

## BOX-MODELING OF BONE AND TOOTH PHOSPHATE OXYGEN ISOTOPE COMPOSITIONS AS A FUNCTION OF ENVIRONMENTAL AND PHYSIOLOGICAL PARAMETERS\*

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A time-dependent box model is developed to calculate oxygen isotope compositions of bone phosphate as a function of environmental and physiological parameters. Input and output oxygen fluxes related to body water and bone reservoirs are scaled to the body mass. The oxygen fluxes are evaluated by stoichiometric scaling to the calcium accretion and resorption rates, assuming a pure hydroxylapatite composition for the bone and tooth mineral. The model shows how the diet composition, body mass, ambient relative humidity and temperature may control the oxygen isotope composition of bone phosphate. The model also computes how bones and teeth record short-term variations in relative humidity, air temperature and  $\delta^{18}\text{O}$  of drinking water, depending on body mass. The documented diversity of oxygen isotope fractionation equations for vertebrates is accounted for by our model when for each specimen the physiological and diet parameters are adjusted in the living range of environmental conditions.

*Keywords:* Body water; Bone; Box-modeling; Oxygen isotope; Paleoenvironment; Phosphate; Tooth

### INTRODUCTION

Stable isotope compositions of phosphatic tissues from terrestrial vertebrates have been recognized as pertinent proxies of their living environments ever since Longinelli's pioneer work [1]. Thereafter numerous studies have attempted to assess the ecological parameters of various fossil animals from their oxygen, carbon or nitrogen isotope contents [2]. Nevertheless, empirical studies of the phosphate oxygen isotope composition of various animals, mainly mammals, revealed complex relationships with environmental parameters such as surface water or rainfall compositions, air temperature, and relative humidity [3–5]. Moreover, the environmental record is disturbed by the metabolic activity and diet of animals [6, 7]. Modeling the isotopic composition of phosphatic tissues is a remarkable approach to con-

\* The code to perform calculations is available upon request to the first author.

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strain the respective influences of the environmental, physiological and behavioural parameters. Indeed, the oxygen isotope records in biogenic apatites (bone, tooth) have been extensively used as proxies of past surface water isotopic values [8], themselves known to be related to mean air temperatures [9–11].

Some authors [7, 12, 13] have already attempted to model the relationships between the environmental parameters and the  $\delta^{18}\text{O}$  value of body water. In the frame of these models, body water is treated as a unique reservoir exchanging oxygen isotopes with the environment, which is itself considered as an infinite reservoir. The  $\delta^{18}\text{O}$  value of the phosphatic tissue is calculated from the body water composition taking into account a temperature-dependent fractionation factor derived from the equation proposed by Longinelli and Nuti [14]. In our time-dependent box-model, the animal reservoirs are the bone mineral, the tooth enamel and the body water, that are connected by elemental and isotopic fluxes of bone accretion, bone resorption, and tooth enamel accretion. These body reservoirs are themselves connected by fluxes with external reservoirs that contain oxygen sources used during the biological activity (Fig. 1). In this study, we investigate how the oxygen isotope composition of hydroxylapatite from bones and teeth is influenced by animal diet, general metabolism, ambient air temperature and relative humidity. We also attempt to quantify how time-dependent variations in the  $\delta^{18}\text{O}$  of external sources (*e.g.* water, food) are recorded in tooth enamel and bone, which are characterized respectively by incremental growth and permanent reworking. Therefore, we explore the properties and limits of this model for predicting oxygen isotope fractionations between animal bone or tooth and its environment through several case studies (fish, rats, horses, deers) for which experimental or empirical fractionation equations already have been established.

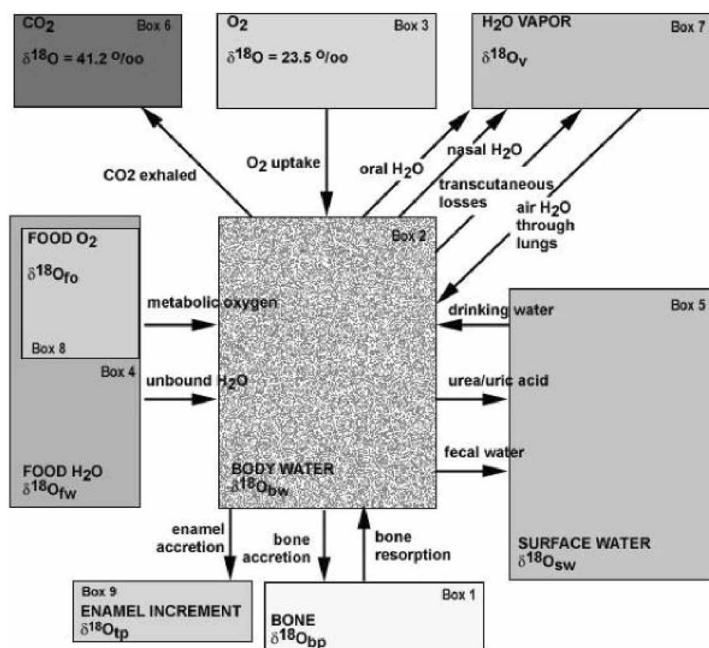


FIGURE 1 Schematic diagram showing the oxygen fluxes connecting the various reservoirs that control the oxygen isotope composition of animal bones. Oxygen fluxes and sizes of the mineral and body water reservoirs are scaled to the animal body mass. The  $\delta^{18}\text{O}$  values of atmospheric carbon dioxide and molecular oxygen may be considered as constant at the scale of animal lives. bp: bone phosphate; tp: tooth phosphate; bw: body water; v: vapor water; fo: food-bound oxygen; fw: food water; sw: drinking surface water.

## FORMULATION OF THE MODEL

### The Mathematical Formalism

The different reservoirs and connecting oxygen fluxes of the model are represented in Figure 1. The mass transfer between reservoirs is treated by first-order kinetic reactions [15]. Using the same formalism as used by Albarède *et al.* [16], the mass conservation of oxygen for a reservoir  $i$  leads to define:

$$\frac{dM_i}{dt} = \sum_{j \neq i} Q_{j \rightarrow i} - \sum_{j \neq i} Q_{i \rightarrow j}, \quad (1)$$

where  $M_i$  is the mass of the box  $i$ ,  $t$  is the time and  $Q_{i \rightarrow j}$  is the mass flux from box  $i$  to box  $j$ . The mass conservation of  $^{18}\text{O}$  with the concentration  $C_i$  in the reservoir  $i$  is

$$\frac{dC_i}{dt} = - \left[ \sum_{i \neq j} \frac{Q_{i \rightarrow j} K_{i \rightarrow j}}{M_i} + \sum_{i \neq j} \frac{Q_{j \rightarrow i} - Q_{i \rightarrow j}}{M_i} \right] C_i + \frac{\sum_{j \neq i} Q_{j \rightarrow i} K_{j \rightarrow i}}{M_i} C_j, \quad (2)$$

where  $K_{i \rightarrow j}$  is the enrichment factor of  $^{18}\text{O}$  due to the fractionation of oxygen isotopes upon transferring from box  $i$  to box  $j$ . All isotopic values are expressed on the  $\delta$  scale in ‰ SMOW:  $\delta^{18}\text{O} = 1000[(R_{\text{sample}}/R_{\text{standard}}) - 1]$ , where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the molar ratios of the heavier to the lighter isotopes.

### Oxygen Fluxes between Reservoirs

Oxygen fluxes are scaled to the animal energetic requirements, according to the method developed by Kohn [12]. The oxygen need of a given animal is estimated on the basis of its metabolic rate that relates with the body mass,  $M$ , as a power function:  $E = aM^b$ . The  $a$  and  $b$  coefficients are assigned depending on the animal and its diet (*e.g.* herbivorous mammal, carnivorous mammal, rodent, reptile, fish), according to equations reported in [17–20] (see Tab. I). Atmospheric oxygen uptake is proportional to the energy requirement by using a given oxygen conversion factor [12]. According to Kohn's model [12], all environmental fluxes are based on this basic scaling law, and are calculated on the basis of the physiological parameters summarized in Table I. Input oxygen fluxes to the animal body are (1) air through the lungs and associated water vapor, (2) food, and (3) drinking water (see more details in Fig. 1). Output oxygen fluxes from the animal body are (1) faeces and urinary water, (2) urea or uric acid, (3) transcutaneous and exhaled water vapor (via respiration or, for some mammals like carnivores, via panting), and (4) expired carbon dioxide.

The oxygen fluxes between body water and bone phosphate have been stoichiometrically calculated from calcium fluxes considering the phosphate group ( $\text{PO}_4^{3-}$ ) of the bone apatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ). We thus estimate the phosphate oxygen content of bone from its calcium content which is 1.65% of the total body weight according to [21]. In the absence of better constrained data, this value was used for all the studied species. Calcium fluxes for different kinds of animals have been compiled [22–29]. Adult vertebrates have similar rates of bone accretion and resorption, leading to a global steady state of the skeleton mass. The calcium accretion and resorption rates, called  $V_{\text{O}+}$  and  $V_{\text{O}-}$ , respectively, are computed from literature data [22–29] after selection of the pathological-free animals:

$$V_{\text{O}+} \text{ (mg/day)} = V_{\text{O}-} = 140.65M^{0.64} \quad (R^2 = 0.68). \quad (3)$$

TABLE I Values Assigned to the Physiological Parameters of the Modeled Animal.

<i>Physiological parameters</i>	<i>Herbivores</i>	<i>Carnivores</i>	<i>Rodents</i>	<i>Reptiles</i>	<i>Ref.</i>
Metabolic scaling coefficient, <i>a</i>	7.96	1.67	5.48	0.196	[19]
Metabolic scaling exponent, <i>b</i>	0.646	0.869	0.712	0.889	
Diet					
% Carbohydrates	85	0	40	0	[12]
% Lipids	5	20	15	10	
% Proteins	1	80	45	90	
% Water in food	65	65	65	65	[12]
Digestibility	0.7	0.9	0.85	0.85	
Energy extraction efficiency	0.9	1	1	0.9	
Water economy index	0.25	0.9	0.15	0.25	
Body water content (% of body weight)	60	60	60	60	
Sweat/pant ratio	0.5	0.25	1	1	
% Water in feces	60	60	60	0	
Body temperature (K)	311.15	311.15	311.15	Equal to environmental ones	

Note: Data sources from Kohn [12] and Nagy *et al.* [19]. Flux assignments use the same equations as those proposed by Kohn [12], except for the bone–body water and tooth–body water exchanges, whose expressions are given in the text.

Considering the biological mechanism of bone reworking that occurs from its outer surface, the relation linking calcium fluxes (accretion and resorption rates) and bone or body mass is expected to have the form of a 2/3 (surface/volume) exponent power relation. Therefore we propose a general scaling relation:

$$Vo+ \text{ (mg/day)} = 140M^{2/3}. \quad (4)$$

Tooth enamel is modeled by incremental growth; each increment is a reservoir with a surface of  $30 \times 0.1 \text{ mm}^2$ . The growth rate is  $3.5 \mu\text{m/day}$  over a period of 10 days, according to available data on the morphology and growth of human teeth [30]. After 10 days, a new increment is added to the existing tooth enamel crown. Similar tooth growth rates are assumed for modeled animals. In the case of rodents, tooth growth rates are of the same order of magnitude as human tooth [31].

### Oxygen Isotope Fractionations through Mass Transfer

The mechanism of bone resorption does not introduce any isotopic fractionation as the degraded tissue is quantitatively destroyed before the synthesis of a new one. On the contrary, the fractionation factor between the newly synthesized bone and body water is calculated from the equation determined by Longinelli and Nuti [14] that relates the  $\delta^{18}\text{O}$  values of trace phosphate present in marine invertebrate carbonate shells and the ambient water. The flux of water through the body of marine invertebrates is so high that the  $\delta^{18}\text{O}$  value of their body water is maintained similar to that of ambient water. It means that for a given body temperature, the fractionation value deduced from Longinelli and Nuti's equation [14] may be used to calculate the bone phosphate–body water fractionation. Isotopic fractionation factors associated with other oxygen fluxes are taken from previously published studies [9, 12, 32, 33]. Some assumptions are also required in the frame of this model.

For example, the  $\delta^{18}\text{O}$  of oxygen from food ingested by herbivorous animals is considered equal to the  $\delta^{18}\text{O}$  of food cellulose, the latter being itself related to the surface water  $\delta^{18}\text{O}$  value. Leaf water and then leaf cellulose are  $^{18}\text{O}$ -enriched relative to stem water during the process of evapotranspiration. The proportions of stem and leaves are the input parameters which can be adjusted depending on the available information related to animal diets.

The  $\delta^{18}\text{O}$  values of atmospheric carbon dioxide and molecular oxygen are +41.2‰ and +23.5‰, respectively [32]. Carbon dioxide is at isotopic equilibrium with ocean water and its value is fixed by the mean Earth surface temperature. As carbon dioxide is a by-product of metabolism, a precise knowledge of its isotopic composition does not influence the isotopic composition of animal tissues. The isotopic composition of molecular oxygen results from the so-called ‘Dole’ effect, *i.e.* the budget between photosynthesis and respiration. If measurements are not available, the  $\delta^{18}\text{O}$  values of surface water are estimated from the linear relationship between the isotopic composition of meteoric waters and the mean air temperature [9]. The water vapor isotope composition is calculated using the vapor–liquid water fractionation equation given by Horita and Wesolowski [33], which is also very close to that of Majoube [34]. Fractionation factors between body water and water vapor and between body water and liquid surface water are calculated from equations  $\zeta$  and  $\psi$  (Tab. II). Isotopic fractionations related to the inhaled water vapor, water and bound oxygen derived from diet depend on temperature, saturated water vapor pressure and relative humidity (Tab. II).

## RESULTS OF THE MODEL

### Aquatic Animals

A preliminary test may be performed to validate the model. Heterothermic aquatic animals such as fish are exposed to a large exchange of water between their bodies and the environment (‘fast water turnover’) and their body temperature is fixed by the temperature of the surrounding waters. We keep in mind that these rules are not checked for some big animals like tunas and large reptiles for which their body temperature may differ from the ambient one because of specific metabolic activities or mass homeothermy. In contrast to the case of terrestrial animals, modeling the aquatic animals requires removing from the model the water vapor reservoir and the related fluxes. Moreover, isotope compositions of dissolved oxygen and carbon dioxide differ from the atmospheric reservoirs.

Assuming a water turnover that is one hundred times higher for aquatic animals than for terrestrial ones [18], phosphate–water fractionation factors are computed for a temperature range from 0 to 30 °C (Fig. 2). In the case of fish, our computed equation

$$T \text{ (}^\circ\text{C)} = -4.31\Delta + 111.3 \quad \text{with } \Delta = \delta^{18}\text{O}(\text{PO}_4) - \delta^{18}\text{O}(\text{H}_2\text{O}) \quad (5)$$

well matches the equation determined by Kolodny *et al.* [35],

$$T \text{ (}^\circ\text{C)} = -4.38\Delta + 113.3 \quad (6)$$

on the basis of  $\delta^{18}\text{O}$  measurements of modern fresh and sea water fish that lived under various latitudes. In the case of aquatic homeotherms (*e.g.* cetaceans), their oxygen isotope compositions are controlled by the composition of the ambient marine water ( $\delta^{18}\text{O} = 0\text{‰}$ ). This property results from a phosphate–water isotopic fractionation value that is set by the internal body temperature close to 38 °C [12]. It is noteworthy that our computed fractionation value of about 17‰ (Fig. 2) is comparable to the value of 17.8‰ deduced from a set of measurements performed on fluvial and marine modern cetaceans [36].

TABLE II Oxygen Isotope Compositions of External Reservoirs and Isotopic Fractionations Associated with Oxygen Fluxes between the Animal Body and the External Reservoirs.

Initial isotopic compositions assigned by default to the reservoirs (‰)		
Body water, $\delta^{18}\text{O}_{\text{BW}}$	0‰	[12]
Surface water, $\delta^{18}\text{O}_{\text{SW}}$	$\delta^{18}\text{O}_{\text{SW}} = 0.69T(^{\circ}\text{C}) - 13.6‰$	[9]
Atmospheric $\text{O}_2$	23.5‰	[32]
Water vapor, $\delta^{18}\text{O}_{\text{V}}$	$\delta^{18}\text{O}_{\text{SW}} + 7.685 - \frac{6.7123 \times 10^3}{T(\text{K})} + \frac{1.6664 \times 10^6}{T(\text{K})^2} - \frac{0.35041 \times 10^9}{T(\text{K})^3}$	[32]
Atmospheric $\text{CO}_2$	$\delta^{18}\text{O}_{\text{CO}_2} = 41.2‰$	[32]
Food water, $\delta^{18}\text{O}_{\text{fw}}$		
Herbivores	$\delta^{18}\text{O}_{\text{fw}} = \frac{\text{leaf}}{\text{leaf} + \text{stem}} \delta^{18}\text{O}_{\text{SW}} + (1 - \text{r.h.}) \cdot [(\delta^{18}\text{O}_{\text{SW}} - \delta^{18}\text{O}_{\text{V}}) + 16] + \left(1 - \frac{\text{leaf}}{\text{leaf} + \text{stem}}\right) \delta^{18}\text{O}_{\text{SW}}$	
Carnivores and reptiles	0.16‰	
Rodents	0.08‰	[12]
Solid food $\delta^{18}\text{O}_{\text{fo}}$		
Herbivores	$\delta^{18}\text{O}_{\text{fo}} = \delta^{18}\text{O}_{\text{fw}} + 27‰$	
Carnivores and reptiles	$\delta^{18}\text{O}_{\text{fo}} = \delta^{18}\text{O}_{\text{fw}} + 7‰$	
Rodents	18.71‰	
Assigned fractionation factors		
$^{18}\alpha_{\text{BW-PO}_4}$	$1 + \left(\frac{111.4 - \text{body}T(^{\circ}\text{C})}{4.3}\right) 10^{-3}$	[14]
$^{18}\alpha_{\text{PO}_4\text{-BW}}$		
$^{18}\alpha_{\text{BW-atmCO}_2}$	$1 + \left(\frac{17,604}{\text{body}T(\text{K})} - 17.93\right) 10^{-3}$	[32]
$^{18}\alpha_{\text{BW-atmO}_2}$	$1 + z(1 - \text{oxygen utilization fraction}) 10^{-3}$ with $z = 10.5\%$ and oxygen utilization fraction = 20%	[12]
$^{18}\alpha_{\text{VW-BW}}$	$\frac{\sum_j Q_{\text{BW} \rightarrow \text{VW}}^j \delta^{18}\alpha_{j\text{-BW}}}{\sum_j Q_{\text{BW} \rightarrow \text{VW}}^j} (\xi)$	$^{18}\alpha_{\text{oral water loss-BW}} = 0.99181$ $^{18}\alpha_{\text{nasal water loss-BW}} = 0.98295$ $^{18}\alpha_{\text{transcutaneous water loss-BW}} = 0.9820$ $^{18}\alpha_{\text{sweat-BW}} = 1$
$^{18}\alpha_{\text{SW-BW}}$	$\frac{\sum_j Q_{\text{BW} \rightarrow \text{SW}}^j \delta^{18}\alpha_{j\text{-BW}}}{\sum_j Q_{\text{BW} \rightarrow \text{SW}}^j} (\psi)$	$^{18}\alpha_{\text{urea-BW}} = ^{18}\alpha_{\text{urine water-BW}}$ $= ^{18}\alpha_{\text{fecal water-BW}} = 1$
	with $Q_{\text{BW} \rightarrow \text{VW}}^j$ as the oxygen flux from body water to vapor water under the form $j$ (sweat, nasal, oral and transcutaneous losses of $\text{H}_2\text{O}$ ); and $^{18}\alpha_{j\text{-BW}}$ the respective fractionation factors	
	with $Q_{\text{BW} \rightarrow \text{SW}}^j$ as the oxygen flux from body water to surface water under the form $j$ (oxygen in urea, urine water, fecal water); and $^{18}\alpha_{j\text{-BW}}$ the respective fractionation factors	

Note:  $^{18}\alpha_{i-j}$  is the oxygen isotope fractionation factor between reservoirs  $i$  and  $j$ . Last column indicates references.

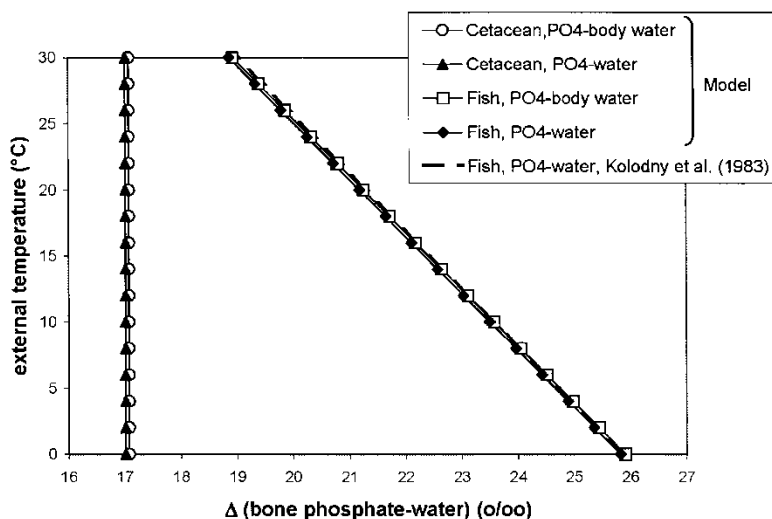


FIGURE 2 Modeled phosphate–environmental water and phosphate–body water oxygen isotope fractionations for fish and cetaceans in relation with the oxygen isotope composition and the temperature of ambient water. The  $\delta^{18}\text{O}$  value of seawater is set to 0‰ and the temperature range covers the temperature variations of seawater at the surface of the Earth. The phosphate–environmental water relation determined by Kolodny *et al.* [35] for fish phosphate (grey line) is reported for comparison with the modeled isotopic fractionations for a fish of 1 kg (black line with filled diamonds) and a cetacean of 1000 kg (black line with filled triangles).

### Terrestrial Animals

Computed oxygen isotope fractionation equations for mammals are compared with those deduced from measurements performed on modern animals. These tests are more pertinent for herbivorous than for carnivorous mammals because the oxygen isotope composition of their diet can be estimated from the environmental parameters, hence limiting the number of unconstrained input parameters.

Two empirical equations for horse bones and teeth were determined by Sanchez-Chillon *et al.* [37] and Bryant *et al.* [38]. In order to obtain reasonable surface water (SW) isotope compositions, we use Bowen *et al.*'s [39] formula [Eq. (7)] that relates the  $\delta^{18}\text{O}$  value of meteoric waters to the latitude and altitude.

$$\delta^{18}\text{O}_{\text{SW}} = -0.0051(|\text{Latitude}|)^2 + 0.1805|\text{Latitude}| - 0.002 \text{Altitude} - 5.247. \quad (7)$$

For a given relative humidity and altitude, the  $\delta^{18}\text{O}$  values are calculated for various latitudes. The ambient temperatures are deduced from Dansgaard's [9] formula:

$$T(^{\circ}\text{C}) = \frac{\delta^{18}\text{O}_{\text{SW}} + 13.6}{0.69} \quad (8)$$

The model  $\delta^{18}\text{O}$  values of phosphates are calculated as a function of water  $\delta^{18}\text{O}$  values and air temperatures by using Eqs. (7) and (8), along with the set of fixed oxygen fluxes and isotopic fractionations given in Tables I and II (Fig. 3). Two model equations are proposed for a diet leaf/(leaf + stem) ratio of 0.5 and 1, respectively. They bracket the two empirical equations and have similar slopes, while the intercept of these model equations clearly depends on the animal diet. In the case of these herbivorous mammals, the oxygen isotope composition

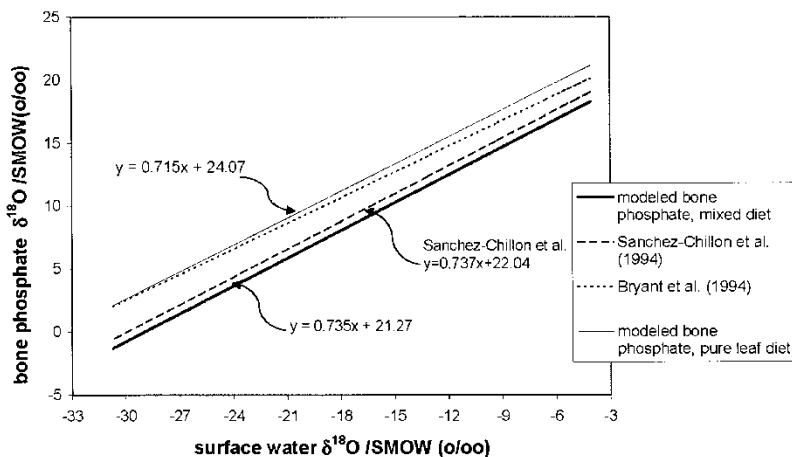


FIGURE 3 Modeled and empirical phosphate  $\delta^{18}\text{O}$  values for a large mammal herbivore (horse) reported against  $\delta^{18}\text{O}$  values of environmental waters. The empirical phosphate–water fractionation equations were determined by Bryant *et al.* [38] and Sanchez-Chillon *et al.* [37] on the basis of horse bones and teeth. The mammal is modeled on the basis of a body weight of 240 kg and a 65% relative air humidity. For a comparison with horse data, water  $\delta^{18}\text{O}$  values are calculated by using Eq. (7) for an altitude of 200 m and longitudes from 0 to 90 °N.

of the food cannot be neglected in the isotopic budget of phosphatic tissues, even though drinking water remains the main source controlling the composition of horse bones.

In addition to the sensitive diet parameter, body mass, relative humidity and temperature of air also influence the oxygen isotope composition of terrestrial mammal apatite as shown in Figure 4. In a general trend of decreasing phosphate–water fractionations ( $\Delta$ ) with increasing temperatures, increasing air humidities are responsible for a strong decrease of  $\Delta$ . With the increase of the animal mass, the slopes of fractionation equations decrease. Respective

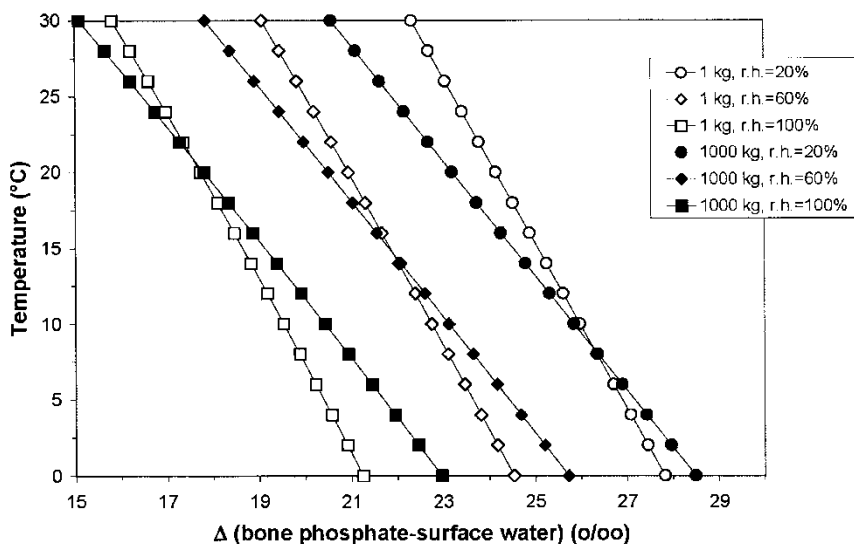


FIGURE 4 Respective influences of the relative air humidity (r.h.), external temperature, and body mass on the phosphate–surface water oxygen isotope fractionation for modeled herbivorous mammals. Increase in the body mass reduces the slopes of the fractionation lines whereas an increase in the air humidity causes a lowering of the phosphate–water fractionation.

contributions of these parameters on both the slope and the intercept of the oxygen isotope fractionation lines may partly explain the scattering commonly observed within the data obtained from animals living in their natural environment.

## DYNAMICS OF BONE AND TOOTH RECORDS

In order to test the validity of our model, we compare our computed results with those of Kohn [12] by using the same set of data and parameters given for an ‘average herbivore’ in New Delhi. We also illustrate the ability of our time-dependent model to predict the  $\delta^{18}\text{O}$  time record as a function of the growth mechanism of phosphatic tissues. Kohn [12] provided a set of monthly temperatures and relative humidities and assigned the surface water isotopic values using the GNIP database [40]. Kohn [12] calculated for every month the body water isotopic composition of a 30 kg herbivore, and deduced the  $\delta^{18}\text{O}$  value of the ‘phosphate’ applying Longinelli and Nuti’s equation [14]. In our study, we use the same physiological and monthly environmental parameters to model simultaneously the body water, bone and tooth phosphate oxygen isotope compositions during 1 year (Fig. 5). As the tooth phosphate increment is small and not reworked after formation, its isotopic compositions mimic the evolution of the body water composition and appear to be in good agreement with Kohn’s [12] results. In contrast to this former oxygen isotope pattern, the bone phosphate record is very smoothed and only reflects a yearly-averaged oxygen isotope composition of the body water.

Considering constant environmental conditions and a set of parameters compatible with the order of rodents, our model shows the time required for body water and phosphate reservoirs to reach steady-state oxygen isotope compositions (Fig. 6). The body water steady-state

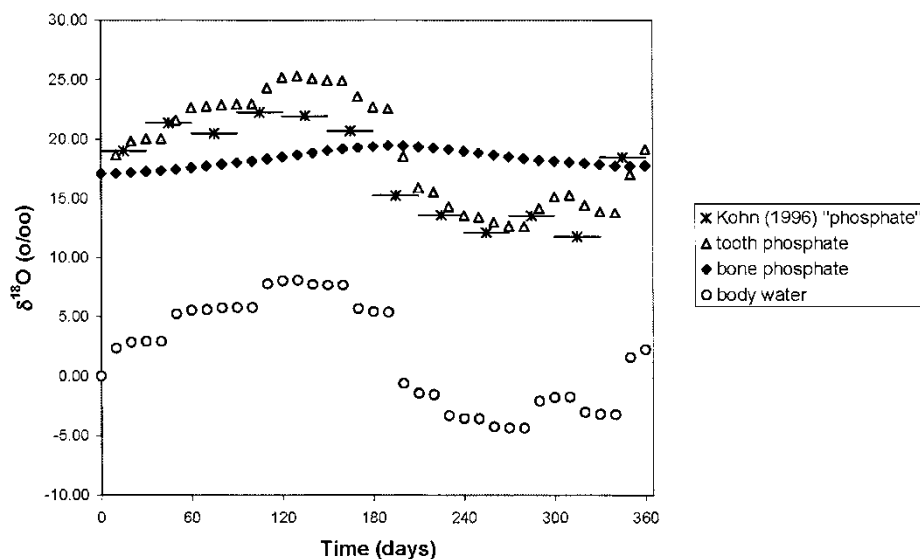


FIGURE 5 Results of our model compared with those of Kohn [12] for a 30 kg average herbivore during 1 year in New Delhi. The model was computed by steps of 10 days using the physiological parameters and the monthly average values of temperature and relative humidity given by Ref. [12]. Surface water (precipitations) oxygen isotope values are taken from Ref. [39] for the location of New Delhi. Note that tooth enamel records accurately the  $\delta^{18}\text{O}$  variations of body water (about 12‰) whereas the  $\delta^{18}\text{O}$  record of bone is very smoothed with less than 2‰ of isotopic variation over 1 year.

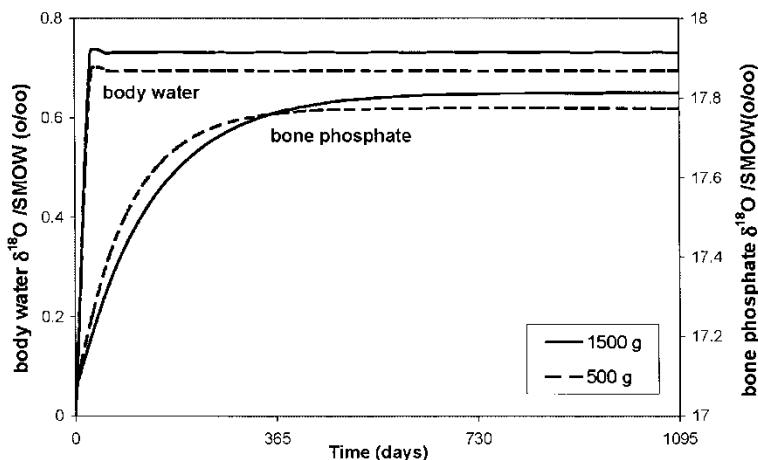


FIGURE 6 Computed evolution over 3 years of oxygen isotope compositions of both body water and bone phosphate from rodents that tend to reach steady-state values depending on the body mass.

value is obtained after a few days whereas several months or years are required for bones, which is in good agreement with the experimental results obtained on rats by Longinelli and Peretti-Padalino [43]. The time required for reaching steady-state isotopic compositions of bones increases with the increasing mass of the animal (Fig. 6).

When simulating sinusoidal seasonal variations in the oxygen isotope composition of surface water, this sinusoidal isotopic signal is preserved in body waters and teeth whatever the animal mass (Fig. 7). The mode of isotopic record through time of the environmental parameters in bones depends on the animal mass. Indeed, the amplitude of the computed isotopic records in bones decreases with the increasing animal mass (Fig. 7). We emphasize that very smoothed

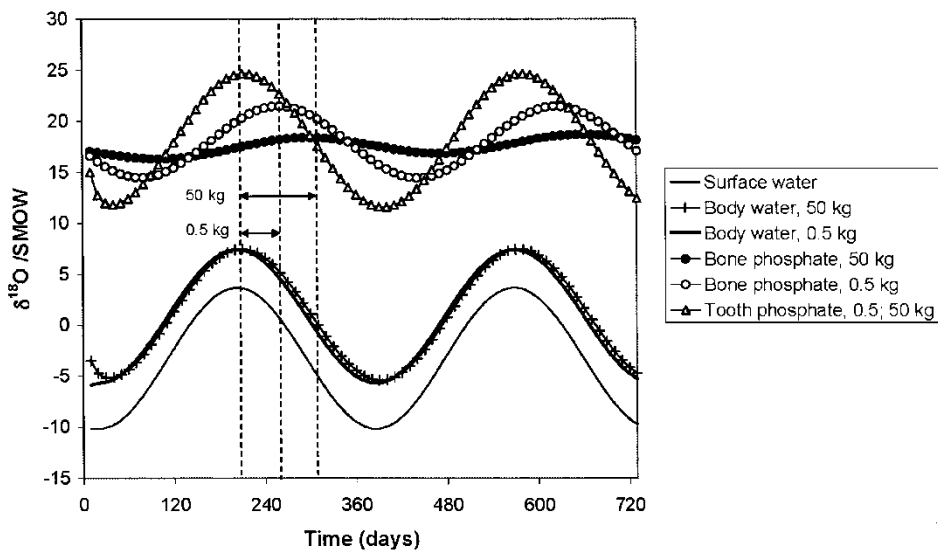


FIGURE 7 Oxygen isotope records in body water and bone phosphate when herbivorous mammals are exposed to seasonal changes in the isotopic composition of drinking water. Note that the amplitude of the original sinusoidal signal decreases with the increasing animal mass. Furthermore, time shifts of about 2–4 months, relative to the input surface water isotopic signal, appear in the records of animal bones with body masses of 0.5 and 50 kg, respectively.

isotopic records in the bones of animals whose mass exceeds 50 kg will be potentially difficult to use as proxies of short-term variations with time in the  $\delta^{18}\text{O}$  of surface waters (Fig. 7).

Our calculations confirm that bones cannot be used as recorders of short-term environmental variations because of their permanent reworking. On the contrary, tooth enamel from hypsodonts (bovidae, equidae, rodents) is able to record seasonal variations in the  $\delta^{18}\text{O}$  of drinking water. Laser ablation techniques are well designed for sampling at the scale of enamel increments and have already given promising results [42, 43].

## CASE STUDIES

### Reconstitution of Herbivore Dietary Preferences

An interesting case study is provided by the data of Cormie *et al.* [44] that were obtained from white-tailed deer and mule deer bones. Indeed, yearly temperatures and relative humidities are given by the authors, only leaving the isotopic compositions of surface waters and the animal mass as unconstrained parameters. The  $\delta^{18}\text{O}$  values of surface water are calculated using Eq. (8) and the average mass of the white-tailed deer was set at 122 kg according to the literature [45]. We selected arbitrarily a leaf/(leaf + stem) ratio in animal diet of 0.5 to model the deer isotopic compositions and to compare them with those measured (Fig. 8). The resulting scattering of results suggests that dietary behaviours vary among individuals of the same species. We adjusted the calculated compositions to data with a precision of 1% changing the leaf/(leaf + stem) ratio. For three data out of thirty, the discrepancy between data and modeled values cannot be resolved. Other factors such as extreme variations in the body mass or migrations of the isolated individuals are not taken into account by this model.

### Respective Influences of Physiology and Diet

Luz and Kolodny [6] have measured the oxygen isotope compositions of bone, teeth and blood of laboratory rats. These animals were maintained at a constant temperature and controlled humidity during the experiments. They were fed on dry pellets and  $^{18}\text{O}$ -enriched or

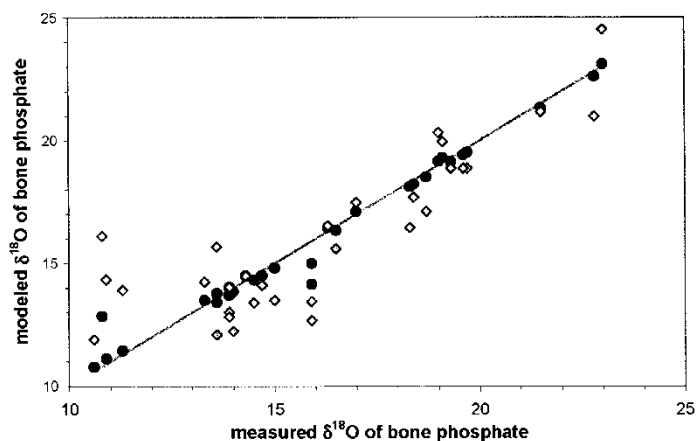


FIGURE 8 Modeled  $\delta^{18}\text{O}$  values reported against the data measured by Cormie *et al.* [44] on white-tailed and mule deers. Grey diamonds: data fitting for an arbitrary dietary leaf/(leaf + stem) ratio of 0.5. Black circles: data fitting with a leaf/(leaf + stem) ratio varying between 0 and 1. The solid line materializes a perfect adjustment between data and modeled oxygen isotope compositions of phosphate from deers.

$^{18}\text{O}$ -depleted drinking water relatively to the SMOW. Assuming the total dryness of food pellets, we compare the model results to the experimental data. For these very small mammals of about 200 g, the body mass scaling relationship of energetic requirements proposed by Nagy *et al.* [19] for rodents was preferred to Kohn's [12] as it provides more realistic values. As shown in Figure 9A, modeled isotopic compositions of rodent bones compare with experimental data even if a few % differences are observed for the two groups of rats. This discrepancy between the modeled and experimental data could result from the presence of water in food. Assuming a plausible  $\delta^{18}\text{O}$  value of 0‰ for food water following Kohn's considerations [12], no more than 20% of water in the food ingested by the rats could reconcile the model results and experimental data (Fig. 9B). We measured a water content of 17% wt in a commercial sample of pelleted food for rats.

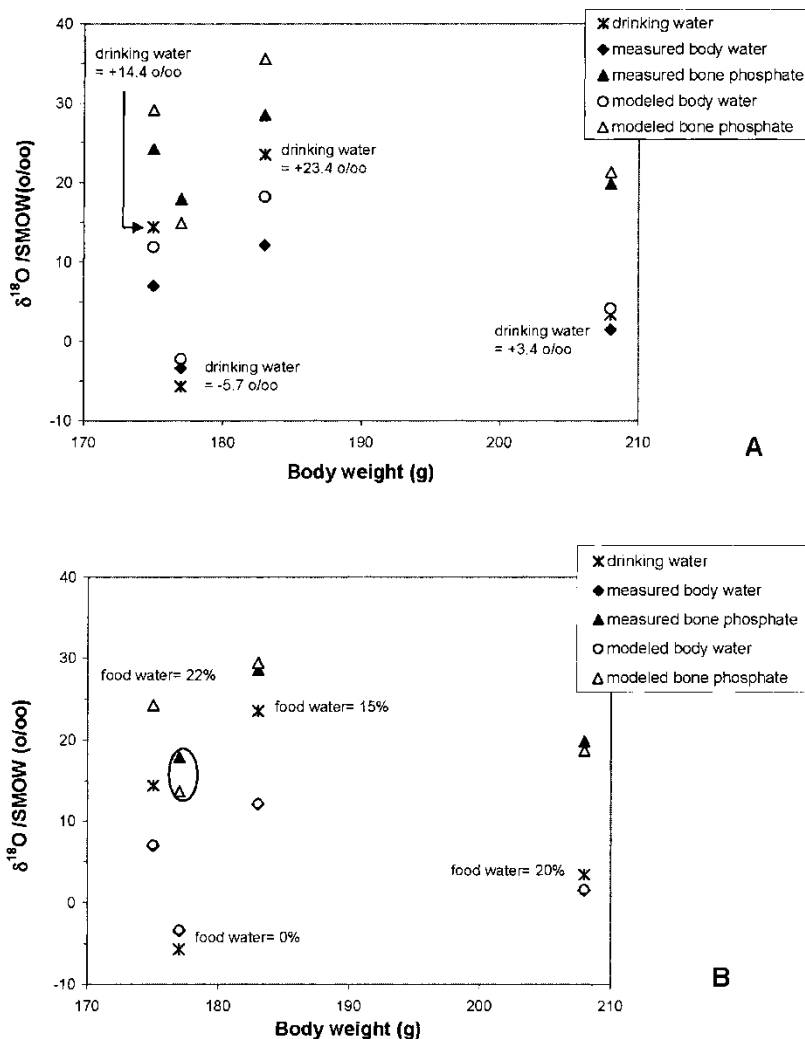


FIGURE 9 Comparison of modeled  $\delta^{18}\text{O}$  values of body water and bone phosphate with data of Luz and Kolodny [6] that were obtained on laboratory rats of known body mass. The rats were maintained under controlled temperature (22 °C) and relative air humidity (50%). They were fed with dry pellets and isotopically-labeled drinking water. A: modeled isotopic compositions assuming that food is totally water-free. B: modeled isotopic compositions assuming that the food contains a fraction of water that is calculated considering that its  $\delta^{18}\text{O}$  value equals 0‰.

## CONCLUDING REMARKS

In the frame of this model, we proposed a general scaling relationship between the bone calcium fluxes and the body mass that were based on data compilation from the literature and a set of oxygen and isotopic fluxes between animal and external reservoirs. When most of the environmental and dietary parameters can be constrained, we obtain a global agreement between the modeled and measured oxygen isotope compositions of body waters and phosphatic tissues from some marine and terrestrial mammals.

The model also provides plausible explanations for some discrepancies previously observed between determined empirical equations that relate the phosphate  $\delta^{18}\text{O}$  value of vertebrates with environmental parameters ( $T$ ,  $\delta^{18}\text{O}_{\text{SW}}$ ). They concern metabolic rates, dietary behaviors, water fluxes with the environment, and isotopic compositions of ingested food and water. For example, the oxygen isotope composition of drinking water is estimated from rainfall compositions, themselves related to mean air temperatures; however, this drinking water could also derive from other sources like groundwater. The use of this model could be improved by a better knowledge of oxygen fluxes connecting the phosphatic tissues with body waters.

Finally, we suggest that such a time-dependent box-modeling approach can be adapted to study the behaviour of any trace element (153-Sm) or isotopic systems (e.g. calcium isotopes) that could help to quantify residence times of elements in bones and body waters or to investigate the mechanisms of bone metabolism.

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