The impact of light intensity and daylength on silicate and nitrate competition among marine phytoplankton

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Abstract

I tested the extent to which differences in light supply could influence the outcome of nutrient (Si and N) competition between marine phytoplankton. Competition experiments were performed with 11 species of marine phytoplankton at Si:N ratios from 16 to 124:1, light intensities from 28 to 225 μmol quanta $m^{-2} s^{-1}$, and three different daylengths. Thus, light supply was the composite result of two components: photoperiod and intensity. Diatoms were dominant competitors at higher Si:N ratios, nonsiliceous flagellates at lower ones. Light had no impact on the transition from flagellate to diatom dominance along the Si:N gradient. However, species within those groups were separated along the light gradient. Contrary to theoretical expectations, changes in light intensity and changes in daylength led to similar shifts in species dominance. Therefore, it was possible to describe the light climate by the integral parameter "daily light dose."

Competition for limiting resources is one of several factors structuring phytoplankton communities. The theoretical and experimental analysis of resource competition gained momentum with Tilman's (1977) competition experiments with the freshwater diatoms *Asterionella formosa* and *Cyclotella meneghiniana*. A largely consistent body of theory (Tilman 1982) and experimental evidence has since developed (*see* Sommer 1989). Tilman's theory makes two major predictions. First, at steady state only as many species can coexist as there are growth rate-limiting resources. Winners of competition (coexisting species) can be identified by zero net growth rates at steady state while losers can be identified by negative net growth rates. Second, the taxonomic identity and the relative abundances of the coexisting species depend on the ratio of the limiting resources (resource ratio hypothesis).

Most of the published experiments have used mineral nutrients as limiting resources (Si and P: e.g. Tilman 1977, 1981; Sommer 1983; Kilham 1986; Si, N, and P: Tilman et al. 1986) and freshwater phytoplankton as test organisms. The resource ratio hypothesis has been used to explain seasonal species replacements

within lakes (Sommer 1993), compositional differences at comparable seasons between lakes (Makulla and Sommer 1993), and long-term species replacements within one lake (Sommer et al. 1993). Also eutrophication-dependent shifts in marine phytoplankton have been explained by decreasing Si:N or Si:P ratios (Riegman 1991; Smayda 1989, 1990). However, species replacements of marine phytoplankton along Si:N or Si:P gradients have not been tested by culture competition experiments so far (except for Antarctic algae: Sommer 1986).

Contrary to nutrients, light has received little attention in published competition studies. In previous studies, light has usually been supplied at supposedly saturating intensities in order to preclude competition for light. Tilman (1990, p. 134) pointed out that

> light competition is conceptually more complex than nutrient competition. We do not yet have either rigorous theoretical predictions or experimental results that indicate that a single number, analogous to R*, can predict the outcome of competition for light. A fuller understanding of light competition remains a major challenge.

There are at least four reasons for the higher complexity of light competition. First, several components of the light field are not subject to exploitation by phototrophic organisms and are, therefore, not a target of competition: surface irradiance, daylength, attenuation by dissolved and dead particulate matter. Second, the theory of competition assumes an even (or at least random) distribution of resources in

space. This assumption may hold for nutrients in a well-mixed water column but it never holds for light. There is always a vertical gradient of light. Third, depending on physical conditions, phytoplankton may circulate within the light gradient or they may be stationary. Some can even actively search for a favorable position within the gradient (buoyant cyanobacteria: Walsby and Brooker 1980; large flagellates: Sommer and Gliwicz 1986). Fourth, physiological adaptation to low light intensities (Richardson et al. 1983) may decrease the intensity below which light becomes limiting, but more nutrients might be required for low light adaptation (e.g. more N for increased chlorophyll synthesis). Thus, nutrient competitive abilities might be influenced indirectly by light adapatation. A theory for light competition in a circulating water column is being developed (Huisman and Weissing 1994). It is considerably more complex than the theory of nutrient competition and does not lead to a prediction as simple as the resource ratio hypothesis.

The competition experiments presented here examine a different scenario. While silicate and nitrate were offered as competitive resources, light was a noncompetitive resource set externally by daylength and surface irradiance. This situation is comparable to a vertically stable water column where the light availability for phytoplankton at each depth is influenced by the overlying populations but not by the nutrient competitors within the same layer.

Material and methods

Organisms—The experimental organisms were 11 unialgal strains of marine algae from six higher taxa. Diatoms were represented by four species (*Nitzschia pungens, Stephanopyxis palmeriana, Neostreptotheca* sp., *Lauderia borealis*), dinoflagellates by *Scrippsiella trochoidea*, chlorophytes by *Dunaliella tertiolecta*, the Prymnesiophyceae by *Chrysochromulina polylepis, Prymnesium parvum,* and *Pleurochrysis carterae*, cryptophytes by *Rhodomonas* sp., and cyanobacteria by the picoplankter *Synechococcus* sp. *Nitzschia* was of Canadian origin (courtesy of R. Pocklington). The other strains originated from the North Sea (courtesy of M. Elbrächter). The inoculum biomass of all species was within one order of magnitude ($0.1–1 \times 10^6 \ \mu m^3 \ ml^{-1}$).

Culture conditions—The medium was artificial seawater with a nutrient salt, trace-element, and vitamin mixture according to the f/2 formulation of Guillard and Ryther (1962), modified to obtain the required silicate:nitrate 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). Si:N ratios were adjusted by varying nitrate concentrations. Other nitrogen sources (e.g. ammonium) were not supplied. The stoichiometric Si:N ratios were 16:1, 24:1, 31:1, 40:1, 74:1, and 124:1, respectively. The ratios reported here are based on chemical analysis (Strickland and Parsons 1968) of the media.

Cultures were maintained in 250-ml Erlenmeyer flasks and kept in suspension by a rotating shaking table. Once per day 30 ml of culture suspension were replaced by fresh medium (semicontinuous culture) which gave a dilution rate of 0.3 d^{-1}. The semicontinuous culture principle is a slight deviation from the assumption of perfect steady state in competition theory. Practical experience [e.g. Tilman's early experiments (Tilman 1977) and my own experiments with Antarctic phytoplankton (Sommer 1986)] has shown that it is an acceptable approximation. Semicontinuous cultures were chosen instead of more perfect chemostats because they are cheaper and easier to maintain, which made it possible to run 24 cultures at the same time.

Light was supplied by fluorescent tubes. To balance the low emission of the white tubes (Osram Biolux) in the red and blue spectral ranges, I combined them with an equal number of purple ones (Osram Fluora). Experimental light intensities were set by distance to the light source. Due to lateral heterogeneities in the light field, light intensities were not exactly identical within each row of the shaking table (Table 1). Light intensities given here are starting intensities (I_0) measured inside culture flasks containing medium but no algae. Light absorption by algae played only a minor role because of the smallness of the cultures. Light intensities in the flasks measured at the end of each experiment were always >0.9 I_0. Three different light/dark cycles were used: 16:8, 12:12, and 8:16 h. The experimental temperature was 15°C.

Table 1. Light intensity (μmol quanta m^{-2} s^{-1} PAR) and Si:N (mol mol^{-1}) ratios in the experimental treatments.

	Si:N					
	16:1	24:1	31:1	40:1	74:1	124:1
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

Sampling and counting—All cultures were sampled twice a week (Tuesday, Friday) and fixed with Lugol's iodine. Ten milliliters of each sample were settled in Utermöhl chambers for counting under an inverted microscope. When enough individuals were present, 100–200 individuals of each species were counted which gives a 95% C.I. of ±14–20% if individuals are randomly distributed. Prymnesiophyceae were identified to species level only during the last three counts. The biomass of each species was estimated as cell volume (V_i) which was calculated as the product of cell number (in No. ml^{-1}) and mean volume of individual cells (in μm^3). The latter was obtained by approximation to the nearest standard geometric solid after microscopic measurements of at least 20 individuals. The relative importance (p_i) of a species was expressed by its contribution to total cell volume (V_i/V_{tot}).

Results

Time-course of experiments—Initially all species increased, thus indicating at least suitable growth conditions. In nearly all experiments, *Dunaliella* and the prymnesiophycean flagellates had the largest initial increases. After ~1 week, the first competitive displacements began. The final species composition began to stabilize after 3–4 weeks. After 5 weeks, the experiments were terminated. At that time, one or two species in the high light treatments and one to three species in the low light treatments formed 95% of the total cell volume. Sometimes losing competitors still had countable abundances at the end of the experiments. They were classified as losers when there was a significant ($P < 0.05$) correlation between log abundance and time. The growth pattern of *Dunaliella* in Fig. 1 is one such example. In the following, I will use two different
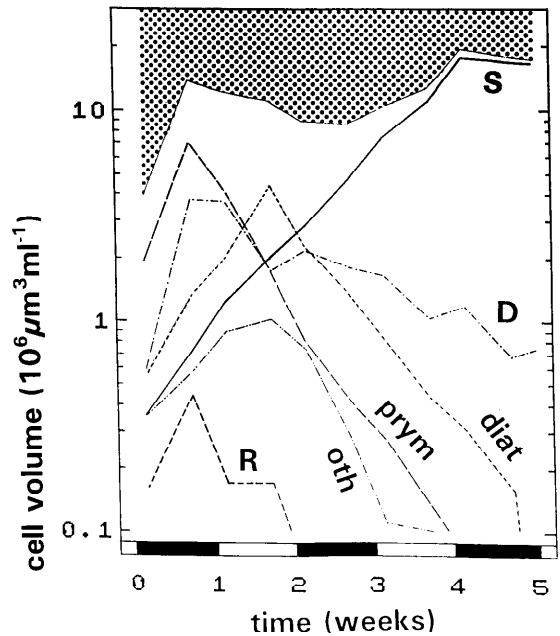

Fig. 1. Example for the time-course of competition experiments. Experiment at Si:N 74:1, $I = 216$ μmol quanta m^{-2} s^{-1}, L/D cycle 16:8. Total cell volume: white area below shading; S—*Stephanopyxis palmeriana*; diat—other diatoms; D—*Dunaliella tertiolecta*; R—*Rhodomonas* sp.; prym—Prymnesiophyceae; oth—other species.

expressions for species composition. The term "final species composition" describes the species composition at the end of an experiment without consideration of winners and losers. The term "equilibrium species composition" is a forecast based on the assumptions that the losers will eventually decline to zero while the biomass ratios between the winners will remain unchanged. The more descriptive Figs. 2–7 are based on "final species composition"; the regression analysis in Table 2 and Fig. 8 are based on "equilibrium species composition."

Only six species ever contributed >10% to final biomass: *S. palmeriana*, *L. borealis*, *N. pungens*, *D. tertiolecta*, *C. polylepis*, and *Rhodomonas* sp. These were the same species that were classified as winners in at least one experiment.

Diatoms and the Si:N ratio—The contribution of diatoms to total cell volume responded only to Si:N ratio, not to daylength or light intensity (Eq. 1 in Table 2). There was a sharp transition between Si:N 16:1 and Si:
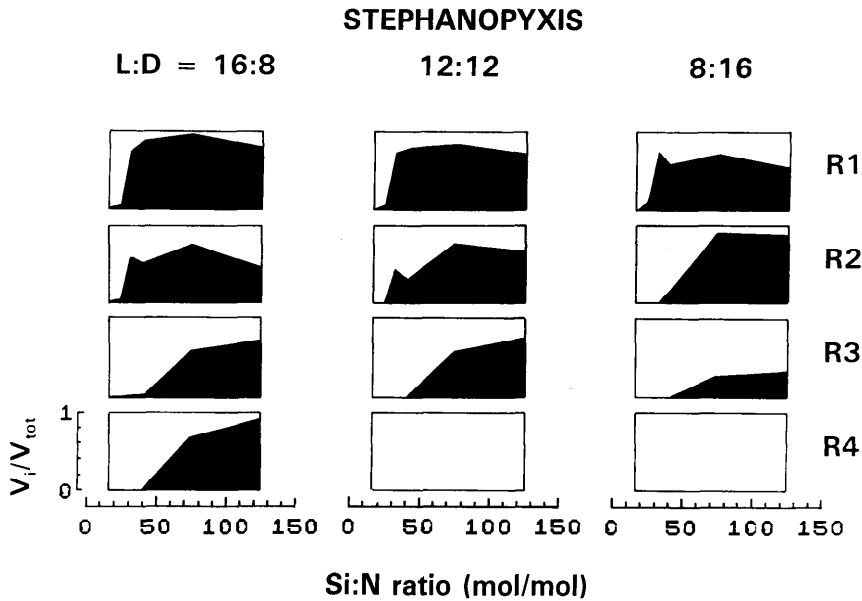
STEPHANOPYXIS

Fig. 2. Contribution of *Stephanopyxis palmeriana* to total cell volume (black area) at the end of competition experiments. Si : N gradient within blocks. Rows: R1—142–225 μmol quanta m^{-2} s^{-1}; R2—72–105 μmol quanta m^{-2} s^{-1}; R3—36–60 μmol quanta m^{-2} s^{-1}; R4—28–40 μmol quanta m^{-2} s^{-1}.

N 31 : 1. At ratios >31 : 1 only diatoms were classified as winners. At ratios <16 : 1 no diatom species could persist. At 31 : 1 diatoms contributed 60–90% to equilibrium biomass; at 24 : 1 they contributed <40%.

Final species composition—S. palmeriana was the dominant alga at high Si : N ratios and high light conditions (Fig. 2). Both reduction of light intensity and daylength decreased its importance relative to other diatoms. At 12- and 8-h daylength, it was completely excluded from cultures with light intensities <40 μmol quanta m^{-2} s^{-1}. *L. borealis* was excluded from the highest (>150 μmol quanta m^{-2} s^{-1}, 16-h daylength) and from the lowest (<40 μmol quanta m^{-2} s^{-1}, 8-h daylength) light conditions. The competitive optimum of this species was a combination of medium Si : N (40 : 1) and medium amount of light (Fig. 3). *N. pungens* was absent or nearly absent from high light cultures, but it was the dominant phytoplankton species at high Si : N and low light conditions (Fig. 4).

Among the nonsilicified taxa, there was a segregation of species along the light gradient. The green alga *D. tertiolecta* was dominant at low Si : N at light intensities >70 μmol quanta m^{-2} s^{-1} under long and medium daylength (Fig. 5). Decreasing light intensity and decreas-

ing daylength led to a decreased importance of *Dunaliella*. *C. polylepis* appeared to be the specialist for medium light among the flagellates (Fig. 6), while *Rhodomonas* was the dominant flagellate under low light conditions (Fig. 7).

Discussion

Nutrient ratios—The general pattern of increasing importance of diatoms with increasing Si : N ratios resembles a similar pattern

Table 2. Multiple regression analysis (stepwise variable selection, backward procedure, F-to-remove = 4) of relative contribution to biomass (p_i) on Si : N ratios and daily light dose. $y = \arcsin p_i$; $x = \log_{10}(Si : N)$; $z = \log_{10}DLD$. All regressions are significant at $P < 0.0001$.

(Eq. 1)	All diatoms: $y = -1.95 + 1.85x$, $r^2 = 0.71$;
(Eq. 2)	*Stephanopyxis*: $y = -1.48 + 1.04x + 0.81z^2$, $r^2 = 0.69$;
(Eq. 3)	*Lauderia*: $y = -6.43 + 7.76x - 2.24x^2 + 1.64z - 1.56z^2$, $r^2 = 0.44$;
(Eq. 4)	*Nitzschia*: $y = -3.51 + 4.93x - 1.34x^2 - 1.81z + 0.90z^2$, $r^2 = 0.43$;
(Eq. 5)	*Dunaliella*: $y = 5.30 - 5.71x + 1.40x^2 + 1.44z - 0.80z^2$, $r^2 = 0.49$;
(Eq. 6)	*Chrysochromulina*: $y = 5.20 - 5.64x + 1.53x^2 - 0.19z^2$, $r^2 = 0.44$;
(Eq. 7)	*Rhodomonas*: $y = 0.86 - 0.33x - 0.43z$, $r^2 = 0.40$.

LAUDERIA

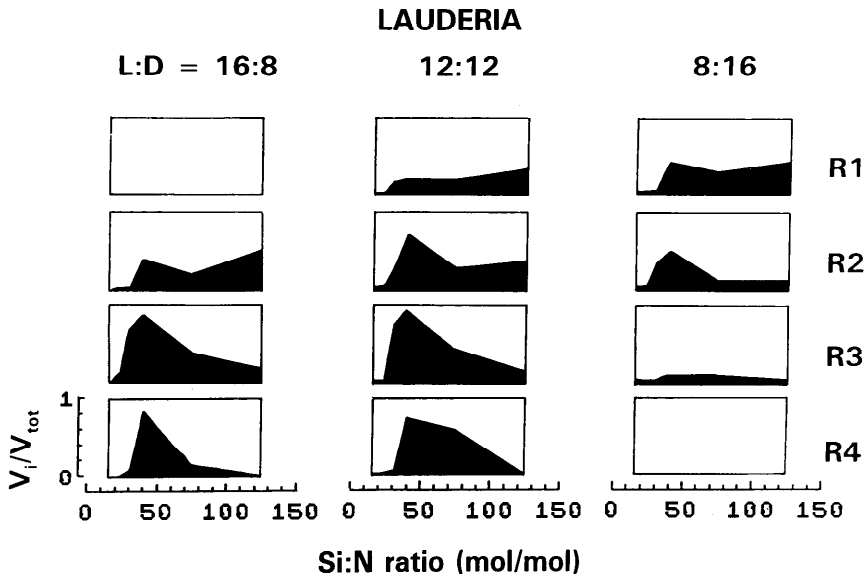L:D = 16:8   12:12   8:16

Fig. 3. As Fig. 2, but of *Lauderia borealis*.

observed along the Si : P gradient in lake phytoplankton (Sommer 1983; Kilham 1986; Tilman et al. 1986). The Si : N ratio has received less attention in freshwater ecology because conventional (but not rigorously tested) wisdom (Hecky and Kilham 1988) considers N limitation in lakes unimportant.

The increase of flagellates with decreasing

Si : N ratios in my experiment seems consistent with Smayda's (1989, 1990) and Riegman's (1991) hypothetical explanation of species shifts in eutrophicated coastal waters. The agreement, however, is only qualitative; at the quantitative level, a major discrepancy remains. In my experiments, the transition took place at much higher ratios (between 16 and
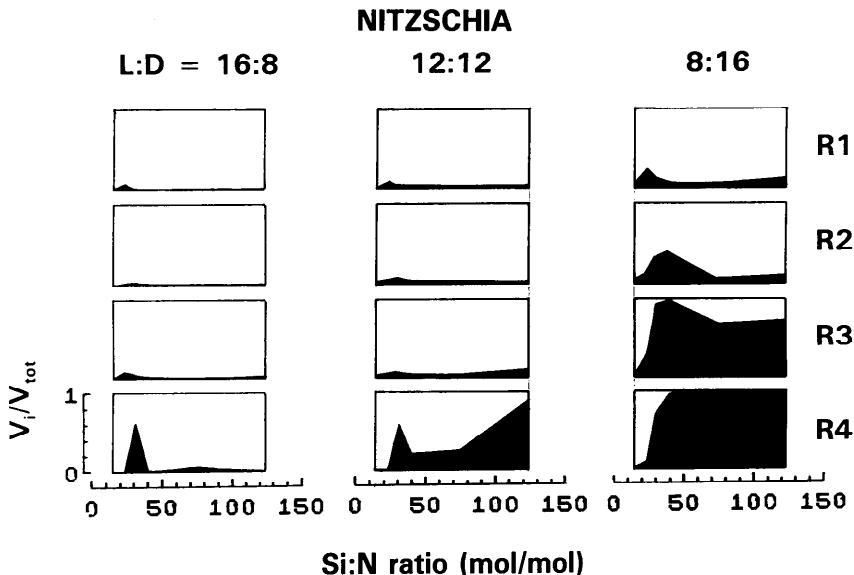
NITZSCHIA

L:D = 16:8   12:12   8:16

Fig. 4. As Fig. 2, but of *Nitzschia pungens*.
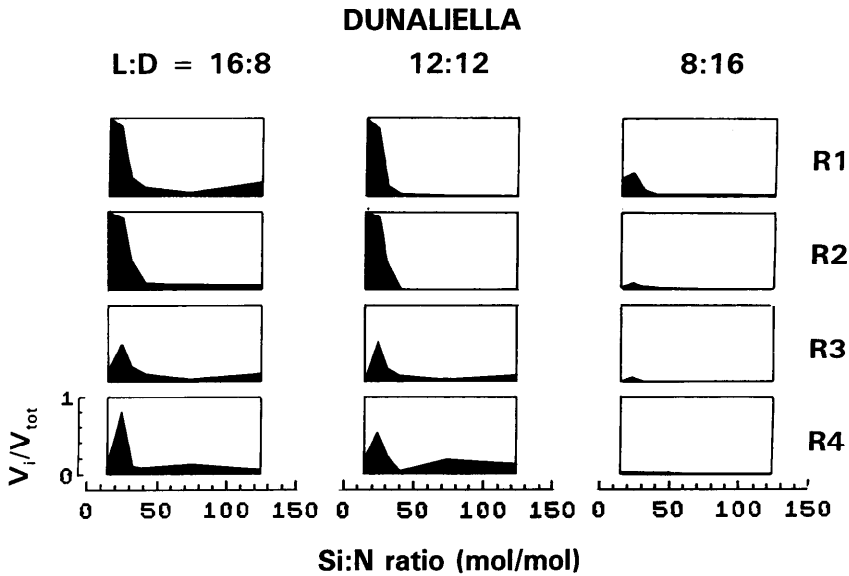
DUNALIELLA

Fig. 5. As Fig. 2, but of *Dunaliella tertiolecta*.

31 : 1) than in eutrophicated coastal seas, such as the southern North Sea, where diatom dominance, usually observed in spring, is being partially replaced by flagellates (Cadée 1986; Cadée and Hegeman 1991; Riegman et al. 1992). In the southern North Sea, winter (prebloom) Si:N was between 1 and 2:1 during the 1960s and has decreased because of increased N loading to 0.1–0.25 at present (Radach et al. 1990).

There are several possible explanations for this discrepancy. Nanoplankton flagellates are subject to higher grazing losses than diatoms. The higher loss rates might compress the com-
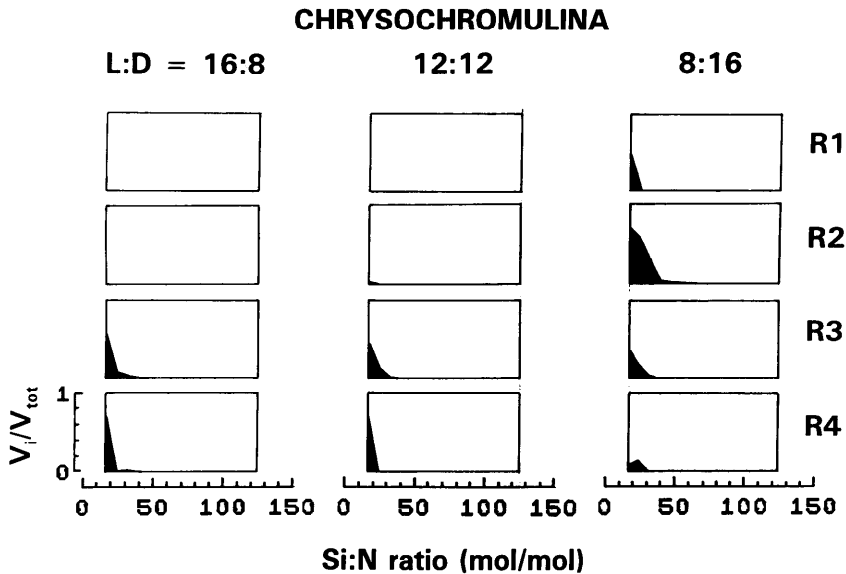
CHRYSOCHROMULINA

Fig. 6. As Fig. 2, but of *Chrysochromulina polylepis*.

RHODOMONAS

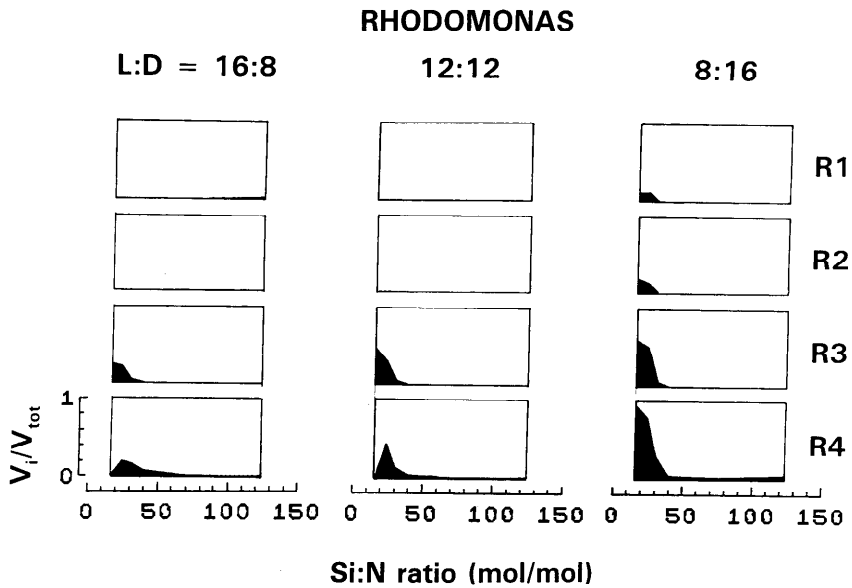L:D = 16:8   12:12   8:16

Fig. 7.   As Fig. 2, but of *Rhodomonas* sp.

petitive niche of flagellates along the Si : N gradient. Environmental variability in situ (e.g. vertical transport through the light gradient) might displace the Si : N ratio of dominance transitions relative to cultures with constant conditions. Such displacements have been found experimentally at several levels of environmental complexity (periodic nutrient supply: Sommer 1985; addition of grazers and nutrient recycling: Sommer 1988). The diatoms used in my experiments have unusually high Si requirements. At present, I consider this explanation most plausible. In a series of ongoing experiments (Sommer unpubl.) in a different context, some diatoms (e.g. *Nitzschia closterium, Asterionella glacialis*) win competition against flagellates at any Si : N > 2 : 1.

Light field—It is obvious from Figs. 2 to 7 that daylength and light intensities had qualitatively similar impacts on the performance of species along the Si : N gradient. No species preferred the combinations of long day-dim light or short day-bright light. This finding leads to the question of whether the two dimensions of the light niche in the experimental design can be combined into one: daily light dose (DLD) calculated as the product of light intensity (I) times the length of the light period (L). Theoretically, this should be incorrect be-

cause of the nonlinear response of growth rate to light intensity. Under light saturation, no increase in intensity could compensate for a decrease in photoperiod. There is no simple way to calculate a "corrected light dose" where light intensity is substituted by a value which takes saturation into account (e.g. by truncating all light intensities at the saturation coefficient). Different species have different saturation curves. Therefore, a particular combination of I and L in one culture could result in a different corrected light dose for different species.

In spite of these theoretical objections, even coarser general indices of light (e.g. the ratio of euphotic depth to mixing depths) have made an acceptable practical compromise in describing the light niche of phytoplankton species in situ (e.g. Reynolds and Reynolds 1985). Therefore, I tried to examine the utility of DLD by a multiple regression analysis (stepwise variable selection, backward procedure, F-to-remove 4.0). The arcsin-square-root-transformed p_i values based on equilibrium species composition were entered as the dependent variable. The independent variables were $\log_{10}(\text{Si}:\text{N})$, $\log_{10}\text{DLD}$, and the squares of both (Table 2) or, alternatively, $\log_{10}I$ and $\log_{10}L$ and the squares of both instead of $\log_{10}\text{DLD}$.
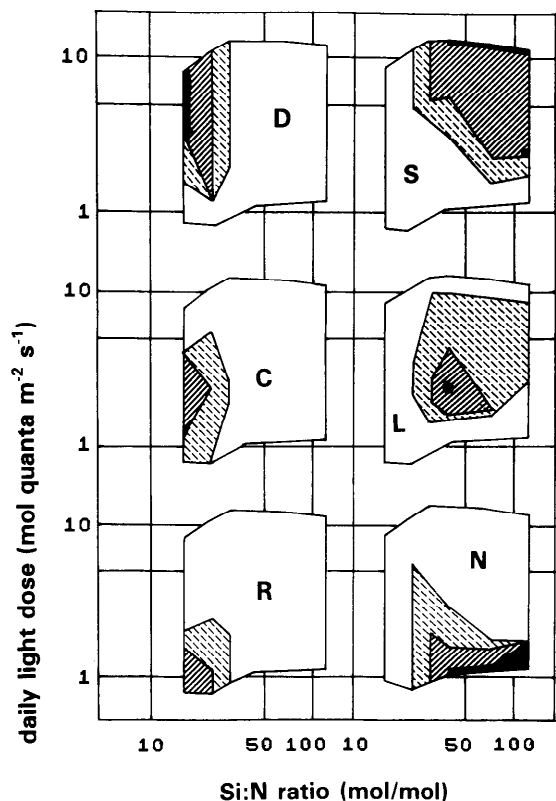
Fig. 8. Contour diagrams of the competitive success of *Dunaliella tertiolecta* (D), *Chrysochromulina polylepis* (C), *Rhodomonas* sp. (R), *Stephanopyxis palmeriana* (S), *Lauderia borealis* (L), and *Nitzschia pungens* at different Si : N ratios and daily light doses; white—excluded; broken hatching—persisting, but <50% of equilibrium cell volume; hatching—>50%; black—100%.

Using light intensity and daylength separately instead of DLD did not significantly improve the goodness-of-fit; r^2-values never increased by more than 0.03.

Having thus established that the use of DLD as a considerable simplification without a significant deterioration of fit, I constructed the contour diagrams in Fig. 8. The contours are based on the original data for equilibrium species composition and not on the regression equations. They are a graphical representation of the competitive niche of each of the six species under discussion with the dimensions Si : N ratio and DLD.

Further experimentation with more species and a wider range of light intensities is needed to test the robustness of the daily light dose concept. A considerable reduction of light cli-

mate complexity would be possible if light variability in the vertical gradient could also be integrated into DLD. On the other hand, I expect that light inhibition at high intensities would strongly restrict the usefulness of DLD.

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