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The biological and biogeochemical consequences of phosphate scavenging onto phytoplankton cell surfaces

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Abstract

Phytoplankton carbon (C) and nitrogen (N) content is commonly normalized to phosphorus (P) quotas as Redfield C : N : P ratios. We examined how surface-bound P pools affect Redfield stoichiometry and P uptake kinetics in cultures and natural blooms of diatoms, raphidophytes, dinoflagellates, pelagophytes, prasinophytes, and cyanobacteria. The amount of surface-adsorbed P on exponential growth phase cultures ranged from 14% to 57% of the total cellular P. The C : total P and N : total P ratios (surface-adsorbed P + intracellular P) for all species were near Redfield values, with lower values for the diatoms and higher ratios for the dinoflagellates and cyanobacteria. However, when corrected for the P adsorbed to exterior surfaces, intracellular or ''biological'' C : P and N : P ratios are 1.2–2 times higher. As with the cultured isolates, the amount of surface-adsorbed P for the natural bloom samples ranged from 15% to 46%. Carbon : total P ratios ranged from 71 to 151, whereas C : intracellular P ratios were 1.3–1.7 times higher. N : P ratios for the natural samples showed similar trends. Phosphate uptake rates into the intracellular pool were as little as half those measured for uptake into total cellular P pools for both the natural blooms and cultures. Cells can, however, access some of the surface-bound P over time to support growth, and the partitioning of P between adsorbed and interior pools is affected by growth stage, cellular P demand, and external phosphate concentrations. We conclude that, as with iron (Fe) and other particle-reactive elements, bulk particulate C : N : P ratios do not necessarily reflect the true biological P quotas of eukaryotic and prokaryotic algae and that P scavenging onto cell surfaces is likely to exert a large influence on the biogeochemical cycling of this nutrient.

Traditionally, the growth of phytoplankton in the marine environment was considered limited by the availability of nitrogen (Ryther and Dunstan 1971). However, the role of phosphorus (P) as a limiting nutrient in the ocean is currently being reexamined. P may be limiting to all or part of the photoautotrophic community in several coastal systems (Thingstad et al. 1998), in the Sargasso Sea (Wu et al. 2000; Sañudo-Wilhelmy et al. 2001; Mills et al. 2004), and in the Mediterranean (Diaz et al. 2001). Recent increases in N_2 fixation in the subtropical North Pacific have apparently driven a shift from nitrogen (N) limitation to P limitation in this regime (Karl et al. 1997).

The P quotas of phytoplankton have long served as a foundation for understanding algal elemental stoichiometry in general. In the Redfield paradigm, all other major cellular components are normalized to P content. The classical values of 1 P : 16 N : 106 carbon (C) (Redfield 1958) have, however, been extensively questioned, with some recent best estimates being 1 : 25 : 117 (Anderson and Sarmiento 1994; Broecker and Henderson 1998). The ratio of N : P is often used as a benchmark to differentiate N limitation from P limitation and is thought to set an upper limit on the nitrate : phosphate ratio in the ocean (Falkowski 1997; Tyrrell 1999). This treatment assumes that phytoplankton are N limited at N : P <16 and P limited at N : P >16 (Geider and La Roche 2002).

However, recent studies amply demonstrate that phytoplankton elemental ratios differ considerably between taxonomic groups and with varying nutrient regimes (Quigg et al. 2003). Broecker and Henderson (1998) suggested that an N : P ratio of 25 : 1 is a critical value, because of the flexibility in the average C : N : P composition of phytoplankton and marine particulate matter (Hecky et al. 1993) and because this N : P ratio marks the transition between N and P limitation (Tyrrell 1999). Geider and La Roche (2002) further confirmed that the critical ratio of N : P for algal growth limitation is significantly higher than the Redfield ratio and suggested a 50 : 1 threshold value.

New evidence demonstrates that phytoplankton Redfield ratios are also strongly affected by partitioning into surface-adsorbed and intracellular P pools (Sañudo-Wilhelmy et al. 2004). Much of the P associated with algal cells is simply

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abiotically adsorbed onto reactive cell surfaces, with the rest being in intracellular biological pools. Some of the reported wide variability in Redfield stoichiometry could therefore be attributable to the differential surface adsorption or scavenging of inorganic phosphate (P_i). Thus, the measurement of both surface-bound and intracellular P pools may be required to assess algal N or P limitation accurately.

Oceanographers have long recognized the need to differentiate particle-reactive trace metals such as iron (Fe) in phytoplankton into intracellular or ''biological,'' and surface-adsorbed or ''scavenged'' pools. Methods such as the titanium (Ti) wash (Hudson and Morel 1989) and oxalate wash (Tovar-Sanchez et al. 2003) are commonly used to remove surface-bound Fe, allowing the determination of true intracellular quotas or uptake rates (Hutchins et al. 1999*a*,*b*; Eldridge et al. 2004). The finding that the oxalate wash can be used in a similar manner to differentiate scavenged and interior pools of P (Sañudo-Wilhelmy et al. 2004) offers an analogous opportunity to better understand the true biological P requirements of phytoplankton.

Although the particle reactivity of P_i in marine environments such as sediments and hydrothermal vents has long been recognized by geochemists (Berner 1973; Wheat et al. 1996; Bjerrum and Canfield 2002), biologists and biogeochemists have not generally appreciated the significance of this process for phytoplankton P stoichiometry. Geochemical studies, however, note that an appreciable amount of dissolved phosphate is removed from seawater by adsorption onto Fe oxides (Berner 1973; Wheat et al. 1996). In the Mediterranean Sea, the unusual ratio of N : P was explained by the removal of phosphate from seawater through inorganic adsorption processes onto mineral particles of Saharan dust (Krom et al. 1991). Similarly, Sañudo-Wilhelmy et al. (2004) suggested that the particle reactivity of phosphate with algal cell surfaces appears to be mediated by ligand-exchange reactions with adsorbed metal (hydr-) oxides, particularly those of manganese (Mn).

We investigated the effects of surface-adsorbed P pools on variations in phytoplankton elemental quotas and P uptake using a wide variety of prokaryotic and eukaryotic algal species. These diverse taxa represent the dominant species found in a broad range of marine environments, from temperate estuaries to the oligotrophic central gyres to the high-nutrient, low-chlorophyll waters (HNLC) of the Southern Ocean. We compared laboratory cultures and/or natural blooms of diatoms, raphidophytes, dinoflagellates, pelagophytes, prasinophytes, and cyanobacteria, providing general insight into how the particle reactivity of P can affect variability in N : P and C : P ratios in phytoplankton. P uptake kinetics in the cyanobacterium *Trichodesmium* and in the diatom *Thalassiosira weissflogii* were reexamined using intracellular levels of P instead of total cell-associated concentrations. We also determined how algal growth rates and external phosphate concentrations can affect the size of the surface-scavenged P pool and investigated the bioavailability of surface-bound P to growing cells. Our results suggest that P scavenging is another crucial factor that may need to be taken into account in any realistic examination of algal elemental stoichiometry or nutrient limitation throughout the world's ocean.

Materials and methods

Culturing methods and natural bloom sampling—Cultured cyanobacteria investigated included *Trichodesmium* strains IMS101 and GBRTRLI101, *Prochlorococcus* CCMP1986, and *Synechococcus* CCMP1334 and PCC7002. IMS101 is an Atlantic isolate (Prufert-Bebout et al. 1993), GBRTRLI101 was isolated from the Great Barrier Reef, Australia (Bell et al. 2005), CCMP1986 originates in the Mediterranean Sea (Bertilsson et al. 2003), and CCMP1334 and PCC7002 are Sargasso Sea and coastal Atlantic isolates, respectively (Rippka et al. 1979; Cuhel and Waterbury 1984).

Eukaryotic algal cultures consisted of two raphidophytes (*Heterosigma akashiwo* CCMP2393 and *Chattonella subsalsa* CCMP2191), three diatoms (*T. weissflogii* CCMP1336, *Thalassiosira* sp., and *Chaetoceros* sp.), one prasinophyte (*Tetraselmis* sp.), one dinoflagellate (*Prorocentrum minimum* CCMP2233), and one pelagophyte (*Aureococcus anophagefferens* CCMP1708). The raphidophytes, prasinophyte, and dinoflagellate were isolated from the estuarine Delaware Inland Bays, U.S.A., the *Chaetoceros* sp. was isolated in 2001 from the SOIREE (Southern Ocean iron enrichment experiment) site in the polar Southern Ocean (Boyd et al. 2000), and the *Thalassiosira* sp. was isolated in 2003 from the subarctic Bering Sea by our laboratory. The other species were obtained from the listed culture collections.

Synechococcus CCMP1334 is axenic, while the other isolates listed are unialgal. All species were cultured using Aquil nutrients and trace metals (Morel et al. 1979) except for *Aureococcus* and *Prochlorococcus*, which were cultured in L1 medium (Guillard and Hargraves 1993) and Pro 99 medium (modified from Chisholm et al. 1992), respectively. All media were made using 0.2-μm filtered, microwave-sterilized natural open-ocean seawater with 20 μmol L^{-1} (P-replete cultures) or 0.2 or 0.5 μmol L^{-1} (P-limited cultures) orthophosphate (P_i) as a P source, except in experiments testing the removal efficiency of dissolved organic P. In these experiments, *Trichodesmium* IMS101, *Synechococcus* CCMP1334, *T. weissflogii* CCMP1336, and *Chaetoceros* sp. were grown using 20 μmol L^{-1} Na-glycerophosphate. *Synechococcus* CCMP1334 was also grown in medium containing 20 μmol L^{-1} P as adenosine triphosphate (ATP). Medium preparation, algal culturing, and sampling were carried out using sterile techniques, and culturing and filtration materials were precleaned with 10% trace metal grade HCl and rinsed with Milli-Q.

All of the algae were grown at 25°C ± 1°C, except for *Thalassiosira*, *Tetraselmis*, and *Prochlorococcus*, which were grown at 18°C ± 1°C, and the polar *Chaetoceros* sp., which was grown at 4°C. A 12 : 12 light : dark cycle with cool-white fluorescent lamps was used for all species. *Trichodesmium* and *Synechococcus* were grown at 45 and 20 μmol quanta m^{-2} s^{-1}, respectively. The other algae were grown at 70–80 μmol quanta m^{-2} s^{-1}. Triplicate culture samples were maintained in exponential growth phase through a minimum of six to seven generations using semicontinuous culture methods before harvesting. The growth of each culture was monitored on a daily basis using in vivo chlorophyll fluorescence or cell counts.

Table 1. Collection information for the field bloom samples. DIB, Delaware Inland Bays; DRE, Delaware River Estuary.

Dominant algal bloom species	Cells L^{-1}	Date and bay	P_i (μmol L^{-1})	Temperature (°C)	Salinity
Heterosigma akashiwo	1.98×10^8	4 Aug 04 DIB	0.47	34	0–0.5
Gymnodinium instriatum	8×10^6	4 Aug 04 DIB	0.15	28	6
Chattonella subsalsa	7×10^6	31 Aug 04 DIB	0.20	29	27
Chattonella verruculosa	6.5×10^7	7 Sep 04 DIB	0.55	25	20
C. verruculosa	6.8×10^6	9 Sep 04 DIB	0.35	25.5	20
Cylindrotheca sp.	1.6×10^7	14 Sep 04 DIB	0.40	24.5	24.1
Synechocystis sp.	1.8×10^{11}	21 Sep 04 DRE	0.26	17	0.04
Leptocylindrus sp.	1.7×10^8	27 Sep 04 DRE	0.62	26	25.9

We also used a number of natural algal bloom samples obtained from local estuarine waters in our experiments. These natural blooms were composed of many of the same major algal groups used in the culture experiments, including cyanobacteria, raphidophytes, dinoflagellates, and diatoms. Bloom samples were collected from the Delaware River or Delaware Inland Bays estuaries and were sampled immediately upon return to the laboratory. Blooms were defined as being dominated by a single species (>90% of cells) using microscopic cell counts and were identified according to the taxonomy of Tomas (1997). Such dense, near-unialgal blooms (many of them harmful species) occur commonly in these highly eutrophic estuarine systems. Table 1 presents the taxa, dates, locations, and cell densities of the natural blooms sampled and the salinity, temperature, and P_i concentrations at the stations where they were collected.

Determination of surface-adsorbed and intracellular P—Triplicate samples of cultures or bloom samples were filtered under low vacuum (<17 kPa) for elemental stoichiometry or $^{33}P_i$ uptake experiments. ''Total'' cell-associated P was determined by rinsing the filters twice with 5 mL of 0.2 μmol L^{-1}–filtered Sargasso seawater containing <0.02 μmol L^{-1} P_i. Oxalate reagent was used to determine ''intracellular'' P as previously described for Fe (Tovar-Sanchez et al. 2003) and P (Sañudo-Wilhelmy et al. 2004). Briefly, filters were incubated for 5 min with 5 mL of oxalate reagent and rinsed twice with 5 mL of Sargasso seawater. ''Surface-adsorbed'' or ''scavenged'' P was defined as the difference between total and intracellular P. Algal cells are not lysed or damaged in any way by the oxalate reagent washing procedure and, in fact, can resume growth soon thereafter (Tovar-Sanchez et al. 2003).

As an additional mechanistic control experiment, removal efficiency of surface-bound P_i by the oxalate reagent was compared with the Ti wash method (Hudson and Morel 1989). Ti washing has been applied as a standard method in many prior studies to remove surface-bound trace elements (Hutchins et al. 1999*a*,*b*; Eldridge et al. 2004) and thus provides a useful comparison to the newer oxalate wash method. Identical volumes of a *T. weissflogii* culture were filtered, followed by a 5-min exposure to the Ti reagent, the oxalate reagent, or seawater alone as described above. Six replicate samples were treated with each wash protocol, and total and intracellular P determinations were performed as described above.

C, N, and P elemental analysis—Triplicate samples for CHN analysis were obtained by filtering 5–20 mL of bloom or culture samples onto precombusted 13-mm Gelman AE GF filters or 25-mm GF/F filters. Samples were dried at 60°C before analysis using a Costech Elemental Combustion System (Hutchins et al. 1998). For particulate organic P (POP) determinations, 10–40-mL cultures were filtered onto precombusted 25-mm GF/F glass fiber filters, rinsed twice with 2 mL of 0.17 mol L^{-1} Na_2SO_4, placed in a combusted glass scintillation vial with 2 mL of 0.017 mol L^{-1} $MgSO_4$, and evaporated to dryness at 95°C. Vials were then baked at 450°C for 2 h. After cooling, 5 mL of 0.2 mol L^{-1} HCl was added to each tightly capped vial and heated at 80°C for 30 min. Dissolved phosphate from digested POP samples and from filtered culture medium samples was measured colorimetrically using a spectrophotometer (Solorzano and Sharp 1980; Fu et al. 2005).

$^{33}P_i$ uptake—For uptake kinetic examinations, *Trichodesmium* IMS101 and *T. weissflogii* CCMP1336 were grown in P-replete (20 μmol L^{-1}) or P-limited (0.2 μmol L^{-1} for *Trichodesmium* or 0.5 μmol L^{-1} for *Thalassiosira*) seawater medium. Rates of phosphate uptake were determined by measuring $^{33}P_i$ incorporation (Fu et al. 2005). Before each experiment, cultures were gently filtered and washed twice with 0.2-μm filtered, <0.02 μmol L^{-1} P_i Sargasso seawater. The cells were floated off the filter in 20 mL of medium containing P_i concentrations ranging from 0.1 μmol L^{-1} to 20 μmol L^{-1} for *Trichodesmium* or from 0.1 μmol L^{-1} to 50 μmol L^{-1} for *T. weissflogii* in 30-mL clear polyethylene flasks.

To measure uptake, 0.06 mBq of carrier-free $^{33}P_i$ (MP Biomedicals) was added to each flask containing P_i adjusted to the desired concentration. Uptake flasks were incubated for 1 h under the same conditions used to grow the cells. Uptake was terminated by rapid filtration through 0.45-μm Millipore polycarbonate filters, with or without oxalate washing as described above. The filters were then counted using a Wallac System 1400 liquid scintillation counter. During each experiment, glutaraldehyde-killed cells were carried through the same procedures as the live cells in order to compare the

amount adsorbed onto dead cells with the amount removed from the exterior of live cells by the oxalate reagent. Each experiment used triplicate-killed control blanks at each phosphate concentration.

The rate of P_i uptake was calculated as previously described (Sañudo-Wilhelmy et al. 2004; Fu et al. 2005). Cell-specific (V) and P-specific (V_p) uptake rates were calculated by normalizing molar P uptake derived from the total ^{33}P-specific activity in each experimental treatment to cell counts or to POP measurements. Kinetic values for both uptake and growth experiments (V_{max} or μ_{max} and K_s or K_m) were determined using the iterative rectangular hyperbolic curve-fitting procedure in Sigma Plot 8.0.

Time-course $^{33}P_i$ uptake experiments were carried out on cultures of *Trichodesmium* IMS101 and GBRTRLI101 to examine the maximum internalization rates of surface-bound P. One hundred milliliters of dense exponential growth-phase cultures grown at nonlimiting P_i concentrations (20 μmol L^{-1}) was filtered and rinsed and then transferred to 50 mL of severely P-limited medium made with unamended <0.02 μmol L^{-1} P_i Sargasso seawater. These cultures were then spiked with tracer levels of carrier-free $^{33}P_i$ (final added $[P_i] < 3 \times 10^{-11}$ mol L^{-1}) and incubated under standard light and temperature conditions. Samples were filtered after 5 min, 21 h, and 66 h and rinsed with seawater or oxalate reagent as described above to determine the changes in surface-bound and intracellular P. Because these cultures were growing rapidly when transferred into P-starved conditions, we assumed that their P demand and thus the internalization rates of surface-bound P represented near-maximal values.

Coupled $^{32}Si/^{32}P$ uptake—We used a coupled ^{32}Si and ^{32}P uptake experiment to demonstrate that a surface-adsorbed pool exists in diatom cells that can be removed by oxalate washing for P but not for Si. Sañudo-Wilhelmy et al. (2004) demonstrated that the wash does not remove cellular C or N, either. Stock cultures of the Bering Sea *Thalassiosira* sp. isolates were filtered and resuspended in Si-free Aquil medium. Eight subsamples received increasing additions of Na_2SiO_3 from 0 to 20 μmol L^{-1}. The kinetic uptake experiment was carried out using the ^{32}Si and ^{32}P method (Tréguer et al. 1991). Samples were spiked with 1,300 Bq of the radioactive isotope silicon-32 (^{32}Si) and incubated for 24 h. Six subsamples from each culture vial were then split equally and filtered onto 0.6-μm polycarbonate filters. The first set of triplicates was rinsed with 0.2 μm of filtered seawater, while the second set of triplicates was rinsed with the oxalate reagent as described previously.

The radioactive isotope ^{32}Si decays into ^{32}P with a half-life of 134 yr. Thus, the ^{32}Si stock solution is always composed of both ^{32}Si and ^{32}P in isotopic equilibrium, the latter having a much shorter half-life of 14 d. Uptake of ^{32}P thus increases in a proportional fashion with increasing concentrations of added ^{32}Si. Biological Si uptake is measured by counting the ^{32}P uptake by Cerenkov effect on a scintillation counter, as described in Tréguer et al. (1991) and modified by Leynaert (1993). Measurement of the radioactivity of samples immediately after filtration represents the sum of both P uptake and Si uptake by diatoms. When remeasured after approximately 5 months, the ^{32}P has disappeared (after >10 times its half-life), and the disintegration rates counted are solely generated by the ^{32}Si that was originally taken up as Si.

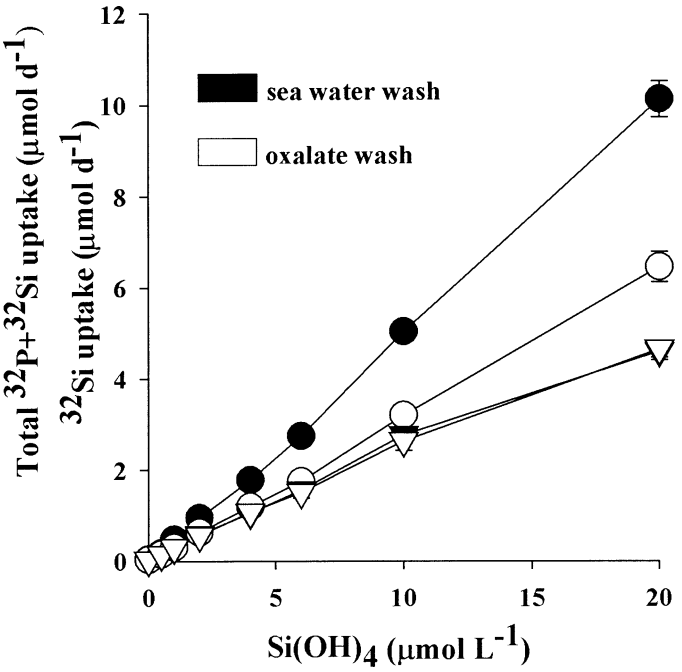

Fig. 1. Removal of surface-adsorbed P but not Si from cultured *Thalassiosira* sp. diatom cells. Uptake of P and Si combined was measured immediately following the 24-h incubation with $^{32}Si/^{32}P$ stock (circles) and 5 months later, after the ^{32}P had completely decayed, yielding uptake of ^{32}Si only (triangles). Two sets of triplicate samples were filtered and rinsed with seawater (filled symbols) or with the oxalate reagent (open symbols).

Results

That P but not Si exists in a surface-bound pool that can be removed from diatom cells by the oxalate wash was illustrated by the Si/P kinetic uptake experiment performed on the centric diatom *Thalassiosira* sp. (Fig. 1). The sum of ^{32}P and ^{32}Si uptake rates was measured immediately after filtration by counting the total radioactivity in each sample. Oxalate washing caused a significant decrease in the total combined Si and P uptake by diatoms. On average, the samples rinsed with oxalate contained only 68% $\pm$ 7% of the radioactivity measured in the samples rinsed with seawater.

After the samples were stored for 5 months to allow all of the ^{32}P to decay, they were recounted a second time, allowing a calculation of ^{32}Si uptake rates alone. As shown in Fig. 1, there was no impact of the oxalate wash treatment on Si uptake rate measurements compared to the seawater rinsing. This experiment indicates that the oxalate reagent does not remove Si from the surface of cells but that it is very efficient at removing adsorbed P. Thus, a substantial surface-adsorbed pool exists for P but not for Si (Fig. 1) or for C and N (Sañudo-Wilhelmy et al. 2004). It is also notable that in this case, P uptake rates would have been overestimated by approximately 30% if the adsorbed phosphate had not been rinsed from the cells before counting.

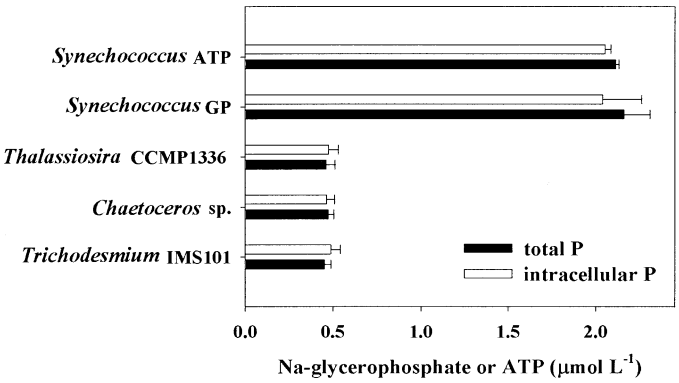

Fig. 2. Cellular phosphorus measured in the intracellular P and total P pools for four algal species (*Synechococcus* CCMP1334, *Thalassiosira* CCMP1336, *Chaetoceros* sp., and *Trichodesmium* IMS101) grown in medium with 20 μmol L^{-1} organic P (Na-glycerophosphate; GP) as a sole P source and for *Synechococcus* CCMP1334 grown in medium with 20 μmol L^{-1} P supplied as ATP for a sole P source.

The difference between counts before and after the decay period gives an estimate of P assimilation by the cultures during the uptake period (data not shown). These show that in our kinetics experiment, P uptake was low and relatively invariant at all low added Si concentrations. P uptake, however, increased somewhat at the highest Si levels, perhaps due to growth stimulation of the diatom cultures by elevated Si levels during the uptake period.

Removal efficiency of surface-adsorbed P_i from *T. weissflogii* cells was not significantly different ($p > 0.05$) using the oxalate wash and the Ti wash (data not shown). Both washes removed 24–28% of the total cellular P when compared to the filtered seawater-washed samples, even though the removal mechanism of the surface-adsorbed pool may have been different (reduction vs. oxalate-promoted dissolution under nonreductive conditions; *see* Hudson and Morel [1989] and Zinder et al. [1986], respectively). However, Zinder et al. (1986) also showed that reductive dissolution from surfaces could be promoted by oxalate. While further studies are required to fully understand the surface-removal mechanisms, our results clearly showed that the Ti wash (Hudson and Morel 1989), which, like the oxalate wash (Tovar-Sanchez et al. 2003), was designed to remove adsorbed metals, also washes off scavenged P with almost identical efficiency.

The oxalate reagent efficiently removed cell surface–associated phosphate but did not remove any cellular P from four algal species grown in medium containing only an organic P source (sodium-glycerophosphate or ATP) (Fig. 2). There were no significant differences in cellular P content between oxalate reagent and seawater-rinsed samples. Similar results were obtained in axenic *Synechococcus* cultures grown using either sodium-glycerophosphate or ATP as the sole P source. This could indicate either that there is no surface-adsorbed dissolved organic P pool or that, if there is, it is not removed by the oxalate reagent. Together, these experiments support the suggestion of Sañudo-Wilhelmy et al. (2004) that the scavenged behavior of phosphate is a property of this P chemical species and that the existence of surface-bound P is diagnostic of cells growing on phosphate P.

Figure 3A shows the distribution of total cellular P into surface-adsorbed and intracellular P in 11 cultured isolates of eukaryotic and prokaryotic marine phytoplankton. The amount of surface-adsorbed P on cells harvested during exponential growth ranged from 14% (*Prorocentrum*) to 57% (*Trichodesmium*) of the total P (Fig. 3A). There was a significant inverse linear correlation between specific growth rates of all the species tested (except for *A. anophagefferens*, for which growth rates were not measured) and the amount of P in scavenged pools ($r^2 = 0.65$; Fig. 3B). The fraction of surface-adsorbed P increased for cultures with lower growth rates. Only a weak correlation ($r^2 = 0.28$) was found between surface to volume ratios of the cultured cells and the ratio of adsorbed to intracellular P (data not shown). This suggests that factors other than just the surface area of the cells available for P scavenging largely determine the relative amounts of surface-bound and interior P (*see following text*).

Molar C : P and N : P ratios calculated using both total and intracellular P pools in the same cultures are shown in Fig. 3C,D. The C : total P (surface-adsorbed P + intracellular P) ratios for all the species were close to Redfield values and varied among the eukaryotes between 80 for the diatom *Thalassiosira* and 129 for the dinoflagellate *Prorocentrum.* Diatoms had the lowest ratios of C : total P (80–88), while cyanobacteria generally had slightly higher C : total P ratios than the eukaryotes, ranging from 108 and 117 for the two *Synechococcus* strains to 129 for *Prochlorococcus* and 131 for *Trichodesmium.* However, for all species, C : intracellular P ratios were 1.2–2 times higher than C : total P ratios and were always much higher than the Redfield model (Fig. 3C).

Like the C : P ratios, the N : total P (surface-adsorbed P + intracellular P) ratios for all the species were close to Redfield values, ranging from 13 for the diatom *Chaetoceros* to 24 for the cyanobacterium *Prochlorococcus.* However, the N : intracellular P ratios were 1.3–2 times higher than in the total P pool (Fig. 3D).

For the eight natural bloom samples, the amount of surface-adsorbed P ranged from 15% to 46% (Fig. 4A). Thus, the partitioning of P into interior and scavenged pools was quite similar to the trends observed in the exponential phase batch cultures, although the growth histories of the natural samples are unknown. These ratios were also very similar to those of the unialgal cultures despite the potential contributions of other organisms (other algae, bacteria, and microzooplankton) and detritus, suggesting that elemental ratios in these dense blooms were dominated by the algal biomass.

The removal of surface-adsorbed P as a function of the ambient P_i concentrations at the collection sites is shown in Fig. 4B. There was a significant positive linear correlation between ambient P_i concentrations and the fraction of total P in the surface-adsorbed pool ($r^2 = 0.80$). Like the laboratory cultures, the natural bloom samples showed significant differences between the molar C : P and N : P ratios measured using the total P and intracellular P pools (Fig. 4C,D). In all eight blooms, C : total P ratios ranged from 71 to 151. The blooms of the diatoms *Cylindrotheca* and *Leptocylindrus* had much lower ratios of C : total P (71 and 76, respectively), while the dinoflagellate bloom (*Gymnodinium*) and cyanobacterial bloom (*Synechocystis*) had higher C : to-

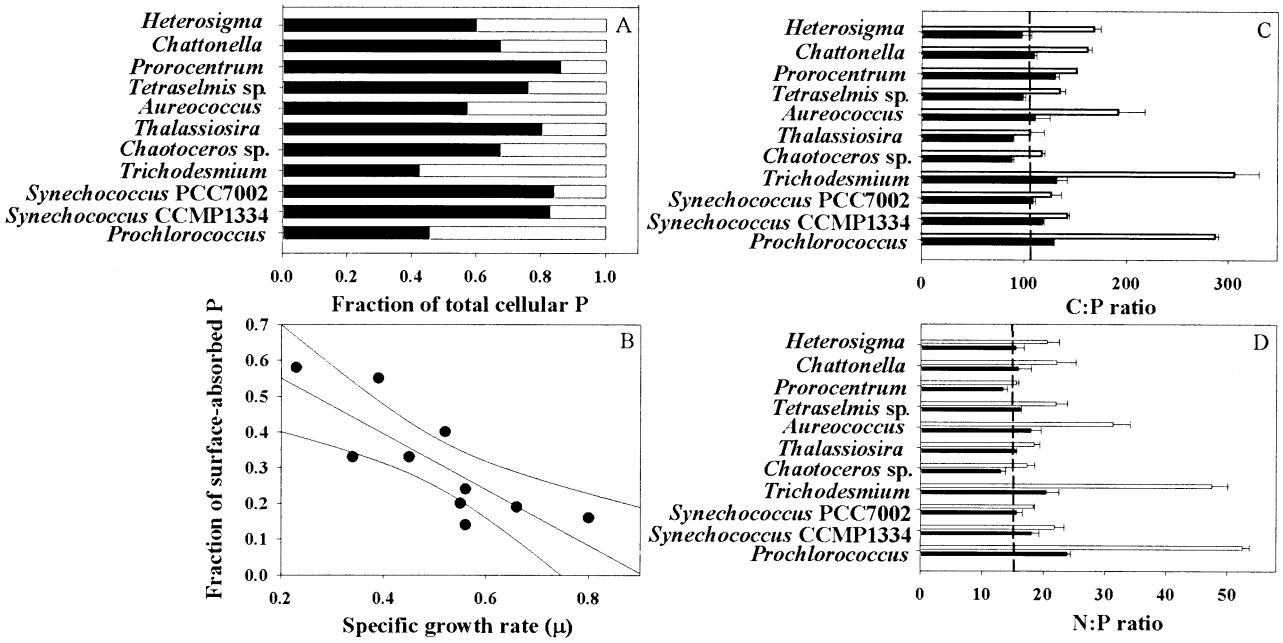

Fig. 3. Effects of phosphate scavenging on cellular partitioning of P and elemental ratios in 11 species of cultured prokaryotic and eukaryotic algae. (A) Fractions of total cell-associated P in the intracellular (filled bars) and surface-adsorbed P pools (open bars) and (B) inverse correlation between the growth rates of the cultures and the fraction of total cellular P in exterior pools. Shown are the linear correlation (solid line, $r^2 = 0.65$; $y = 0.71 - 0.78x$) and 95% confidence limits (dashed lines). (C) C : P and (D) N : P ratios measured using the total P (filled bars) and intracellular P pools (open bars). Error bars represent the standard deviations of triplicate samples, and dashed lines indicate the Redfield ratio.

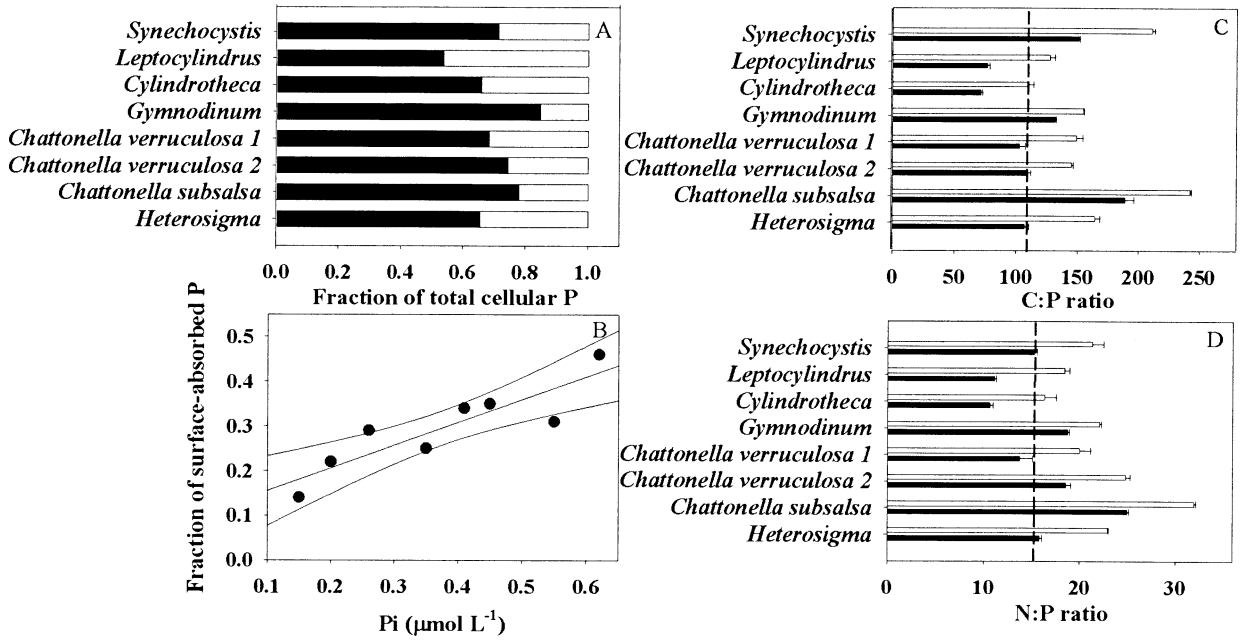

Fig. 4. Effects of phosphate scavenging on cellular partitioning of P and elemental ratios in eight natural estuarine bloom samples of prokaryotic and eukaryotic algae. (A) Fractions of total cell-associated P in the intracellular (filled bars) and surface-adsorbed P pools (open bars) and (B) correlation between the ambient P_i concentrations at the collection sites and the fraction of total cellular P in exterior pools. Shown are the linear correlation (solid line, $r^2 = 0.78$; $y = 0.10 + 0.51x$) and 95% confidence limits (dashed lines). (C) C : P and (D) N : P ratios measured using the total P (filled bars) and intracellular P pools (open bars). Error bars represent the standard deviations of triplicate samples, and dashed lines indicate the Redfield ratio.

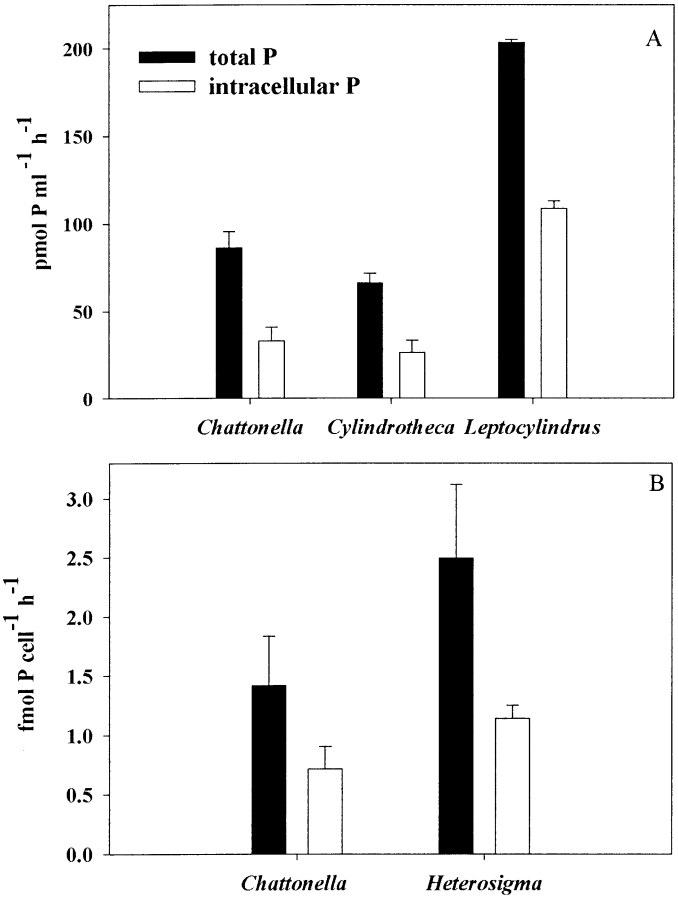

Fig. 5. Phosphate uptake rates ($^{33}P_i$) measured for intracellular (oxalate rinsed, open bars) and total P pools (seawater rinsed, filled bars) in (A) three natural local estuarine bloom samples, including a raphidophyte and two diatoms (pmol P mL^{-1} h^{-1}), and (B) two laboratory raphidophyte cultures isolated from local estuarine waters (fmol P cell^{-1} h^{-1}). Error bars represent the standard deviations of triplicate samples.

tal P ratios (131 and 151, respectively). As in the laboratory cultures, the ratios of C : total P of the raphidophyte blooms were close to Redfield values, except for one bloom of *C. subsalsa* (~190). In every case, however, the ratios of C : intracellular P were 1.3–1.7 times higher than the ratios of C : total P (Fig. 4C). The N : total P ratios were close to Redfield values for all the natural samples, except for the two diatom blooms (~11) and the *C. subsalsa* bloom (~25) (Fig. 4D). For all bloom samples, the N : intracellular P ratios were 1.3–1.6 times higher than those calculated using the total P pool.

Phosphate uptake rates were measured for natural bloom samples of the raphidophyte *C. subsalsa* and the diatoms *Cylindrotheca* sp. and *Leptocylindrus* sp. (Fig. 5A). Removal of the surface-adsorbed P pool resulted in substantially lower uptake rates, ranging from 38% to 53% of the rates measured for uptake into total cellular P pools. Similar to the natural bloom samples, 51% and 46% of total phosphate uptake was incorporated into intracellular pools in our local isolates of the raphidophytes *C. subsalsa* and *H. akashiwo*, respectively (Fig. 5B).

Results of kinetic determinations of phosphate uptake in the cyanobacterium *Trichodesmium* IMS101 are shown in Fig. 6. Cell-normalized rates of P_i incorporation (V) into both total and intracellular pools by P-replete cultures of *Trichodesmium* increased linearly as the phosphate concentrations increased up to 1 μmol L^{-1} (Fig. 6A). The maximum cellular uptake rates (V_{max}) measured after removal of the surface-adsorbed P pool were half as high as those for the total P pool, but the cellular half-saturation constants (K_s) were similar from both measurements (Table 2).

In contrast to the cell-normalized values, P-specific uptake rates (V_P) into the intracellular and total P pools by P-replete cultures followed similar curves (Fig. 6B), and the maximum specific uptake rates (V_{Pmax}) from both measurements were not significantly different ($p > 0.05$) (Table 2). However, the P-specific half-saturation constant for P_i uptake (K_{Ps}) for the intracellular P pool was higher than for the total P pool ($p < 0.01$).

Like the P-replete *Trichodesmium* cultures, V_{max} values of P-limited cells for the total cellular P pool were significantly higher (2.4-fold greater, $p < 0.01$) than those for the intracellular P pool (Fig. 6C; Table 2). K_s was also significantly higher (twofold) for the total P pool ($p < 0.01$) (Fig. 6C; Table 2). Unlike P-replete cultures, the V_{Pmax} values for the total P pool in P-limited cultures were roughly twice as high as for the intracellular P pools ($p < 0.01$; Fig. 6D), but K_{Ps} values for both P pools were not significantly different ($p > 0.05$) (Fig. 6D; Table 2).

The maximum cellular uptake rates (V_{max}) for the total P pool in P-replete cultures of the diatom *T. weissflogii* were 30% higher than for the intracellular P pool ($p < 0.005$), but K_s values were not significantly different ($p > 0.05$) from each other (Fig. 7A; Table 2). In contrast, V_{Pmax} and K_{Ps} values were not significantly different for the two pools in P-replete cultures ($p > 0.05$; Table 2). In P-limited cultures, the V_{max} values for the total P pool were 1.7-fold higher than for the intracellular P pool ($p < 0.001$), and there were significant differences in K_s values ($p < 0.05$) between these two pools (Fig. 7C; Table 2). P-specific uptake kinetic constants (V_{Pmax} and K_{Ps}) in P-limited cultures were significantly different between the two pools ($p < 0.001$ and $p < 0.0005$, respectively) (Fig. 7D; Table 2).

A time course measuring the fraction of intracellular and surface-adsorbed P in P-replete cultures of the centric diatom *T. weissflogii* CCMP1336 and the cyanobacterium *Synechococcus* CCMP1334 is presented in Fig. 8A,B. The cultures were grown into N-limited stationary phase in medium with initial excess P (initial P, 20 μmol L^{-1}; initial N, 200 μmol L^{-1}) for 7–9 days. Both of these species showed similar trends, as the amount of surface-adsorbed P was low during initial rapid growth, but then increased as growth slowed with time. During the exponential growth phase, the amount of surface-adsorbed P was 15% (*Thalassiosira*) and 18% (*Synechococcus*), but these values increased to 40% and 42%, respectively, when the cultures reached final stationary phase.

A time-course determination of the surface-adsorbed phosphate pool in exponentially growing laboratory cultures of *Trichodesmium* strains IMS101 and GBRTRLI101 is shown in Fig. 8C. Cultures were transferred to severely P-limited medium (unamended Sargasso seawater, <0.02 μmol L^{-1} P_i)

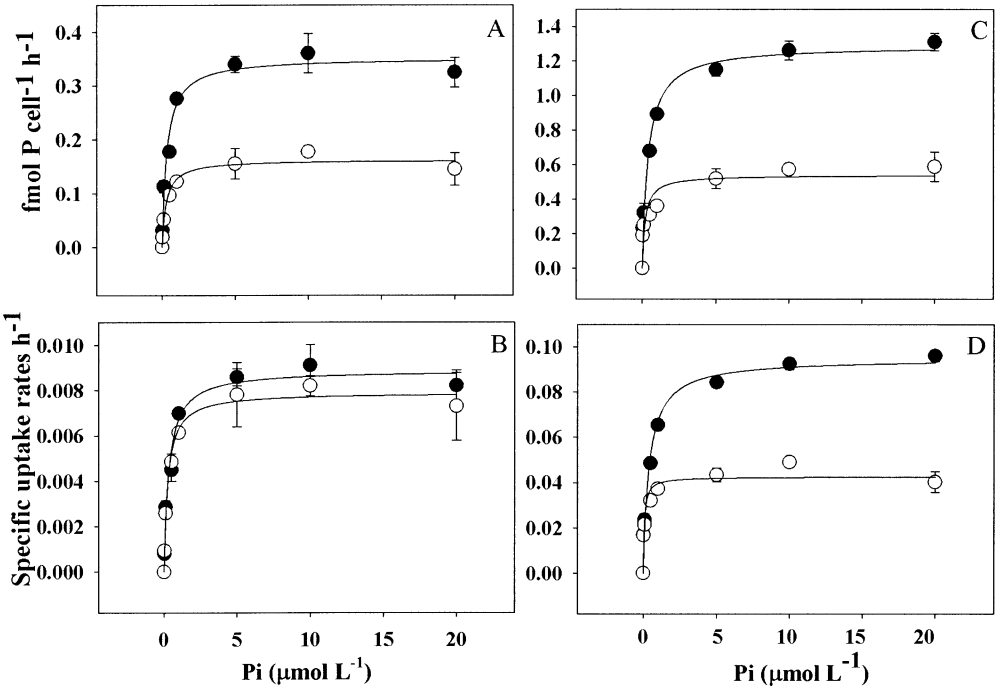

Fig. 6. Phosphate uptake rates measured into intracellular (oxalate rinsed, open circles) and total P pools (seawater rinsed, filled circles) by cultures of the cyanobacterium *Trichodesmium* IMS101 as a function of P_i concentration. (A) Cell-normalized uptake (V) and (B) P-specific uptake (V_P) by P-replete cultures. (C) V and (D) V_P in P-limited cultures. Note Y-axis scale differences for both V (panels A and C) and V_P (panels B and D) between P-replete and P-limited cultures. Rectangular hyperbolic regressions between uptake rates and P_i concentrations were forced through the origin, assuming no uptake at a hypothetical zero P_i concentration. Error bars are the standard errors of triplicate samples.

and then spiked with only tracer levels of ^{33}P to estimate the maximum internalization rates of surface-bound P. The maximum fraction of total cellular P in the surface-adsorbed pool (87%) was recorded immediately (5 min, day 0) after adding the $^{33}P_i$ radiolabel, demonstrating the very rapid kinetics of P scavenging onto the cell surfaces. This cell surface-bound pool represented about 70% of the added $^{33}P_i$ activity, suggesting that fast, efficient scavenging of dissolved P_i can be advantageous to algae growing in P-limited regimes.

By 21 h (day 0.875) later, the fraction of total cellular P on the surface of the cells had been reduced to 20.4% and 35% for IMS101 and GBRTRLI101, respectively (Fig. 8C). At the final 66-h (day 2.75) time point, only 3.4% (IMS101) and 21.4% (GBRTRLI101) remained on the cell surfaces. That no appreciable dissolved ^{33}P activity was detected in the seawater at this time (data not shown) suggests an efficient assimilation of the surface-bound P by the severely P-starved cultures during the 2.75-d experiment. Slopes of regression lines for the internalization of the adsorbed P pool versus time (Fig. 8C) demonstrated that these cultures were able to assimilate 21% (GBRTRLI101) to 27% (IMS101) of this surface pool per day.

Discussion

Our experiments confirm and support the study of Sañudo-Wilhelmy et al. (2004) that focused largely on P scavenging in *Trichodesmium*. We extended these findings to a broad taxonomic range of eukaryotic and prokaryotic phytoplankton in the laboratory and the field. Our results strongly suggest that the elemental composition of many (and probably all) planktonic algae growing on phosphate as a P source is affected by the existence of surface-adsorbed and intracellular P pools.

Molar C : total P ratios from the laboratory cultures and bloom samples for most taxa, except for diatoms (C : total P = 71–88) and cyanobacteria (C : total P = 129–131), were close to Redfield values. However, the molar C : intracellular P ratio was always greater than the Redfield ratio, frequently up to two times higher. Molar N : intracellular P ratios were also up to two times higher than the ratios of N : total P. Clearly, P quotas of phytoplankton that consider only the intracellular, ''biological'' P pools are as little as half those predicted by the traditional Redfield approach that encompasses both intracellular and scavenged P. As with other particle-reactive elements such as Fe, the determination of the true physiological P quotas first requires the removal of the surface-bound P pool from algal cells.

In our laboratory cultures grown under P-replete conditions, N : intracellular P ratios ranged from 17 to 52, consistent with model calculations (Geider and La Roche 2002). Hence, the use of a Redfield ratio of 16 N : total P to estimate algal N or P limitation may be misleading. Future definitions of critical ratios for N and P limitation of phytoplankton

Table 2. Kinetic constants for P_i uptake in P-limited and P-replete cultures of the cyanobacterium *Trichodesmium erythraeum* (IMS101) and the diatom *Thalassiosira weissflogii* (CCMP1336) into the total P and intracellular P pools. Shown are cell-normalized maximum uptake rates (V_{max}, fmol P cell^{-1} h^{-1}) and half-saturation constants (K_s, μmol P L^{-1}); P-specific maximum uptake rates (V_{pmax}, h^{-1}); and half-saturation constants (K_{ps}, μmol P L^{-1}).

Species	Growth condition	Kinetic constants							
		V_{max}		K_s		V_{pmax}		K_{ps}	
		Total P	Intra-P	Total P	Intra-P	Total P	Intra-P	Total P	Intra-P
Trichodesmium erythraeum	P-limited	1.3±0.06	0.54±0.05	0.40±0.03	0.20±0.01	0.095±0.004	0.043±0.003	0.41±0.03	0.47±0.04
	P-replete	0.35±0.015	0.16±0.01	0.32±0.02	0.28±0.03	0.0079±0.0003	0.0089±0.0004	0.25±0.02	0.32±0.03
Thalassiosira weissflogii	P-limited	144.3±1.5	84.3±0	1.1±0.08	0.90±0.08	1.09±0.0006	0.87±0	1.05±0.006	0.90±0
	P-replete	3.5±0.03	2.7±0.09	0.68±0.05	0.71±0.06	0.0013±0.00006	0.0012±0.00015	0.67±0.04	0.65±0.006

should focus on the intracellular P pool, rather than on total cellular P content.

Under our culture conditions, the diatoms *T. weissflogii* and *Chaetoceros* sp. had lower C : total P ratios (81–88) than those of other algae. This observation is in agreement with the study of Quigg et al. (2003), who found that C : total P ratios of diatoms were ~75. In contrast, our C : total P ratios for the cyanobacteria *Synechococcus* CCMP1334, *Prochlorococcus* CCMP1986, and *Trichodesmium* IMS101 and the dinoflagellate *Prorocentrum* were all higher than Redfield values, ranging from 118 to 135. Our results are quite close to ratios observed in previous studies of *Synechococcus* CCMP1334 by Cuhel and Waterbury (1984) and of *Prochlorococcus* CCMP1986 by Bertilsson et al. (2003).

In our cultures, only the C : total P and N : total P ratios of the prasinophyte, pelagophyte, and raphidophytes were close to the Redfield model. These observations generally confirm the studies of Ho et al. (2003) and Quigg et al. (2003), suggesting that variation in elemental composition arises from different requirements for N and P among algal groups (Geider and La Roche 2002). Phytoplankton metal : P ratios that are based on total P measurements (Ho et al. 2003; Quigg et al. 2003) would, however, be considerably higher if they were based on intracellular P quotas.

The ratios of C : intracellular P for the cyanobacteria *Trichodesmium* and *Prochlorococcus* (287 and 305, respectively) were also much higher than for the other species examined. Like C : total P ratios, the C : intracellular P ratios of diatoms are significantly lower than those of the other algal groups, often close to the Redfield ratio. The C : intracellular P ratio of the harmful bloom pelagophyte *Aureococcus* (''brown tide'') was 1.7 times higher than the C : total P ratio of 110. The dinoflagellate *Prorocentrum* had C : intracellular P ratios similar to the raphidophytes (161–178), although the C : total P ratios of *Prorocentrum* were 1.3 times higher than the same ratios in raphidophytes. This suggests possible differences in cell surface-scavenging properties, but not in intracellular P quotas, between the two groups.

As with the C : intracellular P ratios, the N : intracellular P ratios were highest for the cyanobacteria *Trichodesmium* and *Prochlorococcus*, ranging from 47 to 52. The prasinophyte *Tetraselmis* and the raphidophytes also had N : intracellular P ratios substantially higher than the Redfield model, which were relatively constrained between 21 and 31. The same ratios for the diatoms, the dinoflagellate, and the coastal cyanobacterium *Synechococcus* PCC7002 were, however, significantly lower, fairly close to Redfield values.

Similar to the laboratory cultures, high levels of surface-adsorbed P (ranging from 15% to 46% of the total) were also measured in natural estuarine raphidophyte, dinoflagellate, diatom, and cyanobacteria blooms. Sañudo-Wilhelmy et al. (2004) observed even higher values (~60%) for two natural open-ocean diatom blooms. For both estuarine and oceanic samples, there were significant differences between the molar C : P and N : P ratios calculated using the total P or intracellular P measurements. Because the prior growth history and nutrient status of the bloom samples were unknown and can affect P partitioning (*see following text*), it is difficult to compare elemental ratios from different algal groups rigorously. However, the elemental ratios of C : P and

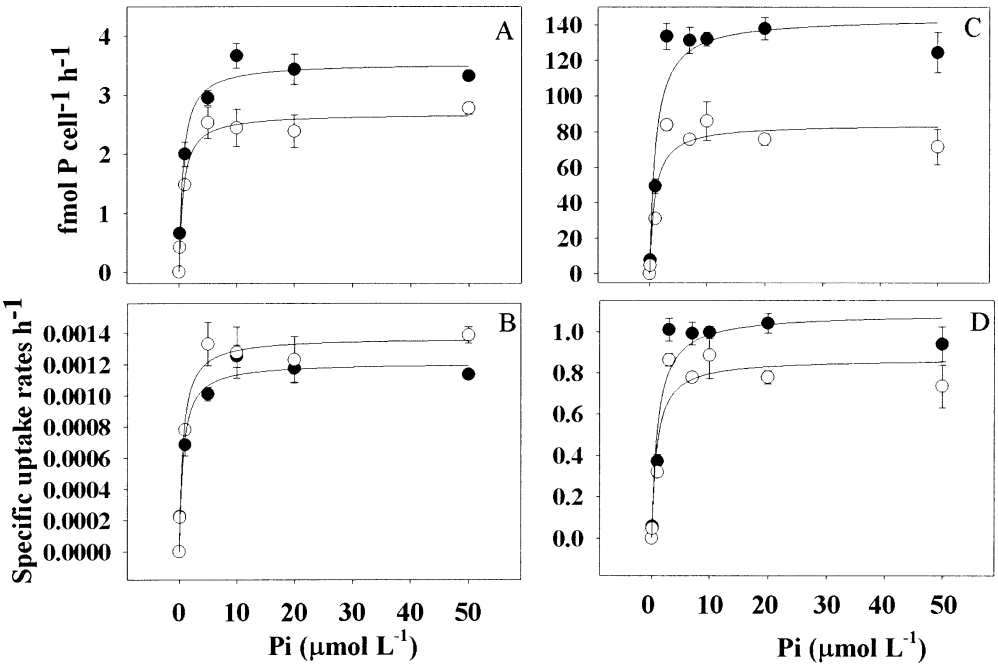

Fig. 7. Phosphate uptake rates measured into intracellular (oxalate rinsed, open circles) and total P pools (seawater rinsed, filled circles) by cultures of the centric diatom *Thalassiosira weissflogii* as a function of P_i concentration. (A) Cell-normalized uptake (V) and (B) P-specific uptake (V_P) by P-replete cultures. (C) V and (D) V_P in P-limited cultures. Note Y-axis scale differences for both V (panels A and C) and V_P (panels B and D) between P-replete and P-limited cultures. Rectangular hyperbolic regressions between uptake rates and P_i concentrations were forced through the origin, assuming no uptake at a hypothetical zero P_i concentration. Error bars are the standard errors of triplicate samples.

N : P (both total and intracellular) from the two diatom blooms were similar to the laboratory culture values and were significantly lower those observed during the blooms of other taxa.

Taxonomic trends in intracellular P-based ratios of N : P and C : P are summarized by plotting the two ratios against each other for all of the major algal groups we examined in all of the natural bloom samples and laboratory cultures (Fig. 9). Two of the species of cultured cyanobacteria we examined had very elevated ratios of both C : P and N : P, while the single field cyanobacterial bloom had an elevated C : P ratio but an N : P ratio that was close to many of the other species examined. Ratios of the two laboratory cultures of *Synechococcus*, however, cluster down with many of the eukaryotic algae. This suggests that some, but not all, cyanobacteria have P quotas that are much lower than those of eukaryotes. Likewise, the single cultured pelagophyte we examined had a low P content relative to both C and N.

At the other extreme, all four natural bloom and culture samples of diatoms had very low ratios of both N : intracellular P and C : intracellular P, suggesting that the P quotas of this group are unusually high (Fig. 9). Ratios of the raphidophytes (except for a single natural bloom sample with very high values), the prasinophyte, and the two dinoflagellate samples all have intermediate elemental ratios ranging from 137 to 175 for C : intracellular P and from 18 to 25 for N : intracellular P. Thus, clear trends are evident when intracellular P-based elemental ratio data are grouped by major algal taxon and seem to reflect P availability during the evolutionary history of phytoplankton (Bjerrum and Canfield 2002; Quigg et al. 2003).

A rather strong relationship was found between the P_i concentrations in the water collected with the natural blooms and the fraction of the total P in surface-adsorbed pools (Fig. 4B). At higher P_i concentrations, greater amounts were found in the scavenged fraction, a correlation that is perhaps surprising, considering the relatively narrow range of P_i concentrations in these environmental samples (0.15–0.62 μmol L^{-1}, Table 1). Our culture results suggest that surface-adsorbed P is apparently not present in cultures grown on medium with dissolved organic P as a sole P source or that, if it is present, it cannot be removed by the oxalate reagent (Fig. 2). Together, these results suggest that the existence of a surface-adsorbed P pool that can be washed off with oxalate is diagnostic of cells growing on orthophosphate as a P source and that the relative amounts in scavenged and intracellular pools reflect the external phosphate concentrations at which the cells were grown. The total amount of adsorbed P should saturate at some external P_i concentration, since the number of cell surface–binding sites is necessarily finite. We did not determine this saturation concentration, and future work will be required to evaluate it.

Like elemental ratios, our results suggest that kinetic constants for phytoplankton phosphate uptake using total P pools (McCarthy and Carpenter 1979; Donald et al. 1997; Fu et al. 2005) also need to be reevaluated using intracellular

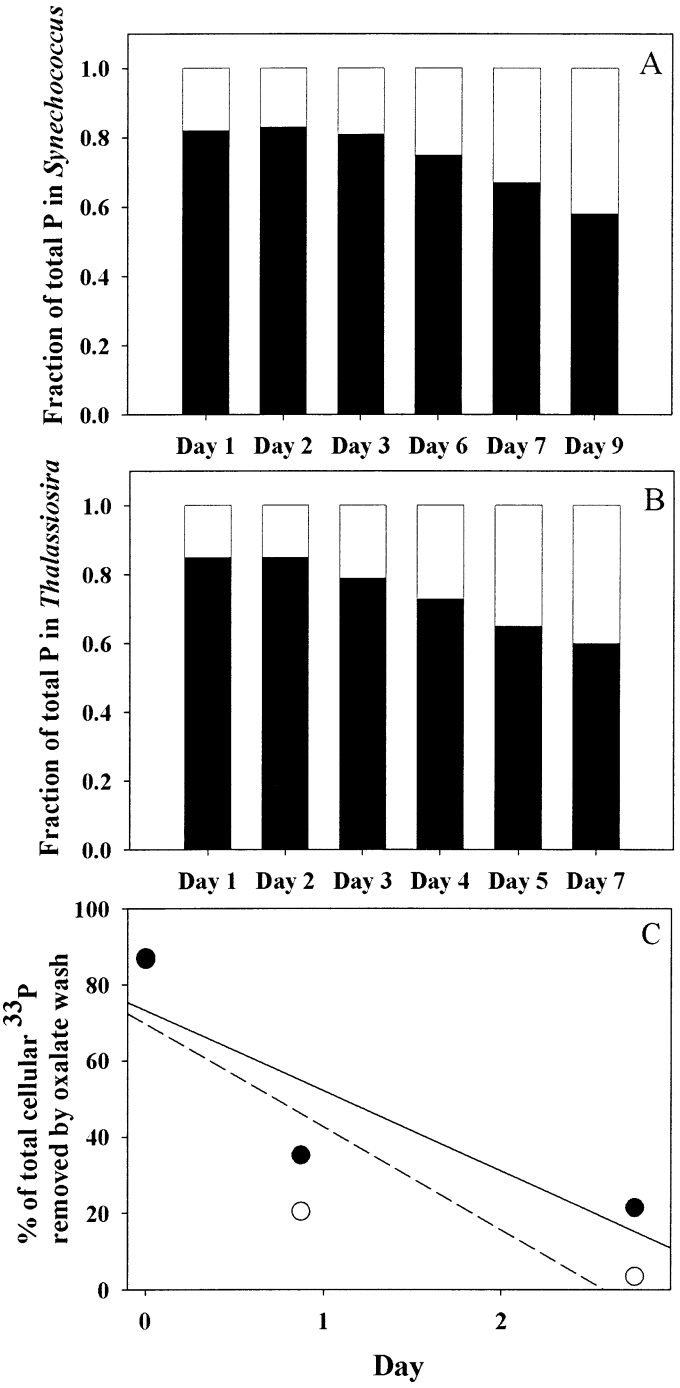

Fig. 8. A time course from early exponential to late stationary growth phase showing the fraction of total cellular P measured in intracellular (filled bars) and surface-adsorbed (open bars) pools in P-replete cultures of (A) the cyanobacterium *Synechococcus* CCMP1334 and (B) the diatom *Thalassiosira weissflogii* CCMP1336. (C) A time course showing the fraction of total cellular ^{33}P activity in the surface-adsorbed pool 5 min, 21 h, and 66 h after tracer levels of $^{33}P_i$ were added to exponentially growing cultures of *Trichodesmium* IMS101 (open circles) and *Trichodesmium* GBRTRLI101 (filled circles) directly after transfer to P-starved medium (unamended Sargasso seawater). Initial symbols overlie each other. Regression lines are shown for internalization of the surface-adsorbed P pool for IMS101 (solid line, $y = 69.7 - 27.1x$, $r^2 = 0.74$) and GBRTRLI101 (dashed line, $y = 73.3 - 21.1x$, $r^2 = 0.74$).

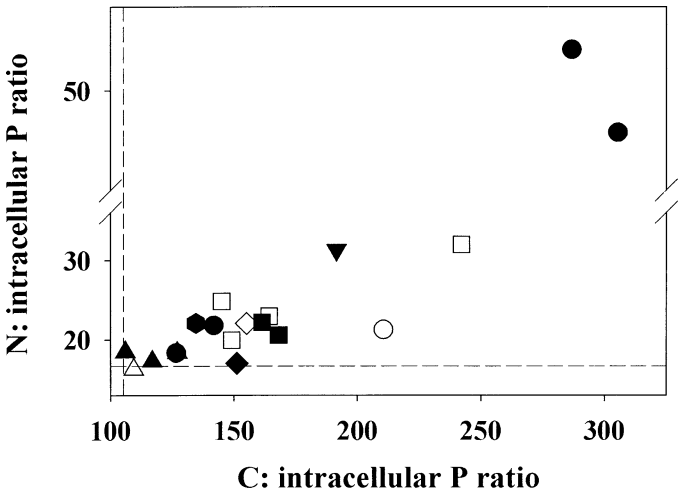

Fig. 9. A plot of N : intracellular P ratios versus C : intracellular P ratios for the major phytoplankton taxa examined in the laboratory experiments (closed symbols) and natural blooms (open symbols). Shown are data for cultures or blooms of five cyanobacteria (circles), four diatoms (triangles), seven raphidophytes (squares), two dinoflagellates (diamonds), one pelagophyte (inverted triangle), and one prasinophyte (hexagon). Dashed lines indicate Redfield N : P and C : P ratios. Note scale break on the Y-axis.

P. Removal of surface-adsorbed P resulted in much lower cell-normalized maximum uptake rates for P-replete and P-limited *Thalassiosira* and *Trichodesmium* cultures—in some cases, less than half those measured using the total cellular P pool. Cell-normalized half-saturation constants measured for the intracellular P pool showed similar trends for both P-replete and P-limited *Trichodesmium* and P-limited *Thalassiosira*, although there was no significant difference for P-replete *Thalassiosira.*

Maximum P-specific uptake rates for both species in P-limited cultures were also much lower after removing the surface-adsorbed pool. In contrast, however, maximum specific uptake rates for both P-replete cultures of *Trichodesmium* and *Thalassiosira* were quite similar for the intracellular P and total P pools. The reason for this is that, in the P-replete cultures used for these experiments, there were initially relatively large amounts of surface-adsorbed phosphate, while in P-limited cultures, there was much less present (*see following text*). After the short ^{33}P uptake period, however, a similar amount of adsorbed isotope is removed by the oxalate reagent from cell surfaces of both types of cultures. Thus, after washing at the end of the uptake period, the numerator in the specific uptake calculation (V_P = ^{33}P uptake rate/cellular P) is reduced by a similarly large factor for both P-replete and P-limited cultures, but the denominator is reduced by a proportional amount only in P-replete cultures. The net result yields similar P-specific uptake rates in P-replete cells, regardless of the presence or absence of the scavenged P pool, but much lower specific uptake rates after oxalate washing in P-limited cultures.

In uptake experiments, killed cell controls are sometimes used to correct for the abiotic adsorption of an isotope onto live cell surfaces (McCarthy and Carpenter 1979; Rivkin and Swift 1982; Fu et al. 2005). Our comparison of the amount

of surface-adsorbed ^{33}P in live cells (total − intracellular) pools with the total (presumably only surface adsorbed) ^{33}P taken up by glutaraldehyde-killed cells does not support the use of this protocol. We found that the amount of ^{33}P adsorbed to the killed cells averaged only 41% of the amount adsorbed to the surface of live cells from the same cultures (data not shown). Hence, these killed controls are not an adequate correction for the amounts of isotope in surface-adsorbed pools of live cells. Glutaraldehyde and other preservatives greatly alter cellular properties; for instance, microscopic examination shows that glutaraldehyde-killed cells have decreased cell biovolumes and frequently present a shrunken or wrinkled appearance. It is possible that exposure to glutaraldehyde also changes the cell surface chemical properties of the killed control cells, relative to live cells, as well as potentially altering dissolved chemistry in the seawater medium. When available, direct measurement methods for abiotic isotope adsorption by live cells (such as the oxalate or Ti washes) are preferable to the use of controls with killed cells.

Although our results suggest that the surface-adsorbed P pool should not be considered part of the intracellular biological P quota of cells, this does not mean that scavenged phosphate is not biologically available to some degree over time. The time course using two strains of severely P-starved *Trichodesmium* showed that the surface-adsorbed P decreased dramatically during the incubation period, suggesting that a large portion of this P pool can be internalized relatively rapidly when cellular demand is high. This observation supports the suggestion of Sañudo-Wilhelmy et al. (2004) that P uptake is a two-step process: adsorption onto the cell surface, followed by internalization. This also suggests that surface-bound phosphate could, in effect, serve as a cellular P reserve, in addition to intracellular biochemical P storage compounds like polyphosphates (Kulaev and Vagabov 1983; Cembella et al. 1984).

Another perspective on how algal P demand can change the size of the scavenged P pool is offered by the experiments that examined changes in cultures grown in N-limited medium with excess P (20 μmol L^{-1}) (Fig. 8A,B). For P-replete cultures of both the cyanobacterium *Synechococcus* and the diatom *T. weissflogii,* the amount of surface-adsorbed P more than doubled from the early exponential growth phase to the N-limited stationary phase. These culture experiments and the ones discussed above together suggest that the amount of P adsorbed to the outside of algal cells varies with cell growth demand (less scavenged P at high cellular P demands) and with exterior phosphate concentrations (more scavenged P at higher exterior concentrations). These conclusions are further supported by the inverse correlation between culture growth rates and adsorbed P (Fig. 3B) and the direct correlation with external phosphate concentrations in the field samples discussed above (Fig. 4B).

The amount of scavenged P in natural populations would therefore be expected to be a function of the proportion of actively growing versus senescent cells and of ambient P_i levels. Phytoplankton growing in oligotrophic regimes, where ambient P_i is frequently at nanomolar levels (Wu et al. 2000; Karl et al. 2001), might be expected to have much less of their total cellular P in scavenged pools than in algae from P_i-rich coastal or HNLC environments. Although few measurements have so far been made on oceanic algae, *Trichodesmium* colonies and two diatom bloom samples collected by Sañudo-Wilhelmy et al. (2004) from the oligotrophic North Atlantic Ocean had surprisingly large amounts of adsorbed P, considering the low P_i concentrations typically found here. More work with field phytoplankton populations from a variety of oceanic regimes is required.

Another factor that could affect the amount of cell surface P scavenging is the availability of metal hydrous oxides to mediate ligand-exchange binding of phosphate. Sañudo-Wilhelmy et al. (2004) observed a strong correlation between the amount of adsorbed Mn on field-collected *Trichodesmium* colonies and the amount of scavenged phosphate (and also another oxyanion, molybdate). This proposed P-scavenging mechanism is supported by the work we did here comparing the P removal efficiency of the oxalate and Ti washes, which both operate by removing surface-bound metals. The amounts of metal cations like Fe and Mn adsorbed to cell surfaces are typically much lower in remote oceanic regions with low ambient metal levels than in coastal regimes (Hutchins and Bruland 1995; Tovar-Sanchez et al. 2003). Thus, in areas like the Southern Ocean where both Mn and Fe levels are quite low, the degree of phosphate scavenging onto phytoplankton surfaces may be correspondingly reduced compared to areas where metal hydroxides are more abundant.

The molar amounts of scavenged metal cations that would be available in most oceanic regimes are also much lower than the ambient levels of phosphate. Our experiments show that two surface wash methods that remove adsorbed trace metals also remove phosphate, although the chemistry and stoichiometry of this proposed binding mechanism remain speculative. Further work by marine chemists is clearly required to elucidate the mechanism of phosphate scavenging onto algal cells, but our work makes the existence and potential biological and biogeochemical significance of this process evident.

P partitioning between the surface-adsorbed and intracellular pools, as we observed in the natural bloom samples, could have implications for the fate of P after algal cells are grazed. P in phytoplankton can be assimilated by bivalve larvae and copepods with efficiencies >50% (Reinfelder and Fisher 1991, 1994). For other scavenged elements like Fe, zooplankton grazers preferentially assimilate intracellular pools and regenerate surface-adsorbed fractions to the dissolved phase (Hutchins and Bruland 1995). It seems likely that the scavenged behavior of P on algal cell surfaces also affects its assimilation and regeneration during grazing by zooplankton.

Two of the natural bloom samples we examined were collected from the extreme upper ends of the estuaries, at the essentially zero salinity (the *Heterosigma* bloom on 4 August 2004 and the *Synechocystis* bloom on 21 September 2004) (Table 1). These freshwater samples showed that surface-adsorbed P also profoundly affects the elemental ratios of freshwater algae. Phytoplankton growth in lakes and rivers is commonly P limited (Schindler 1977; Hecky and Kilham 1988). Thus, future limnology studies need to accurate-

ly assess how the existence of surface-bound and intracellular pools of phytoplankton P affects P limitation and biogeochemical cycling in freshwater ecosystems. Since many of the estuarine blooms we studied (including the raphidophytes and dinoflagellates) are potentially toxic or harmful algal bloom (HAB) species, future HAB studies may also need to consider differences in intracellular P quotas and requirements between these taxa and other algal groups such as diatoms.

In the subtropical North Pacific, it has been proposed that the pelagic ecosystem has shifted from N limitation to P limitation on the basis of a change in the mean N : P ratio of upper water column–suspended particulate matter from ~15 to 21 (Karl et al. 1997). Our results show that the amount of surface-adsorbed P on phytoplankton can range from 15% to 50% of the total cellular P, suggesting that such ratio-based calculations require cautious reassessment. Actual P requirements for many phytoplankton (particularly the cyanobacteria that dominate in this regime) may be considerably lower than previously thought. Lower P quotas could also help explain why primary production rates at both the BATS (Bermuda Atlantic Time-Series Study) in the North Atlantic and the HOT (Hawaii Ocean Time-Series Study) in the North Pacific are generally similar (Karl et al. 2001), despite differences in dissolved P levels between the two regions (Wu et al. 2000).

It is now widely recognized that Redfield's classic reciprocal relationship between algal elemental ratios and deep-water dissolved nutrient ratios represents an averaging of many biological and chemical processes operating throughout the surface and deep ocean. Falkowski and Davis (2004) point out that Redfield ratios are an emergent property of the marine ecosystem, incorporating processes such as algal uptake and requirements, remineralization efficiencies of sinking particles, and elemental loss rates to the sediments. To these various factors, we now need to add the scavenged behavior of phosphate, which, along with actual biological requirements for P, may affect the overall P content of planktonic organisms in the ocean. P scavenging onto sinking biogenic particles could also represent a significant but previously unrecognized loss term for dissolved phosphate in addition to sorption onto mineral phases, burial of organically incorporated P, and formation of authigenic minerals (Baturin 2003). Phosphate particle reactivity with algal biomass is a neglected phenomenon, but it is one that nevertheless has potentially significant consequences for the biogeochemical cycling of P in the sea.

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