AUTECOLOGY OF THE ALGAL FLAGELLATE

CRYPTOMONAS EROSA SKUJA

STUDIES ON THE AUTECOLOGY OF THE FRESHWATER ALGAL FLAGELLATE CRYPTOMONAS EROSA SKUJA

by

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Ph.D. Biology

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Studies on the autecology of the freshwater algal flagellate Cryptomonas erosa Skuja.

ABSTRACT

Cryptomonas erosa Skuja (Cryptophyceae), a member of a common but little studied group of freshwater phytoflagellates, was grown under various light/temperature and dark regimes in batch culture and under phosphorus limitation in chemostat culture. Batch cells grew maximally at moderately high light intensities and temperature, whereas at much lower temperatures severe photic stress limited cell division, resulting in both excretion and storage of excess photosynthate. Phosphate uptake as measured with ^{32}P revealed that in chemostat culture a low K_s (0.14 μM P) allowed the species to survive at low substrate concentrations. The photosynthetic response of C. erosa grown under suboptimal conditions of low light/low temperature or extreme P deficiency was characterized by reduced rates of dark enzyme function, as measured by P_{max}, and by saturation and inhibition at low light intensities. Survival of a lengthy dark period was dependent not on heterotrophy or phagotrophy, but rather on the slow respiration of stored carbohydrate at low temperatures. Results obtained on the physiological ecology of C. erosa are related to the natural abundance and distribution of cryptomonads and other phytoflagellates.

D.Ph. Biologie

Keith Morgan

Etudes sur l'autoécologie de l'algue flagellée dulcicole Cryptomonas erosa Skuja.

ABSTRAIT

Cryptomonas erosa Skuja (Cryptophyceae), appartenant a un groupe commun mais peu étudié de phytoflagellés d'eau douce, fut cultivé sous diverses conditions de lumière/température et à l'obscurité dans des cultures de type "batch" et sous des conditions limitantes en phosphore dans des cultures de type "chemostat". Les cellules cultivées en "batch" réalisèrent une croissance maximale à des intensités lumineuses (0.043 ly · min^{-1}) et des témperatures (23.5°C) modérément élevées tandis qu'à des températures beaucoup plus basses, le stréss photique limita la division cellulaire, résultant à la fois en une excrétion et un storage de l'excès de matière photosynthétisée. Les expériences relatives à l'assimilation du phosphate (^{32}P) ont montré que dans les cultures de type "chemostat", une faible valeur du Ks (0.14 µM P) permet à l'espèce de survivre à une concentration de substrat très faible. La réponse photosynthétique de C. erosa cultivée en conditions sous-optimales de lumière et de température ou en conditions de déficience extrême en phosphore fut caractérisée par une réduction des fonctions enzymatiques de la phase sombre (telle que mesurée par P_{max}) et par une saturation et une inhibition à de faibles intensités lumineuses. La survivance à

une période d'obscurité prolongée fut dépendante non pas des phénomènes d'hétérotrophie ou de phagotrophie, mais plutôt d'une respiration lente des carbohydrates emmagasinés à de basses températures. Les résultats obtenus sur l'écologie physiologique de C. erosa sont reliés à l'abondance et à la distribution naturelle des cryptomonades et autres flagellés.

PREFACE

The thesis is presented as a series of four interconnected papers in publication format as permitted under the regulations of the Graduate Faculty of McGill University. These regulations also require the following statement as to elements of the thesis that are considered to be "contributions to original knowledge".

Although an important component of the plankton community in many lakes, cryptomonads have received little attention in culture work, and their ecology is poorly known. The data presented here on Cryptomonas erosa Skuja represent, to the author's knowledge, the most comprehensive treatment of cryptomonad autecology. The data include information on the interaction of light and temperature on cell division and photosynthesis, the effect of light, temperature, and phosphate deficiency on the P_{max}, I_k and I_I of C. erosa, the nutrient physiology of an oligotroph in chemostat culture, including the K_s for phosphate uptake, and the role of carbon storage in algal dark survival. These are contributions to original knowledge.

ACKNOWLEDGEMENTS

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Acknowledgements are also due to Ms Heddy Kling of the Freshwater Institute, Winnipeg for the taxonomic identification of Cryptomonas erosa Skuja; to Mr. S. Stainton of the Freshwater Institute, for nitrogen and carbon analyses; to Mr. R. Lamarche and Mr. P. Gauthier of the Biology Department for photographic reproduction of the figures; and to Mrs. Anne Soucy and Ms Marilyn Dalton of the Atlantic Regional Laboratory, Halifax for their careful typing of the manuscript.

I wish to especially thank my wife, Pamela, for preparing many of the figures, and for her patience, co-operation and understanding through the course of this study.

This thesis is dedicated to my parents.

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GENERAL INTRODUCTION

In a natural ecosystem the growth of an algal population is controlled by physical, chemical and biological factors such as light intensity, temperature, nutrient availability, sedimentation and grazing. In addition, each of these factors is continually changing while interacting with the others resulting in a complex and variable response in algal growth. Although field studies of algal ecology have the advantage of dealing with "real" events, they must contend with this inherent complexity of the natural system, making an elucidation of the interaction virtually impossible. In order to circumvent these problems and to allow only a single parameter to change at any one time, laboratory microcosms have been successfully used. Yet, because of their simplicity and artificiality, the ecological relevance of laboratory studies is often questioned. It is true that the study of algal ecology in the laboratory requires certain compromises between recreating the natural environment and making the system work. A major compromise is that algal isolates will not grow in natural lake water, but must be supplied with a synthetic medium containing the essential elements at concentrations much higher than those found in nature. Other "unnatural" conditions of laboratory cultures include light quality differences, turbulence differences, and the artificiality of uni-algal or axenic cultures. These conditions in turn might reasonably be expected to modify the morphological, physiological and reproductive characteristics of isolates. The modifications can be genetic and/or physiological. Genetic variation, through the selection

of mutants favoured by the growth conditions imposed, is probably not important with recent isolates. However, populations maintained in culture for many years may bear little resemblance to the original isolate and their use in ecological studies of phytoplankton dynamics is questionable. Since algae are physiologically plastic, physiological variations induced by the growth environment are certain. In the rich milieu of batch cultures, algae grown under optimal light and temperature conditions attain their maximal intrinsic growth rates (μ_{max}). Since such rapid rates rarely occur in nature both the batch environment and the physiology of the organism differ greatly from that in nature. However, under suboptimal growth conditions in culture, the morphological and physiological changes in the cells may well resemble more closely the suboptimal growth conditions normally encountered in nature. For example, phosphorus limited cells in culture do exhibit the lowered pigment content, high C:P and N:P atomic ratios and increased phosphatase activity also manifest by phosphorus-limited cells in nature. This at least suggests that the artificial environment of laboratory cultures does not alter the basic physiology of the cells, and that the μ_{max} achieved in optimum batch cultures is only the extreme of a continuum of physiological change. However, numerous studies in which the temperature, light or nutrient response of an organism in culture is at variance with its known ecology indicates that this is not always the case. The interaction of pH, light and temperature and total salts concentrations are known to shift the tolerance limits of algae, while more subtle factors such as ionic ratios, chelation and trace metal concentrations

may effect a change in the physiology of cultured cells. To the extent that laboratory cultures are changed in ecologically significant ways, caution must be exercised in applying laboratory data to natural populations. In autecological studies the investigator should continually compare his data with field observations and, where possible, test laboratory findings with natural populations.

Although high nutrient concentrations in batch cultures do not preclude meaningful light-temperature studies, the low concentrations of nutrients in most lakes necessitate that nutrient studies employ ecologically relevant concentrations. The measurement of algal growth and transformation at low natural concentrations has only been made truly possible with the use of continuous-flow cultures. Two basic types of continuous-flow systems are the turbidostat and the chemostat. An essential feature of both is the provision for the continuous removal of a culture at a rate equal to the addition of fresh nutrient. However, as growth in a turbidostat is limited not by any element, but only by the prevailing light and temperature, this system is not amenable to nutrient studies. In contrast, in a chemostat an essential nutrient is supplied at a low limiting rate, and steady state growth rates less than maximal are easily achieved. With chemostats it is possible to measure the kinetic constants of nutrient transport and relate change in the physiology of the cells to the degree of nutrient limitation. Since abundant information indicates that most temperate zone lakes are limited by phosphorus, P-limited chemostats are especially valuable in providing insight into the growth of algal species in freshwater systems, and the role of nutrient kinetics in algal succession.

The objective of the present study was to obtain a measure of the physiological ecology of the freshwater algal flagellate Cryptomonas erosa Skuja that would help explain the abundance and distribution of cryptomonads in nature. Cryptomonads are members of a nanoplanktonic and largely motile group of algae that includes many chrysophytes and dinoflagellates, which at all times dominates the plankton of oligotrophic temperate zone lakes, and often forms an important part of the algal community in eutrophic lakes. However, the study of freshwater phytoflagellates in laboratory cultures has been largely ignored and their light, temperature and nutritional requirements are poorly known.

The first problem investigated was the effect of light on the cell division and photosynthesis of C. erosa at different temperatures in batch culture and the results compared with the light-temperature distribution of cryptomonads in nature (Part I).

To achieve growth under low natural phosphate concentrations, steady state populations of C. erosa were subsequently examined in phosphorus-limited chemostat culture (Part II). The nutrient transport kinetics of the alga was determined and change in cell physiology related to the degree of nutrient deficiency.

The third manuscript examines data only briefly considered in Parts I and II on the photosynthesis-light response of C. erosa cultured under a variety of light-temperature conditions in batch culture and various degrees of phosphate deficiency in chemostat culture. In particular this paper examines variation in the photosynthetic capacity of the alga in relation to the light, temperature and nutrient regimes imposed, and discusses

the possible mechanisms involved (Part III).

Finally, because cryptomonads and other phytoflagellates form significant winter populations in snow and ice-covered lakes, the last manuscript examines the physiological mechanisms available to C. erosa for survival during long periods of darkness (Part IV).

The appendices contain data not suitable for inclusion in the preceding manuscripts but relevant to the thesis.

Since this dissertation is presented as four manuscripts to be submitted for separate publication, a certain amount of redundancy is unavoidable, for which the author asks the reader's forbearance.

Part I

The influence of light intensity and temperature on growth.

ABSTRACT

In order to contribute to an understanding of the dynamics of freshwater phytoflagellates, the flagellate Cryptomonas erosa Skuja (Cryptophyceae) was grown under controlled laboratory conditions at different combinations of light intensity and temperature (23.5, 15, 4 and 1°). Measurements included cell division, photosynthetic carbon assimilation vs irradiance, chlorophyll a content, cell volume and cell carbon. Maximum growth (μ = 1.23 div. day^{-1}) occurred at 23.5° and 0.043 ly min^{-1}. At much lower temperatures growth showed a "stress" response with increasing light intensity. Because cell division was more adversely affected by light than carbon uptake, the resulting excess production of photosynthate was either stored or excreted. The alga has an unusual ability to expand in volume to accommodate storage carbohydrate. The stress response of C. erosa in the laboratory contrasts with its much superior growth in cold oligotrophic lakes. Evidence is presented which suggests that cryptomonads and other nanoplankton grow maximally in the summer waters of eutrophic lakes, but fail to achieve a high biomass due to a high cell loss resulting from zooplankton grazing.

INTRODUCTION

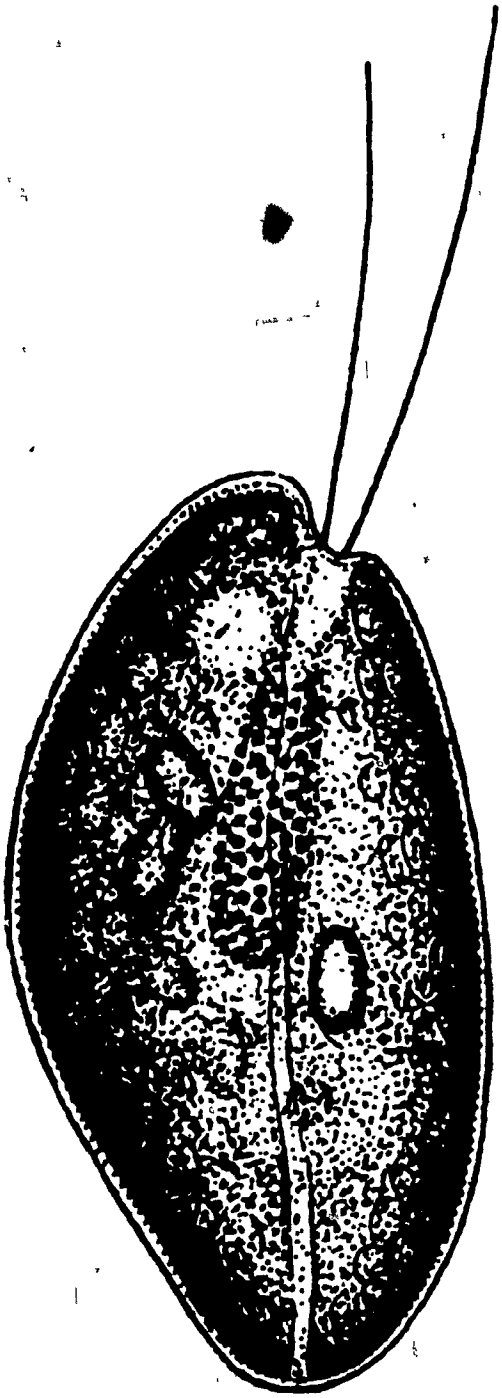
The presence or absence of algal species in lakes and their seasonal cycles are controlled in a complex manner by the interaction of light, temperature and nutrients. This complexity, on which in nature is superimposed the significant controlling effects of grazing and sedimentation, obstructs ready attempts to define a relationship between phytoplankton growth and the natural environment. Such light, temperature and nutrient relationships are, however, more easily defined under controlled laboratory conditions and culture studies thus usefully supplement field investigations. In culture phytoplankton growth is customarily measured either in terms of photosynthesis or cell division, but only a few studies (e.g. McAllister et al. 1964; Eppley and Sloan, 1965) have considered both processes. However, cell division need not respond to change in the same manner as photosynthesis (Droop, 1954; Sorokin and Krauss, 1962). It is reasonable to assume that if the two processes proceed at different rates and the cell becomes stressed by the production of too little or too much photosynthate, it will attempt to bring the two processes in balance. We examine, in the present report, the affect of various combinations of light and temperature on carbon uptake and cell division of the common freshwater flagellate Cryptomonas erosa Skuja (Cryptophyceae).

The Cryptophyceae are biflagellated motile unicells comprising both pigmented and apochlorotic forms. They are usually oval in shape with a tendency to dorsal-ventral flattening and swim in a characteristic spiral motion. They possess a gullet lined with trichocysts (Shuster, 1968), a flexible periplast rather than a cell wall (Faust, 1974) and

pigmented forms contain both chlorophylls a and c and the biloprotein phycoerythrin (Haxo and Fork, 1959). Cryptophyceae reproduce by longitudinal cell division either in the motile stage or in cysts, surrounded by a mucous sheath (Pringsheim, 1944). Cryptomonas erosa Skuja is a typical pigmented form (Fig. 1), larger than most cryptomonads, with a reported size range of 13-45 µm long and 6-26 µm wide (Huber-Pestalozzi, 1950).

The Cryptophyceae are members of a nanoplanktonic and largely motile group of algae which includes many chrysophytes and dinoflagellates that at all times dominate the plankton of oligotrophic lakes (Nauwerck, 1968; Pechlaner, 1971; Kalff et al., 1975). They also contribute a significant percentage of the phytoplankton production in many eutrophic lakes (Gelin, 1971; Kalff, 1972; Granberg, 1973). However, with their generally small size, poor preservation with formalin and difficult taxonomy, cryptomonads and other flagellates are all too often overlooked in lakes. In addition culture studies of freshwater algae have to date dealt almost exclusively either with Chlorella - like species which in many ways are atypical of the general plankton, or with diatom and blue-green species so common in eutrophic lakes. Although a number of culture studies have examined the unique morphology and pigmentation of cryptomonads (Allen et al., 1959; O'hEocha and Raftery, 1959; Hibberd et al., 1971; Gantt, 1971), only a few have considered aspects of their ecology (Pringsheim, 1968). The ecology of other phytoflagellates is also poorly known, yet without such information it is impossible to understand the phytoplankton dynamics of lakes or the range of adaptive mechanisms in algae.

Fig. 1. Cryptomonas erosa Skuja.



The present report on the response of C. erosa to light and temperature in batch culture is part of a larger study which has also examined the growth of the alga in phosphorus - limited chemostats (Part II), its photosynthetic response in relation to the growth environment (Part III), and its survival on incubation in the dark (Part IV). The purpose of this study was to obtain a measure of the physiological ecology of C. erosa in laboratory cultures that would yield insight in explaining the abundance and distribution of cryptomonads in nature.

METHODS

Cryptomonas erosa Skuja was isolated into axenic culture from a small naturally eutrophic lake near Montreal, Quebec. The cells were grown in a relatively dilute (conductivity = 240 µmhos at 25°) mineral salts medium, pH buffered with 100 mg l^{-1} $NaHCO_3$ and chelated with NaEDTA (Table 1). Biotin, thiamine and vitamin B_{12} were added to provide for a probable vitamin requirement (Provasoli and Pinter, 1953; Pringsheim, 1968). Other organics are apparently not needed. The cultures were maintained in 300-400 mls of medium in 500 ml pyrex reagent bottles, and without shaking or aeration, were incubated in constant temperature growth chambers under continuous cool-white fluorescent light. The light intensity was measured at the base of the cultures with a photocell, which was calibrated against a quantum radiometer (Lambda Instruments). Light units are expressed in ly min^{-1} (cal cm^{-2} min^{-1}) of photosynthetically available radiation (PAR) where .01 ly min^{-1} PAR ≃ 230 ft.c. CW illumination ≃ 32 microEinsteins m^{-2} sec^{-1} (Appendix A). Stock cultures were maintained in exponential growth at 8.6 x 10^{-3} ly min^{-1} and 15°.

Table 1. Composition of the medium

			Concentration
	Macronutrients		mg/l
	$Ca(NO_3)_2 \cdot 4H_2O$		67.4
		Ca	11.4
		N	8.0
	K_2HPO_4		5.6
		P	1.0
		K	2.25
	$MgSO_4 \cdot 7H_2O$		28.2
		Mg	2.8
		S	3.7
	$NaHCO_3$		100.0
		HCO_3^-	72.6
	$NaSiO_3 \cdot 9H_2O$		46.0
		Si	4.9
	Micronutrients		µg/l
Trace Metal Mix	Na EDTA		2080
	$FeCl_3$		2400
		Fe	500
	$CuSO_4 \cdot 5H_2O$		24.0
		Cu	6.4
	ZnCl		24
		Zn	12
	H_3BO_3		488
		B	78.4
	$MnCl_2 \cdot 4H_2O$		312
		Mn	77.6
	$CoCl_2 \cdot 6H_2O$		35
		Co	8.8
	$Na_2MoO_4 \cdot 2H_2O$		20
		Mo	6.4
Vitamins	Thiamine		200
	B_{12}		1.0
	Biotin		1.0

pH 8.0

Bacterial contamination of stocks and experimental cultures was routinely checked on nutrient agar, by phrase contrast microscopy and occasionally by erythrosin staining on membrane filters (Kuznetsov, 1959). The stock cultures remained bacteria-free during the course of the study, and bacterial biomass in the experimental cultures was always < 1% of the algal biomass.

For the light-temperature experiments sufficient stock cells were inoculated into 3 or 4 replicate bottles to yield an initial population of 50-200 cells ml^{-1}. The experimental cultures were incubated under a range of light intensities at 23.5, 15, 4 and 1°. At the three lower temperatures the algae were first grown at a relatively high light level (.0194, .0086, and .0086 ly min^{-1}, respectively) and following analysis of the population, the cells, while still in exponential growth, were inoculated into fresh medium and incubated at a lower light level. This process was repeated several times, until finally the cells were grown at, or close to, compensatory light levels. At the highest temperature (23.5°), the experimental procedure was changed so that the cells were incubated all at once under a range of light levels achieved in a light-gradient box covered with different layers of wire mesh screen. The cells were preconditioned for at least one week under each set of conditions.

At each light-temperature regime log-growth populations were analyzed as follows:

Cell Division Rate. Cell numbers were counted at frequent intervals by microscopy in a Palmer-Howard counting chamber. At least 400 cells were counted in each replicate to give ± 10% counting accuracy (Lund et al., 1958). The cell division rate was calculated from the

equation $k = \ln (N_{t_1}/N_{t_2}) \cdot [1/(t \ln 2]$ where k = cell div. day^{-1} and N_t and N_{t-1} are the cell densities at times t and t-1.

Cell Volume. The mean cell volume of the population was determined from measurements on at least 200 cells. The cell volume was calculated by treating the cell as a prolate spheroid.

Carbon Uptake. The photosynthetic rate of the cells was measured three times during log growth by adding $NaH^{14}CO_3$ at 0.125 - 0.25 $\mu Ci\ ml^{-1}$ to two 5 or 10 ml subsamples from two replicate cultures and incubating them under the growth conditions for 1 to 6 hours. The cells were filtered live on 0.45 μm membrane filters at low vacuum ($\frac{2}{50}$ mm Hg) and washed with 1-2 mls of medium prior to assay in a geiger system of known efficiency. Dark uptake was subtracted and the 6% isotope correction was applied (Steemann Nielson, 1952). The total inorganic carbon available for photosynthesis was calculated from the total alkalinity, pH and water temperature following Bachmann (Saunders et al., 1962). Carbon uptake is expressed as picograms (10^{-12} gms) carbon fixed per cell per day. In addition, at a few light-temperature conditions, the photosynthetic rate was measured by the acidification-bubbling technique (Schindler et al., 1972), whereby samples incubated with ^{14}C were acidified to pH 3-4, bubbled 30 min, and 0.5 or 3.0 ml aliquots counted in Aquasol® (New England Nuclear, Boston) in a liquid scintillation system.

To examine the photosynthetic response of C. erosa in more detail, cells grown under light-saturated and light-limited conditions at each temperature (except 1°) were placed in a light-gradient box, and ^{14}C- uptake was measured over a range of light intensities. The cells were filtered, filter activity assayed in the GM system, and the results graphed in the form of photosynthesis-light curves. Regression lines have been fitted

($p \leq 0.05$) to the points to describe each curve at subsaturating and inhibitory light levels, while carbon uptake values along the saturation plateau were averaged to give a mean maximum photosynthetic rate (P_{max}). The lines have been extended to intersect at I_k and I_i, the light intensities at onset of saturation and inhibition, respectively. (See also Part III).

Chlorophyll a. The pigment content of the cells was measured by in vivo fluorescence on a Turner model III fluorometer equipped with a red sensitive R136 photomultiplier (Lorenzen, 1966). A correction for phaeophytin was applied by measuring the amount of fluorescence before and after acidification with dilute HCl (Strickland and Parsons, 1968). The fluorometer was calibrated against the amount of chlorophyll in filtered, acetone-extracted samples as measured by spectrophotometry, using the trichromatic equation of Strickland and Parsons (1968). The equation to relate chlorophyll a concentration to fluorescence was chl-a ($\mu g l^{-1}$) = (2.0) x F x (R_b - R_a) where F is a conversion factor equal to 0.367 for door 30 on the fluorometer, and R_b and R_a are the fluorescence readings before and after acidification (Appendix B).

Cell Carbon. Particulate carbon was analyzed at only some light-temperature conditions. The samples were filtered on pre-ignited Whatman GFC filters, which were combusted in an infrared furnace, with chromatographic separation of the gases in a Carlo-Erba model 1102 CHN analyzer (Stainton et al., 1974).

RESULTS

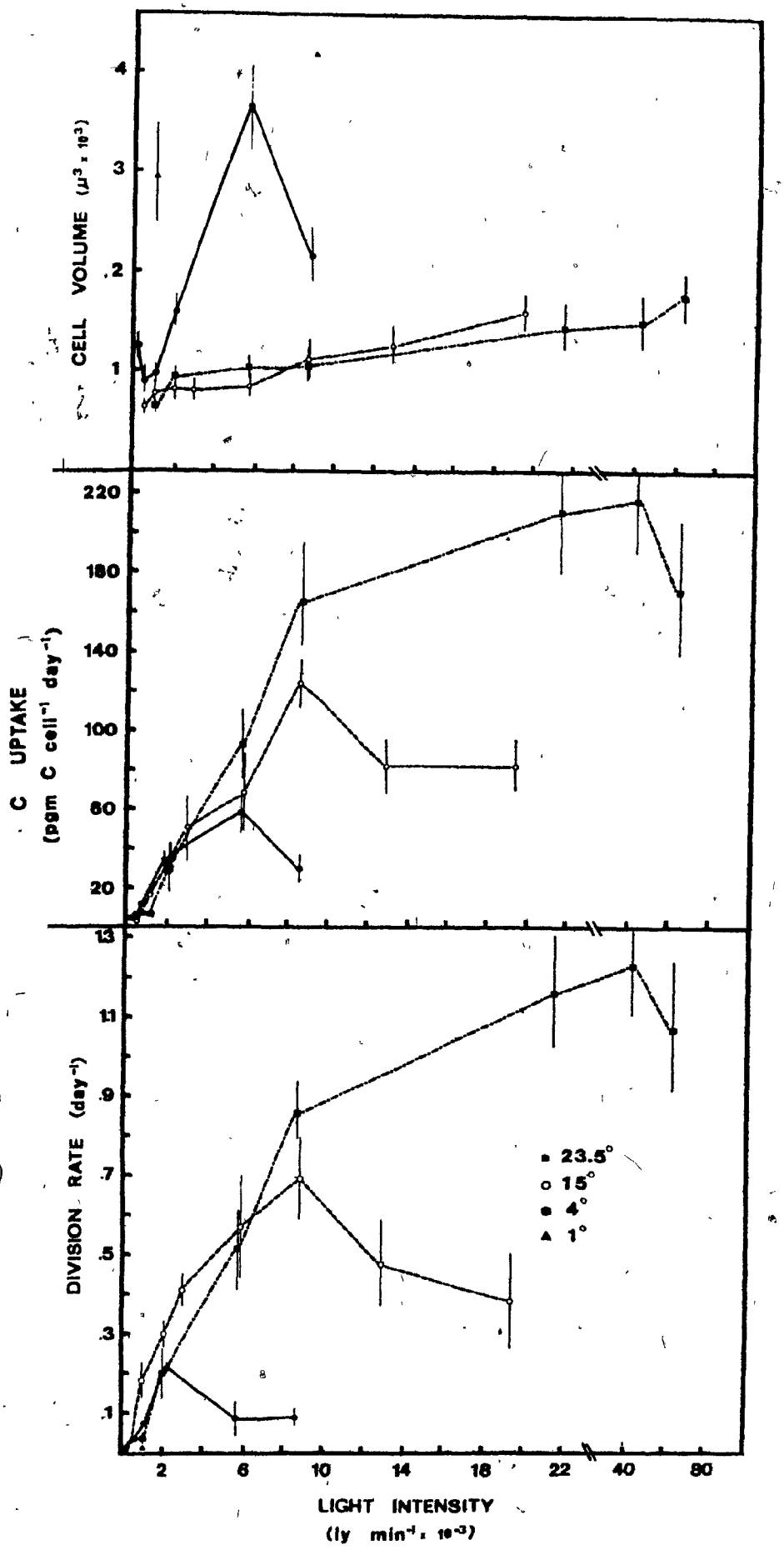
The mean cell division rates, carbon uptake, cell volume and chlorophyll a content of C. erosa at different combinations of light and temperature are shown in Figs. 2 and 5 (see also Appendix C).

Cell division. C. erosa was able to grow over a temperature range of 1 to 23.5°, but no attempt was made to ascertain growth at higher temperatures. The maximum measured growth rate of 1.23 divisions day^{-1} occurred at 23.5° and .042 ly min^{-1}, while the maxima at 15, 4 and 1° were 0.7, 0.21 and 0.025 divisions day^{-1}, respectively. Thus the maximum growth rate decreased sharply with temperature, so that at 1° growth of the alga was severely limited. The cell division rate at 15 and 23.5° increased hyperbolically with increasing light at subsaturating intensities, but linearly at 4° (Fig. 2). At light saturation cell division at the highest temperature (23.5°) was maximal over a broad range of light intensities, with only slight inhibition at the highest light level examined (.065 ly min^{-1}). However, with decreasing temperature the optimum light intensity for growth was lowered, and at higher intensities there was marked inhibition of cell division. At 1° growth was restricted to very low light levels, and attempts to grow C. erosa at only .0086 ly min^{-1} were unsuccessful (Appendix D).

Extrapolation of the light intensity-cell division curves to the abscissa yields compensation light levels for growth of 8.0, 4.1 and 1.3 x 10^{-4} ly min^{-1} at 23.5, 15 and 4°, respectively. At 1° a light level of 0.43 x 10^{-4} ly min^{-1} (≃ 1 ft.c) was just below compensation, with the population slowly disappearing over a 4 month period (Appendix D). Thus the minimum light intensity for growth decreased with decreasing temperature.

Photosynthesis. Carbon uptake in relation to light intensity at each temperature conforms to the typical pattern of a linear increase in the rate at subsaturating levels, followed by light saturation, and at yet higher intensities, by inhibition (Fig. 2). In a pattern similar to

Fig. 2. The influence of light intensity on the cell division rate, carbon uptake rate and cell volume of C. erosa at different temperatures.

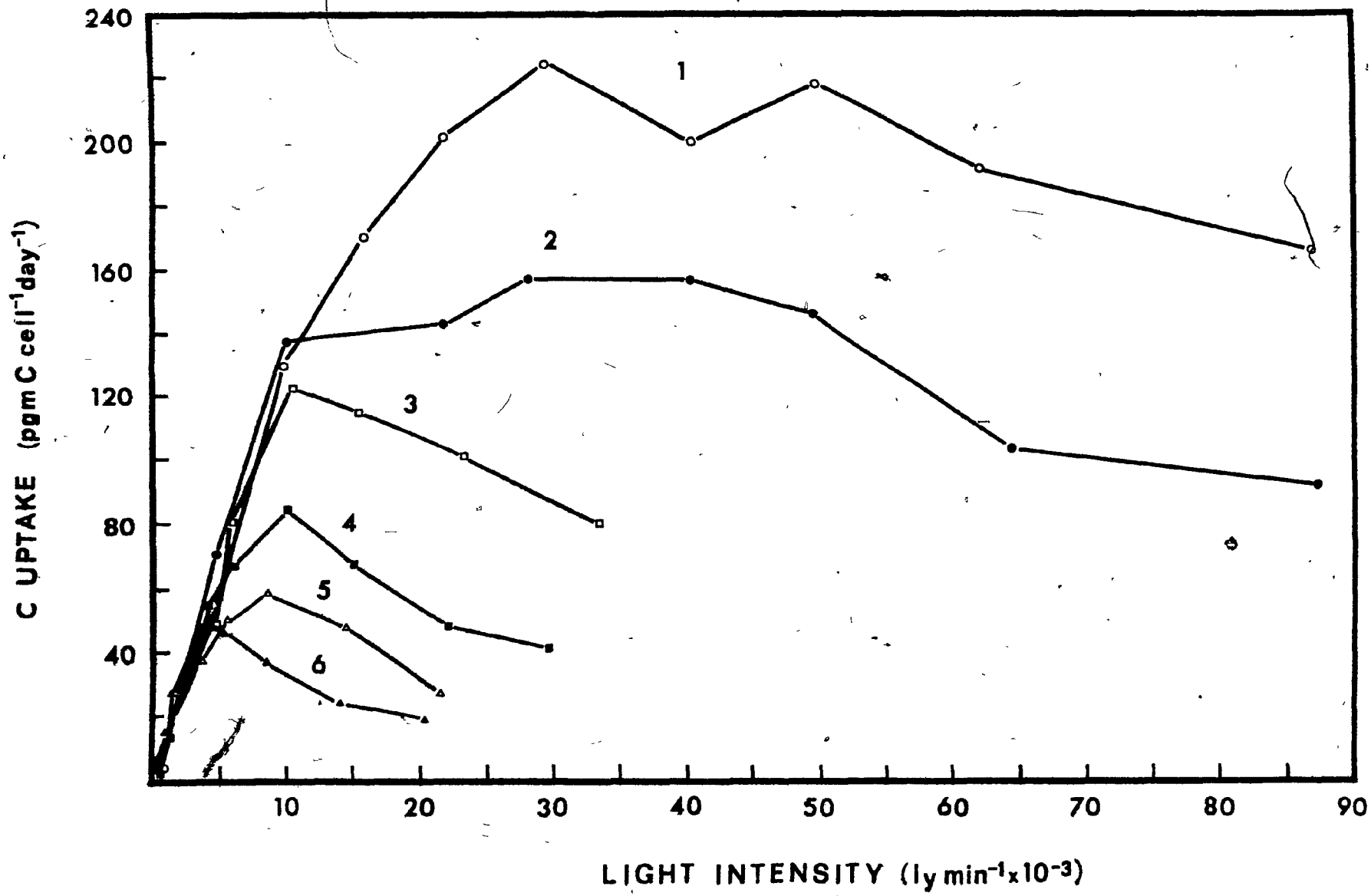

CELL VOLUME ($\mu^3 \times 10^3$)
4
3
2
1
C UPTAKE
(pgm C cell^{-1} day^{-1})
220
180
140
100
60
20
DIVISION RATE (day^{-1})
1.1
1.0
.9
.7
.5
.3
.1
23.5°
15°
4°
1°
2
6
10
14
18
22
40
80
LIGHT INTENSITY
(ly min$^{-1} \times 10^{-3}$)

the light intensity - cell division curves, the maximum measured carbon fixation rate decreased with temperature, as did the light intensity at which carbon uptake was saturated and inhibited. At low subsaturating light intensities the rate of carbon uptake differed little with temperature, except at near compensation light intensities, when the rate declined to very low levels at higher temperatures.

The photosynthetic response of C. erosa to various light-temperature combinations is also described by the photosynthesis-light curves (Fig. 3; see also Part III). The results again show that the initial slope of carbon uptake versus light is relatively independent of the growth conditions, whereas the maximum photosynthetic rate along the saturation plateau (P_{max}) decreases with temperature, characterized by an average temperature coefficient (Q_{10}) of 1.82. In addition, at each temperature cells grown under light-limited conditions have a lower ($\bar{X}$ 25%) P_{max} than cells grown at light-saturation. Since the initial slope of the curves changes very little, the light intensity at onset of saturation (I_k) also decreases with decreasing light and temperature. Furthermore, the light intensity at onset of inhibition (I_i) declines in a similar fashion, so that at the lowest light/temperature examined the I_k and I_i are virtually the same and the saturation plateau disappears (Fig. 3).

Cell volume and carbon content. The cell volume of C. erosa is strongly affected by the growth conditions. A minimum cell size of 300-400μ^3 (cell dimensions ≃ 6 X 14 μm) was observed for cells incubated in the dark, while huge cells of over 9000μ^3 (≃ 22 X 36 μm) occurred under certain low temperature-high light conditions. At each temperature the mean cell volume decreased with decreasing light, and at the higher temperatures had a minimum

Fig. 3. The photosynthesis-light response of C. erosa grown under various light/temperature conditions (ly $min^{-1} \times 10^{-3}$/°C). 1. 21.5/23.5°. 2. 2.0/23.5°. 3. 8.6/15°. 4. 1.0/15°. 5. 5.6/4°. 6. 1.0/4°.


240
200
160
120
80
40
C UPTAKE (pgm C cell^{-1}day^{-1})
1
2
3
4
5
6
10
20
30
40
50
60
70
80
90
LIGHT INTENSITY (ly min^{-1}x10^{-3})

value of approximately $600\mu^3$ at near compensatory light levels (Fig. 2). Low temperature (4 and 1°) cells were 50-400% larger than high temperature (15 and 23.5°) cells, being largest at those light levels that inhibited cell division.

Measures of suspended carbon in some batch cultures and in phosphorus-limited chemostat-cultures permit the determination of carbon content from cell volume (Fig. 4). The results show that 15° batch cells contained approximately 16% carbon (wet weight), whereas 4 and 1° cells and chemostat cells (15°) had a higher carbon content (20%). An increase in cell volume therefore directly reflects an increased carbon content, but the slope of the relationship is significantly changed by the growth conditions. Based on the cell carbon-cell volume regressions low temperature cells contain up to 4 times more carbon than high temperature cells (Table 2). Furthermore, if the relationship also holds for the smallest and largest cells observed, the carbon content of C. erosa ranged from 45 to 1800 pgm C $cell^{-1}$. Cell carbon content calculated from the regression on cell volume can be compared with the estimate obtained from the expression

$$\text{Carbon cell}^{-1} = \frac{\text{C uptake cell}^{-1}\text{day}^{-1}}{\text{cell div. day}^{-1}}$$

The results show (Table 2) that the two measures agree reasonably well, except under high light and temperature (23.5°) conditions and at near compensation light levels.

Chlorophyll a. Phaeophytin was detectable only in those cultures where cell division was inhibited by light (Appendix B). Because

Fig. 4. The linear regression of cell carbon on cell volume for 15° batch cells, ○ , (C = 0.15V + 9.5) and 4 and 1° batch cells, ● , and chemostat cells, △ (C = 0.20V + 3.5).

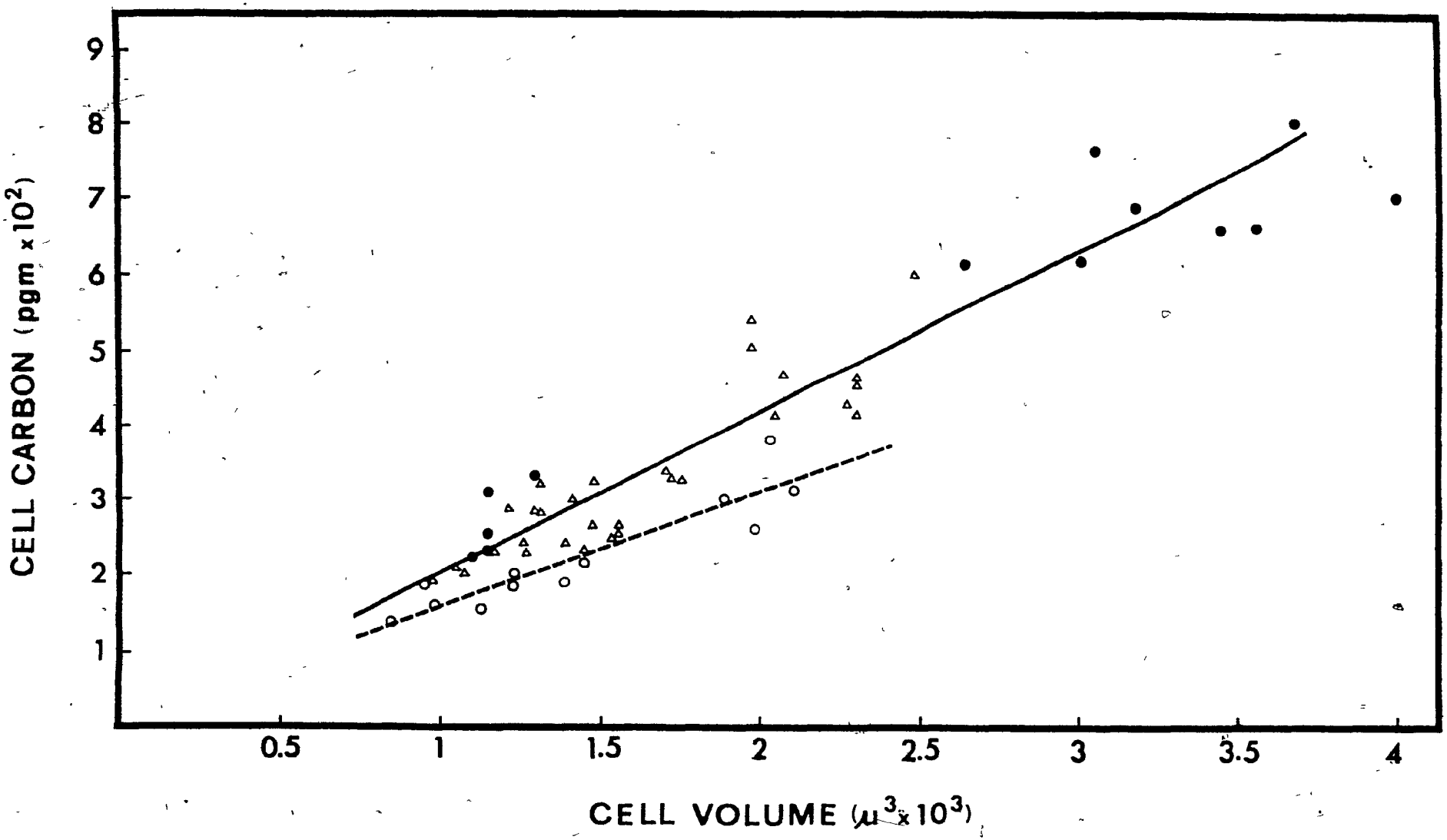
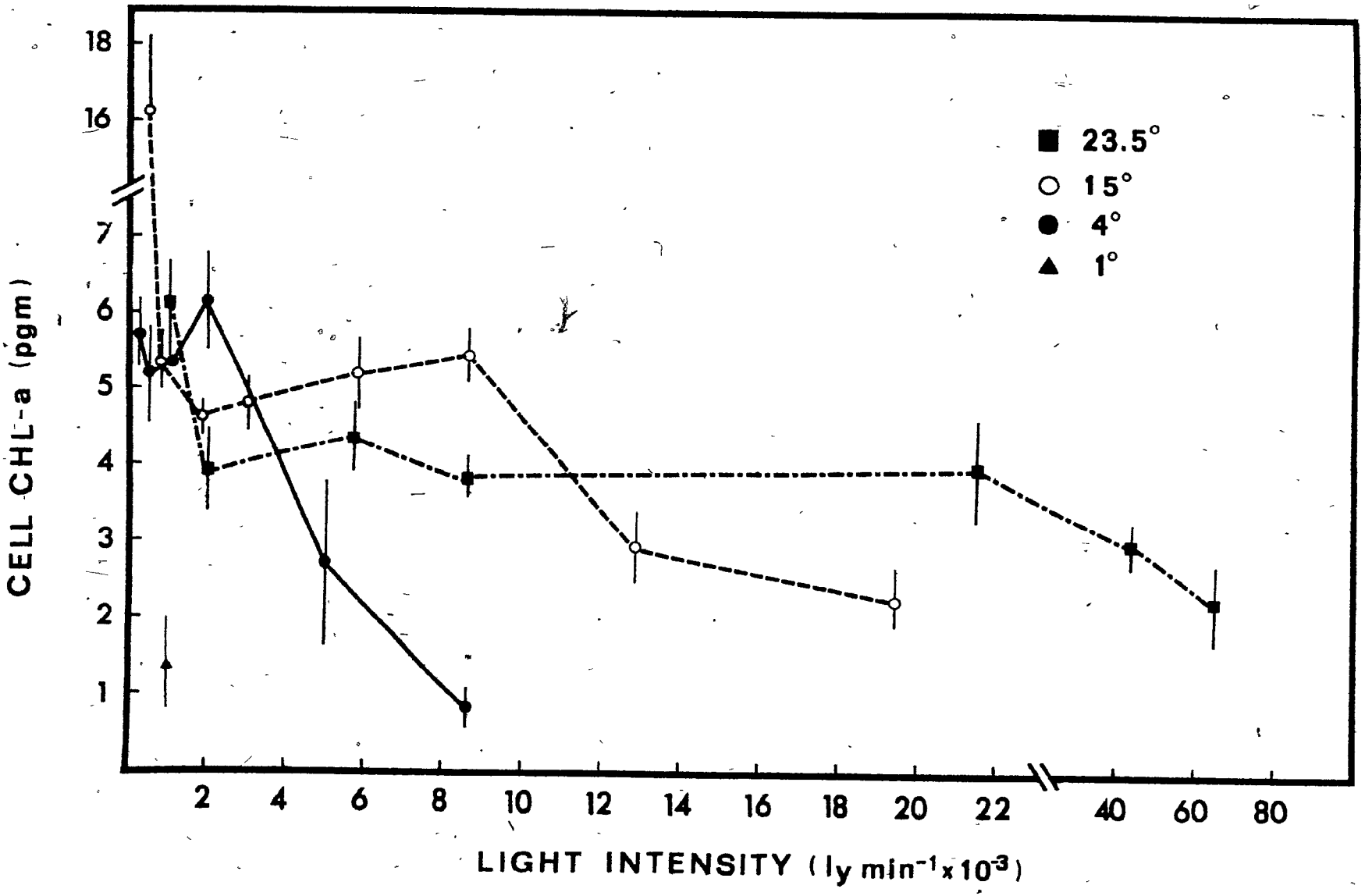

CELL CARBON (pgm x10²)
9
8
7
6
5
4
3
2
1
0.5
1
1.5
2
2.5
3
3.5
4
CELL VOLUME (μ³x10³)

Table 2. Cell carbon content of C. erosa estimated from cell volume and from the ratio of carbon uptake to cell division

Growth conditions	Carbon content (pgm C cell^{-1})	
	Cell volume	Carbon uptake:cell division
23.5°		
64.6 × 10^{-3} ly min^{-1}	273	161
43.1	235	182
21.5	229	181
8.61	170	194
5.60	165	182
2.02	151	156
0.99	106	190
15°		
19.4 × 10^{-3} ly min^{-1}	249	217
12.9	198	174
8.6	174	179
5.6	136	120
3.0	130	122
2.0	132	116
0.99	124	89
0.47	105	147
4°		
8.6 × 10^{-3} ly min^{-1}	452	357
5.6	755	699
2.0	330	155 ?
0.99	205	153
0.47	189	197
0.19	263	680

Fig. 5. The relationship between chlorophyll a content per cell and light intensity at different temperatures.


CELL CHL-a (pgm)
18
16
7
6
5
4
3
2
1
23.5°
15°
4°
1°
2
4
6
8
10
12
14
16
18
20
22
40
60
80
LIGHT INTENSITY (ly min⁻¹ x 10⁻³)

photodestruction of chlorophyll a is unlikely at these still relatively low light levels, the presence of phaeophytin-like pigments suggests that cell death occurred in the cultures and that cell counts underestimated the actual cell division rate.

Chlorophyll a $cell^{-1}$ (phaeophytin-corrected) was lowest at photoinhibitory light levels, and at all temperatures increased to 4-6 pgm $cell^{-1}$ (0.4-0.7% wet weight) at lower light intensities (Fig. 5). Only at higher temperatures did the pigment content increase still further at the lowest light levels. The very large increase in pigment content at 15° may in part reflect increased concentrations of chlorophyll c or phycoerythrin, which also absorb blue light and fluoresce red (Holm-Hansen et al., 1965).

DISCUSSION

Algal growth is subject to stress imposed by the complex interaction of light, temperature and nutrients, with stress defined as an inbalance in cell function. Our results show that with all nutrients in excess, growth of C. erosa in batch culture is strongly affected by an interaction between light and temperature. This is best described by considering the different high and low light and temperature combinations respectively, and relating these to the natural distribution and abundance of cryptomonads.

High Light - High Temperature

The maximum observed growth of C. erosa of 1.23 div. day^{-1} at 23.5° and .045 ly min^{-1} is similar to that of C. ovata of 1.09 div. day^{-1} at 25° and 1000 ft. c. (Brown and Richardson, 1968) and shows that in culture these

cryptomonads grow best at moderately high light levels and temperatures. The results are in contrast to the reported very low light intensities required for growth of cryptomonads on agar or complex liquid media (Allen et al., 1959; Haxo and Fork, 1959), suggesting that very high concentrations of inorganics and/or organics result in increased light stress. The optimum light intensity for growth of C. erosa (23.5°) is nevertheless lower than that of most diatoms and dinoflagellates in culture (Eppley and Strickland, 1968) and is much less than the light fluxes at which many greens grow maximally (Sorokin and Krauss, 1958; 1965). Brown and Richardson (1968) showed that algal groups with greater amounts of accessory pigments have lower optimal light intensities for growth, attributable to the increased range in effective light absorption provided by these pigments. Thus typical high-light species like Chlorella contain chlorophyll a unaccompanied by any major amount of accessory pigments, while the more light-sensitive cryptomonads contain significant amounts of chlorophyll c and phycoerythrin.

At the highest temperature (23.5°), both cell division and carbon uptake were maximal at the same relatively high light levels (Fig. 2). Because the two processes increased at approximately the same rate with increasing light, cell carbon calculated from the cell division to carbon uptake ratio is relatively constant over a broad range of light intensities (Table 2). The not realized increase in carbon content, as determined from cell volume (Fig. 4), is probably the result of increased vacuolization of the cells at high light levels, as shown for Cryptomonas ovata (Brown and Richardson, 1968). In contrast, a real increase in carbon content

was observed at 15°, with high-light cells having twice the carbon content of cells at near compensation light levels. The increase in cell carbon is the result of a greater relative increase in carbon uptake than in cell division at subinhibitory light intensities, and a greater relative decrease in cell division than carbon uptake at inhibitory light levels (Fig. 2).

High loss rates resulting from grazing, sinking and cell lysis, together with only weekly or monthly measures of algal biomass yield aberrantly low values (<0.5 div. day^{-1}) for maximal growth of cryptomonads in nature (Willén, 1961; Pavoni, 1963; Holmgren, 1968; Ilmavirta and Kotimaa, 1974). At the same time the activity coefficients (carbon assimilation:carbon content) computed by Findenegg (1971) provide questionably high values of 1.88 to 4.29 div. day^{-1} for C. erosa in a mountain lake, probably the result of an underestimation of the cell carbon content. More reasonable estimates of maximum growth of cryptomonads were obtained during a diurnal study of cell numbers in a Finnish lake (Ilmavirta, 1974), yielding rates of 0.94 div. day^{-1} at 14° and 1.76 div. day^{-1} at 21°. Similar rates (≃ 1.0 div. day^{-1} at 13°) were obtained in nature by means of autoradiography (Knoechel, pers. comm.). These results indicate that in nature cryptomonads are also capable of excellent growth at relatively high temperatures. Moreover these maximal rates are similar to those recorded for many blue-greens, diatoms, and with the exception of the Chlorococalles, many greens in culture (Hoogenhout and Amesz, 1965), and do not support the results of Moss (1973) that typical oligotrophic species have intrinsic growth rates lower than those of eutrophic species.

High Light -- Low Temperature

With decreasing temperature, cell division and carbon uptake of C. erosa were much reduced, and the cells were saturated and inhibited at very low light intensities. The maximum growth rates observed (0.21 and 0.025 div. day^{-1} at 4 and 1°, respectively) are well below the maxima of 0.65 - 1.32 div. day^{-1} at 0-5° for two obligate psychrophilic diatoms (Bunt, 1968; Durbin, 1974), obtained at light intensities considerably above those found inhibitory for C. erosa. In an arctic lake, too, much superior growth (0.25 div. day^{-1}) was reported for a cryptomonad at 0°, while several chrysophytes and dinoflagellates had maximum growth rates of 0.12 - 0.52 div. day^{-1} (Kalff et al., 1975). The poor growth of C. erosa at low temperatures in batch culture thus contrasts with that recorded for cryptomonads in nature. There are two factors in our cultures which may account for the severe inhibitory effect of light at low temperature. Firstly, C. erosa was grown under continuous illumination. Several studies have shown that at optimal temperatures for growth an increase in daylength generally results in increased cell division (Tamiya et al., 1955; Hobson, 1974), yet at the temperature extremes of an alga, long daylength or continuous illumination inhibit cell division (Jitts et al., 1964; Durbin, 1974). Secondly, although the growth medium was relatively dilute compared to most other batch cultures, a possible nutrient imposed stress cannot be totally precluded. An increased total salt concentration (McCombie, 1960), increased

salinity (Smayda, 1969) and chemical poisoning (Jitts et al., 1964) caused shifts in the lower temperature limit for growth to higher temperatures, and did increase the susceptibility of algae to light stress. Thus the greater the stress imposed by one factor, the less the cell can cope with others. The high total salts concentration of batch media or the high concentration of one or more of their constituents may well be the principal factor why C. erosa and other algae grow so poorly in culture at the same low temperatures at which they thrive in nature.

Although the batch environment at low temperatures is unnaturally extreme, the observed physiological response of C. erosa under the stress imposed must, to a degree, also occur in natural populations. The photosynthetic response of cells at low temperatures is characterized by a lowered rate of carbon uptake at light-saturation and by lower saturating light intensities (Fig. 2). The results of the photosynthetic-light experiments (Fig. 3), only briefly considered here (see Part III), show that with decreasing temperature, carbon uptake is limited not by a reduction in the rate per calorie at light-limiting intensities, but by a lowered maximum photosynthetic capacity (P_{max}). The P_{max} is a measure of the maximum rate of the dark reactions of photosynthesis as determined by the environmental temperature and the cellular concentration of dark enzymes (Steemann Nielsen and Jørgensen, 1968). Although a loss of dark enzymes cannot be precluded, the lowered P_{max} is most reasonably explained as a temperature-dependent reduction in the rate constant of the enzymatic reactions. A direct result of this lowered capacity is that even very

low light intensities saturate the dark reactions, as shown by a shift in the I_k to lower values (Fig. 3). In addition carbon uptake of low temperature cells is inhibited at low quantum flux (low I_i). The association of low I_i with low P_{max} suggests that the condition of photoinhibition is a high rate of light reactions coupled with a low rate of dark reactions (Steemann Nielson and Jørgensen, 1968).

Cell division of C. erosa is even more adversely affected by low temperature than is carbon uptake. For example, at 4° and growth inhibitory light levels of .0056 and .0086 ly min^{-1}, carbon uptake is respectively 1.6 and 5.0 fold less than rates measured at 23.5°, whereas cell division is 6 and 10 times smaller (Fig. 2). At an even lower temperature (1°) cell division is completely inhibited at these fluxes. Thus at low temperatures cell division is more inhibited by light than is carbon uptake, and assumes the principal role in limiting the overall growth process, something also observed in several other studies (Sorokin and Krauss, 1962; Wilson and James, 1966). Our results support the "Master Reaction" theory of Sorokin (1960) which postulates that cell division and carbon accumulation are semi-independent processes responding differently to light and temperature. The photoinhibitory effect of light on cell division has been shown to result from lowered DNA synthesis (Sokawa and Hase, 1968; Soeder and Stengel, in Stewart, 1974), while Cook (1968) proposed the photodestruction of some unit central to cell metabolism, such as the cytochrome system.

Because carbon uptake proceeds at a faster rate than cell division at low temperatures, the cell must either increase its mass or excrete

surplus organic carbon. The marked increase in cell volume and associated cell carbon at 4 and 1° (Fig. 2; Table 2) demonstrates that when cell division of C. erosa is inhibited, excess photosynthate is primarily retained as storage material. Other freshwater and marine algae also commonly show an increase in carbon content when grown at low temperatures (Eppley, 1972). In cryptomonads carbon is stored as starch in ellipsoidal or spheroidal granules enclosed within a double membrane around the pyrenoid (Lucas, 1970). In C. erosa at low temperatures these granules accumulated in such quantity as to obscure all other cell organelles. The unusual ability of cryptomonads to expand in size and retain storage carbon likely results from their possession of an elastic, flexible periplast rather than a cell wall (Faust, 1974). Under extreme photoinhibitory conditions at 1° (.0086 ly min^{-1}), cell division was completely inhibited, so that with continued carbon uptake the mean cell volume increased to over 4000 μ^3, with some giant cells reaching 9000 μ^3 (1800 pgm C). However, shortly thereafter the cells slowly disappeared from culture, suggesting that because synthesis and expansion of the cell membrane could not continue to keep pace with carbon accumulation, the cells literally exploded (Appendix D). A wide range in cell volume - and presumably carbon content - is also reported for C. erosa and other cryptomonads in nature (Huber-Pestalozzi, 1950; Kling and Holmgren, 1972). When applied to nature our results predict a maximum cell size in the surface waters of lakes when the water temperature is low and the light flux high.

A second mechanism by which C. erosa copes with excess photosynthate is evident by comparing carbon uptake as measured by the

acidification-bubbling technique, which measures net plus excreted carbon (Schindler et al., 1972) with that obtained by standard membrane filtration, which appears to measure only the production of particulate carbon (Table 2; also Antia et al., 1963; Eppley and Sloan, 1965). The difference in the rates thus represents carbon excretion. The results show (Table 3) that at subsaturating light levels 11 - 24% ($\bar{x}$18%) of the total carbon fixed is excreted, whereas at two growth inhibitory light levels associated with low temperature, 35 and 50% of the carbon is immediately lost from the cell. Although the greatest quantity of carbon is excreted from rapidly growing cells at 23.5°, the highest percentage of carbon is excreted under conditions that cause a greater relative decrease in cell division than in carbon uptake. Thus increased excretion rates occur when the cells fix more carbon than the rest of the plant enzyme systems can incorporate into growth and storage products. The nature of the excretory products of C. erosa was not examined, but the production of a copious mucilage under inhibitory light conditions suggests that the principal excretion products are polysaccharides. According to Hellebust (in Stewart, 1974) the amounts of polysaccharides released can represent a considerable 15-90% fraction of the photoassimilated carbon of some algae.

High excretion rates have also been measured in natural populations (Fogg et al., 1965; Watt, 1966), but it has not been recognized that a possible mechanism is the interaction of high light with some other environmental factor which inhibits cell division more than photosynthesis. Thus the buildup of organic products during mid-summer stratification

Table 3. A comparison of carbon uptake by C. erosa as measured by the techniques of standard membrane filtration versus acidification-bubbling. Limits are one standard deviation.

	Growth conditions		Carbon uptake (pgm C cell^{-1} day^{-1})			
	Temp. (°C)	Light intensity (ly min^{-1} × 10^{-3})	Filtered	Acidification-bubbling	Carbon excreted (pgm C cell^{-1} day^{-1})	% Carbon excreted / carbon uptake (A-B)
(1)	23.5	21.5	209.6 ± 21.1	253.0 ± 18.8	43.4	17.1
(2)	23.5	2.0	31.1 ± 4.7	38.9 ± 3.6	7.8	20.0
(3)	15	8.6	EXPT #1 98.7 ± 10.3	118.0 ± 8.3	19.3	16.4
			EXPT #2 109.3 ± 12.7	137.7 ± 10.9	28.4	20.6
(4)	15	1.0	EXPT #1 16.2 ± 2.1	19.7 ± 2.1	3.5	17.8
			EXPT #2 18.3 ± 1.4	20.4 ± 1.1	2.1	11.4
(5)	4	5.6†	45.7 ± 6.1	70.1 ± 4.7	24.4	34.8
(6)	4	1.0	12.9 ± 2.7	16.9 ± 0.9	4.0	23.7
(7)	1	1.0†	4.7 ± 0.6	10.9 ± 1.2	6.2	56.9

† Extreme photoinhibition of cell division.

(Round, 1971) and the relatively greater proportion of extracellular release in oligotrophic than in eutrophic waters (Anderson and Zeutschel, 1970; Saunders, 1972), may result respectively from the adverse affects of nutrient limitation and low nutrient/low temperature conditions on cell division. Although the excretion rates of C. erosa measured at the growth conditions (Table 3) cannot be strictly applied to the short-term photosynthesis-light experiments (Fig. 3), they do indicate that the photoinhibition function of the curves must also largely result from increased carbon excretion with increasing light intensity. In nature too the excretion of organics, combined with the movement of light-sensitive flagellates, such as C. erosa, away from brightly-lit surface waters must be responsible, at least partly, for the commonly recorded surface depression of the depth/photosynthesis profile.

Low Light

Under extreme light limitation, a plant cell is stressed not by an excess of photosynthate, but rather by too little carbon to satisfy temperature-dependent rates of respiration and cell division. Although no direct measures of C. erosa respiration were made, dark incubation yielded rates of carbon loss of approximately 54, 36, 14 and 3 pgm C $cell^{-1}$ day^{-1} at 23, 15, 4 and 1°, respectively (Part IV), indicating a marked increase in respiration with increasing temperature. The increased respiration rate elevates the compensation light flux at which the alga can balance a potentially destructive decrease in cell mass. With decreasing light at 15 and 23.5° the mean cell volume approached a

minimum of 500-600 μ^3(Fig. 2). The same lower limit was obtained independently from cells incubated in the dark (Part IV). This value therefore appears to represent a genetically fixed minimum for a viable C. erosa.

The principal adaptive mechanism of algae to light limitation is to maximize light capture through the increased synthesis of cell pigment (Halldal, 1970). The absence of such an increase in C. erosa at low temperatures (Fig. 5) can be interpreted as resulting from a temperature-dependent decrease in the amount of carbon required for cell division and cell respiration, whereas at high temperatures the sharp increase in pigment at the lowest light levels is a physiological response to an increased carbon demand. The ability of C. erosa to grow at low light levels may also depend on its possession of chlorophyll c and phycoerythrin, which increase the range of light wavelengths effective for light absorption (Haxo and Fork, 1959). Although no data were obtained here on the accessory pigments, several studies (see Halldal, 1970) have shown that algae contain maximal concentrations of chlorophyll c and phycoerythrin under low light conditions. In addition phycoerythrin absorbs maximally at those wavelengths (540-570 μm) that penetrate deepest in natural waters, thus favouring growth of cryptomonads in deep waters of clear oligotrophic lakes (Nauwerck, 1966, 1968).

Aspects of the Ecology of Cryptomonads

Cryptomonads are members of a nanoplanktonic and largely motile flora which includes many chrysophytes and dinoflagellates and some small

diatom and green species, which at all times dominate the plankton of oligotrophic, north temperate zone lakes (Pechlaner, 1971; Kling and Holmgren, 1972: Kalff et al., 1975). They are also present throughout the year in eutrophic lakes, but in most contribute a less significant percentage of the summer biomass with the appearance of netplanktonic diatoms and blue-greens, even though the nanoplankton biomass may be greater than in the spring (Pavoni, 1963). Cryptomonad species, although traditionally associated with cold waters, are also reported in large numbers in warm tropical (Lewis, 1974) and desert lakes (Biswas, 1969) and the group is thus ubiquitous, able to thrive under a wide range of light/temperature conditions.

The adaptive features of cryptomonads and other algae for survival in oligotrophic north temperate zone lakes include motility, small size, and the ability to grow under low temperature and/or low nutrient conditions. Motility allows the algae to remain suspended in a stable water column during long periods of ice cover, and to control to some extent, the light flux received. Due to the scarcity of light under the winter and early spring snow and ice cover, the algae concentrate in surface waters (e.g. Wright, 1964). As shown here for C. erosa, the adaptation to low light can be considerable, and in one study in an arctic lake, an increase in algal biomass was recorded during late winter at a light flux of only 0.18 ly day^{-1} (≃ 3 ft.c) (Kalff, unpubl. data).

In spring, with the disappearance of snow and a resulting sudden increase in light climate, the algae appear to suffer considerable light stress, such that in many lakes motile species actively migrate downwards and form a biomass maximum in deep waters (Nauwerck, 1968; Pechlaner,

1971). In comparison to other algal groups, the Cryptophyceae appear to be especially light sensitive, often forming the deepest living populations in clear oligotrophic lakes (Nauwerck, 1966, 1968). Indeed a sharp decrease in cryptomonad biomass at the summer turnover in an arctic lake (Kalff, unpubl. data) suggests photodestruction of the cells, such as observed for C. erosa at 1° in culture.

Pechlaner (1971) and Findenegg (1967) attribute the depth maximum of phytoplankton in high mountain lakes to a better balance between light and nutrients in meta- and hypolimnetic waters. Kalff and Welch (1974) similarly explain change in the photosynthetic response of arctic algal communities in terms of nutrient availability. Growth of C. erosa in phosphate-limited chemostats confirm this suspected photoinhibitory effect of high light under nutrient deficiency (Parts II and III). In addition, our batch culture data suggest that the low temperatures commonly found in arctic and mountain lakes also have a specific detrimental effect on adaptation to high light or, at the very least, synergistically compound a largely nutrient-dependent light stress.

Although cryptomonads and other flagellates dominate the winter plankton of north temperate zone eutrophic lakes, they are typically replaced during the summer, at least in terms of biomass, by large diatoms and blue-greens. To conclude that flagellates are supplanted because of slowed growth under adverse high light/temperature conditions fails, however, to explain the summer maxima of cryptomonads in some eutrophic lakes (Nauwerck, 1963; Ilmavirta et al., 1974) or their reported abundance under intense illumination at 27-30° in tropical and desert

lakes (Biswas, 1969; Lewis, 1974). Direct evidence for light saturation of cryptomonads at high light intensities was obtained for natural populations in a light incubator (at 20°) (Javornický, 1970) and by *in situ* autoradiography in a eutrophic lake (at 13°) (Knoechel, pers comm.). Thus as shown for *C. erosa* in culture, higher temperatures and increased nutrient availability increase the optimal light intensity for growth of cryptomonads in nature. The further observation by Knoechel of doubling times of *Rhodomonas minuta* (Cryptophyceae) of approximately one per day, at a time when the population did not increase, suggests that flagellates only fail to attain high numbers because of a high loss of cells. Since sedimentation of flagellates must be small, the loss can be attributed largely to zooplankton grazing, which limits the abundance of all nanoplankton in eutrophic waters. It is highly probable that the very intermittent flourishing and disappearance of cryptomonad populations in lakes (Nauwerck, 1963; Happey, 1968) is closely coupled to the wax and wane of zooplankton populations rather than to physiological mechanisms.

In batch culture *C. erosa* grows over a wide range of temperature, but severe photic stress limits cell division at low temperatures, forcing the cells to both store and excrete excess carbon. The much superior growth of cryptomonads in arctic and high mountain lakes and their ability to survive throughout the year the multiple shock of changing light, temperature and nutrient conditions attest to a greater physiological plasticity in natural populations than in culture. In nutrient-sufficient batch cultures, adaptation to high light was contingent on higher temperatures, whereas in

chemostat culture the effect of phosphorus limitation was of overriding importance. In nature, too, adaptation to light is largely nutrient dependent (see Kalff and Welch, 1974, Figs. 3 and 5), even though temperature must also play a significant role at very high and low temperatures. Thus the growth of C. erosa is, as in all algae, dependent on the light, temperature and nutrient supply. In addition, the ability of motile species to at least partially self-regulate their growth environment is an important strategy to reduce stress and promote species success. The actual biomass of C. erosa and other phytoflagellates realized is controlled in a complex fashion by the above factors, upon which in nature is superimposed the significant controlling effect of zooplankton grazing.

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Part II

Growth in phosphorus-limited chemostat culture.

ABSTRACT

Cryptomonas erosa Skuja (Cryptophyceae), a member of a common but little studied group of freshwater phytoflagellates, was grown in phosphorus-limited chemostat culture. Under increasing P-deficiency, the cells showed increases in cell volume and carbon content, whereas cell N and chlorophyll a content were little affected. The C:P and N:P atomic ratios also reflect the availability of phosphorus. Photosynthetic carbon assimilation and cell division were differently affected by the rate of P supply. Photosynthesis was limited by P at slow growth rates, but by light intensity at higher growth rates, whereas cell division was P-limited at all growth rates examined. The lowered rate of carbon uptake of severely P-deficient cells was shown the result of a loss in photosynthetic capacity, with the cells light saturated and inhibited at low light intensities.

The cellular phosphorus content decreased only at the slowest rates of P supply. Phosphate (^{32}P) uptake experiments showed this to be the result of a low maximum uptake capacity (v_{max}), limiting the accumulation of P in an internal reservoir. Radiobiological assay showed that growth rate limiting concentrations of phosphate in the chemostats were ≤ 0.10 µg P ℓ^{-1}, substantially lower than chemical measures of soluble reactive phosphorus. The kinetic results are discussed in relation to the growth of cryptomonads in both oligotrophic and eutrophic lakes.

INTRODUCTION

The role of phosphorus as a limiting nutrient in the control of phytoplankton growth and lake eutrophication has received extensive study (for review see Fogg, 1973). Laboratory cultures of algae have been used to examine the relationship of phytoplankton growth rates and biomass yield to phosphorus concentration (Thomas and Dodson, 1968; Müller, 1971), the kinetics of phosphorus uptake (Kuenzler and Ketchum, 1963; Rhee, 1973), and morphological and physiological characteristics of cells which may serve as indices of P limitation in natural systems (Fitzgerald and Nelson, 1966; Healey, 1973a). Since available phosphate as determined by radiobiological assay is in < 1 µg P l^{-1} concentrations in most lakes (Rigler, 1966) and because maximum growth of planktonic algae is achieved at low concentrations (Mackereth, 1953; Fuhs et al., 1972), it is important that culture work employ ecologically meaningful nutrient levels. Although batch cultures have been successfully used to relate growth rate to substrate concentration (Thomas and Dodson, 1968; Eppley and Thomas, 1969), more often the effect of supplying a low concentration of nutrient has been to shorten the duration of the exponential phase rather than reduce the growth rate (Fogg, 1965). However, the use of continuous-flow chemostat cultures readily achieve the long term steady state conditions that allow the measurement of algal growth and transformation at natural substrate concentrations. Moreover, the adaptation of algae to change in nutrient supply can best be analyzed with continuous cultures. The studies to date on P-limited growth of freshwater

algae in chemostats have dealt almost exclusively with diatom, green and blue-green species so common in eutrophic waters, while information is lacking, from either batch or continuous flow culture, on the nutrient physiology of species of algal flagellates so characteristic of lakes. Dugdale (1967) pointed out the need for study of the nutrient kinetics of phytoplankton of different productivity regimes, in order to provide information on the range of adaptive mechanisms of algae to their environment, and their role in phytoplankton succession and competition. The nutrient physiology of an alga will at least partially determine its presence and its abundance in lakes of different trophy. Here we report on the ecology of the common algal flagellate Cryptomonas erosa Skuja (Class Cryptophyceae) in a phosphorus-limited chemostat.

The Cryptophyceae are members of a nanoplanktonic motile group of algae which includes many chrysophytes and dinoflagellates, which at all times dominate the plankton of oligotrophic lakes (Nauwerck, 1968; Pechlaner, 1971; Kalff et al., 1975). They also contribute a significant percentage of the phytoplankton production in many eutrophic lakes (Gelin, 1971; Kalff, 1972; Granberg, 1973). However, except for a number of culture studies that have examined the unique morphology and pigmentation of cryptomonads (Haxo and Fork, 1959; Shuster, 1968; Faust, 1974), only a few have considered aspects of their ecology (Pringsheim, 1968). To our knowledge no cryptomonads have been previously grown in continuous culture.

The present report on the growth of C. erosa in a P-limited chemostat is part of a larger study which has also examined the response

of the alga to a range of light/temperature conditions in batch culture (Part I), its photosynthetic response in relation to the growth environment (Part III), and its survival in the dark (Part IV). The purpose of this study was to obtain a measure of the physiological ecology of C. erosa in laboratory cultures that would help explain the abundance and distribution of cryptomonads in nature.

METHODS

The organism and growth medium. Cryptomonas erosa Skuja (Fig. 1) was isolated into axenic culture from a small naturally eutrophic lake near Montreal, Quebec, Canada. The algae were grown in a relatively dilute mineral salts medium (conductivity = 240 μmhos at 25°) buffered with $NaHCO_3$ to pH 8.0 and chelated with NaEDTA (Part I). Thiamine, vitamin B_{12} and biotin were added as other cryptomonads have required vitamins for growth (Pringsheim, 1968). Stock cultures were maintained at 15° and .0086 ly min^{-1} of continuous cool-white illumination.

For the chemostat work, phosphorus was added at a concentration of either 100 μg or 25 μg Pl^{-1}. Despite the addition of the KH_2PO_4 after autoclaving a precipitate of iron and phosphorus formed to reduce the phosphate concentration in the medium to approximately 80 and 14 μg Pl^{-1}. The resulting atomic ratios of N:P were 221:1 and 1279:1, respectively, with all other nutrients in excess.

Equipment. The chemostat set-up is shown schematically in Fig. 2 (see also Figs. 3 and 4). The growth vessel is a 2 l round-bottom flask equipped with an overflow tube to maintain a constant culture volume, and a sampling port at mid-culture level. In early work C. erosa

Fig. 1. Cryptomonas erosa Skuja.

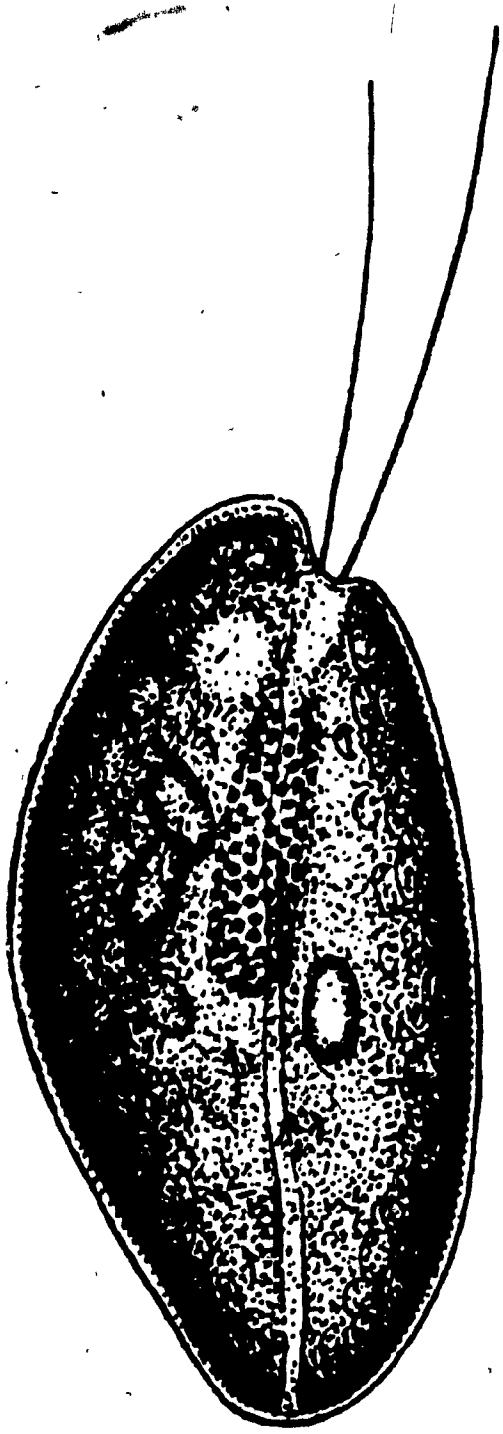
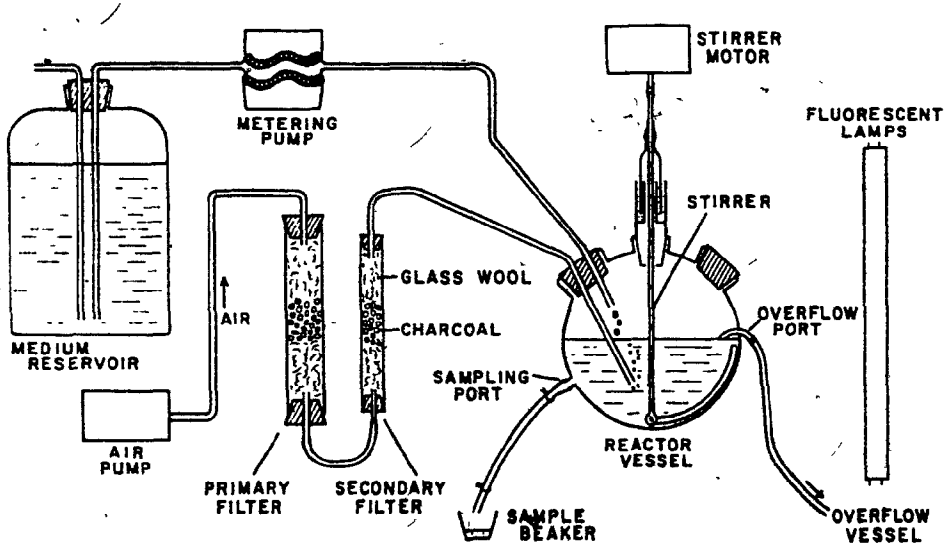


Fig. 2. Diagram of chemostat.


METERING PUMP
STIRRER MOTOR
FLUORESCENT LAMPS
STIRRER
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AIR
MEDIUM RESERVOIR
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SAMPLING PORT
AIR PUMP
PRIMARY FILTER
SECONDARY FILTER
REACTOR VESSEL
SAMPLE BEAKER
OVERFLOW VESSEL

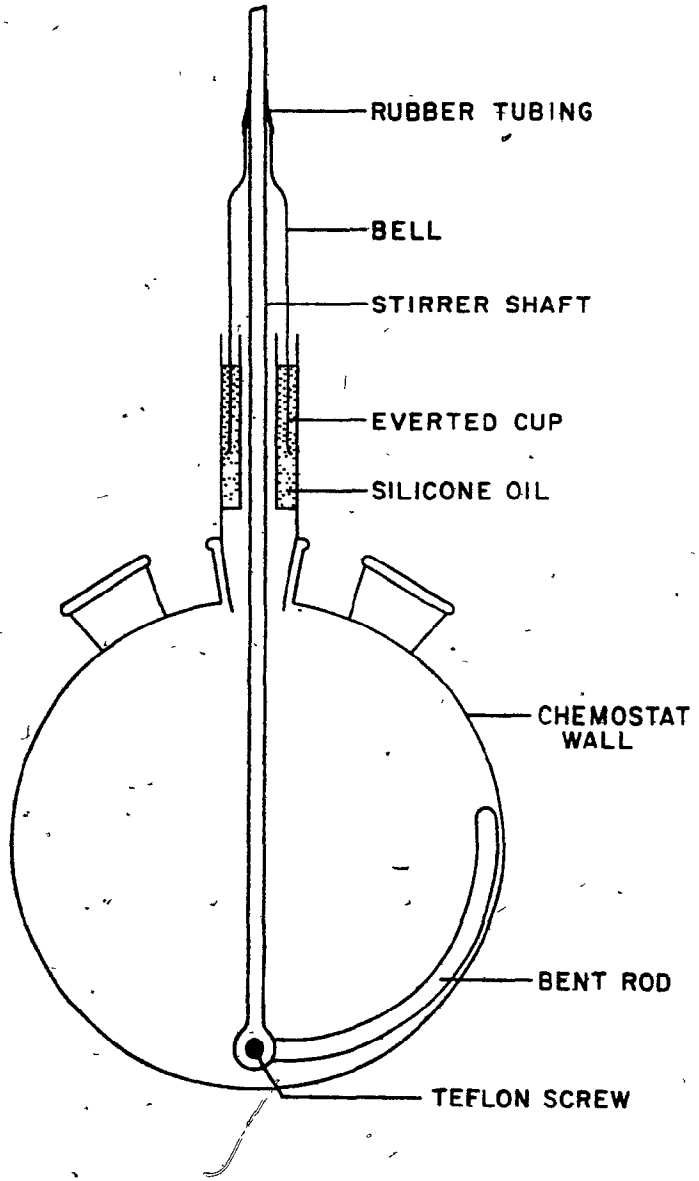

RUBBER TUBING
BELL
STIRRER SHAFT
EVERTED CUP
SILICONE OIL
CHEMOSTAT WALL
BENT ROD
TEFLON SCREW

Fig. 3. Photograph of two chemostat units, showing growth vessels, stirrers, air filters and fluorescent lights.

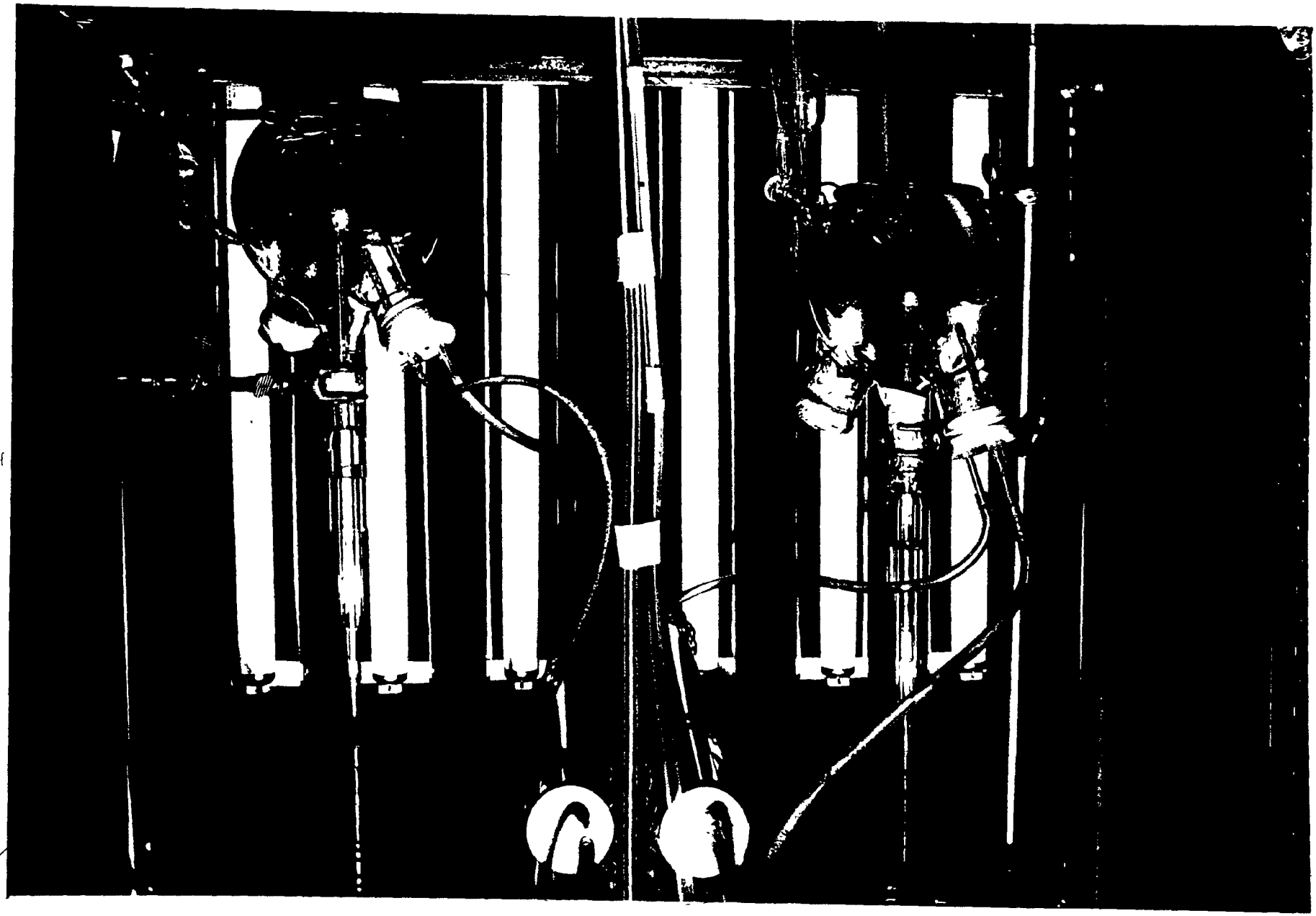
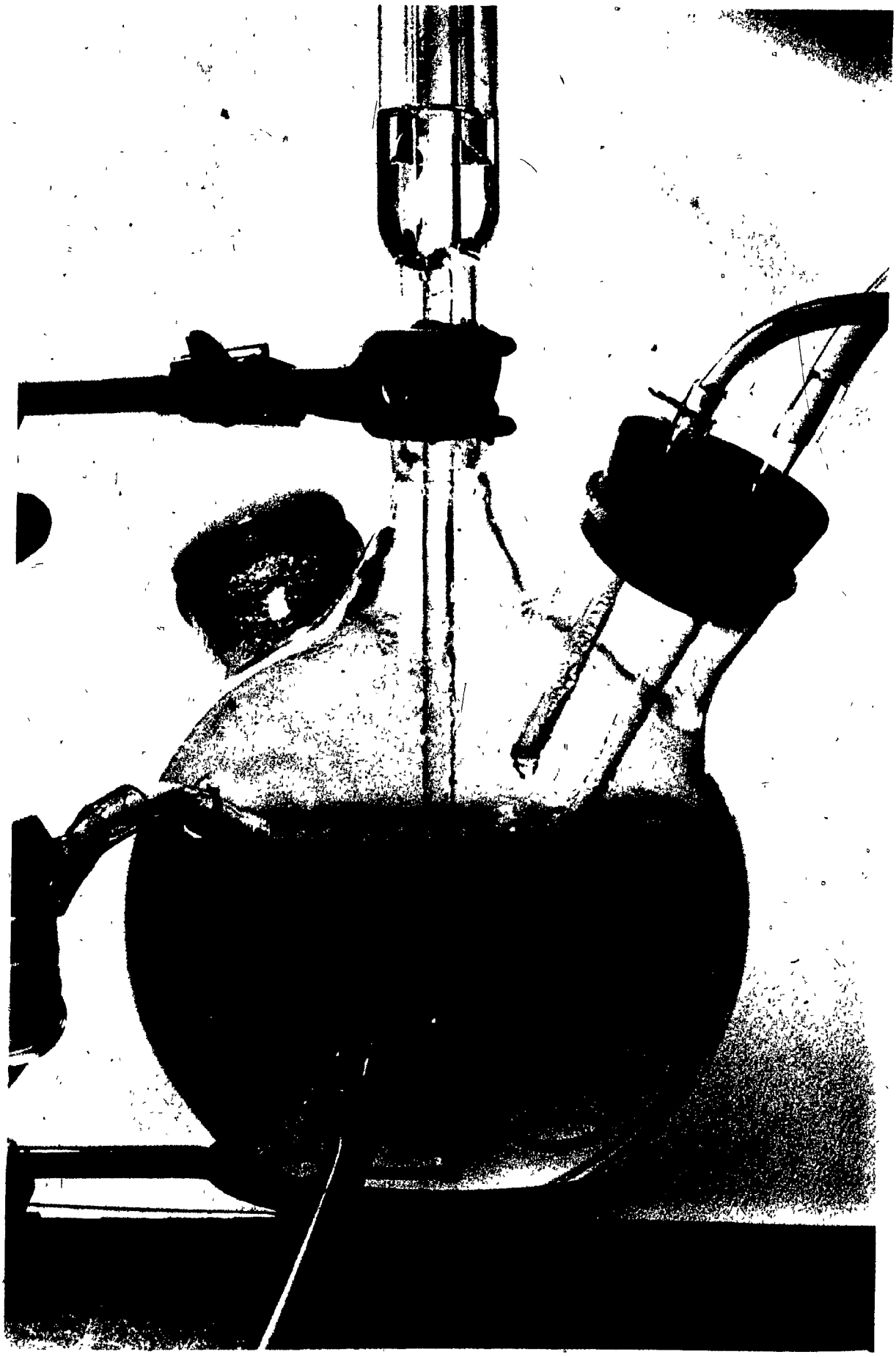


Fig. 4. Photograph of growth vessel, showing "sweep-stirrer".



readily attached in thick layers to the walls of the chemostat by excreting a copious mucilage, whereas those cells in suspension retained their motility. Sufficient vigorous agitation by aeration and stirring with a spin bar to remove the wall growth was lethal to the cells. Such mechanical injury possibly explains why cryptomonads rapidly disappeared in chemostat cultures of natural phytoplankton assemblages (Barlow et al., 1973). The problem of wall growth was minimized by designing a "sweep-stirrer" of glass (Fig. 2), fitted to the inside curvature of the vessel wall and driven by a low speed-high torque motor. Sterility of the cultures was maintained by passing the shaft of the stirrer through a cup filled with heavy silicone oil, which was covered with a bell mounted on the shaft. Although the alga still attached to the stirrer, the walls were kept sufficiently clean at a speed of only one revolution sec^{-1}. The difference between phosphate measured in the reservoirs and total P measured in the outflow indicated that about 10% of the P was lost to the walls and stirrer in the chemostats receiving 80 $\mu g\ Pl^{-1}$ in the feed, and 10-20% in those vessels receiving 14 μgPl^{-1}. Additional mixing in the growth vessels was provided by bubbling charcoal and glass-wool filtered air through the culture at 500 mls min^{-1}. Gassing with CO_2-enriched air was not required as the pH of the cultures remained < 8.6. The growth medium was supplied from 10 liter glass carboys, and metered with a Harvard model 1102 peristaltic pump, which yielded a flow rate precision of ±1%.

The chemostats were operated in a constant temperature incubator at 15° under continuous cool-white illumination located at the rear of the

vessels. Light intensity was measured with a photocell, which was calibrated against a quantum radiometer so that light units are given in ly min^{-1} (cal cm^{-2} min^{-1}) of photosynthetically available radiation (PAR), where .01 ly min^{-1} PAR ≈ 230 ft.c. CW illumination ≈ 32 microEinsteins m^{-2} sec^{-1} (Appendix A). As the light was kept unidirectional by blackening the sidewalls of the supporting cabinets, the light intensity at the chemostat centers was calculated from the extinction coefficient of light through the vessel and, depending on cell density, ranged from 9.3 to 11.3 x 10^{-3} ly min^{-1} (Appendix E). Earlier work in batch cultures at 15° had shown that 8.6 x 10^{-3} ly min^{-1} was optimal for growth of C. erosa, whereas 12.9 x 10^{-3} ly min^{-1} was somewhat inhibitory (Part I). Bacterial contamination in the chemostats was examined in a high-power phase contrast microscope and by plating on nutrient agar. Although the cultures were not strickly axenic, bacterial biomass was estimated to be <1% of the algal biomass, except at the end of the chemostat experiments, when the substrate concentration in the reservoir was increased from 14 to 80 µg P l^{-1}, and the chemostats became heavily contaminated with bacteria.

General design of experiments. With either 80 or 14 µg P l^{-1} in the reservoir, the algae were grown at various growth rates by varying the flow rate of medium into the vessels. By the use of silicone tubing of different internal diameter, a different growth rate was achieved in three chemostats at one time. The experiments were started with 80 µg P l^{-1} in the reservoir, and two growth rates were examined in each vessel (6 in all).

The phosphate concentration was then decreased to 14 µg P l^{-1} and one growth rate was examined in each vessel. When the phosphate concentration was subsequently returned to 80 µg P l^{-1}, the chemostats became contaminated with bacteria. All experiments were run continuously without reinoculation from stock cultures.

Small samples were withdrawn every few days to determine cell numbers and cell volume. The amount of sample withdrawn for other analyses did not exceed one-tenth of the volume of the chemostat. However, at the slowest growth rates this represented up to 100% of the volume of culture displaced per day, and thus several days were allowed between samplings. The establishment of a steady state was judged from cell counts.

Analyses. The following determinations were made on samples removed from the chemostats.

(1) Cell counts and cell volume measurements were made by microscopy in a Palmer-Howard counting chamber. Sufficient replicate samples were taken to give ±10% sampling error. The cell volume was determined by treating the cell as a prolate spheroid.

(2) Suspended nitrogen and carbon was determined by infrared combustion of GFC filtered material, with chromatographic separation of the gases in a Carlo-Erba model 1102 CHN analyzer (Stainton et al., 1974).

(3) Soluble reactive phosphorus was analyzed by the molybdate-blue technique (Murphy and Riley, 1962) and total P and total soluble P as above after persulphate digestion (Johnson, 1971). Particulate P was determined by difference.

(4) Chlorophyll a was measured by in vivo fluorescence in a Turner model III fluorometer (Lorenzen, 1966) with correction for phaeopigments after 1-2 min acidification (Holm-Hansen et al, 1965). The fluorometer was calibrated with samples filtered on GF/A filters, extracted in acetone, and analyzed for chlorophyll a by spectrophotometry, using the trichromatic equation of Strickland and Parsons (1968) (Appendix B).

(5) Carbon uptake was measured by adding $NaH^{14}CO_3$ at .125-.25 μCi ml^{-1} to 10 or 20 ml subsamples which were incubated 1-2 hrs (15°) at the same light intensity as in the center of the chemostats. Depending on the cell density, 0.5 or 5.0 ml aliquots were filtered at 25° mm Hg vacuum through 0.45 μm membrane filters, and filter activity assayed in a windowed GM system of known efficiency. The remainder of the sample was acidified to pH 3-4, bubbled 30 min (Schindler et al., 1972) and 0.5 or 3.0 ml aliquots counted in Aquasol® (New England Nuclear, Boston) in a liquid scintillation system.

To examine the photosynthetic response of C. erosa in more detail, cells removed from the chemostats were placed in a light-gradient box, and ^{14}C uptake measured at several light intensities up to 2 to 3 times the light level at the chemostat centers. In a parallel set of experiments, the photosynthesis-light relationship was determined after a 24 hr enrichment with 50 μg P l^{-1} added phosphate. The cells were filtered on 0.45 μm membrane filters and filter activity assayed in the GM system. The results were graphed in the form of photosynthesis-light curves. Regression lines ($P \leq .05$) have been fitted to the points to describe each curve at subsaturating and inhibitory light levels, while carbon

uptake values along the saturation plateau were averaged to give a mean maximum photosynthetic rate (P_{max}). The lines were extended to intersect at I_k and I_i, the light intensities at onset of saturation and inhibition, respectively (see also Part III).

(6) Phosphorus uptake kinetics: .02-.04 μCi ^{32}P ml^{-1} as H_3PO_4 (New England Nuclear, Boston) and sufficient KH_2PO_4 were added to a series of erlenmeyer flasks to yield 0-50 or 100 μg P l^{-1}. Without any pretreatment, 10-20 ml subsamples from the chemostats were added to each flask and incubated at 15° at mid-culture light levels, with frequent swirling in the first 30 min of incubation, but only occasionally thereafter. After 10 min, 30 min, 1-2 hrs., 5-8 hrs. and, in some experiments, 20-24 hrs. of incubation, 1-5 mls were filtered live at low vacuum (50 mm Hg) through 0.45 μm membrane filters, rinsed with an equal volume of phosphorus-free medium, and the filter activity determined in the geiger system. Michaelis-Menten kinetics were evaluated by the linear transformation S/v vs S, where S is the substrate concentration added (μg P l^{-1}) and v is the uptake velocity (μg P l^{-1} $time^{-1}$). An unweighted least squares line was fitted to the points (Riggs, 1963), and the maximum uptake velocity (V_{max}) determined from the slope, and $K_s + S_o$ from the x-intercept, where K_s is the half-saturation constant for uptake and S_o is the concentration of residual phosphate in the growth vessels. The residual phosphate concentration was estimated by measuring the loss of ^{32}P from solution using a radiobiological procedure described by Rigler (1966). In this method the uptake rate of phosphate is calculated at each concentration from the sum of the original P added plus various estimates for S_o. As the assumed concentration of S_o is decreased, the

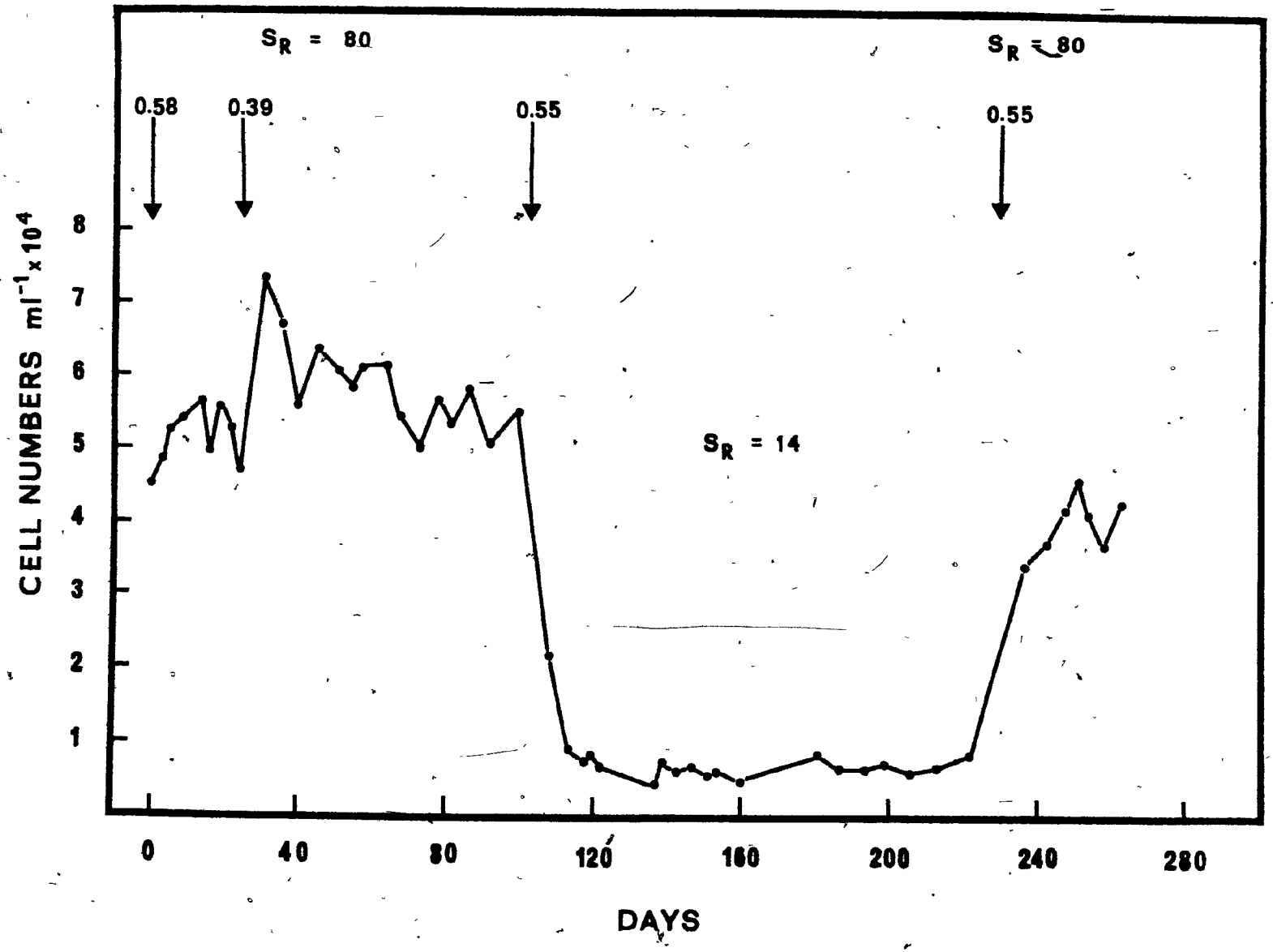
relationship between uptake and concentration more closely approximates the expected hyperbola, until at some concentration of S_o the curve bends smoothly to the x-y intercept (Appendix F).

RESULTS

Steady State Populations and Associated P

Cell densities of C. erosa resulting from changes in the dilution rate and the concentration of phosphorus in the reservoir are presented for one growth vessel in a continuous run lasting about 10 months (Fig. 5 and Appendix G). After each decrease in the dilution rate the cell density increased sharply and then decreased to the final steady state value. With a change in P concentration in the reservoir at high growth rates it took 20 days to re-establish a new steady state, whereas at very slow growth rates the transition stage lasted up to 60 days (Appendix G). Populations of P-limited C. erosa thus adapted only slowly to changes in P supply, but once a steady state was established it was maintained for up to 3 months. Preliminary work demonstrated that increasing the dilution rate to 0.50 day^{-1} resulted in a slow washout of cells from the chemostats and, from the rate of decline in cell density, the maximum growth rate of the alga was estimated as 0.70 div. day^{-1} (Appendix H). Following the temporary greater availability of P after an increase in the concentration of P in the reservoir, the population increased at a similar rate of 0.65 div. day^{-1} (Fig. 5). These rates are similar to those measured in nutrient-rich batch cultures at 15°C,

Fig. 5. Cell density of C. erosa in one growth vessel with changes in growth rate (div. day^{-1}; ↓) and phosphate concentration in the inflow.


S_R = 80
S_R = 80
0.58
0.39
0.55
0.55
S_R = 14
CELL NUMBERS $ml^{-1} \times 10^4$
8
7
6
5
4
3
2
1
0
40
80
120
160
200
240
280
DAYS

where maximal growth increased linearly with temperature between 4 and 23.5° (Part I).

Steady state cell densities and associated phosphorus concentrations at each growth rate in the three vessels are summarized in Table 1. The variation in cell density of 7-10% and 20% of the mean in the 80 μg P l^{-1} (high P) and 14 μg P^{-1} (low P) chemostats, respectively, is due to sampling error as well as to variable loss of P as a precipitate in the reservoir and to wall growth. Since wall growth was not appreciably different between the vessels in the high P chemostats, changes in steady state density reflect growth rate differences. The results show (Table 1) that at growth rates > 0.22 div. day^{-1} cell numbers do not change significantly, but increase 20 and 40% respectively at the two lowest growth rates. In the low P chemostats a greater variation in wall growth and precipitation of P in the reservoir obscure any relationship between growth rate and cell density.

Dissolved organic P (DOP) formed a significant 20-40% fraction of total P (TP). P content per cell (q), calculated from the difference between TP and DOP, did not differ significantly between the high and low P chemostats at similar dilution rates (Table 1). Cellular P content was thus independent of the concentration of P in the reservoir. The relationship between growth rate and P $cell^{-1}$ (Fig. 6) shows that cell division is independent of cellular P at growth rates >0.22 div. day^{-1}, but declines with P $cell^{-1}$ at slower rates of P supply. An extrapolation of the line to the abscissa gives the critically minimum P content needed for growth as 0.30 pgm P $cell^{-1}$.

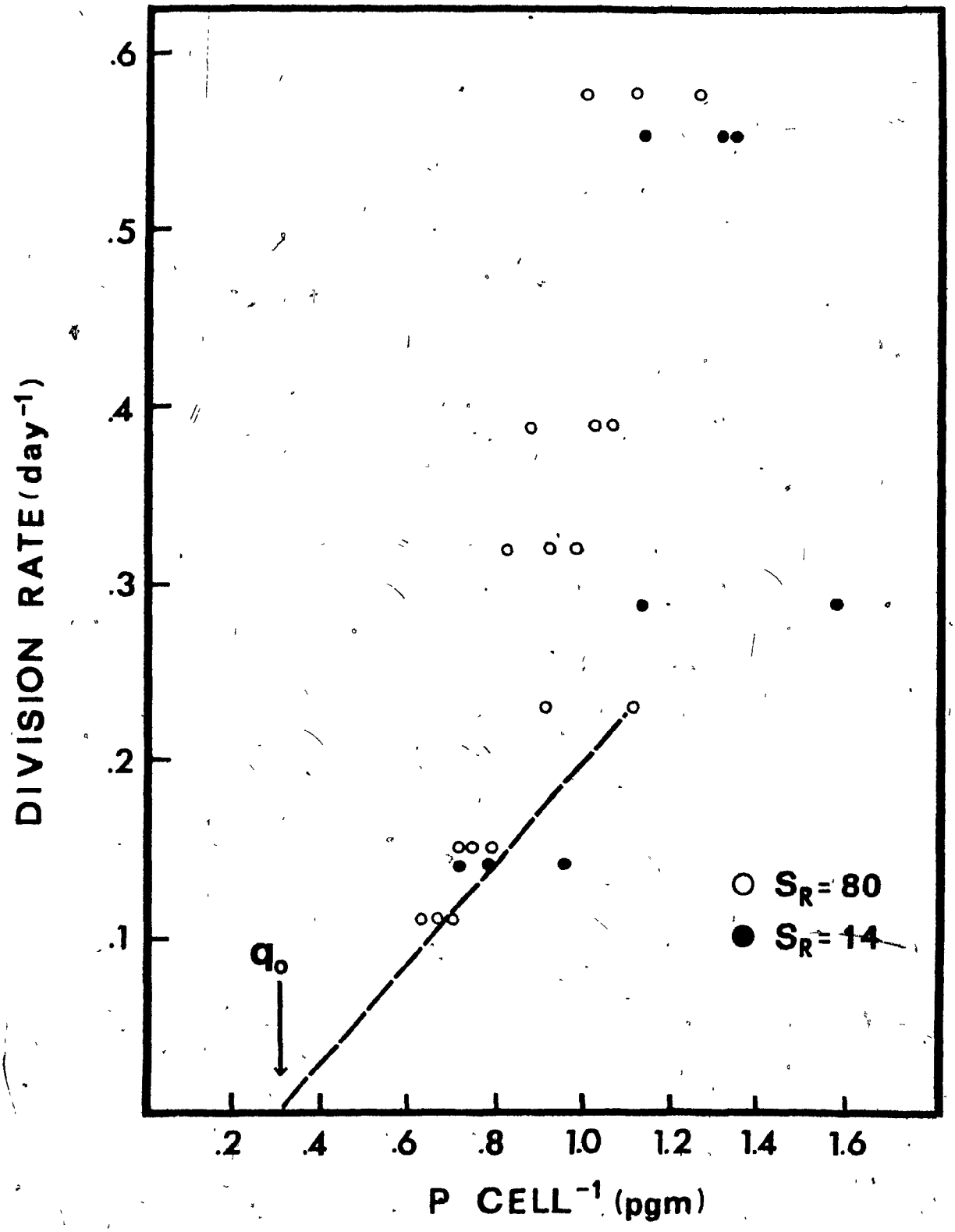
Residual phosphate in the chemostats as measured by chemically

Table 1. Steady state cell densities and associated TP, DOP and P cell^{-1}, and residual phosphorus as measured by SRP and radiobiological ^{32}P assay. Limits are one standard deviation.

Phosphorus in reservoir (µg P ℓ$^{-1}$)	Cell division rate (div. day^{-1})	Cell density (cells ml^{-1})	TP (µg P ℓ$^{-1}$)	DOP (µg P ℓ$^{-1}$)	P cell^{-1} (picograms)	Residual phosphorus (µg P ℓ$^{-1}$)	
						SRP	^{32}P assay
80	0.58	51369 ± 3934	72.7 ± 4.8	14.2 ± 3.3	1.13 ± 0.13	1.63 ± 0.60	-
	0.39	56934 ± 4154	76.7 ± 3.3	20.8 ± 1.9	0.99 ± 0.07	0.94 ± 0.44	<0.10
	0.32	57872 ± 7891	67.1 ± 2.5	15.0 ± 2.9	0.90 ± 0.08	1.17 ± 0.40	-
	0.23	54960 ± 3621	73.1 ± 4.2	21.9 ± 4.2	1.01 ± 0.11	1.22 ± 0.27	<0.10
	0.15	67311 ± 6414	71.0 ± 3.3	21.7 ± 5.3	0.74 ± 0.03	0.82 ± 0.37	-
	0.14	71580 ± 4088	72.6 ± 5.1	22.8 ± 3.1	0.67 ± 0.04	1.88 ± 0.61	<0.10
14	0.55	6534 ± 1172	12.1 ± 1.9	3.8 ± 0.3	1.26 ± 0.11	undetectable* -1.34	
	0.29	6414 ± 1578	9.9 ± 1.2	2.3 ± 0.5	1.36 ± 0.24	undetectable -0.74	<0.10
	0.14	7097 ± 1029	9.7 ± 2.4	3.9 ± 0.2	0.81 ± 0.13	undetectable -1.12	

* Undetectable = <0.50 µg P ℓ$^{-1}$.

Fig. 6. The relationship between cell division rate and cellular phosphorus in the chemostats. The graph is extrapolated to yield the minimum P content per cell (q_o) at zero growth.


DIVISION RATE (day^{-1})
.6
.5
.4
.3
.2
.1
q_o
○ S_R = 80
● S_R = 14
.2
.4
.6
.8
1.0
1.2
1.4
1.6
P CELL^{-1} (pgm)

determined soluble reactive phosphorus (SRP) was undetectable (<0.50 µg P l^{-1}) in some growth vessels and between 0.7 and 1.9 µg P l^{-1} in others (Table 1). However, radiobiological ^{32}P assay consistently indicated substantially less phosphorus available for algal growth. In the example shown (Fig. 7) the rate-concentration curve was first calculated by equating SRP to orthophosphate, and was then recalculated at progressively lower concentrations of assumed residual phosphate. In the absence of sufficient data at <1 µg P l^{-1} additions during the first few minutes of uptake, our estimates lack great precision, but they do indicate that the maximal amount of residual phosphate was less than 0.10 µg P l^{-1} at all growth rates (Table 1 and Appendix F). The chemically determined reactive P therefore overestimates by up to 20 times the concentration of orthophosphate in the chemostats.

Carbon Fixation

The carbon uptake rate (at mid-culture light intensities) is independent of the cell division rate at µ> 0.22 div. day^{-1}, but declines sharply at slower growth rates (Fig. 8). In the low P chemostats, higher light intensities resulting from the decrease in cell density lead to greater carbon uptake than that measured for the high P chemostats. In both sets of chemostats, the amount of carbon fixed as measured by the acidification-bubbling technique is consistently greater ($\bar{x}$ 22%) than that measured on membrane filters (Fig. 8). Finally, when the filtered results are plotted against P $cell^{-1}$ (Fig. 9), it is evident

Fig. 7. Calculation of the residual phosphate concentration in the chemostats by ^{32}P assay: typical results. The curves are the product of the rate constant of uptake and the sum of P added plus various assumed values for residual phosphate. The curve bends sharply upwards at a residual phosphate concentration set equal to SRP (1.22 µg P ℓ^{-1}) and first bends towards the x-y intercept at an assumed residual P concentration of 0.10 µg P ℓ^{-1}

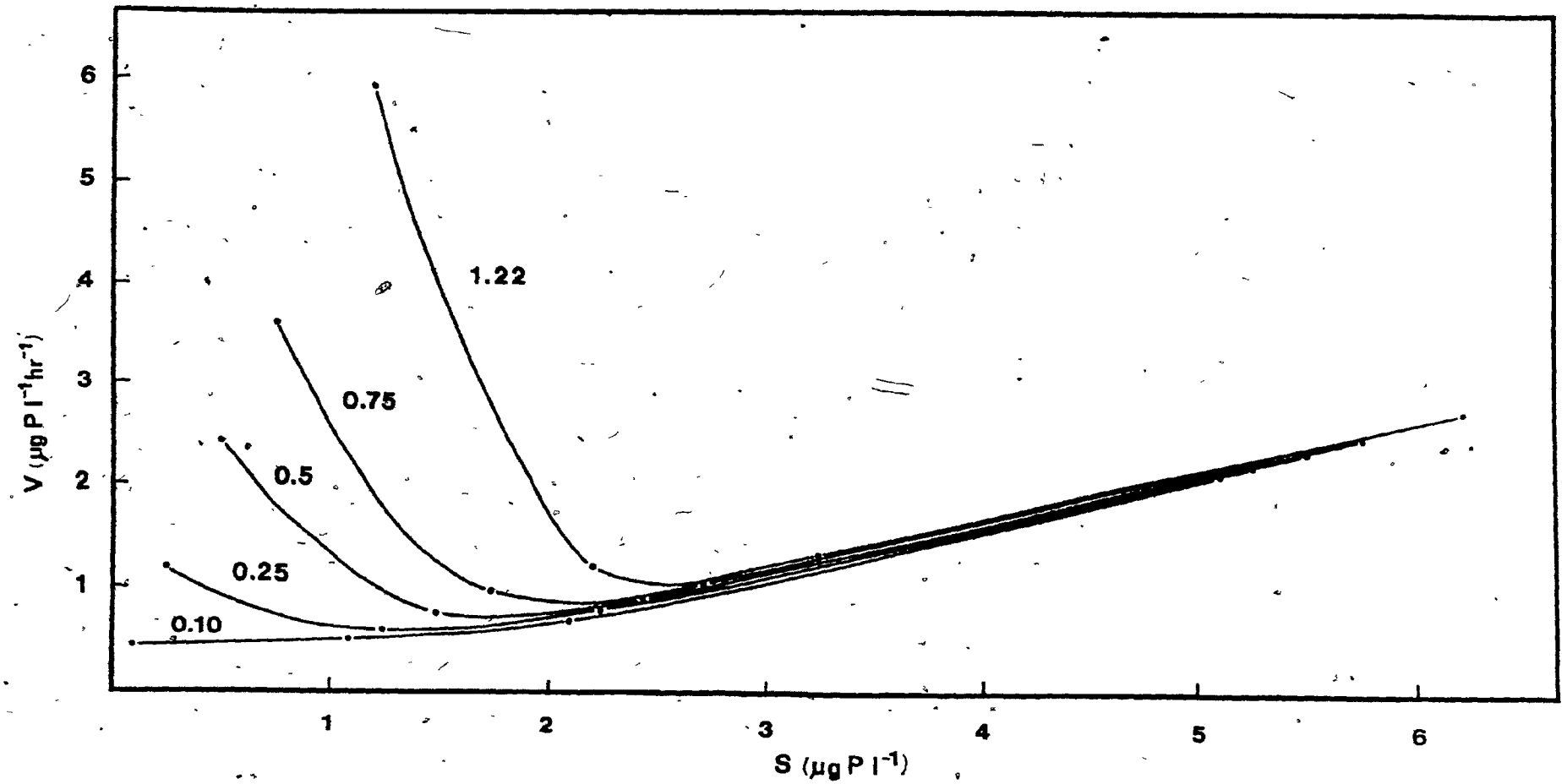

6
5
4
3
2
1
V (µg P l⁻¹hr⁻¹)
1.22
0.75
0.5
0.25
0.10
1
2
3
4
5
6
S (µg P l⁻¹)

Fig. 8. The carbon uptake rate measured by standard membrane filtration (○) and by acidification-bubbling (●) at various growth rates in the chemostats.

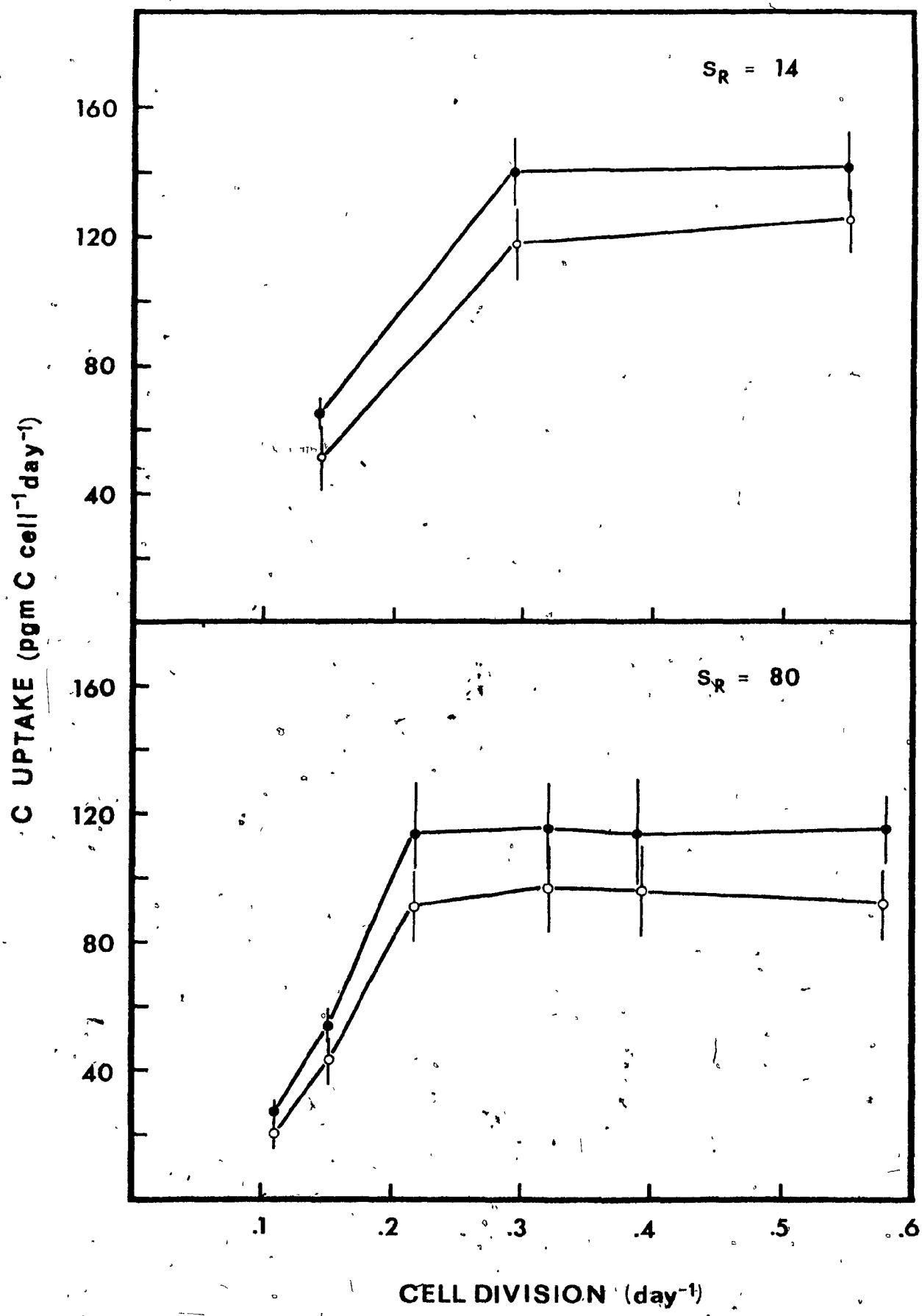
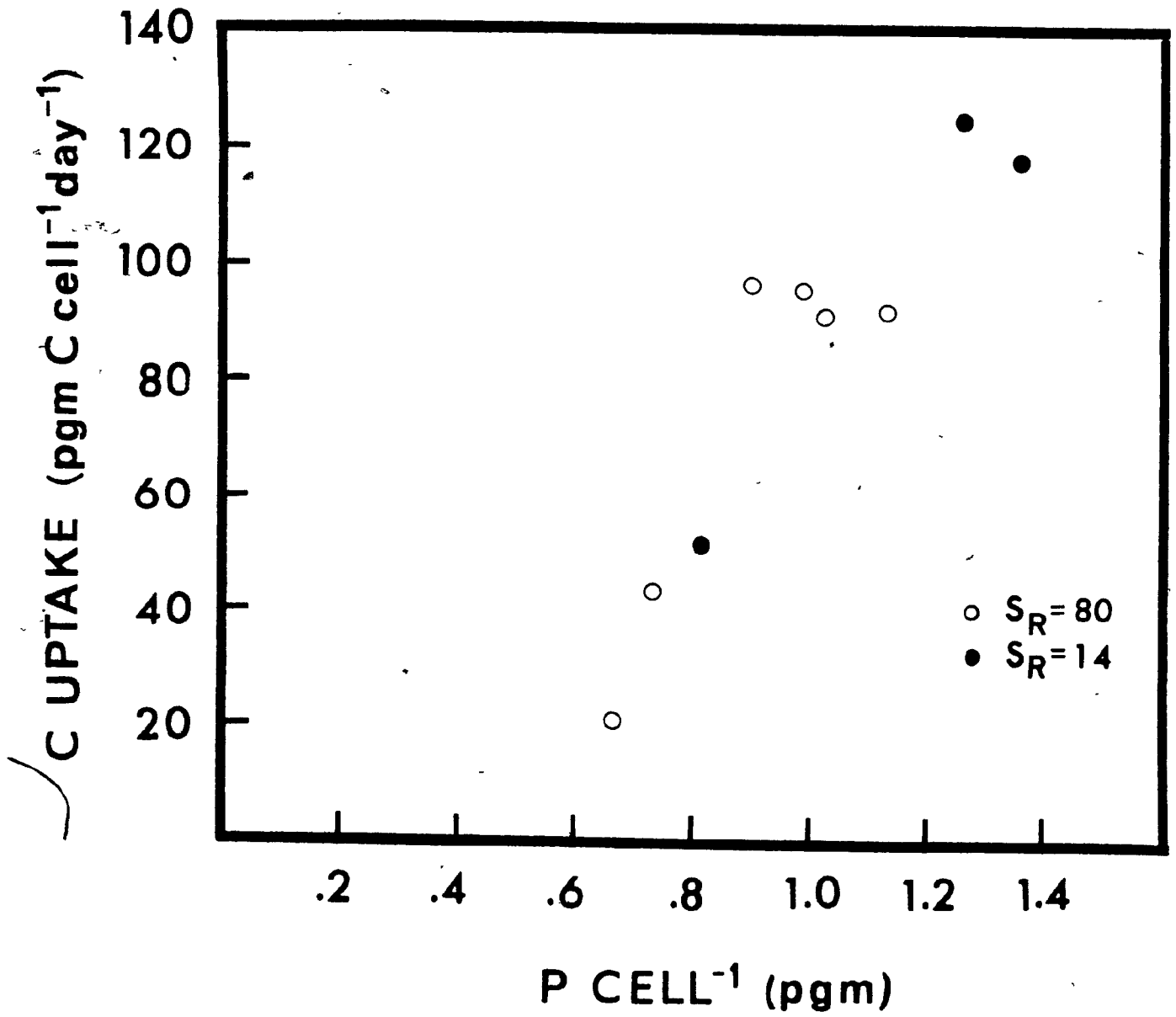

S_R = 14
S_R = 80
160
120
80
40
C UPTAKE (pgm C cell^{-1}day^{-1})
.1
.2
.3
.4
.5
.6
CELL DIVISION (day^{-1})

Fig. 9. The relationship between carbon uptake rate and P content per cell.


140
120
100
80
60
40
20
C UPTAKE (pgm C cell^{-1}day^{-1})
.2
.4
.6
.8
1.0
1.2
1.4
P CELL^{-1} (pgm)
S_R = 80
S_R = 14

that carbon fixation decreases only when the P content is less than an apparently critical 0.8-0.9 pgm P $cell^{-1}$.

The effect of various degrees of P limitation on the photosynthetic response of C. erosa is best described by a series of photosynthesis-light curves (Fig. 10) (also Part III). The results show that: (1) In all experiments but one, the slope of the P-I curve at subsaturating light levels was not significantly affected by the nutrient status of the cells, either before or after enrichment with P, (2) that cells grown at higher rates of P supply had a maximum photosynthetic capacity (P_{max}) greater than cells under severe P limitation, (3) that more P sufficient cells saturated at higher light intensities (I_k) than deficient populations. Moreover when the I_k's were compared to the mid-chemostat light flux ($\simeq 10^{-2}$ ly min^{-1}) it was evident that carbon uptake was light-saturated only at growth rates < 0.22 div. day^{-1}, (4) that at higher rates of P supply the onset of photoinhibition (I_i) usually occurred at higher light intensities than under greater P deficiency, and (5) that a 24 hr enrichment with P had a negligible effect on the photosynthetic response of cells at higher growth rates (>0.22 div. day^{-1}), but increased the P_{max} and I_k of severely P-limited cells (Fig. 11 and Appendix I).

Morphology and Cellular Composition of C. erosa

P-limitation affects not only cellular P content, but also cell morphology and the relative abundance of other cell components.

Cell volume and carbon content. The mean cell volume of C. erosa

Fig. 10. The photosynthesis-light response of C. erosa grown under various degrees of P-limitation in continuous culture. Steady state growth rate (div. day^{-1}): 1 - 0.55; 2 - 0.39; 3 - 0.23; 4 - 0.14; 5 - 0.11.

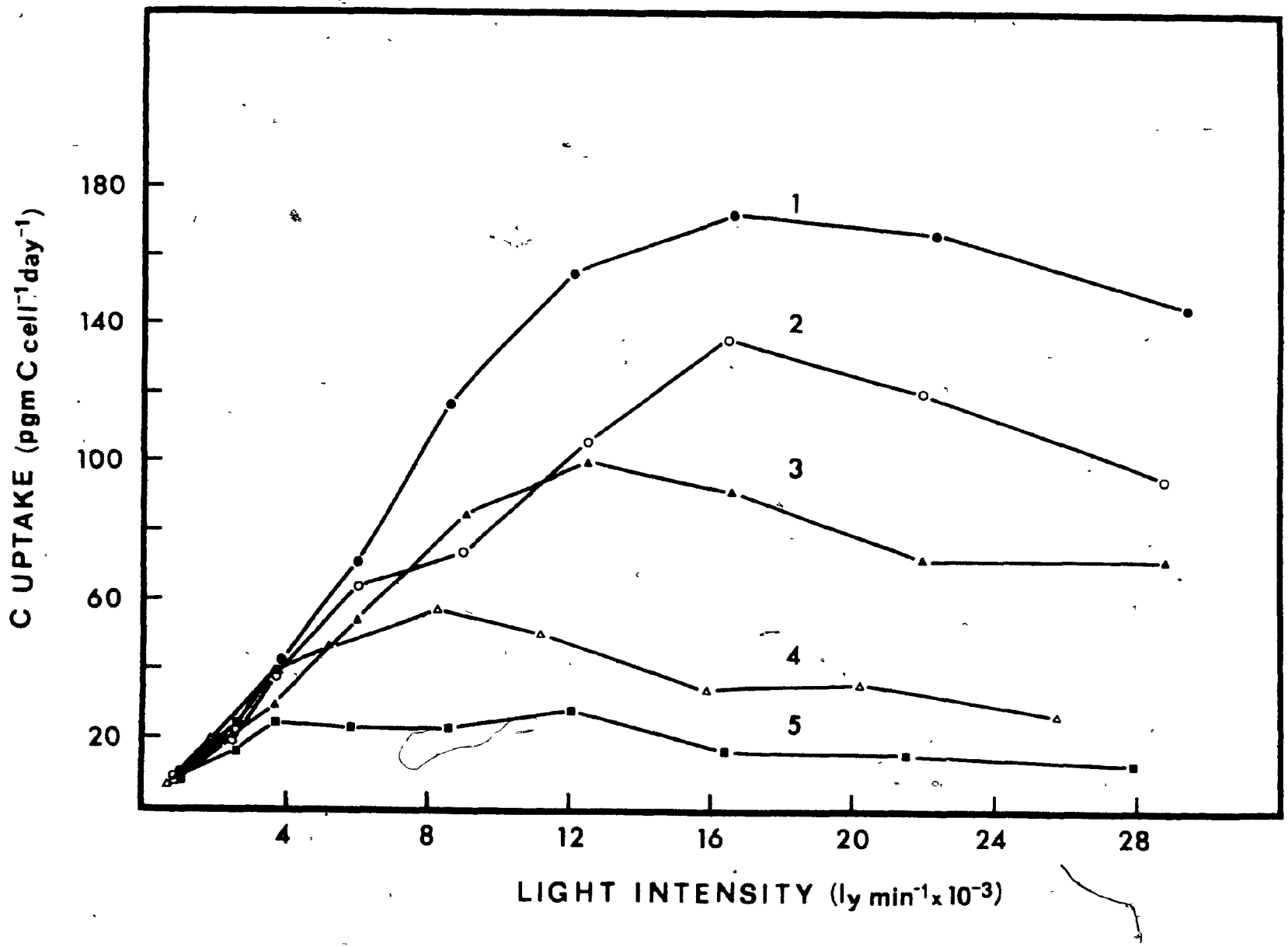
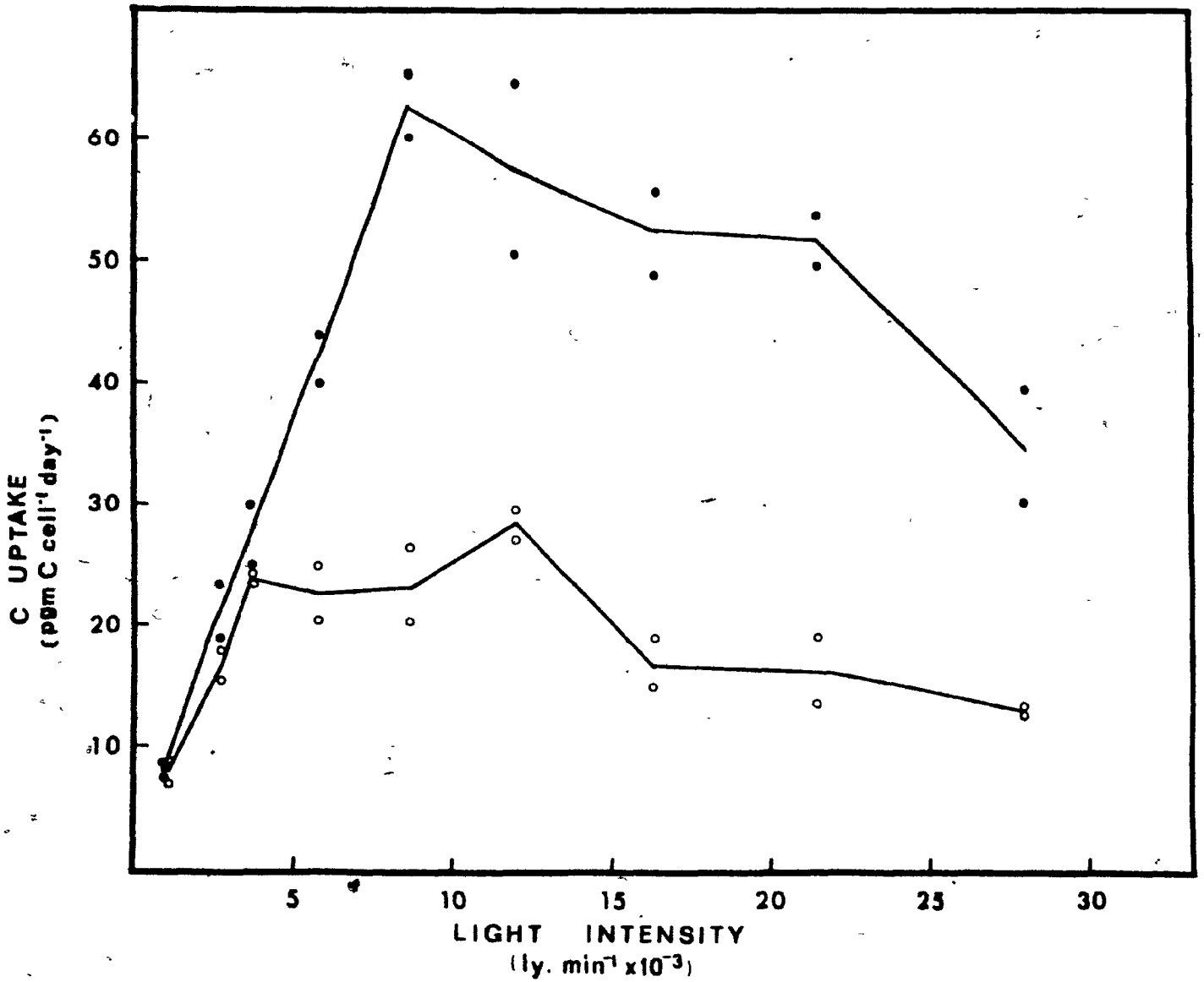

C UPTAKE (pgm C cell^{-1}day^{-1})
180
140
100
60
20
4
8
12
16
20
24
28
LIGHT INTENSITY (ly min^{-1}x 10^{-3})
1
2
3
4
5

Fig. 11. The photosynthesis-light response of C. erosa before (○) and after (●) a 24 hr enrichment with phosphate. The cells were grown at 0.11 div. day^{-1} in the chemostats.


C UPTAKE
(pgm C cell⁻¹ day⁻¹)
60
50
40
30
20
10
5
10
15
20
25
30
LIGHT INTENSITY
(ly. min⁻¹ x10⁻³)

in the low P chemostats increases to a maximum at the slowest growth rate, but in the high P chemostats is maximal at an intermediate growth rate (Table 2). Cells in the low P chemostats are also 50-100% larger. Measures of suspended carbon in some batch cultures and in the chemostats show that carbon is a fairly constant 20% of the wet weight so that an increase in cell volume directly reflects an increase in cell carbon (Fig. 12). Cell carbon content is therefore also maximal at intermediate and slow rates of P supply (Table 2). Direct measures of cell carbon can be compared with estimates obtained from the expression

$$\text{carbon cell}^{-1} = \frac{\text{net C uptake cell}^{-1}\ \text{day}^{-1}}{\text{cell div. day}^{-1}}$$

The results show (Table 2) that at most growth rates the measures are in reasonable agreement, indicating that the uptake of ^{14}C on filters measured the production of particulate carbon.

Cell nitrogen. N cell^{-1} did not change appreciably with growth rate (Table 2) and no relationship was observed between cell nitrogen and cell volume.

Atomic ratios. The relative abundance of C, N and P in C. erosa is strongly affected by the P supply. In nutrient-rich batch cultures the P content of the cells was 2.5-5 fold greater than in the chemostats, presumably the result of polyphosphate storage (Rhee, 1973). The atomic ratio of N:P was 14-16, while C:P was <200, except under certain high light/low temperature conditions when the cells stored vast quantities of carbohydrate. In contrast, in the chemostat cultures the ratios were much higher at all growth rates examined. N:P increased from 54 to 114 while C:P rose to

Table 2. Steady state population characteristics and comparison of cell carbon measured in three different ways. Limits are one standard deviation.

P concentration in reservoir (μg P ℓ^{-1})	Cell division rate (div. day^{-1})	Cell volume (μ^3)	Cell carbon (picograms)			Cell nitrogen (picograms)	Cell chlorophyll (picograms)
			Particulate carbon	Cell volume	C fixed / Cell div.		
80	0.58	1047 ± 92	197 ± 12.2	218	159	26.3 ± 5.3	5.0 ± 0.42
	0.39	1381 ± 100	237	286	245	28.6	5.57 ± 0.34
	0.32	1240 ± 43	251 ± 29	258	302	29.2 ± 4.3	5.82 ± 0.74
	0.23	1580 ± 114	327	327	396	33.0 ± 1.7	6.55 ± 0.31
	0.15	1375 ± 58	271 ± 30	285	293	27.0 ± 8.4	5.89 ± 0.45
	0.11	1337 ± 43	304	277	191	35.7	5.26 ± 0.03
14	0.55	1616 ± 1172	289 ± 46	335	227	-	4.52 ± 0.26
	0.29	2132 ± 149	488 ± 50	440	409	-	5.30 ± 0.40
	0.14	2657 ± 145	470 ± 79	548	369	-	5.05 ± 0.79

Fig. 12. The linear regression of cell carbon on cell volume for 4 and 1° batch cells (●) and chemostat cells (△) (C = 0.20V + 3.5).

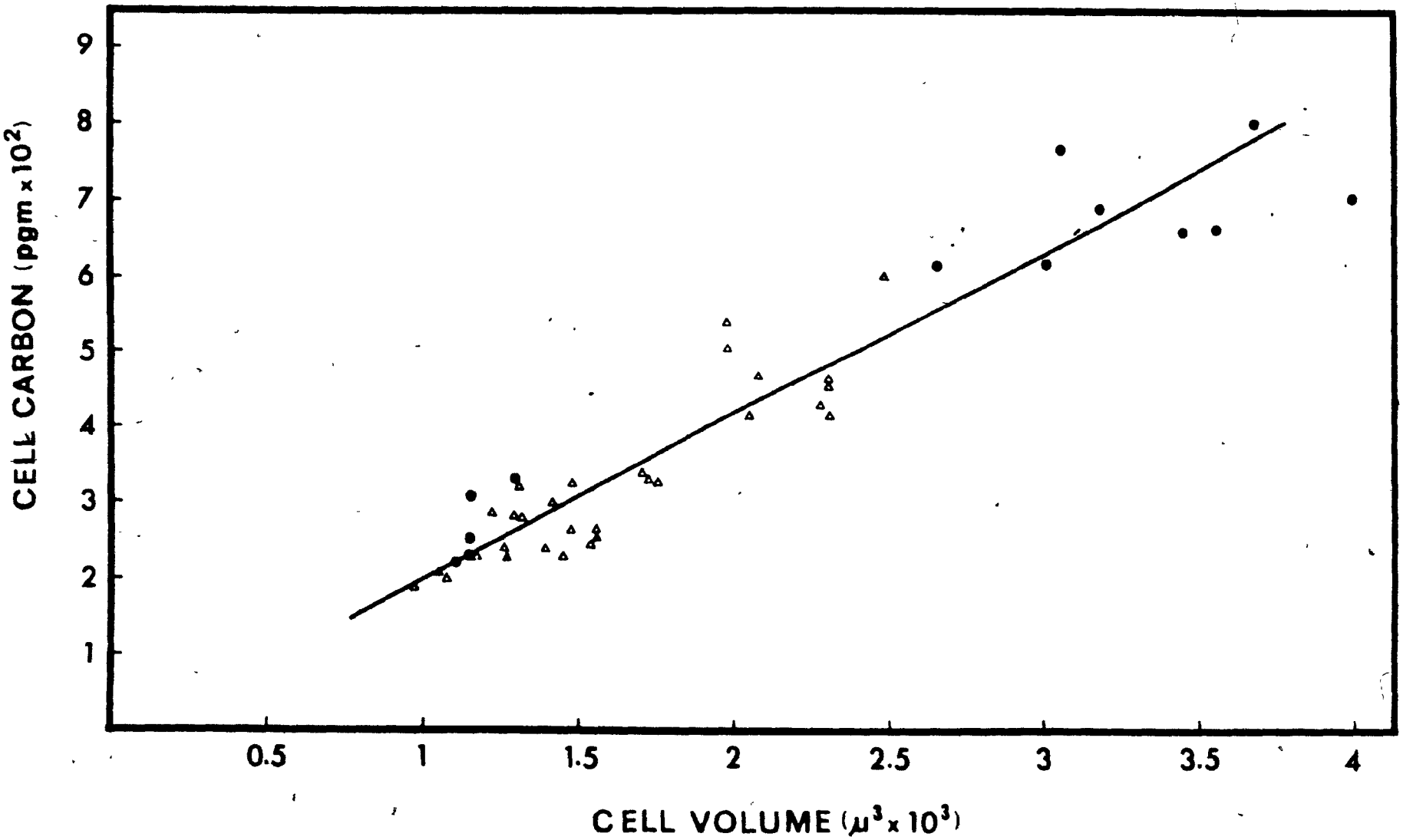
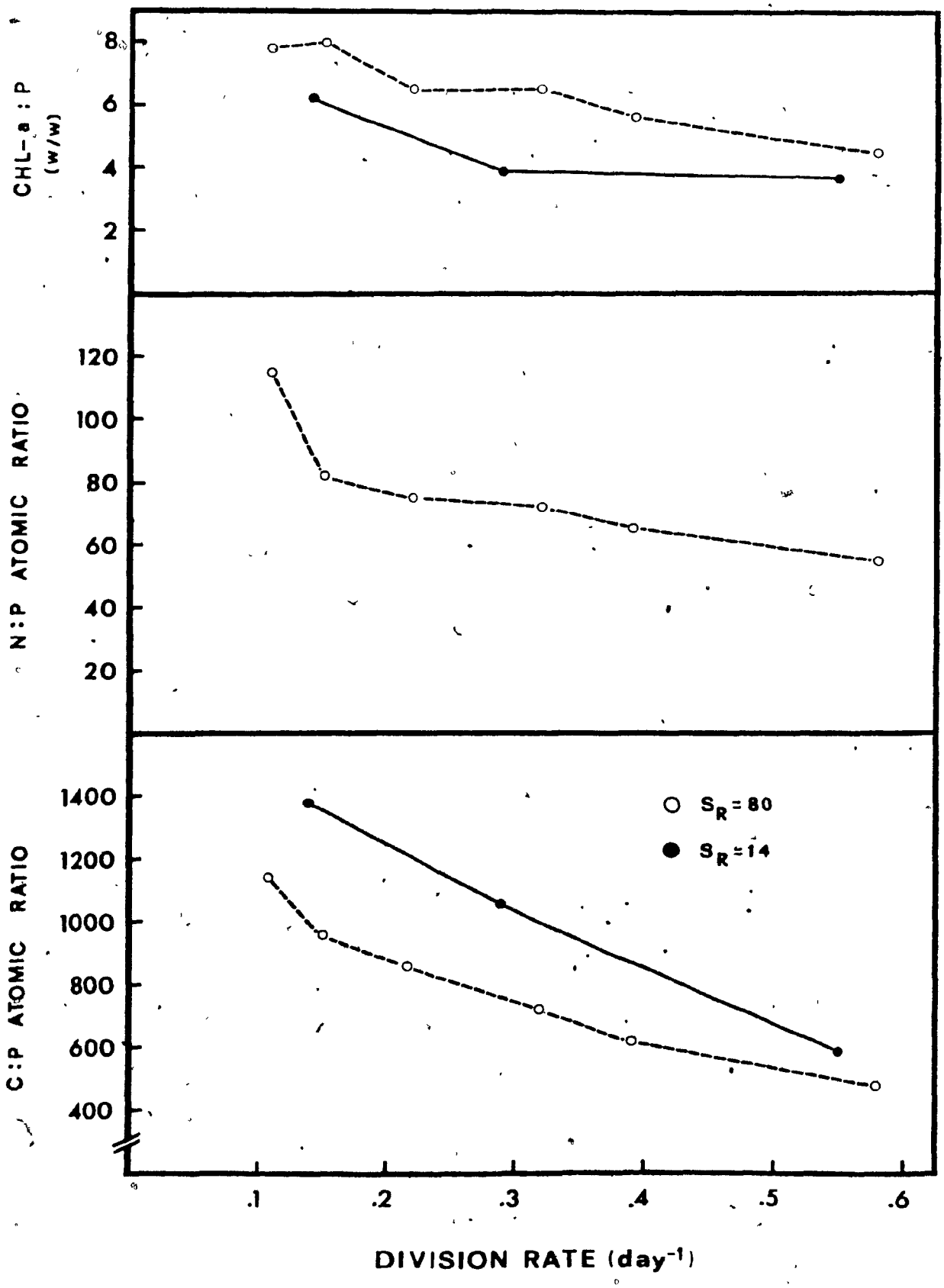

CELL CARBON (pgm x 10²)
9
8
7
6
5
4
3
2
1
0.5
1
1.5
2
2.5
3
3.5
4
CELL VOLUME (μ³ x 10³)

Fig. 13. C:P and N:P atomic ratios and chl-a:P (w/w) in P-limited C. erosa.


CHL-a : P (w/w)
8
6
4
2
N:P ATOMIC RATIO
120
100
80
60
40
20
C:P ATOMIC RATIO
1400
1200
1000
800
600
400
O S_R = 80
● S_R = 14
.1
.2
.3
.4
.5
.6
DIVISION RATE (day^{-1})

over 1000 with increasing P deficiency (Fig. 13).

Chlorophyll a. The pigment content per cell^{-1} was not affected by the rate of P supply (Table 2). Some effect had been anticipated because the cultures appeared quite pale at the slowest growth rates, apparently the result of increased light scattering caused by increased cell size and carbon content (Fuhs et al., 1972). The low P chemostat cells had a consistently lower pigment content, a possible response to their higher light regime. The ratio of chl-a:P (w/w) increased with a decline in P supply to a maximum of 7.9 (Fig. 13).

Phosphorus Uptake Kinetics

Phosphorus uptake by C. erosa and possibly all other algae, as measured by ^{32}P on membrane filters, is subject to two errors in the filtration process. Firstly ^{32}P added to killed samples is also retained by the filters. The nature of this retention was not explored, but may represent ^{32}P adsorbed to colloids and detritus or a small volume of liquid held by the filter matrix. Although blank counts are only 0.3 to 0.5% of the total radioactivity added, they introduce significant error in those samples where high ^{31}P additions result in <1% removal of ^{32}P by the cells. If blank corrections are not made V_{max} is overestimated. A second error is that the amount of ^{32}P in the filtrate increases with filtration pressure, presumably from cell breakage and leakage. Even filtration at only the 50 mm Hg used routinely here, results in an approximately 10% loss of cellular ^{32}P, when compared to the radioactivity of cells concentrated by low speed centrifugation (Appendix J). However, in the

absence of sufficient data the uncorrected filtered results were used. In addition, other work in our laboratory shows that termination of ^{32}P uptake with Lugol's iodine also leads to a significant loss (up to 30%) of ^{32}P incorporated by both C. erosa and a planktonic diatom (Brighten, pers. comm.). Such loss of cellular P was avoided in the present study by live filtration of the cells.

Phosphorus uptake by C. erosa follows Michaelis-Menten kinetics with rate saturation at low 50-100 μg l^{-1} concentrations of P (Fig. 14 and Appendix K). The maximum uptake rate (V_{max}), calculated at each sampling time, decreased within even the first 30 min of uptake. However, by extrapolation of the V_{max} at 10 and 30 min to the ordinate, it was possible to estimate the uptake rate at zero time. The V_{max} (zero-time) was low (< 3 pgm P $cell^{-1}$ day^{-1}) in all experiments, and was not relatable to the steady state growth rate in the chemostats (Table 3 and Appendix K). Although phosphorus uptake was non-linear, the $K_s + S_o$ determined at each sampling time was consistently low, with an average value of 4.25 ± 1.19 μg P l^{-1} for all uptake experiments (Table 3). Since our maximal estimates of S_o by radiobiological assay (<0.10 μg P l^{-1}) are << $K_s + S_o$, the half-saturation constant for phosphorus uptake by C. erosa is approximately 4.3 μg P l^{-1} or 0.14 μM P.

When the concentration of P in the reservoir was increased from 14 to 80 μg P l^{-1}, the three growth vessels became heavily contaminated with bacteria. Although the reason for this is unclear, the new steady state cell density of C. erosa was reduced (Fig. 5 and Appendix G) and the SRP concentration increased by as much as 10 fold. The presumed increase in available

Fig. 14. Short term (10 min) uptake of phosphate (○, left ordinate) and the linear transformation according to the Woolfe plot (●, right ordinate). Cells were grown in chemostat culture at $\mu = 0.39$ div. day^{-1}.

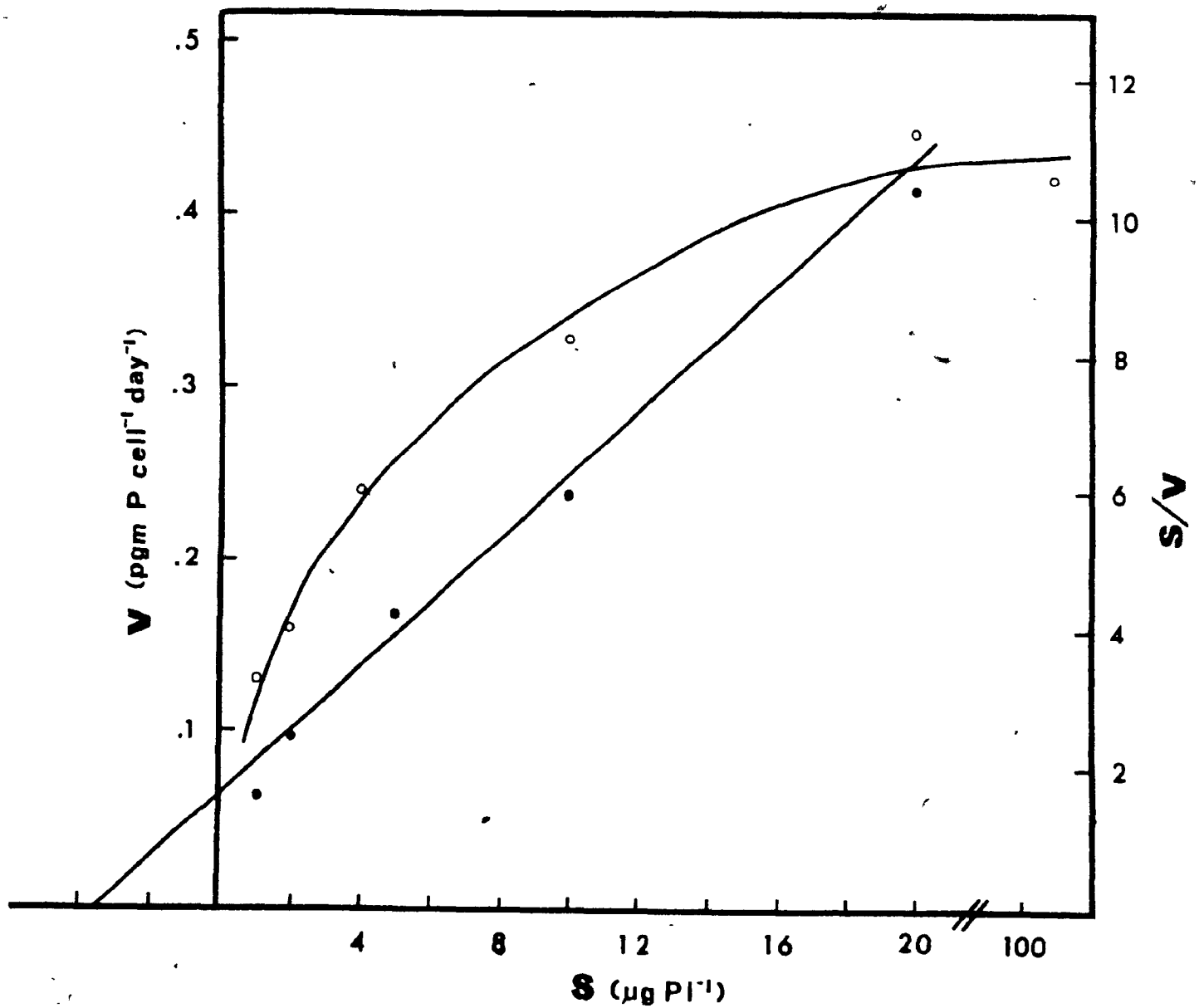

.5
.4
.3
.2
.1
V (pgm P cell⁻¹ day⁻¹)
12
10
8
6
4
2
S/V
4
8
12
16
20
100
S (µg Pl⁻¹)

Table 3. Characterization of P uptake kinetics of C. erosa

S_R μg P ℓ^{-1}	Specific growth rate (day^{-1})	P content $cell^{-1}$ (q) (pgm)	$\hat{V}_{max}$ zero-time (pgm P $cell^{-1}$ day^{-1})	$K_s + S_o$ (μg P ℓ^{-1})	Specific uptake* rate (pgm P $cell^{-1}$ day^{-1})	Predicted uptake† rate (pgm P $cell^{-1}$ day^{-1})
80	0.27	0.99	1.7	4.0	0.27	0.04
	0.16	1.01	1.8	5.6	0.16	0.03
	0.074	0.67	1.2	3.1	0.05	0.04
14	0.38	1.26	1.5	2.7	0.48	0.06
	0.20	1.36	2.8	5.4	0.27	0.05
	0.095	0.81	2.4	4.7	0.08	0.05

* Specific uptake rate from equation $V = \mu q$.

† Predicted uptake rate from Michaelis-Menten equation at S_o = .10 μg P ℓ^{-1}.

Table 4. P uptake kinetics in chemostats following contamination with bacteria.

Data are given in % ^{32}P removed from solution.

Added P (µg P ℓ^{-1})	10 min		30 min		60 min		8 hr	
	Algae*	Bacteria†	Algae	Bacteria	Algae	Bacteria	Algae	Bacteria
0	15.0%	2.8	22.3	5.7	24.7	8.9	39.1	12.2
.5	15.6	2.6	23.8	3.8	27.0	6.1	38.1	8.9
1	15.8	2.0	22.0	3.4	27.7	4.6	38.1	8.2
5	17.7	1.9	29.2	2.8	34.5	4.0	41.8	6.6
10	18.9	1.6	34.7	2.0	39.6	3.6	39.1	6.1
50	14.6	1.4	44.1	2.1	60.6	3.3	60.0	5.0

* Activity retained on 8 µm filter.

† Activity retained on 0.45 µm filter following 8 µm prefiltration.

phosphate coincided with a P uptake no longer consistent with Michaelis-Menten kinetics because the % ^{32}P removed from solution increased with increasing substrate additions (Table 4). The same results were obtained in 8 other such experiments at several growth rates. Differential filtration of algae and bacteria through 8 and 0.45 μm filters showed that this new type of uptake was associated only with C. erosa and could not be attributed to bacterial uptake or filter retention of colloidal or detrital-bound ^{32}P. The results, therefore, show a major change in the transport system of the alga resulting from higher P concentrations.

DISCUSSION

Phosphorus Limitation of Cell Division Versus Carbon Uptake

In the chemostat experiments with C. erosa there is conflicting evidence as to whether phosphorus or light intensity is the limiting factor for growth. It was shown that at higher growth rates (> 0.22 div. day^{-1}) the rate of carbon uptake was independent of the supply rate of P (Fig. 8). Moreover, from the photosynthesis-light curves, carbon uptake of such cells was saturated only at light intensities greater than those present in the growth vessels (Fig. 10). However, carbon uptake was sharply lowered at slower rates of P supply and, from the P-I experiments, was light-saturated in the chemostats. The results thus suggest that growth is regulated by P only at the lowest dilution rates, and by light at higher rates. However, other evidence attests to P as the limiting factor at all growth rates. This evidence includes the reduction of residual phosphate to nanogram levels in all growth

vessels, the demonstration that the cellular P content was independent of the concentration of P in the reservoir, and that the cell density of steady state populations responded to even small changes in reservoir P. Moreover, when the substrate concentration in the inflow was increased from 14 to 80 μg P l^{-1}, the temporary greater availability of substrate caused the population to increase at the maximum cell division rate (0.65 - 0.70 div. day^{-1}) (Fig. 5). The apparent discrepancy in the results can be resolved by considering cell division and carbon uptake to be differently affected by P limitation. That is, at higher rates of P supply the amount of chlorophyll and photosynthetic enzymes present permit carbon uptake to proceed at a rate limited only by light, while P specifically affects components required for cell division. Only at lower rates of supply are both carbon uptake and cell division controlled by P alone. Batch culture studies have shown that low temperature also severely retards cell division of the species, while carbon fixation is less impaired (Part I). Similar results have been reported for other algae grown at low temperatures (Sorokin and Krauss, 1962; Wilson and James, 1966) or under N and P deficiency (Droop, 1954; Schindler, 1971). Such results support the "Master Reaction" theory of Sorokin (1960) which postulates cell accumulation and cell division to be semi-independent processes that can respond differently to the environment. Whereas batch cultures enriched well beyond natural substrate levels readily manifest the effect of light and temperature on algal growth, chemostat cultures illustrate the overriding importance of nutrients at lower and more natural concentrations. However, in nature it is virtually impossible to

measure the effect of nutrient limitation on cell division because the phytoplankton biomass is also significantly affected simultaneously by grazing and sedimentation.

As the results of the P-I curves are discussed in detail elsewhere (Part III), a possible mechanism of P control of carbon uptake is only briefly considered here. The P-I curves show that the initial slope of carbon fixation versus light at low intensities is relatively unaffected by the nutrient status of the cells in comparison with the maximum photosynthetic rate (P_{max}), which is markedly lowered under severe P limitation (Fig. 10). The P_{max} is a measure of the maximum rate at which the dark reactions of photosynthesis can use products from the primary photochemical reactions (Steemann Nielsen and Jørgensen, 1968). Since the maximum rate is dependent on the environmental temperature and the concentration of dark enzymes, we interpret the decrease of the P_{max} in our constant temperature chemostats the result of an inability of the cell to maintain a sufficiently high concentration of dark enzymes. Yentsch and Lee (1966) and Griffiths (1973) have similarly explained a lowered P_{max} in low light and dark cells, while Hellebust and Terborgh (1967) and Morris and Farrell (1971) have related change in the photosynthetic capacity to the concentration of the dark enzyme RuDP carboxylase. With fewer enzyme molecules present, severely P-limited cells are saturated at low light intensities, as shown by a shift in the I_k to lower values (Fig. 10). Thus, in contrast to more P-sufficient cells, the lowered photosynthetic maximum is reached at lower light intensities.

A 24 hr phosphate enrichment had a negligible effect on the photosynthetic

response of C. erosa grown at higher rates of P supply, but increased the P_{max} and I_k of severely P-limited cells (Fig. 11 and Appendix I), indicating that phosphorus accumulated by the cells is channelled into metabolic pathways which restore the level of the dark enzymes. However, restoration of the photosynthetic rate was only 40-60% of the maximum rate of cells grown at higher rates of P supply. Since P deficient C. erosa are rich in carbohydrate even this degree of recovery had not been expected until the previously stored carbon had been utilized in renewed growth (Healey, 1973a). However, within 24 hrs of enrichment many cells had divided (Appendix I) and it was probably only these smaller cells with their now lower carbon content that were photosynthesizing at the maximum rate, while cells which had not yet divided were much less active. The failure of cells grown at higher rates of P supply to respond photosynthetically to added P, despite the observed P-limitation of cell division, may well partly account for the variable response in photosynthesis of natural communities in short-term nutrient bioassays. The results suggest that despite species changes, long-term measures of chlorophyll a concentration and cell numbers better assess nutrient limitation in lakes than short-term bioassays.

Since C. erosa in the chemostats maintains a high rate of carbon uptake despite a reduction in cell division, the cell must either increase its mass or excrete excess organic carbon. It appears from the difference in carbon uptake as measured by the acidification-bubbling and the standard membrane filtration techniques (Fig. 8) that the cell

excretes approximately 20% of the total carbon fixed, yet the proportion remains constant regardless of the degree of inbalance between carbon uptake and cell division. Because cell volume and carbon content are both maximal at the point of greatest imbalance (Table 2), it is evident that excess photosynthate is principally retained within the cell as storage carbon. However, a much more extreme inbalance in batch cultures at low temperatures (1 and 4°) and inhibitory light levels (Part I) resulted in a virtual cessation of cell division and excretion of up to 50% of the total carbon fixed. Because the carbon uptake rate decreases with decreasing rates of cell division (Fig. 8), similar rates of excretion do not occur in the chemostats. The proportion of carbon retained or excreted is thus dependent on the degree of inbalance between carbon uptake and cell division.

A general response of algae to nutrient limitation is the accumulation of carbon storage compounds (Healey, 1973a). In the Cryptophyceae carbon is stored as starch in spheroidal or ellipsoidal starch granules in a sheath around the pyrenoid (Lucas, 1970), which in the chemostat cells were normally deposited in such abundance as to obscure the remainder of the cell contents. Carbohydrate storage has been reported for several N deficient algae (Allen and Smith, 1969; Guérin-Dumartrait et al., 1970) and P deficient algae (Fuhs et al., 1972; Healey, 1973b), while yet other algae accumulate lipids (van Baalen and Marler, 1963).

Although the nature of the excretory products of C. erosa was not examined, its mucilaginous growth on the chemostat walls suggests that the 20% carbon excreted is largely composed of polysaccharides.

According to Hellebust (in Stewart, 1974), the amounts of polysaccharides released may represent a considerable 15-90% fraction of the photoassimilated carbon in some species. That C. erosa does not excrete a higher percentage is the result of its ability to store large amounts of carbon. In general a combination of high light and low nutrients and/or low temperature, inhibiting cell division but not photosynthesis, will result in the excretion of a higher proportion of photosynthate. The higher rates of carbon excretion observed in oligotrophic than in eutrophic waters (Anderson and Zeutschel, 1970; Saunders, 1972) may well be caused by the differential affect of low nutrients and frequently low temperatures on cell division.

Phosphorus Content of C. erosa

Although the phosphorus content of the cells (q) decreased at the slower growth rates, P cell^{-1} was constant at higher growth rates (Fig. 6). Thus cell division cannot be related to cellular P content by a rectangular hyperbola of the form

$$\mu = \frac{\mu_m (q - k)}{q}$$

used by Droop (1968) to describe algal growth on vitamin B_{12}, where k is equivalent to the minimum substrate content of the cells (q_o) in our study. Similar expressions have described phytoplankton growth on iron (Davies, 1970), nitrate (Caperon and Meyer, 1972a) and phosphate (Fuhs, 1969). Nutrient-limited growth of algae generally fits the above model because of variation in the size of the nutrient reservoir of a species, which is nearly empty under severe limitation but fills under higher

rates of nutrient supply. It is the size of this reservoir that controls the growth rate of the cell (Caperon, 1967; Droop, 1968). We can interpret the lack of fit of our data to this hyperbola to result from either a decrease in the amount of incorporated P (protoplasmic P) and an increase in reservoir P, resulting in a constant level of P $cell^{-1}$, or from the lack of filling of the reservoir at higher rates of P supply (Caperon and Meyer, 1972a). Although the present data do not permit a clear choice between these alternatives, the P uptake kinetics of C. erosa (Table 3) suggest that the alga has a low uptake capacity (low V_{max}), limiting the accumulation of large amounts of P in the reservoir (Eppley and Thomas, 1969). Thus at higher rates of P supply the nutrient uptake rate of the cells does not exceed the rate of incorporation into protoplasm, and the additional P is channelled into more biomass.

By extrapolating to q at zero growth the minimum P content of C. erosa (q_o) is 0.30 pgm P $cell^{-1}$ (Fig. 6) and, according to Soeder et al. (1971), such values are species specific. They list the minimum P content of several small green and diatom species as ranging from 0.05-0.14 pgm $cell^{-1}$, while a diatom similar in size to C. erosa had a q_o of 0.39 pgm P (Fuhs et al., 1972), suggesting that minimal P of algae may be more a function of cell volume than of species. Thus one might predict that the maximal biomass to develop in a P-deficient lake would be largely independent of species composition. However, as small cells are more susceptible to predation than large cells, the maximum standing crop in a lake dominated by nanoplankton would be less than that in a lake dominated by non-grazed netplanktonic blue-greens and diatoms.

Cell Composition as a Measure of P Deficiency

Cell composition when expressed as ratios has been used to assess the nutrient status of phytoplankton (Fuhs et al., 1972; Perry, 1976). Under conditions of balanced growth Redfield et al (1963) and Ketchum (1969) cite atomic ratios of C:N:P of 100:15:1 or 106:16:1, whereas C:P and N:P ratios greater than 200 and 20-22, respectively, indicate a P deficiency (Healey, 1975). The high ratios observed in chemostat grown C. erosa (Fig. 13) are thus clearly indicative of P-limitation and, except for certain unusual growth conditions (see results), provide a clear separation between P-rich batch cells and P-deficient chemostat cells. They are also distinctly different from those recorded for other cultured species deficient in nitrogen (Sakshaug et al., 1973; Perry, 1976). However, such ratios are of less use in field assessments of deficiency because of contamination from detritus and heterotrophic organisms (Peterson et al., 1974; Perry, 1976). Moreover, the low phosphate concentrations in natural waters preclude the extensive uptake of surplus P that is possible in enriched laboratory cultures and, with the resulting lower P $cell^{-1}$, the ratios less clearly delineate N from P deficiency. P limitation in lakes is thus best assessed by supplementing information obtained on atomic ratios with measures of kinetic parameters, such as nutrient debt and alkaline phosphatase activity (Healey, 1975).

Steady State Phosphate Concentration

We estimate, using radiobiological ^{32}P assay, that the maximum amount of residual phosphate in the chemostats was 0.10 μg P l^{-1}. The up to 20 fold discrepancy observed in our data between chemically determined SRP and available orthophosphate is consistent with similar measurements in batch cultures (Kuenzler and Ketchum, 1962) and in natural waters (Rigler, 1966, 1968). Rigler (1973) suggests that the strong acidic conditions of the molybdate-blue technique will hydrolyze free phosphate esters and release PO_4-P from fulvic acid-metal phosphates or colloidal-iron phosphate. In addition, the filtration loss of ^{32}P from C. erosa (Appendix J) and other algae (King, 1970) further introduces error when attempts are made to measure P chemically.

Since our measures of steady state residual P are maximal estimates, whether a threshold concentration of phosphate exists in the chemostats is unclear. In other chemostat studies (Button et al., 1973; Perry, 1976) the addition of radioactive tracer to the reservoir suggests a low residual concentration of P, while Caperon and Meyer (1972a) also postulate a threshold concentration of nitrate for several marine algal species. In nature one might predict a high threshold concentration to occur only in communities dominated by high K_s species, which less effectively remove nutrients at low concentrations. However, bioassay measures of phosphate at low .001 μg P l^{-1} concentrations in lake waters (Levine, 1975) most probably characterize the efficiency at which microbial communities normally scavenge phosphorus and other nutrients.

Kinetics of P Uptake

Since at steady state the amount of phosphate taken up by C. erosa in the chemostats must equal the amount used to produce new cells,

$$V = \mu q \qquad (1)$$

where V is the specific uptake rate day^{-1} (pgm P $cell^{-1}$ day^{-1}), μ is the specific growth rate day^{-1}, and q is the amount of phosphorus per cell (pgm P $cell^{-1}$). The uptake kinetics of the population can also be expressed by the familiar Monod equation

$$V = \frac{V_{max}\, S}{K_s + S} \qquad (2)$$

Taking the V_{max} (zero-time) and the mean K_s determined for each uptake experiment (Table 3) it is possible to calculate (equation 2) the uptake rate that would occur in the chemostats at the maximum estimated concentration of available phosphate (0.10 µg P l^{-1}). The calculations show these predicted uptake rates to be 1.3 - 8.0 ($\bar{x}$ 4.7) times less than the specific uptake rate (equation 1) and the discrepancy increases to the extent that S is lower than our maximal estimates (Table 3).

There are two possible reasons why the in vitro measurements of P uptake might underestimate the uptake rate based on growth in the chemostats: (1) The samples for in vitro P uptake were frequently swirled, but the continuous stirring and aeration of the chemostats was not maintained. If the decreased circulation created a local depletion of phosphate at the active surface of the cell, P transport into the cell would be lowered and V_{max} accordingly underestimated. Munk and Riley (1952) and Pasciak and Gavis (1974) have demonstrated the importance

of medium flow on nutrient uptake. In situ incubation of phytoplankton in bottles may, for the same reason, underestimate production in the open waters of lakes, (2) Any change in the environment of cells removed from the chemostats that inhibited growth would also reduce P transport and the V_{max}. Light intensity, temperature and pH were kept constant, but the cells were subject to other stress, such as the possible inhibitory effect of a sudden up to 1000 fold increase in phosphate concentration. Nitrate uptake of a marine alga was linear only if the cells were pre-incubated with a low concentration of nitrate (Eppley and Thomas, 1969), suggesting that some transport systems require "prepping" in order to respond to high levels of substrate. Similar perturbation of the growth conditions must also occur in nutrient bioassays of natural communities and may well account for the frequently reported failure of algae to respond to short-term nutrient additions (Sakamoto, 1971; Kalff and Welch, 1974).

Although it appears that in vitro measurements of phosphate uptake underestimate the actual V_{max} of C. erosa in the chemostats, estimates of K_s were not changed during incubation and appear to be independent of the maximum uptake velocity. Our estimated K_s of approximately 0.14 µM P therefore seems meaningful and we conclude that such a low K_s allows the species to survive at low concentrations of phosphate. Half-saturation constants have been used to assess the relative abilities of phytoplankton to compete for nutrients (Dugdale, 1967). Data collected on K_s for P uptake of freshwater algae (Healey, 1973a) reveal significant interspecific differences in K_s and therefore in nutrient uptake capabilities. The K_s for nitrate uptake by marine algae also show species differences (Eppley et al., 1969; Carpenter and Guillard, 1971). In comparison to the K_s of

green, blue-green and diatom species studied to date (0.58-16 µM P), C. erosa is characterized by a transport system of unusually high affinity for phosphate. Whether other nanoplankton characteristic of oligotrophic freshwaters also have low K_s, and thus a highly developed ability to acquire phosphate from dilute solutions, remains unknown. Although most studies show K_s not to vary as a function of nutrient limitation, Fuhs et al. (1972) report a decreased affinity of algae to P under nutrient sufficient conditions, while Healey (1973b) found K_s to vary with the concentration of Mg ions. In addition Hagen et al (1957) and Müller (1972) found evidence of biphasic (double K_s) P uptake in higher plant roots and in a diatom, respectively, although the break between the first and second absorption isotherms in the diatom was at phosphate concentrations considerably above those found in nature. The P uptake kinetics of C. erosa, following an increase in phosphate in the chemostats (Table 4), was not analyzable by Michaelis-Menten kinetics. However, the greatly increased capacity at this time for P uptake is probably attributable to the operation, at <50 µg P l^{-1} concentrations, of a second higher K_s system. The ability of algae in nature to respond to different nutrient environments with K_s variability is likely more common than recognized today.

Culture studies show that rather than through K_s variation algae more characteristically respond to change in nutrient conditions by varying their uptake capacity (V_{max}) (Caperon and Meyer, 1972b; Eppley and Renger, 1974). The V_{max} is commonly maximal under conditions of severe nutrient depletion and, according to Caperon and Meyer, reflects an increased concentration of transport enzymes (permeases) at the cell surface. The apparent disadvantage of a species with a low affinity (high K_s) system for

growth at low substrate concentrations can thus be offset by increasing the number of uptake sites. In culture high K_s species normally do have high V_{max} and grow as well as low K_s species at low nutrient concentrations (Fuhs et al., 1972; Button et al., 1973). However, the maintenance of turbulent flow in laboratory cultures probably overestimates significantly the V_{max} attainable in nature (Fuhs et al., 1972). Furthermore, the synthesis of various transport enzymes are potentially in competition with one another for energy and materials. In rich culture media deficient in but one element, a cell can readily maximize production of enzyme specific for that limiting substrate. However, in nature the simultaneously low concentration of many essential nutrients probably limits the energy and materials available for the production of any one permease, so that the high V_{max} measured in the laboratory are not realized by natural populations. In addition, the overestimation of PO_4-P by current chemical methods will further result in a significant overestimation of the uptake rates possible in nature.

Aspects of the Ecology of Cryptomonads

Cryptomonads are members of a nanoplanktonic and largely motile flora which includes many chrysophytes and dinoflagellates that at all times dominate the plankton of oligotrophic lakes (Nauwerck, 1968; Pechlaner, 1971; Kalff et al, 1975). They also characterize the winter and early spring plankton of meso- and eutrophic lakes, but typically form a less significant percentage of the biomass during the summer and fall with the appearance of netplanktonic diatoms,

greens and blue-greens (Pavoni, 1963; Gelin, 1971), even though the total flagellate biomass may be no lower than during the spring. Indeed, in some eutrophic and even polluted lakes phytoflagellates form a high percentage of the summer biomass (Tuunainen et al., 1972; Ilmavirta et al., 1974). They thus thrive in lakes of all trophy and are a cosmopolitan, apparently physiologically plastic group.

The particular attributes which allow flagellates to survive in high mountain and polar lakes include motility and/or small size and the ability to grow under low temperature and low nutrient conditions. Motility allows the algae to remain within a stable water column during long periods of ice cover and to control, to some extent, the light flux received. Motility also continuously renews the nutrient supply at the active surface of the cell which is advantageous for growth at low nutrient concentrations (Pasciak and Gavis, 1974).

Our study shows that growth of the typical freshwater oligotroph, C. erosa, at low phosphate concentrations is facilitated by a high affinity transport system. Studies of marine algae in culture have similarly shown that species characteristic of oligotrophic oceanic regions have lower K_s for nitrate and silicate uptake than phytoplankton of eutrophic coastal waters (Eppley et al., 1969; Guillard et al., 1973). Because austere environments apparently select for low K_s species, the energetic cost of maintaining an inefficient high enzyme system (high V_{max}) maybe prohibitive in continuously nutrient poor environments. However, smaller species that thrive in oligotrophic lakes need not have as low a K_s as relatively large C. erosa, since

small cells can offset a higher K_s with a more favourable surface to volume ratio, lower absolute P requirement, and a higher V_{max} per unit surface area (Fuhs et al., 1972). Nevertheless, the range of K_s of characteristically oligotrophic species is most probably lower than that of more eutrophic forms, even though Perry (1976) reported that two oceanic clones of a marine diatom had K_s values for phosphate no lower than that for a neritic clone. Further work on the role of the K_s and V_{max} in algal succession is presently in progress in our laboratory.

The most obvious reason why flagellates and small non-motile cells dominate both the flora of oligotrophic lakes and the winter plankton of eutrophic lakes is the ability to remain in suspension under ice cover. Much less apparent are the factors which cause nanoplankton to be replaced by net plankton during the summer. Evidence obtained by selective filtration of the algal community through membrane filters and screens of different porosity shows that even when net plankton comprise a larger percentage of the biomass, small forms are more efficient primary producers and are responsible for the largest fraction of the photosynthate produced (Malone, 1971; Kalff, 1972). Direct evidence for the high renewal rates of nanoplankton in a naturally eutrophic lake was recently obtained through autoradiography by Knoechel (pers. comm.) who observed doubling times of Rhodomonas minuta (Crytophyceae) of approximately one per day, at a time when the population did not increase. His results suggest that nanoplankton are growing rapidly in eutrophic lakes, but fail to attain large numbers because of a high loss of cells, almost certainly attributable to a high susceptibility to grazing by zooplankton,

whereas the biomass of netplankton is more subject to sedimentation (Knoechel, 1976). In oligotrophic lakes phytoplankton renewal rates are normally far in excess of zooplankton grazing rates, whereas in eutrophic lakes the two rates are comparable (Haney, 1973). The greater grazing pressure in eutrophic lakes probably results not only from higher zooplankton numbers, but also from a shift in dominance with increasing trophy from predacious copepods to filter-feeding cladocerans (Patalas, 1972). We therefore suggest that the absolute abundance of small algae in the summer waters of eutrophic lakes is significantly limited by the numbers and kinds of zooplankton present.

Considering the highly favourable surface/volume ratio of small forms and their low absolute P requirements, it is apparent that with similar values of K_s, nanoplankton will outcompete netplankton at low naturally occurring concentrations of P. In relatively large C. erosa the disadvantage of a low maximum uptake rate per unit surface area is offset by a high affinity K_s system, such that the species can compete successfully for P in both oligotrophic and eutrophic lakes. Although the availability of kinetic constants will provide insight into the relationship between algal growth and nutrient concentrations, models of phytoplankton succession in nature will be of low predictive power without an equal consideration of sedimentation and zooplankton grazing.

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Part III

The effect of growth environment on the photosynthetic response of laboratory cultures.

ABSTRACT

The relationship of photosynthesis to light intensity is examined in the freshwater algal flagellate Cryptomonas erosa Skuja (Cryptophyceae) grown under different light/temperature regimes in batch culture and under phosphate deficiency in chemostat culture. Batch cells grown at low light and temperature have lowered rates of maximal carbon uptake (P_{max}) and are saturated and inhibited at low light intensities (low I_k and I_I). Similar results were obtained for chemostat-grown C. erosa severely deficient in phosphorus. The observed variation in photosynthetic capacity occurred without any accompanying major change in the rate/intensity characteristic at low light levels or in the cell content of chlorophyll a. Thus under a suboptimal environment, the principal stage of photosynthesis affected is the rate of the dark enzyme reactions. It is suggested that at low temperatures the P_{max} is reduced because of a decrease in the rate constant of the dark reactions, whereas at low light or under P-deficiency, dark enzymes are selectively lost from the cell. The data are discussed in relation to light adaptation of algae in nature, and in particular, to the photosynthetic response of cryptomonads and other motile species in cold oligotrophic and in eutrophic lakes.

INTRODUCTION

The photosynthetic response of algae is controlled in a complex manner by light intensity, temperature and nutrients. This complexity on which, in nature, is superimposed a dynamic flux, obstructs ready attempts to establish the relationship between the environment and rates of primary production. Such relationships are, however, more easily defined under controlled laboratory conditions, and culture studies usefully supplement investigations of primary production in natural waters. The present study examines the photosynthetic response of the common freshwater flagellate Cryptomonas erosa Skuja (Cryptophyceae) which is a member of a motile, largely nanoplanktonic group of algae which, besides other cryptomonads, includes many chrysophytes and dinoflagellates. Although these algae dominate the plankton at all times in oligotrophic lakes (Pavoni, 1963; Pechlaner, 1971) and contribute a significant percentage of the production in many eutrophic lakes (Kalff, 1972; Granberg, 1973), they have received little attention in culture studies and their physiological ecology is poorly known. Yet such information is needed for an understanding of the range of adaptive mechanisms of algae, which in turn is needed for an improved understanding of the phytoplankton dynamics of lakes.

The photosynthetic reaction can be separated into two components: the photochemical processes governed by the light absorbing mechanism, and the enzymatic processes governed by the activity of the photosynthetic enzymes (Steemann Nielsen and Jørgensen, 1968). Since these two components are semi-independent, their rates provide clues to the response of the

photosynthetic system to environmental factors. The examination of photosynthesis-light curves (P vs I curves) provides a means of obtaining the relative rates of the two processes. Such curves consist of a light-limited region, the slope of which is a measure of the rate of the primary photochemical reactions, and a light-saturated (plateau) region which is the maximum rate of photosynthesis (P_{max}) and is limited by the rate of the dark reactions. A third, the photoinhibition region, is also observed in many studies.

We have examined the photosynthesis-light curves of C. erosa cultured under a variety of light and temperature conditions in nutrient-sufficient batch cultures and under various degrees of phosphate deficiency in chemostat cultures. In particular we want to examine variation in the photosynthetic capacity of the alga in relation to the light, temperature and nutrient regimes imposed and to discuss the possible mechanisms involved.

METHODS

Cryptomonas erosa Skuja was isolated into axenic culture from a naturally eutrophic lake near Montreal, Quebec, Canada. The algae were grown in batch culture in 500 ml reagent bottles, containing 300 ml of a relatively dilute mineral salts medium (conductivity = 240 μmhos at 25°), with the addition of 3 vitamins (Part I). The cultures were subject to a regime of continuous cool-white fluorescent illumination over a range of light intensities at 23.5, 15, 4 and 1°. Light intensity at the base of the culture bottles was measured with a photocell calibrated against

a quantum radiometer (Lamda Instruments) so that light units are presented in ly min^{-1} (cal cm^{-2} min^{-1}) of photosynthetically available radiation (PAR), where .01 ly min^{-1} PAR ≃ 230 ft. c. CW illumination ≃ 32 microEinsteins m^{-2} sec^{-1} (Appendix A).

The chemostat units have been described previously (Part II) and consist essentially of 2 l round bottom flasks with a sampling port and an overflow tube to maintain a constant culture volume. The units were equipped with a "sweep-stirrer" fitted to the inside curvature of the vessel wall to remove wall growth. The medium was fed through a peristaltic pump, and contained either 80 or 14 µg P l^{-1}. The cultures were maintained at 15° and were continuously illuminated by cool-white fluorescent lamps located at the rear of the growth vessels. The light intensity at the chemostat centers was calculated from the extinction coefficient of light through the cultures and, depending on cell density, ranged between 9.0 and 11.2 x 10^{-3} ly min^{-1}. By varying the dilution rate in the growth vessels, the algae were grown under various degrees of phosphorus limitation at growth rates of 0.11 to 0.58 cell div. day^{-1}.

At a number of growth conditions in both batch and chemostat cultures, the photosynthetic response of the algae was examined over a range of light intensities. For this purpose samples of log-growth cells were removed from culture and $NaH^{14}CO_3$ added at .125-.25 µCi ml^{-1}. 5-10 ml aliquots were then placed in erlenmeyer flasks and incubated at various light intensities in a blackened light-gradient box. After 1-6 hours of incubation, the samples were filtered live at ≃50mm Hg on 0.45 µm membrane filters, washed with a small volume of sterile medium

and filter activity assayed in a windowed GM counter of known efficiency. Dark counts were subtracted and the 6% isotope factor applied. At the time of incubation, cell density was determined by microscope counts in a Palmer-Howard counting cell. In addition, chlorophyll a concentration was measured by in vivo fluorescence on a Turner model III fluorometer (Lorenzen, 1966), which was calibrated by spectrophotometry, using acetone-extracted material and the trichromatic equation of Strickland and Parsons (1968) (Appendix B).

The carbon uptake data are expressed on a per cell basis in the form of photosynthesis-light curves. Regression lines have been fitted ($p \leq 0.05$) to the points to describe each curve at subsaturating and inhibitory light levels, while carbon uptake values along the saturation plateau were averaged to give a mean maximum photosynthetic rate (P_{max}). The lines have been extended to intersect at I_k and I_i, the light intensities at onset of saturation and inhibition, respectively.

RESULTS

The photosynthesis-light intensity curves of the batch and chemostat cultures are shown in Figs. 1 and 2, respectively, and the results summarized in Table 1. The P-I curves conform to the typical pattern of a linear increase in carbon uptake with increasing light at subsaturating intensities, followed by light saturation and ultimately, at still higher intensities, by photoinhibition. The P-I relationship in the batch and chemostat cultures are strikingly similar. The most characteristic feature of the curves is that in all experiments but one, the initial slopes are little

Fig. 1. The photosynthesis-light response of C. erosa grown under various temperature/light conditions (°C/ly $min^{-1} \times 10^{-3}$) in batch culture. 1. 23.5°/21.5. 2. 23.5°/2.0. 3. 15°/8.6. 4. 15°/1.0. 5. 4°/5.6. 6. 4°/1.0.

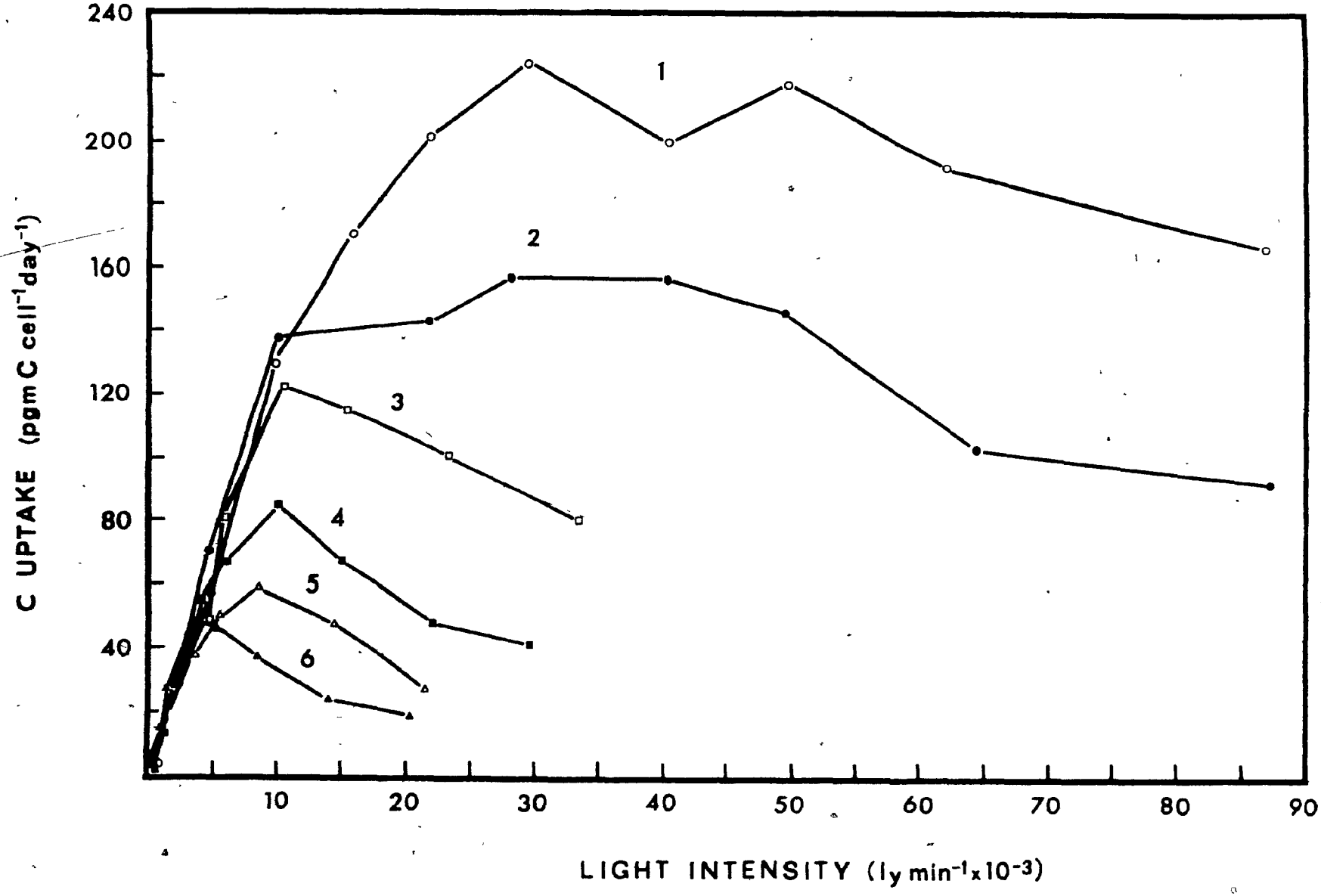

240
200
160
120
80
40
C UPTAKE (pgm C cell^{-1}day^{-1})
1
2
3
4
5
6
10
20
30
40
50
60
70
80
90
LIGHT INTENSITY (ly min^{-1}x10^{-3})

Fig. 2. The photosynthesis-light response of C. erosa grown under various degrees of P-limitation in chemostat culture. Steady state growth rate (div. day^{-1}): 1 - 0.55; 2 - 0.39; 3 - 0.23; 4 - 0.14; 5 - 0.11.

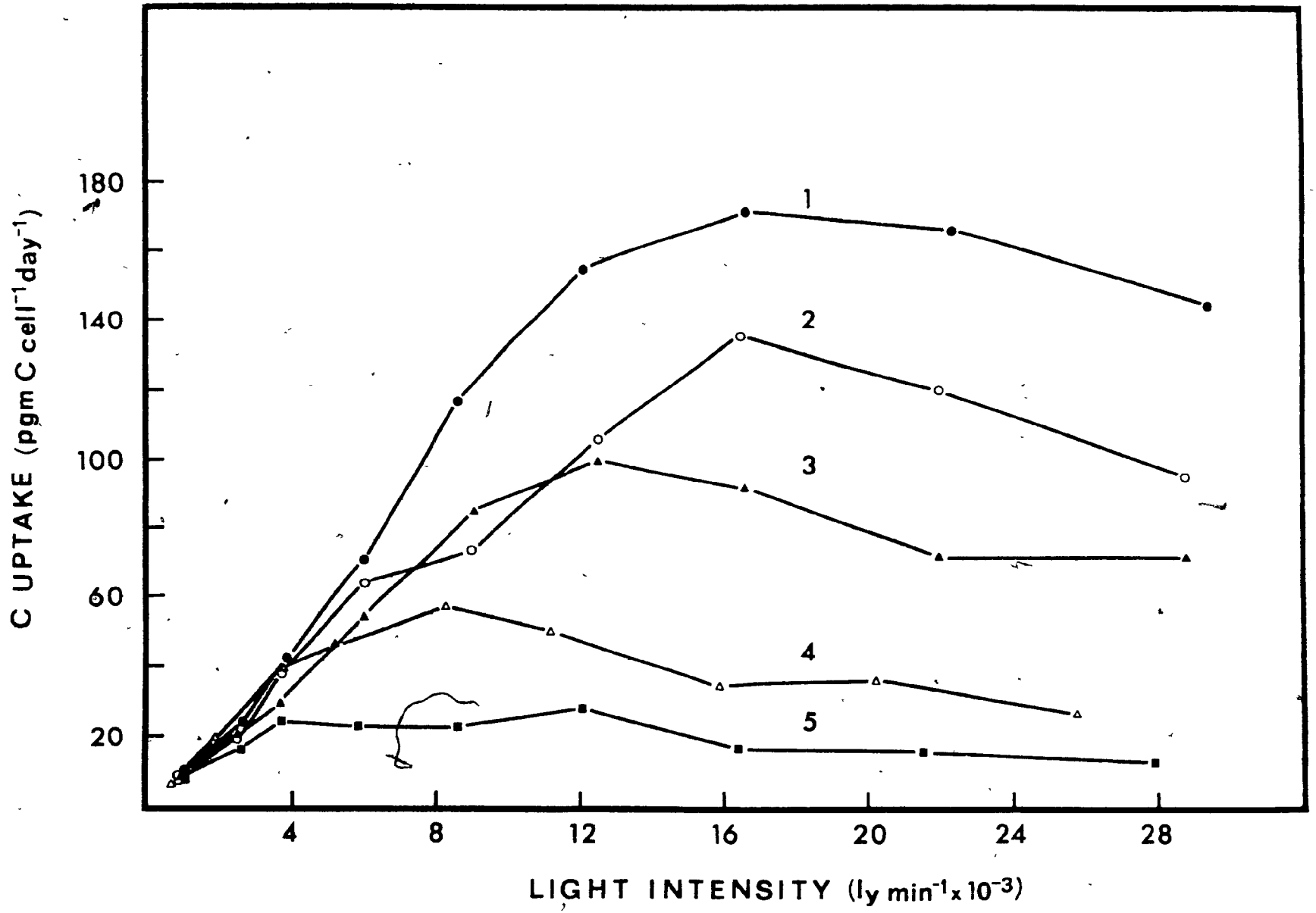

C UPTAKE (pgm C cell^{-1}day^{-1})
180
140
100
60
20
1
2
3
4
5
4
8
12
16
20
24
28
LIGHT INTENSITY (ly min^{-1}x 10^{-3})

Table 1. Summary of photosynthesis-light curves in batch and chemostat cultures

Growth conditions	Initial slope (rate/ly min^{-1})	P_{max} (pgm C $cell^{-1}$ day^{-1})	I_k (ly min^{-1} × 10^{-3})	I_i (ly min^{-1} × 10^{-3})	Inhibitory slope (rate/ly.min^{-1})
A. - BATCH CULTURE					
Temp. (°C) / Light intensity (ly min^{-1} × 10^{-3})					
(1) 23.5 / 21.5	13766X - 5.7	212	16.2	46.5	-1178X + 267
(2) 23.5 / 2.0	14031X - 4.0	157	11.6	36.3	-972X + 191
(3) 15 / 8.6	13116X - 4.2	121	9.5	16.2	-2166X + 153
(4) 15 / 1.0	15058X - 3.4	85	5.8	8.4	-2275X + 104
(5) 4 / 5.6	10673X + 1.0	59	4.7	9.0	-2459X + 82
(6) 4 / 1.0	13976X + 2.9	47	3.7	4.0	-1799X + 54
B. - CHEMOSTAT CULTURE					
Cell division rate (div. day^{-1})					
(1) 0.55	13865X - 9.4	165	12.6	21.9	-2951X + 233
(2) 0.39	11204X - 4.5	136	12.7	16.6	-3295X + 191
(3) 0.23	9457X - 2.2	101	11.0	13.9	-1804X + 120
(4) 0.14	10581X - 0.5	59	5.3	6.0	-1715X + 70
(5) 0.11	5827X + 2.3	25	3.8	12.5	-860X + 35

Regressions for initial and inhibitory slopes are significant at $P < .05$.

Table 2. Chlorophyll a content and P_{max} per unit chlorophyll in batch and chemostat cultures

	Growth conditions		Chl-a (pgm cell^{-1})	$P_{max/chl-a}$ ($\frac{\text{pgm C hr}^{-1}}{\text{pgm chl-a}}$)
	A. - BATCH CULTURE			
	Temp. (°C)	Light intensity (ly min^{-1} × 10^{-3})		
(1)	23.5	21.5	4.1	2.16
(2)	23.5	2.0	4.0	1.64
(3)	15	8.6	5.5	0.91
(4)	15	1.0	5.4	0.65
(5)	4	5.6	2.8	0.87
(6)	4	1.0	5.3	0.37
	B. - CHEMOSTAT CULTURE			
	Cell division rate (div. day^{-1})			
(1)	0.55		4.5	1.53
(2)	0.39		5.6	1.01
(3)	0.23		6.6	0.64
(4)	0.14		5.1	0.48
(5)	0.11		5.3	0.19

different, varying over only a 1.6 fold range, with no consistent pattern apparent. The one exception is a lowered initial slope for the chemostat population grown under the greatest P deficiency (μ = 0.11 cell div. day^{-1}), and even here the decrease is < 50% of the mean for all other slopes. Thus the rate of the photochemical reactions is largely independent of the temperature, light intensity or nutrient conditions under which the cells were grown.

In contrast, the maximum photosynthetic capacity (P_{max}) and the values of I_k and I_i vary markedly with different growth conditions (Table 1; Figs. 1 and 2). In nutrient-rich batch cultures there is a consistent decrease in P_{max} with decreasing temperature, characterized by an average temperature coefficient (Q_{10}) of 1.82 . In addition, at each temperature, cells grown under light-limiting conditions have a lower ($\bar{x}$25%) P_{max} than cells grown at light-saturation. At a constant temperature (15°) and light intensities which vary only 1.2 fold, the P_{max} of chemostat populations decreased with increasing phosphate deficiency. Thus at higher rates of P supply, the P_{max} was at least as great as that for nutrient-rich batch cells grown at similar light intensities and temperature, while under severe P deficiency the P_{max} was lowered 2.5-4.8 fold. Therefore, the dark reactions of photosynthesis as measured by P_{max} are, in contrast to the photochemical reactions, strongly affected by the growth environment.

Since the initial slopes of the P-I curves change very little, the light intensity at onset of saturation (I_k) is determined primarily by variation in the maximum photosynthetic rate at light saturation. When

P_{max} decreases with decreasing light and temperature the value of I_k in the batch cultures also decreases. In a similar fashion, I_k decreases with increasing P deficiency in the chemostat cultures. Thus both low light/low temperature cells and P-starved cells are saturated at much reduced light intensities ($3.7\text{-}5.3 \times 10^{-3}$ ly min^{-1}) (Table 1).

The data also show that at high light and temperature (23.5°) in batch culture, maximal carbon uptake is maintained over a broad range of light intensities, with only slight photoinhibition (0.80 P_{max}) at the highest light level examined. At a somewhat lower temperature (15°) in the chemostats, carbon uptake of cells at higher rates of P supply is also inhibited only at relatively high light levels, approximately the same as those observed in P-rich batch cultures at 15°. However, with a further decrease in light and temperature in batch cultures, the light intensity at onset of photoinhibition (I_i) decreases, with I_i approaching I_k. This approach is nearly complete at the lowest light and temperature when I_i is approximately equal to I_k, and little or no saturation plateau remains (Fig. 1; Table 1). The P-I curve is then pyramid-shaped, described simply by a light limitation and light inhibition slope. Light intensities greater than I_i result in severe photoinhibition with carbon uptake reduced to 0.4-0.5 P_{max} at light intensities of only 0.02 ly min^{-1}. In chemostat cultures a low I_i was also observed in two of three severely P deficient populations with a distinct pyramid-shaped curve in one (Table 1; Fig. 2). The reason for the high value of I_i in the one experiment remains unclear. Thus in most experiments the photosynthetic response of C. erosa grown at suboptimal conditions is

characterized by reduced rates of maximal carbon uptake and by saturation and photoinhibition at low light intensities.

The chlorophyll a content of the cells is shown in Table 2. Although cell pigment increased in batch cultures grown at near compensatory light intensities and decreased under inhibitory light levels (Part I), chlorophyll $cell^{-1}$ in those populations examined by P-I curves varied, with one exception, only between 4.0 and 5.5 pgm $cell^{-1}$. Similarly the pigment content of chemostat cells was unaffected by the degree of phosphate deficiency (Part II). Thus the photosynthetic rate expressed per unit chlorophyll a varies widely between 0.19 and 2.16 pgm C pgm chl-a^{-1} hr^{-1} (Table 2), and the P_{max} is therefore not proportional to the chlorophyll a content of the cells. Only at low subsaturating light intensities was carbon uptake roughly proportional to the product of light intensity and pigment.

DISCUSSION

The photosynthesis-light curves show that the light-saturated rate of carbon uptake (P_{max}) in C. erosa varies considerably under different growth conditions, whereas the photosynthetic rate at low subsaturating light levels is little affected. All these changes in photosynthetic capacity occur without any accompanying change in the chlorophyll a content of the cells. Because the rate/intensity characteristic at low light levels is little changed, the possibility of some form of "chlorophyll inactivation" (Steemann Nielsen and Jørgensen, 1968) can be ruled out and, instead, the results indicate that change in photosynthetic

capacity is largely controlled by the dark reactions of photosynthesis. Although in some studies the light reactions are significantly affected by the growth conditions (Steemann Nielson and Jørgensen, 1968; Jørgensen, 1970), our results are consistent with previous findings that the dark reactions are usually affected to a greater degree in algae grown at suboptimal temperatures (Tamiya et al., 1953; Aruga, 1965; Talling, 1966), low light or dark conditions (Jørgensen, 1964; Yentsch and Lee, 1966) or under nutrient deficiency (Rabinovitch, 1951; Knoechel, 1976).

The maximum rate of the dark reactions is controlled by the environmental temperature and the cellular concentration of dark enzymes (Steemann Nielson and Jørgensen, 1968), which in turn is a function of the growth environment. We interpret the observed decrease in P_{max} with decreasing temperature (Fig. 1) as a reduction in the rate constant of the enzymatic reactions, because a smaller fraction of the enzyme molecules have the necessary activation energy to react. In contrast, the lowered P_{max} of light-limited cells or of extremely P-deficient cells can be interpreted as an inability of the algae to maintain a sufficiently high concentration of dark enzymes. Yentsch and Lee (1966) and Griffiths (1973) have previously explained, in this way, a lowered P_{max} of low light and dark cells, respectively, while Hellebust and Terborgh (1967) and Morris and Farrell (1971) have related changes in the photosynthetic capacity to the concentration of the dark enzyme ribulose diphosphate carboxylase. Our results also show that C. erosa grown at low temperatures or under extreme P deficiency not only have a lower P_{max}, but are saturated at lower light intensities (low I_k) (Table 1).

This was expected, since with the postulated decrease in the rate constant of the enzyme reactions or with the presence of fewer enzyme molecules, even very low light intensities saturate the dark reactions and shift the I_k to lower values.

Low temperature and P-deficient cells are not only saturated at low light intensities, but in most cases, also suffer photoinhibition at low quantum flux (low I_i). In two experiments the algae became saturated and inhibited at virtually the same light intensity and the plateau of the P-I curve disappeared, as has also been observed in nature (Knoechel, 1976). The nature of photoinhibition in C. erosa was explored in other experiments where carbon uptake of batch cells was compared by the techniques of standard membrane filtration and acidification and bubbling (Schindler et al., 1972) to yield, by difference, a measure of carbon excretion. It was found that the proportion of photoassimilated carbon released from the cells was greatest under photoinhibitory light levels associated with low temperature (Part I). These results indicate that inhibition of photosynthesis in the P-I curves largely results from increased carbon excretion at high light intensities. Other culture studies have shown that under conditions of high light intensity and low CO_2 concentration, glycollic acid is often the major extracellular product as part of the phenomenon of photorespiration (Tolbert, in Stewart, 1974). However, the low light levels at which C. erosa is inhibited and, in particular, the association of low I_i with low P_{max} (Table 1) are inconsistent with the high light intensities and high rates of photosynthesis required for the induction of photorespiration (Brown and Weis, 1959; Knoechel, 1976). Although photorespiration cannot be totally precluded in our experiments, the production

of a copious mucilage by C. erosa grown under inhibitory light conditions (Part I) suggests that the photoinhibition function is primarily the result of increased excretion of polysaccharides. The amounts of polysaccharides so released can represent up to 90% of the photoassimilated carbon in some algae (Hellebust, in Stewart, 1974).

Because the dark reactions of photosynthesis are located in the chloroplast lamellae, one might assume that change in the photosynthetic capacity would be closely tied to the chlorophyll content of the cell. Such a relationship has been found in several studies even when photosynthesis is reduced by nutrient deficiencies (McAllister et al., 1964; Eppley and Renger, 1974). Moreover, Eppley and Sloan (1966) obtained a good correlation between growth and light absorption of chlorophyll in several species of marine algae. However, it is apparent that in P-deficient C. erosa the photosynthetic capacity can be reduced to very low levels, without a corresponding decrease in chlorophyll a (Table 2), similar to results obtained by Griffiths (1973) for a marine dinoflagellate incubated at high temperatures in the dark. Similarly Thomas and Dodson (1968) and Jørgensen (1970) found wide variation in the assimilation numbers of N and P deficient cultures, respectively, while Yentsch and Lee (1966) reported a 20 fold range in P_{max}/unit chlorophyll of natural and laboratory populations. The results indicate that in C. erosa, as well as in other algae, dark enzymes are selectively lost from the chloroplast lamellae and that chlorophyll is therefore not a good indicator of the activity of the dark enzymes. The apparent species differences in this relationship make hazardous predictions of algal growth based on assimilation

numbers alone.

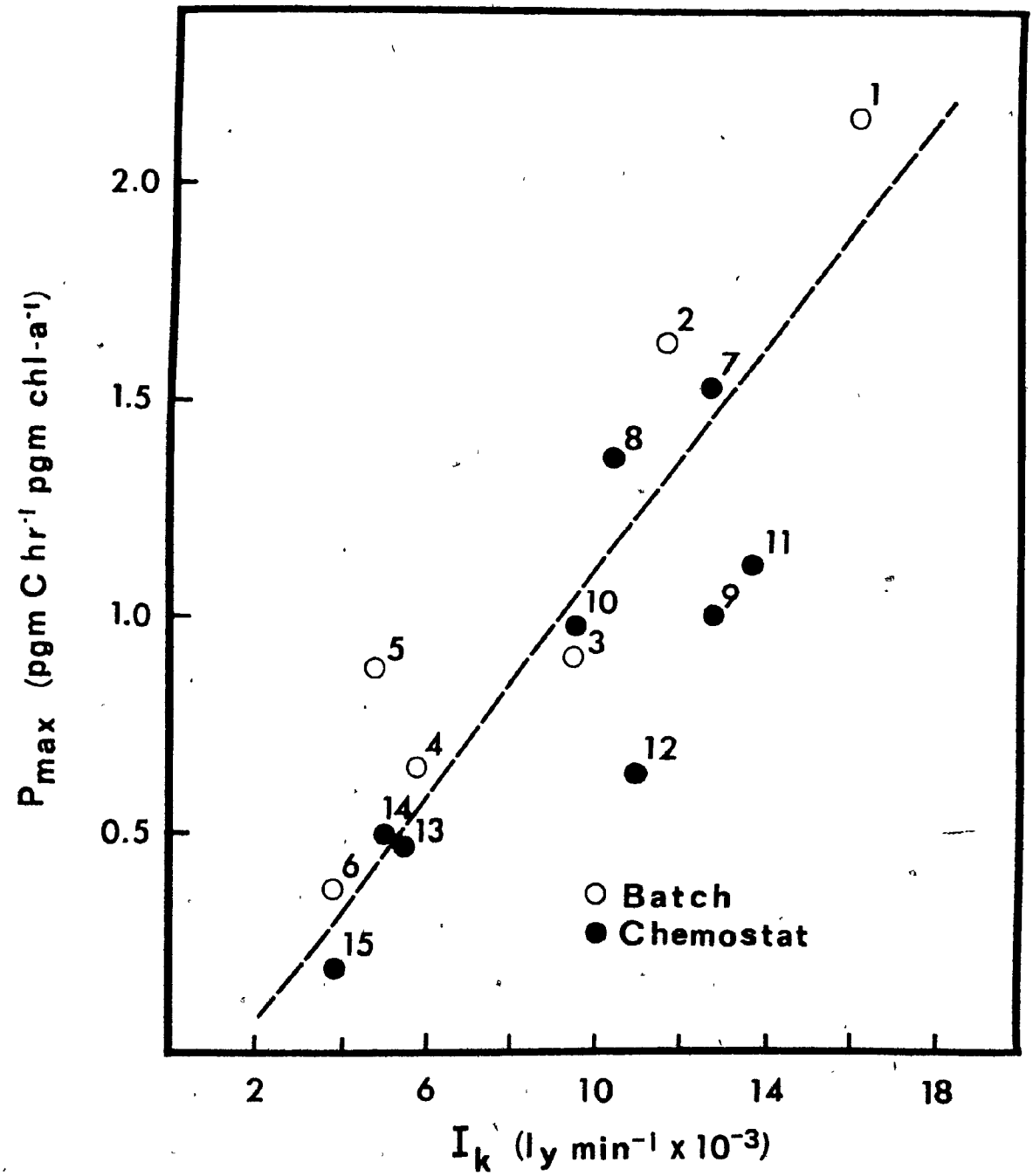
Some algae can adapt extensively to different light regimes by varying their pigment content (Steemann Nielsen and Jørgensen, 1962; Jørgensen, 1964), while other species offset a temperature-dependent decrease in dark enzyme function by the synthesis of more enzymes (Morris and Farrell, 1971). These studies show that changes in I_k and P_{max} characterize cells that are better adapted physiologically to their environment. Our data show that low I_k and P_{max} are found in cells grown under low light/temperature and nutrient deficient conditions (Fig. 3) and therefore characterize a photosynthetic response to a physiologically inferior environment. Thus in contrast to some algae, C. erosa, at least in culture, is unable to extensively adjust its photosynthetic response to stressed conditions. Our results support the conclusion of Yentsch and Lee (1966), based on data collected for cultured and natural populations, that low I_k and P_{max} are normally indicative not of physiological adaptation but of physiological stress.

In nutrient-rich batch cultures of C. erosa, light and temperature determine the photosynthetic response of the cells, whereas in continuous-flow cultures the effect of P limitation is readily manifest (Figs. 1 and 2). In nature, too, a chronic shortage of essential nutrients for growth must be of overriding importance. However, evidence available in the literature is conflicting. Data collected by Yentsch and Lee (1966) and the observations of Talling (1966) and Gelin (1975) show that temperature and/or light significantly affect the P_{max} and I_k of natural populations.

Fig. 3. The relationship between I_k and the maximum rate of photosynthesis per unit chlorophyll. The dashed line is fitted by eye.

Legend: batch populations (°C/ly $min^{-1} \times 10^{-3}$): 1 - 23.5°/21.5; 2 - 23.5°/2.0; 3 - 15°/8.6; 4 - 15°/1.0; 5 - 4°/5.6; 6 - 4°/1.0.

chemostat populations (cell div. day^{-1}): 7,8 - 0.55; 9 - 0.39; 10,11 - 0.29; 12 - 0.23; 13,14 - 0.14; 15 - 0.11.


2.0
1.5
1.0
0.5
P_{max} (pgm C hr^{-1} pgm chl-a^{-1})
2
6
10
14
18
I_k (ly min^{-1} x 10^{-3})
1
2
7
8
11
9
10
3
5
4
12
14
13
6
15
Batch
Chemostat

Similarly Ichimura et al. (1968) found that in a Japanese lake a diatom population at 2 m and 22.6° was saturated at a light flux twice that of a cryptomonad population at 6 m and 12.8°. On the other hand, Lewis (1974) found that under intense illumination in a tropical lake, highest P_{max} occurred only at times of increased nutrient availability. A recent autoradiographical study of species specific carbon uptake in freshwater diatoms (Knoechel, 1976) also reports little change in I_k and P_{max} over a 6-25° temperature range. P_{max} was, however, relatable to the surmised input of nutrients following rain events. In north-temperate zone lakes the June-August period is one of maximal light and temperature and, based on the high P_{max} recorded in batch cultures under such conditions, one might expect that algal populations would wax prolifically. Yet there are many examples of decreased production during mid-summer stratification (Round, 1971). In contrast, despite generally lower light/temperature conditions, increased production occurs when nutrients are returned to the open waters at the spring and fall turnovers, which together with the abundant data now linking eutrophication with high phytoplankton production, indicate that the photosynthetic response of natural populations is largely nutrient-dependent.

The basic light response parameters are the light-limitation and inhibition slopes and the P_{max}. In C. erosa the light-limited slope is little affected by the growth environment, and carbon uptake at higher light intensities is essentially dependent on the magnitude of the P_{max} and the slope of the inhibition function, as has been found for some diatom populations in nature (Knoechel, 1976). Batch cells grown under optimal conditions can maintain a high P_{max} at high light intensities, while P-deficient cells are markedly inhibited. In nature a chronic P-

limitation in most lakes must also result in severe inhibition of carbon uptake in brightly-lit surface waters. The commonly reported movement of flagellates away from the surface is thus an important adaptive mechanism to bring the algae into a zone of more optimal light which permits increased carbon uptake. Moreover those flagellates passing into more nutrient-rich deeper waters have, because of a now increased P_{max}, a growth advantage over non-motile species.

Due to the scarcity of light under the winter snow and ice cover of temperate and arctic lakes, cryptomonads and other flagellates form a surface biomass maximum (e.g. Wright, 1964). At the spring melt, there is considerable evidence that this extremely shade-adapted community is severely stressed by the sudden increase in light (Pechlaner, 1971; Kalff and Welch, 1974). One can postulate that at this time the photosynthetic response of the algae is pyramidal in shape, with the P_{max} at a sharply defined optimal light intensity. Support for this premise is provided by Kalff and Welch who found that under rising light levels in spring in an arctic lake, the depth of maximum photosynthesis (nP_{max}) gradually moved from the surface to deep waters and thus remained associated with a particular optimum light intensity. In other cold oligotrophic lakes there is evidence that motile species react to light stress by a general downward migration (e.g. Pechlaner, 1971), which in combination with diel movements (Tilzer, 1974; Kalff and Welch, 1974) maintains the cells in a zone of optimal light and, presumably, maximal carbon uptake. During the summer a general physiological adaptation to higher light occurs that has been ascribed to the presence of higher nutrient levels in deep waters or to the increased nutrient availability concomitant

with the mid-summer turnover (Findenegg, 1967; Pechlaner, 1971; Kalff and Welch, 1974). Our chemostat studies of C. erosa confirm this nutrient-dependent adaptation to higher light intensities and further indicate that such adaptation is largely dependent on an increased synthesis of dark enzymes.

Some species such as Chlorella are able to adapt extensively to different light intensities by mainly varying the pigment content per cell (Steemann Nielson and Jørgensen, 1968). Other species such as certain diatoms (Jørgensen, 1964) and, in the present study, C. erosa, are able to adapt less extensively and do so primarily by change in the cellular content of dark enzymes. Both adaptive mechanisms involve a matching of the maximum rate of the enzymatic processes to the rates of the photochemical processes and both promote algal survival over a range of growth conditions. Motility adds a further dimension to light adaptation and relieves, at least partly, the necessity of extensive physiological change. Motility thus promotes maximal population success and, in conjunction with physiological changes in enzyme and pigment content, accounts for the cosmopolitan distribution of C. erosa and other phytoflagellates.

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Part IV

A mechanism for dark survival.

ABSTRACT

Changes in cell number and carbon content of the freshwater algal flagellate Cryptomonas erosa Skuja (Cryptophyceae) were followed in complete darkness. Cells incubated in the dark at high temperatures rapidly disappeared from culture, whereas those at low temperatures survived up to 80 days of darkness. Radioactive tracer experiments with organic substrates and growth of the algae in association with bacteria showed that heterotrophic metabolism by C. erosa was negligible. A lengthy dark survival was instead dependent on the slow respiration of stored carbohydrate. The respiration of carbon reserves is suggested as the primary mode for survival of cryptomonads during the long winter darkness in polar lakes.

INTRODUCTION

A number of recent studies have documented the importance of the nanoplankton both in terms of phytoplankton standing crop and primary production in marine (Malone, 1971; Watt, 1971; McCarthy et al., 1974) and freshwaters (Rodhe et al., 1958; Pavoni, 1963; Kalff, 1972). However, culture studies of freshwater algae have dealt almost exclusively with either Chlorella-type algae which, in many respects, are atypical of the natural plankton, or with net planktonic diatoms, green and blue-greens so common in eutrophic lakes. The present study investigates the physiological ecology of the common freshwater flagellate Cryptomonas erosa Skuja (Class Cryptophyceae) which is a member of a nanoplanktonic and largely motile flora that includes, besides other cryptomonads, many chrysomonads and dinoflagellates, as well as a few small diatoms and green species. These algae dominate the plankton of oligotrophic lakes (Nauwerck, 1968; Pechlaner, 1971; Kalff et al., 1975), and also contribute a significant percentage of the primary production in many eutrophic lakes (Gelin, 1971; Kalff, 1972; Granberg, 1973). However, the culture of these algae has been largely ignored, and their ecology is poorly known. Yet, without such information, it is impossible to fully understand the phytoplankton dynamics of lakes, and the range of adaptive mechanisms in algae.

The physiological ecology of C. erosa has been studied under a variety of light-temperature regimes in batch culture (Part I), under various degrees of phosphorus limitation in chemostat

culture (Part II), and its photosynthetic response related to change in the growth environment (Part III). In the present report we examine the response of the alga to incubation in the dark.

Cryptomonads are present in the water column throughout the year in many arctic, high mountain and north temperate lakes. In winter large non-motile diatoms and blue-greens rapidly sediment under ice cover and over-winter as resting spores or cysts. However, cryptomonads, together with other flagellates and small non-motile species, remain within the water column and over-winter in the vegetative state. Under the snow and ice cover in temperate and mountain lakes, algae in surface waters still receive, at least at intervals, sufficient radiation for a measurable amount of primary production (Wright, 1964; Pechlaner, 1971). However, in arctic and sub-arctic lakes light is absent for up to 3 months during the polar night (Kalff and Welch, 1974) and winter survival of algae must be by some non-photosynthetic means. We therefore examined the physiological mechanisms available to C. erosa for survival during long periods of darkness. Some preliminary results were reported in Morgan and Kalff (1975).

METHODS

Cryptomonas erosa Skuja was isolated into axenic culture from a small naturally eutrophic lake near Montreal, Quebec, Canada. The alga was grown in batch culture in a relatively dilute mineral salts medium (conductivity = 240 µmhos at 25°). The only organics added were trace amounts of vitamins and the metal chelater NaEDTA (Part I). The

cultures were maintained in 500 ml pyrex reagent bottles without shaking or aeration and incubated in constant temperature (±0.5°) chambers under continuous cool-white illumination. The alga was grown under a variety of light-temperature conditions, and while still in exponential growth, sufficient cells were inoculated into 2 or 3 bottles of fresh medium to give an initial dark population of between 500 and 2000 cells ml^{-1}. The bottles were wrapped ~~in foil, and cells~~ grown at 23.5 and 15° were incubated at their respective growth temperatures in the dark, while cells grown at 4° were incubated in the dark at 4 and 1°. Changes in cell numbers and mean cell volume were followed in the dark cultures until the population disappeared. Cell numbers were counted in a Palmer-Howard counting chamber and cell volume was determined by treating the cell as a prolate spheroid. At one or more times during the dark incubation, the viability of dark cells was determined by returning the cells to both the pre-dark light intensity and to a low light level of 10^{-3}ly min^{-1} PAR (≃ 20 ft.c).

The possible role of heterotrophy and phagotrophy in the survival of C. erosa at low limiting light levels and in the dark was examined in two ways: (1) In a series of cultures (15 and 4°) C. erosa was grown in association with a mixed bacterial flora isolated from the same lake as the alga. No attempt was made at taxonomic identification of the bacteria, nor were bacterial numbers controlled or routinely measured in the cultures. The bacterial counts that were made were on nutrient agar or by direct microscope examination of filters stained with

erythrosin B (Kuznetsov, 1959). Carbon uptake, cell division and cell volume of C. erosa were compared at several light levels in algal-bacterial versus axenic cultures. The algal-bacterial cultures were incubated in the dark with and without transfer to fresh medium to examine if the resulting difference in bacterial numbers and concentrations of organics would significantly affect the dark survival of C. erosa. Changes in cell numbers and cell volume in the dark were followed as described previously. (2) Heterotrophy by C. erosa was also examined through the uptake of labelled organics under low light and dark conditions. Following procedures of Wright and Hobbie (1965), ^{14}C-glucose or acetate of high specific activity (Amersham-Searle) was added at 1-50 $\mu g l^{-1}$ concentrations and, following an approximately one day incubation, samples were filtered onto 0.45 μm membrane filters and filter activity assayed in a windowed GM system. Heterotrophy was also investigated with a mix of labelled organics that was isolated by sonification and filtration from an algal-bacterial culture grown with $NaH^{14}CO_3$ in our laboratory.

RESULTS

C. erosa retained its motility in the dark and no true resting stage was observed. In a few cultures some cells did become enmeshed in a mucilaginous envelope, yet under the microscope, probing the cells with a needle caused them to swim free. No growth was obtained in the dark in axenic or algal-bacterial cultures, and all populations disappeared within 80 days. Cultures grown at 2.0×10^{-3} ly min^{-1} ($\simeq$ 50 ft.c) at 23.5, 15 and 4°, and incubated in the dark both at these temperatures and 1°, illustrate the typical pattern of decline in cell numbers in relation to

Fig. 1. The decline in cell numbers (——— , left ordinate) and cell volume (– – – – , right ordinate) of C. erosa at various temperatures in the dark.

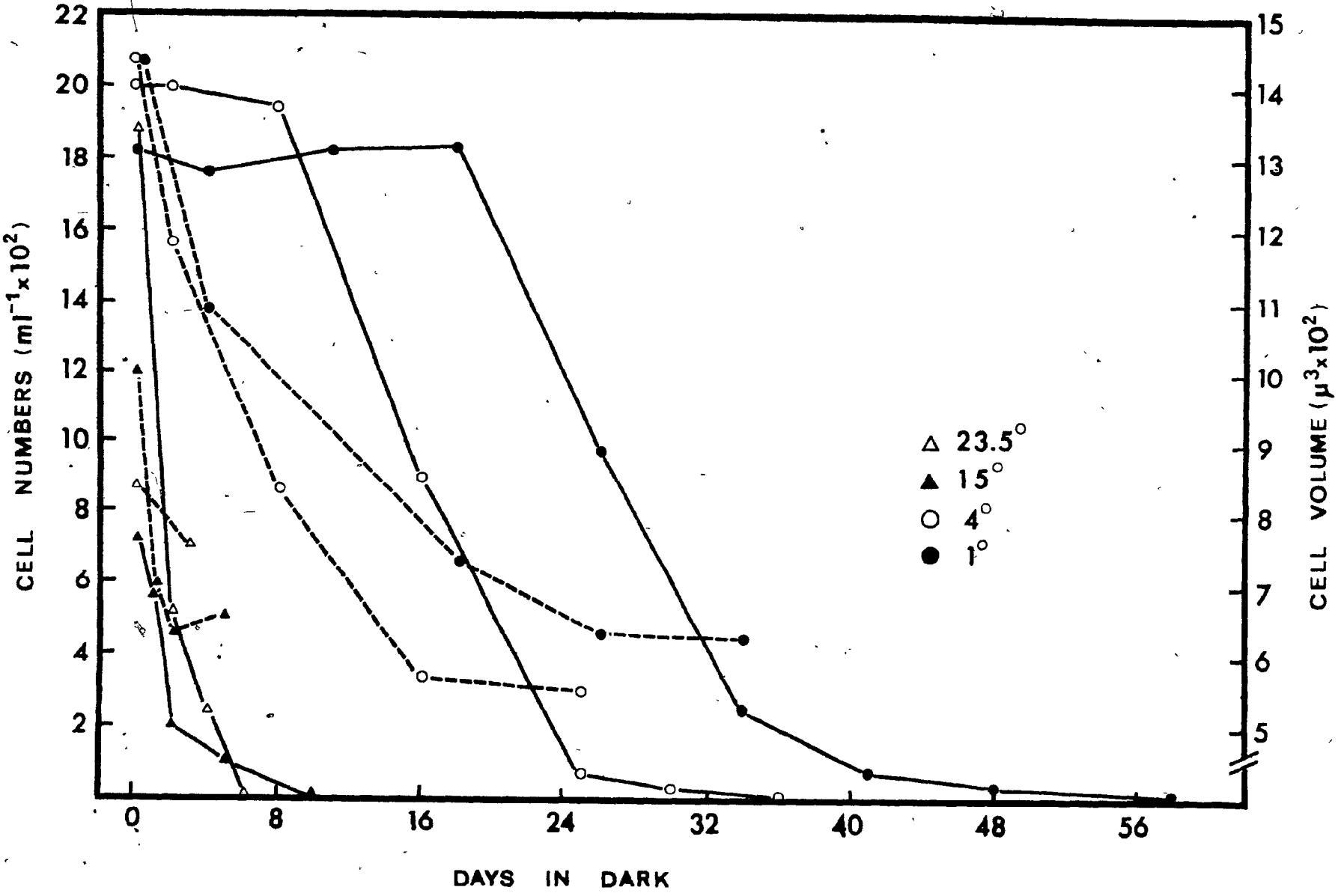

CELL NUMBERS ($ml^{-1} \times 10^2$)
CELL VOLUME ($\mu^3 \times 10^2$)
DAYS IN DARK
0
8
16
24
32
40
48
56
2
4
6
8
10
12
14
16
18
20
22
5
6
7
8
9
10
11
12
13
14
15
△ 23.5°
▲ 15°
○ 4°
● 1°

Table 1. Viability of dark cells on return to the light (+ = growth; - = no growth)

Growth conditions		Dark temperature (°C)	Time to complete disappearance in dark (weeks)	Time when returned to light (weeks)	Viability at 10^{-3} ly min^{-1}	Viability at pre-dark light intensity
Temp. (°C)	(ly $min^{-1} \times 10^{-3}$)					
15	8.6	15	2	1	+	-
				1.5	-	-
15	5.6	15	2	1	+	-
				2	-	-
15	2.0	15	1.3	0.6	+	+
				1.1	-	-
15	1.0	15	0.6	0.3	+	N.A.
4	5.6	4	9-10	2	+	+
				4	+	-
				8	+	-
4	2.0	4	4-5	1	+	+
4	1.0	4	3.5	2	+	N.A.
4	2.0	1	7	2.5	+	+
				3.7	+	+
				6	+	+
4	1.0	1	5.5	2	+	
				4	+	N.A.
				5.3	+	
1	1.0	1	10-12	2.5	+	
				6	+	N.A.
				9	+	

temperature (Fig. 1). The results show that at high temperatures (23.5 and 15°) cell numbers decline almost immediately upon transfer to the dark, with a 100% loss of cells within 4 to 20 days. In contrast, at 4 and 1° cell numbers do not decrease for approximately one and three weeks, respectively, followed by fairly rapid cell death to extinction within 20 to 80 days (Appendix L). Incubation of C. erosa at a light intensity just below compensation at 1° (.062 ly day^{-1}) further retarded cell death and cells were still present after three months (Appendix D).

Cells present in the dark would generally resume normal growth upon transfer to the light (Table 1). C. erosa at 1° were still viable after 7-9 weeks of darkness. However, in some cultures, especially at high temperatures (15°), very small dark cells were not viable in the light. In addition transfer of any cells to relatively high light intensities almost always resulted in cell destruction even though growth was possible at a lower light level (10^{-3} ly min^{-1}).

During the initial period of dark incubation when no cell death was observed in the low temperature cultures, the mean cell volume of the population, nevertheless, declined rapidly in the first 1 to 2 days, but more slowly thereafter, with a 40% decrease in volume in about 1 week at 4° and a 50% decrease in 2-3 weeks at 1° (Fig. 1). The 40-50% loss in cell mass was coincident with the onset of cell death. In contrast, at higher temperatures (15 and 23.5°) the decrease in cell volume was a more rapid 25-30% loss within two days of darkness (Fig. 1). In most cultures the mean cell volume of the population decreased even further to a minimum of approximately 600 μ^3 (Fig. 1) which remained constant

with time because the smallest cells died and disappeared, while larger cells became smaller and approached the minimum cell size. However, after lengthy incubation in the dark at low temperatures, some populations disappeared even though the mean cell volume was significantly greater than the minimum.

From analyses of particulate carbon in some batch cultures in the light and in phosphorus-limited chemostat cultures, cell volume was found to provide a measure of the carbon content of the alga (Part I). The relationship was described by two linear equations:

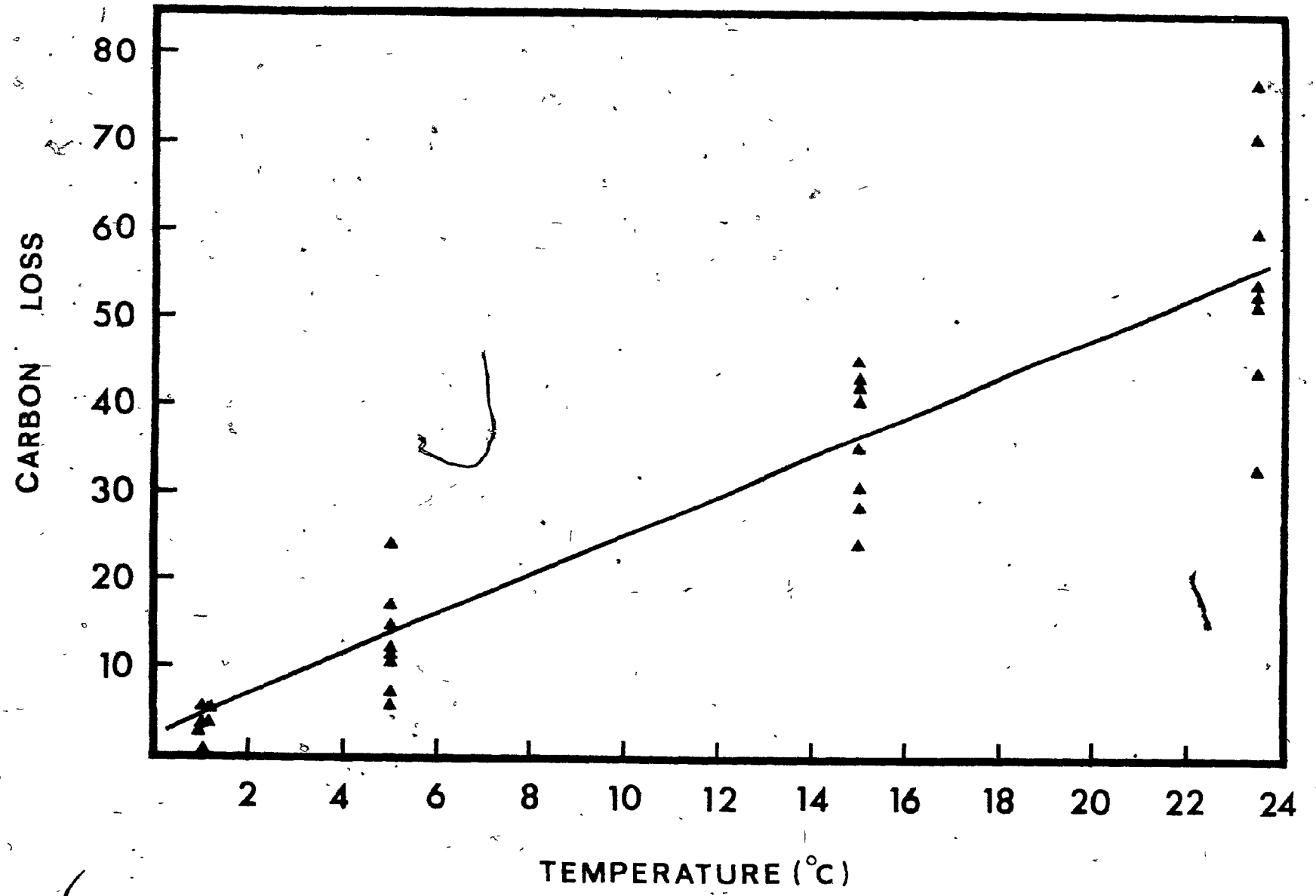
(1) $\text{pgm C cell}^{-1} = 0.1498\ V + 9.5$ (15°)

(2) $\text{pgm C cell}^{-1} = 0.2049\ V + 3.5$ (4 and 1°)

where V is the cell volume in μ^3.

Thus cells at low temperatures contain a higher percentage of carbon per unit cell volume than cells at 15°. On the assumption that the equations also apply to dark cells, the rate of carbon loss per cell in the dark was calculated from the decrease in the mean cell volume of the population during the time in which little or no cell death was observed. At low temperatures the rate of carbon loss was calculated during the period of more slowly declining cell volume (Fig. 1), whereas at high temperatures the rapid decline in cell numbers necessitated that carbon loss be calculated in the first 1-2 days of incubation. The results show that the mean rate of carbon loss (pgm C cell^{-1} day^{-1}) decreased sharply with decreasing temperature, such that the loss rate at 1° was 17 fold less than that at 23.5° (Fig. 2 and Appendix M).

Fig. 2. The relationship between carbon loss per cell (pgm C day^{-1}) in the dark and temperature.


CARBON LOSS
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
24
TEMPERATURE (°C)

Growth of C. erosa under a variety of light-temperature regimes resulted in cells with a wide range in cell volume (400-9000 μ^3) and carbon content (60-1800 pgm C $cell^{-1}$) (Part I). The amount of carbon per cell at high temperatures decreased with decreasing light to a minimum at near compensatory light levels. Under growth inhibitory levels at low temperatures, cell division was more adversely affected than carbon uptake and the resulting excess production of photosynthate was principally retained as stored carbon. The carbon content of light-inhibited cells at low temperatures was thus 3 to 8 times that of light-limited cells at higher temperatures (Part I). The relationship between quantity of carbon stored in the light and subsequent dark survival is shown in Fig. 3. Dark survival is expressed in units of time only, because the percentage of cells dying per day is independent of the initial cell density of the dark population. The results show, firstly, that cells incubated in the dark at low temperatures survive the longest, and secondly, that at each temperature, dark survival increases with increased quantity of stored carbon. Thus at low temperatures, cells with over 500 pgm C $cell^{-1}$ survive 2 to 3 times longer than cells with less than 200 pgm C $cell^{-1}$. Small cells grown at near compensatory light levels at higher temperatures possess a critically low carbon content prior to dark incubation and die in as little as 4 days.

Although the dark survival of C. erosa appears to be primarily dependent on its internal carbon supply, the heterotrophic uptake of organics excreted by the cells or released from lyzed cells cannot be precluded. The possible importance of heterotrophy (and phagotrophy) was determined

Fig. 3. The relationship between quantity of carbon stored in the light and the time to complete disappearance in the dark at various temperatures.

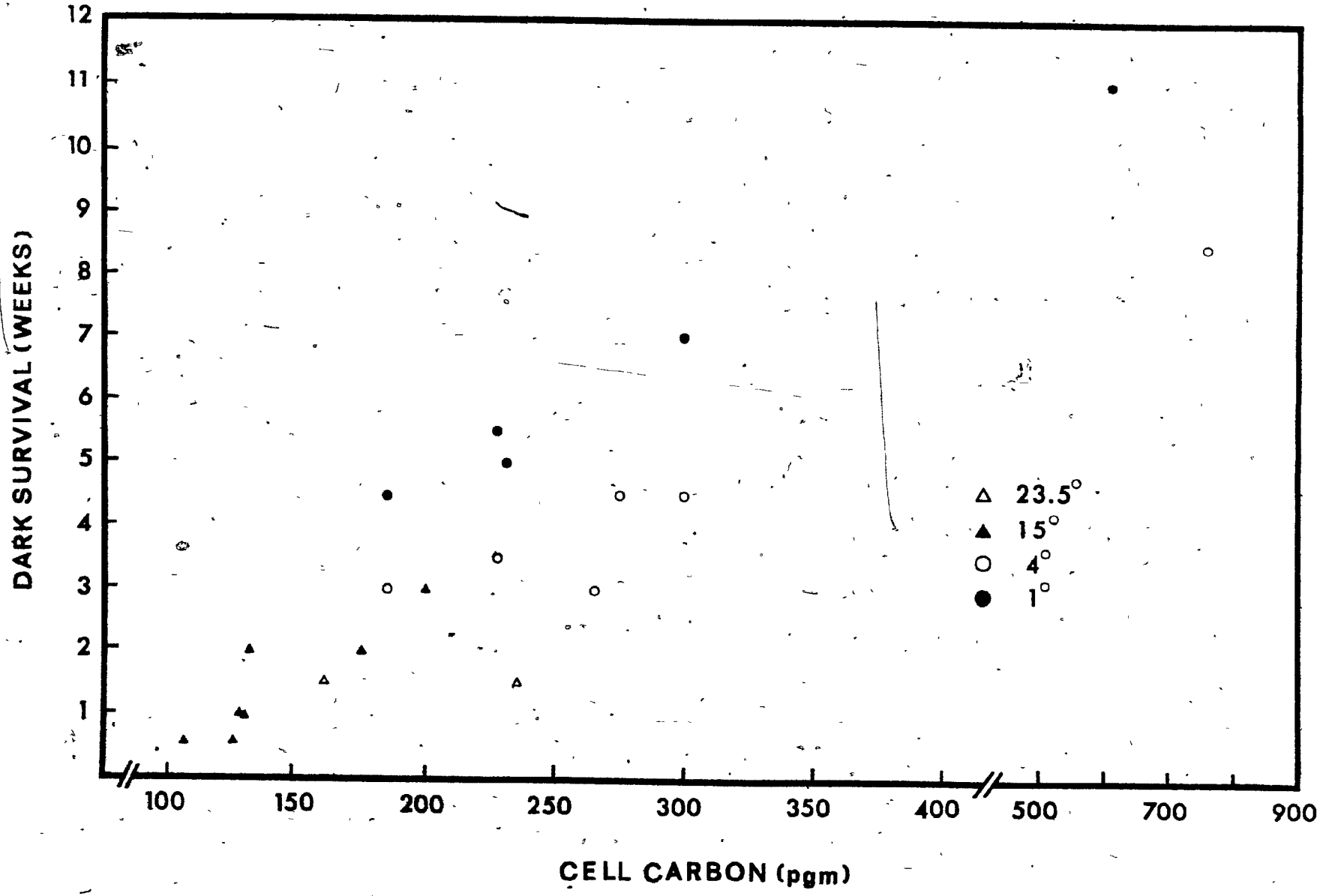

DARK SURVIVAL (WEEKS)
12
11
10
9
8
7
6
5
4
3
2
1
100
150
200
250
300
350
400
500
700
900
CELL CARBON (pgm)
23.5°
15°
4°
1°

Table 2. Growth of C. erosa in axenic versus algal-bacterial cultures, and time to complete disappearance in the dark. A = axenic; B = algal-bacterial; Transfer = dark incubation in "fresh" medium; Non-transfer = dark incubation in "old" medium. Limits are one standard deviation.

	Light intensity (ly $min^{-1} \times 10^{-3}$)		Cell division (div. day^{-1})	C uptake (pgm C $cell^{-1}$ day^{-1})	Mean cell volume (μ^3)	Dark temperature (°C)	Time to complete disappearance in dark (weeks)	
							transfer	non-transfer
Temperature: 15°								
(1)	2.0	A	0.30 ± .03	34.8 ± 8.2	820 ± 79	15	1.3	-
		B	0.25 ± .03	26.9 ± 4.9	964 ± 89	15	2.5	3.5
(2)	1.0	A	0.18 ± .04	16.0 ± 3.8	771 ± 110	15	0.6	-
		B	0.14 ± .01	14.7 ± 2.9	1000 ± 104	15	3	3
(3)	0.62	A	0.022 ± .013	3.2 ± 1.4	640 ± 60	15	0.6	-
		B	0.017 ± .007	4.4 ± 1.8	818 ± 94	15	2.5	3
Temperature: 4°								
(1)	2.0	A	0.21 ± .01	33.3 ± 4.1	1591 ± 175	4	4-5	-
						1	7	-
		B	0.19 ± .01	29.3 ± 10.6	1614 ± 124	4	5-6	5-7
						1	8-9	7-9
(2)	1.0	A	0.072 ± .004	11.0 ± 2.2	982 ± 95	4	3-4	3-4
						1	5-6	4-6
		B	0.086 ± .01	14.7 ± 3.0	917 ± 70	4	3	4
						1	5-7	7-8
(3)	0.62	A	0.03 ± .004	5.9 ± 1.9	905 ± 121	4	3	-
						1	5	-
		B	0.027 ± .01	9.9 ± 4.4	1171 ± 83	4	5-6	6
						1	7-10	7-10
(4)	0.24	A	0.0038 ± .0032	3.7 ± 1.9	1266 ± 110	4	3-4	-
						1	-	-
		B	0.0030 ± .002	3.9 ± 2.1	1508 ± 184	4	5-6	7-8
						1	-	-

Table 3.5 Heterotrophy in C. erosa: typical results. Pretreatment was 1 week in the dark at 4°. Results obtained following a further 24 hr incubation with ^{14}C-glucose and ^{14}C-acetate in the light and dark.

	Substrate concentration ($\mu g \ell^{-1}$)					
	1	2	5	10	20	50
GLUCOSE	CPMS/5 mls at 24 hrs					
Incubated in dark	100	159	230	276	310	396
Incubated at 10^{-3}ly min^{-1}	127	113	239	257	299	406
Killed control*	114	141	210	264	282	370
ACETATE	CPMS/5 mls at 24 hrs					
Incubated in dark	78	93	182	249	247	310
Incubated at 10^{-3}ly min^{-1}	87	96	198	230	257	349
Killed control*	60	79	207	212	260	312

* Killed with Lugol's iodine.

by growing the algae in association with bacteria at low light levels (at 4 and 15°), and comparing the dark survival of axenic versus algal - bacterial cultures. The results show (Table 2) that neither carbon uptake or cell division of C. erosa was significantly affected by the presence of bacteria. Yet, at all light levels at 15° and at the two lowest light levels at 4°, the mean cell volume of the algae was greater in bacterial cultures, due to the occurrence of some especially large carbon-laden cells embedded in mucilage. When incubated in the dark, algal populations grown in association with bacteria showed dramatically improved dark survival at 15° and, to a lesser degree, at low temperatures (Table 2), almost surely the result of improved survival of the large cells. However, no difference was observed in the dark survival of algal-bacterial cultures transferred to fresh medium versus those not transferred, despite the higher concentrations of bacteria and organics in the latter cultures (Appendix N). Other experiments (15 in all) with labelled substrates also showed C. erosa unable to assimilate 1-50 µg l^{-1} concentrations of glucose and acetate, or a mix of organics, in either the light or dark (Table 3).

DISCUSSION

C. erosa can survive a lengthy dark period (up to 80 days) at 1°, but at much higher temperatures (15 and 23.5°) the alga rapidly disappears from culture. Two marine flagellates were similarly shown to rapidly lose their viability at high temperatures (Yentsch and Reichert, 1963; Hellebust and Terborgh, 1967), while several marine species were found to

survive three months darkness at -1.8° (Bunt and Lee, 1972). In contrast to these results, Antia and Cheng (1970) observed that certain marine algae were viable for at least 6 months in the dark at 20°, yet it is most improbable that the cells remained vegetatively active, but rather survived in a resting stage.

In the absence of encystment or sporulation dark survival depends upon maintenance of the respiratory system. Exhaustion of endogenous substrate results in failure of that system and cell death. Respiration of stored carbon by C. erosa was manifested by a decrease in cell volume and, by relating cell volume to carbon content, it was possible to estimate the mean daily carbon loss per cell. The calculation showed that the loss decreased markedly with temperature (Fig. 2). Thus at high temperatures, reserve carbon is quickly exhausted, followed by rapid cell death, whereas at low temperatures the alga survives a lengthy dark period because of slowed respiration of storage materials. When supplied with a low sub-compensatory light intensity, sufficient photosynthesis occurred to further retard carbon loss and prolong survival of the population. Bunt et al. (1966) have previously shown that respiration in marine diatoms was substantially depressed at low temperatures and proposed that this response would contribute to successful survival in the dark. In cryptomonads carbon is stored as starch in spheroidal or ellipsoidal granules (Lucas, 1971), which in C. erosa grown at low temperatures and inhibitory light levels accumulated in such quantity as to obscure the remainder of the cell contents. The unusual ability of cryptomonads to expand in size to accommodate large carbon

reserves may depend on their possession of an elastic periplast rather than a more rigid cell wall (Faust, 1974). This capacity to store carbohydrate is, as shown in C. erosa, (Fig. 3) an additional important mechanism for survival during lengthy dark periods.

The amount of cell carbon available for respiration is limited, and in most dark populations the mean cell volume decreased to a minimum of approximately 600 μ^3. Cells much smaller than 600 μ^3 quickly expired, presumably from failure of the respiratory system. This value therefore appears to represent the genetically fixed minimum necessary for a viable C. erosa. Cell viability on return to the light is also dependent on maintenance of a functional photosynthetic system. Although no measures were made here on the photosynthetic capacity of dark cells, other studies of marine flagellates show that, at high temperatures, photosynthetic enzymes are rapidly lost in the dark (Yentsch and Reichert, 1963; Hellebust and Terborgh, 1967). Dark incubation of C. erosa at low temperatures must also retard loss of photosynthetic capacity. Our results further indicate that dark cells exposed to a relatively high light intensity are destroyed (Table 1), suggesting that in lakes resumption of algal growth following the polar night or the snow cover melt is dependent on slowly increasing light levels.

Laboratory studies have shown that some algae, including cyptomonads (Wright, 1964), are able to grow in the dark on milligram to gram concentrations of organics (Lewin, 1953; Danworth, 1962; Lylis and Trainor, 1973), while Shuster (1968) and Wawrik (1971) report, respectively,

that certain cryptomonad species can ingest bacteria and even other cryptomonads. In our cultures, however, C. erosa was unable to assimilate organics added at more natural microgram concentrations, nor did heterotrophy or phagotrophy appear to play a significant role in dark survival. The presence of bacteria apparently did cause some algal cells to secrete a muciliganous envelope, wherein slowed cell division may have resulted in an increased carbon content which resulted in prolonged dark survival. The nature of the algal-bacterial interaction remains unclear as does the reason why the phenomenon occurred in only some cells. Although our results do not exclude some heterotrophy, C. erosa is apparently an obligate phototroph, as was also found for other cryptomonads (Cheng and Antia, 1970; Faust and Gantt, 1973). Whether C. erosa could assimilate significant amounts of organics at much higher concentrations was not examined since such experiments were considered to have little ecological relevance.

In high arctic lakes cryptomonads, other flagellates, and some small green and diatom species survive complete darkness at 0-1° during the three month polar night (Kalff and Welch, 1974), similar to the maximum survival of C. erosa in culture (Fig. 3). In the absence of photosynthesis, heterotrophy has been postulated as a mechanism for the dark survival of arctic algae (Wilce, 1966). However, in comparison with normally organically rich batch cultures, much lower concentrations of available organics (Schindler et al., 1974) and bacterial numbers (Tilzer, 1972; Morgan and Kalff, 1972) are found in oligotrophic mountain and arctic lakes. Moreover, Wright and Hobbie (1966) have shown that

algae compete inefficiently with aquatic bacteria for organic substrates at low natural concentrations. An autoradiographic investigation of algal populations in arctic sea ice also found that heterotrophic metabolism by the algae was negligible (Horner and Alexander, 1972). Thus as previously suggested by others (Rodhe et al., 1966; Nauwerck, 1966; Pechlaner, 1971), it is most unlikely that either phagotrophy or heterotrophy provides a significant source of carbon for the winter dark survival of C. erosa and probably all other planktonic algae in natural waters. Our study, instead, indicates that long term dark survival during the polar night depends upon the slow respiration of stored carbon.

The carbon content of algae is dependent on the relative rates of net carbon uptake and cell division. In batch and chemostat cultures, the carbon content of C. erosa varied 20 to 30 fold, being maximal under conditions of high light and low temperature or nutrient limitation when cell division is more adversely affected than carbon uptake (Parts I and II). In nature, the wide range in cell volume reported for C. erosa and other cryptomonads (Huber-Pestalozzi, 1950; Kling and Holmgren, 1972), which appears to lack much taxonomic significance, must similarly reflect extreme variation in cell carbon. Since the dark survival of C. erosa increased with the quantity of stored carbon (Fig. 3), cell carbon content must be important in the winter dark survival of algae. Our results suggest that high summer light fluxes in cold and nutrient deficient arctic lakes would result in decreased cell division and increased carbon content. Those cells that contained the largest carbon

reserve would be most likely to survive the long winter darkness and serve as the seed population when the light returns.

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Appendix A

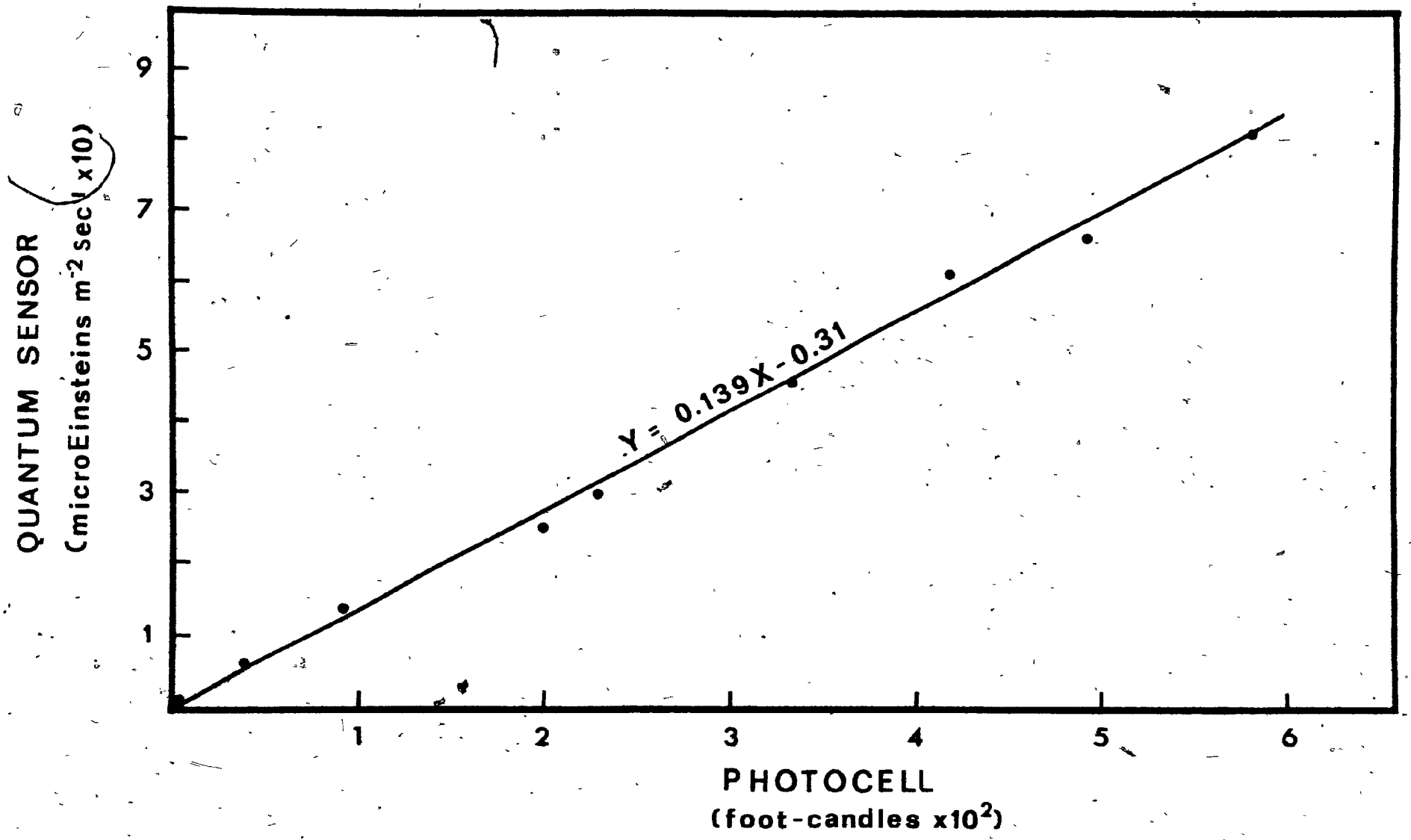
Calibration of Photocell Against Quantum Radiometer

The photocell (G.M.Co.) was calibrated in microEinsteins m^{-2} sec^{-1} with a quantum radiometer (Lambda Instruments). The light source was cool-white fluorescent. The calibration is tabled below and graphed in Fig. 1. The relationship was linear, described by the regression $Y = 0.139X - 0.31$ ($p<0.05$) where X = ft.c. and Y = $\mu E\ m^{-2}\ sec^{-1}$. Light units are presented in ly min^{-1} where 1 $\mu E\ m^{-2}\ sec^{-1}$ = .0003105 ly min^{-1} PAR.

Table 1

Photocell (foot-candles)	Quantum radiometer (microEinsteins m^{-2} sec^{-1})	Light units in ly min^{-1} × 10^{-3} PAR
582	81	25.31
494	66	20.62
429	61	19.06
336	46	14.37
229	30	9.37
200	25	7.81
153	21	6.56
91	13.3	4.15
38	5.6	1.75
16	1.75	0.55
3.1	0.33	0.10

Fig. 1. Calibration of photocell against quantum radiometer.


QUANTUM SENSOR
(microEinsteins m^{-2} sec^{-1} x10)
9
7
5
3
1
Y = 0.139X - 0.31
1
2
3
4
5
6
PHOTOCELL
(foot-candles x10^2)

Appendix B

Calibration of Fluorometer Against Spectrophotometer

Instruments

(1) Turner model 111 Fluorometer with primary filter Corning CS 5-60 and secondary filter Corning CS 2-64, modified for in vivo chlorophyll measurements as follows:

(a) red sensitive (R136) photomultiplier which extends the response of the instrument to 750 mμ

(b) blue fluorescent lamp (F4T5)

(c) high sensitivity door

(2) Bausch and Lomb Spectronic 88

Samples

(1) Fluorometer: Serial dilutions of stock C. erosa populations with growth medium, as well as some experimental populations from batch and chemostat cultures, measured in vivo.

(2) Spectrophotometer: Samples of the above populations, filtered on glass-fibre filters, and ground and extracted in 90% acetone, with chlorophyll a calculated from the trichromatic equation of Strickland and Parsons (1968).

Analysis of phaeopigments. 2-4 Drops of 1N HCl added to both fluorometer and spectrophotometer samples and emission and absorbance, respectively, read after 2-5 min.

The Calibration. Fluorescence measurements can be carried out only on very dilute solutions because of quenching and self-absorption. If either of these factors is significant, a non-linear relationship between chlorophyll concentration and fluorescence will result. This would be most readily seen at higher chlorophyll concentrations on the lower sensitivity scales of the fluorometer. To avoid this problem, samples with a high chlorophyll content (some chemostat samples only) were diluted with the growth medium, so that all samples were read on the two most sensitive scales (door 30 and 10). Over this range a linear relationship between chlorophyll and fluorescence was observed (Fig. 1). The relationship is described by the equation

$$\text{Chl-a } (\mu g l^{-1}) = (R_b)\ (k_x)\ \left(\begin{matrix}\text{dilution}\\ \text{factor}\end{matrix}\right)$$

where R_b is fluorescence before acidification and k_x is the calibration constant equal to 0.367. It should be noted that k_x is a function of the combination of photomultiplier, excitation lamp, high sensitivity door and filters used.

Phaeophytin. Phaeophytin normally is not found in living phytoplankton cells (Patterson and Parsons, 1963), but is apparently produced as a result of zooplankton grazing (Currie, 1962) and cell death. Phaeophytin was not present in most cultures of C. erosa, but low acid ratios were observed in cultures where cell division was inhibited by light (Fig. 2). We interpret this result as a degradation of chlorophyll a to phaeophytin-like products in dead and dying cells. It was observed that fluorometric acid ratios ≥ 2 were always associated with little or no phaeophytin as

Fig. 1. Calibration of fluorometer against spectrophotometer.

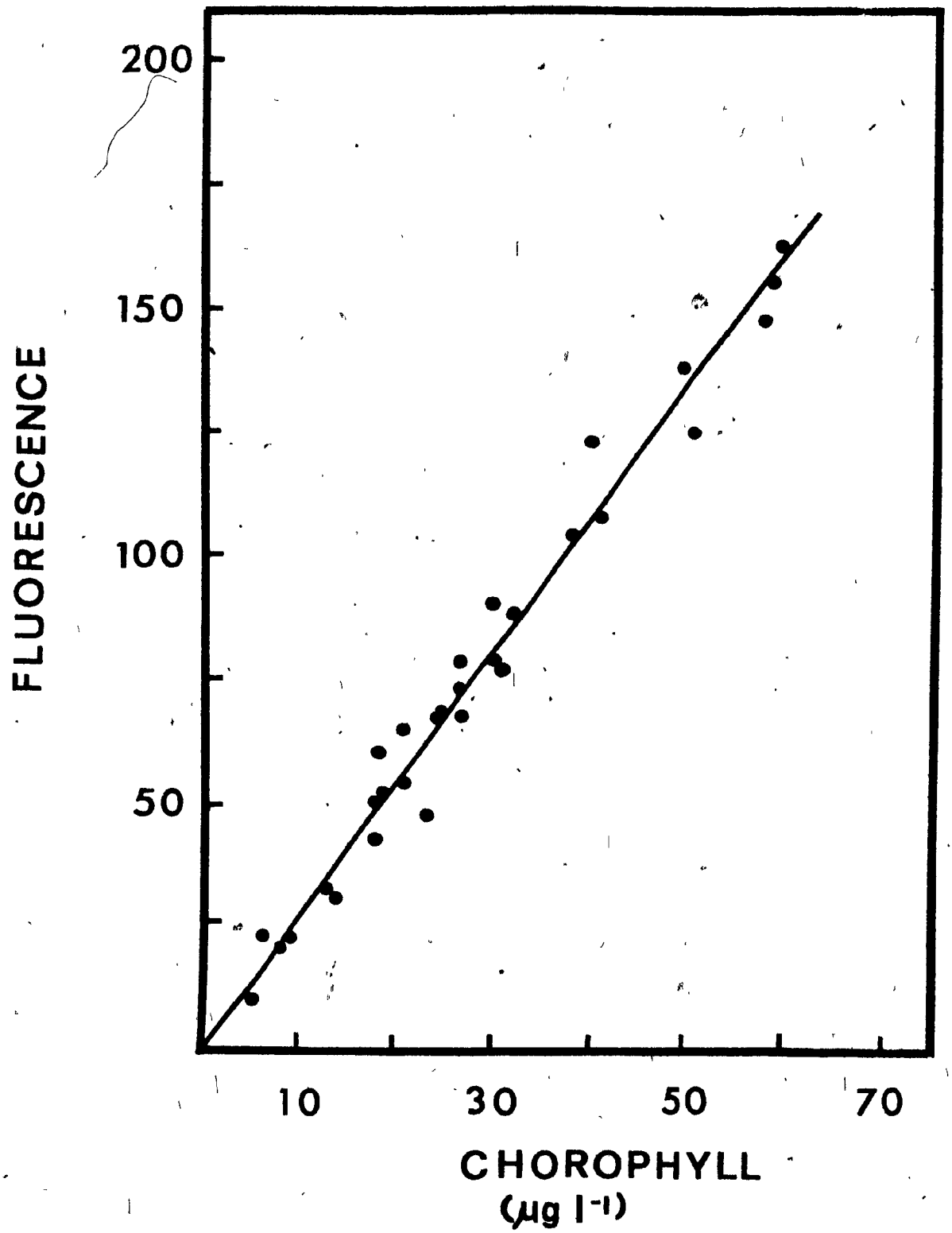

200
150
100
50
FLUORESCENCE
10
30
50
70
CHOROPHYLL
(µg l⁻¹)

Fig. 2. The relationship between fluorometer acid ratio (R_b/R_a) and light intensity in batch cultures of C. erosa at different temperatures.

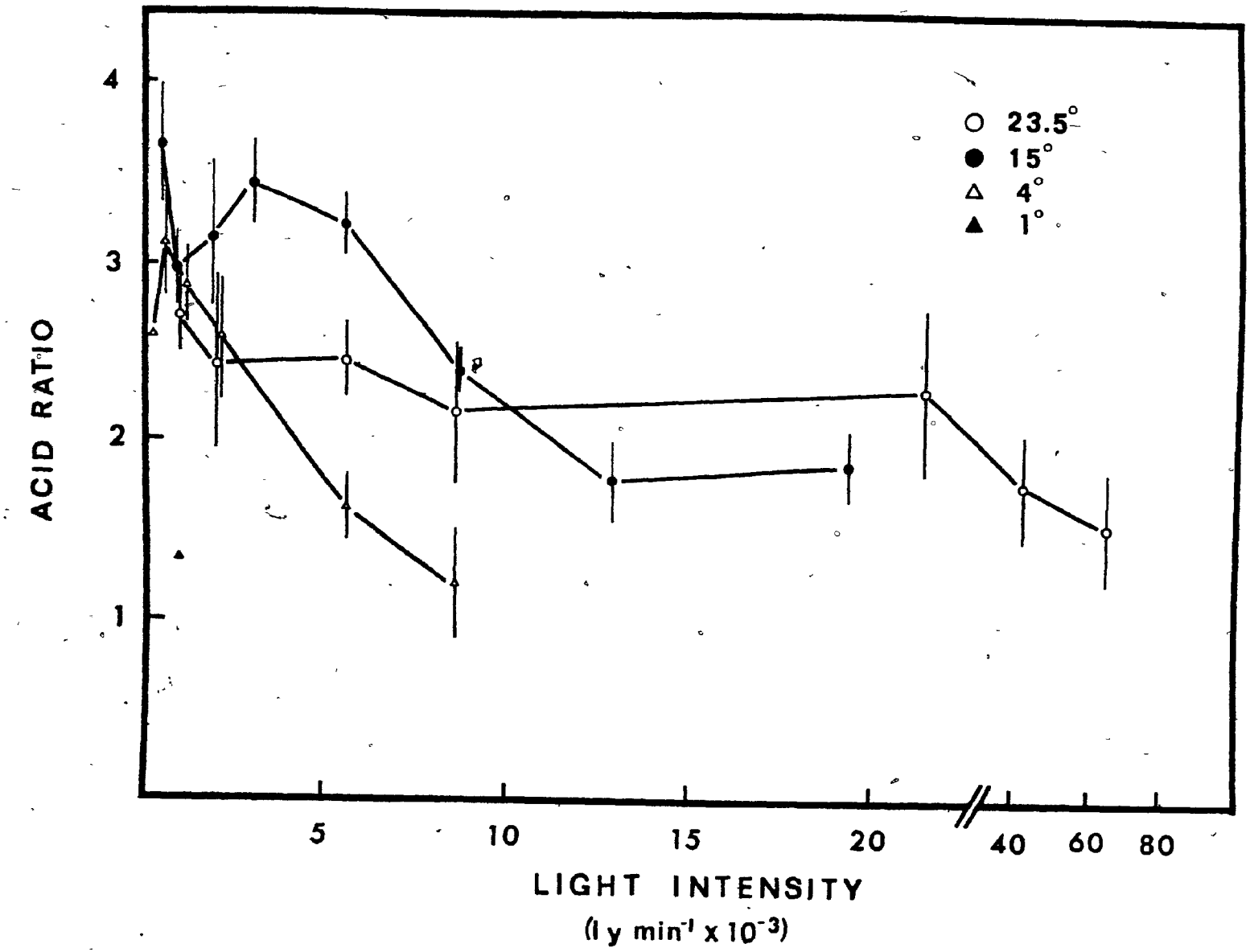
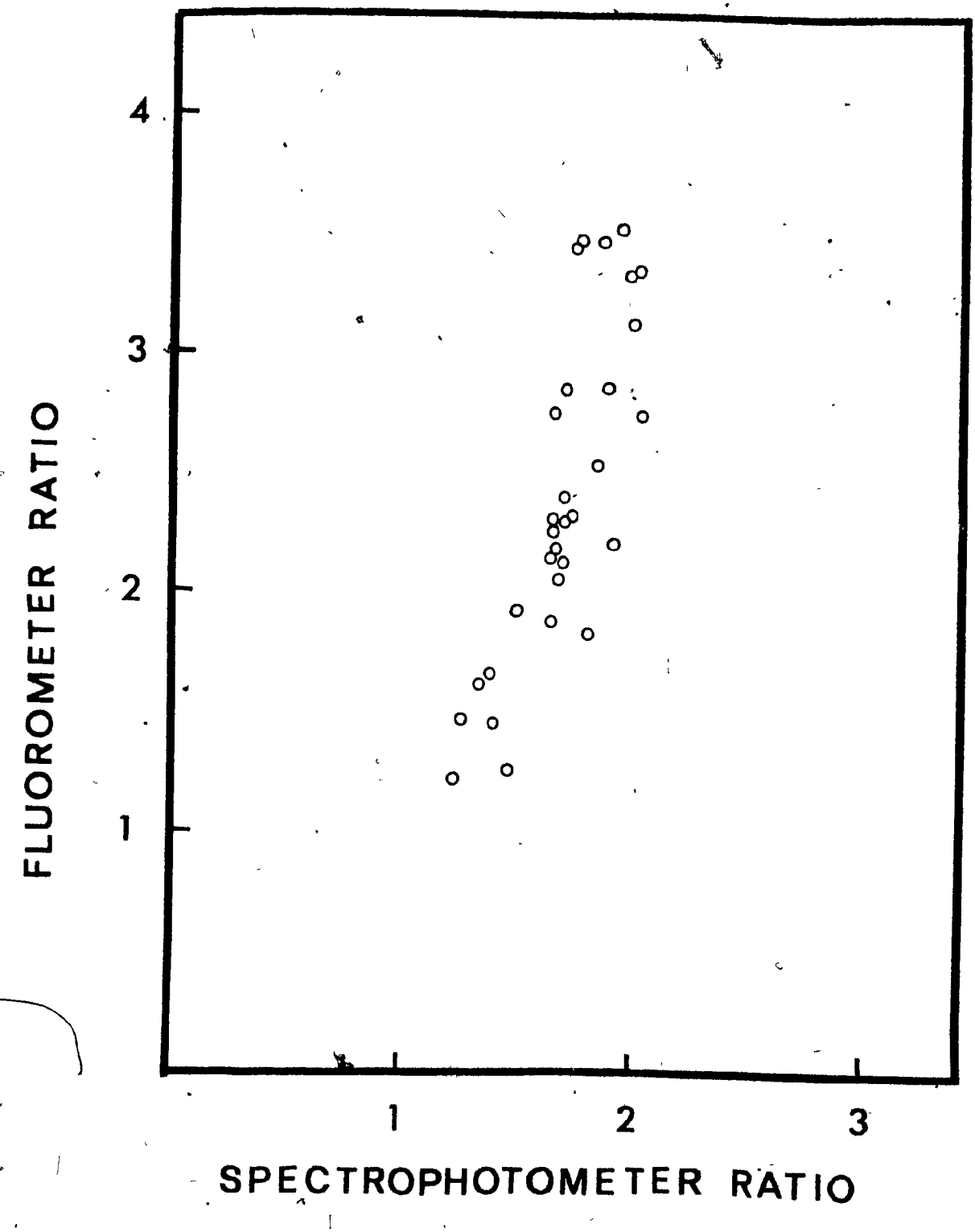

ACID RATIO
4
3
2
1
23.5°
15°
4°
1°
5
10
15
20
40
60
80
LIGHT INTENSITY
(l y min⁻¹ x 10⁻³)

Fig. 3. The relationship between fluorometer acid ratio and spectrophotometer acid ratio.


FLUOROMETER RATIO
4
3
2
1
1
2
3
SPECTROPHOTOMETER RATIO

determined by spectrophotometry (Fig. 3). Therefore in those few samples with acid ratios <2, chlorophyll was corrected for phaeophytin by the equation

$$\text{Chl-a } (\mu g l^{-1}) = (2.0)\ (k_x)\ (R_b - R_a)\ \left(\begin{matrix}\text{dilution} \\ \text{factor}\end{matrix}\right)$$

where R_b and R_a are the fluorescence before and after acidification, respectively.

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Appendix C

Cell Division, Carbon Uptake, Mean Cell Volume and Chlorophyll a of *C. erosa* Under Various Combinations of Light and Temperature in Batch Culture

Light intensity (ly $min^{-1} \times 10^{-3}$)	Cell division (div. day^{-1})	Carbon uptake (pgm C $cell^{-1}$ day^{-1})	Mean cell volume (μ^3)	Chlorophyll a (pgm $cell^{-1}$)
Temperature: 23.5°				
64.6	1.07 ± 0.16	172.5 ± 33.7	1762 ± 235	2.31 ± 0.47 (3.11)*
43.1	1.23 ± 0.13	218.3 ± 27.9	1507 ± 262	3.04 ± 0.31 (3.27)*
21.5	1.16 ± 0.19	209.6 ± 30.1	1464 ± 212	4.06 ± 0.62
8.6	0.86 ± 0.07	166.6 ± 23.6	1069 ± 160	3.89 ± 0.27
5.6	0.51 ± 0.10	92.7 ± 17.2	1038 ± 110	4.40 ± 0.44
2.0	0.20 ± 0.06	31.1 ± 13.6	946 ± 73	3.96 ± 0.51
1.0	0.03 ± 0.01	5.7 ± 2.4	674 ± 60	6.06 ± 0.62
Temperature: 15°				
19.4	0.38 ± 0.12	82.4 ± 13.4	1608 ± 170	2.38 ± 0.37 (2.68)*
12.9	0.47 ± 0.11	81.6 ± 14.0	1265 ± 180	2.98 ± 0.48 (3.89)*
8.6	0.69 ± 0.10	123.8 ± 11.0	1108 ± 205	5.49 ± 0.35
5.6	0.57 ± 0.13	68.4 ± 19.6	851 ± 96	5.23 ± 0.48
3.0	0.41 ± 0.04	50.0 ± 16.4	810 ± 104	4.86 ± 0.31
2.0	0.30 ± 0.03	34.8 ± 8.2	820 ± 79	4.65 ± 0.14
1.0	0.18 ± 0.04	16.0 ± 3.8	771 ± 110	5.35 ± 0.44
0.5	0.022 ± 0.013	3.24 ± 1.4	640 ± 60	16.26 ± 2.92

Light intensity (ly $min^{-1} \times 10^{-3}$)	Cell division (div. day^{-1})	Carbon uptake (pgm C $cell^{-1}$ day^{-1})	Mean cell volume (μ^3)	Chlorophyll a (pgm $cell^{-1}$)
Temperature: 4°				
8.6	0.086 ± 0.023	30.5 ± 6.0	2187 ± 260	0.85 ± 0.27 (3.35)*
5.6	0.084 ± 0.048	58.7 ± 7.5	3666 ± 407	2.75 ± 1.10 (4.60)*
2.0	0.21 ± 0.010	33.1 ± 4.1	1591 ± 175	6.18 ± 0.61
1.0	0.072 ± 0.004	11.0 ± 2.2	982 ± 95	5.34 ± 0.42
0.5	0.030 ± 0.004	5.9 ± 1.9	905 ± 121	5.16 ± 0.17
0.2	0.0055 ± 0.0046	3.7 ± 1.9	1266 ± 110	5.73 ± 0.50
Temperature: 1°				
8.6	cell death	-	>4000	-
1.0	0.025 ± 0.005	6.8 ± 1.3	2961 ± 480	1.41 ± 0.29 (2.99)*
0.04	negative (< compensation pt)	-	-	-

Values are means for log-growth populations in 2-4 replicate bottles. Limits are one standard deviation.

* ( ) = pigment $cell^{-1}$ before phaeophytin-correction.

Appendix D

Growth of C. erosa at 1°

Table 1. Decline in cell numbers and cell volume of C. erosa incubated at a subcompensatory light intensity of 0.043×10^{-3} ly min^{-1} (≃1 ft.c.) at 1°

Time after inoculation (days)	Flask #	Cell numbers (ml^{-1})	Mean cell volume (μ^3)
0	1	650	
	2	790	
	3	880	3091 ± 1203
	4	1090	
39	1	680	
	2	730	
	3	760	2058 ± 703
	4	810	
87	1	290	
	2	390	
	3	320	
	4	420	
127	1	30	
	2	50	
	3	70	1014 ± 361
	4	50	

Table 2. Cell numbers and cell volume of C. erosa grown at an inhibitory light level of 8.6×10^{-3} ly min^{-1} at 1°. Population inoculated at 1100 cells ml^{-1}, with mean cell volume of 1520 ± 369 μ^3

Time after inoculation (days)	Flask #	Cell numbers (ml^{-1})	Mean cell volume (μ^3)
7	1	870	2086 ± 430
	2	940	2193 ± 510
20	1	730	4169 ± 893
	2	810	4031 ± 982
40	1	210	3812 ± 642
	2	140	4310 ± 500
48	1	62	-
	2	20	-

Appendix E

Light Intensity in the Chemostats

The chemostats were illuminated from the rear by cool-white fluorescent lamps which were spaced so as to provide uniform lighting. In addition the light was kept unidirectional by blackening the side-walls of the supporting cabinets. The light intensity at the chemostat centers could thus be calculated from the extinction coefficient of light through the culture. The equation used was

$$\text{Extinction coefficient } (E_v) = \frac{1}{Z_2 - Z_1} (\ln \iota_1 - \ln \iota_2)$$

where Z_1 and Z_2 are the distances of the front and rear surfaces of the growth vessel from the light source, while ι_1 and ι_2 are the light flux measured at these surfaces. The E_v determined was then substituted in the equation to calculate the light flux at the chemostat centers. Light intensity was routinely monitored once a week and typical results are given in Table 1. Samples removed from the chemostats for measurement of ^{14}C uptake were illuminated at these same light intensities.

Table 1. Light intensity at chemostat centers. Data are measures obtained over three consecutive weeks.

Vessel #	ι-rear (ly $min^{-1} \times 10^{-3}$)	ι-front (ly $min^{-1} \times 10^{-3}$)	E_v	ι-center (ly $min^{-1} \times 10^{-3}$)
		$S_R = 80$		
1	16.3	5.9	.064	9.8
1	16.3	5.9	.064	9.8
1	16.8	5.5	.069	9.6
2	16.3	5.9	.064	9.8
2	16.8	5.8	.066	9.9
2	16.3	5.6	.066	9.6
3	16.3	5.2	.070	9.3
3	16.3	5.4	.069	9.4
3	15.9	5.2	.069	9.0
		$S_R = 14$		
1	16.3	7.3	.050	10.9
1	16.8	7.5	.050	11.2
1	16.1	6.9	.053	10.6
2	17.0	7.5	.051	11.2
2	16.3	7.3	.050	10.9
2	16.8	7.4	.051	11.1
3	16.8	7.5	.050	11.2
3	16.8	7.6	.049	11.3
3	16.3	7.2	.051	10.8

Appendix F

Calculation of Residual Phosphate Concentration in the Chemostats

The residual phosphate concentration in the chemostats (S_o) was estimated by a radiobiological procedure described by Rigler (1966). If ^{32}P is added to an algal sample in which the cycling of P is dominated by the exchange of PO_4-P between solution and the cells, the loss of ^{32}P from solution is described by the equation

$$Y_t - Y_\infty = (Y_o - Y_\infty)e^{-kt} \qquad (1)$$

where Y_o is the % ^{32}P in solution at t=0 (i.e. 100%), Y_t is the % ^{32}P in solution at any time (t) after addition, and Y_∞ is the % ^{32}P remaining in solution when equilibrium distribution of tracer has been attained. Thus the difference between % ^{32}P in solution and the asymptote Y_∞ is decreasing exponentially. A plot of $\ln(Y_t - Y_\infty)$ against time will yield a straight line, the slope of which (k) is the rate constant of ^{32}P loss from solution (Riggs, 1963; Rigler, 1973). In the present experiments the loss of ^{32}P from solution was measured in both chemostat samples receiving no added ^{31}P and in several samples receiving up to 10 µg P ℓ^{-1} concentrations of added P. In no sample receiving ^{31}P was an equilibrium distribution of ^{32}P attained, even after 24 hrs of incubation (Appendix K). Therefore, the asymptote was calculated as that value of Y_∞ which when subtracted from Y_t gave the best fit to the regression of $\ln(Y_t - Y_\infty)$ against time.

In the tables below are presented results of ^{32}P loss from solution in two experiments, which are representative of data obtained at all growth rates in the chemostats. The results are shown only for the asymptote Y_∞ which gave the best fit to the regression. Since an equilibrium distribution of tracer was attained in the sample receiving no ^{31}P, a comparison of its rate constant with those of samples receiving ^{31}P must necessarily be made soon after phosphate addition. Thus in each sample, the rate constant of ^{32}P loss from solution was determined for the first 0.5 hrs of uptake (see tables below). The uptake rate in each sample was then determined by the equation

$$V = (k)(S + S_o) \qquad (2)$$

where S is the concentration of ^{31}P added and S_o is the residual phosphate concentration. By assuming various values for S_o a family of rate-concentration curves was described (Part II, Fig. 7). At very high assumed concentrations of S_o (e.g. 1 µg P ℓ^{-1}), the rate-concentration curves bend sharply upwards, away from the x-y intercept. As S_o is set equal to lower values, the relationship between uptake and concentration more closely approximates the expected hyperbola, until at some concentration of S_o the curve bends towards the x-y intercept. Unfortunately our measures of residual phosphate in the chemostats lack great precision since ^{32}P loss was not measured at sufficiently low P additions (<1 µg P ℓ^{-1}) during the first few minutes of uptake. However, the results do show (Part II, Fig. 7; tables below) that the curves bend towards the x-y intercept only at assumed S_o concentrations $\leq$ 0.10 µg P ℓ^{-1}. This result was obtained consistently in replicate experiments at all growth rates in the chemostats.

Table A. $S_R = 80$ $\mu = 0.23$ div. day^{-1}

1. Calculation of rate constant

	Addition of ^{32}P ($\mu g\ \ell^{-1}$)	Time (hrs)	Y_t(%)	Y_∞(%)	$Y_t - Y_\infty$ (%)	ln $Y_t - Y_\infty$	Regression	Rate constant (0-0.5 hrs)
(1)	0.0	0	100		90.7	4.507		
		.17	42.8		33.5	3.512	Y=	
		.50	16.9	9.3	7.6	2.028	-1.0281X	4.895
		2.0	9.6		0.3	-1.204	+3.091	
		6.0	9.4		0.1	-2.303	r=.874	
(2)	1.0	0	100		80	4.382		
		.17	90.3		70.3	4.253	Y=	
		.50	80.9	20	60.9	4.109	-.4043X	0.531
		2.0	53.8		33.8	3.520	+4.337	
		6.0	26.8		6.8	1.917	r=.99	
(3)	2.0	0	100		70	4.248		
		.17	94		64	4.159	Y=	
		.50	87.3	30	57.3	4.048	-.2063X	0.391
		2.0	74.3		44.3	3.791	+4.20	
		6.0	49.4		19.4	2.965	r=.99	
(4)	5.0	0	100		30	3.401		
		.17	97.2		27.2	3.303	Y=	
		.50	93.9	70	23.9	3.174	-.3178X	0.445
		2.0	85.8		15.8	2.760	+3.37	
		6.0	74.3		4.3	1.459	r=.99	
(5)	10.0	0	100		30	3.401		
		.17	98		28	3.332	Y=	
		.50	96.1	70	26.1	3.261	0.1487X	0.271
		2.0	91.1		21.1	3.049	+3.362	
		6.0	81.9		11.9	2.477	r=.99	

2. Calculation of S_o

Substrate added ($\mu g\ P\ \ell^{-1}$)	Rate constant (hr^{-1})	Assumed concentration of S_o ($\mu g\ P\ \ell^{-1}$)					
		1.22*	1.0	0.5	0.25	0.10	0.05
		Uptake velocity ($\mu g\ P\ \ell^{-1}\ hr^{-1}$)					
0	4.895	5.97	4.90	2.45	1.22	0.49†	0.24
1	0.531	1.18	1.06	0.80	0.66	0.58	0.56
2	0.391	1.26	1.17	0.98	0.88	0.82	0.80
5	0.445	2.77	2.67	2.44	2.34	2.27	2.25
10	0.271	3.04	2.98	2.85	2.78	2.74	2.73

*SRP concentration = 1.22 $\mu g\ P\ \ell^{-1}$.

†S_o concentration at which curve first bends towards the x-y intercept.

Table B. S_R = 14 μ = 0.29 div. day^{-1}

1. Calculation of rate constant

	Addition of ^{31}P ($\mu g\ \ell^{-1}$)	Time (hrs)	Y_t (%)	Y_∞ (%)	$Y_t - Y_\infty$ (%)	ln $Y_t - Y_\infty$	Regression	Rate constant (0-0.5 hrs)
(1)	0.0	0.	100		80	4.382		
		.17	87.6		67.6	4.214	Y=	
		.50	76.1	20	56.1	4.027	-.6008X	0.710
		1.38	59.9		37.9	3.686	+4.38	
		6.27	21.8		1.8	0.588	r=0.98	
(2)	1.0	0	100		70	4.248		
		.17	97.6		67.6	4.214	Y=	
		.50	95.8	30	65.8	4.187	-.0501X	0.122
		1.38	93.1		63.1	4.145	+4.223	
		6.27	79.3		49.3	3.898	r=0.99	
		21.18	53.7		23.7	3.165		
(3)	2.0	0	100		40.0	3.689		
		.17	98.5		38.5	3.651	Y=	
		.50	97.4	60	37.4	3.622	-.0680X	0.128
		1.38	96.0		36.0	3.584	+3.674	
		6.27	86.2		26.2	3.266	r=.99	
		21.18	69.3		9.3	2.230		
(4)	5.0	0	100		40.0	3.689		
		.17	99.2		39.2	3.668	Y=	
		.50	98.8	60	38.8	3.658	-.0329X	0.058
		1.38	97.6		37.6	3.627	+3.676	
		6.27	91.9		31.9	3.463	r=.99	
		21.18	79.7		19.7	2.981		
(5)	10.0	0	100		20.0	2.996		
		.17	99.5		19.5	2.970	Y=	
		.50	98.9		18.9	2.939	-.0534X	
		1.38	98.8	80	18.8	2.934	+2.986	0.111
		6.27	94.1		14.1	2.646	r=.99	
		21.18	86.4		6.4	1.856		

2. Calculation of S_o

Substrate added ($\mu g\ P\ \ell^{-1}$)	Rate constant (hr^{-1})	Assumed concentration of S_o ($\mu g\ P\ \ell^{-1}$)					
		1.0	0.74*	0.5	0.25	0.10	0.05
		Uptake velocity ($\mu g\ P\ \ell^{-1}\ hr^{-1}$)					
0	0.710	0.71	0.53	0.36	0.18	0.071†	0.036
1	0.122	0.24	0.21	0.18	0.15	0.13	0.13
2	0.128	0.38	0.35	0.32	0.29	0.27	0.26
5	0.058	0.35	0.33	0.32	0.30	0.29	0.29
10	0.111	1.24	1.21	1.19	1.15	1.14	1.14

*SRP concentration = 0.74 $\mu g\ P\ \ell^{-1}$.

†S_o concentration at which curve bends towards the x-y intercept.

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Rigler, F. 1966. Radiobiological analysis of inorganic phosphorus in lake water. Verh. int. Ver. Limnol. 16: 465-70.

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Appendix G

Cell Densities of C. erosa in Chemostat Culture

Cell densities of C. erosa are presented for the two growth vessels receiving intermediate (Fig. 1) and low (Fig. 2) rates of P supply (see Part II, Fig. 5 for data on the growth vessel receiving the highest rates of P supply). The figures show change in cell numbers resulting from changes in the dilution rate and the concentration of phosphorus in the reservoir. After each decrease in the dilution rate the cell density increased sharply and then decreased to the final steady state value. At very slow growth rates (Fig. 2) a decrease in the P concentration in the reservoir resulted in a transition stage that lasted up to 60 days. Although the existence of such transient phenomena render the use of chemostats a tedious enterprise, they lend support to the doubts about the value of batch culture work at low nutrient levels.

Fig. 1. Cell density of C. erosa in growth vessel receiving intermediate rates of P supply. S_R = concentration of phosphate in reservoir (μg P ℓ^{-1}); ($\downarrow$) = steady state growth rate (cell div. day^{-1}).

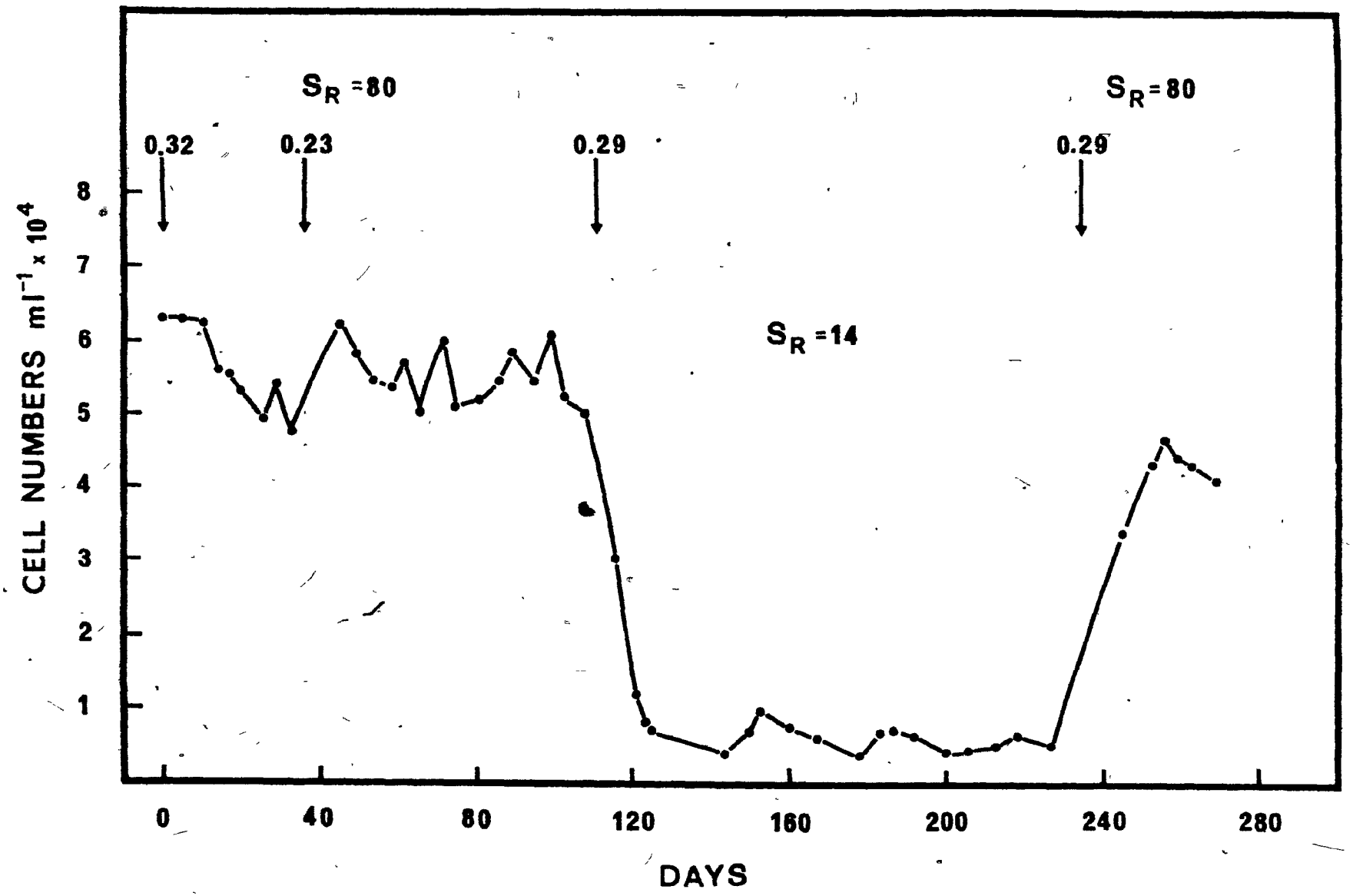
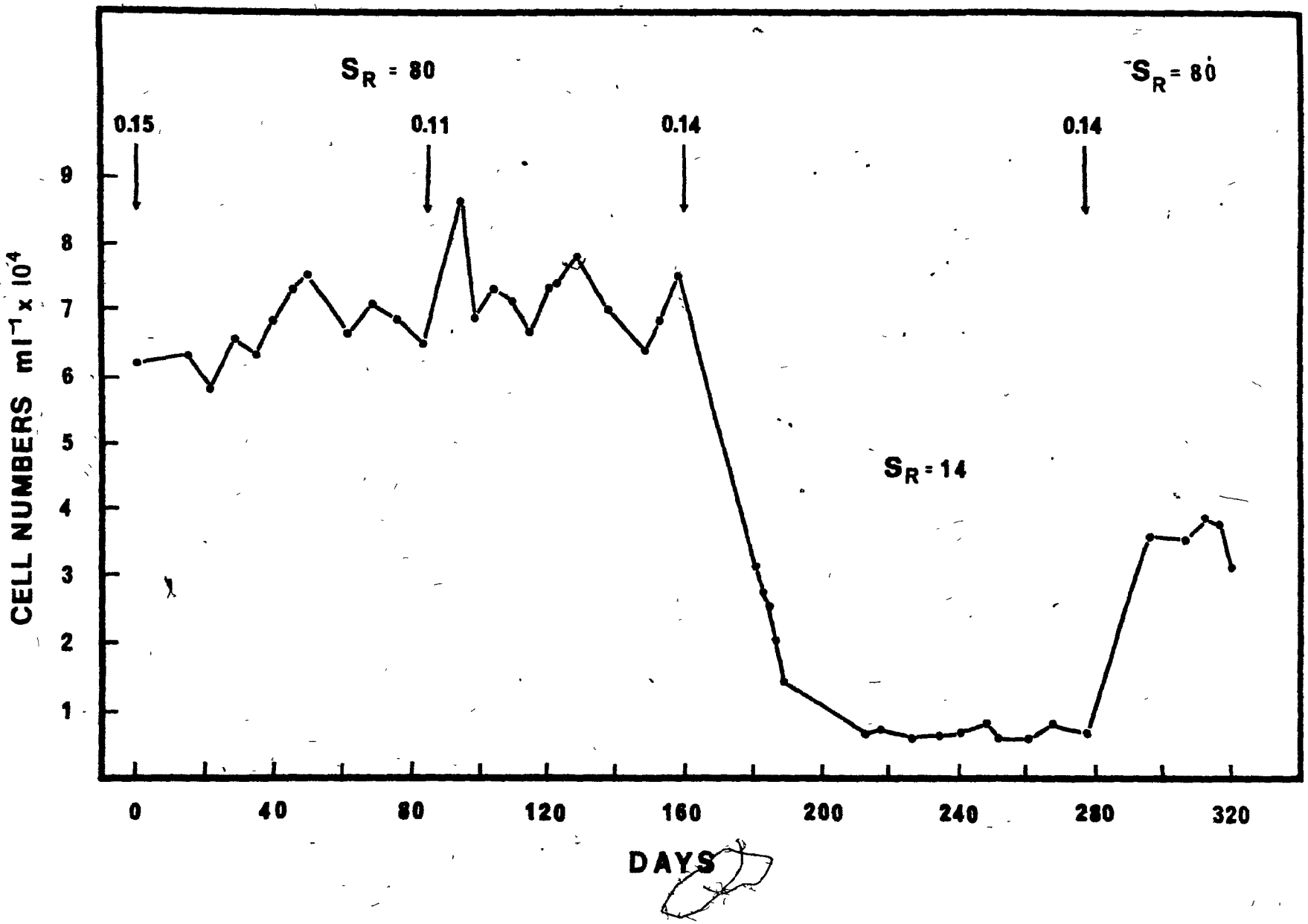

S_R =80
S_R =80
0.32
0.23
0.29
0.29
S_R =14
CELL NUMBERS $ml^{-1} \times 10^4$
8
7
6
5
4
3
2
1
0
40
80
120
160
200
240
280
DAYS

Fig. 2. Cell density of C. erosa in growth vessel receiving low rates of P supply. S_R = concentration of phosphate in reservoir (µg P ℓ^{-1}); (↓) = steady state growth rate (cell div. day^{-1}).


S_R = 80
S_R = 80
0.15
0.11
0.14
0.14
S_R = 14
CELL NUMBERS $ml^{-1} \times 10^4$
1
2
3
4
5
6
7
8
9
0
40
80
120
160
200
240
280
320
DAYS

Appendix H

Maximum Cell Division Rate of C. erosa in Chemostat Culture

Maximal growth was determined by increasing the dilution rate to effect washout of cells. A dilution rate of 0.50 day^{-1} resulted in a slow decline in cell density (Table 1). The cell division rate during this period was calculated from the equation

$$X_2 = X_1 e^{(\mu-D)(t_2-t_1)}$$

where X_1 and X_2 are cell densities (ml^{-1}) at times t_1 and t_2, and μ and D are the specific growth rate and dilution rate, respectively. The maximum growth rate was approximately 0.70 cell div. day^{-1}, similar to the maximum rate measured in batch culture at 15°.

Table 1. Decline in cell density over time at D = 0.50 day^{-1}

Day	Mean cell density (ml^{-1})
0	50,237
5	43,927
7	41,610
10	38,986
12	36,930
14	35,327
18	31,881
21	29,612
24	27,593

Appendix I

Photosynthesis-Light Response of C. erosa in Control Versus Phosphate Enriched Chemostat Populations

The relationship between photosynthetic carbon assimilation and light was determined for C. erosa grown under various degrees of P-deficiency in chemostat culture. Samples removed from the chemostats were divided into control samples, for which a P-I curve was determined immediately, and into enriched samples, which were first incubated 24 hrs with 50 μg ℓ^{-1} added P. To each set of samples was added 0.125-0.25 μCi ml^{-1} $NaH^{14}CO_3$, and replicate (2) aliquots were placed in a light-gradient box at light intensities up to 2-3 times those present at the chemostat centers (Appendix E). Following a 1-2 hr incubation, the cells were filtered on 0.45 μm membrane filters and filter activity assayed in a geiger system. In addition the cell density was determined before and after the 24 hr phosphate enrichment.

The results show (Table below; also Part II, Fig. 11) that phosphate enrichment had little effect on the photosynthetic response of C. erosa grown at higher rates of P supply ($\mu > 0.22$ div. day^{-1}), but significantly increased the P_{max}, I_k and, in most experiments, the I_i of severely P-deficient cells. The initial slope of the curves was, however, unaffected by P enrichment. The cell division rate of enriched cells was almost always greater than the steady state growth rate in the chemostats, with the largest increase again occurring in extremely P-deficient cells (Table below). The cell numbers did not, however, increase at the maximum

growth rate (0.70 div. day^{-1}). Two possible reasons for the submaximal growth rate are that (1) the synthetic processes involved in cell division were not fully activated within 24 hr of enrichment, and (2) that the enrichments operated as <u>in vitro</u> batch experiments, depressing growth.

Growth rate in chemostats (div.day^{-1})	Cells ml^{-1} t_0	Cells ml^{-1} t_{24}	Growth rate in enriched samples (div.day^{-1})	Initial slope (rate/ly min^{-1})	P_{max} (pgm C cell^{-1} day^{-1})	I_k (ly min^{-1} × 10^{-3})	I_i (ly min^{-1} × 10^{-3})	Inhibitory slope (rate/ly min^{-1})
S_R = 80								
(1) 0.39	C 54290	-	-	11204X - 4.5	136	12.7	16.6	-3295X + 191
	E 54290	70361	0.38	10780X - 2.6	130	11.8	NO INHIBITION	
(2) 0.23	C 51942	-	-	9457X - 2.2	101	11.0	13.9	-1804X + 120
	E 51942	63980	0.30	12863X - 3.7	135	9.8	14.5	-2928X + 177
(3) 0.11	C 73443	-	-	5827X + 2.3	25	3.8	12.5	-860X + 35
	E 73443	88917	0.28	6821X + 2.6	60	8.4	10.2	-1245X + 74
S_R = 14								
(1) 0.55	C 6235	-	-	13865X - 9.4	165	12.6	21.9	-2951X + 233
	E 6235	9250	0.57	13175X - 4.5	157	12.2	NO INHIBITION	
(2) 0.55	C 5899	-	-	14147X + 0.3	148	10.4	NO INHIBITION	
	E 5899	9439	0.68	12010X + 3.0	158	12.9	16.7	-4081X + 227
(3) 0.29	C 4333	-	-	14003X - 7.9	125	9.5	14.1	-3341X + 173
	E 4333	6013	0.48	10355X - 4.0	165	16.4	21.5	-4265X + 257
(4) 0.29	C 6506	-	-	10051X + 5.2	143	13.6	16.8	-5219X + 231
	E 6506	9327	0.52	12084X + 3.6	140	11.3	16.6	-5098X + 225
(5) 0.14	C 6942	-	-	10581X - 0.5	59	5.3	6.0	-1715X + 70
	E 6942	8940	0.37	8917X + 1.6	106	11.9	14.7	-3348X + 155
(6) 0.14	C 6580	-	-	11652X + 2.2	60	5.0	10.2	-2208X + 88
	E 6580	9011	0.46	11418X + 1.0	93	8.1	16.3	-2906X + 140

C = control. E = enriched. Regressions of initial and inhibitory slopes are significant at $p \leq 0.05$.
"No inhibition" indicates lack of significance at $p \leq 0.05$.

Appendix J

Cell Loss of ^{32}P on Filtration

In preliminary work ^{32}P uptake by C. erosa was investigated with cells filtered at both high and low vacuums, and concentrated by low speed centrifugation. Low vacuum filtration was at ~~4-5~~ 200-250 mm Hg with the vacuum broken just as the filter sucked dry, whereas high vacuum filtration was at ~~15-20~~ 750-1000 mm Hg, with the filter sucked dry for approximately 5 seconds. The procedure for centrifugation was as follows: (1) A 5 ml subsample at each ^{31}P addition was centrifuged for 2 min at 5300 rpm, which spun down >95% of the cells and essentially terminated ^{32}P uptake in the top 2-3 ml of liquid. (2) The upper 2 ml were immediately removed and recentrifuged for 10 min at 5300 rpm, which removed all cells from suspension. (3) 0.5 ml surface aliquots were placed in 10 ml of Aquasol^{R} and the activity was determined in a liquid scintillation system.

Filtered and centrifuged results are given as % ^{32}P incorporated into the cells. Typical results (Table 1) show that in comparison to centrifuged cells, those filtered at low and high vacuum contained, respectively, approximately 10 and 20% less activity. Thus filtration, especially at high vacuums, results in P loss from the cells, presumably from cell breakage and leakage. Although centrifugation yields the potentially best estimate of P uptake, the technique is subject to considerable error at high P additions when the % P^{32} remaining in solution is only fractionally less than the total amount added. In

addition centrifugation is a more lengthy procedure than filtration and is thus not amenable to routine analysis, nor can short term (e.g. <15 min) ^{32}P uptake be accurately measured unless uptake is terminated by killing the cells. Other work in our laboratory indicates that killing both C. erosa and a planktonic diatom with Lugol's iodine results in an up to 30% loss of incorporated P. We conclude that filtration is the best procedure for routine analysis, but that care must be taken to maintain low vacuum pressures.

Table 1. ^{32}P Uptake compared by low and high vacuum filtration and by centrifugation. Data are expressed in % removal of ^{32}P by the cells

^{31}P Added µg P ℓ^{-1}	Filtration 750-1000 mm (15-20)	Filtration 200-250 mm (4-8)	Centrifugation	% Loss at high vacuum	% Loss at low vacuum
I. S_R = 80 µg P ℓ^{-1}; k = 0.39 div. day^{-1}; 0.5 hr incubation					
0	78.7	84.9	93.2	15.6	7.8
1	11.7	12.9	13.7	14.6	5.9
2	7.6	8.3	9.1	16.5	8.8
5	3.7	4.1	4.2	11.9	2.4
10	2.1	2.4	2.7	22.2	11.1
50	0.54	0.61	0.72	25.0	15.3
II. S_R = 14 µg P ℓ^{-1}; k = 0.55 div. day^{-1}; 1.0 hr incubation					
0	26.3	28.9	32.7	19.6	11.6
1	3.9	4.2	4.3	9.3	2.3
2	2.4	2.8	2.8	14.3	0.0
5	1.0	1.2	1.4	28.6	14.3
10	0.70	0.73	0.83	15.6	12.1
50	0.16	0.2	0.2	20.0	0.0

Appendix K

Phosphorus Uptake Kinetics in C. erosa

Phosphorus uptake kinetics in C. erosa was examined two or three times at each steady state growth rate in the chemostats (14 experiments in all). Michaelis-Menten kinetics were evaluated using a Woolf plot, in which S/v is graphed against S, where S is the phosphate concentration added (μg P ℓ^{-1}) and v is the uptake rate (μg P ℓ^{-1} hr^{-1}), at each concentration. An unweighted least squares regression was fitted to the points. The inverse of the slope provides a measure of the maximum uptake rate (V_{max}), and the x-intercept a measure of $K_s + S_o$, where K_s is the half-saturation constant for uptake and S_o is the concentration of P in the sample prior to substrate addition (i.e. the residual phosphate concentration in the chemostat). If, as in the present study, S_o is much less than K_s, then the x-intercept is an adequate measure of K_s. The kinetic parameters V_{max} and K_s were calculated for each sampling time (0.17, 0.5, 1.0-2.0, ≈6.0 and ≈24 hr). Since the uptake of phosphorus was nonlinear, with V_{max} decreasing over time, the uptake rates at 0.17 and 0.5 hr were graphically extrapolated (in semi-log plots) to zero time. The V_{max} (zero-time) was expressed in terms of pgm P $cell^{-1}$ day^{-1}. Estimates of $K_s + S_o$ did not similarly change with time, permitting the calculation of an average $K_s + S_o$ for each uptake experiment. The data and calculations for a single P uptake experiment at each growth rate are presented below. For each sampling time are given the % ^{32}P removed

from solution, the uptake velocity, v ($\mu g\ P\ \ell^{-1}\ hr^{-1}$) and the calculation S/v (hr^{-1}). All regressions of S/v vs S are significant at $p \leq 0.05$.

(1) S_R = 80 μ = 0.39 div. day^{-1} Cell numbers = 50520 ml^{-1}

Phosphate added (μg P ℓ^{-1})	Incubation time (hr) 0.17 %	v	S/v	0.50 %	v	S/v	2.0 %	v	S/v	6.0 %	v	S/v
0	60.7	-	-	80.7	-	-	90.4	-	-	90.6	-	-
1	10.8	.65	1.54	20.6	.41	2.43	39.3	.20	5.09	60.8	.10	9.87
2	6.9	.83	2.42	13.9	.56	3.60	28.6	.29	6.99	40.7	.14	14.74
5	4.0	1.20	4.16	8.0	.67	6.25	17.2	.43	11.6	23.8	.20	25.2
10	2.8	1.68	5.95	4.9	.98	10.20	10.1	.51	19.8	15.2	.25	39.5
20	1.9	2.28	10.41	2.9	1.16	17.20	8.4	.84	23.8	10.8	.36	55.6
100	0.35	2.14	47.6	0.7	1.40	71.4	1.7	.85	117.6	2.4	.40	250
Regression Y =	.473X + 1.59			0.77X + 2.10			1.12X + 4.99			2.39X + 10.81		
V_{max}	2.11			1.30			0.89			0.42		
$K_s + S_o$	3.36			2.73			4.45			4.52		

$\overline{X}$ $K_s + S_o$ = 3.77 ± 0.87

V_{max} (zero time) = 3.10 μg P ℓ^{-1} hr^{-1}

V_{max} (zero time) cell^{-1} = 1.33 pgm P cell^{-1} day^{-1}

(2) S_R = 80 μ = 0.23 div. day^{-1} Cell numbers = 52,245 ml^{-1}

Phosphate added (μg P ℓ^{-1})	Incubation time (hr) 0.17			0.50			2.0			6.0		
	%	v	S/v	%	v	S/v	%	v	S/v	%	v	S/v
0	57.2	-	-	83.1	-	-	83.7	-	-	90.1	-	-
1	9.7	.58	1.72	19.1	.38	2.62	46.2	.23	4.33	73.2	.12	8.19
2	6.0	.72	2.78	12.7	.51	3.94	25.7	.26	7.79	50.6	.17	11.86
5	2.8	.84	5.95	6.1	.61	8.20	14,2	.36	14.08	25.7	.21	23.34
10	2.0	1.20	8.33	3.9	.78	12.82	8.9	.45	22.47	18.1	.30	33.10
20	1.5	1.80	11.11	2.7	1.08	18.51	5.2	.52	38.46	9.8	.33	61.2
100	0.35	2.10	47.61	0.6	1.20	55.56	1.2	.60	166.7	2.0	.33	300.0
Regression (Y=)	.451X + 2.53			.803X + 2.75			1.62X + 5.05			2.94X + 5.29		
V_{max}	2.21			1.24			0.62			0.34		
$K_s + S_o$	5.61			3.42			3.12			1.80		

$\overline{X}$ $K_s + S_o$ = 3.49 ± 1.58

V_{max} (zero time) = 2.70 μg P ℓ^{-1} hr^{-1}

V_{max} (zero time) cell^{-1} = 1.24 pgm P cell^{-1} day^{-1}

(3) S_R = 80 μ = 0.11 div. day^{-1} Cell numbers = 74,318 ml^{-1}

Phosphate added (μg P ℓ^{-1})	Incubation time (hr) 0.17			0.50			2.0			6.0		
	%	v	S/v	%	v	S/v	%	v	S/v	%	v	S/v
0	60.7	-	-	86.4	-	-	89.8	-	-	91.0	-	-
1	12.1	.73	1.38	20.7	.41	2.42	47.2	.24	4.23	70.4	.11	8.52
2	7.4	.89	2.25	13.8	.55	3.62	29.3	.29	6.83	51.9	.17	11.6
5	5.2	1.29	3.21	7.1	.71	7.04	13.7	.34	14.60	26.9	.22	22.3
10	3.4	1.62	4.90	5.0	1.00	10.0	8.2	.41	24.39	20.1	.34	29.9
20	2.4	2.88	6.94	3.4	1.36	14.7	5.9	.59	33.95	11.2	.37	53.6
100	0.4	2.40	41.7	0.45	1.40	71.4	1.4	.70	143.0	2.4	.40	250.0
Regression (Y=)	.407X + 0.71			0.69X + 2.35			1.37X + 6.27			2.43X + 6.79		
V_{max}	2.46			1.45			0.73			0.41		
$K_s + S_o$	1.74			3.41			4.58			2.80		

$\overline{X}$ $K_s + S_o$ = 3.13 ± 1.19

V_{max} (zero time) = 3.20 μg P ℓ^{-1} hr^{-1}

V_{max} (zero time) cell^{-1} = 1.03 pgm P cell^{-1} day^{-1}

(4) S_R = 14 μ = 0.55 div. day^{-1} Cell Numbers = 6185 ml^{-1}

Phosphate added (μg P ℓ^{-1})	Incubation time (hr) 0.17			0.50			1.0			6.0			24.0		
	%	v	S/v	%	v	S/v	%	v	S/v	%	v	S/v	%	v	S/v
0	9.2	-	-	18.9	-	-	35.2	-	-	68.9	-	-	73.1	-	-
.5	3.1	.09	5.4	4.8	.05	10.5	8.3	.04	12.0	20.5	.017	29.3	51.7	.011	46.4
1	1.8	.11	9.1	3.0	.06	16.6	5.2	.05	19.2	13.4	.022	44.8	37.8	.016	63.5
2	1.2	.14	13.9	2.4	.10	20.8	3.4	.07	29.4	8.7	.029	69.0	25.2	.021	95.2
5	0.6	.18	27.8	1.3	.13	38.5	1.6	.08	62.5	3.9	.033	154	12.7	.026	189
10	0.4	.24	41.7	LOST			1.0	.10	100	3.2	.053	188	8.2	.034	293
50	0.1	.30	167	0.2	.20	250	0.3	.14	370	0.9	.075	667	3.1	.065	774
Regression (Y=)	3.20X + 7.57			4.78X + 11.37			7.10X + 17.78			12.50X + 49.67			14.16X + 81.80		
V_{max}	0.31			0.21			0.14			0.08			0.07		
$K_s + S_o$	2.36			2.37			2.50			3.98			5.78		

$\overline{X}$ $K_s + S_o$ = 3.40 ± 1.50

V_{max} (zero time) = 0.38 μg P ℓ^{-1} hr^{-1}

V_{max} (zero time) cell^{-1} = 1 47 pgm P cell^{-1} day^{-1}

(5) $S_R = 14$ $\mu = 0.29$ div. day^{-1} Cell Numbers = 4030 ml^{-1}

Phosphate added (µg P ℓ$^{-1}$)	Incubation time (hr) 0.17			0.50			1.38			6.27			21.18		
	%	v	S/v	%	v	S/v	%	v	S/v	%	v	S/v	%	v	S/v
0	12.4	-	-	23.9	-	-	40.1	-	-	78.2	-	-	74.8	-	-
1	2.4	.14	6.9	4.2	.084	11.9	6.9	.050	19.9	20.7	.033	30.3	46.3	.022	45.8
2	1.5	.18	11.4	2.6	.104	19.2	4.0	.058	34.4	13.8	.044	45.4	30.7	.029	59.0
5	0.81	.24	30.6	1.2	.12	41.7	2.4	.086	58.5	8.1	.065	77.4	20.3	.048	104
10	0.53	.32	31.4	1.1	.22	45.4	1.2	.087	115	5.9	.094	106	13.6	.064	156
50	0.13	.39	128	0.36	.36	139	0.5	.181	276	1.4	.112	448	3.2	.076	662
Regression (Y=)	2.43X + 6.83			2.44X + 18.2			4.97X + 33.1			8.42X + 27.0			12.48X + 37.24		
V_{max}	0.41			0.41			0.20			0.12			0.08		
$K_s + S_o$	2.81			7.45			6.66			3.21			2.99		

$\overline{X}\ K_s + S_o = 4.02 \pm 2.24$

V_{max} (zero time) = 0.41 µg P ℓ$^{-1}$ hr^{-1}

V_{max} (zero time) cell^{-1} = 2.44 pgm P cell^{-1} day^{-1}

(6) S_R = 14 μ = 0.14 div. day^{-1} Cell Numbers = 7480 ml^{-1}

Phosphate added (μg P ℓ^{-1})	Incubation time (hr) 0.17 %	v	S/v	0.50 %	v	S/v	1.37 %	v	S/v	5.47 %	v	S/v	20.10 %	v	S/v
0	8.42	-	-	23.1	-	-	38.7	-	-	80.7	-	-	86.4	-	-
1	1.39	.083	12.0	4.7	.094	10.6	8.2	.060	16.7	18.7	.031	29.3	39.2	.020	51.3
2	0.85	.102	19.6	2.6	.104	19.2	5.1	.074	26.9	11.2	.041	53.6	24.2	.024	83.1
5	0.57	.171	29.2	1.2	.12	41.7	3.1	.113	44.2	4.6	.043	130	16.1	.040	125
10	0.43	.26	38.8	0.95	.19	52.6	2.4	.175	57.1	3.8	.069	158	12.4	.062	162
50	0.16	.48	104	0.28	.28	179	0.7	.256	196	0.9	.082	608	3.0	.075	670
Regression (Y=)	21.77X + 16.65			3.29X + 15.87			3.53X + 20.1			11.41X + 40.7			12.39X + 49.80		
V_{max}	0.56			0.30			0.28			0.09			0.08		
$K_s + S_o$	9.39			4.82			5.69			3.57			4.02		

$\overline{X}$ $K_s + S_o = 5.50 \pm 2.32$

V_{max} (zero time) = 0.77 μg P ℓ^{-1} hr^{-1}

V_{max} (zero time) cell^{-1} = 2.47 pgm P cell^{-1} day^{-1}

Appendix L

The Decline in Cell Numbers (ml^{-1}) and Cell Volume (μ^3) of C. erosa in the Dark - Additional Results

	Pre-dark conditions				Results							
	Temp. (°C)	Light (ly $min^{-1} \times 10^{-3}$)	Dark Temp. (°C)	Replicate #	Days in the dark							
(1)	15	8.6	15		0	1	7	10	15			
		Cell numbers		1	2000*	2830	420	27	10			
				2	2000*	2550	293	30	10			
		Mean cell volume†			1035	-	770	-	-			
(2)	15	2.0	15		0	2	4	6	8	10		
		Cell numbers		1	1800*	515	250	135	30	0		
				2	1800*	790	380	80	0	-		
		Mean cell volume†			818	-	710	646	-	-		
(3)	15	1.0	15		0	2	4					
		Cell numbers		1	2000*	175	15					
				2	2000*	170	0					
		Mean cell volume†			786	624	-					
(4)	4	5.6	4		0	4	13	20	30	44	54	61
		Cell numbers		1	5330	5800	4590	3070	700	90	30	10
				2	4250	4550	3580	2390	410	150	60	20
				3	5280	5570	4340	2440	830	110	60	30
		Mean cell volume		1	3995	2925	1864	1538	1352	1182	962	-
				2	3450	2451	1713	1655	1422	1293	1080	-
				3	3554	2201	1889	1524	1455	1210	-	-

	Pre-dark conditions		Dark		Results							
	Temp. (°C)	Light (ly $min^{-1} \times 10^{-3}$)	Temp. (°C)	Replicate #	Days in the dark							
(5)	4	2.0	4		0	2	8	16	25	30	36	
		Cell numbers		1	2000*	2120	1989	830	60	20	0	
				2	2000*	1890	1900	950	90	40	10	
		Mean cell volume†			1443	1184	934	571	549	-	-	
(6)	4	1.0	4		0	1	15	25	29			
		Cell numbers		1	2000*	2120	360	10	0			
				2	2000*	1865	400	20	0			
		Mean cell volume†			1128	793	612	-	-			
(7)	4	2.0	1		0	4	11	18	26	34	41	50
		Cell numbers		1	2000*	1880	1770	1920	1080	310	75	0
				2	2000*	1630	1870	1750	890	200	90	0
		Mean cell volume†			1443	1092	-	734	634	624	-	-
(8)	4	1.0	1		0	1	14	29	37	46		
		Cell numbers		1	2000*	1770	1440	160	10	0		
				2	2000*	1985	1445	90	20	0		
		Mean cell volume†			1128	935	765	685	-	-		

Pre-dark conditions				Results					
Temp. (°C)	Light (ly $min^{-1} \times 10^{-3}$)	Dark Temp. (°C)	Replicate #	Days in the dark					
				0	17	44	65	75	85
(9) 1	1.0	1							
	Cell numbers		1	650	730	410	100	10	0
			2	790	860	230	80	0	0
			3	880	930	360	190	40	10
	Mean cell volume		1	3059	2496	1701	1492	-	-
			2	2640	2399	1794	1362	-	-
			3	3141	1987	1630	1294	-	-

*Estimated density of inoculant.

†Mean cell volume of cells from both replicates.

Appendix M

Carbon Loss per Cell in the Dark at Various Temperatures

Carbon loss in the dark was calculated from the decline in cell volume, using the regressions of cell carbon versus cell volume (Part I, Fig. 4). The loss was determined during the time in which little or no cell death occurred. At low temperatures cell volume decreased more rapidly in the first few days of incubation and therefore carbon loss was calculated only for the period of slower decline which followed. The initial rapid loss may represent excretion of carbon, whereas the subsequent slower loss is interpreted to result primarily from the endogenous respiration of stored carbohydrate. A much more rapid decline in cell numbers at higher temperatures necessitated that carbon loss be calculated within the first few days of incubation. These estimates may include an excretion component and thus overestimate the respiration rate of stored carbon. Nevertheless, the data indicate a marked increase in respiration with increasing temperature, which leads to rapid cell death at high temperatures. The calculations are presented below.

	Growth conditions		Dark temp. (°C)	Days in dark	Replicate #	Cell numbers (ml^{-1})	Mean cell volume (μ^3)	Cell carbon (pgm $cell^{-1}$)	Carbon loss (pgm C $cell^{-1}$ day^{-1})
	Temp. (°C)	Light (ly $min^{-1} \times 10^{-3}$)							
(1)	23.5	21.5	23.5	0	1	964	1332	209	-
					2	1040			
					3	984	1680	261	-
					4	947			
				1	1	843	1080	157	52
					2	793	988	149	60
					3	874	1163	184	77
					4	1039	1221	192	71
				2	significant decline in cell numbers				
(2)	23.5	2.0	23.5	0	1	687	950	152	-
					2	704			
					3	747	1050	167	-
					4	730			
				1	1	517	730	119	33
					2	604	657	108	44
					3	555	689	113	54
					4	611	700	114	53
				2	significant decline in cell numbers				
								$\bar{X}$ at 23.5° = 55.5 ± 14.1	
(3)	15	8.6	15	0	1	2030	1605	250	-
					2	1864	1632	254	-
					3	2189	1539	240	-
					4	1793	1465	229	-
					5	1923	1519	237	-
				3	1	1816	786	127	41
					2	1670	734	119	45
					3	1798	699	114	42
					4	1650	764	124	35
					5	1749	657	108	43

	Growth conditions		Dark	Days		Cell	Mean cell	Cell	Carbon loss
	Temp. (°C)	Light (ly $min^{-1} \times 10^{-3}$)	temp. (°C)	in dark	Replicate #	numbers (ml^{-1})	volume (μ^3)	carbon (pgm $cell^{-1}$)	(pgm C $cell^{-1}$ day^{-1})
(4)	15	1.0	15	0	1	1592	1028	163	-
					2	1730	1184	187	-
					3	1618	1051	167	-
				2	1	1310	710	116	24
					2	1465	780	126	31
					3	1301	671	110	29
								$\bar{X}$ at 15° = 36.2 ± 7.7	
(5)	4	5.6	4	0	1	5330	3995	822	-
					2	4250	3450	710	-
					3	5280	3554	732	-
				4	1	5800	2925	603	-
					2	4550	2451	506	-
					3	5570	2201	454	-
				13	1	4590	1864	385	24 [$t_4 - t_{13}$]
					2	3580	1713	354	17
					3	4340	1889	391	7
(6)	4	2.0	4	0	1	est 2000	1443	299	-
					2				
				2	1	1880	1092	246	-
					2	1630			
				8	1	1770	834	174	12.0 [$t_2 - t_8$]
					2	1870			
(7)	4 (Algal-Bacterial)	2.0	4	0	1	est 2000	1595	330	-
					2				
				2	1	2120	1360	282	-
					2	2820			
				8	1	2155	904	189	-
					2	2290			
				16	1	1755	595	125	11.2 [$t_2 - t_{16}$]
					2	1540			

	Growth conditions		Dark temp. (°C)	Days in dark	Replicate #	Cell numbers (ml^{-1})	Mean cell volume (μ^3)	Cell carbon (pgm $cell^{-1}$)	Carbon loss (pgm C $cell^{-1}$ day^{-1})
	Temp. (°C)	Light (ly $min^{-1} \times 10^{-3}$)							
(8)	4	1.0	- 4	0	1	6830	1167	243	-
					2	11750	1162	242	-
					3	9190	1165	242	-
				5	1	8100	1030	215	-
					2	13950	1009	210	-
					3	9710	959	200	-
				7	1	5540	841	176	-
					2	13050	855	179	-
					3	10930	903	188	-
				9	1	6680	819	171	11.0
					2	11380	729	152	14.5 $[t_5 - t_9]$
					3	9170	850	178	5.5
									$\bar{X}$ at 4° = 12.8 ± 5.8
(9)	4	2.0	1	0	1	est 2000	1443	299	-
					2				
				4	1	1880	1092	227	-
					2	1630			
				18	1	1920	734	154	5.21 $[t_4 - t_{18}]$
					2	1750			
(10)	4 (Algal-Bacterial)	2.0	1	0	1	est 2000	1595	330	-
					2				
				3	1	1870	1193	248	-
					2	2190			
				18	1	1895	821	172	5.07 $[t_3 - t_{18}]$
					2	2200			

	Growth conditions		Dark	Days			Mean		
	Temp. (°C)	Light (ly $min^{-1} \times 10^{-3}$)	temp. (°C)	in dark	Replicate #	Cell numbers (ml^{-1})	cell volume (μ^3)	Cell carbon (pgm $cell^{-1}$)	Carbon loss (pgm C $cell^{-1}$ day^{-1})
(11)	4	1.0	1	0	1	est { 2000	{ 1128	234	-
					2				
				1	1	1770	{ 935	195	-
					2	1985			
				14	1	1440	{ 765	160	2.69 $[t_1 - t_{14}]$
					2	1455			
(12)	4 (Algal-Bacterial)	1.0	1	0	1	est { 2000	{ 1041	217	-
					2				
				1	1	1800	{ 886	185	-
					2	1750			
				14	1	1340	{ 669	141	3.38 $[t_1 - t_{14}]$
					2	1555			
(13)	4	0.41	1	0	1	est { 575	{ 891	186	-
					2				
				14	1	510	{ 889	186	0.0 $[t_0 - t_{14}]$
					2	460			
(13)	4 (Algal-Bacterial)	0.41	1	0	1	est { 575	{ 1563	324	-
					2				
				4	1	720	{ 1335	277	-
					2	710			
				26	1	540	{ 1005	209	3.09 $[t_4 - t_{16}]$
					2	600			

$\bar{X}$ at 1° = 3.24 ± 1.9

Appendix N

Bacterial Numbers in the Algal-Bacterial Cultures

Using sterile procedures, samples from the open waters of a nearby lake were centrifuged at speeds sufficiently low to effect a separation of bacteria and algae. Subsamples of the supernatant containing bacteria were inoculated into axenic cultures of C. erosa, and stock algal-bacterial populations were maintained at 15°. Although no attempt was made at taxonomic identification, the bacterial flora which developed was dominated by Vibrio-like rods, approximately 0.3 μm wide by 2 μm long. Experimental cultures of C. erosa grown at various low light levels at 15 and 4° were inoculated from the stocks to yield an initial bacterial density of approximately 10^3 cells ml^{-1}. The number of bacteria which developed in the cultures was not controlled. Total microscope counts of erythrosin-stained material showed the largest populations of bacteria in cultures at the lowest light levels, where very slow growth of C. erosa resulted in a long (up to 6 months) association of bacteria and algae (see Table). The maximum biomass of bacteria was estimated as 30-40% of the algal biomass. It was in these cultures that some C. erosa became enmeshed in a mucilaginous envelope, wherein a slowed cell division rate may have accounted for their increased cell volume and carbon content. Such large, carbon-laden cells almost surely resulted in the enhanced dark survival of the algal populations (Part IV, Table 2). However, the dark survival of C. erosa in algal-bacterial cultures incubated directly in the dark was

no greater than its survival in cultures where bacterial numbers were first diluted 5-10 times with fresh medium. Although this result does not preclude some ingestion of bacteria, phagotrophy is not a significant source of carbon, even at the high bacterial numbers in our cultures. At much lower concentrations of bacteria in the open waters of oligotrophic lakes, phagotrophic nutrition of planktonic algae must be of minor importance.

Table 1. Bacterial numbers in algal-bacterial cultures at the time of dark incubation. Data are direct microscope counts of filters stained with erythrosin B

	Growth conditions		
	Temp. (°C)	Light (ly $min^{-1} \times 10^{-3}$)	Cell counts ($X10^5$ ml^{-1})
(1)	15	2.0	1.8 ± 1.1
(2)	15	1.0	4.9 ± 2.1
(3)	15	0.62	8.7 ± 3.6
(4)	4	2.0	0.7 ± 1.1
(5)	4	1.0	2.0 ± 0.9
(6)	4	0.62	3.7 ± 2.4
(7)	4	0.24	6.3 ± 3.1

GENERAL CONCLUSIONS

Growth of the freshwater phytoflagellate Cryptomonas erosa Skuja in nutrient sufficient batch culture was maximal at moderately high light and temperatures (.045 ly min^{-1}; 23.5°), whereas at low temperatures growth was much reduced, and the cells saturated and inhibited at very low light intensities. The imposed stress of continuous illumination, or more likely, a high total salts concentration may well be responsible for the poor growth in culture at the same low temperatures at which it thrives in nature. In addition the possession of significant quantities of phycoerythrin increases its susceptibility to light stress and may, in part, account for the depth distribution of cryptomonads in nature. Since, in culture, low temperatures more adversely affected cell division than carbon uptake, the resulting excess production of photosynthate was either retained as storage carbohydrate or excreted. The wide range in cell volume of C. erosa (300-9000 μ^3) makes questionable the significance of cell size in cryptomonad taxonomy. Although cryptomonads and other nanoplankton normally grow faster than diatom and blue-green species, their absolute abundance in eutrophic lakes is probably significantly limited by zooplankton grazing.

In chemostat culture carbon uptake and cell division of C. erosa were also differently affected by the rate of phosphorus supply. Photosynthesis was limited by light at high dilution rates, but by phosphorus at slow dilution rates, whereas cell division was P-limited at all growth rates examined. Phosphorus-limited cells show increases

in mean cell volume, carbon content and refractility, whereas chlorophyll and nitrogen content are little affected. The C:P and N:P atomic ratios reflect the availability of phosphorus, but such ratios are less useful in field assessments of deficiency because of contamination from detritus and heterotrophic organisms. Growth of C. erosa at the low phosphate concentrations present in the chemostats ($\leq$0.10 µg P ℓ^{-1}) was facilitated not by a large capacity to transport and store phosphate, but rather by a high affinity, low K_s system (K_s = 0.14µ MP). Since the production of transport enzymes are potentially in competition with one another, the simultaneously low concentrations of many essential nutrients in nature may limit the energy and materials available for the production of any one permease. Thus, the high v_{max} so often measured in laboratory cultures may not be realized in oligotrophic environments, and selection instead favours growth of C. erosa and other low K_s species.

The photosynthetic response of C. erosa grown under low light and temperatures and phosphate deficiency was characterized by a lowered photosynthetic capacity (P_{max}) and by saturation and inhibition at low light levels (low I_k and I_I). The reduction in photosynthetic capacity occurred without any accompanying major change in the rate/intensity characteristic at low light levels or in the chlorophyll content of the cells. Thus in suboptimal environments, the principal stage of photosynthesis affected is the rate of the dark enzyme reactions. It is suggested that at low temperatures, P_{max} is lowered through a decrease in the rate constant of the dark reactions, whereas under low light or P-deficiency, dark enzymes are selectively lost from the cell. In

nutrient-rich batch cultures, light and temperature determined the photosynthetic response of the cells, whereas in chemostat cultures the effect of phosphorus limitation was readily manifest. In nature, too, the abundant data linking eutrophication with high phytoplankton productivity indicates that the photosynthetic response of natural populations is largely nutrient dependent. While some species are able to adapt extensively to different light intensities through variation in pigment content, light adaptation in C. erosa was primarily effected through change in dark enzyme function. In addition, the ability of motile species to maintain themselves in a zone of optimal light intensity and thus maximal carbon uptake, is an important strategy for species success, which facilitates the cosmopolitan distribution of C. erosa and other phytoflagellates.

Incubation of C. erosa in the dark resulted in a decline in cell numbers and cell volume/cell carbon. At high temperatures reserve carbon was quickly exhausted, followed by rapid cell death, whereas at low temperatures the alga survived a lengthy dark period (up to 80 days) because of slowed respiration of storage carbohydrate. The ability of the alga to expand in size to accommodate large carbon reserves is thus an important mechanism for dark survival. In culture, heterotrophic metabolism of C. erosa was negligible. Similarly, the low concentrations of available organics in the open waters of lakes and the inability of most planktonic algae to compete efficiently with aquatic bacteria for organic substrates must also preclude heterotrophy as a significant carbon source for natural populations. Whereas algae in snow and ice-covered temperate lakes may frequently receive sufficient radiation to at least

compensate respiration, the survival of cryptomonads through the three month polar night appears dependent on the respiration of stored carbon.