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Partial characterization of the dissolved organic phosphorus pool in the oligotrophic North Pacific Ocean

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Abstract

The combined application of two independent methods for phosphorus (P) determination in seawater, continuous-flow UV photodecomposition and magnesium-induced coprecipitation (MAGIC), was used to provide a partial characterization of the dissolved P pools at Sta. ALOHA (22°45′N, 158°W) in the oligotrophic subtropical North Pacific Ocean. Comprehensive laboratory analyses of the UV light-induced photodecomposition of a variety of specific organic P compounds dissolved in seawater confirmed that submicromolar concentrations of monophosphate compounds could be analytically separated from nucleotide di- and triphosphates based upon a previously described temperature-controlled low-pressure, mercury vapor UV irradiation treatment. When combined with a recently described high-precision P-detection system, the total dissolved phosphorus (TDP) in seawater could be reproducibly subdivided into three chemically distinct pools: soluble reactive phosphorus (SRP; presumably dominated by orthophosphate), UV-labile phosphorus ($P_{UV\text{-}L}$; containing primarily monophosphate esters), and UV-stable phosphorus ($P_{UV\text{-}S}$; containing primarily nucleotide di- and triphosphates, nucleic acids, and other compounds that are resistant to the UV treatment). Field application of these procedures to samples collected at Sta. ALOHA during the period September 1991 to March 1992 (HOT-30 to HOT-35) revealed the presence of all three operationally defined pools. In the upper portion of the water column (0–100 m) the TDP pool (29.26 ± 2.32 mmol P m^{-2}) contained, on average, 23% SRP, 26% $P_{UV\text{-}S}$, and 51% $P_{UV\text{-}L}$. With increasing depth, the concentration of $P_{UV\text{-}L}$ decreased while that of the $P_{UV\text{-}S}$ pool increased; the $P_{UV\text{-}L}$:$P_{UV\text{-}S}$ ratios decreased from values of 2–5 in the upper water column to ≤1.0 at 200 m.

Phosphorus (P) is an essential macronutrient for all living organisms. In the cell, P is incorporated into a broad spectrum of organic compounds with vital metabolic functions, including structure (membrane lipids), catabolism (sugars and fatty acids), energetics (nucleotides), and cellular coordination (nucleic acids). Under certain environmental conditions more exotic, otherwise nonessential P-containing compounds such as phosphonates, polyphosphates, regulatory nucleotides, and phosphorylated proteins can also be produced by selected groups of marine microorganisms. Consequently, through the normal processes of cell growth, exudation, and cell death by the combined contributions of autolysis, viral infection, and grazing there is a continual production of dissolved P compounds, especially in the upper portion of the world ocean (Orrett and Karl 1987).

In the sea, P growth requirements can be met by the uptake and assimilation of a variety of inorganic and organic P compounds, in addition to the most readily assimilated and favored orthophosphate (HPO_4^{2-}) ion. Because most phosphorylated organic compounds cannot be assimilated intact by microorganisms (although there are notable exceptions; e.g. Ruby et al. 1985), cell membrane-associated enzymes are responsible for facilitating uptake by a variety of coupled hydrolysis-transport mechanisms. Alkaline phosphatase (APase; EC 3.1.3.1) and other phosphohydrolyases present in both *Bacteria* (heterotrophic bacteria and cyanobacteria) and *Eucarya* (eucaryotic algae and zooplankton), and 5-nucleotidase (5NDase; EC 3.1.3.5) predominantly present in *Bacteria* are considered to be the two most important enzyme classes in aquatic environments (Kobori and Taga 1979; Karl and Craven 1980; Ammerman and Azam 1985; Jansson et al. 1988). These enzymatic reactions sustain the P cycle in seawater.

The quantitative analysis of dissolved P in seawater has traditionally relied on the spectrophotometric molybdenum blue assay method (Murphy and Riley 1962). As it is now routinely applied, a quantity termed "soluble reactive phosphorus" (SRP) is derived. SRP is a variable mixture of HPO_4^{2-} and P that is released from acid-labile inorganic and organic compound classes during the requisite acidification procedure. A second SRP determination, following either high-temperature persulfate digestion (Menzel and Corwin 1965), high-intensity ultraviolet (UV) photooxidation (Armstrong et al. 1966), or both (Ridal and Moore 1990), can be used to estimate TDP and, by difference, the magnitude of

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the "soluble nonreactive phosphorus" (SNP) pool. The SNP pool is sometimes referred to as dissolved organic phosphorus (DOP), even though it may contain pyrophosphate, inorganic polyphosphates, and other inorganic derivatives. Although several SNP compound classes have been reported to exist in seawater, including polyphosphates (Solórzano and Strickland 1968), nucleotides (Azam and Hodson 1977; Nawrocki and Karl 1989), nucleic acids (DeFlaun et al. 1986; Karl and Bailiff 1989), and monophosphate esters (Strickland and Solórzano 1966), the SNP pool in seawater remains largely uncharacterized. This uncertainty in TDP pool composition is a major impediment in our attempts to quantify P fluxes in the marine environment.

Recently, Yanagi et al. (1992) developed a method for the partial characterization of the SNP pool in seawater. Their analytical procedure provides an opportunity to distinguish among broad classes of P compounds on the basis of UV light-induced hydrolysis. We adapted this approach and in combination with the magnesium-induced coprecipitation (MAGIC) method described by Karl and Tien (1992) applied it to seawater samples collected at the U.S. JGOFS time-series Sta. ALOHA (22°45′N, 158°W; Karl and Lukas 1996). Our results provide evidence for the existence of at least two distinct pools of SNP compounds; a UV-labile ($P_{UV\text{-}L}$), monophosphate ester-enriched pool that dominates the upper water column (<100 m) and a UV-stable ($P_{UV\text{-}S}$) nucleotide-enriched pool that increases at depths greater than 100 m.

Materials and methods

Laboratory studies—Photodecomposition experiments. Twenty-three organic P compounds were tested in UV-photodecomposition trials (Table 1). All reagents were of (at least) analytical grade with a minimum assay purity of >98% (Sigma). Stock solutions, prepared in oceanic surface seawater were diluted further with surface water to provide test concentrations ~100–400 nM P above the surface seawater background of 200–250 nM total P. These solutions were irradiated with UV light using a continuous flow system described previously (Yanagi et al. 1992; *see Fig. 1*). In this procedure, seawater is pumped through a quartz column that is coiled around a cluster of three low-pressure mercury vapor lamps (110 W each, model UV-110, Sen Light). The reaction vessel was both air and water cooled to a temperature ≤40°C (Fig. 1). The exposure period was varied in preliminary experiments to establish the kinetics of photodecomposition of selected test compounds. Subsamples collected before and after UV irradiation were used to measure SRP and, by difference, to calculate the photolability of each test compound or unknown seawater sample.

P analyses. Several operationally defined P pools were determined in this study (Fig. 2). Seawaters collected before and after UV treatment were assayed for SRP using established protocols (Murphy and Riley 1962). The MAGIC preconcentration method (Karl and Tien 1992, 1997) was used to increase the sensitivity and the precision of the standard SRP analysis (Fig. 2). The SRP concentration difference, before and after a 20-min UV irradiation, is operationally defined as the UV-labile P pool ($P_{UV\text{-}L}$) and will be shown to consist, primarily, of monophosphate ester compounds. A separate subsample of seawater collected after the UV irradiation treatment is reacted with persulfate following the method of Menzel and Corwin (1965). This high temperature oxidation procedure quantitatively converts most combined P compounds to SRP (with the possible exception of compounds with C–P bonds). The SRP concentrations in these UV- and persulfate-treated samples are subsequently measured using the previously described preconcentration/colorimetric assay procedures. This determination is equivalent to the TDP concentration in the sample, and the difference between TDP and [SRP + $P_{UV\text{-}L}$] concentrations is a measure of the UV-stable P pool ($P_{UV\text{-}S}$). A commercially available certified reference solution (CSK standard phosphate solution, Wako Chemicals) was used as the calibration standard throughout this study. These four operationally defined pools (i.e. SRP, $P_{UV\text{-}L}$, $P_{UV\text{-}S}$, and TDP) provide the basis for a partial chemical characterization of the naturally occurring total dissolved P pool at Sta. ALOHA.

Analytical precision estimation. Several experiments were conducted to determine the precision of the SRP, [SRP + $P_{UV\text{-}L}$], and TDP pool determinations (*see Fig. 2*). Because the $P_{UV\text{-}L}$ and $P_{UV\text{-}S}$ pools are calculated (i.e. not directly measured), the precision of these determinations is obtained by the relationship $(A \pm a) - (B \pm b) = (A - B) \pm \sqrt{a^2 + b^2}$, where A and B are the mean values of [SRP + $P_{UV\text{-}L}$] and [SRP] for $P_{UV\text{-}L}$ pool estimation or [TDP] and [SRP + $P_{UV\text{-}L}$] for $P_{UV\text{-}S}$ pool estimation. The terms a and b are the respective error determinations for the measured values of A and B. The routine precision for the directly measured P pools are [SRP], 4.2% (±0.6%); [SRP + $P_{UV\text{-}L}$], 1.9% (±0.7%); and [TDP], 5.2% (±1.6%) based on numerous determinations over the course of this study. The higher uncertainty for the measurement of [SRP] compared to [SRP + $P_{UV\text{-}L}$] is due to the lower concentrations of the [SRP] pool. Accordingly, we estimate the precision of the derived pools, $P_{UV\text{-}L}$ and $P_{UV\text{-}S}$, to be ~2.5 and 2.7%, respectively, for the concentrations typically measured in this study (i.e. 50–100 nM for [SRP], 100–200 nM for [SRP + $P_{UV\text{-}L}$], and 250–350 nM for [TDP]).

A measure of the temporal reproducibility of organic P photodecomposition was also made by measuring the attribution of total dissolved P into $P_{UV\text{-}L}$ and $P_{UV\text{-}S}$ pools for two model compounds: adenosine-5′-triphosphate (ATP), a characteristic $P_{UV\text{-}S}$ compound, and glucose-1-phosphate (G-1-P), a characteristic $P_{UV\text{-}L}$ compound. The quantitative separation of P between the $P_{UV\text{-}L}$ and $P_{UV\text{-}S}$ pools for these standard compound solutions did not change over time. The total variability determined from the six independent sets of photolability experiments of the derived $P_{UV\text{-}L}$ pool (i.e. $P_{UV\text{-}L}$ = [SRP + $P_{UV\text{-}L}$] − [SRP]) was 2.6% for ATP and 3.4% for G-1-P, only slightly larger than the estimated 2.5% precision expected for a single set of determinations.

Field studies—Sample collection and storage. Seawater samples were collected at Sta. ALOHA (22°45′N, 158°W) during the period 18 September 1991–5 March 1992 during regular Hawaii Ocean Time-series (HOT) program cruises (HOT-30 to HOT-35; Karl and Lukas 1996). Seawaters were collected at predetermined depths (0–200 m) using PVC sampling bottles

Table 1. Photodecomposition of a variety of organic P compounds, measured as release of SRP following a 20-min exposure to UV light, as described in the text.

Compound*	P concn added (nM)	SRP released during UV treatment† (nM)	Photodecomposition (% of total P)	TDP after UV treatment† (nM)	Recovery (%)
Monophosphates					
AMP	250	232	92.8	249	99.6
dAMP	200	177	88.5	206	103.0
dGMP	200	159	79.5	160	80.0
dUMP	250	239	95.6	315	126.0
TMP	200	163	81.5	106	53.0
dCMP	200	175	87.5	220	110.0
Rib-P	200	194	97.0	200	100.0
Gl-1-P	100	92	92.0	104	104.0
Gl-6-P	200	193	96.5	178	89.0
Gal-6-P	200	185	92.5	159	80.0
F-6-P	200	164	82.0	188	94.0
F-1,6-P	340	318	93.5	357	105.0
PGA	200	171	85.5	212	106.0
PEP	200	200	100	266	133.0
Phos-tyro	200	183	91.5	178	89.0
PA	300	260	86.7	240	80.0
Mean			90.2		97.0
SD			±6.0		±19.1
n = 16					
Nucleotide di- and triphosphates					
ATP	300	64	21.3	285	95.0
dATP	300	68	22.7	405	135.0
dGTP	150	20	13.3	126	84.0
dGTP	300	17	5.7	243	81.0
ADP	400	136	34.0	452	113.0
dGDP	200	43	21.5	188	94.0
UDP	200	45	22.5	191	95.5
TDP	200	46	23.0	192	96.0
Mean			20.5		99.2
SD			±8.2		±17.3
n = 8					

* Abbreviations: AMP, adenosine-5′-monophosphate; dAMP, deoxyadenosine-5′-monophosphate; dGMP, deoxyguanosine-5′-monophosphate; dUMP, deoxyuridine-5′-monophosphate; TMP, thymidine monophosphate; dCMP, deoxycytidine monophosphate; ADP, adenosine-5′-diphosphate; dGDP, 2′-deoxyguanosine-5′-diphosphate; UDP, uridine-5′-diphosphate; TDP, thymidine diphosphate; ATP, adenosine-5′-triphosphate; dATP, deoxyadenosine-5′-triphosphate; dGTP, 2′-deoxyguanosine-5′-triphosphate; Rib-P, D-ribose monophosphate; Gl-1-P, glucose-1-phosphate; Gl-6-P, glucose-6-phosphate; Gal-6-P, galactose-6-phosphate; F-6-P, D-fructose-6-phosphate; F-1,6-P, D-fructose-1-6-diphosphate; PGA, 3-phosphoglyceric acid; Phos-tyro, *O*-phosphotyrosine; PEP, phosphoenol pyruvate; PA, phytic acid.

† Corrected for control surface seawater diluent samples, processed simultaneously.

with Teflon-coated springs mounted on a conductivity–temperature–depth (CTD)-rosette system. After recovery, subsamples were placed into 500-ml high-density polyethylene (HDPE) bottles and stored frozen for subsequent P pool characterization. Each bottle was washed with 1 M HCl, rinsed with deionized distilled water (DDW), and air dried prior to use. Seawater was subsampled directly, without filtration. Independent measurements of particulate P (>0.7 μm) pools at Sta. ALOHA during this interval demonstrated that they were small relative to the total dissolved pool (0–100 m depth-integrated particulate P pools were ≤2 mmol P m^{-2} compared to ~30 mmol P m^{-2} for the dissolved P pool) and can be ignored for the purposes of this study. The sample bottles were filled about two-thirds full and placed upright in a −20°C freezer to prevent the seawater from freezing out around the cap (Dore et al. 1996). We also evaluated the effect of sample storage on dissolved P pool characterization.

Results

Photodecomposition of organic P Compounds—Exposure of a variety of organic P compounds to UV light generated by the low-pressure mercury vapor lamps in a reproducible and controlled time and temperature regimen can be used to

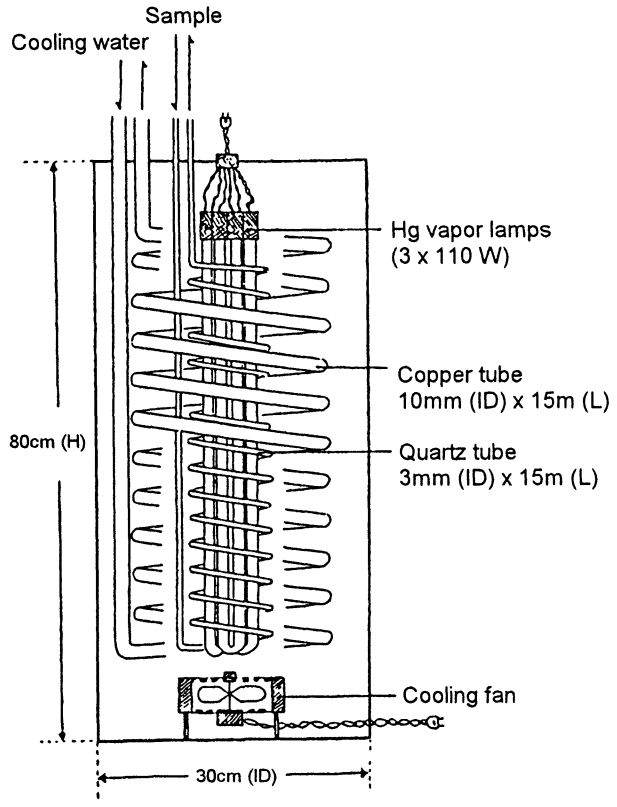


Fig. 1. Schematic diagram of apparatus used for partial UV photodecomposition of organic P compounds in seawater. The system includes a peristaltic pump, quartz flow cell, low-pressure mercury vapor lamps, and temperature controller device. Adapted from Yanagi et al. (1992).

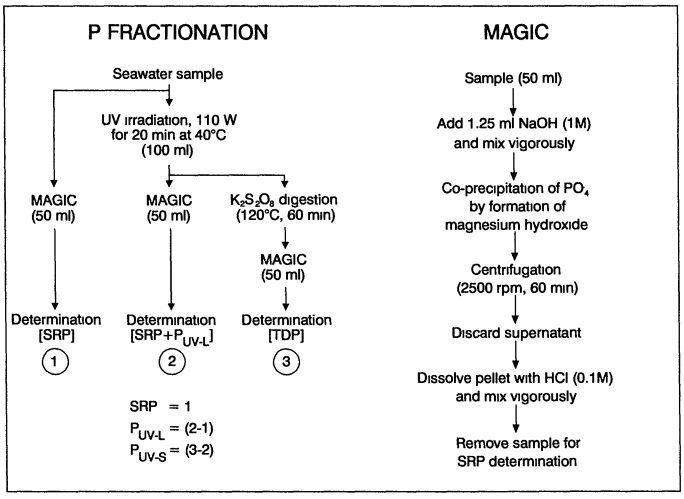


Fig. 2. Flowchart showing the analytical scheme used to estimate four operationally defined P pools: SRP, P_{UV-L}, P_{UV-S}, and TDP. The magnesium-induced coprecipitation (MAGIC) method was used to increase assay sensitivity and precision.

separate the test compounds into two unique categories based on photodecomposition properties (Fig. 3, Table 1). All test compounds containing ester-linked monophosphate moieties, regardless of whether the compound contained one or more PO_4 groups, were readily and nearly quantitatively (mean, 90.2 ± 6%) decomposed (i.e. SNP converted to SRP) during the 20-min UV irradiation period. This result was evident whether the monophosphate ester was a nucleotide (e.g. AMP), a sugar phosphate (e.g. Gl-1-P), a phosphorylated amino acid derivative (e.g. phosphotyrosine), or an organic polyphosphate (e.g. phytic acid). The photodecomposition process was relatively rapid (≤20 min; Fig. 3) and was independent of initial organic P concentration (Table 1; Yanagi et al. 1992). By comparison, nucleotide di- and triphosphate compounds were relatively stable to UV irradiation, even after extended exposure (Fig. 3). Pyrophosphate ($Na_4P_2O_7$) and tripolyphosphate ($Na_5P_3O_{10}$) behave as nucleotide polyphosphates with <5% photodecomposition even at relatively high (3 μM) concentrations (Yanagi et al. 1992). For the compounds tested, this operationally defined separation between these compound classes appears to be robust; photodecomposition of the UV-labile P compounds (P_{UV-L}) was 90.2 (±6.0)% effective compared to 20.5 (±8.2)% hydrolysis for the UV-stable P compounds (P_{UV-S}). Consequently, on the basis of this straightforward and reproducible analytical procedure, SNP compounds in seawater can be partially characterized as the P_{UV-L} or P_{UV-S} subcomponents of the TDP pool (Table 1).

Effects of sample storage on dissolved P-pool characterization—Prior to the application of this method for the partial characterization of dissolved P pools in nature, it was necessary to evaluate the effects of sample storage on the stability and subsequent photolability of TDP from the surface waters of the North Pacific Ocean. Photodecomposition experiments using Sta. ALOHA surface seawater were conducted by comparing fresh samples to replicates that were stored frozen for periods up to 45 d. Without exception, the measured attribution of dissolved P between SRP and P_{UV-L} was unaltered during storage (≤4% overall variability for

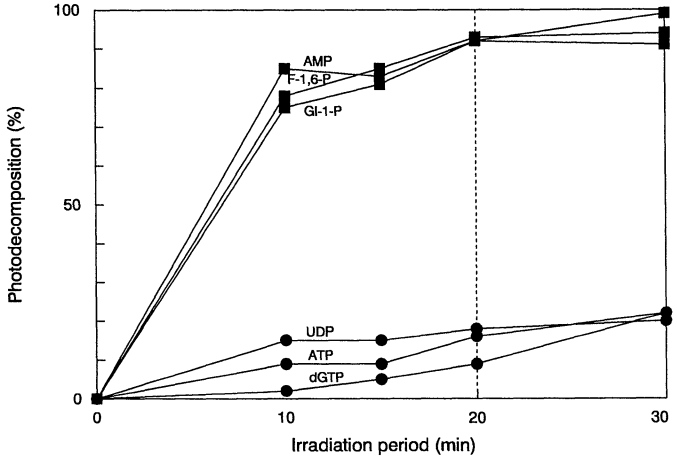


Fig. 3. Time course and extent of the photodecomposition of selected organic compounds using the flowthrough UV-irradiation system described in the materials and methods section. AMP, F-1, 6-P, and G-1-P are defined operationally as P_{UV-L} compounds, and UDP, ATP, and dGTP are examples of P_{UV-S} compounds. An irradiation period of 20 min was used throughout this study.

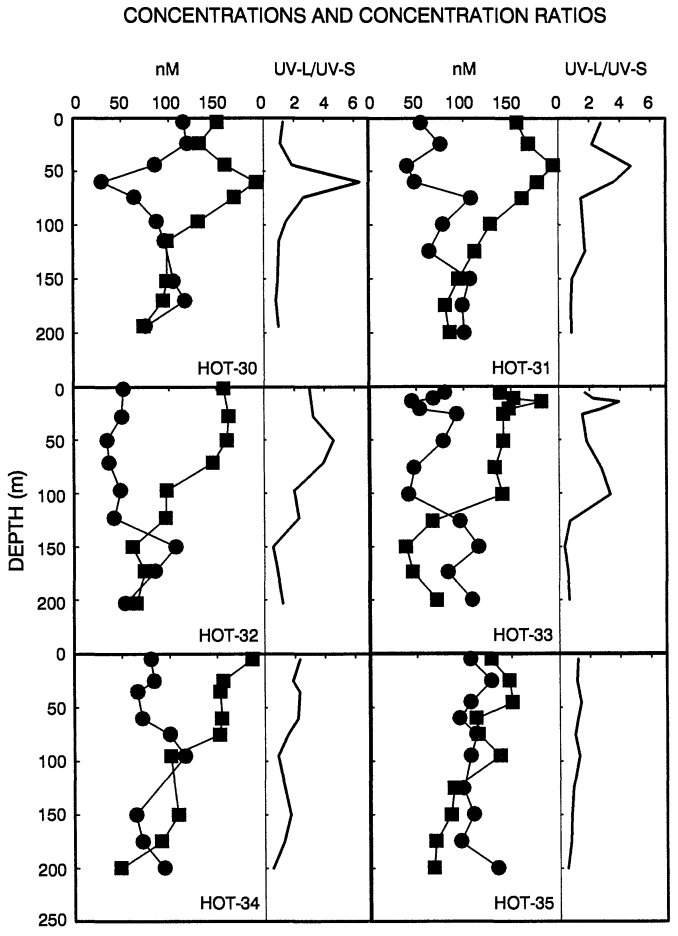


Fig. 4. Concentration vs. depth profiles for P_{UV-L} (■) and P_{UV-S} (●) pools at Sta. ALOHA for the period September 1991 (HOT-30) to March 1992 (HOT-35). The P_{UV-L}:P_{UV-S} concentration ratios are also shown for each cruise. The precision estimation for the P_{UV-L} and P_{UV-S} pool determinations is 2.5 and 2.7%, respectively.

six independent analysis trials each separated by ~1 week). These results indicate that both the complex mixture of organic P compounds in seawater (which is known to exceed SRP in our study area by at least a factor of three) must be well preserved by the frozen storage conditions used in this study. Furthermore, the constant values that we observed for the P_{UV-L} pool suggest that the photodecomposition procedures are not only reproducible with time, but that the chemical composition and reactivity of the original water samples, at least with respect to organic P compounds, are not affected by sample storage.

Dissolved P pools in the oligotrophic North Pacific—We used the described UV irradiation procedures to characterize TDP pools at the U.S. JGOFS North Pacific Ocean benchmark Sta. ALOHA. Samples were collected over a 6-month period from fall 1991 to spring 1992. Both P_{UV-L} and P_{UV-S} compound classes were detected on all six cruises and at all depths sampled (0–200 m). The concentrations of P_{UV-L} ranged from >150 nM P at the surface on HOT-34 (Feb 1992) to <50 nM P at 200 m (Fig. 4). On each cruise there was a distinct near-surface enrichment of P_{UV-L}, and on HOT-30 and HOT-31 (September and October 1991) there was a well-defined subsurface maximum at ~50 m (Fig. 4). By comparison, the concentration of P_{UV-S} generally ranged from 50 to 100 nM P in the upper portion of the water column and increased with depth to >100 nM at 200 m. These generally opposing concentration-vs.-depth trends for the P_{UV-L} and P_{UV-S} pools resulted in P_{UV-L}:P_{UV-S} concentration ratios from 2 to 5 in the upper 100 m, decreasing to near unity at 200 m (Fig. 4).

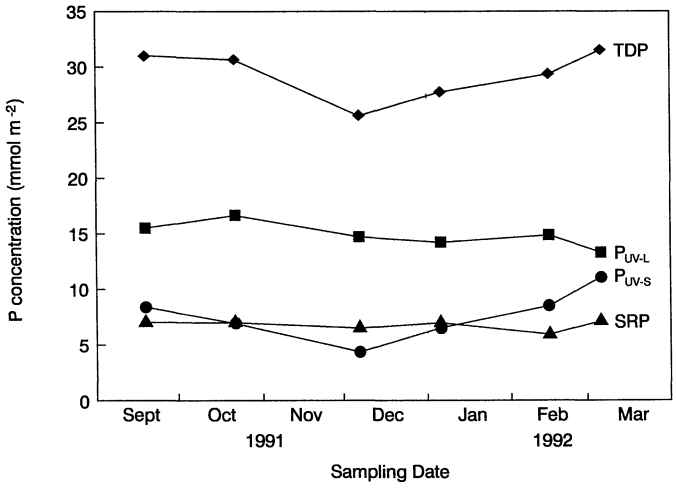


Fig. 5. Dissolved P pool inventories at Sta. ALOHA for the period September 1991–March 1992. Data shown are the 0–100-m depth-integrated P concentrations (mmol m^{-2}) for each of the pools identified: SRP (▲), P_{UV-L} (■), P_{UV-S} (●) and TDP (◆).

Previous analyses have determined that >90% of the total primary production at Sta. ALOHA occurs in the upper 100 m of the water column (Letelier et al. 1996). For this reason, we focused our attention on P pools and P dynamics in the upper euphotic zone. The 0–100-m depth-integrated inventories of all major P pools (SRP, P_{UV-L}, P_{UV-S}, TDP) remained remarkably constant during the 6-month period of observation (Fig. 5, Table 2). The most pronounced trend was for a slightly increasing trend in P_{UV-S} and a corresponding decrease in P_{UV-L}, especially in 1992 (Fig. 5). However, on average, the TDP pool in the upper 100 m of the water column (29.26 ± 2.32 mmol P m^{-2}) can be characterized as consisting of 23% SRP (6.78 ± 0.45 mmol P m^{-2}), 26% P_{UV-S} (7.65 ± 2.26 mmol P m^{-2}), and 51% P_{UV-L} (14.89 ± 1.14 mmol P m^{-2}) during the period of our observations (Table 2).

Discussion

Oligotrophic marine environments in general and the subtropical North Pacific Ocean in particular are unique habitats with regard to the distribution and dynamics of dissolved phosphorus. SRP, the presumed preferred substrate for the growth of microorganisms, represents <25% of the TDP pool. The remaining 75% of the TDP is poorly characterized and it is not apparent whether the relatively large steady-state SNP pool results from a rapid production rate, a slow pool turnover, or both. However, because vertical water col-

Table 2. Upper water column depth-integrated (0–100 m) P inventories at Sta. ALOHA.

HOT cruise No.	Date	SRP* (mmol m^{-2})	P_{UV-L} (mmol m^{-2})	P_{UV-S} (mmol m^{-2})	SNP† (mmol m^{-2})	TDP (mmol m^{-2})	SRP/TDP (×100%)	PP‡ (mmol m^{-2})
30	18 Sep 91	7.04	15.54	8.44	23.98	31.02	22.7	1.78
31	21 Oct 91	7.02	16.67	6.96	23.63	30.65	22.9	1.85
32	6 Dec 91	6.52	14.73	4.38	19.11	25.63	25.4	1.61
33	5 Jan 92	7.00	14.22	6.54	20.36	27.36	25.6	1.65
34	14 Feb 92	5.97	14.87	8.53	23.39	29.36	23.4	1.43
35	5 Mar 92	7.13	13.32	11.07	24.38	31.51	20.3	1.56
Mean		6.78	14.89	7.65	22.48	29.26	23.4	1.65
SD		±0.45	±1.14	±2.26	±2.19	±2.32	±1.96	±0.15

* Soluble reactive phosphorus.
† Soluble nonreactive phosphorus.
‡ Particulate phosphorus.

umn profiles often display elevated near-surface SNP concentrations, contemporary production is assumed.

Precise chemical characterization of the SNP pool has not yet been possible. Strickland and Parsons (1972) list five different classes of dissolved P in seawater that can be distinguished on the basis of reactivity with molybdate and ease of hydrolysis; however, none of these compound classes is structurally defined. Even the inorganic, soluble, and molybdate-reactive P (i.e. SRP) pool may be dominated by compounds other than free orthophosphate ions (Rigler 1968).

Quantitative studies of P pool dynamics in aquatic environments have been hampered by our inability to characterize the TDP pool. This leads to uncertainties in P pool turnover rate estimation and, therefore, P mass fluxes. Despite the availability of two convenient radioisotopic tracers of P (^{32}P and ^{33}P), the results from uptake experiments are also limited because of uncertain isotope dilution and lack of information on the biologically available pools of P in the habitats under investigation (Bossard and Karl 1986). The problem is especially acute in oligotrophic seawaters where uncharacterized SNP dominates the TDP pool.

Research conducted to date has focused on either a direct chemical identification of a generally small portion of the total SNP pool (e.g. ATP, inorganic polyphosphate, DNA) or an indirect characterization of multiple compound classes. P pool characterization has been made by gel chromatography molecular weight fractionation (e.g. Lean 1973; Downes and Paerl 1978) and by measuring SNP pool hydrolysis following the addition of diagnostic hydrolytic enzymes (Herbes et al. 1975; Solórzano 1978; Kobori and Taga 1979). The most commonly used enzyme in these studies, alkaline phosphatase (APase), has broad substrate specificity for monophosphate esters, including sugar-P, linear polyphosphates, and nucleotides (Strickland and Solórzano 1966). Although the database on monophosphate ester P concentrations in seawater is not extensive, independent studies of several coastal marine habitats concluded that APase hydrolyzes ~10–50% of the total SNP pool (Strickland and Solórzano 1966; Taft et al. 1977; Kobori and Taga 1979). Our results from the upper 100 m of the water column at Sta. ALOHA place the monophosphate ester (i.e. P_{UV-L}) pool at an average concentration of 14.89 (±1.14) mmol m^{-2}, a value equivalent to 66% of the SNP and 50% of the TDP pools, respectively.

The SNP pool at Sta. ALOHA exceeds the SRP pool by a factor of 3 and seems to have relatively constant proportions of 2.20 P_{UV-L} : 1.13 P_{UV-S} : 1.0 SRP over the period of investigation (Table 2, Fig. 5). This distribution of dissolved P compounds at Sta. ALOHA, including the relatively large pools of monophosphate esters (i.e. P_{UV-L}) and nucleotides (i.e. P_{UV-S}), implies that the presence of specific cell-associated enzymatic activities may ultimately control the bioavailability of the TDP pool. There are numerous enzymes involved in P transfer and, therefore, in TDP pool dynamics. Acid and alkaline phosphatase, phosphodiesterase, 5′-nucleotidase, nuclease, and ATPase are examples of the classes of enzymes presumed to be important in the marine P cycle. Presumptively, microorganisms growing in oligotrophic ecosystems would have the complement of enzymes required to assimilate the SNP compounds that are available. Phosphatases and nucleotidases are two groups of enzymes with potentially important ecological roles (Ammerman 1991; Thingstad et al. 1993).

Much of our perspective on the role of APase in P metabolism is based on the research performed using *Escherichia coli* as a laboratory model (Wanner 1987). Those results suggest that *E. coli* exhibits a physiologically regulated gene expression of APase in response to phosphate limitation. This induction of APase enables organisms to utilize a broad range of monophosphate ester compounds as a P source when orthophosphate is in limiting supply. The enzyme 5NDase catalyzes the hydrolysis of 5′-nucleotide monophosphates to inorganic phosphate and the corresponding nucleotide and may be an important first step in the nucleotide salvage pathways in microorganisms (Bengis-Garber 1985). Consequently, 5NDase may play an important role in P regeneration, especially from the P_{UV-S} pool. In the marine environment, 5NDase activity is usually localized in the picoplankton (0.2–1.0 μm) size fraction (Ammerman and Azam 1985, 1991), but it is also produced by marine eucaryotic algae (Flynn et al. 1986). Like APase, the 5NDase from marine bacteria has a fairly broad substrate specificity including ribonucleotide and deoxyribonucleotide mono-, di-, and triphosphates, pyrophosphate, glycerol phosphate, and *p*-nitrophenyl phosphate (*p*-NPP), with large variations in

Table 3. Dissolved P pool dynamics at Sta. ALOHA.

HOT cruise No.	Date	Primary production* (mmol C m^{-2} d^{-1})	PP export† (mmol P m^{-2} d^{-1})	P pool‡ turnover time (d)	P pool§ residence time (yr)
30	18 Sep 91	44.4	0.012	16–70	7.5
31	21 Oct 91	40.6	0.007	17–75	12.7
32	6 Dec 91	29.2	0.010	22–88	7.5
33	5 Jan 92	34.3	0.012	20–80	6.6
34	14 Feb 92	58.3	0.008	10–50	10.5
35	5 Mar 92	42.9	0.013	17–73	7.0

* 0–100 m depth-integrated rates calculated from 12-h (dawn-to-dusk) in situ ^{14}C-uptake experiments (Karl et al. 1996).

† Downward flux of particulate P measured using free-drifting sediment traps deployed at a reference depth of 150 m (Karl et al. 1996).

‡ Turnover time calculated using P uptake estimated from primary production, assuming a C:P molar ratio of 100. The minimum and maximum estimates are determined by assuming either SRP (minimum) or TDP (maximum) best approximates the bioavailable pool (*see Table 2*).

§ Residence time of P is calculated from the measured export flux, assuming a C:P molar ratio of 180 (Karl et al. 1996) and the total P pool inventory (TDP + PP; Table 2).

substrate specificity even among closely related species (Neu 1967; Bengis-Garber 1985). Because the TDP substrate pool at Sta. ALOHA seems to include a mixture of numerous compound classes (Table 2, Fig. 5), it is reasonable to assume that both APase and 5NDase activities, and perhaps other combined-P hydrolyzing enzymes, are essential for sustaining the P cycle in the surface waters of the subtropical North Pacific.

A more important and difficult question, that regarding P bioavailability of the various compound classes, was beyond the scope of this initial P characterization study. However, if we assume that the $P_{UV\text{-}L}$ and $P_{UV\text{-}S}$ pools are largely representative of monophosphate esters and nucleotides, respectively, then they should both be readily available to the microbial assemblage. Based on the estimated P requirements for photoautotrophic production (direct measurements of ^{14}C-HCO_3 uptake measurements and an assumed C:P molar ratio of 100:1) and the measured P export rates from sediment trap experiments, we calculate turnover times of 10–80 d for the P pool at Sta. ALOHA (Table 3). The fairly large range in these turnover time estimates is a result of uncertainties in the actual size of the bioavailable P pool. If the measured SRP pool is assumed to be equal to the bioavailable pool, then the P pool turnover times would be in the range of 10–20 d (Table 3), a value similar to that reported by Perry and Eppley (1982) for the central North Pacific gyre. From the measured P inventories and loss rates (downward particulate P fluxes), a P residence time can also be calculated. These values (3.4–7.5 yr) are consistent with estimates of the upward eddy diffusivity of SRP for this habitat. Other potential loss rates, including downward diffusion of SNP and the mesozooplankton "gut flux" (Longhurst and Harrison 1989), do not appear to be significant processes quantitatively in this region of the ocean (Christian et al. 1997). Alternatively, there may be important P-source terms in addition to the eddy-diffusive flux (e.g. atmospheric deposition, upward particle fluxes) that we have not included in this analysis.

From the P residence time estimations one must conclude that any major environmental change that affects nutrient dynamics might take nearly a decade to observe. Since 1988, repeated hydrological, chemical, and microbiological measurements have been made at Sta. ALOHA. On the basis of this time-series, Karl and colleagues (Karl et al. 1995; Karl and Tien 1997) reported what they thought to be a major change in biogeochemical processes, including a shift from a N-limited to a P-limited ecosystem. Fundamental to this change is a change in the frequency and duration of El Niño Southern Oscillation (ENSO) conditions and a resultant increase in the population dynamics of *Trichodesmium,* a potentially important N_2-fixing microorganism (Letelier and Karl 1996). The present 6-month study (HOT-30 to HOT-35) was conducted within this longer ocean time-series measurement program during the period of most intense ENSO forcing (Karl et al. 1995). Consequently, it may be difficult to extrapolate our results from Sta. ALOHA during September 1991 to March 1992 to much larger time or space scales. Finally, it is important to view the P cycle not as an independent set of reactions, but as part of the larger, integrated C-N-P-O biogeochemical dynamics of the ecosystem. By investigating the detailed mechanisms and controls of the P cycle we will gain a better understanding of the coupled N and C cycle processes as well.

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