The oxygen isotopic composition of phosphate in Elkhorn Slough, California: A tracer for phosphate sources

Karen McLaughlin*, Barbara J. Cade-Menun, Adina Paytan

Department of Geological and Environmental Sciences, Stanford University, Stanford, CA 94305-2115, USA

Abstract

Elkhorn Slough, a small seasonal estuary in central California, has been subjected to increased nutrient loading from agricultural and other non-point sources. However, because nutrients do not behave conservatively, tracing nutrient sources and cycling in ecosystems like Elkhorn Slough has been difficult to assess. This is particularly true of phosphorus (P), which has only one stable isotope and cannot be used as an isotopic tracer. However, isotopic fractionation of oxygen in phosphate at surface water temperatures only occurs as a result of enzyme-mediated, biochemical reactions. Thus, if phosphate demand is low relative to input and is not heavily cycled within the ecosystem, the $\delta^{18}O$ of phosphate will reflect the isotopic composition of phosphate sources to the system. We utilized the $\delta^{18}O$ of dissolved inorganic phosphate (DIP) within the main channel of the slough and nearby Moss Landing Harbor and the $\delta^{18}O$ of reactive phosphate from sediment and soil samples collected within the watershed to understand phosphate sources and cycling within Elkhorn Slough. Trends in the $\delta^{18}O$ of DIP were seasonally consistent with high values near the mouth reflecting oceanic phosphate (19.1‰–20.3‰), dropping to a minimum value near Hummingbird Island in the central slough (point source, 14.1‰–14.4‰), and increasing again near the head of the slough, reflecting fertilizer input (18.9‰–19.3‰). Reactive phosphate $\delta^{18}O$ values extracted from sediments and soils in the watershed range from 10.6‰ in a drainage ditch to 22.3‰ in creek sediments near agriculture fields. The wide range in phosphate $\delta^{18}O$ values reflects the variations in land use and application of different fertilizers in this agriculturally dominated landscape. These data suggest that phosphate $\delta^{18}O$ can be an effective tool for identifying P sources and understanding phosphate dynamics in estuarine ecosystems.
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1. Introduction

Agricultural expansion 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 N and P from fertilizer and animal waste enters surface waters and groundwater (Carpenter et al., 1998). Furthermore, these nutrient loads enter the coastal ocean and can stimulate large scale phytoplankton blooms (Rabalais et al., 2002; Beman et al., 2005). Estuaries already receive more nutrients than any other type of ecosystem, with some receiving more than 1000-fold that of heavily fertilized agricultural land (Nixon et al., 1986). Nutrient enrichment in aquatic systems can cause diverse problems such as toxic algal blooms, anoxia, fish kills, loss of biodiversity, and a host of other problems (Carpenter et al., 1998; Tilman et al., 2001). Thus, identifying and understanding nutrient inputs and their effects on estuaries and adjacent coastal areas are of critical importance to managing these ecosystems and for restorative efforts.

Phosphorus is a required element for life; consequently, its availability may impact primary production rates as well as

* Corresponding author. Present address: Department of Chemical Engineering and Materials Science, University of California, Irvine, Irvine, CA 92697-2575, USA.

E-mail addresses: karenm@uci.edu (K. McLaughlin), bjcm@pangea.stanford.edu (B.J. Cade-Menun), apaytan@pangea.stanford.edu (A. Paytan).

species distribution and ecosystem structure (Smith, 1984; Sharp, 1991; Benitez-Nelson, 2000; Karl et al., 2001). Phosphorus may limit primary productivity in some estuarine and marsh environments (Smith, 1984; Howarth, 1988), while in others only certain organisms within the ecosystem may be P-limited (Sundareshwar et al., 2003; Nicholson et al., 2006). Because P has only one stable isotope, P stable isotope ratios cannot be used for studies of nutrient sources, cycling and utilization (as is the case for nitrogen and carbon). However, most of the P found in nature is strongly bound to oxygen (O), which has three stable isotopes; hence, phosphate (PO_4^{3-}) can be analyzed for its O isotopic composition. Oxygen isotopic composition is reported in standard delta notation ($\delta^{18}O$), which is calculated using the following equation:

$$\delta^{18}O = \left[\frac{R_{sample}}{R_{VSMOW}} - 1 \right] \times 1000 \qquad (1)$$

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

The P–O bond in phosphate is resistant to inorganic hydrolysis and, at the temperature and pH of most natural systems, phosphate does not exchange O with water without biological mediation (Longinelli et al., 1976; Blake et al., 1997; O'Neil et al., 2003). Thus, observed variability in the $\delta^{18}O_p$ will either reflect mixing of isotopically distinct sources of phosphate (McLaughlin et al., 2006c) or the alteration of the phosphate $\delta^{18}O$ as the result of the exchange of O during the cycling of phosphate through living cells (Blake et al., 2005). In the latter case, each time a phosphate molecule is cycled (taken up by organisms and processed by enzymes) phosphate O will be exchanged with cellular water, resulting in isotopic equilibrium with the surrounding water at the temperature of reaction.

The expected equilibrium $\delta^{18}O_p$ can be calculated from the temperature and the $\delta^{18}O$ of the environmental water ($\delta^{18}O_w$) using the empirically derived fractionation equation between phosphate and water developed by Longinelli and Nuti (1973):

$$T\,(^{\circ}C) = 111.4 - 4.3\left(\delta^{18}O_p - \delta^{18}O_w\right) \qquad (2)$$

where T is the environmental temperature, $\delta^{18}O_p$ is the isotopic composition of the phosphate, and $\delta^{18}O_w$ is the isotopic composition of the environmental water. Blake et al. (1997) derived a similar equation for phosphate extracted from microbial cultures:

$$T\,(^{\circ}C) = 155.8 - 6.4\left(\delta^{18}O_p - \delta^{18}O_w\right) \qquad (3)$$

Thus, the $\delta^{18}O_p$ can be used to identify isotopically distinct phosphate sources (if phosphate is not heavily cycled within an ecosystem) 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).

Results of several laboratory studies characterizing the exchange and fractionation of phosphate O isotopes suggest that the $\delta^{18}O_p$ of DIP could be used to evaluate the degree of recycling of the DIP pool (Blake et al., 1997, 1998; Paytan et al., 2002; Blake et al., 2005). Experiments assessing the isotope effects of enzyme-mediated turnover of phosphate and microbially-mediated degradation of organic matter demonstrated that significant exchange of O isotopes between phosphate and water accompanies the hydrolytic cleavage and metabolism of both organically bound phosphate and inorganic orthophosphate (Blake et al., 1997). Bacterial metabolic processes significantly alter the $\delta^{18}O_p$ of DIP in laboratory culture experiments, even when P concentrations are high (Blake et al., 1998). Intracellular phosphate cycling results in the temperature-dependent equilibrium O isotope fractionation (Eq. (2)), which imparts the equilibrium $\delta^{18}O_p$ on phosphate recycled within cells (Blake et al., 2005). Furthermore, results of an algae culture experiment indicate that intracellular O isotope exchange between P compounds and water is very rapid (hours to days) (Paytan et al., 2002).

Phosphate oxygen isotope tracer studies in natural environments are extremely limited. However, recent field studies suggest the utility of $\delta^{18}O_p$ of DIP as a tracer for the mixing of riverine versus oceanic phosphate as well as localized phosphate sources in estuarine and coastal ocean ecosystems (Colman, 2002; McLaughlin et al., 2006a,c). In each of these studies, isotopic equilibrium had not been reached and the $\delta^{18}O_p$ could be used to determine distinct P sources and to determine the extent of P cycling (approach to equilibrium) in these systems.

Elkhorn Slough is a coastal wetland and ecological reserve. The main channel of the slough is primarily N-limited and is tidally flushed; consequently, the residence time of water within the main channel is short (Caffrey et al., 2002a). Accordingly, the isotopic composition of phosphate is likely to represent sources of phosphate to the estuary rather than variation in cycling. The objective of this study was to utilize the $\delta^{18}O_p$ in the main channel waters and the $\delta^{18}O_p$ of fertilizer, compost and reactive phosphate from soils and sediments within the watershed to trace phosphate sources to Elkhorn Slough. Results from this study will contribute to the growing body of work utilizing $\delta^{18}O_p$ to understand phosphate dynamics in aquatic environments.

2. Site description

Elkhorn Slough is a small seasonal estuary extending inland approximately 11.4 km from the Monterey Bay (Fig. 1), with a watershed area of approximately 182 km^2 (Caffrey et al., 2002a). The slough and its tidal creeks are shallow, with an average water depth of approximately 1.4 m and a tidal range of approximately 1.7 m along the main channel. The main channel of the slough is bordered by intertidal mudflats and pickleweed marsh, with several pocket marshes and shallow ponds isolated from the main channel by Southern Pacific Railroad tracks (Caffrey et al., 2002b). Tides are the dominant mixing mechanism for the slough, removing 3/4 of the mean high water volume daily (Smith, 1973).

Agricultural land-use dominates the Elkhorn Slough watershed. The land is planted in row crops and used for grazing,
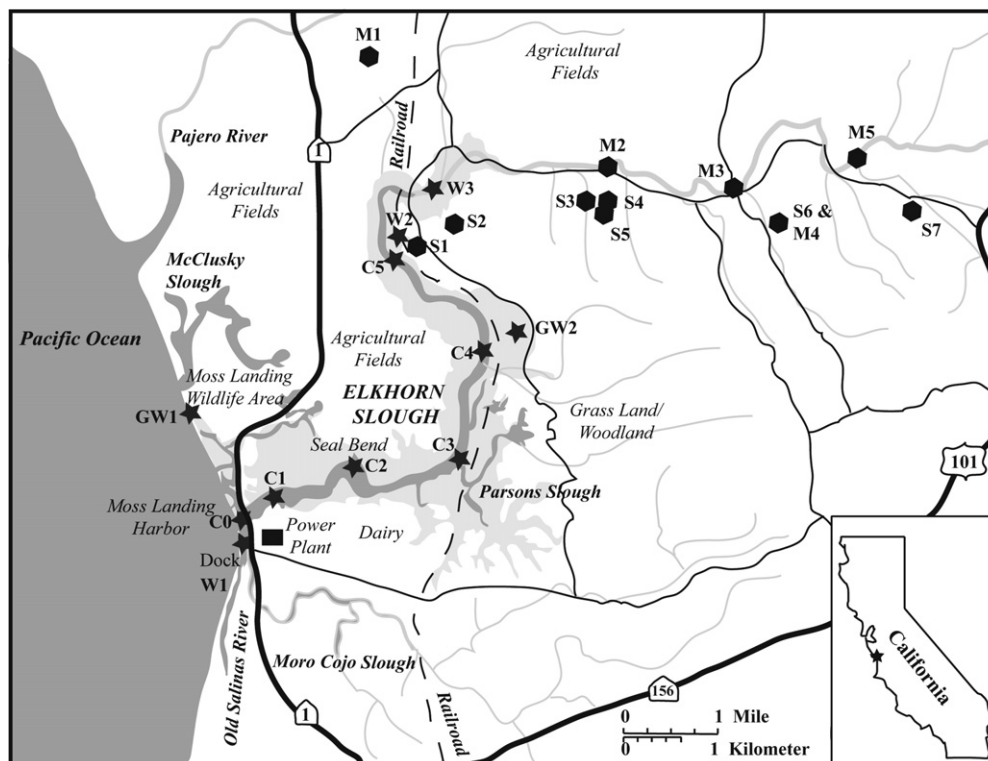
Fig. 1. Map of Elkhorn Slough. The Elkhorn Slough main channel is dark grey and mudflat and creek areas are lighter grey. Major roads and highways are black lines, and the railroad is the dashed line. Stars indicate water samples (those denoted with a C are main channel sites, W are marshes and ponds off the main channel and GW are groundwater samples taken from pits and wells); hexagons indicate sediment (denoted with M) and soil samples (S). Land use is described by the text in italics.

and these areas drain into Elkhorn Slough via Carneros Creek at the head of the estuary and numerous small, ephemeral creeks (Caffrey et al., 2002a). Most of the land is zoned rural-residential, with the majority of the homes using septic systems. Another small portion of the watershed is comprised of urban and commercial land uses concentrated in the towns of Moss Landing and Castroville (Caffrey et al., 2002b). Approximately 10% of the watershed (primarily in proximity to the slough) is protected land managed in its natural state by private conservation groups and public agencies (Caffrey et al., 2002b).

There are three major terrestrial nutrient sources to the Elkhorn Slough: freshwater runoff (highest during the winter rainy season) dominates these inputs, followed by groundwater input, and lastly, atmospheric inputs from dust or nutrients dissolved in rain and fog (Caffrey et al., 2002a). Phosphorus concentrations within the slough have increased dramatically since the 1970s as a result of increased agriculture in the watershed, and can be as high as 135 µM in sites receiving agricultural runoff (Caffrey et al., 1997, 2002a). Dissolved inorganic N to dissolved inorganic phosphate ratios (DIN:DIP) are highly variable throughout the slough, and range from 0.7 to 366, with the highest values in the lower slough and old Salinas River channels and the lowest values in areas with restricted circulation off the main channel. These geographical differences among the ratios have been attributed to local differences in N input and cycling (denitrification) rather than

changes in phosphate levels (Caffrey et al., 2002a, 2003). Concentrations of DIP in the slough water are always measurable and are typically high, suggesting excess phosphate compared to requirements by the biota.

3. Materials and methods

We collected surface water within the main channel of Elkhorn Slough at various stages of a tidal cycle on February 1, 2003, and along transects in the main channel on October 8, 2003 and May 13, 2004, representing different seasons. Water samples were also collected from groundwater wells, marshes and ponds adjacent to the main channel in April 2005. Eight liters of surface water were pumped into acid-washed high-density polyethylene bottles and filtered through Whatman GF/F filters to remove particulates. DIP was stripped from seawater by adding 150 ml of 1 M sodium hydroxide to each sample and vigorously shaking (Karl and Tien, 1992; Thomson-Bulldis and Karl, 1998). Magnesium hydroxide floc was allowed to settle for 1–2 h before supernatant was siphoned off, leaving approximately 1–2 L of floc in seawater. Samples were then processed according to McLaughlin et al. (2004) for $\delta^{18}O$ analysis of DIP ($\delta^{18}O_p$). Isotopic analyses were conducted on a Eurovector Elemental Analyzer coupled to a Micromass (now GVI) IsoPrime mass spectrometer at the U.S. Geological Survey in Menlo Park, California. Results were calibrated and precision monitored using two internal silver

phosphate standards, STDH ($\delta^{18}O_p = 20.0\%_0$) and STDL ($\delta^{18}O_p = 11.3\%_0$) that were run in duplicate after every 10 samples. The isotopic values of these standards were determined previously by comparison to other standards which had also been analyzed by the traditional fluorination method (McLaughlin et al., 2004). Water $\delta^{18}O$ was determined using Finnegan MAT 251, also at the U.S. Geological Survey in Menlo Park. All $\delta^{18}O$ isotopic measurements are reported in the standard delta notation in per mil units ($\%_0$) with respect to Vienna Standard Mean Ocean Water (VSMOW).

Dissolved inorganic phosphate concentrations were determined colorimetrically using HACH phosver 3 reagent powder pillows on a HACH spectrophotometer and are reported in micro moles per liter (μmol L^{-1}) with an analytical precision of ± 0.2 μmol L^{-1}. Temperature and salinity were collected on site using a YSI probe.

Total P in sediment and soil samples was determined by sulfuric acid digest (Parkinson and Allen, 1975) and colorimetric analysis (Watanabe and Olsen, 1965). Reactive P was extracted from solid samples (soils, sediments, compost, and fertilizers) with a dilute acid fluoride solution (0.025 M HCl, 0.03 M NH$_4$F − Bray P1 solution) with a ratio of 1 g solid to 10 ml Bray solution while shaking for 2 min (Bray and Kurtz, 1945). Soluble reactive P was then measured colorimetrically using the molybdenum blue method (Watanabe and Olsen, 1965). Organic P was determined by difference before and after ignition (Saunders and Williams, 1955).

The $\delta^{18}O_p$ of soluble reactive phosphate of solid samples was determined by extracting the reactive phosphate from 20 g of soil and sediment and 1 g of fertilizer and compost in dilute acid fluoride solution in a solid: solution ratio of 1:10. Solids were filtered from the extract solution on 0.8 μm pore size polycarbonate filters and phosphate in the extract solutions was converted to silver phosphate according to the McLaughlin et al. (2004) procedure and analyzed for $\delta^{18}O_p$. A subset of the solid samples was digested in 10 N nitric acid as well as in isotopically enriched 10 N nitric acid and converted to silver phosphate for $\delta^{18}O_p$ of total acid soluble P isotope analysis according to McLaughlin et al. (2006b).

4. Results

The $\delta^{18}O_p$ within the main channel of Elkhorn Slough ranged between 14.1$\%_0$ (central slough) and 20.3$\%_0$ (slough mouth) (Table 1). No significant variations at each station within the slough main channel were observed over the course of the tidal cycle (Fig. 2). A significant difference between low and high tide $\delta^{18}O_p$ at the Monterey Bay Aquarium Research Institute dock in Moss Landing Harbor (17.9$\%_0$ at low tide and 19.7$\%_0$ at high tide) was detected (Table 1). Along the main channel, the highest $\delta^{18}O_p$ values were always seen at the slough mouth (C0), with values decreasing along the transect to a minimum at station C4 and then increasing again at C5 (Fig. 3). The $\delta^{18}O_p$ values at C4 were not significantly different between October 2003 and May 2004 (Fig. 3; 14.1 ± 0.3$\%_0$ in October and 14.4 ± 0.2$\%_0$ in May). The highest $\delta^{18}O_p$ values recorded (greater than 19$\%_0$) approach but are always

Table 1
Summary of Elkhorn Slough tidal cycle variations in water samples

Location	$\delta^{18}O_p$ ($\%_0$)	Equilibrium $\delta^{18}O_p$ ($\%_0$)	DIP[a] (μmol L^{-1})	Temperature (°C)	Salinity
February 1, 2003					
Low Tide					
Dock	17.9 ± 0.3	21.8	4.38	14.2	31.0
Mouth (C0)	19.2 ± 0.2	22.2	0.64	14.1	33.3
Kirby (C5)	19.3 ± 0.1	21.4	2.84	16.5	31.9
High Tide					
Dock	19.7 ± 0.1	22.2	3.43	14.4	32.6
Mouth (C0)	18.8 ± 0.2	23.1	0.46	10.8	33.6
Kirby (C5)	19.3 ± 0.2	21.6	5.03	14.9	33.0
October 8, 2003					
C0	19.1 ± 0.1	22.1	0.72	13.9	33.6
C2	18.6 ± 0.2	22.3	0.85	14.0	33.3
C3	15.9 ± 0.5	21.8	0.55	16.1	33.2
C4	14.1 ± 0.3	21.5	0.59	15.8	33.2
May 13, 2003					
C0	20.3 ± 0.3	22.3	0.91	13.8	34.4
C1	18.8 ± 0.2	22.3	1.21	13.7	33.9
C2	18.0 ± 0.2	22.1	1.81	14.7	33.7
C3	18.4 ± 0.2	21.8	2.32	15.9	33.2
C4	14.4 ± 0.2	21.6	2.94	16.6	29.5
C5	18.9 ± 0.1	21.3	3.01	18.0	32.3

[a] Dissolved inorganic phosphate concentration.

lower than the values predicted from the $\delta^{18}O_p$-temperature equilibrium equation (Eq. (2)) (Figs. 2 and 3).

Water samples outside the tidally-influenced main channel of the slough had low $\delta^{18}O_p$ relative to the seawater values (Table 2). The lowest $\delta^{18}O_p$ sample was collected in Azevedo Pond which is adjacent to the slough and receives runoff from strawberry fields, and main channel water enters the Pond only during high tides (Chapin et al., 2004). The DIP in Carneros Creek water and groundwater sampled at Kirby Park also had low $\delta^{18}O_p$ relative to the $\delta^{18}O_p$ of main channel DIP. Salinas River water and a groundwater sample collected from a beach pit near Moss Landing Harbor had $\delta^{18}O_p$ values similar to the Moss Landing Harbor water sampled at low tide (17.9$\%_0$).
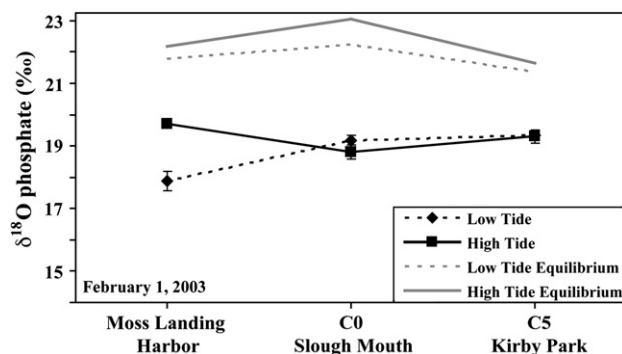

Fig. 2. Phosphate oxygen isotopic composition at three of the sampling locations at low (stippled line) and high (solid line) tide on February 1, 2004. The grey stippled and solid lines represent the expected equilibrium value for the temperature and water $\delta^{18}O$ based on the Longinelli and Nuti (1973) phosphate-water equilibrium expression for low tide and high tide respectively. Error bars represent one standard deviation of duplicate analyses.
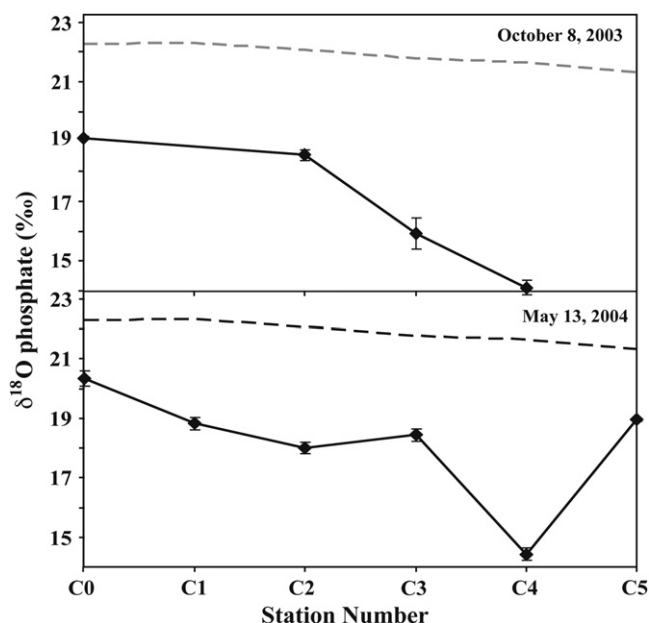
Fig. 3. Phosphate oxygen isotopic composition (solid line) as a function of station number for the two main channel transects on October 8, 2003 (top) and May 13, 2004 (bottom). The dotted line represents the expected equilibrium value for the temperature and water $\delta^{18}O$ based on the Longinelli et al. (1976) phosphate-water equilibrium expression. Error bars represent one standard deviation of duplicate analyses.

The $\delta^{18}O_p$ of reactive phosphate and total phosphate in compost and fertilizer samples span a wide range of isotopic compositions and all have high concentrations of reactive and total P (Table 2). The two commercial fertilizer samples obtained from local farmers have values close to that of marine-derived phosphorites (Shemesh et al., 1983) and fall

within the natural range for seawater $\delta^{18}O_p$ (Longinelli et al., 1976; Colman, 2002; Colman et al., 2005). The soil and sediment samples also have a wide range of $\delta^{18}O_p$ values and P concentrations, most of which fall in between the isotopic compositions of the fertilizer and compost. The exceptions are the drainage ditch and the strawberry field soil, both of which have lower $\delta^{18}O_p$ (possibly representing a different fertilizer used there) and the horse farm soil, which has a higher $\delta^{18}O_p$. Soils had considerably higher reactive P and total P than creek sediments (Table 2) but the $\delta^{18}O_p$ values span similar ranges throughout the watershed.

5. Discussion

Nutrient concentrations within Elkhorn Slough have been found to be related to adjacent land use, with areas closest to agricultural land use having high and variable concentrations of nutrients (Caffrey et al., 2002b). Furthermore, high nutrient runoff has been reported to have a local rather than estuary-wide effect due to longer water residence times at the head of the slough compared to the mouth (Caffrey et al., 2002b). The phosphate $\delta^{18}O$ data for the Elkhorn Slough main channel corroborate these findings, such that $\delta^{18}O_p$ values vary from station to station along the main channel but trends along the transect are consistent through time. This suggests that local effects related to land use, which are consistent throughout the year, are not overwhelmed by tidal or seasonal influences. Phosphate $\delta^{18}O$ may be a recorder of either phosphate cycling, indicated by an approach to isotopic equilibrium, or of phosphate source, when phosphate is not heavily utilized and the source signal is retained. Phosphate concentrations in the slough never drop to very low levels,

Table 2

Summary of data for sediment, soil, and fertilizer samples taken around the Elkhorn Slough and the tributaries to the Elkhorn Slough

Location and Map Identification Number	$\delta^{18}O_{Bray\ P1}$ (‰)	$\delta^{18}O_{total\ PO4}$ (‰)	Total P[a] (mg/kg)	Bray P1 (mg/kg)	Organic P (mg/kg)	N:P$_{org}$ (mol/mol)
Don Crawford commercial compost	14.8	27.0	9598	395.8	739.2	17.1
Maher Farm compost	18.8	23.3	2594	162.0	403.6	36.9
Commercial fertilizer 1	17.6	20.5	23863.4	1446.7	2774.2	53.3
Commercial fertilizer 2	20.2	19.4				2
Azevedo Ranch soil (S1)	11.5		302.2	101.0	86.6	8.4
Blohm Ranch soil (S2)	21.5	23.4	190.0	26.1	80.6	7.5
Horse Farm soil (S3)	24.3		237.9	65.0	54.9	18.6
Elzas bush bean soil (S4)	18.3		441.0	111.9	131.0	7.5
Elzas upper field soil (S5)	16.9		278.3	48.7	109.4	3.0
Maher Farm soil (S6)	17.7		235.2	51.6	58.1	6.0
Carneros Ranch soil (S7)	15.0		732.0	138.9	115.9	4.3
Pajero drainage ditch sediments (M1)	10.6		186.9	3.8	104.8	11.2
Carneros Creek sediment (M2)	18.5		72.3	11.5	26.1	6.5
Carneros Creek sediment (M3)	22.3	19.0	146.4	17.7	6.1	16.3
Maher Pond sediment (M4)	15.5		73.8	15.6	7.7	13.0
Carneros Creek sediment (M5)	21.6		32.9	7.4	25.4	5.4
Salinas River (W1)		17.2				
Azevedo Pond Water (W2)		10.9				
Carneros Creek (W3)		11.5				
Beach Pit Groundwater (GW1)		18.0				
Kirby Marsh Groundwater (GW2)		15.1				

[a] Total phosphorus.

suggesting that P is not a limiting nutrient in this ecosystem (Table 1). In addition, because the majority of the slough is tidally flushed such that phosphate does not have a long residence time, one would not expect significant P turnover by the biomass, resulting in an isotopic equilibrium signal in the $\delta^{18}O_p$. Accordingly, the observed $\delta^{18}O_p$ is likely an indicator of phosphate source and not turnover within the biomass. A similar situation has been observed in nearby San Francisco Bay where input from local point sources of phosphate has been identified using $\delta^{18}O_p$ (McLaughlin et al., 2006c).

We were able to utilize the $\delta^{18}O_p$ of DIP and organic matter phosphate to identify potential phosphate sources to Elkhorn Slough. Our findings indicate that oceanic phosphate, which is brought in by tidal flushing and has a $\delta^{18}O_p$ of ∼20‰ (McLaughlin et al., 2006a), dominates the lower slough (C0 through C2 in October and through C3 in May). The central slough is dominated by a different, low $\delta^{18}O_p$ phosphate source most notably seen at C4 (with a low value also at C3 in October, 2003). Site C4 appears to be strongly influenced by fresh groundwater discharge as indicated by radium isotope analyses (Street et al., 2005). Furthermore, other chemical tracers (Ba, B) have also indicated that there is a significant groundwater input source with a distinct chemical signature in the central slough (Paytan unpublished). Groundwater sampled from a well near Kirby Marsh exhibits a low $\delta^{18}O_p$ similar to that observed at site C4 (15.1‰). Therefore, the low $\delta^{18}O_p$ source at C4 is most likely related to groundwater phosphate, where phosphate has equilibrated with isotopically-depleted freshwaters.

Site C5 in the upper slough does not exhibit the low $\delta^{18}O_p$ values observed at C4. The $\delta^{18}O_p$ values at the head of the slough at station C5 are comparable to those near the mouth of the slough. However, because C5 is only slightly affected by tidal flushing (Largier et al., 1997), it is unlikely that this is an oceanic source. The head of the slough receives direct runoff from agricultural fields or indirectly thorough Carneros Creek (Caffrey et al., 2002b); therefore, it is more likely that the high $\delta^{18}O_p$ seen here is from phosphate from commercial fertilizers with isotopic signatures similar to seawater (e.g. made from phosphate ore of marine origin). Indeed, the fertilizers and compost samples provided from farmers within the Elkhorn Slough watershed all had $\delta^{18}O_p$ similar to seawater value and the DIP at C5 (Table 2).

The lack of tidal cycle variations in $\delta^{18}O_p$ (Fig. 2) indicates the consistency of the phosphate sources into the slough and the dominance of these sources at each site. The mouth of the slough has values typical of $\delta^{18}O_p$ values for Monterey Bay at both low and high tide (McLaughlin et al., 2006a). $\delta^{18}O_p$ at C5 also remains consistently high, due to fertilizer input from agriculture as discussed above, while the mid-slough sites are influenced groundwater phosphate sources. The only site that showed measurable change when sampled at different times in the tidal cycle was the dock station in Moss Landing Harbor. During low tide, $\delta^{18}O_p$ values are low (17.9‰) compared to high tide (19.7‰). This implies mixing of oceanic phosphate, which has a signature of approximately 20‰, with a low $\delta^{18}O_p$ source, namely the Old Salinas River,

which supplies the harbor with high-nutrient, freshwater from sewage effluent and agricultural runoff at low tide (Smith, 1973; Caffrey et al., 2002a). Indeed, $\delta^{18}O_p$ measurements of water within the Salinas River are similar to values seen at the harbor at low tide (17.2‰). Furthermore, groundwater sampled from a beach pit nearby (representing the groundwater in the unconfined aquifer) also had a $\delta^{18}O_p$ similar to the low tide harbor value (18.0‰). Equilibration of phosphate with freshwater and the influence of sewage or a fertilizer with an isotopic signature different from present-day Monterey Bay seawater phosphate will result in low $\delta^{18}O_p$ values for riverine waters and groundwater (Colman, 2002; Gruau et al., 2005). Elkhorn Slough is relatively isolated from the harbor due to the current regime (Smith, 1973); consequently, the influence of the Old Salinas River did not extend into the slough, at least with respect to phosphate loads during the time of our sampling.

The variability in $\delta^{18}O_p$ observed in soils collected throughout the watershed and in sediments from Carneros Creek (Table 2) is indicative of the wide range of land uses and soil types. Soil samples had higher total and reactive P concentrations than Carneros Creek sediments. However, both the creek sediments and soil samples span the same range of $\delta^{18}O_p$, which indicates that P sources to the creek sediments and to the soil samples originate from sources with similar isotopic signatures, most likely fertilizers. The Maher Farm samples (compost, soil and sediment pond) are an example of how phosphate oxygen isotopes may be fractionated within a system. The $\delta^{18}O_p$ of reactive phosphate extracted from Maher Farm compost is low (18.8‰) relative to the $\delta^{18}O_p$ of the nitric-acid-extractable phosphate of that compost (23.3‰), implying that phosphate with low $\delta^{18}O_p$ is more readily mobilized. Furthermore, the $\delta^{18}O_p$ of reactive phosphate in the compost (18.8‰) is higher than the farm soil sample (17.7‰), which in turn is higher than the sediment pond (15.5‰). Thus, low $\delta^{18}O_p$ phosphate is preferentially moved from the compost to the soils and from there into the sediment pond. This is the first time that such an isotope effect has been reported and this observation should be confirmed with more data from other sites. The Carneros Creek samples indicate the prevalence of fertilizer use within the watershed: all samples analyzed have relatively high $\delta^{18}O_p$ values (18.5‰, 22.5‰, 21.6‰), similar to the fertilizers measured. In fact in all cases, the $\delta^{18}O_p$ of creek sediments is higher than that of soils sampled in close proximity (Elzas Ranch soils, Maher Farm and Carneros Ranch respectively), suggesting that most of the P in the creek water and sediments comes directly from the fertilizer rather than through leaching of phosphate from soils. Alternatively, the $\delta^{18}O_p$ of creek sediments could have a higher isotopic signature than the soil P due to preferential removal of low $\delta^{18}O_p$ phosphate into creek waters. Indeed, creek water sampled near the head of the slough had considerably lower $\delta^{18}O_p$ values than sediment samples upstream.

The low $\delta^{18}O_p$ values at the Azevedo Ranch strawberry fields and the Pajero drainage ditch are anomalous in the data set. They are significantly lower than the fertilizer and compost values we analyzed and are similar to DIP values

observed in the San Francisco Bay near the confluence of the Napa River (McLaughlin et al., 2006c). Though different from the other sediments and soils taken within the watershed, they are similar to the $\delta^{18}O_p$ of dissolved inorganic phosphate collected from Azevedo Pond and the Carneros Creek (Table 2). These values could be attained by repeated P turnover in freshwater with low $\delta^{18}O_w$ or the use of fertilizer with a low $\delta^{18}O_p$ signature, which was not available to us for analysis. These results indicate that a more extensive investigation of the isotopic signatures of various fertilizer and compost products used in the watershed is needed to better constrain sources and processes influencing P dynamics in this and other estuarine systems. However, the variability observed within the limited sample set presented here is very promising and indicates that $\delta^{18}O_p$ could be a powerful tool for identifying non-point source P pollution (e.g. groundwater and fertilizer inputs as in this study) into aquatic systems.

6. Conclusion

The phosphate $\delta^{18}O$ of DIP within the main channel of Elkhorn Slough is indicative of variability in phosphate source throughout the channel, which is affected by the surrounding land use. Trends in the $\delta^{18}O$ of DIP in the main channel were consistent between the October 8, 2003 and May 13, 2004 sampling periods, with 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. The $\delta^{18}O_p$ variations within the main channel of the slough indicate that phosphate sources are not strongly influenced by tides or season. The only significant change in $\delta^{18}O_p$ over the course of the tidal cycle occurred within the Moss Landing Harbor, where the influence of the Old Salinas River was apparent at low tide.

Reactive phosphate $\delta^{18}O$ values extracted from sediments and soils in the upper Elkhorn Slough/Carneros Creek watershed span a wide range of isotopic compositions, which reflect the variations in land use and application of different fertilizer types in this agriculturally dominated landscape. Our limited data set indicates that low $\delta^{18}O_p$ phosphate is removed preferentially as DIP moves downstream or is utilized by organisms in soil and sediment samples. These data suggest that phosphate $\delta^{18}O$ can be an effective tool for identifying P sources and for understanding phosphate dynamics in estuarine ecosystems. To fully tie the phosphate in the estuary to potential sources, it is crucial to determine the isotopic signature of all possible end-members and sources in the watershed. Management strategies to control phosphate concentrations in receiving waters can be more effective if non-point sources are identified. The $\delta^{18}O_p$ is a promising tool to identify specific sources and their movement from land to sea.

References

Beman, J.M., Arrigo, K.R., Matson, P.A., 2005. Agricultural runoff fuels large phytoplankton blooms in vulnerable areas of the ocean. Nature 434, 211–214.

Benitez-Nelson, C.R., 2000. The biogeochemical cycling of phosphorus in marine systems. Earth Science Reviews 51, 109–135.

Blake, R.E., O'Neil, J.R., Garcia, G.A., 1997. Oxygen isotope systematics of biologically mediated reactions of phosphate: I. Microbial degradation of organophosphorus compounds. Geochimica et Cosmochimica Acta 61, 4411–4422.

Blake, R.E., O'Neil, J.R., Garcia, G.A., 1998. Effects of microbial activity on the $\delta^{18}O$ of dissolved inorganic phosphate and textural features of synthetic apatites. American Mineralogist 83, 1516–1531.

Blake, R.E., O'Neil, J.R., Surkov, A.V., 2005. Biogeochemical cycling of phosphorus: insights from oxygen isotope effects of phosphoenzymes. American Journal of Science 305, 596–620.

Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Science 59, 39–45.

Caffrey, J., Shaw, S., Silberstein, M., DeVogelaere, A., White, M., 1997. Water Quality Monitoring in Elkhorn Slough. Elkhorn Slough Technical Reports Series, vol. 1997, No. 1, 10 pp.

Caffrey, J., Brown, M., Tyler, W.B., Silberstein, M. (Eds.), 2002a. Changes in a California Estuary: A Profile of Elkhorn Slough. Elkhorn Slough Foundation, Moss Landing.

Caffrey, J.M., Harrington, N., Ward, B., 2002b. Biogeochemical processes in a small California estuary. 1. Benthic fluxes and pore water constituents reflect high nutrient freshwater inputs. Marine Ecology Progress Series 233, 39–53.

Caffrey, J.M., Harrington, N., Solem, I., Ward, B.B., 2003. Biogeochemical processes in a small California Estuary. 2. Nitrification activity, community structure and role in nitrogen budgets. Marine Ecology Progress Series 248, 27–40.

Carpenter, S.R., Caraco, N.F., Correl, D.L., Howarth, R.W., Sharpley, A.H., 1998. Non-point pollution of surface waters with phosphorus and nitrogen. Ecological Applications 8, 559–568.

Chapin, T.P., Caffrey, J.M., Jannasch, H.W., Coletti, L.J., Haskins, J.C., Johnson, K.S., 2004. Nitrate sources and sinks in Elkhorn Slough, California: results from long-term continuous in situ nitrate analyzers. Estuaries 27, 882–894.

Colman, A., 2002. The oxygen isotope composition of dissolved inorganic phosphate and the marine phosphorus cycle. Ph.D. thesis, Department of Geology and Geophysics, Yale university.

Colman, A.S., Blake, R.E., Karl, D.M., Fogel, M.L., Turekian, K.K., 2005. Marine phosphate oxygen isotopes and organic matter remineralization in the oceans. Proceedings of the National Academy of Sciences 102, 13023–13028.

Gruau, G., Legeas, M., Riou, C., Gallacier, E., Martineau, F., Henin, O., 2005. The oxygen isotopic composition of dissolved anthropogenic phosphates: a new tool for eutrophication research? Water Research 39, 232–238.

Howarth, R.W., 1988. Nutrient limitation of primary production in marine ecosystems. Annual Review of Ecology and Systematics 19, 89–110.

Karl, D.M., Tien, G., 1992. MAGIC: a sensitive and precise method for measuring dissolved phosphorus in aquatic environments. Limnology and Oceanography 37, 105–116.

Karl, D.M., Bjorkman, K.M., Dore, J.E., Fujieki, L., Hebel, D.V., Houlihan, T., Letelier, R.M., Tupas, L.M., 2001. Ecological nitrogen-to-phosphorus stoichiometry at station ALOHA. Deep-Sea Research II 48, 1529—1566.

Largier, J.L., Hollibaugh, J.T., Smith, S.V., 1997. Seasonally hypersaline estuaries in Mediterranean-climate regions. Estuarine, Coastal and Shelf Science 45, 789—797.

Longinelli, A., Nuti, S., 1973. Revised phosphate-water isotopic temperature scale. Earth and Planetary Science Letters 19, 373—376.

Longinelli, A., Bartelloni, M., Cortecci, G., 1976. The isotopic cycle of oceanic phosphate. I. Earth and Planetary Science Letters 32, 389—392.

McLaughlin, K., Kendall, C., Silva, S., Stuart-Williams, H., Paytan, A., 2004. A precise method for the analysis of $\delta^{18}O$ of dissolved inorganic phosphate in seawater. Limnology and Oceanography Methods 2, 202—212.

McLaughlin, K., Chavez, F.P., Pennington, J.T., Paytan, A., 2006a. A time series investigation of the oxygen isotopic composition of dissolved inorganic phosphate in Monterey Bay. Limnology and Oceanography 51 (5).

McLaughlin, K., Kendall, C., Silva, S.R., Paytan, A., 2006b. Oxygen isotopes of phosphatic compounds — application for marine particulate matter, sediments and soils. Marine Chemistry 98, 148—155.

McLaughlin, K., Paytan, A., Kendall, C., Silva, S.R., 2006c. Phosphate oxygen isotopes as a tracer for sources and cycling of phosphate in North San Francisco Bay. Journal of Geophysical Research 111, G03003. doi:10.1029/2005JG000079.

Nicholson, D., Dyhrman, S., Chavez, F., Paytan, A., 2006. Alkaline phosphatase activity in the phytoplankton communities of Monterey Bay and San Francisco Bay. Limnology and Oceanography 51, 874—883.

Nixon, S.W., Oviatt, C., Frithsen, J., Sullivan, B., 1986. Nutrients and the productivity of estuarine and coastal marine ecosystems. Journal of the Limnology Society of South Africa 12, 43—71.

O'Neil, J.R., Vennemann, T.W., McKenzie, W.F., 2003. Effects of speciation on equilibrium fractionations and rates of oxygen isotope exchange between $(PO_4)_{aq}$ and H_2O. Geochimica et Cosmochimica Acta 67, 3135—3144.

Parkinson, J.A., Allen, S.E., 1975. Wet oxidation procedure suitable for determination of nitrogen and mineral nutrients in biological material. Communications in Soil Science and Plant Analysis 6, 1—11.

Paytan, A., Kolodny, Y., Neori, A., Luz, B., 2002. Rapid biologically mediated oxygen isotope exchange between water and phosphate. Global Biogeochemical Cycles 16, 1013.

Rabalais, N.N., Turner, R.E., Wiseman, W.J., 2002. Gulf of Mexico hypoxia, A.K.A. "The dead zone". Annual Review of Ecology and Systematics 33, 235—263.

Saunders, W.M.H., Williams, E.G., 1955. Observations on the determination of total organic phosphorus in soils. Journal of Soil Science 6, 254—267.

Sharp, J.H., 1991. Review of carbon, nitrogen, and phosphorus biogeochemistry. Reviews of Geophysics. Supplement 29, 648—657.

Shemesh, A., Kolodny, Y., Luz, B., 1983. Oxygen isotope variations in phosphate of biogenic apatites, II. Phosphorite rocks. Earth and Planetary Science Letters 64, 405—416.

Smith, R.E., 1973. The Hydrography of Elkhorn Slough, a Shallow California Coastal Embayment. Moss Landing Marine Laboratories Technical Publication, vol. 72, No. 2, 88 pp.

Smith, S.V., 1984. Phosphorus versus nitrogen limitation in the marine environment. Limnology and Oceanography 29, 1149—1160.

Street, J.H., Asbeck, L., Moore, W.S., Paytan, A., 2005. 226-Radium activity in bivalve shells as a tracer of paleo-groundwater input into Elkhorn Slough, California. Eos Transactions, American Geophysical Union, Fall Meeting Supplement 86, PP31B—PP1537.

Sundareshwar, P.V., Morris, J.T., Koepfler, E.K., Fornwalt, B., 2003. Phosphorus limitation of coastal ecosystem processes. Science 299, 563—565.

Thomson-Bulldis, A., Karl, D., 1998. Application of a novel method for phosphorus determinations in the oligotrophic North Pacific Ocean. Limnology and Oceanography 43, 1565—1577.

Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W.H., Sinberloff, D., Swackhamer, D., 2001. Forecasting agriculturally driven global environmental change. Science 292, 281—284.

Watanabe, F.S., Olsen, S.R., 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO3 extracts from soil. Soil Science Society of America Proceedings 29, 677—678.