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Bioassay experiments in the Falsterbo Channel – nutrients added daily

E. Granéli

Dept. of Marine Botany, University of Lund, Lund, Sweden

Abstract

In situ enrichment bioassays were performed during the summers of 1978 and 1979 in the Falsterbo Channel, south Baltic Sea. Phosphorus and/or nitrogen was added daily for up to two weeks to 200 l polyethylene bags with unfiltered surface water. Additions of nitrogen or nitrogen plus phosphorus invariably increased the biomass and ^{14}C fixation of phytoplankton. Phosphorus additions had no such effects. Phytoplankton species which reacted most strongly to the enrichment were *Aphanothece* sp., *Nodularia spumigena*, *Skeletonema costatum*, *Nitzschia closterium*, *Chaetoceros wighami* and *Oocystis* sp. The mean C/Chl a quotient was around 70 for chlorophyll a values below 6 mg · m^{-3} but decreased to about 30 for chlorophyll a values above 10.

Introduction

Nitrogen has often been regarded as the most important limiting nutrient for phytoplankton production in marine waters (GOLDMAN 1976). In the Baltic Sea phosphorus was felt to be the most limiting nutrient for primary production by FONSELIUS (1977), while SEN GUPTA (1972) proposed nitrogen. In the Bothnian Bay phosphorus seemed to be in short supply in the central areas while nitrate was depleted by early summer in coastal waters (ALASAARELA 1979). With respect to the Öresund, eutrophication has usually been discussed in terms of phosphorus (see references in EDLER 1977), partly because of the lack of reliable long-term measurements of nitrogen.

In earlier studies in the Öresund and adjacent waters (GRANÉLI 1978, GRANÉLI in prep.), it was shown that nitrogen additions to natural phytoplankton communities usually stimulated phytoplankton growth while phosphorus rarely did so. In these enrichment bioassays nutrients were added only at the beginning of the experiments.

In the present *in situ* bioassays, nutrients were added daily to plastic bags to simulate a permanent increase in the fertility of the water. Because of rupture of the bags, the experiments could not be run for more than about two weeks. Even so, this approach better corresponds to a real situation of nutrient enrichment than only initial nutrient addition. Chlorophyll a, ^{14}C fixation, phosphorus and nitrogen uptake and changes in the phytoplankton populations were studied in the various bags.

Materials and methods

The experiments were carried out in the Falsterbo Channel on the southwest coast of Sweden. The southern end of the channel is widened and protected by breakwaters, which makes it an excellent location for *in situ* experiments.

Three experiments were performed during the following periods: August 23 to September 4, 1978 (12 days), June 23 to July 7, 1979 (15 days) and July 1 to July 7, 1979

(7 days). 200 l double polyethylene bags (0.1 mm thick) were filled with 150 l unfiltered surface water. The bags were fixed to a wooden frame with styrofoam floats which held the open ends about 30 cm above the surface. The frame was anchored to the bottom at 4 m depth. A roof of polyethylene covered the construction. The water in the bags was mixed before sampling with a 30 cm diam. Secchi disc.

Four different bags were used in the enrichment bioassays: 1) control (C) containing untreated surface water, 2) one bag with phosphorus (P) added as KH_2PO_4, 3) one bag with nitrogen (N) added as $NaNO_3$ and 4) one bag with both phosphorus and nitrogen added (PN).

In the bioassays lasting for 12 and 15 days, 20 l water was removed from each of the bags daily and 20 l of unfiltered surface water from the experimental site was added. New spikes of P and N were added with the water. In the experiment lasting 7 days, 5 l of water were taken out daily, but only nutrients and no new water were added.

The phosphorus and nitrogen additions to the bags were:

	Phosphorus ($\mu g\ l^{-1}$)		Nitrogen ($\mu g\ l^{-1}$)	
	Initial	Daily	Initial	Daily
12 day exp. (1978)	24	2.2	400	16
15 day exp. (1979)	35	11	500	154
7 day exp. (1979)	35	11	350	154

Water was filtered through Whatman GF/F filters for chlorophyll a analysis. Chlorophyll a was determined with the spectrophotometric equations of JEFFREY and HUMPHREY (1975) (see also EDLER 1979). For measurement of $H^{14}CO_3^-$ assimilation 30 ml glass bottles with water from the bags were inoculated with 4 μCi-bicarbonate and incubated close to the bags at 0.3 m depth for 2 hours. The contents of the bottles were filtered and ^{14}C activity on filters was determined with Geiger-Müller equipment. ^{14}C activity was transformed to mg $C \cdot m^{-3} \cdot d^{-1}$ using measurements of light, pH, temperature and salinity (GARGAS 1975). Light was measured at Sturup, about 30 km NE of the Falsterbo Channel.

Nutrients (NO_3-N, HN_4-N and PO_4-P) were analysed on unfiltered samples according to the methods in CARLBERG (1972).

Water samples (150 ml) were preserved with Lugol's solution for identification and enumeration of the most frequent phytoplankton species. Nomenclature followed HENDEY (1974) and PARKE and DIXON (1976). The algae were counted in an inverted microscope, and numbers were transformed to plasma volume (PV) using size measurements and formulae in EDLER (1979). Plasma volume was transformed to cell carbon using the factor 0.11 (STRATHMANN 1967), and C/Chl a quotients were calculated (mg/mg).

Results

Weather conditions during the experiments

August 1978 was colder than normal and had lower than normal rainfall. Weather changed to overcast with much precipitation, however, during the last week in August when the bioassay experiment was performed. This continued through the first week in September.

June 1979 was sunny and warm until June 22, when the weather changed to cold and overcast. The weather worsened during the first week in July. Thus weather conditions during both years' experiments were similar, i.e. cold and overcast with rain.

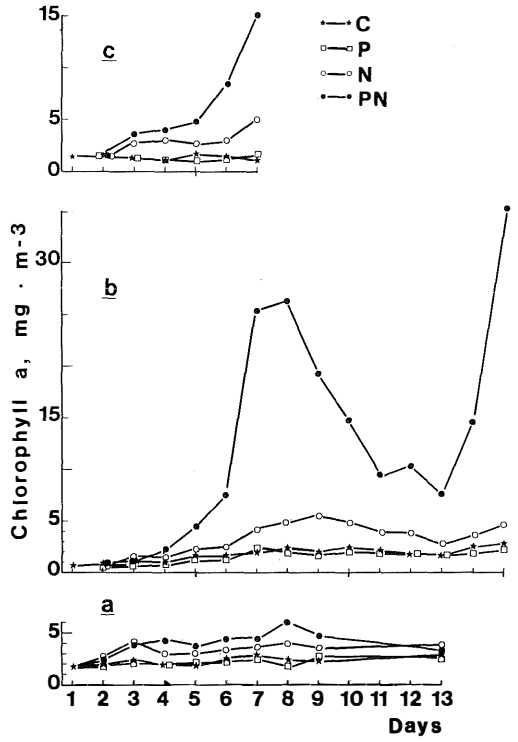

Figure 1

Chlorophyll a concentrations for the three experiments. a = August 23 to September 4, 1978, b = June 23 to July 7, 1979 and c = July 1 to July 7, 1979. C = control bag, P = phosphorus addition, N = nitrogen addition, PN = phosphorus plus nitrogen addition.

Water temperature during the experiments in 1978 varied between 10 and 18°C and in 1979 between 15 and 19°C.

Chlorophyll a (Fig. 1, a–c)

Initial chlorophyll a concentrations were 1.65, 0.68 and 1.38 mg m^{-3} in the water used for experiments a, b and c, respectively. Control and phosphorus bags showed the lowest chlorophyll a values in each experiment. There was an increase in chlorophyll a even in these bags, however. Additions of nitrogen alone gave maximal chlorophyll a values of 2.4, 7.9 and 3.6 times the initial values for a, b and c. Phosphorus and nitrogen added together gave the highest maximal chlorophyll a values in all 3 bioassays: a = 3.7, b = 39 and 52 (2 peaks) and c = 11 times higher than the initial concentrations.

Chlorophyll a concentrations increased even in the control bags. In experiment b the increase was from initial value 0.68 to 2.65 mg m^{-3} on the last day of the experiment. In the other bioassays the increase was less marked. In bags with phosphorus additions chlorophyll a concentrations were similar to or on some occasions even lower than concentrations in control bags.

Maximal chlorophyll a concentrations in the bags with only nitrate added were similar for the three bioassays, 4–5 mg · m^{-3}, in spite of differences in the amounts of added nitrogen. When phosphorus was also provided, chlorophyll a concentrations reached

about 30 mg · m^{-3} in experiment b and about 15 mg · m^{-3} in experiment c. In experiment a, on the other hand, maximal chlorophyll a concentration in the PN bag was only about 5 mg · m^{-3}.

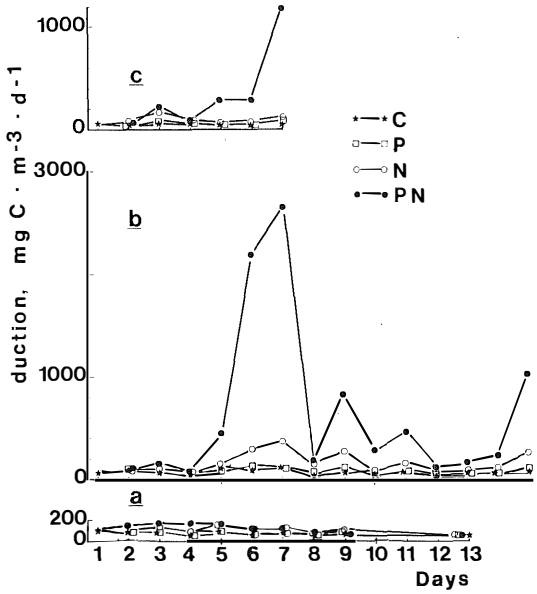

Figure 2

Primary production per day of bag water incubated outside the bags at 0.3 m depth. For identification of experiments a, b, and c and symbols see Fig. 1.

Primary production (Fig. 2, a–c)

Initial ^{14}C fixation in the water used for the experiments was 100, 54 and 58 mg C · m^{-3} · d^{-1} for experiments a, b and c, respectively. Primary production followed a trend similar to that of chlorophyll a in all 3 experiments.

Phosphorus (Fig. 3, a–c)

Initial concentrations of inorganic phosphorus (PO_4-P) in the water used for bioassays were 4.1, 5.8 and 5.0 mg·m^{-3} (a, b and c, respectively). Control and nitrogen bags behaved similarly and showed only small changes in phosphate concentration. In all three experiments there was a slight decrease initially, and in experiment a phosphate concentrations in the C and N bags increased towards the end of the experiment. In the P bags of experiments a and b, phosphate decreased towards the end of the experiments while in experiment c there was a continuous increase. In the PN bags there was a rapid uptake of phosphate, although concentrations approached the values in the C and N bags only in exp. b.

Nitrate (Fig. 4, a–c)

Nitrate was initially present in low concentrations, between 5 and 7 mg · m^{-3} for the three experiments. In the C and P bags nitrate concentrations remained more or less

constant in all 3 bioassays. In the N bags different patterns were observed: in experiment a, nitrate decreased to low levels after about one week, in experiment b nitrate was stable around 500–800 mg $N \cdot m^{-3}$, while in c there was a continuous increase. In the PN bags nitrate was depleted after 5 and 7 days, respectively, in experiments a and b. In experiment c, nitrate in the PN bag increased in the same way

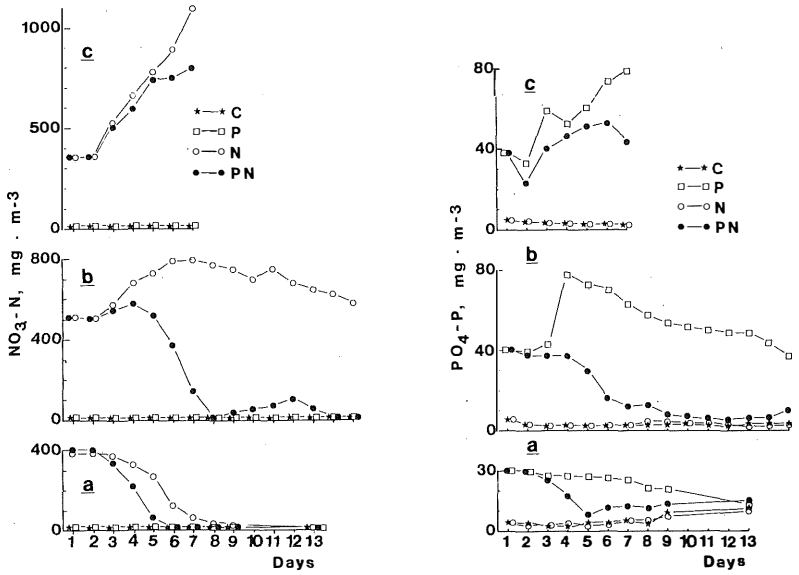

Figure 3

Inorganic phosphorus (PO_4-P) concentrations in the bags. For identification of experiments a, b and c and symbols see Fig. 1.

Figure 4

Nitrate nitrogen (NO_3-N) concentrations in the bags. For identification of experiments a, b and c symbols see Fig. 1.

as in the N bag. However, experiment c was terminated on the 7th day when development of a chlorophyll a peak had begun. Nitrate consumption reached maxima of about 200 and 400 mg $\cdot m^{-3} \cdot d^{-1}$ in the PN bags of experiments a and b (Fig. 5, a–c). Nitrate consumption in the N bags peaked later than in the PN bags.

Phytoplankton composition and biomass (Fig. 6, a–c)

In experiment a the colonial blue-green alga *Aphanothece* sp. reacted most strongly to the addition of nutrients. The biomass of this species increased relative to the control in all bags with nutrient addition. In the PN bag there was also an early peak of the nitrogen-fixing blue-green alga *Nodularia spumigena* and later of the diatom *Nitzschia closterium*. In experiment b, on the other hand, *N. closterium* and *Skeletonema costatum* reached the highest plasma volumes in the PN bag. *N. closterium* was present in small amounts in the other bags. The green alga *Oocystis* sp. developed strongly at the end of the experiment, especially in the N bag. As in experiment a, monads contributed significantly to the total plasma volume in all bags. In experiment

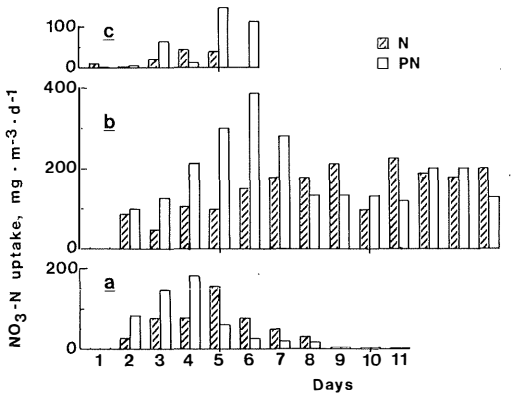

Figure 5

Uptake of NO_3-N in the N and PN bags calculated from concentration in the bags and daily additions. For identifications of experiments a, b and c and symbols see Fig. 1.

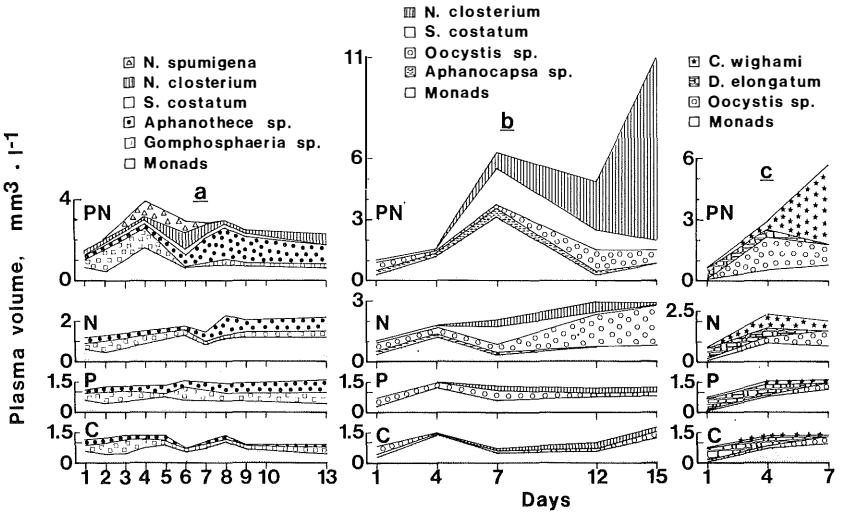

Figure 6

Plasma volumes of the most frequent phytoplankton species during the experiments. For identification of experiments a, b and c and symbols see Fig. 1.

c the diatom *Chaetoceros wighami* and a green alga of the genus *Oocystis* responded positively to nitrogen and nitrogen plus phosphorus enrichment. ''Monads'' and *Diatoma elongatum* dominated the phytoplankton biomass in the C and P bags.

There was a clear dependence of chlorophyll a concentration on phytoplankton plasma volume (Fig. 7). However, the C/Chl a quotient was not constant. The value was roughly 70 : 1 for chlorophyll a values below 6 mg · m^{-3}, but decreased to about 30 : 1 for high chlorophyll a values (> 10 mg · m^{-3}). These high chlorophyll a values were found only in bags enriched with phosphorus and nitrogen (experiments b and c).

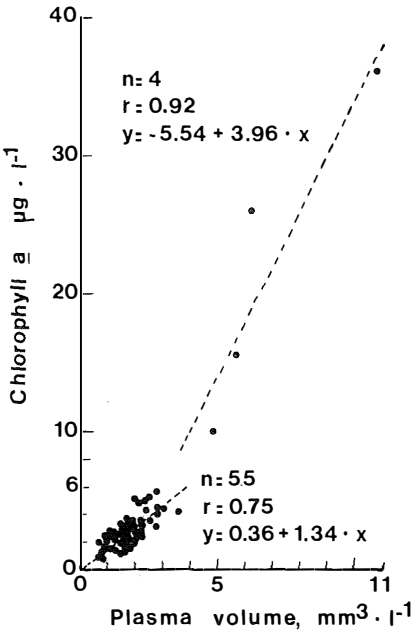

Figure 7
Relation between plasma volume and chlorophyll a concentration for all experiments and bags.

Discussion

Enrichment experiments designed to test the "limiting nutrient concept" always deviate considerably from the natural situation, both with respect to time and space. To approximate the natural situation as well as possible, e.g. to simulate upwelling of nutrient-rich water or eutrophication of coastal waters through sewage discharge, there should be a continuous addition of nutrients to the test organisms and water renewal over a period of at least several weeks. All experiments could not be run this long because of destruction of the plastic bags, although runs for up to two weeks were achieved with bags intact.

As the water volume in the bags was about 150 l, and 20 l was exchanged per day in experiments a and b, the water renewal time was about one week. In the Öresund, where much of the water in the Falsterbo area is ultimately transported, surface water is renewed rapidly and mixed with Kategatt water. A phytoplankton cell in the 0–10 m layer stays in the Öresund an average of 2–3 days (ÖVK 1979). With respect to water exchange the bioassay experiments (a and b) were conducted under conditions similar to those in the Öresund.

Addition of nutrients to the Öresund from municipalities and industries is substantial in spite of recent efforts to treat all sewage at least biologically (ÖVK 1979). The human population influencing the Öresund totals over 2 million, and there are fertilizer industries discharging large quantities of inorganic phosphorus. However, the actual increase in nutrient concentrations in the Öresund is limited by efficient mixing and rapid water renewal. Sustained "excess concentrations" of total N and P in the central part of the Öresund have been estimated to roughly 100 and 10 mg N and P · m^{-3}, respectively (ÖVK 1979).

In experiments a and b, where water was exchanged daily, equilibrium concentrations of total N and P (assuming that nutrients were not lost through adsorption to the bags or denitrification) would be 16.5 mg P · m^{-3} and 120 mg N · m^{-3} (exp. a) and 82.5 mg P · m^{-3}/ 1155 mg N · m^{-3} (exp. b). Thus the nutrient enrichment in experiment a was of the same order of magnitude as that observed in central Öresund. The nutrient additions in experiment b could only approximate those in shallow bays close to sewage outfalls (e.g. in the Lomma and Köge bays). Even the lower additions of N and P produced significant increases in phytoplankton biomass.

It is evident from the present experiments that nitrogen is more limiting to phytoplankton growth (in the sense of LIEBIG's law) than is phosphorus. Thus substantial increases in chlorophyll a and ^{14}C fixation can be achieved through nitrogen enrichment alone. Unfortunately, eutrophication of the Öresund has often been discussed in terms of phosphorus enrichment. This is explained by a) a lack of reliable older data on inorganic and total nitrogen for the Öresund, and b) an uncritical application in brackish water ecology of ideas concerning nutrient limitation which were orginally developed for fresh water. The practical/economic implications are obvious, e.g. with respect to sewage treatment.

If inorganic nitrogen is added in amounts over a few hundred mg · m^{-3}, phosphorus becomes limiting. The generally steep increase in chlorophyll a when both nitrogen and phosphorus are added together indicates that other elements, e.g. trace metals, are not in short supply in the South Baltic.

Mean phytoplankton cell carbon/chlorophyll a quotients for low chlorophyll a values (< 6 mg·m^{-3}) were around 70, which is in agreement with summer ratios calculated by EDLER (1977) for the Öresund from plasma volumes. When chlorophyll a increased to high values (> 10 mg·m^{-3}), which only happened in the PN bags of experiments b and c, plasma volume did not increase proportionately. Thus C/Chl a ratios decreased to roughly 30, which is close to spring and autumn values for the Öresund (EDLER 1977). In the spring nutrient availabilty in the Öresund is high, resembling conditions in the enriched bags. Variable C/Chl a quotients imply that chlorophyll a does not necessarily measure the increase in phytoplankton biomass during a nutrient enrichment (cf. JONGE 1980).

The experiments were made during a part of the year when monads and flagellates often compose a large part of the phytoplankton biomass in the south part of the Öresund. Monads were present in significant amounts in all the experiments but did not react strongly to nutrient enrichment. The greatest increase in biomass (plasma volume) was found for a few species of diatoms and blue-green algae. Among the former *S. costatum* is known to form blooms in the Öresund (EDLER 1977). Although the experiments were of a rather short duration (up to 2 weeks), they show that stability with respect to phytoplankton species composition is not reached even with sustained nutrient enrichment.

In conclusion, the increase in primary production seen in the Öresund since 1930 (GARGAS et al. 1978, ÖVK 1980) has mainly been caused by increased availability of inorganic nitrogen. The hypothesis that inorganic phosphorus also limited phytoplankton production initially in this area cannot be totally ruled out. However, phosphorus does not presently seem to limit phytoplankton growth, except perhaps during occasional late summer blooms of *Nodularia spumigena*.

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