Chaetoceros cells. ○ = 0.25 day^{-1}; ● = 0.70 day^{-1}; □ = 0.95 day^{-1}; and ■ = 1.20 day^{-1} growth rates.


90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
P^B
CHL/CELL

The rate of nitrogen assimilation per cell ($D\underline{q}$) did not equal the rate of nitrate uptake calculated from the disappearance of nitrate from the medium divided by the number of cells present. $D\underline{q}$ consistently underestimated the velocity of nitrate uptake ($\underline{v}$) and was approximately 66% of $\underline{v}$ under all conditions (Fig. 12). The difference between $D\underline{q}$ and $\underline{v}$ might have been an apparent one due to growth on the walls of the culture vessel in which case nitrate could be removed by such cells but the particulate nitrogen would not be measured. This possibility is unlikely to account for all of the difference, however, since when the reactors were scrubbed at the end of each experiment, little wall growth was seen. It is more likely that the difference between uptake and assimilation was due to excretion, either as dissolved organic nitrogen, which was not measured, or as ammonia lost to the atmosphere (Goldman *et al.* 1973).

The correlation between particulate nitrogen and cell number was significant for the entire set of data (Table 6; Fig. 6). The sunset and 24-hour averaged values for each category (Table 5) were not significantly different from each other or from the correlation for the entire set of data. At sunrise the correlation between nitrogen and cell number was not significant in nitrogen-limited cultures, and

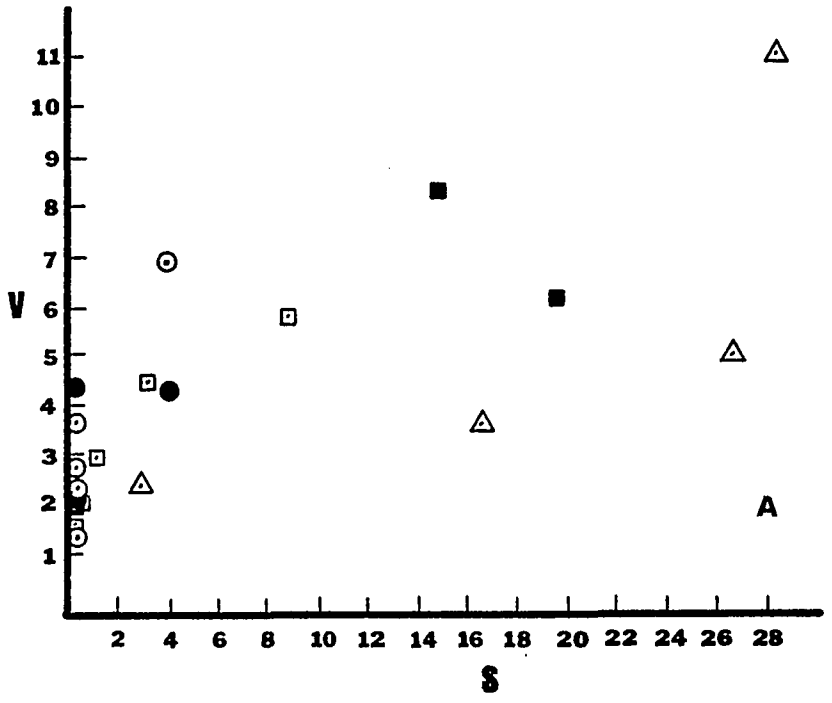
Figure 12. Comparison of velocity of nitrate uptake ($\underline{v}$) calculated by disappearance of nitrate from the culture medium (abscissa) and by the product of dilution rate and nitrogen per cell (ordinate) ($D\underline{q}$). ○ = 100% light intensity; ● = 46%; □ = 30%; ■ = 20%, and △ = 3%. Straight line represents a one-to-one correspondence; dashed line represents $D\underline{q} = 0.66\underline{v}$.

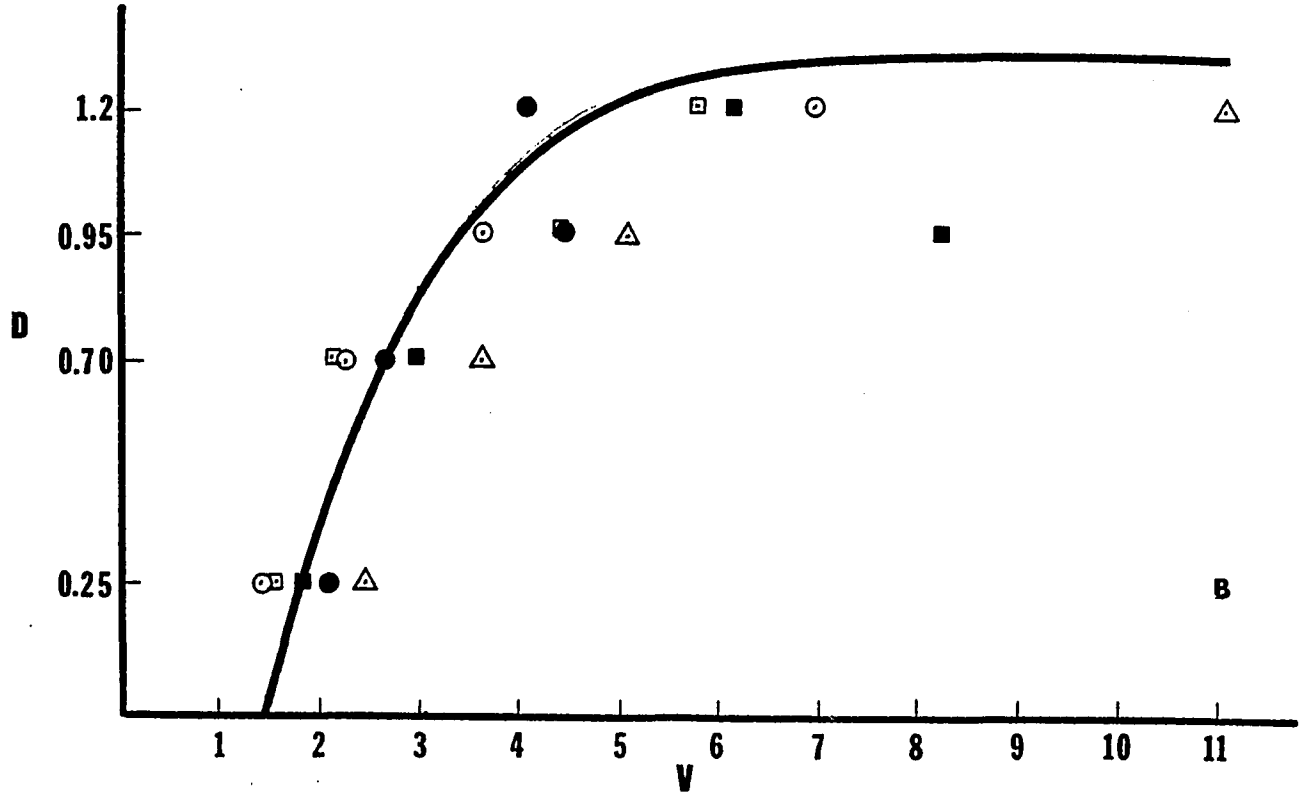

Dq
V
7
6
5
4
3
2
1
0.5
1.5
2.5
3.5
4.5
6.0
7.0
8.0
9.0
10.0
11.0

the correlation coefficient for this category was significantly different from that for the light-limited population. The high intercept of the least-squares data fit line for nitrogen-limited cultures relative to light-limited cultures (Table 6) suggests excretion of nitrogen in a form that would be retained on a glass fiber filter (Mykelstad and Haug 1972). The differences in slope show that nitrogen per cell was lower in nitrogen-limited conditions than in other conditions, although this difference was significant only at sunrise. Thus, while accumulation of nitrogen per cell at the end of each light period was similar under all conditions of the experiment, nitrogen limitation was associated with a metabolism during the dark period that resulted in changes in the cellular nitrogen content including possible excretion of complex nitrogen compounds. This excretion was affected by the light history of the cultures and was higher at high light intensities than at low light intensities. Nitrogen per cell was not related to growth rate and equation 4 was not substantiated in these experiments.

Nitrate uptake per cell ($\underline{v}$) was not related significantly to concentration of nitrate in the medium ($p > 0.10$). Nitrogen-limited cultures had no detectable nitrate and the correlation between the remaining cultures and substrate concentration was not significant (Fig. 13a; $p > 0.10$). Instead, $\underline{v}$ and growth rate were related by a hyperbola with a D_{max} of 1.42 and a half-saturation constant of 3.03 (Fig. 13b). The

Figure 13. Relationships between nitrate uptake, substrate concentration, and growth rate. A. Nitrate uptake per cell (V) in µg-at NO_3 10^{-7} Chaetoceros cells day^{-1} as a function of substrate concentration (S) in µg-at NO_3 l^{-1}. B. Growth rate (D) in dilutions per day as a function of nitrate uptake per cell (V). ○ = 100%; ● = 46%; □ = 30%; ■ = 20%; △ = 3% of incident light intensity.

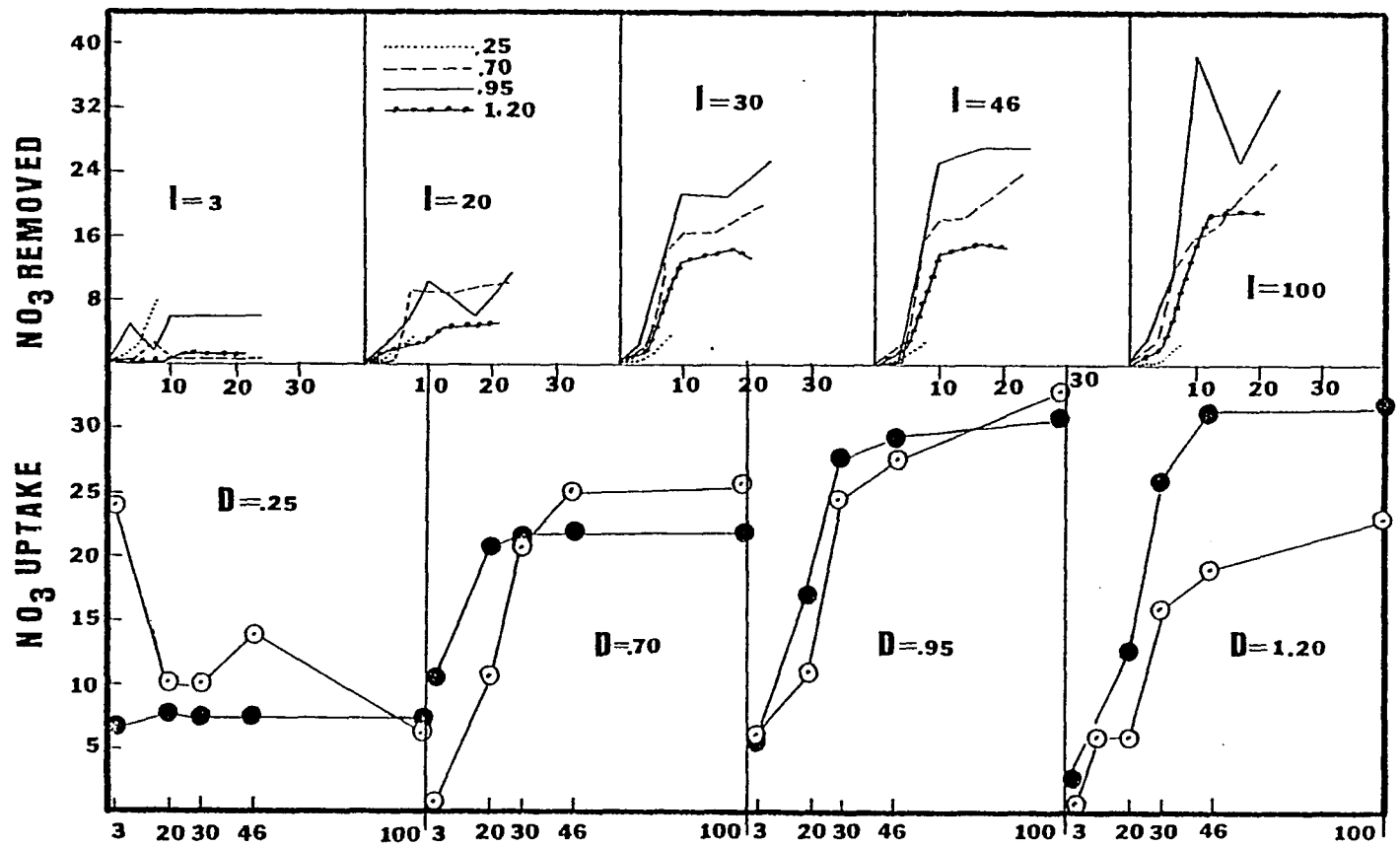

V
S
A
11
10
9
8
7
6
5
4
3
2
1
2 4 6 8 10 12 14 16 18 20 22 24 26 28


D
V
B
1.2
0.95
0.70
0.25
1 2 3 4 5 6 7 8 9 10 11

relationship was strictly empirical. One rate was plotted against another, rather than a rate being plotted against a concentration as would be required to suggest a Michaelis-Menton type of relationship. The data do show, however, that uptake rates greater than 4 µg-at NO_3 per 10^7 cells per day were not related to growth rate under any conditions.

Batch nitrate uptake experiments. At the end of each experiment continuous flow was stopped and all cultures were innoculated to bring the nutrient concentration up to approximately deep water concentration. The inoculations were done without knowing the exact value of the nutrients in the culture medium at the time of inoculation so some deviation away from deep water level occurred, especially for cultures growing at 1.2 day^{-1} where the level after spiking was 19.85 µg-at NO_3 l^{-1}. (Deep water concentration was 30.5 µg-at NO_3 l^{-1} for this experiment.) A time series of nutrient concentration in the medium was done (Fig. 14). Batch culture uptake was calculated as the total amount removed over the incubation period, averaged on a per day basis. These values were close to the values observed in continuous culture except at 0.25 and 1.2 dilutions per day (Fig. 14). The difference at 0.25 day^{-1} may have been due to the shorter incubation period for this experiment (8 hr) and the difference at 1.2 day^{-1} may have been due to

Figure 14. Removal of nitrate from the medium in batch culture, which had been spiked with nutrients to deep water level at the end of each steady state period (top), and rates of nitrate uptake in batch culture compared with rates in steady state culture (bottom). Previous growth rates are labeled for each curve in the top part of the figure. In the bottom part, ○ = batch cultures; ● = steady state.


.25
.70
.95
1.20
I=3
I=20
I=30
I=46
I=100
NO3 REMOVED
NO3 UPTAKE
D=.25
D=.70
D=.95
D=1.20

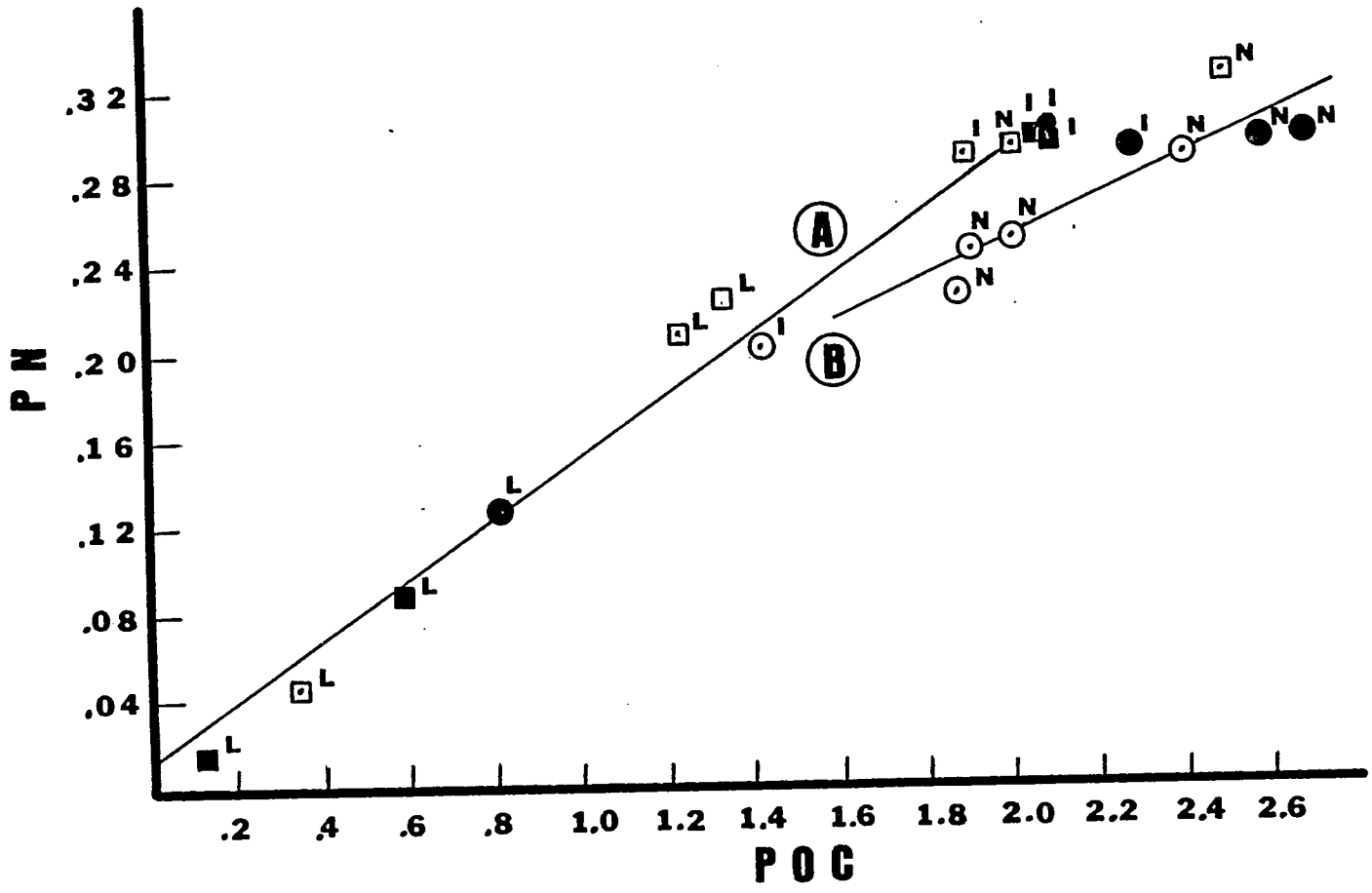
the shorter incubation period for this experiment (8 hr) and the difference at 1.2 day^{-1} may have been due to the low level of the nutrient spike.

However, it is more likely that the differences reflect nutrient history. Cells that had grown in nitrate-depleted cultures consistently showed greater uptake rates in batch culture than in steady state whereas cells from light-limited cultures generally showed greater uptake rates in steady state than in batch culture. This finding is consistent with the concept that "nitrogen-starved" cells will, when introduced into a nitrogen-rich environment, take up nitrogen more rapidly than will cells that are not nitrogen starved (Eppley and Renger 1974).

Nitrogen assimilation, nitrogen uptake, and photosynthesis. To examine the relationship between nitrogen assimilation and photosynthesis, nitrogen was first tested for correlation with carbon for the entire set of data (Fig. 15). The correlation was significant ($p < 0.01$), and a least-squares fit of the data gave a line with a slope of 0.115 N:C.

Separate tests for each category of limitation showed that the correlation between nitrogen and carbon for light-limited cultures was significantly different than the correlation for nitrogen-limited or intermediate cultures both at sunset and averaged over 24 hours (Table 6). At sunrise

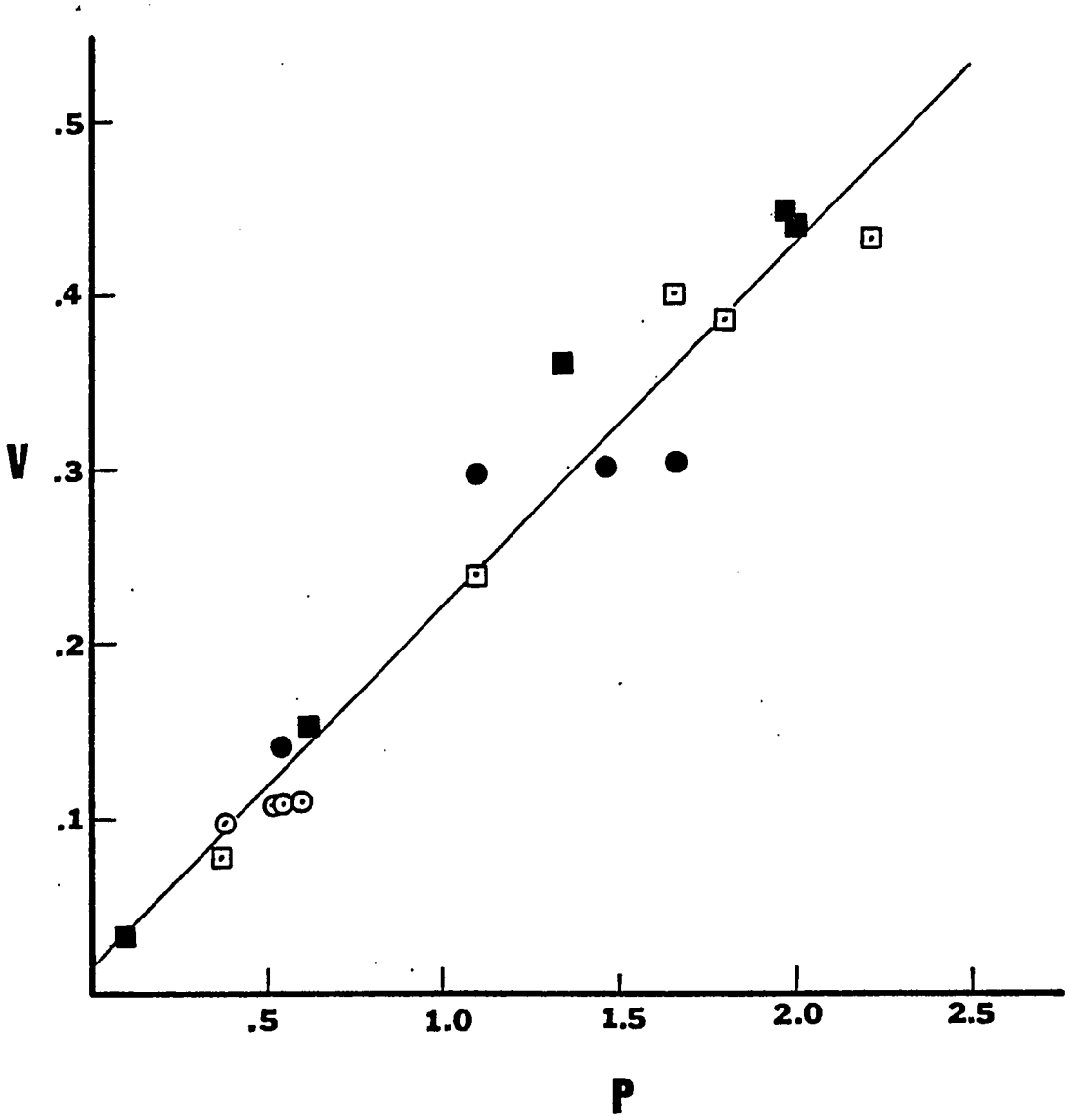
Figure 15. The correlation of particulate nitrogen (PN) and particulate organic carbon (POC) in mg l^{-1} at steady state. Two lines for the least-squares fit to the data are shown. Line A ($y = 0.111 + 0.140\ x$; $r = 0.981$) is for light-limited cultures (L). Line B ($y = 0.068 + 0.090\ x$; $r = 0.858$) is for nitrogen-limited cultures. ○ = 0.25 day^{-1}; ● = 0.70 day^{-1}; □ = 0.95 day^{-1}; and ■ = 1.20 day^{-1} growth rates.


PN
.32
.28
.24
.20
.16
.12
.08
.04
.2
.4
.6
.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
POC
A
B
L
I
N

the difference between light-limited and intermediate cultures was not significant, but the difference between nitrogen-limited and light-limited cultures was significant. The slopes of the least-squares equations showed that the N:C ratio was highest for light-limited cultures and lowest for nitrogen-limited cultures. The N:C ratio within each category of limitation was not significantly related to dilution rate ($p > 0.10$). Thus, either light limitation resulted in a reduction in photosynthesis relative to nitrogen assimilation as revealed in an increased N:C or, inversely, nitrogen limitation resulted in a reduction in nitrogen assimilation relative to photosynthesis as revealed in a decreased N:C.

Nitrate uptake, on the other hand, maintained the same relationship with photosynthesis under all conditions of the experiments ($r = 0.972$; $p < 0.001$; Fig. 16). Correlations between $\dot{V}$ and photosynthesis for the three categories of limitation were not significantly different from each other. These findings demonstrate that while the relationship between nitrogen uptake and photosynthesis was invariant, the incorporation of nitrogen into the cellular material was affected by the limiting conditions. It seemed likely that production of protein would be most affected by nitrogen limitation. Nonprotein carbon production was calculated

Figure 16. The correlation between nitrate uptake (V) in μg-at NO_3 l^{-1} day^{-1} and net photosynthesis (P) in mg POC l^{-1} day^{-1}. Nitrate uptake was calculated as the removal of nitrate from the culture medium. Net photosynthesis was calcuated as the product of dilution rate and the 24-hour averaged particulate organic carbon concentration at steady state. The equation for the line is $y = 0.0157 + 0.2048\ x$. The correlation ($r = 0.972$) was significant ($p < 0.001$).


.5
.4
V
.3
.2
.1
.5
1.0
1.5
2.0
2.5
P

as the product of (POC - 4PN) and the growth rate (Giddings 1977). In nitrogen-limited cultures 49% of total carbon production was in production of non-protein carbon while only 38 and 37% of total carbon production was non-protein carbon in intermediate and light-limited cultures, respectively. Cellular adaptation to nitrogen-limiting conditions therefore occurred not by changing the relationship between uptake and photosynthesis, but by the use to which uptake and photosynthetic products were put.

Effects of Light and Dark Periods

Particulate carbon and cell number tended to increase significantly during the day and decreased at night (Tables 2 and 7; Fig. 17). Nocturnal declines were never as great as expected on the basis of dilution rate in the absence of growth, even at 1.2 day^{-1}. The variations in particulate nitrogen were not significant (Table 2). However, all measures of biomass had the highest ratio of sunset to sunrise values at 1.2 day^{-1}, demonstrating a more pronounced diel effect at this growth rate than at lower growth rates (Table 7). This effect was most pronounced for nonprotein carbon (NPC), with nearly three times as much NPC present at sunset as at sunrise.

The light-dark periods thus appeared to have the greatest effect on

Figure 17. Particulate organic carbon (mg POC l^{-1}) as a function of time of day (sunrise or sunset) during steady state. Each column represents one dilution rate (D) and each row represents one light intensity (% of incident). Heavy black line separates nitrate-depleted cultures (to the left of the line) from cultures with nitrate in the medium (to the right of the line).

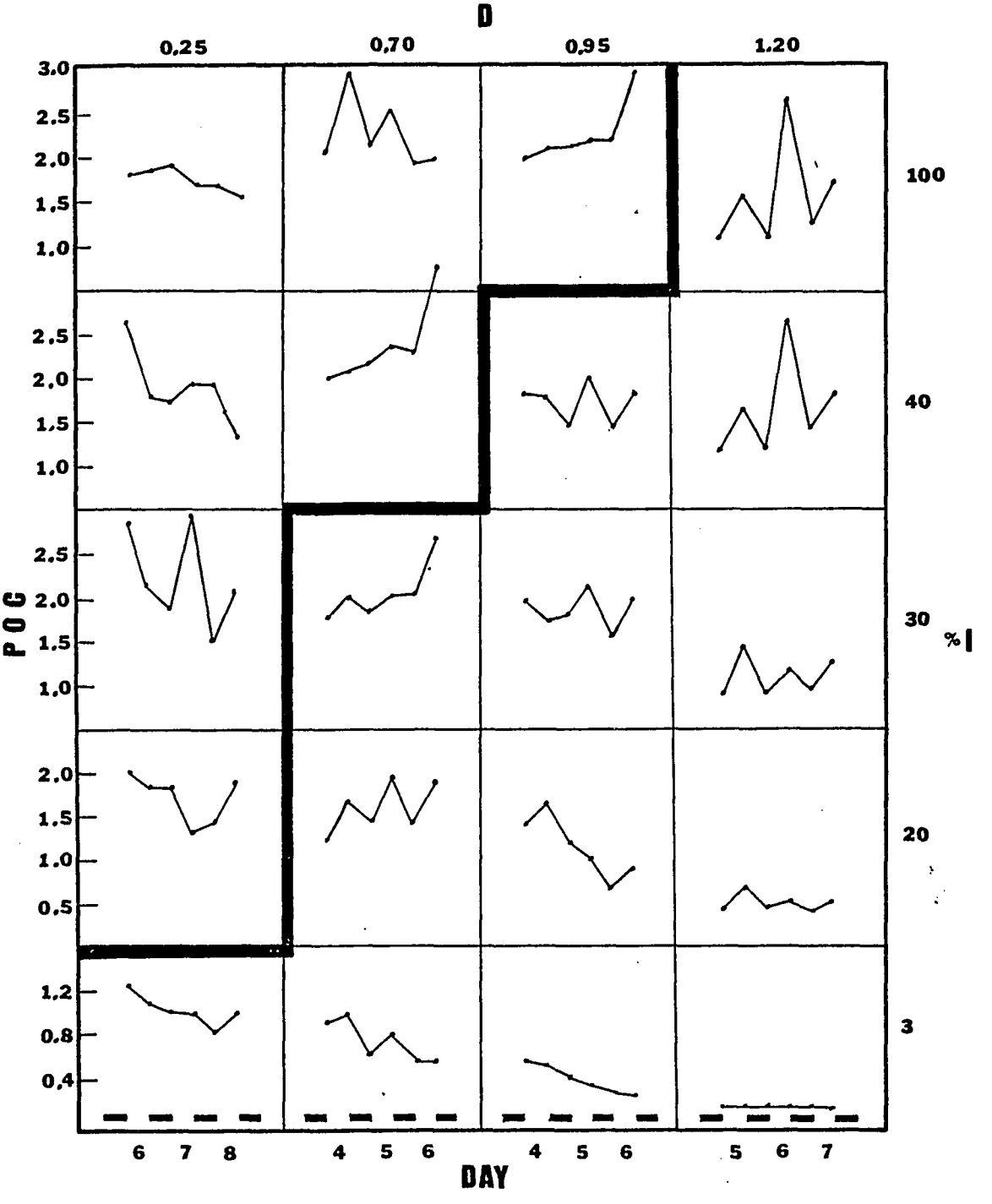

D
0,25
0,70
0,95
1.20
3.0
2.5
2.0
1.5
1.0
100
40
30
20
3
%
POG
0.5
1,2
0,8
0,4
6 7 8
4 5 6
4 5 6
5 6 7
DAY

Table 7. Ratios of sunset to sunrise values of biomass.

Dilution Rate (day^{-1})	Light Intensity (%)	Carbon	Nitrogen	NPC	Chlorophyll	Cell Number	Mean for each Light Intensity
							(carbon)
0.25	100	0.90	0.89	0.92	0.69	1.01	1.27
	46	0.84	0.84	0.84	0.73	0.96	1.23
	30	1.04	1.01	1.08	0.84	1.13	1.22
	20	0.97	0.89	1.08	1.23	1.03	1.21
	3	0.96	0.91	1.02	1.22	1.02	1.06
	X	0.94	0.91	0.99	0.94	1.03	
							(nitrogen)
0.70	100	1.28	0.99	1.64	0.82	0.96	1.03
	46	1.18	0.87	1.68	0.90	1.03	1.03
	30	1.20	0.96	1.63	0.82	1.29	1.08
	20	1.39	1.24	1.73	1.16	1.72	1.08
	3	1.10	1.00	1.42	0.88	1.49	1.01
	X	1.23	1.01	1.62	0.91	1.30	
							(chlorophyll)
0.95	100	1.18	0.98	1.52	0.65	1.19	0.88
	46	1.20	1.15	1.28	1.13	1.22	1.06
	30	1.16	1.03	1.42	1.09	1.51	1.11
	20	1.12	1.12	1.11	1.10	1.02	1.24
	3	0.93	0.84	1.05	0.96	1.22	0.98
	X	1.12	1.02	1.28	0.99	1.23	
							(cell number)
1.20	100	1.70	1.24	3.40	1.37	1.51	1.17
	46	1.68	1.26	2.92	1.48	2.08	1.32
	30	1.49	1.31	2.10	1.67	1.60	1.38
	20	1.36	1.08	2.11	1.45	1.81	1.40
	3	1.23	1.27	1.20	0.84	0.84	1.14
	X	1.49	1.23	2.35	1.36	1.57	

the coupling between uptake of carbon by photosynthesis and the incorporation of that carbon into protein. In nitrogen-limited conditions less protein was formed than in other conditions and the accumulation of non-protein photosynthetic products at the end of the day was not large. As conditions became intermediate, photosynthetic products accumulated during the day at a rate faster than they could be incorporated into protein. As conditions became light-limited, the rate of photosynthesis per volume culture was reduced and the accumulation of photosynthetic products no longer exceeded the rate at which they could be incorporated into protein, so the light-dark cycle had little effect on this coupling.

Light and dark periods also affected the nitrate concentration of the culture medium. Nitrogen-limited cultures by definition contained no detectable nitrate at any time during steady state (Table 2) and thus showed no periodic fluctions. Concentrations in light-limited and intermediate cultures followed patterns inverse to carbon and cell number, with high values at sunrise and low values at sunset. Thus nitrate uptake was independent of the diel cycle in nitrogen-limited cultures but in other cultures it was closely linked to the diel cycle and to changes in carbon biomass.

Chlorophyll decreased during the day in nitrogen-limited

conditions and increased during the day in intermediate cultures (Table 7). It decreased during the day at 3% light intensity but increased during the day in other light-limited cultures. Chlorophyll loss has long been associated with nitrogen-poor environments (Steele 1962) and may be due in part to destruction by light in high-light environments (Steeman Nielson 1975). Chlorophyll loss during the day in these experiments generally occurred under either or both conditions. Chlorophyll gain at a high light intensity (100% light; 1.2 day^{-1}) occurred in an intermediate culture where the rate of photosynthesis (Fig. 5) and particulate nitrogen biomass (Fig. 3) were maximal.

CONCLUSIONS

The results describe a system in which growth rate was under experimental control and in which all other processes were adapted to maintain that growth rate. Light limitation and nitrogen limitation resulted in different adaptations by the cells to maintain growth.

Light-limited Cultures

The major features of the light-limited cultures were decreased biomass and increased chlorophyll per cell relative to other cultures. These features directly reflect the photosynthesis-growth relationship under reduced light: photosynthesis leading to particulate carbon production in a volume of culture was decreased from that in other cultures growing at the same growth rate at higher light intensities (Fig. 5a). Carbon production per unit chlorophyll was low, but chlorophyll per cell increased so carbon production per cell remained in balance with nitrate uptake per cell and was equal to carbon production by cells growing at the same rate at higher light intensities. Consequently, carbon content per cell was not significantly affected by either light intensity or growth rate in any of the cultures. Malone et al. (1975) and Thomas and Dodson (1972) found that carbon content per cell varied with growth rate while Caperon

and Meyer (1972) found that growth rate did not affect carbon content per cell. These diverse findings suggest that the mechanism of maintaining a constant carbon per cell under widely varying environmental conditions may be a common, but not universal, result of cellular adaptation.

Nitrogen-limited Cultures

The major features of nitrogen-limited cultures were a decrease in the N:C ratio (Fig. 15) and in chlorophyll per cell (Fig. 8) relative to light-limited and intermediate cultures. Also, correlations between carbon and cells and between nitrogen and cells went from significant at the end of the light period to not significant at the end of the dark period.

The reduced N:C resulted from a lower production of protein than in other conditions and was not the result of low growth rate per se, since N:C was not related to growth rate in these experiments. From previous studies suggesting a nutrient quota (Droop 1968; Caperon and Meyer 1972a, b) a correlation between either PN per cell or the N:C ratio and growth rate had been expected. The changes in N:C in the Caperon and Meyer study (1972), however, were related to changes in carbon rather than to changes in nitrogen, and the correlation between N:C and growth rate was most likely related to an

increase in protein-bound carbon relative to total carbon with an increase in growth rate (Giddings 1977).

Metabolism of carbon and nitrogen in nitrogen-limited cultures was affected by light history. Carbon and nitrogen concentrations per cell at the end of the dark period were greater for cells growing at low light intensities than at high light intensities, demonstrating a relationship between light history and excretion or respiration rates. This relationship was not seen in light-limited or intermediate cultures.

The low chlorophyll per cell in nitrogen-limited conditions was sufficient to maintain a photosynthesis per cell equal to that in other cultures growing at the same rate and not limited by nitrogen.

Multi-factor Limitation

There was not a sharp distinction between cellular adaptations to nitrogen-limited conditions and light-limited conditions, but gradual shifts occurred along the spectrum of conditions. Droop (1973, 1974) and G-Yull Rhee (1974) examined transitions between limitation by one nutrient to limitation by another. They found gradual changes in metabolic processes even though Droop (1974) found that a mathematical model that assumed a "threshold" response -- one assuming no

influence on uptake and growth by nonlimiting nutrients -- fit the data better than a model that assumed some influence at all times. MacIssac and Dugdale (1972) hypothesized that the energetics of photosynthesis and nitrate uptake were intimately and independently related to light intensity. They assumed a maximum uptake rate under nutrient-saturating conditions to be set by photosynthesis and that the instantaneous rate was limited by substrate concentration near the surface and by light intensity at depth. The cross-over point depended on the nutrient profile but was often at 10% of surface intensity. These findings could be interpreted instead as demonstrating the interdependence of uptake and photosynthesis under widely varying environmental conditions and the adaptation of these rates to maintain certain growth rates. It is possible that growth rate was determined by nutrient supply and rate of removal throughout most of the water column in their study and that it was adaptation to these conditions that made uptake appear to be affected first by photosynthesis and then by substrate concentration.

The response of chlorophyll to light intensity and dilution rate demonstrates the effects of different kinds of limitation (Fig. 3). At a growth rate of 0.20 day^{-1} chlorophyll was highest at 3% light and decreased rapidly with increasing light. At the next highest growth rate chlorophyll was highest

at 20% light and then decreased with increasing light. At the two highest growth rates, chlorophyll was at a maximum at 46% light. Both nutrient-limitation and light-limitation were related to decreases in chlorophyll concentrations. Chlorophyll peaks at each growth rate were identified as "intermediate" cultures. The finding demonstrates clearly that it would not be possible to predict the effect of light intensity on population chlorophyll without also knowing the growth rate. Since photosynthesis per cell was related to growth rate under all experimental conditions, these chlorophyll per cell changes imply that the chlorophyll concentration itself was adjusted to give photosynthetic rates appropriate to growth rates, as suggested by Giddings (1977).

If the per cent light levels used in this study are representative of per cent light depths in a stratified water column, the chlorophyll maximum would be between the surface and the 46% light depth at high growth rates (0.95 and 1.20 day^{-1}). As growth rate decreased, the chlorophyll maximum would increase in depth (20% light depth at 0.70 D and 3% light depth at 0.25 D). This gives a different slant to the deepening of the chlorophyll maximum after a bloom, often interpreted as an increase in biomass, possibly accentuated by an increase in chlorophyll per cell (Venrick et al. 1973). If growth rate throughout the water column declines after a

bloom, the chlorophyll maximum may sink because of a decrease in optimal light (for chlorophyll) with a decrease in growth rate.

The sensitivity of chlorophyll to changes in light and nutrient conditions may be a key factor in the ability of cells to acclimate to a wide variety of environmental conditions. A growth rate may be maintained throughout nearly an entire euphotic zone by simple adjustments in chlorophyll per cell, which maintain a balance between photosynthesis and nutrient uptake, with a resulting uniform growth rate.

Optimal Conditions for the Mass Culture of Algae

One of the purposes of this study was to provide data to help optimize a mariculture system. The N:C ratio reflects the protein:carbohydrate ratio and thus the nutritional value of the phytoplankton. Since complete removal of nitrate from the deep water was associated with a decrease in N:C and a lowered production of protein-bound carbon, nitrogen-limited cultures would provide less protein per cell to shellfish herbivores than would intermediate or light-limited cultures. The maximum production of particulate nitrogen in this study was 0.36 mg PN l^{-1} day^{-1} (or 0.30 g PN m^{-2} day^{-1}) at the highest growth rate and at 100% light intensity. Maximum carbon production was 2.1 g POC m^{-2} day^{-1}. However, 13% of the nitrate was left in the water by this culture. It is

probable that an optimum production by this system would fall between the growth rate at which maximum nitrogen production occurs and the growth rate at which all nitrate is removed from the culture. The culture in these experiments that removed most nitrate and yet had a N:C ratio high enough to provide a high proportion of protein as food was the culture growing at 100% light intensity at 0.95 day^{-1} and producing nitrogen at the rate of 0.32 g PN l^{-1} day^{-1}.

APPENDIX I. Complete characteristics of each culture. Experiments were conducted in the following sequence: (1) June 26 - July 6, 1974: 0.25 · day^{-1}; (2) September 19 - 27, 1974; 1.20 · day^{-1}; (3) December 4 - 12, 1974: 0.70 · day^{-1}; (4) January 7 - 16, 1975: 0.95 · day^{-1}.

Key to tables of data:

Chaet.	=	Chaetoceros curvisetus
Other	=	Other cells in the culture: Flagellates, protozoa, ciliates, amoeba, or bacteria
POC	=	Particulate organic carbon
PN	=	Particulate nitrogen
Chl a	=	Chlorophyll a
NO_3	=	Dissolved inorganic nitrate
NO_2	=	Dissolved inorganic nitrite
NH_4	=	Ammonia
PO_4	=	Reactive phosphate
SiO_4	=	Silicate
(A)	=	A.M. Sample, taken at sunrise
(P)	=	P.M. sample, taken at sunset

When sample 1 = (A), all odd-number samples are sunrise samples. When sample 1 = (P), all odd-number samples are sunset samples.

DILUTION RATE: $0.25 \cdot day^{-1}$

LIGHT INTENSITY: 100%

Sample No.	Cell No. Chaet. ($10^7 \cdot l^{-1}$)	Cell No. Other ($10^7 \cdot l^{-1}$)	POC[1]	PN[1]	Chl a[2]	Phaco[2]	NO_3[3]	NO_2[3]	NH_4[3]	PO_4[3]	SiO_4[3]	Temp.[4]	Light[5]
1(A)	0.35	0	0.3484	0.0442	2.85	3.86	36.59	0.20	NA*	2.04	22.59	29.2	0
2(P)	4.80	0.02	1.6108	0.2938	24.90	16.60	11.64	0.35		0.58	8.34	29.5	282
3	2.35	36.70	1.3339	0.2574	13.84	6.06	8.32	0.40		0.68	6.65	25.0	0
4	3.45	1.75	1.1264	0.2562	13.15	6.95	1.84	0.20		0.57	-.10	29.2	332
5	4.30	0.85	1.9097	0.3458	24.42	5.91	< 0.40	< 0.02		0.60	1.15	25.5	0
6	5.55	3.18	3.0081	0.3037	12.44	9.35	"	"		0.61	2.87	29.2	382
7	6.12	4.42	2.4063	0.3068	15.01	11.59	"	"		0.36	1.19	25.5	0
8	7.00	2.75	2.6637	0.2944	7.56	4.44	"	"		0.38	1.17	28.8	352
9	4.90	3.10	2.8180	0.3291	6.42	4.69	"	"		0.40	1.00	26.5	0
10	5.08	3.78	1.7931	0.2277	2.81	6.57	"	"		0.45	2.32	29.5	368
11	4.02	4.12	1.8244	0.2449	4.61	4.00	"	"		0.44	1.05	25.5	0
12	4.65	3.35	1.8849	0.2460	5.41	3.22	"	"		0.54	1.27	29.5	415
13	2.82	2.25	1.9621	0.2556	5.65	3.67	"	"		0.39	-**	25.5	0
14	3.75	2.70	1.7646	0.2485	8.69	2.52	"	"		0.30	-**	29.8	263
15	3.65	4.65	1.7004	0.2432	11.38	2.62	"	"		0.34	4.09	25.5	0
16	2.45	5.00	1.5413	0.2056	5.17	2.59	"	"		0.41	0.78	27.0	259
17	3.52	6.00	1.5690	0.1883	7.11	2.58	"	"		0.46	1.00	25.0	0
18	2.32	7.22	1.7407	0.1867	3.37	1.47	"	"		0.49	1.45	29.0	358
19	1.90	8.40	1.4703	0.1684	3.95	1.34	"	"		0.50	10.07	25.5	0
20	0.95	3.35	1.0744	0.1171	1.68	2.75	"	"		0.60	1.94	29.2	328

1 ($mg \cdot l^{-1}$)
2 ($\mu g \cdot l^{-1}$)
3 ($\mu g\text{-at} \cdot l^{-1}$)
4 (°C)
5 ($ly \cdot d^{-1}$)
* No analysis
** Sample lost

DILUTION RATE: 0.25 · day^{-1}

LIGHT INTENSITY: 46%

Sample No.	Cell No. Chaet. ($10^7 \cdot l^{-1}$)	Cell No. Other ($10^7 \cdot l^{-1}$)	POC[1]	PN[1]	Chl a[2]	Phaeo[2]	NO_3[3]	NO_2[3]	NH_4[3]	PO_4[3]	SiO_4[3]	Temp.[4]	Light[5]
1(A)	0.42	0.72	0.3317	0.0482	1.54	4.00	36.31	0.20	NA*	2.00	24.02	29.2	0
2(P)	2.22	0.02	1.4691	0.2125	23.04	9.72	14.00	0.33		0.71	18.67	29.5	282
3	2.30	39.20	1.4177	0.2698	25.02	1.92	8.96	0.46		0.73	4.28	25.0	0
4	2.90	1.48	1.7149	0.2119	11.63	7.37	1.46	0.16		0.57	6.18	29.2	332
5	4.80	0.85	1.8861	0.3183	19.97	12.38	< 0.40	<0.02		0.61	3.65	25.5	0
6	4.48	0.80	2.4378	0.2681	10.32	10.24	"	"		0.69	9.97	29.2	382
7	4.12	4.25	2.2726	0.2797	10.67	9.51	"	"		0.43	1.64	25.5	0
8	3.65	3.50	2.5979	0.2705	9.46	7.17	"	"		0.42	1.27	28.5	352
9	4.30	3.18	2.6504	0.3207	13.30	5.74	"	"		0.42	1.40	26.5	0
10	3.02	3.80	1.7529	0.2195	7.92	5.52	"	"		0.37	0.85	28.5	368
11	5.65	4.02	2.6510	0.3016	12.34	6.06	"	"		0.40	0.90	25.5	0
12	4.65	3.02	1.8153	0.2300	8.62	7.00	"	"		0.50	2.34	28.2	415
13	4.78	3.60	1.7583	0.2191	10.63	4.56	"	"		0.36	-**	25.5	0
14	4.45	3.05	1.9472	0.2258	10.43	5.37	"	"		0.32	-**	29.5	263
15	4.00	4.10	1.9139	0.2201	15.10	3.93	"	"		0.32	1.35	25.5	0
16	3.80	7.18	1.3432	0.1821	8.66	4.42	"	"		0.40	1.02	26.8	259
17	3.18	7.70	1.4915	0.1535	9.75	3.09	"	"		0.50	0.95	25.0	0
18	1.58	4.18	1.4858	0.1251	5.11	2.07	"	"		0.45	1.55	28.4	358
19	0.80	4.40	0.9587	0.1171	4.49	1.58	"	"		0.54	2.18	25.5	0
20	0.45	2.10	0.7972	0.0908	4.94	3.70	"	"		0.55	1.25	29.2	328

1 ($mg \cdot l^{-1}$)
2 ($\mu g \cdot l^{-1}$)
3 ($\mu g\text{-at} \cdot l^{-1}$)
4 (°C)
5 ($ly : d^{-1}$)
* No analysis
** Sample lost

DILUTION RATE: 0.25 · day^{-1}

LIGHT INTENSITY: 30%

Sample No.	Cell No. ($10^7 \cdot l^{-1}$) Chaet.	Other	POC[1]	PN[1]	Chl a[2]	Phaeo[2]	NO_3[3]	NO_2[3]	NH_4[3]	PO_4[3]	SiO_4[3]	(°C) Temp.	Light ($ly \cdot d^{-1}$)
1(A)	0.51	0.22	0.3043	0.0466	1.34	3.10	36.04	0.20	NA*	2.10	23.07	29.2	0
2(P)	1.95	0.05	1.5595	0.2222	22.26	9.20	12.94	0.32		0.92	11.66	29.5	282
3	6.60	31.80	1.3980	0.2628	23.44	2.37	9.50	0.44		0.71	11.01	25.0	0
4	2.65	1.92	2.4040	0.2850	13.35	6.38	2.07	0.28		0.65	8.10	29.2	332
5	3.12	0.40	1.8364	0.3149	23.67	14.09	<0.40	<0.02		0.64	2.88	25.5	0
6	6.12	1.42	3.0548	0.3174	14.50	7.91	"	"		0.64	1.95	29.2	382
7	4.80	4.12	2.5804	0.3116	13.73	8.58	"	"		0.38	2.09	25.5	0
8	4.58	3.65	2.6596	0.2926	9.38	8.58	"	"		0.37	1.83	28.5	352
9	5.20	2.85	2.8354	0.3455	8.90	7.55	"	"		0.56	0.90	26.5	0
10	5.15	4.52	2.0218	0.2672	8.97	7.29	"	"		0.40	0.62	28.2	368
11	4.40	4.60	2.8838	0.3386	10.75	7.32	"	"		0.40	1.39	25.5	0
12	5.55	2.28	2.1243	0.2537	10.02	7.48	"	"		0.49	1.64	28.0	415
13	3.32	3.12	1.7888	0.2490	11.69	5.00	"	"		0.38	-**	25.5	0
14	4.85	3.00	3.0785	0.4223	9.71	6.08	"	"		0.29	-**	29.5	263
15	3.20	4.15	1.4539	0.1995	15.87	5.03	"	"		0.31	0.95	25.5	0
16	4.90	5.68	2.1590	0.2178	12.95	5.13	"	"		0.41	2.03	26.8	259
17	3.12	4.52	1.8750	0.2056	12.95	6.55	"	"		0.46	1.22	25.0	0
18	2.98	2.40	1.3785	0.1588	8.58	3.34	"	"		0.48	0.88	28.4	358
19	2.82	2.40	1.5702	0.1708	12.69	3.60	"	"		0.52	2.65	25.5	0
20	2.58	1.65	1.3002	0.1329	5.51	6.63	"	"		0.55	0.88	28.6	328

[1] ($mg \cdot l^{-1}$)
[2] ($\mu g \cdot l^{-1}$)
[3] ($\mu g\text{-at} \cdot l^{-1}$)
* No analysis
** Sample lost

DILUTION RATE: 0.25 · day^{-1}
LIGHT INTENSITY: 20%

Sample No.	Cell Number ($10^7 \cdot l^{-1}$) Chaet.	Other	POC[1]	PN[1]	Chl a[2]	Phaeo[2]	NO_3[3]	NO_2[3]	NH_4[3]	PO_4[3]	SiO_4[3]	Temp. (°C)	Light ($ly \cdot d^{-1}$)
1(A)	0.72	0.85	0.3751	0.0569	2.42	3.86	35.81	0.21	NA*	2.01	22.41	29.2	0
2(P)	5.12	0.20	1.5925	0.2221	19.24	10.60	16.26	0.28		0.86	14.16	29.5	282
3	1.60	17.30	1.0513	0.2054	15.64	5.43	13.10	0.44		0.76	20.88	25.0	0
4	0.88	1.30	1.9072	0.2468	13.06	9.69	3.79	0.30		0.66	7.83	29.2	332
5	3.88	0.35	2.0523	0.3719	22.69	11.40	0.40	0.02		0.83	3.75	25.5	0
6	4.42	2.40	2.5736	0.2877	19.11	10.52	"	"		0.70	1.00	28.4	382
7	3.30	4.00	2.1603	0.2698	15.90	11.28	"	"		0.40	1.82	25.5	0
8	4.70	2.40	2.6082	0.2853	20.25	12.78	"	"		0.37	1.94	28.2	352
9	4.08	1.68	2.7057	0.3185	15.70	13.67	"	"		0.42	1.40	26.5	0
10	5.20	1.88	2.2809	0.2685	12.82	11.33	"	"		0.40	1.15	28.0	368
11	2.60	2.15	2.0739	0.2730	10.26	7.74	"	"		0.38	0.68	25.5	0
12	3.30	2.30	1.8642	0.2405	10.55	5.64	"	"		0.48	2.68	27.8	415
13	2.75	3.30	1.8695	0.2767	9.18	7.06	"	"		0.56	-**	25.5	0
14	2.30	3.58	1.3097	0.1940	12.16	4.57	"	"		0.37	-**	29.2	263
15	2.52	3.70	1.4804	0.2668	10.77	4.74	"	"		0.38	2.66	25.5	0
16	2.85	2.45	1.9496	0.2593	20.55	5.39	"	"		0.45	0.62	26.5	259
17	3.10	2.05	1.6221	0.2657	18.23	5.53	"	"		0.44	1.18	25.0	0
18	3.85	0.68	1.8584	0.2712	22.16	12.71	"	"		0.48	0.80	28.2	358
19	5.25	1.40	2.1131	0.2963	22.00	6.06	"	"		0.48	1.90	25.5	0
20	5.75	1.40	2.1269	0.2688	28.58	7.42	"	"		0.56	0.95	28.4	328

[1] ($mg \cdot l^{-1}$)
[2] ($ug \cdot l^{-1}$)
[3] ($\mu g\text{-at} \cdot l^{-1}$)
*No analysis
** Sample lost

DILUTION RATE: 0.25 · day^{-1}

LIGHT INTENSITY: 3%

Sample No.	Cell Number ($10^7 \cdot l^{-1}$) Chaet.	Other	POC[1]	PN[1]	Chla[2]	Phaeo[2]	NO_3[3]	NO_2[3]	NH_4[3]	PO_4[3]	SiO_4[3]	Temp. (°C)	Light ($ly \cdot d^{-1}$)
1(A)	0.70	0.48	0.2962	0.0470	1.26	3.46	36.10	0.18	NA*	2.08	24.48	29.2	0
2(P)	2.05	0.02	1.0877	0.1421	9.33	3.63	22.88	0.29		1.27	21.28	29.5	282
3	1.68	7.00	0.7035	0.1252	4.64	7.70	21.37	0.30		1.12	13.10	25.0	0
4	1.28	1.50	1.1398	0.1532	10.26	6.24	10.28	0.28		0.88	11.50	29.2	332
5	3.07	1.70	1.2176	0.2200	15.43	9.09	4.60	0.39		0.55	8.22	25.5	0
6	3.50	1.72	2.0968	0.2627	12.10	12.73	<0.40	<0.02		0.62	15.33	28.2	382
7	4.02	2.25	2.2745	0.3165	14.54	8.04	"	"		0.38	1.54	25.5	0
8	4.85	1.72	2.3342	0.2983	30.25	16.74	"	"		0.38	3.60	28.0	352
9	3.60	1.42	2.1054	0.3002	28.75	11.10	"	"		0.34	2.92	26.5	0
10	2.68	0.92	1.6810	0.2415	26.50	11.43	1.10	0.01		0.37	1.29	27.8	368
11	1.65	0.98	1.2648	0.1922	16.79	5.69	3.36	0.07		0.61	2.12	25.5	0
12	2.38	0.58	1.1129	0.1585	26.36	7.47	2.60	0.14		0.57	2.66	27.5	415
13	1.40	0.80	1.0098	0.1657	21.34	6.83	5.56	-**		0.50	-**	25.5	0
14	1.65	1.48	1.0067	0.1526	18.95	8.19	6.41	-**		0.50	-**	29.0	263
15	1.45	2.00	0.8113	0.1299	18.56	2.65	8.61	0.20		0.58	3.91	25.5	0
16	1.92	1.80	1.0257	0.1579	20.22	9.19	8.50	0.29		0.62	2.50	26.2	259
17	1.95	1.25	0.8815	0.1334	18.99	3.45	10.04	0.27		0.68	4.54	25.0	0
18	3.55	0.88	1.0877	0.1839	37.98	8.88	6.62	0.40		0.60	2.58	28.0	358
19	2.62	0.55	1.0722	0.1917	24.45	8.26	8.50	0.36		0.70	5.53	25.5	0
20	4.30	0.65	1.3700	0.2430	38.72	4.07	4.07	0.25		0.60	3.23	28.2	328

[1] ($mg \cdot l^{-1}$)
[2] ($\mu g \cdot l^{-1}$)
[3] ($\mu g\text{-}at \cdot l^{-1}$)
*No analysis
** Sample lost

DILUTION RATE: 0.70 · day^{-1}

LIGHT INTENSITY: 100%

Sample No.	Cell No. ($10^7 \cdot l^{-1}$) Chaet.	Other	POC[1]	PN[1]	Chla[2]	Phaeo[2]	NO_3[3]	NO_2[3]	NH_4[3]	PO_4[3]	SiO_4[3]	Temp (°C)	Light
1(A)	0.85	0.98	0.4936	0.0868	11.06	7.07	32.62	0.26	0.40	1.64	20.00	24.0	0
2(P)	2.78	0.35	1.2816	9.2441	16.71	29.27	8.86	0.42	0.38	0.56	9.83	26.0	280
3	2.15	0.92	1.0854	0.2182	40.76	10.56	13.39	0.27	0.32	0.56	9.71	23.0	0
4	7.38	0.82	2.3664	0.2824	28.52	31.63	<0.40	<0.02	0.30	0.56	1.06	25.5	228
5	9.50	0.25	2.3088	0.3448	38.72	31.24	"	"	0.33	0.60	1.94	23.5	0
6	9.25	0.45	2.3370	0.3106	14.30	26.12	"	"	0.31	0.78	1.74	25.0	185
7	10.60	0.12	2.1498	0.2937	14.66	30.80	"	"	0.42	0.54	2.74	22.0	0
8	10.60	0.70	3.3613	0.3137	23.73	13.29	"	0.02	0.28	0.69	1.33	25.5	223
9	9.58	0.68	2.1558	0.2927	25.88	24.51	"	0.03	0.30	0.54	3.80	23.5	0
10	8.35	0.25	2.6628	0.2896	16.99	13.39	"	0.03	0.30	0.50	1.98	26.5	334
11	10.40	0.22	1.9980	0.2842	30.55	12.83	"	<0.02	0.23	0.42	2.30	23.5	0
12	10.30	0.20	2.0595	0.2579	17.41	15.67	"	0.02	0.27	0.56	2.08	25.9	265
13	8.68	0.15	2.2302	0.3287	30.57	13.30	"	0.02	0.30	0.54	2.37	24.0	0

1 (mg · l^{-1})

2 (µg · l^{-1})

3 (µg-at · l^{-1})

DILUTION RATE: 0.70 · day^{-1}

LIGHT INTENSITY: 46%

Sample No.	Cell Number[1] Chaet.	Other	POC [2]	PN [2]	Chl*a* [3]	Phaeo [3]	NO_3 [4]	NO_2 [4]	NH_4 [4]	PO_4 [4]	SiO_4 [4]	Temp. (°C)	Light ($ly \cdot d^{-1}$)
1(A)	1.08	0.72	0.5639	0.0975	8.05	8.91	31.59	0.24	0.38	1.58	19.75	24.0	0
2(P)	3.38	0.20	1.2066	0.2158	17.55	35.36	9.28	0.34	0.23	0.58	11.00	25.4	280
3	2.82	1.75	1.0995	0.2151	27.27	10.31	14.19	0.24	0.38	0.58	10.63	23.0	0
4	5.25	1.50	2.2893	0.3424	28.91	32.84	< 0.40	< 0.02	0.38	0.56	1.14	24.6	228
5	9.72	0.35	1.9686	0.3120	38.11	30.04	"	"	0.35	0.58	2.19	23.5	0
6	9.12	0.78	2.0071	0.2855	32.34	23.89	"	"	0.32	0.86	1.24	24.5	185
7	6.28	0.20	2.0482	0.3492	31.39	24.80	"	"	0.37	0.56	4.18	22.0	0
8	7.48	0.30	2.0795	0.2693	45.42	11.73	"	0.02	0.32	0.56	0.99	24.5	223
9	7.72	0.27	2.2063	0.3480	37.26	15.01	"	0.02	0.36	0.68	1.88	23.5	0
10	8.50	0.52	2.3977	0.2798	24.28	20.59	"	0.04	0.36	0.54	0.86	26.5	334
11	10.18	0.35	2.3224	0.3127	45.58	14.17	"	< 0.02	0.24	0.44	1.30	23.0	0
12	8.70	0.25	3.3025	0.3329	33.62	18.83	"	0.03	0.25	0.42	3.34	25.0	265
13	8.78	0.12	2.0381	0.3342	39.00	21.96	"	0.02	0.32	0.53	2.09	24.0	0

1 ($10^7 \cdot l^{-1}$)

2 ($mg \cdot l^{-1}$)

3 ($\mu g \cdot l^{-1}$)

4 ($\mu g\text{-at} \cdot l^{-1}$)

DILUTION RATE: 0.70 · day^{-1}

LIGHT INTENSITY: 30%

Sample No	Cell Number[1] Chaet	Other	POC[2]	PN[2]	Chl a[3]	Phaeo[3]	NO_3[4]	NO_2[4]	NH_4[4]	PO_4[4]	SiO_4[4]	Temp. (°C)	Light ($ly \cdot d^{-1}$)
1(A)	0.88	1.05	0.5295	0.0903	8.19	7.02	32.40	0.26	0.42	1.55	19.22	24.0	0
2(P)	3.05	0.15	1.0340	0.1886	4.68	35.95	11.41	0.36	0.28	0.66	10.58	25.0	280
3	2.82	0.90	1.0139	0.2065	25.44	10.37	15.50	0.24	0.35	0.67	11.06	23.0	0
4	5.65	0.55	2.0487	0.3012	34.19	28.52	<0.40	<0.02	0.32	0.68	1.22	24.0	228
5	6.25	0.48	1.7008	0.2745	48.50	21.95	"	"	0.36	0.52	2.70	23.5	0
6	7.92	0.20	2.0523	0.2981	32.98	24.59	0.18	"	0.32	1.07	0.94	24.5	185
7	6.25	0.18	1.7949	0.3113	38.86	16.56	0.39	"	0.38	0.57	3.71	22.0	0
8	11.66	0.28	2.0633	0.2679	36.89	22.04	<0.40	0.02	0.28	0.53	0.66	24.5	223
9	8.60	0.25	1.8680	0.3004	37.99	28.08	"	0.02	0.31	0.84	2.77	23.5	0
10	8.42	0.18	2.0157	0.2650	32.42	21.14	"	0.02	0.31	0.49	0.76	25.0	334
11	4.70	0.28	2.0693	0.2985	46.12	15.78	"	<0.02	0.28	0.45	2.62	23.0	0
12	11.08	0.08	2.8136	0.3407	31.47	15.80	"	0.03	0.37	0.44	1.95	24.8	265
13	7.80	0	1.9650	0.3372	41.98	16.12	"	0.02	0.32	0.55	2.08	24.0	0

1 ($10^7 \cdot l^{-1}$)

2 ($mg \cdot l^{-1}$)

3 ($\mu g \cdot l^{-1}$)

4 ($\mu g\text{-at} \cdot l^{-1}$)

DILUTION RATE: 0.70 · day^{-1}

LIGHT INTENSITY: 20%

Sample No.	Cell Number[1] Chaet.	Other	POC[2]	PN[2]	Chl *a*[3]	Phaeo[3]	NO_3[4]	NO_2[4]	NH_4[4]	PO_4[4]	SiO_4[4]	Temp (°C)	Light ($ly \cdot d^{-1}$)
1(A)	0.95	0.50	0.6130	0.1158	8.90	6.09	32.52	0.28	0.40	1.46	20.58	24.0	0
2(P)	3.60	0.10	0.9802	0.1683	11.59	26.58	9.93	0.34	0.28	0.72	10.83	24.7	280
3	3.62	0.60	0.9633	0.1742	20.56	6.98	15.91	0.24	0.38	0.72	10.31	23.0	0
4	6.10	0.85	2.0653	0.2956	29.56	22.04	<0.40	<0.02	0.34	0.47	0.72	23.8	228
5	6.35	0.45	1.4885	0.2499	43.06	15.91	"	"	0.41	0.74	2.61	24.0	0
6	8.00	0.28	1.9407	0.2975	33.65	32.16	"	"	0.33	0.68	2.60	24.2	185
7	5.00	0.22	1.2061	0.2143	46.94	19.78	1.81	"	0.51	0.69	6.04	22.0	30
8	7.68	0.28	1.7167	0.2636	55.88	13.70	<0.40	0.02	0.26	0.82	1.44	24.2	223
9	3.05	0.12	1.4906	0.2485	40.14	23.18	6.28	0.08	0.32	0.66	6.44	23.5	0
10	6.40	0.08	2.0606	0.3327	47.04	16.89	<0.40	<0.02	0.32	0.50	3.04	25.0	334
11	4.70	0.28	1.4473	0.2659	44.62	14.41	0.74	<0.02	0.22	0.48	4.74	23.0	0
12	8.38	0.12	1.9718	0.3095	49.56	14.63	<0.40	0.02	0.28	0.46	1.26	24.5	265
13	4.70	0.20	1.4209	0.2656	35.26	12.90	1.47	0.02	0.02	0.60	3.83	24.0	0

1 ($10^7 \cdot l^{-1}$)

2 ($mg \cdot l^{-1}$)

3 ($\mu g \cdot l^{-1}$)

4 ($\mu g\text{-at} \cdot l^{-1}$)

DILUTION RATE: 0.70 · day^{-1}

LIGHT INTENSITY: 3%

Sample No.	Cell Number[1] Chaet.	Other	POC[2]	PN[2]	Chla[3]	Phaeo[3]	NO_3[4]	NO_2[4]	NH_4[4]	PO_4[4]	SiO_4[4]	Temp. (°C)	Light ($ly \cdot d^{-1}$)
1(A)	0.88	0.40	0.5090	0.0950	8.13	6.39	32.26	0.27	0.39	1.48	18.95	24.0	0
2(P)	2.40	0.08	0.8843	0.1627	13.51	17.26	14.20	0.32	0.24	0.86	11.33	24.5	280
3	2.98	0.52	0.7539	0.1428	15.95	5.72	18.78	0.20	0.38	0.92	12.32	23.0	0
4	5.75	0.75	1.8381	0.2900	27.74	22.35	<0.40	<0.02	0.38	0.48	1.86	23.5	228
5	5.35	0.18	1.5228	0.2722	43.41	19.61	"	"	0.35	0.56	3.56	24.0	0
6	4.80	0.18	1.5767	0.2729	41.79	19.54	3.46	"	0.44	0.67	8.26	24.2	185
7	2.32	0.18	0.9622	0.1899	32.80	10.96	13.02	0.07	0.48	0.84	12.70	22.0	0
8	3.35	0.50	1.0497	0.1680	32.09	6.55	13.44	0.17	0.48	0.99	11.61	24.0	223
9	1.38	0	0.6600	0.1162	22.52	8.95	15.98	0.15	0.31	0.97	15.49	23.5	0
10	2.72	0	0.7901	0.1328	20.31	6.01	16.40	0.21	0.41	0.86	15.93	25.0	334
11	2.05	0.08	0.5785	0.1044	22.64	4.71	20.98	0.14	0.35	1.16	16.72	23.0	0
12	2.35	0.05	0.5919	0.1081	15.84	5.07	19.67	0.24	0.21	1.09	13.28	24.5	265
13	1.48	0.15	0.3912	0.0701	10.50	2.73	16.58	0.16	0.37	0.98	14.29	24.0	0

1 ($10^7 \cdot l^{-1}$)

2 ($mg \cdot l^{-1}$)

3 ($\mu g \cdot l^{-1}$)

4 ($\mu g\text{-at} \cdot l^{-1}$)

DILUTION RATE: 0.95 · day^{-1}

LIGHT INTENSITY: 100%

Sample No.	Cell Number[1] Chaet.	Other	POC[2]	PN[2]	Chl *a*[3]	Phaeo[3]	NO_3[4]	NO_2[4]	NH_4[4]	PO_4[4]	SiO_4[4]	Temp (°C)	Light ($ly \cdot d^{-1}$)
1(P)	1.08	0.22	1.2144	0.1824	13.55	14.14	25.85	0.23	1.05	1.08	20.0	24.5	-
2(A)	2.40	0.12	0.7516	0.1116	11.68	4.69	26.08	0.20	0.44	1.07	19.7	22.5	0
3(P)	5.78	0	2.2580	0.3532	34.75	35.81	1.20	0.24	1.28	0.42	4.6	24.0	296
4	5.05	0	1.4896	0.2916	39.06	99.96	8.93	0.10	0.48	0.56	7.7	21.2	0
5	5.50	0.18	2.2140	0.3196	29.74	21.78	<0.04	0.04	0.42	0.56	1.0	24.0	257
6	7.40	0.05	1.7704	0.2952	32.34	15.71	0.24	0.34	1.12	0.41	3.2	22.2	0
7	9.88	0.05	1.4260	0.1696	25.51	19.42	<0.04	0.08	1.66	0.48	1.6	25.2	248
8	6.92	0.20	2.0356	0.3188	29.00	23.55	"	<0.02	0.32	0.58	2.2	22.0	0
9	8.82	0.22	2.1072	0.3032	16.69	24.55	"	0.04	0.32	0.59	1.8	24.0	335
10	7.45	0.50	2.1480	0.3204	15.20	67.22	"	"	0.36	0.64	2.2	22.2	0
11	7.55	0.30	2.2032	0.3296	17.99	23.73	"	"	0.36	0.30	1.5	24.6	253
12	6.78	0.05	2.2048	0.3676	38.22	22.08	"	"	0.40	0.30	3.1	22.5	0
13	8.95	0.25	3.2008	0.3508	18.92	18.06	"	"	0.22	0.42	1.4	25.2	303
14	7.70	0.18	2.3692	0.3808	32.22	10.16	"	<0.02	0.28	0.40	2.0	23.2	0

1 ($10^7 \cdot l^{-1}$)

2 ($mg \cdot l^{-1}$)

3 ($\mu g \cdot l^{-1}$)

4 ($\mu g\text{-at} \cdot l^{-1}$)

DILUTION RATE: 0.95 · day^{-1}

LIGHT INTENSITY: 46%

Sample No.	Cell Number[1] Chaet	Other	POC[2]	PN[2]	Chl *a*[3]	Phaeo[3]	NO_3[4]	NO_2[4]	NH_4[4]	PO_4[4]	SiO_4[4]	Temp (°C)	Light ($ly \cdot d^{-1}$)
1(P)	2.02	0.12	1.1000	0.1620	18.92	10.88	25.42	0.25	0.42	1.01	20.2	24.1	-
2(A)	2.30	0.15	0.7516	0.1120	13.28	6.79	26.44	0.06	0.94	1.06	21.0	22.5	0
3(P)	4.98	0.05	1.6948	0.3024	11.99	24.97	2.92	0.29	1.46	0.31	5.4	23.4	296
4	4.80	0.02	1.9028	0.3508	25.15	10.24	10.54	0.11	0.44	0.52	10.0	21.5	0
5	6.02	0.05	2.0336	0.3164	39.12	26.70	0.40	0.02	0.49	0.56	0.9	23.2	257
6	5.03	0.08	1.3524	0.2500	30.66	11.22	"	0.34	1.18	0.41	6.2	22.4	0
7	8.38	0.05	2.9844	0.3748	41.16	24.80	"	0.26	1.41	0.44	1.2	24.2	248
8	7.50	0.20	1.8880	0.3072	37.27	28.33	"	0.02	0.36	0.56	5.5	22.2	0
9	8.10	0.08	1.7824	0.2628	35.82	23.36	"	0.02	0.40	0.62	2.3	23.0	335
10	5.52	0.18	1.4844	0.2312	33.95	12.67	"	0.02	0.38	0.80	8.2	22.2	0
11	6.28	0.25	2.0596	0.3236	31.40	29.45	"	0.05	0.31	0.30	1.2	23.5	253
12	3.98	0	1.4052	0.2284	43.62	10.85	9.68	0.22	0.38	0.30	7.8	22.5	0
13	6.32	0.12	1.8852	0.2976	40.90	23.25	1.04	0.04	0.22	0.44	3.6	24.1	303
14	5.42	0.08	1.4276	0.2468	44.10	51.93	10.62	0.16	0.33	0.42	8.3	23.0	0

1 ($10^7 \cdot l^{-1}$)

2 ($mg \cdot l^{-1}$)

3 ($\mu g \cdot l^{-1}$)

4 ($\mu g\text{-at} \cdot l^{-1}$)

DILUTION RATE: 0.95 · day^{-1}

LIGHT INTENSITY: 30%

Sample No.	Cell No.[1] Chaet.	Cell No.[1] Other	POC[2]	PN[2]	Chl a[3]	Phaeo[3]	NO_3[4]	NO_2[4]	NH_4[4]	PO_4[4]	SiO_4[4]	Temp (°C)	Light ($ly \cdot d^{-1}$)
1(P)	2.32	0.02	1.0160	0.1512	12.26	11.41	25.62	0.22	1.18	0.99	20.8	23.5	-
2(A)	1.95	0.02	0.7872	0.1372	11.84	5.46	26.32	0.07	1.19	1.05	20.6	22.5	0
3(P)	4.45	0.08	2.2044	0.3356	26.25	30.87	4.35	0.32	2.31	0.54	4.8	23.0	296
4	2.55	0.08	1.4680	0.2616	36.22	6.42	11.66	0.14	0.46	0.54	10.4	21.7	0
5	6.08	0	2.0680	0.3220	37.72	21.26	<0.40	0.01	0.28	0.07	1.3	23.1	257
6	4.32	0.02	1.2700	0.2184	24.68	10.80	"	0.02	0.28	0.44	6.2	22.6	0
7	8.62	0.08	2.4148	0.3332	26.73	35.28	"	0.02	0.34	0.51	1.2	23.8	248
8	6.98	0.05	1.9228	0.3264	34.44	20.49	"	0.04	0.38	0.62	6.2	22.2	0
9	7.78	0.12	1.7132	0.2568	29.40	43.30	"	<0.02	0.36	0.62	1.5	23.0	335
10	4.85	0.10	1.7952	0.2984	34.24	11.56	5.79	0.40	0.42	0.65	6.3	22.2	0
11	5.86	0.28	2.3484	0.3460	43.30	14.43	1.36	0.06	0.34	0.28	1.6	23.5	253
12	2.05	0.40	1.5276	0.2376	31.18	7.48	11.04	0.21	0.36	0.36	8.9	22.5	0
13	6.60	1.12	2.0488	0.2864	36.12	8.90	2.10	0.12	0.25	0.44	1.1	24.0	303
14	3.82	0.05	1.5110	0.2248	28.81	7.00	12.10	0.12	0.28	0.50	2.5	22.8	0

1 ($10^7 \cdot l^{-1}$)

2 ($mg \cdot l^{-1}$)

3 ($\mu g \cdot l^{-1}$)

4 ($\mu g\text{-at} \cdot l^{-1}$)

DILUTION RATE: 0.95 · day^{-1}

LIGHT INTENSITY: 20%

Sample No.	Cell Number[1] Chaet.	Other	POC[2]	PN[2]	Chl *a*[3]	Phaeo[3]	NO_3[4]	NO_2[4]	NH_4[4]	PO_4[4]	SIO_4[4]	Temp. (°C)	Light ($ly \cdot d^{-1}$)
1(P)	1.32	0.22	1.0135	0.1464	14.96	13.60	25.20	0.25	0.42	1.06	20.7	23.2	-
2(A)	1.55	0.02	0.7668	0.1308	11.51	4.64	26.26	0.14	1.34	1.06	20.4	22.5	0
3(P)	3.50	0.10	1.9992	0.3132	31.44	27.02	4.84	0.30	2.40	0.52	6.0	22.0	296
4	4.05	0	1.3116	0.2140	37.24	16.72	12.56	0.11	0.50	0.53	11.4	22.0	0
5	6.00	0.08	2.1572	0.3608	28.36	44.14	2.36	0.23	2.04	0.52	2.0	23.0	257
6	3.15	0.05	0.7708	0.1208	27.60	6.58	12.15	0.06	1.37	0.40	12.9	22.7	0
7	5.58	0	1.8720	0.2972	25.44	5.70	2.32	0.06	1.38	0.41	1.7	23.6	248
8	4.02	0	1.4492	0.2316	31.66	16.03	11.72	0.12	0.44	0.56	10.0	22.2	0
9	3.98	0	1.6976	0.2652	37.80	22.68	8.62	0.22	0.40	0.60	7.4	23.0	335
10	2.58	0	1.2016	0.2076	22.85	6.55	16.93	0.17	0.26	0.75	13.6	22.2	0
11	1.32	0.15	1.0796	0.1664	26.30	11.12	13.72	0.30	0.33	0.30	11.2	23.2	253
12	1.65	0.02	0.6678	0.1228	22.32	8.15	21.10	0.22	0.40	0.60	16.6	22.5	0
13	2.88	0.08	0.9370	0.1986	20.72	8.15	15.39	0.31	0.30	0.60	13.0	23.8	303
14	1.15	0.05	0.6496	0.1144	15.70	5.26	22.90	0.12	0.36	1.00	17.0	22.5	0

1 ($10^7 \cdot l^{-1}$)

2 ($mg \cdot l^{-1}$)

3 ($\mu g \cdot l^{-1}$)

4 ($\mu g\text{-at} \cdot l^{-1}$)

DILUTION RATE: 0.95 · day^{-1}

LIGHT INTENSITY: 3%

Sample No.	Cell Number[1] Chaet.	Other	POC[2]	PN[2]	Chl a[3]	Phaeo[3]	NO_3[4]	NO_2[4]	NH_4[4]	PO_4[4]	SiO_4[4]	Temp (°C)	Light ($ly \cdot d^{-1}$)
1(P)	1.98	0	1.0584	0.1528	3.81	2.61	24.75	0.08	0.98	1.02	20.2	23.0	-
2(A)	1.45	0.02	1.0588	0.1976	13.60	5.71	25.94	0.16	0.44	1.06	20.2	22.5	0
3(P)	3.60	0	2.0696	0.3336	24.67	23.20	4.07	0.34	2.10	0.52	4.0	22.5	296
4	3.85	0.02	1.4992	0.2724	34.30	10.18	11.90	0.08	0.41	0.52	11.0	22.2	0
5	1.98	0	2.3572	0.3516	30.02	11.46	14.01	0.28	1.36	0.56	14.8	22.8	257
6	1.32	0.05	1.3756	0.2360	16.58	3.98	20.87	0.20	0.36	1.02	19.4	22.8	0
7	2.30	0	0.9430	0.1558	23.86	5.56	20.36	0.07	1.32	1.04	15.8	23.2	248
8	1.40	0	0.5448	0.0884	11.40	5.94	22.88	0.16	0.46	1.84	20.3	22.5	0
9	1.28	0	0.5276	0.0724	10.71	3.15	24.80	0.20	0.42	1.43	17.5	22.5	335
10	0.78	0.02	0.4124	0.0494	6.27	1.95	26.90	0.17	0.42	1.70	19.2	22.1	0
11	0.85	0.08	0.3626	0.0406	6.76	2.39	27.30	0.18	0.42	1.38	19.2	23.0	253
12	0.28	0	0.2411	0.0305	3.57	1.01	29.01	0.12	0.33	1.40	19.2	22.5	0
13	0.75	0.08	0.2266	0.0281	2.97	1.13	29.41	0.12	0.44	1.57	18.8	23.5	303
14	0.28	0	0.2202	0.0301	1.62	0.55	31.28	0.11	0.28	1.68	19.9	22.5	0

1 ($10^7 \cdot l^{-1}$)

2 ($mg \cdot l^{-1}$)

3 ($\mu g \cdot l^{-1}$)

4 ($\mu g\text{-at} \cdot l^{-1}$)

DILUTION RATE: 1.2 · day^{-1}

LIGHT INTENSITY: 100%

Sample No.	Cell No.[1] Chaet.	Cell No.[1] Other	POC[2]	PN[2]	Chl a[3]	Phaeo[3]	NO_3[4]	NO_2[4]	NH_4[4]	PO_4[4]	SiO_4[4]	Temp. (°C)	Light ($ly \cdot d^{-1}$)
1(A)	0.85	1.22	0.7488	0.0656	2.74	4.42	34.02	0.23	0.54	1.82	20.8	24.5	0
2(P)	2.34	0.18	0.7478	0.1120	9.49	4.72	20.24	0.32	0.70	1.05	13.6	27.1	435
3	0.40	6.62	0.4675	0.6959	1.59	3.06	23.83	0.09	0.87	1.29	14.9	24.8	0
4	1.00	1.12	0.5999	0.0879	5.23	4.00	18.48	0.19	0.26	0.98	15.8	27.2	438
5	1.02	3.25	0.4289	0.0700	4.55	2.57	21.56	0.26	0.40	1.17	16.0	24.5	0
6	1.35	0.92	1.0036	1.1661	17.56	4.99	10.52	0.30	0.46	0.80	10.5	27.5	491
7	2.30	1.42	0.5887	0.1188	13.81	2.92	17.62	0.28	0.67	1.19	13.9	24.3	0
8	3.68	0.48	1.6938	0.2450	24.25	9.16	4.60	0.32	0.71	0.84	7.1	27.3	453
9	1.78	0.25	1.1320	0.2251	11.96	7.34	6.50	0.46	0.95	0.82	11.7	24.4	0
10	4.35	0.52	1.6761	0.2727	23.20	9.88	0.94	0.07	0.96	0.95	6.0	28.2	547
11	5.20	0.72	1.1822	0.2385	20.80	6.85	8.96	0.22	0.34	0.58	9.0	24.8	0
12	6.35	0.15	2.7271	0.3553	33.05	5.60	0.31	0.03	0.52	0.48	3.6	28.0	508
13	2.85	0.20	1.3882	0.2644	28.08	7.76	6.98	0.22	0.48	0.36	8.7	24.7	0
14	5.13	0.10	1.8765	0.2715	27.32	10.28	0.26	0.08	0.38	0.84	1.2	26.0	226
15	4.22	0.20	1.2363	0.2184	24.74	6.97	0.26	0.06	0.38	0.41	6.4	24.2	0

1 ($10^7 \cdot l^{-1}$)

2 ($mg \cdot l^{-1}$)

3 ($\mu g \cdot l^{-1}$)

4 ($\mu g\text{-at} \cdot l^{-1}$)

DILUTION RATE: 1.2 · day^{-1}

LIGHT INTENSITY: 46%

Sample No.	Cell No.[1] Chaet.	Other	POC[2]	PN[2]	Chl *a*[3]	Phaeo[3]	NO_3[4]	NO_2[4]	NH_4[4]	PO_4[4]	SiO_4[4]	Temp (°C)	Light ($ly \cdot d^{-1}$)
1(A)	1.08	0.62	1.5120	0.0802	2.90	3.83	33.96	0.19	0.35	1.77	21.5	24.5	0
2(P)	3.82	0.30	1.4559	0.1775	14.03	11.52	13.52	0.27	0.48	0.54	9.6	26.5	435
3	0.30	12.78	0.7876	0.1062	4.99	2.55	20.13	0.24	0.82	1.10	14.0	24.7	0
4	0.25	5.12	1.1340	0.1334	7.20	6.78	16.06	0.24	0.48	0.94	15.2	26.8	438
5	0.52	1.40	0.5559	0.1007	7.79	4.02	19.45	0.32	0.46	0.97	15.2	24.5	0
6	2.32	0.68	1.1447	0.2028	26.57	7.27	7.67	0.42	0.36	0.64	7.4	26.5	491
7	1.55	0.82	0.7890	0.1481	16.73	4.87	13.94	0.35	0.46	1.06	12.6	24.3	0
8	4.95	0.30	1.2646	0.2140	21.06	11.91	3.74	0.20	0.57	0.82	5.3	26.6	453
9	2.62	0.50	1.5181	0.2195	16.36	3.24	7.16	0.40	0.44	0.89	10.4	24.4	0
10	8.25	0.32	1.7166	0.2678	34.19	8.76	1.48	0.07	0.38	0.88	2.2	26.8	547
11	4.30	0.25	1.1878	0.2200	23.88	6.92	10.12	0.28	0.62	0.64	8.8	24.7	0
12	8.50	0.12	2.7156	0.3275	31.26	17.61	0.26	0.08	0.69	0.50	2.4	26.5	508
13	4.42	0.28	1.4007	0.2586	28.83	10.20	6.90	0.14	0.64	0.41	12.6	24.7	0
14	8.28	0.20	1.8759	0.2862	36.84	20.90	0.44	0.14	0.40	0.51	1.4	25.3	226
15	4.98	0.12	1.2954	0.2510	25.00	-	7.24	0.24	0.28	0.37	8.6	24.2	0

1 ($10^7 \cdot l^{-1}$)

2 ($mg \cdot l^{-1}$)

3 ($\mu g \cdot l^{-1}$)

4 ($\mu g\text{-at} \cdot l^{-1}$)

DILUTION RATE: 1.2 · day^{-1}

LIGHT INTENSITY: 30%

Sample No.	Cell No.[1] Chaet.	Other	POC[2]	PN[2]	Chl a[3]	Phaeo[3]	NO_3[4]	NO_2[4]	NH_4[4]	PO_4[4]	SiO_4[4]	Temp. (°C)	Light[1] ($ly \cdot d^{-1}$)
1(A)	0.90	0.50	2.1277	0.0698	2.30	4.06	34.30	0.20	0.36	1.78	20.0	24.5	0
2(P)	2.30	0.22	1.7060	0.1817	14.46	10.81	12.44	0.27	0.39	0.46	8.7	26.1	435
3	1.35	26.20	1.0107	0.1269	5.53	2.82	19.61	0.22	0.72	1.02	12.8	24.7	0
4	0.65	6.28	0.8266	0.1094	8.18	6.87	16.06	0.27	0.39	0.92	13.0	26.5	438
5	0.85	1.18	0.5910	0.0873	7.53	3.69	20.18	0.33	0.55	0.88	15.6	24.5	0
6	3.48	0.42	0.9794	0.1601	19.06	8.28	10.07	0.46	0.60	0.84	8.1	26.3	491
7	1.10	0.68	0.5882	0.1075	15.25	3.35	17.52	0.33	0.52	1.24	14.4	24.3	0
8	4.35	0.25	1.1283	0.1803	17.34	9.21	6.05	0.64	0.38	0.94	6.2	26.4	453
9	2.60	0.40	0.8796	0.1735	11.17	4.54	12.25	0.44	0.46	1.08	14.2	24.4	0
10	5.72	0.35	1.5128	0.2479	28.48	15.71	5.62	0.19	0.56	0.91	2.0	26.3	547
11	3.62	0.25	0.8712	0.1657	18.35	4.04	15.10	0.28	0.44	0.96	10.4	24.7	0
12	4.85	0.02	1.2167	0.2050	30.89	12.17	1.76	0.08	0.44	0.66	4.2	26.3	508
13	2.58	0.02	0.9784	0.1843	24.90	5.85	11.62	0.16	0.64	0.56	10.2	24.6	0
14	5.72	0.10	1.3503	0.2327	31.64	7.90	6.30	0.26	0.27	0.56	3.2	25.2	226
15	1.92	0.10	0.8892	0.1585	16.96	5.15	14.44	0.22	0.34	0.66	12.0	24.2	0

1 ($10^7 \cdot l^{-1}$)

2 ($mg \cdot l^{-1}$)

3 ($\mu g \cdot l^{-1}$)

4 ($\mu g\text{-at} \cdot l^{-1}$)

DILUTION RATE: 1.2 · day^{-1}

LIGHT INTENSITY: 20%

Sample No.	Cell No.[1] Chaet.	Other	POC[2]	PN[2]	Chl a[3]	Phaeo[3]	NO_3[4]	NO_2[4]	NH_4[4]	PO_4[4]	SiO_4[4]	Temp (°C)	Light ($ly \cdot d^{-1}$)
1(A)	0.65	0.60	1.2077	0.0613	2.83	3.47	34.03	0.22	0.44	1.76	20.2	24.5	0
2(P)	4.95	0.32	1.3575	0.1826	15.67	9.11	12.82	0.26	0.52	0.55	10.9	25.8	435
3	0.70	23.15	0.7430	0.1088	5.08	2.89	19.80	0.25	1.19	1.11	14.4	24.6	0
4	0.52	6.20	0.7810	0.1095	10.81	5.44	17.64	0.24	0.52	0.96	15.8	26.2	438
5	0.62	0.62	0.4197	0.0676	9.23	2.28	22.19	0.21	0.44	0.92	16.0	24.5	0
6	1.52	0.60	0.7747	0.1299	19.57	7.84	14.40	0.44	0.46	1.00	10.6	26.0	491
7	1.08	0.62	0.4660	0.0790	11.67	4.00	20.27	0.26	0.62	1.36	16.6	24.3	0
8	1.98	0.05	0.9848	0.1696	15.90	6.55	13.15	0.24	0.44	1.06	12.4	26.1	453
9	0.82	0.25	0.4964	0.0993	11.94	3.62	17.60	0.24	0.52	1.34	16.3	24.4	0
10	2.50	0.35	0.7264	0.1263	16.78	6.46	15.45	0.22	0.39	1.26	11.1	26.0	547
11	1.22	0.18	0.4197	0.0744	7.49	2.10	22.23	0.12	0.57	1.37	14.6	24.7	0
12	2.82	0	0.5631	0.0922	12.67	2.49	19.59	0.23	0.44	1.10	12.0	26.0	508
13	1.42	0.10	0.4020	0.0658	7.35	1.72	22.42	0.14	0.80	1.22	15.3	24.6	0
14	5.72	0.10	0.5043	0.0400	9.34	2.09	22.19	0.22	0.79	2.56	14.6	25.1	226
15	0.78	0.08	0.2990	0.0502	5.43	1.28	23.38	0.10	0.27	1.46	17.2	24.2	0

1 ($10^7 \cdot l^{-1}$)

2 ($mg \cdot l^{-1}$)

3 ($\mu g \cdot l^{-1}$)

4 ($\mu g\text{-at} \cdot l^{-1}$)

DILUTION RATE: 1.2 · day^{-1}

LIGHT INTENSITY: 3%

Sample No.	Cell No.[1] Chaet.	Other	POC[2]	PN[2]	Chl *a*[3]	Phaeo[3]	NO_3[4]	NO_2[4]	NH_4[4]	PO_4[4]	SiO_4[4]	Temp (°C)	Light ($ly \cdot d^{-1}$)
1(A)	0.92	0.62	0.9264	0.0707	2.76	4.00	34.06	0.16	0.42	1.76	18.8	24.4	0
2(P)	3.88	0.20	1.2507	0.1914	15.69	8.21	13.57	0.30	0.40	0.60	10.6	25.1	435
3	0.40	18.85	0.6385	0.1029	5.36	2.77	20.42	0.20	0.82	1.08	15.6	24.5	0
4	0.68	4.65	0.4608	0.0696	5.81	2.57	22.60	0.22	0.59	1.07	18.0	25.9	438
5	0.15	0.72	0.2603	0.0388	2.64	3.34	25.95	0.10	0.68	1.58	20.0	24.5	0
6	0.12	0.80	0.2389	0.0350	3.02	3.90	26.22	0.16	0.86	1.58	18.4	25.8	491
7	0.30	0.25	0.1571	0.0201	1.82	2.36	27.62	0.10	0.54	1.90	19.2	24.2	0
8	0.40	0.05	0.2282	0.0216	2.31	2.52	27.36	0.26	0.82	1.85	19.1	25.8	453
9	0.18	0.20	0.1344	0.0173	0.68	1.68	28.10	0.07	0.66	1.85	20.0	24.3	0
10	0.25	0.18	0.1490	0.0196	0.71	1.55	27.82	0.06	0.34	1.86	20.9	25.8	547
11	0.18	0.10	0.1055	0.0137	0.48	1.11	28.97	0.04	0.35	1.76	19.9	24.6	0
12	0.18	0.15	0.1233	0.0126	0.38	0.66	29.04	0.09	0.64	1.74	23.3	25.5	508
13	0.22	0.08	0.0811	0.0071	0.27	0.43	28.20	0.14	1.07	1.62	21.2	24.5	0
14	-	-	0.1217	0.0160	0.12	0.38	28.45	0.09	0.51	1.68	27.4	24.9	226
15	0.01	-	0.0840	0.0047	0.11	0.27	28.42	0.04	0.27	1.70	21.7	24.2	0

1 ($10^7 \cdot l^{-1}$)

2 ($mg \cdot l^{-1}$)

3 ($\mu g \cdot l^{-1}$)

4 ($\mu g\text{-at} \cdot l^{-1}$)

APPENDIX II. Mean values for selected characteristics during the steady state period. Based on sunset samples.

Cell Number (10^7 Chaetoceros cells · l^{-1})

%I	Dilution Rate, Day^{-1} 0.25	0.70	0.95	1.20
100	4.59±1.68	9.75±1.22	8.44±0.77	5.28±1.01
46	3.91±0.65	8.23±0.65	6.90±1.04	8.34±0.14
30	5.01±0.37	10.39±1.73	6.74±0.97	5.43±0.50
20	3.67±1.23	7.49±1.00	2.73±1.34	2.28±0.68
3	2.70±1.27	2.80±0.51	0.96±0.28	0.16±0.10

Carbon ($mgPOC \cdot l^{-1}$)

	Dilution Rate, Day^{-1} 0.25	0.70	0.95	1.20
100	1.9295±0.4204	2.6945±0.6515	2.5036±0.6056	2.0932±0.5580
46	1.8913±0.4549	2.5932±0.6345	1.9091±0.1401	2.1027±0.5367
30	2.4086±0.4485	2.2975±0.4476	2.0368±0.3178	1.3599±0.1483
20	2.0025±0.4864	1.9164±0.1785	1.2381±0.4043	0.5979±0.1151
3	1.4321±0.5753	0.8106±0.2296	0.3723±0.1507	0.1313±0.0153

Nitrogen ($mgPN \cdot l^{-1}$)

	Dilution Rate, Day^{-1} 0.25	0.70	0.95	1.20
100	0.2444±0.0328	0.2871±0.0280	0.3279±0.0238	0.2998±0.0480
46	0.2256±0.0315	0.2940±0.0341	0.2947±0.0305	0.2938±0.0306
30	0.2907±0.0784	0.2912±0.0429	0.2964±0.0454	0.2285±0.0218
20	0.2495±0.0350	0.3019±0.0352	0.2101±0.0504	0.0862±0.0435
3	0.2018±0.0654	0.1363±0.0301	0.0470±0.0228	0.0161±0.0035

Chlorophyll (mg Chl a · l^{-1})

%I	Dilution Rate, Day^{-1} 0.25	0.70	0.95	1.20
100	5.93 ± 2.28	19.38 ± 3.78	17.87 ± 1.12	27.86 ± 4.95
46	9.02 ± 0.96	34.44 ± 10.59	40.56 ± 5.73	34.10 ± 2.80
30	10.21 ± 1.58	33.59 ± 2.89	36.27 ± 6.95	30.34 ± 1.65
20	15.27 ± 4.76	50.83 ± 4.55	28.27 ± 8.71	12.93 ± 3.73
3	24.46 ± 4.73	22.75 ± 8.40	6.81 ± 3.87	0.40 ± 0.30

Chlorophyll Per Cell (mg Chl a · 10^7 C. curvisetus cells)

%I	Dilution Rate, Day^{-1} 0.25	0.70	0.95	1.20
100	0.93 ± 0.33	1.86 ± 0.24	2.13 ± 0.25	5.29 ± 0.08
46	2.35 ± 0.44	4.26 ± 1.64	6.04 ± 1.60	4.09 ± 0.39
30	1.87 ± 0.16	3.28 ± 0.52	5.55 ± 1.81	5.63 ± 0.70
20	2.84 ± 0.52	6.85 ± 0.81	8.35 ± 1.63	5.78 ± 1.15
3	10.49 ± 0.84	7.95 ± 1.47	6.76 ± 2.43	2.26 ± 0.21

C:Chl ratio (mg POC·mg Chl^{-1})

%I	Dilution Rate, Day^{-1} 0.25	0.70	0.95	1.20
100	446.0±166.3	139.0±19.7	139.0±26.1	74.33± 6.8
46	235.3± 34.8	81.0±30.3	47.3± 4.5	62.67±21.1
30	240.3± 38.4	69.0±17.6	56.3± 2.1	45.0 ± 7.2
20	161.3± 28.0	38.3± 6.7	43.7± 2.3	47.0 ± 6.1
3	60.7± 17.6	36.3± 3.1	59.7±14.4	267.0 ±80.6

Carbon per cell (mg POC · 10^7 C. curvisetus cells)

%I	Dilution Rate, Day^{-1} 0.25	0.70	0.95	1.20
100	0.380 ± 0.026	0.279 ± 0.068	0.296 ± 0.060	0.393 ± 0.032
46	0.561 ± 0.162	0.313 ± 0.058	0.282 ± 0.056	0.251 ± 0.059
30	0.452 ± 0.112	0.223 ± 0.041	0.310 ± 0.091	0.250 ± 0.014
20	0.520 ± 0.070	0.260 ± 0.054	0.523 ± 0.260	0.274 ± 0.068
3	0.594 ± 0.101	0.286 ± 0.031	0.380 ± 0.068	0.641 ± 0.063

Nitrogen per cell (mg PN·10^7 C. curvisetus cells)

%I	Dilution Rate, Day^{-1} 0.25	0.70	0.95	1.20
100	0.0466 ± 0.0056	0.0298 ± 0.0049	0.0391 ± 0.0047	0.0572 ± 0.0050
46	0.0611 ± 0.0164	0.0357 ± 0.0027	0.0437 ± 0.0100	0.0352 ± 0.0030
30	0.0538 ± 0.0093	0.0284 ± 0.0047	0.0452 ± 0.0131	0.0421 ± 0.0013
20	0.0617 ± 0.0107	0.0411 ± 0.0096	0.0872 ± 0.0337	0.0365 ± 0.0125
3	0.0727 ± 0.0153	0.0484 ± 0.0022	0.0473 ± 0.0096	0.0742 ± 0.0059

NO_3 uptake per cell (μg-at NO_3-N · 10^7 Chaetoceros cells)

%I	Dilution Rate, Day^{-1} 0.25	0.70	0.95	1.20
100	1.43	2.27	3.67	6.97
46	2.11	2.67	4.47	4.28
30	1.53	2.14	4.45	5.79
20	1.82	2.95	5.65	6.12
3	2.45	3.66	5.06	11.06

N:C ratio
(mg PN · mg POC^{-1})

	Dilution Rate, Day^{-1}			
%I	0.25	0.70	0.95	1.20
100	0.127	0.107	0.131	0.143
46	0.119	0.113	0.154	0.140
30	0.121	0.127	0.146	0.168
20	0.125	0.158	0.170	0.120
3	0.141	0.168	0.126	0.123

	Dilution Rate, Day^{-1}			
%I	0.25	0.70	0.95	1.20
100				
46				
30				
20				
3				

	Dilution Rate, Day^{-1}			
%I	0.25	0.70	0.95	1.20
100				
46				
30				
20				
3				

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