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On population dynamics in multi-species cultures of diatoms and dinoflagellates

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ABSTRACT: In order to assess the phenomena possibly underlying population dynamics and species succession in the sea, the following phytoplankton culture experiments were made. In uni-algal and in multi-algal batch cultures, generation times and cell yields gained during logarithmic growth were determined for the diatoms *Biddulphia regia* and *Coscinodiscus concinnus,* as well as for the dinoflagellates *Ceratium horridum* and *Prorocentrum micans.* In multi-species cultures, none of the tested organisms showed any influence on generation time, compared with uni-algal cultures. In contrast, the cell yield of different species showed considerable changes depending on the species concerned and the species-combination used. The dinoflagellates *C. horridum* reached, if cultivated together with *B. regia* or *B. regia* and *C. concinnus,* only 10 % of the cell number of uni-algal cultures. In the combinations tested, *B. regia* produced always more than half of the cell number attained in uni-algal cultures. In multi-species cultures, *C. concinnus* cell production was not affected. Addition of nitrate and phosphate to stationary-phase multi-species cultures induced further growth. Thus it is concluded that growth is limited by nutrient competition in the multi-species experiments conducted. Possible mechanisms of nutrient competition are discussed.

INTRODUCTION

Many efforts have been made to explain the phenomena of phytoplankton succession and population dynamics, either by analysing field data or by studying the autecology of single species in culture experiments. Field data are very important in studying the functioning of ecosystems, but as many components vary simultaneously and very often independently in natural environments, the reason for changes in natural populations are recognizable only in a very few cases. Numerous culture experiments of single phytoplankton species have been conducted in order to obtain data suitable for explaining plankton growth in the natural habitat. In these experiments, algal responses to variations of such parameters as light, temperature or nutrient concentrations were tested. However, in nature, phytoplankton species usually grow together with other species. It is likely that the species interact in one way or the other and, so far, only very few experiments have proved the question of interaction and competition among coexisting species in multi-species cultures.

In order to assess the phenomena responsible for population dynamics and species

succession in the sea, the following aspects were investigated in multi-species culture experiments: (1) The possible effect on generation time due to the interaction between two or more species cultivated together; (2) the possible effects of interaction between two or more species cultivated together on cell production. During all experiments, cell densities corresponded to those prevailing in the natural environment.

MATERIAL AND METHODS

The diatoms *Biddulphia regia* (Schulze) Ostenfeld, isolated from the plankton near Helgoland, and *Coscinodiscus concinnus* W. Smith, isolated off Niewport (Belgium), as well as the dinoflagellates *Ceratium horridum* (Cleve) Gran and *Prorocentrum micans* Ehrenberg, both isolated near Helgoland, were cultivated in batch cultures employing glass Petri dishes of 5 cm diameter, filled with exactly 10 ml medium. The medium was prepared after Stosch & Drebes (1964), but modified by adding only $^1/_{10}$ of the nutrients nitrogen and phosphorus (Table 1). The sea water (31.6 ‰ S) employed was taken off Helgoland in January, 1973. The temperature was kept at 18° C ± 0.2° C, illumination at 3300 lux was supplied by "Philips W33" fluorescent tubes with a light/dark cycle of 14:10 hours. To allow physiological adaptation to

Table 1

Modified 'Stosch & Drebes medium' used in the experiments

Seawater	1.000 l	31.6 ‰ S
$NaNO_3$	4.25 mg	50 µmol
$Na_2HPO_4 \times 12\ H_2O$	1.075 mg	3 µmol
$FeSO_4 \times 7\ H_2O$	278 µg	1 µmol
$MnCl_2 \times 4\ H_2O$	19.8 µg	0.1 µmol
SiO_2	12 mg	200 µmol
$Na_2EDTA \times 2\ H_2O$	3.72 mg	10 µmol
Cobalamin	0.7 µg	0.005 µmol

After sterile filtration adjusted to pH 7.5

culture conditions the species were cultivated under these conditions for at least two months corresponding at least to 5 transfers before starting the experiments. Two controls and at least 3 parallel tests were run in one series of experiments and each series was duplicated at least once.

Each day, the whole population in each Petri dish was counted under the dissecting microscope. Generation times with confidence limits were calculated during the logarithmic growth period. After terminating the experiments, total cell number for each species was counted in the fixed samples. Two different sets of experiments were conducted:

(1) The diatoms *Biddulphia regia* and *Coscinodiscus concinnus* and the dinoflagellate *Ceratium horridum* were inoculated in Petri dishes either singly or in all combinations possible. From each species 4 individuals were inoculated in each Petri

dish, and the generation time and the production of cells in the 10 ml medium was followed. After all species had reached the stationary growth phase, 0.1 ml nitrate solution, corresponding to an enrichment of 500 μg-at NO_3-N l^{-1}, and 0.1 ml phosphate solution, corresponding to an enrichment of 30 μg-at PO_4-P l^{-1} were added in order to test whether nutrient competition was responsible for growth limitation.

(2) In a second set of experiments it was tested whether the dinoflagellate *Prorocentrum micans* will continue to grow in multi-species cultures after the other species have reached the stationary growth period. In these experiments, 10 individuals of *Prorocentrum micans* were inoculated; the other procedures were identical to those described above. In addition, it was tested whether *P. micans* will grow if inoculated into stationary-phase cultures of the other algae. In these experiments, *P. micans* was inoculated with 5, 10 or 50 individuals. By addition of 0.1 ml nitrate solution, corresponding to an enrichment of 500 μg-at NO_3-N l^{-1}, and 0.1 ml phosphate solution, corresponding to an enrichment of 30 μg-at PO_4-P l^{-1}, it was tested whether nutrient competition or perhaps other factors were responsible for the phenomena observed. The small and highly motile cells of *P. micans* could not be counted exactly under the dissecting microscope, thus generation time of this species was only estimated. Final cell yield at the end of the experiments was counted in the inverted microscope in the fixed samples.

RESULTS

Generation time

Generation times with confidence limits (95 % level) of the algae tested in monoculture are presented in Table 2. Generation times with confidence limits for *Biddulphia regia* in monoculture and in multi-species cultures and the same for *Coscinodiscus concinnus* are given in Table 3. Both species revealed no statistically significant differences in generation time neither in monocultures nor in multispecies cultures.

Table 2

Generation time plus confidence limits (95 % level) of the diatoms *Biddulphia regia* (BID), *Coscinodiscus concinnus* (COS) and of the dinoflagellates *Ceratium horridum* (CER) and *Prorocentrum micans* (PRO) in unialgal cultures at 18° C, 3300 lux and a 14 : 10 light-dark cycle

BID	COS	CER	PRO
$19.7 \leqq 20.9 \leqq 22.3$	$43.7 \leqq 50.0 \leqq 58.5$	$49.7 \leqq 58.3 \leqq 73.4$	$45.8 \leqq 50.5 \leqq 56.4$

In *Ceratium horridum* cultivated together with *Coscinodiscus concinnus*, generation time is the same as in unialgal cultures. However if cultivated together with *Biddulphia regia* or with *B. regia* plus *Coscinodiscus concinnus*, *C. horridum* divided only 3 to 4 times. While an exact determination of the generation time is not possible, the data suggest that it is the same in the multi-species cultures as in the controls.

Table 3

Generation time plus confidence limits (95 % level) of the diatoms *Biddulphia regia* (upper part) and *Coscinodiscus concinnus* (lower part) in unialgal and multi-species cultures. (For Culture conditions and abbreviations consult Table 2)

BID	BID + COS	BID + CER	BID + COS + CER
19.7 ≦ 20.9 ≦ 22.3	20.5 ≦ 22.2 ≦ 24.3	20.3 ≦ 21.9 ≦ 23.8	19.9 ≦ 22.6 ≦ 26.1
COS	COS + BID	COS + CER	COS + BID + CER
43.7 ≦ 50.0 ≦ 58.5	40.7 ≦ 45.0 ≦ 50.2	39.1 ≦ 43.1 ≦ 48.1	45.3 ≦ 49.3 ≦ 54.1

The few data from the second set of experiments which can be used for calculating the generation time of *Prorocentrum micans* suggest that this species too remained unaffected, as far as its generation time is concerned by the other species present.

Production

Mean cell density during the stationary phase of *Biddulphia regia* monocultures (number of cultures: n = 12) was 2230 cells 10 ml^{-1}, cell yield varied between 2050 to 2572 cells 10 ml^{-1}. There was no difference if cultivated together with *Ceratium horridum* (n = 6). If cultivated together with *Coscinodiscus concinnus* (n = 6) or with *C. concinnus* plus *C. horridum* (n = 6), cell production was significantly lower, the mean (n = 12) was 1693 cells 10 ml^{-1}; variations ranged from 1540 to 1850 cells 10 ml^{-1} (Fig. 1). If nutrients were added, *B. regia* always started to divide again. It is concluded that nutrient competition caused growth limitation in this species.

Coscinodiscus concinnus showed no difference in cell production in the multi-species cultures as compared with monocultures. There were 12 monocultures, 6 cultures together with *Biddulphia regia*, 6 together with *Ceratium horridum* and 6 together with *B. regia* plus *C. horridum*. The cell density of these 30 cultures varied between 60 and 70 cells 10 ml^{-1}. If nutrients were added to stationary-phase cultures, *C. concinnus* again started cell production; it is concluded that nutrient limitation caused growth limitation (Fig. 1).

Ceratium horridum was extremely sensitive to the presence of *Biddulphia regia* but not to that of *Coscinodiscus concinnus*. In monocultures, this species produced 450 to 750 cells 10 ml^{-1}. If cultivated together with *B. regia*, *C. horridum* divided only once or not at all after *B. regia* had reached the stationary phase. *B. regia* has a generation time of about 21 h, whereas that of *C. horridum* exceeds 2 days; the latter could, therefore, divide only 3 to 4 times before *B. regia* went into stationary phase. As a consequence, *C. horridum* produced only 30 to 50 cells 10 ml^{-1}, i.e. about 10 % of the production of the controls (Fig. 1).

If nutrients were added, only *Biddulphia regia* renewed population growth. In order to decide whether growth limitation was caused by nutrient competition or by inhibiting factors excreted by *B. regia*, medium from the stationary-phase mixed cul-

tures was decanted into a new Petri dish, enriched with nitrate plus phosphate as described, and inoculated with 4 cells of *Ceratium horridum*. The dinoflagellate started to divide without a lag phase, and its generation time equalled that of the controls; the total cell yield was between 300 and 400 cells. Thus it can be concluded that only nutrient limitation is responsible for the lower *C. horridum* cell numbers obtained in mixed cultures with *B. regia*.

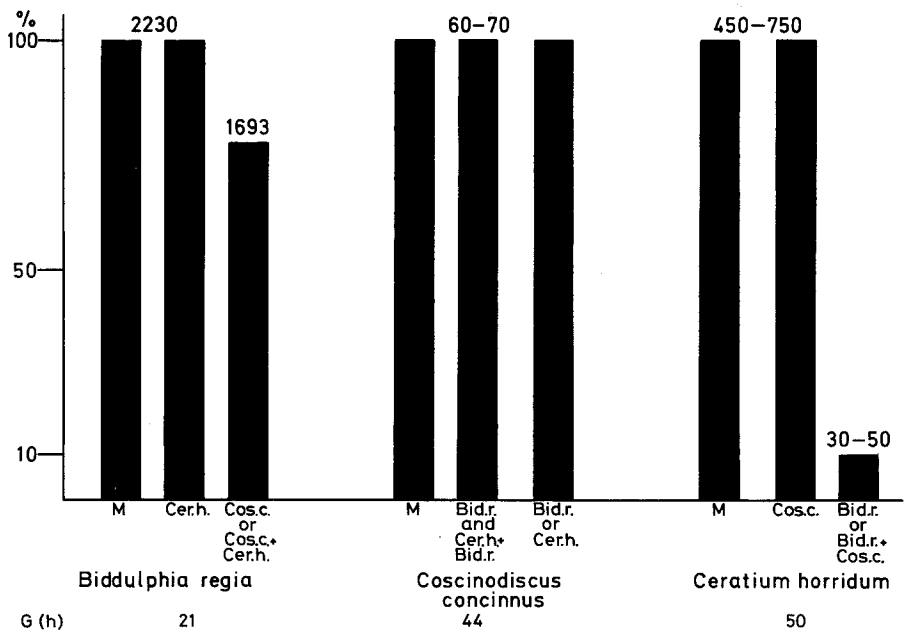

Fig. 1: Cell production gained in stationary-phase cultures of *Biddulphia regia* (Bid. r.), *Coscinodiscus concinnus* (Cos. c.) and *Ceratium horridum* (Cer. h.), expressed as per cent of monocultures, in multi-species cultures and in monocultures (M). Numbers above black columns: number of cells in stationary-phase cultures. G (h): generation time in hours

If cultivated together with *Coscinodiscus concinnus*, *Ceratium horridum* continued to divide even if *C. concinnus* was in the stationary phase. *C. horridum* apparently produced the same cell density as in monocultures, but as the cell number varied to a large extent in the monocultures (450 to 750 cells) only a large number of experiments can reveal possible slight differences.

Prorocentrum micans, if cultivated together with *Biddulphia regia*, divided only once after *B. regia* had reached the stationary growth phase. In mixed cultures of *P. micans* and *Coscinodiscus concinnus*, the dinoflagellate gained each time about 1000 cells 10 ml^{-1}. There was no difference, no matter whether the inoculum consisted of 5 cells, 10 cells or as many as 50 cells. If nutrients were added both, *P. micans* and *C. concinnus* started to divide again.

In order to examine the possibility that inhibition due to algal excretion may have been responsible for limiting population growth, 10 *Prorocentrum micans* cells

were inoculated into monocultures of either *Biddulphia regia* or *Coscinodiscus concinnus* which had entered the stationary growth phase 2 days before. The growth of *P. micans* was followed. In stationary-phase cultures of *B. regia* with no nutrient enrichment, the inoculated cells of *P. micans* divided only 1 to 2 times. After enrichment with nutrients, in *P. micans* as well as in *B. regia* cell division was resumed. If inoculated in stationary-phase monocultures of *C. concinnus, P. micans* attained a final cell density of about 200 to 500 cells, equivalent to at least 4 to 5 cell divisions. Hence, there is no reason to assume that an inhibitioning factor had been excreted into the medium. Consequently, growth limitation must have been caused by nutrient competition.

In conclusion, with respect to cell-production several different growth patterns exist in multi-species cultures (Fig. 1). We distinguish 3 types: (1) No effect on either of the species co-cultivated. This applies to *Coscinodiscus concinnus* and *Ceratium horridum.* (2) No effect on one of the species, but on the others. This applies to *Coscinodiscus concinnus* (no effect) if cultivated together with *Biddulphia regia* or *B. regia* plus *Ceratium horridum* or *Prorocentrum micans.* (3) All species of multi-species cultures failed to yield the same cell density as in monocultures. One species yielded more than half *(Biddulphia regia),* the other less than half the cells as compared to their monocultures *(Prorocentrum micans).*

Theoretically, there are a number of further, possible growth patterns not demonstrated in the present experiments; these may perhaps be found if more combinations are tested.

DISCUSSION

Competition is a factor attracting increasing interest in ecological research. In recent years, some papers dealing with the theoretical background of this phenomenon have appeared, for references and discussion see O'Brien (1974), Wiegert (1974), Vandermeer (1975) and others. Nevertheless, papers dealing with multi-species algal cultures are rare, e.g., Kroess (1971, 1972) and Elbrächter (1976). If two or more algae are cultivated together in a defined volume of water, it can be expected that there will be some influence on the growth of one or more members of mixed cultures. Such an influence might effect growth or cell composition.

In this study, the possible influence on growth is investigated, the parameters measured being generation time and cell production. Generation time is one of the most important factors influencing population dynamics. It deals with the time in which a population of known magnitude can multiply. If there is no growth limitation, this value corresponds to the time in which feeders can use the equivalent of the standing stock as food at a given time without disturbing steady-state conditions. This applies if we regard the water body in which a phytoplankton population is growing as "continuous culture" with no growth limitation of any kind.

If, on the other hand, we regard the water body in which a phytoplankton population grows as "batch culture", growth will be limited at least by nutrients. Feeders use whole cells; therefore, the amount of cells produced in a given water mass with a given nutrient concentration is of interest for modelling ecosystems.

Growth limitation in multi-species cultures might be caused by excretion by one species suppressing growth of other species, e.g. Kroess (1971, 1972), Elbrächter (1976). In the present experiments, none of the results suggest that changes in growth rates were due to inhibition caused by excreted factors.

Carlucci & Bowes (1970) demonstrated that liberation of vitamins produced by one species will enhance growth of other species in mixed cultures. In the present experiments, there was no growth enhancement and this possibility will not be discussed further.

In our experiments, generation time was identical in multi-species cultures and in unialgal cultures. The observed influence on growth was restricted to limitation in respect to cell production and this could be ascribed to nutrient competition. Addition of nutrients to stationary multi-species cultures resulted in further cell division.

There are some possible mechanisms of nutrient competition which cause growth limitation. According to Dugdale (1967), Eppley et al. (1969) and others, nutrient uptake can be described by an equation similar to that of Michaelis-Menton enzyme kinetics. It was demonstrated by various authors that different species can have different uptake rates and half-saturation constants for different nutrients. These authors concluded that cell division and generation time will depend on the external concentration of the limiting nutrients. Fedorov & Kustenko (1972) demonstrated that *Skeletonema costatum* and *Thalassionema nitzschioides* have different affinities to nitrate and phosphate. Changes in the ratio of these nutrients in mixed cultures caused subsequent changes in the biomass of the species concerned.

Droop (1974, 1975) concluded from his experiments with *Monochrysis lutheri* that the internal nutrient status of the cells regulate cell division. In addition, he demonstrated that algal cells have the possibility of luxury consumption. However, Droop also demonstrated that luxury consumption of a non-limiting nutrient is regulated by the concentration of a limiting nutrient. If growth stopped by nutrient limitation of one nutrient, luxury consumption of another nutrient, present in excess, stopped also. This mechanism can significantly effect growth limitation in mixed algal cultures.

Perhaps, one of the phenomena observed during the present experiments may be explained by this last-mentioned mechanism: the growth limitation of *Ceratium horridum* cultivated together with *Biddulphia regia*. The dinoflagellate showed no growth in mixed cultures after nutrient enrichment of stationary phase cultures. In contrast, *B. regia* cells began to divide after nutrient enrichment. If inoculated into enriched medium decanted from stationary-phase *B. regia* cultures, *C. horridum* showed good growth. Thus, inhibition by excreted factors can be excluded. The explanation for this phenomenon might be that *B. regia* has a very high uptake rate and a very high luxury consumption of the nutrients added, so that *C. horridum* had no opportunity to build up an internal substrate concentration high enough to allow cell division. As a reminder *B. regia* has a generation time of about 21 h; *C. horridum*, of more than 2 days.

Extremely different affinities to nutrients, described by Fedorov & Kustenko (1972), may explain why *Coscinodiscus concinnus* and *Ceratium horridum* gained the same cell yield in mixed cultures as in monocultures. No nutrient competition could

be detected in a limited volume of medium with a limited nutrient concentration. When nitrate and phosphate were added to stationary-phase cultures, both algae started once more to grow; hence, it can be concluded that growth of both algae had been nutrient limited.

Although *Coscinodiscus concinnus* exhibit optimal growth below 18° C (personal communication of Dr. Schöne), cell yields for *C. concinnus* obtained under optimal conditions (Schöne, unpublished) did not differ significantly from the results found at 18° C. Therefore, it is unlikely that the results of the multi-species cultures discussed here were influenced by temperature conditions sub-optimal for *C. concinnus*.

Growth limitation due to nutrient competition between *Biddulphia regia* and *Prorocentrum micans* may be explained by equal demands and equal uptake rates for nutrients in both species. In mixed cultures, these two species stopped cell division at the same time and, if nutrients were added, both started to grow again.

Our experiments demonstrated a variety of possible interactions and reactions regarding production of phytoplankton species if cultivated in mixed cultures. Further investigations in the field as well as in the laboratory are required to show whether these or other mechanisms apply to interactions of coexisting phytoplankton species in natural habitats.

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LITERATURE CITED

Carlucci, A. F. & Bowes, P. N., 1970. Vitamin production and utilization by phytoplankton in mixed culture. J. Phycol. **6**, 393–400.

Droop, M. R., 1974. The nutrient status of algal cells in continuous culture. J. mar. biol. Ass. U.K. **54**, 825–855.

— 1975. The nutrient status of algal cells in batch culture. J. mar. biol. Ass. U.K. **55**, 541–555.

Dugdale, R. C., 1967. Nutrient limitation in the sea: dynamics, identification, and significance. Limnol. Oceanogr. **12**, 685–695.

Elbrächter, M., 1976. Population dynamic studies on phytoplankton cultures. Mar. Biol. **35**, 201–209.

Eppley, R. W., Rogers, J. N. & McCarthy, J. J., 1969. Half saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr. **14**, 912–929.

Fedorov, V. D. & Kustenko, N. G., 1972. Competition between marine planktonic diatoms in monoculture and mixed culture. Oceanology **12**, 91–100.

Kroess, H. W., 1971. Growth interactions between *Chlamydomonas globosa* Snow and *Chlorococcum ellipsoideum* Deason and Bold under different experimental conditions, with special attention to the role of pH. Limnol. Oceanogr. **16**, 869–879.

— 1972. Growth interactions between *Chlamydomonas globosa* Snow and *Chlorococcum ellipsoideum* Deason and Bold: the role of extracellular products. Limnol. Oceanogr. **17**, 423–432.

O'Brien, W. J., 1974. The dynamics of nutrient limitation of phytoplankton algae: a model reconsidered. Ecology **55**, 135–141.

Stosch, H. A. von & Drebes, G., 1964. Entwicklungsgeschichtliche Untersuchungen an zentrischen Diatommen IV. Helgoländer wiss. Meeresunters. **11**, 209–257.
Vandermeer, J. H., 1975. Interspecific competition: A new approach to a classical theory. Science, N.Y. **188**, 253–255.
Wiegert, R. G., 1974. Competition: a theory based on realistic, general equations of population growth. Science, N.Y. **185**, 539–542.

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