Chapter 6

Dynamics of DOP

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I. INTRODUCTION

Phosphorus (P) is an essential macronutrient for all living organisms; life is truly built around P (deDuve, 1991). In the sea, P exists in both dissolved and particulate pools with inorganic and organic forms. The uptake, remineralization and physical and biological exchanges among these various pools are the essential components of the marine P cycle (Fig. 1). Compared to the much more comprehensive investigations of carbon (C) and nitrogen (N) dynamics in the sea, P pool inventories and fluxes are less well documented though no less important.

During cell growth, P is incorporated into a broad spectrum of organic compounds with vital functions including structure, metabolism, and regulation. In time, selected P-containing organic compounds are lost from the cells to the surrounding environment by combined exudation and excretion processes. When cells turn over, whether by death/autolysis, grazing, parasitism, or viral infection, there is an enhanced release of intracellular P-containing compounds as both dissolved and particulate organic matter (DOM and POM, respectively). In this broad view, dissolved organic P (DOP) is simply the intermediate between inorganic P (Pi) uptake and Pi regeneration (Fig. 1). For this and other ecological and analytical interdependencies of Pi and DOP, it is impossible to isolate DOP from the remainder of the marine P cycle. It is also imperative to emphasize that the production and cycling of P-containing compounds are inextricably linked to C and N dynamics by virtue of the fact that marine DOM and POM pools include many compounds that contain both C and P (e.g., phospholipids, sugar phosphates and selected vitamins and phosphonates) or C, N, and P (e.g., nucleotides, nucleic acids, and selected phosphonates; see Figs. 2 and 3 and Table 1). It is, therefore, inappropriate to consider DOP as separate from dissolved organic C (DOC) and dissolved organic N (DON) or to view the P-cycle in any similar biogeochemical isolation.

This review will take a holistic approach to the marine P-cycle with an emphasis on the production and turnover of P-containing and N-and-P-containing dissolved organic matter (i.e., DOC-P and DOC-N-P, hereafter collectively referred to as DOP). By design, this chapter will focus on the pelagic environment, especially the open sea. Investigation of the marine sedimentary P cycle is further complicated by the presence of numerous poorly defined P reservoirs (e.g., Ruttenberg, 1992;
Dynamics of DOP

Figure 1  The open-ocean P cycle showing the various sources and sinks of inorganic and organic P, including biotic and abiotic interconversions. The large rectangle in the center represents the upper water column TDP pool composed of Pi, inorganic polyphosphate, and a broad spectrum of largely uncharacterized DOP compounds. Ectoenzymatic activity (Ecto) is critical for microbial assimilation of selected TDP compounds. Particulate P, which includes all viable microorganisms, sustains the P cycle by assimilating and regenerating Pi, producing and hydrolyzing selected non-Pi P, especially DOP compounds, and supporting net particulate matter production and export. Atmospheric deposition, horizontal transport, and the upward flux of low-density organic P compounds are generally poorly constrained processes in most marine habitats. Phosphine (PH₃), shown at the right, is the most reduced form of P in the biosphere and is generally negligible except under very unusual, highly reduced conditions. Redrawn from Karl and Björkman (2001).

Anderson and Delaney, 2000) which precludes a straightforward determination of P inventories and fluxes. While the majority of P-cycle processes occur throughout the world’s oceans, net DOM/POM production is enhanced in the euphotic zone (e.g., the upper 0–100 m of the water column) while net remineralization of DOM/POM generally occurs at greater water depths. This vertical stratification of the marine P cycle (as well as C and N cycles) is an important factor which ultimately controls the distributions and abundances of microbial biomass and rates of global ocean biomass production, and greatly impacts the sources, sinks, and, most likely, chemical composition of marine DOM.
Figure 2  Bar and shield representation of dissolved matter in seawater showing the intersection of C, N, and P compound classes. For example, dissolved P can exist in a variety of inorganic P forms (outside portion of the shield) or as DOC-P and DOC-N-P compounds. Likewise, C and N have both unique and intersecting pools. Compound symbols include: Pi, orthophosphate; PPI, pyrophosphate (pyro-Pi); PPI, inorganic polyphosphate (poly-Pi); PH3, phosphine; RuBP, ribulose bisphosphate; PEP, phosphoenolpyruvate; N2, nitrogen; NO2⁻, nitrite; NO3⁻, nitrate; NH4⁺, ammonium; and N2O, nitrous oxide.

We will present selected information on DOP formation, distribution and turnover in the sea building upon several previous, authoritative reviews by Duursma (1960), Armstrong (1965), Corner and Davies (1971), and Benitez-Nelson (2000) on various aspects of the marine P cycle, as well as nearly one century of field and laboratory research on this subject. For reasons already mentioned, it is impossible to discuss DOP in any useful ecological framework without also considering other DOM/POM pools and related biogeochemical processes. Although dissolved inorganic P concentrations (typically reported as soluble reactive phosphorus or SRP) are routinely measured in physical, chemical, and biological studies of the marine environment, estimates of total P (i.e., the sum of reactive and nonreactive forms of dissolved P, also called total dissolved P or TDP) are rare, despite the existence, for over 50 years, of reliable analytical methods. Although TDP was included as a core measurement during the International Geophysical Year (IGY) Atlantic Basin hydrographic survey of 1957–1958 (McGill, 1963), none
of the “modern” oceanographic sampling programs, including Geochemical Ocean Sections (GEOSECS) and World Ocean Circulation Experiment (WOCE) included TDP as a core measurement. Even the Joint Global Ocean Flux Study (JGOFS) program, which sponsored regional-scale field studies of ocean biogeochemistry, mostly ignored P-cycle processes. Consequently the extant database of high-quality, paired SRP and TDP in the world’s oceans is relatively sparse in comparison to the global coverage of SRP.

II. TERMS, DEFINITIONS, AND CONCENTRATION UNITS

The total phosphorus (TP) fraction in seawater is divided, unequally, among particulate P (PP) and TDP fractions (TP = PP + TDP); both fractions contain inorganic and organic P derivatives. In most open ocean marine environments, the TDP pool greatly exceeds the PP pool, but it is the biogenic PP pool (i.e., cells or living biomass) that ultimately produces and remineralizes DOP, thereby sustaining the marine P cycle.

The inorganic forms of P consist mostly of orthophosphoric acid (in seawater at a salinity of 33‰, 20°C, and pH 8.0 as 1% H₂PO₄⁻ / 87% HPO₄²⁻ / 12% PO₄³⁻; Kester and Pytkowicz, 1967), pyrophosphate (P₂O₇⁴⁻; hereafter abbreviated pyro-P₂), and other condensed cyclic (metaphosphate) and linear (polyphosphate) polymers of various molecular weights (hereafter abbreviated poly-P). The condensed phosphates can exist in the dissolved, colloidal and particulate matter fractions of seawater, whereas Pi and pyro-P₂ are mostly contained in the truly dissolved fraction or within intracellular pools. Of these various inorganic forms, only Pi is quantitatively detected by the standard molybdenum blue assay procedure (see Section V.D for more information on reaction specificity). Therefore the measurements of pyro-P₂ and poly-P₂ pools require sample hydrolysis to yield reactive Pi.

The organic-P fractions include primarily monomeric and polymeric phosphate esters (C–O–P bonded compounds), phosphonates (C–P bonded compounds), and organic condensed phosphates (Table I and Fig. 3). Among the ester-linked DOP compounds, both phosphonomonoesters and phosphodiesters are present (Table I); each compound has unique chemical and physical properties, and each has characteristic phosphohydrolitic enzyme susceptibility. Numerous compound classes (e.g., nucleotides, nucleic acids, phospholipids, phosphoproteins, sugar phosphates, phosphoamides, vitamins) have been detected in seawater and these will be discussed in subsequent sections. Oxidative destruction of the associated organic matter is generally required to convert organic-P to reactive Pi, although certain compound classes are partially hydrolyzed during Pi analysis and thus may contribute to an overestimation of the true Pi concentration. For this reason, the standard molybdenum blue assay measures an operationally defined pool,
BOND TYPE

C-O-P
(Monoester)
Example: Glucose-6-phosphate

C-O-P-O-C
(Diester)
Example: Ribonucleic acid

C-P
(Phosphonate)
Example: Phosphonoformic acid

C-O-P-O-P-O-P
(Polyphosphate monoester)
Example: Adenosine-5'-triphosphate
soluble reactive P (SRP), and the difference between TDP (i.e., equal to SRP following sample hydrolysis) and the initial SRP value has been termed the soluble nonreactive P (SNP) pool. Although SRP is often equated to Pi, in reality SRP only sets an upper constraint on Pi. Depending upon oxidation/hydrolysis conditions that are used for analysis, the SNP pool includes organic-P, pyro-Pi, and poly-Pi. Consequently, SNP concentration is technically not equal to DOP due to the two independent conditions: SRP ≥ Pi and SNP ≥ DOP. This may have important analytical and ecological implications as discussed in subsequent sections.

P in seawater can also be characterized by origin (e.g., biogenic or lithogenic) or by physical characteristics (e.g., molecular weight or photolytic lability). Because many different forms can be used as P sources for marine microorganisms, albeit at variable rates and efficiencies, the most ecologically relevant fraction is biologically available P (BAP) pool. Ideally, BAP consisting of both Pi plus the biolabile fraction of the SNP pool should be measured to constrain oceanic P cycle fluxes, but routine analytical methods do not exist. While it might be argued, a priori, that SNP measurements by the Murphy and Riley (1962) procedure place a lower bound on BAP, because both Pi and acid-labile DOP must be biologically available, this may not always be the case. Pi contained in colloidal associations or adsorbed to nanoparticles would assay as part of the SNP pool but might be unavailable for uptake under ambient conditions. In all likelihood only microbioassay analysis can provide an accurate estimate of BAP (see Section VIII.F). Suffice it to say, we are still lacking a comprehensive chemical description of dissolved P in seawater (see Section VIII).

The measurement of TDP is also operationally defined; typically a high-intensity ultraviolet (UV) photooxidation (Armstrong et al., 1966) or high-temperature wet chemical oxidation (Menzel and Corwin, 1965) or a combined (Ridal and Moore, 1990) pretreatment is used to convert SNP to Pi for subsequent analysis by the standard molybdenum blue assay. However, it is well known that certain P-containing compounds (e.g., poly-Pi, nucleotide di- and triphosphates) are not quantitatively recovered by standard UV photooxidation procedures; neither method quantitatively recovers P from all phosphonate compounds. Depending upon the methods used, the difference between the measurement of TDP and either Pi or SRP can be termed SNP (i.e., SNP = [TDP] - [SRP]) or non-Pi P (non-Pi P = [TDP] - [Pi]), where SNP ≠ N-Pi P (Thomson-Builds and Karl, 1998). As emphasized previously, there is no a priori relationship between these operationally defined pools and the more ecologically relevant BAP pool. Although several SNP compound classes have been reported to exist in seawater, including

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Figure 3 Selected structures of representative DOP pool compound classes with specific examples.
Not shown here are the less well known classes such as phosphoramidates (N-P-bonded) or phospho-
rothionates (S-P-bonded) compounds that could also be present in cells and in seawater.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical formula</th>
<th>(P) (%) by weight</th>
<th>Molar C:N:P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monophosphate esters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ribose-5-phosphoric acid (R-5-P)</td>
<td>(\text{C}_5\text{H}_11\text{O}_5\text{P}) (230.12)</td>
<td>13.5</td>
<td>5:--:1</td>
</tr>
<tr>
<td>Phospho(enol)pyruvic acid (PEP)</td>
<td>(\text{C}_3\text{H}_5\text{O}_3\text{P}) (168)</td>
<td>18.5</td>
<td>3:--:1</td>
</tr>
<tr>
<td>Glyceraldehyde 3-phosphoric acid (G-3-P)</td>
<td>(\text{C}_3\text{H}_2\text{O}_4\text{P}) (170.1)</td>
<td>18.2</td>
<td>3:--:1</td>
</tr>
<tr>
<td>Glycerophosphoric acid (Gly-3-P)</td>
<td>(\text{C}_2\text{H}_5\text{O}_6\text{P}) (172.1)</td>
<td>18.0</td>
<td>3:--:1</td>
</tr>
<tr>
<td>Creatine phosphoric acid (CP)</td>
<td>(\text{C}_4\text{H}_2\text{N}_2\text{O}_5\text{P}) (211.1)</td>
<td>14.7</td>
<td>4:3:1</td>
</tr>
<tr>
<td>Glucose-6-phosphoric acid (Glu-6-P)</td>
<td>(\text{C}_6\text{H}_12\text{O}_6\text{P}) (260.14)</td>
<td>11.9</td>
<td>6:--:1</td>
</tr>
<tr>
<td>Ribulose-1,5-bisphosphoric acid (RuBP)</td>
<td>(\text{C}_5\text{H}<em>8\text{O}</em>{12}\text{P}_2) (304)</td>
<td>20.4</td>
<td>2.5:--:1</td>
</tr>
<tr>
<td>Fructose-1,6-diphosphoric acid (F-1,6-DF)</td>
<td>(\text{C}_6\text{H}<em>12\text{O}</em>{12}\text{P}_2) (340.1)</td>
<td>18.2</td>
<td>3:--:1</td>
</tr>
<tr>
<td>Phosphoserine (PS)</td>
<td>(\text{C}_3\text{H}_6\text{NO}_3\text{P}) (185.1)</td>
<td>16.7</td>
<td>3:1:1</td>
</tr>
<tr>
<td><strong>Nucleotides and derivatives</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosine 5’-triphosphoric acid (ATP)</td>
<td>(\text{C}<em>{10}\text{H}</em>{16}\text{N}<em>5\text{O}</em>{13}\text{P}_3) (507.2)</td>
<td>18.3</td>
<td>3:3:1:7:1</td>
</tr>
<tr>
<td>Uridylic acid (UMP)</td>
<td>(\text{C}<em>9\text{H}</em>{12}\text{N}<em>2\text{O}</em>{9}\text{P}) (324.19)</td>
<td>9.6</td>
<td>9:2:1</td>
</tr>
<tr>
<td>Uridine diphosphate glucose (UDPG)</td>
<td>(\text{C}<em>{15}\text{H}</em>{28}\text{N}<em>2\text{O}</em>{17}\text{P}_2) (566.3)</td>
<td>10.9</td>
<td>7:5:1:1</td>
</tr>
<tr>
<td>Guanosine 5’-diphosphate 3’-diphosphate or “magic spot” (ppGpp)</td>
<td>(\text{C}<em>{10}\text{H}</em>{17}\text{N}<em>2\text{O}</em>{17}\text{P}_4) (603)</td>
<td>20.6</td>
<td>2.5:1:25:1</td>
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<tr>
<td>Pyridoxal 5-monophosphoric acid (PyMP)</td>
<td>(\text{C}<em>6\text{H}</em>{12}\text{NO}_4\text{P}) (247.2)</td>
<td>12.5</td>
<td>8:1:1</td>
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<tr>
<td>Nicotinamide adenine dinucleotide phosphate (NADP)</td>
<td>(\text{C}<em>{22}\text{H}</em>{26}\text{N}<em>4\text{O}</em>{12}\text{P}_2) (662)</td>
<td>9.4</td>
<td>11:3:1</td>
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<td>Ribonucleic acid (RNA)</td>
<td></td>
<td>~9.2%</td>
<td>~9.5:4:1</td>
</tr>
<tr>
<td>Deoxyribonucleic acid (DNA)</td>
<td></td>
<td>~9.5%</td>
<td>~10:4:1</td>
</tr>
<tr>
<td>Inositolhexaphosphoric acid or phytic acid (PA)</td>
<td>(\text{C}<em>5\text{H}</em>{10}\text{O}_{24}\text{P}_6) (660.1)</td>
<td>28.2</td>
<td>1:--:1</td>
</tr>
<tr>
<td><strong>Vitamins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiamine pyrophosphate (vitamin B₁)</td>
<td>(\text{C}<em>{12}\text{H}</em>{19}\text{N}_4\text{O}_7\text{P}_2\text{S}) (425)</td>
<td>14.6</td>
<td>6:2:1</td>
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<tr>
<td>Riboflavin 5’-phosphate (vitamin B₂-P)</td>
<td>(\text{C}<em>{17}\text{H}</em>{21}\text{N}_2\text{O}_6\text{P}) (456.3)</td>
<td>6.8</td>
<td>17:4:1</td>
</tr>
<tr>
<td>Cyanocobalamin (vitamin B₁₂)</td>
<td>(\text{C}<em>{63}\text{H}</em>{89}\text{CoN}<em>{14}\text{O}</em>{14}\text{P}) (1355.42)</td>
<td>2.3</td>
<td>63:14:1</td>
</tr>
</tbody>
</table>

(Continues)
Table I (Continued)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical formula (molecular weight)</th>
<th>$P$ (%) by weight</th>
<th>Molar C: N: P</th>
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<tr>
<td><strong>Phosphonates</strong></td>
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<tr>
<td>Methylphosphonic acid (MPh)</td>
<td>CH$_3$O$_2$P (96)</td>
<td>32.3</td>
<td>1:---:1</td>
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<td>Phosphonoformic acid (FPn)</td>
<td>CO$_2$PH$_3$ (126)</td>
<td>24.6</td>
<td>1:---:1</td>
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<tr>
<td>2-aminoethylphosphonic acid (2-AEPn)</td>
<td>C$_2$H$_6$NO$_4$P (141)</td>
<td>22.0</td>
<td>2:1:1</td>
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<td><strong>Other compounds/compound classes</strong></td>
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<td>Marine fulvic acid* (MFA)</td>
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<td>0.4–0.8</td>
<td>80–100:---:1</td>
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<td>Marine humic acid* (MHA)</td>
<td>Variable</td>
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<td>&gt;300:---:1</td>
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<tr>
<td>Phospholipids (PL)</td>
<td>Variable</td>
<td>≤4</td>
<td>~40:1:1</td>
</tr>
<tr>
<td>Malathion (Mal)</td>
<td>C$<em>9$H$</em>{16}$O$_2$PS (267)</td>
<td>11.6</td>
<td>9:---:1</td>
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<td>&quot;Redfield&quot; plankton</td>
<td>Variable</td>
<td>1–3</td>
<td>106:16:1</td>
</tr>
</tbody>
</table>

*aMarine FA and HA are operationally defined fractions, thus their composition may vary with source. These values are from Nissenbaum (1979).*

poly-Pi (Solórzano and Strickland, 1968), nucleotides (Azam and Hodson, 1977; Nawrocki and Karl, 1989), nucleic acids (DeFlaun et al., 1986; Karl and Bialiff, 1989), and monophosphate esters (Strickland and Solorzano, 1966), the SNP pool in seawater remains largely uncharacterized.

The earliest reports of Pi and TDP in seawater, prior to approximately 1930, all reported P as milligrams of phosphorus pentoxide (P$_2$O$_5$) per cubic meter of seawater (e.g., Atkins, 1923). Ironically, the chemical form P$_2$O$_5$ decomposes in water; the correct form should be P$_4$O$_{10}$ (Olson, 1967). Despite a logical recommendation by Atkins (1925) “to convert the conventional P$_2$O$_5$ values into the more rational values for the phosphate ion the factor 1.338, or very approximately 4/3, may be used to multiply the former,” the P$_2$O$_5$ equivalence reporting practice continued. In 1933, Cooper (1933) made another plea for the importance of consistency in reporting dissolved nutrient and other elemental data. He suggested the gram-atom (or submultiple thereof, e.g., milligram-atom, microgram-atom) of the element under investigation per cubic meter as the most useful and meaningful concentration unit. This would provide for the direct comparison with other elements, and a relatively straightforward calculation of bioelemental atomic stoichiometry (i.e., C:N:P:Si) for dissolved or particulate matter. Atomic, molecular and ionic ratios would all be numerically identical. Cooper (1933) went on to state, “it is felt that such a radical change in the method of reporting results, before being put into service, requires the concurrence of the majority of oceanographical chemists, as uniformity in practice above all else is desirable.” This bold suggestion was not
immediately accepted by the contemporary community of scholars, and even at the present time there is no uniformity of reporting dissolved and particulate matter P concentrations.

A variety of units, all interchangeable, have been used to report DOP in seawater. In preparing this review we have converted all of the reported concentration data to either ng-at P L\(^{-1}\) (nM P) or µg-at P L\(^{-1}\) (µM P) as appropriate. For organic P pools this refers to P only; so 507 ng L\(^{-1}\) of dissolved adenosine 3'–triphosphate (ATP), for example, would be equal to 1 nM ATP, but 3 nM P because each mole of ATP contains three P atoms. This practice of reporting DOP in P molar equivalents is absolutely necessary because the exact chemical composition remains largely unknown. For quantitative measurements of polymeric compounds such as DNA, RNA, and lipid-P we also report the assumptions that we made regarding the mole percentage of P in the specific polymeric compound. Sometimes molality (mol kg\(^{-1}\) of seawater) rather than molarity (mol L\(^{-1}\) of seawater) is used so that one does not have to calculate changes in volume that occur due to variations in temperature, pressure, or salinity but, for the purposes of this review, we will consider these changes to be negligible.

III. THE EARLY YEARS OF PELAGIC MARINE P-CYCLE RESEARCH (1884–1955)

Several pathfinding scientific studies, especially those conducted during the first half of the 20th century, provided a sound foundation for contemporary investigations of the marine P cycle. The creation of the Marine Biological Association of the United Kingdom in 1884 and dedication of their marine laboratory at Plymouth in 1888, and the creation of the Marine Biological Laboratory at Woods Hole, Massachusetts, in 1888 are especially noteworthy because of the major impact these two research centers have had, and continue to have, on the field of marine ecology and biogeochemistry. In 1903, working out of the Plymouth laboratory, Donald J. Matthews began a systematic study of the oceanographic features of the English Channel. His time-series research program that was later continued by Atkins, Cooper, and Harvey, led to a comprehensive understanding of the fundamental links between nutrients, phytoplankton, and fish production in the sea. Matthews (1916, 1917) is also credited with making the first reliable estimations of phosphate in seawater, and with the discovery of oceanic DOP. The colorimetric method that he selected was based on the Pouget and Chouchak reagent (sodium molybdate/strychnine sulfate/nitric acid) which yielded a yellow colored product the intensity of which was proportional to the amount of phosphate in the water sample. Because this method was not very sensitive, Matthews (1917) first had to concentrate the dissolved phosphate by coprecipitation using either ammonia or a mixture of ammonia and an iron salt. The former, a predecessor to the modern
"MAGIC" technique (Karl and Tien, 1992), removed phosphate by adsorption onto magnesium hydroxide, Mg(OH)$_2$, and the latter as ferric phosphate and ferric hydroxide.

Using this laborious but robust method for samples collected near Knap Buoy in the English Channel, Matthews (1916, 1917) made two very important observations: (1) the concentration of phosphate in seawater was approximately 0.85 μM in December 1915, decreasing systematically to a minimum of <0.1 μM between late April to late May, increasing again in January 1917 to a similar winter value, and (2) TDP, measured as phosphate following sample oxidation by potassium permanganate, exceeded the initial concentration of phosphate in the untreated sample by up to a factor of two- to threefold. In other words, Matthews documented for the first time the seasonal dynamics of phosphate during the vernal blooming of phytoplankton and, more significant to the topic of this review, the presence of DOP in coastal seawaters. While he was very careful to emphasize that the reported DOP values were not necessarily quantitative, the organic component was highest when the phosphate was at its maximum, suggesting that DOP formation may be coupled to particulate matter production. He concluded this key discovery paper by stating that "the nature and origin of this organic phosphorus is, of course, quite unknown" (Matthews, 1917). The importance of Matthew’s research cannot be overstated; however, today, nearly a century after his pioneering contributions, we still lack a comprehensive understanding of DOP dynamics in seawater.

Shortly after the completion of these initial studies, Matthews was called into military service and joined the Hydrographic Office of the Navy, where he remained following the end of World War I. A reorganization of research programs at the Plymouth Marine Laboratory led to the formation of a Department of General Physiology and the addition of W. R. G. Atkins and H. W. Harvey to the scientific staff (Southward and Roberts, 1987). Along with L. H. N. Cooper, F. S. Russell, and other staff chemists and plankton biologists at the Plymouth Laboratory, they reestablished in 1921 the monthly time-series sampling program at several key locations in the English Channel. By this time, the phosphate detection system used by Matthews had been replaced by the more sensitive Denigès (1921) method which employed ammonium molybdate, sulfuric acid, and stannous chloride (Atkins and Wilson, 1926). An intense blue color developed in the presence of phosphate. This "molybdenum blue" method, or slight variations thereof, continues to be the method of choice for contemporary studies of the marine P-cycle (see Section V.D). While the seasonal phosphate concentration dynamics using the Denigès-Atkins method revealed trends that were similar to the results published a decade earlier by Matthews, the time-series measurements documented significant interannual variations in both the date of initiation of the spring bloom of phytoplankton and, therefore, the net rate of phosphate uptake (Atkins 1928). There was also significant interannual variation in total phosphate consumed during the spring period, a value that was subsequently related to annual
variations in the potential production of fish. That is to say, knowledge of the rate of phosphate consumption provided a lower limit constraint on annual phytoplankton production. The seasonal net consumption of phosphate was highly correlated to the removal of both silicate and carbon dioxide, suggesting that diatoms were largely responsible for organic matter production in the English Channel ecosystem (Atkins, 1930). However, by comparison to this deliberate focus on net plankton production for fisheries management, studies of organic matter decomposition and phosphate remineralization were generally ignored.

Despite these field successes at Plymouth during the 1920s, research on the nature of the “enigmatic” DOP pools, discovered earlier by Matthews, was placed on hold. Even worse, Atkins considered the observed increase in phosphate following permanganate oxidation to be an analytical artifact due to the presence of arsenite in seawater and concluded that “much of what was formerly considered to be phosphorus in organic combination, in seawater, is in reality arsenic” (Atkins and Wilson, 1927). This inappropriate conclusion from one of the intellectual giants of those times was sufficient to preclude further investigations of DOP at Plymouth or elsewhere, for at least a decade, or more.

H. W. Harvey and L. H. N. Cooper, two of Atkins’ contemporaries at the Plymouth Marine Laboratory who shared common interests in nutrient and plankton dynamics, began systematic studies of the coupled N and P cycles, including nutrient remineralization. It was reasoned that the inorganic nutrients assimilated into organic compounds by marine plankton must eventually be recycled back to nitrate and phosphate by the combined processes of digestion, decay, and chemical hydrolysis. Consequently both dissolved and particulate organic phosphorus must be considered integral components of the marine P cycle. At about this same time studies on the role of marine bacteria as agents of organic matter decomposition were getting underway at several independent marine laboratories worldwide (e.g., Waksman and Renn, 1936; Renn, 1937; ZoBell and Grant, 1943). Furthermore, considerations of coupled particle sinking and decomposition (Seiwell and Seiwell, 1938) provided the incentive for investigations of the role of biological processes in the distributions of nonconservative properties (e.g., nutrients, oxygen and carbon dioxide) as a function of water depth and distance from land masses. Included in these investigations were studies of the release of phosphate during dark storage of water samples with and without added plankton (Gill, 1927; Cooper, 1935) or specific DOP compounds (Harvey, 1940). It was concluded that much of the total P in the sea, especially in the surface waters, was tied up in physiologically important classes of living and nonliving particulate matter which upon the initial period of decomposition were released as DOP. Only after microbial decomposition was the P released back as free phosphate. From these laboratory experiments came a renewed appreciation for the role of DOP in the marine P cycle and a focused effort on field measurements thereof. Independent, but similar, studies on the N:P stoichiometry of plankton production and decomposition conducted by Redfield et al.
Dynamics of DOP

Figure 4 Annual changes in various living and nonliving P pools and total inventories for the samples collected in the English Channel. The annual dominance of TDP (>80% of total P) is evident. The most notable seasonal change in the P inventory is the shift from PI dominance in winter to DOP dominance in late spring–summer. Redrawn with permission from Harvey (1955).

(1937) and Cooper (1938) also accelerated during the 1930s, leading, eventually, to an ecumenical theory of nutrient dynamics in the sea.

By the early 1940s, the fundamental role of DOP in the marine P cycle was firmly established (Atkins, 1930; Kreps, 1934; Cooper, 1938; Redfield et al., 1937; Newcombe and Brust, 1940). The sustained time series investigations of the English Channel provided evidence for a seasonally variable pool of DOP, which at the height of the phytoplankton bloom in late spring to early summer was maximal (Harvey, 1950; Armstrong and Harvey, 1950; Fig. 4). Field studies conducted in the epipelagic waters of the Gulf of Maine (Redfield et al., 1937) and in Chesapeake Bay (Newcombe and Brust, 1940) revealed similar results.

Confirmation of the presence of a significant pool of DOP in seawaters from diverse habitats further stimulated research to ascertain the sources and sinks of
these potentially diverse, but biologically, relevant, compound classes. Until this
time, bacteria had been considered to be the principal agents of DOP remineral-
ization to \( \text{Pi} \), the preferred substrate for phytoplankton growth. However, careful
laboratory experiments conducted by Chu (1946) documented the ability of se-
lected bacteria-free phytoplankton cultures to utilize DOP, thereby providing a
novel, alternative pathway in the marine P cycle. Presumably these microorgan-
isms would be selected for during the summer months when \( \text{Pi} \) concentrations
were low and DOP/Pi ratios were high which could promote a seasonal succession
of phytoplankton species in certain habitats.

It seems appropriate to end this section on “The early years of marine P-cycle
research” with the publication of Harvey’s seminal monograph “The Chemistry and
Fertility of Sea Waters” (Harvey, 1955). While his field observations concentrated
mainly on the English Channel, the conceptual framework presented in this now
classic volume received worldwide attention and provided the incentive for a large
portion of the DOP research which followed during the next half-century.

IV. THE PELAGIC MARINE P CYCLE:
KEY POOLS AND PROCESSES

Compared to the more complex cycles of C, N, and S that are characterized
and sustained by redox transformations, the marine P cycle appears rather simple
(Fig. 1). With few exceptions, P in the sea is present in the pentavalent state
(+5) as \( \text{PO}_4^{3-} \), whether as free orthophosphate or as P incorporated into either
phosphate ester or phosphonate compounds. The presence of phosphite (\( \text{PO}_3^{3-} \))
and phosphine gas (\( \text{PH}_3 \)) has been reported in selected anoxic marine habitats
where they were formed and, at least in the case of \( \text{PO}_3^{3-} \), consumed as part of the
marine P cycle (Dévai et al., 1988; Gassmann, 1994; Schink and Friedrich, 2000).
These reduced Pi derivatives are not likely to be formed in open ocean habitats.
Despite this redox simplicity, Pi is rapidly assimilated to form a diverse spectrum
of organic and inorganic derivatives. These compounds have key structural and
metabolic functions and, therefore, are continually produced by all living
organisms.

Cellular P metabolism in the marine environment is complex. The transfer of
phosphoryl groups is a fundamental characteristic of intermediary metabolism
and is, therefore, crucial for life. Numerous enzymes share the ability to cata-
lyze phosphoryl group transfer including phosphatases, phosphokinases, phos-
phomutases, nucleotidases, nucleases, phosphodiesterases, phospholipases, and
nucleotidyl transferases and cyclases (Knowles, 1980). Of these enzyme classes,
the phosphatases (mono- and diesterases), nucleotidases and nucleases have been
most frequently studied in the marine environment (Fig. 5). The depolymerization
reactions converting high-molecular-weight (HMW) DOP to intermediate- and
Figure 5  Schematic presentation of the role of selected enzymes (both dissolved and cell/organism-associated) in DOP pool dynamics. The production of detrital P, including both particulate and IMW/HMW dissolved inorganic and organic P pools provides key substrates (open boxes) for the specific enzymes (shaded boxes). The continued supply of monomeric compounds (<1 kDa; open circles) fuels additional enzymatic processes that lead to DOP or Pi assimilation by living biomass. C-O-P ester-linked P-compounds; C-P, phosphonates; PPase, inorganic polyphosphatase; PMEase, phosphomonoesterase (including but not limited to APase); SNase, 5' nucleotidase; NTPase, nucleotide triphosphatase; PDEase, phosphodiesterase.

Low-molecular-weight (IMW and LMW, respectively) DOP are probably slow relative to the hydrolysis and direct uptake of monomeric DOP. Consequently, HMW DOP turnover is probably the “bottleneck” in marine P-cycle dynamics (Fig. 5).

Marine microorganisms can assimilate three separate types of P: (1) Pi, (2) ester-linked DOP compounds, and (3) phosphonates. Typically, Pi is the preferred substrate so during Pi-sufficient growth conditions the synthesis of specific enzymes for phosphate ester and phosphonate utilization are usually repressed (see Section IX.C). Most microorganisms synthesize one or more phosphohydrolytic enzyme in order to degrade selected DOP compounds. For DOP compounds that are not able to be transported into the cell directly, hydrolysis prior to transport is necessary. In bacteria, DOP hydrolysis usually occurs at the outer cell membrane or in the periplasmic space, and under certain conditions enzymes are exported from
the cell for hydrolytic activity in the surrounding medium. This may be one source of the reported presence of “dissolved” enzymatic activity in the sea. Often times the Pi that is released upon hydrolysis is transported into the cell and assimilated into new biomolecules for metabolism or cell growth. However, depending upon the DOP substrate in question, the Pi-free organic molecule may be the preferred target substrate and the newly released Pi remains behind in the medium. By example, the hydrolysis of glucose-6-P (glu-6-P) most likely provides Pi, whereas the hydrolysis of AMP probably provides a purine base for nucleic acid synthesis with the Pi remaining in the medium. Consequently, the composition of the DOP pool as well as the biosynthetic needs of the microbial assemblage under investigation will largely determine whether cellular phosphohydrolase activity is a mechanism for net Pi regeneration or net Pi sequestration. In either case, however, the enzymatic activity represents a net sink for DOP compounds and effectively sustains DOP pool turnover.

The combined processes of excretion, exudation, death, and autolysis, including viral lysis, provide a constant flux into the DOP pool, and the combined processes of hydrolysis (including ectoenzymatic activity, chemical hydrolysis and photolysis) and active microbial uptake comprise the major DOP sinks. The ability to transport and metabolize selected DOP compounds appears to be enhanced during conditions when the preferred growth substrate, Pi, is present at limiting concentrations. The regulation of this switch from Pi to DOP assimilation is under complex metabolic control. Suffice it to say that DOP turnover is inextricably linked to Pi supply, and this results in generally negative correlation between Pi and DOP concentrations in the global ocean (see Section VI). For this reason there may be both spatial and temporal decoupling of DOP production and DOP utilization in the marine environment.

In coastal regions there can be both point source (e.g., outfalls, rivers, groundwaters) and nonpoint source (e.g., runoff) inputs of Pi, pyro-Pi, poly-Pi, and DOP; the chemical composition of the latter may be fundamentally distinct from the autochthonous DOP pool. Atmospheric deposition of P is another potential source term but these fluxes, especially for DOP, are poorly constrained at present. Atmospheric inputs are also likely to be regionally variable with the largest fluxes immediately adjacent to (i.e., downwind) major continental, industrial, or volcanogenic source terms. Active volcanoes may be unique in that they can catalyze the formation of gaseous P (P4O10) by the intense heating of basaltic rocks (Yamagata et al., 1991). The gaseous by-products condense in the volcanogenic steam plume and fallout as pyro-Pi and Pi.

Because there is no significant gas phase in the oceanic P cycle, the primary net removal mechanism for P in the open ocean is via gravitational settling of particulate matter, downward diffusion and advection, and, for selected regions of the world’s ocean, horizontal transport. However, for most open ocean habitats where concentration gradients are weak or nonexistent, horizontal advection
represents both a source and sink with a near zero net impact on the P budget. Over sufficiently long time scales (decades to centuries) there is a balance between the sources and sinks, which leads to relatively time-invariant pool inventories and fluxes. However, over seasonal-to-decadal time scales, both the concentrations and fluxes can vary in response to changes in local and regional physical forcing and global climate variability. These perturbations can also lead to changes in the relative proportions of P to DOP, or DOP, to TDP in selected habitats. The North Pacific Subtropical Gyre case study, presented later in this review, will focus on the P cycle in a climate-forced marine ecosystem.

The use of enzymatic biomarkers to assess the P status of natural microbial assemblages has been extensively employed in microbiological oceanography. Constitutive enzymes, those found in cells regardless of nutrient status, inducible enzymes, those produced by an organism when exposed to a specific substrate, and repressible enzymes, those synthesized when the concentration of a specific repressor becomes very low, are the three main classes of enzymes used to efficiently regulate the cell’s biodegradation potential. Consequently, the presence or absence of a specific enzyme can be used as an ecological indicator of metabolic readiness (see Section IX).

The importance of P in microbial metabolism and, therefore, in ecosystem processes is best demonstrated by the impressive ecophysiological response of bacteria to P stress or P limitation. Under such conditions, substantial and coordinated changes occur that prepare the cells for competition and survival, including the synthesis of more efficient and specific Pi-capture systems, and the ability to utilize a broader range of potential P-containing organic substrates, including phosphonates. In the case of phosphonates, enzymatic hydrolysis of the C–P bond requires either a phosphonatase pathway or a C–P lyase pathway. These independent pathways are distinguishable by their substrate specificities (Wanner, 1993); both pathways are stimulated during periods of P limitation (Metcalf and Wanner, 1991).

Recently, a comprehensive analysis of protein synthesis during P limitation and phosphonate metabolism was conducted for Escherichia coli (VanBogelen et al., 1996). Two-dimensional gel electrophoresis was used to identify 413 separate proteins which exhibited differential synthesis upon P limitation. About half (208) of the proteins exhibited enhanced synthesis while the other half (205) displayed reduced synthesis. This family of proteins is referred to as the P limitation stimulon. Growth on phosphonates as the sole source of P altered the synthesis of 257 proteins; 227 displayed induced synthesis and 30 were repressed. The overlap between the P limitation and phosphonate stimulons included 137 proteins, most (>85%) of which displayed induced synthesis. The aggregate mass of proteins responding to P limitation or to growth on phosphonates was 30–40% of the total mass of the cell, once again emphasizing the key role of P in cellular metabolism. This impressive laboratory study can be used as a blueprint for bacterial growth
in the marine environment, at least for the purposes of ecological prediction and hypothesis testing.

Another group of enzymes that are an integral part of the Pi limitation stimolun are phosphatases, a general term used to refer to an enzyme that catalyzes the hydrolysis of esters and anhydrides of phosphoric acid. The most common is a class of alkaline phosphononoesterases, also called alkaline phosphatase (APase; EC 3.1.3.1) which potentially can catalyze the hydrolysis of a broad spectrum of DOP compounds optimally at seawater pH. APase is a relatively nonspecific enzyme that releases Pi from a variety of phosphonoesters, including di-, tri-, and polyphosphate (e.g., nucleotides) organic derivatives. The ecological advantage is obvious; selected DOP compound classes could serve as reliable growth substrates during periods of Pi depletion. APase enzymes are diverse with variable substrate specificities, physical and kinetic properties, and metal ion requirements. Even within a single species, there may be multiple forms of APase (de Prada et al., 1996). Some, but not all, APases also possess mononucleotidase activity. Although APase activity has been reported for numerous marine habitats, most ecological studies lack a detailed description of the enzymes under consideration.

The relative ecophysiological roles of APase versus 5NDase, ATPase, phosphonate lyase, and other free and cell-associated enzymes will undoubtedly vary with DOP pool composition. Together, these enzymes act primarily on LMW, monomeric substrates and undoubtedly help to keep the ambient pools of LMW DOP at low (10^{-3} M, or less) levels. We hypothesize that the DOP pool turnover is controlled largely by the production rate of IMW and LMW substrates from the hydrolysis of HMW DOP, rather than by LMW DOP utilization. This would lead to an enhanced ecological role for those enzymes that are capable of hydrolyzing the HMW DOP, especially nucleases (including DNase and RNase), phosphodiesterases (exonuclease), and proteases (Fig. 5). P-cycle closure would ultimately require the coordinated suite of enzymes, but formation of monomers may be the remineralization bottleneck. If this model is correct, then the MW spectrum of DOP in the marine environment would be skewed toward HMW (>10 kDa). At present, there are few open ocean data available to test this ecological prediction.

V. SAMPLING, INCUBATION, STORAGE, AND ANALYTICAL CONSIDERATIONS

A. SAMPLING

The reliability of any field-collected data set is determined by the methods used for sampling and analysis. Although this chapter focuses on the ecological implications of the field data themselves, it is important to comment briefly on sampling, experimental design for P flux estimations and laboratory analysis of the respective P pools.
There are several general concerns that should be mentioned in regard to the methods reviewed in this chapter. First and foremost, P contamination is a major potential problem. Furthermore, sample contamination by toxic trace metals during sampling and subsampling is also a concern for P-flux studies that require sample incubation. It is recommended that all sampling gear, as well as storage and incubation bottles, be thoroughly acid-cleaned (1 M or ~10% HCl) and distilled water rinsed before use, and sample water rinsed before collection of the target seawater.

Sampling is one of the most important, but often overlooked, aspects of oceanography. Because of the ease with which a seawater or sediment sample is obtained it is tacitly assumed that sampling is a straightforward and simple task; in reality, it is not (Karl and Dore, 2001). Questions of time and space (Dickey, 1991), minimum size and number of samples, sample replication, contamination, and postcollection treatment of the primary samples are all relevant. Most of our conceptual views of the marine environment and, therefore, the basis for our sampling protocols focus on the vertical structure of the marine environment, despite the fact that the ocean is clearly a “horizontal” habitat (e.g., horizontal-to-vertical scale of the North Pacific Ocean is >1000:1; Karl and Dore, 2001). In the open sea there is a well-defined vertical zonation of biological communities based on light (in the near surface) and other physical and chemical properties at depths greater than approximately 200 m. In designing a sampling program for P-cycle research it is essential to consider this identifiable zonation as well as the source and other unique characteristics of each of the unique water masses.

The investigator should be aware of at least three separate areas where variability can be introduced into field measurements: replication at the level of sampling (i.e., multiple water samples collected from a common depth), replication at the level of subsampling (i.e., multiple subsamples from a single sample) and analytical replication (i.e., multiple analyses of a single sample extract). Because of the heterogeneous distribution of microbial communities in nature, and therefore of DOP production, variance between sampling bottles is generally the largest source of error. Replication is most meaningful when performed at the highest level, i.e., multiple samples of water from a given environment (Kirchman et al., 1982). It has also been demonstrated that the overall variance and the precision with which the sample variance can be estimated are functions of the procedure used to subsample the initial sample collection (Venrick, 1971).

**B. USE OF ISOTOPIC TRACERS IN P-CYCLE RESEARCH**

The use of stable and radioisotopic tracers to monitor and quantify the rates of microbial growth, metabolism, and biogeochemical cycling of key elements and compounds has revolutionized the field of microbiological oceanography. For P-cycle research, there are two major categories: (1) the use of naturally occurring,
cosmogenic radioactive isotopes and (2) the use of exogenously supplied radioactive isotopes. There are two radioisotopic tracers for P, $^{32}$P ($E_{\text{max}} = 1.71$ MeV, half life = 14.3 days) and $^{33}$P ($E_{\text{max}} = 0.25$ MeV, half life = 25.3 days), which both exhibit $\beta^-$ particle decay. The detection and quantification of the cosmogenic radiotracers $^{32}$P and $^{33}$P (Lal and Lee, 1988; Lal et al., 1988) are most useful for long-term (day to week) whole ecosystem studies. Two recent applications in the Sargasso Sea and Gulf of Maine have demonstrated the efficacy of using natural cosmogenic $^{32}$P/$^{33}$P radioisotopes in studies of the marine P cycle (Waser et al., 1996; Benitez-Nelson and Buesseler, 1999). The use of exogenously supplied $^{32}$P and $^{33}$P-labeled inorganic and organic compounds, is best suited for short-term (hour to day) studies of metabolic pathways, nutrient fluxes and organic tissue labeling patterns. Several whole lake (pond) ecosystem studies (Hutchinson and Bowen, 1947, 1950; Rigler, 1956) and at least one marine reef flat $^{32}$Pi experiment (Atkinson and Smith, 1987) have been conducted, but direct tracer release has not yet been used in either open ocean patch studies or in mesocosm enclosures.

Although P has only a single stable isotope, $^{31}$P, the oxygen atoms that are in association with both inorganic and organic P pools contain three isotopes: $^{16}$O, $^{17}$O, and $^{18}$O. These could, in theory, assist in a quantitative study of the marine P cycle (Longinelli et al., 1976), although no comprehensive study of oxygen isotopes in DOP has yet been published. Phosphate oxygen is tightly bound to P such that under ambient conditions in the sea, exchange of oxygen between Pi and the surrounding water is negligible (Blake et al., 1997). However, it has been hypothesized that biological cycling of P would act to isotopically equilibrate the phosphate–oxygen with ambient water (A. Colman, pers. commun.). Time and space measurements in the $\delta^{18}$O of Pi and DOP could thus provide invaluable information on P biogeochemistry.

The use of exogenous radioisotopic tracers has become routine for many P-cycle investigations. Often this is the only approach that is sensitive and specific enough to measure the sometimes low fluxes of P that occur in open ocean ecosystems. The details of selected individual methods are discussed elsewhere, however, there are several general considerations regarding the use of $^{32}$P/$^{33}$P radioactive isotope tracers in studies of microbial ecology that merit attention. These include: (1) the overall reliability of the added element (or compound) as a tracer, including an evaluation of the site of labeling and its uniqueness and stability during cellular metabolism and biosynthesis, (2) isotope discrimination factors, (3) the partitioning of the added tracer into existing exogenous and internal pools of identical atoms, molecules, or compounds and the importance of measuring the specific activity of the incorporated tracer, and (4) the design and implementation of experimental procedures and proper kinetic analysis of the resulting data. The underlying assumption of these methods is that the subsequent incubation conditions do not alter the $\text{in situ}$ rates of compound uptake, metabolism, or biosynthesis. This assumption is usually difficult to verify (Karl and Dore, 2001).
In order to ensure that the rates measured during the postcollection incubation procedure are representative of those occurring in nature, several precautions must be taken. First and foremost, the initial sample must be collected with great care so as to minimize chemical and microbiological contamination. Furthermore, exposure of viable microorganisms to environmental conditions that are substantially different from those at the collection site should be avoided so as to minimize any deleterious effects, ranging from short-term transitions in metabolism to death.

A very important but often overlooked principle in the use of radioisotopic tracers in marine ecological studies is the evaluation of the specific activity (radioactivity per unit mass) of the added, incorporated, or metabolized element, molecule, or compound during the incubation/labeling period. The ideal tracer is one that can be added without perturbing the steady-state concentration of the ecosystem as a whole. In ecological studies, an accurate assessment of the specific activity during the incubation/labeling period is further complicated by the dilution of the added tracer with exogenous pools present in the environment and by endogenous pools present in living microbial cells. Without a reliable measurement of the extent of dilution prior to incorporation, tracer uptake data by themselves are of limited use in quantitative microbial ecology (see Section IX.A). Furthermore, isotope-specific activities may change over the course of the labeling period due to the combined effects of depletion (uptake) of the added tracer or isotope dilution by a constant regeneration of the exogenous pools (assuming steady-state conditions). In fact, Pi regeneration rates have been estimated in environmental samples by measuring the extent of isotope dilution during short-term sample incubation periods (Harrison, 1983).

C. Sample Processing, Preservation, and Storage

Early investigations made no attempt to preserve seawater samples prior to analysis for Pi or TDP, even during prolonged storage (weeks to months). The regeneration of Pi with time would have resulted in systematic overestimations of Pi and underestimations of DOP, without greatly affecting TDP. Since that time, there has been a great deal of discussion and some acrimonious debate on the issue of seawater sample processing, preservation, and storage prior to analysis for SRP and TDP.

The first important consideration is whether or not to filter the water sample prior to preservation and long-term storage. The answer to this question is generally site-specific and will depend entirely on the objectives of the study and on the relative proportions of particulate and dissolved P. In most open ocean ecosystems and in all subeuphotic zone habitats PP rarely exceeds 1–5% of the total P in the sample so filtration may not be necessary. However, when sample filtration is desirable,
then the choice of filter matrix (polycarbonate, cellulose acetate, glass fibers, silver, aluminum oxide), porosity, pressure differential employed, and volume-to-area (i.e., filter loading) are equally relevant concerns. Filtration can also increase SRP and DOP concentrations by cell leakage or breakage (Pilson and Betzer, 1973).

No matter what filter is selected, there will be some limitation. For example, glass fiber filters (Whatman GF/F, or equivalent) that are routinely used in oceanography have two potential problems: (1) the porosity (0.7 μm nominal) precludes a unique separation of small particles and colloids from the truly dissolved P pools and (2) in certain habitats there is a significant adsorption of dissolved matter onto the GF/F filter matrix. This latter problem is a special concern in oligotrophic ocean environments where DOP concentrations exceed PP by one to two orders of magnitude. Furthermore, the ability of poly-Pi to quantitatively bind to powdered silica glass (Ault-Riché et al., 1998) suggests that dissolved poly-Pi in seawater may be underestimated if water samples are first filtered through silica glass fiber filters. The advantage of glass fiber filters is that they can be combusted at 450°C and acid-cleaned before use to thoroughly remove any P contamination. They also have favorable flow characteristics and high loading capacity, which are important for the measurements of certain P pools.

If filtration is not employed then PP must also be measured independently to provide the most accurate estimate of DOP (i.e., DOP = TP - (Pi + PP)). The use of independent analytical procedures, one for TP (e.g., UV photooxidation) and another for PP (e.g., high temperature ashing followed by acid hydrolysis), has the potential for large systematic errors if, for example, particulate poly-Pi is present. To the extent possible, the oxidation/hydrolysis methods used for TDP and PP should be matched.

For decades marine chemists have tested the suitability of various preservation techniques, considering the potential effects of storage container, temperature, chemical additions, and radiation on samples of different water types (Murphy and Riley, 1956; Gilmartin, 1967; Maher and Woo, 1998). The considerable body of literature presents varied and often contradictory opinions on the effectiveness of various preservation methods. The most extreme position is that all Pi, SRP, and TDP measurements must be conducted in the field on fresh sample materials. This, of course, is not always possible and sometimes not even desirable even when it is possible. A recent study by Dore et al. (1996) has reevaluated several long-standing criticisms of the seawater sample storage problem. In their hands, immediate freezing (−20°C) of the sample in a high-density polyethylene (HDPE) bottle, stored upright in the dark provides a simple and suitable method for storage of seawater for periods up to one for subsequent analysis of SRP and DOP. Alternatively, preservation with mercuric chloride followed by storage at 4°C in the dark (Kattner, 1999), and acidification to pH 1 followed by storage at 4–5°C (Monaghan and Ruttenberg, 1999) have also been used.
D. DETECTION OF P\textsubscript{i} AND P-CONTAINING COMPOUNDS IN SEAWATER

The analysis of dissolved and particulate P-containing compounds in seawater is neither simple nor straightforward. Olson (1967) has created a glossary for P-containing compounds that includes 75 potential forms of inorganic and organic P that might be found in nature. Strickland and Parsons (1972) have defined eight of the most relevant operational classes of P compounds based on reactivity with the acidic molybdate reagents, ease of hydrolysis, and particle size. These range from “inorganic, soluble and reactive,” presumably P\textsubscript{i}, through “enzyme hydrolyzable phosphate” (P\textsubscript{i} released following treatment with APase), to “inorganic, particulate and unreactive” (presumably P-containing minerals). Some of the operationally defined pools have no convenient analytical method of determination, while others can be estimated only as the difference between two operational classes with partially overlapping specificity. Only a very few specific P-containing compounds or compound classes can be readily detected at the low concentrations typically found in seawater (see Section VIII). Our inability to completely characterize these various dissolved and particulate pools currently limits further progress toward a comprehensive understanding of the marine P cycle.

1. Analysis of P\textsubscript{i}

The quantitative estimation of DOP (as well as TP, TDP, and PP) relies upon the measurement of P\textsubscript{i}, both before and after sample oxidation/hydrolysis (see below). Consequently, the precision and accuracy of DOP pool estimation is tied directly to the specificity and reliability of P\textsubscript{i} analysis. Although P\textsubscript{i} can be measured by any of a number of unrelated analytical techniques (Boltz, 1972), quantitative analysis of P\textsubscript{i} in seawater has traditionally relied upon the formation of a 12-molybdophosphoric acid (12-MPA) complex and its subsequent reduction to yield a highly colored blue solution, the extinction of which is measured by absorption spectrophotometry (Osmond, 1887; Denigès 1920, 1921; Atkins, 1923; Fiske and Subbarow, 1925; Murphy and Riley, 1958, 1962). Ironically, molybdate enhances the hydrolysis of selected organic-P compounds and pyro-P\textsubscript{i} (Wéil-Malherbe and Green, 1951), so the molybdenum blue protocol appears prepositioned for P\textsubscript{i} overestimation in natural seawater samples. However, without knowledge of the precise chemical composition of seawater DOP, we cannot predict the magnitude of this interference.

The measurement of P\textsubscript{i} (i.e., SRP) has a very rich and diverse history which, unfortunately, cannot be fully chronicled here. Over the years, numerous improvements have been introduced to the basic method so that substantial variability now exists in the conditions used for color development, final reduction of the 12-MPA complex, and treatment of potentially interfering compounds; Armstrong (1965), Olson (1967), and Broberg and Petterson (1988) provide comprehensive historical
accounts of these changes. In 1962, the single "mixed reagent" (sulfuric acid, ammonium molybdate, ascorbic acid, potassium antimony tartrate) was introduced and this is the protocol most commonly employed today (Murphy and Riley, 1962). One critical difference between methods is the reducing agent; ascorbic acid in the Murphy–Riley method versus stannous chloride by many other researchers, and the use of antimony (+3) as a reaction catalyst. In addition to being able to mix all required reagents together prior to the single addition of "mixed reagent" to the sample, color development is rapid and relatively stable and salt error, which for the use of stannous chloride is significant (e.g., ≥15%), is <1% (Murphy and Riley, 1962). Both methods, however, are affected by arsenate reactivity and by the acid-catalyzed hydrolysis of labile organic-P compounds. The consequences of these potential interferences for quantitative determinations of Pi, TDP, and, therefore, DOP are discussed below.

Although the stepwise chemical procedure for Pi determination is straightforward and fully amenable to automated analysis, there are many complexities, both analytical and conceptual, inherent in measuring and interpreting Pi concentrations in seawater (Tarapchak, 1983). The SRP pool measured by the standard Murphy–Riley procedure is not necessarily equivalent to the concentration of Pi, but may also include non-Pi P-containing compounds that are hydrolyzed under the acidic reaction conditions (Fig. 6). Rigler (1956, 1973) was the first to demonstrate that the conventional method of SRP determination in aquatic environments can result in a serious overestimation of Pi, especially when the SNP concentration equals or exceeds the Pi pool. Since then, many investigators have struggled with this problem, and it is still a challenge to obtain a reliable measurement of the Pi concentration in seawater. Drummond and Maher (1995) have recently described an adaptation of the Murphy–Riley (1962) procedure that yields full phosphoantimony molybdate color development in less than 1 min. Although they did not comment further on this, it is conceivable that this rapid reaction rate might provide a more accurate estimate of Pi by eliminating or at least reducing the time that is available for DOP hydrolysis, similar to the "6-s" assay (Chamberlain and Shapiro, 1969). Significant differences between SRP and Pi have been observed for nearly every natural aquatic ecosystem where more rigorous and specific methods of Pi analysis have been employed (Kuenzler and Ketchum, 1962; Jones and Spencer, 1963; Rigler, 1968; Pettersson, 1979; Karl and Tien, 1997; Thomson-Blidsis and Karl, 1998), and even different SRP methods return unequal estimates of "Pi" when tested with common seawater samples (Karl and Tien, 1997). As discussed later in this review, accurate determination of Pi is absolutely essential for the application of artificial 32Pi or 33Pi tracer studies if mass flux estimation (Pi uptake or regeneration rates, DOP production rates) is the experimental objective, and for accurate estimation of SNN and DOP, because these pools are difference estimations (i.e., TDP-SRP). Any overestimation of Pi (e.g., by partial DOP hydrolysis) will result in a equimolar underestimation of DOP, and thus will greatly impact the calculated
Figure 6  Schematic presentation of the chemically diverse pool of P-containing compounds in the marine environment. Shown on the left is the subset of compounds that are typically detected using the standard Murphy–Riley molybdenum blue spectrophotometric SRP assay including Pi and certain labile P compounds. The dashed line indicates that the water sample can be pretreated to remove the inhibitory effects of SRAs, but this method is not always employed. Shown at the top is the spectrum of target compounds using the MAGIC-based SRP analysis method and, at right, the TDP procedure which theoretically measures all P-containing compounds. Depending upon the TDP method selected, however, linear polyphosphates or phosphonates may not be quantitatively recovered. Various operational definitions, given at the bottom, are derived from these analyses but none of these chemical techniques yields an accurate estimate of the biologically available P (BAP) pool, which is the pool of greatest interest in studies of the microbially driven P-cycle.

Pi/DOP ratio. Although the use of malachite green oxalate for Pi measurements in seawater may reduce, or even eliminate, DOP hydrolysis (Fernández et al., 1985), this method has not been widely tested under field conditions.

According to Strickland and Parsons (1972), the limit of Pi detection using the standard Murphy–Riley method is approximately 0.03 \( \mu M \) Pi and the precision at the 0.3 \( \mu M \) Pi level is \( \pm 0.02/n^{0.5} \mu M \), where \( n \) is number of determinations (e.g., if triplicate samples are prepared, then precision at 0.3 \( \mu M \) Pi level is 0.012, or 3.8%). For measurements of DOP, which rely upon independent estimates of SRP and TDP, both the detection limit and the precision are degraded. The accuracies of Pi, TDP, and DOP estimations, on the other hand, are not easily determined because reliable, certifiable SNP standards do not exist. Furthermore, the Pi concentration
in many oligotrophic oceanic habitats is below this reported detection limit of 0.03 μM P, so alternate, high-sensitivity Pi detection methods are sometimes required.

Long pathlength spectrophotometry, including liquid waveguide "total reflection" capillary cells (up to tens of meters in length), have been used to enhance detection sensitivity (see Fujita et al., 1984). Ormaza-Gonzalez and Statham (1991) reported a Pi detection limit of 1 nM with a relative standard deviation of 6% with a 0.6-m Pyrex capillary system. More recently, Teflon AF-2400 capillary tubing (ID = 280 μm, length 4.5 m) has been used for seawater absorbance spectroscopy applications (Waterbury et al., 1997), but not yet for the measurement of Pi.

The sensitivity of colorimetric Pi analysis has also been enhanced by thermal lensing and the use of solvents to extract and concentrate the 12-MPA dye, but none of these methods improves the signal-to-noise (blank) ratio for Pi detection. Karl and Tien (1992) devised the magnesium-induced-coprecipitation (MAGIC) method for Pi analysis that improves both the detection limit and assay precision by concentrating the Pi from seawater prior to the addition of the reagents, thereby enhancing the signal-to-noise ratio. This method has been used to reliably detect subnanomolar concentrations of Pi in seawater (Wu et al., 2000). More recently, MAGIC has been combined with a novel luminol chemiluminescence technique to provide a convenient, alternative subnanomolar Pi detection system (Zui and Birsks, 2000).

The MAGIC technique, because it employs an alkaline solution for preconcentration of Pi, rather than an acidic solution, also has the advantage of reducing the potential interference from the hydrolysis of acid-labile DOP compounds during processing if they are not coprecipitated (Karl and Tien, 1992). Although it is conceivable that seawater also contains base-labile DOP which might also be included in the MAGIC-Pi assay (e.g., phosphoproteins), the pH excursion from ambient seawater required for Mg(OH)\(_2\) formation is only about 1 to 1.5 pH units (Mg(OH)\(_2\) buffers seawater at pH 9.4), so Pi isolation from non-Pi SRP and SNP compounds is possible (Thomson-Buldids and Karl, 1998). The MAGIC procedure has also been used to separate Pi from DOP, thereby providing a method for the direct determination of DOP (technically, non-Pi TDP) in seawater samples with high Pi/DOP ratios (Thomson-Buldids and Karl, 1998). Consequently, this improved method enhances the reliability of both Pi and DOP determinations in seawater.

2. Analysis of TDP

The measurement of SNP (and DOP) in seawater requires paired measurements of SRP and TDP, the latter following pretreatment to effect a quantitative conversion of all inorganic and organic, nonreactive P to SRP. The key to successful and accurate SNP (and DOP) estimation is complete oxidation and hydrolysis of the
combined P. Methods for the breakdown of P-containing organic matter can be
classified as: (1) dry combustion with or without subsequent acid hydrolysis of
poly-Pi, (2) wet combustion with permanganate, persulfate, or perchloric acid as
the oxidant, (3) UV photodradiation, or (4) alkali fusion with sodium carbonate or
sodium nitrate, followed by acid digestion. Of these, the first three method classes
have been used extensively to estimate marine DOP. Two recent and compre-
sensive reviews of environmental organic-P measurements have recently appeared
(Robards et al., 1994; Maher and Woo, 1998), so only a few of the many analytical
concerns will be discussed here.

The earliest investigators relied on permanganate oxidation (Matthews, 1917),
Kjeldahl digestion (Jones and Perkins, 1923), fuming sulfuric acid in a nitric/
hydrochloric acid mixture (Juday et al., 1928), and fuming sulfuric acid plus hy-
drogen peroxide (Redfield et al., 1937), before a simpler, safer, and generally more
convenient method for DOP analysis was eventually devised by Harvey (1948).
His method required only the addition of sulfuric acid (0.28 N final concentration)
to a whole or filtered seawater sample followed by autoclaving at 135–140°C for
5–6 h. In his hands, this method quantitatively converted nucleic acids, phospho-
protein, and phosphate esters to Pi (Harvey, 1948). An additional advantage is that
this procedure could be performed at sea for near real time estimation of DOP
(technically SNP) concentrations, if necessary or desirable. Unfortunately, only
a few years later Pratt (1950) reported that TP concentrations obtained using the
Harvey (1948) method were frequently less than the sum of SRP plus PP, so he
concluded that this method was unsatisfactory for seawater analysis; no alternative
method was presented.

Hansen and Robinson (1953) proposed a method based on initial sample oxida-
tion with perchloric acid, followed by treatment with concentrated hydrochloric
acid. Several advantages at that time included a lower blank (compared to sulfuric
acid) and more rapid oxidation of organic matter. However, like the earlier methods,
the Hansen and Robinson method required time-consuming heating to evaporate
the seawater during sample oxidation, not to mention the inherent hazards with the
use of perchloric acid.

In 1964, Menzel and Vaccaro showed the complete oxidation of carbon from
diverse organic compounds using persulfate as an oxidizing agent and a year
later an adaptation of this method was described for the quantitative determi-
nation of TP and TDP in seawater (Menzel and Corwin, 1965). The method
requires only the addition of potassium persulfate (0.7% K₃S₂O₈ final concen-
tration) to a seawater sample followed by autoclaving at 120°C for 30 min, or
boiling (100°C) for 1 h. Microwave digestion has also been used (Woo and Maher,
1995). During the procedure, decomposition of persulfate to sulfuric acid results
in a drop in pH to about 1.5–1.8, with hydrogen peroxide as another key by-
product. This highly oxidizing, hot acidic environment ensured the complete ox-
dation of organic matter. Tests with standard P-containing compounds and P mass
balance reconciliation with diatom cultures confirmed the efficacy of this procedure (Menzel and Corwin, 1965). It should be mentioned that inorganic poly-Pi, if present, would be hydrolyzed to Pi by the Menzel and Corwin (1965) method so its presence would result in a stoichiometric overestimation of DOP if the assumption, TDP-SRP = DOP, is made.

Of the various chemical oxidation methods that require evaporation of seawater, only the magnesium sulfate–hydrochloric acid hydrolysis (Solórzano and Sharp, 1980) and the magnesium nitrate oxidation methods (Cembella et al., 1986) have been employed for DOP estimation. These straightforward, efficient, but somewhat laborious methods involve drying a small volume (~10 mL) sample with exogenous magnesium salt, followed by high-temperature (450–500°C) combustion for 2 h. This decomposes the organic matter and converts some of the DOP to Pi. The residue is then treated with hot (80°C) hydrochloric acid to completely hydrolyze any poly-Pi that may be present to reactive Pi. The Cembella et al. (1986) magnesium nitrate method targeted hydrolysis of the extremely stable phosphonate compound class and was shown to be very effective.

Armstrong et al. (1966) have provided a fundamentally different approach to the same end; namely, photochemical combustion of organic matter by UV radiation. This method, which employs quartz reaction tubes, a 1200-W mercury arc lamp (Hanovia Model 189A, or equivalent), and the addition of only hydrogen peroxide as a source of oxygen, provides an efficient and rapid (1 h) means for the conversion of selected DOP compounds to Pi. A comprehensive review of the probable mechanisms of organic matter destruction during UV treatment, including characteristics of the various UV lamps, effects of pH and choice of oxidant(s) has recently been published (Golimowski and Golimoska, 1996). Several advantages include low (effectively, zero) reagent blank and, therefore, higher precision, and the ability to measure both DON (by oxidation to nitrate plus nitrite) and DOP in a single sample. For the phosphate ester (Gly-3-P, ribose-5-P) and phosphonate compounds (2-AEPn) tested, complete hydrolysis to Pi was observed following an irradiation period of approximately 1 h at 60–80°C (the sample tubes were positioned 7 cm from the UV lamp with an incident actinic energy of 200–250 mW cm⁻²; Armstrong et al., 1966). However, UV photooxidation methods are plagued by aging lamps, hot spots/cold spots in the irradiation apparatus, and the need for adequate temperature control; the use of internal standards and adequate reference materials is highly recommended (see Kérouel and Aminot, 1996).

One unique characteristic of the UV photooxidation method, which may be construed as either an analytical advantage or as a disadvantage, is the inability to degrade inorganic and organic polyphosphates. As mentioned above, the persulfate oxidation procedure will overestimate DOP in the presence of inorganic poly-Pi. In contrast, inorganic poly-Pi will not interfere in the UV method but DOP will be underestimated if organic poly-Pi compounds, such as ATP, are present. Armstrong and Tibbitts (1968), using a lower intensity 380-W UV mercury arc
lamp to decrease the hydrolysis reaction rates, suggested that organic poly-Pi esters initially released inorganic poly-Pi that was slowly hydrolyzed with time. They also suggested that TDP concentration, including poly-Pi, could be determined by including a postirradiation acid hydrolysis step, a method that could theoretically be used to infer relative contributions of monooester-linked P versus poly-Pi. Yanagi et al. (1992) later developed this approach of differential UV-lability into a quantitative assay for the partial characterization of DOP pool (see Section VIII).

TDP has also been measured in seawater using hydride generation and gas chromatography (Hashimoto et al., 1987). This method has several advantages over colorimetry, in particular, detection limit and reaction specificity. More importantly, the analytical basis for this method (reduction of all forms of phosphate to phosphine gas by borohydride reduction) is fundamentally different from the colorimetric procedures. The excellent quantitative correspondence between TP and TDP concentrations measured by the P hydride method and persulfate digestion for samples collected from 0 to 9600 m in the Japan Trench provides assurance that there is probably no large, yet undetected DOP pool in seawater. Such a pool was implicitly hypothesized to occur when Suzuki and colleagues reported the existence of substantial, previously undetected pools of DOC and DON (Suzuki et al., 1985; Sugimura and Suzuki, 1988). To achieve a Redfield reconciliation, there would also need to exist a previously undetected pool of DOP, but no such pool has been found (Hashimoto et al., 1987; Karl et al., 1993).

With this spectrum of potentially available methods for the measurement of TDP (i.e., SNP and, therefore, DOP) it is only natural that individual researchers might endeavor to compare two or more of these methods before adopting the most reliable one for the seawater samples under consideration. It is conceivable, even likely, that the efficiencies of these methods are site-specific due to regional variations in the chemical composition of the ambient SNP pools. It is important to mention that in presenting the DOP concentration data, later in this review, we make no corrections for variable DOP recovery based on the method selected because, quite frankly, there is no way to estimate this unless multiple TDP protocols were employed—and this situation is rare.

Four comprehensive TDP methods comparisons are worthy of mention. Ridal and Moore (1990) compared the persulfate oxidation and UV–irradiation methods against a new method which relies on a sequential UV–persulfate technique. This stepwise UV–acid treatment follows the analytical recommendation made originally by Armstrong et al. (1966) for the quantitative recovery of inorganic and organic poly-Pi compounds. Their results for samples collected from a variety of coastal and open ocean marine habitats suggested that, primarily in open ocean ecosystems, the combined method returned values 1.25–1.50 times higher than either method separately (Ridal and Moore, 1990). A subsequent study was conducted in the northeast subarctic Pacific Ocean (Ridal and Moore, 1992). For this habitat, the UV method returned an average concentration that was only 71 ± 9%
and the persulfate method 83 ± 9% of the "DOP" concentration estimated by the combined UV-persulfate technique. They concluded that the "standard" methods of analysis used for most marine studies should be considered minimum estimates of the ambient DOP concentrations; however, without additional information on the presence of inorganic poly-Pi it is not known which DOP estimation is the most accurate.

Nedashkovskiy et al. (1995) compared the wet persulfate oxidation and the dry magnesium nitrate combustion methods using seawater collected off Vladivostok and in the Northwestern Bering Sea. For samples containing low concentrations of organic P (≤0.5 μM) the two methods were indistinguishable. However, in productive Bering Sea waters, where organic P exceeded 0.5 μM, the mean difference approached 20% in favor of the dry combustion method.

Ormaza-González and Statham (1996) compared five independent methods of TDP analysis. The highest concentrations were obtained by the magnesium nitrate oxidation method while the lowest values were obtained by the UV photooxidation technique. These data suggest that inorganic and organic poly-Pi compounds, that are not detected by the UV method, may comprise a significant percentage of the TDP pool in the North Sea waters used for this comparison.

Monaghan and Ruttenberg (1999) compared the dry combustion method, using two separate oxidants (magnesium nitrate and magnesium sulfate), to the wet chemical persulfate oxidation method. This comprehensive study tested the recovery of Pi from a variety of organic-P compounds as well as the estimation of TDP from continental shelf seawaters collected off California. Although quantitative recovery of Pi from selected phospholipids and phosphonates was only achieved by dry combustion, TDP concentrations for natural seawater samples were comparable for magnesium sulfate oxidation and persulfate and, on average, 7% lower for the nitrate oxidation method. This indicates a negligible presence in these natural samples, of the known organic-P compounds that are poorly recovered by persulfate oxidation, namely phospholipids and phosphonates (Monaghan and Ruttenberg, 1999).

Finally, because many marine biogeochemists seek fundamental information on the coupled N and P pool dynamics in seawater, several methods have been devised to optimize the measurements of DON and DOP or DOC/DON/DOP in a single chemical oxidation treatment. As mentioned above, UV photooxidation can be used to measure both compound classes, though the optimal irradiation time for DOP is 1–2 h, compared to 24 h for DON (Walsh, 1989). A recent comparison of DON estimation using UV photooxidation, persulfate wet chemical oxidation, and high temperature combustion oxidation indicated higher variability and lower recoveries for the UV method (Bronk et al., 2000); however, their inability to recover NH₄⁺ as NO₃⁻ casts some doubt on the efficiency of their UV photooxidation procedures.

The fundamental problem with simultaneous wet chemical oxidation of DON and DOP is that organic N requires an alkaline medium while organic P requires an
E. Analytical Interferences in SRP and TDP Estimation

Of the various potential interferences on the accuracy of Pi and TDP estimation, two in particular deserve mention. The first is the potential overestimation of Pi by "reactive" organic-P compounds (Fig. 6). Their ubiquity in marine ecosystems worldwide, especially surface waters, will contribute to the measurement of SRP, and systematically underestimate SNP and DOP. TDP is unaffected by their presence. An equally insidious analytical interference derives from the nearly ubiquitous presence of arsenic (As) in seawater. Arsenate (AsO$_4^{3-}$), the most oxidized form of As, reacts with the SRP reagents and forms a blue-colored complex of an equivalent molar absorptivity to that of Pi, while arsenite (AsO$_3^{3-}$) does not react at all. Dissolved AsO$_4^{3-}$ can be reduced to AsO$_3^{3-}$ with thiosulfate in acidic medium in the presence of excess metabisulfite (von Schouwenburg and Walinga, 1967; Johnson, 1971) to prevent it from interfering with SRP estimation (Fig. 6). As will be reported later in this chapter, the concentration of soluble reactive As (SRAs) sometimes exceeds the concentration of Pi so attention to this potential analytical problem is imperative. Because the time for full color development is prolonged by the addition of thiosulfate required to eliminate AsO$_4^{3-}$ interference, this procedure is not routinely employed in most automated SRP analyses (Downes, 1978), even though AsO$_3^{3-}$ may still interfere. For accurate TDP determinations, it is also necessary to correct for AsO$_3^{3-}$ that is produced during sample oxidation as a result of the conversion of SNAs to SRAs (e.g., oxidation of organic As), though this is seldom, if ever, done.

More than just an analytical nuisance, As has an important biogeochemical cycle that has many features in common with the marine P cycle. In fact, because of the variable redox states commonly found in seawater (As$^{5+}$ and As$^{3+}$) and the role marine microorganisms have in As oxidation, reduction, and methylation, we can anticipate an active As cycle in most marine habitats. Furthermore, AsO$_3^{3-}$ is a well studied analog of Pi, and it is transported and incorporated by many of the same enzyme systems. It is also a well recognized uncoupler of oxidative and photophosphorylation and, hence, a metabolic poison (Benson, 1984; Francesconi and Edmondson, 1993). When the ambient pool of AsO$_3^{3-}$ is high, relative to Pi, as occurs in many oligotrophic marine habitats (Karl and Tien, 1997), there should be a selection for microorganisms with either high specificity Pi transport systems or high capacity detoxification mechanisms, or both. The former would lead to a
preponderance of As$\text{O}_4^{3-}$ and the latter to a preponderance of DOAs. As mentioned above, both SRP (P) and TDP measurements need to be cognizant of potential interference from all possible forms of As.

Finally, all field data on SRP and TDP concentrations measured using the Denigès-Atkins method and collected prior to about 1940 had an inadvertent analytical error caused by salt interference that required a correction factor of 1.35 (Cooper, 1939a). This correction, when applied to the data collected for the N:P of plankton and water samples collected off Plymouth, UK, resulted in a revised N:P molar stoichiometry of 15:1, rather than the 20:1 that had been reported previously; this revised ratio was termed the “Cooper ratio” (Cooper, 1938, 1939b).

VI. DOP IN THE SEA: VARIATIONS IN SPACE

Biogeochemical cycles of C, N, and P in the sea are ultimately sustained by solar energy via the process of photosynthesis. Consequently, DOP production is highly correlated with the primary formation of organic matter in the euphotic zone. The C:N:P molar stoichiometry of both particulate and dissolved organic matter pools is a key ecological parameter in the sea and will therefore also be considered briefly in this review. However, the primary focus will be on total DOP, which likely contains a broad spectrum of compounds of variable C:P and C:N:P stoichiometry (Fig. 2; Table I).

The instantaneous concentration of DOP in seawater is expected to vary geographically, with depth in the water column and, for a given location, with time. Consequently, the ambient DOP pool must be viewed as a transient that is largely controlled by the balance between local DOP production and DOP removal processes. The physical, chemical, and biological influences on these key ecosystem processes will be discussed later, as will information on the chemical characterization of the heterogeneous DOP pool. In this section, we present the geographical and depth distributions of oceanic DOP to establish generalized concentration patterns and broad correlations with other relevant parameters, including P. We have employed two separate data sources: (1) the U.S. National Oceanic and Atmospheric Administration – National Ocean Data Center’s (NOAA-NODC) online oceanographic profile database (http://www.nodc.noaa.gov/cgi-bin/JOPl/jopl) and (2) our own DOP database collated from the archival literature, which includes many published profiles that are not included in the NOAA-NODC database.

The NOAA-NODC global ocean search acquired all SRP/TDP data entries; the full extracted data set included $n = 250,694$ paired measurements. We then screened these data and removed all entries where the reported SRP exceeded the reported TDP (i.e., “negative” DOP); this decreased the number of measurements to $n = 233,118$ data pairs. This was our primary Global Ocean DOP data set. We also subsampled the Global Ocean DOP data set to obtain a secondary database for
stations where the depth of the water column was \( \geq 200 \) m (i.e., the pelagic marine environment which is the stated focus of this review). This otherwise unedited Global Open Ocean DOP (or GOOD) database, which includes \( n = 139,747 \) measurement pairs is available along with our enhanced DOP summary upon request of the senior author. The GOOD database includes measurements from all major ocean basins but has several large data gaps, most notably the Eastern North Pacific Ocean, the South Pacific Ocean, and the Southern Ocean (Fig. 7). Nevertheless, this \( n = 139,747 \) pairs of SRP/TDP measurements greatly exceeded our initial expectation, especially considering the relatively few open ocean DOP profiles that have been published in the refereed literature. A notable exception, that we highlight here, is the extensive survey of the North and South Atlantic Ocean basins conducted as part of the International Geophysical Year (IGY). During this 2-year (1957–1958) investigation, McGill (1963, 1964) compiled what amounts to the most comprehensive study of oceanic DOP yet attempted. This must be considered the exception to the otherwise sparse open ocean database.

A. Regional and Depth Variations in DOP

There are several general features of the global ocean DOP distributions. First, concentration versus depth profiles in the open ocean almost exclusively reveal
Figure 8  SRP, DOP and DOP as a percent of TDP for the Hawaii Ocean Time-series Sta. ALOHA (22°45′N, 158°W). These data are mean values and 95% confidence intervals based on samples collected during 110 research cruises between October 1988 and December 1999.

elevated DOP in the upper 0–100 m of the water column. For example, the 12-year climatology of SRP and DOP at Station ALOHA (22°45′N, 158°W) documents a characteristic inverse depth relationship with high concentrations of DOP in the surface water, decreasing with water depth, and vice versa for SRP concentrations (Fig. 8). The only possible exception to this general pattern might be for high-latitude habitats in winter where deep vertical mixing and low solar irradiance preclude contemporaneous near-surface DOP production via primary production.

Despite this predictable depth dependence of total DOP in the open sea, individual DOP compounds or compound classes can have one of three fundamentally different distributions as a function of depth in the water column: (1) local enrichments near the sea surface with decreasing concentrations beneath the euphotic zone (similar to total DOP), (2) near surface depletion with increasing concentrations beneath the euphotic zone, or (3) constant concentration with depth. Dissolved
nucleotides (e.g., ATP) conform to the first pattern of depth distribution and dissolved vitamins (e.g., vitamin B₁₂) generally conform to the second pattern. These depth distributions are a result of net production/consumption and import/export processes (see Section VIII).

At the subtropical location of Sta. ALOHA, DOP in the upper 100 m of the water column averages 0.20–0.22 μM or approximately 70–80% of the TDP pool. Below 100 m, DOP decreases with increasing water depth to values <0.05 μM; DOP concentrations at depths ≥300 m are consistently <10% of the corresponding TDP, indicating a deep water dominance by SRP (Fig. 8). Similar patterns are also observed for both the IGY (North and South Atlantic Oceans) and GOOD data sets (Fig. 9), with the exception of a generally lower percentage of DOP in near surface

![Figure 9](image_url)

**Figure 9** Vertical profiles of DOP, expressed as percentage of TDP, for the entire Global Open Ocean DOP (GOOD) database and International Geophysical Year (IGY) subsampled data (see text for more details). The data are presented as mean values and 95% confidence intervals. The data were depth binned, as shown, prior to the determinations of the summary statistics. For the IGY profile, n ranged from 32 (125 m) to 594 (surface) with a median of n = 130. For the GOOD profile, n ranged from 805 (850 m) to 12,234 (25 m) with a median of n = 2700.
waters (i.e., 50–60% of the TDP pool compared to 70–80% for the subtropical North Pacific; Fig. 8). For all oceanic DOP profiles, however, both the absolute DOP concentration and the DOP/TDP ratio vary systematically, and therefore predictably, with water depth.

It is well known that the concentration of SRP, especially in subthermocline (>600 m) waters, increases as the age of the water mass also increases (Levitus *et al.*, 1993). This is a result of the long-term net remineralization of organic matter. These spatial patterns are clearly evident in the IGY data set (Figs. 10 and 11 [see color plate]). A comparison of two zonal sections, one at 40°N and the other at 24.25°S in the Atlantic Ocean documents the following: (1) a contrast between relatively low TDP/low SRP waters at 40°N compared to high TDP/high SRP in the northward flowing Antarctic Bottom Water in the west (>4000 m) and Antarctic Intermediate Water (~1000 m) seen at 24.25°S; (2) nearly homogeneous concentrations of all forms of P in the relatively “young, well mixed” North Atlantic compared to the South Atlantic, especially for the sub euphotic zone waters; and (3) a general increase in surface water DOP concentrations in the South Atlantic basin, especially in the near-surface water (Figs. 10 and 11). For DOP there also appears to be a significant basin-scale east-to-west gradient, at least at 40°N, with higher DOP concentrations in the western North Atlantic, and minimum DOP concentrations in the deep central waters of both basins. These regional variations are superimposed on the general global DOP distributions described previously and are probably related to circulation patterns and processes. A similar Atlantic Ocean intrabasin gradient in DOP was recently reported by Vidal *et al.* (1999) for a transect from the Canary Islands to Argentina (22°N to 31°S). Euphotic zone (0–200 m) DOP concentrations in the western portion of the basin were approximately two to three times greater than they were in the eastern portion of the Atlantic basin (0.2 to 0.3 μM versus <0.1 μM; Vidal *et al.*, 1999).

If the DOP/TDP ratio of the global ocean remains more or less constant for a given water depth, as the GOOD data set implies (Fig. 9), then one might predict a systematic interocean basin increase in DOP concentration with highest values in the North Pacific and lowest concentrations in the North Atlantic, resulting from the ocean’s “conveyor-belt”-like circulation patterns. These interbasin differences in the concentration of DOP, and perhaps in the C:N:P stoichiometry of the DOM pool as well, are anticipated, especially if remineralization processes and long-term accumulation of recalcitrant DOM is an important process. Regional variations within a given ocean basin are also possible.

The extant global data set on subeuphotic zone DOP concentrations reveals an approximately constant DOP:TDP ratio which translates to a higher absolute DOP concentration in the North Pacific compared to the North Atlantic based on the higher concentrations of SRP (and hence, TDP) in the Pacific basin (Fig. 9). This reflects the broad conveyor-belt-like features of ocean circulation; the North Atlantic Ocean is the origin and the North Pacific Ocean is the terminus of the global transport system.
Figure 10  Phosphorus concentration (µM) versus ocean depth contours of TDP (top), SRP (center) and DOP (bottom) for samples collected on a transect at 40°N latitude in the Atlantic Ocean during the International Geophysical Year (IGY). TDP was measured using the method of Harvey (1948). These data were originally presented by McGill (1963) and were obtained by using the NOAA-NODC World Wide Web-based online search program.
Figure 11  As in Figure 10, except for a transect at 24.25°S latitude.
Unfortunately subeutrophic zone DOP measurements, especially where DOP is ≤10% of the TDP pool, are not very reliable given the nature of the paired analyses of SRP and TDP and estimation of DOP by difference. For this reason, several investigators acknowledge the uncertainty of the deep water DOP pool estimates despite reporting them. The global compilation, with individual estimates based on fairly large sample sizes (n>200), indicates: (1) a detectable pool of DOP at all ocean depths and (2) a decreasing DOP percentage with increasing depth. These features would be consistent with the time dependent remineralization of labile and semilabile DOP. While the global open ocean DOP data sets are poorly positioned to evaluate this biogeochemical prediction rigorously, owing to the inherent inaccuracies of DOP estimation in subeutrophic zone waters (see following section), there does appear to be an increase in DOP along the circulation trajectory (Fig. 12). The global pattern of increasing SRP in deep waters has been previously observed, but we believe this is the first evidence for interbasin variations in near-surface DOP.

**Figure 12** Changes in SRP and TDP pools along the global ocean circulation trajectory from the North Atlantic to the North Pacific oceans. North and South Atlantic measurements are from the NOAA-NODC Global Ocean DOP dataset, and the North Pacific measurements are from Station ALOHA (22°45’N, 158°W). Values shown are means and 95% confidence intervals for each respective data set.
This rather robust inverse relationship between SRP and DOP in the surface ocean is a manifestation of Pi uptake, net biomass production, and the rapid re-mineralization of particulate organic matter. The higher the ratio of recycled-to-total production, the higher the turnover rate of particulate and dissolved organic matter. These processes lead to the eventual, local accumulation of semilabile and refractory DOM compounds, resulting in the broad geographical distributions observed for the global DOP data set. While it is not explicitly demonstrated here, there is likely to be a corresponding gradient in the chemical composition of DOP with a higher percentage of labile phosphate esters in high-latitude regions and, for a given location, in near surface waters.

In spite of these broad generalized patterns of DOP in the world ocean, local and regional variations are also evident. DOP is enriched (>0.25 μM) in most coastal and estuarine habitats that have been investigated (Table II). In the semienclosed Baltic Sea and surrounding coastal regions (Fig. 13 [see color plate]), surface DOP concentrations vary considerably from a minimum, “background” concentration of 0.4 to 0.6 μM to values >1 μM in regions that are likely impacted by point source and non-point-source nutrient inputs. In particular, the west coast of the Jutland Peninsula appears to be especially enriched in DOP relative to offshore regions. Even in the open ocean pelagic ecosystem off the west coast of South America, elevated near surface DOP concentrations are apparent (Fig. 14 [see color plate]). Upwelling-induced organic matter production and coupled DOP production, combined with coastal runoff and locally restricted flushing can all contribute to both local and regional elevations in surface DOP.

B. DOP Concentrations in the Deep Sea

The precision of DOP estimation is eroded when SRP is ≥90% of the TDP pool, for example at all ocean depths greater than approximately 500 m in the global open ocean (Fig. 9), as well as in many high-latitude surface waters. Small relative errors in SRP and TDP determinations translate into large errors in the calculation of DOP. Ketchum et al. (1955) presented a comprehensive assessment of the analytical and statistical considerations for samples collected in the equatorial Atlantic Ocean. From a paired Pi and TDP (using the method of Harvey, 1948) measurement suite that consisted of more than 1000 seawater samples, they concluded that unless the difference (i.e., TDP-Pi) exceeded 10% of the TDP value, the DOP (technically, SNP) estimate cannot be considered to be significantly different from zero. For their analyses, 95% of the surface water samples contained significant DOP decreasing through the region of the phosphate-cline where Pi increases and DOP decreases with increasing water depth. At depths greater than 1000 m there was no measurable (statistically significant) DOP present; only 13 out of 259 deep water samples (5%) gave positive differences that exceeded 1 standard deviation.
Figure 13 Compilation of near surface ocean DOP concentrations (μM P) for samples collected in the Baltic Sea and surrounding coastal regions. These data were obtained from the NOAA-NODC using their World Wide Web-based online search program and are neither time-averaged nor contemporaneous measurements. Stations are indicated by white circles.
Figure 14  As in Figure 13, except for samples collected off the west coast of South America.
Dynamics of DOP (Ketchum et al., 1955). It could not be determined whether DOP was present at concentrations below the analytical detection limit, or whether DOP was truly absent. Consequently, few reliable data sets exist for DOP concentrations in the deep mesopelagic and abyssopelagic zones (≥1000 m). This is quite unfortunate because the poorly understood, stepwise conversion of PP and DOP to P in is a key metabolic process in these regions.

This analytical uncertainty, for better or for worse, has not prevented ocean researchers from sampling, analyzing, and reporting subeuphotic zone DOP concentrations. The caution we urge here is to be cognizant of the statistical constraints on the methodologies employed, as they will clearly affect the ecological implications of the data obtained. Examination of these data sets documents a fairly broad range in mesopelagic and deep sea DOP concentrations that cannot be easily reconciled with any known oceanic processes. A difference of just 20–50 nM DOP between these determinations, when scaled to dimensions of the deep sea, creates or eliminates a DOP pool that becomes significant for global ocean P budgets. It is imperative that we obtain reliable deep water DOP measurements if we ever hope to understand subeuphotic zone DOP dynamics or marine P cycle as a whole.

In theory, one might anticipate a small, but finite DOP pool that would represent a balance between local DOP production and utilization processes. The supply of DOP to depth depends to a large extent on the nature of organic matter export processes (e.g., downward diffusion and mixing of DOM versus gravitational settling of POM) and on the pathways and coupling between export and remineralization mechanisms. However, only the process of gravitational settling of particulate matter can export significant amounts of “fresh” organic materials to subthermocline (≥500 m) ocean depths. During the 1- to 2-month-long journey to the seabed in the open ocean, these exported particles are disaggregated, hydrolyzed, and otherwise reworked with a continuous, and sometimes variable, loss of organic C, N, and P. Much of this organic matter is remineralized at depth which accounts for the generally increasing concentrations of dissolved inorganic nutrients (see Fig. 8 and 12). For open ocean habitats, the flux of organic P from the euphotic zone (150 m reference depth) is approximately 5 mmol P m⁻² year⁻¹. This statistical population of sinking particles is attenuated nearly an order of magnitude by the 1000-m depth horizon and nearly two orders of magnitude at the seabed (5000 m). This attrition of organic P from the sinking particulate pool as a predictable function of depth is the primary starting material for the suspended particulate and dissolved organic matter pools beneath the euphotic zone. Only when a sufficient number of determinations are available can the statistical significance of DOP in the deep sea be assured.

Repeated observations of SRP and TDP in a section between Montauk Point, New York, and Bermuda during the period 1958–1961 have demonstrated the appearance, at depth, of a low-salinity subarctic intermediate water mass that covaries with DOP concentration (McGill et al., 1964). For water samples collected
Table II
Selected Marine Dissolved Organic Phosphorus (DOP) Concentrations and DOP as Percentage of Total Dissolved Phosphorus (% of TDP) Reported from a Variety of Geographic Locations

<table>
<thead>
<tr>
<th>Location</th>
<th>Depth (m)</th>
<th>Methoda</th>
<th>DOPb (µM)</th>
<th>% of TDP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coastal/Estuarine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suruga Bay, Japan</td>
<td>0–1000</td>
<td>UV</td>
<td>0.11–0.29</td>
<td></td>
<td>Yanagi et al., 1992</td>
</tr>
<tr>
<td>Prydz Bay, Antarctica</td>
<td>0–200</td>
<td>UV</td>
<td>0.05–0.90</td>
<td></td>
<td>Yanagi et al., 1992</td>
</tr>
<tr>
<td>Florida Bay, USA</td>
<td>Surface</td>
<td>DA-AH</td>
<td>0.37 ± 0.02 Range 0.04–2.03 (n = 183)</td>
<td></td>
<td>Fourquarean et al., 1993</td>
</tr>
<tr>
<td>Sandfjord, Norway</td>
<td>Surface</td>
<td>PO</td>
<td>0.224–0.264</td>
<td>98–98</td>
<td>Thingstad et al., 1993</td>
</tr>
<tr>
<td>Mamala Bay, USA</td>
<td>Surface</td>
<td>PO</td>
<td>0.12–0.17</td>
<td>37–43</td>
<td>Björkman and Karl, 1994</td>
</tr>
<tr>
<td>Chesapeake Bay, USA</td>
<td>Surface</td>
<td>PO-AA</td>
<td>0.2–0.6</td>
<td></td>
<td>Conley et al., 1995</td>
</tr>
<tr>
<td>Bay of Aarhus, Denmark</td>
<td>3</td>
<td>PO</td>
<td>0.48 ± 0.01</td>
<td></td>
<td>Thingstad et al., 1996</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>PO</td>
<td>0.63 ± 0.03</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>16</td>
<td>PO</td>
<td>0.98 ± 0.05</td>
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<td>Continental shelf</td>
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<tr>
<td>Southern California Bight</td>
<td>5</td>
<td>UV</td>
<td>0.21</td>
<td>51</td>
<td>Ammerman and Azam, 1985</td>
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<td>20</td>
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<td>0.22</td>
<td>42</td>
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<tr>
<td>Santa Catalina Basin</td>
<td>0–50</td>
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<td>0.27 to 0.42</td>
<td>~0.15</td>
<td>Holm-Hansen et al., 1966</td>
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<tr>
<td>(33°18.5’ N, 118°40’ W)</td>
<td>75–1300</td>
<td>UV</td>
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<tr>
<td>NE Pacific off</td>
<td>10</td>
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<td>0.126</td>
<td>66</td>
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<td>25</td>
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<td>NE continental shelf slope</td>
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<td>(George Banks)</td>
<td>Surface</td>
<td>UV</td>
<td>0.17</td>
<td></td>
<td>Hopkinson et al., 1997</td>
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<tr>
<td></td>
<td>200</td>
<td>UV</td>
<td>0.06</td>
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<td>Southern NW shelf, Australia</td>
<td>&lt; 25 m</td>
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<td>Eel River Shelf, N. California</td>
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<td>DA-AH</td>
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<th>Method</th>
<th>DOP (µM)</th>
<th>% of TDP</th>
<th>Reference</th>
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<td></td>
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<td>158°14'W)</td>
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<td>0.15–0.20</td>
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<tr>
<td></td>
<td>0–50</td>
<td>UV</td>
<td>0.10–0.35</td>
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<td>Abell et al., 2000</td>
</tr>
<tr>
<td>North Pacific</td>
<td></td>
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<td></td>
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<tr>
<td>Subtropical Gyre</td>
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<tr>
<td></td>
<td>Surface</td>
<td>PO</td>
<td>0.140–0.285</td>
<td>66–90</td>
<td>Björkman et al., 2000</td>
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<td></td>
<td>3600</td>
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<tr>
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<td>PO-AA</td>
<td>0.13</td>
<td>95</td>
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<td></td>
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<td>0.09</td>
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<td>BDL</td>
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<td>Sargasso Sea</td>
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<td>(26°N, 70°W to 31°40'N,</td>
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<td>64°10'W)</td>
<td>125</td>
<td>UV</td>
<td>0.1–0.5</td>
<td>~95–100</td>
<td>Cavender-Bares et al.,</td>
</tr>
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<td>Sargasso Sea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2001</td>
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<tr>
<td>(31°67'N, 64°17' to 26°1'N, 70°W)</td>
<td>Surface</td>
<td>UV</td>
<td>0.074 ± 0.042</td>
<td>~99</td>
<td>Wu et al., 2000</td>
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<td>Southern Ocean</td>
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<tr>
<td>(57°35'S, 57°W)</td>
<td>300</td>
<td>UV</td>
<td>0.16</td>
<td></td>
<td>Sanders and Jickells,</td>
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<tr>
<td></td>
<td></td>
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<td>0.10</td>
<td></td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td></td>
<td>BDL</td>
<td></td>
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</table>

4 UV, ultraviolet photo-oxidation; PO, wet persulfate oxidation; PO-AA, persulfate oxidation under alkaline-acid conditions; DA-AH, dry ashing, acid hydrolysis; AH-PO, ash hydrolysis and persulfate oxidation; UV-PO, ultraviolet photo-oxidation-persulfate oxidation.
5 Data are mean concentrations, mean ± 1 SD or ranges as shown.
6 BDL, below detection limit which for the standard paired SRP/TDP assay is ~20–30 nM.
at hydrostation “S” south of Bermuda statistically significant time variable concentrations of DOP (i.e., TDP-SRP) were evident (Fig. 15). It was concluded that these are advective rather than in situ features and provides a constraint on the interpretation of single-point, Eulerian design time-series experiments. It is possible that these variations in the DOP concentrations of deep North Atlantic Ocean waters are real. McGill et al. (1964) previously reported time-dependent variations of DOP from <0.04 to >0.15 μM for waters below 1500-m at Station “S” in the Sargasso Sea near Bermuda (Fig. 15). From mass balance considerations, they estimated a net in situ DOP consumption rate of 0.1 μM year⁻¹, so it is conceivable that interannual variations may exist even in the deep sea. Significant seasonal and interannual variations in particulate matter export at the 1000-m reference level on the order of ±50% of the long-term climatological mean are not unexpected, even in the subtropical gyres of the world ocean. This could easily lead to the changes observed by McGill et al. (1964) for samples collected at Station S. Even if these “temporal” patterns can be reconciled by spatial variability at this site, one would still need to explain the cause(s) of spatial variability in deep-water DOP.

Recently a novel method has been devised that can provide more accurate and reliable DOP measurements in the presence of a large SRP pool; deep-sea DOP estimation is a perfect application for the modified MAGIC method (Thomson-Buildis and Karl, 1998). This method provides a separation of SRP from most DOP compounds prior to the direct measurement of DOP following oxidation-hydrolysis to Pi. Application to deep Pacific Ocean seawater has indicated an abyssopelagic DOP concentration of <40 nM values that would have been undetectable by the
conventional TDP-SRP difference methodology (Fig. 16). For comparison, we present two other “credible” published profiles for the North Pacific Ocean obtained using different methods for TDP but both based upon the more traditional DOP estimation by difference between SRP and TDP. While there does appear to be a detectable DOP pool in the deep waters of the North Pacific it is uncertain whether it is $<40$ nM as the MAGIC method implies or more than twice that amount (Fig. 16).

An important ecological prediction of this depth-dependent delivery of organic P to the subeuphotic zone waters is the potential selection against DOP utilization
by the relatively high and depth-dependent ambient concentrations of Pi. All else being equal, one would predict a relative "preservation" of DOP in the deep sea due to preferential assimilation of Pi. Of course, if DOP is utilized for reasons other than Pi acquisition, for example, as a biosynthetic precursor for nucleic acids, then there may be a simultaneous utilization of both Pi and DOP in these otherwise Pi-sufficient deep sea habitats. To our knowledge this important aspect of microbial ecophysiology has not been systematically investigated.

C. C:N:P STOICHIOMETRY OF DISSOLVED AND PARTICULATE MATTER POOLS

All known organisms contain a nearly identical suite of biomolecules with common structural and metabolic functions; this biochemical uniformitarianism serves to constrain the bulk elemental composition of life. In a seminal paper, Redfield and his colleagues (Redfield et al., 1963) summarized much of the earlier research on C, N, and P stoichiometry of dissolved and particulate matter pools in the sea and combined these data sets into an important unifying concept that has served as the basis for many subsequent field and modeling studies in oceanic biogeochemistry. As Paul Falkowski has so eloquently stated "the elemental stoichiometry that we call the Redfield ratio is a result of nested processes that have a molecular foundation but are coupled to biogeochemical processes on large spatial and long temporal scales" (Falkowski, 2000). These biogeochemical characteristics of oceanic habitats involve both macro (N, P) and trace nutrient (e.g., iron) limitation, including DOP pool bioavailability.

Despite this perceived uniformity, it is well known that the chemical composition of living organisms can vary considerably as a function of growth rate, energy (including light) availability, and ambient nutrient (including both major and trace elements) concentrations and bioelemental ratios. For example, under conditions of saturating light and limiting N, certain photoautotrophic organisms can store C as lipid or carbohydrate, thereby increasing their C:N and C:P ratios. Likewise, if P is present in excess of cellular demands, it can be taken up and stored as poly-Pi, causing a decrease in the bulk C:P and N:P ratios. Conversely, when the bioavailable N:P ratio is greater than what is present in "average" organic matter (i.e., >16N:1P by atoms), selected groups of microorganisms can exhibit a metabolic "P-sparing" effect and produce biomass with C:P and N:P ratios significantly greater than the hypothesized Redfield ratios of 106:1 and 16:1, respectively. Based on theoretical biochemical arguments, Raven (1994) predicted that the C:N:P ratio in microorganisms is nonscalable with decreasing cell size. He concluded that the C:P ratio in small cyanobacteria should be higher than in average eukaryotic phytoplankton cells. Consequently, there does not appear to be a robust constraint on ecological C:N:P stoichiometry in the marine environment.
Loh and Bauer (2000) have recently assembled one of the most complete biogeochemical data sets, including particulate C, N, P, as well as dissolved C, N, P pool measurements. They used their data from the Eastern North Pacific Ocean to test the hypothesis of preferential remineralization of N relative to P, and of N and P relative to C. Based on C:P and N:P ratios, they concluded that organic P is preferentially remineralized over organic C and N, resulting in increasing C:P and N:P ratios of the DOM pool with increasing water depth. Our inability to provide a comprehensive inventory of DON and DOP compounds has promoted a stoichiometric assessment of the dissolved organic matter pool based on separate measurements of DOC, DON, and DOP. However, this does little to provide a biochemical characterization of the representative compound classes dissolved in seawater.

Cavender-Bares et al. (2001) have measured near-surface (3 m) dissolved inorganic and organic N and P distributions along a >2500-km transect (sampled in March 1998). Their study area included the eastern North Atlantic subtropical gyre, the Gulf Stream and temperate coastal shelf habitats. Throughout the subtropics, the total dissolved N (TDN) and TDP pools were dominated by organic components (Fig. 17). SRP was very low throughout the southern portion of the

![Graph](image)

**Figure 17** Total N and P (TDN and TDP) and organic N and P (DON and DOP) and molar N:P stoichiometry of the respective pools for samples collected along a North Atlantic Ocean transect in March 1998. Vertical striped bars indicate the approximate location of the Bermuda Atlantic Time-series Study (BATS) and the Gulf Stream. The dotted line in the bottom plot shows the Redfield N:P ratio (16:1) as a point of reference. Reprinted with permission from Cavender-Bares et al. (2001).
transect with concentrations between 1 and 10 nM and occasionally below 1 nM (Cavender-Bares et al., 2001). Only north of the Gulf Stream did the dissolved inorganic constituents represent a significant proportion of the total dissolved nutrient pools. Furthermore, the DON and DOP pools were relatively invariant over a broad geographical range (26°–37°N) with mean concentrations of 6.3 μM N and 0.12–0.13 μM P, respectively (Fig. 12). The mean molar N:P ratio of the DOM pool averaged 50:1, a value that is three times greater than the canonical Redfield stoichiometry of 16N:1P. North of the Gulf Stream the molar ratio TDN:TDP pool dropped to 16:1 even though the N:P ratio of the DOM pool remained >25 (Fig. 17). The accumulation of N, relative to P, in the marine DOM pool within the subtropical gyre is likely caused by the addition of “extra” N by the metabolic activities of N2 fixing microorganisms that are selected for during periods of low (<16N:1P) dissolved inorganic nutrient availability, especially south of 31°N (Cavender-Bares et al., 2001). Further implications of this ecological selection process on related aspects of the marine P cycle are discussed in the following section.

VII. DOP IN THE SEA: VARIATIONS IN TIME

Long-term ecological studies are predicated on the straightforward assertion that certain processes, such as succession and climate variability, are long-term processes and must be studied as such (Strayer et al., 1986). Indeed there are many examples in the scientific literature where interpretations from short-term ecological studies are at odds with data sets collected over much longer time scales. Because it is difficult to observe slow or abrupt environmental changes directly, much less to understand the fundamental cause-and-effect relations of these changes, Magnuson (1990) has coined the term “the invisible present” to refer to these complex ecological interactions. Most of what we know about the marine DOP pool is locked up in the invisible present and opaque past. As data accumulate in a long-term ecological context, new phenomena will become apparent and new understanding will be derived.

Our presentation, above, of the compiled GOOD database implies that DOP pools are constant in time for a given location. This is, most likely, an inaccurate assumption. Because the generally inverse relationship between subepuhtotic zone SRP and DOP concentrations is a manifestation of plankton growth in the surface ocean, one might predict a significant but opposite seasonal cycle in the concentrations of SRP and DOP, with DOP maxima following the vernal bloom in temperate ocean habitats (Harvey, 1955; Strickland and Austin, 1960; Butler et al., 1979). During the Research on Antarctic Coastal Ecosystems and Rates (RACER) program, a detailed study of Marguerite Bay from open water to near the fast ice edge revealed a systematic and coherent shift from a SRP-dominated to
a DOP-dominated euphotic zone (Fig. 18). This spatial mosaic was a result of an ice-edge-induced bloom of phytoplankton and the temporal uncoupling of DOP production and DOP utilization processes (Karl et al., 1992). Similar processes are likely to occur wherever and whenever net planktonic biomass is produced. These seasonally phased near-surface ocean production processes should also drive a seasonal cycle in subeuphotic zone processes via a coupled particulate matter production–export cycle. However, for reasons already presented above, we currently lack the analytical tools to observe small changes in DOP within habitats where the DOP concentrations are \(\leq 10\%\) of the corresponding TDP pools.

During the past century, there have been several systematic time-series studies of the marine P cycle; we shall present two case studies in this section. The first is from coastal waters of the English Channel, a study that began in 1916 with the pioneering research of Matthews, Atkins, Cooper, Harvey, and others at the Plymouth Marine Laboratory (see Section III). The second, the Hawaii Ocean Time-series (HOT) study of the subtropical North Pacific Ocean, began in October 1988 and continues to the present. Both research programs have revealed significant time-dependent and climate-driven changes in P biogeochemistry and in the potential role of DOP in ocean productivity.

A. English Channel

The nearly continuous 60-year data set (1923–1987) for Pi and nitrate concentrations at station E1 in the English Channel has recently been summarized and interpreted by Joint et al. (1997) and Jordan and Joint (1998). Winter maxima in the concentrations of Pi varied considerably with significant interannual and, especially, interdecadal frequencies. Independent analyses of these same data had previously suggested a temporal trend in the chemistry and biology of the English Channel waters beginning in the 1930s, one that was broadly related to North Atlantic climate variability (Russell et al., 1971; Southward, 1980). During the period 1924–1929, wintertime surface water Pi concentrations averaged 0.67 \(\mu\)M compared to a mean concentration of 0.48 \(\mu\)M during 1931–1938. After 1969, Pi returned to the 1920s value of 0.62 \(\mu\)M. Coincident with these decadal-scale alterations in wintertime Pi, there was a significant change in fisheries, including the disappearance of herring after 1930. These ecosystem processes were hypothesized to be linked to climate changes, and to the resultant effects on water movements and associated planktonic assemblages. This has become known as the “Russell Cycle” hypothesis.

However, after careful reanalysis, even this heroic data collection effort appears to be inadequate for a rigorous statistical test of this hypothesis (Joint et al., 1997). Changes (improvements) in Pi measurement protocols, uncertain retrospective
"corrections" and the World War II sampling gap all contribute uncertainties to this otherwise incredibly rich data set. From this careful time-series analysis (Joint et al., 1997), one might wonder about the ecological significance of any single Pi or DOP profile collected in the expeditionary mode of most oceanographic investigations!

Irrespective of the temporal trends in Pi concentrations, the N:P stoichiometry of the dissolved inorganic nutrient pool (reported as the nitrate:Pi molar ratio) varies seasonally with significant deviations from the Redfield ratio of 16N:1P (Jordan and Joint, 1998). The most intriguing result was the significant shift in the frequency distribution from a N:P ratio >6 during winter and fall to a N:P ratio <6 during summer (Jordan and Joint, 1998). These relatively low inorganic N:P ratios in summer, in the absence of known water column denitrification appeared enigmatic, and suggested a decoupling of nitrate and Pi regeneration from the organic matter formed during the the vernal bloom of phytoplankton.

From the pioneering research of Harvey and others we would have anticipated a fairly high net rate of DOP (and DON) production during the summertime period (see Fig. 4). If DOP was recycled more rapidly than DON, or if DOP was assimilated directly in preference of Pi, this could account for the shift in dissolved inorganic nutrient ratios in this habitat. A comprehensive 11-year study (1969–1977) of nutrient dynamics in the English Channel, which included measurements of both the dissolved inorganic and total dissolved nutrient pools (and, therefore, DON:DOP as well) clearly reveals the inverse correlations between nitrate/DON and between Pi/DOP during the year (Butler et al., 1979, Fig. 19). Whereas the inorganic N:P ratios varied from a minimum of 3 in summer to a maximum of 13 in winter, the organic N:P ratios varied from a maximum of 42 in summer to a minimum of 25 in winter; TDN:TDP was “buffered” at a value of 19.83 ± 2.25. This ratio is believed to reflect the stoichiometric balance of plankton production and export processes (Butler et al., 1979).

B. NORTH PACIFIC SUBTROPICAL GYRE

The subtropical gyres of the world ocean are extensive, coherent regions that occupy approximately 40% of the surface of the Earth. With a surface area of nearly 2 × 10^7 km^2, the North Pacific Subtropical Gyre (NPSG) is the largest of these regions and, therefore, Earth's largest contiguous biome. Once thought to be homogeneous and static habitats, there is increasing evidence that mid-latitude

Figure 18 SRP and DOP pools for water samples collected along an offshore (■ and ●) -to-ice edge (▲) transect in Marguerite Bay, south of Adelaide Island on the western Antarctic Peninsula (near 68°S, 68°W). (Inset) The negative correlation between SRP and DOP for this region.
gyres exhibit substantial physical and biological variability on several time scales from months to decades.

There is long standing interest and substantive debate over the nature of nutrient control of primary production in the NPSG and in the world’s ocean as a whole. Codispoti (1989) has summarized the key scientific issues, specifically the balance between rates of N2 fixation and denitrification and the bioavailability of P. Extended periods of fixed N-limitation should select for N2-fixing microorganisms and force the ecosystem toward P limitation; P would be the ultimate production rate limiting macronutrient. However, this rather simple conceptual model assumes
that N₂-fixing microorganisms can effectively compete for P and other required trace elements (especially iron) that are required for the activity of nitrogenase, the enzyme responsible for the reduction of N₂ to ammonium. Other environmental factors likely to influence N₂ fixation are temperature, turbulence, and dissolved oxygen concentration; oxygen inhibits nitrogenase activity. The input of new N into the surface ocean can decouple the otherwise linked regional C–N–P cycles, leading to an altered P-deficient stoichiometry in dissolved and particulate matter pools and a pulsed, net sequestration of atmospheric carbon dioxide. The two most crucial environmental controls on N₂ fixation are the bioavailabilities of iron and P, including DOP (Karl et al., 2002).

Pioneering research in the NPSG conducted by Perry (1972, 1976) suggested that P might control microbial growth in the near surface waters. Several physiological parameters, including high particulate C:P ratios and high biomass-normalized rates of APase, were indicative of P-deficiency. Nevertheless, analytical and intellectual assets at this time were directed elsewhere. The Plankton Rate Processes in Oligotrophic Oceans Study (PRPOOS) had a deliberate focus on gross and net rates of primary production in the NPSG, but not on the nutrient controls thereof. In fact, PRPOOS completed its field work on marine production just a few years before the prokaryotic microorganism Prochlorococcus sp. was discovered as the dominant phototrophic component of these open ocean habitats (Chisholm et al., 1988), so one might legitimately wonder, "What else didn’t we know at that time?" The Vertical Transport and Exchange (VERTEX) program conducted extensive studies of coupled primary production and particulate matter export in the NPSG, but again lacked an explicit focus on the ecophysiological controls thereof. The Asian Dust Inputs to Oligotrophic Seas (ADIOS) project focused on the eolian deposition of trace elements, including iron, but did not systematically evaluate the interrelationships between iron deposition, N₂ fixation, and P pool dynamics. In fact, neither of these three major biogeochemical programs included core measurements of DOP; the then unifying concept of new and regenerated production (sensu Dugdale and Goering, 1967), based strictly on nitrate and ammonium pool dynamics, respectively, reigned as biogeochemical dogma. There is presently compelling evidence to suggest that this N-centric view of new and export production in the NPSG is in need of revision to accommodate both N₂ fixation and P biodynamics (Karl, 1999, 2000).

Since October 1988, a comprehensive suite of ocean measurements including SRP, Pi, and TDP determinations have been obtained at the oligotrophic Sta. ALOHA in the NPSG. Core measurements were selected to provide a data set to evaluate existing C–N–P biogeochemical models and, if necessary, to improve them. The emergent data set from Sta. ALOHA is unique, robust, and rich with previously undocumented phenomena. During the initial investigation period it became evident that the NPSG P cycle was unexpectedly complex. SRP pool dynamics were characterized by both high-frequency (weeks to months) and
Figure 20  P pool dynamics at Sta. ALOHA for the period 1989–1994. (Top) SRP was measured both by the standard autoanalyzer technique (SRP-AA; data points connected by solid line) and by MAGIC (SRP-M; data points connected by dashed line). (Bottom) DOP was measured by difference (TDP-SRP) following UV treatment. Each data point represents the 0–100-m depth-integrated inventory of SRP or DOP measured on the cruises, generally from six to seven individual water depths. The model I linear regression fit (bold line) for the SRP-M data set is as follows: SRP-M (mmol m⁻²) = 9.95 – (0.003 × days since 1 January 1989). The model I linear regression fit (bold line) for the DOP data set is as follows: DOP (mmol m⁻²) = 18.98 + (0.003 × days since 1 January 1989). Redrawn from Karl and Tien (1997).

lower-frequency (years) changes that included aperiodic DOP pool inflation (up to 50%) above the longer term mean (Fig. 20). In these investigations, SRP was measured by both the “standard” autoanalyzer method (SRP-AA) and by MAGIC (SRP-M); significant differences were sometimes, but not always, observed between these two procedures (Fig. 20). Because these two methods target for analysis different subcomponents of the TDP pool (Fig. 5) these differences (e.g., the period May 1991 to August 1992; Fig. 20) imply temporal changes to the bulk chemical composition of the SNP pool. For example, an increase in acid-hydrolyzable DOP (e.g., sugar phosphates) could result in a condition where SRP-AA > SRP-M and, conversely, an increase in base-hydrolyzable DOP (e.g., phosphoprotein)
could lead to a condition where SRP-M > SRP-AA. Nevertheless, the most impressive result was the systematic and sustained decreasing upper water column (0–100 m) inventory for both SRP-AA and SRP-M from a value of \(~10\) mmol P m\(^{-2}\) in January 1989 to \(~5\) mmol P m\(^{-2}\) in December 1994, and the sustained net accumulation of SNP from \(~19\) mmol P m\(^{-2}\) in January 1989 to \(~26\) mmol P m\(^{-2}\) in December 1994. This nearly quantitative shift from dissolved "inorganic" to dissolved "organic" P suggested an accumulation of biorefractory materials, a condition that could be a manifestation of enhanced N\(_2\) fixation and a switch from N-limitation to P-limitation as first suggested by Karl et al. (1995). At the end of 1994, it was difficult to predict how long these features could persist without fundamentally disrupting new and export production processes in the NPSG. Continued measurements of SRP and DOP have documented further decreases in the SRP inventory to \(~2.2\) mmol P m\(^{-2}\) by December 1997, but without a concomitant increase in DOP (Karl et al., 2001b; Church et al., 2001). Apparently, the initial DOP pool inflation (Fig. 20) was only a temporary perturbation in the NPSG P-cycle dynamics. In fact, since 1994 DOP has decreased at a rate of approximately 0.37 mmol P m\(^{-2}\) per annum. This enhanced net utilization of semilabile DOP would be predicted under sustained conditions of P limitation. A shift in bioavailable P, from Pi to DOP, could also promote species selection, thereby affecting overall community structure and key ecological processes.

It is possible that Prochlorococcus competes favorably for the utilization of DOP, either directly or following ectoenzymatic activity. During conditions of sustained DOP dominance, like at Sta. ALOHA (DOP/Pi \(\geq 10\)), Prochlorococcus would be selected for and thus would begin to dominate the photoautotroph assemblage. Karl et al. (2001a) have recently hypothesized that the NPSG has selected for Prochlorococcus and against eukaryotic algae over the past several decades and the attendant domain shift has caused significant changes in ecosystem structure and nutrient dynamics including P-cycle processes.

The stoichiometry of the TDN:TDP pools in the upper 0–100 m of the water column (mostly dominated by DON and DOP) also displayed variability on monthly, seasonal, and interannual time scales. For example, monthly observations revealed occasional high frequency changes in the N:P ratio of the total dissolved matter pool between consecutive cruises (e.g., during spring 1989 and spring 1997; Fig. 21). These features were characterized by decreases in the N:P ratio from values that were significantly higher than the Redfield ratio to values approximating it (Fig. 21). When observed, these events always coincided with pulsed inputs of inorganic nutrients as detected by elevated nitrate plus nitrite (N\(+\)-N) inventories (Karl et al., 2001b). In certain years (e.g., 1991, 1993, 1996) these nutrient injections were either absent or, more likely, missed by the relatively coarse monthly frequency of our sampling program. We also observed several time periods of sustained, systematic change in the N:P ratio; e.g., January 1991 to July 1992, where
Figure 21 Nitrogen-to-phosphorus (N:P) ratios for the total dissolved matter pools in the upper 0–100 m of the water column at Sta. ALOHA during the 9-year observation period. (A) TDN:TDP versus sampling date. For each cruise, the mean value ± 1 SD is presented. As a point for reference, the horizontal dashed line is the Redfield ratio of 16N:1P. (B) Frequency histogram of TDN:TDP values for the 9-year data set. As a point for reference, the vertical dashed line is the Redfield ratio of 16N:1P. (C) Seasonal variability in TDN:TDP at Sta. ALOHA. Spring, Mar–May; Summer, June–August; Fall, September–November; Winter, December–February. The values presented are the mean ±1 SD for each data set. (D) Interannual variability in TDN:TDP at Sta. ALOHA. The values presented are the mean ±1 SD for each data set. Redrawn from Karl et al. (2001b).
the N:P decreased from >20 to values equivalent to the Redfield ratio, followed by an approximately 18-month period during which the N:P ratio slowly increased back to values approaching 25:1 (Fig. 21A). These features resulted in significant interannual variations in the TDN:TDP ratio (Fig. 21D), with 1993 standing out as a year with an anomalously high mean TDN:TDP ratio of 22.8 (SD = 1.8). The mean TDN:TDP ratio for the complete 9-year data set was 19.6 (SD = 2.6), well above the 16N:1P Redfield ratio.

Major differences were also observed for the molar N:P stoichiometries of the dissolved inorganic nutrient pools (i.e., N+P:SIP) versus the total dissolved nutrient pools (i.e., TDN:TDP; Fig. 22). The greatest differences were observed in the upper 0–400 m of the water column (and, especially in the upper 0–100 m) where dissolved organic nutrients are present as significant fractions of the TDN and TDP pools. Whereas the dissolved inorganic N:P ratios in the upper water column were significantly lower than the Redfield ratio of 16N:1P, the N:P stoichiometry of the total dissolved pool (inorganic plus organic) was significantly greater than the Redfield ratio by as much as 50% (Fig. 22). Furthermore, there were systematic changes in the N:P stoichiometry as a function of water depth; inorganic N:P increased toward a ratio of approximately 14, while total N:P decreased toward the same value (Fig. 22). In both data sets, the greatest rate of change in N:P with depth was in the 100- to 400-m region of the water column.

The relatively high TDN:TDP ratios in the near-surface waters are consistent with the hypothesis that P, not N, is the (or one of several) production rate limiting nutrient(s) in this ecosystem. This conclusion assumes that the TDN and TDP pools are fully bioavailable (see Smith, 1984; Jackson and Williams, 1985). However, recent research on dissolved organic matter suggests that near-surface pools are composed of at least two components: one that is locally produced and consumed during microbial metabolism (the labile pool), and one that may be more refractory. Although it is impossible to quantify these subcomponents using existing analytical techniques (and in reality there may be a continuum of bioavailabilities) for the sake of the present discussion we will assume that the mean deep-water (>600 m) DON and DOP pools are refractory. If TDN and TDP are corrected for these nonlabile components, the depth profile of N:P ratios assumes the characteristic “T-shape” (Fanning, 1992), but for a fundamentally different reason than the original author suggested. Rather than being a consequence of analytical uncertainties at low surface ocean concentrations, we hypothesize that the T-shaped profile for the corrected TDN:TDP ratios at Sta. ALOHA is a manifestation of an alternation between periods of N limitation (left-hand portion of the T) and periods of P limitation (right-hand portion of the T).

Dinitrogen (N₂) fixation is one of two major microbiological processes (the other being denitrification) that can significantly influence oceanic N:P stoichiometry on global scales. Several lines of evidence from Sta. ALOHA suggest that
Figure 22 Nitrogen-to-phosphorus (N:P) ratios versus water depth for samples collected at Sia ALOHA during the period October 1988 to December 1997. (Left) Molar N:P ratios for dissolved inorganic pools calculated as nitrate plus nitrite (N+N)-soluble reactive phosphorus (SRP). (Center) Molar N:P ratios for the "corrected" total dissolved matter pools (see text for details). (Right) Molar N:P ratios for total dissolved matter pools, including both inorganic and organic compounds, calculated as total dissolved nitrogen (TDN):total dissolved phosphorus (TDP). As a point for reference, the vertical dashed line in each graph is the Redfield molar ratio of 16N:1P. Redrawn from Karl et al. (2001b).

N₂ fixation is an important contemporary source of new nitrogen for the pelagic ecosystem of the NPSG. In addition to the observed secular changes in SRP inventories and the N:P ratios already discussed, other independent measurements include: (1) *Trichodesmium* population abundances and estimates of their potential rates of biological N₂ fixation, (2) seasonal variations in the natural ¹⁵N abundances of particulate matter exported to the deep sea and collected in bottom-moored sediment traps, and (3) increases in the DON pools during the period of increased rates of N₂ fixation (Karl et al., 1997).
At the beginning of the HOT program in 1988, biogeochemical processes in the gyre were thought to be well understood. New and export production were limited by the supply of nitrate from below the euphotic zone, and rates of primary production were thought to be largely supported by locally regenerated nitrogen. The contemporary view recognizes the gyre as a very different ecosystem (Karl, 1999; Karl et al., 2001a). Based on decade-long data sets, we hypothesize that there has been a fundamental shift from N limitation to P limitation (Karl et al., 1995; Karl and Tien, 1997). The ecological consequences of this hypothesized N\textsubscript{2} fixation-forced P limitation, especially on DOP pool dynamics, is presented elsewhere (Karl et al., 2001b; see also Fig. 23). Suffice it to say that enhanced P\textsubscript{i} cycling rates, shifts in the chemical composition of the DOP pool, and microbial biodiversity changes are all relevant features of these decade-scale ecosystem processes. The fundamental role of nutrient dynamics in biogeochemical processes

Figure 23  Schematic presentation of the NPSG alternating ecosystem state hypothesis. This cartoon depicts the contrasting N and P nutrient cycles during periods of low rates of N\textsubscript{2} fixation (e.g., 1970s) and enhanced rates of N\textsubscript{2} fixation (1980–present). It is believed that the increased frequency and duration of the El Niño-Southern Oscillation (ENSO) cycle since the early 1980s is a major cause of the N\textsubscript{2} fixation rate enhancement (see Karl, 1999; Karl et al., 2001a). The small rectangles and ovals at the top of each panel represent the average N:P ratios in particulate and dissolved matter, respectively, and the upward and downward arrows are the N:P stoichiometry of imported (mostly dissolved) and exported (mostly particulate) matter. N\textsubscript{2} fixation (on right) decouples the N:P stoichiometry of the NPSG ecosystem. The center panels depict the inventories of SRP during both phases of the cycle showing a secular decrease in SRP following the selection and growth of N\textsubscript{2}-fixing microorganisms, such as Trichodesmium. Many of these predictions have been confirmed during the 12-year study at Sta. ALOHA (see text).
and ecosystem modeling demands that we have a comprehensive, mechanistic understanding of inventories and fluxes. Although the present ongoing ocean time-series study at Sta. ALOHA has certainly not resolved all of these important matters, it does provide an unprecedented data set to begin the next phase of hypothesis testing.

VIII. DOP POOL CHARACTERIZATION

A major analytical challenge in DOP pool characterization is the detection of individual compounds typically present at pM to nM concentrations dissolved in seawater medium containing approximately 35 g L$^{-1}$ of inorganic salts. Pre-concentration and separation using ion exchange resins, ion exclusion or similar chromatographic procedures or even lyophilization that have proven useful for the characterization of DOP in soil extracts and freshwater habitats (e.g., Minear, 1972; Hino, 1989; Nanny et al., 1995; Espinosa et al., 1999) are generally not applicable for the analysis of marine DOP.

Because abiotic synthesis of organic P is not likely to occur in the marine environment, both the presence of a detectable DOP pool, as well as its molecular weight spectrum and chemical composition are dependent upon biological, mostly microbiological, processes. If marine DOP is derived from living organisms, as it ultimately must be, then the molecular spectrum of P in living cells or in marine particulate matter should be a first-order inventory of DOP sources. The macromolecular composition (by weight percent) of an "average" bacterial cell is as follows: protein, 52%; polysaccharide, 17%; RNA, 16%; lipid, 9.4%; DNA, 3.2%; other, <3% (Stouthamer, 1977). Of these compound classes, only the nucleic acids and, to a lesser extent, lipids are P-rich. Correll (1965) has measured the percentage distribution of P in natural particulate matter from 11 stations in the sub-Antarctic and Antarctic waters and reported a predominance of RNA-P (15–74%) and lipid P (3–29%) with trace amounts (2–15%) of acid-soluble organic P; these results are consistent with expected subcellular pool distributions. Miyata and Hattori (1986) have also investigated the composition of natural plankton populations (i.e., particulate P) in Tokyo Bay via differential chemical extraction. Their results indicated that nucleic acid P and lipid P were the dominant forms of organic P, together accounting for about 60–70% of the total. A large cellular pool of Pi was also detected.

As Waksman and Carey (1935) correctly noted many years ago, the DOM pool in seawater is in a "state of dynamic equilibrium" in which residues and waste products are the source terms and bacterial activity is the sink. The ambient chemical composition of DOM and individual DOP compound concentrations, therefore, reflect the net balance between production and utilization. Nearly 70 years ago, Krogh (1934) first estimated that the amount of DOM present in the sea was nearly
300 times greater than that contained in all living organisms. He believed that this large reservoir of organic matter, including DOP, must represent "waste products" that cannot be recovered and may even be slowly accumulating. Furthermore, partial degradation, including photo- and chemical alteration processes, can potentially create DOP compounds that are not present in the organisms themselves. Consequently, the chemical composition of DOP in a given habitat can vary significantly as a result of either preferential production or preferential utilization of selected organic-P compounds or compound classes.

The marine DOP pool is also expected to vary considerably, ranging from truly dissolved monomeric compounds through polymeric (IMW and HMW) compound classes to small (<0.2 µm) particles and colloids. Colloidal organic matter is extremely abundant in the marine environment (Koike et al., 1990; Wells and Goldberg, 1991), but remains poorly characterized. The cumulative high surfaceto-volume ratio of marine colloids may facilitate complexation, aggregation, or adsorption interactions with truly dissolved compounds. Furthermore, some colloids may be consumed directly by filter-feeding microorganisms. Colloids appear to have relatively short residence times in the upper ocean, based on $^{238}$U/$^{234}$Th disequilibrium measurements (Moran and Buesseler, 1992), so they may play a key role in the interactions between DOM production and removal processes.

Fox et al. (1952) may have been the first to study the distribution and chemical composition of marine colloids, which they termed "leptopel." In their field studies, they collected this material by adsorption onto finely powdered metal oxide/hydroxide matrices. The concentrated colloidal materials were then subjected to chemical extraction and analysis by a variety of techniques. Although no direct estimates of the P content of this leptopel were given, they concluded that "organic leptopel was significant to the biogeochemical cycles of C, N and P."

More recently, the technique of cross-flow filtration (CFF) has been used to isolate and concentrate colloidal marine materials (e.g., Whitehouse et al., 1990; Buesseler et al., 1996) for subsequent analysis. In the absence of selective adsorption or contamination, TP should behave conservatively in CFF systems. However, in practice, large deviations are observed including an enigmatic production of colloidal P during sample processing (Bauet et al., 1996). A similar production of colloidal P during ultrafiltration processing of freshwater samples has also been reported (Nanny and Minar, 1997), suggesting that some caution is advised in the interpretation of colloidal-P data sets. For the purposes of this review and except where otherwise noted, colloids are included in the "dissolved" matter fraction.

Marine DOP can be characterized by a number of independent techniques including, for example, direct chemical analysis of selected molecules or compound classes, the use of specific hydrolytic enzymes or partial photochemical degradation, chromatographic, and molecular weight fractionation, and $^{31}$P NMR. The combined use of molecular weight separation and specific chemical class
characterization by, for example, $^{31}$P NMR or enzymatic hydrolysis is beginning to provide a glimpse of complexity of the DOP pool. From an ecological and biogeochemical perspective, the direct measurements of specific P-containing organic compounds are the most useful data sets because their fluxes can be traced to specific sources and sinks. However, the few studies which have been conducted have identified only a minority fraction of the ambient DOP pool, leaving the majority uncharacterized.

Below, we present several independent approaches to DOP pool characterization, beginning with the least specific method of physical separation by molecular weight/size and ending with specific methods of single compound or compound class characterization. Example marine DOP data sets will also be presented for each major application. We end this section with some speculation on the possible chemical composition of the uncharacterized majority of the DOP pool.

A. Molecular Weight Characterization of the DOP Pool

Various techniques have been used to fractionate DOP by molecular weight, size, ionic charge, or ability to adsorb onto specific resins (e.g., nonionic XAD). When combined with chemical detection systems these procedures can be used to characterize the total DOP pool. However, it is uncertain how much ecological information can be obtained by these separation techniques alone because DOP bioavailability is probably not directly correlated with these physical properties. Although the HMW organic fraction has often been considered to be more biorefractory than LMW matter, recent metabolic evidence has shown just the opposite for selected marine habitats (Amon and Benner, 1996). Currently it is impossible to determine how representative this HMW DOP is of either the total DOP or the more ecologically relevant BAP pool.

A basic problem in studies of DOP pool characterization is the need for preconcentration of selected compounds prior to analysis. A widely used method in freshwater habitats, but less so in marine ecosystems, is gel chromatography, usually employing Sephadex gels. This provides for the separation of HMW-P, including colloids, in the "void volume" from IMW and LMW fractions (Broberg and Persson, 1988).

Matsuda and Maruyama (1985) used Sephadex G-25 gel chromatography to characterize the DOP pool in coastal waters of Tokyo Bay. Several model DOP compounds were coanalyzed to establish the overall efficacy of this method; HMW DOP compounds (MW >5 kDa) were contained in the void volume, LMW monophosphate esters (e.g., Gly-3-P) eluted prior to Pi, and nucleotides eluted after Pi. Prior to separation, the TDP was first concentrated 30- to 35-fold by rotary evaporation and salt exclusion, a method which recovered only approximately 50% of the total P. Separate HMW and LMW DOP pools were observed in all
seawater samples that were analyzed. Both fractions decreased with increasing water depth, suggesting that biochemical lability was independent of molecular weight. In contrast, DOP compounds of IMW (>400 Da but <5 kDa) were relatively constant and, perhaps, biochemically stable (Matsuda and Maruyama, 1985). The IMW DOP fraction also absorbed UV light, consistent with nucleotide bases; no further chemical characterization was given and no ecological implications were discussed.

More recently, the method of tangential flow ultrafiltration has been used to isolate and concentrate DOM for subsequent chemical analysis (e.g., see Benner et al., 1992). However, this method is very selective for the IMW and HMW (generally >1 kDa) compounds, which for many aquatic ecosystems may be ≤35% of the total organic matter pool. In a series of carefully controlled experiments, Nanny et al. (1994) evaluated the behavior of ultrafiltration and reverse osmosis for concentration and molecular size fractionation of SNP in freshwater lakes. They evaluated both exogenously supplied standard compounds and the effects of increasing ionic strength by NaCl addition. Their results demonstrated that membrane type, including pore size, ionic strength, specific DOP test compound and volume concentration factor were all key variables that affected model compound—and presumably natural DOP compound—recovery. Another potential problem with the interpretation of molecular weight separations is the real possibility of LMW, monomeric DOP compound, or Pi association with HMW materials. While the exact mechanism is not well understood, adsorption, hydrogen bonding, metal bridging, and even Maillard reaction are all distinct possibilities (Carlson et al., 1985). A novel and significant seawater application of tangential flow ultrafiltration for HMW DOP pool concentration, coupled with 31P NMR molecular characterization (Clark et al., 1998) is presented in a subsequent section of this review (see Section VIII.C).

**B. DOP Pool Characterization by Enzymatic Characterization**

There are at least three separate applications for the use of specific enzymes in ecological studies of the marine P-cycle: (1) the presence and relative activities of specific enzymes in natural microbial assemblages can be used as physiological indicators of Pi stress, deficiency, or other ecological processes; (2) *in vitro* or *in vivo* measurements of the rates of specific enzymes in natural assemblages of microorganisms can provide relevant information on DOP turnover rates; and (3) exogenous additions of specific enzymes to whole, filtered, or partially purified seawater samples can be used to help characterize the DOP pool composition and to determine its potential bioavailability. The first two applications are discussed in a subsequent section; the third application is discussed below.
In theory, selected DOP compounds or compound classes could be estimated by measurements of Pi accumulation in cell-free seawater samples following a timed incubation with an exogenous purified enzyme or multiple enzyme cocktail. This is a straightforward and versatile approach. For example, Herbes et al. (1975) used three separate enzyme treatments to characterize both the LMW and HMW DOP fractions from a variety of aquatic habitats. This method could also be used to characterize nascent DOP that is produced during 32Pi or 33Pi tracer addition experiments, but to our knowledge this has not yet been attempted. The specificity of the enzyme(s) used will establish the specificity of DOP compound or compound class analysis.

In 1966, Strickland and Solórzano described a quantitative assay for total dissolved phosphonomonoester P (PME-P) using exogenous APase from E. coli. An adaptation of this method using immobilized E. coli APase has also been described (Shan et al., 1994). This assay monitors the appearance of SRP during timed incubations following APase addition, relative to controls without exogenous enzyme. Conversely, the in situ activity of APase can be estimated by adding excess substrates followed by timed measurements of P for other by-products (see Section IX.C.1). Significant PME-P concentrations ranging from 0.05 to 0.45 μM were detected in coastal California seawater samples (Strickland and Solórzano, 1966). APase can also release Pi from pyro-Pi and poly-Pi (Rivkin and Swift, 1980), so technically Pi increase from APase treatment only provides an upper constraint on PME-P concentration in seawater. However, independent estimations of pyro-Pi and poly-Pi concentrations could be made to establish limits on this potential source of interference. Technically, though, this should be termed the APase-hydrolyzable P (APHP) pool, rather than PME-P.

Taft et al. (1977) applied this assay to samples collected in Chesapeake Bay over 1 year. PME-P/APH and APHP was >10% of the total DOP pool in 25 of 61 samples (but typically <20%); PME-P/APH and APHP concentrations covaried with DOP, and both peaked in late summer (Taft et al., 1977). Kobori and Taga (1979) measured PME-P/APH concentrations, APase activity, bacterial cell number and percentage of isolates that are APase positive, and DOP in a variety of Japan coastal habitats. Ambient concentrations of PME-P/APH ranged from 19 to 50% of the total DOP but, in general, appeared to be scavenged to low concentrations, presumably because they are readily used by the APase-containing bacteria that dominated these habitats. At depths greater than 100 m in Sagami Bay, PME-P/APH was undetectable in the environments that were studied.

A similar approach to detecting other components of the marine DOP pool has employed DNase and/or RNase treatments of isolated dissolved nucleic acids to estimate the specific enzyme-hydrolyzable components of these macromolecular fractions by measurements of DNA or RNA before and after specific enzyme treatments (DeFlaun et al., 1987; Siuda and Chrost, 2000). Most, but not all, of the extracellular nucleic acid pool appears to be nuclease-hydrolyzable, which implies
that it is readily assimilated by microorganisms. Direct estimates of \(^{3}H\)-DNA turnover (Paul et al., 1987) have also supported this conclusion. The "residual" nucleic acid fraction that is not degraded by specific nuclease treatment, could be adsorbed to clay particles or otherwise chemically or physically altered. These semilabile or truly refractory nucleic acid fractions could accumulate over time.

McKelvie et al. (1995) devised a method using immobilized phytase that was designed to selectively release Pi from phytic acid and related inositol phosphates. However, in practice the phytase system was less specific than anticipated so the defined pool was termed "phytase hydrolysable P" or PHP (McKelvie et al., 1995). PHP was compared to SRP, and for a variety of aquatic environments including several estuarine but no open ocean habitats. PHP was a significant percentage of the "apparent DOP." However, potential problems with the inability to separate Pi from labile DOP in the standard SRP determination could lead to an underestimation of DOP, and overestimation of the percentage of PHP by the methods employed. This analytical limitation is not unique to the phytase assay. In any case, most of the DOP pool in the environments studied appeared to be enzyme hydrolyzable.

Suzumura et al. (1998) used tangential flow ultrafiltration techniques to isolate LMW and HMW SNP fractions in Tokyo Bay, Japan. These pools were then characterized using two phosphohydrolytic enzymes, APase (as above) and phosphodiesterase (PDEase; EC 3.1.4.1). The former enzyme alone will release Pi from a variety of PMEs, and the combination of the two enzymes will release Pi from nucleic acids. The molecular weight spectrum revealed a dominance of LMW-SNP (54–76% of the bulk SNP); no further characterization of this fraction was reported. Enzymatic characterization of the HMW-SNP fractions revealed the presence of both phosphomonoesters and diesters, with diester compounds in greatest abundance. However, on average, the majority of the isolated HMW-SNP was resistant to the enzyme treatments (Suzumura et al., 1998). Pretreatment with chloroform, converted a portion of HMW-SNP to an enzymatically labile form and, on this basis, the authors suggested the presence of hydrophobic P-containing compounds, perhaps phospholipids. Presumably, this latter pool of enzymatically resistant compounds is also unavailable for microbial decomposition without prior hydrolysis or other diagenetic alteration.

C. DOP POOL CHARACTERIZATION BY \(^{31}P\) NMR

Nuclear magnetic resonance (NMR) spectroscopy has proven to be a powerful analytical tool for the molecular characterization of marine DOM. The abundant, naturally occurring isotope of P, \(^{31}P\), has a magnetic moment that is detectable by dipole resonance in an applied magnetic field. Although resonance is observed that is due solely to the P atom, chemical shifts due to the electron shells of
the other atoms with which P is associated give the diagnostic NMR spectra. Specifically, mono- and di-P esters are readily distinguishable from phosphonates and can be identified in bulk DOP concentrates. Both solution and solid-state magic angle spinning (MAS) $^{31}$P NMR techniques have been employed in marine ecosystems (Ingall et al., 1990). A major limitation with this method is the detection limit; marine DOP must be concentrated many thousand-fold prior to analysis. During the concentration process, DOP can be selectively eliminated (e.g., by molecular weight) or chemically altered. Detection of Pi in the HMW fraction indicated either postconcentration DOP hydrolysis or desorption of Pi from HMW organic/inorganic matter, or both (Nanny and Minear, 1997). Ironically, the NMR measurement technique itself is “nondestructive.”

Nanny and Minear (1997) have combined $^{31}$P NMR with selective chemical hydrolysis and other reaction techniques to further characterize the major compound classes of HMW DOP isolated from aquatic ecosystems. For analysis of Crystal Lake, they detected phosphonates in the HMW DOP, but not in the IMW or LMW fractions.

Using tangential flow ultrafiltration and solid-state cross-polarized magic angle spinning (CPMAS) $^{31}$P NMR, Clark et al. (1998, 1999) demonstrated that approximately 75% of the HMW DOP collected at a station in the South Pacific Ocean (12°S, 134°W) was composed of ester-linked P compounds and the remainder (~25%) were phosphonates (Fig. 24). Both classes could potentially include a broad spectrum of individual compounds with independent sources and sinks and

![Figure 24](image_url)  
**Figure 24** $^{31}$P NMR spectra of HMW DOP from several reference depths in the Pacific Ocean (12°S, 135°W). The peak at 0 ppm is indicative of phosphate esters, and the peak at 25 ppm denotes phosphonates. Asterisks indicate spinning sidebands, an artifact of magic angle spinning. Reprinted with permission from Clark et al., (1998).
variable residence times. Clark et al. (1998) also noted a significant shift in the bulk C:N:P elemental composition of the isolated HMW-DOM with depth (C:N:P molar ratios of 247:15:1, 321:19:1, and 539:31:1 for surface, 375 m, and 4000 m, respectively), indicating a selective remineralization of P from seawater DOM.

The relatively high proportion of phosphonates was unexpected and was interpreted to be a result of the selective retention of phosphonates over time, based on the presumption that these compounds are not readily degraded in the sea. Equally intriguing and enigmatic is the fact that despite a sixfold decrease in HMW DOP concentration from 90 nM in the surface to 15 nM at a depth of 4000 m, the $^{31}$P NMR spectra show P-esters and phosphonates at approximately identical proportions, indicating a coupled utilization (Fig. 24). In the absence of a deep water source of P-esters, the hypothesized higher rates of P-ester biodegradation should have resulted in an increasingly lower proportion of P-esters with depth in the water column; this is contrary to what was observed. From the information presented, one is led to conclude: (1) both compound classes are ubiquitous in the marine environment including the South Pacific abyss, (2) both P esters and phosphonates have similar, probably slow, turnover times in the open ocean, and (3) both compound classes are used in proportion to their ambient concentrations. What is still unresolved from the extant data set is the primary source of the high phosphonate to P-ester DOP at all water depths.

More recently, Kolowith (née Clark) et al. (2001) extended their South Pacific Ocean HMW DOP$^{31}$P-NMR measurement program to a station in the North Atlantic subtropical gyre (32°N, 64°W), three stations in the North Pacific Ocean (10°N, 140°W; 18°N, 134°W; 22°N, 130°W), and two stations in the North Sea. The cumulative result from all 16 individual samples shows a remarkable uniformity in composition regardless of water depth or distance from shore; P-esters and phosphonates are present in similar proportions in the HMW DOP throughout the world ocean (Kolowith et al., 2001). For selected sampling sites simultaneous collections of PP (0.1- to 60-μm fraction) were also obtained, and these were subjected to the same $^{31}$P NMR analysis. Only P-ester compounds were detected in the particulate matter concentrates, reemphasizing an apparent relative enrichment of phosphonates in the DOM pools (Kolowith et al., 2001).

**D. DOP Pool Characterization by Partial Photochemical Oxidation**

Karl and Yanagi (1997) used continuous-flow UV photodecomposition to provide a partial characterization of SNP in the subtropical North Pacific Ocean. TDP was reproducibly subdivided into three chemically distinct pools: SRP (presumably dominated by Pi), UV-labile SNP (P_{UV,L}, containing primarily monophosphate esters), and UV-stable SNP (P_{UV,S}, containing primarily nucleotide di- and
triphosphates, nucleic acids, and other compounds that are resistant to the low-intensity UV treatment that was developed for the purpose of organic-P pool characterization). Field application of these procedures to samples collected at Sta. ALOHA (22°45'N, 158°W) during the period September 1991 to March 1992 revealed the presence of all three operationally defined pools with an upper water column (0–100 m) average of 23% SRP, 26% $P_{UV-S}$, and 51% $P_{UV-L}$ (Karl and Yanagi, 1997). However, the $P_{UV-S}$ pool did vary nearly threefold (4.38 mmol P m$^{-2}$ in December to 11.07 mmol P m$^{-2}$ in March; Fig. 25), suggesting variable production or consumption rates. Depth profiles also revealed near surface enrichments in the $P_{UV-L}$ pool, suggesting a direct coupling with photosynthetic processes (Karl and Yanagi, 1997).

E. DIRECT MEASUREMENT OF DOP COMPOUNDS

Despite recent progress in TDP pool characterization, individual compound analyses are difficult and therefore rare. Only a few organic-P compounds or compound classes have been measured in seawater. These individual compounds probably have multiple sources and sinks, and variable residence times in the marine environment. For example, some compounds are probably excreted from living cells (e.g., vitamins and c-AMP), while others are most likely produced only following cell death or lysis (e.g., nucleic acids). Unfortunately, no comprehensive
study has been conducted to attempt a P mass balance for a given water sample, but it appears that no more than half, and probably less, of the marine DOP pool has been chemically characterized. This uncertainty in SNP pool composition is a major impediment in our attempts to quantify P fluxes in the marine environment.

1. Nucleic Acids

None of the five major nucleic acid bases contain P; only the nucleotide derivatives and nucleic acid polymers thereof are part of the DOP pool (Table I). Pioneering research efforts to measure DNA in the sea began in the late 1960s with the quantitative laboratory and field studies of Holm-Hansen and colleagues (Holm-Hansen et al., 1968; Holm-Hansen, 1969). During these initial investigations it was established that a large proportion of particulate DNA was associated with nonliving organic matter (Holm-Hansen, 1969). Subsequent studies confirmed the presence of a large pool of detrital DNA, in both particulate (Karl and Winn, 1984) and dissolved fractions (DeFlaun et al., 1986). Paul et al. (1990) have also detected the intact gene for the enzyme ribulosebisphosphate carboxylase/oxygenase (rbcL) as part of the dissolved DNA (D-DNA) pool indicating that phytoplankton must be considered a probable source for extracellular DNA. Dissolved RNA (D-RNA) has also been detected in seawater (Karl and Bailiff, 1989; Sakano and Kamatani, 1992). Compared to the volume of research focused on D-DNA very little is presently known about D-RNA, even though it is sometimes the larger of the two pools in seawater and in cells. If viral lysis is a major control on bacterial and phytoplankton populations in the marine environment, and there is still active debate on this matter, then the production of dissolved nucleic acids in seawater may ultimately be controlled by the viral lytic cycle.

The measurement of dissolved nucleic acids requires isolation either by adsorption onto barium sulfate (Pillai and Ganguly, 1972) or hydroxyapatite (Hicks and Riley, 1980), or by precipitation using ethanol (DeFlaun et al., 1986), cetyltrimethylammonium bromide (Karl and Bailiff, 1989), or polyethylene glycol (Maruyama et al., 1993), followed by colorimetric or fluorometric dye detection. Depending on the fluorescent dye selected, either single-stranded DNA or single-plus double-stranded DNA is measured (Sakano and Kamatani, 1992). Some DNA may be bound to histone or similar protein and in this state may be inaccessible to nucleases or to the specific dyes commonly used to quantify nucleic acids. Dissolved nucleic acid can also be measured, indirectly, by high-performance liquid chromatography (HPLC) analysis of the free nucleic acid bases released following polymer hydrolysis (Breiter et al., 1977). There are unique advantages and disadvantages of each method (Siuda and Güde, 1996), but a detailed discussion is beyond the scope of this chapter.

Research conducted over the past decade has indicated that extracellular DNA has at least three forms: (1) naked, free DNA, (2) DNase-resistant naked
DNA possibly adsorbed onto small particles or contained within colloids, and (3) protein–DNA complexes, perhaps virus particles. Though less carefully described, D-RNA should have a similar distribution spectrum. Initially, certain researchers thought that much of the dissolved nucleic acid was essentially virus particles (see Wommack and Colwell, 2000 for a historical account); this important controversy will not be resolved here. Nevertheless, at least three lines of independent evidence argue against this: (1) the cooccurrence of large concentrations of D-RNA (RNA-containing marine viruses are rare; Steward et al., 1992), (2) the broad D-DNA molecular weight spectrum (<0.1 to >36 k base pairs; DeFlaun et al., 1987), and (3) direct measurements of virus particles and quantitative estimations of their potential contributions to the D-DNA pool. Virus particles, if present, would contribute to the "non-DNase digestible D-DNA" that has been measured for many marine habitats (e.g., Maruyama et al., 1993).

During an extensive, time-series investigation in the North Adriatic Sea, Weinbauer et al. (1993) reported that virus particles averaged 17.1% (range, 0.7–88.3%) of the measured D-DNA. Using the method of vortex flow filtration, Paul et al. (1991) demonstrated that viral DNA averaged only 3.7% (range 0.9–12.3%) of the D-DNA for a variety of aquatic habitats of different trophic states. Jiang and Paul (1995) used differential centrifugation to separate seawater D-DNA into truly soluble and bound (viral particles, colloids, adsorbed D-DNA) forms; D-DNA pool averaged 50% soluble, 8–15% viral, and 35–42% other bound D-DNA. Kingdom probing of the isolated D-DNA using 16S rRNA-targeted oligonucleotide probes (universal, eubacterial, and eukaryotic) indicated that D-DNA was a complex domain mixture. However, these results also confirmed a relatively low viral particle contribution to D-DNA in the variety of marine habitats investigated (Jiang and Paul, 1995). Nevertheless, even if the nonliving virus particles did represent a large portion of the dissolved nucleic acid pool in seawater, they would still be "DOM" by our operational definitions and would still need to be considered as part of the dynamic marine P-cycle. The probable fate of free virus particles is to be consumed by protozoan grazers, degraded by cell-associated or free enzymes or adsorption onto sinking particles. Consequently, virus particles are like all other nonliving organic-P pools in the sea from an ecological perspective.

The distribution of dissolved DNA follows the general pattern of DOM in the marine environment; namely, highest concentrations in coastal waters, decreasing with distance from shore and with depth in the water column (DeFlaun et al., 1987; Fig. 26). For samples collected at an oligotrophic North Pacific station, Karl and Bailiff (1989) reported higher concentrations of D-DNA than particulate DNA (P-DNA), and D-RNA to D-DNA ratios ranging from 3 to 10, similar to particulate RNA:DNA ratios found in growing microorganisms. Although no DOP data were presented, the P content of the D-RNA plus D-DNA fractions could have accounted for approximately 30–40 nM DOP in the euphotic zone of their oligotrophic North
Pacific Station; DOP in this habitat is typically 250–400 nM (Orrett and Karl, 1987). For a series of stations in the English Channel, Hicks and Riley (1980) reported that P contained in the dissolved nucleic acid fraction (RNA plus DNA) accounted for 27–49% of the total DOP. A similar estimation for samples collected in Tokyo and Sagami Bays yielded a mean dissolved nucleic acid P of 12.9% of the total DOP (Sakano and Kamatani, 1992). The latter authors also documented significant temporal (seasonal?) changes in the concentrations of both D-RNA and D-DNA and especially in the D-RNA/D-DNA ratio (Fig. 27). In another study of D-DNA dynamics in Tampa Bay, Florida, both seasonal and diel concentration variations were observed (Paul et al., 1988; Jiang and Paul, 1994). The mechanism for the significant diel periodicity was not identified, and it is likely to be a result of the balance between production and utilization processes. D-DNA P averaged
6.6% of the total DOP, but because D-RNA was not measured, this value should be considered a lower constraint on the contribution of dissolved nucleic acids to marine DOP.

A significant research effort has been invested to ascertain extracellular nucleic acid production and utilization processes. Although selected bacterial species produce extracellular DNA during growth, no comparable experimental data are available for D-RNA production. Paul et al. (1987) investigated the dynamics of D-DNA (i.e., production and turnover rates) in subtropical coastal and oceanic environments. Daily D-DNA production by heterotrophic bacteria (using $^3$H thymidine) was $\sim$5% of the ambient pool. Active consumption of radiolabeled E. coli DNA by cell-associated and extracellular nucleases was also demonstrated. The utilization of D-DNA appears to involve hydrolysis by nonspecific cell-associated nucleases, uptake of individual nucleic acid bases, and salvage pathway biosynthesis back into cellular nucleic acids. Likewise, viral RNA seeded into filtered–sterilized coastal seawater was stable for approximately 1 month compared to only 2 days in paired unfiltered samples (Tsai et al., 1995), indicating a probable rapid turnover of D-RNA.
Novitsky (1986) also examined the degradation of $^3$H-labeled detrital DNA and RNA in sedimentary marine ecosystems. He found that: (1) RNA was degraded faster than DNA, (2) both nucleic acids were ultimately degraded to the same extent, and (3) both dissimilation to $^3$H$_2$O and assimilation via salvage pathways into new RNA and DNA were evident.

The production of extracellular nucleic acids may also be a manifestation of microzooplankton grazing processes or viral-induced cell lysis. The concentrations of D-DNA and nanoflagellates were found to co-vary at a station in the Adriatic Sea (Turk et al., 1992); quantitative estimates indicated that most of the ingested DNA was subsequently released into the environment. The turnover of D-DNA was greater in P-limited than in N-limited habitats (Lorenz and Wackernagel, 1994), suggesting that DNA, a P-enriched macromolecule, may be an important source of P for microbial assemblages.

The presence of extracellular nucleic acids and their rapid turnover have important ecological implications. First, RNA and DNA are N- and P-enriched components of the seawater DOM pool (Table I), which could provide nutrients for autotrophic and heterotrophic microorganisms. Second, dissolved nucleic acids could provide a supply of purine and pyrimidine bases for nucleic acid biosynthesis. This would spare the cell the energy that would otherwise be required for *de novo* synthesis of these invaluable precursors. Third, and perhaps most importantly, free DNA could effect genetic transformation under ecologically permissive conditions. Natural transformation by extracellular DNA is a potential mechanism for lateral gene exchange in natural aquatic habitats. Bacteria are the only organisms known to actively take up DNA and recombine it into their genomes, a process called natural transformation (Redfield et al., 1977), and may have evolved it as a means for bacteria to adapt to changing environments. The presence of gene-sized DNA fragments in seawater prompted DeFlaun and Paul (1989) to look for natural transformation among marine microbial assemblages and, later, Paul et al. (1991) were the first to document transformation in seawater samples under ambient environmental conditions. The D-DNA pool may, therefore, represent the "community genome" (DeFlaun and Paul, 1989). The ecological implications of horizontal gene transfer are profound; we believe that this is an important contemporary area of research, poised for rapid progress in the next decade.

2. ATP and Related Nucleotides

ATP is a biologically labile, but chemically stable, compound, with key functions in cellular energetics, metabolism, and biosynthesis. Its inherent chemical stability predicts that there might exist a detectable dissolved ATP (D-ATP) pool in seawater that would exist as a transient between ATP production and utilization. Azam and Hodson (1977) were the first to report the presence of D-ATP in seawater.
Figure 28  Dissolved ATP and c-AMP concentration versus depth profiles for samples collected in the North Pacific Ocean. (Left) D-ATP concentrations: (●, solid line) at 32°42’N, 117°23’W in the San Diego Trough (redrawn with permission from Azam and Hodson, 1977) and (■, dashed line) at Sta. ALOHA 22°45’N, 158°W (Björkman and Karl, 2001) versus depth. (Right) c-AMP versus depth at a station 41 km offshore in the Southern California Bight (redrawn from Ammerman and Azam, 1981).

and since that time it has been detected in all marine and freshwater environments where measurements have been attempted (e.g., Fig. 28, left).

The quantitative determination of D-ATP requires isolation and partial purification (e.g., H$_2$SO$_4$ extraction, adsorption onto activated charcoal, desalting, elution into an ammoniacal ethanol solution, and vacuum evaporation; Hodson et al., 1976) prior to detection by the firefly luciferin–luciferase bioluminescence assay. The recovery of D-ATP by this rather labor-intensive procedure is typically around 30% (Karl and Holm-Hansen, 1978; McGrath and Sullivan, 1981), although higher recovery has also been reported (Hodson et al., 1976). Other adenine and nonadenine nucleotides are also coadsorbed and coconcentrated by the charcoal method. The firefly assay confers a high sensitivity and substrate specificity. Only ATP and, to a lesser extent, guanosine triphosphate (GTP) and uridine triphosphate (UTP) react with crude luciferase enzyme preparations to yield light; however, the presence and relative concentrations of these various substrates can be determined by the kinetics of luciferase light emission (Karl, 1978). More recently, Björkman and Karl (2001) have devised an alternative D-ATP concentration technique that is based upon coprecipitation with magnesium hydroxide (i.e., the MAGIC technique; Karl and
Tien, 1992), followed by firefly bioluminescence. This simple and straightforward technique also routinely yields a high recovery of ATP (≥90%) and, if necessary or desired, can yield quantitative recovery. Nonadenine nucleotide triphosphates (e.g., GTP) are also quantitatively coprecipitated. A HPLC technique has also been described (Admiraal and Veldhuis, 1987), but the relatively poor detection limit precludes its use without some preconcentration method. The advantage of HPLC is that all nucleotides and related derivatives can be separated and quantified from a single run.

D-ATP is typically highest in surface coastal waters, decreasing substantially with distance from shore and with depth (Azam and Hodson, 1977; Hodson et al., 1981; McGrath and Sullivan, 1981; Figure 28, left); however, ambient near-surface ocean concentrations are not static. For example, the ambient D-ATP pool in the Southern Ocean varied considerably during and following the spring bloom of phytoplankton, with a peak abundance of >2.5 nmol L⁻¹ in December-January and minimum concentrations (<0.004 nmol L⁻¹) in March (Nawrocki and Karl, 1989). At the height of the bloom, D-ATP was approximately 60% of the corresponding particulate ATP (P-ATP), but by March P-ATP was much more dominant.

For subeuphotic zone waters off southern California (500 m depth), D-ATP typically exceeds P-ATP, sometimes by an order of magnitude (Azam and Hodson, 1977). However, more recent and, perhaps, more reliable MAGIC-based estimates of subeuphotic zone D-ATP concentrations from the subtropical North Pacific Ocean revealed a much steeper concentration versus depth gradient and much lower deep water concentrations than previously suggested; nevertheless, D-ATP was still detected at a depth of 500 m (Fig. 28, left). This presence of D-ATP in deep seawater argues either for a local source or for a long residence time, or both. While ATP is chemically stable in seawater, with a half-life of approximately 8 years at 21°C (Hulett, 1970), it is microbiologically labile with ambient pool turnover times of hours to days in near-surface temperate waters (Azam and Hodson, 1977; Hodson et al., 1981; McGrath and Sullivan, 1981). D-ATP is used almost exclusively for biosynthesis and less than 2% of the [¹⁴C]ATP tracer was respired (Azam and Hodson, 1977). Marine microorganisms can and do assimilate D-ATP, in part for P and in part for purine salvage and nucleic acid synthesis. Consequently, D-ATP concentration measurements coupled with exogenous radiolabeled [³²P/³³P, ³H, ¹⁴C] D-ATP uptake measurements can be used to estimate D-ATP and mass flux through marine microbial assemblages (Azam and Hodson, 1977). D-ATP is one of the few organic-P compounds that can be measured at ambient pool concentrations and can be tracked through the microbial food web with specific radioisotopic tracers. In this regard, and especially considering its fundamental role in cellular bioenergetics and biosynthesis, ATP might be considered a "model" compound in studies of the marine P cycle.

Because ATP is the primary energy currency in all living cells, the presence of D-ATP, often at concentrations exceeding P-ATP, may seem enigmatic. Why would
cells leak or otherwise lose such a vital intracellular constituent? The potential mechanisms of D-ATP production, including exudation, inefficient grazing, cell death or viral lysis, are not well constrained. It is possible that the presence of non-ATP nucleotides, measured along with D-ATP, might help to identify the major sources. For example, if the grazing of actively growing cells is the main source for D-ATP in seawater, then one might also expect to observe other nucleotide triphosphate compounds that are vital for biosynthesis (e.g., GTP), and a high ratio of ATP relative to total adenylates (sum of ATP + ADP + AMP). If, on the other hand, D-ATP was a result of viral lysis or programmed death and autolysis, then non-ATP adenine nucleotides (e.g., ADP and AMP) would be expected to dominate the total extracellular nucleotide pool.

Nawrocki and Karl (1989) compared the pattern of intracellular and extracellular adenine nucleotides (reported as the adenylate energy charge; ECₐ = [ATP + ½ ADP]/[ATP + ADP + AMP]) for samples collected in Bransfield Strait, Antarctica, as a direct test of the D-ATP source hypothesis. During the early phase of the spring bloom (December), the particulate and dissolved ECₐ ratios were indistinguishable (D-ECₐ/P-ECₐ = 1.01 ± 0.06) at all stations. These results suggested that grazing or excretion could be the primary source of D-ATP in this habitat. A calculation revealed that a loss of approximately 2–3% of the total ATP production by the cells present in the water column would be needed to account for the measured ATP flux. More recently, Björkman and Karl (2001) have reported elevated D-GTP concentrations and high D-GTP/D-ATP ratios (approximately 1:1) in near-surface waters (0–100 m) of the subtropical North Pacific gyre. These field results are consistent with a grazing/excretion source.

3. Cyclic AMP

Adenosine 3’, 5’-cyclic monophosphate (c-AMP) is a ubiquitous cellular compound that is required for the synthesis of a number of inducible catabolic proteins, for bacterial flagellum synthesis, catabolite gene activation, and other key functions (Botsford and Harman, 1992). It is found in both prokaryotes and eukaryotes, including marine bacteria, phytoplankton, and probably Archaea. Intracellular concentrations of c-AMP are carefully regulated by enzymatic synthesis and degradation, as well as by active excretion from the cell.

In 1981, Ammerman and Azam documented the existence of c-AMP in coastal seawater. The dissolved c-AMP was extracted from seawater and concentrated using the H₂SO₄-charcoal adsorption technique used previously by Hodson et al. (1976) for D-ATP measurements. The isolated c-AMP was quantified by a commercially available radioimmunoassay. At one offshore station in the Southern California Bight, c-AMP was maximal (2–3 pM-P) in the near surface water, decreasing to <1 pM-P at 500 m (see Fig. 28, right). In near-shore seawaters, the
concentration of dissolved c-AMP was generally higher and varied approximately fourfold with time of day, suggesting a dynamic production and consumption cycle; concentrations peaked at sunset (Ammerman and Azam, 1981).

Although c-AMP is stable in filter-sterilized (0.2 \( \mu \)m) surface seawater, it is rapidly assimilated by marine microorganisms, especially bacteria (Ammerman and Azam, 1981). Uptake of c-AMP is effected by a high-specificity, high-affinity (\( K_m <10 \) pM) transport system (Ammerman and Azam, 1982). Evidence from well-designed dual labeled ([\( ^{32}P \)]c-AMP/[2,8-\(^3\)H]c-AMP) experiments indicated that c-AMP was assimilated intact and that it remained in the cytoplasm rather than being incorporated into biomolecules. This is fully consistent with its role as a regulatory molecule. More recently, Dunlap et al. (1992) has reported growth of the marine luminous bacterium, *Vibrio fischeri*, on c-AMP as a sole source of C, energy, N, and P (also see Callahan et al., 1995). Growth was effected by a high-specific activity, narrow-substrate-specificity c-AMP phosphodiesterase located in the periplasm of the cell. Ammerman and Azam (1982) suggested that uptake and release of c-AMP may control intracellular levels and, hence, metabolism for microbes in the marine environment. In their ecosystem model, starved or otherwise metabolically debilitated bacterial cells would take up dissolved c-AMP from seawater to supplement or replace *de novo* synthesis of c-AMP from cellular ATP; new catabolic enzymatic pathways would be produced and alternate ambient substrates metabolized (Ammerman and Azam, 1987). To our knowledge, this intriguing model has not yet been evaluated under field conditions.

### 4. Lipids

Lipids are major constituents of all living cells. As a class of biomolecules, lipids have enormous structural diversity that, in part, reflects phylogenetic diversity. For example, whereas bacteria contain phospholipids containing ester-linked fatty acids, the membrane phospholipids in *Archaea* have ether-linked isoprenoid side chains (Tornabene and Langworthy, 1979). At least two different groups of lipids, both cell-membrane-associated, have been detected as constituents of the marine DOP pool: total phospholipid (PL) and lipopolysaccharide (LPS). Despite their billing, certain PL molecules contain as much N as they do P (Table 1), and neither N nor P are in very high relative abundance compared to C (molar C:P ratio is \(~40:1\)).

Phospholipids are ubiquitous in *Bacteria* and *Eukarya* and present to a lesser extent in *Archaea*; they function primarily as structural components of the membrane. The PL content of cells can be quite large. According to Dobbs and Findlay (1993), PL-P in marine bacteria can account for approximately 15–20% of total cellular P—despite the relatively low percent P content—but the exact amount will vary with nutrient status and growth rate. The turnover time of phospholipids following cell death is rapid (2–10 days), at least in aerobic sedimentary
habitats, but lipid turnover has not been investigated in the more dilute, pelagic ecosystem.

Phospholipids are easily separated from other DOP compounds because of their polar nature; although soluble in organic solvents, they have an associated hydrophilic group at the P-bonded end. Phospholipids can be separated and detected by HPLC or by thin-layer chromatography coupled with flame ionization detection (TLC-FID, Chromarod-Iatroscan system; Parrish and Ackman, 1985). Several different types of phospholipids are present but, most often, “total PL” is reported. However, total PL can be further fractionated by altering hydrolysis conditions into diacyl phospholipids, plasmalogens (vinyl ether containing lipids) and phosphonolipids. The inherent stability of the C-P bond in organic phosphonate compounds has been employed, analytically, to separate phosphate ester-linked lipid compounds from phosphonolipids. Using a stepwise treatment with hydrochloric acid (6 M, 120°C, 2 h) to cleave phosphodiesters, followed by APase to cleave monophosphate esters, only phosphonolipids are expected to remain (Snyder and Law, 1970).

Phosphonolipids are produced by eukaryotes, but degraded by prokaryotes. Certain membrane phospholipid derivatives of phosphorylethanolamine (e.g., 2-AEP; Table 1), detected in relatively high concentrations in certain protozoans (Kennedy and Thompson, 1970), may increase the stability of the surface membranes by resistance to degradation. This would also predict a longer residence time in DOM and could lead to an accumulation relative to more labile DOP compounds. They may have an important physiological and ecological role, especially under low nutrient conditions.

Liu et al. (1998) used CFF to concentrate the colloidal fraction of DOM (>10 kDa) from phytoplankton cultures and surface seawater collected in Conception Bay, Newfoundland. As expected, colloidal lipids were always present but, on average, lipids were higher in the <10-kDa fraction. Relative to total lipids, the percentage of PL was nearly twice as high in the colloids than in the truly dissolved fraction (Liu et al., 1998). This indicates that colloidal P may be chemically distinct from truly dissolved compounds.

Probably the most comprehensive study of PL in the marine environment was that conducted in Bedford Basin, Canada, by C. Parrish and colleagues (Parrish, 1986, 1987; Parrish and Wangersky, 1987). The total lipid fraction was extracted with cold dichloromethane, followed by Chromarod-Iatroscan system detection. Repeat analyses of the major classes of both particulate and dissolved lipids leading up to and following the vernal primary production maximum revealed complex dynamics especially for the dissolved lipid classes (Parrish, 1987). Their results for near surface waters (~5 m) collected during the 1982 spring bloom revealed a phytoplankton community with peak chl a values of >30 μg L⁻¹ on Julian day 86; nitrate decreased throughout the bloom period (Fig. 29). Dissolved PL were high
(equivalent to ~50–60 nM-P) prior to the spring bloom, but decreased substantially during the bloom. In contrast, the particulate PL concentration was relatively low (<5 nM-P) before and during the initial stages of the bloom, increasing to ~20 nM-P at the height of the bloom (Fig. 29). Dissolved PL consistently accounted for 15–20% of the total dissolved lipid content of the water column. Similar results, notably the decreased concentrations of dissolved PL at the height of the bloom and a large increase in dissolved PL during the demise phase, were also observed at this location in 1984 (Parrish, 1987). It was suggested that phytoplankton
in this coastal marine habitat can use PL-Pi as a P source for growth. A postbloom resupply of dissolved PL, hypothesized to be a result of intense grazing pressure, was also observed (Parrish, 1987).

5. Vitamins

Vitamins are a class of otherwise unrelated organic compounds that are needed in trace amounts for the normal functioning of all organisms. Both thiamine pyro-Pi, the biologically active form of vitamin B₁, and cobalamin (vitamin B₁₂) have been detected in seawater where they contribute to the DOP pool. It is unlikely that vitamins comprise a significant percentage of the total DOP pool, but there is no question that they are important from an ecological perspective. Vitamin B₁₂, in particular, is an obligate growth factor for many marine phytoplankton, so net growth of these organisms requires vitamin B₁₂ uptake from seawater. Vitamin B₁₂ is synthesized exclusively by microorganisms, predominantly bacteria, so there may be an obligate vitamin syntrophy within the marine microbial world (Provasoli and Pintner, 1953). Metabolic relationships between diatoms and heterotrophic bacteria may effectively exchange utilizable organic matter for vitamin B₁₂, thereby maintaining a viable population of B₁₂-requiring phytoplankton during periods of vitamin deficient growth (Haines and Guillard, 1974), as might occur in low nutrient, subtropical gyres.

Vitamins B₁ and B₁₂ can be measured using rapid and reproducible bioassay procedures based on the incorporation of [¹⁴C] bicarbonate during growth of specific, vitamin-requiring phytoplankton (Carlucci and Silbernagel, 1966a,b). Application of these extremely sensitive (e.g., detection limits for vitamin B₁₂ <0.05 ng of total vitamin per liter which equates to 4×10⁻¹⁴ mol of P) bioassays have revealed low concentrations (sometimes undetectable) in the surface ocean, especially in open ocean oligotrophic ecosystems, and higher concentrations at depth (Carlucci and Silbernagel, 1966c; Fig. 30). The enigmatic increase at depth may be a result of local production and more dependent on vitamin B₁₂ import via sinking particles; however, no comprehensive studies have been conducted.

An extensive survey of dissolved and particulate vitamins (including B₁ and B₁₂) in the Southern Ocean was conducted by Carlucci and Cuhel (1977). At most stations, both vitamins were undetectable in the surface waters (e.g., Fig. 30), and for vitamin B₁ it was usually undetectable throughout the entire water column to depths of at least 3000 m. Particulate vitamin B₁ and B₁₂ peaked in near-surface waters and decreased significantly with water depth (Carlucci and Cuhel, 1977). It was concluded that vitamins may limit eukaryotic phytoplankton growth in these high latitude polar habitats. Likewise, for seawater samples collected in the subarctic Pacific Ocean, Natarajan (1970) documented a stimulation of primary production, as measured by light-dependent [¹⁴C] bicarbonate uptake, by the addition of vitamin B₁, but not B₁₂, especially for samples where the ambient
B\textsubscript{1} concentrations were low. From this data set it was concluded that vitamin B\textsubscript{1} may be limiting these natural phytoplankton assemblages.

Menzel and Spaeth (1962) documented a seasonal cycle in vitamin B\textsubscript{12} concentrations in the Sargasso Sea, with lowest concentrations during the spring–summer period. They also showed that vitamin B\textsubscript{12}-requiring phytoplankton species (mostly diatoms) were absent during periods of undetectable vitamin B\textsubscript{12} concentration, suggesting a key ecological selection pressure. Finally, it is interesting to note that the subdiscipline of “vitamin ecology” seems to have peaked in the early 1970s without any clear resolution of the role of vitamins in oceanic biogeochemistry. We submit that this is an area ripe for progress in the future.

6. Inorganic Poly-P\textsubscript{i} and Pyro-P\textsubscript{i}

Inorganic poly-P\textsubscript{i} are linear polymers of P\textsubscript{i}, joined by phosphoanhydride bonds identical to high-energy P-bonds of ATP (Kulaev and Vagabov, 1983). The poly-P\textsubscript{i} chain length can vary considerably from \( n = 2 \) (pyro-P\textsubscript{i}) to \( n \geq 1000 \). This diversity of form has important implications both for their analysis in seawater and
for their potential ecological role. While these important cell-derived compounds are obviously not part of the DOP pool per se, they do contribute to the TDP pool and, therefore, to the usual operational definition of “DOP” (i.e., [TDP]–[SRP]). Furthermore, both pyro-Pi and poly-Pi can be formed by enzymatic or photolytic hydrolysis of selected organic-P compounds, so as a courtesy we will include them here as probable constituents of the marine DOP pool.

Isolation and partial purification of poly-Pi is difficult because of the variable molecular weight (chain length) and variable chemical properties. For example, laboratory studies have identified at least three poly-Pi fractions: (1) acid-soluble, (2) base-soluble, and (3) other (Clark et al., 1988). Quantitative estimation is usually by difference measurement based on the relative photochemical stability of poly-Pi. This difference approach is inherently unreliable, especially when total poly-Pi is ≤20% of TDP. Furthermore, it is impossible to separate inorganic poly-Pi from organic poly-Pi by these methods.

Solorzano and Strickland (1968) detected poly-Pi in both coastal and open ocean ecosystems. They employed a sequential UV photooxidation followed by hydrochloric acid hydrolysis. The UV-stable, acid-labile Pi was taken as a measure of the sum of organic plus inorganic poly-Pi. Concentrations in surface waters ranged from 200 nM P (25% of SNP) in a red tide off Newport Beach to undetectable levels (<50 nM P) in the East-Central Pacific Ocean.

Isotope dilution has also been proposed as a method for pyro-Pi and poly-Pi estimation in samples containing other potentially interfering P compounds (Quimby et al., 1954) but, to our knowledge, this method has not been applied to seawater samples. More recently, poly-Pi has been detected and quantified using a firefly luciferase-based assay for ATP following incubation with exogenous additions of the enzyme polyphosphate kinase and the substrate ADP (Ault-Riché et al., 1998). To our knowledge this sensitive and specific assay for inorganic poly-Pi has not yet been applied to seawater.

Poly-Pi is ubiquitous in every cell in nature where it participates in multiple, key functions (Kornberg et al., 1999). Pyro-Pi, like poly-Pi and the γ- and β-P groups of ATP, is a “high-energy” P compound that is important in cellular bioenergetics; some researchers believe that pyro-Pi was the evolutionary precursor to ATP (e.g., Lipmann, 1965). Extracellular pyro-Pi can also serve as the sole source of energy for the growth of certain aquatic, anaerobic bacteria (Liu et al., 1982; Varma et al., 1983). Furthermore, the poly-Pi polymer has a clear osmotic advantage over free Pi (i.e., it is osmotically inert), and may also act as a metal-ion chelator or high-capacity buffering system (Kornberg et al., 1999). Perhaps one of the most intriguing potential roles for poly-Pi in marine microorganisms is its role in enabling competence for bacterial transformation by facilitating the cross-membrane transport of extracellular DNA (Reusch and Sadoff, 1988), and perhaps other small and large DOP compounds. Poly-Pi, the “molecular fossil,” appears to have recently come back to life (Kornberg and Fraley, 2000).
The most important function of poly-Pi, however, appears to be regulation of gene expression, especially as it relates to nutritional stresses including P and N limitation. When microorganisms are placed into a Pi-deficient medium they are induced to take up large amounts of Pi when it becomes available again. The Pi is converted into poly-Pi and stored until needed for macromolecular biosynthesis (Jensen and Sicko, 1974). In E. coli, poly-Pi transients, accounting for changes of $\geq$100-fold in periods of minutes to hours, have been observed during shifts from nutrient-sufficient to minimal growth media (Ault-Riché et al., 1998). Cells deficient in poly-Pi are noncompetitive during periods of nutritional stress, whether acute or chronic (Kornberg et al., 1999). For growth in a fluctuating nutrient environment, rapid uptake and storage of Pi would be a key survival strategy. There is also an intracellular transient accumulation of poly-Pi at the onset of Pi depletion which appears to be under control of the Pho regulon discussed below. This process, termed “poly-Pi overplus” (Voelz et al., 1966), is fundamentally distinct from “luxury uptake” of Pi which also results in poly-Pi formation and storage but does not require Pi depletion. Of the two processes, the poly-Pi overplus phenomenon is probably most important in the marine environment and especially so in the open ocean. For example, if near-surface ocean microbes are exposed to alternating periods of high and low Pi, as they probably are (Karl, 1999), then this could lead to a poly-Pi overplus response and intracellular sequestration of P as poly-Pi. Consequently, the current view that poly-Pi would not be expected to exist in low nutrient open ocean seawaters may be terribly incorrect; a focused research effort on this topic should be undertaken.

**F. Biologically Available P**

Regardless of the rigor and precision with which P-containing compound pools are measured, the ecological significance of these analytical determinations will be incomplete until reliable estimates of the BAP pool are routinely available. In addition to Pi, which is generally the preferred substrate for microorganisms, the P contained in a variety of polymeric inorganic compounds, in monomeric and polymeric organic compounds and in selected P containing minerals is available to some or all microorganisms; indeed some microorganisms may prefer ester-linked P sources to free orthophosphate (Tarapchak and Moll, 1990; Cotner and Wetzel, 1992). However, the bioavailability of most organic-P pools depends on ambient Pi pool concentrations and on the expression of specific enzymes that control transport, salvage, and substrate hydrolysis. Because many of these enzymes are induced by low Pi, bioavailability may be a variable, time- and habitat condition-dependent parameter, rather than an easily predicted or measured metric. Assessment of the BAP may also depend on the time scale of consideration; for example, substrates that appear to be recalcitrant on short time scales
(e.g., <1 day) may fuel longer-term (annual to decadal) microbial metabolism, especially as the ambient Pi pool becomes limiting.

Theoretically, only a well-designed bioassay procedure can ever be expected to provide reliable data on bioavailable P; chemical assay systems, regardless of design, cannot replicate the metabolism of a living cell. One of the first P bioassay systems, described by Stewart et al. (1970), used P-starved cells of the N2-fixing cyanobacterium Anabaena, and a response based on acetylene reduction (N2 fixation) to detect bioavailable P in freshwater ecosystems. Björkman and Karl (1994) have assessed the short-term bioavailability of seven organic and two inorganic P compounds to natural marine microbial communities by measuring the sparing effect on the uptake of exogenous 32P relative to unamended (negative control) and Pi-supplemented (positive control) subsamples, following a procedure described by Berman (1988) for freshwater habitats. These short-term bioavailability surveys indicated that there were marked differences among the compounds tested, but confirmed a strong metabolic preference for Pi. Of the compounds tested, nucleotides (ATP, UDP, GDP) were the most available organic-P compounds, followed by poly-Pi and Gly-3-P. Hexose-P compounds had "bioavailability factors" that were consistently <5% those of equimolar additions of Pi (Björkman and Karl, 1994).

Karl and Bossard (1985) estimated BAP using a γ-P labeling technique that they developed for ATP pool turnover. Because the intracellular ATP pool turns over rapidly, the specific radioactivity in the γ-P position of the P-ATP pool will reach equilibrium not only with the extracellular Pi pool but with the potentially larger pool of extracellular P that is available to the microbial assemblage at that time, i.e., the BAP pool. Bossard and Karl (1986) successfully applied this 32P labeling method to a variety of marine and freshwater habitats and reported that BAP was greater than or nearly equal to measured SRP. More recently, Björkman et al. (2000) used the γ-P ATP labeling method to estimate the BAP pool at two stations in the oligotrophic North Pacific Ocean. In their study BAP was generally greater than Pi or SRP but, as in Bossard and Karl (1986), less than the TDP pool. The BAP:TDP molar ratio averaged 0.24, and generally exceeded the Pi:TDP and SRP:TDP ratios, sometimes by more than a factor of two. These results suggest that there exists some fraction of the ambient DOP that has a bioavailability factor equal to, or greater than, Pi in these open ocean habitats.

G. DOP: THE "MAJORITY" VIEW

Although no comprehensive characterization has been attempted using all possible DOP compound or compound class specific detection capabilities simultaneously, it is probable that less than 50% of the total DOP, and perhaps much less,
can be identified. That leaves greater than half of the DOP uncharacterized by existing protocols. What is this “majority DOP?”

Seawater DOM is marine in origin (Lee and Wakeham, 1988; Hedges et al., 2000); however, it appears to be diagenetically altered and chemically distinct from the source materials. This may account, in large part, for our inability to characterize it. Hedges et al. (2000) have recently reviewed the status of what they term the molecularly uncharacterized component of nonliving organic matter, or simply “MUC”. It is generally believed that partial degradation leads to the production of refractory materials that selectively accumulate in seawater. The relatively old mean $^{14}$C age of the DOM pool (of which DOP is a subcomponent) even in surface ocean implies that much of it may be turned over on time scales of centuries to millennia. However, if we assume that the biorefractory deep water DOM (~40 $\mu\text{M C}$, mean age of 6000 years) is also present in surface waters where it represents about 35–50% of the total pool, then the remainder of the surface pool must be zero age (i.e., modern) to reconcile the measured mean surface DOM age estimate of 1500 years. These age estimates may or may not also apply to the P-containing DOM pool because these fractions have never been isolated for direct age determination. The fact is that we simply do not know how the age of the DOM pool scales on mass, class, or other characteristics. Information regarding the age of specific components of the DOP pool would, in our view, be invaluable from an ecological perspective.

There are two general processes potentially leading to the formation of MUC (Hedges et al., 2000): (1) heteropolycondensation reactions whereby small reactive organic intermediates combine to from larger and more complex organic compounds (also known as humification) and (2) differential/partial biodegradation of high-molecular-weight organic matter. The starting materials for the latter process would likely be complex compounds like bacterial peptidoglycan-rich cell walls, DNA-histones, or viral particles. While there appears to be an ongoing paradigm shift from process (1) to process (2), few data currently exist for DOP compounds.

Humification, the process whereby organic materials that are relatively resistant to microbial and free-enzyme mineralization are polymerized, condensed, and otherwise reworked into a diverse suite of compounds called “humic substances,” is widespread in nature (Rashid, 1985). Based on their relative solubilities in acidic or basic solutions, humic substances are further subdivided into humic acids (HA, insoluble in acid, soluble in base), fulvic acids (FA, acid and base soluble), and humins (insoluble in both acid and base). Marine humic substances also have variable molecular weights, ranging from LMW to colloid sized particles, and are isotopically and chemically unique from soil humus. They can adsorb, complex, and chelate most trace metals, and probably adsorb Pi as well. This would be in addition to the actual P content of the compounds themselves.
Dispersed HA and FA compounds may account for some measurable fraction of the marine DOP pool, but the amount remains uncertain. According to Nissenbaum (1979), sedimentary marine HA and FA fractions contain 0.1–0.2 and 0.4–0.8% P by weight, respectively, with corresponding bulk C:P ratios ranging from 300 to 400 in HA to <100 in FA (Table 1). The P-enriched FA fraction is thought to be more recent. Nissenbaum (1979) suggests the following diagenetic scheme: plankton → DOM → FA → HA → kerogen. The fact that FA compounds are enriched in P (relative to C) compared to the average C and P contents of plankton (106C:1P) seems enigmatic. This P-enrichment of FA is especially interesting considering the fact that the mean C:P molar ratio of DOM in surface and deep sea water indicates a P depletion (C:P_surface ~200–400, C:P_deep >800) relative to living biomass. Even many labile DOP compounds have C:P ratios larger than the quoted value of <100 for marine FA (Nissenbaum, 1979). From these analyses we must conclude that the FA fraction of seawater is a probable, but ill-constrained, P trap. Mopper and Schultz (1993) have identified a “humic-type” signature as one of two main fluorescence components of the DOM pool at Sta. ALOHA. While quantitative information on HA/FA abundance is lacking, it is clear that humic substances are present throughout the water column in the global ocean. More careful field work certainly needs to be done to substantiate this claim.

Brophy and Carlson (1989) have documented the microbiological formation of biorefractory HMW DOM from bioactive LMW precursors and suggested that this might be a pathway for local accumulations of organic matter. In similar experiments, labile protein added to seawater and allowed to “age” for 40 days was degraded fourfold more slowly than the non-aged protein (Keil and Kirchman, 1994). The chemical modification was determined to result from abiotic, organic–organic interaction which the authors conclude may be a necessary first step in the formation of biorefractory compounds.

It is probable that a fraction of the marine DOP pool is also derived from cell wall-associated compound classes. McCarthy et al. (1997) suggested that degradation-resistant biomolecules, rather than abiotically produced heterocyclic geopolymers, dominate the marine DOM pool. This refractory DOM pool appears to contain a large percentage of bacterial peptidoglycan (McCarthy et al., 1998). Lipopolysaccharide (LPS) is a component of the cell wall of Gram-negative bacteria including some cyanobacteria (Mayer et al., 1985; Wilkinson, 1996). It is localized in the center portion of the outer membrane, where it contributes to cell integrity. Although LPS is rapidly degraded following cell death and lysis, dissolved LPS (D-LPS) has been detected in aquatic environments and may represent >90% of the total LPS in certain habitats (Karl and Dobbs, 1998). Spontaneous release of LPS has been documented for many bacteria and appears to occur during normal growth (Cadieux et al., 1983). D-LPS may also accumulate as a consequence of protozoan grazing activities or viral lysis.
Phosphorylation of LPS is a probable mechanism of metabolic regulation in the sea (Ray et al., 1994). A striking feature is the presence of a high level of P (2–6%, by weight) which exists as either ethanolamine-P, ethanolamine-pyro-Pi, protein-P, or Pi (Mühlradt et al., 1977). A majority of marine bacteria are Gram-negative and thus may contain LPS-P. Currently it is not known how much P is contained in the D-LPS fraction in seawater (the assay for D-LPS does not measure P content directly), and the P content of individual forms of LPS varies considerably. As discussed above, LPS phosphorylation may serve as a regulatory mechanism in bacteria, so the LPS-P content of seawater could be variable in both space and time. We hypothesize that both LPS and the Gram-positive bacterial cell wall analog compounds teichoic and teichuronic acids (which contain Gly-3-P and ribitol-P polymers) may be present as components of the DOP pool because bacterial cell walls are such a significant percentage of total bacterial cell biomass. This prediction, however, awaits direct experimental evaluation.

The coordination and regulation of cellular metabolism are key ecological processes. Bacteria, for example, impose regulatory mechanisms on most catabolic and biosynthetic processes to ensure that needs are met, but not exceeded (Saier et al., 1995). Large portions of the bacterial and archaeal genomes appear to be used for regulation, so it is conceivable that regulatory molecules are constantly produced and used by marine microorganisms. These compounds could, in theory, be selectively retained in the DOM pool following exudation or cell death.

The phosphorylation and dephosphorylation of proteins is an important metabolic process in living organisms that may be a form of signal transduction and gene regulation (Stock et al., 1989). Both exogenous and endogenous cues can elicit specific adaptive responses that optimize metabolism and maximize survival. The low-temperature dependence of the protein phosphorylation process in certain bacteria (Ray et al., 1994) provides a potential mechanism for a geographical variability in DOP production. The phosphoenolpyruvate:sugar phosphotransferase system (PTS) in bacteria is one of the best characterized examples of phosphoprotein regulation (Saier, 1989); numerous other examples also exist (Bourret et al., 1991). The PTS is used for sugar transport, phosphorylation, and chemoreception; it has been detected in marine bacteria (Hodson and Azam, 1979).

Synthesis of polysaccharides (including LPS) from monosaccharides generally relies on a biosynthetic pathway that involves a nucleoside–sugar intermediate (e.g., UDP-glucose, CDP-fructose). To our knowledge these phosphorylated intermediates have not been reported in seawater, though they undoubtedly exist largely for the same reasons as dissolved nucleotides. Also, a family of nucleotide derivatives, including guanosine-3'-diphosphate-5'-diphosphate (ppGpp) has been implicated in the growth rate-dependent regulation of ribosomal RNA and protein synthesis since its discovery more than three decades ago (Cashel and Gallant, 1969). These regulatory processes have unknown but probably key ecological roles and may, therefore, be present in variable intracellular and extracellular
concentrations in seawater. To our knowledge, they have not yet been detected in seawater.

A novel group of phosphoinositol derivatives has recently been reported in very high concentrations (50–350 mM) in the cytoplasm of certain hyperthermophilic bacteria and Archaea (Scholz et al., 1992; Martins et al., 1997; Ramakrishnan et al., 1997). Initially it was thought that they served as thermoprotectors, but it now appears that they have other, yet undiscovered, functions—perhaps osmolytic (Chen and Roberts, 1998). Inositol is stable to hot hydrochloric acid hydrolysis (6 M, 110°C, 48 h), so it is possible that they have not been quantitatively measured by use of wet chemical oxidation techniques. Inositol isomers have also been detected in marine sediments (White and Miller, 1976; Suzumura and Kamatani, 1995), but not yet in the water column. Another phosphorylated compound, 2,3-diphosphoglycerate, has been shown to be a novel phosphagen in selected Archaea (Kanodia and Roberts, 1983; Matussek et al., 1998). The recent discovery of high concentrations of planktonic Archaea in seawater (DeLong, 1992; Fuhrman et al., 1992; DeLong et al., 1994; Karner et al., 2001), demands that we also consider them as a potential source for marine DOP.

Finally, several organic-P compounds have commercial applications; for example, triphenyl phosphate is a plasticizer and tricresyl phosphate is an additive in gasoline. In addition, organophosphorus pesticides (parathion, malthion, ethion, thimet) are in use worldwide. While most of these are eventually degraded, some compounds persist longer than others, especially when present in low concentrations. Atmospheric, riverine, or outfall introduction into the sea may arrest the degradation processes via substrate dilution. This could lead to the selective accumulation of biochemically refractory or otherwise “unwanted” DOP compounds, in spite of a low relative production or delivery rates.

In summary, a complete molecular characterization of marine DOP has not yet been possible. This situation may be as much a consequence of lack of effort as it is due to lack of methods. When one considers the combined probable contribution from dissolved nucleic acids, dissolved phospholipids, dissolved proteins, low-molecular-weight intermediates (including nucleotides, regulatory molecules, and osmolytes), and HA and FA fractions, budget reconciliation may be possible in the near future.

IX. DOP PRODUCTION, UTILIZATION, AND REMINERALIZATION

The phosphate regulon (Pho) of E. coli includes at least 31 genes arranged in eight separate operons (Wanner, 1993). These genes are coregulated by environmental P concentrations and, working together, facilitate Pi assimilation. During Pi limitation, selected Pho enzymes are turned on, including: (1) a high-affinity,
high-specificity periplasmic permease (Pst) to enhance Pi assimilation capacity, (2) a periplasmic alkaline phosphatase to facilitate monophosphate ester-linked DOP hydrolysis, and (3) specific enzymes to facilitate the uptake and hydrolysis of phosphonates. Expression of the Pi regulon has also been reported for marine bacteria (McCarter and Silverman, 1987), so the much more extensive laboratory studies of E. coli may be an adequate model at least for hypothesis testing in Pi-stressed marine habitats.

DOP production in the sea typically begins with biological Pi uptake and incorporation into one of the many intracellular P-containing compounds in the cell. For this reason, the environmental controls on rates of Pi uptake and pathways of Pi assimilation into organic-P compounds are critical to our understanding of DOP dynamics. Because Pi concentrations in the surface ocean rarely exceed 3 μM, one might anticipate the universal presence of the high-specificity, high-sensitivity Pi transport system for many microorganisms in the sea.

Most of the Pi that is assimilated by microorganisms is locally regenerated back to Pi to sustain in situ production processes. In the open ocean, typically less than 10% of the total P that is assimilated is exported from the system. As for Pi uptake, accurate Pi regeneration or DOP production estimation requires information on the ambient pool of BAP in the habitat under investigation. When combined with independent methods of DOP pool characterization, the radiotracer experiments described below can also be used to quantify the fluxes of specific DOP compounds or compound classes (e.g., nucleotides). A “pulse-chase” experimental design (i.e., preincubation with $^{32}$Pi or $^{33}$Pi radiotracer followed by the addition a 10-fold excess of $^{31}$Pi) could be employed to follow both net DOP production, during the labeling phase, and DOP turnover in the postchase treatments. Dual labeled $^{33}$Pi/$^{32}$Pi pulsed experiments can also be conducted to trace DOP pool dynamics and to estimate DOP pool residence times by models that take advantage of the differential halflives of these independent radiotracers. Finally, the addition of exogenous, $^{32}$P (or $^{33}$P)-radiolabeled DOP compounds (e.g., Glu-6-P, ATP, Gly-3-P) can provide information on the turnover time of individual pools and potentially (if combined with direct measurements of ambient pool concentrations) on mass fluxes (production and utilization rates) through selected organic-P pools.

Our present inability to measure any but a very few DOP compounds (a notable exception is ATP, as discussed above), however, limits the field application of this experimental design.

A. DOP Production and Remineralization

The use of $^{32}$P (or $^{33}$P) as a tracer for Pi uptake, DOP production, and Pi regeneration has been extensive in oceanography to measure the growth and metabolic activities of algal and bacterial assemblages (Rigler, 1956; Watt and Hayes, 1963;
Sorokin and Vyshkovtsev, 1974; Taft et al., 1975; Harrison et al., 1977; Perry and Eppley, 1981; Sorokin, 1985; Atkinson, 1987). Certain P radiotracer field experiments have employed either size fractionation treatments (Harrison et al., 1977), metabolic inhibitors (Krempin et al., 1981) or multiple labeled substrates (Cuhel et al., 1983) to separate algal and heterotrophic bacterial activities. Typically, the radiotracer is added as carrier-free $^{32}$Pi (or $^{33}$Pi) in order to minimize perturbations that may be caused by Pi pool enrichment.

The use of exogenous radiotracers to measure DOP production during timed incubation experiments generally requires a protocol to separate unused or regenerated radiolabeled Pi from the recently produced radiolabeled DOP compounds. This is an analytical challenge in many field studies because most of the radiisotope is generally present as Pi and because of the potential diversity of the individual DOP compounds that are produced. Smith et al. (1985) employed the previously described isobutanol extraction technique to separate the $^{33}$Pi (SRP)–molybdenum blue complex from DO$^{33}$P in solution. To the extent that SRP exceeds Pi, or if any of the DO$^{33}$P compounds are soluble in isobutanol, the solvent extraction method will underestimate the net DO$^{33}$P production rate. Orrett and Karl (1987) compared the isobutanol technique to the much simpler “Bochner and Ames” (1982) technique; the latter involved a procaine–tungstate–tetraethylammonium-dependent selective precipitation of $^{32}$Pi leaving DO$^{32}$P in solution. The mean DO$^{32}$P production rate estimates from an open ocean field application of these two methods showed no significant differences (Orrett and Karl, 1987). More recently, Björkman et al. (2000) have employed the MAGIC technique, described earlier, as an effective means to separate $^{32}$Pi from DO$^{32}$P in sample filtrates.

Once experimental data are available on $^{32}$P/$^{33}$P-labeled DOP accumulation rates (expressed as Bq per volume per time) conversion to the more ecologically meaningful absolute P fluxes requires the assumption/application of a specific P assimilation model to convert P radioactivity to P mass. The key to providing a reliable mass flux is, again, the accurate estimation of the BAP pool. Orrett and Karl (1987) reviewed four independent models that have been used for seawater: (1) SRP (or Pi) model, (2) TDP model, (3) RNA model, and (4) ATP model. Each model differs in the assumptions used for P uptake and assimilation. For example, the SRP model assumes that the exogenous precursor $^{32}$Pi/$^{33}$Pi mixes completely with the SRP pool prior to uptake and incorporation. Consequently the radiolabeled DOP that is produced during the incubation would have a specific radioactivity (radioactive per unit mass) equivalent of that calculated for the SRP pool. However, in most open ocean environments where DOP > Pi, it may be more reasonable to acknowledge the possible role of DOP in microbial metabolism. The TDP model, therefore, assumes that both Pi and DOP are bioavailable during these relatively short (<1 day) incubation experiments. In our opinion, this assumption may also be incorrect and would lead to an underestimation of the true precursor specific radioactivity. The remaining two metabolic models rely on direct measurements of
the specific radioactivity in two independent subcellular constituent pools that are expected to reflect the isotope dilution of the added precursor by all bioavailable extracellular and intracellular P pools (Orrett and Karl, 1987). In the RNA model, the specific radioactivity of nascent RNA is measured directly (using double-labeled $^{3}H$-adenine and $^{32}P$ incubations; Karl, 1981) and this value is taken to represent an average for the entire DOP pool. The final method, the ATP model, is analogous to the RNA model but assumes that the terminal phosphate group of ATP (the $\gamma$-P pool rather than the $\alpha$-P as in the RNA model) accurately reflects isotope dilution. Two key advantages of the ATP model are the rapid turnover of cellular ATP which leads to rapid isotopic equilibration, and the compelling evidence that the $\gamma$-P position of ATP is very likely the precursor for the biosynthesis of most P-containing organic compounds (see Section VIII.F).

Hudson and Taylor (1996) devised and applied a novel method for the direct measurement of DOP production in planktonic communities. Their procedure requires a preincubation with high specific activity $^{33}P$ for 17–76 h to label the metabolically active organisms. A subsequent pulse of nonradioactive $^{31}P$ competitively inhibited further $^{32}P$ uptake and provided a time zero starting point for the timed appearance of $[^{33}]P$DOP from the combined processes of excretion, exudation, grazing, and cell lysis. The addition of the $^{31}P$ pulse is assumed to have no effect on DOP loss by the community of microorganisms. From information on the mean specific radioactivity of the particulate P pool at the beginning of the second incubation, the DOP production (mol P L$^{-1}$ day$^{-1}$) can be estimated. To our knowledge, this method has not yet been applied to marine ecosystems.

Watt and Hayes (1963) and Johannes (1964) were among the first to demonstrate contemporaneous $P$ uptake and DOP production in near surface planktonic assemblages using $^{32}P$ radiotracer incubations. Johannes (1964) also documented a coupled production of DOP by diatoms and DOP uptake by bacteria. It was also shown that mesozooplankton produced substantial amounts of DOP. According to Sato and Pomeroy (1965) zooplankton, in general, have low oxygen consumed to $P$-released ratios, which suggests that they do not completely oxidize their food; this is consistent with the reported high rates of DOP release.

Smith et al. (1985) conducted $^{33}P$ uptake experiments in coastal and offshore Hawaiian waters. They detected net $[^{33}]P$DOP production in all of their incubations, and used both three-component ($P$ → $PP$ → DOP) and four-component ($P$ → $PP_1$/$PP_2$ → DOP) steady-state models to evaluate the observed P pools and fluxes. Their results were consistent with a rapid and coupled production and utilization of DOP in these marine habitats.

Dolan et al. (1995) examined coupled $P$ uptake and passage through a simplified planktonic food web as defined by specific particulate matter size fractions in Villefranche Bay, France. $P$ uptake was dominated (>50%) by the smallest size fraction (0.2–1 $\mu$m), presumably auto- and heterotrophic bacteria. $P$ turnover times were rapid, less than a few hours for most of the 3-month observation period.
Release of incorporated $^{32}$P from various particulate size-fractions was investigated by incubating with $^{32}$Pi for a 3-h period, followed by the addition of an excess of unlabeled AMP (100 $\mu$M). The addition of AMP, they reasoned, would partially inhibit the assimilation of recently produced [$^{32}$P]DOP compound and provide a more accurate estimate of gross DOP fluxes. The measured rates of [$^{32}$P]DOP release in these experiments were low, generally $\leq$1% of the corresponding particulate $^{32}$P activity per hour (Dolan et al., 1995). However, when the concentration of oligotrich ciliates (predators of microorganisms in the 1- to 6-$\mu$m size class) was artificially increased, there was a significant transfer of $^{32}$P from particulate to dissolved pools. These field results confirmed the role of protozoan grazing in nutrient cycling processes (Andersen et al., 1986), including the $\text{Pi} \rightarrow \text{PP} \rightarrow \text{DOP} \rightarrow \text{Pi}$ pathway.

Thingstad et al. (1993) conducted a comprehensive study of microbial transformations of P in P-limited Sandefjord, western Norway, including the coupling of Pi uptake, DOP production, specific DOP compound hydrolysis, and enzymatic hydrolysis. They focused on the production and turnover of nucleotides, and used ATP as a “model” compound. DOP/Pi concentration ratios in this habitat varied considerably but were generally between 10 and 100:1; Pi (reported as SRP) was $\leq$5 nM at selected stations. An averaged flow model was devised to best accommodate the measurements and their underlying assumptions. The most striking result was the presence of a large DOP pool (~200–250 nM) that was dominated (>99%) by polymeric compounds (presumably RNA and DNA) that turned over very slowly compared to the relatively small but rapidly assimilated nucleotide pool. In this regard, their results are consistent with the nucleosome/phosphodiesterase “bottleneck” hypothesis discussed in a previous section of this review (Fig. 5).

Orrett and Karl (1987) reported 0–100 m depth-integrated DOP production rates ranging from 0.3–0.8 mmol P m$^{-2}$ day$^{-1}$ (TDP specific activity model) and 0.5–1.2 mmol P m$^{-2}$ day$^{-1}$ (RNA-specific activity model) for water samples collected in the NPSG. They reasoned that these DOP production rates could be further extrapolated to organic carbon, if the mean C:P molar ratio was either known or correctly assumed. An upper bound on C:P was taken as the whole cell C:P (106C:1P; Redfield et al., 1963), although it is unlikely that DOP compounds are, on average, this carbon-rich (see Table I). They assigned a value of 3C:1P as the theoretical lower bound on this value, a value identical to the nucleotide triphosphate pool. It is equally unlikely that the DOP pool would be that C poor, relative to P. A ratio of 9.5C:1P, the approximate value for RNA, was taken as the most reasonable estimate; the true C:P ratio for DOP is likely to be closer to the lower bound than to the upper bound. The extrapolated rate of DOC production, 24 mmol C m$^{-2}$ day$^{-1}$, was about 50% of net primary production for this region (Karl et al., 1996, 1998). Because this estimate is based on accumulation (net production) of DOP during the incubation period and, therefore, does not include
contemporaneous $^{32}$P-DOP production and $^{32}$P-DOP utilization, gross DOP fluxes will be even larger. These results suggest an important role for DOP in microbial loop processes in these low-nutrient, open-ocean habitats. At steady-state, DOP production and DOP remineralization rates would be in balance. Consequently, given the DOP pool size and estimates of DOP turnover rates, these organic pools are likely to serve as an important source of P, as well as C and N, for microorganisms. If the compound C:P ratio is less than the whole cell C:P ratio, or if C is derived from additional or alternative sources, then Pi is likely to be released into the medium. These coupled processes most likely sustain the marine P cycle in the euphotic zone of the sea.

Finally, Björkman et al. (2000) measured coupled rates of Pi uptake and DOP production at several stations in the NPSG using $^{32}$Pi as a tracer. Pi uptake rates varied from 3 to 8.2 nM Pi day$^{-1}$; Pi pool turnover time was 2–40 days. Net DOP production (i.e., accumulation) was 10–40% of the net Pi uptake. The estimated turnover time for the entire DOP pool, assuming compositional singularity with nascent DOP, was 60–300 days. In all likelihood, the recently produced materials are assimilated much more rapidly than this simple calculation would suggest. Pi regeneration from selected, exogenously added DOP compounds was rapid and efficient; highest rates of Pi release were observed for nucleotides (Björkman et al., 2000).

Although coupled Pi uptake and DOP production is well documented in a variety of marine ecosystems, the actual mechanisms of DOP production remain elusive. Admiral and Werner (1983) investigated the production of DOP by two coastal marine diatom species in laboratory culture. In addition to total DOP production rates, they concentrated a fraction of the DOP pool, using Sephadex G-10 chromatography, and documented partial reabsorption of the isolated DOP compounds by the same two species during Pi-limited growth. The inadvertent diffusive loss of LMW compounds or the active excretion of both LMW and HMW compounds are both feasible; the list of specific compounds that are liberated by growing algae is very large (Fogg, 1966; Hellebust, 1974). Alternatively, DOP release could result from cell autolysis, predator grazing or viral lysis. Each separate pathway might be expected to produce a different spectrum of compounds. Most of the research conducted to date has focused on extracellular production of DOC, not DOP, but suffice it to say that DOP production by healthy microorganisms is probably a universal phenomenon.

**B. DIRECT UTILIZATION OF DOP**

DOP compounds in seawater consist of both labile and refractory compounds. The labile DOP pool includes both transportable and nontransportable organic compounds, either of which can serve as P sources for microbial growth. The
outer membrane of Gram-negative bacteria allows the transport of molecules up to about 600 Da (Weiss et al., 1991). Therefore, many DOP compounds can, and probably are, taken up directly without the need for prior hydrolytic alteration. For example, Gly-3-P and AMP can be assimilated intact by certain bacteria (Wanner, 1993; Ruby et al., 1985), whereas larger DOP compounds must be enzymatically hydrolyzed, either at the cell surface (or in the periplasmic space for bacteria) or in the surrounding medium prior to assimilation. The Pi released is then available for assimilation and biosynthesis.

Therefore, the ability of an organism to grow on one or multiple DOP substrates as the sole source of cellular P can be traced to one of two independent properties: the presence of cell membrane or periplasmic bound enzymes that catalyze the DOP compound dephosphorylation and thereby enhance Pi availability or the presence of a DOP compound- or compound-class-specific uptake system. Both pathways are present in marine microorganisms; growth of both prokaryotic and eukaryotic microorganisms on a variety of different DOP compounds is well documented (Kuenzler, 1965; Cembella et al., 1984a,b; Antia et al., 1991; van Boekel, 1991).

Among other functions, the Pho regulon controls the transport of selected, intact DOP compounds into bacterial cells. Several proteins of the outer membrane of many bacteria (termed "porins") are involved in the formation of aqueous pores through which small hydrophilic molecules (<600 Da, including Pi and selected DOP molecules) can pass through the membrane. Pi concentration regulates the synthesis of these proteins; Pi starvation enhances their biosynthesis (Tommassen and Lugtenberg, 1981; Saier, 2000). Tanoue (1995) has reported that porins, specifically porin-P (a protein that is synthesized during Pi-limitation) may be a major component of the total dissolved protein pool in seawater. Using sodium dodecylsulfate–polyacrylamide gel electrophoresis, Suzuki et al. (1997) detected several distinct bands with molecular weights 14.3–66 kDa; one frequently observed band (MW = 48 kDa) was identified as bacterial porin P. Consequently, it appears that porins may be an ecologically significant potential pathway of Pi and DOP assimilation in low-nutrient, open-ocean habitats.

Laboratory studies conducted with E. coli have documented independent and, fairly specific transport systems for Gly-3-P and Glu-6-P that are derepressed during Pi limitation. Although both systems also cotransport Pi, they are low-affinity in this regard, especially when compared to the Pst system that is also active during periods of Pi stress. These specific DOP transport systems appear to be one example of an anion-exchange mechanism in bacteria (Maloney et al., 1990). The uptake of the DOP compound is linked with the export of Pi and may, therefore, integrate into a vital chemiosmotic circuit or H⁺ pump; in effect, these DOP transport systems are Pi-linked antiporters.

Although neither of these specific DOP uptake systems has yet been detected in marine bacteria, it is very likely that they do exist and may have an important ecophysiological role. The two most potentially significant functions of these
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Anion-exchange mechanisms are: (1) to catalyze the heterologous exchange of Pi and a selected sugar phosphate (e.g., Pi:Gly-3-P and Pi:Glu-6-P) and (2) to exchange intracellular AsO$_4^{3-}$ for either Pi or for a sugar phosphate. The first function, the asymmetric exchange of Pi for a sugar phosphate, may be part of the cell's mechanism to balance the supply of C and P. Because many DOP compounds have excess P relative to C, compared to whole cell C:P stoichiometry (e.g., the molar C:P ratios for Gly-3-P and Glu-6-P are 3 and 6, respectively, compared to a Redfield stoichiometry of 106; also see Table 1) this would provide an efficient system to achieve a physiological C-to-P balance. This control mechanism might be especially important during periods of low growth rate when C demands for maintenance energy generation are high, but biosynthetic demands for P are low, as in the mesopelagic or abyssopelagic zones. The second function, the exchange of AsO$_4^{3-}$ for Pi or the exchange of AsO$_4^{3-}$ for a sugar phosphate may be one of several strategies for As detoxification, especially in open ocean environments where the As/Pi concentration ratio exceeds 10 (Karl and Tien, 1997).

Another potential advantage of the direct uptake of DOP, especially nucleotide monophosphates, is the use of these molecules as biosynthetic precursors. This conservation of preexisting phosphate bonds has significant implications for cellular energetics and for the growth efficiency of microorganisms (Rittenberg and Hespell, 1975).

The availability of water insoluble or hydrophobic DOP compounds, like membrane phospholipids, may require additional, specific enzymes for assimilation. Lemke et al. (1995) evaluated the role of cell surface hydrophobicity, a measurable property of all microbial cells, on the relative utilization rates of hydrophobic and hydrophilic P compounds. Hydrophilic bacteria grew rapidly on Pi and Gly-3-P, but could not assimilate the hydrophobic substrate, phosphatidic acid (PA) or membrane phospholipids (Lemke et al., 1995). Conversely, bacteria with hydrophobic cell surfaces efficiently utilized PA and lipid P. These cell-specific metabolic capabilities could lead to DOP resource partitioning among otherwise competing microheterotrophs, or to species selection and succession following production or exhaustion of one or more key DOP substrates.

An interesting and potentially important study of the effect of fluid motion on the utilization of selected low-molecular-weight DOM (not explicitly DOP compounds) by heterotrophic bacteria revealed that uptake rate was enhanced by advective flow, but only at low subsaturating DOM concentrations (Logan and Kirchman, 1991). One prediction of their results is that particle-bound bacteria, especially those sinking through the water column, might be more important for deep-water DOP remineralization than the solitary microorganisms suspended in the water column.

Finally, much has been written on the competition between bacteria (chemo-heterotrophs) and algae (mostly eukaryotic phototrophs) for Pi and DOP in aquatic environments since the elegant laboratory studies conducted by Rhee (1972).
These investigations have examined uptake affinities, cell quotas, storage capabilities and other ecophysiological parameters under both P-sufficient and P-limited growth conditions. Despite some contradictory field results, most investigations revealed a tight metabolic coupling between the producer and consumer species and explicit nutrient resource-based competition, especially at limiting concentrations of Pi. Less well documented is the ability to switch from Pi-based to DOP-based metabolism or the sequential versus simultaneous utilization of two or more P-containing substrates. In open-ocean environments, the bacterial–algal dichotomy disappears because the “algae” are bacteria (e.g., the picophytoplankton assemblage). Most low nutrient environments are dominated by prokaryotes, e.g., Prochlorococcus or Synechococcus, and may even be supported by mixotrophic growth (e.g., simultaneous utilization of photoautotrophic and chemoheterotrophic metabolic pathways). In any event, all prokaryotes and eukaryotes function as P-traps and ultimately must compete with each other.

C. ENZYMES AS P-CYCLE FACILITATORS

Because Pi starvation causes a significant increase in APase activity it has been suggested that the detection of APase in field collected samples may be an ecophysiological indicator of Pi stress. However, the literature on this topic is confusing and, at times, contradictory. For example, it has also been shown that starvation for nucleic acid bases will induce APase synthesis even under conditions of excess Pi (Wilkins, 1972). Consequently, there may be alternative ecological interpretations for the presence of elevated APase in natural assemblages of microorganisms.

APase activity can be measured by colorimetry (using para-nitrophenyl phosphate as the substrate), fluorometry (using either o-methyl fluorescein phosphate or 4-methylumbelliferyl phosphate as the substrate), or firefly bioluminescence (using ATP as the substrate). Substrate selection determines to a large extent measurement sensitivity and assay specificity. For example, para-nitrophenyl phosphate and ATP are both hydrolyzed by APase and the related enzyme nucleotidase, so these assays are not necessarily specific for APase unless combined with other sample treatments (e.g., Pi additions; Karl and Craven, 1980). Furthermore, the use of artificial substrates has the potential to overestimate the in situ rates of enzymatic activity (Cembella et al., 1984a; Admiraal and Veldhuis, 1987). Only ATP is likely to be a native substrate in natural samples and only the use of ATP provides a detection system that can provide in situ rate estimates. A new insoluble fluorogenic substrate for APase, termed enzyme-labeled fluorescence (ELF), has been used to detect the presence of the enzyme in single microbial cells (González-Gil et al., 1998; Dyhrman and Palenik, 1999). Cell detection is by either epifluorescence microscopy or laser-based flow cytometry.
Laboratory studies of APase in pure cultures of marine bacteria (Hassan and Pratt, 1977) report that APase was completely repressed, partially repressed, or not repressed at all by the presence of 50 mM Pi in the growth medium. This variable response suggested the presence of different APase isozymes, some of which appear to be constitutive. In any case, the relatively high Pi concentrations used in this and other laboratory studies of the Pi repression of APase activity is $10^4$ to $10^6$ times higher than Pi concentrations in most surface ocean waters (open ocean 1–100 nM Pi, coastal ocean <1 μM), so even "relatively high" seawater concentrations must be considered Pi-depleted for the purposes of the microbial APase derepression response. An ecological prediction is that APase should be ubiquitous in seawater.

Morita and Howe (1957) may have been the first to measure APase activity in cultures of marine bacteria. Their interests centered on the effects of ambient hydrostatic pressure on the potential efficiency of Pi regeneration in the deep sea. The first published report of APase activity in seawater was for coastal waters near Taiwan (Wai et al., 1960). They detected and quantified APase activity by several procedures including the measurement of Ca$^{2+}$ increase following a timed incubation with fish bone powder (calcium phosphate) and by an increase in Pi following a timed incubation with Gly-3-P. APase activity was ubiquitous, but varied with sample location (Wai et al., 1960).

Because all living organisms in the sea, from bacteria to marine mammals, can produce APase and other phosphatases it is difficult to determine the exact source of the enzyme activity in most environmental samples. From an ecological perspective it is critical to know which organisms are expressing APase activity under a particular set of environmental conditions. APase activity in the dissolved fraction is also common (Reichardt et al., 1967). Most investigators measure APase activity in either whole water (dissolved plus particulate activity) or filtered (or filter size-fractionated) subsamples. For example, in Toulon Bay, APase in the >90-μm fraction accounted for more than 80% of the total activity, whereas in other coastal regions of the Mediterranean APase was restricted to the picopelag (0.25–5 μm) fraction (Gambin et al., 1999). Boon (1994) has devised a very clever technique to distinguish between prokaryotic and eukaryotic APase activities. His method relies on a differential inhibition by various physical (e.g., thermal deactivation) and chemical (e.g., Zn$^{2+}$ and Cu$^{2+}$ ion inhibition) treatments. To our knowledge, this technique has not yet been applied to the marine environment.

Since the first field report of APase activity in seawater by Wai et al. (1960), numerous studies have been conducted using a variety of increasingly more sensitive and specific assay systems. Because APase synthesis is derepressed following Pi limitation and is not induced by the presence of suitable substrates, in vivo enzyme activity under saturated substrate concentrations is not equivalent to P flux. Theoretically, in situ rates of DOP hydrolysis can be estimated only if the concentration of exogenious substrate is comparable to in situ specific DOP compound
concentrations. Consequently, most APase and other enzyme activity measurements in seawater must be considered "potential" activities.

Perry (1972) was the first to document APase activity in an oligotrophic, open ocean ecosystem. The APase activity in the NPSG was greatest for samples collected in the upper 60 m of the water column and decreased with increasing water depth and ambient Pi concentration. Microbial assemblages lacking detectable APase activity at time of sample collection produced APase within 1–2 days when incubated in bottles; the addition of 5 μM Pi to water samples immediately repressed APase activity (Perry, 1972). Li et al. (1998) measured significant dissolved (<0.2 μm) and particulate APase activities in the Gulf of Aqaba, northern Red Sea. Dissolved APase activities ranged from 40–70% of the total APase activity; most of the particulate APase activity was attributed to the picoplankton fraction. Cell-free activities were stable in the dark at 4°C for extended periods (up to 40 days) and could lead to variable dissolved:particulate ratios, for example, as a consequence of transient periods of Pi-limitation. In contrast to this open-ocean study, Taga and Kobori (1978) reported a positive, not negative, relationship between APase activity and Pi concentrations for samples collected in Tokyo Bay. As mentioned previously, even the highest Pi concentrations found in marine ecosystems (i.e., 3–4 μM and generally much lower) are low relative to the Pi concentrations required to repress APase activity in laboratory cultures of bacteria and eukaryotic algae. Consequently, the positive relationship between APase activity and Pi is probably controlled more by seston biomass than by per cell APase activities. In this regard, Smith et al. (1992) reported intense APase activity in association with marine aggregates compared to surrounding seawater; volume-normalized enhancements were 10^1- to 10^4-fold. The interstitial fluids of large dimension suspended and sinking particles may have chemical compositions that are different from the surrounding bulk fluid environments. This could select for, or against, specific enzyme activities.

There are at least two ecologically relevant postsynthesis controls on in situ APase activities in seawater: trace metal concentrations and UV-B radiation. Reuter (1983) demonstrated that APase activities in phytoplankton cultures, cell-free enzyme preparations and field collected samples are inhibited by free copper ions at concentrations comparable to those found in the marine environment (cupric ion activities of 10^{-9} to 10^{-12} M). This trace metal effect could interfere with or totally block the direct utilization of selected DOP compounds by natural microbial assemblages and therefore alter marine P-cycle dynamics. More recently, Garde and Gustavson (1999) have documented a UV-B radiation (280–320 nm) sensitivity of APase activity in the marine environment. A major implication of this work is that photodegradation of APase activity may limit a cell's ability to obtain Pi from the ambient DOP pool, thereby exacerbating the effects of Pi limitation in well-lighted, near-surface habitats. This UV-B control of APase activity would probably be more important in clear, open-ocean ecosystems than in coastal
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habitats. In more productive coastal-shelf and estuarine habitats there may be a positive photolytic effect that derives from the light-dependent release of APase previously bound (and therefore deactivated) by humic substances (Boavida and Wetzel, 1998; Boavida, 2000). This photodegradation/photoactivation dichotomy may also affect other enzymes in the marine P cycle and, therefore, influence DOP turnover rates.

Finally, Hoppe and Ullrich (1999) have presented a comprehensive assessment of APase activities not only in the euphotic zone but to abyssal ocean depths in the Indian Ocean. Contrary to expectation, the measured total APase activities generally increased with increasing depth despite decreased bacterial biomass, increased ambient concentrations of Pi, and a decrease in the activities of other hydrolytic enzymes. Elevated APase activities in the deep ocean (>1000 m) had previously been reported for particulate matter samples collected in the central North Pacific Ocean (Koike and Nagata, 1997) and may be a general feature of marine ecosystems. Hoppe and Ullrich (1999) hypothesized that the elevated deep water APase activities may be a manifestation of an enhanced carbon acquisition system involving bioavailable DOP compounds. The elevated APase would locally regenerate Pi but, more importantly, would capture the DOP compound carbon skeleton which could be respired to provide energy for cell maintenance. If this intriguing deep sea metabolic model is supported by future field research it would be an important lesson regarding our general ignorance of subeuphotic zone ocean processes. Regardless, the report of elevated cell-specific APase activities in Pi-sufficient deep sea habitats casts doubt on past interpretations of environmental APase activity as being an indicator of Pi stress only.

APase is just one of several potential enzymatic facilitators of DOP turnover in the sea (Fig. 5); we have discussed it at length here as a "case study" because it has been measured extensively in the sea. The enzyme 5NDase is a membrane-bound protein found in most bacteria (Bengis-Garber and Kushner, 1982) and in eukaryotic algae (Flynn et al., 1986); it is responsible for the hydrolysis of extracellular nucleotides (Fig. 5). In nature the substrate specificity of 5NDase overlaps with other enzymes; for example, ATP is also hydrolyzed by APase as well as by more specific ATPases (EC 3.6.1.4) found in many cells. A major difference seems to be in the regulation of enzyme production, especially the effects of Pi limitation. In contrast to APase, the activity and synthesis of 5NDase in E. coli are not inhibited by Pi and in this respect resemble inorganic pyrophosphatase (Neu, 1967). 5NDase may play a key role in nucleotide pool turnover and biosynthetic salvage pathways. Ammerman and Azam (1985) were the first to detect 5NDase in natural marine microbial assemblages and since that time numerous reports have appeared. Ironically, the assay system most frequently employed releases 32Pi from [γ32P]ATP. This would integrate the activities of several different classes of phosphohydrolytic enzymes (5NDase, APase, ATPase), thereby overestimating specific 5NDase activity. The addition of 100 μM of Pi to the assay mixture
should improve the accuracy of 5NDase detection in collected samples (Cotner and Wetzel, 1991). From an ecological perspective, however, it is not so important which enzyme class is active but rather what the total rate of catalysis is under in situ conditions.

Siu and Güde (1994) found that ATP was hydrolyzed much more rapidly by 5NDase than by APase, whereas the rates of AMP and adenosine 5'-diphosphate (ADP) were comparable. If total extracellular nucleotide production in the ocean is controlled by cell exudation or grazing activities, then ATP may be a large portion of the pool and 5NDase activity would be important. If, on the other hand, hydrolysis of polymeric DNA and RNA supplies most of the dissolved nucleotides in seawater, then both APase and 5NDase would be important to the cells (Fig. 5). In fact, Ammerman (1991) concludes that the major function of 5NDase may be to assist in the coupled recycling of RNA and DNA by hydrolyzing the ribo- and deoxyribonucleotides produced by nuclease enzyme activity. As discussed previously, the "bottleneck" in the DOP cycle may be nuclease, rather than 5NDase activity (Fig. 5).

Nucleases and related enzymes catalyze the breakdown of nucleic acids by hydrolysis of the phosphodiester bonds. Some nucleases are specific for RNA (RNases), others act only on DNA (DNases), while still others are nonspecific. Furthermore, nucleases can be classified by mode of substrate attack; polynucleotides can be hydrolyzed at many interior locations in the polymer (endonucleases) or stepwise from one end of the chain (exonucleases). Additionally, the exonuclease attack can be directed from either the 3'→5' or the 5'→3'. Therefore the hydrolysis products from nuclease activity in seawater can result in a mixture of oligonucleotides as well as 3' and 5' mononucleotides (Fig. 5). This is a potentially important point, since 5NDase is specific for 5'-nucleotides and will not hydrolyse 3' compounds (Bengis-Garber and Kushner, 1981). APase, on the other hand, will hydrolyze both 3'- and 5'-nucleotides (Fig. 5). Nuclease activity is also important for control of intracellular RNA concentrations, especially during carbon and energy starvation. In laboratory studies with E. coli, the RNA degradation products—including both 3'- and 5'-nucleotides and oligonucleotides—appear in the medium (Cohen and Kaplan, 1977). Consequently, this may be an important source for DOP production under certain nongrowth conditions (Novitsky and Morita, 1977).

D. DOP INTERACTIONS WITH LIGHT AND SUSPENDED MINERALS

The marine DOP pool is known to react with UV light; in fact, this is one of the many techniques used to characterize (Karl and Yanagi, 1997) and quantify it (Armstrong et al., 1966). Less is known about solar-induced DOP photolysis in
the sea. Francko and Heath (1982) reported the existence of UV-sensitive P (SNP which released SRP following timed exposure to natural UV irradiation) in lake ecosystems and it is probable that similar compound classes also exist in the marine environment (see Kieber et al., 1989). One intriguing implication of photochemical alteration of DOM is the possible cooccurrence of iron photo_reduction (reduction of hydrous iron oxides) and Pi desorption from colloidal particles (Tate et al., 1995). If similar actinic effects occur in the marine P cycle, one might anticipate that they would be restricted to the near surface waters where light fluxes are maximal. Near sea surface enrichments of SRP have recently been reported for samples collected in the North Pacific Ocean (Haury et al., 1994; Karl and Tien, 1997; Haury and Shulenberger, 1998), which could in principle arise via natural DOP photolysis (although other explanations are also possible). The near surface accumulation of lipid-rich, positively buoyant colloidal or particulate material may be important in this hypothesized pathway (i.e., organic matter + light → SRP).

Finally, metal ion catalysis of phosphate ester hydrolysis in aqueous solutions is well-documented (Dixon et al., 1982). However, the generally low concentrations of transition metal ions in open ocean habitats may preclude this process. More important in these habitats, phosphate ester hydrolysis may be facilitated by sus-pended minerals, including amorphous iron and manganese hydroxides (Baldwin et al., 1995). This could lead to an ecologically significant coupling between the atmospheric delivery of bioessential trace metals and the pulsed, abiotic release of Pi from semilabile or biorefractory DOP.

X. CONCLUSIONS AND PROSPECTUS

The biogeochemical cycle of P (as well as C and N) in the sea is sustained by energy supplied to the surface ocean by sunlight. Photosynthetic production of organic matter fuels a complex series of trophic interactions and organic matter export (both as DOM and POM) from the euphotic zone that ultimately sustains life throughout most of the world ocean.

In pelagic marine ecosystems, the supply rates of both N and P exert primary controls on ecosystem productivity (Smith et al., 1986). Over long time scales, P, rather than N, is probably the biomass- and production-rate-limiting nutrient in the global ocean (Redfield, 1958; Codispoti, 1989), due to P removal in shallow-water, carbonate-dominated ecosystems and due to the role of bacterial N2 fixation as a mechanism for relieving the ecosystem of fixed N limitation. Consequently, field studies of P cycling in the epipelagic zone are of fundamental importance for our understanding of microbial processes in oligotrophic oceanic habitats. Nevertheless, P-cycle investigations conducted beyond the continental shelf are rare, especially by comparison to the relatively large body of knowledge on
N cycling in oceanic habitats. This situation is due, in part, to technical limitations in the ability to obtain precise and accurate determinations of low concentrations of dissolved P.

A more complete chemical characterization of the DOM pools in seawater would be a most welcomed addition to contemporary studies of the marine P cycle. Likewise, a better understanding of the ecosystem processes that are responsible for extracellular accumulation of relevant biomolecules such as ATP, GTP, and nucleic acids will help to constrain DOP sources, sinks, and fluxes. Finally, it is imperative that future field studies recognize the P cycle as an integral component of microbiological oceanography and marine biogeochemistry, along with the better studied though no less relevant C and N cycles. As McGill (1963) remarked nearly four decades ago, “The organic portion of the phosphorus cycle will undoubtedly receive much closer scrutiny in the future as its importance becomes more evident to biologists and oceanographers.” While prophetic at that time, we still have some unfinished business. We look forward to more pathfinding contributions on marine DOP pool dynamics in the new millennium.

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