

Are marine diatoms favoured by high Si:N ratios?

Ulrich Sommer*

Institut für Chemie und Biologie des Meeres (ICBM), Carl-von-Ossietzky Universität, Postfach 2503, D-26111 Oldenburg, Germany

ABSTRACT: Competition experiments were performed first with 4, then with 11 species of marine phytoplankton at various ratios of silicate:nitrate and various light intensities. Diatoms became dominant at Si:N ratios > 25:1 while flagellates were the superior competitors at lower ratios. The light supply did not influence the competitive position of diatoms and non-siliceous flagellates in general, while it was important in determining the outcome of competition at the species level. In the 11 species experiments, *Stephanopyxis palmeriana* was the dominant diatom at high light intensities. It shared dominance with *Lauderia annulata* at medium and low light intensities and high Si:N ratios. *Pseudonitzschia pungens* was the dominant diatom at low light intensities and relatively low Si:N ratios. The green alga *Dunaliella tertiolecta* was the dominant flagellate at high light intensities, while at low light intensities the prymnesiophycean *Chrysochromulina polylepis* and the cryptophyte *Rhodomonas* sp. were also important.

KEY WORDS: Phytoplankton · Competition · Nutrients

INTRODUCTION

Anthropogenic eutrophication of coastal seas, such as the southern North Sea and the Baltic Sea, has induced biomass increases and compositional changes of phytoplankton (Cadée 1986, Radach et al. 1990). The most typical compositional change has been a decrease in the relative importance of diatoms in favour of non-silicified algae, particularly flagellates (Cadée & Hegeman 1991). Some of these flagellates, e.g. *Phaeocystis* (Riegman et al. 1992) and *Chrysochromulina polylepis* (Maestrini & Granéli 1991), can form nuisance blooms. An increasing frequency and magnitude of such blooms has been observed with increasing eutrophication (Granéli et al. 1989, Smayda 1989, 1990).

Among others, Riegman (1991) and Smayda (1989, 1990) have offered decreasing Si:N and/or Si:P ratios as a working hypothesis to explain the shifts from diatom dominance to increased importance of flagellates. There is ample evidence for an increase in N and P inputs into coastal seas, but not for a similar increase in Si loading (Andersson & Rydberg 1988, Radach et al. 1990). It is assumed that with decreasing Si:N and/or

Si:P ratios, more N and P remain available for the growth of non-diatom biomass because silicate sets a limit to diatom growth. This hypothesis requires that diatoms be superior competitors for non-silicate nutrients under silicate sufficiency.

This hypothesis is perfectly consistent with Tilman's (1982) theory of resource competition and has been frequently tested in continuous culture competition experiments with freshwater phytoplankton (summarized in Sommer 1989). There have also been several successful attempts to apply the resource ratio hypothesis to species shifts in freshwater phytoplankton (Makulla & Sommer 1993, Sommer 1993, Sommer et al. 1993). The only experimental application of Tilman's competition theory to marine phytoplankton was performed with phytoplankton from the Antarctic Sea (Sommer 1985). Here, I present a total of 48 competition experiments with temperate, marine phytoplankton. They were performed at 6 different Si:N ratios and 4 different light intensities (24 environmental conditions). One full set of environmental conditions was tested with 4 species in each experiment, another full set was tested with 11 species. Among the experimental species were the prymnesiophycean flagellate *Chrysochromulina polylepis*, which formed a toxic bloom in the Scandinavian coastal waters in 1988 (Maestrini & Granéli 1991),

*Present address: Institut für Meereskunde, Düsternbrooker Weg 20, D-24105 Kiel, Germany

and the diatom *Pseudonitzschia pungens* f. *multiseries*, which has formed several toxic blooms along the Canadian Atlantic coast (Smith 1993).

MATERIAL AND METHODS

Organisms. In the first series of experiments (4 species experiments), 1 species was used per division: *Pseudonitzschia* (= *Nitzschia*) *pungens* f. *multiseries* (Hasle) Hasle (Bacillariophyceae), and the flagellates *Dunaliella tertiolecta* Butcher (Chlorophyta) and *Rhodomonas* sp. Karsten (Cryptophyta), *Chrysochromulina polylepis* Manton et Parke (Prymnesiophyceae). In the second series (11 species experiments) diatoms were represented by 4 species [*Pseudonitzschia pungens*, *Stephanopyxis palmeriana* Grunow, *Neostreptotheca subindica* von Stosch, *Lauderia annulata* Cleve (= *L. borealis* Gran)], flagellates were represented by 6 species (the dinoflagellate *Scrippsiella trochoidea* Loeblich III, the chlorophyte *Dunaliella tertiolecta*, the prymnesiophycean *Chrysochromulina polylepis*, *Prymnesium parvum* Carter, *Pleurochrysis carterae* Christensen), and the cryptophyte *Rhodomonas* sp., and immotile picoplankton was represented by the cyanobacterium *Synechococcus* sp. *P. pungens* was a clonal strain of Canadian origin (by courtesy of R. Pocklington); the other clonal strains originated from the North Sea (by courtesy of M. Elbrächter).

Culture conditions. The medium was an artificial seawater medium with a nutrient salt, trace element, and vitamin mixture according to the f/2 medium by Guillard & Ryther (1962) modified in order to obtain the required Si:N ratios and to avoid P limitation even at the highest nitrate concentrations (19.8 μM NO_3^- vs 2 μM P). Silicate concentrations did not vary between experiments (317 μM); experimental Si:N ratios were adjusted by varying nitrate concentrations. Other nitrogen sources (e.g. ammonium) were not supplied. The stoichiometric Si:N ratios in the 4 species experiments were 14:1, 17:1, 22:1, 29:1, 49:1, 95:1; in the 11 species experiments they were 16:1, 24:1, 31:1, 40:1, 74:1, 124:1, respectively. After composing the media, the nutrient concentrations were measured according to standard oceanographic methods (Strickland & Parsons 1968). The ratios reported here are measured ratios rounded off to the nearest integer.

Cultures were maintained in 250 ml Erlenmeyer flasks and kept in suspension by a rotating shaking table. Once per day 30 ml culture suspension was replaced by fresh medium ('semicontinuous culture') which gave a dilution rate of 0.3 d^{-1} . The experimental temperature was 15°C.

Light was supplied by fluorescent tubes. In order to balance the emission weakness of the white tubes

(Osram 'Biolum') in the red and blue spectral ranges they were combined with purple ones (Osram 'Fluora'). The light:dark period was 16:10 h. Experimental light intensities were set by distance to the light source (Table 1). Due to lateral heterogeneities in the light field, light intensities were not exactly identical within each row of the shaking table. Light intensities given in this article are starting intensities (I_0) measured in culture flasks containing medium but no algae. Light absorption by algae played only a minor role because of the small culture volumes. Light intensities within the flasks measured at the end of each experiment were always $>0.9 I_0$.

Sampling and counting. Samples from all experiments were taken twice a week and fixed with Lugol's iodine. Of each sample, 10 ml were settled in Utermöhl chambers for counting under an inverted microscope. If enough individuals were present, 100 to 200 individuals of each species were counted which gives a 95% CL of ± 14 to 20% if individuals are randomly distributed. Biomass of each species was estimated as cell volume (V_i) which was calculated as product of the cell number (in no. ml^{-1}) and the volume of individual cells (in μm^3). The latter was obtained by geometric approximation of microscopic measurements of at least 20 individuals. The relative importance of a species was expressed by its contribution to total cell volume (V_i/V_{tot}).

RESULTS

Time course of experiments

Initially the abundance of all species increased. This implies that growth conditions were sufficient for all species to grow in the absence of competitive pressure.

Table 1. Light intensity ($\mu\text{E m}^{-2} \text{s}^{-1}$ photosynthetically active radiation) and Si:N (mol mol^{-1}) ratios in the 4 species and the 11 species experiments

4 species experiment						
Si:N	14:1	17:1	22:1	29:1	49:1	95:1
Light intensity						
Row 1	142	197	223	225	216	199
Row 2	72	83	94	100	105	99
Row 3	36	38	46	55	55	60
Row 4	28	28	33	37	39	40
11 species experiment						
Si:N	16:1	24:1	31:1	40:1	74:1	124:1
Light intensity						
Row 1	142	197	223	225	216	199
Row 2	72	83	94	100	105	99
Row 3	36	38	46	55	55	60
Row 4	28	28	33	37	39	40

By implication, species replacements occurring later in the experiments can be explained by competitive exclusion. Such displacements began to occur after 1 wk of growth. The species growing fastest at the beginning (most frequently *Dunaliella tertiolecta*) could not retain dominance at high Si:N ratios (Fig. 1). After ca 3 wk clear differences began to emerge between the low- and the high-Si:N cultures. The 4 species experiments were terminated on Day 26, the 11 species experiments on Day 35.

Species composition at the end of experiments

4 species experiments

The relative importance of *Pseudonitzschia pungens* increased with increasing Si:N ratios and was more or less independent of light intensities (Fig. 2). The transition from flagellate to diatom dominance occurred around Si:N = 25:1. *Dunaliella tertiolecta* was the most important non-siliceous alga. At the highest light level it was the only persisting flagellate taxon. At the lower light levels and low Si:N ratios also a population of

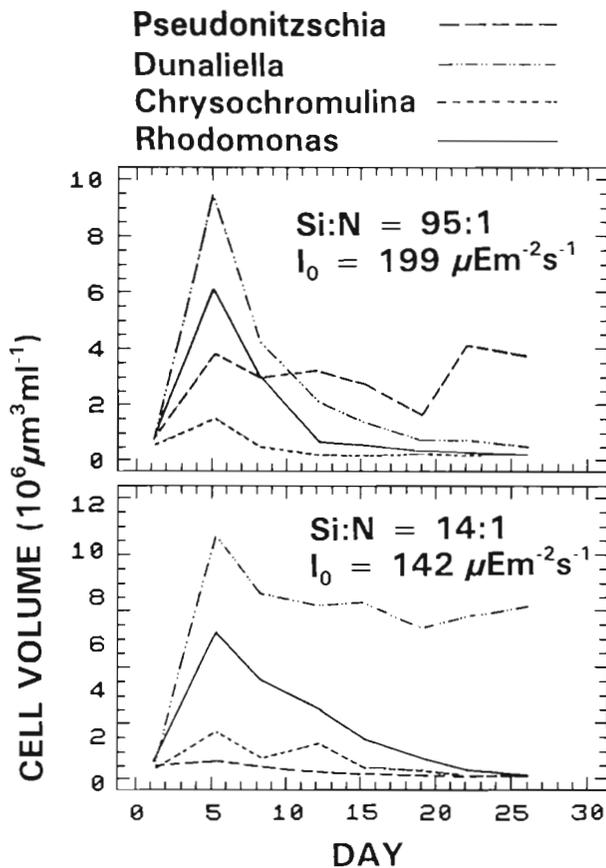


Fig. 1 Time course of two 4 species experiments (row 1, Si:N = 95:1 and 14:1)

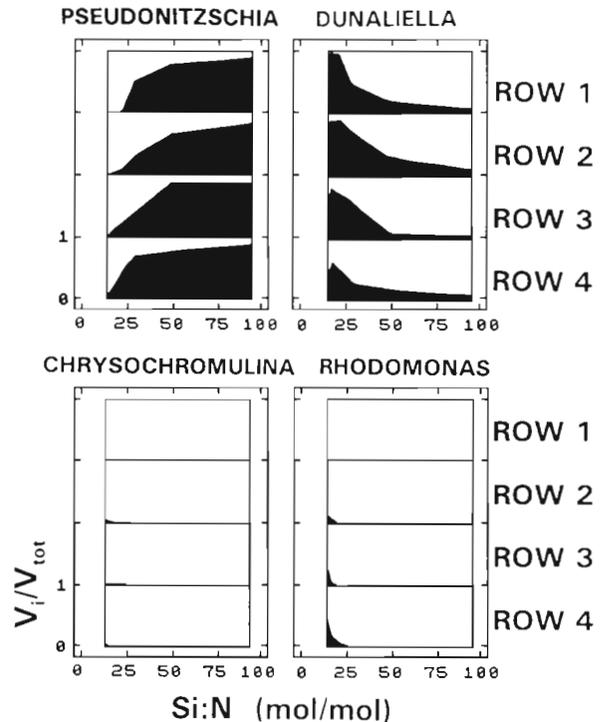


Fig. 2. Species composition (expressed as species contribution to total biomass) at the end of the 4 species experiments

Rhodomonas sp. resisted competitive exclusion. Small residual populations of *Chrysochromulina polylepis* could still be detected at low light intensities and Si:N ratios. It could not be judged whether they were stable or whether prolongation of culture would have led to their exclusion.

11 species experiments

At the coarsest level of taxonomic resolution (diatoms vs flagellates) the final outcome was very similar to the 4 species experiments (Fig. 3). The relative biomass of diatoms increased with increasing Si:N ratios and showed no pronounced dependence on light. Again, the transition from flagellate to diatom dominance occurred at Si:N ratios around 25:1. Five of the 11 species began to decline already after a short growth pulse during the first week in all experiments: *Neostreptothea subindica*, *Scrippsiella trochoidea*, *Prymnesium parvum*, *Pleurochrysis carterae*, *Synechococcus* sp.

Stephanopyxis palmeriana (Fig. 4) was the only persisting diatom at the highest light level but lost importance with decreasing light. In row 2, diatom biomass was composed of *S. palmeriana* and *Lauderia borealis* over the entire range of diatom dominance. In rows 3 and 4 *S. palmeriana* persisted only in cultures with very high Si:N ratios.

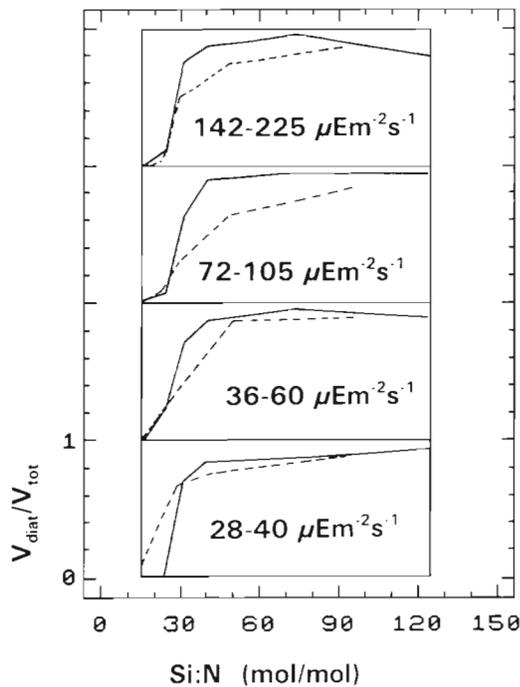


Fig. 3. Contribution of diatoms to total biomass in the 4 species (---) and the 11 species experiments (—). Panels top to bottom are rows 1 to 4, respectively

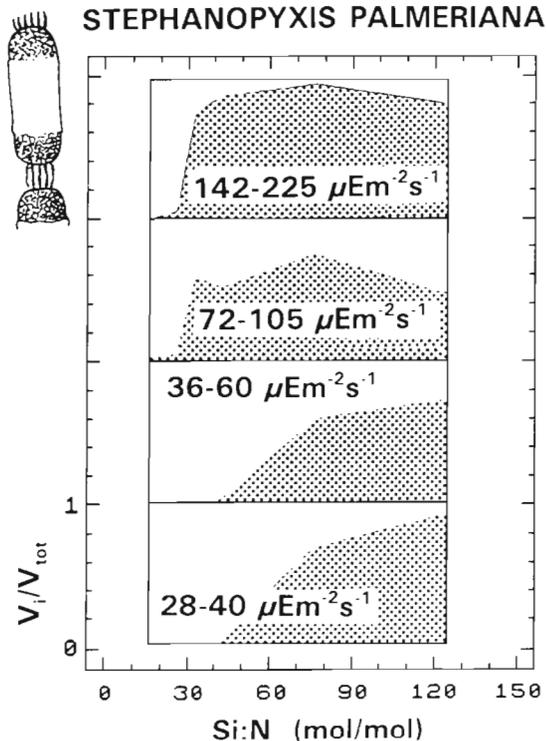


Fig. 4. *Stephanopyxis palmeriana*. Contribution to total biomass at the end of the 11 species experiments. Panels top to bottom are rows 1 to 4, respectively

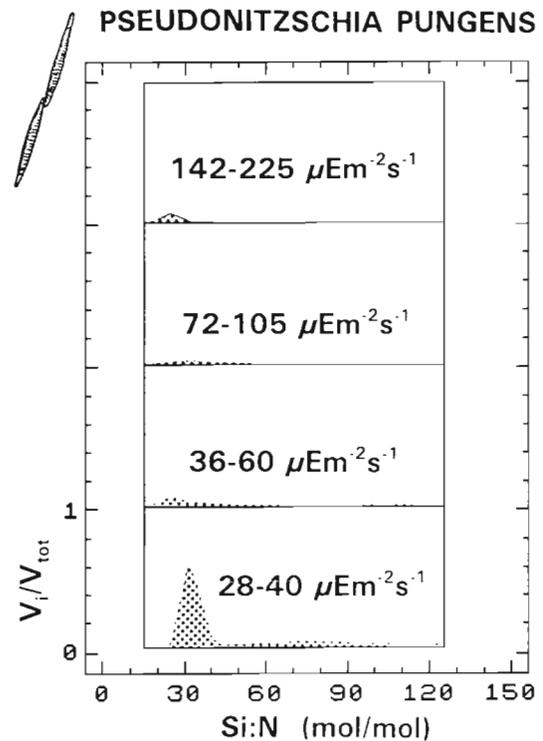


Fig. 5. *Pseudonitzschia pungens*. Contribution to total biomass at the end of the 11 species experiments. Panels top to bottom are rows 1 to 4, respectively

The range of conditions of *Pseudonitzschia pungens* dominance (Fig. 5) was strongly restricted compared to the 4 species experiments. Small residual populations remained in rows 1 to 3 at relatively low Si:N ratios. In row 4 it was the dominant phytoplankton species at Si:N = 31:1 and remained in culture at low biomass levels at all higher ratios.

Lauderia annulata (Fig. 6) did not persist in row 1. In row 2 it was codominant with *Stephanopyxis palmeriana* at all Si:N ratios from 40:1 upwards. In rows 3 and 4 it held an intermediate position between *Pseudonitzschia pungens* and *S. palmeriana* with an optimum at Si:N = 40:1.

Dunaliella tertiolecta (Fig. 7) behaved similarly as in the 4 species experiments. It was the only numerically important flagellate under high light conditions and shared dominance with the other flagellates at low Si:N ratios in rows 3 and 4.

Chrysochromulina polylepis (Fig. 8) was more successful than in the 4 species experiments and contributed > 50% to total biomass at the lowest Si:N ratio in rows 3 and 4. *Rhodomonas* sp. (Fig. 9) held a similar position with a maximal relative importance at low light and low Si:N ratios. The biomass of both species fluctuated widely even during the last week which made it impossible to judge which one would eventually persist and which one would become displaced.

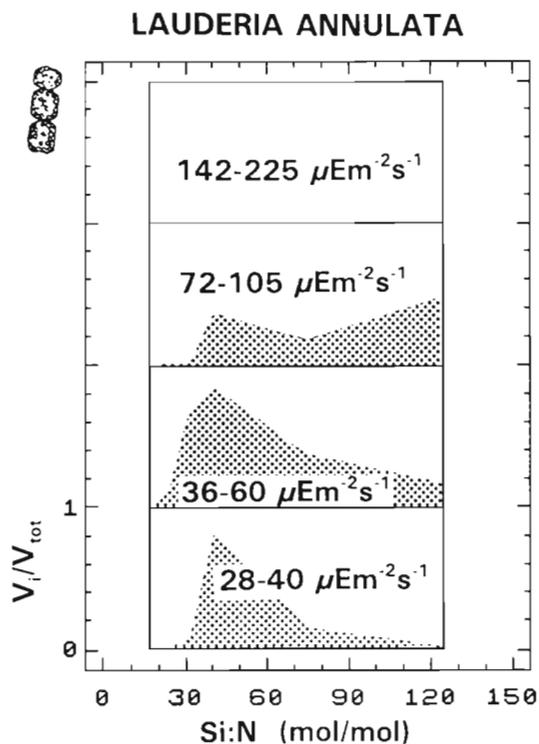


Fig. 6. *Lauderia annulata*. Contribution to total biomass at the end of the 11 species experiments. Panels top to bottom are rows 1 to 4, respectively

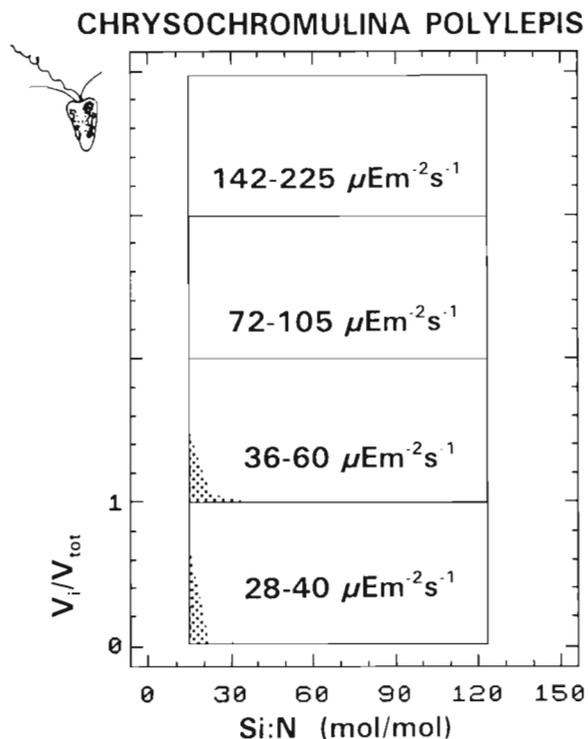


Fig. 8. *Chrysochromulina polylepis*. Contribution to total biomass at the end of the 11 species experiments. Panels top to bottom are rows 1 to 4, respectively

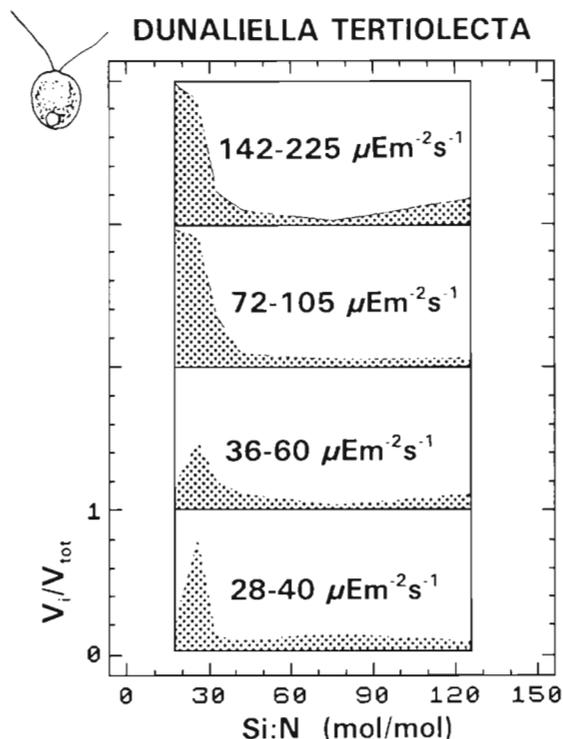


Fig. 7. *Dunaliella tertiolecta*. Contribution to total biomass at the end of the 11 species experiments. Panels top to bottom are rows 1 to 4, respectively

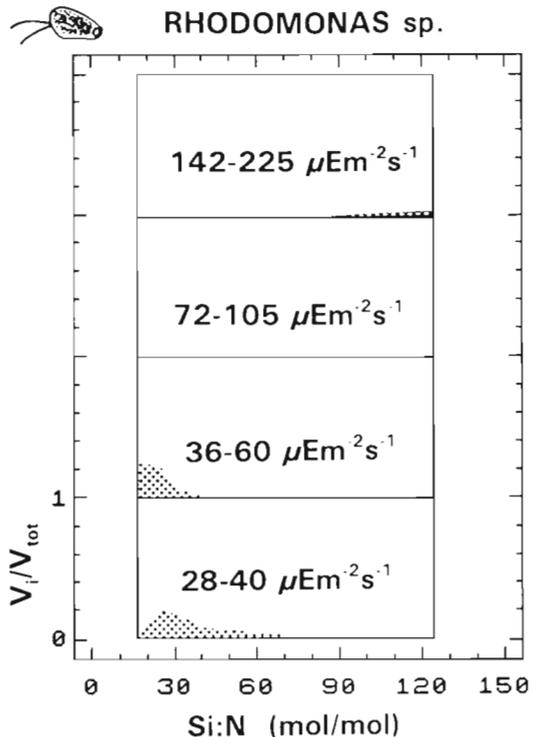


Fig. 9. *Rhodomonas* sp. Contribution to total biomass at the end of the 11 species experiments. Panels top to bottom are rows 1 to 4, respectively

DISCUSSION

Tilman's (1982) theory and other models on competitive exclusion predict that the number of persisting species under steady-state conditions cannot exceed the number of limiting resources. Theoretically, total exclusion of competitive losers requires infinite time. Practically, a duration of 4 to 5 wk has been sufficient in the majority of competition experiments with phytoplankton to discriminate between winners and losers (Sommer 1989). Sometimes it is impossible, however, to distinguish between slow exclusion and persistence. In the case of the experiments reported here, 2 species should persist under light sufficiency and 3 under light limitation: 1 N-limited species, 1 light-limited species, and 1 Si-limited species. The latter has to be a diatom by necessity. General ecophysiological knowledge about planktonic algae (Kohl & Nicklisch 1988) suggests light sufficiency of growth rates for most species in row 1 and possibly row 2. Moderate light limitation of species with high requirements might begin in row 3, but even here physiological low-light adaptation (Jørgensen 1969) should enable many species to grow at maximal growth rates. However, low-light adaptation might have metabolic costs, e.g. a higher N demand for enhanced chlorophyll synthesis. These metabolic costs might lead to shifts in competitive performance.

There was an apparent contradiction to the theoretical expectation at the low Si:N ratios in rows 3 and 4 with 3 non-siliceous algae coexisting. There are 3 potential explanations. First, steady state had not yet been attained. Second, there was an additional unidentified limiting resource (a trace element? a vitamin?). Third, 1 of the 3 flagellates persisted by heterotrophic nutrition, e.g. bacterivory. This would have been most probably *Chrysochromulina polylepis* because bacterivory is widely distributed among pigmented Prymnesiophyceae including several species of this genus. On the other hand, bacterivory is controversial among pigmented Cryptophyta and is unknown among Chlorophyta (Sanders & Porter 1988).

One of the central hypotheses of Tilman's competition theory predicts that ranges of competitive dominance of different species should be sorted along gradients of resource ratios ('resource ratio hypothesis'). Numerous experiments with freshwater phytoplankton (Sommer 1983, Kilham 1986, Tilman et al. 1986; for a review see Sommer 1989) and a few experiments with marine, Antarctic phytoplankton (Sommer 1986) have shown that diatoms are dominant competitors at high ratios of silicate to non-siliceous nutrients. The experiments reported here are the first corresponding result for temperate, marine phytoplankton.

The experimental results qualitatively confirm Riegman's (1991) and Smayda's (1989, 1990) explanation of eutrophication related species shifts in the phytoplankton of coastal seas. Contrary to the qualitative agreement there remains a quantitative discrepancy. In my experiments, the transition from flagellate to diatom dominance took place at Si:N ratios around 25:1. This is much higher than the Si:N ratios prior to spring phytoplankton growth *in situ*. Radach et al. (1990) report winter (pre-bloom) Si:N ratios from 0.5 to 1:1 for the German Bight during the late 1960s and ratios from 0.12 to 0.25 at present. This is the range where the transition from diatom dominance to an increasing importance of flagellates took place *in situ*. Of course, there is no perfect analogy between supply ratios in continuous cultures and concentration ratios before the onset of growth *in situ*. But the slowness of Si recycling relative to other nutrients would rather reduce Si:N supply ratios during the growth period instead of increasing them relative to pre-bloom concentration ratios. It should also be noted that nutrient concentrations prior to the onset of the spring bloom (ca 40 μM Si and 40 μM N) are far from being limiting, which precludes nutrient competition during the starting phase. Only species shifts in the peak period when nutrients are exhausted can potentially be ascribed to competition.

The quantitative discrepancy between experimental results can be explained by a variety of hypotheses which can be tested by further experiments:

(1) The diatom species chosen for my experiments have particularly high silicate requirements and therefore high optimal Si:N ratios. Inclusion of diatoms with lower Si requirements would displace the transition from flagellate to diatom dominance to lower Si:N ratios.

(2) Nanoplankton flagellates are subject to loss factors (e.g. micrograzers) to which some diatoms are less subject. This could compress the competitive niche of flagellates along the Si:N gradient. There is ample evidence for protozoan grazers to feed selectively on pico- and nanoplankton while not feeding on microplankton diatoms (discussed in Riegman et al. 1993).

(3) Several forms of environmental variability, e.g. the vertical transport through the light gradient, might displace the Si:N ratio of dominance transition relative to experiments with constant conditions. Experiments with freshwater phytoplankton have shown such displacements along the Si:P gradient by adding various levels of environmental complexity to the experimental design (periodic supply of 1 nutrient and of 2 nutrients, Sommer 1985; addition of grazers and of nutrient recycling, Sommer 1988).

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