Phosphorus cycling in the Sargasso Sea: Investigation using the oxygen isotopic composition of phosphate, enzyme labeled fluorescence, and turnover times

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Abstract

Dissolved inorganic phosphorus (DIP) concentrations in surface water of vast areas of the ocean are extremely low (<10 nM) and phosphorus (P) availability could limit primary productivity in these regions. We utilized multiple techniques to investigate biogeochemical cycling of P in the Sargasso Sea, Atlantic Ocean. We found that dissolved organic phosphorus (DOP) is extensively utilized by phytoplankton and bacteria to supplement cellular requirements. Remineralization of the DOP pool was most extensive above the thermocline as indicated by expression of alkaline phosphatase, rapid P turnover (4 - 8 hours), and large $\delta^{18}O_p$ deviations from equilibrium. These data suggest that DOP remineralization by extracellular enzymes in the euphotic zone can account on average for 35% (range 10 - 50%) of P utilized. Below the thermocline, alkaline phosphatase expression is reduced, turnover times increase, and $\delta^{18}O_p$ values approach isotopic equilibrium. In the surface waters of the Sargasso Sea, C-fixation supported by regenerated DOP utilization may account for 4×10^9 mol C $year^{-1}$.

Introduction

The oceanic phosphorus (P) cycle is closely coupled with the global carbon cycle through the role of P as a major nutrient supporting ocean primary productivity. The concentrations of dissolved inorganic phosphorus (DIP) in much of the open ocean surface waters are low and can limit or co-limit primary production (Wu *et al.* 2000b, Vidal *et al.* 2003, Lomas *et al.* 2004, Mills *et al.* 2004). In contrast, the dissolved organic phosphorus (DOP) pool is significantly larger than the DIP pool and thus, utilization of this chemically heterogeneous pool as a P source for living organisms can potentially influence carbon sequestration in the ocean via the biological pump (Dyhrman *et al.* 2006, Torres-Valdes *et al.* 2009, Lomas *et al.* 2010). However, the complex biogeochemical cycling of P in the open ocean is poorly characterized and quantitative estimates of the bioavailability and utilization of DOP on large (ecologically relevant) spatial and temporal scales are lacking. In order to better understand P cycling in the open ocean and to estimate the degree of DOP utilization, we use a combination of techniques: $\delta^{18}O_p$ distribution in the water column (Blake *et al.*

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2005, Colman *et al.* 2005, McLaughlin *et al.* 2006), enzyme-labeled fluorescence (ELF) analysis of alkaline phosphatase in single cells (Dyhrman and Palenik 1999, Ruttenberg and Dyhrman 2005), and ^{33}P uptake derived P turnover times (Benitez-Nelson and Buesseler 1999, Sohm and Capone 2010). Analyses were made on samples representing relatively high nutrients near-shore environment as well as open ocean oligotrophic nutrient depleted environments.

Marine organisms cope with low levels of biologically available P in different ways. Some species have lower cellular P requirements (Christian 2005, vanMooy *et al.* 2009). Other species have adapted to utilize P from organic sources (DOP) and from polyphosphates (Dyhrman *et al.* 2006, Dyhrman and Haley 2006), evidenced by the abundance of alkaline phosphatase activity in seawater and phosphorus uptake measurements (Benitez-Nelson and Karl 2002; Vidal *et al.* 2003; Bjorkman and Karl 2003, 2005). A number of studies have shown that P is preferentially remineralized from dissolved organic matter relative to carbon, allowing efficient utilization of P in the euphotic zone (Clark *et al.* 1998, Hopkinson *et al.* 2002, Aminot and Kerouel 2004, Hopkinson and Vallino 2005, Lomas *et al.* 2010). Phosphorus turnover rates obtained using cosmogenic isotopes of P show that P recycling rates in the dissolved and particulate pools in surface waters are very rapid (less than a day to two weeks), suggesting that low P concentrations can support relatively high primary production (Benitez-Nelson and Buesseler 1999, Benitez-Nelson and Karl 2002). Furthermore, P recycling rates vary spatially and temporally (Sohm and Capone 2010) and picoplankton preferentially utilize certain DOP compounds to obtain P and other associated nutrients (Benitez-Nelson and Buesseler 1999). However, quantitative estimates of the contribution of DOP regeneration to carbon fixation in the open ocean is lacking, a data gap addressed by this study.

Methods

Study Site

We collected depth profiles at 6 stations along a transect from the shelf break off the coast of Virginia (USA) through the Sargasso Sea (Figure 1) on March 2004. Profiles ranged in depth from 200 m (at the shelfbreak) to 4,200 m at Station 6. Station 2 (31.9812° N, 64.3488° W) approximately coincides with the existing Bermuda Atlantic Time Series (BATS) Station (34.667° N, 64.167° W).

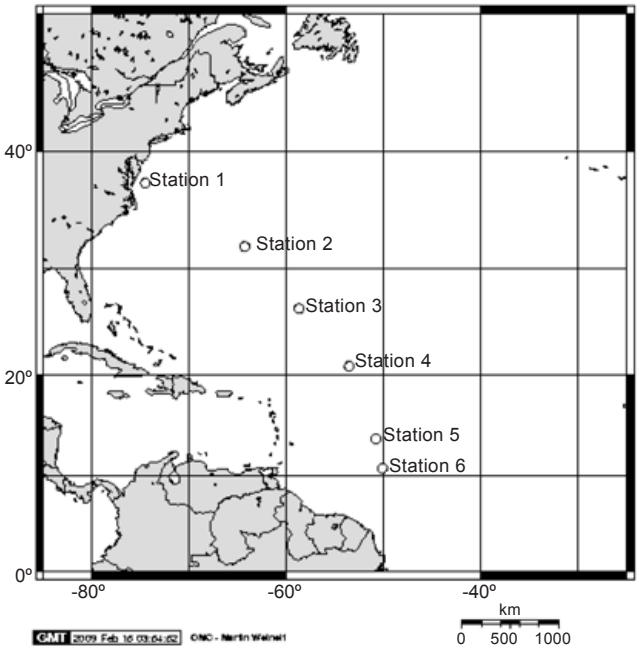

Figure 1. Map of sampling stations.

Sampling Approach

Water samples were collected from Niskin bottles mounted to a 24-place rosette. Water samples at each depth were analyzed for $\delta^{18}O_p$ and soluble reactive phosphate (SRP) concentration (SRP operationally defined and is primarily (87%) DIP, but may also include some easily hydrolyzable inorganic and organic forms of P). Surface water samples were also analyzed for chlorophyll *a* and ELF. Phosphorus turnover time was assessed at Stations 3 through 6.

Chlorophyll *a* (Chl *a*) concentration (mg chl m^{-3}) was measured by a modified fluorometric procedure in which water is filtered through 25 mm Whatmann GF/F filters, filters were extracted in 90% acetone in a freezer overnight, and Chl *a* is measured using a Turner Designs Model-10 fluorometer calibrated with a commercial Chl *a* standard. Approximately 50 ml of water was collected for SRP concentration analysis. These samples were concentrated by the MagIC method of Karl and Tien (Karl and Tien 1992) and analyzed on an Alpkem autoanalyzer. Surface waters (1 L) were collected for alkaline phosphatase (AP) enzyme- labeled fluorescence (Dyhrman and Palenik 1999). This involved collecting plankton samples on a 0.22 µm filter (by low-vacuum filtration), resuspending the sample in an ethanol solution, adding the label (ELF-97), and transferring

the sample to an Epitube. Samples are stored in the dark at 4°C until analysis. Cell counts are performed using a Nikon epifluorescent microscope using a 100-W mercury lamp (DAPI filter set, excitation at 350 nm, and maximum ELF emission at 520 nm) for ELF activity as well as with standard illumination. Slides are scanned, and each identifiable cell is tallied as either positive or negative for ELF labeling, indicating AP activity. A positive tally is given to any cell that had a considerable amount (>10% of cell area) of visible fluorescent ELF labeling. At least 300 individual cells were tallied for each sample. We note that the ELF procedure is not a quantitative measure of enzyme activity, but rather an account of the percentage of cells that have expressed the enzyme over the past week or two (Dyhrman and Palenik 1999, Lomas *et al.* 2004).

Approximately 40 L of water was collected from each depth for oxygen isotope analysis of phosphate ($\delta^{18}O_p$) in high density polyethylene (HDPE) acid washed Jerrycans (two 25 L containers per depth). DIP was stripped from seawater by adding 1 M sodium hydroxide to each sample immediately after collection and rigorously shaking (Karl and Tien 1992, Thomson-Bulldis and Karl 1998). Because two Jerrycans were collected per depth, each container was treated as a separate sample for the initial precipitation. Magnesium hydroxide floc was allowed to settle in each container for 2 hours in a cold room (4°C) before supernatant was siphoned off, leaving approximately 5 L of floc in seawater. The floc from both Jerrycans was then combined into a single container in which the floc was allowed to continue to settle. After another hour additional supernatant was siphoned off leaving approximately 1 - 2 L of floc in seawater. This was then stored in a 2 L HDPE bottle and frozen until analysis for $\delta^{18}O_p$ (McLaughlin*, et al.* 2004). Isotopic analyses were conducted on a Eurovector Elemental Analyzer coupled to a mass spectrometer at the US Geological Survey in Menlo Park, California. Two calibrated internal silver phosphate standards, STDH ($\delta^{18}O_p$ = 20.0‰) and STDL ($\delta^{18}O_p$ = 11.3‰) were analyzed throughout each mass spectrometer run for calibration and drift correction. Results from field replicate analyses of water samples (collected at the same depth and time thus including natural variability and processing reproducibility) fell within acceptance limits of 80 - 120% relative percent difference. Water samples were also collected in 20 ml HDPE-depressed cap scintillation vials with no headspace for $\delta^{18}O_w$ analysis. $\delta^{18}O_w$ was determined using a Finnigan MAT 251 mass spectrometer also at the US Geological Survey in Menlo Park. All oxygen isotopic composition measurements are reported in standard delta notation ($\delta^{18}O$) relative to Vienna Standard Mean Ocean Water (VSMOW).

To measure phosphate turnover times, duplicate 50 ml samples of seawater were placed in 60 ml acid washed polycarbonate bottles with 0.5 - 2 µCi of $H_3{}^{33}PO^{-3}{}_4$ and incubated in 25% light on deck (euphotic zone samples), in a dark incubator on deck (samples >200 m) or in the dark at 4°C incubator (samples >1000 m) for 60-90 minutes. To calculate an instantaneous uptake rate, samples were collected within the linear range of increase of isotope in the cell, rather than after the isotopic equilibration outside the cells. Incubated samples were filtered onto 0.2 µm polycarbonate filters placed into 7 ml plastic scintillation vials which were rinsed with filtered seawater. To control for abiological adsorption, control samples amended with glutaraldehyde were also incubated. Activity of ^{33}P was measured in a scintillation counter after addition of 5 ml of scintillation cocktail. The turnover time of the phosphate pool in a sample is calculated as $T = R_t*t/(R_f-R_k)$, where t is the incubation time and R_t, R_f and R_k are the radioactivity (in counts per minute) of the total pool added, the filter and the killed control, respectively. The data obtained indicates the length of time that would be required for uptake and utilization of all the SRP in each respective sample. The average coefficient of variation associated with the turnover time calculations was 27%. For more detail see Sohm and Capone (Sohm and Capone 2006).

Results and Discussion

Our results indicate that the Sargasso Sea is deficient in DIP such that the biological communities are utilizing extracellular enzymes to access the DOP pool. The first line of evidence is observed $\delta^{18}O_p$ values in the mixed layer (above the thermocline) at most of the stations are significantly lower than values expected for equilibrium (paired t-test; $P < 0.001$), suggesting large scale recycling of the DIP pool (Figure 2). A shift in the isotopic composition towards values less than equilibrium implies isotopic fractionation associated with extracellular enzyme hydrolysis such as alkaline phosphatase or 5′-nucleotidase, or possibly some other uncharacterized enzyme that imparts a negative

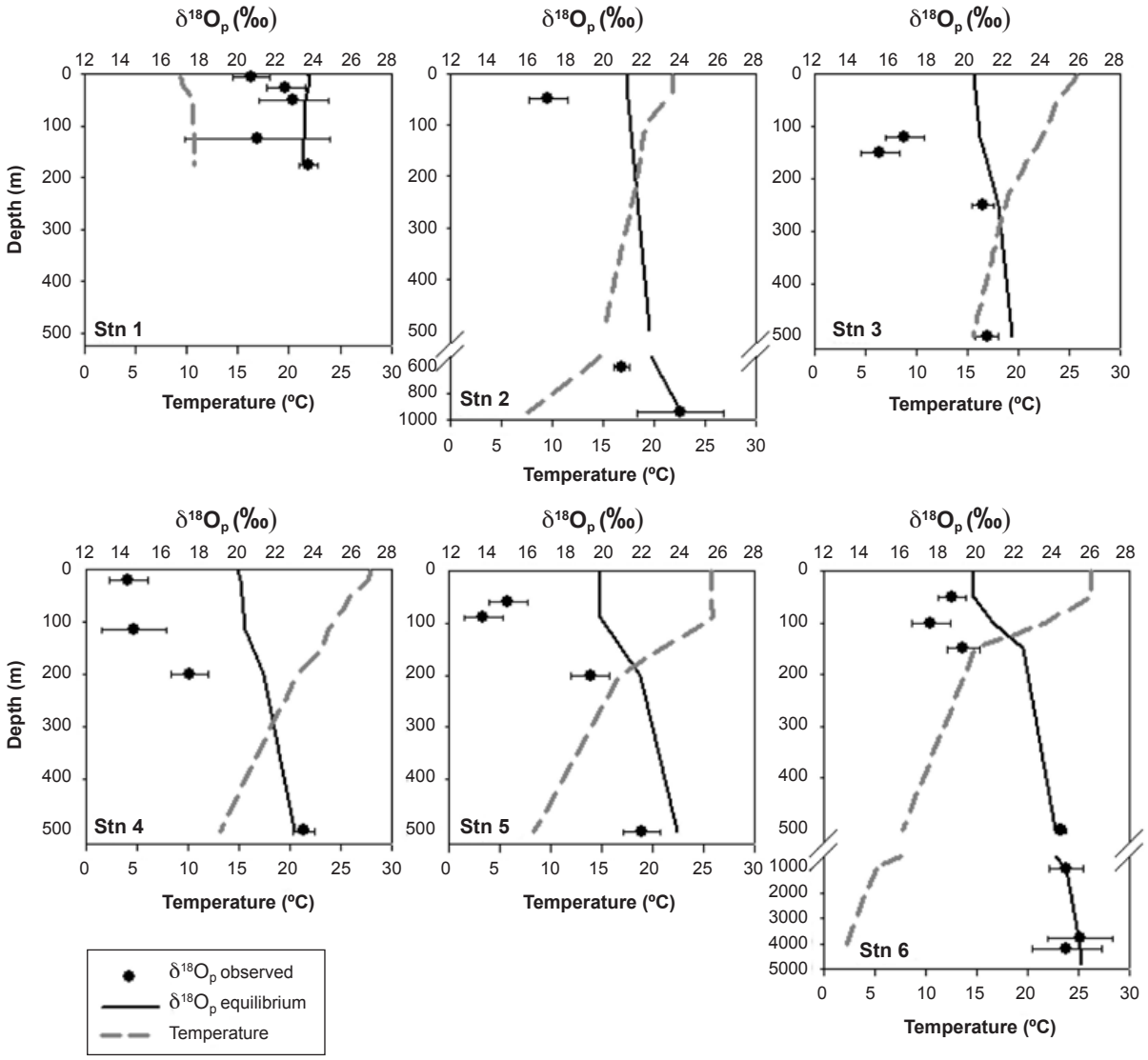

Figure 2. Depth profiles of $\delta^{18}O_p$ along with the calculated equilibrium values and the temperature. The solid line represents the expected equilibrium $\delta^{18}O_p$ calculate based on the oxygen isotope value of seawater and the temperature at the respective depth using the equation for equilibrium (Longinelli and Nuti 1968). Observed $\delta^{18}O_p$ values are significantly lower than equilibrium $\delta^{18}O_p$ ($p < 0.001$) and increasing turnover times are correlated with increasing $\delta^{18}O_p$ ($p = 0.004$).

fractionation at the P-O bond site, is shifting the product (phosphate) isotopic composition towards lower than equilibrium values. While several enzymes and substrates are involved in P cycling in the ocean and the precise isotopic fractionation associated with some of these enzymes is not known (Liang and Blake 2006, 2009), the enzymes and substrates (phosphoesters) for which the activity is characterized and these conclusions are based, are the most abundant in the water column (Kolowith *et al.* 2001, Cotner and Biddanda 2002, Paytan and McLaughlin 2007, Young and Ingall 2009).

The second line of evidence is the high percentage of eukaryotes expressing alkaline phosphatase activity in surface waters at all stations (up to 80%). This is within the range reported by Lomas *et al.* (2004); though the expression is variable among sites and decreases with depth (Figure 3; Table 1). Furthermore, bulk alkaline phosphatase activities in the euphotic zone were also high reaching 80 nmol µg chl a^{-1} h^{-1} (Sohm and Capone 2006; Table 1). High levels of alkaline phosphtase activity are indicative of P-deficiency and the utilization of DOP (Vidal *et al.* 2003). The surface waters where ELF is observed are also characterized by lower $\delta^{18}O_p$ values, suggesting that the disequilibrium in $\delta^{18}O_p$ can largely be attributed to utilization of DOP and likely involves the abundant

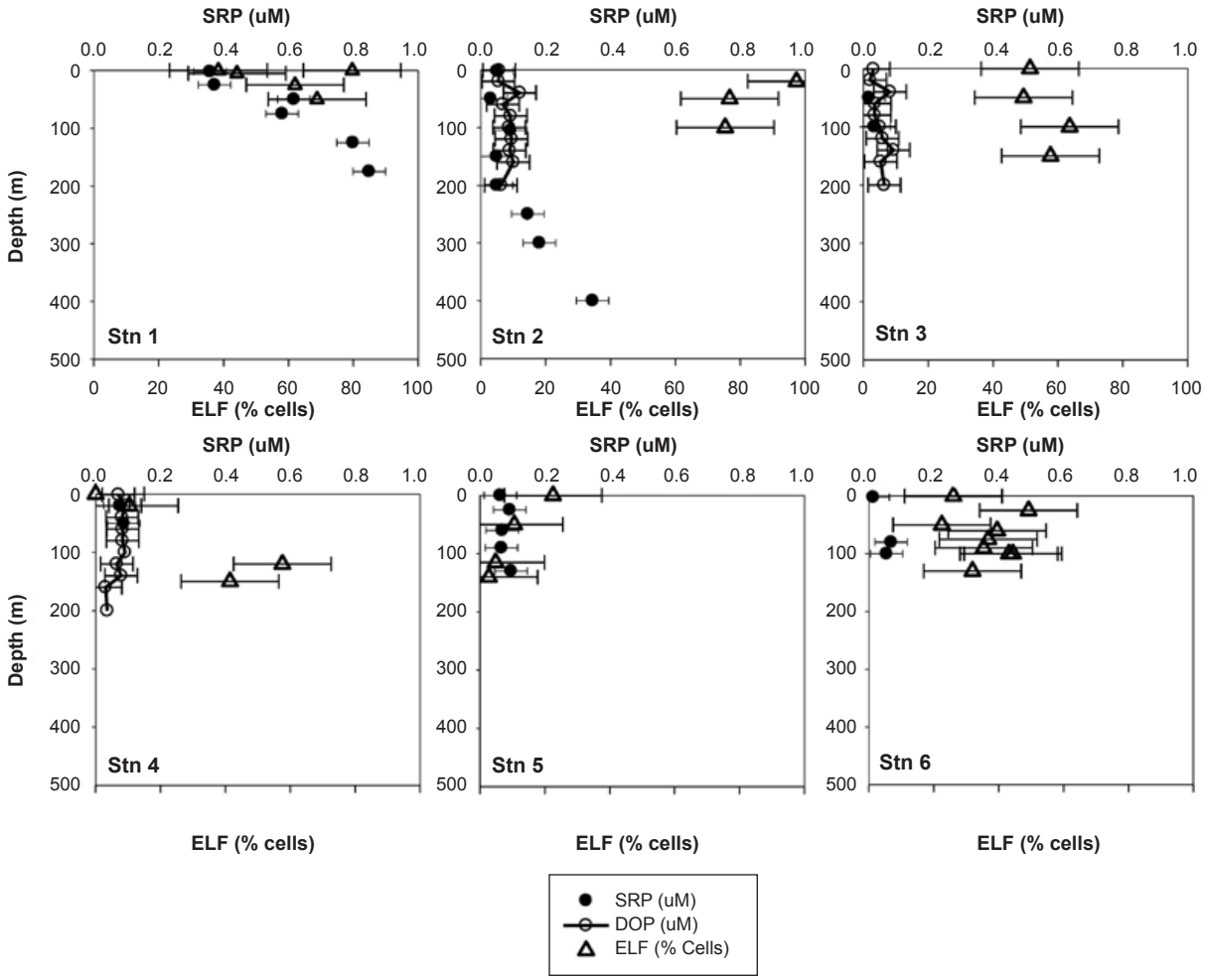

Figure 3. Depth profiles of soluble reactive phosphate concentration and percent cells labeled for alkaline phosphatase expression. DOP data is also shown for some stations (M. Lomas, unpublished). Surface water DOP data for additional stations are shown in Table 1.

Table 1. Data for representative samples collected in the upper water column during this cruise. Additional data for other depths is shown in the figures and given in Table SI-1. Here we present the data for select depths for which additional measures of P dynamics were measured (e.g., turnover time, uptake rates, or Al-P activity) for the same samples. Bulk APA = bulk alkaline phosphatase activity from Sohm and Capone 2006 collected on the same cruise within the euphotic zone. PO_4 uptake rate data from Sohm and Capone 2006 collected on the same cruise within the euphotic zone. PO_4 uptake at station 6 was measured twice $\delta^{18}O_{EQ} = \delta^{18}O_p$ expected for equilibrium with temperature and $\delta^{18}O_w$ (Longinelli and Nuti 1968): Equilibrium $\delta^{18}O_p$ = ((Temperature -111.4)/(-4.3)) + $\delta^{18}O_w$. % P Utilized from DOP based on "best estimate" see text and Table SI-2.

Stn #	Depth (m)	Temp (°C)	Sal	SRP (µM)	DOP (µM)	Chl *a* (mg m^{-3})	ELF (%)	Bulk APA (nmol µgchl^{-1} h^{-1})	PO_4 Uptake (nmol µgchl^{-1} h^{-1})	Turnover (h)	$\delta^{18}O_p$ (‰)	$\delta^{18}O_{EQ}$ (‰)	DOP Utilization (%)
1	5	9.39	34.60	0.36	-	1.54	38	-	-	-	20.7	23.7	22.6
2	50	19.85	36.69	0.05	0.17	0.14	77	17.6	25.0	-	17.1	21.3	32.5
3	120	22.62	37.09	0.02	-	0.02	57	83.9	61.1	16	16.7	20.6	31.1
4	20	25.26	37.24	0.04	0.10	0.05	50	6.5	154.9	4	14.2	20.0	46.8
5	60	25.78	36.59	0.03	0.12	0.04	39	-	74.4	7	15.1	19.9	38.4
6	100	21.65	36.59	0.05	-	0.15	44	3.1	69.5 & 43.8	9	17.6	20.9	25.7

enzyme alkaline phosphatase (and possibly other extracellular enzymes, see below). This is consistent with the observations of Mather *et al.* (2008), who found widespread alkaline phosphatase activity in the North Atlantic. They concluded this activity was due to enhanced utilization of the DOP pool in response to high levels of nitrogen fixation, forcing the system towards P-limitation.

The third line of evidence is rapid turnover times above the thermocline (4 - 8 hours) that increases with depth to ~17 hours just below the thermocline, and up to 6 days at greater depth (Figure 4; Table 1 and Supplemental Table SI-1 in Supplemental Information). Phosphate uptake rates were also high with rates of over 150 nmol µg chl a^{-1} h^{-1} (Sohm and Capone 2006; Table 1)**.** These data are consistent with similar measurements in this region obtained at different times (Sohm and Capone 2010). Comparison of phosphate turnover times and nutrient addition bioassays in the P limited Mediterranean Sea suggest that turnover times of less than 7 hours indicate P-deficiency (Zohary and Roberts 1998). The near surface turnover times seen in this study are comparable to the values seen in the Mediterranean and suggest low phosphate availability compared to demand in the surface waters of the Sargasso Sea (Ammerman *et al.* 2003, vanMooy *et al.* 2009). These conditions are ideal for promoting the utilization of the DOP pool, consistent with the high surface ELF-expression and $\delta^{18}O_p$ values below equilibrium in surface waters that are indicative of the activity of extracellular enzymes.

DOP concentrations were not measured on all samples in this cruise; however, unpublished data at Station 2 (M. Lomas, Bermuda Atlantic Time Series Station), data from Sohm and Capone (2006, 2010) at these and many other stations in the area, as well as additional literature data, indicate that DOP values from this area are consistently highest in surface waters and decrease with depth (Wu *et al.* 2000a,b; Hopkinson and Vallino 2005; *Karl* 2009; vanMooy *et al.* 2009).

In general, in the open ocean sites within the thermocline there is a tendency for lower $\delta^{18}O_p$ values to coincide with lower DIP ($p < 0.001$), higher DOP concentrations ($p < 0.001$), faster turnover rates ($p = 0.004$) and the greatest amount of cells exhibiting ELF ($p = 0.072$; Table 1). This is consistent with the hypothesis that in the absence of sufficient inorganic orthophosphate, the biological communities utilize labile DOP.

Below the thermocline, the situation was reversed. The $\delta^{18}O_p$ values are close to equilibrium, DIP concentrations are higher, DOP concentrations are lower than in the surface, and turnover times are longer. These results indicate utilization of DOP is slower below the euphotic zone, though still present. This is also noted by Colman *et al.* (2005). They suggest that the observed trend at depth was due to the continued slow metabolism of sinking particulate and dissolved organic matter (POM and DOM respectively) by heterotrophic bacteria in the deep ocean likely using hydrolytic phosphoenzymes such as alkaline phosphatase to obtained C (or N) and not P for metabolic needs. Additional processes,

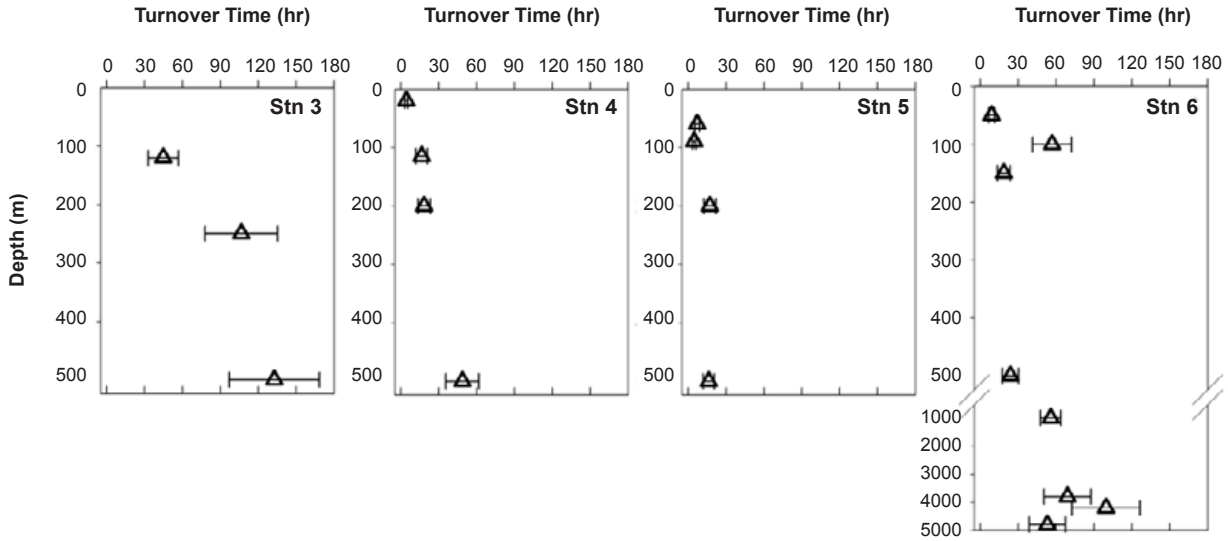

Figure 4. Depth profiles of turnover times for Stations 3 through 6.

including the contribution of preformed DIP derived from surface waters, and oxygen inheritance effects on POM/DOM formed in warmer surface waters but hydrolyzed at depth, also contribute to the observed trend. The $\delta^{18}O_p$ values of the deep water DIP pool may shed light on the relative contribution of different sources and cycling of DIP. Specifically, three processes can be considered: 1) remineralization of sinking particulate organic matter from the surface waters using extracellular enzymes that would most likely tend to shift $\delta^{18}O_p$ towards lower than equilibrium values; 2) DIP processed intracellularly by deep heterotrophic organisms and released to seawater, shifting $\delta^{18}O_p$ values towards equilibrium; and 3) preformed phosphate which will carry $\delta^{18}O_p$ signatures of the DIP pool at the site of deep water formation (e.g., high latitudes). Since deep ocean values approach the equilibrium isotopic composition, we can infer that extracellular remineralization rates (non-equilibrium processes) are less important compared to those in surface waters or to intracellular cycling at depth (which imparts an equilibrium isotopic signature). Our turnover time data indicates phosphate is consistently recycled even at depth (e.g., turnover time 50 - 100 hours; Table SI-1). However, it is recycled more slowly than in surface waters, indicating that the equilibrium values are at least in part achieved by intracellular P cycling.

We used an isotope mass balance model to estimate the fraction of phosphate derived from DOP remineralization based on the available data on fractionation associated with enzyme mediated DOP hydrolysis (Liang and Blake 2006, 2009) and the deviation from equilibrium of $\delta^{18}O_p$ observed in surface water. To obtain a representative estimate for the fraction of phosphate regenerated from DOP utilization we made several assumptions. First, the $\delta^{18}O_p$ of DOP is in isotopic equilibrium with ambient water; this is supported by the few available $\delta^{18}O_p$ data of living and sinking cells (Paytan *et al.* 2002) and the rapid and extensive turnover of P in cells. Second, all of the DOP is in the form of monoesters (monoesters are the most abundant P form of DOP but not the only form). Third, remineralization to DIP is via alkaline phosphatase (it is the most abundant but not the only enzyme in seawater). Based on these simplifying assumptions, during remineralization, one oxygen atom will be incorporated from the ambient water with a fractionation of -30‰ and the resulting $\delta^{18}O_p$ values can be calculated as:

$$\text{Remineralized } \delta^{18}O_p = (0.75 * \text{equilibrium } \delta^{18}O_p) + (0.25 * (\delta^{18}O_w - 30)) \quad \text{Eq. 1}$$

Thus, the measured $\delta^{18}O_p$ values will get lower as the contribution of remineralized DOP increases. We can calculate the fraction of phosphate regenerated from DOP utilization as follows:

$$\%\ \text{DOP utilized} = (\text{observed } \delta^{18}O_p \text{ values} - \text{equilibrium } \delta^{18}O_p \text{ values}) * 100 / (\text{calculated remineralized } \delta^{18}O_p \text{ values} - \text{equilibrium } \delta^{18}O_p \text{ values}) \quad \text{Eq. 2}$$

We realize that DOP is a chemically heterogeneous pool with many compounds (*Clark et al.* 1998, 1999; Kolowith *et al.* 2001; Young and Ingall 2009), and that different enzymes are involved in the regeneration of DOP (Dyhrman and Palenik 1999, Scanlan and Wilson 1999, Ilikchyan *et al.* 2009, vanMooy *et al.* 2009) and these may involve yet unknown isotopic fractionation. However, we feel the values calculated represent the "best estimate" because they are based on the most abundant DOP form (monoesters) and enzyme (alkaline phosphatase; Figure 5). Calculations using a similar approach with different combinations of substrates and enzymes were also made (Table SI-2) with fractionation factors described by Liang and Blake (2006, 2009). Diester RNA as substrate and the combination of phosphodiestrase and alkaline phosphatase will give values similar to the "best estimate" while using monoesters as substrate and the 5' nucleotidase enzyme or a diester RNA substrate and the combination of phosphodiestrase and 5' nucleotidase enzymes will result in a higher percent of DOP utilized (Table SI- 2 "high estimate" values). On the other hand, if all of the DOP is in the form of phosphodiester DNA, then lower fractions of DOP utilized are obtained with the minimum value calculated for the phosphodiester DNA substrate with combination of phosphodiestrase and alkaline phosphatase enzymes (Table 2 "low estimate" values).

Without precise knowledge of the molecular characterization of the DOP pool and all the active enzymes and the respective fractionation factors associated with each of the enzymes, it is impossible to calculate exact values for the DOP utilization rate. However, based on current knowledge and

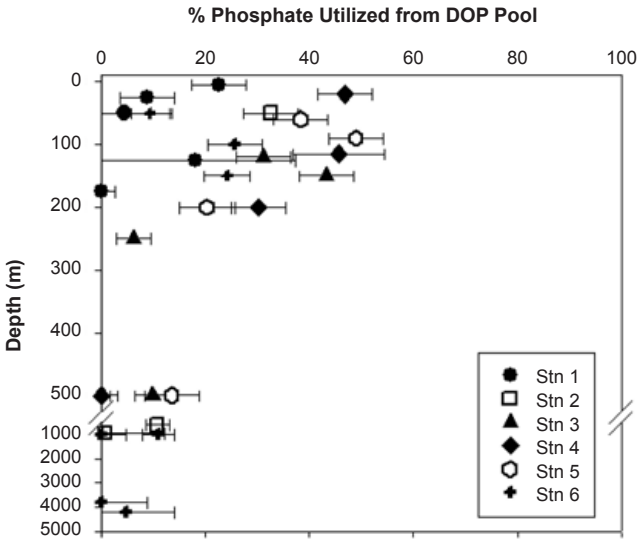

Figure 5. Percentage of phosphate in the water column that was regenerated from DOP utilization for all stations as a function of depth.

understanding of oceanographic processes, we feel that the estimated values are representative and further discussion is based on these values. The DIP fraction that is not from DOP regeneration in the surface waters is the fraction that is in equilibrium with seawater and is released from cells or expelled during cell lysis. In addition, some of the DIP could also be "new" DIP from external sources (upwelling, riverine or atmospheric deposition). In the bottom water, the DIP could also be preformed P.

The above calculations indicate that DOP utilization is greatest in the euphotic zone where photosynthesis occurs, coinciding with high ELF-expression, and most rapid phosphate turnover times (Table SI-1). DOP utilization and cycling can account for close to 50% of the observed DIP in the oligotrophic surface water column (e.g., Stations 4 and 5, using "best estimate" values), whereas near shore (Station 1) DOP remineralization typically accounts for less than 20% of DIP (Figure 4). This suggests that a large fraction of carbon sequestered by phytoplankton was supported by P from DOP and implies strong coupling between P uptake and regeneration processes in oligotrophic ocean surface waters. Importantly, we note that our calculation does not take into account any phosphate that originated from extracellular DOP hydrolysis but was then taken up by organisms, processed intercellularly, and released back to the water column with an equilibrium isotopic overprint. Therefore, our calculations represent the minimum fraction of the total phosphate that originated from DOP. The degree of deviation from equilibrium is related to the ratio of extracellular regeneration of phosphate from DOP to phosphate release from cells (lysis) after processing inside cells and achieving isotopic equilibrium. While existing data on the $\delta^{18}O_p$ of cellular phosphate is very small (Paytan *et al.* 2002), these results suggest that the signature is near equilibrium regardless of growth rate, temperature, algal species, or nutritional state. Thus, we conclude that low values of $\delta^{18}O_p$ deviating from equilibrium indicate extensive utilization of DOP and suggest P limitation. This does not necessarily imply physiological limitation (cellular stress) as the required P is obtained from DOP regeneration (an energetically costly process that involved synthesis of enzymes).

Net primary production in the Sargasso Sea has been estimated by various transient tracer distributions. These studies are consistent , averaging approximately 4.0 mol C m^{-2} $year^{-1}$ (McGillicuddy and Robinson 1997). Using the Redfield ratio of C:N:P = 106:16:1, this is equivalent to 0.038 mol P m^{-2} $year^{-1}$. Our $\delta^{18}O_p$ values indicate that the average percent DOP utilized in surface waters (<200 m) for all stations in the Sargasso Sea is 37 ±6 % (using the best estimate values), which corresponds to 1.5 mol C m^{-2} $year^{-1}$ (0.014 mol P m^{-2} $year^{-1}$) of the production. This translates to roughly 4 x 10^9 mol $year^{-1}$ C fixation supported by regenerated DOP utilization in surface waters of the Sargasso Sea.

Summary and Implications

Multiple lines of evidence suggest that the Sargasso Sea is deficient in DIP such that the biological communities utilize extracellular enzymes to access the DOP pool. This is consistent with an increasing body of data for this region. We also provide a quantitative estimate for the contribution of DOP to P uptake in the water column, which is consistent with recent model- based calculations (Lomas *et al.* 2010). This suggests the bioavailability of the DOP pool is critical for organisms in the euphotic zone and that the bulk of phosphate cycling in these waters is attributed to extracellular enzymatic regeneration. These enzymes are expressed by the biomass to combat the low DIP concentrations in the surface waters (consistent with rapid phosphate turnover times).

An implication of these results is that inorganic nitrogen (N) to P ratios (N:P) would not be a suitable

measure for nutrient limitation. Global assessments of nutrient limitation in oligotrophic systems should include estimates of bioavailable nutrients including those from the DOM pools (Downing 1997, Emerson *et al.* 2001, Klausmeier *et al.* 2004, Krom *et al.* 2004). In the absence of studies such as the one reported here, global estimates of maximum primary productivity based on nutrient availability would considerably underestimate actual productivity and carbon sequestration.

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Supplemental Information

Supplemental Information available at ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2011AnnualReport/ar11_SupplementalInfo_SargassoSeaP.pdf