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SYMPOSIUM

NON-TRADITIONAL ISOTOPE PERSPECTIVES IN VERTEBRATE PALAEOBIOLOGY

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Abstract: The recent development of multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) notably in the disciplines of earth sciences, now allows the precise measurement of isotope ratios, even at low concentration. Non-traditional isotope systems, such as alkaline earth (Ca, Mg) and transition (Cu, Fe, Zn) metals are now being measured in a variety of biological tissues, including bone and teeth. Although our understanding of the environmental and biological mechanisms behind the fractionation of such elements is still in its infancy, some of these isotopes are suspected to fractionate along the food chain, as has been reported in the literature for calcium, magnesium and zinc. Other geochemical methods, such as concentration analyses, permit a prior assessment of diagenesis in the fossils and such an approach indicates that in some circumstances, not only enamel but also dentine or bone can preserve its original biogenic composition. The aims here are to: review the current knowledge surrounding these various isotopic tools; address their potential preservation in biological apatite; and provide the palaeobiologist with a guide to the different toolkits available, including a discussion of their potential applications in vertebrate palaeobiology with a case study involving two mammal assemblages from the Pleistocene of Europe.

Key words: non-traditional isotopes, palaeobiology, calcium, cave bear, Pleistocene.

GEOCHEMICAL tools are a great asset to infer biological characteristics in fossil organisms that otherwise would remain completely unnoticed with morphological observations alone. Among stable isotopes, carbon (C), oxygen (O) and nitrogen (N) have been and are still widely used in ecology, archaeology and palaeoecology. From the vertebrate individual organism to the community, it is possible to: reconstruct body temperatures and infer thermophysiology; reconstruct ambient palaeoenvironmental conditions and infer habitat use and migrations; and last, but not least, to reconstruct diet and trophic structures in ancient food webs. Most isotope approaches rely on stable isotope ratios of carbon and oxygen but also on radiogenic strontium preserved in fossilized tooth and bone. Elemental concentrations have also proved useful in many contexts such as dietary inference. For example, elemental ratios of strontium to calcium or barium to calcium allow the reconstruction of ecosystem structures.

Contrary to light isotope systems, which have a variability beyond 10‰, non-traditional isotopes comprising alkaline earths (Ca, Mg) or transition metals (Cu, Fe, Zn), show isotope variability within a maximum range of 2–3‰. Recent analytical progress in the earth sciences now allow the measurement of such stable isotope systems, which show limited fractionation and require high precision and accuracy of measurements from mass spectrometers. Indeed, insights from non-traditional isotope fractionation are already starting to prove useful in archaeological studies (see review by Jaouen & Pons 2016). As long as diagenesis can be assessed, there should be no temporal restrictions for their applications to fossil samples. Here, the reader will be presented briefly with traditional systems used in palaeobiology and for further information will refer to previous reviews in ecology and palaeobiology (e.g. Kelly 1999; Koch 2007; Newsome *et al.* 2010). We will then summarize the current research on non-traditional isotopes in modern and extinct vertebrates, discuss the different analytical toolkits available, and present new information in a case study using calcium isotopes.

TRADITIONAL ISOTOPES IN PALAEOBIOLOGY

New analytical techniques are now being implemented that allow the measurement of non-traditional isotope

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ratios. Nevertheless, geochemical approaches to investigate the trophic organization of modern vertebrate ecosystems rely primarily on carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and elemental concentration ratios (Sr/Ca and Ba/Ca). Palaeoenvironmental information can be derived from oxygen ($\delta^{18}O$) and radiogenic strontium ($^{87}Sr/^{86}Sr$) but also sulfur isotopes ($\delta^{34}S$). Because each element is independently affected by diagenesis and because environmental or physiological processes may add some noise to the observed isotopic variability, it is important to explore new systems that will be used in conjunction with traditional methods. Traditional isotope systems are briefly presented below, underlining their use in vertebrate palaeobiology. For comprehensive reviews on traditional systems, the reader may consult, among others, Gannes *et al.* (1998), Koch (2007), Newsome *et al.* (2010) and Clementz (2012).

C isotopes

The observation that carbon radioisotopic dates were systematically younger than expected when analysing plants such as corn led to the recognition of an isotopic difference between plants with different photosynthetic pathways (Bender 1968). As a consequence, $\delta^{13}C$ values have been used to address the contribution of C_3 versus C_4 plants in human and animal diets since at least the late 1970s (DeNiro & Epstein 1978; Vogel 1978; van der Merwe 1982). Such a discovery had some major implications in palaeobiology and the study of ancient climates and environments with the recognition that C_4 plants became predominant in terrestrial ecosystems at the end of the Miocene (Cerling *et al.* 1997). Although carbon isotopes can be measured in various soft tissues, palaeontological studies focus on mineral tissues such as teeth or bone, where carbon takes part in the carbonate fraction of bioapatite. Therefore, carbon isotopes also highlight trophic dynamics between mammals and changing environments through deep time (Macfadden *et al.* 1999) and provide specific clues as to diets of extinct hominins (Schoeninger 2012). Across aquatic environments, carbon isotopes can also account for productivity with the highest $\delta^{13}C$ values measured in animal tissues living in upwelling and near-shore marine habitats and the lowest $\delta^{13}C$ values measured in animals living in marine offshore or estuarine/freshwater habitats (Clementz & Koch 2001).

N isotopes

Nitrogen is a major constituent of proteins and, among vertebrate fossil samples, collagen is exclusively retrieved from bone or dentine but not from tooth enamel. Primary producers provide a diversified isotopic baseline to the rest of the food chain but among vertebrates, physiological processes induce isotopic fractionation, which is responsible for the strong relation of $\delta^{15}N$ with trophic levels. For this reason, nitrogen isotopes are commonly used to discriminate between herbivores and carnivores in ecological studies (DeNiro & Epstein 1981). Other physiological processes related to nitrogen isotope abundances involve nursing and weaning. For example, $\delta^{15}N$ time series highlighting transition from mother's milk diet to an adult diet have been monitored on dentinal annuli of sectioned marine mammal teeth (Newsome *et al.* 2010). In the 1980s, it was demonstrated that collagen could be preserved in recent fossil bones (Armstrong *et al.* 1983; Stafford *et al.* 1988) and this discovery opened the way for using $\delta^{15}N$ in palaeodietary investigations (Ambrose 1990). However, nitrogen isotopes are rarely preserved beyond 100 000 years (Koch 2007) hence palaeodietary inferences are limited to 'recent' fossil faunas.

O isotopes

Another important stable isotope system is oxygen with the measurement of its isotope abundances in bone apatite ($\delta^{18}O_p$) allowing the inference of the source of drinking water (Longinelli 1984). Such results can be used to calculate mean air temperatures and reconstruct past climatic conditions, as has been shown in Cretaceous continental ecosystems (Amiot *et al.* 2011). In addition to palaeoenvironmental inferences, this system opened notable perspectives, for example the reconstruction of the thermophysiology of long extinct faunas such as dinosaurs (Barrick & Showers 1994; Amiot *et al.* 2006) or marine reptiles from the Mesozoic (Bernard *et al.* 2010). The measurement of oxygen isotopes has also demonstrated the potential for recording long palaeoclimatic records in incremental tissues such as in fossil elephant tusks or theropod tooth enamel (Koch *et al.* 1989; Goedert *et al.* 2016*a*).

S isotopes

Sulfur enters the composition of collagen, and sulfur isotopes have been measured in bone from a variety of archaeological contexts allowing a discrimination between marine and terrestrial sources (Richards *et al.* 2001). Because sulfur may substitute for phosphate as sulfate in bioapatite, its investigation in fossil bone and teeth offers potential in the fossil record (Koch 2007; Goedert *et al.* 2016*b*).

NON-TRADITIONAL ISOTOPES IN MODERN FAUNAS

Bioessential elements are involved in various metabolic processes and have a recognized vital role in the body. Their concentrations are tightly regulated in blood to lie between the deficiency and toxicity thresholds (Balter *et al.* 2013). Despite many caveats (Ezzo 1994; Burton & Price 2000) this simple rule has often been forgotten in bone-chemistry studies. As such, the concentration of bioessential elements in fossil bones and teeth can potentially be used for tracking palaeo-deficiencies or toxicities, at best (Patterson *et al.* 1987; Rasmussen *et al.* 2008). In addition, the regulation of a given bioessential element is associated with kinetic processes, changes in the molecular configuration of binding molecules, and redox conditions that are generally associated with isotopic fractionation. This has long been recognized for calcium (Skulan *et al.* 1997; Skulan & DePaolo 1999; Heuser & Eisenhauer 2010) and isotopic fractionation of calcium remains a promising tool for tracing dietary change from bone and tooth compositions (Reynard *et al.* 2010, 2011, 2013). In this section, however, we will also review the current understanding of fractionation of trace elements (TEs) such as magnesium, iron, copper and zinc, related to biological processes, with an emphasis on isotopic ratios rather than solely on concentrations.

Ca isotopes

Calcium isotopes were initially used in the earth sciences in the 1970s (Russell *et al.* 1978) but the first studies to provide evidence for isotope variation in biological materials, both modern and fossil, are more recent (Skulan *et al.* 1997; Skulan & Depaolo 1999). It was first recognized that for the same trophic level, calcium isotope values for marine organisms were higher than those reported for continental organisms (Skulan *et al.* 1997). This observation is of importance because it becomes necessary to keep comparisons within well-defined assemblages, the initial source of calcium being environmental and possibly originating from food and/or drinking water. In fact, seawater calcium was originally found to be enriched in heavy isotopes compared to river water calcium, and quite uniform because of the long residence time of calcium in the ocean (Zhu & Macdougall 1998). Subsequent study led to the recognition that trophic levels could be inferred from calcium isotope variability, both in marine and continental assemblages (Skulan & Depaolo 1999) and this was later confirmed in a study focusing on modern and fossil marine mammals (Clementz *et al.* 2003) and more recently on modern and fossil elasmobranchs (Martin *et al.* 2015*a*). As for continental environments, Chu *et al.* (2006) found no significant calcium isotope variability in relation to geological and environmental conditions and suggested that calcium isotope variability might be largely controlled by diet. Moreover, Chu *et al.* (2006) reported that milk has a distinctly light isotope composition. In a later report, Reynard *et al.* (2010) could not recognize any trophic level effect in a continental archaeological assemblage but confirmed a physiological effect related to lactation. Heuser *et al.* (2011) also suggested the existence of calcium isotope fractionation due to physiological differences between mammals, dinosaurs and reptiles. They also suggested minimal diagenetic overprint on calcium isotope values in Mesozoic continental vertebrates. Melin *et al.* (2014) analysed two modern terrestrial mammalian faunas but did not find significant trophic level effects. However, their study highlighted the necessity of using calcium isotopes in conjunction with other isotope proxies, such as carbon. There are certainly differing physiological effects on calcium isotope fractionation and the recent analyses of different mammal organs challenge previous assumptions about the processes behind calcium isotope fractionation (Tacail *et al.* 2014). It should be noted that some organisms, such as fish and elasmobranchs, do fit the trophic level effect hypothesis, and that marine mammals only partly fit that picture, with peculiar physiological processes possibly at play (Martin *et al.* 2015*a*).

The preliminary studies discussed above have shown encouraging perspectives for reconstructing trophic structures from calcium isotopes preserved in osteological remains of modern vertebrates. Certainly, more work is needed to understand the physiological aspects behind calcium isotope fractionation, but used in conjunction with other proxies, $\delta^{44}Ca$ can reveal new palaeobiological information. Here, fractionation processes between the diet and the mineralized tissues of a vertebrate take place at each step of the trophic chain. There is a progressive decrease of calcium isotope ratios from primary producers to higher trophic levels, whether in continental or marine ecosystems.

Mg isotopes

High precision measurements of magnesium isotopes are relatively recent thanks to the advent of multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) technology (Galy *et al.* 2001). As with calcium, magnesium is a bioessential element involved in both plant (Black *et al.* 2006) and animal metabolism (Nadler & Rude 1995). Recent analyses of bone and teeth of two African mammalian faunas showed an increase of about 0.25‰ in the $^{26}Mg/^{24}Mg$ ratio at each step of a trophic chain (Martin *et al.* 2014). Further analysis of enamel

from a mammalian fauna from Gabon has shown a similar ordering according to trophic level, although overlap between dietary categories is present. A possible mechanism explaining such isotopic variability is that faeces leave the body ^{26}Mg-depleted relative to diet, a depletion that is balanced by a ^{26}Mg-enriched muscle reservoir (Martin *et al.* 2015*b*). Used in conjunction with Ba/Ca elemental ratios preserved in enamel, δ^{26}Mg highlighted a distinction between herbivores and omnivores (Martin *et al.* 2015*b*). Also, a similar correlation was highlighted between δ^{26}Mg and δ^{66}Zn in the same samples (Jaouen & Pons 2016). Contrary to calcium, there is a potential effect of the substrate because of the isotopic heterogeneity of geological reservoirs (Martin *et al.* 2014). Hence, it is necessary to analyse faunas from the same provenance. This isotope approach has only recently been explored and necessitates the establishment of a large database of modern ecosystems. Conversely, in the fossil record no data have yet been published but when possible other geochemical proxies, such as radiogenic strontium, if not modified during diagenesis, should be checked to monitor substrate homogeneity. Furthermore, physiological processes responsible for magnesium isotope fractionation have not been explored yet and spatial variability at the scale of tooth, dentition and body reservoirs remain to be looked at in detail. There are also prospects for the study of magnesium isotope variability in marine ecosystems, with magnesium being highly concentrated in seawater. It is suspected that, as for marine calcium, homogeneity in the primary source of magnesium might provide a clear reading of trophic level effects. Its potential is therefore high as a source of information on physiology, ecology and palaeobiology.

Cu, Fe and Zn isotopes

Transition metals including copper (Cu), iron (Fe) and zinc (Zn) show isotope variability in vertebrate soft tissues that encompasses isotope variability observed in earth materials (Walczyk & von Blanckenburg 2005; Balter *et al.* 2010; Jaouen *et al.* 2013). These elements play essential metabolic roles and have the potential to be affected by physiological processes. Of relevance to palaeontological questions is the record of such fractionation processes in bioapatite, which could be preserved in fossils. Iron and copper isotope variability in male versus female blood is different (Walczyk & von Blanckenburg 2005; Jaouen *et al.* 2012) and the fractionation process behind it could be due to menstruation (Jaouen & Balter 2014). Jaouen *et al.* (2012) found that such isotopic differences are also documented in archaeological bones. Although an inventory of such fractionation processes in modern mammal blood and bone has yet to be compiled, interesting perspectives for sex determination of our ancient relatives are possible. Potentially, sex ratios in community structure or sexual dimorphism in the fossil record could be inferred.

Zinc isotopes seem to be of relevance for ecological studies, particularly at inferring trophic levels, as recently demonstrated in continental (Jaouen *et al.* 2013, 2016*a*) and coastal (Jaouen *et al.* 2016*b*) mammal faunas, although some overlap between dietary categories also exists. As for magnesium isotopes, it is suspected that the substrate also influences the heterogeneity of the results (Jaouen *et al.* 2016*a*). Nevertheless, in a study involving marine mammals (Jaouen *et al.* 2016*b*) the use of δ^{13}C and δ^{15}N highlighted a correlation between nitrogen and zinc isotopes. Also, a similar pattern (i.e. an increase of the δ^{66}Zn ratio up trophic chain) has been recorded in both bone and enamel (Jaouen *et al.* 2016*a*), which offers interesting applications to the fossil record.

Sr isotopes

Strontium incorporated in animal tissue is derived from food and drinking water. Radiogenic ^{87}Sr is a decay product of ^{87}Rb, which is highly variable from one geological substrate to another. Hence, the ^{87}Sr/^{86}Sr ratio measured in animal tissues mirrors that of the local geological substrate and many studies, especially in archaeology, use this ratio to explore mobility of past human populations; for a comprehensive review the reader is directed to Bentley (2006). Radiogenic strontium can also be used in palaeontology to discuss the mobility and habitat of Mesozoic vertebrates (Kocsis *et al.* 2009; Martin *et al.* 2016) or to reconstruct palaeoenvironments of even older faunas such as elasmobranchs of the Permian and Triassic (Fischer *et al.* 2012, 2013).

From a palaeodietary perspective, mass-dependent stable strontium isotope composition has recently received attention with $\delta^{88/86}$Sr (involving the ^{88}Sr/^{86}Sr ratio) showing a decrease from herbivores to carnivores, thus providing important information for diet reconstruction in archaeology (Knudson *et al.* 2010) and potentially also in palaeontology. Variations of the ^{88}Sr/^{86}Sr ratio can be measured both with thermal ionization (Krabbenhöft *et al.* 2009) and MC-ICP-MS (Knudson *et al.* 2010).

FROM THE SAMPLE TO THE ISOTOPE RATIO

In comparison to light stable isotopes, non-traditional ones are heavier and show smaller isotopic fractionation in the range of a few parts per mil. For these reasons, assessing the variability of alkaline earth metals such as

calcium (expressed as $\delta^{44}Ca$), magnesium ($\delta^{26}Mg$) and transition metals such as copper ($\delta^{65}Cu$), iron ($\delta^{56}Fe$) and zinc ($\delta^{66}Zn$) is relatively new in the domain of earth sciences, and was made possible thanks to significant improvements in instrumentation (e.g. Albarède & Beard 2004). The different steps from sampling to analysing such non-traditional isotopes in biogenic apatite are summarized in Figure 1.

The quantity needed for analysis is dependent on: (1) the concentration of the element of interest into the sample material (i.e. bone or tooth); and (2) the limits of detection of the mass spectrometer. Both calcium and magnesium are considered to be major constituents of bioapatite making up to nearly 40 wt% and several thousands of ppm respectively. Fe and Zn make between 20 and 100 ppm while Cu is the least concentrated of all, present at about 10 ppm levels. Isotopic ratios are measured on two types of mass spectrometer: thermal ionization mass spectrometers (TIMS) for Ca and Fe; and MC-ICP-MS for Ca, Mg, Cu, Fe, Zn. One measurement on MC-ICP-MS requires about 2 μg of Ca and less than 0.4 μg of Mg, Cu, Fe and Zn. Therefore, considering both elemental concentrations in the sample and the amenable measure, the necessary weight for a sample remains small for Ca and Mg (about 1 mg will provide 100 measures of Ca and between 10 and 50 measures of Mg) but is relatively larger for Cu, Fe and Zn (40 mg of bioapatite is needed for 1 measure of Cu and 10 measures of Fe and Zn). Table 1 shows two examples, the first is barely destructive to the sample and corresponds to the minimal weight allowing a comfortable measurement of Ca and Mg isotope ratios. In the second case, the weight needed is more destructive and corresponds to the typical amount necessary to conduct $\delta^{15}N$ analysis of bone collagen, and is also largely sufficient to allow measurements of Cu, Fe and Zn. This second choice now allows the measurement of isotopic ratios of Cu, Fe and Zn. The advantage of calcium is its high concentration in bioapatite, which allows minimal quantities of sample to be taken. Using a computer-assisted microdrill, it is possible to obtain the desired weight by leaving holes about 350 μm in diameter. This is of particular interest when asking permission to sample curated specimens. Moreover, this leaves plenty of space for spatial sampling along accreting tissues, for example across incremental annuli in teeth or otoliths recording mineralization throughout the life of an animal. At this stage, it is possible to either directly place the samples in Teflon beakers and dissolve them in nitric or hydrochloric acid, or to add a step to remove potential diagenetic carbonates and other associated allochthonous elements through leaching.

Following sampling and dissolution, bone or teeth are subjected to chemical purification in the clean laboratory using acids and ion exchange resins to remove the phosphate matrix and any other elements that could potentially lead to destabilization or interference in the isotopic measurements. Strontium is present in variable concentration in vertebrate apatite (Peek & Clementz 2011) and needs to be removed. Because both doubly charged strontium and single charged calcium have similar mass to charge ratios (m/z) for the main measured isotopes ($^{84}Sr^{++}$, $^{86}Sr^{++}$ and $^{88}Sr^{++}$ interfering with $^{42}Ca^{+}$, $^{43}Ca^{+}$ and $^{44}Ca^{+}$ respectively) the strontium has to be removed from the sample to avoid interference with calcium during the analysis. Here, the goal is to retrieve the element of interest in a separate vial and, depending on the protocol, all other elements composing bioapatite may be discarded or kept in separate vials for further chemical purification. At this stage, it is very important to collect close to 100% of the element (referred to as yield); incomplete collection will cause the sample to become fractionated from its original isotope composition, thus leading to skewed results (Russell & Papanastassiou 1978). Complete collection of a given element is first implemented using elution profiles of standard samples. Each element composing the sample travels with different kinematics through the resin depending on the molarity of the acid used. When the right recipe is established, the element is collected at the end of the column in a Teflon beaker. Details of elution profiles and resin chromatography of vertebrate mineralized tissues are described in the literature for calcium (Tacail *et al.* 2014; Martin *et al.* 2015*a*), for magnesium (Martin *et al.* 2014, 2015*b*) and for copper, iron and zinc (Balter *et al.* 2010). Yields can be assessed by measuring the presence or absence of signals in split solution collected before and after the expected elution of the target element. At the end of the purification step, collected volumes containing the purified element are dried down on a hot plate. Once completely dried, the sample can be retaken in a fixed volume corresponding to the expected concentration for subsequent analysis on the mass spectrometer. According to the instrument of choice, the sample is either kept liquid (MC-ICP-MS) or completely re-dried (TIMS).

Originally, non-traditional isotopic systems were measured on thermal ionization mass spectrometers (TIMS). Isotopic measurements of Ca, Mg, Cu, Fe and Zn can now be achieved on multi-collector inductively coupled plasma mass spectrometers (MC-ICP-MS), the analytical details and guidelines of which are explained in Albarède *et al.* (2004). The two types of spectrometer differ in the introduction method of the sample, but their geometry remains comparable. In TIMS, a drop of the liquid sample is dried on a filament composed of a neutral element such as rhenium or tungsten, which undergoes heating into a vacuum. The solid sample eventually becomes ionized and gets accelerated into the mass spectrometer. As for MC-ICP-MS, the sample is diluted in a low

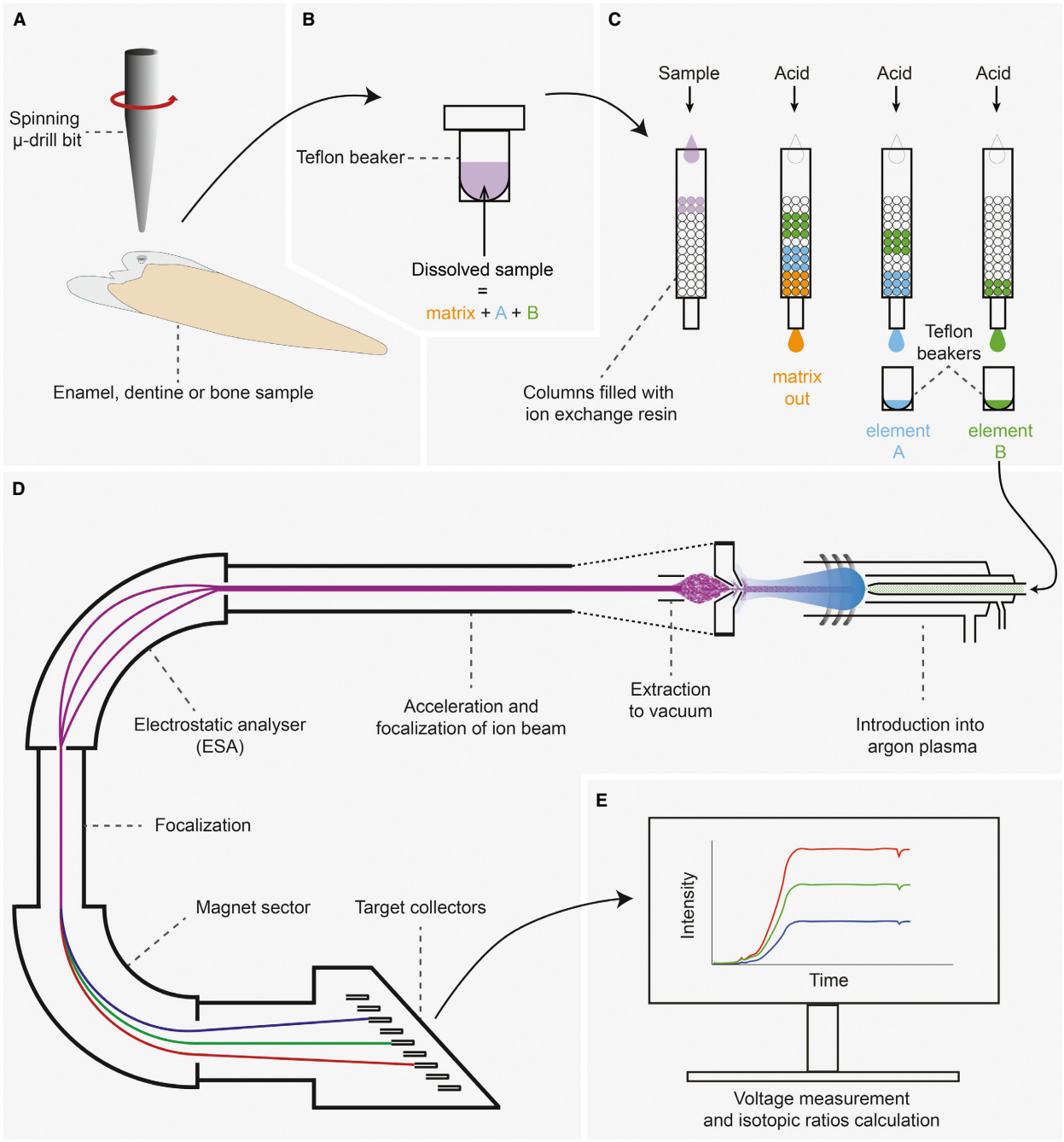

FIG. 1. Illustration of the various steps from fossil sampling to isotope ratio mass spectrometry. A, sampling a few milligrams of fossil apatite using a drill bit mounted on a microdrilling device or a dremel tool. B, sample digestion with acid in a Teflon beaker. C, the dissolved sample is subjected to ion-exchange chromatography in order to be purified for an element of interest such as Ca, Mg, Cu, Fe or Zn. D, MC-ICP-MS analysis consists of ionizing the purified sample into a plasma; the different elements will be accelerated into a vacuum and will pass through an electrostatic analyser that focuses ions according to their kinetic energy, then through a magnetic sector that will sort the isotopes according to their ion mass/ion charge ratios (m/z). E, data acquisition and processing; each mass will hit a different detector, which will convert isotopic abundance into a measurable voltage, allowing calculation of isotope ratios and delta values.

concentrated acid (often 0.05 N HNO_3) gets nebulized into a carrier gas, in this case argon, and is eventually ionized into an argon plasma. At this stage, the sample enters a vacuum space and becomes accelerated into the mass spectrometer. Because plasma-ionized particles have a high energy spread, it is necessary to focus the ion

TABLE 1. Indicative concentrations of Ca, Mg, Cu, Fe and Zn in bioapatite, and the quantity of bioapatite needed for one isotopic measurement on MC-ICP-MS with examples showing the number of analyses (n measures) possible with two given sample amounts.

	Concentration in bioapatite	Amount required for one measure on MC-ICP-MS (μg)	**Example 1** Mini sampling 1 mg bioapatite n measures	**Example 2** Equiv. bone collagen sampling 250 mg bioapatite n measures
Ca	40%	2	100	25 000
Mg	2000–10 000 ppm	0.2	10–50	400–12 500
Cu	10 ppm	0.4	Not measurable	40
Fe	100 ppm	0.4	Not measurable	400
Zn	100 ppm	0.4	Not measurable	400

beam with an electrostatic analyser (ESA), which sets the kinetic energy of the ion beam within a narrower energy spread. The beam then proceeds through a magnetic sector, which separates ions according to their mass to charge ratios (m/z). The different isotopes then hit the detector, which is constituted of several faraday cups (hence the name multi-collector) that turn the different ion beams into measurable voltages. Based on the different voltages, isotope ratios are then calculated. This combination of an ESA and a magnetic sector is known as a double focusing geometry, allowing for a better transmission and mass resolution. The TIMS instrument does not need a double focusing geometry because the ion beam provided by thermal ionization has a limited energy spread thanks to the ionization setup method. Therefore, ions are only sorted according to their masses using a magnetic sector.

Laser ablation systems flash-fire a high-energy laser beam onto the sample surface, resulting in a nearly bulk vaporization of solid sample. These setups can now be connected to the MC-ICP-MS. The advantage of this method is that no chemistry is undertaken, allowing for a direct spatial analysis of samples. It is important to remember that flat surfaces are necessary for such analyses, requiring cutting and/or polishing of samples, which is obviously destructive and unfeasible for precious specimens. The major drawback remains analytical because ablating a sample implies ionizing a wide variety of elements in the plasma, therefore creating a wealth of interferences that need to be corrected. Calcium isotopes can be measured in this way provided a strict matrix-matching method together with a thorough correction of strontium interferences (Li *et al.* 2016; Tacail *et al.* 2016) and analytical implementation for laser ablation of magnesium, copper, iron and zinc isotope ratios in bioapatite is pending.

Isotopic fractionation effects in alkaline earth and transition metals are small; in other words, isotope abundance ratios, which can be measured with high precision, show relatively small variation in nature. As raw isotope ratios are difficult to deal with and are pointless as such, we report normalized deviations from a reference material. Moreover, because variations of isotope compositions are small, we multiply it by a magnifying factor, typically 1000. This is all expressed in a single equation, which applies to any traditional or non-traditional isotopic system:

$$\delta^{i/j}X = \left[\frac{({}^{i}X/{}^{j}X)_{\text{sample}}}{({}^{i}X/{}^{j}X)_{\text{reference material}}} - 1\right] \times 1000$$

where ${}^{i}X$ and ${}^{j}X$ stand for molar abundances of isotopes i and j of element X respectively and the δ value is expressed in ‰ units. For magnesium, the delta notation is given by $\delta^{x}\text{Mg} = [({}^{x}\text{Mg}/{}^{24}\text{Mg})_{\text{sample}}/({}^{x}\text{Mg}/{}^{24}\text{Mg})_{\text{reference}} - 1] \times 10^{3}$ where x = 26 or 25, and the DSM3 solution for the standard. For copper, the delta notation is given by $\delta^{65}\text{Cu} = [({}^{65}\text{Cu}/{}^{63}\text{Cu})_{\text{sample}}/({}^{65}\text{Cu}/{}^{63}\text{Cu})_{\text{reference}} - 1] \times 10^{3}$ with the NIST-SRM976 solution for the standard. For iron, the delta notation is given by $\delta^{x}\text{Fe} = [({}^{x}\text{Fe}/{}^{54}\text{Fe})_{\text{sample}}/({}^{x}\text{Fe}/{}^{54}\text{Fe})_{\text{reference}} - 1] \times 10^{3}$ where x = 56 or 57, and the IRMM14 solution for the standard. For zinc, the delta notation is given by $\delta^{x}\text{Zn} = [({}^{x}\text{Zn}/{}^{64}\text{Zn})_{\text{sample}}/({}^{x}\text{Zn}/{}^{64}\text{Zn})_{\text{reference}} - 1] \times 10^{3}$ where x = 66, 67 or 68, and the JMC3-0749 Lyon for the standard. Different notations are used for Ca where the full isotopic ratio is commonly given in the exponent to express whether ^{40}Ca has been measured or not.

DIAGENESIS OF NON-TRADITIONAL ISOTOPES

Recognizing isotope systems as tracers of biological processes in modern skeletal tissues provides interesting possibilities for their applications in the fossil record. However, delving into the past implies the risk that any original biogenic signal could be overprinted or replaced during diagenesis and this needs to be controlled for. Post mortem modification of the original chemical composition can be challenging to assess when interpreting isotopic compositions of fossil samples. The intensity of diagenesis depends

on the concentration ratio between the diagenetic fluid and the fossil, that is the water/rock (W/R) ratio, and the difference of the isotopic composition between the diagenetic fluid and the fossil. Departures from original isotopic composition have been modeled using simple mass balance for Mg, Ca, Fe, Cu and Zn isotopes, for both continental and marine settings (Fig. 2). The developed model does not involve classical W/R equations, as given in Sharp (2007) for example, because it does not take into account the initial and final stages of bioapatite alteration. Nor does it take into account isotopic fractionation between fluid and bioapatite, which have been otherwise not determined. The equation reads:

$$\delta M_{\mathrm{mix}} = \frac{xM_{\mathrm{bio}} \times \delta M_{\mathrm{bio}} \times [M]_{\mathrm{bio}} + (1-x)M_{\mathrm{dia}} \times \delta M_{\mathrm{dia}} \times [M]_{\mathrm{dia}}}{xM_{\mathrm{bio}} \times [M]_{\mathrm{bio}} + (1-x)M_{\mathrm{dia}} \times [M]_{\mathrm{dia}}}$$

where M is the metal, δM and $[M]$ its isotope composition and concentration, respectively, the subscripts bio and dia for biological and diagenetic fraction, respectively, and x the fraction of altered bioapatite. Departure from original isotopic composition is given by the ratio $\delta M_{\mathrm{mix}}/\delta M_{\mathrm{bio}}$ for a given value of x. Four consequences emerge from these calculations. The first is that mixing of diagenetic Mg and Ca in the terrestrial setting is unlikely because the Mg and Ca concentrations in rivers are negligible relative to those in bioapatite. Second, the mixing of diagenetic Mg in the marine setting is probably very important because the Mg concentration in seawater is similar to that of bioapatite. Third, the diagenesis of transition metals (Fe, Cu and Zn) in the terrestrial environment is likely to be an efficient process because their concentrations are lower in bioapatite than in rivers. And fourth, the diagenesis of transition metals in the marine environment is unlikely because their concentrations in seawater are under the ppb level, while they are 1000 times higher in bioapatite.

Despite the fact that the calcium isotopic composition of bone and tooth is hardly modifiable by diagenesis, there is a risk that the measured calcium and magnesium isotope composition is overprinted due to the presence of secondary calcite infilling the porosity of samples. As a rule, the fossil bone and teeth have to be rid of secondary carbonates, and more generally of any secondary mineral phase. This step (leaching) is achieved using diluted acetic acid (0.1 M), that dissolves secondary carbonates leaving bioapatite, which will be finally dissolved using more concentrated nitric acid (*c.* 30%). There are many leaching procedures and the reader will find some useful references describing the different protocols in Balter *et al.* (2002*a*). Transition metals, such as iron, copper and zinc can be associated with oxyhydroxids that need to be dissolved using reducing agents such as hydroxylamine.

Once calcite and oxyhydroxides have been leached from the fossil bone or enamel samples, it is necessary to proceed to post-hoc tests to check whether the concentrations and the isotope compositions are related or not, and whether the measured elements are derived from a diagenetic or a biogenic pool. Tests of diagenesis on trace elements and their isotopes have recently been reviewed in Reynard & Balter (2014). Tests of diagenesis will need to be constantly implemented. As such, if a sizable pool of a biological metal M is exchanged with a diagenetic component, this would affect the M isotopic composition and concentration and the samples would fall on a $1/M$ vs δM line representing the mixing between the biological and the diagenetic M components. This simple scheme holds if the biological and diagenetic pools are different enough in terms of isotopic composition and concentration to generate a single $1/M$ vs δM mixing line. However, if we emphasize that the diagenetic pool would be quite homogeneous in terms of isotopic composition and concentration, the biological pool can be easily characterized by an important variability. This will generate as many mixing lines as there are original isotopic compositions, leading to an overall blurred $1/M$ vs δM relationship. Anyway, such controls are necessary to ensure we provide isotopic values related to biological fractionation and not diagenesis.

The study of diagenetic influence on the isotopic composition of calcium in fossil bone and teeth has not received a lot of attention. This is also the case for magnesium and zinc, which have only been recently recognized as bearing some trophic level information in modern samples. Heuser *et al.* (2011) conducted tests and discussed the effects of diagenesis on calcium isotopes in phosphatic tissues. They measured similar calcium isotope values for fossil bone, dentine and their surrounding sediment (n = 5) either implying exchange between sediment and skeletal tissue or addition of calcium to the tissue in the form of newly formed minerals. Heuser *et al.* (2011) also proposed the hypothesis that calcium isotopic composition could reflect that of the local substrate during the lifetime of the animal.

Other methods exist to control for the diagenetic alteration of bone, for example, by monitoring the concentration of phosphorous and calcium. Under stoichiometric conditions the ratio Ca/P is close to 2.2 in modern biological apatite (bone or teeth) (Sillen 1986). Nevertheless, the fact that one element is affected by diagenesis does not necessarily mean that others will be.

LATE PLEISTOCENE CASE STUDY WITH Ca ISOTOPES

In order to test for the preservation of biogenic information from calcium isotopes, we measured two faunal assemblages of late Pleistocene age. The importance of

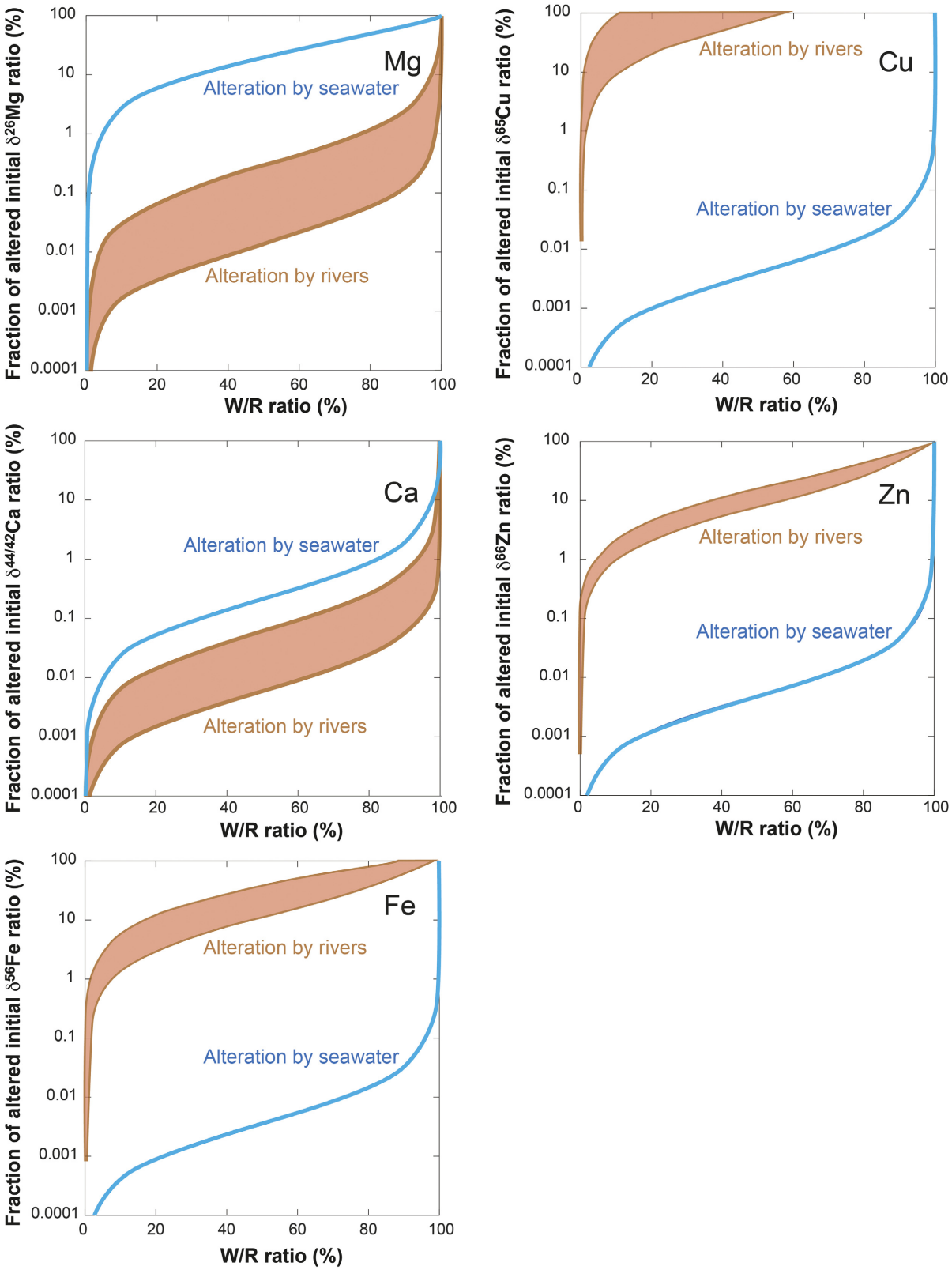

FIG. 2. Departure from the original isotope composition (%) as a function of the water/rock ratio (W/R, %), which is assumed to be representative of the fraction of altered bioapatite. In this simple mass balance, no assumption is made concerning diffusion processes. Values used for the calculation are given in Martin *et al.* (2017, table S1).

analysing a relatively 'recent' fossil fauna is that dietary behaviour can be approximated with a certain level of confidence and our new calcium results can be compared with other geochemical proxies from the literature to test for consistencies in palaeobiological interpretations. In this particular case, bone preserves collagen so calcium isotopes can be compared with nitrogen isotopes derived from the same samples. Moreover, enamel lacks collagen but trace elements such as strontium and barium are less prone to alteration and their elemental concentrations can be compared to calcium isotopes derived from the same samples.

Two Late Pleistocene mammal assemblages recovered from cave deposits were compared for their isotope variability in $\delta^{44/42}$Ca. The specimens from Scladina cave, Sclayn, Belgium originate from layer 1A, dated >36.2 kyr and 38.7 ± 1.5 kyr BP (Gilot 1992). The specimens from Jaurens, Corrèze, France originate from a single cave dated from 29.7 to 32.6 kyr BP (Guerin *et al.* 1979). The fauna from Sclayn is the same as that analysed in previous studies for carbon and nitrogen (Bocherens *et al.* 1997) and for Sr/Ca and Ba/Ca elemental ratios (Balter *et al.* 2001, 2002*b*). The fauna from Jaurens was selected for this study in the collections of Université Lyon 1.

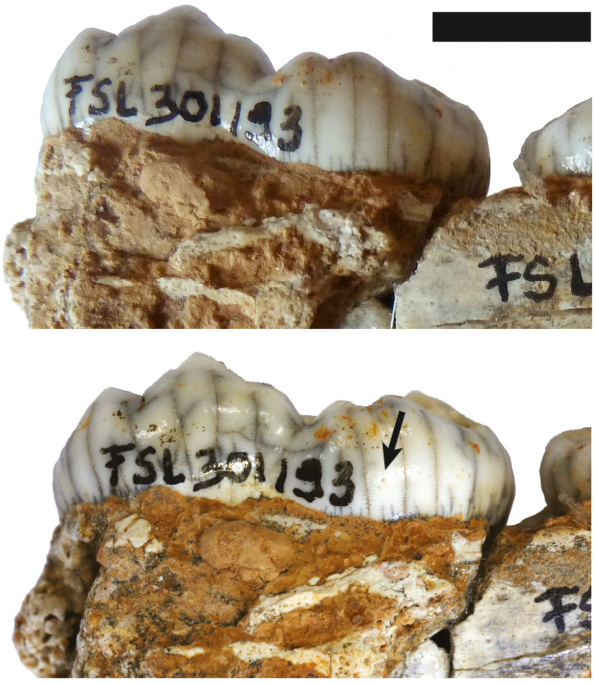

FIG. 3. *Ursus arctos* molar from Jaurens, France sampled in this study. The top picture is before sampling. The arrow in the bottom picture shows the microdrill perforation after sampling highlighting that a minimal amount of sample is needed for calcium isotope measurements. Scale bar represents 1 cm.

Material and analytical methods

The Sclayn assemblage contains 17 specimens all consisting of bone powder representing 7 species: 3 *Bison priscus*, 2 *Crocuta crocuta*, 2 *Equus caballus*, 1 *Mammuthus primigenius*, 2 *Coelodonta antiquitatis*, 1 *Ursus arctos* and 6 *Ursus spelaeus*. The Jaurens assemblage contains 27 enamel specimens and 1 bone specimen (Fig. 3; Martin *et al.* 2017, figs S3, S4). The enamel–bone pair belongs to a single species, *Panthera pardus*, but whether or not it belongs to a single individual cannot be assessed. Species sampled for tooth enamel include 3 *Bison priscus*, 3 *Coelodonta antiquitatis*, 3 *Rangifer tarandus*, 3 *Canis lupus*, 5 *Crocuta crocuta*, 4 *Panthera spelaea* and 4 *Ursus arctos*. About 300 µg of tooth enamel was sampled with a computer-assisted microdrill (New Wave Micromill; https://www.esi.com/) under a binocular microscope (Olympus SZ61).

Calcium from fossil samples was purified using a two-step chemical process involving strontium-specific resin followed by a cation-exchange resin (Tacail *et al.* 2014).

The purified calcium fraction of each sample was measured for isotope ratios on a Neptune Plus MC-ICP-MS at Laboratoire de Géologie de Lyon (e.g. Martin *et al.* 2015*a* for details). Delta values were obtained using the standard-sample bracketing method with ICP Ca Lyon as standard (Tacail *et al.* 2014). As the MC-ICP-MS technique does not allow the measurement of ^{40}Ca due to interference from ^{40}Ar plasma, isotopic compositions are expressed as delta values calculated using the ^{44}Ca/^{42}Ca isotopic ratio as follows:

$$\delta^{44/42}\mathrm{Ca} = \left[\frac{(^{44}\mathrm{Ca}/^{42}\mathrm{Ca})_{\mathrm{sample}}}{(^{44}\mathrm{Ca}/^{42}\mathrm{Ca})_{\mathrm{ICP\,Ca\,Lyon}}} - 1\right] \times 1000$$

We report four independent measurements of NIST standard SRM1486 (average = −1.01‰, see Martin *et al.* 2017, table S2 for details) that are in good agreement with previously published values (−1.04 ± 0.11‰, 2 SD in Martin *et al.* (2015*a*); −1.03 ± 0.13‰, 2 SD in Tacail *et al.* (2016)). Long-term reproducibility as measured for SRM1486 is 0.12‰. When plotted against $\delta^{44/42}$Ca, $\delta^{43/42}$Ca values fall on a line with null *y*-axis origin and a slope of 0.487 ± 0.022 (2 SE) (Martin *et al.* 2017, fig. S1). Variations of isotope compositions depend thus on mass of isotopes, respecting the mass-dependent fractionation of isotopes that predicts a slope of about 0.5.

Leaching experiments were conducted on two samples from Jaurens to assess their isotope values (Martin *et al.* 2017, table S2). For each sample, bone or tooth powder was selected, then divided into two batches. The first batch was treated as such with no pretreatment. The second batch was pretreated using 0.1 N ultrapure acetic acid to leach carbonates or any diagenetic pollutants from

the sample for 30 min. This sample was rinsed three times using MQ water and the remaining sample was ran through the same purification technique and analytical procedure as the untreated sample.

Results

In the Sclayn assemblage, calcium isotope values (Martin *et al.* 2017, table S2; Fig. 4) range from −0.86 to −1.39‰, which is significantly higher than the observed range of values in the Jaurens assemblage from −1.14 to −2.02‰ (Wilcoxon–Mann–Whitney, $p = 0.00000077$). The lowest values of the Sclayn dataset are represented by *Crocuta crocuta* and *Mammuthus primigenius*. In the Jaurens dataset, the lowest values are represented by the *Crocuta* and the *P. pardus* enamel sample. In both datasets, higher values are represented by *Coelodonta antiquitatis*, *Equus caballus*, *Bison priscus*, and *Ursus arctos* and *U. spelaeus*. Intermediate values are represented by *Rangifer tarandus*, *Canis lupus* and *Panthera spelaea* in the Jaurens sample. The value for the calcite encrusting a tooth of *Crocuta crocuta* (FSL 300610) is the highest of the dataset (−0.28 ± 0.05‰, 2 SD, n = 3).

The results of the leaching experiment show a slight difference between calcium isotope values obtained for the bone sample (FSL 301081) with the untreated bone sample showing a slightly higher value (−1.38 ± 0.11‰, 2 SD) than the leached sampled (−1.52 ± 0.03‰, 2 SD). The leached enamel sample (FSL 300918lea) compares well with the untreated enamel sample (FSL 300918) with values of −1.48 ± 0.04‰ (2 SD) and −1.45 ± 0.06‰ (2 SD) respectively.

Concentration analyses for Ca, P, Mg, Sr, Ba and Zn are recorded alongside Ca isotope values in Martin *et al.* (2017, table S2). Ca/P ratios for the Jaurens samples average 1.98 ± 0.13 (2 SD).

Are Ca isotope values of biogenic nature?

The range of Ca/P ratios measured in tooth enamel samples from Jaurens and the bone samples from Sclayn respect the stoichiometry of bioapatite (2.02 ± 0.09, 2 SD) and the values are within the range of expected values for modern bone (Sillen 1986) as well as for the bone standard SRM1486 measured in the same analytical session (1.95). Such results indicate that if any changes

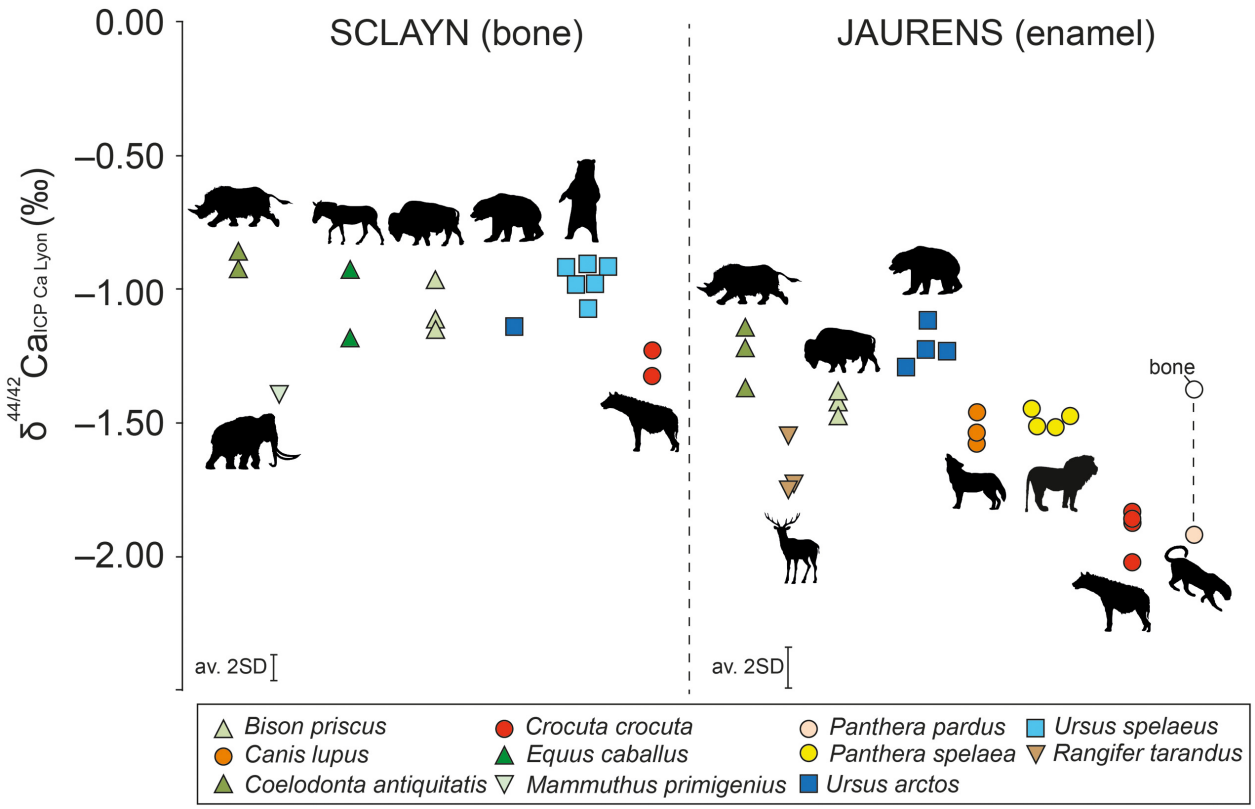

FIG. 4. Fossil mammal $\delta^{44/42}$Ca (in ‰ relative to ICP Ca Lyon standard) variability in two Late Pleistocene assemblages. A, the Sclayn (Belgium) dataset exclusively includes bone samples whereas B, the Jaurens (France) dataset includes mostly enamel samples as well as a single bone sample. To the exception of the felids, all silhouettes are taken from www.phylopic.org (Public Domain licence).

occurred during diagenesis, they affected calcium and phosphorous equally. Here, the average calcium and phosphorous concentrations in tooth enamel are 33.3 ± 4.4% and 16.3 ± 4.0% (1 SD), respectively. The average calcium and phosphorous concentrations in bones from Sclayn are much lower being 13.4 ± 3.5% and 6.4 ± 1.7% (1 SD) respectively, indicating important diagenetic-related leaching of these elements. As a comparison, in the modern bone standard SRM1486, the values are of 44.9% and 23.0%. The leaching experiment conducted here (Martin *et al.* 2017, table S2) on one enamel sample shows that both calcium and phosphorous have been leached away preserving stoichiometry ([Ca] = 20.4%, [P] = 10.8% for the leached sample (FSL 300918lea), [Ca] = 31.8%, [P] = 15.9% for the untreated sample (FSL 300918)). In the case of the bone leached here (FSL 300918lea), almost no calcium or phosphorus were lost, possibly due to the fact that collagen or other material in the porous structure make them less soluble. Although the evidence is indirect, these results show that leaching may have been responsible for the observed lower concentrations of calcium and phosphorous in the fossil bones than in fossil tooth enamel samples.

Although stoichiometry is preserved, does leaching have an impact on measured calcium isotope composition? The measured $\delta^{44/42}$Ca difference may not be significant given the larger error obtained on the untreated sample that falls within the range of the leached sample. Nevertheless, it could also be compatible with a small diagenetic overprint in bone from secondary calcite. Indeed, the calcite sample has a ^{44}Ca-enriched calcium isotope composition and diagenetic contamination of secondary calcite in the pores of fossil bone would tend to lead to heavier isotope compositions. The second sample, which consists of tooth enamel shows both values obtained on untreated and treated samples as undistinguishable (−1.45 ± 0.06‰, 2 SD vs −1.48 ± 0.04‰, 2 SD). We do not observe significant effects of the leaching process on calcium isotope values, although more analyses should be undertaken to confirm this.

Conversely, uptake of diagenetic calcium cannot be confirmed here. One way to look at such diagenetic inclusion is to search for correlations between elements that are absent during the lifetime of an animal and that eventually enter bioapatite during fossilization. Here, we observe no correlation between $\delta^{44/42}$Ca and manganese concentration, which was obviously added into bioapatite after the death of the animal (Martin *et al.* 2017, fig. S2). Manganese is virtually absent from modern bone as indicated here with SRM1486, for which 4 ppm was measured. Manganese is incorporated during fossilization as is often visible in fossils with the characteristic manganese dendrites. Unsurprisingly here, fossil bone is more concentrated in manganese (>600 ppm) than are tooth enamel samples (average = 70 ppm). Other elemental concentrations such as rare earth elements (REE), uranium or thorium diagenetic uptake could not be measured here because the sampled masses were too low to pass detection limits. It should be noted that other elements used in ecological studies (Sr, Ba, Zn, Mg) were measured for concentration and are within the range of observed values in modern samples (see Martin *et al.* 2017, table S2).

Further preservation of an original biogenic calcium isotope value is supported by the calcium isotope values that are significantly different from the value measured on the calcite surrounding fossil specimens. The value of calcite (−0.28 ± 0.05‰) is within the range of expected values for carbonates (e.g. Fantle & Tipper 2014). Given the prominence of secondary calcite surrounding fossil samples in the Jaurens deposit, diagenetic overprinting of calcium isotope values would require a shift of isotope values from fossil samples toward higher values and beyond the range of expected values for modern samples. The fact that mammal tooth or bone values are within the range of values measured in previous studies for modern bone and teeth (Skulan *et al.* 1997; Clementz *et al.* 2003; Reynard *et al.* 2010; Tacail *et al.* 2014; Martin *et al.* 2015*a*) suggests that the $\delta^{44/42}$Ca values recorded in both fossil assemblages reflect a strong biogenic component.

The spread is nearly double in Jaurens (0.88‰) than in Scalyn (0.53‰) and it could be argued that bone samples are diagenetically altered toward higher isotope values reflecting that of the substrate. Nevertheless, the observed scatter in calcium isotope values corresponds to the isotope variability recorded in the bone of modern terrestrial mammals, both in modern and archaeological context and are most likely to be representing biogenic characteristics (Reynard *et al.* 2010; Tacail *et al.* 2014). Whether this is related to dietary or physiological effects is discussed below.

Palaeocological inferences

The analysis of two datasets of similar ages and faunal compositions but of different tissues (the assemblage from Sclayn consisting of bone and the one from Jaurens consisting of tooth enamel) provides some new insights into calcium isotope variability in recently extinct mammal species. The two localities share three broad ecological categories including herbivores with *C. antiquitatis* and *B. priscus*, omnivores with *U. arctos*, and carnivores with *C. crocuta*. The first observation is a systematic difference with higher mean values for bone than for tooth enamel with a $\Delta_{\text{enamel–bone}}$ of −0.39‰ for *C. antiquitatis*, of −0.34‰ for *B. priscus*, of 0.07‰ for *U. arctos* and of

−0.60‰ for *C. crocuta*. This offset is −0.47‰ if all values are averaged per assemblage (−1.06‰ for bone vs −1.53‰ for tooth enamel). This $\Delta_{enamel-bone}$ value is also very close to that between *P. pardus* bone and tooth enamel within the single assemblage at Jaurens (−0.54‰). Previously published offsets are smaller. Heuser *et al.* (2011) found a smaller offset between dentine and tooth enamel of about –0.20‰ for dinosaur and Tacail *et al.* (2014) reported an offset of –0.30‰ between bone and tooth enamel for sheep. Martin *et al.* (2015*a*) reported a positive offset between bone and tooth enamel of +0.20‰ in marine mammals. The enamel–bone spacing observed in the present study supports the conclusion that original biogenic information has been retained. Indeed, a better understanding of the fractionation processes between different mineralized tissues requires systematic pairs within a single individual. Given the different residence times of calcium in bone, enamel and dentine reservoirs, further skeletal variability will have to be explored and the fact that the variety of bone tissues may also reflect further isotopic differences considered. Most important here for the purpose of faunal comparison is to keep in mind that bone and tooth enamel calcium isotope values cannot be readily compared. Comparing fossil assemblages should focus on single tissue analyses.

The present environmental context is continental and although initial studies recognized a trophic ordering of calcium isotopes values in continental ecosystems (Skulan & DePaolo 1999; Skulan *et al.* 1997) a more recent study found that this might be less straightforward (Melin *et al.* 2014). That a trophic level effect has been recognized in the marine environment appears settled, although the offset between trophic levels is not large (Skulan *et al.* 1997; Clementz *et al.* 2003; Martin et al. 2015*a*). Reasons for this limited trophic level effect have been discussed in the light of seawater as a buffering medium, but this study also found that marine mammals do not fully fit in this picture (Martin et al. 2015*a*). Clearly, physiological aspects need to be explored to fully understand its impact, together with diet on the observed values. Here, whether for the bone or tooth enamel dataset, calcium isotope values show low dispersion by taxonomic group. With the exception of mammoths and reindeer, all herbivores possess the highest values in the dataset. Carnivores have systematically lower values, although this is less significant for *Panthera spelaea* and *Canis lupus*, which show some overlap with the *Bison* group. Ingestion of mineralized tissue such as bone in the diet of *Crocuta crocuta* agrees with the interpretation that a diet composed of mineralized tissues, such as that of *Tyrannosaurus rex*, could explain the low isotope values (Heuser *et al.* 2011). But the present low isotope values recovered for *P. pardus*, which is not known to specifically feed on bone, remains puzzling. This requires further datapoints, as admittedly here only two samples of *P. pardus* were available for analysis. An obvious outlier is represented by *Rangifer tarandus*, which presents values in the range of the carnivore group. Given that second molars sampled for *R. tarandus* may form early, a nursing effect may be suspected with a milk-based diet potentially explaining the low values (see Chu *et al.* 2006) observed here.

Comparing our calcium isotope data with other geochemical proxies of interest in ecology provides additional insights on the mechanisms behind calcium isotope fractionation. First of all, nitrogen isotopes extracted from bone collagen allow for trophic inferences and the present dataset from Sclayn provides an opportunity to compare $\delta^{15}N$ values from the same samples analysed for calcium isotopes. The two *Crocuta crocuta* samples, representing the only carnivores of the dataset, are clearly separated from other mammals with the highest $\delta^{15}N$ and lowest $\delta^{44/42}Ca$ values; this would provide a sound argument for a dietary imprint of calcium isotopes (Fig. 5A). Interestingly, the single mammoth datapoint also falls within the range of values for *Crocuta crocuta*. Previous studies based on nitrogen isotopes have underlined the discrepancy between mammoth $\delta^{15}N$ values and those of other herbivores, suggesting that mammoths fed on highly ^{15}N-enriched plants (Schwartz-Narbonne *et al.* 2015) or practiced coprophagy (Clementz *et al.* 2009; van Geel *et al.* 2011) making it look isotopically like a carnivore. The present calcium isotope values could also be linked to specific plant consumption and although more datapoints are needed to confirm this, it is known that $\delta^{44/42}Ca$ is variable in plants, including across different organs (see compilation in Fantle & Tipper 2014). Comparing the Sr/Ca ratios with $\delta^{44/42}Ca$ values in the Jaurens sample reveals an ordering that follows the biopurification of strontium (Balter 2004) (see Fig. 5B). Here, *Crocuta crocuta* and *Panthera pardus* are the most biopurified samples, which could be interpreted as them being taxa occupying the highest trophic position in the ecosystem. This pattern follows their lowest calcium isotope values in the Jaurens dataset and, for this reason, it is tempting to interpret calcium isotope data as a proxy for trophic level. Here, in the Sr/Ca vs $\delta^{44/42}Ca$ space, other apex carnivores such as wolves and lions do overlap with bisons and reindeer, which implies that other factors are at play in the isotope fractionation of calcium. However, rhinoceroses are closely located in a putative herbivore space. Further work is needed to decipher the role of different food sources, seasonality or physiological effects such as milk-feeding or weaning on the record of calcium isotopes in tooth enamel. In contrast to marine ecosystems, the source of calcium in continental environments is probably extremely diverse for vertebrate organisms (e.g. variability in a single plant from roots to leaves) and

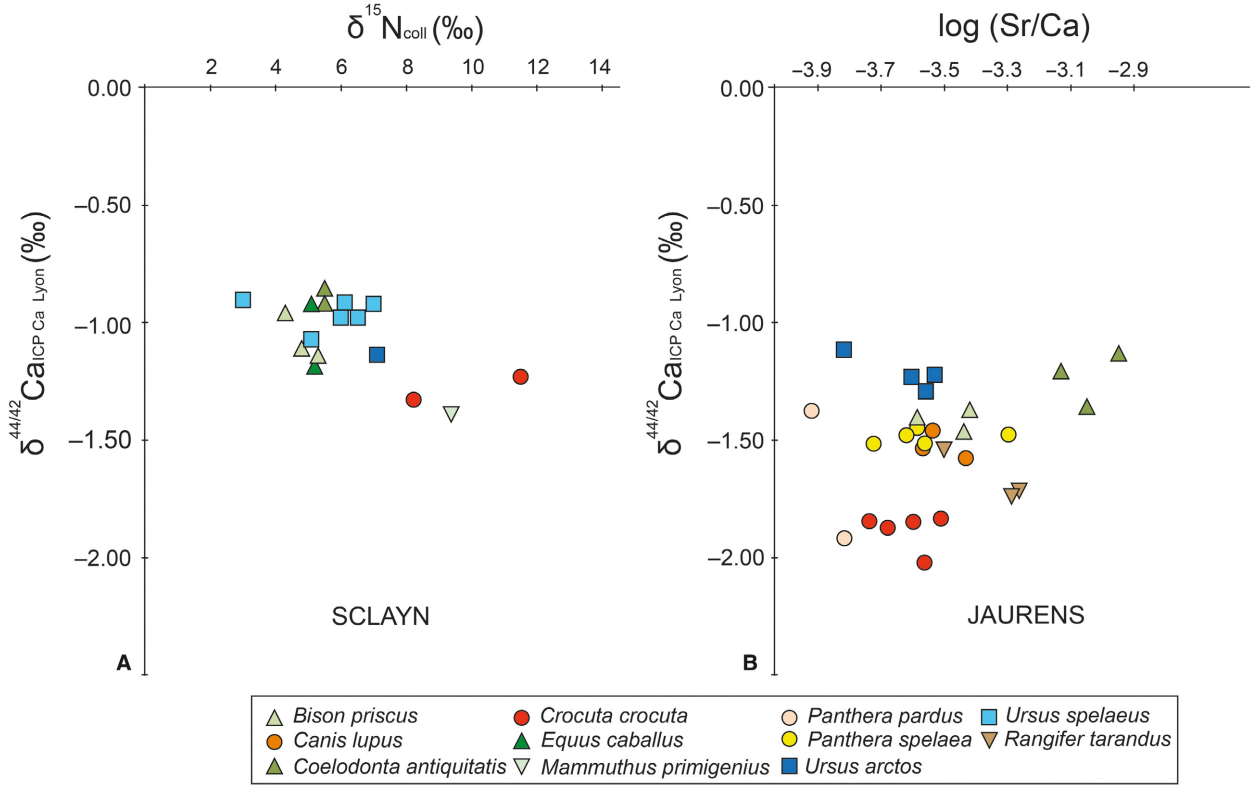

FIG. 5. Fossil mammal $\delta^{44/42}$Ca (in ‰ relative to ICP Ca Lyon standard) variability in two Late Pleistocene assemblages. A, plotted against δ^{15}N (‰ atmospheric N_2) for the bone dataset of Sclayn (Belgium) (Bocherens *et al.* 1997; Balter *et al.* 2002*b*). B, plotted against log (Sr/Ca) for the enamel dataset of Jaurens.

could explain the large variability and overlap observed here.

As demonstrated above, used in conjunction with other proxies, calcium isotopes can provide some insights on the diet of extinct taxa. Relevant to our assemblage is the inferred diet of *Ursus arctos* and *U. spelaeus*, which remains a matter of debate (e.g. Robu *et al.* 2013) although recent studies have underlined mostly plant-based consumption for *U. spelaeus* (e.g. Krajcarz *et al.* 2016). Although at Sclayn, *U. arctos* is represented by a single individual in our dataset, we do not find a significant difference between its calcium isotope value and those of *U. spelaeus*.

The Jaurens tooth enamel dataset where Sr/Ca and $\delta^{44/42}$Ca values are plotted show that *U. arctos* falls in the mid-range values, together with bison, reindeer and some of the wolves and lions (Fig. 5). If we consider those values to represent trophic levels, then *U. arctos* from Jaurens could be interpreted as omnivorous or herbivorous in agreement with modern observations (Robu *et al.* 2013). As for the Sclayn bone dataset, *U. spelaeus* also falls with other herbivores within the δ^{15}N vs $\delta^{44/42}$Ca space. The sole *U. arctos* datapoint is positioned slightly outside the herbivore group in what could correspond to the omnivore group, but admittedly a single datapoint is not enough here to draw conclusions about its dietary habits, and the carnivores are also clearly under-represented. The absence of a clear calcium isotope difference between *U. arctos* and *U. spelaeus* is a preliminary finding, and it should be stressed that the comparison involves two assemblages with different locations and slightly different ages, as well as comparing enamel and bone datasets. Also, as discussed above, diet may not be the only factor affecting calcium isotope fractionation. Deciphering dietary versus physiological influence on calcium isotope fractionation requires more extensive research effort. Several studies based on nitrogen isotopes from bone collagen point to a herbivorous status for *U. spelaeus* (Krajcarz *et al.* 2016). Moreover, similar studies from a single locality recover *U. spelaeus* with significantly lower δ^{15}N values relative to *U. arctos*, implying a distinct herbivorous versus an omnivorous diet for these two taxa (Bocherens *et al.* 2011). Nevertheless, omnivory for *U. spelaeus* has been proposed on the basis of nitrogen and carbon isotopes (Richards *et al.* 2008; Robu *et al.* 2013) and on the basis of microwear (Peigné *et al.* 2009). Dietary heterogeneity at the scale of the population but also in relation to season is to be expected and opens perspectives of research notably with the help of other proxies, including the geochemical proxies presented in this work.

CONCLUSIONS

Traditional isotope approaches to vertebrate palaeobiology have offered, and continue to offer, major insights into the ecology and evolution of extinct faunas. Important information on palaeoenvironments, resource use and physiology can be derived from oxygen, carbon, and sufur isotopes. Indeed, the major limitation on palaeodietary inference is linked to the preservation of collagen, which is a prerequisite for the measurement of nitrogen isotopes. On the other hand, preservation potential of non-traditional isotopes seems high in biomineralized tissues. Moreover, fractionation appears to be controlled by physiological processes and there is increasing evidence that some of these non-traditional isotope systems (e.g. Mg, Ca, Zn) may represent useful palaeodietary tracers. The palaeoecological inferences drawn from calcium isotopes are still in their infancy and among the subjects to be addressed there remains variability linked to physiology and diet. Calcium offers interesting perspectives because it requires only a small quantity of sample (less than half a milligram) and therefore allows for the development of precise time series and the study of seasonal variations at the scale of tissue increments. Moreover, where collagen has completely degraded, calcium (and perhaps magnesium) furnish promising prospects for future research due to their relatively high concentrations and preservation potential in mineralized tissues.

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DATA ARCHIVING STATEMENT

Data for this study are available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.tc28h):

Fig. S1. Triple isotope plot ($\delta^{44/42}$Ca vs $\delta^{43/42}$Ca in ‰) of all data analysed in this study.

Fig. S2. Manganese plotted against $\delta^{44/42}$Ca values for bone and enamel samples measured in this study.

Fig. S3. Photographs of samples analysed for this study; *Canis lupus*, *Crocuta crocuta*, *Panthera spelaea*, *P. pardus*, *Ursus arctos*; Jaurens, Corrèze, France.

Fig. S4. Photographs of samples analysed for this study; *Ursus arctos*, *Coelondonta antiquitatis*, *Rangifer tarandus*, *Bison priscus*; Jaurens, Corrèze, France

Table S1. Data from the literature used in the model of the water/rock ratio presented in Fig. 2.

Table S2. Calcium isotope values (expressed as $\delta^{44/42}$Ca and $\delta^{43/42}$Ca (in ‰) relative to standard ICP Ca-Lyon) measured on two Pleistocene assemblages as well as elemental concentrations for some major and trace elements.

Editor. Andrew Smith

REFERENCES

ALBARÈDE, F. and BEARD, B. 2004. Analytical methods for non-traditional isotopes. *Reviews in Mineralogy & Geochemistry*, **55**, 113–152.

—— TELOUK, P., BLICHERT-TOFT, J., BOYET, M., AGRANIER, A. and NELSON, B. 2004. Precise and accurate isotopic measurements using multiple-collector ICPMS. *Geochimica et Cosmochimica Acta*, **68**, 2725–2744.

AMBROSE, S. H. 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. *Journal of Archaeological Science*, **17**, 431–451.

AMIOT, R., LÉCUYER, C., BUFFETAUT, E., ESCARGUEL, G., FLUTEAU, F. and MARTINEAU, F. 2006. Oxygen isotopes from biogenic apatites suggest widespread endothermy in Cretaceous dinosaurs. *Earth & Planetary Science Letters*, **246**, 41–54.

—— WANG, X., ZHOU, Z., WANG, X., BUFFETAUT, E., LÉCUYER, C., DING, Z., FLUTEAU, F., HIBINO, T., KUSUHASHI, N., MO, J., SUTEETHORN, V., WANG, Y., XU, X. and ZHANG, F. 2011. Oxygen isotopes of East Asian dinosaurs reveal exceptionally cold Early Cretaceous climates. *Proceedings of the National Academy of Sciences*, **108**, 5179–5183.

ARMSTRONG, W. G., HALSTEAD, L. B., REED, F. B. and WOOD, L. 1983. Fossil proteins in vertebrate calcified tissues. *Philosophical Transactions of the Royal Society of London B*, **301**, 301–343.

BALTER, V. 2004. Allometric constraints on Sr/Ca and Ba/Ca partitioning in terrestrial mammalian trophic chains. *Oecologia*, **139**, 83–88.

—— PERSON, A., LABOURDETTE, N., DRUCKER, D., RENARD, M. and VANDERMEERSCH, B. 2001. Were Neandertalians essentially carnivores? Sr and Ba preliminary results of the mammalian palaeobiocoenosis of Saint-Césaire. *Comptes Rendus à L'Académie des Sciences IIA*, **332**, 59–65.

—— SALIEGE, J. F., BOCHERENS, H. and PERSON, A. 2002*a*. Evidences of physico-chemical and isotopic modifications in archaeological bones during controlled acid etching. *Archaeometry*, **44**, 329–336.

—— BOCHERENS, H., PERSON, A., LABOURDETTE, N., RENARD, M. and VANDERMEERSCH, B. 2002*b*. Ecological and physiological variability of Sr/Ca and Ba/Ca in mammals of West European mid-Würmian food webs. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **186**, 127–143.

—— ZAZZO, A., MOLONEY, A. P., MOYNIER, F., SCHMIDT, O., MONAHAN, F. J. and ALBARÈDE, F. 2010. Bodily variability of zinc natural isotope abundances in sheep. *Rapid Communications in Mass Spectrometry*, **24**, 605–612.

—— LAMBOUX, A., ZAZZO, A., TÉLOUK, P., LEVERRIER, Y., MARVEL, J., MOLONEY, A. P., MONAHAN, F. J., SCHMIDT, O. and ALBARÈDE, F. 2013. Contrasting Fe, Cu, and Zn isotopic patterns in organs and body fluids of mice and sheep, with emphasis on cellular fractionation. *Metallomics*, **5**, 1470–1482.

BARRICK, R. E. and SHOWERS, W. J. 1994. Thermophysiology of *Tyrannosaurus rex*: evidence from oxygen isotopes. *Science*, **265**, 222–224.

BENDER, M. M. 1968. Mass spectrometric studies of carbon 13 variations in corn and other grasses. *Radiocarbon*, **10**, 468–472.

BENTLEY, R. A. 2006. Strontium isotopes from the earth to the archaeological skeleton: a review. *Journal of Archaeological Method & Theory*, **13**, 135–187.

BERNARD, A., LÉCUYER, C., VINCENT, P., AMIOT, R., BARDET, N., BUFFETAUT, E., CUNY, G., FOUREL, F., MARTINEAU, F., MAZIN, J.-M. and PRIEUR, A. 2010. Regulation of body temperature by some Mesozoic marine reptiles. *Science*, **328**, 1379–1382.

BLACK, J. R., YIN, Q. and CASEY, W. H. 2006. An experimental study of magnesium-isotope fractionation in chlorophyll-a photosynthesis. *Geochimica et Cosmochimica Acta*, **70**, 4072–4079.

BOCHERENS, H., BILIOU, D., PATOU-MATHIS, M., BONJEAN, D., OTTE, M. and MARIOTTI, A. 1997. Palaeobiological implications of the isotopic signatures (^{13}C, ^{15}N) of fossil mammals collagen in Scladina Cave (Sclayn, Belgium). *Quaternary Research*, **48**, 370–380.

—— STILLER, M., HOBSON, K. A., PACHER, M., RABEDER, G., BURNS, J. A., TÜTKEN, T. and HOFREITER, M. 2011. Niche partitioning between two sympatric genetically distinct cave bears (*Ursus spelaeus* and *Ursus ingressus*) and brown bear (*Ursus arctos*) from Austria: isotopic evidence from fossil bones. *Quaternary International*, **245**, 238–248.

BURTON, J. H. and PRICE, T. D. 2000. The use and abuse of trace elements for paleodietary research. 159–171. *In* AMBROSE, S. H. and KATZENBERG, M A. (eds). *Biogeochemical approaches to paleodietary analysis*. Kluwer Academic/Plenum Publishers.

CERLING, T. E., HARRIS, J. M., MACFADDEN, B. J., LEAKEY, M. G., QUADE, J., EISENMANN, V. and EHLERINGER, J. R. 1997. Global vegetation change through the Miocene/Pliocene boundary. *Nature*, **389**, 153–158.

CHU, N.-C., HENDERSON, G. M., BELSHAW, N. S. and HEDGES, R. E. M. 2006. Establishing the potential of Ca isotopes as proxy for consumption of dairy products. *Applied Geochemistry*, **21**, 1656–1667.

CLEMENTZ, M. T. 2012. New insight from old bones: stable isotope analysis of fossil mammals. *Journal of Mammalogy*, **93**, 368–380.

—— and KOCH, P. L. 2001. Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. *Oecologia*, **129**, 461–472.

—— HOLDEN, P. and KOCH, P. L. 2003. Are calcium isotopes a reliable monitor of trophic level in marine settings? *International Journal of Osteoarchaeology*, **13**, 29–36.

—— FOX-DOBBS, K., WHEATLEY, P. V., KOCH, P. L. and DOAK, D. F. 2009. Revisiting old bones: coupled carbon isotope analysis of bioapatite and collagen as an ecological and palaeoecological tool. *Geological Journal*, **44**, 605–620.

DENIRO, M. J. and EPSTEIN, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, **42**, 495–506.

—— —— 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, **45**, 341–351.

EZZO, J. A. 1994. Putting the "chemistry" back into archaeological bone chemistry analysis: modelling potential paleodietary indicators. *Journal of Anthropological Archaeology*, **13**, 1–34.

FANTLE, M. S. and TIPPER, E. T. 2014. Calcium isotopes in the global biogeochemical Ca cycle: implications for development of a Ca isotope proxy. *Earth-Science Reviews*, **129**, 148–177.

FISCHER, J., VOIGT, S., FRANZ, M., SCHNEIDER, J. W., JOACHIMSKI, M. M., TICHOMIROWA, M., GÖTZE, J. and FURRER, H. 2012. Palaeoenvironments of the late Triassic Rhaetian Sea: implications from oxygen and strontium isotopes of hybodont shark teeth. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **353–355**, 60–72.

—— SCHNEIDER, J. W., HODNETT, J.-P., ELLIOTT, D. K., JOHNSON, G. D., VOIGT, S., JOACHIMSKI, M. M., TICHOMIROWA, M. and GÖTZE, J. 2013. Stable and radiogenic isotope analyses on shark teeth from the Early to the Middle Permian (Sakmarian-Roadian) of the southwestern USA. *Historical Biology*, **26**, 710–727.

GALY, A., BELSHAW, N. S., HALICZ, L. and O'NIONS, R. K. 2001. High-precision measurement of magnesium isotopes by multiple-collector inductively coupled plasma mass spectrometry. *International Journal of Mass Spectrometry*, **208**, 89–98.

GANNES, L. Z., MARTÍNEZ DEL RIO, C. and KOCH, P. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comparative Biochemistry & Physiology Part A*, **119**, 725–737.

GILOT, E. 1992. Sclayn: datation par ^{14}C du Moustérien final. 173–174. *In* OTT, M. (ed.) *Recherches aux grottes de Sclayn. Vol. 1, Le contexte* Etudes et Recherches Archéologiques de L'Université de Liège **27**.

GOEDERT, J., AMIOT, R., BOUDAD, L., BUFFETAUT, E., FOUREL, F., GODEFROIT, P., KUSUHASHI, N., SUTEETHORN, V., TONG, H., WATABE, M. and LÉCUYER, C. 2016*a*. Preliminary investigation of seasonal patterns recorded in the oxygen isotope composition of theropod dinosaur tooth enamel. *Palaios*, **31**, 10–19.

—— FOUREL, F., AMIOT, R., SIMON, L. and LÉCUYER, C. 2016*b*. High-precision 34S/32S measurements in vertebrate bioapatites using purge-and-trap elemental

analyser/isotope ratio mass spectrometry technology. *Rapid Communications in Mass Spectrometry*, **30**, 2002–2008.

GUERIN, C., PHILIPPE, M. and VILAIN, R. 1979. Le gisement Pleistocène supérieur de la grotte de Jaurens à Nespouls, Corrèze, France: historique et généralités. *Nouvelles Archives du Muséum D'Histoire Naturelle de Lyon*, **17**, 11–16.

HEUSER, A. and EISENHAUER, A. 2010. A pilot study on the use of natural calcium isotope ($^{44}Ca/^{40}Ca$) fractionation in urine as a proxy for the human body calcium balance. *Bone*, **46**, 889–896.

—— TÜTKEN, T., GUSSONE, N. and GALER, S. J. G. 2011. Calcium isotopes in fossil bones and teeth — diagenetic versus biogenic origin. *Geochimica et Cosmochimica Acta*, **75**, 3419–3433.

JAOUEN, K. and BALTER, V. 2014. Menopause effect on blood Fe and Cu isotope compositions. *American Journal of Physical Anthropology*, **153**, 280–285.

—— and PONS, M. L. 2016. Potential of non-traditional isotope studies for bioarchaeology. *Archaeological & Anthropological Sciences*, published online 3 November. doi: 10.1007/s12520-016-0426-9

—— BALTER, V., HERRSCHER, E., LAMBOUX, A., TELOUK, P. and ALBARÈDE, F. 2012. Fe and Cu stable isotopes in archeological human bones and their relationship to sex. *American Journal of Physical Anthropology*, **148**, 334–340.

—— PONS, M.-L. and BALTER, V. 2013. Iron, copper and zinc isotopic fractionation up mammal trophic chains. *Earth & Planetary Science Letters*, **374**, 164–172.

—— BEASLEY, M., SCHOENINGER, M., HUBLIN, J.-J. and RICHARDS, M. P. 2016*a*. Zinc isotope ratios of bones and teeth as new dietary indicators: results from a modern food web (Koobi Fora, Kenya). *Scientific Reports*, **6**, 26281.

—— SZPAK, P. and RICHARDS, M. P. 2016*b*. Zinc isotope ratios as indicators of diet and trophic level in Arctic marine mammals. *PLoS ONE*, **11**, e0152299.

KELLY, J. F. 1999. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology*, **78**, 1–27.

KNUDSON, K., WILLIAMS, H. M., BUIKSTRA, J. E., TOMCZAK, P. D., GORDON, G. W. and ANBAR, A. D. 2010. Introducing $\delta^{88}/^{86}Sr$ analysis in archaeology: a demonstration of the utility of strontium isotope fractionation in paleodietary studies. *Journal of Archaeological Science*, **37**, 2352–2364.

KOCH, P. 2007. Isotopic study of the biology of modern and fossil vertebrates. 99–154. *In* MICHENER, R. and LAJTHA, K. (eds). *Stable isotopes in ecology and environmental science*, 2nd edn. Blackwell Publishing.

—— FISCHER, D. C. and DETTMAN, D. 1989. Oxygen isotope variation in the tusks of extinct proboscideans: a measure of season of death and seasonality. *Geology*, **17**, 515–519.

KOCSIS, L., ŐSI, A., VENNEMANN, T., TRUEMAN, C. N. and PALMER, M. R. 2009. Geochemical study of vertebrate fossils from the Upper Cretaceous (Santonian) Csehbánya Formation (Hungary): evidence for a freshwater habitat of mosasaurs and pycnodont fish. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **280**, 532–542.

KRABBENHÖFT, A., FIETZKE, J., EISENHAUER, A., LIEBETRAU, V., BÖHM, F. and VOLLSTAEDT, H. 2009. Determination of radiogenic and stable strontium isotope ratios ($^{87}Sr/^{86}Sr$; $\delta^{88}/^{86}Sr$) by thermal ionization mass spectrometry applying an $^{87}Sr/^{84}Sr$ double spike. *Journal of Analytical Atomic Spectrometry*, **24**, 1267–1271.

KRAJCARZ, M., PACHER, M., KRAJKARZ, M. T., LAUGHLAN, L., RABEDER, G., SABOL, M., WOJTAL, P. and BOCHERENS, H. 2016. Isotopic variability of cave bears ($\delta^{15}N$, $\delta^{13}C$) across Europe during MIS 3. *Quaternary Science Reviews*, **131**, 51–72.

LI, Q., THIRLWALL, M. and MÜLLER, W. 2016. Ca isotopic analysis of laser-cut microsamples of (bio)apatite without chemical purification. *Chemical Geology*, **422**, 1–12.

LONGINELLI, A. 1984. Oxygen isotopes in mammal bone phosphate: a new tool for paleohydrological and paleoclimatological research? *Geochimica et Cosmochimica Acta*, **48**, 385–390.

MACFADDEN, B. J., SOLOUNIAS, N. and CERLING, T. E. 1999. Ancient diets, ecology, and extinction of 5-million-year-old horses from Florida. *Science*, **283**, 824–827.

MARTIN, J. E., VANCE, D. and BALTER, V. 2014. Natural variation of magnesium isotopes in mammal bones and teeth from two South African trophic chains. *Geochimica et Cosmochimica Acta*, **130**, 12–20.

—— TACAIL, T., ADNET, S., GIRARD, C. and BALTER, V. 2015*a*. Calcium isotopes reveal the trophic position of extant and fossil elasmobranchs. *Chemical Geology*, **415**, 118–125.

—— VANCE, D. and BALTER, V. 2015*b*. Magnesium stable isotope ecology using mammal tooth enamel. *Proceedings of the National Academy of Sciences*, **112**, 430–435.

—— DEESRI, U., LIARD, R., WATTANAPITUKSAKUL, A., LAUPRASERT, K., TONG, H., BUFFETAUT, E., SUTEETHORN, V., SUAN, G., TELOUK, P. and BALTER, V. 2016. Strontium isotopes reveal long-term residency of thalattosuchians in the freshwater environment. *Paleobiology*, **42**, 143–156.

—— TACAIL, T. and BALTER, V. 2017. Data from: Non-traditional isotope perspectives in vertebrate palaeontology. *Dryad Data Repository*. 10.5061/dryad.tc28h

MELIN, A. D., CROWLEY, B. E., BROWN, S. T., WHEATLEY, P. V., MORITZ, G. L., YU, F. T. Y., BERNARD, H., DEPAOLO, D. J., JACOBSON, A. D. and DOMINY, N. J. 2014. Calcium and carbon stable isotope ratios as paleodietary indicators. *American Journal of Physical Anthropology*, **154**, 633–643.

NADLER, J. L. and RUDE, R. K. 1995. Disorders of magnesium metabolism. *Endocrinology & Metabolism Clinics of North America*, **24**, 623–641.

NEWSOME, S. D., CLEMENTZ, M. T. and KOCH, P. 2010. Using stable isotope biogeochemistry to study marine mammal ecology. *Marine Mammal Science*, **26**, 509–572.

PATTERSON, C. C., SHIRAHATA, H. and ERICSON, J. E. 1987. Lead in ancient human bones and its relevance to historical developments of social problems with lead. *Science of the Total Environment*, **61**, 167–200.

PEEK, S. and CLEMENTZ, M. T. 2011. Sr/Ca and Ba/Ca variations in environmental and biological sources: a survey of marine and terrestrial systems. *Geochimica et Cosmochimica Acta*, **95**, 36–52.

PEIGNÉ, S., GOILLOT, C., GERMONPRÉ, M., BLONDEL, C., BIGNON, O. and MERCERON, G. 2009. Predormancy diet of the European cave bear: evidence for omnivory from a dental microwear analysis of Late Pleistocene *Ursus spelaeus* from Goyet, Belgium. *Proceedings of the National Academy of Sciences*, **106**, 15390–15393.

RASMUSSEN, K. L., BOLDSEN, J. L., KRISTENSEN, H. K., SKYTTE, L., HANSEN, K. L., MØLHOLM, L., GROOTES, P. M., NADEAU, M.-J. and ERIKSEN, K. M. F. 2008. Mercury levels in Danish Medieval human bones. *Journal of Archaeological Science*, **35**, 2295–2306.

REYNARD, B. and BALTER, V. 2014. Trace elements and their isotopes in bones and teeth: diet, environments, diagenesis, and dating of archeological and paleontological samples. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **416**, 4–16.

REYNARD, L. M., HENDERSON, G. M. and HEDGES, R. E. M. 2010. Calcium isotope ratios in animal and human bone. *Geochimica et Cosmochimica Acta*, **74**, 3735–3750.

REYNARD, L., HENDERSON, G. M. and HEDGES, R. E. M. 2011. Calcium isotopes in archaeological bones and their relationship to dairy consumption. *Journal of Archaeological Science*, **38**, 657–664.

—— PEARSON, J. A., HENDERSON, G. M. and HEDGES, R. E. M. 2013. Calcium isotopes in juvenile milk-consumers. *Archaeometry*, **55**, 946–957.

RICHARDS, M. P., FULLER, B. T. and HEDGES, R. E. M. 2001. Sulphur isotopic variation in ancient bone collagen from Europe: implications for human palaeodiet, residence mobility, and modern pollutant studies. *Earth & Planetary Science Letters*, **191**, 185–190.

—— PACHER, M., STILLER, M., QUILÈS, J., HOFREITER, M., CONSTANTIN, S., ZILHÃO, J. and TRINKAUS, E. 2008. Isotopic evidence for omnivory among European cave bears: Late Pleistocene *Ursus spelaeus* from the Peştera cu Oase, Romania. *Proceedings of the National Academy of Sciences*, **105**, 600–604.

ROBU, M., FORTIN, J. K., RICHARDS, M. P., SCHWARTZ, C. C., WYNN, J. G., ROBBINS, C. T. and TRINKAUS, E. 2013. Isotopic evidence for dietary flexibility among European Late Pleistocene cave bears (*Ursus spelaeus*). *Canadian Journal of Zoology*, **91**, 227–234.

RUSSELL, W. A. and PAPANASTASSIOU, D. A. 1978. Calcium isotope fractionation in ion-exchange chromatography. *Analytical Chemistry*, **50**, 48–51.

—— —— and TOMBRELLO T. A. 1978. Calcium isotope fractionation in earth and other solar system materials. *Geochimica et Cosmochimica Acta*, **42**, 1075–1090.

SCHOENINGER, M. J. 2012. The ancestral dinner table. *Nature*, **487**, 42–43.

SCHWARTZ-NARBONNE, R., LONGSTAFFE, F. J., METCALFE, J. Z. and ZAZULA, G. 2015. Solving the wooly mammoth conundrum: amino acid ^{15}N-enrichment suggests a distinct forage or habitat. *Scientific Reports*, **5**, 09791.

SHARP, Z. 2007. *Principles of stable isotope geochemistry*. Pearson/Prentice Hall, 344 pp.

SILLEN, A. 1986. Biogenic and diagenetic Sr/Ca in Plio-Pleistocene fossils of the Omo Shungura Formation. *Paleobiology*, **12**, 311–323.

SKULAN, J. and DEPAOLO, D. J. 1999. Calcium isotope fractionation between soft and mineralized tissues as a monitor of calcium use in vertebrates. *Proceedings of the National Academy of Sciences*, **96**, 13709–13713.

—— —— and OWENS T. L. 1997. Biological control of calcium isotopic abundances in the global calcium cycle. *Geochimica et Cosmochimica Acta*, **61**, 2505–2510.

STAFFORD, T. W., BRENDEL, K. and DUHAMEL, R. 1988. Radiocarbon, 13C and 15N analysis of fossil bone: removal of humates with XAD-2 resin. *Geochimica et Cosmochimica Acta*, **52**, 2257–2267.

TACAIL, T., ALBALAT, E., TÉLOUK, P. and BALTER, V. 2014. A simplified protocol for measurement of Ca isotopes in biological samples. *Journal of Analytical Atomic Spectrometry*, **29**, 529–535.

—— TÉLOUK, P. and BALTER, V. 2016. Precise analysis of calcium stable isotope variations in biological apatites using laser ablation MC-ICPMS. *Journal of Analytical Atomic Spectrometry*, **31**, 152–162.

VAN DER MERWE, N. J. 1982. Carbon isotopes, photosynthesis, and archaeology. *American Scientist*, **70**, 596–606.

VAN GEEL, B., GUTHRIE, R. D., ALTMANN, J. G., BROEKENS, P., BULL, I. D., GILL, F. L., JANSEN, B., NIEMAN, A. M. and GRAVENDEEL, B. 2011. Mycological evidence of coprophagy from the feces of an Alakan Late Glacial mammoth. *Quaternary Science Reviews*, **30**, 2289–2303.

VOGEL, J. C. 1978. Isotopic assessment of the dietary habits of ungulates. *South African Journal of Science*, **74**, 298–301.

WALCZYK, T. and BLANCKENBURG, F. VON 2005. Deciphering the iron isotope message of the human body. *International Journal of Mass Spectrometry*, **242**, 117–134.

ZHU, P. and MACDOUGALL, J. D. 1998. Calcium isotopes in the marine environment and the oceanic calcium cycle. *Geochimica et Cosmochimica Acta*, **62**, 1691–1698.