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Calcium isotopes in fossil bones and teeth — Diagenetic versus biogenic origin

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Abstract

We present the first systematic study of Ca isotopes ($\delta^{44/40}$Ca) in Late Triassic to Late Cretaceous dinosaur bones and teeth (enamel and dentin) from sympatric herbivorous and carnivorous dinosaurs. The samples derive from five different localities, and data from embedding sediments are also presented. Additional $\delta^{44/40}$Ca in skeletal tissues from modern reptiles and birds (avian dinosaurs) were measured for comparison in order to examine whether the original Ca isotopic composition in dinosaur skeletal apatite was preserved or might have changed during the diagenesis and fossilization process.

$\delta^{44/40}$Ca of fossil skeletal tissues range from −1.62‰ (*Tyrannosaurus rex* enamel) to +1.08‰ (*Brachiosaurus brancai* bone), while values in modern archosaur bones and teeth range from −1.63‰ (caiman enamel) to −0.37‰ (ostrich bone). The average $\delta^{44/40}$Ca of the three types of fossil skeletal tissue analyzed – bone, dentin and enamel – show some systematic differences: while $\delta^{44/40}$Ca in bone exhibits the highest values, while $\delta^{44/40}$Ca in enamel has the lowest values, and dentin $\delta^{44/40}$Ca falls in between. Values of $\delta^{44/40}$Ca in the remains of herbivorous dinosaurs (0.1–1.1‰) are generally higher than those of bones of modern mammalian herbivores (−2.6‰ to −0.8‰) and from modern herbivorous archosaurs, which exhibit intermediate $\delta^{44/40}$Ca (−0.8‰ to −0.4‰). These systematic isotopic shifts may reflect physiological differences between dinosaurs, mammals and reptiles representing different taxonomic groups of vertebrates.

Systematic offsets in skeletal apatite $\delta^{44/40}$Ca between herbivorous and carnivorous dinosaurs are not obvious, indicating a lack of a clear-cut Trophic Level Effect (TLE) shift between herbivores and carnivores in dinosaurs. This observation can be explained if the carnivorous dinosaurs in this study fed mainly on soft tissues from their prey and did not ingest hard (calcified) tissue to much extent. The most striking indication that the primary $\delta^{44/40}$Ca is actually preserved in most of the fossil teeth is a difference in $\delta^{44/40}$Ca of about 0.35 ± 0.10‰ (1SD) between dentin and enamel, based upon 11 of 16 analyzed dentin-enamel pairs. This difference is close to that found in modern reptiles (0.28 ± 0.05‰), and strongly suggests that this tell-tale signature is a primary feature of the fossilized dinosaur material as well. Furthermore, simple mass balance calculations show that changes of the original $\delta^{44/40}$Ca in bones and teeth by diagenetically-formed calcium-bearing minerals are either small or would require implausible high original $\delta^{44/40}$Ca values in the skeletal apatite.

1. INTRODUCTION

Fossil bones and teeth are valuable geochemical archives, and their isotopic and trace elemental compositions can be used to reconstruct diet, trophic level, thermophysiology, paleoclimate and the habitat of vertebrates (see overviews by: Kohn and Cerling, 2002; Hedges et al., 2006; Koch, 2007). However, the applicability of the isotopic and trace elemental compositions of skeletal tissues for these reconstructions is limited, as during fossilization (cf. Kohn, 2008) and diagenetic alteration the original chemical and isotopic composition of bones and teeth is changed

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over time (e.g. Henderson et al., 1983; Ayliffe et al., 1994; Hubert et al., 1996; Kolodny et al., 1996; Kohn et al., 1999; Hedges, 2002; Zazzo et al., 2004; Pfretzschner, 2004). While these changes have been described and investigated in detail for trace elements and the major organic constituents of bones and teeth, virtually no information is available on changes to the major inorganic component – calcium – and its isotopic composition.

Fresh skeletal tissues consist of water, organic matter and a mineral component: nm-sized crystals of a carbonated hydroxylapatite with up to 5% carbonate ions on varying positions in the crystal lattice (cf. Bonucci, 2007). The proportions of these components, as well as the carbonate fraction of the dahllite and its crystal size, vary depending on the type of tissue (cf. Pasteris et al., 2008) and species (cf. Bonucci, 2007). As calcium is the principal inorganic component of skeletal tissues, with concentrations ranging from ~27 wt.% (fresh bone) to ~40 wt.% (fresh enamel), it can be assumed that the original Ca isotopic composition of bones and teeth (enamel and dentin) are at least potentially better preserved during fossilization compared to that of trace elements and other isotope systems.

Over the past decade, calcium stable isotopes have proven to be useful proxies for paleoceanographic reconstructions, and helpful for tracing biological processes in marine animals. For example, the Ca isotopic composition in calcium-bearing mineral phases has been used to reconstruct sea surface temperatures (Nägler et al., 2000; Gussone et al., 2004; Hippler et al., 2006), and the past ocean Ca mass balance (De La Rocha and DePaolo, 2000; Fantle and DePaolo, 2005; Heuser et al., 2005; Farkas et al., 2007), as well as to investigate biomineralization in marine monads (e.g. Gussone et al., 2007; Hippler et al., 2009). Many studies have focused on foraminifera (e.g. Heuser et al., 2005; Gussone et al., 2009; Gussone and Filipsson, 2010; Hippler et al., 2009), coccolithophores (e.g. Gussone et al., 2006; Gussone et al., 2007; Langer et al., 2007) or calcium carbonates from multicellular marine organisms (Böhm et al., 2006; Steuber and Buhl, 2006; Heinemann et al., 2008). A small number of studies have investigated non-$CaCO_3$-based archives such as marine calcium phosphates (Schmitt et al., 2003; Soudry et al., 2004; Arning et al., 2009) or calcium coprecipitated in marine sedimentary barites (Griffith et al., 2008).

To date, only a small amount of work exists exploiting Ca isotopes to study vertebrate metabolism, which has great potential for unraveling calcium pathways through diet, laying-down of bone and excretion (Skulan et al., 1997; Skulan and DePaolo, 1999; Clementz et al., 2003; Chu et al., 2006; Skulan et al., 2007; Heuser and Eisenhauer, 2010; Reynard et al., 2010, 2011). One key observation is that Ca isotopes ($\delta^{44/40}$Ca) appear to be fractionated passing along the food chain by about $-1‰$ per trophic level (DePaolo, 2004). Skulan and DePaolo (1999) presented a simple Ca isotope transport model for vertebrates explaining the cause of this so-called Trophic Level Effect (TLE). According to this model, the Ca isotopic composition of mineralized tissues (bone) becomes fractionated by about $-1.3‰$ ($\delta^{44/40}$Ca) compared to soft tissue (blood) and a Ca isotope fractionation only takes place during the formation of mineralized tissue. According to Skulan and DePaolo (1999), Ca isotope fractionation does not occur during the transport of dietary Ca into the body (blood), nor during the degradation of mineralized tissue, nor during the transport of Ca from blood to excreta (urine).

Here we investigate the Ca isotopic composition of fossil teeth and bone of several dinosaur taxa in order to understand better the preservation of primary Ca isotope signatures in fossilized skeletal tissues. The ultimate aim, however, is to use such fossilized material to reconstruct ancient food chains and trophic levels of now-extinct ecosystems. Perhaps the most important challenge we face in this study is whether or not we can find tell-tale signs of diagenetic alteration so as to identify samples which are not, or are least, affected by diagenetic alteration and thus still preserve original biogenic Ca isotope compositions from the organism. With this in mind, we analyzed fossil skeletal tissues from a large number of dinosaur genera from several localities and ages, as well as samples of the embedding sediment. For comparison with modern ecosystems, bones and teeth of extant reptiles (including birds), the nearest living relatives of dinosaurs, were also analyzed.

2. SAMPLES

We analyzed bones, the cortex (outer compact part of the bone) of mainly long bones, and teeth (enamel and dentin) from several dinosaur taxa (Table 1). These skeletal remains come from five different localities ranging in age from Late Triassic (Trossingen, Germany), Late Jurassic (Tendaguru Formation, Tanzania and Morrison Formation, USA) to Late Cretaceous (Hell Creek Formation, USA and Dinosaur Park Formation, Canada). These fossil sites represent different depositional settings and lithologies: (1) Trossingen: continental red beds, carbonaceous silt-claystones (Sander, 1992), (2) Tendaguru Formation: near-coastal, marl- and sandstones (Bussert et al., 2009), (3) Morrison Formation: floodplain deposits, silt- to sandstones (Dodson et al., 1980; Foster, 2007), (4) Hell Creek Formation: fluvial channel and delta deposits, mud- to sandstones (Hartman et al., 2002), and (5) Dinosaur Park Formation: fluvial deposits, silt- to sandstones (Currie and Koppelhus, 2005). The dinosaur taxa cover a spectrum of different dinosaur clades, saurischia (prosauropods, sauropods, theropods) and ornithischia (stegosaurs, hadrosaurs) to avoid results that might be biased by phylogenetic effects.

The dinosaurs investigated are thought to have been either strictly herbivorous (sauropods, prosauropods, and ornithischia) or carnivorous (theropods) although there is still some debate over the diet of prosauropods, such as for *Plateosaurus*, investigated here, where omnivory is considered a possibility (Barrett, 2000). We also analyzed the embedding sediment of the bones, if available. These sediment samples were taken from the sedimentary matrix or from the marrow cavity of the bones. In both cases, the Ca isotopic composition may give additional information on the diagenetic history of the bones and teeth at a given sample locality and may give insights into Ca exchange processes between fossil tissue and embedding sediment.

Table 1
Ca isotope results of the measurements of fossil and modern skeletal tissues (weighted $\delta^{44/40}$Ca and weighted 2SE).

Sample name	Taxon	Diet	Tissue	$\delta^{44/40}$Ca (‰)	±2SE	*n*
Trossingen[a], Germany, Late Triassic (Norian)						
PL TR 7	*Plateosaurus engelhardti*	Herbivore	Enamel	−0.02	0.07	2
			Dentin	0.35	0.05	3
			Bone	0.48	0.04	5
PL TR 2	*Plateosaurus engelhardti*	Herbivore	Bone	0.67	0.03	7
Morrison Formation (Howe Ranch)[b], USA, Late Jurassic (Tithonian)						
CA MO 11	*Camarasaurus* sp.	Herbivore	Enamel	−0.39	0.03	3
			Enamel	−0.25	0.07	2
			Dentin	0.07	0.11	4
AP MO 11	*Apatosaurus* sp.	Herbivore	Bone	0.26	0.02	3
			Bone	0.14	0.06	2
			Bone	0.10	0.02	6
AP MO 13	*Apatosaurus* sp.	Herbivore	Bone	0.08	0.06	2
AP MO 15	*Apatosaurus* sp.	Herbivore	Enamel	−0.67	0.04	3
			Dentin	0.53	0.03	4
DI MO 13	*Diplodocus* sp.	Herbivore	Enamel	−0.13	0.03	3
		Herbivore	Dentin	0.27	0.03	3
DI MO 7	*Diplodocus* sp.	Herbivore	Bone	0.34	0.02	5
DI MO 8	*Diplodocus* sp.	Herbivore	Bone	0.06	0.03	4
BR MO 11	*Brachiosaurus* sp.	Herbivore	Bone	0.43	0.03	4
ST MO 2	*Stegosaurus* sp.	Herbivore	Dentin	−0.03	0.03	5
ST MO 1	*Stegosaurus* sp.	Herbivore	Bone	0.12	0.04	2
AL MO N23-6	*Allosaurus* sp.	Carnivore	Enamel	−0.39	0.03	3
			Dentin	0.02	0.02	7
TH MO 1	Theropod indet.*	Carnivore	Enamel	−0.32	0.02	4
			Dentin	0.00	0.02	6
Tendaguru Formation (Tendaguru Hill)[c], Tanzania, Late Jurassic (Kimmeridgian/Tithonian)						
BR TE 11	*Brachiosaurus brancai*	Herbivore	Enamel	0.13	0.04	3
	Brachiosaurus brancai	Herbivore	Dentin	0.33	0.03	4
BR TE 9	*Brachiosaurus brancai*	Herbivore	Enamel	0.10	0.04	3
			Dentin	0.51	0.02	3
BR TE 2	*Brachiosaurus brancai*	Herbivore	Bone	1.08	0.06	4
BR TE 10	*Brachiosaurus brancai*	Herbivore	Enamel	0.27	0.05	3
			Dentin	0.52	0.05	3
BR TE 6	*Brachiosaurus brancai*	Herbivore	Bone	0.60	0.02	9
JA TE 1	*Janenschia robusta*	Herbivore	Bone	0.59	0.03	6
DIC TE 1	*Dicraeosaurus sattleri*	Herbivore	Bone	0.46	0.02	6
BA TE 3	*Barosaurus* sp.	Herbivore	Bone	0.67	0.03	5
DY TE 16	*Dryosaurus lettovorbecki*	Herbivore	Bone	0.44	0.03	5
TH TE 1	Theropod indet.*	Carnivore	Bone	0.59	0.03	5
TH TE 3	Theropod indet.*	Carnivore	Enamel	−0.71	0.03	4
			Dentin	0.11	0.03	4
Hell Creek Formation[d], USA, Late Cretaceous (Maastrichtian)						
TY HE 1	*Tyrannosaurus rex*	Carnivore	Enamel	−1.62	0.03	4
			Dentin	−0.57	0.02	7
Dinosaur Park Formation[e], Canada, Late Cretaceous (Campanian)						
FZ DP369	*Albertosaurus* sp.	Carnivore	Enamel	−0.47	0.03	4
			Dentin	−0.63	0.03	3
FZ DP372	*Albertosaurus* sp.	Carnivore	Enamel	−0.60	0.03	4
			Dentin	−0.13	0.02	4
FZ DP381	Theropod indet.*	Carnivore	Enamel	−0.25	0.03	4
			Dentin	−0.01	0.02	3
FZ DP341	Hadrosaur indet.*	Herbivore	Enamel	0.01	0.06	3
			Dentin	0.73	0.05	2
Modern samples						
VA 1	*Varanus niloticus*	Carnivore	Enamel	−1.86	0.02	4
			Dentin	−1.55	0.03	3
KR 1	*Caiman crocodilus*	Carnivore	Enamel	−1.63	0.03	3
			Dentin	−1.38	0.02	6

(*continued on next page*)

Table 1 (*continued*)

Sample name	Taxon	Diet	Tissue	$\delta^{44/40}$Ca (‰)	±2SE	*n*
K StrF5 1	*Struthio camelus*	Herbivore	Bone	−0.39	0.05	2
K StrF5 2	*Struthio camelus*	Herbivore	Bone	−0.45	0.06	2
K StrF5 3	*Struthio camelus*	Herbivore	Bone	−0.37	0.05	2
K StrF5 4	*Struthio camelus*	Herbivore	Bone	−0.43	0.05	2
K StrCS5	*Struthio camelus*	Herbivore	Bone	−0.78	0.05	2
K StrCS4	*Struthio camelus*	Herbivore	Bone	−0.49	0.06	2
K StrCS3	*Struthio camelus*	Herbivore	Bone	−0.43	0.06	2
K Kr26	*Alligator mississippiensis*	Carnivore	Bone	−1.38	0.05	2
K Kr51	*Alligator mississippiensis*	Carnivore	Bone	−1.59	0.05	2

Detailed descriptions of the geological setting and taphonomy of the fossil sites can be found in the following references: [a]Sander (1992); [b]Carpenter et al. (1998), Turner and Peterson (1999); [c]Bussert et al. (2009); [d]Hartman et al. (2002); [e]Currie and Koppelhus (2005).
* Undetermined species.

Furthermore, from one *Apatosaurus* bone (AP MO 11) and one *Camarasaurus* tooth (CA MO 11) – both from the Howe Ranch Quarry, Morrison Formation – multiple bone and enamel samples, respectively, were analyzed to check for intra-tissue $\delta^{44/40}$Ca variability.

In order to interpret better the fossil data we also analyzed bones and teeth from extant reptiles (alligator, Nile monitor) and birds (ostrich) (Table 1), the latter of which are the closest living relatives of dinosaurs and are thus most suited for comparison from a phylogenetic perspective.

3. METHODS

3.1. Sample preparation

Samples of bones and teeth were obtained using a hand-operated drill equipped with a diamond-studded drill bit. Enamel was obtained by carefully scraping off the surface of the tooth using the hand drill to yield 1–5 mg of material. Bones and dentin were prepared by first removing the outermost 1–2 mm of the specimen using the hand drill; further drilling resulted in 5–15 mg of sample powder. Sample preparation and measurement was performed in two different laboratories: the Max-Planck-Institut für Chemie (Mainz, Germany) and the Institut für Mineralogie, Universität Münster (Germany).

In Mainz, samples (0.6–22 mg) were weighed into PFA beakers and a mixture of 1 ml high-purity concentrated HNO_3 and 30 µl of H_2O_2 (Suprapure®, Merck) was added. Dissolution occurred over 12 h on a hot plate at 140 °C. After digestion, a small aliquot of the solution – containing 1.5–3 µg of Ca – was taken and an appropriate amount of ^{43}Ca–^{48}Ca double spike tracer solution was added.

Calcium was separated from the rest of the sample using standard cation exchange techniques with glass chromatographic columns containing ~0.7 ml of AG 50 W-X8 (200–400 mesh) resin and utilizing 2.0 N HCl as eluent. The calcium fraction was dried, and a portion loaded onto pre-degassed single Re filaments along with a Ta_2O_5-based activator, as described in Heuser et al. (2002).

The procedures in Münster were basically the same as those followed in Mainz, except for the following: (1) since Ca was the main cation in the phases studied, no chemical purification was performed by ion exchange, (2) a ^{42}Ca–^{43}Ca double spike tracer was used to correct for instrumental fractionation instead of the ^{43}Ca–^{48}Ca tracer.

3.2. Mass spectrometry and data reduction

All samples as well as double spiked standards (NIST SRM 915a and SRM 1486) were measured using Thermo-Fisher TRITON thermal ionization mass spectrometers (TIMS) located in Mainz and Münster. Details of the mass spectrometric data collection can be found in Heuser and Eisenhauer (2010). Data reduction for the natural Ca isotope fractionation of the sample and standard materials made use of the iterative algorithm previously described by Nägler et al. (2000) and Heuser et al. (2002). This algorithm calculates the ^{44}Ca/^{40}Ca sample ratio from the measured ^{40}Ca/^{48}Ca, ^{43}Ca/^{48}Ca and ^{44}Ca/^{48}Ca ratios (measurements in Mainz) or from the measured ^{40}Ca/^{43}Ca, ^{42}Ca/^{43}Ca and ^{44}Ca/^{43}Ca ratios (measurements in Münster). All Ca isotope data are reported using the δ-notation: $\delta^{44/40}\text{Ca}\ (‰) = [(({}^{44}\text{Ca}/{}^{40}\text{Ca})_{\text{sample}}/({}^{44}\text{Ca}/{}^{40}\text{Ca})_{\text{standard}})-1] \times 1000$, (cf. Eisenhauer et al., 2004). All $\delta^{44/40}$Ca values are expressed relative to the standard reference material NIST SRM 915a which was measured alongside our samples. The average weighted 2SD of the NIST SRM 915a measurements from each session (normally $n = 3$) is ±0.07‰ and range from ±0.02‰ to ±0.21‰. The long-term weighted 2SD of all NIST SRM 915a measurements is 0.04‰ ($n = 84$).

To ensure the compatibility of Ca isotope data between the two laboratories, NIST SRM 1486 was used as a second matrix-matched reference material. The measured $\delta^{44/40}$Ca values of SRM 1486 are in good agreement between the two labs (−1.03‰ in Mainz versus −0.93‰ in Münster), and are both close to the value of −1.01‰ reported by Heuser and Eisenhauer (2008).

One dentin sample (CA MO 11) was processed in both labs in order to test the reproducibility of the two preparation methods (chemical purification and no chemical purification). The results were in good agreement (+0.07‰ in Mainz, +0.03‰ in Münster), which confirms that skeletal tissue samples from which organic material has been removed do not need to be chemically purified further prior to TIMS Ca isotopic measurements (see Heuser and Eisenhauer (2008)).

4. RESULTS

The $\delta^{44/40}$Ca values measured in this study are listed in Table 1 (extant and fossil bones and teeth) and Table 2 (sediment samples). Calcium isotope data of the dinosaur specimens are also plotted in Fig. 1. $\delta^{44/40}$Ca values for all analyzed specimens range from $-1.86‰$ for a modern Nile monitor enamel up to $+1.08‰$ for a Late Jurassic *Brachiosaurus brancai* bone. The $\delta^{44/40}$Ca values of most fossil skeletal apatite samples lie between $-0.5‰$ and $+1.0‰$. The enamel $\delta^{44/40}$Ca of a *Tyrannosaurus rex* sample from the Hell Creek Formation is $-1.62‰$, which is significantly lower than all other fossil enamel $\delta^{44/40}$Ca values measured in this study. We do not have any indication that this value is an outlier, and the reason for this extreme enrichment in "light" Ca compared to that of the other fossil samples will be discussed later (see Section 5.2).

$\delta^{44/40}$Ca of modern skeletal tissues are lower on the whole compared to those of fossils. $\delta^{44/40}$Ca values of extant archosaur bones and teeth range from $-1.9‰$ (Nile monitor enamel) up to $-0.4‰$ (ostrich bone). In general, $\delta^{44/40}$Ca from fossil bone tends to be more positive than $\delta^{44/40}$Ca from fossil dentin while $\delta^{44/40}$Ca is the most negative in fossil enamel.

Only small differences ($<0.5‰$) in the mean Ca isotopic composition of the fossils exist between the respective localities investigated. Tendaguru bone and tooth (dentin and enamel) samples display the highest average $\delta^{44/40}$Ca values (bone: 0.63 ± 0.21; dentin 0.37 ± 0.19; enamel: 0.04 ± 0.25; errors are 1 SD) compared to those from the Morrison Formation which have, on average, the lowest values (bone: 0.19 ± 0.14; dentin: 0.14 ± 0.22; enamel: -0.36 ± 0.18). Due to the lack of data on $\delta^{44/40}$Ca in bone from the Hell Creek and Dinosaur Park Formations, a proper comparison with data from the other localities is not possible. However, the fact that only minimal Ca isotope differences – given the overall scatter in values – are observed between the various sample localities argues against any simple "location dependency" in $\delta^{44/40}$Ca values. Ergo, there is no apparent correlation with the age of the samples, differences in the geologic setting, nor the taphonomy of the sample sites.

Table 2
Ca isotope results of the analyzed sediment samples (weighted $\delta^{44/40}$Ca and weighted 2SE).

Sample	Lithology	Origin	$\delta^{44/40}$Ca (‰)	±2SE	*n*
Trossingen					
SED PL TR 7	Nodular marl	Marrow cavity filling	0.48	0.02	7
Morrison Fm.					
SED WHY 184	Sandstone	Bone bed matrix	0.03	0.04	3
SED WHY 197	Sandstone	Close to bone bed	0.37	0.03	3
Tendaguru Fm.					
SED DY TE 16	Carbonate	Marrow cavity filling	0.68	0.02	2
SED BR TE 2	Marly limestone	Marrow cavity filling	0.71	0.06	5

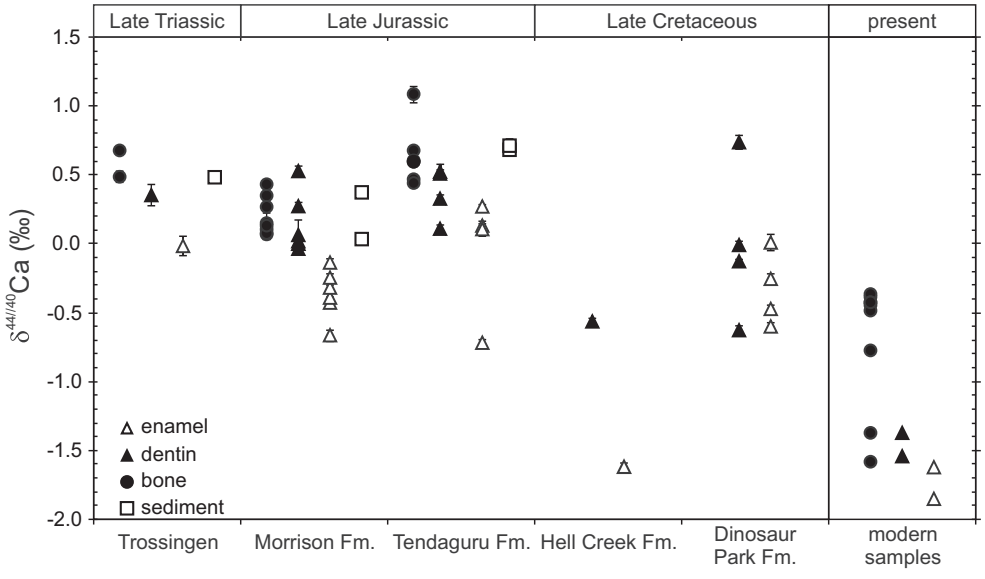

Fig. 1. Ca isotope ratios of enamel (open triangles), dentin (filled triangles) and bones (filled circles) of dinosaurs from five localities and samples from modern reptiles, expressed in terms of $\delta^{44/40}$Ca normalized to NIST SRM 915a. Error bars represent the weighted 2SE.

This result is at least intuitively to be expected given the small range in $\delta^{44/40}$Ca found in basement rocks (e.g. DePaolo, 2004), meaning that the initial $\delta^{44/40}$Ca is likely to have been similar at each site prior to being fractionated by local sedimentary and biological processes.

From a 4.5-cm-thick bone cortex cross section of an *Apatosaurus* long bone (AP MO 11) we analyzed samples from three different positions to investigate possible intra-bone variations of the Ca isotopic composition (Fig. 2). $\delta^{44/40}$Ca of these samples decrease from the rim (+0.26‰) to bone center (+0.14‰), and $\delta^{44/40}$Ca is slightly lower close to the marrow cavity (+0.10‰) and are all within or close to analytical uncertainty. The observed difference in $\delta^{44/40}$Ca between the outer part (rim) and the inner part (close to the marrow cavity) of the bone if substantiated by more precise measurement, would be consistent with the effects of bone remodeling.

During ontogeny, bone is remodeled: primary bone is decomposed and replaced by newly-formed secondary bone tissue of Haversian type, characterized by secondary osteons with small canals through which the blood vessels ramify. The remodeling in this specimen took place preferentially in the area around the marrow cavity – as shown by abundant secondary osteons – and results in lower $\delta^{44/40}$Ca values of the newly formed bone compared to those from the "old" bone (cf. Skulan and DePaolo, 1999).

Two enamel samples from a large *Camarasaurus* tooth (CA MO 11) exhibit $\delta^{44/40}$Ca of −0.25‰ and −0.39‰, respectively, while the dentin has a higher value of 0.07‰ (Table 1). Intra-tissue $\delta^{44/40}$Ca variability within both the *Apatosaurus* bone (difference: 0.16‰) and the *Camarasaurus* tooth (difference: 0.14‰) is thus similar in magnitude. These intra-tissue differences appear to have been generated by metabolic processes during ontogeny rather than the effects of diagenetic alteration which would tend to lead to scatter.

5. DISCUSSION

5.1. $\delta^{44/40}$Ca of dinosaurs compared to those of modern skeletal tissues

There are only a few datasets on Ca isotopes in skeletal tissues from modern vertebrates currently available (Skulan et al., 1997; Skulan and DePaolo, 1999; Clementz et al., 2003; Chu et al., 2006; Reynard et al., 2010; Reynard et al., 2011). If we compare our Ca isotope data from fossil skeletal tissues with those from recent bones (Skulan et al., 1997; DePaolo, 2004; Chu et al., 2006) – see Fig. 3 – it becomes obvious that all the fossil samples tend to have higher $\delta^{44/40}$Ca values. We note, however, that most of the literature $\delta^{44/40}$Ca values are from mammalian bones and only few data points exist from bones of birds or reptiles.

Our $\delta^{44/40}$Ca data from fossil and recent archosaur skeletal tissues are in good agreement with the previously-published data on bones from modern reptiles, extant birds and fossil dinosaurs presented in Skulan et al. (1997). Thus,

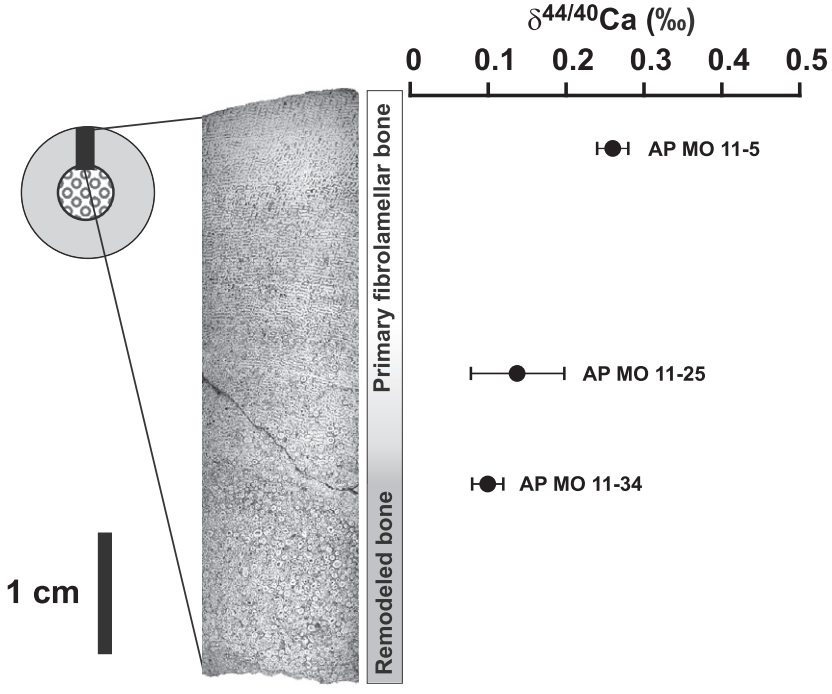

Fig. 2. $\delta^{44/40}$Ca variation within the bone cortex of an *Apatosaurus* long bone. The photograph shows the polished section of a drillcore across the bone cortex. The outer part still consists of primary fibrolamellar bone tissue, while the inner part towards the marrow cavity is completely remodeled, as indicated by abundant secondary osteons (structure consisting of concentric layers, or lamellae, of compact bone tissue which surround a central canal, containing the bone's nerve and blood vessels). Intra-bone $\delta^{44/40}$Ca differences are small ~0.16‰ and no significant difference between the two bone tissue types exists.

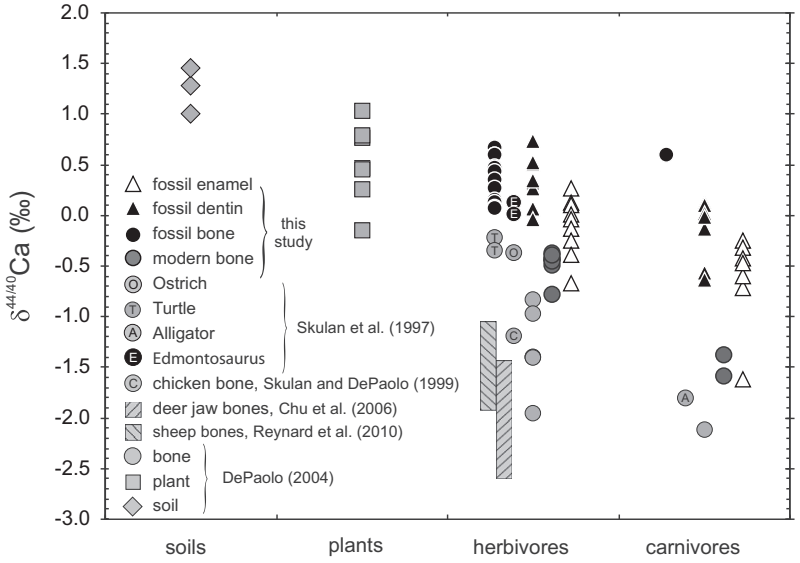

Fig. 3. Comparison of $\delta^{44/40}$Ca from this study (fossil bone, fossil enamel, fossil dentin, bones of modern reptiles and birds) with literature values for soils, plants and mammalian bones (DePaolo, 2004; Chu et al., 2006; Reynard et al., 2010) as well as for two dinosaur bones (Skulan et al., 1997). The data are plotted against the nominal trophic level. Our $\delta^{44/40}$Ca values for dinosaur bones are in general consistent with published values for two bones from the herbivorous dinosaur *Edmontosaurus* (Skulan et al., 1997). All dinosaur bone $\delta^{44/40}$Ca values appear to be higher than those of mammals from the same trophic level. This could be either due to different type of animal involved (reptiles versus mammals) or due to diagenetic overprinting the original $\delta^{44/40}$Ca signal (see text).

we are confident that we can also incorporate these data from the literature in making our comparison of Ca isotopes in skeletal tissues from extinct and extant vertebrates.

The combined dataset, consisting of our new data and those from previous work, reveals that the Ca isotopic composition of dinosaur skeletal tissues tends to be systematically enriched in "heavy" Ca compared to modern samples. For example, while $\delta^{44/40}$Ca of herbivorous mammal bone ranges from −2.6‰ up to −0.8‰, the values for herbivorous dinosaur bones fall between 0.1‰ and 1.1‰. Bone $\delta^{44/40}$Ca values for extant ostriches lie in between these two groups at about −0.5‰ to −0.4‰, while a corresponding value for a single chicken bone is −1.2‰.

Although the data set for carnivores is much smaller than that for herbivores, a similar systematic trend is observed – namely that $\delta^{44/40}$Ca for mammalian bone is lower than that for bone from modern reptiles and dinosaurs. Our interpretation is that these systematic differences between mammals, birds and dinosaurs probably have underlying physiological causes related to reproduction (viviparous versus oviparous) or their respective thermophysiology. A more extensive and focused Ca isotope study of modern fauna is likely to shed more light on this issue.

5.2. Trophic Level Effect (TLE)

Both datasets – fossil and extant – display no obvious discrete ranges of $\delta^{44/40}$Ca values for herbivores as compared to carnivores. This is in sharp contrast to that proposed by DePaolo (2004), whose data compilation at that time suggested a general Ca isotopic offset of around 1‰ between herbivores and carnivores.

The fact we do not see a convincing Ca isotopic difference between herbivores and carnivores as a whole does not a priori disprove the existence of such TLE offsets within a single, local food chain (Skulan et al., 1997; Clementz et al., 2003).

Indeed, it does seem intuitively reasonable that differences of around 1‰ may exist between herbivores and carnivore within a particular ecosystem or food chain, respectively. The problem appears to be lumping together all the data, which tends to obscure the relationships at a local level. Thus, broader Ca isotope comparisons such as across ecosystems, do not appear to be as straightforward as originally envisioned by DePaolo (2004).

Skulan and DePaolo (1999) and Clementz et al. (2003) have reported a decrease in bone $\delta^{44/40}$Ca with each additional trophic level. The existence of such a TLE within a fossil food chain would be a very strong argument against diagenetic overprinting of the original Ca isotopic signal. Conversely, the lack of a clear TLE in a fossil Ca isotope data set does not imply necessarily that the data have been compromised by diagenesis.

Firstly, the $\delta^{44/40}$Ca-trophic level relationship has so far only been established for bones of mammalian herbivores and carnivores (DePaolo, 2004). When combined with the more comprehensive data set of Chu et al. (2006) and Reynard et al. (2010), no convincing TLE between herbivores and carnivores as a whole exists, as the range in bone $\delta^{44/40}$Ca of herbivores now completely overlaps that found in carnivores (cf. Section 5.1 and Fig. 3).

Nevertheless, based upon the simple physiological model of Ca transport presented by Skulan and DePaolo (1999) some sort of TLE should exist for reptilian bones within a given, local food chain. Regrettably, it is not possible to reconstruct such effects in dinosaur ecosystems unambiguously from the fossil bones alone due to potential diagenetic overprinting.

Secondly, using the Skulan and DePaolo (1999) model, it can easily be shown that a TLE between herbivores and carnivores is only likely to exist if – and only if – the carnivore's diet involved digestion of mineralized tissue, in other words "bone" (Fig. 4); indeed, this was also suggested by Clementz et al. (2003). In the model of Skulan and DePaolo (1999), no Ca isotope fractionation takes places during bone decomposition and no Ca isotope fractionation exists between dietary calcium and soft tissue calcium. The latter has been confirmed in a study by Hirata et al. (2008). According to their model, significant Ca isotope fractionation within vertebrates can only occur during formation of mineralized tissue (bone), whereby the mineralized tissue becomes isotopically "light".

Such a model – depending basically on the formation of bone – can readily explain the presence of a TLE between plants (nominal trophic level 1) and herbivores (nominal trophic level 2). However, the TLE between sympatric herbivores and carnivores from the same ecosystem will depend on the intricacies of how, exactly, the carnivore consumes its prey. If a carnivore's diet only consists of herbivore soft tissue (i.e., meat) the carnivore's soft tissue will

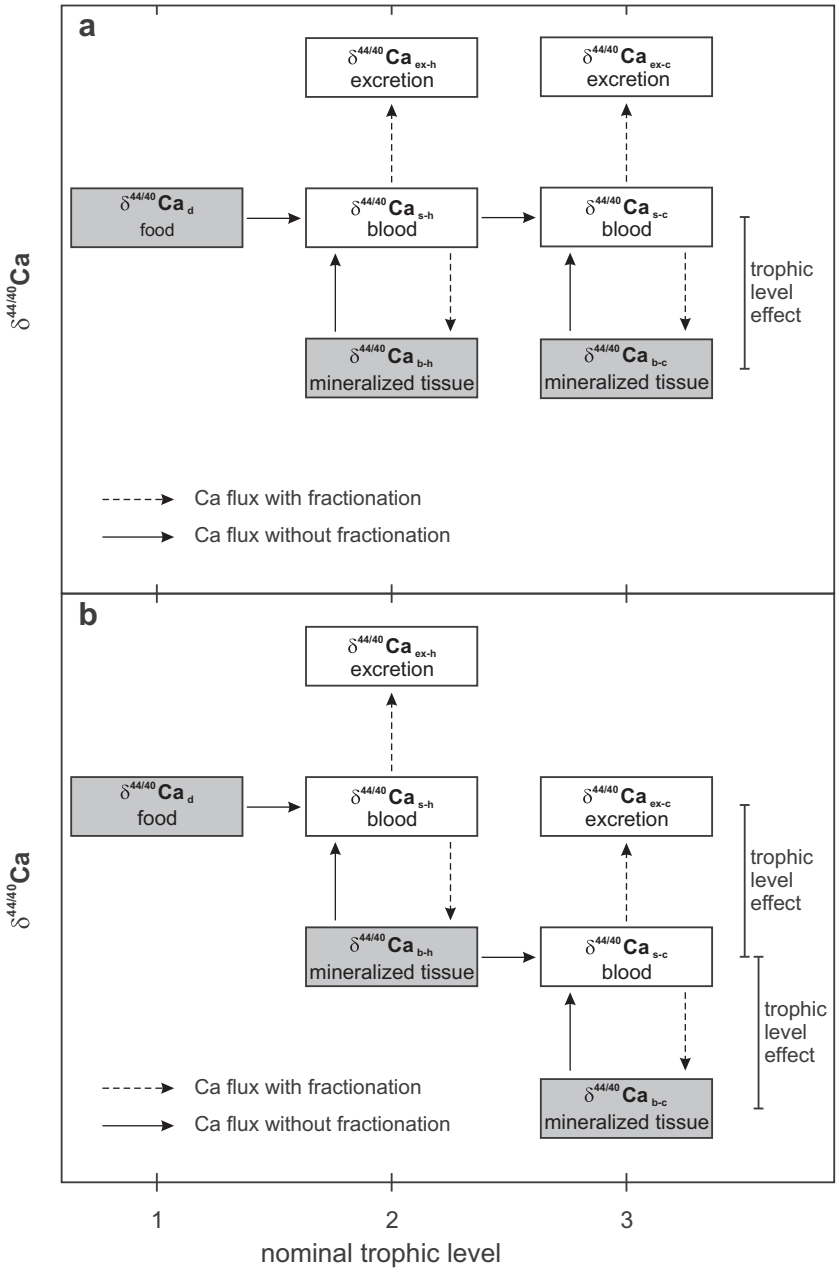

Fig. 4. Simple box model (after Skulan and DePaolo, 1999) illustrating that any Trophic Level Effect (TLE) between carnivores (nominal trophic level 3) and herbivores (nominal trophic level 2) will depend on the type of food that the carnivore ate. If the carnivores mainly devoured soft tissue, no TLE would then exist between herbivores and carnivores (Fig. 4a). If the carnivore additionally consumes mineralized tissue, the carnivore's Ca becomes isotopically "lighter" than that in herbivores, generating a TLE (Fig. 4b).

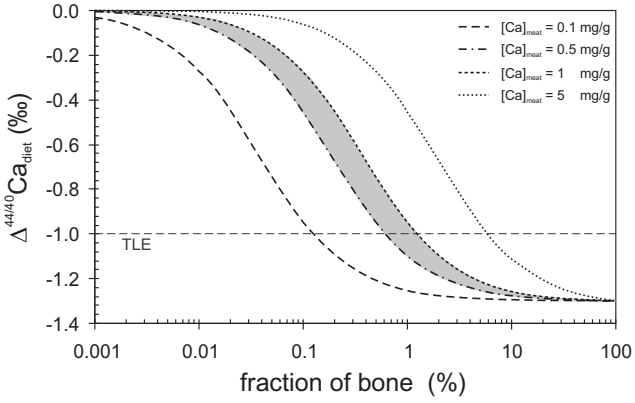

Fig. 5. Model showing how the $\delta^{44/40}$Ca of a carnivore's diet as a whole depends critically on the amount of ingested bone, as well as differing Ca concentrations in meat. Plotted is the $\Delta^{44/40}Ca_{diet}$ which is the difference of the Ca isotopic composition between a bone containing diet and a bone free diet. The meat is assumed to have Ca concentrations ranging from 0.5 to 1 mg/g Ca (grey shaded area). Due to the high Ca content in bone (~270 mg/g), incorporation of about 0.6–1.5% of mineralized tissue in bulk in the food will result in a Trophic Level Effect of about −1‰, as expected from Fig. 4b, which is significant.

inherit the same Ca isotopic signature as the herbivore soft tissue. Thus the mineralized tissues (i.e., bone) of carnivores (nominal trophic level 3) and their prey – the herbivores – would not differ (Fig. 4a). The situation will change dramatically if the carnivore's diet contains mineralized tissue of its prey as well as flesh – such as by consuming the prey whole – since the bone is such a large reservoir of "light" calcium (cf. Clementz et al., 2003). In such circumstances, a prominent TLE can be anticipated, with the predators having isotopically far lighter calcium in their bones than their prey (Fig. 4b).

It is quite straightforward to model the amount of bone needed to develop a marked TLE, where the bulk dietary $\delta^{44/40}$Ca is changed by −1‰. Assuming a difference of ~1.3‰ in $\delta^{44/40}$Ca between meat and bone (Skulan and DePaolo, 1999) along with a Ca concentration of 26.8 wt.% in fresh bone, we can calculate the $\delta^{44/40}$Ca in "food" following Chu et al. (2006) as:

$$\delta^{44/40}Ca_{diet} = \frac{([Ca]_{meat} \cdot \delta^{44/40}Ca_{meat} \cdot (1 - x_{bone})) + ([Ca]_{bone} \cdot (\delta^{44/40}Ca_{meat} - 1.3) \cdot x_{bone})}{([Ca]_{meat} \cdot (1 - x_{bone})) + ([Ca]_{bone} \cdot x_{bone})} \quad (1)$$

where x_{bone} is the fraction of bone in the food, $\delta^{44/40}Ca_{meat}$ is the Ca isotopic composition of meat (soft tissue) and $[Ca]_{bone}$ and $[Ca]_{meat}$ are the Ca concentrations of bone and meat, respectively.

Fig. 5 shows that the modeled Ca isotopic composition of the bulk diet depends on the fraction of bone ingested and the Ca concentration of soft tissue, here designated as $[Ca]_{meat}$. Due to the considerable differences in the Ca concentration and Ca isotopic composition between mineralized tissue and soft tissue, only small amounts of bone digestion are needed to dominate the calcium budget in the animal overall and to result in a TLE of about 1‰. For typical $[Ca]_{meat}$ concentrations, which are between 0.5 and 1 mg/g, only 0.6–1.5% bone by mass in the food are sufficient to create a dominant TLE due to bone ingestion.

The ingestion and digestion of mineralized tissue may be responsible for the "light" Ca isotopic composition of the *T. rex* enamel sample. Several authors (e.g. Chin et al., 1998) report that *T. rex* was able to crunch and ingest bones.

Therefore, we argue here that the $\delta^{44/40}$Ca data are likely to be the most unambiguous proxy in deciding whether the carnivores devoured some bone alongside the meaty parts of their prey or, alternatively, whether they were more discriminating eaters.

However, the $\delta^{44/40}$Ca values measured in bones from herbivorous and carnivorous dinosaurs indicate no clear TLE at all, as illustrated in Fig. 6a. Please note, however, that there is only one sample of carnivore bone and, therefore, the existence of a TLE cannot be fully ruled out. In contrast, the $\delta^{44/40}$Ca values of dentins (Fig. 6b) and $\delta^{44/40}$Ca values in tooth enamel (Fig. 6c) appear to show a small offset in carnivore teeth relative to herbivore teeth. We consider this offset to represent the first tentative evidence of a true TLE in dinosaur ecosystems. But the observed differences in enamel and dentin between herbivorous and carnivorous dinosaurs of about +0.2‰ are much smaller than the reported difference of +1‰ between modern herbivorous and carnivorous mammalian bones suggested by DePaolo (2004).

5.3. $\Delta_{dentin\text{-}enamel}$ as a possible monitor of diagenetic alteration

When comparing the $\delta^{44/40}$Ca values of enamel and dentin from the same teeth, dentin $\delta^{44/40}$Ca are more positive than those of the enamel in nearly every case – i.e., the dentin is enriched in "heavy" Ca (cf. Table 1 and Fig. 7). The difference between dentin and enamel ($\Delta_{dentin\text{–}enamel}$ =

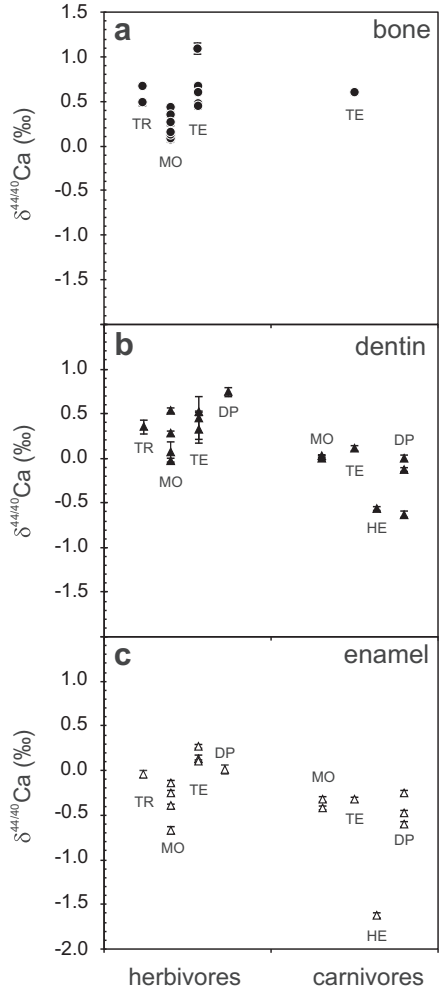

Fig. 6. Comparison of $\delta^{44/40}$Ca value between herbivores and carnivores: (a) $\delta^{44/40}$Ca in bones, (b) $\delta^{44/40}$Ca in dentin and (c) $\delta^{44/40}$Ca in enamel. The samples are sorted by locality and decreasing geological age: TR, Trossingen (Late Triassic); MO. Morrison Fm. (Late Jurassic); TE, Tendaguru Fm. (Late Jurassic); HE, Hell Creek Fm.; and DP, Dinosaur Park Fm. (both Late Cretaceous).

$\delta^{44/40}Ca_{dentin}$–$\delta^{44/40}Ca_{enamel}$) is about +0.4‰ for most of the teeth (11 of 16 samples) studied (Fig. 7); it also appears to be independent of the taphonomic setting, sample age or whether the dinosaurs were herbivores or carnivores.

By comparison, in modern reptiles $\Delta_{dentin-enamel}$ is also positive, and lies at about +0.3‰ (Fig. 7). Considering this observation, we suggest, tentatively, that a $\Delta_{dentin-enamel}$ offset of +0.3‰ to +0.4‰ might constitute a "biogenic signal" originating from differences in the biomineralization of the enamel and dentin in the ameloblasts (cells that deposit enamel) and odontoblasts (cells that deposit dentin), respectively. The exact fractionation mechanism remains to be established, though.

Tissues such as dentin and bone are more susceptible than enamel to diagenetic alteration (e.g. Ayliffe et al., 1994; Koch et al., 1997; Budd et al., 2000) due to their greater organic matter and proportionally lesser mineralization (cf. Pasteris et al., 2008). Hence, subsamples of fossil teeth with $\Delta_{dentin-enamel}$ of +0.3‰ to +0.4‰ are most easily interpreted in terms of an intrinsic "biogenic" signal rather than being a product of alteration. Moreover, it is extremely unlikely that diagenesis would so systematically shift the Ca isotopic composition of these two tooth tissues in every instance, and in the same direction. For this reason, we suggest that $\Delta_{dentin-enamel}$ – at least for reptile teeth – can be used as a convenient measure of overprinting of the Ca isotopic signal by diagenetic alteration. If the $\Delta_{dentin-enamel}$ values at a given fossil site are close to +0.3‰, it would mean that at least the enamel has probably undergone little diagenetic alteration of its Ca isotopic composition. A difference close to 0‰ would then indicate sample contamination during sampling – i.e., the enamel was contaminated by dentin from the same tooth or vice versa.

5.4. $\delta^{44/40}$Ca in fossil skeletal tissues compared to that in sediment

Comparison of $\delta^{44/40}$Ca in fossil skeletal tissues with those in surrounding sediments potentially provides a further, useful indicator of alteration of the calcium isotopic signature of the skeletal apatite. When compared, average $\delta^{44/40}$Ca of the sediments exhibit similar values as bones and dentin samples from the same locality; however it must be pointed out that only five sediment samples were analyzed in the present study (Table 2). The fact that $\delta^{44/40}$Ca are similar provides tentative support for some degree of Ca (isotope) exchange between sediment and skeletal tissue or, alternatively, addition of calcium to the skeletal tissue in the form of newly-formed calcium-bearing minerals. It also cannot be excluded that the Ca isotopic composition in the skeletal tissues somehow reflects the local Ca isotopic composition of the sediment which was transported somehow into the skeletal tissues during the lifetime of the dinosaur.

Unfortunately, very little is known about the susceptibility of Ca isotopes to diagenesis in geological materials from which to draw some guidelines. One important study is by Fantle and DePaolo (2007) who documented changes in the Ca isotopic composition of calcareous nanofossil oozes and chalks from a marine drill-core on the Ontong Java Plateau resulting from diagenetic carbonate recrystallization of carbonate with depth in the core. They calculated changes in the original calcium isotopic composition of biogenic carbonate resulting from recrystallization (dissolution and reprecipitation) as a function of depth using a numerical model. According to this model, the effects on $\delta^{44/40}$Ca can be quantified if the reaction rates, $\delta^{44/40}$Ca in pore fluid and solid, the fractionation factor (α) and the sedimentation rates through time are all known.

Based on their diagenetic model, Fantle and DePaolo (2007) suggested that negligible alterations in $\delta^{44/40}$Ca values (<0.15‰ or so) occurs during recrystallization of biogenic carbonates. Although their model is not directly applicable to diagenesis of phosphatic skeletal remains in continental sediments, it is safe to assume that recrystallization is only likely to have minor effects on bioapatite $\delta^{44/40}$Ca. This line of reasoning is supported, at least on

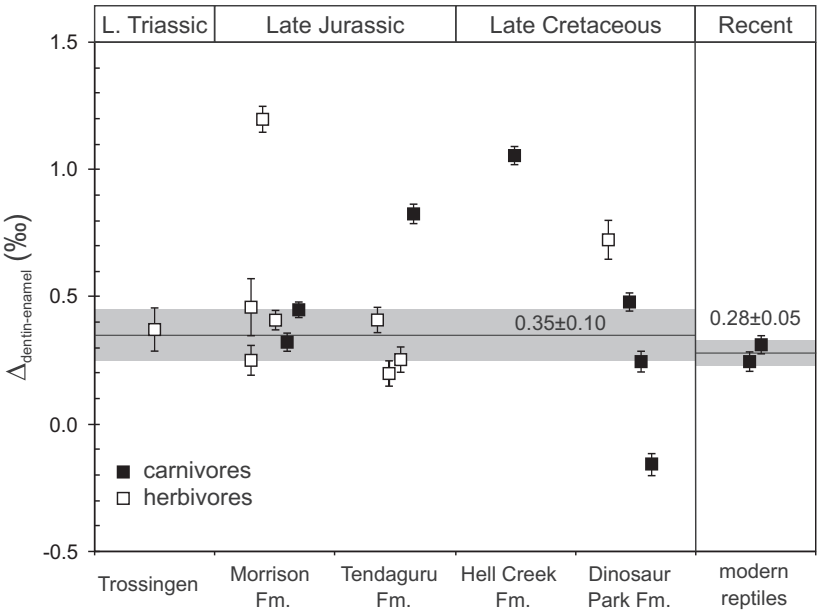

Fig. 7. The difference in Ca isotopic composition between dentin and enamel is denoted as $\Delta_{dentin-enamel}$ ($=\delta^{44/40}Ca_{dentin} - \delta^{44/40}Ca_{enamel}$). As can be seen, the average $\Delta_{dentin-enamel}$ offset of +0.35‰ for fossil teeth is close to that found in modern teeth (+0.28‰). We suggest that this offset is of biogenic origin and independent of diet. During diagenesis, such a Ca isotope offset between dentin and enamel is unlikely to be retained, since dentin is much more susceptible to diagenetic alteration than enamel. Therefore $\Delta_{dentin-enamel}$ is a potential monitor of diagenetic alteration of the Ca isotopic composition in fossil tooth specimens.

shorter time scales, by Reynard et al. (2011), who presented several lines of evidence that diagenetic alteration of bioapatite $\delta^{44/40}$Ca is negligible.

5.5. Estimating the influence of secondary precipitates

During early diagenesis, organic matter from skeletal tissues becomes degraded (e.g. Pfretzschner, 2004, 2006), changes porewater chemistry and makes way for newly formed minerals (either diagenetic apatite or other mineral phases) called "secondary precipitates". Since the combined volume of organic matter and bound water in bone (49 vol.%) and dentin (50 vol.%) is larger than in enamel (10 vol.%) (cf. Pasteris et al., 2008), changes in the $\delta^{44/40}$Ca due to secondary precipitates can be expected to be potentially greater for bone and dentin than for enamel. Using simple mass balance calculations we can estimate the effect of secondary precipitates on the $\delta^{44/40}$Ca in skeletal tissue.

In order to replace the non-mineralized components of bone and dentin completely, at most half of the volume of fresh bone or dentin can be filled by secondary precipitates. We assume these secondary precipitates to be a mixture of calcium-rich phases, which represents a "worst case" scenario, since apart from fluorapatite (e.g. Hubert et al., 1996; Kolodny et al., 1996; Elorza et al., 1999) and calcium carbonates, calcium-free secondary precipitates such as pyrite, pyrolusite, iron hydroxides, quartz and other minerals are also commonly formed in the pore space of fossil bones (e.g. Hubert et al., 1996; Pfretzschner 2001a,b; Pfretzschner and Tütken, 2011). In this simplified view, the final bone or dentin represents a roughly 1:1 mixture by volume of original skeletal tissue with secondary precipitates. If the secondary minerals are also calcium rich, then the mass balance of Ca isotopes can be approximated as:

$$\delta^{44/40}Ca_{measured} = 1/2(\delta^{44/40}Ca_{primary} + \delta^{44/40}Ca_{secondary}). \quad (2)$$

Depending on the source(s) of calcium for the secondary precipitates, we consider two scenarios: (1) sedimentary and secondary calcium originate from the same source (e.g. pore fluid), or (2) calcium from the sediment itself is the sole source of the secondary calcium. In both scenarios, the measured sediment Ca isotopic composition can be used to calculate back the primary Ca isotopic composition originally in the bone and dentin.

In the first scenario, calcium originating from a single source (e.g. pore fluids) is fractionated to a different extent in sediment and in secondary precipitates. This results in a shift in Ca isotopic composition in the secondary precipitates versus that in the sediment:

$$\delta^{44/40}Ca_{sediment} = \delta^{44/40}Ca_{source} + \Delta_{sed} \quad (3)$$

$$\delta^{44/40}Ca_{secondary} = \delta^{44/40}Ca_{source} + \Delta_{sec} \quad (4)$$

where Δ_{sed} and Δ_{sec} represent the Ca isotope fractionation occurring during precipitation in the sediment and in secondary precipitates, respectively. Combining Eqs. (3) and (4) leads to:

$$\delta^{44/40}Ca_{secondary} = \delta^{44/40}Ca_{sediment} - \Delta_{sed} + \Delta_{sec}. \quad (5)$$

The average analyzed $CaCO_3$ is 5.6 wt.% in our bone and dentin samples. Assuming this $CaCO_3$ resides only in secondary precipitates, which make up 50 wt.% of the total

sample by mass, it follows that about 11 wt.% of the secondary precipitates are Ca carbonates and the remaining 89 wt.% must be Ca phosphates. Thus, in this instance the Ca isotope fractionation of any secondary precipitates would be dominated by Ca phosphate precipitation ($\Delta_{sec} \approx \Delta_{phosphate}$).

The magnitude of Ca isotope fractionation during precipitation of Ca phosphates from aqueous solutions ($\Delta_{phosphate}$) is not well known, however. Schmitt et al. (2003) have measured the Ca isotopic compositions of marine phosphorites (seawater precipitates), and reported that the phosphates are isotopically lighter than seawater by about 1‰ or so. Therefore, it seems reasonable to assume here in the absence of further data that $\Delta_{phosphate}$ is around −1‰ in our case.

The sediment analyzed from the Morrison Formation is a fine-grained sandstone with low carbonate (and calcium) content. In this instance, precipitation of secondary calcite cannot have played a major role, and any Ca isotope fractionation was presumably minimal. Calcium fractionation factors for other calcium-bearing minerals present in the sediment are unknown at the present time. However, we can reasonably assume, for the time being, that the Ca isotope fractionation in the sediment and in the secondary precipitates are nearly the same ($\Delta_{sed} = \Delta_{sec}$) and thus $\delta^{44/40}Ca_{secondary} = \delta^{44/40}Ca_{sediment}$.

By contrast, the sediment samples investigated from the Tendaguru Formation and Trossingen are mainly composed of marly limestones and marls. So here we are forced to consider Ca isotope fractionation during the formation of calcite. Marriott et al. (2004) found a fractionation of −0.77‰ for inorganically precipitated calcite at 25 °C, which we consider appropriate to use for Δ_{sed} in lieu of more detailed information. Using Eq. (5) and with $\Delta_{phosphate}$ around −1‰ (see above), $\delta^{44/40}Ca_{secondary}$ for the cases of the Tendaguru Formation and Trossingen are calculated to be 0.46‰ and 0.25‰, respectively.

If sedimentary calcium is considered to be the source of the secondary precipitates (second scenario above), then $\delta^{44/40}Ca_{secondary}$ and $\delta^{44/40}Ca_{sediment}$ are interconnected via:

$$\delta^{44/40}Ca_{secondary} = \delta^{44/40}Ca_{sediment} + \Delta_{phosphate}. \quad (6)$$

In toto, re-arranging Eq. (2) and inserting either Eqs. (5), (6), we obtain the following equations for estimates of $\delta^{44/40}Ca_{primary}$ values:

$$\text{scenario 1}: \quad \delta^{44/40}Ca_{primary} = 2 \cdot \delta^{44/40}Ca_{measured} - \delta^{44/40}Ca_{sediment} + \Delta_{sed} - \Delta_{phosphate} \quad (7)$$

$$\text{scenario 2}: \quad \delta^{44/40}Ca_{primary} = 2 \cdot \delta^{44/40}Ca_{measured} - \delta^{44/40}Ca_{sediment} - \Delta_{phosphate}. \quad (8)$$

The calculated effects are presented in Fig. 8, expressed in terms of shifts in $\delta^{44/40}Ca_{primary}$ values from $\delta^{44/40}Ca_{measured}$. In general, $\delta^{44/40}Ca_{measured}$ values are lower than corresponding $\delta^{44/40}Ca_{sediment}$, while $\delta^{44/40}Ca_{primary}$ are shifted towards "heavier", more positive values for this reason. In some cases, though, the opposite is true and $\delta^{44/40}Ca_{measured}$ is higher than $\delta^{44/40}Ca_{sediment}$ at the sample

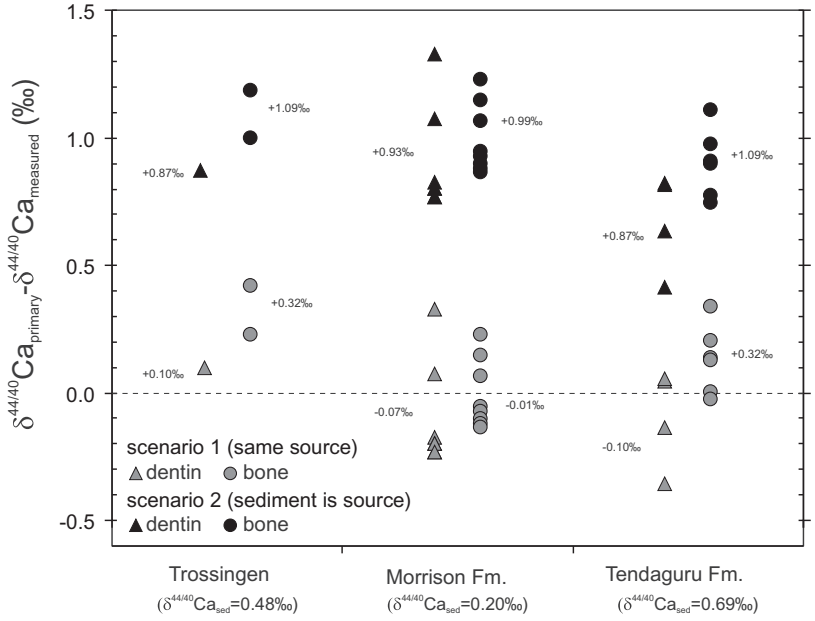

Fig. 8. Differences between calculated primary $\delta^{44/40}Ca$ values and those measured in bones (circles) and dentin (triangles) predicted to occur during formation of calcium-bearing secondary precipitates. Two scenarios are considered (see text for details). Numbers indicate the average changes of $\delta^{44/40}Ca_{primary}$ compared to $\delta^{44/40}Ca_{measured}$ for the different scenarios and skeletal tissues. In scenario I, sedimentary calcium and that of the secondary precipitates are from the same source, resulting in slightly lower $\delta^{44/40}Ca$ being observed. In scenario II, the sediment is source of the calcium in the secondary precipitates, causing a shifted to higher $\delta^{44/40}Ca$ values. However, such a shift is improbable, since $\delta^{44/40}Ca$ of bones from modern herbivorous mammals and birds all have $\delta^{44/40}Ca$ <0‰ while those from modern carnivorous mammals and reptiles are less than −1.2‰ (cf. Fig. 3).

locality. As a consequence, the span of minimum to maximum values inferred for $\delta^{44/40}Ca_{primary}$ is double that of our actual measured data and is independent of the sample locality. The average shift in $\delta^{44/40}Ca_{primary}$ relative to $\delta^{44/40}Ca_{measured}$ is not only dependent on the scenario chosen, but also on the type of tissue involved, the sample location (see Fig. 8) and on the chosen fractionation factors (Δ_{sed} and Δ_{sec}).

The fact that the dataset of calculated $\delta^{44/40}Ca_{primary}$ exhibits more scatter than the raw, measured data in Fig. 8 is a strong indication that either our correction for diagenesis is hopelessly inadequate, or else the measured dataset requires far less correction than implied in our "worse case" scenario. In the second scenario, in which sediment is the source of secondary calcium, the inferred $\delta^{44/40}Ca_{primary}$ values are high and quite unlike those found in modern bones. Furthermore, since the herbivores derive their calcium from plant matter, the plants would necessarily require extremely high $\delta^{44/40}Ca$ of around +2.5‰ as well. However, the highest $\delta^{44/40}Ca$ reported in the literature for plants thus far are lower at around +1.5‰ (Holmden and Bélanger, 2010). For both of these reasons, we consider the second scenario estimates for $\delta^{44/40}Ca_{primary}$ to be quite unrealistic and inaccurate. Only in the case of the Morrison Formation samples do the applied corrections make some degree of sense, assuming the Ca source for secondary precipitates and sediment are the same (using the first scenario), resulting in slightly lower primary $\delta^{44/40}Ca$ values.

Overall, our modeling suggests that the original Ca isotopic composition of the dinosaur materials was not significantly changed by diagenesis/fossilization, but this cannot be stated with 100-percent certainty. Nevertheless, the fact that the overall scatter in the dataset is doubled by applying a rudimentary correction for secondary precipitates does suggest that the $\delta^{44/40}Ca$ values measured today (and not the corrected values) lie close to those originally present in the samples.

6. CONCLUSIONS

In order to interpret $\delta^{44/40}Ca$ values in fossil bones and teeth in terms of reconstructing the paleobiology of extinct vertebrates and their ecosystems, the $\delta^{44/40}Ca$ signal must be shown to be primary beyond reasonable doubt and not changed post-mortem. In the present study, we have attempted to assess the degree to which $\delta^{44/40}Ca$ in dinosaur fossils is "primary," as well as which skeletal parts – bone, dentin or enamel – are most likely to have retained useful Ca isotope information.

First, there is no striking evidence that the $\delta^{44/40}Ca$ values of the fossils studied were seriously overprinted by diagenesis after death. Rather, we found some strong indications against significant diagenetic alteration. The most convincing is the apparent preservation of systematic $\delta^{44/40}Ca$ differences of around +0.3‰ to +0.4‰ between dentin and enamel from the same tooth. Such an offset is found in extant reptiles, and its observation in dinosaur teeth would appear most improbable unless the $\delta^{44/40}Ca$ were primary.

Similarly, we have tried to assess the impact of secondary precipitates on the original Ca isotopic composition of bone and dentin, based upon $\delta^{44/40}Ca$ of sources and simple mass balance. Secondary precipitates are concluded, overall, to have had a negligible effect on $\delta^{44/40}Ca$. While secondary precipitates have the potential for producing shifts, this would require that primary $\delta^{44/40}Ca$ in fossil bones and teeth were higher than $\delta^{44/40}Ca$ measured, and higher than $\delta^{44/40}Ca$ found in modern reptile skeletal matter, which appears unlikely.

Since we can never entirely rule out diagenetic influence on the calcium isotopic composition, we strongly recommend using $\delta^{44/40}Ca$ values from enamel rather than from either dentin or bone, as has already been recommended in the case of other isotope systems (e.g. Wang and Cerling, 1994; Koch et al., 1997; Budd et al., 2000). This is simply because enamel contains more original mineralized tissue (90 vol.-%), and so is proportionally less affected by any secondary precipitates formed. Calcium isotope data from dentin or bone alone should probably be treated with extreme caution in reconstructing paleo-food chains and paleo-diet of extinct organisms or in estimating paleo-environmental conditions.

Our $\delta^{44/40}Ca$ data for tooth enamel from dinosaurs, along with those for bones and teeth of modern reptiles and birds, are suggestive of – but do not overwhelmingly support – the presence of a calcium isotope Trophic Level Effect for these animal groups, which has previously only been suggested for mammals. Nevertheless, analysis of enamel from a *T. rex* specimen would support a Trophic Level Effect in dinosaurs since it is the most isotopically "light" calcium found thus far in fossil samples; it also suggests that *T. rex* consumed some bone along with the flesh of its prey. Nevertheless, this result should be considered tentative, since the "light" calcium in *T. rex* might be due to a locality effect or the taphonomic setting, for example; further Ca isotope data on *T. rex* will hopefully settle this issue.

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