Limnol. Oceanogr., 32(2), 1987, 383–395
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Dissolved organic phosphorus production in surface seawaters[1]

K. Orrett[2] *and D. M. Karl*
Department of Oceanography, University of Hawaii, Honolulu 96822

Abstract

Measurements were made of the accumulation of [^{32}P]DOP during seawater sample incubations with $^{32}PO_4^{3-}$. Accumulation rates (i.e. nCi [^{32}P]DOP liter^{-1} h^{-1}) were extrapolated to total DOP mass fluxes (i.e. ng P liter^{-1} h^{-1}) with two independent P-assimilation models. The first model assumed that the [^{32}P]DOP produced had the same specific radioactivity (i.e. nCi ^{32}P per ng P) as the total dissolved phosphorus pool measured at the beginning of the incubation period. The second model assumed that the [^{32}P]DOP produced had the same specific radioactivity as the P contained in recently synthesized RNA. Two North Pacific Ocean stations were investigated in detail. DOP production rates measured in mesotrophic waters off Manzanillo, Mexico, varied substantially with depth and time of day, whereas the production rates measured in the oligotrophic North Pacific gyre were more constant. DOP production rates, integrated over the depth of the euphotic zone, averaged 237 mg P m^{-2} d^{-1} for the mesotrophic environment and 31 for the oligotrophic. Estimates of DOC flux, extrapolated from the DOP production rates by assuming a C:P ratio of 9.5:1, suggest that a significant amount of the carbon fixed in oceanic environments may be recycled through soluble substrate pools.

Phosphorus is an essential macronutrient for all microorganisms, and in certain marine environments the availability of P may ultimately control organic production (Smith 1984). In the ocean, total dissolved phosphorus (TDP) is partitioned between a dissolved inorganic phosphorus pool (DIP; also referred to as soluble reactive phosphorus, SRP, or phosphate, PO_4^{3-}), and a dissolved organic phosphorus pool (DOP; also operationally referred to as soluble nonreactive phosphorus, SNRP). The steady state concentration of DOP in surface seawater often exceeds that of SRP, sometimes by an order of magnitude (Strickland and Austin 1960; Smith et al. 1985). Recent evidence, based on geochemical mass balance arguments, suggests that DOP is biochemically labile and is most likely available as a source of P for microbial metabolism (Jackson and Williams 1985; Smith et al. 1986). Nevertheless, we have only a limited understanding of the pathways of DOP production, the chemical composition and concentration of individual constituents of the total DOP pool, and the in situ rates of DOP turnover.

Most recent studies of P dynamics in the marine environment have used radioisotopic tracers (^{32}P and ^{33}P) to monitor the movement of P among the various particulate and dissolved pools (e.g. Harrison et al. 1977; Perry and Eppley 1981; Harrison 1983; Herbland 1984; Sorokin 1985; Harrison and Harris 1986). In general, the results of these investigations have revealed a rapid uptake of P by phytoplankton and bacteria and an equally rapid exchange (recycling) of P among various inorganic and organic compartments. Unfortunately it has been difficult to obtain accurate estimates of SRP or DOP mass flux from radiotracer experiments due to uncertainties in the absolute value of the specific radioactivity (radioactivity per unit mass) of the various precursor and product pools.

The present study was undertaken in the North Pacific Ocean as part of the vertical transport and exchange (VERTEX) experiment and was designed to estimate the rates of DOP production and recycling. The approach used was to measure rates of [^{32}P]DOP accumulation in surface seawaters after adding $^{32}PO_4^{3-}$. The resulting accumulation rates (nCi ^{32}P liter^{-1} h^{-1}) were converted to absolute fluxes (ng P liter^{-1} h^{-1}) with two different models of P assimilation.

We thank E. Laws, P. Bossard, S. Smith, G. Knauer, M. Pace, and D. Lean for their comments, criticism, and discussions of microbial P dynamics, the scientists and crew members of the RV *Wecoma* and RV *Cayuse* (VERTEX 3 and 4) for their co-opera-

[1] This research was supported by NSF grants OCE 82-16673 and PYI 83-51751 to D. M. Karl.
[2] Deceased.

tion and assistance in the field, and D. Burns for technical assistance.

Materials and methods

Phosphorus analyses—SRP analyses were performed on a Technicon AutoAnalyzer II with the procedure described by Strickland and Parsons (1972). TDP was determined with the hot persulfate oxidation method of Menzel and Corwin (1965). Dissolved organic phosphorus was taken as the difference between TDP and SRP concentrations.

Nucleic acid synthesis—Rates of RNA and DNA synthesis were measured as described by Karl (1981). The procedure was modified only by the simultaneous addition of $^{32}PO_4^{3-}$ (carrier-free, as $H_3{}^{32}PO_4$, New England Nuclear Corp., final activity 0.1 μCi ml^{-1}, in most cases) and [2-^{3}H]adenine (15.5 Ci mmol^{-1}, New England Nuclear Corp., final activity 0.1 μCi ml^{-1}, in most cases). The particulate material from 150–300-ml subsamples was collected onto Whatman GF/F filters and frozen for subsequent determination of ^{32}P and ^{3}H incorporation into RNA and DNA.

ATP and [^{3}H/^{32}P]ATP—ATP was measured by the firefly bioluminescence reaction (Karl and Craven 1980). Seawater samples of 150–500 ml were filtered through GF/F filters, and ATP was extracted from the collected particulate material by immersion of the filters into boiling 60 mM phosphate buffer (pH 7.4). The extracts were stored frozen for subsequent analysis. [^{3}H/^{32}P]ATP (as required for nucleic acid synthesis rate calculations) was purified with the PEI thin-layer chromatographic separation procedure described by Karl and Bossard (1985).

Isolation of [^{32}P]DOP—Microbial production of DOP was assessed with $^{32}PO_4^{3-}$ as a radioactive tracer. The resultant [^{32}P]DOP was purified and measured by two different procedures. Both analytical techniques require chemical complexation of residual inorganic phosphate ($^{32}PO_4^{3-}$) and subsequent removal of the bound complex, either by solvent extraction or by precipitation. The technique of [^{32}P]DIP-complex solvent (isobutanol) extraction is identical in principle to that described by Strickland and Parsons (1972) for measuring low concentrations of SRP. In our method, the blue phosphomolybdate complex (i.e. [^{32}P]DIP) is partitioned into isobutanol, with [^{32}P]DOP remaining in the aqueous phase. Additional details of the "isobutanol method," which was used in this study for the VERTEX 3 [^{32}P]DOP determinations off Manzanillo, Mexico, have been described by Kuenzler (1970) and Lemasson and Pages (1981).

For VERTEX 4 [^{32}P]DOP determinations in the central North Pacific Ocean, an alternative method of separating inorganic from organic phosphorus was used to expedite sample processing, minimize handling of ^{32}P, and minimize the possibility of hydrolysis of labile organophosphorus compounds which has been imputed frequently with the standard phosphate analysis reagents. This technique was originally described by Bochner and Ames (1982*a*) for use with bacterial cultures and was evaluated and adapted in our lab for oceanographic field studies.

The Bochner-Ames technique involves complexation of inorganic phosphate with tungsten and procaine. Under acidic conditions the [^{32}P]DIP complex is insoluble and can be separated quantitatively from [^{32}P]DOP by low-speed centrifugation. The Bochner-Ames reagent was prepared by combining 400 mM sodium tungstate, 500 mM tetraethylammonium hydrochloride, and 500 mM procaine in 5 : 4 : 1 proportions to yield final concentrations of 200 mM tungstate, 200 mM tetraethylammonium, and 50 mM procaine. Before precipitation, 1 ml of filtered seawater sample was removed for determination of total radioactivity by liquid scintillation counting. Duplicate 5-ml portions of each sample filtrate were transferred to 15-ml polystyrene culture tubes, chilled to 2°C, and acidified with 11 M formic acid to a final concentration of 1 M (~pH 2). Bochner-Ames reagent (0.5 ml) was immediately added to each tube and the tubes were shaken vigorously. A white precipitate formed instantly. The samples were centrifuged at 4,000 × *g* in a refrigerated centrifuge (4°C) for 7–10 min. Without removing the supernatant or disrupting the pellet, a second 0.5-ml volume of Bochner-Ames reagent was added to each tube, and the above centrifugation step was

Table 1. Outline of the various methods available to estimate the specific radioactivity of the P contained in recently synthesized DOP in oligotrophic seawaters.

Method of data analysis	Assumptions	Calculation of [^{32}P]DOP specific activity
1. SRP model	$^{32}PO_4^{3-}$ mixes with SRP pool Newly formed [^{32}P]DOP has a specific radioactivity equivalent to SRP pool	$\frac{^{32}PO_4^{3-}\text{ (nCi added liter}^{-1})}{\text{SRP (nmol liter}^{-1})}$
2. TDP model	$^{32}PO_4^{3-}$ mixes with TDP pool Newly formed [^{32}P]DOP has a specific radioactivity equivalent to TDP pool	$\frac{^{32}PO_4^{3-}\text{ (nCi added liter}^{-1})}{\text{TDP (nmol liter}^{-1})}$
3. RNA model	Specific radioactivity of the P in cellular RNA reflects ^{32}P isotope dilution by all extracellular P pools Newly formed [^{32}P]DOP has a specific radioactivity equivalent to that of the P contained in nascent RNA	$\frac{[^{32}\text{P]RNA (nCi added liter}^{-1})}{\text{total P in RNA (nmol liter}^{-1})}$
4. ATP model	Specific radioactivity of the γ-positioned P in cellular ATP reflects ^{32}P isotope by all extracellular and intracellular P pools Newly formed [^{32}P]DOP has a specific radioactivity equivalent to that of the γ-P in ATP	$\frac{[^{32}\text{P]ATP in }\gamma\text{-P (nCi liter}^{-1})}{\text{ATP (nmol liter}^{-1})}$

repeated. This two-step precipitation procedure resulted in the complete sedimentation of the white precipitate, leaving a clear supernatant. A portion of this solution was removed for determination of [^{32}P]DOP by either Cerenkov or liquid scintillation counting. For each set of samples, several $^{32}PO_4^{3-}$ standards were prepared in 0.2 μm filtered seawater to evaluate the efficacy of the DIP precipitation procedure. Under the reaction conditions specified, [^{32}P]-labeled nucleic acids are quantitatively removed with the [^{32}P]DIP fraction; hence, the [^{32}P]DOP isolated by the Bochner-Ames procedure probably underestimates the actual DOP flux.

Metabolic models—When [^{32}P]DOP accumulation rates are measured (e.g. nCi ^{32}P liter^{-1} h^{-1}), the specific radioactivity [i.e. nCi ^{32}P (ng P)$^{-1}$] of the product pool(s) must be known in order to calculate absolute flux rates (e.g. ng P liter^{-1} h^{-1}). Since the specific radioactivity of the DOP produced is neither known nor easily measured directly, one must rely on certain assumptions to extrapolate nCi ^{32}P to ng P. We considered four independent metabolic models for P assimilation in surface seawaters (Table 1).

The first model assumes that SRP is the only readily available source of P for microorganisms in the sea and, consequently, the [^{32}P]DOP produced during the incubation period would have a specific radioactivity [nCi ^{32}P (ng P)$^{-1}$] equivalent to that calculated for the SRP pool. Although this assumption may be true for water samples where SRP is the dominant portion ($\leq$90%) of the TDP pool, we feel that other P pools are also important in most surface seawaters, especially where the concentration of DOP exceeds that measured for SRP. Both bacteria and algae are capable of assimilating DOP and probably do so under in situ conditions. Data presented herein will demonstrate that DOP is actively consumed by microorganisms in the oligotrophic North Pacific Ocean.

The second model assumes that both SRP and DOP are consumed by marine microorganisms and that the [^{32}P]DOP produced during the incubation period has the same specific radioactivity as the TDP pool. It is questionable whether all P-containing organic compounds are equally available for assimilation, but there is compelling evidence, both short-term microbiological and long-term geochemical (Jackson and Williams 1985; Smith et al. 1985; Smith et al. 1986), that the DOP pool in the surface waters of the ocean is biogeochemically la-

bile. For these reasons, we will accept the assumptions of this model and will adopt it as one of the two independent methods we will use to calculate DOP production rates from the measurements of [^{32}P]DOP accumulation. This method of ^{32}P assimilation and extrapolation is subsequently referred to as the "TDP specific radioactivity model."

The remaining two metabolic models (Table 1) both rely on direct measurement of the specific radioactivity [nCi ^{32}P (ng P)$^{-1}$] of individual cellular constituents which are expected to reflect isotope dilution of the added $^{32}PO_4^{3-}$ by all potential extracellular and intracellular P pools. In the RNA model we have selected ribonucleotide bases as representative organic compounds for labeling cellular P. The specific radioactivity of nascent RNA is measured directly and this value is taken to represent an average for the entire DOP pool. RNA bases were selected, in part, because they comprise a major reservoir of phosphorus in microbial cells under normal growth (i.e. nonpolyphosphate forming) conditions and because the rate of total microbial RNA synthesis can be quantified by measuring [^{3}H]adenine incorporation, thereby providing the additional data required to make this extrapolation from [^{32}P]DOP (nCi) production to DOP (ng) flux. From double-labeled ([^{3}H]adenine and $^{32}PO_4^{3-}$) incubations, the rate of RNA synthesis (Karl 1981) and the accumulation of ^{32}P into RNA and DOP were measured. If we assume that adenine is 25 mol% of total microbial RNA (an assumption well supported by literature values: Pace 1973), the specific radioactivity of P in the recently synthesized RNA (i.e. nCi ^{32}P per ng, or nmol, P) and, hence, absolute P flux can be calculated. This method was also adopted for the calculations presented here and is subsequently referred to as the "RNA specific radioactivity model."

The final method, referred to as the "ATP model," is somewhat analogous to the RNA model but assumes that the terminal phosphate group of ATP (i.e. the γ-positioned P), rather than the P contained in RNA bases, will accurately reflect isotope dilution from all extracellular and intracellular sources. Two important advantages of measuring the specific radioactivity of the γ-P of ATP are that the γ-P of ATP turns over very rapidly (Chapman and Atkinson 1977) and consequently is expected to achieve isotopic equilibrium in periods of several minutes and that there is compelling evidence from past literature on microbial metabolism that the γ-positioned P of ATP is likely the precursor for most P-containing organic compounds of the cell. Unfortunately, this superior method was not completely developed at the time of our field study. Consequently, we present only a few preliminary data on application of the ATP model to estimate the concentration of biologically available phosphorus (BAP: Bossard and Karl 1986) in near-surface waters of the VERTEX 4 station (*see below*).

Field investigations—DOP production experiments were conducted during the VERTEX 3 and 4 sediment trap study. VERTEX 3 was conducted in mesotrophic waters off the west coast of Mexico (15°40′N, 107°30′W) from 27 October to 17 November 1982; VERTEX 4 was conducted in the oligotrophic waters of the central North Pacific Ocean (27°59′N, 154°59′W) from 11 to 23 August 1983. Surface water samples (0–150 m) were collected with standard 10-liter Niskin bottles. Each water sample was prescreened through 90-μm Nitex mesh to remove large zooplankton, transferred to 2.5-liter polycarbonate bottles, and incubated with [^{3}H]adenine and $^{32}PO_4^{3-}$ each at final radioactivities of 0.1 μCi ml^{-1}. The samples were either returned to their original depth (in situ) or incubated in a constant temperature on-deck incubator, which was covered with neutral density shade cloth and blue filters (Rohm-Hass 2609) designed to match the in situ light intensity and spectral irradiance characteristics, respectively.

Results

Development and evaluation of methods—Application of the isobutanol technique consistently yielded an average of 99.35–99.57% removal of $^{32}PO_4^{3-}$ from 0.2-μm filter-sterilized seawater (range of individual determinations was 99.0–99.7%: Orrett 1984). Criticism that the standard phosphate analysis reagents overestimate PO_4^{3-} levels due to hydrolysis of labile or-

ganic compounds (Rigler 1968) also applies to the [^{32}P]DOP extraction technique used here. The potential for nonenzymatic hydrolysis of DOP was tested with the presumably heterogeneous [^{32}P]DOP produced by a cutrophic freshwater community of microorganisms. No difference in total [^{32}P]DOP activity was observed between 1 and 30 min of exposure to the molybdate reagents before washing with isobutanol.

The procaine/tungstate/tetraethylammonium precipitation of phosphate was tested to determine the conditions for optimal efficiency in seawater medium. Removal of $^{32}PO_4^{3-}$ was maximal in samples acidified to a final concentration of 1 M formic acid (pH ~2) with a 0.1-volume addition of 11 M formic acid. Two consecutive additions of the Bochner-Ames reagent to each sample, each followed by centrifugation, increased the efficiency of $^{32}PO_4^{3-}$ precipitation. Removal of $^{32}PO_4^{3-}$ internal standards by the two-step Bochner-Ames procedure was typically 99.4% efficient and varied by no more than 0.2% among separate experiments. The isobutanol and Bochner-Ames techniques were compared with a [^{32}P]DOP sample collected from a freshwater microbial community. The mean [^{32}P]DOP values estimated by the two independent purification procedures were not significantly different ($P > 0.05$, t-test for paired samples).

A field experiment was done to evaluate whether the [^{32}P]DOP we observed was actually due to the passage of small cells through our GF/F filters. Duplicate samples were analyzed for [^{32}P]DOP in GF/F (nominal pore-size 0.7 μm) and GS (pore-size 0.22 μm) filtrates (Fig. 1). A t-test for paired samples indicates that there was no significant difference ($P > 0.05$) between the two procedures. GF/F filters have been found to retain particles smaller than the nominal porosity, including 50% of particles of 0.4-μm size (Sheldon 1972).

VERTEX 3—At the VERTEX 3 site, vertical profiles of SRP and DOP were measured at two different times on the same day (Fig. 2). The ratio of DOP:SRP exceeded 1.0 in the surface waters and decreased with increasing water depth to a value of 0.1 at 100 m. SRP concentrations ranged from about 0.2–2.5 μm and DOP from about 0.02–0.8. These data are typical of oceanic profiles measured at other Pacific Ocean stations (Jackson and Williams 1985; Smith et al. 1986).

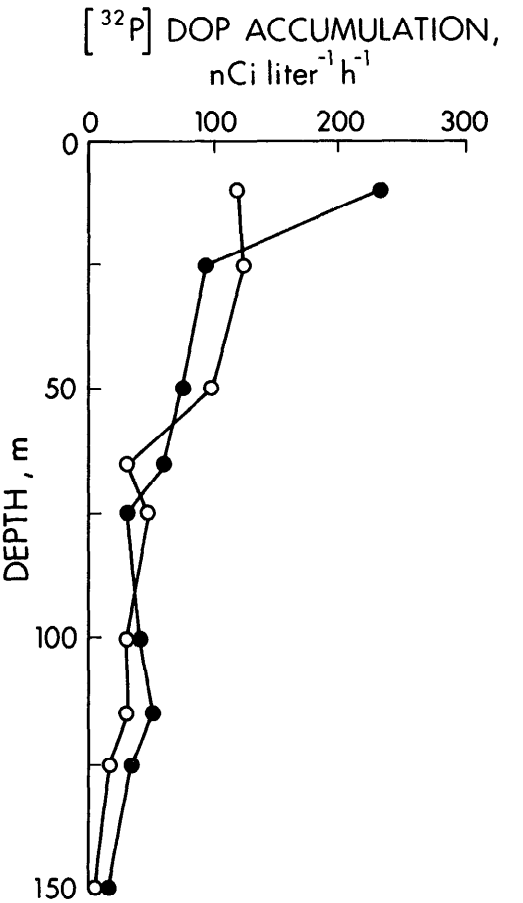


Fig. 1. Comparison of [^{32}P]DOP accumulation for samples processed with Whatman GF/F (O) and Millipore GS (●) filters.

Two separate in situ experiments were performed to measure rates of DOP production for water samples collected during VERTEX 3 (Fig. 3). The data presented use the models of both TDP and RNA specific activity (discussed previously) to calculate the absolute rates of P production. A salient feature of these data is the peak in DOP production at 75 m during the morning incubation (0500–1000), which was not observed for the samples incubated later in the day (1000–1400 hours). Table 2 presents the

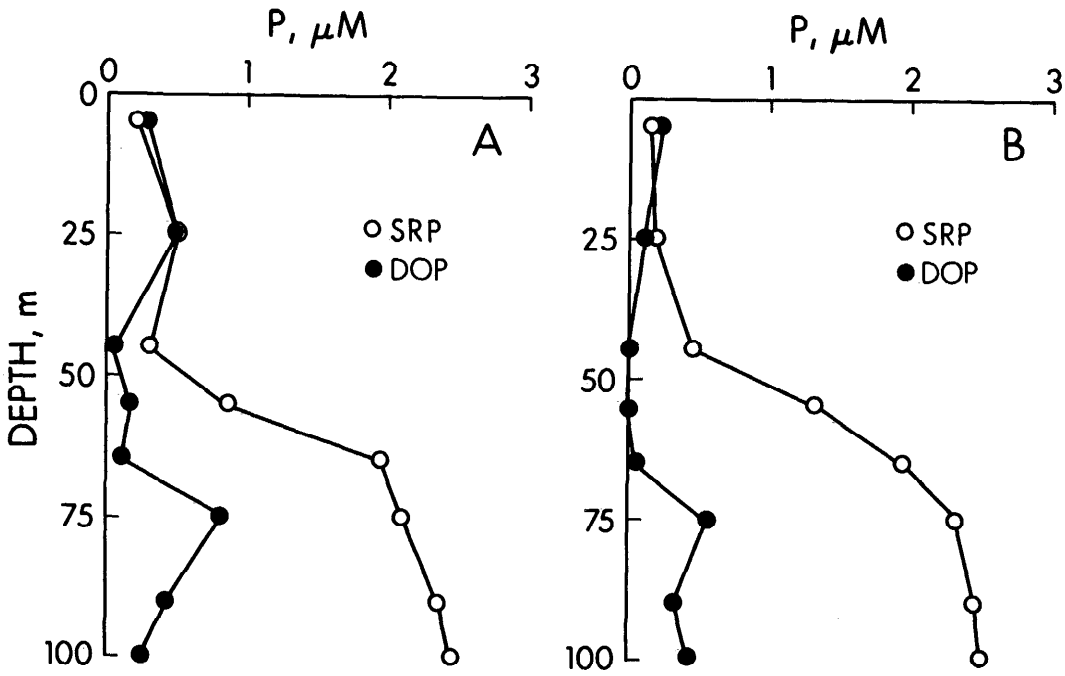


Fig. 2. Vertical profiles of DOP and SRP at the VERTEX 3 station, 11 November 1982. A. Samples collected at 0500 hours. B. Samples collected at 1000 hours.

depth-integrated values of DOP production and net P accumulation for the 0–60-m and 60–100-m portions of the water column. The morning incubation period yielded significantly higher rates of P incorporation and DOP production, regardless of the metabolic model used to extrapolate the ^{32}P incorporation data. Furthermore, the DOP production rates exceed the estimated values of net P incorporation by an order of magnitude, which suggests that the gross assimilation of P greatly exceeds the measured net fluxes. This would be expected if, for example, microbial grazing rates and nutrient recycling rates are rapid. Mean values of P incorporation of 19.6 mg P m^{-2} d^{-1} during the two time intervals divided into a mean steady-state biomass of 38.4 mg P m^{-2} indicates a turnover time of 1.96 d for total microbial cellular P. We estimate mean turnover time of DOP to be 3.3 d.

VERTEX 4—At the VERTEX 4 site, vertical profiles of SRP and DOP were also taken at two different times of the same day (Fig. 4). DOP concentrations were about equal to SRP concentrations in most samples to a depth of about 100 m, below which SRP exceeded DOP.

Two separate in situ experiments were performed to measure rates of DOP production for water samples collected during VERTEX 4 (Fig. 5). The extrapolated rates of DOP production ranged from 2 to 20 ng P $liter^{-1}$ h^{-1} with the model of TDP specific activity, to values from 6 to 193 ng P $liter^{-1}$ h^{-1} by the model of RNA specific activity. Differences between the two assimilation models were greatest below 115 m. At these greater depths, the estimates of DOP production based on the method of RNA specific activity exceeded those calculated from the model of TDP specific activity. As in VERTEX 3, afternoon rates of production, extrapolated from either model, were lower than morning rates. DOP production peaked at 75–85 m in both the morning and afternoon casts in calculations with the TDP specific activity model.

At the VERTEX 4 station, depth-integrated production of DOP generally exceeded the estimated rates of net P assimilation (Table 3). In all cases, rates of DOP

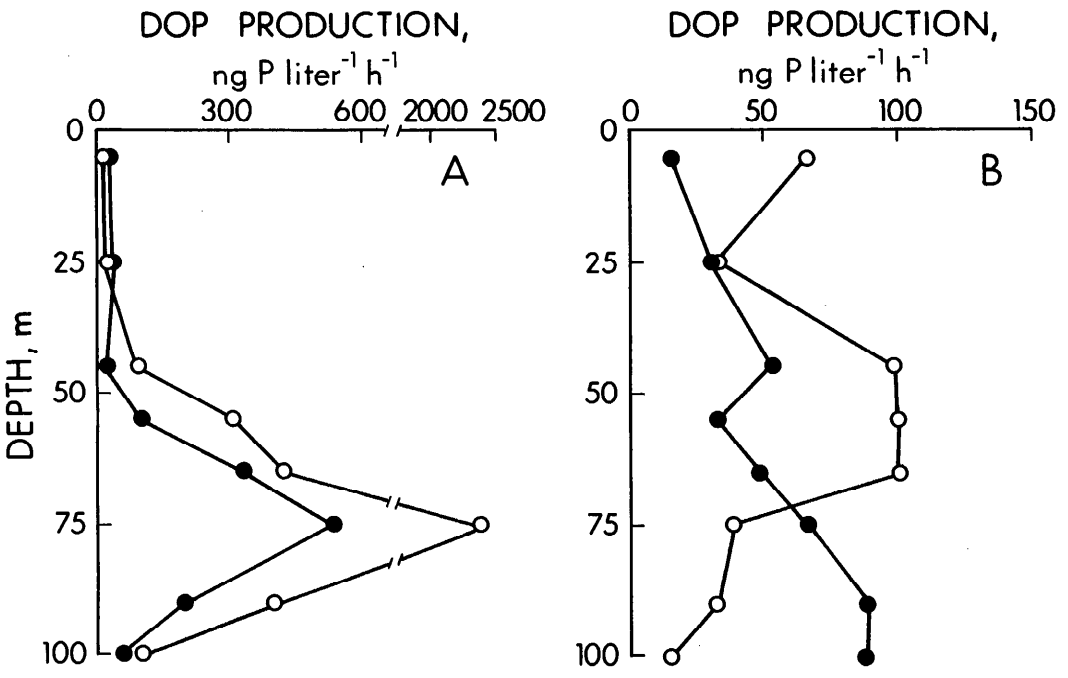


Fig. 3. DOP production rates at the VERTEX 3 station, 11 November 1982. Calculations based on TDP specific activity method—●; calculations based on RNA specific activity method—O. A. Incubation period, 0500–1000 hours. B. Incubation period, 1000–1400 hours.

production extrapolated by the model of RNA specific activity exceed the production estimates based on the model of TDP specific activity. Comparison of DOP production, net P assimilation, and microbial biomass on two separate dates (11 and 16 August) indicates excellent agreement, suggesting that the system was most likely in steady state during our experimental observation period. Mean P incorporation for 11 August of 15 mg P m^{-2} d^{-1} divided into a microbial biomass of 44 mg P m^{-2} indicates a turnover time of 3 d for total cellular P. DOP turnover time is estimated to be 11 d.

During VERTEX 4, we also had an opportunity to estimate the concentrations of biologically available phosphorus (BAP) for water samples where SRP concentrations were <50% of the TDP pool. At all depths where the measurements were performed, the BAP concentration, as estimated by direct measurement of the γ-positioned P of the pool of total microbial ATP (Bossard and Karl 1986), exceeded the SRP pool by factors ranging from 1.8 to 6.7 (Table 4). These data suggest that sources of P other than the SRP pool must be available for microbial metabolism and are consistent with the stated assumptions for the use of the models of TDP and RNA specific ra-

Table 2. Depth-integrated DOP production (mg P m^{-2} d^{-1}) and P incorporation (mg P m^{-2} d^{-1}) for water samples collected off Mexico (VERTEX 3, 11 November 1982).

	DOP production		Net P assimilation*	Microbial biomass† (mg P m^{-2})
	TDP model	RNA model		
Incubation 1 (0500–1000)				
0–60 m	64	131	16.6	30.6
60–100 m	297	952	4.2	10.1
Total	361	1,083	20.8	40.7
Incubation 2 (1000–1400)				
0–60 m	48	92	16.0	25.6
60–100 m	65	48	2.4	10.5
Total	113	140	18.4	36.1

* Extrapolated from measurements of total microbial DNA synthesis, assuming a C:DNA (wt/wt) ratio of 50:1 and a C:P (mol/mol) ratio of 106:1.

† Extrapolated from measurements of total microbial ATP, assuming a C:ATP (wt/wt) ratio of 250:1 and a C:P (mol/mol) ratio of 106:1.

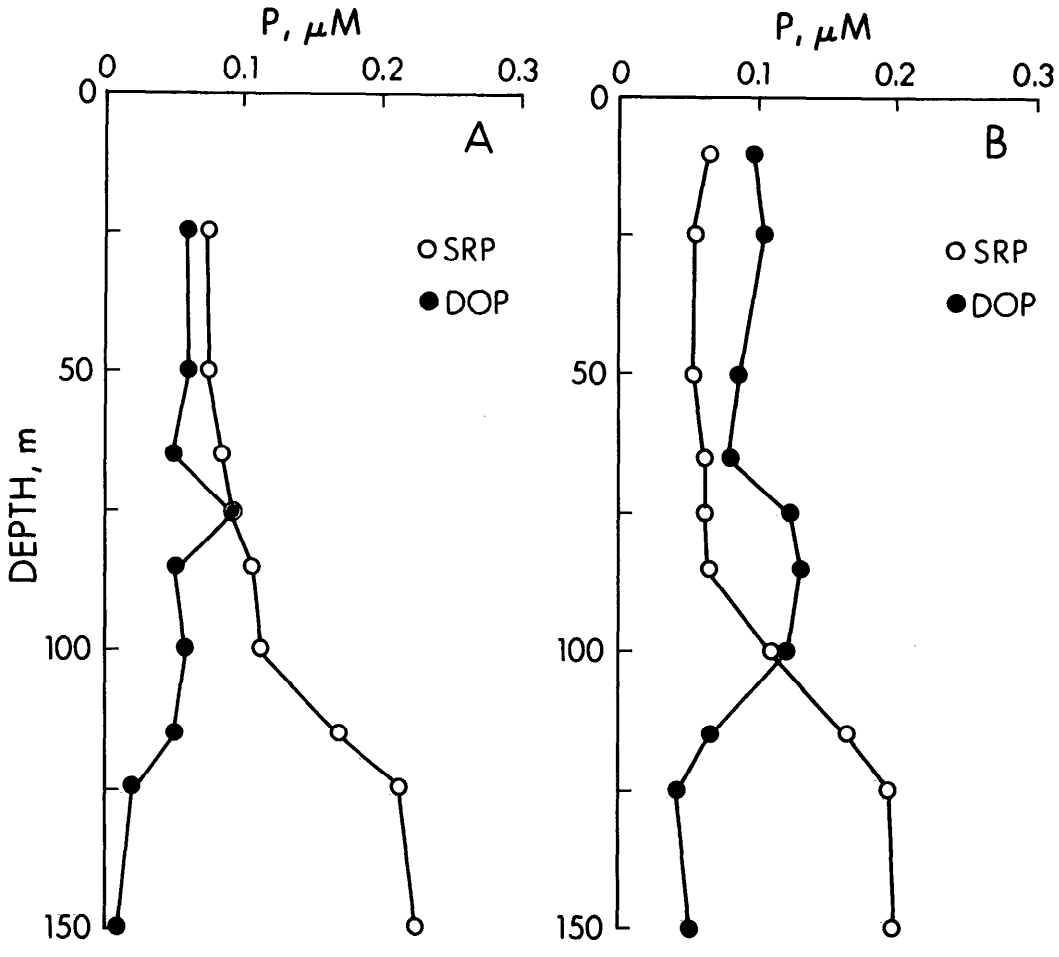


Fig. 4. Vertical profiles of DOP and SRP at the VERTEX 4 station, 10 August 1983. A. Samples collected at 0530 hours. B. Samples collected at 1130 hours.

dioactivity (*see Table 1*). In the surface waters (i.e. ≤50m), the estimated concentrations of BAP ranged from 60 to 87% of the measured pools of TDP. However, at greater water depths, the estimated concentrations of BAP were up to three times greater than the measured pools of TDP. It is conceivable that additional reservoirs of P, not included in the estimates of the TDP pool (e.g. particulate P, endogenous cellular pools, etc.), were important for the growth and metabolism of microorganisms at these depths. Unfortunately, our method was not sensitive enough to quantify concentrations of BAP at depths >75 m due to the increased pools of SRP and the corresponding decrease in the uptake of the added $^{32}PO_4^{3-}$.

Discussion

The approach used here, i.e. the selection of an appropriate model compound to represent the specific radioactivity of the entire DOP pool is, at least in principle, analogous to that used by Storch and Saunders (1975) to estimate the mass flux of carbon during phytoplankton excretion. This information is required to extrapolate the measured radiotracer data (nCi ^{32}P liter^{-1} h^{-1}) to reliable estimates of P flux (ng P liter^{-1} h^{-1}). The model of TDP specific activity might be expected to yield an accurate estimate of the true rate of DOP production if uptake of phosphorus occurs from both the SRP and DOP pools, if the ratio of SRP uptake to DOP uptake equals the ratio of SRP to DOP

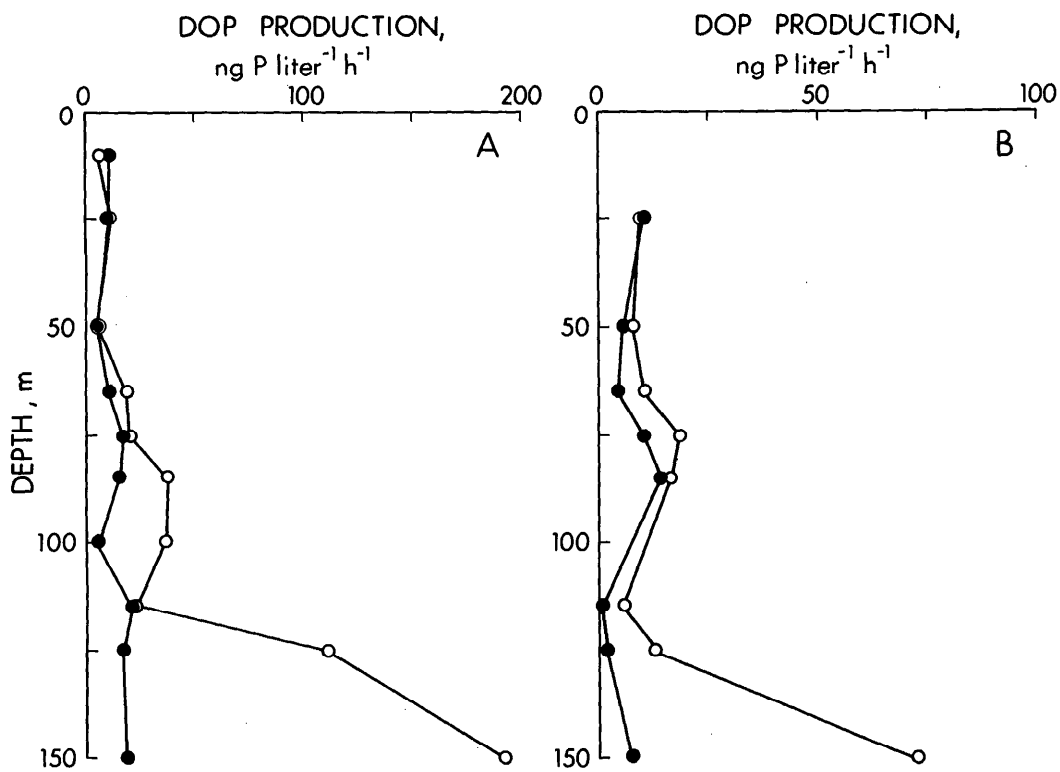


Fig. 5. DOP production rates at the VERTEX 4 station, 10 August 1983. Symbols as in Fig. 3. A. Incubation period, 0530–1130 hours. B. Incubation period, 1130–1730 hours.

concentration (i.e. nonselective use of P), and if the radioactive label is uniformly distributed among all molecular forms of DOP. Under these conditions one could be assured that the specific activity of the assimilated P was the same as the specific activity of the TDP, and it is obvious that the specific activity of the recently assimilated P is an upper bound on the specific activity of the DOP released by the cells. However, if microorganisms preferentially assimilate a particular portion of the TDP pool that has a higher or lower specific activity than the entire TDP pool, then the use of the model of TDP specific activity would not be expected to yield an accurate estimate of DOP production.

By comparison, the model of RNA specific radioactivity might be expected to provide more reliable rates of DOP production since it is based on fewer assumptions. Most P-containing low and high molecular weight pools in microbial cells are expected to have specific activities equivalent to that of ATP, which is also the precursor to the RNA measured in our experimental protocol. By measuring the specific radioactivity of the P in newly formed RNA we are, in effect, measuring the labeling pattern of cellular ATP, as well as all intracellular DOP compounds that have ATP as their P precursor. At the time of our study we did not include direct measurements of the specific radioactivity of the P contained in ATP; however, a convenient field method now exists (Karl and Bossard 1985). This method could be used to test the above-mentioned hypothesis regarding nonselective uptake of P from pools of SRP and DOP.

The rates of DOP production measured here, ranging from 3 to 328 ng P liter^{-1} h^{-1} (TDP specific activity model) and 2 to 193 ng P liter^{-1} h^{-1} (RNA specific activity model), coupled with the general observation that concentrations of DOP are not rapidly increasing in oceanic waters, imply an equally

Table 3. Depth-integrated DOP production (mg P m^{-2} d^{-1}) and P incorporation (mg P m^{-2} d^{-1}) for water samples collected in the North Pacific Ocean (VERTEX 4, August 1983).

	DOP production		Net P assimilation*	Microbial biomass* (mg P m^{-2})
	TDP model	RNA model		
Incubation 1; 11 Aug (0530–1130)				
0–100 m	25	38	11.5	39.4
100–150 m	21	118	4.2	8.8
Total	46	156	15.7	48.2
Incubation 2; 11 Aug (1130–1730)				
0–100 m	12	16	12.2	35.1
100–150 m	3	28	2.8	8.9
Total	15	44	15.0	44.0
Incubation 3; 16 Aug (1130–1730)				
0–100 m	10	18	14.7	36.5
100–150 m	5	18	1.5	8.6
Total	15	36	16.2	45.1

* Values derived from measurements of microbial ATP and rates of ATP and rates of DNA synthesis as described in Table 2.

Table 4. Calculation of concentration of biologically available P for surface waters of the North Pacific Ocean.

Depth (m)	SRP	DOP	TDP	ATP pool, -P* (pCi ^{32}P pmol^{-1} P)	Estimated BAP† (μmol P liter^{-1})
	(μmol P liter^{-1})				
10	0.064	0.126	0.190	230	0.102
				189	0.124
25	0.075	0.171	0.246	137	0.171
				161	0.145
50	0.078	0.141	0.219	123	0.190
				123	0.190
65	0.088	0.165	0.253	53	0.442
				63	0.371
75	0.094	0.120	0.214	38	0.616
				36	0.650

* ^{32}P contained in γ-positioned P of total microbial ATP pool was measured with the methods described by Karl and Bossard (1985) following a 6-h in situ incubation with $^{32}PO_4^{3-}$ added at a concentration of 23.4 μCi liter^{-1}. Data are from incubation 1, VERTEX 4 (August 1983).
† Biologically available phosphorus, as defined by Bossard and Karl (1986).

rapid uptake or hydrolysis of DOP within the epipelagic zone. Even these relatively high rates of DOP production measured with $^{32}PO_4^{3-}$ are probably conservative estimates of the actual gross rates of DOP production under in situ conditions since we make no effort to correct for [^{32}P]DOP assimilation during the in situ incubation period.

In principle, extracellular DOP might arise from excretion (exudation), cell death and autolysis, or losses resulting from inefficient feeding or predation. Each pathway might be expected to give rise to a different spectrum of organic-P compounds. Several previous studies of P cycling in aquatic ecosystems have attempted to characterize the chemical nature of the DOP pool (Minear 1972; Lean 1973; Herbes et al. 1975; Downes and Paerl 1978; Francko and Heath 1979). The methodology used for most of these investigations has relied on molecular weight fractionation, and the results indicate the presence of a heterogeneous mixture of DOP constituents of both low (<500) and high molecular weight (>50,000). A more detailed chemical characterization of the total DOP pool might ultimately provide useful information regarding the mechanisms and pathways of DOP production in nature.

The rates of DOP production measured here could, in principle, be extrapolated to rates of DOC production if the average C:P ratio of the DOP pool is known. An upper bound would be the whole cell C:P (atomic) ratio of 106:1 (Redfield et al. 1963), but it is unlikely that DOP compounds are, on the average, this rich in carbon. The theoretical minimum for any class of intracellular organic compounds would be set by the nucleotide triphosphates (NTPs), which have an average C:P ratio of ~3:1. However, we do not expect NTPs to represent a significant percentage (i.e. generally <15%) of the total organic P in living cells (Bieleski 1973; Bochner and Ames 1982*b*). Since our rates of DOP production are calculated, in part, using newly formed RNA as a model compound and since nucleic acids are the largest organic-P reservoir in the cell, we selected the average C:P ratio of RNA monomers (i.e. a C:P ratio of 9.5:1) for our extrapolation to rates of DOC production. Because the ribonucleotide C:P is much closer to the lower than to the upper bound, we suggest that our rates of DOC production are likely to be conservative estimates. The rates of DOC production thus calculated are equivalent to 2,252 and 285 mg C m^{-2} d^{-1} for VERTEX 3 and VERTEX 4. By comparison, the rates of total microbial carbon production, derived from mea-

surements of DNA synthesis with [^{3}H]adenine, were 795±241 and 686±16 mg C m^{-2} d^{-1} for VERTEX 3 and 4 (D. M. Karl unpubl. data). These data suggest that organic carbon, as well as phosphorus, is turning over on time scales of hours in oligotropic ocean waters. The inability to detect this substantial DOC flux with standard $H^{14}CO_3$ procedures (*see* Sharp 1977) might be interpreted as an indication that phytoplankton plays a minor role in DOP production. However, it is also conceivable that much of the carbon incorporated into DOP compounds (in particular nucleotides and nucleic acids) is salvaged directly from extracellular sources and consequently would not be expected to have achieved isotopic ($^{14}CO_2$) equilibrium with respect to intracellular pools that are synthesized de novo from the precursor pool ($^{14}CO_2$). In any case, we feel that accurate estimates of DOP production may be used, independently, to set limits on production and recycling of soluble organic carbon and should prove useful in future studies of the microbial food web in oligotrophic ocean waters.

Table 5 presents summaries of the P budgets for the euphotic zone of the water column at the VERTEX 3 and 4 stations. During VERTEX 3, DOP comprised 18% of the TDP pool and living P accounted for 48% of the total particulate P pool. Assimilation of 19.6 mg P m^{-2} d^{-1} into a living biomass of 38 mg P m^{-2} extrapolates to a specific growth rate (μ) of 0.36 d^{-1}. Conservative (TDP specific activity model) estimates of DOP production indicate a flux of 237 mg P m^{-2} d^{-1}. If this production is also considered to represent organic material which had been produced by microbial metabolism and subsequently lost through the combined effects of exudation, grazing, or lysis, the actual rates of gross P incorporation must be even greater than the measured values. When we include rates of both DOP production and net P assimilation, we conclude that microbial P has a turnover time of ~4 h. The loss of particulate P from the euphotic zone via sinking particles was equivalent to about 3% of the net accumulation of microbial P.

At the VERTEX 4 station, DOP comprised 40% of the TDP, and living P accounted for 62% of the total particulate P pool. The estimated proportion of living-to-nonliving P was higher than that observed during VERTEX 3 and may indicate a more rapid remineralization of particulate P from the euphotic zone which averaged <1% of the net microbial P production. Accumulation into a living biomass of 46 mg P m^{-2}, extrapolated from measurements of microbial DNA synthesis, was 15.6 mg P m^{-2} d^{-1}, which yields a specific growth rate (μ) of 0.24 d^{-1}. This rate exceeds the value reported by Perry and Eppley (1981) for the central North Pacific of 0.14 doublings d^{-1} (μ = 0.10), but is lower than the rate measured during the PRPOOS experiment in oligotrophic waters near Hawaii (Laws et al. 1984). If we compare our estimates of DOP production in the central North Pacific Ocean (1,000 μmol P m^{-2} d^{-1}) to estimates of the net downward flux of DOP for a similar oligotrophic ocean site (~4 μmol P m^{-2} d^{-1}: Smith et al. 1986), we obtain independent evidence for the lability of the DOP pool in epipelagic waters.

Our results indicate the importance of DOP in studies of microbial nutrient cycling in the sea. As presented in the two summary P budgets (Table 5), production of DOP can exceed net assimilation of P (i.e. P retention

Table 5. Summary budgets for the distribution and transformations of phosphorus in the euphotic zone of the water column at two oceanic stations. All concentrations are expressed as mg P m^{-2} and fluxes as mg P m^{-2} d^{-1}.

	VERTEX 3	VERTEX 4
Steady state pools		
Soluble P		
SRP	3,581	520
DOP	791	343
Total	4,372	863
Particulate P		
Living	38	46
Nonliving	41	28
Total	79	74
Total P	4,451	937
Fluxes		
Net microbial P assimilation	19.6	15.6
DOP production	237	31
Particulate loss from euphotic zone*	3	<1

* Measured by free-floating sediment traps.

in cells) and thus indicates a much faster cycling of P through microbial communities than certain previous estimates might have suggested. If these observations are confirmed as a general oceanic feature, we must conclude that the production and uptake of DOP is rapid and consequently must represent an important source of P for microbial communities in surface ocean waters.

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Submitted: 2 January 1985
Accepted: 16 May 1986