

**Early Pliocene Terrestrial
Paleotemperatures and an FTIR
Framework for Assessing the
Reliability of Stable Isotopes from
Fossil Bone and Enamel:
Alligators, Turtles, and Fish from the
Gray Fossil Site, Tennessee**

Travis Durham, Chris Widga,
Blaine W. Schubert, Alan Wanamaker



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Cover Photograph: Examples of Gray Fossil Site individuals sampled for this research. Top specimen is the right lower jaw of ETMNH 3582 (*Alligator* sp.). Turtle carapaces from left to right are ETMNH 11643 (*Trachemys haugrudi*), ETMNH 4687 (*Sternotherus palaeodorus*), and ETMNH 12491 (*Chrysemys corniculata*). Photograph © Blaine Schubert.

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Early Pliocene Terrestrial Paleotemperatures and an FTIR Framework for Assessing the Reliability of Stable Isotopes from Fossil Bone and Enamel: Alligators, Turtles, and Fish from the Gray Fossil Site, Tennessee

Travis Durham^{1†}, Chris Widga^{1,2*}, Blaine W. Schubert¹, Alan Wanamaker³

Abstract - The Gray Fossil Site (GFS), in the Appalachian Highlands of northeastern Tennessee, is an early Pliocene (4.9–4.5 Ma) Lagerstätte with an abundant and diverse vertebrate fauna. The hypothesized short duration of the accumulation and chronological placement of the GFS potentially provides valuable insights into a period of elevated atmospheric CO₂ and the validation of deep-time climate models. To this end, alligator enamel and turtle and fish bones from GFS were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) and stable isotope analyses to estimate terrestrial, environmental paleotemperatures during this time. Although $\delta^{18}\text{O}$ of bone and enamel CO₃ provide reasonable estimates of GFS paleotemperatures, FTIR analyses indicate potential alteration of the PO₄ fraction. Alligator and turtle $\delta^{18}\text{O}$ values likely reflect local growing season conditions and new analyses of previously published $\delta^{18}\text{O}$ of mammal enamel CO₃ provides a robust estimate of GFS mean annual temperature ($16.6 \pm 1.9^\circ\text{C}$) during the early Pliocene. Lower $\delta^{18}\text{O}$ values of GFS fish suggest hydrological connections of the GFS sinkhole pond with deeper local groundwater and the presence of a strong thermocline.

Introduction

Models of future climate change where atmospheric CO₂ levels approach and possibly exceed those of the known paleontological record underscore the importance of understanding Pliocene climates (Brown et al. 2018, Burke et al. 2018). However, the continued development of these paleoclimate models requires validation through comparison with proxy data from the paleontological record (Haywood et al. 2016). Well-dated Pliocene paleoclimate records for eastern North America are rare outside of coastal areas. The Gray Fossil Site (GFS) in northeastern Tennessee provides a rich record of paleontological and paleoclimate data for this time in the form of records of vertebrate and plant species, as well as stable isotopes from fossil materials. Here, we explore the reliability of paleotemperature reconstructions based on oxygen isotope paleothermometry of vertebrate tissues from GFS. Our work underscores the importance of embedding independent measures of quality control, such as FTIR, in stable isotope studies of bone and tooth enamel.

The GFS is a fossil-rich paleontological locality in Washington County, northeastern Tennessee, USA (36.5°N, 82.5°W) (Fig. 1). Fossil-bearing deposits at the GFS fill an early Pliocene-aged paleo-sinkhole where sediments from the upper part of a paleo-sinkhole contain an early Pliocene fauna that is the subject of ongoing of ongoing geological and paleontological research (e.g., Bourque and Schubert 2015, Czaplewski 2017, Doughty et al. 2018, Jasinski 2018, Jasinski and Moscato 2017, Keenan and Engel 2017, Ochoa et al. 2012, Odom et al. 2025; Samuels et al. 2018, Samuels et al. 2025, Shunk et al. 2009, Shunk et al. 2006, Zobaa et al. 2011). The sinkhole fill sequence consists of up to 42 m of dark, well-laminated, organic-rich lacustrine silts and clays, as well as chert and dolomite gravel lenses (Clark et al. 2005). Sediment cores revealed a series of rhythmites in the upper lacustrine strata, which alternate between fine-grained silty clay layers and coarse-grained, organic-rich layers (Shunk et al. 2006; 2009). Biostratigraphically, the GFS is close to the Hemphillian–Blancan transition (early Pliocene, 4.9–4.5 Ma) (Samuels et al. 2018).

A taxonomically-rich herpetofauna has been recovered from the GFS. These taxa represent both aquatic and terrestrial environments and include: frogs (Schattauer 2012); salamanders (Boardman and Schubert 2011, Darcy 2015, Gunnin et al. 2025); turtles (Bourque and Schubert 2015, Conley 2022, Jasinski 2018, Jasinski 2023), alligator (Gunnin 2023, Turala 2026); lizards (Mead et al. 2012); and snakes (Jasinski and Moscato 2017, Jurestovsky 2016). The presence of these poikilothermic taxa are useful environmental indicators, as well as ideal materials for biogeochemical studies to infer early Pliocene ecological conditions, including quantitative assessment of temperature at the GFS.

¹ Center of Excellence in Paleontology and Department of Geosciences, East Tennessee State University, Johnson City, TN.

² Earth and Mineral Sciences Museum and Art Gallery, Penn State University, University Park, PA. ³ Department of Earth, Atmosphere, and Climate, Iowa State University, Ames, IA. [†] Deceased. *Corresponding author: cxw5766@psu.edu.

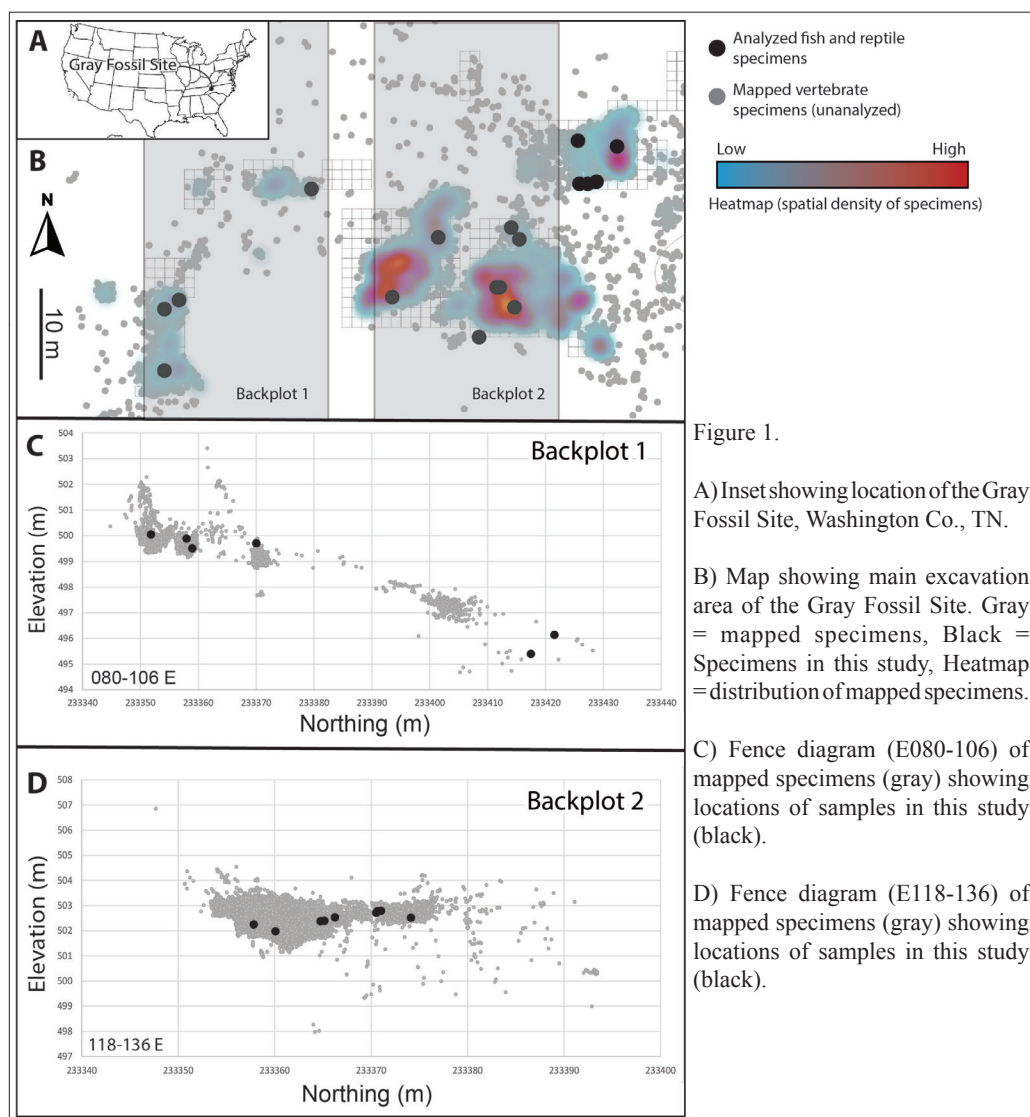


Figure 1.

A) Inset showing location of the Gray Fossil Site, Washington Co., TN.

B) Map showing main excavation area of the Gray Fossil Site. Gray = mapped specimens, Black = Specimens in this study, Heatmap = distribution of mapped specimens.

C) Fence diagram (E080-106) of mapped specimens (gray) showing locations of samples in this study (black).

D) Fence diagram (E118-136) of mapped specimens (gray) showing locations of samples in this study (black).

Previous paleoecological research at the GFS indicates an ecosystem dominated by C3 plants was present throughout the period of deposition (Shunk et al. 2006; DeSantis and Wallace 2008). Pollen and macrobotanical fossils represent a non-analog, successional oak-hickory-pine woodland (Ochoa et al. 2012, Quirk and Hermesen 2021, Siegert and Hermesen 2020, Zobaa et al. 2011). The role of seasonal drought and fire in this woodland remains unclear. Although some researchers suggested a seasonal fire regime based on plant taxa and sedimentary charcoal clasts within the GFS sinkhole fill (Zobaa et al. 2011), other studies highlighted the closed-forest, low fire regime of the small mammal community (Samuels et al. 2018). These conflicting interpretations are unresolved and highlight the need for additional paleoecological research.

The Pliocene presence of certain reptiles provided key elements to climate reconstruction at GFS. Specifically, the extra-limital presence of alligator (*Alligator*) and tortoise (Testudinidae) indicate warmer mean annual temperatures, milder cold seasons, or both (DeSantis and Wallace 2008, Shunk et al. 2006). This study examines stable isotopes from these taxa and more (noted in Methods section) to extend and calibrate paleoclimate parameters from the GFS. In so doing, we address key questions about the fidelity of

biological signals in stable isotopes of fossil vertebrate tissues, reconstruct the early Pliocene temperature of shallow groundwater reservoirs and temperatures during the growing season, and provide additional context for discussions of early Pliocene seasonality patterns in inland, terrestrial environments.

Materials

This study sampled structural carbonate (CO₃) and phosphate (PO₄) of bone and tooth enamel from GFS reptiles and fish for Fourier Transform Infrared spectrometry (FTIR), stable carbon, and stable oxygen isotope analyses. Although stable isotope analyses of GFS mammals and sediments have been previously reported (Shunk et al. 2006; DeSantis and Wallace 2008), herpetofauna and fish have not yet been integrated into our isotopic understanding of the GFS assemblage. All modern and fossil specimens in this study come from the East Tennessee State University Museum of Natural History (ETMNH) collection at the Gray Fossil Site & Museum near Gray, Tennessee. Catalog numbers are prefixed with ETMNH for fossil materials, and ETMNH-Z for modern osteological specimens.

Six samples from five fossil specimens of *Alligator* were collected. A well-preserved skull, ETMNH 3582 was sampled twice (two separate teeth) to explore replicability of analyses within a specimen with a complex diagenetic history (Table 1). Specimens with well-preserved enamel and minimal damage to the tooth crown were selected for sampling. A total of 12 turtles and four fish were also sampled (Table 1). These included six carapace fragments from *Trachemys haugrudi*, one carapace fragment from a tortoise (Testudinidae), two musk turtles (*Sternotherus palaeodorus*), and three painted turtles (*Chrysemys corniculata*). Four fish vertebrae from locations spread throughout the site were also sampled. Although not identifiable to genus level, size and morphology of these specimens are consistent with small Centrarchidae, the only fish family present in GFS deposits (Maden 2023). With the exception of ETMNH 3582, all turtle and *Alligator* samples are from partial skeletons or skeletal elements that represent different adult individuals

Table 1. Specimens analyzed in this study. An * indicates that subsequent stable isotope and FTIR studies found this sample to be diagenetically modified and it was not included in the final dataset.

ETMNH	Lab Number	Taxon	Material	Element
6935	ETSU2019-18	<i>Trachemys haugrudi</i>	bone	carapace
12988	ETSU2019-19	<i>Trachemys haugrudi</i>	bone	carapace
12834	ETSU2019-20	<i>Trachemys haugrudi</i>	bone	carapace
3562	ETSU2019-21	<i>Trachemys haugrudi</i>	bone	carapace
11643	ETSU2019-22	<i>Trachemys haugrudi</i>	bone	carapace
17285	ETSU2019-23	<i>Trachemys haugrudi</i>	bone	carapace
1000	ETSU2022-11*	<i>Sternotherus palaeodorus</i>	bone	carapace
3595	ETSU2022-12	<i>Sternotherus palaeodorus</i>	bone	carapace
4687	ETSU2022-13	<i>Sternotherus palaeodorus</i>	bone	carapace
20544	ETSU2022-14	<i>Chrysemys corniculata</i>	bone	carapace
12491	ETSU2022-15	<i>Chrysemys corniculata</i>	bone	carapace
3559	ETSU2022-16	<i>Chrysemys corniculata</i>	bone	carapace
22205	ETSU2019-17	Testudinidae	bone	carapace
7409	ETSU2022-17	Fish	bone	vertebra
8665	ETSU2022-18	Fish	bone	vertebra
30712	ETSU2022-19	Fish	bone	vertebra
31177	ETSU2022-20a	Fish	bone	vertebra
3582	ETSU2019-30*	<i>Alligator</i>	enamel	tooth
3582	ETSU2019-28	<i>Alligator</i>	enamel	tooth
18124	ETSU2019-24	<i>Alligator</i>	enamel	tooth
5150	ETSU2019-25	<i>Alligator</i>	enamel	tooth
11776	ETSU2019-26	<i>Alligator</i>	enamel	tooth
3434	ETSU2019-27	<i>Alligator</i>	enamel	tooth

based on size or dental eruption stage. Although it is not possible to demonstrate that different individual fish were sampled on the basis of duplicated elements (all sampled specimens were vertebrae), samples come from very different locations within the 1.8 hectare Gray Fossil Site deposit, therefore it is likely that these samples represent different individuals.

Four samples of *Alligator* enamel and three samples of turtle carapace (*Gopherus polyphemus*, N = 2; *Trachemys scripta*, N=1) from modern individuals prepared as osteological specimens were also used as FTIR standards to better understand fossilization pathways (Supplemental Table S1).

Methods

A carbide drill bit (1.5 mm diameter) attached to a handheld rotary tool was used to obtain a 10-15 mg powdered enamel or bone sample from each tooth and carapace fragment, taking care to sample only the outermost surface of the tooth to avoid contaminating enamel powder with underlying dentin. All samples were split into three aliquots for FTIR analyses, preparation of the CO₃ fraction, and preparation of the PO₄ fraction.

FTIR Spectroscopy

FTIR analysis was performed on a Vertex 80v FTIR instrument (Bruker) at the Penn State Materials Characterization Lab equipped with a Praying Mantis diffuse reflectance accessor. Powdered bone samples were diluted (ca. 2 to 5 wt%) with SpectroGrade™ KBr powder and ground to fine powder using an agate mortar and pestle. The sample was loaded in the microsampling cup and inserted into the Praying Mantis accessory, purged with dry and carbon dioxide free air.

The spectra were recorded in Kubelka-Munk unit using an external MIR source, a broad band LN-MCT detector (scanner velocity: 40 kHz) with 256 scans, 4cm⁻¹ resolution and atmospheric compensation. Data acquisition and integration was performed using OPUS (v7.8) software.

A series of indices were calculated to assist in the characterization of diagenetic processes operating across geologic time scales in bone and enamel samples. The CO₃/PO₄ ratio is often used as an indication of CO₃ alteration. For our purposes, we calculate this ratio from baseline corrected spectra as follows (Dal Sasso et al. 2018, Simpson et al. 2023)

$$\frac{(\text{peak height at } 1400 \text{ cm}^{-1} + \text{peak height at } 1500 \text{ cm}^{-1})}{(\text{peak height } 1000 \text{ cm}^{-1} + \text{peak height } 604 \text{ cm}^{-1})}$$

The amount of unmodified structural CO₃ was evaluated. (Scaggion et al. 2024)

$$\frac{(\text{peak height at } 875 \text{ cm}^{-1})}{(\text{peak height at } 1035 \text{ cm}^{-1})}$$

An estimate of the degree to which CO₃ had been removed and remineralized (i.e., secondary CO₃ formation) was also measured. (Scaggion et al. 2024)

$$\frac{(\text{peak height at } 712 \text{ cm}^{-1})}{(\text{peak height at } 1035 \text{ cm}^{-1})}$$

Finally, the potential impact of microbially mediated decomposition processes on bone samples was examined by monitoring peak heights of biomarkers of methanogenic bacteria (Loron et al. 2023):

$$\frac{(\text{peak height at } 2960 \text{ cm}^{-1})}{(\text{peak height at } 2925 \text{ cm}^{-1})}$$

Although not exhaustive, these indices reflect different potential diagenetic pathways that may be operating at the GFS and have real-world implications for the evaluation, analysis and interpretation of potential biological signals in vertebrate stable isotope datasets.

Sample Pretreatment and Instrumental Analyses

Structural Carbonate (CO₃). Five milligrams of powdered enamel or bone apatite were placed in 1.5 mL microcentrifuge tubes and treated with 2.5% NaOCl to remove organic contaminants. After 24 hours, the NaOCl was decanted from the microcentrifuge tubes and rinsed to neutral (4x) with deionized water. Samples were then treated with 0.1 N acetic acid to remove secondary carbonates. After 4 hours the acetic acid was decanted from the microcentrifuge tube and rinsed to neutral (4x) with deionized water. All samples were then air dried in a vacuum desiccation chamber for 48 hours.

Pretreated carbonate samples (~5 mg) were measured on a ThermoFinnigan Delta Plus XL mass spectrometer coupled with a GasBench II with a CombiPal autosampler at the Iowa State University Stable Isotope Laboratory (Ames, IA). All results were reported relative to VPDB based on international reference material (NBS-18 and IAEA 603) using a regression based method. The average analytical uncertainty across all measurements were $\pm 0.11\text{‰}$ for $\delta^{18}\text{O}$ and $\pm 0.07\text{‰}$ for $\delta^{13}\text{C}$. These results were combined with previously reported stable isotope results from mammalian enamel CO₃ (DeSantis and Wallace 2008).

Bone Phosphate (PO₄). Preparation of the phosphate fraction of bioapatite (bone and tooth enamel) follows Shabaga et al. (2018). Powdered bone or enamel (~5 mg) samples were pretreated with 2.5% NaOCl and 0.125 M NaOH for removal of organic material prior to digestion in 2 M HF (12 hr). Samples were neutralized with 0.74N NH₄OH, digested samples were titrated with a 2M AgNO₃ solution at 50°C to precipitate Ag₃PO₄, decanted and dried.

Ag₃PO₄ crystals were analyzed using a Thermo Finnigan Delta Plus XL mass spectrometer in continuous flow mode connected to a Thermo-Chemical Elemental Analyzer at Iowa State University. Reference standards (CBS [Caribou Hoof Standard], USGS42 [Tibetan Human Hair Powder], KHS [Kudu Horn Standard]) were used for isotopic corrections, and to assign the data to the appropriate isotopic scale VSMOW. The combined uncertainty (analytical uncertainty and average correction factor using a regression-based method) for $\delta^{18}\text{O}$ is $\pm 0.28\text{‰}$ (VSMOW).

Stable Isotope Data Analyses

Reported $\delta^{13}\text{C}$ (VPDB) and $\delta^{18}\text{O}$ values (VPDB) were corrected to account for the change in $\delta^{13}\text{C}$ of atmospheric carbon dioxide (CO₂) since the early Pliocene (Zachos et al. 2001, Tipple et al. 2010), as per Whiting et al. (2016)

$$\begin{aligned}\delta^{13}\text{Cc} &= \delta^{13}\text{CVPDB} - 0.5\text{‰} \\ \delta^{18}\text{Oc} &= \delta^{18}\text{OVPDB} + 1.2\text{‰}\end{aligned}$$

All $\delta^{13}\text{C}$ values were further adjusted to account for enrichment between enamel and diet following tissue-diet corrections in Table 2.

Measured $\delta^{18}\text{O}$ values of bone and enamel carbonate ($\delta^{18}\text{Oc}$) were converted to VSMOW, then transformed into estimates of $\delta^{18}\text{O}$ phosphate $\delta^{18}\text{O}$ ($\delta^{18}\text{Op}$) following Zazzo et al. (2004)

$$\delta^{18}\text{Op} = 0.973 * \delta^{18}\text{Oc} (\pm 0.014) - 8.121 (\pm 0.363)$$

These values, and results from measured bone and enamel phosphate ($\delta^{18}\text{Op}$) were subsequently converted to $\delta^{18}\text{O}$ estimates of ingested water ($\delta^{18}\text{O}_{\text{water}}$) using equations from Table 3.

Table 2. Tissue-diet corrections used in this study.

Taxon $\delta^{13}\text{C}$	Tissue	Tissue-diet fractionation	Source
Alligator	Enamel	11.42	Cullen, 2023
Turtles	Bone	8.4	Cullen et al., 2019
Fish	Bone	9	Cullen et al., 2019
Terrestrial mammals	Enamel*	14.1	Cerling and Harris, 1999
<i>Mammut</i>	Dentin	9.5	Ambrose and Norr, 1993

These $\delta^{18}\text{O}_{\text{water}}$ values were used to estimate Mean Annual Temperature (MAT) using the following equation.

$$\text{MAT} = (\delta^{18}\text{O}_{\text{water}} + 14.178) / 0.442 \text{ (Tütken et al. 2006)}$$

A standard error was estimated for MAT values using 1σ uncertainty estimates of instrumental error (a2; 0.16‰), individual variability in the calculated offset between CO_3 and PO_4 (b2; 0.36‰ from Zazzo et al. 2004), error in estimates of $\delta^{18}\text{O}_{\text{water}}$ from $\delta^{18}\text{O}_{\text{p}}$ (c2; 1.6‰ from Tütken et al. 2006) and estimates of MAT (d2; 3.8°C from Tütken et al. 2006). This error was propagated using the equation

$$\sqrt{(a^2 + b^2 + c^2 + d^2)}$$

The combined 1σ error estimate for MAT is $\pm 1.9^\circ\text{C}$.

The $\delta^{18}\text{O}$ values of CO_3 and PO_4 fractions were also compared to each other, and combined with FTIR analyses to interrogate the impact of diagenesis on isotopic results. All statistical tests were performed in R Studio.

Results

Post-depositional Alteration of the Isotopic Signal

Oxygen isotope values of CO_3 and PO_4 bioapatite fractions of the same samples are significantly different, leading to different paleoclimate reconstructions of fossils from the Gray Fossil Site (Fig. 2, Table 4, Supplemental Table 2). These differences indicate that there is some degree of diagenetic alteration occurring in the CO_3 or PO_4 fraction of bone and enamel bioapatite. It is also possible that both fractions have been altered.

FTIR analyses further illustrate similarities and differences between the GFS samples and bone that has not been diagenetically altered. Bone CO_3/PO_4 indices are significantly different between fossil and modern bones indicating either the CO_3 or the PO_4 portion was altered. Although the Structural CO_3 and Secondary CO_3 indices are also significantly different between GFS and modern bones, extensive overlap remains in the distribution of values between modern and ancient samples. Microbial biomarkers are more common in fossil bones (Table 5, Supplemental Table 3; Fig. 3). However, all enamel indices of fossil and modern samples are similar, and are not significantly different, highlighting the long-term integrity of enamel CO_3 and PO_4 (Table 5, Fig. 3).

Although these FTIR data should be considered a coarse-grained reflection of diagenetic alteration in bone bioapatite and tooth enamel, they provide some guidance for potential factors at work in depositional environments that may impact the biological signals of stable isotope information in vertebrates.

Two samples are outliers in the FTIR and stable isotope datasets. A turtle (ETMNH 1000; *Sternotherus palaeodorus*) exhibited high CO_3/PO_4 values (1.74) while a replicated sample of an *Alligator* (ETMNH 3582) showed a very high $\delta^{13}\text{C}$ value (5.4‰). These values are far outside of the 2-sigma range of their respective datasets and indicate poor sample quality (e.g., contamination), extensive diagenetic alteration, or both. These results were removed from the analyzed isotopic dataset.

Table 3. Equations used to estimate $\delta^{18}\text{O}_{\text{water}}$ in different taxonomic groups from GFS. Mammal data from DeSantis and Wallace, 2008, but re-analyzed in this paper.

Taxon $\delta^{13}\text{C}$	Equation
Alligator	$= 0.82 * (\delta^{18}\text{O}_{\text{p}}) - 19.13$ (Amiot et al. 2007)
Turtles and Fish	$= (1.01 * \delta^{18}\text{O}_{\text{p}}) - 22.3$ (Barrick et al. 1999)
Tapirs	$= (\delta^{18}\text{O}_{\text{p}} - 25.09) / 1.31$ (Tütken et al. 2006)
Rhino	$= (\delta^{18}\text{O}_{\text{p}} - 22.06) / 0.77$ (Delgado Huertas et al. 1995 , Tütken et al. 2006)
Peccary	$= (\delta^{18}\text{O}_{\text{p}} - 22.61) / 0.85$ (Longinelli 1984 , Tütken et al. 2006)
Camel	$= (\delta^{18}\text{O}_{\text{p}} - 24.9) / 1.01$ (D'Angela and Longinelli 1990)
Mastodon	$= (\delta^{18}\text{O}_{\text{p}} - 23.3) / 0.94$ (Ayliffe et al. 1992)

Table 4. Summary of Gray Fossil Site turtle, alligator, and fish stable isotope values.

Taxon	$\delta^{13}\text{C}_{\text{VPDB}}$ (sd)	$\delta^{13}\text{C}$ diet (sd)	$\delta^{18}\text{O}_{\text{c VPDB}}$ (sd)	$\delta^{18}\text{O}_{\text{c}}$ (sd)	$\delta^{18}\text{O}_{\text{c-p}}$ (sd)	$\delta^{18}\text{O}_{\text{c-water}}$ (sd)	$\delta^{18}\text{O}_{\text{c MAT}}$ (sd)	$\delta^{18}\text{O}_{\text{p}}$ (sd)	$\delta^{18}\text{O}_{\text{p water}}$ (sd)	$\delta^{18}\text{O}_{\text{p MAT}}$ (sd)
<i>Alligator</i>	-3.78 (5.17)	-15.7 (5.17)	-3.1 (0.69)	26.42 (0.71)	17.59 (0.69)	-4.71 (0.56)	21.43 (1.28)	13.84 (4.7)	-7.78 (3.86)	14.47 (8.73)
<i>Chrysemys corniculata</i>	-2.82 (2.14)	-11.72 (2.14)	-4.25 (0.22)	25.24 (0.23)	16.44 (0.22)	-5.7 (0.22)	19.19 (0.5)	14.54 (1.13)	-7.62 (1.14)	14.84 (2.59)
<i>Sternotherus palaeodorus</i>	-3.45 (1.64)	-12.35 (1.64)	-4.39 (1.13)	25.1 (1.16)	16.3 (1.13)	-5.84 (1.14)	18.87 (2.58)	12.03 (1.74)	-10.15 (1.76)	9.12 (3.99)
<i>Trachemys haugrudi</i>	-0.7 (2.03)	-9.6 (2.03)	-4.44 (1.71)	25.04 (1.77)	16.25 (1.72)	-5.89 (1.74)	18.75 (3.93)	13.96 (4.16)	-8.2 (4.2)	13.51 (9.5)
Testudinidae	0.7	-8.2	-3.2	26.32	17.49	-4.64	21.59	10.8	-11.39	6.31
All Turtles	-1.57 (2.21)	-10.47 (2.21)	-4.28 (1.26)	25.21 (1.3)	16.41 (1.26)	-5.73 (1.27)	19.12 (2.88)	13.52 (3.13)	-8.65 (3.16)	12.51 (7.16)
Camelidae	-13.8	-28.4	-1.7	27.87	19	-5.84	18.85			
<i>Mammot</i>	-14.1	-24.1	-4.2	25.29	16.49	-7.25	15.68			
<i>Teleoceras</i>	-13.3 (0.26)	-27.9 (0.26)	-4.82 (0.73)	24.65 (0.75)	15.86 (0.73)	-8.05 (0.95)	13.87 (2.14)			
<i>Tapirus polkensis</i>	-13.06 (0.91)	-27.66 (0.91)	-4 (0.67)	25.5 (0.69)	16.69 (0.67)	-6.41 (0.51)	17.57 (1.15)			
Tayassuidae	-13.1 (0.82)	-27.7 (0.82)	-4.4 (0.44)	25.09 (0.45)	16.29 (0.44)	-7.44 (0.51)	15.25 (1.16)			
All Mammals	-13.15 (0.83)	-27.6 (1.03)	-4.08 (0.8)	25.42 (0.83)	16.61 (0.81)	-6.72 (0.82)	16.87 (1.85)			
All Fish	-5.71 (0.6)	-15.21 (0.6)	-7.17 (1.1)	22.23 (1.13)	13.51 (1.1)	-8.66 (1.11)	12.49 (2.52)	13.54 (0.26)	-8.62 (0.27)	12.57 (0.6)
All Taxa Combined	-8.98 (5.54)	-21.56 (7.81)	-4.24 (1.28)	25.25 (1.32)	16.45 (1.28)	-6.42 (1.3)	17.55 (2.95)	13.61 (3.23)	-8.41 (2.99)	13.06 (6.77)

Table 5. Pairwise Comparisons of Modern and Fossil Bone and Enamel FTIR indices. W represents the sum of the ranks for the differences between paired observations in a Wilcoxon test. Bold indicates significant p-values.

Pair Comparison	CO_3/PO_4	CH_3/CH_2	Structural CO_3	Secondary CO_3
Bone (Fossil: Modern)	$W = 0$; $p = 0.000$	$W = 60$; $p = 0.005$	$W = 11$; $p = 0.050$	$W = 8$; $p = 0.022$
Enamel (Fossil: Modern)	$W = 10$; $p = 0.762$	$W = 21$; $p = 0.067$	$W = 7$; $p = 0.352$	$W = 7$; $p = 0.35$

Stable Isotopes

The CO_3 and PO_4 fractions of 21 specimens were analyzed for $\delta^{13}\text{C}$, $\delta^{18}\text{O}_\text{C}$, and $\delta^{18}\text{O}_\text{P}$ (Table 3). Although $\delta^{18}\text{O}_\text{P}$ values are provided (Table 4, Supplemental Table S2), FTIR analyses of the dataset demonstrate

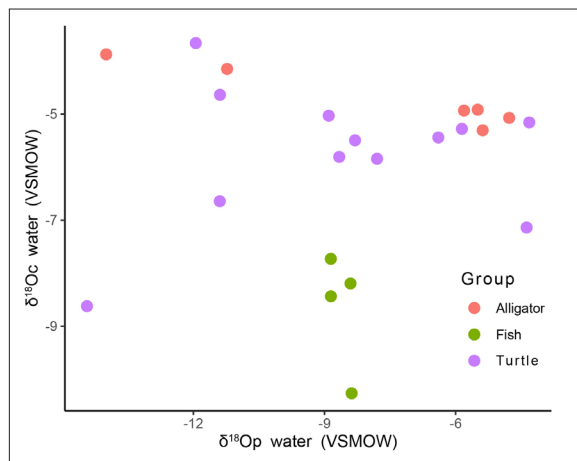


Figure 2. Relationship between $\delta^{18}\text{O}_\text{C}$ water and $\delta^{18}\text{O}_\text{P}$ water (all data). Samples do not plot along line of equilibrium, which is what would be expected if original $\delta^{18}\text{O}$ values were unaltered as has been demonstrated for fish (Sisma-Ventura et al., 2019) and mammals (Iacumin et al. 1996).

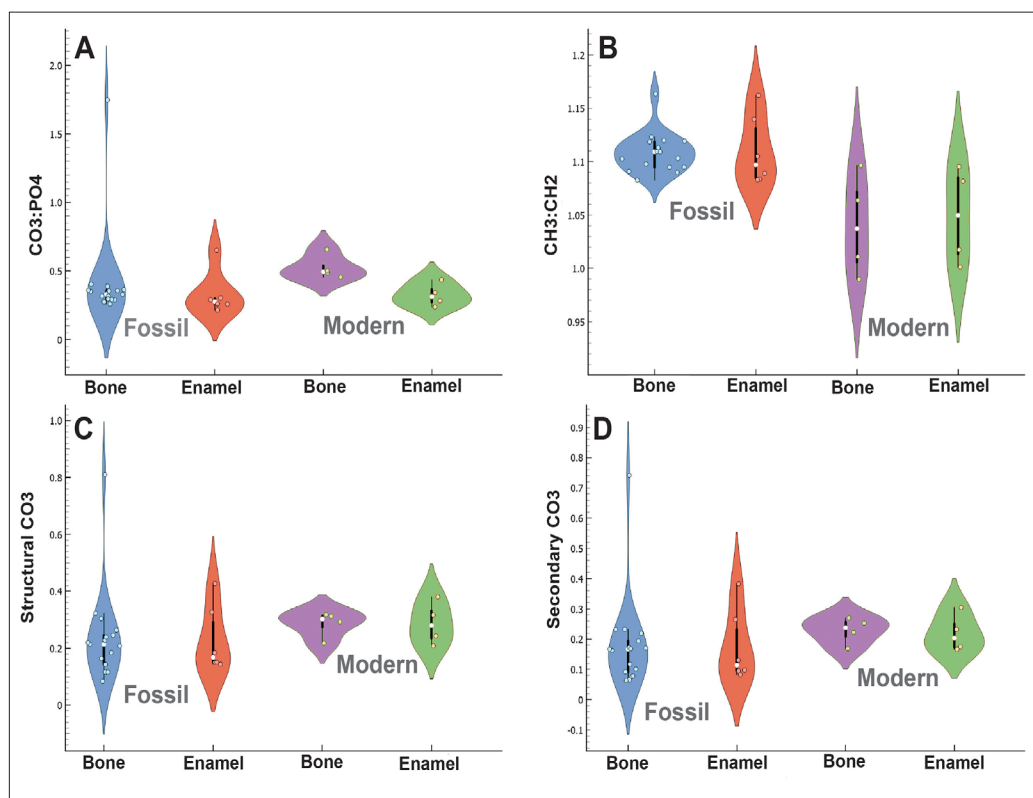


Figure 3. FTIR analyses of fossil and modern alligator enamel, turtle bone, and fish bone samples. A) CO_3/PO_4 index of GFS and modern, bone and tooth enamel. B) Index of microbial biomarkers (CH_3/CH_2). C) Structural CO_3 index of GFS and modern, bone and tooth enamel D) Secondary CO_3 index of GFS and modern, bone and tooth enamel. Comparisons between GFS and modern bone samples were statistically different in all FTIR indices. However, all enamel samples showed similar measures of preservation.

statistically significant differences between microbially altered ($\text{CH}_3\text{:CH}_2$) fossil samples and modern bones. We hypothesize that microbial communities colonizing GFS fossil bones during burial created PO_4 , shifting the $\delta^{18}\text{O}_\text{p}$ values of bone and tooth enamel away from their original stable isotope values. Therefore, it is likely that these values have been diagenetically altered and do not reflect the original biological signals.

All herpetofauna and aquatic animals (alligators, turtles, and fish) have $\delta^{13}\text{C}$ values significantly less negative than terrestrial mammals (Fig. 4).

The mean $\delta^{18}\text{O}_\text{c}$ -water values from mammals, turtles, and alligator are very similar and small differences do not reach the level of statistical significance. *Alligator* enamel $\delta^{18}\text{O}_\text{c}$ -water ranges from -4.1‰ to -5.3‰, with a mean value of -4.7 (sd = 0.6)‰ (Fig. 4). Growing season temperature (= MAT) calculations, on the basis of *Alligator* enamel CO_3 range from $20.1 \pm 1.9^\circ\text{C}$ to $22.7 \pm 1.9^\circ\text{C}$ with a mean of 21.4°C (sd = 1.3) (Fig. 5, Fig. 6).

Twelve turtle samples from four different taxa have an average $\delta^{18}\text{O}_\text{c}$ -water of -5.7‰ (sd = 1.3), indicative of an average temperature during bone formation of $19.1 \pm 1.9^\circ\text{C}$ (sd = 2.9). *Trachemys haugrudi* exhibits the largest range of $\delta^{18}\text{O}_\text{c}$ -water values, from -3.7‰ to -8.6‰. *Sternotherus palaeodorus* and *Chrysemys corniculata* both have a more constrained range, -5.0‰ to -6.6‰ and -5.4‰ to -5.8‰, respectively. The single result from a land tortoise (Testudinidae) is -4.6‰.

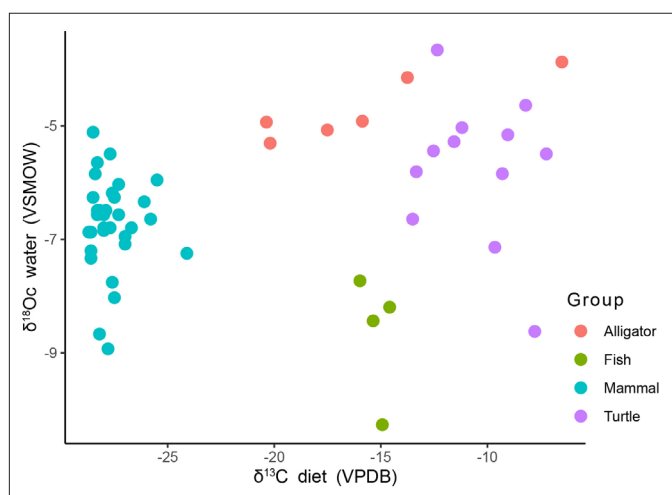


Figure 4. Comparison of $\delta^{13}\text{C}_\text{diet}$ and $\delta^{18}\text{O}_\text{water}$ of GFS fauna. Terrestrial mammals are distributed in C3-woodland isospace while aquatic turtles and alligators are more positive due to dominance of less negative $\delta^{13}\text{C}$ values in limestone-dominated foodwebs.

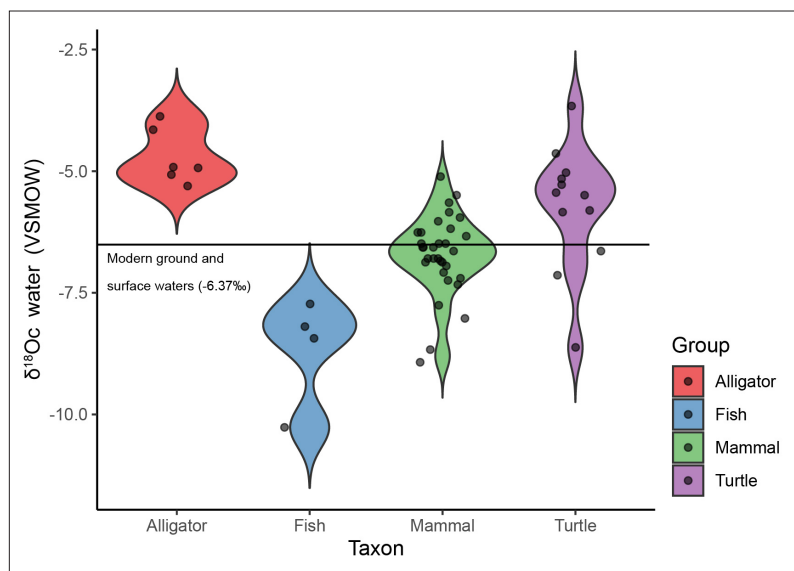


Figure 5. Estimated $\delta^{18}\text{O}_\text{c}$ -water of fossil samples from the Gray

Fish $\delta^{18}\text{O}$ -water values are generally more negative than mammals and reptiles. The average $\delta^{18}\text{O}$ -water of four samples is -8.7‰ (sd = 1.1) indicating a calculated water temperature of $12.5 \pm 1.9^\circ\text{C}$ (Fig. 5, Fig. 6).

Today, the average ground and surface water values within 30km of the GFS average -6.37‰ (sd = 0.26) (N = 6) (data from waterisotopes.org). This is similar to the average $\delta^{18}\text{O}$ -water for all GFS taxonomic groups which, collectively, have a mean $\delta^{18}\text{O}$ -water of -6.4‰ (sd = 1.3). However, not all taxonomic groups track this metric with equal fidelity. Mammals have mean $\delta^{18}\text{O}$ -water value of -6.7‰ (sd = 0.8), while turtles (mean $\delta^{18}\text{O}$ -water = -5.7‰ ; sd = 1.3) and alligators (mean $\delta^{18}\text{O}$ -water = -4.71‰ ; sd = 0.6) are less negative. Fish, however, have more negative values (mean $\delta^{18}\text{O}$ -water = -8.7‰ ; sd = 1.1).

Discussion

Biogeochemistry and Fossilization at the Gray Fossil Site

Stable isotope results from fossil bones and teeth must be interpreted within appropriate taphonomic and geochemical contexts. This includes an evaluation of whether isotopic results represent biological/ecological signals or whether they have been diagenetically altered, and instead, reflect local depositional histories. The depositional context of the GFS, a deep sinkhole pond that is potentially open to ground-water flow or microbial influences, must be taken into account. It is possible that the structural CO_3 or PO_4 fractions of bone or enamel could be altered, or both.

The average $\delta^{18}\text{O}$ spacing between CO_3 and PO_4 in pristine bioapatite is $\sim 9\text{‰}$ (Bryant et al. 1996). In this dataset, only the fish have $\delta^{18}\text{O}$ -water CO_3 - PO_4 spacing that approximate estimates of unaltered bioapatite (GFS fish CO_3 - PO_4 = 8.7‰). The CO_3 - PO_4 spacing for alligator (12.6‰) and turtles (11.9‰) is much higher, indicating some degree of diagenetic alteration has occurred. Also, the lack of a strong linear relationship between the $\delta^{18}\text{O}$ -water of CO_3 and PO_4 from multiple taxonomic groups strongly suggests that either the CO_3 or PO_4 fraction of bone and enamel has been diagenetically modified (following Iacumin et al. 1996).

Altered $\delta^{18}\text{O}$ values can occur when primary apatite is dissolved, then replaced by secondary apatite (Zazzo et al. 2004). Within the burial environment, two different models may explain divergence from equilibrium: 1) enamel altered by inorganic kinetic effects with local groundwater, which usually affects CO_3 or CO_2 dissolution of primary apatite followed by microbially-mediated reprecipitation of secondary apatite, which can alter the $\delta^{18}\text{O}$ values of PO_4 (Zazzo et al. 2004).

FTIR results show strongly overlapping CO_3/PO_4 values between the Gray Fossil Site and modern, unaltered sample datasets. However, this index could still be affected by alteration of either (or both) CO_3 and PO_4 fractions. Consideration of CH_3/CH_2 index indicates the possibility of microbial alteration in the

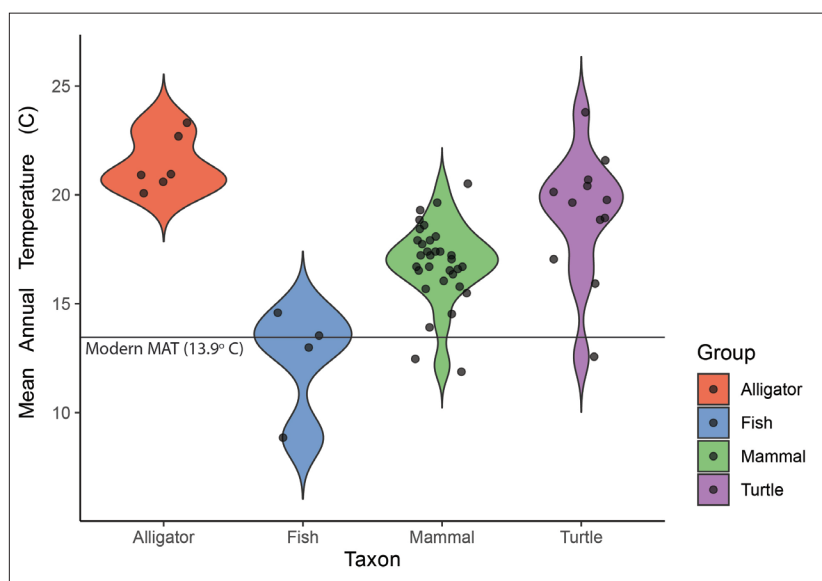


Figure 6. Estimated MAT of fossil samples from the Gray Fossil Site.

GFS samples, as compared to modern osteological samples. This suggests that the isotopic values of bone PO_4 was subject to microbially-mediated alteration during the course of fossilization at the GFS.

These results build on and generally complement a previous study of diagenesis at the GFS that note early diagenesis and potential microbial alteration of GFS bones (Keenan and Engel 2017). Keenan and Engel (2017) also indicated some dissolution and recrystallization of the CO_3 portion, although our comparisons to modern osteological samples suggest this may not have been significant enough to have altered $\delta^{18}\text{O}$ of GFS specimens.

Finally, although the PO_4 fraction of bone and enamel bioapatite is generally considered more robust to diagenetic alteration (Clementz 2012; Vennemann et al. 2002), results of the current study suggest that this generalization cannot be universally applied to all depositional environments.

Hydrodynamics of the Gray Fossil Site sinkhole

Despite their recovery from stratigraphically and spatially different locations within the sinkhole deposits, all four fish samples have generally lower $\delta^{18}\text{O}_{\text{water}}$ values. Since fish $\delta^{18}\text{O}_{\text{water}}$ is in equilibrium with their environment, it is likely that this is an accurate estimate for the $\delta^{18}\text{O}$ of the sinkhole waters. However, the more positive values in mammals and reptiles indicate more ingestion of warmer or evaporatively enriched surface waters, either in the sinkhole pond or from other surface sources. These differences suggest different source waters for the taxonomic groups, with fish living at levels of the pond that are buffered by deeper, colder groundwater. Turtles and alligators, occupying shallower depths at the surface and edges of the pond would have $\delta^{18}\text{O}_{\text{water}}$ values that are more influenced by surface water.

This pattern suggests that the GFS pond is buffered against variability in hydroclimate due to groundwater connections, and that there was likely a significant thermocline within the pond.

Species-specific Narratives of Climate and Growth

There are species-specific trends in the preservation of a $\delta^{18}\text{O}_{\text{water}}$ signal. These differences may be due to 1) temporal averaging in different sampled tissues (e.g., enamel vs. bone) and 2) variable behavior and landscape-use of the sampled taxonomic groups.

Higher $\delta^{13}\text{C}$ values in alligators, turtles, and fish compared to mammals suggests that $\delta^{13}\text{C}$ in aquatic food webs is strongly influenced by the dissolution of local limestone which generally has higher $\delta^{13}\text{C}$ values than atmospheric CO_2 (Phillippsen, 2013). Mammal $\delta^{18}\text{O}$ values are closest to modern surface waters in the region, while the $\delta^{18}\text{O}$ of turtles and alligators may better reflect water sources during the season of growth (for alligators from nearby North Carolina, this is April to October; Lance 2003). GFS fish values reflect the deepest parts of the sinkhole system.

The GFS *Chrysemys* and *Sternotherus* exhibit $\delta^{18}\text{O}$ reconstructed temperature estimates that are almost 7°C greater than the mean estimate for fish (Fig. 6). Modern stinkpots (*S. odoratus*) are generally nocturnal and do not bask, while painted turtles are diurnal and engage in extensive basking behavior (Ernst and Barbour 1972). Although we might expect to see similar evidence of these behavior patterns in the fossil record, in which *Chrysemys* exhibited generally higher temperature estimates due to basking behavior than *Sternotherus*, this is not the case. Reconstructed body temperatures of the two taxa are very similar (Table 4). Potential reasons for this pattern include: 1) taxon-specific fractionation effects that are still poorly understood, 2) basking behaviors do not significantly impact body water values, 3) body water $\delta^{18}\text{O}$ is more sensitive to seasonal growth than species-specific behaviors, or 4) fossil *Chrysemys* from the GFS did not behave in the same way as their modern counterparts.

Early Pliocene Climate at the Gray Fossil Site

The reconstructed early Pliocene groundwater temperature at the GFS on the basis of $\delta^{18}\text{O}$ in fish CO_3 is $12.5 \pm 1.9^\circ\text{C}$. The reconstructed surface water temperature on the basis of mammal $\delta^{18}\text{O}$ CO_3 is $16.9 \pm 1.9^\circ\text{C}$. This latter estimate is likely a more accurate measure of local MAT. The modern MAT calculated from a multi-year weather station 10 km to the east of the site (Tri-Cities airport, Washington Co., TN) is 13.9°C (Fig. 6). Although the Paleoclim model does not have a regional estimate for the early Pliocene, this is similar to the model prediction for GFS during the mid-Pliocene Warm Period (Brown et al. 2018; Dolan et al. 2015; Karger et al. 2017) (16.6°C), and similar to estimates derived from patterns in rodent and lagomorph hypsodonty (16.8°C ; Schap et al. 2021) and leaf margin analyses (16.6°C ; Simpson 2018).

Conclusions

This combination of stable isotope analyses and FTIR provided a robust framework for the assessment of diagenetic modification and potential modification of biological and/or ecological signals in vertebrate enamel and bone. In this dataset, which reflects unique diagenetic conditions in an early Pliocene-aged sinkhole fauna from northeastern Tennessee, isotopes of bone and enamel CO₃ seem to better reflect underlying climatic and biological signals in the stable isotopes of mammals and reptiles than bone and enamel PO₄. This is not a universal pattern, but underlies the complexity of diagenetic processes at the local scale and the importance of independent means of assessing sample quality.

Poikilothermic animals at GFS are variable paleothermometers. This variability may capture local changes in ecosystems through space and time. The GFS dataset shows different patterns for mammals, alligators, turtles, and fish. Variability in stable oxygen and carbon isotopes reflects different diets, home range sizes, life modes, and seasons of growth. Although climate factors were an important influence on vertebrate isotope values, they were by no means the only factor. A more holistic view of taxonomic trends provided better understanding of local climate, hydrological, and ecological conditions.

The reconstructed early Pliocene MAT at the GFS is $16.9 \pm 1.9^\circ\text{C}$ based on $\delta^{18}\text{O}$ of mammalian enamel CO₃. This is consistent with other estimates of MAT calculated for the GFS by a range of different techniques. This temperature is 3°C higher than the modern MAT calculated from a nearby weather station, indicating that this region of the southern Appalachians was slightly warmer than today during the time of deposition.

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Supplemental Data

Table S1 is available online at <https://www.eaglehill.us/papaonline/suppl-files/papa-003-durham-s1.pdf>

Table S2 is available online at <https://www.eaglehill.us/papaonline/suppl-files/papa-003-durham-s2.pdf>

Table S3 is available online at <https://www.eaglehill.us/papaonline/suppl-files/papa-003-durham-s3.pdf>

Literature Cited

- Ambrose, S.H., and L. Norr. 1993. Experimental Evidence for the Relationship of the Carbon Isotope Ratios of Whole Diet and Dietary Protein to Those of Bone Collagen and Carbonate. In *Prehistoric Human Bone*. Ed. J.B. Lambert and G. Grupe. Springer Verlag. https://doi.org/10.1007/978-3-662-02894-0_1.
- Amiot, R., C. Lécuyer, G. Escarguel, J-P Billon-Bruyat, E. Buffetaut, C. Langlois, S. Martin, F. Martineau, and J-M. Mazin. 2007. Oxygen Isotope Fractionation between Crocodilian Phosphate and Water. *Palaeogeography, Palaeoclimatology, Palaeoecology* 243(3–4): 412–20. <https://doi.org/10.1016/j.palaeo.2006.08.013>.
- Ayliffe, L. K., A. M. Lister, and A. R. Chivas. 1992. The Preservation of Glacial-Interglacial Climatic Signatures in the Oxygen Isotopes of Elephant Skeletal Phosphate. *Palaeogeography, Palaeoclimatology, Palaeoecology* 99(3):179–91. [https://doi.org/10.1016/0031-0182\(92\)90014-V](https://doi.org/10.1016/0031-0182(92)90014-V).
- Barrick, R.E., A.G. Fischer, and W.J. Showers. 1999. Oxygen Isotopes from Turtle Bone: Applications for Terrestrial Paleoclimates? *PALAIOS* 14(2):186. <https://doi.org/10.2307/3515374>.

- Boardman, G.S., and B.W. Schubert. 2011. First Mio-Pliocene salamander fossil assemblage from the southern Appalachians. *Palaeontologia Electronica*, 14(2), 16A.
- Bourque, J.R., and B.W. Schubert. 2015. Fossil Musk Turtles (Kinosternidae, *Sternotherus*) from the Late Miocene–Early Pliocene (Hemphillian) of Tennessee and Florida. *Journal of Vertebrate Paleontology* 35(1):e885441. <https://doi.org/10.1080/02724634.2014.885441>.
- Brown, J.L., D.J. Hill, A.M. Dolan, A.C. Carnaval, and A.M. Haywood. 2018. PaleoClim, High Spatial Resolution Paleoclimate Surfaces for Global Land Areas. *Scientific Data* 5(1):180254. <https://doi.org/10.1038/sdata.2018.254>.
- Burke, K. D., J.W. Williams, M.A. Chandler, A.M. Haywood, D.J. Lunt, and B.L. Otto-Bliesner. 2018. Pliocene and Eocene Provide Best Analogs for Near-Future Climates. *Proceedings of the National Academy of Sciences* 115(52):13288–93. <https://doi.org/10.1073/pnas.1809600115>.
- Bryant, D.J., P.L. Koch, P.N. Froelich, W.J. Showers, and B.J. Genna. 1996. Oxygen Isotope Partitioning between Phosphate and Carbonate in Mammalian Apatite. *Geochimica et Cosmochimica Acta* 60(24):5145–48. [https://doi.org/10.1016/S0016-7037\(96\)00308-0](https://doi.org/10.1016/S0016-7037(96)00308-0).
- Cerling, T.E., and J.M. Harris. 1999. Carbon Isotope Fractionation between Diet and Bioapatite in Ungulate Mammals and Implications for Ecological and Paleoecological Studies. *Oecologia* 120(3):347–63. <https://doi.org/10.1007/s004420050868>.
- Clark, G.M., M. Kohl, H.L. Moore, and I.D. Sasowsky. 2005. The Gray Fossil Site: A Spectacular Example in Tennessee of Ancient Regolith Occurrences in Carbonate Terranes, Valley and Ridge Subprovince, Southern Appalachians USA. Sinkholes and the Engineering and Environmental Impacts of Karst, September 22, 82–90. [https://doi.org/10.1061/40796\(177\)10](https://doi.org/10.1061/40796(177)10).
- Clementz, M.T. 2012. New Insight from Old Bones: Stable Isotope Analysis of Fossil Mammals. *Journal of Mammalogy* 93(2):368–80. <https://doi.org/10.1644/11-MAMM-S-179.1>.
- Conley, J. 2022. Community Structure Analysis of Turtles with Application to the Early Pliocene Gray Fossil Site. M.S. Thesis, East Tennessee State University. <https://dc.etsu.edu/etd/4121>.
- Cullen, T.M., F.J. Longstaffe, U.G. Wortmann, M.B. Goodwin, L. Huang, and D.C. Evans. 2019. Stable Isotopic Characterization of a Coastal Floodplain Forest Community: A Case Study for Isotopic Reconstruction of Mesozoic Vertebrate Assemblages. *Royal Society Open Science* 6(2):181210. <https://doi.org/10.1098/rsos.181210>.
- Cullen, T. 2023. Stable Isotope Analyses of Living and Extinct Crocodylians: Implications for Understanding Their Ecology, Environments, and Physiology. In *Ruling Reptiles: Crocodylian Biology and Archosaur Paleobiology*. Indiana University Press.
- Czaplewski, N.J. 2017. First Report of Bats (Mammalia: Chiroptera) from the Gray Fossil Site (Late Miocene or Early Pliocene), Tennessee, USA. *PeerJ* 5 (April): e3263. <https://doi.org/10.7717/peerj.3263>.
- Dal Sasso, G., Y. Asscher, I. Angelini, L. Nodari, and G. Artioli. 2018. A Universal Curve of Apatite Crystallinity for the Assessment of Bone Integrity and Preservation. *Scientific Reports* 8(1): 12025. <https://doi.org/10.1038/s41598-018-30642-z>.
- D’Angela, D., and A. Longinelli. 1990. Oxygen Isotopes in Living Mammal’s Bone Phosphate: Further Results. *Chemical Geology: Isotope Geoscience Section* 86(1):75–82. [https://doi.org/10.1016/0168-9622\(90\)90007-Y](https://doi.org/10.1016/0168-9622(90)90007-Y).
- Darcy, H.E. 2015. Additional Research and Taxonomic Resolution of Salamanders (Amphibia: Caudata) from the Mio-Pliocene Gray Fossil Site, TN. M.S. Thesis, East Tennessee State University. <https://www.proquest.com/docview/1846960805/abstract/D30F6AFC4F1D45CAPQ/1>.
- Delgado Huertas, A., P. Iacumin, B. Stenni, B. Sánchez Chillón, and A. Longinelli. 1995. Oxygen Isotope Variations of Phosphate in Mammalian Bone and Tooth Enamel. *Geochimica et Cosmochimica Acta* 59(20):4299–305. [https://doi.org/10.1016/0016-7037\(95\)00286-9](https://doi.org/10.1016/0016-7037(95)00286-9).
- DeSantis, L.R.G., and S.C. Wallace. 2008. Neogene Forests from the Appalachians of Tennessee, USA: Geochemical Evidence from Fossil Mammal Teeth. *Palaeogeography, Palaeoclimatology, Palaeoecology* 266(1–2):59–68. <https://doi.org/10.1016/j.palaeo.2008.03.032>.
- Dolan, A.M., A.M. Haywood, S.J. Hunter, et al. 2015. Modelling the Enigmatic Late Pliocene Glacial Event: Marine Isotope Stage M2. *Global and Planetary Change* 128 (May):47–60. <https://doi.org/10.1016/j.gloplacha.2015.02.001>.

- Doughty, E.M., S.C. Wallace, B.W. Schubert, and L.M. Lyon. 2018. First Occurrence of the Enigmatic Peccaries *Mylohyus elmorei* and *Prosthennops serus* from the Appalachians: Latest Hemphillian to Early Blancan of Gray Fossil Site, Tennessee. *PeerJ* 6 (November):e5926. <https://doi.org/10.7717/peerj.5926>.
- Ernst, C.H. and R.W. Barbour. 1972. *Turtles of the United States*. University Press of Kentucky.
- Gunnin, D. 2023. Femoral Osteohistology in American Alligators (*Alligator mississippiensis*) Reveals High Variation in Growth and Facilitates Interpretation of an Early Pliocene Alligator. M.S. Thesis, East Tennessee State University. <https://www.proquest.com/docview/2890698012/abstract/DC00DDDFDACA4870PQ/1>.
- Gunnin, D., B.W. Schubert, J.X. Samuels, K.E. Bredehoeft, and S. Maden. 2025. A New Plethodontid Salamander from the Early Pliocene of Northeastern Tennessee, USA, and its Bearing on Desmognathan Evolution. *Historical Biology*, May 16, 1–25. <https://doi.org/10.1080/08912963.2025.2501332>.
- Haywood, A.M., H.J. Dowsett, and A.M. Dolan. 2016. Integrating Geological Archives and Climate Models for the Mid-Pliocene Warm Period. *Nature Communications* 7(1):10646. <https://doi.org/10.1038/ncomms10646>.
- Iacumin, P., H. Bocherens, A. Mariotti, and A. Longinelli. 1996. Oxygen Isotope Analyses of Co-Existing Carbonate and Phosphate in Biogenic Apatite: A Way to Monitor Diagenetic Alteration of Bone Phosphate? *Earth and Planetary Science Letters* 142(1):1–6. [https://doi.org/10.1016/0012-821X\(96\)00093-3](https://doi.org/10.1016/0012-821X(96)00093-3).
- Jasinski, S.E. 2018. A New Slider Turtle (Testudines: Emydidae: Deirochelyinae: *Trachemys*) from the Late Hemphillian (Late Miocene/Early Pliocene) of Eastern Tennessee and the Evolution of the Deirochelyines. *PeerJ* 6 (February):e4338. <https://doi.org/10.7717/peerj.4338>.
- Jasinski, S.E. 2023. A New Species of *Chrysemys* (Emydidae: Deirochelyinae) from the Latest Miocene-Early Pliocene of Tennessee, USA and its Implications for the Evolution of Painted Turtles. *Zoological Journal of the Linnean Society* 198(1):149–83. <https://doi.org/10.1093/zoolinnean/zlac084>.
- Jasinski, S.E., and D.A. Moscato. 2017. Late Hemphillian Colubrid Snakes (Serpentes, Colubridae) from the Gray Fossil Site of Northeastern Tennessee. *Journal of Herpetology* 51(2):245–57. <https://doi.org/10.1670/16-020>.
- Jurestovsky, D.J. 2016. New Records of Colubrids from the Late Hemphillian Gray Fossil Site of Northeastern Tennessee. MS Thesis, East Tennessee State University.
- Karger, D.N., O. Conrad, J. Böhner, T. Kawohl, H. Kreft, R.W. Soria-Auza, N.E. Zimmermann, H.P. Linder and M. Kessler. 2017. Climatologies at High Resolution for the Earth's Land Surface Areas. *Scientific Data* 4(1):170122. <https://doi.org/10.1038/sdata.2017.122>.
- Keenan, S.W., and A.S. Engel. 2017. Reconstructing Diagenetic Conditions of Bone at the Gray Fossil Site, Tennessee, USA. *Palaeogeography, Palaeoclimatology, Palaeoecology* 471:48–57. <https://doi.org/10.1016/j.palaeo.2017.01.037>.
- Lance, V. 2003. Alligator Physiology and life history: The importance of temperature. *Experimental Gerontology* 38(7):801-805.
- Longinelli, A. 1984. Oxygen Isotopes in Mammal Bone Phosphate: A New Tool for Paleohydrological and Paleoclimatological Research? *Geochimica et Cosmochimica Acta* 48(2):385–90. [https://doi.org/10.1016/0016-7037\(84\)90259-X](https://doi.org/10.1016/0016-7037(84)90259-X).
- Loron, C.C., E. Rodriguez Dzul, P.J. Orr, A.V. Gromov, N.C. Fraser, and S. McMahon. 2023. Molecular Fingerprints Resolve Affinities of Rhynie Chert Organic Fossils. *Nature Communications* 14(1):1387. <https://doi.org/10.1038/s41467-023-37047-1>.
- Maden, S. 2023. An Early Pliocene Fish Assemblage from the Southern Appalachians: Ichthyofauna of the Gray Fossil Site. M.S. Thesis, East Tennessee State University. <https://dc.etsu.edu/etd/4262>.
- Mead, J.I., B.W. Schubert, S.C. Wallace, and S.L. Swift. 2012. Helodermatid lizard from the Mio-Pliocene oak-hickory forest of Tennessee, eastern USA, and a review of monstrosaurian osteoderms. *Acta Palaeontologica Polonica*, 57(1), 111-121.
- Ochoa, D., M. Whitelaw, Y. Liu, and M. Zavada. 2012. Palynology of Neogene Sediments at the Gray Fossil Site, Tennessee, USA: Floristic Implications. *Review of Palaeobotany and Palynology* 184 (September):36–48. <https://doi.org/10.1016/j.revpalbo.2012.03.006>.

- Odom, W.E., D.E. Granger, S.C. Wallace. 2025. $^{10}\text{Be}/^{9}\text{Be}$ and $^{26}\text{Al}/^{10}\text{Be}$ Support a Late Miocene Burial Age for Basal Gray Fossil Site Sediments. *Pan-American Paleontology* 1:1–20.
- Phillippsen, B. 2013. The freshwater Reservoir Effect in radiocarbon dating. *Heritage Science* 1:24. <https://doi.org/10.1186/2050-7445-1-24>
- Quirk, Z.J., and E.J. Hermesen. 2021. Neogene *Corylopsis* Seeds from Eastern Tennessee. *Journal of Systematics and Evolution* 59(3):611–21. <https://doi.org/10.1111/jse.12571>.
- Samuels, J.X., K.E. Bredehoeft, and S.C. Wallace. 2018. A New Species of *Gulo* from the Early Pliocene Gray Fossil Site (Eastern United States); Rethinking the Evolution of Wolverines. *PeerJ* 6 (April):e4648. <https://doi.org/10.7717/peerj.4648>.
- Samuels, J.X., O.R. Williams, S. Maden, B.W. Schubert. 2025. Early Pliocene Deer from the Gray Fossil Site, Appalachian Highlands, Tennessee, USA. *Palaeontologia Electronica*, 38(2), 1-24.
- Scaggion, C., M. Marinato, G. Dal Sasso, L. Nodari, T. Saupe, S. Aneli, L. Pagani, C.L. Scheib, M. Rigo and G. Artioli. 2024. A Fresh Perspective on Infrared Spectroscopy as a Prescreening Method for Molecular and Stable Isotopes Analyses on Ancient Human Bones. *Scientific Reports* 14(1):1028. <https://doi.org/10.1038/s41598-024-51518-5>.
- Schap, J.A., J.X. Samuels, and T.A. Joyner. 2021. Ecometric Estimation of Present and Past Climate of North America Using Crown Heights of Rodents and Lagomorphs. *Palaeogeography, Palaeoclimatology, Palaeoecology* 562 (January):110144. <https://doi.org/10.1016/j.palaeo.2020.110144>.
- Schattauer, S.A. 2012. Non-Ranid Anurans of the Mio-Pliocene Gray Fossil Site, Tennessee. M.S. thesis, East Tennessee State University. <https://dc.etsu.edu/etd/1454>.
- Shabaga, B.M., H. Gough, M. Fayek, and R.D. Hoppa. 2018. A Simplified Silver Phosphate Extraction Method for Oxygen Isotope Analysis of Bioapatite. *Rapid Communications in Mass Spectrometry* 32(15):1237–42. <https://doi.org/10.1002/rcm.8149>.
- Shunk, A.J., S.G. Driese, and G.M. Clark. 2006. Latest Miocene to Earliest Pliocene Sedimentation and Climate Record Derived from Paleosinkhole Fill Deposits, Gray Fossil Site, Northeastern Tennessee, U.S.A. *Palaeogeography, Palaeoclimatology, Palaeoecology* 231(3–4):265–78. <https://doi.org/10.1016/j.palaeo.2005.08.001>.
- Shunk, A.J., S.G. Driese, and J.A. Dunbar. 2009. Late Tertiary Paleoclimatic Interpretation from Lacustrine Rhythmites in the Gray Fossil Site, Northeastern Tennessee, USA. *Journal of Paleolimnology* 42(1):11–24. <https://doi.org/10.1007/s10933-008-9244-0>.
- Siegert, C., and E.J. Hermesen. 2020. *Cavilignum pratchettii* Gen. et Sp. Nov., a Novel Type of Fossil Endocarp with Open Locules from the Neogene Gray Fossil Site, Tennessee, USA. *Review of Palaeobotany and Palynology* 275 (April):104174. <https://doi.org/10.1016/j.revpalbo.2020.104174>.
- Simpson, E. 2018. Estimating the Temperature at the Gray Fossil Site from the Paleobotanical Record. Paper presented at the Geological Society of America, Southeastern Section: 67th Annual Meeting - 2018, Knoxville, TN. April 13. <https://gsa.confex.com/gsa/2018SE/webprogram/Paper312393.html>.
- Simpson, E., B.E. Crowley, and D.M. Sturmer. 2023. Is the Damage Worth It? Testing Handheld XRF as a Non-Destructive Analytical Tool for Determining Biogenic Bone and Tooth Chemistry Prior to Destructive Analyses. *Frontiers in Environmental Archaeology* 1 (January). <https://doi.org/10.3389/fearc.2022.1098403>.
- Sisma-Ventura, G., T. Tütken, S. T., Peters, O. M. Bialik, I. Zohar, and A. Pack (2019). Past aquatic environments in the Levant inferred from stable isotope compositions of carbonate and phosphate in fish teeth. *PLoS One*, 14(7), e0220390.
- Tipple, B.J., S.R. Meyers, and M. Pagani. 2010. Carbon Isotope Ratio of Cenozoic CO_2 : A Comparative Evaluation of Available Geochemical Proxies. *Paleoceanography* 25(3). <https://doi.org/10.1029/2009PA001851>.
- Turala, M. M. (2026). Estimating Body Size and Biogeography in the Genus *Alligator*. M.S. Thesis, East Tennessee State University. <https://dc.etsu.edu/etd/4687/>
- Tütken, T., T. W. Vennemann, H. Janz, and E. P. J. Heizmann. 2006. Palaeoenvironment and Palaeoclimate of the Middle Miocene Lake in the Steinheim Basin, SW Germany: A Reconstruction

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- from C, O, and Sr Isotopes of Fossil Remains. *Palaeogeography, Palaeoclimatology, Palaeoecology* 241(3):457–91. <https://doi.org/10.1016/j.palaeo.2006.04.007>.
- Vennemann, T.W., H.C. Fricke, R.E. Blake, J.R. O’Neil, and A. Colman. 2002. Oxygen Isotope Analysis of Phosphates: A Comparison of Techniques for Analysis of Ag_3PO_4 . *Chemical Geology* 185(3–4):321–36. [https://doi.org/10.1016/S0009-2541\(01\)00413-2](https://doi.org/10.1016/S0009-2541(01)00413-2).
- Whiting, E.T., D.W. Steadman, and J. Krigbaum. 2016. Paleoeecology of Miocene Crocodylians in Florida: Insights from Stable Isotope Analysis. *Palaeogeography, Palaeoclimatology, Palaeoecology* 451 (June):23–34. <https://doi.org/10.1016/j.palaeo.2016.03.009>.
- Zachos, J., M. Pagani, L. Sloan, E. Thomas, and K. Billups. 2001. Trends, Rhythms, and Aberrations in Global Climate 65 Ma to Present. *Science* 292(5517):686–93. <https://doi.org/10.1126/science.1059412>.
- Zazzo, A., C. Lécuyer, S.M.F. Sheppard, P. Grandjean, and A. Mariotti. 2004. Diagenesis and the Reconstruction of Paleoenvironments: A Method to Restore Original $\delta^{18}\text{O}$ Values of Carbonate and Phosphate from Fossil Tooth Enamel. *Geochimica et Cosmochimica Acta* 68(10):2245–58. <https://doi.org/10.1016/j.gca.2003.11.009>.
- Zobaa, M.K., M.S. Zavada, M.J. Whitelaw, A.J. Shunk, and F.E. Oboh-Ikuenobe. 2011. Palynology and Palynofacies Analyses of the Gray Fossil Site, Eastern Tennessee: Their Role in Understanding the Basin-Fill History. *Palaeogeography, Palaeoclimatology, Palaeoecology* 308(3–4):433–44. <https://doi.org/10.1016/j.palaeo.2011.05.051>.