Coastal plankton response to nutrient enrichment: an experimental system
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Abstract

The approach of a model system was chosen to simulate the natural nutrient status, the biological processes of the Gulf of Trieste (Adriatic Sea) and the effects of nutrient addition. Nutrient enrichment experiments were conducted in June 1994 and April 1995 to estimate the effects of different nutrient concentrations and ratios on coastal plankton dynamics and the production of organic matter. Natural phytoplankton were incubated in situ with the addition of four artificial nutrient sources (phosphate, nitrogen as nitrate+ammonium, silicate, a mixture of all nutrients) and natural nutrient sources (rain water and river water). In both experiments additions of phosphate and the mixture of all nutrients had the most profound effects on biomass increase and on production of particulate and dissolved organic matter. Bacterial biomass and production increased shortly after nutrient addition while phytoplankton passed over a 2-3 day lag phase. Size-fractionated primary production showed the largest enhancement in the >10 μm and 2-10 μm size fractions, while picoplanktonic cyanobacteria were outcompeted. The rates of released extracellular organic carbon (EOC) and the percentage of extracellular release (PER) were higher in the first experiment. Our results indicate that the effects on nutrients' pulses depended not only on different nutrient ratios but also on the initial structure of the biotic component in the marine system.

Introduction

Marine eutrophication is perceived to be a problem in several coastal areas which have been subjected for some decades to influences from different
sources, mainly antrophogenic in origin. The northern Adriatic has long been known as a very productive region, mainly due to the nutrient inputs from the river Po and other small rivers. During the last twenty years several nuisance events have occurred including red tides, toxic phytoplankton, bottom-layer anoxia and mucilage accumulation. The last outbreak of the mesoscale mucus accumulations in the northern Adriatic during late 80's and early 90's intensified the questions concerning the eutrophication of this area. Several hypotheses were put forward concerning the origin and fate of the mucilage phenomenon.

There is now a general consensus that mucilages are built-up of organic material in a matrix, which originates from phytoplankton exudates. The most likely explanation for the excessive release of phytoplankton exudates seems to be the depletion in nutrients or the disequilibrium of nutrient ratios. However, other factors like high light intensities and the taxonomic composition of phytoplankton assemblages are also significant.

The present paper describes the development of natural plankton assemblage and dissolved organic matter dynamics in a model system after the addition of different combinations of artificial nutrients and natural sources of nutrients, such as rain water and river water. Two such experiments were carried out (June/July 1994, April 1995) during periods which have been considered critical for the development of mucilage (early spring-early summer) in the northern Adriatic.

**Material and methods**

**Sampling.** Seawater was collected in the south-eastern part of the Gulf of Trieste at 2 m depth and was pre-screened through 200 µm mesh to remove zooplankton grazers. A 70 l sample was poured into a large container, thoroughly mixed and subsamples taken for initial measurements.

**Experimental system.** Based on our previous experience and knowledge of nutrient concentration in the studied area we decided to use seven treatment regimes: besides the control without any addition we applied the following nutrient, rain water and river water additions (Tab. 1). The respective concentrations and volume of freshwater additions reflect the highest nutrient

<table>
<thead>
<tr>
<th>Treatment label</th>
<th>Nutrient addition</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>control without addition</td>
</tr>
<tr>
<td>B</td>
<td>0.6 µM phosphate</td>
</tr>
<tr>
<td>C</td>
<td>5.1 µM nitrate + 1.8 µM ammonium</td>
</tr>
<tr>
<td>D</td>
<td>11 µM silicate</td>
</tr>
<tr>
<td>E</td>
<td>15% v/v rain water</td>
</tr>
<tr>
<td>F</td>
<td>15% v/v river water</td>
</tr>
<tr>
<td>G</td>
<td>mixture (B+C+D)</td>
</tr>
</tbody>
</table>

Table 1: Summary of the treatment regimes.
concentrations and natural rainfall and river water dilutions in the Gulf of Trieste.

Nalgene bottles containing natural plankton assemblages in prescribed nutrient treatments were incubated in situ at 2 m depth. Samples were withdrawn daily or every second day. We followed inorganic nutrients, particulate and dissolved organic nitrogen and carbon, particulate and dissolved carbohydrates, phytoplankton structure and pigment biomarkers, bacterioplankton abundance, and primary and bacterial production during 6 and 10 day experiments. Some of these parameters are presented.

Two enrichment experiments were carried out: 27 June-4 July 1994 (PALEX 1), and 3-12 April 1995 (PALEX 2).

**Nutrients** were analyzed in filtered (NO\textsubscript{2}, NO\textsubscript{3}, N-total, PO\textsubscript{4}, P-total) and unfiltered (NH\textsubscript{4}, Si) samples using standard colorimetric procedures.\textsuperscript{6} Chlorophyll \textit{a} (Chl \textit{a}) concentrations were determined fluorometrically.\textsuperscript{7} 25 ml subsamples were filtered onto 0.22 \textmu m Millipore filters, extracted in 90% acetone and the fluorescence of extracts measured on a Turner fluorometer 112.

**Cell counts.** Samples for phytoplankton were preserved with neutralized formaldehyde (1.5% final concentration). Micro- and nanoplankton were identified on an inverted microscope using the technique of Utermöhl.\textsuperscript{8} A subsample was settled for 24 hours and either 100 or 50 fields of the bottom chamber were examined at 200x or 400x magnification.

Cells of pico- and bacterioplankton were counted with an epifluorescence microscope in formalin preserved (2%) water samples. Cyanobacteria and 2-3 \textmu m eucaryotic cells were counted in green excitation light (magnification of 1250x) according to the protocol by Takahashi et al.\textsuperscript{9} and heterotrophic bacteria in UV light using DAPI according to Porter.\textsuperscript{10}

**Phytoplankton productivity** was measured by the \textsuperscript{14}C technique.\textsuperscript{11} Subsamples were incubated in light and dark 75 ml polycarbonate bottles after the addition of 6 \mu Ci of NaH\textsuperscript{14}CO\textsubscript{3} and were suspended close to the in situ experimental bottles for three hours. At the end of the incubation, cells in different size classes were collected on 10 \textmu m, 2 \textmu m, 0.6 \textmu m and 0.2 \textmu m polycarbonate filters, as well as subsamples of 0.2 \textmu m filtrate (exudate). Scintillation cocktail was added and the activity measured on a Canberra TriCarb 2500 scintillation counter. Assimilation of carbon was calculated as described by Gargas.\textsuperscript{12}

To determine extracellular organic carbon (EOC) the 0.2 \textmu m filtrates were acidified and left open in the hood for 24 hours. Data were not corrected for bacterial uptake. Percentage of extracellular release (PER) was calculated as EOC x 100/total primary production.

**Bacterial production** was measured by modified \textsuperscript{3}H-Thymidine method and production based on the amount of thymidine incorporated into DNA was calculated according to Fuhrman & Azam.\textsuperscript{13}
Results

In both experiments the inoculated plankton assemblages were taken from an environment that seemed to be P-limited (TIN/P>40), but the initial phytoplankton biomass was much lower in PALEX 1 (0.68 μg Chl a l⁻¹) compared to PALEX 2 (3.39 μg Chl a l⁻¹).

The responses of coastal phytoplankton to different nutrient additions and to natural rainfall and riverine dilutions, were controlled by different parameters such as phytoplankton biomass in terms of Chl a, primary production, abundance and taxonomic structure. Results obtained by different methods showed good agreement. The most evident common feature was a strong stimulating effect, that the addition of all nutrients (G treatment), and of phosphate (B treatment) exerted on plankton production and biomass. Responses to other treatments as compared to the control without any addition was smaller; still the impact of rain water (E treatment) and river water (F treatment) addition was significant.

Figure 1: Chlorophyll a biomass in different nutrient treatments expressed as a percentage relative to control during PALEX 1 (a) and PALEX 2 (b). The bold line denotes the percentage of Chl a biomass in control (100%).

The phytoplankton response to different nutrient treatments in terms of biomass (Fig. 1), productivity (Fig. 2), and abundance (Fig. 3, 4) passed
through a 2-3 day lag phase in both experiments, but the developments were rather different in PALEX 1 and PALEX 2.

**Phytoplankton biomass and production of particulate and dissolved organic carbon**

In both experiments the highest increase of Chl a, expressed as a percentage relative to control was observed in treatments B and G (Fig. 1). The stimulating effect of phosphate addition is especially evident in PALEX 1. However, phytoplankton biomass had grown up to 730% (B treatment) relative to control at the end of the first experiment, while in the second one the highest increase of Chl a was around 550% on day 5 in treatment G. Despite these differences in Chl a biomass in PALEX 1 and PALEX 2, the maximum total primary production in G and B treatments were of approx. the same rate in both experiments (around 16 and 12 μg C l⁻¹h⁻¹, respectively; Fig. 2). In other treatments the peak values varied from 1.18 and 3.28 μg C l⁻¹h⁻¹ in PALEX 1, and from 2.17 to 6.89 μg C l⁻¹h⁻¹ in PALEX 2. However, the specific production, expressed as primary production per biomass unit, was much higher in PALEX 1, indicating the higher activity of June/July phytoplankton population compared to April senescent population. The EOC rates and PER values were higher in the first experiment. In different treatments PER averaged from 25 to 61% and from 23 to 34% in PALEX 1 and PALEX 2, respectively. In both experiments the highest mean PER was calculated in control and in treatment with the addition of all nutrients, while the lowest one was found in the treatment with the river water addition.

Size-fractionated production rates showed the strongest response in 2-10 μm fraction: in different treatments values varied from 0.13 to 9.70 μg C l⁻¹h⁻¹ (the highest in G treatment), while rates in fractions >10 μm and 0.6-2 μm varied between 0.05 and 3.71 μg C l⁻¹h⁻¹ and from 0.01 to 3.97 μg C l⁻¹h⁻¹. On the contrary, in PALEX 2 the largest response was recorded in fraction >10 μm (0.56 to 13.81 μg C l⁻¹h⁻¹) and only a slight initial increase was observed for 2-10 μm fraction (0.12 to 2.60 μg C l⁻¹h⁻¹). The 0.6-2 μm fraction exhibited negligible reaction to nutrient additions (<0.01 to 0.47 μg C l⁻¹h⁻¹).

**Plankton abundance and taxonomic structure**

Increased primary production in the 2-10 μm fraction in PALEX 1 correlated with an expansion of nanoflagellates (Fig. 3a) and small 2-3 μm eucaryotic cells (not shown in Fig.3). Nanoflagellates dominated in the initial sample and increased the most in the G treatment from the initial abundance of 1.9x10⁵ cells l⁻¹ to 1.2x10⁷ cells l⁻¹ at the end of the experiment. Cyanobacteria decreased constantly from the beginning of the experiment (Fig. 3b). However, on the last experimental day an elevated abundance of diatom *Chaetoceros sp.* was observed in the G treatment.

Bacterial abundances had already reached maximal values on the second and third experimental day, preceding the autotrophic component of the system.
Figure 2: Total primary production and percentage of phytoplankton extracellular release (PER %) in different nutrient treatments during PALEX 1 (a) and PALEX 2 (b).
Figure 3: Abundance of phytoplankton (micro- and nanoplankton) (a), cyanobacteria (b) and heterotrophic bacteria (c) in different nutrient treatments during PALEX 1 (note the difference in scales).

From initial values (0.9 x 10⁹ L⁻¹) bacterial abundance doubled in 24-48 hours (Fig. 3c) to attain a maximum of 2.0x10⁹ cells l⁻¹ in the B treatment and 1.8x10⁹ cells l⁻¹ in the G treatment, but decreased thereafter. The average growth of bacteria in treatments B and G was 32 µg C l⁻¹ day⁻¹.

Initial phytoplankton assemblage (micro- and nanoplankton) in PALEX 2 was dominated by diatoms (6.46x10⁶ cells l⁻¹) mainly *Pseudonitzschia delicatissima* comp. (5.63x10⁶ cells l⁻¹) and *Skeletonema costatum* (0.65x10⁶ cells l⁻¹) reaching an abundance of over 7.6 x 10⁸ cells l⁻¹ in G treatment at the end of the experiment (Fig. 4a).
Figure 4: Abundance of phytoplankton (micro- and nanoplankton) (a), cyanobacteria (b) and heterotrophic bacteria (c) in different nutrient treatments during PALEX 2 (note the difference in scales).

The relative contribution of nanoflagellates to total phytoplankton biomass was negligible (5-9%). Determination of the relative increase of particular species indicated that the highest relative growth was attained by *Skeletonema costatum*, *Chaetoceros sp.* and *Cylindrotheca closterium* although *Pseudonitzschia delicatissima comp.* remained most numerous. The abundance of cyanobacteria decreased during the course of the experiment in all treatments (Fig. 4b).

Initial bacterial abundance was lower ($0.5 \times 10^9$ cells l$^{-1}$) than in PALEX 1 and neither reached particularly high values (max. in B and G treatment $1.1 \times 10^9$ cells l$^{-1}$ and $1.4 \times 10^9$ cells l$^{-1}$; Fig. 4c), even though the bacterial production was
similar in both experiments: average for G treatment in PALEX 2 was 30.2 μg C l⁻¹ day⁻¹, in other treatments production rates were lower (1.3-19.1 μg C l⁻¹ day⁻¹).

Discussion and conclusions

In both experiments the most marked response of plankton assemblage was observed in the treatment receiving all nutrients (G treatment), followed by the addition of phosphate (B treatment), river (F treatment) and rain (E treatment) water. The additions of the latter two in approx. natural proportions notably enhanced production and biomass accumulation suggesting the importance of these sources of nutrients in the northern Adriatic. The results from our experimental system largely agree with observation in the field influencing the results.

Limiting the comparison to G treatments in two experiments, we observed some differences in the developments. A very high autotrophic biomass (max. over 20 μg Chl a l⁻¹) associated with > 10 size fraction, mainly diatoms, developed in PALEX 2, while the heterotrophic biomass was comparatively low (less than 30 μg C l⁻¹). In contrast, a considerably lower autotrophic (max. Chl a 4.3 μg l⁻¹, dominance of nanoflagellates) and higher (over 45 μg C l⁻¹) heterotrophic microbial biomass developed during PALEX 1.

PER (percentage of production released as dissolved organic matter) ranged between < 1 % to over 70 % on one occasion. Mean PER was higher in PALEX 1 (40 %) than in PALEX 2 (28 %). Nevertheless, the majority of PER values in PALEX 2 were below 30 % with a mean of 17 % comparing well with an average of 12 % reported by Baines & Pace for marine-estuarine systems.

In both experiments the response of bacterioplankton to nutrient (mainly phosphate) addition preceded the response of autotrophs (mainly 2-10 μm in PALEX 1, and >10 μm in PALEX 2) indicating the importance of competition for inorganic nutrients. Interestingly, picoautotrophs were outcompeted in both cases.

Finally, our results indicate that the approach of a model system can help to understand some biological processes, which are probably crucial for the development of mucilage.

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