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Northern Adriatic mesocosm experiment Rovinj 2003: Nutrient dynamics

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Abstract

Background and Purpose: *Nutrient dynamics and influence on the plankton community of the northern Adriatic were studied during a month in 25 L mesocosms, enriched with nutrients in different ratios (»balanced«, N/P=16 and »P-deficient«, N/P=100), with the aim to contribute to understanding the production mechanism of microparticles, considered as precursors of the mucilage phenomenon.*

Materials and Methods: *Seawater for the experiment was collected in May 2003 in a frontal zone between oligotrophic and eutrophic waters in the northern Adriatic, 20 Nm W off Rovinj (Istria). Seawater in 25 L bottles was enriched with nutrients and incubated floating on the sea surface. Analyses of nutrients and other relevant parameters were performed with methods widely used in oceanography.*

Results: *Orthophosphate uptake occurred already in conditions of low phytoplankton biomass. Uptake of nitrate and orthosilicate was delayed for about a week in respect to orthophosphate, i.e. started only when phytoplankton biomass developed significantly. The bloom intensity was lower in »P-deficient« conditions, but not as low as predictable from the different orthophosphate enrichment in the two mesocosms. However, much higher alkaline phosphatase activity was induced in the »P-deficient« than in »nutrient balanced« mesocosm.*

Conclusions: *In »P-deficient« mesocosm, orthophosphate was used more efficiently than in »nutrient balanced«, due to rapid recycling by alkaline phosphatase. Furthermore, the N/P ratio of produced organic matter differed considerably between the two mesocosms, suggesting that phytoplankton in the northern Adriatic can grow over a broad range of N/P ratios.*

INTRODUCTION

The northern Adriatic is one of the most eutrophic regions of the Mediterranean Sea *(1, 2)*, mainly due to the Po River (average flow rate 1500 $m^3 s^{-1}$) nutrient discharges, representing up to 70 % of the annual total nutrient loads in the region *(3)*.

The nutrient concentrations in the Po River are two orders of magnitude higher than in the oligotrophic part of the Adriatic *(4)*. The N/P ratio varies considerably in the Po waters, but on average it is also elevated compared to the Adriatic seawater, unaffected by riverine discharges. Moreover, this ratio changed significantly during the past decades, as concluded from historical data *(5–10)*. From these data, a mean N/P value of 40 was calculated for the period 1972–1985. This

value increased by at least 50 % in the second part of the eighties and at least doubled during the nineties (~ 100 in 1997 and 1999–2002). Changes were due to a gradual decrease of orthophosphate concentrations, compared to the early eighties values, mainly as a result of a drastic reductions of detergent polyphosphate content in Italy in the period 1984–1988 *(6, 8)*. In contrast, the Po inorganic nitrogen content was not significantly modified.

The Po discharges greatly increase the surface layer nutrient content and affect their ratios over large northern Adriatic areas. An average N/P ratio about 60 was estimated for the most productive waters (salinity <35) in the period 1972–1981 *(11)*, i.e. much higher that the »optimal« for phytoplankton (N/P=16; *12*) or bacteria (N/P=9; *13*) »balanced« requirements. The N/P ratio of the freshened layer increased further since the mid eighties, due to decrease of orthophosphate and concomitant increase of inorganic nitrogen concentrations, which probably were not assimilated by a more markedly P-limited phytoplankton *(14)*.

The changes in nutrient ratios in the surface layer of the northern Adriatic, influenced by the Po River discharges, coincided with an increased frequency of mucilage events. These events were scientifically documented in the region since the late 19th century with a frequency of approximately 10–50 years *(15)*. But, since the late eighties the phenomenon exploded several times (1988, 1989, 1991, 1997, 2000–2003; *16, 17*).

There is a wide consensus that the mucilage is a build-up of organic material entrapped in a matrix, generated primarily by gelling of phytoplankton polysaccharide exudates (see *16* for review and references). Furthermore, laboratory experiments have shown that N/P ratio significantly different from 16 favors polysaccharide excretion in phytoplankton cultures *(18, 19)*. Consequently, the assumption that the N/P changes in the northern Adriatic can be one of the important factors triggering the mucilage phenomenon is probably justified.

To assess a possible role of extremely high N/P ratio on production of exudation particles, considered as precursors of mucilage aggregation, a mesocosm experiment was designed, with different P enrichments *(20)*. In this paper, nutrient concentrations and N/P ratio dynamics during the experiment were described in relation to phytoplankton and bacteria biomass development, with the aim to contribute the understanding of mechanisms involved in production of mucilage precursors.

MATERIAL AND METHODS

The seawater for the experiment was collected on 06 May 2003 in a frontal zone between eutrophic and oligotrophic waters in the northern Adriatic (station SJ105; $\phi = 45° 2{,}0'$ N and $\lambda = 13° 9.3'$; *21*). After collection, seawater was filtered through a 250 μm net and redistributed in seven 25 L transparent nalgene bottles. One bottle was kept as control, without nutrient addition, while the remaining six were enriched with approximately 100 μmol L^{-1} of nitrate and orthosilicate, vitamins, and, in triplicates, with 1 μmol L^{-1} (»P-deficient« mesocosm) and 6.3 μmol L^{-1} of orthophosphate (»nutrient balanced« mesocosm). The bottles were incubated floating at the sea surface. Subsamples were collected from all bottles: daily for chlorophyll *a* determination or less frequently (2–3 days interval) for other parameters. During the last week sampling was limited to two bottles for each mesocosm and performed in longer time intervals. The experiment set-up and measurement protocols are described in detail elsewhere *(20)*.

Nutrient measurements were performed in duplicate for each bottle in filtered water (Millipore 0.45 μm), using spectrophotometric methods widely used in oceanography *(22, 23)*. Total phosphorus was determined as orthophosphate, after sample treatment with 250 nm UV radiation *(24)*. This method gives satisfactory results for total phosphorus when the living and non living particles are present in concentrations lower than 4 μg L^{-1} *(25)*. The total variability (CV) between bottles was lower than 5.4 %. Total inorganic nitrogen (TIN) was calculated as a sum of nitrate, nitrite, and ammonium concentrations.

Alkaline phosphatase activity was measured with fluorogenic substrate analog *(26)* using methylumbelliferyl-phosphate at a final concentration of 125 μmol L^{-1}. Fluorescence was measured on a 96 wells fluorometer (Victor II) on 12 wells for each sample, at 355 nm ex/460 nm em. Activity was calculated after calibration of the fluorometer with methylumbelliferone. After the tenth day, measurements were performed in duplicate for each bottle using a Turner TD-700 fluorometer, with P/N 10–069 ex/P/N 10-110R-C em. No significant differences were found between these two techniques for a number of 36 samples.

Chlorophyll *a* was determined fluorometrically after extraction of material collected on Whatman GF/C filters with 90% acetone *(22)*. Bacteria total counts were determined by epifluorescence microscopy using 4', 6-diamidino-2-phenylindole (DAPI) to stain the cells *(27)*.

Cumulative N/P ratio in organic matter (Norg/Porg) produced during the experiment was calculated as the difference between nitrate and orthophosphate concentrations measured each day, and their respective initial values (Table 1).

RESULTS

Concentrations of nutrients and total phosphorus in bottles were measured for the first time about 40 hours after nutrient additions (day 2; Table 1). In the control bottle nutrient concentrations did not change significantly compared to the initial values in seawater used. In the enriched mesocosms the orthophosphate concentrations on day 2 were significantly lower than expected, while total phosphorus values were close to the added orthophosphate. Considerable quantities of this nutrient can be taken by phytoplankton in P-limited environment and stored in intracellular pools, before assimilation pro-

TABLE 1

Nutrient and total phosphorus concentrations measured in the collected seawater (SW) immediately before nutrient additions (6 May 2003) and on day 2 of the experiment (8 May) in the control and enriched bottles

Bottle number[b]	Parameters[a]					
	$c(PO_4)$	c(TP)	$c(SiO_4)$	$c(NO_3+NO_2)$	$c(NO_2)$	$c(NH_4)$
SW	0.04		3	5	0.3	1.2
2	0.00	0.15	6	4	0.4	0.6
3	0.4	1.0	122	87	0.4	0.7
4	0.4	1.1	123	86	0.4	0.1
5	0.4	1.1	125	89	0.4	0.6
6	5.4	6	122	88	0.5	0.4
7	5.5	6	130	90	0.4	0.7
8	5.5	6	126	88	0.5	0.7

[a] PO_4-orthophosphate, TP-total phosphorus, SiO_4-orthosilicate, NO_3-nitrate, NO_2-nitrite, NH_4-ammonium (μmol L^{-1}).

[b] SW-seawater used for the mesocosm experiment (6 May 2003); control (bottle 2), »P-deficient« (bottles 3–5), and »nutrient balanced« (bottles 6–8) mesocosms on day 2 of the experiment (8 May 2003).

cesses and growth start *(28)*. Since the organic phosphorus initially present in collected seawater was very low (about 0.15 μmol L^{-1}), the total phosphorus was taken as a good measure for the initial value of the added orthophosphate.

For nitrate and orthosilicate the concentrations measured on day 2 were taken as the initial values, since the chlorophyll *a* (Chl *a*) concentration (0.9 μg L^{-1}) and net primary production (1 μg C L^{-1} d^{-1}) were low and did not differ significantly from the day of collection. In a previous enrichment experiment with northern Adriatic seawater, it was shown that no significant uptake of these nutrients occurred in conditions of low phytoplankton biomass and activity, as opposed to phosphorus compounds *(29)*.

Bloom development phase

The nutrient dynamics in replicate bottles was similar during the whole experiment. The orthophosphate uptake occurred already within the first two days (0.5–0.7 μmol L^{-1}; Figure 1a), both in »P-deficient« and »nutrient balanced« mesocosms, before any significant phytoplankton or bacterial growths were observed (Figure 1e,f). During these initial days the alkaline phosphatase activity (APA) was lower than in the control bottle; Figure 1d). While in »P-deficient« conditions, after the initial uptake, the orthophosphate concentration (0.4–0.5 μmol L^{-1}) did not change significantly up to day 10, in the »nutrient balanced« bottles a gradual decrease was observed (Figure 1a). In both mesocosms this nutrient was exhausted after the phytoplankton (days 9 and 10) and heterotrophic bacteria biomass maximum developed (on day 12 in »nutrient balanced« and days 15–17 in »P-deficient« conditions).

Orthophosphate decrease and related phytoplankton and bacteria increases in the »P-deficient« mesocosm were coupled with a substantial increment of APA (Figure 1d). APA reached the highest values after orthophosphate exhaustion, in correspondence to the maximum abundance of heterotrophic bacteria (Figure 1f). In »nutrient balanced« conditions, APA varied with a similar pattern, but the maximum value was two orders of magnitude lower than in »P-deficient« mesocosm (Figure 1d). In the control bottle APA remained constant.

In the first four days of the experiment, significant uptake of added orthosilicate and nitrate did not occur (Figure 1b,c), but started in the successive days, concurrently with phytoplankton growth (Figure 1e). During this phase 61–63% of nitrate and 30–37 % of orthosilicate initial concentrations were assimilated in both mesocosms.

During the bloom phase nitrite (0.4–1.1 μmol L^{-1}) and ammonium (0.1–1.8 μmol L^{-1}) concentrations in the enriched mesocosms varied significantly between bottles, but accounted mostly for only 0.5–1% of the total inorganic nitrogen (TIN).

Post bloom phase

After the phytoplankton bloom decay (day 17), orthophosphate concentrations in the »P-deficient« mesocosm continued to be minimal up to the end of the experiment (Figure 1a), while nitrate (Figure 1b) and orthosilicate (Figure 1c) values slightly increased and then remained approximately at the same levels since the day 17 (~40 μmol L^{-1} and ~100 μmol L^{-1}, respectively). APA also decreased significantly, but remained up to the end of the experiment at a level much higher than in the initial days, and, higher than in the »nutrient balanced« mesocosm and control (Figure 1d).

In »nutrient balanced« conditions, during the decline of phytoplankton bloom, a release of orthophosphate was observed, followed by its reuptake after a week (Figure 1a), when a new moderate accumulation of Chl *a* occurred (Figure 1e). This process coincided with assimilation of practically all added nitrate (Figure 1b), while, at

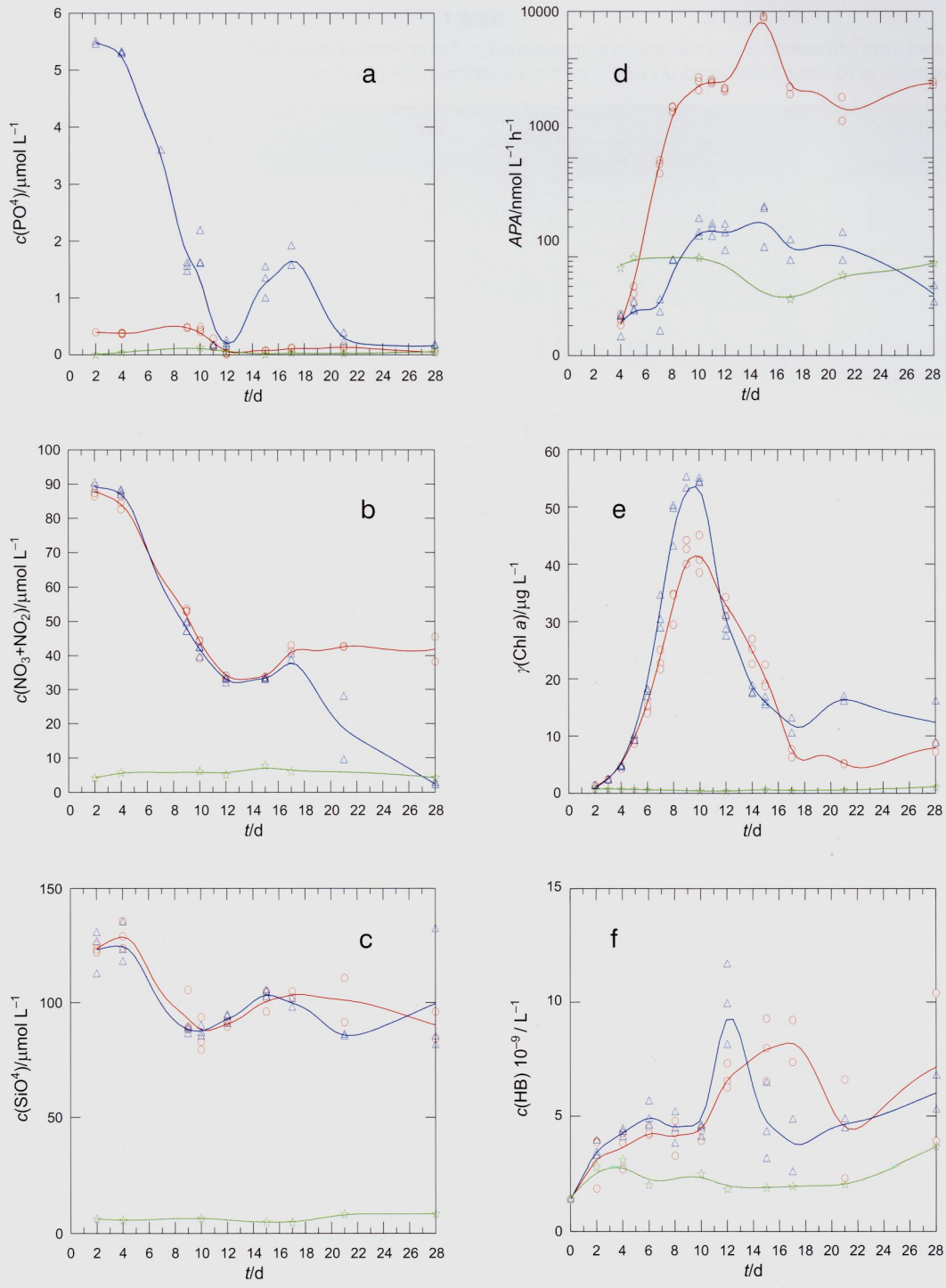

Figure 1. *(a) Orthophosphate (PO_4), (b) nitrate+nitrite (NO_3+NO_2), (c) orthosilicate (SiO_4) concentrations, (d) alkaline phosphatase activity (APA), (e) chlorophyll a (Chl a) concentration and (f) heterotrophic bacteria abundance (HB) in control (☆), »P- deficient« (●) and »nutrient balanced« mesocosms (△).*

the same time, the orthosilicate concentration decrease was relatively small (<15 µmol L^{-1} on day 21), and was replaced by prevailing regeneration processes on day 28 (Figure 1c).

Differently from nitrite (0.3–1.5 µmol L^{-1}), ammonium concentrations in all bottles (0.9–9 µmol L^{-1}) varied more than during the bloom phase. This indicates that nitrogen regeneration occurred at very different rates, even in duplicate bottles. Due to nitrate depletion in the »nutrient balanced« mesocosm, ammonium contributions to TIN increased with time, becoming even more important than nitrate on day 28.

DISCUSSION

Orthophosphate uptake, in both enriched mesocosms, occurred when phytoplankton biomass and primary production were still as low as in the used seawater. It is known that phytoplankton cells growing in P-limiting conditions and in changing environmental nutrient conditions, as in the northern Adriatic, can store large quantities of phosphorus into intracellular pools *(28, 30, 31)*. Heterotrophic bacteria might also contribute to the orthophosphate uptake. In fact, bacteria started to grow immediately, at least doubling their abundance in respect to the initial seawater already on day 2.

Faster uptakes of orthophosphate compared to nitrate and orthosilicate were also observed in earlier experiments *(29)*. This agrees with results of bioassays, performed with both oligotrophic and eutrophic northern Adriatic waters *(32, 33)*, indicating phosphorus as a principle limiting element of primary production. Moreover, N/P ratios much higher than 16 in the more productive northern Adriatic waters *(11)*, as well as the N/P regeneration rates of 40, calculated from yearly estimates for total N and P *(3)*, further support this finding.

In accordance with the phosphorus limiting role, phytoplankton blooms were more marked in »nutrient balanced« than in »P-deficient« conditions. However, the peak values (Chl *a* ~ 50 μg l^{-1} and ~30 μg l^{-1}, respectively) were not so distinct as expected from an initial six fold orthophosphate availability difference. Obviously, phosphorus deficit in the »P-deficient« mesocosm was partly compensated by more rapid recycling rates, mainly due to induction of extracellular alkaline phosphatase, both in bacteria and phytoplankton *(34, 35)*.

Remarkably, APA in the »nutrient balanced« mesocosm remained low even when orthophosphate was depleted. Phytoplankton APA may not only be mediated by external orthophosphate concentrations, but also by internal N/P ratios. Inhibition of APA by intracellular N/P ratio is species depending, but generally occurs at values below 14 *(36)*. The calculated N/P ratios in produced organic matter (Norg/Porg) in the »nutrient balanced« mesocosm were lower than 13 (Figure 2). In this mesocosm the organic phosphorus pool was probably enough, not only to satisfy the bacterial demand, but also to support some primary production with regenerated orthophosphate, without involving APA. In contrast, in »P-deficient« conditions Norg/Porg was much higher (40–80), except during a few initial days, due to orthophosphate storage in intracellular pools. The maximum value was reached at the peak of phytoplankton bloom (Figure 2). According to Parslow et al. *(37)* phytoplankton can still grow even if its cellular P-content is several times lower than the optimal, when inorganic nitrogen compounds and orthosilicate are available. These results suggest that the northern Adriatic phytoplankton community can grow in waters with very high N/P inorganic ratios, using quite efficiently the available phosphorus, mainly rapidly recycled by alkaline phosphatase.

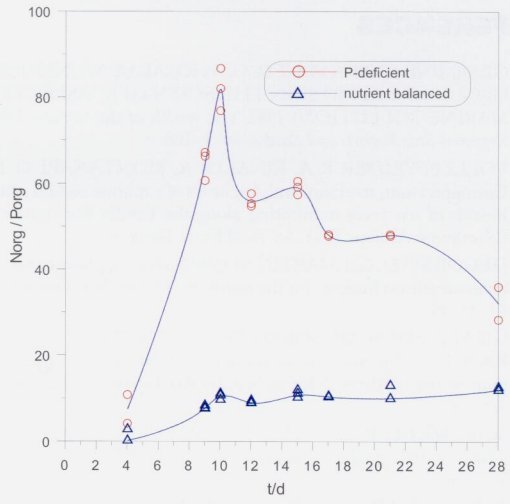

Figure 2. *Calculated N/P ratio in organic matter produced during the experiment in »P-deficient« (○) and »nutrient balanced« mesocosms (△).*

In both mesocosms the first bloom was dominated by the diatom *Dactylosolen fragilissimus (38)*, while the autotrophic nanoflagellate abundance was not significant *(39)*. In these conditions, significant assimilation of orthosilicate occurred, concomitantly with orthophosphate and nitrate. During the initial phase of the second bloom, more intense in the »nutrient balanced« mesocosm, the diatom *Cylindroteca closterium* dominated the microphytoplankton, although with much greater importance of the autotrophic nanoflagellate contribution than during the first bloom. This could be a reason of the relatively low orthosilicate uptake, compared to nitrate, observed during the second bloom.

In the control, the phytoplankton biomass remained low during the whole experiment. The orthophosphate concentrations varied near the analytical zero and obviously limited the phytoplankton growth, as nitrate and orthosilicate values were relatively high (4–8 μmol L^{-1} and 5–8 μmol L^{-1}, respectively). Consequently, the alkaline phosphatase activity remained approximately at the same level up to the end of the experiment. Although this level was relatively low, compared to that reached in the »P-deficient« mesocosm, it was sufficiently high to indicate a significant P limitation. This is also supported by the fact that during the experiment initial days APA in control bottle (Figure 1d) was much lower than in the enriched ones, where orthophosphate was abundant.

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