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Characterization of alkaline phosphatase activity in the North and South Pacific Subtropical Gyres: Implications for phosphorus cycling

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Abstract

We characterized alkaline phosphatase (AP) activity (APA) and report AP kinetic parameters in the North and South Pacific Subtropical Gyres (NPSG and SPSG, respectively) to evaluate the relative importance of APA in dissolved organic P (DOP) remineralization. APA potential hydrolysis rates were low but measurable (0.10 ± 0.06 nmol L^{-1} h^{-1}, $n = 12$, and 0.13 ± 0.08 nmol L^{-1} h^{-1}, $n = 6$) in surface samples from the NPSG and central SPSG, respectively. When compared to dissolved inorganic P (DIP) uptake rates, the potential utilization of DOP by APA may be important for microbial communities in these oligotrophic habitats. Assuming an upper (80%) and a lower (10%) fraction of the DOP to be AP-hydrolyzable, APA could represent 25% ± 18% ($n = 11$) and 4% ± 3% ($n = 11$) of the DIP uptake, respectively, in surface waters of the NPSG and 147% ± 86% ($n = 6$) and 20% ± 12% ($n = 6$), respectively, in surface waters of the central SPSG. The ratio of APA to DIP uptake was significantly larger in the SPSG compared to the NPSG despite having higher DIP and lower DOP. The half-saturation constant (K_m) was in the range of DOP concentrations measured in the two gyres, meaning that microorganisms can adjust their DOP utilization according to DOP availability. Dissolved APA was a large fraction of total APA in the NPSG, contributing between 12% and 100% (41 ± 23%, $n = 23$) of total APA at Sta. ALOHA. Utilization of the AP-hydrolyzable fraction of the DOP probably contributes to production in the extensive Pacific oligotrophic gyres.

Phosphorus (P) is essential to life and its role to sustain ocean productivity is fundamental (Perry 1972; Karl 2000; Dyhrman et al. 2007). The most bioavailable form is dissolved inorganic P (DIP) as orthophosphate, but many compounds of dissolved organic P (DOP) pool can be used as a P source, even under P-sufficient microbial growth (Orrett and Karl 1987; Björkman and Karl 1994; Karl 2007*a*). An illustration of DOP bioavailability is its disappearance in the mesopelagic zone while DIP is high (Karl 2007*a*). Björkman and Karl (2003) showed that in the North Pacific Subtropical Gyre (NPSG), the microbial community simultaneously utilizes DIP and DOP compounds for their P nutrition and, on average, use them to the same extent as DIP in the upper water column. The authors suggested that the simultaneous utilization of P from both DIP and DOP pools could indicate resource partitioning: some microorganisms may be adapted for use of DOP, whereas others use DIP regardless of P concentrations. Recent studies showed that DIP and DOP are taken up simultaneously even at the organism level (Beversdorf et al. 2010; White et al. 2010).

In the subtropical ocean, DOP represents the larger fraction of the total P in the upper 100 m of the water column, for example, 70–80% in the NPSG (Karl and Björkman 2002) and > 80% in the Sargasso Sea (Cavender-Bares et al. 2001; Lomas et al. 2010). Small DOP compounds can be assimilated directly by certain microorganisms while the majority of DOP compounds must be remineralized prior to uptake (Cembella et al. 1984). DOP remineralization is mediated by enzymatic hydrolysis (Berman 1970; Kobori and Taga 1979; Hoppe 2003). Alkaline phosphatase (AP) is one of several potential DOP hydrolyzing enzymes, but it is the best studied and arguably the most important for nutrition since it has the potential to hydrolyze a broad spectrum of DOP compounds (e.g., the phosphomonoesters [P esters]). For example, Berman (1970) showed that when phosphatases were incubated with unfiltered sample fixed with chloroform, the amount of DIP released was large enough to fulfill the P needs of phytoplankton in Lake Kinneret. Moreover, AP is produced by a majority of marine microorganisms (Cembella et al. 1984; Hoppe 2003). Since AP activity (APA) is enhanced with DIP starvation, its detection in the field is often used as an indicator of DIP stress or limitation (Perry 1972; Van Wambeke et al. 2002; Dyhrman and Ruttenberg 2006). Nevertheless, some studies have shown contradictory results, and there may be alternative ecological interpretations for the presence of APA in natural environments (Karl and Björkman 2002; Hoppe 2003). While being mindful of the methodological caveats, APA measurements can be used as a metric of the potential utilization of the AP-hydrolyzable fraction of the DOP.

Perry (1972) adapted a clinical fluorometric assay, based on the release of fluorescein, to assay low levels of APA in

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oligotrophic seawater. Previous colorimetric methods could not easily detect APA without extended assays or preconcentration of cells. Perry (1972) showed that in the NPSG, APA was detectable but very low when DIP was close to or below the detection limit of the method used at that time (i.e., ≤ 30 nmol DIP L^{-1}). No APA was detected in the filtrate (< 0.45 μm; Perry 1972). To our knowledge, since this study, only two other papers report APA data for the NPSG (Koike and Nagata 1997; Duhamel et al. 2010), and no data are available for the South Pacific Subtropical Gyre (SPSG). Consequently, our knowledge of APA and its relative importance in the P cycling of the largest area of the world ocean is very limited.

Herein, we characterize APA in the NPSG and SPSG. We report the spatial variability in surface samples from the NPSG and along a zonal transect in the SPSG as well as with depth (euphotic layer) within the NPSG. For comparison, we also present results from higher-biomass regimes, such as a phytoplankton bloom in the NPSG and mesotrophic to eutrophic areas to the west and east of the central part of the SPSG, including the waters within the upwelling region of the Chilean coast. We also report interseasonal APA variability and kinetic responses. The goals of this study were to provide information on APA at the otherwise well-characterized Sta. ALOHA and its surrounding waters as well as for the undersampled SPSG and to examine the significance of APA in DOP remineralization and P cycling.

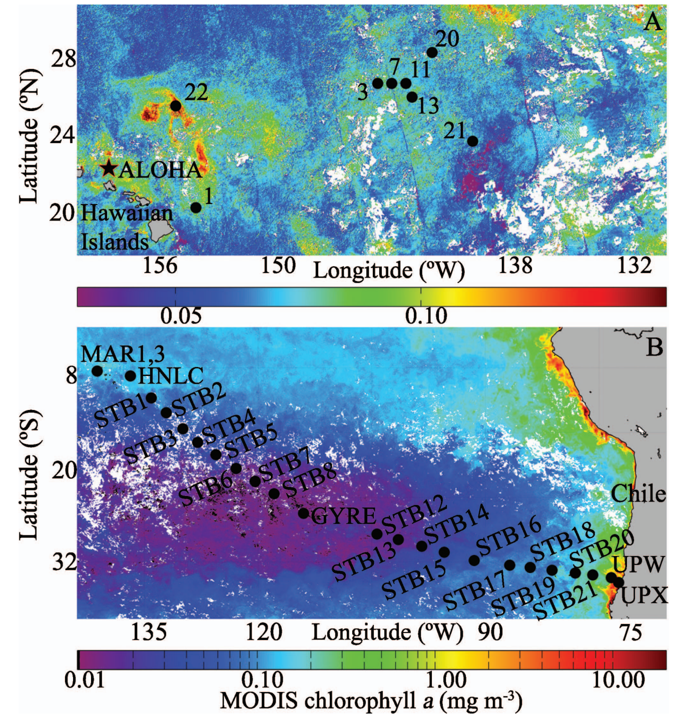

Fig. 1. Stations location. (A) Study areas in the North Pacific Subtropical Gyre with Sta. ALOHA (black star) and the POOB2 cruise stations (black dots) on average moderate-resolution imaging spectroradiometer (MODIS) Chl *a* (mg m^{-3}) for 05 August–13 August 2009. (B) The BIOSOPE cruise transect in the southeast subtropical Pacific Ocean (on average MODIS Chl *a* for November 2004) between Marquesas Islands (Sta. MAR) and the Chilean upwelling (Sta. UPW-UPX). Note the different chlorophyll *a* scales.

Methods

Sampling—Samples were collected at the station "A Long-Term Oligotrophic Habitat Assessment" (Sta. ALOHA; 22°45′N, 158°00′W; Fig. 1A) in the NPSG during several Hawaii Ocean Time-series cruises (Table 2): HOT-200 (February 08), -201 (May 08), -206 (December 08), -210 (April 09), -211 (May 09), and -216 (November 09). Samples were also acquired during the Pacific Open Ocean Bloom 2 (POOB2, 29 July–14 August 2009) cruise in the NPSG (Fig. 1A). In addition, samples were collected during the BIogeochemistry and Optics South Pacific Experiment (BIOSOPE; October–December 04) cruise, which crossed different biogeochemical provinces, including the SPSG (Fig. 1B). The central portion of the oligotrophic SPSG is located between Sta. STB6 (20.45°S; 122.9°W) and Sta. STB14 (30.04°S, 98.4°W). Data for nutrients, biomass, and DIP uptake and turnover rates from the BIOSOPE cruise were published elsewhere (Moutin et al. 2008; Duhamel and Moutin 2009).

Nutrient analysis—DIP concentrations were determined on triplicate 50-mL samples by the magnesium-induced coprecipitation (MAGIC) method (Karl and Tien 1992) followed by the molybdenum blue reaction (Murphy and Riley 1962). Total dissolved P (TDP) was measured using wet persulfate oxidation (Menzel and Corwin 1965) followed by MAGIC as described in Björkman et al. (2000) and DOP estimated as (TDP-DIP).

Biomass and cell counts—Samples for chlorophyll *a* (Chl *a*) were filtered onto microfiber glass-fiber filters (Whatman grade GF/F) at Sta. ALOHA or on polycarbonate filters (0.2 μm) for the POOB2 cruise. Chl *a* samples were extracted in cold 100% acetone (7 d, −20°C). The fluorescence was measured using a Turner Design 10-AU fluorometer.

Samples for picoplankton enumeration were fixed in a final concentration of 0.2% paraformaldehyde, flash frozen in liquid nitrogen, and stored at −80°C. Picophytoplankton and heterotrophic bacterial abundances were determined using a Cytopeia Influx flow cytometer.

DIP uptake rate—DIP uptake rates were determined as in Björkman et al. (2000). Results are presented as the average of duplicate samples. The DIP uptake rate and turnover time were calculated as follows: DIP uptake = $[((Rf - Rb):Rt) \times [DIP]]:t$ and DIP turnover = $[DIP]$:DIP uptake, with the incubation time (t, h), the radioactivity on the filter (Rf, Bq), the radioactivity of the blank (Rb, Bq), the total tracer added (Rt, Bq), and the DIP concentration ([DIP], nmol L^{-1}).

Alkaline phosphatase activity—Total APA was measured using the fluorogenic phosphatase substrate 4-methylumbelliferyl phosphate (MUF-P; Sigma-Aldrich). Measure-

Table 1. List of abbreviations.

ALOHA	A Long-Term Oligotrophic Habitat Assessment
AP	Alkaline phosphatase
APA	Alkaline phosphatase activity
BIOSOPE	BIogeochemistry and Optics SOuth Pacific Experiment
Chl *a*	Chlorophyll *a* (μg L^{-1})
DIP	Dissolved inorganic phosphorus (nmol L^{-1})
DIP turnover	Turnover time of the ambient DIP (month)
DIP uptake	DIP uptake rate (nmol L^{-1} h^{-1})
DOP	Dissolved organic phosphorus (nmol L^{-1})
HOT	Hawaii Ocean Time-series
K_m	Half-saturation constant (nmol L^{-1})
MAGIC	MAGnesium-Induced Co-precipitation
MUF	4-Methylumbelliferone
MUF-P	4-Methylumbelliferyl phosphate
NPSG	North Pacific Subtropical Gyre
P	Phosphorus
POOB	Pacific Open Ocean Bloom
V_{DOP}	Potential hydrolysis rate of DOP at ambient concentrations (nmol L^{-1} h^{-1})
SPSG	South Pacific Subtropical Gyre
TDP	Total dissolved phosphorus (nmol L^{-1})
V	Hydrolysis rate (nmol L^{-1} h^{-1})
$V_{DOP}:DIP$	Fraction of DIP potentially released by APA relative to the ambient concentration of DIP (% d^{-1})
V_m	Maximum (potential) hydrolysis rate of DOP (nmol L^{-1} h^{-1})
Z_{eu}	Euphotic layer

ments made at saturating concentration of the substrate (1 μmol L^{-1}) allowed for the determination of the maximum hydrolysis rate (V_m). To determine other kinetic parameters, a series of six to eight different MUF-P concentrations, ranging from 0.025 to 1 μmol L^{-1} (final concentration), were measured. The kinetic parameters were determined using the Hanes–Woolf plot graphical representation of the rearrangement of the Michaelis–Menten equation as follows: $S:V = K_m:V_m + S:V_m$, with the MUF-P concentration (S), the hydrolysis rate (V), the maximum hydrolysis rate (V_m), and the half-saturation constant (K_m). With the maximum concentration of MUF-P added (1 μmol L^{-1}), two types of samples were analyzed to distinguish dissolved activity (< 0.2 μm seawater) from whole-seawater activity. All samples were incubated in the dark at in situ temperature in an incubator (BIOSOPE and POOB2 cruises) or at room temperature (HOT cruises). Hydrolysis of MUF-P to 4-methylumbelliferone (MUF; excitation and emission: 359 and 449 nm, respectively) was measured on a PerkinElmer LS-5 and on a Kontron SFM25 spectrofluorometer in the NPSG and SPSG, respectively. At least four measurements were obtained within 12 h to verify the linearity of the assay. A standard curve using MUF (Sigma-Aldrich) from 0 to 500 nmol L^{-1} in 0.2-μm-filtered and boiled seawater was used to calculate MUF-P hydrolysis rates. Blanks (i.e., ultrapure water) and killed controls (i.e., sample fixed with 0.2% paraformaldehyde, final concentration) were run periodically at saturating concentration and indicated no significant autohydrolysis of the substrate. The AP-hydrolyzable fraction of DOP was not measured in this study. The maximum

Table 2. Characteristics of the surface seawaters samples from the NPSG (at ~ 5-m depth for HOT and POOB2 cruises). The latitudes (Lat.) and longitudes (Long.) are given in decimal units (dec.). DIP: the dissolved inorganic phosphorus concentration (nmol L^{-1}); DOP: the dissolved organic phosphorus concentration (nmol L^{-1}); Chl *a*: the chlorophyll *a* concentration (μg L^{-1}), DOP : TDP: the concentrations ratio (%) of DOP to total dissolved phosphorus (TDP); DIP uptake: the uptake rate of DIP (in nmol L^{-1} h^{-1}); DIP turnover: the turnover time of the DIP pool (month).

Cruise	Date	Sta.	Lat. (dec. N)	Long. (dec. W)	DIP (nmol L^{-1})	DOP (nmol L^{-1})	Chl *a* (μg L^{-1})	DOP : TDP (%)	DIP uptake (nmol L^{-1} h^{-1})	DIP turnover (month)
HOT-201	28 May 08	ALOHA	22.75	158.00	111	—	0.040	—	0.15±0.02*	1.0*
HOT-206	01 Dec 08	ALOHA	22.75	158.00	33	207	0.160	86	0.14	0.3
HOT-210	30 Apr 09	ALOHA	22.75	158.00	85	175	0.066	67	0.15±0.02*	0.8*
HOT-211	29 May 09	ALOHA	22.75	158.00	21	221	0.063	91	0.15±0.02*	0.2*
HOT-216	04 Nov 09	ALOHA	22.75	158.00	79	222	0.095	74	0.15±0.02*	0.7*
POOB2	30 Jul 09	1	20.50	153.70	121	152	0.066	56	0.15	1.1
POOB2	02 Aug 09	3	26.10	144.70	31	141	0.068	82	0.10	0.4
POOB2	03 Aug 09	7	26.10	144.00	17	137	0.057	89	0.08	0.3
POOB2	04 Aug 09	11	26.10	143.30	90	141	0.085	61	0.10	1.3
POOB2	05 Aug 09	13	25.50	143.00	12	135	0.076	92	0.09	0.2
POOB2	07 Aug 09	20	27.50	142.00	107	148	0.086	58	0.13	1.1
POOB2	09 Aug 09	21	23.50	140.00	153	151	0.090	50	0.28	0.7
POOB2	12 Aug 09	22	25.10	154.70	27	161	0.236	85	0.23	0.2

* No DIP uptake are data available for these cruises, so the average value (average ± SD) for 16 cruises made between March 2000 and November 2001 at Sta. ALOHA are shown (K. M. Björkman unpubl. data). DIP turnover was calculated using the average DIP uptake value.

potential hydrolysis rate of DOP, calculated as $V_{DOP} = (V_m \times [DOP]) : ([DOP] + K_m)$ (nmol L^{-1} h^{-1}), would occur if the ambient DOP concentration is fully AP hydrolyzable. The same equation was used to estimate DOP hydrolysis if 80% (V_{DOP}[80]) or 10% (V_{DOP}[10]) of the DOP was AP hydrolyzable.

Calculation and statistical analysis—Results are reported as mean ± 1 standard deviation. Daily rates were calculated by multiplying the hourly rate by 24. Total picoplankton abundances were used to calculate cell-abundance-specific V_m.

The daily fraction of DIP potentially released by APA, at ambient DOP concentrations, was calculated as the V_{DOP} (nmol L^{-1} d^{-1}) to DIP concentration (nmol L^{-1}) ratio and expressed as a fraction of the ambient DIP pool (V_{DOP} : DIP, in % d^{-1}). V_{DOP} : DIP uptake (%) is the V_{DOP} (nmol L^{-1} h^{-1}) to uptake rate of DIP (nmol L^{-1} h^{-1}) ratio. This ratio is also calculated assuming that only 80% or 10% of the ambient DOP concentration is AP-hydrolyzable (i.e., V_{DOP}[80] : DIP uptake or V_{DOP}[10] : DIP uptake, respectively).

We used Sigmastat 3.1 (Systat Software) for the statistical analysis. Significance is reported where $p \leq 0.05$.

Results

Study area description—Samples were collected in the NPSG at Sta. ALOHA and during the POOB2 cruise. Sta. ALOHA has been studied since October 1988, and its characteristics and dynamics are described elsewhere (Karl and Lukas 1996; Karl 1999; Karl et al. 2001*b*). During the POOB2 cruise, a phytoplankton bloom formed west of the sampling area, and one station (Sta. 22) was sampled within the bloom (Fig. 1A). This station presented very different characteristics compared to the rest of the stations sampled in the NPSG. Cholorophyll *a* (Chl *a*) concentration measured at 5 m at Sta. 22 was 0.24 μg L^{-1}, that is, three times higher than the mean value measured in the NPSG at the same depth (0.08 ± 0.03 μg L^{-1}; Table 2). Moreover, 59% of the Chl *a* measured at Sta. 22 was found in the fraction larger than 10 μm, while this fraction represented only 8% ± 3% (n = 7) of the Chl *a* in the other stations of the POOB2 cruise (data not shown). Samples collected in the SPSG during the BIOSOPE cruise were representative of a broad variety of environmental conditions (for more details describing this area, *see* Claustre et al. 2008). For comparison with the NPSG, we will focus mainly on the most oligotrophic waters from the SPSG area (between Sta. STB6 and Sta. STB14), where Chl *a* concentrations measured at 50% of surface irradiance (i.e., between 20 and 30 m depth) were 0.024 ± 0.08 μg L^{-1} (Duhamel and Moutin 2009). In the SPSG, nitrate was undetectable (i.e., ≤ 3 nmol L^{-1}) in the euphotic zone (Raimbault et al. 2008). The NPSG also shows barely detectable nitrate concentrations (< 10 nmol L^{-1}) in the upper 0–100 m (Karl et al. 2008), but the HOT long-term study at Sta. ALOHA has recorded both seasonal and interannual variability (Karl et al. 2001*a*, 2008) resulting from stochastic mixing events (Johnson et al. 2010). DIP concentrations measured in the NPSG surface waters (i.e., 5 m) varied between 12 and 153 nmol L^{-1} (68 ± 46 nmol L^{-1}, n = 12; Table 2). In the SPSG, DIP concentrations measured in the 50% of surface irradiance layer (130 ± 10 nmol L^{-1}; Moutin et al. 2008) were significantly higher than in the NPSG (Mann–Whitney test, p = 0.003). On the contrary, DOP concentrations in the NPSG (166 ± 33 nmol L^{-1}, n = 12; Table 2) were generally higher than in the SPSG (159 ± 28 nmol L^{-1}; Moutin et al. 2008), although, when taking the entire data set of DOP concentrations into account, there was no significant difference between the two areas (Mann–Whitney test, p = 0.422). On average, DOP represented 74% ± 15% (n = 12; Table 2) and 55% ± 4% (Moutin et al. 2008) of the TDP in the surface waters of the NPSG and SPSG, respectively.

Kinetic parameters—The kinetic parameters of APA were assessed within the euphotic zone (Z_{eu}: 0–125 m) at Sta. ALOHA on six separate occasions over 2 yr (Fig. 2). Results showed that V_m was consistently low (i.e., < 0.4 nmol L^{-1} h^{-1}) over the entire Z_{eu} and throughout the year (Fig. 2A). V_m, cell-abundance-specific V_m, and DIP concentration generally increased with depth (Fig. 2-A,D,F), while total and *Prochlorococcus* cell abundance and DOP concentration decreased (Fig. 2E,G,H). V_m and cell-abundance-specific V_m were higher in winter (Fig. 2A,F). Most of the profiles showed maximum V_m and cell-abundance-specific V_m at 75 m, where the lowest DIP concentrations were also measured (Fig. 2 A,D,F). K_m values were the lowest at 5 m and 100 m (236 ± 80 nmol L^{-1}, n = 10) and were generally the highest at 75 m (469 ± 168 nmol L^{-1}, n = 5). No significant correlations were found between V_m or K_m and DIP or DOP concentrations (Fig. 2I,J). *Prochlorococcus* and heterotrophic bacteria represented more than 98% of the total cell abundance throughout the euphotic layer at Sta. ALOHA (Fig. 2G,H). *Prochlorococcus* represented 29% ± 7% (n = 25) of the total cell abundance between 5 and 100 m, while it represented 12% ± 9% (n = 5) of the total cell abundance at 125 m. *Prochlorococcus* abundance did not show seasonal pattern (Fig. 2G). No significant correlation was found between V_m and total cell abundance (Spearman rank order correlation $p > 0.05$, n = 30).

The kinetic parameters of APA were also assessed in surface waters (i.e., ≥ 50% of surface irradiance) in both the NPSG and the SPSG (Table 3). With the exception of Sta. 22 from the POOB2 cruise, all V_m estimates were lower than 0.61 nmol L^{-1} h^{-1}, which was the maximum value found in the SPSG. At Sta. 22, V_m increased to 3.12 nmol L^{-1} h^{-1}. No significant correlation was found between V_m and DIP, DOP, or Chl *a* concentrations (Spearman rank order correlation $p > 0.05$). K_m values were of the same order of magnitude as DOP concentrations. No significant correlations were found between V_m or K_m and DIP or DOP for the NPSG (Fig. 3A,B) or between V_m and DIP or DOP for the SPSG (Fig. 3C). Only K_m in the SPSG increased with increasing DIP and DOP concentrations (Spearman rank order correlation, $p < 0.05$, n = 28; Fig. 3D).

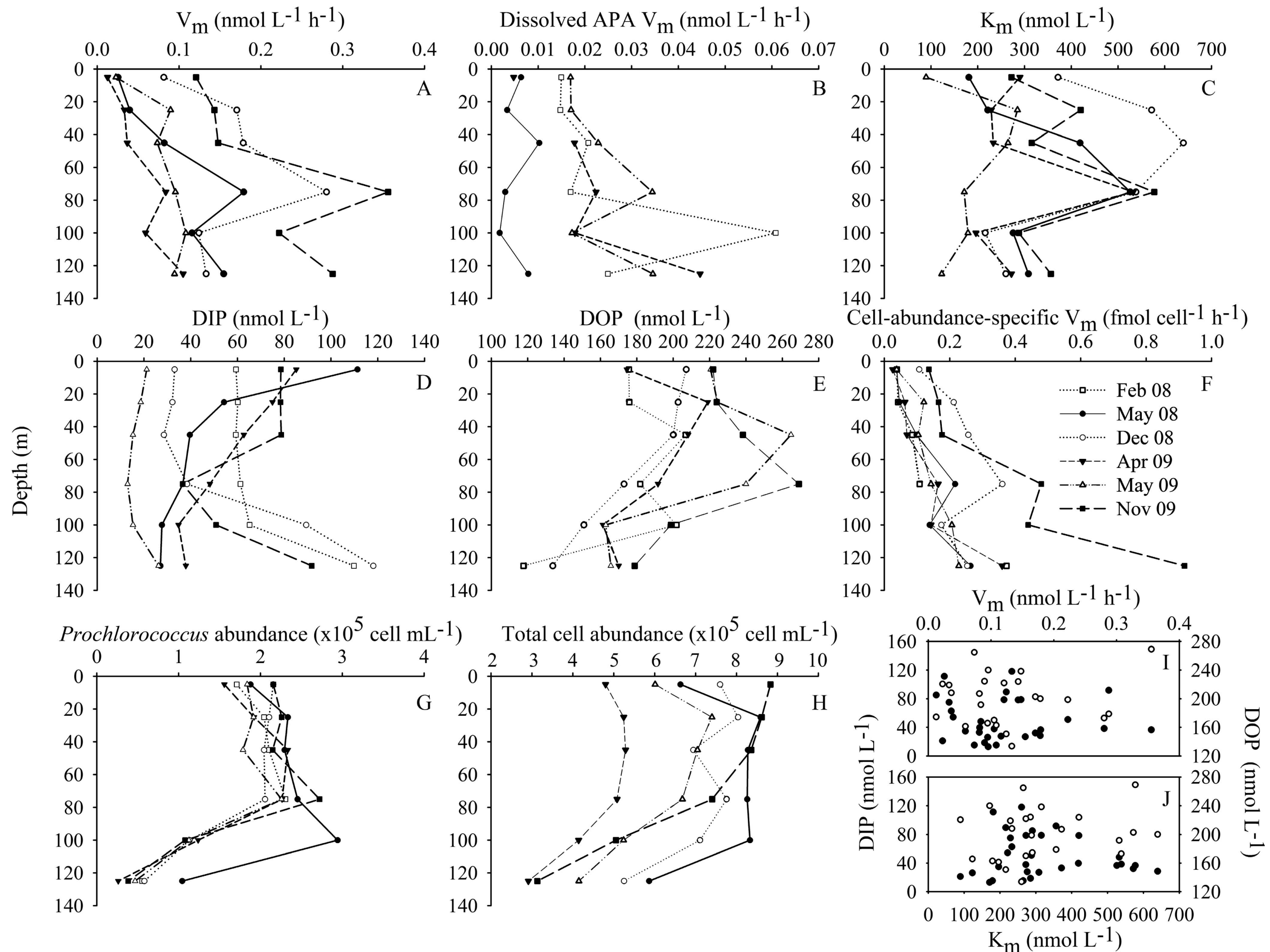

Fig. 2. Kinetic parameters of alkaline phosphatase activity at Sta. ALOHA, measured between February 2008 and November 2009 along the euphotic layer. (A) V_m: the maximum hydrolysis rate (i.e., measured at saturating concentration of the substrate) in nmol of MUF-P hydrolyzed per liter and per hour (nmol L^{-1} h^{-1}); (B) dissolved APA V_m: the maximum hydrolysis rate in the dissolved fraction (i.e., < 0.2 μm) in nmol of MUF-P hydrolyzed per liter and per hour (nmol L^{-1} h^{-1}); (C) K_m: the half-saturation constant (nmol L^{-1}); (D) DIP: the dissolved inorganic phosphorus concentration (nmol L^{-1}); (E) DOP: the dissolved organic phosphorus concentration (nmol L^{-1}); (F) the cell-abundance-specific maximum hydrolysis rate (i.e., ratio between V_m and the total picoplankton cell abundance determined by flow cytometry; fmol $cell^{-1}$ h^{-1}); (G) *Prochlorococcus* abundance ($\times 10^5$ cell mL^{-1}); (H) Total cell abundance: the total picoplankton cell abundance ($\times 10^5$ cell mL^{-1}); (I) relationship between V_m and DIP (black circles) and DOP (open circles) concentrations; (J) relationship between K_m and DIP (black circles) and DOP (open circles) concentrations.

Metric for APA significance in P cycling—The daily fraction of DIP potentially released by APA represented between 0.1% d^{-1} and 63.2% d^{-1} of the DIP measured in the corresponding surface sample (Table 3). V_{DOP} : DIP ratio was typically higher in the NPSG (2.3% $\pm$ 2.6% d^{-1}, n = 11, excluding Sta. 22) than in the SPSG (0.8% $\pm$ 0.4% d^{-1}, n = 6) because of the lower DIP concentrations measured in the NPSG compared with the SPSG. In the NPSG, V_{DOP} represented 3–77% (28% $\pm$ 20%, n = 11) of the DIP uptake measured at the same stations, excluding bloom Sta. 22. The highest values were recorded at Sta. 22 of the POOB2 cruise (312%) and in the SPSG (172% $\pm$ 101%, n = 6). No significant correlation was found between V_{DOP} and DIP uptake. On average, the hydrolysis rate at ambient DOP concentration (V_{DOP}) was one-third of V_m. Since the entire stock of DOP is not entirely available to AP utilization, these estimates are an upper limit of the potential role of APA in P flux. In order to provide more reasonable estimates, we also calculated V_{DOP} estimates assuming that either 10% or 80% of the DOP concentration is APA reactive. $V_{DOP}(10)$ represented 4% $\pm$ 3% (n = 11) and 20% $\pm$ 12% (n = 6) of DIP uptake in the NPSG (excluding Sta. 22 = 32%) and SPSG, respectively, while $V_{DOP}(80)$ represented 25% $\pm$ 18% (n = 11) and 147% $\pm$ 86% (n = 6) of DIP uptake in the NPSG (excluding Sta. 22 = 261%) and SPSG, respectively (Table 3).

Dissolved activity—Dissolved activity measured at saturating concentration of the substrate (i.e., dissolved APA

Table 3. Kinetic parameters of APA and values of several proxies scaling the kinetic parameters of APA to the uptake rates of DIP in surface samples from the North (HOT and POOB2 cruises) and South (BIOSOPE cruise) subtropical Pacific Ocean. V_m: the potential velocity in nmol of MUF-P hydrolyzed per liter and per hour (nmol L^{-1} h^{-1}); K_m: the half-saturation constant (nmol L^{-1}); V_{DOP}: potential hydrolysis rate of DOP at ambient concentrations, considering that the ambient DOP concentration is fully bioavailable (nmol L^{-1} h^{-1}); V_{DOP} : DIP uptake: the ratio of V_{DOP} to uptake rate of DIP (%); $V_{DOP}(80)$: DIP uptake: the ratio of the potential hydrolysis rate of DOP, considering that 80% of the DOP is fully bioavailable, to the uptake rate of DIP (%); $V_{DOP}(10)$: DIP uptake: the ratio of the potential hydrolysis rate of DOP, considering that 10% of the DOP is fully bioavailable, to the uptake rate of DIP (%); V_{DOP} : DIP: the ratio of V_{DOP} (nmol L^{-1} d^{-1}) to DIP concentration (nmol L^{-1}), that is, the DIP potentially released by APA in 24 h at ambient DOP concentrations expressed as a fraction of the ambient DIP pool (% d^{-1}).

Cruise	Sta.	Depth (m)	V_m (nmol L^{-1} h^{-1})	K_m (nmol L^{-1})	V_{DOP} (nmol L^{-1} h^{-1})	V_{DOP} : DIP uptake (%)	$V_{DOP}(80)$: DIP uptake (%)	$V_{DOP}(10)$: DIP uptake (%)	V_{DOP} : DIP (% d^{-1})
HOT-201	ALOHA	5	0.02	204	—	—	—	—	—
HOT-206	ALOHA	5	0.08	372	0.03	21	18	2	2.1
HOT-210	ALOHA	5	0.01	290	0.005	3*	3	0.4	0.1
HOT-211	ALOHA	5	0.02	136	0.01	9*	9	2	1.6
HOT-216	ALOHA	5	0.12	261	0.06	37*	32	5	1.7
POOB2	1	5	0.21	390	0.06	39	33	4	1.2
POOB2	3	5	0.13	462	0.03	32	27	3	2.4
POOB2	7	5	0.12	122	0.06	77	69	12	8.9
POOB2	11	5	0.11	343	0.03	32	27	4	0.8
POOB2	13	5	0.14	560	0.03	30	25	3	5.2
POOB2	20	5	0.06	316	0.02	14	12	2	0.4
POOB2	21	5	0.14	335	0.04	15	13	2	0.7
POOB2	22	5	3.12	538	0.72	312	261	32	63.2
BIOSOPE	MAR1	5	0.10	422	—	—	—	—	—
BIOSOPE	HNLC	5	0.12	432	0.05	24	21	3	0.4
BIOSOPE	STB1	10	0.15	334	0.06	13	11	2	0.5
BIOSOPE	STB2	15	0.61	583	0.20	100	86	12	2.3
BIOSOPE	STB3	15	0.39	313	0.18	204	180	28	2.3
BIOSOPE	STB4	15	0.17	259	0.08	99	87	14	0.8
BIOSOPE	STB5	20	0.12	271	0.06	143	127	21	0.9
BIOSOPE	STB6	25	0.08	330	0.03	92	79	11	0.5
BIOSOPE	STB7	20	0.09	310	0.04	187	162	23	0.7
BIOSOPE	STB8	30	0.11	255	0.05	269	234	34	0.9
BIOSOPE	GYRE	30	0.10	305	0.03	90	77	10	0.6
BIOSOPE	STB12	30	0.29	406	0.08	313	264	33	1.5
BIOSOPE	STB14	20	0.09	259	—	—	—	—	—
BIOSOPE	STB15	15	0.05	283	0.03	81	70	9	0.5
BIOSOPE	STB16	15	0.03	332	0.02	31	27	4	0.3
BIOSOPE	STB17	15	0.10	704	—	—	—	—	—
BIOSOPE	STB18	15	0.04	669	0.02	8	7	1	0.2
BIOSOPE	STB19	15	0.05	432	0.01	3	2	0.3	0.1
BIOSOPE	STB20	5	0.08	777	0.02	15	13	2	0.1
BIOSOPE	STB21	5	0.06	450	0.02	54	45	6	0.2
BIOSOPE	UPX	5	0.26	1255	0.02	5	4	1	0.1

* No DIP uptake data are available for these cruises, so the average value (average ± SD) of DIP uptake rates measured on 16 cruises made between March 2000 and November 2001 at Sta. ALOHA (K. M. Björkman unpubl. data) have been used to calculate the V_{DOP} : DIP uptake ratios.

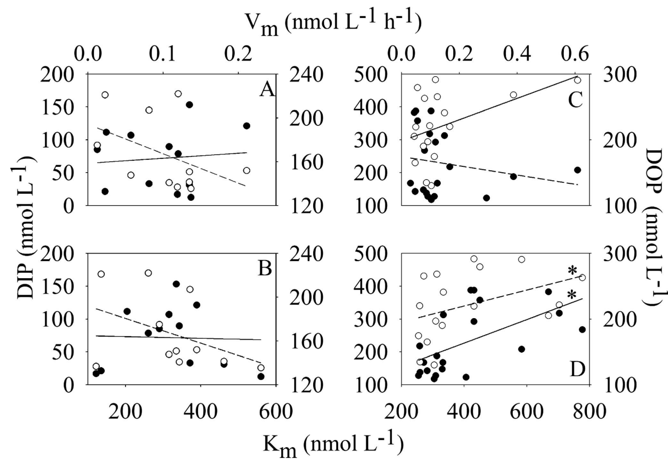

Fig. 3. Relationship between the kinetic parameters V_m and K_m of alkaline phosphatase activity and the pools of DIP (black circles) or DOP (open circles) (A, B) in the NPSG and (C, D) along the BIOSOPE transect. (A, C) Relationship between V_m (nmol L^{-1} h^{-1}) and DIP and DOP concentrations (nmol L^{-1}). (B, D) Relationship between K_m (nmol L^{-1}) and DIP and DOP concentrations (nmol L^{-1}). Significant relationships are identified by an asterisk.

V_m; Fig. 2 B) contributed between 12% and 100% (41% ± 23%, n = 23) of the total activity at Sta. ALOHA. The kinetic parameters of dissolved APA were assessed in surface samples (i.e., 5 m) during the POOB2 cruise (Table 4). At this depth, dissolved activity represented 6–30% of the total APA V_m with the lower value at Sta. 22. The V_m parameter varied between 0.02 and 0.17 nmol L^{-1} h^{-1} with a maximum at Sta. 22 (0.04 ± 0.02 nmol L^{-1} h^{-1}, n = 6, excluding Sta. 22). Dissolved APA at ambient DOP concentrations (V_{DOP}) represented between 4% and 30% of the uptake rate of DIP. K_m varied between 177 and 593 nmol L^{-1} (337 ± 150 nmol L^{-1}, n = 7), which is of the same magnitude as the measured DOP concentrations (Table 2).

Discussion

We measured relatively low APA throughout the year and the euphotic zone (V_m < 0.4 nmol L^{-1} h^{-1}) at Sta. ALOHA. *Prochlorococcus* and heterotrophic bacteria represented the majority (> 98%) of the total cell abundance throughout the euphotic layer at Sta. ALOHA. Using 3-O-methylfluorescein phosphate at 100 nmol L^{-1} Perry (1972) showed that in the NPSG (30–31°N and 136–147°W), APA varied between 1.14 and 7.80 nmol L^{-1} h^{-1} in surface samples when DIP was equal to or less than 30 nmol L^{-1}. These values are relatively high compared to our measurements, except for Sta. 22 and the stations located in the Chilean upwelling, and are typical of P-limited areas such as the eastern Mediterranean Sea (Van Wambeke et al. 2002) and the Sargasso Sea (Lomas et al. 2010) or of high-biomass systems dominated by eukaryotic phytoplankton (Labry et al. 2005; Dyhrman and Ruttenberg 2006). Karl et al. (2001*b*) showed that Chl *a* concentration and primary production in surface waters of the NPSG have more than doubled between 1967 and 1997, while the concentrations of dissolved silicate and phosphate have decreased. The authors hypothesized that these changes were due to the phylogenic "domain shift" from a phytoplankton population dominated by eukaryotic cells to one dominated by pigmented prokaryotes. Perry (1972) measured high APA when Sta. ALOHA was still dominated by eukaryotes. That may explain why Perry's (1972) APA were in the same range as the ones we measured at Sta. ALOHA only in blooming conditions (Duhamel et al. 2010 and Sta. 22 this study) or in the Chilean upwelling. Using MUF-P, Koike and Nagata (1997) measured rates of APA in the central Pacific that were in the same range as those reported in this study.

APA is often considered to be an indicator of P stress (i.e., physiological response to reduced P supply) or P limitation (i.e., production or growth limitation; Van Wambeke et al. 2002; Hoppe 2003; Dyhrman and Ruttenberg 2006) since DIP starvation significantly increases APA while excess DIP is thought to suppress APA (Chróst and Overbeck 1987; Tanaka et al. 2006; Duhamel et al. 2010). With low V_m and relatively high K_m throughout the euphotic zone, APA parameters at Sta. ALOHA are characteristic of non–DIP-limited environments. Nevertheless, the hydrolysis rates V_m and cell-abundance-specific V_m were always measurable and varied seasonally and with depth. They were higher throughout the euphotic zone in winter and at 75 m, where a decrease in DIP concentrations is often observed at Sta. ALOHA. However, as previously demonstrated for other marine habitats (Sebastián et al. 2004; Dyhrman and Ruttenberg 2006; Cao et al. 2010), these changes were not significantly correlated to variations in DIP and DOP concentrations or cell abundance (Figs. 2,

Table 4. Kinetic parameters of dissolved APA (i.e., in the < 0.2 μm as a fraction) in surface samples (~ 5 m) measured during the POOB2 cruise. V_m: the potential velocity in nmol of MUF-P hydrolyzed per liter and per hour (nmol L^{-1} h^{-1}); K_m: the half-saturation constant (nmol L^{-1}); dissolved APA V_m: the fraction of dissolved APA compared to the whole seawater APA (%); V_{DOP} : DIP uptake: the ratio of V_{DOP} (nmol L^{-1} h^{-1}) to the uptake rate of DIP (nmol L^{-1} h^{-1}).

	Sta.						
	1	7	11	13	20	21	22
V_m (nmol L^{-1} h^{-1})	0.08	0.03	0.02	0.04	0.02	0.03	0.17
K_m (nmol L^{-1})	451	593	229	403	279	177	230
Dissolved APA V_m (%)	30	24	16	28	27	15	6
V_{DOP} : DIP uptake (%)	13	7	8	11	5	4	30

3). Indeed, constitutive phosphatases have been found in many microorganisms, and their activity does not reflect the ambient pool of DIP (Hassan and Pratt 1977; Cembella et al. 1984; Hoppe 2003). Moreover, these enzymes could potentially hydrolyze organic compounds not only for P but also for carbon or nitrogen (Nicholson et al. 2006; Cao et al. 2010). In an upwelling system, Sebastián et al. (2004) found no response to phosphate amendments, which they interpreted as a greater importance of constitutive enzymes in DIP-rich habitats (Hassan and Pratt 1977) and/or as bacterial response to insufficient labile organic carbon (Chróst and Overbeck 1987; Hoppe 2003; Nicholson et al. 2006). Consequently, the presence of APA in the natural environment can have different ecological interpretations.

V_m and cell-abundance-specific V_m were still relatively high at 125 m at Sta. ALOHA, where DIP concentrations were higher (Fig. 2). Although these higher specific rates are also driven by lower cell abundances at 125 m (due mostly to lower *Prochlorococcus* abundance), they can explain part of the decrease in DOP and increase in DIP concentrations recorded at 125 m (Fig. 2E,F). Koike and Nagata (1997) also reported an increase of activity around 100–125 m at their subtropical station. In addition, we measured significant hydrolysis rates in the mesopelagic zone at Sta. ALOHA ($V_m = 0.03 \pm 0.02$ nmol L^{-1} h^{-1} at 1000 m), as previously observed in other areas of the ocean (Koike and Nagata 1997; Hoppe and Ullrich 1999; Tamburini et al. 2009). The presence of APA in high-DIP-concentration areas such as the mesopelagic zone or coastal upwellings can be due to the assimilation of P-ester compounds for growth and biosynthesis rather than P salvage per se.

The DOP pool contains a spectrum of various DOP compounds classes with different relative bioavailability, ranging from refractory material with slow turnover time (years to centuries) to labile material with fast turnover time (minutes to hours; Karl 2007*b*; Mather et al. 2008). The kinetic parameter K_m is a measure of the affinity of an enzyme for its substrate, and a low K_m value means high affinity (and strong binding) of the enzyme for its substrate. We found K_m values in the range of the total DOP concentrations measured in the NPSG and SPSG areas, meaning that the enzymes have a relatively low affinity for the DOP stocks found in these areas. This result also indicates that microorganisms are adapted to their environment and can adjust their uptake rates according to the availability of DOP. For example, the Pacific gyres are very stable environments that are believed to be supported by stochastic event phenomena, such as upwelled nutrient pulses (Karl and Letelier 2008; Johnson et al. 2010). With $K_m \geq$ DOP concentrations, microorganisms must be adapted to use pulsed AP-hydrolyzable DOP inputs. Moreover, high DOP concentrations are expected near microorganisms that are decomposing or excreting organic matter or near detrital particles or aggregates that are undergoing hydrolysis (Azam and Hodson 1981). With high K_m, microorganisms must be ready to benefit from such intermittent pulses of DOP. Mather et al. (2008) found very high K_m values in both the North and the South Atlantic Subtropical Gyres (789 ± 387 and 565 ± 114 μmol L^{-1}, respectively), while the DOP concentrations were in the range of those measured in the present study (80 ± 10 and 240 ± 10 nmol L^{-1} in the North and South Atlantic, respectively). Nevertheless, Mather et al. (2008) used very high MUF-P concentrations (i.e., 1–750 μmol L^{-1} substrate) compared to the in situ DOP concentrations (lower than 350 nmol L^{-1}) and long incubation time (i.e., 24–48 h), which could have led to the high estimate of the K_m parameter. Indeed, Azam and Hodson (1981) showed multiphasic kinetics for uptake of glucose. The authors demonstrated that V_m and K_m rose gradually as the range of glucose concentration was increased. Consequently, using high MUF-P concentrations, the authors measured APA of enzymes adapted to high substrate concentrations (Sebastián and Niell 2004; Nedoma et al. 2006). The interpretation of K_m should also be taken with caution since the affinity of an enzyme for a substrate is a compound-specific, not bulk, property and is the combined effect of different K_m of a consortium of enzymes. Since DOP is a mixture of various compounds, it would be relevant to test APA affinity from various organisms for various artificial (e.g., MUF-P, 6,8-di-fluoro-4-methylumbelliferyl phosphate) and natural (e.g., glucose-6-phosphate, adenosine monophosphate) substrates.

An approach to determine the potential in situ hydrolysis rate of the AP-hydrolyzable DOP is to calculate V_{DOP}. For comparison with the literature, we calculated V_{DOP} assuming that all the DOP is AP hydrolyzable. To scale the relative importance of this rate within the P demand, we compared V_{DOP} with the uptake rate of DIP in surface samples. Interestingly, V_{DOP} represented 3–77% (median 30%) of the uptake of DIP in the NPSG, while it represented $> 81\%$ of the uptake of DIP in the SPSG (median 139%). Nevertheless, only labile P esters are potentially hydrolyzable by alkaline phosphatase enzymes. Consequently, these estimates of the DOP hydrolysis rates (i.e., V_{DOP}) are overestimations. Using ultraviolet (UV) light–induced photodecomposition of TDP, Karl and Yanagi (1997) showed that UV-labile P (containing primarily monophosphate esters) represented half the TDP pool at Sta. ALOHA. More recently, using phosphorus nuclear magnetic resonance analysis on samples collected from a broad range of marine environments, Young and Ingall (2010) showed that 80–85% of the DOP pool is P esters. We thus calculated $V_{DOP}(80)$, which corresponds to V_{DOP} if 80% of the DOP is AP hydrolyzable. Since the fraction of labile P esters is unknown, $V_{DOP}(80)$ corresponds to an upper limit of the rates of DOP hydrolysis by APA. Using AP from bovine intestinal mucosa, Suzumura et al. (1998) showed that nonreactive DOP was a significant fraction (up to 67%) of the high-molecular-weight DOP. Consequently, the fraction of DOP that can be hydrolyzed by APA could be small. Using the method of Strickland and Parsons (1972) based on P release by adding *Escherichia coli* AP, Moutin et al. (2008) and Duhamel et al. (2009) found that the AP-hydrolyzable fraction of the DOP was below the detection limit of 20 nmol L^{-1} in the SPSG and NPSG, respectively. Nevertheless, this method can be criticized since in natural

environments, DOP is hydrolyzed by an enzyme consortium (not one enzyme from a single organism, here *E. coli*, biochemical properties of which might be very different from marine bacterial phosphatases), and, as discussed earlier, different enzymes will have singular specificities to different DOP compounds present in variable concentrations. In addition, different enzymes might work in synergy, explaining why the addition of several hydrolytic enzymes leads to higher DIP release from DOP than single enzyme additions (Feuillade and Dorioz 1992; Turner et al. 2002). Duhamel et al. (2010) argued that regardless of the standing stock, DOP is also produced during DIP transfer through the food webs (Orrett and Karl 1987), so the daily supply of labile DOP (including P esters) can exceed the ambient concentration at any given time. As a lower limit of the rates of DOP hydrolysis by APA, we calculated $V_{DOP}(10)$, which corresponds to V_{DOP} if 10% of the DOP is AP hydrolyzable. Even considering this lower limit, the APA would provide a significant fraction of DIP to fulfill P demand in the SPSG ($V_{DOP}[10]$: DIP uptake = 10–33% in surface). The fraction of DIP potentially released by APA was significantly larger in the SPSG compared to the NPSG ($V_{DOP}[10]$: DIP uptake = 0.4–12% in surface). This finding underlines the fact that the AP-hydrolyzable fraction of the DOP pool is more efficiently recycled in the central part of the SPSG than in the NPSG. This result is particularly interesting considering that the DIP concentrations were lower in the NPSG than in the SPSG, while DOP concentrations were higher in the NPSG. This is also illustrated by higher values of Chl *a*–specific V_m (i.e., the ratio of V_m of APA to Chl *a* concentration; data not shown) in the SPSG than in the NPSG. The microbial composition in the NPSG and SPSG were similar, with heterotrophic bacteria and *Prochlorococcus* representing the essential of picoplankton abundances, *Prochlorococcus* contributing to almost a third of total abundances, and *Synechococcus* and picophytoeukaryotes abundances being almost negligible (Grob et al. 2007; Fig. 2G,H). It would be interesting to combine molecular approaches (e.g., fluorescent in situ hybridization) to the single-cell ELF97 phosphate labeling technique in order to link microbial diversity to the actors of APA in both gyres. Such an approach might provide essential information in order to understand why the fraction of DIP potentially released by APA was significantly larger in the SPSG compared to the NPSG.

Microbial alkaline phosphatases can be released into the environment and contribute to the pool of "free enzymes" (Lazzaroni et al. 1985; Hoppe 2003). In the NPSG, we consistently measured relatively high dissolved (< 0.2 μm) activity (Fig. 2B; Table 4). In contrast, Perry (1972) did not detect APA in the filtrate (< 0.45 μm). This result can be due to lower sensitivity of the 3-O-methylfluorescein assay compared with MUF. Indeed, more recent studies also report high dissolved APA that could reach 100% of the total activity, for example, 42–74% in the northern Red Sea (Li et al. 1998), 13–44% in the Bay of Biscay (Labry et al. 2005), and 71–100% in the NPSG (Duhamel et al. 2010). These observations are supported by a metagenomic analysis that predicted that up to 30% of marine bacterial AP enzymes are extracellular (Luo et al. 2009). Dissolved APA represented a lower fraction of the total activity at Sta. 22, where the bloom was recorded. This result is consistent with previous findings showing that the fraction of dissolved APA decreased with increasing P limitation, total APA, and Chl *a* in nitrogen enrichment experiments (Duhamel et al. 2010). In cultures, it was shown that P-limited batch cultures of *Emiliania huxleyi* release less AP from the cell surface into the medium at exponential growth (Xu et al. 2006). Both in situ and culture observations showed that when cells are actively growing (e.g., when nitrogen limitation is relieved), the proportion of dissolved APA decreases. The pool of "free dissolved" phosphatases is still fairly unexplored, and the study of its origin and ecological role in aquatic ecosystems would be a significant area of future research (Hoppe 2003; Duhamel et al. 2010).

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