Limnol. Oceanogr., 51(3), 2006, 1381–1390

Presence and regulation of alkaline phosphatase activity in eukaryotic phytoplankton from the coastal ocean: Implications for dissolved organic phosphorus remineralization

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Abstract

The biologically important constituents of the dissolved organic phosphorus (DOP) pool, their bioavailability, and their cycling in coastal systems are still poorly understood. Here we use the enzyme alkaline phosphatase as a metric of DOP bioavailability and track the activity of this enzyme in a coastal system. We observed alkaline phosphatase activity (APA) in the >0.2-μm size fraction of all surface samples tested during an Oregon coast cruise in August 2001. Although there was not a significant trend between APA and phosphate concentration in the data set as a whole, chlorophyll *a*–normalized APA was elevated at the station with the lowest dissolved inorganic phosphate (DIP) concentration. Activity was also elevated in nutrient-addition experiments in which nitrate amendments were used to force community drawdown of DIP. These data are consistent with phosphate regulation of APA. A cell-specific APA assay revealed that the percentage of diatoms with APA mimicked the trend in the hydrolytic rate, but such a trend was not observed for the dinoflagellates. Further, the percentage of dinoflagellate cells with APA was routinely higher than the percentage of diatom cells with activity. In nutrient-addition experiments designed to evaluate the regulation of APA, diatom taxa expressed APA less frequently than dinoflagellates, but they displayed a tighter regulation of the activity by DIP than dinoflagellates. The variability observed in the presence and regulation of APA in these eukaryotic phytoplankton indicates that DOP bioavailability is a potential driver of phytoplankton nutrition and species composition in the coastal ocean.

Of the nutrient reservoirs in the ocean, the dissolved organic nutrient pools are increasingly recognized as important, as numerous studies have demonstrated the ability of phytoplankton to use dissolved organic phosphorus (DOP) and nitrogen (DON) as sources of phosphorus and nitrogen (e.g., DOP: Cembella et al. 1984*a*,*b*; DON: Antia et al. 1991). Not only are DOP and DON often available to phytoplankton, but their concentrations can exceed those of dissolved inorganic phosphate (DIP) and nitrogen (DIN) in some instances, particularly in the photic zone (Monaghan and Ruttenberg 1999; Karl and Björkman 2002; Ruttenberg and Dyhrman 2005). The influence of nutrient flux on primary production in the coastal ocean is a topic that is currently under intense scrutiny, as the coastal margins are recognized as critical sources of carbon export (Hales et al. 2005*b*). Yet the extent to which organic nutrients such as DOP are bioavailable and contribute to primary production in the coastal zone is still poorly understood.

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Acknowledgments

We thank Sheean Haley, Tori Ziemann, Ellen Roosen, Mary Derr, and Christie Haupert for valuable technical assistance with sample collection and processing and also Pat Wheeler for data on chlorophyll *a* concentrations. Our thanks go to the Captain and crew of the RV *Thompson* and the PIs of the COAST Program. Funds for this work were provided by the National Science Foundation (NSF) Chemical Oceanography Program (OCE 0119134).

DOP bioavailability depends on its composition, the phytoplankton present, their physiology, and microbial regeneration processes, among a suite of variables. The molecular-level composition of DOP in seawater is poorly characterized, although recent studies on the high-molecular-weight DOP pool indicate a predominance of phosphonate (C-P) and phosphomonoester (C-O-P) bond classes (Clark et al. 1998; Kolowith et al. 2001). Of these two bond classes, the phosphomonoesters are typically considered more labile and available to primary producers, although recent genomic evidence indicates that phosphonates may be available to *Synechococcus* (Palenik et al. 2003).

Phytoplankton employ various enzyme systems to access DOP compounds through hydrolytic cleavage of phosphate from DOP; most DOP compounds cannot be assimilated directly (Cembella et al. 1984*a*; Chróst 1991). Of the suite of enzymes that may be involved in the breakdown of DOP in seawater (e.g., diesterase, phytase, C-P lyase, 5′ nucleotidase, and alkaline phosphatase), alkaline phosphatase is perhaps the best studied. Alkaline phosphatase can hydrolyze phosphomonoesters into bioavailable phosphate at alkaline pH (Kuenzler and Perras 1965). The enzyme is commonly regulated by phosphate supply, such that activity is increased when DIP drops below critical threshold levels (Cembella et al. 1984*a*,*b*; Jansson et al. 1988). Because of this property, alkaline phosphatase activity (APA) has been used to invoke P stress, or P limitation, in both marine and freshwater systems (Dyhrman and Palenik 1999; Beardall et al. 2001; Dyhrman et al.

2002). Here we use the term P limitation to indicate a limitation of biomass (Liebig-type limitation), and the term P stress to indicate a physiological response to P supply, but not necessarily a limitation in biomass. For example, under low P conditions, phytoplankton may up-regulate proteins or enzymes to aid in P scavenging (a P stress response). The extent to which these P-stressed phytoplankton are able to scavenge P will result in either P limitation or recovery.

Many subtleties exist in the intrinsic ability of different taxa to hydrolyze phosphomonoesters and in the regulation of the enzymes (e.g., alkaline phosphatase) that allow them to do so (Riegman et al. 2000; Hoppe 2003; Moore et al. 2005). An example of this heterogeneity comes from the recent genome sequences of picocyanobacteria and diazotrophs (www.jgi.doe.gov), in which some genomes indicate a capacity for hydrolysis of phosphomonoesters, while others do not (Scanlan and West 2002; Orchard et al. 2003). For example, the genome of *Prochlorococcus* MED4 has a gene encoding a putative alkaline phosphatase (*phoA*), whereas *Prochlorococcus* MIT9313 does not (Scanlan and West 2002). When present, there are also many differences between organisms in their regulation of APA (Beardall et al. 2001). This complicates interpretation of community APA as a P deficiency indicator and necessitates the use of multiple indicators and/or nutrient-addition experiments in order to confidently assign the nutrient-deficiency status of a population (Healey and Hendzel 1979; Rose and Axler 1998; Beardall et al. 2001).

The aims of this study were to examine whether phytoplankton in the upwelling-dominated coastal Oregon system experience P stress and to examine the extent to which phytoplankton may access the DOP reservoir to meet their nutritional P demand. As highlighted above, DOP bioavailability is dependent on both the chemical composition of DOP and the intrinsic ability of different phytoplankton to utilize different DOP compounds. Depending on these factors, DOP may influence both phytoplankton growth and species composition under conditions of P stress, where phytoplankton may be required to make use of DOP as a P source. In this study we compare the cell-specific APA (enzyme-labeled fluorescence assay) of key phytoplankton groups to community APA rate data generated for the >0.2-μm size fraction. We also examine how APA responds to inorganic nutrient additions in shipboard incubation experiments. These incubation studies provide information on enzyme regulation that is essential for determining to what extent APA is an indicator of P stress in a given system.

Methods

Field site—The water column was sampled during an August 2001 cruise off northern Oregon as part of the Coastal Advances in Shelf Transport (COAST) Program. Surface samples (typically 5 m) from a nearshore to offshore transect along the Cape Perpetua (CP) line, located on Heceta Bank, spanning nearshore station CP-1 (44°13.5′N, 124°08.8′W) to offshore station CP-11 (44°13.5′N 125°0.9′W), were collected with a pump on a towed sled (Hales et al. 2005*a*). A detailed discussion of the study site can be found elsewhere (Ruttenberg and Dyhrman 2005). At designated stations along the transect, water from the pump-profiler was collected into 20-liter carboys. These carboys were sampled for filtration and subsequent analysis of discrete samples for total dissolved phosphorus (TDP), DIP, DOP, chlorophyll *a* (Chl *a*), phytoplankton species composition, and enzymatic activity. After first inverting carboys to homogenize water samples, water was subsampled for these analyses into acid-cleaned (10% HCl) bottles.

Table 1. Physical characteristics for the two sites at which nutrient incubations were performed.

Characteristics	Station CP-1	Station CP-6
Transect position	Nearshore	Mid-shelf
Coordinates	44°13.499′N, 124°08.797′W	44°13.493′N, 124°36.76′W
Total water depth (m)	35	109.4
Upwelling or relaxation?	Upwelling	Relaxation
Sample depth (m)	5	3
Salinity	33.44	32.72
Temperature (°C)	11.14	12.48

Nutrient-addition experiments—Surface water was sampled from two stations, one a nearshore station (CP-1) and the other a mid-shelf station (CP-6), for nutrient-addition experiments (Table 1). Physical parameters of the water column at each location are listed in Table 1. At each site water was collected into an acid-cleaned 50-liter carboy, prescreened through a 100-μm sieve, and collected into 4-liter polycarbonate containers. Triplicate containers were either not amended (control) or were amended to final concentrations as follows: phosphate, 30 μmol L^{-1} (DIP) or nitrate, 100 μmol L^{-1} (DIN). Each treatment was incubated for 5 d. On day 5, each treatment was sampled for the abundance of different phytoplankton genera, APA, and Chl *a*. Pairwise comparisons between treatments and controls were performed using a *t*-test (degrees of freedom = 5), and significance is reported where $p \leq 0.05$.

Nutrient analyses—TDP was determined on 0.2-μm filtrates using a high-temperature ashing/hydrolysis method (Solórzano and Sharp 1980), with modification (Monaghan and Ruttenberg 1999). The detection limit of the TDP assays was 0.06 μmol L^{-1}. Soluble reactive phosphorus (SRP) was determined by the standard phosphomolybdate blue method (Koroleff 1983). The detection limit of the SRP assays was 0.03 μmol L^{-1}. Herein, DIP and SRP are considered synonymous (*see* Ruttenberg and Dyhrman [2005] for a discussion), and DOP is defined as the TDP concentration minus the SRP concentration.

Phytoplankton analyses—Chl *a* was measured on samples collected onto GF/F filters from the water column (typically 5 m) or from incubation treatments, using a 95% methanol extraction and a Turner10-AU fluorometer. The water column data were kindly provided through the

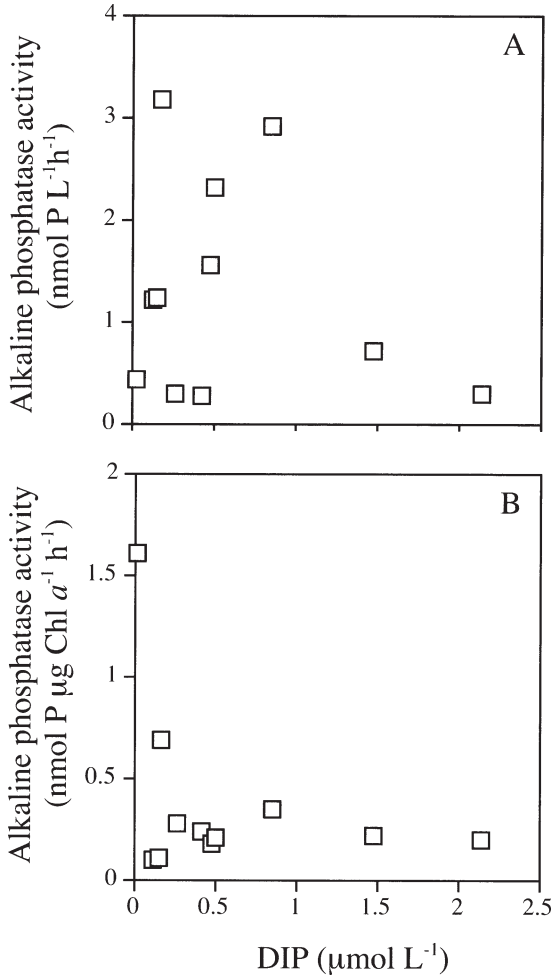

Fig. 1. (A) Surface water column alkaline phosphatase activity (APA) from an August 2001 cruise at all stations as a function of DIP concentration. (B) The same data set plotted with APA normalized to Chl *a* as a function of DIP concentration. Note difference in the scale of the y-axis.

collaborative COAST Program (Wetz and Wheeler 2005). Phytoplankton abundance was assessed to genus level, or to species level when possible, on 60-mL grab samples preserved with 1 mL of 25% glutaraldehyde and analyzed by microscopy through an outside contractor (www.phycotech.com).

APA—To assay APA, 0.5 liters of seawater was filtered onto an acid-cleaned 0.2-μm polycarbonate filter (47 mm) and stored frozen at -30°C in an acid-cleaned Petri dish prior to analysis. The rationale for using a small pore size was to examine cell-associated activity for the whole community, as opposed to only one size fraction of the community, or dissolved APA. The cell-associated APA will be more closely related to the cell-specific APA measured with the enzyme-labeled fluorescence (ELF) assay (*see below*). Further, by assaying activity of the whole community, we avoided ambiguities associated with traditional size-fractionation approaches.

Table 2. Community alkaline phosphatase activity (APA), cell-specific APA (reported as % positive), dissolved inorganic phosphate (DIP), and dissolved organic phosphorus (DOP) concentration for surface-water samples following a nearshore (CP-1) to offshore (CP-11) Oregon coast transect in August 2001. Cell-specific APA is reported as the percentage of positive cells for all dinoflagellates, the dinoflagellate genus *Gonyaulax*, all diatoms, and the diatom genera *Thalassiosira* and *Coscinodiscus*.

	CP-1	CP-5	CP-6	CP-11
DIP (μmol L^{-1})	0.85	0.17	0.5	0.02
DOP (μmol L^{-1})	0.27	0.20	0.25	0.73
APA (nmol P L^{-1} h^{-1})	2.93	3.18	0.94	0.24
Dinoflagellate APA (% positive)	100	36	90	100
Gonyaulax APA (% positive)	96	33	83	100
Diatom APA (% positive)	63	ND*	10	2
Thalassiosira APA (% positive)	53	ND	13	3
Coscinodiscus APA (% positive)	96	ND	0	ND

* ND, no data.

APA was measured fluorometrically (Perry 1972) as detailed below. Sample filters were incubated at room temperature in 2-mL sterile, phosphate-free artificial seawater (ASW) (Lyman and Fleming 1940) on a shaker table (200 rpm) for 10 min prior to the start of the assay. The fluorogenic phosphatase substrate 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP, Molecular Probes) was added to a final concentration of 10 μmol L^{-1} to start the analysis. This substrate has a maximal sensitivity at neutral pH. Initial kinetic studies done in this study area indicated that this concentration is saturating (data not shown). Hydrolysis of DiFMUP to the fluorescent product 6,8-difluoro-7-hydroxy-4-methylcoumarin (DiFMU) was measured on a temperature-controlled (25°C) CytoFluor multiwell plate reader (Perseptive Biosystems) at an excitation equal to 360 nm and an emission equal to 460 nm. Subsamples were removed and assayed immediately and then at intervals adjusted to the activity of the sample, such that all readings fell within the linear range of the assay (at least four measurements within 4 h or less). A standard curve from 0 to 4.8 μmol L^{-1} DiFMU (Molecular Probes) in ASW was generated and used to calculate the rate of DiFMUP hydrolysis.

Samples for cell-specific APA (ELF assay) were collected from surface water and incubation samples, then assayed with ELF, as previously described (Dyhrman and Palenik 1999). Eukaryotic phytoplankton in the ELF samples were identified using brightfield or DIC optics on a Zeiss Axioplan microscope. Each cell was also scored as positive or negative with regard to the presence or absence of the green fluorescent precipitate indicative of APA using a DAPI long-pass filter set. Some variability in the distribution of the precipitate can occur in certain cases. For the purposes of this work, a cell was scored as positive if 30% or more of the cell surface appeared green.

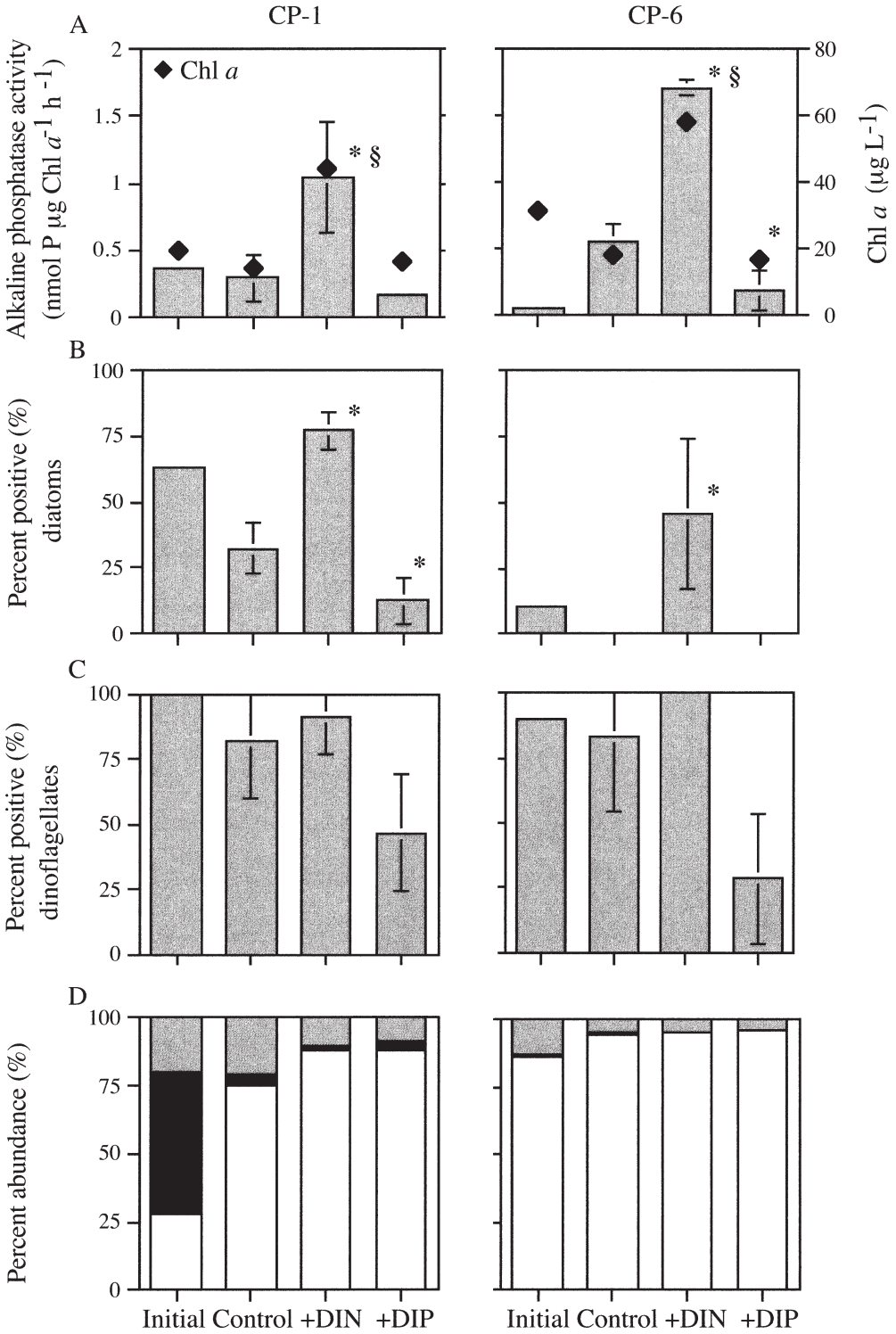

Fig. 2. Plots from two nutrient-addition experiments (nearshore CP-1 and mid-shelf CP-6) during an August 2001 cruise along the Oregon coast. All graphs are plotted with initial water column conditions (initial) as well as control (control), nitrate-amended (+DIN), and phosphate-amended (+DIP) treatments from a 5-d incubation. In all cases, error bars denote standard deviations ($n = 3$). No replicate samples were available for initial conditions. (A) Chl *a* concentration (diamonds) and alkaline phosphatase activity (APA, bars) in the initial water column sample and the different treatments. Significance (*t*-test, $p \leq 0.05$) is indicated (*, APA; §, Chl *a*) for pairwise comparisons between the +DIN or the +DIP treatment and the control. (B) The percentage of ELF-assayed diatoms with cell-specific APA in the initial water column sample and the different treatments. Significance (*t*-test, $p \leq 0.05$) is indicated (*) for pairwise comparisons between the +DIN or the +DIP treatment and the control. (C) The percentage of positive ELF-assayed dinoflagellates as a function of treatment or initial conditions. (D) Percent abundance (cell mL^{-1}) of different classes of eukaryotic phytoplankton present

Results

Community APA was observed in the surface samples of all stations assayed along the COAST sampling grid (Fig. 1A,B). There were substantial variations in activity over a range of DIP concentrations (Fig. 1A), but normalizing the enzyme activity to Chl *a* concentrations (Fig. 1B) minimized most of this variability. Normalized APA was observed over a broad range of DIP concentrations but was especially elevated at one station, where DIP concentration was lowest (0.2 μmol L^{-1}) (Fig. 1B). Surface stations along one of the cruise transects (CP line) were examined in detail for cell-specific APA (ELF assay) and were used as sites for nutrient-addition experiments (Table 1). The percentage of ELF-assayed cells with cell-specific APA in all diatom genera followed the same trend as the community APA, with the highest percentages of positive cells in samples with the highest community APA (Table 2). This was also the case for representative individual diatom genera *Thalassiosira* and *Coscinodiscus* (Table 2). This was not the case for all dinoflagellate genera nor for representative specific genera (e.g., *Gonyaulax*) (Table 2).

To better characterize the regulation of community (e.g., >0.2-μm fraction) and cell-specific APA along this transect, nutrient-addition experiments were performed at Station CP-1 (nearshore) and at CP-6 (mid-shelf). The two sites were similar in salinity but differed in both location and in the apparent upwelling–relaxation sequence, with strong upwelling conditions and a temperature of 11.14°C at CP-1 and relaxation conditions at CP-6, with a higher temperature of 12.48°C. Details regarding upwelling and relaxation events in this system and their influence on P biogeochemistry can be found elsewhere (Ruttenberg and Dyhrman 2005). Ambient DIP was 0.85 μmol L^{-1} and 0.5 μmol L^{-1} at CP-1 and CP-6, respectively (Table 2). DIN (nitrate and nitrite) was estimated from shipboard, real-time autoanalyzer results during the time frame of this study to be roughly 10 μmol L^{-1}. In both incubations there was a significant increase ($p = 0.00042$, $p = 6.4 \times 10^{-6}$ for CP-1 and CP-6, respectively) in Chl *a* concentration in the +DIN treatments (Fig. 2A). In the CP-1 incubation, community APA significantly ($p = 0.050$) increased in the +DIN treatment relative to the control, with no significant change in the +DIP community APA relative to the control (Fig. 2A). In the CP-6 incubation there was a significant ($p = 0.0005$) community APA increase in the +DIN treatment relative to the control, with a significant decrease ($p = 0.045$) in the +DIP treatment relative to the control (Fig. 2A). There was substantial drawdown of DIP (to <0.04 μmol L^{-1}) in the +DIN treatments of both experiments, but not in the control or +DIP treatments (data not shown).

In these same nutrient-addition experiments, the percentage of diatoms with cell-specific APA was significantly elevated ($p = 0.002$, $p = 0.050$ for CP-1 and CP-6, respectively) in +DIN treatments relative to controls (Fig. 2B). In contrast, the percentage of diatoms with APA was depressed in +DIP treatments relative to the control treatments (Fig. 2B). This difference was significant ($p = 0.003$) for CP-1 (Fig. 2B). There was no cell-specific diatom APA observed in either the control treatments or the +DIP treatments in the CP-6 incubation (Fig. 2B). The percentage of dinoflagellates with cell-specific APA was higher and more variable between replicates than that observed for the diatom genera (Fig. 2C). The average percentage of dinoflagellates with cell-specific APA was depressed, but not significantly so, in +DIP treatments relative to control treatments and in +DIN treatments for both experiments (Fig. 2C).

Dinoflagellate genera dominated the initial sample for CP-1, but their abundance was substantially reduced in the incubation treatments after 5 d (Fig. 2D). Diatom genera dominated the eukaryotic phytoplankton taxa in the initial water sample for CP-6, and they also dominated all the experimental treatments for both incubations (Fig. 2D). There was no significant shift in the dominance of diatoms or diatom taxa with the addition of DIN or DIP in either experiment, relative to the control (Fig. 2D). Detailed analyses of two diatom genera (*Thalassiosira* and *Coscinodiscus*) and one dinoflagellate genus (*Gonyaulax*) were performed to examine cell-specific APA in the CP-1 nutrient-addition experiment. Representative images of ELF-assayed cells for these genera illustrate positive and negative cells (Fig. 3). The average percentage of *Thalassiosira* cells with cell-specific APA was significantly elevated ($p = 0.049$) in +DIN treatments and significantly depressed ($p = 0.0167$) in +DIP treatments (Fig. 4). Similar results were observed for *Coscinodiscus*, where there was a significant ($p = 0.012$) increase in the percentage of this genus with cell-specific APA in the +DIN treatments relative to the control (Fig. 4). As with *Thalassiosira*, the percentage of *Coscinodiscus* cells with cell-specific APA decreased in the +DIP treatment relative to the control, although the decrease was not significant. The cell-specific APA results for the dinoflagellate, *Gonyaulax* sp., were less distinct as a result of higher variability between triplicate treatments, although they followed the same general trend as the diatoms (Fig. 4).

Discussion

Studies of APA in different systems can provide insight into both phosphomonoester hydrolysis and physiological P stress responses. There are currently two major ways to assay APA in situ; with soluble DOP analogs for assays of hydrolytic rate or with ELF for assays of cell-specific activity. ELF assays are documented as a percentage of cells with activity but not as a rate. In our study of an upwelling-dominated Oregon coastal system, we performed

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in initial field samples and the different treatments. Black bars denote dinoflagellate abundance, white bars show diatom abundance, and the gray bars show the abundance of other eukaryotic phytoplankton.

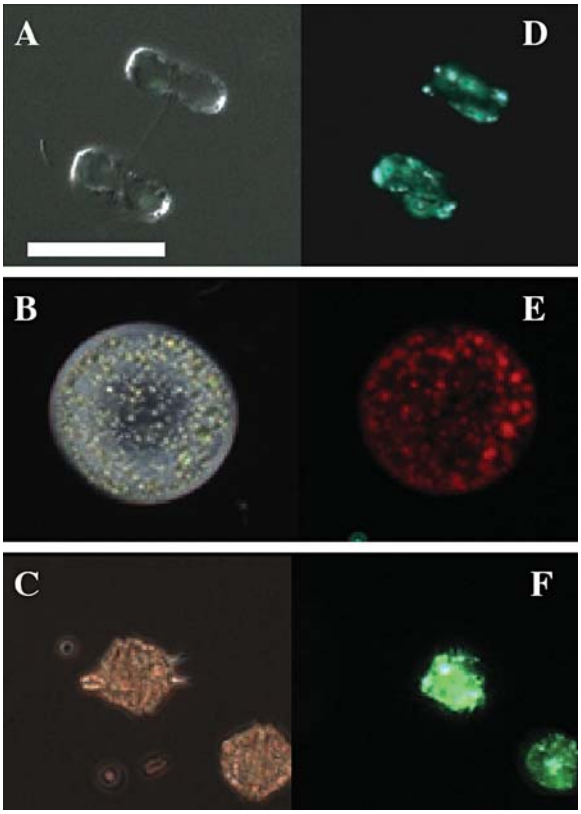

Fig. 3. ELF-assayed samples of representative eukaryotic phytoplankton taxa imaged with DIC optics or brightfield optics (A–C) and with a DAPI long-pass filter set (D–F). (A, D) Images of the diatom *Thalassiosira* sp.; note the green fluorescence, indicating that this cell has alkaline phosphatase activity. (B, E) Images of the diatom *Coscinodiscus* sp. with no green fluorescence, only red chlorophyll autofluorescence, indicating a lack of alkaline phosphatase activity. (C, F) Images of *Gonyaulax* sp. with the green fluorescence indicative of alkaline phosphatase activity. Scale bar 100 μm.

the first type of assay to identify trends in the community capacity for phosphomonoester hydrolysis, and we augment this community APA data with cell-specific APA derived from ELF assays. In this study we complement these field observations with shipboard incubations to examine the sensitivity of APA (both community APA and cell-specific APA) to changes in DIP supply. In the absence of regulation information provided by either culture studies or such incubation experiments, the presence of APA cannot be unequivocally interpreted as evidence for P stress or P limitation.

We observed APA and, thus, a community capacity for phosphomonoester hydrolysis in all the samples (>0.2-μm size fraction) we examined, ranging up to 3.3 nmol P L^{-1} h^{-1}. This hydrolytic rate is comparable to rates (roughly 2–10 nmol P L^{-1} h^{-1}) observed in the >0.2-μm size fraction during a study of phosphorus biogeochemistry along the French Atlantic coast (Labry et al. 2002). This community APA is also in good agreement with calculations of V_{max} (up to 2.1 nmol P L^{-1} h^{-1}) in a northwest African upwelling system (Sebastián et al. 2004), but it is substantially lower that the APA observed in areas heavily influenced by nitrogen-rich freshwater input, such as the Louisiana Gulf Coast, with hydrolytic rates of near 500 nmol P L^{-1} h^{-1} (Ammerman and Glover 2000).

In culture studies with phytoplankton, APA is commonly regulated by DIP, with a robust inverse correlation between DIP and activity (Riegman et al. 2000) or between DIP and the percentage of cells with ELF-assayed activity (Dyhrman and Palenik 1999). As such, APA is commonly used as an indicator of P stress. However, the relationship between DIP and APA is not always observed in field studies (Rengefors et al. 2003; Ruttenberg and Dyhrman 2005) nor in culture studies with model organisms (González-Gil et al. 1998). Community APA along the Oregon coast was present over a wide range (0.02–2.7 μmol L^{-1}) of DIP concentrations, with no significant correlation between DIP concentration and activity. This was true in an analysis of the whole data set (Fig. 1) and with examination of a nearshore to offshore gradient in DIP (Table 2). In an earlier study of this coastal system, a similar pattern was observed shelf-wide, although a significant inverse correlation between APA and DIP concentration was seen in a subset of the data, along a transect where the water had a longer residence time (Ruttenberg and Dyhrman 2005). The disconnect between DIP concentrations and APA observed in this study is potentially the result of many different factors, including the cycling rate of DIP, the potential for P storage, differences in the presence and regulation of the APA in different microbes, and the physiological history of the cells, which may not track with instantaneous DIP concentrations in well-mixed systems (Ruttenberg and Dyhrman 2005, and references therein).

Community rate measurements of APA, such as those discussed above, provide insight into DOP hydrolysis and cycling. However, the enzyme is commonly found in many organisms, including both heterotrophic bacteria and phytoplankton, making it difficult to estimate the extent to which the phytoplankton community may be specifically contributing to the observed hydrolytic rates. ELF-based assays have become a powerful tool for identifying which members of the phytoplankton community have APA, offering substantially enhanced resolution over size fractionation. This method is being increasingly used to identify cell-specific APA in field populations from both freshwater (Rengefors et al. 2001, 2003) and marine environments (Dyhrman and Palenik 1999; Dyhrman et al. 2002; Lomas et al. 2004). Table 3 provides a summary of marine dinoflagellates and diatoms in which cell-specific APA has been examined with the ELF method.

Using the ELF method, we were able to identify cell-specific APA in a number of dinoflagellates and diatoms. The presence of cell-specific APA in different genera (expressed as a percentage of the total cells) was highly variable between stations and between taxa. In the case of the diatoms, the percentage of ELF-positive cells generally tracked with the community APA assayed in the >0.2-μm fraction: the highest percentages of cells with cell-specific APA were present at stations with the highest hydrolytic rates. This was not the case for dinoflagellate genera, in which, for example, the percentage of cells with activity was

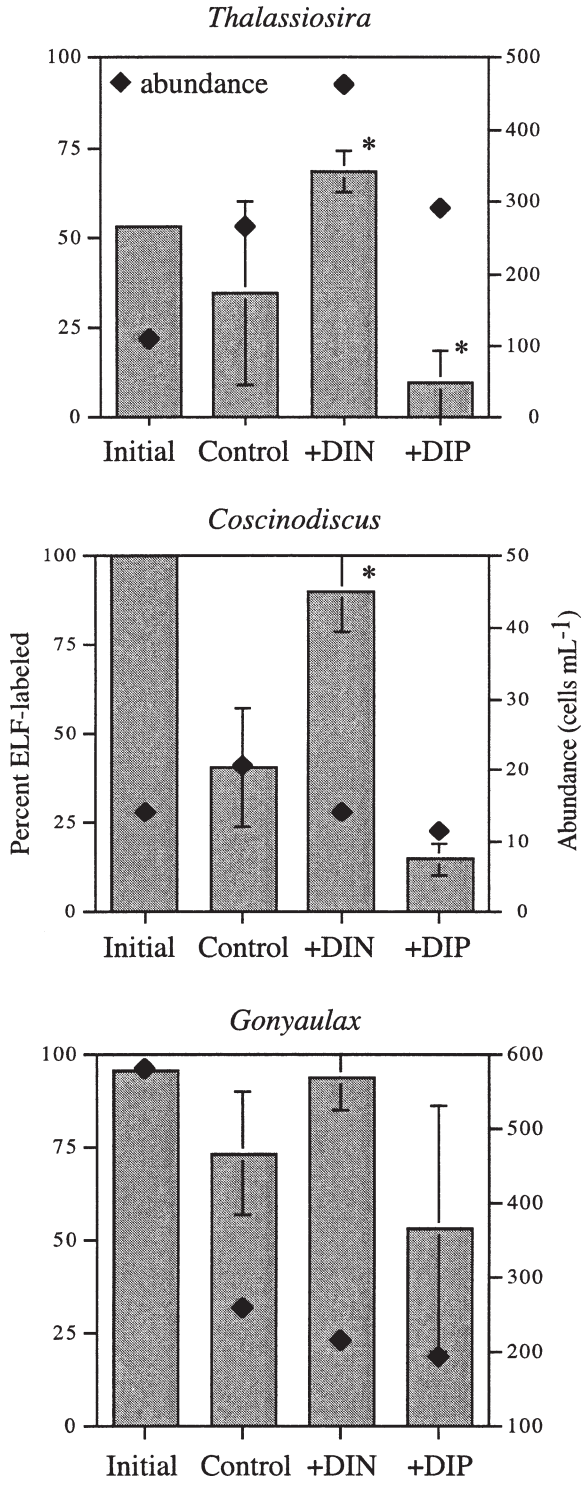

Fig. 4. Graphs of the percentage of ELF-assayed cells with cell-specific alkaline phosphatase activity (APA, gray bars) present in initial (initial) surface water and after a 5-d incubation (site CP-1) with no amendment (control) or with amendments of nitrate (+DIN) or phosphate (+DIP). Significance (*t*-test, $p < 0.05$) is indicated (*) for pairwise comparisons between the cell-specific APA in +DIN or +DIP treatments and the control. Error bars denote standard deviations ($n = 3$). No replicate samples were available for initial conditions. The abundance (cells mL^{-1}) of each genus (diamonds) is also plotted for the initial conditions and treatments. Note differences in the axes. Error bars were not plotted on the abundance data for clarity of the figure, but typically encompassed no more than 3% variability.

high at stations with comparatively low hydrolytic rates. In fact, a higher percentage of dinoflagellates were consistently identified with cell-specific APA than diatoms. Differences in activity between taxa have also been observed in the Sargasso Sea (Lomas et al. 2004). As highlighted above, such differences may be a function of enzyme regulation, alternative nutritional strategies (e.g., heterotrophy, as discussed in Lomas et al. [2004]), or other factors, and this topic certainly warrants further study. Inconsistencies between the activity trends in whole community rates and the activity trends in individual taxa highlight the fact that phosphomonoester hydrolysis and utilization may not be equivalent for all taxa in a given system. As such, changes in phosphomonoester concentration may disproportionately affect some organisms and influence community composition. In this study, changes in phosphomonoester concentration are more likely to disproportionately influence dinoflagellates relative to diatoms, since the dinoflagellates consistently had higher percentages of cells with APA. In summary, the data from these two different assays demonstrate that APA is present in phytoplankton along the Oregon coast and indicate that DOP bioavailability contributes to phytoplankton growth and community composition in this system. In addition, the coupled ELF and rate assays reveal substantial heterogeneity in both the rate of hydrolysis and the degree to which the enzyme is present in different eukaryotic phytoplankton. This heterogeneity has potentially profound consequences for phytoplankton P physiology and community composition in this system.

In order to fully interpret the capacity for phosphomonoester hydrolysis and to evaluate the extent to which the APA indicates P stress at our Oregon coast field site, we pursued regulation studies using shipboard nutrient-addition experiments. Without such regulation studies, assays of P quota, P uptake, or other measures of P physiology, the presence of APA is permissive but not conclusive evidence of P stress. With these incubations we examined how sensitive both the community hydrolytic rate (community APA) and the proportion of single cells with activity (cell-specific APA) were to changes in DIP supply. Nutrient-addition experiments were performed at two different sites (CP-1, nearshore; and CP-6, mid-shelf; *see Table 1*). CP-1 was sampled under upwelling conditions and CP-6 during a relaxation event. At both sites there was drawdown of DIP in +DIN treatments relative to controls (data not shown). This decrease in DIP in the +DIN treatments corresponded to a significant increase in community APA relative to control treatments for both incubation experiments (Fig. 2). These data show that the community APA is sensitive to declines in DIP concentration and that the activity can be driven higher than the rates observed in controls, demonstrating phosphate regulation of the enzyme. At CP-6, in addition to a positive

Table 3. A summary of cell-specific detection of alkaline phosphatase activity (APA) and its down-regulation in the presence of elevated phosphate in different marine dinoflagellate and diatom taxa.

Taxa	Cell-specific APA detected with ELF	P regulation of cell-specific APA*	Reference
Akashiwo sanguinea	Yes	Yes	Lomas et al. 2004
Alexandrium fundyense	Yes	No	González-Gil et al. 1998
Amphidinium sp.	Yes	Yes	González-Gil et al. 1998
Ceratium sp.	Yes	ND†	This study; Lomas et al. 2004
Chaetocerous sp.	Yes	ND‡	This study
Coscinodiscus sp.	Yes	Yes	This study
Dinophysis sp.	Yes	ND	Dyhrman and Palenik 1999
Heliotheca thamesis	Yes	Yes	Lomas et al. 2004
Gonyaulax sp.	Yes	No	This study
Prorocentrum micans	Yes	ND	Dyhrman unpubl. data
Prorocentrum minimum	Yes	Yes	Dyhrman and Palenik 1999
Prorocentrum sp.	Yes	ND‡	This study
Pseudo-nitzschia sp.	Yes	ND‡	This study
Skeletonema sp.	Yes	ND‡	This study
Thalassionema sp.	Yes	ND‡	This study
Thalassiosira sp.	Yes	Yes	This study

* As assayed by changes in the presence or absence of positive cells or by a change in the percentage of positive cells with phosphate supply (e.g., a decrease in the percentage of positive cells when a population is refed with phosphate).
† ELF, enzyme-labeled fluorescence; ND, not determined.
‡ In some cases, cell-specific APA was observed, but the genus was not present with a high enough frequency in the nutrient-addition experiments to examine regulation.

community APA response to diminished DIP, there was a significant decrease in community APA with DIP addition relative to the control treatments. Taken together, the incubation experiment data indicate that observed in situ activities may be the result of a physiological response to DIP supply (P stress), where drawdown of DIP increased activity and where resupply of DIP decreased the need for DOP hydrolysis and resulted in a decline in the enzyme activity. The occurrence of a P stress response indicates a sensitivity to DIP and potential hydrolysis of DOP, but it is distinct from a primary P limitation of biomass, which was not observed (*see below*). The results of these nutrient-addition experiments clearly show that community APA is sensitive to changes in DIP supply, and they thus provide a critical contextual framework for interpreting the water column observations, in that the low-level rates observed in controls could be driven higher but could also be reduced. In short, the in situ activity is at an intermediate rate relative to its capacity in this system. These results highlight the importance of incubation studies, or other measures of P physiology, for interpretation of low-level community APA as a P stress indicator. For example, if low-level APA in a system cannot be driven lower with DIP addition, then it is not indicative of a P stress response.

Nutrient-addition experiments, such as those described above, are classically used to examine Liebig-type nutrient-limitation scenarios, although there are many caveats to the interpretation of these types of experiments (Beardall et al. 2001). In both incubations from our study system, Chl *a* standing stock was most responsive to the +DIN addition, and there was no significant Chl *a* increase in the +DIP treatments relative to the controls. Although these data are still consistent with an ongoing P stress response, they indicate a primary N limitation of biomass. The marked increase in community APA in the +DIN treatments, however, indicates that P could be secondarily limiting in this system. The absence of an incubation treatment with combined +DIN and +DIP prevents us from definitively evaluating the degree to which P may influence growth.

Cell-specific APA was also examined in the incubations. The proportion of diatoms (all genera combined) with cell-specific APA was regulated by P supply. This sensitivity to DIP was also observed in the genera *Thalassiosira* and *Coscinodiscus*. In brief, the percentage of diatoms with activity was driven higher by DIP drawdown in the +DIN treatment and was decreased by the +DIP addition relative to the no-addition control. This is again consistent with an interpretation of P stress. In this study we were unable to demonstrate significant differences in the percentage of cells with APA as a function of ambient DIP levels for the dinoflagellates (all genera), nor were we able to demonstrate significant P-related changes in specific genera, such as *Gonyaulax*. P regulation of cell-specific APA has been demonstrated for the dinoflagellate *Prorocentrum minimum* (Dyhrman and Palenik 1999); however, this regulation has not been examined in detail with many other dinoflagellates (Table 3). A definitive evaluation of cell-specific APA regulation with cultures of *Gonyaulax* or other dinoflagellates from this system would help to clarify how the ELF data for the dinoflagellates should be interpreted in the field. At this juncture, the Oregon coast field data indicate that the cell-specific APA of dinoflagellates in this community may not be strongly P regulated. Unlike the diatoms, the presence of cell-specific APA does not support an interpretation of P stress for dinoflagellates in this system.

Observations from this study and those highlighted earlier show that many marine diatom and dinoflagellate genera have APA that is detectable with the cell-specific

ELF method (Table 3) and that the ELF method is a robust tool for examining cell-specific APA for these taxa in field populations. This is an important point, as microbes can respond differently to unique alkaline phosphatase substrates (e.g., ELF vs. DiFMUFP), and not all phytoplankton with APA can be easily detected with the ELF method (Dyhrman unpubl. data). However, it is critical to note that the sensitivity of ELF-assayed activity to DIP supply has not been confirmed for many marine dinoflagellates and diatoms (Table 3). For example, activity has been observed in field populations of *Dinophysis* (Dyhrman and Palenik 1999) and *Gonyaulax* (this study), but there is no evidence that the activity in these genera is regulated by DIP supply. If the proportion of cells with APA in a population or a particular group is not demonstrated to be sensitive to P supply, then the presence of cells with activity may not be interpreted as a P stress response. It is thus critical to examine the regulation of ELF-assayed activity before invoking its presence as an indicator of phytoplankton P deficiency.

With our enzymatic approach we observed APA that indicates a community capacity for phosphomonoester remineralization at our study site. Further, the cell-specific assays of APA clearly show that a portion of this activity can be attributed to dominant eukaryotic taxa such as diatoms and dinoflagellates. DOP is not typically examined as a major driver of phytoplankton growth or community composition in upwelling-dominated coastal systems, as these environments have traditionally been assumed to be strictly under N control. Identification of APA along the Oregon coast, however, indicates the possibility of P stress among some phytoplankton and emphasizes the potential importance of DOP as a source of P to primary producers in these environments. Using shipboard incubation studies to examine the sensitivity of APA to P supply at two different locations along a shelf transect, the community activity and the cell-specific diatom activity were shown to be P regulated, whereas the dinoflagellate activity was consistently in a high proportion of cells and was not clearly P regulated. These data highlight that P physiology and the capacity for DOP hydrolysis can differ substantially between phytoplankton in the same system. As such, DOP bioavailability may influence primary production and the dominance of diatoms versus dinoflagellates in this coastal system. Further work on the constituents of the DOP pool and their bioavailability in the coastal ocean is crucial for advancing our understanding of how dissolved organic nutrient reservoirs influence coastal production and phytoplankton community structure.

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Received: 20 May 2005
Accepted: 3 November 2005
Amended: 12 December 2005