View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by College of William & Mary: W&M Publish

W&M ScholarWorks

Dissertations, Theses, and Masters Projects
Theses, Dissertations, & Master Projects

1980

Aspects of 14CO2 uptake in cyclostat grown Chlorella sp population exposed to varying lengths of photoperiods

Steven J. Hastings
College of William and Mary - Virginia Institute of Marine Science

Follow this and additional works at: https://scholarworks.wm.edu/etd

Part of the Botany Commons

Recommended Citation
Hastings, Steven J., "Aspects of 14CO2 uptake in cyclostat grown Chlorella sp population exposed to varying lengths of photoperiods" (1980). *Dissertations, Theses, and Masters Projects.* Paper 1539617510.
https://dx.doi.org/doi:10.25773/v5-ppm3-gc20

This Thesis is brought to you for free and open access by the Theses, Dissertations, & Master Projects at W&M ScholarWorks. It has been accepted for inclusion in Dissertations, Theses, and Masters Projects by an authorized administrator of W&M ScholarWorks. For more information, please contact scholarworks@wm.edu.

ASPECTS OF $^{14}CO_2$ UPTAKE IN CYCLOSTAT GROWN *CHLORELLA SP.* POPULATION EXPOSED TO VARYING LENGTHS OF PHOTOPERIODS

A Thesis

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

---

by

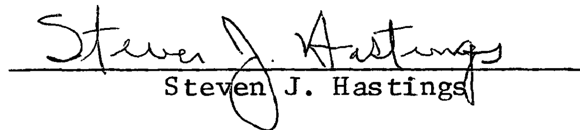
Steven J. Hastings

1980

APPROVAL SHEET

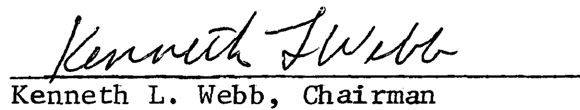
This thesis is submitted in partial fulfillment of the requirements for the degree of

Master of Arts

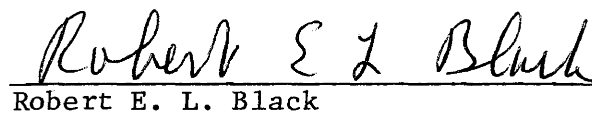


Steven J. Hastings

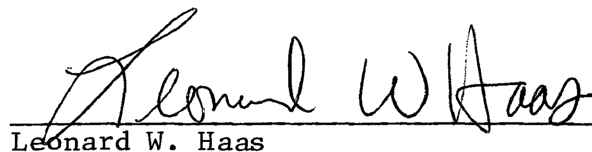
Approved,



Kenneth L. Webb, Chairman



Robert E. L. Black



Leonard W. Haas

Robert A. Jordan

Bruce J. Neilson

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	iv
LIST OF TABLES	v
LIST OF FIGURES	vi
ABSTRACT	vii
INTRODUCTION	2
MATERIALS AND METHODS	5
GENERAL CULTURE AND SAMPLING METHODS	5
BATCH CULTURE GROWTH	8
CYCLOSTAT GROWTH	9
BATCH CULTURE PMAX DETERMINATIONS	12
CYCLOSTAT CULTURE PMAX AND C/CELL DETERMINATIONS	12
^{14}C UPTAKE METHODOLOGY	13
RESULTS	15
BATCH CULTURES	15
CYCLOSTAT CULTURES	23
DISCUSSION	36
LITERATURE CITED	46
APPENDIX I. MEDIA PREPARATION FOR CYCLOSTAT CULTURES	51
APPENDIX II. THEORY OF THE CHEMOSTAT	52
APPENDIX III. LIGHT VS. ^{14}C UPTAKE CURVES	56
VITA	57

ACKNOWLEDGMENTS

The advice of Drs. Kenneth Webb and Leonard Haas throughout the course of this study was invaluable. Appreciation is extended to: Donald Abernathy, Barry Kilch, Rebecca Matheson, Majorie Petty, Jane Wingrove and Marston Youngblood for laboratory and culturing assistance. Margaret Peoples and Kay Stubblefield are gratefully acknowledged for their art work and Linda Jenkins for typing the thesis. My sincere appreciation goes to Harry Walthall for his glass blowing abilities that went into the construction of the cyclostat. Thanks goes to my committee for their editorial efforts. This work would not have been possible had it not been for the group of people I am privileged to call my friends.

LIST OF TABLES

Table		Page
I	McLachlan's Artificial Seawater	6
II	f/2 media	7
III	I_k Values From Cyclostat.	35
IV	Cyclostat Cell Population Values.	44

LIST OF FIGURES

Figure		Page
1.	Schematic Diagram of Cyclostat Unit	10
2.	Growth Curve of *Chlorella* Including pH, cell/nitrogen and cell number	16
3.	Growth Curve of *Chlorella*, 8L/16D Photoperiod	18
4.	Growth Curve of *Chlorella*, 16L/8D Photoperiod	19
5.	Photosynthesis vs. Light Curves From Batch Cultures . .	20
6.	Changing Pmax Values of *Chlorella* Batch Cultures in Different Phases of Growth	21
7.	Change in Cell Characteristics Upon Approach to Steady State.	22
8.	Variation in Pmax of Cyclostat *Chlorella* Cultures (C/cell)	24
9.	Variation in Pmax of Cyclostat *Chlorella* Cultures (C/chl *a*).	25
10.	Variation in Total Carbon Production/light period of Cyclostat *Chlorella* Cultures.	27
11.	Variation in Chlorophyll *a*/cell of Cyclostat *Chlorella* Cultures.	28
12.	Time Course of Change in Rates of C/cell/hr. and chl *a*/cell with a change in Photoperiods of Cyclostat *Chlorella* cultures.	29
13.	Linear Portion of the Photosynthesis vs. Light Curve (C/cell/hr.).	30
14.	Linear Portion of the Photosynthesis vs. Light Curve (C/chl *a*/hr.)	31
15.	Variation in I_k of Cyclostat *Chlorella* Cultures (C/cell).	33
16.	Variation in I_k of Cyclostat *Chlorella* Cultures (C/chl *a*)	34

ABSTRACT

Chlorella sp. (isolated from the York River, Virginia) was cultured with varying lengths of light dark periods using batch and cyclostat ($u=.288^{-day}$) techniques. Chl *a*/cell, N/cell and ^{14}C uptake was found to be 4-6 times greater for batch cultures in logarithmic growth compared to stationary growth phase. These values changed on a day to day basis throughout the growth cycle from lag to stationary growth. The cyclostat cultures were similar to the stationary growth phase with respect to chl *a*/cell, N/cell and ^{14}C uptake which was attributed to the low dilution rate utilized.

The effect of 8L/16D, 10L/14D, 12L/12D, 14L/10D and 16L/8D photoperiods upon the pattern of Pmax in cyclostat cultures was investigated. Except for the 10L/14D and 14L/10D photoperiods a bell shaped pattern of Pmax was observed. The maximum value occurred during the middle of the light period. A diurnal variation in alpha was not observed. It was concluded that photoperiod alone would not alter the diurnal pattern of Pmax in an algal population.

As the length of the photoperiod increased (as well as the total light energy/day) chl *a*/ cell and ^{14}C uptake/cell/hr. decreased. It was concluded that the cells adapted to the increase in light energy/day by decreasing carbon production/day, respiration and chl *a*/cell to maintain a constant C/N value.

ASPECTS OF $^{14}CO_2$ UPTAKE IN CYCLOSTAT GROWN *CHLORELLA SP.*

POPULATIONS EXPOSED TO VARYING LENGTHS OF PHOTOPERIODS

INTRODUCTION

The daily periodicity in the ability of algae to photosynthesize has been cited by Holmes and Haxo (1958), Yentsch and Ryther (1957), Verduin (1957), Taguchi (1976b), Platt and Jassby (1976) and Gargas et al. (1979) in natural populations and Eppley et al. (1971) and Eppley and Coatsworth (1968) in cultures. Variation in the light saturated $^{14}CO_2$ uptake ability of phytoplankton is of ecological importance because of its effect on primary production measurements and therefore, estimates of productivity of the ocean. Lorenzen (1963) points out that the variation cannot be directly related to fluctuations in light intensity observed in the environment from sunrise to sunset. Gargas et al. (1979) and MacCaull and Platt (1977) found that their estimates of daily primary productivity varied up to two-fold depending upon whether or not they corrected their ^{14}C uptake measurements for diel variation.

Doty and Oguri's (1957) paper is one of the most often cited papers on diurnal variation in a natural algal population's ability to take up carbon under saturating light (Pmax). Doty (1959) published data that showed a correlation between latitude and the degree of variation in Pmax seen from sunrise to sunset. The lower the latitude that the measurements were made the greater the ratio of the maximum observed light saturated rate over the minimum observed value for a given day. Lorenzen (1963), using the Gaarder and Gran (1927) oxygen

method to measure photosynthesis, published evidence that daylength, which varies with latitude and season, not latitude directly, was responsible for Doty's (1959) observations.

Stross (1973) and MacCaull and Platt (1977) concluded that photosynthetic rhythms observed in the field result from intrinsic oscillations within the phytoplankton cells and fluctuating environmental factors. Diurnal variation in an algal cell's physiological parameters that might influence photosynthetic rhythms include: nitrogen uptake (Goering et al., 1964), chlorophyll synthesis (Yentsch and Rhyther, 1957; and Shimada, 1958), nitrate reductase activity (Eppley, Packard and MacIsaac, 1970), internal nitrate concentration (Collos and Slawyk, 1976), ammonia uptake (Goering, Dugdale and Menzel, 1964) and cell division (Smayda, 1975).

Malone (1971) provides data that demonstates the role of the nutrient regime in controlling diurnal photosynthetic production. He reports that net-phytoplankton exhibited a maximum carbon uptake in the after regardless of the nutrient environment. Nanoplankton were found to show a morning maximum in a eutrophic environment. Malone's hypothesis is that phasing of the diurnal cycles of the plankton reflects the inherent differences in the kinetics of carbon and nitrogen uptake and storage of photosynthate and nitrogen between the two phytoplankton size fractions under varying nutrient concentrations.

Paerl and Mackenzie (1977) published data showing high rates of carbon uptake by nanoplankton relative to netplankton during the early daylight hours. During the afternoon and early evening the

netplankton showed an increasingly relative higher carbon uptake. These workers did not provide nutrient data but do support Malone's observations. Both studies show the potential effect of community structure, i.e. the relative abundance of net and nanoplankton, on the diurnal rhythm of photosynthesis.

Haas (1975) observed that the daily pattern of *in situ* rate of photosynthesis by York river phytoplankton population changed with seasons. Maximum *in situ* rates of photosynthesis did not always occur during the time of maximum incident light intensity.

The above studies document the occurrence of diel variation in photosynthetic carbon uptake by marine phytoplankton. Elucidation of the causative factors of the diel variation is hindered by numerous environmental variables which exhibit cycles that continuously impinge upon the natural populations. The primary objective of this study was to determine whether varying photoperiods can cause a systematic change in the time of occurrence of a cyclostat algal population's maximum light-saturated ability to take up $^{14}CO_2$. A secondary objective was to determine the variation in Pmax, alpha and I_k within and among photoperiods and whether quantitative values of chl *a*/cell, C/N, and carbon production/biomass/hour are correlated with the length of the photoperiod to which algal population is exposed. Finally, it was intended that this study provide chemical and physiological data on a cyclostat grown alga at a fixed growth rate and various lengths of light/dark cycles.

MATERIALS AND METHODS

Batch culture experiments provided physiological data on *Chlorella sp*. throughout its growth cycle to compare with subsequent values from steady state populations. Steady state cultures provided a physiologically identical population from one day to the next. Although the steady state population's physiological characteristics changed from hour to hour the pattern of change was the same for any given day. The steady state populations were exposed to various photoperiods and Pmax determined on the algae every two hours over a 24 hour period for each photoperiod utilized.

General Culture and Sampling Methods

The experimental organism, IA66 *Chlorella sp*., was isolated by Dr. Perkins from the York river. Growth media were enrichments of filtered York river water for all experiments except those done for growth rates of batch cultures and all cyclostat culture work. In the latter two cases artificial sea water (McLachlan, 1974; Table I) was prepared, salinity 15 o/oo. The enrichments consisted of f/2 media (Guillard and Ryther, 1962; Table II) added aseptically after the seawater had been autoclaved. Bacterial contamination was low in both the cyclostat culture and batch culture experiments and did not rise in batch cultures until static growth phase was reached. Before initiation of any experiments the algae were serially diluted in autoclaved media.

TABLE I

McLachlan's artificial seawater

NaCl	11.58 g
$MgSO_4$	1.19 g
$MgCl_2$	2.01 g
$CaCl_2$	0.725
KCl	0.37

H_3BO_4	0.5 ml of solution with 24.74 g/l
KBr	0.5 ml of solution with 200.5 g/l
$NaHCO_3$	3.5 ml of solution with 50.4 g/l

add to 1 liter of DH_2O

TABLE II

f/2 media

Salt solution: To 200 ml DH_2O add:

$NaNO_3$	1.5 g (varies)
$NaH_2PO_4 \cdot H_2O$	0.1 g
Fe sequestrene	0.1 g
$Na_2SiO_3 \cdot 9H_2O$	0.1 g
Thiamine HCl	0.002 g
Biotin	0.001 g
Vitamin B_{12}	0.001 g

Trace metal solution: To 1000 ml DH_2O add:

$CuSO_4 \cdot 5H_2O$	0.0196 g
$ZnSO_4 \cdot 7H_2O$	0.044 g
$CoCl_2 \cdot 6H_2O$	0.20 g
$MnCl_2 \cdot 4H_2O$	0.36 g
$Na_2MoO_4 \cdot 2H_2O$	0.0126 g

To 1 liter of sterile seawater add 10 ml of sterile salt solution and 5 ml trace metal solution.

Six replicate counts were made on a given sample preserved in Lugol's solution using a Spencer Bright-line hemacytometer for counting. In some cases cell lengths were measured using an ocular micrometer (Taguchi (1976a) found no statistical differences in the size of cells preserved with Lugol's and unpreserved samples. Chlorophyll a concentrations was determined on three replicate one ml samples. The samples were filtered onto Whatman 25mm GF/C filters and frozen until analyzed according to the method of Yentsch and Menzel (1963). A Turner model 111 fluorometer was used. Nitrate analysis was by the method of Wood et al. (1967).

The algae were cultured at 15°C in a temperature controlled growth chamber (Controlled Environments LTD. Winnipeg Canada, model G-30). The light intensity was 95 μ Ein$\cdot m^{-2}s^{-1}$ except for the batch culture diurnal Pmax experiments which were grown at 40 μ Ein$\cdot m^{-2}s^{-1}$ (12L/12D). A Lamda LiCor model LI-185A quantum/radiometer/photometer was used to measure light when expressed as μ Ein$\cdot m^{-2}s^{-1}$ where light is given in klux a panlux meter was used. The photoperiods used for the growth rates and cyclostat populations are given in the results section. The light source was two banks of six 40 watt "cool-white" fluorescent tubes.

Batch Culture Growth

For batch cultures, an inoculum was transferred into sterile 1000 ml erelenmeyer flasks containing 500 ml of growth media. Culture contents were mixed with Teflon-coated magnetic stirring bars (a magnetic stirrer was placed under each flask) and bubbled with air. the air passed through a moisture trap and then through sterile cotton

filters and tubes, into the flask.

Nitrogen/cell from the batch culture experiment was determined by dividing the nutrient concentration on day zero by the number of cells on the day no nitrate was present. This value was considered a good approximation of cellular nitrogen in the cells when they were in the first day of lag phase or two or three days into stationary phase. The preceding cell nitrogen value was taken times the number of cells inoculated into the media on day zero. This value was added to the nitrate concentration of the media on day zero yielding the total amount of nitrogen available on day zero and then the difference divided by the cell concentration for the day to give N/cell.

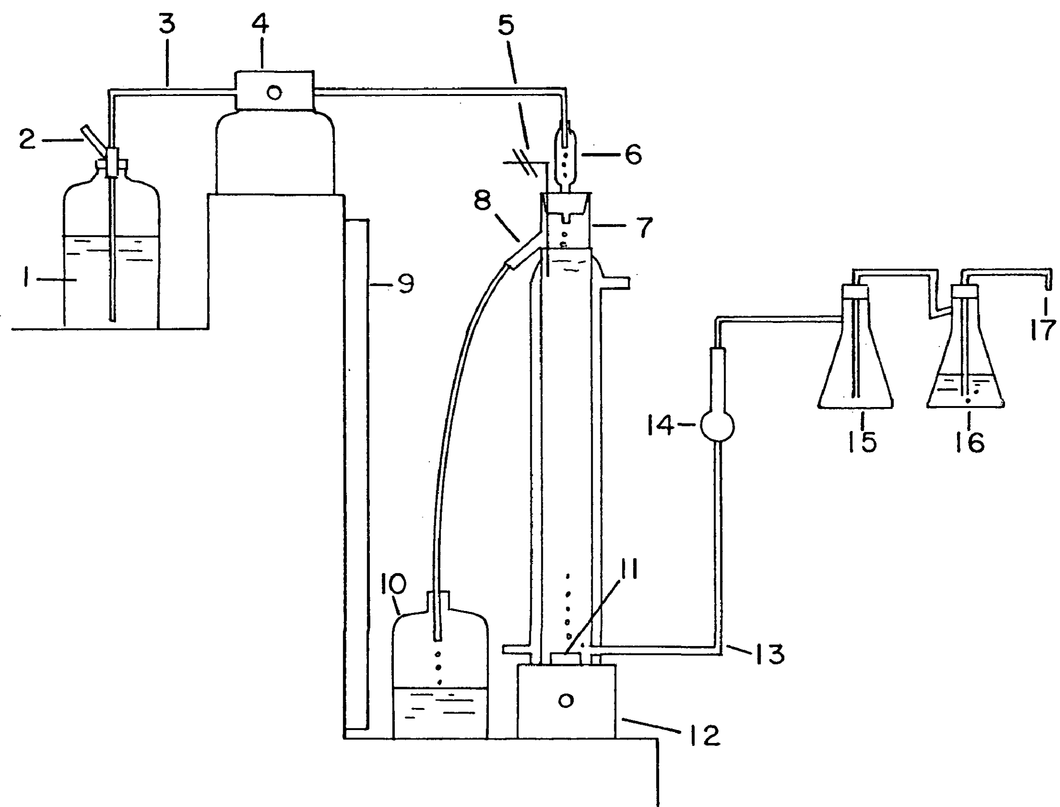
Cyclostat Growth

The cyclostat culture vessel (Figure 1) had a volume of 2.5 liters and was similar in design to that of Brewer and Goldman (1976). Air was passed through a .1N H_3PO_4 bath for ammonia removal, a moisture trap and finally a cotton wool filter. Air entered the vessel via a glass tube connected to a port at the base of the chamber. A magnetic stirrer and stir bar facilitated mixing. Media was peristaltically pumped (Manostat Cassette Pump, Junior model) into the unit from 21 liter supply bottles, each containing eight liters of media. Details of media preparation and introduction into the cyclostat can be found in Appendix I. All glassware and associated tubing was autoclaved before a cyclostat experiment and isopropanol applied before joining cork to glass or tubing to glass. At no time during the runs were bacteria observed to build up in the supply lines leading into the culture vessel. Any time the chamber showed

Figure 1

Schematic Diagram of Cyclostat Unit

Figure from Brewer and Goldman (1977). 1. media supply bottle; 2. cotton plug air intake; 3. fed lines; 4. peristaltic pump; 5. clamped sampling tube; 6. break line; 7. 2.5 liter vessel; 8. overflow port; 9. "cool-white" fluorescent lights; 10. overflow bottle; 11. magnetic stirring bar; 12. magnetic stirrer; 13. air supply line; 14. cotton wool filter; 15. moisture trap; 16. .01N H_3PO_4 acid wash bottle; 17. to air supply.



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

bacteria growth on the walls, or at the end of an experiment, the whole assembly was broken down, washed and autoclaved. A new inoculum of algae was then used to start up the system again.

The cyclostat culture was started by filling the unit with medium, adding a small inoculum of Chlorella sp. culture and then maintaining it as a batch culture for one-two days until an observable population developed. At this time media was pumped in at a flow rate of 30 ml/hr (held constant throughout all experiments) resulting in a turnover time of 3.47 days, growth rate μ of $.288^{-day}$. The doublings/ day was equal to .416 calculated according to Goldman, McCarthy and Peavey, 1979). The cyclostat culture was maintained until steady state levels of cell populations were reached. Steady state was defined as the time when variation in cell number was less than $\pm$ 10% for at least two consecutive days. Diel variation in cell numbers was found to be of no significance in determining steady state. The overflow was collected in a storage bottle for daily measurement of flow rate.

Cell nitrogen was estimated using equation 5 in Appendix II. Collos and Slawk (1979) found that PN measurements made on the overflow agreed quite well with the nitrogen concentration in the inflow. Carbon content of the cells was estimated by multiplying the average Pmax (per cell basis) value of the population (as determined from carbon vs. light experiments) by the total hours of light exposure and dividing by the dilution rate. Eppley and Renger (1974) found this method of calculation to agree well with PC measurements of the cells. The theory on which chemostat and cyclostat growth is based can be found in Appendix II.

Batch Culture Pmax Determinations

A batch culture of algae was preadapted to a 12L/12D photoperiod by subjecting the culture to the photoperiod for one growth cycle. After serially diluting the cells with sterile media the beginning concentration of cells was adjusted to $8x10^5$ cells/ml. Day one was equivalent to the day the original population had increased tow fold. On days two and four a light vs. ^{14}C uptake curve was generated from samples taken at 0600 (lights on) 0900, 1200, 1500, and 1800 hours (lights off). The theory on which Pmax is determined from light vs. carbon uptake curves from batch and cyclostat cultures is given in Appendix III. Carbon uptake was measured after diluting (using fresh media, 18.5 μM nitrate) a sample from the culture to $2x10^5$ cells/ml after cell number, chlorophyll a and alkalinity samples had been taken. Ten mls of diluted culture was placed in 30 ml capacity screw top culture tubes. One dark and two light bottles were prepared for each of the following light intensities; 10, 5, 3, .3 and 0 Klux. The light incubators contained two 40 watt "cool-white" fluorescent bulbs which were attenuated by neutral density filters. The temperature of the incubators was controlled by circulating water through it from a constant temperature bath (Precision Scientific Co.) set at 15°C.

Cyclostat Culture Pmax and C/cell Determination

An algal population that had reached steady state at one of the following photoperiods: 8L/16D, 10L/14D, 12L/12D, 14L/10D or 16L/8D, was sampled every two hours for 24 hours. Approximately 15 mls (0.6% of cyclostat volume) of culture was withdrawn from the chemostat at each sampling time. After chlorophyll a, cell number

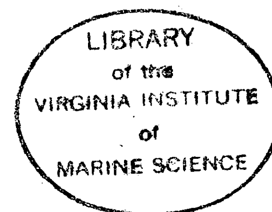
and alkalinity samples were taken it was diluted to a cell concentration of $1x10^6$ cells/ml using fresh media without nitrate. Duplicate ten ml sam-les were placed in 30 ml capacity screw top cultures tubes for incubation at the following light intensities: 100, 50, 25, 15, and 0 μ $Ein \cdot m^{-2}s^{-1}$. Light and temperature control of the light incubators was the same as in the batch culture experiments.

Alpha was determined from the slope of the line defined by carbon uptake/biomass/hr vs. light at 15 and 25 μ $Ein \cdot m^{-2}s^{-1}$. ^{14}C uptake vs. light was found to be linear at these light intensities. Pmax was equal to the ^{14}C uptake value at 100 μ $Ein \cdot m^{-2}s^{-1}$. In all cases the ^{14}C uptake value was only slightly greater at 100 vs. 50 μ $Ein \cdot m^{-2}s^{-1}$. When the algae were exposed to 250 μ $Ein \cdot m^{-2}s^{-1}$ the ^{14}C uptake value was equal to the value determined at 100 μ $Ein \cdot m^{-2}s^{-1}$. The derivation of I_k can be found in Appendix III as well as the theory on which alpha and Pmax are based.

Carbon/cell values for the cyclostat populations were determined by taking the summation of the Pmax values (C/cell/hr) measured every two hours beginning with the value determined at lights on of a particular photoperiod and continuing on through lights off but not including this last value. The sum was then multiplied by two equalling C uptake/cell/day. This product taken times the turnover time of the culture in days (1/.288 days or 3.47 days) yields C/cell.

^{14}C Uptake Methodology

One μCi of $Na_2{}^{14}CO_3$ was added to the ten mls of sample and incubated at the appropriate light level for one hour. The incubation


LIBRARY
of the
VIRGINIA INSTITUTE
of
MARINE SCIENCE

was terminated by filtering the algae onto prewetted metrical membrane filters (Gelman GA-8 .2μm 25 mm). The algae were rinsed one time with fresh media and the filter placed in a scintillation vial to which .3 ml of NCS was added and allowed to digest overnight. Ten ml of cocktail (50 mg POPOP, 4g PPO per liter of toluene) was added to each vial which were to sit for several hours before counting on a Beckman model LS150 liquid scintillation counter. Counting efficiency was determined to be 90% using the internal standards method. Alkalinity analyses and carbon uptake rate calculations were performed according to the methods of Strickland and Parsons (1972).

RESULTS

Batch Cultures

Preliminary batch culture experiments were carried out to ensure that suitable growth conditions were met for Chlorella sp. Fogg (1965) lists five phases of growth characteristics of batch cultures of unicellular algae. The first three phases are shown in the growth curve for a population of Chlorella sp. grown on a 12L/12D cycle at 15°C (Figure 2) i.e. lag, logarithmic and linear.

Nitrogen/cell throughout the growth cycle is presented in Figure 2 for a batch culture of Chlorella sp. There is an initial increase in nitrogen from .02 to .12 pico gram-at-N/cell prior to any increase in cell number. Maximum N/cell occurred just before logarithmic growth, followed by an asymptotic decrease throughout the remaining growth phases.

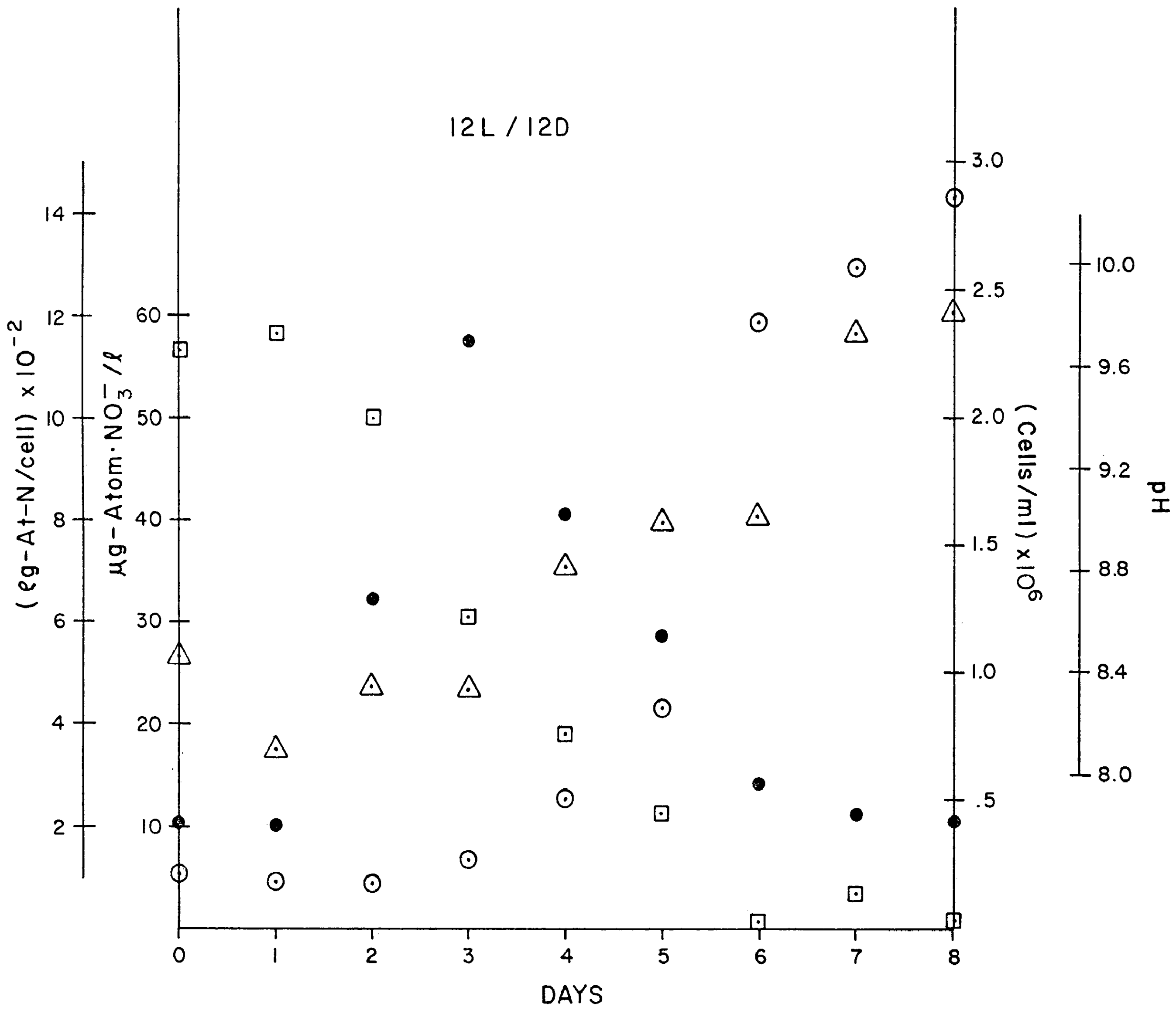
Cell number and pH are essentially a mirror image of the nitrate concentration curve. The increase in pH is attributed to the uptake of nitrate ions with a concomitant release of ^{-}OH ions to maintain a charge balance within the cell (Fogg, 1965). If CO_2 is present in sufficient quantity, its uptake by an algal population would be expected to buffer any change in pH. This is attributed to the equilibrium CO_2 maintains with HCO_3^{-2}, CO_3^{-2} and H_2CO_3 in water. The culture, whose growth curve is shown in Figure 2, was not bubbled with air (a source of CO_2) and the change in pH was almost two units. Subsequent cultures had air bubbled through them which decreased the

Figure 2

Growth curve of _Chlorella_ including

pH, cell/nitrogen and cell number

Cell number (○, cells/ml.x10^6), nitrate concentration in the culture (□, µg at-NO_3^-/1), cell nitrogen (●, pg at-N/cell) and pH (Δ) with time for a _Chlorella_ population with a 12L/12D photoperiod.


12L / 12D
(ℓg-At-N/cell) x 10^-2
14
12
10
8
6
4
2
µg-Atom·NO3^- /ℓ
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
DAYS
3.0
2.5
2.0
1.5
1.0
.5
(Cells/ml) x10^6
10.0
9.6
9.2
8.8
8.4
8.0
pH

pH change to less than one.

Growth curves for batch cultures subjected to 8L/16D and 16L/8D photoperiods are shown in Figures 3 and 4 respectively. The 12L/12D (Figure 2) exhibited the greatest growth rate while the 8L/16D the lowest. Growth rate values were 0.685, 0.533 and 0.463 divisions per day for 12L/12D, 16L/8D and 8L/16D populations, respectively.

In both the 8L/16D and 16L/8D batch cultures, cell size and chl a/cell increased before and/or during maximum growth phase, returning to lag phase levels once stationary phase was approached (Figures 3 and 4). The length of the cells varied from 2.8 to 3.5 µm. Chlorophyll a/cell varied from 0.013 to 0.052 pico-grams/cell.

Representative light vs. carbon uptake/chl a/hr curves are presented in Figure 5 for day two and day four of exponential growth (12L/12D). I_k was 4.5 and 5 Klux for day two and day four, respectively. Values of both Pmax and alpha were much greater on day two than on day four (Figure 5). Pmax values measured on day two were 0.61-0.86 while on day four they were 0.11-0.23 pico-grams C/cell/hr (Figure 6).

The transient physiological characteristics of batch cultures were compared to cyclostat grown algae using changes in cell number, chlorophyll a/cell and cell size. A batch culture was inoculated in the cyclostat chamber. Once the cells began dividing the pumping of media at 30 ml/hr into the chamber was initiated. Figure 7 shows the change from batch culture to steady state once the pump was turned on. Chlorophyll a/cell peaked at 0.036 pico-grams chl a/cell, five days before a cell number maximum of 2.4×10^7 cells/ml. Once steady state was achieved cell number and chlorophyll a/cell remained constant at 9.1×10^6 cells/ml and .0086 pico-grams chl a/cell, respectively.

Figure 3

Growth curve of *Chlorella*, 8L/16D photoperiod

Cell number (○ , cells/ml.x10^6), chl *a*/cell (□ , pg chl *a*/cell) and cell length (Δ, μm) with time for a population of *Chlorella* with a photoperiod of 8L/16D.

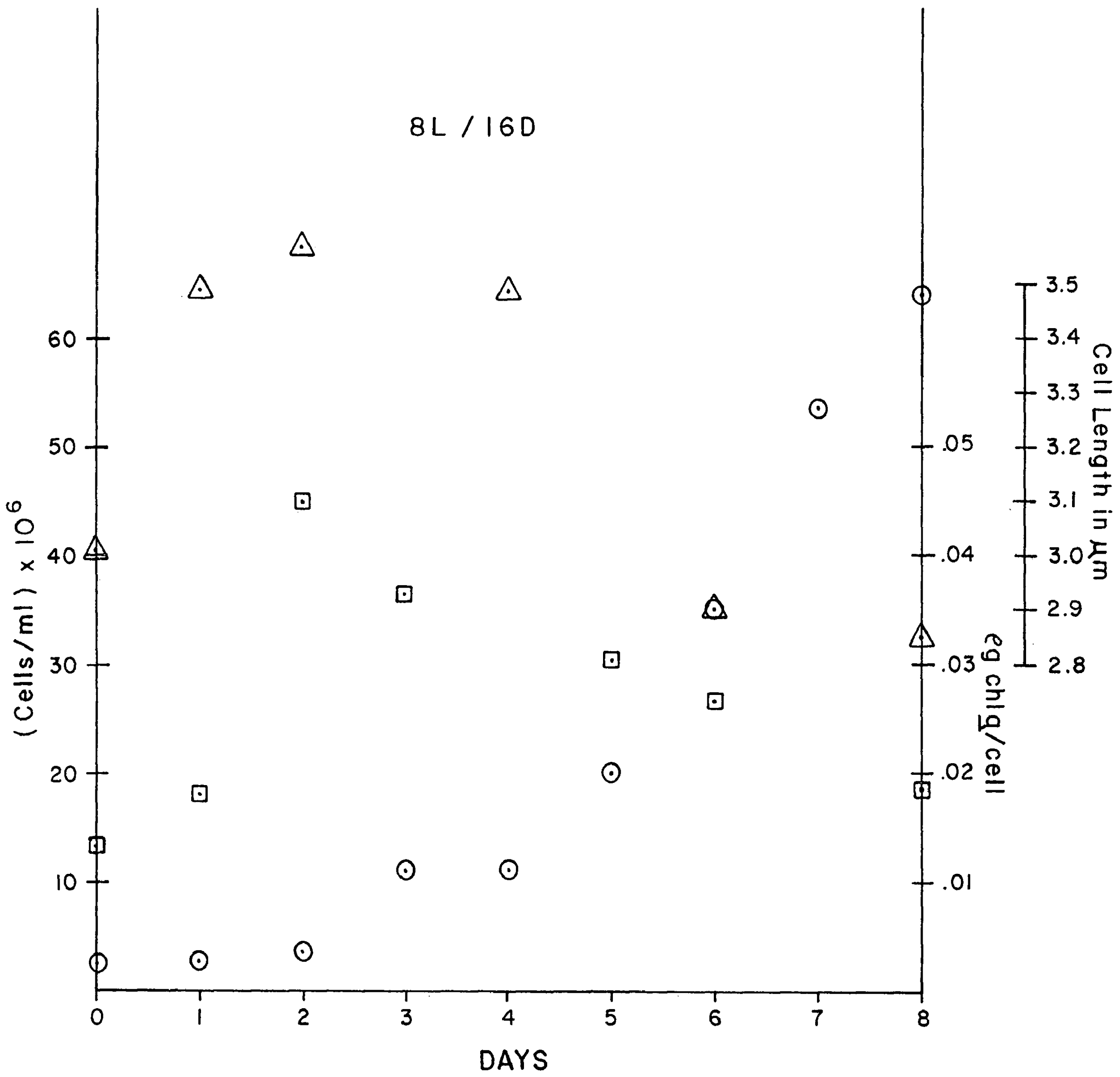

8L / 16D
(Cells/ml) x 10^6
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
DAYS
.05
.04
.03
.02
.01
eg chla/cell
3.5
3.4
3.3
3.2
3.1
3.0
2.9
2.8
Cell Length in µm

Figure 4

Growth curve of *Chlorella*, 16L/8D photoperiod

Cell number (○, cells/ml.x10^6), chl *a*/cell (□, pg chl *a*/cell) and cell length (Δ, μm) with time for a population of *Chlorella* with a photoperiod of 16L/8D.

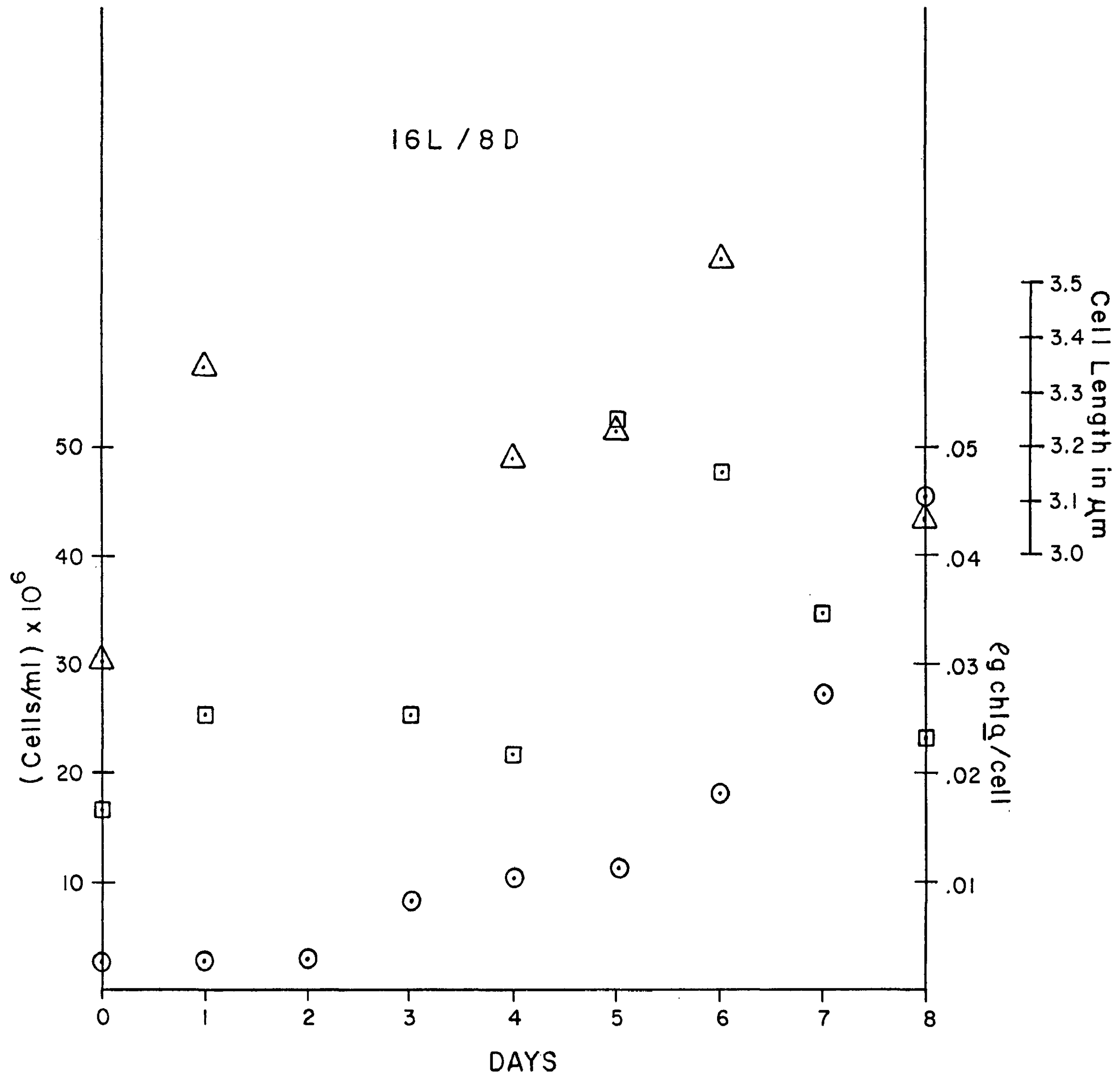

16L / 8D
(Cells/ml) x 10^6
50
40
30
20
10
0
1
2
3
4
5
6
7
8
DAYS
.05
.04
.03
.02
.01
ρg chl a/cell
3.5
3.4
3.3
3.2
3.1
3.0
Cell Length in μm

Figure 5

Photosynthesis vs. light curves from batch cultures

Carbon uptake (pgC/pg chl *a*/hr) vs. light (Klux) for a batch culture of *Chlorella* in day 2 (○) and day 4 (□) of logarithmic growth, 12L/12D photoperiod.

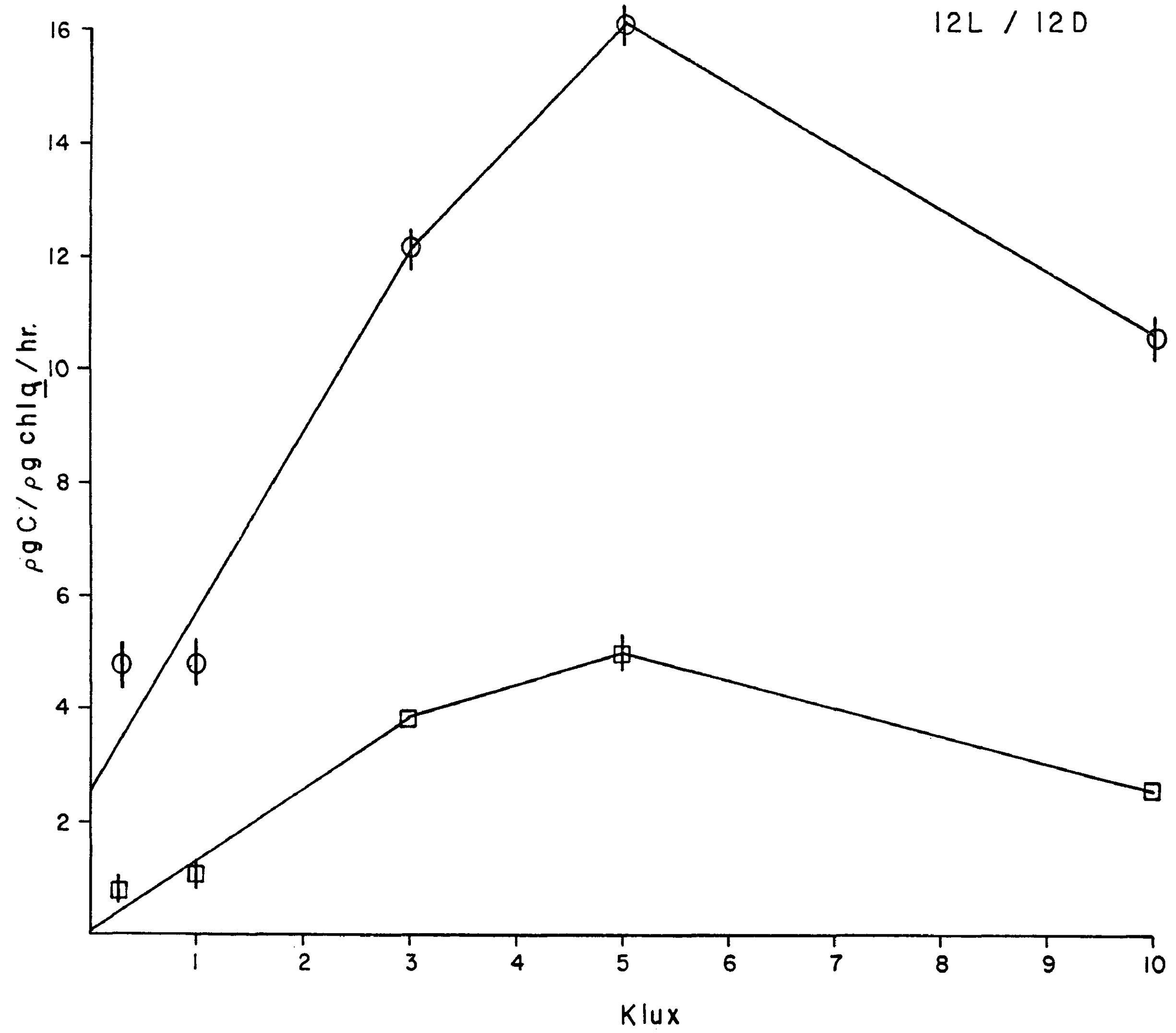

12L / 12D
ρgC/ρg chl a/hr.
16
14
12
10
8
6
4
2
1
2
3
4
5
6
7
8
9
10
Klux

Figure 6

Changing Pmax values of *Chlorella* batch cultures in differen phases of growth

Light saturated carbon uptake (O , pgC/cell hr) and chl *a*/cell (□ , pg chl *a*/cell) for a batch culture of *Chlorella* in day 2 (-----) and day 4 (———) of logarithmic growth vs. time in a 12L/12D photoperiod beginning at 0600.

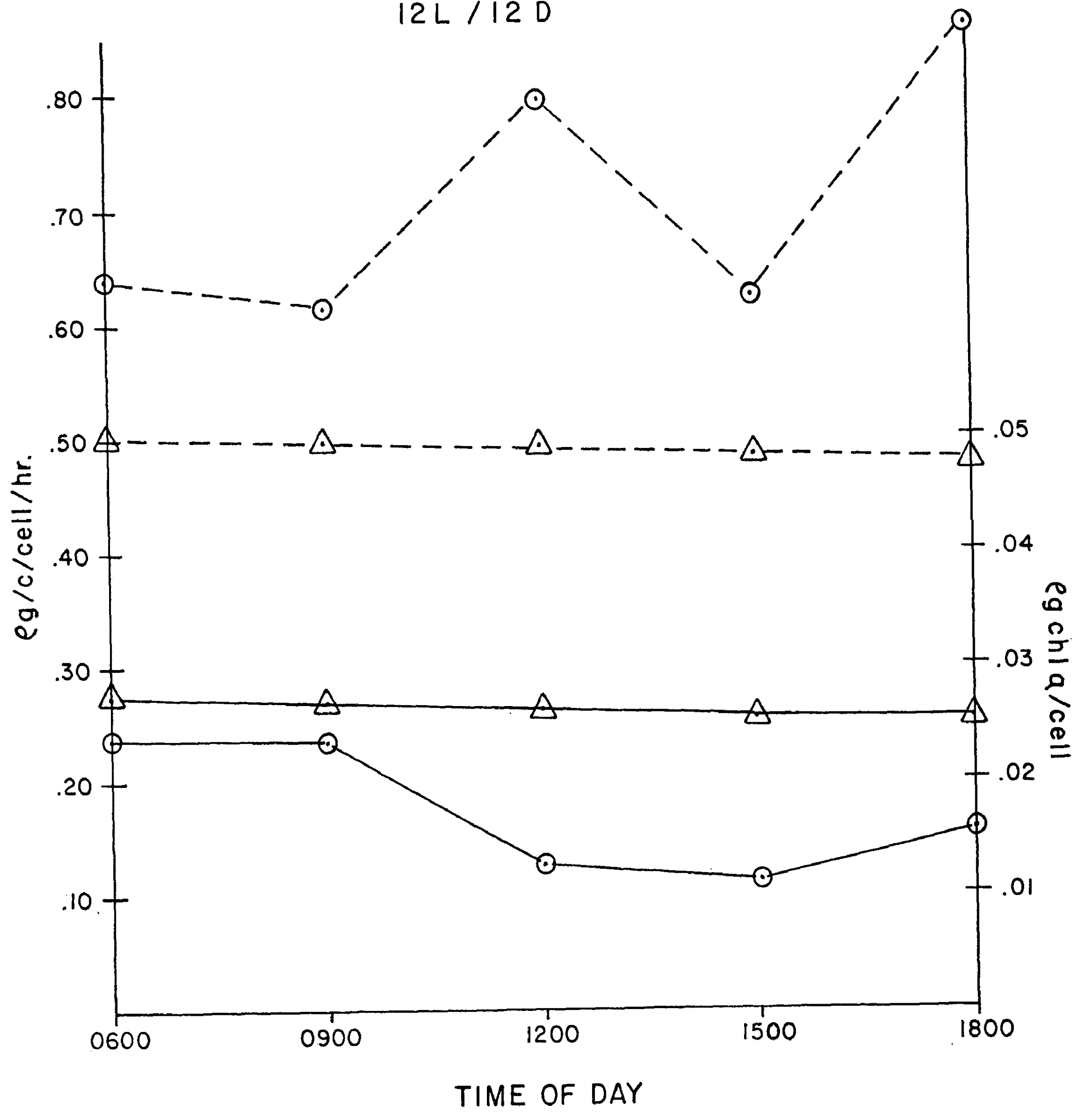
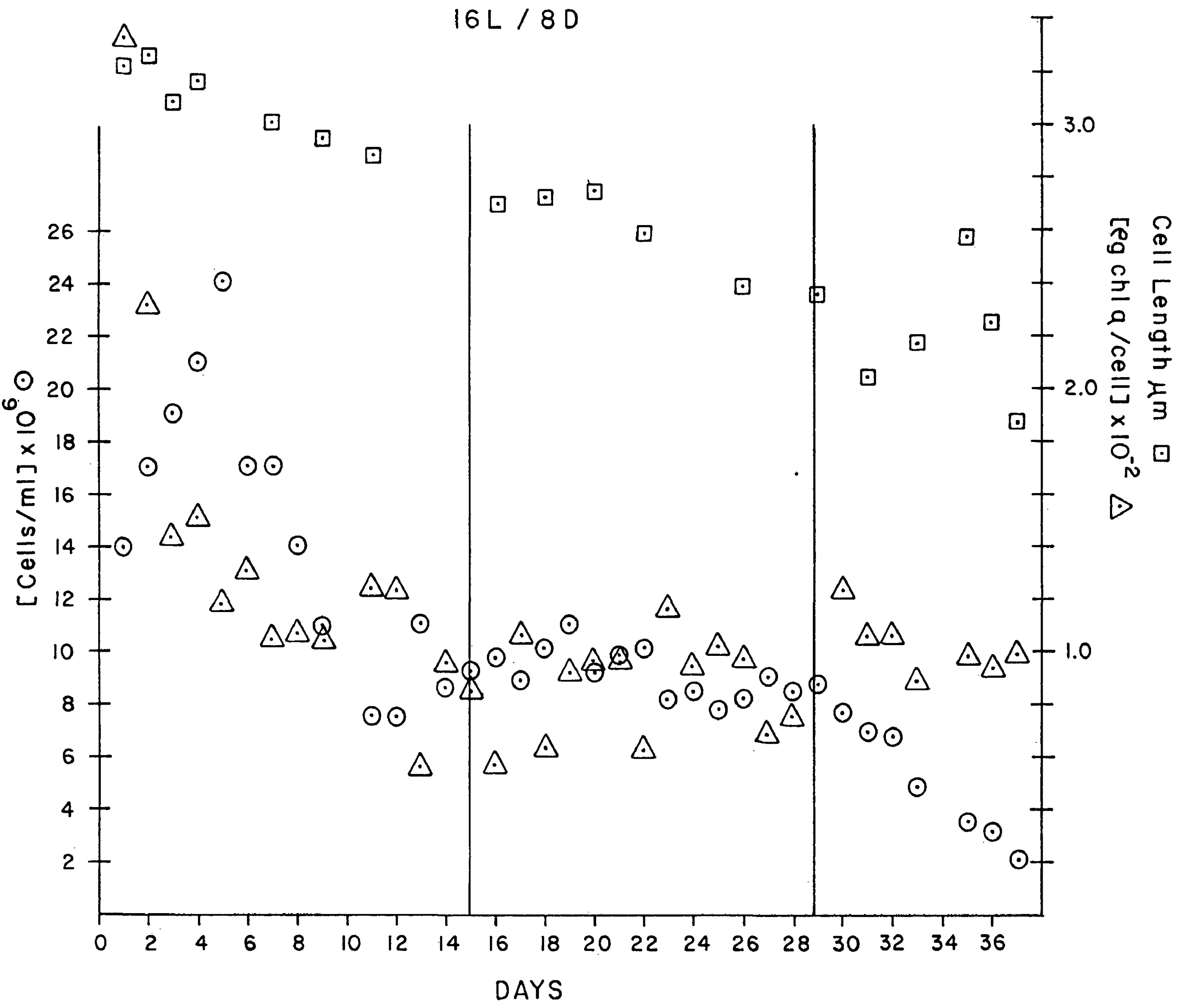

12L / 12D
.80
.70
.60
.50
.40
.30
.20
.10
ℓg/c/cell/hr.
.05
.04
.03
.02
.01
ℓg chl a/cell
0600
0900
1200
1500
1800
TIME OF DAY

Figure 7

Change in cell characteristics upon approach to steady state

Cell number (○ , cell/ml.x10^6), chl *a*/cell (Δ, pg chl *a*/cell) and cell length (□ , μm) vs. time (days) as a batch culture of *Chlorella* approaches and achieves steady state, followed by washout.


16L / 8D
[Cells/ml] x 10^6 ⊙
Cell Length µm ⊡
[eg chl a /cell] x 10^-2 △
DAYS
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
3.0
2.0
1.0

Cell number/ml decreased linearly with time during washout attributed to depletion of the nitrate within the supply bottle by bacteria. The range in cell length on days on-five (batch culture phase, 3.1-3.3 μm) was statistically different ($P<.001$) from the length of the cells in steady state or washout phase (1.9-3.0 μm).

Cyclostat Cultures

Nitrate limitation of the *Chlorella sp.* cyclostat culture was verified by observing a linear relationship, passing through the origin, betweeen nitrate added to the medium and steady state cell number produced (r 0.998). A cyclostat culture on an 8L/16D cycle yielded cell concentrations of $8x10^9$ and $1/10^{10}$ cells/liter at 160 μm and 185 μm added nitrate, respectively.

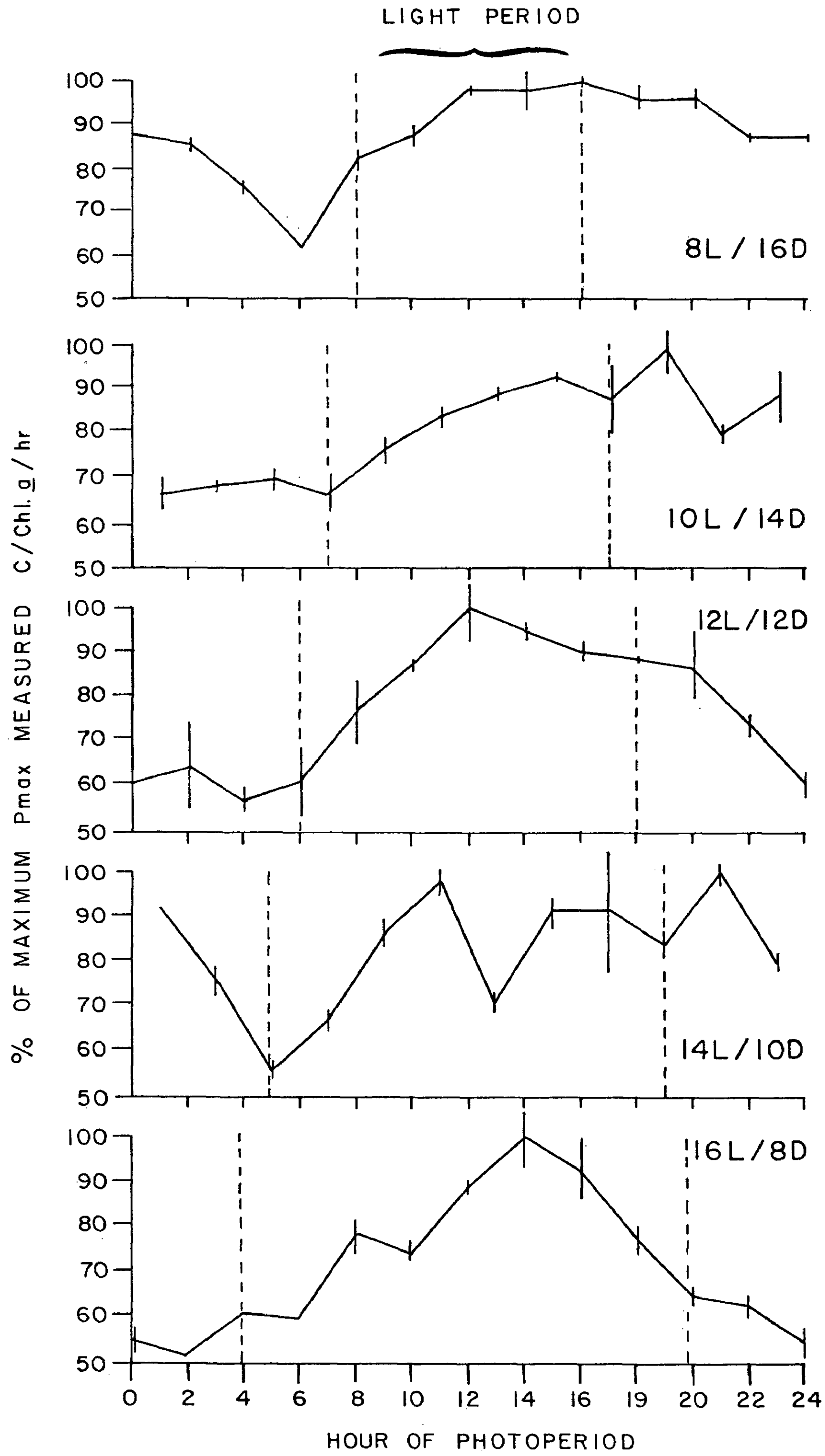
The pattern of diel variation of Pmax in cyclostat cultures was investigated as a function of the following photoperiods: 8L/16D, 10L/14D, 12L/12D, 14L/10D and 16L/8D. Figures 8 and 9 show the variation in Pmax expressed as pico-gram C/cell/hr and pico-gram C/ pico-gram chl *a*/hr respectively within a given photoperiod. The 100% value of Pmax for each photoperiod is given in figure headings 8 and 9. By inspection it can be seen that Pmax typically increases during the light portion of the photoperiod and decreases during the dark. A runs test, to test for randomness, was made on the data. The variation in Pmax expressed as C/cell/hr was found to be non-random at the 0.05 level for all the photoperiods except 14L/10D. When Pmax is expressed as C/chl *a*/hr all the photoperiods except 10L/14D and 14L/10D were found to be non-random at the 0.05 level.

The total amount of carbon fixed/cell in the cyclostat

Figure 8

Variation in Pmax of cyclostat *Chlorella* cultures (C/cell)

Variation in Pmax (rate of light saturated photosynthesis calculated ad carbon/cell/hr) for a specific photoperiod (beginning and end of photoperiod marked by vertical dotted lines) measured every two hours within a given photoperiod as a percent of the maximum Pmax measured for the entire photoperiod. 100% Pmax values are .18, .12, .11, .13, and .05 pgC/cell/hr for 8L/16D, 10L/14D, 12L/12D, 14L/10D and 16L/8D photoperiods, respectively.

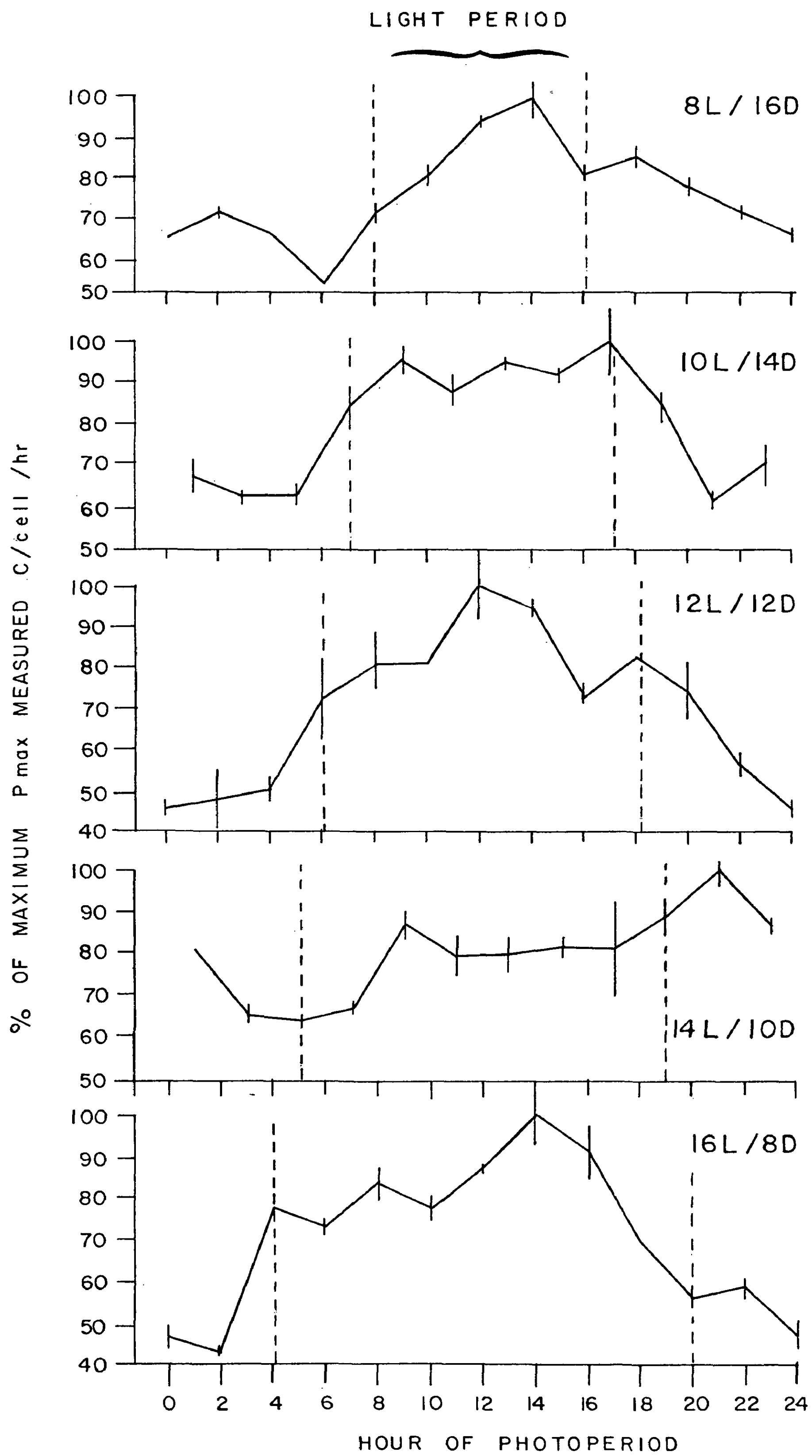


LIGHT PERIOD
8L / 16D
10L / 14D
12L/12D
14L/10D
16L/8D
% OF MAXIMUM Pmax MEASURED C/Chl. g / hr
HOUR OF PHOTOPERIOD

Figure 9

Variation in Pmax of cyclostat *Chlorella* cultures (C/chl *a*)

Variation in Pmax (rate of light saturated photosynthesis calculated as mgC/mg chl *a*/hr) for a specific photoperiod (beginning and end of photoperiod marked by vertical dotted lines) measured every two hours within a given photoperiod as a percent of the maximum Pmax measured for the entire photoperiod. 100% Pmax values are 7.2, 7.1, 5.3, 8.4, and 2.7 pgC/pg chl *a*/hr for 8L/16D, 10L/14D, 12L/12D, 14L/10D, and 16L/8D photoperiods, respectively.


LIGHT PERIOD
8L / 16D
10L / 14D
12L / 12D
14L / 10D
16L / 8D
% OF MAXIMUM P max MEASURED C/cell /hr
HOUR OF PHOTOPERIOD

populations during a given photoperiod was estimated from the carbon uptake measurements made during the light portion of the photoperiods. This value decreased linearly with an increase in the length of the light portion of the photoperiod (Figure 10). A linear regression of the data revealed that the slope was significantly different from zero at the 0.005 level. Values ranged from 1.16 to 0.77 pico-grams C/cell/day for an 8L/16D and 24L/0D photoperiod, respectively. Chlorophyll a/cell also decreased in a linear fashion ($P<.005$) with an increase in the length of the light period (Figure 11). Values ranged from 0.0085-0.0189 pico-grams chl a/cell.

The amount of time required for a cyclostat population to adapt to an altered photoperiod was determined by monitoring chl a/cell and carbon uptake/cell/hr as the photoperiod was changed. Chlorophyll a/cell exhibited a similar variation with respect to time and direction of change (increase or decrease in value) with carbon uptake (Figure 12). Approximately two-three days were required for complete adaptation.

The mean of the linear portion of all the light curves, from a cyclostat population, within a given photoperiod is graphed on a C/cell/hr and C/chl a/hr basis (Figures 13 and 14, respectively). Although there are only two values of x (i.e. two light levels) used to determine the slopes, in a majority of cases there are 22-24 y values (two reps, 12 sampling periods) for each x. An inspection of the means and standard deviation for each y value suggest that on a C/cell/hr basis the slopes between photoperiod treatments were different. The slopes graphed on a C/chl a/hr basis appear to be equal. However,

Figure 10

Variation in total carbon production/light period of cyclostat *Chlorella* cultures

This graph shows the relationship between pgC uptake/cell/light period as a function of the length of the light period. The values were obtained by taking the summation of the Pmax values measured every two hours beginning with the value determined at lights on of a particular photoperiod and continuing on through to lights off but not including this value. The sum was then multiplied by two.

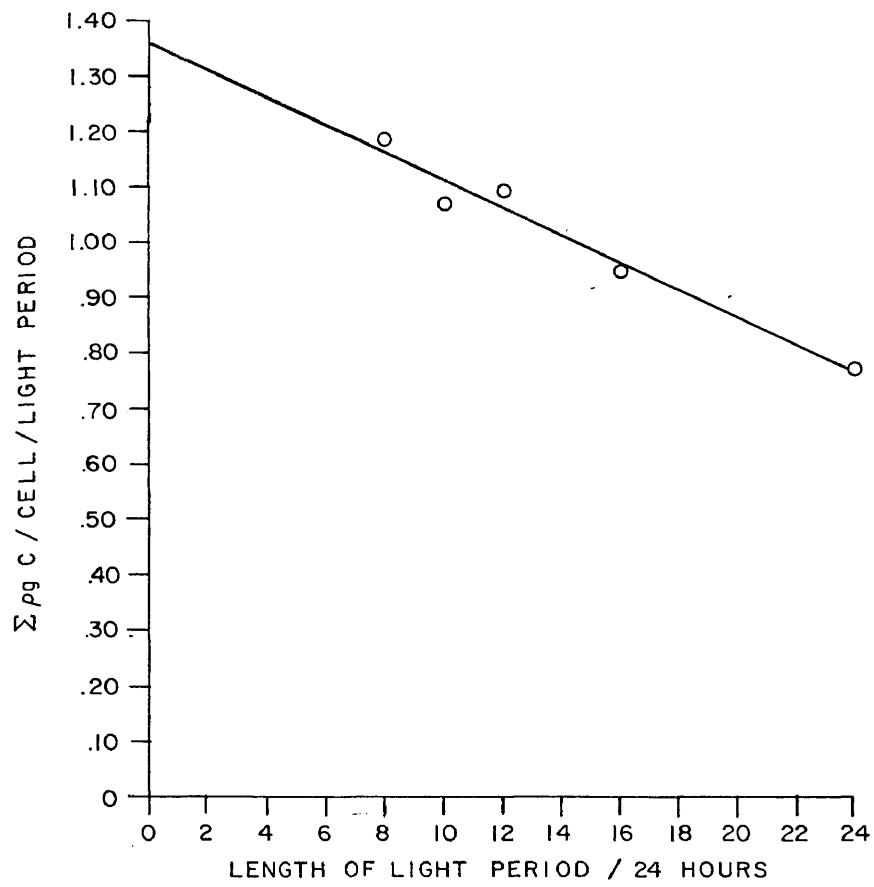
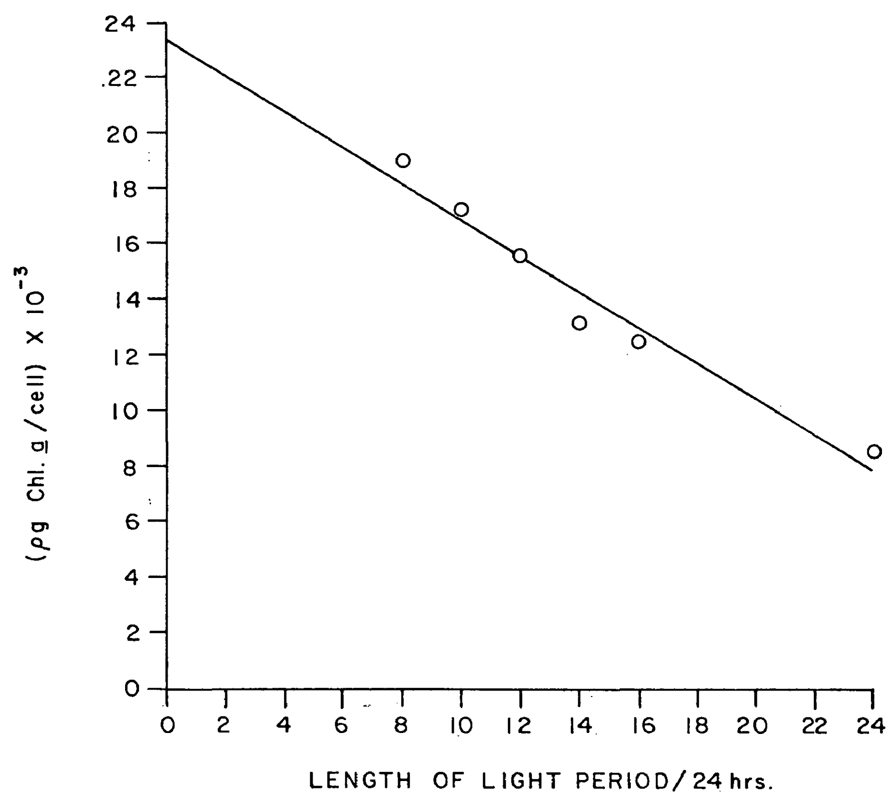

1.40
1.30
1.20
1.10
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.10
0
Σ ρg C / CELL / LIGHT PERIOD
0
2
4
6
8
10
12
14
16
18
20
22
24
LENGTH OF LIGHT PERIOD / 24 HOURS

Figure 11

Variation in chl *a*/cell of
cyclostat *Chlorella* cultures

This figure shows the variation in chl *a*/cell (pg Chl *a*/cell) $x10^{-3}$ with the length of the light period/twenty-four hr. Variation in measured chl *a*/cell values was less than 10% for a given photoperiod.



24
.22
20
18
16
14
12
10
8
6
4
2
0
(ρg Chl. a /cell) X 10-3
0 2 4 6 8 10 12 14 16 18 20 22 24
LENGTH OF LIGHT PERIOD/24 hrs.

Figure 12

Time course of change in rates of C/cell/hr and chl *a*/cell with a change in photoperiods of cyclostat cultures

This figure shows the change in pgC/cell/hr ( ) and pg chl *a*/cell ( ) vs. time when the photoperiod was adjusted to 16L/8D (first arrow), 8L/16D (second arrow) and 24L/0D (third arrow).

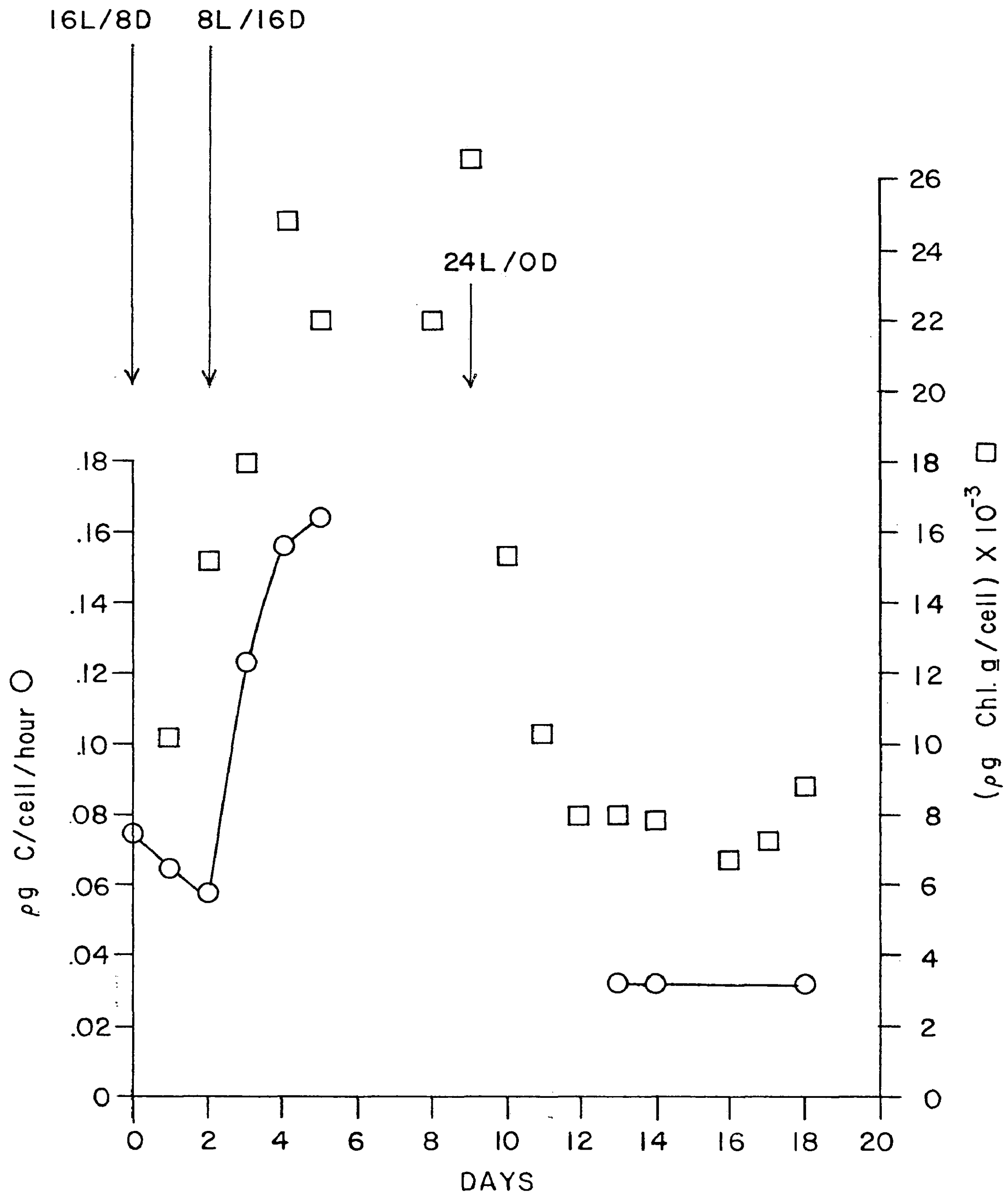
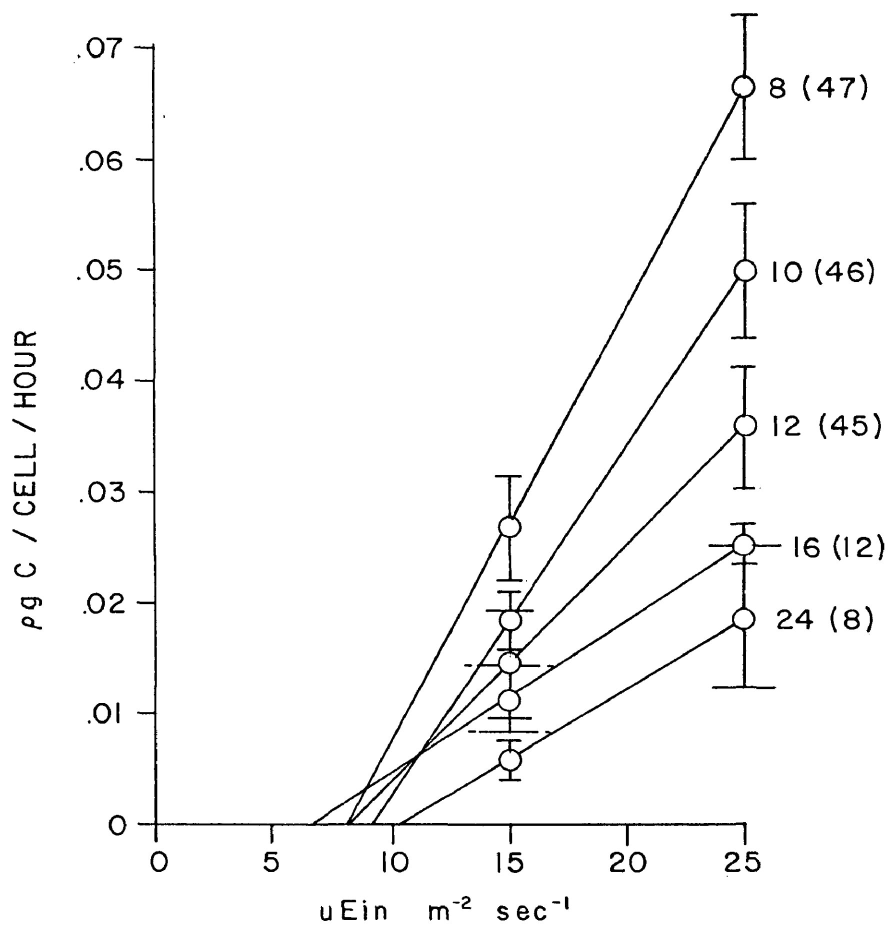

16L/8D
8L/16D
24L/0D
ρg C/cell/hour ○
(ρg Chl. a/cell) X 10^-3 □
DAYS
0
2
4
6
8
10
12
14
16
18
20
.18
.16
.14
.12
.10
.08
.06
.04
.02
0
26
24
22
20
18
16
14
12
10
8
6
4
2
0

Figure 13

The linear portion of the photosynthesis vs. light curve (C/cell/hr)

In this figure each line is labelled with respect to the photoperiod and the number of determinations used in plotting the two points making the line (number in parenthesis). For the 8L/16D, 10L/14D and 12L/12D photoperiods the values were obtained every two hours throughout the light and dark phases of the photoperiod and the mean (, pgC/cell/hr) and standard deviation (vertical lines) are shown. For the 16L/8D and 24L/0D, replicate values done on the populations during the midpoint of the light phase (not applicable to 24L/0D) on three and two consecutive days respectively are plotted.

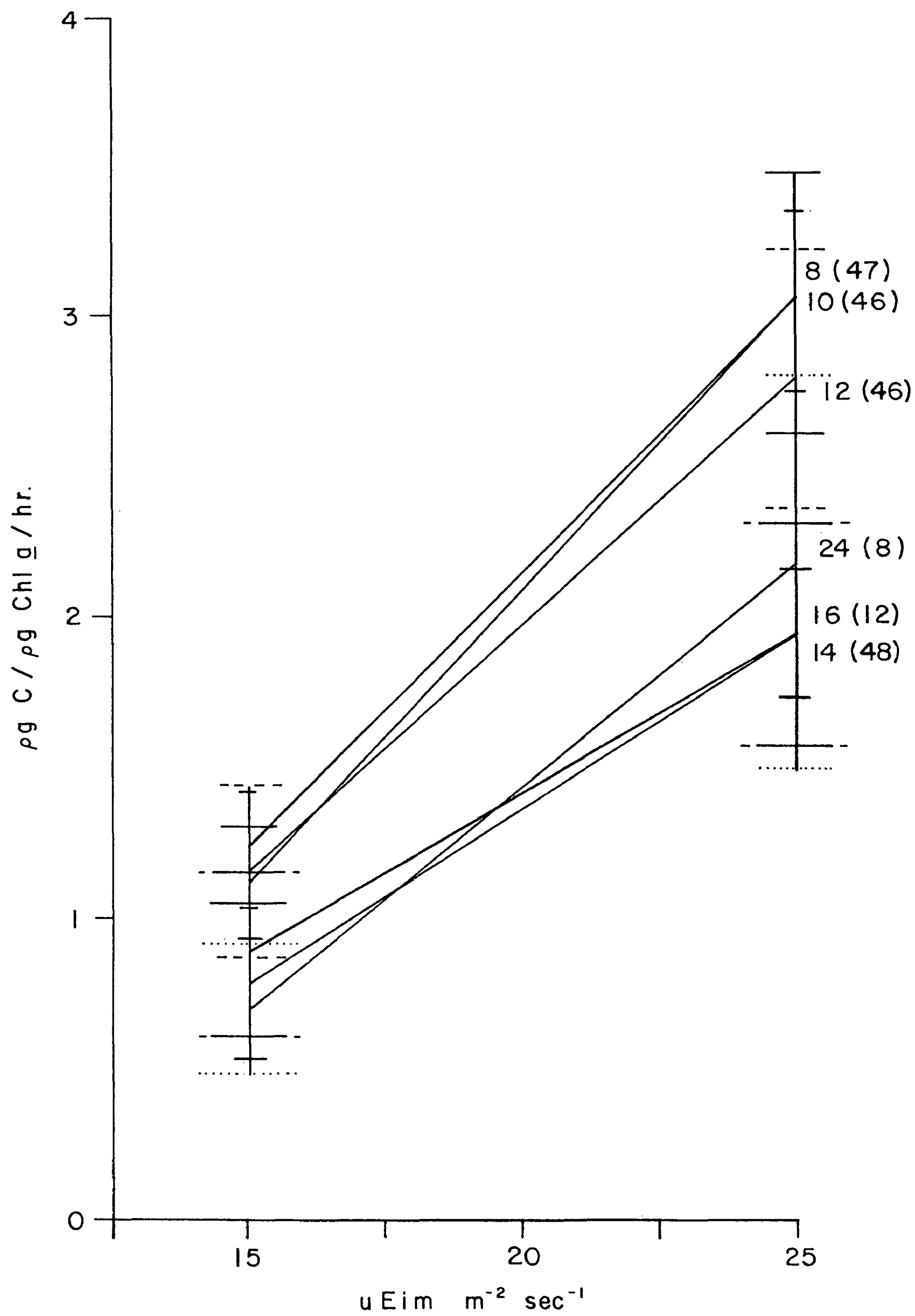


.07
.06
.05
.04
.03
.02
.01
0
ρg C / CELL / HOUR
0
5
10
15
20
25
uEin m⁻² sec⁻¹
8 (47)
10 (46)
12 (45)
16 (12)
24 (8)

Figure 14

The linear portion of the photosynthesis vs.
light curve (C/chl *a*/hr)

In this figure each line is labelled with respect to the photoperiod and the number of determinations used in plotting the two points making the line (number in parenthesis). For the 8L/16D, 10L/14D, 12L/12D and 14L/10D photoperiods the values were obtained every two hours throughout the light and dark phases of the photoperiod and the mean (, pgC/cell/hr) and standard deviation (vertical lin-s) are shown. For the 16L/8D and 24L/0D photoperiod, replicate values done on the populations during the midpoint of the light phase (not applicable to 24L/0D) on three and two consecutive days respectively are plotted.



4
3
2
1
0
ρg C / ρg Chl a / hr.
8 (47)
10 (46)
12 (46)
24 (8)
16 (12)
14 (48)
15
20
25
u Eim m⁻² sec⁻¹

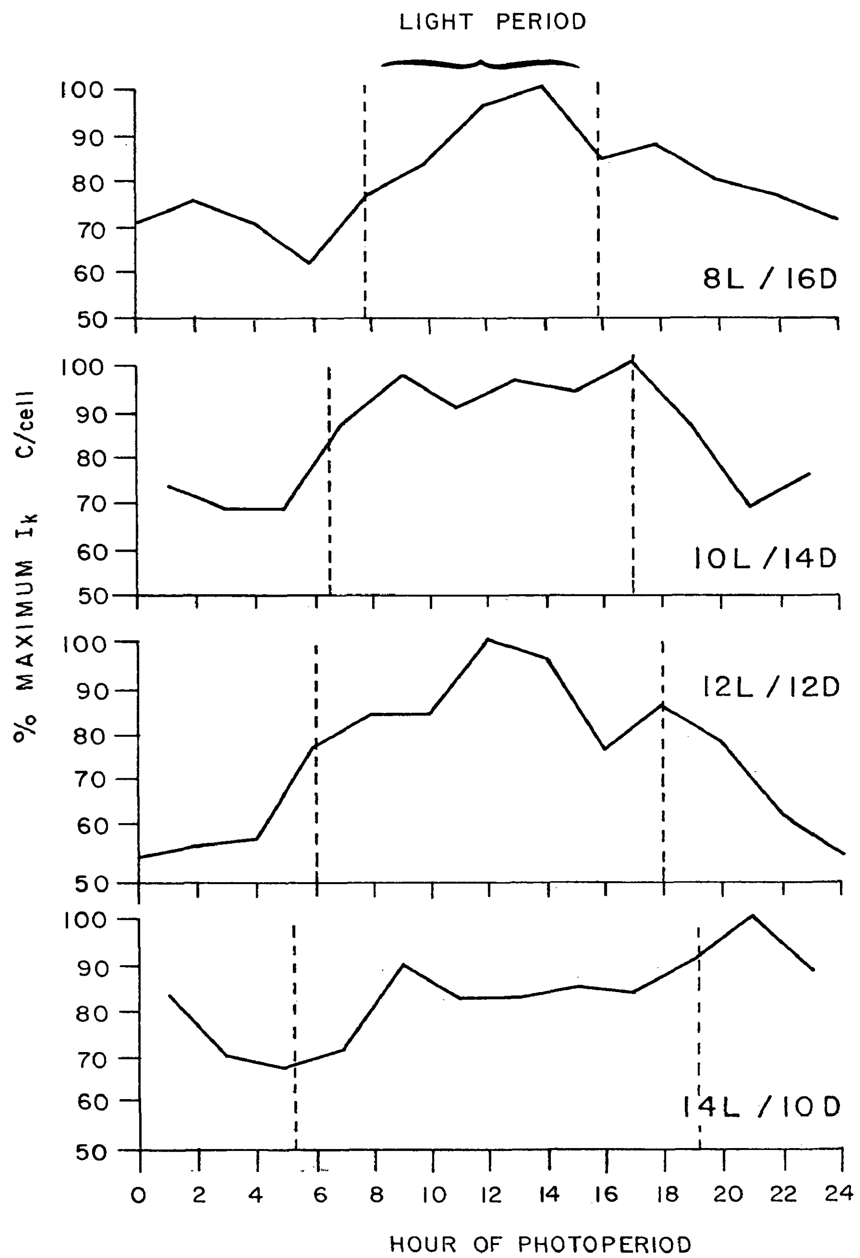
an analysis of covariance of k regression lines revealed that in neither case were the slopes equal at the 0.001 level. Within any given photoperiod the slope (alpha) was found to vary randomly (P .05) based on a runs test made on the alpha values measured every two hours over a 24 hour period.

I_k was found to vary in a diel fashion (Figures 15 and 16) for all the cyclostat populations entrained in a light dark photoperiod. During the light period higher I_k's were observed than in the dark. A runs test on the data showed non-randomness in all cases (P 0.05) except for the 14L/10D photoperiod when I_k was determined using C/cell/hr and 10L/14D and 14L/10D when calculated as C/chl _a_/hr. Table III lists the mean and standard deviation of I_k for each photoperiod.

Figure 15

Variation in I_k of cyclostat *Chlorella* cultures (C/cell)

This figure shows the variation in I_k obtained from pgC/cell/hr vs. light curves for a specific photoperiod (labelled on graph) generated every two hours over 24 hours for a given photoperiod. Values are a percent of the maximum I_k observed during each photoperiod.



LIGHT PERIOD
% MAXIMUM I_k C/cell
8L / 16D
10L / 14D
12L / 12D
14L / 10D
100
90
80
70
60
50
0
2
4
6
8
10
12
14
16
18
20
22
24
HOUR OF PHOTOPERIOD

Figure 16

Variation in I_k of cyclostat _Chlorella_ cultures (C/chl _a_)

This figure shows the variation in I_k obtained from pgC/pg chl _a_/hr vs. light curves for a specific photoperiod (labelled on graph) generated every two hours over 24 hours for a given photoperiod. Values are a percent of the maximum I_k observed during each photoperiod.

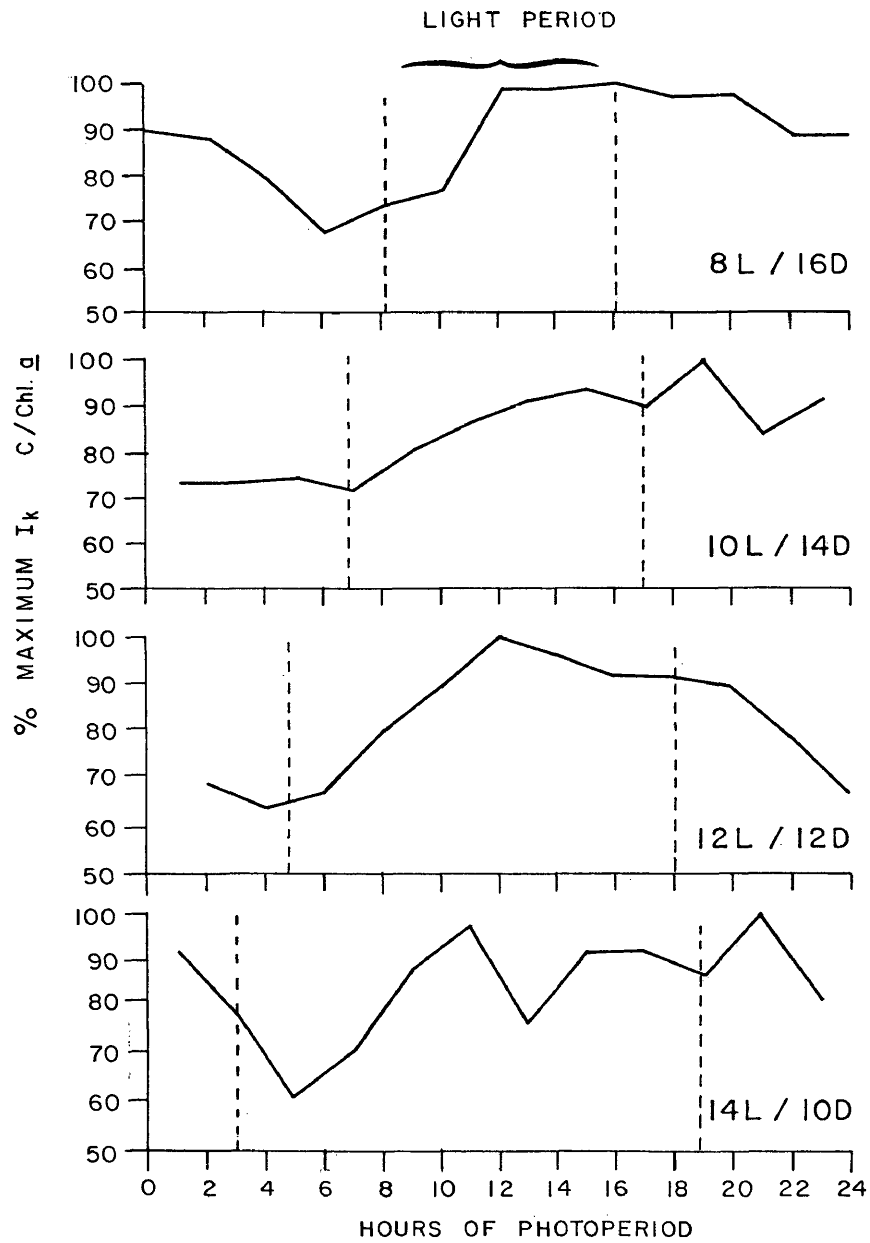

LIGHT PERIOD
8L / 16D
10L / 14D
12L / 12D
14L / 10D
% MAXIMUM I_k C/Chl. a
HOURS OF PHOTOPERIOD

TABLE III

I_k Values from Cyclostat

$I_k (\mu E/m^2/sec)$

Photoperiod	C/cell intersect	C/chl *a* intersect
8/16	43.3 ± 5.9	43.2 ± 4.5
10/14	39.0 ± 5.6	38.8 ± 4.2
12/12	44.6 ± 9.9	44.5 ± 7.1
14/10	50.7 ± 5.6	50.4 ± 7.0
16/8	55.9 ± 5.9	59.4 ± .8
24	36.0 ± .2	36.4 ± 1.5

DISCUSSION

The original question of this study concerned the effect of varying photoperiods on an algal population's pattern of Pmax throughout a 24 hour day. In this study the illumination provided for the laboratory experiments was held constant while the photoperiod was varied resulting in an increase in total light energy with the lengthening of the light period.

Three out of five of the cyclostat culture response to light/dark periods was a bell shaped pattern of Pmax during the light period. This was observed in cultures exposed to 8L/16D, 12L/12D and 16L/8D (Figures 8 and 9) with peak values occurring during the middle of the light period. Minimum values of Pmax occurred most often during the middle of the dark period. Similar patterns of Pmax were reported for nitrate limited cyclostat cultures (12L/12D) of *Thalassiosira psedonana* Hasle (Eppley and Renger, 1974), *Thalassiosira allenii* (Laws and Wong, 1978) and batch cultures (12L/12D) of *Glenodinium sp.*, *Ceratium furca* Ehrenberg, *Gonyaulax polyedra* Stein (Prezelin, Meeson and Sweeney, 1977) and *Skeletonema costatum* (Jorgensen, 1966). The pattern of Pmax in the cyclostat cultures exposed to 10L/14D and 14L/10D was erratic. This is similar to Haas's (1975) data from natural populations exposed to ca. 14 hr plus photoperiods with respect to *in situ* photosynthesis. The present data set is lacking in qualitative aspects to adequately explain the non bell shaped pattern

of Pmax in the cyclostat cultures exposed to 10L/14D and 14L/10D photoperiods.

Haas (1975) reported three basic patterns of diel _in situ_ photosynthesis by York river phytoplankton populations. During short days the algal poplations exhibited a peak value of _in situ_ photosynthesis at noon. On days of intermediate length the major peak shifted to the afternoon with a secondary peak occurring in the morning as well as a noon decrease. During the longest days equal mid-morning and mid-afternoon peaks were separated by relatively low values at noon. The fact that maximum observed _in situ_ photosynthesis did not always occur at the midpoint of the photoperiods might be attributed to zooplankton grazing, tidal influence, and product inhibition, inhibitory light levels or the existing nutrient regime. Present data are not in agreement with the hypothesis that varying photoperiods alone will cause a change in the diel pattern of Pmax.

A model of diurnal variation can be put forth to explain the pattern of light saturated photosynthesis observed in this data set. In the model light provides an outside source of energy which results in energized compounds of ATP, NADH and fixation of CO_2, thus providing sufficient substrate for the operation of the Calvin cycle. Decreased activity of the Calvin cycle during the dark period is attributed to the termination of an outside energy source and a switch to total reliance upon energy and substrate reserves from within the cell. Incorporation of Sweeney's (1969) hypothesis that changes in membrane permeabilities cause changes in the dark reactions of photosynthesis might explain the exact timing of maximum Pmax during the

middle of the light period. Sweeney (1969) postulates that light induced changes in membrane permeabilities could affect membrane bound enzyme activities either directly or indirectly by modulation of the flow of some critical substrate. Njus, Sulzman and Hastings (1974) suggest that changes in membrane permeabilities could provide the proper time course to control rhythms of at least 24 hours.

The present model is based on Prezelin and Sweeney's (1977) model; both require a change in membrane permeability. However, Prezelin and Sweeney's (1977) model requires inactivation of the photosynthetic units (Psu, i.e. photosysystems I and II) associated with the thylakoid membrane. The result is that although the chl a concentration within a cell does not change, some of its light (energy) capturing ability is deactivated. This causes a lowering of the alpha value and a concomitant lowering of Pmax without requiring any change in the dark reaction enzyme activities or substrate concentrations (if I_k does not change, see Appendix III). The present data set does not indicate that photosynthetic units are inactivated which concurs with Mishkind and Beal's (1979) work on Ulva lactuca.

There are three hypotheses in the literature that address the issue of variation in the linear portion of the light vs. $^{14}CO_2$ uptake/cell hr curve (alpha) by phytoplankton. Banister (1974) and Dunstan (1973) assume alpha should be a relative stable parameter based on the fact that the quantum yield at low irradiances depend on the general photochemical reactions of chloroplast pigments and should therefore be independent of both species and temperature. Prezelin and Sweeney (1977) provide evidence that alpha varies throughout the day in an algal culture entrained on a light/dark cycle due

to activation and inactivation of the photosynthetic units (photosystems I and II) associated with the thylakoids. Taguchi (1976b) hypothesized that alpha would vary as a result of self-shading of the chloroplast which would effectively reduce the number of photosynthetic units that would be activated by the light.

A runs test on alphas from the cyclostat populations indicated a random variation within a given photoperiod. This is contrasted with a non-random variation observed in I_k. Dunstan (1973), Banister (1974), Walter and Edmunds (1973), Yentsch and Lee (1966), Gargas et al. (1979) and MacCaull and Platt (1977) point out that I_k is a derived parameter from the ratio of Pmax and alpha. If both Pmax and alpha show a similar diurnal variation then I_k would be expected to vary randomly. However, Gargas et al. (1979) note that if alpha is constant then I_k should reflect a similar non-random change as Pmax, which is the case in this study (Figures 15 and 16).

Platt and Jassby (1976) observed a five fold seasonal variation in alpha. The highest values of alpha were associated with higher values of ambient light. Taguchi (1976a) and Platt and Jassby (1976) hypothesize that alpha is a function of cell size, pigment composition, cellular architecture and light quality. They suggest that higher light intensities decreased the chlorophyll content of the cell thus decreasing self-shading and increasing alpha. If the self-shading hypothesis applied to the present data set a linear relationship would be expected between paired values of alpha (C/chl a/hr/light unit) and chl a/cell from the six photoperiod treatments which encompass a three fold variation in total light intensity/day. The regression

coefficient, r, of the cyclostat data was only 0.495. The lack of the self-shading effect might be attributed to the fact that though chl *a*/cell does vary among the cyclostat populations, the maximum value was one-third the maximum value of the batch cultures. Taguchi (1976a) limited the self-shading hypothesis to cells packed with chlorophyll.

Batch cultures at various stages of the growth cycle (Figure 5, log and stationary) exhibited different alpha values. Senger and Bishop (1967) using batch cultures of *Scenedesmus* report different values of quantum yield of photosynthesis as a function of the physiological age of the algae. However, the present data set from cyclostat cultures does not support the hypothesis that variation in alpha can account for variation in Pmax over a light/dark cycle. Had this been the case then alpha would have varied non-randomly over the day and I_k randomly.

Senft (1978) found that *Anabaena* cf. *wisconsinense* and *Chlorella pyrenoidosa* exhibited increasing rates of light saturated photosynthesis with an increasing nutrient cell quota. Batch cultures in this study indicated that nitrogen/cell changed from one day to the next throughout the growth curve (Figure 2) with a maximum value occurring just before log phase growth. These results are similar to Daley and Brown's (1973) for *Anacystis nidulans* and *Phormidium molle* in batch cultures. The high batch culture Pmax values observed early in log phase of growth vs. later (0.80 vs. 0.20 pico-gram C/cell/hr, Figure 6) agrees with Senft's hypothesis that the nutrient quota of a cell controls the light saturated photosynthetic rate. The cyclostat

population exhibited a somewhat lower cell quota than the batch culture in lag phase (0.0185 vs. 0.02 pico-gram atom-N/cell, respectively) and a concomitant lower Pmax (0.032-0.18, cyclostat populations vs. 0.20 pico-gram C/cell/hr, batch cultures). These results indicate that carbon production/cell/hr and the nutrient quota of the algal cell are intricately related.

Highest chlorophyll content per cell of the batch culture populations was measured during the log phase of its growth cycle and exceeded chl a/cell values calculated for the cyclostat cultures by a factor of three; light environments were identical. However, the chl a/cell in the stationary phase of the batch cultures and the cyclostat populations was similar. These results can most likely be explained by the fact that the batch cultures in log phase were not limited by nutrients while the cyclostat and stationary phase populations were. Eppley and Sloan (1966) and Eppley and Renger (1974) observed decreasing chl a/cell with increasing nutrient deficiency and the present data clearly supports this.

In cyclostat populations an inverse relationship exists between chl a/cell and the length of the photoperiod (Figure 11). Hobson et al. (1979) reported similar results using batch cultures of *Isochrysis galbana* Parke exposed to six, twelve and eighteen hours of light. Valanne (1977) using moss found an analogous relationship between chlorophyll content and photoperiod. The present dats is in agreement with Hobson et al. (1979) and Valanne (1977) that plants (algae) adapt to shorter photoperiods by an increase in the chlorophyll content of the cell(s).

Carbon/chl a ranged from 222 in cyclostat populations with an 8L/16D photoperiod to 342 in 24L/0D (Table IV) compared to Ch/chl a value of 91 for other algal populations under steady state conditions and similar dilution rates (Eppley and Renger, 1974). Laws and Wong report C/chl a values of 311 for Thalassiosira alleni grown at growth rates similar to those utilized in this study. Eppley (1972) reports a typical C/chl a value of 30-40 for a Peru upwelling region while 90-100 is more typical of the low-nutrient surface waters off southern California. Thus, present results are compatible with the suggestion that the cells have been stressed by a low nutrient environment.

It has been suggested that the photosynthetic assimilation number of natural phytoplankton communities is a function of the degree of nutrient deficiency (Curl and Small, 1965). They found assimilation ratios in nutrient depleted waters of 0-3, borderline cases of 3-5 and nutrient rich areas with ratios of 5-10. In this study the batch cultures in log phase (nutrient sufficient) exhibited assimilation ratios of 12-18. Algae in near stationary phase or cyclostat populations (previously shown to be nutrient limited) were found to have ratios of 4-9 and 4-8, respectively. Although the ratios do not agree exactly with Curl and Small (1965) they do show a decrease with increased nutrient deficiency.

Carbon/nitrogen ratios were calculated for the cyclostat algal populations. Meyers (1951) reported a constant C/N value of 5.7 for Chlorella pyrenoidosa grown under conditions limited only by light. Nitrogen depleted cells typically show a much higher ratio (Eppley and Thomas, 1969; Eppley, Rogers and McCarthy, 1969; and Prochazkova,

Blazka and Kralova, 1970). Ratios from the cyclostat populations, which varied between 16 and 10, are similar to the value of 14.14 reported for Thallassiosira pseudonanna under similar growth conditions (Eppley and Renger, 1974). Caperon and Ziemann (1976) have shown that increasing nutrient deficiency was paralleled by increasing C/N values. The decrease in C/N with the length of the photoperiod (Table IV) could then be interpreted to mean that the population in continuous light was the least nutrient deficient. However, nitrogen content of the cells did not change (cell number and nitrate concentration within the reservoir remained constant) regardless of the light period. Therefore, the carbon content must have decreased with increasing length of the photoperiod. More will be said with respect to the C/N value in what follows.

Total carbon fixed/cell/day decreased linearly with increasing photoperiods (Figure 10). Hobson et al. (1979) using batch cultures reported higher daily net photosynthetic rates for populations entrained in 12L/12D compared to 6L/18D and 18L/6D photoperiods. The exact relationship was a function of temperature. Eppley and Coatsworth (1968) and Ferguson et al. (1976) report longer daylengths increased growth. Other studies suggest that short day lengths (8L/16D) or intermediate day lengths are more conducive to growth than long daylengths or continuous light (Castenholz, 1964; Hodson, 1974; and Foy, Gibson and Smith, 1976). The primary difference between the studies cited and the present work is that the latter used steady state populations of nutrient deficient algae. There is an alternate interpretations of the decreasing total carbon fixed/cell/day with increasing photoperiods.

TABLE IV

Cyclostat Cell Population Values

Photoperiod	pgC/cell/day	pgC/pgV	C/chl	Assimilation
8	1.160	15.6	222	8
10	1.118	14.9	229	6.6
12	1.062	14.2	236	5.7
14	1.013	13.5	246	5.1
16	.964	12.8	257	4.6
24	.768	10.4	342	4.1

It is hypothesized that if the present data set is corrected for respiration in the dark period that total net photosynthesis/cell/day would remain constant regardless of the total light energy/day as long as the light energy was neither limiting or inhibiting for a given dilution rate. The 8L/16D cyclostat population, relative to the other photoperiods, would have to respire in the dark period the largest percentage of its carbon fixed during the light period, i.e. up to 33%. This is not an unrealistic value as Laws and Wong (1978) report dark respiration (at comparable dilution rates) varied from 19-30% of the carbon fixed during the light periods of their cyclostat cultures. If the respiration corrections are made on the present data set the C/N value for all the photoperiods would be equal to 10.4. Goldman, McCarthy and Peavey (1979) point out that there is a direct relationship between the nutrient influenced growth rate and the elemental composition of oceanic phytoplankton. Their work would not have predicted a change in C/N as a function of photoperiod if the growth rate remained constant. Giddings (1977) reported that nitrogen limited chemostat cultures of *Scenedesmus abundans* did not change with respect to C/N values when the light intensity was increased (24L/0D).

The nutrient limited cyclostat cultures in this study are believed to have adapted to increasing levels of light energy/day by decreasing daily carbon uptake/cell, cell respiration, and chl *a*/cell. The result is that a constant C/N value is maintained within the cells for a particular dilution rate.

LITERATURE CITED

Banister, T. T. 1974. Production equations in terms of chlorophyll concentration, quantum yield, and upper limits to production. Limnol. Oceanogr. 19: 1-12.

Brewer, P. G. and J. C. Goldman. 1976. Alkalinity changes generated by phytoplankton: Some experiments with Phaeodactylum tricornutum. Mar. Biol. 37: 377-387.

Caperon, J. and D. A. Ziemann. 1976. Synergistic effects of nitrate and ammonium ion on the growth and uptake kinetics of Monochrysis lutheri in continuous culture. Mar. Biol. 16: 73-84.

Castenholz, R. W. 1964. The effect of daylength and light intensity on the growth of littoral marine diatoms in culture. Physiologia Plant. 17: 951-963.

Chisholm, S. W. and P. A. Nebbs. 1975. Simulation of algal growth and competition in a phosphate-limited cyclostat. In: Canale, R. P. (Ed.) Modeling Biochemical Processes in Aquatic Ecosystems. Ann Arbor Press, Ann Arbor, Michigan, 355-377.

Collos, Y. and G. Slawk. 1979. ^{13}C and ^{15}N uptake by marine phytoplankton. I. Influence of nitrogen source and concentration in laboratory cultures of diatoms. J. Phycol. 15: 186-190.

Curl, H., Jr. and L. F. Small. 1965. Variations in photosynthetic assimilation ratios in natural, marine phytoplankton communities. Limnol. Oceanogr. 10(Suppl.): R67-R73.

Daley, R. J. and S. R. Brown. 1973. Chlorophyll, nitrogen and photosynthetic patterns during growth and senescence of two blue-green algae. J. Phycol. 9: 395-401.

Doty, M. S. 1959. Phytoplankton photosynthetic periodicity as a function of latitude. Journal of the Marine Biological Association of India. 1: 66-68.

Doty, M. S. and M. Oguri. 1957. Evidence for a photosynthetic daily periodicity. Limnol. Oceanogr. 2: 37-40.

Dunstan, W. M. 1973. A comparison of the phytosynthesis-light intensity relationship in phylogenetically different marine microalgae. J. Exp. Mar. Biol. Ecol. 13: 181-187.

Eppley, R. W. 1972. Temperature and phytoplankton growth in the sea. Fish. Bull. 70: 1063-1085.

Eppley, R. W., A. F. Carlucci, O. Holm-Hansen, D. Kiefer, J. J. McCarthy, E. Venrick and P. M. Williams. 1971. Phytoplankton growth and composition in shipboard cultures supplied with nitrate, ammonium or urea as the nitrogen source. Limnol. Oceanogr. 16: 741-751.

Eppley, R. W. and J. L. Coatsworth. 1968. Culture of the marine phytoplankton Dunaliella tertiolecta with light/dark cycles. Arch. Mikrobiol. 55: 66-80.

Eppley, R. W., T. T. Packard and J. J. MacIsaac. 1970. Nitrate reductase in Peru current phytoplankton. Mar. Biol. 6: 195-199.

Eppley, R. W. and Renger. 1974. Nitrogen assimilation of an oceanic diatom in nitrogen-limited continuous culture. J. Phycol. 10: 15-23.

Eppley, R. W. and R. R. Sloan. 1966. Growth rates of marine phytoplankton: correlation with light absorption by cell chlorophyll a. Physiol. Plant. 19: 47-59.

Eppley, R. W. and W. H. Thomas. 1969. Comparison of half-saturation constants for growth and nitrate uptake of a marine phytoplankton. J. Phycol. 5: 375-379.

Ferguson, R. L., A. Collier and D. A. Meeter. 1976. Growth response of Thalassiosira pseudonana Hasle and Heimdal clone 3H to illumination, temperature and nitrogen source. Ches. Sci. 17: 148-158.

Fogg, C. F. 1965. Algal Cultures and Phytoplankton Ecology. University of Wisconsin Press, Madison Wisconsin. pp. 126.

Foy, R. H., C. E. Gibson and R. V. Smith. 1976. The infleunce of daylength, light intensity and temperature on the growth rates of planktonic blue-green algae. Br. Phycol. 11: 151-163.

Gaarder, T. and H. H. Gran. 1927. Investigations of the production of plankton in the Oslo Fjord. Rapp. P.-V. Reun. Cons. Perm. Int. Explor. Mer. 42: 1-48.

Gargas, E., I. Hare, P. Martens and L. Edler. 1979. Diel changes in phytoplankton photosynthetic efficiency in brackish waters. Mar. Biol. 52: 113-122.

Giddings, J. M. 1977. Chemical composition and productivity of Scenedesmus abundans in nitrogen limited chemostat cultures. Limnol. Oceanogr. 22: 911-918.

Goering, J. J., R. C. Dugdale and D. W. Menzel. 1964. Cyclic dirunal variations in the uptake of ammonia and nitrate by photosynthetic organisms in the Sargasso Sea. Limnol. Oceanogr. 9: 448-451.

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

Guillard, R. R. L. and J. H. Ryther. 1962. Studies of marine plankton diatoms. I. Cyclotella nanna (Hustedt) and Detonula confervacea (Cleve.) Gran. Can. J. Microbiol. 8: 229-239.

Haas, L. W. 1975. Plankton dynamics in a temperate estuary with observations on a variable hydrographic condition. Ph.D. College of William and Mary, Williamsburg, Virginia.

Hobson, L. A. 1974. Effects of interactions of irradiance, daylength and temperature on division rates of three species of marine unicellular algae. J. Fish. Res. Bd. Can. 31: 391-395.

Hobson, L. A., F. A. Hartley and D. E. Ketchum. 1979. Effects of variations in daylength and temperature on net rates of photosynthesis, dark respiration and excretion by Isochrysis galbana. Plant Physiol. 63: 947-951.

Holmes, R. W. and F. T. Haxo. 1958. Dirunal variations in the photosynthesis of natural phytoplankton populations in artificial light. U.S. Fish and Wildlife Service, Special Scientific Report: Fisheries. 279: 73-76.

Jorgensen, E. G. 1966. Photosynthetic activity during the life cycle of synchronous Skeletonema cells. Physiol. Plant. 19: 789-799.

Laws, E. A. and D. C. L. Wong. 1978. Studies of carbon and nitrogen metabolism by three marine phytoplankton species in nitrate-limited continuous culture. J. Phycol. 14: 406-416.

Lorenzen, C. J. 1963. Diurnal variations in photosynthetic activity of natural phytoplankton populations. Limnol. Oceanogr. 8: 56-62.

MacCaull, W. A. and T. Platt. 1977. Diel variations in the photosynthetic parameters of coastal marine phytoplankton. Limnol. Oceanogr. 22: 723-731.

McLachlan, J. 1964. Some considerations of the growth of marine algae in artificial media. Can. J. Microbiol. 10: 769-782.

Malone, T. C. 1971. Diurnal rhythms in netplankton and nanoplankton assimilation ratios. Mar. Biol. 10: 285-289.

Mishkind, M., D. Mauzerall and S. I. Beale. 1979. Dirunal variation in situ of photosynthetic capacity in Ulva is caused by a dark reaction. Plant Physiol. 64: 896-899.

Morris, I. and H. E. Glover. 1974. Questions on the mechanism of temperature adaptation in marine phytoplankton. Mar. Biol. 24: 147-154.

Myers, J. 1951. Physiology of the algae. Ann. Rev. Microbiol. 5: 157-180.

Njus, D., F. M. Sulzman and J. W. Hastings. 1974. Membrane model for the circadian clock. Nature. 248: 116-120.

Paerl, H. W. and L. A. Mackenzie. 1977. A comparative study of the diurnal carbon fixation patterns of nanoplankton and netplankton. Limnol. Oceanogr. 22: 732-738.

Parsons, T. and M. Takahashi. 1973. Biological Oceanographic Processes. Pergamon Press, N.Y. pp. 186.

Platt, T. and A. D. Jassby. 1976. The relationship between photosynthesis and light for natural assemblages of coastal marine phytoplankton. J. Phycol. 12: 421-430.

Prezelin, B. B., B. W. Meeson and B. M. Sweeney. 1977. Characterization of photosynthetic rhythms in marine dinoflagellates. I. Pigmentation, photosynthetic carpacity and respiration. Plant Physiology. 60: 384-387.

Prezelin, B. B. and B. M. Sweeney. 1977. Characterization of photosynthetic rhythms in marine dinoflagellates. II. Photosynthesis-irradiance curves and in vivo chlorophyll a fluorescence. Plant Physiol. 60: 388-392.

Prochazkova, L., P. Blazka and M. Kralova. 1970. Chemical changes involving nitrogen metabolism in water and particulate matter during primary production experiments. Limnol. Oceanogr. 15: 797-807.

Senft, W. H. 1978. Dependence of light-saturated rates of algal photosynthesis on intracellular concentrations of phosphorus. Limnol. Oceanogr. 23: 709-718.

Senger, H. and N. I. Bishop. 1967. Quantum yield of photosynthesis in synchronous Scenedesmus cultures. Nature 214: 140-143.

Shimada, B. M. 1958. Diurnal fluctuations in photosynthetic rate and chlorophyll a content of phytoplankton from Eastern Pacific waters. Limnol. Oceanogr. 3: 336-339.

Smayda, T. J. 1975. Phased cell division in natural populations of the marine diatom Ditylum brightwellii and the potential significance of diel phytoplankton behavior in the sea. Deep-Sea Res. 22: 151-165.

APPENDIX I

Media Preparation for Cyclostat Cultures

Artificial seawater media was prepared in 24 liter batches at a salinity of 15 o/oo. After the f/2 enrichment (except for nitrate) nitrate was added via a 700 mM nitrate spike to give a final concentration of 185 μM. Eight liter aliquots were then placed into 21 liter supply bottles which were corked with cotton plugs and autoclaved. Media prepared in this manner could be stored for months without any observable bacterial contamination or change in nutrient concentration. A number 12 cork containing two glass tubes was placed in the top of the supply bottles. One tube containing cotton wool served as an air supply for the bottle. The other tube extended to the bottom of the bottle with the other end connected to silicon tubing (Silicone medical tubing, A. H. Thomas) which attached to a peristaltic pump. The cork and associated tubing were autoclaved and fitted into the supply bottle under an aseptic laboratory hood equipped with a UV light. The tubing was connected to a glass tube that then joined to the pump tubing. At the outflow of the pump tubing another bent glass tube ran to a 25 ml volumetric pipette that ran into the cyclostat where it was held in place by a number 12 stopper. The pipette served as a break line to prevent any bacteria or algae from contaminating the nutrient media.

APPENDIX II

Theory of the Chemostat

A continuous culture (chemostat) of algae utilizes a culture chamber supplied with a nutrient medium at a constant rate (via a peristaltic pump) which displaces a fixed portion of the culture by way of an overflow. A critical nutrient in the culture medium such as nitrogen is supplied at a rate which is limiting relative to other nutrients. Steady state is defined by a zero change in cell concentration (X) over time. The rate of change of X will equal the net rate of change due to population growth (UX) minus the less of cells due to overflow (DX):

$$dX/dt = (U-D)X \tag{1}$$

It follows then that if dX/dt equals zero at steady state U must be equal to D.

Another requirement of steady state is that the standing stock of the chemostat is controlled by the limiting nutrient concentration of the inflow. In steady state the change in limiting nutrient in the growth chamber during an infinitesimal time interval is equal to the input minus output minus consumption or:

$$ds/dt = Ds_i - Ds_o - U\bar{X}/Y \tag{2}$$

where ds, change in limiting nutrient concentration; D, dilution rate; s_i, limiting nutrient concentration of input; s_o, limiting nutrient concentration of output; $\bar{X}$, steady state cell concentration; Y, yield coefficient or cell concentration divided by the limiting nutrient utilized. At steady state D=U, ds/dt = 0 and Y is a constant for a particular growth rate. Therefore equation 2 becomes:

$$0 = U(s_i - s_o) - U\bar{X}/Y \qquad (3)$$

$$\bar{X}/Y = s_i - s_o \qquad (4)$$

It should be noted that the cell quota (q, limiting nutrient per cell where q=1/Y) can be determined from equation 4 by rearrangement:

$$q = (s_i - s_o)/\bar{X} \qquad (5)$$

Therefore at a fixed steady state growth rate a linear relationship exists between $\bar{X}$ (x axis) and $s_i - s_o$ (y axis) with the y intercept equal to zero and the slope of the line equal to q.

In the present study the continuous culture technique was utilized but the cell population was exposed to alternating light and dark periods. Chisholm and Nebb (1975) call such an apparatus a cyclostat. Chisholm and Nebb (1975) note that if a population of cells is synchronized cell division takes place at one point in time and equation 1 (this study) becomes;

$$\frac{dX}{dt} = -DX_t \qquad \text{if } t \leq t_1 \qquad (6)$$

if cell division is completed and after integration:

$$X_t = X_{t_o} e^{-Dt} \tag{7}$$

At t_1 all the cells ready to divide do so and the proportion dividing:

$$Ue =]X_{(t_1+\Delta t)} - X_{t_1}]/X_{t_1} \quad \text{where } \Delta t \to 6 \tag{8}$$

where Ue is the relative growth rate.

From equation 7

$$X_{t_1} = X_{t_o} e^{-Dt_1} \tag{9}$$

combined with equation 8 yields

$$Ue = \frac{X_{(t_1+\Delta t)} - X_{t_o} e^{-Dt_1}}{X_{t_o} e^{-Dt_1}} \quad \text{where } \Delta t \to 0 \tag{10}$$

In rhythmic steady state:

$$X_{(t_1+\Delta t)} = X_{t_o} \quad \text{where } \Delta t \to 0 \tag{11}$$

and it follows that

$$Ue = e^{Dt_1} - 1$$

If t_1 equals one day the proportion of cells dividing in a perfectly phased population over a 24 hour period is expressed as

$$Ue = e^D - 1$$

A dilution rate of .288 day^{-1} was used in the present study which is equal to a Ue of .33 day^{-1}. The low dilution rate utilized requires that a distinction be made between 28.8% of the population dividing per day (cell division randomly distributed) and 33.3% (perfect phasing) with a 33.3% increase in cell concentration at time t followed by a decay every 24 hours. It would be difficult to conclude whether or not complete phasing was exhibited by the cells in the present study at such a low dilution rate utilized since the difference between maximum and minimum cell concentration over a 24 hour period would be so low. Chisholm and Nebbs (1975) note that if partial phasing is induced in the population U is less than Ue and the difference between maximum and minimum cell concentrations would be even smaller.

APPENDIX III

Light vs. ^{14}C Uptake Curves

Carbon uptake increases in a linear fashion with increased light intensity, up to some asymptotic value, Pmax, where light becomes saturating. The linear portion of the light curve is a reflection of the photochemical reaction of the chlorophyll molecules (Parsons and Takahashi, 1973). The initial slope of the curve is called alpha expressed as mgC/mg Chl _a_/hr/light unit (Platt and Jassby, L976). When Pmax is reached this is indicative that the dark reactions of photosynthesis can no longer accept all of the CO_2 available even though the photochemical reactions may be providing sufficient energy compounds such as ATP and NADH. A change in the level of light saturated photosynthesis indicates a change in the concentration of the enzymes associated with the dark reactions of photosynthesis (Morris and Glover, 1974). The light intensity at which alpha intersects a line parallel to the x axis at a y value equal to Pmax is the I_k value first described by Talling (1958) and is indicative of the type of light environment to which the algal population is adapted.

VITA

Steven John Hastings

Born in Lincoln, Nebraska, on September 18, 1952. Graduated from the University of Nebraska in December, 1974, B.S. in Zoology. In September, 1975, the author entered the College of William and Mary, School of Marine Science. Graduate assistant, Department of Ecology and Pollution, January 1976, until August, 1979.