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Article

Anomalous ^{13}C enrichment in Mesozoic vertebrate enamel reflects environmental conditions in a "vanished world" and not a unique dietary physiology

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Abstract.—Biogeochemical analyses of organisms' tissues provide direct proxies for diets, behaviors, and environmental interactions that have proven invaluable for studies of extant and extinct species. Applying these to Cretaceous ecosystems has at times produced anomalous results, however, as dinosaurs preserve unusually positive stable carbon isotope compositions relative to extant C_3-feeding vertebrates. This has been hypothesized to be a unique property of dinosaur dietary physiology, with potential significance for our interpretations of their paleobiology. We test that hypothesis through multi-taxic stable carbon isotope analyses of a spatiotemporally constrained locality in the Late Cretaceous of Canada, and compare the results to a modern near-analogue environment in Louisiana. The stable carbon isotope anomaly is present in all sampled fossil vertebrates, dinosaur or not. This suggests another more widespread factor is responsible. Examinations of diagenetic effects suggest that, where present, they are insufficient to explain the isotope anomaly. The isotope anomaly is therefore not primarily the result of a unique dietary physiology of dinosaurs, but rather a mix of factors impacting all taxa, such as environmental and/or source-diet differences. Our study underscores the importance of multi-taxic samples from spatiotemporally constrained localities in testing hypotheses of extinct organisms and ecosystems, and in the use of modern data to "ground truth" when evaluating analogue versus non-analogue conditions in greenhouse paleoecosystems.

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Introduction

Stable isotopic analyses have been used to test a wide range of ecological questions pertaining to modern animal groups, and closely related or ecologically similar fossil lineages throughout the Cenozoic (Janis et al. 2002; Koch 2007; Angst et al. 2014; Whiting et al. 2016). In recent years, these methods have been more frequently applied to more ancient ecosystems, such as those from the Mesozoic. Applications of these methods are often hampered by: (1) the potential for diagenetic overprinting; (2) the lack of close living relatives and resulting potential for unique dietary physiologies and associated trophic enrichment factors (TEFs; Δ, or $\Delta = \delta_{tissue} - \delta_{diet}$; also referred to as a vital effect, trophic discrimination factor, tissue-diet fractionation, etc.) (Stanton Thomas and Carlson 2004; Tütken 2011; Montanari et al. 2013; Amiot et al. 2015). In addition, coastal floodplains, a habitat type closely associated with deposits rich in

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terrestrial and freshwater vertebrate fossils during much of the Mesozoic, have considerable terrestrial–aquatic resource intermixing that may hamper attempts to reconstruct ecology from isotopic data in the absence of additional contextual data (Cullen et al. 2019).

Despite these challenges, numerous studies have applied stable isotope methods to Mesozoic dinosaurs, analyzing bioapatite samples from a relatively wide range of taxa (Straight et al. 2004; Fricke et al. 2008; Tütken 2011; Amiot et al. 2015). Independent studies have noted that stable carbon isotope compositions of dinosaur bioapatite ($\delta^{13}C_{bioapatite}$) are anomalously positive (~ −8‰ to −2‰) when compared with expected values for a large terrestrial vertebrate (e.g., a large herbivorous mammal; ~ −18‰ to −8‰) feeding primarily on terrestrial C_3 plants (Stanton Thomas and Carlson 2004; Fricke et al. 2008). These compositions have been found in material from multiple dinosaur clades from sites on multiple continents and time periods (Fricke et al. 2008; Amiot et al. 2015). This inevitably leads to the question of whether this positive "carbon isotope anomaly," hereafter abbreviated as CIA, is the result of: (1) inherent differences in dinosaur dietary physiology relative to extant vertebrates; (2) diagenetic overprinting in the $\delta^{13}C$ measured from these fossils; (3) differences in the mean and range of $\delta^{13}C$ composition of coastal floodplain plants in the Mesozoic relative to modern coastal floodplain systems; (4) other nonanalogous features of the broader environment in the Mesozoic relative to modern systems; or (4) a mixture of some or all of these factors.

With respect to the first of those hypotheses, one must consider the relationship between dietary physiology and TEFs. In modern systems, the TEF between $\delta^{13}C_{diet}$ and $\delta^{13}C_{bioapatite}$ will vary depending on the dietary physiology of the organism (influenced by its phylogenetic history, source diet, and trophic level) and can be experimentally determined via controlled feeding experiments and comparisons from multi-tissue sampling in extant taxa (Passey et al. 2005; Koch 2007; Fig. 1A). For extinct organisms, one option for reconstructing $\delta^{13}C_{diet}$ from measured $\delta^{13}C_{bioapatite}$ is the application of TEFs from extant relatives.

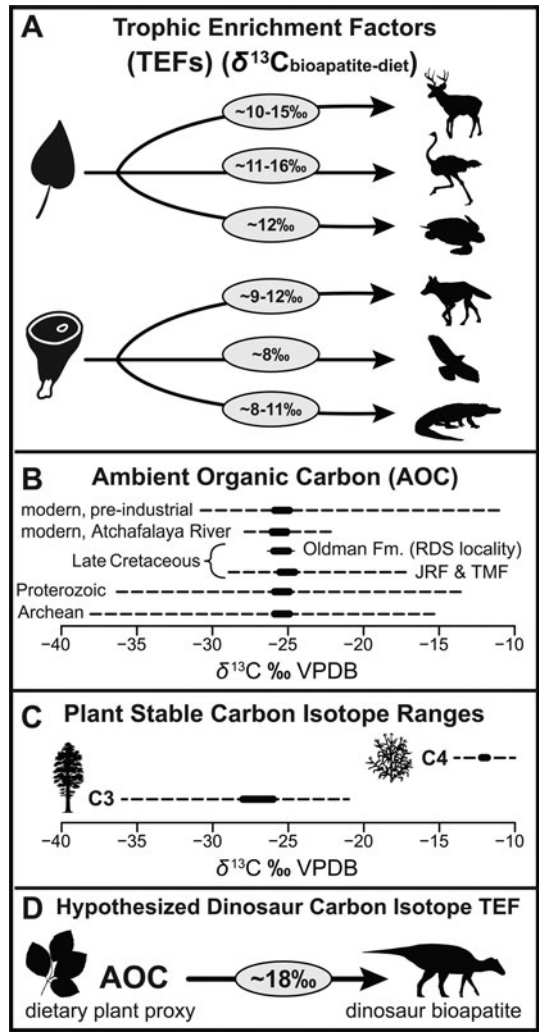

FIGURE 1. Summary of stable isotopic ranges from environmental and dietary sources and associated trophic enrichment factors (TEFs) in consumers. A, $\delta^{13}C_{bioapatite\text{-}diet}$ TEFs from extant herbivores (mammal, *Odocoileus*; bird, *Struthio*; reptile, *Chelonia*) and carnivores (mammal, *Canis*; bird, "raptor"; reptile, *Alligator*) reported in per mil (‰). B, Comparison of ambient organic $\delta^{13}C$ (AOC) ranges of modern and ancient systems. Late Cretaceous examples are subdivided into ranges of AOC measured from the "Rainy Day Site" (RDS) in the Oldman Formation of Alberta (site examined in this study) and those from multiple localities in the Judith River Formation (JRF) and Two Medicine Formation (TMF) of Montana (as reported in Fricke et al. 2008). Also noted are AOC ranges recorded throughout the Proterozoic and Archean (before the evolution of plants; as reported in Garcia et al. 2021). C, Approximate $\delta^{13}C$ ranges of C_3 and C_4 plants. D, Previously hypothesized high-magnitude TEF of dinosaurs, calculated as the difference between measured dinosaur $\delta^{13}C_{bioapatite}$ and $\delta^{13}C_{AOC}$ (assumed to be a proxy for $\delta^{13}C$ of terrestrial plants consumed by these animals). TEF sources, AOC values, and other data provided in Supplementary Table S2.

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Alternatively, one can identify another measure to use as a proxy for $\delta^{13}C_{diet}$, and then use the difference between that proxy and measured $\delta^{13}C_{bioapatite}$ compositions to estimate a TEF for the extinct species, independent of data from their extant relatives. To estimate a TEF for dinosaurs, previous authors (e.g., Stanton Thomas and Carlson 2004; Fricke et al. 2008) have used the average value of ambient organic $\delta^{13}C$ (AOC) measured from Cretaceous sediments (~−26‰ to −25‰) (Fig. 1B) as a proxy for terrestrial C_3 plants (Fig. 1C) forming herbivore $\delta^{13}C_{diet}$, and the difference between this value and measured dinosaur $\delta^{13}C_{bioapatite}$ to calculate a TEF of ~18‰. This estimated dinosaur TEF value represents a substantially higher offset between assumed diet and $\delta^{13}C_{bioapatite}$ than experimentally determined TEFs for most extant vertebrates, including those from the closest living relatives of dinosaurs (birds or crocodilians) (Stanton Thomas and Carlson 2004; Fricke et al. 2008; Montanari et al. 2013; Amiot et al. 2015; Fig. 1A,D), with previous authors (Stanton Thomas and Carlson 2004; Fricke et al. 2008) hypothesizing that a unique dinosaurian dietary physiology is responsible for such high-magnitude TEF values and, by association, the positive CIA present in dinosaur $\delta^{13}C_{bioapatite}$.

Alternate hypotheses for the CIA include a combination of non-analogue environmental factors influencing the $\delta^{13}C$ of dietary plants, alongside potential differences in plant community composition (with resulting differences in average $\delta^{13}C$), resulting in $\delta^{13}C_{bioapatite}$ compositions of dinosaur tooth enamel that are anomalously positive relative to extant terrestrial herbivores feeding on C_3 plants (Stanton Thomas and Carlson 2004). Isotopic resetting due to diagenetic alteration has also been suggested to explain the CIA in dinosaur $\delta^{13}C_{bioapatite}$, especially in bone and dentine, although multiple stable isotope studies of dinosaurs (and other fossil taxa) have concluded that primary isotopic signals can be preserved, along with ecological/environmental proxy data (Stanton Thomas and Carlson 2004; Fricke et al. 2008).

Understanding the source of the CIA is paramount for in-depth and meaningful stable isotope investigations of Mesozoic paleocommunity ecology. A priori or experimentally obtained TEFs do not exist for extinct non-avian dinosaurs. Therefore, we test the competing hypotheses for the CIA through analysis of an assemblage of dinosaurs and co-occurring non-dinosaur vertebrate taxa (including those with close living relatives with known TEFs), all collected from the same site, and compare them with similar data from a modern near-analogue ecosystem. Vertebrate microfossil bonebed sites are ideal for this study, because they represent spatially constrained wetland/river deposits with minimal time averaging, have shared fossilization and diagenetic processes, and contain abundant and diverse fossil samples representative of a local paleocommunity (Cullen and Evans 2016). We analyzed $\delta^{13}C_{bioapatite}$ compositions of a range of vertebrate taxa, while also measuring local AOC and performing multiple tests to assess for potential diagenetic alteration, in order to test the previously proposed hypothesis that the CIA in $\delta^{13}C_{bioapatite}$ corresponds to a distinct TEF and physiology in non-avian dinosaurs. We find that the CIA is not unique to non-avian dinosaurs, but is present in the $\delta^{13}C_{bioapatite}$ compositions of a phylogenetically diverse suite of taxa with living and physiologically similar representatives (such as mammals, fish, and crocodilians) (Stanton Thomas and Carlson 2004; Chinsamy and Hurum 2006; Amiot et al. 2007; Köhler et al. 2012; Brito et al. 2017). We also find that the CIA is not present in any vertebrate taxa in the near-analogue modern ecosystem, and our preservational tests of the fossil samples determine that diagenetic overprinting, if present, is not the primary driver of the CIA. Thus, we find that the CIA does not result from a unique dietary physiology of dinosaurs, as previously hypothesized. It may instead represent a combination of changes in the carbon isotope baseline of this system, as well as related differences in dietary sources relative to extant systems, reflecting non-analogue conditions that existed in greenhouse conditions but that are not seen in modern icehouse systems.

Material and Methods

Geological Setting and Paleoenvironmental Conditions.—Fossil material analyzed in this study was sampled from the "Rainy Day Site" (or RDS), a vertebrate microfossil bonebed in the Campanian-aged (78–75.5 Ma) uppermost

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Oldman Formation, a part of the Belly River Group, and geographically located in the Milk River/Manyberries region of southeastern Alberta, adjacent to the border with Montana (Peng et al. 2001; Brinkman et al. 2004; Eberth 2005; Arbour and Evans 2017). The Oldman Formation is subdivided into three informal units, with the lower and upper preserving wetter, more coastally influenced environments, and the middle ("Comrey sandstone") preserving a more inland and seasonally drier environment (Brinkman et al. 2004; Cullen and Evans 2016). The geology of the sampled RDS locality (including a stratigraphic section) is provided in a prior study of the paleoecology of the site by Cullen et al. (2020). In brief, the sedimentology of RDS consists of a mix of sand, silt, and dark mudstone; abundant bivalve mollusk shells; and a series of sublayers spanning approximately 160 cm. It is situated stratigraphically about 1 m above the top of a set of twin bentonite layers and approximately 44 m above the top of the Comrey sandstone (denoting the contact between the informal upper and middle units of the Oldman Formation). This distance is based on the measured distance between the lower twin bentonite and the Canal Creek bentonite as exposed beneath RDS (see measured stratigraphic section in Cullen et al. 2020: fig. 1D) and the distance between the Canal Creek bentonite and the top of the Comrey sandstone as exposed at the nearby McPheeter's Bonebed site (Chiba et al. 2015). The interval containing the RDS locality is consistent with the more organic-rich, warm subtropical, relatively humid and wet, seasonally flooded coastal plain deposits of the uppermost Oldman Formation (Eberth and Hamblin 1993; Eberth 2005, 2015; Chiba et al. 2015; Arbour and Evans 2017; Cullen et al. 2020), and lacks the caliche nodules (among other features) that characterize the more inland and seasonally arid environments of the middle Oldman Formation (Mack and Jerzykiewicz 1989; Chiba et al. 2015; Zelenitsky et al. 2016). The upper Oldman Formation preserved in the Milk River/Manyberries region is equivalent to the middle Coal Ridge Member of the Judith River Formation in northern Montana (Freedman Fowler and Horner 2015; Arbour and Evans 2017), while also being time-equivalent to the lower Dinosaur Park Formation as exposed further north in Dinosaur Provincial Park (Eberth and Hamblin 1993; Cullen and Evans 2016).

The site was originally sampled in 1993 and 1996, and the assemblage first described by Peng et al. (2001), with further depositional, taphonomic, and paleoecological research performed on RDS and associated microfossil bonebed sites in the Belly River Group by Brinkman et al. (2004), Cullen and Evans (2016), and Cullen et al. (2020, 2022). For a discussion of the formational processes of vertebrate microfossil bonebeds, see Rogers and Brady (2010) and Rogers et al. (2017). The material sampled for isotopic analysis was primarily collected from the lowest sublayers of the bonebed in 2013, consistent with the earlier sampling of RDS (D. Brinkman personal communication). The sampled material was screen-washed with water at the Royal Tyrrell Museum of Palaeontology (RTMP) in Drumheller, Alberta, Canada, and sent to the Royal Ontario Museum (ROM) for sorting and identification by the authors.

Sample Selection from Cretaceous and Modern Localities.—Fossil specimens from RDS for stable carbon isotope analysis were selected from both the 2013 sampling ($N = 28$) and the original 1993/1996 sampling ($N = 18$). A taxonomically broad sample was used to test the hypotheses relating to the CIA, consisting of hadrosaurid tooth enamel (dinosaur, $N = 7$), tyrannosaurid tooth enamel (dinosaur, $N = 7$), *Saurornitholestes* tooth enamel (dinosaur, $N = 6$), multituberculate tooth enamel (mammal, $N = 3$), *Leidyosuchus* tooth enamel (crocodilian, $N = 6$), low and bulbous non-*Leidyosuchus* alligatoroid tooth enamel (crocodilian, $N = 6$), lepisosteid scale ganoine (fish, $N = 9$), and bivalve shell carbonate (bivalve, $N = 2$). Specimens used in this study from the 1993/1996 sampling were identified in Peng et al. (2001), with those identifications, as well as later identifications from the 2013 sampling confirmed by the lead author of this study (T.M.C.), as well as by D. Brinkman and D. Larson (for a subset of the specimens), based on preserved diagnostic characters (for details on individual taxon ID characters, see

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Peng et al. 2001; Brinkman et al. 2004; Cullen et al. 2016). The distinction between *Leidyosuchus* and "alligatoroid" taxon categories are kept here to maintain consistency with other studies of these assemblages. Representative examples of specimens from each of the fossil taxa included in these analyses are provided in Supplementary Figure S1.

These fossil samples were compared with a multi-taxic sample collected from a modern coastal floodplain in Louisiana: the Atchafalaya River Basin. This extant sample is ecologically, physiologically, and phylogenetically diverse, and includes representatives of taxa that are functionally/ecologically analogous (and phylogenetically related) to members of the fossil RDS assemblage (e.g., crocodilians, lepisosteid fish, small mammals). Originally sampled and analyzed in Cullen et al. (2019), the taxa used for modern comparison include terrestrial herbivorous mammals (*Odocoileus*), aquatic herbivorous mammals (*Myocastor*), omnivorous terrestrial metatherian and placental mammals (*Didelphis* and *Procyon*, respectively), faunivorous terrestrial mammals (*Canis*), semi-aquatic faunivorous crocodilians (*Alligator*), and aquatic faunivorous lepisosteid fish (*Lepisosteus* and *Atractosteus*).

Geochemical Analyses.—All isotopic analyses were performed in the Laboratory for Stable Isotope Science (LSIS) at the University of Western Ontario. The primary analysis dataset was prepared and analyzed using laser-ablation gas chromatography–isotope ratio mass spectrometry, following the methods outlined in Cullen et al. (2019), itself based on the methods of Cerling and Sharp (1996) and Larson and Longstaffe (2007). Analytical method details, including pretreatment specifics, calibration data, and notational information, are reported in Supplementary Methods S1, with primary isotopic data reported in Supplementary Tables S1 and S5.

A secondary subset of bioapatite structural carbonate carbon and oxygen isotope compositions were determined at LSIS to test for potential diagenetic overprinting by secondary carbonates. This involved powdering of previously analyzed teeth to perform comparisons of pretreated versus untreated samples, with parallel comparisons to Fourier transform infrared (FTIR) spectroscopy of each sample, following the approach of Webb et al. (2014). Stable carbon isotope compositions, crystallinity indices (CIs), and carbonate/phosphate ratios (CO_3/PO_4, shortened as C/P hereafter) of this subset were compared to assess the impact of diagenetic alteration, recrystallization, and/or secondary carbonate deposition in pretreated and untreated samples. See Supplementary Methods S1 for a detailed description of analytical procedures, and Supplementary Table S3 for tabulated results for each specimen.

In addition to these measurements, AOC was also determined from the RDS locality, based on samples from sediments collected alongside the RDS vertebrate fossil materials. These data are recorded in Supplementary Table S3, and presented in Figure 1B, alongside literature values of global modern preindustrial AOC (Stanton Thomas and Carlson 2004; Garcia et al. 2021), AOC ranges from the Atchafalaya River in Louisiana (Rosenheim et al. 2013), AOC ranges from other nearby Late Cretaceous sites (Fricke et al. 2008), and mean AOC ranges from the Proterozoic and Archean (Garcia et al. 2021). See Supplementary Methods S1 for further details of this procedure.

In addition, a further subset analysis was performed at LSIS comparing the $\delta^{13}C$ and $\delta^{18}O$ of enamel versus dentine in hadrosaur tooth bioapatite from this site. This material was pretreated and analyzed via the same laser-ablation approach used for the primary analyses. If enamel and dentine isotopic compositions are substantially different (and particularly in a consistent manner, e.g., higher $\delta^{13}C$ and lower $\delta^{18}O$ in dentine vs. enamel), this difference could indicate relatively greater alteration in the more porous dentine tissues relative to the more alteration-resistant enamel (Owocki et al. 2020). Relatedly, detection of heterogeneities across the tooth tissues when sampled via laser ablation can provide an additional indicator of the preservation of primary isotopic signals, whereas consistent intra-tooth homogenization can be indicative of diagenetic overprinting (Sharp and Cerling 1998). Thus, if differences are relatively minor and heterogeneity remains, comparison of dentine versus enamel signals via repeated laser ablation measurements provides an additional potential

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indicator that diagenetic alteration is comparatively minor (or at least insufficient to remove primary signals). See Supplementary Figure S2 and Supplementary Table S4 for data from these tests.

Finally, X-ray diffraction (XRD) and cathodoluminescence (CL) were performed on bivalve shell fragments at RDS to assess for potential impacts of diagenesis by testing for the presence of shell aragonite and for evidence of elements consistent with recrystallization (or conversely, the absence of luminescence, indicating the likely preservation of primary microstructure). Given the susceptibility of shell aragonite to alteration (and particularly its greater susceptibility when compared with bioapatite tissues generally and enamel specifically), detecting a lack of alteration via CL or XRD is useful not only for interpreting their preservation but also as a proxy for the preservation of the more alteration-resistant enamel and bioapatite materials (Dettman and Lohmann 2000; Fricke et al. 2008). See Supplementary Methods S1 for further details and Supplementary Figure S2 for results of these tests.

TEFs.—Values for $\delta^{13}C_{diet}$ were calculated using TEFs (Δ, with $\Delta = \delta_{bioapatite} - \delta_{diet}$) gathered from the literature (Krueger and Sullivan 1984; Lee-Thorp and Van der Merwe 1987; Lee-Thorp et al. 1989; Johnson et al. 1998; Koch 1998; Cerling and Harris 1999; Biasatti 2004; Passey et al. 2005; Sare et al. 2005; Koch 2007; Angst et al. 2014; O'Connell and Hedges 2017; Cullen 2023) and are compiled as taxon–diet means in Supplementary Table S2 and Figure 1. Extant TEFs represent mean values of species-specific, experimentally derived TEFs and are applied to non-dinosaur $\delta^{13}C_{bioapatite}$ compositions to calculate $\delta^{13}C_{diet}$ based on the TEFs of extant taxa that are phylogenetically, ecologically, and physiologically most similar to those in the RDS sample. The previously hypothesized dinosaur-unique TEF, which was derived from the offset between dinosaur $\delta^{13}C_{bioapatite}$ and $\delta^{13}C_{AOC}$ and hypothesized to account for the high CIA (Stanton Thomas and Carlson 2004; Fricke et al. 2008), is applied to stable carbon compositions of dinosaurs only and presented alongside the bird, mammal, and reptile TEF scenarios for $\delta^{13}C_{diet}$ in dinosaurs.

Results

Positive CIA Present in All Sampled Fossil Taxa, Including Non-dinosaurs.—Stable carbon isotope analyses of 46 fossil specimens from 7 vertebrate taxa from the upper Oldman Formation RDS vertebrate microfossil bonebed reveal the positive CIA is present in $\delta^{13}C_{bioapatite}$ of all specimens, both dinosaur and non-dinosaur (Fig. 2A,B), consistent in magnitude with the previously reported positive CIA of Cretaceous dinosaur bioapatite (Stanton Thomas and Carlson 2004; Fricke et al. 2008; Amiot et al. 2015). No $\delta^{13}C_{bioapatite}$ compositions from any vertebrate sampled from the modern subtropical coastal floodplain preserve a similar CIA, regardless of trophic position, dietary physiology, habitat/environmental preference, or taxonomy (Fig. 2C). AOC from RDS is similar in mean composition to other nearby Cretaceous sites (Fricke et al. 2008), AOC from the Atchafalaya River of Louisiana (Rosenheim et al. 2013) (i.e., the region of our modern near-analogue comparison), as well as modern mean AOC and mean AOC from throughout Earth history (Garcia et al. 2021). See Supplementary Table S1 for $\delta^{13}C_{bioapatite}$ for each specimen and Supplementary Table S5 for the mean $\delta^{13}C_{bioapatite}$ for each taxon.

Isotopic Signals in Material from This Site Are Not Overprinted by Diagenetic Alteration.—Isotopic analyses and FTIR spectroscopy of enamel bioapatite from RDS show similar isotopic compositions in pretreated and untreated samples, while also remaining largely consistent in both CI and C/P (Supplementary Table S3). As well, these samples exhibit a range of CIs (~2.5–3.0) similar to unaltered bones and enamel, well below the threshold (>4.3) considered indicative of extensive recrystallization (Webb et al. 2014). The C/P of these samples ranges from 0.43 to 0.68 and has a mean of ~0.52, broadly consistent with unaltered bone and enamel (with potential for the presence of some secondary carbonate) (Webb et al. 2014). It should be noted that much higher CI values have also been reported as representing unaltered bioapatite (and enamel specifically) in mammals (Roche et al. 2010), although reptile enamel CI ranges in that same study were consistent with the CI

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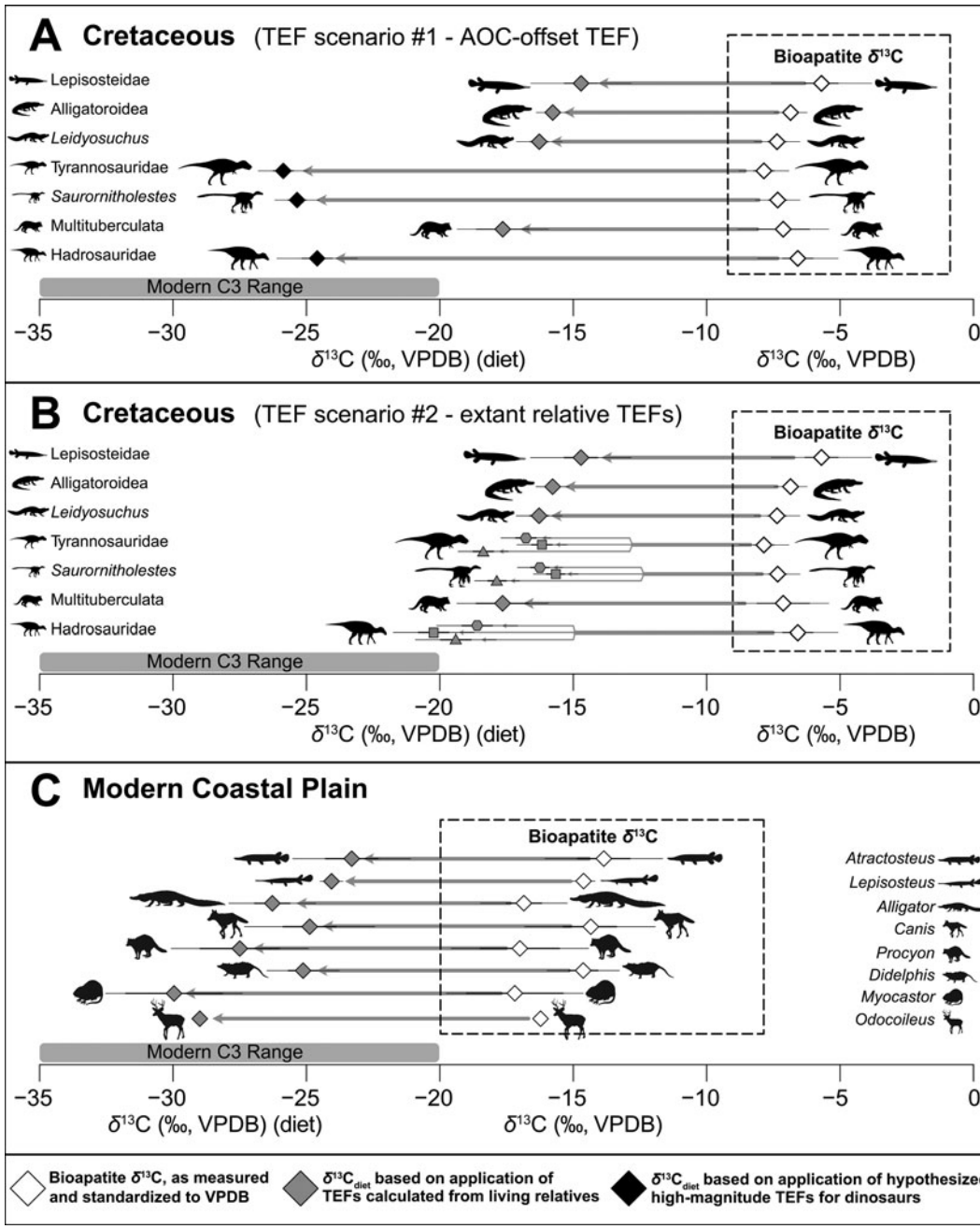

FIGURE 2. Range and mean of $\delta^{13}C_{bioapatite}$ of sampled fossil and extant taxa, along with hypothesized $\delta^{13}C_{diet}$ reconstructions. A, Isotopic distribution of Cretaceous "Rainy Day Site" (RDS) system when $\delta^{13}C_{diet}$ is calculated using hypothesized high-magnitude trophic enrichment factors (TEFs) for dinosaurs and mean TEFs from extant relatives for non-dinosaur taxa. B, Isotopic distribution of Cretaceous RDS system when $\delta^{13}C_{diet}$ is calculated using mean TEFs from extant organisms for all taxa. In B, dinosaurs are reconstructed under three extant TEF scenarios: as mammals (triangles), as birds (squares), and as reptiles (hexagons). Ranges of these extant TEFs are listed in Fig. 1, and exact values are listed in Supplementary Tables S2 and S5. C, Isotopic distribution of a modern subtropical coastal floodplain ecosystem sampled from the Atchafalaya River Basin of Louisiana, with $\delta^{13}C_{diet}$ calculated from mean TEFs from extant taxa. Extant mean TEFs are also described in Fig. 1 and Supplementary Table S2 and further discussed in Cullen et al. (2019). Thick horizontal lines extending from mean values represent standard error, with thin horizontal lines representing standard deviation. Stable isotope compositions also provided in Supplementary Table S1 (individual samples) and Supplementary Table S5 (taxon mean values).

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ranges recorded for reptile taxa at RDS. As a result, although FTIR results are broadly consistent with unaltered bioapatite, the prospect of RDS samples having some degree of alteration cannot be fully ruled out (and/or may reflect the inclusion of dentine alongside enamel in the RDS powders analyzed for FTIR and pretreatment tests). Similarly, comparisons of dentine and enamel samples obtained from laser ablation analyses of a subset of hadrosaur teeth preserve a combination of results broadly consistent with the preservation of original isotopic compositions, including: (1) relatively minor differences between measured enamel and dentine $\delta^{13}C$ and $\delta^{18}O$ (Supplementary Fig. S2); (2) preservation of heterogeneity in isotopic signals across the tooth surface (Supplementary Table S4); and (3) where minor differences are present, they represent directional differences in mean composition consistent with unaltered enamel and minor to moderate alteration to dentine (e.g., the latter possessing higher $\delta^{13}C$, −7.0‰ vs. −6.8‰, and lower $\delta^{18}O$, 17.2‰ vs. 15.4‰, respectively) (Sharp and Cerling 1998; Owocki et al. 2020). Taken together, the consistency between pretreated and untreated isotopic compositions, the CI and the C/P from FTIR, and the dentine to enamel comparisons, are all indicative of the general preservation of original isotopic compositions in the sampled specimens, alongside related ecological/environmental proxy signals, with these signals not substantially impacted by diagenetic overprinting where it may be present. In addition, XRD and CL microscopy confirms the aragonitic mineralogy and general lack of alteration of mollusk shell material found co-occurring with the vertebrate fossils at RDS, providing additional indirect support for the more limited overall impact of diagenetic overprinting on sampled RDS materials (Supplementary Fig. S2). While not the focus of this study, the oxygen isotope ranges in bioapatite from taxa sampled from this site (Cullen et al. 2020) are also consistent with ranges expected from bioapatite of related species living in similar modern environments (Cullen et al. 2019) and distinct from what would be expected if these samples were actively equilibrating with oxygen isotopes from precipitation or groundwater from SE Alberta (Longstaffe 1984) before excavation. Additional relative indicators of the preservation of original isotopic signal at this locality include: (1) the like-with-like groupings of samples with their particular taxa (rather than being relatively homogenized as one would expect after complete diagenetic overprinting) and (2) preservation of expected predator–prey offsets in $\delta^{13}C_{bioapatite}$ among relevant taxa in the RDS dataset (e.g., tyrannosaurs and hadrosaurs) (Bocherens 2000; Fricke et al. 2008; Cullen et al. 2020; Supplementary Table S1).

CIA and Isotopic Distributions of Vertebrates under Hypothesized TEF Scenarios.—Under TEF scenario 1 (Fig. 2A), applying the previously proposed higher-magnitude TEF (~18‰; derived from the previously observed offset between dinosaur $\delta^{13}C_{bioapatite}$ and $\delta^{13}C_{AOC}$) to dinosaurs at RDS shifts their estimated $\delta^{13}C_{diet}$ into a range consistent with extant C_3 feeders, but leaves the $\delta^{13}C_{diet}$ ranges of co-occurring vertebrates (based on application of TEFs from their closest extant relatives) anomalously positive. Under TEF scenario 2 (Fig. 2B), dinosaur $\delta^{13}C_{diet}$ is instead estimated by applying bird, mammal, or reptile TEFs (each plotted separately in Fig. 2B), resulting in the relative positions of all taxa in isotope-space forming a gradient of resource use and exhibiting a reduced (though still present) positive CIA. In a near-analogue modern environment (subtropical coastal plain of Louisiana), the $\delta^{13}C_{diet}$ of sampled vertebrates (both terrestrial and aquatic) all fall within C_3 ranges (Fig. 2C), with no sampled extant taxon preserving a CIA in its $\delta^{13}C_{bioapatite}$ comparable to those observed in every vertebrate in the Cretaceous dataset.

Isotopic Community Ranges of Both Systems Are of Similar Magnitude but Shifted.—The extant TEF-inferred $\delta^{13}C_{diet}$ of taxa analyzed from the Cretaceous coastal plain of the RDS (Fig. 2B) shows a similar range (~10‰ to 13‰, depending on the TEF applied for dinosaurs) to data for the extant coastal plain environment of the Atchafalaya River Basin of Louisiana (Cullen et al. 2019; Fig. 2C). Despite being of similar magnitude, the mean $\delta^{13}C_{diet}$ is shifted upward by ~10‰ in the RDS (~ −17.5‰ to −16.5‰, depending on the dinosaur TEF scenario) compared with the Atchafalaya community (~ −27‰), reflecting the CIA.

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Discussion

Dinosaur Dietary Physiology as a Cause of the Positive Stable CIA.—The positive CIA has been hypothesized to be the result of either dinosaurs possessing a unique dietary physiology and consequently a higher-magnitude TEF than present in extant vertebrates, or as the result of external factor(s) influencing the $\delta^{13}C_{bioapatite}$ composition (i.e., diagenetic overprinting, substantial dietary source differences, non-analogue environmental features, or a mixture of some or all of these). The primary argument for the former hypothesis is based on the offset observed between AOC and $\delta^{13}C_{bioapatite}$ of dinosaurs and relies on the following assumptions to be correct: (1) mean AOC is a meaningful proxy for the plant $\delta^{13}C$ consumed by dinosaurs, and therefore the difference between $\delta^{13}C_{AOC}$ and $\delta^{13}C_{bioapatite}$ should reflect an accurate TEF estimate for dinosaur diets; and (2) the $\delta^{13}C_{bioapatite}$ compositions of co-occurring vertebrates with extant relatives of known dietary physiology are not also anomalously positive in the Cretaceous system relative to their ranges in the modern system.

The first assumption, is, on its face, a reasonable one. The terrestrial plant community in the Late Cretaceous of North America (and Campanian of Alberta/Montana in particular) was predominantly C_3-based (Koch 1998; Stanton Thomas and Carlson 2004), and while individual C_3 plants cover a very wide range of $\delta^{13}C$ (Fig. 1C), their mean composition was (and remains) similar to that of AOC (Fig. 1B). However, it is worth considering that ambient/bulk organic carbon is just that, a bulk sample related to the total organic carbon in the terrestrial and aquatic systems, and may not accurately reflect the $\delta^{13}C$ of the specific terrestrial plant community of a given location or the individual plant tissues or plant species that form the diet of a particular organism (Grocke 2002; Hong and Lee 2013; Garcia et al. 2021). Indeed, while AOC excursions are useful proxies for examining major environmental changes in deep time as part of chemostratigraphic studies, the measured mean composition of AOC has a relatively consistent baseline value (~ −26‰ to −25‰) throughout the last 3.5 billion years of Earth history, despite major changes in both plant and animal ecology and evolution occurring over this time (not the least of which includes the initial evolution of plants themselves) (Hong and Lee 2013; Nordt et al. 2016). This would suggest that for finer-scale measurements of trophic habits at ecologically relevant timescales, background AOCs should not be used uncritically, particularly when considering the diet of individual species and/or when other data exist that are in conflict with the assumptions necessitated by using background AOCs, as is the case in this present study. Indeed, the modern near-analogue dataset provides an example of this issue. In the modern system, an unusually low-magnitude TEF (relative to the known experimentally determined mammalian herbivore TEFs) would be required to estimate a $\delta^{13}C_{diet}$ composition of *Odocoileus* (white-tailed deer) that is in line with the difference between mean $\delta^{13}C_{AOC}$ and *Odocoileus* $\delta^{13}C_{bioapatite}$. The $\delta^{13}C_{diet}$ for *Odocoileus* estimated from the application of a mean of known mammalian herbivore TEFs plots in the C_3 range but is several per mil more negative than mean AOC. Consequently, the first assumption necessary for the hypothesis of unique dinosaur dietary physiology and related higher-magnitude TEFs is rendered uncertain at best.

The second assumption can be considered through examination of the multi-taxic $\delta^{13}C$ data measured from both the Cretaceous and modern systems. Although applying the AOC-derived TEF to dinosaurs in the RDS locality does shift their estimated $\delta^{13}C_{diet}$ range to be consistent with extant C_3 feeders (Fig. 2A), all other vertebrates in the system, with their $\delta^{13}C_{diet}$ estimated by applying TEFs from their closest living relatives, remain in $\delta^{13}C$ ranges that are more positive than extant C_3 feeders generally and their own close relatives/ecological analogues specifically (Fig. 2A,C). As a consequence, the isotopic niche distributions within this ecosystem are reconstructed as dichotomous, with dinosaurs completely distinct in resource use from all other vertebrates and a zone of unused carbon isotope resources existing between the dinosaur and non-dinosaur $\delta^{13}C$ ranges. While this is not problematic per se, given the

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potential dietary carbon inputs for the mammals, reptiles, and fish in this Cretaceous system that could result in them not plotting in C_3 ranges, it does become an issue when considered in the context of modern near-analogue data, and particularly with respect to the sampled taxa with close relatives (and ecological analogues) in both systems (e.g., lepisosteid fish, crocodilians, and mammals). This is because while these taxa all exhibit the same positive shift in their Cretaceous $\delta^{13}C_{bioapatite}$ samples as is observed in the dinosaurs, they do not exhibit the same pattern in their $\delta^{13}C_{bioapatite}$ in the modern subtropical coastal plain, where the $\delta^{13}C_{diet}$ of all of these modern taxa falls within C_3 ranges (Fig. 2C). These taxa are also not exclusively aquatic or faunivorous, as small Late Cretaceous terrestrial mammals are reconstructed as having a range of omnivorous to herbivorous diets (Grossnickle et al. 2019), relatively similar to the small omnivorous mammals sampled from the modern system.

If the CIA in dinosaurs is indeed explained by a unique dietary physiology (and associated higher-magnitude TEF), then the CIA also being present in Cretaceous fish, mammals, and crocodilians (but not in their living relatives in similar environments with similar ecologies) would require the implausible assumption that all of these distantly related groups of taxa either completely shifted their dietary carbon intakes (despite filling similar ecological niches in both systems) or independently evolved similar dietary physiologies to dinosaurs in the Cretaceous only to completely diverge to their disparate extant dietary physiologies (and TEFs) at a later point. Given how comparatively unlikely this is, and how applying the hypothesized high-magnitude TEF to herbivorous dinosaurs alone does not explain similar differences in non-dinosaurs or the resulting differences in relative isotopic distributions in the ancient and modern coastal plain systems, the alternative hypothesis that these phenomena are the result of one or multiple broader external factors impacting all taxa in the system emerges as the most parsimonious explanation.

Diagenetic Overprinting as a Cause of the Positive Stable CIA.—If a unique dinosaur dietary physiology is unlikely to be responsible for the CIA, and it is present in all sampled Cretaceous fossil vertebrate taxa despite not being present in any of the sampled extant vertebrate taxa, then diagenetic alteration and a shift in preserved isotopic compositions (at least in an absolute sense) would appear a plausible explanation. However, our suite of tests indicate that diagenetic alteration, if/where present, is insufficient to fully explain the CIA. This is consistent with the result of other studies considering diagenetic impacts on the preservation of original isotopic signals in vertebrate bioapatite from the Belly River Group of Alberta and lateral equivalents in Montana (Fricke et al. 2008; Cullen et al. 2020, 2022), as well as with studies more broadly describing the preservation of fossils with minimal taphonomic alteration from these strata (Choi et al. 2022). To be clear, however, it does not fully rule out the potential that diagenetic alteration exists and may be contributing to some extent to the CIA. Indeed, some studies that have reported anomalously positive $\delta^{13}C$ compositions in other taxa and concluded they were the result of diagenetic alteration, such as from a multi-taxic sample from the Eocene Messel Pit (Tütken 2014), where enamel $\delta^{13}C_{enamel}$ compositions of a mammal taxon were broadly in the range of modern C_3-feeding herbivorous mammals, but $\delta^{13}C_{dentine}$ and $\delta^{13}C_{bone}$ compositions from multiple other taxa were very positive and appeared to be the result of diagenetic overprinting. Unlike in the RDS example, however, the anomalous $\delta^{13}C$ compositions in the Messel Pit were ~15‰–20‰ more positive than expected, a substantially greater magnitude of difference than observed here at RDS (whether via the difference between "expected" values and the CIA itself, or in our dentine–enamel comparisons), potentially indicating a greater degree of diagenetic impacts in samples from Messel Pit compared with RDS. Similarly, diagenetic tests and leachate comparisons performed during $^{87}Sr/^{86}Sr$ analyses of enamel bioapatite from a multi-taxic sample from RDS suggest relatively limited impacts of diagenetic exchange (Cullen et al. 2022), as well as general consistency with expected regional values of substrates these animals would likely

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have lived upon (Armstrong et al. 1998; Terrill et al. 2020). This appears further unlike the Messel Pit dataset, where evidence of diagenetic Sr exchange is more substantial and further distinguishes their enamel versus bone/dentine samples. It is also possible that the positive $\delta^{13}C$ ranges measured at Messel Pit were the result of multiple factors, as here, but with diagenetic alteration being the greatest relative contributor when compared with environmental or source diet differences, with the RDS representing an inverse situation wherein diagenetic alteration is present but its impact is relatively small when compared with the proportional impacts of environmental factors and source diet differences. As those Messel Pit materials derive from an oil shale formed in the anoxic bottom waters of a meromictic lake, it is also conceivable that they experienced substantially different depositional and diagenetic histories when compared with the mixed fluvial and wetland settings of Cretaceous vertebrate microfossil bonebeds, with that partially responsible for the apparent differences in relative diagenetic impact.

Potential Isotopic Baseline Shifts Driving the Stable Carbon Isotope Anomaly.—If the CIA is unlikely to be primarily the result of dinosaur dietary physiology or diagenetic overprinting, then it is plausible that a combination of environmental and other factors may instead be responsible. In this Cretaceous system, multiple possible factors exist that may have positively shifted the $\delta^{13}C$ of dietary plant materials from comparative averages in a modern system so as to result in the observed CIA. For example, studies of fossil plant resins from the Late Cretaceous of Alberta have reported $\delta^{13}C$ compositions enriched by ~2‰–3‰ relative to modern resin and, given the close link between $\delta^{13}C$ of resin and bulk plant matter, suggested that many of the plants in this system also likely exhibited less negative $\delta^{13}C$ compositions (Tappert et al. 2013). This enrichment was found to be unrelated to diagenetic or other preservational issues and was hypothesized to be a result of ^{13}C fractionation differences in resin-producing plants stemming from lower pO_2 during this time (Tappert et al. 2013). Additionally, recent work has demonstrated that lacustrine aquatic C_3 plants can have more positive $\delta^{13}C$ compositions depending on whether they use bicarbonate or dissolved atmospheric CO_2 (Plint et al. 2019). Given this, and the evidence of numerous aquatic plant taxa in this region/time (Braman et al. 2005), consumption of aquatic plants offers an additional potential input of more positive $\delta^{13}C$ in the diets of herbivores (and consequently other taxa at higher trophic levels) in this system. Other factors that may have influenced the $\delta^{13}C$ compositions of plants (and the tissues of herbivorous dinosaurs consuming those plants) include higher $\delta^{13}C_{atm}$ (relating to higher atmospheric CO_2, higher by +1.5‰–2‰), more enriched dietary plant $\delta^{13}C$ (relating to feeding primarily on particular gymnosperm taxa, +1‰–2‰), and most or some of these plants being osmotically stressed (+2‰–3‰) (Fry and Sherr 1989; Arens et al. 2000; Onstad et al. 2000; Stanton Thomas and Carlson 2004).

When these various factors are taken into consideration, the dietary plant composition shifts into a range that is consistent with the stable carbon range of sampled hadrosaurs when applying any of the three TEF scenarios based on extant data from herbivorous reptiles, birds, or mammals (Fig. 2B). This baseline shift allows the majority of the anomalously positive $\delta^{13}C$ compositions of the sampled taxa to be explained, with diagenetic alteration effects potentially responsible for the remainder of the difference (with the amount of unexplained variation somewhat contingent on the actual TEF of herbivorous dinosaurs). This approach also produces a gradient of $\delta^{13}C$ compositions in the Cretaceous community (Fig. 2B) that is similar in relative pattern to that of the modern ecosystem (Fig. 2C), unlike the dichotomous isotopic distribution produced when applying the high-magnitude dinosaur TEF (Fig. 2A).

Conclusions

We demonstrate that the CIA, previously hypothesized as reflecting unique dinosaur dietary physiology, is in fact present in all vertebrate taxa sampled from the Cretaceous coastal plain and is not present in any related taxa in near-analogue modern coastal plains. This refutes the original hypothesis that the

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positive stable carbon compositions are chiefly the result of a unique dietary physiology and associated high-magnitude TEF. What is causing the CIA remains at least partially uncertain, but is evidently related to broader external factors impacting the stable carbon compositions of all vertebrates in this system, rather than the unique physiology of a single group. Our diagenetic tests indicate original stable isotope compositions are likely preserved and diagenetic overprinting, where present, is of secondary impact. As a result, the CIA is likely the result of a suite of factors, primarily related to environmental differences and differences in dietary plant sources, and potentially impacted to a more minor extent by diagenetic signals, with all of these factors operating in concert to positively shift the isotopic baseline in this greenhouse ecosystem relative to expectations from similar modern systems. Given the importance of potential unique physiologies to our understanding of the paleobiology of dinosaurs, refuting that hypothesis represents a significant step in our ongoing attempts to understand Cretaceous ecosystems and environments, one that would not be possible without this sort of spatiotemporally constrained multi-taxic analysis. This does not fully rule out the possibility of dinosaur groups possessing higher-magnitude TEFs, but does suggest that if they are present, they are more modest than previously proposed and a direct measure of the offset from bioapatite $\delta^{13}C$ to local bulk organic $\delta^{13}C$ cannot be assumed to produce an accurate estimate of the specific TEFs for those taxa. Our work underscores the need for additional research on these ancient greenhouse systems, both to facilitate more accurate reconstructions of the species, ecosystems, and environments in these deep-time contexts and, more broadly, to allow us to understand and predict long-term trends in these partially non-analogous systems. As the climate of the Earth continues to change, potentially shifting from current icehouse into greenhouse conditions, comprehensive data from the Cretaceous and other similar periods will be invaluable for understanding and managing changes and threats to species, ecosystems, and environments in the future.

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Declaration of Competing Interests

The authors declare no competing interests.

Data Availability Statement

Analytical data contained in attached Supplementary Material and available from the Figshare Digital Repository: https://doi.org/10.6084/m9.figshare.21585660.

Supplementary Methods S1. Expanded details of analytical procedures

Supplementary Figure S1. Representative examples of specimens from RDS locality

Supplementary Figure S2. Additional tests for potential of diagenetic overprinting

Supplementary Table S1. Measured stable carbon isotope compositions and specimen data

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Supplementary Table S2. TEF source data used in $\delta^{13}C_{diet}$ calculations

Supplementary Table S3. Preservational, pretreatment, and FTIR data

Supplementary Table S4. Enamel vs. dentine stable isotope comparisons

Supplementary Table S5. Mean $\delta^{13}C_{diet}$ compositions for studied taxa

Literature Cited

Amiot, R., C. Lécuyer, G. Escarguel, J.-P. Billon-Bruyat, E. Buffetaut, C. Langlois, S. Martin, F. Martineau, and J.-M. Mazin. 2007. Oxygen isotope fractionation between crocodilian phosphate and water. Palaeogeography, Palaeoclimatology, Palaeoecology 243:412–420.

Amiot, R., X. Wang, Z. Zhou, X. Wang, C. Lécuyer, E. Buffetaut, F. Fluteau, Z. Ding, N. Kusuhashi, and J. Mo. 2015. Environment and ecology of East Asian dinosaurs during the Early Cretaceous inferred from stable oxygen and carbon isotopes in apatite. Journal of Asian Earth Sciences 98:358–370.

Angst, D., C. Lécuyer, R. Amiot, E. Buffetaut, F. Fourel, F. Martineau, S. Legendre, A. Abourachid, and A. Herrel. 2014. Isotopic and anatomical evidence of an herbivorous diet in the Early Tertiary giant bird *Gastornis*. Implications for the structure of Paleocene terrestrial ecosystems. Die Naturwissenschaften 101:313–322.

Arbour, V. M., and D. C. Evans. 2017. A new ankylosaurine dinosaur from the Judith River Formation of Montana, USA, based on an exceptional skeleton with soft tissue preservation. Royal Society Open Science 4:161086.

Arens, N. C., A. H. Jahren, and R. Amundson. 2000. Can C3 plants faithfully record the carbon isotopic composition of atmospheric carbon dioxide? Paleobiology 26:137–164.

Armstrong, S. C., N. C. Sturchio, and M. J. Hendry. 1998. Strontium isotopic evidence on the chemical evolution of pore waters in the Milk River Aquifer, Alberta, Canada. Applied Geochemistry 13:463–475.

Biasatti, D. M. 2004. Stable carbon isotopic profiles of sea turtle humeri: implications for ecology and physiology. Palaeogeography, Palaeoclimatology, Palaeoecology 206:203–216.

Bocherens, H. 2000. Preservation of isotopic signals (13C, 15N) in Pleistocene mammals. Pp. 65–88 *in* S. H. Ambrose and M. A. Katzenberg, eds. Biogeochemical approaches to paleodietary analysis. Kluwer Academic Publishers, New York.

Braman, D. R., E. B. Koppelhus, and P. Currie 2005. Campanian palynomorphs. Pp. 101–130 *in* P. J. Currie and E. B. Koppelhus, eds. Dinosaur Provincial Park: a spectacular ancient ecosystem revealed. Indiana University Press, Bloomington.

Brinkman, D. B., A. P. Russell, D. A. Eberth, and J. H. Peng. 2004. Vertebrate palaeocommunities of the lower Judith River Group (Campanian) of southeastern Alberta, Canada, as interpreted from vertebrate microfossil assemblages. Palaeogeography, Palaeoclimatology, Palaeoecology 213:295–313.

Brito, P. M., J. Alvarado-Ortega, and F. J. Meunier. 2017. Earliest known lepisosteoid extends the range of anatomically modern gars to the Late Jurassic. Scientific Reports 7:17830.

Cerling, T. E., and J. M. Harris. 1999. Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. Oecologia 120:347–363.

Cerling, T. E., and Z. D. Sharp. 1996. Stable carbon and oxygen isotope analysis of fossil tooth enamel using laser ablation. Palaeogeography, Palaeoclimatology, Palaeoecology 126:173–186.

Chiba, K., M. J. Ryan, D. R. Braman, D. A. Eberth, E. E. Scott, C. M. Brown, Y. Kobayashi, and D. C. Evans. 2015. Taphonomy of a monodominant *Centrosaurus apertus* (Dinosauria: Ceratopsia) bonebed from the upper Oldman Formation of southeastern Alberta. Palaios 30:655–667.

Chinsamy, A., and J. H. Hurum. 2006. Bone microstructure and growth patterns of early mammals. Acta Palaeontologica Polonica 51:325–338.

Choi, S., N.-H. Kim, H.-I. Kim, J. J. Kweon, S. K. Lee, S. Zhang, and D. J. Varricchio. 2022. Preservation of aragonite in Late Cretaceous (Campanian) turtle eggshell. Palaeogeography, Palaeoclimatology, Palaeoecology 585:110741.

Cullen, T., F. Longstaffe, U. Wortmann, L. Huang, F. Fanti, M. Goodwin, M. Ryan, and D. Evans. 2020. Large-scale stable isotope characterization of a Late Cretaceous dinosaur-dominated ecosystem. Geology 48:546–551.

Cullen, T. M. 2023. Stable isotope analyses of living and extinct crocodylians: implications for understanding their ecology, environments, and physiology. *In* H. N. Woodward and J. O. Farlow, eds. Ruling reptiles: crocodylian biology and archosaur paleobiology. Indiana University Press, Bloomington, Indiana (forthcoming).

Cullen, T. M., and D. C. Evans. 2016. Palaeoenvironmental drivers of vertebrate community composition in the Belly River Group (Campanian) of Alberta, Canada, with implications for dinosaur biogeography. BMC Ecology 16:1–35.

Cullen, T. M., F. Fanti, C. Capobianco, M. J. Ryan, and D. C. Evans. 2016. A vertebrate microsite from a marine-terrestrial transition in the Foremost Formation (Campanian) of Alberta, Canada, and the use of faunal assemblage data as a paleoenvironmental indicator. Palaeogeography, Palaeoclimatology, Palaeoecology 444:101–114.

Cullen, T. M., F. J. Longstaffe, U. G. Wortmann, M. B. Goodwin, L. Huang, and D. C. Evans. 2019. Stable isotopic characterization of a coastal floodplain forest community: a case study for isotopic reconstruction of Mesozoic vertebrate assemblages. Royal Society Open Science 6:181210.

Cullen, T. M., S. Zhang, J. Spencer, and B. Cousens. 2022. Sr-O-C isotope signatures reveal herbivore niche-partitioning in a Cretaceous ecosystem. Palaeontology 65:e12591.

Dettman, D. L., and K. C. Lohmann. 2000. Oxygen isotope evidence for high-altitude snow in the Laramide Rocky Mountains of North America during the Late Cretaceous and Paleogene. Geology 28:243–246.

Eberth, D. A. 2005. The geology. Pp. 54–82 *in* P. J. Currie and E. B. Koppelhus, eds. Dinosaur Provincial Park: a spectacular ancient ecosystem revealed. Indiana University Press, Bloomington, Indiana.

Eberth, D. A. 2015. Origins of dinosaur bonebeds in the Cretaceous of Alberta, Canada. Canadian Journal of Earth Sciences 52:655–681.

Eberth, D. A., and A. P. Hamblin. 1993. Tectonic, stratigraphic, and sedimentologic significance of a regional discontinuity in the upper Judith River Group (Belly River wedge) of southern Alberta, Saskatchewan, and northern Montana. Canadian Journal of Earth Sciences 30:174–200.

Freedman Fowler, E. A., and J. R. Horner. 2015. A new brachylophosaurin hadrosaur (Dinosauria: Ornithischia) with an intermediate nasal crest from the Campanian Judith River Formation of north-central Montana. PLoS ONE 10:e0141304.

Fricke, H. C., R. R. Rogers, R. Backlund, C. N. Dwyer, and S. Echt. 2008. Preservation of primary stable isotope signals in dinosaur remains, and environmental gradients of the Late Cretaceous of Montana and Alberta. Palaeogeography, Palaeoclimatology, Palaeoecology 266:13–27.

Fry, B., and E. B. Sherr. 1989. δ13C measurements as indicators of carbon flow in marine and freshwater ecosystems. Pp. 196–229 in P. W. Rundel, J. R. Ehleringer, and K. A. Nagy, eds. Stable isotopes in ecological research. Ecological Studies 68. Springer, New York.

https://doi.org/10.1017/pab.2022.43 Published online by Cambridge University Press

Garcia, A. K., C. M. Cavanaugh, and B. Kacar. 2021. The curious consistency of carbon biosignatures over billions of years of Earth-life coevolution. ISME Journal 15:2183–2194.

Grocke, D. R. 2002. The carbon isotope composition of ancient CO_2 based on higher-plant organic matter. Philosophical Transactions of the Royal Society of London A 360:633–658.

Grossnickle, D. M., S. M. Smith, and G. P. Wilson. 2019. Untangling the multiple ecological radiations of early mammals. Trends in Ecology and Evolution 34:936–949.

Hong, S. K., and Y. I. Lee. 2013. Contributions of soot to $\delta^{13}C$ of organic matter in Cretaceous lacustrine deposits, Gyeongsang Basin, Korea: implication for paleoenvironmental reconstructions. Palaeogeography, Palaeoclimatology, Palaeoecology 371:54–61.

Janis, C. M., J. Damuth, and J. M. Theodor. 2002. The origins and evolution of the North American grassland biome: the story from the hoofed mammals. Palaeogeography, Palaeoclimatology, Palaeoecology 177:183–198.

Johnson, B. J., M. L. Fogel, and G. H. Miller. 1998. Stable isotopes in modern ostrich eggshell: a calibration for paleoenvironmental applications in semi-arid regions of southern Africa. Geochimica et Cosmochimica Acta 62:2451–2461.

Koch, P. L. 1998. Isotopic reconstruction of past continental environments. Annual Review of Earth and Planetary Sciences 26:573–613.

Koch, P. L. 2007. Isotopic study of the biology of modern and fossil vertebrates. Pp. 99–154 *in* R. Michener and K. Lajtha, eds. Stable isotopes in ecology and environmental science. Wiley, Malden, Mass.

Köhler, M., N. Marín-Moratalla, X. Jordana, and R. Aanes. 2012. Seasonal bone growth and physiology in endotherms shed light on dinosaur physiology. Nature 487:358.

Krueger, H. W., and C. H. Sullivan. 1984. Models for carbon isotope fractionation between diet and bone. Pp. 205–220 in J. R. Turnlund and P. E. Johnson, eds. Stable isotopes in nutrition (ACS Symposium Series 258). ACS Publications, Washington, D.C.

Larson, T. E., and F. J. Longstaffe. 2007. Deciphering seasonal variations in the diet and drinking water of modern white-tailed deer by in situ analysis of osteons in cortical bone. Journal of Geophysical Research: Biogeosciences 112(G4).

Lee-Thorp, J., and N. J. Van der Merwe. 1987. Carbon isotope analysis of fossil bone apatite. South African Journal of Science 83:712–715.

Lee-Thorp, J. A., J. C. Sealy, and N. J. van der Merwe. 1989. Stable carbon isotope ratio differences between bone collagen and bone apatite, and their relationship to diet. Journal of Archaeological Science 16:585–599.

Longstaffe, F. J. 1984. The role of meteoric water in diagenesis of shallow sandstones: stable isotope studies of the Milk River aquifer and gas pool, southeastern Alberta: Part 1. Concepts and principles. Pp. 81–98 *in* D. A. McDonald and R. C. Surdam, eds. Clastic diagenesis. AAPG Memoir 37. American Association of Petroleum Geologists, Tulsa, Okla.

Mack, G. H., and T. Jerzykiewicz. 1989. Detrital modes of sand and sandstone derived from andesitic rocks as a paleoclimatic indicator. Sedimentary Geology 65:35–44.

Montanari, S., P. Higgins, and M. A. Norell. 2013. Dinosaur eggshell and tooth enamel geochemistry as an indicator of Mongolian Late Cretaceous paleoenvironments. Palaeogeography, Palaeoclimatology, Palaeoecology 370:158–166.

Nordt, L., J. Tubbs, and S. Dworkin. 2016. Stable carbon isotope record of terrestrial organic materials for the last 450 Ma yr. Earth-Science Reviews 159:103–117.

O'Connell, T., and R. Hedges. 2017. Chicken and egg: testing the carbon isotopic effects of carnivory and herbivory. Archaeometry 59:302–315.

Onstad, G. D., D. E. Canfield, P. D. Quay, and J. I. Hedges. 2000. Sources of particulate organic matter in rivers from the continental USA: lignin phenol and stable carbon isotope compositions. Geochimica et Cosmochimica Acta 64:3539–3546.

Owocki, K., B. Kremer, M. Cotte, and H. Bocherens. 2020. Diet preferences and climate inferred from oxygen and carbon isotopes of tooth enamel of *Tarbosaurus bataar* (Nemegt Formation, Upper Cretaceous, Mongolia). Palaeogeography, Palaeoclimatology, Palaeoecology 537:109190.

Passey, B. H., T. F. Robinson, L. K. Ayliffe, T. E. Cerling, M. Sponheimer, M. D. Dearing, B. L. Roeder, and J. R. Ehleringer. 2005. Carbon isotope fractionation between diet, breath CO2, and bioapatite in different mammals. Journal of Archaeological Science 32:1459–1470.

Peng, J., A. P. Russell, and D. B. Brinkman. 2001. Vertebrate microsite assemblages (exclusive of mammals) from the Foremost and Oldman Formations of the Judith River Group (Campanian) of southeastern Alberta: an illustrated guide. Provincial Museum of Alberta, Natural History Occasional Paper 25:1–54.

Plint, T., F. J. Longstaffe, and G. Zazula. 2019. Giant beaver palaeoecology inferred from stable isotopes. Scientific Reports 9:1–12.

Roche, D., L. Ségalen, E. Balan, and S. Delattre. 2010. Preservation assessment of Miocene–Pliocene tooth enamel from Tugen Hills (Kenyan Rift Valley) through FTIR, chemical and stable-isotope analyses. Journal of Archaeological Science 37:1690–1699.

Rogers, R. R., and M. E. Brady. 2010. Origins of microfossil bonebeds: insights from the Upper Cretaceous Judith River Formation of north-central Montana. Paleobiology 36:80–112.

Rogers, R. R., M. T. Carrano, K. A. Curry Rogers, M. Perez, and A. K. Regan. 2017. Isotaphonomy in concept and practice: an exploration of vertebrate microfossil bonebeds in the Upper Cretaceous (Campanian) Judith River Formation, north-central Montana. Paleobiology 43:248–273.

Rosenheim, B. E., K. M. Roe, B. J. Roberts, A. S. Kolker, M. A. Allison, and K. H. Johannesson. 2013. River discharge influences on particulate organic carbon age structure in the Mississippi/Atchafalaya River System. Global Biogeochemical Cycles 27:154–166.

Sare, D. T. J., J. S. Millar, and F. J. Longstaffe. 2005. Nitrogen- and carbon-isotope fractionation between mothers and offspring in red-backed voles (*Clethrionomys gapperi*). Canadian Journal of Zoology 83:712–716.

Sharp, Z., and T. Cerling. 1998. Fossil isotope records of seasonal climate and ecology: straight from the horse's mouth. Geology 26:219–222.

Stanton Thomas, K. J., and S. J. Carlson. 2004. Microscale $\delta^{18}O$ and $\delta^{13}C$ isotopic analysis of an ontogenetic series of the hadrosaurid dinosaur *Edmontosaurus*: implications for physiology and ecology. Palaeogeography, Palaeoclimatology, Palaeoecology 206:257–287.

Straight, W., R. Barrick, and D. Eberth. 2004. Reflections of surface water, seasonality and climate in stable oxygen isotopes from tyrannosaurid tooth enamel. Palaeogeography, Palaeoclimatology, Palaeoecology 206:239–256.

Tappert, R., R. C. McKellar, A. P. Wolfe, M. C. Tappert, J. Ortega-Blanco, and K. Muehlenbachs. 2013. Stable carbon isotopes of C3 plant resins and ambers record changes in atmospheric oxygen since the Triassic. Geochimica et Cosmochimica Acta 121:240–262.

Terrill, D. F., C. M. Henderson, and J. S. Anderson. 2020. New application of strontium isotopes reveals evidence of limited migratory behaviour in Late Cretaceous hadrosaurs. Biology Letters 16:20190930.

Tütken, T. 2011. The diet of sauropod dinosaurs: Implications from carbon isotope analysis of teeth, bones, and plants. Pp. 57–79 *in* N. Klein, K. Remes, and M. Sander, eds. Biology of the sauropod

https://doi.org/10.1017/pab.2022.43 Published online by Cambridge University Press

dinosaurs: Understanding the life of giants. Indiana University Press, Bloomington.

Tütken, T. 2014. Isotope compositions (C, O, Sr, Nd) of vertebrate fossils from the Middle Eocene oil shale of Messel, Germany: implications for their taphonomy and palaeoenvironment. Palaeogeography, Palaeoclimatology, Palaeoecology 416:92–109.

Webb, E. C., C. D. White, and F. J. Longstaffe. 2014. Investigating inherent differences in isotopic composition between human bone and enamel bioapatite: implications for reconstructing residential histories. Journal of Archaeological Science 50:97–107.

Whiting, E. T., D. W. Steadman, and J. Krigbaum. 2016. Paleoecology of Miocene crocodylians in Florida: insights from stable isotope analysis. Palaeogeography, Palaeoclimatology, Palaeoecology 451:23–34.

Zelenitsky, D. K., F. Therrien, K. Tanaka, P. J. Currie, and C. L. DeBuhr. 2016. Latest Cretaceous eggshell assemblage from the Willow Creek Formation (upper Maastrichtian–lower Paleocene) of Alberta, Canada, reveals higher dinosaur diversity than represented by skeletal remains. Canadian Journal of Earth Sciences 54:134–140.

https://doi.org/10.1017/pab.2022.43 Published online by Cambridge University Press