Limnol. Oceanogr., 48(3), 2003, 1049–1057

Bioavailability of dissolved organic phosphorus in the euphotic zone at Station ALOHA, North Pacific Subtropical Gyre

Karin M. Björkman[1] *and David M. Karl*
Department of Oceanography, University of Hawaii, Honolulu, Hawaii 96822

Abstract

The biologically available phosphorus (BAP) and soluble reactive phosphorus (SRP) concentrations and phosphorus (P) uptake rates within the euphotic zone (0–175 m) were measured on eight cruises between October 2000 and November 2001 to Sta. ALOHA (22.75°N, 158°W) in the North Pacific Subtropical Gyre. The SRP concentrations in the upper 100 m ranged from 7 to 84 nmol P L^{-1}, with a mean concentration of 41 nmol P L^{-1} (SE = 4, $n = 40$). The BAP pool consistently exceeded the SRP pool by factors of 1.4–2.8 in the upper 100 m, the additional P amounting to 7–15% of the dissolved organic P (DOP) pool, assuming that the measured SRP pool is fully bioavailable. Mean P uptake rates, based on SRP concentrations, ranged from 0.8 to 4.0 nmol P L^{-1} d^{-1}, with the highest rates in the surface waters decreasing with increasing depth. Mean P uptake rates that are based on BAP concentrations ranged from 0.6 to 8.0 nmol P L^{-1} d^{-1}, with a maximum at ~45 m, the depth corresponding to the highest adenosine-5′-triphosphate concentrations and highest DOP : SRP ratios. In the 125–175-m depth interval, SRP concentrations increased from 74 to 200 nmol P L^{-1} and DOP declined by an average of 70 nmol P L^{-1}. Because of the lower DOP concentrations at these depths, the contribution to BAP from the DOP pool increased to ~25%. These results indicate that the microbial community utilizes combined P compounds for their P nutrition simultaneously and, on average, uses them to the same extent as SRP in the upper water column. These results have important implications for P biogeochemistry in low–inorganic nutrient environments.

Phosphorus (P) is an essential element required for all living organisms. It is found in a range of biomolecules with a variety of cellular roles, from structural components in cell membranes, to cellular energetics by adenosine-5′-triphosphate (ATP)–adenosine diphosphate (ADP)–adenosine monophosphate (AMP) interconversions, to genetic information in nucleic acids (RNA, DNA). P is frequently the limiting nutrient for phytoplankton growth in freshwater ecosystems (Schindler 1977; Hecky and Kilham 1988; Hudson et al. 2000) and in many marine environments (Rivkin and Anderson 1997; Wu et al. 2000; Sañudo-Wilhelmy et al. 2001).

The North Pacific Subtropical Gyre (NPSG), has recently undergone a regime shift, believed to be the result of climatological changes over the past decades (Karl 1999). This ecosystem is currently characterized by a community based on prokaryotic primary production and an increasing dependency on nitrogen (N_2) fixation as a source of new N (Karl et al. 1997). Concomitant with the increase in available nitrogen (N) in the upper water column, a decrease in the soluble reactive phosphorus (SRP) pool has been observed over the last decade (Karl et al. 2001*a*,*b*). Additionally, the P in the particulate matter pool has declined in both absolute terms (Hebel and Karl 2001) and in relation to the carbon (C) and N content of the particulate material (Karl et al. 2001*b*), resulting in an ecological stoichiometry far from the traditional C : N : P Redfield ratio of 106 : 16 : 1 (Redfield et al. 1963).

Dissolved inorganic phosphate (DIP), considered to be the most readily available form of P to microorganisms, continues to decline in the upper water column in this environment (Karl et al. 2001*a*,*b*). Consequently, the cycling of DIP must be intensified or other sources for P exploited if the microbial community is to maintain its current production. Recent evidence from the NPSG has shown that primary production, instead of declining, appears to be higher today than was previously believed and could be the result of the abovementioned regime shift from predominantly eukaryotic primary producers to a prokaryote-based system (Karl 1999; Karl et al. 2001*a*).

The dissolved organic P (DOP) concentration in this environment has averaged 200–220 nmol P L^{-1} over the past decade in the upper 100 m of the water column (Karl and Björkman 2002). This constitutes ~75–80% of the total dissolved P (TDP) pool, and the DOP pool is frequently 5–10-fold larger than the measured SRP pool (Karl and Björkman 2002). Considering the large DOP pool relative to the DIP pool in the upper water column, the simultaneous utilization of this source of P should be important for the P nutrition of the extant microbial community.

In this study, eight cruises to Sta. ALOHA in the NPSG over a 13-month period, we investigated the P pool uptake dynamics, with specific emphasis on the biologically available P (BAP) pool, in the top 175 m of the water column. The BAP pool was compared to the analytically determined SRP pool, presumed to represent DIP, in order to assess the microbial community's potential dependency and utilization characteristics on combined P compounds relative to DIP.

Materials and methods

Sampling and station location—Samples for incubation experiments and nutrient analyses were collected at Sta. ALOHA (22°45′N, 158°00′W) during Hawaii Ocean Time-

[1] Corresponding author (bjorkman@hawaii.edu).

Acknowledgments

We thank the HOT program staff and the captain and crew of the R/V *Kaimikai-O-Kanaloa* for their support at sea. This research was supported by National Science Foundation grants OCE 96-17409 and 99-06820 awarded to D.M.K. This is SOEST contribution 6077 and US-JGOFS contribution 849.

series (HOT) cruises from October 2000 to November 2001 (HOT 119, 120, 125–128, 131, 132; $n = 8$).

Seawater samples were routinely collected from eight depths (5, 25, 45, 75, 100, 125, 150, and 175 m) with Niskin-type polyvinyl chloride bottles. Seawater was transferred into acid-cleaned, sample-rinsed 250-ml polycarbonate (PC) incubation bottles for P uptake experiments and 500-ml PC bottles for determination of BAP pool concentrations. Samples for SRP analysis were collected into 125-ml high-density polyethylene bottles. The SRP samples were tightly capped, frozen upright, and kept at −20°C until analyzed (Dore et al. 1996).

Phosphorus uptake rate experiments—Phosphorus uptake experiments were conducted with $^{32}PO_4$ as a radioactive tracer (orthophosphoric acid, carrier free; ICN Biomedicals, catalog #64014L). Duplicate PC bottles were incubated in the light at each depth. The incubations were typically labeled to a final ^{32}P activity of 75–150 kBq L^{-1}. The P uptake experimental bottles were incubated in situ at their respective collection depth from dawn to dusk on a free-floating array. After retrieval of the array, the samples were processed immediately. During the processing time, typically less than 2 h, the incubations were kept in the dark at 22–24°C. For total radioactivity determination, 1 ml of seawater was collected from each incubation bottle. A 100-ml portion of the remaining volume was filtered onto a polycarbonate membrane filter (Nuclepore, 0.2-μm pore size). The filters and samples for total activity were placed into borosilicate scintillation vials for liquid scintillation counting (LSC).

P uptake rates (nmol L^{-1} d^{-1}) were determined from the turnover time (T_t) of the radiotracer (T_t = total ^{32}P activity/^{32}P uptake rate) and the measured SRP and BAP pool concentrations (P uptake = [SRP or BAP]/T_t).

BAP pool measurements—Experiments to determine the BAP pool employed the technique developed by Karl and Bossard (1985). This method uses the specific $^{32}PO_4$ labeling of the terminal P group (γ-P) of the intracellular ATP pool, which at isotopic equilibrium equals the specific activity of the external available P pool (i.e., the γ-P–specific labeling reflects the isotope dilution effect from all available P compounds used by the microbial community; Fig. 1). The BAP pool size thus can be determined from the total radioactivity of the sample and the measured intracellular γ-P specific activity (Karl and Bossard 1985; Bossard and Karl 1986; Karl 1993). This determination will give a measure of the P pool that is as available as DIP and will not reflect processes that occur over timescales longer than the incubation period.

Briefly, the samples were spiked with $^{32}PO_4$ and incubated from dawn to dusk in on-deck incubators under three light levels and controlled temperature regimes. The entire content was then filtered through a Whatman GF/F filter and plunged into boiling Tris buffer for cellular ATP extraction (Holm-Hansen and Booth 1966). The extract was stored frozen until further processing, following procedures described elsewhere (Karl and Bossard 1985; Karl et al. 1987; Karl 1993). In short, the ATP extract was concentrated by vacuum evaporation and purified using polyethyleneimine (PEI) thin-layer chromatography. The isolated ^{32}P-ATP was eluted from the PEI matrix and selectively hydrolyzed with apyrase (Sigma Chemical, #A6132), and the terminal P was separated by activated charcoal extraction (Karl and Bossard 1985). The specific radioactivity (SA, nCi γ-^{32}P [pmol ATP]$^{-1}$) of the γ-P position of the intracellular ATP pool was used to calculate BAP pool concentration: BAP = (total ^{32}P activity in whole water)/SA.

The possible experimental outcomes are as follows. (1) $^{32}P/^{31}P$ of the γ-P of ATP is equal to the measured extracellular $^{32}P/^{31}SRP$. Conclusion: the SRP pool is equal to the BAP pool and P is not supplied from the DOP pool. (2) $^{32}P/^{31}P$ of the γ-P of ATP is less than $^{32}P/^{31}SRP$ but more than zero. Conclusion: P is supplied from both SRP and DOP simultaneously. (3) $^{32}P/^{31}SRP$ of the γ-P of ATP is more than $^{32}P/^{31}SRP$. Conclusion: The BAP pool is smaller than the measured SRP pool; that is, the measured SRP is not fully bioavailable.

Sample analysis—SRP concentrations were determined by the magnesium-induced coprecipitation (MAGIC) method (Karl and Tien 1992) followed by the molybdenum blue reaction described by Murphy and Riley (1962). All samples were corrected for arsenate interference (Johnson 1971), and the concentration was determined colorimetrically using a Beckman DU 630 spectrophotometer (λ = 880 nm). DOP concentrations were determined from the difference of the total dissolved P (TDP) and SRP concentrations. TDP concentrations were determined by the ultraviolet (UV) photooxidation method (Walsh 1989) and were part of the core measurements of the HOT program.

Radioactivity was determined using a Packard Tri-Carb® LSC. Aquasol II (Packard, #NEF 952) was used as the fluor and the samples were quench corrected using Packard instrument SIS protocols.

Water column particulate concentrations of ATP were determined by the firefly bioluminescence technique (Holm-Hansen and Booth 1966) with peak height analysis (Karl and Holm-Hansen 1978). Particulate P (PP) concentrations were part of the HOT core measurements. P inventories and uptake rates integrated over depth were calculated by the trapezoidal method. Information about HOTS core measurements and protocols are available on the World Wide Web at http://www.hahana.soest.hawaii.edu/hot/hot-dogs/interface.html.

Results

Dissolved phosphorus inventories in the upper water column—The SRP concentration in the upper water column (0–100 m) ranged over one order of magnitude among cruises, with a low of 7 ± 1 nmol P L^{-1} ($n = 2$) to 84 ± 2 nmol P L^{-1} ($n = 2$), with an average concentration of 41 nmol P L^{-1} (SE = 4 nmol P L^{-1}, $n = 40$). BAP pool concentrations exceeded the SRP concentrations in the top 100 m and ranged from <20 to 180 nmol P L^{-1} with a mean concentration of 74 nmol P L^{-1} (SE = 6 nmol P L^{-1}, $n = 40$). The DOP pool concentrations ranged from ~170 to 280 nmol P L^{-1}, with an average concentration of 268 (SE = 6 nmol P L^{-1}, $n = 40$; Table 1; Fig. 2). Below 125 m, the SRP concentrations increased with increasing depth at ~2.5 μmol

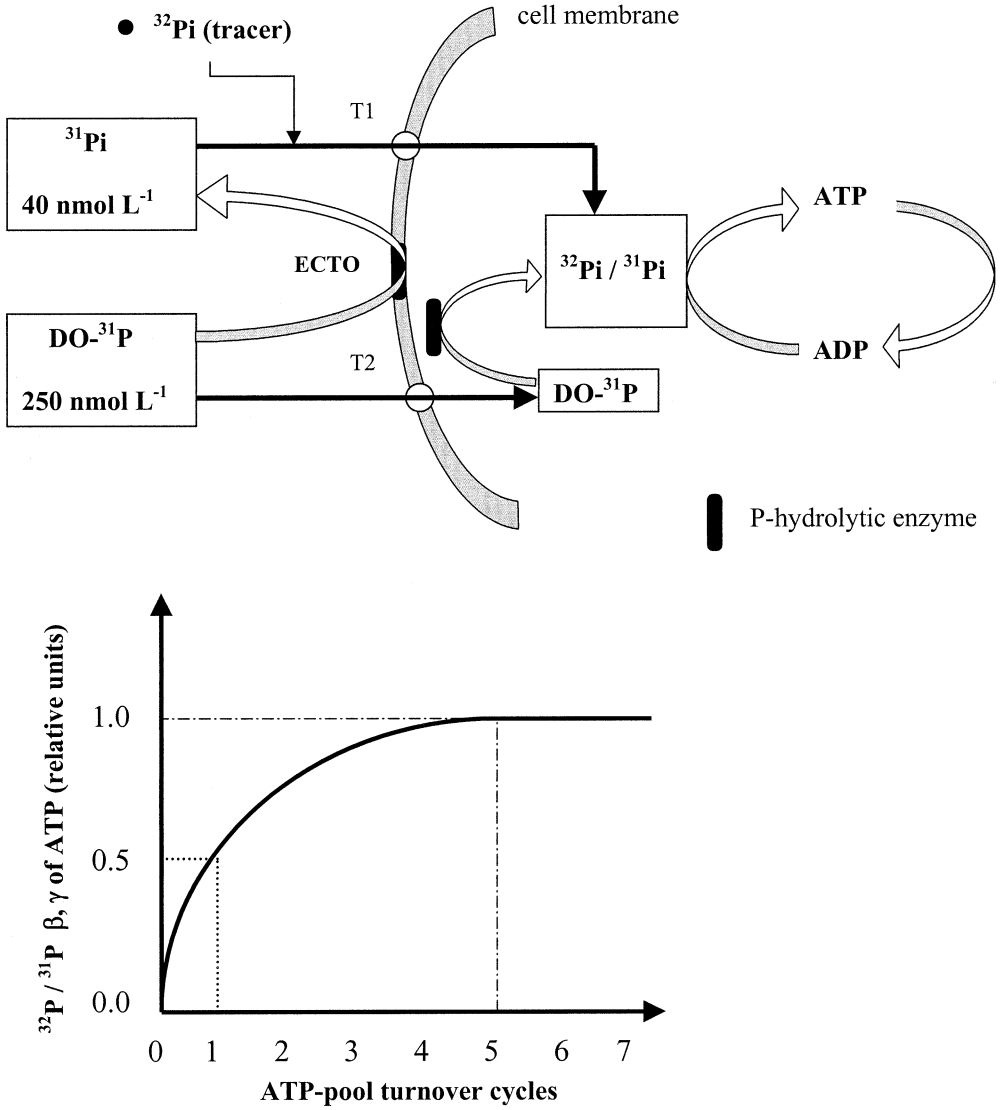

Fig. 1. Theoretical background to BAP pool determination by specific labeling of cellular ATP. (Top panel) The P is taken up via cross-membrane P transport systems (T1 for inorganic P (Pi), and T2 for DOP). DOP compounds can be hydrolyzed to Pi by membrane-associated ectoenzymes (ECTO), or if transported intact, by intracellular P hydrolases. Direct cross-membrane transport is known for a few DOP compounds; however, this pathway is probably minor relative to ectoenzyme hydrolysis of DOP prior to Pi uptake. The Pi will be incorporated into ATP by phosphorylation of ADP, which is a relatively rapid intracellular process (seconds to minutes). (Bottom panel) The dilution of the ^{32}P tracer into the available P pool is reflected in the ratio of ^{32}Pi/^{31}Pi of the terminal P (γ) of the intracellular ATP pool. Isotopic equilibrium is reached after approximately five turnover cycles of the intracellular ATP pool and is equal to the external ^{32}Pi/^{31}P available.

m^{-4}, and the DOP pool decreased by ~1.4 μmol m^{-4}. The molar ratio of DOP : SRP was highest in the upper water column, with a subsurface maximum between 45 and 75 m of 8 : 1, then declined with depth and approached a 1 : 1 ratio in the 150–175-m depth interval (Fig. 3). The BAP : SRP range was 1.4–2.3 : 1 in the top 75 m, with maxima corresponding to those of DOP : SRP, and the ratio declined with depth. At 175 m, the SRP pool was larger than the BAP pool (Fig. 3). Assuming that all of the measured SRP is bioavailable, the contribution to the BAP pool from DOP ranged from 7 to 15% of DOP pool inventories in the top 100 m and then increased to 25% at 125–150 m as the DOP pool concentrations decreased (Table 1). The BAP : SRP concentration ratios based on water column inventories also varied over time, with the highest ratios occurring when SRP inventories were <2 mmol P m^{-2} (integrated 0–75 m; Table 2) as seen in October 2000 and June 2001 (Fig. 4).

PP and ATP inventories—PP concentrations ranged from 8 to 25 nmol P L^{-1} in the top 100 m, with a mean concentration of 14 nmol P L^{-1} (SE = 0.8, n = 40). PP decreased with depth and, compared to the mean inventories in the top

Table 1. Water column inventories of soluble reactive phosphorus (SRP), biologically available phosphorus (BAP), dissolved organic phosphorus (DOP), particulate phosphorus (PP) and particulate adenosine-5′-triphosphate (P-ATP) at Sta. ALOHA.*

Depth (m)	SRP (nmol L^{-1})	BAP (nmol L^{-1})	DOP (nmol L^{-1})	PP (nmol L^{-1})	P-ATP (pmol L^{-1})
5	45(8)	64(10)	263(9)	15(2)	53(3)
25	45(8)	79(14)	251(13)	16(2)	68(3)
45	33(8)	76(16)	279(11)	16(2)	73(6)
75	31(7)	70(17)	257(12)	14(2)	72(3)
100	50(9)	80(17)	237(17)	12(1)	59(5)
125	74(11)	132(31)	242(22)	9(1)	48(7)
150	142(12)	193(44)	192(19)	5(1)	33(4)
175	200(17)	120(24)	172(22)	5(1)	24(4)

* Values are the mean concentrations with standard errors in parentheses ($n = 8$).

100 m, only a third remained at 150 m (Table 1; Fig. 5A). Particulate ATP concentrations ranged from 42 ± 2 to 103 ± 6 pmol ATP L^{-1}, with a mean concentration in the top 100 m of 65 pmol ATP L^{-1} (SE = 2.2, $n = 40$). Below 100 m, ATP concentrations declined on average by 0.5 nmol ATP m^{-4} (Table 1; Fig. 5B).

Phosphorus uptake rates and turnover times—Phosphorus uptake rates were based on the particulate ^{32}P activity retained on 0.2-μm PC filters and were highest in the upper (0–75 m) water column, declining with depth from the combined result of lower biomass and lower light levels (Table 3; Fig. 6). P uptake rates from the BAP pool measurements, when integrated over the top 175 m, were approximately twice the rates estimated when applying SRP pool concentrations.

BAP uptake rates, in general highest between 0–100 m, declined to rates similar to those for the SRP pool (Table 3; Fig. 6). The BAP uptake rates also showed a subsurface (45 m) maximum that was not present in the rates based on the

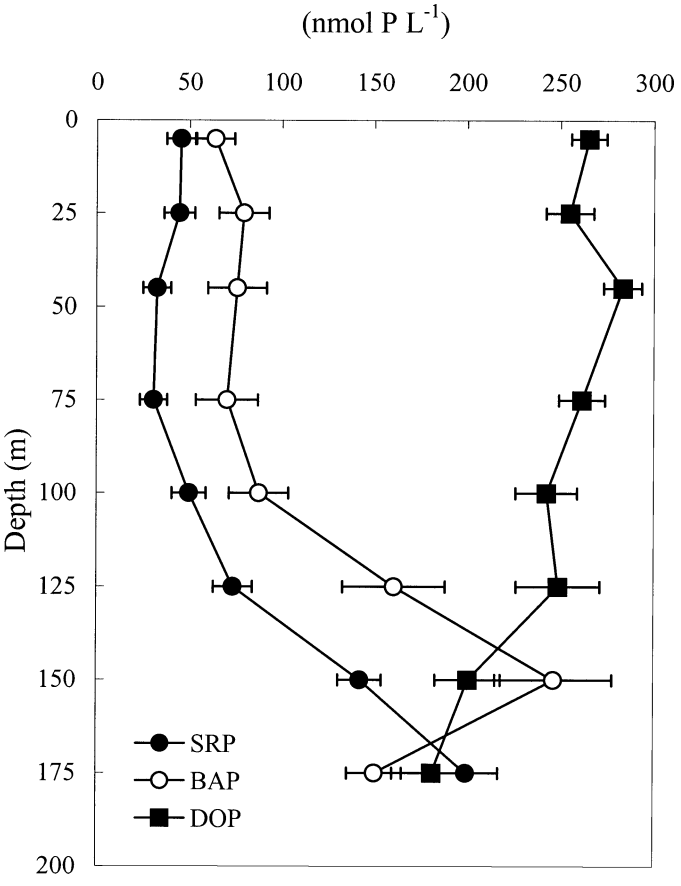

Fig. 2. Mean concentrations of three different dissolved P pools in the top 175 m of the water column at Sta. ALOHA. Data are from October 2000 to November 2001. Error bars are the standard errors of means ($n = 8$).

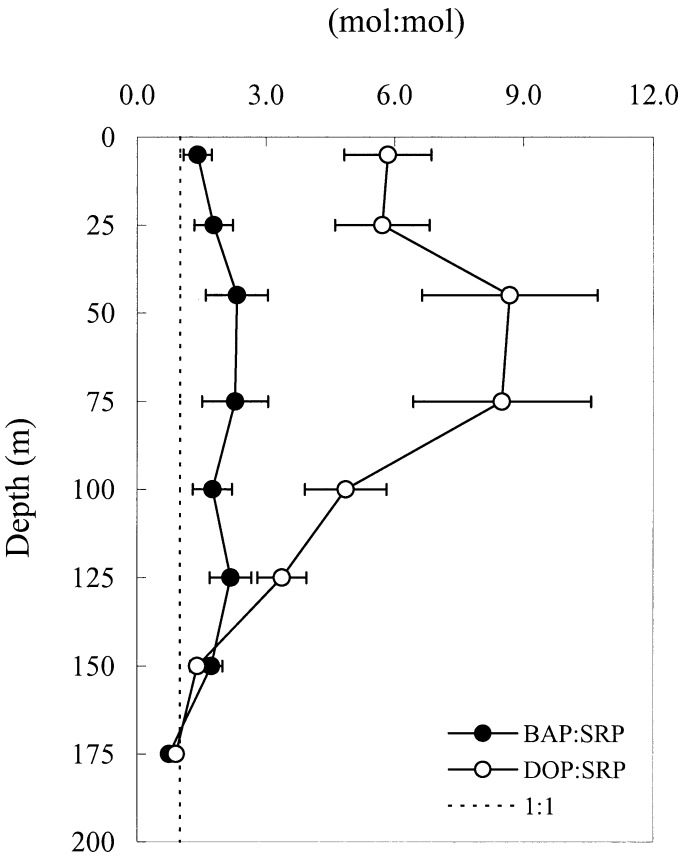

Fig. 3. The mean concentration ratios of BAP : SRP and DOP : SRP in the top 175 m of the water column at Sta. ALOHA. Error bars are the standard errors of the means ($n = 8$).

Table 2. Water column inventories from uptake rates of SRP, BAP, P-ATP, SRP, or BAP and from PP at Sta. ALOHA. Abbreviations as in Table 1.

Cruise	Date	SRP (mmol m^{-2})	BAP (mmol m^{-2})	P-ATP (μmol m^{-2})	SRP uptake (μmol P m^{-2} h^{-1})	BAP uptake (μmol P m^{-2} h^{-1})	PP (mmol m^{-2})
0–75 m							
HOT 119	Oct	0.95±0.04	2.89±0.24	4.8±0.3	7.2±0.3	21.6±0.8	0.96±0.02
HOT 120	Nov	3.96±0.22	6.86±0.56	4.8±0.3	8.7±1.1	15.2±1.3	0.84±0.04
HOT 125	Apr	5.61±0.04	10.62±0.76	5.0±0.2	11.1±0.6	20.9±1.5	0.87±0.04
HOT 126	May	2.56±0.01	4.87±0.20	4.3±0.2	9.6±0.2	18.3±0.3	1.32±0.03
HOT 127	Jun	1.83±0.02	5.22±0.20	4.6±0.4	9.9±0.1	30.9±0.2	0.77±0.01
HOT 128	Jul	3.33±0.13	7.16±0.44	5.5±0.4	12.6±0.4	28.3±0.7	1.11±0.02
HOT 131	Oct	1.48±0.15	2.35±0.14	6.2±0.4	13.2±1.4	24.9±3.2	1.64±0.01
HOT 132	Nov	3.07±0.03	4.02±0.16	5.2±0.6	9.7±0.6	12.7±0.9	1.56±0.01
Mean (SD)		2.85±1.50	5.50±2.68	5.1±0.6	10.2±2.0	21.6±6.2	1.13±0.33
075–175 m							
HOT 119	Oct	7.84±0.41	14.58±1.10	4.5±0.2	3.7±0.6	9.6±1.1	0.63±0.05
HOT 120	Nov	8.12±0.56	13.75±1.59	5.4±0.3	4.3±0.5	8.6±0.8	0.58±0.02
HOT 125	Apr	8.90±0.14	16.93±1.22	5.9±0.3	6.8±0.4	14.8±0.6	1.04±0.02
HOT 126	May	8.14±0.18	15.46±2.26	6.3±0.4	5.1±0.2	9.9±0.6	0.83±0.05
HOT 127	Jun	6.52±0.59	16.13±1.19	5.6±0.5	5.1±0.2	19.5±0.7	0.77±0.00
HOT 128	Jul	10.70±0.08	13.98±0.73	5.9±0.4	6.1±0.2	9.9±0.3	0.94±0.09
HOT 131	Oct	13.19±0.14	6.95±0.36	3.2±0.5	4.8±0.4	3.2±0.2	1.06±0.06
HOT 132	Nov	12.97±0.09	2.13±0.06	2.7±0.6	3.4±0.7	2.1±0.4	0.96±0.06
Mean (SD)		9.55±2.47	12.49±5.18	4.9±1.3	4.9±1.1	9.7±5.6	0.85±0.18

Values are integrated over 0–75 m and 75–175 m.

measured SRP concentration. There was a significant positive correlation between the mean BAP uptake rates and the mean DOP : SRP ratio ($r^2 = 0.9$, $p < 0.001$), as analyzed by Model II regression analysis, as well as with the mean ATP concentrations ($r^2 = 0.8$, $p < 0.01$) and DOP concentrations ($r^2 = 0.7$, $p < 0.01$). However, SRP uptake rates, although also significantly correlated with the above parameters ($p < 0.05$), were not significantly correlated to SRP concentrations.

The mean turnover time for the dissolved phosphate pool ranged from 10 to 14 d in the top 75 m, becoming increasingly longer with depth and increasing SRP inventories (Table 3). The turnover time of the PP pool, on the basis of the SRP or BAP uptake rates, ranged from approximately 4–10 days (SRP based rates) or from <2 to ~7 d for BAP-based rates, with the faster turnover times in the upper 75 m of the water column (Table 3).

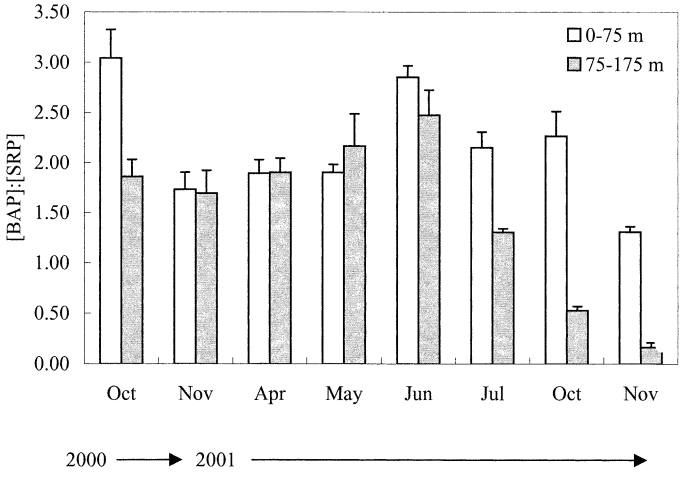

Fig. 4. Variation over time in BAP : SRP concentration ratios. Values are integrated over 0–75 m and 75–175 m. Error bars are ±1 SD.

Discussion

A challenging problem in marine ecology has long been to obtain accurate estimates of the fluxes of bioelements through the microbial community because they require knowledge of the bioavailability of the nutrient pool constituents. In oligotrophic open ocean ecosystems, much of the ambient N and P is in the dissolved organic nutrient pool; for P, the organic : inorganic concentration ratios typically exceed 5 : 1 in the upper water column at Sta. ALOHA (Karl and Tien 1992; Karl and Björkman 2002). Our knowledge to date of the chemical composition of the DOM pool remains limited, so there are no readily available methods to ascertain the bioavailability of the DOM pool.

This research problem has been approached in a variety of ways. In the case of P contained within the DOM pool, selective hydrolysis of natural DOP compounds with commercially available phosphohydrolytic enzymes (Kobori and Taga 1978; Suzumura et al. 1998) has been used to constrain the proportion of the DOP pool that is enzymatically hydrolyzable and, hence, potentially available to the microbial community. Others have investigated the community's natural enzymatic capability by means of artificial substrates, the hydrolysis of which can be selectively detected and quantified. Most commonly, these studies have used fluorochrome-tagged substrate proxies for alkaline phosphatase

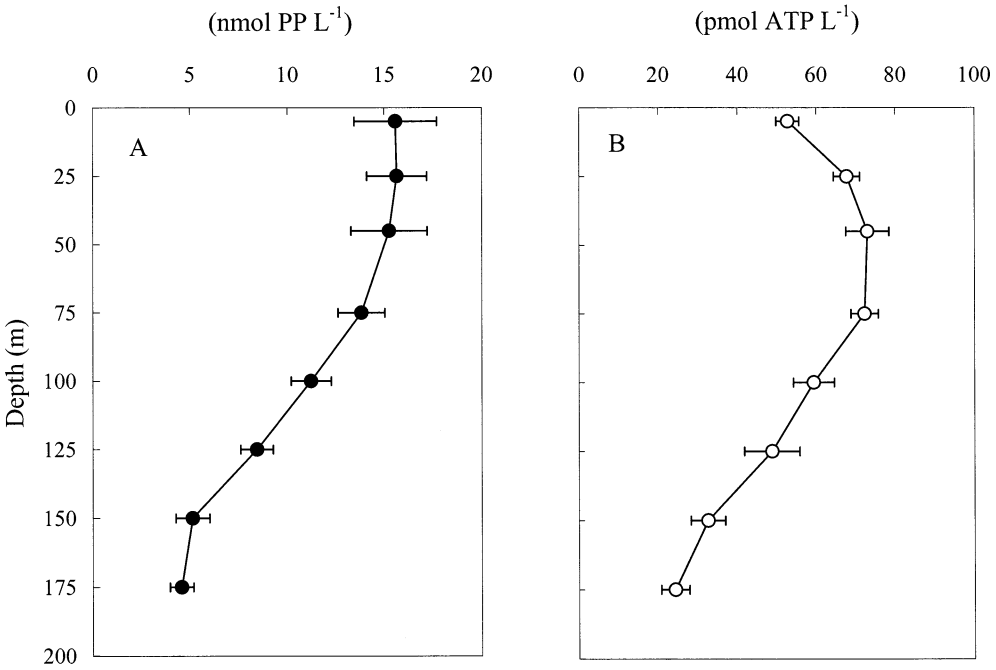

Fig. 5. Mean particulate P concentrations in the top 175 m of the water column at Sta. ALOHA. (A) Particulate phosphorus concentrations (PP). (B.) Particulate ATP concentrations. Error bars are the standard errors of the means ($n = 8$).

(APase) activity, indicating the microbial community's potential rate of hydrolysis of P esters (Perry 1972; Kobori and Taga 1978; Chróst and Overbeck 1987; Martinez and Azam 1993; Hernández et al. 1996). Because the test substrate is added at saturating concentrations, this approach yields only a potential rate of DOP hydrolysis—usually an upper constraint. The decomposition of nucleotides, presumably mediated by a bacterial enzyme 5′-nucleotidase, has also been assessed by the uptake of natural compounds such as ATP that can be measured at ambient pool concentrations (Ammerman and Azam 1985). Karl and Yanagi (1997) partially characterized the DOP pool by measuring its susceptibility to hydrolysis by low-energy UV radiation for seawater samples at Sta. ALOHA. They identified three major classes of P within the TDP pool on the basis of their photolytic properties. Clark et al. (1998) used ^{31}P-NMR to characterize in part the high–molecular weight DOP pool. Their results documented an unexpected presence of phosphonates (organic compounds containing a C-P bond) in addition to the anticipated P ester–linked (C-O-P) compound classes. However, it can be assumed that individual substrates within each of the major P classes revealed in these various studies, although chemically similar, might have very different bioavailabilities. Consequently, these approaches do not necessarily describe the activities of the extant microbial community and its hydrolysis rates of natural DOP at ambient concentrations or quantify to what extent the DOP pool is utilized.

The method employed in this study to assess the size of the BAP pool does not alter the system in terms of substrate additions significantly above the natural level (the tracer concentrations never exceeded 5 pmol P L^{-1}) or make any a priori assumption regarding the size of the enzymatically labile pool. Hence, it allows for a good approximation of

Table 3. Phosphorus uptake rates from SRP and BAP pool concentrations and the turnover time of the dissolved and particulate P pools. Abbreviations as in Table 1.

Depth (m)	SRP-based rate (nmol P L^{-1} d^{-1})	BAP-based rate (nmol P L^{-1} d^{-1})	Turnover time P pool (d)	Turnover time PP pool	
				SRP (d)	BAP (d)
5	4.0(0.4)	5.5(0.5)	12(2)	3.9(0.7)	2.8(0.7)
25	3.6(0.3)	7.3(1.0)	13(2)	4.3(0.5)	2.2(0.5)
45	3.2(0.2)	8.0(1.0)	10(2)	4.8(0.7)	1.9(0.4)
75	2.2(0.2)	6.1(1.3)	14(3)	6.3(0.8)	2.3(0.4)
100	1.4(0.2)	3.0(0.8)	44(13)	7.8(1.4)	3.7(0.8)
125	0.9(0.1)	1.9(0.4)	104(27)	10.2(1.6)	4.5(1.3)
150	0.8(0.2)	1.1(0.2)	184(18)	5.9(1.4)	4.7(1.2)
175	0.8(0.1)	0.6(0.2)	268(48)	5.4(1.0)	7.4(2.6)

Values are the mean rates, and standard errors in parentheses are ($n = 8$).

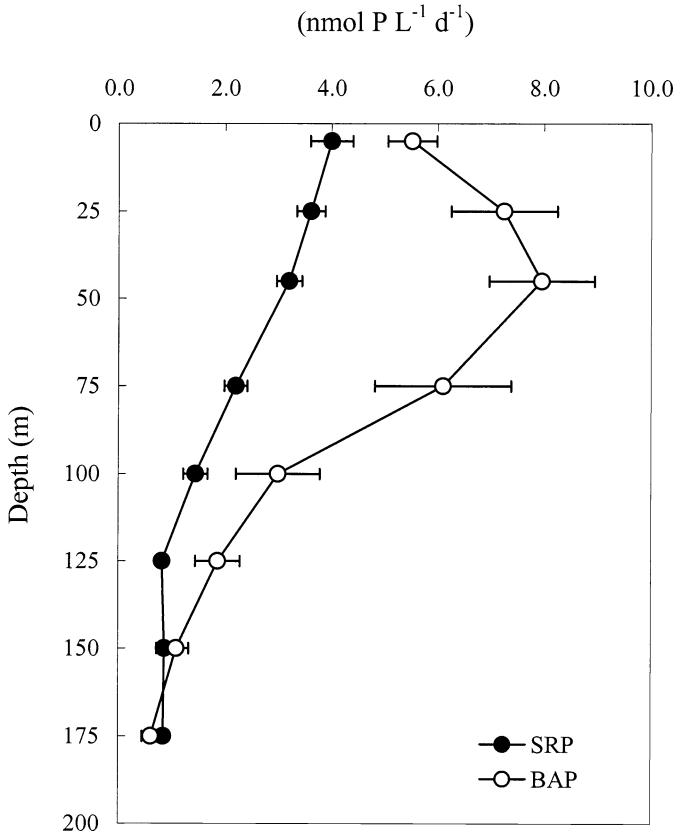

Fig. 6. Mean phosphorus uptake rates (nmol P L^{-1} d^{-1}) as measured by the turnover time (T_t) of the radiotracer $^{32}PO_4$ and the measured P pool concentrations (P uptake rate = [SRP or BAP]/T_t). Error bars are the standard errors of the means (n = 8).

the community's P utilization. The primary assumptions of the method employed here are that the $^{32}PO_4$ tracer, when assimilated into the cell, will dilute with all P compounds taken up regardless of whether it originated from the SRP or DOP pool. This isotope dilution effect is manifested in the specific activity of the γ-P of ATP, measured as described above, and is used to assess the overall BAP pool size. The method also assumes that particulate ATP is only associated with live metabolizing cells; no violation to this has yet been observed. A limitation to this approach is sensitivity. If the concentration of ATP is low (<10 pmol L^{-1}) or if the incorporation rates of ^{32}P into ATP are low, then the signal will be too low for a reliable determination of the size of the BAP pool and thus the BAP uptake rates for the procedures employed here. This occurred at 175 m on several occasions and during HOT 131 and 132 below 100 m. It also contributed to the <1 : 1 BAP : SRP concentration ratio observed at 175 m (Fig. 3), which in theory should not be possible unless a portion of the measured SRP pool is unavailable. However, the sensitivity can be increased, if desired, by using higher concentrations of ^{32}P, larger sample volumes, or both.

The determination of the size of the BAP pool is independent of the measured SRP concentrations. However, when calculating the respective contribution of SRP and DOP to the BAP pool, it is important for the interpretation of the results to remember that SRP is determined analytically and known to overestimate the DIP pool it is to represent (Thomson-Bulldis and Karl 1998). Additionally, DOP is derived by the difference between SRP and TDP; hence, it will depend on the accuracy of both methods. This means that the relative utilization of the DOP pool estimated from the measured BAP pool might change with the ability to determine DIP concentrations more accurately.

The BAP concentrations measured here were, on average, twice the measured SRP concentration in the upper portion of the euphotic zone. Provided that the SRP pool was fully available and representative of DIP, equimolar portions of the DOP and SRP pools are used simultaneously at all times at Sta. ALOHA. If the measured SRP contains P derived from DOP, then the proportional use of DOP would be even greater. Ratios of BAP : SRP ranging from 1–2 have previously been observed for surface waters in this region (Björkman et al. 2000), and Orrett and Karl (1987) employed the ATP-specific labeling technique and reported BAP : SRP concentration ratios in the North Pacific of 1.6–2.4 within the upper euphotic zone, thus falling within the same range observed in this study.

The simultaneous utilization of P from both SRP and DOP sources could indicate a resource partitioning that appears independent of P concentrations. Some microorganisms may be adapted for use of DOP, whereas others use DIP. This could produce the oscillations in DOP and SRP pools previously observed at Sta. ALOHA (Karl and Tien 1997) and seen in this study as well, depending on the relative contribution of the respective microbial groups. Because BAP supplies, on average, are turning over on timescales of 1–2 weeks in the upper water column, there should not be any immediate P limitation for the community as a whole. However, individual groups or species could well be P stressed, and several independent lines of evidence support this conclusion, including the P-depleted (relative to C and N) stoichiometry of dissolved and particulate matter, for example (Hebel and Karl 2001; Karl et al. 2001*b*). Attempts to separate functional groups by size fractionation have proven difficult in these environments because the majority of the microbial community, including the phototrophic component, passes through a 1-μm–pore size filter. Estimates of BAP concentrations in preincubation size-fractionated samples (<0.8 μm, <0.4 μm) compared to whole-water samples were almost identical (data not shown), indicating no clear separation of preferential utilization of SRP or DOP by any size-dependent component of the community.

Benitez-Nelson and Karl (2002) have shown, by analyzing the natural abundance of the $^{33}P/^{32}P$ ratios of dissolved and particulate P material, that during summer months, the microbial community relied more on recycled P, presumably through the utilization of DOP compounds, relative to winter months. This is consistent with the relative ratios of BAP : SRP inventories observed here, which were highest in months where the SRP inventory was <2 mmol m^{-2} (integrated over 0–75 m). The high ratio seen in October 2000 can be explained by the very low SRP inventory in the top 75 m (mean = 13 nmol P L^{-1}, SE = 1 nmol P L^{-1}, n = 8). There was also a strong positive correlation between DOP : SRP ratios and BAP uptake rates, indicating that as

SRP supplies become scarce in relation to DOP, a larger fraction of the DOP pool is utilized.

The correlation observed between ATP concentration and P uptake rates can be explained as a phenomenon related to biomass because ATP is a biomarker for living organisms, and it would be expected that higher biomass would result in an overall higher P consumption rate per volume sample, if not necessarily per unit biomass. The lack of correlation between SRP concentration and P uptake rate might appear counterintuitive in a P-limited system where the microorganisms presumably will respond with higher uptake rates as more SRP is introduced; however, the surprisingly uniform P uptake rates observed, relative to the fluctuations in the SRP concentration, indicate that the extant community is either incapable of capitalizing on new inorganic P, or a predominant part of their P demand is being met by DOP utilization. It also indicates that the present prokaryote-dominated system could be P replete at current growth rates, and the low ambient P concentration could be a selection factor against the establishment of large eukaryotic primary producers.

The portion of BAP uptake contributed from the DOP pool would correspond to a hydrolysis rate of 1.5–4.8 nmol P L^{-1} d^{-1}, which is similar to the range of DOP production rates measured in surface waters at Sta. ALOHA (Björkman et al. 2000). This could imply that the freshly produced DOP and the DOP contributing to the labile BAP pool are one and the same and are turning over on daily timescales. These compounds could include exudates or by-products of protozoan grazing, viral lysis, or autolytic processes. Compared to the ~1-d turnover time for these components, turnover time for the bulk DOP pool at these utilization rates would be in the range of 2–6 months. Because the DOP concentration in deeper, older waters is lower than in the euphotic zone, it is very likely that most of the marine DOP pool ultimately is bioavailable (Karl and Björkman 2002).

Church et al. (2002) reported that DOP inventories within the euphotic zone did not show any net change over the last decade, contrary to the observed net increases in both DOC and DON inventories in the NPSG. Furthermore, the molar ratios of C, N, and P of the dissolved pools are high, especially in C (~430 : 27 : 1; Church et al. 2002), relative to the C : N : P Redfield stoichiometry of 106 : 16 : 1 (Redfield et al. 1963), as are the bulk suspended particulate material (~160 : 26 : 1). The difference between particulate and dissolved pool stoichiometries would imply that DOP is selectively recycled relative to DOC compounds. However, it is important to bear in mind that the nomenclature of DOC, DON, and DOP is a misnomer of the chemical nature of these pools because there are compounds that simultaneously belong to two or even all categories (Karl and Björkman 2002). Strictly speaking, within the DOM pool, DOC, DOC-N, DOC-P, and DOC-N-P compounds are all represented. The selective recycling of P may well indicate a community limited by P relative to C and N and could in part help explain the increase in the DOC and DON inventories observed by Church et al. (2002).

The DOP utilization rates derived from the BAP pool were substantially lower than APase activities measured previously in this environment would imply (Perry 1972; 27–190 nmol L^{-1} P d^{-1}). Björkman et al. (2000) also showed that certain DOP compounds, when added in excess, could be hydrolyzed by the extant microbial community at rates 50 times or more of ambient P uptake rates. However, these hydrolysis rates are only indications of the community potential, rather than in situ rates. As such, P hydrolysis and uptake do not appear to be tightly coupled when highly bioavailable DOP compounds are available in excess. However, at ambient P concentrations, this coupling might be tighter. Furthermore, it has been demonstrated that certain DOP compounds, in particular nucleotides, appear to be highly bioavailable to the microbial community in these oligotrophic marine environments (Björkman and Karl 1994; Björkman et al. 2000). It is plausible that nucleotides have short turnover times and could be largely responsible for the additional P making up the BAP pool. Preliminary results suggest that the flux through the dissolved ATP pool might be five times more rapid than for bulk DOP and could contribute 5–10% of the assimilated P (Björkman 1999). This suggests that DOP utilization might be controlled by the chemical composition of the DOP pool and might ultimately determine its bioavailability.

It is necessary to know the flux of bioavailable P and how it varies in time and space to adequately understand the P dynamics in the oligotrophic NPSG, or in any aquatic system for that matter. The methodology employed here lends itself well to determining the BAP pool because it makes no assumptions about the chemical constituents or their relative contribution to this pool, nor what microorganisms are involved. Insight into P fluxes through the microbial community should also contribute to a better understanding of C flux in these systems.

References

AMMERMAN, J. W., AND F. AZAM. 1985. Bacterial 5′-nucleotidase in aquatic ecosystems: A novel mechanism of phosphorus regeneration. Science **227:** 1338–1340.

BENITEZ-NELSON, C. R., AND D. M. KARL. 2002. Phosphorus cycling in the North Pacific Gyre using cosmogenic ^{32}P and ^{33}P. Limnol. Oceanogr. **47:** 762–770.

BJÖRKMAN, K. 1999. Nutrient dynamics in the North Pacific Subtropical Gyre: Phosphorus fluxes in the upper oligotrophic ocean. Ph.D. thesis, Stockholm Univ. (ISBN 91-87272-73-3).

———, AND D. M. KARL. 1994. Bioavailability of inorganic and organic phosphorus compounds to natural assemblages of microorganisms in Hawaiian coastal waters. Mar. Ecol. Prog. Ser. **111:** 265–273.

———, A. L. THOMSON-BULLDIS, AND D. M. KARL. 2000. Phosphorus dynamics in the North Pacific Subtropical Gyre. Aquat. Microb. Ecol. **22:** 185–198.

BOSSARD, P., AND D. M. KARL. 1986. The direct measurement of ATP and adenine nucleotide pool turnover in microorganisms: A new method for environmental assessment of metabolism, energy flux and phosphorus dynamics. J. Plankton Res. **8:** 1–13.

CHRÓST, R. J., AND J. OVERBECK. 1987. Kinetics of alkaline phosphatase activity and phosphorus availability for phytoplankton and bacterioplankton on Lake Plußsee (North German Eutrophic Lake). Microb. Ecol. **13:** 229–248.

CHURCH, M. J., H. W. DUCKLOW, AND D. M. KARL. 2002. Multiyear increases in dissolved organic matter inventories at Station

ALOHA in the North Pacific Subtropical Gyre. Limnol. Oceanogr. **47:** 1–10.

CLARK, L. L., E. D. INGALL, AND R. BENNER. 1998. Marine phosphorus is selectively remineralized. Nature **393:** 426.

DORE, J. E., T. HOULIHAN, D. V. HEBEL, G. TIEN, L. TUPAS, AND D. M. KARL. 1996. Freezing as a method of sample preservation for the analysis of dissolved nutrients in seawater. Mar. Chem. **53:** 173–185.

HEBEL, D. V., AND D. M. KARL. 2001. Seasonal, interannual and decadal variations in particulate matter concentrations and composition in the subtropical North Pacific Ocean. Deep-Sea Res. II **48:** 1669–1696.

HECKY, R. E., AND P. KILHAM. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. Limnol. Oceanogr. **33:** 796–822.

HERNÁNDEZ, I., S.-J. HWANG, AND R. T. HEATH. 1996. Measurement of phosphomonoesterase activity with a radiolabelled glucose-6-phosphate. Role in the phosphorus requirement of phytoplankton and bacterioplankton in a temperate mesotrophic lake. Arch. Hydrobiol. **137:** 265–280.

HOLM-HANSEN, O., AND C. R. BOOTH. 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnol. Oceanogr. **11:** 510–519.

HUDSON, J. J., W. D. TAYLOR, AND D. W. SCHINDLER. 2000. Phosphate concentrations in lakes. Nature **406:** 54–56.

JOHNSON, D. L. 1971. Simultaneous determination of arsenate and phosphate in natural waters. Environ. Sci. Tech. **5:** 411–414.

KARL, D. M. 1993. Adenosine triphosphate (ATP) and total adenine nucleotide (TAN) pool turnover rates as measures of energy flux and specific growth rate in natural populations of microorganisms, p. 483–494. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], Handbook of methods in aquatic microbial ecology. Lewis Publishers.

———. 1999. A sea of change: Biogeochemical variability in the North Pacific Subtropical Gyre. Ecosystems **2:** 181–214.

———, AND K. M. BJÖRKMAN. 2002. Dynamics of DOP, p. 249–366. *In* D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter. Elsevier Science.

———, AND P. BOSSARD. 1985. Measurement and significance of ATP and adenine nucleotide pool turnover in microbial cells and environmental samples. J. Microbiol. Methods **3:** 125–139.

———, AND O. HOLM-HANSEN. 1978. Methodology and measurement of adenylate energy charge ratios in environmental samples. Mar. Biol. **48:** 185–197.

———, AND G. TIEN. 1992. MAGIC: A sensitive and precise method for measuring dissolved phosphorus in aquatic environments. Limnol. Oceanogr. **37:** 105–116.

———, AND ———. 1997. Temporal variability in dissolved phosphorus concentration in the subtropical North Pacific Ocean. Mar. Chem. **56:** 77–96.

———, AND K. YANAGI. 1997. Partial characterization of the dissolved organic phosphorus pool in the oligotrophic North Pacific Ocean. Limnol. Oceanogr. **42:** 1398–1405.

———, D. R. JONES, J. A. NOVITSKY, C. D. WINN, AND P. BOSSARD. 1987. Specific growth rates of natural microbial communities measured by adenine nucleotide pool turnover. J. Microbiol. Methods **6:** 221–235.

———, R. LETELIER, L. TUPAS, J. DORE, J. CHRISTIAN, AND D. HEBEL. 1997. The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. Nature **388:** 533–538.

———, R. R. BIDIGARE, AND R. M. LETELIER. 2001*a*. Long-term changes in plankton community structure and productivity in the subtropical North Pacific Ocean: The domain shift hypothesis. Deep-Sea Res. II **48:** 1449–1470.

———, AND OTHERS. 2001*b*. Ecological nitrogen-to-phosphorus stoichiometry at Station ALOHA. Deep-Sea Res. II **48:** 1529–1566.

KOBORI, H., AND N. TAGA. 1978. Phosphatase activity and its role in the mineralization of organic phosphorus in coastal waters. J. Exp. Mar. Biol. Ecol. **36:** 23–39.

MARTINEZ, J., AND F. AZAM. 1993. Periplasmic aminopeptidase and alkaline phosphatase activities in a marine bacterium: Implications for substrate processing in the sea. Mar. Ecol. Prog. Ser. **92:** 89–97.

MURPHY, J., AND J. P. RILEY. 1962. A modified single solution method for determination of phosphate in natural waters. Anal. Chim. Acta **27:** 31–36.

ORRETT, K., AND D. M. KARL. 1987. Dissolved organic phosphorus production in surface seawaters. Limnol. Oceanogr. **32:** 383–395.

PERRY, M. J. 1972. Alkaline phosphatase activity in subtropical central North Pacific waters using a sensitive fluorometric method. Mar. Biol. **15:** 113–119.

REDFIELD, A. C., B. H. KETCHUM, AND F. A. RICHARDS. 1963. The influence of organisms on the composition of seawater, p. 26–77. *In* M. N. Hill [ed.], The sea. Interscience.

RIVKIN, R. B., AND M. R. ANDERSON. 1997. Inorganic nutrient limitation of oceanic bacterioplankton. Limnol. Oceanogr. **42:** 730–740.

SAÑUDO-WILHELMY, S. A., AND OTHERS. 2001. Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean. Nature **411:** 66–69.

SCHINDLER, D. W. 1977. Evolution of phosphorus limitation in lakes. Science **195:** 260–262.

SUZUMURA, M., K. ISHIKAWA, AND H. OGAWA. 1998. Characterization of dissolved organic phosphorus in coastal seawater using ultrafiltration and phosphohydrolytic enzymes. Limnol. Oceanogr. **43:** 1553–1564.

THOMSON-BULLDIS, A., AND D. M. KARL. 1998. Application for a novel method of phosphorus determinations in the oligotrophic North Pacific Ocean. Limnol. Oceanogr. **43:** 1565–1577.

WALSH, T. W. 1989. Total dissolved nitrogen in seawater: A new-high-temperature combustion method and a comparison with photo-oxidation. Mar. Chem. **26:** 295–311.

WU, J., W. SUNDA, E. A. BOYLE, AND D. M. KARL. 2000. Phosphate depletion in the western North Atlantic Ocean. Science **289:** 759–762.

Received: 14 July 2002
Accepted: 12 December 2002
Amended: 30 December 2002