ALGAL NUTRIENT COMPETITION IN CONTINUOUS CULTURE

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INTRODUCTION

Autotrophic planktonic algae require essentially the same mineral nutrients. Depletion of dissolved phosphate, but also of silicate and nitrate, is a common phenomenon in stratified lakes during the summer. Under such conditions the ability to grow efficiently at low nutrient concentrations becomes critical for competitive superiority of algal species. Steady-state relationships between growth rates and limiting nutrient concentrations are generally described by a Michaelis - Menten type of equation (MONOD, 1950)

$$\mu = \mu_{max} \cdot \frac{S}{S + k_s}$$

where μ is the growth rate, μ_{max} the maximum growth rate, S the concentration of the limiting nutrient, k_s the concentration at which half of the maximum growth rate can be achieved ('half saturation constant'). Increased competition for a single nutrient should lead to exclusion of all species but one ('principle of competitive exclusion', HARDIN (1960)).

Under the condition of continuous resupply of nutrients and elimination of cells, as in chemostat culture, competitive superiority of species 1 over species 2 is defined by the following conditions :

$$\frac{k_{s1}}{\mu_{max1} - D} < \frac{k_{s2}}{\mu_{max2} - D}$$

where D is the dilution rate of the continuous culture. TILMAN (1977) demonstrated by two-species-experiments that coexistence in steady-state equilibrium is possible, when the different species are limited by different nutrients. The result of interspecific competition could be predicted from the Michaelis - Menten parameters of the species and the nutrient ratio between Si and P. Tilman proposed his findings as an 'equilibrium' solution of HUTCHINSON's (1961) paradox of the plankton (numerous species of phytoplankton coexist in even small volumes of water, although they compete for the same resources). Alternative explanations have emphasized that competitive exclusion is not achieved, because environmental conditions that favour one species do not persist long enough and spatial heterogeneity in plankton distribution protects species inferior in competition ('disequilibrium hypothesis', RICHERSON et al., 1970).

In this study it was attempted to extend TILMAN's approach from a two species system to natural assemblages of phytoplankton. Phosphate and silicate were chosen as potentially limiting nutrients, because they are the only macronutrients which are depleted down to limiting levels in the course of the vegetation period in Lake Constance (MOHAMMED and MÜLLER, 1981; SOMMER, 1981). The following questions were asked. Can competitive exclusion be achieved, when the inoculum consists of numerous species ? Is the result of competition experiments predictable at the species level or only at a more general level (diatoms, green algae, etc.) ? Is the result independent of the inoculum ? Can the results be compared to real lake situations ?

METHODS

Experiments were carried out at a dilution rate of 0.3 d^{-1} in a 20 l chemostat. The nutrient solution was Chu No. 12 as modified by MÜLLER (1972) with all nutrients but phosphate and silicate in excess. The concentration of phosphate was 75 μg $P.l^{-1}$, whereas the silicate concentrations varied among experiments. The temperature was 18 °C and the surface light intensity 1.6×10^{16} quanta. $cm^{-2}.s^{-1}$ PAR (Photosynthetic Active Radiation). Tests with twice and half this light intensity indicated light saturation. pH was regulated at 7.0 by addition of CO_2 gas in order to prevent CO_2 limitation. Samples for cell counts and dissolved nutrient analysis were taken daily. 400 individuals of each species were counted, thus giving a counting accuracy of approximately 10 %. Algal biomass was estimated as cell volume, for which 50 individuals of each species were measured. The detection level for silicate was 40 μg $Si.l^{-1}$ and for phosphate 1 μg $P.l^{-1}$. The degree of nutrient depletion was defined as

$$d = \frac{S_0 - S_1}{S_0} \times 100 \, [\%]$$

where S_0 is the concentration of the nutrient in the influx and S_1 the concentration of the dissolved nutrient in the culture vessel. The experiments were continued until competitive equilibrium was achieved. This was defined as the moment when the final winner or winning combination of species comprises at least 95 % of the total biomass and all other remaining populations decrease continuously for more than two flowthrough periods (6 2/3 days).

Molar P : Si ratios were 1 : 4, 1 : 10, 1 : 20, 1 : 30, 1 : 40, 1 : 80. Every nutrient ratio was used twice for experiments. Inocula were 10 l of natural water from Lake Constance. For the duplicate runs inocula differing as much as possible in species composition were chosen.

RESULTS

The time course of experiments.
Three consecutive phases could be distinguished in all experiments (Fig. 1). During the first 3 to 7 days (phase I) there was a mass development of algae that were already either abundant in the inoculum or just about to develop a bloom in the lake. The growth rates of all algae, except naked flagellates, were close to values for μ_{max} reported in the literature. A considerable fraction of nutrients remained in the dissolved phase and the total algal biomass was markedly below the equilibrium level.

During phase II the algal biomass was already at or near the equilibrium level but the nutrient consumption had not yet reached its final level. There were consecutive mass developments of several species with no regular pattern. As far as these algae were important in Lake Constance, they were characteristic for periods with concentration of dissolved PO_4- P of above 10 $\mu g.l^{-1}$ (Table 1). All dominant species of this phase were r-strategists sensu KILHAM and KILHAM (1980), with small cells, quickly growing and highly nutrient demanding. Growth rates were markedly below their maximum. Phase III was characterized by an asymptotic approach to the equilibrium. All species except the final winners declined in population size.

The final equilibrium.
Only two diatoms (Synedra acus, Asterionella formosa) and two green algae (Mougeotia thylespora, Koliella spiculiformis) were successful competitors in the steady-state phase (Fig. 2). At the P : Si ratio of 1 : 80, none of the species was limited by silicate, since about half of the silicate remained unconsumed in the dissolved pool, and Synedra acus turned out to be the most successful competitor for phosphate. At the
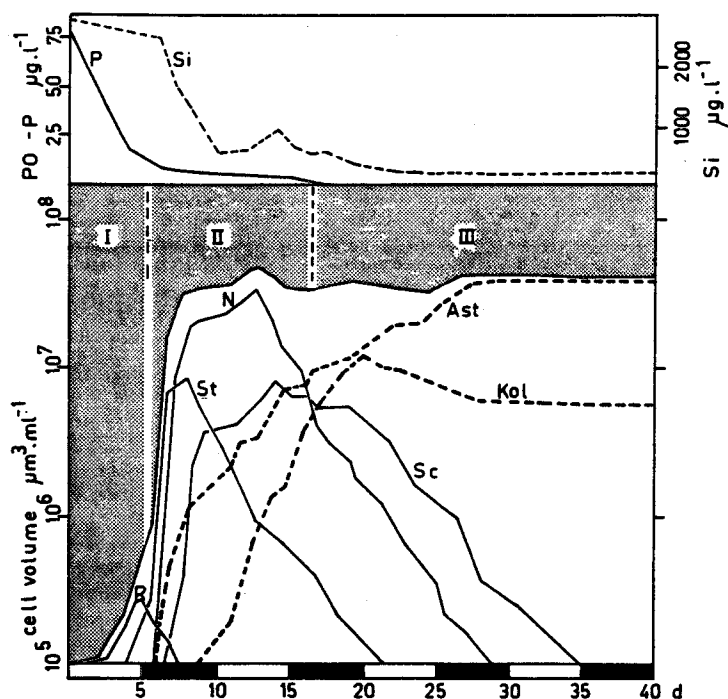
Fig. 1. Example of the temporal evolution of the competition experiments (P : Si = 1 : 40). Upper section: concentration of dissolved P and Si in the culture suspension ($\mu g.l^{-1}$). Lower section: total algal biomass and biomass of dominant species ($\mu m^3.ml^{-1}$, log scale). R : *Rhodomonas minuta*; St : *Stephanodiscus hantzschii*; N : *Nitzschia actinastroides*; Sc : *Scenedesmus communis*; Ast : *Asterionella formosa*; Kol : *Koliella spiculiformis*.

P : Si ratio of 1 : 4 the concentration of silicate was too low, the silicate remained unconsumed and the steady-state assemblage was a monoculture of *Mougeotia thylespora*. At the intermediate P : Si ratios both nutrients were probably limiting as indicated by more than 95 % depletion. As expected in most cases there was a two species coexistence with increasing proportions of diatoms with increasing silicate concentrations. Only at the P : Si ratio of 1 : 30 three species seemed to coexist. Possibly competitive exclusion between *A.formosa* and *S.acus* was undetectably slow.

DISCUSSION

Predictability of results and independence of inoculum.

Competitive exclusion and coexistence of a few species limited by different resources were in agreement with theory. In all cases the duplicate experiments yielded the same equilibrium species composition, independent of the abundance of species in the inoculum. Even extreme rareness (= undetectability) of a species did not prevent it from becoming dominant, only the time required for competitive exclusion varied considerably in dependance on the inoculum. At the P : Si ratio of 1 : 40 the final *A.formosa - K.spiculiformis* association stabilized within 3 weeks, when *A.formosa* had comprised 65 % of the total biomass in the inoculum. But it took 6 weeks, when *A.formosa* had been present in the inoculum in undetectably low numbers. The only result that seems to be inconsistent with the Monod model

Table 1. Dominant algae in competition experiments; 1 algae dominating total phytoplankton biomass at least once during seasonal succession in Lake Constance; 2 algae with least 5 % of total biomass once during succession; $^+$algae capable of growth in the lake under moderate P-depletion (<5µg PO_4 - P.l.$^{-1}$); $^{++}$ algae capable of growth under strong P-depletion (<2µg PO_4 - P.l^{-1}).

molar P : Si ratio	PHASE I	PHASE II	PHASE III
1 : 4	1 Pandorina morum 1 Cryptomonas ovata 1 Rhodomonas minuta	2 Chlorella sp. Scenedesmus communis 2 Monoraphidium contortum	1 Mougeotia thylespora $^+$
1 : 10	1 Pandorina morum 1 Cryptomonas ovata		Koliella spiculiformis $^+$ (70 %) 2 Synedra acus $^{++}$ (30 %)
1 : 20	1 Rhodomonas minuta 1 Stephanodiscus hantzschii	2 Chlorella sp. Scenedesmus communis	2 Synedra acus $^{++}$ (75 %) Koliella spiculiformis $^+$ (25 %)
1 : 30	1 S.astraea 1 S.binderanus $^{++}$ 1 Asterionella formosa $^{++}$ 1 Fragilaria crotonensis $^{++}$	2 Monoraphidium contortum 1 Stephanodiscus hantzschii Nitzschia actinastroides	2 Synedra acus $^{++}$ (35 - 40 %) 1 Asterionella formosa $^{++}$ (35 - 40 %) Koliella spiculiformis $^+$ (25 %)
1 : 40			1 Asterionella formosa $^{++}$ (92 %) Koliella spiculiformis $^+$ (8 %)
1 : 80			2 Synedra acus $^{++}$

75 ──────────────────── P
Si input $\mu g.l^{-1}$

| 270 | 675 | 1350 | 2025 | 2700 | 5400 |

| 95 | 96 | 97 | 99 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | P depletion % |
| 7 | 11 | 95 | 95 | 97 | 98 | 96 | 96 | 94 | 95 | 52 | 50 | Si depletion % |

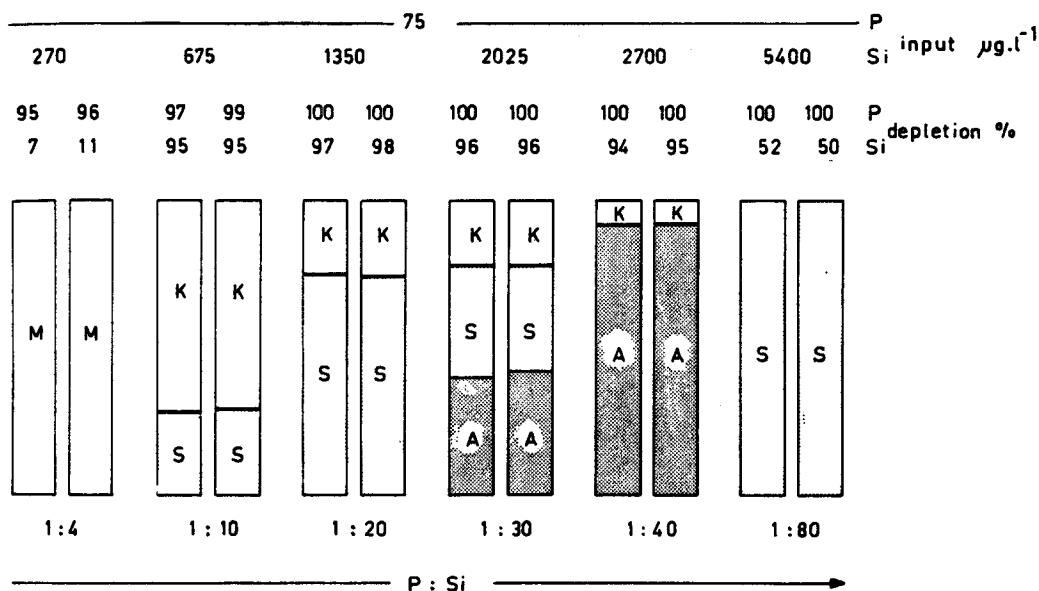1:4   1:10   1:20   1:30   1:40   1:80

P : Si ──────────────────────►

Fig. 2. The final equilibrium in competition experiments. Upper lines: input concentration ($\mu g.l^{-1}$) and degree of depletion (%) of P and Si. Bars: species composition in terms of biomass; unshaded : green algae; shaded : diatoms; M : *Mougeotia thylespora*; K : *Koliella spiculiformis*; S : *Synedra acus*; A : *Asterionella formosa*.

was the superiority of *S.acus* over *A. formosa,* both at the highest and the lower silicate concentrations, whereas *A. formosa* was successful at the intermediate P : Si ratios.

At the P : Si ratio of 1 : 80 silicate was an excess factor for all species, while at the lower silicate concentrations *A. formosa* and *S.acus* had to compete for silicate. According to the MONOD model only one species should be the successful competitor for silicate in all experiments with the same dilution rate.

At the moment only a hypothetical explanation for the observed phenomenon is possible. It has been observed both in the filed (LUND, 1950; SOMMER and STABEL, 1983) and in cultures (MOED, 1973) that severe silicate limitation leads to increased mortality of *A. formosa.* If *S.acus* has a higher k_s for silicate-limited growth but is less affected by mortality at low silicate concentrations than *A. formosa,* than the observed pattern of the competition results can be explained.

Comparability with real lake situations.
Growth of Cryptophyceae, Chlorophyceae and small centric diatoms in Lake Constance is generally associated with concentrations of soluble reactive phosphorus of more than 10 $\mu g.l^{-1}$, whereas pennate and filamentous centric diatoms are capable of mass growth at phosphorus concentrations below detection level, as long as silicate concentrations are sufficient. The onset of phosphorus depletion, generally occurring in July, is regularly followed by a shift from a Chlorophyceae - Chryptophyceae plankton to a diatom plankton (Fig. 3). Shifts in species composition of natural phytoplankton are not only controlled by growth factors but also by loss factors such as grazing, sedimentation, cell death and parasitism. The chemostat imposes a uniform loss rate (= dilution rate) on all species, therefore loss resistence has no selective importance in the experiments and the competition for growth factors (in our case nutrients) can be studied seperately.
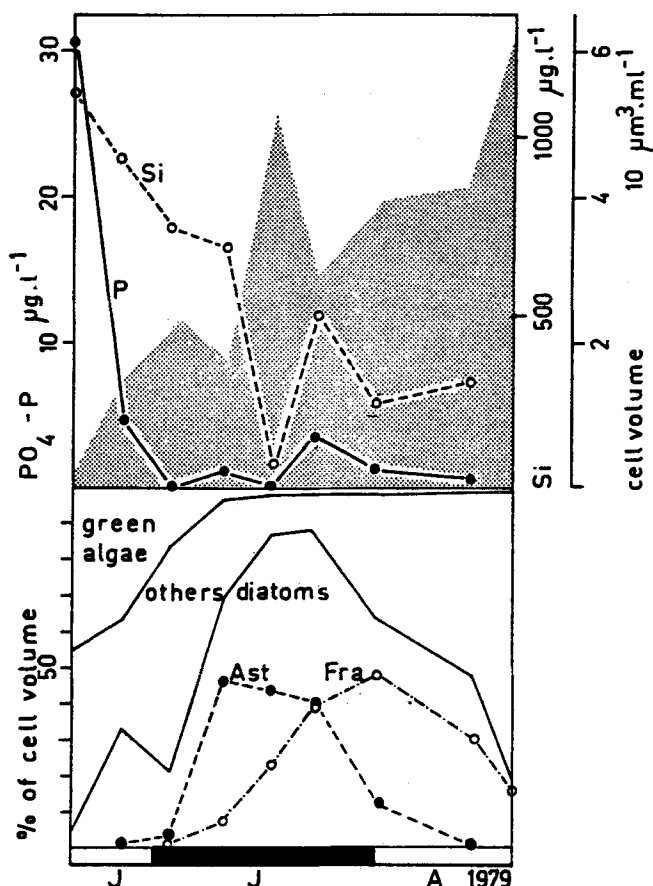
Fig. 3: Early summer transition from a Chryptophyceae - Chlorophyceae to a diatom phytoplankton in Lake Constance. Upper section: concentration of dissolved P and Si (μg.l^{-1}, average of the euphotic zone); shaded area: total algal biomass ($10^3\mu m^3$.ml^{-1}, average of the euphotic zone). Lower section: composition of algal biomass (% of cell volume); Ast: *Asterionella formosa;* Fra: *Fragilaria crotonensis.*

On the other hand species sensitive to mechanical stress (stirring of the cultures) are discriminated in the experiments. Consequently the chemostat can only provide a model system for nutrient competition, but it cannot be expected that the same species are as successful as in the lake. Among the successful competitors in the experiments reported here, *A.formosa* and *M.thylespora* are dominant species in the lake plankton, *S.acus* is a subdominant species in diatom associations and *K.spiculiformis* is a rare, but regularly occurring species in Chryptophyceae - Chlorophyceae associations.

The most serious limitation for the application of steady-state models to the species succession of natural phytoplankton is the long time required to obtain competitive equilibrium. It is emphasized that the relatively low dilution rate of 0.3 d^{-1} is in the same range as the elimination rates of algal species of Lake Constance summer plankton after their maximum abundance (from 0.15 d^{-1} for *Ceratium hirundinella* to 0.45 d^{-1} for *Aphanizomenon flos-aquae*). Therefore the 3 to 6 weeks needed to obtain equilibrium associations, that are only dependent on the experimental conditions but not on their 'history' (i.e. the inoculum), are no experimental artifact. At least in midlatitude lakes such a long time of identical environmental conditions is improbable. The plankton associations observed in the lake are no equilibrium associations but transitional stages between preceding associations and equilibrium associations, that are rarely reached. Consequently TILMAN's concept of steady-state coexistence of algae limited by different resources contributes less to the solution of HUTCHINSON's 'paradox of the plankton' than the various 'disequilibrium' concepts.

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SUMMARY

Natural phytoplankton from Lake Constance was exposed to competition experiments in continuous culture at 6 different molar rations of P : Si as potentially limiting nutrients. As predicted by theory there was competitive exclusion of all species but one at single nutrient limitation, and coexistence of two species in steady state when P was limiting for one and Si for the other species. In one case coexistence of three species occurred. Equilibrium species composition, which stabilized after 3 to 6 weeks of cultivation, was independent of the species composition of the inoculum and only controlled by the nutrient regime. Comparability of experimental results and field observations is discussed.

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