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J. Phycol. **12**, 246–252 (1976)

KINETICS OF SILICIC ACID UPTAKE AND RATES OF SILICA DISSOLUTION IN THE MARINE DIATOM *THALASSIOSIRA PSEUDONANA*[1,2]

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SUMMARY

Tracer techniques using the stable isotope ^{30}Si were used to measure rates of silicic acid uptake and silica dissolution in silicon replete and silicon depleted populations of 2 clones of the marine diatom Thalassiosira pseudonana *Hasle & Heimdal. Uptake kinetics were describable using the Michaelis-Menten equation for enzyme kinetics, and no threshold concentration for uptake was evident. The maximum specific uptake rate of the estuarine clone 3H (0.062–0.092 · h^{-1}) was higher than that of the Sargasso Sea clone 13-1 (0.028–0.031 · h^{-1}), but half-saturation constants for uptake by the 2 clones were not measurably*

[1] *Accepted: 13 February 1976.*
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different (0.8–2.3 μM for 3H; 1.4–1.5 μM for 13-1). There was little or no light dependence of uptake in populations grown under optimal light conditions prior to the experiment. Exponentially growing populations released silicic acid to the medium by dissolution of cellular silica at rates ranging from 6.5 to 15% of the maximum uptake rate.

Key index words: silica; silicic acid; Thalassiosira; *uptake kinetics*

INTRODUCTION

Studies of the kinetics of nutrient uptake and nutrient-limited growth in algae have produced a number of rate equations which provide a framework for describing both the response of an algal population to nutrient limitation and the concentration range within which nutrient limitation exists. Briefly, the specific uptake rate of a nutrient, V (27), and the specific growth rate of the population, μ (20), have been related to the intracellular or extracellular concentration of the limiting nutrient by 4 similar but distinct kinetic equations.

For several nutrients it has been shown that the specific uptake rate is a function of the external substrate concentration, S (1,5–8,11,14,18,25,27), and that the concentration dependence may be described by the Michaelis-Menten equation for enzyme kinetics[4]:

$$V = V_{max} \frac{S}{K_t + S} \tag{1}$$

where V_{max} is the maximum specific uptake rate and K_t the substrate concentration at which $V = V_{max}/2$. Equation 1 has been modified for silicic acid and nitrate uptake to allow for a threshold concentration, S_o, below which no uptake takes place (4,25). By this new equation:

$$V = V_{max} \frac{S - S_o}{K'_t + (S - S_o)} \tag{2}$$

where K'_t is the value of $(S - S_o)$ at which $V = V_{max}/2$.

Monod (20) used an equation of the Michaelis-Menten form to describe the nutrient-limited growth of bacteria, and this growth rate equation has been extended to algae (6,9,10,13,14,24,26):

$$\mu = \mu_{max} \frac{S}{K_s + S} \tag{3}$$

where μ_{max} is the maximum specific growth rate of the population and K_s the substrate concentration at which $\mu = \mu_{max}/2$. Caperon and Meyer (3) found that Equation 3 did not fit their experimental data on N-limited growth of 4 algae, and suggested a substantially different mechanism for nutrient limitation of algal growth:

$$\mu = \mu_{max} \frac{Q - Q_o}{K_q + (Q - Q_o)} \tag{4}$$

where Q is the cellular content of the limiting nutrient (the cell quota, 6), Q_o the cell quota at or below which no growth takes place, and K_q the value of $(Q - Q_o)$ at which $\mu = \mu_{max}/2$. Equation 4 implies an intracellular pool of the limiting nutrient which has been taken up by the cell but not yet transformed into a final cellular product. This pool is therefore available for use in cell growth. At Q_o the pool is empty and no growth takes place. But as $(Q - Q_o)$ which is a measure of the size of the intracellular pool increases, the growth rate increases in a manner describable by Monod-type growth kinetics.

A number of studies of the kinetics of silicic acid uptake (1,5,11,14,25,26) and Si limited growth (13,14, 24,26) in diatoms have been reported, with results which are remarkably consistent with one another considering the differences in organisms studied and experimental procedures employed. Maximum specific uptake rates of silicic acid and maximum specific growth rates both tend to fall within the range of 0.02 to 0.12 · h^{-1}. Uptake kinetic studies have all generated data which can be fit by Equation 1 or 2 and indicate that the uptake rate is dependent upon the external substrate concentration in the range of *ca.* 0–5 μM. Growth kinetic studies have produced contradictory data on the precise nature of the limitation imposed on the growth rate of diatoms at low silicic acid concentration (i.e., whether Equation 3 or 4 or neither describes Si limited growth kinetics). However, they tend to show growth rates which are less than maximum at external silicic acid concentrations below 2 μM.

Guillard, Kilham and Jackson (13) studied the Si limited growth kinetics of *Thalassiosira pseudonana* Hasle & Heimdal in batch culture, using 2 physiologically distinct races, one from a coastal estuary (clone 3H) and one (clone 13-1) from the Sargasso Sea (12). They suggest that physiological differences between the clones represent genetic adaptations to high and low nutrient environments, respectively, and that the 2 clones conform to the hypothesis of Kilham (15) which predicts that diatoms adapted to high Si environments should have high values of μ_{max} and K_s when compared to diatoms adapted to low Si environments. Paasche (24) studied the kinetics of Si limited growth in clone 3H using a chemostat and obtained kinetic constants which are very similar to those of Guillard, *et al.* (13) if calculated according to Equation 3. He found, however, that his data were fitted poorly by Equation 3 at low growth rates and that an hyperbola of the form of Equation 4 provided a better description. Divergences of Si limited growth rate from Equation 3 in

[4] Uptake kinetic data can be fit equally well by any other equation which generates a rectangular hyperbola (13). All such equations are strictly curve-fitting devices and use of the Michaelis-Menten equation, although its derivation is biochemical, is not intended to imply any understanding of biochemical pathways involved in nutrient uptake.

chemostats at low growth rates have also been reported for *Skeletonema costatum* (Grev.) Cleve (14).

Paasche (25) determined kinetic parameters of silicic acid uptake in 5 diatom species, including *T. pseudonana* (clone 3H) in batch culture. His data are best fit by Equation 4, with a value of S_o ranging from 0.32 to 1.33 μM. He recognized that S_o may be an artifact in experiments where the uptake rate is determined from a change in the external substrate concentration, because of possible masking of low uptake rates by dissolution of cellular silica. However, he attempted to correct for dissolution by measuring dS/dt in a medium containing cells killed by freezing. He found that dissolution rates were high, on the order of 10–20% of V_{max}, but that a positive value of S_o persisted even after his data were corrected for dissolution. He interpreted S_o to be the concentration of some chemical form of Si which is reactive in a colorimetric acid-molybdate analysis but not available to the cell. Subsequent experiments by Paasche have indicated no threshold concentration for silicic acid uptake in *Thalassiosira nordenskioldii* Cleve (26).

Tracer methods using stable isotoypes of Si (^{29}Si, ^{30}Si) have recently been developed to measure rates of silicic acid uptake and silica dissolution in natural phytoplankton populations (11,21–23). This paper describes a study of silicic acid uptake kinetics in *T. pseudonana* clones 3H and 13-1, using ^{30}Si tracer techniques, and reports some direct measurements of silica dissolution rates in healthy, growing unialgal diatom populations.

MATERIALS AND METHODS

Clones 3H and 13-1 of *Thalassiosira pseudonana*, maintained in the culture collection at the Woods Hole Oceanographic Institution, were grown from small inocula (< 10^3 cells · ml^{-1}) to densities of *ca.* 10^6 cells · ml^{-1} in high nutrient (f/2 (10)) medium at *ca.* 20 C and 3500 lx under a 14:10 h LD cycle using Sylvania fluorescent cool-white lights. Experiments were performed on cells that were in 2 presumably different physiological states: Si replete, cells harvested directly from f/2 medium and Si depleted, cells allowed to grow in Si-free f/2 medium for 2–3 days prior to harvest for the experiment. A concentrated cell suspension for use as inoculum was prepared by filtering each culture at 170–260 g · cm^{-2} vacuum until a cell density of *ca.* 10^8 cells · ml^{-1} was obtained (cell counts in a hemocytometer). This provided a particulate Si concentration of 2–4 μmol Si · ml^{-1} in the inocula. Microscopic examination of the inocula indicated that the cells were intact and undamaged after concentration by filtering. Inocula of Si depleted cells were added to the experimental vessels immediately after concentration, while inocula of Si replete cells were maintained in the light for *ca.* 2 h, during which the silicic acid concentration of the medium was reduced to < 1 μM.

Low Si seawater was prepared by allowing diatoms to remove Si from Sargasso Sea surface water stored in polyethylene bottles and subsequently removing the cells by filtration through 0.8 μm polycarbonate membrane filters. Two batches of low Si water were obtained; one was measured by colorimetric acid-molybdate analysis (28) to have a silicic acid concentration of 0.8 μM and the other 0.3 μM. The 0.3 μM water was used in experiments where labeled silicic acid was to be added at concentrations of 7.0 μM or less, while the 0.8 μM water was used in experiments conducted at higher silicic acid concentrations. ^{30}Si isotope dilution analyses performed as part of the dissolution rate measurements indicated that the silicic acid concentration of the "0.8 μM" water was actually 0.2–0.3 μM and that the "0.3 μM" water contained no detectable silicic acid. We believe the isotope dilution analyses are less subject to errors resulting from reagent blanks, and so have used these analyses in calculating the silicic acid concentrations at which experiments were conducted.

Table 1. *Silicic acid concentrations, inoculum volumes and medium volumes employed in silicic acid uptake kinetic experiments on* T. pseudonana, *clones 3H and 13-1.*

Silicic acid concentration μM	Inoculum volume ml	Medium volume l	Flask material	Parameter measured[a]
0.25	1.0	2.0	pyrex	V
0.50	1.0	2.0	pyrex	V
1.50	1.0	1.0	pyrex	V
2.50	1.0	1.0	pyrex	V
3.50	1.0	1.0	pyrex	V
7.00	1.0	1.0	pyrex	V
10.0	2.0	2.0	polycarbonate	V, V_{dis}
15.0	2.0	2.0	polycarbonate	V, V_{dis}

[a] See text for details.

Rates of silicic acid uptake (V) and silica dissolution (V_{dis}) were measured by the ^{30}Si tracer techniques of Nelson and Goering (21–23). Cultures were incubated in media containing ^{30}Si-labeled (95.55 atom % ^{30}Si) silicic acid. In uptake rate experiments the cells were harvested by filtration through 0.8 μm polycarbonate membrane filters and the isotopic composition of the particulate Si measured by conversion to barium fluosilicate followed by solid sample mass spectrometry. In experiments in which both the uptake and the dissolution rate were measured, the initial and final isotopic compositions of the dissolved silicic acid were measured by conversion to silicomolybdic acid, extraction into *n*-butanol and conversion to barium fluosilicate followed by solid sample mass spectrometry. Uptake rates were calculated from the increase in the ^{30}Si content of the cellular silicon, and dissolution rates from the increase in the ^{28}Si content of the dissolved silicic acid during the incubation. Details of the isotopic analyses and rate calculations are given by Nelson (21) and Nelson and Goering (22,23).

For each uptake kinetic experiment a series of eight 2*l* fernbach flasks containing ^{30}Si-labeled silicic acid was prepared as summarized in Table 1. The medium in each of the 2 polycarbonate flasks was divided immediately, half was retained for incubation and the remainder filtered through a 0.8 μm polycarbonate membrane filter. The filter was retained for particulate Si analysis and the filtrate analyzed to determine the initial isotopic composition of the dissolved silicic acid. The 8 flasks were then covered with pyrex petri dishes and incubated 4 h at *ca.* 20 C and 3500 lx. To keep the incubation time constant, flasks were inoculated in groups of 2 or 3 at 30 min intervals. By this procedure each sample could be filtered immediately following incubation. To minimize depletion of silicic acid in the medium during the incubation, samples incubated at silicic acid concentrations of 0.25 and 0.50 μM contained half the cell density (10^8 cells · 2l^{-1} vs. 10^8 cells · l^{-1}) of the other samples. We estimated, based upon the data of Paasche (25; Table 1) that under these conditions less than 30% of the silicic acid in each flask would be consumed in a 4 h incubation. However, uptake rates in these experiments were somewhat higher than those Paasche measured so it is possible that as much as 40% of the silicic acid initially present was consumed in some experiments (see Table 2).

TABLE 2. *Predicted (ΔS_p) and estimated actual depletion (ΔS_e) of the initial silicic acid concentration (S_i) during 4 h incubation under conditions in these experiments.*

S_i μM	V(predicted)[a] pg Si · cell⁻¹ · h⁻¹	ΔS_p μM	ΔS_p %S_i	V(highest measured)[b] pg Si · cell⁻¹ · h⁻¹	ΔS_e μM	ΔS_e %S_i
0.25	0	0	0	0.015	0.10	40.0
0.50	0	0	0	0.021	0.15	30.0
1.50	0.029	0.40	28.0	0.040	0.56	37.4
2.50	0.043	0.59	24.0	0.056	0.78	31.2
3.50	0.050	0.70	20.0	0.052	0.73	21.4
7.00	0.060	0.84	12.0	0.065	0.91	13.0
10.0	0.063	0.88	8.8	0.077	1.08	10.8
15.0	0.067	0.93	6.2	0.096	1.34	8.9

[a] From Equation 2 using values of V_{max} (0.073 pg Si · cell⁻¹ · h⁻¹), S_o (0.67 μM), K_t (1.39 μM) for clone 3H (25).
[b] Using value of 1.0 pg Si · cell⁻¹ for clone 3H (13).

At the end of the 4 h incubation period each sample was filtered through a 0.8 μm polycarbonate membrane filter and the filter retained for analysis of ^{30}Si uptake. The filtrate from the 6 pyrex flasks was discarded and that from the 2 polycarbonate flasks analyzed to determine the final isotopic composition of the dissolved silicic acid.

Two experiments were performed to determine the light dependence of silicic acid uptake in Si depleted populations of clone 3H. One ml of inoculum at *ca.* 10^8 cells · ml⁻¹ was added to each of 16 pyrex erlenmeyer flasks (250 ml) containing 200 ml of low Si seawater. Sufficient ^{30}Si-labeled silicic acid was added to achieve a concentration of 2.5 μM in 8 flasks and 25.0 μM in the other 8. These experiments were designed to determine the light dependence of instantaneous rates of silicic acid uptake under Si limited and Si unlimited conditions, respectively. One flask of each series was incubated 4 h at each of the following light intensities: 0, 75, 350, 650, 1300, 2700, 4300 and 10,300 lx. Incubations were initiated in groups of 4 at 30 min intervals to allow immediate filtration of each sample at the end of the incubation. The filters were retained for ^{30}Si analysis and the filtrates discarded.

In all kinetic experiments V was plotted vs. S, and in those plots where it was determined visually that the dependence of V upon S was describable by a rectangular hyperbola the kinetic parameters V_{max} and K_t were determined by a least squares fit of a straight line to a plot of $S \cdot V^{-1}$ vs. S, which is based upon a linearized form of Equation 1:

$$\frac{S}{V} = \frac{K_t + S}{V_{max}} \quad (5)$$

where the value of S at the X-intercept is $-K_t$ and the value of $S \cdot V^{-1}$ at the Y-intercept is $K_t \cdot V_{max}^{-1}$ (11).

RESULTS

Concentration dependence of uptake rate. Plots of V vs. S for Si replete and depleted clone 3H, and Si replete and depleted clone 13-1 are given in Fig. 1. All 4 plots are approximately hyperbolic, although if the dependence of V upon S in Si replete 3H is hyperbolic, V_{max} does not occur within the concentration range used in these experiments. The kinetic parameters V_{max} and K_t, computed according to Equation 5, are presented in Table 3. A threshold concentration for silicic acid uptake is not evident in any of these kinetic experiments.

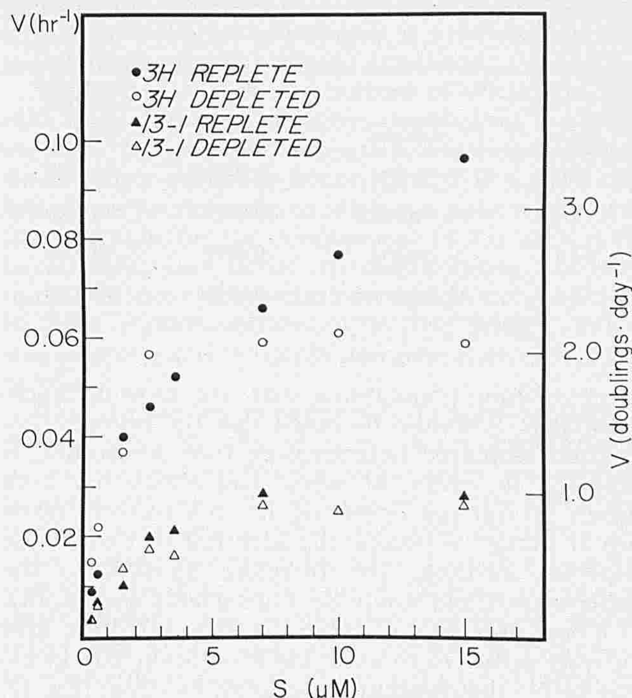

FIG. 1. Plot of specific uptake rate of silicic acid (V) vs. silicic acid concentration (S) for clones 3H and 13-1 of *T. pseudonana*.

Light dependence of uptake rate. Plots of V vs. light intensity for Si depleted 3H under Si unlimited (S = 25.0 μM) and Si limited (S = 2.5 μM) conditions are presented in Fig. 2. These data clearly do not conform to a rectangular hyperbola, so the kinetic parameters V_{max} and K_{lt} (5,11,19) are not applicable. The uptake rate of silicic acid is independent of light intensity at low silicic acid concentration, and only weakly light dependent under conditions of saturating substrate concentration.

Dissolution of silica from exponentially growing populations. Results of the dissolution rate experiments are presented in Table 4. Error estimates (21, 23) are large because of the relatively short (4 h) incubation time employed. However, even with the large uncertainties imposed by the short incubation time it is evident that there is dissolution of silica

TABLE 3. *Kinetic parameters* V_{max} *and* K_t *of silicic acid uptake.*

Clone	Initial condition	V_{max} h⁻¹	K_t μM
3H	Si replete	0.092[a]	2.3[a]
3H	Si depleted	0.062	0.8
13-1	Si replete	0.031	1.4
13-1	Si depleted	0.028	1.5

[a] Response may not be hyperbolic for Si replete clone 3H, so kinetic parameters V_{max} and K_t may have little meaning.

TABLE 4. *Specific dissolution rates of exponentially growing populations.*

Clone	Initial condition	V_{dis} h^{-1}	Maximum analytical error h^{-1}
3H	Si replete	0.0075	±0.0040
3H	Si depleted	0.0085	±0.0040
13-1	Si replete	0.0020	±0.0040
13-1	Si depleted	0.0043	±0.0040

from diatom populations that are growing exponentially. It should be noted that the isotopic procedures employed here measure true dissolution, as opposed to release of silicic acid which has been taken up but not deposited, because recently taken up Si would be isotopically identical to that in the external medium, and therefore its flux to the external medium would be undetectable analytically.

These populations were all taking up silicic acid at rates sufficient to mask the dissolution rate in experiments that measure dissolution by dS/dt (e.g. 17, 25).

DISCUSSION

Threshold concentration for silicic acid uptake. Fig. 1 indicates that the rate of silicic acid uptake is dependent upon the external substrate concentration in the range of 0.25–15.0 μM and that this dependence may be described by Equation 1. The data of Paasche (25) which indicated a threshold silicic acid concentration of *ca.* 0.67 μM, below which no uptake takes place in *T. pseudonana,* are not confirmed by these experiments. Paasche recognized that S_o can be an experimental artifact in studies measuring uptake from dS/dt if the raw data are not corrected for dissolution of cellular silica or if the dissolution rate is underestimated. Comparison of Tables 3 and 4 indicates that V_{dis} ranged from 6.5 to 15% of V_{max} in these tracer experiments, which is in good general agreement with Paasche's estimate of 10–20%. If anything, the measured values of V_{dis} are somewhat lower than Paasche's estimates, making it unlikely that his observation of a threshold concentration is a simple artifact arising from failure to correct his data adequately for silica dissolution. However, Paasche's interpretation of S_o as the concentration of some molybdate-reactive but biologically unavailable form of Si is difficult to reconcile with the present understanding of the inorganic aqueous chemistry of Si (29). Both Paasche's (25) observations of a threshold substrate concentration for silicic acid uptake in *T. pseudonana* and those reported here of the absence of such a threshold are based upon small numbers of experiments, so the question must be considered unresolved.

Silicic acid uptake kinetics and Si limited growth kinetics. Results of silicic acid uptake kinetic studies reported here (Fig. 1 and Table 2) appear to support one of the 2 conclusions drawn by Guillard, *et al.* (13) concerning adaptation of clones 3H and 13-1 to high and low silicon environments, respectively. V_{max} for the estuarine clone 3H clearly exceeds that of the Sargasso Sea clone 13-1 and by approximately the same amount (*ca.* 1 doubling · day^{-1}) that μ_{max} for clone 3H was found to exceed μ_{max} for clone 13-1. Thus in a high Si environment which initially contains a mixture of the 2 clones of *T. pseudonana,* the ratio of 3H to 13-1, expressed either in number of cells or in Si content of the population, should roughly double each day, resulting eventually in a population dominated by clone 3H regardless of its initial composition.

Although the difference between V_{max} for clone 3H and V_{max} for clone 13-1 is almost identical to the difference in μ_{max} reported for the 2 clones, comparison of the 2 sets of data shows that for both 3H and 13-1 V_{max} is somewhat lower than μ_{max}. Paasche (24, 25) reported a similar difference between V_{max} and μ_{max} for clone 3H. The reason for this difference is not clear, but it is possible that some degree of synchrony develops in the division cycle of *T. pseudonana* in response to the 14:10 LD cycle in which the cells are maintained. This synchrony would cause both V_{max} and μ_{max} to vary during the day, but its effects would be observable only in uptake experiments, which have incubation times considerably shorter than a division cycle. It is thus possible, although we believe unlikely, that the observed difference between V_{max} values of clones 3H and 13-1 results from performing short term experiments on populations that were at different positions in a synchronized division cycle, rather than any inherent difference between the uptake rate capacities of the clones. However, V_{max} was less than μ_{max} for both 3H and 13-1 so it is more likely that any effect of synchrony on V_{max} was similar in both experiments and that the observed difference between the clones is genuine.

The observation of Guillard, *et al.* (13) that clone 13-1 has a significantly lower K_s for Si limited growth than clone 3H is not reflected by any corresponding difference between K_t values for silicic acid uptake in the clones. Although clone 3H demonstrates higher rates of both uptake and growth at high silicic acid concentrations, clone 13-1 fails to demonstrate any greater efficiency than does clone 3H for uptake at silicic acid concentrations down to 0.25 μM. The raw data (13) actually show that the growth rate of clone 13-1 does not exceed that of clone 3H at silicic acid concentrations down to 0.32 μM, so it is possible that their intersection of the growth kinetic curves of the clones is an artifact generated by curve fitting. Perhaps a truncated hyperbola model (5,14) provides

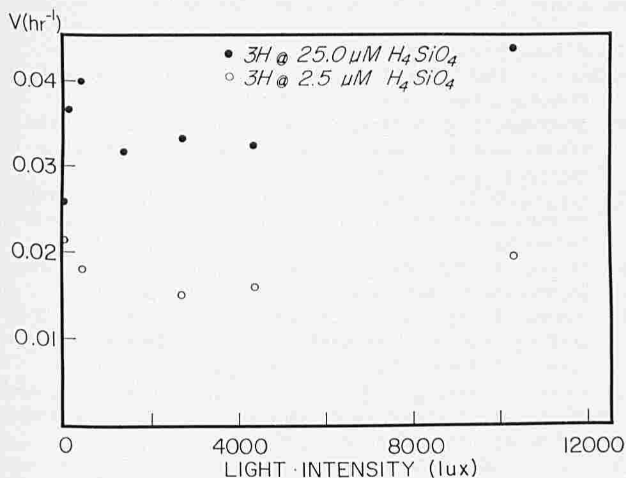

FIG. 2. Plot of specific uptake rate of silicic acid (V) vs. light intensity for clone 3H when substrate concentration is limiting (2.5 μM) and unlimiting (25.0 μM) to uptake.

a better description of Si limited growth in *T. pseudonana*.

Light dependence of silicic acid uptake. Results of experiments to determine the light dependence of silicic acid uptake in clone 3H indicate that the specific uptake rate is independent of light intensity at a silicic acid concentration of 2.5 μM and only weakly light dependent at 25.0 μM (Fig. 2). These results are in sharp contrast with the data of Davis (5) on *Skeletonema costatum*, which indicated a hyperbolic response of uptake rate to light intensity. Davis' data were obtained in 5–7 day perturbation experiments in continuous culture, while those reported here result from 4 h tracer experiments in batch culture. Thus Davis was determining something very close to the steady state response of silicic acid uptake to variations in light intensity, whereas our experiments measured the effect of light intensity on something which approximates instantaneous uptake rates. The absence of light dependence in 4 h incubations, and the presence of strong light dependence in 5–7 day incubations suggests that a stress, affecting the cells' ability to take up silicic acid, develops with time in a diatom population maintained at sub-optimal light intensity. There is a reasonable mechanism for the development of such a stress. Lewin (16) reported that Si assimilation by diatoms is closely linked to aerobic respiration and ATP production. This implies that the cell is expending photosynthetically fixed energy to take up silicic acid. Thus a population transferred from optimal to sub-optimal light conditions should continue to take up silicic acid at a high rate initially, but as the cells' energetic reserves become depleted with time, the uptake rate should become dependent upon the rate of fixation of new energy. If this reasoning is accurate then the degree of light independence of the instantaneous rate of silicic acid uptake in a diatom population is a measure of the population's general energetic condition.

The degree of light dependence of silicic acid uptake in natural phytoplankton populations is highly variable (21), and has been attributed to the population's recent metabolic history (2,21). If a stress developing with time affects nutrient uptake rates, then the time scale for the development of this stress may be an important factor in understanding nutrient uptake in natural populations, which are subjected to light regimes influenced by day length, water transparency, and the depth and time scale of vertical mixing.

Silica dissolution in exponentially growing diatom populations. Results of these tracer experiments on exponentially growing populations are similar to those obtained by Paasche (25) cells killed by freezing, a process in which the cells' protective organic coating remains intact (17). Paasche's assumption that the dissolution rate of dead but physically intact diatom cells is also characteristic of living cells, from which dissolution cannot be measured by observing changes in silicic acid concentration, is thus supported experimentally. Simultaneous silicic acid uptake and silica dissolution have also been observed in natural phytoplankton populations (21, 23). Lewin (17) detected little or no dissolution of silica from living cells, and suggested that living diatoms may have some mechanism, beyond the production of an organic coating, to prevent silica dissolution. Lewin recognized that masking of dissolution by silicic acid uptake was an alternative explanation of her experimental results, and this second interpretation is consistent with our direct measurement of simultaneous uptake and dissolution in *T. pseudonana*.

ACKNOWLEDGMENTS

This research was supported by the Oceanography Section, National Science Foundation Grants GA-37963 to the University of Alaska, GB-41315 to the University of Michigan, and GB-33288 to the Woods Hole Oceanographic Institution, and by the National Science Foundation International Decade of Ocean Exploration Coastal Upwelling Ecosystems Analysis Grant GX-33502 to the University of Washington.

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J. Phycol. **12**, 252–254 (1976)

SEXUAL ISOLATION BETWEEN A EUROPEAN AND AN AMERICAN POPULATION OF *ECTOCARPUS SILICULOSUS* (PHAEOPHYTA)[1]

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SUMMARY

Sexual plants of Northeastern American Ectocarpus siliculosus *(Dillw.) Lyngb. have been isolated and cultured. Female gametes produce a volatile sex hormone acting on male gametes. Combination of American and European gametes shows that the initial step of the sexual reaction (attraction of male gametes) works normally, whereas interaction of cell surfaces and fusion of gametes is prevented.*

[1] *Accepted: 17 February 1976.*

Key index words: chemotaxis; Ectocarpus; *incompatibility; sexual attraction*

INTRODUCTION

Systematic treatment of the genus *Ectocarpus* is highly complicated and still at the stage of morphological description of field material. Phenotypic variation seems to be rather large. This is expressed by the diverging opinions of Russell (9), who main-

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