A study of phytoplankton in the Lesser Antilles region. *Bull. Mar. Sci.* 20:331–49.
10. Hargraves, P. E. & Miller, B. 1974. The ebridian flagellate *Hermesinum adriaticum* Zach. *Arch. Protistenk.* 116: 280–4.
11. Hasle, G. R. 1974a. Some marine plankton genera of the diatom family Thalassiosiraceae. *Nova Hedwigia Beih.* 45:1–68.
12. ——— 1974b. The "mucilage pore" of pennate diatoms. *Nova Hedwigia Beih.* 45:167–94.
13. ——— 1975. Some living marine species of the diatom family Rhizosoleniaceae. *Nova Hedwigia Beih.* 53:99–152.
14. Heiden, H. & Kolbe, R. W. 1928. Die marinen Diatomeen der Deutschen Südpolar Expedition, 1901–03. *Dtsch. Südpol. Exped.* 8:450–714.
15. Hendey, N. I. 1937. The plankton diatoms of the southern seas. *Discovery Rep.* 16:151–364.
16. ——— 1974. A revised check-list of British marine diatoms. *J. Mar. Biol. Assoc. U.K.* 54:277–300.
17. Hustedt, F. 1930. Die Kieselalgen. *In* Rabenhorst's *Kryptogamen–Flora von Deutschland, Oesterreich und der Schweiz.* 7(1):1–920.
18. ——— 1956. *Kieselalgen (Diatomeen).* Franckh'sche Verlagshandlung, Stuttgart. 70 pp.
19. Karsten, G. 1907. Das Indische Phytoplankton. *Wiss. Ergebn. Deutsch. Tiefsee-Epex. (Valdivia).* 2(2):1–548, Taf. 35–54.
20. Lebour, M. V. 1930. The Plankton Diatoms of Northern Seas. Ray Society Publ. London, 116:1–244.
21. Nel, E. A. 1968. The microplankton of the South-West Indian Ocean. *S. Afr. Div. Sea Fish. Invest. Rep.* 62:1–106.
22. Ricard, M. & Gasse, F. 1972. *Ethmodiscus appendiculatus* et *E. gazellae* en microscopic electronique a balayage. *Rev. Algol.* n.s. 10:312–8.
23. Ross, R. & Sims, P. A. 1974. Observations on family and generic limits in the Centrales. *Nova Hedwigia Beih.* 45: 97–130.
24. Simonsen, R. 1972. Ideas for a more natural system of the centric diatoms. *Nova Hedwigia Beih.* 39:37–54.
25. ——— 1974. The diatom plankton of the Indian Ocean Expedition of RV Meteor, 1964–1965. *"Meteor" Forschungergebn.* D(19):1–66. Gebrüder Borntraeger, Berlin.
26. Swift, E. 1973. The marine diatom *Ethmodiscus rex*: its morphology and occurrence in the plankton of the Sargasso Sea. *J. Phycol.* 9:456–60.
27. Taylor, F. J. R. 1966. Phytoplankton of the South Western Indian Ocean. *Nova Hedwigia* 12:433–76.
28. Venrick, E. L. 1969. The distribution and ecology of oceanic diatoms in the North Pacific. Ph.D. Dissertation, University California, San Diego. 653 pp.
29. Wood, E. J. F. 1965. Protoplankton of the Benguela-Guinea current region. *Bull. Mar. Sci.* 15:475–9.
30. ——— 1966. A phytoplankton study of the Amazon region. *Bull. Mar. Sci.* 16:102–23.

J. Phycol. **12**, 291–300 (1976)

CONTINUOUS CULTURE OF MARINE DIATOMS UNDER SILICATE LIMITATION. II. EFFECT OF LIGHT INTENSITY ON GROWTH AND NUTRIENT UPTAKE OF *SKELETONEMA COSTATUM*[1,2]

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SUMMARY

Two replicate experiments were conducted to investigate the effect of light intensity on the growth and nutrient uptake of Skeletonema costatum *(Grev.) Cleve in silicate-limited continuous culture. Each experiment began with 4 identical chemostat cultures of* S. costatum *growing at the normal laboratory light (0.14 ly·min^{-1}, continuous illumination) under strong silicate limitation. Screens were placed over 3 cultures reducing them to light intensities of 0.042, 0.021 and 0.0018 ly·min^{-1}.*

Based on growth rates, nutrient uptake rates, cell morphology and chemical composition, the cultures receiving 0.021, and 0.0018 ly·min^{-1} appeared to be light-limited, whereas the culture receiving 0.14 ly·min^{-1} was silicate-limited. The 0.021 and 0.0018 ly·min^{-1} cultures were not able to grow as fast as the dilution rate (0.04 h^{-1}), and they washed out of the reactor. The 0.042 ly·min^{-1} culture showed some characteristics of both light and silicate-limitation, maintaining ca. 70% of the population of the 0.14 ly·min^{-1} culture. In both of these cultures the effluent silicate concentration was 0.3–0.4 μg-at Si·l^{-1}. In the 0.042 ly·min^{-1} culture the population was still silicate-limited in the sense that the silicate concentration in the inflow medium controlled the number of cells grown; however, the growth characteristics, chemical composition and morphology of these cells were typical of light-limited cells and very different from those characteristics of the 0.14 ly·min^{-1} light population.

Key index words: chemostats; light; silicate; Skeletonema

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[2] Contribution No. 915 from the Department of Oceanography, University of Washington, Seattle. This work represents a portion of a dissertation submitted in partial fulfillment of the requirements for the Ph.D. degree.

INTRODUCTION

Phytoplankton growing in the surface layers of the ocean consume nutrients which are advected or mixed into the euphotic zone from depth. When the water column is stable, a gradient of nutrient concentration with the lowest values near the surface is established. An opposite gradient of light intensity, decreasing with depth, also exists. Thus the euphotic zone is frequently divided into two regions: i) an upper, nutrient-limited region, and ii) a lower, light-limited region.

Nutrient limitation and light limitation have been studied separately in many batch culture and field experiments, but only a few studies have looked at both limitations at the same time. The effect of reduced light intensity on the growth of N-starved and P-starved phytoplankters was examined by McAllister, *et al.* (26). Coombs, *et al.* (7) studied the photosynthetic rate of silicate-starved *Navicula pelliculosa* (Breb.) Hilse at different light intensities in batch culture. The interaction of light and inorganic N in controlling the uptake of N was the subject of recent field work by MacIsaac and Dugdale (25).

Numerous interactions between N and light limitation have been observed. Nitrate and ammonium uptake rates are higher in the light than in the dark (1,15–17,25). Conversely, chlorophyll *a* and photosynthetic enzymes all contain N and hence photosynthesis may be directly affected by N depletion. Similar interactions have been demonstrated for P and light limitation (23,24,26). No such interactions have been observed between silicate and light limitation. The objective of this study was to examine the effect of light intensity on nutrient-limited phytoplankton populations with as few complicating factors as possible. Therefore, it was decided to study the effect of light intensity on silicate-limited continuous cultures of phytoplankton to establish the effect of the 2 competing limitations, instead of examining the more complicated light-N and light-P cases.

Two replicate experiments were conducted using *Skeletonema costatum* (Grev.) Cleve is silicate-limited continuous culture. The chemostat-type continuous culture was chosen for this study as it allows the researcher maximum control over the growth rate and nutrient environment of the cell when silicate concentrations are very low (20). In this way it was possible to examine the long-term response of *S. costatum* to change in light intensity while still maintaining a controlled chemical environment for up to 32 days. During the course of the experiments a variety of standing stock, growth rate and uptake rate measurements were made to assess the physiological state of the populations.

METHODS

The chemostat system, sampling techniques, nutrient analyses, fluorometer and cell counting techniques have been described previously (11,12,27). Chlorophyll *a* analysis was done according to the SCOR-UNESCO procedure except that the filters were placed immediately into 90% acetone. Samples were stored in the cold and dark and analyzed within 24 h (4).

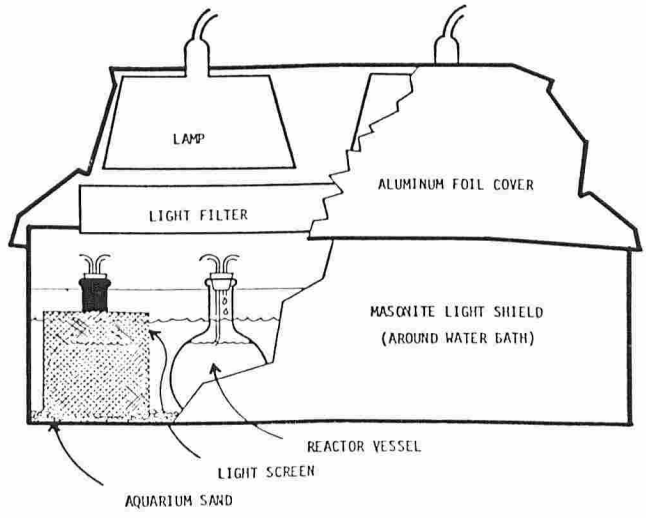

FIG. 1. Chemostat system as modified for use in light experiments. Light shield partially removed to show the interior. See text for details.

Chemostat experiments were initiated with an axenic inoculum of *Skeletonema costatum* (clone *Skel.* obtained from Dr. Joyce Lewin, University of Washington). All equipment was autoclaved, and the media was filtered through an HA Millipore filter. Low levels of bacteria (1 bacterium/100 *S. costatum*) were frequently observed, but no other algal species were observed.

The lighting system as described previously (12), provided 0.14 ly·min^{-1} color corrected to simulate 5 m underwater light under sunny conditions for Jerlov type 3 costal water. The chemostat system (12) was modified for use in light studies by the addition of light shields around the water bath containing the reactor vessels (Fig. 1). The shield was ⅛ inch thick, tempered masonite, painted flatblack on the side facing the reactor vessels. The surface on which the water bath rested was also flatblack. Spaces around the lights were covered with aluminum foil so that the entire system of lights, light filter and water bath with reactor vessels was sealed *ca.* 99% lighttight.

To obtain reduced light intensities, nickel screens (Perforated Products, Inc., Brookline, Massachusetts) were placed over the individual reactor vessels (Fig. 1). All joints or other possible light leaks were sealed with black electrical tape. Around the reactor on the bottom of the water bath was placed 1 cm aquarium sand as a base for the screens. The lower edge of the screen was imbedded in the sand producing a lighttight seal.

The light screens were initially identified by percent open space as claimed by the manufacturer. The actual amount of light passing through the screens was measured with an ISCO model SR Spectroradiometer and was slightly different (Table 1). When discussing the present work, the different light intensities are referred to as the 100, 30, 15 and 1% light levels. When comparisons are made with other workers' results, actual values in ly·min^{-1} are used.

All glassware was borosilicate glass and was rinsed in 1N HCl and soaked in distilled de-ionized water. The soaking water was changed frequently and analyzed for silicate released from the glassware. Only glassware showing no measurable release of silicate in 24 h was used.

Previous workers (e.g., 10) have shown that diatoms only

TABLE 1. *The 4 light levels used in this study. (Labels in column 2 are used in the text except where absolute values are required as ly·min⁻¹.)*

Nominal % light	Actual % light	ly·min⁻¹
100	100	0.14
25	30.0	0.042
10	15.0	0.021
1	1.32	0.0018

require silicon during those phases of the cell cycle when the cell wall is being laid down. Thus if the cells have a synchronized division cycle one would observe a pulse of silicate uptake. In the present study, conducted under continuous illumination, there was no indication of synchronous cell division. During a 5-day period in each experiment, uptake rates and growth rates were calculated every 8 h and only small random variations in the rates were observed at steady state.

During periods of adaptation to reduced light intensity when there were significant changes in cell numbers, fluorescence and nutrient concentrations in the reactor, the following equation was used to calculate growth rates (12):

$$\mu = D + (1/t)\ ln\ X/X_o \qquad (1)$$

where: t = time in h; X = population density; X_o = population density at $t = 0$; μ = growth rate (h^{-1}) and D = dilution rate (h^{-1}).

Experimental design. Two experiments were conducted to look at the effect of light intensity on silicate-limited populations. Virtually identical procedures were used in both studies. Each study was started with a single chemostat culture growing at the normal light intensity (0.14 ly·min⁻¹) and silicate limitation (dilution rate = 0.04 h^{-1}, substrate concentration (S) = 0.3 μg-at Si·l^{-1}). The culture was divided into 4 chemostats and grown under the original conditions until the 4 cultures reached steady state. At that time, light screens were placed over 3 of the chemostats, reducing them to 30, 15 and 1% of the normal experimental light. The fourth chemostat was kept at 100% light as a control. Chemostats were maintained at the reduced light levels for 2–3 weeks to study the long-term or adaptive response to the new light intensities.

Inflow and outflow nutrients (NO_3^-, NH_4^+, PO_4^{-3}, and Si $(OH)_4$), cell numbers, fluorescence and flow rates were measured daily during both studies. Nutrient concentrations, fluorescence and cell numbers were measured every 8 h on days 10–15 of both experiments. Chlorophyll *a* measurements were made every 1–2 days during both experiments.

A basic difference in the 2 experiments was the physiological state of the *S. costatum* cultures. In the first experiment an older population which had not undergone synchronized sexual reproduction for several months was used (12). These cells required 5 days to adapt to the reduced light intensities. In the second experiment cells which had undergone synchronized sexual reproduction 12 days prior to the beginning of the study were used. These cells adapted to the reduced light intensities in a few hours.

On 3 occasions during the second experiment (days 11, 21, 23), samples were taken from chemostats at steady state, enriched with nutrients (50 ml chemostat effluent + 50 ml inflow water enriched to give 10 μg-at Si·l^{-1}), and incubated in 125 ml Pyrex bottles at the 4 different light intensities as before and the samples were incubated 9 h. In all 3 experiments nutrient uptake rates were calculated from the difference between initial and final nutrient analyses using Equation 1 (D = 0). In one experiment (day 23), initial and final cell counts and fluorometer readings were also used to calculate growth over the 9 h period.

To describe the silicate uptake kinetics of *S. costatum*, it was necessary to obtain estimates of K_s and V_{max} for the particular population and culture conditions being studied. To obtain this information at a given point in time the perturbation technique of Caperon and Meyer (5) was used. At the end of each light experiment a perturbation experiment was conducted on the 100 and 30% light populations.

In a perturbation experiment, estimates of the kinetics parameters V_{max} and K_s for silicate uptake can be made by adding silicate to a silicate-limited chemostat reactor at steady state and following the decline in the dissolved silicate concentration with time. Uptake rates can be calculated from these concentration changes. Chemostats at steady state, with a large cell population (~3 × 10^8 cells·l^{-1}) and a low dissolved silicate concentration (0.3–0.4 μg-at Si·l^{-1}) were used. The amount of silicate added was approximately 10 μg-at Si·l^{-1}. Phosphate and nitrate were also added if it appeared they could become limiting during the experiment. Unlike (5) the nutrient pump was not turned off and the dilution rate was maintained throughout the experiment.

After nutrient addition, samples of the effluent were taken for nutrient analysis (NO_3^-, NH_4^+, PO_4^{-3}, Si $(OH)_4$) *ca.* every 30 min. Sampling frequency was increased to every 15 min when nutrient levels approached the estimated value of K_s. Samples for cell counts and *in vivo* chlorophyll *a* fluorescence were taken 5 times during each experiment which lasted 28 h.

Details of the calculation of perturbation results and a comparison of technique used here and that of Caperon and Meyer (5) are given elsewhere (6). Confidence intervals (±2 SD) were calculated for V based on the measured error term for the nutrient measurements, propagated according to the standard theory of error (3).

RESULTS

Changes in cell numbers with time during the first experiment are presented in Fig. 2 which is annotated to indicate when light screens were placed over the reactors and when perturbation experiments were conducted. Changes in effluent silicate are presented in Fig. 3. On day 22 cells were observed sticking to the walls of the reactor flasks. The flasks were changed for new autoclaved ones as soon as possible, but this still resulted in a serious perturbation of the system. Fortunately, this problem arose at a noncritical time in the experiment. The experiment was extended 7 days until a steady state was again established before the final perturbation experiments were conducted.

Using Equation 1 growth rates in terms of cell number change, fluorescence and N, P and Si were calculated for days 12–17 and 17–22 for the 30, 15 and 1% levels (Table 2). In these cultures a dramatic increase in growth rate was observed on day 17; in the 30% population the increase was sufficient to reverse their washout trend and the population again increased to a steady state condition by day 22. The other 2 populations continued to washout, but at a significantly slower rate. Average growth rates for the 2 periods are presented in Table 2. Chlorophyll *a*, N, P and Si per cell and the uptake ratio of N:Si:P have been calculated for the experimental populations (Table 3). In the 15 and 1% light populations, the ratios are for day 15 of the experiment, which was the last day there was a sufficient population

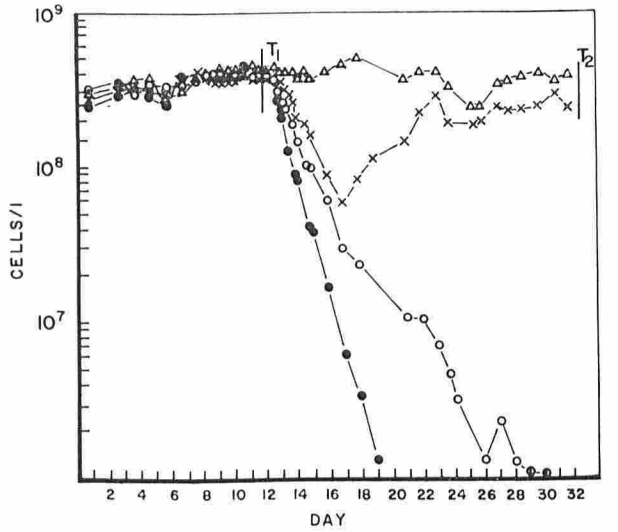

FIG. 2. Changes in cell numbers with time during experiment 1. Cultures growing at 100% light until time T_1, when light screens were placed on 3 reactors: ● = 1% light, ○ = 15% light, × = 30% light, △ = 100% light. At time T_2 perturbation experiments were conducted on the 30 and 100% light populations.

left in the reactor to make the appropriate measurements. The values for the 30 and 100% reactors are based on samples taken the day before the perturbation experiments were performed.

Changes in cell numbers with time during the second experiment are presented in Fig. 4 which is annotated to indicate when batch uptake and perturbation experiments were conducted during the study. Changes in reactor nutrient levels are presented in Fig. 5. In this experiment there was initially

TABLE 2. *Average growth rates (h^{-1}) based on changes in cell numbers, fluorescence and average uptake rates (h^{-1}) calculated from changes in nutrient concentrations in reactor after light screens were placed over reactor flasks. Experiment 1 is divided in 2 time periods as explained in the text. Silicate utilization rates from perturbation experiments are included where appropriate.*

Experiment	% light	Perturbation Si V_i	μ (cell numbers)	μ (fluorescence)	V_{PO_4}	$V_{Si(OH)_4}$	$V_{\Sigma N}$
1	100	0.074					
Not adapted	30		0.020	0.022	0.021	0.029	0.027
(day 12–17)	1		0.000	0.002	0.006	0.002	0.000
1	100	0.074					
Adapted	30	0.055	0.068	0.050	—	0.050	0.050
(day 18 on)	15		0.010	0.015	0.011	0.016	0.012
	15		0.028	0.019	—	0.025	—
	1		0.002	—	—	0.000	0.000
2	100	0.088					
	30	0.051					
	15		0.033	0.020	0.022	0.024	0.020
	1		0.003	0.003	0.007	0.000	0.002

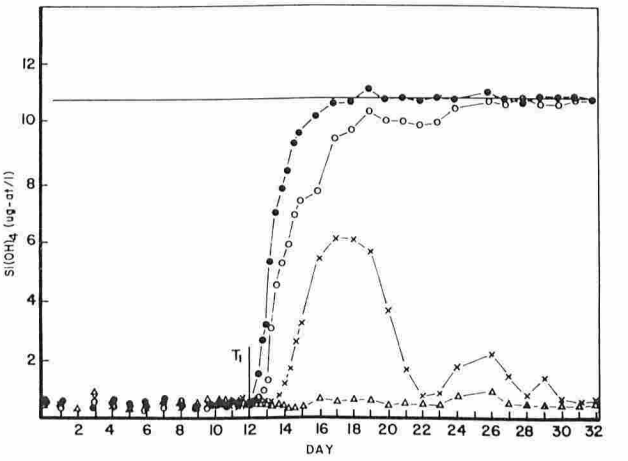

FIG. 3. Changes in effluent silicate concentration during experiment 1. Cultures growing at 100% light intensity until time T_1, when light screens placed on 3 reactors (symbols as Fig. 2). Solid line is concentration of silicate in inflow medium.

a problem with maintaining a constant level of PO_4^{-3} in the inflow medium. However, phosphate levels were always saturating and it does not appear to have been a significant factor in the outcome of the experiment.

Growth rates in terms of change in cell numbers, fluorescence and N, P and Si were calculated for the

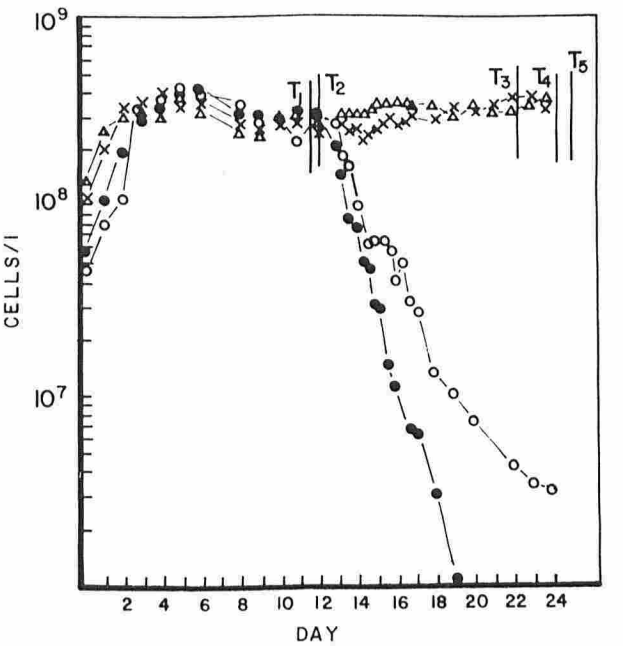

FIG. 4. Changes in cell numbers with time during experiment 2. Cultures growing at 100% light intensity until time T_2, when light screens placed on 3 reactors (symbols as Fig. 2). At times T_1, T_3 and T_4 batch nutrient uptake experiments conducted. At time T_5 perturbation experiments conducted on the 30 and 100% light populations.

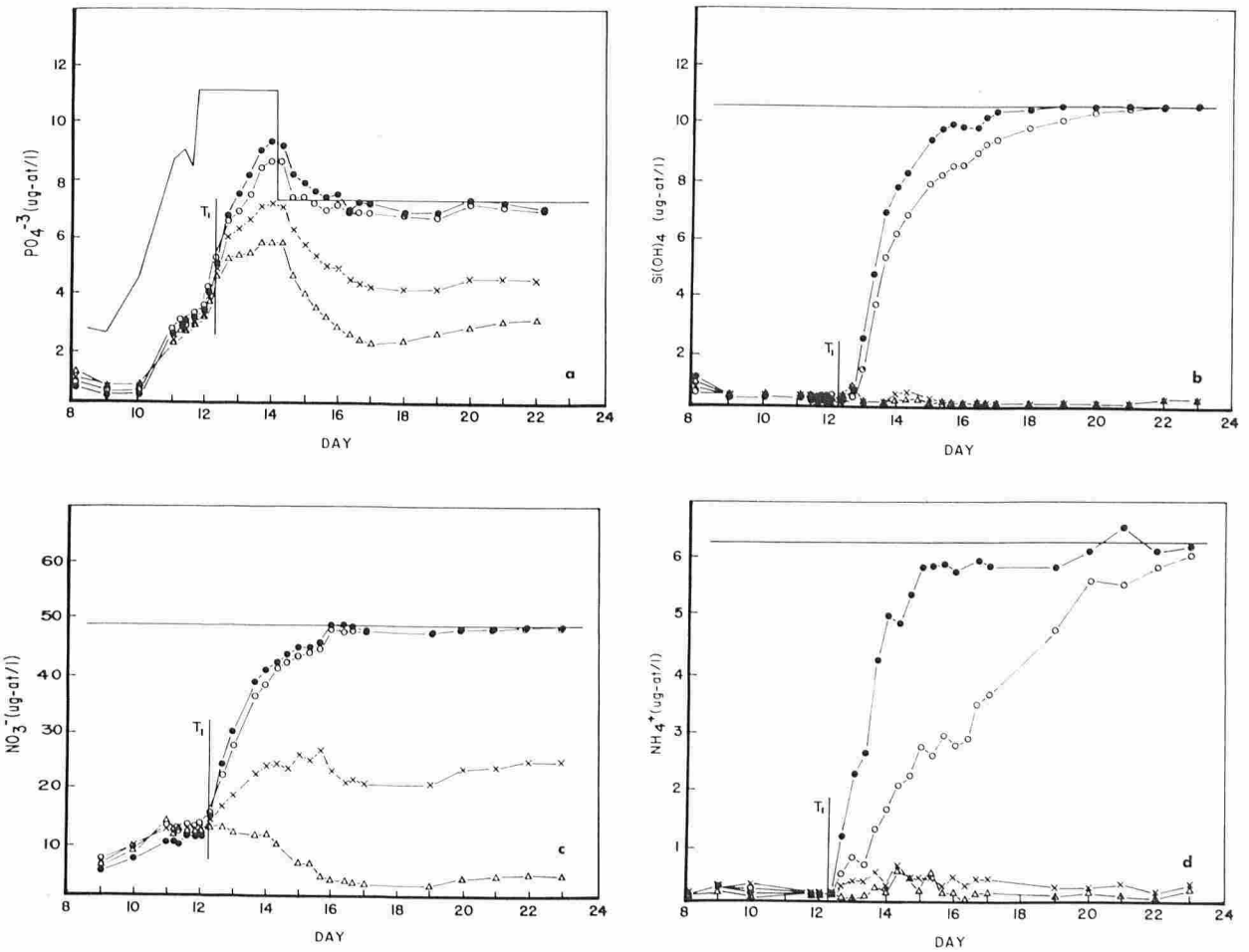

FIG. 5. Changes in nutrient concentrations in reactor vessels during experiment 2. Cultures growing at 100% light intensity until time T_1, when light screens placed over 3 reactors (symbols as Fig. 2). Solid line is concentration of nutrient in inflow medium: Fig. 5a. Phosphate. Fig. 5b. Silicate. Fig. 5c. Nitrate. Fig. 5d. Ammonium.

15 and 1% populations using Equation 1 for days 12–23 of the experiments (Table 2) with average values given. Per cell ratios of N, P, Si, chlorophyll *a* and fluorescence, etc., were calculated as described for experiment 1 (Table 3).

The major difference in the 2 experiments is in the time taken for the populations to adapt to light intensity change: 5 days in experiment 1 vs. a few hours in experiment 2. Based on the understanding of the effect of sexual cycle on growth rate and adaptability (12), the gross difference in the results of the 2 studies can be explained in terms of the age or time since auxospore formation of the populations. The experiment 2 populations, which had undergone synchronized sexual reproduction 12 days prior to when the screens were placed over the reactors, were able to respond to the change in light intensity almost immediately. The populations of experiment 1 which had not undergone synchronized sexual reproduction for several months took 5 days to respond. However, the end result of adaptation is the same as measured by the growth rate and nutrient uptake rate of the fully adapted cells (Table 2). The nature of the adaptive process—either adaptation of the individual cells, or sorting of the mixed population in experiment 1 for the few faster-growing cells that were initially present—is not yet clear and will require further study.

A plot of silicate uptake as a function of light intensity from data in Table 2 is shown in Fig. 6. Perturbation results are used for the 0.14 and 0.042 ly·min^{-1} levels, and $V_{Si(OH)_4}$ values for the lower light levels. In the 0.021 and 0.0018 ly·min^{-1} reactors, the silicate concentration had increased to sufficient levels so that $V \rightarrow V_{max}$. The non-adapted populations in experiment 1 (days 12–17) had very low uptake rates. However the same populations after the 5 day adaptation period showed good agreement with experiment 2 populations.

The curve is a least squares fit to the light adapted values of a Michaelis-Menten type equation for light-limited algal cultures (14):

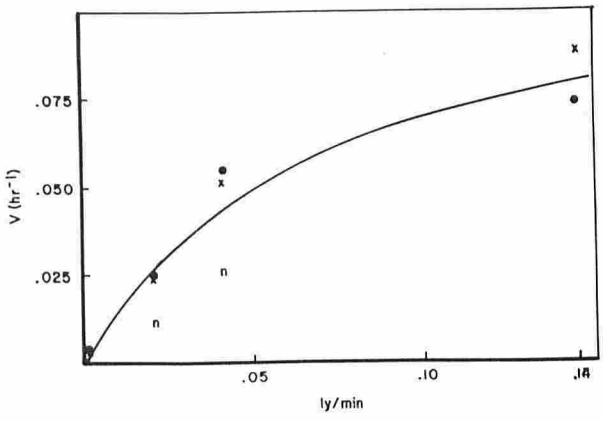

FIG. 6. Maximal silicate uptake rates (Table 2) as function of light intensity. n = values for days 12–17 of experiment 1; • = values for experiment 1 after day 17; × = values for experiment 2. Curve is a least squares fit of Equation 2 to light adapted values: $\mu_{max} = 0.12$ h^{-1}; $K_I = 0.07$ ly·min^{-1}.

$$\mu = \mu_{max}\,(\bar{I}/K_I + \bar{I}) \qquad (2)$$

where: μ = growth rate (h^{-1}), μ_{max} = the growth rate at light saturation (h^{-1}), $\bar{I}$ = mean light intensity in the culture (ly·min^{-1}), K_I = light intensity at which μ is ½ μ_{max}. The fit is not very good especially around the 30% light level.

Several studies of the growth rate of *S. costatum* as a function of light intensity have been made (8,21,22, 26). These studies measured growth as either $^{14}CO_2$ uptake rate or increase in cell numbers or both, and in general all 4 show similar results. McAllister, *et al.* (26) present data particularly suitable for direct comparison with the present study. Figure 7 shows their curve for ^{14}C uptake vs. light intensity for *S. costatum* growing at 20 C with ample nutrients, together with the data points from Fig. 6, superimposed on their curve. McAllister, *et al.* also calculated growth rates based on an increase in carbon and increase in cell numbers which agreed well with this curve. Similarly, values given here in Table 2 for growth in terms of cell numbers or N or P are also in agreement with silicate uptake values. The result of this study fall on McAllister's curve, with the exception of the 100% light data points which fall below the curve. The 100% light populations had been living in a low silicate environment for 25 days or more. It appears that this continued stress reduced the maximum growth potential of *S. costatum* compared to that of cells which remained in a high nutrient environment, such as the unlimited cells used by McAllister, *et al.* In that study they also tested N and P starved cells and found the maximal growth rates were dramatically reduced to less than 20% of the normal cells. In the present study the normal growth vs. light intensity curve has been truncated at the level where silicate becomes the limiting factor.

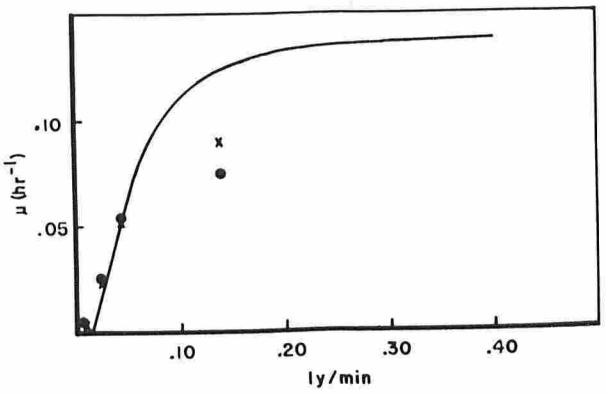

FIG. 7. Growth rate as function of light intensity. Curve is from McAllister, *et al.* (27) for $^{14}CO_2$ uptake by *S. costatum* growing in batch culture at 20 C with ample nutrients. • = silicate V_{max} values from experiment 1 after day 17; × = silicate V_{max} values from experiment 2 replotted from Fig. 6.

The chemical composition of *S. costatum* growing at the different light levels also suggests that it is silicate-limited at 100% light, but not at the other light levels (Table 3). There is roughly 30% less Si/cell in the 100% light population compared to the other populations. The uptake ratios by atoms for the 100% light population compare with those for other Si-limited chemostat cultures while that of the 30% light population were similar to the ratios for nonnutrient-limited *S. costatum* (19). The 1 and 15% light populations showed a marked increase in P/cell compared to the higher light intensities while Si and N stayed fairly constant.

Batch uptake experiments. Results of the batch uptake experiments from experiment 2 (Table 4) clearly show that cells from the 100% light reactors were silicate-limited. Even when incubated at 1% light intensity these cells had high silicate uptake rates. The average silicate uptake rate for all light intensities was 0.075 h^{-1} or almost twice the normal growth rate of 0.04 h^{-1}, as set by the dilution rate

TABLE 3. *Cellular composition and uptake ratios of cells growing at different light levels. In all,* D = *0.04 h*$^{-1}$*: uncertainty values are* ± *2 SD. See text for details.*

Experiment % light	$Si(OH)_4$ in reactor μg-at·l^{-1}	Cells/l × 10^7	Chl *a* (μg/10^7 cells)	N (μg-at/10^7 cells)	Si (μg-at/10^7 cells)	P (μg-at/10^7 cells)	Uptake ratio by atoms N:Si:P
1							
100	0.3 ± .08	40.0	2.0	1.02	0.26	0.16	6.2:1.6:1
30	0.4 ± .1	21.4	3.2	1.15	0.48	0.13	8.8:3.7:1
15	7.2 ± .25	8.0	1.8	0.62	0.43	0.23	2.6:1.8:1
1	9.4 ± .28	3.0	4.7	1.0	0.33	0.33	3.0:1.0:1
2							
100	0.3 ± .08	36.2	5.7	1.42	0.29	0.15	9.5:1.9:1
30	0.3 ± .08	33.8	3.8	0.90	0.32	0.09	10.0:3.5:1
15	8.4 ± .26	6.0	3.8	1.56	0.40	0.20	5.3:1.4:1
1	10.0 ± .31	1.4	2.8	2.42	0.36	0.42	5.6:0.8:1

TABLE 4. *Nutrient uptake rates and growth rates from 9 h batch uptake experiments conducted during experiment 2. Rates are h^{-1}; samples taken from chemostats at steady state at D = 0.04 h^{-1} and light intensity indicated.*

Day	Source light %	Incub. light %	V_{PO_4}	V_{SiO_4}	V_{NO_3}	V_{NH_4}	$V_{\Sigma N}$	μ (cell no.)	μ (fluoro)
11	100	100		0.095	0.027				
		100		0.089	0.026				
	100	30		0.085	0.009				
		30			0.010				
	100	15		0.080	0.004				
		15		0.067	0.003				
	100	1		0.069	0.002				
		1		0.044	0.003				
21	100	100	0.043	0.081	0.049	0.011	0.060		
		30	0.027	0.077	0.030	0.010	0.040		
		15	0.032	0.078	0.022	0.019	0.041		
		1	0.025	0.073	0.015	0.017	0.032		
	30	100	0.052	0.057	0.062	0.017	0.079		
		30	0.024	0.030	0.031	0.020	0.051		
		15	0.020	0.009	0.016	0.018	0.034		
		1	0.013	0.024	0.023	0.011	0.034		
23	100	100	0.052	0.077	0.044	0.000	0.044	0.026	0.008
		30	0.042	0.080	0.031	0.000	0.031	0.031	0.008
		15	0.035	0.065	0.022	0.000	0.022	0.024	−0.004
		1	0.028	0.067	0.016	0.000	0.016	0.033	0.012
	30	100	0.060	0.064	0.055	0.002	0.057	0.035	0.047
		30	0.033	0.039	0.012	0.002	0.014	0.021	0.002
		15	0.023	0.036	−0.005	0.002	−0.003	0.004	−0.016
		1	0.014	0.028	−0.015	0.002	−0.013	0.002	−0.028

of the chemostat. By contrast, the uptake of N and P was *ca.* equal to the dilution rate of 0.04 h^{-1} when incubated at 100% light and considerably lower when incubated at lower light intensities. Nitrate was the added N source, and ammonium was added only as a contaminant. Thus in some instances zero ammonium uptake was observed because no ammonium was present at the beginning of the experiment.

Phytoplankton from the 30% light reactor did not take up silicate preferentially (Table 4). All nutrients were taken up at *ca.* equal rates. In this the short-term nutrient uptake rate was linked to light. When incubated at the normal light intensity (30%), the 30% light population took up nutrients at *ca.* their normal growth rate of 0.04 h^{-1}. When these cells were incubated at the 100% light intensity increased uptake rates were observed for all nutrients. At the lower light intensities, the uptake rates were reduced.

Perturbation experiments. Perturbation experiments were conducted on the 30 and 100% light populations at the end of both experiments (see Fig. 2, 4). The results were similar (Table 5) and plots of silicate uptake velocity (V) vs. substrate concentration (S) for the perturbations at the end of experiment 2 are shown in Fig. 8. For the 100% light population the ability to take up silicate became critical at S = 3–4 μg-at Si·l^{-1}. A Michaelis-Menten type uptake hyperbola has been fit to the data

TABLE 5. *Kinetic parameters obtained from perturbations conducted on the 30 and 100% light population at end of each experiment. V_{max} and K_s values are given ± 1 SD. See text for explanation of V_i.*

Experiment	Light level %	V_{max} (h^{-1})	K_s μg-at Si·l^{-1}	V_i (h^{-1})
1	100	0.101 ± 0.02	1.81 ± 0.89	0.074
	30	—	—	0.055
2	100	0.127 ± 0.01	1.1 ± 0.2	0.088
	30	—	—	0.051

from S = 0.3 to S = 2.5 for experiment 2, 100% light population (Fig. 8a). Above those substrate levels, V oscillates about some mean value which will be called $V_i = V$ internally controlled, meaning that uptake no longer follows the uptake hyperbola but has been limited to this level by some kind of internal feedback control mechanism. This definition is based on the results of numerous perturbation experiments with both silicate-limited and ammonium-limited cultures (6). V_i is the maximal silica utilization rate and is comparable to the results of batch uptake experiments and the uptake rates calculated from population changes in Table 2. V_i is the maximum uptake rate the cell can sustain for a period of hours or days whereas V_{max} is the maximal uptake rate attainable by the permease system. V_{max} can only be maintained for a few minutes before the internal pools are saturated and uptake becomes regulated by the utilization of silica to form the cell wall. This analysis of the perturbation results has been presented in detail elsewhere (6).

In general the results of the 100% light perturbation experiments in this study agree with those of Caperon and Meyer (5). They used smaller spike additions of nutrient increasing the limiting nutrient concentration to 2–6 μg-at·l^{-1} (vs. 10 μg-at·l^{-1} in this study) and the nutrient pump was turned off. Working in this lower nutrient range (generally below 3 μg-at·l^{-1}) Caperon and Meyer's results were adequately explained by Michaelis-Menten kinetics as were the 100% light results for the same range of nutrient concentrations. However, in the latter the 10 μg-at·l^{-1} spike additions were sufficient to put the populations into an internally controlled situation where uptake was controlled by the utilization of silica within the cell to form the cell wall.

The 30% light populations showed very little response to the addition of silicate to the reactor compared to the 100% light populations (Fig. 8). At no time was the uptake of silicate the limiting factor. The maximal uptake rates observed (V_i) were 0.051 and 0.055 h^{-1} only slightly greater than the dilution rate of 0.04 h^{-1}. At the end of the experiment (28 h) the 30% light populations had approximately 1.2 μg-at Si·l^{-1} left, about triple their steady state value. By comparison the 100% light population returned

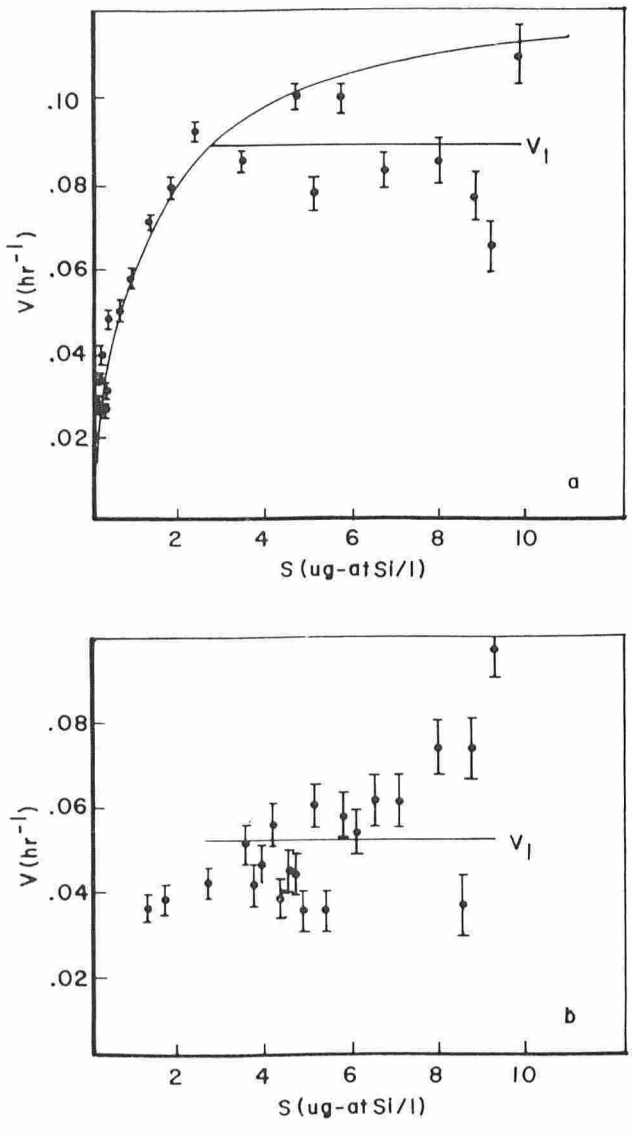

FIG. 8. Silicate uptake rate as a function of silicate concentration in reactor during perturbation experiments of days 24–25 of experiment 2. Fig. 8a. 100% light reactor: curve is a least squares fit of a Michaelis-Menten hyperbola ($V = V_{max}\, S/(K_s + S)$) to the points between $S = 0.3$ and $S = 2.5$ μg-at Si·l^{-1}; $V_{max} = 0.127 \pm 0.01$; $K_s = 1.1 \pm 0.2$. V_i is average V for $S > 2.5$ μg-at Si·l^{-1}. Fig. 8b. 30% light reactor: V_i is average value for all values of V: brackets are ± 2 SD about the velocity.

to their steady state value in 12 h. If there is a switching from internal control to external control by silicate no effects of silicate limitation would be expected until V_i intersects the uptake hyperbola (Fig. 8) which would be *ca.* 0.6 μg-at·l^{-1} for the 30% light populations.

The 30% light population was still silicate-limited in the sense that the amount of silicate in the inflow medium controlled the number of cells formed. At steady state, the chemostat system was still regulating on silicate, which was 0.4 μg-at Si·l^{-1} in the reactor whereas all other nutrients were present in excess ($NO_3^- > 20$ μg-at N·l^{-1}, $PO_4^{-3} > 4$ μg-at P·l^{-1}). However, in the perturbation experiment the 30% light population did not respond to added silicate but the 100% light population, which was clearly silicate-limited, responded to added silicate by taking it up at near maximal rates.

DISCUSSION AND CONCLUSIONS

Comparison of the 30 and 100% light populations provides a striking contrast (Table 6). The 2 populations were living in virtually the same nutrient environment and yet responded quite differently to changes in light intensity and silicate concentration in batch uptake and perturbation experiments. Clearly, the 100% light population was silicate-limited but the 30% light population appeared to be light-limited. The 30% light population had the chemical composition and morphology of nutrient sufficient cells whereas the 100% light cells had the composition of a typical Si-limited *S. costatum* (19).

The response of the 2 populations to increased light and added silicate is also a good indicator of the type of limitation present. In batch uptake experiments, the 100% light population responded to added silicate by approximately doubling its silicate uptake rate regardless of incubation light intensity. These results are similar to the findings of Coombs, *et al.* (7) that during Si starvation rates of CO_2 fixation, O_2 evolution and ^{32}P incorporation fell to low levels, and the light saturation level fell from 0.15 to 0.027 ly·min^{-1}. The reintroduction of Si led to immediate transients in all parameters studied, and the added Si was rapidly taken up and incorporated into new tests.

By contrast, the 30% light population showed no increase in silicate uptake rate when given additional amounts of silicate. Only when incubated at 100% light did the cells take up silicate at greater than their steady state rate. Under those conditions increased uptake rates for nitrate and phosphate (Table 4) also occurred and no preference for silicate was indicated.

The 15 and 1% light populations were clearly light-limited. In these, the light level was so low that the maximal growth rate was less than the dilution rate of the chemostat and the populations washed out of the reactor (Table 2, Fig. 2, 4). The 30% light population was maintained in the reactor because its maximal growth rate was 0.053 h^{-1}, or 0.013 h^{-1} greater than the dilution rate. The population size was, however, still controlled by the silicate concentration in the inflow. The silicate concentration in the reactor was reduced to about the same level as in the 100% light reactor (0.35 ± 0.1 vs. 0.3 ± 0.08 μg-at Si·l^{-1}). However under the stress of low light intensity, the 30% light population could not tolerate the added stress of Si-limitation. Fewer cells were

TABLE 6. *Comparison of the 30 and 100% light populations; both growing in chemostats with same medium (10.5 μg-at Si·l⁻¹) and dilution rate (0.04 h⁻¹). Average results for experiments 1 and 2.*

	100% light (0.14 ly·min⁻¹)	30% light (0.042 ly·min⁻¹)
Effluent $Si(OH)_4$ (μg-at Si·l⁻¹ ± 2 SD)	0.3 ± 0.08	0.35 ± 0.1
Cells·l⁻¹ × 10⁻⁷	38.1	27.6
Chemical composition μg-at/10⁷ cells	N = 1.22; Si = 0.28; P = 0.15	N = 1.03; Si = 0.40; P = 0.11
Uptake ratio N:Si:P (by atoms)	8.13:1.9:1	9.4:3.6:1
Perturbation experiment results V_i (h⁻¹)	0.081	0.053
Batch uptake results; growth with excess of all nutrients incubated at 100, 30, 15, 1% light levels	High Si uptake rate = 0.075 h⁻¹ regardless of incubation light intensity	Takes all nutrients at *ca.* equal rate (h⁻¹); nutrient uptake rate controlled by light
Microscope observations	Cells thin-walled; Si spines between cells not observed, cells touching end to end	Si spines between cells visible, 1–2 μm space between cells

produced with the same amount of silicate taken up, and the cells retained a more normal chemical composition and showed no signs of Si-limitation.

The present results agree with recent studies of multiple nutrient limitation by Droop (13; B_{12} and P) and Rhee (28; N and P) where they found a sharp transition from one limitation to the other with no intermediate state. In those studies as here, the nature of the limitation was defined by the chemical composition of the cells and their nutrient uptake and growth abilities rather than the ambient concentration of the limiting nutrient. For example Rhee (28) observed that *Scenedesmus* sp. switches from P to N limitation and vice versa at an N:P ratio of 30 and that the instantaneous V_{max} for phosphate for P-limited cells was *ca.* 8 times that of N-limited cells where V_{max} for P was equal to the steady state P utilization rate. These results showed the same pattern as the results of the present study and both illustrate the point that the physiological state of the cell (i.e., is it limited, and if so by what factor) is best characterized by the chemical composition and morphology of the cell, and by its ability to take up nutrients.

Droop (13) notes that most of the present chemostat results can be explained by a cell quota type model where nutrient uptake is a Michaelis-Menten function of ambient nutrient concentration and growth is a hyperbolic function of the concentration of that nutrient in the cell. He cites several studies in the literature and his own works that have found this to be a reasonable approximation. The present steady state data can be adequately described by such a model, however there are actually only 2 levels of Si limitation the 100% light populations, and the others all of which are not Si-limited according to their cell quotas. On the other hand, the perturbation experiment results are not adequately explained by the present cell quota models. In particular V_i (the internally controlled substrate velocity) is not adequately explained by the cell quota models, nor is the initial spike of uptake or the switching from externally controlled uptake to internally controlled uptake and then back to external control. These features have been observed repeatedly in perturbation experiments with both ammonium- and silicate-limited cultures of *S. costatum* (6).

To deal with these unsteady state results and to describe natural populations where not only unsteady state conditions but also multiple N sources and multiple species, etc. occur, it will be necessary to use a biochemically based model, where each nutrient is treated according to the best understanding of its biochemical pathways in the cell. This idea was first suggested by Williams (30) and an initial model of this type has been developed for N (18). Rhee (28) also has seen the usefulness of this approach and has investigated which form of phosphate within the cell acts as the inhibitor to phosphate uptake, a key piece of information for the development of this type of model. The present understanding of the biochemistry of Si utilization by diatoms (2,9,29) appears to be sufficient for such purposes and a biochemically based computer simulation model for Si uptake by diatoms has been developed to simulate both steady state and perturbation experiment results. The preliminary results suggest that this will be a fruitful approach.

This study was a first investigation of the combined effect of light and nutrient limitation under chemostat steady state and perturbed conditions. The results suggest that light can be treated as another potentially limiting factor for phytoplankton growth in a manner similar to the way the major nutrients are treated. Light then, would have a unique set of equations similar to those for N and Si and the effects of all the potentially limiting factors would be interconnected through the cells growth rate. Many of the differences between the observed characteristics of nutrient-limited algal cultures and natural populations may in part be due to the varying natural light regime and the fact that even in near

surface waters phytoplankton may be light-limited for a significant part of the day. Thus natural populations may not show the typical characteristics of nutrient-limited cells we expect to see based on the results of culture work.

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REFERENCES

1. Anonymous. 1971. *Eutrophication in coastal waters: nitrogen as a controlling factor.* Water Pollution Control Research Project, Environmental Protection Agency, Washington, D.C. Project #16010 EHC. 67 pp.
2. Azam, F., Hemmingsen, B. B. & Volcani, B. E. 1974. Role of silicon in diatom metabolism. *Arch. Microbiol.* 97:103–14.
3. Beers, Y. 1957. *Introduction to the Theory of Error.* Addison-Wesley, Reading, Massachusetts. 66 pp.
4. Blasco, D. 1973. Estudio de las variaciones de la relación fluorescencia *in vivo*/clorofila *a*, y su aplicación en oceanografía. Influencia de la limitación de deferentes nutrientes, efecto del día y noches y dependencia de la especie estudiada. *Invest. Pesq.* 37:533–56.
5. Caperon, J. & Meyer, J. 1972. Nitrogen-limited growth of marine phytoplankton. Part II. Uptake kinetics and their role in nutrient limited growth of phytoplankton. *Deep-Sea Res.* 19:619–32.
6. Conway, H. L., Harrison, P. J. & Davis, C. O. 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation. II. Transient response of *Skeletonema costatum* to an addition of the limiting nutrient. *Marine Biol.* 35:187–96.
7. Coombs, J., Spanis, C. & Volcani, B. E. 1967. Studies on the biochemistry and fine structure of silica shell formation in diatoms. Photosynthesis and respiration in silicon-starvation synchrony of *Navicula pelliculosa*. *Plant Physiol.* 42:1607–11.
8. Curl, H., Jr. & McLeod, G. C. 1961. The physiological ecology of a marine diatom *Skeletonema costatum* (Grev.) Cleve. *J. Mar. Res.* 19:70–88.
9. Darley, W. M. 1970. Silicon and the division cycle of the diatoms *Navicula pelliculosa* and *Cylindrotheca fusiformis*. *In* Loeblich, A. R., Jr. [Ed.] *Proc. of the North American Paleontological Convention*, 1969. Allen Press, Lawrence, Kansas, Part 6, 994–1009.
10. ——— & Volcani, B. E. 1969. Role of silicon in diatom metabolism. A silicon requirement for deoxyribonucleic acid synthesis in the diatom *Cylindrotheca fusiformis* Reimann and Lewin. *Exp. Cell Res.* 58:334–42.
11. Davis, C. O. 1973. Effects of changes in light intensity and photoperiod on the silicate-limited continuous culture of the marine diatom *Skeletonema costatum* (Grev.) Cleve. Ph.D. Dissertation, University of Washington, Seattle. 122 pp.
12. ———, Harrison, P. J. & Dugdale, R. C. 1973. Continuous culture of marine diatoms under silicate limitation. I. Synchronized life cycle of *Skeletonema costatum*. *J. Phycol.* 9:175–80.
13. Droop, M. R. 1974. The nutrient status of algal cells in continuous culture. *J. Mar. Biol. Assoc. U.K.* 54:825–55.
14. Eppley, R. W. & Dyer, D. 1965. Predicting production in light-limited continuous cultures of algae. *Appl. Microbiol.* 13:833–7.
15. Eppley, R. W., Carlucci, A. F., Holm-Halsen, O., Kiefer, D., McCarthy, J. J., Venrick, E. & Williams, P. M. 1971. Phytoplankton growth and composition in shipboard cultures supplied with nitrate, ammonium, or urea as the nitrogen source. *Limnol. Oceanogr.* 16:741–51.
16. Eppley, R. W., Rogers, J. N., McCarthy, J. J. & Sournia, A. 1971. Light-dark periodicity in nitrogen assimilation of the marine phytoplankters *Skeletonema costatum* and *Coccolithus huxleyi* in N-limited chemostat culture. *J. Phycol.* 7:150–4.
17. Grant, B. R. & Turner, I. M. 1969. Light-stimulated nitrate and nitrite assimilation in several species of algae. *Comp. Biochem. Physiol.* 29:995–1004.
18. Grenney, W. J., Bella, D. A. & Curl, H. C. 1973. A mathematical model of the nutrient dynamics of phytoplankton in a nitrate-limited environment. *Biotechnol. Bioeng.* 15: 331–58.
19. Harrison, P. J., Conway, H. L. & Dugdale, R. C. 1976. Marine diatom growth in chemostats under silicate or ammonium limitation. I. Cellular chemical composition and steady state growth kinetics of *Skeletonema costatum*. *Marine Biol.* 35:177–86.
20. Herbert, D., Elsworth, R. & Telling, R. C. 1956. The continuous culture of bacteria: a theoretical and experimental study. *J. Gen. Microbiol.* 14:601–22.
21. Jitts, H. R., McAllister, C. D., Stephens, K. & Strickland, J. D. H. 1964. The cell division rates of some marine phytoplankton as a function of light and temperature. *J. Fish. Res. Board Can.* 21:139–57.
22. Jorgensen, E. G. & Steemann Nielsen, E. 1965. Adaptation in plankton algae. *In* Goldman, C. R. [Ed.] *Primary Productivity in Aquatic Environments.* University of California Press, Berkeley. 39–46.
23. Kuenzler, E. 1970. Dissolved organic phosphorus excretion by marine phytoplankton. *J. Phycol.* 6:7–13.
24. ——— & Ketchum, B. H. 1962. Rate of phosphorus uptake by *Phaeodactylum tricornutum*. *Biol. Bull.* 123:134–45.
25. MacIsaac, J. J. & Dugdale, R. C. 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. *Deep-Sea Res.* 19:209–32.
26. McAllister, C. D., Shah, N. & Strickland, J. D. H. 1964. Marine phytoplankton photosynthesis as a function of light intensity: a comparison of methods. *J. Fish. Res. Board Can.* 21:159–81.
27. Pavlou, S. 1972. Phytoplankton growth dynamics. Technical Series I. *Chemostat Methodology and Chemical Analyses.* Department of Oceanography, University of Washington, Seattle. Special Report 52. 130 pp.
28. Rhee, G. 1974. Phosphate uptake under nitrate limitation by *Scenedesmus* sp. and its ecological implications. *J. Phycol.* 10:470–5.
29. Werner, D. 1966. Die kieselsäure im stoffwechsel von *Cyclotella cryptica* Reimann, Lewin und Guillard. *Arch. Mikrobiol.* 55:278–308.
30. Williams, F. M. 1971. Dynamics of microbial populations. *In* Patten, B. C. [Ed.] *Systems Analysis and Simulation in Ecology.* Academic Press, New York, 197–267.