Rapid biologically mediated oxygen isotope exchange between water and phosphate

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[1] In order to better constrain the rate of oxygen isotope exchange between water and phosphate via biochemical reactions a set of controlled experiments were conducted in 1988 at the Aquaculture Plant in Elat, Israel. Different species of algae and other organisms were grown in seawater tanks under controlled conditions, and the water temperature and oxygen isotopic composition ($\delta^{18}O_w$) were monitored. The oxygen isotopic composition of phosphate ($\delta^{18}O_p$) in the organisms' food source, tissues, and the $\delta^{18}O_p$ of dissolved inorganic phosphate (DIP) were measured at different stages of the experiments. Results indicate that intracellular oxygen isotope exchange between phosphorus compounds and water is very rapid and occurs at all levels of the food chain. Through these reactions the soft tissue $\delta^{18}O_p$ values become 23–26‰ higher than $\delta^{18}O_w$, and $\delta^{18}O_p$ values of DIP become ~20‰ higher than $\delta^{18}O_w$. No correlation between $\delta^{18}O_p$ values and either temperature or P concentrations in these experiments was observed. Our data imply that biogenic recycling and intracellular phosphorus turnover, which involves kinetic fractionation effects, are the major parameters controlling the $\delta^{18}O_p$ values of P compounds dissolved in aquatic systems. This information is fundamental to any application of $\delta^{18}O_p$ of dissolved organic or inorganic phosphate to quantify the dynamics of phosphorus cycling in aquatic systems. *INDEX TERMS:* 1615 Global Change: Biogeochemical processes (4805); 4825 Oceanography: Biological and Chemical: Geochemistry; 4845 Oceanography: Biological and Chemical: Nutrients and nutrient cycling; 4870 Oceanography: Biological and Chemical: Stable isotopes; *KEYWORDS:* phosphate, oxygen isotopes, nutrient cycling

1. Introduction

1.1. Marine Phosphorus Cycle

[2] Phosphorus (P) is considered to be a limiting nutrient in some oceanic systems [*Karl et al.*, 1995; *Cotner et al.*, 1997; *Wu et al.*, 2000] and is possibly the ultimate limiting macronutrient for marine productivity over long timescales [*Delaney*, 1998; *Tyrrell*, 1999]. An extensive summary of the global biogeochemical cycle of P in the ocean with emphasis on sources and sinks is given by *Delaney* [1998], and *Benitez-Nelson* [2000] reviewed the biogeochemical cycling of P within the oceans with particular focus on the composition and recycling rates of P. Despite the major role P plays in controlling marine productivity, relatively little is known about the rates of the biogeochemical and physical processes that control availability and recycling of P in marine systems.

[3] *Orrett and Karl* [1987] and *Karl and Tien* 1997, [and references therein] examined the concentration and turnover rates of soluble reactive P (SRP), soluble nonreactive P (SNP) and total dissolved P (TDP) in the North Pacific Subtropical Gyre. The major conclusions from this long-term effort are that the marine P cycle is unexpectedly complex and that the dynamics of the different P pools is likely to have an important influence on rates of total and export production and on the potential sequestration of atmospheric CO_2. *Lal and Lee* [1988], *Lee et al.* [1991], and *Benitez-Nelson and Buesseler* [1999] have measured the activities of cosmogenically produced ^{32}P and ^{33}P in TDP, SRP, and particulate P. They concluded that P turnover rates within the dissolved and particulate pools are rapid (<1 day to ~2 weeks) and vary over seasonal timescales, that certain dissolved organic P (DOP) compounds are preferentially utilized, and that P plays a significant role in supporting gross and export productivity. These studies, however, are very elaborate, require sampling of large quantities of seawater (>1000 L) and cannot be applied to all water masses because of the relatively short half-lives of the P radionuclides. An alternative easy analysis that will yield globally representative estimates of in situ P turnover rates in the oceans is needed.

[4] It has recently been suggested that variations in space and time of the oxygen isotopic composition of dissolved inorganic phosphate (DIP) in the oceans ($\delta^{18}O_p$) may provide an efficient tracer for quantifying the rates of biological and physical cycling of phosphate in oceans and estuaries [*Colman et al.*, 2000; *McLaughlin et al.*, 2000; *Stern and Wang*, 2000]. The principal concept here is that the isotopic signature ($\delta^{18}O_p$) of DIP entering the euphotic zone from rivers or from deepwater upwelling is considerably different from that of DIP in the euphotic zone (due to equilibration in water with different $\delta^{18}O_w$ and/or temperature). The isotopic signature of the DIP delivered into the euphotic zone will be gradually eliminated as phosphate is recycled through the biomass; therefore the $\delta^{18}O_p$ of DIP in the euphotic zone should be an efficient tracer of marine phosphorus cycling. The $\delta^{18}O_p$ value of DIP in the euphotic zone at any given place and time would depend on the relative rate of input of phosphate into the system and rate of isotopic exchange by turnover in the biomass. Therefore knowledge of the rates of isotopic exchange in biochemical processes and the parameters affecting this exchange

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($\delta^{18}O_w$, temperature, and P concentrations) is crucial for evaluating the applicability of $\delta^{18}O_p$ as a tracer for water column P dynamics. Results presented here aim to quantify these rates and parameters.

1.2. Oxygen Isotopic Composition of Phosphate

[5] Unlike carbon or nitrogen, P has only one stable isotope and therefore cannot be used as an isotopic tracer. However, because P in nature is found mainly as orthophosphate (PO_4^{3-}) and its derivatives [*Bieleski*, 1973], the oxygen atoms bound to P may prove to be useful isotopic tracers. The P-O bond in phosphate is resistant to inorganic hydrolysis over the range of Earth's surficial temperatures, so oxygen atoms bound to P do not readily exchange with the surrounding water and are relatively resistant to diagenetic alterations in sediments without biological mediation [*Tudge*, 1960; *Brodskii and Sulima*, 1953]. However, the P-O bond can be easily broken in enzyme-mediated biochemical reactions [*Dahms and Boyer*, 1973; *Boyer*, 1978]. Oxygen isotope ratios of phosphate in apatite from marine and terrestrial vertebrates and invertebrates indicate that biogenic apatite precipitates in isotopic equilibrium with environmental water at the temperature of deposition [*Longinelli*, 1966; *Longinelli and Nuti*, 1968; *Kolodny et al.*, 1983; *Shemesh et al.*, 1983; *Luz et al.*, 1984]. Bacterially mediated fractionation between P-bound oxygen and water appears to be governed by equilibrium reactions rather than kinetic factors, and the temperature-dependent isotopic equilibrium of apatite precipitated in microbial culture experiments with ambient water is the same as for apatite in marine and terrestrial vertebrates and invertebrates [*Blake et al.*, 1998].

[6] In soft tissues of marine organisms in their natural environment and in dissolved phosphate in seawater, no variation of $\delta^{18}O_p$was observed with either depth or latitude in the Atlantic and Pacific Oceans [*Longinelli et al.*, 1976], although for dissolved phosphate an isotopic difference was detected between Pacific (20.6‰) and Atlantic (19.7‰) samples. These values, which are ~20‰ higher than the seawater $\delta^{18}O$ ($\delta^{18}O_w$), appear to reflect steady state conditions for each oceanic basin and are controlled by kinetic isotopic fractionation attained in biological processes. However, it must be recognized that *Longinelli et al.* [1976] extracted P from seawater without prefiltration and used Fe-coated fibers that adsorb both inorganic and organic P, thus complicating any interpretation of their results. The $\delta^{18}O_p$ of organic tissues of various marine animals measured by *Longinelli et al.* [1976] were on average 3‰ higher than the $\delta^{18}O_p$ dissolved in seawater at the same location (thus ~23‰ higher than $\delta^{18}O_w$). Once more, this fractionation was attributed to biological metabolism of phosphate. No temperature dependences of the isotopic composition of oxygen in seawater dissolved phosphate or soft-tissue P compounds were observed.

[7] In order for $\delta^{18}O_p$ of phosphate dissolved in water to be an applicable tracer for phosphate cycling and dynamics, significant differences in space and time of the $\delta^{18}O_p$ of DIP should be observed. Such variations may result from differences in the $\delta^{18}O$ of the water with which the phosphate oxygen has exchanged and possibly from differences in the temperature of equilibration. Examples for such settings include input of phosphate that equilibrated in waters with significantly different $\delta^{18}O_w$ than seawater (rivers or glacial ice melt) or input of phosphate from sources with significantly different temperatures (upwelling of cold deep water). Furthermore, the rate of isotopic exchange that occurs via turnover of phosphate within the dissolved and particulate pools (through the biomass) will determine how close to equilibrium with water the DIP will be at any given temperature. The faster the phosphate turnover and oxygen exchange rates are, relative to the input rate of new phosphate into the euphotic zone, the closer the $\delta^{18}O_p$ of DIP is to the equilibrium value. Therefore some knowledge of the rate of oxygen isotope exchange between DIP and ambient water through biochemical reactions is required for better appreciation of the potential applicability of this new tracer. Moreover, the degree of temperature dependence of the isotopic equilibration should be determined for evaluation of possible temporal changes in $\delta^{18}O_p$ of DIP at any given location. Therefore the objectives of this research were to get an appreciation of the oxygen isotope exchange rate, or how long it takes for phosphate in organic matter or DIP to achieve isotopic equilibrium with surrounding water. We have conducted a set of experiments in controlled systems; some of the parameters that could potentially influence this exchange were monitored, and the effect they have on the isotope exchange was evaluated. Although such experiments are gross oversimplifications of natural systems, it is simple to estimate the isotopic exchange rate and to get a general appreciation for the parameters affecting this exchange in these systems because all the P input sources (rates and isotopic composition) are well controlled. Results provide a first approximation for oxygen isotope exchange rates and their sensitivity to environmental parameters in natural systems.

2. Experimental Setup

[8] Algae (*Ulva sp*. and *Gracilaria conforta*) were grown in 600 L plastic containers with water exchange rate of 100 L/h, as part of an experiment to identify biofilters for marine fishpond effluents (see *Neori et al.* [1991] for details). The water supply for some of the containers came directly from the Gulf of Aqaba without filtration, containing ~0.5 μM SRP (as well as bacteria and potentially other microorganisms). SRP is defined here as material which passes through a 0.2 μm pore size filter and forms phosphomolybdate under acidic conditions following the procedure of *Strickland and Parsons* [1972]. SRP may thus include acide-labile DOP compounds, while DIP pertained only to the inorganic fraction of the dissolved P pool [*Benitez-Nelson*, 2000]. Operationally, DIP in this work is the dissolved phosphate that binds to the chitosan-ammonium-molybdate (CAM) columns that contain no surplus molybdate at pH of ~4 (see section 3 and *Muzzarelli and Spalla* [1972]). The water supply to the containers was stopped once a week for 18 hours, and a fertilizer (0.15 mM NaH_2PO_4 with a $\delta^{18}O_p$ value of 6‰) was added to each container. The $\delta^{18}O_p$ of DIP in seawater from the Gulf of Aqaba was 20.6‰. The rest of the containers received recycled water from fishponds (containing 3–7.5 μMSRP); no fertilizer was added to these containers, and the algae grew at steady state conditions utilizing the P dissolved in the fishpond water. *Nannochloropsis*microalgae were grown in plastic "sleeves" in an enriched medium containing 320 μM of the fertilizer described above. Algal biomass in all settings doubled in less than a week.

[9] Rotifers were grown for 4 days in 20 L tanks and given food sources (algae and yeast) with different $\delta^{18}O_p$ values. The rotifer growth rate was ~20% per day (E. Lubznes, personal communication, 1988), indicating that after 4 days, the major P source for the rotifers was from the food.

[10] Two 100 m^3 ponds containing fish (*Sparus aurata*), macroalgae, phytoplankton, and clams were monitored throughout the year (see *Neori et al.* [1989] for operation protocol). The ponds were filled with seawater pumped from the Gulf of Aqaba (300 m offshore, 20 m depth) at a rate of 2 m^3/h (water residence time in the ponds ~2 days). Once a day, the fish in the pond were fed 5 kg of fish meal pellets. The dissolved P did not come directly from dissolution of pellets, which were rapidly consumed. Previous work suggests that 85% of the P in these ponds is recycled (A. Neori and M. D. Krom, personal communication, 1988). The SRP concentrations in the ponds ranged between 3 and 7.5 μM depending predominantly on phytoplankton bloom conditions [*Krom and Neori*, 1989].

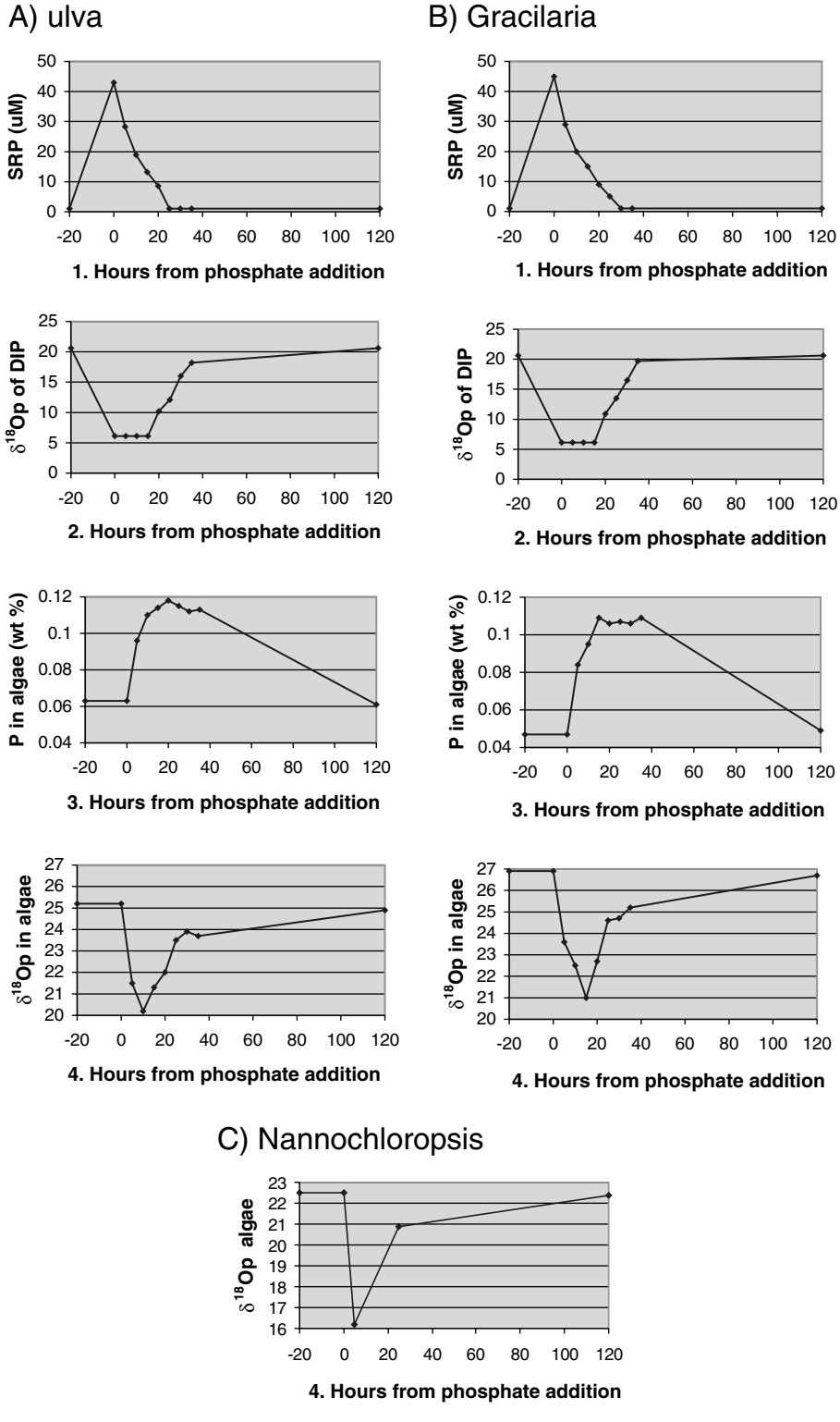

Figure 1. Changes in P concentrations (μM) and $\delta^{18}O_p$ (‰) values in the water (1 and 2, respectively) and algae (3 and 4, respectively) through time after fertilizer addition in the 600 L growth containers. Time zero indicates the time of fertilizer addition, and the scale is in hours from fertilization. (a) *Ulva* sp. growth experiments, (b) experiments with *Gracilaria conforta*, and (c) *Nannochloropsis*. The $\delta^{18}O_w$ in these experiments was 1.3‰.

Table 1. Phosphorus Concentrations and $\delta^{18}O_p$ of Algae and of DIP in the Water in Containers with Algae Growing at Steady State Conditions in Recycled Fishpond Water (No Fertilization)[a]

Date	Temperature, °C	SRP Water, μ*M*	$\delta^{18}O_p$ SRP, ‰	P Concentration *Ulva*, wt %	$\delta^{18}O_p$ *Ulva*, ‰	$\delta^{18}O_p$ *Gracilaria*, ‰
28 March 1988	21	3.6	18.2	0.185	24.2	25.2
20 April 1988	17	3.3	18.4	0.187	24.8	25.6
13 May 1988	22	3.2	18.6	0.182	24.7	25.0
22 May 1988	20	3.6	17.6	0.196	23.7	24.8
Mean (±SD)		3.4 (0.2)	18.2 (0.4)	0.187 (0.005)	24.3 (0.4)	25.1 (0.3)

[a] The $\delta^{18}O_w$ in the containers was 1.3‰.

[11] The $\delta^{18}O_p$ values of alga, fish, rotifers, and clams as well as that of feed pellets were analyzed monthly between January and April of 1988 (see Tables 3a and 3b). In the alga growth experiments the P concentrations in the algae (weight %) were also measured. The $\delta^{18}O_p$ of phosphate dissolved in seawater from the growth containers and the SRP concentration in these waters were determined, and the growth water temperature and oxygen isotopic compositions ($\delta^{18}O_w$) were monitored.

3. Analytical Methods

[12] Phosphate from fish meal, apatite, and NaH_2PO_4 used as the fertilizer in the algae ponds was separated and purified for $\delta^{18}O_p$analysis using sequential precipitation as ammonium-phosphomolybdate (APM), magnesium-ammonium-phosphate (MAP); the P conversion yield for this procedure is 100% [*Tudge*, 1960; *Longinelli*, 1966; *Kolodny et al.*, 1983]. Organic matter was washed with distilled water, dried in a 50°C oven, and treated with 10 *M* HNO_3 at 60°C prior to phosphate separation and purification; all of the P associated with organic matter in samples used here was converted to PO_4 in this leaching step [*Longinelli et al.*, 1976]. This operationally defined organic phosphorus fraction includes also intracellular DOP and DIP, which are considered here as part of the biogenic P pool in algae. Dissolved phosphate was separated from seawater (in the growth containers and the Gulf of Aqaba) using CAM columns [*Muzzarelli and Spalla*, 1972; *Paytan*, 1989]. "Beads" were prepared from chitosan flakes dissolved in acetic acid and dripped into an ammonium-molybdate bath. The beads were loaded into 5 × 55 cm PVC columns, and 150–400 L of prefiltered (through a 0.5 μm Hytrex filter) and acidified (with 0.002 *M* H_2SO_4 to pH 4) water was passed at a rate of ~300 mL/min to recover enough dissolved phosphate from the samples (~60% yield). This procedure involves P complexation to molybdate without surplus molybdate at a pH of 4 (not too acidic), thus reducing the potential of DOP hydrolysis [*Dick and Tabatabai*, 1977]; however, any reactive DOP that is converted to PO_4^{3-} as a result of acidification of seawater to pH 4 will also be adsorbed. Phosphate was leached off the columns with ammonia and reacted with HNO_3 at 50°C to precipitate APM that was purified and precipitated as $BiPO_4$ [*Tudge*, 1960]. Oxygen was liberated from $BiPO_4$ by reaction with BrF_5 in a vacuum line at 250°C and converted to CO_2 for mass spectrometer measurements (Micromass V.G. 602). The reproducibility of the $\delta^{18}O_p$ isotope analysis is 0.4‰; this was the typical analytical error involved with this method ~15 years ago when much of these data were accumulated [*Shemesh et al.*, 1983]. All the results reported here are the mean values of three or more analyses of the same sample.

[13] SRP concentrations were measured in the samples by the method of per sulfate oxidation at low pH, using an autoanalyzer [*Afghan*, 1982; *Krom et al.*, 1985]. Water samples were filtered through a 0.7 μm GFF filter before analysis.

4. Results and Discussion

[14] The extent and rate of oxygen isotopic exchange between phosphate and water during uptake and biosynthesis in different phyla of primary producers was evaluated in the algal growth experiments. Changes in SRP concentrations in the growth water and their δ^{18}Op values as well as those of intracellular P in the algae through time after fertilization are presented in Figure 1. The SRP concentrations and $\delta^{18}O_p$ values in water and intracellular P of algae growing at steady state conditions in recycled fishpond water (no P fertilization) are presented in Table 1.

[15] In the fertilization experiments, upon addition of fertilizer (time = 0) and stopping the water circulation, the SRP concentrations increased from <1 to 43 μ*M*, and within the first 18 hours (before circulation was resumed) the concentration dropped down to 8.6 μ*M* (Figures 1a, 1, and 1b, 1). At the same time the P concentration in the algae increased dramatically (from 0.063 to 0.118% in *Ulva* and from 0.047 to 0.098% in *Gracilaria*) (Figures 1a, 3, and 1b, 3). This indicates that the algae consumed ~80% of the added phosphate within the first 18 hours after fertilization. As circulation resumed, phosphate concentrations dropped to background levels due to both continuous uptake by algae and dilution by low phosphate seawater (washout). Over the next few days (without further fertilizer addition), cellular P concentration (per gram algae) decreased, and after a week, it reached levels of 0.06 and 0.05% in *Ulva*and *Gracilaria*, respectively. The $\delta^{18}O_p$ of fertilizer added was 6‰, and that of seawater phosphate, prior to fertilizer addition, was 20.6‰. As expected, upon addition of the fertilizer the $\delta^{18}O_p$ of DIP in the containers dropped to 6.1‰, as the fertilizer contributed over 97% of the phosphate in the system. It stayed at this ratio until circulation resumed, the fertilizer was washed out, and the main P source was from the circulating seawater, thus controlling the $\delta^{18}O_p$ value. The $\delta^{18}O_p$ of the algae also changed with time. First, it decreased sharply because of uptake of the fertilizer phosphate ($\delta 18O_p$ 6‰), and then it increased as oxygen was exchanged via intracellular phosphate turnover and processing. This increase started before circulation was resumed (between 10 and 15 hours after fertilization) and continued to increase after the circulation recommenced. A similar increase was observed in the *Nannochloropsis* growth experiment where water was not exchanged (aeration by bubbling only). Within ~30 hours from fertilization the $\delta^{18}O_p$ in algae almost equaled the original prefertilization level. In the steady state growth experiments (Table 1) the P content and $\delta^{18}O_p$ in the algae remained relatively constant throughout the sampling period, with *Gracilaria*being ~1‰ higher than *Ulva* grown at the same conditions. No correlation between $\delta^{18}O_p$of the algae or that of SRP in the growth containers with temper-

Table 2. The $\delta^{18}O_p$ in Rotifers After 4 days of Food Ingestion and That of Their Food Source

Food Source	$\delta^{18}O_p$ Food, ‰	$\delta^{18}O_w$ Water, ‰	$\delta^{18}O_p$ Rotifiers, ‰
Yeast	20.1	1.3	19.4
Algae	22.2	1.3	19.4
Algae	29.8	1.3	19.4

ature was observed over the small temperature ranges represented in these experiments (17°–22°C).

[16] The $\delta^{18}O_p$ values of the algae in these experiments were considerably higher than those of the fertilizer or those of SRP in the growth water. Simple mass and isotope balance considerations suggest that the $\delta^{18}O_p$ of the algae cannot be explained solely by mixing of two P reservoirs with different $\delta^{18}O_p$ values, the fertilizer (6‰) and the algae P pool before fertilization (25.2 or 26.9‰ for the different algae). The P concentration in the algae doubled (from 0.063 to 0.118% in *Ulva* and from 0.047 to 0.098% in *Gracilaria*) within the first few hours from fertilizer addition; therefore simple mixing would result in $\delta^{18}O_p$ of ~16‰. However, the observed $\delta^{18}O_p$values in the algae throughout the experiment are higher than expected from just mixing, indicating very rapid exchange of intracellular P-bound oxygen with that of water. As phosphate entered the cells initially, some but not all of the oxygen atoms were exchanged. Hence some fraction of the O atoms of the intracellular P pool is from the phosphate source utilized (fertilizer in our case). Over time, the P inside the cells was continuously processed and cycled, resulting in oxygen isotope exchange and resetting the initial fertilizer's oxygen isotope ratio. Within 30 hours from fertilizer addition the $\delta^{18}O_p$ of algae P reached constant values that were ~23–25‰ higher than the surrounding $\delta^{18}O_w$. This trend was similar for all three organisms examined (algae of different phyla). Therefore the $\delta^{18}O_p$ in the algae at any given time appears to depend on the relative rates of phosphate uptake and intracellular P cycling. In our experiments the initial uptake rate was relatively high, resulting in a sharp minimum in $\delta^{18}O_p$ soon after the fertilizer was added. This was followed by an increase in $\delta^{18}O_p$ due to continuous P turnover in the cell and reduction in the uptake rate of fertilizer phosphate due to washout. Complete exchange was achieved within the first 35 hours from fertilization, and no trace of the original fertilizer $\delta^{18}O_p$ was left. This is consistent with results obtained in small-scale laboratory experiments: *Avron et al.* [1964], *Bieleski* [1972, 1973], and *Boyer* [1978] investigated the O exchange between P and water during biochemical processing in algae using labeled phosphate ($P^{18}O_4$) and/or water ($H_2{}^{18}O$) and found that all the oxygen in P exchanged with water within 48 hours.

[17] If the above scenario of complete and rapid exchange is correct, it is expected that once all the phosphorus-bound oxygen in the cells has exchanged and equilibrated with the surrounding water, a constant $\delta^{18}O_p$ will be attained for all organisms at stable conditions of temperature and $\delta^{18}O_w$. A cautious look at the results indicates, however, that this was not the case; for example, $\delta^{18}O_p$ values of *Ulva* sp. and *Gracilaria conforta* grown at the same conditions (Table 2) showed a difference of ~1‰. Even for the same species of algae grown at stable conditions of temperature and $\delta^{18}O_w$ (for example, *Ulva* in the fertilization experiments), the $\delta^{18}O_p$ value seemed to increase by ~2‰ between 48 hours after fertilization (presumably complete equilibrium) and the next few days (Figures 1a, 4, and 1b, 4). The reason for the observed variability is embedded in the sample processing and the nature and amount of P compounds in the cells. Phosphorus in living organisms is present in a whole range of compounds [*Bieleski*, 1972]; in some of these compounds the phosphorus to oxygen ratio is not 1 to 4 (for example, polyphosphates) or the phosphorus is bound to less than four oxygen atoms (for example, phosphonates with C-P bonds). The preparation of samples for isotope analyses involves conversion of all phosphorus to orthophosphate (P bound to four oxygen atoms). Therefore some oxygen from the reagent solutions used (typically with low $\delta^{18}O$ values as they are prepared with fresh water) was incorporated into the sample [*Blake et al.*, 1997]. The effect of this incorporation depends on the content of P compounds not bound to four oxygen atoms in the cell, and this will vary from species to species and even within one species depending on the nutritional status and phosphate availability. For example, when P-deficient algae are exposed to high levels of phosphate, they rapidly take up any available phosphate in excess of their nutritional requirements [*Aitchinson and Butt*, 1973] and store this P in the cell as polyphosphate chains [*Perry*, 1976]. As the algae grow, if phosphate concentrations in the medium are low, they utilize the stored polyphosphate and transform it to other compounds such as nucleic acids, lipids, etc., until the polyphosphate storage is completely exhausted [*Fitzgerald and Nelson*, 1966]. Using reagents prepared with ^{18}O-spiked water and simple mass balance calculations, we have confirmed that up to 10% (in algae with abundant polyphosphate) of the oxygen bound to the phosphate analyzed originated in the reagent [*Paytan*, 1989]. This explains the apparent increase in $\delta^{18}O_p$ after complete exchange has been achieved (by 48 hours from fertilization) as well as the differences in $\delta^{18}O_p$ of different algae grown in similar conditions. Specifically what one records is partial incorporation of reagent oxygen into the orthophosphate analyzed; the degree of this incorporation depends on the proportions and concentrations of different P compounds in the cell which differ between different organisms and in any organism with time. In the future this problem could potentially be overcome by treating homogenous splits of each sample using spiked reagents with

Table 3a. The $\delta^{18}O_p$ of Organisms in Intensive Seawater Fishponds[a]

Organism	Date	Temperature, °C	$\delta^{18}O_p$, ‰
Fish bone	8 Jan. 1988	20.0	21.1
Fish bone	31 Jan. 1988	18.5	21.7
Fish bone	18 Feb. 1988	19.0	21.9
Fish bone	28 March 1988	21.0	22.3
Mean (±SD)			21.7 (0.5)
Fish flesh	8 Jan. 1988	20.0	25.1
Fish flesh	31 Jan. 1988	18.5	23.8
Fish flesh	18 Feb. 1988	19.0	24.3
Fish flesh	28 March 1988	21.0	24.0
Fish flesh	20 April 1988	17.1	23.0
Mean (±SD)			24.0 (0.7)
Clamshell	8 Jan. 1988	20.0	21.0
Clamshell	31 Jan. 1988	18.5	21.6
Clamshell	8 Feb. 1988	19.0	21.4
Mean (±SD)			21.3 (0.3)
Clam flesh	8 Jan. 1988	20.0	24.0
Clam flesh	31 Jan. 1988	18.5	23.2
Mean (±SD)			23.6 (0.6)
Algae			
Pond B1	8 Jan. 1988	20.0	21.3
Pond B3	8 Jan. 1988	20.0	22.7
Pond B3	31 Jan. 1988	18.5	20.5
Pond B3	18 Feb. 1988	19.0	22.2
Pond B3	28 March 1988	21.0	19.6
Mean (±SD)			21.3 (1.2)

[a] The $\delta^{18}O_p$ of fish meal given to the fish was 20.8‰.

Table 3b. The $\delta^{18}O_p$ of SRP in Water

Date	Pond	Temperature, °C	SRP Water, μ*M*	$\delta^{18}O_p$ Water, ‰
20 Nov. 1987	B3	22.5	3.0	20.6
7 Jan. 1988	B3	20.0	5.4	19.9
7 Jan. 1988	B1	19.0	7.5	20.6
31 Jan. 1988	B1	18.5	4.2	20.4
18 Feb. 1988	B3	19.0	5.8	20.8
18 Feb. 1988	B3	19.0	5.8	21.2
18 Feb. 1988	B3	19.0	5.8	21.1
18 Feb. 1988	B3	19.0	5.8	20.7
18 Feb. 1988	B3	19.0	5.8	20.7
28 March 1988	B3	21.0	6.3	18.9
28 March 1988	B1	21.0	6.0	19.4
20 April 1988	B3	17.1	3.7	20.6
20 April 1988	B1	16.8	3.5	20.0
Mean (±SD)				20.4 (0.7)

known and different isotope values and correcting for the incorporation.

[18] To examine whether similar exchange between phosphate and water occurs during P processing in higher levels of the food chain, we analyzed the $\delta^{18}O_p$ of rotifers that were given food sources with different $\delta^{18}O_p$ values (Table 2). The $\delta^{18}O_p$ of rotifers after 4 days of food ingestion was 19.4‰ regardless of the $\delta^{18}O_p$ of food source, indicating that the $\delta^{18}O_p$ in rotifers was not affected by the food source. These results suggest that isotopic exchange between water and P occurs through biochemical reactions at higher levels of the food chain.

[19] We also analyzed the $\delta^{18}O_p$ of different organisms grown in fishponds (Table 3a) and that of DIP in the ponds (Table 3b); the temperature and SRP concentrations in the ponds were monitored. The fishponds' systems were somewhat more representative of natural conditions: they included more than one organism; they were maintained for many months (without harvesting or draining); and the temperature and other parameters (nutrient concentrations, biomass, oxygen levels, etc.) were allowed to fluctuate "naturally" within these systems. The P dissolved in the ponds, which ranged between 3 and 7.5 μ*M*, was predominantly from fish meal that has been recycled through the biomass (Neori and Krom, personal communication, 1988); Gulf of Aqaba water typically contains < 0.5 μ*M* SRP. The $\delta^{18}O_p$ of this food source (20.3 ± 0.4‰) was almost identical to that dissolved in the fishpond water (20.4‰). Although it was clear that the phosphate dissolved in these ponds has undergone some biochemical processing within the pond's biomass before entering the dissolved phosphate pool [*Krom et al.*, 1985], it was impossible to estimate the degree of P cycling in these ponds using $\delta^{18}O_p$ because the isotopic composition of P added to the ponds (as feed pellets) was similar to the $\delta^{18}O_p$ of the phosphate that has undergone isotopic exchange by biochemical processes and which now comprised the dissolved phosphate in the pond's water. However, experiments to evaluate the influence of food $\delta^{18}O_p$ on fish bone, done by *Kolodny et al.* [1983], show that $\delta^{18}O_p$ in equilibrium with $\delta^{18}O_w$ was sharply different from that of the food. The temperature in the ponds during the sampling period varied between 16.8° and 22.5°C, and the SRP concentrations ranged between 3 and 7.5 μ*M*. No obvious correlation between the $\delta^{18}O_p$ of soft tissue or of DIP with either temperature or SRP concentrations represented by the relatively small range in the ponds was observed. These results, if indeed representative of natural systems, are in agreement with the conclusion arrived by *Longinelli et al.* [1976]: that the $\delta^{18}O_p$ of both SRP and organic phosphorus compounds is controlled by kinetic, non-temperature-dependent, biogenic fractionation rather than equilibrium isotopic exchange between phosphate and water or that the temperature effect on fractionation, if it exists, is not apparent and could not be resolved in the small range of temperatures examined here considering the precision of our measurements.

[20] Consider now the $\delta^{18}O_p$ values of the different organisms living in the ponds. The mean $\delta^{18}O_p$ of fish bone (21.8‰) and clamshells (21.3‰) indicated isotope equilibration at a temperature of ~22°C (using $t = 111.4 - 4.3(\delta^{18}O_p - \delta^{18}O_w)$ from *Longinelli and Nuti* [1973]). This is a reasonable estimate for the mean temperature to which the fish and clams were exposed over their life span. The $\delta^{18}O$ of the apatite in the fish bone and clamshells was ~1.4‰ higher than the $\delta^{18}O_p$ of phosphate dissolved in pond water (20.4‰). This could be due to the higher turnover rates of DIP (relative to bone or shell phosphate); thus DIP may represent equilibrium at temperature conditions averaging a much shorter time span (days to weeks). However, the temperatures of the pond water during the sampling months (January to April) were consistently lower than 22°C. If indeed $\delta^{18}O_p$ of DIP is governed by equilibrium reactions with the same temperature dependence as for apatite, we would expect higher $\delta^{18}O_p$ for the DIP contrary to observations. Alternatively, this may indicate that the isotope exchange reactions involved in determining the $\delta^{18}O_p$ of DIP are not dependent exclusively on temperature, at least within the narrow temperature range recorded in these ponds or that different fractionation factors are involved in the formation of different P-bearing species. The above conclusions differ from those arrived by *Blake et al.* [1997] for microbially mediated apatite precipitation experiments. However, those experiments represent a much wider temperature range (20°–35°C) compared to the temperature range recorded in our work (16.8°–22.5°C) and were conducted at higher P concentrations (5–10 m*M*) than observed in seawater. Moreover, results from experiments in inorganic phosphate growth medium at 25°C after 68 days record a wide range in the fractionation factor (α), suggesting that these reactions are not governed solely by simple thermodynamic fractionation factors [*Blake et al.*, 1997] (Table 1) and that more complex processes of oxygen isotope fractionations are observed (consistent with *Blake et al.* [1998] and *O'Neil et al.* [2001]).

[21] Soft-tissue $\delta^{18}O_p$ values in these organisms were ~2‰ higher than $\delta^{18}O_p$ of their hard parts (apatite). The difference in the $\delta^{18}O_p$between skeletal phosphate and phosphorus compounds in organic tissue in a single organism might indicate that the biochemical/physiological processes and related isotope fractionation effects involved in the synthesis of these compounds are different. These results could not be explained by partial incorporation of reagent oxygen into the organic compounds because this would result in lower $\delta^{18}O_p$, contrary to the observed results. All $\delta^{18}O_p$ values measured for different organisms in the pond (Table 3a) were higher than the $\delta^{18}O_p$ of DIP in pond water, and the latter was ~20‰ higher than $\delta^{18}O_w$ ($\delta^{18}O_w$ = ~1.5‰). This difference suggests that some degree of isotope exchange occurs between organic phosphorus compounds and water during the regeneration of these compounds and conversion into the SRP pool. Such exchange has also been observed in microbial culture experiments [*Blake et al.*, 1998].

[22] Finally, to compare results obtained from our experiment with observations from a natural system, the $\delta^{18}O_p$ values of DIP in waters from the Gulf of Aqaba were determined. During two different cruises aboard the RV *Trevos* in the summer months of 1988, 400 L of water from a depth of 550 m were processes through CAM columns (SRP concentration were 0.5 μ*M*). The $\delta^{18}O_p$ value measured in the first cruise was 19.8‰, and in the second it was 20.4‰. These values are similar to results reported by *Longinelli et al.* [1976] for the Tyrhenian Sea

(20.0‰) and also for the Atlantic (19.7‰). It is not possible to draw many conclusions regarding the effects of temperature, phosphate concentration, or productivity on $\delta^{18}O_p$ of marine DIP from the two samples analyzed here. However, the similarity to results obtained for very different locations and periods is encouraging and generally supports the conclusions arrived at by *Longinelli et al.* [1976], which suggest that biological kinetic fractionation processes affect these isotope ratios. To get a better understanding of the coupling between $\delta^{18}O_p$ in DIP and the marine P cycle, a systematic, extensive sampling program to determine $\delta^{18}O_p$ of DIP in seawater at different locations, depths, and seasons is required along with more controlled laboratory experiments to determine the degree of temperature influences on the exchange reactions. Such experiments are currently underway.

5. Conclusions

[23] Oxygen isotope exchange between P compounds and water occurs in different organisms, at all levels of the food chain. These reactions are very rapid; complete exchange of all P-bound oxygen in algae occurred in <48 hours. The exchange mechanisms involved are most likely different from the equilibrium exchange observed for biogenic and nonbiogenic apatite. Within the small range of temperatures sampled, considering the precision of our measurements, no temperature effect on the isotopic fractionation was resolved. The $\delta^{18}O_p$ values of organic tissue P were ~23–26‰ higher than those of the surrounding water. The $\delta^{18}O_p$ of dissolved inorganic phosphate were ~20‰ higher than $\delta^{18}O_w$.

[24] Phosphorus cycling and turnover are complex processes that involve transformation between and within the POP, DOP, and DIP pools, all of which involve oxygen isotope exchange reactions. These processes, which most likely control the $\delta^{18}O_p$ values of DIP in seawater and other aquatic systems, seem to be determined either by kinetic isotope effect or by thermodynamic fractionation which involves a relatively small temperature effect. Data obtained here provide estimates for the rates by which oxygen isotope exchange with surrounding water occurs via enzyme-mediated reactions (fast) and determines some of the environmental processes influencing this exchange ($\delta^{18}O_w$). These results are important for assessing the utility of $\delta^{18}O_p$ of DIP as a tracer for P cycling in the ocean and suggest that this proxy is most likely to work only in areas where the input rate of new phosphate into the euphotic zone is substantially faster than the uptake and equilibration rate. Moreover, applications of $\delta^{18}O_p$ of DIP for studying oceanic P cycling relay on temperature-dependent equilibrium effects [*et al.*, 2000] that may be hard to resolve in the temperature range of surface seawater considering the complex processes of biologically mediated oxygen isotope fractionations involved [*Blake et al.*, 1998]. On the other hand, $\delta^{18}O_p$ of DIP or POP will reflect the $\delta^{18}O$ of the water in which they cycle through biochemical reactions.

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