ABSTRACT

Thermoregulation and Dental Isotopes Reveal the Behavior and Environment of Pleistocene Megafauna at Waco Mammoth National Monument

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Waco Mammoth National Monument (WMNM) in central Texas is a significant Pleistocene paleontological site, containing at least 16 Columbian mammoths and specimens of 12 other vertebrate taxa. Interpreting this site, however, is contingent on understanding the environment Pleistocene animals lived in and how they interacted with that environment behaviorally. Actualistic studies of modern analogs can be used to better understand the behavior and geographic range of a Pleistocene animal and thus increase their usefulness as paleoclimate and paleoenvironmental indicators. Mass and thermal modeling studies done on modern tortoises have been used to determine the temperature tolerance of the giant tortoise species of central Texas, constraining the climate present at WMNM during its formation. Understanding the long-term movements of a fossil organism can reflect the environment it lives in. Strontium isotope ratio analysis of megafaunal teeth from WMNM have shown that not all of the mammoths at the site shared a geographic origin. The behavior revealed – mammoths congregated at WMNM from a wide area – necessitated a reconsideration of the long-standing mechanism of death for the megafauna there. Serial analysis of carbon and oxygen isotopes from the same teeth revealed that while the animals at WMNM shared a diet, some may have had distinct sources of drinking water. They also reveal that the WMNM megafauna lived in a drier, more drought-prone world that previously thought. Taking a multi-proxy approach to better understand interactions between Pleistocene megafauna and the environmental changes they experienced should inform our attempts to conserve our remaining megafauna.

Thermoregulation and Dental Isotopes Reveal the Behavior and Environment of Pleistocene Megafauna at Waco Mammoth National Monument by

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CHAPTER ONE

Introduction

Three separate scholarly articles have been organized as chapters to make up this dissertation. Donald A. Esker was the lead author on each, but he worked with people from three separate institutions to produce this volume. Without this collaboration, this volume would not have come to be.

Chapter Two is titled, "Reconstructing the mass and thermal ecology of North American Pleistocene tortoises," and was co-authored by Donald A. Esker, Steven L. Forman, and Dava K. Butler. This study adjusts the way fossil tortoises are used as paleoclimate proxies by developing numerical models for their mass and thermal properties. The project was conceived of and lead by DAE. DAE and DKB collected morphometric data on live tortoises, and DAE collected morphometric data on fossil tortoises. DAE analyzed the morphometric data with help and advice from SLF. The manuscript was written by DAE with input from SLF. SLF and DKB both suggested changes and improvements to the paper.

Chapter Three is titled, "Home range of the Columbian mammoths (*Mammuthus columbi*) and grazing herbivores from the Waco Mammoth National Monument, (Texas, USA) based on strontium isotope ratios from tooth enamel bioapatite." The purpose of the research was to determine the geographic range of the megafauna at Waco Mammoth National Monument (WMNM) using ⁸⁷Sr/⁸⁶Sr ratios in dental enamel and native vegetation. It was co-authored by Donald A. Esker, Steven L. Forman, Chris Widga, J.

Douglas Walker, and Joseph E. Andrew. The project was conceived and lead by DAE. Sample collection was done by DAE with equipment, instruction, and advice from CW. Mass spectrometry was done by JDW and JEA. Data analysis was done by DAE with advice from JDW. The manuscript was written by DAE with input from SLF and CW. SLF, CW, JDW, and JEA all made editorial contributions.

Chapter Four is titled, "Environmental constraints from serial δ^{13} C and δ^{18} O analysis of teeth from Waco Mammoth National Monument, TX USA," and was coauthored by Donald A. Esker, Steven L. Forman, Chris Widga, and Ren Zhang. This research was done to examine sub-seasonal scale changes in δ^{13} C and δ^{18} O of fossil teeth from WMNM. This shed light on the movements, diet, climate, and death of the megafauna preserved there. The project was conceived of and lead by DAE. Sample collection was done by DAE with equipment and advice from CW. Mass spectrometry was done by RZ. Data analysis was done by DAE with advice from CW. The manuscript was written by DAE with input from SLF and CW. SLF, CW, and RZ all made editorial contributions.

Chapter Five is the summary of the work so far and suggests directions for continuing research.

CHAPTER TWO

Reconstructing the Mass and Thermal Ecology of North American Pleistocene Tortoises

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Abstract

Researchers often interpret the presence of tortoises in Pleistocene assemblages as evidence of an interglacial age, based on an assumption that these fossils indicate thermic climates, as modern giant tortoises require. Since the Paleocene, tortoises have been common components of terrestrial fossil assemblages and have repeatedly evolved species of giant size. Whereas extant giant tortoises are found only on islands off the coasts of South America and Africa, at least two species persisted in North America until the terminal Pleistocene. These tortoises, *Hesperotestudo crassiscutata* and *Gopherus* "hexagonatus," both of which reached carapace lengths of >1 m, were distributed across the southern United States. This study provides new metrics to derive quantitative weight estimates from measurements of the tortoise shell. The linear measurements of 69 anatomical features of the shells of 108 live tortoises indicate that the regression between straight carapace length and weight is most significant, with a maximum $r^2 > 0.99$. This regression is useful for tortoises that weigh between 1.8 and 339 kg (Table A2). This mass estimate, coupled with a heat dissipation rate derived from thermoregulation modeling, provides estimates of how long tortoises can maintain a viable body temperature at low ambient temperatures. Depending on size, a tortoise can survive a

maximum of 2.3 to 33 hours of freezing temperatures, which corresponds to a mean annual temperature \geq 22°C and a mean winter low temperature \geq 7.5°C. This analysis infers warmer temperatures at Pleistocene sites with fossil tortoise occurrences than previous qualitative estimates.

Introduction

Many intervals in geological history have served as case studies in the biological effects of climate change, including the Paleocene-Eocene thermal maximum and the Miocene thermal optimum (Wolfe 1994; Kraus and Riggins 2007). The evidence for past climate change comes from a variety of paleoclimate proxies such as ice and sediment cores, palynology, speleothem records, and the isotopic composition of soil carbonate and organic matter in paleosols (Curry and Baker 2000; Serefiddin et al. 2004; Tripsanas et al. 2007; Oerter et al. 2016). However, the most commonly used proxies for the Quaternary are the composition of ancient faunas. Many Quaternary taxa are still extant, and their climatic requirements are thought to be mostly unchanged, so Quaternary fossils can be interpreted as paleoclimate proxies (Hashemi et al. 2015). One taxon commonly used is the family Testudinidae: tortoises. Modern North American tortoises prefer a mean annual temperature of approximately 22°C, and it is assumed that this held true throughout the Quaternary (Hibbard 1960). Statistical studies on the diversity, body size, range size, and distribution of turtles as a whole seem to provide support for this idea (Angielczyk et al. 2015). As a result, fossil tortoises have even been used as biostratigraphic indicators of interglacial conditions (Nordt et al. 2015). Recent studies on potential behavioral and physiological thermoregulation in tortoises have questioned this association (Moll and Brown 2017). No attempt has been made to quantify the thermal

properties of each fossil tortoise taxa, however. This study evaluates the quantitative basis for a fossil tortoise-based paleoclimate proxy.

The Quaternary tortoise genera found in the southern United States and northern Mexico were the high-domed *Hesperotestudo*—previously assigned to *Geochelone*—and the vertically compressed *Gopherus* and *Xerobates*. Of these, only small (<10 kg) members of *Gopherus* and *Xerobates* persist in the region today (Kazmaier et al. 2001). Modern North American tortoises are only found south of 34°N, but fossil tortoises occur as far north as Ellesmere Island, Canada (82°N) in the Paleogene and as far north as Pennsylvania and Indiana (40°N) during the Pleistocene (Parris and Daeschler 1995; Eberle et al. 2009; Czaplewski et al. 2013). Modern North American tortoises live in mesic to xeric habitats and are almost exclusively herbivorous (Rostal et al. 2014). Most of the ~20 tortoise genera outside North America live in warm climates with low seasonality (Lagarde et al. 2002; Fritz et al. 2012).

Hibbard (1960) used the existence of non-analogue herpetofaunas as evidence of a qualitatively less seasonal climate during parts of the Pleistocene. Some sites, such as Pipe Creek Sinkhole in Indiana, contain extraliminal reptilian and amphibian taxa with modern ranges to the north or south of the sinkhole, indicating taxa adapted to a mesic climate with broad latitudinal ranges (Hibbard 1960). Hibbard thought that the existence of "*Geochelone*" *crassiscutata* at Pipe Creek implied that climate in the region at the time of deposition had less extreme lows than those occurring in the upper Great Plains in the modern day (Hibbard 1960).

Extant species of "*Geochelone*" live in warmer environments than current conditions in the southern United States or northern Mexico. Early twentieth-century

attempts to transplant these species to the Gulf Coastal plains, Mexican Plateau, and the Sonoran and Mojave Deserts failed (Hibbard 1960). Furthermore, behaviors for coping with temperature extremes, such as burrowing and salivating, documented in genera like Gopherus and Xerobates, are absent in Geochelone. The interpretation of the equable climate tolerance of tortoises (Hibbard, 1960) was extended to infer low temperature variability, even in the absence of extraliminal taxa living in cooler and warmer modern climates (Jackson and Kaye 1975; Holman 1980; Nordt et al. 2015; Biewer et al. 2016). Molecular and morphological phylogenetics has revealed *Geochelone* contains only two species, and both are restricted to south Asia (Crumly 1984; Le et al. 2006; Fritz and Bininda-Emonds 2007). North American fossil tortoises are not in the subfamily Testudininae with Geochelone, so environmental and behavioral inferences based on the habits of these south Asian animals may lack merit (Fig. 2.1A–C). Tortoises' significance as a paleoclimate indicator in the late Pleistocene should be reexamined, given their present distribution across Eurasia, Africa, and the Americas and broad latitudinal range of ~40°S to ~40°N (Leidy 1868; Cope and Drinker 1895; Sellards 1916, 1917; Hay 1917, 1926; Simpson 1930; Gidley and Gazin 1938; Brattstrom 1954, 1961; Auffenberg 1958, 1962; Crook and Harris 1958; Holman 1958, 1959a,b, 1962; Mecham 1958; Dalquest 1962; Bonifay and Bonifay 1963; Gut and Ray 1963; Edmund 1965; Schultz 1969; M.-F. Bonifay 1973; Converse 1973; Bachmayer 1975; Bachmayer and Symeonidis 1975; Malatesta 1980; Mangili 1980; Roth and Laerm 1980; Parmley 1986; Holman and Winkler 1987; Westgate 1989; Bon and Sala 1991; E. Bonifay 1991; de Broin 1991; Amani and Geraads 1993; Raynal et al. 1993; Rhodes 1994; Barbato and Gliozzi 1995; Emslie 1995; Meylan 1995; Morgan and Hulbert 1995; Dundas et al. 1996; Pajak et al.

1996; Salotti et al. 1997; Agusti et al. 1999; Hervet and Salotti 2000; Cantalamessa et al. 2001; Wagner and Prothero 2001; Meers and Hulbert 2002; Bianucci and Landini 2005; Cisneros 2005; Steadman et al. 2007; Brinkman et al. 2008; Dundas 2013; Harvati et al. 2013; Thomas et al. 2014)



Figure 2.1. Selected phylogenies of tortoises. a) Morphological phylogeny of extant tortoises. Modified from Crumly, 1984. b) Molecular phylogeny of extant tortoises. Modified from Le et al., 2006. c) Morphological phylogeny of modern xerobatine tortoises and their fossil relatives. Adapted from Reynoso and Montellano-Ballesteros, 2004.

Modeling the thermal biology and ecology of individual extinct taxa yields a quantitative basis to assess the temperature tolerance of extinct vertebrates, independent of modern analogues. Thermodynamic modeling has provided insights into the ecology and climate requirements of early synapsids, dinosaurs, and a variety of smaller poikilotherms (Bennett 1996; Head et al. 2009; Sander et al. 2011; Clarke et al. 2013). One consistent result of thermodynamic modeling was the high thermal inertia of large bodies (>25 kg), which enhances the organism's tolerance to diurnal temperature fluctuations (Sander et al. 2011; Seymour 2013). This research focused on reconstruction of the thermobiology of extinct tortoises. To this end, estimates of size and mass are needed for extinct tortoises. This contribution provides straightforward numeric analysis and models to estimate tortoise mass and thermal inertia, which yield new quantitative relations for paleoclimatic inferences.

Methods

Biometrics of Living Tortoises

Linear morphometric measurements were taken of living tortoises to evaluate the correlation between measurable external features of tortoises and their weight. Previous approaches identified straightforward osteological measurements that correlate significantly with mass in extant relatives of the extinct organism under study (Cassini et al. 2012). Various measurements were taken of shell attributes of living tortoises, analogous to counterparts in the fossil record (Table A1).

Tortoise shells are composed of a dorsal carapace and a ventral plastron. These shell parts, often well preserved, consist of an inner layer of osteoderms connected by a thin sheet of innervated fibrous tissue to an outer layer of keratinous scutes (Morales Pérez and Serra 2009; Bramble and Hutchison 2014). These scutes have sulci around their perimeters that anchor them to the osteoderms (Chiari et al. 2009). Therefore the dimensions of the scutes are measurable even when only the osteoderms remain. After the weight of a live tortoise was determined, 8 measurements were taken of the whole shell and 61 measurements were taken of the dimensions of individual scutes (Table A1). In total, 108 tortoises of 10 genera and 18 species were examined for a total of ~7000 measurements of carapaces and scutes (Table A2). This study excluded six tortoises of the genera *Malacochersus* and *Kinixys* from analysis, because their shell morphologies

were unlike any other genus of tortoises. Measurements of the carapace and carapacial scutes were completed on all tortoises, but plastral measurements were restricted to tortoises that weighed \leq 50 kg, because of safety concerns for all involved. These size measurements were regressed against the tortoise's live weight to determine the mathematical function to estimate a fossil tortoise's corresponding weight.

Linear Measurement Versus Mass Regressions

Estimating the live weight of an extinct organism is a matter of geometry. The mass of solid objects of identical shapes and differing sizes scales with the cube of any linear measurement.

The formula is:

Equation 2.1. Equation for determining the mass of an object based on a linear dimension

$$y = a(x^b)$$

where x is the linear measurement, y is the mass, a is a constant for that measurement, and b, the rate function, is 3. This is called isometric growth, rare among vertebrates.

Most vertebrates undergo allometric growth, in which different parts of the organism grow at different rates, leading to varying proportions of body parts over time. The degree of allometry for different body parts is described by the variable *b* in equation (1), such that *b* can be >3 or <3. If the mass of the animal increases slowly in reference to a linear measurement that deviates from isometric growth, then *b* < 3. If the mass of the animal increases comparatively quickly in reference to a linear measurement, then *b* > 3. The rate variable (*b*) in the length versus weight regressions for the tortoises varied between 2.5 and 3.5, reflecting anatomical features that become proportionally smaller

and larger through ontogeny, respectively. By measuring the dimensions of whole shells and individual scutes on multiple tortoises, this method allows us to produce equations of allometry for each measurement. In turn, these equations can be used to estimate mass.

Thermal Modeling

There is a lack of studies on the thermal tolerances of tortoises. Specifically, the effect of low temperatures on tortoise viability is undocumented. Freezing is an obvious danger; tortoise tissue starts to freeze at -1.6°C, but North American tortoises survive momentary supercooling to -6.0°C in only <5% of trial runs (Spotila et al. 2014). Tortoises rely on hindgut fermentation to extract nutrients from food. Low temperatures can reduce the activity of a tortoise's gut flora until it starves or dies of gastroenteritis (Hibbard 1960). For this reason, veterinarians caution that small captive North American tortoises should be brought indoors when the nighttime temperature drops below ~4.4°C (Bailey 1992; Nussear et al. 2007). Conservatively, we choose 5.0°C as the viable limit for modeling.

An empirical thermal model was developed to estimate the temperature tolerance of extinct tortoises. Tortoise temperatures were taken with an infrared thermometer and a FLIR thermal imaging camera, thus there was no physical interaction with the tortoises during measurements. The temperature of nine tortoises of seven species was monitored and recorded at regular intervals of 3 or 9 minutes for 45 to 720 minutes. For six of the tortoises, the initial body temperature was \sim 30°C after basking outdoors, with cooling of the tortoise monitored indoors with an air temperature of \sim 20°C. The ambient indoor temperature was recorded simultaneously with tortoise temperatures. The remaining three tortoises, >100 kg members of the genera *Chelonoidis* and *Aldabrachelys*, were

monitored over 12 hours in their outdoor enclosure as ~30°C daytime temperatures dropped to nighttime temperatures of ~20°C. As moving between habitats with different temperatures is a regular part of reptile behavior, approval from the Baylor Institutional Animal Care and Use Committee was unnecessary. Ultimately, 11 time series were recorded for cooling of tortoises (Table A3). The tortoise temperatures were regressed simultaneously against time and ambient air temperature to produce a 3D curve (Fig. 2.2).

R#18 Aldabrachelys gigantea 3D Cooling Curve: Time (s) and Ambient Temperature (° C) vs. Tortoise Temperature (° C)



Figure 2.2. The 3D regression describing cooling from R#18, *Aldabrachelys gigantea*. The 'x' axis represents time in seconds, the 'y' axis represents ambient temperature in ° C, and the 'z' axis represents tortoise temperature in ° C. R2 = 0.961, RMSE = 0.1696.

These data lead to the formulation for cooling of tortoises:

Equation 2.2. Equation for determining the cooling time of a tortoise.

$$T = a(t^{0.5} \times (\ln T_a)^2) + b$$

where *T* is the tortoise's temperature, *t* is the time in seconds, and T_a is the ambient air temperature. The constant *a* is determined by the mass of the tortoise:

Equation 2.3. Equation for determining the mass constant for equation 2.2.

$$a = -0.00347 \times (m^2 - 0.16067m) / (m^2 - 0.27616m + 0.01853)$$

where m is the tortoise's mass in kilograms. The constant b is determined by the starting temperature of the tortoise:

Equation 2.4. Equation for determining the temperature constant for equation 2.2.

$$b = 0.7062T_{init} + 9.17$$

where T_{init} is the tortoise's initial temperature in degrees Celsius. The *a* and *b* values of the cooling curves for each live tortoise in the thermal modeling study, along with the r^2 values, are shown in Table 2.1.

Tortoise #	Tortoise Name	а	b	r^2
R#115	Oliver 7~27~17*	-0.0095	32.6315	0.947
R#115	Oliver 7~28~17*	-0.0131	34.0439	0.965
R#115	Oliver 7~29~17*	-0.0191	36.9864	0.992
R#2	Aegis	-0.0036	30.7876	0.754
R#23	Miss Sokatra	-0.0055	30.0421	0.996
UN#	Male A. radiata	-0.0035	26.2365	0.787
R#18	Skipper	-0.0035	31.5722	0.988
R#19	Professor	-0.0035	31.2584	0.978
R#20	Gilligan	-0.0035	30.1991	0.966
R#1	Bob	-0.0037	28.1589	0.880
R#4	Plushy	-0.0036	28.0412	0.891

Table 2.1. Constants for Equation 2.2 for each living tortoise used to develop the thermal model, along with an r^2 value for the correlation between the model and the measured temperatures. Asterisk (*) indicates that the animal belongs to the corresponding author; all other tortoises are property of the Cameron Park Zoo. The R# refers to the 'Record Numbers' listed for each tortoise in Appendix 1.2.



Example cooling curves are shown in Fig. 2.3.

Figure 2.3. Cooling curves for four living tortoises. Dots indicate measured temperatures during cooling, lines indicate cooling curves calculated according to Equation 2.2. a) R#23 Pyxis planicauda, a = -0.0055, b = 30.0421, correlation = 0.996, RMSE = 1.007, b) R#1 *Xerobates berlandieri*, a = -0.0037, b = 28.1589, correlation = 0.938, RMSE = 0.685, c) Un# CPZ *Astrochelys radiata*, a = -0.0035, b = 26.2365, correlation = 0.887, RMSE = 0.352, d) R#18 *Aldabrachelys gigantea*, a = -0.0035, b = 31.5723, correlation = 0.994, RMSE = 0.595.

Results

Estimating Weight of Extinct Testudinids

Sixty-nine mathematical functions were determined between tortoise weight and associated linear measurements of the shells and scutes. Sixty-two regressions returned an $r^2 > 0.90$, of which 48 had an $r^2 > 0.95$, and 2 had an $r^2 > 0.99$. The straight carapace length, curved carapace width, medial height, and straight width of the first vertebral scute all had r^2 values >0.95 (see Fig. 2.4A–D, Table 2.2).



Figure 2.4. Four linear measurement vs. weight regressions that yielded high (>0.95) r2 values. Solid line is the regression, dashed lines denote 95% confidence interval. Plotted in log-log space. a) straight carapace length, b) curved carapace width, c) straight width of 1st vertebral scute, d) medial height. Insets show graphs without log-scaled X-axes.

Category	a	b	r ²	
Curved Carapace	0.0002	2.827	0.9925	
Length (mm)				
Curved Carapace	0.0003	2.8374	0.9899	
Width (mm)				
Medial Height	0.006	2.7402	0.9624	
(mm)				
Straight Width of	f 0.0086	2.9655	0.9816	
5th Vertebral				

Table 2.2. , Selected regressions with high (>0.95) r^2 values. All equations take the form of y=ax^b, where 'a' is a scaling constant, 'b' is the exponent of allometry, and 'x' is the linear measurement.

Regressions with lower r^2 values reflect measurements of sexually dimorphic features such as the humeral and anal scutes (Fig. 2.5A, B, Table 2.3; McRae et al. 1981). Sexual dimorphism arising from differences in allometry are recognized in tortoises (Regis and Meik 2017).



Figure 2.5. Two linear measurement vs. weight regressions that yielded low (<0.90) r2 values. Solid line is the regression, dashed lines denote 95% confidence interval. Plotted in log-log space. a) length of anals, b) straight width across humerals. Insets show graphs without log-scaled x-axes.

Category	a	b	r ²
Gular Scute	0.4925	2.4722	0.8659
Length (mm)			
Anal Scute Length	2.1029	2.3021	0.6537
(mm)			

Table 2.3. Selected regressions with low (<0.90) r2 values. All equations take the form of y=axb, where 'a' is a scaling constant, 'b' is the exponent of allometry, and 'x' is the linear measurement.

These regressions can be applied to tortoise genera with typical one-half triaxial ellipsoid shell morphologies, to yield estimates on tortoise mass. This analysis excludes only the hingeback tortoises in the genus *Kinixys*, whose carapace can shorten when the tortoise is alarmed, and the pancake tortoises in the genus *Malacochersus*, whose shell is discoid. These regressions can be used to estimate a fossil tortoise's mass, when the outline of a single scute is visible. This mass estimate provides a basis for the thermal modeling of North American Pleistocene tortoises. Thus, four specimens of fossil tortoises from Texas are targets for thermal modeling: an *H. crassiscutata* from Zesch Cave UT-VPL 40685-B15F4; an *H. wilsoni* from Friesenhahn Cave UT-VPL 9333-732; a giant *Gopherus* specimen cf. *Gopherus "hexagonatus*" from Willacy County UT-VPL 971-1; and a *Xerobates* cf. *X. berlandieri* from Lake Creek Lake MM# P-13453. The reconstructed weights range from 1.8 to ~340 kg (Fig. 2.6, Table 2.4).



Figure 2.6. Relative size of tortoises found in Texas, extinct and extant.

Table 2.4. Modeled weights for four fossil tortoises from Texas. UT-VPL = University of Texas Vertebrate Paleontology Laboratory, MMC = Mayborn Museum Center.

Collection	Collection #	Taxon	Weight (g)
UT-VPL	40685-B15F4	Hesperotestudo crassiscutata	131,736.84
UT-VPL	971-1	Gopherus cf. 'hexagonatus'	339,385.18
UT-VPL	933-3732	Hesperotestudo wilsoni	1,800.49
MMC	P-13453	Xerobates cf. berlandieri	17,981.47

Modeled Heat Loss Curves for Extinct Texas Testudinids.

Heat loss when exposed to 0°C was modeled for four tortoises. Three of these are fossil specimens of the genera *Gopherus* and *Hesperotestudo*, and the fourth is an extant specimen of the genus *Pyxis*. In each model the tortoise starts with a body temperature of

~30°C. The heat-loss curve for the largest tortoise reconstructed, the cf. *Gopherus "hexagonatus"* (UT-VPL 971-1), (Fig. 2.7A, Table 2.5) indicates that without behavioral thermoregulation, ~32.9 hours is needed to reach a nonviable body temperature; this was the longest cooling time modeled. The shortest cooling time was modeled with an adult *Pyxis planicauda* (R#25) approximately the same mass (~150 g) as a hatchling giant tortoise. When exposed to freezing temperatures, this *P. planicauda* specimen would drop to a nonviable body temperature in ~2.3 hours (Blake et al. 2013) (Fig. 2.7B, Table 2.5).



Figure 2.7. Hypothetical cooling curves for Pleistocene tortoises. a) *Gopherus cf. 'hexagonatus'* UT-VPL 971-1 reaching an unviable temperature in ~33 hours. b) *Pyxis planicauda* R#25 reaching an unviable temperature in ~2.4 hours.

MUSEUM	ACCESSION #	NAME	а	b
UT-VPL	971-1	Noman	-0.003	30.356
UT-VPL	933-3732	Leona	-0.004	30.356
MMC	R-13453	Not Named	-0.003	30.356
CPZ	R#25	DoomBringer*	-0.013	30.356

Table 2.5. The 'a' and 'b' values of hypothetical cooling curves of fossil tortoises. Tortoises with asterisks (*) are live animals being treated as fossils for the purposes of the study. They were not actually subjected to low temperatures, however.

Discussion

This project developed 69 linear measurement versus weight regressions to provide a quantitative basis to reconstruct mass based on partial preservation of a tortoise carapace and diagnostic scutes (Table A2). The regressions developed here provide reliable weight estimates for all tortoises with domed, non-kinetic carapaces. The wholecarapace measurements all have regressions with an $r^2 \ge 0.95$ (Table A4). Curvilinear measurements produced slightly higher r^2 values than linear ones, as seen below (Table A2). Complete tortoise shells make up a small percentage of tortoise fossils (Behrensmeyer and Turner 2017). To allow for continuous growth, the bones of the tortoise shell never fuse entirely, but are held together by fibrous tissue and crenulated sutures. If not buried promptly, the shell disarticulates into individual osteoderms (Jackson et al. 2015).

Fragmentary material is sufficient to reconstruct tortoise weights. Most regressions based on the dimension of individual scutes typically had r^2 values >0.90 and a normalized root-mean-square error (nRMSE) of ~0.07 (Table A.2). On the carapace,

the scutes that yield the highest r^2 values are the vertebrals, located at the dorsal midline (Fig. 2.8).



Figure 2.8. Tortoise measurement diagram with abbreviations. Adapted from Chiari et al., 2009. Explanation of abbreviations is in Table A4.

The underlying neural osteoderms are connected in a series by the vertebral column and fibrous connective tissue at the sutures. As a result, the complete outlines for vertebral scutes are often preserved. The pleural scutes, which stretch between the vertebrals and the marginals, also produce regressions with high r^2 values and low nRMSE (Fig. 2.8, Table A2). The regressions for the outer ring of carapacial scutes, the marginals and caudal scute (Fig. 2.8), are the least statistically significant for reconstructing a fossil tortoise's weight. The presence of a cervical scute, at the anterior end of the carapace above the neck, is variable between conspecific individuals in many tortoise species (Rostal et al. 2014). As a result, the proportions of the marginal and caudal scutes on the edge of the carapace vary more than for other carapacial scutes. In addition, when the tortoise fossil is fragmentary, the shape of the marginal scutes differs very little, possibly leading to misidentification.

The carapace of a tortoise varies less with sex and ontogeny than the plastron does. Thus, regressions using carapacial measurements have higher r^2 values and higher mean percent errors than plastral regressions (Table A2). If no carapacial material is measurable, plastron length and width across the humerals both yield r^2 values >0.90 and mean percent errors of <20%. The straight carapace length regression predicts that a tortoise with a 1-m-long carapace should weigh ~194 kg, though within two sigma errors the potential weight range is between 155 and 233 kg. This range is partially attributable to the gut content of the tortoise (Sadeghayobi et al. 2011).

Modeling the heat dissipation physiology of a fossil tortoise is possible with a statistically accurate estimate of its live weight. We modeled the thermoregulation and temperature tolerance for four Pleistocene tortoises. The results of the thermal model

indicate that even large tortoises could not have survived more than ~33 hours of continuously freezing temperatures. These results are consistent with the prior assertion that fossil tortoises indicate hyperthermic to thermic paleoclimates, though not entirely frost-free environments. This analysis indicates that giant tortoises would be in imminent danger at below-freezing temperatures after 20 to 30 hours. This inference is consistent with data on the geographic distribution of most, but not all, modern tortoises. Using national and international climate databases, it was possible to determine which regions typically have \geq 33 consecutive hours of freezing temperatures and then match these to the mean annual temperature (MAT) and mean winter low temperature (MWLT) for each region (National Weather Service 2017; National Centers for Environmental Information 2018; U.S. Department of Commerce 2019a,b). Xerobates berlandieri is restricted to south of 29°N. Concordant with model results, this region rarely has >33 consecutive hours of freezing conditions that would kill giant tortoises, and it has a mean annual temperature \geq 22°C (Table 2.6). Of the four fossil tortoises modeled here, one comes from a latitude of 31.5° N, now too cold for tortoises at MAT = 19° C (Table 2.6).

County	NOAA Station	Mean Summer High	Mean Winter Low	MAT
Val Verde	Del Rio International Airport	35.61	5.56	21.39
Dimmit	Carizo Springs	36.50	5.39	21.72
Webb	Laredo 2	37.28	8.72	23.44
Medina	Hondo	34.78	4.72	20.61
La Salle	Fowlerton	35.89	4.78	21.17
Zapata	Escobas	36.06	8.11	22.89
Atascosa	Pleasanton	35.17	5.22	20.94
Jim Hogg	Hebbronville	35.89	7.83	22.44
Brooks	Falfurrias	36.00	6.83	22.11
Hidalgo	McCook	36.39	9.89	23.50
Bee	Beeville 6	34.28	7.33	20.00
San Patricio	Sinton	34.17	7.56	21.83
Jim Wells	Mathis 4	34.78	7.61	21.78
Nueces	CC Int Air.	33.78	9.28	22.33
Kleberg	Kingsville	34.89	8.67	22.56
Cameron	Harlingen	34.78	10.56	23.28
Calhoun	Port La Vaca	33.61	7.78	21.61
Aransas	Rockport	32.94	8.72	22.28
	MEAN	35.15	7.48	21.99
McLennan	Waco AP	35.00	3.17	19.33

Table 2.6. Climate data from 1981 - 2010 for 18 Counties within the modern range of *Gopherus berlandieri*. Counties without weather stations were excluded (National Climatic Data Center 2010). Modern climate data for the Lake Creek Lake site is listed at the bottom.
The modern *Chelonoidis chilensis* is found at even higher latitudes, 40°S, and colder average temperatures, MAT = 14°C, where the model indicates that tortoises should not survive (Fritz et al. 2012; Servicios Climáticos Arg 2017). The presence of *Chelonoidis* sp. indicates that behavioral thermoregulation or physiological differences between species may allow tortoises greater tolerance to low temperatures than indicated by this study. If so, the extinction of giant tortoises from North America and the significant range reduction in the smaller species may reflect other factors.

Some modern tortoises of the genus *Xerobates* have specialized diets with a dependence on plants with a crassulacean acid metabolism (CAM plants) such as *Opuntia* (Rostal et al. 2014). Recent reductions in suitable browse have caused three of five North American tortoises species to be listed as "vulnerable" by the International Union for the Conservation of Nature (IUCN 2018). Changes to the moisture regimes and temperature variability of North America that adversely affected CAM plant populations would result in a trophic cascade for the animals dependent on them, including tortoises. Other factors that might influence tortoise populations are the presence of soil types that can be burrowed, access to natural burrows or caves suitable for bromating—entering a state of lowered metabolic activity to survive cold weather—or environmental disturbances to the reproductive cycle (Laloë et al. 2017). If Pleistocene tortoises could burrow or make use of preexisting caves or burrows to thermoregulate, it would weaken the association between tortoises and warm climates.

The thermal model and associated paleoclimate inferences should be considered within the context of tortoise ecology and behavior. Thus, tortoises may be an indicator of warmer, less seasonal conditions in the Pleistocene, but only within the context of

other fossil species (Hibbard 1960; Holman 1980). This numerical heat-loss model makes it possible to quantify the maximum survivable number of consecutive freezing hours at a site, thus improving the usefulness of a vertebrate assemblage containing tortoises as a paleoclimate proxy.

Conclusions

- 1. The weight of extinct and extant tortoises can be estimated from 69 individual or combined linear measurements of the carapace, plastron, and individual scutes. The regressions between weight and shell linear measurements were significant for 15 of 17 taxa, except for two extant tortoise genera, Malacochersus and Kinixys, which are absent in the Pleistocene fossil record. The 15 most common taxa yielded 66 significant regressions between weight and a linear shell measurement, with r^2 values >0.90 and nRMSE values between 0.006 and 0.16, with a mean of 0.071. The regression between curved carapace length and weight has the highest r^2 (0.993), with an nRMSE of 0.040. This regression has been found to be applicable to fossil tortoises with weights between 1.8 and \sim 339 kg. Seven measurements produce regressions with r^2 values <0.90: gular length, anal length, length across the first pair of marginals, length across the second pair of marginals, cervical scute width, anterior carapace thickness, and posterior carapace thickness (Table A2). Future work to determine whether these regressions work with other taxa of turtles should be straightforward.
- The empirical thermal model produced results consistent with temperature measurements of living tortoises. Heat-loss measurements of a living tortoise show that the simplifying assumptions of the model concerning geometry, composition, and

metabolism of tortoises result in agreement within two sigma errors between the model-derived values and measurements.

3. This numeric analysis indicates that at 0°C tortoises not engaging in behavioral or metabolic thermoregulation will reach nonviable temperatures in 2 to 30 hours, depending on size. This temperature tolerance corresponds to an MAT ≥ 22°C and an MWLT ≥ 7.5°C, which approximates the preferred habitat temperature of current American tortoise genera (National Climatic Data Center 2010). This analysis infers higher MAT and MWLT for the survivability of tortoises in a Pleistocene context than previous estimates, which may reflect warmer micro-environments and/or geographically widespread past warm states. It is ill advised to use a single fossil occurrence for paleoclimatic inference, but an assemblage framework, including tortoise fossils, provides an improved context for evaluating the warmth of interglaciations.

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CHAPTER THREE

Home Range of the Columbian Mammoths (Mammuthus columbi) and Grazing Herbivores from the Waco Mammoth National Monument, (Texas, USA) Based on Strontium Isotope Ratios from Tooth Enamel Bioapatite

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Abstract

Waco Mammoth National Monument (WMNM) is a central Texas Late

Pleistocene fossil site dating to ~67 ka. At least 23 Columbian mammoths (*Mammuthus columbi*) along with the remains of 12 other vertebrate genera have been discovered at this locality. Mammoth teeth were micro-sampled at a high resolution with a computerized micromill while bison and horse were serially sampled with a handheld rotary tool. Serial samples from these individuals offer insights into the movement patterns (87 Sr/ 86 Sr) of WMNM herbivores. Regional vegetation and local sediments were also collected to construct an 87 Sr/ 86 Sr isoscape to track past movement of mammals, derived from Sr analyses of teeth enamel. 87 Sr/ 86 Sr in WMNM herbivores often reflects multiple grazing provenances, and sources. A series of mixing models are proposed to understand the contribution of different surface Sr sources to herbivore diets. These data indicate one mammoth spent some time in the Llano uplift ~180 km to the SSW during the formation of molar enamel. The remaining individuals (three mammoths, a bison and a horse) lived in an area more proximal to the WMNM, ≤ 70 km SE of the site. 87 Sr/ 86 Sr

from WMNM mammoths indicate individual fidelity to at least two geographic ranges, with at least one mammoth, potentially unrelated to the potential matriarchal herd, challenging the assertion of a sole matriarchal herd. Including the horse and bison, it appears that at least 25 mammals congregated at the confluence of Brazos and Bosque rivers at ca. 67 ka ago, prior to their demise. A resource shortage – such as a drought – is consistent with this pattern.

Introduction

Waco Mammoth National Monument (WMNM) is a Late Pleistocene vertebrate fossil site that has produced at least 23 Columbian mammoths (Mammuthus columbi) and the remains of 12 other vertebrates (Nordt et al., 2015). The high density of fossil remains makes the site a konzentrat-lagerstätten in Seilacher's original broad definition (Seilacher et al., 1985). The demographics of the mammoth remains are consistent with the simultaneous death and eventual burial of a matriarchal herd. If correct, this is the only such herd yet found in the Americas (D'Arcy et al., 2008; Haynes, 1987). Taphonomic and geologic studies yielded insight on movement and environmental conditions prior to burial of the mammoth herd (Nordt et al., 2015; Wiest et al., 2016; 2017). Recent studies hypothesize that drought was a factor in the congregation of mammoths at a diminishing watering hole (Wiest et al., 2016), which is counter to previous interpretations that a catastrophic flood killed and buried these mammoths (Nordt et al. 2015). Understanding the movement and geographic origin of mammoths may assist in evaluating these different hypotheses for the demise of this hypothetical mammoth herd ~67 ka ago (Nordt et al., 2015; Wiest et al. 2016).

The measurement of strontium isotopes $({}^{87}\text{Sr}/{}^{86}\text{Sr})$ on the apatite fraction of teeth has been instrumental in reconstructing the movement of Pleistocene mammals, including mammoths and mastodons (Capo et al., 1998; Feranec et al., 2007; Frei and Price, 2012; Hoppe, 2004; Kootker et al., 2016; Saunders et al., 2010). The movement of large mammals has been inferred with knowledge of the geologic variations of strontium isotopes in the native bedrock, sediments, soil, and water as reflected in biologic uptake in the overlying vegetation by grazing animals (Ben-David and Flaherty, 2012). The geographic variation of Sr isotopes from the measurements of soils, sediments or vegetation is the basis for the contoured Sr isoscape which is a fundamental metric to infer mammoth movement and home areas (Bowen, 2010; Hedman et al., 2018; Widga et al., 2017). Early studies sampled mammoth and mastodon teeth in bulk with the apatite from multiple growth increments spanning 10 to 15 years. The resultant Sr isotopes provided insights on the home range of mammals and whether they were endemic to the area where they were found, but not movement on a sub-annual scale (Hoppe et al., 1999). Such bulk testing was done on mammoth teeth from the future WMNM and concluded that all of the ⁸⁷Sr/⁸⁶Sr ratios were consistent with local sources (Hoppe, 2004). In contrast micromill sampling of tooth enamel for Sr analysis often achieves subannual resolution during the period of enamel deposition, allowing for more detailed records of the geographic range of large mammals. For example, the migration history with a sub-month resolution was documented from serial, finely-sampled teeth of horse, bison, primate, and a sloth (Birch and Dean, 2009; Glassburn et al., 2018; Larmon et al., 2019; Le Roux et al., 2014; Widga et al., 2010). Analyses of enamel accretion rates for proboscidean molars indicate the potential for an environmental record of up to 15 years

(Dirks et al., 2012; Metcalfe and Longstaffe, 2012). Micromilling of a *Mammuthus* from Missouri tooth for Sr isotopes revealed a movement of ~300 km between the ⁸⁷Sr/⁸⁶Sr signature of enamel and local sedimentary values (Kolis et al., 2019; Widga et al., 2017).

This study applies serial sampling for Sr isotope analysis to mammoth tooth enamel from the Waco Mammoth National Monument (WMNM). The aim of these analyses is to better understand the cause of this mass accumulation of fossils, the geographic range of the mammoths, and provide insight into the paleoenvironment at the time of fossil accumulation. Non-mammoth megafaunal teeth from the site were also serially sampled, both as a point of comparison for the mammoths, and to better understand Late Pleistocene niche partitioning.

Geologic Setting

WMNM is in central Texas near the Balcones Escarpment on the edge of the Edwards Plateau. The wider region under study is included in a circular area with a 300 km radius with WMNM in the center. This encompasses most of eastern Texas and small areas of Oklahoma and Louisiana (Hartmann et al., 1992). The elevation is highest in the west and lowest in the southeast, sloping from 600 m to sea level, draining into the Gulf of Mexico by six major river systems (Gray, 1919). Each major river valley is filled with Quaternary sediment, with an active floodplain and one or more fluvial terraces (Blum and Aslan, 2006). Much of this area is a monocline dipping to the southeast, with Pleistocene sediment on the Gulf Coast and Cretaceous carbonate and siliciclastic rocks running along the U.S. Interstate Highway 35 corridor between Dallas and San Antonio. In between these two bands are deposits of Paleogene and Neogene clastic rocks and unconsolidated sediments. In the northwest are Carboniferous and Permian siliciclastic rocks, some richly fossiliferous. In the southwest is the Llano Uplift, a dome ~150 km in diameter. Precambrian intrusive, felsic igneous rocks are exposed in the center of the dome, ringed by progressively younger Lower Paleozoic metamorphic and sedimentary rocks. The only extrusive igneous rocks in the region surround the extinct Cretaceous volcano at Pilot Knob in Austin, covering only 8 km² (Ewing and Caren, 1982; Hartmann et al., 1992).

WMNM lies in the interfluve of the Bosque and Brazos rivers at 31.6° N, 97.2° W, ~2.7 km northwest of the modern confluence of those rivers. It is excavated into the T2 river terrace, one of the three terraces in the area above the modern active floodplain (Bongino, 2007). The sediments are predominately fine silts and sands, with a detrital carbonate content of ~15% by volume, and with pedogenic carbonate in some of the upper soil horizons (Nordt et al., 2015a). Directly below the fossil-bearing deposits at the WMNM is a thick gravel channel-fill deposit cut 4 meters into the Cretaceous Austin Chalk (Bongino, 2007; Hartmann et al., 1992; Nordt et al., 2015a). Both the terrace sediments and the gravel are more mineralogically similar to the carbonate-dominated sediments of the modern Bosque River than the primarily siliciclastic sediments of the modern Brazos River. The site was therefore in and adjacent to the ancient Bosque River, or a tributary thereof (Nordt et al., 2015a).

Mammoths and other Pleistocene Mammals of Waco Mammoth National Monument

The species of mammoth present at the site is the Columbian mammoth, *Mammuthus columbi*, the largest of the three species of mammoth living in North America at the end of the Pleistocene (Agenbroad, 1994). Based on site demographics and phylogenetic bracketing, mammoths are thought to have been social animals, living

in matriarchal herds (Agenbroad and Barton, 1991; The Proboscidea. Evolution and Palaeoecology of Elephants and Their Relatives., 1996). Both genera of modern elephants live in matriarchal herds, and *Mammuthus* is nested within this clade (Fernando and Lande, 2000; Shannon et al., 2008). The strongest fossil evidence for this inference is the existence of single-sex mass accumulations of mammoths, like that seen in Hot Springs South Dakota (Lister and Agenbroad, 1994). The demographics of the site have been interpreted to imply that the main bone occurrence at WMNM may be an *M. columbi* matriarchal herd (Table 3.1) (Haynes, 1992). The minimum number of individuals (MNI) based on ulnae counts indicate at least 23 mammoths at WMNM, 20 of which were deposited in the same stratum and may thus have constituted a herd. There are three skeletons 1 m higher in the section where the overlying sediment has been OSL dated to ~53 ka (Nordt et al., 2015a).

WMNM DEMOGRAPHICS								
Animal Number	Tooth Wear Stage	AEY	Sex					
0	VII	4 to 5	J					
2	XX	~33	F					
3	Х	10 to 11	J					
4	XXIII-XXIV	~45	F					
12	XVIII	~25	F					
h1	-	≥32	F					
h2	-	≥32	F					
h3	-	12 to 13	J					
h4	-	~20	F					
h5	-	16 to 20	F					

Table 3.1. Partial demographic data for WMNM mammoths (Haynes, 1992). All listed individuals are from the 'matriarchal herd' stratum. The rest of the animals found in that stratum have not been aged, but other osteological measurements indicate that they were adult females (Fox et al., 1992).

There are also remains of other Pleistocene megafauna within the strata including *M. columbi* skeletons. The most complete non-mammoth fossil is a *Camelops* specimen. This camelid was 2.1 meters tall in life, and is partially articulated. Disassociated bones exhibiting carnivore modification suggest potential scavenging activity (e.g. Wiest et al. 2016) but the skeleton is ~90% complete. Most of the other megafauna from the site are identified from a small number of isolated specimens; including *Bison* sp., *Equus* or *Harringtonhippus*, *Capromeryx* sp., and *Platygonus* sp. (Benedict, 2010; Heintzman et al., 2017).

Methods

Sampling Enamel from Teeth

Enamel is the hardest material in a vertebrate skeleton, mostly composed of micrometer-sized, densely-packed crystals of hydroxyapatite, also called 'rods' or 'prisms' (Ungar, 2010). The large crystal size of hydroxyapatite in enamel, in comparison with the other mineral tooth components, dentin and cementum, results in a smaller surface-area-to-volume ratio and thus, results in a lower isotopic exchange. Combined with the tight crystal packing and resulting low permeability of enamel, it is much less susceptible to diagenesis than any other vertebrate fossil tissue (Gage et al., 1989; Lee-Thorp and van der Merwe, 1991; Zazzo et al., 2012).

The enamel components of mammoth teeth are an important environmental archive, with potentially sub-annual resolution. During tooth formation enamel is deposited starting at the anterior portion of the crown of the tooth and progresses posteriorly and rootward until it reaches the junction between the crown and root (Fig. 3.1A). In animals like *Bison* and *Equus* the process of enamel deposition takes between 12 and 18 months, while in proboscideans like *M. columbi* deposition of all of the enamel in a tooth can take up to 15 years (Dirks et al., 2012; Uno, 2012). In *Mammuthus columbi* and *M. primigenius*, enamel is accreted at a mean rate of ~13 mm yr⁻¹, with fluctuations in growth rate within and between teeth in the same individual (Dirks et al., 2012; Metcalfe and Longstaffe, 2012). Enamel crystals form an oblique angle to dentin and to the surface of the tooth, angling towards the crown and away from the dentine (Fig. 3.1B). These crystals are broken into shorter segments by growth lines called the Striae of Retzius. The segment of a crystal bounded by two striae represents a single period of

deposition of ~0.5 months (Metcalfe and Longstaffe, 2012). Sampling of teeth was confined to the innermost layer of enamel, at the enamel-dentine contact. This position is composed of aprismatic enamel, secreted in a single 100 µm thick layer.



Figure 3.1. Macrostructure and microstructure of a typical mamn oth, genus *Mammuthus*. A) Parasagittal cross-section of WMNM 368, mammoth 'N.' B) Diagrammatic view of the enamel-dentin junction of the same tooth, showing targeted aprismatic enamel sample.

There are multiple systems for naming proboscidean teeth; herein we use P1-P3 and M1-M3 for upper molars and p1-p3 and m1-m3 for lowers (Agenbroad, 1994). In order to ensure that the records for each of the WMNM teeth were roughly contemporaneous, each tooth was sampled from a posterior loph near the junction of crown and root. This is the part of the tooth most likely to still be accreting when the mammoths died. The first tooth sampled was a P3 from mammoth 'N,' an eight-year-old juvenile. This tooth was sampled with a Seimens CNC machine housed in the metal shop at Baylor University. The smallest bit available for this machine is 1/16" (1.58 mm). As a result, each 1.58 mm occlusal-basal sub-sample time-averages \geq 44 days (Dirks et al., 2012; Metcalfe and Longstaffe, 2012). The tooth was clamped into the CNC machine, and the bit was positioned using a Dino-Lite Edge AM7515MT2A field microscope. The bit was lowered 100 µm into the enamel, and the powder was extracted using an infant nasal aspirator. After each sub-sample was taken, the tooth and aspirator were blown out with compressed air, rinsed twice with deionized water, and dried with compressed air. The powdered sub-sample was placed directly into a labeled 2-dram vial. The CNC machine was programmed to be lowered a further 100 µm, and the process was repeated until a change in powder color or drill noise indicated that the bit had penetrated the full thickness of the enamel and dentin was being extracted. The penultimate subsample, containing the aprismatic enamel, was retained for analysis.

Collecting the aprismatic enamel layer of the remaining mammoths was done via a computer-controlled large-volume micromill at Eastern Tennessee State University (ETSU). The specimen to be sampled was strapped to a platform attached to a computercontrolled stage via a ball-swivel mount. An armature above the stage held a rotary tool with a 0.5 mm diameter end mill (Widga, 2018). The reduced diameter of the bit decreased the time averaging to ~14 days. Next a clay dam was built around the region of the tooth to be sampled and filled with 20-200 μ L reverse-osmosis filtered, laboratorygrade DI water. Leeching of Sr is possible, but would not have induced fractionation in the remaining Sr (Graustein and Armstrong, 1983). The clay used is insoluble in water and therefore unlikely to introduce contamination to the enamel samples (Rush, 2015). At this point the rotary tool was activated and set to 7,000 – 10,000 RPM, and software (Galiltools v. 1.6) actuated the stage so that the rotary tool cut a groove 0.5 mm wide by

0.5 cm long by 100 µm deep. A pipette was used to collect the water and the powdered sub-sample, which was placed in a labeled micro-centrifuge tube. Then the area of the tooth within the clay dam was rinsed with five changes of DI water to prevent contamination between subsamples. This process was continued until a change in the color of the powder or the sound of the rotary tool indicated that the machine was now cutting through softer dentine. At this point the previous subsample, containing enamel from the innermost aprismatic layer, was set aside for isotopic analysis (Kolis et al., 2019). Ten to twenty such grooves were cut along the growth axis of each tooth, approximately 1 mm apart.

The sampling strategy for non-mammoth megafauna was simpler. *Equus* and *Bison* teeth form quickly over a shorter time period than *Mammuthus* teeth (Glassburn et al., 2018; Widga et al., 2010). Like the mammoth tooth samples, these were taken perpendicular to the growth axis of the tooth. As a result, sampling across multiple layers of prismatic enamel results in a time averaging 7-14 days and can be done by hand using a rotary tool with a 2.5 mm bit (Britton et al., 2009). The tooth's surface to be sampled was washed with ethyl alcohol, dried, and then placed on unused 20 cm² piece of aluminum foil, within a fume hood. After drilling a 0.5 to 2.0 cm groove across the frontal plane of the tooth, the resultant powder on the aluminum foil was transferred to a labeled vial (Table 3.2). The fume hood was blown out with compressed air so the excess enamel powder could be removed by the exhaust fan, the entire interior was wiped down with ethyl alcohol, and the used piece of aluminum foil was discarded between sampling routines.

Table 3.2. Data on the enamel sampling done for this study. 'Sample set length' refers to the distance between the most crownward and rootward samples on a tooth in mm, and is thus measured parallel to the growth axis of the tooth. 'Sample width' refers to the width of each individual sample groove, also measured parallel to the growth axis of the tooth and perpendicular to the length of the growte. 'Approximate duration of sample set deposition' is an estimate of the time required for each tooth to grow the length of the 'sample set length,' based on published growth rates (Gadbury et al., 2000; Hoppe et al., 2004; Metcalfe and Longstaffe, 2012). 'Approximate duration of individual enamel sample deposition' is an estimate of the time averaging in each sample, based on the published growth rates cited above.

Specimen #	WMNM 368	WMNM 72	WMNM 318	WMNM 366	MMC P1600	WMNM 1229	WMNM 1190
Individual	Mammoth 'N'	Mammoth 'C'	Mammoth 'L'	Mammoth 'M'	Gravel Pit Mammoth	Equid	Bison
Sample Set Length (mm)	84.7 mm	15 mm	20.9 mm	22 mm	17.8 mm	51 mm	54.9 mm
Sample Width (mm)	1.55 mm	0.5 mm	0.5 mm	0.5 mm	0.5 mm	2.4 mm	2.9 mm
Approx. Duration of Sample Set Enamel Deposition	~2,380 days	~420 days	~590 days	~620 days	~500 days	~175 days	~362 days
Approx. Duration of Individual Enamel Sample Deposition	≥44 days	≥14 days	≥14 days	≥14 days	≥14 days	≥8 days	≥19 days

Preparation of Enamel Samples for Sr Analysis

The vials containing DI water and enamel samples from the *M. columbi* teeth were placed in a centrifuge at 3,000 RPM for 30 minutes; this pelletized the enamel powder at the tip of the vial. Most of the DI water was then siphoned by pipette, with disposable tips changed between vials. Finally, the vials were placed in a Carolina

Scientific 6" vacuum desiccator loaded with color-changing silica gel desiccant and left over-night. Sealed vials were shipped to the University of Kansas Isotope Geochemistry Laboratory for Sr isotopic analysis. The non-mammoth powdered tooth enamel was shipped without further processing.

Collection of Vegetation and Sediment Sample for the Sr Isoscape

To this end, regional vegetation was sampled to construct an ⁸⁷Sr/⁸⁶Sr isoscape. The specific area of interest is a circular region with a radius of 300 km, centered on WMNM. This range was selected because it is the longest distance deduced for a North American mammoth to move during a lifetime (Hoppe, 2004; Kolis et al., 2019). Previous Sr isoscapes have been made by analyzing bedrock, ground / tap-water, modern rodent teeth, or soil strontium (Bataille and Bowen, 2012; Brennan et al., 2016; Chesson et al., 2012; Feranec et al., 2007; Hoppe et al., 1999; West et al., 2009). The source of strontium in terrestrial vertebrate herbivore teeth is from the ingested vegetation (Ben-David and Flaherty, 2012). M. columbi was a grazer, so for this study grasses were collected (Bern et al., 2005; Hedman et al., 2018; Mead et al., 1986; Stewart et al., 1998). An age-lithology model was used to generate the isoscape. Age-lithology models can be used to produce an isoscape by assuming that rocks of the same geologic age and rock type have the same ⁸⁷Sr/⁸⁶Sr ratios. Age-lithology models have been shown to explain more of the observed variance than age-only models of ⁸⁷Sr/⁸⁶Sr ratios (Bataille and Bowen, 2012). In rock, ⁸⁷Sr/⁸⁶Sr ratios can vary predictably by age and lithology (Ben-David and Flaherty, 2012). Not all strontium is biologically available, so sampling the bedrock is impractical. However, the vegetation Sr ratio reflects the soil parent material, which should be derived from the underlying rock or sediment. The exception would be

parts of some river valleys with thick Late Quaternary and Holocene sediment sequences. (Bern et al., 2005; Graustein and Armstrong, 1983). Satellite imagery was used in conjunction with geologic maps to find soils developed directly on bedrock – thus above modern river valleys and terraces – and unaffected by human buildings and agriculture (Google, 2017; Hartmann et al., 1992). Three sampling sites were chosen for each of twelve age-lithology categories; sites for each category were between 20 and 200 meters of one another. The coordinates for each sampling site is available in Table B1.

Small samples of the Smithson Paleosol from the WMNS were collected to determine whether the ⁸⁷Sr/⁸⁶Sr ratios of modern soils are comparable to buried counterparts developed on the same bedrock residuum (Bongino, 2007; Nordt et al., 2015a). The paleosol was placed in a seed-starting tray, sown with corn (*Zea*) and peas (*Pisum*) and watered only with DI water for six weeks. The resulting seedlings were then collected and prepared for Sr analyses, like the wild grass samples. Some of the paleosol was also kept for Sr isotope analysis.

Vegetation and Sediment Sample Preparation for Sr Analysis

The thirty-eight vegetation samples were washed to remove any adhering particles. Collected plants were place in polyethylene bags, filled with DI water, sealed, agitated vigorously for 30 seconds, and lastly the fluid decanted; this process was repeated four times. Each sample was then transferred to a separate, labeled cheesecloth bag and placed in a drying oven at 60° C for 24-48 hours after which these vegetation samples were ready for Sr analyses. A small quantity of the Smithson Paleosol was also sent as a point of comparison with the *Zea* and *Pisum* seedlings and modern vegetation from the Brazos River valley.

Enamel ⁸⁷Sr/⁸⁶Sr Analyses

The size and density of enamel crystals in mammoth teeth result in a resistance to diagenesis (Bongino, 2007; Widga, 2017). Thus, acidification of enamel samples was unnecessary because of the minimal permineralization of fossil remains at WMNM (Bongino, 2007; Widga, 2017). Instead, the powdered enamel samples were dissolved in 7.5 N HNO₃ and the Sr was extracted with a cation-exchange column loaded with strontium-specific resin. The extracted strontium was analyzed using a thermal ionization mass spectrometer (TIMS) with an automated VG Sector54, an eight-collector system, and a twenty-sample turret. The results were calibrated by adjusting the readings so the NBS-987 standard yielded an ⁸⁷Sr/⁸⁶Sr ratio of 0.71025 and an ⁸⁶Sr/⁸⁸Sr ratio of 0.1194. About 30 standards were analyzed during the course of this work.

Vegetation and Sediment ⁸⁷Sr/⁸⁶Sr Analyses

The dried plant matter of each sample was powdered using a mortar and pestle, and all fragments larger than 0.25 mm were filtered out. The powder was poured into quartz crucibles, then placed in a muffle furnace for 2 hours at 80° C, another 2 hours at 200° C, and 24 hours at 600° C. Each sample of vegetation ash was then put in a solution of 7 M nitric acid heated to 80° C to dissolve the strontium into solution. The liquid was pipetted into a new vial and reacted with 0.3 ml of hydrogen peroxide heated to 80° C to ensure that all organic components were removed. The samples were allowed to dry before being dissolved in 0.5 mL of 3.5 M nitric acid, then run through a cation-exchange column and TIMS as above (West et al., 2009; Widga et al., 2017).

The samples of the Smithson Paleosol from the Waco Mammoth Site, which represents the landscape for penecontemporaneous grazers, were analyzed for Sr, as one potential end-member source, working on the assumption that the mammoths fed in the region before death. Given their daily food requirements of ~180 kg, this seems probable (Lister, 2007). Samples of \leq 30 g were dried overnight in a desiccating oven at 60° C. From this a ~1.0 g subsample was leached with 2.5 mL of ammonium nitrate, shaken for 8 hours, then centrifuged at 3,000 rpm for 15 minutes. The supernatant liquid was decanted and moved to a new container, then allowed to completely dry. The remaining solids were redissolved in 2 mL of 2 M nitric acid. The sample was then passed through the cation exchange columns and TIMS as above (Willmes et al., 2014).

Two-end-member Mixing Model of Sr Values to Infer Grazing Areas of Mammoths

The geographic origin of the strontium found in each enamel subsample was ascertained by comparing the subsample's ⁸⁷Sr/⁸⁶Sr ratio with the vegetation ⁸⁷Sr/⁸⁶Sr ratios. Those enamel samples that did not fall within the range of any of the age-lithology categories had their geographic origins estimated via a two-end-member mixing model (Faure, 1998). The model used here is presented in Equation 3.1:

Equation 3.1. Equation for a two-end-member mixing model. X_1 is the % of Sr from the first age-lithology category, R_{mix} is the ⁸⁷Sr/⁸⁶Sr ratio in the enamel sample, R_1 is the ⁸⁷Sr/⁸⁶Sr ratio from the first age-lithology category, and R_2 is the ⁸⁷Sr/⁸⁶Sr ratio from the second age-lithology category (Faure, 1998).

$$X_1 = \frac{R_{mix} - R_2}{R_1 - R_2} * 100$$

The first end member used for every enamel subsample was the Smithson Paleosol ratio. This was selected because it is the age-lithology category at WMNM, where the mammoths and other animals are known to have spent time, immediately prior to demise. The second end member for each subsample was the geographically nearest age-lithology category that when mixed with the Smithson Paleosol Sr could explain the ⁸⁷Sr/⁸⁶Sr ratio in the subsamples. As such our results represent a lower bound on the geographic range of the animal, rather than a unique solution. In every case the ⁸⁷Sr/⁸⁶Sr ratio could be explained by the animal spending time further away from the site than we estimate here, but not closer.

Results

⁸⁷Sr/⁸⁶Sr of Vegetation and Associated Isoscape in East Texas

Our sampling strategy employed twelve age lithology categories, eleven of which were sampled three times and one of which was sampled four. The ⁸⁷Sr/⁸⁶Sr ratios partially overlap at 2σ, so that there are five isotopically distinguishable regions of vegetation isotope ratios (Fig. 3.2, Table B2). The degree of overlap is the result of two age-lithology categories: Lower Paleozoic clastic rocks and Upper Paleozoic carbonate rocks. These rocks, the Hickory Sandstone and Winchell Limestone, respectively have a standard deviation an order of magnitude higher than in the other Phanerozoic rocks measured. The other age-lithology category with a high standard deviation is the pre-Cambrian granite (Town Mountain) but the mean ⁸⁷Sr/⁸⁶Sr ratio is sufficiently elevated at 0.728, and is thus statistically distinct from other lithologies (Fig. 3.2).



Figure 3.2. Range of ⁸⁷Sr/⁸⁶Sr values of vegetation growing on each of 12 age/lithology categories of rock, oldest first. Lower Paleozoic clastic rocks and Upper Paleozoic carbonate rocks have wide ratio distributions, but geographically limited ranges. The isotope ratio range of the Smithson Paleosol (~67 kya) is represented by a teal dashed line, and the mean ⁸⁷Sr/⁸⁶Sr ratio for mammoth 'N' is represented as a red dashed line. The color scale used here is used in the isoscape as well.

The Zea and Pisum plants grown in the Smithson Paleosol samples produced ⁸⁷Sr/⁸⁶Sr ratios of 0.7089 and 0.7079 respectively. The exchangeable strontium directly from the paleosol had a ratio of 0.7082 (Fig. 3.2, Table B3). The heavier Sr ratio in the plants is the result of parental Sr in the seeds' cotyledons of 0.7094 and 0.7099 respectively. The paleosol strontium ratio can be attributed to ~70% of the soil strontium derived from the local Upper Mesozoic carbonate rocks and the remaining ~30% reflecting Upper Paleozoic clastic rocks from the headwaters of the Bosque River, 140 km to the northwest (Hartmann et al., 1992).

The initial research focus was to define a strontium isoscape for most of eastern Texas. The mapping was done with Google Earth Pro in conjunction with a geologic map and gazetteer of Texas (Gray, 1919; Hartmann et al., 1992). Each rock formation occurring in the 283,000 km² study area was placed into one of the twelve age-lithology categories to broadly characterize the spatial variability in Sr ratios. The result was an isoscape that displays approximated ⁸⁷Sr/⁸⁶Sr ratios found in shallow-rooted grasses across the region (Fig. 3.3). An implicit assumption is that the distribution of bedrock lithologies and associated Sr ratios in grasses has not changed significantly in the past ~ 67 ka. Other sources for bioavailable Sr include airborne dust and sea-salt aerosols might have conceivably been different at the time of deposition and thus influenced the enamel isotope ratios, but research shows that bedrock isotopic composition dominates in most settings (Bataille et al., 2012; Bondy et al., 2017). For the purposes of the present study, this was assumed to be the case across the study area.



Figure 3.3. Map of study location and isoscape. Sampled area is 283,000 km². Color scale for ⁸⁷Sr/⁸⁶Sr ratios shown here is used in all following figures. See (Sup Info 1) for vegetation sample site locations. Mammoth silhouettes represent minimum range from WMNM based on a two-end-member mixing model, not the locations where the mammoths spent the most time.

Sr Isotopes for Waco Mammoth 'N', 'M', 'L', and 'C' and Inferred Grazing History

WMNM 368 is a P3 molar from mammoth 'N.' Mammoth 'N' is a juvenile mammoth between 8 and 10 years old at death (Haynes, 1992). Five subsamples were analyzed for ⁸⁷Sr/⁸⁶Sr ratios. The range of ratios of these subsamples was 0.0041 (Table B4). Of these, only one could be caused by ingesting vegetation within ~70 km of WMNM (Figs. 3.2, 3.4).



Figure 3.4. Aggregate 87 Sr/ 86 Sr ratios of sampled teeth. X axis shows 87 Sr/ 86 Sr, length of boxes shows range of ratios from each tooth, color and vertical line show the median 87 Sr/ 86 Sr value for each tooth, whiskers show 1 σ for each tooth. The color scale matches that in figures 3.2 and 3.3.

All other ⁸⁷Sr/⁸⁶Sr ratios appear to reflect vegetation growing on the Lower Cenozoic clastic deposits of the Smithson Paleosol, and pre-Cambrian intrusive, felsic igneous rocks or unidentified lithologies with elevated ⁸⁷Sr/⁸⁶Sr ratios at the eastern margin of the igneous intrusion (Fig. 3.3). These high ratios may reflect that this mammoth spent a portion of each ~40-day sampling period taking in strontium from plants grown in soil developed on granitic rock or mixed lithologies at least 180 km south-by-southwest of WMNM, in the Llano Uplift. Early in the formation of the tooth, this would be taken prenatally by the mother and passed to 'N' (Metcalfe et al., 2010). This should not alter the interpretation of the data, as strontium does not fractionate biologically (Ericson, 1985). The largest percentage of time spent feeding within the granitic province was ~36%. (Figs. 3.4, 3.5).



Figure 3.5. ⁸⁷Sr/⁸⁶Sr ratios recorded in serial samples, mammoth 'N' (WMNM 368). X-axis shows sample numbers, with those to the right having been deposited most recently. Y-axis shows ⁸⁷Sr/⁸⁶Sr ratios. Pie charts show two-end-member mixing required to explain the isotopic data. Large pie charts show the highest and lowest ⁸⁷Sr/⁸⁶Sr ratios for the tooth. Unlike every other tooth sampled, the high ⁸⁷Sr/⁸⁶Sr ratios in WMNM 368 indicate that it spent some time feeding on vegetation growing on granitic soils. As in all following pie charts, this figure reconstructs the ⁸⁷Sr/⁸⁶Sr ratios assuming the minimum possible distance from WMNM. The data can also be explained by a more distant home range, but not by a nearer one.

It is possible that the presence of granitic Sr in the last deposited growth interval indicates that travel time between the granitic province and WMNM was within < 40 days. Alternately, the last deposited enamel may be missing owing to damage to the root/crown transition. If this is the case, the isotopically lighter Sr might not be from the Smithson Paleosol, but from Phanerozoic rocks nearer the Llano Uplift. Either scenario indicates that mammoth 'N' must have spent time far from WMNM.

Mammoth 'C,' (WMNM 72) is a fragmentary M2 molar from an adult female

~33 years old at time of death (Haynes, 1992). Seven samples were analyzed for

strontium isotope ratios. The range of variation of these samples is lower than seen in teeth from Mammoth 'N' at 0.0011 (Table B4). Unlike Mammoth 'N,' the ⁸⁷Sr/⁸⁶Sr ratios in WMNM 366 indicate that mammoth 'C' fed within a radius of at least 70 km from WMNM prior to its death (Figs. 3.3, 3.6A).



Figure 3.6. ⁸⁷Sr/⁸⁶Sr ratios recorded for serial samples of mammoth 'C,' 'L,' and, 'M' teeth from the WMNM, showing a similar range of values. X-axis shows sample numbers, with those to the right having been deposited most recently. Y-axis shows ⁸⁷Sr/⁸⁶Sr ratios. Pie charts show two-end-member mixing model that partitions the isotopic data. Large pie charts show the highest and lowest ⁸⁷Sr/⁸⁶Sr ratios for each tooth. A) mammoth 'C.' B) mammoth 'L.' C) mammoth 'M.' This two-end-member mixing model represents the minimum distance from the WMNM traveled to explain the observed ⁸⁷Sr/⁸⁶Sr ratios. A more distant home range is always possible.

The Sr ratios necessitates this mammoth to have ingested vegetation growing above parent material of at least two nearby Sr isoscape categories (Figs. 3.3, 3.4). This mammoth may have fed on plants growing in the Smithson Paleosol or equivalent as much as 65% of the time and as little as 25% of the time during tooth formation. (Fig. 3.6A). These results differ by $< 2\sigma$ from WMNM 366 (mammoth 'M') and WMNM 318 (mammoth 'L'), indicating the possibility that the three were herd-mates.

WMNM 318 tooth is a fragmentary P3 or M1 molar from mammoth 'L,' an adult female between 15 and 29 years old at time of death (Lee et al., 2012). Eleven serial samples were analyzed for strontium. The range of variation of Sr isotopes are lower in 'L' than for mammoth 'N': 0.0015 (Table B4). These Sr ratios for tooth mammoth 'L' are consistent with the inference that mammoth 'L' grazed in a \geq 70 km region around WMNM. These ratios are congruous with grazing on landscapes rooted in the Smithson Paleosol or equivalent soils and within valleys Upper Cenozoic clastic sediments. Modeling iterations indicate that as much as 82% to as little as 37% of mammoth L's time was spent grazing on vegetation rooted in to the Smithson Paleosol or an equivalent landscape (Fig. 3.6B). This is broadly similar to mammoth 'M' and is consistent with the inference that mammoths 'M' and 'L' shared a geographic range, and could in principle have been herd-mates.

366 WMNM is a fragmentary M1 or M2 molar from an adult female ~25 years old at time of death; mammoth 'M,' (Haynes, 1992). Ten samples were analyzed for strontium. The range of variation of strontium isotope ratios – 0.0015 – is lower than in mammoth 'N' at 0.0041, but are similar to mammoths 'C' and 'L' at 0.0011 and 0.0012 respectively (Table B4). Mammoth 'M' fed on plants growing in the Smithson Paleosol

or equivalent level as much as 76% of the time and as little as 23% during each of the 10to 14-day periods assessed, and spent the remainder feeding above Upper Cenozoic Clastic sediments (Fig. 3.6C). It does not appear to share a feeding profile with mammoth 'N,' but is similar to mammoths 'C' and 'L.'

Sr Isotopes for Bison and Equid Tooth from WMNM and non-WMNM Mammoth

One *M. columbi* from outside WMNM was also sampled as a control analysis. This was done to ascertain whether or not Sr isotope ratios could be used to differentiate between WMNM mammoths and other late Pleistocene congeners that lived in the same area. MMC P1600 – the 'Brazos Gravel Pit' mammoth – was found ~100 years ago at 31°28'59"N by 97° 1'12"W, ~20 km southeast of WMNM. This fossil is late Pleistocene in age, and possibly older than the WMNM, found in a 4m-higher river terrace. The variation in ⁸⁷Sr/⁸⁶Sr ratios at 0.00033 for this tooth is the lowest recorded of any of the mammoths in this study (Table B4). The isotope ratios may indicate that this 'control' mammoth spent time grazing on vegetation rooted in the Smithson Paleosol or equivalent soils, and Upper Cenozoic clastics, but the time spent in each area varied little from subsample to subsample. The 'control' mammoth spent between 100% and 88% of its time feeding on vegetation growing in the Smithson Paleosol or equivalent (Fig. 3.7A). The 'Brazos Gravel Pit' (BGP) mammoth distinguishes itself from mammoth 'N' by yielding Sr ratios indicative of grazing within ~70 km from the WMNM, similar to mammoths, 'C,' 'L,' and 'M.' However, it differs from those three in that the range of variation of its Sr ratios is ~75% lower than the WMNM mammoths. The BGP mammoth has a Sr serial record distinct from the WMNM mammoths, despite this assessment of a similar minimum geographic grazing range.



Figure 3.7. ⁸⁷Sr/⁸⁶Sr ratios recorded in serial samples from three teeth from WMNM. X-axis shows sample numbers, with those to the right having been deposited most recently. Y-axis shows ⁸⁷Sr/⁸⁶Sr ratios. Pie charts show two-end-member mixing required to explain the isotopic data. Large pie charts show the highest and lowest ⁸⁷Sr/⁸⁶Sr ratios for each tooth. A) MMC P1600, the 'Brazos Gravel Pit' mammoth ~10 km ESE of WMNM. Age uncertain. ⁸⁷Sr/⁸⁶Sr ratios are less variable than in the WMNM material. B) WMNM 1190, a bison from the A#1 strata at WMNM. Ratios are broadly similar to the three WMNM mammoths in Fig. 3.5. C) WMNM 1229, a horse tooth from the 'matriarchal herd strata at WMNM. Shows relatively less time was spent feeding above Late Cenozoic clastic sediment than the Bison in Fig. 3.6b and mammoths in Fig.3.5, and spent some time feeding on grass growing on Upper Mesozoic carbonate sediments in the uplands above the site. Two-end-member mixing model represents the least distance from WMNM traveled to produce the observed ⁸⁷Sr:⁸⁶Sr ratios. A more distant home range is always possible.

The sampled bison tooth (WMNM 1190) was a fractured first or second lower right molar associated with a partial right mandible, exhibiting moderate wear. The tooth was found in 1978 in the same stratum as the mammoth aggregation and thus is of approximately the same age. This animal shows similar variability in Sr ratios – 0.0013 – to the WMNM mammoths (Table B4). The variation in ⁸⁷Sr/⁸⁶Sr ratios – 0.0010 – is slightly lower than the range seen in mammoths 'C,' 'L,' and 'M.' The bison fed on plants grown in the Smithson Paleosol or equivalents as much as 93% and as little as 55% of each 10-14 day sampling interval (Fig. 3.7B). The remaining strontium may have been from plants grown in Upper Cenozoic clastic sediments within valleys; along the Brazos or Bosque river valleys and tributaries, for example.

The sampled horse tooth (WMNM 1229) was a lower left m3 molar exhibiting considerable wear to the occlusal surface. The tooth was originally described as *Equus*, but *Equus* and *Haringtonhippus* cannot be distinguished based solely on dental characters (Heintzman et al., 2017). The tooth was found in 1986 in the same strata as the mammoth aggregation and it is likely of the same age. The ⁸⁷Sr/⁸⁶Sr ratios are comparatively low for this horse but the range of values is 0.0010, comparable with both the bison tooth and with mammoths 'C,' 'L,' and 'M,' but not 'N' (Table B4). Two of the eleven samples taken from the horse tooth – 7n11 and 7n1 – had ⁸⁷Sr/⁸⁶Sr ratios that could be explained by mixing Sr from plants growing in the Smithson Paleosol or equivalent with Sr from plants is seen in the bison, and in mammoths 'C,' 'L,' and 'M.' The ⁸⁷Sr/⁸⁶Sr ratios in the other nine samples may be explained by portioning of Sr from plants growing in the Smithson Paleosol or equivalent service in the Smithson Paleosol or equivalent with Sr from plants growing in the Smithson Paleosol or equivalent service in the samples may be explained by portioning of Sr from plants growing in the Smithson Paleosol or equivalent with Sr from plants growing in the Smithson Paleosol or equivalent with Sr from plants growing in the Smithson Paleosol or equivalent with Sr from plants growing in the Smithson Paleosol or equivalent by portioning of Sr from plants growing in the Smithson Paleosol or equivalent with Sr from plants growing in the Smithson Paleosol or equivalent with Sr from plants growing in the Smithson Paleosol or equivalent with Sr from plants growing in the Smithson Paleosol or equivalent with Sr from plants growing in the Smithson Paleosol or equivalent with Sr from plants growing in the Smithson Paleosol or equivalent with Sr from plants growing in the Smithson Paleosol or equivalent with Sr from plants growing in the Smithson Paleosol or equivalent with Sr from plants growing in the Smithson Paleosol or equivalen

close proximity to Upper Mesozoic carbonate lithologies, potentially 1 km to the south and 10 m higher in elevation than WMNM (Fig 2.7c).

Discussion

The original interpretation of the fossil assemblage at WMNM is that it represents the catastrophic death of an entire matriarchal herd of mammoths (Haynes, 1992). This Sr isotopic data on four of the twenty-three mammoths indicates at least two geographic ranges. Mammoth 'N,' the juvenile produced a series of ⁸⁷Sr/⁸⁶Sr ratios that differ at 25 from other sampled mammoths. The strontium ratios in mammoths 'C,' 'L,' and 'M,' can all be explained by feeding in areas that needn't have been further than 70 km away from the modern WMNM. This comports well with home-range studies of other extinct proboscideans (Baumann and Crowley, 2015; Hoppe et al., 1999). Mammoth 'N' had much higher strontium ratios than the other WMNM mammoths; the difference can only be explained by the input of strontium from granitic rocks ~ 180 km away. Movement this far is less commonly observed among extinct proboscideans, but not unprecedented (Kolis et al., 2019). The sampling method differed slightly for Mammoth 'N' compared to all other teeth in that the former had \sim 3 times the time averaging as the latter. The longer time averaging should have dampened intratooth variability in Sr isotopes, but its variability is actually greater than the other mammoth teeth sampled (Fig. 3.5). This Sr data with the two-end member modeling using the presented Sr isoscape for east Texas indicates that mammoth 'N' has a distinctly different migratory history than other mammoths. Mammoth 'N' was a juvenile, and would have been under normal circumstances too young to have left his/her parentage herd (Lister and Agenbroad, 1994; Shannon et al., 2008). Mammoth 'N' may have joined a hypothetical larger herd shortly

before its demise, as modern elephant herds do change in membership over time (Turkalo and Fay, 2001). Alternately more than one matriarchal herd might be present at the site. The BGP mammoth has the lowest variability in ⁸⁷Sr/⁸⁶Sr ratios of the sampled megafaunal teeth. If diagenetic change can be ruled out, it would indicate limited movement of BGP mammoth in contrast to the WMNM mammoths, showing that the WMNM mammoths can be potentially isotopically distinguished from other local mammoths.

The *Bison* and equid teeth came from highly disarticulated, incomplete, and scattered remains. It is possible that these teeth were transported to the site by fluvial processes from some distance up-stream. The isotopic ratios of both animals are consistent with dietary sources of strontium within ~70 km of WMNM. Moreover, the very low ⁸⁷Sr/⁸⁶Sr ratios seen in nine of the horse-tooth samples demonstrate that it spent time on the uplands, where none of the other animals under study have shown evidence of grazing. The last enamel deposited before death shows a more local signal, as was seen in the bison, 'Brazos Gravel Pit' mammoth, and mammoths 'C,' 'L,' and 'M.' This suggests that changes in resource availability and/or herd dynamics lead the equid to the area shortly before its death.

The presence of multiple species is inconsistent with a mammoth-specific catastrophic flood-kill. Rather, an *M. columbi* aggregation at WMNM would be consistent with changes in environmental conditions prior to the demise of the hypothetical Waco herd (cf. Nordt et al., 2015; Wiest et al. 2017). Modern African elephant (*Loxodonta africana*) herds can briefly gather into meta-herds when they encounter each other by chance (Viljoen, 1989). However, the presence of twelve other

megafaunal taxa at WMNM may indicate another scenario. *L. africana* herds – or individual elephants – often congregate along with other African megafauna during times of drought (Dudley et al., 2001; Foley et al., 2008; Wato et al., 2016). This inference is consistent with taphonomic and ichnological evidence for drought conditions and exposure of carcasses to decay and scavenging for months to years after demise (Wiest et al., 2016).

Conclusions

In light of this serial isotope study the longstanding hypothesis that the mammoths at WMNM represent a single matriarchal herd is no longer the sole plausible hypothesis. While three of the mammoths – 'C,' 'L,' and 'M' – could conceivably have been long-term members of a single social unit, mammoth 'N' need not have been. The higher ⁸⁷Sr/⁸⁶Sr ratios observed in 'N' can only be explained by Sr input from granitic soils. Unlike the other mammoths, the *Bison*, and the equid, mammoth 'N' – or its pregnant mother – must have spent time at least 180 km away from WMNM – or beyond. It is possible that 'N' or its mother joined the hypothetical matriarchal herd sometime after the last sampled enamel was laid down; modern African elephant herds are observed to fission and fuse on occasion. Just as plausibly, the accumulation at WMNM might represent more than one matriarchal herd from more than one region. This scenario has the advantage of producing the same demographic percentages as a single herd.

Testing this hypothesis will require sampling of the remainder of the mammoths at WMNM; one or more might share mammoth 'N's' ⁸⁷Sr/⁸⁶Sr signature. If this hypothesis is borne out, it would mean that animals were converging on the future WMNM from a wide area, perhaps hinting at resource shortages. Similar programs of study at other proboscidean cites around North America could be vital in understanding the extinction of the Pleistocene megafauna there. Changing patterns in range size, both temporally and geographically, could answer questions about the role of habitat fragmentation and extreme climate events in the megafaunal extinction.

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CHAPTER FOUR

Environmental Constraints from Serial δ^{13} C and δ^{18} O Analysis of Teeth from Waco Mammoth National Monument, TX USA

Abstract

Waco Mammoth National Monument (WMNM) is a Late Pleistocene - ~67 ka konzentrat-lagerstätte in central Texas, in the interfluve of the South Bosque and Brazos Rivers. The site consists of at least 23 Columbian mammoths - Mammuthus columbi along with a minimum of 12 other taxa. Sixteen of these animals were found on a single bedding plane and demographically resemble a single, matriarchal herd. Through stable isotope analysis, teeth can serve as a proxy for an organism's movement, diet, and climate in which it lived. Bulk sampling of WMNM mammoth teeth for Sr, C, and O, was done in the 1990s, but new serial sampling methods allow reconstruction of changes in isotope ratios over the period of enamel deposition. Here we present the results of the serial isotopic analysis of four *M. columbi* teeth from WMNM, an equid tooth from the site, and an *M. columbi* tooth from ~20 km ESE of WMNM. The δ^{13} C showed no seasonal signal in vegetation preference but, given the non-selective diet of proboscideans and equids, did indicate a higher percentage of C3 plants were present than in modern times. δ^{18} O analysis of the teeth show up to six annual wet/dry cycles in the mammoths and a little over one cycle in the equid. In three of the mammoth teeth and the equid tooth, the last deposited enamel appears to be relatively enriched in ¹⁸O, indicating that they were drinking more heavily evaporated water prior to their death. Three of them also indicate that regional surface water 67 kya was much heavier in ¹⁸O than it is at

present, indicating an overall more arid climate. These results are consistent with a recently proposed hypothesis that the WMNM mammoths were killed by a drought. Not all of the teeth studied follow these trends; however, leaving the question unsettled until more data is collected.

Introduction

Waco Mammoth National Monument is an important paleontological site in central Texas., This site preserves over a dozen Late Pleistocene taxa, most notably at least 23 individuals of Mammuthus columbi dating to ~67 ka (Bongino 2007; Nordt et al., 2015). Taphonomic and stratigraphic studies indicate that the demise of sixteen Mammuths was probably penecontemporaneous and may have constituted a matriarchal herd (Haynes 1992; Nordt et al. 2015; Wiest et al., 2017). Bulk isotopic analysis completed in past decades showed that the sampled mammoths from the site all had similar 87 Sr/ 86 Sr, δ^{13} C and δ^{18} O values, not falsifying this conclusion (Hoppe et al., 1999; Hoppe, 2004). The longstanding interpretation of the kill mechanism for the accumulation, a flash flood (Fox et al., 1992), has recently been challenged on taphonomic grounds in favor of a drought (Wiest et al., 2016). Recent serial analysis of ⁸⁷Sr/⁸⁶Sr ratios of four WMNM mammoths have shown that one had a different geographic origin than other the three mammoths tested (Esker et al., 2019). This study further evaluates the environmental conditions prior to demise of this herd by analyses of δ^{13} C and δ^{18} O of serially sampled teeth from four WMNM mammoths, a 'control' mammoth from elsewhere, and an equid. The intent is to determine whether the environmental conditions at WMNM were consistent with a resource shortage like a drought.

Modern Conditions

Geologic Context

Waco Mammoth National Monument (WMNM) is at 31°36'21.05"N by 97°10'27.53"W, on the northwestern edge of the central Texas city of Waco (Hartmann et al., 1992). The study area is a circular region 300 km in radius, with WMNM at its center and reflects the longest distance a Columbian mammoth has been demonstrated to have traveled (Kolis et al., 2019). This site is surrounded by Cretaceous marine and coastal facies, striking to the northeast. These rocks are part of a monocline dipping to the southeast, with Quaternary sediment along the Gulf Coast and Paleocene sediment ~170 km inland. Rocks 140 km northwest of the site are of Carboniferous and Permian age. The Llano Uplift occurs 150 km to the southwest of the site and is composed of a dome with Proterozoic intrusive igneous rocks at the center surrounded by successively younger Lower Paleozoic sedimentary formations. There is a small outcrop of Upper Cretaceous extrusive igneous rocks in the southern part of Austin (Ewing and Caren, 1982).

WMNM is in the interfluve of the South Bosque and Brazos rivers (Fig 4.1). The topography is predominantly a modern floodplain and three successively higher and older fluvial terraces. The site is within the second river terrace, originally exposed by erosion from an ephemeral tributary of the South Bosque River (Bongino, 2007). The site is composed of at least two fossil-bearing strata. The oldest of these has been dated to $67,000 \pm 5,000$ yr via optically stimulated luminescence dating (OSL) (Nordt et al., 2015b). Thus far this stratum has produced the remains of sixteen Columbian mammoths (*Mammuthus columbi*). There is another bone-bearing stratum 1 m above the 'lower

bone-bearing bed,' including the only adult male mammoth from the site, with the enclosing sediments yielding an OSL date of $53,000 \pm 5,000$ yr (Nordt et al., 2015).



Figure 4.1. Location of Waco Mammoth National Monument. The study area is circled; the coordinates are of WMNM

Modern Regional Climate

Understanding the relationship between climate and stable isotope data requires a modern baseline. For this study we will use the modern climate of the study area. The study area is \sim 283,000 km², and thus encompasses significant climate variability. Annual rainfall in the region decreases westward, with the highest rainfall – \sim 1,518 mm yr⁻¹ – near Jasper, Texas, along the Louisiana border and the lowest (538 mm yr⁻¹) near San Angelo, Texas (National Climatic Data Center, 2010; The National Map: Printable

Maps). The highest mean annual temperatures (MAT) within the study area are in the south near Victoria, Texas, at 21° C and the lowest MAT is near Seymour, Texas, at 17° C (National Climatic Data Center, 2010; The National Map: Printable Maps).

Modern Regional Carbon Isoscape

Photosynthesis discriminates against ¹³C, with different photosynthetic pathways – C3, C4, and CAM – discriminate to different degrees. C3 vegetation discriminates the most with values of -27 to 24‰, C4 plants discriminates less with ratios between 10 and 15‰ and CAM plants are intermediate (Ben-David and Flaherty, 2012; Bender, 1971). Vegetation for the study region varies in δ^{13} C between ~-15‰ and ~-20‰, with the immediate environs around WMNM being ~-18‰ (Firmin, 2016). Based on a simple two-end-member mixing model, the inferred highest modern percentage of C3 vegetation in the study area is ~50%, the lowest is ~14%, and in the ~100 km² region around WMNM vegetation is approximately 36% C3 (Fig. 4.2). Based on analysis of paleosol organic matter, vegetation %C3 for the WMNM at the time of the mammoths' deaths was between 52 and 67% (Bongino, 2007).



Figure 4.2. Texas vegetation δ^{13} C VPDB modified from Firmin (2016). Study area is circled.

Modern Regional Oxygen Isoscape

Modern meteoric water δ^{18} O measurements in Texas are preferable to surface water δ^{18} O measurements as a point of comparison, because the latter are strongly affected by anthropogenic effects (Dimarco et al., 2012; Van Plantinga et al., 2017)(Fig 3.3). At present, meteoric δ^{18} O within 300 km of WMNM is between -6.3‰ and -3.3‰ VSMOW, with precipitation being isotopically lighter with increasing distance from the Gulf of Mexico (Bowen et al., 2007). δ^{18} O in surface water becomes heavier through evaporation, thus this is a lower bound for modern surface water δ^{18} O values. An upper bound can also be put on the Brazos River where it enters the Gulf of Mexico: 0.5‰ to - 1.25% VSMOW (Dimarco et al., 2012). Longer residence times in impounded rivers lead to isotopically heavier water. The modern study area – without anthropogenic modification of its surface water – would have a δ^{18} O between -6.3‰ and 0.5‰.



Figure 4.3. δ^{18} O VSMOW of Texas precipitation. Modified from Bowen et al. (2007). The study area is circled.

Materials and Methods

Teeth Sampled

For this study, six mammal teeth, stored at the Mayborn Museum Complex, were serially sampled for δ^{13} C and δ^{18} O analyses (Benedict, 2010). Of those, four were Columbian mammoths from the 'lower bone-bearing' stratum at WMNM, one was an equid tooth from the same stratum, and one was a Columbian mammoth tooth found ~20 km ESE of WMNM in the late 19th century (Table 4.1, Table C1).

Table 4.1. Teeth sampled for this study using the P2-P4, M1-M3 nomenclature for proboscidean teeth.

Specimen #	Specimen Name	Specimen Description Provenance		
WMNM 72	Mammoth 'C'	M2 molar	WMNM 'lower bone-	
			bearing' stratum	
WMNM 318	Mammoth 'L'	P4 or M1	WMNM 'lower bone-	
			bearing' stratum	
WMNM 366	Mammoth 'M'	M1 or M2	WMNM 'lower bone-	
			bearing' stratum	
WMNM 368	Mammoth 'N'	P4	WMNM 'lower bone-	
			bearing' stratum	
P1600	'Gravel Pit' Mammoth	M2 or M3	Gravel pit at	
			31°28'59"N by 97°	
			1'12"W	
WMNM 1229	Equid tooth	m3 heavily worn	WMNM 'lower bone-	
			bearing' stratum	

Enamel is the most impermeable hydroxyapatite tissue in a vertebrate tooth (Ungar, 2010). The other two substances, dentin and cementum, are composed of hydroxyapatite crystals an order of magnitude smaller and more loosely packed; they are thus both highly permeable. Enamel's relative impermeability makes it resistant to diagenetic change (Gage et al., 1989; Lee-Thorp and van der Merwe, 1991; Zazzo et al., 2012).

Mammal teeth – and proboscidean teeth in particular – form over an extended period of time, making them valuable potential records (Lee et al., 2012). In mammals, tooth mineralization begins at the leading edge of the crown and proceeds rootwards and rearwards (See Esker and others. 2019, Fig. 1a). In members of the Equidae, enamel is deposited over a period of 1.0 to 1.5 years, while in modern elephants – and mammoths – formation of the last tooth might take 15 years (Dirks et al., 2012; Uno, 2012). In Late Pleistocene North American mainland mammoths, the rate of enamel extension is ~13 mm yr⁻¹ (Dirks et al., 2012; Metcalfe and Longstaffe, 2012). The outer layer of enamel crystals in a mammal tooth do not form parallel to the surface of the tooth, but at a low angle to it (cf. Esker et al. 2019 Fig. 1B). The innermost layer of enamel, 100 μ m thick, does form parallel to the tooth surface, so this was the layer sampled. This aprismatic enamel represents a single growth period of 1 to 2 weeks (Blumenthal et al., 2014; Dirks et al., 2012; Metcalfe et al., 2011; Zazzo et al., 2012).

Mammoths and modern elephants have two sets of three teeth of teeth over a lifetime, giving the impression of six sets of teeth. The standard nomenclature for proboscideans is P1-P3 and M1-M3 for upper molariforms and p1-p3 and m1-m3 for lowers (Agenbroad, 1994). Here we will use the more general nomenclature for mammal

teeth, P2-P4 and M1-M3 for upper molariforms and p2-p4 and m1-m3 for lowers (Ungar, 2010). Tooth enamel was sampled from near the posterior-most and rootward-most part of each tooth such that each enamel record was approximately contemporaneous.

Initial analysis was of a P4 from mammoth 'N,' an ~8-year-old juvenile. This tooth was sampled at Baylor University with a Seimens CNC machine. A 1/16" (1.58 mm) bit was used in sampling, as this was the smallest diameter available. Each sample time-averaged about 44 days assuming enamel growth rate of ~13 mm yr⁻¹ (Dirks et al., 2012; Metcalfe and Longstaffe, 2012). The tooth was secured to a stage within the CNC machine and a Dino-Lite Edge AM7515MT2A field microscope was used to position the drill head. Each time the bit was lowered 100 μ m into the enamel, an infant nasal aspirator was used to collect the powdered hydroxyapatite subsample. The tooth and the equipment were cleaned after each subsample to prevent cross-contamination (Esker et al., 2019). The programmed depth of the drill-head was lowered by 100 μ m, and the process repeated until changes to the sample color or sound of the CNC machine indicated that the full thickness of the enamel had been penetrated. We retained the second-to last subsample for analysis, as it contained the aprismatic enamel.

A computer-controlled micromill, designed and built by CW, was used to sample the other mammoth teeth. After being secured to a computer-controlled stage, a rotary tool with a 0.5 mm diameter diamond bit was clamped into an armature above it (Widga, 2018). The smaller bit allowed for time-averaging of ~14 days. To prevent sample loss we built a clay dam around the sampling area and filled it with 20-200 μ L laboratorygrade DI water. After turning on the rotary tool and setting it to 7,000 – 10,000 RPM, software (Galiltools v. 1.6) moved the stage and tooth so that the rotary tool removed a

subsample of enamel 0.5 mm wide by 0.5 cm long by 100 µm deep. The water and powdered subsample were removed from the dammed area with a disposable-tip pipette and placed in a plastic centrifuge tube. The tooth and equipment were then thoroughly cleaned to prevent cross-contamination (Esker et al., 2019). As with the CNC machine, these steps were repeated until the full thickness of the enamel had been sampled. The second-to-last subsample was kept for isotopic analysis (Kolis et al., 2019). Between ten and twenty samples were taken from along the growth axis of each tooth, ~1.0 mm apart.

Serial Sampling Non-mammoth Enamel with a Rotary Tool

Computer-controlled machinery was not necessary for the non-mammoth sampled. Equid teeth form more quickly than proboscidean teeth (Glassburn et al., 2018; Widga et al., 2010). Time averaging is ~14 days when samples are taken perpendicular to the growth axis of the tooth with a rotary tool using a 2.5 mm bit (Britton et al., 2009). The surface of the tooth was cleaned prior to sampling, then a ~0.5 cm long, 2.5 mm wide sample was taken from the buccal surface of the tooth, the resultant powder stored in a labeled vial. The tooth, sampling apparatus, and sampling area were cleaned after each sample to avoid cross-contamination (Esker et al., 2019).

Sample Preparation for Isotope Ratio Mass Spectrometry

The vials containing powdered mammoth enamel samples in DI water were centrifuged at 3,000 RPM for 30 minutes; compressing the powder to a pellet in the tip of the vial. Most of the water was pipetted and disposed of, with the plastic pipette tips discarded between subsamples. Each subsample was completely dried after being placed in a Carolina Scientific 6" vacuum desiccator for ~12 hours. These vials were sent to the

Baylor University Stable Isotope Laboratory for carbon and oxygen analysis. The powdered equid tooth enamel and powdered enamel from mammoth 'N' were sent with no further preparation.

Approximately 1.0 to 0.5 mg of each subsample was placed in a Thermo Finnigan gas-bench automated sampling device and reacted with ortho-phosphoric acid at 90° C. It was analyzed by a Thermo-Electron Δ ta V Advantage IRMS in dual inlet mode (Metcalfe et al., 2010). The machine was calibrated with the NBS-18 (n=4), IAEA-CO-1 (n=4), and Merck Carbonate (n=12) isotopic standards.

Results

$\delta^{13}C$ WMNM Mammoth Teeth

Data from the IRMS enamel analysis for the WMNM mammoth teeth was corrected assuming a 14.1‰ enrichment in ¹³C versus the δ^{13} C value of their food plants and a 1.4‰ (Keeling and Piper, 2001). The percent C3 in their diet was then calculated with a two-end-member mixing model assuming a C3 end member with a δ^{13} C of -27.4‰ and a C4 end member with a δ^{13} C of -12.7‰ (Bender, 1971). Fractionation in C3 plants changes when under drought stress, becoming -24.6‰ (Cerling and Harris, 1999; Passey et al., 2002). In order to account for this possibility, C3% reconstructions were done using both normal (-27.4‰) and drought-stressed (-24.6‰) C3 end members. Mean percentage C3 for the WMNM mammoths' diet was 43.8 ± 4.7% under normal conditions and 54.1 ± 5.8% under water stress (Fig. 4.4, Table C1, File C2). Mammoth 'N,' the animal found to have a different geographic origin than the other WMNM mammoths had a diet consisting of ~44.4 ± 3.7% C3 under normal conditions and ~54.8

 \pm 4.6% C3 under drought conditions. The other three WMNM mammoths collectively had an average diet of ~43.4% \pm 5.3% C3 vegetation under normal conditions and ~53.7% \pm 6.5% C3 under drought stress. Mammoth 'N's diet appears not to have differed significantly from that of the other WMNM mammoths.



Figure 4.4. Time series of %C3 vegetation in the diets of WMNM fauna. X axis is the sample number, with youngest samples to the right; Y axis is %C3 vegetation. Blue line shows normal conditions, magenta line indicates drought stressed conditions. Calculated from δ^{13} C via a two-end member mixing model. A) is mammoth 'C' (WMNM 72). B) is mammoth 'L' (WMNM 318). C) is mammoth 'M' (WMNM 366), D) is mammoth 'N' (WMNM 368). E) is the 'Gravel Pit' mammoth (MMC P1600). F) is the WMNM equid (WMNM 1229).

$\delta^{13}C$ 'Gravel Pit' Mammoth Tooth

The δ^{13} C for MMC P1600, the mammoth from the gravel pit ~20 km to the ESE of WMNM, was corrected using the same 14.1‰ and enrichment as above, and the percentage C3 was calculated using the same end-member values for C3 and C4 plants as above. Mean percentage of C3 vegetation for the 'Gravel Pit' mammoth's diet 31.4 ± 2.7% C3 plants under normal conditions and 38.8 ± 3.4% C3 (Fig. 4.4). It thus differs significantly from the WMNM mammoths. Its provenance on a higher terrace of the Brazos river suggests that it is older than the WMNM megafauna (Google, 2017).

$\delta^{13}C$ WMNM Equid Tooth

Equid tooth WMNM 1229 was corrected using the same enrichment and endmember values mentioned previously. Mean %C3 for the equid's diet was $44.9\% \pm 4.7\%$ under normal conditions and $55.5\% \pm 5.8\%$ when the C3 plants are drought-stressed. (Fig. 4.4). This is within uncertainty of the WMNM mammoths, but not the mammoth from the gravel pit in the region.

$\delta^{18}O$ WMNM Mammoth Teeth

The biogeochemistry of δ^{18} O in tooth enamel is used to track of the δ^{18} O of an animal's drinking water. The drinking water δ^{18} O in turn is a result of several factors, including local humidity, temperature, and residence time in lakes and rivers. Variations in δ^{18} O reflect seasonal changes in the evaporative balance of drinking water for large mammals whose tooth enamel formed over months to years. Biological fractionation of oxygen between ingested water and enamel can be accounted for as follows (Equation 4.1) (Barrick et al., 1999; Kohn et al., 1996):

Equation 4.1. Equation for estimating the $\delta^{18}O(\delta_w)$ of water based on the $\delta^{18}O(\delta_p)$ of mammalian tooth enamel. -22.7 and 0.78 are constants related to mammalian metabolism (Luz et al., 1984).

$$\delta_w = \frac{\delta_p - 22.7}{0.78}$$

Mammoth 'C' (WMNM 72) is a fragmentary M2 molar from an adult female ~33 years old at time of death (Haynes, 1992). Eight subsamples were analyzed for carbon and oxygen isotopes, with the distance between the first and last samples being 15 mm. Based on an assumed enamel extension rate (13 mm/yr) the period of enamel deposition may be ~1.2 years (Table C3). In agreement with this, isotopic analysis shows one local minimum in δ^{18} O and two local maxima in the record for this tooth (Fig 3.5A). Corrected for biological fractionation, the lightest subsample is 1.14‰ δ^{18} O and the heaviest is 5.54‰ δ^{18} O. The most recently deposited subsample analyzed is the heaviest.



Figure 4.5. Dual plots of ⁸⁷Sr/⁸⁶Sr (magenta triangles and dashed lines) and δ^{18} O (blue dots and solid lines) versus sample position in mm for three of the teeth under study. Sample position is measured from the crown/root junction, therefore samples further to the right on each graph are from later in the animal's lifetime. A) is mammoth 'C' (WMNM 72). B) is mammoth 'L' (WMNM 318). C) is mammoth 'M' (WMNM 366). ⁸⁷Sr/⁸⁶Sr given as a proxy for geographic origin. See Esker et al. 2019 for further explanation.

Mammoth 'L' (WMNM 318) tooth is a fragmentary P4 or M1 molariform from mammoth 'L,' an adult female between 15 and 29 years old upon demise (Lee et al., 2012). Seven serial samples were analyzed for carbon and oxygen isotopes. The distance between the first and last samples is 20.9 mm (Table C3). Based on the estimate for enamel extension rates (13 mm/yr), the period of enamel deposition may be ~1.6 years. Three of the subsamples for this tooth – including the oldest two – proved too small to analyze, thus the full 1.6-year period was not present. The isotopic analysis shows only one minimum and two maxima, indicating a record length of ~1 year. The subsamples are all isotopically light compared to the other mammoths; when corrected for biological fractionation, the lightest is -10.2‰ δ^{18} O and the heaviest is -0.2‰ δ^{18} O. The most recently accreted enamel subsample is the heaviest (Fig 4.5B).

Mammoth 'M' (WMNM 366) is a partial M1 molar from an adult female ~25 years old at time of death (Haynes, 1992). Eight subsamples were successfully analyzed for carbon and oxygen. An additional two subsamples – including the last deposited – proved too small to analyze. The full length of the sample set was 22 mm (Table C3). Based on the standard enamel extension rate for proboscideans (13 mm/yr), this should represent a record of ~1.7 years. The presence of three local maxima indicates a somewhat longer record than this (Fig 4.5C). When corrected for biological fractionation the lightest subsample is -4.0‰ δ^{18} O and the heaviest is 3.4‰ δ^{18} O. The most recently deposited subsample analyzed is heavy at 1.1‰ δ^{18} O.

WMNM 368 is a P4 molariform from mammoth 'N' who was eight years old at death (Haynes 1992). Ten subsamples of enamel underwent isotopic analysis for C and O, with the distance between the first and last samples being 84.7 mm (Table C3). Two

additional subsamples were too small for analysis. The enamel-dentin junction is not preserved on this tooth, so the last deposited enamel has not been sampled. Based on an average rate of enamel accretion of ~13 mm yr⁻¹, the period of deposition represented may be ~6.5 years (Dirks et al., 2012; Metcalfe and Longstaffe, 2012). Isotopic analysis shows four local δ^{18} O maxima in the record for this tooth (Fig. 4.6A). Corrected for biological fractionation, the isotopically lightest subsample is 1.29‰ δ^{18} O VSMOW and the heaviest is 6.38‰ δ^{18} O. The last subsample analyzed was relatively light at 1.35‰ δ^{18} O.



Figure 4.6. Dual plots of ⁸⁷Sr/⁸⁶Sr (magenta triangles and dashed lines) and δ^{18} O (blue dots and solid lines) versus sample position in mm for three of the teeth under study. Sample position is measured from the crown/root junction, therefore samples further to the right on each graph are from later in the animal's lifetime. A) is mammoth 'N' (WMNM 368). B) is the 'Gravel Pit' mammoth (MMC P1600). C) is the WMNM equid (WMNM 1229). ⁸⁷Sr/⁸⁶Sr given as a proxy for geographic origin. See Esker et al. 2019 for further explanation.

$\delta^{18}O$ 'Gravel Pit' Mammoth Tooth

MMC P1600 is an M2 or M3 molar from the 'Gravel Pit' mammoth found ~20 km ESE of WMNM. It was between 29 and 40 years old at the time of its death (Lee et al., 2012). Eight subsamples were successfully analyzed for carbon and oxygen isotopes. Two further subsamples – including the last deposited enamel on the tooth – were too small for analysis. The total length of the sample set was 17.8 mm (Table C3). Based on the standard enamel extension rate for proboscideans, this should represent a record of ~1.4 years. The record contains two local maxima and three local minima, indicating a somewhat more rapid extension rate. When corrected for biological fractionation the isotopically lightest sample is -1.7‰ δ^{18} O VSMOW and the heaviest is 7.7‰ δ^{18} O VSMOW. The youngest subsample analyzed is the lightest (Fig 4.6B).

$\delta^{18}O$ WMNM Equid Tooth

WMNM 1229 is a left m3 for either an *Equus sp.* or *Harringtonhippus sp.*; the two genera are not distinguishable on the basis of dental morphology (Heintzman et al., 2017). The occlusal surface is significantly worn, making it impossible to assess the age of the animal at death. Horse teeth grow over a shorter period of time than mammoth teeth, and the enamel extension rate can be as high as ~106 mm yr⁻¹ (Hoppe et al., 2004; de Winter et al., 2016). Ten samples were analyzed for carbon and oxygen isotopes, an additional sample was too small for analysis. The total length of the sample set is 51 mm, thus the tooth represents a period of at least ~0.47 years (Table C3). There are five local maxima in the record and four local minima. The isotopically lightest sample is -3.5‰ δ^{18} O and the heaviest is 6.1‰ δ^{18} O (Fig. 4.6C). The youngest sample analyzed is comparatively heavy at 3.7‰ δ^{18} O.

Discussion

$\delta^{13}C$ Seasonal Pattern

Carbon isotopes indicate no seasonal pattern in the dietary C3:C4 ratio for the WMNM megafauna. (Fig. 4.7, 4.8). Neither modern equids nor modern proboscideans show a strong preference for vegetation using a particular photosynthetic pathway, and this appears to have been the case for the animals at WMNM ca. 67 ky ago (Macfadden et al., 1999; Sukumar, 2003; Turkalo and Fay, 2001).



Figure 4.7. In solid black lines, δ^{13} C of the vegetation for three of the serially-sampled megafauna teeth in this study. In dashed red lines, δ^{18} O values of drinking water for the same samples. Left Y axis shows δ^{13} C in ‰VPDB. The right Y axis shows δ^{18} O ‰VSMOW. The X axis measures distance from the crown/root junction in mm, and is a proxy for sample age, with the youngest deposited samples to right. A) is mammoth 'C' (WMNM 72). B) is mammoth 'L' (WMNM 318). C) is mammoth 'M' (WMNM 366).

After accounting for biological fractionation and atmospheric differences, the δ^{13} C for the WMNM teeth should reflect the available vegetation at the time. The mean percentage of C3 plants for the region ca. 67 kya was $43.8 \pm 4.7\%$ assuming normal conditions, and $54.1 \pm 5.8\%$ under drought conditions (File C2). This is somewhat higher than the current percentage of C3 plants in the immediate area of WMNM today, but well within the variation seen within 300 km of WMNM (Firmin, 2016). It is within error of the %C3 based on pedogenic carbonate δ^{13} C of 41% (Bongino, 2007). The noncontemporaneous 'gravel pit' mammoth molar (MMC P1600) looks very different from the WMNM megafauna, with a mean %C3 of $31.4 \pm 2.7\%$ under normal conditions and of $38.8 \pm 3.4\%$ when the C3 plants are water stressed (Fig 4.8D; File C2). Based on ⁸⁷Sr/⁸⁶Sr ratios, MMC P1600 appears to have been a local animal, though its source on an upper Brazos river terrace indicates it is probably older than the WMNM mammoths (Esker et al., 2019). This implies either a more arid climate than present – and during WMNM 'lower bone-bearing' strata deposition – during the period of time that the 'Gravel Pit' mammoth was alive (Lehmann et al., 2016; Szabó et al., 2017).



Figure 4.8. In solid black lines, δ^{13} C of the vegetation for three of the serially-sampled megafauna teeth in this study. In dashed red lines, δ^{18} O values of drinking water for the same samples. Left Y axis shows δ^{13} C in ‰VPDB. The right Y axis shows δ^{18} O ‰VSMOW. The X axis measures distance from the crown/root junction in mm, and is a proxy for sample age, with the youngest deposited samples to right. A) is mammoth 'N' (WMNM 368). B) is the 'Gravel Pit' mammoth (MMC P1600). C) is the WMNM equid (WMNM 1229).

WMNM Drinking Water $\delta^{18}O$ *Compared to Modern Surface and Meteoric Water*

The modern δ^{18} O of meteoric water within the study area is -6.3‰ to -3.3‰ (Bowen et al., 2007; Mcchesney, 2017). The δ^{18} O of modern Brazos River water where it enters the Gulf of Mexico is between 0.5 and -1.25‰ (Dimarco et al., 2012). The -6.3‰ to 0.5‰ range expected for the modern study region is less enriched in ¹⁸O than those calculated for mammals from WMNM. The mean δ^{18} O of those animals is 1.11‰, and the summer mean δ^{18} O is 3.54‰ implying drier conditions at 67 kya than at present (Table 4.2, Figs. 4.3, 4.6, 4.9; Table C4).

$\delta^{18}O$ Seasonal Signal

Serial δ^{18} O enamel samples can be used to develop a chronology by noting seasonal signals: high δ^{18} O for summer, and low δ^{18} O for winter (Gadbury et al., 2000; Gigleux et al., 2017). Mammoths 'C,' 'L,' and 'M,' and the WMNM equid were all depositing enamel relatively heavy in ¹⁸O at or near the time of their deaths. In turn, this data can be used to determine the season in which the last sampled enamel was deposited (Fisher et al., 2014). In contrast, the ~6.5 years of record for mammoth 'N' were analyzed and the youngest sampled enamel was light in ¹⁸O, indicating cool conditions during the interval of deposition for the enamel nearest the break in the crown. Mammoth 'N's last recorded maximum, however, represents the highest δ^{18} O recorded in any WMNM sample at 6.38‰ (Fig. 4.6).

Table 4.2. δ^{18} O of the maxima for each of the teeth considered in this study. These samples represent the most heavily evaporated drinking water in each record, and thus represent 'summer' values, except in the equid (Gigleux et al., 2017). Given the high enamel accretion rate in equids, less than a year is recorded, so only the highest maximum was used to calculate the WMNM summer mean (Hoppe et al., 2004).

Mammoth 'C'	Mammoth 'L'	Mammoth 'M'	Mammoth 'N'	Gravel Pit' Mammoth	Equid
5.54	0.71	1.05	5	5.42	3.71*
5.54	-1.11	3.37	4.94	3.99	3.37*
		-0.29	5.22	7.72	2.61*
			6.38		3.56*
					2.4*
					1.63*
					6.1



Figure 4.9. δ^{18} O of the drinking water consumed by the six animals under study. The boxes represent the interquartile range of δ^{18} O for each tooth, the horizontal line in each box represents the mean δ^{18} O for each tooth, and the whiskers represent the 95% confidence interval for each tooth. The cyan bar shows the 95% confidence interval for the lightest quartile of δ^{18} O values in modern meteoric water in the study region (Table C5, Bowen et al., 2007; IAEA/WMO, 2019). The magenta line shows the 95% confidence interval for the heaviest quartile of δ^{18} O of Brazos River water between 2007 and 2008 (Dimarco et al., 2012).

Inferred Continental-Scale Environmental Conditions Associated with the Demise of the WMNM Megafauna

Recent research on the taphonomy and ichnology of WMNM is counter to the inference that the mammoths there were killed in a flood (Bongino, 2007; Nordt et al., 2015b). Instead, evidence of prolonged surface exposure under arid conditions suggests the possibility that a drought might have contributed to the accumulation (Wiest et al., 2016). The 'lower bone-bearing' stratum accumulated during Oxygen Isotope Stage (OIS) 4, a glacial interval between 71,000 and 57,000 years ago (Taylor and Aitken, 1997). The δ^{13} C and δ^{18} O speleothem record from Crevice Cave, Missouri, shows the southeastern US became hotter and drier during this period, and Illinois pollen studies show the period between 90 and 60 ka as being unusually dry (Curry and Baker, 2000; Dorale et al., 1998). Looking more broadly at glacial intervals, phytoliths records for Texas during OIS 2 show lower-than-modern mean annual precipitation as well (Ellwood and Gose, 2006).

A recent ⁸⁷Sr/⁸⁶Sr study of four teeth from the site indicates the movement of mammoths just prior to their demise. One juvenile mammoth migrant traveled at least 180 km to the confluence of the Brazos and Bosque rivers, possibly joining an exogenous group. The remaining three mammoths appear to have migrated from at least 70 km down the Brazos River valley (Esker et al., 2019). This movement of two distinct mammoth populations may reflect megafaunal herds from over a wide area converging on a more viable water source at the convergence of the Brazos and Bosque rivers during a dry period or possibly an extended drought (Dudley et al., 2001; Foley et al., 2008; Wato et al., 2016).

Synthesis: WMNM

Combining the δ^{13} C, δ^{18} O, and 87 Sr/ 86 Sr serial sampling results allows us to make more definitive statements about each WMNM animal's environmental conditions and behavior during the time before their death. In turn this casts light on the drought hypothesis as a kill mechanism.

WMNM 72, mammoth 'C' grazed on a mixture of C3 and C4 vegetation between $43.3 \pm 5.1\%$ C3 under normal conditions, or $53.4 \pm 6.3\%$ C3 under drought conditions. The former estimate is lower than the 52% to 67% C3 vegetation that grew during MIS 4 in the immediate vicinity of the 'lower bone-bearing' stratum (Bongino, 2009). The high percentage of C3 plants at the site during OIS 4 is probably the result of broad-leaf riparian vegetation growing along the Bosque River and its tributaries. The implication is that mammoth 'C' generally fed away from the watercourse, though the relatively high first and last %C3 may represent an increased intake in riparian vegetation (Fig 4.4A). This is consistent with the ⁸⁷Sr/⁸⁶Sr results that shows mammoth 'C' to have spent time a short distance (at least 70 km) away from WMNM, where the vegetation makeup was presumably different (Fig. 4.5A, Esker et al., 2019). The δ^{18} O shows that the climate overall was more arid than present, with mammoth 'C' laying down its last bit of enamel during the summer months.

WMNM 318, mammoth 'L,' grazed on plants of $50.3 \pm 4.4\%$ C3 under normal conditions, and $51.3 \pm 5.4\%$ C3 when the food plants were under drought stress, making it comparable to mammoth 'C,' and make the 'normal' estimate lower in C3 plants than the soil organic carbon indicates that the site was (Fig. 4.7B). The ⁸⁷Sr/⁸⁶Sr results are consistent with the same home-range of mammoth 'C,' as well (Fig. 4.5B). The overall

 δ^{18} O signal for this mammoth differs significantly from 'C' (and every other animal in the study) in in resembling the isotopically light modern surface water in the study area (Fig. 4.9). This indicates either a distinct water source for mammoth 'L,' or diagenetic alteration of the enamel by modern water. The first supposition would indicate that 'L,' had a different geographic origin, while the latter would demonstrate the patchy nature of diagenetic change within a single bonebed (Wang and Cerling, 1994).

WMNM 366, mammoth 'M,' consumed a mix of vegetation similar to what is seen in mammoths 'C,' and 'L,' containing $46.8 \pm 6.0\%$ C3 plants under normal conditions, and $57.8 \pm 7.4\%$ C3 under drought stress (Fig. 4.4C). The strontium ratio analysis shows a similar range of movement to 'C,' and 'L,' as well (Fig. 4.5C). δ^{18} O is intermediate between mammoths 'C,' and 'L,' implying either that it drank from a different combination of water sources than either, or that it has been diagenetically altered (Fig 4.9). The maximum δ^{18} O is still higher than is seen in the study region at present, implying a drier climate as seen in mammoths 'C,' 'N,' and the WMNM equid (Figs. 4.3,4.9).

WMNM 368, mammoth 'N,' had a diet broadly similar to mammoths 'C,' 'L,' and 'M,' made up of $44.4 \pm 3.7\%$ C3 plants if the plants were under normal conditions, or $54.8 \pm 4.6\%$ C3 if they were drought stressed (Fig. 4.4D). The ⁸⁷Sr/⁸⁶Sr results differ significantly from every other animal in the study (Fig. 4.8A). The strontium ratio is so high that the animal must have consumed some plants growing in granite-derived soils to achieve it (Esker et al., 2019). Thus, the animal must have spent some time at least 180 km away from WMNM, in the Llano Uplift, where such soils are found. The δ^{18} O has a nearly identical range to that of mammoth 'C,' but the last sample taken from the broken

base of the tooth is depleted in ¹⁸O. This indicates that this enamel was deposited during the winter, an unknown interval before the mammoth 'N's death.

WMNM 1229, the equid from the 'lower bone-bearing' stratum, had a diet containing slightly less C3 vegetation than mammoths 'C,' 'L,' 'M,' and 'N' (Figs. 4.4, 4.8B), with C3 plants making up $36.9 \pm 4.7\%$ C3 of its diet under normal conditions, or $45.6 \pm 5.8\%$ C3 if they were drought stressed. This is consistent with the diets hypothesized for both Pleistocene elephantids and equids (Macfadden et al., 1999; Mead et al., 1986). Like mammoths 'C,' 'L,' and 'M,' the strontium ratio analysis indicates that the equid needn't have migrated more than 70 km to arrive at WMNM (Fig. 4.6B, Esker et al., 2019). Unlike these animals, however, some enamel samples from WMNM 1229 must have been laid down after eating vegetation growing in soil developed on Cretaceous carbonates. The nearest such deposits are less than 2 km from WMNM, but none of the WMNM mammoths can be shown to have eaten from them (Hartmann et al., 1992). The WMNM equid's range of δ^{18} O values is most similar to mammoth 'M.' As a result of rapid deposition of enamel in equids, the record for WMNM 1229 has a higher resolution than any of the mammoths, with the enamel in the 10 samples taken representing just ~ 175 days of enamel deposition – less than a full seasonal cycle. The fluctuations in δ^{18} O therefore represent either multiple water sources that the equid made use of during this time, or fluctuations in the δ^{18} O of a single water source over the same period (Figs. 4.6B; 4.9). Together these results indicate an animal that was living in the same drier-than-modern climate as mammoths, 'C,' 'M,' and 'N,' but was not always in close proximity to them before they met their mutual end at WMNM.

Taken together, the isotopic data from WMNM reveals that the relationship of the animals to the site, the climate, and each other is more complex than previously supposed (Nordt et al., 2015b). While δ ¹³C shows that the WMNM megafauna had similar diets, differing δ ¹⁸O and ⁸⁷Sr/⁸⁶Sr signatures imply that they had differing geographic origins. In addition, several of the teeth record that some WMNM fauna had been drinking water far more enriched in ¹⁸O than is present in surface water in the study area, strongly indicating a drier climate. In the 'lower bone-bearing' strata at WMNM we see animals from different herds and different species crossing a desiccated landscape to converge on the last of a dwindling resource: water.

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CHAPTER FIVE

Summary and Future Research

Multiple approaches can be taken when putting the field of vertebrate paleontology in the service of terrestrial paleoclimatology. Understanding the climate tolerances of individual taxa of a fossil assemblage can be used toward reconstructing the paleoclimate during the period of assemblage formation. Strontium isotope ratios in mammal teeth can be used to reconstruct the home range and movement of individual animals in the months and years prior to their internment. These patterns of movement can be matched with climatic conditions known to generate them in the present. Carbon and oxygen isotope analyses of mammal teeth can be used to reconstruct the diet of individual animals, and the evaporative balance of their drinking water, over the last few years of their lives. Both are a function of climate. In this dissertation, all three methods have been applied to a single site: Waco Mammoth National Monument.

Reconstructing the Mass and Thermal Ecology of North American Pleistocene Tortoises

- The largest fossil tortoise known from Texas weighed ~339 kg.
- Tortoises of this size could survive no more than ~33 hours of freezing temperatures.
- The presence of tortoise remains at WMNM implies a milder climate than at present, where temperature never drop to freezing for more than ~33 hours.

Home Range of the Columbian Mammoths (Mammuthus columbi) and Grazing Herbivores from the Waco Mammoth National Monument, (Texas, USA) Based on Strontium Isotope Ratios from Tooth Enamel Bioapatite

- Of the six sampled teeth from the 'lower bone-bearing' strata, three of the mammoths, as well as the bison, show an ⁸⁷Sr/⁸⁶Sr ratio that could have been achieved by feeding on vegetation as close as 70 km away.
- The equid's tooth has an ⁸⁷Sr/⁸⁶ Sr ratio more consistent with feeding on soil developed directly on Late Mesozoic carbonate rocks. The sample deposited closest in time to when the equid died is similar to the three mammoths and bison, however.
- One of the mammoths, a juvenile, has an ⁸⁷Sr/⁸⁶Sr ratio so high that it must have fed at least 180 km from WMNM. It represents a recent addition to the herd, or a member of a second herd at the site.
- The movements of the animals in the study indicate that they converged at the WMNM from over a wide area before perishing. This is consistent with a resource shortage, as could be caused by a drought.

Environmental Constraints from Serial $\delta^{13}C$ and $\delta^{18}O$ Analysis of Teeth from Waco Mammoth National Monument, TX USA

- None of the animals showed a strong seasonal signal in the C3/C4 ratio of their diet.
- The δ^{13} C of the plants in the WMNM animals' diet is within the range of variation of vegetation in the modern study area.

- The youngest sampled enamel was deposited in the summer for two of the WMNM mammoths and the WMNM equid, and during the fall and winter in two other WMNM mammoths.
- For at least part of the record, three of the WMNM mammoths and the equid drank water more enriched in ¹⁸O than any surface water in the modern study area. This implies more heavily evaporated drinking water, and a more arid climate.
- One of the WMNM mammoths lived its entire life in a region comparatively depleted in ¹⁸O, implying that it came to WMNM from a moister or more inland location.

Overall Conclusions

Taken together the results here imply an arid, less seasonal climate relative to today, and reinforce the idea that the animals at WMNM congregated there from over a wide area. This is consistent with a major drought central Texas during MIS 4.

This approach can be expanded on at WMNM to further improve our understanding of site formation there. Other ectotherms from the site could benefit from a rigorous thermoregulatory analysis, and there are a minimum of 60 additional mammoth teeth to examine from the 'lower bone-bearing' stratum, each containing a record of movement, diet, and climate. Both approaches could also be used beyond WMNM to see how the diet and range of Pleistocene megafaunal taxa changed with geography and time. This in turn could answer questions about the precise mechanism behind the end-Pleistocene megafaunal extinction, and inform conservation policy to prevent similar extinctions in the future.

APPENDICES

APPENDIX A

Supplementary Data for Chapter Two

Table A1. Linear measurement and ancillary data for all live tortoises. Observations and measurements in red represent have been affected by congenital irregularities or pathologies of the shell; they were not used in calculating the weight regressions in this study, but have been kept for completeness. The missing measurements are those of tortoises too large to allow for plastral measurements, or too recalcitrant in behavior to allow us to take complete measurements.

Esker, D. Appendix A. (2019) doi:10.18738/T8/9VMGJJ. Located at: https://dataverse.tdl.org/privateurl.xhtml?token=79416230-1932-4a6e-8a05-9da72d4c79d4

Table A2. Regressions for every linear measurement taken. The constant for a particular regression is 'a' and the rate function is 'b.' Esker, D. Appendix A. (2019) doi:10.18738/T8/9VMGJJ. Located at: https://dataverse.tdl.org/privateurl.xhtml?token=79416230-1932-4a6e-8a05-9da72d4c79d4

Table A3. Temperature loss measurements for live tortoises and temperature loss models for fossil tortoises.

Esker, D. Appendix A. (2019) doi:10.18738/T8/9VMGJJ. Located at: https://dataverse.tdl.org/privateurl.xhtml?token=79416230-1932-4a6e-8a05-9da72d4c79d4

Table A4. The linear measurement worksheet used by the authors. Includes explanations for the abbreviations in Fig. 2.8.

Esker, D. Appendix A. (2019) doi:10.18738/T8/9VMGJJ. Located at: https://dataverse.tdl.org/privateurl.xhtml?token=79416230-1932-4a6e-8a05-9da72d4c79d4
APPENDIX B

Supplementary Data for Chapter Three

Table B1. Latitude and longitude of vegetation sampling sites. Esker, D. Appendix B. (2019) doi:10.18738/T8/KLJVUP. Located at: https://dataverse.tdl.org/privateurl.xhtml?token=d7bda3f3-67be-4112-bfea-e0ef6d3ec125

Table B2. Vegetation ⁸⁷Sr/⁸⁶Sr results. Esker, D. Appendix B. (2019) doi:10.18738/T8/KLJVUP. Located at: https://dataverse.tdl.org/privateurl.xhtml?token=d7bda3f3-67be-4112-bfea-e0ef6d3ec125

Table B3. Crop, soil, and paleosol ⁸⁷Sr/⁸⁶Sr results. Esker, D. Appendix B. (2019) doi:10.18738/T8/KLJVUP. Located at: https://dataverse.tdl.org/privateurl.xhtml?token=d7bda3f3-67be-4112-bfea-e0ef6d3ec125

Table B4. Tooth enamel ⁸⁷Sr/⁸⁶Sr results. Esker, D. Appendix B. (2019) doi:10.18738/T8/KLJVUP. Located at: https://dataverse.tdl.org/privateurl.xhtml?token=d7bda3f3-67be-4112-bfea-e0ef6d3ec125

APPENDIX C

Supplementary Data for Chapter Four

Table C1. Recorded δ^{13} C, δ^{18} O, and 87 Sr/ 86 Sr for each enamel sample in this study. Includes enrichments from herbivore fractionation, differing Pleistocene atmospheric δ^{13} C, conversion from δ^{13} C to %C3 (normal and arid) and apatite δ^{18} O VPDB to drinking water δ^{18} O VSMOW.

Esker, D. Appendix C. (2019) doi:10.18738/T8/QO8M7B. Found at: https://dataverse.tdl.org/privateurl.xhtml?token=b18e265b-8432-42a9-b936-1dd4a97b0e2a

File C2. %C3 vegetation making up the diet of the WMNM mammoths, 'Gravel Pit' mammoth, and WMNM equid. This is a '.dat' file for use in conjunction with the paleontological statistics package 'PAST.' Program available at: https://folk.uio.no/ohammer/past/ Esker, D. Appendix C. (2019) doi:10.18738/T8/QO8M7B. Found at: https://dataverse.tdl.org/privateurl.xhtml?token=b18e265b-8432-42a9-b936-1dd4a97b0e2a

Table C3. Sample set length, sampling interval, estimated enamel accretion time, and estimated time-averaging per sample for every tooth in this study. Esker, D. Appendix C. (2019) doi:10.18738/T8/QO8M7B. Found at: https://dataverse.tdl.org/privateurl.xhtml?token=b18e265b-8432-42a9-b936-1dd4a97b0e2a

Table C4. Drinking water δ^{18} O VSMOW local maxima for each tooth in this study, approximating mean summer drinking water. For WMNM 1229, only the highest peak was used, as the time represented by the sample series was ~175 days, and did not record more than one summer.

Esker, D. Appendix C. (2019) doi:10.18738/T8/QO8M7B. Found at: https://dataverse.tdl.org/privateurl.xhtml?token=b18e265b-8432-42a9-b936-1dd4a97b0e2a

Table C5. Lightest quartile of precipitation δ^{18} O as recorded by IAEA/WMO (2019), and used in Bowen et al. (2007). Esker, D. Appendix C. (2019) doi:10.18738/T8/QO8M7B. Found at: https://dataverse.tdl.org/privateurl.xhtml?token=b18e265b-8432-42a9-b936-1dd4a97b0e2a

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