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Multigeochemical proxies for paleobiological assessment of thermoregulation, hard part mineralization, climate, and ecology of Mesozoic non-avian dinosaurs and their relatives

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy in Geology

by

Jade Lauren-Nicole Knighton

2026

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2026

ABSTRACT OF THE DISSERTATION

Multigeochemical proxies for paleobiological assessment of thermoregulation, hard part mineralization, climate, and ecology of Mesozoic non-avian dinosaurs and their relatives

by

Jade Lauren-Niolce Knighton

Doctor of Philosophy in Geology

University of California, Los Angeles, 2026

Professor Aradhna K. Tripathi, Co-Chair

Professor Robert Alfred Eagle, Co-Chair

The first chapter of this thesis evaluates the preservation and geochemical fidelity of eight Late Cretaceous fossil eggshell samples from Mongolia and Alberta using polarized-light petrography, electron backscatter diffraction (EBSD), cathodoluminescence, Raman spectroscopy, conventional stable isotopes, carbonate clumped isotopes (Δ_{47}), and triple oxygen isotopes ($\Delta^{17}\text{O}$). Most specimens retain recognizable microstructure, yet petrographic preservation is not consistently coupled to isotopic fidelity. The two Alberta samples yield elevated apparent Δ_{47} temperatures (48.5–61.5 °C), consistent with burial-related resetting. Several Mongolian samples yield lower apparent temperatures (21.2–31.5 °C) and the strongest convergence among preservation screens; these values are treated as candidate biomineralization temperatures. Carbon-isotope values are comparatively reproducible within specimens, whereas oxygen-isotope and $\Delta^{17}\text{O}$ results indicate variable fluid interaction. These results show that eggshell geochemistry can retain primary information, but only when interpreted within a multi-proxy preservation framework. $\delta^{13}\text{C}$ values (–13.7‰ to –3.7‰ VPDB)

prove highly robust and consistent with a C3 vegetation-dominated diet. In the best-preserved material, primarily from Mongolia, primary signatures remain intact: $\delta^{13}\text{C}$ ranges from -6.16‰ to -4.18‰ VPDB, $\delta^{18}\text{O}$ ranges from -12.4‰ to -8.2‰ VPDB, and Δ_{47} temperatures yield reliable physiological estimates of 21.2 °C to 27.8 °C for oviraptorids and 31.5 °C for a hadrosaur.

The second chapter applies Δ_{47} thermometry and bond-reordering models to fourteen paleosol-carbonate samples from the Permian Bernal Formation and Triassic Anton Chico Member at Bernal Mesa, New Mexico. Apparent temperatures range from 10.9 ± 3.2 to approximately 93 °C . Two Permian samples yield low apparent temperatures consistent with late, cool recrystallization; the remaining samples yield $52.4\text{--}93.1\text{ °C}$ and are interpreted as variably reset during burial. Forward models using the disordered calcite-reordering framework of Hemingway and Henkes (2021), conditioned on a prescribed Late Cretaceous peak-burial time and subsequent cooling, yield maximum burial temperatures of approximately $164\text{--}178\text{ °C}$ for most of the burial-reset population. These temperatures imply roughly $4.1\text{--}6.5\text{ km}$ of overburden for geothermal gradients of $25\text{--}30\text{ °C km}^{-1}$, consistent with regional Laramide exhumation histories.

The final chapter compares thermophysiological signals in marine vertebrates from two temporally distinct assemblages: a late Campanian–early Maastrichtian succession in New Jersey and the Turonian Codell Sandstone of Kansas. Phosphate oxygen isotopes ($\delta^{18}\text{O}_{\text{P}}$), structural-carbonate Δ_{47} thermometry, Fourier-transform infrared spectroscopy, and carbonate–phosphate isotope offsets were applied to tooth enamel/enameloid and co-occurring carbonate shells. New Jersey oysters and belemnites yield an ambient-water temperature near 20 °C . Mosasaur $\delta^{18}\text{O}_{\text{P}}$ estimates average $32.6 \pm 0.7\text{ °C}$ in New Jersey and $33.8 \pm 0.8\text{ °C}$ in Kansas; New Jersey mosasaur Δ_{47} data yield $39.5 \pm 6.6\text{ °C}$. In Kansas, *Cretoxyrhina mantelli* yields $36.0 \pm 0.6\text{ °C}$ from $\delta^{18}\text{O}_{\text{P}}$ and $37.7 \pm 5.6\text{ °C}$ from Δ_{47} . These patterns are compatible with

elevated heat retention in mosasaurs and *C. mantelli*, whereas several other taxa track local ectothermic or environmental baselines more closely.

Taken together, these chapters underscore how diagenesis and taphonomic alteration do not act uniformly across different fossil substrates or isotope systems. Across the dissertation, the central methodological result is that visible microstructural preservation does not guarantee geochemical fidelity, and no single screening proxy is sufficient. Integrating multiple methods, including petrography, crystallography, spectroscopy, conventional isotopes, clumped isotopes, and explicit kinetic or thermodynamic models can identify plausible primary signals while revealing where burial, fluid exchange, mineralogy, or analytical limitations preclude strong biological, tectonic, or climatic inference. Ultimately, while this approach recovers paleobiological insights, such as variable regional endothermy in apex sharks, and the high metabolic demands that preceded the selective extinction of Cretaceous marine giants, its primary value lies in demonstrating how advanced taphonomic evaluation can unlock paleoclimates, paleobiological change, and post-depositional tectonic histories.

The dissertation of Jade Knighton is approved.

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University of California, Los Angeles

2026

DEDICATION

I dedicate this thesis to my daughters, who I hope will follow their passions to the ends of the Earth as I have. I also dedicate this work to every person who lent me a helping hand, whether it was babysitting or taking a chance on me, I could have never accomplished this without your help.

CONTENTS

ABSTRACT OF THE DISSERTATION.....	ii
DEDICATION	vi
LIST OF FIGURES	xiv
LIST OF TABLES	xv
ACKNOWLEDGEMENTS.....	xvi
VITA.....	xvii
PUBLICATIONS	xvii
FIELD OF STUDY	xvii
INTRODUCTION	1
Chapter 1 INTEGRATED ISOTOPIC (Δ_{47} , $\Delta^{17}\text{O}$), PETROGRAPHIC, AND RAMAN SPECTROSCOPIC ANALYSES OF DIAGENETIC VERSUS PALEOBIOLOGICAL SIGNALS IN CRETACEOUS FOSSIL EGGSHELLS.....	4
Abstract	4
Plain Language Summary.....	5
1 Introduction	5
1.1 Background on clumped isotope paleothermometry	7
1.1.1 Challenges in applying clumped isotopes to eggshells	8
1.2 Overview of this study	12
2 Methods	13
2.1 Sample locality and geological setting	13
2.1.1 Udan Sayr, Mongolia (Djadokhta Formation)	13
2.1.2 Alberta, Canada (Oldman Formation).....	14

2.1.3 Comparative context.....	14
2.1.4. Comparative physiology of taxa.....	15
2.2 Sample preparation	15
2.3 Material preservation analyses	16
2.3.1 Petrography	16
2.3.2 Cathodoluminescence (CL)	16
2.3.3 Scanning electron microscopy (SEM).....	17
2.3.4 Raman spectroscopy	17
2.4 Stable isotope analysis	17
2.4.1 Clumped isotope analysis.....	17
2.4.2 Triple oxygen analysis	19
2.5 Data handling	21
2.5.1 Tripathi-Eagle Lab standards and procedures.....	21
2.5.2 Data cleaning and quality control.....	21
3 Results	22
3.1 Petrographic sample preservation analyses	22
3.2 Geochemical analyses	24
4 Discussion	26
4.1 Preservation and Signal Fidelity.....	26
4.2 Geochemical Reliability and Diagenetic Overprints	28
4.3 Dual-clumped Isotope Insights.....	28
4.4 Implications for Dinosaurian Thermophysiology, Diet, and Paleoenvironments ...	29
4.5 Limitations and Future Directions.....	29

5 Figures.....	31
6 Tables.....	36
Supporting information for: Chapter 1. Diagenetic and biological signals in fossil eggshells	40
Supplementary material	40
Supplementary figures	43
Chapter 2 CLUMPED-ISOTOPE (Δ_{47}) THERMOMETRY OF PALEOSOL CARBONATES REFLECTS MULTI-KILOMETER MESOZOIC BURIAL AND LARAMIDE EXHUMATION OF THE PERMIAN BERNAL FORMATION, NEW MEXICO	56
Abstract	56
Introduction.....	56
1.1 Geologic Setting	57
1.2 Tectonic Setting.....	58
1.3 Paleontology and the Permian-Triassic Boundary	58
1.4 Post-Depositional and Burial History	59
Materials and methods	59
2.1 Sampling	59
2.2 Plane Polarized and Cross-Polarized Light Petrography	59
2.3 Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (EDAX/EDS).....	59
2.4 Isotope Ratio Mass Spectrometry	60
2.5 Modeling.....	60

Results.....	61
3.1 Petrography	61
3.2 Clumped and Bulk Isotopes	61
3.3 Elemental Mapping (EDAX/EDS)	61
3.4 Geochemical Kinetic Modeling	62
Discussion	63
4.1 Interpretation of Temperature Variability	63
4.2 Interpretation of Isotopic Relationships and Clustering	64
4.3 Limitations	65
4.4 Local Geological Context and Contributions to Regional Understanding.....	66
Acknowledgements	66
References	67
Figures.....	71
Tables.....	78
Chapter 3. THERMOREGULATION AND ECOLOGY OF GIANT MARINE	
PREDATORS OF THE MESOZOIC	81
Key Points:	81
Abstract	82
Plain Language Summary:	84
1 Introduction.....	85
1.1 Background	88
1.1 Challenges in applying clumped isotopes to bioapatite.....	91
1.2 Overview of this study	92

2 Methods.....	93
2.1 Sample locality and geologic setting	93
2.2. Comparative Context.....	95
2.3 Sample preparation	98
2.3.1 Sample collection.....	98
2.3.2 Sample cleaning	99
2.3.3 Sample preparation for clumped isotope paleothermometry	99
2.3.4 Sample preparation for oxygen phosphate analysis.....	99
2.4 Material preservation analyses.....	100
2.4.1 Fourier transform infrared spectroscopy (FTIR)	100
2.4.2 Comparative geochemistry	101
2.4.2.1 $\delta^{18}\text{O}_{\text{phosphate}}$ ($\delta^{18}\text{O}_p$)	101
2.4.2.2 Seawater temperature estimation.....	102
2.4.2.3 Statistical analyses	102
2.5 Stable isotope analysis.....	103
3 Results.....	105
3.1 Evaluation of sample preservation by FTIR	105
3.2 Evaluation of preservation through geochemical analyses	106
3.2.1 Stable isotope results.....	108
3.2.2 Ocean paleotemperature estimation from carbonate clumped isotopes	108
3.2.3 Vertebrate enamel carbonate moiety geochemistry and body temperature estimation	109
3.2.4 Statistical analyses.....	111

4 Discussion	113
4.1 Evaluation of Sample Preservation and Diagenetic Signals	113
4.2 Late Cretaceous Lamniform Bioenergetics in Macroevolutionary Context.....	114
4.3. Carbon Isotope ($\delta^{13}\text{C}$) Signatures	116
The carbon isotope ($\delta^{13}\text{C}$) values obtained from the enameloid phases provide complementary evidence regarding carbon sourcing within these Late Cretaceous marine ecosystems. Because $\delta^{13}\text{C}$ signatures vary minimally during trophic transfer, they primarily reflect the isotopic baseline of primary producers at the base of the local food web, which varies dynamically between nearshore, offshore, pelagic, and benthic environments.	
4.4 Ecological stratification and niche partitioning in Late Cretaceous oceans.....	117
4.6. Comparative Thermophysiology Across Extinct and Modern Marine Amniotes and Chondrichthyans	122
ACKNOWLEDGEMENTS.....	124
OPEN RESEARCH	124
5 Figures.....	124
6 Tables.....	131
Supporting information for:	141
Supplementary figures	141
Supplementary tables.....	142
References	150
SUMMARY	165
REFERENCES.....	167

APPENDIX I	167
APPENDIX II	184
APPENDIX III	203

LIST OF FIGURES

Figure 1.1	31
Figure 1.2	33
Figure 1.3	34
Figure 1.4	35
Figure 1.5	36
Figure 2.1	71
Figure 2.2	72
Figure 2.3	74
Figure 2.4	75
Figure 2.5	76
Figure 2.6	77
Figure 3.1	125
Figure 3.2	126
Figure 3.3	127
Figure 3.4	128
Figure 3.5	129
Figure 3.6	130
Figure 3.7	131

LIST OF TABLES

Table 1.1	37
Table 1.2	37
Table 1.3	38
Table 2.1	78
Table 2.2	79
Table 2.3	79
Table 2.4	80
Table 3.1	131
Table 3.2	132
Table 3.3	133
Table 3.4	138

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McCormack, J., Maisch IV, H., Becker, M. A., Griffiths, M. L., **Knighton, J.**, Eagle, R. A., ... and Shimada, K. (2026). Sharks as apex predators in the Late Cretaceous Western Interior Seaway empirically revealed by zinc isotope analyses. *Gondwana Research*. Volume 155, July 2026, Pages 227-239.

FIELD OF STUDY

Major Field: Geology

Studies in Paleobiology and Paleoecology

Professor Aradhna K. Tripathi

INTRODUCTION

Paleobiology has traditionally relied on morphology and bone histology to reconstruct the biology of extinct organisms, while the evolution of sedimentary facies and their mineralogy has been used to study past climate and tectonics. Geochemical analyses now provide complementary information because minerals can record aspects of formation temperature and fluid composition, and in biominerals, these conditions as well as diet and trophic ecology. However, diagenesis, recrystallization, kinetic isotope effects, and uncertain physiological fractionations can modify the measured signal. The chapters that follow therefore pair isotope measurements with independent preservation screens and explicitly evaluate the assumptions required for paleobiological or tectonic interpretation.

Chapter 1 examines conventional carbon- and oxygen-isotope ratios together with carbonate clumped isotopes (Δ_{47} , dual-clumped isotopes ($\Delta_{47}-\Delta_{48}$), and triple oxygen isotopes ($\Delta^{17}\text{O}$) in fossil eggshells from Mongolia and Devil's Coulee in the Oldman Formation of Alberta, Canada. Petrography, cathodoluminescence, EBSD, Raman spectroscopy, and trace-element measurements are used to assess preservation. The objective is to determine which specimens, if any, retain interpretable biomineralization-temperature, body-water, and dietary signals.

Chapter 2 investigates the post-depositional thermal history of paleosol carbonates from the Permian Bernal Formation and the Triassic Anton Chico Member at Bernal Mesa, New Mexico. Rather than reconstructing the end-Permian extinction itself, the chapter uses petrography, elemental mapping, Δ_{47} thermometry, and reordering models to evaluate burial resetting and to estimate plausible maximum burial temperatures and overburden across strata that bracket the Permian–Triassic unconformity.

Chapter 3 evaluates thermophysiological and ecological hypotheses for Late Cretaceous marine vertebrates from New Jersey and Kansas. Phosphate oxygen isotopes and structural-

carbonate Δ_{47} measurements from teeth are compared with co-occurring ectothermic vertebrates and carbonate-shell temperature estimates. FTIR spectra and carbonate–phosphate isotope offsets provide preservation checks. The two assemblages differ in age and depositional setting, so locality-specific baselines and uncertainty in seawater composition are treated explicitly.

Together, these studies ask a common question: under what conditions can geochemical measurements from ancient carbonates and bioapatites be interpreted as primary environmental or biological signals? The dissertation emphasizes screening, uncertainty propagation, and comparison among independent proxies. This framework is broadly relevant to deep-time climate, physiology, ecology, and basin evolution, especially where later alteration can mimic or obscure the signal of interest.

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Renne, P. R., et al. (2013). "Time scales of critical events around the Cretaceous–Paleogene boundary." *Science*, 339(6120), 684-687.

Chapter 1 INTEGRATED ISOTOPIC (Δ_{47} , $\Delta^{17}\text{O}$), PETROGRAPHIC, AND RAMAN
SPECTROSCOPIC ANALYSES OF DIAGENETIC VERSUS PALEOBIOLOGICAL SIGNALS IN
CRETACEOUS FOSSIL EGGSHELLS

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Abstract

Fossil eggshells are potentially valuable archives of dinosaur and early bird physiology, diet, and paleoenvironments, yet their geochemical fidelity is often compromised by diagenesis. Here we utilize a combined approach of petrographic (polarized light microscopy, electron back scatter diffraction, cathodoluminescence), Raman spectroscopy, and stable isotope Δ_{47} , $\Delta^{17}\text{O}$ to evaluate preservation of 8 Late Cretaceous eggshell samples from North America and Mongolia. Petrographic analyses show that the majority of samples retain some well-preserved microstructural features, with the preservation in specimens from Devil's Coulee (Alberta, Canada) demonstrating this the most clearly in EBSD analysis. Clumped isotope (Δ_{47}) temperatures range from 21.2°C to 61.5°C, with elevated values (48.5, 61.5°C) even in petrographically well preserved fossils, indicating partial resetting during burial. Oxygen isotope and $\Delta^{17}\text{O}$ data are indicative of diagenetic interaction with meteoric fluids in many, but not all, eggshells with some specimens preserving negative $\Delta^{17}\text{O}$ values. In contrast, carbon isotope ($\delta^{13}\text{C}$) values (−13.71‰ to −3.66‰ VPDB) are more robust and consistent with a C3 vegetation-dominated diet. In the best preserved samples, including those with negative $\Delta^{17}\text{O}$ values, $\delta^{13}\text{C}$ was measured at −6.16‰ to −4.18‰ VPDB. $\delta^{18}\text{O}$ for these same samples ranged from −12.43‰ to −8.21‰ VPDB, and clumped isotope temperatures range from 21.2°C to 31.5°C. These results emphasize the importance of rigorous multi-proxy screening for eggshell isotope studies, but also indicate that diagenesis does not obscure primary isotopic signatures in all cases in fossils of this age. The Mongolian material provides the most reliable petrographic

signals, as evidenced by good petrographic preservation as well as negative $\Delta^{17}\text{O}$ values, with Δ_{47} temperatures estimated at 21.2 to 27.8 °C for oviraptorids, and 31.5 °C for the hadrosaur.

Plain Language Summary

Dinosaur eggshells can preserve chemical clues about how these ancient animals lived, what they ate, and the environments they inhabited. In this study, we examined 8 eggshells from the Late Cretaceous period (about 75–66 million years ago) using advanced microscopic and chemical techniques to check how well they had been preserved over millions of years. Many eggshells still show their original microscopic structure, especially those from Mongolia. However, almost all the fossils examined have been chemically altered to some degree by groundwater and burial processes after they were buried. This alteration makes it difficult to interpret temperature readings taken from the shells as primary, as some appear much hotter than is biologically plausible. The most reliable information comes from carbon isotopes, which suggest the dinosaurs mostly ate plants similar to modern trees and shrubs. The best-preserved eggshells from Mongolia give the clearest picture and point toward hadrosaurs that maintained relatively warm body temperatures, and oviraptorids which had cooler temperatures. This research shows that while dinosaur eggshells are promising for studying ancient life, scientists must carefully check each sample for damage before drawing conclusions. With proper screening, these fossils can still reveal important details about dinosaur biology and ecosystems.

1 Introduction

Stable carbon and oxygen isotope ratios preserved in fossil eggshell have long been utilized as proxies for reconstructing dinosaur paleobiology, diet, and paleoenvironmental conditions. However, the inherent porosity of eggshells, which facilitates gas exchange during embryonic development, also renders it highly susceptible to post-depositional diagenetic

alteration by groundwater. In their study of dinosaur eggshell from the Campanian–Maastrichtian Baruungoyot and Nemegt formations of the Gobi Desert, Mongolia, Graf et al. (2018) documented pervasive diagenesis through petrographic, cathodoluminescence, and stable isotope analyses. Their results revealed that $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values in the eggshells plot along trends indicative of vadose and phreatic zone alteration, often converging with paleosol carbonate signatures influenced by interaction with older calcareous rocks. Consequently, none of the analyzed samples retained sufficiently pristine isotopic compositions for reliable paleoenvironmental or paleoecological interpretations using conventional techniques, highlighting the critical need for rigorous diagenetic screening when working with fossil eggshell material.

Thermoregulation in non-avian dinosaurs and their close relatives, the basal Aves, has long been an enduring question in vertebrate paleobiology. Early studies suggested that non-avian dinosaurs primarily utilized an ectothermic modality similar to modern reptiles (Bakker, 1972; Farlow, 1990). In more recent decades, studies on paleobiology and isotopic geochemistry of dinosaurs have revealed a more nuanced picture, indicating that many dinosaurian clades possessed the ability to regulate their own body temperature, to varying degrees, ranging from homeothermy to regional or sustained endothermy (Eagle et al., 2015; Dawson et al., 2020; Grady et al., 2014). Even more recently, biomolecular evidence suggests that the evolution from ectothermy to endothermy or mesothermy for those clades may not have been totally linear, and that secondary ectothermy may have evolved in some ornithischian lineages (Wiemann et al., 2022). While the study of thermoregulation in ancient Aves and their relatives is a broad question with many lines of potential interrogation, the prominence of this group throughout the Mesozoic makes this study important in the evolution and history of life on Earth. Because eggshells are secreted at or near maternal body temperature, and are abundant

across clades and paleoenvironments, they provide a valuable archive for testing these hypotheses (Eagle et al., 2015; Dawson et al., 2020).

Traditional reconstructions of thermoregulatory strategy have relied heavily on bone histology and conventional oxygen-isotope paleothermometry ($\delta^{18}\text{O}$). These approaches can provide valuable constraints but depend on assumptions about growth, environmental-water composition, physiology, and diagenesis (Dawson et al., 2020; Eagle et al., 2015). Carbonate clumped-isotope (Δ_{47}) thermometry can estimate mineral-formation temperature without independently specifying the $\delta^{18}\text{O}$ of the parent water, but it remains sensitive to kinetic effects, mixing, solid-state reordering, and fluid-mediated recrystallization. It is therefore complementary to, rather than a replacement for, other thermophysiological and preservation evidence (Dawson et al., 2020; Eagle et al., 2020; Eiler, 2007; Ghosh et al., 2006).

1.1 Background on clumped isotope paleothermometry

Carbonate clumped isotope paleothermometry is based on the temperature-dependent excess abundance of carbonate groups containing both ^{13}C and ^{18}O relative to a stochastic isotope distribution (Ghosh et al., 2006; Shauble et al., 2006). At lower temperatures, the lower zero-point vibrational energy of heavy-isotope bonds favors greater isotopic ordering; the excess abundance of mass-47 CO_2 released during acid digestion therefore generally decreases as formation temperature increases (Eiler, 2007). Because the temperature signal is encoded in intramolecular isotope ordering, Δ_{47} thermometry does not require an assumed bulk $\delta^{18}\text{O}$ value for the parent fluid. Primary ordering can be overprinted by post-depositional diagenetic effects such as solid-state reordering or fluid-mediated recrystallization, which must be evaluated when interpreting ancient carbonates (Dennis and Schrag, 2010; Eagle et al., 2015; Passey and Henkes, 2012; Stolper and Eiler, 2015).

1.1.1 Challenges in applying clumped isotopes to eggshells

The successful application of clumped isotope paleothermometry (Δ_{47}) to fossil eggshells hinges on the ability to recover accurate *in vivo* mineralization temperatures reflecting the body temperature of the egg-laying animal. Because eggshell calcite forms through biological processes at relatively low temperatures, even minor diagenetic overprinting can reset clumped isotope values toward higher apparent temperatures characteristic of burial conditions. Consequently, rigorous, multi-proxy diagenetic screening is indispensable before interpreting Δ_{47} data (Dennis and Schrag, 2010; Eagle et al., 2015; Passey and Henkes, 2012; Stolper and Eiler, 2015). Landmark studies have combined petrographic observations, geochemical analyses, and advanced microstructural techniques to distinguish primary biogenic signals from secondary alteration (Choi et al., 2020; Choi et al., 2021; Choi et al., 2022; Dawson et al., 2020; Eagle et al., 2015; Ingalls, 2021).

Traditional screening methods remain foundational. Petrographic thin-section analysis and scanning electron microscopy (SEM) reveal textural evidence of diagenesis, including crystal coarsening, pore infilling by secondary minerals, dissolution-reprecipitation features, and destruction or recrystallization of original eggshell microstructures such as mammillary cones, prismatic columns, or squamatic ultrastructure. Cathodoluminescence (CL) microscopy and trace elemental analysis (e.g., via electron probe microanalysis or LA-ICP-MS) further identify diagenetic fluids: elevated manganese (Mn) or iron (Fe) concentrations often indicate interaction with meteoric or anoxic pore waters, while strontium (Sr) depletion signals open-system exchange with burial fluids that can alter both elemental and isotopic compositions (Dawson et al., 2020; Eagle et al., 2015). These approaches effectively flag many altered samples but may miss cryptic or low-temperature diagenesis that nevertheless affects clumped isotope distributions.

In recent years, Raman spectroscopy has emerged as a powerful, non-destructive tool for detecting thermal alteration and organic matter maturation in fossil eggshells. In a seminal 2020 study, Choi et al. applied micro-Raman spectroscopy to an enigmatic dark-banded egg from the Upper Cretaceous Wido Volcanics of South Korea. Raman spectra revealed prominent D (disordered) and G (graphite) bands characteristic of amorphous carbon, demonstrating that the dark coloration and banding resulted from thermally altered organic matter rather than primary pigmentation or mineralogical features. This work established Raman spectroscopy as a reliable method for identifying preserved amorphous carbon in fossil eggshells and introduced the use of Raman parameters (e.g., band positions, intensities, and widths) to estimate the thermal maturity and maximum paleotemperatures experienced by specimens. Subsequent research by the same group generalized these observations across volcanic-influenced deposits, showing that Raman spectroscopy can detect thermally altered organic matter even in eggshells that appear macroscopically well-preserved (Choi et al., 2020; Choi et al., 2021). In their 2021 paper on fossil eggshells of amniotes as a paleothermometry tool, Choi and colleagues integrated Raman data with FTIR spectroscopy and EBSD to develop a multi-proxy framework. They demonstrated that Raman spectroscopy not only identifies diagenetic carbon but also supports quantitative paleotemperature estimates via Raman spectroscopy of carbonaceous material (RSCM) techniques, previously applied more commonly to metamorphic rocks. These studies underscore that eggshells, with their intimate association of calcite and organic matrix, are particularly well-suited for RSCM because the organic component can record thermal histories that complement mineralogical proxies.

Complementing Raman work, electron backscatter diffraction (EBSD) has provided unprecedented insight into the crystallographic and microstructural integrity of eggshells. Seung Choi and colleagues have been at the forefront of applying EBSD to both modern and fossil eggshells. In a 2019 study, Choi et al. conducted detailed EBSD mapping of maniraptoran

dinosaur eggshells, focusing on calcite grain orientations, grain boundary geometries (low-angle vs. high-angle, linear vs. rugged), and crystallographic preferred orientations across different ultrastructural zones. This approach allows us to distinguish primary biogenic growth patterns, such as the radiating c-axis orientations in mammillary layers or the characteristic squamatic textures, from diagenetic recrystallization, which typically produces more equant grains, randomized orientations, or anomalous grain boundary migrations. Building on this, Choi's group published a dedicated protocol for EBSD analysis of fossil eggshells (Choi et al., 2023), outlining best practices for sample preparation, data collection, and interpretation to avoid artifacts. Their work has demonstrated EBSD's ability to detect subtle diagenetic changes invisible under standard SEM or petrography, such as partial recrystallization that could affect clumped isotope values without wholesale textural destruction. When integrated with Raman spectroscopy, EBSD provides a robust crystallographic and thermal maturity assessment, as seen in studies where both techniques corroborate the presence or absence of significant burial alteration (Choi et al., 2021).

Triple oxygen isotope ($\Delta^{17}\text{O}$) analysis offers an independent geochemical avenue for assessing the fidelity of primary isotopic signals in eggshell carbonate. Unlike conventional $\delta^{18}\text{O}$, which is sensitive to both temperature and water composition and easily reset by diagenetic fluids, $\Delta^{17}\text{O}$ captures mass-independent fractionation effects that are highly resistant to equilibrium exchange. Eggshell carbonate derives its oxygen from the mother's body water, which incorporates a strong atmospheric O_2 signal during respiration. Atmospheric molecular oxygen carries a characteristic negative $\Delta^{17}\text{O}$ anomaly (typically on the order of hundreds of per meg) resulting from stratospheric photochemical reactions and terrestrial biosphere influences (Hare et al., 2025; Passey, 2021). This anomaly is transferred to eggshell calcite, with additional modulation by evaporative processes in body water and plant transpiration, though the latter primarily affects mass-dependent ($\delta^{18}\text{O}$) components. Diagenetic alteration by pore waters,

which generally lie near the global meteoric water line (GMWL) with near-zero $\Delta^{17}\text{O}$, tends to erase or dilute this atmospheric signature. Therefore, the preservation of a primary negative $\Delta^{17}\text{O}$ value in fossil eggshells serves as compelling evidence of minimal isotopic resetting and retention of original body-water chemistry (Hare et al., 2025; Wostbrock et al., 2020).

Recent work by Hare et al. (2025) has substantially advanced the application of triple oxygen isotopes to eggshells, analyzing modern and fossil ratite eggshell carbonate to reconstruct late Cenozoic atmospheric pCO_2 and global primary productivity (GPP). Their study demonstrated that eggshell $\Delta^{17}\text{O}$ reliably records atmospheric influences mediated through animal physiology, while also serving as a sensitive diagenetic filter. When combined with traditional $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ data, $\Delta^{17}\text{O}$ helps “see through” alteration by identifying samples that retain the distinctive non-mass-dependent signature absent in most meteoric or burial fluids. Earlier foundational studies on triple oxygen isotopes in carbonates and biominerals (e.g., Wostbrock et al., 2020) provided the theoretical and empirical framework showing how $\Delta^{17}\text{O}$ vs. $\delta^{18}\text{O}$ trends can diagnose fluid-rock interaction, further supporting its use in eggshell screening.

A recent study by Tagliavento, et al. found that *Troödon* was able to achieve body temperatures comparable to modern endotherms, but also employed a heterothermic strategy like extant birds (2023). Dual-clumped data is also able to give insight on the mineralization of the eggshell during formation (Tagliavento et al., 2023). Tagliavento et al. found that the mineralization of *Troödon* eggshells was more similar to reptile eggshells than birds, meaning that their reproductive structure likely reflected this with two functional ovaries that produced a limited number of eggs in each clutch (2023). Such information is invaluable in the study of extinct species.

This study builds upon these advances by conducting a comprehensive, integrated analysis of microscopic (petrography, SEM, CL, Raman spectroscopy, EBSD) and geochemical

(trace elements, triple oxygen isotopes) techniques across a diverse suite of Cretaceous eggshells. The specimens span varied depositional environments and ootaxa, enabling evaluation of how diagenetic pathways differ by setting and eggshell type. By establishing refined, multi-proxy criteria for signal fidelity, this work aims to provide a robust methodological foundation for confident application of clumped isotope thermometry to well-preserved fossil eggshells, ultimately advancing our understanding of dinosaurian and avian thermoregulation in deep time.

1.2 Overview of this study

Cretaceous eggshells across a variety of global localities representing diverse depositional environments and ootaxa were collected from two key locations including Udan Sayr, Mongolia (Djadokhta Formation) and Alberta, Canada (Oldman formation) (e.g., Eagle et al., 2015; Dawson et al., 2020; Zelenitsky et al., 1996). Both units are contained within Late Cretaceous strata, with some oospecies overlapping between localities for more direct comparison (Czepiński, 2020; Chiappe et al., 2001; Díaz-Martínez et al., 2022; Eberth and Hamblin, 1993; Jerzykiewicz and Russell, 1991; Krumenacker, 2010; Krumenacker et al., 2016; Rogers, 1998; Rogers, 2016; Rogers, 2024; Salgado et al., 2007; Saneyoshi et al., 2021). This design allows for in-depth examination of diagenetic and thermal alteration, while respecting factors related to depositional environment or possible species-level effects. This study constitutes a particularly comprehensive examination of assessment fidelity, by including microscopic and geochemical methodology, both standard and emerging. The resulting screening framework will allow identification of a subset of well-preserved specimens suitable for reliable clumped isotope (Δ_{47}) paleotemperature reconstructions and thermoregulatory inferences. Ultimately, this study contributes to past and future paleobiology studies of non-avian dinosaurs through eggshell analyses by elucidating the strengths and limitations of different proxies, and may serve to advise future studies in design for optimal biological signal

clarity. Here, we apply a comprehensive multi-proxy diagenetic screening including petrography, EBSD, Raman, CL, and $\Delta^{17}\text{O}$ to 8 eggshell samples spanning three continents

2 Methods

This study utilizes a suite of complementary methods for diagenetic assessment of the fossil material, including petrography, cathodoluminescence (CL), electron backscatter diffraction (EBSD), and Raman spectroscopy. Each method was applied to each specimen studied.

2.1 Sample locality and geological setting

Two sample localities were investigated in this study including the Djadokhta Formation of Mongolia and the Oldman Formation of North America (Table 1.1). Both of these localities represent distinct paleoecological and depositional settings, allowing for carbonate preservation comparisons across differing terrestrial environments.

2.1.1 Udan Sayr, Mongolia (Djadokhta Formation)

The Djadokhta Formation in the Gobi Desert of southern Mongolia is dated to the Campanian, roughly 75 Ma based on biostratigraphic and magnetostratigraphic data (Czepiński, 2020; Jerzykiewicz and Russell, 1991; Saneyoshi et al., 2021; Watabe et al., 2010). The formation is dominated by reddish-orange to buff-colored aeolian sandstones, interdune mudstones, and minor fluvial deposits, representing a semi-arid to arid desert environment with large dune fields, ephemeral streams, and occasional playa lakes (Dingus et al., 2008). This depositional setting includes both aeolian-dominated facies with localized fluvial influences (Dingus et al., 2008).

Abundant fossil eggshells are known from this location, primarily attributed to oviraptorids (*Elongatoolithidae*) and protoceratopsians, along with associated skeletal material

of *Protoceratops andrewsi* and *Pinacosaurus* (Dingus et al., 2008). The arid climate and rapid burial in an aeolian landscape or flash-flood deposits provided an ideal preservation potential of delicate structures (Dingus et al., 2008).

2.1.2 Alberta, Canada (Oldman Formation)

Three fossil eggshells were recovered from Devil's Coulee in the Oldman Formation, which is an exposed badland in southern Alberta (Currie, 1988). This unit is middle to late Campanian and represents a coastal alluvial plain setting along the western margin of the Western Interior Seaway. Sediments are primarily mudstones, siltstones, and fine- to medium-grained sandstones, indicative of overbank floodplain deposits, crevasse splays, and minor channel fills. Periodic flooding buried dinosaur nests in mud and silt, facilitating preservation potential through rapid burial (Eberth and Hamblin, 1993).

Devil's Coulee is a significant dinosaur nesting site in Canada, yielding primarily hadrosaur and theropod nests (*Spheroolithus*, *Prismatoolithus*). The fossils occur in fine-grained overbank facies where calcareous nodules and pedogenic features can also be found. The humid, seasonally wet climate and location along the seaway edge likely influenced early diagenesis in fossils from Devil's Coulee (Dingus, 2008; Zelenitsky and Hills, 1997; Zelenitsky et al., 1996). The Oldman formation is part of the broader Judith River Group, which crops out in northern Montana and represents a time-equivalent, laterally adjacent unit on the U.S. side (Rogers, 2016; Zelenitsky and Hills, 1997; Zelenitsky et al., 1996). Records from this region indicate a humid, seasonally wet climate influenced by proximity to the seaway, with abundant vegetation to support herbivorous and carnivorous dinosaur communities (Rogers, 1998; Rogers, 2016).

2.1.3 Comparative context

The suite of eggshells within this study constitute a range of terrestrial deposition settings with various degrees of coastal influence. The Oldman samples represent a humid, coastal-influenced floodplain, in contrast with semi-arid to arid paleoenvironments of Southern Mongolia (Dingus et al., 2008; Eberth and Hamblin, 1993; Rogers, 1998; Rogers, 2016) . These differences in depositional setting provide a useful framework for evaluating how local deposition patterns and early diagenetic conditions affect the preservation of eggshell carbonate and the geochemical signals with the fossils.

2.1.4. Comparative physiology of taxa

Oviraptorids (*Elongatoolithus* and *Macroolithus*) and hadrosaurs (*Sphaeroolithus* spp.) are interpreted as having differed in their reliance on drinking water based on body size, dietary ecology, and habitat preferences reported in the literature (Horner et al., 2004; Weishampel and Horner, 1990; Eagle et al., 2015). Smaller-bodied oviraptorids are often considered more capable of obtaining water from food sources and metabolic water in arid to semi-arid environments, consistent with interpretations of maniraptoran physiology (Eagle et al., 2015; Dawson et al., 2020). In contrast, large-bodied hadrosaurs, as herbivores, likely required more regular access to surface water (Horner et al., 2004; Weishampel and Horner, 1990). Because several Mongolian specimens are listed only as “Mongolia / Cretaceous” without precise formation-level provenance, it is not possible to assign all samples to a single paleoclimatic regime. Therefore, drinking-mode interpretations are treated cautiously and used primarily as a framework for evaluating isotopic patterns rather than a definitive classification. This approach helps contextualize body-water signals without over-relying on unverified locality-specific climate assumptions.

2.2 Sample preparation

Each sample underwent preparation consisting of sonication with ultra-pure deionized water to remove any loose matrix or debris. Sonication was performed for 5 minutes in 1 minute bursts. Eggshell fragments were then dried in an oven at 40 °C overnight, broken in half, and one half was sent for polished sectioning. The other halves were pulverized using an agate mortar and pestle if they were loose, if they were attached to a matrix layers were carefully drilled off using a dental drill at low speed. All samples underwent light drilling at a low speed on the outer layers as well to remove any potential pore infill. Powders for clumped isotope analysis underwent an additional hydrogen peroxide wash for 3h to remove any organics. H₂O₂ treated powders were then triple washed using ultra-pure deionized water and dried in an oven at 40 °C.

2.3 Material preservation analyses

2.3.1 Petrography

Polished sections were photographed in the Experimental Petrology (PTX) Lab at the University of California, Los Angeles (UCLA) using an Olympus microscope equipped with a camera and Stream Basic software. The lens was adjusted to 5x to 10x magnification, depending on the thickness of the eggshell cross-section.

2.3.2 Cathodoluminescence (CL)

Cathodoluminescence (CL) was performed at the Corsetti Lab at the University of Southern California (USC) using an Olympus BX60 microscope equipped with an ELM3R Luminoscope CL system. Careful consideration was taken to image the same sections of each eggshell to match with petrographic photomicrographs for optimal comparison between figures. Images were taken at 4x magnification, and exposure times varied between samples depending on the amount of activator elements (such as Mn), with lower exposure times

needed for very bright, high activator elements visible in the section (Barbin and Gaspard, 1995).

2.3.3 Scanning electron microscopy (SEM)

Scanning electron microscopy was performed at Seoul National University in the lab managed by Dr. Sungkeun Lee. Sections were imaged in electron backscatter diffraction (EBSD), grain boundary, inverse pole figure (IPF, X, Y, and Z axes), and Euler modes. Similarly, sections were imaged in the same sites as petrographic and CL images for direct comparison.

2.3.4 Raman spectroscopy

Raman spectroscopy was performed on each polished section as well to further probe thermal maturity of specimens. This technique relies on looking for evidence of microstructure destruction and possible geochemical reordering, which is suggested by the presence of disordered (D) and graphite (G) bands. Absence of these bands is typically interpreted to be suggestive of well-preserved specimens (Choi et al., 2020). Raman was performed at Seoul National University in the Lee Lab as well.

2.4 Stable isotope analysis

2.4.1 Clumped isotope analysis

Each sample was powdered for stable isotope analysis. Each sample underwent $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, Δ_{47} , and $\Delta^{17}\text{O}$ analysis. Aliquots of cleaned sample were measured from 0.42-0.52 mg or 5.8-6.5 mg depending on mass spectrometer, with aliquots being measured on three different mass spectrometers within the Triпати-Eagle Isolab. Samples were measured using a Thermo-MAT Finnegan 253 dual-inlet gas source mass spectrometer as well as two Nu Perspective machines as described in Upadhyay, et al., 2021 and Lucarelli et al., 2023, which have been shown to yield results statistically indistinguishable from one another to ensure high rates of

reproducibility. Samples were analyzed from 2020 to 2026. Every 4 to 5 sample measurements, a carbonate standard was used and Δ_{47} values are reported on the Inter-Carbon Dioxide Equilibrium Scale (I-CDES90, Bernasconi et al. 2021) at 90 °C. Nine standards were used, including ETH-1, ETH-2, ETH-3, ETH-4, CM Tile, Carmel Chalk, TV-ST, TV-BT, and Veinstrom (Upadhyay et al., 2021; Lucarelli et al., 2023). ETH-1 and ETH-2 were used for non-linearity corrections while ETH-1, ETH-2, and ETH-3 and the remaining in-house standards were utilized for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ drift corrections and empirical transfer function (ETF) calculations. ETH-4 was used as a consistency standard and not included in any standard corrections. For drift, nonlinearity, and ETF corrections either 10 or 20-point moving averages were used for each correction interval, following published studies, depending on the drift correction interval (Meckler et al., 2014; Upadhyay et al., 2021; Lucarelli et al., 2023). The Brand parameter set was used for calculations (Brand et al., 2010; Brand et al., 2014; Daëron et al., 2019; Petersen et al., 2019). Calculations to determine Δ_{47} , $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$ use publicly available software (Easotope, John and Bowen, 2016).

Combining Δ_{47} data with Δ_{48} data allows for study of kinetic effects as well as refine temperature data, due to the characteristic relationship the proportions have to disequilibrium (Tripathi et al., 2015; Fiebig et al., 2019; Guo, 2020; Bajnai et al., 2020; Lucarelli et al., 2023). Dual-clumped isotope measurements (Δ_{47} and Δ_{48}) were performed on a subset of well-preserved eggshell samples as well as extant Reptilian and Avian taxa to further evaluate kinetic effects during biomineralization and potential diagenetic alteration. Δ_{48} provides an independent constraint on disequilibrium processes because it is sensitive to both temperature and kinetic fractionation during CO_2 hydration and hydroxylation (Hill et al., 2014; Bajnai et al., 2020; Lucarelli et al., 2023). When combined with Δ_{47} , dual-clumped data can distinguish between equilibrium formation at body temperature and disequilibrium effects or partial reordering (Tagliavento et al., 2023; Parvez et al., 2024). Analyses followed protocols

established in Parvez et al. (2023) and Lucarelli et al. (2023). Powdered eggshell samples (0.4–0.6 mg) were reacted with 105% phosphoric acid at 70°C in a common acid bath. Evolved CO₂ was purified cryogenically and analyzed on a Thermo MAT 253 or Nu Perspective isotope ratio mass spectrometer in the Triпати-Eagle Isolab. Data were corrected for nonlinearity, drift, and scale compression using 10- or 20-point moving averages, following Meckler et al. (2014), Upadhyay et al. (2021), and Lucarelli et al. (2023). All values are reported on the I-CDES90 scale (Bernasconi et al., 2021). Δ_{48} values were calculated using the Brand et al. (2010, 2014) parameters as implemented in Easotope (John and Bowen, 2016). A full list of extant species data points are available in supplementary information (Table S1).

2.4.2 Triple oxygen analysis

Triple oxygen isotope compositions were measured on powdered eggshell carbonate from two specimens from Devil's Coulee, Alberta, Canada, and six specimens from Udan Sayr, Mongolia. All analyses were performed in collaboration with Dr. Vincent J. Hare and Dr. Sangbaran Ghoshmaulik at the Stable Light Isotope Laboratory, University of Cape Town (UCT), South Africa.

Measurements were taken via tunable infrared laser absorption spectroscopy (TILDAS). Prior to analysis, eggshell fragments were cleaned mechanically to remove adhering sediment. Small subsamples (~5–10 mg) were powdered using an agate mortar and pestle. To minimize potential diagenetic contamination while preserving structural carbonate, powders underwent a mild pretreatment consisting of ultrasonication in ultrapure water for 5 minutes in 1 minute bursts, followed by thorough rinsing and drying at 40°C. This protocol targets removal of organic residues and loosely bound contaminants without significant alteration of the carbonate lattice.

Approximately 0.9–2 mg of pretreated powder was reacted with 100% phosphoric acid (H₃PO₄) at 70°C in a Thermo GasBench II to liberate CO₂ which was then analyzed for $\delta^{13}\text{C}$,

$\delta^{18}\text{O}$, and $\Delta^{17}\text{O}$ by TILDAS using an Aerodyne Research Inc. instrument, following methods described in Hare et al. (2022). This laser-based technique measures the isotopologues of CO_2 with high sensitivity, requiring only $\sim 8\text{--}9\ \mu\text{mol}$ of CO_2 per analysis and offering excellent throughput while achieving external reproducibility of $\sim 4\text{--}10$ per meg ($0.004\text{--}0.010\text{‰}$) for $\Delta^{17}\text{O}$. $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values were measured from laser 1, and $\Delta^{17}\text{O}$ values were measured from laser 2. Samples were measured in duplicate along with NBS18 and IAEA-603 reference standards with $\Delta^{17}\text{O}$ values taken from Wostbrock et al. (2020).

Raw data were corrected for concentration dependence resulting from scale-offset errors due to gas concentration mismatches between sample and working gas, as well as for pressure and temperature offsets, and standardized to the VSMOW-SLAP scale using the calibrated carbonate reference materials (Hare et al., 2022; Griffith et al., 2012; Bajnai and Hare, 2025). Replicates whose pressure mismatch exceeded 0.15 Torr (absolute value), whose temperature mismatch exceeded 0.01 K (absolute value), or that produced fewer than 8 aliquots were excluded. $\Delta^{17}\text{O}$ values were calculated using the linearized form:

$$\Delta^{17}O_{bw} = \delta^{17}O_{bw} - 0.528 \times \delta^{18}O_{bw} \quad (1)$$

where δ' denotes the linearized delta notation ($\ln(1 + \delta)$) and 0.528 is the reference slope for high-temperature terrestrial equilibrium processes. All $\Delta^{17}\text{O}$ values are reported in per meg (parts per million) relative to the VSMOW-SLAP scale.

The same fragments were analyzed for clumped isotopes (Δ_{47}) and conventional stable isotopes ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) to enable paired reconstructions of formation temperature and parent body water composition. $\delta^{13}\text{C}$ values are reported on the VPDB-LSVEC scale. Analytical uncertainties and replicate measurements are reported alongside individual sample results.

$\delta^{18}\text{O}_{\text{BW}}$ at UCT in the Stable Light Isotope Laboratory uses the fundamental conversion framework published in Passey, et al., 2014. To determine the isotopic composition of the body water pool (bw) from which the eggshell precipitated, the triple-oxygen isotope anomaly ($\Delta^{17}\text{O}$) is extracted and expressed in parts per million (per meg). This can then be used to directly map from the eggshell to the fluid without biomineralization overprint, using a tightly defined mineral-fluid triple-oxygen scaling exponent ($\theta_{\text{calcite-water}} \approx 0.524$).

The result is an isolated body water endmember, which can then be projected back along the physiological evaporation trajectory ($\theta_{\text{evaporation}} \approx 0.518$) to determine the unevaporated environmental baseline where it intersects the Local Meteoric Water Line (LMWL). Calculations for body water were performed at UCT.

2.5 Data handling

2.5.1 Tripathi-Eagle Lab standards and procedures

Standard values for each instrumental configuration are typically within 0.005‰ of values reported by Bernasconi et al. (2021) as described in Upadhyay et al. (2021) and Lucarelli et al. (2023). Long-term absolute mean error in standard Δ_{47} across instruments is 0.0002‰ (or 0.03% as described in percentage), and is normally distributed (Upadhyay et al., 2021; Lucarelli et al., 2023). The average Δ_{47} standard deviation (1 s.d.) among standard replicates for all instruments is 0.021‰ (Upadhyay et al., 2021; Lucarelli et al., 2023).

Similar values are reported for samples. For samples, average Δ_{47} reproducibility is 0.007‰ (1 s.e.) (Supplemental Table 1.S.1). The ranges for samples are 0.003-0.012‰ (1 s.e.). Standard and sample data for new measurements are provided following reporting style for Eagle et al. (2015) and will be archived online with manuscript acceptance.

2.5.2 Data cleaning and quality control

Data quality control followed published procedures described in Parvez et al. (2023). All samples are routinely screened based on Δ_{48} or Δ_{49} values that are indicative of high organic content, no samples reported for this study were flagged on these bases. Replicates with incomplete gas transfer and Δ_{47} , $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$ values that fall outside of a SD range of $\pm 0.5\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ and $\pm 0.05\text{‰}$ for Δ_{47} are also flagged. Similar screening thresholds for Δ_{47} were reported by Meckler et al. (2014), Upadhyay et al. (2021), and Lucarelli et al. (2023). Data were screened based on instrument source stability, pumpdown times and leaks in the acid digestion system, and standard data quality during correction intervals as described in Parvez et al. (2023). Of the new analyses conducted for this study, 155 replicates were included, and 8 replicates excluded.

3 Results

3.1 Petrographic sample preservation analyses

Samples underwent rigorous testing to separate thermoregulatory and biomineralization signals from diagenetic signals. Each specimen was imaged using plane light and cathodoluminescence (CL) petrography, as well as via SEM-EBSD and Raman spectroscopy. Of the 8 eggshells that were imaged, the majority (5) show varying degrees of alteration or yield inconclusive results (Figure 1.1, 1.2, 1.3, S1, and S2). A full table of petrographic results is included in Table 1.1.

Petrographic and microstructural analyses were conducted on eggshell samples using electron backscatter diffraction (EBSD) for crystallographic orientation and microstructure, Raman spectroscopy for mineralogical composition and organic matrix preservation, cathodoluminescence (CL) for detecting diagenetic overgrowths or recrystallization, and trace element geochemistry as diagenetic proxies. Final preservation determinations follow the criteria of Eagle et al. (2015), with ratings of “Apparently Well Preserved,” “Moderately

Preserved,” “Poorly Preserved,” or “Uncertain/Inconclusive.” The majority of eggshell samples exhibit variable but generally good structural preservation at the microstructural scale despite evidence of isotopic alteration in the geochemical dataset. No samples were rated as pristine, and all show at least minor evidence of diagenetic processes. EBSD and Raman analyses frequently indicate retention of original eggshell ultrastructure, such as columnar or prismatic calcite layers with preferred crystallographic orientation, while CL and trace element data reveal more heterogeneous diagenetic influence (Figure 1.2, 1.3, S1, S2, and S3).

Eggshells from Devil’s Coulee, Alberta, Canada (*Continuoolithus*, *Prismatoolithus*, and *Spheroolithus* spp.) displayed well-preserved microstructure by EBSD and Raman but yielded mostly “Inconclusive” or “Poorly Preserved” ratings for CL due to patchy luminescence suggestive of minor calcite overgrowth. Final determinations for these samples are predominantly “Uncertain” or “Inconclusive” (Figure 1.2, 1.3, S1).

Preservation among Mongolian samples (various Cretaceous–Campanian formations) was more variable across multiple taxa, including *Macroolithus rugustus*, *Protoceratopsidovum* spp., *Spheroolithus* spp., and *Elongatoolithus* spp (Table 1.1). Several samples, particularly *Macroolithus rugustus* and some *Protoceratopsidovum* specimens, were rated “Well Preserved” by EBSD, Raman, and CL analyses, indicating strong microstructural integrity and limited diagenetic alteration that corresponds with geochemical data (Figure 1.1, 1.2, 1.3, 1.5, S2, S3, Table 1.1). However, the calculated $\delta^{18}\text{O}$ body water values from $\Delta^{17}\text{O}$ data in the Mongolian set trend toward possible meteoric water signals, suggesting interaction with diagenetic fluids despite the good microstructural preservation observed in some samples (Figure 1.1). In contrast, other Mongolian samples, such as certain *Spheroolithus* and *Macroelongatoolithus* specimens, showed disrupted crystal orientation or organic matrix degradation and were rated “Poorly Preserved” or “Inconclusive” (Figure 1.2, 1.3, S2, Table 1.1), Final determinations for

the Mongolian eggshells range from “Well Preserved” to “Uncertain,” “Inconclusive,” or “Poorly Preserved” (Table 1.1).

Strong microstructural preservation (EBSD and Raman) was observed in approximately 60–70% of samples, particularly from Mongolian localities, with several well-preserved Mongolian samples also yielding possibly reliable geochemical signals (Figure 1.3, S2, S3). However, CL data indicate that many eggshells experienced at least partial interaction with diagenetic fluids (1.2, S2). There is no consistent correlation between petrographic preservation quality and isotopic values across the full dataset, although samples rated These petrographic results, when combined with the geochemical data, indicate that while primary eggshell fabrics are largely retained, all samples have undergone some degree of diagenetic alteration. Interpretations of stable and clumped isotope data are therefore presented with appropriate caution. Detailed sample-level ratings are provided in Table 1.1.

3.2 Geochemical analyses

Geochemical analyses also reflect varying degrees of preservation (Figure 1.1). Eight eggshell specimens from multiple Late Cretaceous localities were analyzed for $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and Δ_{47} . Results are reported as means $\pm$ 1 standard deviation (s.d.) for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (in ‰ relative to VPDB), and as means $\pm$ 1 standard error (s.e.) for Δ_{47} -derived temperatures ($T \Delta_{47}$ in °C). Derived $\delta^{18}\text{O}$ water values (VSMOW) are also presented. Across the full dataset, ($n = 4\text{--}10$ per specimen), $\delta^{13}\text{C}$ was measured at -13.71‰ to -3.66‰ VPDB, $\delta^{18}\text{O}$ ranges from -12.88‰ to -4.22‰ VPDB, and apparent Δ_{47} temperatures range from 21.2°C to 61.5°C . The narrower ranges of -6.16‰ to -4.18‰ for $\delta^{13}\text{C}$, -12.43‰ to -8.21‰ for $\delta^{18}\text{O}$, and $21.2\text{--}31.5^\circ\text{C}$ for Δ_{47} refer only to the subset of Mongolian specimens judged best preserved by the combined screening criteria.

Devil's Coulee, Alberta, Canada samples include *Spheroolithus albertensis* and *Continuoolithus canadensis* (n=4–5 per specimen). $\delta^{13}\text{C}$ values measured $-13.71 \pm 0.05\text{‰}$ and $-10.84 \pm 0.06\text{‰}$ VPDB for *Spheroolithus albertensis* and *Continuoolithus canadensis* respectively. $\delta^{18}\text{O}$ values measured $-9.88 \pm 0.02\text{‰}$ to $-4.22 \pm 0.03\text{‰}$ VPDB. T Δ_{47} values measured $61.5 \pm 1.9^\circ\text{C}$ and $48.5 \pm 2.8^\circ\text{C}$, with $\delta^{18}\text{O}$ water values from $-0.93 \pm 0.30\text{‰}$ to $2.58 \pm 0.47\text{‰}$ VSMOW (Figure 1.1).

Multiple taxa were analyzed from various localities in Mongolia. Taxa analyzed include *Macroolithus rugustus*, *Spheroolithus* spp., and *Elongatoolithus* spp. (n=6–10). $\delta^{13}\text{C}$ values were generally more positive than Canada samples in some taxa, ranging from $-7.85 \pm 0.07\text{‰}$ (*Elongatoolithus*) to $-3.66 \pm 0.12\text{‰}$ (*Spheroolithus*). $\delta^{18}\text{O}$ values ranged from $-12.88 \pm 0.14\text{‰}$ to $-8.21 \pm 0.09\text{‰}$ VPDB (Figure 1.1).

T Δ_{47} temperatures on Mongolian eggshells varied widely from $21.2 \pm 2.2^\circ\text{C}$ to $36.9 \pm 2.8^\circ\text{C}$. $\delta^{18}\text{O}$ water values ranged from $-10.87 \pm 0.47\text{‰}$ to $-4.55 \pm 0.30\text{‰}$ VSMOW. Dual-clumped data were obtained and plotted for samples with the best petrographic preservation (Mongolian oviraptorid and hadrosaur eggshells and two North American specimens) (Figure 1.5). Δ_{47} temperatures ranged from $21.2 \pm 2.2^\circ\text{C}$ to $31.5 \pm 1.5^\circ\text{C}$ for plotted set. Δ_{48} values ranged from 0.201 ± 0.019 to $0.251 \pm 0.011\text{‰}$ (I-CDES, fossil specimens), with ST-F1R (*Spheroolithus tenuicorticus*, hadrosaur) and EF-F1R (*Elongatoolithus frustrabilis*, oviraptorid) plotting within the equilibrium field for low-temperature calcite formation when compared to theoretical predictions (Figure 1.5; Hill et al., 2014; Lucarelli et al., 2023). Most samples fall along the theoretical equilibrium line in Δ_{47} – Δ_{48} space, similar to modern reptiles (0.192 ± 0.017 to $0.275 \pm 0.023\text{‰}$, Δ_{48} reptiles, this study) and distinct from strong kinetic effects observed in some modern birds (Figure 1.5; Tagliavento et al., 2023). The best-preserved Mongolian samples (e.g., MR-F1L and EF-F1R) show Δ_{48} values consistent with equilibrium precipitation at

body temperature, supporting limited diagenetic resetting in these specimens (Figure 1.5, Table S1).

Calculated body-water triple-oxygen anomalies ($\Delta^{17}\text{O}_{\text{bw}}$) are near zero for several specimens (MR-F1L: -11 ± 12 per meg; SM-F1R: $+5 \pm 19$ per meg; ST-F1R: -8 ± 9 per meg). More negative values occur in EF-F1R (-34 ± 9 per meg) and ES-F1R (-77 ± 33 per meg), although ES-F1R is poorly preserved petrographically. Near-zero values are compatible with meteoric-water-like endmembers, but they do not by themselves distinguish primary body-water composition from diagenetic exchange. Modestly negative values increase confidence in primary signal retention only where petrographic, Δ_{47} , and other geochemical screens also agree.

Full sample-level data, including standard errors and multi-proxy preservation metrics, are provided in Table 1.1. Intra-taxon variability was generally low for $\delta^{13}\text{C}$ within individual catalog entries (s.d. typically $<0.3\text{‰}$, average 0.14‰), while $\delta^{18}\text{O}$ and Δ_{47} showed greater dispersion in several groups.

4 Discussion

The multi-proxy petrographic and geochemical investigation of 8 Late Cretaceous eggshell samples demonstrates that, while many specimens retain excellent microstructural preservation, diagenetic processes have variably compromised some of the primary isotopic signals (Eagle et al., 2015; Graf et al., 2018). This pattern of strong preservation of ultrastructure coupled with evidence of isotopic alteration highlights both the potential and the inherent challenges of using fossil eggshells as paleobiological and paleoenvironmental archives.

4.1 Preservation and Signal Fidelity

Petrographic analyses (EBSD, Raman, CL) confirm that eggshells from the Mongolian specimens represent the highest quality material in the dataset (Figure 1.2, 1.3, S2, S3). These specimens frequently preserve primary calcite fabrics and crystallographic orientation, supporting their use for interpreting original formation conditions (Choi et al., 2020, 2021, 2022, 2023). In contrast, many other Mongolian samples and the Devil's Coulee eggshells exhibit more pronounced diagenetic features, ranging from minor fluid interaction to complete microstructural degradation (Figure 1.2, 1.3, S1).

The lack of direct correlation between microstructural preservation and geochemical integrity is noteworthy. Several samples rated “Well Preserved” by EBSD and Raman still display $\Delta^{17}\text{O}$ trends consistent with meteoric water interaction (Figure 1.1, Table 1.1; Passey et al., 2014; Hare et al., 2025). This decoupling suggests that recrystallization or isotopic exchange can occur without wholesale destruction of eggshell ultrastructure, a phenomenon previously documented in calcite biominerals (Eagle et al., 2015; Kelson et al., 2020). Consequently, rigorous multi-proxy screening remains essential for distinguishing primary from diagenetic signals in eggshell studies (Dawson et al., 2020; Choi et al., 2021).

The near-zero to slightly positive $\Delta^{17}\text{O}$ values in well-preserved Mongolian samples, particularly the hadrosaur eggshells (SM-F1R and ST-F1R), are consistent with body waters that closely track local meteoric compositions, potentially reflecting limited evaporative enrichment or direct meteoric influence. The contrast between SM-F1R and ST-F1R could suggest these specimens may record different meteoric water sources rather than strong differential evaporation or pervasive diagenesis (Passey et al., 2014; Wostbrock et al., 2020). For oviraptorid samples such as MR-F1L and EF-F1R, the combination of modestly negative $\Delta^{17}\text{O}$ anomalies, low Δ_{47} temperatures (21–28 °C), and strong EBSD/Raman preservation supports greater retention of primary signals. Overall, these results reinforce the importance of

multi-proxy screening (including $\Delta^{17}\text{O}$) because microstructural preservation alone does not guarantee isotopic fidelity (Choi et al., 2021; Hare et al., 2025). When integrated with clumped-isotope temperatures and petrographic data, the $\Delta^{17}\text{O}$ results strengthen confidence in primary paleobiological signals from the best-preserved Mongolian material while highlighting the pervasive influence of meteoric fluids across the broader dataset.

4.2 Geochemical Reliability and Diagenetic Overprints

Clumped isotope (Δ_{47}) temperatures range broadly from $21.2 \pm 2.2^\circ\text{C}$ to $61.5 \pm 1.9^\circ\text{C}$, with the highest values representing evidence diagenetic resetting (Dennis and Schrag, 2010; Passey and Henkes, 2012; Stopler and Eiler, 2015). Even among the best-preserved samples, some elevated temperatures exceed plausible body temperatures, suggesting partial resetting during burial. Oxygen isotope data show wide variability, indicating a dynamic hydrological environment and varied drinking water sources, with potential diagenetic influence (Amiot et al., 2023; Montanari et al., 2013). Carbon isotope values ($\delta^{13}\text{C}$: -13.71‰ to -3.66‰ VPDB) exhibit lower intra-taxon variability and appear more robust to diagenesis, consistent with a predominantly C3 vegetation diet.

$\Delta^{17}\text{O}$ results from Mongolian samples trend toward meteoric water compositions rather than leaf-water evaporation arrays, implying interaction with diagenetic fluids despite good microstructural preservation in several specimens (Figure 1.1, Passey et al., 2014; Wostbrock et al., 2020; Hare et al., 2025). Most primary signals derive from the remaining Mongolian samples that preserved $\Delta^{17}\text{O}$ anomalies.

4.3 Dual-clumped Isotope Insights

Dual-clumped ($\Delta_{47}-\Delta_{48}$) measurements provide an additional test of kinetic effects during mineralization (Hill et al., 2014; Bajnai et al., 2020; Lucarelli et al., 2023; Parvez et al.,

2023). Proximity to a predicted equilibrium array is consistent with limited kinetic fractionation at the time of carbonate formation, but it does not independently rule out later mixing or partial reordering. The Mongolian specimens plot closer to the low-temperature equilibrium field than several modern birds, supporting equilibrium-like mineralization in those samples. Any inference that oviraptorids had lower body temperatures than the hadrosaur remains provisional because the comparison involves few specimens, uncertain taxonomic assignment, and residual preservation uncertainty.

4.4 Implications for Dinosaurian Thermophysiology, Diet, and Paleoenvironments

The lower apparent Δ_{47} temperatures in the best-supported Mongolian specimens are compatible with biologically plausible eggshell mineralization temperatures and with prior evidence for heterogeneous dinosaur thermoregulation (Eagle et al., 2015; Dawson et al., 2020; Tagliavento et al., 2023). They should not, however, be used to assign metabolic categories without independent physiological constraints. Carbon-isotope values may retain dietary or habitat information more readily than oxygen-bearing systems in this dataset, but diet-specific interpretation requires calibration of diet-to-body-water and body-carbon fractionation. The combined isotope and preservation data therefore support candidate paleobiological interpretations while also showing that local depositional and burial histories exert first-order control on signal fidelity.

4.5 Limitations and Future Directions

This study is limited by the small number of samples per ootaxon and locality, as well as the inherent challenges of working with diagenetically sensitive biominerals. Future work should expand sampling from well-constrained, low-burial-temperature localities and integrate complementary proxies such as trace metal ratios and histological analysis of associated

skeletal material. Application of emerging techniques, including high-resolution secondary ion mass spectrometry (SIMS) mapping and advanced $\Delta^{17}\text{O}$ modeling, may further improve our ability to isolate primary signals.

A potential source of bias in our seawater $\delta^{18}\text{O}$ estimates arises from the use of mixed biogenic carbonate material (oyster and belemnite). Non-linear mixing effects can occur when end-members differ in bulk $\delta^{13}\text{C}$ and/or $\delta^{18}\text{O}$ composition, producing offsets in measured Δ_{47} (and Δ_{48}) that are independent of formation temperature and can be misinterpreted as kinetic or environmental signals (Defliese & Lohmann, 2015; White & Defliese, 2023). Although the magnitude of such bias depends on the isotopic contrast between the mixed components, even modest heterogeneity (approximately $\geq 2\text{‰}$ in $\delta^{18}\text{O}$) can generate measurable Δ_{47} offsets relative to modern analytical precision. Because oyster and belemnite material may record different bulk isotopic compositions or growth conditions, homogenization of these materials prior to analysis could introduce a small systematic uncertainty into the reconstructed water compositions. Future work employing taxa-specific or micro-sampled analyses would help quantify and minimize this potential mixing bias.

In conclusion, carefully screened Late Cretaceous eggshells, particularly those from Mongolia, retain valuable primary geochemical information capable of informing debates on dinosaur thermoregulation, diet, and paleoenvironments. However, the pervasive influence of diagenesis documented here reinforces the importance of conservative interpretation and multi-proxy approaches in eggshell isotope geochemistry.

5 Figures

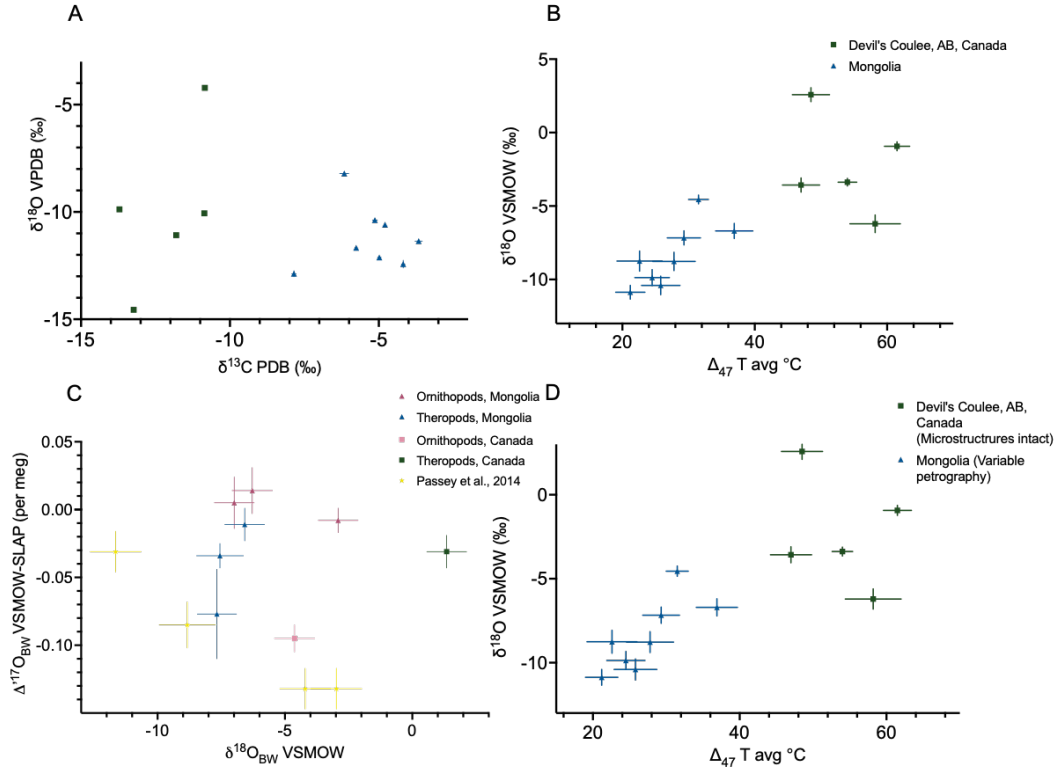
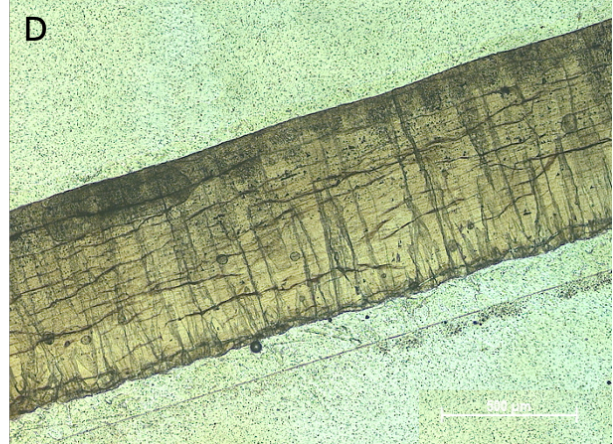
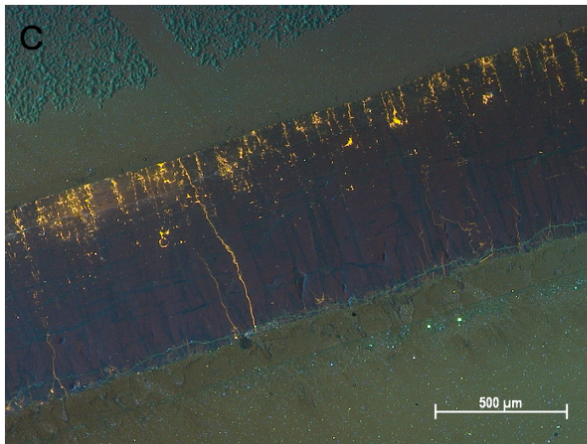
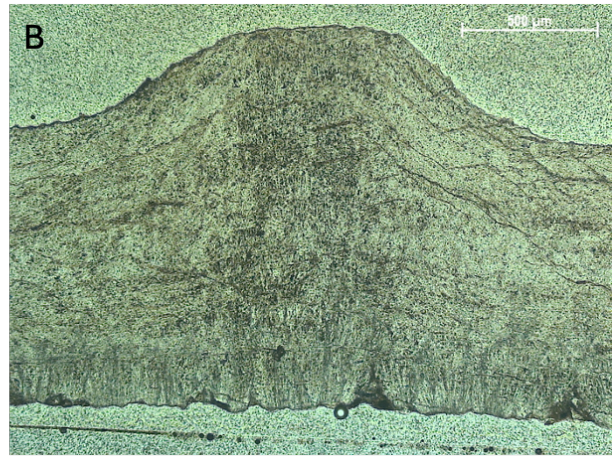
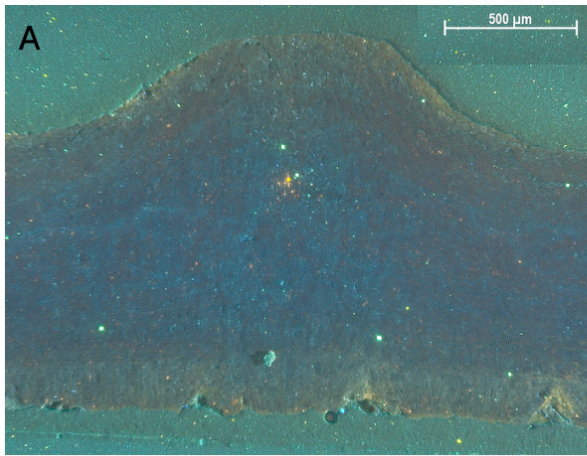


Figure 1.1

Examples of isotopic data used to assess preservation. (A) $\delta^{13}\text{C}$ vs $\delta^{18}\text{O}_{\text{mineral}}$ crossplot for all eggshells included in this study. Both isotopic signatures are highly variable in general, and amongst individual localities. Data points with error bars represent mean $\pm$ 1 s.d. where multiple analyses were performed per specimen. (B) $\delta^{18}\text{O}_{\text{water}}$ vs Δ_{47} temperatures for all eggshells included in this study. Data points with error bars represent mean $\pm$ 1 s.d. where multiple analyses were performed per specimen. (C) $\delta^{18}\text{O}_{\text{bodywater}}$ vs $\Delta^{17}\text{O}_{\text{bodywater}}$ values for all samples tested for $\Delta^{17}\text{O}$ in this study (Mongolian eggshells, blue/magenta triangles for theropods and ornithopods respectively, and Devil's Coulee eggshells, dark green squares and pink squares for theropods and ornithopods respectively), compared to values from Passey et al., 2014

(yellow stars). It can be observed that the data in this study follow trends in contrast to those found to be consistent with *in vivo* signals in the Passey et al., 2014 study, with positively-trending, near-meteoric water values. Data points with error bars represent mean $\pm$ 1 s.d. where multiple analyses were performed per specimen. (D) $\delta^{18}\text{O}_{\text{water}}$ vs Δ_{47} temperatures for samples in this study. Data points with error bars represent mean $\pm$ 1 s.d. where multiple analyses were performed per specimen.



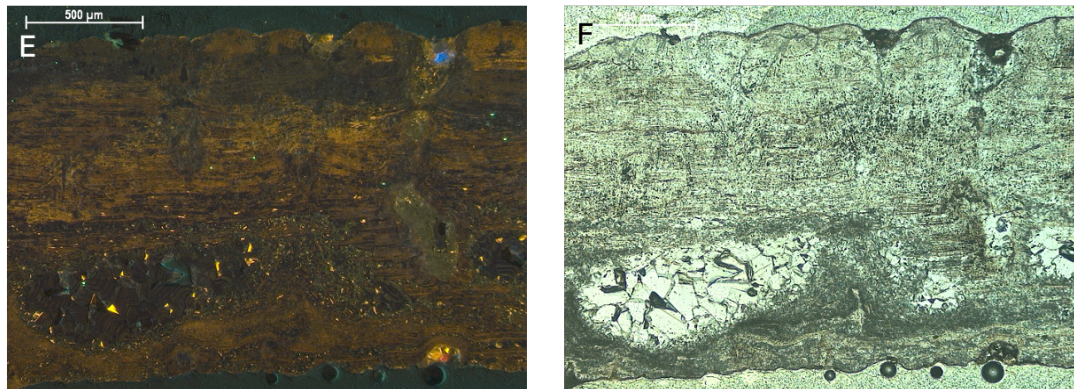
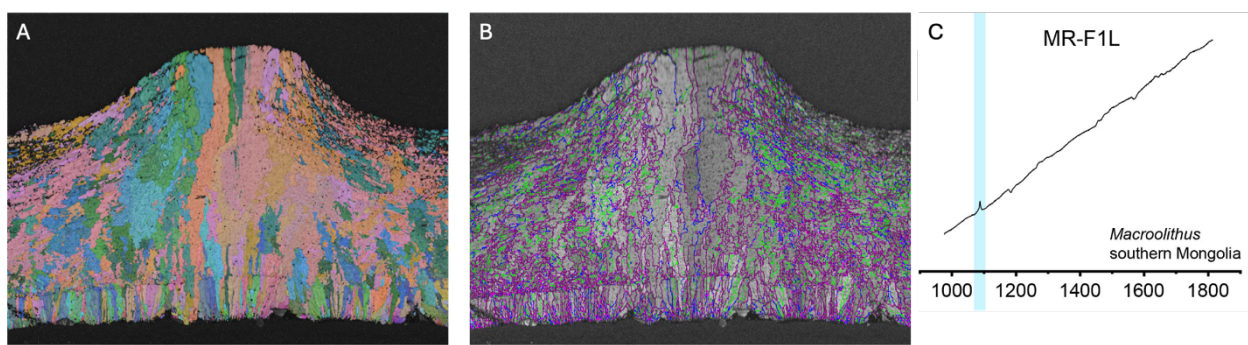


Figure 1.2

Petrographic microscopy characterization of eggshells. (A-B) CL and optical light images of a petrographically “Well preserved” specimen, MR-F1L from Mongolia. Sample is largely non-luminescent throughout. (C-D) CL and optical light images of a petrographically “Inconclusive” specimen, PLEGG-DC-1F1 from Devil’s Coulee, AB, Canada. Outer edge shows some luminescence, and some pore canals luminesce as well. It is unclear how much material is altered, but it appears most of the eggshell is non-luminescent. (E-F) CL and optical light images of a petrographically “Poorly preserved” specimen, SI-F1R from Mongolia. Sample is luminescent throughout, with some layers revealing blocky infilled areas. All specimens photographed at 4x magnification.



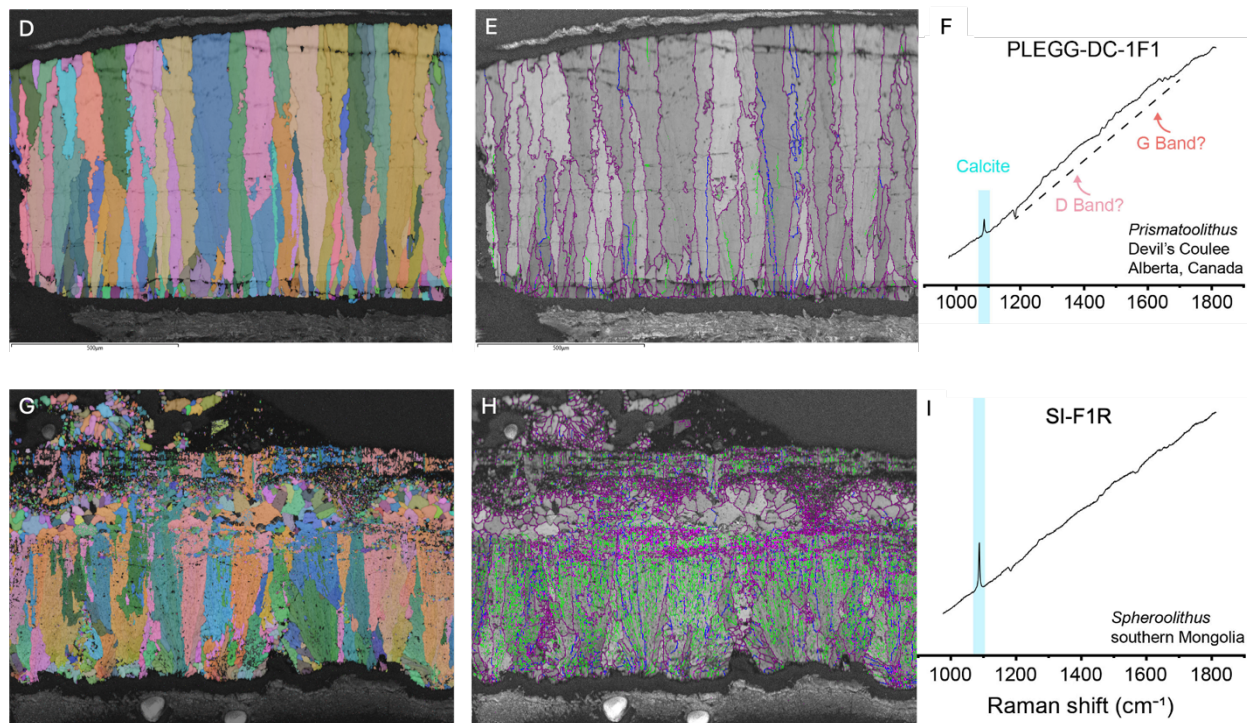


Figure 1.3

Electron backscatter diffraction (EBSD) images and Raman spectra characterization of eggshells. (A-B) Euler and grain boundary (GB) images of “Well preserved” eggshell from Mongolia, MR-F1L. EBSD images show microstructures are very well preserved and there is no obvious sign of fluid mediated alteration. (C) Raman spectra for “Well preserved” eggshell from Mongolia, MR-F1L. Calcite band can be observed in blue, however no disordered (D) or graphite (G) bands are present, indicating no apparent solid-state reordering. (D-E) Euler and GB images of “Inconclusive” PLEGG-DC-1F1 from Devil’s Coulee, AB, Canada. EBSD images show intact microstructures, and no apparent evidence of fluid mediated alteration. (F) Raman spectra for “Inconclusive” PLEGG-DC-1F1 from Devil’s Coulee, AB, Canada show possible weakly developed D and G bands, indicating possible solid state reordering. (G-H) Euler and grain boundary images for “Poorly preserved” eggshell from Mongolia, SI-F1R. EBSD images show evidence of fluid mediated alteration and extensive recrystallization. (I) Raman spectra for

“Poorly preserved” eggshell from Mongolia, SI-F1R show calcite band in blue, however no disordered (D) or graphite (G) bands are present, indicating no apparent solid-state reordering.

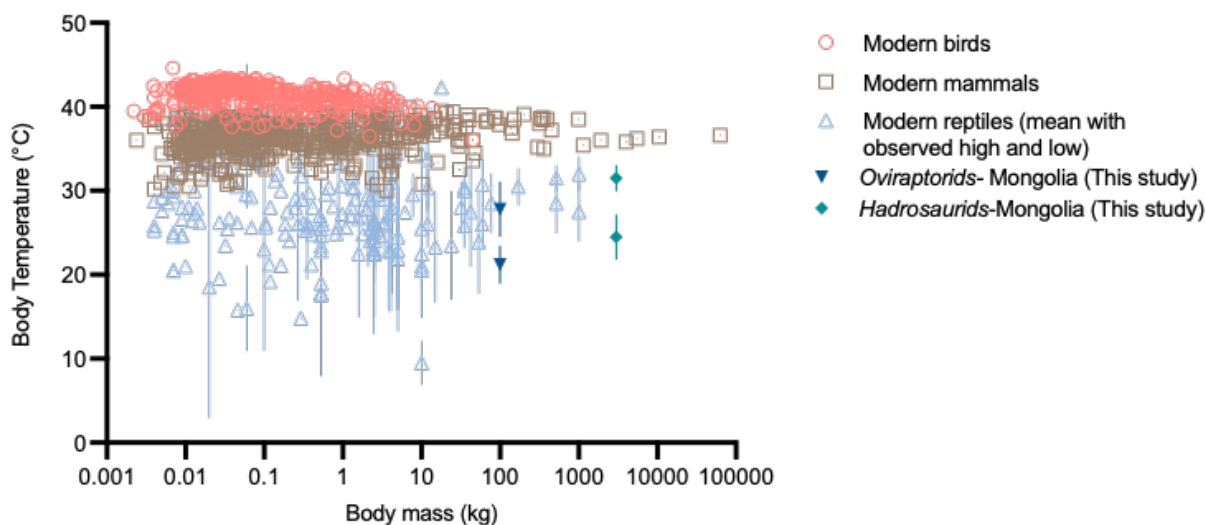


Figure 1.4

Body temperature vs mass estimates of well preserved Oviraptorids (*Elongatoolithus frustrabilis*, *Macroolithus rugustus*; dark blue inverted triangles) and Hadrosaurids (*Spheroolithus maiasauroides*, *Spheroolithus tenuicorticus*; green diamonds) from Mongolia from this study compared to modern taxa. Data compiled from Eagle et al., 2015 for modern measurements of body mass vs temperature for modern birds (pink circles), modern mammals (brown squares) and modern reptiles (blue triangles). Data from well-preserved specimens in this study was compared against the modern data, demonstrating that Oviraptorids in this study fall within the same range as modern reptiles. Hadrosaur body mass estimate is from Weishampel and Horner (1990) and Oviraptorid body mass estimate is from Ma et al., 2017).

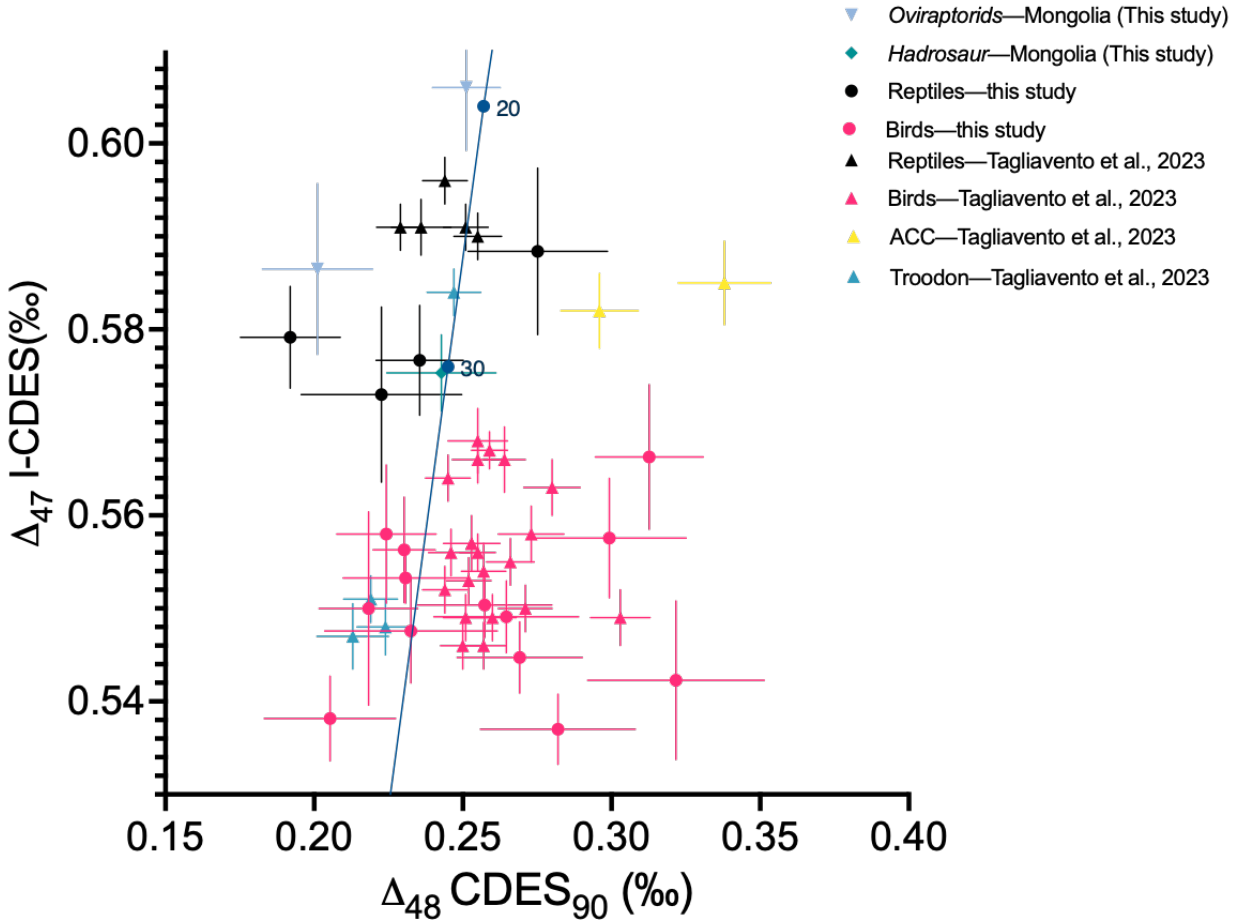


Figure 1.5

Modern reptile (black) and bird (hot pink) Δ_{47} and Δ_{48} data (this study, circles) compared to well-preserved fossil taxa (Oviraptorids, inverted blue triangles, Hadrosaurids, green diamonds).

Data are compared to Tagliavento, et al., 2023 (triangles). Blue line is theoretical Δ_{48}/Δ_{47} values after Hill et al., 2014 and Lucarelli et al., 2022. It can be observed that endotherms and reptiles fall into two distinct areas in Δ_{48}/Δ_{47} space, with our non-avian dinosaur data falling within the reptile space.

6 Tables

Table 1.1

Sample locality information and compiled diagenetic screening results. Samples were categorized following the convention from Eagle et al., 2015.

Sample ID	Genus and Species	Material type	Stratigraphy and locality	Epoch	EBSD	Raman	CL	$\Delta^{17}\text{O}$ ¹ s.e.	Final
CCEGG-DC-1F1	<i>Continuoolithus canadensis</i>	eggshell	Devil's Coulee, Canada	Late Cretaceous	Well Preserved	Inconclusive	Well Preserved	- 0.031	0.012 Uncertain
SAEGG-DC-1F1	<i>Spheroolithus albertensis</i>	eggshell	Devil's Coulee, Canada	Late Cretaceous	Well Preserved	Inconclusive	Inconclusive	- 0.095	0.010 Uncertain
MR-F1L	<i>Macroolithus rugustus</i>	eggshell	Mongolia	Cretaceous	Well Preserved	Well Preserved	Well Preserved	- 0.011	0.012 Well Preserved
SI-F1R	<i>Spheroolithus irenensis</i>	eggshell	Mongolia	Cretaceous	Poorly Preserved	Well Preserved	Poorly Preserved	0.014	0.017 Poorly Preserved
SM-F1R	<i>Spheroolithus maiaesauroides</i>	eggshell	Mongolia	Cretaceous	Well Preserved	Well Preserved	Well Preserved	0.005	0.019 Well Preserved
ST-F1R	<i>Spheroolithus tenuicorticus</i>	eggshell	Mongolia	Cretaceous	Well Preserved	Well Preserved	Inconclusive	- 0.008	0.009 Well Preserved
EF-F1R	<i>Elongatoolithus frustrabilis</i>	eggshell	Mongolia	Cretaceous	Inconclusive	Well Preserved	Well Preserved	- 0.034	0.009 Well Preserved
ES-F1R	<i>Elongatoolithus subitectorius</i>	eggshell	Djadokhta Formation, Udan-Sayr, Mongolia	Campanian	Poorly Preserved	Poorly Preserved	Well Preserved	- 0.077	0.033 Poorly Preserved

Table 1.2

Clumped isotope data for fossil eggshells.

*Represents the number of distinct CO₂ extractions from each sample that was separately purified and analyzed. ** Values given on the ‘absolute reference frame’ or ARF. All eggshells are calcite. Green text rows are ootaxa which appear well preserved by most criteria.

Sample ID	n*	$\delta^{13}\text{C}$ VPDB	1 s.d.	$\delta^{18}\text{O}$ VPDB	1 s.d.	Δ_{47}	1 s.e.	T Δ_{47} [°C]**	1 s.e.	$\delta^{18}\text{O}$ water VSMOW	1 s.d.
CCEGG-DC-1F1	5	-10.84	0.06	-4.22	0.03	0.532	0.007	48.5	2.8	2.58	0.47
SAEGG-DC-1F1	4	-13.71	0.05	-9.88	0.02	0.503	0.004	61.5	1.9	-0.93	0.3
MR-F1L	6	-5.76	0.04	-11.67	0.06	0.587	0.009	27.8	3.2	-8.77	0.62
SI-F1R	6	-3.66	0.12	-11.36	0.06	0.561	0.007	36.9	2.8	-6.7	0.51
SM-F1R	6	-4.98	0.06	-12.12	0.04	0.596	0.008	24.5	2.6	-9.87	0.54
ST-F1R	6	-6.16	0.15	-8.21	0.09	0.575	0.004	31.5	1.5	-4.55	0.3
EF-F1R	10	-4.18	0.06	-12.43	0.18	0.606	0.007	21.2	2.2	-10.87	0.47
ES-F1R	9	-7.85	0.07	-12.88	0.14	0.593	0.009	25.8	2.9	-10.4	0.63

Table 1.3

$\Delta^{17}\text{O}$ data for eggshells. For $\Delta^{17}\text{O}$, uncertainties are reported as expanded uncertainties, by combination of analytical error and systematic error, in quadrature. All measurements are corrected (empirically) for concentration dependence due to scale offset errors, (Hare et al., 2022; Griffith et al., 2012; Bajnai and Hare, 2025). Analytical error is 1σ in the case of triplicates, and the difference between values, in the case of duplicates. Systematic error is the average of the 1σ repeatabilities of NBS18 and IAEA603 from Wostbrock et al. (2020) (0.008 per meg). Samples from each run were corrected to the VSMOW-SLAP scale using within-run IAEA-603 and NBS18 standards. Known values were from Wostbrock (2020), with $\lambda_{\text{RL}} = 0.528$. $\delta^{13}\text{C}$ is reported on VPDB-LSVEC scale.

Sample			$\Delta^{17}\text{O}$			$\delta^{18}\text{O}$			$\delta^{13}\text{C}$				$\delta^{18}\text{O}_{\text{bw}}$		$\Delta^{17}\text{O}_{\text{bw}}$		
ID	Material	LAB	(‰)	1 σ	$\mu\Delta^{17}\text{O}$	(‰)	1 σ	n	(‰)	1 σ	Region	Age	VSMOW	±	SLAP	±	
MR-F1L	eggshell	UCT	-	0.098	0.006	0.010	19.041	0.037	2	-5.785	0.037	Mongolia	Cretaceous	-6.58	0.78	-0.011	0.012
ES-F1R	eggshell	UCT	-	0.163	0.023	0.024	17.919	0.023	2	-7.878	0.041	Mongolia	Cretaceous	-7.67	0.77	-0.077	0.033
EF-F1R	eggshell	UCT	-	0.121	0.002	0.008	18.048	0.374	2	-4.097	0.205	Mongolia	Cretaceous	-7.55	0.92	-0.034	0.009
SI-F1R	eggshell	UCT	-	0.073	0.011	0.013	19.350	0.104	2	-3.724	0.023	Mongolia	Cretaceous	-6.28	0.79	0.014	0.017
SM-F1R	eggshell	UCT	-	0.082	0.013	0.015	18.621	0.061	2	-4.959	0.039	Mongolia	Cretaceous	-6.99	0.78	0.005	0.019
ST-F1R	eggshell	UCT	-	0.094	0.002	0.008	22.804	0.013	2	-6.192	0.129	Mongolia	Cretaceous	-2.91	0.78	-0.008	0.009
SAEGG-DC-1-F1	eggshell	UCT	-	0.181	0.004	0.009	21.047	0.103	2	-13.734	0.068	Alberta, Canada	Cretaceous	-4.62	0.79	-0.095	0.010
CCEGG-DC-1-F1	eggshell	UCT	-	0.118	0.007	0.011	27.173	0.035	2	-10.936	0.026	Alberta, Canada	Cretaceous	1.35	0.78	-0.031	0.012

Supporting information for: Chapter 1. Diagenetic and biological signals in fossil eggshells

Supplementary material

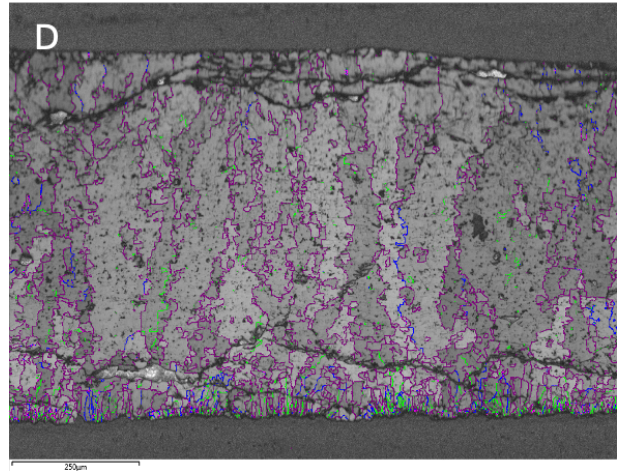
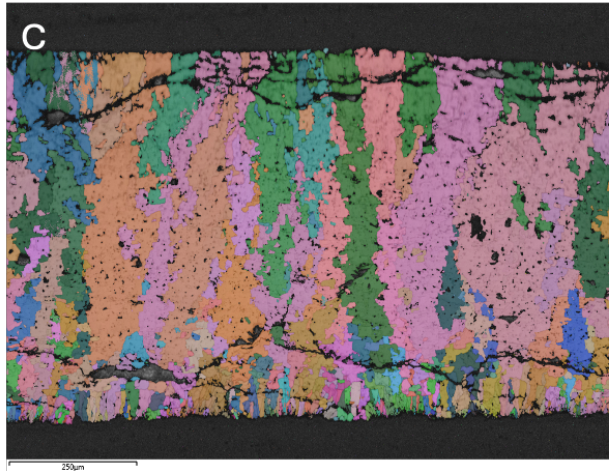
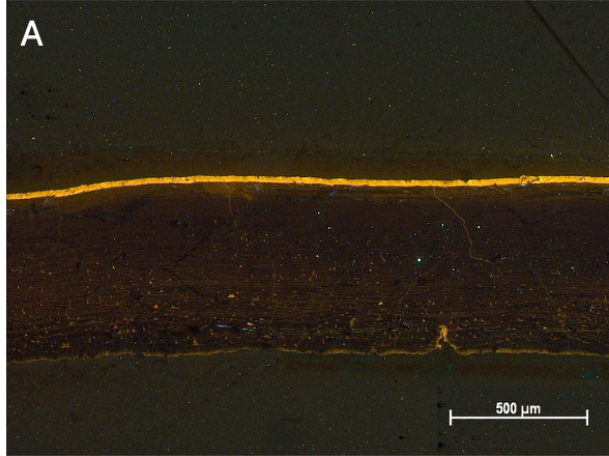
Table S1.1 Complete modern bird and reptile data for this study.

Sample Name	Common Name	n Replicates	H ₂ O ₂ Treated	$\delta^{13}\text{C}$ PDB avg	1 s.d.	$\delta^{18}\text{O}$ PDB avg	1 s.d.	Δ_{47} avg	1 s.e.	Δ_{48} avg	1 s.e.	Average T [°C]	1 s.e.	$\delta^{18}\text{O}$ water SMOW avg	1 s.d.
AP-MOD-2	King Penguin	6	x	-16.053	0.092	-3.327	0.045	0.568	0.009	0.253	0.022	34.54	3.12	0.909	0.614
CAA-MOD-2	American Crocodile	6	x	-12.502	0.064	-3.972	0.040	0.584	0.007	0.285	0.019	28.46	2.24	-0.901	0.459
CG-AMOD-2	Alligator	7	x	-13.486	0.064	-16.756	0.136	0.586	0.009	0.376	0.084	27.97	2.95	-13.842	0.546
CNN-CMOD-2	Nile Croc	7	x	-9.284	0.053	-2.764	0.029	0.590	0.006	0.252	0.023	26.33	1.95	-0.119	0.387
DN-MOD-2	Emu	6	x	-6.740	0.051	-7.645	0.063	0.557	0.007	0.280	0.033	38.72	2.79	-2.637	0.505
MA-MOD-2	Golden-fronted Woodpecker	4	x	-8.920	0.070	0.780	0.035	0.542	0.005	0.299	0.041	44.47	1.92	6.904	0.351
AA-MOD-1	Common Kiwi	6		-12.002	0.037	-9.063	0.037	0.556	0.003	0.220	0.020	38.81	0.98	-4.024	0.189
AC-MOD-1	Ruby-throated Hummingbird	5		-11.754	0.049	-2.946	0.103	0.592	0.019	0.794	0.079	26.64	7.06	-0.344	1.401
AP-MOD-1	King Penguin	7		-16.044	0.115	-3.283	0.080	0.566	0.008	0.301	0.016	35.06	2.94	1.055	0.571
AT-MOD-1	Tree Pipit	11		-15.958	0.084	-4.165	0.091	0.543	0.004	0.267	0.024	43.96	1.50	1.828	0.278

BVP-MOD-1	Great Horned Owl	7		-	12.310	0.054	-3.550	0.065	0.549	0.003	0.227	0.023	41.46	1.30	2.004	0.226
CAAMOD-1	American Crocodile	6		-	12.495	0.066	-3.920	0.058	0.579	0.005	0.177	0.018	30.20	1.92	-0.505	0.389
CC-MOD-1	Costa's Hummingbird	4		-	-6.335	0.079	1.888	0.160	0.583	0.010	0.700	0.296	29.10	3.37	5.094	0.746
CG-AMOD-1	Alligator	6		-	13.433	0.047	16.827	0.058	0.573	0.009	0.422	0.016	32.61	3.36	-13.015	0.653
CH-MOD-1	Cooper's Hawk	7		-	-9.629	0.036	-2.873	0.044	0.554	0.006	0.230	0.011	39.75	2.20	2.359	0.417
CNNCMOD-1	Nile Croc	6		-	-9.270	0.070	-2.715	0.050	0.577	0.006	0.156	0.023	31.11	2.14	0.882	0.408
DN-MOD-1	Emu	7		-	-6.601	0.090	-7.590	0.059	0.558	0.006	0.299	0.029	38.29	2.46	-2.658	0.474
MA-MOD-1	Golden-fronted Woodpecker	7		-	-9.040	0.046	0.700	0.034	0.537	0.004	0.266	0.021	46.43	1.59	7.174	0.275
MACN-PV-94a	<i>Enanornithes</i>	10		-	-4.555	0.125	-6.854	0.095	0.572	0.004	0.399	0.107	32.96	1.64	-2.917	0.326
MACN-PV-94a-2	<i>Enanornithes</i>	7		-	-4.656	0.079	-6.701	0.091	0.574	0.007	0.257	0.024	32.32	2.63	-2.897	0.503
MACN-PV-94a-3	<i>Enanornithes</i>	7		-	-3.461	0.042	-4.380	0.083	0.570	0.003	0.259	0.025	33.53	1.28	-0.314	0.236
MACN-PV-94a-4	<i>Enanornithes</i>	9		-	-4.939	0.099	-7.184	0.167	0.541	0.015	0.256	0.020	46.16	6.83	-0.995	1.161
PB-MOD-1	Gray Peacock Pheasant	7		-	2.641	0.045	-5.101	0.060	0.534	0.009	0.343	0.040	48.18	3.62	1.608	0.668
PP-MOD-1	Cliff Swallow	7		-	13.884	0.026	-5.519	0.065	0.556	0.008	0.218	0.018	38.93	2.98	-0.468	0.559

RADT-MOD	Radiated Tortoise	5		- 14.640	0.099	-5.594	0.059	0.588	0.009	0.268	0.019	27.06	3.07	-2.815	0.637
RR-MOD-1	Crested Wood Partridge	7		-7.539	0.021	-4.327	0.040	0.541	0.004	0.274	0.022	44.65	1.45	1.794	0.250
SCOMOD-1	Ostrich	6		-8.003	0.125	-3.733	0.036	0.538	0.005	0.175	0.022	45.96	1.95	2.623	0.348
SS-MOD-1	Allen's Hummingbird	3		- 12.110	0.014	0.190	0.028	0.572	0.019	0.611	0.172	33.10	6.79	4.164	1.331
TV-MOD-1	Turkey Vulture	7		-7.170	0.068	-3.399	0.072	0.547	0.006	0.226	0.029	42.53	2.24	2.338	0.384
ZA-MOD-1	White Winged Dove	11		-4.443	0.063	-3.743	0.083	0.542	0.004	0.277	0.035	44.38	1.60	2.331	0.282
KB-MOD-1	Bell's Hingeback Tortoise	7		- 11.320	0.037	-5.429	0.038	0.596	0.007	0.219	0.041	24.50	2.36	-3.165	0.493

Supplementary figures



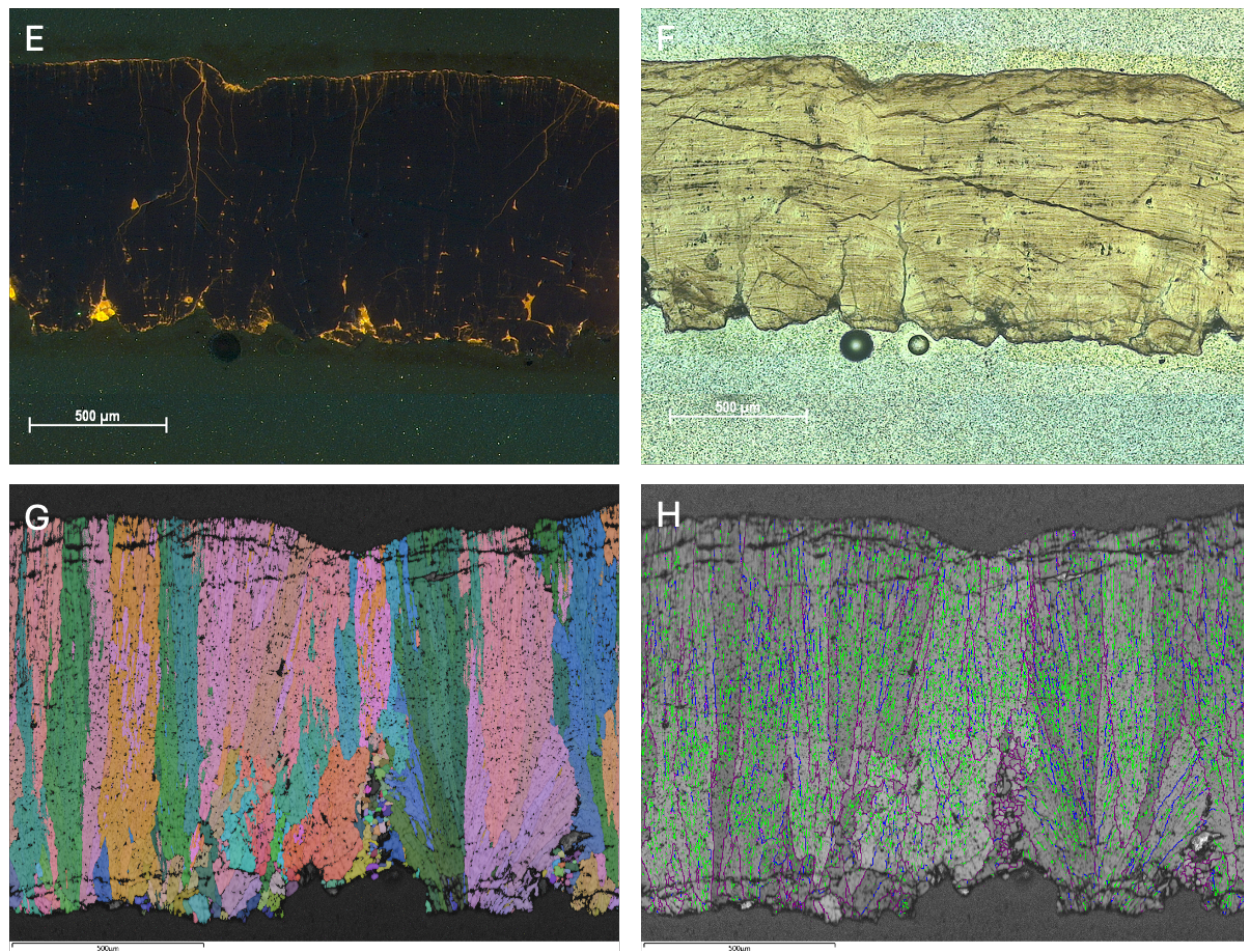
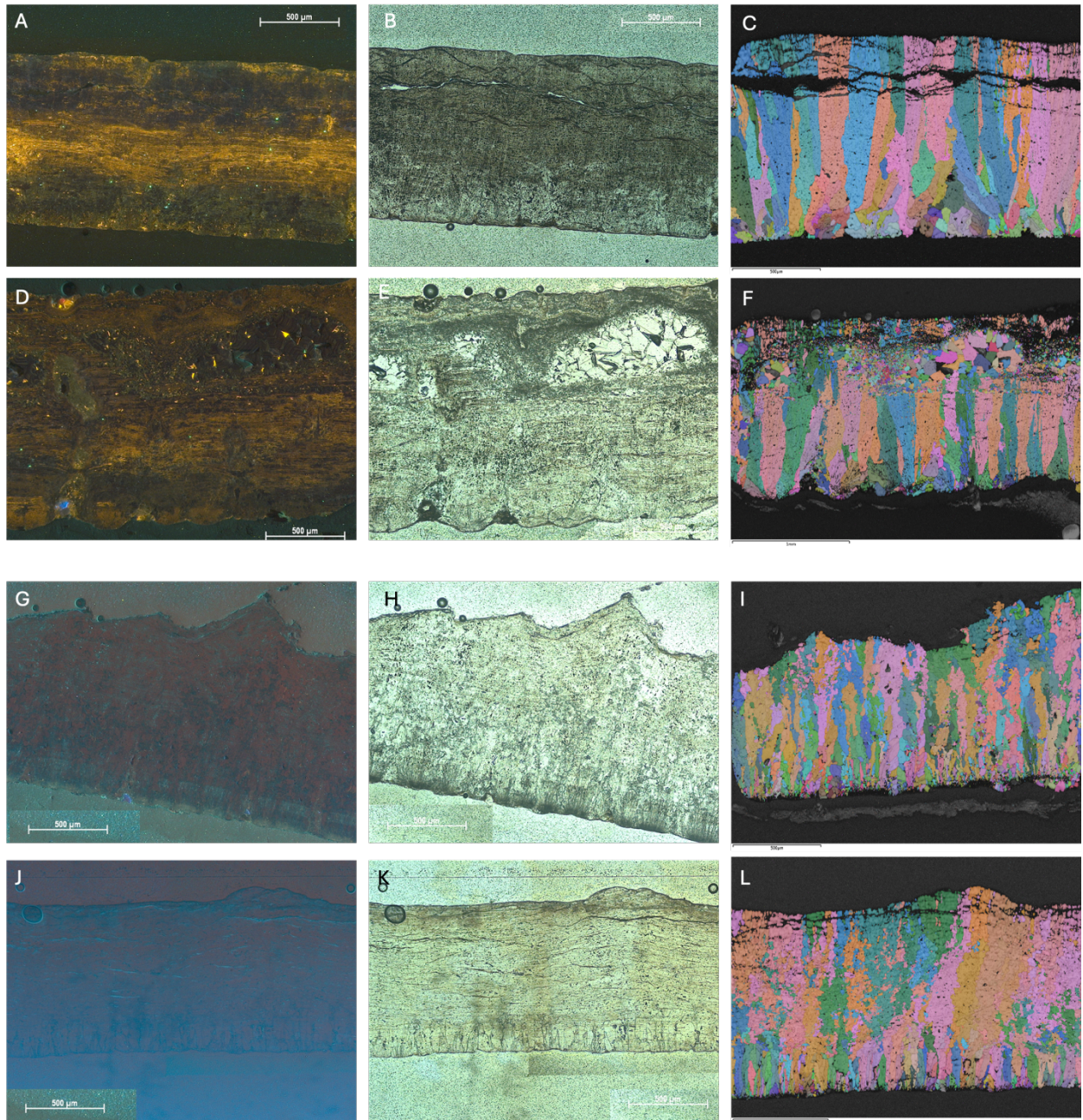


Figure S1.1 Petrographic characterization of Devil's Coulee, AB, Canada eggshells. (A-B)

CL (left) and optical images (right) of CCEGG-DC1-F1. Luminescence is concentrated to the outermost layer, therefore should have minimal effect on geochemistry as outer layers are taken off prior to pulverization. Since it is not known whether all the recrystallized material was removed, CL preservation is considered inconclusive. (C-D) EBSD (Euler, left; GB, right) images of CCEGG-DC1-F1. Evidence of fluid mediated alteration or widespread recrystallization is not observed in EBSD images. (E-F) CL and optical images of SAEGG-DC1-F1.

Luminescence is concentrated to the outermost layer, therefore should have minimal effect on geochemistry as outer layers are taken off prior to pulverization. Since it is not known whether all the recrystallized material was removed, CL preservation is considered inconclusive. (G-H)

EBSD images of SAEGG-DC1-F1. Abiogenic calcites are observed within some pore canals, however overall microstructure is intact. All CL and optical images taken at 5x magnification.



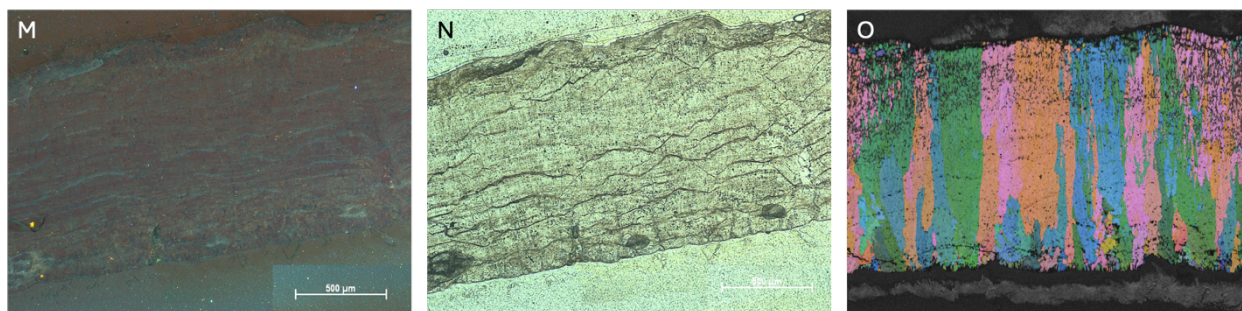


Figure S1.2 Petrography of Mongolian eggshells. (A-B) CL (left) and optical (right) images of ST-F1R. Luminescent layers are observed throughout the eggshell. (C) EBSD (Euler) image of ST-F1R. EBSD images show no evidence of fluid mediated alteration or recrystallization, although abiogenic calcite can be observed in pore canals. (D-E) CL and optical images of SI-F1R. Luminescent layers are observed throughout the eggshell, and abiogenic material can also be observed. (F) EBSD (Euler) image of SI-F1R. Very poor preservation, abundant recrystallization, evidence of fluid mediated alteration. (G-H) CL and optical images for EF-F1R. No luminescence observed. (I) EBSD (Euler) image of EF-F1R. Some recrystallization observed, indicating possible fluid mediated alteration. (J-K) CL and optical images of ES-F1R. No luminescence observed. (L) EBSD (Euler) image of ES-F1R. EBSD images show no evidence of fluid mediated alteration or recrystallization; the sample appears well preserved. (M-N) CL and optical images of SM-F1R. No widespread luminescence observed. (O) EBSD (Euler) image of SM-F1R. EBSD images show no evidence of fluid mediated alteration or recrystallization; the sample appears well preserved. All CL and optical images taken at 5x magnification.

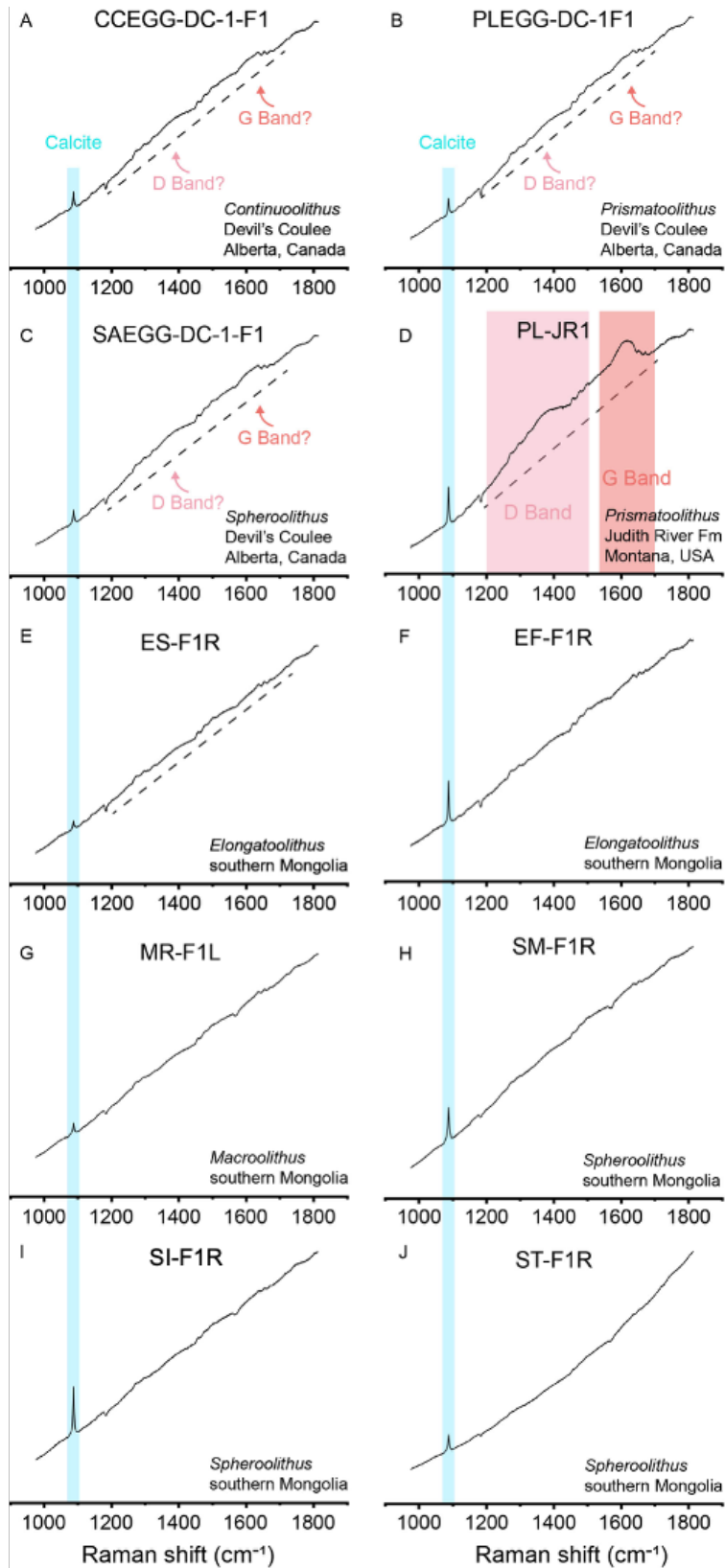


Figure S1.3 Full Raman spectral data. (A-E) Eggshells observed to have evidence of solid state reordering. PL-JR-1 shows well-developed D and G bands, indicating a high probability of solid state reordering. Devil's Coulee eggshells show possible D and G bands (poorly developed) which could be indicative of solid state reordering. No specimens from other localities, such as Mongolia showed D or G bands.

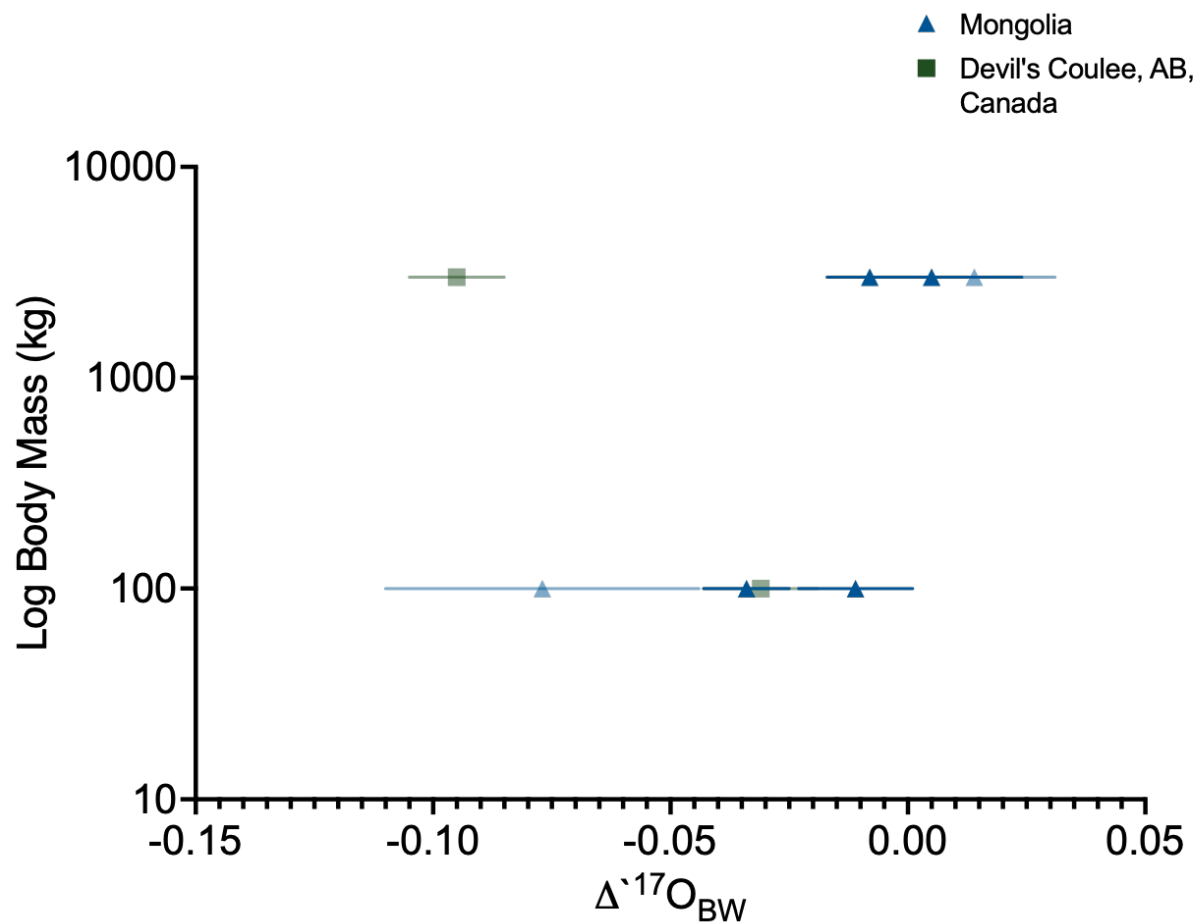


Figure S1.4 Triple oxygen results versus estimated body mass (kg).

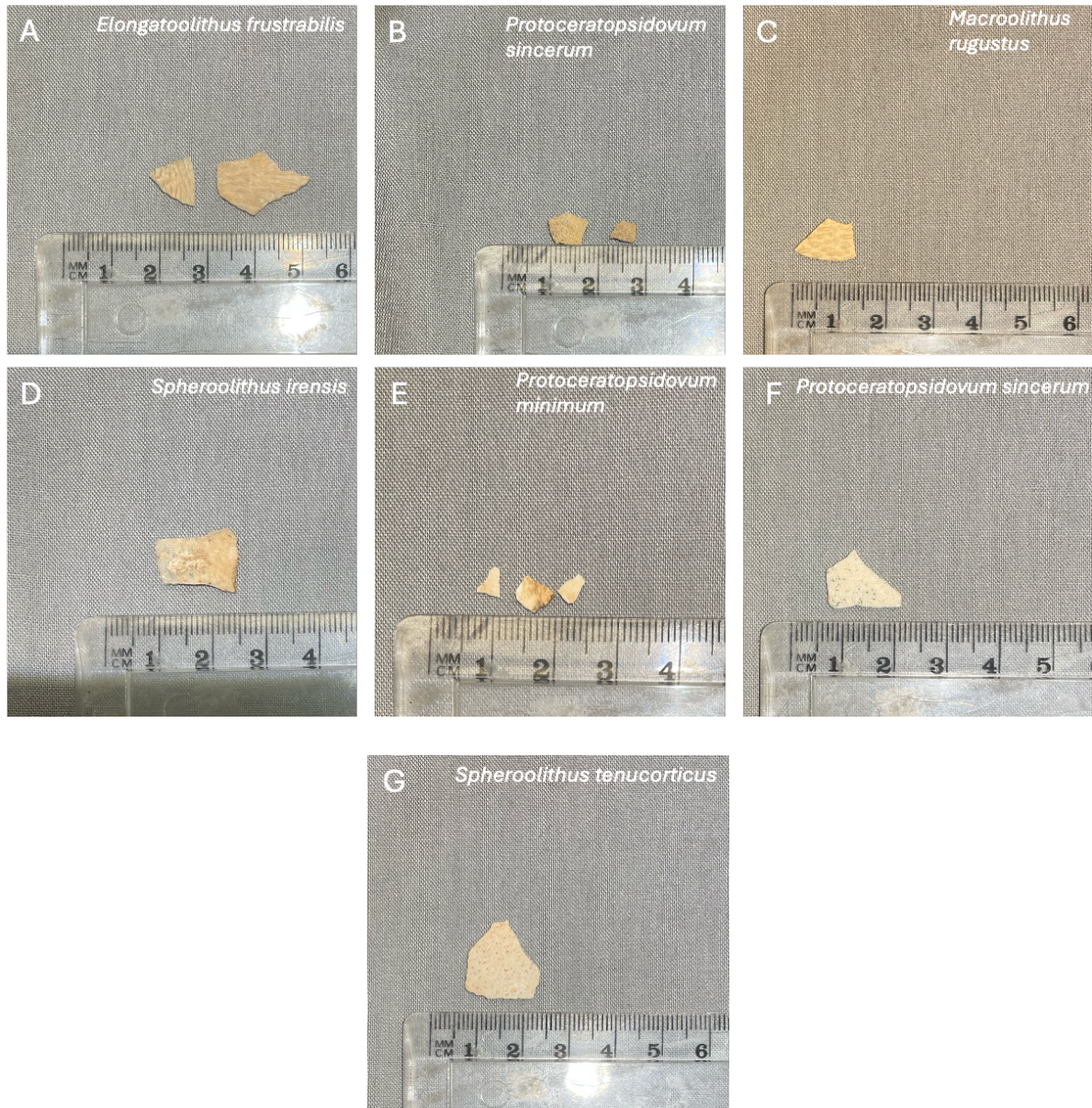


Figure S1.5 Mongolian eggshells in this study in hand specimen.

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Chapter 2 CLUMPED-ISOTOPE (Δ_{47}) THERMOMETRY OF PALEOSOL CARBONATES REFLECTS MULTI-KILOMETER MESOZOIC BURIAL AND LARAMIDE EXHUMATION OF THE PERMIAN BERNAL FORMATION, NEW MEXICO

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Abstract

Carbonate clumped-isotope (Δ_{47}) thermometry can record primary pedogenic temperatures as well as post-depositional thermal resetting. We report data from fourteen carbonate samples collected from the Permian Bernal Formation and the Triassic Anton Chico Member at Bernal Mesa, New Mexico. Apparent temperatures range from 10.9 ± 3.2 to 92.9 ± 4.6 °C. Two Permian samples yield low apparent temperatures (10.9 and 18.8 °C) and depleted calculated water oxygen isotope compositions, and because they are stratigraphically below the thermally altered samples, probably represent post Mesozoic groundwater rather than original paleosol temperatures. The other twelve samples yield 52.4–92.9 °C and are interpreted as variably reset during burial. Forward models using the disordered calcite-reordering framework of Hemingway and Henkes (2021), conditioned on a prescribed Late Cretaceous peak-burial time and subsequent cooling, yield maximum burial temperatures of approximately 164–178 °C for most of the burial-reset population. These temperatures imply approximately 4.1–6.7 km of overburden for geothermal gradients of 25–30 °C km⁻¹ and are constructed using regional Laramide exhumation onset and cooling at 75–80 Ma. The study demonstrates the potential of Δ_{47} reordering models in continental red-bed successions for the Permian-Triassic boundary strata in the southern Western Interior.

Keywords: clumped isotopes, pedogenic carbonate, burial history, Laramide orogeny, Permian–Triassic boundary, New Mexico

Introduction

Burial histories are key to understanding thermal maturation, fluid flow, and economic potential of sedimentary basins. Recent advances have demonstrated the power of carbonate clumped-isotope (Δ_{47}) thermometry combined with solid-state reordering kinetics, for reconstructing burial and exhumation histories in sedimentary basins, including pedogenic carbonates (Eiler 2007; Huntington and Lechler 2015; Kelson et al. 2020; Li et al. 2021; Ryb et al. 2021). Clumped-isotope (Δ_{47}) thermometry, pioneered by Eiler (2007), measures the abundance of mass-47 CO₂ (¹³C¹⁸O¹⁶O) relative to a stochastic distribution and provides temperature estimates independent of the isotopic composition of the parent fluid. Subsequent reviews have highlighted its growing utility in continental settings, particularly for pedogenic carbonates and burial history reconstructions (Huntington and Lechler 2015; Kelson et al. 2020). These advances have enabled quantitative constraints on maximum burial temperatures and exhumation histories in both marine and terrestrial sedimentary successions (e.g., Li et al. 2021; Ryb et al. 2021).

Ryb et al. (2021) applied this approach to Paleozoic marine carbonates across the Colorado Plateau, using the distribution of measured Δ_{47} values and forward modeling of reordering to constrain peak burial temperatures and total exhumation. Similarly, Li et al. (2021) developed kinetic models to reconstruct maximum burial temperatures in the Sichuan Basin using Δ_{47} data from Permian and Upper Triassic limestones and dolostones.

The Bernal Formation of New Mexico's upper contact with the Anton Chico Member of the Moenkopi Formation is recognized to represent the Permian—Triassic boundary (Lucas and Hayden, 1991). Independent constraints on the burial and thermal history of the Bernal and Anton Chico Formations are limited. Previous regional studies have used vitrinite reflectance and apatite fission-track thermochronology to document Mesozoic burial and Laramide exhumation across northern and central New Mexico (Kelley et al., 1992; Cather 2004), but few data exist specifically for the Bernal Mesa area. Detrital zircon U-Pb geochronology from overlying Upper Triassic Chinle Group strata provides maximum depositional age constraints for the regional Triassic succession (Rasmussen et al. 2021), yet direct chronological or thermal maturity data from the Permian—Triassic boundary interval at Bernal Mesa remain sparse.

This study tests whether Δ_{47} measurements from paleosol carbonates, interpreted with petrography, elemental mapping, and kinetic models, can constrain the post-depositional thermal history of the Bernal—Anton Chico succession. Paleosol carbonates form at low near-surface temperatures, which provides a useful initial condition, but they may contain multiple generations of calcite or dolomite and can undergo early or late fluid-mediated alteration (Quade et al. 2007, 2013; Zamanian et al. 2016). Fourteen samples are therefore used to evaluate the range of diagenetic histories and to estimate model-dependent maximum burial temperatures rather than to assume a single preserved paleoclimate signal.

1.1 Geologic Setting

The Bernal Formation is a Permian sedimentary unit exposed in north-central New Mexico, particularly around the town of Bernal in San Miguel County (Figure 2.1), where it forms part of the landscape on Glorieta Mesa and nearby buttes including Bernal Mesa (Baldwin and Muehlberger 1962; Lucas and Hayden 1991). Although the Bernal name has been recommended for discontinued usage, the unit is locally retained for the type area (Lucas and Hayden 1991). It consists primarily of siltstone, fine-grained sandstone, and shale, with minor components such as gypsum, dolomitic limestone, and anhydrite, representing clastic shelf deposits (Baldwin and Muehlberger 1962; Lucas and Hayden 1991). The formation is thin, averaging about 100 feet (30 m) thick, with a maximum of 126 feet (38 m) near Ocate, and thins southward. At its type section, it measures 42.5 m (139 ft) (Lucas and Hayden 1991). It unconformably overlies the San Andres Formation (including the Glorieta Sandstone Member) and is unconformably overlain by the Middle Triassic Anton Chico Member of the Moenkopi Formation, marking the Permian-Triassic boundary in this region Baldwin and Muehlberger 1962; Lucas and Hayden 1991). Permian and Triassic greenhouse spikes of the basal Roadian, mid-Capitanian, and late Changsingian are recorded in this section (Retallack, 2009; Retallack et al., 2011) by paleosols with unusual depth to Bk horizon (Fig. 2.2B) and unusually negative $\delta^{13}\text{C}$ of pedogenic carbonate (Fig. 2.2C). The Bernal Formation is equivalent to the lower part of the Artesia Group (including the Grayburg and Queen Formations) and possibly the Seven Rivers Formation, but its usage has been restricted or recommended for abandonment in some

areas due to historical misapplication to Triassic rocks (Tait et al. 1962; Lucas and Hayden 1991).

The Bernal Formation records a desert to shallow marine depositional environment, with fine silt, salt, and gypsum indicating arid conditions and evaporite precipitation in restricted basins or sabkhas (Baldwin and Muehlberger 1962; Lucas and Hayden 1991). Deposition began after the underlying San Andres Limestone, which formed in a warm shallow sea, as sea levels fluctuated due to global glaciation cycles (Kottlowski 1963). The lithology includes brownish-red siltstone and fine-grained sandstone, with a single bed of gray to brown medium-grained sandstone, massive gypsum 10 m above the base, and a thin dolomitic limestone near the top, suggesting periodic marine incursions amid predominantly continental clastic input (Lucas and Hayden 1991). In the Glorieta Mesa area near Bernal village, it features purplish-red sandstone and siltstone with thin gypsum beds, reflecting a transition from marine to desert settings (Baldwin and Muehlberger 1962). Overall, the formation captures the expansion of continental red beds southward during the Permian, with arid climate supporting evaporite deposition similar to other Permian units like the Castile and Salado Formations in deeper basins (Tait et al. 1962; Kottlowski 1963).

Stratigraphically, the Bernal Formation is part of the Permian Artesia Group in northeast and east-central New Mexico and is the equivalent to the lower half of this group (Tait et al. 1962; Lucas and Hayden 1991). It overlies the Glorieta Sandstone Member of the San Andres Formation and underlies the Santa Rosa Formation (Baldwin and Muehlberger 1962). Lithologically, it is dominated by red-brown to red-orange siltstone with minor quartzose sand, feldspar, mica, anhydrite, and calcareous material; in southern areas, it includes a 1-foot-thick sandstone bed, massive gypsum, and dolomitic limestone (Lucas and Hayden 1991). The formation is finer-grained than the overlying Moenkopi Formation, with unconformities at both contacts indicating erosional hiatuses (Lucas and Hayden 1991). In broader Permian stratigraphy, it fits amid cyclic marine-continental units like the Abo and Cutler red beds below and evaporite-dominated sequences in adjacent basins (Tait et al. 1962).

1.2 Tectonic Setting

During the Permian, New Mexico experienced relative tectonic quiescence following the Pennsylvanian uplift of the Ancestral Rocky Mountains (~300 Ma), with subsidence in basins separated by uplifts (Kottlowski 1963; Lucas 2018). The Bernal Formation was deposited in a shallow marine to continental transition zone on the clastic shelf of the Permian sea, influenced by transgressions and regressions linked to global sea-level fluctuations from glaciation (Lucas and Hayden 1991). In the Pecos area, Aridisol paleosols of the Bernal Formation reflect arid to semiarid desert conditions (Retallack, 2009). Ongoing subsidence without tectonic deformation allowed for evaporite and red bed accumulation (Baldwin and Muehlberger 1962; Kottlowski 1963). The formation's northern facies is thinner and finer-grained, representing a marginal shelf environment prograding southward from continental red beds like the Abo Formation and Cutler Group (Lucas and Hayden 1991).

1.3 Paleontology and the Permian-Triassic Boundary

Fossil content in the Bernal Formation is limited, with no records of vertebrates, invertebrates, or plants, though underlying San Andres Limestone contains abundant marine fossils (Baldwin and Muehlberger 1962). Nearby Permian units like the Cutler Group preserve tetrapod fossils, indicating early land vertebrate evolution in continental settings (Lucas 2018). The formation's upper contact with the Anton Chico Member of the Moenkopi Formation represents the Permian-Triassic boundary, a major unconformity spanning the end-Permian mass extinction (~252 Ma), with no continuous record across the boundary in this area (Lucas and Hayden 1991; Retallack, 2009).

1.4 Post-Depositional and Burial History

After deposition, the Bernal Formation experienced erosion during the Triassic, creating the unconformable contact with overlying units, followed by burial under Mesozoic and Cenozoic strata (Lucas and Hayden 1991). The Laramide orogeny (~70 Ma) uplifted much of New Mexico, exposing Permian rocks on mesas like Glorieta Mesa through differential erosion (Kottowski 1963). In the Bernal area, the formation's preservation on elevated mesas reflects resistance to erosion due to its siltstone and sandstone composition, with gypsum dissolution potentially influencing local karst features (Baldwin and Muehlberger 1962). This history highlights the Bernal Formation's role in documenting the transition from Permian paralic deserts to Triassic continental environments in north-central New Mexico (Tait et al 1962; Lucas and Hayden 1991).

Materials and methods

2.1 Sampling

Fourteen samples were collected by GJR in Late October of 2005 from Bernal Mesa, New Mexico (Figure 2.1, Table 2.1). Detailed stratigraphic data is depicted in Figure 2.2. Samples are archived in the Condon Collection of the Museum of Natural and Cultural History of the University of Oregon in Eugene. Samples were subsequently sectioned in paired polished and thick sections. Paleosols were drilled from thick sections using a Dremel tool and treated with dilute H₂O₂ to remove organic matter prior to analysis.

2.2 Plane Polarized and Cross-Polarized Light Petrography

Polished thin sections were examined under both plane light (PPL) and cross-polarized light (XPL) to assess fabric and search for any signs of recrystallization or replacement. Samples were examined using an Olympus petrographic microscope at 5X magnification, equipped with a camera and imaging software.

2.3 Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (EDAX/EDS)

To better understand the relationship between clumped isotope temperatures and diagenetic history, selected samples were characterized using scanning electron microscopy

with energy-dispersive X-ray spectroscopy (EDAX/EDS) for elemental mapping and phase identification. Analyses were performed at UCLA's Ion Microprobe Laboratory using a Vega Tescan scanning electron microscope equipped with EDAX detectors and software.

2.4 Isotope Ratio Mass Spectrometry

All isotopic analyses were performed in the Tripati-Eagle IsoLab using a Thermo Finnegan MAT 253 gas source mass spectrometer and a Nu Perspective IS mass spectrometer. Stable carbon ($\delta^{13}\text{C}$), oxygen ($\delta^{18}\text{O}$), and carbonate clumped (Δ_{47}) isotope ratios of the samples were measured using methods described previously (Defliese and Tripati 2020; Upadhyay et al. 2021; Lucarelli et al. 2023). Briefly, samples were digested at 90 °C in a common phosphoric acid bath. CO_2 gas was first purified by a cryogenic trap containing liquid nitrogen, which removes water and other gases with low vapor pressures, followed by an adsorption trap which passes the sample gas through a gas chromatograph (GC) column with Porapak Type-QTM 50/80 mesh at -20 °C to remove organic contaminants and silver wool (Sigma-Aldrich) to remove sulfur compounds (Upadhyay et al. 2021). Carbonate standards ETH-1, ETH-2, ETH-3, and ETH-4 (Bernasconi et al. 2018), as well as in-house standards bracketed samples to establish reproducibility and calculate the empirical transfer function (ETF) and nonlinearity correction (Dennis et al. 2011). The average external reproducibility (1 SD) of Δ_{47} measurements presented here is 0.008‰. Table S1 contains raw data, and ETF and nonlinearity slopes and intercepts for all standards and samples. Apparent temperatures were calculated from measured Δ_{47} values using the unified calibration of Anderson et al. (2021):

$$T\ (^{\circ}\text{C}) = 10^6 \times \left(\frac{0.04459}{\Delta_{47} + 0.1262} \right) - 273.15 \quad (1)$$

2.5 Modeling

Kinetic models from Hemingway and Henkes (2021) were used to simulate solid-state clumped isotope bond reordering based on measured Δ_{47} values. These models treat reordering as a continuum of parallel first-order processes with a distribution of activation energies (disordered kinetics), unifying earlier approaches such as the transient defect model of Passey and Henkes (2012) as special cases. The disordered model was implemented to back-calculate maximum burial temperatures (MBT) by forward-modeling thermal histories that reproduce the observed Δ_{47} , accounting for progressive heating during burial and partial re-equilibration during exhumation.

The equilibrium reference used by the reordering workflow is represented by the following polynomial relation:

$$\Delta_{47(eq)} = -3.407 \times 10^9 T^{-4} + 2.365 \times 10^7 T^{-3} - 2.67 \times 10^3 T^{-2} - 5.880 T^{-1} + 0.280 \quad (2)$$

Reordering was simulated with the disordered kinetic framework of Hemingway and Henkes (2021) as implemented in the isotopylog package. The primary calculations use the published dry-calcite parameters and an initial carbonate-formation temperature of 25 °C. Because several samples contain high-Mg carbonate or possible dolomite and show petrographic evidence of fluid-mediated recrystallization, the resulting maximum burial temperatures should be regarded as calcite-model approximations rather than mineralogy-independent estimates.

Thermal histories were parameterized with linear heating from deposition at approximately 252 Ma to a prescribed peak-burial time of approximately 77–80 Ma, followed by linear cooling to the present. The 75–80 Ma onset of cooling was selected from regional stratigraphic and thermochronologic constraints (Cather, 2004; Kelley et al., 1992; Thacker et al., 2021). It is therefore a model input, not an independently estimated parameter. Sensitivity tests used a shorter heating duration and alternative kinetic assumptions to evaluate how strongly maximum burial temperature depends on the prescribed history.

Maximum burial depths were estimated from the modeled MBT values using a linear geothermal gradient:

$$Depth (km) = \frac{MBT - T_{surface}}{\Gamma} \quad (3)$$

where $T_{surface} \approx 15 \text{ }^{\circ}\text{C}$ and $\Gamma = 25\text{--}30 \text{ }^{\circ}\text{C km}^{-1}$ (Meyer, 1992; Kelley et al., 1992).

The disordered kinetic model was explored for the paleosol-carbonate dataset and compared with regional overburden constraints. Application of dry-calcite kinetics is best justified for samples dominated by calcite and showing evidence for solid-state reordering. Results were compared against independent regional burial constraints from nearby Zuni Mountains, Nacimiento arch, and Sangre de Cristo Mountains (Kelley et al. 1992; Cather 2004; Thacker 2021).

Results

3.1 Petrography

Each polished section was examined using plane polarized and cross-polarized light petrography to pre-assess degree of diagenetic alteration, and to assess whether Δ_{47} -derived temperatures were likely reflective of Earth surface temperatures or reflective of thermal resetting. Polished sections show variable but pervasive diagenetic alteration, including coarse sparry calcite, cross-hatching, dissolution-remineralization features, and loss of micritic matrix in the majority of samples (Figure 2.3). Alteration is heterogeneous, consistent with variable fluid-rock interaction during burial and exhumation.

3.2 Clumped and Bulk Isotopes

Apparent temperatures (Table 2.2, Figure 2.4) range from $10.9 \pm 3.2 \text{ }^{\circ}\text{C}$ in R4944 to $92.9 \pm 4.6 \text{ }^{\circ}\text{C}$ in R4964. R4944 and R4948 yield 10.9 ± 3.2 and $18.8 \pm 4.2 \text{ }^{\circ}\text{C}$, respectively; their low apparent temperatures and, for R4944, depleted calculated water composition are consistent with cool recrystallization from post-Triassic groundwater, rather than original paleosol formation temperatures. The remaining twelve samples yield $52.4\text{--}92.9 \text{ }^{\circ}\text{C}$ and are interpreted as variably reset during burial.

3.3 Elemental Mapping (EDAX/EDS)

Sample R4944 ($T_{\Delta_{47}} = 10.9 \pm 3.2$ °C), which records the lowest apparent temperature and strongly depleted $\delta^{18}\text{O}_{\text{water}}$ (-8.1 ± 0.7 ‰ SMOW), contains patchy calcite and high-Mg carbonate domains (Ca up to 26 wt%, as determined by EDAX point analysis) intergrown with quartz, aluminosilicates, and minor organic carbon (Table 2.3, Figure 2.5). Multiple spots also show Mg-rich phases (up to 17 wt% Mg). This mineralogy, combined with the very low clumped-isotope temperature, supports late-stage meteoric reordering under cool, near-surface conditions following Laramide exhumation, or possibly high-dolomite composition. The heterogeneous distribution of carbonate suggests localized dissolution and reprecipitation by meteoric fluids.

In contrast, sample R4950 ($T_{\Delta_{47}} = 84.3 \pm 1.4$ °C) contains abundant Ca-rich carbonate phases (up to ~30 wt% Ca, as determined by EDAX map) intimately intergrown with quartz, feldspars, and clay minerals. Elemental maps reveal clear domains of sparry calcite, supporting petrographic evidence of significant recrystallization during burial and consistent with thermal resetting at elevated temperatures.

Samples R4959L ($T_{\Delta_{47}} = 52.6 \pm 4.7$ °C) and 4959U ($T_{\Delta_{47}} = 56.9 \pm 3.9$ °C) both display abundant carbonate. These samples contain prominent Ca-rich phases (often 20–30 wt% Ca, EDAX map) with significant Mg content (up to 16 wt% in Mg-rich domains), suggestive of high-Mg calcite or dolomite components typical of pedogenic carbonates in arid environments (Regnet et al., 2024). The carbonate is intergrown with a silicate matrix (Si, Al, Mg, minor Na), indicating partial recrystallization (Zamanian et al. 2016).

Sample R4964 ($T_{\Delta_{47}} = 92.9 \pm 4.6$ °C, EDAX point analysis), a high-temperature reset sample, is dominated by siliciclastic phases including quartz and K-bearing silicates, with only minor and patchy Ca detected. This is consistent with either preferential analysis of the non-carbonate matrix or partial dissolution and recrystallization of carbonate during burial diagenesis.

Overall, EDS data reveal a clear link between mineralogical characteristics and Δ_{47} temperatures, with samples preserving lower temperature estimates shown to contain abundant pedogenic (often Mg-bearing) carbonate, whereas high-temperature reset samples show evidence of more extensive sparry recrystallization or variable carbonate preservation, and the meteoric-reset sample is largely devoid of carbonate in analyzed areas.

The EDS observations document heterogeneous Ca- and Mg-bearing domains intergrown with siliciclastic phases, but the small number of mapped areas and the use of elemental weight percentages do not establish a quantitative relationship between carbonate abundance and Δ_{47} temperature. The maps are consistent with variable mineralogy and recrystallization and are used as qualitative preservation evidence rather than as proof of a single alteration mechanism.

3.4 Geochemical Kinetic Modeling

Solid-state reordering was modeled with the disordered kinetic framework of Hemingway and Henkes (2021) using the isotopylog implementation in Python (Table 2.4, Figure 2.6). The preferred history uses approximately 175 Myr of heating followed by approximately 75 Myr of cooling; an alternative 150 Myr heating duration provides a sensitivity test. Across the modeled samples, maximum burial temperatures range from approximately 138 to 183 °C, with most of the burial-reset population clustering near 164–178 °C. Lower measured Δ_{47} values require higher peak temperatures under the imposed time–temperature path.

Using an initial present-day surface temperature of 15 °C, the full modeled range corresponds to approximately 4.9–6.7 km of burial for a 25 °C km⁻¹ geothermal gradient or 4.1–5.6 km for a 30 °C km⁻¹ gradient. These values overlap regional estimates of Mesozoic overburden. The two cool-recrystallized samples yield lower modeled peak temperatures, but because their Δ_{47} values may reflect open-system alteration, they should not be weighted equally with the burial-reset population when estimating maximum burial.

Most burial-reset samples yield similar model-dependent peak temperatures, consistent with substantial thermal modification during prolonged burial. The degree of re-equilibration cannot be established from the modeled temperatures alone, particularly where mineralogy and fluid-mediated recrystallization are uncertain.

Sensitivity tests using faster (“wet”) and dolomite kinetic parameters produce shifts of derived MBT corresponding to minimal burial depth differences (.05-1+ km, depending on sample gradient). The first-order multi-kilometer burial conclusion is robust to these alternatives; therefore only dry-calcite results are reported in detail here.

Overall, modeled maximum burial temperatures converge around 160–170 °C for most samples, supporting solid-state re-equilibration of Δ_{47} during prolonged burial under the Laramide thermal regime.

Discussion

4.1 Interpretation of Temperature Variability

The heterogeneity in apparent Δ_{47} temperatures reflects variable preservation of isotopic signals within the thin Bernal Formation and overlying Anton Chico Member, possibly affected by dolomitization of some carbonates in early diagenesis making these sections more resistant to reordering with continued burial (Hemingway and Henkes 2021; Regnet et al. 2024). The main cluster of twelve samples, spanning both Permian (BMP) and Triassic (BMT) paleosols, yields apparent temperatures of ~52–93 °C, consistent with solid-state isotopic reordering during prolonged burial. Modeling indicates partial re-equilibration at peak burial temperatures of ~164–178 °C for these samples (Table 2.4). Notably, the two low-temperature outliers (R4944 and R4948) occur at the very base of the studied stratigraphic section (Figure 2.2; 1 m and 16 m, respectively), presenting an apparent inversion of the expected burial geothermal gradient. This stratigraphically localized low-temperature signature is best explained by preferential post-exhumation fluid flow (Bergman et al. 2013; Ghosh et al. 2016; Mangenot et al. 2018; Li et al. 2026), because that part of the section was buried even more deeply than the thermally reset upper part of the section. The base of the Bernal Formation unconformably overlies the highly permeable Glorieta Sandstone Member of the San Andres Formation (Scholle 2003; Lucas and Zeigler 2004). This contact likely acted as a major lateral conduit for cool, unheated meteoric groundwaters during or after Laramide exhumation (Broadhead 1984; Basabivazo 1997). The proximity of the basal paleosols to this underlying aquifer system may have facilitated localized open-system dissolution and recrystallization, resetting the clumped-isotope signatures of these lowest-temperature samples while leaving the upper, more isolated mudrock-dominated intervals of the formation protected from meteoric alteration, preserving the solid-state reordering signal.

These MBT values correspond to maximum burial depths of approximately 4.9–6.7 km (using a 25 °C km⁻¹ geothermal gradient) or 4.1–5.6 km (using a 30 °C km⁻¹ gradient). This is substantially deeper than burial of 0.6 km inferred from measured thickness of known overlying formations nearby (Retallack, 2009). Our revised depths are consistent with independent regional estimates of Mesozoic overburden in north-central New Mexico. Two samples with the highest Δ_{47} values (R4944 and R4948) record the lowest MBT (~138–148 °C) and may reflect incomplete resetting or minor late-stage meteoric influence, whereas the most reset samples (lowest Δ_{47} indicate the deepest burial conditions).

Maximum burial temperatures calculated assuming 175 Myr versus 150 Myr of heating differ by only ~5–12 °C on average (paired t-test, $p < 0.001$), with a very strong Pearson correlation of $r = 0.999$. These statistical analyses demonstrate that there is no statistically significant difference between the two populations of data, and the two groups change with synchronicity. This modest difference (typically <7% relative) translates to an uncertainty of less than 200–300 m in estimated burial depth (using a 25 °C km⁻¹ geothermal gradient). These results demonstrate that our overburden reconstructions (approximately 4.1–6.7 km) are relatively insensitive to reasonable variations in burial duration. The modeled thermal histories were constructed assuming the onset of Laramide-related exhumation around 75–80 Ma, aligning closely with regional thermochronological constraints from the Zuni Mountains, Nacimiento arch, and Sangre de Cristo Mountains (Cather 2004; Thacker et al. 2021).

These data provide new quantitative evidence for significant Mesozoic burial followed by rapid unroofing of the southern Western Interior. Overall, modeled maximum burial temperatures converge around 160–170 °C for most samples, supporting solid-state re-equilibration of Δ_{47} during prolonged burial under the Laramide thermal regime. Importantly, the reset samples from both the Permian Bernal Formation and the Triassic Anton Chico Member (which together straddle the Permian-Triassic boundary) display highly consistent maximum burial temperatures. This similarity demonstrates that both units experienced the same multi-kilometer Mesozoic burial history following Triassic deposition, despite the major unconformity and environmental disruption at the PT boundary. The tight clustering across stratigraphic levels reinforces that Δ_{47} thermometry effectively captures the post-depositional thermal evolution of the entire Permo-Triassic succession.

4.2 Interpretation of Isotopic Relationships and Clustering

The stable and clumped isotope data reveal two distinct populations consistent with variable degrees of diagenetic resetting (Fig. 2.7). In $\delta^{18}\text{O}$ – $\delta^{13}\text{C}$ space (Fig. 2.7A), most samples form a broad cluster with $\delta^{18}\text{O}$ values ranging from approximately –10 to +1‰ and $\delta^{13}\text{C}$ from –9 to +1‰. This distribution is most like pedogenically modified marine detrital carbonate (Melim et al., 2004; Retallack et al., 2021). In contrast, the two samples with the lowest apparent temperatures (R4944 and R4948) exhibit more negative $\delta^{18}\text{O}$ values, more like pedogenic carbonate (Retallack, 2025).

The relationship between Δ_{47} and calculated $\delta^{18}\text{O}_{\text{water}}$ (Figure 2.4) further supports this interpretation. Samples that experienced significant burial heating ($T\Delta_{47} = 52$ –93 °C) are characterized by more positive calculated $\delta^{18}\text{O}_{\text{water}}$ values (+2 to +9‰), indicating contamination with marine carbonate clasts. The strong clustering of these twelve samples, combined with their coherent maximum burial temperatures (~164–178 °C), demonstrates solid-state re-equilibration at peak burial conditions. The clear separation between the burial-reset

cluster and the meteoric-reset outliers highlights the heterogeneous nature of diagenesis within this thin formation and underscores the robustness of the kinetic modeling results derived from the reset population. These reset samples record prolonged Mesozoic subsidence to ~4.1–6.7 km burial depth followed by rapid Laramide unroofing. Together, these patterns affirm that Δ_{47} thermometry, when paired with stable isotope data, can effectively disentangle burial and post-exhumation overprints in continental red-bed successions.

4.3 Limitations

Several assumptions and sources of uncertainty inherent to the approach warrant consideration. First, our models rely on the disordered kinetic framework of Hemingway and Henkes (2021). While petrographic and elemental mapping evidence supports dominantly solid-state reordering with limited open-system fluid interaction, the presence of high-Mg calcite and possible dolomite components in some samples could result in slightly different reordering behavior (Brenner et al. 2018). We note, however, that sensitivity tests using 150 Myr to 175 Myr burial durations in modeled maximum burial temperatures.

Second, we assumed linear heating and cooling paths and a fixed initial formation temperature of 25°C. Real burial histories are rarely perfectly linear, and short-term temperature excursions (e.g., hydrothermal events or variations in geothermal gradient) could influence the degree of reordering. Similarly, although 25 °C is consistent with warm-season soil temperatures in Permian equatorial Pangea (Mack 2003; Tabor 2007), seasonal or depth-related variability in pedogenic carbonate formation could introduce modest uncertainty in the starting Δ_{47} value.

Third, the heterogeneous nature of diagenesis in red-bed paleosols is evident from variable petrographic alteration and EDS results. While most samples show clear evidence of burial resetting, the preservation of both low-temperature (meteoric) and high-temperature signals within the same thin formation highlights that Δ_{47} records can be complex at the hand-sample scale. Finally, maximum burial depths were calculated using a range of continental geothermal gradients (25–30 °C km⁻¹); actual gradients may have varied spatially and temporally.

Lastly, mineralogy exerts a first-order control on clumped isotope reordering kinetics, and this likely contributes to the observed variability in our dataset. While most samples are dominated by calcite, several contain detectable dolomite or high-Mg calcite components. Dolomite is known to reorder more slowly than calcite under equivalent thermal conditions (e.g., Brenner et al. 2018; Hemingway and Henkes 2021). It is therefore plausible that some dolomite-bearing samples formed earlier in the diagenetic history (prior to peak Mesozoic burial) and experienced less extensive reordering due to intrinsically slower kinetics (Regnet et al. 2024). This interpretation is consistent with the slightly lower maximum burial temperatures recorded by certain samples. Future work combining detailed petrography, elemental mapping, and mineral-specific kinetic modeling will help disentangle the relative contributions of formation timing, mineralogy, and burial temperature in controlling the final Δ_{47} signatures.

Despite these limitations, the tight clustering of modeled maximum burial temperatures (~164–178 °C) across multiple samples and sensitivity tests provides confidence in the first-order burial and exhumation history. Future work incorporating multi-phase thermal histories, dolomite-specific kinetics, or integration with other low-temperature thermochronometers (e.g., apatite (U-Th)/He) would further refine these reconstructions.

4.4 Local Geological Context and Contributions to Regional Understanding

The Bernal Mesa succession in north-central New Mexico occupies a key position along the Permo-Triassic boundary, where the Bernal Formation is unconformably overlain by the Anton Chico Member of the Moenkopi Formation (Lucas and Hayden 1991). This contact marks a major stratigraphic hiatus coinciding with the end-Permian mass extinction and the subsequent Triassic recovery interval (Lucas 2005; Retallack, 2009; Haque et al. 2021). Recent stratigraphic and geochronologic studies have significantly refined the regional framework. Lucas and Rasmussen et al. have provided updated correlations and depositional models for the Moenkopi and Chinle Groups (Lucas 2018; Rasmussen et al. 2021), while high-resolution U-Pb zircon studies have clarified the timing of post-boundary continental sedimentation across the Colorado Plateau and southern Western Interior (Rasmussen et al. 2021).

Our clumped-isotope results provide the direct quantitative constraints on the post-depositional thermal history of this boundary interval. By documenting maximum burial temperatures of ~138–183 °C and corresponding overburden depths of 4.1–6.7 km, this study supports previous regional reconstructions that relied primarily on vitrinite reflectance, apatite fission-track, and basin modeling (Kelley et al. 1992; Cather 2004; Thacker et al. 2021). The thermal histories recorded in both Permian Bernal and Triassic Anton Chico paleosols demonstrate that the entire Permo-Triassic succession experienced the same multi-kilometer Mesozoic burial episode, despite the prominent unconformity at the boundary (Lucas and Hayden 1991). These findings refine the timing of maximum burial and support Laramide-related exhumation onset around 75–80 Ma, consistent with low-temperature thermochronological data from the Zuni Mountains, Nacimiento arch, and Sangre de Cristo Mountains (Thacker et al. 2021).

This work contributes to a growing body of recent research applying Δ_{47} thermometry to continental successions for burial history reconstruction (Ryb et al. 2021; Li et al. 2021) and extends these methods to pedogenic carbonates in red-bed systems. By integrating multi-proxy diagenetic screening (petrography, EBSD, and elemental mapping) with disordered kinetic modeling (Hemingway and Henkes 2021), the study establishes a framework for using clumped isotope paleothermometry to model maximum burial temperature and quantitatively constrain burial depths and exhumation in paleosol carbonates. The results underscore the value of Δ_{47} thermometry for resolving basin evolution in complex continental settings where traditional proxies are limited by diagenesis or sparse thermochronologic data, while also linking local stratigraphy at the PT boundary with broader tectonic evolution of the southern Western Interior (Lawton 2020; Lawton et al. 2022).

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Declaration of interest statement

The authors report there are no competing interests to declare.

Data availability statement: The data that support the findings of this study are openly available in EarthChem at [http://doi.org/\[doi\]](http://doi.org/[doi]), reference number [reference number] and at <https://blogs.uoregon.edu/gregr/detailed-webpage/downloadable-data/>.

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Figures

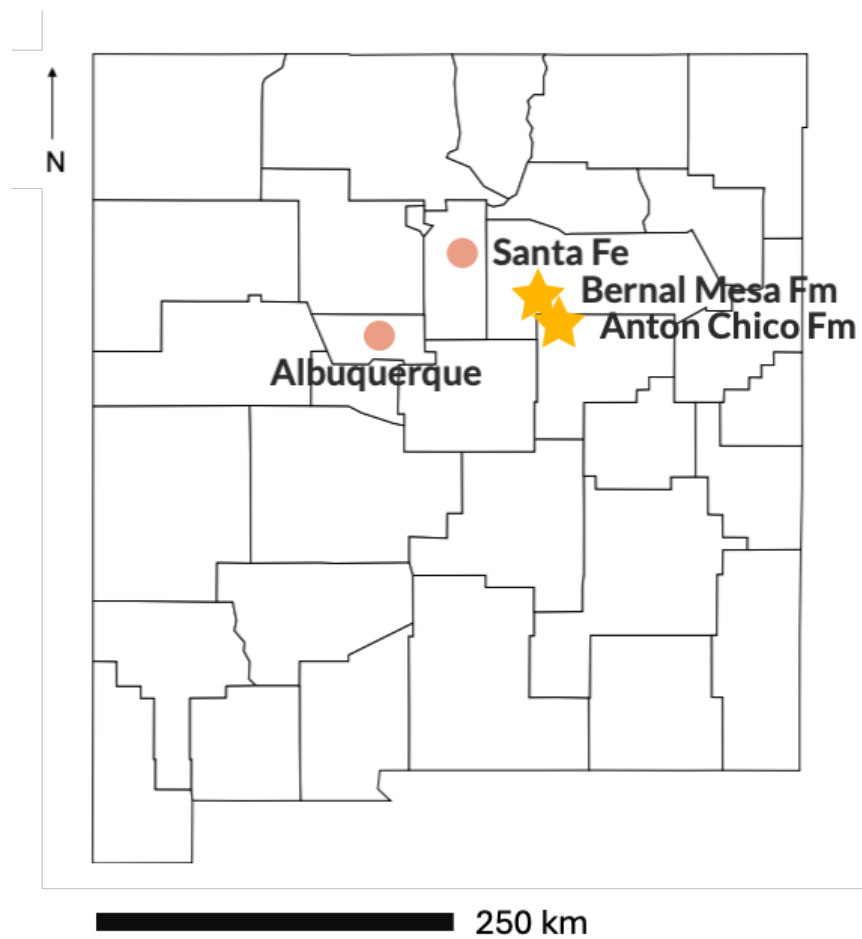


Figure 2.1

Bernal Mesa Formation, New Mexico. Samples were collected from the study area (red circle).

Full collection site data available in Table 1.

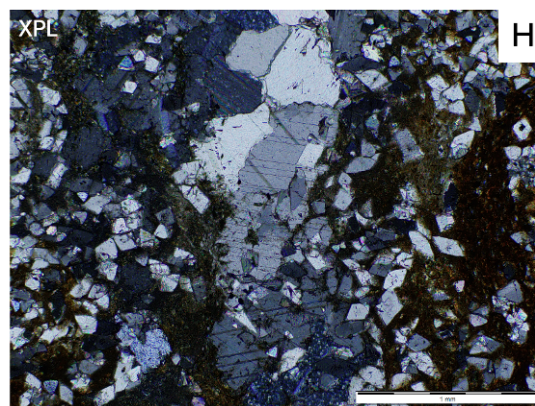
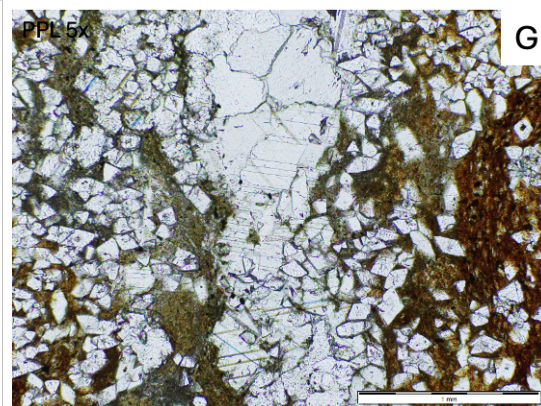
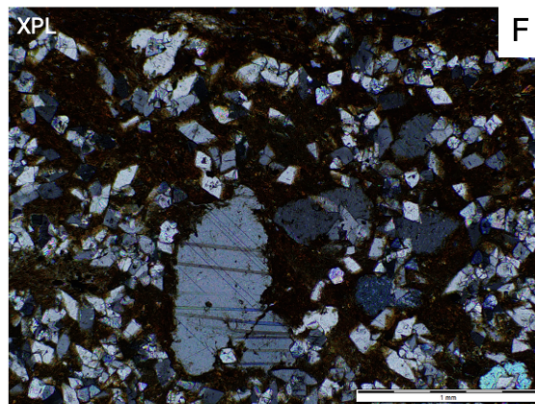
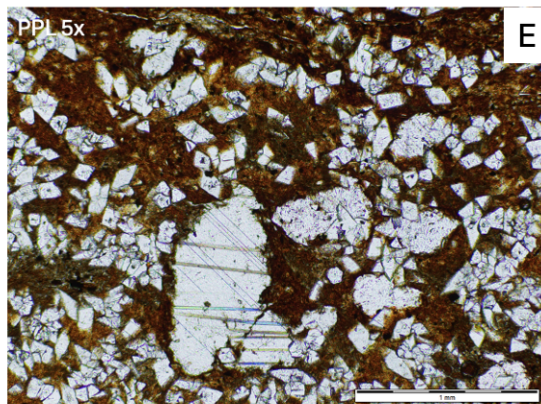
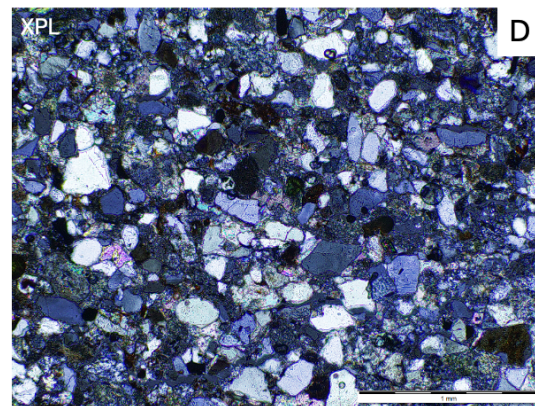
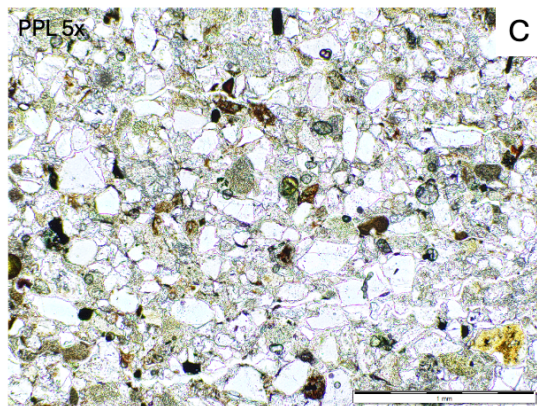
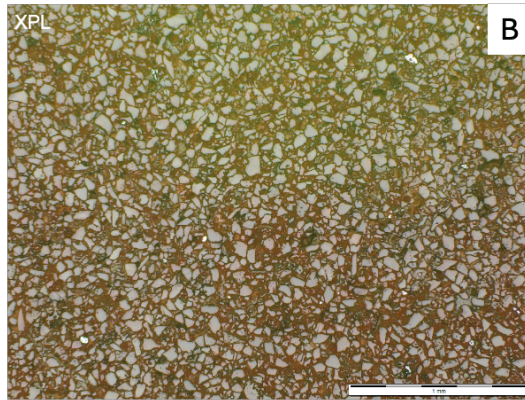
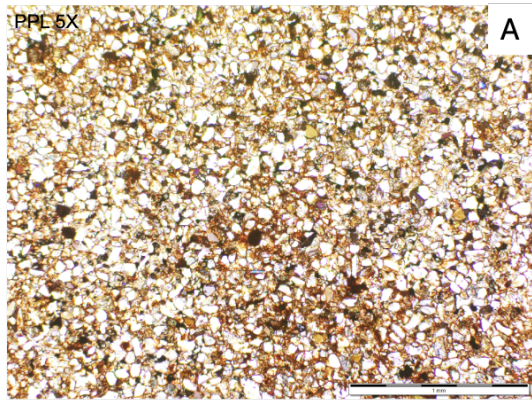


Figure 2.3

Plane polarized and cross polarized petrographic images. (A-B) Images of R4944 (Permian-aged). Significant coarsening. Δ_{47} estimated temperature at 10.9 ± 3.2 °C and $\delta^{18}\text{O}_{\text{water}}$ values of -8.1 ± 0.7 . Low Δ_{47} estimates and significantly depleted $\delta^{18}\text{O}_{\text{water}}$ value are supportive of late meteoric recrystallization. Photomicrographs taken at 5x magnification. (C-D) Plane polarized and cross polarized petrographic images of R4964 (Triassic-aged). Significant coarsening, clear sparry characteristics, no micritic matrix remaining. Δ_{47} estimated temperature at 92.9 ± 4.6 °C and $\delta^{18}\text{O}_{\text{water}}$ values of 3.2 ± 0.6 . High Δ_{47} estimates are supportive of thermal recrystallization. Photomicrographs taken at 5x magnification. (E-F) Plane polarized and cross polarized petrographic images of R4959-L (Permian-aged). The polished section features large sparite crystals with characteristic cross-hatching patterns and angular, rhomboid grains (possible dolomite) in a darkened groundmass. Δ_{47} estimated temperature at 52.6 ± 4.7 °C and $\delta^{18}\text{O}_{\text{water}}$ values of 7.0 ± 0.8 . Photomicrographs taken at 5x magnification. (G-H) Plane polarized and cross polarized petrographic images of R4959-U (Permian-aged). The polished section features large sparite crystals with characteristic cross-hatching patterns and angular, rhomboid grains (possible dolomite) in a darkened groundmass. Δ_{47} estimated temperature at 56.9 ± 3.9 °C and $\delta^{18}\text{O}_{\text{water}}$ values of 7.6 ± 0.8 . Photomicrographs taken at 5x magnification.

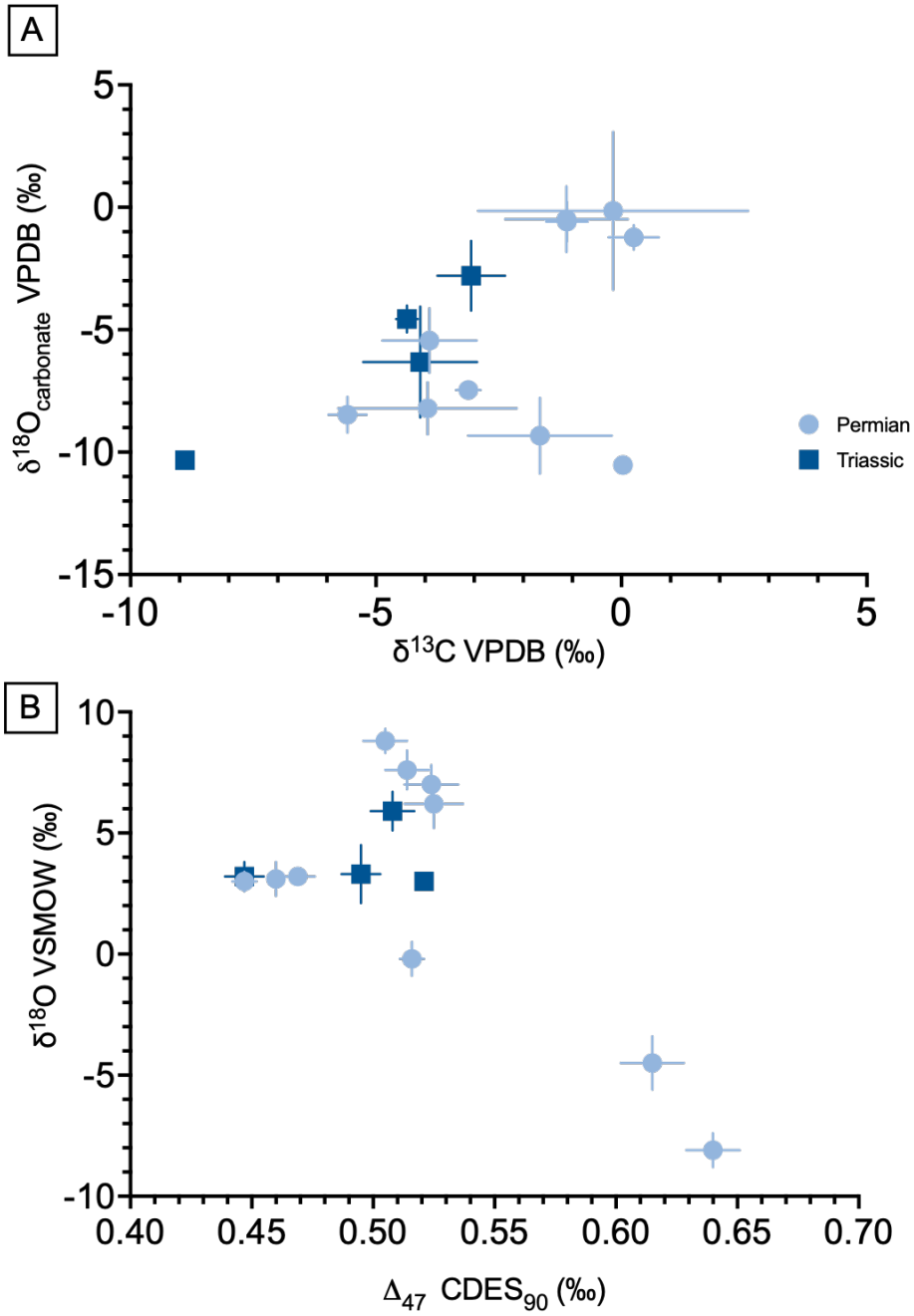


Figure 2.4

Clumped and bulk isotope results from Bernal Formation paleosols. (A) $\delta^{18}\text{O}$ versus $\delta^{13}\text{C}$ (both in ‰ VPDB). (B) $\delta^{18}\text{O}$ of formation water (‰ VSMOW, calculated using the Anderson et al.

(2021) calibration and measured $T\Delta_{47}$ versus Δ_{47} (‰). Blue circles = Permian (BMP) samples; dark blue squares = Triassic (BMT) samples. Error bars represent 1 s.e. The main cluster of samples (high $T\Delta_{47}$, moderate-to-positive $\delta^{18}\text{O}_{\text{water}}$) reflects burial resetting, while the two outliers with low $T\Delta_{47}$ and strongly negative $\delta^{18}\text{O}_{\text{water}}$ (R4944 and R4948) record late meteoric recrystallization.

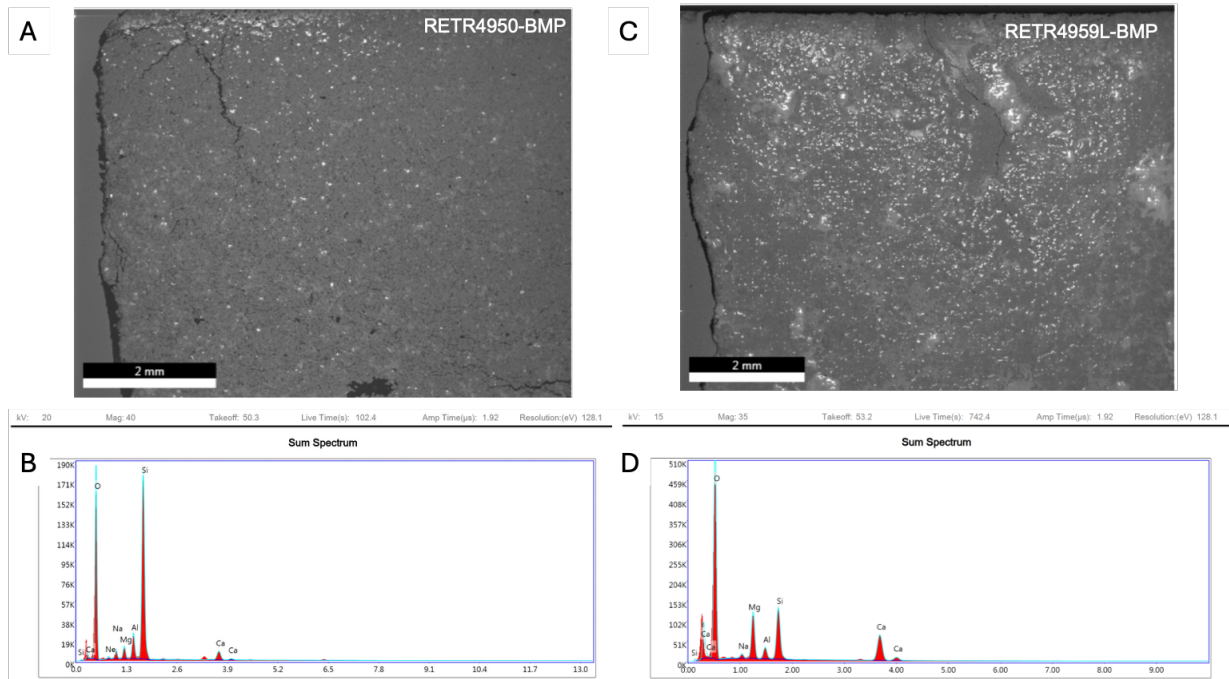


Figure 2.5

Elemental mapping results of carbonate paleosols.

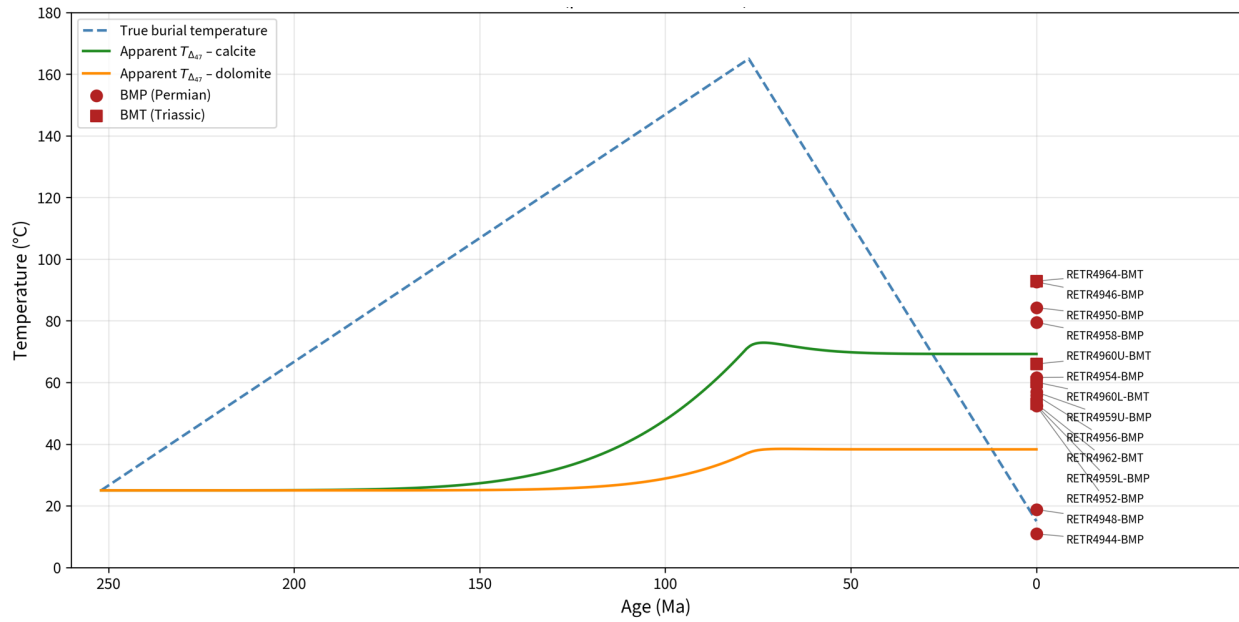


Figure 2.6

Temperature evolution of paleosol carbonates from the Permian Bernal Formation at Bernal Mesa, New Mexico. The blue dashed line shows the modeled burial temperature path assuming linear heating from ~25 °C at deposition (~252 Ma) to a maximum burial temperature of ~165 °C (representative median MBT) over ~175 Myr, followed by Laramide cooling to surface temperatures (~15 °C) over the last ~75 Myr. The green line shows the forward-modeled apparent $T_{\Delta 47}$ evolution for calcite using the disordered solid-state reordering kinetics of Hemingway and Henkes (2021). A lag between the actual burial temperature and the apparent $T_{\Delta 47}$ is expected, as reordering is a time- and temperature-dependent process that does not occur instantaneously. Red points show the measured Δ_{47} -derived apparent temperatures for the 14 paleosol carbonate samples in this study.

Tables

Table 2.1

Collection site data for samples included in this study. All samples were collected from the south face and western end of the Bernal Mesa near Chapelle, San Miguel County, New Mexico (N35.39339 W105.299970).

Museum number	Field number	Eyeheight (in)	Formation	Age
R4964	MU435	45	Anton Chico Formation	Triassic
R4962	MU433	50.1	Anton Chico Formation	Triassic
R4960-U	MU439-U	30	Anton Chico Formation	Triassic
R4960-L	MU439-L	30	Anton Chico Formation	Triassic
R4959-U	MU440-U	29.7	Bernal Formation	Permian
R4959-L	MU440-L	29.7	Bernal Formation	Permian
R4958	MU431	28.8	Bernal Formation	Permian
R4956	MU429	25.8	Bernal Formation	Permian
R4954	MU427	21.5	Bernal Formation	Permian
R4952	MU427	21	Bernal Formation	Permian
R4950	MU423	19.9	Bernal Formation	Permian
R4948	MU419	12.8	Bernal Formation	Permian
R4946	MU419	6.1	Bernal Formation	Permian

R4944	MU419	0.6	Bernal Formation	Permian
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Table 2.2

Clumped isotope paleothermometry and bulk isotope results from carbonate paleosols.

Catalog number	Age	Fm	n	$\delta^{13}\text{C}$ VPDB (‰)	1 s.d.	$\delta^{18}\text{O}$ VPDB (‰)	1 s.d.	Δ_{47} I-CDES (‰)	1 s.e.	T Δ_{47} (°C)	1 s.e.
R4944	Permian	Bernal	7	-3.12	0.25	-7.46	0.27	0.640	0.011	10.9	3.2
R4946	Permian	Bernal	5	0.03	0.12	-10.53	0.10	0.447	0.005	92.6	3.3
R4948	Permian	Bernal	10	-3.91	0.96	-5.45	1.32	0.615	0.013	18.8	4.2
R4950	Permian	Bernal	5	-1.66	1.46	-9.33	1.55	0.460	0.002	84.3	1.4
R4952	Permian	Bernal	7	0.25	0.51	-1.23	0.50	0.525	0.012	52.4	5.2
R4954	Permian	Bernal	13	-0.17	2.75	-0.15	3.23	0.505	0.009	61.6	4.8
R4956	Permian	Bernal	7	-3.95	1.82	-8.21	1.07	0.516	0.005	55.8	2.1
R4958	Permian	Bernal	3	-5.58	0.39	-8.47	0.73	0.469	0.007	79.5	4.0
R4959L	Permian	Bernal	8	-1.12	1.25	-0.49	1.34	0.524	0.011	52.6	4.7
R4959U	Permian	Bernal	9	-1.11	0.42	-0.59	0.81	0.514	0.009	56.9	3.9
R4960L	Triassic	Anton Chico	12	-3.06	0.69	-2.80	1.43	0.508	0.009	60.1	3.9
R4960U	Triassic	Anton Chico	9	-4.10	1.16	-6.32	2.27	0.495	0.008	66.0	4.0
R4962	Triassic	Anton Chico	7	-4.37	0.22	-4.56	0.55	0.521	0.003	53.1	1.4
R4964	Triassic	Anton Chico	9	-8.89	0.12	-10.33	0.17	0.447	0.008	92.9	4.6

Table 2.3

Summary of elemental mapping results of selected samples of varying preservation.

Sample	Category	Carbonate Abundance	Key Features	Interpretation
R4944	Surface / Meteoric	< 26 wt%	Quartz, K-feldspar, Ti-oxides	Late cool meteoric resetting
R4950	Reset (Burial)	< 30 wt%	Abundant sparry Ca-carbonate	Burial resetting
R4959L	Possible incomplete resetting	20-30 wt%	Carbonate + Mg phases	Burial resetting
R4959U	Possible incomplete resetting	20-30 wt%	Strong Ca-carbonate	Burial resetting
R4964	Reset (Burial)	0-4 wt%	Silicates dominant	Burial resetting

Table 2.4

Summary results of burial and exhumation model. Low-temperature outliers are highlighted in red text.

Sample	TΔ_{47} (°C)	MBT (°C)	Depth (25°C/km)	Depth (30°C/km)
R4944	10.9	138.2	4.93	4.11
R4946	92.6	182.7	6.71	5.59
R4948	18.8	147.9	5.32	4.43
R4950	84.3	177.9	6.52	5.43
R4952	52.4	163.5	5.94	4.95
R4954	61.6	169.8	6.19	5.16
R4956	55.8	167.2	6.09	5.07
R4958	79.5	175.1	6.4	5.34
R4959L	52.6	164.1	5.96	4.97
R4959U	56.9	166.3	6.05	5.04
R4960L	60.1	168	6.12	5.1
R4960U	66.0	171.5	6.26	5.22
R4962	53.1	164.8	5.99	4.99
R4964	92.9	182.7	6.71	5.59

Chapter 3. THERMOREGULATION AND ECOLOGY OF GIANT MARINE PREDATORS OF THE MESOZOIC

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Key Points:

- Ancient ocean temperature: Eastern US Cretaceous coastal seawater is estimated as 20°C based on clumped isotope measurements on oysters ($19.9 \pm 1.4^\circ\text{C}$) and belemnites ($20.3 \pm 1.0^\circ\text{C}$) from New Jersey
- Mosasaurs' body temperature: Averaged $32.6 \pm 0.7^\circ\text{C}$ in New Jersey and $33.8 \pm 0.8^\circ\text{C}$ Kansas specimens via $\delta^{18}\text{O}_p$, and averaged $39.9 \pm 6.6^\circ\text{C}$ via clumped isotope paleothermometry (New Jersey), roughly 12–20°C warmer than the seawater.
- Other marine animals: Most sharks and fish had body temperatures much closer to seawater temperature, consistent with being cold-blooded, however, the largest shark (*Cretoxyrhinia mantelli*, Codell Sandstone, KS) demonstrates higher temperatures ($37.7 \pm 5.6^\circ\text{C}$, clumped; $36.0 \pm 0.60^\circ\text{C}$, $\delta^{18}\text{O}_p$).
- *Cretalamna*, a relative to the Otodontid shark lineage and a taxon that survived the K-Pg mass extinction event measured $19.8 \pm 0.8^\circ\text{C}$, clumped; $23.7 \pm 0.4^\circ\text{C}$, $\delta^{18}\text{O}_p$ in New Jersey, and $44.3 \pm 3.7^\circ\text{C}$, clumped (from only 3 replica analyses); $33.3 \pm 0.5^\circ\text{C}$, $\delta^{18}\text{O}_p$ in Kansas .
- Evidence for endothermy: Mosasaurs maintained relatively high and stable body temperatures across different locations and times, supporting the conclusion that they

were warm-blooded (endothermic). The larger sharks included in this study, *Cretoxyrhina mantelli*, also maintained relatively high body temperatures, suggesting that they may have been regionally- or fully endothermic.

- Carbon isotopes suggest mosasaurs and *Enchodus* (a bony fish) fed closer to shore or in bottom waters, while some sharks lived in open ocean (pelagic) environments.
- Method: Used phosphate oxygen isotopes ($\delta^{18}\text{O}_p$), with carbonate clumped isotopes (Δ_{47}) for confirmation. Diagenesis (fossil alteration) was checked and ruled out for most samples.
- Implications: Endothermy may have provided Mosasaurs and larger Cretaceous sharks (such as *C. mantelli*) a competitive advantage as apex predators but may have made them more sensitive to major ocean changes during the K/Pg mass extinction.

Abstract

Recent studies of giant marine predators, such as *Otodus megalodon*, have shown that these giants not only achieved the highest trophic levels in their respective ecosystems, but they also achieved high body temperatures above that of their surroundings, suggesting they may have been regionally or fully endothermic. Mosasaurs were also dominant Late Cretaceous marine predators, yet their thermoregulatory strategy remains debated as well. We applied phosphate oxygen isotopes ($\delta^{18}\text{O}_p$), carbonate clumped isotopes (Δ_{47}), and supporting analyses (Fourier Transform Infrared Spectroscopy for diagenesis) to tooth enamel/enameloid and mollusk shells from a Campanian–Maastrichtian assemblages (Wenonah-Mt. Laurel/Navesink contact, New Jersey). Seawater temperatures, constrained by Δ_{47} measurements on benthic oysters ($19.9 \pm 3.3^\circ\text{C}$) and pelagic belemnites ($21.4 \pm 2.8^\circ\text{C}$), averaged $\sim 20^\circ\text{C}$. $\delta^{18}\text{O}_p$ -derived body temperatures were $19.9 \pm 0.3^\circ\text{C}$ for *Enchodus* sp., $19.9 \pm 0.5^\circ\text{C}$ for *Scapanorhynchus texanus*, $22.7 \pm 0.4^\circ\text{C}$ for *Cretalamna appendiculata*, $26.7 \pm 0.6^\circ\text{C}$ for *Squalicorax pristodontus*,

and $32.6 \pm 0.7^{\circ}\text{C}$ for *Mosasaurus* sp. from the New Jersey specimens. Similar species from the Codell Sandstone of Kansas were also tested. $\delta^{18}\text{O}_\text{p}$ -derived body temperatures were $25.3 \pm 0.4^{\circ}\text{C}$ for *Enchodus petrosus*, $30.8 \pm 0.4^{\circ}\text{C}$ for *Cretalamna* sp., $36.0 \pm 0.6^{\circ}\text{C}$ for *Cretoxyrhina mantelli*, and $33.8 \pm 0.8^{\circ}\text{C}$ for *Mosasaurus* sp. Whilst not reaching a high level of replication allowing statistical tests of significant differences Δ_{47} data was overall in alignment with $\delta^{18}\text{O}_\text{p}$ data, yielded similar patterns of endothermy versus ectothermy between species (e.g., $19.8 \pm 0.8^{\circ}\text{C}$ for *C. appendiculata*, $\sim 39.5 \pm 6.6^{\circ}\text{C}$ for *Mosasaurus* from New Jersey). Both clumped and phosphate oxygen stable isotope proxies provide marginal evidence for low level regional endothermy in the ancestor of the gigantic regional endotherm *Otodus megalodon*; *C. appendiculata*. Stronger evidence for regional endothermy was found in the medium to gigantic sized mackerel sharks *S. pristodontus* and *C. mantelli* but not the Mitsukurinidae *S. texanus*. This result is consistent with prior work on Miocene and Pliocene *Otodontid* and *Carcharodon* sharks with the highest trophic feeders and largest body mass sharks demonstrating regional endothermy. A lack of regional endothermy in both modern and Cretaceous Mitsukurinidae is also conserved. Combined prior work on geochemical trophic level reconstruction ($\delta^{66}\text{Zn}$) and our thermometry data seem to place Cretaceous Mosasaurs in an ecologically distinct space to sharks with higher levels of endothermy than most sharks of the time ($\sim 9\text{--}20^{\circ}\text{C}$ above seawater) but slightly lower levels of trophic feeding than the most gigantic sharks. In total these observations may inform the persistence of smaller generalist feeding shark species at the end of the Cretaceous, but extinction of sharks that were larger regional endotherms and higher trophic level feeders as well as Mosasaurs. By utilizing carbonate-clumped isotope paleothermometry (Δ_{47}), this study demonstrates that the elevated body temperature of the apex predator *Cretoxyrhina mantelli* ($37.7 \pm 5.6^{\circ}\text{C}$) relative to the ectothermic *S. raphiodon* and *S. texanus* ($29.9 \pm 4.5^{\circ}\text{C}$; $15.4 \pm 5.2^{\circ}\text{C}$, respectively) mirrors zinc trends from both the Cretaceous

and Neogene, indicating that high metabolic demands and regional endothermy drove the selective extinction of Late Cretaceous shark lineages.

Plain Language Summary:

Mosasaurus were giant marine reptiles that ruled the oceans during the Late Cretaceous period, just before the dinosaur extinction and K/Pg boundary. Research has long debated whether they were cold-blooded like modern reptiles or warm-blooded like some sharks and marine mammals. In this study, the chemical composition (stable oxygen and carbon isotopes) preserved in fossil teeth from mosasaurs and other sea creatures from two different locations in the United States (New Jersey and Kansas) was analyzed. By comparing the isotopes in predator teeth to those in co-occurring invertebrate shell remains and other animals that record ancient seawater temperature, we estimated body temperatures. The results show that mosasaurs had body temperatures around 33–40°C, body temperatures significantly warmer (about 12–20°C hotter) than the surrounding ocean water, which averaged about 20–25°C. These elevated temperatures were consistent across different environments, suggesting mosasaurs had some form of endothermy (internal heat production), similar to modern warm-blooded animals. This ability likely helped them thrive as top predators in varied ocean conditions. The study supports the idea that mosasaurs were active, high-energy hunters and may help explain both their success during the Cretaceous and their vulnerability to the massive environmental changes at the end of the Mesozoic Era. This study also demonstrates that the elevated body temperature of the apex predator *Cretoxyrhina mantelli* (37.7 ± 5.6 °C relative to the generalist *Cretalamna* (19.8 ± 0.8 °C) mirrors zinc and clumped isotope trends, both in coeval taxa and Neogene taxa, indicating that high metabolic demands and regional endothermy drove the selective extinction of Late Cretaceous shark lineages.

1 Introduction

The Mesozoic saw the domination of clade Reptilia on land, in the sky, and in the oceans. Marine megafauna included plesiosaurus, ichthyosaurus, and mosasaurus. Recent oxygen isotope studies have demonstrated that ichthyosaurs and plesiosaurs commonly possessed body temperatures above that of the surrounding waters, indicating an endothermic thermoregulatory strategy for these taxa, but the strategy for mosasaurs remains less clear (Bernard et al., 2010; Séon et al., 2024). At least some oxygen isotope data and bone growth rates suggest that mosasaurs were endotherms like the giant marine reptiles that they displaced ecologically (Harrell et al., 2016; Séon et al., 2024). Their giant size, tooth morphology, and lack of any apparent predators would suggest that mosasaurs sat atop the food web in Late Cretaceous oceans, which would be made significantly more difficult if the group was constrained by ectothermic habits.

Mosasaurs are squamate reptiles, a group whose living members include snakes and monitor lizards (Russell et al., 1964; Bernard et al., 2010). Of these, mosasaurs are most closely related to the monitor lizards (Russell et al., 1964; Bernard et al., 2010). They represent a large, diverse clade of prolific predators that were integral to marine ecosystems across the globe. They are represented by a rich fossil record from North America, Africa, Europe, and the Middle East (Russell, 1967; Polcyn et al., 2014; Bardet et al., 2015; Carpenter 2017). As the Mesozoic Era ended and the waters began to cool, the fact that the mosasaurs remained persistently ubiquitous suggests that they were possibly able to employ thermoregulatory strategies such as endothermy or mesothermy (i.e. the ability to generate and maintain a high, relatively constant body temperature through internal metabolic heat production versus an intermediate strategy in which body temperature is elevated above ambient conditions but shows greater variability and partial reliance on environmental heat sources). Additionally, having an elevated body temperature would have allowed mosasaurs to take advantage of

cooler circumpolar habitats—as their fossils are widespread, it is possible that they had physiological advantages that allowed them versatility in terms of water temperature ranges they were able to inhabit (Bernard et al., 2010; Polcyn et al., 2014; Harrell et al., 2016). Lastly, it is thought that the mosasaurus inherited their ecological position from the ichthyosaurs. Ichthyosaurs, due to their physiology and sophisticated fusiform anatomy, are thought to have been very active predators, like modern sport fish (Motani, 2002). Due to this, it is thought they are similarly able to raise their body temperature for hunting and pursuit (Bernard et al., 2010; Harrell et al., 2016). The paleobiology and extinction of marine megafauna is interesting as the largest and most prolific of predators have often hailed from the marine realm. This is true even in the modern day, as the cetaceans represent the dominant clade of the Cenozoic. Recent work using triple oxygen analyses ($\Delta^{17}\text{O}$) in combination with $\delta^{18}\text{O}$ analysis suggests that giant marine reptiles may have had more moderate body temperatures, with mosasaur estimates ranging from $23\text{--}26 \pm 5^\circ\text{C}$ and plesiosaur estimates around 24°C , suggesting that these taxa were possibly regionally endothermic rather than fully endothermic (Sambuichi et al., 2025).

The mosasaurs possessed a variety of physical characteristics which helped them attain diversity and success during the Late Cretaceous. Narrow tails and long bodies allowed for lateral undulations to provide propulsive force for swimming (Lindgren et al., 2011a). Tail morphology also suggests that mosasaurs adapted from terrestrial to near-shore environments, and later to pelagic sea dwellers (Lindgren et al., 2013). Early forms show higher intervertebral mobility, and later forms possess a downturn in the posterior caudal segment to become more fin-like as mosasaurs transition from anguilliform to carangiform locomotion, making them more suitable for pelagic environments, although recent multi-proxy approaches demonstrate that mosasaurs may have inhabited or frequently ventured to freshwater, riverine environments in the Western Interior Seaway during the latest Cretaceous (Lindgren et al., 2013; Carpenter, 2017; During et al., 2025).

Mosasaur also possessed double-hinged jaws like those of snakes, whose utility is thought to have been for predation (Caldwell et al., 2006; Lindgren et al., 2011a). Mosasaur teeth were rooted deep in their mandible and were constantly shed and replaced throughout their lifetime. Also like their relatives, soft tissue impressions show that mosasaurs had scaly integument (Lindgren et al., 2009; Lindgren et al., 2011b). Their non-reflective, keeled scales may have provided some advantage to them as stealth or ambush predators (Lindgren et al., 2011b). Previous research also shows that mosasaurs were countershaded, with dark backs and light underbellies (Lindgren et al., 2014).

However, mosasaurs and other marine reptiles of the time were not the only taxonomic group to gain widespread apex predator status in Late Cretaceous oceans. Sharks were also dominant predators of the time, with genera such as *Cretoxyrhina*, *Cretalamna*, *Squalicorax*, and *Scapanorhynchus* representing key lamniform and mitsukurinid taxa frequently recovered from Late Cretaceous deposits, including those of the Atlantic Coastal Plain of the USA.

Cretoxyrhina is a large lamniform mackerel shark that inhabited Late Cretaceous oceans, and is thought to have been similar physiologically and ecologically to modern Great White sharks (*Carcharodon carcharias*) (Shimada, 2008). These sharks were relatively large, averaging about 7 meters in length and weighing up to 3,400 kg (Shimada, 2008). However, unlike the great white shark, whose teeth are coarsely serrated for cutting large prey, *Cretoxyrhina* possessed large, robust teeth with smooth cutting margins (or only very fine serrations in some specimens). This dentition suggests it was adapted for grasping and slicing rather than the pronounced sawing action seen in extant white sharks (Shimada, 2008; Shimada et al., 2010). *Cretalamna* (e.g., *C. "appendiculata"*) was another prominent lamniform of Late Cretaceous time. Dentition is characterized by strong central cusps and lateral cusplets, likely indicative of a generalist strategy in neritic to oceanic waters, with some evidence suggesting possible mesothermic physiology to support active predation (McCormack et al.,

2026). *Squalicorax* was a medium-to-large (up to 5 meters) lamniform thought to be an opportunistic predator/scavenger with coarsely serrated teeth (Becker and Chamberlain, 2012). Its widespread, near-global distribution and varied diet as evidenced by bite marks on fossils suggest it was capable of taking down large prey, including marine reptiles (Schwimmer 1997; Becker and Chamberlain, 2012). *Scapanorhynchus* (e.g., *S. texanus*) is an ancient mitsukurinid goblin shark relative that had slender teeth and an elongated snout. Abundant in shallow marine settings, they likely specialized in grasping small fish and soft-bodied prey (Cappetta, 2012). Recent zinc isotope work highlights dietary niche partitioning among these and other large coeval sharks, revealing that *Cretoxythrina* achieved high trophic level positions while *Otodus* ancestor, *Cretalamna* shows broader, more generalist foraging habits underscoring structured trophic hierarchies in the Western Interior Seaway prior to the K-Pg mass extinction event (McCormack et al., 2026).

1.1 Background

An assemblage of fossils from the Wenonah-Mount Laurel–Navesink Formation contact of Monmouth County, New Jersey spanning trophic levels and thermoregulatory strategies presents a unique opportunity to study the ecology of this ancient environment. This formation has produced a wealth of Cretaceous marine fossils that have been examined in previous studies (Becker et al., 2000; Becker et al., 2005; Becker et al., 2006; Becker and Chamberlain, 2012). These previous studies have revealed much about the paleoecology of sharks in the Late Cretaceous of the North Atlantic, including possible pathologies and feeding habits (Becker et al., 2000; Becker et al., 2006; Becker and Chamberlain, 2012). Other recent studies of giant marine predators from this area focused away from skeletal morphology and towards geochemistry revealed that specimens in this formation may retain their isotopic integrity (Kast et al., 2022; Griffiths et al., 2023). Dietary proxy studies have produced reconstructions of

relative trophic position using nitrogen and zinc isotopes on tooth enameloid (Kast et al., 2022; McCormack et al., 2022, McCormack et al., 2026).

Recent isotope paleothermometry investigations have reached further back in time to the Mesozoic to estimate body temperatures in marine megafauna to yield interesting results about the paleobiology of extinct megapredators (Séon et al., 2020; Griffiths et al., 2023; During et al., 2025; Sambuichi et al., 2025). These developments provide an opportunity to reconstruct ancient ecosystems in greater resolution than was previously possible. Oxygen phosphate measurements have been useful to reconstruct body temperature in mosasaurs, and recent findings suggest an endothermic thermophysiology for this group, contrary to previous studies (Séon et al., 2020; Griffiths et al., 2023). Recent oxygen isotope studies have demonstrated that ichthyosaurs and plesiosaurs commonly possessed body temperatures above ambient waters, indicating endothermy, but mosasaur thermophysiology remains debated (Séon et al., 2020). Some $\delta^{18}\text{O}_\text{p}$ data and bone histology suggest endothermy, consistent with their giant size, predatory morphology, and apex position in Late Cretaceous oceans, traits difficult for strict ectotherms to sustain. As squamates most closely related to monitor lizards, mosasaurs' global distribution and persistence into cooling terminal Mesozoic waters imply thermoregulatory flexibility (endothermy or mesothermy), potentially inherited from active ichthyosaur-like ancestors. The key to the recent findings was the inclusion of seabirds in the study to constrain an endothermic range. This allowed for a more direct comparison of isotopic signatures (Harrell et al., 2016). However, recent work with combined $\Delta^{17}\text{O}$ and $\delta^{18}\text{O}$ analyses suggests that mosasaur body temperatures may have been cooler than originally thought, making regional endothermy more likely than full endothermic physiology (Sambuichi et al., 2025).

More recently, oxygen phosphate has been used to determine *Otodus megalodon* thermophysiology relative to coeval sharks (Griffiths et al., 2023). This study combined oxygen

phosphate measurements with Δ_{47} measurements to demonstrate that *megalodon* was an endothermic apex predator (Griffiths et al., 2023). The shark species included in this study also present an interesting suite of taxa to examine Cretaceous food webs. The Late Cretaceous marine ecosystem was dominated by a diverse assemblage of lamniform sharks, including the apex macropredator *Cretoxyrhina mantelli*, the opportunistic scavenger *Squalicorax*, the deep-water goblin shark relative *Scapanorhynchus*, and the highly resilient generalist *Cretalamna*. Recent advancements in zinc isotope ($\delta^{66}\text{Zn}$) analysis by McCormack et al. (2022) provide a robust proxy for mapping the trophic positions and dietary flexibility of these extinct taxa through deep geologic time. This chemical framework reveals a striking pattern regarding which lineages survived the Cretaceous-Paleogene (K-Pg) extinction event. Gigantic, fast-swimming apex predators like *Cretoxyrhina* and highly specialized macro-predatory ecomorphs of *Squalicorax* may have possessed elevated metabolic demands consistent with regional endothermy (Shimada et al., 2021). When the K-Pg impact and preceding large-scale climate change collapsed the marine food web, these high-trophic, high-energy demanding specialists went extinct, whereas *Cretalamna* survived by virtue of its smaller size, lower metabolic threshold, and dietary opportunism (McCormack et al., 2026). This dynamic directly parallels macroevolutionary trends seen in the late Miocene and Pliocene, where similar energetic constraints, declining prey availability, and fierce dietary competition between emerging apex predators ultimately decimated the megatooth lineages (Paredes-Aliaga and Herraiz, 2024). Furthermore, the evolutionary trajectory of *Cretoxyrhina* showcases a remarkable case of convergent evolution with the Neogene *Otodus megalodon*; despite being separated by tens of millions of years, both independent lineages evolved massive body sizes, specialized robust shearing teeth, and apex trophic status to exploit abundant marine megafauna, only to suffer identical fates when their respective ecosystems fractured (Cooper et al., 2022; McCormack et al., 2022).

1.1 Challenges in applying clumped isotopes to bioapatite

While carbonate clumped isotope (Δ_{47}) thermometry is well-established and routinely applied to relatively pure carbonate minerals such as calcite and aragonite in mollusk shells and foraminifera (Ghosh et al., 2006; Huntington et al., 2009; Tripathi et al., 2011), its application to bioapatite (carbonate-bearing apatite) in tooth enamel and enameloid presents several analytical and interpretive challenges.

Bioapatite contains only a minor structural carbonate component, typically 3–6 wt% CO_3^{2-} (LeGeros, 1981; Wacker et al., 2016), substantially lower than pure carbonates. This low carbonate yield necessitates significantly larger sample sizes— often 5–20 mg or more per replicate— compared to the sub-milligram quantities sufficient for shell carbonate (Eagle et al., 2010; Löffler et al., 2019; Tagliavento et al., 2023). This requirement limits the number of replicate analyses possible from individual fossil specimens, particularly when only small amounts of well-preserved enamel are available.

One major complication arises from the intimate association of bioapatite with residual organic matter. Organics can contaminate the evolved CO_2 gas during acid digestion, potentially causing isobaric interferences that artificially elevate Δ_{47} values and yield erroneously low temperature estimates (Eagle et al., 2010; Wacker et al., 2016). Various oxidative pre-treatments, such as hydrogen peroxide (H_2O_2) or sodium hypochlorite, are commonly employed to remove organics (Eagle et al., 2010; Suarez and Passey., 2014), yet these steps carry the risk of partial dissolution or isotopic alteration of the structural carbonate. In this study, we applied hydrogen peroxide and acetic acid pre-treatment following protocols published in Eagle et al. (2010), as well as FTIR spectroscopy to monitor potential diagenetic alteration and residual organic content prior to analysis.

Acid digestion kinetics of bioapatite also differ from those of pure carbonates. Bioapatite reacts more slowly and may require higher temperatures or longer reaction times, which can introduce kinetic fractionation effects if not carefully controlled (Löffler et al., 2019; Tagliavento et al., 2023). Recent methodological advances, such as the use of Kiel IV devices equipped with water sinks, have helped reduce sample size requirements and improve reproducibility for enamel and enameloid (Tagliavento et al., 2023), but inter-laboratory standardization remains an ongoing challenge.

Furthermore, bioapatite, while generally more resistant to alteration than bone or dentin, is still susceptible to diagenetic overprinting, particularly through recrystallization or carbonate exchange with burial fluids. Enamel's large crystal size and low porosity provide better preservation potential than other bioapatites (Suarez and Passey, 2014; Kohn and Cerling, 2002), yet even subtle alteration can reset Δ_{47} values toward ambient burial temperatures. Traditional screening tools (e.g., XRD crystallinity indices) may not detect early-stage diagenetic changes, making independent constraints from phosphate oxygen isotopes ($\delta^{18}\text{O}_p$) particularly valuable for validation.

Despite these challenges, careful sample screening, appropriate pre-treatment protocols, and methodological refinements allow Δ_{47} analysis of bioapatite to produce robust body temperature estimates that are consistent with $\delta^{18}\text{O}_p$ -derived temperatures, as demonstrated in this study for mosasaurs and other Cretaceous marine vertebrates.

1.2 Overview of this study

Using bony fish and belemnites as ectothermic endmembers, lamnid sharks as mesothermic benchmarks, and oysters as sea temperature proxies, our group aims to use stable isotope geochemistry in the tooth enamel and shells of these taxa to compare against the

mosasaur enamel as a paleotemperature proxy for body temperature. Additionally, similar species were selected from an additional Cretaceous location with a different paleoenvironment to check for environmental effects in paleothermometry measurements. Clumped isotope paleothermometry, oxygen phosphate analysis, and FTIR are employed to examine the suite of fossils. Through these investigations, we hope to characterize the role that mosasaurus and other coeval sharks played in Late Cretaceous ecologies and perhaps offer an explanation as to why they were prone to extinction at the end of the Mesozoic.

2 Methods

2.1 Sample locality and geologic setting

An assemblage of fossils from the Wenonah-Mt. Laurel/Navesink Formation contact of Monmouth County, New Jersey spanning trophic levels and thermoregulatory strategies presents a unique opportunity to study the ecology of this ancient environment. This formation has produced a wealth of Cretaceous marine fossils that have been examined in previous studies (Becker et al., 2000; Becker et al., 2005; Becker et al., 2006; Becker and Chamberlain, 2012). These previous studies have revealed much about the paleoecology of sharks in the Late Cretaceous of the North Atlantic, including possible pathologies and feeding habits (Becker et al., 2000; Becker et al., 2006; Becker and Chamberlain, 2012).

The Wenonah-Mt. Laurel/Navesink contact in northeastern New Jersey is part of a long, discontinuous belt of Cretaceous sediments extending from New Jersey to Alabama (Becker et al., 2005; Becker and Chamberlain, 2012). Its age is constrained primarily using index fossils, including genera such as *Exogyra*, *Belemnitella*, *Baculites*, and *Gryphaea* (Weller, 1905; Cobban, 1974). Despite the nature of the sands, the stratigraphic column in this area as a whole appears to be intact due to paleontological evidence (Weller, 1905). $\text{Sr}^{87}/\text{Sr}^{85}$ dating of fossils from this formation reveals an age of 80-76 Ma (Becker, 1997). Additionally, a study using K-Ar

dating from glauconites combined with biostratigraphic evidence also placed these formations within the Late Cretaceous, yielding ages of 59.2 ± 1.9 to 63.2 ± 2.0 Ma for the Navesink Formation, 63.2 ± 1.9 to 70.3 ± 2.5 Ma for the Mt. Laurel Sand, and an age of 72.9 ± 2.7 for the Wenonah Formation (Owens and Sohl, 1973). Vertebrate fossils in this formation are considered overall to be well preserved (Martino and Curran, 1990).

In Monmouth County, stream erosion exposes Upper Cretaceous lag deposits containing a wealth of marine fauna (Becker et al., 2005; Becker and Chamberlain, 2012). The sequence has been subjected to reworking by exhumation and reburial associated with transgressive and regressive sea level cyclicity, however the vertebrate fauna remains in distinct stratigraphic horizons (Becker et al., 2006). The suite of fossils includes taxa from a spread of trophic levels and feeding strategies, including oysters, belemnites, predatory bony fish, mackerel sharks (including an otodontid), lamnid sharks, and mosasaurs.

The Codell Sandstone is a member of the Upper Cretaceous (Turonian) Carlile Shale, deposited along the eastern margin of the Western Interior Seaway (Hattin, 1962). It was named for exposures near Codell in Rooks County, Kansas, where it forms the uppermost unit of the Carlile Shale. In Kansas, the Codell Sandstone Member overlies the Blue Hill Shale Member of the Carlile Shale and is unconformably overlain by the Fort Hays Limestone Member of the Niobrara Formation (Bass, 1926; Hattin, 1962). Thickness in western Kansas typically ranges from 3 to 40 feet (1–12 m), consisting primarily of very fine- to fine-grained, heavily bioturbated, calcareous sandstone and sandy siltstone (Bass, 1926; Hattin, 1962). The unit was deposited during a regressive phase of the Greenhorn cyclothem in the Late Cretaceous, representing a regional clastic pulse into the epicontinental seaway (Glenister and Kauffman, 1985). The Codell Sandstone was deposited in a shallow-marine shelf to shoreface setting, influenced by storm waves and currents (Hattin, 1962; Sonnenberg, 2015). It is characterized by intense bioturbation, hummocky cross-stratification, and a mixture of quartz sand with carbonate

material. In Kansas, it is interpreted as reworked shelf sands deposited during a period of relative sea-level fall, immediately preceding the major transgression that deposited the overlying Niobrara carbonates (Bice et al., 2016). This sandstone reflects dynamic paleogeographic conditions in the Western Interior Seaway during the Turonian.

2.2. Comparative Context

The fossils discussed in this chapter come from two distinct Upper Cretaceous marine depositional settings in North America: the Codell Sandstone Member of the Carlile Shale in Kansas and the Wenonah/Mount Laurel–Navesink interval in New Jersey (Figure 3.1). Although these units are separated by significant differences in age, geography, and tectonic setting, both preserve mosasaur remains within shallow-marine, clastic-influenced environments along the margins of major Cretaceous seaways. To isolate potential biological metabolic heat retention from background environmental fluctuations, shark isotopic signatures ($\delta^{18}\text{O}_p$ and Δ_{47}) must be calibrated against localized, stratigraphically constrained ectothermic baselines. This sampling design controls for the pronounced spatial, temporal, and depth-stratified thermal gradients characteristic of the Late Cretaceous greenhouse climate. The selection of our reference taxa is structurally validated by established paleothermometry frameworks for the Western Interior Seaway (WIS) and the Atlantic Coastal Plain (ACP).

During the Late Cretaceous, the WIS in Kansas and the Atlantic Coastal Plain in New Jersey represented contrasting marine settings, with the WIS being a broad, relatively shallow epicontinental sea and the Atlantic coast representing a more open ocean-influenced continental shelf environment (Kaufman, 1984; Lowery et al., 2018; Curley et al., 2025). Recent studies highlight significant differences in sea-surface and shallow-water temperatures. In the WIS, particularly during the mid-Cretaceous Thermal Maximum in the Cenomanian-Turonian, clumped isotope data from oyster fossils indicate extreme mid-latitude warmth, with mean

temperatures of approximately 28-34 °C and evidence of strong seasonality in sub-annual sclerochronology (Henehan et al., 2022; Hoffman et al., 2025). These findings show that the WIS was likely a hot epicontinental sea under greenhouse conditions. The Late Cretaceous Atlantic Coast encompassing New Jersey records relatively cooler shallow marine temperatures. Clumped isotope analyses from stratigraphically similar coastal plain sites yield values more similar to modern subtropical conditions, reflecting greater oceanic exchange and potentially less extreme warmth than WIS localities (Meyer et al., 2018; Suarez et al., 2024). These thermal contrasts likely influenced local ecology, faunal composition, and depositional patterns, providing important context for paleoecological studies in North America.

The Codell Sandstone Member is middle to late Turonian (~93–91 Ma) in age and forms the uppermost unit of the Carlile Shale, deposited along the eastern margin of the Western Interior Seaway (Hattin, 1962; Bice et al., 2016). Intense bioturbation and mixed quartz-carbonate composition indicate relatively slow sedimentation rates in a turbid, nearshore setting. Mosasaur remains, primarily early ruseselosaurines, occur here within a diverse vertebrate assemblage that includes sharks, bony fish, plesiosaurs, and turtles, reflecting a productive epicontinental sea ecosystem during the early radiation of mosasaurs (Bice et al., 2016). For the Turonian Codell Sandstone assemblages of Kansas, ambient water-column conditions are constrained using the co-occurring teleost *Enchodus*. Decades of oxygen isotope ($\delta^{18}\text{O}_p$) and organic (TEX₈₆) paleothermometry within the mid-latitude WIS indicate a warm, sub-tropical epicontinental sea, with mixed-layer temperatures routinely spanning 26°C to 32°C, and occasionally reaching 34°C during Turonian thermal maxima (Kaufman, 1984; Wright, 1987; Fassell and Bralower, 1999; Petersen et al., 2016; Lowery et al., 2018; Henehan et al., 2022; Hoffman et al., 2025). Deeper, restricted bottom-waters during this maximum transgression remained cooler but stable, ranging from 18°C to 23°C. Sampling *Enchodus* from the exact

same lithostratigraphic horizon provides a direct, non-endothermic environmental control representing the ambient upper-water column.

In contrast, the Wenonah Formation, Mount Laurel Formation, and overlying Navesink Formation in New Jersey are late Campanian to early Maastrichtian (~77–69 Ma) in age and were deposited on the passive-margin Atlantic Coastal Plain along the western edge of the Atlantic Ocean (Gallagher, 2005). The Wenonah–Mount Laurel contact and the Mount Laurel–Navesink contact record a coarsening-upward regressive sequence followed by transgression. The Mount Laurel Formation consists of glauconitic, fine- to medium-grained quartz sand, while the Navesink Formation comprises highly fossiliferous, glauconitic sandy marl and clay deposited in middle to outer shelf settings (Owens et al., 1970; Gallagher, 2005). These units reflect shallow subtidal to shelf environments with periodic storm influence, where higher glauconite content indicates slower sedimentation and more open-marine conditions compared to the Codell. Mosasaurs are well documented from these strata, particularly the Mount Laurel and Navesink, representing later, more derived taxa during the peak diversity of the group prior to the end-Cretaceous extinction (Gallagher, 2005). For the open-ocean, marginal marine shelf environments recorded in the Wenonah and Mt. Laurel Formations of New Jersey, baseline calibrations account for the subsequent global cooling trend of the latest Cretaceous. Existing paleoceanographic reconstructions utilizing $\delta^{18}\text{O}$ from exceptionally preserved benthic foraminifera, oyster shells, and *Belemnitella americana* rostra along the Atlantic Coastal Plain establish an average open-shelf thermal baseline fluctuating between 16°C and 22°C (Sugarman et al., 1995; Miller et al., 2004).

Although the Codell Sandstone (Turonian) preserves some of the oldest known North American mosasaurs and offers insight into the early diversification of the group (Bice et al., 2016), the New Jersey interval (late Campanian–Maastrichtian) captures significantly later

stages of mosasaur evolution, including larger and more specialized forms (Gallagher, 2005). Paleogeographically, the Kansas material originates from an interior epicontinental seaway (Hattin, 1962), whereas the New Jersey fossils reflect a passive-margin, open-ocean-influenced coastal plain setting (Gallagher, 2005). Depositionally, both units represent relatively shallow, clastic-influenced marine environments conducive to mosasaur preservation; however, the Codell is more proximal with higher terrigenous input and bioturbation (Hattin, 1962; Bice et al., 2016), while the New Jersey sequence shows stronger glauconitic influence and a clearer transgressive signature at the Mount Laurel–Navesink contact (Owens et al., 1970; Gallagher, 2005). Taphonomically, both yield mosasaurs within diverse marine vertebrate assemblages, but the New Jersey sites frequently feature higher concentrations of material due to slower sedimentation rates and phosphatic/glauconitic lag deposits (Gallagher, 2005).

This comparison illustrates that mosasaurs occupied broadly similar shallow-marine ecological niches across different seaways throughout approximately 20–25 million years of Late Cretaceous time, providing important context for their paleoecology and evolutionary history. By processing pooled benthic oyster shells (*Agerostrea* and *Pycnodonte*) alongside pelagic cephalopods (*Belemnitella americana*) from these units, the methodology captures a vertically integrated profile of the water column. Establishing these concurrent benthic and pelagic baseline values ensures that any positive thermal deviations measured in the target shark enameloid can be mathematically evaluated against true ambient environmental endpoints rather than localized depth or seasonal overprints

2.3 Sample preparation

2.3.1 Sample collection

All fossil samples were collected and sampled under the relevant institutional permissions. New Jersey specimens are repositied at William Paterson University, and Kansas

specimens are deposited at the Sternberg Museum of Natural History, Fort Hays State University. Museum catalog numbers, taxonomic identifications, sampled tissue, and destructive-sampling permissions are listed in the supplementary tables.

2.3.2 Sample cleaning

Samples were prepared by ultrasonication in ultrapure deionized water for 5 minutes in 1 minute intervals. All teeth were then subsequently dried in an oven at 40 °C overnight. Tooth enameloid was then drilled off at low speed using a dental drill and carbide microdrill bit. Drilled sample was pulverized using an agate mortar and pestle to homogenize grain size.

2.3.3 Sample preparation for clumped isotope paleothermometry

For clumped isotope analysis, the resulting sample powder was treated using dilute hydrogen peroxide for 3 h, triple-rinsed with ultrapure deionized water, dried in an oven at 40 °C overnight. Following the protocol of Eagle et al. 2010, bioapatite samples were then soaked in 0.1M acetic acid, buffered to pH 4.6 in 1M sodium acetate for 14 h, and then rinsed three times with deionized water prior to isotopic analysis. Once treated samples were completely dry, aliquots (0.42-6.6 mg, depending on mass spectrometer for pure carbonates, 4.0-22.0 mg for bioapatites, depending on carbonate wt%) were measured and run on either the Thermo Finnegan MAT 253 or Nu Perspective mass spectrometer in previously published configurations (Defliese and Tripathi, 2020; Upadhyay, et al., 2021; Lucarelli et al., 2023).

2.3.4 Sample preparation for oxygen phosphate analysis

Untreated powders were prepared for oxygen phosphate analysis using the protocol described in Mine et al., 2017. Bioapatite was dissolved in hydrofluoric acid, phosphate ions isolated in solution, and silver phosphate was precipitated with a silver amine solution. Precipitated silver phosphate crystals were then rinsed five times with ultrapure deionized water

and dried overnight at 50 °C. Microprecipitations were carried out at the Stable Isotope Ecosystem Laboratory (SIELO) at UC Merced, as well as the Tripati-Eagle IsoLab at UCLA.

2.4 Material preservation analyses

2.4.1 Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was used to evaluate the mineralogical composition and extent of diagenetic alteration in tooth samples from the Codell Sandstone (Kansas) and the Wenonah/Mount Laurel–Navesink interval (New Jersey). All analyses were performed by Dr. Robin Trayler at the SIELO Lab (University of California, Merced) using a Vertex 70 spectrometer (Bruker, Billerica, USA) housed in the Nuclear Magnetic Resonance Facility (University of California, Merced). Small fragments (~1–5 mg) of dense enameloid were sampled from each taxon. Samples were cleaned ultrasonically in deionized water and analyzed using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy. Spectra were collected in the mid-infrared range (typically 4000–400 cm^{-1}) with a resolution of 4 cm^{-1} . Multiple scans were averaged per sample to improve signal-to-noise ratio. Spectral processing included baseline correction and normalization. Key absorption bands were examined for phosphate (ν_1 , ν_3 ~960–1100 cm^{-1}), carbonate (ν_3 ~1400–1450 cm^{-1}), and amide (protein/collagen) bands (amide I ~1650 cm^{-1} ; amide II ~1550 cm^{-1}). The apatite crystallinity index (CI) was calculated from the splitting of the phosphate ν_4 band (565–605 cm^{-1}), and additional indices such as the carbonate-to-phosphate (C/P) ratio and Water-Amide-on-Phosphate Index (WAMPI) were used to assess diagenetic alteration and organic matter preservation (following methods in Trayler et al., 2023). All samples were analyzed in replicate, with spectra compared against modern tooth standards and previously published marine datasets to evaluate relative preservation between the Turonian (Codell) and Campanian–Maastrichtian (New Jersey) specimens.

2.4.2 Comparative geochemistry

Basic statistical analyses were performed on data between the two locations. P-values were calculated using two-tailed independent samples t-tests assuming unequal variances (Welch's t-test). Sample sizes for mosasaurs are small (n=7 for NJ, n=3 for KS), which limits statistical power. Additionally, larger errors in clumped isotope paleothermometry temperatures and low replication in some taxa also limit statistical power.

2.4.2.1 $\delta^{18}\text{O}_p$ phosphate ($\delta^{18}\text{O}_p$)

Depending on sample availability, measured paleotemperature estimates from both $\delta^{18}\text{O}_p$ and Δ_{47} for taxa were directly compared to assess consistency. Further, comparing both $\delta^{18}\text{O}_p$ and Δ_{47} directly in specimens allows us to compare the moieties of carbonate-bearing phases in bioapatite. Statistically, the moiety difference between these two carbonate-bearing phases in extant taxa will fall between 7-11‰ in 99% of samples, and within 6-10‰ in 95% of samples (Eagle et al., 2010). This method is employed in other bioapatite studies as a diagenetic pre-screening (Eagle et al., 2010). Paleotemperatures were estimated for $\delta^{18}\text{O}_p$ following methods published in Lécuyer et al., 2013, as implemented in comparable recent studies (Griffiths et al., 2023).

To reconstruct paleotemperatures from the shark teeth, the phosphate-water oxygen isotope fractionation equation was applied to measured $\delta^{18}\text{O}_p$ values.

$$T(^{\circ}\text{C}) = 111.4 - 4.3 \times (\delta^{18}\text{O}_p - \delta^{18}\text{O}_{xw}) \quad (1)$$

where T is the temperature in degrees Celsius, $\delta^{18}\text{O}_p$ is the oxygen isotope composition of the phosphate (in ‰ VSMOW), and $\delta^{18}\text{O}_{\text{w}}$ is the oxygen isotope composition of the ambient seawater (in ‰ VSMOW; Longinelli and Nuti, 1973).

To generate a range of plausible seawater $\delta^{18}\text{O}_{\text{w}}$ values, we integrated (1) literature-based estimate for Late Cretaceous seawater from Shackleton and Kennett of -1 for an ice-free world and (2) $\delta^{18}\text{O}_{\text{w}}$ values derived independently from clumped isotope paleothermometry on co-occurring biogenic carbonates from New Jersey (1975). This approach produced a range of seawater compositions that accounts for potential regional variability between the WIS and open Atlantic. Body temperatures were then calculated across this range of $\delta^{18}\text{O}_{\text{w}}$ end-members to provide minimum to maximum temperature estimates (Figure 2).

To account for uncertainties in seawater $\delta^{18}\text{O}_{\text{w}}$ and propagate errors in temperature estimates, we employed a Bayesian statistical approach as applied in similar previous studies (Griffiths et al., 2023).

2.4.2.2 Seawater temperature estimation

Three taxa, *Belemnitella americana*, *Pycnodonte convexa*, and *Agerostrea mesentericia* from New Jersey were included as ectothermic endmembers to provide sea temperature estimates and comparison to fish, sharks, and mosasaurs in this set. Since the rostra and shells of these taxa, respectively, are pure calcium carbonate, only the sonication and hydrogen peroxide cleaning steps were performed.

2.4.2.3 Statistical analyses

All statistical analyses were performed in Microsoft Excel. For comparison of averaged values (e.g., mean temperatures) between two values, a Welch's two-tailed t-test was used, as it does not assume equal variances between groups. For comparisons involving paired arrays of measurements (e.g., clumped vs. phosphate-derived temperatures), a two-way ANOVA was applied. P-values were generated directly in Excel using built-in functions T.TEST(array1, array2, 2, 3) for Welch's t-tests and statistical significance was considered at $\alpha = 0.05$.

2.5 Stable isotope analysis

Clumped isotope analyses (Δ_{47} , $\delta^{18}\text{O}$, $\delta^{13}\text{C}$) were conducted in the Eagle-Tripati lab at the University of California, Los Angeles, between 2022 and 2026. Samples were analyzed on a Nu Perspective IS mass spectrometer (bioapatites) and a Thermo MAT Finnegan 253 dual inlet gas source mass spectrometer (carbonates; Thermo Fisher, Bremen, Germany) which have been demonstrated to produce statistically indistinguishable results for Δ_{47} (Upadhyay et al., 2021). Both instruments prepared samples for analysis through digestion in a common acid bath containing 103 wt% phosphoric acid (phosphates) and 105 wt% phosphoric acid (carbonates) reacted at 90°C followed by multiple steps of cryogenic purification and passage through a GC column packed with Porapak Type-QTM 50/80. Following purification, sample CO_2 gas was then introduced into the IRMS system via an automated changeover block, allowing for continuous alternating measurements of sample and reference CO_2 gas on detectors configured to measure isotopologues of masses 44, 45, 46, 47, 48, and 49. Measurements taken in bellows mode (~100 to 300 mg of sample material) consisted of four blocks of 20 cycles totaling in 1,600 s of integration with balancing to maintain 80 nA on mass 44 at every acquisition, while samples conducted in microvolume mode (30 to 100 mg of sample material) consisted of three blocks of 20 cycles for a total of 1,200 s of integration and a range of 80 nA to 30 nA over the course of each acquisition. Carbonate standards ETH-1 and ETH-2 were used to perform a nonlinearity

correction on sample unknowns. These nonlinearity-corrected values were then projected into the I-CDES reference frame of Bernasconi et al. (2021) using ETH-1,2, and 3 along with three in-house standards. ETH-4 was not included in the corrections and was instead used as a check for data quality. In-house bioapatite standards (Elephant E, WE-CA1) of extant species tooth enameloid were also included to check data quality during sample runs. Corrections were applied to sample and standard data over a moving average of 10 standards on either side of a given sample and were calculated using the Easotope software package, using the calibration outlined in Anderson et al., 2021 (John et al., 2016).

Once standardized to the I-CDES framework, absolute paleotemperatures are calculated using the unified, multi-laboratory community calibration equation presented by Anderson et al. (2021), which forms the operational thermal framework utilized by Lucarelli et al. (2023). This calibration spans an expansive temperature range (0.5 °C to 1100 °C) and utilizes strict carbonate-based standardization to reconcile previous interlaboratory slope discrepancies. The core polynomial relationship used to translate standardized Δ_{47} (I-CDES) values into absolute temperatures in Kelvin (T , in K) is defined as:

$$\Delta_{47}(I - CDES) = \frac{0.0391 \times 10^6}{T^2} + 0.1552 \quad (2)$$

The resulting temperatures in Kelvin were subsequently converted to degrees Celsius ($^{\circ}\text{C} = T - 273.15$).

To reconstruct the oxygen isotope composition of ambient body water ($\delta^{18}\text{O}_{\text{BW}}$), independent temperature estimates were derived from clumped isotope measurements coupled with oxygen isotope signatures of the concurrent mineral phases ($\delta^{18}\text{O}_{\text{mineral}}$ VPDB). Because analytical standard for carbonates and water differ (VPDB vs VSMOW scales), all mineral values were first converted to VSMOW using the standard IUPAC conversion equation:

$$\delta^{18}O_{\text{mineral (VSMOW)}} = 1.03092 \times \delta^{18}O_{\text{mineral (VPDB)}} + 30.92 \quad (3)$$

Body water fluid compositions ($\delta^{18}O_w$ VSMOW) were then calculated using the calcite-water equilibrium fractionation equation from Kim and O'Neil (1997):

$$1000 \ln \alpha_{c-w} = \frac{18.03 \times 10^3}{T} - 32.42 \quad (4)$$

Where T represents the absolute temperature in Kelvin (K). An average ambient water temperature of 20°C (293.15 K) was used as calculated from coeval New Jersey belemnites and oysters. The final isotopic composition of the $\delta^{18}O_{BW}$, VSMOW was then mathematically isolated and solved using the exponential inversion of the fractionation factor (α_{c-w}):

$$\delta^{18}O_w (\text{VSMOW}) = \frac{\delta^{18}O_c (\text{VSMOW}) + 1000}{\exp\left(\frac{1000 \ln \alpha_{c-w}}{1000}\right)} - 1000 \quad (5)$$

$\delta^{18}O_p$ was weighed into ~0.2 mg Ag_3PO_4 aliquots and packed into silver capsules. The analyses were measured in triplicate using a Thermal Conversion Elemental Analyzer (TC/EA)-ConFlo IV-Delta V Plus continuous flow isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) at SIELO, University of Merced (California, USA). Silver phosphate reduction to CO gas was achieved by heating the TC/EA graphite column at 1,450 C. Raw measurements were corrected for drift and linearity effects using USGS 80 and USGS 81 (USGS; >99% purity) silver phosphate reference materials. A 2-point calibration is applied to calibrate corrected $\delta^{18}O_p$ measurements to Vienna Standard Mean Ocean Water (VSMOW) also using the USGS reference materials. Enameloid $\delta^{18}O_p$ values are reported here as mean $\pm 1\sigma$.

3 Results

3.1 Evaluation of sample preservation by FTIR

To assess the preservation of the tooth enamel(oid) in our fossil specimens, we performed FTIR spectroscopy and calculated two key indices. The A-Carbonate on Phosphate Index (A-C/PI), reflects carbonate substitution in the apatite lattice while the Phosphate Crystallinity Index (PCI) indicates crystal order and potential diagenetic recrystallization (Sponheimer and Lee-Thorp, 1999; Miller et al., 2018). As shown in Figure 3.3, most fossil samples plot in a cluster with relatively low A-C/PI (0.15-0.30) and moderate PCI values (3.0-4.0), consistent with well-preserved Cretaceous and Miocene shark bioapatite (Enault et al., 2015; Davesne et al., 2022; Ziegler et al., 2021; Lebourg et al., 2022). These values overlap with modern and Miocene alligator enamel references, which serve as proxies for minimally altered phosphatic material (Enault et al., 2015; Ziegler et al., 2022; Lebourg et al., 2022). Samples show good preservation, with limited evidence of extensive carbonate-to-phosphate substitution or recrystallization.

3.2 Evaluation of preservation through geochemical analyses

To assess whether fossil teeth have experienced diagenetic alteration we use an internal check involving the two different oxygen-bearing moieties in bioapatite, the carbonate group (CO_3) and the phosphate group (PO_4). In well-preserved bioapatite, these two phases should be in isotopic equilibrium with each other because they formed together at the same temperature *in vivo*. If they fall out of equilibrium, it is strong evidence that one or both phases have been altered by later fluids or recrystallization (Eagle et al., 2010). Samples from this study are compared to moiety differences between carbonate phases in extant taxa, where 95% of extant taxa will fall in the 7-10‰ range, whereas 99% of extant taxa will fall in the 6-11‰ range. All samples in our study fall within the 99th percentile range, with all but *Pycnodontid indet.* and *S. pristodontus* from New Jersey falling within the 95th percentile (Figure 3.4).

Geochemical signatures were consistent between $\delta^{18}\text{O}_p$ and Δ_{47} paleotemperature measurements across all taxa except *Pycnodontiformes indet.*, which also showed an elevated

temperature in Δ_{47} analyses. $\delta^{18}\text{O}_p$ and Δ_{47} paleotemperature estimates were similar to each other across all other taxa, and yielded plausible body temperatures. While FTIR analysis did not flag *Cretalamna* from Kansas, Δ_{47} analysis reveals at this time a higher temperature inconsistent with New Jersey Δ_{47} analyses and both Kansas and New Jersey $\delta^{18}\text{O}_p$, however restricted sample size ($n=3$) may influence this discrepancy.

To evaluate the consistency and reliability of our paleotemperature reconstructions, independent two-sample Welch's t-tests were performed to cross-compare the temperature estimates derived from phosphate oxygen ($\delta^{18}\text{O}_p$) and carbonate-clumped isotopes (Δ_{47}) within identical taxonomic groups from the Codell Sandstone dataset. For *Cretoxyrhina mantelli* (Kansas), the mean estimates yielded by both proxies ($36.0 \pm 0.6^\circ\text{C}$ vs. $37.7 \pm 5.6^\circ\text{C}$, respectively) were statistically indistinguishable ($p=0.781$). Similarly, paleotemperatures calculated for the co-occurring Codell Sandstone teleost *Enchodus* ($25.3 \pm 0.4^\circ\text{C}$, $\delta^{18}\text{O}_p$ vs. $28.3 \pm 3.3^\circ\text{C}$, Δ_{47}) showed no statistically significant difference ($p=0.421$). This statistical agreement between two independent thermodynamic proxies demonstrates internal consistency, the preservation of offsets between species is also an indication that secondary diagenetic overprinting has not obscured primary signals and aids to validate the physiological fidelity of the recorded thermal signatures. Similar patterns can be observed in New Jersey specimens. T-tests for individual taxa show no significant difference between two groups as a whole, however some taxa consistently show an offset (*Cretalamna* spp.), while offsets are location-dependent in other taxa (New Jersey *Enchodus*, $p<0.01$).

To test whether statistically significant differences exist between the New Jersey and Codell Sandstone $\delta^{18}\text{O}_p$ estimates, equivalent taxa from both locations were tested using Welch's t-test. The test found no statistically significant difference between the two locations for *Mosasaurus* ($p=0.313$, $\delta^{18}\text{O}_p$), or *Enchodus* ($p=0.943$, clumped) however there was a statistically significant difference in the measurements of the two *Cretalamna* populations using

both paleothermometers ($p=0.016$, clumped; $p<0.01$, $\delta^{18}\text{O}_p$) and *Enchodus* populations ($p<0.01$, $\delta^{18}\text{O}_p$), indicating that differences in measurements between the two locations may be tied to individual species rather than being strictly dictated by location.

3.2.1 Stable isotope results

$\delta^{18}\text{O}_p$ values were used to estimate paleotemperature following methods in Longelli and Nuti (1973). Seawater $\delta^{18}\text{O}$ was assumed to be -1‰ , following Shackleton and Kennett (1975). Despite the depositional and paleoecological differences between the two sample localities and possibility that the two locations had different $\delta^{18}\text{O}$ seawater values, this literature-derived seawater $\delta^{18}\text{O}$ (-1‰) was used for temperature calculations, therefore results should be considered in the context of a potential offset from this assumption between the two localities (Shackleton and Kennett, 1975).

Mosasaur enamel yielded a mean $\delta^{18}\text{O}_p$ value of $17.8 \pm 0.5\text{‰}$ ($n = 7$) and a mean $\delta^{18}\text{O}_p$ paleotemperature of $32.6 \pm 0.7^\circ\text{C}$. Other taxa showed lower temperatures: *Cretalamna* averaged $23.7 \pm 0.4^\circ\text{C}$, *Squalicorax* averaged $26.7 \pm 0.6^\circ\text{C}$, and *Pycnodontidae* averaged $24.8 \pm 0.6^\circ\text{C}$ for samples from New Jersey.

Mosasaur material from the Codell Sandstone produced a mean $\delta^{18}\text{O}_p$ value of $17.7 \pm 0.4\text{‰}$ ($n = 3$) and a mean clumped-isotope paleotemperature of $33.8 \pm 0.8^\circ\text{C}$. Associated taxa yielded the following mean temperatures: *Cretoxyrhina mantelli* at $36.0 \pm 0.6^\circ\text{C}$, *Enchodus* at $25.3 \pm 0.4^\circ\text{C}$, and *Cretalamna* at $30.8 \pm 0.4^\circ\text{C}$. Carbonate $\delta^{18}\text{O}$ values in Kansas samples were generally more negative, ranging from -2.6 to -1.7‰ VPDB.

3.2.2 Ocean paleotemperature estimation from carbonate clumped isotopes

The stable isotope and clumped isotope composition of the Campanian/Maastrichtian Wenonah-Mt. Laurel Formation varies distinctly across the sampled taxa and material types.

Among the invertebrates, the five belemnite rostrum samples (*Belemnitella americana*, BA-NJ-1 through BA-NJ-5) yielded relatively uniform $\delta^{13}\text{C}$ values ranging from -0.11‰ to 0.73‰ VPDB, with tightly clustered $\delta^{18}\text{O}$ values between -0.35‰ and 0.21‰ VPDB. Their corresponding Δ_{47} values ranged from 0.600‰ to 0.611‰ , translating to calculated clumped isotope temperatures ($T\Delta_{47}$) between 19.6°C and 23.3°C and calculated ambient water oxygen values ($\delta^{18}\text{O}_{\text{water}}$) of 1.2‰ to 2.2‰ . In contrast, the two oyster species exhibited divergent carbon signatures: *Agerostrea mesenterica* (NJ-OM-1) displayed a heavily enriched $\delta^{13}\text{C}$ value of 2.58‰ , while *Pycnodonte convexa* (NJ-PC-1) showed a depleted value of -0.79‰ . Despite this carbon variation, both oyster species possessed similar oxygen signatures (0.06‰ and 0.10‰), nearly identical Δ_{47} values (0.609‰ and 0.610‰) and seawater temperature estimates (19.9°C and 20.0°C), and an identical calculated $\delta^{18}\text{O}_{\text{water}}$ value of 1.4‰ VSMOW.

3.2.3 Vertebrate enamel carbonate moiety geochemistry and body temperature estimation

Vertebrate enameloid samples from New Jersey demonstrated much greater geochemical variability, particularly in their stable isotope ratios. Carbon signatures ranged from highly depleted values in the marine reptile *Mosasaurus* (-10.50‰) and the predatory fish *Enchodus* (-7.13‰) to heavily enriched values in the sharks *Cretalamna appendiculata* (6.94‰) and *Squalicorax pristodontus* (2.46‰). Oxygen isotope ($\delta^{18}\text{O}$) values for all enameloid samples were consistently negative, spanning from -3.05‰ (*Scapanorhynchus texanus*) to -0.83‰ (*C. "appendiculata"*).

Clumped isotope thermometry separated the vertebrate taxa into two distinct thermal groups in New Jersey taxa. A lower-temperature cluster, tracking closely with the ambient invertebrate data, was comprised of *S. texanus* ($n=4$; $15.4\pm 5.2^{\circ}\text{C}$), *C. "appendiculata"* ($n=3$; $19.8\pm 0.8^{\circ}\text{C}$), and *S. pristodontus* ($n=7$; $19.9\pm 3.7^{\circ}\text{C}$). Conversely, a higher-temperature

cluster consisted of *Enchodus* (n=8; $28.0 \pm 1.2^\circ\text{C}$), an indeterminate *Pycnodontid* (n=2; $37.5 \pm 8.4^\circ\text{C}$), and *Mosasaurus* (n=7; $39.5 \pm 6.6^\circ\text{C}$).

The $\delta^{18}\text{O}_{\text{BW}}$ calculated range from $-1.55 \pm 0.55\text{‰}$ (*Scapanorhynchus*) to $0.67 \pm 0.10\text{‰}$ (*Cretalamna*) in New Jersey specimens and $-6.75 \pm 1.07\text{‰}$ (*Scapanorhynchus*) to $-2.01 \pm 0.19\text{‰}$ (*Enchodus*). The benthic and pelagic baseline controls within the Wenonah-Mt. Laurel/Navesink Formation samples display high-level internal consistency. Body water (‰, V-SMOW) compositions calculated from *B. americana* rostra (n=5) yield tightly clustered values ranging from $1.15 \pm 0.25\text{‰}$ to $1.71 \pm 0.25\text{‰}$. This serves as an independently derived baseline and closely follows measurements from co-occurring oysters *A. mesenterica* ($1.56 \pm 0.07\text{‰}$) and *P. convexa* ($1.60 \pm 0.09\text{‰}$). Teleost *Enchodus* ($-0.74 \pm 1.50\text{‰}$) from New Jersey also demonstrated $\delta^{18}\text{O}_{\text{BW}}$ values near 0‰ VSMOW. This uniform background framework is the backdrop against which apex marine predator *Mosasaurus* can be compared, yielding a slightly depleted fluid value of $(-0.32 \pm 0.08\text{‰})$. In contrast, *Scapanorhynchus* exhibits the most depleted fluid composition in the New Jersey group ($-1.55 \pm 0.55\text{‰}$)— a feature present in both New Jersey and Kansas *Scapanorhynchus* teeth.

Data from the older, Turonian-aged Codell Sands Formation consisted exclusively of vertebrate enameloid samples, which generally displayed enriched carbon signatures and severely depleted oxygen signatures compared to their Campanian/Maastrichtian counterparts. The carbon isotope values (‰, VPDB) ranged from a moderate 0.80‰ in *Enchodus* to heavily enriched values in the shark taxa, including *Scapanorhynchus raphiodon* ($5.17 \pm 0.82\text{‰}$), *Cretalamna* sp. ($6.52 \pm 0.28\text{‰}$), and *Cretoxyrhinia mantelli* ($7.27 \pm 0.23\text{‰}$). Every taxon within this formation demonstrated strongly negative $\delta^{18}\text{O}$ values, ranging from -3.60‰ (*Enchodus*) down to -8.24‰ (*S. raphiodon*) (Figure 5).

Calculated enameloid clumped isotope temperatures ($T\Delta_{47}$) for the Turonian specimens revealed some interspecies divergence, however it does fail to reach the threshold of statistical significance due to low sample sizes. Lower, closely aligned temperatures were recorded for *Enchodus* (28.3°C $\Delta_{47}=0.585\text{‰}$) and *S. raphiodon* (29.9°C $\Delta_{47}=0.581\text{‰}$). *Cretoxyrhina mantelli* yielded an intermediate temperature of 37.7°C ($\Delta_{47}=0.560\text{‰}$).

Fluid values calculated from the Codell Sandstone mirror ecological patterns observed in New Jersey specimens, but generally shift toward more depleted values, likely reflecting the differing paleoenvironment of the WIS. The local pelagic baseline in this region is constrained by teleost *Enchodus*, with a calculated $\delta^{18}\text{O}_{\text{BW}}$ of $-2.01\pm0.19\text{‰}$. Pelagic species in this region such as *Cretoxyrhina* and *Cretalamna* converge with slightly more depleted values $-3.20\pm0.33\text{‰}$ and $-3.95\pm0.31\text{‰}$, respectively), while deep-water taxon *Scapanorhynchus* exhibits the most severely depleted value ($-6.75\pm1.07\text{‰}$)

3.2.4 Statistical analyses

To evaluate the metabolic strategies and potential endothermic capabilities of Late Cretaceous shark lineages, two-sample Welch's t-tests were performed using phosphate oxygen isotope paleotemperatures ($\delta^{18}\text{O}_{\text{p}}$) and clumped isotope temperatures (Δ_{47}). Taxa were statistically compared against co-occurring ectothermic teleost baselines (*Enchodus*) and regional ambient marine invertebrate baselines (pooled *Belemnitella* and oysters from New Jersey) across two distinct geographic systems: the Campanian/Maastrichtian of New Jersey and the Turonian Codell Sandstone of Kansas (Table 3.1, Table 3.2, Table 3.3).

A two-sample t-test comparing mosasaur $\delta^{18}\text{O}_{\text{p}}$ paleotemperatures between New Jersey ($32.6 \pm 0.7^{\circ}\text{C}$) and Kansas ($33.8 \pm 0.8^{\circ}\text{C}$) revealed no statistically significant difference ($p = 0.313$). Across both sites, mosasaurs consistently recorded the high $\delta^{18}\text{O}_{\text{p}}$ paleotemperatures

among all analyzed taxa (overall mosasaur mean = $33.0 \pm 0.6^{\circ}\text{C}$), significantly warmer than co-occurring teleosts (mean $\sim 24\text{--}27^{\circ}\text{C}$; $p < 0.01$ for mosasaurs vs. teleosts in both KS and NJ). This pattern supports the interpretation of mosasaurs as active, mobile predators occupying the warmest surface or near-surface waters.

In the Codell sandstone *Cretoxyrhina mantelli* also recorded high $\delta^{18}\text{O}_p$ paleotemperatures at $36.0 \pm 0.6^{\circ}\text{C}$, significantly higher than that of coeval teleosts ($25.3 \pm 0.4^{\circ}\text{C}$; $p = < 0.01$) and co-occurring lamniform shark *Cretalamna* spp. ($30.8 \pm 0.4^{\circ}\text{C}$; $p = 0.005$)

The otodontid relative *Cretalamna* demonstrates significant environmental or geographic variation in its thermal signature, fluctuating between ectothermic and endothermic profiles. In the Campanian/Maastrichtian of New Jersey, *Cretalamna* “*appendiculata*” exhibits an internal body temperature profile that tracks significantly cooler than the co-occurring teleost baseline (*Enchodus*) under Δ_{47} ($p > 0.01$) and warmer under $\delta^{18}\text{O}_p$ proxies ($p > 0.01$). Furthermore, its body temperatures are statistically indistinguishable from regional pelagic cephalopods (*Belemnitella*, $p = 0.433$) and benthic oysters ($p = 0.940$), suggestive of an ambient, ectothermic lifestyle in this setting. Conversely, in the warmer Turonian greenhouse system of Kansas, *Cretalamna* sp. displays a highly significant positive thermal elevation, maintaining internal temperatures significantly warmer than *Enchodus* across both the clumped proxy (Δ_{47} , $p = 0.012$) and the phosphate proxy ($\delta^{18}\text{O}_p$, $p > 0.01$). In the Kansas data in particular we must consider the possibility of a degree of alteration particularly of the clumped isotope signature.

Statistical analysis establishes *Squalicorax pristodontus* (New Jersey) as an ectotherm that uniformly tracks local seawater conditions. While the $\delta^{18}\text{O}_p$ paleotemperature proxy detects a significant segregation from the teleost baseline ($p < 0.01$), this difference is driven by *Squalicorax* maintaining a colder internal profile than *Enchodus*. When contrasted with ambient environmental indicators, *Squalicorax* shows statistical overlap with both pelagic cephalopods

(Δ_{47} , $p=0.811$) and benthic oysters (Δ_{47} , $p=0.999$), confirming it possessed no capacity for metabolic heat retention (Table 3.3).

The goblin shark lineage *Scapanorhynchus* exhibits a cool thermal signature indicative of a cold- or deep-water ectothermic ecological niche, mirroring its modern deep-sea relatives. In the New Jersey system, *Scapanorhynchus texanus* is statistically segregated from the surface-dwelling teleost baseline. The $\delta^{18}\text{O}_p$ proxy establishes no significance ($p=0.504$) that *Scapanorhynchus* maintained an internal temperature profile significantly different from *Enchodus*. The Δ_{47} test reflects overlap with the thermal profile of *Enchodus* ($p=0.775$) and statistically overlaps with thermal profiles of the pooled cephalopod ($p=0.373$) and oyster ($p=0.098$) baselines. Within the shallower Western Interior Seaway depositional environment of Kansas, *Scapanorhynchus raphiodon* also follows the standard ectothermic teleost baseline (Δ_{47} , $p=0.775$), indicating a reliance on ambient water temperatures.

4 Discussion

4.1 Evaluation of Sample Preservation and Diagenetic Signals

The fidelity of geochemical signatures in fossil bioapatite and carbonate is essential to reconstructing accurate paleoecological and physiological traits. In this study, the alignment between $\delta^{18}\text{O}_p$ and Δ_{47} paleotemperatures across almost all taxa suggest the fossils are well-preserved. This suggestion is further evidenced by analyzing the moiety difference between the different carbonate phases, although caution should be exercised for interpretations of shark taxa on this basis (Karnes et al., 2024). Samples within this study all plot within the 99th percentile range, with all but Pycnodontidae indet. and *S. pristodontus* from New Jersey falling within the 95th percentile (Figure 3.4). FTIR spectroscopy also suggests that the majority of samples retain their original mineralogic composition (Figure 3). However, the indeterminate

pycnodontid (Pycnodontiformes indet.) represents an anomaly in both the FTIR spectroscopy and clumped isotope analyses. All screening methods flag this taxon as possibly non-primary, with the Δ_{47} yielding an elevated, biologically implausible temperature of 37.5°C to 37.7°C for a shallow-water, typically ectothermic teleost fish. The elevated pycnodontid Δ_{47} temperatures are best interpreted as the result of diagenetic alteration and partial recrystallization in the presence of fluids at elevated burial temperatures, rather than a primary metabolic signal. This highlights the necessity of multi-proxy validation, ensuring that enigmatic thermal signatures in co-occurring taxa are not misattributed to physiology when they stem from taphonomic artifacts.

4.2 Late Cretaceous Lamniform Bioenergetics in Macroevolutionary Context

Our study finds that apex predator *Cretoxyrhina mantelli* from the Turonian Codell Sandstone shows a body temperature above that of the ambient seawater. Our data suggest *C. mantelli* regulated a core body temperature ($36.0 \pm 0.6^\circ\text{C}$ via $\delta^{18}\text{O}_p$ and $37.7 \pm 5.6^\circ\text{C}$ via Δ_{47}) ~6 to 9°C above co-occurring teleost fish, *Enchodus* ($30.8 \pm 0.4^\circ\text{C}$ via $\delta^{18}\text{O}_p$ and $28.3 \pm 3.3^\circ\text{C}$ via Δ_{47}). This statistically significant separation, as determined by a Welch's t-test ($p < 0.01$), provides evidence for regional endothermy.

Furthermore, *C. mantelli* also maintained a significantly higher core metabolic temperature than co-occurring lamniforms like *Cretalamna* sp. ($33.3 \pm 0.5^\circ\text{C}$ $\delta^{18}\text{O}_p$; Welch's $p = 0.005$). This pattern indicates that while *Cretalamna* may have relied on a metabolic approach more similar to other ectotherms, *Cretoxyrhina* employed a more energy-intensive strategy. This directly parallels the bioenergetic strategy of the Neogene megatooth shark *Otodus megalodon*, which utilized specialized strategies to elevate its core body temperature for enhanced swimming efficiency and rapid visual processing (Griffiths et al., 2023). Finding a structurally parallel, thermal elevation in *Cretoxyrhina* may be suggestive that regional endothermy was an ecologically advantageous strategy utilized by Cretaceous lamniforms,

allowing them to optimize foraging and dominate pelagic food webs alongside contemporary endothermic marine reptiles before their high metabolic demands ultimately exceeded resources during climate shifts.

Although the closest relative in our sample set to *Otodus megalodon* are the *Cretalamna* specimens, they do not show the same degree of metabolically-controlled body temperature as *C. mantelli* consistently, in either the New Jersey ($32.6 \pm 0.7^\circ\text{C}$ via $\delta^{18}\text{O}_p$ and $19.8 \pm 0.8^\circ\text{C}$ via Δ_{47}) or Kansas ($33.3 \pm 0.5^\circ\text{C}$ via $\delta^{18}\text{O}_p$ and $44.3 \pm 3.7^\circ\text{C}$ via Δ_{47}) sets. The relatively small sample size for the Kansas Δ_{47} ($n=3$) estimate may not be reflective of the measurement once additional replicates are provided or may imply evidence of selective alteration of the clumped isotope signature but not the $\delta^{18}\text{O}_p$. At this time, the data do not clearly point to the same level of endothermy suggested by *Cretoxyrhina* data.

The metabolic strategy of *Cretoxyrhina* is apparently similar to the physiological model recently established for the Neogene megatooth shark *Otodus megalodon* (McCormack et al., 2022; Griffiths et al., 2023). Through convergent evolution, it appears that both independent lamniform lineages evolved massive body sizes and macropredatory dentition to thrive in pelagic food webs. By elevating its core body temperature, *Cretoxyrhina* optimized its physiology, allowing *C. mantelli* to operate as a fast-swimming apex predator, successfully occupying niches alongside contemporaneous apex predators such as *Mosasaurus* ($33.0 \pm 0.6^\circ\text{C}$). In contrast to *Cretoxyrhina*, coeval *Cretalamna* sp. recorded a significantly lower internal temperature ($30.8 \pm 0.4^\circ\text{C}$ $\delta^{18}\text{O}_p$; $p=0.005$), measuring closer to environmental baselines. These data indicates that *Cretalamna* may have exhibited a more metabolically conservative, ectothermic strategy.

During the Late Cretaceous, sharks faced severe ecological pressure (Myers and Lieberman, 2010). *Cretoxyrhina mantelli* disappeared during the Campanian amid rising

competition from marine reptiles, taxa like *Squalicorax* that persisted until the close of the Cretaceous before the K-Pg mass extinction (Siversson and Lindgren, 2005; Myers and Lieberman, 2010). Conversely, *Cretalamna* survived the K-Pg extinction (Siversson, et al., 2013). Its smaller body size, lower estimated body temperature, and flexible trophic positioning may have played a role in its persistence into the Paleogene. This selective macroevolutionary filter directly parallels the subsequent faunal turnovers observed during the Neogene. In the late Miocene and Pliocene, dropping global temperatures and shifting marine mammal distributions disturbed the pelagic food supply, resulting in decline of the regionally endothermic megatooth *Otodus megalodon* lineages while smaller, more ecologically flexible or metabolically conservative generalists persisted (McCormack et al., 2022; Paredes-Aliaga and Herraiz, 2024). Ultimately, our dataset demonstrates that the energetic constraints of modern and Neogene apex predators were already operating as the primary drivers of macroevolutionary selection within the greenhouse world of the Late Cretaceous.

4.3. Carbon Isotope ($\delta^{13}\text{C}$) Signatures

The carbon isotope ($\delta^{13}\text{C}$) values obtained from the enameloid phases provide complementary evidence regarding carbon sourcing within these Late Cretaceous marine ecosystems. Because $\delta^{13}\text{C}$ signatures vary minimally during trophic transfer, they primarily reflect the isotopic baseline of primary producers at the base of the local food web, which varies dynamically between nearshore, offshore, pelagic, and benthic environments.

In the Turonian Codell Sandstone, *Cretoxyrhina mantelli* and *Cretalamna* sp. exhibit highly enriched, overlapping $\delta^{13}\text{C}$ values ($7.27 \pm 0.23\text{‰}$ VPDB and $6.52 \pm 0.28\text{‰}$ VPDB, respectively), which point toward shared foraging grounds in highly productive, well-mixed pelagic zones where dissolved inorganic carbon (DIC) was strongly influenced by intense marine primary productivity. In stark contrast, co-occurring *Enchodus* records an relatively

depleted carbon signature ($0.80 \pm 0.10\text{‰}$ VPDB), suggesting spatial or dietary separation, such as foraging on deeper-water organic matter or localized benthic food webs that were decoupled from the open-surface pelagic system.

A similar offset is observed in the Campanian/Maastrichtian units of New Jersey, where taxa like *Cretalamna* “*appendiculata*” ($6.94 \pm 0.18\text{‰}$ VPDB), *Squalicorax pristodontus* ($2.46 \pm 0.57\text{‰}$ VPDB) and *Scapanorhynchus texanus* ($1.32 \pm 0.46\text{‰}$ VPDB) maintain distinct carbon signatures from the depleted signatures of *Enchodus* ($-7.13 \pm 0.72\text{‰}$ VPDB) and *Mosasaurus* ($-10.50 \pm 0.07\text{‰}$ VPDB) (Figure 3.5). The depletion observed in these latter taxa implies an influx of ^{12}C -enriched food sources potentially linked to being in a localized upwelling regime, terrestrial carbon inputs, or specialized benthic habitats and appears to reflect a distinct environment from open ocean pelagic feeding shark taxa. Ultimately, these $\delta^{13}\text{C}$ groupings demonstrate that despite sharing the same macro-localities, these apex marine vertebrates may have actively partitioned into distinct ecological niches and environments, potentially preventing direct ecological competition through fine-scale habitat utilization.

These $\delta^{13}\text{C}$ observations are reinforced by patterns observed in $\delta^{66}\text{Zn}$ studies which show clear niche partitioning among Cretaceous shark taxa (McCormack et al., 2026). This recent study found that taxa studied were distinct from one another, thus forming distinct dietary niches within the Late Cretaceous of the Codell Sandstone. Of taxa within our study, the highest value was determined from *Mosasaurus* indet. ($0.34 \pm 0.03\text{‰}$ 2SD, $n = 3$), while the lowest values come from *Cretalamna* spp. ($-0.19 \pm 0.22\text{‰}$ 2SD, $n = 6$) and *C. mantelli* ($-0.43 \pm 0.36\text{‰}$ 2SD, $n = 9$). *Squalicorax* ($-0.01 \pm 0.04\text{‰}$ 2SD, $n = 9$) and *Scapanorhynchus* ($-0.14 \pm 0.01\text{‰}$ 2SD, $n = 7$) both demonstrate relatively low values as well (McCormack et al., 2026).

4.4 Ecological stratification and niche partitioning in Late Cretaceous oceans

$\delta^{18}\text{O}_{\text{BW}}$ reveals additional valuable insights. Deep-water signals from *Scapanorhynchus* in both New Jersey and Kansas evidenced by relative depletion compared to other coeval taxa in this dataset, could be suggestive of habitat partitioning. Since modern goblin sharks are sluggish, deep-water benthic specialists, stratified seaways or open shelves may have deep-water masses that are markedly decoupled from surface waters which are heavily depleted, cold, dense and influenced by different bottom-water currents (Yano et al., 2007; Finney et al., 2024). A depleted fluid value in both localities suggests that *Scapanorhynchus* may have been restricted to a deep, benthic sub-habitat, effectively isolated from the pelagic surface zones. These calculated values suggest that the Late Cretaceous oceans may have been partitioned, with lower-energy benthic species residing primarily in separate deep water zones.

4.5. Synthesizing trophic positioning from zinc isotopes and clumped isotope paleothermometry into a model of comparative Late Cretaceous physiology with implications for extinction or survival during end Cretaceous climate variability and the K-Pg mass extinction.

By integrating carbonate-clumped isotope paleothermometry (Δ_{47}) and phosphate oxygen values ($\delta^{18}\text{O}_{\text{p}}$) with the recent zinc isotope ($\delta^{66}\text{Zn}$) trophic framework established by McCormack et al. (2026), we can construct a model of Late Cretaceous marine trophic physiology (Figure 3.6, 3.7). Historically, paleoecological studies have treated body size, trophic level, and metabolic strategy as independent variables. However, combining these biogeochemical systems reveals that body temperature and trophic position are coupled and are capable of influencing both the macroevolutionary success and possibly predicting the extinction vulnerability of apex lamniform sharks.

The isotopic data from *Cretoxyrhina mantelli* represents a possible manifestation of pelagic predatory specialization. Clumped isotope data demonstrates a core body temperature

($37.7 \pm 5.6^\circ\text{C}$) consistent with modern mammalian baselines and Neogene superpredators like *Otodus megalodon* (Griffiths et al., 2023). When viewed alongside the low $\delta^{66}\text{Zn}$ values characteristic of apex macropredators, a possible physiological relationship emerges (McCormack et al., 2022; McCormack et al., 2026). Regional endothermy is an energy-expensive strategy, requiring a multi-fold increase in caloric intake compared to an ectothermic counterpart of equal mass. To meet metabolic demands, *Cretoxyrhina* likely required high-velocity, high-efficiency foraging capabilities to continuously target calorie-dense marine megafauna (such as teleosts and marine reptiles). Elevated core temperatures may have facilitated active hunting strategies capable of capturing larger prey and subsequently promoting the selection for gigantism. This evolutionary trajectory may represent an example of convergent evolution with *O. megalodon*. Separated by roughly 80 million years, both lineages independently integrated apex trophic positions (low $\delta^{66}\text{Zn}$), regional endothermy (high Δ_{47} temperatures), and gigantism to dominate the pelagic open-ocean environment.

Conversely, the data for *Cretalamna* points to a highly successful, alternative thermophysiological strategy. Our data show *Cretalamna* sp. body temperature estimated to be $30.8 \pm 0.4^\circ\text{C}$ ($\delta^{18}\text{O}_p$, Kansas), significantly lower than *Cretoxyrhina* ($36.0 \pm 0.6^\circ\text{C}$ $\delta^{18}\text{O}_p$, Kansas), indicating an ectothermic physiology, more similar to co-occurring ectotherm *Enchodus* ($28.3 \pm 3.3^\circ\text{C}$, Kansas). Per recent zinc isotope analyses, generalist lineages like *Cretalamna* exhibit intermediate-to-high $\delta^{66}\text{Zn}$ values, reflecting a lower, more flexible trophic position and a more varied diet (McCormack et al., 2026).

This dataset also elucidates the metabolic and trophic strategies of other prominent Late Cretaceous predators, notably the lamniform shark *Squalicorax* and the large mosasaurid *Mosasaurus*. Across both locations, *Squalicorax* shows consistent ectothermic to moderately elevated signals. The New Jersey teeth yield enameloid phosphate oxygen values

corresponding to body temperatures around 27°C, while Δ_{47} temperatures reveal a slightly cooler 20°C. These temperatures align closely with co-occurring ectothermic taxa. In the context of the zinc isotope framework, *Squalicorax* likely occupied a relatively high trophic position consistent with opportunistic/scavenging ecology (McCormack et al., 2026). This moderate metabolic demand would have allowed greater resilience to prey fluctuations compared to more hyper-specialized or more endothermic groups.

Mosasaurus in contrast, exhibits warmer body temperatures suggestive of elevated metabolism. The New Jersey specimens show oxygen phosphate derived temperatures averaging at $32.6 \pm 0.7^\circ\text{C}$, while Kansas falls similarly at $33.8 \pm 0.8^\circ\text{C}$. Clumped results for the New Jersey *Mosasaurus* yield an elevated temperature of $39.9 \pm 6.6^\circ\text{C}$ as well. These clumped isotope values place *Mosasaurus* between *Cretoxyrhina* and low-temperature generalists like *Cretalamna*, although zinc isotope values place *Mosasaurus* comparatively low trophically (higher $\delta^{66}\text{Zn}$ values). As massive marine reptiles, mosasaurs combined gigantothermy and potentially regional endothermy, enabling effective pursuit or ambush predation. Furthermore, this high metabolic activity combined with relatively low trophic levels as determined by zinc isotope ratios may indicate that *Mosasaurus* maintained a more broad, flexible diet while consuming sufficient prey biomass to support the elevated metabolic requirements for its large body size. However, the elevated energetic requirements still tied them closely to productive marine food supplies, possibly contributing to their extinction at the K-Pg boundary (Polcyn et al., 2014).

By pairing a lower trophic standing with a low metabolic rate, *Cretalamna* minimized its energetic requirements, and likely did not require the same continuous, high-volume diet that *Cretoxyrhina* likely relied upon. While this may have restricted *Cretalamna* to smaller body sizes and prevented it from occupying the highest trophic levels during the Turonian, it could have

insulated the lineage from fluctuating prey availability. This integrated trophic-physiological model provides a mechanistic explanation for the highly selective faunal turnover observed leading up to and at the K-Pg boundary. Mass extinction events act as ecological filters that disproportionately target high-energy specialists. When the rapidly shifting climate induced collapsed global marine primary productivity, the trophic cascade propagated upward rapidly. Because *Cretoxyrhina mantelli* operated at the apex of the food web (lowest $\delta^{66}\text{Zn}$) and possessed the highest baseline metabolic demands (highest endothermic temperatures), *C. mantelli* was likely at a disadvantage surviving sudden scarcity in the pelagic zone (McCormack et al., 2026). As *C. mantelli* went extinct in the upper Santonian to the lower Campanian, their metabolic strategy may have become a liability when the marine ecosystem could no longer supply the massive caloric thresholds required to sustain their gigantism (Siversson and Lindgren, 2005).

In contrast, *Cretalamna* successfully survived the K-Pg extinction event (Siversson, et al., 2013). Its low-energy ectothermic baseline and trophic position (higher $\delta^{66}\text{Zn}$) may have allowed it to weather the prolonged scarcity of pelagic biomass by subsisting on a broader, opportunistic array of smaller, surviving low-trophic organisms. This exact macroevolutionary dynamic repeated itself in Neogene fauna during the late Miocene and Pliocene, changing oceanic circulation and declining prey availability created an identical energetic trap, likely driving the endothermic apex predator *O. megalodon* to extinction while smaller, more metabolically plastic carcharhinid and lamnid generalists survived (McCormack et al., 2022; Paredes-Aliaga and Herraiz, 2024). Ultimately, our combined dataset highlights that across deep-time, gigantism and regional endothermy represent a highly vulnerable evolutionary position that is heavily affected by the climate and broader ecosystem.

4.6. Comparative Thermophysiology Across Extinct and Modern Marine Amniotes and Chondrichthyans

To fully evaluate the evolutionary significance of our stable isotope data, the thermal profiles of Late Cretaceous lamniforms and mosasaurs must be contextualized alongside both the fossil record of other marine vertebrates and modern analogous taxa. The evolution of metabolic heat retention in marine environments represents an example of evolutionary convergence, appearing independently across multiple distinct lineages of reptiles, mammals, and fish. Placing our results within this broader comparative framework highlights how distinct anatomical groups solved energetic challenges during the Mesozoic.

Our data suggest that *Mosasaurus* maintained a stable, elevated core body temperature ($33.0 \pm 0.6^\circ\text{C}$) that was independent of geographic and environmental baselines. This suggestion of endothermy aligns with oxygen isotope work on pelagic marine reptiles from both the Jurassic and Cretaceous (Bernard et al., 2010; Séon et al., 2020), which demonstrated that advanced mosasaurids, ichthyosaurs, and plesiosaurs all independently developed endothermic traits. While early branch mosasauroids likely relied on ectothermy within shallow, warm coastal waters, the gigantism achieved by Late Cretaceous forms like *Mosasaurus* was structurally supported by a high metabolic rate. This elevated thermal ability may have facilitated other traits conducive to active hunting strategies, such as prolonged fast swimming. Maintaining a stable 33°C body temperature gave *Mosasaurus* a distinct metabolic advantage over coeval ectothermic prey, establishing it as a highly mobile, apex predator more similar to the ecological roles of Neogene odontocetes (killer whales) and modern pinnipeds.

This elevated metabolic strategy, however, may have also increased vulnerability to extinction. Recent modeling by Payne et al. (2026) demonstrates that heat production scales more rapidly with body size than heat loss in mesothermic vertebrates, creating an “overheating

predicament” for large-bodied forms. As a result, many mesothermic predators are restricted to cooler, deeper, or higher-latitude environments. Continued ocean warming can impose disproportionate physiological stress on such taxa by elevating metabolic costs and narrowing thermal safety margins, thereby substantially increasing extinction risk (Payne et al., 2026). Applying this to *Mosasaurus*, it suggests that its high core body temperature and gigantism, while advantageous during the relatively stable Late Cretaceous greenhouse climate, could have become more of a liability during the environmental perturbations associated with the end-Cretaceous mass extinction, including rapid warming and ecosystem disruption.

The 10.7°C thermal separation between *Cretoxyrhina mantelli* and the teleost *Enchodus* represents a reference frame for understanding deep-time evolution of chondrichthyan physiology. In the modern ocean, regional endothermy (or mesothermy) is restricted to a small, highly specialized subset of active pelagic fishes, most notably within the family Lamnidae (e.g., the great white shark *Carcharodon carcharias*), as well as tunas. These modern taxa retain metabolically generated heat, elevating their core body temperatures anywhere from 3°C to 14°C above ambient seawater. *Cretoxyrhina* achieved an internal temperature of ~36-38°C to , 10°C offset with ectotherms, even in the warm Turonian greenhouse seawater (~28°C recorded by *Enchodus*). Contrastingly, *Squalicorax* displays a different thermoregulatory strategy. Oxygen isotope data ($\delta^{18}\text{O}_p$) successfully segregates *Squalicorax* from the teleost baseline with extreme statistical significance ($p < 0.01$), this difference is driven by *Squalicorax* demonstrating a cooler thermal profile. When compared against regional environmental baselines, *Squalicorax* displays statistical overlap with both pelagic cephalopods (Δ_{47} , $p = 0.811$) and benthic oysters (Δ_{47} , $p = 0.999$), suggesting that this widespread predator lacked any mechanism for metabolic heat retention. This suggests that the complex internal vascular physiology required for regional endothermy was fully realized in the Late Cretaceous but restricted to specific lineages, driving the ecological success of gigantic lamniforms alongside endothermic marine reptiles while

ectothermic macro-predators successfully partitioned adjacent ecological niches. It later arose independently in the Otodontid and *Carcharodon* sharks of the Cenozoic.

Internal contrasts within our dataset can further elucidate the paleoecology of these taxa. The fact that *Cretalamna* sp. ($19.8 \pm 0.8^\circ\text{C } \Delta_{47}$, New Jersey), *Squalicorax pristodontus* ($19.9 \pm 3.7^\circ\text{C } \Delta_{47}$, New Jersey), and *Scapanorhynchus texanus* ($15.4 \pm 5.2^\circ\text{C } \Delta_{47}$, New Jersey) recorded significantly lower internal temperatures suggests that regional endothermy was not a universal trait shared by all Cretaceous sharks and instead may represent a size-dependent specialization. This comparative framework ultimately demonstrates that the Late Cretaceous pelagic environment supported two distinct evolutionary pathways to apex predatory dominance. *Mosasaurus* and *Cretoxyrhina mantelli*, independent lineages of reptile and shark that convergently adopted high-energy, high-endothermic strategies to optimize trophic positioning, while metabolically conservative ectotherms (*Cretalamna*, *Squalicorax*, and teleosts) measure closer to ambient environmental temperatures. While the endothermic strategy allowed *Mosasaurus* and *Cretoxyrhina* to dominate the apex niches of the Late Cretaceous, it tied their survival to a constant, high-volume abundance of calorie-dense prey. When shifting climates and the Chicxulub impact at the K-Pg boundary restricted food supply, the metabolically conservative shark *Cretalamna* survived the collapse, demonstrating that over macroevolutionary timescales, conservative, low-energy metabolic approaches may serve as a more resilient evolutionary strategy.

ACKNOWLEDGEMENTS

OPEN RESEARCH

5 Figures

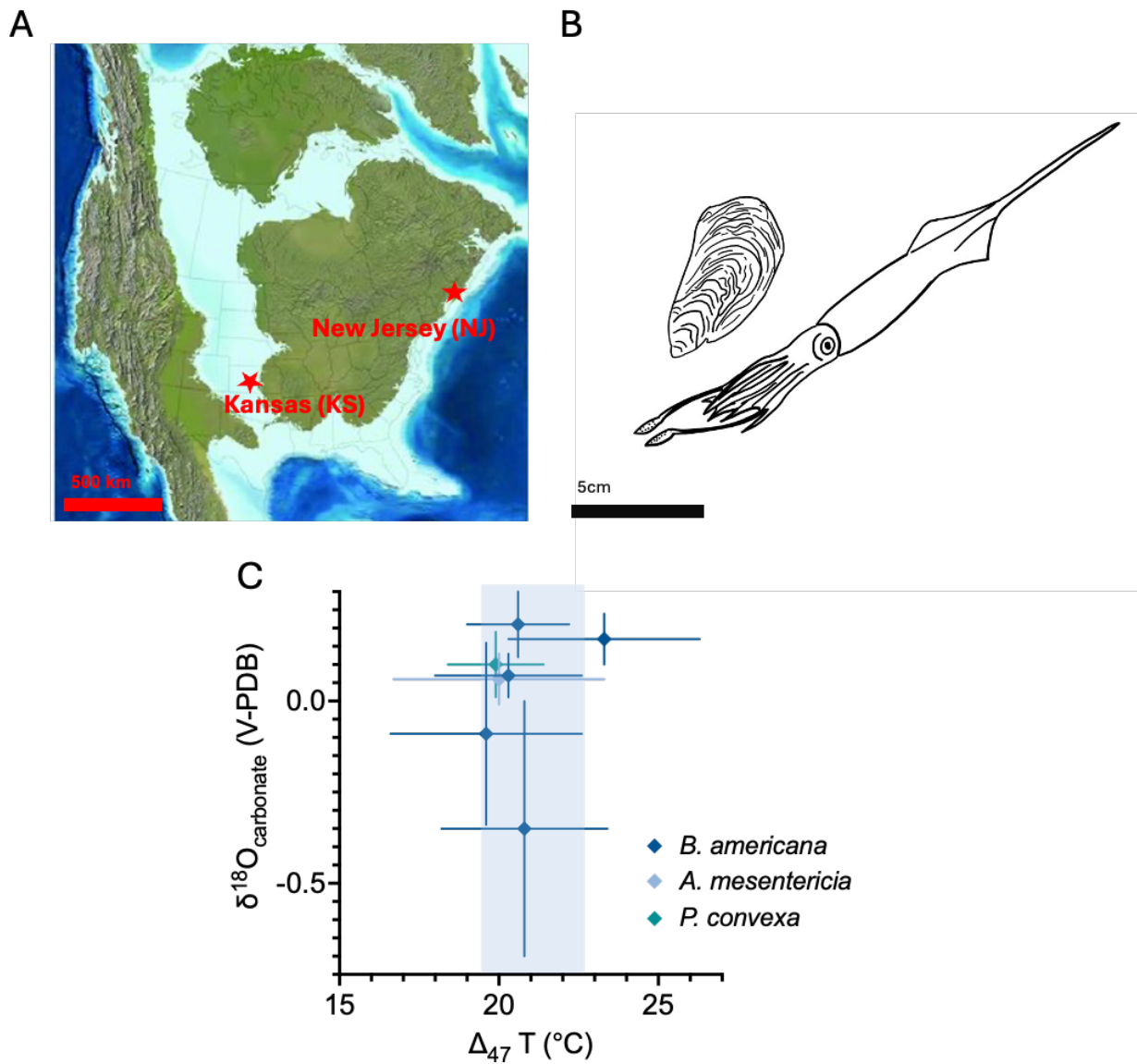


Figure 3.1

(A) Paleomap of sample locations included in this study: Wenonah/Mt. Laurel-Navesink contact, Monmouth County, New Jersey, and Codell Sandstone, Kansas (Modified from Gates et al. (2012) after original maps by Ron Blakey (Colorado Plateau Geosystems / Deep Time Maps). (B) Paleoartist rendering of carbonate material taxa, oysters and *Belemnitella americana*. By Jade Knighton. (C) Seawater paleotemperature and $\delta^{18}O_{\text{mineral}}$ estimates based on clumped

isotope analyses of oysters and belemnite rostra from the Wenonah/Mt. Laurel-Navesink Formation of New Jersey. Blue shaded region represents the midpoint between clumped estimated seawater temperatures of the Atlantic Coastal Plain (ACP) at the Navesink Formation (39 °N paleolatitude) and TEX₈₆ seawater estimates from the Shuqualak-Evans Borehole (35 °N paleolatitude). Clumped ACP seawater estimates a 3-14 °C seawater temperature, and TEX₈₆ estimates a seawater temperature of between 28 and 31 °C (Linnert et al., 2014; Meyer et al., 2018).

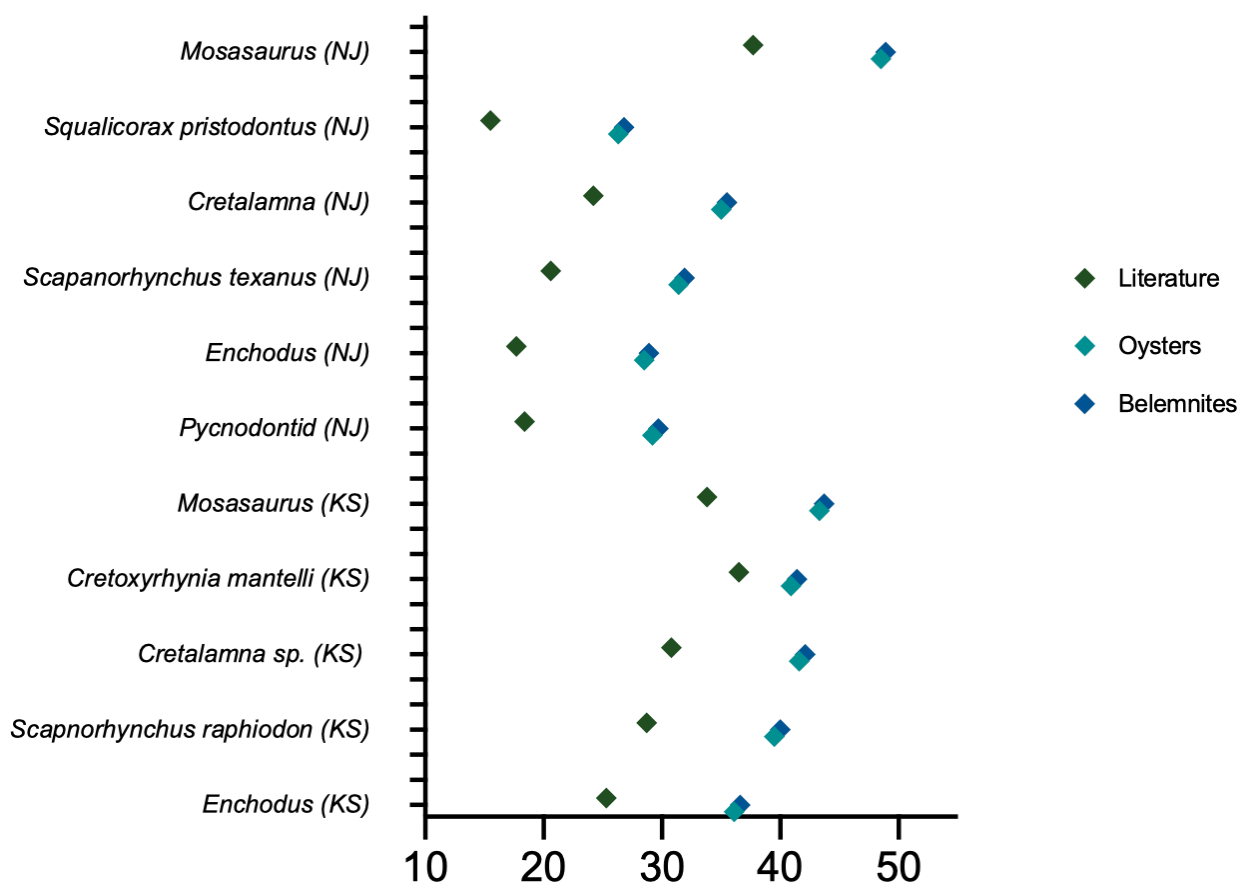


Figure 3.2

$\delta^{18}\text{O}_p$ calculations based on 1) literature (dark green), 2) belemnites (dark blue), and 3) oysters (sea green). Literature values are based on a $\delta^{18}\text{O}$ seawater value of -1‰ for an ice-free world (Shackleton and Kennett, 1975).

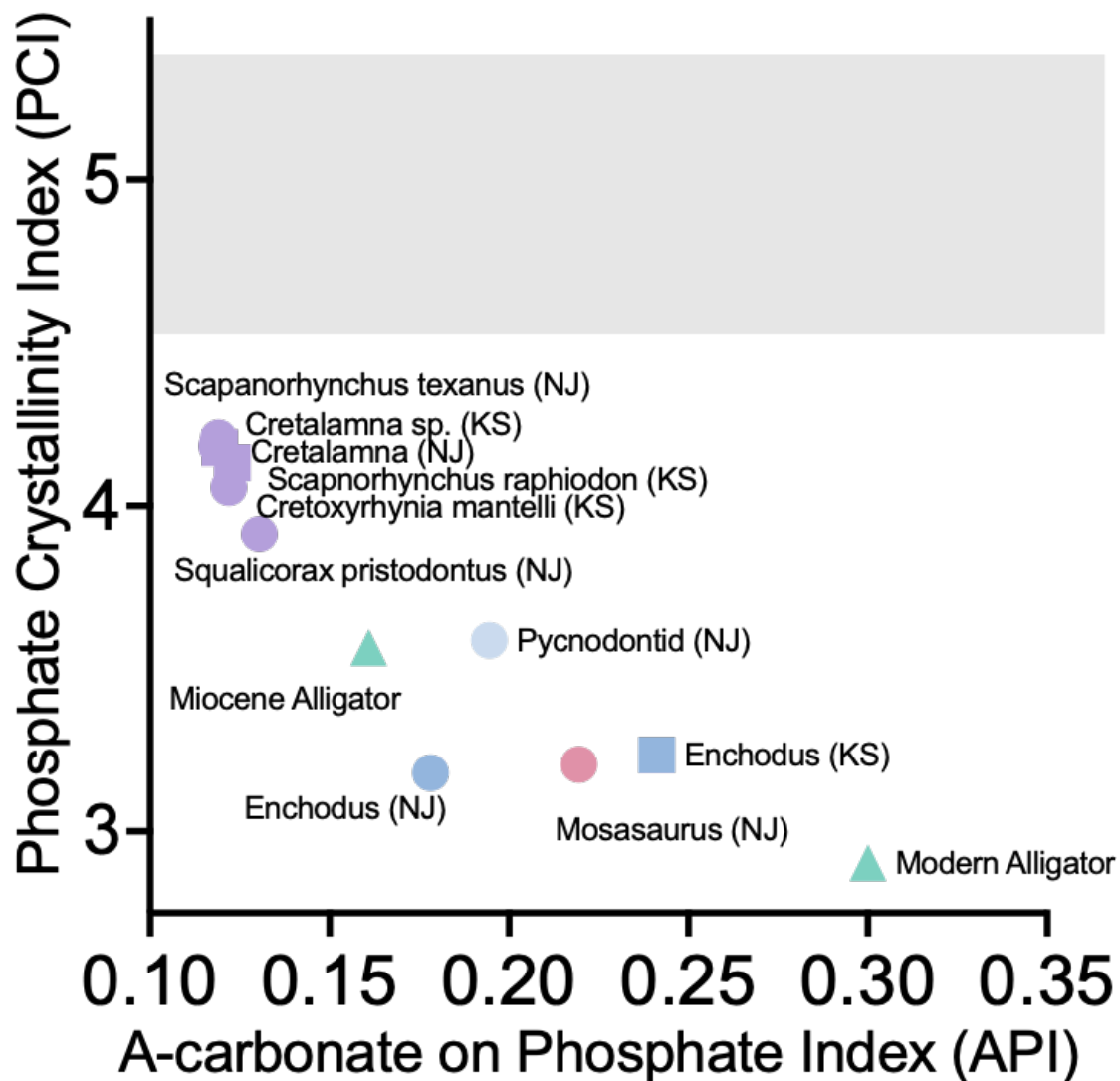


Figure 3.3

Fourier Transform Infrared Spectroscopy (FTIR) results for New Jersey and Kansas teeth. Blue symbols represent bony fish taxa, purple symbols represent shark taxa, pink symbols represent mosasaur taxa, and green symbols represent alligators. Faded symbols represent taxa which were found to be altered by geochemical analyses. Squares represent taxa from the Codell Sandstone, Kansas, circles represent taxa from the Wenonah-Mount Laurel/Navesink Contact, triangles represent Cenozoic taxa for comparison. Gray shaded region represents heavily-

recrystallized region of $PCI=4.5-5.4$ as determined from previous studies (Labs-Hochstein and MacFadden, 2006).

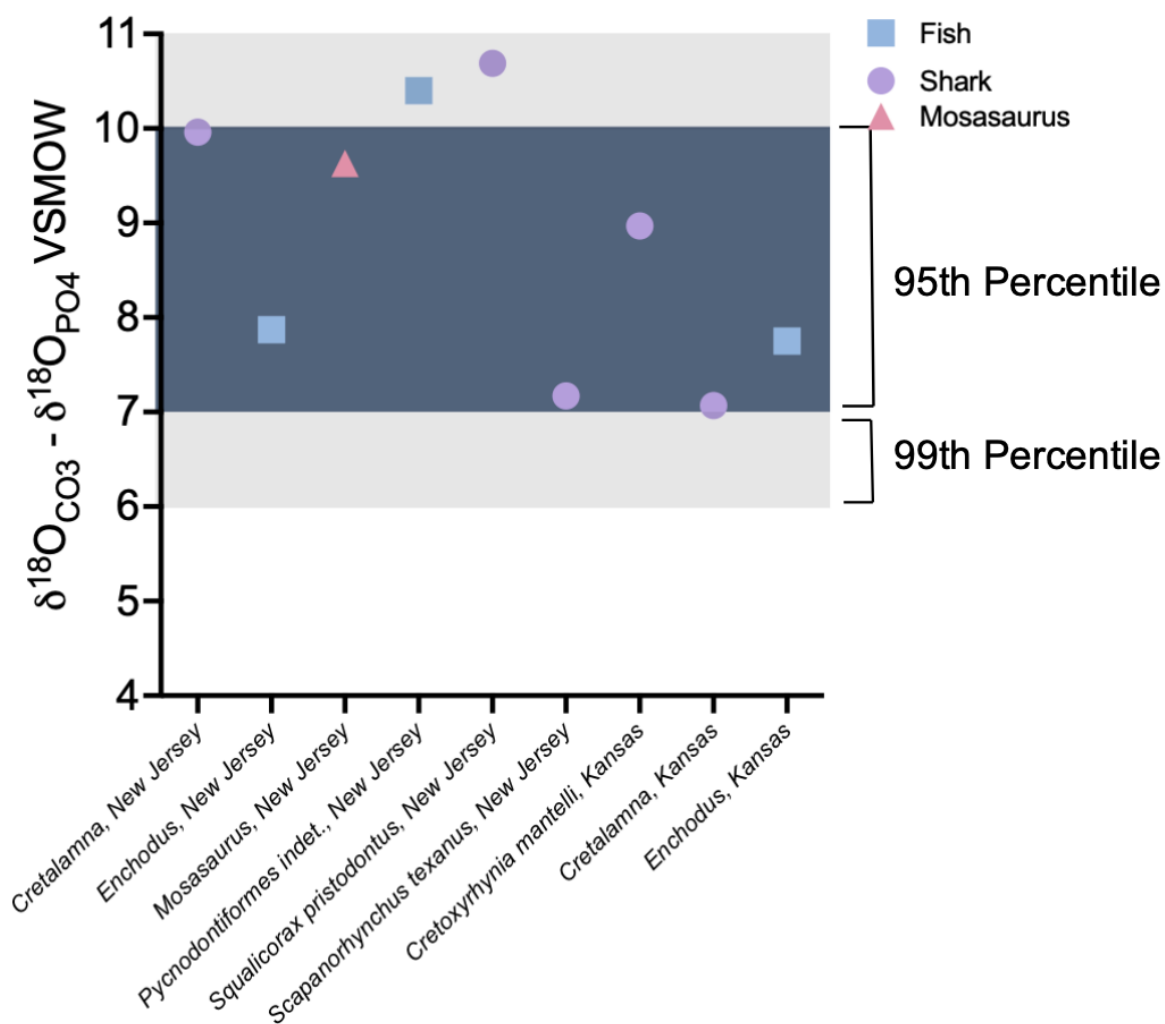


Figure 3.4

Moiety offset between carbonate group (CO_3) and the phosphate group (PO_4) in bioapatite samples in this study. Samples from this study are compared to moiety differences between carbonate phases in extant taxa, where 95% of extant taxa will fall in the 7-10‰ range (dark blue shading), whereas 99% of extant taxa (gray shaded regions) will fall in the 6-11‰ range.

This internal check follows methodology presented in Eagle et al. (2010) to flag possible recrystallization in fossil bioapatites. All samples in our study fall within the 99th percentile range, with all but *Pycnodontid* indet. and *S. pristodontus* from New Jersey falling within the 95th percentile.

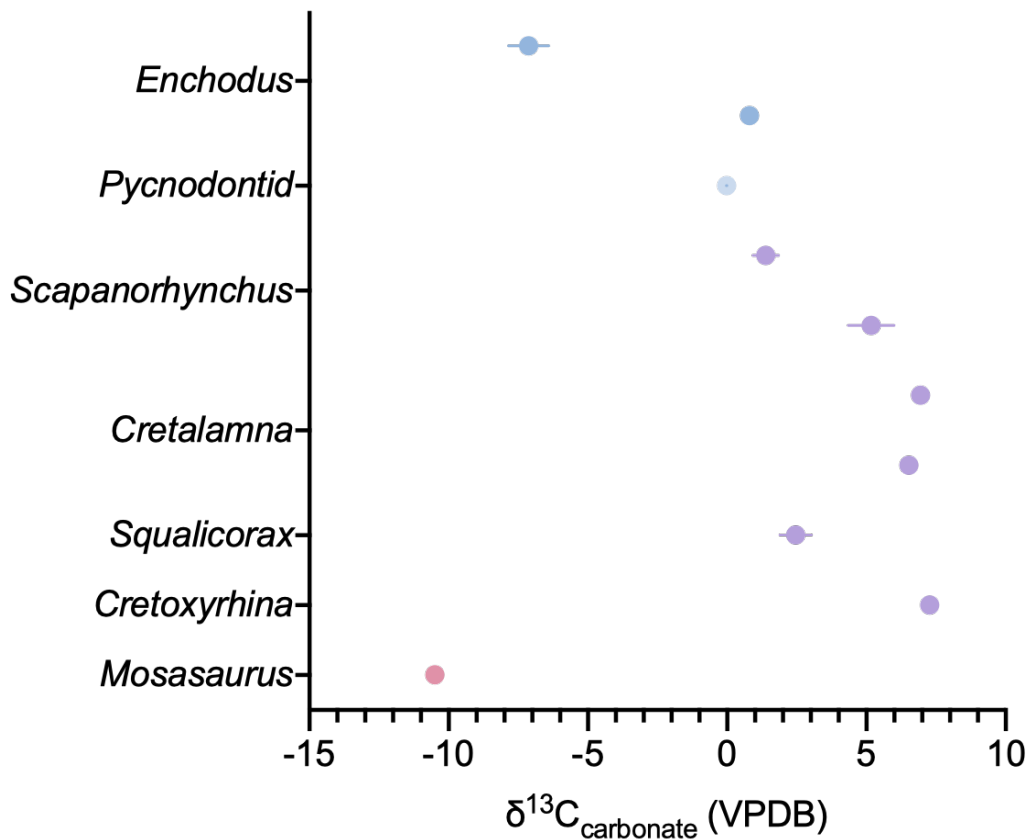


Figure 3.5

Carbon signatures for samples included in this study. Carbon signatures ranged from highly depleted values in the marine reptile *Mosasaurus* (-10.50‰) and the predatory fish *Enchodus* (-7.13‰) to heavily enriched values in the sharks *Cretalamna* “*appendiculata*” (6.94‰) and *Squalicorax pristodontus* (2.46‰).

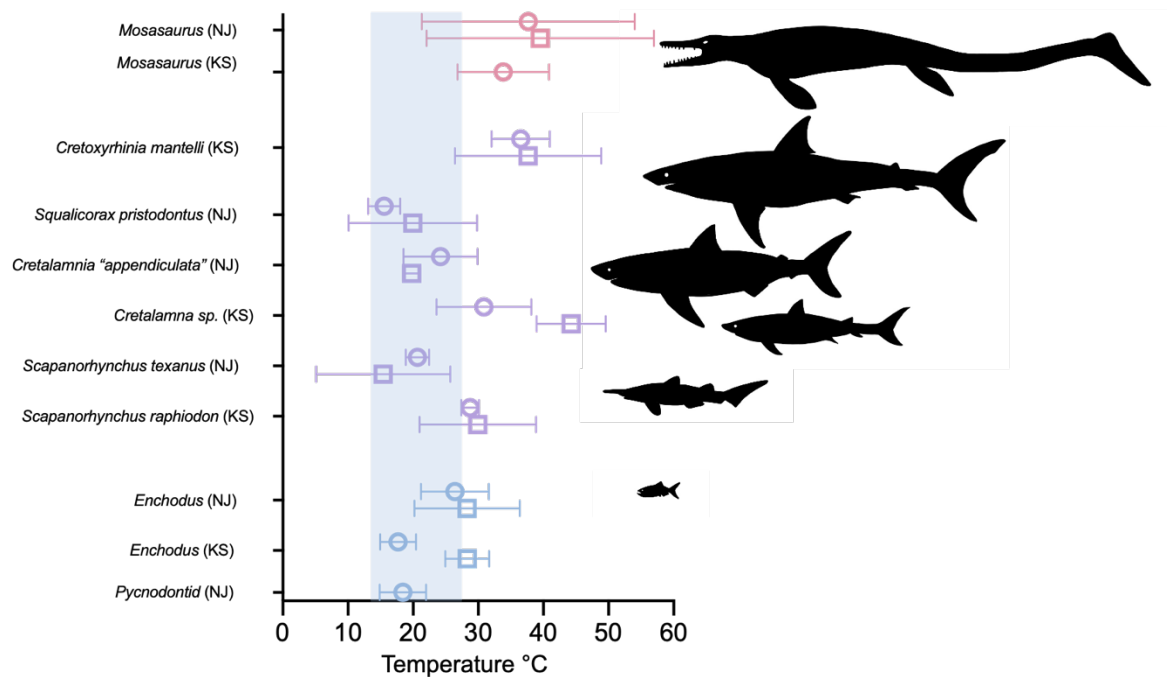


Figure 3.6

Paleotemperature data for species included in this study. $\delta^{18}O_p$ derived temperatures (circles) and Δ_{47} -derived temperatures (squares) for each taxon included in this study, 1 SE error indicated by whiskers. Telosts are shown in blue, sharks in purple, and mosasaurs in pink. Blue shaded region represents the midpoint between clumped estimated seawater temperatures of the Atlantic Coastal Plain (ACP) at the Navesink Formation (39 °N paleolatitude) and TEX_{86} seawater estimates from the Shuqualak-Evans Borehole (35 °N paleolatitude). Clumped ACP seawater estimates a 3-14 °C seawater temperature, and TEX_{86} estimates a seawater temperature of between 28 and 31 °C (Linnert et al., 2014; Meyer et al., 2018).

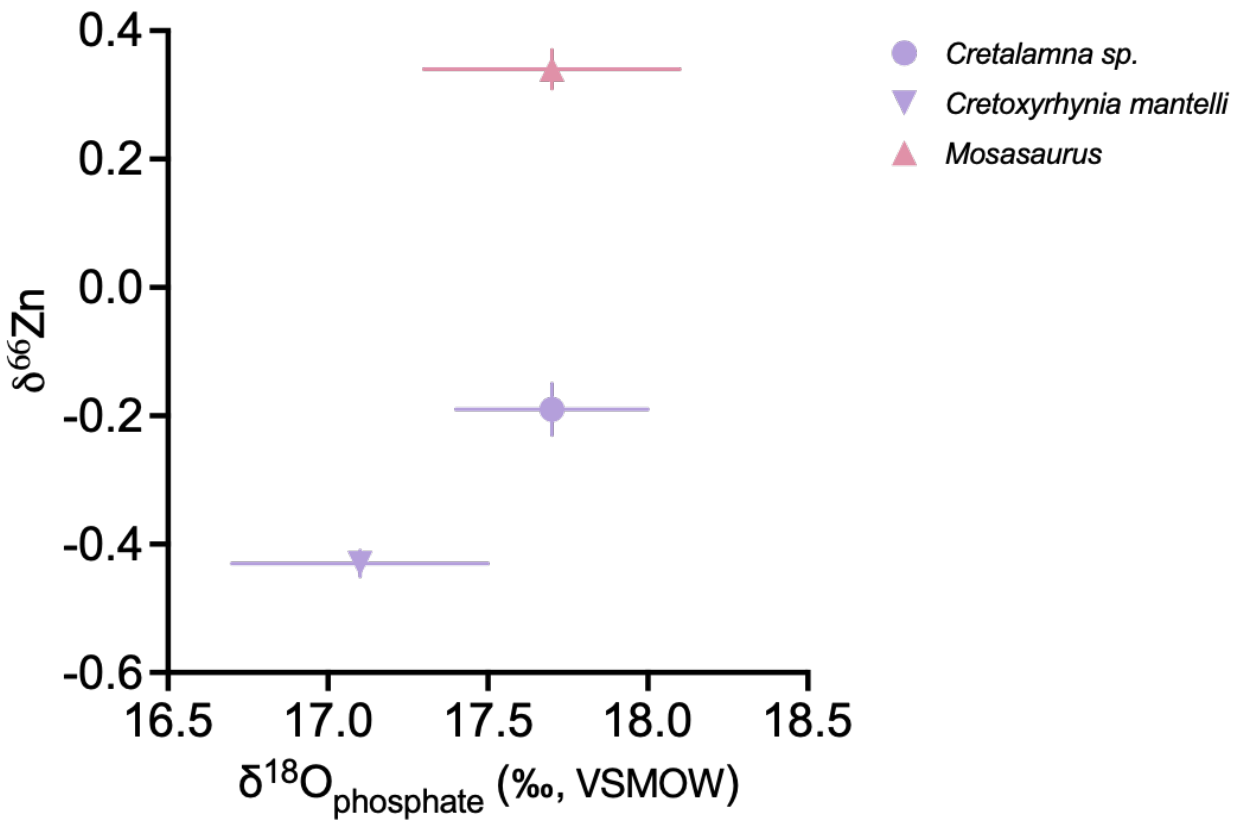


Figure 3.7

$\delta^{18}\text{O}_p$ vs Zinc isotope composition. Zinc (^{66}Zn) data after McCormack et al.(2026). $\delta^{18}\text{O}_p$ analyses were performed on the same specimens found in McCormack et al.(2026). Clear dietary niche partitioning can be observed between *Mosasaurus*, *Cretoxyrhina*, and *Cretalamna*. Faded symbols represent individual teeth zinc isotope composition, while the dark symbols are the average of all the teeth sampled.

6 Tables

Table 3.1

Summary of statistical comparisons (t-tests) for Mosasaurs.

Comparison	Parameter	Locality/Taxa Involved	p-value	Statistical Significance	Interpretation
Geographic Variation	$\delta^{18}\text{O}_p$ Paleotemperature	Mosasaurus (NJ vs. KS)	0.313	Not Significant ($p > 0.05$)	No difference in mosasaur measurements between NJ and KS (O phos)
Taxonomic Variation	$\delta^{18}\text{O}_p$ Paleotemperature	Mosasauroids vs. Teleosts (Enchodus)	< 0.01	Significant ($p < 0.01$)	Mosasaurus (NJ and KS) warmer than teleosts (O phos)
Regional Belemnite baseline	$\delta^{18}\text{O}_p$ Paleotemperature	NJ Mosasaur vs NJ pooled belemnites	< 0.0001	Significant ($p < 0.01$)	Mosasaurus warmer than co-occurring belemnites
Regional benthic baseline	$\delta^{18}\text{O}_p$ Paleotemperature	NJ Mosasaur vs NJ pooled oysters	< 0.0001	Significant ($p < 0.01$)	Mosasaurus warmer than co-occurring oysters

Table 3.2

Summary of statistical comparisons (t-tests) for *Cretoxyrhina*.

Comparison	Parameter	Locality/Taxa Involved	p-value	Statistical Significance	Interpretation
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Local Teleost Baseline	$\delta^{18}\text{O}_p$ Paleotemperature	Codell Sandstone (Turonian) Cretoxyrhina mantelli vs. Enchodus	< 0.0001	Extremely Significant ($p < 0.01$)	Cretoxyrhina warmer than co-occurring ectothermic fish in KS (O phos),
Co-occurring Lamniform	$\delta^{18}\text{O}_p$ Paleotemperature	Codell Sandstone (Turonian) Cretoxyrhina mantelli vs. Cretalamna sp.	< 0.01	Significant ($p < 0.01$)	Cretoxyrhina warmer than Cretalamna in KS (O phos).
Local Teleost Baseline	$\Delta 47$ Temperature	Codell Sandstone (Turonian) Cretoxyrhina mantelli vs. Enchodus	0.200	Not Significant ($p > 0.05$)	C. Mantelli not (significantly) different from Enchodus in KS (clumped)
Regional Cephalopod Baseline	$\Delta 47$ Temperature	NJ (Campanian/Maast.) vs. KS Cretoxyrhina mantelli vs. Pooled Belemnitella	0.043	Statistically Significant ($p < 0.05$)	KS Cretoxyrhina warmer than belemnites in NJ (clumped)
Regional Benthic Baseline	$\Delta 47$ Temperature	NJ (Campanian/Maast.) vs. KS Cretoxyrhina mantelli vs. Pooled Oysters	0.040	Statistically Significant ($p < 0.05$)	NJ Cretoxyrhina warmer than NJ oysters (clumped)

Table 3.3

Summary of statistical comparisons (t-tests) for Cretalamna, Squalicorax, and Scapanorhynchus.

Comparison	Parameter	Locality/Taxa Involved	p-value	Statistical Significance	Interpretation
Local Teleost Baseline	Δ_{47} Temperature	NJ (Campanian/Maast.) <i>Cretalamna</i> "appendiculata" vs. <i>Enchodus</i>	0.0003	Extremely Significant (p < 0.01)	New Jersey <i>Cretalamna</i> cooler than NJ <i>enchodus</i> (Clumped)
Local Teleost Baseline	$\delta^{18}\text{O}_p$ Paleotemperature	NJ (Campanian/Maast.) <i>Cretalamna</i> "appendiculata" vs. <i>Enchodus</i>	< 0.01	Extremely Significant (p < 0.01)	NJ <i>Cretalamna</i> warmer than NJ <i>Enchodus</i> (O phos)
Regional Cephalopod Baseline	Δ_{47} Temperature	NJ (Campanian/Maast.) <i>Cretalamna</i> "appendiculata" vs. Pooled <i>Belemnitella</i>	0.4332	Not Significant (p > 0.05)	NJ <i>Cretalamna</i> temp not significantly different from Belemnites

Regional Benthic Baseline	Δ_{47} Temperature	NJ (Campanian/Maast.) <i>Cretalamna</i> "appendiculata" vs. Pooled Oysters	0.9401	Not Significant (p > 0.05)	NJ <i>Cretalamna</i> not significantly different from NJ oysters (clumped)
Local Teleost Baseline	Δ_{47} Temperature	KS Codell Sandstone (Turonian) <i>Cretalamna</i> sp. vs. <i>Enchodus</i>	0.0121	Statistically Significant (p < 0.05)	<i>Cretalamna</i> warmer than <i>Enchodus</i> in KS (clumped)
Local Teleost Baseline	$\delta^{18}\text{O}_p$ Paleotemperature	KS Codell Sandstone (Turonian) <i>Cretalamna</i> sp. vs. <i>Enchodus</i>	< 0.01	Statistically Significant (p < 0.05)	<i>Cretalamna</i> warmer than <i>Enchodus</i> in KS (O phosphate)
Local Teleost Baseline	Δ_{47} Temperature	NJ (Campanian/Maast.)	0.0790	Not Significant (p > 0.05)	NJ <i>Squalicorax</i> not significantly different from <i>Enchodus</i> (clumped)

		<i>Squalicorax pristodontus</i> vs. <i>Enchodus</i>			
Local Teleost Baseline	$\delta^{18}\text{O}_p$ Paleotemperature	NJ (Campanian/Maast.) <i>Squalicorax pristodontus</i> vs. <i>Enchodus</i>	< 0.0001	Extremely Significant (p < 0.01)	phosphate oxygen data distinctly separates <i>Squalicorax</i> from <i>Enchodus</i> in NJ (cooler)
Regional Cephalopod Baseline	Δ_{47} Temperature	NJ (Campanian/Maast.) <i>Squalicorax pristodontus</i> vs. Pooled <i>Belemnitella</i>	0.9989	Not Significant (p > 0.05)	Shows no discernible thermal elevation above the pelagic invertebrate baseline.
Regional Benthic Baseline	Δ_{47} Temperature	NJ (Campanian/Maast.) <i>Squalicorax pristodontus</i> vs. Pooled Oysters	0.9401	Not Significant (p > 0.05)	alignment with ambient bottom-water conditions

Local Teleost Baseline	Δ_{47} Temperature	NJ (Campanian/Maast.) <i>Scapanorhynchus texanus</i> vs. <i>Enchodus</i>	0.7754	Not Significant (p > 0.05)	No difference from NJ <i>Scapanorhynchus</i> and <i>Enchodus</i> (clumped)
Local Teleost Baseline	$\delta^{18}\text{O}_p$	NJ (Campanian/Maast.) <i>Scapanorhynchus texanus</i> vs. <i>Enchodus</i>	0.5038	Not Significant (p > 0.05)	<i>S. texanus</i> no different than <i>Enchodus</i> (O phos) .
Regional Cephalopod Baseline	Δ_{47} Temperature	NJ (Campanian/Maast.) <i>Scapanorhynchus texanus</i> vs. Pooled <i>Belemnitella</i>	0.3725	Not Significant (p > 0.05)	No difference <i>S. texanus</i> v belemnites (clumped)
Regional Benthic Baseline	Δ_{47} Temperature	NJ (Campanian/Maast.)	0.4658	Not Significant (p > 0.05)	No difference <i>S. texanus</i> v oysters (clumped)

		<i>Scapanorhynchus texanus</i> vs. Pooled Oysters			
Local Teleost Baseline	Δ_{47} Temperature	KS Codell Sandstone (Turonian) <i>Scapanorhynchus raphiodon</i> vs. <i>Enchodus</i>	0.7755	Not Significant (p > 0.05)	KS <i>Scapanorhynchus</i> not different from KS <i>Enchodus</i> (clumped)

Table 3.4

Δ_{47} data for this study.

Catalog number	Genus and Species	Material type	Stratigraphy and locality	Stage	n	$\delta^{13}\text{C}$ VPDB	$\delta^{18}\text{O}$ VPDB	$\delta^{18}\text{O}$ VPDB	Δ_{47}	s.e.	T Δ_{47} [°C]*	s.e.	$\delta^{18}\text{O}$ water VSMOW	s.d.	
BA-NJ-1	<i>Belemnitella americana</i>	rostrum	Wenonah-Mt. Laurel/Navesink Fm	Campanian/Maastrichtian	5	0.01	5	0.2	0.0	0.60	0.00	20.6	1.6	1.7	0.4

BA-NJ-2	<i>Belemnitella americana</i>	rostrum	Wenonah-Mt. Laurel/Navesink Fm	Campanian/Maastrichtian	5	0.73	2	7	7	0	9	23.3	3.0	2.2	0.6
BA-NJ-3	<i>Belemnitella americana</i>	rostrum	Wenonah-Mt. Laurel/Navesink Fm	Campanian/Maastrichtian	5	0.16	5	7	6	8	7	20.3	2.3	1.5	0.5
BA-NJ-4	<i>Belemnitella americana</i>	rostrum	Wenonah-Mt. Laurel/Navesink Fm	Campanian/Maastrichtian	6	-0.11	5	5	5	7	8	20.8	2.6	1.2	0.6
BA-NJ-5	<i>Belemnitella americana</i>	rostrum	Wenonah-Mt. Laurel/Navesink Fm	Campanian/Maastrichtian	5	0.54	7	9	5	1	9	19.6	3.0	1.2	0.7
NJ-OM-1	<i>Agerostrea mesenterica</i>	oyster shell	Wenonah-Mt. Laurel/Navesink Fm	Campanian/Maastrichtian	5	2.58	3	6	7	0	0	20.0	3.3	1.4	0.7
NJ-PC-1	<i>Pycnodonte convexa</i>	oyster shell	Wenonah-Mt. Laurel/Navesink Fm	Campanian/Maastrichtian	5	-0.79	6	0	9	9	5	19.9	1.5	1.4	0.4
CA-NJ-E	<i>Cretalamna "appendiculata"</i>	enameloid	Wenonah-Mt. Laurel/Navesink Fm	Campanian/Maastrichtian	3	6.94	8	3	0	0	2	19.8	0.8		

ES-NJ-E	<i>Enchodus</i>	enameloid	Wenonah-Mt. Laurel/Navesink Fm	Campanian/Maastrichtian	8	-7.13	2	-	0.7	2.2	1.5	0.58	0.00	28.0	1.2		
MS-NJ-E	<i>Mosasaurus</i>	enameloid	Wenonah-Mt. Laurel/Navesink Fm	Campanian/Maastrichtian	7	-10.5	7	-	0.0	1.8	0.0	0.55	0.01	39.5	6.6		
PI-NJ-E	<i>Pycnodontid</i>	enameloid	Wenonah-Mt. Laurel/Navesink Fm	Campanian/Maastrichtian	2	-0.02	1	-	0.0	0.9	0.3	0.56	0.02	37.5	8.4		
SP-NJ-E	<i>Squalicorax pristodontus</i>	enameloid	Wenonah-Mt. Laurel/Navesink Fm	Campanian/Maastrichtian	7	2.46	7	-	0.5	1.4	0.6	0.61	0.01	19.9	3.7		
ST-NJ-E	<i>Scapanorhynchus texanus</i>	enameloid	Wenonah-Mt. Laurel/Navesink Fm	Campanian/Maastrichtian	4	1.38	6	-	0.4	3.0	0.5	0.62	0.01	15.4	5.2		
CM-CSKS-E	<i>Cretoxyrhinia mantelli</i>	enameloid	Codell Sandstone	Turonian	5	7.27	3	-	0.2	4.7	0.3	0.56	0.01	37.7	5.6		
CS-CSKS-E	<i>Cretalamna sp.</i>	enameloid	Codell Sandstone	Turonian	3	6.52	8	-	0.2	5.4	0.3	0.54	0.00	44.3	3.0		

EP- CSKS- E	<i>Enchodus</i>	enameloid	Codell Sandstone	Turonian	6	0.80	0	0	0.1	3.6	0.1	0.58	0.01	28.3	3.3		
SR- CSKS- E	<i>Scapanorhynchus raphiodon</i>	enameloid	Codell Sandstone	Turonian	4	5.17	2	4	0.8	8.2	1.0	0.58	0.01	29.9	4.5		

Supporting information for:

Chapter 3. Thermoregulation and ecology of giant marine predators of the Mesozoic

Supplementary figures



Figure S3.1 Photo of teeth from New Jersey sampled in this study. A) *Cretalamna*, B) *Scapanorhynchus texanus*, C) *Squalicorax pristodontus*, D) *Mosasaurus*, E) *Enchodus*, F) *Pycnodontiformes* indet. Scale is in mm.

Supplementary tables

Table S3.1. Complete $\delta^{18}\text{O}_p$ data for this study.

Sample	Common Name	n	T (°C)	T 1 S.E.	T belemnite (°C)	T oyster (°C)	$\delta^{18}\text{O}_p$ (‰)	$\delta^{18}\text{O}_p$ S.D. (‰)	$\delta^{18}\text{O}_p$ S.E. (‰)
CA-NJ-1	<i>Cretalamna "appendiculata"</i>	3	22.7	0.4	33.9	33.5	20.05	0.28	0.09
CA-NJ-2	<i>Cretalamna "appendiculata"</i>	2	22.9	1.6	34.1	33.7	20.00	0.72	0.36
CA-NJ-3	<i>Cretalamna "appendiculata"</i>	3	19.3	0.3	30.6	30.1	20.79	0.19	0.06
CA-NJ-4	<i>Cretalamna "appendiculata"</i>	3	19.2	0.1	30.5	30.0	20.82	0.09	0.03
CA-NJ-44-25	<i>Cretalamna "appendiculata"</i>	3	19.5	0.1	30.7	30.3	20.76	0.08	0.03
CA-NJ-5	<i>Cretalamna "appendiculata"</i>	3	18.8	0.2	30.0	29.6	20.92	0.14	0.05
CA-NJ-6	<i>Cretalamna "appendiculata"</i>	3	21.1	0.5	32.3	31.9	20.40	0.34	0.11

CA-NJ-4-25	<i>Cretalamna "appendiculata"</i>	1	34.5	---	45.7	45.3	17.43	---	---
CA-NJ-5-25	<i>Cretalamna "appendiculata"</i>	2	29.2	0.5	40.5	40.0	18.60	0.20	0.10
CA-NJ-6-25	<i>Cretalamna "appendiculata"</i>	2	30.2	0.1	41.5	41.0	18.37	0.04	0.02
MS-NJ-1	<i>Mosasaurus</i>	3	25.2	0.5	36.4	36.0	19.50	0.34	0.11
MS-NJ-2	<i>Mosasaurus</i>	3	26.3	0.2	37.6	37.1	19.24	0.10	0.03
MS-NJ-3	<i>Mosasaurus</i>	2	33.4	0.7	44.7	44.2	17.66	0.31	0.16
MS-NJ-4	<i>Mosasaurus</i>	1	56.9	---	68.1	67.7	12.45	---	---
MS-NJ-5	<i>Mosasaurus</i>	3	23.1	0.2	34.4	33.9	19.95	0.12	0.04
MS-NJ-6	<i>Mosasaurus</i>	3	34.4	2.6	45.6	45.2	17.45	1.71	0.57
MS-NJ-7	<i>Mosasaurus</i>	1	64.4	---	75.6	75.2	10.79	---	---
MS-NJ-4-25	<i>Mosasaurus</i>	3	20.7	0.5	31.9	31.5	20.50	0.37	0.12
MS-NJ-6-25	<i>Mosasaurus</i>	3	17.9	0.8	29.2	28.7	21.10	0.54	0.18
MS-NJ-7-25	<i>Mosasaurus</i>	3	24.1	0.2	35.4	34.9	19.72	0.16	0.05

ST-NJ-1	<i>Scapanorhynchus texanus</i>	3	20.7	1.0	31.9	31.5	20.49	0.69	0.23
ST-NJ-2	<i>Scapanorhynchus texanus</i>	3	21.5	0.0	32.8	32.3	20.31	0.03	0.01
ST-NJ-3	<i>Scapanorhynchus texanus</i>	3	22.7	0.1	34.0	33.5	20.04	0.07	0.02
ST-NJ-4	<i>Scapanorhynchus texanus</i>	3	21.9	0.1	33.1	32.7	20.23	0.09	0.03
ST-NJ-5	<i>Scapanorhynchus texanus</i>	3	19.3	0.4	30.5	30.1	20.81	0.29	0.10
ST-NJ-6	<i>Scapanorhynchus texanus</i>	3	17.8	0.0	29.1	28.6	21.13	0.02	0.01
ST-NJ-44-25	<i>Scapanorhynchus texanus</i>	2	18.6	1.3	29.8	29.4	20.96	0.56	0.28
ST-NJ-45-25	<i>Scapanorhynchus texanus</i>	2	20.7	1.3	32.0	31.5	20.49	0.56	0.28
ST-NJ-4-25	<i>Scapanorhynchus texanus</i>	1	19.9	---	31.1	30.7	20.67	---	---
ST-NJ-5-25	<i>Scapanorhynchus texanus</i>	1	18.7	---	29.9	29.5	20.94	---	---
ST-NJ-6-25	<i>Scapanorhynchus texanus</i>	3	17.7	0.6	28.9	28.5	21.16	0.42	0.14
ES-NJ-1	<i>Enchodus</i>	3	15.7	0.4	27.0	26.5	21.59	0.25	0.08
ES-NJ-2	<i>Enchodus</i>	3	19.9	0.3	31.2	30.7	20.66	0.18	0.06

ES-NJ-3	<i>Enchodus</i>	3	20.4	0.2	31.7	31.2	20.55	0.14	0.05
ES-NJ-4	<i>Enchodus</i>	3	14.5	0.6	25.8	25.3	21.86	0.38	0.13
ES-NJ-5	<i>Enchodus</i>	3	20.2	0.1	31.5	31.0	20.60	0.04	0.01
ES-NJ-6	<i>Enchodus</i>	3	15.3	0.5	26.6	26.1	21.68	0.33	0.11
ES-NJ-4-25	<i>Enchodus</i>	2	30.2	0.3	41.4	41.0	18.38	0.13	0.06
ES-NJ-45-25	<i>Enchodus</i>	2		0.3			20.63	0.12	0.06
ES-NJ-5-25	<i>Enchodus</i>	1	20.3	---	31.5	31.1	20.58	---	---
ES-NJ-6-25	<i>Enchodus</i>	2	19.0	0.1	30.3	29.8	20.86	0.04	0.02
PI-NJ-1	Pycnodontiformes	3	43.0	1.2	54.3	53.8	15.53	0.79	0.26
PI-NJ-2	Pycnodontiformes	3	32.0	0.1	43.3	42.8	17.97	0.09	0.03
PI-NJ-3	Pycnodontiformes	3	39.7	0.8	50.9	50.5	16.27	0.53	0.18
PI-NJ-4	Pycnodontiformes	3	17.3	0.3	28.6	28.1	21.24	0.22	0.07
PI-NJ-5	Pycnodontiformes	3	15.5	0.4	26.8	26.3	21.64	0.27	0.09

PI-NJ-6	Pycnodontiformes	3	22.4	0.9	33.7	33.2	20.11	0.58	0.19
PI-NJ-4-25	Pycnodontiformes	1	19.7	---	31.0	30.5	20.71	---	---
PI-NJ-5-25	Pycnodontiformes	2	14.0	0.3	25.3	24.8	21.97	0.15	0.07
PI-NJ-6-25	Pycnodontiformes	1	19.6	---	30.8	30.4	20.74	---	---
SP-NJ-1	<i>Squalicorax pristodontus</i>	3	18.1	0.6	29.3	28.9	21.07	0.41	0.14
SP-NJ-2	<i>Squalicorax pristodontus</i>	1	15.4	---	26.6	26.2	21.67	--	--
SP-NJ-42-25	<i>Squalicorax pristodontus</i>	2	17.7	0.1	28.9	28.5	21.16	0.03	0.02
SP-NJ-3	<i>Squalicorax pristodontus</i>	3	13.1	0.5	24.4	23.9	22.17	0.33	0.11
SP-NJ-4	<i>Squalicorax pristodontus</i>	3	39.3	1.2	50.5	50.1	16.36	0.78	0.26
SP-NJ-5	<i>Squalicorax pristodontus</i>	3	42.7	0.5	53.9	53.5	15.60	0.34	0.11
SP-NJ-6	<i>Squalicorax pristodontus</i>	3	40.5	0.4	51.7	51.3	16.10	0.29	0.10
Kansas				0.0					
MS-CSKS-1	<i>Mosasaurus</i>	3	36.7	1.0	47.9	47.5	16.94	0.65	0.22

MS-CSKS-2	<i>Mosasaurus</i>	3	25.8	0.8	37.1	36.6	19.35	0.57	0.19
MS-CSKS-3	<i>Mosasaurus</i>	3	39.0	0.6	50.3	49.8	16.42	0.39	0.13
CM-CSKS-51	<i>Cretoxyrhynia</i>	3	41.1	0.4	52.4	51.9	15.95	0.24	0.08
CM-CSKS-52	<i>Cretoxyrhynia</i>	3	32.2	0.3	43.4	43.0	17.94	0.23	0.08
CM-CSKS-53	<i>Cretoxyrhynia</i>	3	36.3	0.8	47.5	47.1	17.02	0.55	0.18
CM-CSKS-61	<i>Cretoxyrhynia</i>	3	42.2	0.9	53.4	53.0	15.72	0.58	0.19
CM-CSKS-62	<i>Cretoxyrhynia</i>	1	29.3	---	40.6	40.1	18.57	---	---
CM-CSKS-63	<i>Cretoxyrhynia</i>	3	34.8	0.4	46.0	45.6	17.36	0.25	0.08
EP-CSKS-1	<i>Enchodus</i>	3	21.6	0.5	32.8	32.4	20.30	0.35	0.12
EP-CSKS-2	<i>Enchodus</i>	3	27.0	0.0	38.2	37.8	19.09	0.03	0.01
EP-CSKS-51	<i>Enchodus</i>	3	22.7	0.8	34.0	33.5	20.04	0.55	0.18
EP-CSKS-52	<i>Enchodus</i>	3	30.1	0.2	41.4	40.9	18.40	0.13	0.04
CS-CSKS-1	<i>Cretalamna sp.</i>	3	25.7	0.4	37.0	36.5	19.37	0.26	0.09

CS-CSKS-3	<i>Cretalamna sp.</i>	3	36.0	0.4	47.2	46.8	17.10	0.26	0.09
CS-CSKS-1-25	<i>Cretalamna sp.</i>	3	25.7	0.4	37.0	36.5	19.37	0.26	0.09
CS-CSKS-2-25	<i>Cretalamna sp.</i>	3	43.0	0.7	54.2	53.8	15.54	0.48	0.16
CS-CSKS-3-25	<i>Cretalamna sp.</i>	3	36.0	0.4	47.2	46.8	17.10	0.26	0.09

Table S3.2. Museum reference numbers for samples in this study.

Museum number	Catalog number	Genus and Species	Material type	Stratigraphy and locality	Epoch	Stage
n/a	BA-NJ-1	<i>Belemnitella americana</i>	rostrum	Wenonah-Mt. Laurel/Navesink Fm	Late Cretaceous	Campanian/Maastrichtian
n/a	BA-NJ-2	<i>Belemnitella americana</i>	rostrum	Wenonah-Mt. Laurel/Navesink Fm	Late Cretaceous	Campanian/Maastrichtian
n/a	BA-NJ-3	<i>Belemnitella americana</i>	rostrum	Wenonah-Mt. Laurel/Navesink Fm	Late Cretaceous	Campanian/Maastrichtian
n/a	BA-NJ-4	<i>Belemnitella americana</i>	rostrum	Wenonah-Mt. Laurel/Navesink Fm	Late Cretaceous	Campanian/Maastrichtian

n/a	BA-NJ-5	<i>Belemnitella americana</i>	rostrum	Wenonah-Mt. Laurel/Navesink Fm	Late Cretaceous	Campanian/Maastrichtian
n/a	NJ-OM-1	<i>Agerostrea mesenterica</i>	oyster shell	Wenonah-Mt. Laurel/Navesink Fm	Late Cretaceous	Campanian/Maastrichtian
n/a	NJ-PC-1	<i>Pycnodonte convexa</i>	oyster shell	Wenonah-Mt. Laurel/Navesink Fm	Late Cretaceous	Campanian/Maastrichtian
n/a	CA-NJ-E	<i>Cretalamna appendiculata</i>	enameloid	Wenonah-Mt. Laurel/Navesink Fm	Late Cretaceous	Campanian/Maastrichtian
n/a	ES-NJ-E	<i>Enchodus</i>	enameloid	Wenonah-Mt. Laurel/Navesink Fm	Late Cretaceous	Campanian/Maastrichtian
n/a	MS-NJ-E	<i>Mosasaurus</i>	enameloid	Wenonah-Mt. Laurel/Navesink Fm	Late Cretaceous	Campanian/Maastrichtian
n/a	PI-NJ-E	<i>Pycnodontid</i>	enameloid	Wenonah-Mt. Laurel/Navesink Fm	Late Cretaceous	Campanian/Maastrichtian
n/a	SP-NJ-E	<i>Squalicorax pristodontus</i>	enameloid	Wenonah-Mt. Laurel/Navesink Fm	Late Cretaceous	Campanian/Maastrichtian
n/a	ST-NJ-E	<i>Scapanorhynchus texanus</i>	enameloid	Wenonah-Mt. Laurel/Navesink Fm	Late Cretaceous	Campanian/Maastrichtian

FHSM VP-19827	CM-CSKS-E	<i>Cretoxyrhynia mantelli</i>	enameloid	Codell Sandstone	Late Cretaceous	Turonian
FHSM VP-19828	CS-CSKS-E	<i>Cretalamna sp.</i>	enameloid	Codell Sandstone	Late Cretaceous	Turonian
FHSM VP-19831	EP-CSKS-E	<i>Enchodus petrosus</i>	enameloid	Codell Sandstone	Late Cretaceous	Turonian
FHSM VP-19825	SR-CSKS-E	<i>Scapanorhynchus raphiodon</i>	enameloid	Codell Sandstone	Late Cretaceous	Turonian

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SUMMARY

This dissertation integrates clumped isotope (Δ_{47}) thermometry, triple oxygen isotopes ($\Delta^{17}\text{O}$), petrographic analysis, Raman spectroscopy, and electron backscatter diffraction (EBSD) to evaluate the preservation and paleobiological fidelity of Cretaceous fossil eggshells and to reconstruct the thermal physiology and paleoecology of Late Cretaceous marine vertebrates.

Chapter 1, a multi-proxy screening of eggshells from Mongolia and Alberta, Canada, reveals that while many specimens retain excellent microstructural preservation (particularly Mongolian oviraptorid and hadrosaur eggs), some show evidence of variable diagenetic alteration. Clumped isotope temperatures range from $\sim 21\text{--}62\text{ }^{\circ}\text{C}$, with elevated values in some well-preserved samples indicating partial reordering during burial. Carbon isotopes ($\delta^{13}\text{C}$) are the most robust proxy, indicating a predominantly C_3 vegetation-based diet across taxa, with minor positive excursions in likely carnivorous forms. Triple oxygen isotope ($\Delta^{17}\text{O}$) data help identify samples retaining primary body-water signals, but some show interaction with meteoric fluids. The best-preserved Mongolian material offers the strongest opportunity for reliable physiological inferences, highlighting the necessity of rigorous multi-proxy screening (petrography, EBSD, Raman, CL, $\Delta^{17}\text{O}$) for confident interpretation of eggshell geochemistry.

Chapter 2 uses clumped-isotope thermometry of paleosol carbonates from the Permian Bernal and Triassic Anton Chico Formations at Bernal Mesa and records maximum burial temperatures of $\sim 138\text{--}183\text{ }^{\circ}\text{C}$, corresponding to 4.1–6.7 km of Mesozoic overburden. Petrographic and elemental mapping confirm primarily solid-state reordering with limited fluid interaction. Forward modeling using disordered kinetics (Hemingway and Henkes, 2021) supports Laramide exhumation onset around 75–80 Ma. These results provide new quantitative constraints on post-depositional thermal evolution across the Permian–Triassic boundary in the

southern Western Interior and demonstrate the utility of Δ_{47} thermometry in continental red-bed successions.

Chapter 3 integrates the use of oxygen ($\delta^{18}\text{O}_p$) and clumped isotope (Δ_{47}) data from shark and mosasaur enameloid to reveal distinct thermoregulatory strategies and niche partitioning. Gigantic, fast-swimming *Cretoxyrhina mantelli* maintained elevated core body temperatures ($\sim 36\text{--}38^\circ\text{C}$), consistent with regional endothermy and high metabolic demands. In contrast, *Cretalamna*, *Squalicorax*, and many teleosts record lower, more ambient temperatures indicative of ectothermy or limited mesothermy. Mosasaurus exhibits intermediate-to-high body temperatures ($\sim 33^\circ\text{C}$), supporting gigantothermy combined with regional endothermy. When integrated with zinc isotope trophic data (McCormack et al., 2026), these results reveal coupled metabolic–trophic strategies: high-energy endothermic apex predators (*Cretoxyrhina*, advanced mosasaurs) were vulnerable to ecosystem collapse at the K–Pg boundary, while lower-metabolic, more generalist taxa (*Cretalamna*) persisted. This pattern parallels Neogene extinctions of megatooth sharks and underscores the macroevolutionary risks of gigantism and regional endothermy during climate-driven perturbations.

Overall, this dissertation advances methodological frameworks for evaluating diagenetic alteration in fossil bioapatites and carbonates while providing new insights into dinosaurian and marine vertebrate physiology, ecology, and extinction selectivity across the Late Cretaceous.

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APPENDIX I

Disentangling Greenhouse Warmth and Burial History: Clumped Isotope Thermometry of Paleosol Carbonates from the latest Cretaceous from the North Horn Formation, Utah

J. L. Knighton, W. Defliese, G. J. Retallack, R. A. Eagle, A. E. Tripathi

Abstract

Clumped-isotope (Δ_{47}) thermometry was applied to six paleosol-carbonate samples from the North Horn Formation at North Horn Mountain, Utah. Four samples preserve predominantly micritic textures and yield apparent formation temperatures of 24.9 ± 1.5 to 37.5 ± 1.4 °C (mean 32.1 °C), consistent with warm-season pedogenic carbonate formation. Two samples yield temperatures near 51 – 52 °C and contain more extensive sparry carbonate, consistent with diagenetic resetting. The available sample-level age control is insufficient to resolve an immediate temperature change across the K–Pg boundary; the results instead provide warm-season temperature constraints for the sampled interval of the formation.

Calculated water values for the four minimally altered samples range from approximately -5.6 to -2.4‰ VSMOW, and $\delta^{13}\text{C}$ values range from -8.4 to -4.5‰ VPDB. These compositions are compatible with meteoric water and C_3 -dominated soil-respired carbon, respectively, but the small sample set and uncertain stratigraphic positions limit regional climate interpretation. The results demonstrate the potential of integrated petrography and Δ_{47} thermometry for separating candidate surface-temperature signals from burial overprints.

Keywords: clumped isotopes, Δ_{47} , paleosol carbonate, North Horn Formation, K-Pg boundary, Laramide orogeny, paleotemperature,

Introduction

The North Horn Formation spans the Cretaceous–Paleogene transition in parts of Utah, although the exact boundary is difficult to locate at North Horn Mountain. This study uses six paleosol-carbonate samples to evaluate candidate Earth-surface temperatures and diagenetic resetting. Because sample-level ages and stratigraphic distances from the boundary are not yet resolved, the dataset is not used to test the climatic response to the mass extinction itself.

Clumped isotope (Δ_{47}) paleothermometry is a geochemical tool used to reconstruct formation temperatures from carbonate (CaCO_3) minerals. It relies on high-precision measurements of mass-47 CO_2 (the $^{13}\text{C}^{18}\text{O}^{16}\text{O}$ isotopologue) relative to the dominant mass-44 isotopologue ($^{12}\text{C}^{16}\text{O}_2$) using specialized gas-source mass spectrometers. Because the clumping of heavy isotopes (^{13}C and ^{18}O) within the carbonate lattice is thermodynamically favored at lower temperatures and deviates from a stochastic distribution, the measured Δ_{47} value can be converted directly to temperature, independent of the bulk $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$ composition of the precipitating fluid. The method is applicable to both biogenic and abiogenic carbonates and has proven valuable for paleoclimate reconstructions as well as assessments of biomineralization processes and physiology in extinct organisms.

Geologic Setting

The North Horn Formation is a widespread non-marine sedimentary unit exposed across central and eastern Utah, spanning an approximately 140 km (87 mi) east-west transect from the Wasatch Plateau in the west to the Book Cliffs near Green River in the east, interrupted by the San Rafael Swell (Lawton, 1986, Lawton et al., 1993). Named after its type section at North Horn Mountain in Emery County, the formation unconformably overlies the Late Cretaceous Price River Formation west of the San Rafael Swell and the Tuscher Formation to the east, while itself is overlain by the late Paleocene-early Eocene Flagstaff Formation (also called the Flagstaff Limestone) (Spieker, 1946; Fouch et al., 1983; Lawton et al., 1993). Its sediments range in age from Late Cretaceous (Maastrichtian, ~71 Ma) to early Paleocene (Danian, ~66–64 Ma), with some extensions into the early Eocene in related sections, making it one of the few continental sequences in North America that preserves the Cretaceous–Paleogene (K-Pg) boundary (Fouch et al., 1983; Cifelli et al., 1999; Difley, 2007). The formation is time-transgressive, with a Maastrichtian base in western sections (such as near Price Canyon) and a Paleocene base in eastern areas, reflecting variable depositional onset across the region (Fouch et al., 1983; Lawton et al., 1993). Thickness varies dramatically, exceeding 1,100 m (3,600 ft) in complete western sections like North Horn Mountain, but thinning to 15–40 m (50–130 ft) eastward near Green River due to differential subsidence and erosion (Lawton et al., 1993; Difley, 2007).

Tectonic Setting

The North Horn Formation was deposited in a proximal foreland basin at the eastern front of the Sevier orogenic belt during the transition from thin-skinned to thick-skinned deformation associated with the Laramide orogeny (Lawton et al., 1993). West-vergent thrust faults, active from the late Campanian (~75 Ma) through early Eocene (~50 Ma), controlled

sedimentation patterns, creating additional basins on thrust hanging walls and influencing clastic dispersal (Lawton et al., 1993). Pre-depositional thrusting resulted in folding and overturning of older Jurassic-Cretaceous strata (e.g., Indianola Group), forming angular unconformities at the base (Lawton et al., 1993). Syndepositional deformation is evident in progressive unconformities, thickness variations, and paleocurrent shifts due to early fluvial systems from eastward to westward/northwestward (later, off thrust uplifts) (Lawton et al., 1993). The San Rafael Swell and Sevier highlands restricted drainage to an interior system, with the Gunnison thrust system in the San Pitch Mountains exemplifying intermittent uplift that sourced clasts from Precambrian quartzites, Paleozoic carbonates, and Mesozoic sandstones (Lawton et al., 1993). Deposition shifted westward over time, with late Paleocene beds onlapping deformed Mesozoic rocks (Lawton et al., 1993).

Depositional Environments and History

Sedimentation occurred in terrestrial, non-marine settings dominated by fluvial, lacustrine, floodplain, and alluvial fan systems within a high-energy environment (Lawton et al., 1993; Fouch et al., 1983). Early, late Campanian- to Maastrichtian-aged deposition began along the eastern Wasatch Plateau, featuring basal conglomerates filling incised valleys from braided streams and alluvial fans, with debris flows in redbed units (Lawton et al., 1993). As thrusting continued, environments evolved to meandering fluvial channels and overbank floodplains (sheet sandstone units), marshy bogs with coal accumulation (coal-bearing units), and braided rivers with dunes and bars (Big Mountain unit) (Lawton et al., 1993). Lacustrine deltas prograded westward in the Coal Canyon unit, while shallow lakes and lake margins dominated the calcareous siltstone unit, with open-lacustrine conditions in the intercalated Wales Tongue of the Flagstaff Limestone (dolomitic micrite, claystone, oncolites) (Lawton et al., 1993). Upper redbed units represent subaerial plains with channels and ponds (Lawton et al., 1993). Overall,

depositional patterns reflect synorogenic conditions, with rapid sedimentation fluctuations, bioturbation in distal braid plains, and clast mixing from diverse sources (Lawton et al., 1993).

Stratigraphy and Lithology

The formation is informally divided into three main units: the lower variegated (basal) unit, middle coal (with K-Pg boundary), and upper variegated unit (Fouch et al., 1983; Difley, 2007). These are further subdivided into eight lithofacies: (1) basal conglomerate (~100 m thick, upward-fining conglomerates and sandstones); (2) lower redbed (~138–240 m, red siltstone with conglomeratic lenses); (3) sheet sandstone (interbedded sandstones and carbonaceous siltstones); (4) coal-bearing (40–114 m, coal beds and micrite); (5) Big Mountain (~230 m, conglomerates and trough cross-bedded sandstones); (6) Coal Canyon (~60 m, foreset-bedded conglomerates and sandstones); (7) calcareous siltstone (~254 m, bioturbated siltstone with sandstone beds); and (8) upper redbed (10–120 m, mottled siltstone) (Lawton et al., 1993). Dominant lithologies include sandstone, shale, conglomerate, siltstone, limestone, and coal, with intraformational unconformities indicating episodic uplift (Lawton et al., 1993; Fouch et al., 1983).

Paleontology and the K-Pg Boundary

The North Horn records a major faunal turnover at the K-Pg boundary (~66 Ma), located in the middle coal unit in thicker sections, marked by a shift from dinosaur-dominated Maastrichtian assemblages to mammal-rich Paleocene faunas (Cifelli et al., 1999; Difley, 2007). Maastrichtian strata yield squamates (e.g., *Polyglyphanodon sternbergi*), turtles (*Adocus*, *Basilemys*), crocodyliforms (*Pinacosuchus mantiensis*), fishes, amphibians, early mammals (*Alphadon*, *Cimolomys*), dinosaurs (*Torosaurus utahensis*, *Utetitan zellaguymondeweyae*, *Tyrannosaurus rex*, hadrosaurids), eggshells, tracks (*Pteraichnus*), mollusks, plants, and palynomorphs (Gilmore, 1946; Sampson and Loewen, 2005; Difley, 2007). Paleocene sections

feature over 70 taxa, including frogs, multituberculates, periptychids, phenacodontids, crocodyliforms, choristoderes, palynomorphs, and marsupials (*Alphadon*-like) (Cifelli et al., 1999; Difley, 2007). The boundary lacks a clear iridium layer but is identified using biostratigraphic evidence, namely the faunal shift, palynology, and charophytes, as well as environmental changes from semi-arid to wetter conditions, with no major stratigraphic gap apparent (Difley and Ekdale, 2002; Cifelli et al., 1999). However, there remains some controversy in the literature on whether the type section contains the K-Pg boundary.

Post-Depositional and Burial History

Post-deposition, the formation experienced continued Laramide deformation, with angular unconformities beneath the overlying Flagstaff Limestone due to pre-Flagstaff uplifts (Lawton et al., 1993). Burial histories vary regionally, with deeper subsidence in western basins preserving thicker sections and the K-Pg boundary, while eastern areas show erosion and thinner strata (Lawton et al., 1993; Fouch et al., 1983). Magnetostratigraphy reveals hiatuses and normal/reversed polarity zones, indicating episodic exhumation (Lawton et al., 1993). This history underscores the formation's role in understanding terrestrial responses to the K-Pg extinction and Cordilleran tectonics (Cifelli et al., 1999; Difley, 2007).

Methods

Sampling

Samples were collected by Dr. G. J. Retallack in early November of 2004 from North Horn Mountain, Utah. Samples were subsequently sectioned in paired polished thin and thick sections. Paleosols were drilled from thick sections using a dremel and treated with dilute H₂O₂ to remove any organic matter prior to analysis. Sparite veins, where evident in the hand sample, were selectively drilled in two samples (RETR4654-NHK, RETR4658-NHK). Micrite was also re-sampled at the same time, with care taken to exclude any sparite visible in the hand sample. A

dental drill with a microdrill bit was used under a microscope to identify the sparite and micrite areas. Samples were then drilled under the microscope and analyzed on the mass spectrometer.

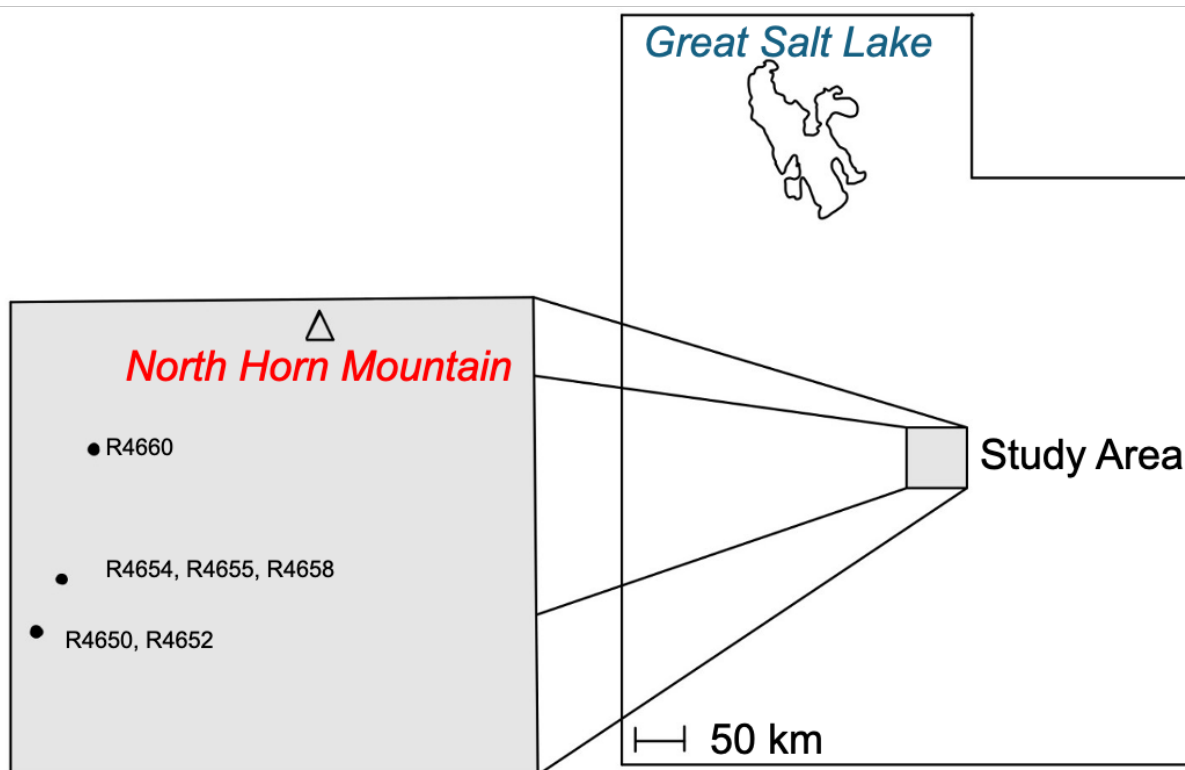


Figure 1. North Horn Mountain Study area and collection sites for paleosols. This map shows paleosol specimens were collected for study. Full latitude and longitude data available in Table S1.

Analysis

All measurements clumped isotope (Δ_{47}) analyses were performed in the Tripathi-Eagle IsoLab using a Thermo Finnegan MAT 253 gas source mass spectrometer and a Nu Perspective IS mass spectrometer. Samples were digested at 90 °C in a common phosphoric

acid bath. CO₂ gas was then purified by a cryogenic trap containing liquid nitrogen, which removes water and other gases with low vapor pressures. The CO₂ gas was then purified using an adsorption trap which passes the sample gas through a Porapak Type-QTM 50/80 mesh GC column at -20 °C to remove potential organic contaminants and silver wool (Sigma-Aldrich) to remove sulfur compounds. Stable carbon ($\delta^{13}\text{C}$), oxygen ($\delta^{18}\text{O}$), and carbonate clumped (Δ_{47}) isotope ratios of the samples were measured using methods previously described (**Defliese and Tripathi, 2020, Upadhyay et al., 2021, Lucarelli et al., 2023**). Carbonate standards were analyzed every 3-5 analyses. The average external reproducibility (one SE) of Δ_{47} measurements presented here is . Standards of ETH-1/2/3/4 as well as in-house standards were run with each sample to establish reproducibility and calculate empirical transfer function (ETF). Table S1 contains standard slope and intercept values, as well as secondary transfer function slope and intercepts.

Modeling for Maximum Burial Temperature (MBT)

For samples demonstrating evidence of thermal alteration, kinetic models from Passey and Henkes (2012) were used to simulate reordering based on measured Δ_{47} , using assumed initial formation temperatures compiled from literature, and estimated burial duration from stratigraphic and geochronologic data, also from existing published data. These models then are used to back-calculate the maximum burial temperature (MBT) by iterating thermal histories that match the observed $T_{\Delta_{47}}$, accounting for heating during burial and partial re-equilibration during exhumation. Measured Δ_{47} values were compared against the geothermal gradient for continental lithosphere, yielding an estimated burial depth for samples.

Results

Petrography

Each polished section was examined using plane polarized and cross-polarized light petrography to pre-assess degree of diagenetic alteration, and to assess whether Δ_{47} -derived temperatures were likely reflective of Earth surface temperatures or reflective of thermal resetting.

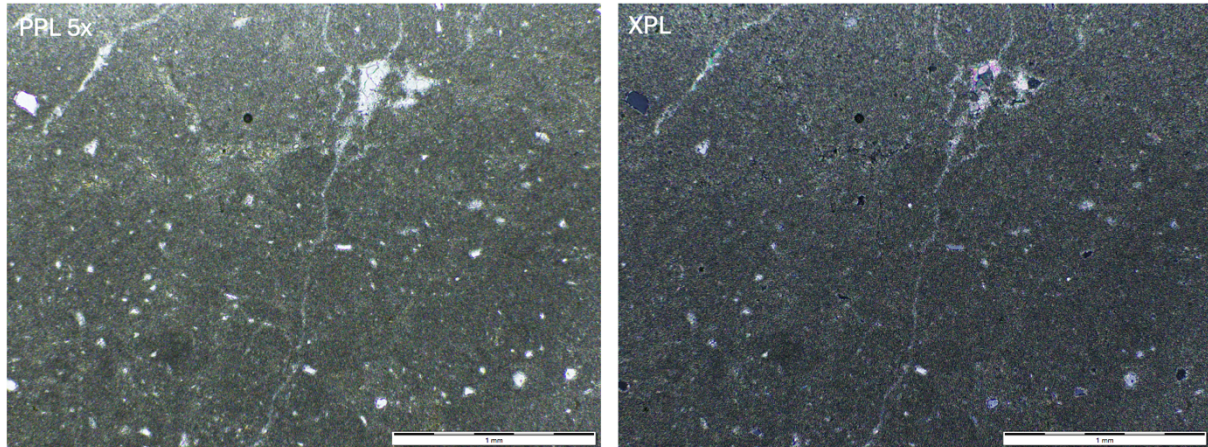


Figure 3. Plane polarized and cross polarized petrographic images of R4650 (26.2 ± 2.5 °C). Voids appear to be infilled with sparite, and some coarsening can be seen in the matrix. However, the majority of the sample appears to be intact. Photomicrographs taken at 5x magnification.

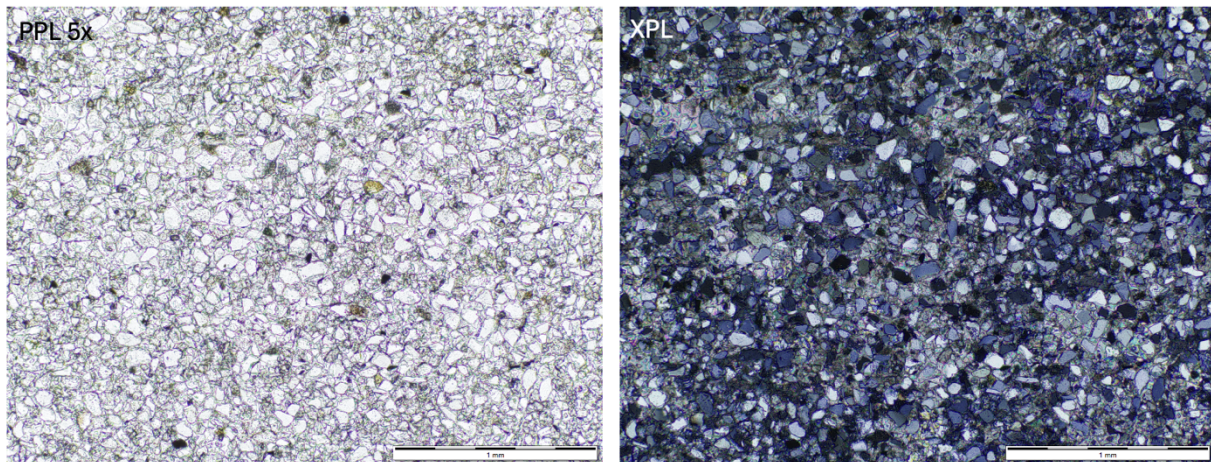


Figure 4. Plane polarized and cross polarized petrographic images of R4652 ($52.3 \pm 4.7^\circ\text{C}$). Significant coarsening, clear sparry characteristics, no micritic matrix remaining, consistent with elevated temperatures observed. Photomicrographs taken at 5x magnification.

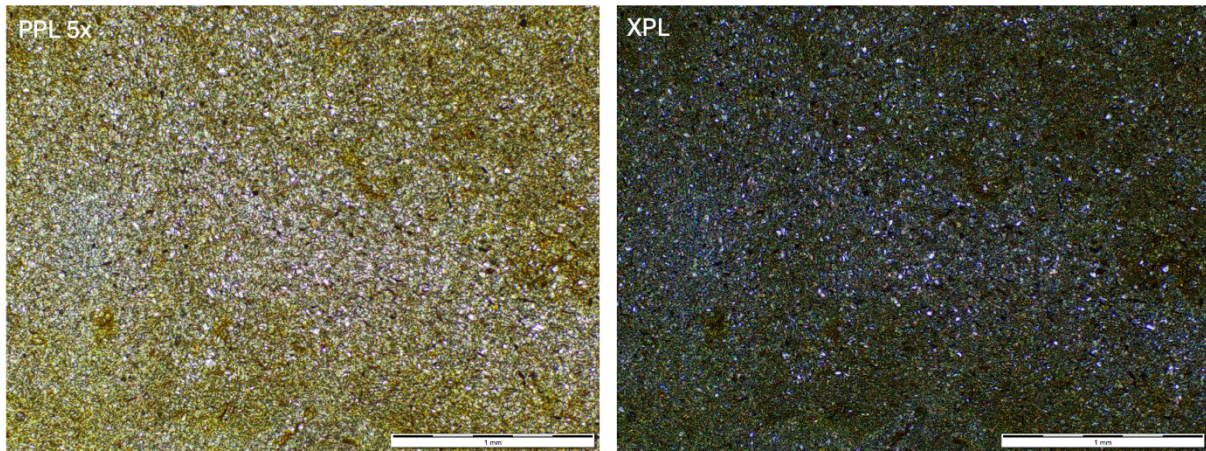


Figure 5. Plane polarized light and cross polarized light images of R4654 ($35.0 \pm 2.4^\circ\text{C}$).

Some degree of coarsening can be seen throughout the specimen, with sparite grains luminescing under crossed polars. Matrix remains overall micritic. Photomicrographs taken at 5x magnification.

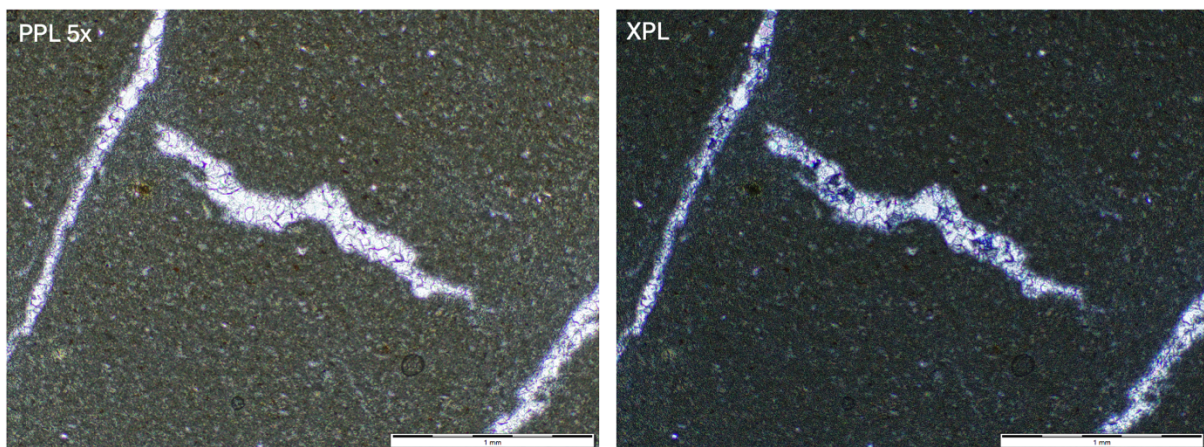


Figure 6. Plane polarized and cross polarized petrographic images of R4655 ($37.5 \pm 1.4^\circ\text{C}$). Sparite veins can be observed within the micritic groundmass. Photomicrographs taken at 5x magnification.

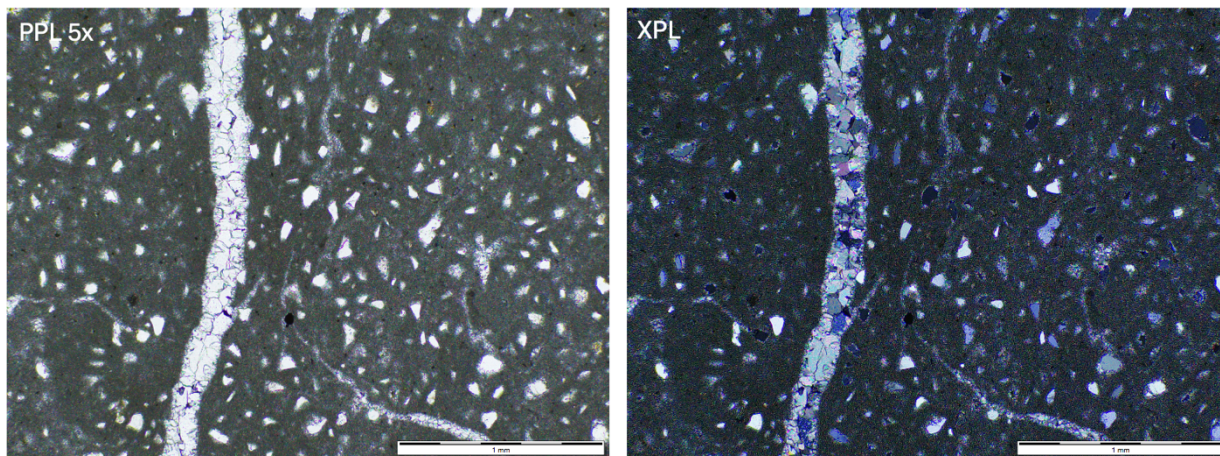


Figure 7. Plane polarized and cross polarized petrographic images of R4658 (24.9 ± 1.5 °C). Sparite veins of various sizes can be seen throughout the section, as well as angular, coarsened sparry grains with evidence of dissolution around edges, however micrite is between sparry grains. Photomicrographs taken at 5x magnification.

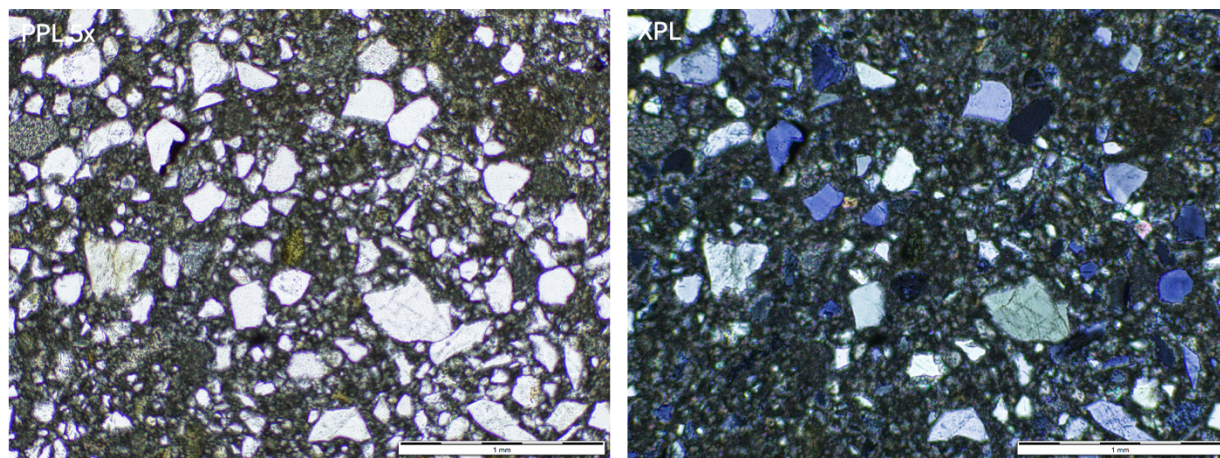


Figure 8. Plane polarized and cross polarized petrographic images of R4660 (51.2 ± 2.0 °C). The polished section features large sparite crystals with characteristic cross-hatching patterns with sub-angular grains in a coarsened groundmass. Groundmass shows evidence of dissolution as well. Photomicrographs taken at 5x magnification.

Clumped Isotope Paleothermometry

Four of the six samples preserve micritic textures and yield Δ_{47} temperatures between 24.9 ± 1.5 and 37.5 ± 1.4 °C (mean 32.1 °C), consistent with primary warm-season pedogenic carbonate formation (O'Connor, 2019). One sample (RETR4652-NHK) shows elevated temperatures (52.3 ± 4.7 °C) and fully sparry fabric indicative of diagenetic resetting. Sparite veins were selectively drilled in two samples (RETR4654-NHK, RETR4658-NHK). Micrite was also re-sampled during these drilling sessions, with care taken to exclude any sparite visible in the hand sample.

Catalog number	Material type	Stratigraphy and locality	Age	n	$\delta^{13}\text{C}$ VPD B	1 s.d .	$\delta^{18}\text{O}$ VPD B	1 s.d .	Δ_{47}	1 s.e.	T Δ_{47} [°C] **	1 s.e.	$\delta^{18}\text{O}$ water VSMOW	1 s.d.
RETR4650-NHK	carbonate paleosol	North Horn Mountain, Utah	Cretaceous	4	- 8.10	0.1 4	- 6.77	0.1 6	0.59 1	0.00 7	26.2	2. 5	-4.2	0.5
RETR4652-NHK	carbonate paleosol	North Horn Mountain, Utah	Cretaceous	5	- 8.21	0.1 3	- 12.4 6	0.2 3	0.52 4	0.01 1	52.3	4. 7	-5.1	0.7
RETR4654-NHK	carbonate paleosol	North Horn Mountain, Utah	Cretaceous	6	- 7.95	0.0 5	- 7.32	0.2 0	0.56 6	0.00 6	35.0	2. 4	-3.0	0.4

RETR4654MI C-NHK	carbon	North	Cretaceous	3	-	0.0	-	0.0	0.56	0.00	34.8	0.7	-3.3	0.1
	ate	Horn												
	paleoso	Mountain,												
RETR4654SP AR-NHK	I	Utah	Cretaceous	5	-	0.5	-	0.2	0.56	0.00	35.5	3.3	-3.2	0.7
	carbon	North												
	ate	Horn												
RETR4655- NHK	paleoso	Mountain,	Cretaceous	8	-	0.2	-	0.1	0.55	0.00	37.5	1.4	-2.4	0.2
	I	Utah												
	carbon	North												
RETR4658- NHK	ate	Horn	Cretaceous	7	-	0.0	-	0.2	0.59	0.00	24.9	1.5	-5.6	0.3
	paleoso	Mountain,												
	I	Utah												
RETR4660- NHK	carbon	North	Cretaceous	3	-	0.4	-	0.7	0.52	0.00	51.2	2.0	-5.3	0.6
	ate	Horn												
	paleoso	Mountain,												
	I	Utah												

Table 1. Summary of results from clumped isotope analyses.

Discussion

The four minimally altered samples record warm-season soil carbonate formation temperatures of 24.9–37.5 °C. Because pedogenic carbonates in semi-arid to monsoonal settings precipitate predominantly during warm, dry periods (Breecker et al., 2009; Ringham et al., 2016), these values represent warmest-month or warmest-quarter soil temperatures. After a conservative +0–3 °C correction for radiative soil heating (Burgener et al., 2019), implied air temperatures are ~25–35 °C, which is consistent with independent Campanian reconstructions from micritic paleosol nodules in the Kaiparowits Formation of southern Utah (30–35 ± 4 °C in the warmest month; Burgener et al., 2019) and Campanian fluvial/pond waters along the western margin of the Western Interior Seaway (mean 30 ± 2.7 °C; Curley et al., 2025).

The four candidate primary samples indicate warm-season soil temperatures over the sampled interval. Without sample-level age control that places measurements immediately below and above the K–Pg boundary, the dataset cannot establish whether cooling occurred across the boundary. The observed range may reflect microhabitat, seasonality, stratigraphic age, or residual alteration.

Calculated $\delta^{18}\text{O}_{\text{water}}$ values for the four candidate primary samples range from -5.64 ± 0.32 to $-2.39 \pm 0.24\text{‰}$ VSMOW. These values are compatible with meteoric waters in a continental interior and may include effects of orographic rainout from western highlands. The $\delta^{13}\text{C}$ values (-8.4 to -4.5‰ VPDB) are compatible with pedogenic carbonate influenced by C_3 soil respiration, although quantitative vegetation or precipitation reconstructions would require additional constraints.

Collectively, these data demonstrate that clumped-isotope thermometry in carefully screened paleosol carbonates can recover primary Earth-surface temperatures even in

tectonically active foreland basins, while also recording burial overprints useful for reconstructing exhumation history.

Conclusion

Clumped-isotope (Δ_{47}) thermometry of paleosol carbonates from the North Horn Formation yields primary warm-season formation temperatures of 24.9–37.5 °C for four of six samples, confirming hot mid-latitude summers during the latest Cretaceous greenhouse and providing a terrestrial counterpart to marine TEX₈₆ and foraminiferal records. Two samples record diagenetic resetting, highlighting the importance of petrographic screening. Ongoing kinetic modeling will constrain maximum burial temperatures and exhumation timing, contributing to understanding Laramide basin dynamics and the environmental context of the K-Pg boundary in western North America. This study underscores the power of clumped (Δ_{47}) and petrographic approaches for disentangling surface climate signals from burial histories in continental successions.

Future work

This work would benefit from trace elemental analysis to fully assess diagenetic influence, as well as search for possible retained ecological and climate signals.

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APPENDIX II

Dual Clumped Isotope Thermometry of Enantiornithes Eggshells Indicates High Body Temperatures with Equilibrium Mineralization Patterns Similar to Modern Reptiles

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Abstract

Enantiornithes are a diverse clade of Cretaceous stem birds that occupied an important phylogenetic position between Archaeopteryx and crown-group Aves (Neornithes). Their thermophysiology remains debated, with evidence suggesting elevated metabolic rates comparable to modern arboreal birds. Here, we apply dual clumped isotope thermometry (Δ_{47} and Δ_{48}), alongside trace element ratios (Sr/Ca, Mn/Ca, Fe/Ca, Mg/Ca, Ba/Ca) and preservation assessments (cathodoluminescence and electron backscatter diffraction), to four partial Enantiornithes eggshells from the Cretaceous Neuquén Basin, Patagonia.

Cathodoluminescence and EBSD indicate variable preservation: two specimens (MACN-PV-94a and MACN-PV-94a-3) show minimal recrystallization and intact prismatic layers, while others exhibit substantial alteration. Trace elements reveal low heavy metal concentrations but elevated Mn in some samples, consistent with partial diagenetic influence.

Δ_{47} -derived temperatures for best-preserved specimens average 30.1–33.8°C (± 1.1 –2.2°C), with one altered sample at 39.5 $\pm$ 3.2°C. These fall within ranges for modern endotherms. Dual Δ_{47} - Δ_{48} data show well-preserved specimens plot near equilibrium (similar to modern reptiles), while altered ones deviate (bird-like disequilibrium). This suggests Enantiornithes achieved high body temperatures akin to endothermic relatives but mineralized eggshells under primarily temperature-controlled (equilibrium) conditions, unlike the kinetically influenced process in crown birds.

These results support heterothermic endothermy as a plesiomorphic trait in Aves, with torpor-like flexibility likely ancestral. High metabolic capability, combined with precocial development and dietary specialization, may have influenced Enantiornithes' ecological success and eventual extinction at the K-Pg boundary.

Keywords: Enantiornithes, basal birds, clumped isotope thermometry, Cretaceous avians, dual clumped isotopes, eggshell mineralization, fossil eggshells, heterothermic endothermy, Mesozoic avians, Neuquén Basin, paleophysiology, thermoregulation

Introduction

Enantiornithes is an extinct stem bird within the Aves class to the extant Neornithes clade that constitutes modern birds (O'Connor, 2009). Enantiornithes' phylogenetic position as an intermediate between *Archaeopteryx* and modern birds and their success during the Mesozoic as demonstrated by their remarkable diversity and long taxonomic range (Early Cretaceous through end Cretaceous) make this group important for understanding the early evolution of birds and the relationship between the Aves and Dinosauria (O'Connor, 2009; 2022; O'Connor and Chiappe, 2011). While Enantiornithes has no living descendants, it forms the sister group to Ornithuromorpha, the clade which gives rise to crown birds (Chiappe and Cyril, 2002; O'Connor, 2009; O'Connor and Chiappe, 2010; Zelenkov, 2017).

Fossil evidence suggests precocious development in Enantiornithines relative to Ornithuromorphines (O'Connor, 2009; O'Connor, 2022). Ecological diversity likely expanded by the Late Cretaceous however, with secondarily flightless Enantiornithines such as *Elsornis*. Smaller enantiornithines are thought to have used intermittent flapping styles in flight, such as bounding and flap-gliding (O'Connor, 2022). Larger, Late Cretaceous forms lack enough fossil material for aerodynamic analyses, however more derived flight-related structures such as enlarged sternal keels could have facilitated more diverse flight styles near the End-Cretaceous (O'Connor, 2009).

The reproductive system of Enantiornithines is similar to that of modern birds, with a single functional ovary and oviduct. Additionally, Enantiornithines demonstrate rapid bone deposition early in life, another feature they share with modern birds (O'Connor, 2022). One

major difference is that Enantiornithines were highly precocial, reaching sexual maturity before skeletal maturity and having sufficient skeletal ossification for flight soon after hatching (O'Connor, 2022).

The enantiornithines possessed a wide variety of skull shapes and dentitions, implying that species had different dietary specializations (O'Connor and Chiappe, 2010). Some species are thought to have been piscivorous due to their long recurved claws, similar to modern-day piscivorous raptors (Wang et al., 2014). Some enantiornithines had large, robust jaws suitable for eating hard shell invertebrates, while others had small rounded teeth suggestive of insectivory (Miller et al., 2022; O'Connor, 2022). Unfortunately, specimens with stomach contents lack skulls to correlate with dentition. The only stomach contents found suggest that enantiornithines ate aquatic crustaceans, and gymnosperm seeds have been found in enantiornithine digestive tracts (O'Connor et al., 2022; O'Connor et al., 2024; Sanz et al., 1996). The lack of fossil evidence of gastroliths and therefore a gastric mill, suggests that Enantiornithines had a more specialized diet than modern birds, possibly soft foods (O'Connor, 2022; O'Connor and Chiappe, 2010). It is postulated that high degrees of specialization in diet may have contributed to their extinction as a response to habitat loss during the Late Cretaceous (O'Connor, 2022).

Fortunately, there exists fossil evidence from various ontogenetic stages in enantiornithines, including eggs, embryos, hatchlings, and juveniles (Elżanowski, 1981; 1995; Mikhailov, 1991; Sanz et al., 1997; Zhou et al., 2004; Kurochkin et al., 2013; O'Connor et al., 2024). This wealth of fossil evidence of early life stages allows for comparison of growth patterns to modern Aves. Enantiornithines appear to demonstrate precocial to superprecocial development when compared to modern birds with longer incubation times (Elżanowski, 1995; Kurochkin et al., 2013; O'Connor, 2014; O'Connor, 2022). Studies of bone histology show that on average, enantiornithines had a rapid growth rate in early stages, but that growth slowed as

adult sizes were approached (Cambra-Moo, 2006). This is consistent with observations in living birds that have precocial development patterns (O'Connor, 2014).

Although there is debate in the literature regarding the metabolic strategy of the Enantiornithines, it has been determined that their resting metabolic rate (RMR) is significantly higher than the RMR of extant ectotherms and is comparable to that of modern arboreal birds of similar size (Cubo et al., 2022). When considered with what is known about the physiology, diet, and ontogeny of Enantiornithines and that patterns similar to modern arboreal bird species are observed in these categories as well, it seems reasonable to rule out ectothermy as a possible metabolic strategy and instead focus on meso- and endothermy as likely metabolic modalities. It must be mentioned, however, that due to the sparsity of material, there is still debate about most of the enantiornithines' life strategies. To directly constrain body temperatures and infer thermophysiological strategies in extinct vertebrates, clumped isotope paleothermometry (Δ_{47}) provides a powerful proxy. This technique measures the abundance of rare ^{13}C - ^{18}O bonds ("clumps") in carbonate minerals, which form in thermodynamic equilibrium with temperature during mineralization (Eiler, 2011). In biogenic carbonates such as teeth or eggshells, Δ_{47} -derived temperatures reflect the in vivo body temperature of the parent organism, independent of environmental water $\delta^{18}\text{O}$ (Eagle et al., 2015). Applied to fossil eggshells, which mineralize internally in the oviduct, this approach has successfully reconstructed thermoregulation in non-avian dinosaurs and early birds, revealing elevated body temperatures comparable to modern endotherms in some taxa (Eagle et al., 2015; Dawson et al., 2020). Recent advances in dual clumped isotope analysis (combining Δ_{47} and Δ_{48}) further distinguish equilibrium vs. kinetic effects during eggshell formation, enabling insights into mineralization processes and heterothermy (Tagliavento et al., 2023). For instance, dual-clumped data on Troodon eggshells indicated high, endotherm-like body temperatures alongside reptile-like equilibrium mineralization, suggesting flexible thermoregulation ancestral to birds (Tagliavento et al., 2023).

Here, we apply this dual Δ_{47} - Δ_{48} approach, alongside trace element and preservation assessments, to *Enantiornithes* eggshells from the Neuquén Basin to test for heterothermic endothermy and characterize eggshell mineralization in this pivotal stem-bird clade.

Heterothermy and torpor are noted in the evolutionary history of modern birds to as far back as the Paraves, however it is still unclear which groups retained this feature as a plesiomorphy and which groups evolved high metabolism later (Chinsamy, et al., 2016; Lovegrove, 2016).

The Neuquén Basin is the geologic setting for the fossil taxon. As a whole this location represents a nearly complete geologic time series spanning the Early Mesozoic to the Early Cenozoic, recording the tectonic evolution of the Andes and the formation of the Basin itself. The thick infill of the Basin includes a remarkably complete Jurassic-Early Cretaceous marine fossil succession, as well as ample terrestrial vertebrates (Aguirre-Uretta, 2024). The richness of sedimentary deposits capturing different environments over time interspersed with volcanic layers to provide absolute dates allows the Neuquén Basin to provide a unique opportunity for paleobiologists. The fossil specimens are from the Cretaceous Period of the Mesozoic, roughly ~136 Ma-66 Ma.

Isotopic proxies such as clumped isotope thermometry as applied to teeth and eggshell fossils can place quantitative constraints on body temperatures of extinct species. Constraining body temperatures via this method relies on measuring the proportions of multiply-substituted (^{13}C - ^{18}O bearing; ‘clumped’) carbonate in eggshells (Eagle et al., 2015). Since proportions of clumping in carbonates is thermodynamically defined, temperatures can be derived from the measured proportions in the mineral (Eiler, 2011). The proportion of measured mass-47 (^{13}C , ^{18}O , ^{16}O) clumped molecules in excess of the stochastic distribution is then expressed as Δ_{47} (Eiler, 2011). Furthermore, as eggshells mineralize in the lower oviduct of Aves, this formation temperature should reflect the *in vivo* body temperature of the animal (Eagle et al., 2015).

Thermophysiology of extinct vertebrates has been studied in fossil eggshells using clumped isotope paleothermometry (Eagle et al., 2015; Dawson et al., 2020). Due to the fact that eggshells mineralize in the oviduct in egg-laying species, the isotopic ordering reflected in CaCO_3 in eggshells should be reflective of core body temperature (Eagle et al., 2015). Previous studies have employed Δ_{47} signatures in CO_2 gases liberated from carbonates as a proxy for body temperature of the host animal to successfully approximate the thermoregulatory strategies in members of clade Dinosauria (Eagle et al., 2015; Dawson et al., 2020).

Recent studies have demonstrated the ability to approximate body temperatures in dinosaurs and make inferences about eggshell mineralization using eggshell fossils using dual-clumped isotope methods (Tagliavento et al., 2023). Dual-clumped isotope analysis involves measuring both Δ_{47} and Δ_{48} components in carbonate minerals (Lucarelli et al., 2023). Δ_{48} is found by measuring the proportion of measured mass-48 (^{12}C , ^{18}O , ^{18}O) clumped molecules in excess of the stochastic distribution (Lucarelli et al., 2023). Clumping for Δ_{48} occurs due to a mass-dependent relationship between ^{18}O and ^{17}O (Lucarelli et al., 2023, Daëron et al., 2016). Combining Δ_{47} data with Δ_{48} data allows for study of kinetic effects as well as refine temperature data, due to the characteristic relationship the proportions have to disequilibrium (Tripathi et al., 2015; Fiebig et al., 2019; Guo, 2020; Bajnai et al., 2020; Lucarelli et al., 2023). A recent study by Tagliavento, et al. found that *Troödon* was able to achieve body temperatures comparable to modern endotherms, but also employed a heterothermic strategy like extant birds (2023). Dual-clumped data is also able to give insight on the mineralization of the eggshell during formation (Tagliavento et al., 2023). Tagliavento et al. found that the mineralization of *Troödon* eggshells was more similar to reptile eggshells than birds, meaning that their reproductive structure likely reflected this with two functional ovaries that produced a limited number of eggs in each clutch (2023). Such information is invaluable in the study of extinct

species, especially those that lie at evolutionary transitions between major clades, such as basal birds like the *Enantiornithines*.

Here, we utilize clumped isotope proxies on 4 fossil specimens of *Enantiornithes* using Δ_{47} and Δ_{48} clumped isotope analysis, as well as multi-geochemical tracers including Sr/Ca, Fe/Ca, Mg/Ca, Mn/Ca, and Ba/Ca to characterize the conditions under which the eggshells mineralized, including paleotemperature and metabolic conditions.

Results

Cathodoluminescence (CL) of specimens

CL microimaging reveals that MACN-PV-94a as well as MACN-PV-94a-3 show very low amounts of recrystallization. MACN-PV-94a-2 as well as MACN-PV-94a-4 show high amounts of recrystallization in both the eggshell itself and the groundmass surrounding the fossil.

Electron Backscatter Diffraction (EBSD) Data

EBSD data was collected to assess the preservation of the fossil specimens. Data was only able to be collected from 3 of the 4 eggshell samples. The fourth eggshell, MACN-PV-94a-4, was not analyzed. MACN-PV-94a-3 was the best preserved sample. Two separate regions of the specimen were analyzed and each showed similar degrees of preservation. The prismatic layer is intact, however the mammillary layer is not observed. MACN-PV-94a-2 has undergone substantial dissolution and recrystallization. The original shell microstructure and textures are not discernable. MACN-PV-94a-1 has some distinguishable microstructure and texture, but also appears to have undergone dissolution and recrystallization.

Trace element data

MACN-PV-94-a-1, MACN-PV-94-a-2, and MACN-PV-94-a-4 were tested for trace element ratios of Sr, Mn, Fe, Mg, and Ba/Ca. MACN-PV-94-a-2 was additionally tested for

concentrations of Li, B, Cd, and U/Ca. MACN-PV-94-a-1 and MACN-PV-94-a-4 were not included because there was not an adequate amount of sample for the additional trace metals. Table X shows the results of these analyses.

Heavy metal concentrations of Sr, Fe, Mg, and Ba are all relatively low in samples MACN-PV-94-a-1, at 0.233, 1, 7.4, and 16.5 $\mu\text{mol/mol}$ respectively, MACN-PV-94-a-2, at 0.252, 1, 4.0, and 41.4 $\mu\text{mol/mol}$, with slight elevations in Fe and Mg observed in MACN-PV-94-a-4, at 10 and 13.5 $\mu\text{mol/mol}$ respectively, compared to MACN-PV-94-a-1 and MACN-PV-94-a-2—however the overall levels of these elements are still overall low, and Fe levels are consistent with background levels (Dawson et al., 2020). All three samples demonstrate elevated levels of Mn concentrations, with MACN-PV-94-a-2 showing the highest level at 3509.55 $\mu\text{mol/mol}$.

Single isotope and clumped temperature data

Comparison of Δ_{47} signatures in modern eggshells versus fossil specimens has been used in previous studies to infer thermoregulatory strategies in extinct species (Eagle et al., 2015; Dawson, 2020). Here, we compared Enantiornithine fossil eggshells with modern endotherms and ectotherms. It was found that MACN-PV-94-a-1, MACN-PV-94-a-2, and MACN-PV-94-a-3 have an average Δ_{47} -temperature 33.8 ± 1.9 °C, 30.1 ± 2.2 °C, and 31.1 ± 1.1 °C respectively. MACN-PV-94-a-4 demonstrated a temperature of 39.5 ± 3.2 °C. Each average is based on at least seven replicates, with MACN-PV-94-a-1 having an additional eighth replicate. Δ_{47} measurements for the Enantiornithes eggshells yielded 1 S.E. values of 0.003–0.015‰ (average 0.007‰ across the four specimens), corresponding to temperature uncertainties of 1.28–6.83 °C (average ~ 3.1 °C). Best-preserved samples showed the lowest uncertainties (e.g., 0.003‰ for Δ_{47} and 1.28 °C for temperature in MACN-PV-94a-3).

A one-way ANOVA performed on the three groups found a statistically significant difference between the three populations (modern bird, modern reptile, and fossil) with a p value of $p = 0.037 < 0.05$.

A Tukey test to look at how each pair differs found no significant difference between the fossil population and bird population, or the fossil population and the reptile population. A summary of the results for both the ANOVA and the Tukey tests is presented in Table 3.

Dual clumped data (modern and fossil bird)

Modern reptiles and Enantiornithine specimens MACN-PV-94-a and MACN-PV-94-a-3 plot closely to the Δ_{47} - Δ_{48} equilibrium line. Modern bird species and MACN-PV-94-a-2 and MACN-PV-94-a-4 plot off the Δ_{47} - Δ_{48} equilibrium line (Figure 2).

Discussion

Controls on eggshell mineralization in modern species.

Figure 1 shows that MACN-PV-94-a, MACN-PV-94-a-2, and MACN-PV-94-a-3, the specimens showing intermediate and best preservations respectively appear to have mineralized in Δ_{47} - Δ_{48} equilibrium and more closely resemble values of modern reptiles in the dataset figure 1. Modern reptile eggshells typically mineralize in isotopic equilibrium, reflecting ambient or maternal temperatures without strong kinetic fractionation. In contrast, bird eggshells often show disequilibrium in Δ_{47} - Δ_{48} space due to rapid, physiologically driven calcification in the oviduct. Well-preserved Enantiornithes specimens (MACN-PV-94a, MACN-PV-94a-3) align closely with the equilibrium line and modern reptile values (Figure 2), despite Δ_{47} temperatures consistent with modern bird eggshells (Figure 3). This indicates temperature as the dominant control during mineralization, with limited kinetic effects. Recrystallized samples (e.g., MACN-

PV-94a-2, -4) deviate toward bird-like disequilibrium, likely reflecting diagenetic overprinting rather than primary biology. MACN-PV-94-a-4 shows a disequilibrium pattern more similar to modern birds, apparently influenced by disequilibrium processes (Figure 2). This observation suggests that temperature was the primary control of clumped isotope composition when Enantiornithine eggshells initially mineralized, while the recrystallized specimens have clumped isotope compositions reflective of disequilibrium effects.

Elevated Mn in trace elements supports some alteration, though low Fe, Sr, Mg, and Ba suggest overall good preservation in key samples, consistent with prior dinosaur eggshell studies. To further interrogate this anomaly, matrix was subsequently microdrilled from the sample, and tested using clumped isotope paleothermometry and trace elements. Additionally, the interior of the eggshell was microdrilled and tested for trace elements and clumped isotopes as well.

Enantiornithes thermophysiology.

Calculated Δ_{47} values of the best preserved Enantiornithes specimens are consistent with the measured values for modern bird eggshells (Figure 3), however the same specimens plot closely to the Δ_{47} - Δ_{48} equilibrium line similar to modern reptiles (Figure 2). This suggests that Enantiornithes was able to achieve high body temperatures similar to modern endothermic relatives, but did not experience the same mode of eggshell mineralization as modern Aves. Heterothermic endothermy is a widespread modality of thermoregulation in modern birds in the form of torpor states, where an endothermic organism drops its body temperature intermittently. These states may be entered on diurnal or seasonal cycles and are typically in response to environmental changes, while the degree to which the animal's metabolism is variable is dependent on the species and how the torpor state is used (Levesque, 2023). The usage of torpor states is evidenced in ancestral forms and in juveniles in both clade Mammalia and clade

Aves, leading researchers to believe that heterothermy is a plesiomorphic trait to the apomorphic rigid endothermy observed in some modern species (Lovegrove, 2011; Geiser, 2008). The tendency of juveniles to transition from poikilothermy to heterothermic endothermy or true endothermy in adulthood is largely considered to be determined by the adult's body size, with smaller species being more likely to exhibit torpor than species with large adults (Geiser, 2008). Therefore, the suggestion that basal birds employed a heterothermic strategy is supported by phylogenetic and modern observations.

Δ_{47} temperatures ($\sim 30\text{--}34^\circ\text{C}$ in best-preserved material) exceed typical ectotherm ranges and align with modern endothermic birds of comparable size, corroborating histological inferences of elevated resting metabolic rates. Combined with equilibrium Δ_{48} patterns, this supports heterothermic endothermy: sustained high temperatures (possibly basal or activity-driven) with flexibility for torpor, as seen in modern small birds and inferred plesiomorphically in Paraves.

This contrasts with stricter endothermy in crown birds and aligns with Troodon findings (heterothermy + reptile-like mineralization). Enantiornithes' precocial ontogeny, rapid early growth, and arboreal-like ecology further support metabolic similarity to modern small endotherms capable of heterothermy.

Effects of H_2O_2 treatment on isotopic values.

Paired modern analyses confirm H_2O_2 affects Δ_{48} , which is variable by taxon, but not Δ_{47} , validating temperature reconstructions while highlighting caution in dual interpretations for treated fossils.

Paired analyses of treated and untreated eggshells were performed on selected species *Crocodylus acutus* (CAA-MOD-1, CAA-MOD-2), *Crocodylus niloticus* (CNN-MOD-1, CNN-MOD-2), *Alligator mississippiensis* (CGAMOD-1, CGAMOD-2), *Melanerpes aurifrons* (MA-MOD-1, MA-MOD-2), *Aptenodytes patagonicus* (AP-MOD-1, AP-MOD-2), and *Dromaius novaehollandiae* (DN-MOD-1, DN-MOD-2) (Table 3). The analyses show that while the Δ_{48} signature is affected by the peroxide treatment, the Δ_{47} signature is not affected by H_2O_2 treatment (Figure 4).

Implications for evolution of endothermy

The suggestion that heterothermic endothermy was the metabolic mode employed by Enantiornithines supports the hypothesis that this strategy is a plesiomorphy within Aves. Heterothermic endothermy in Enantiornithes strengthens evidence that variable thermoregulation is ancestral to Aves, with obligate homeothermy evolving later in Ornithuromorpha or Neornithes. This flexibility may have conferred advantages in variable Mesozoic environments but, paired with dietary specialization (such as the lack of gastric mill), contributed to vulnerability during K-Pg habitat disruption.

Limitations include small sample size ($n=4$, genus-level identification only), potential diagenetic bias in altered specimens, and lack of direct environmental temperature constraints (though Neuquén Basin paleoclimate was warm). Future work should target additional specimens and pair with bone histology or other proxies.

Materials and Methods

Specimens

Modern eggshell specimens were sourced from the Western Foundation of Vertebrate Zoology, Colorado Gators, the Los Angeles Zoo, the San Francisco Zoo, and Gemarkenhof

Farm. Four partial *Enantiornithes* eggshells were provided by the Los Angeles Museum of Natural History (Museum ID number MACN-PV-94-a). The fossil specimens were sourced from the Neuquén Basin of Patagonia, Argentina, where they were then repositied with the Museo Argentino de Ciencias Naturales (MACN) and loaned to the University of California, Los Angeles by way of collaborators at the Natural History Museum of Los Angeles County. The specimens were only identified to the genus level, therefore the taxonomic range and species information could not be further constrained.

Organic matter.

Portions of the penguin, woodpecker, emu, ostrich, Nile crocodile, American crocodile, and alligator eggshells were treated for organic matter using a dilute 3% H₂O₂ solution. The data show that peroxide treatment does appear to affect Δ_{48} values, however the degree to which data are affected appears to vary by species (Figure 4).

Cathodoluminescence.

Fossil eggshells were cut into thin sections 30 μ m thick and polished. Specimens were analyzed at the University of Southern California in the Corsetti Laboratory. The beam was set to 15 kV and exposure time was variable depending on the specimen, with recrystallized specimens having quicker exposure times around 11 seconds and specimens with little to no alteration having exposure times upwards of 19 seconds.

EBSD.

Fossil eggshells were sectioned, mounted in epoxy, and coated with a thin layer of gold to be screened for alteration. Electron backscatter diffraction was performed using a scanning electron microscope housed at University of Alabama Geological Science Department in Tuscaloosa, Alabama. Two areas were analyzed from both MACN-PV-94a-1 and MACN-PV-

94a-3, while only one area was analyzed from MACN-PV-94a-2. MACN-PV-94a-4 was unsuitable for analysis.

Trace element distributions.

All data presented here were collected using a Varian Vista Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) setup in the Tripathi-Eagle IsoLab at University of California, Los Angeles. MACN-PV-94a-1, MACN-PV-94a-2, and MACN-PV-94a-4 were measured for trace elemental concentrations of Sr/Ca, Mn/Ca, Fe/Ca, Mg/Ca, and Ba/Ca.

Dual clumped isotope thermometry.

As mentioned above, a selection of modern specimens were pretreated for organic matter using a dilute 3% H₂O₂ solution. After pretreatment, eggshells were pulverized with a mortar and pestle. Fossil eggshells were sonicated for 5 minutes in deionized water in 1 minute bursts, then left to dry overnight. When dry, the outermost layer was drilled off, discarded, and the sample was taken from underneath the outer layer. This was to remove any contaminants that may have precipitated in pore spaces which were not removed during sonication.

Aliquots of 0.40-0.53 mg were reacted for 20 minutes in a common phosphoric acid bath heated to 90 °C. CO₂ gas was then purified by a cryogenic trap containing liquid nitrogen, which removes water and other gases with low vapor pressures. The CO₂ gas was then purified using an adsorption trap which passes the sample gas through a Porapak Type-QTM 50/80 mesh GC column at -20 °C to remove potential organic contaminants and silver wool (Sigma-Aldrich) to remove sulfur compounds. Stable carbon ($\delta^{13}\text{C}$), oxygen ($\delta^{18}\text{O}$), and carbonate clumped ($\Delta 47$ and $\Delta 48$) isotope ratios of the samples were measured using a dual-inlet Nu Perspective IS mass spectrometer at the University of California Los Angeles using methods previously described (Defliese and Tripathi, 2020, Upadhyay et al., 2021, Lucarelli et al., 2023).

Carbonate standards were analyzed every 5–6 analyses. The average external reproducibility (one standard error) of Δ_{47} measurements across all modern and fossil eggshell samples in this study was 0.0073‰ (n = 31 samples; range 0.003–0.019‰). This corresponds to an average temperature uncertainty of 2.7 °C on the derived body temperatures. Table X contains standard slope and intercept values, as well as secondary transfer function slope and intercepts.

Future work

Sample set would benefit from additional trace elements testing on micritic matrix only to determine if elevated heavy metal values are signals from small amounts of matrix incorporated into powder. Additionally, set has been sent to collaborators in South Korea for Raman analysis.

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APPENDIX III

Clumped Isotopes in Bioapatite Tooth Enameloid Indicate Endothermy in Mesozoic Marine Reptiles from the Callovian–Kimmeridgian of Europe

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Abstract

Recent oxygen isotope studies have suggested that some Mesozoic marine reptiles, including ichthyosaurs and plesiosaurs, exhibited endothermy, but debates persist due to uncertainties in seawater $\delta^{18}\text{O}$ and fractionation factors. Here, we apply carbonate clumped isotope paleothermometry (Δ_{47}) to well-preserved tooth enameloid from Jurassic marine reptiles (ichthyosaurs, plesiosaurs/pliosaurs, metriorhynchids, teleosaurs) and ectothermic endmembers (fish, sharks) from three European localities: the Lower Kimmeridge Clay (England), Oxford

Clay (England), and Marnes de Dives (France). Preservation was assessed via $\delta^{18}\text{O}$ carbonate – $\delta^{18}\text{O}$ phosphate offsets (mostly 6–10‰, consistent with modern bioapatites) and $\delta^{13}\text{C}$ patterns distinguishing air-breathing reptiles from aquatic ectotherms (Eagle et al., 2011).

Δ_{47} -derived body temperatures for most reptiles range from 34–43°C, consistently elevated above coeval ectotherms (24–36°C), supporting endothermy. Ichthyosaurs and plesiosaurs/pliosaurs show stable high temperatures indicative of homeothermy or poikilothermic endothermy, while metriorhynchids are more variable, with some outliers likely exhibiting diagenetic isotopic overprinting. These independent estimates align with and refine prior $\delta^{18}\text{O}$ phosphate findings, confirming ichthyosaurs as likely homeothermic endotherms and plesiosaurs as regionally/regionally elevated thermoregulators.

This work demonstrates Δ_{47} as a powerful, assumption-reduced proxy for marine reptile thermophysiology, offering new insights into Jurassic pelagic ecology and the evolutionary success of these predators.

Keywords: clumped isotopes, Δ_{47} paleothermometry, Mesozoic marine reptiles, endothermy, Jurassic, ichthyosaurs, plesiosaurs

Introduction

Recent $\delta^{18}\text{O}$ studies have provided strong evidence that members of some clades of marine reptiles, including ichthyosaurs and plesiosaurs, likely exhibited at least some degree of endothermy^{44,47,48,73,74}. These exciting developments present opportunities for further isotopic study using other proxies, like clumped (Δ_{47}) isotope paleothermometry for further paleotemperature and ecological reconstruction in these ancient sea predators, as much is still unknown about their paleobiology.

Two of the four major groups of giant marine reptiles (up to 15 m) known from the Jurassic include Ichthyopterygia (ichthyosaurs), Sauropterygia (plesiosaurs, nothosaurs) (Martill et al., 2023). An additional group of medium-sized marine reptiles (2-7m), Thalattosuchia (teleosaurs, metriorhynchids), is also known, and the rest of the marine reptile groups were smaller (Young et al., 2011, Young et al., 2016). Ichthyopterygia and Sauropterygia are first recorded from the Early Triassic. Thalattosuchia is recorded as early as Jurassic time and is considered to have been less successful than its larger relatives. The Ichthyopterygians and Sauropterygians each survived for over 150 million years, while the thalattosuchians existed for only about 60 million years.

Phylogeny

Sauropterygia encompasses the largest and most prolific group of Mesozoic marine reptiles⁷⁵. Of the major groups, they were the most widespread both geographically and in time⁷⁵. They are noted in the fossil record by the Early Triassic until the end Cretaceous, outliving many other famous and dominant contemporaries, such as dinosaur species⁷⁵. The most derived clade of this group is the plesiosaurs⁷⁵. They appear to have evolved in the Jurassic, exhibiting rigid, well-ossified flippers in contrast to other Sauropterygians. These adaptations suggest they used these upgraded flippers for faster swimming. Plesiosaurs are widely distributed geographically and are known from all continents. Plesiosaurs exhibit two distinct body types, plesiosauromorph (long-necked) and pliosauromorph (short-necked) types, with each body type reflecting their different lifestyles⁷⁵. Pliosauromorphs are thought to have been pursuit hunters, while plesiosauromorphs are thought to have used less swimming during hunting^{75,79,80}. It should also be noted that intermediate forms between these two extreme forms existed as well⁷⁵. Viviparity is known among plesiosaur species; however, it is unknown whether this is the ancestral reproductive strategy of Sauropterygia or if this is unique among plesiosaurs^{75,81}.

The second largest group, the Ichthyopterygians produced some of the largest species⁷⁵. They appear in the fossil record in the Late Triassic and became extinct in the Late Cretaceous, spanning a taxonomic range only about 25 million years shorter than that of the Sauropterygians⁷⁵. The Ichthyopterygians get their “fish lizard” moniker from their characteristic caudal fluke, however the basal Ichthyopterygians were more lizard-shaped than fish-shaped⁷⁵. Fish-shaped forms did not evolve until the Norian, about 30 million years after the first Ichthyopterygians show up in the fossil record. Aside from being the first tetrapods of record to evolve a fish-shape, the Ichthyopterygians had the largest eyes of all vertebrates, as well as the highest finger count among vertebrates^{75,82}. The large eyes of this group were adapted to see in low light, presumably for hemispheric sleep and deep diving, both as adaptations to pelagic life^{75,82}. Additionally, Ichthyopterygians are known to have been viviparous, even in the most basal forms^{75,83,84,85}.

Geologic Setting

Samples in this set come from three localities in Europe. The first is an English locality, Smallmouth Sands, Dorset, from the Lower Kimmeridge Clay Formation dated to the Lower Kimmeridgian^{74,86}. The second English locality is from the Oxford Clay Formation Peterborough clay pits dated to the middle Callovian⁷⁴. This site is known for its ample marine and terrestrial fossils^{74,87}. The third locality is the Marnes de Dives Formation in Le Vaches Noires of Normandy, France, dated to the upper Callovian, a formation also known to be rich in marine fossils^{74,88}. The final location is the Slottsmøya member of the Agardhfjellet Formation of Svalbard, Norway. This section is primarily black shale deposits, and its low-oxygen environment make it a desirable location for fossils⁸⁹. Most specimens have been previously investigated using $\delta^{18}\text{O}$ paleothermometry in Séon et al., 2020, save for the Norwegian samples. This previous study revealed two key findings—1) the enameloid appears to be intact for isotopic study and 2) ichthyosaurs, plesiosaurs, and possibly metriorhynchids, likely

exhibited endothermy⁷⁴. Other marine reptile teeth from Slottsmøya were measured for $\delta^{18}\text{O}$ in Séon et al., 2024, however it was found that these samples appeared to have undergone noticeable erosion due to frost weathering⁴⁷. Under further analysis using Raman and $\delta^{18}\text{O}_{\text{carbonate}} - \delta^{18}\text{O}_{\text{phosphate}}$ modeling however, it did not appear that the enamel had been isotopically reordered or affected by the mechanical wear⁴⁷. Ultimately, the study found that ichthyosaurs appear to demonstrate body temperatures consistent with homeothermic endothermy while plesiosaurs and metriorhynchids present with body temperatures that covary with environmental temperatures, but are consistently above ambient water temperature, indicating poikilothermic endotherm strategies⁴⁷.

Previous Research

Although more paleotemperature reconstructions have been done for marine reptiles in recent years, clumped isotope paleotemperature reconstructions are sparse. For this reason, we seek to explore marine reptile thermophysiology using this proxy. In contrast to $\delta^{18}\text{O}$ palaeothermometry, it does not require seawater temperature estimates for paleotemperature reconstruction^{90,91}. This fact makes it an ideal complement to $\delta^{18}\text{O}$ palaeothermometry studies. Using this isotopic proxy, our group seeks to further elucidate the Jurassic ecosystem in which these marine reptiles lived.

Methods

Sampling

Samples were obtained directly from the Laboratoire de Géologie de Lyon, Terre, Planètes et Environnement within the Université Claude Bernard Lyon, where the fossil material was sampled. Powders were soaked in a dilute hydrogen peroxide for 4 h, rinsed with deionized water and dried overnight in an industrial oven set to 40 °C, then subsequently soaked in 0.1M acetic acid, buffered to pH 4.6 in 1M sodium acetate for 14 h, and then rinsed three times with

deionized water, and dried in the oven again prior to analysis. This bioapatite pre-treatment was effectuated following the procedure described in Eagle et al., 2011.

Analysis

All clumped isotope (Δ_{47}) analyses were performed in the Tripathi-Eagle IsoLab at the University of California, Los Angeles using a Thermo Finnegan MAT 253 gas source mass spectrometer and a Nu Perspective IS mass spectrometer. Samples were digested at 90 °C in a common phosphoric acid bath. CO₂ gas was then purified by a cryogenic trap containing liquid nitrogen, which removes water and other gases with low vapor pressures. The CO₂ gas was then purified using an adsorption trap which passes the sample gas through a Porapak Type-QTM 50/80 mesh GC column at -20 °C to remove potential organic contaminants and silver wool (Sigma-Aldrich) to remove sulfur compounds. Stable carbon ($\delta^{13}\text{C}$), oxygen ($\delta^{18}\text{O}$), and carbonate clumped (Δ_{47}) isotope ratios of the samples were measured using methods previously described (Defliese and Tripathi, 2020, Upadhyay et al., 2021, Lucarelli et al., 2023). Carbonate standards were analyzed every 3-5 analyses. The average of Δ_{47} values across all 16 samples in this study was 0.557 ± 0.005 ‰ (1 SE of the mean). For enamel/enameloid samples only (n=10), it was 0.548 ± 0.006 ‰ (1 SE). Individual sample means had typical analytical uncertainties of ~0.007 ‰ (average 1 s.e. from replicates). Standards of ETH-1/2/3/4 as well as in-house standards were run with each sample to establish reproducibility and calculate empirical transfer function (ETF). Table S1 contains standard slope and intercept values, as well as secondary transfer function slope and intercepts. Samples were analyzed on a Nu Perspective IS mass spectrometer using protocols and methods previously described (Upadhyay et al., 2021).

Results and Discussion

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Single isotope and clumped temperature data

Due to the limited number of samples in the set, the entire sample set was run with the exception of duplicate fish and shark species. All marine reptiles were run, and one or two ectotherms for each formation were run as endmembers. As a first pass at determining preservation, $\delta^{18}\text{O}_{\text{carbonate}}$ values measured in our lab were compared against published $\delta^{18}\text{O}_{\text{phosphate}}$ for this sample set in Séon et al., 2020. Figure 1 shows the results of this analysis.

The largest portion of the sample set came from France, with two pliosaurs, each measuring at 38.18 ± 3.06 °C and 40.22 ± 1.36 °C respectively, three metriorhynchids, measuring at 34.48 ± 2.50 °C, 41.40 ± 4.03 °C and 59.20 ± 7.27 °C, one plesiosaur, measuring at 42.77 ± 2.98 °C, one teleosaur measuring at 40.12 ± 5.43 °C and one shark measuring at 36.62 ± 3.43 °C. Smallmouth sands consists of one ichthyosaur, measuring at 39.44 ± 1.06 °C, one metriorhynchid, measuring at 34.71 ± 2.70 °C, one teleosaur, measuring at 30.00 ± 2.32 °C, one shark measuring at 35.37 ± 2.04 °C and one fish measuring at 40.37 ± 1.63 °C. The Oxford Clay consists of one plesiosaur, one shark and one fish, measuring at 52.73 ± 1.50 °C, 31.70 ± 1.01 °C and 24.38 ± 3.28 °C, respectively. The full list of sample data included in Table 1.

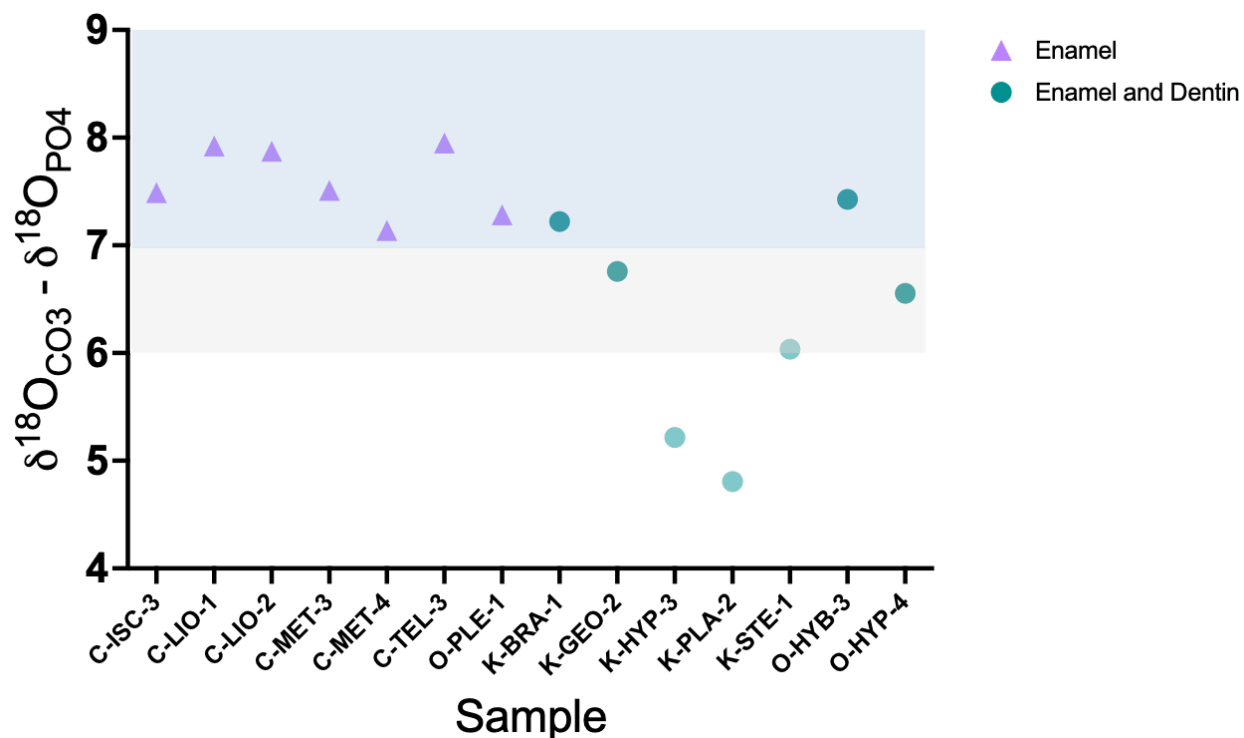


Figure 1. Carbonate content offset for marine reptiles, fish, and sharks. Blue shaded region represents the 99th percentile of modern data, while the gray shaded region represents the 95th percentile. Unshaded regions are most likely altered specimens, and faded symbols represent samples that are suspected to be altered. Only samples that fell within the 95th or 99th percentile were considered to be well-preserved. Purple symbols represent enamel only samples, while green symbols represent mixed enamel and dentin samples. VN6 and VN7 are not plotted because they were not analyzed for $\delta^{18}\text{O}_{\text{phosphate}}$ in Séon et al., 2020.

Sample Name	Material	Common Name	Location	$\delta^{13}\text{C}$ nC	1 s.d.	$\delta^{18}\text{O}$ O	1 s.d.	$\Delta 47$ avg	1 s.e.	$\Delta 48$ avg	1 s.e.	Avg T [°C]	1 s.e.
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				PDB avg		PDB avg								
C-ISC-3	Enameloid	Shark	Marnes de Dives, France	80	0.31	7	2.63	3	3	9	6	7	2	3
C-LIO-1	Enamel	Pliosaur	Marnes de Dives, France	67	7.54	6	2.67	9	8	8	8	9	8	6
C-LIO-2	Enamel	Pliosaur	Marnes de Dives, France	78	4.40	3	2.64	2	2	3	5	7	2	6
C-MET-3	Enamel	Metriorhynchid	Marnes de Dives, France	35	5.53	4	2.09	1	7	7	8	1	8	0
C-MET-4	Enamel	Metriorhynchid	Marnes de Dives, France	44	4.28	1	2.85	5	0	0	4	7	0	3
C-TEL-3	Enamel	Teleosaur	Marnes de Dives, France	46	6.93	7	1.83	7	4	4	8	2	2	3
K-BRA-1	Enamel	Ichthyosaur	Smallmouth Sands	55	5.78	3	4.07	0	4	3	5	8	4	6

K- GEO-2	Bulk	Metriorhynchid	Smallmouth Sands	-	0.2	-	0.5	0.56	0.00	1.16	0.22	34.7	2.7
				64.87	5	3.99	5	7	7	6	2	1	0
K- HYP-3	Bulk	Fish	Smallmouth Sands	-	0.2	-	0.5	0.55	0.00	2.50	0.79	40.3	1.6
				81.89	6	5.09	8	2	4	0	0	7	3
K- PLA-2	Bulk	Shark	Smallmouth Sands	-	0.0	-	0.1	0.56	0.00	2.48	0.45	35.3	2.0
				51.73	2	4.48	8	5	5	6	8	7	4
K- STE-1	Bulk	Teleosaur	Smallmouth Sands	-	0.2	-	0.6	0.58	0.00	0.79	0.23	30.0	2.3
				63.87	7	4.31	2	0	7	9	7	0	2
O- HYB-3	Bulk	Shark	Oxford Clay	-	0.0	-	0.0	0.57	0.00	0.31	0.03	31.7	1.0
				54.48	4	3.29	7	5	3	8	9	0	1
O- HYP-4	Bulk	Fish	Oxford Clay	-	0.2	-	0.5	0.59	0.01	2.01	0.42	24.3	3.2
				61.95	6	3.41	7	7	0	5	5	8	8
O- PLE-1	Enamel	Plesiosaur	Oxford Clay	-	0.0	-	0.2	0.52	0.00	0.61	0.06	52.7	1.5
				48.05	0	3.82	1	2	3	6	2	3	0
VN6	Enamel	Metriorhynchid	Marnes de Dives, France	-	0.0	-	0.0	0.50	0.01	0.73	0.33	59.2	7.2
				26.29	6	1.76	4	9	6	7	6	0	7
VN7	Enamel	Plesiosaur	Marnes de Dives, France	-	0.2	-	0.5	0.54	0.00	0.37	0.12	42.7	2.9
				57.65	1	2.94	5	6	8	8	6	7	8

Discussion

$\delta^{18}\text{O}_{\text{carbonate}} - \delta^{18}\text{O}_{\text{phosphate}}$ offsets range 3.6–8.1‰ (mean 5.7‰), with enamel/enameloid from marine reptiles (plesiosaurs, pliosaurs, metriorhynchids, teleosaurs) mostly 6–8.1‰, consistent with modern bioapatite biogenic range of ~6–10‰ (Séon et al., 2020, Vennemann et al., 2001; Lehn et al., 2020). Bulk ectotherm samples trend lower (3.6–5.8‰), potentially reflecting material differences or minor diagenetic effects on carbonate. Combined with Δ_{48} anomalies near 0–1‰ in enamels and Raman crystallinity (Séon et al., 2020, 2024), this supports primary preservation in key reptile specimens for clumped isotope thermometry.

Fish and shark samples from Smallmouth Sands exhibit higher temperatures reflective of isotopic reordering due to diagenesis, as the temperatures reported here are higher than those reported for $\delta^{18}\text{O}_{\text{phosphate}}$ (Séon et al., 2020). Figure 1 shows the offset between the carbonate and phosphate-derived $\delta^{18}\text{O}$ temperatures, indicating that the majority of the marine reptile specimens are likely well-preserved, and Δ_{47} temperatures likely reflect primary body water temperatures rather than high-temperature resetting during burial. In modern bioapatites the offset of $\delta^{18}\text{O}_{\text{carbonate}} - \delta^{18}\text{O}_{\text{phosphate}}$ is known to be roughly between 6 to 10 ‰, therefore specimens which fall within this range—while they cannot be unambiguously declared as unaltered—are more likely to have intact isotopic signatures²⁴. However, it is less clear whether the stenosaur and geosaurus are affected. It does not appear based on the paleotemperature reconstruction that the fish from the Oxford Clay has been altered, however. Therefore, it is possible that some of the samples which fall within the 95th percentile of modern samples may not be altered but instead may still be isotopically intact. Since their isotopic signatures appear plausible, it makes sense for them to remain in the dataset.

Across the three formations, the ichthyosaurs, plesiosaurs, and pliosaurs all consistently give temperatures above the ectothermic endmembers. The metriorhynchids are less reliable in this respect, sometimes giving very high temperatures (possibly due to diagenetic heating), and other times measuring on par with ectothermic contemporaries.

$\delta^{13}\text{C}_{\text{carbonate}}$ in figure 3 shows the expected distribution of all marine reptiles having low $\delta^{13}\text{C}$ values, due to incorporation of atmospheric CO_2 resulting from breathing air at the surface, while the fish and sharks are less $\delta^{13}\text{C}$ -depleted, resulting from dissolved organic carbon. This expected distribution indicates that the primary isotopic signature is likely preserved.

The Δ_{48} results from our clumped isotope analyses of Jurassic marine reptile teeth and ectothermic endmembers provide a valuable secondary proxy for assessing preservation and potential kinetic or diagenetic effects in bioapatite carbonate. Across the 16 samples, Δ_{48} values range from -0.87‰ to $+2.50\text{‰}$ (mean $+0.93\text{‰}$, standard deviation 0.97‰), with enamel and enameloid samples showing tighter clustering (mean $+0.57\text{‰}$) compared to bulk material (mean $+1.55\text{‰}$). This pattern suggests denser enamel/enameloid is generally more resistant to contamination or alteration, consistent with its compact structure limiting exchange or organic interference. Overall, most Δ_{48} values fall near 0‰ to $+1.2\text{‰}$, aligning with expected near-stochastic distributions at Earth-surface temperatures in well-preserved bioapatite (after acid digestion corrections), as reported in modern and fossil apatite studies. Elevated Δ_{48} ($> +1.5\text{‰}$ to $+2.5\text{‰}$) occurs primarily in bulk samples (e.g., fish K-HYP-3 at $+2.50\text{‰}$, shark K-PLA-2 at $+2.49\text{‰}$), potentially indicating minor kinetic fractionation, organic residues, or isobaric interferences during analysis, though these do not systematically correlate with Δ_{47} -derived temperatures (weak negative correlation, $r \approx -0.17$). Ectothermic taxa (sharks, fish, teleosaurs) average $+1.13\text{‰}$ in Δ_{48} , slightly higher than reptiles ($+0.78\text{‰}$), but without strong evidence of widespread disequilibrium. High-temperature outliers (plesiosaur O-PLA-1 at $52.7 \pm 1.5^\circ\text{C}$ with $\Delta_{48} +0.62\text{‰}$; metriorhynchid VN6 at $59.2 \pm 7.3^\circ\text{C}$ with $+0.74\text{‰}$) show moderate Δ_{48} values, suggesting diagenetic overprinting (partial carbonate reordering) rather than kinetic artifacts as the primary cause of inflated Δ_{47} -derived temperatures. These Δ_{48} patterns reinforce the integrity of enamel samples for primary thermophysiological signals, complementing $\delta^{18}\text{O}$

carbonate-phosphate offsets and supporting elevated body temperatures in ichthyosaurs, plesiosaurs, and pliosaurs as biological rather than diagenetic features.

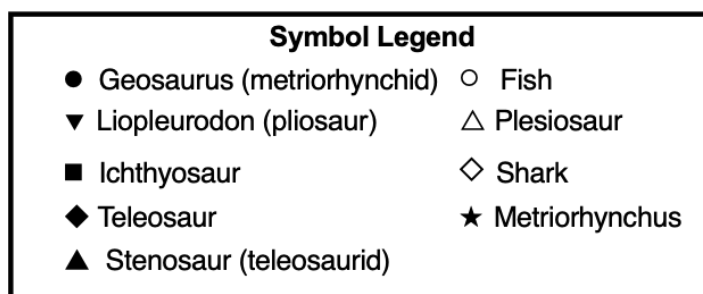
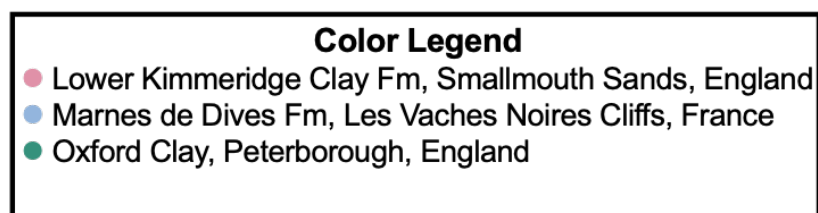
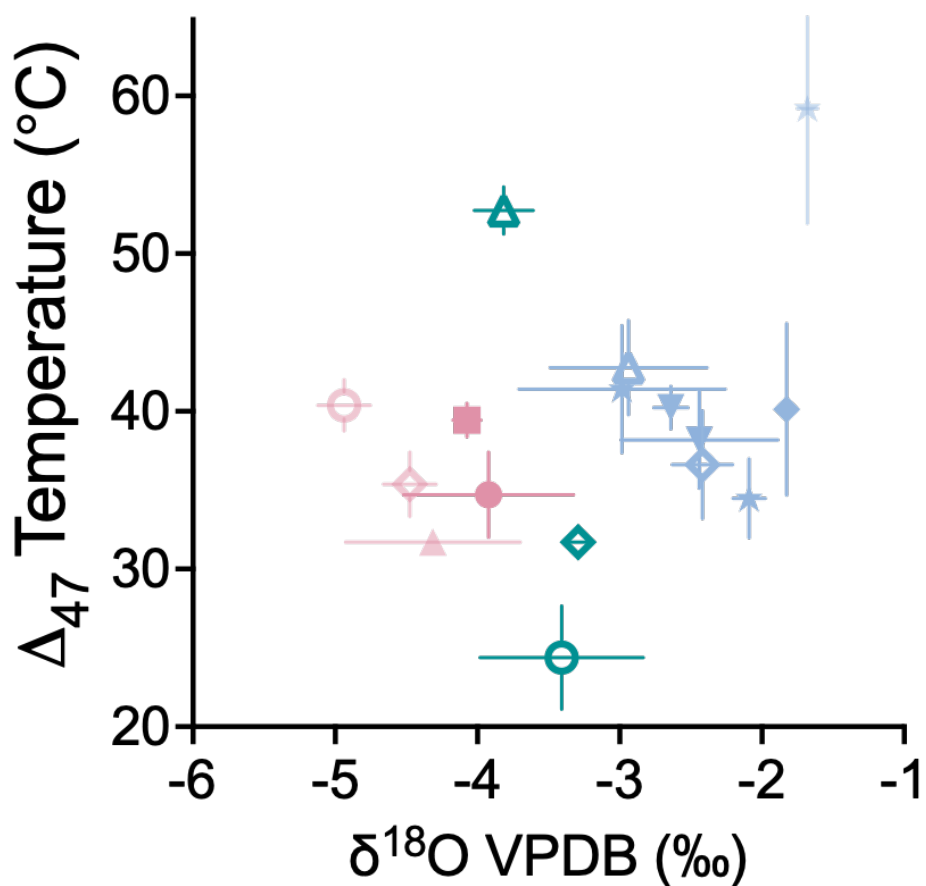


Figure 2. Paleotemperature reconstruction of marine reptiles with fish and sharks as ectothermic endmembers. Faded symbols represent suspected altered samples.

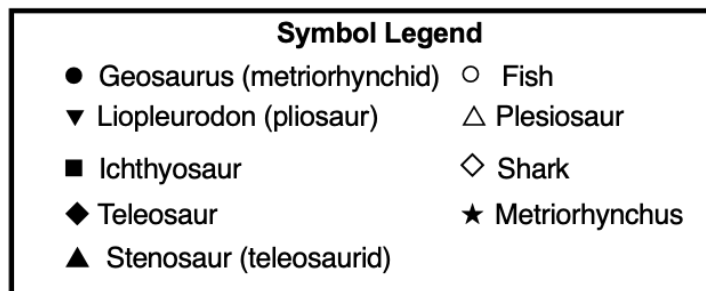
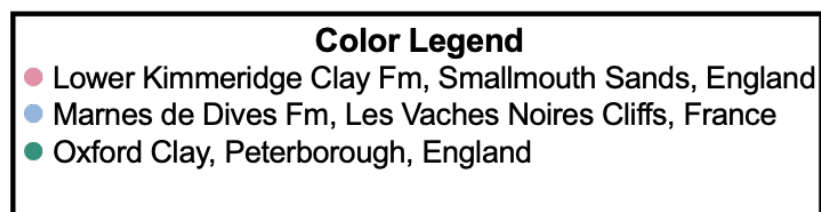
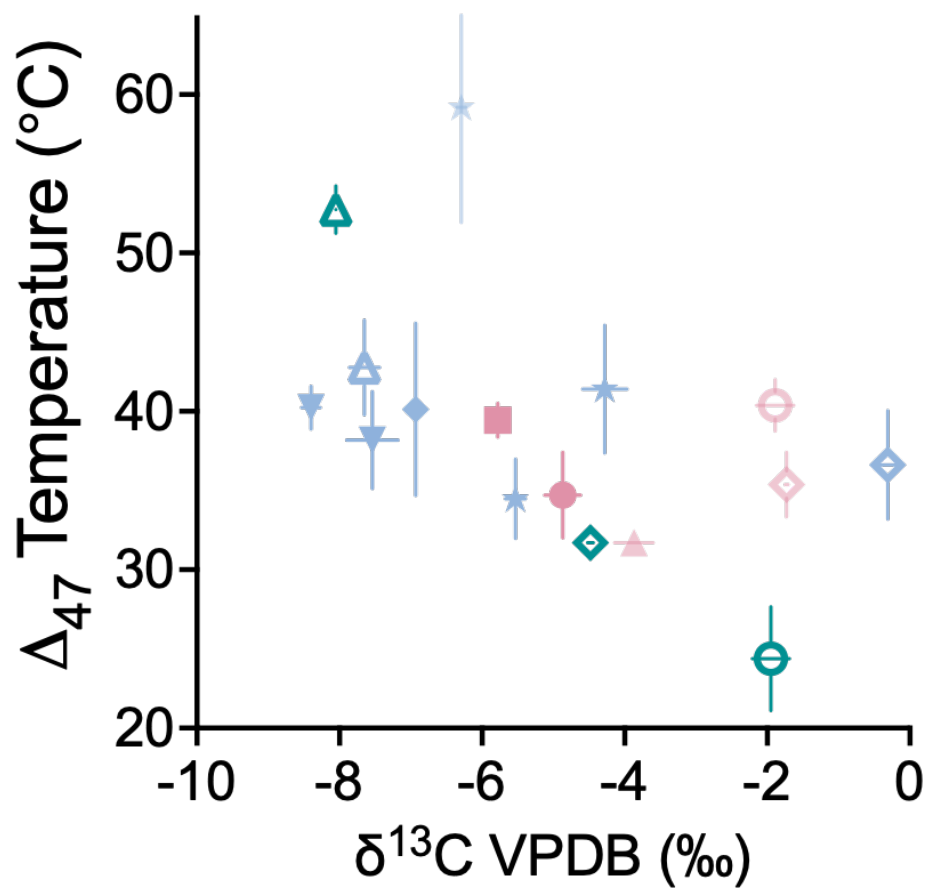


Figure 13. Carbon isotope data for marine reptiles. Faded symbols represent suspected altered samples.

Conclusion

Our clumped isotope (Δ_{47}) paleotemperature reconstructions from Jurassic marine reptile teeth provide independent evidence supporting elevated body temperatures and likely endothermy in key clades, complementing and strengthening prior $\delta^{18}\text{O}$ phosphate studies (Séon et al., 2020, 2024). Most well-preserved specimens, identified via carbonate-phosphate offsets within the 6–10‰ modern biogenic range and plausible $\delta^{13}\text{C}$ depletion in air-breathers, yield temperatures of 34–43°C for ichthyosaurs, plesiosaurs, and pliosaurs, consistently 5–15°C above ectothermic fish and sharks (24–36°C) from the same formations. This pattern holds across localities, despite environmental variability, suggesting metabolic heat production enabled these predators to maintain elevated core temperatures in pelagic settings.

Ichthyosaurs exhibit the most stable high temperatures (39–40°C), consistent with homeothermic endothermy and adaptations like large eyes, viviparity, and fusiform bodies for sustained activity and deep diving. Plesiosaurs and pliosaurs show similarly elevated but slightly more variable values (38–43°C), aligning with poikilothermic endothermy where body temperature covaries somewhat with ambient conditions but remains above seawater. Metriorhynchids are less consistent, with some anomalously high values (59°C) attributable to diagenetic overprinting, while others match ectotherms, indicating limited or variable heat retention in this semi-pelagic group.

These findings refine thermoregulatory models for Jurassic marine ecosystems, where endothermy likely conferred advantages in predation, migration, and thermal niche expansion amid fluctuating Mesozoic oceans. The Δ_{47} proxy's independence from seawater $\delta^{18}\text{O}$

assumptions reduces uncertainties inherent in traditional $\delta^{18}\text{O}$ paleothermometry, making it particularly valuable for bioapatite archives prone to partial alteration. Future work integrating Δ_{47} with $\delta^{66}\text{Zn}$ trophic proxies, larger sample sets, and dual clumped (Δ_{47} – Δ_{48}) screening could further elucidate paleoecological dynamics and test for regional heterothermy.

Ultimately, this study highlights the potential of clumped isotopes to resolve longstanding questions in Mesozoic marine reptile paleobiology, contributing to a more nuanced view of thermophysiological diversity among ancient apex predators.

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