Chapter 21
Tracing the Sources and Biogeochemical Cycling of Phosphorus in Aquatic Systems Using Isotopes of Oxygen in Phosphate

Adina Paytan and Karen McLaughlin

Abstract Phosphorous (P) is an essential nutrient for all living organisms and when available in surplus could cause eutrophication in aquatic systems. While P has only one stable isotope, P in most organic and inorganic P forms is strongly bonded to oxygen (O), which has three stable isotopes, providing a system to track phosphorus cycling and transformations using the stable isotopes of O in phosphate (PO_4), $\delta^{18}O_p$. This isotope system has only recently been utilized in aquatic environments. Available data obtained from different settings indicate that $\delta^{18}O_p$ of dissolved phosphate in aquatic systems can be applied successfully for identifying sources and cycling of phosphate in a broad range of environments. Specifically, work to date indicates that $\delta^{18}O_p$ is useful for deciphering sources of phosphate to aquatic systems if these sources have unique isotopic signatures and if phosphate cycling within the system is limited compared to input fluxes. In addition, because various processes are associated with distinct fractionation effects, the $\delta^{18}O_p$ tracer can be utilized to determine the degree of phosphorous cycling within the biomass and shed light on the processes imprinting the isotopic signatures. As a better understanding of the systematics of and various controls on $\delta^{18}O_p$ is gained, it is expected that $\delta^{18}O_p$ would be extensively applied in research geared to understand phosphorous dynamics in many environments.

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21.1 Introduction

Phosphorus (P, atomic number 15, relative atomic mass 30,9738) is a multivalent nonmetal element of the nitrogen group. Although 23 isotopes of phosphorus are known (all possibilities from ^{24}P up to ^{46}P), only ^{31}P is stable. Two radioactive isotopes of phosphorus have half-lives which make them useful for scientific experiments. ^{32}P has a half-life of 14.26 days and ^{33}P has a half-life of 25.34 days. Phosphorous compounds (organic and inorganic) are found with phosphorous oxidation states ranging from −3 to +5, however the most common oxidation states are +5, +3 and −3. Phosphorous abundance in earth's crust is 1,050 ppm by weight (730 ppm by moles) and the abundance in the solar system is 7 ppm by weight (300 ppb by moles) (Emsley 2000). Due to its high reactivity, phosphorus does not occur as a free element in nature, but it is found in many different minerals (e.g. apatite) and organic compounds (e.g. DNA, RNA, ATP, phospholipids) essential for all living cells. It is produced commercially from calcium phosphate (phosphate rock). Large deposits of phosphate rock are located in the Middle East, China, Russia, Morocco and the United States of America. Based on 2010 estimates, at the current rate of consumption, the supply of phosphorus is estimated to run out in about 300 years. Peak P consumption will occur in 30 years and reserves will be depleted in the next 50–100 years (Vaccari 2009).

Phosphorus, being an essential plant nutrient, is predominantly used as a constituent of fertilizers for agriculture. Phosphorus is also used as a precursor for various chemicals, in particular the herbicide glyphosate and to make organophosphorus compounds which have many applications, including in plasticizers,

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flame retardants, pesticides, extraction agents, and water treatment. It is an important component in steel production, utilized in the making of special glasses and fine china, a component in some laundry detergents, baking powder, matchbook strikers, flares, and for military use in incendiary bombs and grenades.

Phosphorus is a key element in all known forms of life. Inorganic phosphorus in the form of phosphate (PO_4^{3-}) plays a major role in biological molecules such as DNA and RNA where it forms part of the structural framework of these molecules. Living cells also use phosphate to transport cellular energy in the form of adenosine triphosphate (ATP). Nearly every cellular process that uses energy obtains it in the form of ATP. ATP is also important for phosphorylation, a key regulatory event in cells. Phospholipids are the main structural components of all cellular membranes and calcium phosphate salts assist in stiffening bones. Due to its biological role phosphorous is an essential macromineral (nutrient) for terrestrial plants and for marine phytoplankton, algae, and sea-grasses. In ecological terms, phosphorus is often a limiting nutrient in many environments; i.e. the availability of phosphorus governs the rate of growth of many organisms. Indeed, it has been suggested that phosphorous availability may limit primary productivity in some aquatic systems (Bothwell 1985; Hecky and Kilham 1988; Howarth 1988; Karl and Tien 1997; Karl et al. 2001; Krom et al. 1991; Wu et al. 2000), and may be co-limiting in others (Nicholson et al. 2006; Sundareshwar et al. 2003). However, at times an excess of phosphorus can be problematic causing eutrophication and algal blooms (Sharp 1991; Smith and Kalff 1983; Smith 1984).

Agricultural expansion over the next 50 years is expected to be accompanied by a 2.4- to 2.7-fold increase in nitrogen (N)- and phosphorus (P)-driven eutrophication of terrestrial, freshwater, and near-shore marine environments (Tilman et al. 2001). Much of the P from fertilizer and animal waste enters surface waters and eventually also groundwater (Carpenter et al. 1998) and these nutrient loads can stimulate large scale macroalgal and/or phytoplankton blooms in receiving waters (Beman et al. 2005; Rabalais et al. 2002). Phosphorus enrichment in aquatic systems can cause diverse problems such as harmful algal blooms, anoxia, fish kills, and loss of habitat and biodiversity (Carpenter et al. 1998; Tilman et al. 2001). Thus, identifying and understanding phosphorous input and cycling and the effects phosphorous limitation or enrichment may have on aquatic ecosystems are of critical importance to management and restoration efforts.

Phosphorous is continuously and rapidly cycled in aquatic environments. Figures 21.1 and 21.2 represent the global biogeochemical cycle of P in the ocean and lakes, respectively and Fig. 21.3 illustrates the various pools and processes involved in the P cycle in aquatic systems.

21.1.1 Stable Isotope Use to Study P Sources and Cycling

Monitoring P sources and transformations in natural environments using stable isotopes has been difficult to do because, in contrast to C, N, O and S, P has only one stable isotope (^{31}P) thus the use of P stable isotope tracing is not an option. Although radioactive P isotopes (^{32}P, ^{33}P) can and have been used for investigation of P transformations in aquatic systems (Benitez-Nelson and Buessler 1998, 1999; Benitez-Nelson and Karl 2002; Lal et al. 1988; Lal and Lee 1988; Lee et al. 1991) there are many complications involved with this procedure. The use of natural stable isotope signatures has advantages as this approach does not perturb the system (e.g. by adding phosphate) and integrates processes over longer time scales. While P has only one stable isotope, P in most organic and inorganic P forms is strongly bonded to oxygen (O), which has three stable isotopes, providing a system to track phosphorus cycling and transformations using the stable isotopes of O in phosphate ($\delta^{18}O_p$).

Since the pioneering study of Longinelli and Nuti (1973) and several subsequent publications (Fricke et al. 1998; Longinelli et al. 1976; Longinelli 1984; Luz et al. 1984; Luz and Kolodny 1985; Shemesh et al. 1983, 1988), oxygen isotope ratios of bioapatite in teeth and bones have been widely used as paleoenvironmental proxies. The oxygen isotope paleothermometer is based on an empirical equation that is assumed to represent equilibrium fractionations between phosphate and water as a function of temperature as follows (Longinelli and Nuti 1973):

$$T\ (^{\circ}C) = 111.4 - 4.3(\delta^{18}O_P - \delta^{18}O_w) \qquad (21.1)$$

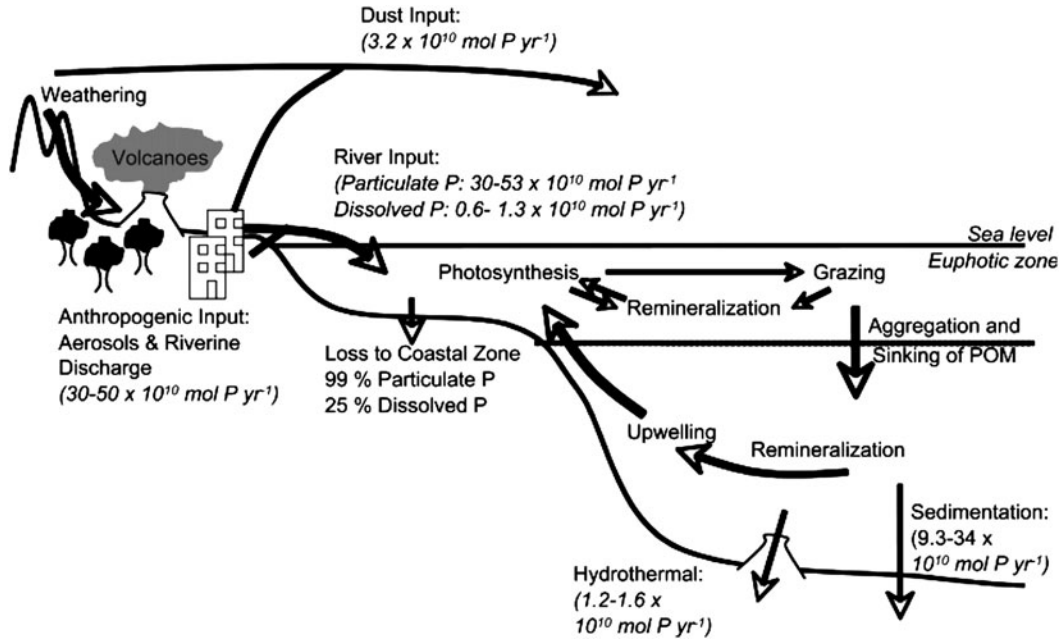

Fig. 21.1 The marine phosphorus cycle. Fluxes are given in italics. Flux data are from Benitez-Nelson (2000) and Follmi (1995). Continental weathering is the primary source of phosphorus to the oceanic phosphorus cycle. Most of this phosphorus is delivered via rivers with a smaller portion delivered via dust deposition. In recent times, anthropogenic sources of phosphorus have become a large fraction of the phosphorus delivered to the marine environment, effectively doubling the pre-anthropogenic flux. The primary sink for phosphorus in the marine environment is loss to the sediments. Much of the particulate flux from rivers is lost to sediments on the continental shelves, and a smaller portion is lost to deep-sea sediments. Hydrothermal systems constitute an additional small sink for P. Figure modified from Paytan and McLaughlin (2007)

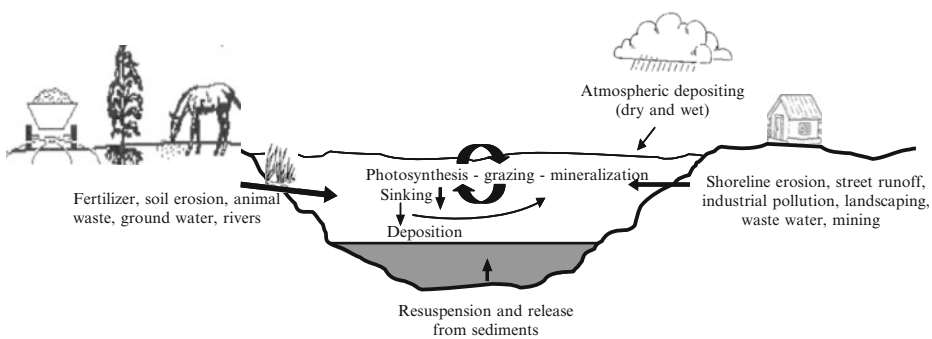

Fig. 21.2 Phosphate sources to lakes include fertilizers, animal waste, soil erosion, industrial and mining waste water input as well as atmospheric deposition. Phosphate enters lakes through rivers, groundwater, direct disposal and runoff. Plants and algae utilize the phosphate as a nutrient. Phosphate is transferred through the food web and some of this particulate matter is remineralized in the water column. Some phosphate is deposited in the sediment. Under anoxic conditions phosphate from the sediments may be recycled back into the water

where $\delta^{18}O_P$ and $\delta^{18}O_W$ are the oxygen isotopic composition of phosphate and water, respectively, in equilibrium with environmental temperature T (°C). Importantly, at most earth-surface temperatures (<80°C) and pressures, the P-O bond is resistant to inorganic hydrolysis and does not exchange O without biological mediation (Blake et al. 1997; Lecuyer et al. 1996) preserving the signature of the temperature and water isotope ratio of the solution from which the minerals precipitated.

However, due to the large sample size required for isotope analysis and the low concentrations of

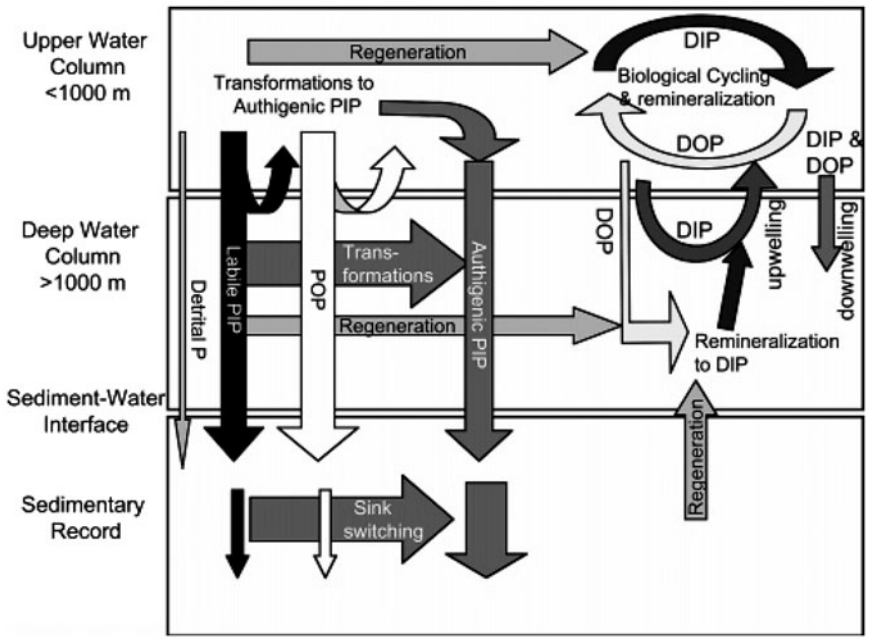

Fig. 21.3 Transformations between P pools in the water column and sediments. Abbreviations are as follows: PIP, particulate inorganic phosphorus; POP, particulate organic phosphorus; DIP, dissolved inorgranic phosphorus; DOP, dissolved organic phosphorus. Particulate phosphorus forms can undergo transformations throughout the water column and within sediments. Particulate phosphorus forms may also undergo regeneration into dissolved forms. Particulate phosphorus is lost from surface waters via sinking. Biological cycling and remineralization are the primary mechanisms of tranformations of the dissolved phases and are dominant in surface waters, though microbial remineralization continues at depth. Dissolved phosphorus forms are lost from surface waters via downwelling and biological uptake (into POP) and are returned to surface waters via upwelling and mixing. Regeneration form sediment can add more dissolved phosphate to deep water. Figure modified from Paytan and McLaughlin (2007)

dissolved phosphate in most water bodies, the oxygen isotopic composition of phosphate, $\delta^{18}O_p$, has only recently been applied systematically for tracking dissolved phosphate in water bodies. Pioneering work by Longinelli et al. (1976) found no variation in the $\delta^{18}O_p$ of dissolved phosphate in seawater with either depth or latitude in the Atlantic and Pacific Oceans, although there was a significant difference between the two ocean basins. The $\delta^{18}O_p$ values were thought to reflect kinetic–biological isotopic fractionation. Longinelli et al. (1976) extracted and concentrated P from large volumes of water without pre-filtration using iron-coated fibers that absorb inorganic and organic P indiscriminately. Analysis of mixed organic and inorganic P samples may have confounded interpretation of the results (Blake et al. 2005) and because of the analytical limitations few attempts to follow up on this work have been made for over a decade (Paytan 1989). These complications have been overcome with current technologies, several detailed protocols for isolating, purifying and precipitating small quantities of phosphate from complex matrix solutions such as fresh and ocean waters were published and this system has now been applied to various water bodies including oceans (Colman et al. 2005; McLaughlin et al. 2006b, 2011), estuaries (McLaughlin et al. 2006a, d) and lakes (Elsbury et al. 2009; Markel et al. 1994). In addition, extensive and innovative laboratory studies have been conducted to carefully determine the fractionation associated with various biogenic and abiotic transformations of P (Blake et al. 1997, 1998, 2001, 2005; Liang 2005; Liang and Blake 2006a, b, 2007, 2009).

21.1.2 Isotopic Signatures of Potential Phosphate Sources to Aquatic Systems

Identifying point and non-point nutrient sources is important for understanding ecosystem health, and

has implications for designing best management practices, industry regulation and allocation of water discharge permits. P sources can be separated into point sources, such as sewage and industrial discharge sites, and non-point sources like urban and agricultural run-off (Young et al. 2009). Phosphate oxygen isotope tracer studies in natural environments are limited. However, recent field studies have demonstrated the utility of $\delta^{18}O_p$ as a tracer of various phosphate sources to lakes, rivers, estuaries and the coastal ocean (Coleman 2002; McLaughlin et al. 2006b, d). A wide range of $\delta^{18}O_p$ values from 6 to 27‰ has been documented in these various studies (Fig. 21.4a). A significant portion of these samples are not in isotopic equilibrium with the surrounding water, indicating that complete intracellular biological cycling of the orthophosphate had not taken place, and a source signature may have been partially retained. In addition, the $\delta^{18}O_p$ of some potential end-member sources (wastewater treatment plant effluent, fertilizers, soaps,

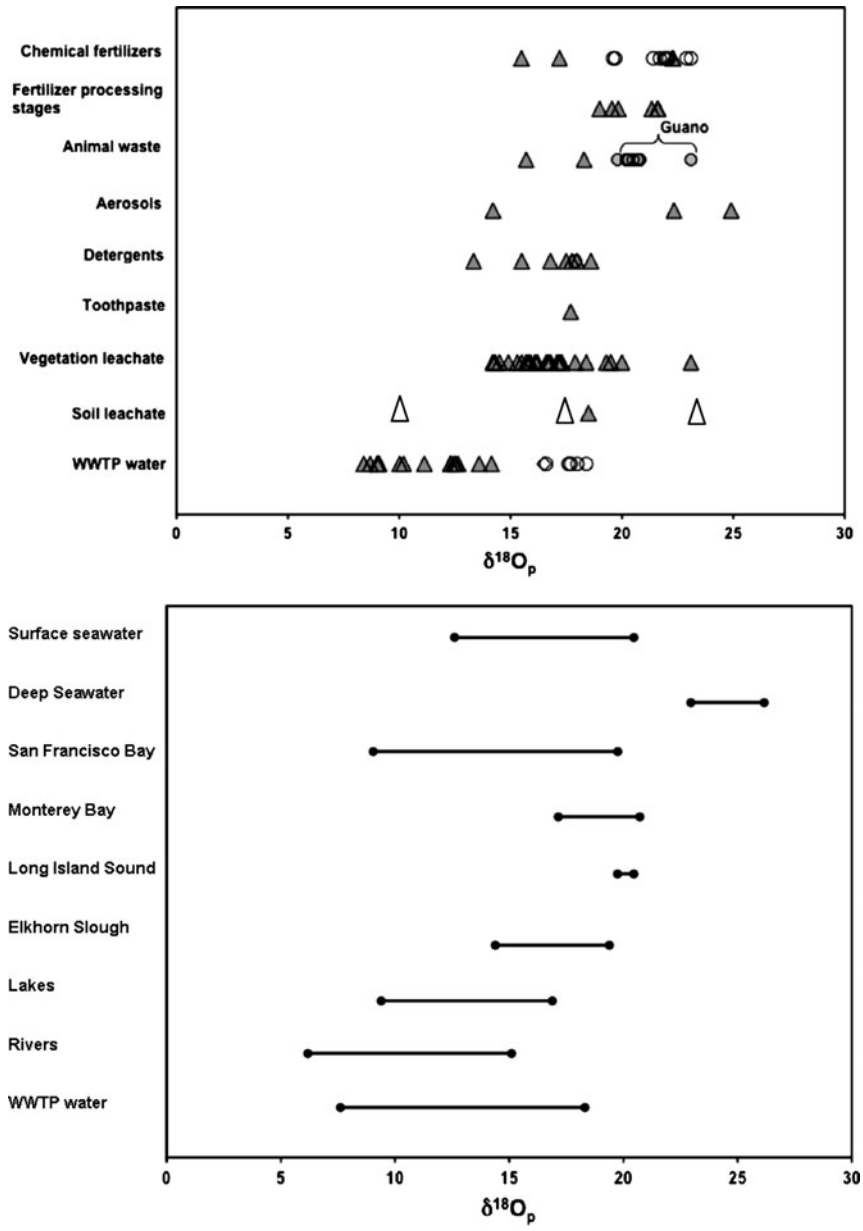

Fig. 21.4 (**a**) $\delta^{18}O_p$ of some potential end-member sources. *Full triangles* from Young et al. (2009); *Open circles* from Gruau et al. (2005); *Full circles* from Avliffe et al. (1992); *Diamonds* from Coleman (2002); *Empty triangles* from Zohar et al. (2010a, b). Figure modified from Young et al. (2009). (**b**) Range of $\delta^{18}O_p$ values observed in different water systems

soil extracts, etc.) has been published (Young et al. 2009) (Fig. 21.4b).

A considerable range of $\delta^{18}O_p$ values has been measured in various P sources and the differences observed among sources are much larger than the analytical precision (±0.3‰) associated with this technique. Although there is considerable overlap in $\delta^{18}O_p$ measured in the various groups of samples, these results indicate that in specific geographic regions, different P source types may span a narrower range and have distinct signatures, and in these cases, the $\delta^{18}O_p$ could be useful for identifying the contribution of the different sources. For example, while the entire range of reported $\delta^{18}O_p$ values for worldwide wastewater treatment plant effluent overlaps with the values measured for multiple types of detergents, organic fertilizers, and chemical fertilizers, all measured $\delta^{18}O_p$ values for the Palo Alto Regional Water Quality Control Plant are significantly lower than any of the measured fertilizers and detergents (Young et al. 2009). Thus, if phosphate is not heavily cycled within an ecosystem such that the source signature is reset, $\delta^{18}O_p$ can be used to identify isotopically distinct phosphate sources and/or the extent of phosphate cycling in aquatic systems (i.e. the deviation from the isotopic composition of the source towards the expected equilibrium value).

21.1.3 Isotope Fractionations Involved in P Cycling

Isotope fractionations associated with several of the important reactions and transformations operating in the P cycle have been determined in controlled laboratory experiments (Table 21.1). This information provides the basis for interpretation of isotope data ($\delta^{18}O_p$) obtained from phosphate in the natural environment. In the absence of biological activity at ambient temperatures, pH, and pressure, isotope exchange between phosphate oxygen and water (or other solutions) is slow and can be considered negligible for the time scales of concern of most environmental applications (Blake et al. 1997; Longinelli and Nuti 1973, Longinelli et al. 1976; O'Neil et al. 2003). Studies of precipitation

Table 21.1 Isotope fractionation effects associated with various biogeochemical processes

Process	Fractionation (Δ or ε)	Reference
Precipitation/dissolution of P minerals (apatite)	+0.7‰ to +1‰ Heavy isotope in mineral phase	Blake et al. (1997)
Adsorption/desorption of P to/from mineral surfaces	~+1‰ Heavy isotope in mineral phase	Liang and Blake (2007)
Precipitation with sesquioxides and hydroxides	~+1‰ Heavy isotope in mineral phase	Jaisi et al. (2009)
Abiotic hydrolysis of polyphosphate (O:P = 3.33), pyrophosphate (O:P = 3.5), phosphonates (O:P = 3.0), monoesters (O:P = 3.0) and diesters (O:P = 2.0)	No fractionation or temperature effect, however incorporation of oxygen from water during formation of PO_4 (O:P = 4) occurs	McLaughlin et al. (2006a)
Transport by water or air	No fractionation or temperature effect	Longinelli (1965)
Assimilation by phytoplankton	Light isotopes preferentially utilized, enrichment of the residual solution (ε = −3‰)	Blake et al. (2005)
Intracellular processing such as inorganic pyrophosphatase (PPase) catalysis	Equilibrium isotopic exchange T and $\delta^{18}O_w$ impact (21.2)	Blake et al. (2005)
Alkaline phosphatase (APase) hydrolization of phosphomonoesterase (extracellular)	Kinetic isotope effects ε = −30‰ effecting only the newly incorporated oxygen	Liang and Blake (2006a, b)
5′-nucleotidase hydrolization (extracellular)	Kinetic isotope effects ε = −10‰ effecting only the newly incorporated oxygen	Liang and Blake (2006a, b)
First step of DNAse hydrolization	Kinetic isotope effects ε = −20‰ effecting only the newly incorporated oxygen	Liang and Blake (2009)
First step of RNAse hydrolization	Kinetic isotope effects ε = +20‰ effecting only the newly incorporated oxygen	Liang and Blake (2009)
Transport from roots to leaves (by transporters)	Enrichment in the process foliage heavier than roots	

and dissolution of various P bearing minerals and studies of P adsorption and desorption onto/from mineral surfaces indicate that the fractionation associated with these processes (given equilibration time of more than a few hours) is small – in the range of 1‰ (Jaisi et al. 2009; Liang 2005; Liang and Blake 2006b). Typically the heavier isotopes in these reactions are associated with the mineral phase while the solution retains phosphate with lighter isotopes. Precipitation or dissolution of apatite minerals (inorganically) will be accompanied by a small oxygen isotope fractionation in the range of +0.7 ‰ to +1‰ (Blake et al. 1997). Similarly, adsorption or precipitation with sesquioxides and hydroxides imprints a small positive isotope effect (Jaisi et al. 2009). In contrast, enzyme mediated biological activity could break the P-O bond in processes that involve large isotopic fractionation. Intracellular as well as extracellular enzymes are expressed by various organisms for the uptake and utilization of P and may play a role in determining the oxygen isotopic composition of phosphate in aquatic systems. Different enzymatic processes induce different isotopic fractionations (Table 21.1). The most dominant enzymatic process controlling $\delta^{18}O_p$ in the environment is the intracellular activity of pyrophosphatase (PPase) (Blake et al. 2005), which involves equilibrium isotopic exchange. Blake et al. (2005) found that this enzymatic activity results in isotopic equilibrium of oxygen in phosphate similar to that described by Longinelli and Nuti (1973). The equation for phosphate extracted from microbial cultures was described by Blake et al. (1997):

$$T(^{\circ}C) = 155.8 - -6.4(\delta^{18}O_p - \delta^{18}O_w) \quad (21.2)$$

These equilibrium relations have been observed in tissues of a variety of organisms, including fish, mammals (Kolodny et al. 1983), bacteria and algae (Blake et al. 1997, 2005; Paytan et al. 2002). Results of an algae culture experiment indicate that intracellular oxygen isotope exchange between phosphorus compounds and water within cells is very rapid (Paytan et al. 2002). These processes are expected to occur in all organisms and phosphate released from cells to the environment will carry this equilibrium signature and impact dissolved phosphate $\delta^{18}O_p$ values leading to equilibrium values. Extracellular remineralization and hydrolization of organic P (P_o) compounds by phosphohydrolase enzymes such as alkaline phosphatase (APase) and 5′-nucleotidase, involves incorporation of one or more oxygen atoms from the ambient water with an isotope fractionation of −30 and −10‰, respectively (Liang and Blake 2006b). A summary of published fractionation values to date is given in Table 21.1. The resulting phosphate from such processes will reflect the fractionation and would typically shift $\delta^{18}O_p$ towards values that are lower than equilibrium. Work by several groups is currently ongoing to determine the isotope fractionation associated with additional enzymes, and will enable better interpretation of field data. Uptake and utilization (assimilation) of phosphate by aquatic plants, algae, and microorganisms is also associated with isotope fractionation. The phosphate with lighter isotopes is preferentially utilized, a process that could enrich the residual solution with phosphate that has heavy isotopes (Blake et al. 2005).

The isotopic composition of dissolved phosphate and particularly the degree of isotope equilibrium or deviation from equilibrium of phosphate in various aquatic systems has been used for deciphering the extent of biological utilization and turnover of phosphate in aquatic systems (Colman et al. 2005; Elsbury et al. 2009; McLaughlin et al. 2006b, d, 2011). This application is based on the assumption that extensive recycling and turnover will lead to isotopic equilibrium while deviation from equilibrium may reflect source signatures or other processes that do not result in isotopic equilibrium such as expression of extracellular enzymes or phosphate uptake (Fig. 21.5). The following sections will describe the methodology (sample preparation and analysis), give examples of application of this system in various settings and address the needs for future progress in this field.

21.2 Materials and Methods

For analysis of $\delta^{18}O_P$ by isotope ratio mass spectrometry (IRMS), it is necessary to convert the phosphate into a pure solid phase without isotopic alteration. The purification steps are of great importance, since the presence of oxygen sources other than phosphate compromises the results (Weidemann-Bidlack et al. 2008). The final compound analyzed should be non-hygroscopic, stable under laboratory conditions, and should decompose to form carbon monoxide (CO) at temperatures attainable in a lab furnace. Silver phosphate (Ag_3PO_4) has been proven a convenient phase for this purpose

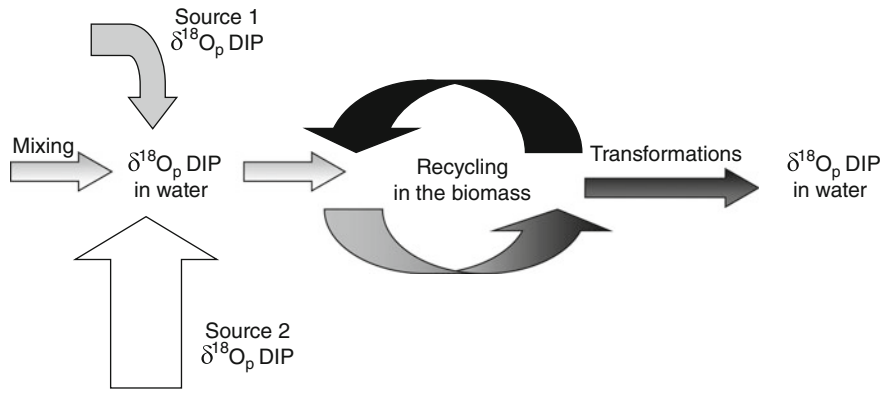

Fig. 21.5 A graphical representation of P mixing and cycling in the water illustrating the utility of $\delta^{18}O_p$ for identifying sources if biological transformations do not erase the source signatures or the degree of intracellular biological cycling and turnover by determining the difference between the source signature and expected equilibrium values (see McLaughlin et al. 2006a, b, c, d)

(Firsching 1961; O'Neil et al. 1994) and has gradually substituted the earlier hazardous fluorination technique (Kolodny et al. 1983; Tudge 1960). Ag_3PO_4 is reduced with carbon in an oxygen free atmosphere at high temperature (>1,300°C) using a thermal combustion elemental analyzer (TCEA) to yield carbon monoxide for analysis by IRMS (Coleman 2002, McLaughlin et al. 2004, O'Neil et al. 1994). The TCEA and mass spectrometer are linked via a continuous flow interface, and the CO gas is measured instantaneously after formation (Kornexl et al. 1999). Prior to mass spectrometric analysis phosphate has to be concentrated (phosphate concentrations are low in many environments), isolated, purified, and precipitated as Ag_3PO_4.

Several detailed protocols for isolating, purifying and precipitating small quantities of phosphate from complex matrix solutions such as fresh and ocean waters have been published (Coleman 2002; Goldhammer et al. 2011; Jaisi and Blake 2010, McLaughlin et al. 2004; Tamburini et al. 2010, Weidemann-Bidlack et al. 2008; Zohar et al. 2010a). Most of these procedures involve a concentration step to collect sufficient amounts of phosphate and remove some of the dissolved organic phosphate and interfering ions from the sample. This is done through a series of precipitations and/or resin treatments followed by a final precipitation as Ag_3PO_4 (Table 21.2). It is important to ensure that the concentration and preparation process does not introduce any isotopic fractionation and all of the above methods report that authentic signatures are preserved. Problems with the final precipitation of silver phosphate have been experienced when working with water samples containing very high concentrations of dissolved organic matter. Several promising approaches for addressing this problem have been explored, including UV radiation of the sample (Liang and Blake 2006b), passing the sample through phosphate-free activated carbon (Gruau et al. 2005), using resins such as DAX-8 to remove organics (Tamburini et al. 2010), precipitation of humic acids (Zohar et al. 2010a) and treatment with H_2O_2 (Goldhammer et al. 2011; Zohar et al. 2010a). Published procedures report that these methods to remove organic matter retain the original isotopic signature of phosphate.

As mentioned above it is very important that the only source of oxygen analyzed (as Ag_3PO_4) originates from the "authentic" phosphate in the sample. There are however two separate processes that may compromise this requirement. If not all of the organic matter is removed or if other minerals that contain oxygen (such as $AgNO_3$) precipitate along with the Ag_3PO_4 (e.g. the Ag_3PO_4 is not pure), then the oxygen contributing to the CO gas will not reflect that of phosphate. Data has to be monitored to ensure that this does not occur. This is done by monitoring the oxygen yield (peak area compared to the pure silver phosphate standards) expected based on the weight of the Ag_3PO_4 sample. The oxygen content per unit weight of Ag_3PO_4 is 15.3% and samples which deviate from this value particularly towards higher oxygen yield should be suspected of contamination. Plotting the oxygen yield (or peak area) of analyzed pure Ag_3PO_4 standards along with the samples should yield a linear relation with weight (Fig. 21.6). It is also advised to include a step to remove tightly sorbed

Table 21.2 Published procedures for the concentration and purification of phosphate from water samples and the precipitation of Ag_3PO_4 for analysis of $\delta^{18}O_p$

McLaughlin et al. (2004, 2006a, b, c, d); (Elsbury et al. (2009); (Young et al. 2009)	Colman (2002); Colman et al. (2005); Goldhammer et al. (2011)	Tamburini et al. (2010); Tudge (1960); Kolodny et al. (1983); Paytan et al. (2002); Liang and Blake (2007)
Magnesium-induced coprecipitation (MagIC, Karl and Tien 1992)	Magnesium-induced coprecipitation (MagIC, Karl and Tien 1992)	Magnesium-induced coprecipitation (MagIC, Karl and Tien 1992)
Dissolution in acetic and nitric acids and buffering at pH 5.5 with 1M potassium acetate	Dissolution in 0.1 M HNO_3	Dissolution in 1 M HCl
Precipitation as cerium phosphate	Anion removal (AG1X8) in $NaHCO_3$ form	Precipitation as ammonium phosphomolybdate
Rinses to remove chloride	HCO_3 removal in acid	Dissolution in citric-acid NH_4OH
Dissolution in 0.2 M nitric acid	Cation removal (AG50X8)	Precipitation of magnesium ammonium phosphate
Cation removal (AG50X8)	Volume reduction by evaporation at 60°C	Rinse and dissolve in 0.5 M HNO_3
Ag_3PO_4 fast precipitation	Ag_3PO_4 slow micro precipitation in P:Ag:NO_3:NH_4OH molar ratios of 1:10:30:75	Cation removal (AG50X8)
		Ag_3PO_4 slow precipitation in P:Ag:NO_3:NH_4OH molar ratios of 1:100:300:750

Note that in water rich in dissolved organic matter (DOM) a step to remove DOM either from the water before the MagIC step or right after dissolution of the magnesium hydroxide is needed. This could be done be repeat MagIC co-precipitation (Goldhammer et al. 2011), DAX-8 Amberlite resin (Tamburini et al. 2010), activated char (Gruau et al. 2005), or precipitation (Zohar et al. 2010a, b)

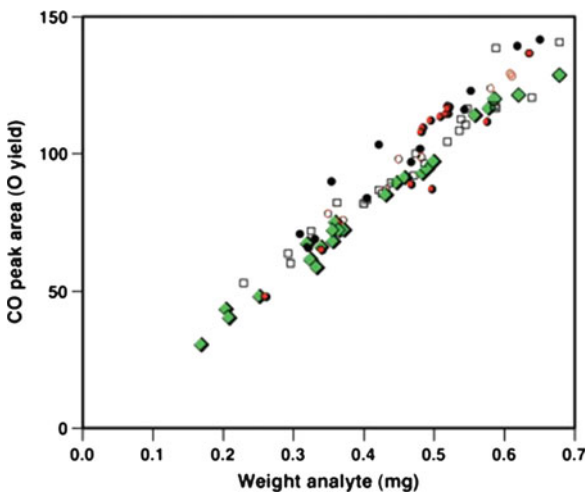

Fig. 21.6 CO peak area for silver phosphate standards (*green diamonds*) and various samples (*other symbols*) relative to sample weight introduced into the mass spectrometer. The expected oxygen yield from pure silver phosphate is 15.3%. If samples fall off the line defined by the standards the sample is likely contaminated by an external source of oxygen and might not represent the oxygen isotope ratios in phosphate. Figure modified from Tamburini et al. (2010)

water molecules from silver phosphate. This can be done by heating the silver phosphate samples to ~ 450°C to get strongly adsorbed water off.

Another potential process by which data could be compromised is contribution of phosphate which is hydrolyzed from condensed forms or organic forms of phosphate for which the O:P ratio is less than 4 during sample processing (this is an analytical artifact) (McLaughlin et al. 2006c). Using ^{18}O-labled and unlabeled reagents on replicates of the same sample these artifacts could be monitored and corrected. If hydrolysis takes place, oxygen from the acid solution is incorporated into the phosphate group, and because the phosphate in the labeled acid solution will have a higher isotope value than phosphate in the unlabeled solution it could be tracked (McLaughlin et al. 2006c). In this case, the use of a simple equation allows the correction and determination of the isotope value of the extracted phosphate (McLaughlin et al. 2006c).

While all of the concentration, purification, separation and precipitation methods published (Table 21.2) were tested for this potential artifact and report that any

impact, if exists, is below analytical error, it is important to note that because of the vast array of organic P compounds in nature and the huge variability in their concentration and relative abundance in different environmental samples each new set/type of samples should be tested to ensure that such artifacts do not compromise the data.

For mass spectrometric analysis about 200–600 μg of Ag_3PO_4 should be weighed into silver capsules. Some laboratories also add a small amount of finely powdered glassy carbon or nickel-carbide, to improve the reaction between the silver phosphate and carbon during pyrolysis. The samples are introduced into the TCEA via a zero blank autosampler. The TCEA furnace is kept at a constant and consistent temperature (1,375 and 1,450°C have been used). The furnace itself consists of a ceramic tube filled with glassy carbon chips encased in a glassy carbon tube. The produced reaction gases are carried by constantly flushing with a high purity helium stream through a GC column held at fixed temperature (e.g. 80°C) to purify the sample from trace contaminants. The gas is admitted to the mass-spectrometer via a Conflow interface. Some systems also include a copper tube which removes oxygen from the helium carrier gas. The ion currents of masses *m*/*z* 28, 29 and 30 are registered on the Faraday cups and converted to $\delta^{18}O$ values relative to a carbon monoxide standard gas for which $\delta^{18}O$ has been calculated relative to SMOW. Each sample is run for 300 s with a CO reference peak preceding the sample peak.

Calibration and corrections for instrumental drifts are accomplished by repeated measurements of internal standards. The standard deviation of the analysis based on repeated measures of the standards is typically less than ±0.4‰. In order to capture instrumental drift with time, delta value linearity, and sample size variability, working standards with known $\delta^{18}O$ values are weighed out in a range of sizes and analyzed along with the samples during each run (for example at ten sample increments). Raw $\delta^{18}O$ values are then corrected to the range of standards for drift and off set of the delta values and sample-size linearity.

The oxygen isotopic composition of phosphate is reported in standard delta notation ($\delta^{18}O$), which is calculated using the following equation:

$$\delta^{18}O = \left[\frac{R_{sample}}{R_{VSMOW}} - 1\right] \times 1,000 \qquad (21.3)$$

where R_{sample} is the ratio of $^{18}O/^{16}O$ in a sample and R_{VSMOW} is the ratio of $^{18}O/^{16}O$ in the isotopic standard for O, Vienna Standard Mean Ocean Water (VSMOW).

Currently there are no certified international Ag_3PO_4 standards and various laboratories use different "home-made" internal standards for which the $\delta^{18}O_p$ has been determined via fluorination (McLaughlin et al. 2004; Vennemann et al. 2002).

21.3 Applications

The use of $\delta^{18}O_p$ of dissolved inorganic phosphate (DIP) to study phosphate sources and cycling is relatively new and it is not yet widely used. In the past decade it has been applied in a variety of aquatic systems including estuaries, coastal water, lakes, rivers, and the open ocean. A brief summary of representative examples is given below. These examples demonstrate the great utility of this system and it is likely that now that the methodology has been worked out extensive application of this tool will take place.

21.3.1 Use of $\delta^{18}O_p$ as a Tracer for Phosphate Sources is Estuaries

In a study of North San Francisco Bay, McLaughlin et al. (2006d) used $\delta^{18}O_p$ to assess mixing of dissolved inorganic phosphate (DIP) sources along an estuarine flow path. Due to different sources of phosphate, temperatures, and $\delta^{18}O_w$ the $\delta^{18}O_p$ signatures of oceanic and riverine phosphate sources are distinct. Based on salinity and $\delta^{18}O_w$, waters in the North San Francisco Bay can be described as a two end-member mixing system between Pacific Ocean waters and the freshwaters of the San Joaquin and Sacramento Rivers (Ingram et al. 1996; McLaughlin et al. 2006d). This mixing can be used to calculate an expected mixing line for $\delta^{18}O_p$. Such a trend will be observed if phosphate in the Bay is not being cycled extensively through the biomass or affected by processes that may alter the source $\delta^{18}O_p$ signatures. Deviations from the mixing-line are observed and attributed to contribution of phosphate with unique $\delta^{18}O_p$ signatures at various locations along the estuary (from point

and non-point sources) such as the discharge points of tributaries or wastewater treatment plants inputs.

The general lack of isotopic equilibrium in DIP throughout the Bay indicates that phosphate cycling is not rapid compared to phosphate input (low utilization rate, short residence time), and that source $\delta^{18}O_p$ contributed to the observed signature at most, if not all, stations. The deviations from the $\delta^{18}O_p$ mixing model have been interpreted to represent inputs of phosphate from local point sources within the North Bay (e.g. at the Napa River confluence) (Fig. 21.7).

At another estuary, Elkhorn Slough (McLaughlin et al. 2006a), the phosphate $\delta^{18}O_p$ within the main channel also indicates variability in phosphate sources throughout the channel, which are related to the surrounding land use. Trends in $\delta^{18}O_p$ show high values near the mouth reflecting phosphate of an oceanic origin, a minimum value near Hummingbird Island in the central slough reflecting phosphate input from groundwater, and high values near the head of the slough reflecting fertilizer input. A clear change in the relative contribution of these sources is observed and linked to water mixing during changing tidal conations at the mouth of the Slough.

In these studies, McLaughlin et al. (2006a, d) demonstrated that it is possible to use $\delta^{18}O_p$ to identify point and non-point source phosphate inputs to aquatic systems and suggest that this may be applied in other impacted systems to identify specific anthropogenic sources, such as fertilizer and sewage phosphate, or to trace natural sources of phosphate. This information is crucial for mitigation of pollution impacts and successful restoration of estuaries and other aquatic systems.

21.3.2 Phosphate Sources and Cycling in Lakes

Phosphorous loading in freshwater lakes has been identified as one of the leading causes for eutrophication and thus linked to hypoxia, harmful algal blooms and other adverse impacts (Schlesinger 1991; Sharp 1991). Despite the clear and wide spread impacts of phosphate loading, relatively few studies have used $\delta^{18}O_p$ to track sources and learn about P cycling in lakes. A study by Markel et al. (1994) focuses on sediments and suspended matter in Lake Kinneret, Israel. The isotope data show that about 70% of the particulate phosphate come to the lake from a basaltic source ($\delta^{18}O_p = 6‰$) with the balance being of sedimentary/anthropogenic origin ($\delta^{18}O_p = 18–25‰$).

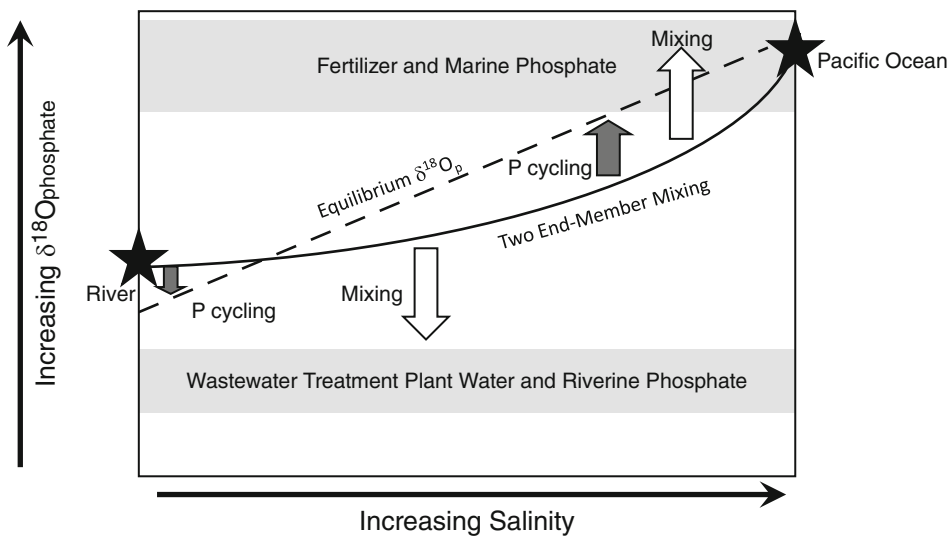

Fig. 21.7 Diagram indicating two end-member mixing (*black line*) and the expected equilibrium line (*dashed line*). Values below both the two end-member and the equilibrium line (*white* down facing *arrows*) indicate mixing with either riverine or wastewater treatment plant effluent. Deviations which move off the two end-member mixing line in the direction of equilibrium line be indicative of phosphate cycling, though they may also represent mixing with fertilizer phosphate. Deviations which fall off the two-end member mixing line in the direction of equilibrium, but in excess of equilibrium are indicative of mixing with fertilizer phosphate or treatment plant effluent depending on location along the salinity gradient

This study also alludes to some internal cycling (precipitation and dissolution) of phosphate in the lake. A study by Elsbury et al. (2009) records the distribution of $\delta^{18}O_p$ in water samples from the western and central basins of Lake Erie along with several potential sources (rivers, waste water treatment plants, atmospheric deposition). $\delta^{18}O_p$ of lake water is largely out of equilibrium with ambient conditions, indicating that source signatures may be discerned. $\delta^{18}O_p$ values in the lake range from +10 to +17‰, whereas the equilibrium value is expected to be around +14‰ and riverine weighted average $\delta^{18}O_p$ value is +11‰ (Fig. 21.8). Therefore, they conclude that some of the lake $\delta^{18}O_p$ values could not be explained by any known source or process. This indicates that there must be one or more as yet uncharacterized source(s) of phosphate with a high $\delta^{18}O_p$ value. In this study the authors speculate that a likely source may be the release of phosphate from sediments under reducing conditions that are created during anoxic events in the hypolimnion of the central basin of Lake Erie.

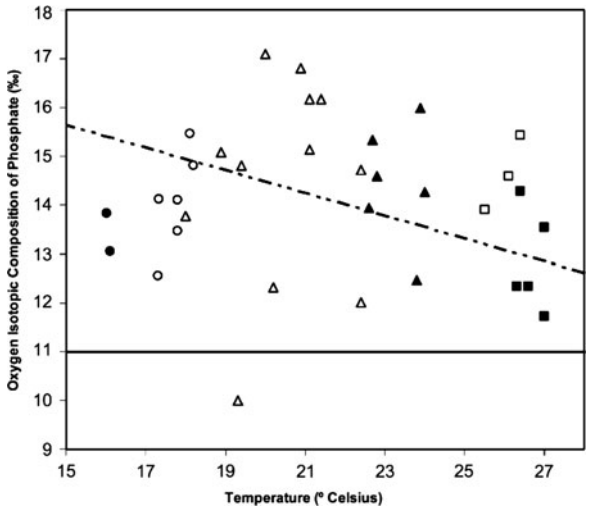

Fig. 21.8 Oxygen isotopic composition of phosphate (δ^{18}Op) of Lake Erie surface water. Symbols refer to different sampling trips. The line at +11‰ represents the weighted average riverine δ^{18}Op. The *dashed line* represents the expected δ^{18}Op value calculated based on the temperature for each sample and at the average lake surface δ^{18}Ow of −6.78‰ (standard deviation 0.3‰). Samples plotting between the river and equilibrium lines could be explained by P cycling a process that would tend to erase source signature and bring the δ^{18}Op values towards equilibrium. Lines above the equilibrium line suggest a source with δ^{18}Op higher than 17‰. Figure modified from Elsbury et al. (2009)

21.3.3 Phosphate Sources and Cycling in Riverine Systems

The range of potential $\delta^{18}O_p$ values for DIP in riverine systems is much greater than the range expected for open-ocean and coastal waters due to the wider range of temperatures, $\delta^{18}O$ water values, and phosphate sources found in riverine systems. Furthermore, land use patterns are thought to have a significant impact on nutrient stoichiometry and concentrations in riverine environments (Harris 2001; Lehrter 2006; Neill et al. 2001), thus, differences in land use could provide unique $\delta^{18}O_p$ signatures with which to trace the relative influence of specific sources to receiving waters. Common phosphate sources for rivers include wastewater treatment effluent, agricultural and urban runoff, manure, leaking septic systems, and natural rock and soil weathering. In addition, river discharge can be viewed as a source of phosphate in relation to other systems; for example, tributaries entering larger rivers, lakes, estuaries, or coastal waters. Although the $\delta^{18}O_p$ of river water will usually be controlled by a complex combination of source inputs, if the $\delta^{18}O_p$ of the sources are known, this can be used to trace the phosphate as it moves down the river's flow path. There are not many data sets for $\delta^{18}O_p$ of rivers but the few that are available report values that do not represent equilibrium and thus most likely reflect changes in source contribution along the river flow path (Fig. 21.9).

Water samples collected from the San Joaquin River (SJR), a hypereutrophic river in the major agricultural region of the California Central Valley, span a range $\delta^{18}O_p$ values greater than the analytical error, and only one sample fell along the expected equilibrium line. The samples do not show a consistent offset from equilibrium, indicating that the $\delta^{18}O_p$ at least partially reflects inputs of phosphate sources with different $\delta^{18}O_p$ signatures, rather than full biological cycling and complete oxygen exchange with water (Young et al. 2009).

Rivers flowing into Lake Erie range in isotope values from +10.5 to +15.2‰. These values range from 4.0‰ lower than the expected isotopic equilibrium to 1.0‰ higher, with two samples falling within range of the expected equilibrium (~ 14‰) and in general are lower than lake values (Elsbury et al. 2009). Tributaries to Lake Tahoe, CA, are also not at equilibrium with values from 8.2 to 12.2‰ (equilibrium ~11‰). While more research is needed, the

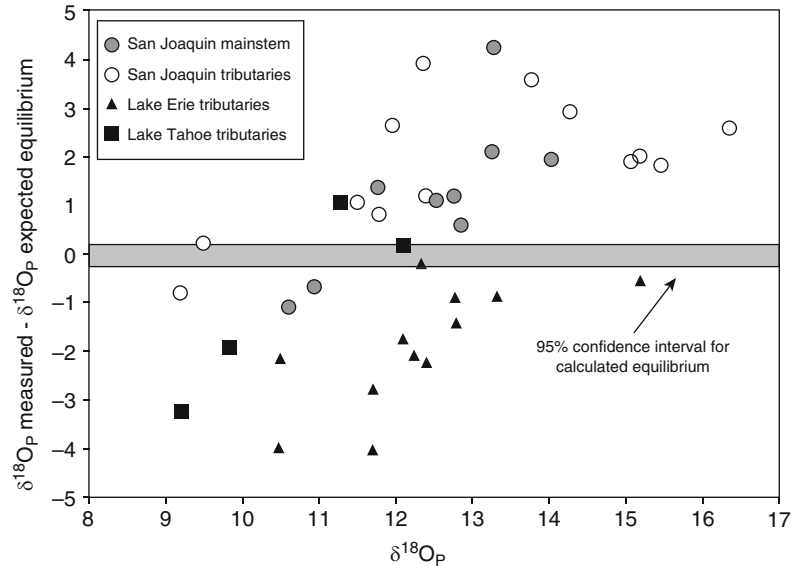

Fig. 21.9 Offset from isotopic equilibrium of various river samples. *Gray bar* represents the range of values that would be at equilibrium considering analytical error and calculation errors associated with determining the equilibrium value. Figure modified from Young et al. (2009)

results of river studies in California and the Lake Erie area (Michigan and Ohio) demonstrate two important factors for using $\delta^{18}O_p$ as a source tracer in river systems. The $\delta^{18}O_p$ value of the majority of water samples are not in isotopic equilibrium, indicating that source signatures are not being rapidly overprinted by equilibrium signatures within the river, and in several instances, certain tributaries had $\delta^{18}O_p$ values that are distinct from those of other tributaries, indicating that the contribution of phosphate from specific tributaries to the receiving water body could be identified using this isotope tracing approach.

21.3.4 Phosphorous Cycling in a Coastal Setting

Phosphate in many coastal systems is not the limiting nutrient for productivity, yet is heavily utilized, thus it is expected that the source signature will be at least partially overprinted and that the $\delta^{18}O_p$ will shift towards equilibrium values. If this is indeed the case the degree of deviation from the source signature could be used as a measure of phosphate turnover rate relative to new phosphate input. This principle has been used in California coastal waters (Monterey Bay) (Fig. 21.10). In this system, $\delta^{18}O_p$ tracks seasonal changes in phosphate cycling through the biomass (e.g. phosphate utilization rates) with the greatest phosphate oxygen isotope exchange occurring during the upwelling season (McLaughlin et al. 2006b). Spatially the greatest percent of phosphate oxygen exchange, and thus the greatest phosphate utilization relative to input, occurs at the locus of upwelling. Episodes of higher phosphate turnover occurs simultaneously throughout the upper 200 m of the water column and on a broad spatial scale. $\delta^{18}O_p$ data also suggest that deep water (~500 m) may be a source of phosphate to the euphotic zone in Monterey Bay.

The degree of P cycling differs among different coastal systems. Colman (2002) concluded that the large deviations in $\delta^{18}O_p$ between riverine and coastal waters in the Long Island Sound reflects extensive equilibration with local coastal water and indicates that in this geographic area rapid microbial cycling overprints source $\delta^{18}O_p$ values on a timescale of weeks.

21.3.5 Phosphorous Cycling in Open Ocean Settings

Dissolved inorganic phosphorus (DIP) concentrations in the open ocean have a typical nutrient profile with low concentrations in surface water due to extensive uptake by primary producers and increasing concentration with depth resulting from regeneration of DIP from sinking particulate matter. The deep Pacific has higher DIP concentration than the deep Atlantic due to

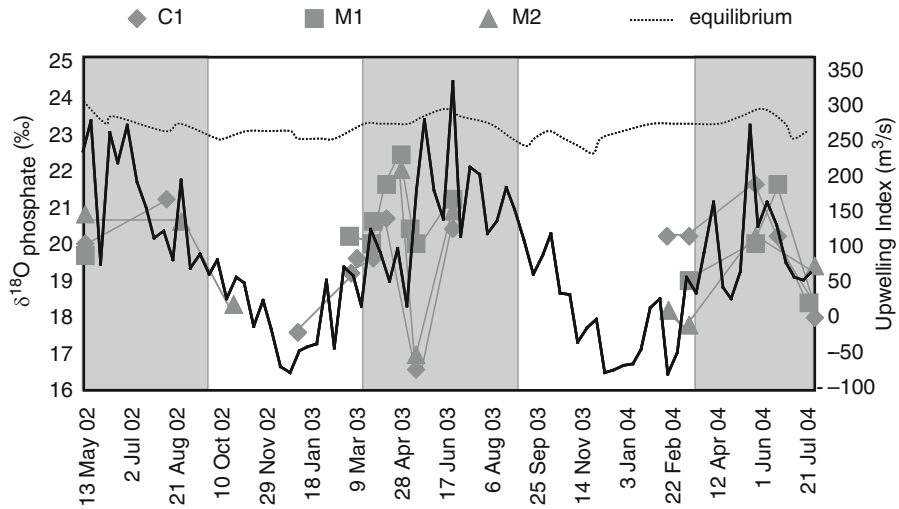

Fig. 21.10 Observed phosphate δ^{18}Op variability from May 2002–August 2004. *Solid line* is the 10-d running mean of the NOAA upwelling index as a function of time; δ^{18}Op is for samples collected at 10-m depth at three monitoring stations (C1, M1, M2); *dashed line* is the expected equilibrium phosphate δ^{18}Op; The δ^{18}O of the phosphate source from deep water upwelling is ~17‰. Data from McLaughlin et al. (2006a, b, c, d)

aging of the water along the circulation pathway (Broecker and Peng 1982). It would thus be expected that the $\delta^{18}O_p$ in open ocean waters be primarily a function of biological turnover with potentially some impact of circulation. Colman et al. (2005) measured the $\delta^{18}O_p$ depth distributions in the Atlantic and Pacific Oceans. At both basins $\delta^{18}O_p$ values were close to, but slightly offset from, the expected equilibrium values (calculated from equation (21.1) and the seawater temperature and $\delta^{18}O_w$). Because seawater values at intermediate depths approaches the equilibrium isotopic composition, intracellular cycling at these depths is suggested as the main process affecting the isotopic signatures. The offset at depth is attributed to differences between the deep water temperature and high latitude surface water temperatures, where DIP is equilibrated and transported along the circulation path (Colman et al. 2005) (Fig. 21.11).

In oligotrophic systems, such as the surface waters of the Sargasso Sea, DIP concentrations are extremely low. Consequently, P is thought to limit or co-limit primary productivity in this region. McLaughlin et al. (2011) investigated the biogeochemical cycling of P in the Sargasso Sea, utilizing multiple techniques including $\delta^{18}O_p$, alkaline phosphatase enzyme-labeled fluorescence (ELF), and ^{33}P uptake derived phosphate turnover rates. Results from these studies indicate that dissolved organic phosphorus (DOP) is utilized by phytoplankton and bacteria to supplement cellular requirements for this vital nutrient. They show that remineralization of the DOP pool is most extensive above the thermocline, as indicated by a large fraction of eukaryotes producing alkaline phosphatase, rapid phosphorus turnover times, and a large deviation from equilibrium of $\delta^{18}O_p$ towards lighter values. These data suggest that DOP remineralization by extracellular enzymes is prevalent and that DOP can account for up to 60% of P utilized and support a corresponding amount of primary production. Below the thermocline, alkaline phosphatase expression is reduced, turnover times increase, and $\delta^{18}O_p$ values approach equilibrium, all of which are indicative of intracellular phosphate cycling and slower turnover of the DOP pool. This study highlights the importance of bioavailable organic P to primary productivity in oligotrophic systems and has implications for the global carbon cycle.

21.4 Future Directions

For a more rigorous interpretation of $\delta^{18}O_p$ data from environmental samples several gaps in our understanding of how phosphate oxygen is fractionated in aquatic systems must be addressed. Particularly, characterization of the isotopic fractionation of phosphate oxygen associated with additional processes including

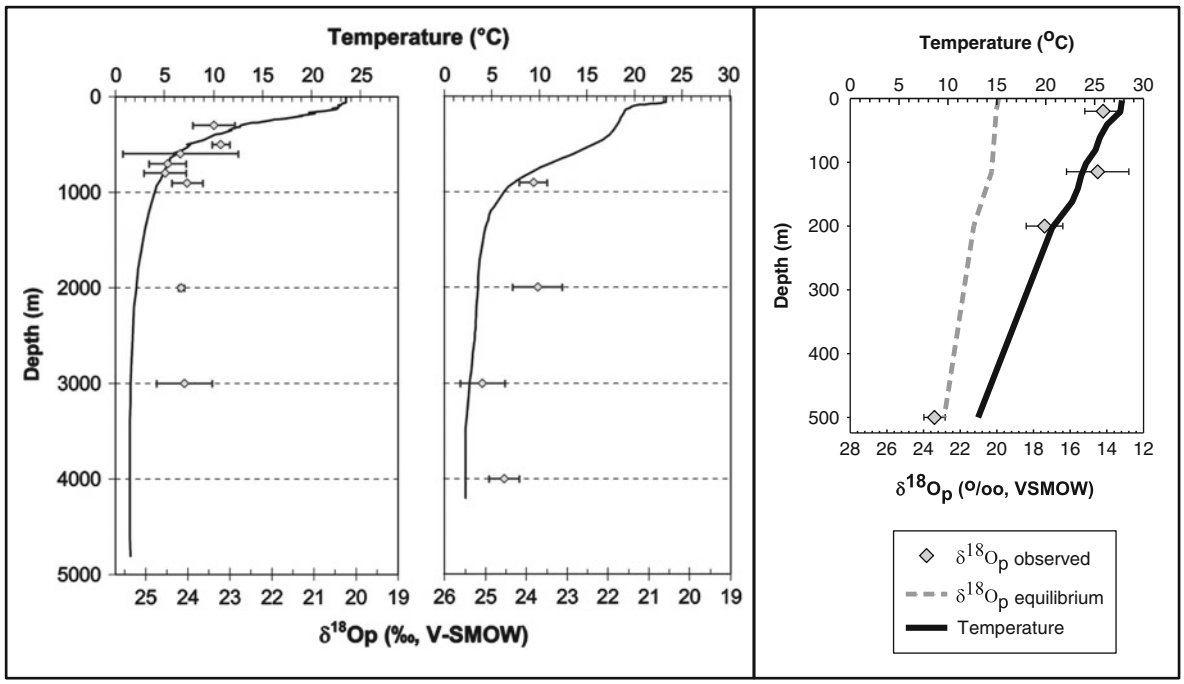

Fig. 21.11 *Left panel* represented the depth profile of $\delta^{18}O_p$ in the Pacific (**a**) and Atlantic (**b**). *Solid lines* represent the temperature depended equilibrium values and *open circles* are measured values. *Error bars* represent 95% confidence intervals based on replicate mass spectrometric analyses of single samples. Note the approach to equilibrium values at intermediate depth and off sets in the deep ocean (modified from Colman et al. 2005). On the right is a depth profile in the upper 500 m of the oligotrophic Sargasso Sea (data from McLaughlin et al. 2011). *Circles* are measured values and 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 relation

those associated with different enzymes as well as inorganic processes (desorption from particles and sediment regeneration). Specifically, there is a dearth of data on the fractionation associated with freshwater periphyton (soft algae and diatoms) and freshwater heterotrophic bacteria. Research has suggested that bacteria are superior competitors for phosphate in aquatic systems compared to phytoplankton (Currie and Kalff 1984); however, differences in isotopic fractionation associated with bacterial cycling of phosphorus compared to algal cycling have not been fully defined. More research is needed to understand how various organisms fractionate phosphate oxygen under a variety of temperature and phosphorus concentration regimes.

Isotopic fractionation associated with sorption onto particulate matter and in co-precipitation of phosphate with various minerals must also be further explored. Phosphate interactions with sediments and co-precipitates in lakes and streams have been found to be an important factor in controlling the dissolved phosphate pool (Fox 1989; House 2003). Such effects are assumed to be negligible in most systems but could potentially play a role in hardwater systems where co-precipitation of phosphate can result in the removal of up to 30% of the dissolved P pool (House 2003). Finally, fractionation associated with remineralization and sedimentary fluxes also needs to be defined.

Procedures for the analysis of oxygen isotopes in organic phosphate compounds have not been fully tested. The only published procedure is of McLaughlin et al. (2006c) in which an $\delta^{18}O$ enriched isotope spike is used to correct for incorporation of reagent oxygen during hydrolysis of organic phosphate. While data presented in this paper is promising, fractionation effects associated with hydrolysis have not been fully evaluated and work on natural samples is limited. The signatures of dissolved organic phosphate compounds and plant material may be important yet these values are mostly unknown.

The database characterizing source signatures is also relatively limited. Specifically, groundwater, atmospheric deposition and agriculture and urban runoff which are known sources to many aquatic systems have not been measured and only limited information regarding isotope signatures of phosphate regenerated from sediments is available. Similarly the observation that waste water treatment effluents from different locations and treatment plants have different $\delta^{18}O_p$ values warrants further work to determine how specific treatment protocols impact the isotope ratio. Thus a more extensive data base at a wide range of locations and settings is needed.

In addition, it is vital that a certified international silver phosphate standard be prepared, characterized and distributed to a wider scientific community. This would permit comparison of results among laboratories and will enable laboratories to establish better QA/QC protocols.

Finally, it could be interesting to extend the utility of this isotope system to other areas of environmental research including environmental forensics, climate research, and to study phosphorous cycling in vegetation and soils. Indeed a few attempts to move in that direction have taken place (Jaisi and Blake 2010; Tamburini et al. 2010; Zohar et al. 2010b). The development is rapid and it is expected that this system will see a great expansion in application to a broad range of problems in the near future.

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