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TEMPERATURE EFFECTS ON SILICON- AND PHOSPHORUS-LIMITED GROWTH AND COMPETITIVE INTERACTIONS AMONG THREE DIATOMS[1]

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ABSTRACT

Three diatom species, Stephanodiscus hantzschii *(Ehr.) Grun.,* Asterionella formosa *Hass. and* Fragilaria crotonensis *Kitt. Hass. were isolated from Lake Maarsseveen where they are dominant and show a successional sequence. The physiological responses of each species to temperature and limitation by silicon and phosphorus were determined over the temperature range of 5° to 20° C using short-term batch culture methods.* Stephanodiscus hantzschii *had higher maximum growth rates than the other two species at all temperatures, and the maximum growth rates of all species increased with increasing temperature. Temperature affected not only maximum growth rates but also half-saturation constants (K_s) and the minimum cell quotas.* S. hantzschii *had low silicon requirements for growth under Si-limiting conditions, and* A. formosa *and* F. crotonensis *had higher and nearly identical silicon requirements. The K_s values for silicon for* S. hantzschii *were essentially constant from 5° to 20°C but varied greatly for the other two species.* A. formosa *had the lowest requirements for growth under phosphorus limitation,* F. crotonensis *was intermediate and* S. hantzschii *had the highest growth requirements for phosphorus. The K_s values for phosphorus were constant over the temperature range for both* A. formosa *and* F. crotonensis *and were much higher and variable for* S. hantzschii.

Nutrient competition experiments were performed in continuous cultures at four temperatures and various Si:P ratios. The results generally, but not always, confirmed the predictions based on the Monod relationships for each species. Results not in agreement with predictions were usually because of similar physiological properties of A. formosa *and* F. crotonensis *or because of decreased loss rates for* F. crotonensis *due to wall growth. In cultures with all three species phosphorus-limited (Si:P > 75),* A. formosa *often dominated as predicted, although* F. crotonensis *was sometimes the most abundant species. As predicted,* S. hantzschii *never dominated at high Si:P ratios. At intermediate Si:P ratios when* A. formosa *and* F. crotonensis *were both Si-limited and* S. hantzschii *P-limited, all three species coexisted because* A. formosa *and* F. crotonensis *have almost identical silicon requirements, although sometimes* F. crotonensis *was more abundant than predicted. At 10°C the results agreed best with the predictions;* A. formosa *dominated at high Si:P ratios and* S. hantzschii *dominated as predicted at low Si:P ratios when all three species were Si-limited.*

Key index words: Asterionella formosa; *cell quota; competition; diatoms;* Fragilaria crotonensis; *growth kinetics; phosphorus; silicon;* Stephanodiscus hantzschii

Diatoms predominate in the phytoplankton of Lake Maarsseveen (The Netherlands) during early

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spring and late summer as part of a successional sequence (Dorgelo et al. 1981, Dorgelo and de Graaf Bierbrauwer 1981, van Donk 1983, van Donk and Ringelberg 1983, van Donk et al. 1988). For a long time temperature was thought to play a relatively minor role in seasonal succession because only maximum growth rate was considered to be set by temperature, although in nature this maximum is probably seldom reached due to growth limitation by nutrients (Eppley 1972). Nevertheless, the effect of temperature on nutrient-limited growth is complex (Paasche 1975, Ahlgren 1978, 1987, Rhee and Gotham 1981, Tilman et al. 1981, Mechling and Kilham 1982, Kilham 1984). The kinetics of nutrient-limited growth can be described by a number of models including the Monod and Droop models (Kilham 1975). Temperature can alter the maximum growth rate (μ_m), the half-saturation constant for growth (K_s) and the minimum requirements of a cell for a particular resource (the minimum cell quota, Q_0). Ahlgren (1987) reviewed the various formulations used to describe the effects of temperature on algal growth constants and concluded that 1) the effect of temperature on μ_m was essentially linear over the range 0°–40° C, 2) K_s and yield at maximum growth rate were independent of temperature, and 3) Q_0 had a complex relationship with temperature, usually being higher at low temperatures. In P- and N-limited *Scenedesmus* sp., *Asterionella formosa* (Rhee and Gotham 1981), *Monochrysis lutheri* Droop (Goldman 1979) and *Oscillatoria agardhii* Gom. (Zevenboom 1980), the minimum cell quota increased with decreasing temperatures. These increases in Q_0 indicate that suboptimal temperatures increase resource requirements, and thus may also alter optimum ratios. The same may be true for supra-optimal temperatures (Tilman et al. 1981, Rhee 1982).

When species exhibit differential resource utilization for growth, it is possible to make predictions about how they will interact when grown together in continuous culture (Veldkamp and Jannasch 1972, Tilman 1977, 1982, Tilman et al. 1982). Any environmental factor which alters substrate utilization can affect the outcome of competition. Under constant environmental conditions, competition for a single limiting resource in a continuous culture should result in selection of only one species. Coexistence in competitive equilibrium is possible if several resources are in relatively short supply and the growth of each species is limited by a different resource (Tilman 1977, 1982). Parameters used to make model predictions are the physiological resource kinetic constants for growth of each species for each resource. The ratios of these constants are important for determining whether a species will be growth-rate limited by one or another resource under particular conditions. There is a particular resource ratio at which a species switches from being limited by one resource to being limited by another. This switching point, or species optimum point, is thought to be unique for each species (Rhee and Gotham 1980, Tilman 1980).

To elucidate the significance of temperature and resource interactions in the seasonal succession of the phytoplankton in Lake Maarsseveen, we studied growth in relation to nutrients and temperature of three dominant species isolated from this lake. This paper reports Si- and P-limited growth for the diatoms *Asterionella formosa*, *Stephanodiscus hantzschii* and *Fragilaria crotonensis* at 5°, 10°, 15° and 20° C. We made predictions about the outcome of competition based on the physiological constants and tested these predictions using continuous cultures at various Si:P ratios at the four temperatures.

MATERIALS AND METHODS

Unialgal clonal cultures of *Asterionella formosa*, *Fragilaria crotonensis* and *Stephanodiscus hantzschii* were isolated from Lake Maarsseveen (The Netherlands) two months before performing the experiments, using the pipet technique of Guillard (1973). The lake is described by Ringelberg (1981) and van Donk (1987). Stock cultures were maintained in 25 mm × 150 mm Pyrex tubes in culture boxes at 5°, 10°, 15° and 20° C, with illumination provided by cool-white fluorescent bulbs at 55 $\mu E \cdot m^{-2} \cdot s^{-1}$ on a 14:10 h LD cycle. Experiments were performed under the same conditions but with the illumination increased to 100 $\mu E \cdot m^{-2} \cdot s^{-1}$. Guillard's (1975) WC medium, modified as outlined below for individual experiments, was used for all studies. Additional K was added as KNO_3 when K_2HPO_4 concentrations were decreased in the medium.

Samples for cell enumeration were preserved with Lugol's solution and counted using the calibrated Whipple-disk method. Growth rates were calculated by a linear least squares regression of log transformed data. Growth rate values and nutrient concentration data were fit to the Monod relationship by an iterative, non-linear regression method (see Kilham 1975).

Nutrients were measured after filtration through 0.4 μm polycarbonate filters. Phosphate and silicate were determined by the methods of Strickland and Parsons (1972) using potassium phosphate and sodium silicofluoride standards, respectively. Our detection limits were ca. 0.02 μM Si and 0.02 μM P.

Acclimated maximum growth rates. To measure maximum growth rates of the diatoms at 5°, 8°, 10°, 15° and 20° C, we used the method of daily *in vivo* chlorophyll fluorescence (Brand et al. 1981) with a Turner Designs 10-000R Fluorometer. The species were cultured separately in 25 mm × 150 mm tubes filled with 20 mL of medium and capped with polypropylene caps. Tubes were inoculated from stock cultures growing at the experimental temperatures. Every day at the same time the tubes were removed from the culture box, mixed on a vortexer and fluorescence measured. A minimum of three transfers was made to fresh tubes before the populations approached stationary phase (continuous batch culture method) to ensure an accurate determination of acclimated maximum growth rates. A minimum of four successive days was used to determine the slope for each transfer.

Batch culture growth experiments. Short-term batch culture growth experiments were used to evaluate the relationship between external nutrient concentrations and growth rates of each species at 5°, 10°, 15° and 20° C. This method was shown to be useful in previous growth kinetic experiments (Guillard et al. 1973, Kilham 1975, Klaveness and Guillard 1975, Tilman and Kilham 1976, Holm and Armstrong 1981, Tilman 1981, Mechling and Kilham 1982). When very low initial cell densities in large volumes are used for relatively short time periods, the cultures approximate steady-state conditions because the algae cannot affect the nutrient concentration to any significant degree.

TABLE 1. *List of symbols, units and equations.*

Symbol	Explanation	Units
μ	Growth rate	day^{-1}
μ_m	Maximum growth rate	day^{-1}
K_s	Monod half saturation constant for growth	μM
S	External nutrient concentration	μM
Q_0	Minimum cell quota	$\mu M \cdot cell^{-1}$
$\mu_m:K_s$	Initial slope of the Monod growth equation	$day^{-1} \cdot \mu M^{-1}$
R*	Equilibrium resource concentration at a particular steady state growth rate	μM
D	Steady state dilution (= mortality) rate	day^{-1}
$\mu = (\mu_m S)/(K_s + S)$	Monod equation	
$\mu = \mu_m(Q - Q_0)/Q$	Droop equation	

Because diatoms can store excess phosphorus (Tilman and Kilham 1976), the algae were starved prior to the batch growth experiments. Exponentially growing cells were inoculated into tubes containing medium without phosphorus. Growth was followed by daily fluorescence measurements. Cells were allowed to grow until they were phosphorus depleted (entered stationary phase). Cells from these cultures were inoculated into 1 L flasks containing 900 mL of sterile medium with varying concentrations of P (0.06–5 μM P). The three species were inoculated together into each flask to a total initial density of ca. 50 cells·mL^{-1}. The low cell density and large volume allowed experiments to proceed for 4–5 days without the initial concentration becoming measurably reduced. Each day at the same time during the light cycle, the cultures were mixed and a 100 mL sample was decanted from each flask into 100 mL graduated cylinders. Lugol's solution was added and the cells were allowed to settle for 24 h. The top 95 mL were then aspirated out and the remaining 5 mL thoroughly mixed. The sample, concentrated 20 fold, was then counted. Phosphate was measured at the beginning and end of each experiment.

Diatoms cannot store silicon in large quantities, so starvation of cells was not necessary for the Si-limitation experiments. Moreover, long periods of Si depletion result in a considerable lag phase for growth (Holm and Armstrong 1981). Exponentially growing cells of the three species were inoculated together into 1 L polycarbonate flasks containing 900 mL of sterile medium with varying concentrations of Si (0.07–32 μM Si). The initial total cell density was ca. 50 cells·mL^{-1}. After inoculation we waited 3 days before taking the first samples for counting (day 0). As in the phosphorus experiments, the cells were concentrated before counting. Each experiment was continued for 5 days. Cell counts were made daily and silicate was measured at the start and end of each experiment. The growth kinetic constants μ_m and K_s were determined by the Monod equation relating growth rate to external nutrient concentrations (see Table 1 for explanation of symbols and units).

Minimum cell quota experiments. In order to study the effect of temperature on the minimum cell quotas for Si (Q_0Si) and P (Q_0P) for the three species, the diatoms were grown separately in tubes to stationary phase under Si or P limitation. Nutrient concentrations and cell densities (initially ca. 50 cells·mL^{-1}) were determined initially and at stationary phase. The difference in nutrient concentration divided by the difference in cell number gives the minimum cell quotas (Rhee and Gotham 1980). We made four replicate measurements for each species at each temperature.

Competition experiments. The competition experiments were performed in continuous cultures in 500 mL polycarbonate flasks as outlined in Kilham (1986). They were placed on rotary shaker tables (agitated at 180 rpm for 10 s·min^{-1}) in culture boxes at the experimental temperatures. The 'WC' medium with various amounts of Si and P (Table 2) was supplied from reservoir polycarbonate flasks. Six experimental cultures were run at each temperature. The Si:P always refers to the influent molar ratio. The three species were inoculated together into 400 mL of experimental medium. The cells were not preconditioned. The dilution rate was 0.2 d^{-1} at 10°, 15° and 20° C. At 5° C we used a dilution rate of 0.11 d^{-1} because of the lower maximum growth rates at this temperature. Growth was followed by daily fluorescence of the effluent. Every 3 days samples for counting were taken directly from the culture flasks. To oppose wall growth, the cultures were decanted into fresh sterile flasks every 7 days. Each experiment was continued for 39 days.

RESULTS

With the acclimated batch growth method, *S. hantzschii* had the highest maximum growth rates at the five experimental temperatures, followed by *A. formosa* and *F. crotonensis* (Table 3). For all three species μ_m increased with increasing temperature, with highest values measured at 20° C (Fig. 1).

The calculated Monod growth constants (μ_m, K_s) were determined under Si and P limitation for each species at each temperature (Table 4, Figs. 1, 2). Under Si limitation, *S. hantzschii* had the lowest K_s values, and the other two species were overlapping in their requirements. Under P limitation, *A. formosa* had the lowest K_s values, *F. crotonensis* was inter-

TABLE 2. *Silicon and phosphorus concentrations (μM) and supply ratios in the reservoir flasks for the six continuous cultures at each temperature.*

20°			15°			10°			5°		
Si	P	Si:P	Si	P	Si:P	Si	P	Si:P	Si	P	Si:P
197	0.48	410	246	0.56	440	196	0.47	417	205	0.39	531
97	0.50	194	131	0.45	289	101	0.48	210	146	0.38	380
95	0.97	98	129	0.38	155	94	1.00	94	127	0.68	188
30	0.75	40	123	1.36	91	30	0.86	35	138	1.41	98
62	2.34	26	60	1.63	37	62	2.18	28	72	1.33	54
6.6	1.69	3.9	6.5	2.74	2.4	5.3	2.06	2.5	6.3	1.62	3.9

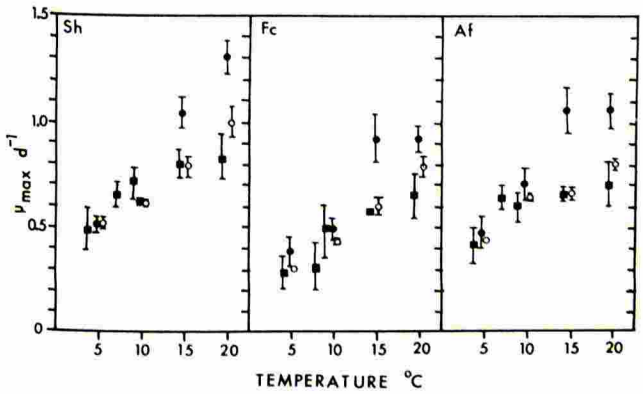

FIG. 1. Maximum growth rates as a function of temperature for the three diatoms using acclimated growth experiments (■) and calculated from batch growth experiments (● = Si-limited; ○ = P-limited). The 95% confidence intervals are indicated. Sh = *S. hantzschii*; Fc = *F. crotonensis*; Af = *A. formosa*.

TABLE 3. *Maximum growth rates (μ_m) of the three diatoms at five temperatures, as determined using the acclimated growth rate method. Standard deviations in parentheses.*

Species	°C	μ_m (day^{-1})
Stephanodiscus hantzschii	5	0.49 (±0.11)
	8	0.65 (±0.06)
	10	0.70 (±0.08)
	15	0.80 (±0.07)
	20	0.83 (±0.12)
Fragilaria crotonensis	5	0.29 (±0.08)
	8	0.31 (±0.11)
	10	0.47 (±0.12)
	15	0.57 (±0.02)
	20	0.65 (±0.09)
Asterionella formosa	5	0.42 (±0.08)
	8	0.64 (±0.05)
	10	0.58 (±0.07)
	15	0.65 (±0.03)
	20	0.71 (±0.10)

mediate and *S. hantzschii* had the highest P requirements.

Healey (1980) suggested that the initial slope of the Monod growth curve (μ_m/K_s) was useful for comparing relative competitive abilities of species, with higher values indicative of superior competitive ability. Tilman (1981) suggested that ranking of competitive ability is possible using R* values at particular steady state growth rates:

$$R^* = (D \cdot K_s)/(\mu_m - D) \quad (1)$$

(see Table 1 for explanation of symbols and units). The species with the lowest R* value will be the superior competitor for the limiting resource. This method is especially useful for species whose μ_m values are quite different. Using both methods of ranking, initial slope (μ_m/K_s) and R*, *S. hantzschii* was predicted to be the superior competitor for Si at all four temperatures, with the other two species being very similar to each other in Si requirements (Table 4). Under P limitation, *A. formosa* was predicted to be the superior competitor at all four temperatures, with *F. crotonensis* somewhat lower and *S. hantzschii* being very inferior.

The minimum cell quotas (Q_0) for phosphorus and silicon for *A. formosa* and *F. crotonensis* varied little over the temperature range studied (Table 5). The Q_0 values for P for *S. hantzschii* increased sharply at higher temperatures.

The individual physiological characteristics can be used to rank the species according to their relative R* values or their initial slopes (Table 4). These data, along with the optimum ratios (Q_0Si:Q_0P; Table 5), can be used to make predictions about the outcome of competitive interactions (Table 6). A more precise method is to use Tilman's (1980) graphical models (Figs. 3, 5) which combine all available physiological information and are explicit for the imposed mortality (dilution) rate. The models establish predicted areas of dominance, coexistence and extinction of each species. Similar kinetic constants for Si-limited growth make it impossible to predict dominance between *A. formosa* and *F. crotonensis* under Si-limitation.

At 20° C, *A. formosa* was predicted to be the superior competitor for P and *S. hantzschii* for Si; *F. crotonensis* was predicted to be eliminated in all cases (Fig. 3). The results of the six competition experiments at 20° C showed that *S. hantzschii* was essentially eliminated from all chemostats except for the lowest Si:P ratio (= 3.9), where it maintained about 5–8% of the cell numbers (Fig. 4). However, these chemostats were infected with a protozoan, *Asterocaelum algophilum* (described by Canter 1973) which was a specific predator on *S. hantzschii*. The demise of this species in the 20° C experiments was probably the result of selective predation, not competitive interactions. *A. formosa* dominated, as predicted, in the highest Si:P ratios (410 and 194), but *F. crotonensis* dominated at the four lower Si:P ratios. *F. crotonensis* apparently has an advantage over *A. formosa* when both species are Si-limited, although we were unable to distinguish them physiologically.

At 15° C, *A. formosa* was predicted to dominate under high Si:P ratios (440, 289, 155, 91), *A. formosa* (Si-limited) and *F. crotonensis* (P-limited) should coexist at Si:P = 37, and *S. hantzschii* (P-limited) and *F. crotonensis* (Si-limited) should coexist at Si:P = 2.4. However, *F. crotonensis* was more abundant than predicted in all of the competition experiments at 15° C (Table 6). *A. formosa* coexisted longer under higher Si:P ratios, and *S. hantzschii* dominated at the lowest Si:P ratio (= 2.4) until after day 30, but none of the results were strictly in accordance with the predictions at 15° C. No significant differences were noted between *A. formosa* and *F. crotonensis* in their P and Si kinetics at 15° C. Wall growth of *F. crotonensis* was a problem in this set of experiments and may be part of the reason for the very erratic changes in percent

TABLE 4. *Kinetic data for batch growth experiments. The 95% confidence intervals are given in parentheses. Also shown are the calculations for the initial slope (μ_m:K_s) and the R* values for a growth rate of 0.2 day^{-1}.*

Species	°C	μ_m	K_s	μ_m:K_s	R*
		Silicon			
Stephanodiscus hantzschii	5	0.51 (0.47–0.55)	0.19 (0.10–0.29)	2.7	0.12
	10	0.62 (0.59–0.64)	0.21 (0.14–0.27)	2.9	0.10
	15	1.05 (0.97–1.12)	0.47 (0.28–0.67)	2.2	0.11
	20	1.31 (1.23–1.39)	0.35 (0.21–0.49)	3.7	0.06
Fragilaria crotonensis	5	0.38 (0.31–0.45)	1.30 (0.38–2.58)	0.29	1.44
	10	0.49 (0.44–0.54)	1.08 (0.56–1.69)	0.45	0.74
	15	0.93 (0.81–1.04)	2.37 (1.43–3.51)	0.39	0.65
	20	0.92 (0.86–0.98)	2.17 (1.69–2.70)	0.42	0.60
Asterionella formosa	5	0.47 (0.40–0.55)	0.94 (0.29–1.59)	0.50	0.70
	10	0.71 (0.63–0.78)	1.54 (0.89–2.29)	0.46	0.61
	15	1.06 (0.95–1.17)	4.43 (3.22–5.88)	0.24	1.03
	20	1.06 (0.97–1.14)	2.35 (1.71–3.07)	0.45	0.55
		Phosphorus			
Stephanodiscus hantzschii	5	0.52 (0.49–0.55)	0.20 (0.16–0.25)	2.6	0.126
	10	0.61 (0.59–0.64)	0.32 (0.27–0.37)	1.9	0.160
	15	0.79 (0.73–0.84)	0.31 (0.27–0.39)	2.5	0.105
	20	1.00 (0.93–1.08)	0.16 (0.12–0.20)	6.2	0.040
Fragilaria crotonensis	5	0.30 (0.29–0.30)	0.034 (0.029–0.039)	8.8	0.071
	10	0.44 (0.43–0.45)	0.049 (0.042–0.056)	8.9	0.041
	15	0.60 (0.56–0.64)	0.050 (0.032–0.070)	12.0	0.025
	20	0.79 (0.74–0.83)	0.045 (0.033–0.059)	17.5	0.015
Asterionella formosa	5	0.44 (0.43–0.45)	0.020 (0.017–0.022)	22.0	0.017
	10	0.65 (0.64–0.66)	0.026 (0.022–0.030)	25.0	0.011
	15	0.66 (0.63–0.69)	0.035 (0.025–0.046)	18.8	0.015
	20	0.80 (0.77–0.83)	0.028 (0.021–0.035)	28.5	0.009

composition that occurred, depending on when the new flasks were attached.

At 10° C, *A. formosa* was predicted to dominate at the highest Si:P ratios (417, 210, 94), *A. formosa* (Si-limited) and *S. hantzschii* (P-limited) to coexist at intermediate ratios (35, 28), and *S. hantzschii* to dominate at the lowest ratio (2.5) (Fig. 5). The results of these experiments (Fig. 6) were consistent with the predictions. *A. formosa* dominated at the highest ratios (417, 210, 94). The rise of *F. crotonensis* at the end was certainly due to wall growth. *S. hantzschii* dominated at the lowest ratio (2.5). The dominance of *F. crotonensis* at ratios of 35 and 28 was not predicted but is likely because we could not distinguish *F. crotonensis* and *A. formosa* on the basis of Si-limited growth kinetics.

At 5° C, *A. formosa* was predicted to dominate in all cases except the lowest Si:P ratio, where *S. hantzschii* should be most abundant. The results of these experiments (Table 6) did not confirm the predictions. *F. crotonensis* dominated under all but the lowest ratio, where *A. formosa* was most abundant.

DISCUSSION

Our results indicate that temperature interacts with nutrient limitation and affects μ_m, K_s and Q_0 of the three diatoms studied. The interactions were not multiplicative but were nutrient and species specific.

The relationship between the maximum growth rate of algae and temperature has been described by a number of functions although it is generally linear between 0–40° C (Ahlgren 1987). Table 7 gives a literature survey of μ_m, K_s and Q_0 values for Si- and P-limited growth of *A. formosa*, *F. crotonensis* and *S. minutus*. Others found a linear increase in μ_m with increasing temperature for *A. formosa* in the range of temperatures studied (Rhee and Gotham 1981, Tilman et al. 1981). Mechling and Kilham (1982) found no increase in maximum growth rate with increasing temperature from 10° to 20° C for *S. minutus* (perhaps the same species as *S. hantzschii*; the taxonomy is in confusion). In our experiments μ_m of all three species increased with increasing temperature. Below 20° C no real optimum was reached.

We observed significant differences in the calcu-

TABLE 5. *Minimum cell quotas (Q_0 = μmol·cell^{-1}) and calculated optimum Si:P ratios (Q_0Si : Q_0P) for each species at each temperature.*

Species	°C	Q_0Si (× 10^{-5})	Q_0P (× 10^{-6})	Q_0Si:Q_0P
Stephanodiscus hantzschii	5	1.19	1.35	8.8
	10	1.36	2.51	5.4
	15	1.28	8.55	1.5
	20	1.19	69.2	0.2
Fragilaria crotonensis	5	7.78	2.44	32
	10	6.55	1.27	52
	15	5.55	2.08	27
	20	5.85	0.91	64
Asterionella formosa	5	5.01	0.95	53
	10	5.64	0.74	76
	15	4.95	0.86	57
	20	4.70	0.86	54

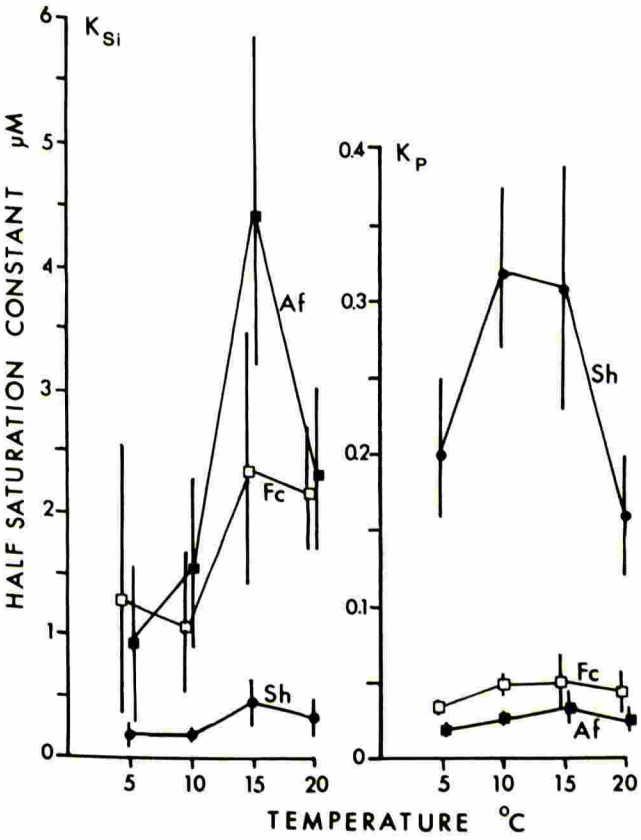

FIG. 2. Half saturation constants (K_s) for the three diatoms for silicon (K_{si}) and phosphorus (K_p) as a function of temperature. The 95% confidence intervals for each value are given. Sh, Fc, Af as defined in Fig. 1.

lated Monod μ_m values for Si- and P-limitation experiments at 15° and 20° C for all three species (Fig. 1). Theoretically, μ_m should be identical, but in fact was higher in the silicon than in the phosphorus experiments. This phenomenon was also observed by Tilman and Kilham (1976), Tilman et al. (1981) and Holm and Armstrong (1981). It is not simply a result of curve-fitting because the higher growth rates were actually observed at non-limiting concentrations of silicon. The maximum growth rates measured by the fluorometric method (Table 3) agree with those determined in the phosphorus experiments. We can offer no explanation for the apparent 'enhanced' maximum growth rates observed in the silicon experiments.

The effect of temperature on K_s and Q_0 is complex. Tilman et al. (1981) found constant K_s values for Si-limited *A. formosa* from 5° to 12.5° C (with higher values at 20° and 24° C). We also found somewhat higher K_s values for Si at 15° and 20° than at 5° and 10° C. In the batch growth experiments of Mechling and Kilham (1982), the K_s values for Si in *S. minutus* were lowest at 10° C and significantly higher at 15° and 20° C. Kilham (1984) found K_s values for Si-limited *Synedra* sp. to be similar for the range 10°–20° C. In the literature, various patterns of changes in K_s with temperature can be found (Thomas and Dodson 1974, Reynolds et al. 1975, Ahlgren 1978, 1987). Paasche (1975) suggested that there is no physiological or biochemical basis for expecting either an increase or decrease in K_s with temperature. Tilman et al. (1982) suggested a

TABLE 6. *Predictions of the outcome of competition along the Si:P gradient among the three diatoms based on the physiological characteristics of each species at each temperature. AF* = Asterionella formosa, *FC* = Fragilaria crotonensis, *SH* = Stephanodiscus hantzschii.

	Si:P	Predicted	Observed	Notes
5° C	531	AF	FC	All spp. P-limited
	380	AF	AF/SH	All spp. P-limited
	188	AF	FC	All spp. P-limited
	98	AF	FC	All spp. P-limited
	54	AF	FC/AF	AF = opt; FC, SH = P-lim
	3.9	SH	AF	All spp. Si-limited
10° C	417	AF	AF	All spp. P-limited
	210	AF	AF	All spp. P-limited
	94	AF	AF/FC	All spp. P-limited
	35	AF = FC/SH	FC/AF	AF, FC = Si-lim; SH = P-lim
	28	AF = FC/SH	FC/AF	AF, FC = Si-lim; SH = P-lim
	2.5	SH	SH	All spp. Si-limited
15° C	440	AF	AF/FC	All spp. P-limited
	289	AF	FC	All spp. P-limited
	155	AF	FC	All spp. P-limited
	91	AF	FC	All spp. P-limited
	37	FC/AF	FC	AF = Si-lim; FC, SH = P-lim
	2.4	FC/SH	FC/SH	AF, FC = Si-lim; SH = P-lim
20° C	410	AF = FC	AF/FC	All spp. P-limited
	194	AF = FC	AF/FC	All spp. P-limited
	98	AF = FC	FC/AF	All spp. P-limited
	40	AF = FC/SH	AF/FC	AF, FC = Si-lim; SH = P-lim
	26	AF = FC/SH	FC/AF	AF, FC = Si-lim; SH = P-lim
	3.9	AF = FC/SH	FC	AF, FC = Si-lim; SH = P-lim

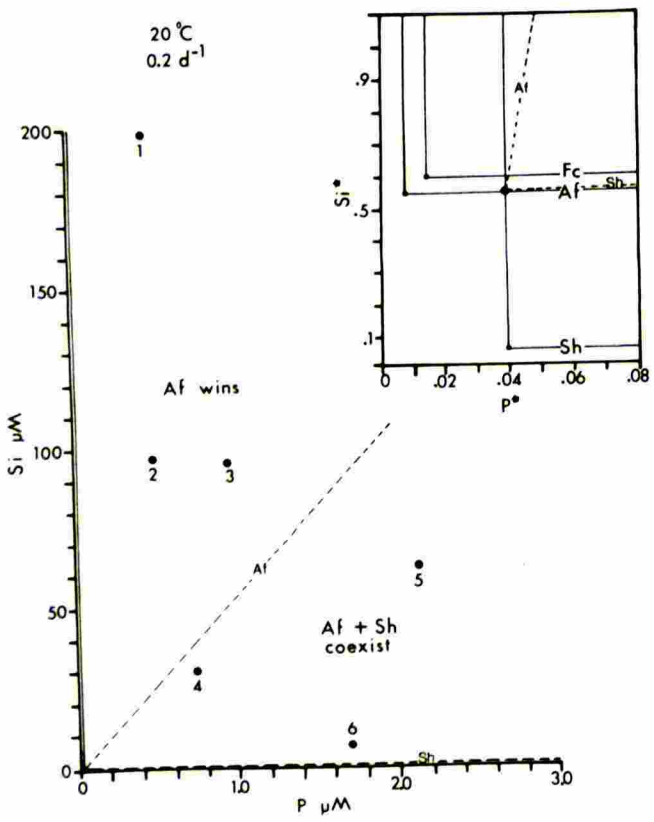

FIG. 3. Predicted competitive outcome based on the zero net growth isoclines for the three species and two resources at a temperature of 20° C and dilution rate of 0.2 day^{-1}. Inset shows the region near the origin. The resource supply points for the six continuous cultures are shown.

U-shaped relationship, and Ahlgren (1987) thought K_s might be relatively constant over a wide range within a species.

In our experiments the K_s values appear to be quite constant from 5° to 20° C for the nutrient for which the species is a good competitor (i.e. phosphorus for *A. formosa* and *F. crotonensis* and silicon for *S. hantzschii*, Fig. 2). The K_s values vary greatly across the temperature range only for the nutrient for which they are not good competitors. This may help to explain the mixed results reported in the literature. It appears that we need to determine the physiological kinetics for a number of potentially limiting resources for particular species.

The minimum cell quota often increases with decreasing temperature (Goldman 1979, Zevenboom 1980, Rhee and Gotham 1981, Kilham 1984), indicating that the cell requires more of the limiting resource to sustain growth. We observed relatively constant Q_0 values over the temperature range studied except for an increase in Q_0-Si for *F. crotonensis* with decreasing temperatures and an increase in Q_0-P for *S. hantzschii* with increasing temperatures.

Among the three species we isolated, *S. hantzschii* grew best under Si limitation. Kilham (1971) showed a correlation between low Si concentrations in a lake and dominance by *S. astraea*. Mechling and Kilham (1982) found for *S. minutus* at 10° C an initial slope of 2.3 and our value for *S. hantzschii* is comparable (2.9). However, at higher temperatures *S. hantzschii* displayed higher affinities. Species in the genus *Stephanodiscus* are generally excellent at growing at very

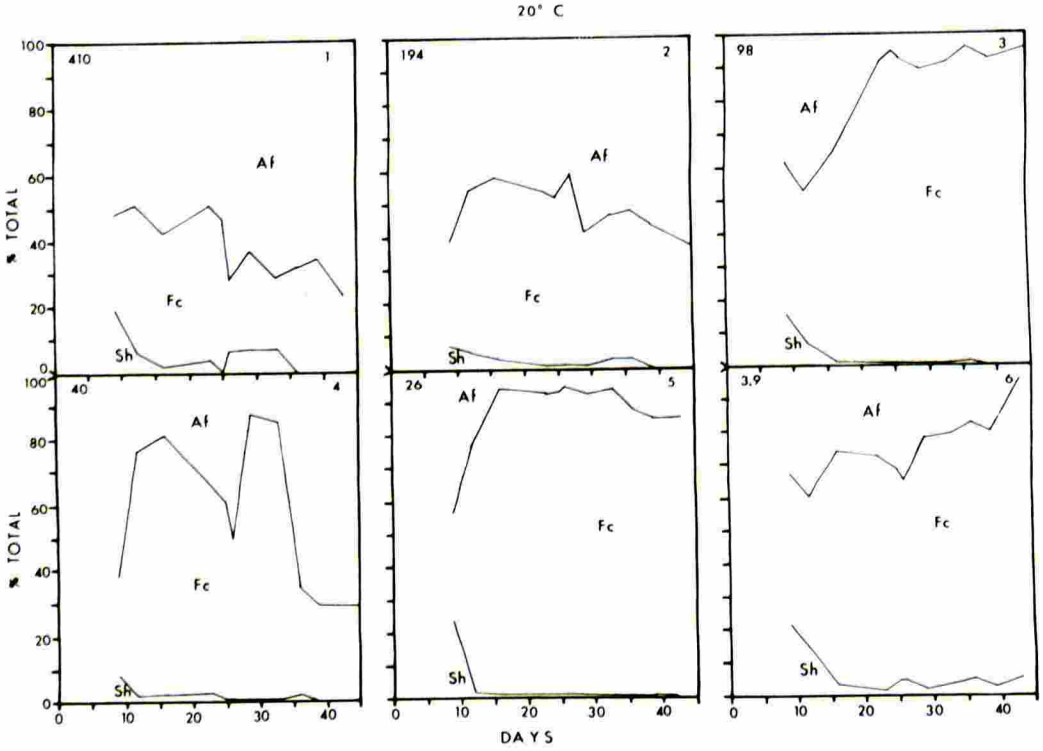

FIG. 4. Results of the six continuous cultures at 20° C and 0.2 day^{-1} run for 45 days. Percent composition of the three species is presented for each culture. The influent Si:P ratio is given in the upper left of each panel.

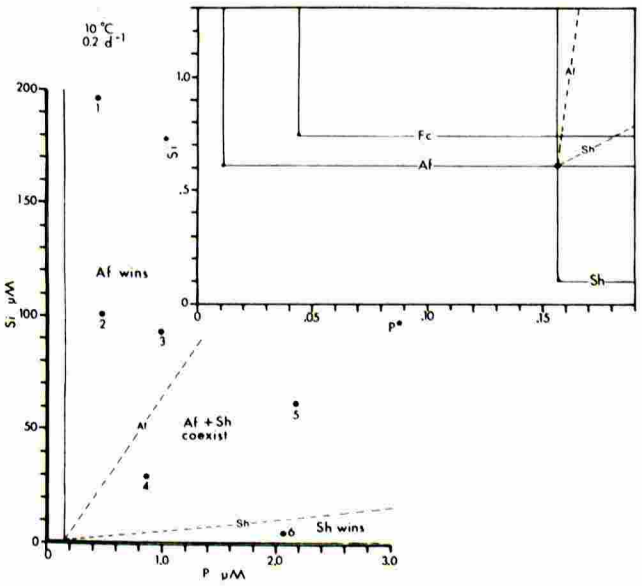

FIG. 5. Predicted competitive outcome based on the zero net growth isoclines for the three species and two resources at a temperature of 10°C and dilution rate of 0.2 day^{-1}. Inset shows the region near the origin. The resource supply points for the six continuous cultures are shown.

low Si concentrations, which supports Kilham's (1971) hypothesis. *Stephanodiscus* species can be characterized as having the best growth abilities at low Si:P ratios (Tilman et al. 1982, Kilham 1984, Kilham et al. 1986).

The quantitative results of the competition experiments were often not consistent with the predictions (Table 6). When species are ranked according to R* (at 0.2 d^{-1}) for Si, the order is *S. hantzschii* < *F. crotonensis* = *A. formosa*. For phosphorus, the order is *A. formosa* < *F. crotonensis* < *S. hantzschii*. *A. formosa* and *F. crotonensis* have very similar requirements for both Si and P, and because the rate of competitive displacement depends on the differences in R* of the species, they may co-occur for a long time (Tilman et al. 1981). Qualitative trends in the competition experiments were that *A. formosa* dominated at the highest ratios (all species P-limited) and *S. hantzschii* reached appreciable abundances only at the lowest Si:P ratios (most Si-limited), with *F. crotonensis* being intermediate. The dominance of *F. crotonensis* was in most cases not predicted, although it could not be ruled out. We offer two possible explanations for this observation. If the mortality rate of *F. crotonensis* were lower because of sticking to the reactor walls, then the predicted areas of dominance and coexistence of the three species would be altered (Fig. 7). *F. crotonensis* would in such a case be expected to dominate especially in the intermediate range of ratios. The experiments conducted at 10° C were shaken manually several times each day in addition to the mechanical mixing. Perhaps this is why those experiments more closely approximated the predicted relationships. The second possibility is that the techniques we used to determine physiological characteristics were not sufficient to distinguish *F. crotonensis* and *A. formosa*, especially under Si-limitation. If one calculates the R* values

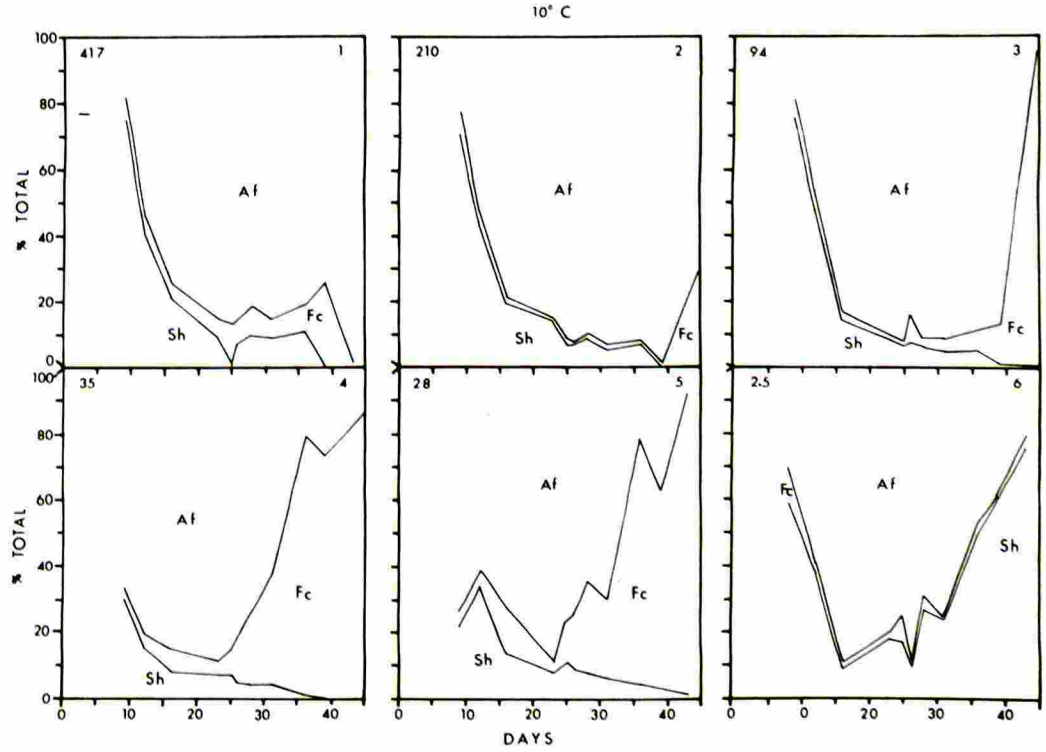

FIG. 6. Results for the six continuous cultures at 10° C and 0.2 day^{-1} run for 45 days. Percent composition of total cell number for three species is presented for each culture. The influent Si:P ratio is given in the upper left of each panel.

TABLE 7. *Growth kinetics for* Asterionella, Fragilaria *and* Stephanodiscus *from the literature. Substrate types (S): Si = silicon; P = phosphorus. Experiment types (E): B = batch; C = continuous culture; T = turbidostat. References (Ref.): 1 = Tilman et al. (1981); 2 = Tilman and Kilham (1976); 3 = Holm and Armstrong (1981); 4 = Tilman (1981); 5 = Gotham and Rhee (1981); 6 = Rhee and Gotham (1981); 7 = Mechling and Kilham (1982); 8 = Kilham (1984).*

Species	S	E	°C	μ_m	K_s	Q_0	Ref.
A. formosa	Si	B	4	0.35	1.3		1
			8	0.52	1.6		
			13	0.79	2.5		
			20	0.73	3.7		
A. formosa	Si	B	20	0.73	3.94	2.96×10^{-7}	2
	P	B	20	0.60	0.02	1.75×10^{-9}	
A. formosa	Si	B	20	0.81	2.91	2.83×10^{-7}	3
	P	B	20	0.67	0.07	3.02×10^{-9}	
A. formosa	Si	B	20	0.78	2.2	1.5×10^{-6}	4
	P	B	20	0.59	0.006	2.6×10^{-8}	
F. crotonensis	Si	B	20	0.62	1.5	1.5×10^{-6}	4
	P	B	20	0.80	0.01	2.6×10^{-8}	
A. formosa	P	C	20	1.01		4.56×10^{-9}	5
F. crotonensis	P	C	20	0.90		3.36×10^{-9}	
A. formosa	P	T	10	0.27			6
			15	0.66			
			20	1.05			
S. minutus	Si	B	10	0.71	0.31	2.01×10^{-7}	7
			15	0.80	1.03	1.05×10^{-7}	
S. minutus	P	B	20	0.75	0.88	1.68×10^{-7}	8
			10	0.52	0.13	1.9×10^{-7}	
			20	0.73	0.14	1.2×10^{-7}	

for Si and P using the lower limit of the 95% confidence intervals for the K_s values for *F. crotonensis* (Fig. 7), the result is an increased region of predicted dominance by *F. crotonensis* at intermediate Si:P ratios.

We believe that the wall growth problem was the major reason for the discrepancies observed between predicted and actual results. More vigorous mixing might have reduced the problem, but continuous mixing of cultures with colonial diatoms will cause clumping and distorted colonial growth. Some clones of *A. formosa* will not grow with continuous agitation (S. Kilham, pers. obser.).

In nature, the assumption that all species experience the same mortality rate is unrealistic. O'Brien (1974) theoretically explored the question of differential mortality on the abilitites of species to compete for nutrients and found that species-specific mortality can change the outcome of nutrient dependent competition between algae. We demonstrated this accidentally in our experiments, with *F. crotonensis* having apparently lower mortalities because of wall growth, and *S. hantzschii* having higher mortalities at 20° C because of selective predation. Differential mortality can be as important as differential competitive ability in determining the species composition, population dynamics and productivity of phytoplankton communities (Tilman 1978, Tilman et al. 1982, Kilham 1988).

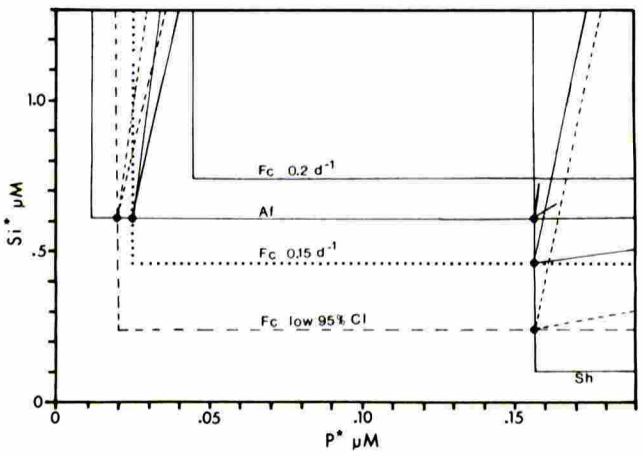

FIG. 7. Zero net growth isoclines for the three species at D = 0.2 d^{-1} and 10° C are indicated by the narrow solid lines (identical to inset of Fig. 5). No area of dominance by *F. crotonensis* is predicted at 0.2 day^{-1}. The effect of lowering the mortality rate for *F. crotonensis* to 0.15 day^{-1} is shown by the dotted line, and the effect of using the lower limits of the 95% confidence intervals for the K_s values is shown by the dashed line. In both cases, an area of dominance by *F. crotonensis* is predicted at intermediate Si:P ratios.

The observed reverse ranking of competitive ability (R*) under Si and P limitation are consistent with observations made by Tilman et al. (1982) and Suttle and Harrison (1988) that there appear to be tradeoffs in relative resource utilization among species. That is, a species such as *A. formosa* has a low R* for P, but a high R* for Si, whereas *S. hantzschii* has a low R* for Si and a high R* for P. The mechanism for such a tradeoff is possibly related to the reason why a 'superspecies' (having the lowest R* for several resources) is unlikely to evolve (Tilman 1982).

A more complete predictive model of phytoplankton dynamics in Lake Maarsseveen would include not only the nutrient physiology and specific mortality rates, but also the resource supply rates and the dependence of all of these factors on elements of the physical environment, including temperature, light, major ions and the mixing regime (see Sommer 1985). During the spring bloom in Lake Maarsseveen, phosphorus is the only growth-rate limiting nutrient. The Si:P ambient concentration ratio >300, bioassay results and phosphorus uptake kinetics all indicate this (van Donk 1983, van Donk et al. 1988, 1989). Over the growing season, bioassays showed that *S. hantzschii* was always P-limited and that *F. crotonensis* was more often P-limited than *A. formosa* (van Donk 1983, van Donk et al. 1988). The species which we found to be the superior competitor for P, *A. formosa*, is usually an order of magnitude more abundant than the other species. However, *A. formosa* is subject to attack by a species-specific fungal parasite (van Donk and Ringelberg 1983), and in years when the parasite decimates the *A. formosa* population, *F. crotonensis* can become dom-

inant (van Donk 1983, 1989, van Donk et al. 1988). This result would be expected if interactions such as those observed in the competition experiments also take place in Lake Maarsseveen.

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PROTEIN TURNOVER AND HETEROCYST DIFFERENTIATION IN THE CYANOBACTERIUM *ANABAENA VARIABILIS*[1]

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ABSTRACT

Under conditions of starvation for fixed nitrogen, cells of the filamentous cyanobacterium Anabaena variabilis *Kütz, degrade much of their protein prior to heterocyst differentiation. Cells starved for a source of fixed nitrogen initially degraded about 2% of their protein per hour; by 24 h after nitrogen stepdown about 40% of the protein was degraded. Most of the acid-soluble radiolabeled material was excreted into the medium. Proteolysis was completely inhibited by chloramphenicol, by cyanide, or in the dark, but was only partially inhibited in the presence of dichlorophenyl dimethylurea. Methionine sulfoximine (MSX) (an inhibitor of glutamine synthetase) in the presence of ammonia caused heterocysts to form. MSX treated cells degraded protein; however, the amount of protein degraded was much less than in cells starved for ammonia. Glutamine, which can serve as a nitrogen source for this strain, did not prevent starvation-induced proteolysis and did not prevent the differentiation of heterocysts.*

Key index words: Anabaena; *blue-green algae; cyanobacteria; heterocyst, nitrogen metabolism; protease; protein turnover*

In the cyanobacterium *Anabaena variabilis,* starvation for a source of fixed nitrogen initiates a series of events that culminates in the differentiation of certain vegetative cells in the filament into heterocysts, which are the sole sites of aerobic nitrogen fixation (Fleming and Haselkorn 1973, Peterson and Wolk 1978). Heterocyst differentiation involves a complex series of morphological and physiological changes that results in a cell specialized for nitrogen fixation in an aerobic environment (reviewed by Haselkorn et al. 1980). Morphological changes indicative of differentiation are discernable within a few hours after removal of a source of fixed nitrogen; however, functional ability to fix nitrogen is not detected until the heterocyst is mature (Fleming and Haselkorn 1973). Although starvation for a source of fixed nitrogen is known to be the primary signal for differentiation, the biochemical mechanisms by which starvation mediates control of differentiation are not known. One of the earliest detectable events upon nitrogen step-down is degradation of protein (Wood and Haselkorn 1979, 1980), and Fleming and Haselkorn (1974) suggested that the products of proteolysis may regulate heterocyst differentiation. At least two enzymes are known to degrade proteins during nitrogen starvation: a soluble, non-specific, Ca^{++}-requiring protease that acts early (Wood and Haselkorn 1979), and the enzyme that appears somewhat later and specifically degrades phycocyanin (Wood and Haselkorn 1980). A Ca^{++}-requiring protease, whose function in differentiation is not yet known, has been purified from *Anabaena variabilis* (Lockau et al. 1988). An enzyme that specifically degrades the storage polypeptide, cyanophycin, in both unicellular (Allen et al. 1984) and filamentous cyanobacteria is preferentially active in heterocysts (Gupta and Carr 1981). What role, if any, protein turnover has in the process of heterocyst differentiation is not known. This study attempts to define some of the factors that control the protein breakdown that begins very shortly after nitrogen stepdown, and to determine whether there is a direct correlation between protein turnover and heterocyst differentiation.

MATERIALS AND METHODS

Anabaena variabilis Kütz. ATCC 29413 was grown in an eightfold dilution of the medium of Allen and Arnon (1955) (AA/8) supplemented with 10 mM N-Tris(hydroxymethyl) methyl-2-ami-

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