Paper I

Algal – bacterial competition for phosphorus from dissolved DNA, ATP, and orthophosphate in a mesocosm experiment

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Abstract

We measured the turnover of phosphorus (P) from radioactive labelled dissolved DNA (dDNA), ATP and orthophosphate, and the partitioning of P from these sources into different size fractions of algae and bacteria in nutrient-manipulated mesocosms. There was a transition from uptake dominated by larger organisms during balanced enrichment towards uptake dominated by smaller organisms during nitrogen (N) enrichment (P-starvation). Contrary to expectation, this effect was counteracted by glucose enrichment, probably because bacterial cells increased in size in a glucose-amended mesocosm. During P-starvation, estimates of biomass-specific affinity for all substrates were consistent with uptake becoming limited by molecular diffusion transport towards the cells. Dissolved organic phosphorus (DOP) turnover times (T) fell to ~5 min for ATP and ~1.5 hour for dDNA (compared to 1.1 and 15.6 hours, respectively, during balanced enrichment), and coincided with little inorganic P liberated from DOP in the water, and reflected a tight coupling between hydrolysis and uptake in this situation. At one time during the experiment, the ability of algae and bacteria to compete for P was also assessed by the combination of isotope dilution experiments and affinity estimates. High affinity and low values of the term $K+S_n$ (the half saturation constant + the natural concentration of bioavailable substrate) when the 1 – 0.2 μm size fraction was compared to the >1 μm size fraction for all substrates indicated bacterial supremacy while competing for both inorganic and organic P. No significant shift in algal – bacterial competition for DOP relative to dissolved inorganic phosphate (DIP) was found.

Introduction

As osmotrophic organisms, both phytoplankton and bacteria can meet their need for phosphorus (P) by the uptake of dissolved forms of P through the cell membrane, probably mainly in the form of orthophosphate (PO_4^{3-}) (Cembella et al. 1984). When free PO_4^{3-} is depleted from the environment, PO_4^{3-} needs to be released from dissolved organic P (DOP) by extracellular enzymes prior to intracellular metabolism (Chróst 1990). The released molecule may be intermediately mixed into the ambient background pool of free PO_4^{3-}, and the entire competition for P then centres around the single molecule. Alternatively, the released molecule may be physically connected to the cell before its uptake, which potentially shifts P competition to favour those organisms that possess membrane-bound DOP-hydrolyzing enzymes.

Bacteria often dominate the uptake of free PO_4^{3-}, both in marine and limnic systems, when ambient PO_4^{3-} concentrations are low (Paerl and Lean 1976; Currie and Kalff 1984; Thingstad et al. 1993). It has thus been speculated that phytoplankton may use DOP as an alternative source of P (cf. Cotner and Wetzel 1992). However, previous studies on the utilization of different P sources by phytoplankton and bacteria seem contradictory. Autoradiographic methods on whole lake water provided evidence that initial PO_4^{3-} uptake was by bacteria and that phytoplankton subsequently utilized DOP that had been excreted from bacteria (Paerl and Lean 1976; Currie and Kalff 1984). However, similar methods demonstrated that most of the PO_4^{3-} uptake was by algae, and that the excreted P was taken up by bacteria in Lake Michigan (Tarapchak and Moll 1990). Using an isotope dilution of $^{32}PO_4^{3-}$ with unlabelled DOP compounds, Berman (1988) demonstrated that bacteria dominated DOP uptake in Lake Kinneret. Studies in estuarine and marine environments have shown that the activity of the membrane bound 5'-nucleotidase (5PN), which hydrolyzes 5'-

nucleotides and regenerates PO_4^{3-}, is usually concentrated in the bacterial size fraction (Ammerman and Azam 1985; Ammerman and Azam 1991*a*). Some of the PO_4^{3-} released by 5PN may be taken up directly without intermediate mixing (Tamminen 1989, Ammerman and Azam 1991*a*), which potentially favours bacteria in the competition for P. This corresponds to the findings that 5'-nucleotides and dissolved DNA (dDNA) are taken up primarily by bacteria in marine (Paul et al. 1987; Turk et al. 1992) and freshwater environments (Siuda and Güde 1996; Siuda et al. 1998).

At low nutrient concentrations, the uptake efficiency and competitive ability of the organism is characterized by the biomass-specific affinity, defined as the volume of water cleared for substrate per unit biomass per unit time. Biomass-specific affinity is thus analogous to the clearance rate of a phagotrophic organism. Both bacterial and algal uptake of nutrients is usually described using the Michaelis-Menten type of saturation kinetics (see Li 1983; Bentzen and Taylor 1991), which is a hyperbolic function of uptake velocity versus substrate concentration. Here, affinity (α) corresponds to the slope of this function and approach to its maximum as the substrate concentration approaches zero. Maximum biomass-specific affinity (α_{max}) can thus be related to the familiar Michaelis-Menten parameters of maximum uptake rate (V_{max}) and half saturation constant (K) through the equation $\alpha_{max} = V_{max}/KB$, where B is the biomass. V_{max} and α_{max} describes how efficient organisms take up substrates at high and low substrate concentrations, respectively. K, however, has no such clear function (Aksnes and Egge 1991). Maximum diffusive transport towards a spherical cell of radius r is given by the expression $4\pi DrS$, where D is the molecular diffusion constant for the substrate in water and S the substrate concentration at an infinite distance from the cell. Assuming that the cell is diffusion-limited, i.e., that the cell's uptake system is so efficient (and the bulk nutrient concentration so low) that all substrate molecules hitting the cell

surface are captured, it is possible to derive a theoretical expression for α_{max} for a spherical cell of radius r (Thingstad and Rassoulzadegan 1999):

$$\alpha_{max} = 3D/(\sigma r^2) \qquad (1)$$

where σ is the volume-specific content of the element in question. The inverse proportionality of α_{max} to the second power of the cell radius indicates that spherical bacteria should be superior competitors to phytoplankton because of the difference in size. However, under specific environmental conditions, a reduction of σ may increase the affinity of larger osmotrophic organisms to a level comparable to that of 'normal-sized' bacteria (Thingstad et al. 2005).

Of the studies that have examined competition for PO_4^{3-} and DOP by means of the relative uptake in 'algal' and 'bacterial' size fractions, most concluded that heterotrophic bacteria acquire P more effectively than phytoplankton at low concentrations in nature, whereas, as concentrations increase, an increasing proportion is acquired by phytoplankton (Cotner and Wetzel 1992; Güde et al. 1992; Thingstad et al. 1993). Güde et al. (1992) hypothesized that the quantity of P compounds, and not the quality, is the decisive factor for the competition between phytoplankton and bacteria, i.e., that bacteria are superior competitors at constantly low concentrations and phytoplankton at high. However, estimated biomass-specific values for PO_4^{3-} affinity in bacteria and phytoplankton in P-starved systems may be comparable (Vadstein 1998; Tanaka et al. 2003), which suggests that the putative superiority of heterotrophic bacteria should be viewed with some caution. Although some attempts to compare the abilities of algae and bacteria to acquire P from sources other than PO_4^{3-} have been made, limited information exists on algal – bacterial competition for DOP as a function of their biomass-specific affinities. Most research has focused on competition for

dissolved inorganic P (DIP; i.e. PO_4^{3-}), and it is not yet known whether competition for PO_4^{3-} reflects competition for DOP. If competition for DOP does indeed shift relative to competition for PO_4^{3-}, the ratio between organic and inorganic forms of P released from the food web would be expected to influence strongly the outcome of algal – bacterial competition for P, and thus the structure and function of P-limited microbial food webs.

In the present study, ATP was used as a model substrate for monomeric DOP and DNA as a model substrate for polymeric DOP. Turnover and partitioning of radiolabelled P from these two sources between different size fractions of marine osmotrophs, as well as the estimated affinity of bacteria and algae, were compared to corresponding data for PO_4^{3-} during a mesocosm experiment. This gave us the opportunity to compare the algal – bacterial competition for inorganic and organic dissolved P in systems manipulated to different P, N, and organic-C status. We also performed isotope dilution experiments and P uptake size fractionation, using ^{33}P-labeled ATP, DNA and PO_4^{3-}, to explore the kinetic parameters of algal and bacterial P uptake.

Materials and methods

Experiment

The data were obtained from an enclosure experiment conducted during July 2003 outside the archipelago zone off Tvärminne Zoological Station, Hanko Peninsula on the SW coast of Finland in the Baltic Sea. Experimental setup and sampling procedures are described elsewhere (Tanaka et al. in press). The data were obtained from three enclosures (volume ~50 m^3) from 01 to 15 July. From 01 - 05 July, referred to as the boosting period, all mesocosms were supplemented daily with inorganic N and P in a ratio (mol: mol) of 16 in order to induce phytoplankton blooms. During the remainder of the experiment (henceforth “the experimental period”), inorganic nutrients and organic carbon were added daily to each mesocosm. One

mesocosm, referred to as NP, received N and P in a molar ratio of 16. The second mesocosm, referred to as 5N, received an increased amount of N five times greater than that added to NP. The third mesocosm, referred to as 5NC, received N as in 5N, and labile organic C. 5N and 5NC did not receive P during the experimental period. N, P, and organic C were added as aqueous solutions of NH_4Cl, KH_2PO_4, and glucose, respectively. Silica was added as $Si(OH)_4$ when the ambient Si concentration dropped below 3 μmol L^{-1} to avoid Si limitation of diatoms. Nutrient additions during the experimental period are shown in Table 1. To circumvent unexpectedly rapid increases of DIN concentration, N addition was reduced in 5N and 5NC during the experimental period as described in Table 1.

Phosphate measurements and biomass estimations

Soluble reactive P (SRP) was measured according to Koroleff (1983). Since SRP measured by the molybdenum blue method may include compounds other than free PO_4^{3-}, the ambient PO_4^{3-} concentrations were estimated by multiplying turnover time (T) of PO_4^{3-} by PO_4^{3-} uptake rate as derived from stoichiometric conversion of carbon based primary and bacterial production (Moutin et al. 2002). This method, including a detailed description of the theory and analytical model leading to the estimates, is described in Tanaka et al. (in press).

The bacterial P-biomass was calculated after microscopic counting of DAPI-stained preparates (Tanaka et al. in press). A constant biomass of 18.5 fg C $cell^{-1}$ and a C:P molar ratio of 50 was assumed for coccoid bacteria (Fagerbakke et al. 1996). For filamentous bacteria, a conservative C biomass of 0.22 pg C μm^{-3} (Bjørnsen 1986) and a C:P molar ratio of 150, which is in the upper range for P limited bacteria (Vrede et al. 2002), was assumed. Chlorophyll *a* (Chl *a*) in the 10 – 1 μm and the >10 μm size fractions was measured according to Jespersen and Christoffersen (1987) and used for estimating phytoplankton P-biomass in the corresponding size fractions. A C:Chl *a* ratio of 20 (w: w) and a C:P molar ratio of 106

was assumed. Total Chl *a* was measured every day, whereas size fractionation of Chl *a* was done irregularly. Therefore, linear extrapolation between adjacent sampling points was performed three times; once during the boosting period and twice during the experimental period. The correspondent size fractionation between ^{33}P uptake measurements and Chl *a* measurements alleviated the error-prone differentiation between the two size fractions of phytoplankton that would occur if the phytoplankton P-biomass was based solely on phytoplankton counts. We therefore chose to base phytoplankton P-biomass on Chl *a* measurements. Phytoplankton P-biomass estimates based on counting and conventional conversion factors (Tanaka et al. in press) correlated well with the Chl *a*-based estimates (r = 0.83±0.06, mean ± SD for the three mesocosms) with the Chl *a*-based biomass being, on average, approximately 20% higher than the estimates based on counting.

Radiolabelling of DNA

Radiolabelling of DNA was performed by random oligonucleotide primed synthesis (ROPS) with the DecaLabel DNA labelling kit (Fermentas K0621) in accordance with the manufacturer's instructions. *Hind*III digests of phage λ DNA (Fermentas) were used as DNA templates and deoxyadenosine 5`-[α-^{33}P]triphosphate (92.5 TBq mmol^{-1}; Amersham Biosciences) was used as the radioactive precursor. This procedure results in products of various lengths. However, the average length was estimated to 0.48 kilobase pairs (kb) using the experimentally derived equation described by Hodgson and Fisk (1987). This length is at the lower end of the range for naturally occurring dDNA (0.12-35.2 kb) in aquatic environments (DeFlaun et al. 1987). We did not correct for potential shortening of the DNA chain length by radiochemical decay, but the radiolabelled DNA was used well within one half life of the radioactive precursor in order to avoid significant shortening.

In order to remove primers, unincorporated deoxynucleotide triphosphates, and other low molecular weight (LMW) material, labelled DNA was pooled in 100 µL aliquots and purified using Amicon Microcon-PCR centrifugal filter devices (Millipore) in accordance with the manufacturer's instructions. The final product was stored in 20 µL aliquots at -20°C until used. The percentage of label incorporation into the DNA, the amount of DNA generated in the reaction, and the specific activity of the product were calculated from the DE-81 filter-binding assay (Sambrook and Russell 2001). The synthesis of radiolabelled DNA resulted in [^{33}P]DNA with a specific activity of approximately 3×10^7 counts per minute (cpm) μg^{-1} and 95.1±3.9% (mean ± SD of 3 replicates) incorporation of label after the purification step. The calculated concentration of DNA in the final product was 13.0±0.5 ng μL^{-1} (mean ± SD of 3 replicates).

Incubation

[^{33}P]DNA was diluted in distilled water and 50 µL aliquots of the dilution were added to 12 mL subsamples to give a final concentration between 1.8-2.2 µg L^{-1}, which corresponds to 6.0-7.3 nmol L^{-1} of nucleotide P assuming an average molecular weight of monophosphorylated nucleotides of ~300 g mol^{-1} (Ammerman and Azam 1991*b*). Zero-time samples were used as blanks in DNA uptake studies for the subtraction of background and abiotic adsorption. Previous studies indicate that blanks obtained by the chemical killing of samples should be avoided in DNA uptake studies (Paul et al. 1987, Jørgensen and Jacobsen 1996). Adenosine 5`-[γ-^{33}P]triphosphate (AT^{33}P; Amersham Biosciences) was diluted in distilled water and 50 µL aliquots of the dilution were added to 12 mL subsamples to give a final concentration of 125 pmol L^{-1}. Carrier-free $^{33}PO_4^{3-}$ (Amersham Biosciences) was diluted in distilled water and 50 µL aliquots of the dilution were added to 10 mL subsamples to give a final concentration of 105 pmol L^{-1}. Fixed samples were used as blanks in ATP and PO_4^{3-}

uptake studies. All substrates were added at concentrations corresponding to a total count of ~10^5 cpm mL^{-1}. Incubations were done separately in 15 mL Falcon tubes at subdued (laboratory) light and in situ temperature (approx 16°C). When the incubation time was ≤10 min, they were done at room temperature. Samples were incubated according to the expected turnover time. For samples incubated with $^{33}PO_4^{3-}$, AT^{33}P, and [^{33}P]DNA, the respective incubation times varied between 30 s and 1 hour, 3 min and 2.5 hours, and 45 min and 5 hours, respectively. Incubations were stopped by cold chase by the addition of cold KH_2PO_4 (1 mmol L^{-1} final concentration) to experiments with $^{33}PO_4^{3-}$ and the addition of cold KH_2PO_4 and cold ATP (1 mmol L^{-1} final concentration) to experiments with AT^{33}P and [^{33}P]DNA. For experiments with [^{33}P]DNA, filtrations were performed immediately after the cold chase. For experiments with $^{33}PO_4^{3-}$ and AT^{33}P, filtrations were performed within 30 min after the addition of cold chase.

Size fractionation and measurement of radioactivity

Bacteria and phytoplankton were separated into different size fractions by filtration onto polycarbonate filters (Poretics) with pore sizes of 0.2, 1, and 10 µm. Polycarbonate filters were supported on Whatman GF/C filters soaked with 10 mmol L^{-1} KH_2PO_4. Filtrations for ^{33}P-studies were done as parallel filtration by using a Millipore manifold. In $^{33}PO_4^{3-}$ uptake studies, portions of 3.3 mL were filtered on each filter. In [^{33}P]DNA and AT^{33}P uptake studies, portions of 2 mL were filtered. To minimize cell breakage and subsequent loss of incorporated label, the vacuum was kept low (< 0.05 bar) during filtration through the 1 and 10 µm filters, increased to 0.2 bar during filtration through the 0.2 µm filters, and finally increased to >0.6 bar to remove any water remaining on the filters. Suction was left on during filter removal. Taken together, this alleviates the need for rinsing and result in consistently low blanks when filtering samples incubated with $^{33}PO_4^{3-}$ (Suttle et al. 1990) and AT^{33}P (cf.

Ammerman and Azam 1991*a*). Filters from samples incubated with [^{33}P]DNA were washed under low vacuum (<0.2 bar) with 3 mL of 0.2 µm filtered and autoclaved sea water amended with 10 µg LMW salmon sperm DNA (Sigma) per mL to minimize abiotic binding of labelled DNA to particulate matter and obtain low blanks (Paul et al. 1987). Filters were transferred to scintillation vials with 3 mL Ultima Gold scintillation cocktail (Packard) and radioassayed with a Lumi-One portable scintillation counter (Bioscan Inc.). Aliquots (50 µL) from the subsamples incubated with $^{33}PO_4^{3-}$ and AT^{33}P, and 100 µL aliquots from the subsamples incubated with [^{33}P]DNA, were transferred directly to scintillation vials and mixed with 3 mL scintillation cocktail to measure the total added radioactivity. The inherent difficulties in obtaining appropriate controls and realistic blanks in dDNA uptake studies (cf. Paul et al. 1987; Jørgensen and Jacobsen 1996), necessitated samples incubated with [^{33}P]DNA to be treated differently from those incubated with $^{33}PO_4^{3-}$ and AT^{33}P. Activity in the 1 – 0.2 µm size fraction is calculated by subtracting activity on the 1 µm filter from the activity on the 0.2 µm filter. Because cells trapped on the 0.2 µm filter are presumably more susceptible to damage and ruptures during the initial filtration (due to the higher vacuum applied) and subsequent loss of label during the washing procedure than those trapped on the 1 and 10 µm filter, a potential bias is thus likely to be represented as an underestimate of dDNA-P uptake compared to PO_4^{3-} and ATP-P uptake in the 1 – 0.2 µm size fraction.

After size fractionation, the remaining subsamples incubated with [^{33}P]DNA and AT^{33}P were assayed for dissolved inorganic ^{33}P (^{33}Pi) with a slight modification of the method outlined by Ammerman and Azam (1991*a*). In brief, the samples were filtered through a 0.2 µm polycarbonate filter. A 1 mL aliquot of filtrate was mixed with 10 mL scintillation cocktail and assayed for total dissolved ^{33}P. A 5 mL aliquot of the filtrate was acidified with H_2SO_4 to a final concentration of 25 mmol L^{-1} and mixed with ~10 mg activated charcoal powder. The charcoal was removed from solution by filtration through a

Dynagard 0.2 µm hollow fibre syringe filter (Spectrum Laboratories). A 1 mL aliquot of the second filtrate was mixed with 10 mL scintillation cocktail and assayed for dissolved ^{33}Pi. Autoclaved samples that were cooled on ice before assays were used as blanks to correct for artificial hydrolysis. The use of boiled samples as blanks is essential in cases where hydrolytic enzyme activity is high (Ammerman and Azam 1991*a*). The efficiency of AT^{33}P and [^{33}P]DNA removal by acidified, activated charcoal were 88.8±2.3% (mean ± SD, n = 7) and 98.8±0.9% (mean ± SD, n = 6), respectively, based on the results obtained from the blanks.

Estimation of turnover time and biomass-specific affinity

Turnover times (T; h) were calculated by the equation (Thingstad et al. 1993): $T = \frac{t}{-\ln(1-R)}$, where t = incubation time and R = consumed fraction of added $^{33}PO_4^{3-}$ or consumed fraction of added ^{33}P + the fraction of ^{33}Pi released from AT^{33}P and [^{33}P]DNA. The measurement of DOP turnover times is thus based on the observation that osmotrophs do not take up intact DOP-molecules, but that it is hydrolyzed before phosphates are assimilated into cellular material (Chróst 1990).

Biomass-specific affinity (α; L nmol P^{-1} h^{-1}) was estimated from the equation (Thingstad and Rassoulzadegan 1999; Tanaka et al. 2003):

$$\alpha = f / (TB) \qquad (2)$$

where f is the fraction of uptake in the respective size fraction, B is the biomass (nmol P L^{-1}) in the respective size fraction, and T is the turnover time (h) for the respective substrate. The contribution of filamentous heterotrophic bacteria to the total biomass seemed negligible in NP and 5N, but became significant in 5NC towards the end of the experimental period in that it increased from <2 % on 01 July to >20 % of the total biomass on 15 July. The biomass of

filamentous heterotrophic bacteria was arbitrarily contributed to the 10 – 1 μm size fraction, because they would most likely not pass the 1 μm filter due to their large size, as determined by epifluorescence microscopy (Tammert, H. pers. comm.). Since some of the filaments were as long as 9 μm, they can be assumed to also have contributed to the >10 μm size fraction (Tammert, H. pers. comm.).

Isotope dilution experiments

The kinetics of orthophosphate uptake was determined by the Rigler bioassay (1966). Unlabelled KH_2PO_4 was added to 10 mL samples (0, 25, 50, 75, and 100 nmol L^{-1}, respectively) prior to the addition of $^{33}PO_4^{3-}$. The incubation was stopped after 5 min by cold chase, and the uptake of ^{33}P was measured as outlined above.

The kinetics of ATP and DNA hydrolysis and uptake were measured according to Thingstad et al. (1993). For ATP kinetics experiments, unlabelled ATP was added to 12 mL samples (0, 25, 50, 75, and 100 nmol L^{-1}, respectively) prior to the addition of $AT^{33}P$. The incubation was stopped after 20 min by cold chase, and hydrolysis of $AT^{33}P$ and uptake of ^{33}P was measured as outlined above. For DNA kinetics experiments, unlabelled *Hind*III digests of phage λ DNA were added to 12 mL samples (0, 8, 16, 24, and 32 μg L^{-1} corresponding to ~0, ~27, ~53, ~80, and ~107 nmol L^{-1} of nucleotide P, respectively) prior to the addition of [^{33}P]DNA. The incubation was stopped after 4 hours by cold chase, and hydrolysis of [^{33}P]DNA and uptake of ^{33}P was measured as outlined above.

T, maximum uptake rates (V_{max}), and the sums of $K_{PO4}+S_n$, $K_{ATP}+S_n$, and $K_{DNA}+S_n$ (the half-saturation constant and the natural concentration of bioavailable orthophosphate, bioavailable ATP and compatible monomeric DOP, and bioavailable DNA and compatible polymeric DOP, respectively) were calculated according to the method of Wright and Hobbie (1966) as modified by Thingstad et al. (1993). The goodness of fit was assessed

independently by comparing $(K + S_n)/V_{max}$ to the ambient T (no substrate added; T_0 in Table 4) (Bentzen and Taylor 1991).

Statistical analysis

Statistical significance for the difference in relative share (percentage of total uptake) of radiolabelled P, and biomass-specific affinity between size fractions were tested by paired Student's *t*-tests (Sokal and Rohlf 1995). The statistical significance of regression lines was tested by the Student's *t*-test (Sokal and Rohlf 1995). The confidence level for all analyses was set at 95 %.

Results

Nutrients

Ambient concentrations of SRP, measured chemically by the molybdenum blue method (Koroleff 1983), and PO_4^{3-}, estimated according to Moutin et al. (2002) following subtraction of background values, are shown in Fig. 1. The SRP concentrations were stable at a low level in the experimental period in all three mesocosms with values of 46±34, 22±31, and 26±40 nmol L^{-1} (mean ± SD, n = 5) for NP, 5N and 5NC, respectively (Fig. 1A). The corresponding estimated PO_4^{3-} concentrations (following subtraction of background values) were 36.6, 8.8, and 6.6 nmol L^{-1} in NP, 5N, and 5NC, respectively, on day 7 (the first day of the experimental period) and decreased gradually to 2.3, 0.6, and 0.3 nmol L^{-1}, respectively, on day 15 (Fig. 1B). Ammonium concentrations were low throughout the experimental period in NP (<0.3 µmol L^{-1}), but accumulated in 5N and 5NC (Tamminen, T. pers. comm.). Nitrate + nitrite concentrations varied between 0.2 and 0.3 µmol L^{-1} during the experimental period (Olli et al. 2005).

Turnover times (T)

Turnover time data are presented in Fig. 2. In NP, $T_{[PO4]}$ decreased from 2.8 hours at the beginning of the experimental period to 0.2 hours at its end (Fig. 2A). $T_{[PO4]}$ in 5N and 5NC were significantly shorter; 0.4 hours (both mesocosms) at the beginning of the experimental period, and 0.04 and 0.02 hours, respectively, at its end (Fig. 2A). The same pattern can be observed for $T_{[ATP]}$ and $T_{[dDNA]}$ (Fig. 2B and 2C, respectively), although the decrease in T was smaller for both ATP and DNA than for PO_4^{3-} in the NP mesocosm. The shortest $T_{[ATP]}$ were 0.7 hours (NP), 0.1 hour (5N), and 0.07 hour (5NC) and occurred on 15 July (Fig. 2B). The shortest $T_{[dDNA]}$ was 14.1 hours in NP and occurred on 09 July, whereas in 5N and 5NC, the shortest times were 2.6 hours and 1.4 hours and occurred on 15 July (Fig. 2C).

Uptake distributions of added ^{33}P-substrates

The distribution of added activity in the three size fractions and that liberated from DOP to the water phase is shown in Fig. 3. Only small amounts of $^{33}P_i$ hydrolyzed from AT^{33}P and [^{33}P]DNA were found free in the water, even following short incubation times (down to <0.1 hour and <1 hour for AT^{33}P and [^{33}P]DNA, respectively, in 5N and 5NC). This result indicates that tight coupling between hydrolysis and uptake occurred. In NP, incubation times were generally two to four times longer.

Means of the relative share (percentage of total uptake) of radiolabelled P during the experimental period are shown as inserted pie charts in Fig. 3. There is a small, but consistent, tendency for the smallest size fraction (1 – 0.2 μm) to take a slightly larger share of P from ATP than they do when the substrate is PO_4^{3-}, and a slightly larger share of P when the substrate is DNA than when it is ATP. Relatively few of the differences were statistically significant (i.e., did not deviate significantly from a 1:1 relationship). Most evident was the

situation in NP, where the portion of ATP-P and DNA-P incorporated into the 1 – 0.2 µm size fraction was significantly larger than that of PO_4^{3-} (p=0.017 and 0.031, respectively). The 1 – 0.2 µm size fraction increased the relative share (percentage of total uptake) of ATP-P and DNA-P compared to PO_4^{3-}. This was reflected as a decrease in relative share of ATP-P and DNA-P compared to PO_4^{3-} consumed by the >10 µm size fraction (p = 0.055 and 0.021 for ATP-P and DNA-P, respectively) (Table 2). In 5N, no significant difference was found between the relative share of PO_4^{3-} and ATP-P in any of the size fractions. However, in the case of DNA-P, the relative share of the larger algae decreased compared to PO_4^{3-} (p=0.018) and to ATP-P (p= 0.006) (Table 2). In 5NC, no statistically significant differences were found between any P-source in any of the size fractions (Table 2). However, it should be noted that, as in NP and 5N, the larger algae seemed to be deprived of DOP.

Biomass-specific affinity

The development of biomass-specific affinity during the course of the experiment is shown in Fig. 4. The highest observed biomass-specific affinity for PO_4^{3-} in the bacterial size fraction was 0.136 L nmol P^{-1} h^{-1} (5NC; 13 July). This value corresponds to the theoretical maximum affinity for PO_4^{3-} in spherical bacterial cells with a diameter of ~0.8 µm, assuming diffusion transport to the cell to be the rate-limiting process and a constant concentration of 0.5 fmol μm^{-3} in bacterial cells (cf. Thingstad and Rassoulzadegan 1999) (Eq. 1). The highest observed biomass-specific affinity for PO_4^{3-} in the 10 – 1 µm and the >10 µm size fractions were 0.092 L nmol P^{-1} h^{-1} (5NC; 15 July) and 0.121 (5NC; 15 July), respectively. These values correspond to the theoretical maximum affinity for PO_4^{3-} in algal cells with a diameter of ~1.4 µm and ~1.2 µm, respectively, assuming a constant concentration of 0.24 fmol μm^{-3} in algal cells (cf. Thingstad and Rassoulzadegan 1999).

Specific affinity for PO_4^{3-} uptake, normalized for the summed P biomass of phytoplankton and bacteria (S-affinity$_{[PO4]}$), was used as an indicator to split the dataset according to Tanaka et al. (in press) into P-deficiency (S-affinity$_{[PO4]}$ <0.02 L nmol-P^{-1} h^{-1}) and P-limitation (S-affinity$_{[PO4]}$ >0.02 L nmol-P^{-1} h^{-1}). In our set of data, the latter is represented by data obtained from 5N and 5NC from 09 to 15 July, and the former is represented by all other data. The term 'deficiency' is here related to a situation where some osmotrophs are under physiological limitation but others are not or an osmotroph community is under systemic limitation, and the term 'limitation' to one where most osmotrophs are under physiological limitation. Physiological limitation refers to a situation where growth rate of the existing organisms is reduced due to the limiting nutrient, and systemic limitation reflects the ability of a system to convert additional nutrients to new biomass (Tanaka et al. in press). At P-deficiency, no differences were found for the biomass-specific affinity for PO_4^{3-} between the three different size fractions (Table 3). For both ATP-P and DNA-P, the 1 – 0.2 μm size fraction had a significantly higher biomass-specific affinity than both the 10 – 1 μm and the >10 μm size fractions. The 10 – 1 μm size fraction also had a higher biomass-specific affinity for DNA-P than the >10 μm size fraction. At P-limitation, the 1 – 0.2 μm size fraction had a significantly higher biomass-specific affinity for PO_4^{3-} than the 10 – 1 μm size fraction (Table 3). In this phase, the 1 – 0.2 μm size fraction had a marginally, but not statistically significant (p=0.053) higher biomass-specific affinity for ATP-P than the 10 – 1 μm size fraction. The 1 – 0.2 μm and the 10 – 1 μm size fractions had a significantly higher biomass-specific affinity for DNA-P than the >10 μm size fraction. The 1 – 0.2 μm size fraction also had a marginally, but non-significant (p=0.062) higher biomass-specific affinity for DNA-P than the 10 – 1 μm size fraction.

Isotope dilution experiments: Kinetic parameters

Kinetic parameters calculated from the turnover data obtained from the isotope dilution experiment on NP in the middle of the experimental period (11 July) are shown in Table 4. The V_{max}'s for all substrates were higher in the >1 μm size fraction than in the 1 – 0.2 μm size fraction, while the opposite was the case for $(K+S_n)/V_{max}$ and biomass-specific affinity. Uptake in both size fractions was well described by the linear model (Table 4), and the independent assessment of turnover time, T_0, corresponded well to the turnover time estimated from $(K+S_n)/V_{max}$. All regression lines were statistically significant (data not shown).

Discussion

Orthophosphate (PO_4^{3-}) is considered the form of P preferentially utilized by osmotrophs, but the available fraction of the DOP pool is also actively utilized (Bentzen et al. 1992; this study). It is generally accepted that aquatic bacteria and phytoplankton can utilize DOP by splitting off the phosphate moiety through hydrolysis before taking up the inorganic phosphate. There is strong evidence that hydrolysis of both monomeric and polymeric DOP is, indeed, the key factor in maintaining growth rates when PO_4^{3-} is scarce (cf. Chróst 1990 and references therein; Bentzen et al. 1992 and references therein). The objective of the current study, was to improve our knowledge of how uptake of organic and inorganic P-compounds are distributed between marine osmotrophs, the competitive abilities of different osmotroph groups, and how this may change with different nutrient (inorganic N and P, organic-C) status in the environment.

Algal – bacterial competition

Competitive ability among osmotrophs is often linked to cell size, with the traditional view being that small, spherical cells with a large surface-to-volume ratio take up substrates more efficiently than larger organisms. In our study, there were trends showing that PO_4^{3-} uptake was dominated by the two largest size fractions (>1 μm) during balanced enrichment (NP), whereas PO_4^{3-} uptake was dominated by the two smallest size fractions (<10 μm) in N-enriched (P-starved) 5N and 5NC mesocosms (Fig. 3). Similar trends were observed for the organic P substrates. Due to the temporal variations in uptake distribution, this is visualized in the inserted pie charts of Fig. 3, where it becomes clear that the mean proportion of labelled substrate going into the >10 μm size fraction is smaller in 5N and 5NC compared to NP. Competitive ability, however, can not be properly evaluated without considering biomass. If competitive ability is assessed only from size fractionation (Fig. 3), one may envisage that the 10 – 1 μm size fraction are superior to the >10 μm size fraction in the competition for DOP. The estimated biomass-specific affinity values for these two size fractions may however be comparable (Fig. 4). Taken together, Fig. 3 and Fig. 4 highlight the need to evaluate competitive ability, not only by size fractionation (as in Fig. 3), but also by taking biomass into account (as in Fig. 4).

Our scope was to compare bacterial and algal biomass-specific affinity for the three P-substrates according to Eq. 2. A crucial assumption made for this approach is that bacterial and algal processes can be approximated via mechanical separation to obtain *f* terms (Eq. 2) corresponding to the respective compartments. The attempt was not entirely successful because of the development of large, filamentous bacteria in 5NC. Therefore, a correction of the affinity estimates concerning 5NC is needed, as explained and discussed below.

Filamentous heterotrophic bacteria accumulated in the glucose-amended 5NC mesocosm, but were not observed in NP and 5N (Tammert, H. pers. comm.). The addition of

glucose to mesocosm experiments has previously been found to induce the growth of large colony forming and filamentous heterotrophic bacteria (Havskum et al. 2003). The biomass-specific affinity of the large filamentous heterotrophic bacteria of 5NC can be estimated by assuming that the biomass-specific affinity of phytoplankton of the >1 μm fractions in 5NC were comparable to the biomass-specific affinity of the >1 μm size fractions in 5N. We thus calculated that the filamentous heterotrophic bacteria were responsible for up to over 50% of the total uptake of all substrates towards 15 July. Filamentous heterotrophic bacteria had a mean biomass-specific affinity during the experimental period of ~ 0.2 L nmol-P^{-1} h^{-1} for PO_4^{3-}, ~ 0.05 L nmol-P^{-1} h^{-1} for ATP-P and ~ 0.003 L nmol-P^{-1} h^{-1} for DNA-P with maximum values of ~ 0.4, 0.1 and 0.05 L nmol-P^{-1} h^{-1}, respectively. All these values were significantly higher than equivalent values for any other group. This finding supports the hypothesis that a portion of heterotrophic bacteria may maximize uptake of the limiting nutrient by using the nonlimiting element (e.g., organic C) to increase their size without thereby increasing their cellular quota of the limiting element (Thingstad et al. 2005). To illustrate this, Eq. 1 can be expressed as

$$\alpha_{max} = (4\pi Dr)/Q_N \qquad (3)$$

where Q_N is the amount of the limiting nutrient needed to make a new cell. For a conservative substrate not lost by leakage or respiration, Q_N will be the cell quota of the limiting element. The maximum affinity increases with increasing r:Q_N ratio. The hypothesis thus predicts that cells with a large surface-to-'quota of limiting element' ratio, rather than cells with a large surface-to-volume ratio, will be superior competitors. Any strategy to increase size without thereby increasing the cellular requirement of the limiting nutrient proportionally will give a competitive advantage.

Figure 5 is compiled from the dataset of Table 3 termed P-limitation (S-affinity$_{[PO4]}$ >0.02 L nmol-P^{-1} h^{-1}), with the affinity of the filamentous bacteria corrected for and attributed to the 1 – 0.2 µm size fraction. This probably represents a best estimate for the competition between heterotrophic bacteria and phytoplankton when P is the primary limiting nutrient and diffusion is the rate limiting step for uptake. Although biomass-specific affinity for P-uptake from ATP and dDNA is lower than that for PO_4^{3-} in both heterotrophic bacteria and phytoplankton, Fig. 5 illustrates that the competition for monomeric and polymeric DOP follows the same trend as that for PO_4^{3-}, which indicates that the algal – bacterial competition for DOP is not much different from the competition for PO_4^{3-}.

This experiment show no significant differences between the algal – bacterial competition for DOP compared to the competition for PO_4^{3-}. Because bacteria often obtain significant quantities of ambient phosphate, it has been speculated that phytoplankton may use DOP as an alternative source for P. This ability was confirmed by the present study. However, we suspect that bacteria had a greater ability to compete for DOP than algae, but not to an extent that could lead to drastic changes in the distribution of P-uptake, depending on whether the P-substrate was organic or inorganic (Fig. 5). Algae in the >10 µm size fraction obtained relatively smaller quantities of their P requirement from DOP, particularly from dDNA (Table 2), which indicates that competition for this form of P is shifted to favour smaller osmotrophs.

Assuming diffusion towards the cell to be the rate-limiting step for uptake, one would expect a low affinity for dDNA compared to the smaller PO_4^{3-} and ATP molecules. The diffusion constant in water (D) for a small molecule like PO_4^{3-} is assumed to be ~10^{-5} cm^2 s^{-1}. The D of ATP is ~3×10^{-6} cm^2 s^{-1} (Diehl et al. 1991). The D of DNA is related empirically to its size: $D = 4.9 \times 10^{-6}$ cm^2 s^{-1} × (basepair size)$^{-0.72}$ (Lukacs et al. 2000). Thus, the D of dDNA

the size employed as a tracer here (mean size 0.48 kb), can be calculated to ~6×10^{-8} cm^2 s^{-1}. Using these values, the relative affinity for a 0.48 kb dDNA molecule compared to a PO_4^{3-} molecule ($affinity_{[dDNA]}$:$affinity_{[PO4]}$) and an ATP molecule ($affinity_{[dDNA]}$:$affinity_{[ATP]}$) would be ~0.006 and ~0.02, respectively. The calculated $affinity_{[ATP]}$:$affinity_{[PO4]}$ ratio would be ~0.3. Typical estimates from our data corresponding to diffusion limitation gives values of ~0.01 for $affinity_{[dDNA]}$:$affinity_{[PO4]}$ and ~0.05 for $affinity_{[dDNA]}$:$affinity_{[ATP]}$ (by inspection of data in Table 3; S-$affinity_{[PO4]}$ >0.02 L nmol-P^{-1} h^{-1}). Thus, the estimated affinity for dDNA is relatively high compared to the estimated affinities for PO_4^{3-} and ATP assuming a diffusion limitation of uptake. However, including the contamination with unincorporated deoxyadenosine 5`-[α-^{33}P]triphosphate in the [^{33}P]DNA stock solution (see Matherials and methods; Radiolabelling of DNA), which results in a ~4 pmol L^{-1} contamination in the final incubations, is sufficient to explain the difference between our estimates and the model for diffusion limited uptake. The ratio of estimated affinity for ATP to estimated affinity for PO_4^{3-} (~0.2) is close to the expected value (~0.3) and indicates that the model for diffusion limited uptake provides a good approximation to the experimental situation.

It has been indicated that the degradation of dDNA involves more complex enzymatic processes than the degradation of monomeric DOP compounds and that hydrolysis is the rate-limiting step for dDNA-P uptake (Siuda and Güde 1996). However, this interpretation was based on direct comparison of dDNA-P and nucleotide-P uptake rates and ignored the vast differences between dDNA and nucleotide diffusion constants. Our data show that the affinity for dDNA-P is close to the theoretical maximum for diffusion-limited uptake. Thus, diffusion transport to the cell may be regarded as the limiting step for utilizing P from dDNA in P-limited environments, rather than hydrolysis.

Kinetic parameters

The kinetics experiments (Table 4) clearly showed that the $K+S_n$ values of the 1 – 0.2 µm size fraction were smaller than those of the >1 µm size fraction. This result indicates that a higher affinity for both PO_4^{3-} and P derived from organic compounds occurs in bacteria when compared to algae at the ambient substrate concentrations. V_{max} corresponds to the maximal ambient uptake rate. Algal uptake of both phosphate and DOP became saturated at higher substrate concentrations than did bacterial uptake. Accordingly, our results thus reflect that phytoplankton are potentially better competitors for all substrates at high substrate concentrations. These results agree with previous data from limnic systems (Bermann 1988; Cotner and Wetzel 1992; Siuda and Güde 1996), and support the qualitative conclusion that heterotrophic bacteria are superior competitors at constantly low substrate concentrations and phytoplankton at high (Güde et al. 1992).

Our values of maximum DNase activity estimated from samples from NP (1.8 µg DNA hydrolyzed L^{-1} h^{-1}) correspond well to those reported by Paul et al. (1987) in estuarine waters (1.8 - 8.0 µg L^{-1} h^{-1}) and Siuda et al. (1998) in a mesotrophic lake (1.4 - 4.0 µg L^{-1} h^{-1}). In previous studies involving limnic systems, Siuda and Güde (1996) found that ATP, AMP and other DNA degradation products were predominantly utilized as a P source by the bacterial size fraction, and that dDNA-P was exclusively taken up by the bacterial size fraction and was primarily not detectable in the 'algal' size fractions. This differs from our results, which showed that the size fractions from 10 – 1 µm and >10 µm took up significant proportions of the added dDNA-P (Table 2, Fig. 3) and could indicate a more dominant role of phytoplankton in phosphorus uptake in marine systems.

Methodological considerations in DNA-P uptake experiments

We did not measure the ambient dDNA concentrations in the current study. However, based on previous reports (DeFlaun et al. 1987; Ammerman and Azam 1991*b*; Jørgensen and Jacobsen 1996), we estimate it to be above 5 μg L^{-1}. Assuming dissolved RNA (dRNA) to be compatible to dDNA as a P source to osmotrophs, and a dRNA:dDNA ratio >4 (Karl and Bailiff 1989), we speculate that [^{33}P]DNA was added at tracer concentrations (i.e., <10% of ambient concentrations). If, however, [^{33}P]DNA was added at above tracer concentrations, our affinity$_{[dDNA]}$ values would be underestimated (Bentzen and Taylor 1991), which seems unreasonable since they were close to the theoretical maximum. Individual components of the dDNA pool are cycled at different rates (Brum 2005). We acknowledge that $T_{[dDNA]}$ and affinity$_{[dDNA]}$ values reported here are estimates of the turnover times and affinity, respectively, of the ambient pool of enzymatically hydrolyzable dDNA, and not all components of the dDNA pool (cf. Brum 2005).

Since the uptake of small DNA pieces for nutritional purposes may not require that activation of an enzyme apparatus like that needed for large DNA fragments (Albano et al. 1987), the different size of tracer DNA, naturally occurring dDNA, and that of added substrate DNA in isotope dilution experiments, must be taken into account when interpreting the results. Washing of filters from samples incubated with [^{33}P]DNA (see Materials and methods) may have biased the comparison between the three P sources. Assuming that the blanks represents the true level of abiotic binding, a potential bias is likely to be reflected as an underestimate of dDNA-P uptake compared to uptake from the two other sources in the 1 – 0.2 μm size fraction. Although the precision of our estimates might be questioned, that does not change the overall conclusion of this investigation.

Three main conclusions can be drawn from this study. First, osmotroph organisms in all size-classes investigated support their growth rates by efficient hydrolysis of both monomeric and polymeric DOP compounds when P is the limiting nutrient. Inorganic P is the preferred source for both bacteria and phytoplankton, while monomeric DOP is used preferentially to polymeric DOP. Second, reduced access to bioavailable P may induce a shift from larger organisms dominating the uptake to smaller organisms dominating the uptake that may again be modified in favour of larger organisms by increased availability of labile DOC, presumably by inducing the growth of large, heterotrophic bacteria that use C to increase size and optimize their competitive abilities. Third, there is no conclusive evidence for a shift in terms of algal – bacterial competition when P is available in the form of either monomeric or polymeric DOP, contrary to PO_4^{3-}. However, there may be a shift in favour of small organisms (<10 μm), when P is available in the form of polymeric DOP.

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Table 1

Table 1. Nutrient supply rates to the mesocosms during the experimental period. Nitrate (N); 1 µmol N L^{-1}, phosphate (P); 0.06 µmol P L^{-1}, silicate (Si); 3 µmol Si L^{-1} and glucose (C); 13.3 µmol C L^{-1}. 5×N and 5×P correspond to five times the amount of N and P, respectively.

Day	Mesocosm		
(July 2003)	NP	5N	5NC
06	N+P	5×N	5×N+C
07	N+P	5×N	5×N+C
08	N+P	5×N	5×N+C
09	N+P	N	5×N+C
10	N+P	N	5×N+C
11	N+P	N	N+C
12	N+P+Si	N+Si	N+C+Si
13	N+P	N	N+C
14	N+P	N	N+C
15	N+P	N	N+C

Table 2

Table 2. Percent uptake and ratios for the partitioning of labelled P from different sources into different size fractions in NP, 5N, and 5NC as estimated from the mean percentage of total uptake in the different size fractions during the experimental period (cf. Fig. 3). Statistically significant differences (p <0.05) are indicated by bold print, marginally significant differences (0.06 > p > 0.05) are indicated by underlining. n = 5 comparisons in all cases.

Mesocosm	Size fraction	%			ratio		
		PO_4	ATP	dDNA	PO_4:ATP	PO_4:dDNA	ATP:dDNA
NP	1-0.2 µm	21.8	31.4	32.8	**0.69**	**0.66**	0.94
	10-1 µm	37.3	40.4	42.4	0.93	0.88	0.95
	> 10 µm	40.9	28.2	24.8	<u>1.45</u>	**1.64**	1.14
5N	1-0.2 µm	33.8	34.4	36.8	0.98	0.92	0.93
	10-1 µm	41.0	48.0	48.8	0.96	<u>0.84</u>	0.88
	> 10 µm	25.2	17.6	14.4	1.10	**1.75**	**1.59**
5NC	1-0.2 µm	25.6	28.5	29.3	0.87	0.91	1.05
	10-1 µm	46.5	51.1	55.9	0.94	<u>0.86</u>	0.92
	> 10 µm	27.9	20.4	14.8	1.32	<u>1.56</u>	1.18

Table 3

Table 3. Comparison of biomass-specific affinity (L nmol-P^{-1} h^{-1}; $\times 10^3$) for different substrates between the 1 – 0.2 µm [A], the 10 – 1 µm [B] and the > 10 µm [C] size fractions during P-deficiency (S-affinity$_{[PO4]}$ <0.02 L nmol-P^{-1} h^{-1}) and P-limitation (S-affinity$_{[PO4]}$ >0.02 L nmol-P^{-1} h^{-1}). The column labelled 'Sign. diff.' lists those size fractions in the same row whose biomass-specific affinity is significantly different (p < 0.05) from each other. See text for details.

S-affinity$_{[PO4]}$ (L nmol-P^{-1} h^{-1})	Substrate	1 – 0.2 µm [A]	10 – 1 µm [B]	> 10 µm [C]	Sign. diff.	n
		Mean (Min. – Max.)	Mean (Min. – Max.)	Mean (Min. – Max.)		
< 0.02	PO_4	3.7 ± 5.1 (0.04 – 12.8)	2.7 ± 4.1 (0.05 – 14.0)	2.2 ± 3.0 (0.2 – 10.4)	----	16
	ATP	1.9 ± 1.4 (0.2 – 4.0)	1.2 ± 0.9 (0.2 – 3.4)	1.0 ± 0.4 (0.4 – 1.6)	A>B, A>C	13
	dDNA	0.2 ± 0.1 (0.05 – 0.4)	0.1 ± 0.05 0.04 – 0.2)	0.04 ± 0.02 (0.02 – 0.1)	A>B, A>C, B>C	10
> 0.02	PO_4	64.7 ± 35.0 (24.7 – 135.9)	42.2 ± 29.6 (12.6 – 91.7)	48.4 ± 44.3 (12.9 – 121.2)	A>B	8
	ATP	16.9 ± 14.0 (3.2 – 48.3)	10.0 ± 10.0 (2.6 – 32.6)	9.6 ± 11.1 (1.8 – 33.4)	----	8
	dDNA	0.9 ± 0.3 (0.4 – 1.3)	0.7 ± 0.4 (0.2 – 1.3)	0.4 ± 0.4 (0.1 – 1.3)	A>C, B>C	8

Table 4

Table 4. Estimates of kinetic parameters and biomass-specific affinity (α) from NP on 11 July.

		Size fraction			
		1 – 0.2 µm	> 1 µm	total uptake (> 0.2 µm)	total hydrolysis
PO_4^{3-}	V_{max} (nmol L^{-1} h^{-1})	8.0	87.1	88.0	
	$K+S_n$ (nmol L^{-1})	12.1	50.7	33.1	
	$(K+ S_n)/ V_{max}$	1.5	0.6	0.4	
	T_0 (h)	1.7	0.5	0.4	
	r^2 (*n*)*	0.998 (3)	0.904 (5)	0.947 (5)	
	Biomass (nmol P L^{-1})	94.1	457.1	551.2	
	α (L nmol P^{-1} h^{-1})†	0.0061	0.0042	0.0045	
ATP	V_{max} (nmol L^{-1} h^{-1})	2.2	10.8	12.9	20.2
	$K+S_n$ (nmol L^{-1})	9.2	29.6	23.1	16.2
	$(K+ S_n)/ V_{max}$	4.1	2.7	1.8	0.8
	T_0 (h)	3.6	1.7	1.1	0.8
	r^2 (*n*)*	0.947 (5)	0.937 (5)	0.962 (5)	0.950 (5)
	α (L nmol P^{-1} h^{-1})†	0.0033	0.0013	0.0017	0.0023
dDNA	V_{max} (µg L^{-1} h^{-1})	0.3	1.7	1.5	1.8
	$K+S_n$ (µg L^{-1})	18.9	73.4	35.9	38.4
	$(K+ S_n)/ V_{max}$	58.7	43.6	23.9	21.9
	T_0 (h)	50.9	42.6	22.2	20.6
	r^2 (*n*)*	0.917 (4)	0.971 (4)	0.928 (4)	0.935 (4)
	α (L nmol P^{-1} h^{-1})†	0.00016	0.00006	0.00008	0.00009

*) *n* (numbers in brackets) refers to the number of points that the regression line is drawn through.

†) α is calculated from Eq. 2.

Figure captions

Fig.1. (A) Concentrations of SRP and (B) estimated PO_4^{3-} concentrations (nmol L^{-1}) without background SRP. Note that the y-axis in B is in logarithmic scale. Data from Tanaka et al. (in press).

Fig. 2. Turnover time (logarithmic scale) of (A) PO_4^{3-}, (B) ATP, and (C) DNA in the mesocosms. Data on $T_{[PO4]}$ from Tanaka et al. (in press).

Fig. 3. The bar charts show the relative fractions of incorporated radioactivity in the three size fractions and that liberated from DOP but not taken up by the organisms during the course of the experimental period. The inserted pie charts show the mean percentage of total uptake in the three particulate fractions.

Fig. 4. Biomass-specific affinity (logarithmic scale) during the course of the experiment.

Fig. 5. Scatter diagram of estimated biomass-specific affinity of bacteria and phytoplankton. Pooled data corresponding to the P-limited situation (S-affinity$_{[PO4]}$ >0.02 L nmol-P^{-1} h^{-1}; see text). The dotted line denotes a 1:1 relationship.

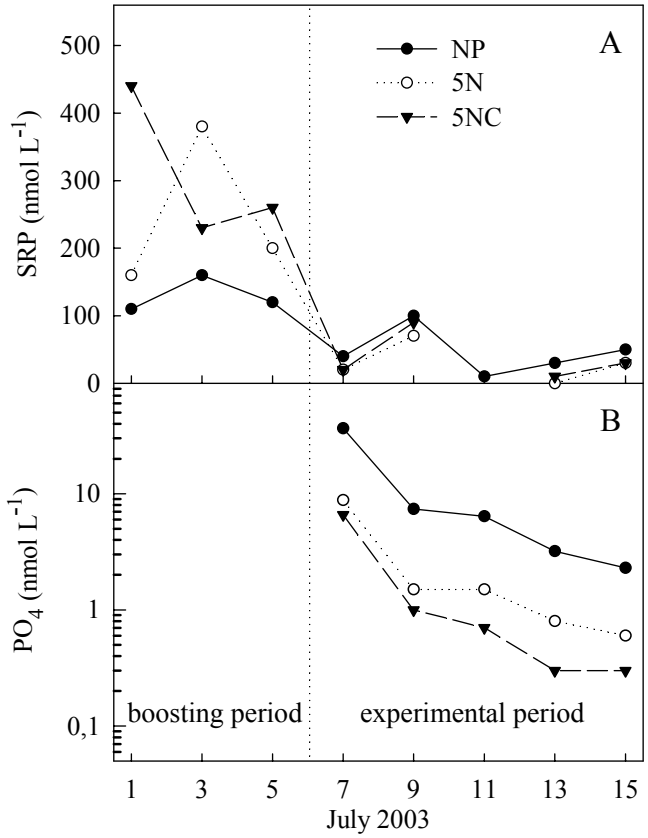
A
B
NP
5N
5NC
SRP (nmol L-1)
PO4 (nmol L-1)
500
400
300
200
100
0
10
1
0,1
boosting period
experimental period
1
3
5
7
9
11
13
15
July 2003

Figure 1

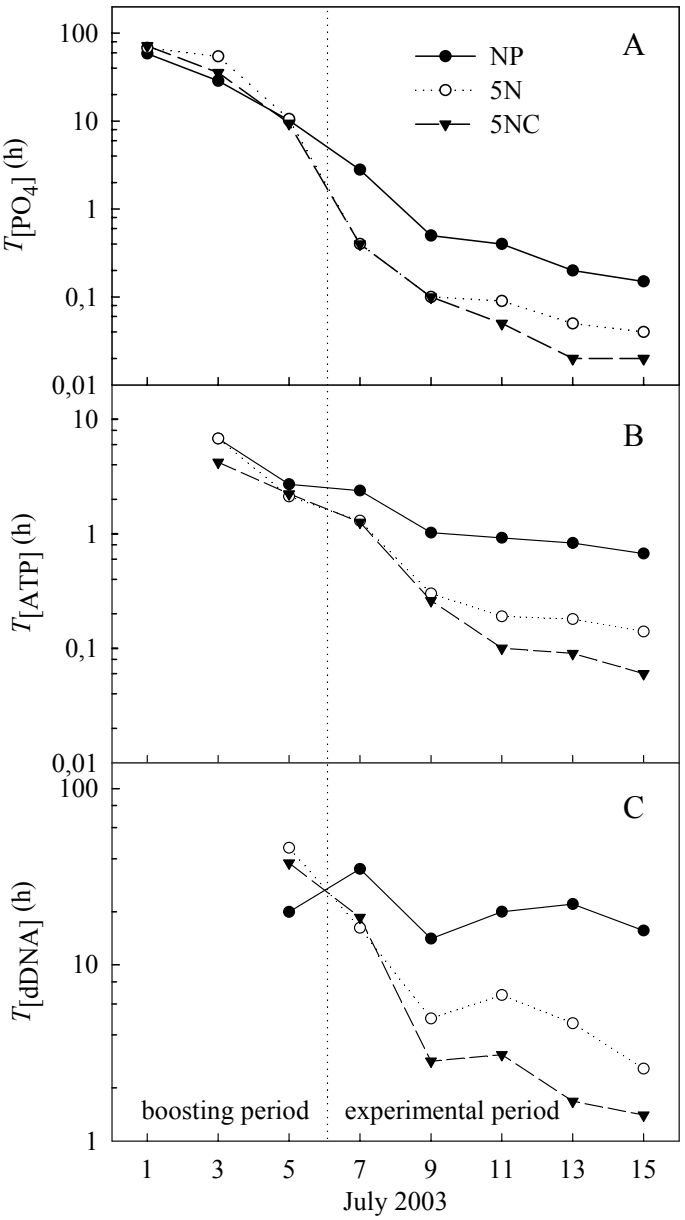
A
B
C
NP
5N
5NC
$T_{[PO_4]}$ (h)
$T_{[ATP]}$ (h)
$T_{[dDNA]}$ (h)
100
10
1
0,1
0,01
boosting period
experimental period
1
3
5
7
9
11
13
15
July 2003

Figure 2

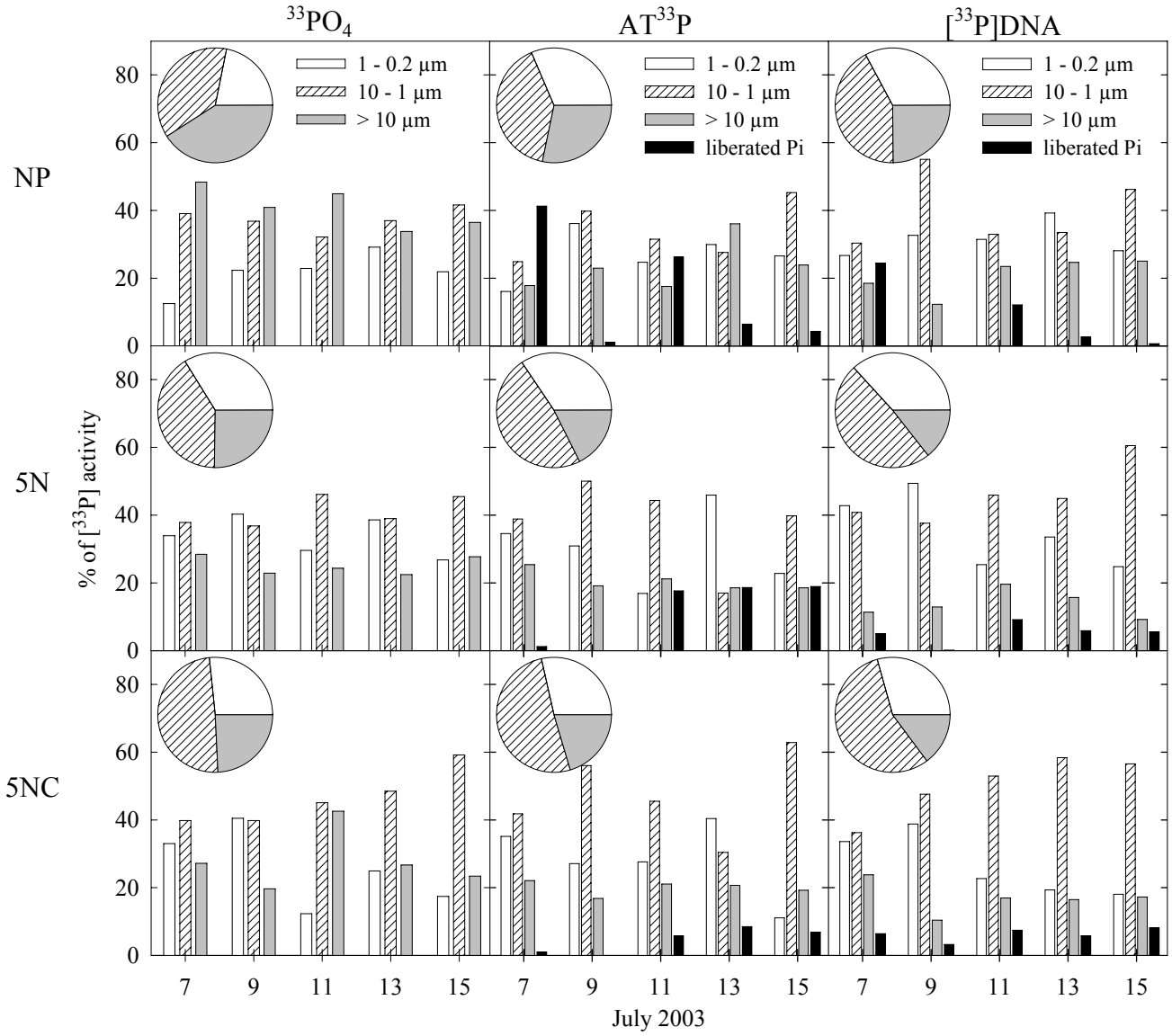
$^{33}PO_4$
AT^{33}P
[^{33}P]DNA
1 - 0.2 µm
10 - 1 µm
> 10 µm
liberated Pi
NP
5N
5NC
% of [^{33}P] activity
0
20
40
60
80
7
9
11
13
15
July 2003

Figure 3

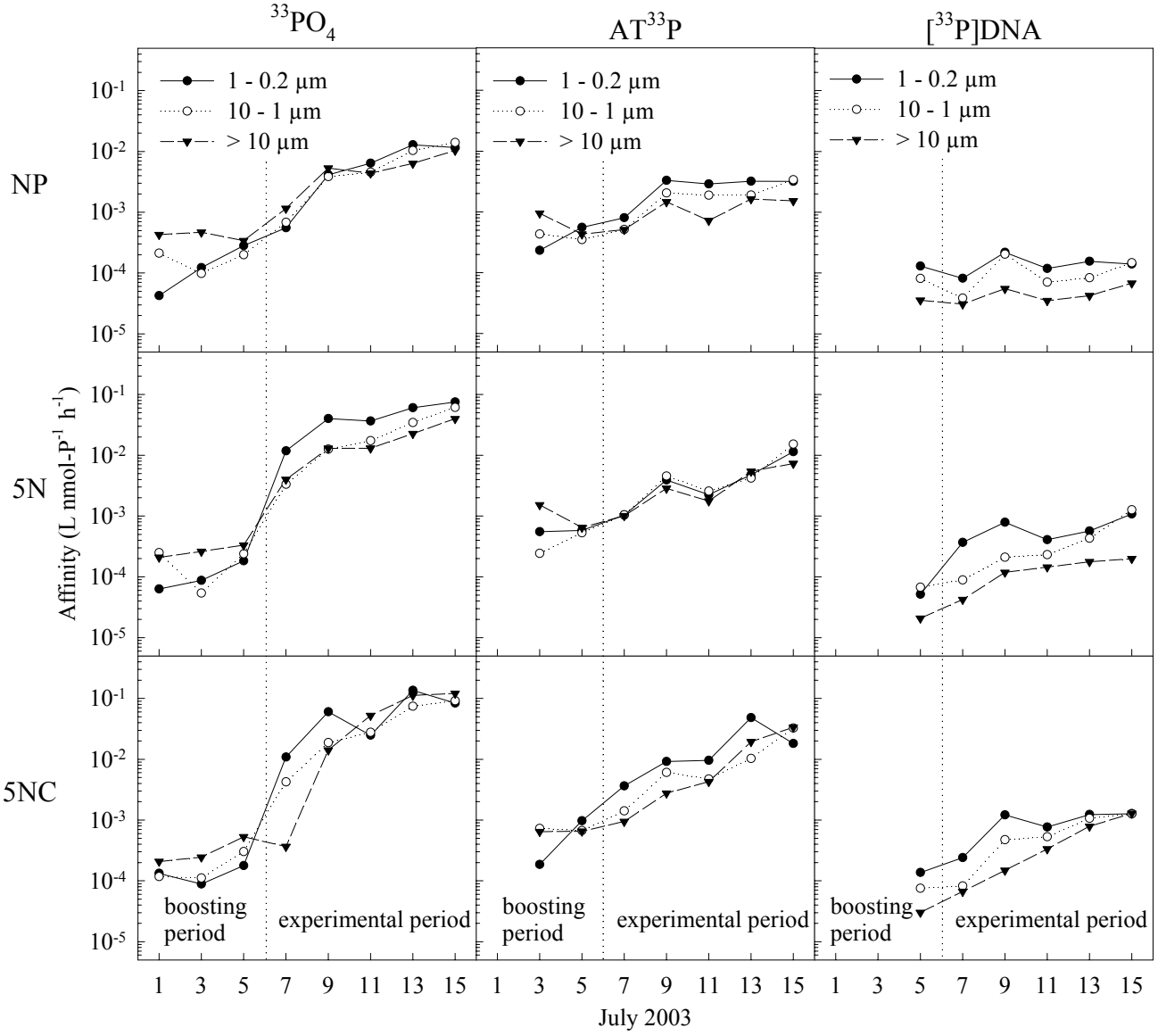
$^{33}PO_4$
AT^{33}P
[^{33}P]DNA
1 - 0.2 µm
10 - 1 µm
> 10 µm
NP
5N
5NC
Affinity (L nmol-P^{-1} h^{-1})
boosting period
experimental period
July 2003

Figure 4

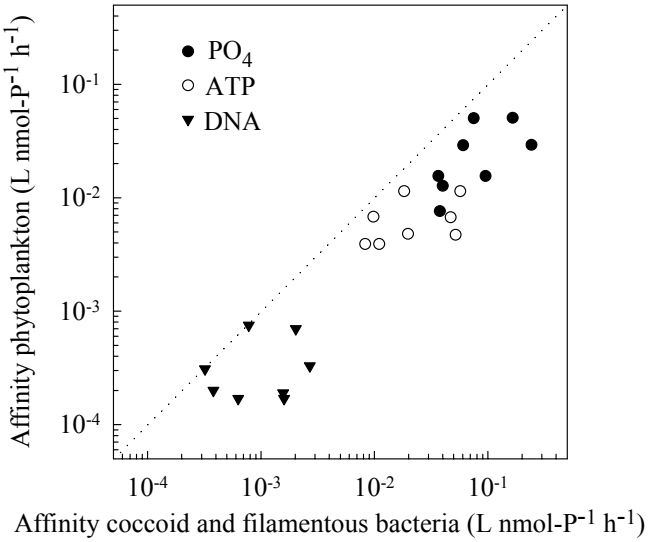
PO4
ATP
DNA
Affinity phytoplankton (L nmol-P-1 h-1)
Affinity coccoid and filamentous bacteria (L nmol-P-1 h-1)
10-1
10-2
10-3
10-4
10-4
10-3
10-2
10-1

Figure 5