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

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## Abstract

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

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

## Introduction

## Background information

Vertebrate evolution has been many times punctuated by ecological transitions between terrestrial and aquatic (freshwater vs seawater) environments resulting in major radiation events: during the Late Devonian-Early Carboniferous, early tetrapods left

aquatic environments and colonised terrestrial ones (Ahlberg and Milner 1994); during the Jurassic-Cretaceous, various crocodylomorphs belonging to the thalattosuchians, the pholidosaurids, the dyrosaurids and the eusuchians, radiated in the marine environments (Martin et al. 2014); One hundred million years later, during the Cenozoic (Eocene), early cetaceans also experienced a secondary adaptation to aquatic environments (Gingerich et al. 2001). Reconstruction of a thorough picture of

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

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

58 The sediments in which vertebrate fossils are embedded also  
59 constitute an important source of information. The detailed study  
60 of the lithology, petrology and geochemistry, along with sedi-  
61 mentary structures, allows precise reconstruction of the environ-  
62 mental conditions that prevailed during the deposition of the  
63 sediments. However, the living environment of vertebrates does  
64 not necessarily represent the depositional environment in which  
65 they were embedded (e.g. anoxic bottom waters). This is particu-  
66 larly true for vertebrates that travel long distances or migrate  
67 (e.g. anadromous and catadromous fish). Furthermore, carcasses  
68 can be transported over long distances after death resulting in a  
69 mismatch between the environment deduced from the sediment  
70 of the taphocoenosis and the genuine living environment.

71 Those problems have raised the need for other methods to  
72 reconstruct living environments independently of vertebrate mor-  
73 phology and depositional environments. For instance, stable car-  
74 bon, oxygen or strontium isotope compositions of bones and  
75 teeth have been used as direct tracers of the living environment  
76 and applied to fossilised remains, such as those of early tetrapods  
77 (Goedert et al. 2018), early cetacean (Roe et al. 1998; Clementz  
78 et al. 2006) or crocodylian taxa (Martin et al. 2016), to get a better  
79 picture of these major ecological transitions. Here, we propose a  
80 new method to determine past living environments of vertebrates  
81 based on the combined analysis of oxygen and sulphur isotope  
82 compositions of their biogenic apatite.

### 83 Oxygen isotope composition of vertebrate apatite

84 Oxygen isotope composition of surface waters ( $\delta^{18}\text{O}_w$ ) is mainly  
85 controlled by evaporation and condensation processes during  
86 which isotopic fractionation takes place (Craig and Gordon  
87 1965; Dansgaard 1964). Marine environments have relatively  
88 uniform  $\delta^{18}\text{O}_w$  values of  $0 \pm 1\%$  except at high latitudes, where

$\delta^{18}\text{O}_w$  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  $\delta^{18}\text{O}_w$  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  $\delta^{18}\text{O}_w$  values higher than  $+2\%$  (e.g., Gat and Levy  
1978).

98 The  $\delta^{18}\text{O}_w$  values of freshwaters mainly derive from those of  
99 meteoric waters (groundwater contributions being possible)  
100 whose ultimate source is seawater. Evaporation of seawater at  
101 low latitudes, distillation and cooling of the humid air mass dur-  
102 ing its transport towards high latitudes are responsible for the  
103 negative  $\delta^{18}\text{O}$  values of meteoric waters (Dansgaard 1964). At  
104 the global scale, the higher the latitude and altitude, the lower the  
105  $\delta^{18}\text{O}$  values of rainfall and snow. These values are comprised  
106 between  $-6$  and  $-2\%$  at low latitudes and decrease down to  
107 about  $-15\%$  at high latitudes, polar caps excluded. Oxygen  
108 isotope compositions of vertebrate biogenic apatite phosphate  
109 ( $\delta^{18}\text{O}_p$ ) are linearly correlated with the oxygen isotope composi-  
110 tion of their environmental waters (Longinelli 1984; Luz et al.  
111 1984). Consequently, vertebrates living or ingesting different  
112 environmental waters will record in their bones distinct oxygen  
113 isotope compositions. Nonetheless, it is worth to note that phys-  
114 iological factors such as evaporative transcutaneous water loss  
115 and thermo-metabolism, which are species-specific, also impact  
116 the oxygen isotope compositions recorded in bioapatites (e.g.  
117 Kohn 1996; Levin et al. 2006).

### 118 Sulphur isotope composition of vertebrate apatite

119 Sulphur isotope composition of sulphates ( $\delta^{34}\text{S}$ ) is highly vari-  
120 able in modern aquatic environments. Marine environments have  
121 high and relatively uniform sulphate  $\delta^{34}\text{S}$  values close to  $+21.0\%$   
122 (Böttcher et al. 2007). Most freshwater environments  
123 (e.g. rivers, lakes, ponds, precipitations) have comparatively low-  
124 er sulphate  $\delta^{34}\text{S}$  values, ranging from  $-20.0$  to  $+20.0\%$  (Krouse  
125 1980; Kaplan 1983; Nehlich 2015). It has been shown that the  
126 sulphur isotope composition of food is recorded in vertebrate  
127 organic tissues (e.g. muscles, hairs) or molecules (e.g. bone col-  
128 lagen) with low isotopic fractionation ( $+0.5\% \pm 2.4\%$ , Nehlich  
129 2015), especially when compared to the oxygen isotopic system.  
130 A recent study also measured very low sulphur isotope fraction-  
131 ation values between the collagen of sub-fossil red fox and that of  
132 its preys (ranging from  $-0.54$  to  $+0.03\%$ , with a mean analyt-  
133 ical error of  $\pm 0.4$ ; Krajcarz et al. 2019). Notably, this study  
134 further allows such low sulphur isotope fractionation to apply  
135 for carnivores.

136 Sulphur isotope analysis of vertebrate organic tissues is, there-  
137 fore, particularly relevant to differentiate between freshwater and  
138 seawater environments. In particular, this method has been suc-  
139 cessfully used to determine the living environment exploited by

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  $\delta^{34}\text{S}$  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  $\delta^{34}\text{S}$   
Q2 151 values close to those of marine environment (+ 20.3‰; Nielsen  
152 1974; Norman et al. 2006). Consequently, the  $\delta^{34}\text{S}$  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 ( $^{34}\text{S}/^{32}\text{S}$ ) of sulphate compound in calcium  
161 phosphate minerals (analytical precision equals 0.5‰ (1 $\sigma$ ))  
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‰ $\pm$   
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

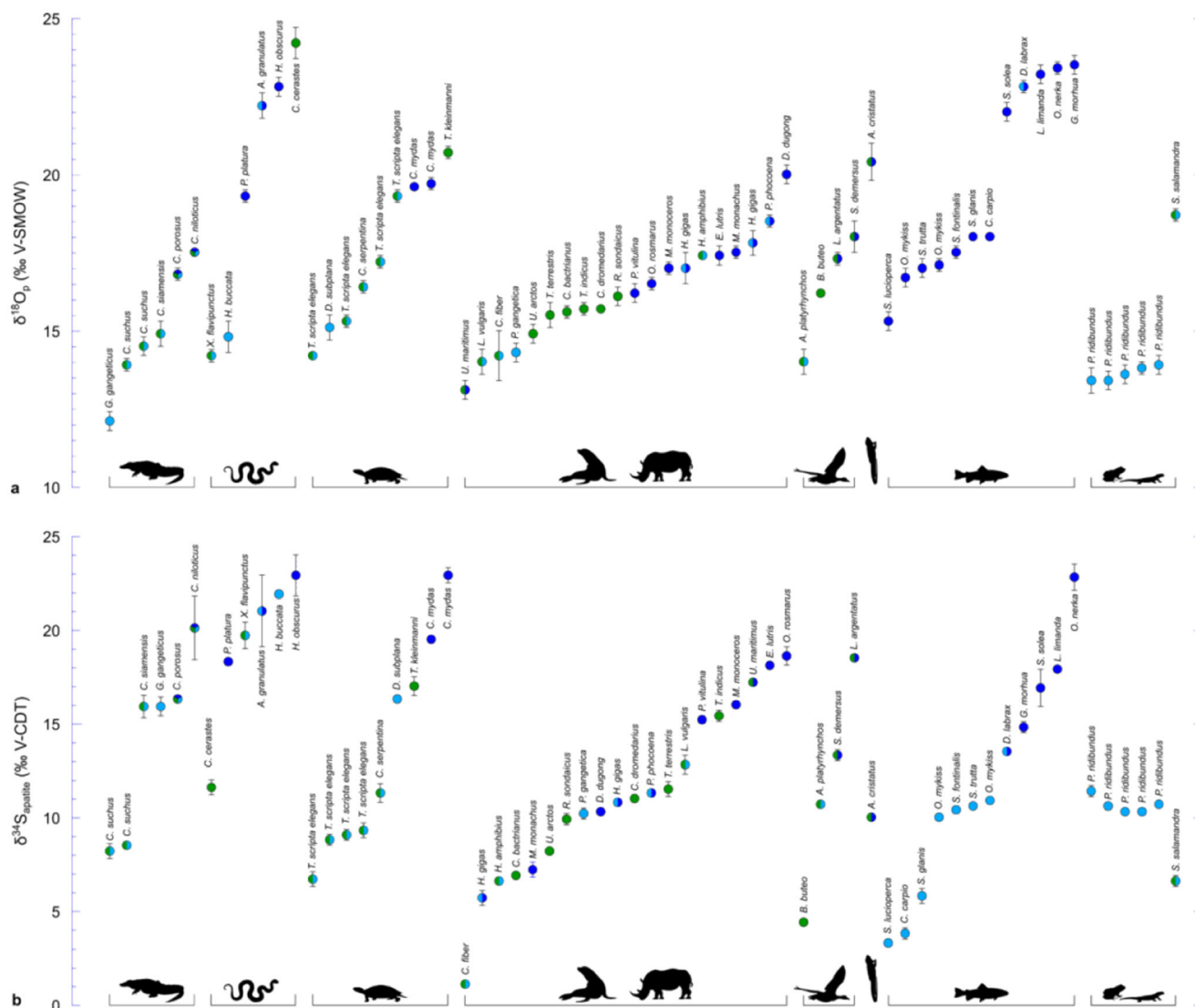
190 and their oxygen isotope composition measured in this study. 190  
191 For each specimen, about 100 mg of bone powder was sam- 191  
192 pled using a spherical diamond-tipped drill bit. The surface of 192  
193 the bone, which may have been chemically treated for curato- 193  
194 rial purpose (samples coming from the 'Musée des 194  
195 Confluences'), was removed prior to sampling. 195

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

## 201 Oxygen isotope analysis

202 Bone apatite samples were treated following the wet chem- 202  
203 istry protocol described by (Crowson et al. 1991) and 203  
204 slightly modified by (Lécuyer et al. 1993). This protocol 204  
205 consists in the isolation of phosphate ( $\text{PO}_4^{3-}$ ) from apatite 205  
206 using acid dissolution and anion-exchange resin. For each 206  
207 sample, 30 mg of enamel powder was dissolved in 2 mL of 207  
208 2 M HF overnight. The  $\text{CaF}_2$  residue was separated by 208  
209 centrifugation, and the solution was neutralised by adding 209  
210 2.2 mL of 2 M KOH. 2.5 mL of Amberlite™ anion- 210  
211 exchange resin was added to the solution to separate the 211  
212  $\text{PO}_4^{3-}$  ions. After 24 h, the solution was removed and the 212  
213 resin was eluted with 27.5 mL of 0.5 M  $\text{NH}_4\text{NO}_3$ . After 213  
214 4 h, 0.5 mL of  $\text{NH}_4\text{OH}$  and 15 mL of an ammoniacal 214  
215 solution of  $\text{AgNO}_3$  were added, and the samples were 215  
216 placed in a thermostated bath at 70 °C during 7 h, allowing 216  
217 the precipitation of silver phosphate ( $\text{Ag}_3\text{PO}_4$ ) crystals. 217  
218 When only a few mg of apatite powders could be collected, 218  
219 the wet chemistry procedure was adapted following 219  
220 (Bernard et al. 2009) for small sample weights (about 220  
221 3 mg). 221

222 Oxygen isotope compositions were measured using a 222  
223 high-temperature pyrolysis (Py) technique involving a 223  
224 VarioPYROcube™ elemental analyser (EA) interfaced in 224  
225 continuous flow (CF) mode to an Isoprime™ isotopic ratio 225  
226 mass spectrometer (IRMS) (EA-Py-CF-IRMS technique 226  
227 (Fourel et al. 2011; Lécuyer et al. 2007) at the 227  
228 Laboratoire de Géologie de Lyon (UMR 5276, Université 228  
229 Claude Bernard Lyon 1). For each sample, 5 aliquots of 229  
230 300  $\mu\text{g}$  of  $\text{Ag}_3\text{PO}_4$  were mixed with 300  $\mu\text{g}$  of pure graph- 230  
231 ite powder and loaded in silver foil capsules. Pyrolysis was 231  
232 performed at 1450 °C. Measurements were calibrated 232  
233 against the NBS120c (natural Miocene phosphorite from 233  
234 Florida:  $\delta^{18}\text{O} = 21.7\text{‰}$  (V-SMOW), (Lécuyer et al. 1993) 234  
235 and the NBS127 (barium sulphate,  $\text{BaSO}_4$ :  $\delta^{18}\text{O} = 9.3\text{‰}$  235  
236 (V-SMOW), (Hut 1987). Silver phosphate samples precip- 236  
237 itated from standard NBS120c were repeatedly analysed 237  
238 ( $\delta^{18}\text{O}_p = 21.6\text{‰}$ ;  $1\sigma = 0.4$ ;  $n = 16$ ) along with the silver 238  
239 phosphate samples derived from fossil bioapatites to en- 239  
240 sure that no isotopic fractionation took place during the 240



Q3

**Fig. 1**  $\delta^{18}\text{O}_p$  and  $\delta^{34}\text{S}_{\text{apatite}}$  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 ( $\delta^{18}\text{O}_p$ ) as variations in parts per mille from the ratio of  $^{18}\text{O}/^{16}\text{O}$  in Vienna Mean Ocean Water (‰ V-SMOW) **b** Sulphur isotope composition of bone apatite ( $\delta^{34}\text{S}_{\text{apatite}}$ ) as variations in parts per mille from the ratio of  $^{34}\text{S}/^{32}\text{S}$  in Vienna Canyon Diablo Troilite (‰ V-CDT). For **a**, **b**, each data point represents a biologically independent animal ( $n = 64$ ) and

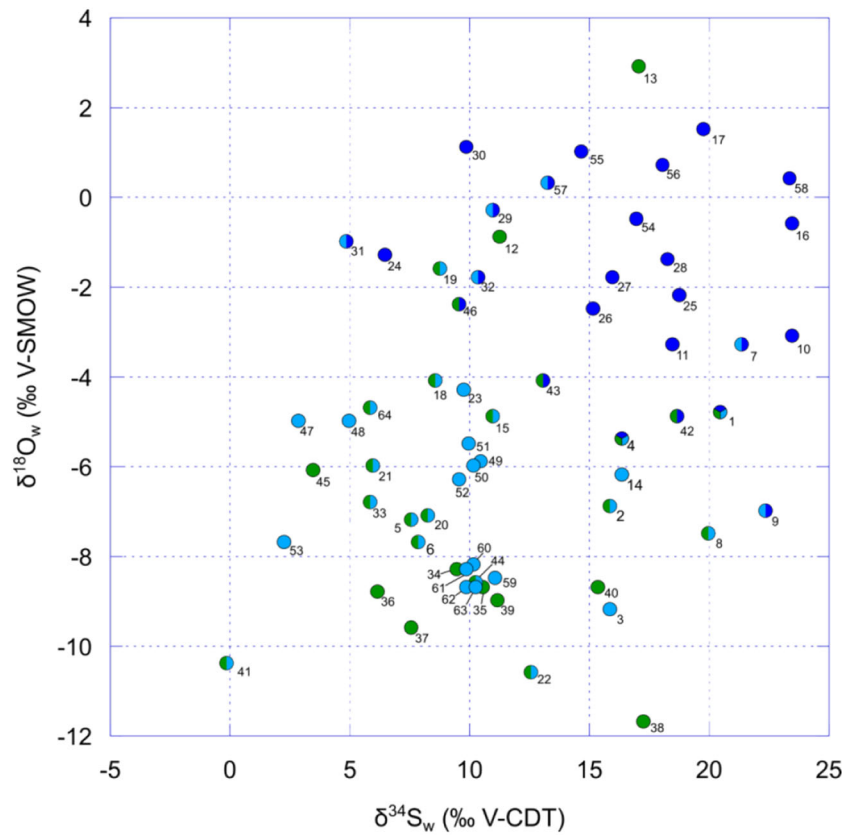
corresponds to the average value of five and three repeated measurements for oxygen and sulphur isotope analysis, respectively (see “Material and Methods”). Each error bar corresponds to 1 s.d. (Online Information 1). For both panels, light blue, dark blue and green colours indicate that the species lives in freshwater, seawater or terrestrial environments, respectively (see Supplementary Information). The name of each species is indicated close to the corresponding dot

241 wet chemistry. The NBS120c average standard deviation  
 242 equals  $0.29 \pm 0.14\text{‰}$ . Data are reported as  $\delta^{18}\text{O}_p$  in ‰  
 243 values vs V-SMOW.

244 **Sulphur isotope analysis**

245 Sulphur isotope compositions were measured using a  
 246 VarioPYROcube™ elemental analyser in NCS combustion  
 247 mode interfaced in continuous-flow mode with an Isoprime  
 248 100™ isotope ratio mass spectrometer hosted by the plat-  
 249 form ‘Ecologie Isotopique’ of the ‘Laboratoire d’Ecologie  
 250 des Hydrosystèmes Naturels et Anthropisés’ (LEHNA,

UMR 5023, Villeurbanne, France). For each bone apatite  
 251 sample, 3 aliquots of 7 mg of bioapatite powder were  
 252 mixed with 20 mg of pure tungsten oxide ( $\text{WO}_3$ ) powder  
 253 and loaded in tin foil capsules. Tungsten oxide is a power-  
 254 ful oxidant ensuring the full thermal decomposition of ap-  
 255 atite sulphate into sulphur dioxide ( $\text{SO}_2$ ) gas (Goedert et al.  
 256 2016). Measurements have been calibrated against the  
 257 NBS127 (barium sulphate,  $\text{BaSO}_4$   $\delta^{34}\text{S} = +20.3\text{‰}$  (V-  
 258 CDT), (Halas and Szaran 2001) and S1 (silver sulphide,  
 259  $\text{Ag}_2\text{S}$   $\delta^{34}\text{S} = -0.3\text{‰}$  (V-CDT), (Robinson 1995) interna-  
 260 tional standards. For each analytical run of bone samples,  
 261 we have also analysed BCR32 samples as a compositional  
 262



**Fig. 2** Reconstructed oxygen and sulphur isotope composition of the environmental waters ( $\delta^{18}\text{O}_w$ ,  $\delta^{34}\text{S}_w$ ) 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  $^{18}\text{O}/^{16}\text{O}$  in Vienna Mean Ocean Water (‰ VSMOW) for oxygen and  $^{34}\text{S}/^{32}\text{S}$  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*

263 and isotopic standard ( $S\% = 0.72$ , certified value  
 264 ((Community Bureau of Reference 1982);  $\delta^{34}\text{S} = 18.4\%$   
 265 (V-CDT), (Fourel et al. 2015; Goedert et al. 2016) to en-  
 266 sure that analytical conditions were optimal to perform  
 267 sulphur isotope analyses of samples with low-S content.  
 268 The sample average standard deviation for  $\delta^{34}\text{S}$  measure-  
 269 ments is  $0.34\% \pm 0.34\%$ . Data are reported as  $\delta^{34}\text{S}$  in ‰  
 270 vs V-CDT.

**Results**

271

**Oxygen isotope**

272

The different vertebrates analysed had oxygen isotope com-  
 273 positions ranging from + 12.1 to + 24.2‰ V-SMOW (Online  
 274 Information 1; Fig. 1a), which mainly reflect the variability of  
 275 oxygen isotope compositions of environmental waters. On the  
 276

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

## 291 Sulphur isotope

292 The different vertebrates analysed had sulphur isotope composi-  
 293 tions apatite ( $\delta^{34}\text{S}_{\text{apatite}}$ ) ranging from  $+1.1$  to  $+22.9\%$  V-  
 294 CDT (Online Information 1; Fig. 1b). On the whole, verte-  
 295 brates living or foraging in marine environments had signifi-  
 296 cantly higher  $\delta^{34}\text{S}$  values than those living or foraging in con-  
 297 tinental (freshwater or terrestrial) environments (median  
 298  $\delta^{34}\text{S}_{\text{apatite}} = +16.9\%$ ,  $1\sigma = 4.4$ ,  $n = 18$  vs  $\delta^{34}\text{S}_{\text{apatite}} = +$   
 299  $10.4\%$ ,  $1\sigma = 4.4$ ,  $n = 40$ ;  $P = 0.0001357$ ). This isotopic pat-  
 300 tern reflects an almost systematic  $^{34}\text{S}$ -enrichment of marine  
 301 environments compared to continental ones. It is again worth  
 302 to note that vertebrates living in freshwater to seawater envi-  
 303 ronment had intermediate median  $\delta^{34}\text{S}_{\text{apatite}}$  values ( $\delta^{34}\text{S} = +$   
 304  $13.8\%$ ,  $1\sigma = 6.0$ ,  $n = 6$ ), although the difference was not sig-  
 305 nificant with that of continental or marine environments ( $P =$   
 306  $0.1063$  and  $P = 0.5264$ ). Sulphur isotope analysis of fossilised  
 307 apatite can, therefore, help to detect the presence or proximity  
 308 of seawater in the living environments of extinct vertebrates.

## 309 Discussion

### 310 Oxygen isotope composition

311 Oxygen isotope analysis of vertebrate biogenic apatite has  
 312 been widely applied to fossilised apatite of extinct vertebrates  
 313 to get information on their living environment (e.g. Clementz  
 314 et al. 2003, 2006; Tütken et al. 2006; Amiot et al. 2015, 2009,  
 315 2010; Pouech et al. 2014; Guy et al. 2018). As illustrated by  
 316 our results, this analysis can be particularly useful to distin-  
 317 guish vertebrates living or foraging in marine environments  
 318 from those living or foraging in continental (freshwater or  
 319 Q6 terrestrial) ones (e.g. sharks: Gates 2019; mosasaurs: Makádi  
 320 et al. 2012; coelacanths: Simon et al. 2003).

321 It can also be used to further differentiate aquatic or semi-  
 322 aquatic lifestyle from a terrestrial one in the case of sympatric  
 323 vertebrates (e.g. Amiot et al. 2010). Indeed, terrestrial animals

lose more water than semi-aquatic animals through transcuta- 324  
 neous evaporation or sweat. Water lost during this process as 325  
 vapour is preferentially  $^{16}\text{O}$ -enriched, resulting in a relative 326  
 $^{18}\text{O}$ -enrichment of the residual body water (Cerling et al. 327  
 2008). Although the different vertebrates sampled come from 328  
 different region of the world, it should be noted for instance 329  
 that the Eurasian otter (*Lutra lutra*) and the Eurasian beaver 330  
 (*Castor fibre*), both having a semi-aquatic lifestyle, have re- 331  
 corded lower oxygen isotope ratios in their bones than fully 332  
 terrestrial mammals (Online Information 1 and Fig. 1a). This 333  
 is also the case for the semi-aquatic mallard duck (*Anas* 334  
*platyrhynchos*), which recorded in its bones lower oxygen 335  
 isotope ratios than the common buzzard (*Buteo buteo*) 336  
 (Online Information 1 and Fig. 1). In the latter case, it is 337  
 worthy to note that both specimens come from the same geo- 338  
 graphic area and therefore rely on environmental waters of 339  
 comparable oxygen isotope compositions. 340

On the contrary, it can be used to detect desert lifestyle 341  
 (Lécuyer et al. 1999). For instance, the horned desert viper 342  
 (*Cerastes cerastes*) and the Kleinmann's tortoise (*Testudo* 343  
*kleinmanni*), had both recorded high oxygen isotope ratios in 344  
 their bones. 345

Nonetheless, for low-latitude environments, oxygen iso- 346  
 tope compositions of freshwater and marine environments 347  
 can display significant overlap. Consequently, water oxygen 348  
 isotope compositions recorded in vertebrate apatites may not 349  
 always be a diagnostic tracer of their living environment (e.g. 350  
 Pouech et al. 2014). 351

### Sulphur isotope composition 352

Compared to oxygen, sulphur isotopes have been less applied 353  
 to question the ecology of extinct vertebrates, principally due 354  
 to technical difficulties. Due to the large amplitude of natural 355  
 isotopic variations, particularly observed between terrestrial 356  
 and marine environments, it remains a particularly relevant 357  
 environmental tracer (cf. Background information). 358

However, as discussed in the "Introduction" section, the 359  
 'sea spray' effect may complicate interpretation concerning 360  
 the living environment of vertebrates for terrestrial environ- 361  
 ment located in the influenced of marine ones. Moreover, 362  
 some freshwater settings may have sulphur isotope composi- 363  
 tions close to that of marine environments. For instance, rivers 364  
 draining basins in which marine evaporites are exposed may 365  
 have elevated dissolved sulphate content (more than 200 mg/L 366  
 for the Colorado River system (Shope and Gerner 2014)) and 367  
 $\delta^{34}\text{S}$  values (up to seawater-like 19.5‰ for the Mackenzie 368  
 River system (Hitchon and Krouse 1972)). Therefore, verte- 369  
 brates living in such environments are expected to have high 370  
 sulphur isotope compositions that could be misinterpreted as 371  
 reflecting an aqueous environment at least submitted to some 372  
 marine influences. Finally, vertebrate species living in aquatic 373  
 environments submitted to the influences of both fresh and 374

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

378 **Combined oxygen and sulphur isotope composition**

379 On the whole, the combined use of oxygen and sulphur isotope  
 380 compositions of bone apatite allows, in most cases, environmen-  
 381 tal identification for the present-day vertebrates after the conver-  
 382 sion of the measured  $\delta^{18}\text{O}$  and  $\delta^{34}\text{S}$  values of apatite into envi-  
 383 ronmental water  $\delta^{18}\text{O}$  value and dissolved environmental sulphate  
 384  $\delta^{34}\text{S}$  values using known isotopic fractionation equations  
 385 (Fig. 2; Online Information 3 and 4).

386 The complementarity of these two isotopic systems lies in the  
 387 different abundance ratios of oxygen and sulphur, respectively, in  
 388 seawater and freshwater bodies. Indeed, oxygen is equally pres-  
 389 ent (as  $\text{H}_2\text{O}$ ) in both marine and freshwater reservoirs whereas  
 390 sulphur content (as  $\text{SO}_4^{2-}$ ) of seawater is generally 100 to 1000  
 391 higher than in freshwater (Fry and Chumchal 2011).  
 392 Consequently, sulphur isotopes will be particularly relevant to  
 393 detect the presence of seawater in the environment, even if only  
 394 a small quantity of seawater intrudes freshwater environment,  
 395 and oxygen isotopes will be relevant to quantify the amount of  
 396 freshwater in the environment, in particular in aquatic environ-  
 397 ments where freshwater and seawater are mixing, like in deltas or  
 398 estuaries (Goedert et al. 2018).

399 Vertebrates living or foraging in marine environments tend to  
 400 have higher oxygen and sulphur isotope compositions recorded  
 401 in their bone apatite than those from freshwater and terrestrial  
 402 habitats. This rule is especially valid when we compare verte-  
 403 brates of close phylogenetic affinity. For instance, the wild ghar-  
 404 rial (*Gavialis gangeticus*), living in freshwater streams, and the  
 405 two captive specimens of desert crocodiles (*Crocodylus suchus*),  
 406 kept in freshwater at the Zoo of Lyon, have recorded in their bone  
 407 apatite  $\delta^{18}\text{O}_p$  and  $\delta^{34}\text{S}_{\text{apatite}}$  values (+ 12.1‰ and + 15.9‰, +  
 408 14.5‰ and + 8.2‰, and + 13.9‰ and + 8.5‰, respectively)  
 409 lower than those measured in bones of the wild Nile crocodile  
 410 (*Crocodylus niloticus*; + 17.5‰ and + 20.1‰) and saltwater  
 411 crocodile (*Crocodylus porosus*; + 16.8‰ and + 16.3‰), both  
 412 known to undertake incursions in brackish waters to seawaters  
 413 (cf. Supplementary Information). Similarly, the sea otter  
 414 (*Enhydra lutris*), fully adapted to life in seawater, has higher  
 415  $\delta^{18}\text{O}_p$  and  $\delta^{34}\text{S}_{\text{apatite}}$  values (+ 17.4‰ and + 18.1‰) than those  
 416 of the Eurasian otter (*Lutra lutra*) ( $\delta^{18}\text{O}_p$  = + 14.0‰ and  
 417  $\delta^{34}\text{S}$  = + 12.8‰), inhabiting freshwater environments. In a simi-  
 418 lar way, the marine narwhal (*Monodon monoceros*) has higher  
 419  $\delta^{18}\text{O}_p$  and  $\delta^{34}\text{S}_{\text{apatite}}$  values (+ 17.0‰ and + 16.0‰) than those of  
 420 the South Asian river dolphin (*Platanista gangetica*; + 14.3‰  
 421 and + 10.2‰).

422 The general picture we have of major ecological transitions  
 423 that took place during vertebrate evolution are incomplete and  
 424 potentially biased as it corresponds to the final stages of these  
 425 transitions. For instance, the colonisation of terrestrial

environments by early tetrapods at the beginning of the 426  
 Carboniferous gave rise to a wide evolutionary radiation of ter- 427  
 restrial tetrapods that are still present on lands today. Similarly, 428  
 the multiple iterations of secondary adaptation to the aquatic 429  
 environment are well illustrated by the numerous species of ver- 430  
 tebrates belonging to different groups (crocodiles, snakes, turtles, 431  
 lizards, birds and mammals), which live in present-day aquatic 432  
 environments. All these vertebrates testify that different groups 433  
 adapted to new environments from a common ancestor. 434  
 However, the way these major ecological transitions proceeded, 435  
 especially during their early stages, is difficult to infer and often 436  
 remained elusive. Indeed, morpho-functional adaptations to a 437  
 specific environment can be diachronous with its effective use 438  
 (exaptation); the diagnose of living environment of vertebrates 439  
 from morpho-functional analysis is thereby limited. Therefore, 440  
 the combined use of  $^{18}\text{O}/^{16}\text{O}$  and  $^{34}\text{S}/^{32}\text{S}$  ratios of skeletal apatite 441  
 should be particularly promising and powerful to document ma- 442  
 jor ecological transitions in the fossil record for any phylogenetic 443  
 group of vertebrates. For instance, this method has already been 444  
 successfully applied to determine the aquatic environment of 445  
 some Devonian early tetrapods and their associated vertebrate 446  
 fauna (Goedert et al. 2018). Furthermore, it could also help to 447  
 precise the ecology of some present-day aquatic vertebrates and 448  
 shed light on the modalities of transition between terrestrial and 449  
 aquatic environments during the course of vertebrate evolution 450  
 over the Phanerozoic. It is also worthy to note that this method 451  
 has the potential to shed light on the ecology of numerous 452  
 present-day vertebrates living in transitional environments, and 453  
 for which the ecology remains unclear. 454

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475 **Compliance with ethical standards** 475

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## References

- 479 Ahlberg PE, Milner AR (1994) The origin and early diversification of  
480 tetrapods. *Nature* 368:507
- 481 Amiot R, Buffetaut E, Lécuyer C et al (2009) Oxygen isotope composi-  
482 tion of continental vertebrate apatites from Mesozoic formations of  
483 Thailand; environmental and ecological significance. *Geol Soc*  
484 *Lond, Spec Publ* 315:271–283
- 485 Amiot R, Buffetaut E, Lécuyer C et al (2010) Oxygen isotope evidence for  
486 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  
488 Asian dinosaurs during the Early Cretaceous inferred from stable  
489 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 temper-  
491 ature variations recorded in the  $\delta^{18}\text{O}$  of *Bison priscus* teeth. *Earth*  
492 *Planet Sci Lett* 283:133–143
- 493 Bocherens H, Drucker DG, Haidle MN et al (2016) Isotopic evidence (C,  
494 N, S) for a high aquatic dietary contribution for a Pre-Dorset muskox  
495 hunter from Umingmak (Banks Island, Canada). *J Archaeol Sci Rep*  
496 6:700–708
- 497 Böttcher ME, Brumsack H-J, Dürselen C-D (2007) The isotopic composi-  
498 tion of modern seawater sulfate: I. Coastal waters with special  
499 regard to the North Sea. *J Mar Syst* 67:73–82. [https://doi.org/10.](https://doi.org/10.1016/j.jmarsys.2006.09.006)  
500 [1016/j.jmarsys.2006.09.006](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  
502 common hippopotamus. *J Zool* 276:204–212
- 503 Clementz MT, Hoppe KA, Koch PL (2003) A paleoecological paradox:  
504 the habitat and dietary preferences of the extinct tethythere  
505 *Desmostylus*, inferred from stable isotope analysis. *Paleobiology*  
506 29:506–519
- 507 Clementz MT, Goswami A, Gingerich PD, Koch PL (2006) Isotopic  
508 records from early whales and sea cows: contrasting patterns of  
509 ecological transition. *J Vertebr Palaeontol* 26:355–370
- 510 Coates MI, Clack JA (1990) Polydactyly in the earliest known tetrapod  
511 limbs. *Nature* 347:66–69. <https://doi.org/10.1038/347066a0>
- 512 Community Bureau of Reference (1982) Certified reference material cer-  
513 tificate of analyses for BCR No. 32. Commission of the European  
514 Communities, Report No. 541
- 515 Craig H, Gordon LI (1965) Deuterium and oxygen 18 variations in the  
516 ocean and the marine atmosphere. In: Tongiorgio E, Spoleto CNR  
517 (eds) *Stable Isotopes in Oceanographic Studies and*  
518 *Paleotemperatures*. Lab. of Nuclear Geology, Pisa
- 519 Crowson RA, Showers WJ, Wright EK, Hoering TC (1991) Preparation  
520 of phosphate samples for oxygen isotope analysis. *Anal Chem* 63:  
521 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  
524 metriorhynchid crocodyliform and their physiological significance.  
525 *Lethaia* 33:269–276
- 526 Fourel F, Martineau F, Lécuyer C et al (2011)  $18\text{O}/16\text{O}$  ratio measure-  
527 ments of inorganic and organic materials by elemental analysis-  
528 pyrolysis-isotope ratio mass spectrometry continuous-flow tech-  
529 niques. *Rapid Commun Mass Spectrom* 25:2691–2696
- 530 Fourel F, Martineau F, Seris M, Lécuyer C (2015) Measurement of  $34\text{S}/$   
531  $32\text{S}$  Ratios of NBS 120c and BCR 32 Phosphorites Using Purge and  
532 Trap EA-IRMS Technology. *Geostand Geoanal Res* 39:47–53.  
533 <https://doi.org/10.1111/j.1751-908X.2014.00297.x>
- 534 Fry B (2002) Stable isotopic indicators of habitat use by Mississippi River  
535 fish. *J N Am Benthol Soc* 21:676–685
- 536 Fry B, Chumchal MM (2011) Sulphur stable isotope indicators of resi-  
537 dency in estuarine fish. *Limnol Oceanogr* 56:1563–1576
- 538 Gat JR (1984) The stable isotope composition of dead sea waters. *Earth*  
539 *Planet Sci Lett* 71:361–376
- 540 Gat JR, Levy Y (1978) Isotope hydrology of inland sabkhas in the  
541 Bardawil area, Sinai. *Limnol Oceanogr* 23:841–850
- Gat JR, Shemesh A, Tziperman E et al (1996) The stable isotope com- 542  
position of waters of the eastern Mediterranean Sea. *J Geophys Res* 543  
*Oceans* 101:6441–6451 544
- Gates TA, Gorscak E, Makovicky PJ (2019) New sharks and other 545  
chondrichthyans from the latest Maastrichtian (Late Cretaceous) of 546  
North America. *J Palaeontol* 93:512–530 547
- Gingerich PD, ul Haq M, Zalmout IS et al (2001) Origin of whales from 548  
early artiodactyls: hands and feet of Eocene Protocetidae from 549  
Pakistan. *Science* 293:2239–2242 550
- Goedert J, Fourel F, Amiot R et al (2016) High-precision  $34\text{S}/32\text{S}$  mea- 551  
surements in vertebrate bioapatites using purge-and-trap EA-IRMS 552  
technology. *Rapid Commun Mass Spectrom* 553
- Goedert J, Lécuyer C, Amiot R et al (2018) Euryhaline ecology of early 554  
tetrapods revealed by stable isotopes. *Nature* 558:68–72 555
- Guy S-V, Thomas T, Irit Z et al (2018) Tooth oxygen isotopes reveal Late 556  
Bronze Age origin of Mediterranean fish aquaculture and trade. *Sci*  
557 *Rep* 8:1–10. <https://doi.org/10.1038/s41598-018-32468-1> 558
- Halas S, Szaran J (2001) Improved thermal decomposition of sulfates to 559  
 $\text{SO}_2$  and mass spectrometric determination of  $\delta^{34}\text{S}$  of IAEA SO-5, 560  
IAEA SO-6 and NBS-127 sulfate standards. *Rapid Commun Mass*  
561 *Spectrom* 15:1618–1620. <https://doi.org/10.1002/rcm.416> 562
- Hesslein RH, Capel MJ, Fox DE, Hallard KA (1991) Stable isotopes of 563  
sulphur, carbon, and nitrogen as indicators of trophic level and fish 564  
migration in the lower Mackenzie River basin, Canada. *Can J Fish*  
565 *Aquat Sci* 48:2258–2265 566
- Hitchon B, Krouse HR (1972) Hydrogeochemistry of the surface waters 567  
of the Mackenzie River drainage basin, Canada—III. Stable isotopes 568  
of oxygen, carbon and sulphur. *Geochim Cosmochim Acta* 36:  
569 1337–1357. [https://doi.org/10.1016/0016-7037\(72\)90066-X](https://doi.org/10.1016/0016-7037(72)90066-X) 570
- Hut G (1987) Stable isotope reference samples for geochemical and hy- 571  
drological investigations. Consultant Group Meeting IAEA, Vienna, 572  
16–18 September 1985, Report to the Director General. 573  
International Atomic Energy Agency, Vienna 42 574
- Kaplan IR (1983) Stable isotopes of sulphur, nitrogen and deuterium in 575  
recent marine environments. In: Arthur MA, Anderson TF, Kaplan 576  
IR, Veizer J, Land LS (eds) *Stable isotopes in sedimentary geology*. 577  
Society of Sedimentary Geology, pp 2.1–2.108 578
- Kohn MJ (1996) Predicting animal  $\delta^{18}\text{O}$ : accounting for diet and phys- 579  
iological adaptation. *Geochim Cosmochim Acta* 60:4811–4829 580
- Krajcarz MT, Krajcarz M, Drucker DG, Bocherens H (2019) Prey-to-fox 581  
isotopic enrichment of  $34\text{S}$  in bone collagen: implications for 582  
paleoecological studies. *Rapid Commun Mass Spectrom* 583
- Krouse H (1980) Sulphur isotopes in our environment. In: *Handbook of* 584  
*Environmental Isotope Geochemistry*, Fritz and Fontes. pp. 435–472. 585
- Lécuyer C, Grandjean P, O'Neil JR et al (1993) Thermal excursions in the 586  
ocean at the Cretaceous—Tertiary boundary (northern Morocco): 587  
 $\delta^{18}\text{O}$  record of phosphatic fish debris. *Palaeogeogr Palaeoclimatol* 588  
*Palaeoecol* 105:235–243 589
- Lécuyer C, Grandjean P, Mazin J-M, De Buffrénil V (1999) Oxygen 590  
isotope compositions of reptile bones and teeth: a potential record 591  
of terrestrial and marine paleoenvironments. In: Hoch E (ed) 592  
*Secondary Adaptation to Life in Water II*, Geologisk Museum, 593  
University of Copenhagen. Brantsen, A.K., Denmark, p 33 594
- Lécuyer C, Fourel F, Martineau F et al (2007) High-precision determina- 595  
tion of  $18\text{O}/16\text{O}$  ratios of silver phosphate by EA-pyrolysis-IRMS 596  
continuous flow technique. *J Mass Spectrom* 42:36–41 597
- Levin NE, Cerling TE, Passey BH et al (2006) A stable isotope aridity 598  
index for terrestrial environments. *PNAS* 103:11201–11,205. 599  
<https://doi.org/10.1073/pnas.0604719103> 600
- Longinelli A (1984) Oxygen isotopes in mammal bone phosphate: a new 601  
tool for paleohydrological and paleoclimatological research? 602  
*Geochim Cosmochim Acta* 48:385–390. [https://doi.org/10.1016/](https://doi.org/10.1016/0016-7037(84)90259-X)  
603 [0016-7037\(84\)90259-X](https://doi.org/10.1016/0016-7037(84)90259-X) 604
- Luz B, Kolodny Y, Kovach J (1984) Oxygen isotope variations in phosphate 605  
of biogenic apatites, III. Conodonts. *Earth Planet Sci Lett* 69:255–262 606

<p>607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 634</p>	<p>Makádi L, Caldwell MW, Ósi A (2012) The first freshwater mosasauroid (Upper Cretaceous, Hungary) and a new clade of basal mosasauroids. <i>PLoS One</i> 7:1–16</p> <p>Martin JE, Amiot R, Lécuyer C, Benton MJ (2014) Sea surface temperature contributes to marine crocodylomorph evolution. <i>Nat Commun</i> 5:4658</p> <p>Martin JE, Deesri U, Liard R et al (2016) Strontium isotopes and the long-term residency of thalattosuchians in the freshwater environment. <i>Paleobiology</i> 42:143–156</p> <p>Nehlich O (2015) The application of sulphur isotope analyses in archaeological research: a review. <i>Earth Sci Rev</i> 142:1–17. <a href="https://doi.org/10.1016/j.earscirev.2014.12.002">https://doi.org/10.1016/j.earscirev.2014.12.002</a></p> <p>Nehlich O, Barrett JH, Richards MP (2013) Spatial variability in sulphur isotope values of archaeological and modern cod (<i>Gadus morhua</i>). <i>Rapid Commun Mass Spectrom</i> 27:2255–2262</p> <p>Pierce SE, Clack JA, Hutchinson JR (2012) Three-dimensional limb joint mobility in the early tetrapod <i>Ichthyostega</i>. <i>Nature</i> 486:523</p> <p>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. <i>Palaeogeogr Palaeoclimatol Palaeoecol</i> 410:290–299</p> <p>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</p>	<p>Roe LJ, Thewissen JGM, Quade J et al (1998) Isotopic approaches to understanding the Terrestrial-to-Marine transition of the Earliest Cetaceans. In: <i>The Emergence of Whales</i>. Springer, Boston, pp 399–422</p> <p>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</p> <p>Simon T, Hagdorn H, Hagdorn MK, Seilacher A (2003) Swimming trace of a coelacanth fish from the Lower Keuper of south-west Germany. <i>Palaeontology</i> 46:911–926</p> <p>Spor F, Bajpai S, Hussain ST et al (2002) Vestibular evidence for the evolution of aquatic behaviour in early cetaceans. <i>Nature</i> 417:163–166. <a href="https://doi.org/10.1038/417163a">https://doi.org/10.1038/417163a</a></p> <p>Trembaczowski A (2011) Use of sulphur and carbon stable-isotope composition of fish scales and muscles to identify the origin of fish. <i>Mineralogia</i> 42:33</p> <p>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. <i>Palaeogeogr Palaeoclimatol Palaeoecol</i> 241:457–491</p>	<p>632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651  652 653 654</p>
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