

Dissolved organic phosphorus in the coastal ocean: Reassessment of available methods and seasonal phosphorus profiles from the Eel River Shelf

E. J. Monaghan and K. C. Ruttenberg¹

Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Abstract

Methods for quantifying total dissolved phosphorus (TDP) were reevaluated. High-temperature ashing/hydrolysis (Ash/Hydrol.) and Acid Persulfate methods were examined for their ability to quantitatively convert a range of organic-P compounds to orthophosphate. The Ash/Hydrol. method recovered on average 99% of organic-P compounds tested, including phosphonates (compounds with C-P bonds). Contrary to concerns voiced in prior studies, we found no P loss due to volatilization in seawater samples. Poor recovery by the Acid Persulfate method was observed for two compound classes: phosphonates and phospholipids. The Ash/Hydrol. method is recommended for routine TDP analyses.

The hydrolysis of dissolved organic phosphorus (DOP) during the phosphomolybdate-blue reaction and the subsequent overestimation of dissolved inorganic phosphorus (DIP) have long been a concern. Extent of hydrolysis of standard DOP compounds was evaluated both during this reaction and over long-term acidified (pH 1.0)/refrigerated and unacidified/frozen storage. Eleven of 12 compounds tested hydrolyzed <2% during the phosphomolybdate-blue reaction. Phospholipid, phosphosugar, and nucleotide mono- and diphosphate standards stored acidified/refrigerated over 2.5 months were <5% hydrolyzed; compounds with higher energy phosphate bonds (e.g., phosphocreatine) hydrolyzed to a greater extent. A natural seawater sample showed no hydrolysis over 80 d, however, suggesting that refrigerated storage of acidified samples is a viable option.

Seasonal DOP profiles from the Eel River Shelf quantified by the Ash/Hydrol. and Acid Persulfate methods yielded comparable results, indicating that the DOP pool at this site did not contain significant phospholipids or phosphonates, which are underrecovered by the Persulfate method. Recoveries using a third method, Nitrate Oxidation, were on average 7% lower. Surface-water DOP exceeded DIP in summer and was apparently related to biological productivity, underscoring the need to include organic species when evaluating nutrient limitation and creating nutrient budgets.

Despite early studies indicating the potential importance of DOP as a nutrient source to primary producers in marine systems (Redfield et al. 1937; Butler et al. 1979; Jackson and Williams 1985; Rivkin and Swift 1985; Orrett and Karl 1987), many past assessments of nutrient inventories and nutrient limitation have ignored dissolved organic nutrient reservoirs. DOP can be a significant fraction of the TDP pool in surface waters—in some cases, seasonally surpassing levels of DIP (Butler et al. 1979; Orrett and Karl 1987; Cotner et al. 1997; Karl and Tien 1997). Exclusion of DOP may lead to erroneous estimates of the bioavailable P reservoir, negatively affecting assessments of nutrient limitation and nutrient budgets. Two factors account for the lack of routine DOP data collection: (1) lack of appreciation of the role of P as a limiting nutrient in oceanic systems (e.g., Krom et al.

1991; MacRae et al. 1994; Cotner et al. 1997; Karl et al. 1997), and (2) the analytical difficulty associated with measuring relatively low concentrations of DOP and the absence of a definitively proven, widely accepted, quick and convenient method of measurement.

Most available methods determine DOP as the difference between TDP and DIP, where TDP and DIP are quantified in separate splits of a single sample of filtered seawater. DIP is quantified directly on an untreated split as that portion of dissolved P that is present as orthophosphate and thus readily forms the phosphomolybdate-blue complex (e.g., Strickland and Parsons 1972; Koroleff 1983). This fraction is sometimes referred to as “soluble reactive phosphorus” (SRP), a term that makes no assumptions about the nature of this molybdate-reactive P pool, since it is suspected that some easily hydrolyzable DOP compounds may be converted to orthophosphate upon contact with the acidic colorimetric reagents (e.g., Rigler 1968). The second split of sample for TDP determination is subjected to some form of oxidation, often followed by a hydrolysis step, to convert all forms of combined dissolved P (DOP and polyphosphates) to orthophosphate. Subsequent quantification of orthophosphate is by the phosphomolybdate-blue colorimetric reaction and spectrophotometric analysis. A recently published method (Thomson-Bulldis and Karl 1998) that separately quantifies DIP and DOP still requires a TDP oxidation step identical to those used in the more common difference methods.

In recent years, a number of studies have evaluated the

¹ To whom correspondence should be addressed.

Acknowledgments

This manuscript was significantly improved by the thorough and thoughtful reviews of David Karl, an anonymous reviewer, and editorial handling by Patrick Mulholland. Anne Giblin and Claudia Benitez-Nelson made helpful comments on an earlier version of the paper, and we gratefully acknowledge their input. So Yung Morris and Kirsten Laarkamp provided much appreciated assistance in sample analysis. We also acknowledge the ONR Strataform project and Strataform scientists, particularly Chuck Nittrouer, for making the Eel River Shelf fieldwork possible. This work was supported by NSF and ONR. Woods Hole Oceanographic Institution contribution 9731.

relative ease and efficiency of TDP oxidation by published methods, but there is still no consensus about which method should be adopted for routine use. A reexamination of previously published methods studies led us to select the high-temperature combustion/hydrolysis method of Solórzano and Sharp (1980), hereafter referred to as the Ash/Hydrol. method, as our method of choice for TDP determination. It is analytically simple and recovers $100 \pm 5\%$ of standard organic-P compounds (Solórzano and Sharp 1980; Kérouel and Aminot 1996; Ormaza-González and Statham 1996; this study).

We report results of a reevaluation of the Ash/Hydrol. method in comparison with the frequently used Acid Persulfate method. Concerns about the ability of the Ash/Hydrol. method to digest phosphonates (Cembella et al. 1986) and the potential loss of P due to volatilization (Karl and Tien 1992; Ormaza-González and Statham 1996) are addressed and shown to be invalid. In addition, loss of DOP due to hydrolysis is assessed both during determination of DIP using the acidic phosphomolybdate-blue method and over long-term storage under acidic (pH 1) conditions.

Finally, seasonal water-column DOP profiles obtained by the Ash/Hydrol. and Acid Persulfate methods are presented from the Eel River Shelf, a coastal system in northern California influenced by nutrient inputs from upwelled deep-water and riverine sources. Tests of standard DOP compounds reveal that the Acid Persulfate method underrecovers two potentially important compound classes. Comparison of recoveries obtained by this and the Ash/Hydrol. method is used to derive compositional information about the DOP pool in these coastal waters and to evaluate possible seasonal variations in DOP composition.

Experimental design and procedures

Recovery experiments—The purpose of the recovery experiments was threefold: (1) to determine the ability of the Ash/Hydrol. method to convert organic P to orthophosphate, (2) to compare the degradative ability of the Ash/Hydrol. method when using two different oxidants: MgSO_4 and $\text{Mg}(\text{NO}_3)_2$, and (3) to contrast the relative degradative ability of the Ash/Hydrol. and Acid Persulfate methods.

The recovery experiments were carried out by dissolving 19 commercially available organic-P test compounds and (inorganic) triphosphate in artificial seawater (ASW: 0.43 M NaCl and 0.03 M MgSO_4 ; Strickland and Parsons 1972). Final compound concentrations of ca. 10 μM P, a factor of 10 or more higher than natural of DOP levels, facilitated concentration determinations on experimental solutions. An effort was made to select naturally occurring compounds with varying types and strengths of phosphate bonds. In total, three phosphosugars, six nucleotides, two nucleic acids, three phospholipids, three phosphonates, two reactive biochemical intermediates, and one polyphosphate were analyzed (Table 1). Test solutions were acidified to pH 1 using HCl so that treatment of standards and field samples would be identical, and TDP concentrations were determined the same day they were made up.

An additional recovery experiment was carried out to de-

termine whether the Ash/Hydrol. method could be used for low salinity samples. High-concentration (5, 10, 15, and 20 μM) orthophosphate solutions were acidified to pH 1.0 using HCl in freshwater (milli-Q H_2O) and in ASW. A third set of orthophosphate standards in freshwater (milli-Q H_2O) was amended by adding a 1-ml aliquot of 10 times concentrated ASW to standard splits in the TDP vial, for a final salinity equivalent to seawater, before drying the sample down.

Hydrolysis experiments—The purpose of the hydrolysis experiments was twofold: (1) to determine which organic-P compounds were susceptible to hydrolysis during the phosphomolybdate-blue reaction, and (2) to determine the extent of degradation of organic-P compounds when stored over an extended period of time under two conditions: acidified (pH 1.0)/refrigerated (4.5–5°C) and unacidified/frozen (–30°C).

Organic-P compound standards were dissolved in ASW to concentrations of ca. 100 μM P. A split was taken, acidified to pH 1.0 in a reaction tube, and reacted immediately to determine the DIP concentration. Another split of the solution was acidified to pH 1.0 and stored refrigerated in either high-density polyethylene (HDPE) or glass bottles. A third split of each solution was left unacidified and stored frozen in HDPE. Subsequent splits were taken from the acidified/refrigerated and unacidified/frozen solutions at varying time intervals (days to weeks), and the DIP concentration was measured to determine the extent of hydrolysis during long-term storage. In addition to the organic-P standard compounds tested, a natural surface seawater sample from the Eel River Shelf was acidified and refrigerated in a manner identical to the standards, and DIP was measured over time, to determine the extent of hydrolysis of organic-P compounds in a natural sample. TDP was measured on the Eel River Shelf sample by the Ash/Hydrol. method to determine the initial quantity of DOP.

TDP in Eel River Shelf samples—Water-column samples were collected on the Eel River Shelf off the coast of northern California in spring (May 1996), summer (July 1996), and winter (January 1997) as part of the STRATAFORM project. Surface-, mid-, and deep-water samples were collected using niskin bottles on a conductivity–temperature–depth rosette, filtered through 0.4- μm polycarbonate filters, acidified to pH 1.0 with high-purity (Fisher-OPTIMA) hydrochloric acid, and stored refrigerated in HDPE bottles. All filters, bottles, filtration apparatus, and niskins were acid cleaned prior to use. DIP was measured using the Koroleff (1983) version of the phosphomolybdate-blue method for acidified samples.

Forty-eight samples from four stations (C30, C40, G40, and G50; Fig. 1) were analyzed to compare TDP concentrations obtained using the Ash/Hydrol., Acid Persulfate, and Nitrate Oxidation methods. Thirty-nine samples were measured in duplicate by both the Ash/Hydrol. and Acid Persulfate methods. Six samples were also measured using the Nitrate Oxidation method.

TDP methods—Ash/Hydrol. method: We employed the method of Solórzano and Sharp (1980) with minor modifications. Samples were ashed in the presence of MgSO_4 to

Table 1. Recovery of organic-P compounds using three different TDP methods: Ash/Hydrol. with MgSO₄, Ash/Hydrol. with Mg(NO₃)₂, and Acid Persulfate. NT, not tested.*

Compound	Ash/Hydrol.—MgSO ₄ % recovery [†]		Ash/Hydrol.—Mg(NO ₃) ₂ % recovery [†]		Acid Persulfate % recovery [†]	
Phosphosugars						
β-D-Glucose-6-phosphate monosodium salt	99.0	(0.6)	NT		98.8	(0.8)
Glycerophosphate disodium salt hydrate [‡]	101.5	(0.4)	99.1	(0.6)	NT	
Phytic acid dipotassium salt [‡]	102.1	(0.5)	102.1	(0.2)	NT	
Nucleotides						
(-)-adenosine 5'-monophosphate monohydrate	102.2	(1.0)	NT		101.2	(2.2)
Adenosine 5'-diphosphate, sodium salt hydrate	103.8	(0.6)	NT		102.9	(2.9)
Adenosine 5'-triphosphate, disodium salt hydrate ^{‡§}	95.3	(0.3)	94.3	(0.4)	95.0	(1.7)
Guanosine 5'-monophosphate disodium salt	95.8	(0.5)	NT		93.8	(1.7)
Guanosine 5'-diphosphate tris salt	97.0	(0.4)	NT		102.7	(6.4)
Guanosine 5'-triphosphate sodium salt	93.4	(0.5)	NT		101.9	(9.8)
Nucleic acids						
Deoxyribonucleic acid sodium salt	97.4	(0.4)	98.2	(0.2)	95.9	(0.8)
Ribonucleic acid	98.7	(0.5)	98.3	(0.4)	95.0	(1.8)
Phospholipids						
L-α-phosphatidylethanolamine	103.5	(2.8)	103.4	(1.4)	55.7	(4.0)
Cardiolipin sodium salt	98.3	(3.0)	102.2	(4.4)	NT	
L-α-phosphatidyl-DL-glycerol sodium salt	103.9	(2.2)	102.4	(4.3)	NT	
Phosphonates/Phosphonic acids						
2-aminoethylphosphonic acid	95.4	(0.7)	NT		73.5	(1.6)
Phosphonoacetic acid	97.8	(0.6)	97.7	(1.2)	NT	
DL-2-amino-3-phosphonopropionic acid	99.9	(0.4)	101.2	(0.2)	NT	
Reactive biochemical intermediates						
Phosphocreatine disodium salt hydrate	100.2	(1.3)	101.4	(1.2)	94.1	(2.7)
Phospho(enol)pyruvate, monosodium salt, hydrate	97.2	(0.2)	98.3	(0.4)	NT	
Polyphosphate						
Tripolyphosphate pentasodium salt hexahydrate	97.5	(0.5)	97.1	(0.3)	98.4	(2.0)
Mean % recovery	99.0		99.7		93.0	
Stdev [¶]	3.1		2.7		13.5	
n	20		13		13	

* Values in parenthesis are relative % reproducibility errors calculated over quadruplicates.

[†] % recovery = (mean P concentration measured/mean P concentration of the standard) × 100. Recoveries > 100% are assumed due to orthophosphate impurities.

[‡] Standard was not acidified for the Ash/Hydrol. test using MgSO₄ or Mg(NO₃)₂.

[§] Mean of quadruplicate recovery results from two separate runs for Ash/Hydrol.—MgSO₄.

^{||} Mean % recovery = mean of all recoveries.

[¶] Stdev = stdev about mean of all recoveries.

oxidize organic P, then acid-hydrolyzed to cleave polyphosphate bonds that persist after high-temperature ashing. 0.2 ml of 0.17 M MgSO₄ was added to each acid-cleaned/muffled 20-cc borosilicate vial, followed by 10 ml of HCl-acidified (pH 1.0) sample. Uncovered samples were dried in an 80°C oven for 2 d. When samples appeared dry, the oven temperature was increased to 130°C for 3 h to drive off residual HCl. After cooling (slowly to avoid spattering), the vials were covered tightly with solvent-rinsed aluminum foil, placed in a muffle furnace, and ashed at 500°C for 2 h. Post-ashing hydrolysis was accomplished by adding 3 ml of 0.75 M HCl to each sample, tightly capping with Teflon-lined caps, homogenizing on a vortex mixer, and oven-heating at 80°C for 20 min. Samples were then removed from the oven, allowed to cool, and, after adding 7 ml of milli-Q H₂O to each vial, were recapped and heated for an additional 10 min

at 80°C to complete the hydrolysis step. When the hydrolyzed samples had reached room temperature, they were adjusted to pH 1.0 (with 2 M NaOH) and measured via the colorimetric method of Koroleff (1983). Modifications include the use of acidified samples and the oven-heating of samples to 130°C before ashing.

Acid Persulfate method: We employed the Acid Persulfate method of Koroleff (1983) with a modified autoclave time. Samples are autoclaved under acidic conditions with potassium persulfate to oxidize organic P. A 4-h autoclave time was used, because Ridal and Moore (1990) demonstrated that maximal DOP recovery occurs only after 3 h. 0.2 ml of 0.18 M potassium-persulfate solution was added to each acid-cleaned/muffled 20-cc borosilicate vial. Ten milliliters of HCl-acidified (pH 1.0) sample was added, and vials were

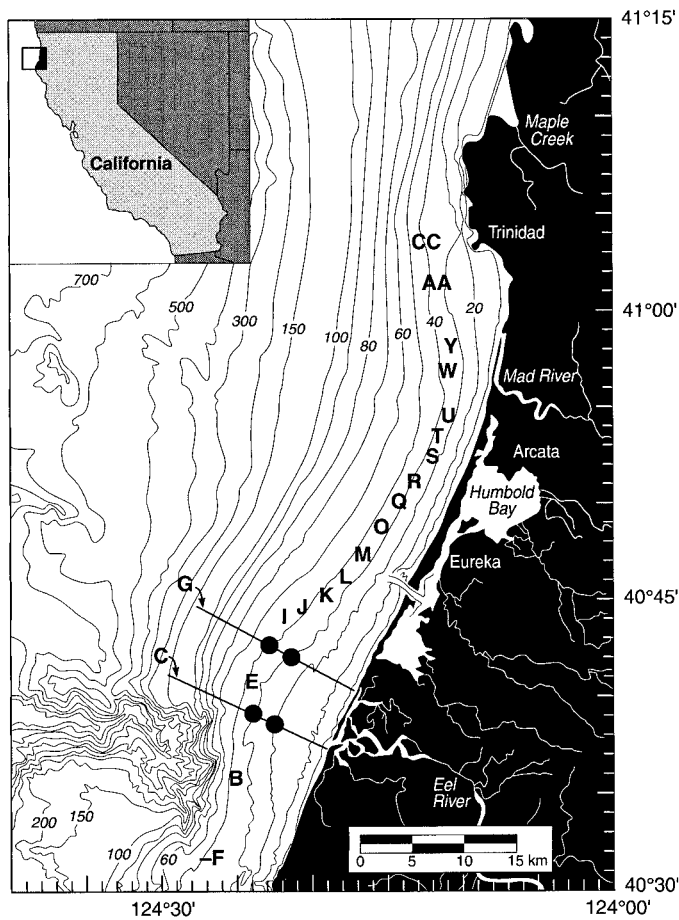


Fig. 1. Station location map for Eel River Shelf samples. Filled circles indicate stations for which data are presented (C30, C40, G40, and G50). Letters indicate STRATAFORM project transect lines. Isobaths are in meters.

covered tightly with Teflon-lined caps and autoclaved at 130°C for 4 h. After cooling to room temperature, samples were vortexed and reacted for orthophosphate using the mixed reagent and ascorbic acid solution specified for Acid Persulfate samples (Koroleff 1983).

Nitrate Oxidation method: The Nitrate Oxidation method of Cembella et al. (1986), with numerous modifications, was followed. This method involves heating the samples in the presence of $\text{Mg}(\text{NO}_3)_2$ to oxidize organic P, followed by an acid hydrolysis step to cleave polyphosphate bonds. 0.4 ml of a $\text{Mg}(\text{NO}_3)_2$ solution (20% $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ w/v in 95% ethanol) was added to each acid-cleaned/muffled 20-cc borosilicate vial containing acid-clean glass beads for boiling control. Ten milliliters of HCl-acidified (pH 1.0) sample was added to each vial, and sample volume was reduced by boiling in a dry block at 120°C. When salt crystals began to appear on the sides of the vials, the temperature was decreased to 80°C to prevent spattering. Once dry, samples were transferred to a hot plate, partially covered with microscope cover slips to prevent spattering, and heated at 300°C, which caused evolution of brown nitrogen dioxide gas. After the brown gas completely burned off (ca. 3 h),

the samples were allowed to cool, and 10 ml of 0.1 N HCl was added to each. Samples were covered with tight fitting Teflon-lined caps and heated in an 80°C oven for 30 min. After cooling, samples were adjusted to pH 1.0 and reacted for orthophosphate using the Koroleff (1983) version of the phosphomolybdate-blue method for acidified samples.

Results

Blanks and standards—(1) High-concentration standards (0–20 μM PO_4): Due to the relatively high concentrations of experimental solutions, we employed high-concentration orthophosphate standards that could be measured in a 1-cm cell. For the Ash/Hydrol. method using MgSO_4 as an oxidant, procedural blanks ($0.07 \pm 0.11 \mu\text{M}$, $n = 14$) were indistinguishable from the phosphomolybdate-blue reagent blank ($0.08 \pm 0.12 \mu\text{M}$, $n = 12$). Procedural blanks were higher ($0.22 \pm 0.11 \mu\text{M}$, $n = 14$) for the Ash/Hydrol. method using $\text{Mg}(\text{NO}_3)_2$ as an oxidant and for the Acid Persulfate method ($0.24 \pm 0.15 \mu\text{M}$, $n = 48$). The standard curve for orthophosphate standards in ASW, which were taken through the Ash/Hydrol. method using MgSO_4 and $\text{Mg}(\text{NO}_3)_2$ (internal standards), was indistinguishable from that obtained with standards that were not taken through the Ash/Hydrol. procedure (external standards) (Fig. 2A). The standard curve for orthophosphate standards that were taken through the Acid Persulfate method, however, was steeper than that obtained from external standards (Fig. 2A).

(2) Low-concentration standards (0–3 μM PO_4): Due to the low orthophosphate concentration of natural samples, we employed low-concentration orthophosphate standards that required the use of a 10-cm cell for spectrophotometric determination. Procedural blanks of the Ash/Hydrol. method measured in a 10-cm cell were indistinguishable from external reagent blanks (absorbance = 0.000–0.005 at 880 nm). Low-concentration internal orthophosphate standards again produced the same standard curve as external standards (Fig. 2B). Procedural blanks of the Acid Persulfate method measured in a 10-cm cell averaged $0.14 \pm 0.01 \mu\text{M}$ ($n = 6$). Similar to high-concentration standards, low-concentration internal orthophosphate standards that were taken through the Acid Persulfate method produced a steeper curve than external standards (Fig. 2B). Procedural blanks of the Nitrate Oxidation method measured in a 10-cm cell averaged $0.06 \pm 0.01 \mu\text{M}$ ($n = 4$). Internal orthophosphate standards taken through the Nitrate Oxidation method were indistinguishable from external standards (Fig. 2B).

Recovery experiments—(1) Ash/Hydrol. method: Recovery (% P in the compound recovered as orthophosphate) of 19 organic-P compounds and tripolyphosphate by the Ash/Hydrol. method was evaluated. All compounds were run in quadruplicate at ca. 10 μM P. On average, P recovery by the Ash/Hydrol. method was $99.0 \pm 3.1\%$ (Table 1). Guanosine 5'-triphosphate gave the lowest recovery (93.4%). The mean relative error over quadruplicates was 0.9%.

Results of recovery tests of orthophosphate standards made up in different solution matrices are as follows. Recovery of orthophosphate standards in ASW taken through

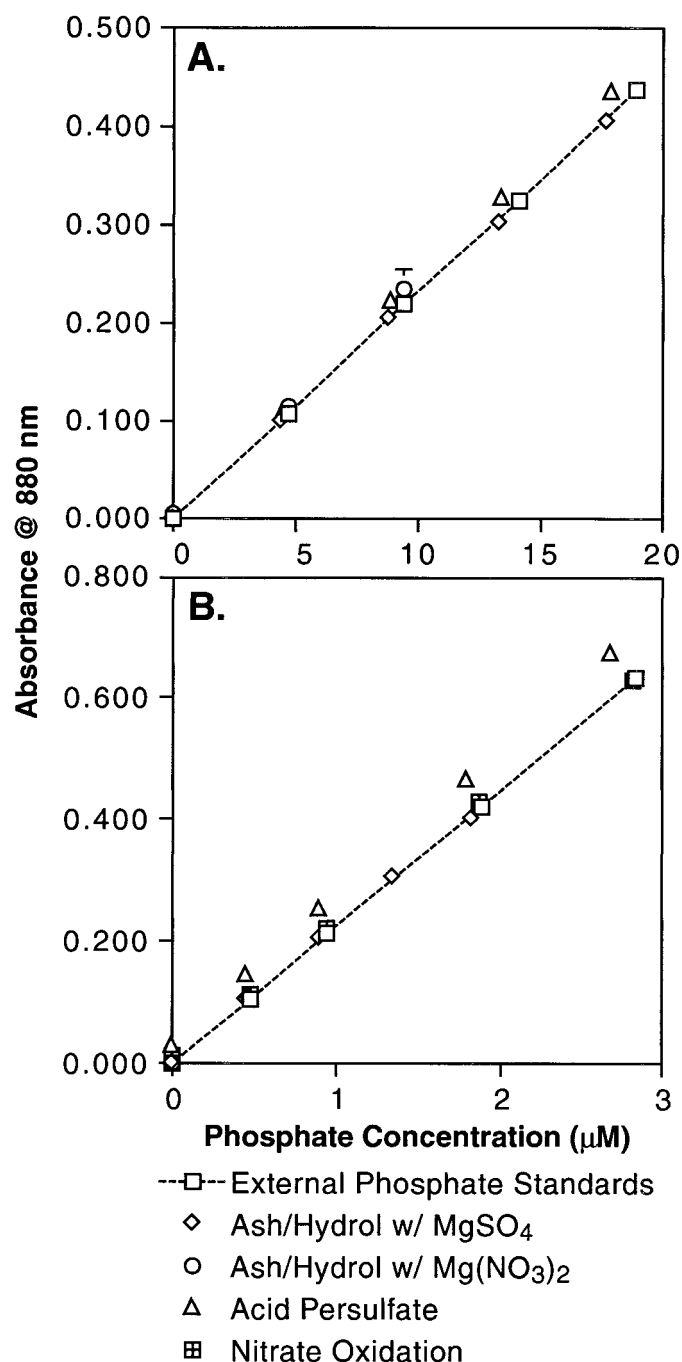


Fig. 2. Standard curves. External orthophosphate standards are those directly reacted using the Koroleff (1983) version of the phosphomolybdate-blue method for acidified samples. All others are orthophosphate standards taken through the TDP method indicated prior to measuring concentrations. Error bars are standard deviations about mean absorbance measurements and, where not visible, are smaller than the symbol. (A) High-concentration standards (0–20 μM) measured in a 1-cm cell. (B) Low-concentration standards (0–3 μM) measured in a 10-cm cell.

the Ash/Hydrol. method averaged $98.5 \pm 0.7\%$, while those in freshwater (milli-Q H_2O) averaged only $85.9 \pm 1.5\%$. In contrast, recovery of orthophosphate standards made up in freshwater (milli-Q H_2O) that had a 1-ml aliquot of 4.3 M NaCl and 0.3 M MgSO_4 added directly to the sample in the TDP vial, bringing it to a final salt concentration equivalent to artificial seawater, averaged $98.8 \pm 0.9\%$.

(2) Ash/Hydrol. method using MgSO_4 vs. $\text{Mg}(\text{NO}_3)_2$: Recovery of 12 organic-P compounds and tripolyphosphate by the Ash/Hydrol. method was evaluated using $\text{Mg}(\text{NO}_3)_2$ as the oxidant instead of MgSO_4 . The Ash/Hydrol. method outlined in the Experimental Design and Procedures section was followed exactly, except the 0.2-ml aliquot of 0.17 M MgSO_4 was replaced with a 0.2-ml aliquot of 50% w/v $\text{Mg}(\text{NO}_3)_2$ solution (as specified by Aspila et al. 1976). On average, the Ash/Hydrol. method using $\text{Mg}(\text{NO}_3)_2$ as the oxidant recovered $99.7 \pm 2.7\%$ of the P (Table 1), statistically indistinguishable from recoveries achieved using MgSO_4 . The mean relative error over quadruplicates was 1.2%.

(3) Acid Persulfate method: Recovery of 12 organic-P compounds and tripolyphosphate was evaluated by the Acid Persulfate method. On average, the Acid Persulfate method recovered $93.0 \pm 13.5\%$ of the P (Table 1). However, only 73.5% of 2-aminoethylphosphonic acid (a phosphonate) and 55.7% of L- α -phosphatidylethanolamine (a phospholipid) was recovered. In contrast, >95% recoveries were obtained for these compounds using the Ash/Hydrol. method. For all other compounds tested, the Acid Persulfate method recovered >93.8% of the combined P. The mean relative error over quadruplicates was 2.9%, considerably poorer than that of the Ash/Hydrol. method.

Hydrolysis experiments—(1) Hydrolysis of organic-P compounds during the phosphomolybdate-blue reaction: Extent of hydrolysis of 12 organic-P compounds and inorganic tripolyphosphate in ASW was evaluated during the time between addition of the acidic (pH 1.0) phosphomolybdate-blue reagents and subsequent measurement (ca. 30 min) (Table 2). Only two compounds, adenosine 5'-diphosphate (2.2% detected as orthophosphate) and phosphocreatine (4.6% detected as orthophosphate), showed >1% hydrolysis of the parent compound.

(2) Long-term hydrolysis of organic-P compounds: The same compounds evaluated for hydrolysis during the phosphomolybdate-blue reaction were monitored for continued hydrolysis over 80 d (Fig. 3). For standards in ASW stored acidified/refrigerated, monophosphate nucleotides, phosphosugars, and phosphonates (seven compounds total) showed no significant hydrolysis (<1%) over the period tested. Tripolyphosphate hydrolyzed 17% and phosphocreatine was 89% hydrolyzed in the first month. The rates of nucleotide hydrolysis follow a coherent pattern: monophosphates show no hydrolysis, diphosphates hydrolyze up to 3%, and triphosphates hydrolyze up to 10% in the first month. Standard splits stored unacidified/frozen displayed low rates of hydrolysis similar to rates observed for acidified/refrigerated splits

Table 2. Hydrolysis of organic-P compounds and tripolyphosphate.

	Compound	During the phosphomolybdate-blue reaction			After 13–16 days of storage	
		*Calculated P Concentration (μM)	† [DIP] (μM) measured	σ % hydrolyzed	‡ % hydrolyzed acidified/refrigerated	‡ % hydrolyzed unacidified/frozen
□	adenosine 5'-monophosphate monohydrate	92.69	0.01	0.01	0.01	0.00
▽	adenosine 5'-diphosphate, sodium salt hydrate	98.65	2.17	2.20	1.31	1.31
○	adenosine 5'-triphosphate, disodium salt hydrate	100.03	0.55	0.55	4.62	0.03
△	guanosine 5'-monophosphate disodium salt	95.00	0.07	0.07	0.02	0.00
●	guanosine 5'-diphosphate tris salt	56.22	0.30	0.53	4.68	0.69
◆	guanosine 5'-triphosphate sodium salt	74.29	0.60	0.81	9.30	0.47
■	tripolyphosphate pentasodium salt hexahydrate	122.00	0.92	0.75	9.36	0.53
★	DL-2-amino-3-phosphonopropionic acid	99.97	0.03	0.03	0.01	0.00
⊕	2-aminoethylphosphonic acid	99.81	0.58	0.58	0.00	0.04
▼	phosphocreatine disodium salt hydrate	58.43	2.68	4.59	86.33	43.03
▣	β -D-glucose-6-phosphate monosodium salt	98.13	0.49	0.50	0.00	0.00
▷	glycerophosphate disodium salt hydrate	100.11	0.08	0.08	0.01	0.00
▣	phytic acid dipotassium salt	91.90	0.42	0.46	0.01	0.05

* Calculated P concentration was obtained using vendor-provided information on formulas and water content except for guanosine 5'-diphosphate, guanosine 5'-triphosphate, phosphocreatine, glucose-6-phosphate, glycerophosphate, and phytic acid where %P was provided by the vendor.

† Compounds were measured for DIP immediately after they were brought into solution.

σ % hydrolyzed = ([DIP] measured/calculated P concentration) \times 100

‡ % hydrolyzed = {([DIP] measured after 13-16 days) - ([DIP] measured during the phosphomolybdate-blue reaction) / calculated P concentration} \times 100

of monophosphate nucleotides, phosphosugars, and phosphonates (Table 2). However, hydrolysis rates of tripolyphosphate, phosphocreatine, and di- and trinucleotides were slowed significantly in unacidified/frozen samples; none of these compounds (except phosphocreatine) exceeded 3.5% hydrolysis over the 80-d test.

The initial ($t = 0$) DIP concentration measured in the acidified/refrigerated Eel River Shelf sample was $1.27 \mu\text{M}$ and remained invariant during the storage period, measuring $1.26 \mu\text{M}$ DIP after 84 d. This result indicates that the DOP present in the sample ($0.20 \mu\text{M}$) did not hydrolyze to any significant extent over the 84-d storage period.

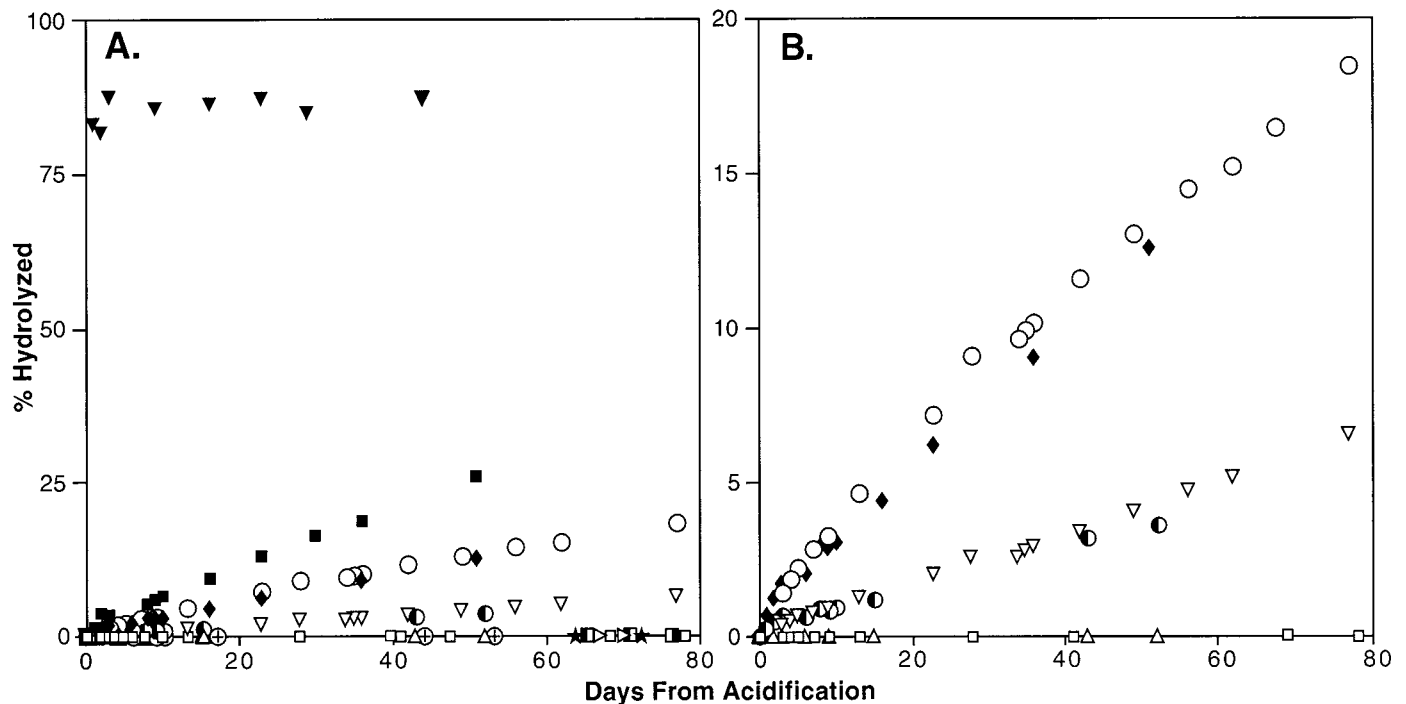


Fig. 3. Hydrolysis experiment. Percent hydrolysis ($\{(\text{measured [DIP] during storage} - [\text{DIP}] \text{ measured prior to storage}) / \text{calculated P concentration of starting solution}\} \times 100$) of compounds stored acidified (pH 1.0) and refrigerated over 80 d. See Table 2 for symbol key. (A) Hydrolysis of all compounds tested. (B) Hydrolysis of nucleotides only, shown with an expanded y-axis.

Table 3. Eel River Shelf water-column TDP measured by the Ash/Hydrol. and Acid Persulfate methods.

Cruise	Station	Water depth (m)	Ash/Hydrol.			Acid Persulfate			Δ of mean [TDP] \ddagger			
			Mean [TDP] (μM) $n = 2$	Δ^*	Rel % error \dagger	Mean [TDP] (μM) $n = 2$	Δ^*	Rel % error \dagger				
Spring	C30	25	1.48	0.00	0.33	1.50	0.00	0.00	-0.02			
		15	1.15	0.01	1.26	1.12	0.01	0.85	0.02			
		3	0.98	0.00	0.49	1.00	0.00	0.00	-0.02			
	C40	35	1.83	0.01	0.79	1.93	0.16	8.13	-0.10			
		20	1.39	0.00	0.35	1.46	0.01	0.65	-0.07			
		10	0.89	0.02	2.17	0.86	0.11	12.66	0.02			
Summer	G40	3	0.78	0.00	0.62	0.84	0.00	0.00	-0.06			
		30	2.27	0.00	0.21	2.43	0.00	0.20	-0.16			
		21	2.25	0.00	0.21	2.25	0.00	0.21	-0.01			
	G50	10	0.75	0.01	1.93	0.79	0.01	1.81	-0.04			
		3	0.73	0.00	0.66	0.69	0.02	2.77	0.04			
		41	2.32	0.01	0.42	2.46	0.00	0.19	-0.15			
		31	2.20	0.01	0.44	2.18	0.14	6.53	0.02			
		21	1.73	0.00	0.28	1.79	0.06	3.11	-0.06			
		10	1.09	0.01	0.72	1.08	0.07	6.86	0.01			
	C30	3	0.41	0.01	3.58	0.39	0.06	14.10	0.01			
		18	1.79	0.02	1.08	1.81	0.01	0.51	-0.03			
		10	0.79	0.00	0.62	0.82	0.00	0.56	-0.04			
		3	0.66	0.07	10.27	0.66	0.00	0.71	0.01			
		C40	31	1.68	0.00	0.00	1.73	0.00	0.27	-0.04		
			20	1.13	0.00	0.43	1.08	0.01	1.28	0.05		
	10		0.58	0.00	0.84	0.59	0.01	1.57	-0.01			
	Winter	G40	3	0.51	0.02	3.84	0.52	0.00	0.00	-0.01		
			37	0.95	0.00	0.51	0.96	0.01	0.97	0.00		
			20	0.85	0.07	8.64	0.92	0.02	2.01	-0.07		
			10	0.82	0.01	1.20	0.89	0.10	11.47	-0.07		
			3	0.83	0.04	4.65	0.77	0.00	0.60	0.06		
			G50	45	0.99	0.01	1.47	0.96	0.01	1.45	0.03	
		30		0.89	0.06	6.56	0.98	0.18	18.03	-0.09		
		20		0.95	0.13	13.82	0.89	0.00	0.52	0.06		
10		0.85		0.01	1.72	0.79	0.00	0.59	0.06			
3		0.80		0.00	0.61	0.79	0.01	1.18	0.01			
C30		20		0.79	0.01	1.22	0.78	0.01	1.19	0.01		
		10	0.80	0.00	0.61	0.77	0.06	7.20	0.03			
		3	0.83	0.00	0.58	0.78	0.01	1.19	0.04			
		C40	42	1.02	0.00	0.00	0.98	0.00	0.47	0.04		
			25	0.93	0.02	2.07	0.92	0.00	0.00	0.01		
			10	0.77	0.01	1.25	0.73	0.01	1.90	0.04		
			3	0.76	0.00	0.63	0.80	0.00	0.00	-0.04		
Mean \S				0.02		1.98		0.03		2.87		
Mean abs. diff.											0.04	

* Δ = difference between duplicate measurements.

\dagger Rel % error = $\{(\Delta/\text{mean [TDP]}) \times 100\}$.

\ddagger Δ of mean [TDP] = mean [TDP] measured by Ash/Hydrol minus mean [TDP] measured by Acid Persulfate.

\S Mean = mean difference between duplicate measurements and mean relative % error in about 39 samples.

|| Mean absolute difference = mean of the absolute delta values in about 39 samples.

TDP in Eel River Shelf samples—Comparison of TDP methods on natural seawater samples: Of the 39 samples measured by both methods, the Ash/Hydrol. and Acid Persulfate methods gave statistically identical TDP concentrations for 21 samples (Table 3). The Acid Persulfate method reports statistically higher TDP concentrations in 10 samples, and the Ash/Hydrol. method reports statistically higher concentrations in eight. Relative percentage error about duplicate TDP measurements averaged 2.0% by the Ash/Hydrol. method and 2.9% by the Acid Persulfate method.

Six samples were also measured by the Nitrate Oxidation

method. On average, the difference between TDP concentrations measured by the Ash/Hydrol. and Nitrate Oxidation methods was 0.06 μM (Table 4). However, in contrast to the Acid Persulfate method comparison, all Ash/Hydrol. method measurements were higher, indicating an average of a 7% under-recovery by the Nitrate Oxidation method. Precision of the Nitrate Oxidation and Ash/Hydrol. methods was similar.

Seasonal DOP profiles: DOP concentrations in surface waters were equal to or less than DIP in spring and winter

(Fig. 4). In summer, however, DOP exceeded DIP concentrations in surface waters at all stations. The DOP and DIP profile structure was most pronounced in summer, with elevated DOP concentrations in surface waters (mean = 0.49 μM) decreasing with depth, mirrored by low DIP (mean 0.12 μM) in surface waters, steeply increasing with depth. Although surface-water DOP concentrations were elevated in spring, they never exceeded DIP. There was no discernible structure to DOP profiles in winter and little evidence of DIP gradients with depth.

Discussion

Review of TDP method development and evaluation of methods—A number of studies have evaluated the relative ease and efficiency of TDP oxidation by the five most commonly used methods: ultraviolet (UV) photooxidation, Acid and Alkaline Persulfate digestion, Ash/Hydrol., and Nitrate Oxidation. Ridal and Moore (1990) recommended a combined UV photooxidation/Acid Persulfate oxidation method. K erouel and Aminot (1996) recommended the Alkaline Persulfate method for seawater, but they also believe that the Ash/Hydrol. method of Sol rzano and Sharp (1980) effects 100% conversion of combined-P to orthophosphate. Ormaza-Gonz lez and Statham (1996) preferred the Nitrate Oxidation method. There is agreement that UV photooxidation alone is not sufficient for the oxidation of a number of important organic-P compounds (e.g., those with a polyphosphate moiety, such as nucleotide polyphosphates: Sol rzano and Sharp 1980; Ormaza-Gonz lez and Statham 1996). One of the earliest methods employed for TDP determination, Perchloric Acid Digestion (Hansen and Robinson 1953; Strickland and Parsons 1972), has been discounted by most workers because of its hazardous nature (Menzel and Corwin 1965; Cembella et al. 1986). Our findings indicate that the Ash/Hydrol. method yields equivalent or superior TDP recovery of both standards and seawater when compared with other available methods.

TDP Recovery Experiments—The primary question in validating TDP measurements is whether the method employed converts all organic P to orthophosphate. To evaluate measured TDP recovery, however, the necessity of running internal vs. external blanks and standards must first be determined.

Blanks and standards: The agreement of external and internal orthophosphate standards for the Ash/Hydrol. method (Fig. 2) permits calculation of sample concentrations by external standards, which greatly simplifies and streamlines the method. Blank absorbance for internal standards taken through the Ash/Hydrol. method, even when measured in a 10-cm cell, is below detection limits. The absence of a blank results in a low detection limit for the Ash/Hydrol. method (0.02 μM). Triplicate internal blanks and standards are routinely run in parallel with samples, however, to verify recovery.

The Acid Persulfate method has a rather high blank (0.14 \pm 0.01 μM) when measured in a 10-cm cell, making resolution of the low DOP levels typically observed in seawater

Table 4. Comparison of TDP measurements made using the Ash/Hydrol., Acid Persulfate, and Nitrate Oxidation methods.

Cruise	Station	Water depth (m)	Ash/Hydrol.			Acid Persulfate			Nitrate Oxidation		
			Mean [TDP] (μM) n = 2	Δ^*	Rel % error†	Mean [TDP] (μM) n = 2	Δ^*	Rel % error†	Mean [TDP] (μM) n = 2	Δ^*	Rel % error†
Summer	C40	20	1.13	0.00	0.43	1.08	0.01	1.28	1.04	0.04	3.77
		10	0.58	0.00	0.84	0.59	0.01	1.57	0.53	0.00	0.92
		3	0.51	0.02	3.84	0.52	0.00	0.00	0.47	0.00	1.04
Winter	C40	25	0.93	0.02	2.07	0.92	0.00	0.00	0.86	0.00	0.57
		10	0.77	0.01	1.25	0.73	0.01	1.90	0.72	0.02	2.19
		3	0.76	0.00	0.63	0.80	0.00	0.00	0.72	0.01	1.36

* Δ = difference between duplicate measurements.
 † Rel % error = $\{(\Delta/\text{mean [TDP]}) \times 100\}$.

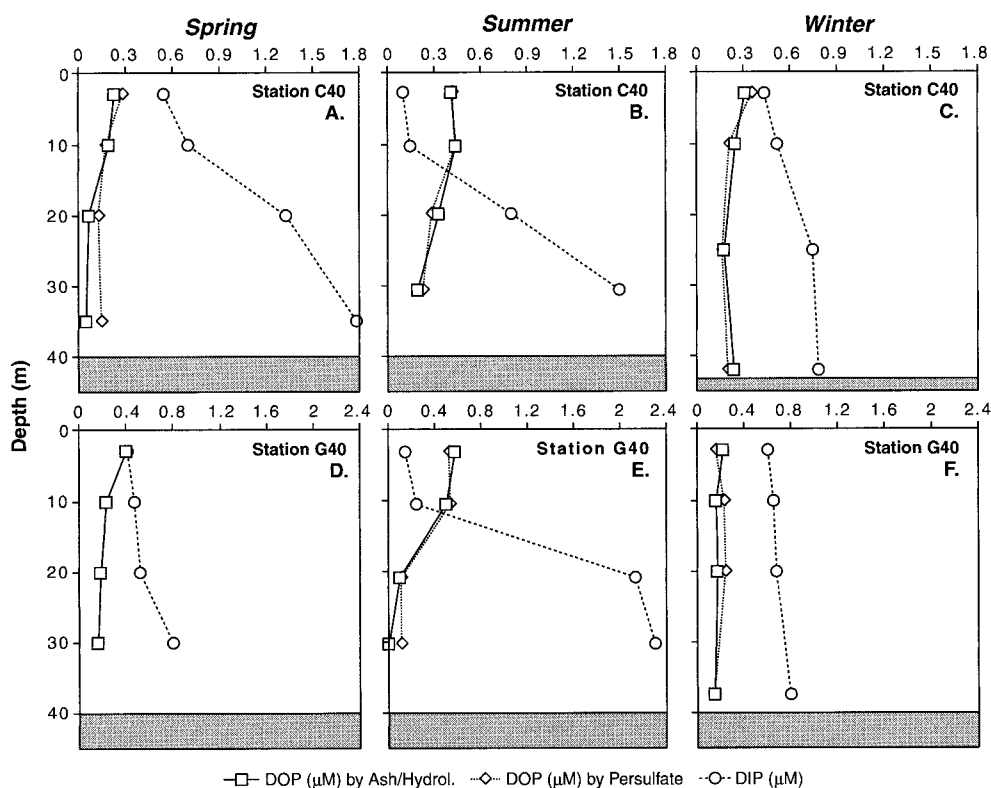


Fig. 4. Seasonal Eel River Shelf dissolved phosphorus profiles. Comparison of DOP in Eel River Shelf samples obtained from the Ash/Hydrol. vs. the Acid Persulfate methods and DIP: (A) Sta. C40—spring; (B) Sta. C40—summer; (C) Sta. C40—winter; (D) Sta. G40—spring (no persulfate data available); (E) Sta. G40—summer; and (F) Sta. G40—winter. Profiles observed at these two stations are typical of shelf-wide seasonal DOP and DIP distributions.

problematic. This blank could be reduced by reagent purification or reduction to minimal required reagent levels. Samples and standards taken through the Acid Persulfate method require molybdate reagents different from the standard DIP reagents, resulting in a discrepancy in slope between internal and external standards (Fig. 2). As a result, internal standards must be run routinely. This, in conjunction with the high internal blank, makes it necessary to run many more standards and blanks than is necessary for the Ash/Hydrol. method. The Nitrate Oxidation method has an intermediate blank ($0.07 \pm 0.01 \mu\text{M}$), creating the same detection limit problem incurred with the Acid Persulfate method.

Recovery by the Ash/Hydrol. method: Recovery for the organic phosphorus compounds assayed averaged $99 \pm 3\%$ by the Ash/Hydrol. method, and the minimum recovery observed was 93% (Table 1). Similar recovery tests on the Ash/Hydrol. method reported by Ormazza-González and Statham (1996) yielded an average of $95 \pm 6\%$ recovery of organic-P standards in seawater. Kérouel and Aminot (1996) found $>90\%$ recovery for all organic-P standards tested except guanosine 5'-monophosphate (GMP: apparent recovery of 76%). The authors attributed low GMP recovery to inflation of original compound weight due to moisture content and impurities. Contrary to the speculation of Cembella et al. (1986), the Ash/Hydrol. method was able to recover 95% of

a phosphonate (2-aminoethylphosphonic acid) that contains a difficult to break P-C bond; Kérouel and Aminot (1996) reported 94% recovery of this compound. Since the Ash/Hydrol. method successfully recovered organic-P compounds at concentrations of $10 \mu\text{M P}$ (at least one order of magnitude above normal seawater DOP concentrations), it is assumed that this method is able to oxidize, hydrolyze, and retain the low DOP concentrations typically found in natural waters.

There is no evidence of P volatilization from organic- and inorganic-P standards in seawater using the Ash/Hydrol. method. In freshwater, however, we observed underrecovery of orthophosphate standards. This problem is remedied by adding salt to freshwater samples before drying down. The quantity of salt contained in the 0.2-ml aliquot of the procedural oxidant (0.17 M MgSO_4) is not adequate. However, we have demonstrated that adding a 1-ml aliquot per 10-ml sample of a concentrated salt solution (4.3 M NaCl and 0.3 M MgSO_4) directly to freshwater samples in the TDP vial, and then following the standard Ash/Hydrol. protocol, eliminates underrecovery. The addition of this quantity of brine solution brings the final concentration of salts in the standard up to the level present in ASW. The resulting 99% recovery of orthophosphate standards is comparable to that obtained in natural and artificial seawater. The presence of salts promotes cation-phosphate complexation, which prevents formation of volatile phosphate species.

Recovery by the Ash/Hydrol. method using $\text{Mg}(\text{NO}_3)_2$: One element recommending the Ash/Hydrol. method is its similarity to the method of Aspila et al. (1976), routinely used to oxidize sedimentary organic P (Ruttenberg 1992), which is likely more refractory than seawater DOP. The Ash/Hydrol. and Aspila methods operate by the same mechanisms: dry ashing/oxidation with post-ashing acid hydrolysis. However, the Aspila method uses $\text{Mg}(\text{NO}_3)_2$ instead of MgSO_4 as an oxidant. No significant difference in recovery of organic-P standards was observed with $\text{Mg}(\text{NO}_3)_2$ (Table 1), even though NO_3^- is a stronger oxidant than SO_4^{2-} (e.g., Stumm and Morgan 1981). The fact that NO_3^- is a stronger oxidant might argue for its routine use in lieu of MgSO_4 . However, the higher blank associated with $\text{Mg}(\text{NO}_3)_2$ leads us to recommend use of MgSO_4 , as initially proposed by Solórzano and Sharp (1980). It is unlikely that the oxidizing potential of MgSO_4 would be exceeded in natural waters, which typically have low DOP levels.

Recovery by the Acid Persulfate method: Twelve of the organic-P compounds and inorganic tripolyphosphate tested for recovery by the Ash/Hydrol. method were tested for recovery by the Acid Persulfate method. For the majority of compounds tested, recovery by the acid persulfate method was comparable to the Ash/Hydrol. method (Table 2). The exceptions to this are 2-aminoethylphosphonic acid (a phosphonate) and L- α -phosphatidylethanolamine (a phospholipid). The Acid Persulfate method converted only 73.5% of the P in 2-aminoethylphosphonic acid to orthophosphate. The most likely explanation for the low recovery is that Acid Persulfate is incapable of complete destruction of the C-P bond found in phosphonates. The same phenomenon was observed by Kérouel and Aminot (1996), who obtained only 77% recovery of 2-aminoethylphosphonic acid in seawater by the Acid Persulfate method. The Acid Persulfate method recovered only 55.7% of a phospholipid, L- α -phosphatidylethanolamine (Table 1). Cembella and Antia (1986) and Menzel and Corwin (1965) reported apparently acceptable recoveries of lecithin (phosphatidylcholine), another phospholipid (96.2 and 101% recovery, respectively). However, the former study used a combined enzyme hydrolysis–persulfate method, and the latter did not report absolute recoveries but instead, normalized to the amount recovered by a sulfuric acid digestion method, which they assumed to yield 100%. These results are therefore not a clean test of the Acid Persulfate method and are not directly comparable to the results reported here, which indicate that phospholipids are underrecovered by the Acid Persulfate method.

The effect that under-recovery of these compounds will have on natural samples depends on the composition of the DOP pool, which may vary seasonally and across different habitats (Thomson-Bulldis and Karl 1998). If seawater DOP has an appreciable phospholipid or phosphonate component, which may be the case in some environments (Clark et al. 1998), there is potential for underestimation of DOP by the Acid Persulfate method. In addition, the error associated with quadruplicate recovery measurements (2.94%) is considerably poorer than the Ash/Hydrol. method (0.87%), possibly due to evaporation during autoclaving. The combination of underrecovery of standards from two compound

classes (phosphonates and phospholipids) and the larger errors in precision associated with the Acid Persulfate method (a problem also reported by Solórzano and Sharp 1980) leads us to refrain from recommending its routine use with natural samples.

Hydrolysis of organic-P compounds—Hydrolysis of organic-P compounds during the phosphomolybdate-blue reaction: Concerns about hydrolysis of organic-P compounds during analysis and their subsequent measurement as DIP have been raised (e.g., Rigler 1968; Ridal and Moore 1990; Karl and Tien 1997; Thomson-Bulldis and Karl 1998). We tested the extent of hydrolysis of organic-P compounds during the Koroleff (1983) version of the phosphomolybdate-blue reaction for acidified samples. Of the compounds tested, only two yielded >1% orthophosphate (Table 2): adenosine 5'-diphosphate (2.20%) and phosphocreatine (4.59%). These should be considered maximum estimates of DOP hydrolysis during reaction, since the original standard may have contained orthophosphate impurities that would inflate estimates of the extent of parent compound hydrolysis. Similar levels of DOP hydrolysis during the acid–molybdate reaction were observed by Thomson-Bulldis and Karl (1998) for 10 of the 13 compounds we tested, and Kérouel and Aminot (1996) found that only 2 out of 10 DOP compounds yielded >2% orthophosphate: phytic acid (14%) and riboflavin monophosphate (7%). We observed lower percentage orthophosphate on similar compound types (Table 2) and suspect that the higher levels detected by Kérouel and Aminot (1996) may have been due to orthophosphate impurities. Our results indicate that if DIP is measured immediately after collection, only a small fraction (<5%) of organic P will be detected as DIP.

Long-term hydrolysis of organic-P compounds during storage: Since it is often not possible to measure samples immediately, we tested the extent of hydrolysis over time under two storage conditions: acidified (pH 1.0)/refrigerated and unacidified/frozen (Table 2). Prolonged storage before measurement of DIP increases the chance for hydrolysis of organic P. Results of the hydrolysis experiments (Fig. 3A) suggest that the extent of DOP degradation during storage will be determined by the constituents of the DOP pool. If the DOP pool is made up of “high-energy” compounds (e.g., phosphocreatine) and triphosphate nucleotides, there could be significant hydrolysis. However, if it is comprised mainly of phosphosugars, monophosphate nucleotides, phosphonates, phospholipids, and phosphonic acids, acidified/refrigerated samples could be stored for ≥ 1 yr without significant hydrolysis.

The Eel River Shelf sample that was monitored for hydrolysis over the same 84-d period as the standards showed no evidence of hydrolysis. Therefore, while typical storage protocols call for frozen storage of samples for phosphate analysis (Karl and Tien 1992; Dore et al. 1996; Kérouel and Aminot 1996; but *see also* Chapman and Mostert 1990), we have found that refrigerated storage of filtered samples acidified to pH 1 with HCl is a viable alternative. This storage protocol is also advantageous for natural waters from reducing environments (e.g., anoxic water-column or pore-water

samples), where it will prevent oxidation of dissolved ferrous iron and sorptive removal of P onto newly formed iron oxyhydroxide phases. In addition, when collected using trace metal clean methods and preserved using high-purity acid, this sample can also be analyzed for trace metals.

This result suggests the potential to store filtered/acidified natural samples for prolonged periods without DOP hydrolysis, opening up the possibility for remote, mooring-based time-series collection of water-column DOP data. However, the possibility exists that the oceanic DOP pool composition varies geographically and that some environments may contain a higher quantity of more easily hydrolyzed DOP compounds. Until the natural DOP pool is characterized, it is not possible to correct for potential hydrolysis of organic-P compounds and subsequent overestimation of DIP. The most conservative approach is therefore to measure DIP as soon as possible.

The order of hydrolysis rates observed in our study—for example, that nucleotides are more susceptible to breakdown than are phosphoesters and phosphosugars—is consistent with the relative bioavailabilities of DOP compounds as determined by Björkman and Karl (1994). Thus, our results provide a rough guide for evaluating the reactivity of a range of aquatic DOP compound classes. The relative stability of the DOP compounds studied (Fig. 3A) may be considerably different in seawater at pH 8, however, since their rates and mechanisms of hydrolysis are pH-dependent (Admiraal and Herschlag 1995). In addition, natural waters potentially contain phosphohydrolytic enzymes that are absent in our experimental solutions.

The abiotic reactivities of these compounds are also consistent with the observed order of hydrolysis rates. The difference between the mono-, di-, and triphosphate rates can be attributed to the relative pKa's of the leaving groups in their respective reactions (Admiraal and Herschlag 1995). For example, phosphate release from AMP and GMP was below detection, while ADP, ATP, GDP, and GTP showed significant phosphate release (Fig. 3B). This is because AMP and GMP have only a phosphoester bond, while the di- and triphosphates (ADP, GDP, ATP, and GTP) have one or two phosphoanhydride bonds in addition to the phosphoester bond. Phosphoanhydride bonds are more prone to hydrolysis, and the triphosphates with two phosphoanhydride bonds are hydrolyzed approximately threefold faster than the diphosphates, which have only one.

DOP in coastal waters of the Eel River Shelf—Little information exists on the specific chemical composition of DOP in seawater. Because it is comprised of excreta from in situ biota plus terrestrial DOP that enters coastal waters via rivers, the chemical composition of coastal DOP might reasonably be expected to vary seasonally. This is particularly true in a system like the Eel River Shelf, which receives tremendous riverine input during winter but is dominated in summer by upwelling-driven biological productivity.

Comparison of TDP measurements by different methods on natural seawater samples: Field measurements of TDP were used to explicitly evaluate the occurrence of seasonal changes in DOP composition. If the make-up of the DOP

pool in summer, when upwelling fueled large algal blooms, is different from that in winter, when algal productivity is low and the potential for terrestrial DOP input from rivers is high, we may observe differences in the relative recoveries by the different TDP methods. The Ash/Hydrol. and Acid Persulfate methods yielded comparable TDP concentrations during spring, summer, and winter (Table 3), however, suggesting that phospholipids and phosphonates (which are underrecovered by the Acid Persulfate method) must not be significant components of the DOP reservoir in this region. If seasonal variability in Eel River Shelf DOP occurs, it does not involve these two compound classes. Further, the absence of an important phosphonate component in Eel River shelf DOP contrasts with results from an oligotrophic region (Clark et al. 1998), where phosphonates are a dominant component. This finding highlights the possibility of significant spatial/environmental heterogeneity in DOP composition (Thomson-Bulldis and Karl 1998). The rather high blank associated with the Acid Persulfate method did not adversely affect this comparison because of the relatively high TDP levels in these coastal waters.

We observed an average of 7% TDP underrecovery with Nitrate Oxidation when compared to the Ash/Hydrol. method (Table 4). This is counter to results reported by Ormazá-González and Statham (1996), who, while obtaining comparable recovery of organic-P standards by both methods, found higher TDP recovery in three seawater samples by Nitrate Oxidation and assumed this discrepancy reflected loss by volatilization during Ash/Hydrol. While this discrepancy is difficult to assess because of low sample numbers, we maintain that our results provide no evidence that P is lost by volatilization, or any other mechanism, during the Ash/Hydrol. method.

Dynamic structure of DOP profiles on the Eel River Shelf: The seasonal features of DOP and DIP profiles on the Eel River Shelf are consistent with seasonal changes in biological activity, as inferred from water-column chlorophyll *a* (Chl *a*) concentrations. Elevated DOP concentrations in summer coincide spatially with highly productive (Chl *a* = 10.36–19.60 $\mu\text{g liter}^{-1}$; Ruttenberg unpubl. data) surface waters. The decrease in DOP concentrations with depth is mirrored by a steep increase in DIP immediately beneath the Chl *a* maximum (Ruttenberg unpubl. data). Surface-water DOP concentrations are somewhat elevated in spring, a time of moderately productive surface waters (Chl *a* = 3.29–5.51 $\mu\text{g liter}^{-1}$; Ruttenberg unpubl. data), but this feature is less pronounced than in summer. Unlike summer profiles, spring DOP concentrations never exceed DIP.

The coincidence of highly productive surface waters with high DOP and low DIP concentrations in summer suggests that DOP may be an important P source to the biological community. This is supported by the presence of alkaline phosphatase (APase) in summer surface waters at sites with low DIP (Ruttenberg et al. 1998). APase is an inducible enzyme in algae and bacteria whose presence indicates phosphate limitation and suggests that organisms are hydrolyzing DOP to satisfy their nutritional phosphate demand. The relative rates of DOP production and consumption will control the period over which the high-concentration surface-water

DOP pool persists. Although our data suggest variability in DOP inventories and depth distributions on the time scale of seasons, determination of short-term variation of surface-water DOP during a seasonal algal bloom would require high-resolution time-series monitoring during the lifetime of the bloom. The absence of structure in winter DOP profiles is consistent with low productivity ($\text{Chl } a = 0.01\text{--}1.25 \mu\text{g liter}^{-1}$; Ruttenberg unpubl. data).

Conclusions

The Ash/Hydrol. method introduced by Solórzano and Sharp (1980), with minor modifications, provides an accurate and precise way of measuring TDP in natural waters. Contrary to earlier concerns, efficient recovery of phosphonates is achieved by this method (2-aminoethylphosphonic acid recovery > 95%). We found no evidence to suggest that P is volatilized from seawater during the Ash/Hydrol. procedure. However, we did observe loss of P in a freshwater matrix and recommend a salt addition step to remedy this problem. We refrain from endorsing the Acid Persulfate method for routine measurement of TDP in natural samples because it was unable to fully recover DOP compounds from two compound classes: phosphonates and phospholipids. In addition, unless the blank associated with the Acid Persulfate method can be lowered and its precision improved, it will be difficult to obtain accurate concentration data in waters with low TDP levels.

Concerns about underestimation of DOP through hydrolysis of organic-P compounds during the phosphomolybdate-blue reaction for DIP are valid; however, the degree of hydrolysis was minimal for most compounds tested. Long-term (months) storage under acidic conditions also resulted in minimal hydrolysis of a number of DOP compounds and a natural water sample, raising the possibility of remote, mooring-based time-series DOP data collection. Until we have a better understanding of the compositional make-up of seawater DOP and how it varies geographically and seasonally, long-term storage remains an intriguing but potentially risky proposition.

The comparable TDP concentrations obtained by the Ash/Hydrol. and Acid Persulfate methods in Eel River Shelf waters indicate that phospholipids and phosphonates, which are under-recovered by the Acid Persulfate method, were likely not dominant components of the DOP reservoir. This contrasts with the apparent importance of phosphonates in oligotrophic regions (Clark et al. 1998) and suggests that there may be significant spatial/environmental heterogeneity in DOP composition.

The relative abundance of DIP and DOP in surface waters of the Eel River Shelf displays significant seasonal variation. Surface DOP levels surpassed levels of DIP during the highly biologically productive summer season, at which time alkaline phosphatase was detected in surface waters, suggesting that DOP was being utilized as a P source. These observations underscore the necessity of quantifying dissolved organic nutrients when attempting to characterize bioavailable nutrient inventories and assessing nutrient limitation.

References

- ADMIRAAL, S., AND D. HERSCHLAG. 1995. Mapping the transition state for ATP hydrolysis: Implications for enzymatic catalysis. *Chem. Biol.* **2**: 729–739.
- ASPILA, K. I., H. AGEMIAN, AND A. S. Y. CHAU. 1976. A semi-automatic method for the determination of inorganic, organic, and total phosphate in sediments. *Analyst* **101**: 187–197.
- BJÖRKMANN, K., 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.
- BUTLER, E. I., S. KNOX, AND M. I. LIDDICOAT. 1979. The relationship between inorganic and organic nutrients in seawater. *J. Mar. Biol. Assoc. U.K.* **59**: 239–250.
- CEMBELLA, A. D., AND N. J. ANTIA. 1986. The determination of phosphonates in seawater by fractionation of the total phosphorus. *Mar. Chem.* **19**: 205–210.
- , ———, AND F. J. R. TAYLOR. 1986. The determination of total phosphorus in seawater by nitrate oxidation of the organic component. *Water Res.* **20**: 1197–1199.
- CHAPMAN, P., AND S. A. MOSTERT. 1990. Does freezing of nutrient samples cause analytical errors? *S. Afr. J. Mar. Sci.* **9**: 239–247.
- CLARK, L. L., E. D. INGALL, AND R. BENNER. 1998. Marine phosphorus is selectively mineralized. *Nature* **393**: 426.
- COTNER, J., J. AMMERMAN, E. PEELE, AND E. BENTZEN. 1997. Phosphorus-limited bacterioplankton growth in the Sargasso Sea. *Aquat. Microb. Ecol.* **13**: 141–149.
- DORE, J. E., T. HOULIHAN, D. V. HEBEL, G. TIEN, L. TUPAS, AND D. KARL. 1996. Freezing as a method of sample preservation for the analysis of dissolved inorganic nutrients in seawater. *Mar. Chem.* **53**: 173–185.
- HANSEN, A. L., AND R. J. ROBINSON. 1953. The determination of organic phosphorus in sea water with perchloric acid oxidation. *J. Mar. Res.* **12**: 31–42.
- JACKSON, G. A., AND P. M. WILLIAMS. 1985. Importance of dissolved organic nitrogen and phosphorus to biological nutrient cycling. *Deep-Sea Res.* **32**: 223–235.
- KARL, D. M., L. LETELIER, J. 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.
- , AND G. TIEN. 1992. MAGIC: A sensitive and precise method for measuring dissolved phosphorus in aquatic environments. *Limnol. Oceanogr.* **37**: 77–96.
- , AND ———. 1997. Temporal variability in dissolved phosphorus concentrations in the subtropical North Pacific Ocean. *Mar. Chem.* **56**: 77–96.
- KÉROUEL, R., AND A. AMINOT. 1996. Model compounds for the determination of organic and total phosphorus dissolved in natural waters. *Anal. Chem. Acta* **318**: 385–390.
- KOROLEFF, F. 1983. Determination of phosphorus. *In* *Methods of seawater analysis*. Verlag.
- KROM, M. D. N. KRESS, S. BRENNER, AND L. I. GORDON. 1991. Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. *Limnol. Oceanogr.* **36**: 424–432.
- MACRAE, M., W. GLOVER, J. AMMERMAN, R. SADA, AND B. RUVALCABA. 1994. Seasonal phosphorus deficiency in the Mississippi River Plume: Unusually large areal extent during the record flood of 1993. *EOS Trans. Am. Geophys. Union* **75**: 30.
- MENZEL, D. M., AND C. CORWIN. 1965. The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulfate oxidation. *Limnol. Oceanogr.* **10**: 441–451.
- ORMAZA-GONZÁLEZ, F. I., AND P. J. STATHAM. 1996. A comparison

- of methods for the determination of dissolved and particulate phosphorus in natural waters. *Water Res.* **30**: 2739–2747.
- ORRETT, K., AND D. M. KARL. 1987. Dissolved organic phosphorus production in surface seawaters. *Limnol. Oceanogr.* **32**: 383–395.
- REDFIELD, A. C., H. P. SMITH, AND B. KETCHUM. 1937. The cycle of organic phosphorus in the Gulf of Maine. *Biol. Bull.* **73**: 421–443.
- RIDAL, J. J., AND R. M. MOORE. 1990. A re-examination of the measurement of dissolved organic phosphorus in seawater. *Mar. Chem.* **29**: 19–31.
- RIGLER, F. H. 1968. Further observations inconsistent with the hypothesis that the molybdenum blue method measured orthophosphate in lake water. *Limnol. Oceanogr.* **13**: 7–13.
- RIVKIN, R. B., AND E. SWIFT. 1985. Phosphorus metabolism of oceanic dinoflagellates: Phosphate uptake, chemical composition, and growth of *Pyrocystis noctiluca*. *Mar. Biol.* **88**: 189–198.
- RUTTENBERG, K. C. 1992. Development of a sequential extraction method for different forms of phosphorus in marine sediments. *Limnol. Oceanogr.* **37**: 1460–1482.
- , E. J. MONAGHAN, AND N. E. KEON. 1998. Seasonal cycle of dissolved organic phosphorus in a coastal marine system: The Eel River Shelf. *EOS Trans. Am. Geophys. Union* **79**: OS52.
- SOLÓRZANO, L., AND J. H. SHARP. 1980. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. *Limnol. Oceanogr.* **25**: 754–758.
- STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. Determination of total phosphorus. *In* Practical handbook of seawater analysis. Fisheries Research Board of Canada.
- STUMM, W., AND J. J. MORGAN. 1981. *Aquatic chemistry: An introduction emphasizing chemical equilibria in natural waters*, 2nd ed. Wiley.
- THOMSON-BULLDIS, A., AND D. KARL. 1998. Application of a novel method for phosphorus determination in the oligotrophic North Pacific Ocean. *Limnol. Oceanogr.* **43**: 1565–1577.

Received: 11 June 1998

Accepted: 10 May 1999

Amended: 14 May 1999