Alkaline phosphatase activity in the phytoplankton communities of Monterey Bay and San Francisco Bay

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Abstract

Enzyme-labeled fluorescence (ELF) and bulk alkaline phosphatase (AP) activity enzyme assays were used to evaluate the phosphorus (P) status of phytoplankton communities in San Francisco and Monterey bays. Both regions exhibit spatial and temporal variability in bulk AP activity with maximum activities during the early spring and summer periods of high biological productivity. ELF analysis revealed pronounced differences in the makeup of organisms responsible for AP activity in these two environments. In Monterey Bay dinoflagellates are responsible for the bulk of the AP activity. Diatoms infrequently exhibited AP activity. Dinoflagellates that comprised only 14% of all cells counted in Monterey Bay accounted for 78% of AP-producing cells examined. The presence of AP activity in this group suggests that changes in P sources, concentrations, and bioavailability could disproportionably influence this group relative to diatoms in Monterey Bay. In San Francisco Bay, AP production, indicated by ELF, was associated primarily with bacteria attached to suspended particles, potentially used to hydrolyze organic compounds for carbon, rather than to satisfy P requirements. Our results highlight the importance of organic P as a bioavailable nutrient source in marine ecosystems and as a component of the marine P cycle.

All organisms require phosphorus (P) for energy transport and growth purposes (Benitez-Nelson 2000), but P may limit primary production in some oceanic systems (Karl et al. 1995; Wu et al. 2000). Phosphorus may be the ultimate limiting nutrient for primary production over geological time scales (Tyrrell 1999). Therefore, understanding the P cycle is essential in determining the coupling between marine primary productivity and the global carbon cycle.

Phytoplankton typically utilize dissolved orthophosphate in order to satisfy cellular P requirements (Cembella et al. 1984). However, many phytoplankton taxa have the capability to utilize dissolved organic P (DOP) forms (Cotner and Wetzel 1991; Björkman and Karl 1994) as well as other inorganic P compounds such as polyphosphate (Palenik and Dyhrman 1998; Scanlan and Wilson 1999). In order for up-take of most organic P compounds to occur, they must be first converted into orthophosphate. The primary means by which marine phytoplankton can convert organic P to bioavailable orthophosphate is induction of alkaline phosphatase (AP) (Cotner and Wetzel 1991), an enzyme that has broad substrate specificity and hydrolyzes ester bonds between P and organic molecules, making organic P available for cellular assimilation by converting it to P (Cembella et al. 1984).

Traditionally, an ecosystem is described as being P- or nitrogen (N)-limited on the basis of relative N:P ratios dissolved in the water. Marine phytoplankton, on average, require N and P at a ratio of 16:1 (Redfield Ratio). If the ratio of dissolved inorganic nitrogen (DIN) to soluble reactive phosphate (SRP) in a marine system is greater than 16:1 the system is considered P-limited. However, such characterization may not necessarily represent real P stress of the entire phytoplankton community for several reasons: (1) different phytoplankton species may have nutrient requirements different from the Redfield Ratio (Guildford and Hecky 2000; Geider and La Roche 2002; Quigg et al. 2003); (2) the analytical determination of SRP and DIN do not account for other available forms of P and N, such as organic sources, or include compounds that are not bioavailable (Karl and Tien 1997; Baldwin 1998; Benitez-Nelson 2000); and (3) low concentrations of a nutrient may not necessarily indicate deficiency; rather, it may represent efficient recycling and

utilization (Cañellas et al. 2000; Hudson et al. 2000; Karl and Björkman 2002).

To more directly determine community P status in natural aquatic ecosystems it has been suggested that because in many phytoplankton species AP is regulated (induced or repressed) by ambient inorganic P concentrations or by intracellular concentrations (Vargo and Shanley 1985; Chrost and Overbeck 1987), AP activity can be used as an index of P status. Indeed, AP activity has been used to indicate P stress in freshwater plankton communities (Chrost 1991; Hernandez et al. 1996) and in the marine environment as well (Cotner et al. 2000; Stihl and Sommer 2001; Vidal et al. 2003).

AP activity in marine ecosystems has been studied using two primary techniques. The first is the use of a quantitative enzyme assay of bulk AP activity of a given water sample or cell mass (Koike and Nagata 1997; Li et al. 1998; Thingstad et al. 1998) in which a specific phosphomonester substrate (e.g., *p*-nitrophenyl phosphate or 4-methylumbelliferyl phosphate) is added to a water sample and is stoichiometrically hydrolyzed in the presence of the enzyme AP. The second method is a qualitative, cell-specific assay using enzyme-labeled fluorescence (ELF) (Gonzalez-Gil et al. 1998; Dyhrman and Palenik 1999; Rengefors et al. 2003). This method also involves the addition of a phosphomonoester substrate (ELF-97 phosphatase substrate; Molecular Probes) to the sample; however, instead of a soluble product that is released to the medium, a fluorescent precipitate forms at the site of AP hydrolysis, thus, fluorescently tagging cells that exhibit AP activity.

Much previous research on marine AP activity utilized the bulk enzyme assay method and focused on areas that have low SRP concentrations or elevated N : P ratios such as freshwater-influenced marine systems (Nausch 1998; Cotner et al. 2000) or oligotrophic settings such as the Red Sea, Mediterranean Sea, Sargasso Sea, and central Pacific Ocean (Li et al. 1998; Van Wambeke et al. 2002; Ammerman et al. 2003).

The ELF method has recently been applied to study P status in laboratory cultures (Gonzalez-Gil, 1998; Dyhrman and Palenik 1999) and for examining natural marine populations (Carlsson and Caron 2001; Dyhrman and Palenik 2001, Lomas et al. 2004). Results from laboratory cultures typically—but not always—show a dependence of AP activity on orthophosphate concentration. For example, dinoflagellates grown in P-replete conditions did not exhibit fluorescence that is indicative of AP activity (ELF labeling), whereas cultures in orthophosphate-depleted media showed >90% of cells to be ELF-labeled (Dyhrman and Palenik 1999). Furthermore, this study reports a 50% decrease in cells with AP activity following orthophosphate addition to P-depleted cultures. In field populations, ELF labeling has shown variability in AP activity between a wide range of species in waters of Narragansett Bay and the Sargasso Sea (Dyhrman and Palenik 1999; Lomas et al. 2004).

While most oceanographic research has focused on AP activity as a proxy for P status in marine phytoplankton, AP is also produced by bacteria (Sundareshwar et al. 2003; Sebastien et al. 2004). It has been suggested that AP activity is induced by bacteria to hydrolyze organic P molecules to access reduced carbon, P, or both (Kirchman et al. 2000; Van Wambeke et al. 2002). Heterotrophic bacteria in particular are likely to be P-limited because they have higher P requirements than phytoplankton (Cotner and Wetzel 1991, Pomeroy et al. 1995). Using bulk AP activity estimates, it is not possible to distinguish between activity attributed to phytoplankton or heterotrophic bacteria, thus potentially overestimating the activity attributed to phytoplankton.

This study utilizes a combination of the two methods—bulk enzyme assays and ELF—to investigate AP dynamics in Monterey Bay and San Francisco Bay. Although bulk AP activity assays provide estimates for the total amount of enzyme being produced by all organisms in a sample, it reveals nothing about which species in a diverse field population contribute to this production. To better understand the nutrition role of organic P compounds and their utilization by natural communities, ELF was used to investigate species-specific AP activity.

Methods

Sampling sites—Surface water samples were collected in San Francisco Bay, from the upper few meters of the water column, along a 10-station north-south transect using a flow-through system (Fig. 1A). This occurred on 12 cruises from November 2001 through April 2003. South San Francisco Bay is characterized by higher turbidity, high levels of wastewater input from several municipal water treatment plants, and longer water residence times than the northern and central parts of the bay (Walters et al. 1985). The northern bay is primarily influenced by influx of water from the Sacramento River (Walters et al. 1985). The middle of this transect (Sta. 21) close to the Golden Gate Bridge, represents the greatest input from open ocean water. During the cruises, temperature, salinity, chlorophyll *a* (Chl *a*), turbidity, and dissolved inorganic nutrient concentrations (SRP, nitrate, nitrite, ammonia, and silica) were determined. More details about the sampling sites, instrumentation used, and analytical procedures can be found through the U.S. Geological Survey (USGS) Web site (www.sfbay.wr.usgs.gov).

In Monterey Bay, samples were collected during eight cruises from December 2001 to April 2003 at three stations located about 2, 25, and 50 km from shore (C1, M1, and M2 respectively) (Fig. 1B). Samples were taken from the surface, 20-m, and 60-m depths. Temperature, salinity, oxygen, Chl *a*, phytoplankton species abundance, and dissolved inorganic nutrient concentrations were determined as part of ongoing time series data collected by Monterey Bay Aquarium Research Institute (MBARI). More information about these sites, and the instrumentation and analytical procedures used, can be obtained at the MBARI Web site (www.mbari.org).

ELF-97 cell-specific assay—Water samples (1 liter) were processed as described by Dyhrman and Palenik (1999). This involved collecting plankton samples on a 0.45-μm filter (by low-vacuum filtration), resuspending the sample in an ethanol solution adding the label (ELF-97), and transferring the sample to an epitube. Samples were stored in the dark at 4°C until analysis. Cell counts were performed using a Nikon epifluorescent microscope using a 100-W mercury lamp
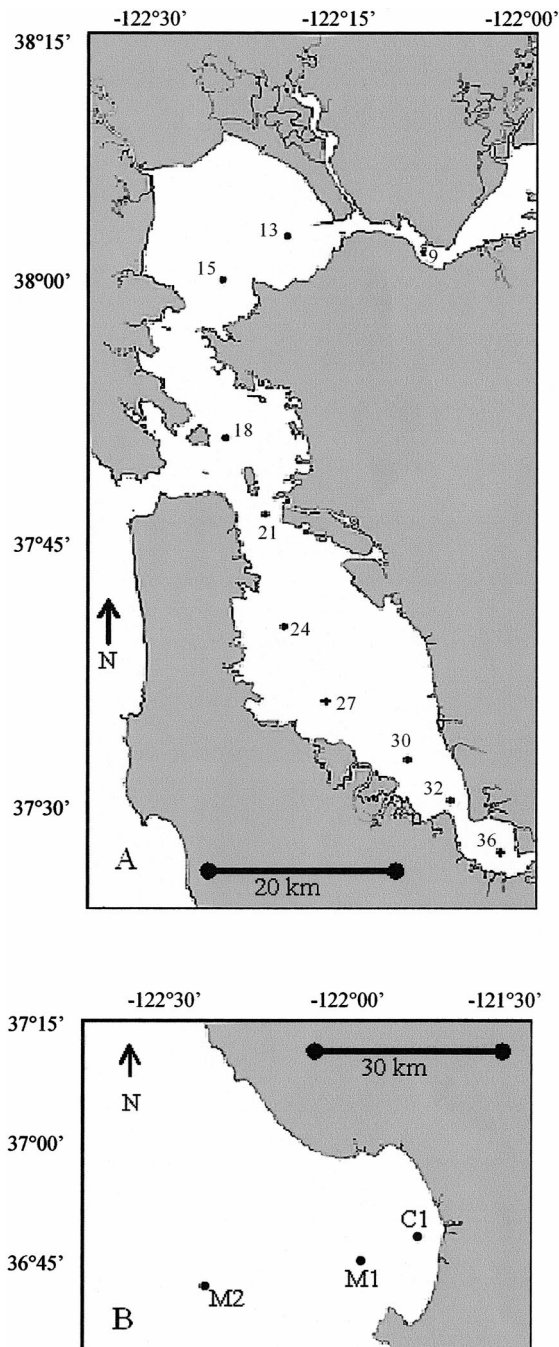
Fig. 1. (A) Sampling stations for San Francisco Bay cruises. Samples were collected from Stas. 9, 13, 15, 18, 21, 24, 27, 30, 32, and 36 from the surface layer. (B) Sampling stations for Monterey Bay cruises. Samples were collected at Stas. C1, M1, and M2 at depths of 60 m, 20 m, and at the surface.

(DAPI filter set, excitation at 350 nm, and maximum ELF emission at 520 nm) for ELF activity as well as with standard illumination. Slides were scanned, and each identifiable cell was tallied as either positive or negative for ELF labeling, indicating AP activity (Fig. 2). A positive tally was given to any cell that had a considerable amount of visible fluorescent ELF labeling.

ELF analysis was performed on surface-water samples from San Francisco Bay collected during June and August 2002 at Stas. 36, 21, and 9, to represent different regions in the bay. An abundance of suspended sediment, however, precluded the ability to identify and count cells; thus, only qualitative observations were recorded. ELF analysis was also performed on surface-water samples collected 3 June 2002, 8 August 2002, and 26 August 2002 for Monterey Bay Stas. M1, M2, and C1. Fifteen of the most commonly observed diatom and dinoflagellate taxa were monitored for each sample. Species composition varied greatly from cruise to cruise, and from station to station. Because species often were present at one time or site and not at the next, and other species had very few individuals in any one sample, it was difficult to track temporal or spatial changes for each of the individual species identified here. Because of this variance, we evaluated the combined data for all diatom species and all dinoflagellates from each sample as well as the data for all three cruises (Tables 1 and 2).

Bulk enzyme assays—Samples for AP assays were collected in 125-mL polyethylene Nalgene bottles and refrigerated until analysis (within 24 h of collection). Activities in sample splits analyzed upon collection or after refrigeration for 24 h were identical within analytical error, suggesting that this storage did not influence the results. AP activity was measured using a *p*-nitrophenyl phosphate (PNP) substrate (Fisher Scientific). Activity was measured on both filtered (0.2-μm polycarbonate filter) and unfiltered fractions of the samples in which unfiltered samples represent the total activity and filtered samples account for activity of enzyme that is released to the water. The difference between the two, total activity minus dissolved activity, represents particle-associated AP activity.

For each sample, 2.5 mL of sample water and 2.5 mL of pH 8.5 Modified Universal Buffer were mixed in 15-mL glass centrifuge tubes, and 1 mL of 25 mmol L^{-1} PNP-phosphate substrate was added to yield a final substrate concentration of ~4 mmol L^{-1} during incubation. After incubation and before spectrophotometric measurement, 4 mL of NaOH (0.1 mol L^{-1}) was added to the solution (resulting in concentrations of 2.5 mmol L^{-1}). These treatments are consistent with previously published protocols. Samples were covered and incubated for 24 h at room temperature on an orbital shaker at 50 rpm. Incubations of this period are necessary due to relatively low AP activity in the samples and are consistent with other marine studies. This time course was observed to be within the linear range of response as determined by hourly analyses of both standards and natural samples. All samples were tested in duplicate along with controls using autoclaved deionized water. Absorbance at 410 nm was measured to determine PNP concentrations. Standards made using p-nitrophenol at concentrations ranging from 0 to 10 μmol L^{-1} were used for calibration. Because other studies have used different substrate concentrations and equilibration temperatures, it is difficult to directly compare levels of activity between studies, but the method used is internally consistent and could be used to demonstrate the temporal and spatial variability of AP in the study area.
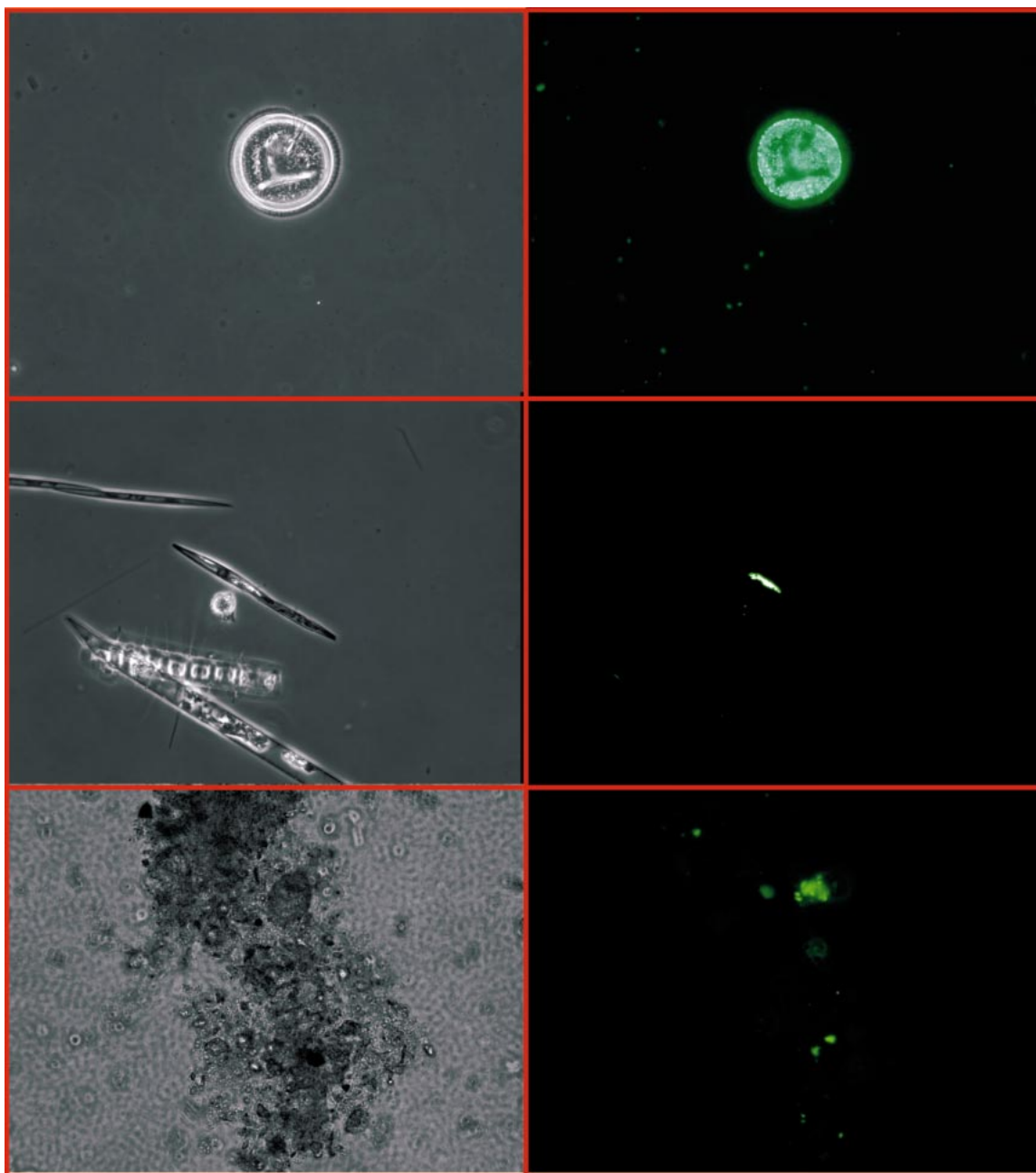
Fig. 2. Examples of ELF samples. Left panels show brightfield images, whereas the right panels show the corresponding epifluorescence image with a DAPI long-pass filter set. Top pair: ELF-labeled *Prorocentrum rostratum.* Middle pair: Example of ELF-labeled diatom (*Pseudonitzschia*) and unlabeled diatoms (*Pseudonitzschia and Chaetoceros*). Bottom pair: bacteria on sediment particles and aggregates showing ELF labeling in San Francisco Bay.

Ancillary data—Phytoplankton species abundance was determined from high-performance liquid chromatography (HPLC) pigment analyses and a set of equations that convert the HPLC data to the chlorophyll concentration (μg L^{-1}) associated with various taxa as described by Andersen et al. (1996); HPLC data are from Chavez (unpubl. data). Chl *a* concentration (mg chl m^{-3}) was measured by fluorometry after water samples were filtered onto 25-mm Whatmann GF/F filters and extracted in acetone. Approximately 10 mL of water was collected for dissolved nutrient concentrations

from each depth, frozen, and analyzed on a nutrient autoanalyzer.

Results

The relative abundance of the 15 most common diatom and dinoflagellate species and the fraction of cells for each species that was labeled by cell-specific (ELF) AP activity for the three cruises and from the three sites at Monterey Bay are given in Table 1. The percentage of ELF labeling

Table 1. Cell-specific AP activity from Monterey Bay. The results include summed data from three cruises from June 2002 through August 2002, and from three sites (see Fig. 1B).

Genera	Group	Positive	Negative	Total	Labeled (%)	Relative abundance
Asterionellopsis glacialis	Diatom	1	41	42	2.4	0.88%
Chaetoceros	Diatom	6	675	681	0.9	14.28%
Eucampia zodiacus	Diatom	2	397	399	0.5	8.36%
Pseudonitzschia	Diatom	2	2,352	2,354	0.1	49.35%
Round diatom	Diatom	29	219	248	11.7	5.20%
Thalsasiostra	Diatom	0	202	202	0.0	4.23%
Ceratium	Dinoflagellate	76	1	77	98.7	1.61%
Ceratium lineatum	Dinoflagellate	24	47	71	33.8	1.49%
Dinophysis	Dinoflagellate	13	1	14	92.9	0.29%
Dissodinium pseudolumula	Dinoflagellate	14	0	14	100.0	0.29%
Gymnodinium	Dinoflagellate	109	26	135	80.7	2.83%
Procentrum rostratum	Dinoflagellate	31	4	35	88.6	0.73%
Prorocentrum minimum	Dinoflagellate	310	111	421	73.6	8.83%
Protoperidinium	Dinoflagellate	45	11	56	80.4	1.17%
Unidentified nonthecate	Dinoflagellate	20	1	21	95.2	0.44%
Totals		682	4,088	4,770	14.30	
	Diatoms	40	3,886	3,926	1.0	82.31%
	Dinoflagellates	642	202	844	76.1	17.69%

for all diatoms and for all dinoflagellates for each sampling period are summarized in Table 2, which shows the preferential labeling of dinoflagellates at all sites throughout the year. Figure 2 shows ELF as observed under microscopy for a few representative samples. In San Francisco Bay, ELF analysis was complicated by high amounts of suspended sediment. Because the size of suspended particles was similar to the size of phytoplankton, it could not be separated by filtration. This resulted in large amounts of particulate matter, and relatively few phytoplankton cells on the filters. Because not enough phytoplankton cells were present to obtain statistically significant results, species data were not statistically analyzed. We note, however, that most phytoplankton (regardless of species) observed in San Francisco Bay during our sampling period were not ELF-labeled. The particulate matter in San Francisco Bay, however, was laced with ELF-labeled microorganisms, indicating substantial amounts of AP activity associated with bacteria attached to the particulate matter (Fig. 2C).

Bulk AP activity exhibited spatial and temporal variability in both San Francisco Bay (Fig. 3) and Monterey Bay (Fig. 4). Typically, the activity was much greater in the unfiltered fraction, suggesting that the activity in most samples was particle-associated, although on occasion, the dissolved portion (data not shown) accounted for a substantial fraction of the activity observed at a station (e.g., Sta. C1 in April 2002). AP activities in Monterey Bay were typically higher at stations closer to shore (C1 and M1) and in the upper water column (0 and 20 m) compared with the 60-m samples (Fig. 4). Maximum activities tend to occur in the early spring and summer periods. In San Francisco Bay, Stas. 32 and 36, in South Bay, exhibited the highest activity through much of the year (Fig. 3). Although the temporal distribution of activity was quite variable, the highest levels tended to occur between February and May in both 2002 and 2003, a period in the year when phytoplankton blooms are common (Cloern pers. comm. 2005). This also corresponds to periods of high turbidity and large amounts of suspended sediments (Hollibaugh, 1996). The maximum activities observed were 148 μmol PNP L^{-1} h^{-1} and 30 μmol PNP L^{-1} h^{-1} in San Francisco Bay and Monterey Bay, respectively. In San Francisco Bay, AP activity showed a weak but statistically significant ($p < 0.05$) positive correlation with Chl a, and weaker but still significant positive correlation with SRP (Fig. 5). In

Table 2. ELF labeling of diatoms versus dinoflagellates from three cruises from June 2002 through August 2002, in Monterey Bay.

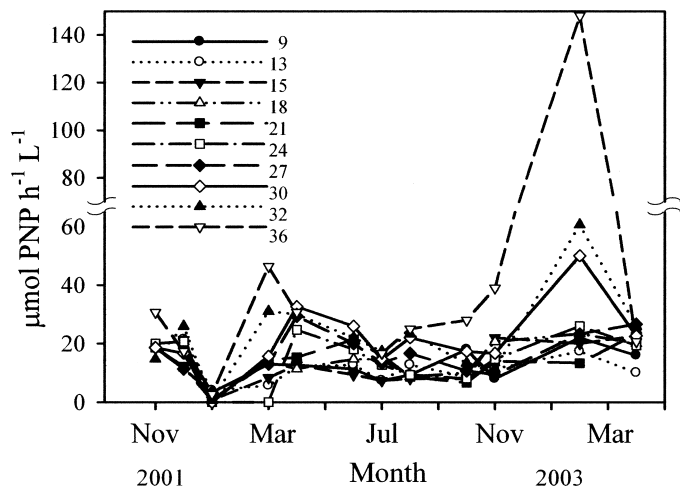| Date	Station	Diatoms Positive	Diatoms Negative	Diatoms % Labeled	Dinoflagellates Positive	Dinoflagellates Negative	Dinoflagellates % Labeled
3 Jun	C1	5	513	1.0%	75	16	82.4
3 Jun	M1	8	480	1.6%	77	31	71.3
3 Jun	M2	0	202	0.0%	231	73	76.0
8 Aug	C1	0	205	0.0%	20	8	71.4
8 Aug	M1	27	564	4.6%	102	8	92.7
8 Aug	M2	0	582	0.0%	11	4	73.3
26 Aug	C1	0	760	0.0%	4	14	22.2
26 Aug	M1	0	580	0.0%	122	48	71.8

Fig. 3. Bulk AP activity in San Francisco Bay from November 2001 through April 2003. Sta. 9 is at Suisun Bay in northern San Francisco Bay and Sta. 36 is at the most southern station (see Fig. 1A).

Monterey Bay, a similar weak positive correlation with Chl *a* was evident, but no correlation with SRP was observed. The bulk AP activity in Monterey Bay is plotted against dinoflagellate abundance determined from HPLC pigment analyses (Fig. 6). The positive correlation suggests that this group is responsible for the bulk of the AP activity.

Discussion

The results of ELF analysis revealed different P regimes in Monterey and San Francisco bays. Above background AP activity (background is defined here as 3 μmol PNP L^{-1} h^{-1} based on maximum levels observed in blanks) was measured throughout the year in San Francisco Bay and in Monterey Bay, both systems which, on the basis of nutrient analysis, have high P (always measurable) and Redfield ratios, which do not indicate P deficiency (on average 12 in Monterey Bay and 7 in San Francisco Bay, during our sampling period). AP activities were observed in other coastal high-P systems such as Tokyo Bay, some fjords, and in an upwelling system off northwest Africa (Kobori and Taga, 1979; Sebastien et al. 2004). When the taxa responsible for the AP activity in our study are examined (using ELF; Tables 1 and 2), it is clear that the activity is associated with specific components in the community and does not indicate that the phytoplankton as a whole are P-deficient.

The dominant phytoplankton in most of the samples from Monterey Bay were the diatom genera *Pseudonitzschia, Chaetoceros,* and *Eucampia* (accounting for 72% of all cells observed for the combined data). Diatoms (all species) comprised 82% of the cells counted in the samples, and dinoflagellates accounted for the remainder (18%). On average, 76% of dinoflagellates exhibited ELF labeling (range, 33% to 100% for various species). In comparison, on average, only 1.0% of diatoms were labeled (range, 0–12%). Dinoflagellates overall accounted for >90% of all ELF-labeled cells, despite their low relative abundance in the phytoplankton population (Table 1, Fig. 2). Our results are consistent
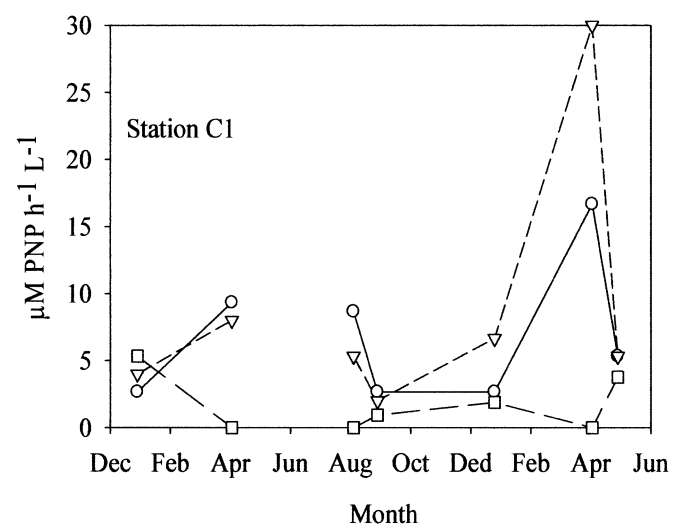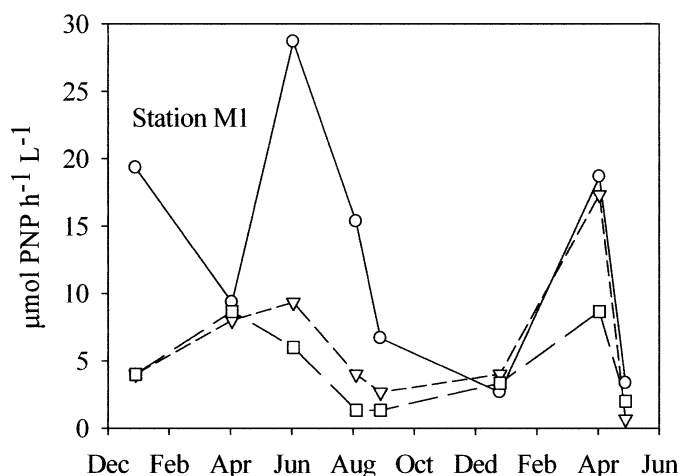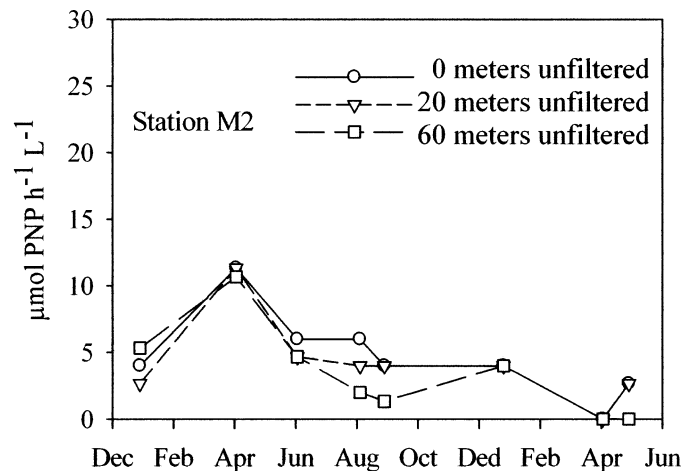

Fig. 4. Bulk AP activity for Stas. M2, M1, and C1 in Monterey Bay from December 2001 through April 2003. Stations show seasonal increases in AP activity during spring phytoplankton blooms and higher activities in stations closer to shore.
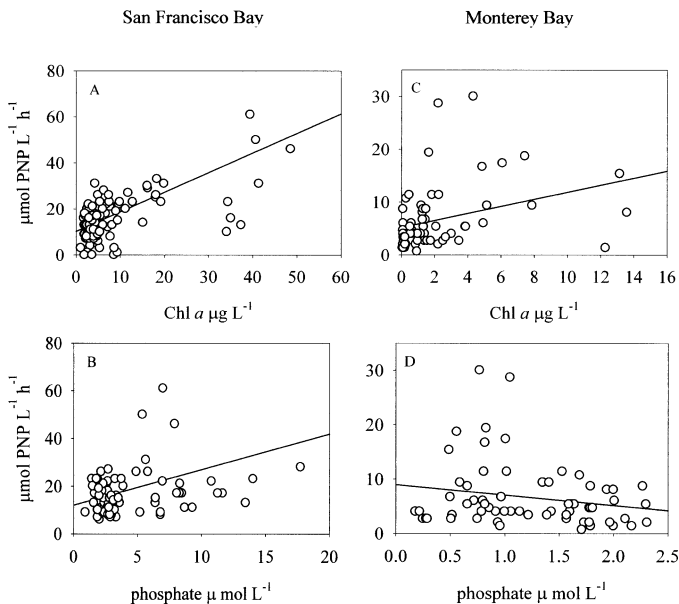
Fig. 5. Bulk AP activity plotted against Chl *a* and SRP for (A, B) San Francisco Bay and (C, D) Monterey Bay. (A, C) Both San Francisco and Monterey bays show significant weak positive correlations between AP activity and Chl *a* concentration ($r^2 = 0.283$ and 0.113, respectively). (B, D) San Francisco Bay samples show a slight positive correlation between SRP and AP activity; no correlation was observed in Monterey Bay ($r^2 = 0.084$ and 0.038, respectively). Significant correlations were calculated on the basis of linear regressions (least square method) using the *f* observed value, the relevant degrees of freedom, and a 0.05 confidence level.

with those of other studies (Kobori and Taga, 1979; Sebastien et al. 2004) that suggest that interpretation of bulk AP activity as a whole-phytoplankton-community P-deficiency indicator cannot be indiscriminately applied. Moreover, our results imply that high SRP (typically >0.5 μmol L^{-1} in surface waters in our study) and low N:P ratios (consistently lower than 16) cannot be used to suggest that the phytoplankton community as a whole is not P-deficient. For example, although nutrient enrichment grow-out experiments in Monterey Bay indicated that additions of nitrate provided the most potential for growth and biomass accumulation and P additions did not have any measurable effects (Kudela and Dugdale 2000), our data indicate that the dinoflagellates may be P-deficient, this is despite measurable SRP concentrations and low N:P ratios. It is possible that the P demands by dinoflagellates are higher than those of diatoms, or that some P component that is included in the SRP analysis is not accessible to dinoflagellates. Our work emphasizes the importance of identifying specific organisms within a community that exhibit AP activity. Moreover, data from San Francisco Bay, where AP activity is primarily associated with heterotrophic bacteria, stresses that when studying phytoplankton nutrition status it is crucial to assess the contribution of heterotrophic bacteria to the bulk AP activity. If this, for example, was not done in San Francisco Bay, the observed AP activity could be wrongly interpreted as reflecting phytoplankton P deficiency.

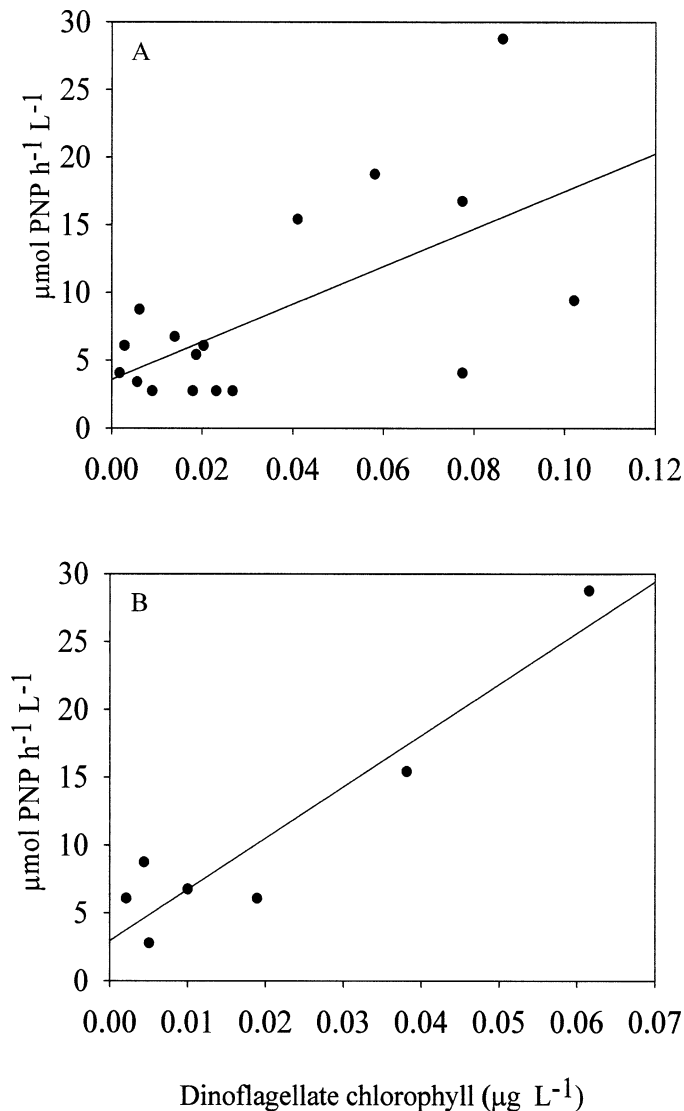In Monterey Bay, diatoms, the dominant class of phyto-

Dinoflagellate chlorophyll (μg L^{-1})

Fig. 6. (A) Bulk AP activity is correlated with dinoflagellate abundance as estimated from the dinoflagellate chlorophyll concentration ($r^2 = 0.39$), and (B) more strongly correlated with dinoflagellate abundance multiplied by the percentage of dinoflagellates exhibiting ELF labeling, which represents the abundance of ELF-labeled dinoflagellates ($r^2 = 0.89$).

plankton in the bay waters, generally did not exhibit much AP activity. The percentage labeling for different diatom species ranged from 0% to 12% with the most abundant species (*Pseudonitzschia* and *Chaetoceros*) exhibiting the least AP activity as determined by ELF labeling. Diatoms do have the ability to synthesize AP as was shown in culture experiments (Lomas pers. comm. 2004), and by instances in which individual diatoms were observed to be ELF-labeled in our and other field samples (Nicholson 2003; Lomas et al. 2004; Fig. 2, Table 1). In contrast, a large fraction of the dinoflagellate taxa in our samples exhibited AP activity; 33–100% of the cells for various species were labeled throughout the year. It is interesting that some variability between dinoflagellate taxa tendency to be labeled was observed; with *Dissodinium,* when found in the water column, exhib-
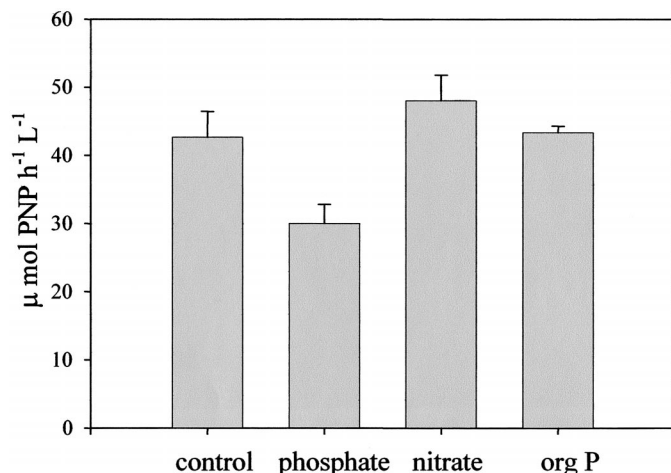
Fig. 7. Bulk AP activity in nutrient-addition incubation experiments with Monterey Bay samples. SRP addition treatment has lower AP activity compared with control, nitrate, or organic phosphate additions. Most of the activity observed was associated with dinoflagellates in the samples as determined by ELF labeling.

Fig. 8. Dinoflagellate pigment concentrations that are proportional to dinoflagellate abundance in relation to SRP concentrations in Monterey Bay.

iting labeling for 100% of the observed cells. The more abundant dinoflagellate groups, *Prorocentrum minimum* and *Gymnodinium,* typically showed less labeling (74% and 81% of cells labeled, respectively). The only dinoflagellate that exhibited labeling of less than 50% of the cells was *Ceratium lineatum.* As expected from the above results, the bulk AP activity in Monterey Bay is positively correlated with dinoflagellate abundance ($r^2 = 0.39$) (Fig. 6A). The correlation is even better (Fig. 6B, $r^2 = 0.89$) when only the ELF-labeled fraction of dinoflagellates is considered (e.g., dinoflagellate abundance multiplied by the percentage of dinoflagellates exhibiting ELF labeling), confirming that this group is responsible for the bulk of the AP activity.

AP is regulated by P in some dinoflagellates (Dyhrman and Palenik 1999); if indeed this AP induction is universal among dinoflagellates, our results indicate that despite presumably replete P conditions (high SRP concentrations) and relatively low N:P ratios, bioavailable inorganic P did not fulfill P demand by the dinoflagellates. However, it is possible that AP is not regulated by P availability for all dinoflagellate taxa (Gonzalez-Gill 1998); culture enrichment experiments should be conducted to establish taxa-specific regulation. Preliminary incubation experiments (Nicholson 2003) of a natural sample (mixed taxa collected in May 2003) from Monterey Bay (Sta. M1) with orthophosphate, nitrate, and glucophosphate (organic P) additions, show lowering of bulk AP activity and activity associated with dinoflagellates (determined using ELF) when inorganic P is added (compared with the control and other additions), suggesting that the AP activity indeed reflects P status (Fig. 7).

The observed difference in AP activity in diatoms and dinoflagellates in Monterey Bay (e.g., Table 1) suggests that changes in nutrient input, and particularly P loading, may not only affect overall phytoplankton abundance, but could also influence species composition. Indeed, the positive correlation between SRP concentrations and dinoflagellate chlorophyll concentrations (which are expected to be proportion-
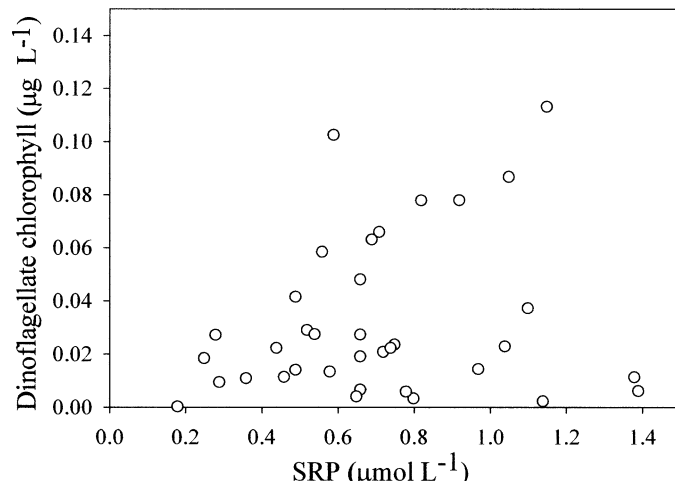
al to their abundance) during some months of the year in Monterey Bay (Fig. 8) suggests that this group may become more abundant under higher SRP conditions, at least under conditions characteristic of these months/cruises (mostly winter months and nonupwelling conditions). Additional research needs to occur to clearly describe and understand the specific interactions between SRP, other environmental parameters, and dinoflagellate abundance in Monterey Bay.

AP activities in Monterey Bay were typically higher in the upper 20 m of the water column compared with activities in the 60-m samples (Fig. 4). Although we did not observe a strong correlation between AP activity and SRP, we would expect lower activity in deeper samples where SRP concentrations are typically higher. The AP activity is also higher closer to shore (Sta. C1); this may be due to the typically higher dinoflagellate abundances closer to shore in Monterey Bay (e.g., Sta. C1; Fig. 9). This is evident from both the higher absolute dinoflagellate chlorophyll concentrations and in the fraction of dinoflagellate chlorophyll to total chloro-
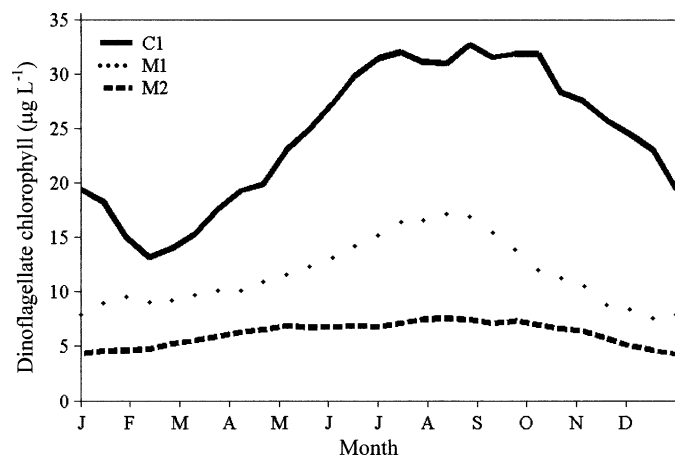

Fig. 9. The seasonal cycle for dinoflagellate chlorophyll concentrations at time series Stas. C1, M1, and M2 in Monterey Bay. Monthly averages for the years 1989–2000 are shown.

phyll at this site (data not shown). Maximum activities tend to occur in the spring and summer; it is possible that during this high productivity season the phytoplankton demand for SRP is high compared with the bioavailable pool, resulting in higher utilization of DOP, and thus higher AP activities. This is also the time of year when a peak in dinoflagellate abundance is observed (Chavez pers. comm. 2005), which may in turn, account for the higher activities.

In San Francisco Bay, ELF assays show that practically all of the AP activity is associated with bacteria and not phytoplankton. The highest activity through much of the year was recorded in Stas. 32 and 36 in the South Bay (Fig. 3). The South Bay is characterized by abundant suspended organic matter, and high labile particulate and dissolved organic carbon concentrations (Conomos 1979) that could provide substrates for bacterial growth. Indeed, bacterial counts tend to be higher in the South Bay, particularly near Sta. 36, which is closest to both land runoff and sewage inputs (Hollibaugh 1996). It is not clear why these bacteria are synthesizing AP; however, because SRP concentrations in the water column are high (between 1.5 and 15 μmol L^{-1}), it is unlikely that this is used to access P. In addition, the absence of a relationship between SRP and AP activity in San Francisco Bay questions the use of AP as a proxy for P deficiency. It is most likely that AP is used for hydrolysis of dissolved organic matter for carbon utilization as has been suggested for other places (Kirchman et al. 2000; Van Wambeke et al. 2002). Indeed, the highest activity levels are observed in the South Bay (Stas. 32 and 36), where DOC and POC concentrations are higher, as mentioned above. These results from San Francisco Bay further support our conclusion that SRP concentrations, N:P ratios, or bulk AP activities alone cannot be used to determine P status of natural eukaryotic phytoplankton populations, and that field and culture-based ELF assays may enhance our understanding of ecosystem response to P availability. The results also suggest that organic P compounds may have an important role in the environment as potential sources for both P and carbon to various organisms.

It is important to further examine the differences in P demand and utilization between species in order to understand how eukaryotic phytoplankton respond to nutrient availability in coastal environments and their ability to utilize various pools of nutrients. Our results imply that organic P compounds may have an important role in the P cycle and the availability of this P pool as a nutrient source that supports primary production and carbon uptake should not be ignored.

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