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Oxygen isotope fractionation between crocodilian phosphate and water

Romain Amiot [a,*], Christophe Lécuyer [a,1], Gilles Escarguel [a], Jean-Paul Billon-Bruyat [b], Eric Buffetaut [c], Cyril Langlois [a], Samuel Martin [d], François Martineau [a], Jean-Michel Mazin [a]

[a] *Laboratoire Paléoenvironnements and Paléobiosphère, CNRS UMR 5125, Université Claude Bernard Lyon I, 27-43 bld du 11 novembre 1918, 69622 Villeurbanne, France*
[b] *Section d'archéologie et paléontologie, Office de la Culture, République et Canton Jura, Hôtel des Halles, 2900 Porrentruy, Switzerland*
[c] *CNRS UMR 5125, 16 cours du Liégat 75013 Paris, France*
[d] *La Ferme aux Crocodiles, Les Blachettes, 26700 Pierrelatte, France*

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Abstract

Oxygen isotope compositions of phosphate ($\delta^{18}O_p$) were measured in tooth enamel from captive and wild individuals of 8 crocodilian species. A rough linear correlation is observed between the $\delta^{18}O_p$ of all the studied species and the oxygen isotope composition of ambient water ($\delta^{18}O_w$). Differences in mean air temperature, diet and physiology could contribute significantly to the large scatter of $\delta^{18}O_p$ values. The combination of these parameters results in a fractionation equation for which the slope (0.82) is lower than that expected (≥ 1) from predictive model equations that assume temperature and diet as fixed parameters. Taking into account large uncertainties, the observed oxygen isotope fractionation between phosphate and ambient water does not statistically differ from that formerly established for aquatic turtles. Case studies show that $\delta^{18}O_p$ values of fossil crocodile tooth enamel can be used to discriminate between marine and freshwater living environments within a precision of about ±2‰ only.

Keywords: Crocodile; Turtle; Oxygen isotopes; Fractionation; Paleoecology

1. Introduction

Only a few continental proxies are available to quantify Pre-Cenozoic environmental or climatic parameters, whereas a major effort is made to better constrain Mesozoic climates through models such as GCM (Global Circulation Models, e.g., Valdes et al., 1999) or LAM (Limited Area Numerical Climate Model, e.g., Haywood et al., 2004). These existing proxies include paleovegetation studies that allow reconstructions of terrestrial paleotemperatures by using a transfer function based on the present-day relationship between leaf margin morphology and air temperature (CLAMP method; Wolfe and Upchurch, 1987; Wolfe, 1993). The fragility of plant tissues, however, limits the conservation of macroremains

* Corresponding author.
E-mail address: Romain.Amiot@univ-lyon1.fr (R. Amiot).
[1] Also at the Institut Universitaire de France, 103 bld Saint-Michel, 75005 Paris, France.

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in most depositional environments. Due to their great abundance in the fossil record and far better resistance to chemical alteration processes, reptile phosphatic remains are potentially reliable proxies of the oxygen isotope composition of surface waters during the Mesozoic. The oxygen isotope composition of these estimated meteoric waters ($\delta^{18}O_w$) can then be used to estimate past air temperatures (Barrick et al., 1999; Amiot et al., 2004). A linear phosphate–water relationship for living turtles was established in order to identify the source of their ingested water (marine, brackish or fresh water) and was applied to fossil counterparts in order to estimate the oxygen isotope composition of environmental water (Barrick et al., 1999). However, fossil bone such as turtle plate can be sensitive to isotopic exchange with aqueous fluids during diagenetic processes (Kolodny et al., 1996; Zazzo et al., 2004b). Indeed, secondary precipitation of apatite and isotopic exchange during microbially mediated reactions may strongly disturb the primary isotopic record (Blake et al., 1997; Zazzo et al., 2004a). Several case studies (Kolodny and Raab, 1988; Picard et al., 1998; Lécuyer et al., 2003; Pucéat et al., 2003) have shown that tooth enamel remains the best biomineral to reconstruct Pre-Cenozoic environments because apatite crystals that make up tooth enamel are large and densely packed, therefore offering more resistance to diagenesis than bone.

Table 1
Oxygen isotope composition of phosphate from tooth enamel of present-day wild (W) and captive (C) crocodilians

Sample	Taxon	Family	Origin	Country	$\delta^{18}O_p$ (‰ SMOW)	$\delta^{18}O_w$ (‰ SMOW)	Data source
CR001	*Crocodylus niloticus*	Crocodylidae	(C) Lyon	France	13.8	−7.3[m]	This study
CR002	*Crocodylus niloticus*	Crocodylidae	(C) Lyon	France	12.9	−7.3[m]	This study
CR004	*Crocodylus niloticus*	Crocodylidae	(C) Parc culturel du Phare	Tunisia	14.9	−5.6[m]	This study
CR005	*Crocodylus niloticus*	Crocodylidae	(C) Parc culturel du Phare	Tunisia	14.7	−5.6[m]	This study
CR007	*Crocodylus porosus/ siamensis*	Crocodylidae	(C) Samutprakarn	Thailand	19.4	−1.8[e]	This study
CR008	*Crocodylus porosus/ siamensis*	Crocodylidae	(C) Samutprakarn	Thailand	18.8	−1.8[e]	This study
CR009	*Crocodylus niloticus*	Crocodylidae	(C) Pierrelatte	France	18.9	−2.1[m]	This study
CR011	*Crocodylus niloticus*	Crocodylidae	(C) Pierrelatte	France	18.3	−2.1[m]	This study
CR012	*Crocodylus niloticus*	Crocodylidae	(C) Pierrelatte	France	19.3	−2.1[m]	This study
CR010	*Crocodylus niloticus*	Crocodylidae	(C) Pierrelatte	France	16.2	−5.5[m]	This study
CR013	*Crocodylus niloticus*	Crocodylidae	(C) Pierrelatte	France	17.1	−5.5[m]	This study
CR024	*Crocodylus niloticus*	Crocodylidae	(C) Pierrelatte	France	16.9	−5.5[m]	This study
CR025	*Crocodylus niloticus*	Crocodylidae	(C) Pierrelatte	France	15.6	−5.5[m]	This study
CR014	*Alligator mississipiensis*	Alligatoridae	(C) Pierrelatte	France	13.3	−8.8[m]	This study
CR015	*Melanosuchus niger*	Alligatoridae	(C) Pierrelatte	France	13.8	−8.8[m]	This study
CR016	*Melanosuchus niger*	Alligatoridae	(C) Pierrelatte	France	13.6	−8.8[m]	This study
CR017	*Tomistoma schlegelii*	Gavialidae	(C) Pierrelatte	France	12.6	−8.8[m]	This study
CR018	*Tomistoma schlegelii*	Gavialidae	(C) Pierrelatte	France	14.3	−8.8[m]	This study
CR019	*Tomistoma schlegelii*	Gavialidae	(C) Pierrelatte	France	12.5	−8.8[m]	This study
CR020	*Crocodylus niloticus*	Crocodylidae	(C) Rivière des anguilles	Mauritius	20.3	−3.0[e]	This study
CR021	*Crocodylus niloticus*	Crocodylidae	(C) Rivière des anguilles	Mauritius	19.2	−3.0[e]	This study
CR022	*Crocodylus niloticus*	Crocodylidae	(C) Lusaka	Zambia	19.0	−4.7[m]	This study
CR023	*Crocodylus niloticus*	Crocodylidae	(C) Lusaka	Zambia	18.7	−4.7[m]	This study
CR026	*Crocodylus niloticus*	Crocodylidae	(C) Etang Salé	Reunion Island	17.4	−6.1[m]	This study
CR027	*Crocodylus niloticus*	Crocodylidae	(C) Etang Salé	Reunion Island	18.5	−6.1[m]	This study
CR029	*Paleosuchus palpebrosus*	Alligatoridae	(C) Pierrelatte	France	13.7	−8.8[m]	This study
CR030	*Crocodylus porosus*	Crocodylidae	(C) Pierrelatte	France	12.9	−8.8[m]	This study
CR031	*Crocodylus niloticus*	Crocodylidae	(C) Benmore	South Africa	23.3	0.9[e]	This study
CR032	*Crocodylus niloticus*	Crocodylidae	(C) Benmore	South Africa	24.3	0.9[e]	This study
–	*Alligator mississipiensis*	Alligatoridae	(C) Florida	U.S.A.	19.2	−3.6[m]	Stoskopf et al. (2001)
–	*Alligator mississipiensis*	Alligatoridae	(W) Northern Florida	U.S.A.	21.1	−3.7[m]	Stoskopf et al. (2001)
–	*Osteolaemus tetraspis*	Crocodylidae	(W) Oubangui river	Central Africa	19.4	−4.5[e]	Lécuyer et al. (2003)
–	*Alligator mississipiensis*	Alligatoridae	(W) Florida	U.S.A.	17.4	−5.0[e]	Lécuyer et al. (1999)
–	*Crocodylus rhombifer*	Crocodylidae	(W)	Brazil	20.1	−2.6[e]	Lécuyer et al. (1999)
–	*Crocodylus niloticus*	Crocodylidae	(?) Jordan Valley	Israel	15.3	−5.2[m]	Kolodny et al. (1996)

Sample identification and location are reported along with the oxygen isotope composition of their environmental water which was measured (m) or estimated (e) by using mean weighted $\delta^{18}O$ values of local meteoric waters from IAEA/WMO data (IAEA/WMO, 2001).

We propose that fossil crocodilian tooth enamel, commonly occurring in Mesozoic fossil vertebrate assemblages, may also be used to reconstruct paleoenvironments. By using an oxygen isotope fractionation equation determined with 29 data obtained from wild or captive individuals belonging to 8 crocodile species, it is demonstrated that the $\delta^{18}O$ values of tooth enamel from Mesozoic crocodiles indicate aquatic environments in agreement with their known paleoecology.

2. Material and methods

2.1. Sample collection

Most present-day crocodilians are restricted to tropical areas due to their ecological requirements. As a large range of $\delta^{18}O_w$ values is needed to establish an oxygen isotope fractionation equation, captive crocodilians living in farms and zoos at various latitudes provide the best sampling opportunities, despite captivity conditions that may not precisely reflect those observed in the wild (for example, average $\delta^{18}O$ values of source waters, temperature of skeletal formation, indoor humidity for crocodilians reared in greenhouses, or stress may be different from those experienced under natural conditions).

Twenty-nine teeth from crocodilians (Table 1) living in farms and zoos at various latitudes (Fig. 1) were analysed along with ambient water when available. Otherwise, water $\delta^{18}O$ values were estimated using mean weighted $\delta^{18}O$ values of local meteoric waters from IAEA/WMO data (IAEA/WMO, 2001). Among the 23 existing species of crocodilians (Huchzermeyer, 2003), tooth samples from 8 species belonging to the three main families (Crocodylidae, Alligatoridae and Gavialidae) were analysed in order to test possible variations in their $\delta^{18}O_p$ values in response to specific ecological or physiological patterns. Teeth of the Nile crocodile *Crocodylus niloticus* come from France, Tunisia, Mauritius, Zambia, Reunion Island and South Africa. Teeth of the Indo-Pacific crocodile *Crocodylus porosus* are from France and Thailand, whereas those from the American alligator *Alligator mississippiensis*, the black caiman *Melanosuchus niger*, the false gharial *Tomistoma schlegelii* and Cuvier's dwarf caiman *Paleosuchus palpebrosus* come from France. Additional $\delta^{18}O_p$ values from the literature complement the database with *C. niloticus* from Israel (Kolodny et al., 1996), bones and teeth of wild and captive *A. mississippiensis* from Florida (Lécuyer et al., 1999; Stoskopf et al., 2001), teeth of the dwarf crocodile *Osteolaemus tetraspis* from Central Africa (Lécuyer et al., 2003) and of the Cuban crocodile *Crocodylus rhombifer* from Brazil (Lécuyer et al., 1999). As crocodile teeth continuously grow over several months by incremental layers with a duration depending upon the size of the crocodile (Erickson, 1996), enamel was sampled with a microdrill from the base to the apex, and several teeth from each species were analysed at all localities in order to minimize possible seasonal variations in $\delta^{18}O$ values. Blood and urine samples were also taken from six Nile crocodiles from "la Ferme aux Crocodiles" at Pierrelatte, France.

2.2. Analytical techniques

Measurements of oxygen isotope ratios of apatite consist of isolating PO_4^{3-}, using acid dissolution and anion-exchange resin, according to a protocol derived from the original method published by Crowson et al.

Fig. 1. Map showing the sampling locations of crocodilian teeth and waters: (1) Pierrelatte, France; (2) Lyon, France; (3) Tunisia; (4) Thailand; (5) Mauritius; (6) Reunion Island; (7) Zambia; (8) South Africa; (9) Florida; (10) Central Africa; (11) Brazil; (12) Israel.

Table 2
Oxygen isotope composition of blood plasma and ambient waters of crocodiles (*Crocodylus niloticus*) and turtles (*Chrysemys* sp.)

Sample	Taxon	Origin	Country	T_{body} (°C)	Body mass (kg)	$\delta^{18}O_{blood}$	$\delta^{18}O_{water}$	$\Delta^{18}O_{blood-water}$	Data source
SG001	*Crocodylus niloticus*	Pierrelatte	France	26	4–5	−6.8	−8.8	2.0	This study
SG002	*Crocodylus niloticus*	Pierrelatte	France	26	4–5	−7.1	−8.8	1.7	This study
SG003	*Crocodylus niloticus*	Pierrelatte	France	26	4–5	−7.5	−8.8	1.3	This study
SG004	*Crocodylus niloticus*	Pierrelatte	France	26	4–5	−7.2	−8.8	1.6	This study
SG005	*Crocodylus niloticus*	Pierrelatte	France	26–29	1.4	−6.8	−6.2	~0	This study
SG006	*Crocodylus niloticus*	Pierrelatte	France	26–29	1.2	−5.4	−6.2	0.8	This study
172	*Chrysemys* sp.	Lee Metcalf Res, MT	U.S.A.	31–33 [a]	–	−11.2	−15.0	3.8	Barrick et al. (1999)
502	*Chrysemys* sp.	Lee Metcalf Res, MT	U.S.A.	31–33 [a]	–	−11.4	−15.0	3.6	Barrick et al. (1999)
211	*Chrysemys* sp.	Fern Hammock, FL	U.S.A.	31–33 [a]	–	−1.2	−3.8	2.6	Barrick et al. (1999)
156	*Chrysemys* sp.	Cedar River, IA	U.S.A.	31–33 [a]	–	−2.3	−6.2	3.9	Barrick et al. (1999)
Urine sample SG005:						$\delta^{18}O_{urine}$			
U1	*Crocodylus niloticus*	Pierrelatte	France	26–29	1.4	−6.4	−6.2	~0	This study

[a] Calculated value (see text).

(1991) and slightly modified by Lécuyer et al. (1993). Silver phosphate is quantitatively precipitated in a thermostatic bath set at a temperature of 70 °C. After filtration, washing with double deionised water, and drying at 50 °C, 15 mg of Ag_3PO_4 are mixed with 0.8 mg of pure graphite powder. $^{18}O/^{16}O$ ratios are measured by reducing silver

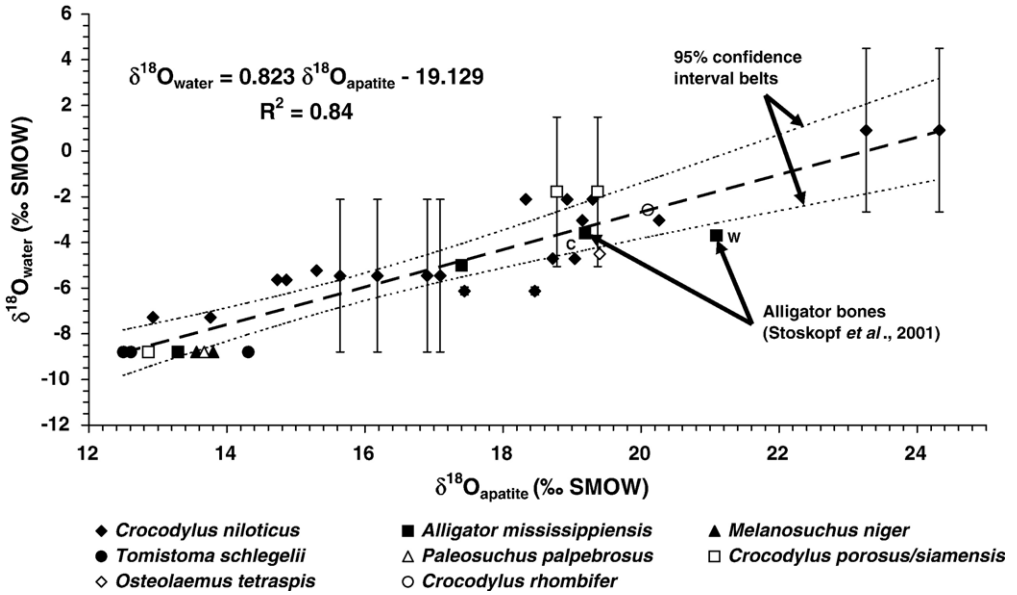

Fig. 2. Variations in $\delta^{18}O_P$ values of apatite phosphate from eight crocodilians reported against the $\delta^{18}O_W$ values of their ambient waters. For several specimens, high evaporations of water ponds that are regularly re-filled by new water led to important variations in the $\delta^{18}O_W$ values, hence, large uncertainties represented by the vertical error bars. The corresponding linear regression line (dashed line) is drawn along with associated bootstrapped 95% confidence interval belts (dotted lines). Concerning Alligator bone values, "C" is for captive and "W" for wild animal.

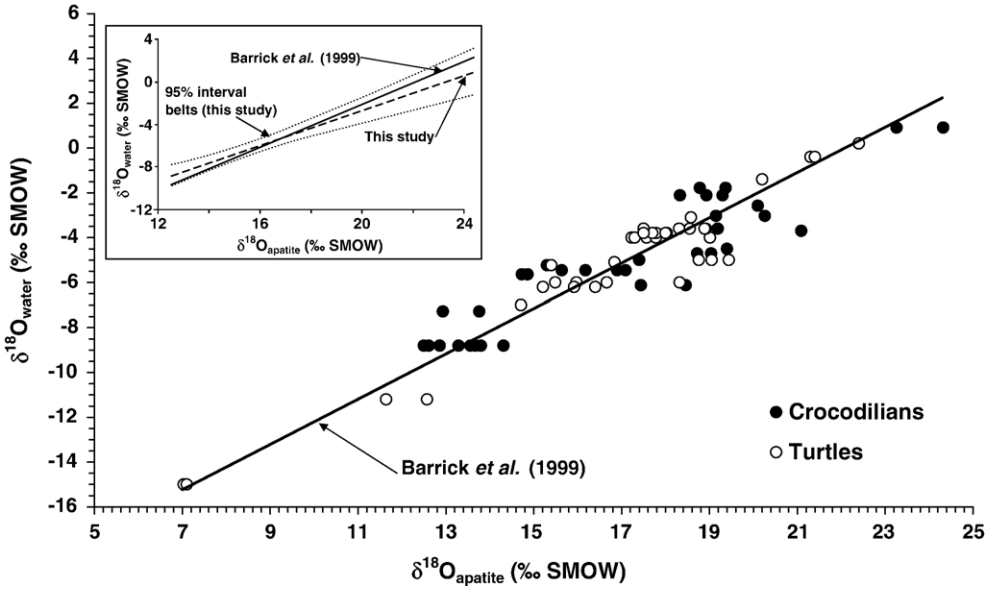

Fig. 3. Same data as in Fig. 1 are plotted along with published turtle values (Barrick et al., 1999). The dashed line corresponds to the equation established for aquatic turtles (Barrick et al., 1999). In the top left caption: comparison between the relationships established for crocodilians (this study) and turtles.

phosphates to CO_2 using graphite reagent (O' Neil et al., 1994; Lécuyer et al., 1998). Samples are weighed into tin reaction capsules, loaded into quartz tubes and degassed for 30 min at 80 °C under vacuum. Each sample was heated at 1100 °C for 1 min to promote the redox reaction. The CO_2 produced was directly trapped in liquid nitrogen to avoid any kind of isotopic reaction with quartz at high temperature. CO_2 was then analyzed with a GV Isoprime™ mass spectrometer at the Laboratory UMR CNRS 5125 'PEPS', University Claude Bernard Lyon 1. Isotopic compositions are quoted in the standard δ-notation relative to V-SMOW. Silver phosphate precipitated from standard NBS120c (natural Miocene phosphorite from Florida) was repeatedly analyzed ($\delta^{18}O = 21.7 \pm 0.2$‰; $n = 6$) along with the silver phosphate samples derived from the crocodilian tooth enamel.

Water analyses correspond to single random sampling performed during the years 2003 and 2004. Three milliliters of water from each farming basin was equilibrated with 20 μmol of CO_2 at 25 °C for 48 h. $\delta^{18}O$ values of water were calculated using the isotopic fractionation value $\alpha_{CO_2-water}$ of 1.0412 at 25 °C determined by O' Neil et al. (1975). Reproducibility of oxygen isotope measurements is better than 0.1‰. Crocodile blood samples were centrifugated two times at 3000 rpm during 10 min to separate the plasma for which the same analytical method as for water was applied, as well as for the urine sample.

3. Results

Oxygen isotope compositions of crocodilian tooth enamel, ambient water, urine and blood are reported in Tables 1 and 2. The four sub-adult *C. niloticus*, with a body mass of 4–5 kg, have a mean $\Delta^{18}O_{blood-water}$ of 1.7±0.3‰ for a body temperature (T_b) of 26 °C (Table 2). Similar blood and urine measurements performed on two juvenile *C. niloticus* of 1.2 kg and 1.4 kg do not show any ^{18}O-enrichment of blood and urine relative to drinking water (Table 2).

$\delta^{18}O_p$ values of phosphate (from 12.5‰ to 24.3‰) from all studied crocodilian species are roughly linearly correlated to those of ambient water (from −8.8‰ to 0.9‰; Fig. 2), leading to the following regression equation:

$$\delta^{18}O_w = 0.82\ \delta^{18}O_p - 19.13 \quad (1)$$

Associated bootstrapped 95% confidence intervals are [0.583; 1.077] and [−23.429;−15.064] for the slope and intercept, respectively. These intervals were estimated using a nonparametric bootstrap (10,000 iterations) coupled with a parametric (Gaussian) resampling of the $\delta^{18}O_P$ and $\delta^{18}O_W$ values of each point constituting the bootstrapped pseudo-samples. This equation is compared to the fractionation equation determined for living turtles (Barrick et al., 1999), another group of semi-aquatic to

aquatic reptiles. Bootstrapped confidence interval belts for predicted values associated to Eq. (1) reveal that the two regression lines cannot be distinguished at the 95% confidence level (Fig. 3). Random and single sampling strategy of ambient waters must be considered as the largest source of uncertainties associated with the determination of the oxygen isotope fractionation equation between crocodilian phosphate and water.

4. Discussion

4.1. Meaning of the oxygen isotope fractionation equation

Crocodilians are carnivorous semi-aquatic to aquatic ectothermic reptiles with aerial respiration. Their oxygen isotope composition should thus reflect, amongst others, the oxygen isotope composition of their drinking water and their body temperature, themselves related to their immediate environment. Therefore, there are several sources of uncertainties in the determination of the oxygen isotope fractionation between crocodilian phosphate and water. One main source is the uncertainty associated with the $\delta^{18}O$ values of water resources that may differ from those of ambient waters. For example, differences in diet, seasonal variations or evaporation of local waters, the use of mountain lake waters or groundwater may explain such discrepancies between the composition of ingested water relative to rainfall. Other sources of uncertainties are related to the physiology of crocodilians.

The "activity" body temperature of crocodilians is restricted to the 26–36 °C range (see Markwick, 1998, and references therein). In order to keep their body temperature within this range, in colder environments, crocodilians will bask in the sun to raise their body temperature above this minimum, and conversely, in warmer environments, they will cool down in water or shade to keep their body temperature below their critical maximum of 39–40 °C. It is emphasized that it is impossible to know the temperature of tooth formation, which can be different from body temperature because of the peripheral position of teeth. Jaw temperature is most likely dominated by ambient temperature.

Studied samples come from captive crocodilians that live in zoos or farms which are mostly located in tropical regions, or, when at temperate latitudes, in conditions where ambient temperatures are maintained within the 25–35 °C range, roughly reproducing the conditions of their tropical environments. Considering crocodilian thermal strategies, it is expected that the oxygen isotope composition of crocodilian phosphate does not only depend on the composition of drinking water but also on temperature. Oxygen isotope fractionation between phosphate and water increases with decreasing temperatures. This basic principle is illustrated by the following example: a captive alligator that was reared at a near constant temperature of 32±2 °C has a mean $\delta^{18}O_p$ value of bones 2‰ lower than its wild counterpart for which body temperatures were 26±8 °C (Stoskopf et al., 2001). More generally, the range of crocodilian activity temperature (about 10 °C) corresponds to $\delta^{18}O$ variations of 2–3‰ according to the phosphate–water temperature scale (Longinelli and Nuti, 1973) and this isotopic range is comparable to the observed data scattering (Fig. 2).

Oxygen isotope measurements of blood samples (Table 2) show that $\Delta^{18}O_{blood-water}$ values of crocodilians range from about 0‰ to 2‰, being responsible for $\delta^{18}O_p$ variations. Body fluids of small crocodilians have oxygen isotope compositions similar to those of environmental waters whereas the largest studied specimens have $\Delta^{18}O_{blood-water}$ from 1.3‰ to 2‰ (Table 2). These

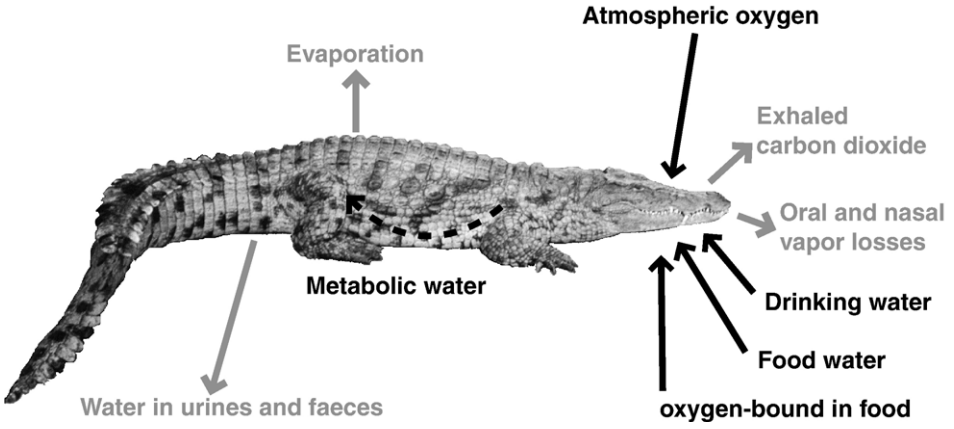

Fig. 4. Main oxygen fluxes that control the oxygen isotope composition of crocodilian body water.

results suggest that the ^{18}O-enrichment of body fluids are related to the body mass and, hence, the metabolic activity, more data being needed to confirm this tendency. This isotopic enrichment is expected to be the result of the balance (Fig. 4) between input (drinking water, food and air intakes), output (evaporation, breathing, urine) oxygen fluxes, and metabolic activity (Luz and Kolodny, 1985; Bryant and Froelich, 1995). The measured body temperature of 26 °C and blood water $\delta^{18}O_w$ values (Table 2) are put into the phosphate–water temperature equation (Longinelli and Nuti, 1973) to calculate phosphate $\delta^{18}O_p$ values that range from 12.5‰ to 14.5‰. These calculated $\delta^{18}O_p$ values match those measured for crocodilians from the Pierrelatte farm (Table 1) living in waters with $\delta^{18}O$ values of −8.8‰ (Table 2 and Fig. 2). Consequently, we can consider that these crocodiles precipitate their phosphate in isotopic equilibrium with their body water.

On the basis of the few isolated data presented in this study, crocodiles have $\Delta^{18}O_{blood-water}$ values of 1.7± 0.3‰ significantly lower than the values of 3.5±0.5‰ obtained for several aquatic turtles belonging to the genus *Chrysemys* (Barrick et al., 1999). Surprisingly, the oxygen isotope fractionation equation established for crocodiles does not statistically differ from that formerly established for aquatic turtles. This means that crocodilians should form their phosphatic skeletons at a lower temperature than turtles; however, both reptiles display similar temperature ranges of apatite synthesis: 20–35 °C for turtles (Barrick et al., 1999) and 26–36 °C for crocodilians (Markwick, 1998). More blood isotopic data for these two semi-aquatic reptiles are needed to solve this apparent paradox.

Linear isotopic fractionation equations that were determined for turtles and crocodilians are characterized by slopes lower or equal to unity. These results are not supported by box models (Kohn, 1996) that predict slopes equal or higher than unity for breathing animals. However, model equations, that assume temperatures and diet as fixed parameters, constitute an oversimplification of natural conditions. In the case of empirical determinations of fractionation equations, differences in mean air temperature, diet and physiology may contribute significantly to the large scattering of observed $\delta^{18}O_p$ values. Crocodiles, being semi-aquatic reptiles, have body water isotopically buffered by a large flux of ambient water, as suggested by the small values of $\Delta^{18}O_{blood-water}$. Consequently, the slope of the fractionation equation is expected to be close to 1, a value compatible with Eq. (1) when taking into account the large associated error envelope defined by the bootstrap analysis.

Despite sizable isotopic uncertainties of as much as ±2‰ generated by Eq. (1) fossil crocodilian remains can be used to determine the oxygen isotope composition of their environmental waters. They can help to discriminate between marine, brackish or freshwater living environments by comparison with other fossil taxa of well known ecology. With this aim in view, the validity of Eq. (1) is tested with a few tooth enamel $\delta^{18}O_p$ values of Mesozoic crocodilians in order to estimate the isotopic source of ingested local waters.

4.2. Mesozoic crocodile case studies

Tooth enamel $\delta^{18}O_p$ values of four marine crocodilians belonging to the genus *Steneosaurus* from the Early Tithonian of France and Germany, for which primary oxygen isotopic preservation has been demonstrated, range from 20.3‰ to 21.7‰ (Billon-Bruyat et al., 2005), indicating $\delta^{18}O_w$ values of −1.8±2‰. This range brackets the $\delta^{18}O_w$ value of about −1‰ estimated for seawater considering an ice-free world where ^{18}O-depleted waters (stored today as polar ice) were added to the ocean.

Oxygen isotope ratios of five tooth enamel samples from the Late Campanian continental fossil crocodilians of Alberta (Canada) range from 12.0‰ to 14.7‰ (Fricke and Rogers, 2000; Amiot et al., 2004), which can be compared to those measured from 10.6‰ to 14.8‰ in turtle plates of the freshwater genus *Aspideretes* (Barrick et al., 1999; Amiot et al., 2004). The high paleolatitude (about 60°N) of the localities, where these fossils were recovered, is compatible with the calculated negative $\delta^{18}O_w$ values ranging from −9.3±2‰ to −7.0±2‰. These $\delta^{18}O_w$ values are, however, less negative than the present-day meteoric water values of about −13‰ from a similar latitude (IAEA/WMO, 2001), likely in response to the warmer climates that prevailed during the Cretaceous.

The continental vertebrate assemblage from the Berriasian locality of Cherves-de-Cognac (France) was deposited in an estuarine paleoenvironment, after being reworked from the land and transported during exceptional events such as storms (El Albani et al., 2004). Aridity markers (such as the occurrence of reworked palygorskite) indicate that dry environments prevailed in this region. $\delta^{18}O_p$ values of freshwater crocodilians (*Goniopholis* and *Pholidosaurus*) and turtles (Pleurosternidae) recovered from this locality range from 21.6‰ to 23.0‰ for turtles and from 21.3‰ to 22.4‰ for crocodilians (Amiot et al., 2006). The resulting $\delta^{18}O_w$ values range from −1.6±2‰ to +0.9±2‰, supporting that these taxa were living in waters submitted to high

evaporation rates. These case studies indicate that oxygen isotope compositions of ambient waters can be calculated to determine their sources.

5. Conclusions

Oxygen isotope compositions of tooth enamel phosphate from various present-day crocodilians are roughly linearly correlated to those of ambient waters. This study underlines the following considerations:

- Variations of about 10 °C in the body temperature of crocodilians corresponding to their activity body temperature range could partly explain the observed mean scatter of 3‰ in the $\delta^{18}O_p$ values of tooth enamel.
- The oxygen isotope fractionation equation determined between crocodilian phosphate and water does not statistically differ from the one established for turtles (Barrick et al., 1999). This similarity may be fortuitous when considering the observed differences in blood ^{18}O-enrichment relative to ambient waters. It may also be related to different temperatures of skeletal formation, differences in diet, various budgets of cutaneous evaporation, continentality or altitude effects for some localities.
- Because of uncertainties of as much as ±2‰ due to the difficulty in determining average $\delta^{18}O_w$ values of source waters and of temperatures of skeletal formation, the oxygen isotope fractionation equation established for crocodilians can be used to identify the source of ingested water, and can thus help to establish whether their aquatic paleoenvironments were freshwater, brackish or marine.

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