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THE USE OF OUTDOOR PHYTOPLANKTON CONTINUOUS CULTURES TO ANALYSE FACTORS INFLUENCING SPECIES SELECTION

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Abstract: Natural phytoplankton populations have been grown in outdoor continuous cultures at three dilution rates ($D = 0.5$, 0.25, and $0.1 \cdot \text{day}^{-1}$) under nitrogen (N) or silicon (Si) limitation and two light intensities. At a high specific nutrient flux (high dilution rate) under N limitation an assemblage of primarily small, fast growing centric diatoms such as *Skeletonema costatum* (Grev.) Cleve and *Chaetoceros* spp. dominated with a low percentage of flagellates. At a low specific nutrient flux, a mixture of larger, slower growing centric diatoms, small flagellates, and pennate diatoms was obtained. Similar trends were observed under silicate limitation. Decreasing the light intensity at the lowest dilution rate selected for an assemblage similar to that observed at the high dilution rate and high light intensity.

The results of these competition experiments suggest that specific nutrient flux (dilution rate) is an important factor in determining between group dominance (e.g., centric and pennate diatoms and small flagellates). Successful competitors representing broad phytoplankton groups can be arranged along a resource gradient of specific nutrient flux (dilution rate), with groups such as centric and pennate diatoms, represented as high and medium flux species, respectively.

INTRODUCTION

The ability to manipulate a planktonic food web by changing factors such as nutrients and light in controlled experimental ecosystems (CEEs) (Menzel & Case, 1977) has been suggested as an important tool in furthering our understanding of food chain transfer efficiencies (Parsons *et al.*, 1978). Because of the large volume of these enclosures, each experiment is expensive and only a limited number of experiments can be performed. To expand our ability to test the many factors affecting phytoplankton species succession, we conducted small scale, outdoor experiments using 2-l continuous and semi-continuous cultures.

Jannasch (1967) used continuous cultures for selection of specific species of bacteria from natural assemblages by the use of different dilution rates. Since then microbiologists have made extensive use of this technique to study species competition in ecological studies (Veldkamp & Jannasch, 1972; Veldkamp & Kuenen, 1973; Harder *et al.*, 1977).

The use of continuous cultures to study competition in mixed species phytoplankton populations has been confined largely to indoor experiments (Dunstan & Menzel, 1971; Peterson *et al.*, 1974; Goldman, 1977; Tilman, 1977; Mickelson *et al.*, 1979; Mickelson, in press). Large scale outdoor continuous flow cultures have been used by Goldman & Ryther (1976) to study how species succession is affected by sea-water sewage mixtures and temperature. Recently, other studies have used small scale outdoor continuous cultures to study species succession in natural phytoplankton assemblages grown under conditions where temperature and the inflow medium concentration are held constant (Jones *et al.*, 1978; Mickelson, in press; Turpin & Harrison, 1979).

Factors affecting species competition in natural phytoplankton assemblages in small controlled outdoor continuous cultures are not nearly as complex as in natural environments (Goldman & Ryther, 1976). Most factors can be adequately controlled: 1) sinking can be reduced or eliminated by stirring, 2) temperature can be controlled by a cooling–heating unit, 3) grazing by herbivores can be reduced by selecting dilution rates which are higher than the reproductive rates of herbivores and by filtering out large herbivores, and 4) fluctuating nutrient concentrations can be eliminated by fixing the pumping rate of an inflow medium containing a constant nutrient concentration. Light intensity is the most difficult factor to control in outdoor cultures.

In this study cultures were grown outdoors to facilitate comparisons between species succession in these experiments and others conducted in the CEEs. In competition experiments lasting two to three weeks, nitrogen- (N) and silicon- (Si) limiting conditions were imposed on natural phytoplankton populations growing in outdoor continuous or semi-continuous cultures. Results of species selection due to the following treatments are reported: 1) type of nutrient limitation (N or Si) or nutrient ratio, 2) decreased light intensity, and 3) change in dilution rate (specific nutrient flux).

Methods and Materials

An outdoor continuous culture system was used with 2-l, borosilicate, flat-bottomed boiling flasks as reactors. Temperature was maintained at $10.5 \pm 0.5\,^{\circ}C$ in a water bath. The sides, ends, and top of the Plexiglas water bath were covered with blue Plexiglas, 0.32 cm thick (Rohm & Hass No. 2093). This decreased the incident radiation by $\approx 50\%$, from a noon value of 600 ly $\cdot$ day^{-1}, to ≈ 300 ly $\cdot$ day^{-1}. The blue Plexiglas also modifies the spectral distribution (400–700 nm) making it similar to that of light at 5 m underwater (Davis *et al.*, 1973). Incident radiation was measured hourly and daily average values are given in Fig. 1.

The inoculum was a natural phytoplankton assemblage collected from 4 to 8 m in CEE 77-3 on 2nd June, 1977 and filtered through a 143-μm net to remove any

large zooplankton. The inoculum for the Si-limited experiment was collected from the same CEE three weeks later. The inflow medium was prepared by filtering deep water from the CEE through an HA Millipore filter. It was stored in 20-l carboys in the cold and dark until required. Nutrients were added to obtain the desired nutrient ratio for the particular experiment. In the N-limited experiment, the nutrient concentration was 8.8, 3.4, 19.9, and 0.93 μg-at $\cdot$ l^{-1} of nitrate, ammonium, silicate, and phosphate, respectively (13 : 21 : 1, N : Si : P by atoms). Concentrations in the Si-limited inflow medium were 35.1, 3.94, 13.4, and 3.58 μg-at $\cdot$ l^{-1} of nitrate, ammonium, silicate, and phosphate, respectively (11 : 3.7 : 1, N : Si : P by atoms). Initially, nutrients were added to the 2-l cultures to obtain nutrient ratios similar to the inflow medium. The cultures were allowed to grow as batch cultures for one or two days and then continuous or daily additions of the inflow medium were initiated.

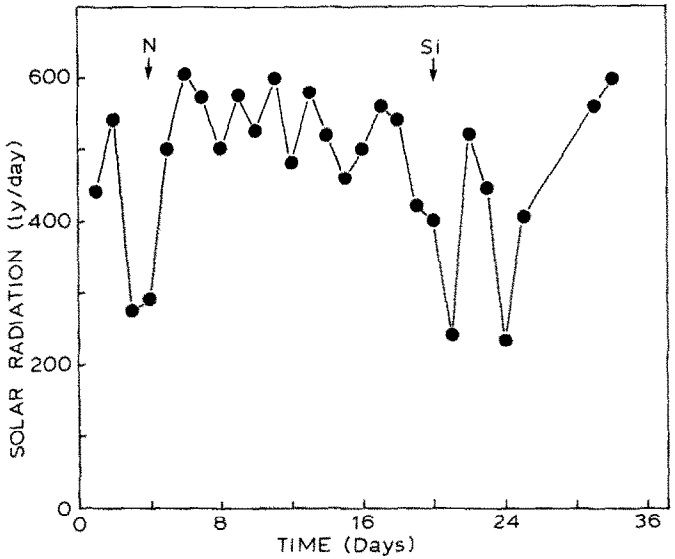

Fig. 1. Total solar radiation (400–700 nm) during the experimental period: arrows indicate the beginning of the nitrogen (N) and silicon (Si) experiments; generally, cultures received 50% of these values (see text for details).

Piston pumps (Fluid Metering Inc.) were used to obtain continuous additions at dilution rates of 0.25 to 0.5 $\cdot$ day^{-1}. The lowest dilution rate ($D = 0.1 \cdot \text{day}^{-1}$) was achieved by making a manual, daily dilution of 10% by volume of the culture at 1300 h. Cultures were gently stirred with a large magnetic stirring bar at 60 rpm (Davis *et al.*, 1973).

Two main experiments were done, one under nitrogen limitation and the other under silicon limitation. Within each experiment three, 2-l cultures were used. They were grown at three different dilution rates (0.5, 0.25, and 0.1 $\cdot$ day^{-1}). In the N-limited experiment a fourth culture was grown at a dilution rate of 0.1 $\cdot$ day^{-1}, but

at 15% incident light intensity (mid-day value was ≈ 90 ly · day^{-1}). This reduction in light intensity was obtained by wrapping the flask with four layers of nylon screening.

Experiments were run for about two or three weeks for high and low dilution rate cultures, respectively. During this period, cultures were sampled daily or every other day and the following measurements were made on the samples: 1) cell numbers and species identification; 2) in vivo fluorescence; and 3) nitrate, ammonium, silicate, and occasionally phosphate. The dilution rate and nutrient concentrations in the inflow medium were also measured.

Cell counts and species identification were made by settling a live sample and counting with an inverted microscope. Samples with numerous small flagellates were preserved in Lugol's solution, settled, and counted. Cell volumes were measured using an eyepiece micrometer and assuming species approximated certain geometrical shapes. In vivo fluorescence was used only to monitor the daily progress of the cultures and it was determined using a fluorometer (Model 111 Turner) with a high sensitivity door. Nutrient analyses were done using a Technicon AutoAnalyzer and by methods described previously (Davis *et al.*, 1973). Particulate nitrogen and silicon were estimated from a nutrient mass balance at steady state (amount of nutrient in cell = inflow–outflow nutrient concentration in the medium).

After several weeks the experiment was terminated and the following measurements were made: 1) cell counts, volumes, and species identification; 2) in vivo fluorescence; and 3) nutrient uptake rates. Nutrient uptake rates were determined using a perturbation technique (Caperon & Meyer, 1972). The experiments were conducted between 11.30 and 15.30 h and for the semi-continuous culture this represented the interval just prior to the normal daily addition. For the determination of the maximal uptake rate, a known amount of the limiting nutrient (nitrate or silicate) was added to a 125 ml subsample to give a concentration of 6 μg-at · l^{-1} and the decrease in the nutrient concentrations was followed for 1 h.

In another flask, the uptake rate at limiting concentrations was determined by adding a smaller amount of the limiting nutrient to a 300 ml subsample of the culture which raised the concentration to 2 μg-at · l^{-1}. Additions of the non-limiting nutrients were also made at the beginning of these experiments to ensure that these nutrients would remain at saturating levels during the experiment. The decline in limiting nutrient was followed until no further decrease in its concentration was observed (2–3 h). Samples were taken every 6–12 min using an automatic sampling valve and were filtered through an in-line HA Millipore filter to remove cells before nutrient analysis using an AutoAnalyzer.

RESULTS

NITROGEN-LIMITED EXPERIMENTS

In each culture a definite species succession was observed and the pattern varied with dilution rate and light intensity (Fig. 2). At the end of the experiment, it was observed that total cell numbers decreased by a factor of 8.5 with decreasing dilution rate, partially due to the dominance of large-celled species at the lower dilution rate (Fig. 3A, B). Recent results by Turpin & Harrison (in prep.) suggest that the selection of these large-celled species at the lowest dilution rate may be due in part to the fact that the limiting nutrient was added semi-continuously (once per day) rather than continuously which was the case with the higher dilution rates.

Leptocylindrus danicus Cleve, was the dominant species in the initial inoculum. One day after pumping was initiated, $\approx 86\%$ of the *L. danicus* population produced resting spores. This event occurred simultaneously in all four reactors and in the CEEs and will be described in a subsequent paper (Davis *et al.*, in prep.). The spores did not germinate in the reactor and subsequently the decrease in their number was proportional to the dilution rate. Some vegetative cells of *L. danicus* remained in all of the reactors and they recovered in the three lower dilution rate reactors, but not at the highest dilution rate of ($0.5 \cdot \text{day}^{-1}$).

Skeletonema costatum (Grev.) Cleve and *Chaetoceros* spp. increased dramatically ($\approx$ 10-fold) in the culture at the highest dilution rate (Fig. 2A) and at the end of the experiment *Skeletonema costatum* clearly became the dominant species, comprising $\approx 74\%$ of the total cell numbers. At the intermediate dilution rate ($D = 0.25 \cdot \text{day}^{-1}$) *S. costatum* was only slightly more dominant than *Chaetoceros* spp., comprising $\approx 28\%$ of the total cell population. The population at $D = 0.25 \cdot \text{day}^{-1}$ had a greater diversity than the 0.5 day^{-1} culture due to an increase in other species such as *Leptocylindrus danicus, Nitzschia delicatissima* Cleve, *Cylindrotheca closterium* Reimann & Lewin (= *Nitzschia closterium* (Ehrenberg) W. Smith), and unidentified, small flagellates. In contrast to the high dilution rate culture, at the lowest dilution rate ($D = 0.1 \cdot \text{day}^{-1}$) *Skeletonema costatum* and *Chaetoceros* spp. were out-competed by such species as *Leptocylindrus danicus* and *Cylindrotheca closterium*. Small flagellates comprised a larger percentage of the total population by numbers at the lower dilution rates because, as noted above, the total cell numbers were lower at the lower dilution rate (Fig. 3C).

Reducing the light intensity from 50 to 15% of incident radiation in the second of two cultures growing at $D = 0.1 \cdot \text{day}^{-1}$, resulted in increased numbers of *Skeletonema costatum* and *Nitzschia delicatissima* and a small decrease in unidentified flagellates. Total cell numbers also increased due to the increase in small-celled species such as *Skeletonema costatum* (Fig. 3A).

At the end of the experiment the ambient nitrate and ammonium concentrations were 0.1 $\mu\text{g-at} \cdot \text{l}^{-1}$ in all cultures, while phosphate and silicate were 0.3 and 2.0 $\mu\text{g-at} \cdot \text{l}^{-1}$, respectively.

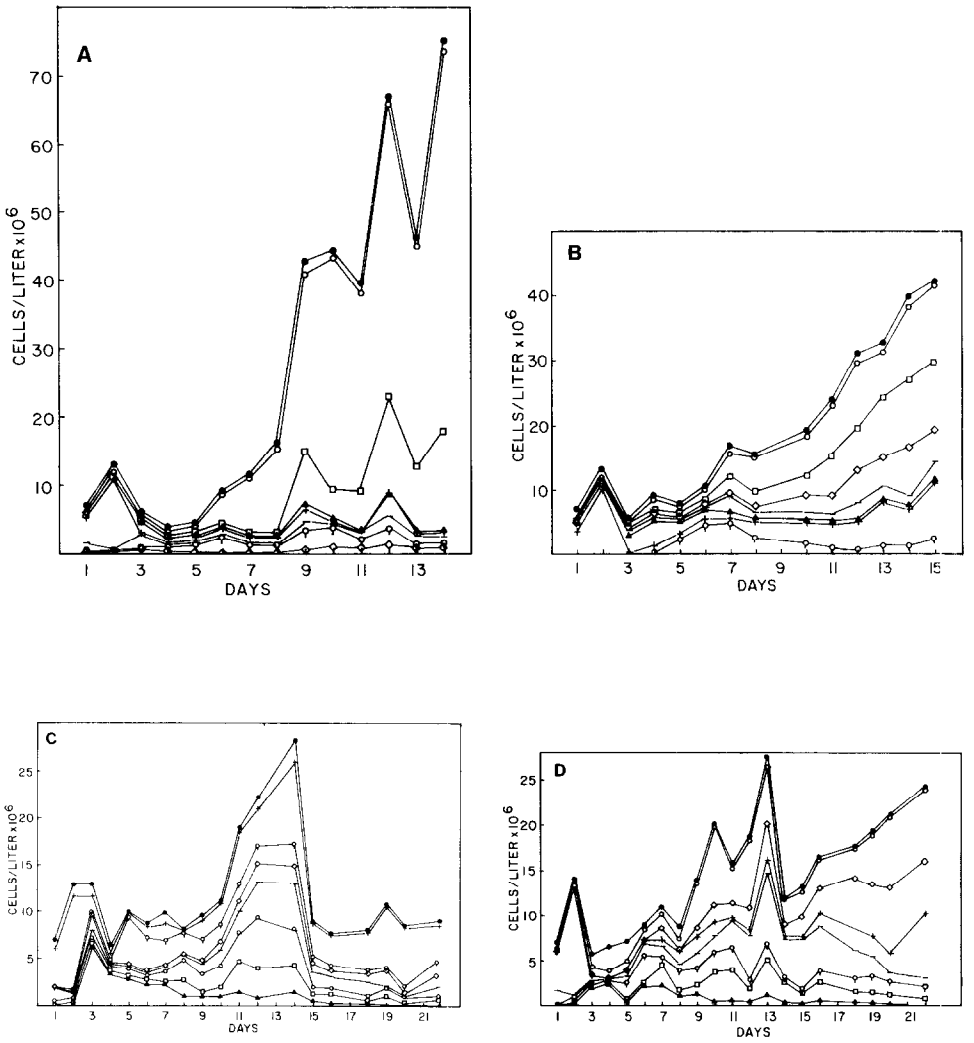

Fig. 2. Species succession of N-limited continuous or semi-continuous cultures with dilution rates (day^{-1}) of 0.5 (A), 0.25 (B), 0.1 (C), and 0.1 and 15% of incident light intensity (D): the cell numbers are given as cumulative values; ◊, *Nitzschia delicatissima*; ♀, total flagellates; –, *Cylindrotheca closterium*; +, *Leptocylindrus danicus*, vegetative cells; ▲, *Leptocylindrus danicus*, resting spores; □, *Chaetoceros* spp.; ○, *Skeletonema costatum*; ●, other species of diatoms or flagellates.

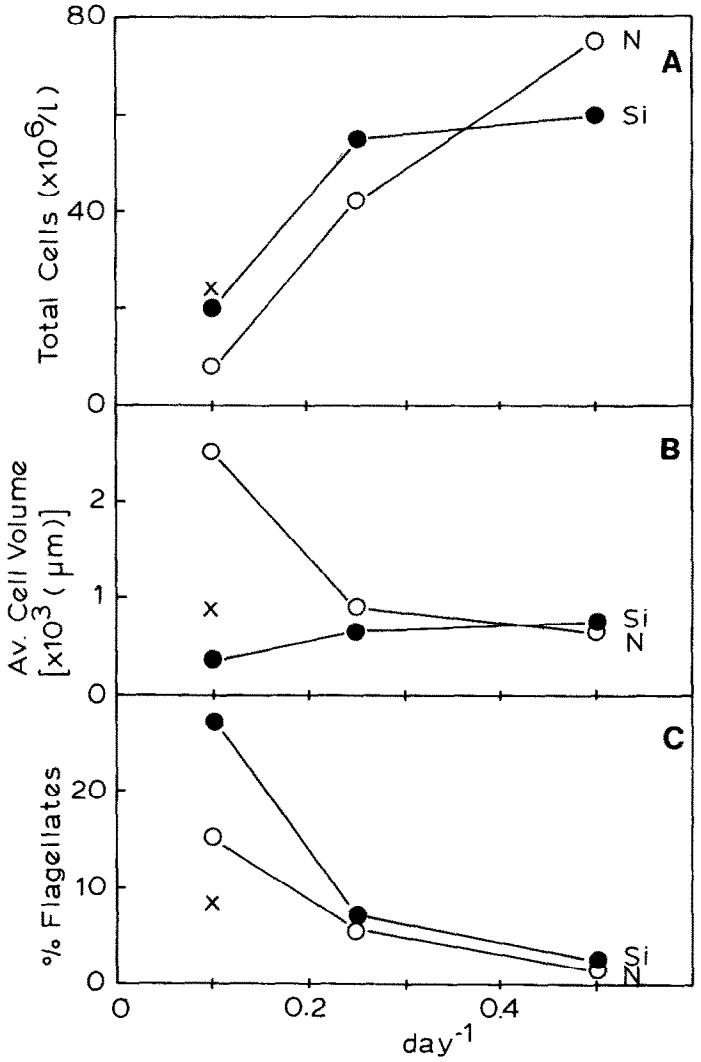

Fig. 3. Total cell numbers (A), average cell volume of 2 or 3 dominant species (B), and percentage flagellates (C), of successful competitors in N- (○) and Si-limited (●) cultures, as a function of dilution rate: X represents the low light N-limited culture (15% of incident radiation) at $D = 0.1 \cdot \text{day}^{-1}$.

The results of the nutrient uptake experiments conducted at the end of the experiment on the mixture of species that were selected by the different treatments, are shown in Fig. 4. The rate at which the populations took up ammonium was proportional to the dilution rate at which they were growing. A decrease in the light intensity increased the rate of nutrient uptake.

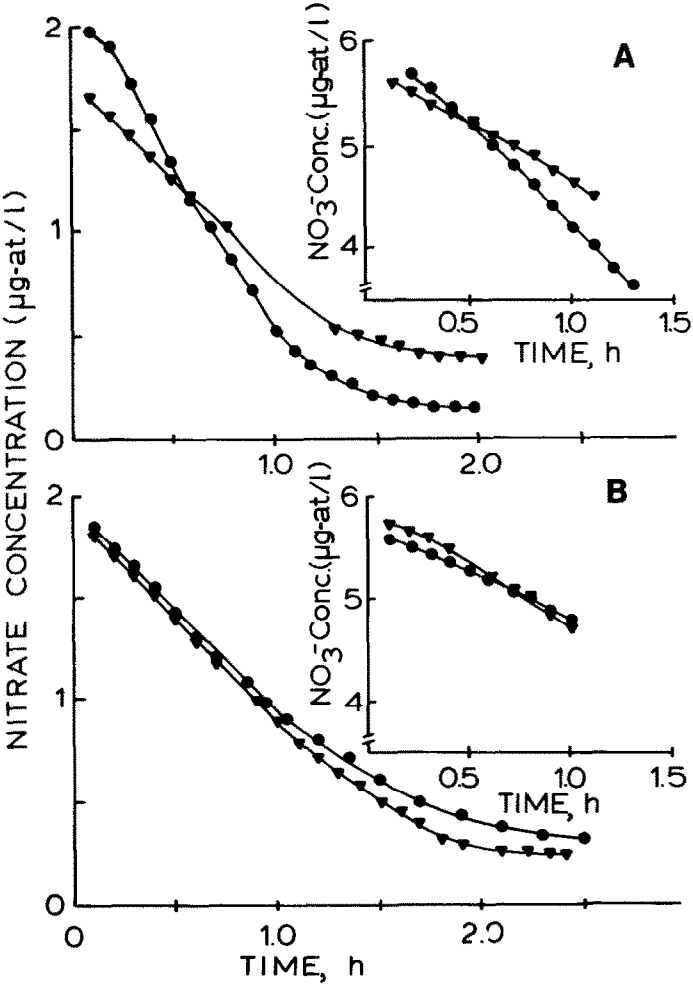

Fig. 4. Disappearance of nitrate during a perturbation experiment on the final populations of four N-limited continuous or semi-continuous cultures, with dilution rates (day^{-1}) of 0.5 (●) and 0.25 (▲) (A), and 0.1 (●) and 0.1 and 15% of incident light intensity (▲) (B): inset figures show rate of nitrate disappearance at higher nitrate concentrations.

SILICATE-LIMITED EXPERIMENTS

The three dilution rates used in the Si-limited experiment resulted in differences in species composition and total cell numbers in the three reactors (Fig. 5). At the end of the experiment, total cell numbers decreased with decreasing dilution rate, but only by a factor of 3 instead of 8.5 which occurred under nitrogen limitation (Fig. 3A). Mean cell volume of the dominant species increased only slightly with increasing dilution rates (Fig. 3B).

In the culture growing at the highest dilution rate ($D = 0.5 \cdot \text{day}^{-1}$), *Chaetoceros*

spp. dominated and comprised ≈ 60% of the total cell numbers (Fig. 5A). *Cylindrotheca closterium* was the second most dominant, making up 24% of the total cell numbers. After an initial increase, *Leptocylindrus danicus* and *Skeletonema costatum* declined to a very low concentration by the end of the experiment.

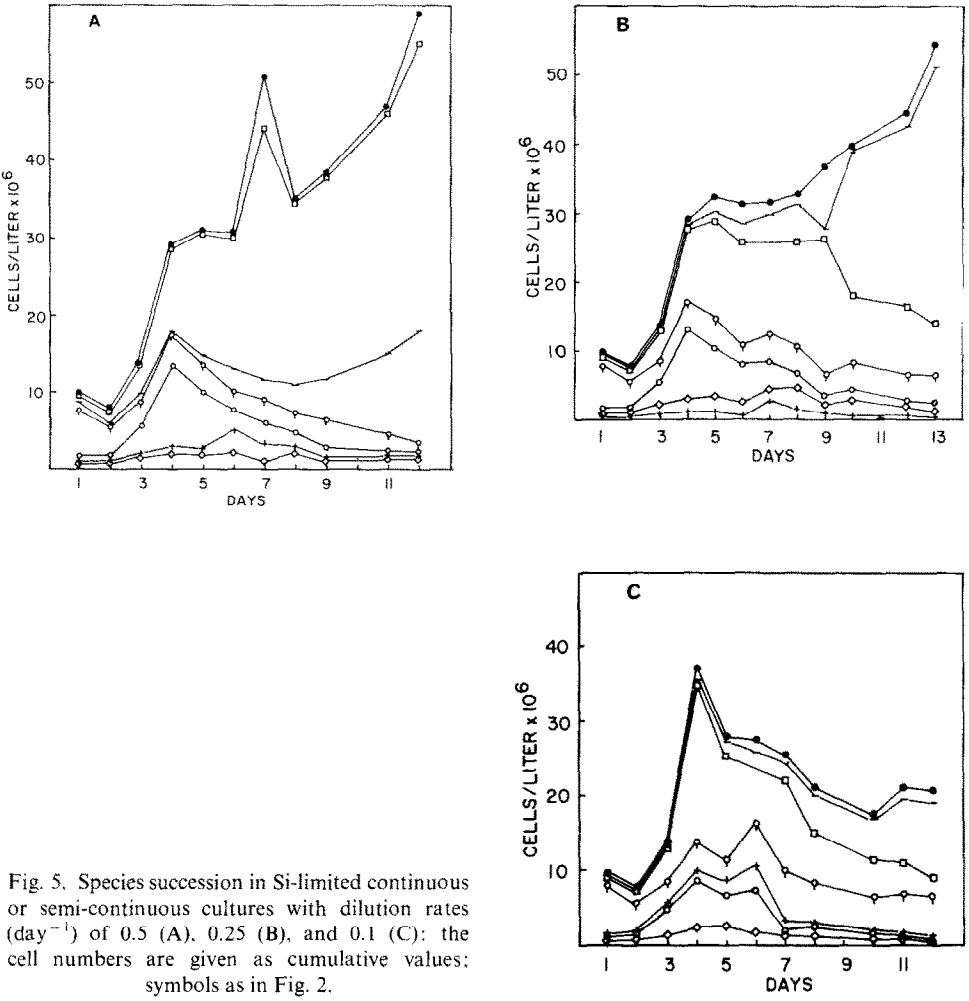

Fig. 5. Species succession in Si-limited continuous or semi-continuous cultures with dilution rates (day^{-1}) of 0.5 (A), 0.25 (B), and 0.1 (C): the cell numbers are given as cumulative values; symbols as in Fig. 2.

The chemostat population growing at the intermediate dilution rate ($D = 0.25 \cdot day^{-1}$) was dominated by *Cylindrotheca closterium* and it made up 69% of the total cell numbers at the end of the experiment (Fig. 5B). It was generally observed to be clumped around dead or nearly dead diatom cells. At this lower dilution rate *Chaetoceros* spp. were reduced to 14% of the total cell population.

At the lowest dilution rate ($D = 0.1 \cdot day^{-1}$), again the population was dominated by *Cylindrotheca closterium* and comprised 50% of the total cell numbers (Fig. 5C). Total flagellates continued to increase during the experiment and at the end of the experiment they were the second most dominant group (27% of the total cell numbers). Diatoms such as *Leptocylindrus danicus*, *Skeletonema costatum* and *Chaetoceros* spp. decreased rapidly as silicate limitation became severe, after six or seven days. *Leptocylindrus danicus* did not form resting spores under silicate limitation as it did under nitrogen limitation.

In cultures grown at three different dilution rates, there was no significant difference in the effluent concentration of NO_3^-, NH_4^+, SiO_4^{4-} and PO_4^{3-} and they were

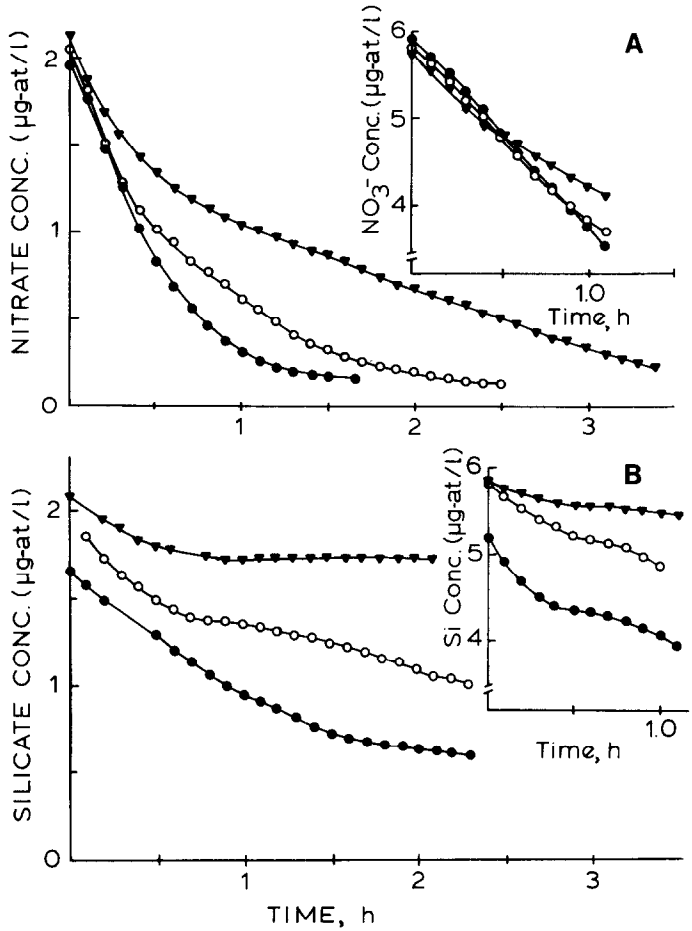

Fig. 6. Nitrate (A) and silicate (B) disappearance during a perturbation experiment on the final populations of three Si-limited continuous or semi-continuous cultures with dilution rates (day^{-1}) of 0.5 (●), 0.25 (○), 0.1 (▼): inset figures show rate of nitrate or silicate disappearance at higher concentrations.

0.1, 0.1, 0.4, and 0.3 μg-at $\cdot l^{-1}$, respectively. This suggests that possibly the diatoms were Si-limited while the other species (e.g., small flagellates) were N-limited.

Both nitrogen and silicon uptake rates were measured on the final assemblages (Fig. 6A and B). The rate of nitrogen uptake decreased with decreasing dilution rate. An S_0 value (the substrate concentration where $V = 0$) of 0.2 μg-at N $\cdot l^{-1}$ was found for all three cultures. In these same populations, the pattern of silicate uptake was different from that found for nitrate (Fig. 6B). For the two higher dilution rate cultures, uptake rates were high for the first 10–30 min and then the rate decreased; the S_0 value was highest for the 0.25 day^{-1} dilution rate culture. For the lowest dilution rate culture ($D = 0.1 \cdot$ day^{-1}) there was little uptake of silicate.

Discussion

There was selection in the natural phytoplankton assemblages in this study due to differences in the dilution rate or specific nutrient flux through the culture. At a high specific nutrient flux under nitrogen limitation, an assemblage of small, fast growing centric diatoms such as *Skeletonema costatum* and *Chaetoceros* spp. dominated with a low percentage of flagellates. At a low specific nutrient flux, a mixture of larger, slower growing centric diatoms, pennate diatoms, and flagellates was obtained. Similar general trends were observed under silicate limitation, but a notable exception was the absence of large centric diatoms at the low specific flux rate. From the results of these competition experiments (Table I), successful phytoplankton groups (e.g. diatoms, flagellates, etc.) can be arranged along a resource gradient represented by the specific nutrient flux (see Fig. 10 in Turpin & Harrison, 1979).

Decreasing the light intensity at the lowest dilution rate selected for *Skeletonema costatum* which was also selected for at a high dilution rate and a high light intensity. This observation suggests that the reduction in light intensity reduced the maximal growth rate (i.e., reduced nutrient demand) and therefore lessened the effect of nutrient limitation at that particular dilution rate. The arrangement of successful species along a gradient of specific nutrient flux, is dependent on light intensity with the succession pattern response to low light intensity being similar to an increase in the specific flux from $D = 0.1$ to 0.25 day^{-1} (Fig. 2B & D; Table I).

Although the experiments in this study were not replicated, the general trend in species succession observed here, agrees with other similar experiments conducted recently (Turpin & Harrison, 1979) and with other workers running indoor mixed species chemostats. Dunstan & Menzel (1971) observed *S. costatum* to dominate most frequently ($D = 0.5$ to $1.0 \cdot$ day^{-1} at 16 °C), while Mickelson (in press) observed dominance by *S. costatum*, *Chaetoceros septentrionale* and *Nitzschia delicatissima* at a high dilution rate ($D = 1.13 \cdot$ day^{-1} at 18 °C), and dominance by *N. delicatissima*, *Leptocylindrus*, *Cylindrotheca closterium* and a small-celled *Chaetoceros sociale*, at low dilution rates ($D = 0.2 \cdot$ day^{-1} at 18 °C). Jones *et al.* (1978)

found large centric diatoms (e.g., *Leptocylindrus danicus*) with pennates and small flagellates in their low dilution rate continuous culture ($D = 0.2 \cdot \text{day}^{-1}$ at 10 °C). After 30 days the diversity of their population declined and the culture was dominated by pennates such as *Nitzschia* and *Phaeodactylum*.

TABLE I

The percentage dominance (% of total number of cells) of three phytoplankton groups (C = centric diatoms, P = pennate diatoms, and F = flagellates) in natural phytoplankton populations, grown in outdoor continuous cultures at three dilution rates under N- or Si-limitation, and two light intensities: data on the temporal distribution of the limiting nutrient (patchiness) are from Turpin & Harrison (1979).

Conditions		Phytoplankton groups	Dilution rate or specific nutrient flux (day^{-1}) 0.1	0.25	0.5
N : Si = 0.6 : 1		C	56	75	95
High light		P	26	19	2
		F	16	6	1
N : Si = 3 : 1		C	8	18	64
High light		P	55	70	26
		F	27	7	3
N : Si = 0.6 : 1		C	65		
Low light		P	29		
		F	3		
N : Si = 0.2 : 1 High light & limiting nutrient patchiness	Homogeneous	C		61	
		P		18	
		F		16	
	High frequency patchiness	C		75	
		P		17	
		F		5	
	Low frequency patchiness	C		87	
		P		6	
		F		4	

Nutrient ratios are known to be important in explaining species competition and coexistence (Tilman, 1977; Rhee, 1978). A large range in the N : Si ratio, 0.6 : 1 to 3 : 1 (by atoms) was deliberately chosen in order to observe species selection under nitrogen- and silicate-limiting conditions, respectively. Even though the initial inoculum for N- and Si-limited experiments was collected at different times, the dominant species were similar in both inocula, with only the ratios of the species differing. Changing the N/Si ratio appeared to determine within group dominance at a high dilution rate and between group dominance (centric compared with pennate diatoms) at a lower dilution rate (Table I). For example, within the centric diatoms at a high dilution rate, *Skeletonema costatum* dominated under nitrogen li-

mitation (N : Si = 0.6 : 1) and *Chaetoceros* spp. dominated under silicate limitation (N : Si = 3 : 1). The switch from *Skeletonema costatum* dominance under ammonium limitation to *Chaetoceros* spp. dominance under silicate limitation is supported by laboratory nutrient uptake experiments. Si-limited *C. debilis* took up silicate at a higher rate than Si-limited *Skeletonema costatum*, while under nitrogen limitation *S. costatum* was more efficient at taking up nitrogen than *Chaetoceros debilis* (Conway & Harrison, 1977).

Results from this study demonstrate the ephemeral nature of silicate limitation. With the ratios employed (N : Si = 3 : 1), silicate was the first nutrient to become limiting, while saturating concentrations of ambient nitrogen and phosphorus remained. The non-diatom component (e.g., small flagellates) and weakly silicified diatoms such as *Cylindrotheca closterium*, however, soon increased, possibly driving the culture into dual limitation, with the diatom component remaining Si-limited and the non-diatom component becoming N-limited. If further increases were to be made in the amount of N and P added, this would probably result in an increase in the ratio of non-diatoms/diatoms. As long as some silicate is supplied at least a small diatom population will, however, persist and utilize that supply.

Uptake kinetics were not calculated from the nutrient disappearance data because of the problems noted by Harrison *et al.* (in prep.). The data do show that the rate of nutrient disappearance and the specific uptake rate (since particulate nitrogen or silicon remained constant with dilution rate) decreased with decreasing dilution rate, suggesting that high dilution rates selected for faster growing species, which is the expected result from classical chemostat theory.

No significant difference was observed in the effluent concentration of the limiting nutrient (N or Si) and this concentration was near the limit of detection. It appears that competition for the limiting nutrient by marine species in steady state growth competition experiments (represented by crossing μ vs S curves) is frequently below the limit of detection (Caperon & Meyer, 1972; Mickelson *et al.*, 1979) particularly for ammonium limitation.

To explain the reason for the dominance of a particular species obtained in mixed species competition experiments, the dominant species occurring at the end of the experiment should be isolated and a μ vs S curve obtained under controlled laboratory conditions (Harder *et al.*, 1977). Since many marine species can, however, grow near maximal growth rates at nearly undetectable levels of the limiting nutrient (Paasche, 1975; Goldman & McCarthy, 1978; Steemann Nielsen, 1978), this approach does not appear to be fruitful. The results of this study also suggest that some species may not compete for the limiting nutrient in the classical manner. For example, the dominance of the diatom *C. closterium* even under severe silicate limitation may be due to two unique characteristics of species in this genera: 1) a weakly silicified frustle, with each valve consisting of silicified strips and large unsilicified areas (Reimann *et al.*, 1965), and 2) an ability to form clumps around dead or dying cells (mostly diatoms), giving it the advantage of being close to a regenerating source

of N, Si, P, dissolved organics, etc. It is suspected that if growth kinetics for this species were determined under these unusual conditions, the results would have been misleading. In fact, this observation may explain why little silicate uptake was observed in the nutrient uptake experiment for the low dilution rate Si-limited culture in which *C. closterium* dominated.

The residual external substrate concentration may best be viewed as a dependent variable which at steady state is in equilibrium with supply rate and uptake rate, i.e. $S = f(\mu)$ rather than $\mu = f(S)$, (see discussion by Nyholm in Kilham, 1978). Even though it is easier to measure S in the natural environment than to estimate the supply rate, the effect of the supply rate on species succession in natural populations can be carefully studied in controlled outdoor continuous cultures where the supply rate or specific nutrient flux is set by the dilution rate.

Our results from the competition experiments suggest that successful competitors representing broad phytoplankton groups can be arranged along a resource gradient of specific nutrient flux (see Fig. 10, Turpin & Harrison, 1979) similar to the arrangement of species along a nutrient ratio gradient, as demonstrated by Tilman (1977). An evaluation of other factors (Table I) in this study (light intensity, nutrient ratio) and in another recent study (temporal distribution of the limiting nutrient, Turpin & Harrison, 1979), suggest that specific nutrient flux, nutrient ratio, and light intensity are important in determining between group dominance, while the temporal distribution of the limiting nutrient appears to determine within group dominance (i.e., species within centric diatoms, etc.).

Acknowledgements

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References

Caperon, J. & J. Meyer, 1972. Nitrogen-limited growth of marine phytoplankton. II. Uptake kinetics and their role in nutrient-limited growth of phytoplankton. *Deep-Sea Res.*, Vol. 19, pp. 619–632.

Conway, H. L. & P. J. Harrison, 1977. Marine diatoms grown in chemostats under silicate or ammonium limitation. IV. Transient response of *Chaetoceros debilis*, *Skeletonema costatum* and *Thalassiosira gravida* to a single addition of the limiting nutrient. *Mar. Biol.*, Vol. 43, pp. 33–43.

Conway, H. L., P. J. Harrison & C. O. Davis, 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation. II. Transient response of *Skeletonema costatum* to a single addition of a limiting nutrient. *Mar. Biol.*, Vol. 35, pp. 187–199.

DAVIS, C. O., P. J. HARRISON & R. C. DUGDALE, 1973. Continuous culture of marine diatoms under silicate limitation. I. Synchronized life cycle of *Skeletonema costatum*. *J. Phycol.*, Vol. 9, pp. 175–180.

DUNSTAN, W. M. & D. W. MENZEL, 1971. Continuous cultures of natural populations of phytoplankton in dilute treated sewage effluent. *Limnol. Oceanogr.*, Vol. 16, pp. 623–632.

GOLDMAN, J. C., 1977. Biomass production in mass cultures of marine phytoplankton at varying temperatures. *J. exp. mar. Biol. Ecol.*, Vol. 27, pp. 161–169.

GOLDMAN, J. C. & J. J. MCCARTHY, 1978. Steady state growth and ammonium uptake of a fast-growing marine diatom. *Limnol. Oceanogr.*, Vol. 23, pp. 695–703.

GOLDMAN, J. C. & J. H. RYTHER, 1976. Temperature influenced species competition in mass cultures of marine phytoplankton. *Biotechnol. Bioeng.*, Vol. 18, pp. 1125–1144.

HARDER, W., J. G. KUENEN & A. MATIN, 1977. A review: microbial selection in continuous culture. *J. appl. Bact.*, Vol. 43, pp. 1–24.

JANNASCH, H. W., 1967. Enrichment of aquatic bacteria in continuous culture. *Arch. Mikrobiol.*, Vol. 59, pp. 165–173.

JONES, K. J., P. TETT, A. C. WALLIS & B. J. B. WOOD, 1978. The use of small, continuous multispecies cultures to investigate the ecology of phytoplankton in a Scottish sea-loch. *Mitt. int. Verein. theor. angew. Limnol.*, Vol. 21, pp. 398–412.

KILHAM, S. S., 1978. Nutrient kinetics of freshwater planktonic algae using batch and semicontinuous methods. *Mitt int. Verein. theor. angew. Limnol.*, Vol. 21, pp. 147–157.

MENZEL, D. W. & J. CASE, 1977. Controlled ecosystem pollution experiment: concept and design. *Bull. mar. Sci.*, Vol. 27, pp. 1–7.

MICKELSON, M. J., in press. Marine phytoplankton ecology: test of a nutrient based hypothesis using continuous cultures. *Proc. int. Symp. Cont. Cultures*, Prague, Czechoslovakia.

MICKELSON, M. J., H. MASKE & R. C. DUGDALE, 1979. Nutrient determined dominance in multispecies chemostat cultures of diatoms. *Limnol. Oceanogr.*, Vol. 24, pp. 298–315.

PAASCHE, E., 1975. Growth of the plankton diatom *Thalassiosira nordenskioeldii* Cleve at low silicate concentrations. *J. exp. mar. Biol. Ecol.*, Vol. 18, pp. 173–183.

PARSONS, T. R., P. J. HARRISON & R. WATERS, 1978. An experimental simulation of changes in diatom and flagellate blooms. *J. exp. mar. Biol. Ecol.*, Vol. 32, pp. 285–294.

PETERSON, B. J., J. P. BARLOW & A. E. SAVAGE, 1974. The physiological state with respect to phosphorus of Cayuga Lake phytoplankton. *Limnol. Oceanogr.*, Vol. 19, pp. 396–408.

RIEMANN, B. E. F., J. C. LEWIN & B. E. VOLCANI, 1965. Studies on the biochemistry and fine structure of silica shell formation in diatoms. I. The structure of the cell wall of *Cylindrotheca fusiformis* Reimann and Lewin. *J. Cell Biol.*, Vol. 24, pp. 39–55.

RHEE, G-Y., 1978. Effects of N : P atomic ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake. *Limnol. Oceanogr.*, Vol. 23, pp. 10–25.

STEEMANN NIELSEN, E., 1978. Growth of plankton algae as a function of nitrogen concentration, measured by means of a batch technique. *Mar. Biol.*, Vol. 46, pp. 185–189.

TILMAN, D., 1977. Resource competition between planktonic algae: an experimental and theoretical approach. *Ecology*, Vol. 58, pp. 338–348.

TURPIN, D. H. & P. J. HARRISON, 1979. Limiting nutrient patchiness and its rôle in phytoplankton ecology. *J. exp. mar. Biol. Ecol.*, Vol. 39, pp. 151–166.

VELDKAMP, H. & H. W. JANNASCH, 1972. Mixed culture studies with a chemostat. *J. appl. Chem. Biotechnol.*, Vol. 22, pp. 105–123.

VELDKAMP, H. & J. G. KUENEN, 1973. The chemostat as a model system for ecological studies. *Bull. Ecol. Res. Comm.* (Stockholm), Vol. 17, pp. 347–355.