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GROWTH OF SKELETONEMA COSTATUM (BACILLARIOPHYCEAE) IN A CYCLOSTAT: COMPARISON OF DIURNALLY CONSTANT AND FLUCTUATING LIGHT INTENSITIES

City University of New York PH.D. 1981

GROWTH OF *SKELETONEMA COSTATUM* (BACILLARIOPHYCEAE) IN A CYCLOSTAT: COMPARISON OF DIURNALLY CONSTANT AND FLUCTUATING LIGHT INTENSITIES

by

ELIZABETH COSPER

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1981

Elizabeth Cosper

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

January 9, 1981
Date

Chairman of Examining Committee
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ABSTRACT

GROWTH OF SKELETONEMA COSTATUM (BACILLARIOPHYCEAE) IN A CYCLOSTAT: COMPARISON OF DIURNALLY CONSTANT & FLUCTUATING LIGHT INTENSITIES

by

Elizabeth Cosper

Dr. T.C. Malone - Advisor

The effects of variations in light intensity on the photodaptive characteristics and efficiency of growth of Skeletonema costatum (Grev.) Cleve were evaluated. The relative importance of changes in carbon specific rates of respiration and organic release to the efficiency of growth was determined. Light intensity was either constant during the light period at levels from 1500 - 15 μEinstein$\cdot_m^{-2}\cdot_s^{-1}$ or fluctuated throughout the light period from 500 to 10 μEinstein$\cdot_m^{-2}\cdot_s^{-1}$.

Carbon specific particulate production rates were a saturating function of incident light intensity. Particulate carbon production rates per unit chlorophyll a were a linear function of light intensity up to 650 μEinstein$\cdot_m^{-2}\cdot_s^{-1}$. Full sunlight condition, 1500 μEinstein$\cdot_m^{-2}\cdot_s^{-1}$, did not induce photoinhibition of growth or gross production. Daily rates of growth remained uniform at approximately 1.00 day^{-1} under comparable constant and fluctuating light regimes.

Under the diurnally constant light regimes the effects of variations in light intensity on cellular biomass characteristics

were dominated by changes in the pigment content of cells. Chlorophyll *a* cell^{-1} decreased from a maximal value at the lowest light intensity to a minimum at 650 μEinstein$\cdot$m$^{-2}\cdot$s^{-1}. Cell size as daily mean carbon cell^{-1}, nitrogen cell^{-1},and cellular volume was unvarying under diurnally constant light conditions from 38 - 1500 μEinstein$\cdot$m$^{-2}\cdot$s^{-1}. In contrast, under diurnally varying light cell size decreased whereas daily mean chlorophyll *a* cell^{-1} was unaffected.

Periodicity in cell division was observed only at light intensities of 130 μEinstein$\cdot$m$^{-2}\cdot$s^{-1} or greater and was decreased under diurnally varying light. Under all light conditions carbon and pigment growth were maximal during the light period but relatively well coupled throughout the 24 hr period. Carbon production during the dark period varied from 19 to 34% of total daily production and was a linear function of growth rate.

Net growth efficiency varied from 0.69 to 0.38 and was maximal at 130 μEinstein$\cdot$m$^{-2}\cdot$s^{-1}. Variations in light reduced net growth efficiency from this optimal level mainly as a consequence of relative changes in respiratory activity. Total daily respiration was a positive linear function of growth rate except at the highest light intensity and under fluctuating light when respiration was greatly enhanced. A disparity between respiratory loss and total respiration increased with light intensity and the refixation of respired carbon was proposed as the process which could account

for the observed conservation of carbon within the cell. Organic carbon release increased with light intensity and under fluctuating light but remained less than 10% of gross production under all light conditions. When carbon production and division were most in phase, efficiency was maximized. Cellular chemical fractionation indicated that under high or variable light conditions, when growth efficiency was reduced, fixed carbon was stored during the light period for subsequent synthesis of protein and pigments, and division at night.

ACKNOWLEDGEMENTS

I am grateful to Dr. T. C. Malone for his encouragement, guidance and support during this study and in preparation of this document. The apparatus to fluctuate the light intensity was designed and built by Mr. H. C. Cosper to whom I am extremely thankful as well as Mr. D. Boardman who constructed the nuclepore filter holder. Dr. J. J. Lee generously loaned me his refrigerated fraction collector.

All the committee members have been instrumental to the success of this research project. However, I would particularly like to thank Dr. J. Marra for his advice and help concerning continuous culture techniques and light sources. Also, Dr. I. Morris' suggestion to do cell fractionation, although not immediately heeded, proved invaluable.

Dr. J. Bunt's continued interest in this research project despite thousands of miles distance has been inspiring. Dr. J. Anderson of Lamont Doherty Geological Observatory also offered valuable thoughts and criticism. Without the support of many wonderful people at Lamont this project would not have been completed.

To tcc, the one person whose efforts and understanding have been integral to the success of this research, I dedicate this disseration.

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INTRODUCTION

Assessment of the role of light in controlling the growth of marine phytoplankton has centered on photosynthetic processes (Parsons et al., 1977). Under optimal conditions both the rates of growth (Thomas, 1966; Beardall and Morris, 1976) and the rate of photosynthesis (Vollenweider, 1965; Jassby and Platt, 1976) can vary with light intensity in a similar fashion: increasing linearly at low intensities and saturating at high intensities. Differences between the photosynthetic and growth responses are dependent on the rates of respiration and extracellular release of organic carbon and on the relationship between time-dependent variations in photosynthesis and growth. These differences are poorly understood.

Respiration as a proportion of total photosynthesis can vary from 5% to greater than 50% even in actively growing cells (Brown and Richardson, 1968; Humphrey, 1975; Burris, 1977; Falkowski and Owens, 1978). There are few studies comparing respiration to growth rate under well controlled culture conditions. A linear relationship between dark respiration and growth rate was found for Chlorella pyrenoidosa and C. ellipsoidea (Myers and Graham, 1959, 1961; Pickett, 1975). A similar relationship was initially found for Monochrysis lutherii (Laws and Caperon, 1976) but further study indicated that night-time carbon losses were diminished at high growth rates in several microalgae thus altering the linear relationship (Laws and Wong, 1978). Extracellular release rates have

been found to increase with growth rate (Ignatiades and Fogg, 1973; Hellebust, 1974) but can be a greater proportion of photosynthesis when photosynthesis and growth are uncoupled (Hellebust, 1974), such as under high light (Ignatiades and Fogg, 1973).

Phytoplankton in surface waters of the sea are continuously exposed to variations in light intensity. Waves (Snyder and Dera, 1970; Gordon et al., 1971), diurnal cycles and meteorological changes (clouds, storms, etc.)(Holmes, 1957; Parsons et al., 1977) vary light over seconds to hours. These variations in light interact with the exponential attenuation of light with depth in the water column (Jerlov, 1974). Turbulent motion in surface waters moves phytoplankton cells through continuously varying light fields (Steeman Nielsen, 1974). Photoadaptation has been shown in involve changes in photosynthetic parameters accompanied by modifications in cellular concentrations of pigments and carboxylating enzymes (eg., Ryther and Menzel, 1959; Steeman Nielsen and Hansen, 1959; Steeman Nielsen and Jørgensen, 1968 a & b; Jørgensen, 1969; Beardall and Morris, 1976; Prézelin and Sweeney, 1979; Falkowski and Owens, 1980). However, the time course of photoadaptation is affected by the light history of cells (Sargent, 1940; Sorokin, 1958; Steeman Nielsen and Jørgensen, 1968 a & b) and response times can vary from a few hours to days (McAllister, 1961; Steeman Nielsen et al., 1962; Steeman Nielsen and Park, 1964; Hitchcock, 1977; Marra,

1980). How the photoadaptive response to continuously fluctuating light compares to the repsonse to discrete and constant light levels is not known.

Recent studies with natural phytoplankton populations have shown that short term fluctuation in light can modify the photosynthetic response. Marra (1978 b) compared in situ production estimates derived from incubations at fixed depths with estimates from incubations in which vertical movements simulating Langmuir circulation was supplied. He found, at times, an increase up to 80% in depth integrated production in the incubations with vertical movement. Fréchette and Legendre (1978) found that flashing light simulating the effects of waves (0.1 to 10 sec) generally lowered photosynthetic rates relative to constant intensity but at light levels below the saturation level for photosynthesis frequently a 4 fold enhancement in the photosynthetic rate was observed. Both in the field and the laboratory, under light fluctuations simulating mixing in the water column phytoplankton can display a reduced rate of photosynthesis after exposure to high light (Harris, 1973; Harris and Lott, 1973; Falkowski and Owens, 1978).

Although fluctuations in light may correlate well with variations in the rate of photosynthesis (Marra, 1978 a), these variations are not necessarily reflected in variations in growth. The uncoupling of photosynthesis, growth and division could lead to increased rates of organic release (Hellebust, 1974) and respiration

(Jackson and Volk, 1970; Harris, 1973; Tolbert, 1974; Lehninger, 1975). The time scales over which variations in light intensity are relevant to growth rates of marine microalgae are poorly understood (Marra 1977).

The relationship between phytoplankton photosynthetic gains and respiratory losses is important to net productivity of the pelagic zone. Only when the depth of vertical mixing of the water column is less than the depth at which integrated water column photosynthesis and respiration are equal can net phytoplankton production occur (Sverdrup, 1953). In evaluating water column respiration it is usually assumed that phytoplankton respiration is constant with depth. However, how respiration varies with depth is unknown (Parsons et al., 1977). The relationship between respiration, growth and photosynthesis under continuously fluctuating light has never been determined.

Marine phytoplankton populations growing under light-dark cycles can exhibit diel variations in cellular biomass characteristics, and rates of photosynthesis, growth and division (Sournia, 1974). Diel variations in photosynthetic capacity have been extensively studied (the light saturated rate of photosynthesis per unit chlorophyll a) both in the field (Doty and Oguri, 1957; Shimada, 1958; Lorenzen, 1963; McAllister, 1963; Newhouse et al., 1967; Harris, 1973; MacCaull and Platt, 1977; Gargas et al., 1979) and in laboratory cultures (Eppley and Coatsworth, 1966; Jørgensen, 1966;

Eppley et al., 1967). Variations in photosynthetic capacity may be controlled, in part, by an endogenous circadian rhythm (eg., Hastings et al., 1961; Sweeney, 1969; Walther and Edmunds, 1973; Prézelin et al., 1977; Prézelin and Ley, 1980); but, environmental variables such as the magnitude and length of diurnal light cycles (Lorenzen, 1963; Kalff, 1969) as well as algal type and nutrient supply (Malone, 1971; Stross et al., 1973; Sournia, 1974; Malone et al., 1980) can also influence the pattern of diel variation in photosynthetic capacity. Little is known about how the photosynthetic response and photoadaptive characteristics of populations subjected to continuous fluctuations in light intensity might modify the pattern of diel variation in photosynthetic capacity (Harris, 1973; Marra, 1978 a and b).

Periodicity in cell division has been demonstrated for many but not all marine phytoplankton species growing under a light-dark regime. In general, diatoms are more flexible in the timing of division and can divide more during the light period compared to other microalgae (Chisholm et al., 1978; Nelson and Brand, 1979). The timing of maximum division and the degree of synchrony can be affected by photoperiod (Eppley and Coatsworth, 1966; Paasche, 1967, 1968), nutrient supply (Jørgensen, 1966; Eppley et al., 1971), temperature (Paasche, 1967, 1968; Williamson, 1980) and growth rate (Chisholm et al., 1975). The uptake of nutrients, synthesis of cellular constitutents and division can exhibit diel variations

which are not necessarily in phase (Jørgensen, 1966; Eppley and Coatsworth, 1966; Eppley et al., 1967; Paasche, 1968; Eppley et al., 1971; Sundberg and Nilshammer-Holmvall, 1975). The few studies which have correlated diel variations in carbon fixation with variations in other growth rate parameters (Eppley and Coatsworth, 1966; Jørgensen, 1966; Eppley et al., 1967; Eppley et al., 1971) have found that carbon production and net pigment synthesis are well coupled and maximal during the light period; whereas, division maxima occurred both during the light and dark period. The effect of variations in light intensity on the diel pattern of coupling between photosynthesis, growth and division is unknown.

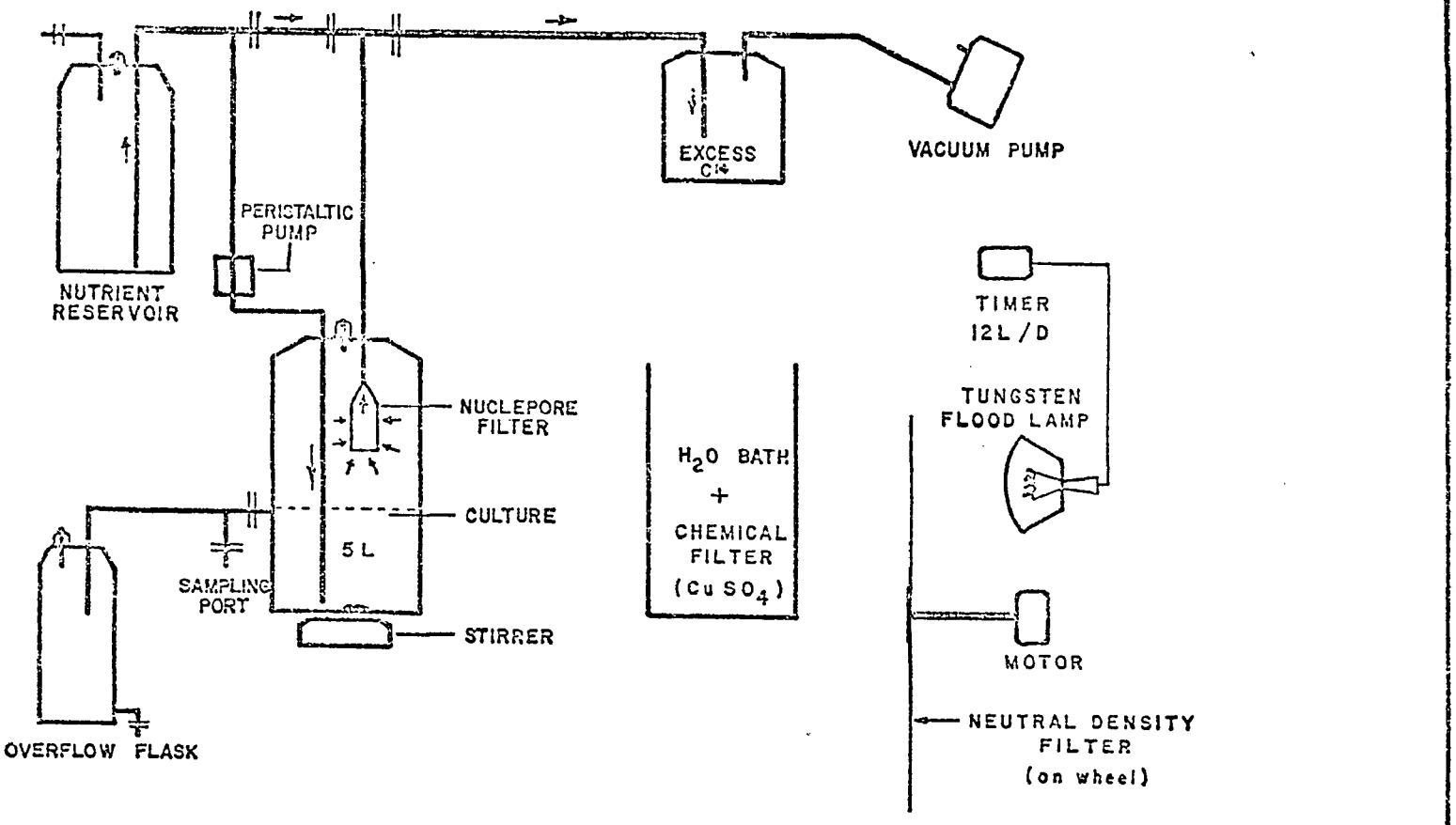
There are few studies of diel variations in respiration and none on the rates of organic release (Sournia, 1974). In well synchronized cultures a diel periodicity in respiration has been observed which can be related to particular stages of the cell cycle (Nihei et al., 1954; Curnutt and Schmidt, 1964; Hochachka and Teal, 1964; Prézelin et al., 1977). Natural populations of phytoplankton also exhibit variations in respiratory activity with maxima generally occurring during the afternoon and early evening (Ryther et al., 1958; Lorenzen, 1963; Harris, 1973). An understanding of the extent to which variations in respiration and organic release correspond with diel variations in photosynthesis and growth is lacking but important to an evaluation of factors influencing the dynamics and efficiency of growth in marine phytoplankton.

In this study, the effects of variations in light intensity on the efficiency of carbon specific growth in a common marine diatom, Skeletonema costatum, were measured and evaluated in terms of the relative importance of respiration and organic carbon release. Variations in light intensity simulated a range of light levels found in the photic zone and cultures were grown under a light-dark cycle in which light was either constant during the light period or fluctuated continuously. Variations in the efficiency of growth were compared to changes in photoadaptive characteristics. The photoadaptive characteristics of constant and diurnally varying light regimes were contrasted. The extent to which variations in light modified the phasing of growth processes and the importance of diel variations to the efficiency of growth were elucidated. Culture conditions allowed for attainment of steady state conditions under each light regime, indicating a stabilization of the adaptive response.

MATERIALS AND METHODS

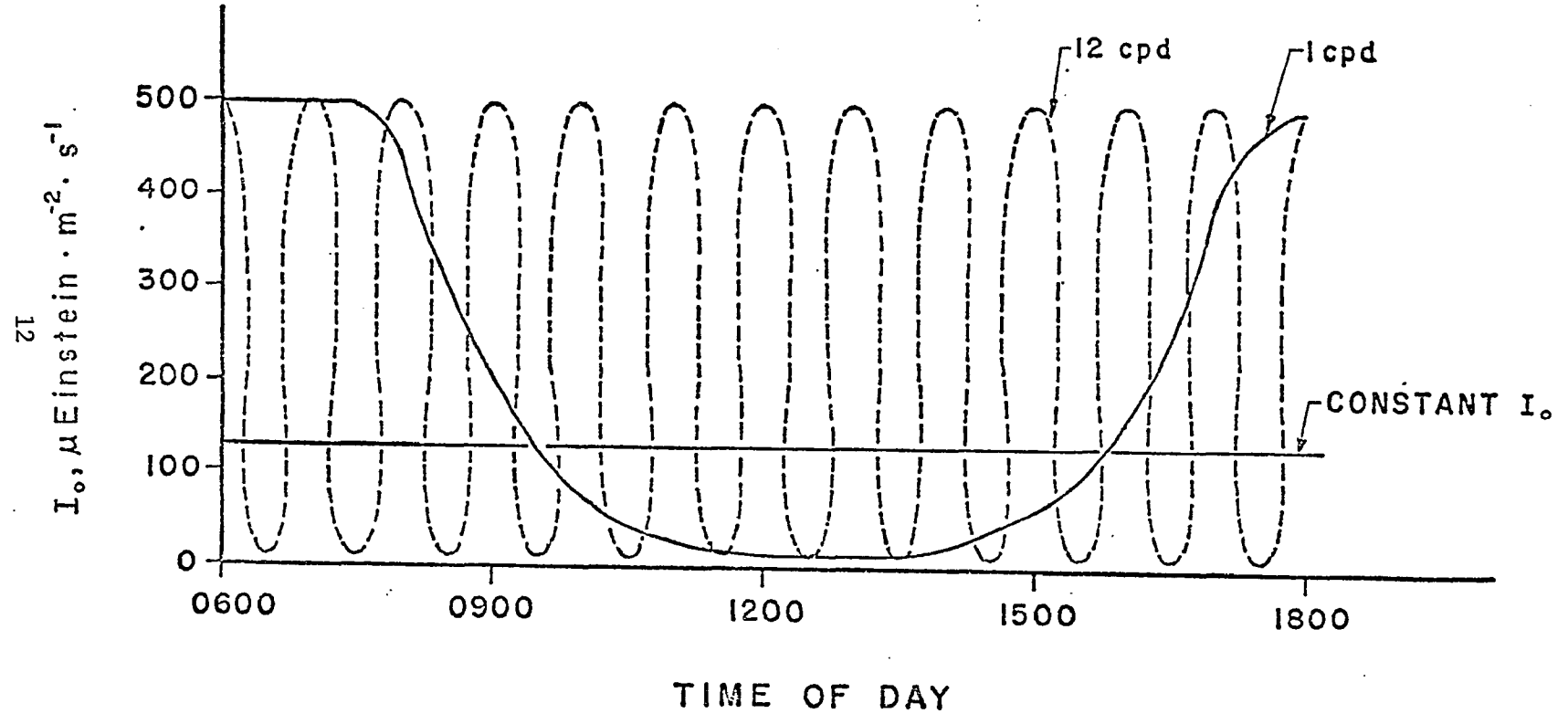
The culture apparatus (Fig. 1) consisted of a glass carboy in which a 5 l continuous culture of S. costatum (clone Skel from the Woods Hole Culture Collection) was maintained in a temperature controlled room (20°C). A magnetic stirring bar on the bottom kept the cells in suspension and well mixed. Guillard's f medium at f/4 concentration (Guillard and Ryther, 1962) was continuously supplied at a specific rate from a nutrient reservoir using a Buchler peristaltic pump and the culture volume was kept constant by an overflow port. Light was supplied on a 12 hour light-dark cycle by a 500 W tungsten lamp and attenuated with nickel plated neutral density filters. A water bath with 0.2% $CuSO_4$ was placed between the light source and the culture in order to better simulate the spectral distribution of sunlight incident on the ocean. Light was varied in two ways: (1) incident light intensity (I_o) was constant during the light period and cultures were grown over a range of discrete light intensities, simulating light levels throughout the photic zone from full sunlight down to approximately the 1% level (1500, 650, 130, 38 and 15 μEinstein$\cdot m^{-2} \cdot s^{-1}$); (2) incident light was varied from 500 to 10 μEinstein$\cdot_m{}^{-2} \cdot_s{}^{-1}$ throughout the light period by rotating a wheel of neutral density filters at either 1 or 12 cycles day^{-1}, simulating the exponential change in light throughout the photic zone (Figure 2). Under the fluctuating light regimes

Figure 1. Culture apparatus



CULTURE APPARATUS

Figure 2. Variations in light intensity (I_0) incident on the culture during the light period under constant and fluctuating light regimes of 1 or 12 cycles day^{-1} (cpd).


12 cpd
1 cpd
CONSTANT I_o
500
400
300
200
100
0
0600
0900
1200
1500
1800
TIME OF DAY
I_o, μEinstein · m^{-2} · s^{-1}

total daily radiation was kept constant at 5.6 Einstein·m^{-2} day^{-1} which was comparable to the amount of daily light received by the culture grown under the constant light regime at 130 μEinstein·m^{-2}·s^{-1}. This allowed for a direct comparison between the culture grown at 130 (constant) μEinstein·m^{-2}·s^{-1} and those grown under fluctuating light of 1 and 12 cycles day^{-1}. Under each light condition the dilution rate of the culture was adjusted to obtain steady state cell densities of 3-5 X 10^4 cells/ml (daily mean) with the highest possible growth rate. Cellular concentrations were low enough to ensure that the demand for major nutrients was at least an order of magnitude less than ambient concentrations and that density dependent effects on growth rate were not manifest (Yoder, 1979). Comparable cell densities minimized variations in scattering of available light in the culture. Steady state was defined for consecutive days between which variations in daily mean biomass were not significant (ANOVA, Model I, $p>0.05$, see Appendix I). The light-dark cycle precluded steady state conditions during the diel cycle (Chisholm et al., 1975). At those times when steady state prevailed daily growth rates equalled one daily dilution of the culture. The steady state dilution rates achieved for each respective light intensity were: 0.10, 0.32, 1.00, 1.99 and 1.68 day^{-1} at 15, 33, 130, 650 and 1500 μEinstein·m^{-2}·s^{-1}, respectively. For the variable light regimes of 1 and 12 cycles per day the steady state dilution rates were 1.08 and 1.15 day^{-1}, respectively.

Samples were obtained from the overflow port and processed immediately or collected in a Buchler refrigerated (3°C) fraction collector and processed within 6 hours of collection.

Each experiment was performed in two stages. Once steady-state was achieved the following biomass characteristics were measured at 0600 (lights on), 1200, 1800 (lights off) and 2400 hours for a minimum of 3 days: cell number and volume, particulate organic carbon (POC) and nitrogen (PON), and chlorophyll $\underline{a}$, $\underline{c}_1$, $\underline{c}_2$ and carotenoids. Once biomass characteristics of the population were defined, 1 mCi of ^{14}C as $NaH^{14}CO_3$ was added to the culture and uptake into the particulate fraction was measured every 3 to 6 hours for the next 36 hours until the cells were in isotopic equilibrium, i.e., the ratio between particulate (P) ^{14}C and ^{12}C was relatively constant. During this time interval changes in organic and inorganic ^{14}C were also determined in order to estimate rates of organic release and to monitor the decrease in the specific acticity of ^{14}C as the label was diluted out of the culture. $P_{^{14}C:\ ^{12}C}$ ranged from 2.8-4.8 x 10^{-4} under diurnally constant light but under diurnally varying light was 1.0 x 10^{-3} when uptake was terminated. This was accomplished by gently flushing the radioactive media from the culture through a nuclepore filter with pores 1 μm in diameter and subsequent replacement with cold media (Fig. 1). This process retained the radioactive cells in the culture and over a period of about 4 hours reduced the activity in the media to approximately 1%

of the initial level. Once steady-state was reestablished (6 to 24 hours), particulate and dissolved ^{14}C were measured at 0600, 1200, 1800 and 2400 hours for at least 3 days. The experiment at 15 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ had to be terminated before the culture was flushed because a tear developed in the nuclepore filter.

Dissolved organic carbon release was determined from changes in the dissolved organic ^{14}C fraction and respiration was calculated as the difference between total losses and dissolved organic release. ^{14}C in the particulate fraction was determined from 10 ml samples filtered onto HA 0.45 μm Millipore filters, fumed for 5 min with HCl and placed in scintillation vials with 10 ml of Aquasol. Dissolved organic ^{14}C was measured on 5 ml aliquots of filtrate collected in scintillation vials which were acidified to pH 3 and bubbled with air for 45 min in order to remove the inorganic ^{14}C (Thomas, 1971). Similarly, 10 ml of Aquasol was added to each scintillation vial prior to counting. Another 5 ml of filtrate was placed in scintillation vials and treated with phenethylamine to convert the inorganic carbon into non-volatile carbamates (Iverson et al., 1976) with subsequent addition of 10 ml Aquasol in order to estimate the total dissolved ^{14}C. Measurements of ^{14}C activity were made in a liquid scintillation counter (Beckman LS 100) and the efficiency of counting was determined for each sample using an external standard and previously determined quench characteristics for each type of sample. Coefficients of variation (C.V.) of

triplicate samples were generally less than 10%, but never exceeded 30%. After accounting for changes in the specific activity of ^{14}C, changes in carbon were calculated over specific time intervals and carbon specific rates (hr^{-1}) were calculated using the following general equation:

$$\frac{1}{t} \ln \left(\frac{C_o + \Delta C}{C_o} \right) + D$$

where t = time, C_o = initial concentration, ΔC = change in concentration, D = dilution rate of culture. The same equation was used to calculate carbon specific growth from changes in POC with time.

For the highest and lowest light intensities and the variable light regimes the ^{14}C label was also followed within the cellular fractions using a modification of methods outlined by Wallen and Geen (1971), Bergmeyer (1974), Morris et al. (1974), and Hollibaugh et al. (1980). The samples were processed similarly to those described above but extra samples were obtained in triplicate and either treated for 10 min with 5% ice-cold tricloroacetic acid (TCA) or for 1 hour with hot (boiling) TCA before filtration. The cold TCA removed the small molecules from the cells; the hot TCA hydrolyzed and removed polysaccharides leaving a filterable residue of mostly protein.

POC and PON samples were measured in triplicate by combustion and gas chromatography in a HP-185 CHN Analyzer. Cell density (8 replicates) was estimated from hemacytometer counts and cell

volume (10 cells/count) from microscopic measurements. pH and total alkalinity were determined using methods in Strickland and Parsons (1972) in order to estimate dissolved inorganic carbon concentrations. Chlorophyll *a*, c_1, c_2 and carotenoids (single samples) were determined using the spectrophotometric methods of Jeffrey and Humphrey (1975) and chlorophyll *a* (triplicate samples) was also measured fluorometrically using a Turner 111 fluorometer (Strickland and Parsons, 1972). *In vivo* fluorescence (duplicate samples) was measured in a Turner Designs Fluorometer as fluorescent yield after 5 sec (a time adequate for the fast fluorescent change but not the slow change to become manifest) using a blue excitation filter (Corning, CS 5-60) and red emission filter (Corning, CS 2-64) (Kiefer, 1973; Loftus and Seliger, 1975). In addition, the effect of variations in the rate of photosynthetic electron transport on *in vivo* fluorescence yield was measured in duplicate in a Perkin Elmer Model 204 Spectrofluorometer at 680 nm after 5 sec excitation (440 nm wavelength, 6.5 μEinstein$\cdot m^{-2} \cdot s^{-1}$) of a dark adapted sample (15 min dark) (Prézelin and Sweeney, 1977; Samuelson and Öquist, 1977). Measurement of fluorescent yield within the first 5 sec of illumination is necessary in order for variations in fluorescent yield to correspond to changes in the rate of photosynthetic electron transport (Papageorgiou, 1975; Samuelson and Öquist, 1977). Measurements of the 3- (3, 4 dichlorophenyl) -1, 1-dimethylurea (DCMU)-induced fluorescence increase were similarly made with each

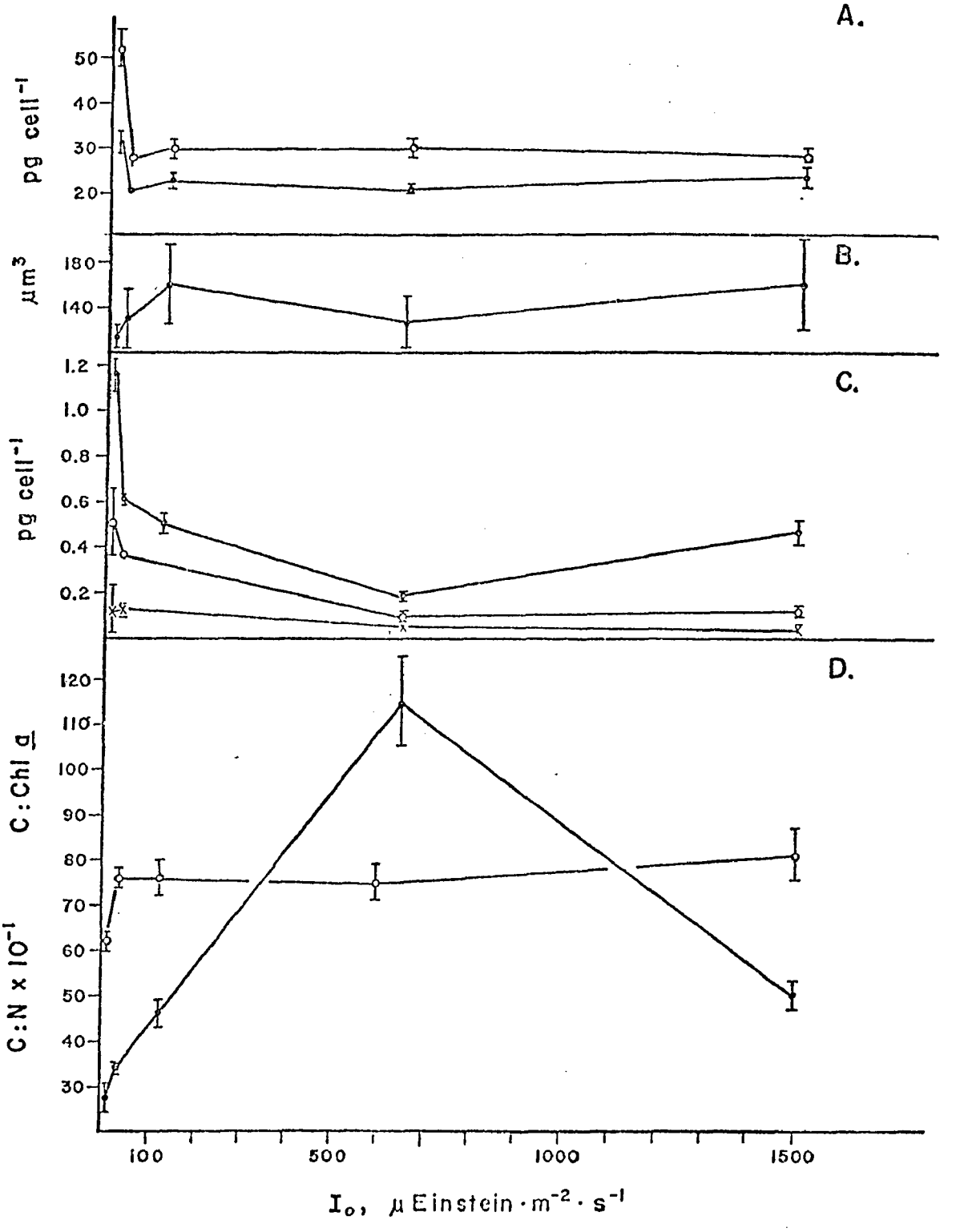
fluorometer at 10^{-6}M DCMU, a concentration saturating for inhibition of photosynthesis in S. costatum over the range of chlorophyll a concentrations observed in the cultures (Falkowski and Owens, 1978).

PART I. EFFICIENCY OF GROWTH UNDER DIURNALLY CONSTANT LIGHT

RESULTS AND DISCUSSION

Cellular biomass characteristics. The effects of light intensity on cellular biomass characteristics of S. costatum were dominated by changes in the pigment content of cells (Figure 3). Cellular concentrations of carbon and nitrogen, respectively 32.5 and 5.2 pg $cell^{-1}$, were maximal at 15 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ and were reduced at higher light intensities but did not vary significantly from 38-1500 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ (Figure 3A). The C:N ratio increased from 6.2 at 15 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ to values of 7.5-8.1 at higher light intensities mainly as a consequence of a decrease in cellular nitrogen (Figure 3D). Mean daily cell volume was not significantly affected by light intensity (Figure 3B). The cellular concentration of chlorophyll *a* decreased from a maximal value of 1.16 pg $cell^{-1}$ at the lowest light intensity to a minimum of 0.19 pg $cell^{-1}$ at 650 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ (Figure 3C). At the highest light intensity chlorophyll *a* $cell^{-1}$ did not decrease further but increased to 0.47 pg $cell^{-1}$. Cellular levels of carotenoids and chlorophyll c_1 and c_2 were also maximal at the lowest light intensities and paralleled variations in chlorophyll *a* $cell^{-1}$ with increasing light except at the highest light intensity (Figure 3C). The C:Chl*a* ratio varied from 27-115 primarily as a consequence of variations in chlorophyll *a* $cell^{-1}$. The ratio was highest at 650 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ and lowest at 15 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ (Figure 3D).

Figure 3. Relationship between mean daily cellular biomass characteristics and incident light intensity (μEinstein$\cdot m^{-2}\cdot s^{-1}$) as: (A) carbon, (·), nitrogen (o) x 10^{-1}, (pg $cell^{-1}$); (B) volume (μm^3 $cell^{-1}$); (C) chl *a*, (·), chl c_1c_2, (x), carotenoids, (o), (pg $cell^{-1}$); (D) C:Chl *a*, (·), C:N, (o); vertical bars are 95% confidence limits.


A.
pg cell⁻¹
50
40
30
20
B.
µm³
180
140
C.
pg cell⁻¹
1.2
1.0
0.8
0.6
0.4
0.2
D.
C:Chl a
C:N x 10⁻¹
120
110
100
90
80
70
60
50
40
30
100
500
1000
1500
I₀, µ Einstein · m⁻² · s⁻¹

Variations in the biochemical composition of S. costatum with changes in light intensity observed in this study are, in general, consistent with the results of other investigators. However, comparisons for the two highest light levels used in this study are unavailable. The relationship between cellular carbon and volume corresponded well with the values predicted by the equation Strathman (1967) developed for diatoms except at the lowest light intensity when carbon $cell^{-1}$ was slightly elevated relative to cell volume. Chlorophyll a $cell^{-1}$ has similarly been found to decrease as light intensity increased from 15 to 250 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ with a concomitant increase in the C:Chl a ratio (Yoder, 1979; Falkowski and Owens (1980). Disparities in cell size most likely can account for any differences in absolute concentrations of carbon and chlorophyll a $cell^{-1}$ since the C:Chl a ratios for similar light levels and temperature compare well between the results reported here and the values obtained by Yoder (1979) and Falkowski and Owens (1980).

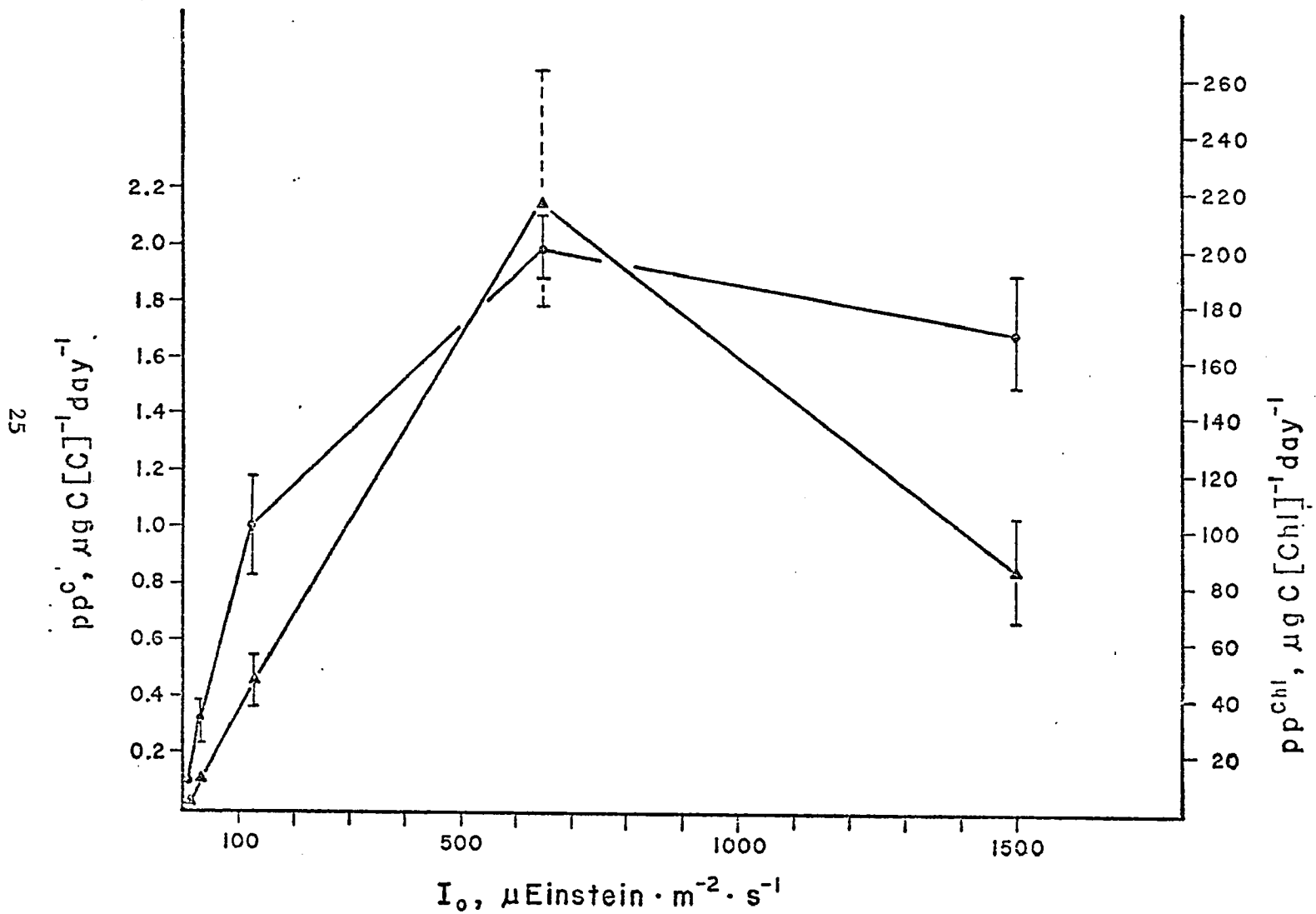
C:N ratios similar to the values reported above were observed by Sakshaug and Holm-Hansen (1977) and Yoder (1979) when S. costatum was grown under nutrient saturated conditions. However, Falkowski and Owens (1980) generally found lower values for C:N with S. costatum. Changes in the C:N ratio with variations in light intensity were relatively small in comparison to the several fold changed observed under nitrogen limitation (Sakshaug and Holm-Hansen,1977). Similar to the above results the lowest values for C:N were observed at low light intensities (Yoder, 1979; Falkowski and Owens,

1980). In contrast to the increase in carbon and nitrogen cell^{-1} at 15 μEinstein$\cdot$m$^{-2}\cdot$s^{-1} in the study reported here, Yoder (1979) found a decrease, particularly in carbon cell^{-1}, at light intensities below 25 μEinstein$\cdot$m$^{-2}\cdot$s^{-1} despite little significant change between 25 and 250 μEinstein$\cdot$m$^{-2}\cdot$s^{-1}. The increases in carbon and nitrogen per cell at the lowest light intensity were not related to changes in cell volume but did correspond well with the increase in cellular pigment content. Nitrogen per cell increased to a greater extent, reducing the C:N ratio, perhaps reflecting increases in the proteins associated with pigment molecules in the membranes of the chloroplast (Thornber, 1975; Alberte et al., 1977).

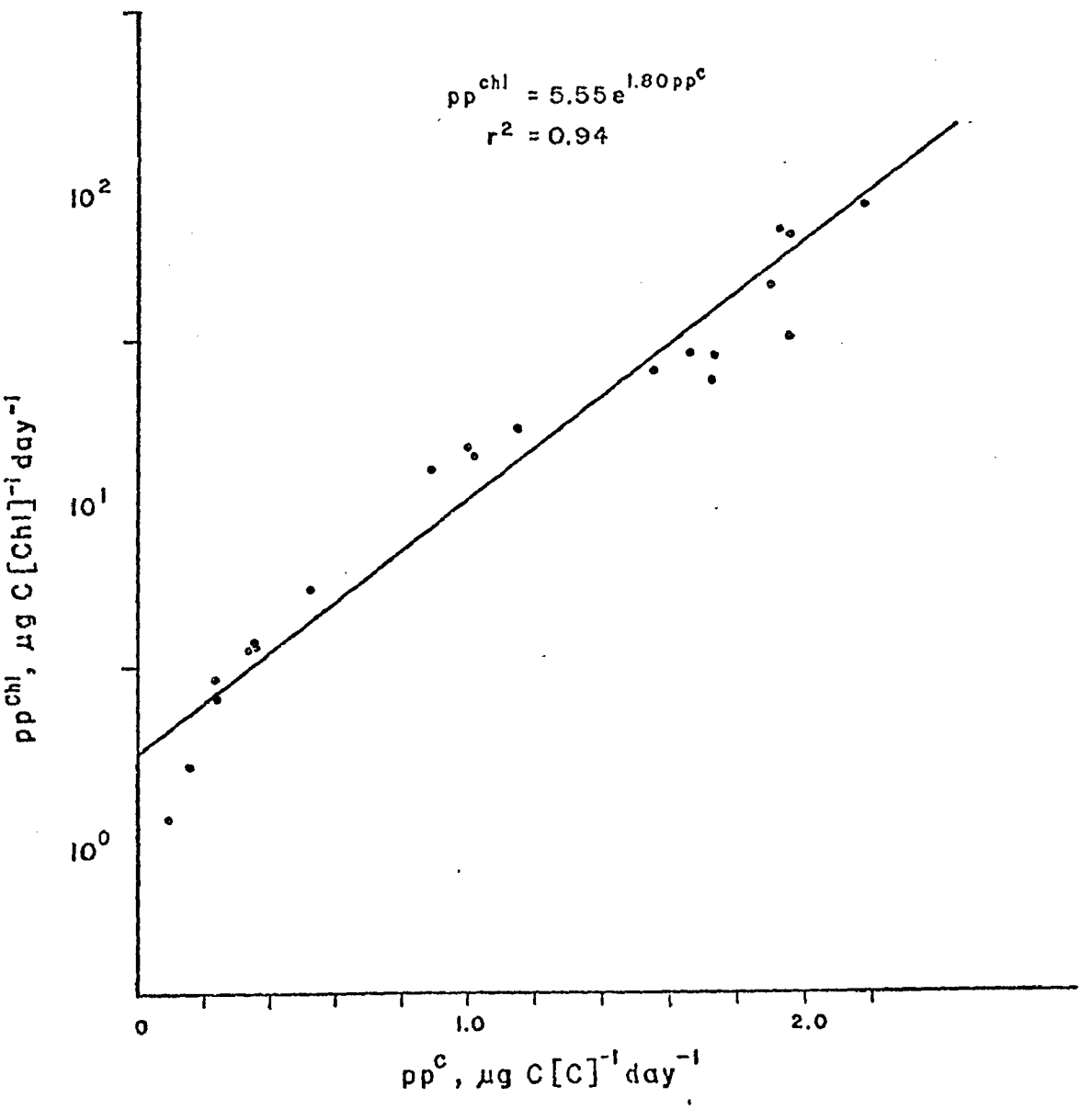
Daily particulate rates of production. Daily biomass specific rates of production were a saturating function of incident light intensity with maxima at 650 μEinstein$\cdot$m$^{-2}\cdot$s^{-1} (Figure 4). Carbon specific particulate production rates (pp^C) (calculated from changes in POC) increased with light from 0.10 to 1.99 day^{-1} and was not significantly decreased at 1500 μEinstein$\cdot$m$^{-2}\cdot$s^{-1}. Chlorophyll a specific particulate production rates (pp^{chl}) varied from 3.5 to 215.0 μgC (chla)$^{-1}\cdot$day^{-1} and, in contrast with carbon specific growth, decreased significantly at the highest light intensity. pp^{chl} increased exponentially with pp^C (Figure 5), mainly as a consequence of variations in chlorophyll a cell^{-1}.

An understanding of the relationship betwen PP^{chl} and PP^C is important to an interpretation of field production estimates since

Figure 4. The effect of incident light intensity (μEinstein$\cdot m^{-2}\cdot s^{-1}$) on carbon specific rates of growth (μg C$\cdot$(C)$^{-1}\cdot$day^{-1} ($\cdot$) and chl _a_ specific particulate production (μg C$\cdot$(chl _a_)$^{-1}\cdot$ day^{-1}) (▲); vertical bars are 95% confidence limits.


2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
260
240
220
200
180
160
140
120
100
80
60
40
20
100
500
1000
1500
I_o, μEinstein · m^{-2} · s^{-1}
pp^C, mg C [C]$^{-1}$ day^{-1}
pp^{Chl}, μg C [Chl]$^{-1}$ day^{-1}

Figuere 5. Relationship between chlorophyll _a_ specific particulate production (μg C$\cdot$ (chl _a_)$^{-1}\cdot$ day^{-1}) and carbon specific rates of growth (μg C$\cdot$(C)$^{-1}\cdot$ day^{-1}); each value represented is for a single day under each of five light treatments.


$pp^{chl} = 5.55e^{1.80pp^c}$
$r^2 = 0.94$
10^2
10^1
10^0
pp^{chl}, µg C [Chl]$^{-1}$ day^{-1}
0
1.0
2.0
pp^c, µg C [C]$^{-1}$ day^{-1}

it is difficult to accurately estimate phytoplankton carbon in the natural environment while estimates of PP^{chl} are easily obtained. Although C:Chl *a* is positively related to growth rate under light limitation, it is inversely related to growth rate under nutrient limitation (Laws and Wong, 1978; Laws and Bannister, 1980). In considering this interaction between light and nutrient limitation Eppley (1972) has reasoned that high growth rates and high C:Chl *a* are mutually exclusive in most natural environments since nutrient and light saturation rarely coincide. He found that even sewage outfalls along the California coast could not maintain such high growth rates.

pp^{chl} comparable to the high rate of 215 $\mu gC \cdot (chl\ a)^{-1} \cdot day^{-1}$ found in this study have been reported for field populations when similar conditions for temperature and nutrient saturation prevail, i.e., 236 $\mu gC \cdot (chl\ a)^{-1} \cdot day^{-1}$ for blooms of *S. costatum* in Tokyo Bay (Hogetsu *et al.*, 1959) and 200 $\mu gC \cdot (chl\ a)^{-1} \cdot day^{-1}$ for mixed assemblages of netplankton diatoms in the Hudson River estuary (Malone, personal communication). From extrapolation of the results reported here these field populations were probably growing at rates well over 2 doublings per day and close to the theoretical maximum growth rate for a temperature of 20°C (Eppley, 1972). Thus, in areas of large nutrient inputs and where there is adequate removal of biomass, pp^{chl} can be maintained at maximal rates analogous to a continuous culture under nutrient saturated conditions.

pp^{chl} was a linear function of light intensity from 15-650 μEinstein$\cdot m^{2} \cdot s^{-1}$ (r^2 = 0.97, $pp^{chl} = -0.67+0.33\ I_o$) as a consequence of variations in pigment content (Figure 3). Chlorophyll *a* $cell^{-1}$ was adjusted so as to compensate for variations in light availability, consistent with the findings reported for many microalgae (Eppley and Sloan, 1966; Steeman Nielsen and Jørgensen, 1968*a* and *b*; Bannister, 1974, 1979; Beardall and Morris, 1976; Laws and Bannister, 1980). The increased concentrations of accessory pigments at the lower light intensities also compensated for the decrease in light intensity and, by transferring their absorbed energy to chlorophyll *a* (Tanada, 1951; Shimura and Fujita, 1975), helped to maintain a uniform rate of change in production per unit chlorophyll *a*. The importance of accessory pigments in enhancing light collection at low light intensities has been documented previously for other microalgae (Tanada, 1951; Haxo, 1960; Shimura and Fujita, 1975, Prézelin, 1976).

In contrast to pp^{c}, pp^{chl} at the highest light intensity, an intensity comparable to full sunlight, was reduced (Fig. 4). Since pp^{c} remained relatively high (Fig. 4), this reduction in pp^{chl} was a consequence of the increase in chlorophyll *a* $cell^{-1}$. Few laboratory studies have used such a high light intensity for growth and the exposure of batch cultures grown at lower light to full sunlight has resulted in decreased photosynthetic rates and a time dependent increase in photo-oxidative damage (Curl and McLoed, 1961;

McAllister, 1961; Belay and Fogg, 1978). Photoinhibitory effects of photosynthesis have been observed at light intensities much lower than full sunlight (Myers and Burr, 1940; Ryther, 1956 a; Ryther and Menzel, 1959; Curl & McLoed, 1961; Steeman Nielsen, 1962; Takahashi, et al., 1971); however, the time for adaptation is an important factor. McAllister (1961) found that after several days at full sunlight the cococolithophore, Syracosphaera carterae, was able to adapt and photoinhibition of photosynthesis was greatly reduced. Farmer (1977) observed no inhibition of either growth rate or photosynthesis in Chaetoceros curvisetus growing in an outdoor continuous culture under full tropical sunlight. But even after a week of exposure to full incident radiation such adaptation was not found for other species growing in batch culture, including S. costatum (Ryther, 1956 a). The use of a continuous culture in this study allowed for the selection of a population of cells which had time for full adaptation to such a high light intensity. After inoculation of the culture and commencement of dilution, step-wise increases in the rate of dilution resulted in an increase in chlorophyll a $cell^{-1}$ whereas carbon per cell remained unchanged. An increase in the dilution rate to 1.94 day^{-1} resulted in a decrease in the C:Chla ratio from 94 to 64 within 36 hours as a consequence of a 50% increase in chl a $cell^{-1}$. Such a selection process in conjunction with time for adaptation is most likely an important reason for the differences between the results discussed above.

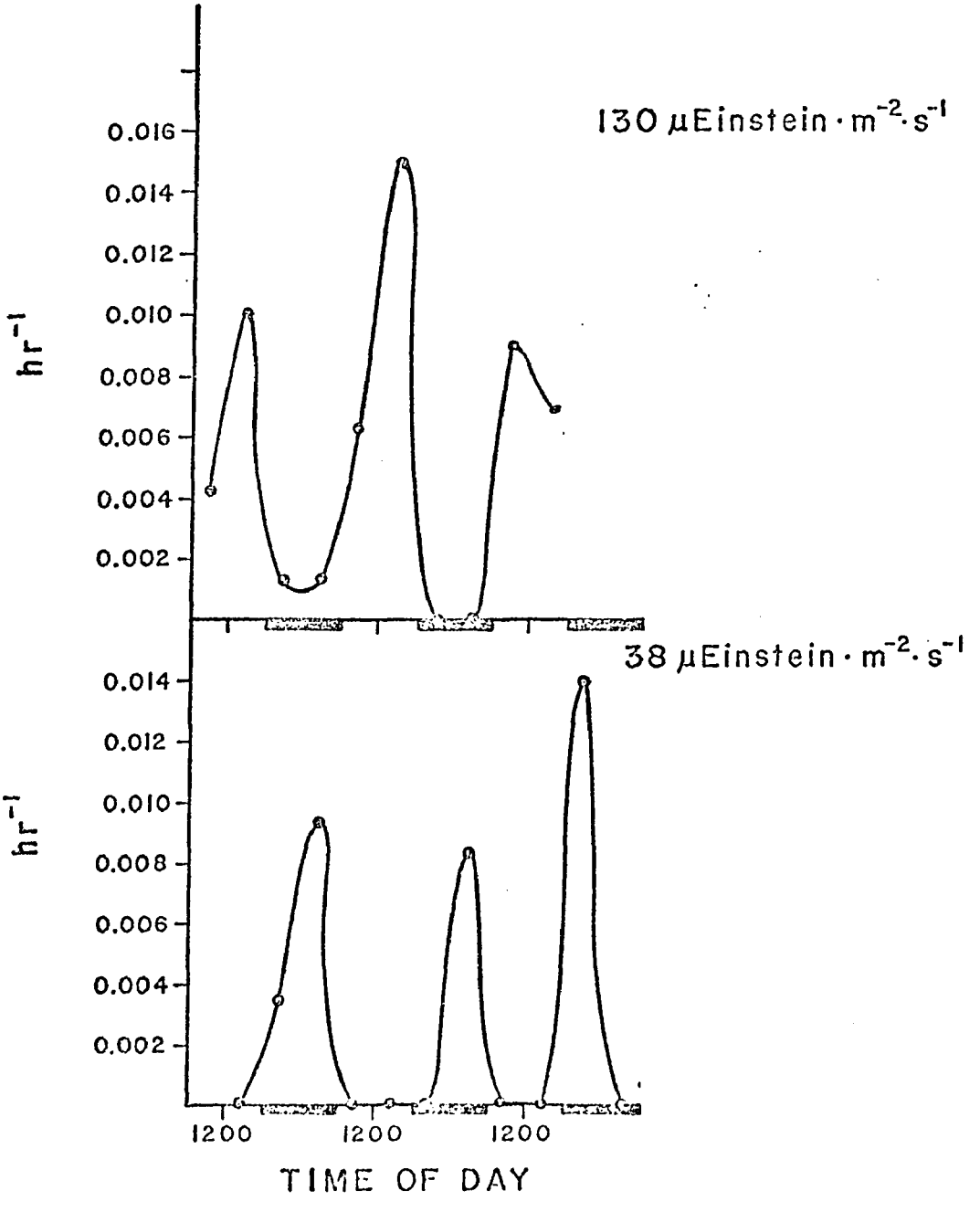
A possible explanation for this unanticipated finding of increased chl*a* cell^{-1} and decreased pp^{chl} at 1500 μEinstein$\cdot m^{-2}\cdot s^{-1}$ is derived from the results of *in vivo* fluorescence. *In vivo* fluorescence per unit chlorophyll *a* measured in the Turner Designs fluorometer decreased to a mean $\pm$ S.E. of 72 $\pm$ 2 (n=24) at the highest light intensity from 282 $\pm$ 10 (n=14) and 148 $\pm$ 17 (n=11) at 650 and 15 μEinstein$\cdot m^{-2}\cdot s^{-1}$, respectively. The ratio between initial fluorescence and DCMU induced fluorescence (F_I/F_{DCMU}), has been found to indicate the extent to which photosynthesis is decreasing fluorescence yield; the closer the ratio approaches one the less photosynthesis is depressing the fluorescence yield (Papageorgiou, 1975). F_I/F_{DCMU} increased slightly to a mean of 0.5 $\pm$ 0.01 (n=24) at 1500 μEinstein$\cdot m^{-2}\cdot s^{-1}$ relative to 0.4 $\pm$ 0.01 at 650 μEinstein$\cdot m^{-2}\cdot s^{-1}$ (n=14) and 15 μEinstein$\cdot m^{-2}\cdot s^{-1}$ (n=11). Similarly, in the Perkin Elmer spectrofluorometer F_I/ F_{DCMU} remained unchanged between 650 and 1500 μEinstein$\cdot m^{-2}\cdot s^{-1}$ with mean values of 0.6 $\pm$ 0.04 (n=14) and 0.5 $\pm$ 0.03 (n=29), respectively. However, the ratio increased substantially to 0.9 $\pm$ 0.05 (n=3) at 15 μEinstein $\cdot m^{-2}\cdot s^{-1}$ reflecting the decrease in photosynthetic rate. Thus, the decrease in fluorescence at 1500 μEinstein$\cdot m^{-2}\cdot s^{-1}$ is not related to variations in photosynthesis and might have represented a decrease in light availability within the cell. Further explanation of the increase in chlorophyll *a* cell^{-1} at 1500 μEinstein$\cdot m^{-2}\cdot s^{-1}$ is not possible. Adaptive mechanisms to prevent photo-oxidative damage under high

light have been observed in other microalgae such as the role attributed to certain carotenoids (Haxo, 1960; Thornber, 1975) and the self-shading induced by contraction of chloroplasts (Kiefer, 1973; Owens and Esaias, 1976).

In contrast to the positive relationship between variations in C:Chl *a* and growth rate observed in this study, C:N remained relatively constant over a wide range of growth rates. In nutrient limited chemostats C:N decreases with increasing growth rate (eg., Caperon and Meyer, 1972; Thomas and Dodson, 1972; Malone *et al.*, 1975; Laws and Bannister, 1980). In addition, Goldman *et al.* (1979) have observed that the C:N ratio approached at maximum growth rates is the Redfield ratio ($C_{106}:N_{16}$), which is typical of the composition of marine particulate matter. This finding prompted Goldman *et al.* (1979) to propose that phytoplankton populations in oceanic waters may be growing at near maximal rates. However, caution is advised in such an extrapolation, since, as found in this study, variations in light intensity can modify growth rates greatly with little change in the C:N ratio.

Respiration and Light Intensity. Respiratory loss of carbon was detectable only at the lower light intensities. Large diel variations in respiratory loss of carbon were observed with a shift in the occurrence of maximum rates from the dark period to the light period as light intensity increased from 38 to 130 μEinstein$\cdot m^{-2} \cdot s^{-1}$ (Fig. 6). Laws and Wong (1978) similarly found for Monochrysis

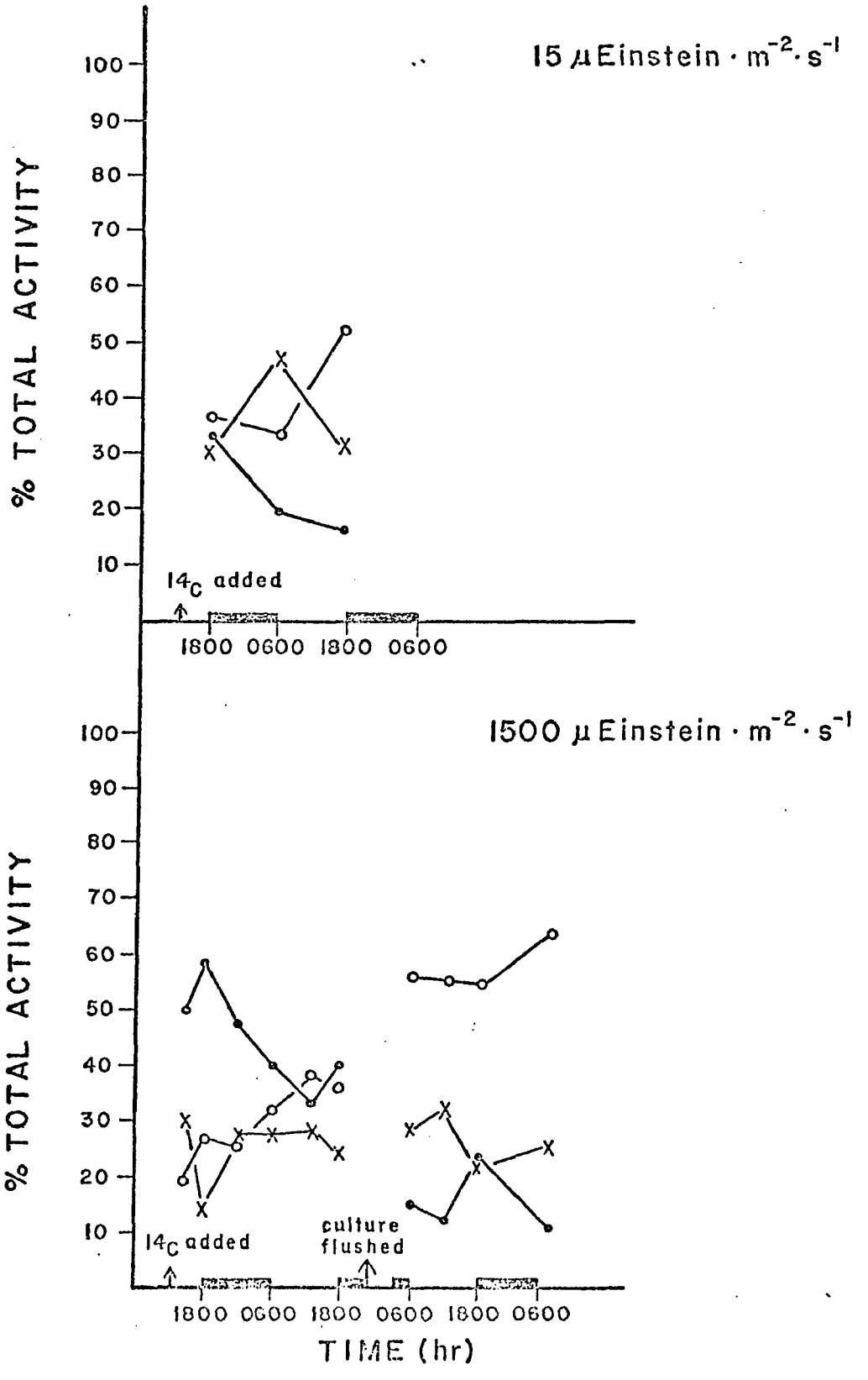
Figure 6. Hourly carbon specific rates of respiratory loss at specific time intervals over several days for cultures grown at incident light intensities of 38 μEinstein$\cdot m^{-2} \cdot s^{-1}$ (lower part) and 130 μEinstein$\cdot m^{-2} \cdot s^{-1}$ (upper part); the lines indicate trends in data not an extrapolation of rates between points.


130 μEinstein · m⁻² · s⁻¹
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
hr⁻¹
38 μEinstein · m⁻² · s⁻¹
0.014
0.012
0.010
0.008
0.006
0.004
0.002
hr⁻¹
1200
1200
1200
TIME OF DAY

Wong (1978) similarly found for Monochrysis lutherii and Dunaliella tertiolecta that at high growth rates in a nitrogen limited chemostat night-time losses of carbon decreased. Also, under field conditions respiratory losses have been seen to be the inverse of in growth rate. Ketchum et al. (1958) found that the ratio between net and gross photosynthesis was highest for inshore waters relative to offshore waters and that nutrient additions could increase the ratio for oceanic populations. Eppley and Sharp (1975) also found that dark carbon losses were a substantial part of net production in the oligotrophic waters of the central gyre of the North Pacific. Thus, it appears that under conditions in which the nutrient supply is adequate, respiratory losses, particularly during the night, can be diminished relative to increases in growth rate.

The lack of respiratory loss of carbon at the highest light intensities did not appear to relate to a sequestering of the ^{14}C label into metabolically inactive carbon pools. A comparison of the time course of the labelling of the various cell fractions (Fig. 7) under high and low light indicated that the ^{14}C label appeared to a greater extent and remained longer in the small molecular weight fraction under high light. Even after several days, 10% of the total ^{14}C label was still in a small molecular weight fraction. Protein and polysaccharide fractions were more rapidly labeled under low light, whereas several days were required at high light before these cellular fractions were similarly labeled. The refixation of

Figure 7. Time course of distribution of ^{14}C activity in cellular fractions as small molecular weight (·), polysaccharide (x) and protein (o), upper part - culture grown under low light (15 μEinstein·m^{-2}·s^{-1}), lower part - culture grown under high light (1500 μEinstein·m^{-2}·s^{-1}).


15 μEinstein · m⁻² · s⁻¹
% TOTAL ACTIVITY
100
90
80
70
60
50
40
30
20
10
14C added
1800 0600 1800 0600
1500 μEinstein · m⁻² · s⁻¹
% TOTAL ACTIVITY
14C added
culture flushed
1800 0600 1800 0600 1800 0600
TIME (hr)

respired carbon within the cell would explain the continued presence of label within the small molecular weight fraction and the lack of respiratory loss of carbon at high light intensities.

Indication of the recycling of respired carbon within the cell is seen in the ^{14}C uptake rates. ^{14}C uptake was monitored over two light periods and one intervening dark period. The initial rates of uptake were always greater than pp^C whereas the rates for the second light period approached pp^C and at the two highest light intensities actually were less than pp^C (Table 1). An endogenous source of carbon, reducing the rate of ^{14}C uptake, would explain such a result. The difference between the first and second 24-hour periods of ^{14}C uptake along with differences in organic release (E^C) during the same time periods gives an estimate of respiratory activity during the light (R^C_I). R^C_I was added to measurable night time losses of carbon (R^C_D) during the intervening dark period to obtain a total estimate of daily respiration, R^C (Table 1). R^C, thus, underestimated total daily respiratory activity by the extent to which respiratory losses during the dark period, R^C_D, underestimated the true respiratory rate. R^C was linearly related to pp^C for all light intensities except the highest ($r^2 = 0.96$, $R^C = 0.068 + 0.47\ pp^C$). A linear relationaship between respiration and growth rate has been observed by other investigators (Myers and Graham, 1959, 1961, 1971; Pickett,

TABLE 1.

Effect of light intensity (I^0) on: the rate of particulate production (pp^C), estimates of pp^C from ^{14}C uptake, rate of organic release (E^C), rate of respiration in the light (R^C_I), respiratory loss in the dark (R^C_D) and total respiration (R^C), rate of gross production (GP^C), net growth efficiency (NGE) and the rate of recycling of carbon (C^r). R^C_I calculated as (A+C)-(B+D); R^C_D measured as losses of ^{14}C from prelabelled cells during dark period between 1st and 2nd light periods ^{14}C uptake.

Io	pp^C	^{14}C UPTAKE		E^C		$R^C I$	$R^C D$	R^C	GP^C	NGE	C^r
		1 st 24 HRS	2 nd 24 HRS	1 st 24 HRS	2 nd 24 HRS						
		A	B	C	D						
μ EINSTEIN$\cdot m^{2}\cdot s^{-1}$	DAY-1	DAY-1	DAY-1	DAY-1	DAY-1	DAY-1	DAY-1	DAY-1	DAY-1	pp^C/Gp^C	DAY-1
1500	1.68	2.97	1.04	0.12	0.26	1.79	0.00	1.79	3.73	0.45	1.79
650	1.99	2.73	1.70	0.11	0.21	0.93	0.11	1.04	3.24	0.61	0.93
130	1.00	1.65	1.21	0.02	0.02	0.43	0.00	0.43	1.45	0.69	0.34
38	0.32	0.75	0.54	0.02	0.01	0.21	0.08	0.29	0.62	0.52	0.21
15	0.10	0.14	0.10	0.00	0.00	0.05	0.06	0.11	0.21	0.49	0.05

1975; Laws and Caperon, 1976; Laws and Wong, 1978; Laws and Bannister, 1980). A comparison of such findings with the parameters of the relationship (intercept and slope) found for S. costatum in this study (Table 2) indicates values which are within the range of estimates found for other species. R^c_I was higher at 1500 μEinstein$\cdot m^{2} \cdot s^{-1}$ than expected on the basis of pp^c, similar to the light enhancement of respiration which can occur as a consequence of photorespiration (Jackson and Volk, 1970; Tolbert, 1974).

Organic Carbon Release and Light Intensity. The rate of organic carbon release (E^c) increased with light intensity from no release at 15 μEinstein$\cdot m^{2} \cdot s^{-1}$ to rates of 0.12 and 0.26 day^{-1}, at the highest light intensity (Table 1). The rates of E^c observed during the ^{14}C uptake were not statistically different ($p>0.05$) than those observed for several days following the flushing of the culture. Since R^c for reasons discussed above (see page 38) was calculated from the ^{14}C uptake data then the values for E^c during the same time period will be used for further calculations. Increased rates of organic release with increases in light is consistent with findings not only for many other microalgae (Hellebust, 1974) but also for S. costatum (Ignatiades and Fogg, 1973). Ignatiades and Fogg similarly found that at high light intensities S. costatum released 10-13% of the carbon fixed into the particulate fraction as organic carbon, whereas this could be reduced to 2% at low light intensities.

TABLE 2.

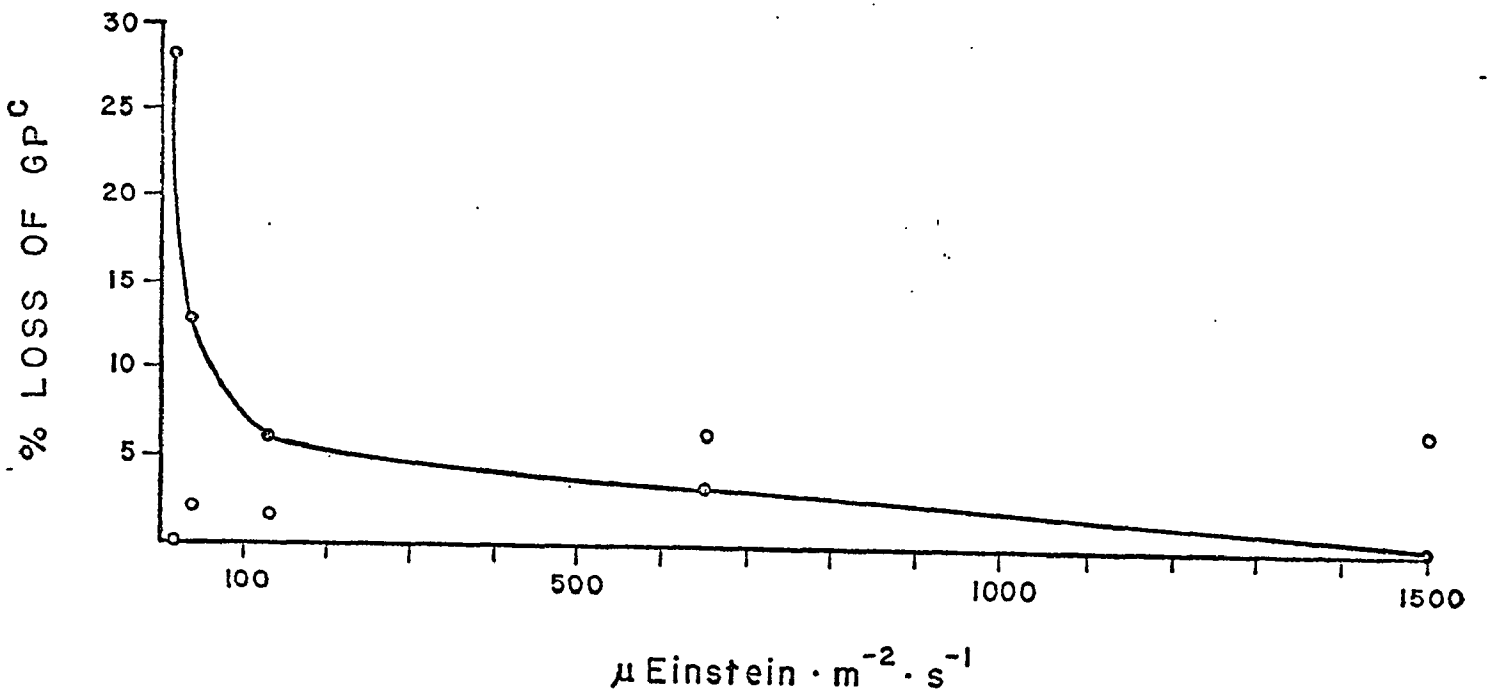
Comparison of parameters describing the linear relationship between carbon specific growth (pp^C) rates and respiration (R^C) or night-time losses of carbon in several microalgae; intercept indicates non-growth rate dependent fraction, slope indicates the growth rate dependent fraction; 95% confidence limits indicated in parentheses; * denotes studies in which data was originally reported $\mu l\ O_2$ (mg dry wt.) $^{-1}h^{-1}$ and have been converted to carbon equivalents assuming a respiratory quotient of 1.0 and a carbon content of 40% of the dry weight; hourly rates have been converted to daily rates.

GROWTH RATE RANGE DAY^{-1}	INTERCEPT Day^{-1}	SLOPE	SPECIES	TEMP. ^oC	SOURCE
0.10 -1.99	0.068 (±0.062)	0.470 (±0.018)	Skeletonema costatum	20	Cosper, this study
0.22 - 1.75	0.038	0.141	Chlorella pyrenoidosa	25	*Myers & Graham, 1959
0.28 - 0.61	0.058	0.415	C. pyrenoidosa	25	*Pickett, 1975
0.35 - 2.40	0.089	0.126	C. pyrenoidosa	25	*Myers & Graham, 1971
0.37 - 1.60	-0.051	0.228	C. ellipsoidea	25	*Myers & Graham, 1961
0.19 - 0.78	0.082	0.18	Monochrysis lutheri	20	Laws & Caperon, 1976
0.13 - 0.47	0.090	0.478	M. lutheri	20	Laws & Wong, 1978
0.11 - 1.04	0.037	0.204	Thalassiosira allenii	20	Laws & Wong, 1978
0.13 - 0.52	0.029	0.742	Dunaliella tertiolecta	20	Laws & Wong, 1978

Efficiency of Growth. Gross production (GP^C) was calculated as the sum of pp^C, R^C and E^C, and net growth efficiency (NGE) as the ratio between pp^C/GP^C (Table 1). GP^C was a saturating function of light intensity similar to pp^C, and NGE varied from 0.45 to 0.69 with an optimum at 130 μEinstein$\cdot m^2 \cdot s^{-1}$. The relative importance of respiration and organic release to GP^C varied greatly with light intensity (Fig. 8). Respiratory loss was the dominant process under low light but decreased rapidly from a maximum of 28.2% with increases in light; organic carbon release increased slightly with light intensity to a level of 7% of GP^C at 1500 μEinstein$\cdot m^2 \cdot s^{-1}$.

Thus, carbon was conserved to a greater extent as light intensity was increased. Carbon recycling reduced respiratory losses of carbon, and, although organic release increased with light intensity, the relative rate remained less than 10% of GP^C even at an intensity simulating full sunlight. Ignatiades and Fogg (1974) observed photoinhibition of photosynthesis in S. costatum under full sunlight conditions in contrast to the continued high rates of photosynthesis and growth observed in this study. Although they attributed the increased excretion rates to photodamage and increased membrane permeability, that is not the likely explanation in this study. The labeling pattern of ^{14}C observed in the cell fractionation studies indicated that the small molecular weight pool was larger under high light conditions than under low. Such an increase in the soluble cytoplasmic fraction could increase organic carbon release by

Figure 8. Relationship between percent loss of gross production as respiration (.) and organic release (o) and incident light intensity (μEinstein$\cdot m^{-2} \cdot s^{-1}$).


% LOSS OF GP^C
30
25
20
15
10
5
100
500
1000
1500
μ Einstein · m^-2 · s^-1

diffusion, a process which has been found to influence the rate of organic carbon release (Ignatiades & Fogg, 1973). An additional explanation, since Skeletonema can excrete large quantities of glycollic acid (Hellebust, 1965), is that photorespiration was enhanced at high light with the concomitant release of glycollic acid (Jackson and Volk, 1970; Tolbert, 1974). Such an interpretation is consistent with the excessive increase in R^C_I at the highest light intensity.

An estimate of the compensation light intensity, I_c, indicated that S. costatum can also grow at extremely low levels of light. I_c was derived from extrapolating pp^C and pp^{chl} to zero growth rate to obtain values of 4.0 and 2.0 $\mu Einstein \cdot m^{2} \cdot s^{-1}$, respectively. Similarly low values for I_c have been found for S. costatum by other investigators (Falkowski and Owens, 1978; Yoder, 1979). A comparison of these I_c values with total daily incident radiation estimated for temperate areas (Kimball, 1924) indicates that such a light intensity would most likely be below the 1% light level throughout the year and approximately equivalent to the 0.2% light level throughout much of the summer. Thus, given the time for adaptation to low light and the fact that I_c for S. costatum is not temperature dependent (Yoder, 1979) it appears that S. costatum could be growing, although slowly, well below the 1% light depth.

CONCLUSIONS

S. costatum minimized losses of carbon with increases in growth rate. Recycling within the cell of respired carbon back into photosynthetic pathways was hypothesized to account for this finding. A comparison of R^C with measurable losses of carbon gave an estimate of the rates at which carbon might have been recycled (C^r) (Table 1). C^r was 44% of total respiration even at the lowest light intensity and increased hyperbolically with light intensity reaching 100% at 1500 μEinstein$\cdot m^{2} \cdot s^{-1}$.

Raven (1972 a and b; 1974) has emphasized the importance of endogenous sources of inorganic carbon for photosynthesis and anaplerotic pathways and has calculated a potential recycling of respired carbon of up to 100% in microalgae. The light dependent suppression of the loss of ^{14}C as CO_2 from labelled cells is a well known phenomenon (Ryther, 1956 b; Hoch et al., 1963; Bunt, 1965; Brown and Tregunna, 1967) and has been related to the supply of CO_2 in Chlorella (Bidwell, 1977) as well as in microalgae and higher aquatic plants (Hough and Wetzel, 1972; Hough, 1976). Tolbert (1974) has suggested that the efficient refixation of CO_2 derived from photorespiration which occurs in terrestrial C_4-plants is also true of algae. In addition to the Calvin cycle of photosynthesis another pathway of carbon assimilation involving phosphoenol pyruvate carboxylase (PEPCase) is present in these C_4-plants and is commonly referred to as C_4 metabolism. Recently, C_4

metabolism has been demonstrated in marine phytoplankton (Beardall et al., 1976) and the activity has been measured to be extremely high in S. costatum relative to other marine microalgae (Morris, 1980). The importance of PEPCase activity to the recycling of respired carbon in marine microalgae needs to be investigated. However, if PEPCase were involved in carbon recycling then such high activity in S. costatum could explain the high rates of recycling observed in this study. Although the refixation of respired carbon does not appear to be exclusive of any particular plant group, relative rates vary considerably. Perhaps the recycling of respired carbon is an important mechanism allowing for high growth rates at high light intensities and might explain, in part, the ability of some marine microalgae, particularly, S. costatum to bloom under field conditions.

The effects of variations in light intensity on growth rate were largely manifest as alterations in the concentrations of cellular pigments, particularly chlorophyll a. Changes in the C:Chl a ratio (except at the highest light intensity) were positively related to growth rate, whereas other cellular characteristics, particularly the C:N ratio, were unreflective of variations in growth rate. The import of the increased cellular chlorophyll a concentration under a light intensity simulating full sunlight is unclear, however, it is significant that growth was not inhibited

and gross production remained high under such a high light intensity. Rates of organic release increased under high light but never exceeded 10% of gross production.

Net growth efficiency decreased with variations in light intensity from an optimal level at 130 μEinstein$\cdot m^{2} \cdot s^{-1}$ mainly as a consequence of variations in rates of respiration relative to growth rate. Calculations of respiration rates indicated that observed respiratory losses of carbon were not necessarily reflective of respiratory activity and the disparities between the two measures were enhanced as growth rate and light intensity increased.

PART II. EFFICIENCY OF GROWTH UNDER DIURNALLY VARYING LIGHT

RESULTS AND DISCUSSION

Daily Growth Rates and Cellular Biomass Characteristics

Daily rates of growth were unaffected by diurnal variations in light intensity (Table 3). Thus, growth rate reflected the total amount of daily radiation despite fluctuations in light levels throughout the light period. However, cell size was modified by diurnal variations in light intensity so that cell volume decreased relative to values under diurnally constant light (Table 3). Carbon per cell decreased significantly ($p<0.05$) from a daily mean of 23.1 pg $cell^{-1}$ under constant light to 17.9 pg $cell^{-1}$ under one cycle day^{-1}. Cellular nitrogen similarly decreased under diurnal variations in light and the C:N ratio remained relatively constant. Cellular volume reflected the decrease in carbon per cell and carbon per unit volume remained consistent with the relationship found by Strathman (1967). Daily mean chlorophyll *a* $cell^{-1}$ was unaffected by diurnal variations in light. Carotenoids and chlorophyll c_1 & c_2 decreased relative to chlorophyll *a* concentrations under diurnally varying light, and this was reflected in an increase in Chl*a*:Carotenoids and Chl*a*:Chl c_1 & c_2. The C:Chl*a* ratio was reduced significantly under diurnal variations in light intensity, mainly as a consequence of reduced carbon per cell.

TABLE 3.

Comparison of daily mean cellular biomass characteristics and growth rates under diurnally constant and variable light regimes; C.L. - 95% confidence limits.

I_o:	Constant		12 Cycles day^{-1}		1 Cycle day^{-1}	
Variable	$\bar{X}$	C.L.	$\bar{X}$	C.L.	$\bar{X}$	C.L.
Divisions, Day^{-1}	0.91	± 0.17	1.12	± 0.28	1.01	± 0.16
Carbon cell^{-1} pg cell^{-1}	23.1	± 1.68	20.3	± 1.45	17.9	± 0.95
Nitrogen cell^{-1} pg cell^{-1}	3.0	± 0.21	2.6	± 0.20	2.6	± 0.14
C:N	7.6	± 0.42	7.9	± 0.41	7.2	± 0.20
Cell Vol. μm^3	161	±35	116	±20	120	±25
Chlorophyll *a* cell^{-1} pg cell^{-1}	0.51	± 0.04	0.55	± 0.04	0.57	± 0.04
Chl*a*:Carotenoids	1.4	-	1.95	± 0.10	1.98	± 0.10
Chl*a*:Chlc_1&c_2	3.5	-	14.9	±10.8	9.2	± 3.8
C:Chl*a*	46	± 3.0	37	± 2.6	32	± 1.8

Continuous fluctuations in light intensity during the light period with photoperiod and total daily radiation kept constant did not alter mean daily cellular chlorophyll *a* concentrations. Cellular chlorophyll *a* content was related to mean daily light intensity and not to the maximum incident intensity as Eppley and Dyer (1965) found for *Dunaliella tertiolecta*. These variations in cellular characteristics are in marked contrast to the 6 fold variation in pigment content of cells but uniform cell size observed when *S. costatum* was grown at discrete light levels over the same range of light variation (Fig. 3).

Smaller cell size but uniform levels of chlorophyll *a* $cell^{-1}$ allowed division and carbon specific growth to be maintained by cultures grown under fluctuating light at rates comparable to constant light even though pp^{chl} (as indicated by C:Chl*a*) declined. Rates of division rather than carbon production were conserved. Quraishi and Spencer (1971) found for several marine microalgae that division rates were unaffected in some species but reduced in others by diurnal variations in light intensity as compared to a constant intensity during the light period. However, they did not compare variations in cell size between the two groups. Marra (1978 *b*) also found that division rates were reduced in *Lauderia borealis* by short term fluctuations (hourly) in light intensity. Perhaps the ability to vary cell size and consequently maintain growth rates proportional to total daily radiation is a species specific characteristic

which could have adaptive significance in the marine pelagic environment. The ability to maximize division rates in an environment where variations in light intensity can be rapid and extreme would confer a selective advantage on a species.

Respiration, Organic Release, and Efficiency of Growth

Rates of respiration and organic release were both elevated under diurnally varying light in comparison to constant light (Table 4). Total respiration (R^C) was twice as high under 1 cycle day^{-1} and elevated 4 fold under 12 cycles day^{-1}. Night-time respiratory losses (R^C_D) were similarly increased under both variable light regimes in contrast to no detectable loss under constant light. However, respiration in the light (R^C_I, as calculated on p.38) was substantially increased under 12 cycles day^{-1} but only slightly under 1 cycle day^{-1}. Rates of organic release (E^C) were 4 to 7 fold higher under variable light than under constant light. Consequently, although particulate rates of production (pp^C) remained unchanged under the variable light regimes, gross production ($GP^C = pp^C + R^C + E^C$) was higher than under the constant light regime (Table 4). GP^C doubled under 1 cycle day^{-1} and was 2.5 times higher under 12 cycles day^{-1}. Net growth efficiency ($NGE = pp^C/GP^C$) decreased from 0.69 under constant light to 0.38 and 0.50 under 12 and 1 cycle day^{-1}, respectively. The lower NGE under 12 cycles day^{-1} was a consequence of the increased respiration during the light period. Since daily growth rates were unchanged and NGE decreased under

TABLE 4.

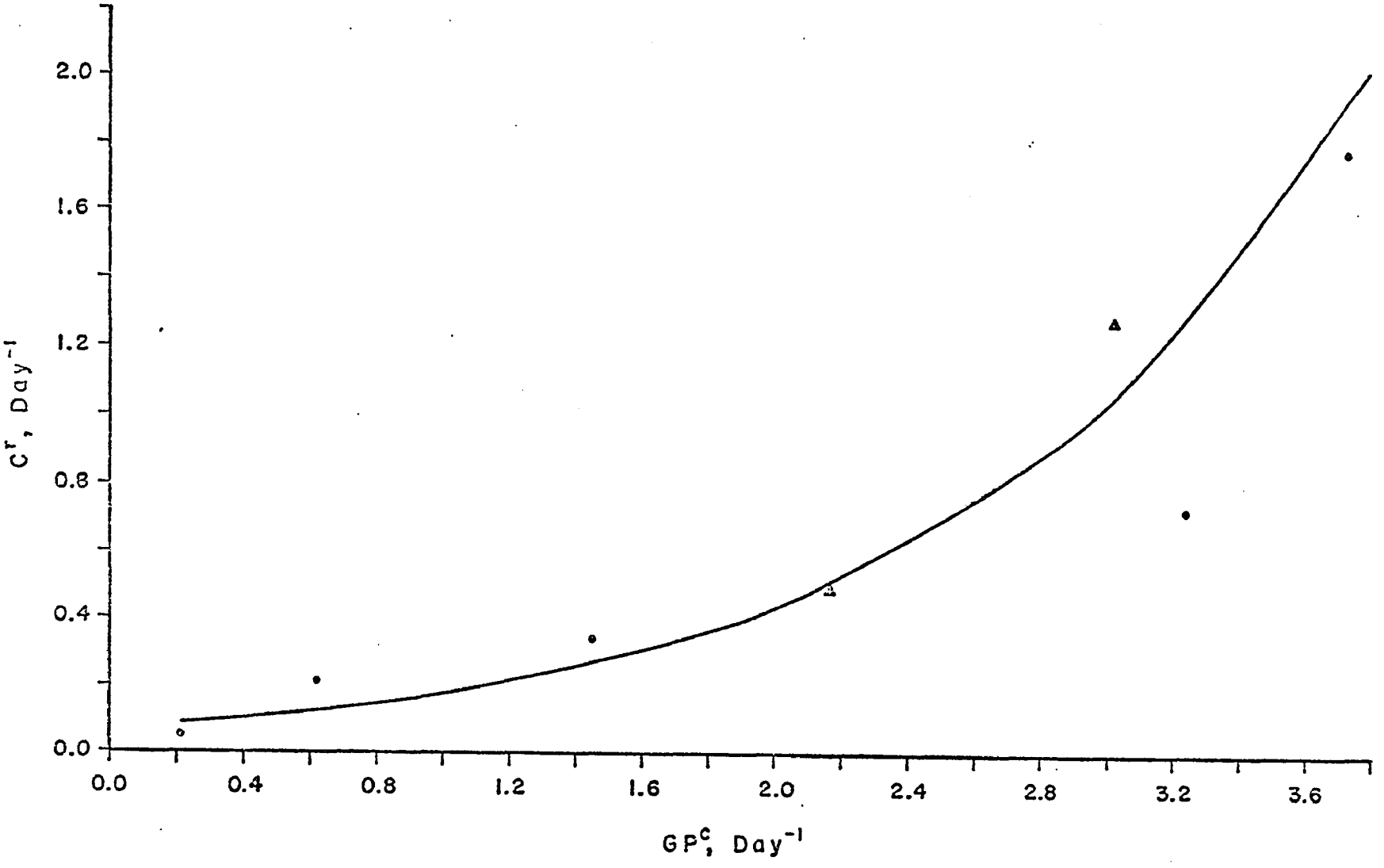
Effect of fluctuations in light intensity (I_o) on: the rate of particulate production (pp^C), estimates of pp^C from ^{14}C uptake, rate of organic release (E^C), calculated rates of respiration in the light (R^C_I) observed respiratory loss in the dark (R^C_D) and estimates of total respiration (R^C), rate of gross production (GP^C), net growth efficiency (NGE), the proportion of R^C recycled (C^r), and the proportion of GP^C as E^C loss or respiration loss (Resp. Loss).

I_o		^{14}C Uptake		E^C									
		1st	2nd	1st	2nd								Resp.
5.6 Einstein $m^{-2} \cdot day^{-1}$	pp^C day^{-1}	24 hrs day^{-1}	24 hrs day^{-1}	24 hrs day^{-1}	24 hrs day^{-1}	R^C_I day^{-1}	R^C_D day^{-1}	R^C day^{-1}	GP^C day^{-1}	NGE pp^C/GP^C	C^r/R^C %	E^C/GP^C %	Loss/GP^C %
CONSTANT	1.00	1.65	1.21	0.015	0.024	0.43	0.000	0.43	1.45	0.69	78	1.7	6.2
12 CYCLES DAY^{-1}	1.15	2.31	0.81	0.074	0.170	1.40	0.300	1.70	3.02	0.38	76	5.6	13.6
1 CYCLE DAY^{-1}	1.08	1.83	1.19	0.074	0.160	0.55	0.370	0.92	2.16	0.50	54	7.4	19.5

diurnally fluctuating light regimes relative to the constant regime then the increase in GP^c indicates that photosynthetic efficiency was increased.

The amount of carbon which was released as a proportion of GP^c increased from 7.9% under constant light to 19.2% under 12 cycles day^{-1} and 26.9% under 1 cycle day^{-1}. Loss of carbon due to organic release was increased under the variable light regimes but still remained less than 10% of GP^c (Table 4). However, respiratory loss of carbon increased 2 to 3 fold under variable light and was the greatest under 1 cycle day^{-1}. Calculations of the rate of recycling of carbon (C^r), determined by comparing respiratory loss of carbon to total respiration (the difference between the two estimates equals C^r), revealed that under 12 cycles day^{-1} rates of C^r remained high, 76% of R^c (Table 4). Under 1 cycle day^{-1} C^r was reduced to 54% of R^c. Relative losses of fixed carbon were not as great under 12 cycles day^{-1} as under 1 cycle day^{-1} because C^r was increased. This is consistent with observations of increased R^c_I and C^r as light intensity is increased under constant light regimes from 15-1500 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ (Table 1). C^r increased exponentially with GP^c under all light regimes ($C^r = 0.074e^{0.87GPc}$, $r^2 = 0.91$) (Fig. 9). Thus, high rates of photosynthesis appeared to be related to increased rates of carbon recycling.

Figure 9. Relationship between the rates of recycling of carbon (C^r) and the rate of daily gross production (GP^c) for cultures grown under diurnally constant light intensity (.) and diurnally fluctuating light (▲); line represents relationship: $C^r = 0.074e^{0.87GP^c}$, $r^2 = 0.91$.


2.0
1.6
1.2
0.8
0.4
0.0
C^r, Day^{-1}
0.0
0.4
0.8
1.2
1.6
2.0
2.4
2.8
3.2
3.6
GP^c, Day^{-1}

CONCLUSIONS

Growth rates were maintained at equivalent rates under diurnal fluctuations in light despite decreased growth efficiency because gross production was enhanced. Decreased efficiency of growth, particularly under the 12 cpd light regime, related to enhanced respiration in the light. Although in this study evidence is lacking, such an increase in respiration in the light is consistent with the effects of photorespiratory activity (Jackson and Volk, 1970; Tolbert, 1974). Harris (1973) and Harris and Lott (1973) have suggested that short term (hours) fluctuations between high and low light intensities can induce higher rates of photorespiration. It is well documented for many plants that photorespiration decreases the efficiency of growth (Zelitch, 1971).

The C:Chl *a* ratio decreased under fluctuating light as a consequence of a decrease in cell size and resulted in a relative increase in carbon fixed per cell. Increased rates of GP^C were manifested as greater rates of ^{14}C fixation during the initial uptake period under the diurnally varying light regimes in comparison to the constant light regime (Table 4). This result is consistent with field observations (Marra, 1978 *b*; Frechette and Legendre, 1978) in which short term measurements of ^{14}C uptake over several hours were enhanced under fluctuating light. In contrast, Marra

(1978 a) found that photosynthesis (O_2 production) in Lauderia borealis correlated well with total daily radiation under a variety of rates of light fluctuation. Similar findings were obtained by McCree and Loomis (1969) for cucumber plants. The ability to increase GP^C under fluctuating light may be species specific. An extrapolation of these results and the findings for S. costatum to an interpretation of ^{14}C uptake measurements with natural populations indicates that fluctuating light could influence short term (less than a photoperiod) incubations to a greater extent than 24 hour incubations. Short term measurements would estimate gross production to a greater extent, whereas the longer incubation would be more reflective of daily growth rates (Savidge, 1978).

PART III. DIEL VARIATIONS IN GROWTH, RESPIRATION AND ORGANIC RELEASE: COMPARISON OF CONSTANT AND DIURNALLY FLUCTUATING LIGHT

RESULTS AND DISCUSSION

Cell Division Periodicity

Under the diurnally constant light regimes diel variations in cell number were significant ($p<0.05$) only at light intensities of 130 μEinstein$\cdot m^{-2}\cdot s^{-1}$ and greater when growth rates were 1.00 day^{-1} or higher (Table 5). Maximal division rates (μ) occurred between 1200 and 1800 hr at 650 and 130 μEinstein$\cdot m^{-2}\cdot s^{-1}$ but extended into the dark period at 1500 μEinstein$\cdot m^{-2}\cdot s^{-1}$ (Figure 10). As light intensity increased the amount of division during the dark period was enhanced.

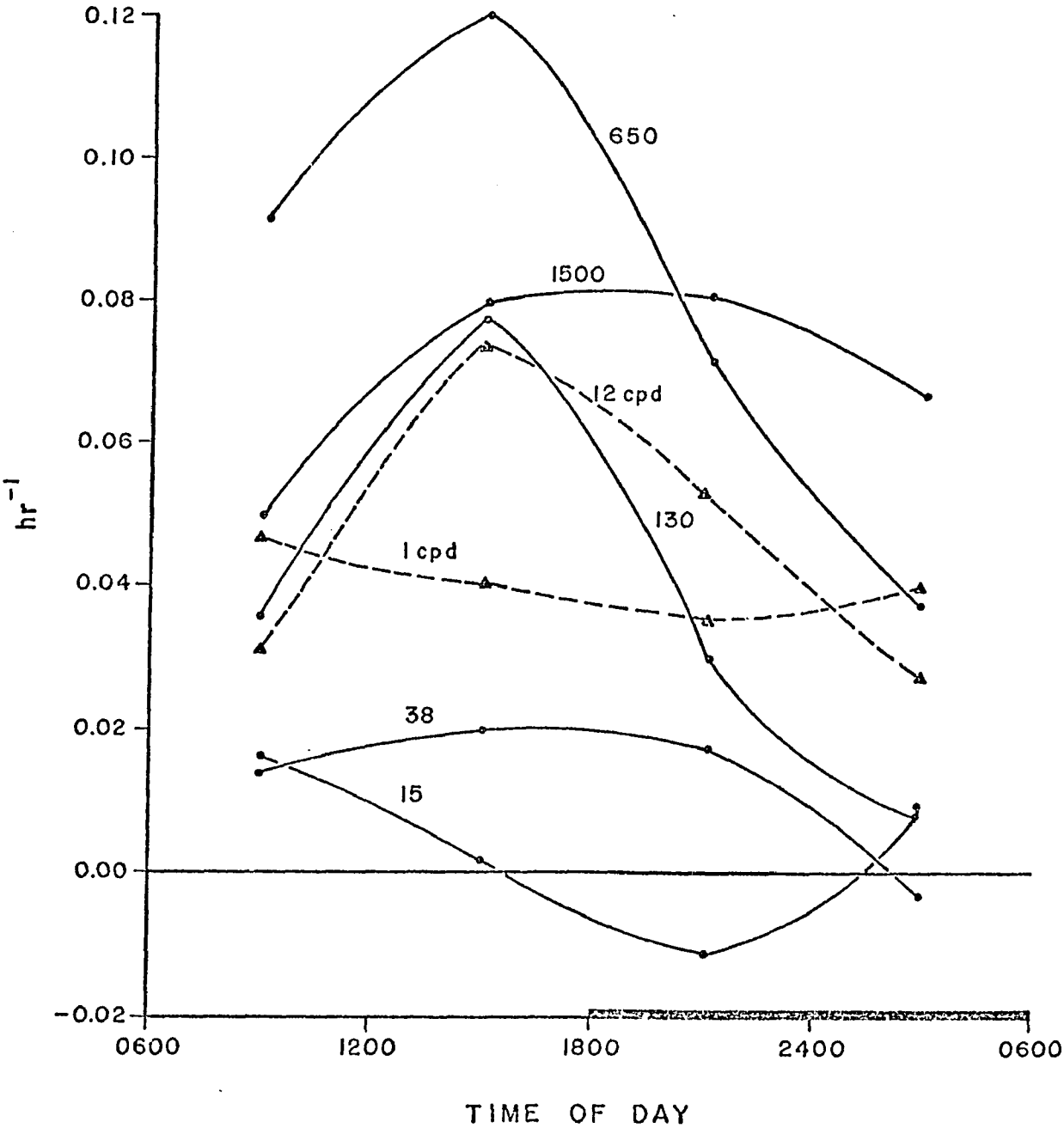
Under the variable light regimes daily growth rates remained comparable to the rate under the constant light of same diurnal magnitude (130 μEinstein$\cdot m^{-2}\cdot s^{-1}$), but diel variations in cell number were reduced (Table 5). Under 1 cycle day^{-1} division rates were uniform throughout the day and under 12 cycles day^{-1} division was maximal between 1200 and 1800 hr (Figure 10). Division during the dark period was increased under both variable light regimes relative to the constant regime. Thus, division rates at night were enhanced by high light intensities and by fluctuating light.

TABLE 5.

Diel Variations in cell number, POC and chlorophyll *a* for each light regime. I_o = μEinstein·m^{-2}·s^{-1}; μ = daily mean growth rate; $\bar{X}$ = mean value for each time of day; I---I indicates groups of values which are not significantly different (P>0.05, Student-Newman-Keuls test of difference, see Appendix II for ANOVA Tables).

	I_o:	15				38				130				650				1600				12 cycles day^{-1}				1 cycle day^{-1}			
	μ:	0.10				0.32				1.00				1.99				1.68				1.15				1.03			
VARIABLE																													
CELL NUMBER																													
Time		2400	0600	1200	1800	0600	1200	1800	2400	0600	1200	2400	1800	0600	1200	2400	1800	1200	1800	2400	0600	1200	0600	1800	2400	0600	2400	1200	1800
$\bar{X} \times 10^6 ml^{-1}$		2.898	2.958	3.235	3.250	3.704	3.964	3.790	4.075	3.756	3.866	4.714	4.755	2.802	3.011	3.740	3.895	4.034	4.356	4.727	4.978	3.213	3.501	3.730	3.963	3.963	4.043	4.202	4.203
		I----------			----------I	I----------			----------I	I---------		I---------		I---------		I---------		I----------			----------I	I---------				I----------			----------I
																			I----------		----------I		I----------		----------I				
POC																													
Time		0600	2400	1200	1800	0600	2400	1200	1800	0600	1200	2400	1800	0600	1200	2400	1800	0600	1200	2400	1800	0600	1200	2400	1800	2400	0600	1200	1800
$\bar{X}$ $\mu g ml^{-1}$		1.009	1.016	1.031	1.107	0.778	0.798	0.812	0.834	0.846	0.968	0.979	1.167	0.581	0.644	0.754	0.830	0.803	0.960	0.990	1.220	0.635	0.673	0.705	0.835	0.626	0.667	0.735	0.855
		I----------			----------I	I----------			----------I	I----------			I--I	I---------		I---------		I----------			I--I	I----------			I--I	I---------			I--I
Chle																													
Time		2400	0600	1200	1800	0600	1200	2400	1800	0600	1200	2400	1800	0600	1200	2400	1800	0600	1200	2400	1800	0600	1200	2400	1800	0600	2400	1200	1800
$\bar{X}$ $\mu g \times 10^{-2} ml^{-1}$		2.74	2.86	3.42	4.10	2.16	2.33	2.37	2.72	1.53	1.98	2.00	2.53	0.45	0.50	0.59	0.70	1.61	1.95	1.99	2.41	1.51	1.90	1.95	2.47	1.91	2.24	2.47	2.88
		I--------I		I--I	I--I	I--I	I--------I		I--I	I--I	I--------I		I--I	I--I	I--I	I--I	I--I	I--I	I--------I		I--I	I--I	I--------I		I--I	I--I	I--------I		I--I

Figure 10. Effect of variations in incident light intensity (I_o) on hourly rates of cell division (hr^{-1}) during 24 hour period; I_o : (1) constant during light period at intensities of 15, 38, 130, 650 & 1500 μEinstein$\cdot m^{-2}\cdot s^{-1}$ (———.———), (2) fluctuating during light period from 500 to 10 μEinstein$\cdot m^{-2}\cdot s^{-1}$ at rates of 1 or 12 cycles day^{-1} (cpd) (---▲---); black bar indicates dark period.

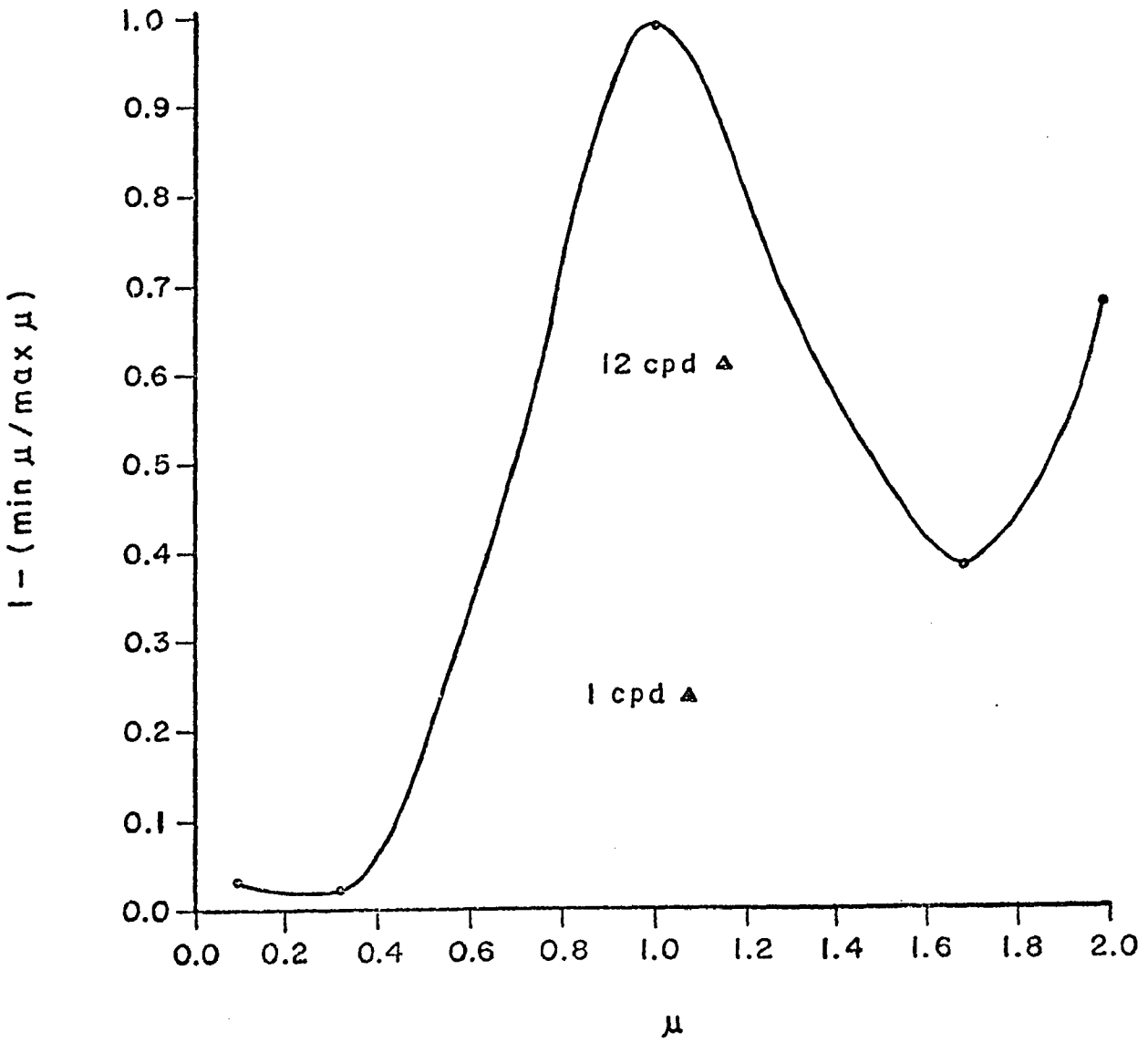

0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
hr⁻¹
650
1500
12 cpd
130
1 cpd
38
15
0600
1200
1800
2400
0600
TIME OF DAY

Jørgensen(1966) also found with S. costatum grown under an identical photoperiod at approximately 52 μEinstein$\cdot m^{-2} \cdot s^{-1}$ that division rates (approximately 1 division day^{-1}) were highest during the second half of the light period and that at a higher light intensity, approximately 175 μEinstein$\cdot m^{-2} \cdot s^{-1}$, more division occurred during the dark period.

Growth of S. costatum under nitrogen limitation caused most division to take place during the dark period (Eppley et al., 1971). This flexibility in the timing of division has been documented for many other diatoms (Eppley et al., 1967; Paasche, 1968; Nelson and Brand, 1979). The distinguishing feature of cell division periodicity in diatoms in comparison to other groups of microalgae is that division is not necessarily confined to the dark period (Paasche, 1967, 1968; Chisholm et al., 1975; Nelson and Brand, 1979) and environmental factors such as light (the intensity and diurnal variation) and nutrient supply are important influences on the timing of division in diatoms.

The degree of periodicity in cell division, calculated as (1 - minimum μ/maximum μ), varied with growth rate (Figure 11). Periodicity oscillated with increasing growth rate with peaks at 1.00 and 1.99 day^{-1}. Under the variable light regimes cultures grew at 1.00 day^{-1} but periodicity in division was reduced, particularly under 1 cycle day^{-1}. In contrast, Quraishi and Spencer (1971) found no reduction in synchrony under diurnally varying light relative to that under diurnally constant light for several marine microalgae.

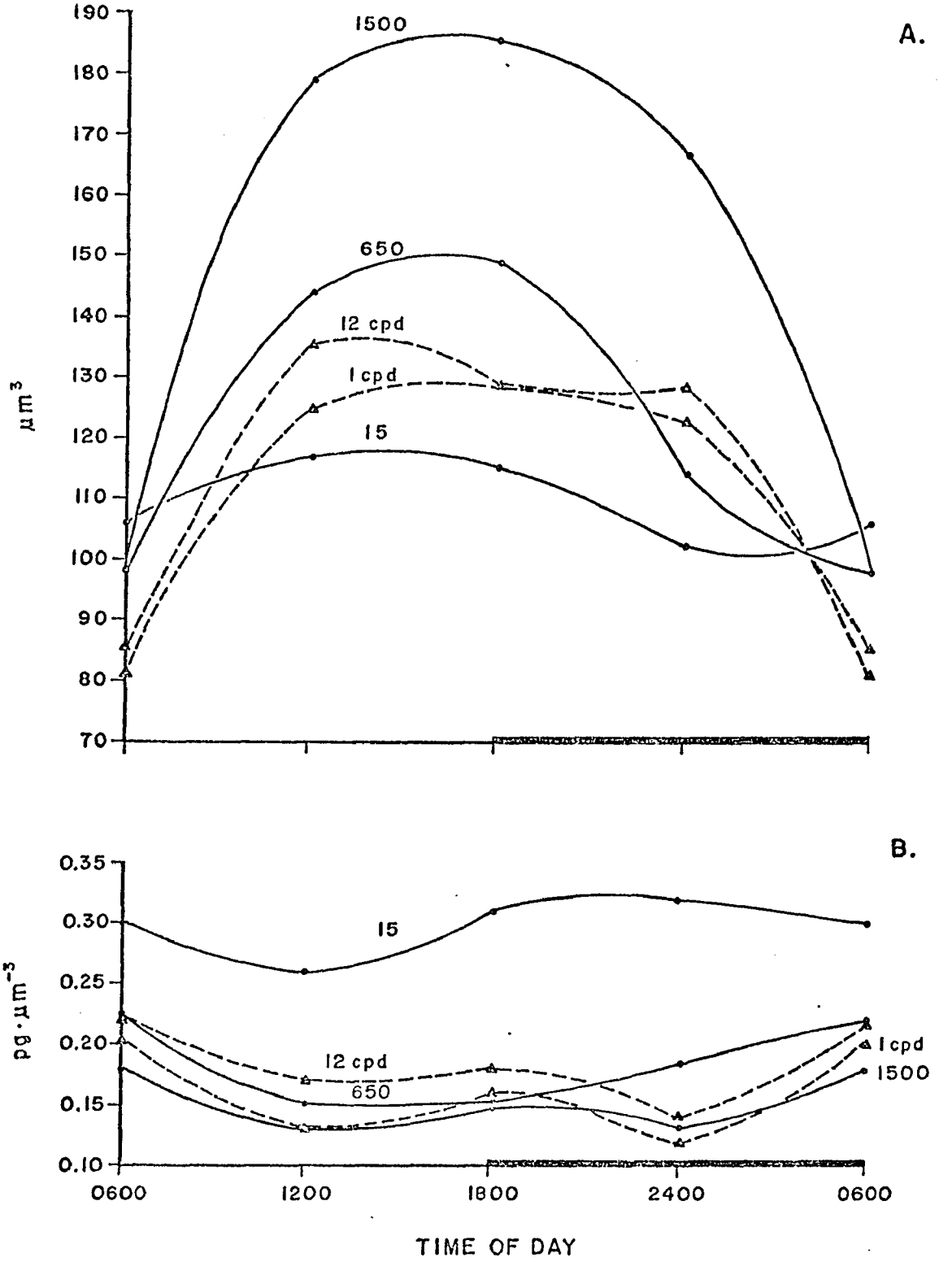
Figure 11. Relationship between mean daily growth rate (μ) and degree of periodicity in cell division (1 - (minimum μ/ maximum μ)) for cultures grown under diurnally constant (——.——) and fluctuating light (▲); cpd - cycles per day. Lines connecting data points indicate patterns of vatiation not an extrapolation of results.


1 - (min μ / max μ)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
12 cpd ▲
1 cpd ▲
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
μ

However, the magnitude of light intensity variation to which they exposed the cultures was less with a maximum of only approximately 100 μEinstein$\cdot m^{-2}\cdot s^{-1}$ in comparison to 500 μEinstein$\cdot m^{-2}\cdot s^{-1}$ used in this study. Jørgensen (1966) also found periodicity in cell division when S. costatum grew at one doubling per day in batch culture under diurnally constant light. Paasche (1968) reported for two other marine diatoms that synchrony increased with growth rate with a maximum at 1 division day^{-1}. Chisholm et al. (1975) similarly observed an oscillation in the degree of synchrony with variations in growth rate in Euglena gracilis. Good synchrony was observed even at less than one division per day. Cell division in Euglena is restricted to the dark period which induces more periodicity in division. The results of this study with S. costatum indicate that the degree of periodicity in division can be related to both division rate and diurnal fluctuations in light intensity. Environmental conditions which enhance growth rates and minimized diurnal fluctuations in light intensity might be expected to enhance periodicity in cell division. Such conditions might exist during stratification of the water column in the summer if nutrient supply remained adequate to maintain high growth rates.

Cellular volume increased during the light period, particularly as light intensity increased, and cell division maxima tended to occur during the latter part of the light period and during the dark (Figure 12A). Cell volume was minimal at 0600 hr for all light regimes. The most rapid increases in volume occurred from

Figure 12. Effect of variations in incident light intensity (I_o) on : (A) cellular volume (μm^3) (B) Carbon:Volume ($pg \cdot \mu m^{-3}$) during 24 hour period; I_o : (1) constant during light period at intensities of 15, 650 & 1500 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ (——. ——), (2) fluctuating during light period from 500 to 10 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ at rates of 1 or 12 cycles day^{-1} (cpd) (---▲---); black bar indicates dark period.


A.
1500
650
12 cpd
1 cpd
15
190
180
170
160
150
140
130
120
110
100
90
80
70
µm³
B.
0.35
0.30
0.25
0.20
0.15
0.10
pg·µm⁻³
15
12 cpd
650
1 cpd
1500
0600
1200
1800
2400
0600
TIME OF DAY

0600 to 1200 hr and were not totally a consequence of increases in carbon cell^{-1} since carbon: volume decreased (Figure 12B). Myers (1953) predicted such a temporal separation between division and other growth parameters under diurnal illumination on the basis of theoretical considerations of the efficiency of growth. Myers termed his theory the "huff and puff" phenomenon. In other words, division is delayed during the light period, while the cell increases in volume, and then a burst in cell division occurs. Shortly thereafter, Tamiya et al. (1953) confirmed the validity of such a concept in Chlorella ellipsoidea and it was also reported for Dunaliella tertiolecta (Eppley and Coatsworth, 1966). Not only did this phenomenon hold true for S. costatum but it was more related to light intensity than growth rate. Diel variations in cell volume were much greater under 1500 μEinstein$\cdot$m$^{-2}\cdot$s^{-1} when the growth rate was 1.68 day^{-1} than under 650 μEinstein$\cdot$m$^{-2}\cdot$s^{-1} when the growth rate was 1.99 day^{-1} (Figure 12A).

Diel Variation of Carbon and Pigments

The pattern of variation throughout the day was similar for POC and chlorophyll a for all light regimes: maximal values occurred at 1800 hr and minimal values at 0600 hr (Table 5). Significant ($P<0.05$) diel variations in chlorophyll a were observed under all light conditions. Under diurnally constant light POC varied significantly ($P<0.05$) only at light intensities of 130 μEinstein$\cdot$m$^{-2}\cdot$s^{-1} and greater, similar to diel variations in cell density. Under the variable light regimes diel variations in POC remained large, unlike

variations in cell density. Variations in C:Chl *a* were relatively small throughout the day except at 130 μEinstein$\cdot m^{-2} \cdot s^{-1}$ and 1 cycle day^{-1} (Table 6). The magnitude of diel variation in C:Chl *a* was greater under the constant light regime than under 1 cycle day^{-1} but even then the amount of variation was only 22% of the maximum value. Thus, the biomass characteristics indicated that particulate carbon production and net chlorophyll *a* synthesis, averaged over 6 hour intervals, were correlated with maximal rates occurring during the light period (Figure 13A&B). As growth rate increased there was an increase in night-time synthesis of both carbon and chlorophyll *a*. Only at 15 μEinstein$\cdot m^{-2} \cdot s^{-1}$ and 1 cycle day^{-1} was night-time loss of carbon observed but chlorophyll *a* loss was observed during the early part of the dark period at both 15 and 38 μEinstein$\cdot m^{-2} \cdot s^{-1}$. Carbon production during the dark period (pp^{C}_{D}, calculated from changes in POC during the dark period) varied from 19 to 34% of total daily production and increased linearly with growth rate even under the diurnally varying light regimes ($pp^{C}_{D} = -0.104 + 0.37\ pp^{C}$, $r^2 = 0.97$) (Figure 14). Night-time chlorophyll *a* synthesis was increased under variable light regimes relative to the constant regime from 2% of the daily rate under constant light to 8% under 12 cycles day^{-1} and 20% under 1 cycle day^{-1}. The low rate of chlorophyll *a* accumulation during the dark period in comparison to pp^{C}_{D} under constant light of 130 μEinstein$\cdot m^{-2} \cdot s^{-1}$ accounted for the greater diel variation in C:Chl*a* observed under this light regime.

TABLE 6.

Diel Variations in C:Chla under different light intensities; Analysis of Variance (ANOVA): C:Chla versus Time of Day (see Appendix III for ANOVA Tables); ns = not significant, $p>0.05$; $\bar{X}$ - mean value; S.E. - Standard Error; n = number of samples.

I_o						ANOVA F Value	ANOVA Significance
$\mu E \cdot m^{-2} \cdot s^{-1}$		0600	1200	1800	2400		
15	$\bar{X}$	28	25	26	30	-	-
	S.E.	-	-	-	-		
	n	1	1	1	1		
38	$\bar{X}$	36	35	31	34	2.44	ns
	S.E.	0.8	1.6	0.8	1.4		
	n	8	8	8	8		
130	$\bar{X}$	54	44	42	45	10.26	$p<0.001$
	S.E.	1.4	1.5	2.7	1.0		
	n	5	4	4	4		
650	$\bar{X}$	130	113	102	108	2.64	ns
	S.E.	5.7	4.8	3.9	12.1		
	n	4	4	4	4		
1500	$\bar{X}$	51	50	49	48	0.18	ns
	S.E.	2.0	2.0	3.7	4.0		
	n	6	6	6	4		
12 cycles day^{-1}	$\bar{X}$	41	35	34	37	2.02	ns
	S.E.	2.9	1.6	2.3	3.1		
	n	10	10	10	6		
1 cycle day^{-1}	$\bar{X}$	36	30	31	29	3.45	$p<0.05$
	S.E.	1.7	1.0	2.0	1.4		
	n	11	10	10	7		

Figure 13. Effect of variations in incident light intensity (I_o) on : (A) carbon specific rates of growth (hr^{-1}); (B) net chlorophyll _a_ synthesis during 24 hour period; I_o : (1) constant during light period at intensities of 15, 38, 130, 650 and 1500 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ (——.——) (2) fluctuating during light period from 500 to 10 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ at rates of 1 or 12 cycles day^{-1} (cpd) (---Δ---); black bar indicates dark period.

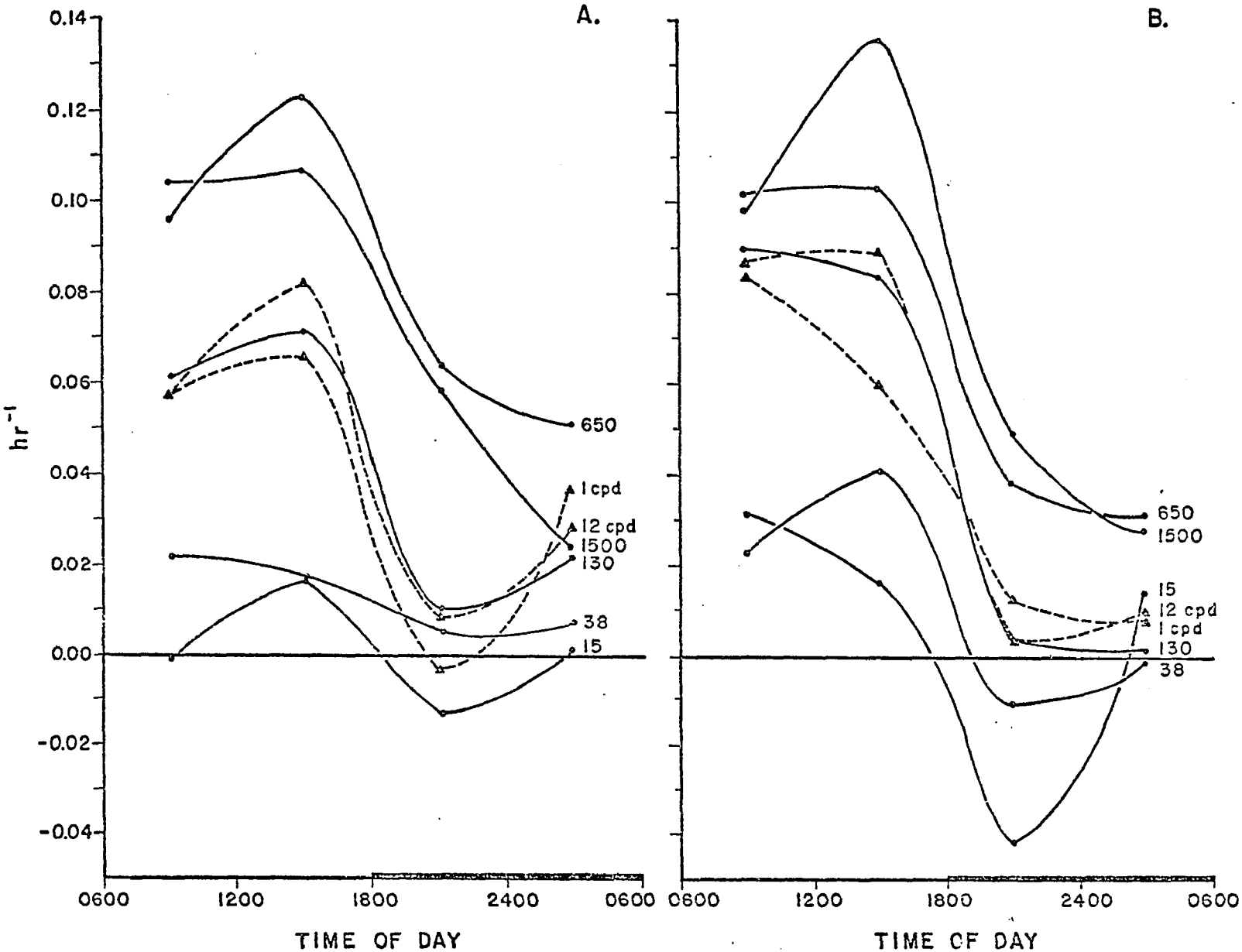
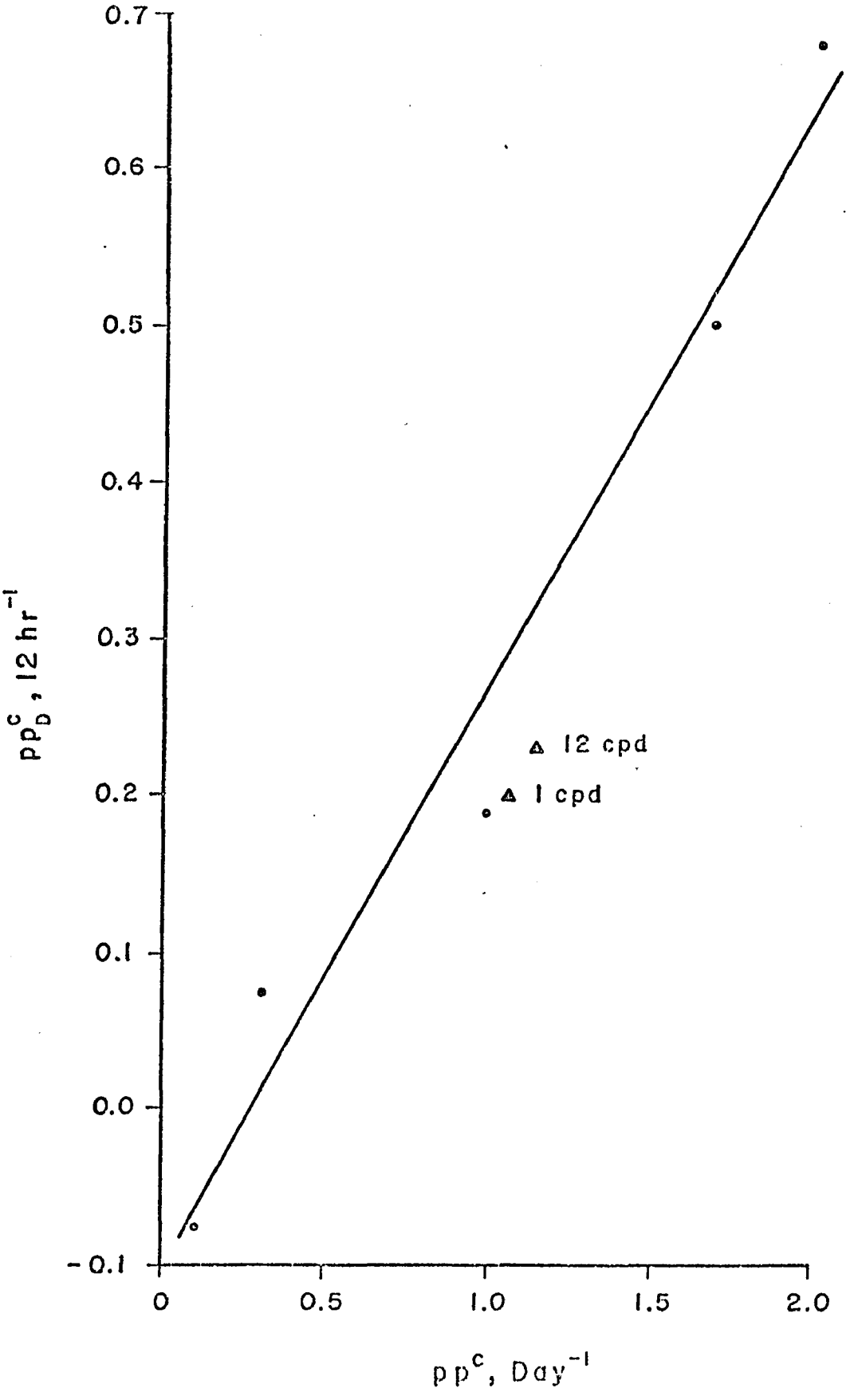

A.
B.
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
hr⁻¹
650
1 cpd
12 cpd
1500
130
38
15
0600
1200
1800
2400
0600
TIME OF DAY

Figure 14. Relationship between dark period carbon specific production (pp^{c}_{D}, 12 hr^{-1}) and daily mean carbon specific production (pp^{c}, day^{-1}) for cultures grown under diurnally constant (.) and varying light (▲) at 1 and 12 cycles day^{-1} (cpd); linear regression : pp^{c}_{D} = $-0.104 + 0.37\ pp^{c}$, $r^2 = 0.97$.

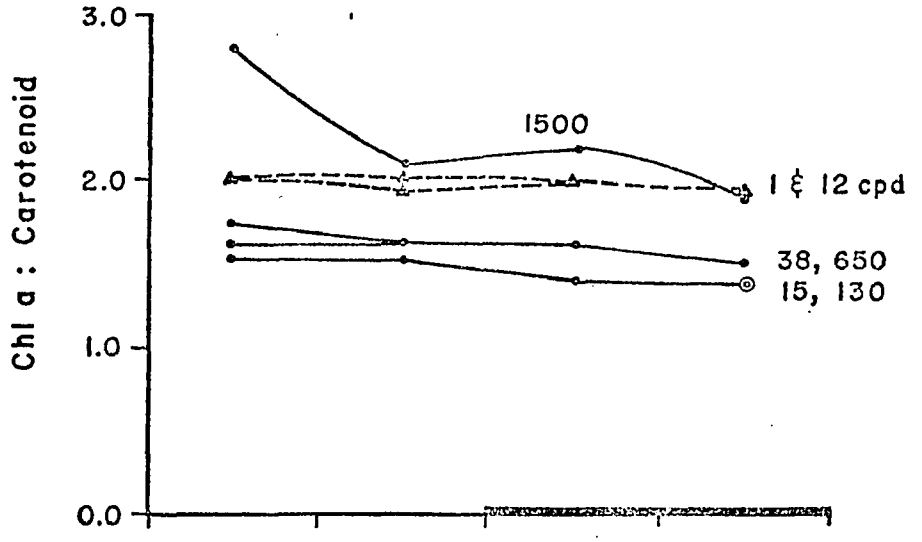

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0
0.5
1.0
1.5
2.0
pp^c_D, 12 hr^{-1}
pp^c, Day^{-1}
12 cpd
1 cpd

Diel variations in carotenoids paralleled variations in chlorophyll _a_ as evidenced by relatively uniform ratios of Chl _a_: Carotenoids throughout the day (Figure 15A). Chl _a_:Chl $\underline{c}_1$ & $\underline{c}_2$ was unvarying during the light period under the constant light regimes but elevated during the night at 15 and 1500 μEinstein$\cdot m^{-2} \cdot s^{-1}$, mainly as a result of a decrease in chlorophyll $\underline{c}_1$ & $\underline{c}_2$ (Figure 15B). Under the diurnally varying light regimes the Chl _a_:Chl $\underline{c}_1$ & $\underline{c}_2$ ratio was highest during the first part of the light period, again due to a decrease in chlorophyll $\underline{c}_1$ & $\underline{c}_2$, and decreased throughout the light period to a minimum during the dark period. Although diel variations in chlorophyll _a_ and carotenoids were well correlated, net synthesis of chlorophyll _a_ and chlorophyll $\underline{c}_1$ & $\underline{c}_2$ was not always as tightly coupled.

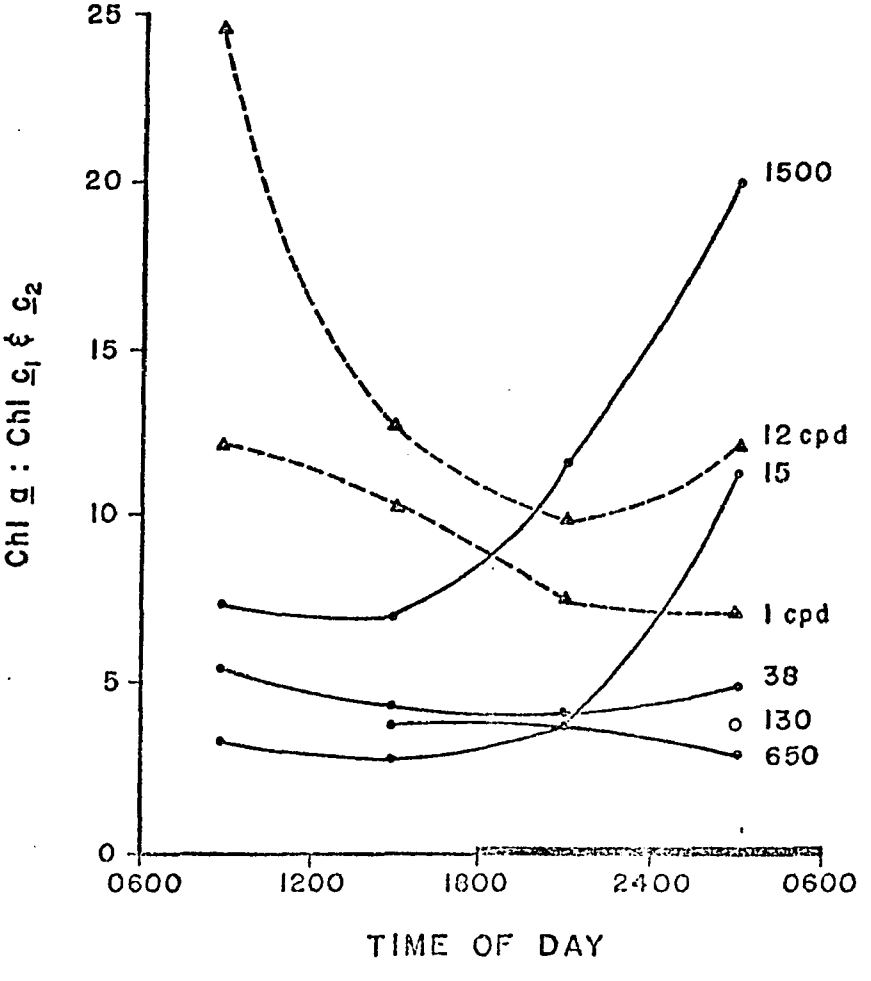
Jørgensen (1966) found diel variations of photosynthetic pigments to be well correlated in _S. costatum_. The coordination of particulate carbon production and pigment synthesis has been documented for other marine microalgae (Eppley and Coatsworth, 1966; Eppley _et al._, 1967). Eppley and Coatsworth (1966) noted that this is a reassuring finding for the interpretation of field estimates of phytoplankton growth since assessment of growth under natural conditions is largely based on chlorophyll _a_ as a biomass indicator. The results of this study extend these observations to include growth under a variety of light conditions. Differences in the relationship between carbon and pigments were more affected by light intensity than time of day (Parts I and II). Under the variable

Figure 15. Effect of variations in incident light intensity (I_o) on : (A) Chl *a*:Carotenoid; (B) Chl *a*:Chl c_1 & c_2 during 24 hour period; I_o : (1) constant during light period at intensities of 15, 38, 650 & 1500 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ (——.——) and 130 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ (o, single values); (2) fluctuating during light period from 500 to 10 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ at rates of 1 or 12 cycles day^{-1}(cpd) (---▲---); black bar indicates dark period.

A


3.0
2.0
1.0
0.0
Chl a : Carotenoid
1500
1 & 12 cpd
38, 650
15, 130

B

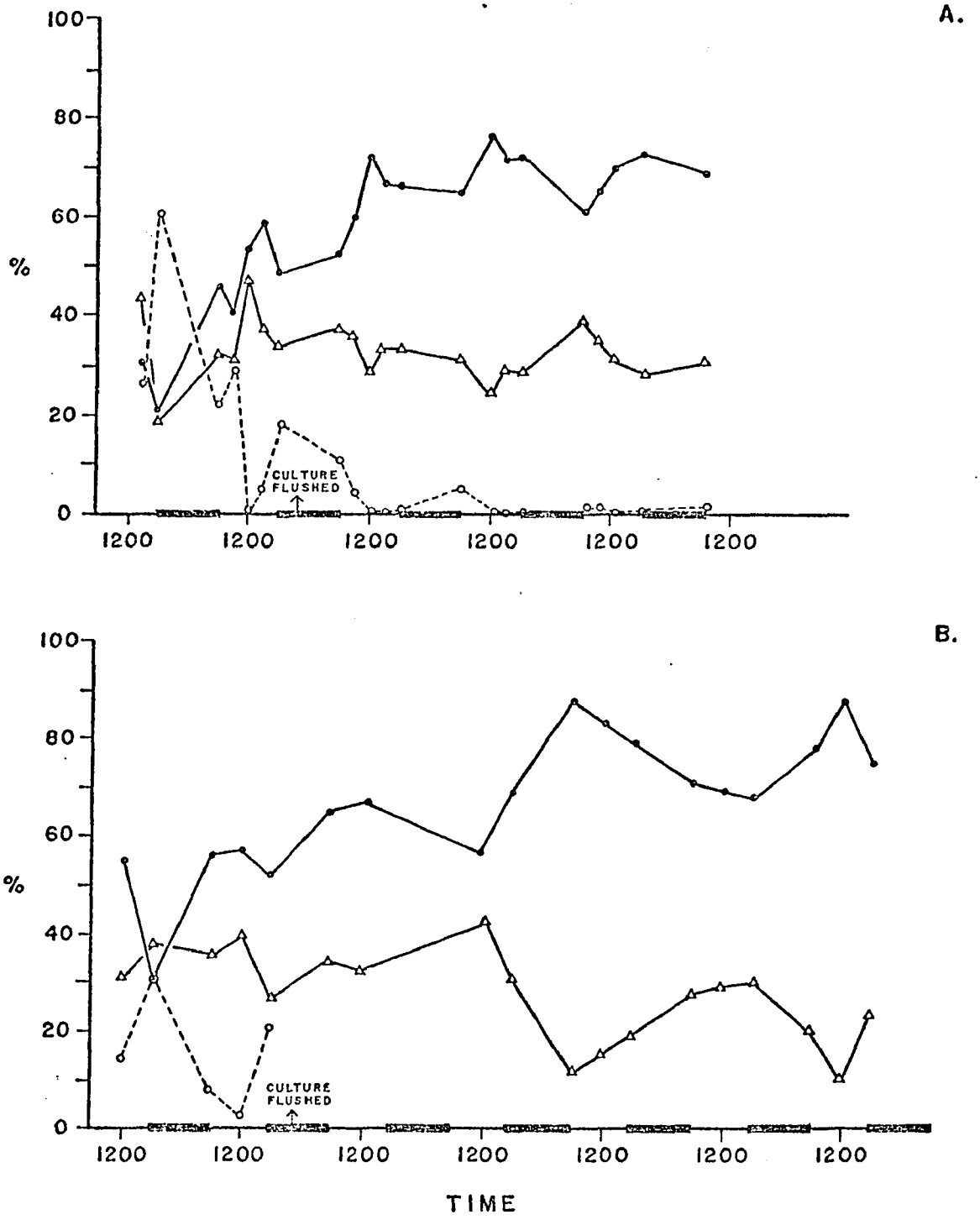

25
20
15
10
5
0
Chl a : Chl c1 & c2
1500
12 cpd
15
1 cpd
38
130
650
0600
1200
1800
2400
0600
TIME OF DAY

light regimes diel variations between carbon and chlorophyll *a* were relatively small compared to variations observed between constant light regimes. Thus, variations in the relationship between carbon and chlorophyll *a* are relatively small under continuously varying light in contrast to the large and rapid variations in chlorophyll *a* concentrations observed when cultures are exposed to different discrete light levels (Hitchcock, 1977).

Net pigment synthesis was largely confined to the light period under all light regimes, consistent with findings of Jørgensen (1966), Eppley and Coatsworth (1966), Eppley *et al.* (1967) and Marra (1978 *a* and 1980). However, Paasche (1967) found little diurnal phasing of chlorophyll *a* synthesis in *Coccolithus huxleyi* under a variety of light-dark regimes. Chlorophyll *a* synthesis is a highly dynamic process with turnover rates on the order of several hours throughout the 24 hr period (Grumbach *et al.*, 1978; Riper *et al.*, 1979). At the two lowest light intensities, 15 and 38 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$, chlorophyll *a* accumulation during the light period was greater than the daily growth rate. This was adjusted by concomitant net losses of chlorophyll *a* during the dark period as would be expected from the rapid turnover of the chlorophyll *a* pool. Also, as relatively more division occurred during the dark period, net synthesis of chlorophyll *a* increased during the dark period, indicating that the chlorophyll *a* pool was in dynamic equilibrium with net growth.

The ability of plants and marine algae to fix carbon during dark periods has been known for a long time (Craigie, 1963) and has been found to be generally true of autotrophic algae (Raven, 1974). Dark bottle fixation of ^{14}C has unfortunately been considered an artifact of the ^{14}C method and thus ignored. However, it is a real autotrophic process and can be particularly pronounced in oligotrophic waters (Morris et al., 1971) in contrast to the growth rate dependence of night-time carbon fixation found in the study reported here. A recent study by Gieskes et al. (1979) also reported dark fixation of carbon in the field, particularly for surface populations, which might be more indicative of a growth rate dependency. Particulate carbon production during the night has been reported for Chaetoceros sp. growing in continuous culture under natural light (Malone et al., 1975; Farmer, 1977) and for mixed chlorophyceae (Church et al., 1980). Raven (1974) attributed dark fixation to β-carboxylation involving anaplerotic pathways which relate to the biosynthetic function of the tricarboxylic acid cycle. The positive relationship found in this study between dark fixation and growth rate is consistent with rates of biosynthesis as they would relate to β-carboxylation. β-carboxylation enzymes have recently been found to be relatively active in carbon fixation in marine microalgae, particularly in S. costatum (Beardall et al., 1976; Appleby et al., 1980; Morris, 1980). Also, the cell fractionation studies revealed that under high light (1500 μEinstein$\cdot m^{-2}\cdot s^{-1}$) (Part 1) and the variable light regimes (Figure 16) ^{14}C label,

Figure 16. Time course of distribution of ^{14}C activity (% of total activity) in cellular fractions as small molecular weight (---o---), polysaccharide (—— Δ ——) and protein (—— .——) for cultures grown under diurnally varying light at rates of (A) 1 cycle day^{-1} (cpd) (B) 12 cpd; black bar indicates dark period.


A.
100
80
60
40
20
0
%
CULTURE FLUSHED
1200
1200
1200
1200
1200
1200
B.
100
80
60
40
20
0
%
CULTURE FLUSHED
1200
1200
1200
1200
1200
1200
1200
TIME

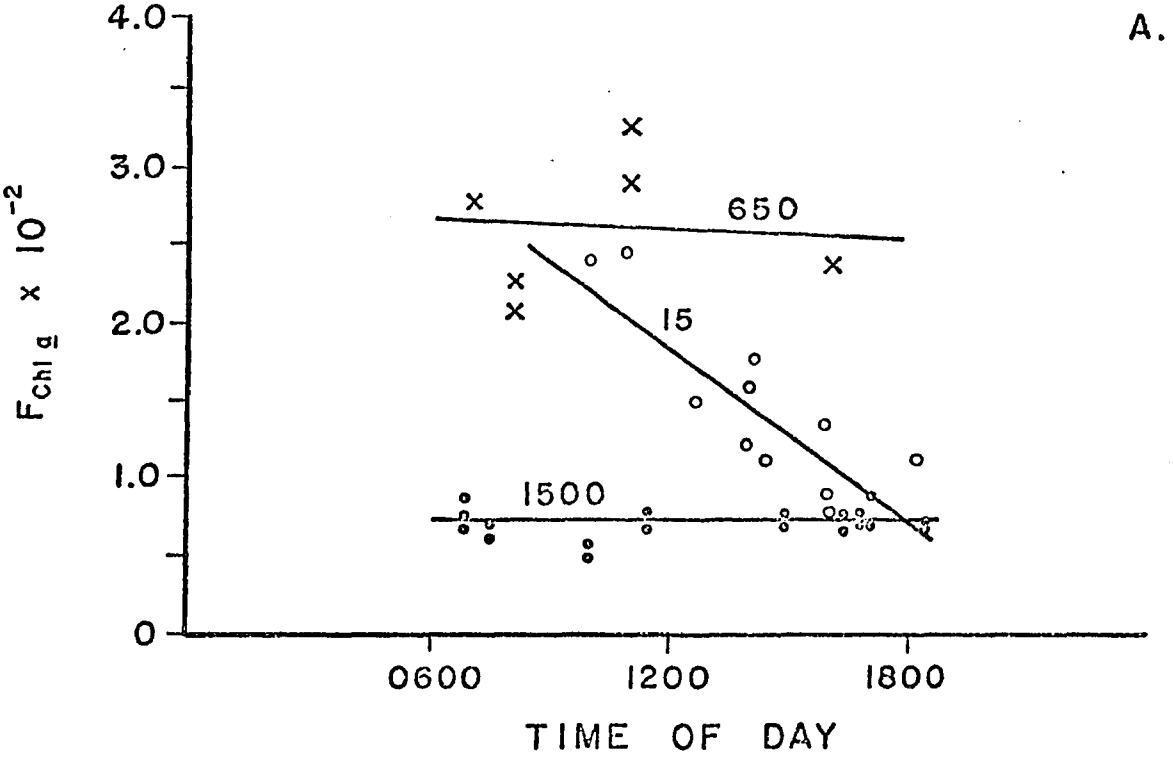
which initially appeared during the light period in small molecular weight compounds, was transferred to protein during the dark period. Protein as well as chlorophyll a synthesis at night indicate that biosynthetic pathways were active, as well as carbon fixation at night, suggest that β-carboxylation activity was involved.

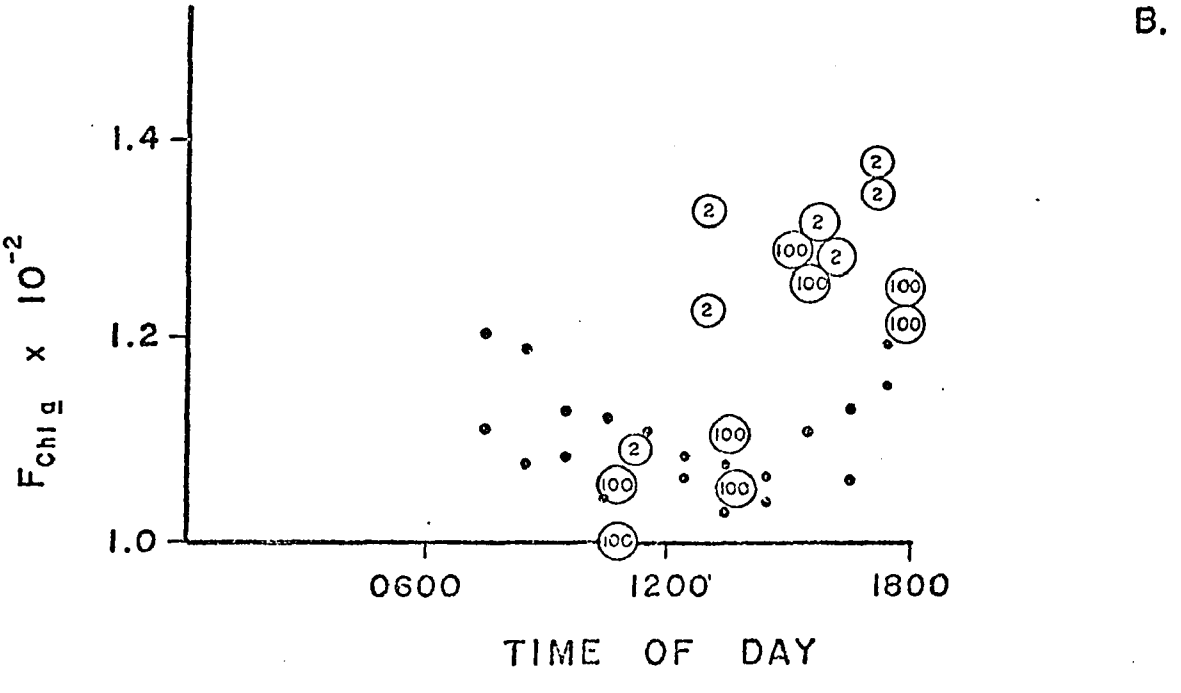
Chlorophyll a and Photosynthetic Activity

In vivo fluorescent measurements indicated that chlorophyll a synthesis was also well coupled to photosynthetic activity. At 650 and 1500 μEinstein$\cdot m^{-2}\cdot s^{-1}$, the fluorescent yield per unit chlorophyll a (F_{chl}) and the ratio between initial and DCMU-induced fluorescence (F_I/F_{DCMU}) varied only slightly throughout the light period ($p>0.05$) (Figure 17A). F_I/F_{DCMU} is an indicator of the extent to which photosynthetic activity is depressing fluorescent yield (Papageorgiou, 1975) and variations in the ratio would indicate a changing relationship between chlorophyll a and the rate of photosynthesis. However, F_{chl} at 15 μEinstein$\cdot m^{-2}\cdot s^{-1}$ decreased throughout the light period ($r=-0.86$, $p<0.01$) (Figure 17A) and this decrease correlated well with a decrease in F_I/F_{DCMU} ($r=0.91$, $p<0.01$). Thus, chlorophyll a fluorescence was less during the latter part of the light period due to increased photosynthetic activity. This is consistent with an increase in pp^{chl} to 0.3 μg C[Chl a]$^{-1}$hr^{-1} in the afternoon at this light intensity, from -0.04 μg C[Chl a]$^{-1}$hr^{-1} in the morning.

Variations of F_{chl} during the light period were relatively small under the diurnally varying light regimes (Figure 17B) (note

Figure 17. Variations of *in vivo* fluorescence per unit chlorophyll *a* ($F_{chl\ a}$) during the light period for cultures grown under (A) diurnally constant light of 15 (o), 650 (x) and 1500 (.) $\mu Einstein \cdot m^{-2} \cdot s^{-1}$; (B) diurnally varying light from 500 to 10 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ at rates of 1 cycle day^{-1} (cpd) (.) or 12 cpd (100% I_o: 100, 2% I_o: 2).


A.
4.0
3.0
2.0
1.0
0
$F_{Chl\ a}$ x 10^{-2}
650
15
1500
0600
1200
1800
TIME OF DAY


B.
1.4
1.2
1.0
$F_{Chl\ a}$ x 10^{-2}
2
100
0600
1200
1800
TIME OF DAY

scale change in y-axis). Under 12 cycles day^{-1} F_{chl} increased to a maximum at the end of the light period but variations in F_{chl}, even as light varied from 100% to 2%, did not correspond to variations in F_I/F_{DCMU} ($r=0.28$, $p>0.05$). Under such rapid fluctuations in light intensity chlorophyll *a* still remained well coupled to photosynthesis. Under 1 cycle day^{-1} F_{chl} varied directly with light intensity with a minimum at 1300 hr when the light intensity was 10 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ and maxima at 0700 and 1800 hr when light intensity was 500 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$. Variations in F_{chl} correlated well with variations in F_I/F_{DCMU} ($r=0.68$, $p<0.01$), indicating that photosynthesis was depressing fluorescent yield to a greater extent as light intensity decreased. Chlorophyll *a* was relatively more active photosynthetically during the low light part of the cycle. Thus, under 1 cycle day^{-1} the efficiency of production expressed as pp^{chl}/I_o ($\mu gC(Chl\ a)^{-1}/Einstein \cdot m^{-2}$) was inversely related to variations in light intensity such that $pp^{chl}/I_o = 8.6\ e^{-0.98Io}$ ($r^2=0.98$) (Figure 18). In contrast, production efficiencies were similar during the light period under constant light of 130 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$. Only under 1 cycle day^{-1} and 15 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ were changes in F_{chl} related to a changing relationship between chlorophyll *a* and photosynthesis.

Diel Variations in Respiration

Respiratory activity during the light period (R^c_L) increased relative to total daily respiration (R^c) as growth rate increased (Figure 19). Diel variations in respiratory loss of carbon indicated

Figure 18. Relationship between production efficiency (μg C(Chl a)$^{-1}$/Einstein$\cdot$m^{-2}) and incident light intensity (I_o, Einstein$\cdot$m$^{-2}\cdot$hr^{-1}) for cultures grown under diurnally constant I_o (.) of 130 μEinstein$\cdot$m^{-2}.s^{-1} or fluctuating I_o at a rate of 1 cycle day^{-1} (cpd) (▲); linear regression for 1 cpd values : (μg C(chl a)$^{-1}$/Einstein$\cdot$m^{-2}) = 8.6 e$^{-0.98\ I_o}$, r^2 = 0.98).

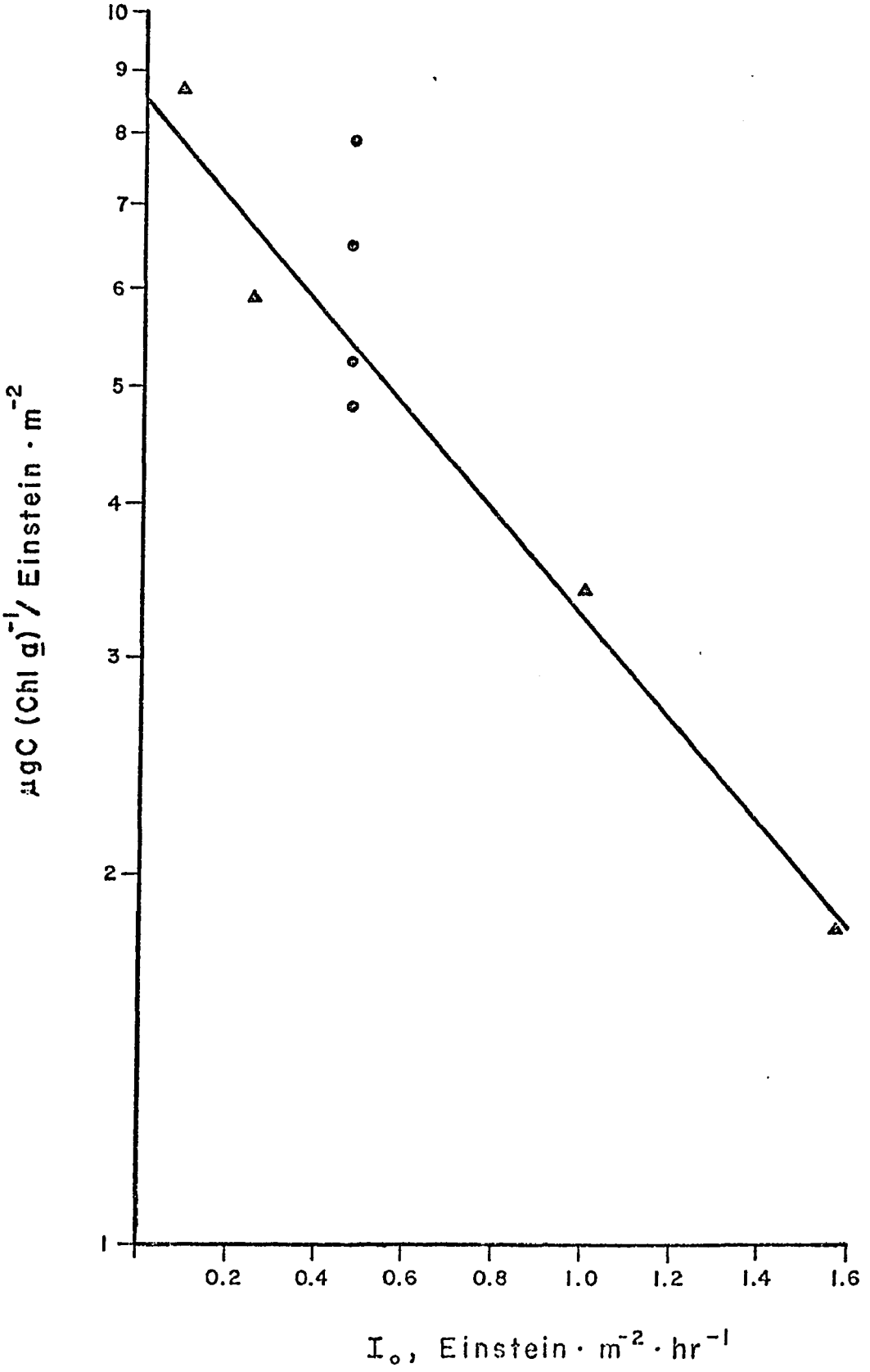
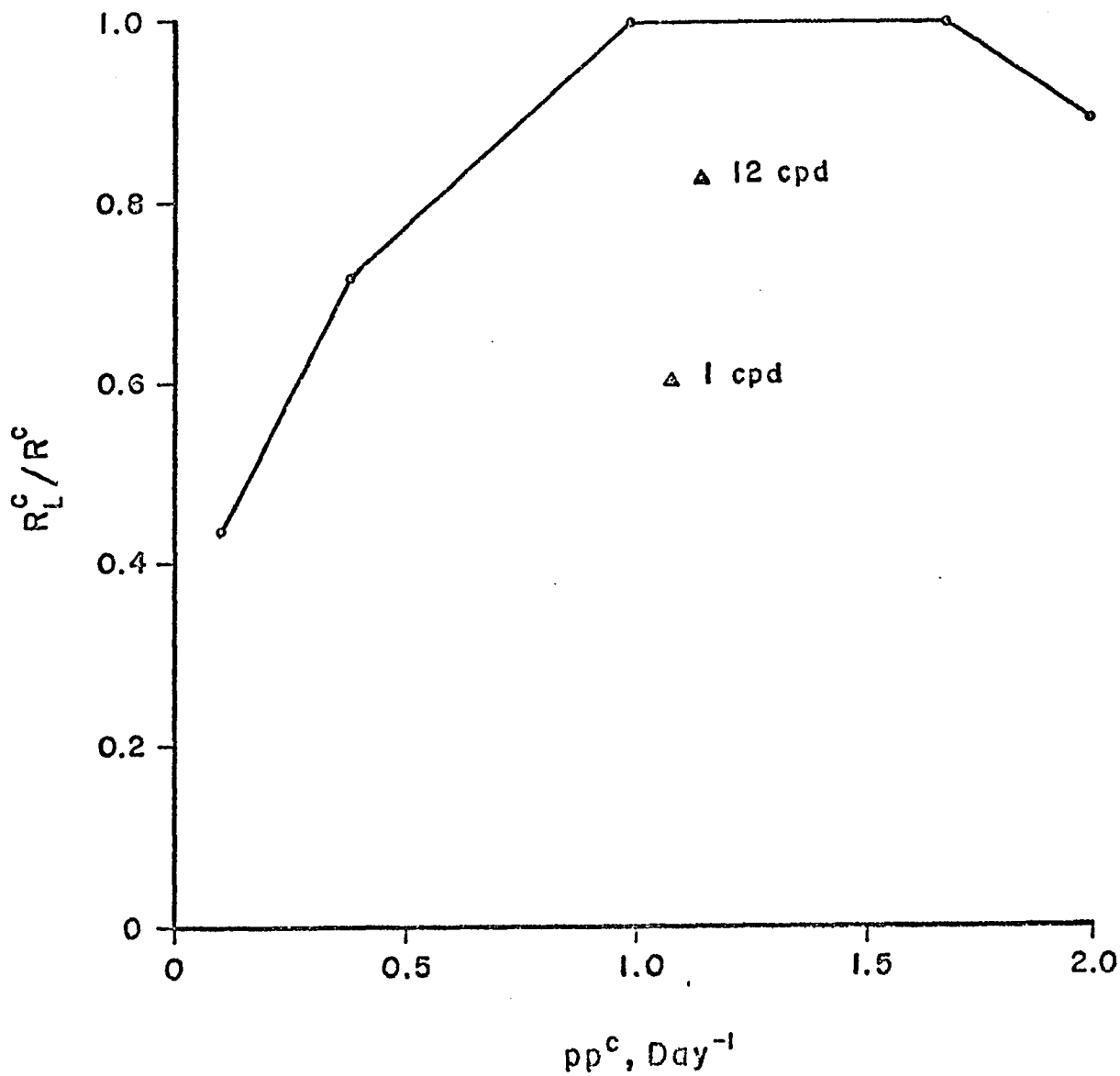

10
9
8
7
6
5
4
3
2
1
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
μgC (Chl a)⁻¹ / Einstein · m⁻²
I₀, Einstein · m⁻² · hr⁻¹

Figure 19. Relationship between proportion of respiration in the light (R^C_L) to total daily respiration (R^C) and daily carbon specific rate of growth (pp^C, day^{-1}) for cultures grown under diurnally constant (_____._____) and varying light (▲) at rates of 1 or 12 cycles day^{-1} (cpd).


1.0
0.8
0.6
0.4
0.2
0
R_L^c / R^c
▲ 12 cpd
▲ 1 cpd
0
0.5
1.0
1.5
2.0
pp^c, Day^{-1}

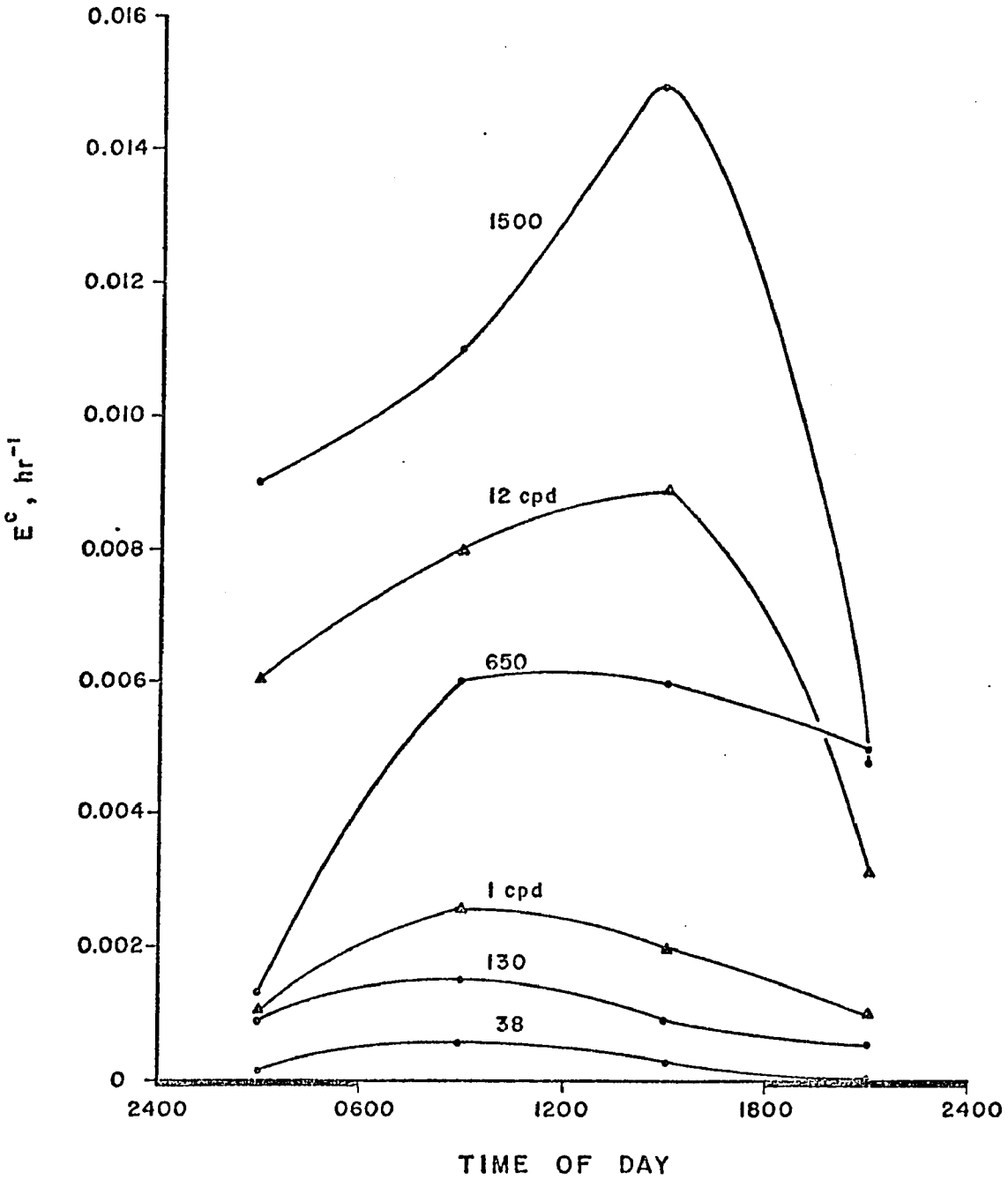
maximal rates were nocturnal at 38 μEinstein$\cdot m^{-2}\cdot s^{-1}$ but diurnal at 130 μEinstein$\cdot m^{-2}\cdot s^{-1}$ (Figure 6). The increase in pp^{C}_{D} with growth rate corresponded with this decrease in night-time respiratory loss. Possibly the refixation of respired carbon during the night was influencing the pattern of respiratory loss of carbon (Part I). There was good agreement between the pattern of carbon losses at night with variations in light as determined from losses of ^{14}C from pre-labelled cells and from changes in POC.

Additional evidence for diminished night-time loss of respired carbon with increasing growth rate is available from nitrogen limited chemostat studies (Laws and Wong, 1978) as well as field studies (Ketchum et al., 1958). Perhaps this interaction between dark carbon fixation and respiratory loss of carbon is generally true of marine microalgae. Under the diurnally fluctuating light regimes in comparison to constant light of 130 μEinstein$\cdot m^{-2}\cdot s^{-1}$ night-time respiratory loss (R^{C}_{D}) was increased relative to R^{C} (Table 4). The relationship between pp^{C}_{D} and growth rate remained the same ($p>0.05$) under the diurnally fluctuating light regimes relative to the constant regime but metabolic activity (division, chlorophyll a synthesis and respiration) increased during the dark period. pp^{C}_{D} no longer compensated for respiration at night and R^{C}_{D} increased.

Diel Variations in Organic Release

Rates of organic release (E^{C}) were maximal during the light period and diel variations consistently increased with light intensity (Figure 20). Fluctuating light, particularly the faster rate

Figure 20. Effect of variations in incident light intensity (I_o) on hourly rates of organic release (E^c, hr^{-1}) during 24 hour period; I_o: (1) constant during light period at intensities of 38, 130, 650 & 1500 μEinstein$\cdot m^{-2} \cdot s^{-1}$ (——. ——) (2) fluctuating during light period from 500 to 10 μEinstein$\cdot m^{-2} \cdot s^{-1}$ at rates of 1 or 12 cycles day^{-1} (cpd) (---▲---); black bar indicates dark period.


0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0
E^c, hr^{-1}
1500
12 cpd
650
1 cpd
130
38
2400
0600
1200
1800
2400
TIME OF DAY

of 12 cycles day^{-1}, increased E^c relative to constant light. A relatively large proportion of carbon (60% of total ^{14}C label) was fixed into a small molecular weight fraction during the light period under high light (Figure 7) and the variable light regime of 1 cycle day^{-1} (Figure 16A). The subsequent transfer of the ^{14}C label to protein at night indicated that under these light conditions the incorporation of recent products of photosynthesis into biosynthetic pathways was delayed.

Hitchcock (1977) similarly found that S. costatum produced relatively more carbohydrate during the day with subsequent protein formation at night when grown at high light intensities and temperatures. Handa (1969) has identified a reserve carbohydrate, β-1,3-glucan, in S.costatum which is preferentially metabolized when cells are placed in the dark. These results indicate that under high light and fluctuating light intensities carbon production can become less coupled to other growth processes so that fixed carbon is stored during the light period for subsequent growth at night.

Such an uncoupling of photosynthesis and growth has been shown to increase rates of organic release (Hellebust, 1974) and the increase in small molecular weight compounds with high E^c is consistent with a diffusion-dependent mechanism of release (Ignatiades and Fogg, 1973). In addition, photorespiratory activity might have been enhanced at the highest light intensity (Part I) and under the 12 cycles day^{-1} fluctuating light (Part II) and could have led to glycollic acid production and its subsequent release

(Tolbert, 1974). Diurnal increases in dissolved carbohydrate in the field have been related to photosynthetic activity and high light (Walsh, 1965). In general, under field conditions dissolved organic release is correlated with photosynthetic activity but increases as a percent loss from eutrophic to oligotrophic areas (Anderson and Zeutschel, 1970; Thomas, 1971; Ignatiades, 1973; Berman and Holm-Hansen, 1974). This pattern could be explained by nutrient limitation uncoupling growth and photosynthesis and subsequently increasing small molecular weight pool size. Rates of organic release would then be dependent on density dependent factors which affect rates of diffusion. The results reported here indicate that organic release could also be enhanced by fluctuating light and high light intensities.

CONCLUSIONS

The flexibility in the timing of division in S. costatum allowed for modifications in the phasing of division and other growth processes with variations in light intensity. Maximum growth efficiency corresponded with maximum periodicity in division (Figure 11 and Table 1) suggesting that the timing of division and pattern of growth under a light-dark cycle is important to the efficiency of growth. When the diurnal phasing of carbon production and division was similar as in cultures grown under 130 and 650 μEinstein$\cdot m^{-2}\cdot s^{-1}$, then efficiency was maximized (Figures 10 & 13 and Table 1). Under 1500 μEinstein$\cdot m^{-2}\cdot s^{-1}$ and fluctuating light relatively more division occurred at night and photosynthesis was less in phase with other growth processes. Under these conditions daily rates of both respiration and organic release were increased. Respiration and organic release were greatly enhanced during the light period under 1500 μEinstein$\cdot m^{-2}\cdot s^{-1}$ and fluctuating light of 12 cycles per day. Night-time carbon production appeared to compensate for increased rates of respiration during the dark period under 1500 μEinstein$\cdot m^{-2}\cdot s^{-1}$. However, under the variable light regimes night-time respiratory activity increased without concomitant increases in rates of dark carbon fixation and the efficiency of carbon assimilation decreased (Table 4). Tamiya et al. (1953) similarly found in Chlorella ellipsoidea a decrease in growth efficiency when photosynthesis was out of phase with other growth processes. However, night-time carbon production appears to be an important process by

which carbon is conserved through refixation of respired carbon, enhancing the efficiency of carbon assimilation with increasing growth rate.

Although the differential phasing of photosynthesis and other growth processes was less efficient, growth rates under the fluctuating light conditions were maintained at rates comparable to the rate under constant light of same diurnal magnitude and photoinhibition of growth was not observed under full sunlight conditions (Parts I & II). This suggests that uncoupling of photosynthesis from other growth processes was of importance in maintaining high growth rates under high or variable light. Such a flexibility in the phasing of division and growth would be advantageous in the pelagic environment when variations in light intensity are rapid and extreme. Such conditions prevail particularly during the early spring in temperate coastal waters when S. costatum and diatoms in general are found to bloom.

SUMMARY AND CONCLUSIONS

The purpose of this study was to measure the effects of variations in light intensity on the efficiency of carbon specific growth in Skeletonema costatum in terms of the relative importance of respiration and organic carbon release. Photoadaptive characteristics and diel variations in growth processes between diurnally constant and varying light regimes were contrasted.

The effects of light intensity on cellular biomass characteristics under diurnally constant light were dominated by changes in the pigment content of cells so that the C:Chl a ratio increased with light intensity, primarily as a consequence of variations in chlorophyll a $cell^{-1}$. In contrast to the constant light regimes, cell size decreased under the variable light regimes but chlorophyll a $cell^{-1}$ remained the same decreasing the C:Chl a ratio.

Daily carbon specific particulate production rates (PP^C) were a saturating function of light intensity with a maximum at 650 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ and no photoinhibition of growth at 1500 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$, a light intensity simulating full sunlight conditions. Daily chlorophyll a specific particulate carbon production (PP^{chl}) was a linear function of light intensity from 15-650 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ and PP^{chl} decreased at 1500 $\mu Einstein \cdot m^{-2} \cdot s^{-1}$ as a consequence of increased chlorophyll a $cell^{-1}$. Cellular concentrations of chlorophyll a and other pigments were adjusted

from 15-650 μEinstein$\cdot m^{-2}.s^{-1}$ to compensate for variations in light availability. Daily rates of growth were unaffected by diurnal variations in light intensity. Smaller cell size and higher chlorophyll a/cell volume allowed division to continue at rates comparable to constant light; rates of division rather than carbon production were conserved.

Respiratory losses of carbon, particularly during the dark period, were diminished relative to increases in growth rate so that losses were undetectable at the highest light intensities. However, estimates of total daily respiratory activity revealed a linear relationship between respiration and growth rate between 15 and 650 μEinstein$\cdot M^{-2}.s^{-1}$. At 1500 μEinstein$\cdot M^{-2}.s^{-1}$ and under diurnally varying light respiration rates were higher than would have been predicted from growth rate. The disparities between respiratory loss of carbon and respiratory activity increased as light intensity and growth rate increased and appeared to relate to the recycling of respired carbon back into photosynthetic pathways. Daily rates of organic release (E^C) increased with light intensity and under fluctuating light but remained less than 10% of GP^C under all light conditions.

Net growth efficiency (NGE) varied from 0.38 to 0.69 and decreased from an optimum at 130 μEinstein$\cdot M^{-2}.s^{-1}$ with variations in light conditions. Changes in NGE were mostly a consequence of variations in respiratory activity not organic release. Under

diurnally varying light, even though rates of gross production were enhanced relative to constant light, growth efficiency was decreased because respiratory rates were increased.

Diel variations in cell division were observed only at light intensities of 130 μEinstein$\cdot M^{-2}.s^{-1}$ and greater and were reduced under fluctuating light. Particulate carbon production and net chlorophyll _a_ synthesis were generally well correlated under all light regimes with maximal rates during the light period. As growth rate increased there was an increase in night-time synthesis of both carbon and chlorophyll _a_.

Respiratory activity during the light period (R_L^C) increased relative to total daily respiration (R^C) as growth rate increased. A decrease in night-time respiratory loss (R_D^C) corresponded with increased dark period carbon production (PP_D^C). Under fluctuating light regimes PP_D^C did not balance respiration at night and so R_D^C increased. Rates of organic release (E^C) were maximal during the light period and diel variations increased with light intensity and fluctuating light. Under high and variable light, when E^C was elevated, a large proportion of carbon was fixed into a small molecular weight fraction during the light period and E^C could have been increased just by diffusion-dependent processes.

The timing of division and pattern of growth under a light-dark cycle was important to the efficiency of growth. When carbon production and division were most in phase then efficiency was

maximized. Under the highest light intensity and fluctuating light relatively more division occurred at night and photosynthesis was less well coupled to other growth processes, increasing respiration and organic release, and decreasing NGE. Also, night-time carbon production appeared to be an important process by which carbon was conserved through the refixation of respired carbon, enhancing the efficiency of carbon assimilation with increasing growth rates.

S. costatum adjusted to variations in light by modifying pigment concentrations and cell size so as to optimize growth rates. The conservation of carbon within the cell at high light intensities appeared to allow for the maintenance of high growth rates so that photoinhibition was not observed even under full sunlight conditions. The flexibility in the phasing of division and growth was important to the efficiency of growth but also related to the continuation of high growth rates under high or variable light. Such physiological adaptations would be advantageous in the pelagic environment where variations in light can be large and rapid, and perhaps, in part, explain the tremendous success of S. costatum as a phytoplankter.

Appendix I. Analysis of variance (ANOVA): carbon specific growth rates (pp^C, hr^{-1}) versus day and time of day for cultures grown under diurnally constant light intensity (I_o) or variable light (500 to 10 μEinstein$\cdot m^{-2} \cdot s^{-1}$) at rates of 1 or 12 cycles day^{-1}.

ANOVA TABLE

I_o, μEinstein·m^{-2}·s^{-1}	Source of Variation	SS	df	MS	F
15	Day	2.064	3	0.688	0.32 ns
	Time	6.557	3	2.186	1.02 ns
	Residual	8.534	4	2.134	
	Total	17.725	10	1.773	
38	Day	22.832	11	2.076	0.47 ns
	Time	22.983	3	7.661	1.84 ns
	Residual	133.624	32	4.176	
	Total	178.151	46	3.876	
130	Day	2.000	7	0.286	0.43 ns
	Time	25.000	3	8.333	13.16 ***
	Residual	13.000	21	0.619	
	Total	40.000	31	1.290	
650	Day	14.450	8	1.806	0.17 ns
	Time	285.867	3	95.289	8.69***
	Residual	263.320	24	10.972	
	Total	563.637	35	16.104	
1500	Day	2.767	4	0.692	1.62 ns
	Time	8.237	3	2.746	6.42*
	Residual	3.422	8	0.428	
	Total	14.997	15	1.000	
1 cycle day^{-1}	Day	12.655	10	1.266	0.18 ns
	Time	201.793	3	67.264	9.35***
	Residual	179.842	25	7.194	
	Total	407.359	38	10.720	
12 cycles day^{-1}	Day	14.842	8	1.855	0.36 ns
	Time	237.984	3	79.328	15.25***
	Residual	104.015	20	5.201	
	Total	354.284	31	11.429	

ns - not significant, * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$

Appendix II Analysis of variance (ANOVA): cell number (CELL NO), particulate organic carbon (POC) and chlorophyll *a* (CHL*a*) concentrations versus Time of Day for cultures grown under diurnally constant light intensity (I_o) or variable light (500 to 10 μEinstein$\cdot m^{-2} \cdot s^{-1}$) at rates of 1 or 12 cycles day^{-1}. (ns - not significant, * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$).

I_0, 15 μEinstein$\cdot m^{-2} \cdot s^{-1}$

ANOVA TABLE

Variable	Source of Variation	SS	df	MS	F
CELL NO	Time of Day	4.41	3	1.47	1.96 ns
	Error	129.00	172	0.75	
	Total	133.41	175		
POC	Time of Day	0.018	3	0.006	1.00 ns
	Error	0.055	9	0.006	
	Total	0.073	12		
CHL*a*	Time of Day	21.55	3	7.18	17.79***
	Error	29.08	72	0.40	
	Total	50.63	75		

I_0, 38 μEinstein$\cdot m^{-2} \cdot s^{-1}$ ANOVA TABLE

Variable	Source of Variation	SS	df	MS	F
CELL NO	Time of Day	5.49	3	1.85	1.85 ns
	Error	403.07	403	1.00	
	Total	408.74	406	1.01	
POC	Time of Day	4.79	3	1.60	1.48 ns
	Error	158.74	147	1.08	
	Total	163.53	150	1.09	
CHL*a*	Time of Day	4.05	3	1.35	38.16***
	Error	3.25	92	0.04	
	Total	7.30	95		

I_o, 130 μEinstein$\cdot m^{-2} \cdot s^{-1}$ ANOVA TABLE

Variable	Source of Variation	SS	df	MS	F
CELL NO	Time of Day	98.10	7	14.01	6.86***
	Error	504.69	247	2.04	
	Total	602.80	254		
POC	Time of Day	0.42	3	0.14	9.02***
	Error	0.44	28	0.02	
	Total	0.86	31		
CHL*a*	Time of Day	0.20	3	0.067	65.53***
	Error	0.01	12	0.001	
	Total	0.21	15		

I_0, 650 μEinstein$\cdot m^{-2} \cdot s^{-1}$ ANOVA TABLE

Variable	Source of Variation	SS	df	MS	F
CELL NO	Time of Day	62.15	3	20.72	13.67***
	Error	430.43	284	1.52	
	Total	492.59	287		
POC	Time of Day	1.00	3	0.33	12.98***
	Error	2.67	104	0.03	
	Total	3.67	107		
CHL*a*	Time of Day	0.44	3	0.15	103.63***
	Error	0.06	44	0.001	
	Total	0.51	47		

I_o, 1500 μEinstein·m^{-2}·s^{-1} ANOVA TABLE

Variable	Source of Variation	SS	df	MS	F
CELL NO	Time of Day	19.80	3	6.60	4.35***
	Error	206.45	136	1.52	
	Total	226.26	139		
POC	Time of Day	1.17	3	0.39	13.07***
	Error	1.37	46	0.03	
	Total	2.55	49		
CHL*a*	Time of Day	4.23	3	1.41	43.78***
	Error	1.48	46	0.03	
	Total	5.71	49		

I_o, 1 cycle day^{-1}

ANOVA TABLE

Variable	Source of Variation	SS	df	MS	F
CELL NO	Time of Day	3.04	3	1.01	0.75 ns
	Error	412.31	304	1.36	
	Total	415.35	307		
POC	Time of Day	0.83	3	0.28	17.71***
	Error	1.71	110	0.02	
	Total	2.53	113		
CHL*a*	Time of Day	12.74	3	4.25	28.20***
	Error	15.06	100	0.15	
	Total	27.81	103		

I_o, 12 cycles day^{-1} ANOVA TABLE

Variable	Source of Variation	SS	df	MS	F
CELL NO	Time of Day	18.78	3	6.26	4.96**
	Error	328.02	260	1.26	
	Total	346.80	263		
POC	Time of Day	0.60	3	0.20	10.89***
	Error	1.68	92	0.02	
	Total	2.28	95		
CHL*a*	Time of Day	12.19	3	4.06	48.16***
	Error	7.93	94	0.08	
	Total	20.12	97		

Appendix III. Analysis of variance (ANOVA): C:Chl*a* versus Time of Day for cultures grown under diurnally constant light intensity (I_0) or variable light (500 to 10 μEinstein$\cdot m^{-2} \cdot s^{-1}$) at rates of 1 or 2 cycles day^{-1}.

I_o, μEinstein·m^{-2}·s^{-1} ANOVA TABLE

	Source of Variation	SS	df	MS	F
38	Time of Day	85.13	3	28.38	2.44 ns
	Error	324.75	28	11.60	
	Total	409.88	31		
130	Time of Day	386.94	3	128.98	10.26***
	Error	163.30	13	12.56	
	Total	550.24	16		
650	Time of Day	1729.50	3	576.50	2.64 ns
	Error	2621.50	12	218.46	
	Total	4351.00	15		
1500	Time of Day	27.00	3	9.00	0.18 ns
	Error	892.50	18	49.58	
	Total	919.50	21		
1 cycle day^{-1}	Time of Day	264.79	3	88.26	3.45*
	Error	869.11	34	25.56	
	Total	1133.90	37		
12 cycles day^{-1}	Time of Day	338.20	3	112.73	2.02 ns
	Error	1785.80	32	55.81	
	Total	2124.00	35		

ns - not significant, * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$

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