Harmful and Toxic Algal Blooms
Yasumoto, T., Oshima, Y and Fukuyo, Y. (Eds)
Intergovernmental Oceanographic Commission of UNESCO 1996

EFFECT OF DIFFERENT NUTRIENT COMBINATIONS ON PHYTOPLANKTON DEVELOPMENT IN MICROCOSMS

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ABSTRACT

Two sets of microcosm experiments were carried out, during winter and autumn 1993, to assay the effect of nutrient loadings, with different ratios among N, P and Si, on the succession patterns of enclosed natural communities of phytoplankton. The experimental setup consisted of 8 Perspex tubes which were filled with 30 L of coastal water. Four nutrient treatments, NPSi, NP, NSi and PSi, were randomly assigned to replicate tubes and added on the first day of the experiment: each pair of tubes received nitrate (N), phosphate (P) and silicate (Si) as indicated. A major chlorophyll a concentration peak appeared 2-3 days after enclosure in all treatments; the magnitude of the peak was highest in the NPSi tubes and lowest in the PSi and NSi ones. Centric diatoms formed the bulk of this initial peak, while dinoflagellates presented their maxima in later phases of the experiment. A principal component analysis showed that successional changes and the initial composition of the inoculum were the most important factors explaining phytoplankton variability in the pooled data set for both experiments. Discriminant analyses based on the abundance of the most frequent taxa could be used to identify phytoplankton composition differences among treatments, within each experiment. However, there were no obvious regularities concerning species or group-specific effects of nutrient enrichment ratios. This points out that the outcome of fertilization events is highly dependent on the initial conditions of the community.

INTRODUCTION

The importance of macronutrient (N, P, Si) concentration ratios in controlling phytoplankton biomass and growth rates is an open question in relationship with eutrophication phenomena. Nitrogen is considered to be the most common limiting nutrient in the oceans and phosphorus in freshwaters. However, in some marine areas, like the Mediterranean, phosphorus appears to be the main limiting nutrient [1, 2]. Experimental investigations carried out with freshwater phytoplankton have suggested that differences in nutrient ratios may lead to changes in phytoplankton species dominance [3, 4]. In marine waters, high phosphate concentrations relative to nitrate are supposed to favour nitrogen fixation (by Trichodesmium, for example); while low phosphate concentrations relative to nitrate have been associated with decreased bacterial activity and increased excretion of organic matter which could lead to noxious mucilage accumulations [5]. However, few experiments have been focused on testing the effect of nutrient ratios on the species composition of marine phytoplankton communities [6]. The purpose of this study was to assay the effect of nutrient loadings, with different ratios among N, P and Si, on the succession patterns of enclosed natural communities of phytoplankton.

MATERIAL AND METHODS

Two sets of microcosm experiments were carried out, lasting for about three weeks; the first experiment (experiment 1 or "winter experiment") was conducted during January/February 1993 and the second one (experiment 2 or "autumn experiment") during October/November of the same year. The experimental setup [7] consisted of 8 Perspex tubes which were filled with 30 L of water from Masnou, a coastal locality 20 km north of Barcelona, and kept indoors at constant temperature and under a 12 h light: 12 h dark cycle. Four nutrient treatments, NPSi, NP, NSi and PSi, were randomly assigned to replicate tubes and added on the first day of the experiment: each pair of tubes received nitrate (N), phosphate (P) and silicate (Si) as indicated in Table 1. In addition, all treatments received a metal supplement (1 ml of the metal stock solution of the f/2 medium [8]). Nutrient determinations were carried out with an Alliance autoanalyzer using the methods of Strickland and Parsons [9]. Chlorophyll a was determined by fluorimetry of acetone extracts [7]. Phytoplankton composition was examined by means of the inverted microscope technique, as described in [7]. To summarize the variability of the phytoplankton data, two (one for each experiment) factorial discriminant analyses (DA) and one principal component analysis (PCA) (for the pooled data of both experiments) were carried out [10] using a logarithmic transform of the abundances of the most frequent taxa (in general, those taxa present in >20% of the cases for the discriminant analyses and in >10% of the cases of each experiment for the principal component analyses).

Table 1. Nutrient concentrations in each treatment, after nutrient enrichments were performed.

Treatment	Nitrate (µM)	Phosphate (µM)	Silicate (µM)
Experiment 1. Jan. 19-Feb. 9 (Winter).			
Nutrient-balanced	46.3	2.36	69.1
Si-def.	46.3	2.36	39.1
P-def.	46.3	0.44	69.1
N-def.	16.9	2.36	69.1
Experiment 2. Oct. 18-Nov. 11 (Autumn)			
Nutrient-balanced	39	2.36	35.5
Si-def.	39	2.36	5.5
P-def.	39	0.44	35.5
N-def.	9.6	2.36	35.5

RESULTS

A major chlorophyll a concentration peak appeared 2-3 days after enclosure in all treatments; in the winter experiment, a minor chlorophyll a peak appeared in most treatments on day 13. The first chlorophyll a peak was highest in the NPSi tubes, followed by the NP ones; the lowest peaks were found in the PSi and NSi tubes (Fig. 1). In both experiments, the end of the initial peak of chlorophyll a coincided with the decrease of P to undetectable levels in all cases, followed by exhaustion of nitrogen (in the N-deficient vessels) and of silicate (immediately after the chlorophyll peak in all Si-deficient treatments and in later days in the other treatments of experiment 2). In experiment 1, high levels of silicate lasted longer due to the high concentration of this nutrient in the initial water (data not shown).

Total phytoplankton numbers showed a major maximum a few days after enclosure, coinciding with that of chlorophyll a (data not shown). In both experiments, centric diatoms, with *Skeletonema costatum* and several *Chaetoceros* species, among others, formed the bulk of the phytoplankton community in the initial peak (Fig. 2). *Skeletonema costatum* and lightly-silicified pennate diatoms (*Amphiprora* sp.) were the main contributors to the second chlorophyll peak of the winter experiment. Flagellates and dinoflagellates tended to increase in the later phases of the winter experiment, with or without a small initial peak (Fig. 2). In the second experiment, flagellates showed a major initial peak accompanying that of diatoms, while dinoflagellates presented a maximum about 5 days later and reached the highest numbers in the NPSi tubes (Fig. 2). As could be expected, in both experiments, diatoms showed the lowest abundances in the NP treatments (Fig. 2). However, in the initial phases of the experiments, the differences between the Si-receiving treatments were small (data not shown).

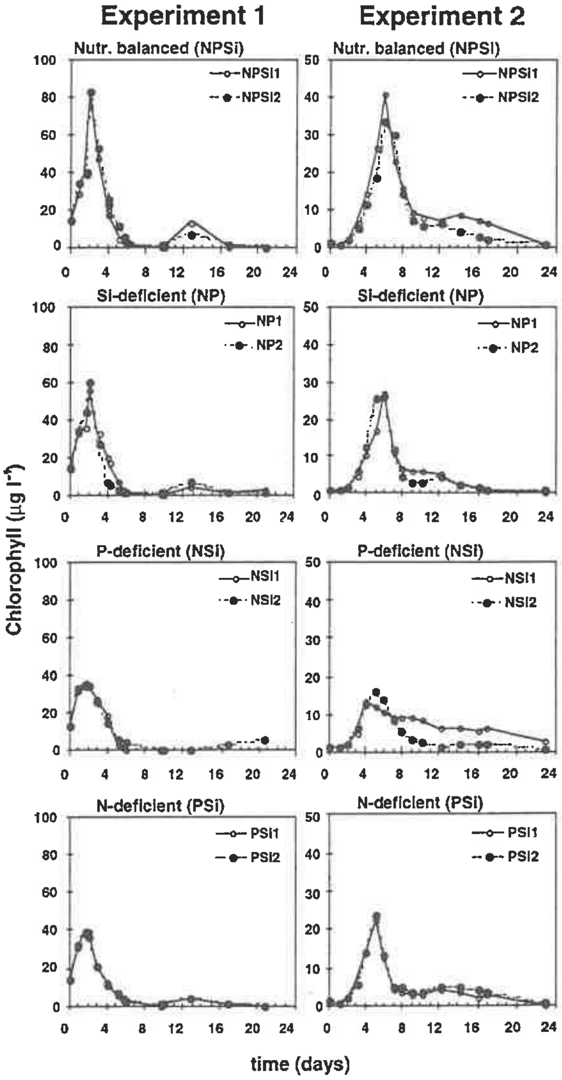

Figure 1.- Temporal variation of chlorophyll a concentration in the different treatments of experiments 1 and 2.

The distribution of the scores of a PCA based on the abundance of 23 selected taxa in both experiments is given in Fig. 3. The second component (C2) separated the two experiments, while the first component (C1) reflected the phytoplankton succession from the beginning of the experiments (positive side of C1) to the initial maximum (negative side of C1) and back to the positive side. In this representation, the differences betwen treatments within each experiment were not

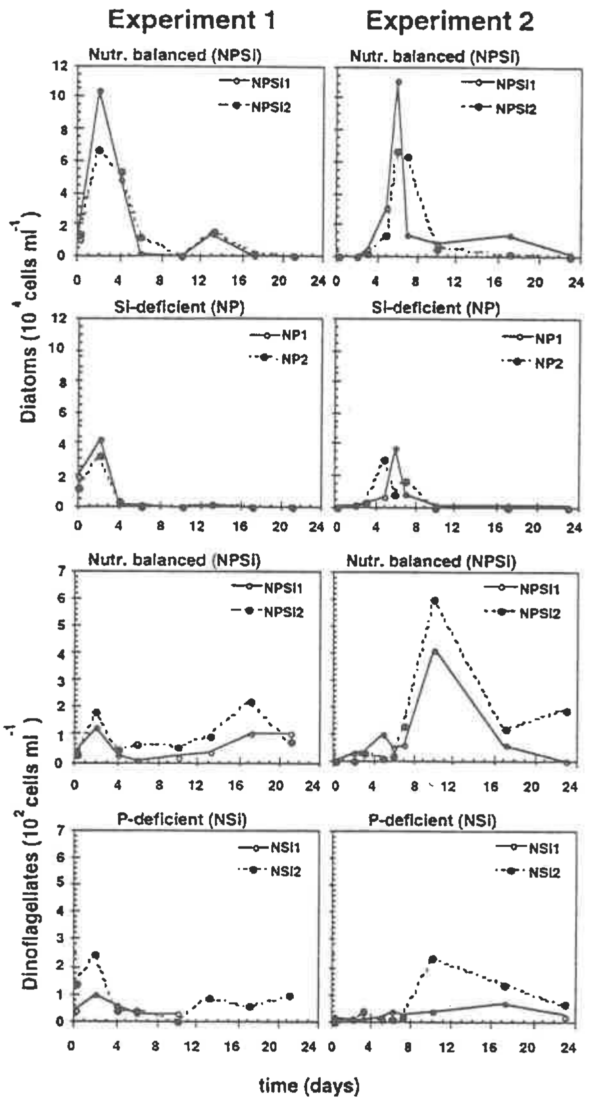

Fig. 2.- Temporal variation of diatom concentration on the NPSi and NP treatments of experiments 1 and 2 and, for dinoflagellates on the NPSi and NSi treatments.

apparent. Centric diatoms were negatively correlated with C1, which showed positive correlations with *Amphiprora* sp. and several dinoflagellates. C2 separated taxa with more representation in experiment 1 (positive side) or experiment 2 (negative side).

The number of taxa selected for the DA was 30 for experiment 1 and 34 for experiment 2. The distribution of the scores of the samples in the space of the two first discriminant variates (DV1 and DV2, respectively) is shown in Fig. 4. In both experiments, the samples corresponding to the different treatments appeared well separated. In the winter experiment, DV1 separated N-deficient from N-sufficient treatments, while DV2 separated P-sufficient from P-deficient ones. In the autumn experiment, DV1 opposed P-sufficient to

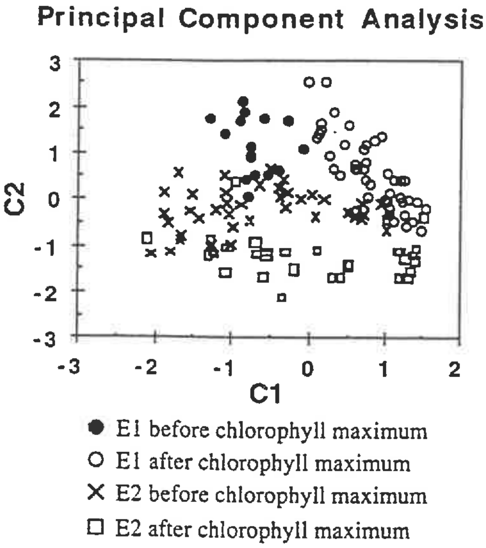

Fig. 3.- Position of the samples in the space of the two first principal components of the PCA.

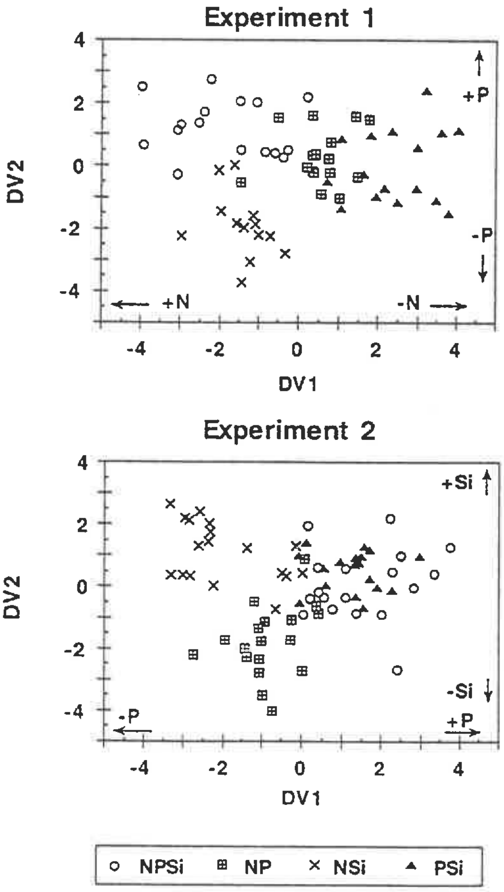

Fig. 4.- Position of the samples in the space of the two first discriminant axes (DV1 and DV2) of the discriminant analysis of Experiments 1 and 2.

P-deficient treatments, while DV2 separated Si-sufficient from Si-deficient ones. Although the overall variance induced by the treatments was significant (Wilks' lambda statistic, $p<0.05$), the correlation coefficients of the taxa with the discriminant variates were in general low. In experiment 2, diatoms showed in general positive correlation with DV2 (reflecting silicate enrichment); however, there were no clear trends of group affinity concerning the other nutrients.

DISCUSSION

A general feature of enclosure experiments, when nutrients are available in the medium, is the occurrence of a phytoplankton succession producing an initial chlorophyll peak [7, 11]. This phenomenon, which occurs even without artificial nutrient additions, may be favoured by better average illumination and a decrease of both physical losses and predator pressure in the enclosures. As shown in this and previous work, the magnitude of the initial peak may be influenced by fertilization treatments [6], and the highest maxima were obtained with additions including both N and P (with or without Si). An interesting point, in the experiments reported here, is the influence of silicate availability. With similar initial (after fertilization) concentrations of nitrate and phosphate, the concentration of chlorophyll of the initial maxima, mainly contributed by diatoms, was more than twice as high in the experiment 1 (with much higher silicate concentrations in the initial water) than in experiment 2 (see table 1). This suggests, if chlorophyll is taken as an indicator of phytoplankton carbon, that diatoms may be more efficient producers of autotrophic biomass than other groups of phytoplankton.

The response of phytoplankton cell numbers to the different treatments showed less clear trends than that of chlorophyll (with the exception of the higher abundance of diatoms in the Si-sufficient treatments). Apart of counting errors, this could be due to differences in cell sizes and to the high numerical contribution of small forms (*Skeletonema costatum*, *Nitzschia closterium*, flagellates) in the enclosures. In this context, it should be noted that the processes affecting nutrient concentration in the vessels are complex, and that undetectable (relative to our analytical methods) concentrations in the medium may mask a high turnover. Recycling of nutrients (faster for P) may explain the maintenance of relatively high chlorophyll levels in late phases of some of the tubes.

As shown by the discriminant analyses, the two first discriminant functions were related, respectively, to N and P availability in experiment 1 and to P and Si availability in experiment 2. The case for Si may be related to the higher silicate concentration in the initial water of the first experiment. Changes in the relative response to N and P may be associated to differences in the initial community and to the fact that limitation by both nutrients appears to have ecological importance in Mediterranean waters [1].

The results of the PCA (Fig. 3) show that, within the framework of seasonal and successional variability, the changes in qualitative composition of phytoplankton induced by single fertilization treatments (within the levels considered here) are relatively small compared with those related to the initial inoculum. However, a closer study of the phytoplankton response (Fig. 4) points out that such treatments produce identifiable changes in the community composition and that the overall effect of a treatment on a particular species may depend on the initial conditions of the community. This may be specially important in the case of potentially noxious taxa.

ACKNOWLEDGEMENTS

This work was funded by UE grant no. EV5V-CT92-0215 and the CSIC. L. A. was supported by a fellowship of the Instituto de Cooperación Iberoamericana and M. M. by a postdoctoral fellowship of the Spanish Ministerio de Educación y Ciencia. E. Quintana provided technical support.

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