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Growth and Competition of the Marine Diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*. I. Nutrient Effects*

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Abstract

Two marine diatoms, *Phaeodactylum tricornutum* (Bohlin) and *Thalassiosira pseudonana* (Hasle and Heimdal), were grown both separately and together in batch cultures on a mixture of waste water and seawater enriched with different components of *f* medium. At 17°C, the maximum division rates of the two species were statistically indistinguishable. The waste water-seawater mixture used proved to have insufficient Si, relative to N and P, for the growth of *T. pseudonana*, which requires approximately 5 x 10^{-14} g-at Si $cell^{-1}$ to divide at a maximum rate. *P. tricornutum*, on the other hand, although capable of taking up nearly 9 x 10^{-15} g-at Si $cell^{-1}$, could sustain maximum rates of division with 4.3 x 10^{-18} g-at Si $cell^{-1}$ or less. No allelopathic interaction between the two species could be detected. We conclude that *P. tricornutum* enjoys a considerable competitive advantage over *T. pseudonana* in a waste water-seawater-based mariculture system that is not supplemented with Si. Although Si proved necessary for *T. pseudonana* to complete more successfully with the other diatom, the presence of excess amounts of Si is not necessarily sufficient for the maintenance of *T. pseudonana* in mixed continuous culture with *P. tricornutum*: other factors, such as light-related or photoperiod-related growth response, are believed to determine the ultimate outcome of competition between these algae in light-limited continuous culture.

Introduction

A central theoretical objective in marine ecology has been to understand the mechanisms of resource partitioning and competition among phytoplankton species and to invoke that knowledge to help explain the observed geographical distributions and temporal successions of planktonic algae. With the development of experimental mariculture systems, this objective has taken on more than purely theoretical interest: in order to provide the best possible algal food to populations of cultured herbivores, one must understand and control competition among species of phytoplankton. Results of aquacultural experiments to date have shown clearly that this practical goal is not close at hand (Goldman and Ryther, 1976).

The successful maintenance of desired algal species has proved elusive in a tertiary sewage treatment-mariculture system which has otherwise shown great promise; in this system, phytoplankton are grown in continuous culture on a mixture of seawater and secondarily treated sewage and then fed to marine bivalves (see Ryther, 1977 for details). Within a few days, inoculated species have inevitably been replaced by other, usually less desirable, species. Although the specific mechanisms by which the inoculated algae are supplanted by other species are unknown, Goldman and Ryther (1976) and Goldman (1977a) have shown that temperature plays an important role. When temperatures range between 10° and 20°C, *Phaeodactylum tricornutum* (Bohlin), a small, pennate diatom, displaces other species; this diatom is a poor bivalve food (Davis and Guillard, 1958; Walne, 1970; Hartman *et al.*, 1973). At high temperatures, *Nitzschia closterium*

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[(Ehrenberg) Wm. Smith], also a pennate diatom, and small green algae (some of uncertain taxonomic position) are likely to predominate. Only for a short period in the winter, at temperatures below 10°C, does *Skeletonema costatum* [(Greville) Cleve], a chain-forming centric diatom of good food value to bivalves (Walne, 1970), often prevail.

We have observed that, regardless of when *Thalassiosira pseudonana* (Hasle and Heimdal) is inoculated into pond cultures, it is inevitably replaced rapidly by *Phaeodactylum tricornutum*. This is disappointing, since this small centric diatom is an excellent bivalve food (Tenore and Dunstan, 1973) and is capable of prodigious maximum rates of division between 10° and 25°C (Guillard and Ryther, 1962). Here we report experiments which examine the competition between the two species from the aspects of nutritional requirements and allelopathic interaction. In the following paper (Nelson *et al.*, 1979) we deal with the aspects of light intensity and photoperiod.

Materials and Methods

Algae

Two sources of *Phaeodactylum tricornutum* (Bohlin) were used. One, designated ESL1, was taken from mass, outdoor cultures continuously fed 10% sewage in seawater at the Environmental Systems Laboratory (ESL), Woods Hole Oceanographic Institution, Massachusetts. This source, containing a small percentage of other algal species and microorganisms, was used in the sewage enrichment experiments. We did not have an axenic isolate of this species available, so for experiments which required this, we used the clone Pet Pd, for which we had an axenic isolate. This clone appears to be physiologically similar, if not identical to ESL1, and was isolated from similar phytoplankton ponds at Aquacultural Research Corporation, Dennis, Massachusetts, USA. Axenic *Thalassiosira pseudonana* (Hasle and Heimdal) (Clone 3H, from an estuarine environment) was used in all experiments.

Enrichment Experiments

For the study of growth and competition of the two diatoms under different nutrient regimes, we added different combinations of *f* medium (Guillard and Ryther, 1962) additives (Table 1) to two series of 125 ml borosilicate flasks in triplicate; the "seawater series" contained 33.0 ml seawater without added sewage; the "sewage series" contained 33.0 ml of 20% secondarily treated sewage (from Wareham, Massachusetts) in seawater. The seawater used had been filtered (0.45 µm Millipore) but not autoclaved; its inorganic N, P, and Si content was negligible. The 20% sewage in seawater was also filtered and not autoclaved; its inorganic nutrient content, contributed virtually in entirety by the sewage, was ca. 330 µM NO_3^-, 50 µM NH_4^+, 50 µM PO_4^{3-}, and 5 µM H_4SiO_4. The NO_2^- content was negligible. To the first of three identical media for each nutrient regime in each series we added *Phaeodactylum tricornutum* (ESL1) at a density of 1 x 10^3 cells ml^{-1}; to the next medium we added *Thalassiosira pseudonana* (1 x 10^3 cells ml^{-1}); and to the final medium we added both species together (1 x 10^3 cells ml^{-1} each). The flasks were capped with 50 ml beakers, and placed in an incubation room held at 17°C under a 14 h light:10 h dark cycle. Illumination, provided by "cool-white" (Sylvania) fluorescent bulbs was 0.064 ly min^{-1}, as measured by an Eppley Co. (Newport, Rhode Island) Model 6-90 radiometer (pyranometer) which measures essentially only photosynthetically active light. This corresponds to ca. 13,000 lux as measured by a General Electric model 213 light meter.

Si Requirements

Si-free *f*/2 medium was prepared as follows. Autoclaved Sargasso Sea water ($[H_4SiO_4]$ = 1.75 µM) was enriched with all *f*/2 medium nutrients except sodium metasilicate, and inoculated with *Thalassiosira pseudonana* (ca. 100 cells ml^{-1}) which was allowed to grow and remove residual Si until division ceased (ca. 5 x 10^4 cells ml^{-1}). The cells were harvested from the "Si-free" medium by filtration (0.2 µm, Nuclepore) using a stainless steel filtration apparatus. The filtrate, collected in a polycarbonate Fernbach flask, was immediately sampled for reactive Si determination, autoclaved, cooled quickly, inoculated with axenic *Phaeodactylum tricornutum* (Clone Pet Pd) at an initial density of 65.5 cells ml^{-1}, and divided into four 100 ml aliquots in 250 ml polycarbonate Erlenmeyer flasks. Two of these were enriched with sodium metasilicate at the *f*/2 level (107 µg-at Si l^{-1}). The 4 flasks were capped with polycarbonate beakers and incubated at 17°C under the illumination conditions described above. The cultures were agitated daily and sampled for cell counts daily, beginning 3 days after inoculation. After 12 days, the cells from

Table 1. Nutrient-enrichment treatments used in both sewage and seawater series. Dash indicates that component was not added, otherwise addition gave enrichment of one-half (*f*/2) or one-quarter (*f*/4) of Medium *f* (Guillard and Ryther, 1962). (Full strength Medium *f* contains: NO_3^-, 1.765 mM; PO_4^{3-}, 72.5 µM; H_4SiO_4, 214 µM; Fe^{3+}, 23.3 µM; thiamine x HCl, 0.2 mg l^{-1}; biotin, 1.0 µg l^{-1}; vitamin B_{12}, 1.0 µg l^{-1}; Cu^{2+}, 79 nM; Zn^{2+}, 153 nM; Co^{2+}, 85 nM; Mn^{2+}, 1.83 µM; MoO_4^{2-}, 52 nM; made in seawater.) V + T = vitamins and trace metals

Treatment no.	Nutrient additions			
	NO_3^-	PO_4^{3-}	H_4SiO_4	V + T
1	-	-	-	-
2	*f*/4	*f*/4	*f*/2	*f*/4
3	-	-	-	*f*/4
4	-	-	*f*/2	-
5	-	-	*f*/2	*f*/4
6	*f*/4	*f*/4	*f*/2	-
7	*f*/4	*f*/4	-	*f*/4

a 50 ml aliquot of each culture were collected for particulate Si determination, using a 0.4 µm Nuclepore filter in a polypropylene syringe filtration apparatus. The filtrate was then treated for reactive Si determination. The amount of Si per cell was determined both from the decrease in reactive Si in the medium during the experiment and from the particulate Si on the filters.

Silicon Determination

Soluble reactive Si was determined by the acid-molybdate method (Strickland and Parsons, 1972). Samples for particulate Si were subjected to sodium carbonate fusion (Nelson and Goering, 1977) and diluted in distilled water for reactive silicon determination as above.

Allelopathic Effects

To test the effect of filtrate from cultures of *Phaeodactylum tricornutum* on the growth of *Thalassiosira pseudonana*, *P. tricornutum* (Clone Pet Pd) was grown in *f*/2 medium from a cell density of 3.50 x 10^2 cells ml^{-1} to a final density of 2.54 x 10^6 cells ml^{-1}. Algal and bacterial cells were removed by double filtration (under pressure not suction) using 3.0 µm, then 0.2 µm Nuclepore filters; the filtrate was enriched with *f*/2 levels of all nutrients, and duplicate 50 ml aliquots of this medium were placed in 125 ml borosilicate Erlenmeyer flasks. Controls were flasks containing 50 ml duplicate aliquots of complete and Si-free *f*/2 medium in which *P. tricornutum* had not been grown. All flasks were inoculated with *T. pseudonana* (1.07 x 10^2 cells ml^{-1}) and placed in a 20°C incubator illuminated as above. Beginning 3 days after inoculation, cell counts were made daily until exponential growth ceased.

Growth

Hemocytometer cell counts were used for division rate determination. At cell densities below 10^5 cells ml^{-1}, Speirs-Levy counting chambers were used; at greater densities, counting chambers 0.1 mm deep with improved Neubauer ruling were used. At least 100 cells were tallied per count, enabling us to have confidence statistically that 95% of the time it would be within ± 20% of the actual cell density (Guillard, 1973). Growth rates, in divisions day^{-1} (k) were calculated by fitting a least-square linear regression to the equation

$$\log_2 C_t = \log_2 C_o + kt, \qquad (1)$$

where t is time in days, and C_t and C_o are cell concentrations at times t and o, respectively. The standard error (SE) of the division rate (i.e., of the regression coefficient) was determined as described by Sokal and Rohlf (1969). The 95% confidence interval on each estimate of k is ± 2 SE.

Results

Enrichment Experiments

Sewage Series

During the first 5 days of the experiment, regardless of treatment, *Phaeodactylum tricornutum* monocultures grew at a constant, exponential rate in the sewage series (Fig. 1A); the mean division rate (k) for all treatments was 1.80 (2 SE = 0.06) division day^{-1}. In Treatment No. 4, after Day 5, although cell density increased, it did so at a rate reduced relative to that previously and to that of the other treatments. Subsequent results suggest that this decline in division rate was due to factors other than nutrient limitation and thus should be ignored; predation by holozoic flagellates, which are often present in the ponds, may have been responsible. In the other 6 treatments, final and highest cell densities achieved ranged from 1.93 x 10^6 to 4.45 x 10^6 cells ml^{-1} (Table 2).

Under identical conditions, *Thalassiosira pseudonana* in monoculture did not fare as well as *Phaeodactylum tricornutum*. In no treatment did exponential growth con-

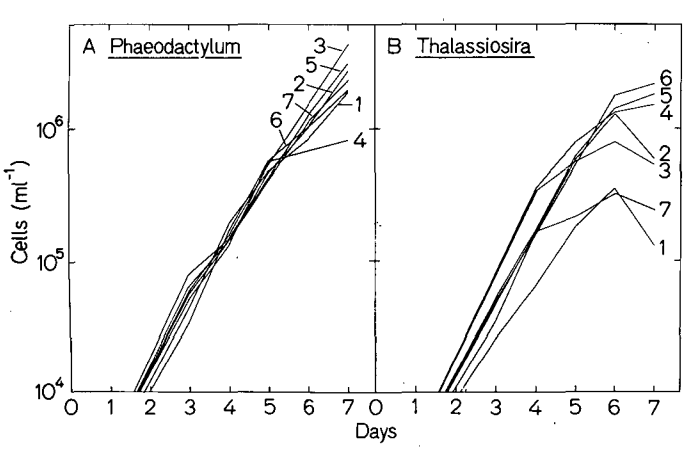

Fig. 1. *Phaeodactylum tricornutum* (A) and *Thalassiosira pseudonana* (B). Cell density in monoculture sewage enrichment experiment. Numbers correspond to treatments listed in Table 1

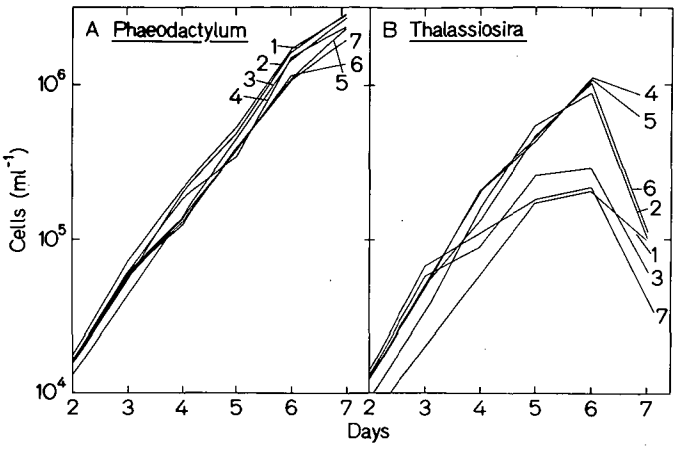

Fig. 2. *Phaeodactylum tricornutum* (A) and *Thalassiosira pseudonana* (B). Cell density in mixed-culture sewage enrichment experiment. Numbers correspond to treatments listed in Table 1

Table 2. *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*. Greatest cell densities (10^4 cells ml^{-1}) achieved in different treatments. N: nitrogen; P: Phosphorus; Si: silicon; V + T: vitamins and trace metals

Treatment	Monoculture		Mixed culture	
	P. tricornutum	*T. pseudonana*	*P. tricornutum*	*T. pseudonana*
Sewage series				
1 (+ O)	193.	28.9	282.	21.0
2 (+ N, P, Si, V+T)	280.	129.	269.	87.0
3 (+ V+T)	445.	80.0	282.	29.0
4 (+ Si)	81.3	152.	232.	109.
5 (+ Si, V+T)	318.	184.	232.	106.
6 (+ N, P, Si)	196.	218.	139.	103.
7 (+ N, P, V+T)	228.	32.0	196.	21.3
Seawater series				
1 (+ O)	4.50	3.08	5.00	0.65
2 (+ N, P, Si, V+T)	43.3	91.0	71.5	57.0
3 (+ V+T)	7.44	0.70	3.60	0.70
4 (+ Si)	5.20	2.25	6.30	1.60
5 (+ Si, V+T)	2.62	3.10	4.20	1.38
6 (+ N, P, Si)	-[a]	69.0	103.	29.0
7 (+ N, P, V+T)	63.2	9.20	109.	7.90

[a] Treatment lost.

Table 3. *Phaeodactylum tricornutum*. Initial and final silicic acid concentration ($[H_4SiO_4]_o$ and $[H_4SiO_4]_t$, respectively) in µM, final cell densities (C_t) in 10^7 cells ml^{-1}, division rates (k) in divisions day^{-1}, and final cell content of Si in g-at Si $cell^{-1}$, calculated from change in silicic acid concentration ($[\Delta H_4SiO_4]$) and particulate Si for diatoms growing in Si-enriched and Si-free *f*/2 medium

Medium	$[H_4SiO_4]_o$	$[H_4SiO_4]_t$	C_t	k[a]	Cell Si content	
					From $[\Delta H_4SiO_4]$	From particulate Si
f/2	107	5.40	1.03	1.85 ± 0.08	9.86×10^{-15}	8.54×10^{-15}
f/2	107	5.19	1.03	1.88 ± 0.10	9.89×10^{-15}	8.96×10^{-15}
f/2, no Si	0.05[b]	0.03[b]	1.16	1.78 ± 0.07	6.07×10^{-18}[c]	4.64×10^{-18}
f/2, no Si	0.09[b]	0.03[b]	1.24	1.77 ± 0.09	1.71×10^{-17}[c]	4.29×10^{-18}

[a] ± 2 SE of the mean (95% confidence interval).

[b] Near limit of analytical detection.

[c] Determined from difference between two almost undetectable concentrations, hence precision very poor.

tinue past the 6th day (Fig. 1B), and the maximum cell density achieved (2.18 x 10^6 cells ml^{-1}), was less than for *P. tricornutum* (4.45 x 10^6 cells ml^{-1}). Much higher cell densities were attained in those treatments containing added Si (Nos. 2, 4, 5, 6) than in those without it (Nos. 1, 3, 7; Table 2). This indicates that the sewage, containing an N:P:Si atomic ratio of 38:5:1, provided a substantial excess of N and P relative to Si, for the growth requirements of *T. pseudonana*. Under conditions such that Si limitation was clearly not a factor (Nos. 2, 4, 5, 6, through Day 5), the mean division rate of 1.87 (2 SE = 0.08) divisions x day^{-1} was not significantly different from that of *P. tricornutum*.

In mixed culture, growth of each diatom was similar to what it had been in monoculture (Fig. 2). However, there were some contrasts. *Phaeodactylum tricornutum* exhibited a constant, exponential growth in all treatments only until Day 6; subsequently, the growth rate declined. Highest cell densities reached by this species ranged from 1.39 x 10^6 to 2.82 x 10^6 cells ml^{-1}, approximately the range seen in the monoculture experiment (Table 2). *P. tricornutum* division rate did not decline in Treatment No. 4 after Day 5, supporting the conclusion that the effect observed in the monoculture experiment was, as suggested, anomalous.

As in the monoculture experiment, the growth of *Thalassiosira pseudonana* in mixed culture appeared to depend on Si availability. During the first 5 days in Treatments Nos. 2, 4, 5, 6, which contained *f*/2 levels of Si, *T. pseudonana's* growth rate equalled that of *Phaeodactylum tricornutum* (Fig. 2). There was no evidence of any inhibitory effect on growth of rate of *T. pseudonana* as a result of the presence of *P. tricornutum* during that time. However, the cell densities achieved by *T. pseudonana* in mixed cultures were consistently less than in monocultures with the same initial nutrient concentrations (Table 2). Growth of this species ceased by Day 6, despite the fact that enough N and P were present to support additional division — as was shown by the subsequent increase in *P. tricornutum* density in those media. This suggests that *P. tricornutum* had incorporated and thereby made unavailable some Si that would otherwise have enabled additional growth of *T. pseudonana*.

Although the cell density of *Phaeodactylum tricornutum* was greatest by Day 7 in both the monoculture and mixed culture treatments (Figs. 1A, 2A), in most cases (11 of 14 treatments) *Thalassiosira pseudonana* reached its maximum density by Day 6; subsequently, a decline in cell density occurred (Figs. 1B, 2B). This decline was most evident in mixed culture (Fig. 2B). A decline in numbers in a diatom batch culture is unusual; cells in non-dividing cultures (or preserved cells) remain recognizable and countable for many months. Therefore, the shells must have disappeared from the medium either by fragmentation or dissolution.

Seawater Series

Most treatments in the seawater series contained inadequate amounts of nutrients to sustain exponential growth past Day 3, when daily counts were initiated; hence, we merely present the maximum cell densities achieved in each treatment (Table 2). The addition of Si, if N and P were also added, had a salutary effect on the growth of *Thalassiosira pseudonana*, regardless of whether that species was alone or in the presence of *Phaeodactylum tricornutum* (compare Treatments Nos. 2 and 6 with No. 7). The omission of sewage and its N and P content clearly prevented either species from attaining the cell densities reached in comparable treatments in the sewage series.

Si Requirement of Phaeodactylum tricornutum

To determine whether *Phaeodactylum tricornutum* removes silicic acid from the medium or requires Si for growth, we measured that species' accumulation of silicic acid from complete *f*/2 medium and its division rate in Si-free *f*/2 medium. Initial and final silicic acid concentration ($[H_4SiO_4]_0$ and $[H_4SiO_4]_t$, respectively), final cell densities obtained (C_t), division rates (k), and cellular Si content for *P. tricornutum* growing in these media are presented in Table 3. No effect of initial silicic acid concentration on either k or C_t could be detected, i.e., *P. tricornutum* had no demonstrable Si requirement for cell division. Nonetheless, *P. tricornutum* cells growing in complete *f*/2 medium took up substantial amounts of silicic acid and contained more than 10^3 times the Si per cell of those growing in the lowest Si medium we could provide (Table 3).

Allelopathic Effects

None was detected. *Thalassiosira pseudonana* (Clone 3H) grew as well in re-enriched medium in which *Phaeodactylum tricornutum* (Clone Pet Pd) had been grown to a density of 2.54 x 10^6 cells ml^{-1} as it did in freshly prepared medium (Fig. 3). However, a labile, short-lived growth-inhibiting substance may have been pro-

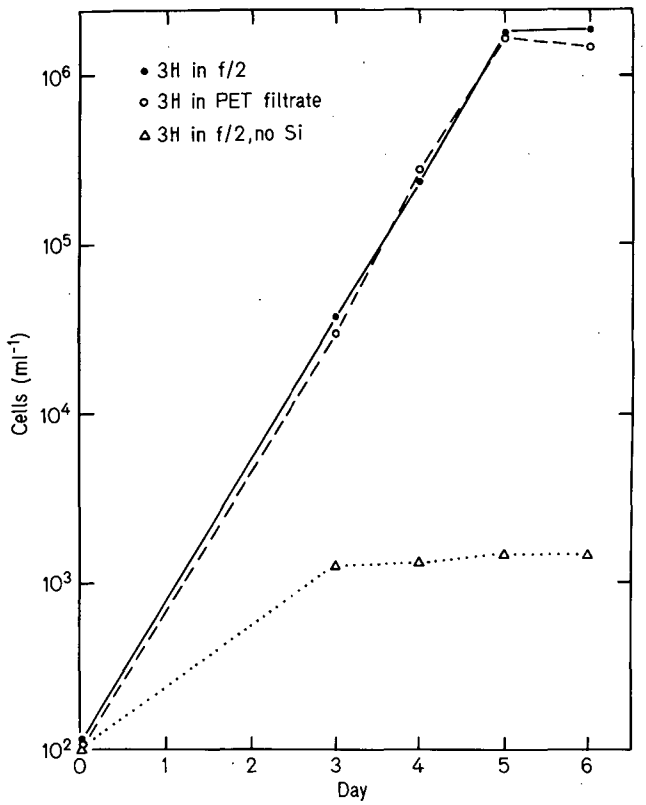

Fig. 3. *Thalassiosira pseudonana* (Clone 3H) growth in *Phaeodactylum tricornutum* filtrate enriched with *f*/2 nutrients (open circles); in complete *f*/2 medium (filled circles); and in *f*/2 medium, Si omitted (triangles)

duced by *P. tricornutum* and become inactive before the filtrate was re-enriched and inoculated with *T. pseudonana*.

Discussion

Of the 14 experimental treatments in the sewage and the seawater enrichment series, 8 (all 7 in the sewage series and No. 7 in the seawater series) apparently contained media with a substantial excess of N and P relative to Si for the growth requirements of *Thalassiosira pseudonana*. The remaining 6 (Nos. 1-6, seawater series) contained a higher relative amount of Si, and were more likely to be N- or P-limited. To verify this, we performed least-squares linear regressions of highest cell density achieved in the different treatments versus initial inorganic N, P, and Si concentrations in the media: one would expect the stronger correlations to exist between yield and the initial concentrations of limiting nutrients. As anticipated, in those treatments considered to contain excess N and P, *T. pseudonana*, whether alone or in mixed culture with *Phaeodactylum tricornutum*, showed a strong positive correlation between initial Si and cell yield, but not between N or P concentration, and cell yield (Table 4). The same comparison made for *P. tricornutum* showed little correlation; this is consistent with our inability to demonstrate a Si requirement for the latter species. The reduced cell yields of *T. pseudonana* [0.74 x 10^{13} versus 1.2 x 10^{13} cells (g-at Si)$^{-1}$] in the presence of *P. tricornutum* is presumably due to Si incorporation by the latter; this Si is thereby made unavailable to support the growth of *T. pseudonana*. Thus, such consumption may represent a strategy used by *P. tricornutum* when in competition with diatoms requiring Si.

In the treatments low in N and P relative to Si (Nos. 1-6, seawater series), strong positive correlations existed between both initial inorganic N and P concentrations and cell yields of both species (Table 4). In this group of treatments, correlation coefficients for N and P pairs were identical, reflecting the fact that the N:P supply ratios were the same regardless of treatment – only absolute levels varied between treatments. Therefore, we cannot ascertain from these correlations whether N or P was the limiting nutrient with respect to cell yield. However, the initial Si concentration clearly did not correlate with yields of either alga in the seawater series.

From values reported in the literature, we can determine the approximate ratios of N, P, and Si required by these species for growth. For *Thalassiosira pseudonana* (Clone 3H), N cell content at maximum division rate (k_{max}) is ca. 5 x 10^{-14} g-at N cell^{-1} (Guillard and Cassie, 1963); P cell content at k_{max} is ca. 6 x 10^{-15} g-at P cell^{-1} (Fuhs, 1969; Fuhs *et al.*, 1972); and Si cell content at k_{max} is ca. 5 x 10^{-14} g-at Si cell^{-1} (Guillard *et al.*, 1973; Paasche, 1973). Thus, at k_{max}, *T. pseudonana* should require a supply of nutrients with an N:P:Si atomic ratio of roughly 1:0.12:1. Sewage used in this study contained an N:P:Si ratio of 38:5:1, quite obviously deficient in Si relative to N and P, even if our supposed requirement ratio is substantially in error.

Although we are unaware of studies specifically relating cellular content of N, P, or Si to the growth of *Phaeodactylum tricornutum*, it does appear from a review of the literature that its relative content of N and P is roughly similar to that shown above for *Thalassiosira pseudonana* (e.g., Ketchum, 1939; Kuenzler and Ketchum, 1962; Goldman, 1977b). Thus, N to P supply ratios probably had little bearing on the outcome of competition in our experiments.

Table 4. *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*. Results of least-squares linear regressions of initial nutrient concentrations with highest cell densities in enrichment experiment. Strong correlations are underlined

Treatment	Nutrient	*P. tricornutum*				*T. pseudonana*			
		Alone		Mixed		Alone		Mixed	
		r[a]	m[b]	r	m	r	m	r	m
Nos. 1-7 sewage series and No. 7 of seawater series	N	0.007	0.004	-0.28	-0.083	0.19	0.066	0.14	0.028
	P	0.18	2.2	0.025	-0.16	0.33	2.5	0.28	1.2
	Si	-0.022	-0.047	0.050	0.056	0.92	1.2	0.99	0.74
Nos. 1-6 seawater series	N[c]	0.71	0.44	0.97	0.19	1.00	0.21	0.93	0.094
	P[c]	0.71	10.7	0.97	4.6	1.00	5.3	0.93	2.3
	Si	0.35	0.88	0.50	0.39	0.50	0.45	0.47	0.2

[a] Correlation coefficient.

[b] Slope of regression of highest cell density obtained against initial nutrient concentration; data shown as 10^{13} cells (g-at N, P, or Si)$^{-1}$.

[c] Always supplied in same ratio, hence identical r values were obtained.

Our demonstration of little or no Si requirement for cell division in fusiform and triradiate *Phaeodactylum tricornutum* is consistent with the observations of Harvey (1955), Lewin *et al.* (1958) and Kuenzler and Ketchum (1962). Table 3 indicates that if a Si requirement exists, it is remarkably low (5×10^{-18} g-at Si cell^{-1}). *P. tricornutum* growing in high Si medium has been reported to contain between 0.4 and 1% SiO_2 on a dry weight basis (Lewin *et al.*, 1958); our results correspond to a range of from 0.0006 to 1%. This large variation in Si content is not surprising, inasmuch as Si content appears to reflect supply more than need.

Results reported here appear to be inconsistent with Darley's statements (1974) that all diatoms have an absolute requirement for Si. However, our experiments were not performed with ovate cells, which produce siliceous shells similar to those of Si-requiring diatoms (Lewin *et al.*, 1958). The question of a Si requirement for division of ovate *Phaeodactylum tricornutum* cells has not, to our knowledge, been directly addressed experimentally.

Under the experimental conditions we used, *Thalassiosira pseudonana* grew as rapidly as *Phaeodactylum tricornutum*, even in the latter's presence. Si availability clearly had substantial bearing on the outcome of competition in the sense that an adequate Si supply was necessary for the perpetuation of *T. pseudonana*; nonetheless, an abundance of Si did not allow displacement of *P. tricornutum* in mixed culture. It is tempting to predict that maintenance of the two diatoms in mixed culture is readily attainable in large outdoor ponds such as those at ESL, however, there are several additional factors to be considered.

Firstly, classical theoretical models of competition hold that stable maintenance (coexistence) of two species occurs only if the growth of each is limited by different resources (e.g. Titman, 1976). Otherwise, one species will displace the other either by "exploitation competition," i.e., using the limiting resource before the other does, or by "interference competition," i.e., actively suppressing the other's growth, as in allelopathy.

Secondly, allelopathic suppression of *Thalassiosira pseudonana* by *Phaeodactylum tricornutum*, although unlikely, is not entirely ruled out. We have shown that if *P. tricornutum* does produce a growth-inhibiting substance, it must be short-lived and sufficiently labile that it was totally inactivated in our experiment by the time *T. pseudonana* was grown in the *P. tricornutum* filtrate. We view this possibility as unlikely, since in our mixed-culture enrichment experiments both species showed exponential growth at k_{max} to cell densities in excess of 10^6 cells ml^{-1}, after which nutrient and light limitation became a factor.

Finally, although our study shows that neither species appears able to outcompete the other at k_{max}, it must be borne in mind that outdoor, continuous cultures such as those at ESL are maintained at dilution rates at which the algae are growing at considerably less than k_{max}. It is for this reason that k_{max} is a poor predictor of success in such systems. Such phytoplankton ponds are intended to be light-limited, inasmuch as the objective is to produce as much biomass on an areal basis as possible (see D'Elia *et al.*, 1977, for details). Thus, in the absence of allelopathic interaction, of nutrient limitation, or of the effects of additional

species, species dominance will most likely be determined by light-intensity-related or photoperiod-related growth response. In the following paper (Nelson *et al.*, 1979), we show that light-related phenomena may provide the key to competition between *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* at low growth rates.

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