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1 3 2 ORIGINAL PAPER

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5 6 # Combined oxygen and sulphur isotope analysis—a new tool to unravel vertebrate (paleo)-ecology

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11 **Abstract**
12 Reconstructing the living environment of extinct vertebrates is often challenging due to the lack of proxies. We propose a new
13 proxy to the living environment based on the combined oxygen and sulphur stable isotope analysis of vertebrate hydroxyapatite.
14 We tested this isotopic proxy to 64 biogenic apatite (bones) samples that represent a wide spectrum of the extant vertebrate
15 phylogenetic diversity including crocodiles, snakes, turtles, mammals, birds, lizards, fish and amphibians. We show that the
16 combination of these two isotopic systems allows the living environment of all these vertebrates to be unambiguously distin-
17 guished between freshwater (aquatic vs semi-aquatic), seawater (aquatic vs semi-aquatic) and terrestrial. The main goal of this
18 study is to provide a present-day isotopic reference frame and to discuss methodological issues that will serve to interpret future
19 oxygen and sulphur isotope results obtained either from fossil or modern skeletal material. This new isotopic approach of
20 combined oxygen and sulphur isotope analysis will be particularly useful to document major aquatic-terrestrial transitions in
21 the fossil record but also to better constrain the living environment of some present-day species.

22 **Keywords** Geochemistry · Stable isotope · Biogenic apatite · Ecology · Fossil

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24 ## Introduction

25 ### Background information

26 Vertebrate evolution has been many times punctuated by ecolog-
27 ical transitions between terrestrial and aquatic (freshwater vs sea-
28 water) environments resulting in major radiation events: during
29 the Late Devonian-Early Carboniferous, early tetrapods left
30 aquatic environments and colonised terrestrial ones (Ahlberg
31 and Milner 1994); during the Jurassic-Cretaceous, various
32 crocodylomorphs belonging to the thalattosuchians, the
33 pholidosaurids, the dyrosaurids and the eusuchians, radiated in
34 the marine environments (Martin et al. 2014); One hundred mil-
35 lion years later, during the Cenozoic (Eocene), early cetaceans
36 also experienced a secondary adaptation to aquatic environments
37 (Gingerich et al. 2001). Reconstruction of a thorough picture of

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these ecological transitions requires detailed knowledge of the living environment of the extinct taxa involved.

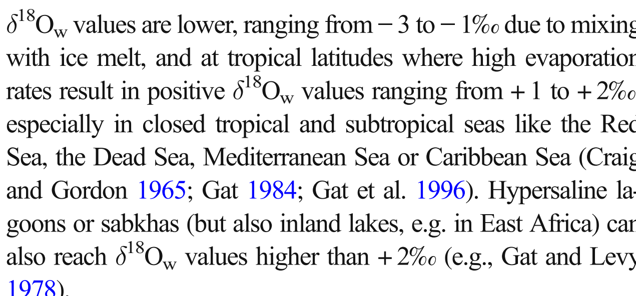
Terrestrial, freshwater and marine environments have different physical and chemical properties such as density, viscosity and salinity, resulting in specific morphological and physiological adaptations of living species. Consequently, the morpho-functional analysis of skeletal remains of fossil taxa has often been used to elucidate their living environment. (Coates and Clack 1990; Fernández and Gasparini 2000; Pierce et al. 2012; Spoor et al. 2002). However, skeletal remains sometimes may be incomplete or may have lost their original shape during post-depositional events such as burial and tectonic deformation or compaction. Such processes preclude a reliable interpretation of anatomical features in terms of morpho-functionality. Furthermore, soft tissues indicative of specific environments such as salt glands are easily degraded, and delicate ossified structures such as the semicircular canal system of the inner ear are rarely preserved in the fossil record. Finally, morphological features can predate functional adaptation (exaptation process) so that it can be misinterpreted in terms of living environment.

The sediments in which vertebrate fossils are embedded also constitute an important source of information. The detailed study of the lithology, petrology and geochemistry, along with sedimentary structures, allows precise reconstruction of the environmental conditions that prevailed during the deposition of the sediments. However, the living environment of vertebrates does not necessarily represent the depositional environment in which they were embedded (e.g. anoxic bottom waters). This is particularly true for vertebrates that travel long distances or migrate (e.g. anadromous and catadromous fish). Furthermore, carcasses can be transported over long distances after death resulting in a mismatch between the environment deduced from the sediment of the taphocoenosis and the genuine living environment.

Those problems have raised the need for other methods to reconstruct living environments independently of vertebrate morphology and depositional environments. For instance, stable carbon, oxygen or strontium isotope compositions of bones and teeth have been used as direct tracers of the living environment and applied to fossilised remains, such as those of early tetrapods (Goedert et al. 2018), early cetacean (Roe et al. 1998; Clementz et al. 2006) or crocodilian taxa (Martin et al. 2016), to get a better picture of these major ecological transitions. Here, we propose a new method to determine past living environments of vertebrates based on the combined analysis of oxygen and sulphur isotope compositions of their biogenic apatite.

Oxygen isotope composition of vertebrate apatite

Oxygen isotope composition of surface waters ($\delta^{18}O_w$) is mainly controlled by evaporation and condensation processes during which isotopic fractionation takes place (Craig and Gordon 1965; Dansgaard 1964). Marine environments have relatively uniform $\delta^{18}O_w$ values of 0 ± 1‰ except at high latitudes, where



$\delta^{18}O_w$ values are lower, ranging from − 3 to − 1‰ due to mixing with ice melt, and at tropical latitudes where high evaporation rates result in positive $\delta^{18}O_w$ values ranging from + 1 to + 2‰, especially in closed tropical and subtropical seas like the Red Sea, the Dead Sea, Mediterranean Sea or Caribbean Sea (Craig and Gordon 1965; Gat 1984; Gat et al. 1996). Hypersaline lagoons or sabkhas (but also inland lakes, e.g. in East Africa) can also reach $\delta^{18}O_w$ values higher than + 2‰ (e.g., Gat and Levy 1978).

The $\delta^{18}O_w$ values of freshwaters mainly derive from those of meteoric waters (groundwater contributions being possible) whose ultimate source is seawater. Evaporation of seawater at low latitudes, distillation and cooling of the humid air mass during its transport towards high latitudes are responsible for the negative $\delta^{18}O$ values of meteoric waters (Dansgaard 1964). At the global scale, the higher the latitude and altitude, the lower the $\delta^{18}O$ values of rainfall and snow. These values are comprised between − 6 and − 2‰ at low latitudes and decrease down to about − 15‰ at high latitudes, polar caps excluded. Oxygen isotope compositions of vertebrate biogenic apatite phosphate ($\delta^{18}O_p$) are linearly correlated with the oxygen isotope composition of their environmental waters (Longinelli 1984; Luz et al. 1984). Consequently, vertebrates living or ingesting different environmental waters will record in their bones distinct oxygen isotope compositions. Nonetheless, it is worth to note that physiological factors such as evaporative transcutaneous water loss and thermo-metabolism, which are species-specific, also impact the oxygen isotope compositions recorded in bioapatites (e.g. Kohn 1996; Levin et al. 2006).

Sulphur isotope composition of vertebrate apatite

Sulphur isotope composition of sulphates ($\delta^{34}S$) is highly variable in modern aquatic environments. Marine environments have high and relatively uniform sulphate $\delta^{34}S$ values close to + 21.0‰ (Böttcher et al. 2007). Most freshwater environments (e.g. rivers, lakes, ponds, precipitations) have comparatively lower sulphate $\delta^{34}S$ values, ranging from − 20.0 to + 20.0‰ (Krouse 1980; Kaplan 1983; Nehlich 2015). It has been shown that the sulphur isotope composition of food is recorded in vertebrate organic tissues (e.g. muscles, hairs) or molecules (e.g. bone collagen) with low isotopic fractionation (+ 0.5‰ ± 2.4‰, Nehlich 2015), especially when compared to the oxygen isotopic system. A recent study also measured very low sulphur isotope fractionation values between the collagen of sub-fossil red fox and that of its preys (ranging from − 0.54 to + 0.03‰, with a mean analytical error of ± 0.4; Krajcarz et al. 2019). Notably, this study further allows such low sulphur isotope fractionation to apply for carnivores.

Sulphur isotope analysis of vertebrate organic tissues is, therefore, particularly relevant to differentiate between freshwater and seawater environments. In particular, this method has been successfully used to determine the living environment exploited by

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140 fish at the species and population levels (Fry 2002; Fry and 141 Chumchal 2011; Hesslein et al. 1991; Nehlich et al. 2013; 142 Trembaczowski 2011) or in archaeological studies to know if 143 ancient human populations relied on freshwater or marine food 144 resources (e.g. Bocherens et al. 2016). More generally, terrestrial 145 environments (including freshwater ones) and animals living 146 there have generally relatively low $\delta^{34}S$ values compared to ma- 147 rine environments. Nonetheless, it is worth to note that coastal or 148 island environments may be substantially influenced by sulphate 149 from marine environments, which can be redeposited as rain or 150 aerosols (the so-called 'sea spray' effect) with sulphate $\delta^{34}S$ Q2 151 values close to those of marine environment (+ 20.3‰; Nielsen 152 1974; Norman et al. 2006). Consequently, the $\delta^{34}S$ values of 153 vertebrates living in those terrestrial environments submitted to 154 sea spray effect can be relatively high and may complicate inter- 155 pretation concerning the living environment.

156 Due to technical difficulties, sulphur isotope analyses have 157 been only applied to organic tissues that easily degrade after 158 animal death and are rarely preserved in the fossil record. A 159 new method has been recently developed to measure the sul- 160 phur isotope ratios ($^{34}S/^{32}S$) of sulphate compound in calcium 161 phosphate minerals (analytical precision equals 0.5‰ (1σ)) 162 with a low-S concentration (0.14% to 1.19%) such as verte- 163 brate bioapatites (Fourel et al. 2015; Goedert et al. 2016). 164 Previous results indicated that sulphur isotope compositions 165 of environmental waters are recorded in vertebrate inorganic 166 tissues (bone apatite) with low isotopic fractionation (0.8‰ ± 167 0.8‰, $n = 5$; Goedert et al. 2018). Therefore, sulphur isotope 168 analysis of bioapatite from extinct vertebrates can provide 169 estimates of the salinity of their aqueous environments 170 (Goedert et al. 2018).

171 Material and methods

172 Sixty-four vertebrate bone apatite samples have been col- 173 lected and analysed in this study (Online Information 1). 174 Samples were selected to encompass a broad ecological 175 and taxonomic spectrum of vertebrates (crocodiles, snakes, 176 turtles, mammals, birds, lizards, fish and amphibians). For 177 each taxonomic group, vertebrates of distinct ecology such 178 as aquatic (freshwater vs marine), semi-aquatic and terres- 179 trial were selected (Online Information 2). Oxygen and 180 sulphur isotope analyses have been performed on each 181 bone sample of the 64 vertebrates.

182 Forty vertebrate bone apatite samples were collected in the 183 osteological collections of the 'Musée des Confluences' of 184 Lyon, France. Samples were further selected in historical col- 185 lections to ensure a wild provenance. Specimens with a la- 186 belled precise localisation were prioritised when possible. In 187 addition, 24 vertebrate bone apatite samples for which sulphur 188 isotope composition have been previously published (Goedert 189 et al. 2016, 2018; cf. Table 1) have been added to the dataset 190 and their oxygen isotope composition measured in this study. 191 For each specimen, about 100 mg of bone powder was sam- 192 pled using a spherical diamond-tipped drill bit. The surface of 193 the bone, which may have been chemically treated for curato- 194 rial purpose (samples coming from the 'Musée des 195 Confluences'), was removed prior to sampling.

196 All statistical tests were performed using Past 3.22 soft- 197 ware. We used Mann-Whitney U test to compare the different 198 median values and give the associated P value (P). Data of 199 Figs. 1 and 2 were plotted using KaleidaGraph 4.5.3 software. 200 Figures were drawn using Inkskape 0.92.3.

Oxygen isotope analysis 201

202 Bone apatite samples were treated following the wet chem- 203 istry protocol described by (Crowson et al. 1991) and 204 slightly modified by (Lécuyer et al. 1993). This protocol 205 consists in the isolation of phosphate (PO_4^{3-}) from apatite 206 using acid dissolution and anion-exchange resin. For each 207 sample, 30 mg of enamel powder was dissolved in 2 mL of 208 2 M HF overnight. The CaF_2 residue was separated by 209 centrifugation, and the solution was neutralised by adding 210 2.2 mL of 2 M KOH. 2.5 mL of Amberlite™ anion- 211 exchange resin was added to the solution to separate the 212 PO_4^{3-} ions. After 24 h, the solution was removed and the 213 resin was eluted with 27.5 mL of 0.5 M NH_4NO_3. After 214 4 h, 0.5 mL of NH_4OH and 15 mL of an ammoniacal 215 solution of $AgNO_3$ were added, and the samples were 216 placed in a thermostated bath at 70 °C during 7 h, allowing 217 the precipitation of silver phosphate (Ag_3PO_4) crystals. 218 When only a few mg of apatite powders could be collected, 219 the wet chemistry procedure was adapted following 220 (Bernard et al. 2009) for small sample weights (about 221 3 mg).

222 Oxygen isotope compositions were measured using a 223 high-temperature pyrolysis (Py) technique involving a 224 VarioPYROcube™ elemental analyser (EA) interfaced in 225 continuous flow (CF) mode to an Isoprime™ isotopic ratio 226 mass spectrometer (IRMS) (*EA-Py*-CF-IRMS technique 227 (Fourel et al. 2011; Lécuyer et al. 2007) at the 228 Laboratoire de Géologie de Lyon (UMR 5276, Université 229 Claude Bernard Lyon 1). For each sample, 5 aliquots of 230 300 µg of Ag_3PO_4 were mixed with 300 µg of pure graph- 231 ite powder and loaded in silver foil capsules. Pyrolysis was 232 performed at 1450 °C. Measurements were calibrated 233 against the NBS120c (natural Miocene phosphorite from 234 Florida: $\delta^{18}O$ = 21.7‰ (V-SMOW), (Lécuyer et al. 1993) 235 and the NBS127 (barium sulphate, $BaSO_4$: $\delta^{18}O = 9.3‰$ 236 (V-SMOW), (Hut 1987). Silver phosphate samples precip- 237 itated from standard NBS120c were repeatedly analysed 238 ($\delta^{18}O_p = 21.6‰$; $1\sigma = 0.4$; $n = 16$) along with the silver 239 phosphate samples derived from fossil bioapatites to en- 240 sure that no isotopic fractionation took place during the

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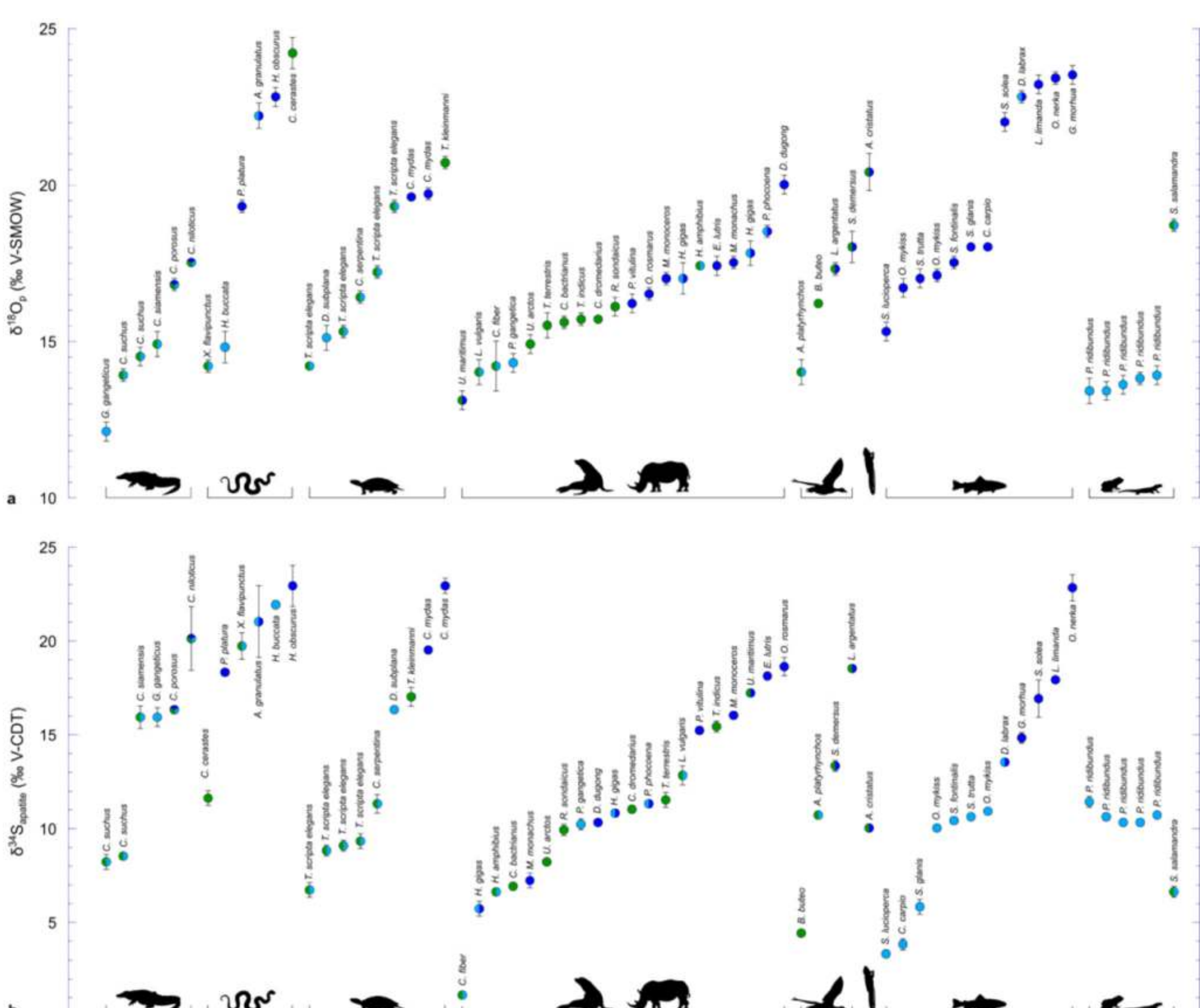

Fig. 1 $\delta^{18}O_p$ and $\delta^{34}S_{apatite}$ values of modern vertebrates including (from left to right) crocodiles, snakes, turtles, mammals, birds, lizards, fish and amphibians. **a** Oxygen isotope composition of bone phosphate ($\delta^{18}O_p$) as variations in parts per mille from the ratio of $^{18}O/^{16}O$ in Vienna Mean Ocean Water (‰ VSMOW) **b** Sulphur isotope composition of bone apatite ($\delta^{34}S_{apatite}$) as variations in parts per mille from the ratio of $^{34}S/^{32}S$ in Vienna Canyon Diablo Troilite (‰ VCDT). For **a**, **b**, each data point represents a biologically independent animal (n = 64) and corresponds to the average value of five and three repeated measurements for oxygen and sulphur isotope analysis, respectively (see "Material and Methods"). Each error bar corresponds to 1 s.d. (Online Information 1). For both panels, light blue, dark blue and green colours indicate that the species lives in freshwater, seawater or terrestrial environments, respectively (see Supplementary Information). The name of each species is indicated close to the corresponding dot

wet chemistry. The NBS120c average standard deviation equals 0.29 ± 0.14‰. Data are reported as $\delta^{18}O_p$ in ‰ values vs V-SMOW.

Sulphur isotope analysis

Sulphur isotope compositions were measured using a VarioPYROcube™ elemental analyser in NCS combustion mode interfaced in continuous-flow mode with an Isoprime 100™ isotope ratio mass spectrometer hosted by the platform 'Ecologie Isotopique' of the 'Laboratoire d'Ecologie des Hydrosystèmes Naturels et Anthropisés' (LEHNA, UMR 5023, Villeurbanne, France). For each bone apatite sample, 3 aliquots of 7 mg of bioapatite powder were mixed with 20 mg of pure tungsten oxide (WO_3) powder and loaded in tin foil capsules. Tungsten oxide is a powerful oxidant ensuring the full thermal decomposition of apatite sulphate into sulphur dioxide (SO_2) gas (Goedert et al. 2016). Measurements have been calibrated against the NBS127 (barium sulphate, $BaSO_4$ $\delta^{34}S = +20.3$‰ (V-CDT), (Halas and Szaran 2001) and S1 (silver sulphide, Ag_2S $\delta^{34}S = -0.3$‰ (V-CDT), (Robinson 1995) international standards. For each analytical run of bone samples, we have also analysed BCR32 samples as a compositional

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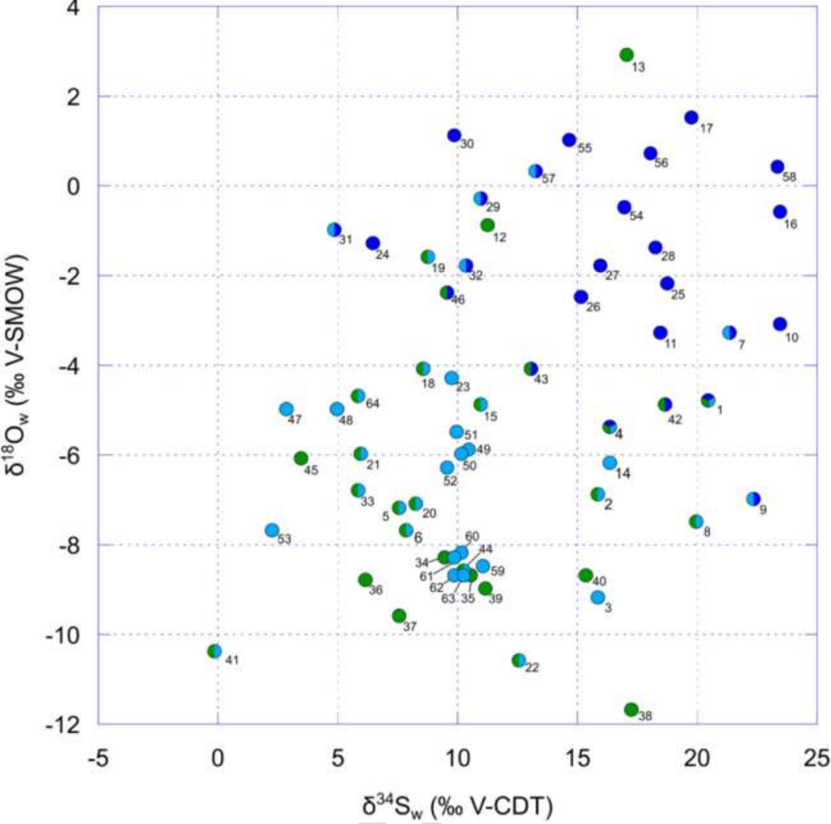

Fig. 2 Reconstructed oxygen and sulphur isotope composition of the environmental waters ($\delta^{18}O_w$, $\delta^{34}S_w$) of the modern vertebrates. For oxygen, the isotopic composition of water was calculated using published isotopic fractionation equations for different groups of vertebrates (Online Information 3). For sulphur, the isotopic composition of water is very close to that recorded in bone apatite (i.e., almost no isotopic fractionation) and was calculated using published values of sulphur isotope composition of bone apatite and associated environmental water measured in present-day vertebrates (Goedert et al. (2018); Online Information 4). Each data point represents a biologically independent animal ($n = 64$) and corresponds to the average value of five and three repeated measurements for oxygen and sulphur isotope analysis, respectively (see "Material and Methods"). Each dot is numbered according to the species it represents (cf. Table 1). Error bars of each individual data point are given in Table S2 and S3 for oxygen and sulphur respectively. Results are given as variations in parts per mille from the ratio of $^{18}O/^{16}O$ in Vienna Mean Oean Water (‰ VSMOW) for oxygen and $^{34}S/^{32}S$ in Vienna Canyon Diablo Troilite (‰ VCDT) for sulphur. Species living in freshwater are represented by light blue dots; those living in seawater are represented by dark blue dots, and green dots are used for terrestrial species.(1): *Crocodylus niloticus*; (2): *Crocodylus siamensis*; (3): *Gavialis gangeticus*; (4): *Crocodylus porosus*; (5): *Crocodylus suchus*; (6): *Crocodylus suchus*; (7): *Acrochordus granulatus*; (8): *Xenochrophis flavipunctus*; (9): *Homalopsis buccata*; (10): *Hydrophis obscurus*; (11): *Pelamis platura*; (12): *Cerastes cerastes*; (13): *Testudo kleinmanni*; (14): *Dogania subplana*; (15): *Chelydra serpentina*; (16): *Chelonia mydas*; (17): *Chelonia mydas*; (18): *Trachemys scripta elegans*; (19): *Trachemys scripta elegans*; (20): *Trachemys scripta elegans*; (21): *Trachemys scripta elegans*; (22): *Lutra lutra*; (23): *Platanista gangetica*; (24): *Monachus monachus*; (25): *Odobenus rosmarus*; (26): *Phoca vitulina*; (27): *Monodon monoceros*; (28): *Enhydra lutris*; (29): *Phocoena phocoena*; (30): *Dugong dugon*; (31): *Hydrodamalis gigas*; (32): *Hydrodamalis gigas*; (33): *Hippopotamus amphibius*; (34): *Rhinoceros sondaicus*; (35): *Camelus dromedarius*; (36): *Camelus bactrianus*; (37): *Ursus arctos*; (38): *Ursus maritimus*; (39): *Tapirus indicus*; (40): *Tapirus terrestris*; (41): *Castor fibre*; (42): *Larus argentatus*; (43): *Spheniscus demersus*; (44): *Anas platyrhynchos*; (45): *Buteo buteo*; (46): *Amblyrhynchus cristatus*; (47): *Cyprinus carpio*; (48): *Silurus glanis*; (49): *Oncorhynchus mykiss*; (50): *Salmo trutta*; (51): *Salvelinus fontinalis*; (52): *Oncorhynchus mykiss*; (53): *Sander lucioperca*; (54): *Solea solea*; (55): *Gadus morhua*; (56): *Limanda limanda*; (57): *Dicentrarchus labrax*; (58): *Oncorhynchus nerka*; (59): *Pelophylax ridibundus*; (60): *Pelophylax ridibundus*; (61): *Pelophylax ridibundus*; (62): *Pelophylax ridibundus*; (63): *Pelophylax ridibundus*; (64): *Salamandra salamandra*

and isotopic standard (S% = 0.72, certified value ((Community Bureau of Reference 1982); $\delta^{34}S = 18.4‰$ (V-CDT), (Fourel et al. 2015; Goedert et al. 2016) to ensure that analytical conditions were optimal to perform sulphur isotope analyses of samples with low-S content. The sample average standard deviation for $\delta^{34}S$ measurements is 0.34‰ ± 0.34‰. Data are reported as $\delta^{34}S$ in ‰ vs V-CDT.

Results

Oxygen isotope

The different vertebrates analysed had oxygen isotope compositions ranging from + 12.1 to + 24.2‰ V-SMOW (Online Information 1; Fig. 1a), which mainly reflect the variability of oxygen isotope compositions of environmental waters. On the

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whole, vertebrates living or foraging in marine environments had significantly higher $\delta^{18}O_p$ values than animals living or foraging in continental (freshwater or terrestrial) environments (median $\delta^{18}O_p = +19.8‰$, $1\sigma = 3.0$, $n = 18$ vs median $\delta^{18}O = +15.4‰$, $1\sigma = 2.4$, $n = 40$; $P = 4.244e\text{-}5$ (Mann-Whitney *U* test)). It also worth to note that vertebrates which live in both freshwater to seawater environment had intermediate median $\delta^{18}O_p$ values ($\delta^{18}O_p = +17.7‰$, $1\sigma = 0.9$, $n = 6$), although the difference was only significant compared to continental environments and not seawater ones ($P = 0.01255$ and $P = 0.1611$, respectively). One exception concerns the horned desert viper (*Cerastes cerastes*) and the Kleinmann's tortoise (*Testudo kleinmanni*), which had both recorded high oxygen isotope ratios in their bones due to their desert lifestyle.

Sulphur isotope

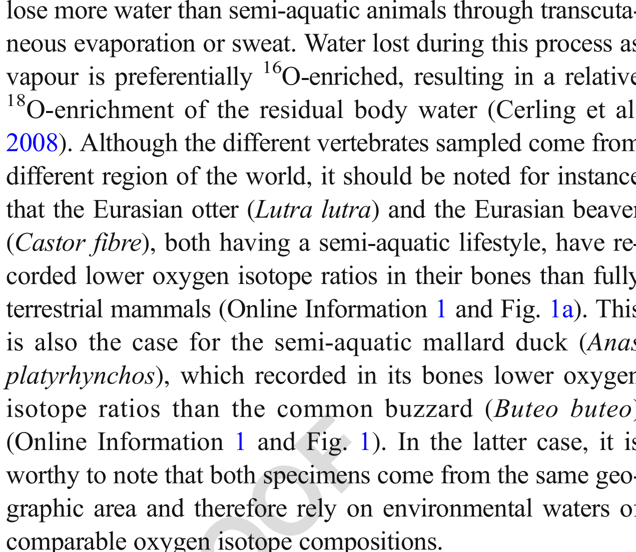
The different vertebrates analysed had sulphur isotope compositions apatite ($\delta^{34}S_{apatite}$) ranging from + 1.1 to + 22.9‰ V-CDT (Online Information 1; Fig. 1b). On the whole, vertebrates living or foraging in marine environments had significantly higher $\delta^{34}S$ values than those living or foraging in continental (freshwater or terrestrial) environments (median $\delta^{34}S_{apatite} = +16.9‰$, $1\sigma = 4.4$, $n = 18$ vs $\delta^{34}S_{apatite} = +10.4‰$, $1\sigma = 4.4$, $n = 40$; $P = 0.0001357$). This isotopic pattern reflects an almost systematic ^{34}S-enrichment of marine environments compared to continental ones. It is again worth to note that vertebrates living in freshwater to seawater environment had intermediate median $\delta^{34}S_{apatite}$ values ($\delta^{34}S = +13.8‰$, $1\sigma = 6.0$, $n = 6$), although the difference was not significant with that of continental or marine environments ($P = 0.1063$ and $P = 0.5264$). Sulphur isotope analysis of fossilised apatite can, therefore, help to detect the presence or proximity of seawater in the living environments of extinct vertebrates.

Discussion

Oxygen isotope composition

Oxygen isotope analysis of vertebrate biogenic apatite has been widely applied to fossilised apatite of extinct vertebrates to get information on their living environment (e.g. Clementz et al. 2003, 2006; Tütken et al. 2006; Amiot et al. 2015, 2009, 2010; Pouech et al. 2014; Guy et al. 2018). As illustrated by our results, this analysis can be particularly useful to distinguish vertebrates living or foraging in marine environments from those living or foraging in continental (freshwater or terrestrial) ones (e.g. sharks: Gates 2019; mosasaurs: Makádi et al. 2012; coelacanths: Simon et al. 2003).

It can also be used to further differentiate aquatic or semi-aquatic lifestyle from a terrestrial one in the case of sympatric vertebrates (e.g. Amiot et al. 2010). Indeed, terrestrial animals lose more water than semi-aquatic animals through transcutaneous evaporation or sweat. Water lost during this process as vapour is preferentially ^{16}O-enriched, resulting in a relative ^{18}O-enrichment of the residual body water (Cerling et al. 2008). Although the different vertebrates sampled come from different region of the world, it should be noted for instance that the Eurasian otter (*Lutra lutra*) and the Eurasian beaver (*Castor fibre*), both having a semi-aquatic lifestyle, have recorded lower oxygen isotope ratios in their bones than fully terrestrial mammals (Online Information 1 and Fig. 1a). This is also the case for the semi-aquatic mallard duck (*Anas platyrhynchos*), which recorded in its bones lower oxygen isotope ratios than the common buzzard (*Buteo buteo*) (Online Information 1 and Fig. 1). In the latter case, it is worthy to note that both specimens come from the same geographic area and therefore rely on environmental waters of comparable oxygen isotope compositions.



On the contrary, it can be used to detect desert lifestyle (Lécuyer et al. 1999). For instance, the horned desert viper (*Cerastes cerastes*) and the Kleinmann's tortoise (*Testudo kleinmanni*), had both recorded high oxygen isotope ratios in their bones.

Nonetheless, for low-latitude environments, oxygen isotope compositions of freshwater and marine environments can display significant overlap. Consequently, water oxygen isotope compositions recorded in vertebrate apatites may not always be a diagnostic tracer of their living environment (e.g. Pouech et al. 2014).

Sulphur isotope composition

Compared to oxygen, sulphur isotopes have been less applied to question the ecology of extinct vertebrates, principally due to technical difficulties. Due to the large amplitude of natural isotopic variations, particularly observed between terrestrial and marine environments, it remains a particularly relevant environmental tracer (cf. Background information).

However, as discussed in the "Introduction" section, the 'sea spray' effect may complicate interpretation concerning the living environment of vertebrates for terrestrial environment located in the influenced of marine ones. Moreover, some freshwater settings may have sulphur isotope compositions close to that of marine environments. For instance, rivers draining basins in which marine evaporites are exposed may have elevated dissolved sulphate content (more than 200 mg/L for the Colorado River system (Shope and Gerner 2014)) and $\delta^{34}S$ values (up to seawater-like 19.5‰ for the Mackenzie River system (Hitchon and Krouse 1972)). Therefore, vertebrates living in such environments are expected to have high sulphur isotope compositions that could be misinterpreted as reflecting an aqueous environment at least submitted to some marine influences. Finally, vertebrate species living in aquatic environments submitted to the influences of both fresh and

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marine water, like in estuaries, may record a sulphur isotope composition in their bioapatite difficult to correctly interpret in terms of living environment.

Combined oxygen and sulphur isotope composition

On the whole, the combined use of oxygen and sulphur isotope compositions of bone apatite allows, in most cases, environmental identification for the present-day vertebrates after the conversion of the measured $\delta^{18}O$ and $\delta^{34}S$ values of apatite into environmental water $\delta^{18}O$ value and dissolved environmental sulphate $\delta^{34}S$ values using known isotopic fractionation equations (Fig. 2; Online Information 3 and 4).

The complementarity of these two isotopic systems lies in the different abundance ratios of oxygen and sulphur, respectively, in seawater and freshwater bodies. Indeed, oxygen is equally present (as H_2O) in both marine and freshwater reservoirs whereas sulphur content (as SO_4^{2-}) of seawater is generally 100 to 1000 higher than in freshwater (Fry and Chumchal 2011). Consequently, sulphur isotopes will be particularly relevant to detect the presence of seawater in the environment, even if only a small quantity of seawater intrudes freshwater environment, and oxygen isotopes will be relevant to quantify the amount of freshwater in the environment, in particular in aquatic environments where freshwater and seawater are mixing, like in deltas or estuaries (Goedert et al. 2018).

Vertebrates living or foraging in marine environments tend to have higher oxygen and sulphur isotope compositions recorded in their bone apatite than those from freshwater and terrestrial habitats. This rule is especially valid when we compare vertebrates of close phylogenetic affinity. For instance, the wild gharial (*Gavialis gangeticus*), living in freshwater streams, and the two captive specimens of desert crocodiles (*Crocodylus suchus*), kept in freshwater at the Zoo of Lyon, have recorded in their bone apatite $\delta^{18}O_p$ and $\delta^{34}S_{apatite}$ values (+ 12.1‰ and + 15.9‰, + 14.5‰ and + 8.2‰, and + 13.9‰ and + 8.5‰, respectively) lower than those measured in bones of the wild Nile crocodile (*Crocodilus niloticus*; + 17.5‰ and + 20.1‰) and saltwater crocodile (*Crocodilus porosus*; + 16.8‰ and + 16.3‰), both known to undertake incursions in brackish waters to seawaters (cf. Supplementary Information). Similarly, the sea otter (*Enhydra lutris*), fully adapted to life in seawater, has higher $\delta^{18}O_p$ and $\delta^{34}S_{apatite}$ values (+ 17.4‰ and + 18.1‰) than those of the Eurasian otter (*Lutra lutra*) ($\delta^{18}O_p$ = + 14.0‰ and $\delta^{34}S$ = + 12.8‰), inhabiting freshwater environments. In a similar way, the marine narwhal (*Monodon monoceros*) has higher $\delta^{18}O_p$ and $\delta^{34}S_{apatite}$ values (+ 17.0‰ and + 16.0‰) than those of the South Asian river dolphin (*Platanista gangetica*; + 14.3‰ and + 10.2‰).

The general picture we have of major ecological transitions that took place during vertebrate evolution are incomplete and potentially biased as it corresponds to the final stages of these transitions. For instance, the colonisation of terrestrial environments by early tetrapods at the beginning of the Carboniferous gave rise to a wide evolutionary radiation of terrestrial tetrapods that are still present on lands today. Similarly, the multiple iterations of secondary adaptation to the aquatic environment are well illustrated by the numerous species of vertebrates belonging to different groups (crocodiles, snakes, turtles, lizards, birds and mammals), which live in present-day aquatic environments. All these vertebrates testify that different groups adapted to new environments from a common ancestor. However, the way these major ecological transitions proceeded, especially during their early stages, is difficult to infer and often remained elusive. Indeed, morpho-functional adaptations to a specific environment can be diachronous with its effective use (exaptation); the diagnose of living environment of vertebrates from morpho-functional analysis is thereby limited. Therefore, the combined use of $^{18}O/^{16}O$ and $^{34}S/^{32}S$ ratios of skeletal apatite should be particularly promising and powerful to document major ecological transitions in the fossil record for any phylogenetic group of vertebrates. For instance, this method has already been successfully applied to determine the aquatic environment of some Devonian early tetrapods and their associated vertebrate fauna (Goedert et al. 2018). Furthermore, it could also help to precise the ecology of some present-day aquatic vertebrates and shed light on the modalities of transition between terrestrial and aquatic environments during the course of vertebrate evolution over the Phanerozoic. It is also worthy to note that this method has the potential to shed light on the ecology of numerous present-day vertebrates living in transitional environments, and for which the ecology remains unclear.

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Data availability All data generated or analysed during this study are included in this published article [and its online information files].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Q7 478 ## References

479 Ahlberg PE, Milner AR (1994) The origin and early diversification of tetrapods. Nature 368:507

481 Amiot R, Buffetaut E, Lécuyer C et al (2009) Oxygen isotope composition of continental vertebrate apatites from Mesozoic formations of Thailand; environmental and ecological significance. Geol Soc Lond, Spec Publ 315:271–283

485 Amiot R, Buffetaut E, Lécuyer C et al (2010) Oxygen isotope evidence for semi-aquatic habits among spinosaurid theropods. Geology 38:139–142

487 Amiot R, Wang X, Zhou Z et al (2015) Environment and ecology of East Asian dinosaurs during the Early Cretaceous inferred from stable oxygen and carbon isotopes in apatite. J Asian Earth Sci 98:358–370

490 Bernard A, Daux V, Lécuyer C et al (2009) Pleistocene seasonal temperature variations recorded in the δ 18 O of Bison priscus teeth. Earth Planet Sci Lett 283:133–143

493 Bocherens H, Drucker DG, Haidle MN et al (2016) Isotopic evidence (C, N, S) for a high aquatic dietary contribution for a Pre-Dorset muskox hunter from Umingmak (Banks Island, Canada). J Archaeol Sci Rep 6:700–708

497 Böttcher ME, Brumsack H-J, Dürselen C-D (2007) The isotopic composition of modern seawater sulfate: I. Coastal waters with special regard to the North Sea. J Mar Syst 67:73–82. https://doi.org/10.1016/j.jmarsys.2006.09.006

501 Cerling TE, Harris JM, Hart JA et al (2008) Stable isotope ecology of the common hippopotamus. J Zool 276:204–212

503 Clementz MT, Hoppe KA, Koch PL (2003) A paleoecological paradox: the habitat and dietary preferences of the extinct tethythere Desmostylus, inferred from stable isotope analysis. Paleobiology 29:506–519

507 Clementz MT, Goswami A, Gingerich PD, Koch PL (2006) Isotopic records from early whales and sea cows: contrasting patterns of ecological transition. J Vertebr Palaeontol 26:355–370

510 Coates MI, Clack JA (1990) Polydactyly in the earliest known tetrapod limbs. Nature 347:66–69. https://doi.org/10.1038/347066a0

512 Community Bureau of Reference (1982) Certified reference material certificate of analyses for BCR No. 32. Commission of the European Communities, Report No. 541

515 Craig H, Gordon LI (1965) Deuterium and oxygen 18 variations in the ocean and the marine atmosphere. In: Tongiorgo E, Spoleto CNR (eds) Stable Isotopes in Oceanographic Studies and Paleotemperatures. Lab. of Nuclear Geology, Pisa

519 Crowson RA, Showers WJ, Wright EK, Hoering TC (1991) Preparation of phosphate samples for oxygen isotope analysis. Anal Chem 63:2397–2400

522 Dansgaard W (1964) Stable isotopes in precipitation. Tellus 16:436–468

523 Fernández M, Gasparini Z (2000) Salt glands in a Tithonian metriorhynchid crocodyliform and their physiological significance. Lethaia 33:269–276

526 Fourel F, Martineau F, Lécuyer C et al (2011) 18O/16O ratio measurements of inorganic and organic materials by elemental analysis–pyrolysis–isotope ratio mass spectrometry continuous-flow techniques. Rapid Commun Mass Spectrom 25:2691–2696

530 Fourel F, Martineau F, Seris M, Lécuyer C (2015) Measurement of 34S/32S Ratios of NBS 120c and BCR 32 Phosphorites Using Purge and Trap EA-IRMS Technology. Geostand Geoanal Res 39:47–53. https://doi.org/10.1111/j.1751-908X.2014.00297.x

534 Fry B (2002) Stable isotopic indicators of habitat use by Mississippi River fish. J N Am Benthol Soc 21:676–685

536 Fry B, Chumchal MM (2011) Sulphur stable isotope indicators of residency in estuarine fish. Limnol Oceanogr 56:1563–1576

538 Gat JR (1984) The stable isotope composition of dead sea waters. Earth Planet Sci Lett 71:361–376

540 Gat JR, Levy Y (1978) Isotope hydrology of inland sabkhas in the Bardawil area, Sinai. Limnol Oceanogr 23:841–850

542 Gat JR, Shemesh A, Tziperman E et al (1996) The stable isotope composition of waters of the eastern Mediterranean Sea. J Geophys Res Oceans 101:6441–6451

545 Gates TA, Gorscak E, Makovicky PJ (2019) New sharks and other chondrichthyans from the latest Maastrichtian (Late Cretaceous) of North America. J Palaeontol 93:512–530

548 Gingerich PD, ul Haq M, Zalmout IS et al (2001) Origin of whales from early artiodactyls: hands and feet of Eocene Protocetidae from Pakistan. Science 293:2239–2242

551 Goedert J, Fourel F, Amiot R et al (2016) High-precision 34S/32S measurements in vertebrate bioapatites using purge-and-trap EA-IRMS technology. Rapid Commun Mass Spectrom

554 Goedert J, Lécuyer C, Amiot R et al (2018) Euryhaline ecology of early tetrapods revealed by stable isotopes. Nature 558:68–72

556 Guy S-V, Thomas T, Irit Z et al (2018) Tooth oxygen isotopes reveal Late Bronze Age origin of Mediterranean fish aquaculture and trade. Sci Rep 8:1–10. https://doi.org/10.1038/s41598-018-32468-1

559 Halas S, Szaran J (2001) Improved thermal decomposition of sulfates to SO2 and mass spectrometric determination of δ34S of IAEA SO-5, IAEA SO-6 and NBS-127 sulfate standards. Rapid Commun Mass Spectrom 15:1618–1620. https://doi.org/10.1002/rcm.416

563 Hesslein RH, Capel MJ, Fox DE, Hallard KA (1991) Stable isotopes of sulphur, carbon, and nitrogen as indicators of trophic level and fish migration in the lower Mackenzie River basin, Canada. Can J Fish Aquat Sci 48:2258–2265

567 Hitchon B, Krouse HR (1972) Hydrogeochemistry of the surface waters of the Mackenzie River drainage basin, Canada—III. Stable isotopes of oxygen, carbon and sulphur. Geochim Cosmochim Acta 36:1337–1357. https://doi.org/10.1016/0016-7037(72)90066-X

571 Hut G (1987) Stable isotope reference samples for geochemical and hydrological investigations. Consultant Group Meeting IAEA, Vienna, 16–18 September 1985, Report to the Director General. International Atomic Energy Agency, Vienna 42

575 Kaplan IR (1983) Stable isotopes of sulphur, nitrogen and deuterium in recent marine environments. In: Arthur MA, Anderson TF, Kaplan IR, Veizer J, Land LS (eds) Stable isotopes in sedimentary geology. Society of Sedimentary Geology, pp 2.1–2.108

579 Kohn MJ (1996) Predicting animal δ18O: accounting for diet and physiological adaptation. Geochim Cosmochim Acta 60:4811–4829

581 Krajcarz MT, Krajcarz M, Drucker DG, Bocherens H (2019) Prey-to-fox isotopic enrichment of 34S in bone collagen: implications for palaeoecological studies. Rapid Commun Mass Spectrom

584 Krouse H (1980) Sulphur isotopes in our environment. In: Handbook of Environmental Isotope Geochemistry, Fritz and Fontes. pp. 435–472.

586 Lécuyer C, Grandjean P, O'Neil JR et al (1993) Thermal excursions in the ocean at the Cretaceous—Tertiary boundary (northern Morocco): δ18O record of phosphatic fish debris. Palaeogeogr Palaeoclimatol Palaeoecol 105:235–243

590 Lécuyer C, Grandjean P, Mazin J-M, De Buffrénil V (1999) Oxygen isotope compositions of reptile bones and teeth: a potential record of terrestrial and marine paleoenvironments. In: Hoch E (ed) Secondary Adaptation to Life in Water II, Geologisk Museum, University of Copenhagen. Brantsen, A.K., Denmark, p 33

595 Lécuyer C, Fourel F, Martineau F et al (2007) High-precision determination of 18O/16O ratios of silver phosphate by EA-pyrolysis-IRMS continuous flow technique. J Mass Spectrom 42:36–41

598 Levin NE, Cerling TE, Passey BH et al (2006) A stable isotope aridity index for terrestrial environments. PNAS 103:11201–11,205. https://doi.org/10.1073/pnas.0604719103

601 Longinelli A (1984) Oxygen isotopes in mammal bone phosphate: a new tool for paleohydrological and paleoclimatological research? Geochim Cosmochim Acta 48:385–390. https://doi.org/10.1016/0016-7037(84)90259-X

605 Luz B, Kolodny Y, Kovach J (1984) Oxygen isotope variations in phosphate of biogenic apatites, III. Conodonts. Earth Planet Sci Lett 69:255–262

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607 Makádi L, Caldwell MW, Ősi A (2012) The first freshwater mosasauroid (Upper Cretaceous, Hungary) and a new clade of basal mosasauroids. PLoS One 7:1–16

610 Martin JE, Amiot R, Lécuyer C, Benton MJ (2014) Sea surface temperature contributes to marine crocodylomorph evolution. Nat Commun 5:4658

613 Martin JE, Deesri U, Liard R et al (2016) Strontium isotopes and the long-term residency of thalattosuchians in the freshwater environment. Paleobiology 42:143–156

616 Nehlich O (2015) The application of sulphur isotope analyses in archaeological research: a review. Earth Sci Rev 142:1–17. https://doi.org/10.1016/j.earscirev.2014.12.002

619 Nehlich O, Barrett JH, Richards MP (2013) Spatial variability in sulphur isotope values of archaeological and modern cod (*Gadus morhua*). Rapid Commun Mass Spectrom 27:2255–2262

622 Pierce SE, Clack JA, Hutchinson JR (2012) Three-dimensional limb joint mobility in the early tetrapod Ichthyostega. Nature 486:523

624 Pouech J, Amiot R, Lécuyer C et al (2014) Oxygen isotope composition of vertebrate phosphates from Cherves-de-Cognac (Berriasian, France): environmental and ecological significance. Palaeogeogr Palaeoclimatol Palaeoecol 410:290–299

628 Robinson BW (1995) Variations in the sulphur isotope composition of CDT. In: Reference and intercomparison materials for stable isotopes of light elements, Proceedings of a consultants meeting held in Vienna, 1–3. Dec. 1993. IAEA, Vienna, Austria, pp 39–45

632 Roe LJ, Thewissen JGM, Quade J et al (1998) Isotopic approaches to understanding the Terrestrial-to-Marine transition of the Earliest Cetaceans. In: The Emergence of Whales. Springer, Boston, pp 399–422

635 Shope CL, Gerner SJ (2014) Assessment of dissolved-solids loading to the Colorado River in the Paradox Basin between the Dolores River and Gypsum Canyon. US Geological Survey, Utah

638 Simon T, Hagdorn H, Hagdorn MK, Seilacher A (2003) Swimming trace of a coelacanth fish from the Lower Keuper of south-west Germany. Palaeontology 46:911–926

641 Spoor F, Bajpai S, Hussain ST et al (2002) Vestibular evidence for the evolution of aquatic behaviour in early cetaceans. Nature 417:163–166. https://doi.org/10.1038/417163a

644 Trembaczowski A (2011) Use of sulphur and carbon stable-isotope composition of fish scales and muscles to identify the origin of fish. Mineralogia 42:33

647 Tütken T, Vennemann TW, Janz H, Heizmann EPJ (2006) Palaeoenvironment and palaeoclimate of the Middle Miocene lake in the Steinheim basin, SW Germany: a reconstruction from C, O, and Sr isotopes of fossil remains. Palaeogeogr Palaeoclimatol Palaeoecol 241:457–491

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