Alkaline phosphatase activity detected in distinct phytoplankton communities in the northern Gulf of Alaska

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ABSTRACT: Alkaline phosphatase activity (APA) has been proposed as a proxy to determine phosphorus (P) stress in marine phytoplankton. Phytoplankton typically meet P requirements by incorporating dissolved inorganic P (DIP), but can use reservoirs of dissolved organic P (DOP) by expressing alkaline phosphatase (AP). Enzyme-labeled fluorescence (ELF) was used to determine group-specific APA within 4 phytoplankton communities associated with mesoscale eddies, which are common to the northern Gulf of Alaska (nGoA). Nutrient stress in relation to eddies in the nGoA has not been well established and these anti-cyclonic eddies may be influencing P status in phytoplankton by isolating distinct biological communities. In the nGoA (fall 2007), APA was greatest in the northwest transitional waters of the Sitka eddy and the western edge of the Kenai eddy compared to coastal (shelf), eddy core waters, and open ocean waters. The APA signal was dominated by picoplankton (0.6 to 5 µm) and nanoplankton (5 to 20 µm), primarily nanoflagellates, though AP-producing phytoplankton were not the dominant phytoplankton at any station. APA did not correlate with DIP levels, and macronutrient concentrations suggest that DIP was not limiting for the bulk phytoplankton assemblage. Dissolved organic P (DOP) was not measured. These results suggest that P status within a community is more nuanced than typical Redfield or inorganic nutrient indices indicate and that organic P sources could influence community composition of phytoplankton.

KEY WORDS: Phosphorus limitation · Dissolved organic phosphorus · Spectrofluorometry · Enzyme-labeled fluorescence · Mesoscale eddy

INTRODUCTION

While most ocean systems are not considered phosphorus (P) limited except over geological timescales (Tyrrell 1999), P is an essential nutrient for all phytoplankton growth, energy transfer, and reproduction (Benitez-Nelson 2000). Marine ecosystems are usually limited (sensu Liebig) by nitrogen (N) or iron (Fe) (Moore et al. 2001), but 2 major P-limited environments include coastal areas influenced by seasonal freshwater inputs (Labry et al. 2002) and oligotrophic gyres that routinely have P levels too low to support maximal phytoplankton growth rates (Li et al. 1998, Hoppe 2003, Oh et al. 2005). Nutrient limitation can be difficult to determine because there are often syn-

ergistic or antagonistic effects between individual nutrients (Wei et al. 2003), ecosystems may shift seasonally between N or P limitation, and mixed communities of phytoplankton may have variable nutrient requirements (McComb et al. 1981, González-Gil et al. 1998).

Marine P is typically present as various dissolved organic P (DOP) compounds and as orthophosphate, dissolved inorganic P (DIP). The canonical molar Redfield ratio of 16:1 (N:P) in the water column or the C:N:P ratio (106:16:1) in phytoplankton biomass has traditionally been considered the determining factor for whether there is potential for N or P limitation in phytoplankton for a specific ecosystem (Redfield 1958, Beardall et al. 2001). However, the Redfield

ratio does not account for organic nutrients, and thus it is not always an accurate assessment of nutrient availability (Benitez-Nelson 2000). Furthermore, while the Redfield ratio may hold true for globally averaged phytoplankton biomass, there can be variable, species-specific P demands (González-Gil et al. 1998, Klausmeier et al. 2004, Nicholson et al. 2006). This may result in potentially subtle shifts in community composition in response to nutrient limitation (Dyhrman & Palenik 2001). Since many phytoplankton are capable of nutrient storage and luxury uptake, each species' nutrient requirements must be based on species-specific stoichiometry (Rhee & Gotham 1980).

Phytoplankton incorporate P by direct assimilation of DIP (Cembella et al. 1984, Chróst & Overbeck 1987, González-Gil et al. 1998). In marine waters, the pool of DOP can comprise 25 to 80% of the dissolved P pool (Hoppe 2003, Suzumura et al. 2012), though not all of it is biologically available (Karl & Bjorkman 2002), When DIP concentrations are depleted, phytoplankton may use reservoirs of DOP, like sugar phosphates and esters, for P deficiencies (Jackson & Williams 1985, Cotner & Wetzel 1992, Yamaguchi et al. 2006). There is evidence to suggest that DOP compounds such as phosphomonoesters and less-labile phosphonates play an important role in phytoplankton (Dyhrman & Haley 2006, Dyhrman & Ruttenberg 2006), though the production of P-cleaving enzymes is more energetically costly than incorporation of DIP (Meseck et al. 2009). In phytoplankton, phosphomonoesters cannot be directly assimilated, and must first be converted to DIP outside the cell (Cembella et al. 1984). Alkaline phosphatase (AP) is the enzyme most widely used by marine phytoplankton and heterotrophic bacteria to achieve this because of its ability to hydrolyze many different DOP compounds (Cembella et al. 1984, Štrojsová & Vrba 2006, Duhamel et al. 2010).

Previous culture work suggests that when DIP is present in Redfield proportions with other macronutrients, most phytoplankton species do not or cannot hydrolyze DOP (Hino 1988, González-Gil et al. 1998, Dyhrman & Palenik 1999). Cultures have shown a direct dependency on DIP concentration for AP activity (APA; Vargo & Shanley 1985, González-Gil et al. 1998), with many phytoplankton not producing AP until the N:P ratio is close to 30:1 (Dyhrman & Palenik 1999). Some phytoplankton will not produce AP even when DIP is limiting (Cembella et al. 1984, González-Gil et al. 1998, Rengefors et al. 2003, Meseck et al. 2009). Reductions in APA have also been shown with additions of DIP to P-starved algal cultures (Dyhrman & Palenik 1999). Laboratory studies thus indicate that APA is triggered by low DIP concentrations and,

because of this, APA is often used as an indicator of P stress or limitation (Kuenzler & Perras 1965, Rivkin & Swift 1980, Cembella et al. 1984, Dyhrman & Palenik 1999, Yamaguchi et al. 2005), a physiological response to low levels of DIP (Duhamel et al. 2010). While this response is robust for many cultured algae, field measurements demonstrate a wide range of variability within phytoplankton assemblages (Li et al. 1998, Dyhrman & Palenik 1999, Nicholson et al. 2006), suggesting that AP production may be more closely related to differences in species' nutrient stoichiometry than to absolute DIP availability, or that low concentrations of DIP may also be indicative of fast regeneration of P rather than DIP limitation (Chróst & Overbeck 1987, Hoppe 2003).

Two methods are routinely used to determine APA in marine environments. The first, a bulk-assay enzyme method, where a specific phosphomonoester substrate is hydrolyzed in the presence of AP (Nicholson et al. 2006), determines total APA (dissolved or particulate) for mixed assemblages (Koike & Nagata 1997, Li et al. 1998) by measuring the hydrolyzed ester substrate in the water (González-Gil et al. 1998, Yamaguchi et al. 2006). It cannot readily distinguish species-specific contributions to APA (González-Gil et al. 1998) and frequently overestimates phytoplankton APA by attributing heterotrophic bacterial APA, which is often greater than phytoplankton production, to the phytoplankton community (Sebastián et al. 2004). It is also difficult to compare between studies (Ou et al. 2006). The second method, enzyme-labeled fluorescence (ELF), attaches a phosphomonoester substrate (probe) to the site of enzyme activity and produces a fluorescent precipitate (González-Gil et al. 1998). Both eukaryotes and prokaryotes can be labeled (González-Gil et al. 1998, Meseck et al. 2009, Duhamel et al. 2010, Grégori et al. 2011). It is not clear if the label is physically located on the exterior of the cell or just under the cell membrane (Dyhrman & Palenik 1999, Díaz-de-Quijano & Felip 2011), but it can be easily visualized. The labeled cells are identified as producing AP in an analogous fashion to fluorescent in situ hybridization (FISH). The ELF method can be visualized with microscopy for eukaryotes, flow cytometry for prokaryotes (González-Gil et al. 1998, Dignum et al. 2004, Meseck et al. 2009, Díaz-de-Quijano & Felip 2011), or spectrofluorometry for estimates of APA intensity (Peacock & Kudela 2012), though each method has limitations (Štrojsová & Vrba 2006). We used the spectrofluorometric method described by Peacock & Kudela (2012), as labeled cells for the northern Gulf of Alaska (nGoA) had an extremely

low abundance and could not be counted microscopically (Nedoma et al. 2003, Nicholson et al. 2006, Peacock & Kudela 2012).

The nGoA is a highly productive coastal environment despite the low macronutrients over the continental shelf, a result of prevailing downwelling winds. Regardless, it is not classified as a P-limited system, and one would not expect to find P stress in phytoplankton in this region. The prevalence of downwelling winds suggests that other mechanisms for increased productivity over the shelf area are at play (Stabeno et al. 2004, Ware & Thomson 2005). Mesoscale anti-cyclonic eddies often form along the shelf break and act to exchange water from the shelf to the slope (Whitney et al. 2005). Biological productivity is typically enhanced along the tracks of these eddies due to greater mixing and entrainment of nutrients (Fiechter et al. 2009). As part of a larger investigation focusing on the role of eddies in cross-shelf transport and stimulation of biological communities, we utilize an ELF spectrofluorometric method to evaluate APA within the nGoA.

MATERIALS AND METHODS

Field collection

Water samples for nGoA APA were collected from the surface and the chlorophyll *a* (chl *a*) maximum (determined *in situ* by fluorescence) from an instrumented CTD rosette aboard the RV 'Thomas Thompson' from August 17 to September 20, 2007 at 32 stations (Fig. 1). Sample locations were chosen based on the paths of 2 anti-cyclonic eddies that were present during the cruise (Fig. 2). Sample sites include the transition edges and cores of the eddies, along with the coastal water and basin water not associated with either eddy. AP samples were filtered through 0.6, 5 and 20 µm polycarbonate filters and stored frozen at −80°C. Ancillary samples collected included nutrients, size-fractionated chl *a*, flow cytometric samples, and whole water for phytoplankton enumeration. Temperature, salinity, and oxygen profiles for each sample site were obtained from the CTD.

Laboratory cultures

Non-axenic cultures of the dinoflagellate *Amphidinium carterae*, obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP, strain 1332), were grown in Pyrex
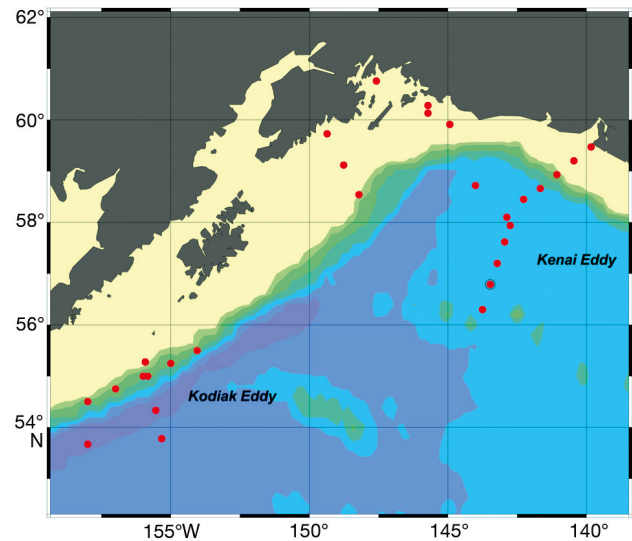

Fig. 1. Station locations (●) in the northern Gulf of Alaska sampled for alkaline phosphatase activity between August 17 and September 20, 2007

2 l flasks with sterile-filtered artificial seawater (ASW); these cultures were used to create APA standard curves by varying the P stress in the culture conditions (Peacock & Kudela 2012). The cultures were grown under P-replete (CCMP L1 nutrients) and P-deplete (CCMP L1 nutrients without P) conditions. Cells grown under P-deplete conditions were transferred at least twice to fully deplete residual DIP before they were used as standards. Cells were used as standards when they reached stationary phase (2 to 3 wk). Further details of the culture conditions and APA methods are included in a separate manuscript (Peacock & Kudela 2012).

ELF analysis

Both culture and field samples were labeled with ELF-97 (Endogenous Phosphatase Detection Kit, Invitrogen) prepared in sterile ASW (González-Gil et al. 1998, Dyhrman & Palenik 1999, Peacock & Kudela 2012). Samples were examined with a Zeiss Axio imager manual A1 microscope using a DAPI filter set with excitation at 350 nm and emission at >450 nm, as well as standard (brightfield) illumination. For culture standards, >400 cells were counted in triplicate to determine presence of APA. Labeled cells were identified by multiple attachment sites of the bright green fluorescing ELF precipitate (González-Gil et al. 1998). Unlabeled cells only displayed autofluorescence (Fig. 3). Standards grown with P-deplete media exhibited 99.9% ELF labeling, as determined
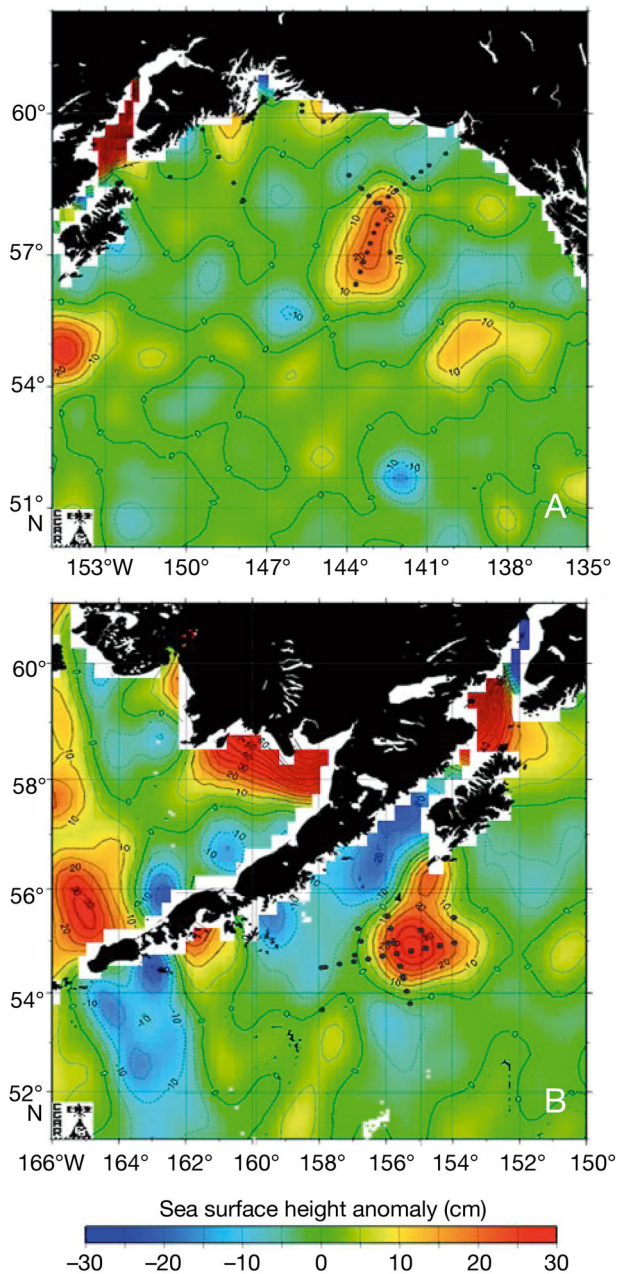
Fig. 3. Enzyme-labeled fluorescence (ELF)-labeled *Amphidinium carterae* cells used for standards grown in media without dissolved inorganic phosphorus. Green: fluorescent ELF precipitate, red: autofluorescence from chlorophyll

Samples were run on a 96-well opaque plate using an M2^e SpectraMax spectrofluorometer internally standardized with fluorescein isothiocyanate (FITC) to 3 fmol per well. Excitation was set to 365 nm with a wide emission band from 400 to 700 nm at 5 nm resolution. Automatic mixing for 20 s before reading was used to minimize particle sinking. Each well contained 70 to 100 µl of sample and was averaged over 8 readings. Temperature control (±0.5°C) was used during the analysis. The maximum emission wavelength, 525 nm, corresponds to the emission used for ELF microscopy (González-Gil et al. 1998). All samples and standards were adjusted for biomass by normalizing the ELF fluorescence to 450 nm. For our intensity analysis, we determined the area under the emission curve from 470 to 620 nm. Intensity was measured in arbitrary units (A.U.) for the nGoA samples, where 0 is no expression and 1 is maximum expression for the nGoA (i.e. 1 = the highest A.U. for the unknown nGoA field samples). Field samples were also qualitatively examined by microscopy to determine presence/ absence of APA, but cell counts were not performed. As per previously published protocols (Dyhrman & Palenik 1999, González-Gil et al. 1998), cells with any ELF labeling (regardless of the number of attached sites per cell) were considered positive for APA.

Ancillary data

Inorganic nutrient samples were collected in the field, 0.7 µm filtered, frozen, and later analyzed using a Lachat autoanalyzer with QuickChem standard co-

Fig. 2. (A) Sitka and (B) Kenai eddies are indicated by positive sea surface height anomaly (SSHa; cm). Station locations (●) are overlayed. Alkaline phosphatase activity was not collected at each station. SSHa data represent a temporal average from the cruise period. SSHa data was from Colorado Center for Aerodynamic Research (CCAR)

by labeled cells normalized to total cells, and standards grown under P-replete media exhibited <0.1% ELF labeling. Standards for spectrofluorometric determination of APA were prepared from the cultures using serial dilutions of cells harvested during the stationary phase (Peacock & Kudela 2012).
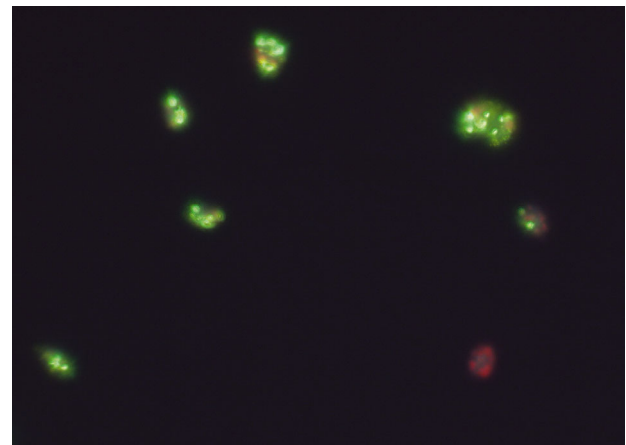
lorimetric methods for nitrate + nitrite (hereafter referred to as nitrate), P, and silicic acid (Knepel & Bogren 2002, Smith & Bogren 2001a, b). External precision was <0.2 µM for all nutrient species. DOP was not analyzed. Water was filtered from either the surface or the chl *a* maximum onto GF/F filters for total chl *a* and using 5 and 20 µm polycarbonate filters for size-fractionated chl *a* (referred to as pico-, nano-, and microplankton for <5, 5 to 20, and >20 µm fractions), extracted in 90% acetone and analyzed on a Turner 10-AU fluorometer (Welschmeyer 1994). Primary productivity data were obtained using standard ^{14}C deckboard incubation methods of whole water (Kudela et al. 2006). Flow cytometry samples were collected from the surface and chl *a* max, preserved with 4% paraformaldehyde and stored at −80°C until counted for *Synechococcus*, picoeukaryotes, and heterotrophic bacteria. Analysis was performed on a Cytopeia Influx flow cytometer with greater than 200 000 events counted per sample. Heterotrophic bacteria samples were prepared by staining the cells with SYBR Green I DNA stain. Qualitative enumeration of microplankton was conducted shipboard with unpreserved samples using a Fluid Imaging FlowCAM.

RESULTS

Characterization of water masses in the nGoA

Both the Sitka and Kenai eddies, named for their formation region, had hydrographic properties similar to previously described nGoA eddies (Tabata 1982, Ladd et al. 2007, Rovegno et al. 2009). Algorithms developed by Rovegno et al. (2009) using satellite altimetry to identify and track nGoA eddies determined that both eddies were about 8 mo old at our sampling time. Sampling sites were categorized by hydrographic data: basin, coastal, transitional, and eddy core water (Fig. 4). Sea surface height anomaly (SSHa) for both eddies was 30 to 40 cm (Rovegno et al. 2009). There were 2 distinct SSHa maxima for the Sitka eddy during the sampling period, with the northernmost SSHa showing clearly defined eddy core water and the southern lobe having a signature more closely related to transitional water (Rovegno et al. 2009). We identified the southern lobe as the Sitka secondary eddy core for distinction from the primary core water. Core water for both eddies was warmer and fresher than the surrounding transitional water and closely resembled the coastal end members sampled for these eddies. The sampled basin water was outside the influence of the mesoscale anomalies and
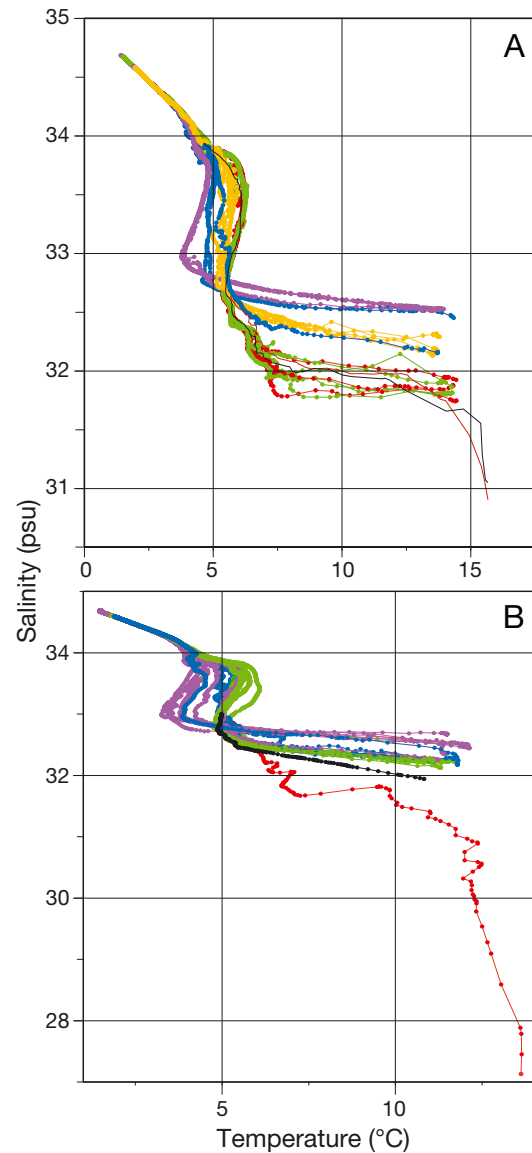

Fig. 4. (A) Sitka and (B) Kenai temperature–salinity diagrams. Core water (green), basin water (purple), coastal water (red), transitional water (blue), secondary core water for the Sitka eddy (orange), and the coastal source water for each eddy (black) are indicated

was high nutrient, lower than expected chlorophyll (HNLC) water associated with the GoA basin.

The N and P concentrations of the Sitka and Kenai eddies were depressed at the surface with concentrations generally increasing at the pycnocline. The transitional water (especially the coastal edges) had enhanced N (~8 µM) and DIP (~1.2 µM) compared to the eddy core. The basin water had >10 µM N and ~1.3 µM DIP. Reactive Fe (bio-available) was depleted in the eddy core and basin waters compared to the shelf waters (Lippiatt et al. 2011). The surface

core water had <1 µM nitrate and <0.4 µM DIP. For both eddies, transitional water had stoichiometric ratios of 8:1 (N:P) indicating N deficiency (Tyrrell 1999, Mackey et al. 2007), while core water was still indicative of potential N limitation (10 to 12:1 N:P), but closer to conventional Redfield proportions.

Phytoplankton assemblages as related to water masses

The dominant phytoplankton for the nGoA in September 2007 were nanoplankton-sized (5 to 20 µm) centric diatoms. Each of the 4 water types identified displayed distinctly different phytoplankton community assemblages based on flow cytometry, FlowCAM imaging, and size-fractionated chl a. On average, the Sitka eddy transitional water had lower depth-integrated biomass (mean ± SD, 38.9 ± 3.4 mg m^{-2} chl a; n = 4) compared to the eddy core and coastal water with concentrations of 39.1 ± 9.7 mg m^{-2} chl a (n = 3) and 56.9 ± 38.2 mg m^{-2} chl a (n = 4), while the HNLC basin water was 48.3 ± 0.9 mg m^{-2} chl a (n = 2). The transitional water and the HNLC water depth-integrated biomass were significantly different from each other (p < 0.001; ANOVA). There were also lower rates of depth-integrated primary productivity within the transitional water (52.6 to 59.2 mg C m^{-2} d^{-1}; n = 2) compared to the shelf and the basin water (67.8 to 170.0; n = 4 and 49.3 to 164.6 mg C m^{-2} d^{-1}; n = 3, respectively; not significantly different). Dinoflagellates were never the dominant phytoplankton, but exhibited greater relative numbers in the transitional water compared to other water masses (as determined by FlowCAM data). *Synechococcus* was also identified in greater numbers within the transitional water (10^5 cells ml^{-1}) compared to the other water masses (10^3 to 10^4 cells ml^{-1}) for both eddies (though the Kodiak eddy had 2-fold more *Synechococcus* than the Yakutat eddy). Picoplankton (0.6 to 5 µm) exhibited the highest biomass

within the transition and basin water while nano- and microplankton (>20 µm) were the dominant size classes in the eddy core and coastal stations.

APA in the nGoA

Of the 32 nGoA stations, 23 exhibited ELF labeling (Table 1). APA was more intense at the chl a maximum regardless of the physical depth. Visual examination by microscopy determined that ELF-labeled diatoms were in the Sitka secondary eddy core and the Yakutat coast (Stns 16, 17, 30; Table 1), but not in any other stations. All other

Table 1. Stations in the northern Gulf of Alaska where alkaline phosphatase activity (APA) was assessed, listed as either positive or negative for enzyme-labeled fluorescence (ELF) by microscopy. Their respective relative APA (spectrofluorometry) for the total size fraction (>0.6 µm) is also given, where 0 = no activity and 1 = the most activity (i.e. the highest APA recorded was given a value of 1). Discrepancies between microscopy and spectrofluorometry are attributed to greater sensitivity of the spectrofluorometric method. HNLC: high nutrient, lower than expected chlorophyll; nd: not detected

Station no.	Sample depth (m)	Description	ELF (+/−)	Relative APA
2	0	Coastal, on shelf	+	0.92
2	25	Coastal, on shelf	+	0.62
3	0	Coastal, on shelf	+	0.38
3	10	Coastal, on shelf	+	0.88
7	10	Sitka transitional, eddy edge	+	0.52
8	5	Sitka transitional, eddy edge	+	0.80
8	25	Sitka transitional, eddy edge	+	0.98
9	5	Sitka core	+	0.41
9	25	Sitka core	+	0.73
11	7	Sitka core	−	0.39
11	25	Sitka core	+	0.12
12	10	Sitka transitional, eddy edge	−	0.19
16	5	Sitka secondary core	+	0.44
16	25	Sitka secondary core	+	0.45
17	25	Sitka secondary core	+	0.32
20	0	Sitka core	+	0.54
23	25	HNLC basin	+	0.46
25	20	Coastal, on shelf	+	0.82
27	25	Prince William Sound	+	nd
29	25	Shelf break	−	0.65
30	25	Mid-shelf	+	0.98
31	0	Coastal, on shelf (Kenai source water)	+	0.72
31	25	Coastal, on shelf (Kenai source water)	+	1.00
54	25	Basin	−	0.69
55	25	Kenai core	−	nd
56	25	Kenai core	+	0.18
57	25	Kenai transitional, eddy edge	−	0.66
58	25	Kenai core	−	0.52
59	25	Kenai core	+	0.56
60	0	Basin	−	0.41
60	25	Basin	−	0.96
62	10	Basin	+	0.80

labeled cells were dinoflagellates or unidentified picoplankton, possibly haptophytes. Based on qualitative microscopic examination, dinoflagellates were more likely to express APA compared to either diatoms or unidentified picoplankton.

There were clearly varying amounts of APA with ELF labeling present in all 4 water masses. Multivariate tests and canonical correlation did not identify significant relationships between any measured environmental factors and APA (temperature, salinity, oxygen, inorganic nutrients, chl a, and fluorescence), while the 4 water masses were distinguishable based on the environmental data. Maximum APA was seen in the Kenai eddy source water (Stn 31) while the least APA was observed in both the Sitka eddy primary core and Kenai eddy core (Fig. 5). Stations identified as transitional or coastal water had greater APA compared to either eddy core or basin water. There was a weak, nearly significant relationship between APA and distance from the eddy core ($r^2 = 0.29$; p = 0.07). When samples were grouped by water mass type, transitional waters exhibited significantly higher APA values compared to eddy core waters (p < 0.05, 1-way t-test).

Size-fractionated APA determined that microplankton contributed to a significant portion of the APA at only the 2 Sitka eddy primary core water stations, a Kenai basin water station, and a coastal station near the Yakutat coast not related to either eddy (Stns 16, 17, 62, and data not shown; Fig. 6). For all other stations APA was documented in the picoplankton or nanoplankton samples. APA and DIP concentrations were not significantly correlated for size fractions (Fig. 7A–C). There was no correlation between chl a and APA (Fig. 7D–F) or DIP and chl a concentrations (data not shown) for any of the size fractionated data.

DISCUSSION

APA in the nGoA detected by spectrofluorometry

ELF spectrofluorometric analysis detected APA throughout the nGoA, even though nutrient indices (dissolved inorganic nutrient measurements, stoi-
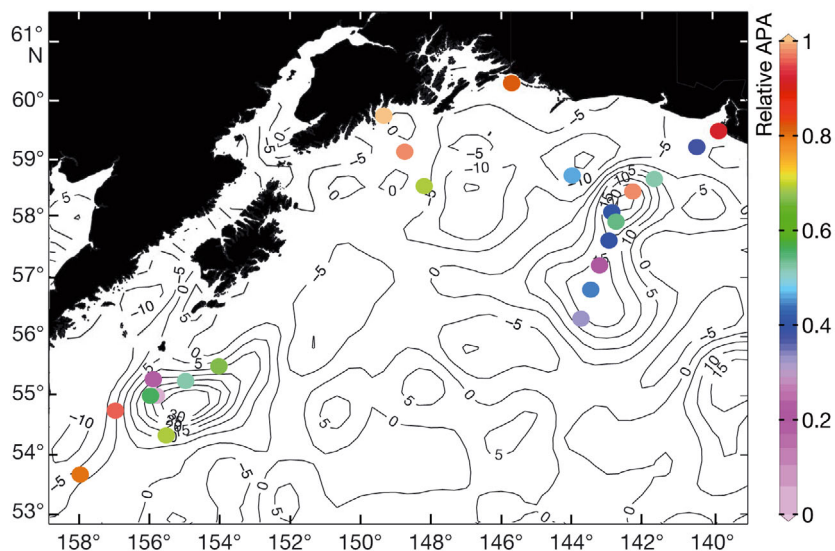

Fig. 5. Relative alkaline phosphatase activity (APA) from selected stations (at the chl a max) for the northern Gulf of Alaska as determined by spectrofluorometry. APA is higher in the transitional water, and lower within the core water

chiometric ratios compared to the Redfield ratio) indicated that the phytoplankton should not be P stressed (Fig. 5). The Sitka and Kenai eddy cores exhibited the lowest concentration of P, though on average, stoichiometric proportions were still indicative of N limitation rather than P limitation. Typically, phytoplankton APA has an inverse relationship with inorganic P (Cembella et al. 1984); therefore, one might expect that APA would be most prominent in the eddy cores where DIP was low. In contrast, our results show that APA was highest near the Yakutat coastline, the northwest transitional water of the Sitka eddy, and the western Kenai eddy transitional water, where nutrients were replete (Fig. 5). Compared to the nearby transitional water, APA was low in the eddy cores and HNLC basin water not influenced by the eddies. APA in the secondary Sitka eddy core was greater compared to either the primary Sitka core or the Kenai core water, but this water mass had hydrographic properties more closely attributed to transitional water than to core water (Rovegno et al. 2009).

The transitional waters had N:P ratios that would seemingly preclude expression of AP by phytoplankton, as sufficient DIP concentrations have been reported to stop gene transcription of AP (Ray et al. 1991), yet these stations demonstrated the greatest amount of APA. Dinoflagellates are strong AP producers (González-Gil et al. 1998), and the greatest APA signal was seen in the transitional water masses with a higher number of dinoflagellates. Regardless,
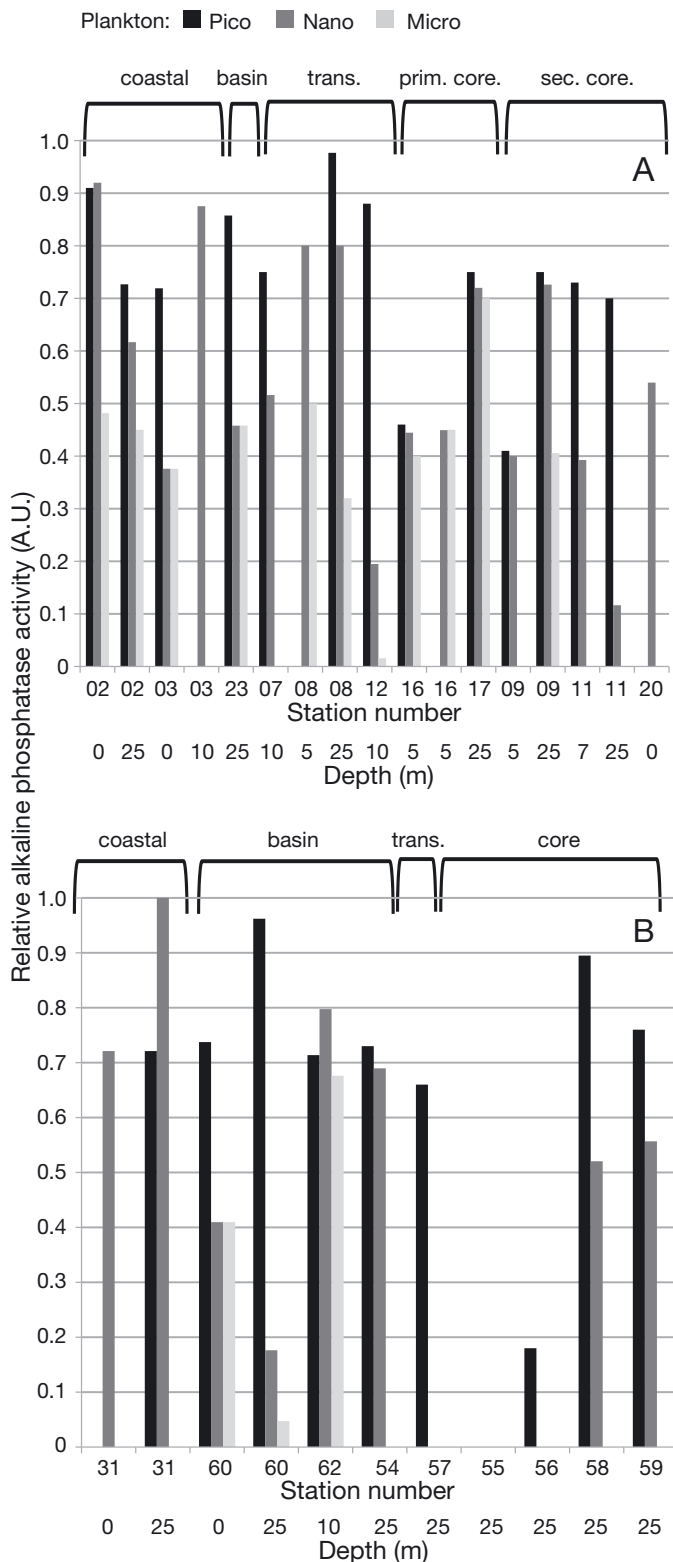
Fig. 6. Size-fractionated relative alkaline phosphatase activity (APA) in arbitrary units (A.U.) for the northern Gulf of Alaska stations in the (A) Sitka eddy and (B) Kenai eddy. Most of the APA was associated with the pico- and nanoplankton size fractions

dinoflagellates never outnumbered diatoms (i.e. diatoms were always the dominant taxon).

There was no correlation between DIP and APA in any of the size fractions (Fig. 7A–C), likely because of the diatom-dominated communities. We did not specifically determine the fraction of total chl *a* attributed to dinoflagellates, but hypothesize that APA would be correlated with dinoflagellate biomass as has been seen in other studies (Nicholson et al. 2006, Meseck et al. 2009). We did not measure DOP for this study; however, previous DOP estimates in the nGoA indicate that DOP is small relative to DIP (Ridal & Moore 1992). If DOP is rapidly recycled and is being actively hydrolyzed by phytoplankton as well as heterotrophic bacteria, then absolute concentrations of DOP may be low but still important for nutrient acquisition of some phytoplankton groups.

Size-fractionated APA

The transitional water stations, where APA was greatest, had proportionally more microplankton than other stations. The dominant size class at the Sitka eddy transition stations was picoplankton, an almost equal mixture of picoeukaryotes and *Synechococcus* (10^3 cells ml^{-1} as determined by flow cytometry); the Sitka eddy transitional water was also the most consistently labeled with ELF, but there was low total biomass (chl *a* < 0.5 mg m^{-3}). We could not assess APA by flow cytometry, either due to the addition of ethanol during processing of the samples (Dignum et al. 2004, Štrojsová & Vrba 2006) or because labeling of *Synechococcus* by ELF is rare as some strains of cyanobacteria are not able to cleave P from DOP (Meseck et al. 2009). Unlike some strains of cyanobacteria, many diatoms (the dominant group in the nGoA during our study) do have the ability to hydrolyze DOP. However, diatoms often express AP under extremely low DIP concentrations compared to dinoflagellates (Dyhrman & Palenik 1999, Nicholson et al. 2006, Meseck et al. 2009). The small number of nano- and microplankton cells labeled by ELF made statistical microscopy counts of phytoplankton unreliable. This has been reported in other field studies and can be problematic when the dominant taxon is not the species producing AP (Dyhrman & Ruttenberg 2006, Nicholson et al. 2006, Duhamel et al. 2010). Regardless, based on size-fractionated APA and microscopy it appears nGoA AP production was most often expressed in the pico- and nanoplankton (dinoflagellates) rather than by the dominant diatom assemblage in the nano- and microplankton size classes.
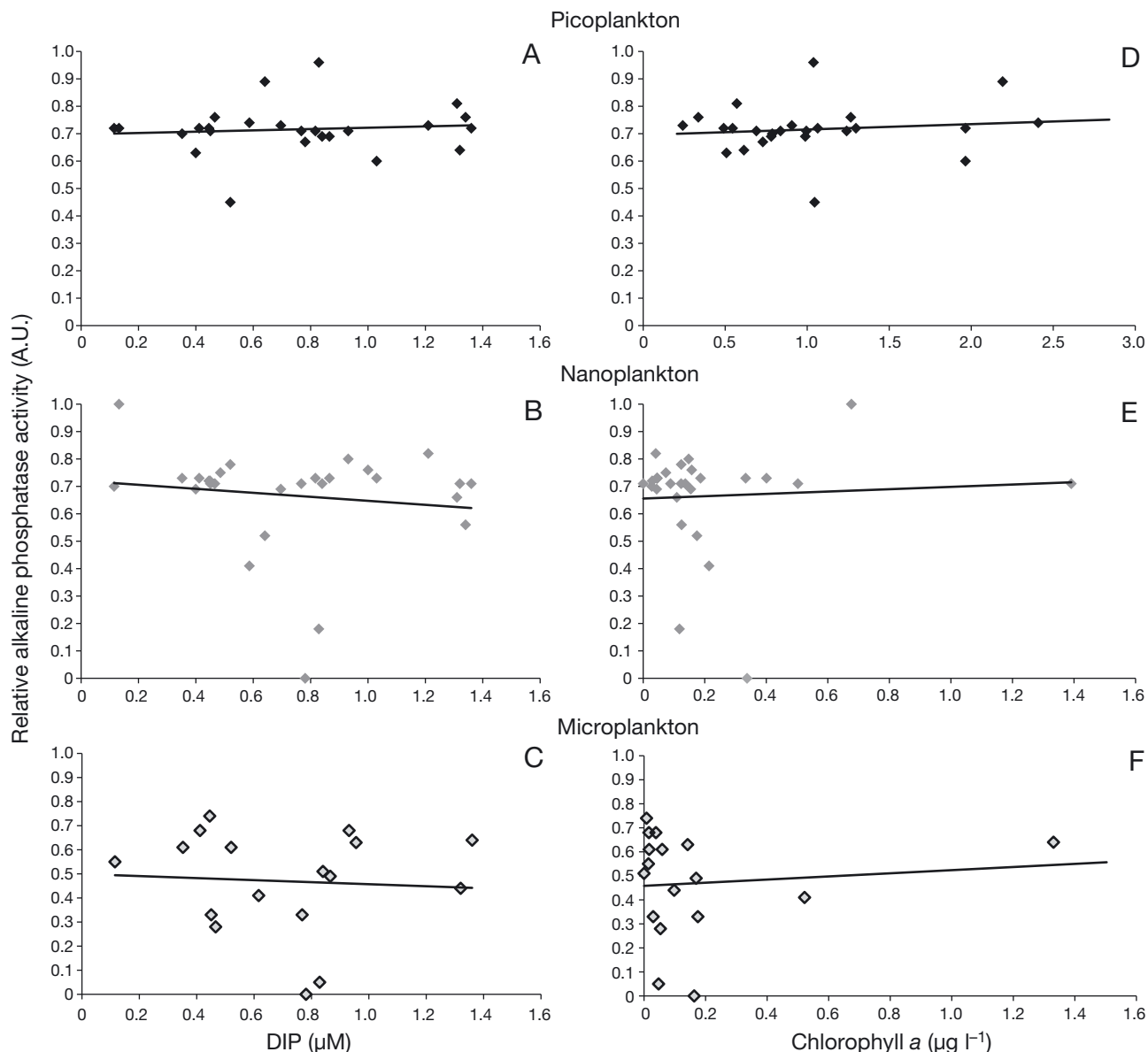
Fig. 7. Relative alkaline phosphatase activity (APA) in arbitrary units (A.U.) plotted against (A, B, C) dissolved inorganic P (DIP) and (D, E, F) chlorophyll *a* for (A, D) picoplankton, (B, E) nanoplankton, and (C, F) microplankton

Community APA

An increasing body of literature confirms the superior ability of dinoflagellates and haptophytes to produce AP compared to other phytoplankton (Cembella et al. 1984, González-Gil et al. 1998, Dyhrman & Palenik 1999, Nicholson et al. 2006, Meseck et al. 2009). While size-fractionation of APA samples has not been used to differentiate between micro- and nanoplankton, it has been used to separate *Synechococcus* and heterotrophic bacteria (Li et al. 1998, Duhamel et al. 2010) and the principle is the same: to differentiate APA within a phytoplankton assemblage

to better understand group dynamics, rather than contributing P stress to the bulk plankton community. Nanoplankton dinoflagellates were synthesizing AP when microplankton dinoflagellates at the same station were not, suggesting that larger cells contain sufficient internal stores of P to avoid synthesizing AP (John & Flynn 2000). It is also possible these non-responding cells may resist synthesizing AP until their internal stores of P are very depleted (Meseck et al. 2009) and assimilation of DOP may be an adaptation based on the cell's ability to store P internally.

In our study, P deficiency is clearly not attributed to the entire community of phytoplankton. APA in the

nGoA appears to be a group- or species-specific response to P stress rather than a response characteristic of the entire assemblage, although we note that we do not have sufficient resolution to identify species-specific patterns of APA. This is consistent with the lack of obvious P limitation using indices such as the stoichiometric ratio (N:P was between 0 and 13 for the nGoA during our sampling period) or DIP concentrations, and classical nutrient criteria do not accurately portray what is happening within the phytoplankton assemblage. The shelf and transitional water in particular indicated N limitation, yet the highest APA was recorded in these water masses. Other coastal high-P systems such as Monterey and San Francisco Bay, California (Nicholson et al. 2006), Tokyo Bay Japan (Kobori & Taga 1979), and off NW Africa (Sebastián et al. 2004) also exhibit APA when classic P deficiency is not present. Furthermore, recent culture studies contradict the classical idea that uptake of DOP is based on a lack of DIP in the media or seawater. APA was detected in uni-algal cultures of 5 species of phytoplankton grown in DIP concentrations >10 μM (Meseck et al. 2009), which is >10× the amount of P we recorded in the nGoA. This study reinforces the idea that AP production is group- or species-specific. Individual cells may also exhibit luxury uptake or internal P pools resulting in differential P limitation under the same environmental conditions (Rengefors et al. 2003), while a possible lag in AP production in response to cellular P stress (Litchman & Nguyen 2008) may make snapshot determination of P stress in populations in dynamic P environments like the nGoA difficult to interpret.

As it appears that AP production is not always linked to depleted DIP concentrations, particularly in coastal environments, other mechanisms for AP regulation should be explored. During our study of the Kenai and Sitka eddies, there was little or no mixing between biological communities of the core water and basin water, except at the transition edges. The transition water for the eddies is a mixture of Fe-deplete, HNLC basin water and Fe-replete, macro-nutrient-deplete water drawn off the coastal shelf (Okkonen et al. 2003, Crawford et al. 2005). Chl *a* is often transported off the shelf along with the core eddy water, wrapped around the edges of eddies and carried several hundred km into the interior of the GoA (Crawford et al. 2005, Whitney et al. 2005, Ladd et al. 2007). The original core water of the eddies characterized by this study is similar in physical, nutrient, and biological properties to putative source water on the shelf (Ladd et al. 2007, Rovegno et al. 2009)—but as the eddy ages there is nutrient mixing

(Lippiatt et al. 2011) and biological properties change quickly, forming distinct communities of phytoplankton. It has been noted previously in laboratory studies that phytoplankton growth is retarded during periods of AP production (Litchman & Nguyen 2008). This corresponds with observations of transitional water where APA increased while biomass and primary productivity were minimal compared to water masses with little or no APA.

CONCLUSION

Conditions that influence regulation of AP by dinoflagellates are not well understood on a community level, and laboratory experiments to establish P threshold limits for AP production have only been conducted with uni-algal cultures (González-Gil et al. 1998, Dyhrman & Palenik 1999, Meseck et al. 2009). Laboratory studies indicate that APA is activated only when under P stress (González-Gil et al. 1998, Dyhrman & Palenik 1999, Dignum et al. 2004, Meseck et al. 2009), but our investigation and previous work by others indicate that AP is actively produced by dinoflagellates (Nicholson et al. 2006, Dyhrman 2008) or haptophytes (Ranhofer et al. 2009) in diatom-dominated assemblages, when P stress is not evident. It is unclear what role AP production plays in modulating the community structure in these mixed assemblages. Using classical nutrient indices, the phytoplankton communities in the nGoA should not have been P stressed, yet small dinoflagellates were clearly synthesizing AP. This suggests that it may be more informative to assess nutrient stress at a group or species level rather than at a bulk community level.

Nutrient concentrations (inorganic and organic) and chl *a* measurements without corresponding APA data make the P status of the community difficult to interpret (Dyhrman & Ruttenberg 2006). The same can be said for measuring only dissolved APA (bulk-enzyme assays), a common APA measurement, and assigning P status to a community without determining group- or species-specific contributions. ELF labeling has improved understanding of AP production in marine phytoplankton but it can be difficult to interpret for samples with low biomass (Nicholson et al. 2006, Štrojsová & Vrba 2006). P status of eukaryotic communities in waters influenced by mesoscale eddies appears to be complex and variable; if APA is indicative of P stress, then some portions of the nGoA phytoplankton assemblage are likely P stressed under typical summertime conditions. The near-

ubiquitous (but low percentage) ELF labeling of cells throughout the nGoA, with enhanced APA in transitional waters, strongly suggests that a more nuanced approach to nutrient acquisition and limitation in these systems needs to be applied as part of future studies.

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