


# Oxygen isotope fractionation between bird bone phosphate and drinking water

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Received: 23 December 2016 / Revised: 27 April 2017 / Accepted: 1 May 2017  
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**Abstract** Oxygen isotope compositions of bone phosphate ( $\delta^{18}\text{O}_p$ ) were measured in broiler chickens reared in 21 farms worldwide characterized by contrasted latitudes and local climates. These sedentary birds were

raised during an approximately 3 to 4-month period, and local precipitation was the ultimate source of their drinking water. This sampling strategy allowed the relationship to be determined between the bone phosphate  $\delta^{18}\text{O}_p$

Communicated by: Sven Thatje

**Electronic supplementary material** The online version of this article (doi:10.1007/s00114-017-1468-2) contains supplementary material, which is available to authorized users.

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values (from 9.8 to 22.5‰ V-SMOW) and the local rainfall  $\delta^{18}\text{O}_w$  values estimated from nearby IAEA/WMO stations (from −16.0 to −1.0‰ V-SMOW). Linear least square fitting of data provided the following isotopic fractionation equation:  $\delta^{18}\text{O}_w = 1.119 (\pm 0.040) \delta^{18}\text{O}_p - 24.222 (\pm 0.644)$ ;  $R^2 = 0.98$ . The  $\delta^{18}\text{O}_p$ – $\delta^{18}\text{O}_w$  couples of five extant mallard ducks, a common buzzard, a European herring gull, a common ostrich, and a greater rhea fall within the predicted range of the equation, indicating that the relationship established for extant chickens can also be applied to birds of various ecologies and body masses. Applied to published oxygen isotope compositions of Miocene and Pliocene penguins from Peru, this new equation computes estimates of local seawater similar to those previously calculated. Applied to the basal bird *Confuciusornis* from the Early Cretaceous of Northeastern China, our equation gives a slightly higher  $\delta^{18}\text{O}_w$  value compared to the previously estimated one, possibly as a result of lower body temperature. These data indicate that caution should be exercised when the relationship estimated for modern birds is applied to their basal counterparts that likely had a metabolism intermediate between that of their theropod dinosaur ancestors and that of advanced ornithurines.

**Keywords** Bird · Phosphate · Oxygen isotope · Fractionation equation

## Introduction

Phosphate oxygen isotope compositions ( $\delta^{18}\text{O}_p$ ) of bioapatite from vertebrate remains such as reptile and mammal teeth or bones are increasingly used to reconstruct terrestrial environmental conditions during the Phanerozoic (e.g., Amiot et al. 2011; Suarez et al. 2014; Tütken 2014; Bojar et al. 2015; Rey et al. 2016). These reconstructions are mainly based on existing phosphate–water isotopic fractionation equations established between bioapatite and drinking water of extant vertebrates and applied to their fossil relatives (e.g., Bryant et al. 1994; Barrick et al. 1999; Amiot et al. 2007; Royer et al. 2013). The  $\delta^{18}\text{O}_p$  value of vertebrate hard tissues (bones, teeth, fish scales) is a function of both the  $\delta^{18}\text{O}_{bw}$  value of the animal's body water and its body temperature  $T_b$  (Kolodny et al. 1983; Longinelli 1984; Luz et al. 1984). The  $\delta^{18}\text{O}_{bw}$  value is related to the  $\delta^{18}\text{O}_w$  value of water ingested through the animal's food and drinking water, the magnitude of the oxygen isotope fractionation between the two reservoirs depending on the body water turnover, the physiology of the animal, and its drinking behavior (e.g., obligate or not drinker). In the case of terrestrial vertebrates, the system of connected reservoirs exchanging oxygen is rather complex. Input oxygen fluxes to the animal body are air through the lungs and associated water vapor, food, and drinking water. Output oxygen fluxes from the animal body are feces and urinary water,

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urea or uric acid, transcutaneous and exhaled water vapor (via respiration, transcutaneous evaporation, transpiration, or, for some mammals such as carnivores, via panting), and expired carbon dioxide (Kohn 1996). The ecology of vertebrates may also influence the oxygen isotope budget of their body water. For example, semi-aquatic vertebrates such as crocodilians reduce the output flux of water lost through aerial evapotranspiration, thus decreasing the  $^{18}\text{O}$  enrichment of body water relative to the drinking water (e.g., Amiot et al. 2007, 2010). For most continental vertebrates, the main source of ingested oxygen is drinking, plant or prey water, which is meteoric water or derived from it (D'Angela and Longinelli 1990; Cormie et al. 1994; Kohn et al. 1996). As the  $\delta^{18}\text{O}_w$  value of meteoric water depends on climatic parameters such as air temperature, humidity, and amount of precipitation (Dansgaard 1964), vertebrates indirectly record in their phosphatic tissues the climatic conditions of their living environment.

During burial and throughout their post-depositional history, animal tissues are potentially subjected to diagenetic processes that may alter their pristine chemical and isotopic compositions. The recrystallized mineral may acquire a new isotopic composition if the interacting aqueous fluids have a temperature or a  $\delta^{18}\text{O}$  value distinct from those that originally formed the biominerals during the animal's life. Vertebrates are also characterized by hard tissues of various resistance against diagenetic alteration. While bones are easily susceptible to pronounced alteration in their host sediments, tooth enamel is very resistant to dissolution processes and may preserve its original  $\delta^{18}\text{O}_p$  values for tens up to hundreds of millions of years (e.g., Kolodny et al. 1996; Amiot et al. 2011; Rey et al. 2016). Therefore, tooth enamel constitutes a material of choice and has been generally preferred by geochemists, paleobiologists, and archeologists for stable isotope studies. This strategy partly explains why no oxygen isotope fractionation equation for bird phosphatic remains is available in the literature so far. Indeed, the only fossil-mineralized body parts left by birds are bones (with the exception of some Mesozoic birds that had teeth), and most of them are hollow and thin to meet lightness requirement for flying. However, fossil bird remains are common in the fossil record, and the determination of an isotopic fractionation equation may have applications in archeology and paleontology to reconstruct local climate conditions by analyzing naturally or human-made mummified birds as those found along the Nile valley during the Coptic and Greco-Roman periods (e.g., ibises), or fossilized remains recovered from Late Mesozoic (Cretaceous) and Cenozoic deposits.

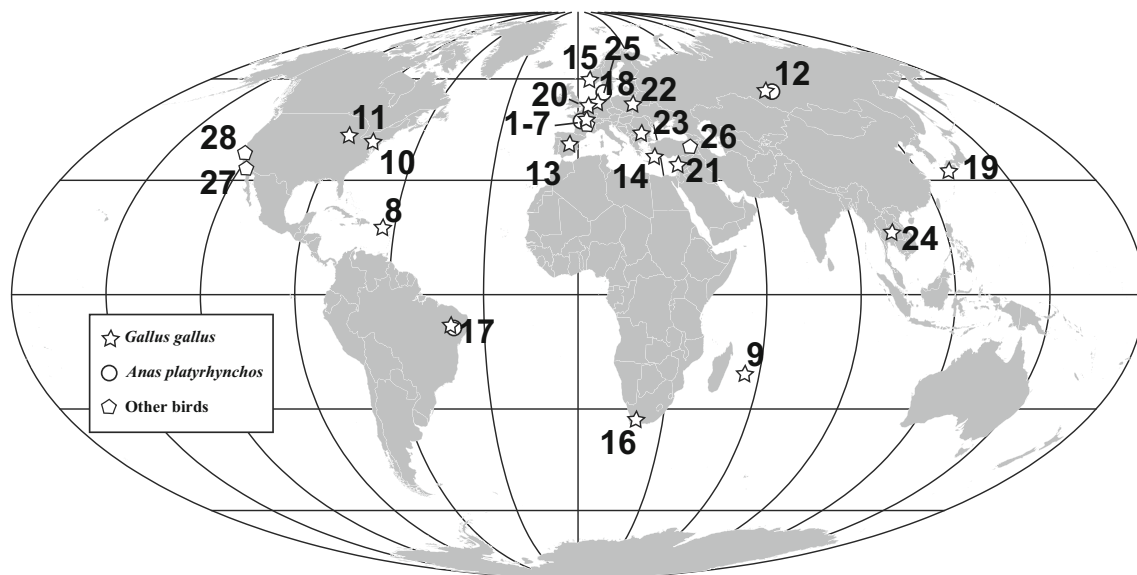
In this study, we established a phosphate–water oxygen isotope fractionation equation for extant birds. As birds are homeothermic, the determination of such an equation requires

the analysis of a collection of specimens that are living at various latitudes. Indeed, the quality of the expected linear fit between bird phosphate and environmental water depends on the spread and precise knowledge of the  $\delta^{18}\text{O}_w$  values of meteoric waters which are the ultimate source of the water ingested by birds. The oxygen isotope fractionation equation has been established using bird bones. For convenience, we used chickens (*Gallus gallus*), the most commonly found domestic bird worldwide, and established a relationship based on this species only, in order to minimize potential interspecific variations in  $\delta^{18}\text{O}_p$  values as a result of ecological and physiological traits. Being sedentary birds, chickens are reared for food production in farms where they drink local water of meteoric origin. As the variations in  $\delta^{18}\text{O}_w$  values are strongly seasonally controlled at intermediate to high latitudes, we only have sampled bones of chickens for which the date of death was known in order to correctly determine the  $\delta^{18}\text{O}_w$  values of their drinking water. This allows us to establish a relationship between the bone phosphate  $\delta^{18}\text{O}_p$  values and the rainfall  $\delta^{18}\text{O}_w$  ones. Finally, the obtained equation has been tested to estimate the drinking water  $\delta^{18}\text{O}_w$  values of extant and fossil birds having various ecologies and occupying different environments.

## Material and methods

### Sample collection

Twenty-one bone samples from chickens raised in outdoor captivity were collected from local farmers of 15 countries characterized by contrasted latitudes ranging from 34° S to 59° N and local climates (Fig. 1; Table 1). These birds intended for consumption were raised with local food and water during a minimum period of 81 days before being slaughtered. As in situ sampling of drinking water was not possible, mean annual  $\delta^{18}\text{O}_w$  values of meteoric waters from nearby IAEA/WMO stations (IAEA/WMO 2016) were used instead, as well as published values of nearby stations for the three localities of Penn State (Coplen and Huang 2000), Kumamoto (Matsubaya and Kwaraya 2014), and Dumbrava de Jos (Bojar et al. 2017). The monthly estimates provided by the Online Isotope in Precipitation calculator (Bowen and Revenaugh 2003) was used for the Thai locality of Maha Sarakham and the Norwegian locality of Tønsberg (Table 1; Supplementary Table). In order to test the effect of some ecological traits (semi-aquatic vs terrestrial, herbivore vs carnivore) on the oxygen isotope composition of phosphate, the bones of five mallard ducks (*Anas platyrhynchos*), a common buzzard (*Buteo buteo*), and a European herring gull (*Larus argentatus*), recovered from France, Denmark, Brazil, Russia, and Turkey (Table 1; Fig. 1), have also been analyzed. We also added to the dataset published  $\delta^{18}\text{O}_p$  values



**Fig. 1** Distribution map of extant bird samples. Numbers refer to localities described in Table 1. “Other birds” correspond to one *Buteo buteo* (7) and *Larus argentatus* (26), as well as published values of

*Struthio camelus* (27; Stanton-Thomas and Carlson 2004) and *Rhea americana* (28; Stanton-Thomas and Carlson 2004)

of a common ostrich (*Struthio camelus*) and a greater rhea (*Rhea americana*) coming from two zoos in California, USA (Stanton-Thomas and Carlson 2004).

### Analytical techniques

Bone powders collected from the mid-shaft of bird long bones were rinsed three times with double-deionized water (DDW) and dried in an oven at 50 °C. Bird bone samples were treated following the wet chemistry protocol described by Crowson et al. (1991) and slightly modified by Lécuyer et al. (1993). This protocol consists in the isolation of phosphate  $\text{PO}_4^{3-}$  from apatite as  $\text{Ag}_3\text{PO}_4$  crystals using acid dissolution and anion-exchange resin. After dissolution of 15 to 20 mg of powdered bone in 2 M HF at 25 °C for 24 h, the  $\text{CaF}_2$  that precipitated was separated from the solution that included the phosphate by centrifugation. The  $\text{CaF}_2$  precipitate was rinsed three times using DDW, and the rinse water was added to the solution that was neutralized with a 2 M KOH solution. Cleaned Amberjet™ resin (2 mL) was added to the neutralized solution in polypropylene tubes. The tubes were placed on a shaking table for 12 h to promote the phosphate ion fixation. Excess solution was discarded, and the resin was washed five times with DDW to remove the traces of ionic contaminants. To elute the phosphate ions quantitatively from the resin, 25–30 mL of 0.5 M  $\text{NH}_4\text{NO}_3$  was added to bring the pH of the solution to 7.5–8.5, and the tubes were gently shaken for about 4 h. The solution was placed in a 250-mL Erlenmeyer flask, and about 0.5 mL of concentrated  $\text{NH}_4\text{OH}$  was added to raise the pH to 9–10. Fifteen milliliters of ammoniacal  $\text{AgNO}_3$  solution was added to the flask. Upon

heating this solution to 70 °C in a thermostatic bath, millimeter-sized yellowish crystals of  $\text{Ag}_3\text{PO}_4$  were quantitatively precipitated. The crystals of silver phosphate were collected on a Millipore™ filter, washed three times with DDW, and air dried at 50 °C.

The oxygen isotope data were obtained using a high-temperature pyrolysis continuous flow technique developed by Fourel et al. (2011). For each sample, five aliquots of 300 to 400 µg of silver phosphate were mixed with 400 µg of pure carbon in silver foil capsules. Pyrolysis was performed at 1450 °C using an Elementar-varyoPYROcube™ elemental analyzer, and CO gas was analyzed using a Isoprime™ isotope ratio mass spectrometer at the stable isotope facility of the Laboratoire de Géologie de Lyon (UMR 5276, France). Oxygen isotope compositions were calibrated against silver phosphate samples precipitated from the NIST SRM 120c standard, for which the  $\delta^{18}\text{O}$  value was fixed at 21.7‰ V-SMOW for correction of instrumental mass fractionation during CO isotopic analysis. The average value of 21.7‰ obtained for the standard SRM 120c was proposed by Lécuyer et al. (1993) who fluorinated silver phosphate crystals with bromine pentafluoride and calibrated the oxygen isotope ratios against the NBS28 quartz international standard. Recently, Halas et al. (2011) confirmed the absence of any sizeable isotopic fractionation effect during the conversion of silver phosphate into CO and suggested a mean  $\delta^{18}\text{O}$  value of  $21.8 \pm 0.2\text{‰}$  for SRM 120c on the basis of an interlaboratory calibration. Chenery et al. (2010) likewise proposed a value of  $21.7 \pm 0.7\text{‰}$  after a 6-month period of repeated measurements of SRM 120c calibrated against NBS127 barium sulfate. SRM 120c standards that were converted into silver phosphate

**Table 1** Oxygen isotope compositions of bird phosphate reported along with their sample number, taxonomy, ecology, geographical origin, and local meteoric water mean annual  $\delta^{18}\text{O}_w$  values using either the closest IAEA/WMO station (IAEA/WMO 2016), published  $\delta^{18}\text{O}_w$  values (Yates et al. 1990; Coplen and Huang 2000; Kennedy et al. 2011; Matsubaya and Kawaraya 2014; Dean et al. 2015; Bojar et al. 2017), or the Online Isotopes in Precipitation Calculator (OIPC; Bowen and Revenaugh 2003)

Sample	Taxon	Ecology	Locality	Country	Figure 1 ref. number	Sampling date	$\delta^{18}\text{O}_p$ (‰ V-SMOW)	$\delta^{18}\text{O}_w$ (‰ V-SMOW)			Closest station	Data sources
								Annual				
								Mean	Min	Max		
P05	<i>Gallus gallus</i>	Terr. herb.	Loué, Sarthe	France	1	2012–12	16.4	–6.4	–8.1	–4.9	Orléans-la-Source	IAEA/WMO
P22	<i>G. gallus</i>	Terr. herb.	Schleithal, Bas Rhin	France	2	2012–12	15.5	–7.9	–10.0	–5.4	Karlsruhe	IAEA/WMO
P48	<i>G. gallus</i>	Terr. herb.	Le Mans, Sarthe	France	3	2012–12	15.5	–6.4	–8.1	–4.9	Orléans-la-Source	IAEA/WMO
P29	<i>G. gallus</i>	Terr. herb.	Carcassonne, Aude	France	4	2012–12	16.7	–6.5	–10.2	–4.5	Toulouse (Univ/Ecolab)	IAEA/WMO
P02	<i>G. gallus</i>	Terr. herb.	Guadeloupe	France	8	2012–12	19.9	–1.0	–2.5	0.8	Barbados (Seawell Airport)	IAEA/WMO
P42	<i>G. gallus</i>	Terr. herb.	La Réunion	France	9	2012–02	16.7	–3.0	–4.8	–2.1	Haut de St Rose (Reunion)	IAEA/WMO
P19	<i>G. gallus</i>	Terr. herb.	Penn State, PA	USA	10	2012–11	14.2	–10.0	–15.3	–4.7	Penn State (PA)	Coplen and Huang (2000)
P55	<i>G. gallus</i>	Terr. herb.	Chicago, IL	USA	11	2012–11	13.1	–7.1	–14.0	–2.8	Chicago (Midway II)	IAEA/WMO
P60	<i>G. gallus</i>	Terr. herb.	Novosibirsk	Russia	12	2012–12	9.8	–16.0	–25.4	–10.5	Novosibirsk	IAEA/WMO
P25	<i>G. gallus</i>	Terr. herb.	Madrid	Spain	13	2012–12	16.7	–5.7	–9.1	–1.5	Madrid-Retiro	IAEA/WMO
P11	<i>G. gallus</i>	Terr. herb.	Rhodes	Greece	14	2012–12	16.1	–5.2	–6.9	–3.6	Rhodes (Maritsai)	IAEA/WMO
P53	<i>G. gallus</i>	Terr. herb.	Tømsberg	Norway	15	2012–12	14.2	–10.6	–12.7	–8.0	Tømsberg	OIPC
P61	<i>G. gallus</i>	Terr. herb.	Western Cap	South Africa	16	2012–11	17.3	–2.6	–4.0	–1.3	Cape Town University	IAEA/WMO
P64	<i>G. gallus</i>	Terr. herb.	Crato, Ceara	Brazil	17	2012–12	22.5	1.2	–4.3	–0.1	Cachimbo + Serra Talhada	IAEA/WMO
P38	<i>G. gallus</i>	Terr. herb.	Oldenburg	Germany	18	2012–12	15.3	–7.0	–8.6	–6.0	Cuxhaven	IAEA/WMO
P45	<i>G. gallus</i>	Terr. herb.	Kumamoto, Kyushu	Japan	19	2013–03	16.4	–6.9	–8.9	–5.1	Ibusuki	Matsubaya and Kawaraya (2014)
P65	<i>G. gallus</i>	Terr. herb.	Amsterdam	Netherlands	20	2012–11	14.8	–7.2	–8.6	–5.2	De Bilt	IAEA/WMO
P07	<i>G. gallus</i>	Terr. herb.	Beirut	Lebanon	21	2013–01	15.6	–3.6	–5.9	–1.1	Green	IAEA/WMO
PhLu	<i>G. gallus</i>	Terr. herb.	Lublin Upland	Poland	22	2014–09	13.0	–10.0	–14.5	–5.4	Brest (Belarus)	IAEA/WMO
PhDuLo	<i>G. gallus</i>	Terr. herb.	Dumbrava de Jos	Romania	23	2014–09	15.7	–9.9	–15.2	–4.9	Dumbrava region	Bojar et al. (2017)
P01	<i>G. gallus</i>	Terr. herb.	Maha Sarakham	Thailand	24	2012–12	15.6	–5.1	–8.2	–2.4	Mahasarakham	OIPC
C04	<i>Anas platyrhynchos</i>	Semi-aqu. herb.	Dianalund	Denmark	25	2012–12	13.5	–9.3	–12.2	–7.3	Taastrup	IAEA/WMO
C08	<i>A. platyrhynchos</i>	Semi-aqu. herb.	Novosibirsk	Russia	12	2012–12	9.5	–16.0	–25.4	–10.5	Novosibirsk	IAEA/WMO
C14	<i>A. platyrhynchos</i>	Semi-aqu. herb.	Crato, Ceara	Brazil	17	2012–12	19.3	–1.2	–7.7	0.6	Cachimbo + Serra Talhada	IAEA/WMO
C19	<i>A. platyrhynchos</i>	Semi-aqu. herb.	Nevers	France	5	?	15.8	–6.4	–8.1	–4.9	Orléans-la-Source	IAEA/WMO
1681	<i>A. platyrhynchos</i>	Semi-aqu. herb.	Miribel	France	6	?	13.2	–7.3	–9.5	–4.9	Miribel	OIPC
1682	<i>Larus argentatus</i>	Semi-aqu. carn.	Van	Turkey	26	?	17.2	–5.4 <sup>a</sup>	–10.7	–0.1	Van Lake and local springs	Dean et al. (2015)
1696	<i>Buteo buteo</i>	Terr. carn.	Villard-les-Dombes	France	7	?	15.8	–7.6	–9.8	–5.1	Villard-les-Dombes	OIPC
UCMP-125001	<i>Struthio camelus</i>	Terr. herb.	San Diego Zoo	USA	27	?	13.7 ± 0.2 <sup>d</sup>	–8.5 <sup>b</sup>	–10.4	–9.0	San Diego tap water	Kennedy et al. (2011); Stanton-Thomas and Carlson (2004)



**Table 1** (continued)

Sample	Taxon	Ecology	Locality	Country	Figure 1 ref. number	Sampling date	$\delta^{18}\text{O}_p$ (‰ V-SMOW)	$\delta^{18}\text{O}_w$ (‰ V-SMOW)			Closest station	Data sources
								Annual				
								Mean	Min	Max		
UCMP 129668	<i>Rhea americana</i>	Terr. herb.	San Francisco Zoo	USA	28	?	$14.7 \pm 0.8^d$	-9 <sup>c</sup>	-14.0	-4.0	San Francisco tap water	Stanton-Thomas and Carlson (2004); Yates et al. (1990)

*Terr.* terrestrial, *semi-aqu.* semi-aquatic, *herb.* herbivorous, *carn.* carnivorous

<sup>a</sup> Mid-range  $\delta^{18}\text{O}_w$  value between local spring waters' minimum value and Van Lake maximum value was used (Dean et al. 2015)

<sup>b</sup> Mid-range  $\delta^{18}\text{O}_w$  value between San Diego tap water (-9.7‰) and local rainwater (-7.2‰; Kennedy et al. 2011)

<sup>c</sup> Mid-range  $\delta^{18}\text{O}_w$  value between San Francisco tap water (min ~-14‰) and local rainwater (max ~-4‰; Yates et al. 1990)

<sup>d</sup> Mean values with standard deviation calculated from four measured values on the same bone (Stanton-Thomas and Carlson 2004)

along with the samples from each chemistry batch have a mean  $\delta^{18}\text{O}_p$  value of  $21.72 \pm 0.18\text{‰}$  ( $n = 8$ ).

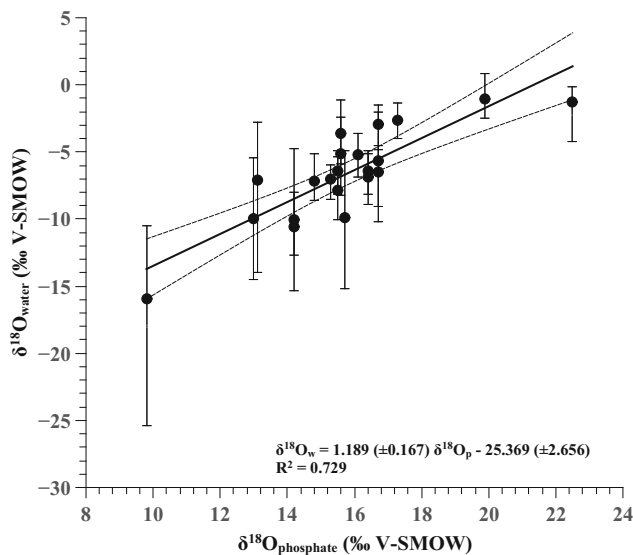
## Results

Oxygen isotope compositions of phosphate from chicken (*G. gallus*) bones range from 9.8 to 22.5‰ (Table 1). The studied birds record the highest  $\delta^{18}\text{O}_p$  values (19.9 to 22.5‰) for the tropical areas of Brazil and Caribbean Islands (Guadeloupe), whereas the lowest  $\delta^{18}\text{O}_p$  values of 9.8 to 14.2‰ correspond to birds that grew at relative high latitudes in coastal areas (Tønsberg, Norway) and within the Eurasian continent far inland (Novosibirsk, Russia, or Lublin, Poland). Intermediate  $\delta^{18}\text{O}_p$  values mostly correspond to bird specimens that lived in mid-latitude settings such as Europe, USA, and South Africa, except those from tropical areas of Reunion Island, France, and Maha Sarakham, Thailand. The observed relatively low  $\delta^{18}\text{O}_p$  values of 16.7 and 15.6‰ are explained in the first case by an altitude effect, while in the second case, the isotopic composition results from an “amount effect” related to the monsoon climate that prevails in Southeast Asia. An overall range in  $\delta^{18}\text{O}_p$  values of 12.7‰ is observed for the bones of investigated present-day birds, which attests their high sensitivity to the  $\delta^{18}\text{O}_w$  of rainfall, the range of which is 14.9‰ (Table 1) for the geographic settings from which the birds originate.

Because the water the birds have ingested may come from reservoirs in which water collected over longer periods are mixed or from groundwater, a least square regression was calculated between chicken bone phosphate  $\delta^{18}\text{O}_p$  values and their corresponding mean annual meteoric water  $\delta^{18}\text{O}_w$  values. The following equation was obtained (Fig. 2; Supplementary Table):

$$\delta^{18}\text{O}_w = 1.189(\pm 0.167)\delta^{18}\text{O}_p - 25.369(\pm 2.656) \\ \text{with } R^2 = 0.73 (n = 21; p = 1.2\text{E}-07) \quad (1)$$

However, ingested waters by the birds during their about 4-month growing period may also correspond to seasonal meteoric waters having  $\delta^{18}\text{O}_w$  values averaging a shorter time period, different from the mean annual value. For this reason, we have considered the minimal and maximal  $\delta^{18}\text{O}_w$  values of meteoric waters within the whole year of each sampling site (Table 1) and used these ranges to maximize the phosphate–water relationship. We have estimated  $\delta^{18}\text{O}_w$  values on the basis of regression coefficients of the least square regression between mean annual  $\delta^{18}\text{O}_w$  and  $\delta^{18}\text{O}_p$  of Eq. (1). If the estimated value was included in the minimum–maximum interval, then it was used for calculating a maximized  $R^2$ ; otherwise, the minimum or maximum value was retained (see Supplementary Material for calculation details). The



**Fig. 2** Phosphate oxygen isotope compositions of chicken bioapatite plotted against the local mean annual rainwater  $\delta^{18}\text{O}_w$  values, along with the corresponding ordinary least square regression line (*plain black line*) and 95% confidence interval belts (*dashed curves*). For individuals with unknown sampling period, mid-range value of the whole year was used instead. Vertical bars correspond to the maximum intra-annual range in  $\delta^{18}\text{O}_w$  values

maximized regression equation improves to (Fig. 3; Supplementary Table):

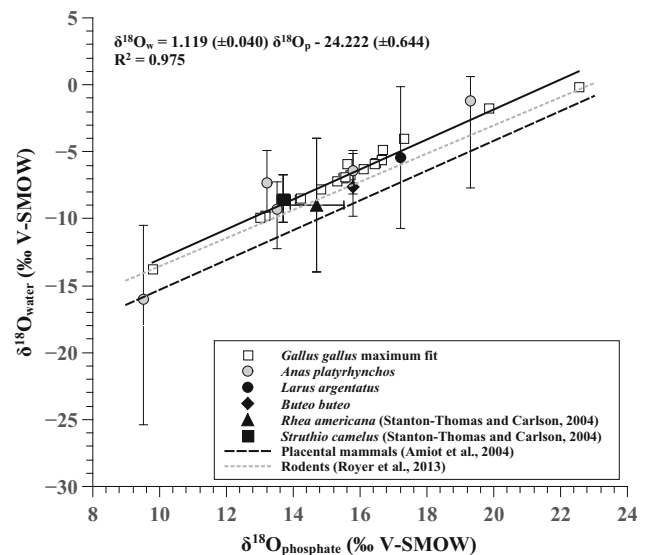
$$\delta^{18}\text{O}_w = 1.119(\pm 0.040)\delta^{18}\text{O}_p - 24.222(\pm 0.644)$$

with  $R^2 = 0.98$  ( $n = 21$ ;  $p = 9.5\text{E-}19$ ;  $\sigma_{\text{est}} = 0.45$ ) (2)

It is noteworthy that this refined Eq. (2) is very close to the first (Eq. (1)), thus confirming that local rain was indeed the main source of drinking water. Moreover, the  $\delta^{18}\text{O}_w$  value of  $-9.68\text{‰}$  predicted by Eq. (2) for the chicken raised in Lublin Upland (sample PhLu; Table 1) matches the value of  $-9.73\text{‰}$  V-SMOW measured on a water sample collected in August 2014 from the pipe water system of Lublin Upland. Oxygen isotope composition of the five ducks, the common buzzard, the European herring gull, the common ostrich, and the greater rhea bone phosphates yielded  $\delta^{18}\text{O}_w$  values close to or matching those predicted by Eq. (2) (Fig. 3).

## Discussion

According to the present dataset, the oxygen isotope compositions of bird phosphates are linearly correlated with the  $\delta^{18}\text{O}_w$  values of their drinking water, approximated by local mean annual  $\delta^{18}\text{O}_w$  value of precipitations. Prior to test this new equation with extinct birds, its validity and meaning need to be discussed in the light of known biological fractionations



**Fig. 3** Oxygen isotope compositions of chicken apatite phosphates plotted against adjusted local rainwater  $\delta^{18}\text{O}_w$  values (see text), along with the corresponding ordinary least square regression line (*plain black line*). For comparison, the  $\delta^{18}\text{O}_p$  values of apatite phosphates from other birds are plotted against the local rainwater  $\delta^{18}\text{O}_w$  mid-range values for the 4 months of chicken growing period. For individuals with unknown sampling period, mid-range value of the whole year was used instead. The *dashed black line* corresponds to the fractionation equation established for placental mammals (Amiot et al. 2004), and the *dashed grey line* to the fractionation equation established for rodents (Royer et al. 2013)

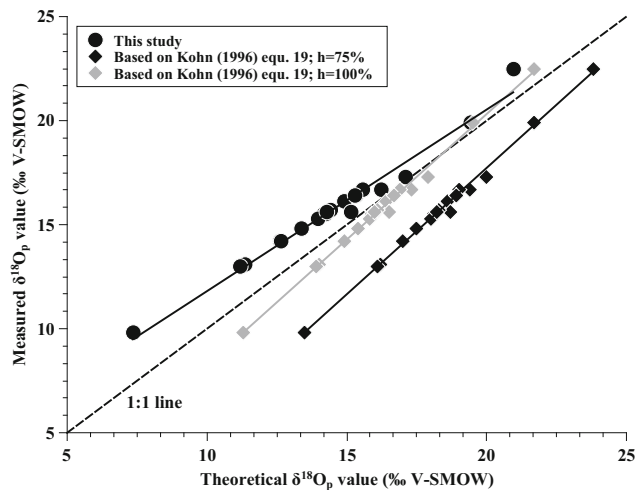
and physiological traits affecting the oxygen isotope compositions of vertebrate bioapatite phosphates.

## Self-consistency of $\delta^{18}\text{O}_p$ – $\delta^{18}\text{O}_w$ pairs

Chickens maintain an average body core temperature  $T_b$  within the  $41\text{--}42\text{ °C}$  range (Kadono and Besch 1978). Therefore, the oxygen isotope composition of their bone phosphate must reflect their body temperature as well as the  $\delta^{18}\text{O}_{\text{bw}}$  value of their body water. In turn,  $\delta^{18}\text{O}_{\text{bw}}$  value reflects the  $\delta^{18}\text{O}_w$  value of ingested water with a fractionation factor  $\alpha_{\text{bw-w}} = 1.00421 \pm 0.00161$  (Lazzerini et al. 2016). This  $\delta^{18}\text{O}_{\text{bw}}$  value and body temperature can be consequently substituted into the phosphate–water–temperature oxygen isotope fractionation equation formerly established by Longinelli and Nuti (1973) and recently checked by Lécuyer et al. (2013):

$$T_b (\text{°C}) = 117.4 - 4.5 (\delta^{18}\text{O}_p - \delta^{18}\text{O}_{\text{bw}}) \quad (3)$$

Theoretical  $\delta^{18}\text{O}_p$  values calculated using the  $\delta^{18}\text{O}_w$  values used to build Eq. (2) (maximum fit  $\delta^{18}\text{O}_w$  values; Supplementary Table) and chicken body temperature compare well with measured  $\delta^{18}\text{O}_p$  values (Fig. 4). However, measured values tend to be higher than theoretical ones toward cold environments that are characterized by lower  $\delta^{18}\text{O}$  values in both phosphate and local waters. The likely reason is that birds



**Fig. 4** Measured  $\delta^{18}\text{O}_p$  values of chickens are reported against theoretical ones. (1) Estimated using known isotopic fractionation factors between drinking water and body water (Lazzerini et al. 2016) and between phosphate and body water (Lécuyer et al. 2013) (black circles). (2) Estimated using the equation modeled by Kohn (1996) for herbivorous birds using a relative humidity ( $h$ ) of 75% (black diamonds) and 100% (gray diamonds)

submitted to environmental temperatures below their comfort zone (or thermoneutral zone) raise their metabolic rates by shivering thermogenesis and increase their breathing oxygen consumption to keep their body at 41–42 °C (West 1965; Van Kampen et al. 1979). This would result in more positive  $\delta^{18}\text{O}_p$  values than expected, because of a higher degree of oxygen inhalation with a  $\delta^{18}\text{O}$  value of 23.88‰ (Barkan and Luz 2005).

### Comparison with the physiological model of Kohn (1996)

A physiological model predicting the  $\delta^{18}\text{O}_p$  values of vertebrates was previously established, and a relationship has been proposed for herbivorous birds (Kohn 1996):

$$\delta^{18}\text{O}_p = 29.3 - 8.6 h + 0.71 \delta^{18}\text{O}_w \quad (4)$$

This equation, based on known input and output fluxes of oxygen through bird body measured on some extant representatives, assumes that most herbivorous birds drink a very small amount of free water, most of their ingested water coming from plant water, the latter having a  $\delta^{18}\text{O}$  value controlled by relative humidity ( $h$ ). Applying an average relative humidity of 75% to this equation predicts overestimated  $\delta^{18}\text{O}_p$  values. However, by setting relative humidity in the equation at 100%, which amounts a bird drinking free water, Eq. (4) reproduces measured  $\delta^{18}\text{O}_p$  values of chickens quite well (Fig. 4). Indeed, chickens drink a notable amount of water, up to about 0.5 l per day for fully adult chickens, with an average water/food consumption ratio of 1.77 g/g (Pesti et al. 1985). Another significant difference between the model proposed by Kohn (1996) and the present chicken dataset is

that Kohn's model overestimates  $\delta^{18}\text{O}_p$  values by assuming a body temperature of 38 °C for birds. This corresponds to the lower range in body temperature for resting birds, whereas during “activity” and “high activity,” birds such as galliforms or columbiforms can raise their body temperature up to 46–47 °C (Prinzinger et al. 1991). The good consistency between measured and theoretical values calculated for a body temperature of 41.5 °C (Fig. 4; this study) would imply that the  $\delta^{18}\text{O}_p$  value of chicken bone phosphate records the animal activity temperature rather than its resting one.

### Comparison with mammals

Birds and mammals are the only two groups of extant endothermic vertebrates, and comparing phosphate–water–oxygen isotope fractionations between birds and terrestrial mammals is therefore relevant. Equation (2) has a slope close to that previously established for extant placental mammals ( $1.113 \pm 0.003$ ; Amiot et al. 2004), but a higher intercept ( $-26.441 \pm 0.051$  for mammals). Within the measured range of chicken  $\delta^{18}\text{O}_p$  values (from 9.8 to 22.5‰), the difference between the  $\delta^{18}\text{O}_w$  values predicted by Eq. (2) and the one for placental mammals ranges from 1.9 to 3.2‰. Several factors may account for this ~2–3‰ difference, the most obvious being the difference in average body temperature between mammals (36–38 °C) and chicken (41–42 °C). Indeed, according to Eq. (3), a body temperature difference of 4–5 °C would lead to a  $\delta^{18}\text{O}_p$  difference of about 1‰. Body mass could also account for the observed difference between chicken and mammals, as a significant part of mammals'  $\delta^{18}\text{O}_p$  values used to establish the equation of Amiot et al. (2004) correspond to large animals with a body mass several orders of magnitude higher than that of chickens (equids, bovids, cervids, and elephants). For a larger body mass, body water residence time is longer, resulting in an increase in body water drinking water  $^{18}\text{O}$  enrichment mainly operating through evaporative water loss and metabolic water input. This process is responsible for higher  $\delta^{18}\text{O}_p$  values as evidenced by the intercept difference of ~2.4‰ observed between the inverted least square regression equation ( $\delta^{18}\text{O}_w = 1.052 \delta^{18}\text{O}_p - 24.073$ ) recalculated for rodents using the dataset of Royer et al. (2013) and that for large mammals (Amiot et al. 2004) (Fig. 3; Supplementary Table).

### Application of the bone phosphate–water oxygen isotope calibration to fossil birds

#### *Penguins from the Miocene and Pliocene Pisco Fm. of Peru*

Published  $\delta^{18}\text{O}_p$  values of marine vertebrates recovered from five fossil sites of the Pisco Formation (Peru) ranging from the

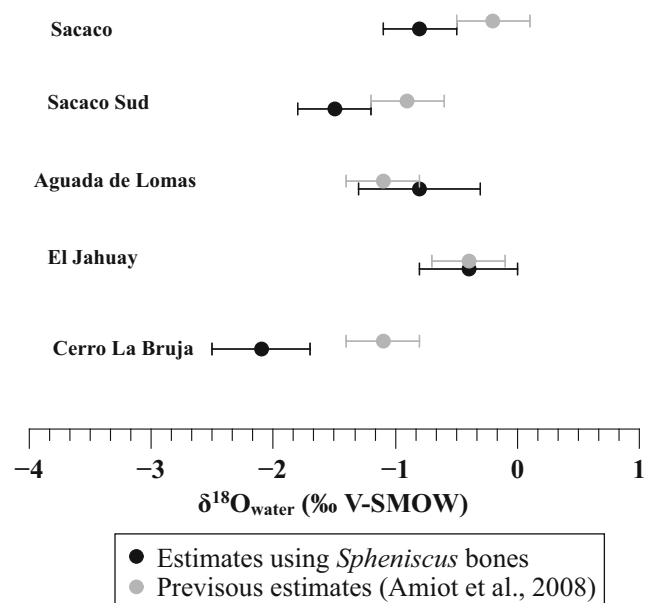


**Table 2** Phosphate oxygen isotope composition of fossil penguin bones from Peru reported along with the  $\delta^{18}\text{O}_w$  value of water estimated using Eq. (2), sample number, bone type, and geographical and stratigraphical origins

Sample no.	Bone	Taxon	Locality	Formation	$\delta^{18}\text{O}_p$ (‰ V-SMOW)	Estimated $\delta^{18}\text{O}_w$ (‰ V-SMOW)	Data source
SAO 69-AW	Femur	<i>Spheniscus urbinai</i>	Sacaco, Peru	Sacaco Fm.	20.7	−1.1	Amiot et al. (2008)
SAO 70-AX	Femur	<i>S. urbinai</i>	Sacaco, Peru	Sacaco Fm.	20.9	−0.8	Amiot et al. (2008)
SAS 1380w-A	Tibiotarsus	<i>S. urbinai</i>	Sacaco Sud, Peru	Sacaco Fm.	20.4	−1.4	Amiot et al. (2008)
SAS 1036-B	Tibiotarsus	<i>S. urbinai</i>	Sacaco Sud, Peru	Sacaco Fm.	19.8	−2.1	Amiot et al. (2008)
SAS 1380u-C	Femur	<i>S. urbinai</i>	Sacaco Sud, Peru	Sacaco Fm.	20.5	−1.3	Amiot et al. (2008)
SAS 305-D	Coracoid	<i>S. urbinai</i>	Sacaco Sud, Peru	Sacaco Fm.	20.2	−1.6	Amiot et al. (2008)
SAS 126-E	Tibiotarsus	<i>Spheniscus</i> sp.	Sacaco Sud, Peru	Sacaco Fm.	20.3	−1.5	Amiot et al. (2008)
AGL 145F-AE	Carpometacarpus	<i>S. urbinai</i>	Aguada de Lomas, Peru	Sacaco Fm.	21.2	−0.5	Amiot et al. (2008)
AGL PPI 145G-AY	Distal femur	<i>S. urbinai</i>	Aguada de Lomas, Peru	Sacaco Fm.	20.4	−1.4	Amiot et al. (2008)
AGL PPI 145C-AZ	Humerus	<i>S. urbinai</i>	Aguada de Lomas, Peru	Sacaco Fm.	21.0	−0.7	Amiot et al. (2008)
ELJ PPI 141B-BA	Prox. radius	<i>S. urbinai</i>	El Jahuay, Peru	Sacaco Fm.	21.5	−0.2	Amiot et al. (2008)
ELJ PPI 141C-BB	Carpometacarpus	<i>S. urbinai</i>	El Jahuay, Peru	Sacaco Fm.	21.4	−0.3	Amiot et al. (2008)
ELJ PPI 141D-BC	Dist. femur	<i>S. urbinai</i>	El Jahuay, Peru	Sacaco Fm.	20.8	−0.9	Amiot et al. (2008)
ELJ PPI 142-BD	Tibiotarsus	<i>S. urbinai</i>	El Jahuay, Peru	Sacaco Fm.	21.1	−0.6	Amiot et al. (2008)
CLB PPI 151-AA	Femur	<i>Spheniscus muizoni</i>	Cerro la Bruja, Peru	Sacaco Fm.	19.5	−2.4	Amiot et al. (2008)
CLB PPI 147f-AB	Tibiotarsus	<i>S. muizoni</i>	Cerro la Bruja, Peru	Sacaco Fm.	20.1	−1.7	Amiot et al. (2008)
CLB PPI 147e-AC	Femur	<i>S. muizoni</i>	Cerro la Bruja, Peru	Sacaco Fm.	19.5	−2.4	Amiot et al. (2008)

latest Middle/earliest Late Miocene to the Early Pliocene allowed the investigation of paleoenvironmental conditions and paleoecologies of the studied vertebrates (Amiot et al. 2008). The sample collection consists of isolated remains of coexisting seals, dolphins, whales, sharks, crocodilians, turtles, and penguins that had preserved their pristine oxygen isotope compositions of bone and tooth phosphates (Amiot et al. 2008). The  $\delta^{18}\text{O}_p$  values of marine mammals, sharks, crocodilians, and turtles were interpreted in terms of seawater  $\delta^{18}\text{O}_{sw}$  values and marine temperatures thanks to existing phosphate–water fractionation equations established for their nearest living relatives (Kolodny et al. 1983; Yoshida and Miyazaki 1991; Barrick et al. 1999; Amiot et al. 2007). Seawater  $\delta^{18}\text{O}_{sw}$  values were estimated to have varied from  $-1.1 \pm 0.3$  to  $-0.2 \pm 0.3\text{‰}$  during the studied interval. At the time, the drinking water  $\delta^{18}\text{O}$  values of penguins (*Spheniscus* sp.; Table 2) could not be estimated from the  $\delta^{18}\text{O}_p$  values of their remains because of the lack of a proper fractionation equation. Using Eq. (2), drinking waters of these marine birds had  $\delta^{18}\text{O}_w$  values ranging from  $-2.1 \pm 0.4$  to  $-0.4 \pm 0.4\text{‰}$  and match previous estimates by less than 1‰ differences (Fig. 5). As marine semi-aquatic predators, penguins ingest a significant amount of seawater along with their fish prey during hunting, thus recording the  $\delta^{18}\text{O}$  value of their foraging environment in their phosphatic remains. Accordingly, Eq. (2) accurately estimates the oxygen

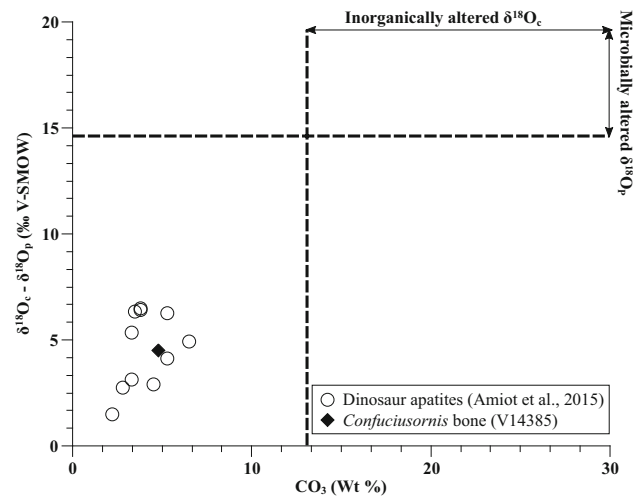
isotope composition of drinking water of extinct birds probably having physiologies closely resembling those of extant ones.



**Fig. 5** Seawater  $\delta^{18}\text{O}_w$  values estimated for five Miocene and Pliocene localities of the Pisco Formation using mammal and reptile  $\delta^{18}\text{O}_p$  values (gray circles; Amiot et al. 2008) and using penguin (*Spheniscus*)  $\delta^{18}\text{O}_p$  values and the newly established Eq. (2) for birds (black circles)

## 2. *Confuciusornis* from the Early Cretaceous Yixian Fm. of China

A humerus belonging to an adult specimen (humerus length 55 mm; IVPP collection number V14385) of the Early Cretaceous tree-dwelling, perching bird *Confuciusornis* housed at the Institute of Vertebrate Paleontology and Paleoanthropology, Beijing, China, was sampled and analyzed to test the applicability of Eq. (2) to a basal bird belonging to the order Confuciusornithiformes (Hou et al. 1995). Phylogeny places *Confuciusornis* between the most primitive *Archaeopteryx* and the derived Ornithothoraces (Wang et al. 2016), the latter possessing a highly advanced anatomy of the thorax that gave them improved flight capability. Moreover, it has been argued that *Confuciusornis* could not achieve efficient flapping flight due to the sideways rather than angled upward orientation of its shoulder joint (Senter 2006). Phosphate oxygen isotope measurement of a humerus fragment from specimen V14385 yielded a value of 15.3‰. In order to test the primary isotopic preservation of specimen V14385, apatite carbonate oxygen isotope composition ( $\delta^{18}\text{O}_c$ ) and the carbonate content of the bone (in wt%) have also been measured and yielded a  $\delta^{18}\text{O}_c$  value of 20.1‰ (V-SMOW) and a weight content of 4.5%. Previous studies have shown that the carbonate content in apatite of modern vertebrates typically ranges from less than 1 to 13.4%, with an average content of 4–5% (Brudevold and Soremark 1967; Rink and Schwarcz 1995; Vennemann et al. 2001; Tarnowski et al. 2002). Thus, samples that have a carbonate content exceeding 13.4 wt% likely contains additional inorganic carbonate precipitated from diagenetic fluids that would affect the measured  $\delta^{18}\text{O}_c$  value. This is clearly not the case for the bone specimen V14385 that contains 4.5% of carbonate and fall within the range measured on dinosaur apatites from the nearby locality of Lujiatun belonging to the Yixian Formation (Fig. 6; Amiot et al. 2015). In modern vertebrates, the oxygen isotope composition of apatite carbonate is higher than that of co-occurring apatite phosphate (7–9‰ in mammals), and up to 14.7‰ in sharks (Vennemann et al. 2001). Experimental (Zazzo et al. 2004a) and empirical studies (Zazzo et al. 2004b) have shown that microbially mediated diagenetic alteration of apatite phosphate results in a higher difference between  $\delta^{18}\text{O}_c$  and  $\delta^{18}\text{O}_p$  values. Therefore, fossil samples exhibiting  $\delta^{18}\text{O}_c - \delta^{18}\text{O}_p$  differences larger than 14.7‰ are most likely altered (Fig. 6). Inorganic alteration at low temperature has little effect on the  $\delta^{18}\text{O}_p$  values of phosphates, even at geological timescales (Lécuyer et al. 1999), so samples affected by inorganic diagenetic alteration of carbonates (resulting either in a high overall carbonate content or anomalous  $\delta^{18}\text{O}_c - \delta^{18}\text{O}_p$  differences) may still preserve the original oxygen isotope composition of their phosphate (Fig. 6). Using both the  $\delta^{18}\text{O}_c - \delta^{18}\text{O}_p$  difference and the carbonate content of the bone V14385 that lie within the range of



**Fig. 6**  $\delta^{18}\text{O}_c - \delta^{18}\text{O}_p$  differences of *Confuciusornis* bone (black diamond) and other dinosaurs from the Yixian Fm. (white circles; Amiot et al. 2015) plotted against their structural carbonate content (wt%) of apatite. Samples that have  $\delta^{18}\text{O}_c - \delta^{18}\text{O}_p$  differences higher than 14.7‰ or carbonate contents (wt%) higher than 13.4% are likely to have lost their pristine oxygen isotope composition of either phosphate or carbonate as a result of inorganic or bacterially mediated alteration (see text)

other samples from the same formation (Fig. 6), we can expect that the bone sample of *Confuciusornis* may have mostly preserved its original oxygen isotope composition of phosphate.

According to Eq. (2), the drinking water of this specimen would have a  $\delta^{18}\text{O}_w$  value  $-7.1 \pm 0.5\text{‰}$ . This value is close to the local water  $\delta^{18}\text{O}_w$  values ranging from  $-11.0$  to  $-7.4\text{‰}$  (mean  $= -9.2 \pm 1.8\text{‰}$ ) that were estimated for the Yixian Formation based on the oxygen isotope compositions of vertebrate remains (dinosaurs, turtles, and choristoderes) recovered from the five localities of Lujiatun, Zhangjiagou, Baicaigou, Beipiao, and Hejiaxin (Amiot et al. 2011). Assuming that diagenetic alteration was negligible, a first hypothesis is that *Confuciusornis* bones correspond to a young adult specimen that have recorded a growing period corresponding to the summer when the  $\delta^{18}\text{O}_w$  value of local precipitations were higher than the average value previously estimated. Indeed, osteohistology indicates that *Confuciusornis* could have reached maturity in about 8 to 13 weeks (de Ricqlès et al. 2003). If specimen V14385 is indeed a young adult 3–4 months old that hatched during spring time, then its elevated  $\delta^{18}\text{O}_p$  value makes sense. A second hypothesis is that *Confuciusornis* had a metabolic status different from that of modern birds. From an osteohistological point of view, the metabolic status of basal birds has remained controversial (Chinsamy et al. 1994; 1995; Zhang et al. 1998; Padian et al. 2001; de Ricqlès et al. 2003); *Confuciusornis* probably had growth rates lower than those of modern birds of similar body mass but within the range of larger birds or relatively slowly growing birds such as tinamous (de Ricqlès et al. 2003). As ratites and tinamous possess lower metabolic rates and body temperatures than carinate birds (Withers et al.

1987), it can be suspected that *Confuciusornis* possessed metabolic rates within a similar range, or even within the range of extant endothermic mammals. Having a metabolic rate within the range of extant mammals would explain the slightly high  $\delta^{18}\text{O}_\text{p}$  value of *Confuciusornis*. For instance, if *Confuciusornis* had a body temperature of 37–38 °C (mammal range) instead of 40–42 °C, then this metabolic difference would result in a  $\sim 1\text{‰}$  more positive value of *Confuciusornis* bone phosphate relative to a modern bird. Thus, decreasing the value of 15.3 to 14.3‰ would generate a  $\delta^{18}\text{O}_\text{w}$  value of  $-8.2\text{‰}$ , within the expected range for the Yixian Formation. A dedicated study of the oxygen isotope composition of basal birds including more specimens of *Confuciusornis* should clarify the applicability of Eq. (2) to investigate Cretaceous environments in terms of  $\delta^{18}\text{O}_\text{w}$  values of local surface waters.

## Conclusion

Oxygen isotope compositions of bone phosphate measured from broiler chickens sampled from 21 farms worldwide show a linear correlation with the  $\delta^{18}\text{O}_\text{w}$  values of local precipitation. The empirical phosphate–water oxygen isotope fractionation equation determined for chicken also provides accurate estimates with a mean standard error of 0.45‰ ( $\sigma_\text{est}$ ) of the drinking water  $\delta^{18}\text{O}_\text{w}$  value of semi-aquatic birds (mallard ducks, European herring gull), of terrestrial birds of prey (common buzzard), and of large ground birds (common ostrich, greater rhea). These results suggest, at the first order, that the established relationship can be used to estimate the drinking water  $\delta^{18}\text{O}_\text{w}$  value of extant birds regardless of their ecological or physiological specificity. A pilot test on published oxygen isotope compositions of fossil penguins (*Spheniscus*) from Miocene and Pliocene localities in Peru shows that this new equation accurately reproduces local seawater  $\delta^{18}\text{O}_\text{w}$  values previously estimated from the  $\delta^{18}\text{O}_\text{p}$  values of co-occurring marine mammals and reptiles. Applied to one bone sample of the basal bird *Confuciusornis*, from the Early Cretaceous of Northeastern China, this equation gives a slightly overestimated  $\delta^{18}\text{O}_\text{w}$  value compared to the previously proposed range, possibly reflecting either a seasonal record, or that *Confuciusornis* had low metabolic rates out of the range of modern birds and within a similar range as mammals. A dedicated isotopic study of basal birds of various phylogenetic affinities and origin should clarify the applicability of the established relationship to the whole avian clade, and maybe to immediate bird ancestors, the maniraptoran theropod dinosaurs.

**Acknowledgements** Dr. Stanislaw Halas, co-author of this study, passed away the 3rd may 2017. Our thoughts are with his family and colleagues during these difficult times. The authors would like to thank V.

Paulet, P. Touzeau, M. Mathis, D. Viscaino, I. Buffetaut, J. Barnoud, M. and W. Halverson, G. and C. von Hahn, A. and O. von Lilienfeld, S. and G. Caillard, J., and J. and P. Angst for providing chicken bones and D. Berthet from the Musée des Confluences, Lyon, France, for providing the bone samples of *Buteo buteo* (50.001696), *Larus argentatus* (50.001682), and *Anas platyrhynchos* (50.001681). We also would like to thank the five anonymous reviewers for their constructive comments that greatly helped to improve the manuscript. This study was supported by the CNRS PICS project no. PIC07193, the National Basic Research Program of China grant 2012CB821900 (RA), and the Institut Universitaire de France (CL).

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