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PHOSPHATE AND SILICATE GROWTH AND UPTAKE KINETICS OF THE DIATOMS *ASTERIONELLA FORMOSA* AND *CYCLOTELLA MENEGHINIANA* IN BATCH AND SEMICONTINUOUS CULTURE[1]

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SUMMARY

Information on the nutrient kinetics of Asterionella formosa *Hass. and* Cyclotella meneghiniana *Kutz. under either phosphate or silicate limitation was obtained for use in a Monod model and in a variable internal stores model of growth. Short-term batch culture growth experiments were fit to the Monod model and long-term semicontinuous culture experiments and short-term uptake experiments were fit to the variable internal stores model. Mathematical analysis indicates that the parameters of the 2 models may be expressed in terms of each other at steady state.*

The qualitative results of both batch and steady state culture methods agree. For limiting phosphate experiments, A. formosa *is better able to grow at low* PO_4*-P concentrations than* C. meneghiniana, *as shown by its lower* K *for* PO_4*-P limited growth. The* k_Q *of* A. formosa *compared to* C. meneghiniana *found in long-term semicontinuous culture indicates that* A. formosa *is almost an order of magnitude more efficient at using internal phosphate for growth. The qualitative results under silicate-limiting conditions also agree between the 2 methods. For short-term batch culture,* K *for silicate-limited growth of* C. meneghiniana *is less than that of* A. formosa. *The* k_Q *from semicontinuous culture experiments indicates that* C. meneghiniana *is the more efficient at using internal silicate for growth. Nutrient uptake experiments showed more variability from a Michaelis-Menten relationship than short-term growth experiments. There were no significant differences between the 2 species in half saturation constants for either phosphate or silicate uptake. We observed a marked dependence of the coefficient of luxury consumption (*R*) of phosphate on the steady state growth rate.* A. formosa *has a higher* R *than* C. meneghiniana.

Key index words: Asterionella; Cyclotella; *diatoms; growth kinetics; phosphate; silicate; uptake kinetics*

INTRODUCTION

A major hypothesis of phytoplankton ecology is that interspecific competition for nutrients determines the species composition and seasonal succession of phytoplankton in lakes (5,12,19,21,23). Numerous physiologically-based models of competition have been formulated which explicitly state the relationships between external nutrient concentration, nutrient supply rate, internal nutrient concentration, and growth rates for one or more species using the same resources. Such work can proceed no further than the physiological information upon which it is based. For instance, Lehman, *et al.* (14) describe a model of phytoplankton population dynamics which includes interspecific competition for nutrients in a manner similar to that proposed in a physiological model by Droop (4). However, Lehman, *et al.* (14) had to estimate necessary nutrient kinetic parameters because this information was not available for major freshwater species.

The present paper reports nutrient kinetic information on 2 species of freshwater diatoms for both phosphate and silicate. These experiments were designed to test whether 2 different experimental and

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theoretical approaches provide comparable information on the relationship between external nutrient concentration and growth. Short-term batch culture growth experiments provide direct information on the dependence of growth rate on external nutrient concentration. These results are summarized using the Monod model for growth (11). This model has been used by ecologists to describe the interactions of several species all competing for the same nutrients. The other type of model used to describe interspecific interactions can be called a variable internal stores model. This type of model includes the process of nutrient uptake and the process of conversion of internal nutrient to growth. Short-term batch culture nutrient uptake experiments were used to establish the relationship between external nutrient concentration and nutrient uptake rates. Long-term, semicontinuous cultures were used to establish the relationship between internal nutrient and steady state growth rate. Together these 2 types of experiments allow determination of the relationship between external nutrient concentration and growth rate. By including variable internal stores, these models allow for the effects of luxury consumption and variable nutrient supply rates, thus making them potentially more predictive under nonsteady state conditions.

The diatoms studied, *Asterionella formosa* Hass. and *Cyclotella meneghiniana* Kutz., are common in mesotrophic, mid-latitude lakes. Phosphate and silicate were chosen for these studies because these are the nutrients most commonly found to be growth rate limiting in such lakes.

MATERIALS AND METHODS

Axenic clones were used in the experiments; *Asterionella formosa* (clone FraAf) was isolated from Frains Lake, Michigan, in October 1973 and *Cyclotella meneghiniana* (clone CyOc2) was isolated from Lake Ohrid, Yugoslavia, in May 1973. A freshwater medium (WC, 9) was used for all cultures (without buffer or NH_4Cl). Cultures were maintained in a culture box at 20 C and 55 $\mu Ein \cdot m^{-2} \cdot s^{-1}$. Illumination was provided by cool-white fluorescent bulbs on a 14:10 LD cycle. Experiments were conducted under these conditions, but with 100 $\mu Ein \cdot m^{-2} \cdot s^{-1}$ illumination.

Counting was done with a Sedgwick-Rafter chamber on samples preserved with Lugol's solution using the calibrated Whipple-disk method. Growth rates for batch culture experiments were calculated in doublings$\cdot$day^{-1} by a linear least squares regression through log transformed data (7).

The kinetic constants (for growth or uptake) were determined by an iterative, nonlinear regression to the Michaelis-Menten equation (2). A Fortran IV program (10) was used to calculate the half saturation constants and maximal rates of growth or uptake.

Silicate was measured with a silicomolybdate method (22) modified for a reduced sample volume. For batch growth experiments, readings were made using a 10 mm path length flow-through cell in a Bausch & Lomb Spectronic 100, allowing accurate determinations to *ca.* 0.05 μM SiO_2-Si. For batch uptake experiments, a 100 mm path length cell was used, allowing accurate determinations to *ca.* 0.01 μM SiO_2-Si. For semicontinuous culture experiments a 50 mm path length cell was used, allowing accurate determination to *ca.* 0.01 μM SiO_2-Si. Calibration standards were sodium silicofluoride solutions.

Phosphate was determined by the Strickland and Parsons method (22), with all samples filtered through 0.45 μm filters, presoaked in distilled water. Absorbance was measured as for silicate. A 2.5 μM PO_4-P standard was used each time for calibration. Phosphate was measured to within 0.01 μM PO_4-P at low concentrations using the 100 mm cell. Intracellular phosphate was measured by a persulfate digestion process (15) modified to use smaller samples (20 ml) when needed. Samples were collected on 25 mm, 0.45 μm pore size filters, resuspended in distilled water and digested. Phosphate was then measured as mentioned.

Batch culture growth experiments:

Phosphate. Exponentially growing diatom cultures were inoculated into flasks containing WC with *ca.* 2 μM PO_4-P and allowed to grow until they had been phosphate depleted for 3 wk. At this time the extracellular PO_4-P concentration was essentially 0 (< 0.01 μM PO_4-P). Cells from these stationary phase cultures were inoculated into flasks containing sterile medium (200 ml in 500 ml flasks) with varying concentrations of PO_4-P (0.04–10 μM PO_4-P). The initial cell density was *ca.* 500 cells$\cdot$ml^{-1}. This allowed experiments to run 4 or 5 days without initial phosphate concentrations being reduced more than 25%. Cell counts were made daily and PO_4-P measured at the start and end of the experiments.

Silicate. Exponentially growing diatom cultures were inoculated into polycarbonate flasks containing WC with *ca.* 5 μM SiO_2-Si and allowed to grow until they were silicate depleted, about 5 days. At this time the ambient SiO_2-Si concentration was less than 0.5 μM Si. Polycarbonate 250 ml flasks were used for all batch growth experiments containing 150 ml of WC minus SiO_2-Si. A stock solution of $Na_2Si_3 \cdot 9H_2O$ containing 0.5 mM SiO_2-Si was used for adding various amounts of SiO_2-Si to each flask, with initial concentrations of 0.4–22 μM. The flasks were then autoclaved. Cells from the stationary phase cultures were inoculated into each flask to a density of *ca.* 200–500 cells$\cdot$ml^{-1}. Each experiment was continued for 4 days. Cell counts were made daily and SiO_2-Si was measured at the start and end of the experiments. For the *A. formosa*, 2 separate experiments were conducted 10 days apart, with 8 SiO_2-Si concentrations in each experiment. The data were pooled for determining the kinetic constants. One experiment was conducted for *C. meneghiniana* with 10 SiO_2-Si concentrations.

Calculations. Data obtained from batch growth experiments were fit to the following Monod equations:

$$\mu = \mu_m \frac{S}{K + S} \tag{1}$$

or, the equation shifted from an intercept at 0, as

$$\mu = \mu_m \frac{S - S_t}{K + S - S_t} \tag{2}$$

where μ and μ_m are the growth rate and maximal growth rate; S is the nutrient concentration; S_t the concentration at which growth rate equals 0; K the half saturation constant for growth.

The concept K_{11m} (6) is useful in relating kinetic information to field observations (13). K_{11m}, defined as the nutrient concentration at which $\mu/\mu_m = 0.9$, is a measure of the concentration at which a population comes under the control of a limiting resource.

Short-term uptake experiments:

Phosphate. The diatoms were conditioned as described for the batch growth experiments. The depleted culture was diluted to have 5×10^5 cells$\cdot$ml^{-1}, and divided among several flasks. Various initial PO_4-P concentrations were used, ranging from 0.08 to 8.0 μM PO_4-P. The PO_4-P in each flask was measured immediately after addition of PO_4-P and at 1, 2, and 4 h.

Silicate. The diatoms were conditioned as described for the batch growth experiments. The depleted culture was divided among several polycarbonate flasks, with the initial cell density

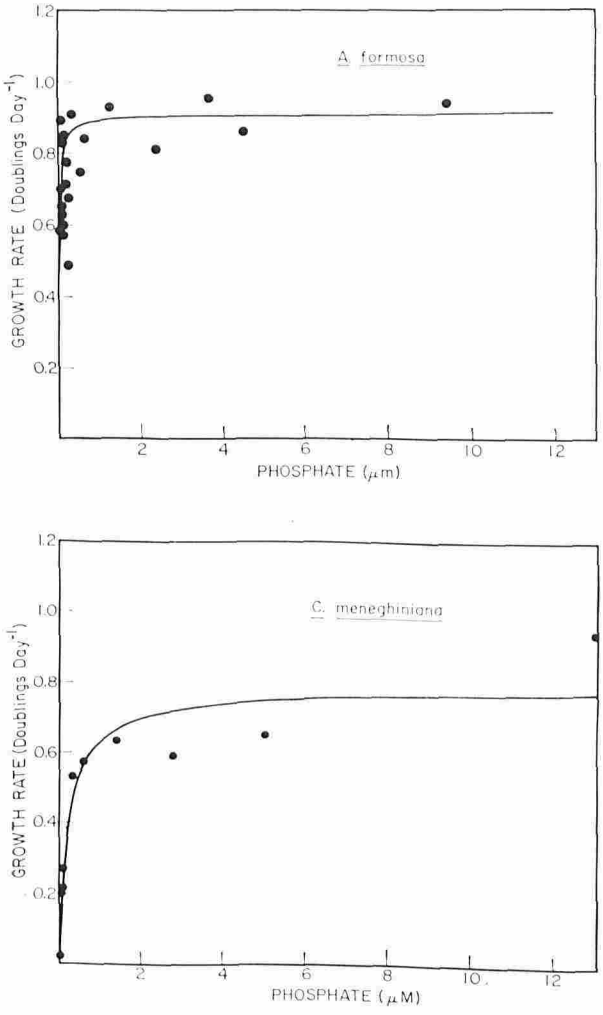

FIG. 1. Growth rates of *Asterionella formosa* (2 separate experiments) and *Cyclotella meneghiniana* as a function of phosphate concentration in short-term batch culture.

at *ca.* 5×10^4 cells·ml^{-1}. Various amounts of SiO_2-Si were added to each flask to give initial values of 0.5 to 30 μM SiO_2-Si. Silicate in each flask was measured immediately after nutrient addition and at 0.5, 1, 2 and 4 h.

Calculations. Uptake rate at each initial nutrient concentration was estimated by a least squares linear regression through the observed nutrient concentrations. Data points were fit to the Michaelis-Menten equation:

$$V = V_m \frac{S}{k + S} \tag{3}$$

where V and V_m are the velocity and maximum velocity of nutrient uptake; S is the nutrient concentration; k is the half saturation constant for uptake.

Semicontinuous culture experiments. Each species was allowed to grow to steady state in semicontinuous cultures. For the phosphate experiments the influent concentrations were 0.10, 0.22 and 0.7 μM PO_4-P (with 100 μM SiO_2-Si). For the silicate experiments the influent concentrations were 8.4 and 9.3 μM SiO_2-Si (with 50 μM PO_4-P). For each influent concentration, cultures were run at several (5–6) flow rates (f) from 0.05 to 0.6·day^{-1}. Cultures were manually diluted each day by removing a portion of the culture suspension and replacing it with an equal volume of sterile medium. Flow rates are expressed as the volume removed/day to the total culture volume. Cell counts and nutrient concentration measurements were performed periodically. Experiments were terminated after steady state nutrient concentrations and cell numbers had been observed for a week, usually after *ca.* 25 days. Some experiments were allowed to continue 45 days. Cell quotas were estimated for the steady state cultures either by direct measurement or by calculation. The persulfate digestion process (15) adequately measured the cell quota of PO_4-P, but the method of Paasche (16) for cell quota of SiO_2-Si was unacceptably variable. For calculation, the relation used is: (influent nutrient concentration − reactor nutrient concentration)/(number of cells at steady state).

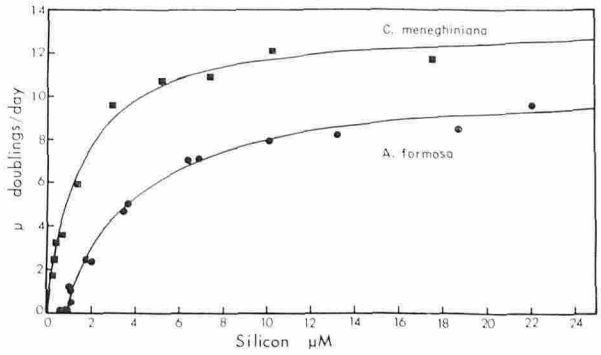

FIG. 2. Growth rates as a function of silicate concentration in short-term batch culture.

The steady state results were fit to Droop's equation (4) relating steady state growth rate to cell quota. For semicontinuous cultures diluted once a day (assuming growth to be a continuous exponential process), steady state growth rate, D, depends on f as:

$$D = \ln(1/(1 - f)) \tag{4}$$

Using this equation to calculate D, observed cell quotas of the limiting nutrient were fit to Equation 5, expressed in the linearized form of Equation 6.

$$D/D_m = 1 - k_Q/Q \tag{5}$$

$$DQ = D_m Q - D_m k_Q \tag{6}$$

where Q is cell quota; D is the steady state growth rate; D_m is the maximal growth rate, i.e., the value of D when Q is infinite; k_Q is the minimal cell quota, i.e., cell quota at which growth ceases.

RESULTS

Short-term batch growth experiments. Figure 1 gives the growth rate vs. initial PO_4-P concentration for *A. formosa* and *C. meneghiniana*. The inocula for the *Asterionella* experiments were grown in low phosphate medium for 3 wk. Essentially all the cells appeared viable after such starvation, but occasional dead cells (< 1%) were not counted. The *C. meneghiniana* used to inoculate the experimental cultures were phosphate starved for 3 wk. Figure 2 shows growth rate vs. initial SiO_2-Si concentration for both species.

Table 1 gives kinetic information on the growth

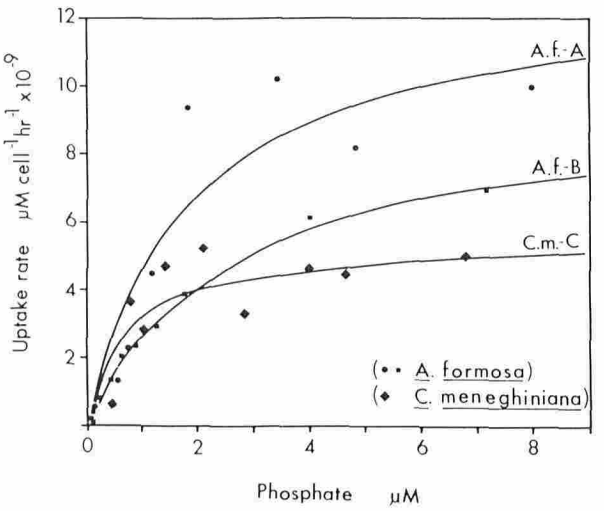

FIG. 3. Short-term uptake rates of *A. formosa* (A.f.-A, A.f.-B) and *C. meneghiniana* (C.m.-C) as a function of phosphate concentration. Experiment A.f.-A performed 2 mo prior to expt. A.f.-B.

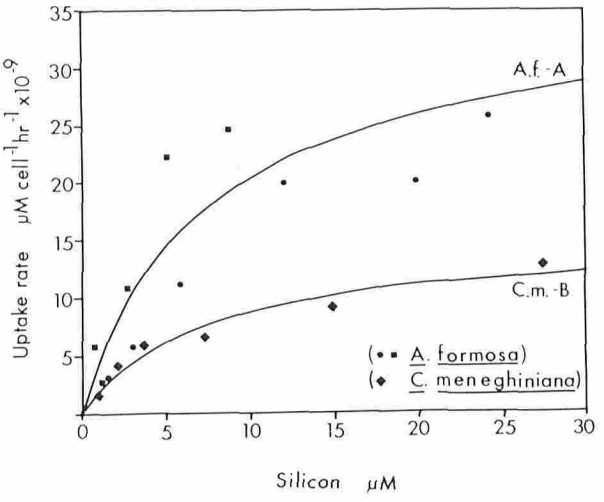

FIG. 4. Short-term uptake rates of *A. formosa* (A.f.-A) and *Cyclotella meneghiniana* (C.m.-B) as a function of silicate concentration.

response of each species to both nutrients. The maximum growth rates in the phosphate experiments were not significantly different for the 2 species (here and elsewhere significance is for $p \geq 0.95$). The half saturation constant of *A. formosa* was significantly lower than that of *C. meneghiniana*. In the silicate experiments *C. meneghiniana* had a higher maximal growth rate and lower half saturation constant than *A. formosa*. The K_{lim} values in the phosphate experiments were lower for *A. formosa* than for *C. meneghiniana* (Table 1). In the silicate experiments K_{lim} was lower for *C. meneghiniana* than for *A. formosa*.

Short-term uptake experiments. Figure 3 gives the uptake rate (V, μmol·cell^{-1}·h^{-1}) vs. initial PO_4-P for both species. Two experiments (A.f.-A, A.f.-B) shown for *A. formosa*, illustrate the variability in replicate experiments done 2 mo apart. Figure 4 shows the uptake rate vs. initial silicate concentration for both species. Two separate experiments are combined for *A. formosa*. Table 1 gives the kinetic parameters for these batch uptake experiments. Maximum uptake rates and half saturation constants were not significantly different for these 2 species in response to phosphate. For the silicate experiments, the half saturation constants were not significantly different, but the maximal uptake rate of *A. formosa* was barely significantly higher. The scatter in the

TABLE 1. *Kinetic information for silicate- and phosphate-limited growth and uptake experiments.*

Expt. type Diatom	Nutrient	Kinetic constant	Confidence interval 95%	Maximum rate	Confidence interval 95%	S_t μM	K_{lim} μM	Y cells·μmol^{-1}
Batch growth		μM		Doublings·day^{-1}				
Asterionella	PO_4-P	$K = 0.02$	0.01–0.03	$\mu_m = 0.88$	0.83–0.93	0	0.16	2.2×10^8
Cyclotella	PO_4-P	$K = 0.25$	0.09–0.45	$\mu_m = 0.78$	0.64–0.91	0	2.25	2.6×10^7
Asterionella	SiO_2-Si	$K = 3.94$	3.28–4.79	$\mu_m = 1.06$	0.97–1.14	0.78	29.2	2.5×10^8
Cyclotella	SiO_2-Si	$K = 1.44$	0.91–2.07	$\mu_m = 1.33$	1.19–1.48	0	13.0	4.2×10^8
Batch uptake		μM		μmol·cell^{-1}·h^{-1}				
Asterionella	PO_4-P (3A)[a]	$k = 1.9$	0.1–7.1	$V_m = 13.2 \times 10^{-9}$	$6.3–20.1 \times 10^{-9}$			
	PO_4-P (3B)[b]	$k = 2.8$	2.3–3.4	$V_m = 9.85 \times 10^{-9}$	$9.0–10.7 \times 10^{-9}$			
Cyclotella	PO_4-P (3C)[c]	$k = 0.8$	0.0–3.2	$V_m = 5.51 \times 10^{-9}$	$2.8–8.3 \times 10^{-9}$			
Asterionella	SiO_2-Si	$k = 7.7$	2.4–21.1	$V_m = 3.58 \times 10^{-8}$	$2.0–5.2 \times 10^{-8}$			
Cyclotella	SiO_2-Si	$k = 7.5$	3.2–14.6	$V_m = 1.51 \times 10^{-8}$	$1.1–1.9 \times 10^{-8}$			
Steady state growth		μmol·cell^{-1}		day^{-1}	Corr. coef. r			
Asterionella	PO_4-P	$k_Q = 1.75 \times 10^{-9}$		$D_m = 0.70$	1.00			
Cyclotella	PO_4-P	$k_Q = 10.7 \times 10^{-9}$		$D_m = 0.69$	0.99			
Asterionella	SiO_2-Si	$k_Q = 2.96 \times 10^{-7}$		$D_m = 1.21$	0.95			
Cyclotella	SiO_2-Si	$k_Q = 1.57 \times 10^{-7}$		$D_m = 1.16$	0.97			

[a] Refers to Fig. 3, curves A.f.-A, A.-f.-B, C.m.-C, respectively.

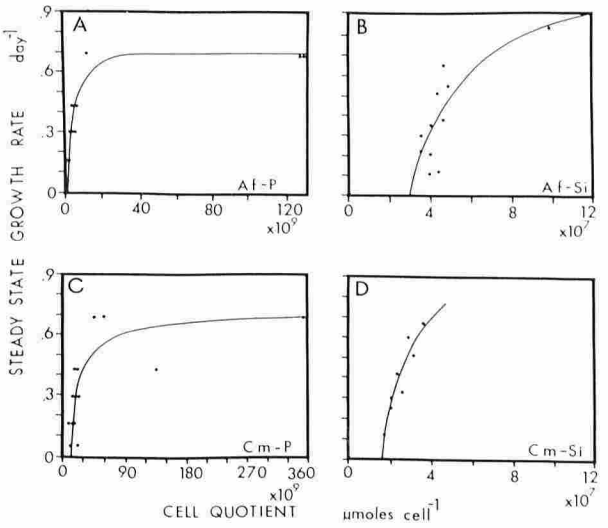

FIG. 5. Long-term steady state growth rate as a function of cell quota for *A. formosa* under phosphate (A) and silicate (B) limiting conditions, and for *C. meneghiniana* under phosphate (C) and silicate (D) limiting conditions (line is the fitted curve using Equation 5 from Droop (4)).

data as indicated by the broad confidence limits (Table 1) may result from the short term batch uptake method used.

Semicontinuous culture experiments. The relationship between cell quota (Q) and growth rate for both species grown to steady state under either silicate or phosphate limitation is shown in Fig. 5. Each point is a data point from one semicontinuous culture. Curves shown are fitted to Droop's equation (Equation 5) with a least squares regression to Equation 6. The calculated minimal cell quotas (k_Q) and maximal steady state growth rates (D_m) for each species and each nutrient are in Table 1.

Internal phosphate levels were measured for silicate-limited cells at various steady state growth rates. These are compared to the cell quotas of phosphate for phosphate-limited cells at the same growth rates. The ratio of internal phosphate levels when silicate-limited to that when phosphate-limited at the same steady state growth rate is R, the coefficient of luxury consumption of phosphate. R is an order of magnitude higher for *A. formosa* than for *C. meneghiniana*. Figure 6 compares R to D/D_m for each species. The line shown is a least squares linear regression. For *A. formosa*, the y-intercept (R_m, the maximal luxury consumption) is 82, the slope –96, and the correlation coefficient –.96. For *C. meneghiniana*, R_m is 6.6, the slope –6.4, and the correlation coefficient –.99.

DISCUSSION

The qualitative results of both batch and steady state culture methods agree. For limiting phosphate experiments, *A. formosa* is better able to grow at low extracellular PO_4-P concentrations than *C. meneghiniana*, as shown by its lower half saturation constant for PO_4-P-limited growth. The lower k_Q of *A. formosa* compared to *C. meneghiniana* found in long-term semicontinuous culture indicates that *A. formosa* is almost an order of magnitude more efficient at using internal phosphate for growth. The qualitative results under silicate limiting conditions also agree between the 2 methods. For short-term batch culture, the half saturation constant for SiO_2-Si-limited growth of *C. meneghiniana* is less than *A. formosa*. The k_Q from semicontinuous culture experiments indicates that *C. meneghiniana* is more efficient at using intracellular silicate for growth than is *A. formosa*. The general qualitative similarities of these 2 methods do not mean that their quantitative predictions are similar. For the 2 methods to be compared, it is necessary to establish a mathematical relationship between the 2 models of growth, as will be done later.

Benndorf (1) reported a half saturation constant for PO_4-P-limited growth of *A. formosa* of 0.02 μM PO_4-P, with a maximal growth rate of 0.90 doublings·day^{-1}. The results reported here for short-term batch culture experiments agree well with those. Kilham (13) reports short-term batch culture kinetic constants for silicate-limited clones of *A. formosa* isolated from Lake Ohrid, Yugoslavia and Lake Windermere, England. The clone from Lake Ohrid had a $K + S_t$ of 1.93 μM SiO_2-Si and the Lake Windermere clone a $K + S_t$ of 1.09 μM SiO_2-Si. The maximal growth rates of these 2 clones were 1.11 and 0.61 doublings·day^{-1}, respectively. There are significant differences among these 3 clones of *A. formosa*. The $K + S_t$ of both the Lake Ohrid and the Lake Windermere clones are significantly lower than that reported here for *A. formosa* from Frains Lake. The Lake Windermere clone has a maximal growth rate significantly less than both the Lake Ohrid and Frains Lake clones. The Lake Ohrid and Frains Lake clones are not significantly different in their maximal growth rates.

The 2 clones Kilham (13) used were not axenic, whereas the Frains Lake clone of *A. formosa* is. It is not known if the kinetic differences found among these clones may have been due to the presence of bacteria. We consider the possibility of direct effects doubtful, because silicate-using bacteria are very rare. However, Paasche (16) noted that *Thalassiosira pseudonana* Hasle & Heimdal used silicate more efficiently in a bacteria contaminated chemostat than in an axenic chemostat. If the differences we observed are not caused by bacteria, it would appear that there are clonal differences in the kinetics of silicate-limited growth for *A. formosa*. Guillard, *et al.* (8) have reported clonal differences in the half saturation constants and maximal growth rates of the marine diatom *T. pseudonana* under SiO_2-Si limitation. If clonal differences are common, researchers must be careful to interpret nutrient kinetic information on a particular species only for the lake from

which the clone was isolated, especially if nutrient physiological information is to be used to interpret interspecific interactions in a lake.

In the silicate-limited growth experiments reported by Kilham (13) for *A. formosa*, significant death rates were observed at nutrient concentrations less than S_t. Death rates were not observed for the axenic Frains Lake clone (Fig. 2). At concentrations less than S_t, the Frains Lake clone failed to grow but did not die. These differences may be caused by the bacteria in the Lake Windermere and Lake Ohrid clones. It may be that bacteria are able to infect the nongrowing, silicate-limited cells, causing death. Such bacterial effects may be less important at higher growth rates.

Nutrient uptake experiments showed more variability from a Michaelis-Menten relationship than short-term growth experiments. The method used for phosphate uptake experiments revealed no significant differences in k or V_m between *A. formosa* and *C. meneghiniana*. Short-term silicate uptake experiments indicate that the half saturation constants are not significantly different. Maximal rates of silicate uptake are different, with *A. formosa* having a higher V_m than *C. meneghiniana* (Table 1).

Rhee (20) reports that the rate of phosphate uptake by *Scenedesmus* sp. depends both on external and internal concentrations. This dependence on cell quota indicates that cell quotas are important in a short-term regulatory feedback mechanism that influences the rate of nutrient uptake. Such control would tend to make short-term uptake experiments such as those we did variable and possibly deviate from a good fit to the Michaelis-Menten equation. Close control of the physiological state prior to uptake experiments is desirable. This problem has been discussed by Davis (3) for silicate uptake of marine diatoms.

Short-term growth experiments provide direct information on the quantitative dependence of growth rate on external nutrient concentration. Such experiments are usually fit to a Monod model. Long-term semicontinuous culture information, together with short-term uptake information can be used to predict the relationship of growth rate to external nutrient concentration. To do this, the Monod model must be equated with the variable internal stores model of growth. Droop (4) has provided empirically-based equations for a variable internal stores model of growth. A simplified version of Droop's 8 equations was used by Lehman, *et al.* (14). We use a modified form of these equations to describe the dependence of growth rate on external nutrient concentration, and apply them to the physiological information we have reported. The equations are simplified from Droop to illustrate the interactions of two important processes: nutrient uptake, and use of internal nutrient for growth. This variable internal stores model requires 3 simultaneous differential equations:

$$\dot{N} = dN/dt = N[D_m(1 - k_Q/Q) - D] \quad (7)$$

$$\dot{Q} = dQ/dt = V_m S/(k + S) - D_m(Q - k_Q) \quad (8)$$

$$\dot{S} = dS/dt = D({}_oS - S) - NV_m S/(k + S) \quad (9)$$

variables of time—N, number of cells·l^{-1}; Q, cell quota, μmol of nutrient/cell; S, external nutrient concentration: *physiological constants*—D_m, maximal growth rate; k_Q, minimal cell quota; V_m, maximal rate of nutrient uptake; k, half saturation constant for nutrient uptake: *experimentally manipulable constants*—D, dilution rate/day (which equals growth rate at steady state); ${}_oS$, concentration of the growth rate limiting nutrient in the influent medium.

According to Equation 7, growth ceases when the cell quota is less than k_Q. At cell quotas larger than k_Q, growth increases with Q in a manner saturating as a hyperbolic function (Fig. 5) with a half saturation constant k_Q, displaced from the origin by an amount k_Q. Equation 8 describes the changes in cell quota which result from uptake and growth. Equation 9 is a mass balance equation. These 3 simultaneous differential equations may be used to describe the dynamics of growth of a single species potentially limited by a single nutrient. Although these equations omit both a maximal cell quota and the effect of cell quota on uptake rate, they allow an analytical discussion of the interrelationships of the 2 models.

By setting the time derivatives equal to 0, Equations 7, 8 and 9 provide the following steady state predictions:

$$Q_v^* = k_Q D_m/(D_m - D) \quad (10)$$

$$S_v^* = D_m k_Q k D/[V_m(D_m - D) - D_m k_Q D] \quad (11)$$

$$N_v^* = ({}_oS - S_v^*)/Q_v^* \quad (12)$$

The steady state cell quota, Q_v^*, is independent of the kinetic parameters of nutrient uptake. The steady state external nutrient concentration, S_v^*, and the steady state cell number, N_v^*, both depend on all 4 physiological constants. These 3 equations (Equations 10–12) describe the steady state characteristics of a population under nutrient limitation in terms of k_Q, D_m, k, V_m and D.

The Monod model may also be used to express steady state values for Q, S and N in terms of K, Y, μ_m and D. The Monod equations for continuous culture (11) give the following steady state relations:

$$S_c^* = DK/(\mu_m - D) \quad (13)$$

$$N_c^* = ({}_oS - S^*)Y \quad (14)$$

where S_c^* is the steady state external nutrient concentration and N_c^* is the steady state standing crop predicted by the Monod model. By setting $S_v^* = S_c^*$, $N_v^* = N_c^*$, and $D_m = \mu_m$, the following interrelationships are obtained:

$$Q_v^* = 1/Y \quad (15)$$

$$\hat{K} = k_Q[kD_m(D_m - D)/(V_m(D_m - D) - D_m D k_Q] \quad (16)$$

Equation 16 can be approximated by Equation 17 for D much less than the washout rate.

$$\hat{K} \doteq k_Q D_m k/V_m \quad (17)$$

Equation 17 is the relationship that Rhee (20) used to calculate the kinetics of growth from the kinetic constants for nutrient uptake.

This illustrates that the half saturation constant for growth determined from short-term batch culture experiments is a summary statistic which includes both the process of nutrient uptake (k, V_m) and the process of utilization of internal nutrient for growth (k_Q, D_m). Because growth rate equals dilution rate only at steady state, some deviation from the Monod equation may be expected in short term batch enrichment growth experiments. The observed dependence of Q_v^* on steady state growth rate (Fig. 5) means that the Monod equation may not be as good a predictor of the steady state standing crop as the variable internal stores model. However, as a summary statistic, $\hat{K}$ may be used to determine the relative importance of uptake vs. efficiency of conversion of internal nutrient to growth in the growth response of a species to external nutrient concentration.

The approximation to $\hat{K}$ (Equation 17) was applied to the steady state growth and to the batch uptake data of Table 1. The estimated half saturation constants for phosphate- and silicate-limited growth of *A. formosa* and *C. meneghiniana* are listed in Table 2. A similar analysis was made for *Thalassiosira pseudonana* using the steady state chemostat data from Paasche (16) and the uptake kinetic data from Paasche (17), and comparing it to the half saturation constant for silicate-limited growth of this clone of 0.98 μM SiO_2-Si reported by Guillard, *et al.* (8). The 0.95 confidence intervals on the uptake kinetic parameters of Table 1 for *A. formosa* and *C. meneghiniana* were used to estimate confidence intervals about $\hat{K}$. This was not done for *T. pseudonana*. The variance in the uptake process alone can account for more variance in $\hat{K}$ than was observed in K. If this range approximates the 0.95 confidence interval about $\hat{K}$, the estimated half saturation constants for growth are not significantly different from those observed (Table 2). This indicates that both the Monod model and the variable internal stores model of growth make similar predictions about the dependence of growth rate on external nutrient concentrations.

If nutrient kinetic information is to be used to compare species as to their relative ability to grow under nutrient limitation, the experimentally observed K from short-term batch culture may be preferable to $\hat{K}$ because of the greater variance in $\hat{K}$. When the Monod model is applied to several species competing for the same limiting nutrient (25), for species which do not differ in their maximal growth rates, the species with the lower K should competitively displace all others at steady state. Silicate-limited, long-term competition experiments between *A. formosa* and *C. meneghiniana* revealed that *C. meneghiniana* was the superior competitor (24,25). This agrees with the prediction based on the observed K, but not with the estimated $\hat{K}$. The observed K predicts that *A. formosa* should be dominant when both species are phosphate-limited; $\hat{K}$ reflects this prediction, but doesn't show significant differences between the two estimated half saturation constants for growth. *A. formosa* was observed to be competitively dominant in long-term, phosphate-limited competition experiments between the 2 species, as predicted by K.

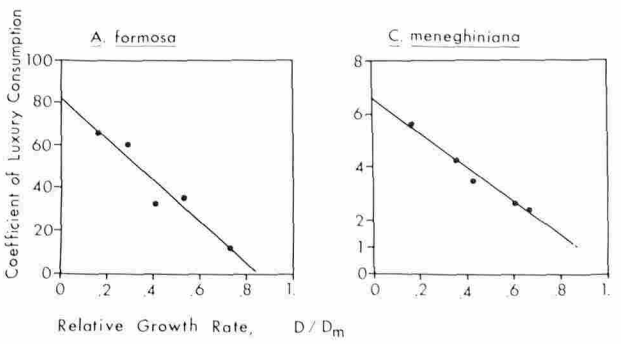

FIG. 6. Coefficient of luxury consumption as a function of relative growth rate (D/D_m).

Droop (4) has defined luxury consumption (R) as the ratio of the cell quota of a nutrient when it is not limiting to that when it is limiting. We have observed a marked dependence of R on steady state growth rate (Fig. 6). *A. formosa* can have up to 82 times more internal phosphate than needed; *C. meneghiniana* can have up to 6.6 times more. The upper limit on internal nutrient levels must act as

TABLE 2. *Estimated half saturation constants for growth* ($\hat{K}$) *calculated from steady state parameters* (k_Q, D_m) *and from short-term uptake parameters* (k, V_m) *using Equation 17.*

Species	Nutrient	$\hat{K}$ μM	Estimated range, μM	S.D. from observed
A. formosa	PO_4	0.01	0.003–0.06	NO
A. formosa	Si	3.21	0.69–15.7	NO
C. meneghiniana	PO_4	0.04	0–0.35	NO
C. meneghiniana	Si	3.76	1.28–10.1	NO
T. pseudonana[a]	Si	1.74		NO

[a] From Paasche (16,17).

an element in a feedback system that controls nutrient uptake. The observed linear decrease in luxury stores with relative growth rate (D/D_m), down to a value of $R = 1$ at $D/D_m = 1$, can be expressed at steady state as:

$$Q_m = k_Q[R_m - (R_m - k_Q)(1 - k_Q/Q)] \qquad (18)$$

where R_m is the coefficient of maximal luxury consumption, and Q_m is the maximal cell quota of nutrient for a specific growth rate, D. Under nonsteady state conditions, nutrients may be taken up at rates which exceed the rate at which they can be converted into new growth. The maximum cell quota calculated above (Q_m) may act as a limit on the rate of nutrient uptake. The rate of uptake must decrease in some manner as cell quota approaches the maximal levels, Q_m. Equations 7–9 may be modified to include such a feedback system that allows for the effect of cell quota on nutrient uptake rate, using the observed limit as a proportional damping force (in a manner analogous to that of Lehman, *et al.*, 14).

The regression we used to calculate k_Q and D_m (4) and the other possible linearization of Equation 6 do not allow a valid computation of confidence limits about k_Q and D_m. Such a computation must best come from a nonlinear regression, similar to that used for the Michaelis-Menten nonlinear regression (2,10). Such a tool would greatly aid in understanding the goodness-of-fit of results to the model. It would also allow comparison of Droop's model with possible alternatives. For instance, Fig. 5 shows several data points which indicate that maximal growth rate is approached more rapidly than predicted by Droop's model. Nonlinear regressions of data to Droop's and other possible models would allow testing of the relative goodness-of-fit.

General discussion. Two experimental approaches to the relationship of phytoplankton growth to nutrient limitation have been presented. Each approach embodies certain physiological assumptions. The Monod model assumes that growth depends on external concentration in a hyperbolic fashion and that yield is independent of growth rate. The short-term batch culture method used to establish K and μ_m provided results that fit the model acceptably. Short-term batch culture estimates of K and μ_m are dependent on the physiological state of the starting inoculum. A method proposed by Paasche (18) may alleviate this complication.

Combination of short-term uptake experiments and long-term growth experiments provide information on the relationship of growth to external nutrient concentration comparable, at steady state, to that of the short-term batch growth experiments. The variable internal stores model based on these experiments (Equations 7, 8, 9) may be more accurate at describing the dynamics of growth in nonsteady state conditions than the Monod model, because it includes luxury consumption. Although short-term batch culture information (K and μ_m) appears to be a more precise predictor of steady state competitive interactions (25), such information may not be as good a predictor of the dynamics of approach to steady state. However, short-term growth experiments are easily and rapidly done. The summary of uptake and efficiency of utilization of nutrients provided in K and μ_m makes short-term growth experiments a valuable tool in interpreting the possible role of nutrients in the interactions of several species. For natural situations that are near steady state, the Monod model may prove satisfactory. For nonsteady state conditions, a variable internal stores model, requiring the less easily obtained parameters k_Q, D_m, k, V_m and possibly R_m, may prove more useful, possibly providing better predictions of the population dynamics of the species involved.

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REGULATION OF GAMETOGENESIS IN *SCENEDESMUS OBLIQUUS* (CHLOROPHYCEAE)[1]

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SUMMARY

The effects of nutrients, temperature and light on gametogenesis in Scenedesmus obliquus *(Turp.) Kütz. were studied in culture. Concentrations of nitrogen in the medium employed showed a marked influence on gamete production. Gametogenesis is inhibited by N excess but is not a response to N starvation or depletion. A drop in N level from that of the growth medium is not required, nor does it* per se *trigger gametogenesis. The N concentration satisfying growth requirements is sufficiently low to permit sexual differentiation. Nitrogen level in the growth medium has no effect on subsequent gamete production, so long as there is sufficient N to maintain a typical culture. Number of gametes present at maximum production time is inversely related to N concentration, but neither time of onset of gametogenesis, nor time of maximum gamete production is affected by N concentration. Cultures incubated at 15 C in medium lacking N take a minimum of 20–24 h to develop cells irreversibly committed to gamete formation. At the concentrations tested, no medium component other than the N-containing salt affected gametogenesis. Temperature influences both time of maximum production and numbers present at maximum production time. Time of maximum production is inversely related to incubation temperature; a 15 C incubation temperature yielded highest gamete production. Light enhances gametogenesis, but gamete formation can occur in absence of light. Achievement of a light-saturated response is dependent upon illumination given at two critical periods: one occurs shortly after N withdrawal; the other occurs later, when cells are becoming irreversibly committed to gamete formation. Ability to produce gametes diminished with prolonged laboratory culture.*

Key index words: Gamete; gametogenesis; light; nitrogen; Scenedesmus*; sexuality; temperature*

INTRODUCTION

The colonial green alga *Scenedesmus obliquus* (Turp.) Kütz., a common freshwater species, is

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