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Dissolved organic phosphorus regeneration by bacterioplankton: 5'-nucleotidase activity and subsequent phosphate uptake in a mesocosm enrichment experiment

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ABSTRACT: Bacterial 5'-nucleotidase activity was studied during a mesocosm enrichment experiment on the SW coast of Finland, Baltic Sea. 5'-nucleotidase activity and uptake of $^{32}PO_4$ hydrolyzed from 5'-nucleotide was compared to direct $^{32}PO_4$ uptake measurements and other nutrient data. The enzyme was not repressed by elevated PO_4 concentrations, and it hydrolyzed orthophosphate from dissolved organic phosphorus in amounts which were quantitatively significant in the phosphorus dynamics of the community. Bacteria partly assimilated the hydrolyzed PO_4, but depending on nutritional conditions, 0 to 80 % of the hydrolyzed PO_4 was released to the environment. Temporal uncoupling between nutrient assimilation and growth was evident for both bacteria and algae. 5'-nucleotidase represented a mechanism of phosphorus regeneration which supports the concept of closely integrated micro-aggregates having an important role in nutrient cycles of nutrient-depleted surface waters.

INTRODUCTION

Although nitrogen is usually considered the principal limiting nutrient for oceanic planktonic communities (Ryther & Dunstan 1971), concentrations of both inorganic nitrogen and phosphorus are often very low, close to or below the detection limits of analytical methods. Therefore, planktonic productivity should be essentially regulated by remineralization processes (Eppley et al. 1973, Furnas et al. 1986). If the prevailing view of nitrogen limitation is valid, the mechanisms of organic phosphorus regeneration should be effective in relation to organic nitrogen regeneration.

Traditionally zooplankton has been considered the main organism group in nutrient remineralization. Johannes (1964) suggested that mineralization rates should be inversely related to the body size of the organisms. Direct remineralization measurements have supported and extended this view, as microplankton (< 100 µm) has usually contributed the bulk of recycled nutrients, both nitrogen and phosphorus (Harrison 1978, 1983, Paasche & Kristiansen 1982, Andersson et al. 1985, Furnas et al. 1986, Andersen et al. 1986). However, the relative roles of protozoa (mainly ciliates and nanoflagellates) and bacteria in net remineralization fluxes remain unclear.

Phytoplankton are able to synthetize cell-surface alkaline phosphatases in phosphate-depleted waters, which allows them to utilize dissolved organic phosphorus (Perry 1972). These enzymes are readily repressed by the presence of orthophosphate, and alkaline phosphatase is therefore likely to represent a temporally restricted strategy of phytoplankton to overcome serious phosphorus depletion.

Bengis-Garber & Kushner (1981, 1982) purified a bacterial membrane-bound enzyme (5'-nucleotidase) which dephosphorylated 5'-nucleotides, and Ammerman & Azam (1985) reported 5'-nucleotidase activity in marine bacterioplankton. The enzyme allowed bacteria to hydrolyze 5'-nucleotides thus releasing orthophosphate. This mechanism for dissolved organic phosphorus regeneration showed 2 ecologically important characteristics: firstly, it was not repressed by the presence of orthophosphate; and secondly, it was shown that bacteria not only could largely fulfill their cellular phosphorus need with 5'-nucleotidase, but also could liberate significant amounts of orthophosphate into the surrounding medium. According to tentative estimates

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by Ammerman & Azam (1985), this pathway of phosphorus regeneration could supply half the orthophosphate required by plankton.

In this study, results from 5'-nucleotidase activity measurements are presented from the brackish water Baltic Sea. Our previous studies have shown that for most of the season, nitrogen is the main limiting nutrient for the planktonic community in the area, but co-limitation by phosphorus has also been repeatedly demonstrated at some stages of the plankton succession (Tamminen 1982, Tamminen et al. 1985, Kivi et al. unpubl.). Therefore the regeneration mechanisms of phosphorus are of specific interest.

The diel cycle of 5'-nucleotidase activity and subsequent uptake of the released phosphate by both bacteria and phytoplankton was studied during a mesocosm experiment. The effect of the nutritional status of the planktonic community on phosphorus regeneration and uptake was studied after additions of inorganic nutrients (PO_4 and NH_4) and organic carbon (sucrose) to experimental units. The results are discussed together with measurements of direct $^{32}PO_4$ uptake, primary and bacterial productivity, chlorophyll *a* and inorganic and organic nutrients. The diel study was a part of a 3 d joint ecosystem experiment by project PELAG, Tvärminne Zoological Station, and the team of Åke Hagström, University of Umeå, Sweden.

MATERIAL AND METHODS

Experiment. The data was obtained from an enclosure experiment on the SW coast of Finland, Baltic Sea. The experiment started on 11 August 1986; the 100 l mesocosms were filled the previous evening with water collected from the surface layer outside the archipelago zone off Tvärminne Zoological Station, Hanko peninsula. Enclosures were incubated in situ in floating racks in close vicinity to the Zoological Station.

The 4 experimental units were manipulated according to Table 1 with inorganic nutrient and organic carbon additions, and samplings took place at 8 h intervals over the diel cycle (11 August at 09:00, 17:00 h, and 12 August at 01:00 and 09:00 h). The first sampling took place prior to substrate additions.

Table 1. Manipulations in the diel experiment. Substrate additions were 20 µg PO_4-P l^{-1}, 80 µg NH_4-N l^{-1} and 200 µg sucrose-C l^{-1}

Unit	P+N	Sucrose
1	–	–
2	+	–
3	–	+
4	+	+

5'-nucleotidase activity. ATP was used as a model substrate in 5'-nucleotidase activity measurements. ^{32}P-ATP hydrolysis and subsequent $^{32}PO_4$ uptake was assayed with the method reported by Ammerman & Azam (1985). Adenosine 5'-[γ-^{32}P]triphosphate (PB.168, Amersham International) was added in 0.1 nM concentrations except for a kinetic assay, where a concentration range of 0.05 to 1.0 nM was used. Incubations lasted for 2 h except for a time series assay (20 min to 7 h). Incubations were performed in situ in 20 ml bottles, in duplicate with a formaldehyde-killed blank (100 µl 38 % formaldehyde/20 ml sample). Time course incubations were done in triplicate without blanks. Incubations were terminated by a corresponding formaldehyde addition.

After incubations, samples were filtered onto 0.2 and 0.8 µm Nuclepore polycarbonate filters (8 ml onto each; Fractions A and B, respectively) and rinsed with an exact volume of prefiltered (Whatman GF/F) brackish water. The 0.2 µm filtrate was collected directly into a scintillation vial, and a 1 ml portion of it was diluted into 10 ml with distilled H_2O and measured directly for ^{32}P (Fraction C). The remaining portion of the filtrate was acidified with H_2SO_4 (0.06 N final concentration) and mixed with activated charcoal (ca 20 mg), after which a 8 ml portion of it was filtered onto 0.45 µm Gelman membrane filter and rinsed with 2 ml of distilled water. This filtrate was measured for ^{32}P as Fraction D.

In the kinetic assay, the filtered volumes and filtrate dilutions were adjusted on the basis of expected activities in each fraction because of the high levels of ^{32}P radioactivity.

All ^{32}P measurements from filtrates were performed with a LKB-Wallac 1219 RackBeta liquid scintillation counter directly from aqueous phase (Cerenkov radiation). The particulate radioactivities were counted after 10 ml addition of PCS scintillation cocktail (Amersham International) with the external channel ratio method. Counting efficiencies were ca 98 % (liquid scintillation) and 47 % (Cerenkov radiation).

The measured ^{32}P fractions (A to D) represented following processes: Fraction A (particulate 0.2 µm), total uptake of $^{32}PO_4$ released from ^{32}P-ATP; Fraction B (particulate 0.8 µm), algal uptake of released $^{32}PO_4$ (see Results); Fraction C (first filtrate), together with Fraction A the total amount of label added; Fraction D (after acidification and activated charcoal adsorption), the $^{32}PO_4$ released from ^{32}P-ATP but not taken up by the organisms (Ammerman & Azam 1985).

When treating a stock dilution of ^{32}P-ATP similarly to the filtrates (Fractions C and D), it was observed that 18.7 % of the ^{32}P was found in Fraction D after the

acidification and adsorption procedure (n=8, SD=1.1 %). This percentual blank value (% of label added) was subtracted from measurements of Fraction D in the time series assay. In the diel study, formaldehyde blanks were subtracted from Fraction D dpm in normal fashion. In the kinetic assay, blanks for Fraction D produced erroneous results for unknown reasons, and therefore only particulate fractions were calculated.

Total 5'-nucleotidase activity was calculated as the sum of hydrolyzed ^{32}P-ATP (A+D) divided by the label added (A+C), expressed per unit time as turnover rate (1/T; expressed as % h^{-1}). Different fractions (A, B and D) were calculated correspondingly.

Other measurements. Uptake of carrier-free $^{32}PO_4$ (PB.4, Amersham International) was measured with tracer (picomolar) additions, in 2 size fractions (0.2 and 0.8 µm; Nuclepore filters). Incubations were duplicates with a formaldehyde-killed blank (100 µl 38 % formaldehyde/20 ml sample), and incubations were terminated after 2 h with a corresponding formaldehyde addition. ^{32}P radioactivities were counted similarly to particulate ^{32}P fractions in ^{32}P-ATP assays, presented above, and calculated as turnover rate (1/T; expressed as % h^{-1}). Absolute uptake rates (v = units P l^{-1} h^{-1}) were calculated by multiplying PO_4 concentration by $^{32}PO_4$ turnover rate (h^{-1}).

Phosphate and ammonium were determined in duplicate for each sampling according to Koroleff (1976). Particulate and dissolved organic phosphorus were determined at the beginning and end of the diel study according to Solorzano & Sharp (1980). Particulate organic carbon and nitrogen were determined with a Hewlett-Packard 185B CHN analyzer. All glassware and glass-fibre filters (Whatman GF/F) in organic nutrient analyses were acid-washed and precombusted (450 °C). Prefiltration through 1.0 µm Nuclepore polycarbonate filters was used for fractionated particulate analyses. Chlorophyll *a* was determined fluorometrically (Turner 111) in 2 size fractions (total and 0.8 µm; Nuclepore polycarbonate filters) after ethanol extraction of duplicate samples (24 h at room temperature).

Primary productivity was measured with the ^{14}C method as apparent net productivity (dissolved and particulate organic ^{14}C; acidification of a 4 ml subsample) and particulate productivity in 2 size fractions as with $^{32}PO_4$ uptake. For details, see Niemi et al. (1983) and Bell & Kuparinen (1984). Bacterial productivity was measured with the ^{3}H-thymidine method (Fuhrman & Azam 1982) as in Bell & Kuparinen (1984). All incubations in bacterial and primary productivity measurements were duplicates with a blank, and they were performed in situ in 20 ml incubation vials. Blanks were dark (primary productivity) or formaldehyde-killed (bacterial productivity) replicates. Incubation time was 2 h and incubations were terminated by 38 % formaldehyde addition (100 µl/20 ml sample).

RESULTS

Size fractionation

Activity measurements were fractionated by filtrations onto 0.2 and 0.8 µm filters. The intention was to approximate bacterial and algal processes to the extent possible via mechanical separation. Contrary to previous results from the area and season, the attempt was not entirely successful.

Because of the expected (and observed; H. Kuosa, pers. comm.) occurrence of small cyanobacteria, the separation threshold was set low (0.8 µm). Mean size of bacterioplankton during the whole experiment was, however, exceptionally high for the area and season (0.078 μm^3; Å. Hagström pers. comm.), causing on average 69 % of bacterial productivity to remain in the >0.8 µm fraction at the start of the experiment. Virtually no attached bacteria could be observed by microscopy (Kuosa et al. 1989), and the fractionation result therefore reflected the size spectrum of free-living bacteria.

On average, only 5 % of both chlorophyll *a* and primary productivity passed 0.8 µm at the start of the experiment. Consequently, the <0.8 µm fraction represented bacterial activity, but as a significant underestimate. The >0.8 µm fraction represented (particulate) algal activity, but as a significant overestimate for nutrient uptake measurements, where the >0.8 µm portion of bacterioplankton interfered. When discussing 5'-nucleotidase activity and $^{32}PO_4$ uptake fractionations, these limitations apply. However, qualitative differences between the <0.8 µm and >0.8 µm fractions are attributed to the difference between bacterial and algal metabolism.

Hydrographical events prior to the experiment explain the size-fractionation results. A strong upwelling occurred just prior to the experiment, and surface temperature decreased by 7 to 8 °C. The mixed flagellate community typical for the season was replaced by a diatom community dominated by *Chaetoceros wighamii* and *Skeletonema costatum* (Kuosa et al. unpubl.). These are typical spring species in the area, and also the increased mean cell size of bacterioplankton closely resembles the normal spring situation.

Time course and kinetics of ^{32}P-ATP hydrolysis and hydrolyzed $^{32}PO_4$ uptake

During the diel experiment, time-series and kinetic assays were performed with ^{32}P-ATP from the control

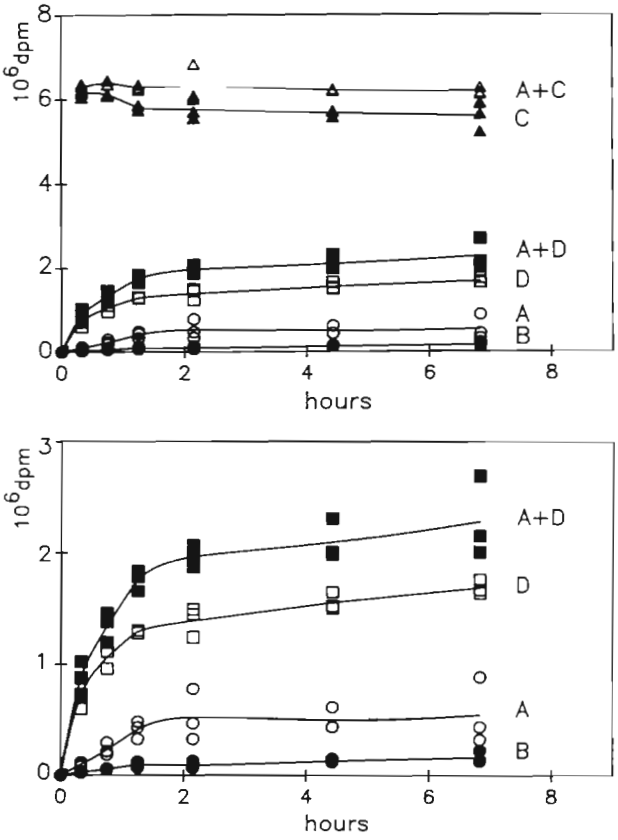

Fig. 1. Time series of the accumulation of radioactivity in different fractions in 5'-nucleotidase assay. Upper graph: all measured fractions of ^{32}P; lower graph: 5'-nucleotidase activity fractions. A: total particulate uptake of hydrolyzed $^{32}PO_4$ (>0.2 μm); B: fractionated (>0.8 μm) particulate uptake of hydrolyzed $^{32}PO_4$; C: 0.2 μm filtrate; A + C: total label added; D: hydrolyzed $^{32}PO_4$ not taken up by organisms; A + D: total hydrolysis. Curves are drawn through time-point averages

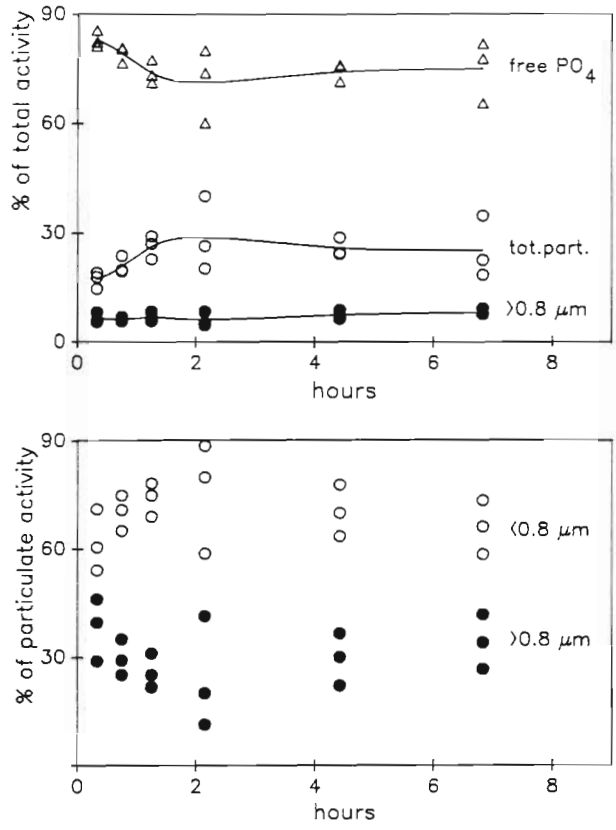

Fig. 2. Percentage distribution of total 5'-nucleotidase activity (upper graph) and particulate uptake of hydrolyzed $^{32}PO_4$ (lower graph) in the time-series assay. Curves are drawn through time-point averages. Free $^{32}PO_4$ = hydrolyzed $^{32}PO_4$ not taken up by organisms; tot.part. = total uptake of hydrolyzed $^{32}PO_4$ (>0.2 μm); >0.8 μm and <0.8 μm = size-fractionated uptake of hydrolyzed $^{32}PO_4$

unit, starting at 14:00 h. The time course of each ^{32}P fraction is presented in Fig. 1. The accumulation of free orthophosphate far exceeded particulate uptake during the first hour, after which it leveled to a linear increase. A corresponding shift was observed in total particulate uptake (Fraction A), where no increase was measured after 2 h. The algal fraction (B) increased steadily during the 7 h period.

The relative distribution of total activity and total particulate activity illustrates these shifts (Fig 2). After initial $^{32}PO_4$ release, the total particulate uptake steadily increased its share until a balance was achieved at around 2 h (Fig. 2A). The algal fraction (>0.8 μm) showed a constant proportion of total activity. When total particulate activity was divided into fractions below and above 0.8 μm (Fig. 2B), it could be seen that small free-living bacteria (<0.8 μm) dominated uptake of hydrolyzed $^{32}PO_4$ throughout the whole time-series experiment.

Particulate uptake kinetics were determined with added substrate concentrations of 0.05 to 1 nM (Fig. 3). Uptake followed Michaelis-Menten kinetics reasonably well in both size fractions ($r_{0.2\,\mu m} = 0.856$; $r_{0.8\,\mu m} = 0.801$). Maximum uptake rate was 3-fold in the total particulate fraction compared to the >0.8 μm fraction (V_{max}, 0.016 and 0.005 nmol l^{-1} h^{-1}, respectively). Corresponding half-saturation constants ($K+S_n$) were 0.62 and 0.72 nM.

Diel study: 5'-nucleotidase activity

The first sampling of the diel study took place prior to substrate additions. Total 5'-nucleotidase activity varied between 2.8 and 4.0 % h^{-1}, and the source of variation between the experimental units was the free $^{32}PO_4$ fraction (Fig. 4). Particulate uptake fractions were all uniform at the start of the experiment.

A general diel cycle expressed itself, as night-time

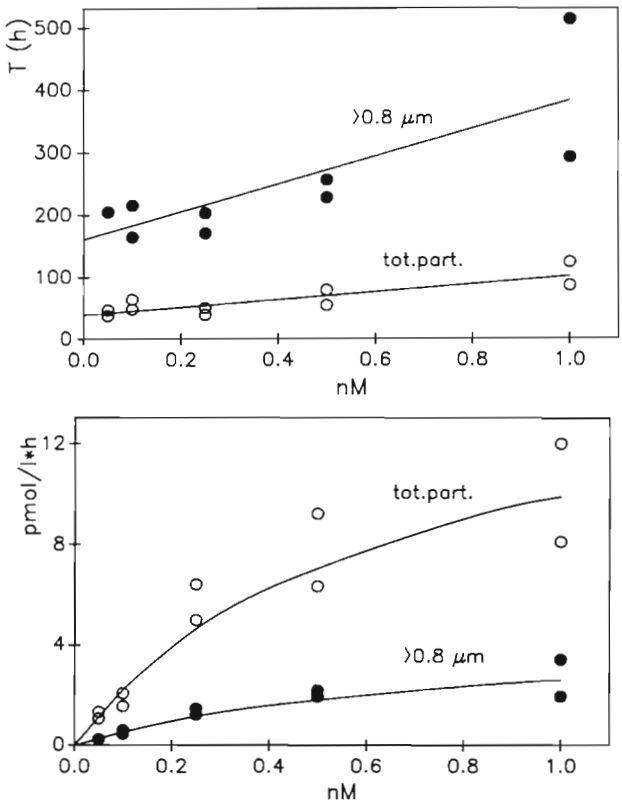

Fig. 3. Particulate uptake kinetics of hydrolyzed $^{32}PO_4$. Upper graph: turnover time (T) vs added ^{32}P-ATP (A); lower graph: uptake rate (v = A/T) vs added ^{32}P-ATP. Linear transformation of Michaelis-Menten kinetics (upper graph, T vs A) was applied to calculate the saturation curves (v vs A) in the lower graph.

activities were low in all units. In the control unit, the free $^{32}PO_4$ fraction was negligible at midnight, but the relative distribution of activity fractions, as well as the activity levels, of the next morning closely resembled the start situation (Fig. 4).

With inorganic nutrient and sucrose additions, distinct changes in this pattern were observed. In both units with added phosphate, the particulate uptake fractions decreased steadily during the experiment (Fig. 4). Total nucleotidase activity did not decrease correspondingly, however, as the free $^{32}PO_4$ fraction remained high also during night-time. The next morning, the free $^{32}PO_4$ fraction was the highest in the phosphate added units.

In the unit with sucrose addition, the free $^{32}PO_4$ fraction disappeared altogether during the first light period. Total nucleotidase activity, consisting entirely of particulate uptake, increased at the beginning of the second light period (Fig. 4).

When viewing the relative proportions of different fractions of total nucleotidase activity, the trends are evident (Fig. 5). With phosphate additions, the particulate fractions decreased and free $^{32}PO_4$ increased steadily. With only organic substrate added, the trends were the opposite (Fig. 5). Only the control unit showed a somewhat balanced diel cycle without trends in the distribution of the total activity.

Diel study: comparison of 5'-nucleotidase activity and $^{32}PO_4$ uptake

Half the added phosphate (20 µgP l^{-1}) had been depleted during the first 8 h of the experiment in both units with inorganic nutrient additions (Fig. 6). Because of the elevated PO_4 concentrations still prevailing, $^{32}PO_4$ turnover rates were low in these units. However, the absolute uptake rates (v) were still greatly enhanced (Fig. 6). At midnight, only negligible $^{32}PO_4$ uptake was measured in these units, and consequently phosphate concentrations remained high. At the beginning of the second light cycle, uptake rates (v) had recovered approximately to start levels in the phosphate-added units in spite of very low turnover rates.

PO_4 concentrations remained at low levels (0.9 to 2.4 µgP l^{-1}) in the units without inorganic nutrient additions (Fig. 6). The diel cycle of $^{32}PO_4$ turnover rate in the control unit resembled corresponding particulate values in 5'-nucleotidase activity measurements (Fig. 4), with a minimum at midnight. The succession of $^{32}PO_4$ turnover rate in the sucrose addition unit (Fig. 6) was almost identical to corresponding particulate values in 5'-nucleotidase activity measurements (Fig. 4), with maximum $^{32}PO_4$ turnover rates at the beginning of the second light cycle. Phosphate uptake rate (v) was clearly elevated in the sucrose addition unit in spite of very low PO_4 concentration.

The relation between 5'-nucleotidase activity and $^{32}PO_4$ uptake measurements was examined in different size fractions during the diel study (Fig. 7). Total 5'-nucleotidase activity was on average 3.1 times higher than $^{32}PO_4$ uptake (turnover rates), and the correlation between the measurements was modest (Fig. 7A). Total particulate ^{32}P in 5'-nucleotidase activity measurements correlated clearly better with $^{32}PO_4$ uptake (Fig. 7A).

Corresponding correlation between bacterial size fractions was nonexistent (Fig. 7B). In contrast, the size fraction of > 0.8 µm showed very good fit between the 2 measures, with their relation practically unity (Fig. 7C).

Diel study: nutritional background

The experiment started during a stage of low biomass and high turnover of the community after a recent upwelling. Chlorophyll *a* values were ca 2 µg l^{-1}, primary productivity ca 6 µgC l^{-1} h^{-1} and bacterial productivity ca 0.2 µgC l^{-1} h^{-1}, corresponding to ca 4 % of

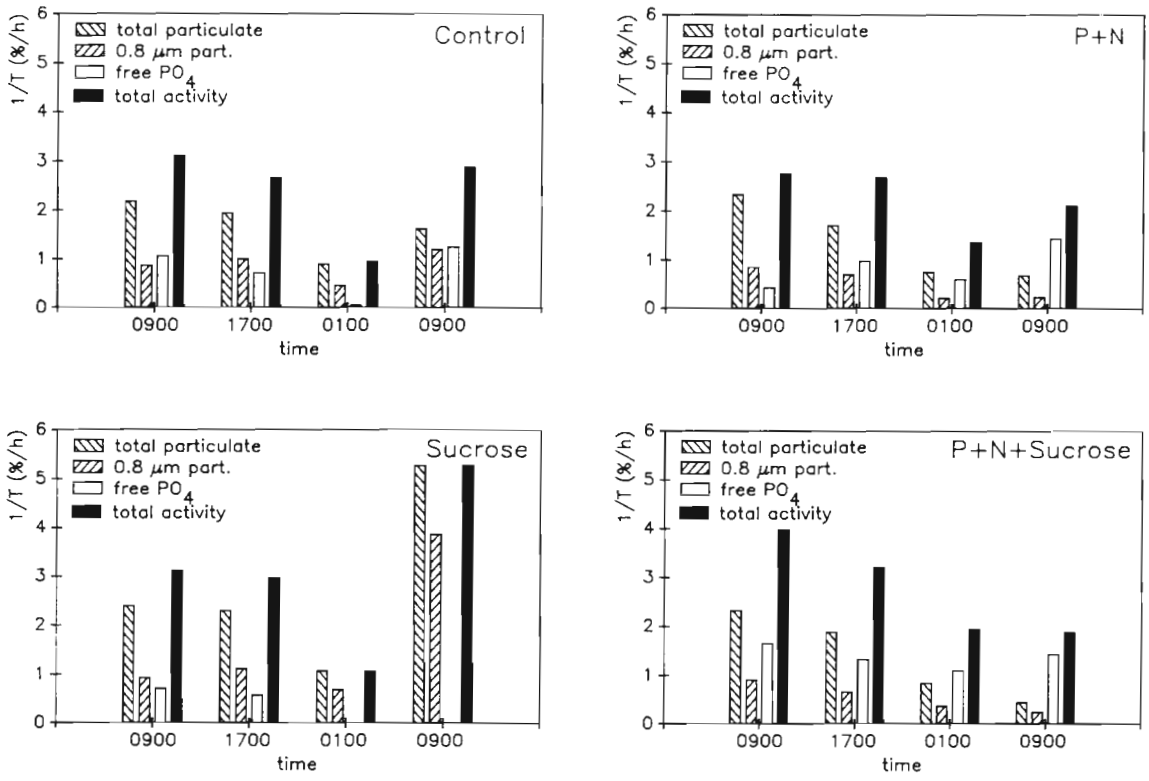

Fig. 4. Fractions of 5'-nucleotidase activity in different experimental units during the diurnal study. Control = no manipulations; P+N = PO_4 (20 µgP l^{-1}) and NH_4 (80 µgN l^{-1}) additions; Sucrose = 200 µgC l^{-1} sucrose addition; P+N+Sucrose = all additions, respectively. Total particulate = total uptake of hydrolyzed $^{32}PO_4$; 0.8 µm part. = > 0.8 µm particulate uptake of hydrolyzed $^{32}PO_4$; free PO_4 = hydrolyzed $^{32}PO_4$ not taken up by organisms; total activity = total 5'-nucleotidase activity

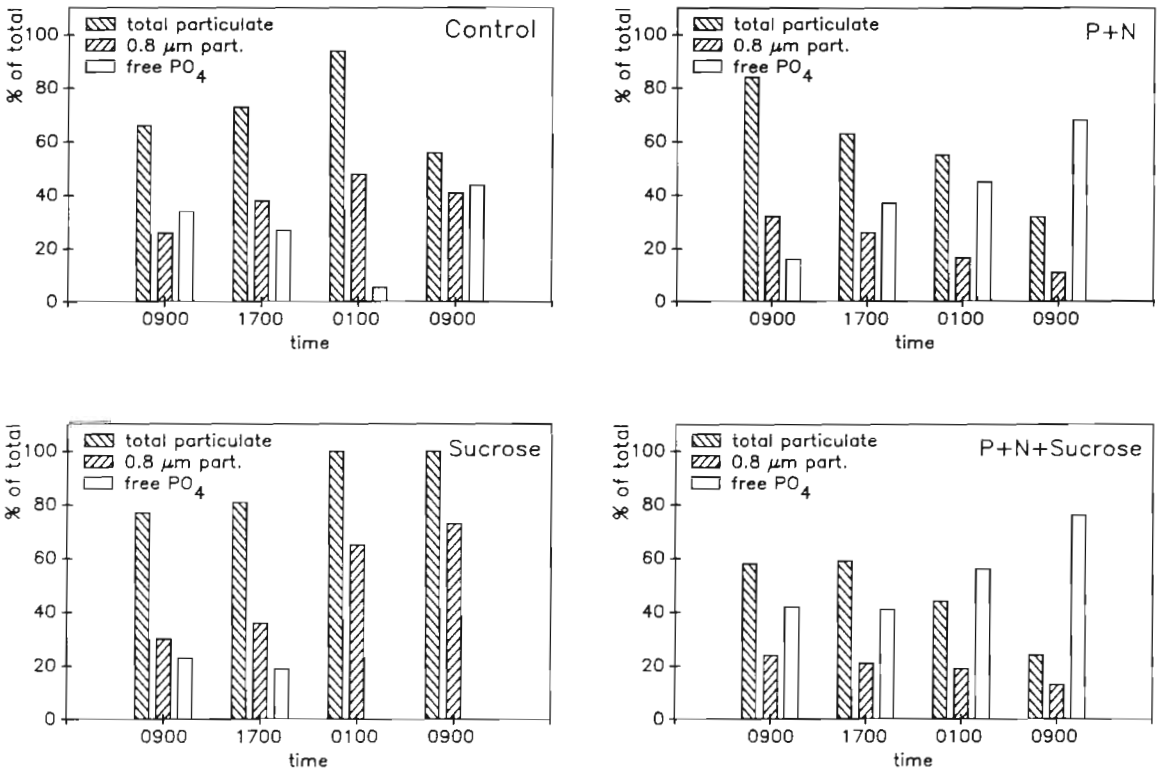

Fig. 5. Percentage distribution of total 5'-nucleotidase activity in different experimental units during the diurnal study. Figure denotations as in Fig. 4

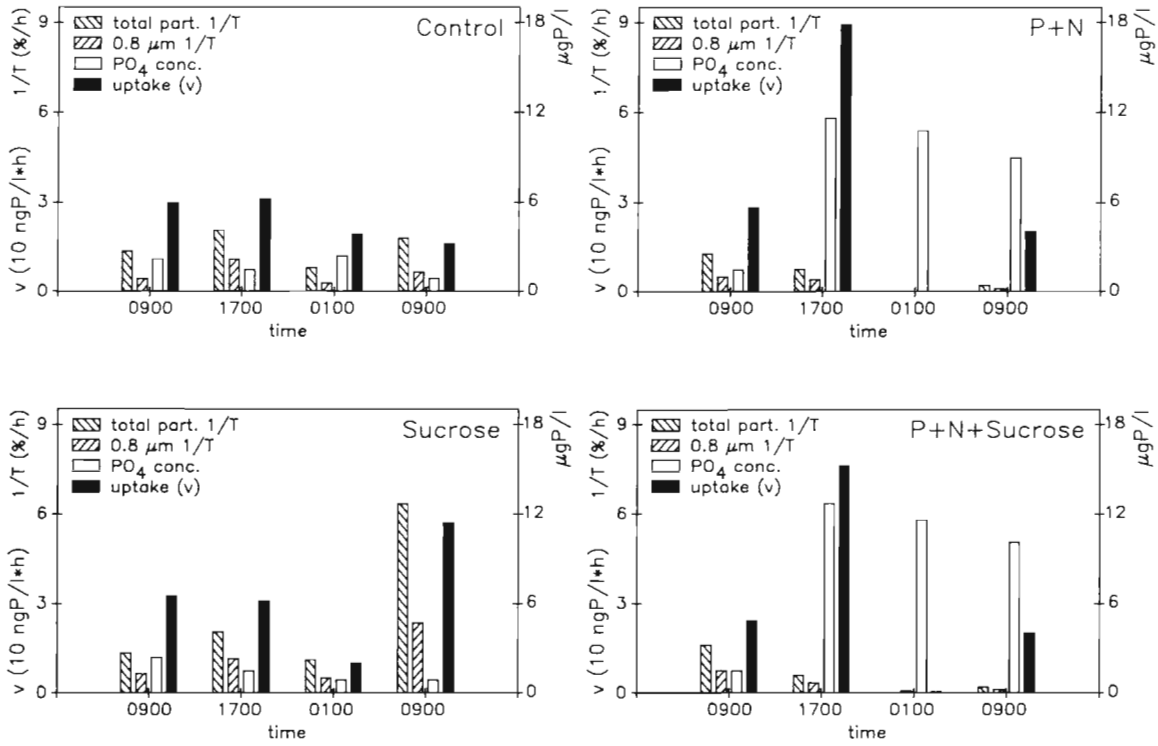

Fig. 6. Uptake of added $^{32}PO_4$ and PO_4 concentrations in different experimental units during the diel study. Total part. 1/T = total turnover rate (% h^{-1}) of $^{32}PO_4$; 0.8 µm 1/T = turnover rate (% h^{-1}) of $^{32}PO_4$ due to > 0.8 µm organisms; PO_4 conc. = chemically analyzed orthophosphate concentration; uptake (v) = PO_4 concentration (µgP l^{-1}) multiplied by $^{32}PO_4$ turnover rate (h^{-1}), scaled to ngP l^{-1} h^{-1}

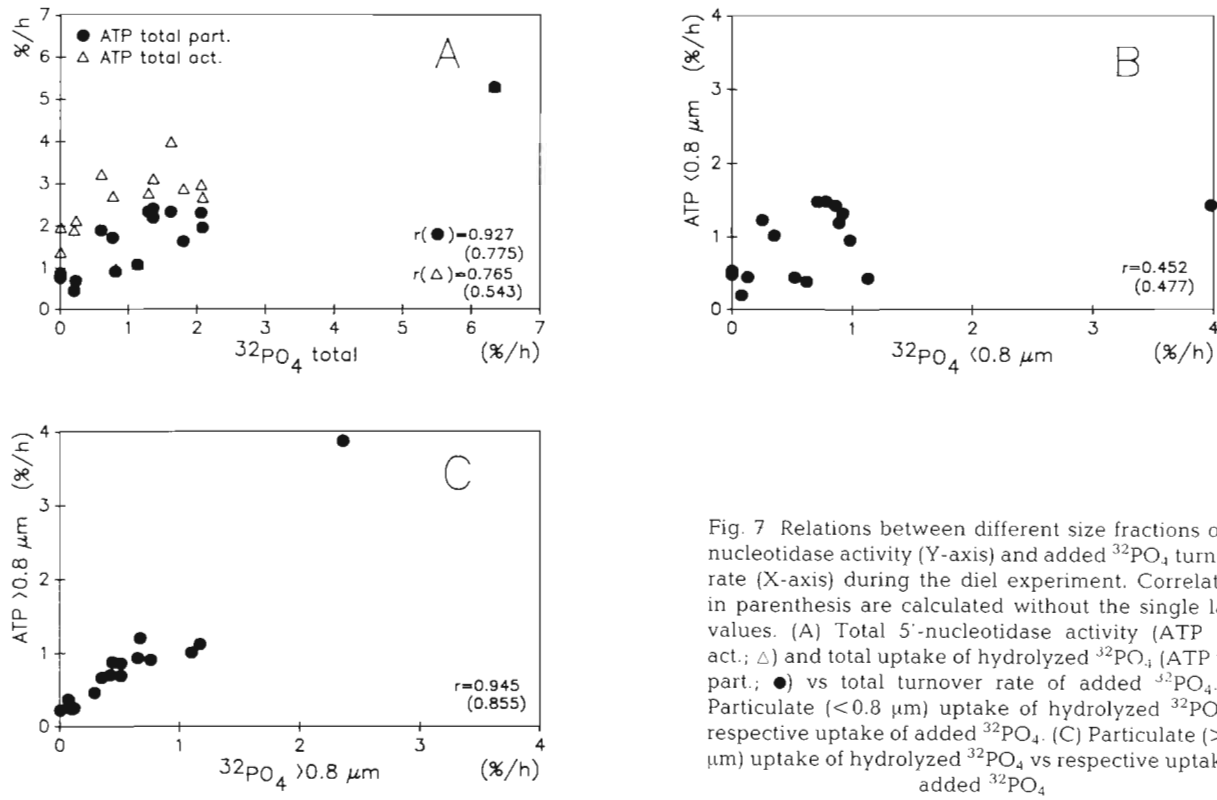

Fig. 7 Relations between different size fractions of 5'-nucleotidase activity (Y-axis) and added $^{32}PO_4$ turnover rate (X-axis) during the diel experiment. Correlations in parenthesis are calculated without the single large values. (A) Total 5'-nucleotidase activity (ATP total act.; △) and total uptake of hydrolyzed $^{32}PO_4$ (ATP total part.; ●) vs total turnover rate of added $^{32}PO_4$. (B) Particulate (< 0.8 µm) uptake of hydrolyzed $^{32}PO_4$ vs respective uptake of added $^{32}PO_4$. (C) Particulate (> 0.8 µm) uptake of hydrolyzed $^{32}PO_4$ vs respective uptake of added $^{32}PO_4$

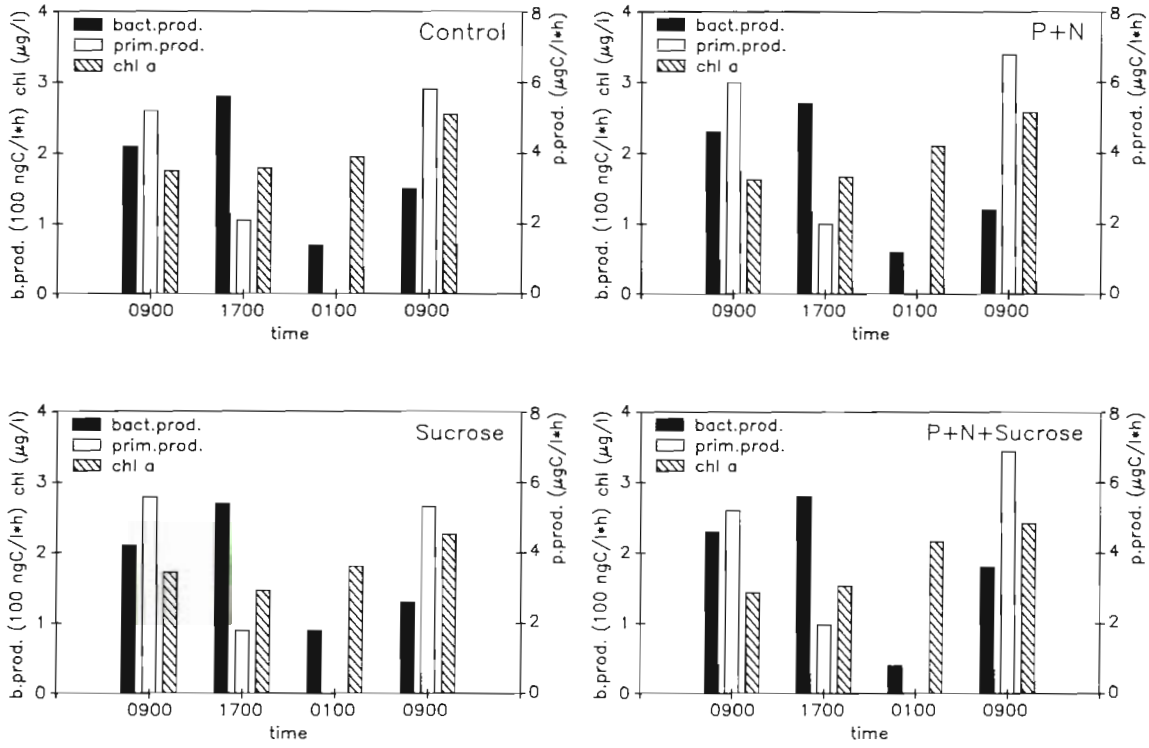

Fig. 8. Bacterial productivity, primary productivity (apparent net productivity) and chlorophyll *a* in different experimental units during the diel study

primary productivity. None of these variables responded to the substrate additions during the first day of the experiment (Fig. 8).

During the second light period, however, distinct stimulations of all these variables evolved, leading to significant changes in the structure and functioning of the ecosystem (Kuosa et al. unpubl.). The highest values at the beginning of the third light period were for chlorophyll *a*, 7 µg l^{-1} (Units 2 and 4); for primary productivity, 18 to 21 µgC l^{-1} h^{-1} (Units 2 and 4); for bacterial productivity, 1 to 1.7 µgC l^{-1} h^{-1} (Units 2 and 4); and for $^{32}PO_4$ uptake rate, 0.77 µgP l^{-1} h^{-1} (Unit 3) (project PELAG, unpubl.). Nutrient dynamics during the first 24 h form the basis for these stimulations.

At the beginning of the experiment, phosphate represented on average 10 % and particulate P 29 % of total phosphorus (Table 2). The inorganic nutrient additions approximately doubled the total phosphorus pool. After 24 h, half the phosphate had been depleted, and particulate phosphorus percentage concentrations had increased to the start level (Table 2).

The particulate nutrient ratios reflected this change (Table 3). In the beginning of the experiment, particulate ratios deviated from Redfield ratios (Redfield et al. 1963) towards N and P shortage compared to C. The size fraction of < 1 µm showed a more pronounced lack of phosphorus compared both to N and C. After 24 h, both units with inorganic nutrient additions had clearly

Table 2. Percentage distribution of total phosphorus during the diel study. DOP: dissolved organic phosphorus; POP: particulate organic phosphorus. Start = mean of the enclosures before enrichments

	PO_4	DOP	POP
Start (09:00 h)	10	61	29
After P+N enrichments	55	31	14
24 h (09:00 h)			
Control	4	59	37
P+N	27	44	28
Sucrose	5	67	28
P+N+Sucrose	31	39	29

Table 3. Particulate nutrient ratios (w:w:w) during the diurnal study. Start = mean of the enclosures before enrichments

	C:N:P
Redfield ratios	*41·7.2.1*
Start (09:00 h)	
Total	79:8.0.1
< 1 µm	98:12:1
24 h (09:00 h)	
Control	85:8.5:1
P+N	66:7.5:1
Sucrose	106:12·1
P+N+Sucrose	66:7.3:1

approached Redfield ratios, with the control unit remaining at start levels (Table 3). The unit with sucrose addition showed ratios which indicated increased phosphorus shortage. The ratios in this unit were practically identical to the start values of the <1 µm size fraction (Table 3).

The ratios of inorganic C:P in uptake were compared in different size fractions (Table 4). In the beginning of the experiment, carbon greatly exceeded phosphorus in uptake, compared both to the corresponding Redfield ratio and that of particulate nutrients (Table 3). This was especially pronounced in the algal size fraction (>0.8 µm). Towards evening, uptake ratios clearly decreased in all units and size fractions, to below the ratios of particulate nutrients. Most significant changes were observed in the units with phosphate additions. In these units, phosphate was assimilated at rates which were double the Redfield ratio in relation to carbon.

The next morning, the control unit showed again very high inorganic carbon uptake compared to phosphate, especially in the >0.8 µm size fraction (Table 4). Phosphate added units had lower uptake ratios, but the unit with sucrose addition deviated clearly from others. The total particulate uptake ratio was about half the corresponding ratio of particulate nutrients (Table 3).

Depletion rates for added inorganic nutrients (Table 5) were calculated on the basis of nutrient concentrations (concentration change per unit time). During the first 8 h after enrichments, the average depletion rates of phosphate were very high, compared to the next 8 h periods (Table 5), to $^{32}PO_4$ uptake rates after 8 h (v; Fig. 6), or to corresponding NH_4 depletion rates (Table 5). The N:P ratio in nutrient depletion during the first 8 h period was 3.1 to 3.4, and for the whole 24 h period, 3.2 to 3.7 (Table 5). These values indicate very effective phosphate uptake (depletion) in relation to nitrogen, as they are approximately half the corresponding Redfield ratio (7).

DISCUSSION

5'-nucleotidase activity

The measurement of 5'-nucleotidase activity is based on the observation that bacteria do not take up intact

Table 4. Inorganic uptake ratios (C:P, w:w) in different size fractions during the diel study. C: primary productivity; P: $^{32}PO_4$ uptake rate (v). Start = mean of the enclosures before enrichments

		C = apparent net P = tot.part.	C = tot.part. P = tot.part.	C = >0.8 µm P = >0.8 µm
Start	(09:00 h)	190:1	160:1	330:1
8 h	(17:00 h)			
	Control	67:1	58:1	82:1
	P+N	23:1	19:1	27:1
	Sucrose	59:1	51:1	69:1
	P+N+Sucrose	26:1	21.1	24:1
24 h	(09:00 h)			
	Control	360:1	260:1	520:1
	P+N	330:1	220:1	380:1
	Sucrose	93:1	66:1	150:1
	P+N+Sucrose	340:1	230:1	300:1

Table 5. Depletion rates (µg l^{-1} h^{-1}) and ratios (w:w) of added inorganic nutrients during the diel study. Nutrient additions were 20 µg PO_4-P l^{-1} and 80 µg NH_4-N l^{-1}

		09:00–17:00 h	17:00–01:00 h	01:00–09:00 h	*24 h*
PO_4					
	P+N	1.33	0.11	0.23	*0.55*
	P+N+Sucrose	1.10	0.14	0.19	*0.48*
NH_4					
	P+N	4.11	0.71	0.48	*1.77*
	P+N+Sucrose	3.73	0.69	0.80	*1.74*
N:P					
	P+N	3.1	6.3	2.1	*3.2*
	P+N+Sucrose	3.4	5.0	4.3	*3.7*

ATP, but that it is hydrolyzed on the cell surface or in the periplasmic space (Bengis-Garber & Kushner 1981, 1982). The γ-, β-, and α-phosphates of ATP are cleaved sequentially by the 5'-nucleotidase, and only after hydrolysis, can phosphates or the adenosine moiety of ATP be assimilated into cellular material (Bengis-Garber & Kushner 1981, 1982).

The time-course results illustrate the functioning of 5'-nucleotidase activity (Figs. 1 and 2). The free hydrolyzed $^{32}PO_4$ fraction dominated activity fractions at the beginning of the incubation, but particulate uptake steadily increased its share of the total activity. The smallest size-fraction clearly responded first to the available free $^{32}PO_4$, and the >0.8 μm fraction showed far slower accumulation. Uptake kinetics of the particulate fractions (Fig. 3) confirmed the significantly higher affinity of the <0.8 μm fraction for hydrolyzed $^{32}PO_4$.

The size distribution of 5'-nucleotidase activity clearly differed from corresponding bacterial productivity measurements with the ^{3}H-thymidine incorporation method. Over 2/3 of thymidine incorporation took place in the >0.8 μm fraction at the beginning of the diel experiment. Less than 50 % of the uptake of hydrolyzed $^{32}PO_4$ occurred in the corresponding fraction at the beginning of the diel experiment, in the time series or in the kinetic assay. When considering that $^{32}PO_4$ uptake in the >0.8 μm fraction represents also algal uptake, these results indicate significantly higher nutrient uptake of small, recently divided bacterial cells, compared to large cells incorporating thymidine into DNA and undergoing cell division.

Whether the smallest cells also initially dominated ATP hydrolysis cannot be rigorously stated on the basis of the data, as size-fractionations were performed after incubations. However, the comparison of the size fractions with direct $^{32}PO_4$ uptake measurements supports this likely assumption (Fig. 7). While uptake of hydrolyzed and added $^{32}PO_4$ in the >0.8 μm fraction correlated very well (Fig. 7C), indicating that they were measuring the same process, no correlation was found between the <0.8 μm fractions (Fig. 7B). The smallest bacteria obviously attained their PO_4 largely from the newly hydrolyzed $^{32}PO_4$ before it mixed with bulk-phase PO_4.

5'-nucleotidase activity results after the enrichments supported the observation by Ammerman & Azam (1985) of the varying degree of coupling between 5'-nucleotide hydrolysis and the uptake of hydrolyzed $^{32}PO_4$. Phosphate additions caused clear inhibition of hydrolyzed $^{32}PO_4$ uptake, but not of 5'-nucleotide hydrolysis (Figs. 4 and 5). This situation represented significant uncoupling between $^{32}PO_4$ hydrolysis and uptake, and up to 80 % of the hydrolyzed phosphate was released to the environment.

The uptake inhibition was not, however, as severe as corresponding inhibition of added $^{32}PO_4$ uptake (Fig. 6). This further suggests that hydrolyzed $^{32}PO_4$ was to a large extent immediately taken up by the hydrolyzing bacteria.

Sucrose addition greatly stimulated 5'-nucleotidase activity (Fig. 4). All hydrolyzed $^{32}PO_4$ was immediately taken up (Figs. 4 and 5), the situation thus representing complete coupling between $^{32}PO_4$ hydrolysis and uptake.

P dynamics in mesocosms

The ratios between particulate nutrients at the beginning of the experiment indicated shortage of both N and P (Table 3). High depletion rates of both NH_4 and PO_4 after enrichments (Table 5) confirmed this, and particulate nutrient ratios had clearly approached Redfield ratios the next morning as a result of intensive nutrient assimilation (Table 3).

This surge-like nutrient uptake did not lead to immediate stimulation of either bacterial or primary productivity, or chlorophyll *a* (Fig. 8). Also, phosphate was depleted far in excess of the Redfield ratio (N:P), both during the first 8 h period and over the whole diel cycle (Table 5). These results illustrate the nutrient storage capacity of the community and consequently, the temporal uncoupling of nutrient assimilation and growth processes.

This temporal uncoupling could also be seen in measures specific both for algae and bacteria. $^{32}PO_4$ uptake increased considerably in relation to primary productivity in all size fractions towards evening, especially in the phosphate-addition units (Table 4), and continued through the night in the control unit (Fig. 6). Although the algal size fraction (>0.8 μm) showed inorganic C:P uptake ratios which were twice as large as total particulate uptake ratios in morning incubations, this difference decreased significantly towards evening (Table 4). These results show that algal nutrient uptake ratios oscillated drastically around the particulate (biomass) nutrient ratios over the diel cycle. Phosphate assimilation during periods of low or nonexistent photosynthesis was essential to retain balanced nutrient ratios in biomass.

The temporal uncoupling of bacterial nutrient assimilation and cell division (growth), observed in the size-distribution discrepancy between ^{3}H-thymidine incorporation and hydrolyzed $^{32}PO_4$ uptake, was further illustrated by the responses in the unit with sucrose addition. Sucrose addition resulted in significant carbon excess in relation to phosphorus in the particulate nutrient pools after 24 h (Table 3). As a consequence, clearly the lowest C:P ratios in uptake were found in the beginning of the second light cycle (Table 4). Initial carbon assimilation by bacterioplank-

ton may have caused relative shortage of phosphorus in biomass, and the high values of both $^{32}PO_4$ uptake (Fig. 6; Table 4) and 5'-nucleotidase activity (Fig. 4) reflected the attempt to balance the nutritional conditions in bacterial biomass. Both enhanced carbon and, successively, phosphorus assimilation were thus observed before any response in bacterial growth (^{3}H-thymidine incorporation) was detected (Fig. 8).

Depletion ratios for added nutrients indicated more effective phosphate assimilation compared to nitrogen (Table 5). Phosphate depletion rates were among the highest observed in the area (unpubl.). They were comparable to maximum values previously obtained during late stages of spring bloom, when algal biomass and primary productivity were an order of magnitude higher than during this experiment. The recent upwelling had created an exceptional situation for the late summer period, when phytoplankton in the area is normally clearly nitrogen-limited (Kivi et al. unpubl.).

Conclusions: 5'-nucleotidase in P dynamics

Ammerman & Azam (1985) demonstrated that bacterial 5'-nucleotidase was not sensitive to prevailing PO_4 concentrations, in contrast to algal alkaline phosphatase. This ecologically important observation was supported by the results from our enrichment experiment.

The coupling between 5'-nucleotide hydrolysis and uptake of hydrolyzed PO_4 was, however, dependent on the nutritional conditions of the community. As suggested by Ammerman & Azam (1985), elevated ambient PO_4 concentrations loosened the coupling, leading to the release of up to 80 % of the hydrolyzed PO_4 into the environment. Although the planktonic community appeared to be limited at least partially by phosphorus, the control unit showed daytime release values of 20 to 40 % of the hydrolyzed PO_4. When bacterioplankton was supplied with organic carbon, 100 % of the hydrolyzed PO_4 was taken up. The amount of excess hydrolyzed PO_4, available to phytoplankton, thus seemed to be a sensitive indicator of the severity of bacterioplankton P limitation.

Direct comparison between quantities of orthophosphate uptake and the uptake of PO_4 hydrolyzed from 5'-nucleotides is not possible because concentration data on dissolved 5'-nucleotides in the area are not available. For order-of-magnitude comparison, literature values were used. Concentrations of total dissolved 5'-nucleotides in seawater approximate 10 to 20 nmol l^{-1} (Ammerman & Azam 1985 and references therein), which would represent ca 2 to 6 % of total dissolved phosphorus during the experiment. Assuming this concentration range, the observed 5'-nucleotidase activity in daytime incubations during the experiment (2 to 5 % h^{-1}) would have released PO_4 on the order of 6 to 30 ng PO_4–P l^{-1} h^{-1}. Corresponding uptake rates of added $^{32}PO_4$ were on the order of 15 to 90 ng PO_4–P l^{-1} h^{-1}.

It seems therefore that bacterial 5'-nucleotidase represented a mechanism of organic phosphorus regeneration which was quantitatively significant in the phosphorus dynamics of the whole planktonic community. A large fraction of the hydrolyzed PO_4 could be available to phytoplankton, depending on the nutritional conditions for bacterioplankton – which are in turn regulated by DOM release by nanoflagellates and algal exudation. The results therefore support the concept of tight metabolical coupling between pico- and nanoplanktonic bacteria, algae and flagellates, and these microaggregates having an important position in the nutrient cycles of nutrient-depleted surface waters (Goldman 1984).

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