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PALEOECOLOGY OF NORTHERN GREAT PLAINS BISON: INFERENCES FROM
STABLE ISOTOPES AND DENTAL CALCULUS

by

Gaimi Lynn Davies

Bachelor of Science, University of North Carolina Wilmington, 2014

A Thesis

Submitted to the Graduate Faculty

of the

University of North Dakota

in partial fulfillment of the requirements

for the degree of

Master of Science

Grand Forks, North Dakota

December 2019

This thesis, submitted by Gaimi Lynn Davies in partial fulfillment of the requirements for the Degree of Master of Science from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

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Dean of the School of Graduate Studies

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Gaimi Lynn Davies
5 December 2019

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DEDICATION

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For Larry and his Relatives

ABSTRACT

The purpose of this study is to infer Northern Great Plains bison (*Bison bison*) paleoecology through the analysis of stable isotopes and dental calculus from bison skeletal remains. Bison used to roam in the millions throughout most of North America but today they are primarily kept in confined and isolated populations. Little is known about their ancestral ecology. The abundance of bison in the archeological record provides a unique opportunity to study their remains on a broad temporal and spatial scale. This research uses the archeological record of bison in North Dakota to study bison diet and environment from the Late Pleistocene up to modern day bison in Theodore Roosevelt National Park (TRNP). Chapter 1 reviews the history and current status of bison in North America and introduces the paleoecological techniques used in this research. Chapter 2 compares the stable isotopes of carbon and nitrogen found in bison remains to temporal episodes in the climatic record to infer bison diet and potential nutritional stress. Chapter 3 studies bison tooth enamel using serial sampling methods to collect seasonal data from the stable isotopes of carbon and oxygen. Individual bison are analyzed to decipher the potential for migratory movement. Finally, Chapter 4 includes the analysis of the contents of dental calculus found on bison teeth. Microfossils are observed under light and scanning electron microscopy to supplement the isotopic data with qualitative observations. This research is possible through collaboration with the National Park Service and TRNP.

CHAPTER I

A REVIEW OF GREAT PLAINS BISON HISTORY AND PALEOECOLOGICAL TECHNIQUES

Bison History in the Great Plains

Bison are large, terrestrial, even-toed ungulates that belong to the family Bovidae, the subfamily Bovinae, and the genus *Bison*. There are two extant subspecies in North America, the wood bison (*Bison bison athabascae*) and the plains bison (*Bison bison bison*). The plains bison once grazed in the tens of millions on the North American landscape but currently they are limited to restricted rangelands (Gates et al. 2010). The history of North American bison is complex and has great cultural significance for the different eras of human history within their historical range. Bison are ingrained in indigenous peoples' cultures and traditions as a holy and self-sacrificing animal that provides materials to aid in their survival. Today they are also known as a symbol of the "American spirit" and the National Mammal of the United States. Bison are an emblem of strength, wildness, and resiliency and their restoration to the grasslands is an amelioration for many people and for struggling ecosystems. The return of bison to indigenous peoples' tribal lands is an exercise of sovereignty for tribal governments and begins to rectify the bison's near extermination by European settlers. In turn, bison's presence on the grasslands prompts a cascade of events leading to greater biodiversity and healthy ecosystem functioning (Gates et al. 2010).

The bison's original passage into North America began in the Middle Pleistocene. Over time, they have experienced several speciation events and the path to modern bison is not perfectly resolved. However, it is believed that *Bison priscus* (steppe bison), ancestor of the plains bison, entered North America from Eurasia around 200,000 years ago via the Bering land bridge (Froese et al. 2017, Martin et al. 2018)). From there, bison diversified into several morphological forms with large variation in body size and horn length. *Bison priscus* and *Bison latifrons* (long-horned bison) are known as the sister taxa group to extant bison and were 37% larger (Martin et al. 2018). *Bison antiquus* became abundant after *Bison latifrons* went extinct (~20,000 years ago). *Bison occidentalis* and *Bison antiquus* evolved into the extant species, *Bison bison*, which can be traced back to the Middle Holocene (~5,000 to 6,000 years before present) (McDonald 1981, Froese et al. 2017, Martin et al. 2018).

During the Earth's transition from the Late Pleistocene into the Holocene epoch, a global mass megafaunal extinction ensued (Meltzer 2015, Mann 2015). While bison ultimately persisted, there is evidence that they experienced a population bottleneck during this time (Heintzman et al. 2016). However, they overcame the adverse environmental conditions and successfully repopulated North America. Bison numbers grew to the tens of millions up until the late 1800's when they were nearly extirpated from the landscape by European settlers (Lott 2002).

All modern plains bison are descended from a group of less than 100 individuals (Hedrick 2009). Today, approximately 20,000 plains bison (and 10,000 wood bison) are heavily managed in isolated conservation herds, primarily within relatively small fenced boundaries and with no natural predators (Freese et al. 2007, Gates et al. 2010). This is concerning to conservationists because, as a keystone species, their presence in prairie ecosystems supports

natural disturbance regimes, increasing biodiversity (Gates et al. 2010). Bison grazing patterns create distinct differences between grazed and ungrazed vegetation and disrupt competition between plant species, allowing for a more complex vegetational community that can support diverse fauna (Knapp et al. 1999). Bison hoof traffic and wallowing behavior create indentations in the ground that collect moisture and form small nurseries for young plants and amphibians, increasing the overall heterogeneity of their habitat. As a result, more pioneer plant species can mosaic amongst the well-established grasses (Butler 2006). Their ability to traverse a broad range of terrain also promotes seed dispersal and soil fertilization through fecal matter, further benefitting ecological communities (McHugh 1958).

Little is known about bison's ancestral ecology and observations from modern herds may not be representative of their historical ethology and range of adaptability. Conservation herds stem from a small gene pool and are not often ecologically challenged across ecotypes and drivers of evolution, nor can these herds shape the landscape by moving freely on large and contiguous rangelands. By investigating ancient bison ecology, we can illuminate their degree of ecological plasticity and use this knowledge in modern bison restoration and management.

The Earth is currently undergoing another period of rapid climate and environmental change as temperatures rise and wild space becomes increasingly limited (Collinge 1996, Reidmiller et al. 2018). More robust methods in conservation strategies are needed if we are to be successful in maintaining biodiversity and ecosystem functions. Advancements in paleontology are allowing the collection of quantitative and qualitative data from vertebrate history, including stable isotopes, ancient DNA (aDNA), and the study of microfossils. Such data facilitate a management approach called "conservation paleobiology" (Dietl and Flessa 2009). The research in this thesis uses stable isotopes in bison bones and teeth and microfossils in bison

dental calculus to gain insight into Northern Great Plains bison paleoecology and will later be combined with the analysis of aDNA obtained from the same specimens.

Sample Assemblage

Ancient bison bone and tooth samples were collected from four North Dakota museum collections: North Dakota Heritage Center and State Museum (State Historical Society of North Dakota), Knife River Indian Villages National Historic Site (NPS permit number: KNRI-2015-SCI-0006), University of North Dakota's Department of Anthropology, and University of North Dakota's Biology Museum. Samples included in this project were collected as part of twenty-one previously excavated archaeological sites in North Dakota and one previously excavated site in northern South Dakota (Figure 1). The context of the archaeological sites encompasses a large temporal scale from the Late Pleistocene throughout the Holocene and likely represent several species of bison in North American history. Samples from the Late Pleistocene are derived from the Beacon Island archeological site where remains of *Bison antiquus* have been identified (Mandel et al. 2014). Holocene sites are associated with each North Dakota Native American cultural tradition as defined by long standing archaeological schema including the Equestrian Period (1780 – 1880), Plains Village (AD 1200 – 1780), Plains Woodland (400 BC to AD 1700's), Plains Archaic (5500 BC to 400 BC) and Paleo-Indian (9500 to 5500 BC) (Figure 2). To avoid repeat sampling of bison individuals, the right 3rd molar was selected whenever possible. In other cases, the molars or large premolars were taken from the right side of the jawbone. Specimens were also chosen from different stratigraphic layers in the archaeological site context. While every effort was made to not repeat samples in the assemblage, there is a minute chance that repeats were made in some cases. Additionally, one bison tooth from the Red River Valley

was obtained from a private collection and one tooth was extracted from a bison skeleton that eroded from a butte within TRNP in the summer of 2017. Modern counterparts were obtained through TRNP from animals sampled ancillary to this research.

Isotopes and Skeletal Material

Isotopes are atoms with the same number of electrons and protons but differing numbers of neutrons, resulting in different atomic weights. These variations in mass have different bond energies, with heavier isotopes reacting slower and remaining more stable (Metcalf 2011). Isotopic compositions are calculated using the measured ratio between heavy and light isotopes in the sample relative to an accepted standard. This is notated as delta (δ) in units of parts per mil (‰) according to the formula given by McKinney et al. (1950).

$$(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where R = heavy/light isotope of a given element.

Isotopes are assimilated into skeletal tissue through the animal's diet and leave a distinct signature based on the chemical composition of ingested material (Tykot 2004). Stable isotopes can be used to study ancient populations because their values will remain constant in a well-preserved fossil (Schwarcz et al. 2010). The way in which isotopes separate as they are assimilated into living tissue is called "fractionation" and varies by species as well as tissue type (Dalerum and Angerbjörn 2005). This study uses collagen from bone and dentin for carbon and nitrogen analysis, and carbonate from tooth enamel for oxygen and carbon analysis. Collagen is formed from the protein in an animal's diet while carbonate from enamel is assembled through a

mixture of protein, carbohydrates, and fats (Tykot 2004). By selecting for these different tissues, a more complete representation of the animal's diet and environment can be attained.

Collagen in bone has a slow turnover rate, therefore, its isotopic value represents the average of several years of the animal's life (Ambrose and DeNiro 1986, Manolagas 2000, Dalerum and Angerbjörn 2005). However, the collagen in dentin from teeth is not continuously replaced so its isotopic value represents the period in which it initially formed (Metcalf 2011). The formation time of dentin in bovids varies between teeth and can range between time in utero for the 1st molar to ten months of age for the 3rd molar (Brown et al. 1960). Bison are fully weaned after the first year so the 3rd molar is the only tooth that is a reliably representative of the individual's diet and not the mother's (Larson et al. 2001). Isotopes could fractionate differently from mother to offspring, but this is thought to be species dependent and there are no studies, to date, that have looked at the difference in values between a bison cow and her offspring (Larson et al. 2001). Studies on other species have shown mixed results. Some have observed no differences between isotopic values between mothers and offspring (Newsome et al. 2006, Ducatez et al. 2008) while others have shown differing values in infant collagen relative to adults (Witt and Ayliffe 2001).

Mature enamel is comprised of 97% inorganic bioapatite and is less prone to diagenesis than collagen in skeletal tissues (Nanci 2017). Enamel undergoes a process of mineralization incremental in nature and the time frame for its accretion varies by species (Zazzo et al. 2012). Bison have high crowned (hypsodont) teeth that mineralize over a period of approximately 18 months (Julien et al. 2012). The isotopic composition of enamel represents the time of the materials formation because once it is created, it does not remodel (Julien et al. 2012). Therefore,

sequential samples from root to crown of bison tooth enamel represent short periods of time (a few weeks to a few months) and can be used to obtain seasonal values (Kohn et al. 1996).

Carbon Stable Isotopes

Measuring the ratio of carbon isotopes ^{13}C to ^{12}C ($\delta^{13}\text{C}$) in bone collagen allows for the study of diet through primary producers in the food web (Bocherens et al. 1994). Plants use atmospheric CO_2 during photosynthesis and will favor ^{12}C , the degree of this favoring is based on their photosynthetic pathway (Metcalf 2011). Therefore, $\delta^{13}\text{C}$ values in fossils are controlled by the photosynthetic pathways used by plants (Clementz 2012) and have distinctive signatures that can be classified as C_3 , C_4 , or Crassulacean acid metabolism (CAM) (Wang et al. 2013). Northern Plains grassland ecosystems are composed mainly of plants with C_3 and C_4 pathways and very few CAM plants (Wang et al. 2013), so only C_3 and C_4 will be considered in this study. The C_3 pathway is used by cool season grasses and C_4 is the photosynthetic pathway for warm season grasses and browse material (Ambrose and DeNiro 1986). Today, the Northern Great Plains has a mixture of C_3 and C_4 grasses with trees and shrubs abundant in riparian areas. As a predominately grazing species, more than 90% of bison diet is composed of grasses and sedges, browse material, lichens and mosses are selected for the remaining 10% (Coppedge et al. 1998, Leyden and Oetelaar 2001).

$\delta^{13}\text{C}$ values are reported relative to a Cretaceous marine fossil found near the Pee Dee River in North Carolina, the Vienna Pee Dee Belemnite (VPDB) standard. C_3 plants are distinguished by $\delta^{13}\text{C}$ values that range from -30‰ to -24‰ and C_4 plants have a range from -15‰ to -11‰ (Lohse et al. 2014). However, we must consider the amount in which $\delta^{13}\text{C}$ is enriched in specific tissues as it moves through trophic levels. Most herbivores have $\delta^{13}\text{C}$

collagen enrichment values of 5‰ to 6‰ (Ambrose and DeNiro 1986), however, a study on modern bison in Yellowstone National Park (Feranec 2007) found there is an average enrichment of 6.3‰ in bison bone collagen. Accordingly, bison living in a 100% C₃ ecosystem would have expected $\delta^{13}\text{C}$ collagen values between -23.7‰ and -17.7‰ and those in a 100% C₄ ecosystems would have expected values between -8.7‰ and -4.7‰. Additionally, there are other environmental factors which could alter expected values.

When interpreting $\delta^{13}\text{C}$ values to reconstruct animal diet, it is important to remember that atmospheric values of CO₂ can vary under different environmental conditions. In closed canopy forest, CO₂ released in respiration is not able to mix with the atmosphere. The ambient air becomes depleted in CO₂, allowing carbon to be transferred to other trophic levels resulting in more negative values for C₃ and C₄ plants than expected (Tieszen 1994).

Anthropogenic contributions of CO₂ into the atmosphere through the burning of fossil fuels and reductions in the removal of CO₂ from the atmosphere due to agriculture and deforestation also need to be considered (Tieszen 1994). $\delta^{13}\text{C}$ in Antarctic ice cores prior to the industrial revolution has a value of -6.5‰ and modern estimates are -8.0‰. For this reason, it is necessary to adjust $\delta^{13}\text{C}$ values by -1.5‰ from samples earlier than 1800 AD when making comparisons to modern samples (Tieszen 1994).

Nitrogen Stable Isotopes

Stable isotopes for nitrogen include ¹⁴N and ¹⁵N ($\delta^{15}\text{N}$) which are reported relative to the isotopic composition of air. The ratio in which plants assimilate nitrogen isotopes is based on their soil chemistry (Lohse et al. 2014). Most terrestrial plants have $\delta^{15}\text{N}$ values between -5‰ to 2‰ (Fry 1991), these values could be lower if a plant obtains nitrogen through a relationship

with nitrogen fixing bacteria (Koch et al. 1994). Values can also differ for different parts of the plant, i.e. roots versus leaves (Hedges et al. 2004, Schwartz et al. 1982).

Nitrogen increases by 2‰ to 5‰ as it moves through each trophic level, therefore herbivores tend to have lower $\delta^{15}\text{N}$ values than secondary consumers (Schwartz-Narbonne et al. 2015). Nitrogen levels in soil are largely based on precipitation amounts. In general, areas with less precipitation will have more nitrogen in the soil and in turn, the plants growing there will have higher nitrogen values (Schwarcz et al. 1999).

In addition to the plants' nitrogen values, physiological stress from drought could alter the signature of $\delta^{15}\text{N}$ in drought resistant animals, including bison (Tykot 2004). Bison are able to make physiological adaptations to conserve water and allow them to produce higher concentrations of urea, which is argued to result in higher nitrogen values in collagen $\delta^{15}\text{N}$ signatures (Fizet et al. 1995). Additionally, altitude may play a factor in $\delta^{15}\text{N}$ values but is a negligible factor in the current study area (Tykot 2004). Study sites have a net elevational change of ~820 meters. The lowest points are located in the eastern portion of North Dakota in the Red River basin area on the eastern side of the state and highest points are found in the southwest.

Oxygen Stable Isotopes

The ratio of ^{16}O to ^{18}O ($\delta^{18}\text{O}$) values of enamel carbonate are relative to the isotopic composition of meteoric water which is partially dependent on water temperature (Longinelli 1984). Meteoric water (water derived from precipitation) is ingested through drinking water as well as plant tissue (Luz et al. 1984). $\delta^{18}\text{O}$ values in skeletal tissue are reflective of body water composition, which is made at a constant temperature in equilibrium with ingested water (Luz et al. 1984).

$\delta^{18}\text{O}$ of meteoric water varies with temperature and environment (i.e. air pressure, aridity, and altitude) (Dansgaard 1964). In general, lower $\delta^{18}\text{O}$ values indicate colder temperatures (Dansgaard 1964). Due to the incremental formation of enamel, short time periods can be tracked in $\delta^{18}\text{O}$ values with a sequential sampling method. When used in parallel with incremental $\delta^{13}\text{C}$ values, the type of variability seen in the carbon during different seasonal conditions give insight into the potential movement of bison across their range (Widga et al. 2010).

Migratory behavior is an area of contention in bison ecology. Only three of the more than fifty extant bison populations are considered free ranging (Boyd 2003). In free-range herds, migration is observed as an altitudinal movement (Reynolds et al. 2003, Plumb et al. 2009, Kauffman et al. 2018). Historical accounts support the idea of bison being a long-distance migratory animal (Widga et al. 2010). However, recent studies of ancient and modern free-range bison have found less evidence of large scale migrations (Julien et al. 2012, Widga et al. 2010). Therefore, one of the main goals for this thesis is to add to the knowledge of ancient bison migratory behavior.

Microfossils in Bison Dental Calculus

Ancient bison tooth specimens often contain a large amount of dental calculus. Dental calculus is the mineral buildup on teeth from bacteria that is composed mainly of calcium phosphate (Gobet et al. 2001). It accumulates at different rates throughout an animal's lifetime and entraps microfossils, therefore; by studying the composition of this material, we can obtain a more complete dietary and environmental record (Gobet et al. 2001).

Microfossils such as pollen, phytoliths, and starch grains may become incorporated into the matrix of calculus during mastication (Middleton and Rovner 1994). This material preserves well on ancient teeth because it is a hard, mineralized deposit and may actually shield entrapped microfossils from the effects of diagenesis (Weber and Price 2016). Microfossils can be extracted and analyzed by demineralizing the calculus and viewing samples using light microscopy and scanning electron microscopy (SEM) (Middleton and Rovner 1994, Power et al. 2014). This may allow for more precise identification of species that are either incorporated into bison diet or occur commonly in the animal's environment.

The collection of qualitative data from bison dental calculus can help strengthen inferences made with the data collected from stable isotopes. It can also help put constraints on paleoclimate models if material is observed from plants that are known to only exist under certain environmental conditions. Additionally, there is potential to learn more about ancient humans since bison have been an important resource for Plains people and have lived alongside them.

OBJECTIVES OF STUDY

North American bison ecology prior to their near extirpation in the late 19th century is not well understood. This work aims to illuminate the ecological history of bison using stable isotopes and microfossils found in dental calculus. The main goals are to:

1. Interpret bison diet and identify nutritional stress from the Late Pleistocene and throughout the Holocene through the comparison of climatic records with carbon and nitrogen isotopic signatures in bison bones and teeth.
2. Infer migratory behavior of bison through the use of serial sampling bison tooth enamel for oxygen and carbon isotopic signatures.
3. Evaluate the utility of the analysis of microfossils in bison dental calculus for describing diet and habitat use of historic bison using scanning electron and light microscopy.

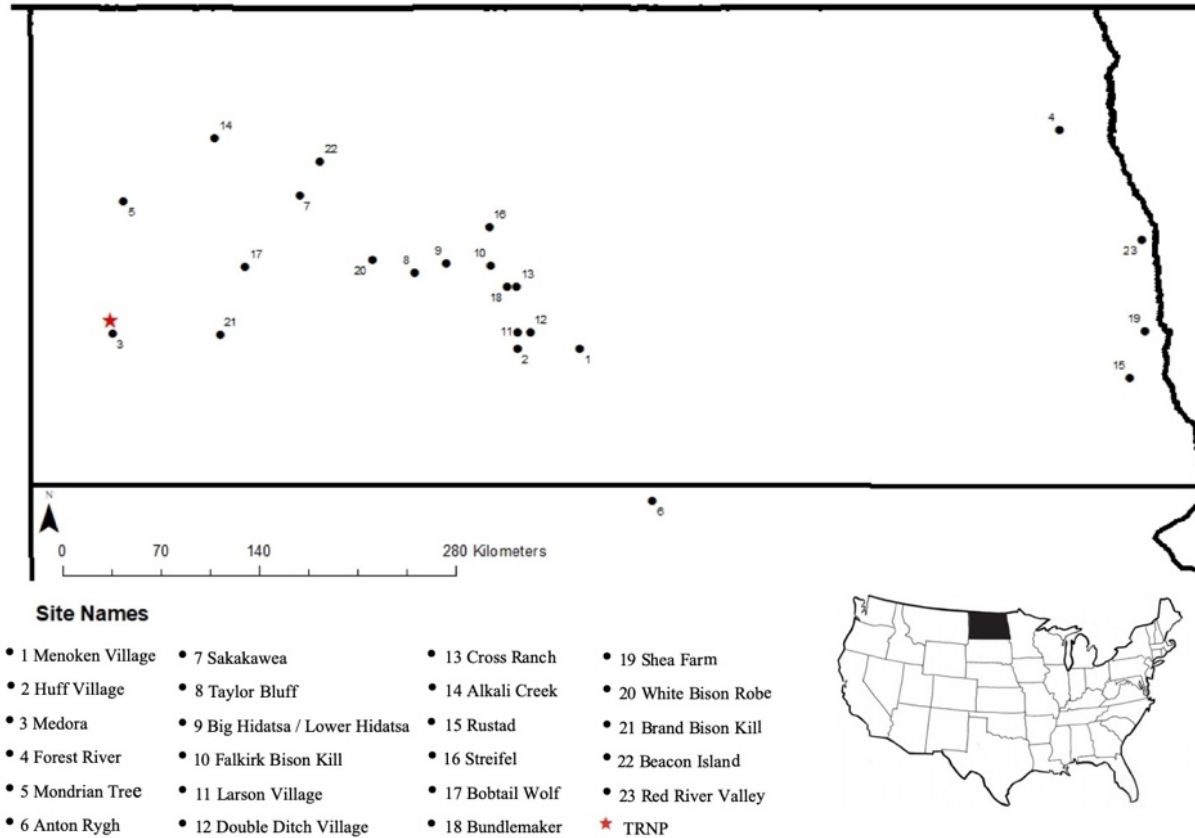


Figure 1. Map of sample sites bison specimens were derived from, modern bison samples were obtained from Theodore Roosevelt National Park (red star).

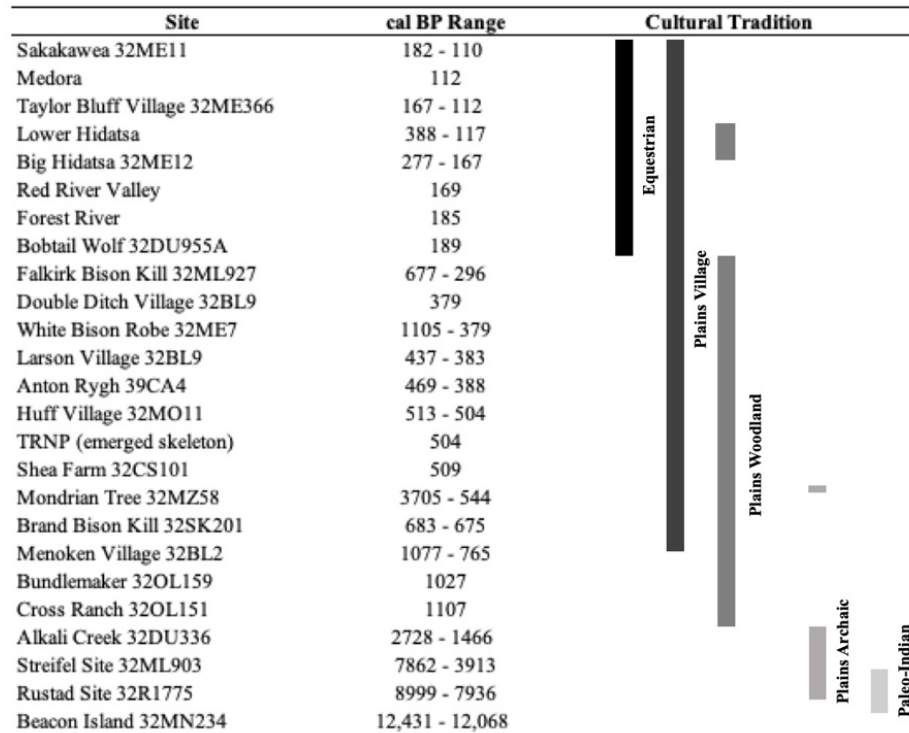


Figure 2. Cultural traditions associated with archeological sites and their calBP age ranges. The Beacon Island Site is derived from the Late Pleistocene, Rustad site is dated in the Early Holocene, Streifel Site represents the Middle Holocene, and all other sites are derived from the Late Holocene.

CHAPTER II

ISOTOPIC PALEOECOLOGY OF NORTHERN GREAT PLAINS BISON DURING THE HOLOCENE

ABSTRACT

Bison (*Bison bison*) are one of the few terrestrial megafauna to survive the transition from the Pleistocene into the Holocene and provide a unique opportunity to study a species on a broad spatiotemporal scale. Today, bison are primarily managed in small and isolated herds and their ability to adapt to a radically changing climate under artificial conditions is in question. We studied the carbon and nitrogen isotopes of Northern Great Plains bison from the terminal Pleistocene and throughout the Holocene to gain insight into bison paleoecology. This time span is contemporary with the first population bottleneck experienced by bison at the end of the Pleistocene and includes the second bottleneck which occurred in the late 19th century. Results were compared with modern bison herd isotopic values from Theodore Roosevelt National Park (TRNP). Patterns of isotopic variation found in bison over time indicate significant ($\delta^{13}\text{C}$ $p = 0.007$, $\delta^{15}\text{N}$ $p = 0.001$) differences in diet composition and correlate with climatic periods throughout the Holocene. Modern bison were most similar to Late Pleistocene bison in isotope values but with considerably less variability. Isotopic relationships described here reveal the plasticity of ancient bison in unrestricted rangelands during climatic fluctuations and may be used as a guide to inform modern bison restoration efforts when identifying bison's physiological limits and suitable reintroduction areas.

INTRODUCTION

The transition from the Pleistocene into the Holocene epoch (~11.7 thousand years ago) marked the disappearance of many North American megafauna. As this mass extinction event is more thoroughly investigated it has become clear that the cause cannot be attributed to any singular explanation for all species or regions (Lorenzen et al. 2011, Meltzer 2015, Mann et al. 2015, Cooper et al. 2015). Bison (*Bison bison*) are one of the few terrestrial megafauna to survive the late Pleistocene extinction in North America. Because of their longevity on the landscape and widespread historical range, they provide a unique opportunity for species study across broad temporal and spatial scales.

Bison entered North America from Eurasia via the Bering Land Bridge during two separate windows of time (195 to 135 thousand years ago and 45 to 21 thousand years ago) when the area's ice sheets retreated and the exposed ground was above sea level (Froese et al. 2017). Bison rapidly colonized lower latitudes of North America when an ice-free corridor between the Cordilleran and Laurentide ice sheets opened around 14 to 13.5 thousand years ago (Mann et al. 2015, Heintzman et al. 2016). In North America, the stable climate during interglaciation led to the rapid spreading of peatlands and dense forests, limiting connectivity of suitable habitat and the ability of megafauna to disperse when coping with climate change (Mann et al. 2015, Gilmour et al. 2015). This issue was confounded by rising sea levels that flooded former dispersal corridors (Mann et al. 2015, Cooper et al. 2015). Fossil records show that animal populations were dwindling before evidence of human presence, though some researchers have attributed the loss of Pleistocene megafauna at least in part to human activity (Mann et al. 2015, Cooper et al. 2015).

Ancient DNA data reveal that bison experienced a drastic reduction in numbers during the terminal Pleistocene, resulting in a genetic bottleneck (Lott 2002, Shapiro et al. 2004). Subsequently, they successfully repopulated North America in the tens of millions by the Early Holocene, spanning from Alaska to Mexico (Lott 2002, Heintzman et al. 2016). Near the end of the 19th century, bison suffered a second bottleneck when they were reduced to a few hundred individuals as a result of European settlement of the continent (Lott 2002, Heintzman et al. 2016). Through conservation efforts enacted during the last century, North American bison have been brought back from the brink of extinction, but their long-term viability as a species remains threatened due to restricted rangelands, artificial selection within confined herds, and a lack of gene flow between herds. Questions remain about the genetic diversity currently found in conservation herds and how the species will respond to environmental change within restricted range. Analysis of stable isotopes in bison remains may help shed light on bison diet and foraging habitat selection over time, providing insights regarding physiological plasticity of the species relevant to management.

Isotopic biogeochemistry of collagen found in ancient bones and teeth is increasingly used in the construction of paleoecology and paleoenvironments. In addition to recording climatic variables such as temperature and precipitation, stable isotopic signatures encapsulate feeding strategies of animals from the past (Tieszen 1994, Hoppe et al. 2006, Lohse et al. 2014). Isotopic ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) are assimilated into herbivore skeletal collagen and tooth dentin through diet, recording the isotopic composition of plant material consumed (Schwarcz et al. 2010). These values will remain the same over time in well-preserved specimens (Tykot 2004). Bone collagen has a slow turnover rate and records isotopic relationships for several years of the animal's life (Ambrose and DeNiro 1986, Manolagas 2000,

Dalerum and Angerbjörn 2005). Tooth dentin forms at a specific point in development and records a shorter window of time, in the order of months (Balasse et al. 2001, Metcalf 2011). The carbon isotopic signature depends on the proportion of plants having C₃ and C₄ photosynthetic pathways in ecosystems (Clementz 2012, Cotton et al. 2016) as well as changes in atmospheric CO₂ from canopy cover in forested areas (van der Merwe and Medina 1991) or post-industrial revolution CO₂ emissions (Tieszen 1994). The values of C₃ plants in North America range from -30‰ to -24‰ and C₄ plants fall between -15‰ and -11‰ (Coppedge et al. 1998). This allows us to distinguish between grazers, browsers, and mixed feeders due to the depletion in ¹³C (i.e. lower δ¹³C values) observed in grazing diets (Ambrose and DeNiro 1986). Bison exhibit an enrichment factor of 6.3‰ (Feranec 2007) when carbon isotopes are assimilated into their skeletal tissue and this value needs to be factored in when calculating the percentage of C₃ and C₄ plants in bison diet. Therefore, the expected δ¹³C values of bison feeding primarily on C₃ plants would be between -23.7‰ and -17.7‰, while C₄ bison diets would range from -8.7‰ and -4.7‰. Throughout the Holocene, the Great Plains have been largely dominated by Poaceae (grass) communities (Valero-Garcés 1997), which can exhibit C₃ or C₄ photosynthetic pathways. The climate in the Northern Great Plains has predominately favored the C₃ subfamily Pooideae with a smaller amount of C₄ subfamilies, Panicoideae and Chloridoideae. The abundance of C₄ grasses increases as warm seasons get longer, allowing us to interpret climatic changes in the diets of grazers (Cotton et al. 2016). Nitrogen isotopic values provide insight into moisture level and nutritional stress due to an observed increase in δ¹⁵N in animal tissue from the recycling of urea under conditions of drought (Ambrose and DeNiro 1986, Hobson et al. 1993, Fizet et al. 1995, Tykot 2004). However, there are other factors that contribute to nitrogen values in herbivores. Higher nitrogen can indicate warmer temperatures and a diet composed of more

graminoids and herbs than trees and shrubs (Tieszen 1994, Bocherens et al. 1996, Fox-Dobbs et al. 2008, Carlson et al. 2016, Hofman-Kamińska et al. 2018). In modern European bison, it was found that canopy cover had the biggest influence on $\delta^{15}\text{N}$ where less light will decrease nitrogen values in plants (Hofman-Kamińska et al. 2018).

North American isotopic studies of bison to date have primarily focused on Pleistocene paleoecology (Bocherens et al. 1996, Feranec and MacFadden 2000, Coltrain et al. 2004, Fox-Dobbs et al. 2008, Feranec et al. 2009, Zazula et al. 2009), climatic interpretations (Lohse et al. 2014, Carlson et al. 2016), and values from modern herds (Hobson et al. 1993, Hoppe et al. 2006, Berini and Badgley 2017). At present, there are few isotopic studies of Holocene bison in North America. Existing research covers relatively short time periods or small sample sizes (Cannon 1997, Butler 2006, Lohse et al. 2014). The limited data on North American bison from the Holocene may be in part due to the prior perception of a relatively stable climate during this epoch, though the most recent studies of paleoclimate portray the Holocene as a dynamic period with fluctuations in temperature and precipitation (Alley et al. 1997, Mayewski 2004, Marsicek et al. 2018). An analysis of approximately fifty paleoclimatic records of greenhouse gases, glacial coverage, and pollen profiles determined that the Holocene had several periods of sudden climate change punctuated by variations in atmospheric circulation, moisture, and temperature changes (Marsicek et al. 2018). For instance, a study of pollen records found three periods in which climate rapidly heated during the Holocene (Marsicek et al. 2018) and evidence of a steep drop in temperature at ~8.2 thousand years ago as well as other large fluctuations in temperature throughout the Holocene are supported by the analysis of Greenland ice cores (Alley et al. 1997). Therefore, bison in North America during the Holocene had to adapt to a wider range of climatic conditions than previously thought. The amount of variation seen in bison isotopic values

throughout specific time periods provides insight into their use of different resources as the environment changed (Jahren et al. 1998, Widga et al. 2010).

This chapter aims to illuminate Northern Great Plains bison ecology from the Late Pleistocene through the Holocene and compare these findings with extant bison from Theodore Roosevelt National Park (TRNP) (Figure 1). The objectives are to: 1) identify $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variation indicative of environmental change across time, and 2) utilize isotopic signatures to elucidate feeding ecology of historic bison in context of their modern counterparts in North Dakota.

METHODS

Sample Assemblage

Samples in this chapter are derived from the 22 archeological sites in North and South Dakota, and one tooth from the Red River Valley in Grand Forks, North Dakota obtained from a private collection (Figure 1). The integrity of skeletal elements was observed under stereo microscopy, revealing well preserved tissues at surfaces and on cross sections of bones and teeth. Preservation quality was determined by a clear delineation between cortical and spongy material with little discoloration, compact tooth dentin and opalescent enamel (Figure 3).

Modern bison teeth were obtained from animals (n=5) culled for management purposes or those dying of natural causes within TRNP. TRNP is located in the Badlands of southwestern North Dakota. Bison are kept in two separate populations within the park, the North Unit and the South Unit. The North Unit is smaller (97 km²) and sustains between 100 and 300 bison at a time. The South Unit is much larger (187 km²) and supports 300 to 500 bison. There are no large predators of bison in the park and fencing surrounds the perimeters; with no possibility for

expansion, bison numbers are managed through round ups to prevent overgrazing. Most of the bison are transported to tribal lands and a small fraction are transferred to other conservation populations within the park system. Average annual precipitation is 38.1 cm and the park experiences a wide range of temperatures with a high mean of 29°C and a low mean of -18°C. Vegetation consists of mostly mixed grass prairie (*Salvia*) with areas of sage (*Artemisia*) and juniper (*Juniperus*). Riparian areas are characterized by ash (*Fraxinus pennsylvanica*) and cottonwood (*Populus deltoides*). Grazing lands are shared with other large herbivores in the park (i.e. feral horses (*Equus caballus*), longhorn cattle (*Bos taurus*), elk (*Cervis elaphus*), pronghorn antelope (*Antilocapra Americana*), mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), moose (*Alces alces*), and bighorn sheep (*Ovis Canadensis*) as well as small mammals such as black-tailed prairie dogs (*Cynomys ludovicianus*).

Sample Preparation and Isotope Analysis

Sections of cortical bone or tooth dentin weighing 1-3 grams were cut from bison specimens using a band saw. The saw blade was cleaned with isopropyl alcohol between samples to prevent cross contamination. Tooth dentin samples were taken in 0.5 to 1 inch contiguous pieces from dentin underlying enamel in the orientation of crown to cusp. Some tooth dentin portions included root material. Samples were then sent to the University of California Irvine (UCI) Keck Carbon Cycle Accelerator Mass Spectrometry Laboratory where they were decalcified in 1N HCl and then gelatinized at 60°C, pH of 2, and ultrafiltered to select for a high molecular weight fraction (>30 kDa). Aliquots of ultrafiltered collagen were measured on a Fisons NA1500NC elemental analyzer/Finnigan Delta Plus isotope ratio mass spectrometer to obtain $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values at a precision of < 0.1‰ and < 0.2‰, respectively. Stable isotope

measurements were determined on amino acid hydrolysate samples. Samples with C:N atomic ratios between 2.9 and 3.6 are indicative of well-preserved collagen (Ambrose and Norr, 1993, Van Klinken 1999) and were then measured for ^{14}C dating at the UCI Keck AMS facility. At this facility, AMS samples were combusted in vacuum sealed quartz tubes at 900°C for three hours. H_2 and a Fe catalyst was used to reduce the sample to graphite at 550°C and reaction water was drawn off. The graphite was then placed in Al boats onto a target wheel with oxalic acid standards, known age bone comparisons, and ^{14}C free Pleistocene whale bone. Carbon dating measurements were made using a National Electronics Corporation compact spectrometer with a 0.5 MV accelerator (NEC 1.55DH-1). The error range for ^{14}C ages (BP) in this study is $\pm 15\text{-}35$ years.

^{14}C Calibration and Temporal Episodes

^{14}C ages were calibrated using OxCal 4.3 (Ramsey 2009) and the IntCal13 curve (Reimer et al. 2013) for the Northern Hemisphere. All bison sample ages are reported in calibrated years before present (cal BP) and ranges are within a 95% confidence interval. The median of the confidence interval was used as the sample date for separation into temporal episodes. All $\delta^{13}\text{C}$ values dated before 1800 AD were adjusted by -1.5‰ to account for the reduction in atmospheric CO_2 due to the increased burning of fossil fuels after the Industrial Revolution (Tieszen 1994).

The temporal range was split into episodes to allow comparisons between bison from the Late Pleistocene to modern. The episodes follow formal Holocene subdivisions recognized by the International Union of Geological Sciences (IUGS) and is based on data from Greenland ice cores, pollen records, lake sediments, and Global Stratotype Section and Points (GSSPs) (Walker

et al. 2018). The Pleistocene-Holocene boundary was marked at 11.7 thousand years ago. The Holocene was split into four episodes, Early Holocene (11.7 to 8.2 thousand years ago), Middle Holocene (8.2 to 4.2 thousand years ago), and Late Holocene (4.2 to 100 years ago) (Walker et al. 2018). Modern bison are also considered an episode.

$\delta^{13}C$ and $\delta^{15}N$ Data Analysis

We used generalized additive models (GAMs) illustrate bison carbon and nitrogen isotopes over time, created with R statistical software and the package “mgcv” (Wood 2011). This method allows the estimation of isotope values between data points, providing a continuous view of bison isotopic fluctuations throughout the Holocene (95% confidence interval). GAMs were modeled with a gaussian distribution, an identity link function, and a smoothing parameter on time (k=5 for $\delta^{13}C$ and k=8 for $\delta^{15}N$).

The $\delta^{13}C$ means of temporal episodes were also used to calculate the percentage of C₃ and C₄ grasses in bison using equation 1, modified from Carlson et al. (2016).

$$1. C_3 (\%) = [(\delta^{13}C_{\text{collagen}} - 6.3_{\text{trophic level fractionation}} - \delta^{13}C_{C4}) / (\delta^{13}C_{C3} - \delta^{13}C_{C4})] \times 100$$

$$C_4 (\%) = 100 - C_3 (\%)$$

Where $\delta^{13}C_{\text{collagen}}$ includes a 6.3‰ adjustment for trophic level fractionation specific to bison (Mandel et al. 2014), $\delta^{13}C_{C4} = -12.5\text{‰}$ and $\delta^{13}C_{C3} = -26.5\text{‰}$.

RESULTS

Seventy-three of the seventy-seven samples of ancient bison bones and teeth yielded sufficient collagen (>1%) to produce reliable data (Table 1). All samples included in the analysis have C:N ratios between 2.9 and 3.6, relative carbon (%C) >30%, and relative nitrogen (%N) between 11 and 16%, indicative of well-preserved collagen (Table 1) (DeNiro 1985, Van Klinken 1999). Five modern bison tooth samples (Bison 100 – Bison 104) from TRNP herds returned carbon and nitrogen isotopic ratios for comparison with ancient samples.

The age of ancient bison specimens ranged from the Late Pleistocene to the Late Holocene, 12,344 to 104 calibrated years before present (cal BP) with samples from the Late Pleistocene (~12.5 cal BP, n=4), Early Holocene (11,700 – 8,200 cal BP, n=1), Middle Holocene (8,200 – 4,200 cal BP, n=7), and Late Holocene (4,200 – 100 cal BP, n=61) (Table 2) (Walker et al. 2018).

As previously stated, dentin and bone collagen represent different lengths of time in the bison's life. The sampling method in this study for dentin likely captures less than a year (Balasse et al. 2001) while the collagen from bone samples describes the average over several years of the animal's diet (Ambrose and DeNiro 1986, Manolagas 2000, Dalerum and Angerbjörn 2005). To compare the variation found in each tissue type, t-tests were conducted for the dentin and bone samples as a whole and also separated into temporal groups. No significant differences were found in isotopic values by sample tissue types (t-tests, $\delta^{13}\text{C}$ p = 0.76, $\delta^{15}\text{N}$ p = 0.91), therefore, they are treated functionally the same for the purpose of this analysis.

Generalized additive models (GAMs) identified significant changes in bison $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values over time (p = 0.007 and p = 0.001, respectively) (Figure 4). Model fit values are included in Table 3. Bison dentin $\delta^{13}\text{C}$ ranged from -21.5‰ to -10.7‰ and dentin $\delta^{15}\text{N}$ ranged from 4.6‰ to 9.3‰. Bison bone $\delta^{13}\text{C}$ values ranged from -21.1‰ to -14.6‰ and bone $\delta^{15}\text{N}$

ranged from 4.9‰ to 8.5‰ (Table 2). Overall, considerable variability was observed among temporal episodes for isotope values $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Table 2, Figures 5, 6). Only one data point is categorized into the Early Holocene, however GAM models estimate the increase in bison's carbon and nitrogen isotopic values throughout this sub-epoch (Figure 4).

Modern bison had the least variation in carbon and nitrogen values and the lowest mean $\delta^{15}\text{N}$ value, 4.9‰. The highest variation for both isotopes was seen in Middle Holocene bone samples although they are derived from the same archaeological site and are close in age (Table 1). The highest mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, -16.8‰ and 8.2‰ respectively, were observed in Middle Holocene bison dentin. Late Pleistocene and modern bison dentin were the most depleted in ^{13}C with a mean $\delta^{13}\text{C}$ of -20.6‰ and -20.5‰, respectively (Table 2, Figure 6).

Ratios of $\text{C}_3:\text{C}_4$ plants demonstrated that bison diet for each temporal episode predominately included C_3 plants (Table 2). Late Pleistocene bison consumed entirely C_3 vegetation with an increase in the consumption of C_4 plants in the Early Holocene, a peak abundance of C_4 material in the Middle Holocene and a small decrease again in the Late Holocene (Table 2, Figure 4a). Modern bison in TRNP exhibited an entirely C_3 diet, congruent with the vegetation found in the park (Longinelli 1984).

DISCUSSION

This project uses the archaeological record of bison in the Northern Plains to understand their evolutionary responses to environmental change and provide insight for best practices in bison conservation and management. The analysis of isotopic values from bison remains spans the terminal phase of the Late Pleistocene to present, representing post-glacial changes in bison diet and vegetation associated with changing climate during the recent natural history of the species. The sample assemblage is contemporary with two bison population bottlenecks. The first bottleneck occurred during the terminal Pleistocene and the second in the late 19th century when bison were nearly extirpated by humans (Lott 2002, Heintzmen et al. 2016).

The Late Pleistocene in the Northern Great Plains is described as a time of sudden environmental change and a significantly wetter landscape after the recent retreat of the Laurentide ice sheet (Yansa 2006). Pollen records from the study region indicate an abundance of *Picea* (evergreen) species and relatively low amounts of herbaceous plants (grasses and forbs) (Yansa 2006, Grimm et al. 2006). Late Pleistocene Bison samples are derived from Beacon Island, a Paleoindian kill site in the Agate basin (Figure 1). The concurrent stratigraphic layer at this site exhibits C₃ dominated plant material (Mandel et al. 2014) and is in agreement with the 100% C₃ diet recorded in the $\delta^{13}\text{C}$ of bison remains (Table 2). However, bison $\delta^{15}\text{N}$ values from the Late Pleistocene appear surprisingly low considering environmental conditions in the Late Pleistocene were adverse enough to wipe out the majority of megafaunal species while causing bison to undergo their first recorded population bottleneck (Shapiro et al. 2004, Mann et al. 2015). One explanation for this discrepancy could be a heavier dependence on the nitrogen poor browse material in bison diet (Ambrose and DeNiro 1986). Substantial incorporation of browse is reported by other Late Pleistocene bison paleoecology studies in North America based on

stable isotopes and bison dentition wearing patterns (Coltrain et al. 2004, Rivals et al. 2007). In which, low $\delta^{13}\text{C}$ values are recorded and indicate more browse in canopied forests where atmospheric CO_2 is more concentrated (van der Merwe and Medina 1991). A grazing diet is more abrasive than a browsing diet and is depicted on the surface of bison teeth as deeper and more frequent pits and scratches (Rivals et al. 2007). However, it cannot be ignored that this could also be an effect of small sample size from only bison dentin for this temporal period.

Evergreen forests south of the Laurentide ice sheet were rapidly succeeded by other vegetative communities in the transition between the Pleistocene and the Holocene (Yansa 2006, Jacobson et al. 2015). By the Early Holocene, new deciduous forest south of the evergreens formed and bordered along riparian areas while grasslands spread throughout the open landscape (Jacobson et al. 2015). As the Great Plains quickly became dominated by prairie (Pielou, 1991, Yansa 2006, Jacobson et al. 2015) bison migrated northward into the developing terrain and became plentiful at this time (Pielou 1991). Yet only one data point from the sample assemblage falls within the Early Holocene boundaries. This single bison indicates an increase in C_4 vegetation incorporated into diet (Table 2), consistent with rising temperatures and the presence of more C_4 Chloridoideae grasses (Mandel et al. 2014, Shuman and Marsicek 2016). Additional evidence of an increase in C_4 grasses are recorded in bison living during the Early Holocene within present day Yellowstone National Park and the state of Nebraska (Jahren et al. 1998, Cannon et al. 2010). Nitrogen levels are higher than what is seen in Late Pleistocene bison despite an increase in effective moisture in the Early Holocene (Shuman and Marsicek 2016), further supporting the argument that bison selected more browse material from lower light areas in evergreen forests during the Late Pleistocene.

The Middle Holocene climate is summarized as highly variable with an overall shift towards warmer, drier conditions and patchiness of resources (Meltzer 2015). Herbaceous plants fluctuated throughout the Middle Holocene, alternating between Poaceae and Ambrosia (ragweed) communities, indicating frequent changes in precipitation (Grimm et al. 2011, Commerford et al. 2018). Bison from this sub-epoch exhibit the highest mean values for carbon and nitrogen as well as large variation in bone samples (Table 2, Figures 5). Although the two bone samples from the Middle Holocene (Bison 22 and 98) coincide in both time and space, they exhibit large isotopic differences (Table 1, Figure 5). This could be representative of the vastly fluctuating climate, the migration of bison from outside areas, or a remnant of different feeding strategies among bison sexes (Berini and Badgley 2017). In any case, the greatest amount of C₄ vegetation is observed in bison diet at this time for both tissue types (24%), indicating a trend towards longer and warmer growing seasons (Widga et al. 2010). This is corroborated by bison from the Eastern Great Plains during the Middle Holocene (Lohse et al. 2014).

Climatic conditions in the Northern Great Plains during the Late Holocene generally followed a cooling trend with increasing moisture up to modern day (Valero-Garcés 1997, Kauffman et al. 2018). However, climate proxies provide evidence that severe arid conditions occurred at intervals throughout this time period (Luz et al. 1984, Valero-Garcés 1997). No obvious indicators of drought are evident in bison samples from the Late Holocene but changes in vegetation type may lower the amount of nitrogen available in soils and dampen the signal of physiological stress (Craine et al. 2018). A wide range of $\delta^{13}\text{C}$ values and more C₄ plant material was signaled in Late Holocene bison (Table 2, Figure 5), suggesting diverse vegetation consumed and longer, warmer growing seasons (Cannon 1997, Widga et al. 2010). One bison (Bison 83) from Larson Village exhibits the highest $\delta^{13}\text{C}$ value recorded in this analysis

(-10.7‰). Either this bison selected more browse material than its counterparts or it traveled further to encounter more C₄ vegetation. Bison from Larson Village that are similar in age (Bison samples 32, 33, 77, 80) all exhibit much lower $\delta^{13}\text{C}$ than this particular bison. Berini and Badgley (2017) determined that male bison will consume more nutrient poor vegetation during the mating season, however, the observed differences were not as large as seen in the Larson bison group. Nitrogen values would be elevated on a nutrient poor diet and that is not what is seen in this bison, therefore, it is likely this bison came from an outside area. Whether it was through migration or trade deals cannot be determined by the information at hand.

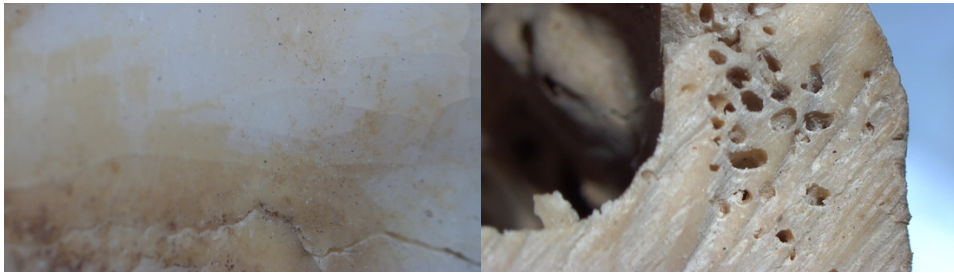
Pollen records indicate that Poaceae increased in abundance during the Late Holocene and the first appearance of *Salaginella densa*, or spikemoss, is documented in the Northern Great Plains (Grimm et al. 2011). The ground cover provided by spikemoss and its ability to persist in dry conditions provides protection from erosion and forage to subsist on during lean winter months. Its expansion likely increased foraging capacity and may have contributed to the immense presence of bison on the prairie during the Late Holocene.

Coinciding with the environmental changes that took place during the Late Holocene is the rise of a more complex human ecosystem throughout North America. How humans influenced landscapes, bison behavior, and available food supplies during the Holocene are currently not well understood but research suggests that, much like today, humans had a large impact on the Great Plains ecosystem. Charcoal deposits from Montana reveal that Native Americans likely used fire to increase grass productivity in an effort to lure bison into their hunting basins, potentially providing quality forage to bison during the Late Holocene that may have impacted their isotopic signatures (Roos et al. 2018). The bison's past response to changing composition of habitat through farming and fire regimes remains unclear but their ability to adapt

and exploit a variety of resources is certainly attributed to the species' long-term survival over other megafauna.

Finally, isotopic values of modern bison dentin from TRNP were compared with ancient bison from the Late Pleistocene through the Late Holocene. Comparatively low variability in modern bison stable isotopes are observed (Table 2, Figure 5). TRNP bison have a more restricted rangeland than their ancient counterparts as well as a presumably shorter window for sample collection than other temporal episodes. Modern bison are depleted in ^{13}C , similar to Late Pleistocene bison (Figure 5), indicating a diet of 100% C_3 plant material (Table 2) despite vastly different climatic conditions experienced by the temporal groups. Nitrogen values are notably lower than other bison groups (Table 2, Figure 6), indicating no evidence of nutritional stress and adequate available moisture (Hobson et al. 1993). While low variation may be attributed to small sample size, other studies have found similar results in several living herds (Feranec 2007). Tieszen (1994) showed that the Wind Cave National Park bison herd in South Dakota had a diet with more C_4 plants but bison also contained a small amount of $\delta^{13}\text{C}$ variability within the herd. The Catalina Island bison population exhibits a comparable $\delta^{13}\text{C}$ average and low variability (Cotton et al. 2016). Modern Yellowstone National Park bison exhibit low variability in $\delta^{13}\text{C}$ values and a similar mean to the TRNP bison in this study despite their ability to cover much larger areas and complete substantial elevational migrations (Berini and Badgley 2017, Kauffman et al. 2018). Whether this trend in low variability in modern bison diet is due to restricted rangelands and herd management practices or if it is a result of a narrowing in plasticity from the recent genetic bottleneck in bison history is still not clear. We would expect that if it were only due to the habitat restrictions imposed upon modern bison, we would see some more variability in Yellowstone herds (Table 4).

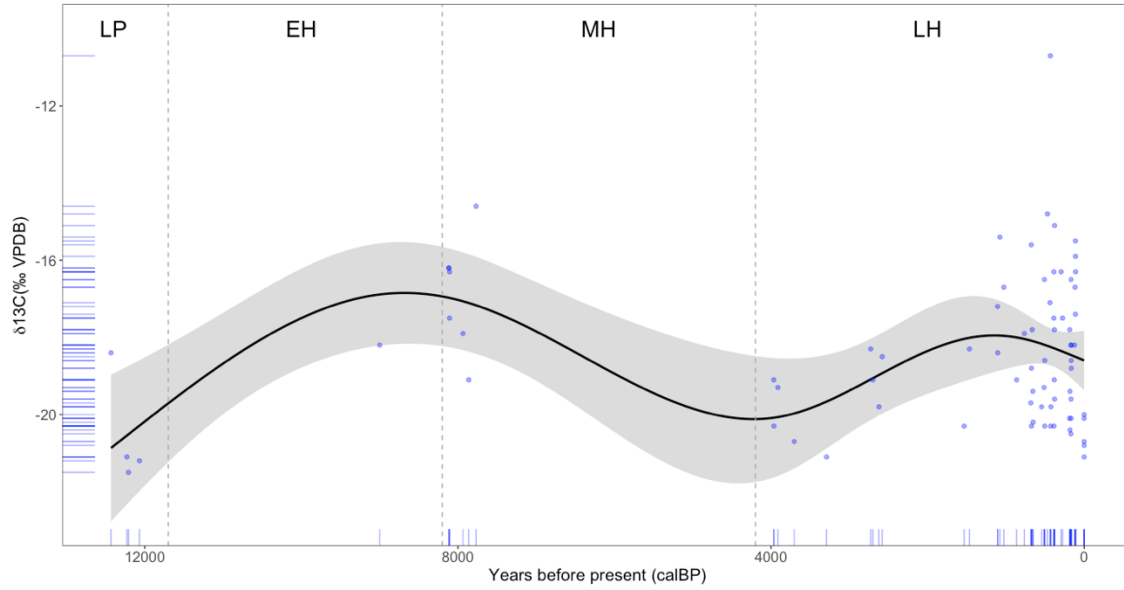
Understanding the predecessors of present-day bison may unlock new views for reintroducing them more broadly to the North American landscape. These techniques are already influencing management decisions for European bison. European bison had to overcome similar environmental challenges as North American species during the terminal Pleistocene and are also predominately constricted to limited rangelands today (Bocherens et al. 2015). Most habitat of modern European bison is forested but their morphological adaptations suggest they evolved in open grasslands (Kerley et al. 2012) and then moved into forested areas as the forests expanded and pressure from humans increased (Hofman-Kamińska et al. 2018). Isotopic studies of the ancient Eurasian steppe bison (*Bison priscus*) are informing conservation strategies for their ecological successor, *Bison bonasus* (Stuart 1999). Several studies have found that *Bison priscus* did rely heavily on grazing, with more browse incorporated over time, as woody vegetation became more accessible (Rivals et al. 2007, Bocherens et al. 2015). This information makes the introduction of European bison to more open grassland habitats a plausible strategy for large scale restoration and is an example of the value of the relatively new field of conservation paleobiology for current species management. This knowledge can be carried over to North American bison with the expansion of isotopic studies and a better understanding of the potential plasticity of this resilient species.



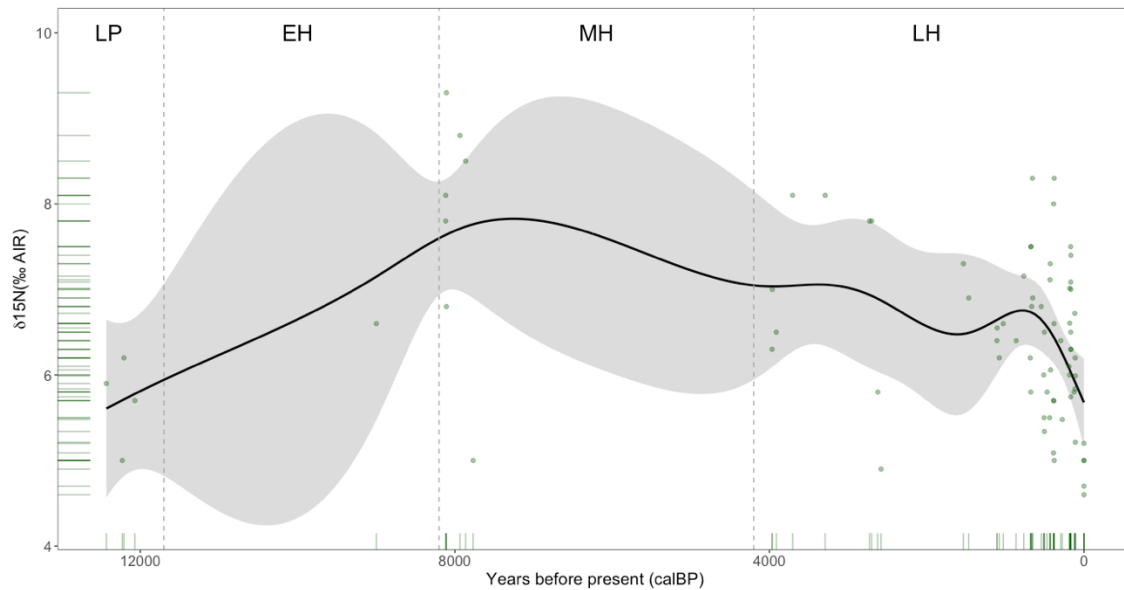
a.

b.

Figure 3. Stereomicroscopy images to determine diagenesis in **a.** tooth enamel (Bison 13) and **b.** bone (Bison 4).



a.



b.

Figure 4. Generalized additive models for **a.** carbon and **b.** nitrogen isotopes in bison tissue over time (calBP age). Shaded region represents the 95% confidence interval. Dashed lines delineate temporal episodes (LP “Late Pleistocene”, EH “Early Holocene”, MH “Middle Holocene”, LH “Late Holocene”) and lines along the axis’ show density of sample points.

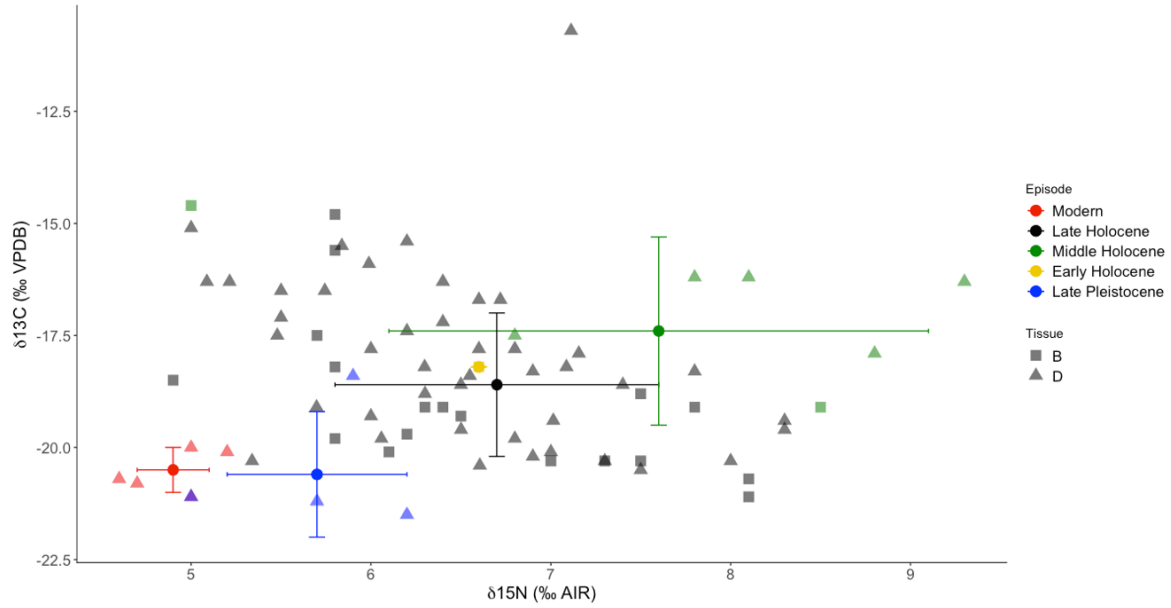
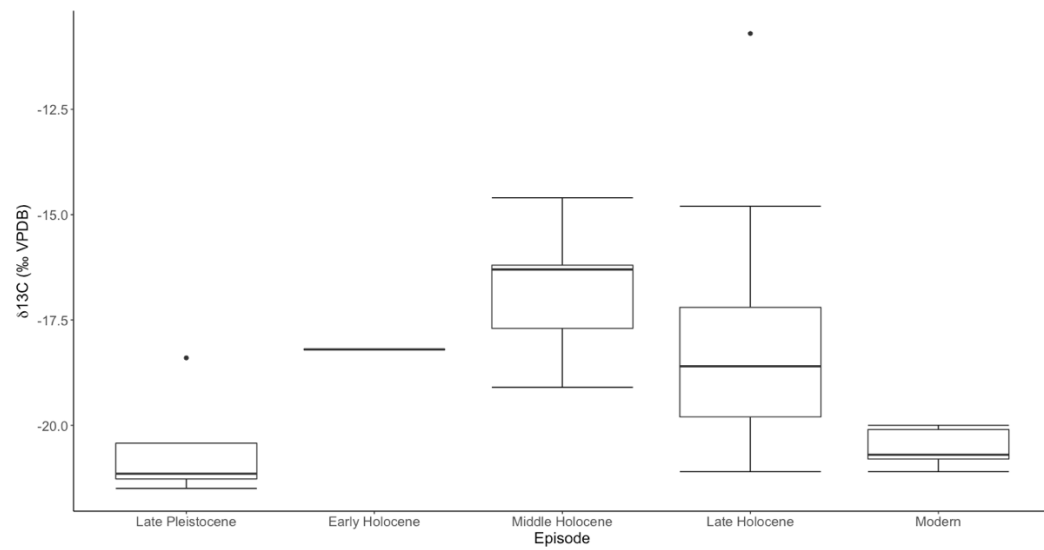
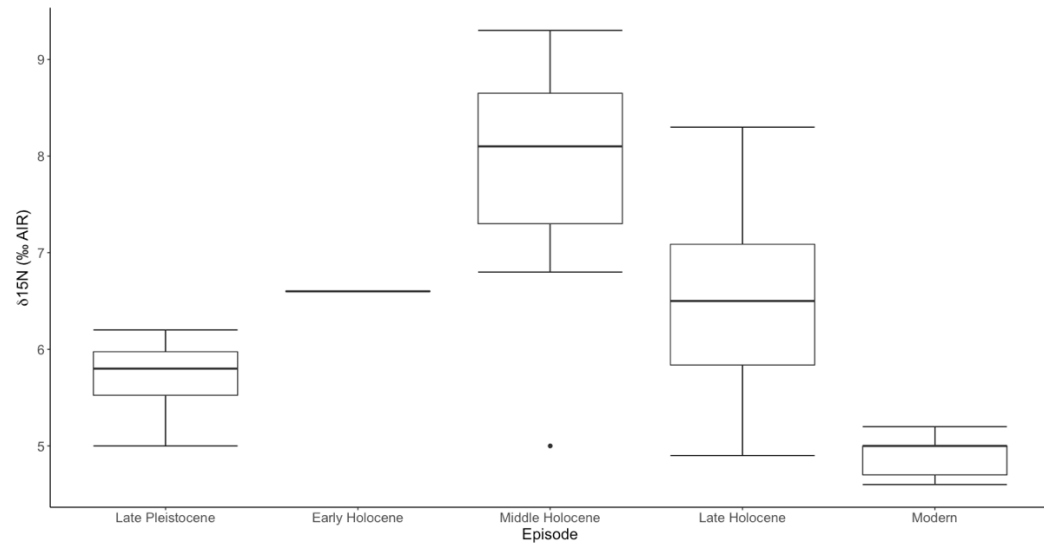


Figure 5. Scatterplot of carbon and nitrogen stable isotopes, means and standard deviation for temporal groups. Colors represent temporal groups while shapes differentiate tissue type, bone (B) or dentin (D).



a.



b.

Figure 6. Boxplots for **a.** carbon and **b.** nitrogen stable isotopes in bison from each temporal episode. Bars represent the range of data with outliers depicted outside of their spread and horizontal lines within the boxes represent the median value

Table 1. Carbon and nitrogen isotopic values of all bison samples, calibrated ¹⁴C date ranges and median, collagen preservation checks, and the percent of C₄ vegetation in bison diet. Samples which did not yield enough collagen are listed here, values not obtained are labeled as “na”. No carbon dating was necessary for modern samples, “na” is used in the modern bison calBP columns.

Bison	Location	δ13C	δ15N	Date Range (calBP)		Median Age	%N	%C	% Collagen	C:N	Episode	Tissue	Element
1	Medora	-17.4	6.2	270	12	112	16.3	45.0	4.4	3.2	LH	D	tooth
2	Mondrian Tree 32MZ58	-21.1	8.1	3357	3241	3291	16.0	46.9	4.5	3.4	LH	B	fragment
3	Mondrian Tree 32MZ58	-18.5	4.9	2724	2497	2580	14.9	42.6	3	3.3	LH	B	phalanx
4	Anton Rygh, SD	-14.8	5.8	501	332	469	15.1	42.9	6.3	3.3	LH	B	ant. cannon
5	Anton Rygh, SD	-17.5	5.7	481	318	388	16.7	45.6	5.3	3.2	LH	B	carpal
6	Menoken Village 32BL2	-19.1	6.4	910	787	863	16.3	45.9	2.6	3.3	LH	B	phalanx
10	White Bison Robe 32ME7-959	-17.8	6.8	679	571	665	16.5	44.4	2.5	3.1	LH	D	tooth
11	White Bison Robe 32ME7-991	-17.8	6.6	460	313	381	17.0	45.9	4.7	3.2	LH	D	M
12	Huff Village 32MO11	-19.3	6.0	527	501	513	16.8	46.0	5	3.2	LH	D	tooth
13	Huff Village 32MO11	-18.6	6.5	518	487	504	15.3	41.6	9.9	3.2	LH	D	M3
14	Alkali Creek 32DU336	-18.3	6.9	1529	1412	1466	14.5	39.8	3	3.2	LH	D	tooth
18	Sakakawea 32ME11	-18.8	6.3	302	7	166	16.7	46.4	5.4	3.2	LH	D	M3
20	Rustad Site 32R1775	-16.3	9.3	8176	8041	8107	14.3	40.2	1.4	3.3	MH	D	tooth
22	Streifel Site 32ML903	-14.6	5.0	7834	7701	7768	15.0	41.9	3.8	3.3	MH	B	femur head
25	Big Hidatsa 32ME12	-18.6	7.4	294	14	167	16.5	45.4	5.9	3.2	LH	D	M
26	Big Hidatsa 32ME12	-20.5	7.5	290	14	170	16.4	44.0	4.3	3.1	LH	D	M3
29	Falkirk Bison Kill 32ML927	-15.6	5.8	694	661	677	14.9	43.1	8.2	3.4	LH	B	vertebrae
30	Falkirk Bison Kill 32ML927	-16.3	6.4	309	156	296	16.7	44.9	3.5	3.1	LH	D	M
31	Bobtail Wolf 32DU955A	-20.1	6.1	283	5	189	15.6	44.9	6.2	3.4	LH	B	phalanx
32	Larson Village 32BL9	-17.1	5.5	491	320	437	16.9	45.3	3.8	3.1	LH	D	M3
33	Larson Village 32BL9	-20.3	7.3	485	319	429	16.0	43.9	5.4	3.2	LH	B	ant. cannon
34	Double Ditch Village 32BL8	-15.1	5.0	465	315	379	16.5	44.2	2.5	3.1	LH	D	M
35	Bundlemaker 32OL159	-16.7	6.6	1173	979	1027	15.2	42.3	6.2	3.2	LH	D	M
36	Cross Ranch 32OL151	-17.2	6.4	1175	1010	1107	16.1	44.2	5	3.2	LH	D	M3
37	Taylor Bluff Village 32ME366	-18.2	5.8	272	11	125	14.9	41.9	6.2	3.2	LH	B	phalanx
38	Taylor Bluff Village 32ME366	-20.1	7.0	294	14	167	16.4	44.8	3.6	3.2	LH	D	M
39	Falkirk Bison Kill 32ML927	-19.4	8.3	673	566	656	14.3	40.4	1.5	3.3	LH	D	M
40	Shea Farm 32CS101	-16.5	5.5	527	492	509	15.6	43.0	5.2	3.2	LH	D	tooth

Table 1 continued.

Bison	Location	δ13C	δ15N	Date Range (calBP)		Median Age	%N	%C	% Collagen	C:N	Episode	Tissue	Element
41	White Bison Robe 32ME7	-19.6	8.3	465	315	379	16.5	44.7	3.6	3.2	LH	D	M3
42	White Bison Robe 32ME7	-20.3	8.0	458	307	386	15.5	42.3	6.3	3.2	LH	D	M3
43	Menoken Village 32BL2	-15.4	6.2	1175	989	1077	15.3	41.9	9.7	3.2	LH	D	M
44	Brand Bison Kill 32SK201	-19.7	6.2	703	668	683	16.5	45.8	3.5	3.2	LH	B	atlas
45	Brand Bison Kill 32SK201	-20.3	7.5	688	665	675	16.0	44.8	2.5	3.3	LH	B	atlas
46	Forest River	-17.8	6.0	284	22	185	16.4	44.9	3.7	3.2	LH	D	M
47	Falkirk Bison Kill 32ML927	-20.2	6.9	670	565	653	16.3	45.4	2.8	3.3	LH	D	M
48	Rustad Site 32R1775	-17.9	8.8	7972	7860	7936	15.8	43.6	7.2	3.2	MH	D	M
49	Alkali Creek 32DU336	-18.3	7.8	2750	2539	2728	15.8	42.6	5	3.2	LH	D	M
50	Rustad Site 32R1775	-18.2	6.6	9029	8786	8999	15.0	42.0	2.1	3.3	EH	B	mandible
51	Mondrian Tree 32MZ58	-19.8	6.8	631	516	544	15.6	42.5	12	3.2	LH	D	M
52	Alkali Creek 32DU336	-20.3	7.3	1567	1416	1533	16.0	43.0	5.7	3.1	LH	D	tooth
53	Streifel Site 32ML903	-20.3	7.0	4079	3897	3964	15.4	42.0	11.3	3.2	LH	B	fragment
54	Beacon Island 32MN234	-21.5	6.2	12389	12030	12209	13.0	37.5	1	3.4	LP	D	M
55	Streifel Site 32ML903	-19.1	6.3	4082	3892	3966	15.5	42.0	12.6	3.2	LH	B	ant. cannon
56	Mondrian Tree 32MZ58	-19.1	7.8	2744	2502	2701	15.3	42.6	9.3	3.2	LH	B	fragment
57	Mondrian Tree 32MZ58	-19.8	5.8	2744	2497	2622	15.0	41.6	6.2	3.2	LH	B	phalanx
58	Beacon Island 32MN234	na	na	na	na	na	na	na	< 1	na	na	D	M
59	Beacon Island 32MN234	-21.1	5.0	12396	12056	12229	14.9	41.7	3.7	3.3	LP	D	M
60	Beacon Island 32MN234	-21.2	5.7	12373	11845	12068	15.0	41.9	2	3.3	LP	D	M
61	Beacon Island 32MN234	na	na	na	na	na	na	na	< 1	na	na	D	M3
62	Beacon Island 32MN234	-18.4	5.9	12547	12140	12431	15.2	41.4	4.2	3.2	LP	D	M3
63	Beacon Island 32MN234	na	na	na	na	na	na	na	< 1	na	na	D	M
64	Mondrian Tree 32MZ58	-20.7	8.1	3825	3640	3705	14.3	40.3	5.6	3.3	LH	B	phalanx
65	Brand Bison Kill 32SK201	-18.8	7.5	691	661	675	16.7	45.6	8.7	3.2	LH	B	horncore
66	Rustad Site 32R1775	-16.2	8.1	8191	8042	8116	14.9	41.7	3.8	3.3	MH	D	M
67	Rustad Site 32R1775	-17.5	6.8	8178	8036	8107	14.7	40.5	5.1	3.2	MH	D	M
68	Rustad Site 32R1775	-16.2	7.8	8187	8042	8116	14.9	41.5	5	3.3	MH	D	M3
74	White Bison Robe 32ME7	-18.4	6.5	1177	1007	1105	16.2	44.0	11.8	3.2	LH	D	M3 root
77	Larson Village 32BL9	-19.1	5.7	469	311	383	16.1	43.6	10.6	3.2	LH	D	M

Table 1 continued.

Bison	Location	δ13C	δ15N	Date Range (calBP)		Median Age	%N	%C	% Collagen	C:N	Episode	Tissue	Element
80	Larson Village 32BL9	-19.8	6.1	498	317	425	16.1	43.5	8.4	3.1	LH	D	M
83	Larson Village 32BL9	-10.7	7.1	495	319	432	16.0	42.9	2.8	3.1	LH	D	M
92	Menoken Village 32BL2	-17.9	7.2	894	728	765	15.9	43.1	5.4	3.2	LH	D	M
97	Streifel Site 32ML903	-19.3	6.5	4084	3998	3913	15.2	42.4	10.5	3.3	LH	B	phalanx
98	Streifel Site 32ML903	-19.1	8.5	7932	7793	7862	14.9	42.3	3.8	3.3	MH	B	fragment
99	Streifel Site 32ML903	na	na	na	na	na	na	na	< 1	na	na	D	M
106	Big Hidatsa 32ME12	-17.5	5.5	310	12	277	15.9	43.3	7.1	3.2	LH	D	M3 root
108	Big Hidatsa 32ME12	-20.4	6.6	287	24	182	16.1	43.8	15.1	3.2	LH	D	M
111	Taylor Bluff Village 32ME366	-15.5	5.8	269	14	112	16.4	44.0	13.6	3.1	LH	D	M root
113	Sakakawea 32ME11	-16.3	5.2	259	25	110	16.1	43.8	12.2	3.2	LH	D	M3
117	Sakakawea 32ME11	-19.6	6.5	305	12	169	16.1	43.1	14.1	3.1	LH	D	M3 root
120	Sakakawea 32ME11	-19.4	7.0	287	24	182	16.1	43.2	6.7	3.1	LH	D	M
121	Sakakawea 32ME11	-15.9	6.0	266	22	111	16.3	44.5	16.0	3.2	LH	D	M3
122	Sakakawea 32ME11	-16.5	5.7	300	14	169	16.6	44.8	14.1	3.2	LH	D	M3
123	Sakakawea 32ME11	-18.2	6.3	302	14	168	16.1	43.8	13.9	3.2	LH	D	M3
125	Lower Hidatsa	-16.3	5.1	458	306	388	15.8	42.5	9.2	3.1	LH	D	M root
126	Lower Hidatsa	-16.7	6.7	271	11	117	16.0	42.8	13.5	3.1	LH	D	M3
131	TRNP, emerged skeleton	-20.3	5.3	521	479	504	16.0	43.6	17.1	3.2	LH	D	M3
132	Red River Valley	-18.2	7.1	300	14	169	15.3	41.5	8.2	3.2	LH	D	M
100	TRNP Bull	-21.1	5.0	na	na	na	15.6	42.4	13.3	3.2	Mod.	D	M3
101	TRNP Bull	-20.1	5.2	na	na	na	15.5	42.9	16.9	3.2	Mod.	D	tooth
102	TRNP Cow, "Wanda"	-20.7	4.6	na	na	na	15.1	41.9	17.4	3.2	Mod.	D	M3
103	TRNP Cow	-20.0	5.0	na	na	na	15.6	42.7	15.8	3.2	Mod.	D	M3
104	TRNP Cow	-20.8	4.7	na	na	na	15.5	42.3	20.4	3.2	Mod.	D	M

Table 2. Statistical summaries for bison dentin and bone and %C₄ in each temporal episode.

Episode (cal BP)	Tissue	n	Mean $\delta^{13}\text{C}$	SD $\delta^{13}\text{C}$	$\delta^{13}\text{C}$		Mean $\delta^{15}\text{N}$	SD $\delta^{15}\text{N}$	$\delta^{15}\text{N}$		% C ₄
					low	high			low	high	
Modern	Dentin	5	-20.5	0.47	-21.1	-20.0	4.9	0.24	4.6	5.2	0
Late Holocene (4,200 – 100)	Dentin	43	-18.0	1.90	-20.5	-10.7	6.5	0.82	5.0	8.3	15
	Bone	18	-19.0	1.67	-21.1	-14.8	6.6	0.94	4.9	8.1	9
	Combined	61	-18.3	1.90	-21.1	-10.7	6.53	0.85	5.0	8.3	14
Middle Holocene (8,200 – 4,200)	Dentin	5	-16.8	0.82	-17.9	-16.2	8.2	0.96	6.8	9.3	24
	Bone	2	-16.9	3.18	-19.1	-14.6	6.8	2.47	5.0	8.5	24
	Combined	7	-17.4	2.10	-19.1	-14.6	7.6	1.5	5.0	9.3	20
Early Holocene (11,700 – 8,200)	Bone	1	-18.2	na	-18.2	-18.2	6.6	na	6.6	6.6	14
Late Pleistocene (12,500 – 11,700)	Dentin	4	-20.6	1.44	-21.5	-18.4	5.7	0.51	5.0	6.2	0

Table 3. Model fit values for generalized additive models (GAMs).

Smoothed Function	k-index	p-value
s(time) $\delta^{13}\text{C}$	0.89	0.12
s(time) $\delta^{15}\text{N}$	0.86	0.12

Table 4. Carbon isotopic values (VPDB) for modern bison compiled from other studies.

Location	n	$\delta^{13}\text{C}$ Mean	$\delta^{13}\text{C}$ SD	Reference
Wichita Mountains National Wildlife Refuge	4	-17.48	1.17	Tieszen (1994), Leyden (2004)
Yellowstone National Park, WY	15	-19.76	0.23	Feranec (2007)
Konza Prairie, KS	7	-16.27	0.53	Tieszen et al. (1994)
Catalina Island, CA	20	-24.75	0.43	Cotton et al. (2016)
Saskatoon, SK	4	-20.63	0.66	Leyden (2004)

CHAPTER III

SEASONAL CHANGES INFERRED FROM OXYGEN AND CARBON STABLE ISOTOPES IN BISON TOOTH ENAMEL

ABSTRACT

The presence of bison in North America throughout history has shaped the evolution of the Great Plains ecosystem and fostered diversity in flora and fauna. After their near extirpation in the late 1800's, bison are functionally absent from the majority of their original range. It would benefit bison conservation managers to know the historical space use of bison when restoring them to the landscape. With a lack of systematic studies on bison prior to their near extirpation, we don't know what migratory patterns were exhibited by their ancestors. In this chapter, a serial sampling method of carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) stable isotopes from bison tooth enamel is used to infer seasonal changes in diet that may occur due to climatic variability and migration patterns. One hundred and eighty-nine samples were serially collected from twenty bison teeth (18 ancient and 2 modern) and fourteen samples were collected from one Late Holocene horse tooth. Samples represent ^{14}C date ranges spanning the Terminal Pleistocene to modern bison in Theodore Roosevelt National Park (TRNP). Analysis of Variance (ANOVA) detects significant differences in isotopic values between all temporal groups apart from the Late Pleistocene and modern bison. Late Holocene bison values were compared to the Late Holocene horse tooth and found significant differences in diet composition. Bison isotopic trends in this study do not indicate large scale migratory movements, apart from potentially two bison. One bison in the Late Pleistocene exhibits comparably high $\delta^{13}\text{C}$ variability, indicating a more varied

diet. The other bison that may have been migratory occurred in the Middle Holocene and had an unpredictable trend in $\delta^{18}\text{O}$ values, suggesting either a dependence on water sources not affected by precipitation or the avoidance of temperature extremes through migratory movement. These results differ from the historical accounts of European settlers on bison migrations. Further research should be pursued to accurately define migratory movements by isotopic values.

INTRODUCTION

Bison have evolved with the North American landscape, fostering the unique biodiversity of flora and fauna that can be found in the Great Plains. Patterns of movement for bison prior to their management by humans, is not well understood. The majority of bison today are kept in confined areas and the question of how far a free-ranging herd would actually migrate or disperse, given the opportunity, remains unclear (Gates et al. 2010). Any observance of bison behavior prior to their near extirpation is based on conjecture and was unsystematically recorded during a time when the natural order of the Great Plains ecosystem was severely disrupted by the expansion of European settlers and aggressive overhunting (Cannon 2001). Nonetheless, these accounts have recorded longitudinal migrations of bison associated with seasonal changes (Seton 1929, Leopold and Roe 1970) and east to west migrations from open grassland to the protection of the Rocky Mountains in winter (Garretson 1938). Unlike dispersals, migrations are prompted by seasonal changes, are not necessarily dependent on population density, and imply that the animal will return to the original location (Soubrier et al. 2016).

Free-ranging extant plains bison (*Bison bison bison*) can only attempt elevational migrations due to the constraints of current management practices (Larter and Gates 1991, Reynolds et al. 2003, Daleszczyk et al. 2007). Conversely, free-ranging wood bison (*Bison bison*

athabascae), whose current range is predominately in Alaska and Canada, have not been documented to complete any large-scale migratory movements (Larter and Gates 1991). It has been shown that the elevational distance moved by bison is dependent on the severity of local conditions. During Yellowstone winters with deep snowpack, bison will move further down in elevation than winters with less precipitation and will follow the mountain down with each snowstorm (Kauffman et al. 2018). When population density is high and resources are limited (or in particularly harsh winters), Yellowstone bison will attempt to move outside of the park, but most of their efforts are thwarted by human intervention (Gates et al. 2010). As a result, the bison behavior we observe today is still largely influenced by humans. Fencing, hazing, and culling practices generally keep bison from venturing into open landscapes (Peacock 1997).

Given the limited connectivity of wilderness for animals to navigate and the artificial conditions bison are kept under, it is not surprising that they generally do not exhibit the type of migratory behavior described by early explorers. Whether ancient bison actually did use migration as a survival strategy is still in question. Insight into the movement of ancient bison could be recorded in the enamel carbonate in bison teeth, where stable isotopes of carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) can be observed in increments small enough to capture seasonal changes in diet.

The finer resolution of time recorded in bison tooth enamel can provide insight into habitat use by bison and also allows the investigation of interspecies interactions on a smaller scale. Because a horse tooth was available in the sample assemblage, it is included into this chapter to see if comparisons could be made between bison and horse. Modern studies on horse and bison diet show that horses incorporate up to fifty percent of browse material while bison usually consume less than ten percent of browse (Feranec 2007). Previous isotopic studies

assessing habitat use by bovids and equines during the Pleistocene have found evidence of niche partitioning between the two species (Feranec 2007, Britton et al. 2012).

Bison have hypsodont (high-crowned) teeth with enamel that forms in two steps. In the first step, a matrix that is high in organic material but lacks minerals is deposited along the tooth's surface. This matrix undergoes maturation and becomes enamel in the second step (Zazzo et al. 2012). Enamel builds up from the root to crown incrementally and isotopically represents the time period in which it was created, a few weeks to a few months (Passey and Cerling 2002, Hillson 2005). These values will not differ from the point in which they are created because, unlike collagen, enamel does not undergo remodeling (Lee-Thorp and Sponheimer 2003). The maturation process is slower than the initial deposition and may allow for complex patterns of formation, resulting in attenuation of the isotopic signal when samples are taken perpendicular to the tooth axis. However, modeling work has proven that the signal is not lost (Passey and Cerling 2002).

Adding to the complexity of tooth formation are the stages in which bison molars form in their lives. The first molar (M_1) starts growing in utero and completes mineralization shortly after birth. As M_1 finishes growth, the second molar (M_2) starts forming and mineralizes during the first year of the animal's life (Gadbury et al. 2000). The third molar (M_3) erupts at 9 to 10 months of age and will complete mineralization at 15 to 18 months. Because the M_3 forms after weaning, it is the only reliable molar to truly represent the diet of the individual and not time in utero or nursing on the mother's milk (Gadbury et al. 2000, Hoppe et al. 2004). While the exact effect nursing has on isotopic fractionation is unclear, it is argued that the effect is minimal compared to environmental influences (Zazzo et al. 2012). Horse teeth and their enamel forms in a similar pattern to bison but will fully mineralize after three years (Hoppe et al. 2004). Because

horse tooth mineralization takes longer than bison, the isotopic signal is dampened in comparison (Higgins and MacFadden 2004).

The Northern Great Plains are dominated by plants using the C₃ photosynthetic pathway, but times of drought could increase the availability of C₄ plants (MacFadden and Cerling 1999, Cannon 2007). Mammals have a consistent carbon fractionation of +14.6‰ in tooth enamel, which translates to all δ¹³C values above zero equating to a pure C₄ diet. Values between 0‰ and -8‰ represent a mixed C₃/C₄ composition and values below -8‰ indicate a pure C₃ diet (Feranec et al. 2009).

δ¹⁸O in enamel carbonate, documents meteoric water values (Dansgaard 1964). These isotopic signatures are assimilated into bison enamel, and in northern latitudes, oxygen isotopic values represent local temperatures (Luz et al. 1984, Hoppe et al. 2004, Britton et al. 2009). Therefore, δ¹⁸O in Northern Great Plains bison tissues change with the temperature and record seasonal variations (Britton et al. 2009). Lower δ¹⁸O represent cold seasons and higher values indicate warm seasons (Dansgaard 1964), creating a sinusoidal pattern in connection with the seasons. Dampening of this sinusoidal pattern may be explained by the animal drinking from water sources less affected by precipitation (i.e. lakes, groundwater and large rivers) but could also be attributed to large scale migratory movements and the avoidance of temperature extremes (Widga et al. 2010, Julien et al. 2012).

The objective for this chapter is to determine if Northern Great Plains bison were migratory during the Holocene. Based on the information described above, I expect non-migratory bison in this study to reflect a C₃ dominated diet and a distinct sinusoidal pattern (i.e. smooth wave pattern) in δ¹⁸O indicative of seasonal changes in temperature. Bison which deviate from these expected patterns potentially underwent seasonal migrations.

METHODS

Sample Assemblage

Tooth enamel samples were taken from a subset of bison specimens previously ^{14}C dated and analyzed for carbon and nitrogen stable isotopes (Table 1). In total, twenty bison molars and one horse molar were selected. The horse molar (median calBP age of 177) was included from the antler, horn, and bone collection at the Medora Visitor's Center for interspecies comparisons. Bison samples were chosen to represent time from the Late Pleistocene and throughout the Holocene (12,229 calBP to modern bison); two Late Pleistocene, three Middle Holocene, thirteen Late Holocene, and two modern. Eleven bison third molars (M_3) and nine unspecified bison molars (either M_1 or M_2), and one horse (unspecified molar) were serially sampled for carbonate from enamel to analyze carbon and oxygen stable isotopes. Specimens were derived from eleven archaeological sites within North Dakota; Big Hidatsa, White Bison Robe, Larson Village, Huff Village, Menoken Village, Rustad, Beacon Island, Falkirk Bison Kill, Bundlemaker, Double Ditch Village, and Alkali Creek, spanning all cultural traditions (Figures 1, 2).

Sampling & Pretreatment

Prior to collecting enamel, the surfaces of the teeth were cleaned using a dental pick and sandpaper. Using a Dremel handheld rotary tool with a 3/32-inch tapered diamond file, small samples of powdered enamel (~3 mg) were taken parallel to the tooth's occlusal surface starting from the enamel root junction to the tooth apex. The thickest and most intact area of enamel was selected for sampling. Depending on the tooth, the most suitable area for sampling was along the

protoconid, hypoconid, metaconid, or entoconid. The enamel powder was collected over sheets of aluminum foil and transferred to 2 mL flip-top plastic centrifuge tubes. Powder samples were then bathed in 0.1 molar acetic acid for 1 hour to remove all non-structural carbonate from the sample. The acetic acid was removed with pipettes and the sample was rinsed 2x with 1 mL of distilled water. Samples were vortexed for 5 seconds and then allowed to settle after rinses. After the final DI water rinse, all of the liquid except for one small drop was pulled off of the powder to minimize sample loss. Flip top caps on the tubes were left open and the samples were placed in an oven to dry at 37°C for 12-24 hours.

The pre-treated samples were then sent to the University of Arizona Stable Isotope Laboratory where they were reacted with dehydrated phosphoric acid under vacuum at 70°C. Measurements were made on an automated carbonate preparation device (KIEL-III) coupled to a gas-ratio mass spectrometer (Finnigan MAT 252). The isotope ratio measurement was calibrated based on repeated measurements of NBS-18 ($\delta^{18}\text{O} = -22.96\text{‰}$, $\delta^{13}\text{C} = -5.00\text{‰}$ VPDB) and NBS-19 ($\delta^{18}\text{O} = -2.20\text{‰}$, $\delta^{13}\text{C} = 1.95\text{‰}$ VPDB) with a precision of $\pm 0.1\text{‰}$ for $\delta^{18}\text{O}$ and $\pm 0.08\text{‰}$ for $\delta^{13}\text{C}$.

Calibration and Analysis

The $\delta^{13}\text{C}$ values of all ancient teeth dated prior to the Industrial Revolution (older than 150 calBP) were corrected by -1.5‰ for the reduction in atmospheric CO_2 due to the increased burning of fossil fuels⁴⁰. Samples were separated by tooth type (third molar or unspecified molar) and were categorized into the formal subdivisions of the Holocene (Walker et al. 2018). Due to the different times of formation of bison teeth and potential influence from the mother's

milk (Metcalf 2011), samples will also be labeled by tooth type (M= unspecified, M₁ and M₂, M₃= third molar) but will be analyzed as a whole.

Analysis of variance (ANOVA) was used to detect significant differences in isotopes between bison temporal groups. A t-test was used to compare the difference between the Late Holocene horse and Late Holocene bison. ANOVA and t tests were performed in Rstudio version 3.5.3.

RESULTS

One hundred and eighty-nine samples were taken from 20 bison teeth (eighteen ancient and two modern) and fourteen samples were taken from the horse tooth (Tables 5, 6). The number of samples per bison tooth ranged from 6 to 13 with an average of 9.5 from each tooth. Most bison with more than 30 mm of sampling distance display a sinusoidal pattern of $\delta^{18}\text{O}$ values, indicating the capture of at least a full year of seasonal changes (Figure 7).

$\delta^{13}\text{C}$ values for third molars ranged from -12.8‰ to -5.1‰ and had a mean value of -8.8‰. $\delta^{13}\text{C}$ for the unspecified molars ranged from -12.4‰ to -4.2‰ with a mean of -7.9‰. $\delta^{18}\text{O}$ for third molars ranged from -17.4‰ to -5.0‰ and had a mean of -10.9‰. $\delta^{18}\text{O}$ values for the unspecified molars ranged from -15.3‰ to -4.7‰ and had a mean of -10.8‰. Amplitude for bison $\delta^{13}\text{C}$ ranged from 0.5 to 3.4 with an average of 1.3 and amplitude for $\delta^{18}\text{O}$ ranged from 2.4 to 8.2 with a mean of 5.0. The horse tooth amplitude was comparable to the bison, 2.9 and 3.5 for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, respectively (Figure 8, Table 7). However, the horse tooth was statistically different from Late Holocene bison in $\delta^{13}\text{C}$ values (t-test, $p < 0.001$).

Analysis of Variance (ANOVA) determined significant differences in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ between bison by temporal episode. Middle Holocene bison were significantly different from

Late Holocene ($\delta^{13}\text{C}$, $p < 0.001$, $\delta^{18}\text{O}$, $p < 0.001$) and modern ($\delta^{13}\text{C}$, $p < 0.001$, $\delta^{18}\text{O}$, $p = 0.003$) bison for both isotopes. Late Pleistocene and modern bison were most similar with no significant differences in either isotope ($\delta^{13}\text{C}$, $p = 0.99$, $\delta^{18}\text{O}$, $p = 0.80$). All other comparisons were significantly different in $\delta^{13}\text{C}$ ($p < 0.001$) but not $\delta^{18}\text{O}$ (Table 8).

Overall, Modern and Late Pleistocene bison had diets dominated by C_3 vegetation (i.e. carbon values lower than -8‰). Some Middle and Late Holocene bison had diets that were C_3 dominated while others showed a mix of C_3 and C_4 vegetation composition (Figure 9).

DISCUSSION

The sampling strategy in this study aims to capture at least a year of seasonal changes in bison diet. Bison molar teeth will wear down with age and some teeth in the sample assemblage were more worn than others and may not represent a full year of growth. Overall, bison teeth with at least 30 mm of sampling length likely depicted a full year of changes (Higgins and MacFadden 2004).

$\delta^{13}\text{C}$ profiles indicate a C_3 dominated diet with some incorporation of C_4 vegetation in bison from all temporal episodes except for modern TRNP bison (Figure 9). A few bison from the Late Holocene have more C_4 plants into their diet during the summer season, when an increase in available C_4 grasses would be expected (Figures 7d, 7g). However, there are also several bison that record an increase in C_4 plants during the winter (Figures 7i, 7p, 7t). This can be explained by the increased consumption of ^{13}C depleted lichen in bison diet during the lean winter months (Widga et al. 2010, Julien et al. 2012).

The mean values of $\delta^{18}\text{O}$ varied widely but net changes in amplitude are similar amongst all bison and are comparable to changes that can be seen in modern meteoric water sources in

Northern latitudes (Table 7) (Widga et al. 2010). A sinusoidal pattern representative of the seasons is shown in all bison with a sufficient sampling distance except for one. Bison 67 from the Middle Holocene records more frequent and unpredictable shifts in $\delta^{18}\text{O}$ than other bison (Figure 7b). The deviation from sinusoidal seasonal curves of the $\delta^{18}\text{O}$ isotopic signal in this bison could indicate a reliance on water sources not closely tied to precipitation or avoidance of temperature extremes from large-scale migratory movements (Widga et al. 2010).

The two Late Pleistocene bison exhibit the most variability in isotopic values (Table 7). Despite having a short sampling distance for Bison 62, it exhibits the largest variation in $\delta^{13}\text{C}$ that decreases as temperatures warm (Figure 7a). The drop in $\delta^{13}\text{C}$ may be explained by the bison spending more time in heavily forested areas to escape the day's heat but this trend is not shared with its temporal cohorts (van der Merwe 1991). Bison 59 likely consumed lichens during the winter months as higher $\delta^{13}\text{C}$ values are observed when temperatures drop (Julien et al. 2012) (Figure 7l). The variability in this bison's carbon values suggest that it came across more diverse vegetation than its counterparts. Whether this is influenced by local phenology or if the bison expanded its range to reach more vegetation is unclear. Late Pleistocene bison were derived from the Beacon Island archeological site. This site records a C_3 dominated plant community in other proxies studied. While Bison 59 did incorporate C_4 plants into its diet, it is not enough to confirm that it came from a different vegetational community (Mandel et al. 2014).

Middle Holocene bison had diets with mixed composition and significantly differ from all other temporal groups in $\delta^{13}\text{C}$ (Figure 9, Table 8). Bison 67 and Bison 68 (Figures 7b, 7m, respectively) consumed more C_4 plants in colder months but this trend is not observed in Bison 48 (Figure 7n). This could be due to its later occurrence at Rustad (~200 years later) and the large climatic fluctuations associated with Middle Holocene climate that would alter the

composition of vegetational communities (Grimm et al. 2011, Commerford et al. 2018). Climatic records indicate an overall shift towards longer and warmer growing seasons and supports the inclusion of more C₄ plants in the Middle Holocene bison diet (Widga et al. 2010).

Most Late Holocene bison exhibit a C₃ dominated diet and small changes in $\delta^{13}\text{C}$ despite seasonal changes being well documented by oxygen, with the exception of a few individuals. Bison 43 (Figure 7c) increases C₄ plants into their diet when temperature cools and were likely subsisting on lichens. Conversely, Bison 47 (Figure 7r) only shows an increasing trend in C₄ plants as temperatures get warmer. Bison 39 (Figure 7q) and Bison 47 were likely herd cohorts given they were both derived from the Falkirk Bison Kill site, share very similar ¹⁴C ages, and have similar isotopic trends. However, the short sampling distance on Bison 47 makes it difficult to make inferences for all seasons.

Another likely cohort group are three bison from White Bison Robe, Bison 11 (Figure 7g), Bison 41 (Figure 7h), Bison 42 (Figure 7f) (Table 7). While Bison 42 records some lower oxygen values than the others, they all exhibit very small changes in $\delta^{13}\text{C}$ throughout the seasons. Such small changes in $\delta^{13}\text{C}$ has been associated with non-migratory behavior and are consistent for all Late Holocene bison in this study. Hanson (1984) suggested that bison living in North Dakota during the Late Holocene had access to enough quality forage for migratory movements to be an obsolete endeavor. However, Chisholm et al., (1986) proposed that Late Holocene bison from Canada were likely migrating to North Dakota in the winter to obtain better forage based on the incorporation of more C₄ plants than expected for the local vegetative community. If Chisholm's hypothesis was correct, there is a possibility that some bison from the current sample assemblage could have originated from Canada and would show more variation

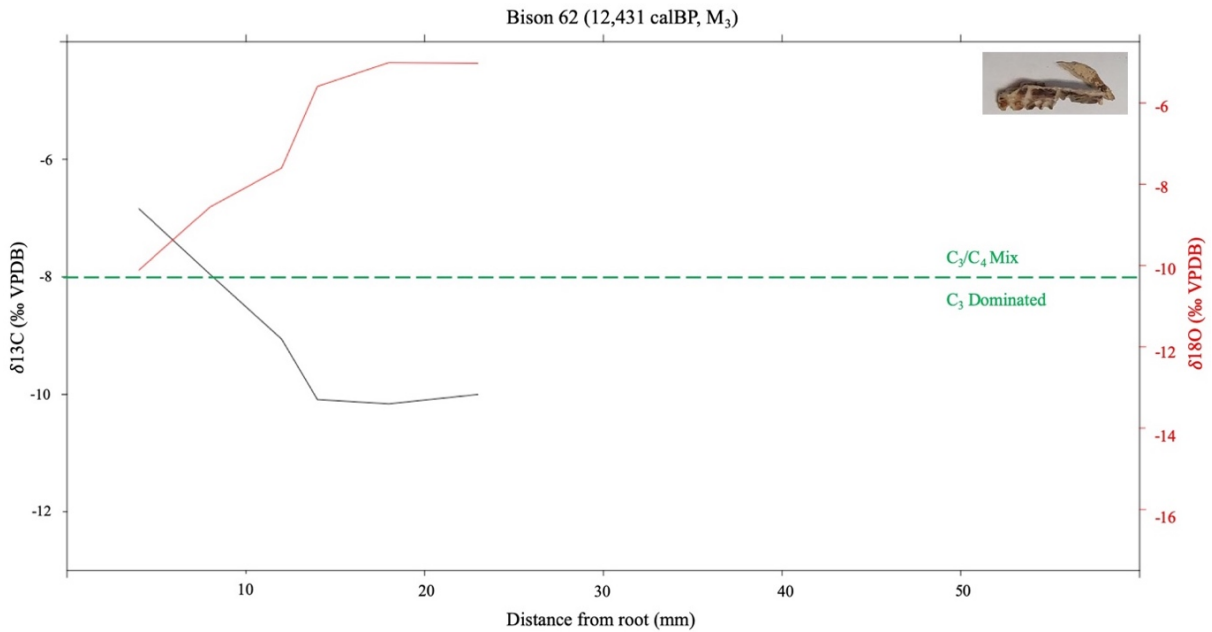
than the bison who lived year-round in present day North Dakota. This is not detected in Late Holocene bison from North Dakota by this sample set (Table 7).

The Late Holocene horse tooth has a weaker sinusoidal signal in oxygen values than most bison sampled in this study (Figure 8). This is likely due to the longer time averaging that takes place during horse enamel formation (Hoppe et al. 2004, Higgens and MacFadden 2004). The horse incorporated significantly different vegetation than bison (t-test, $p < 0.0001$). Late Holocene bison frequently incorporated C_4 vegetation into their diet but this horse fed strictly on C_3 plants. This is consistent with horses incorporating more trees and shrubs into their diet (~50%) as those materials are more depleted in ^{13}C than herbaceous plants. However, there is no clear signal for resource partitioning here because some bison during the Late Holocene also displayed strictly C_3 dominated profiles (Figure 9).

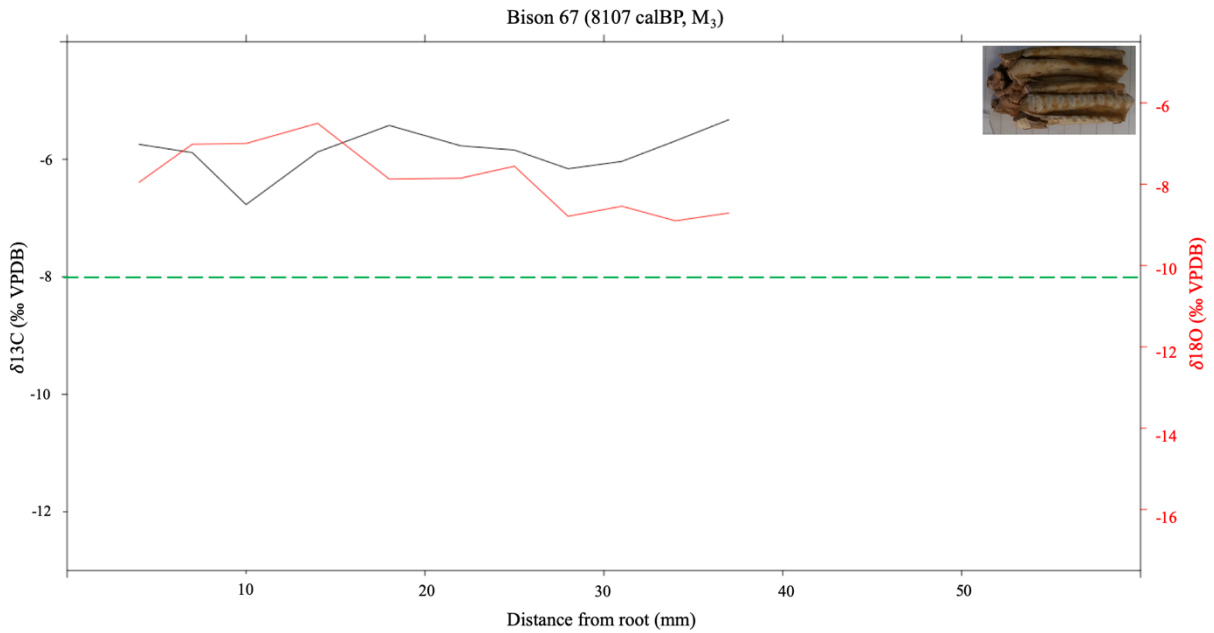
Overall, the sample assemblage is indicative of non-migratory bison with a couple of exceptions, such as Bison 62 in the Late Pleistocene and Bison 67 from the Middle Holocene (Figures 7a, 7b). However, there is still uncertainty if these bison came from an outside area or just had access to more diverse vegetation in the highly mosaiced landscaped associated with the Late Pleistocene and the frequent climatic changes during the Middle Holocene (Cooper et al. 2015). Sampling more bison would help elucidate bison ecology during this time.

These results challenge the observations of early explorers in which bison were reported to migrate with the seasons in mass numbers. The image of bison thundering in large herds across the prairie was more likely driven by abrupt changes in the landscape and the intense pressure from hunters who were motivated to kill as many bison as possible. However, it must be considered that the methods to infer migratory movements from isotopic signatures are still limited. The lack of contiguous lands in the modern world leaves us no migratory counterparts to

compare to ancient bison and limits our ability to define the values that indicate large scale movements. The only animals in North America that are still able to complete large scale migrations belong to the Cervidae (deer) family and they have different diets than bison. The collection of more data from expanded geographical areas can help obtain a better resolved picture. For instance, bison who lived at the edge of their historical range should be studied to observe how the stress of living at the extent of suitable habitat affected their isotopic values. The incorporation of more isotopic data and continued studies of recently reintroduced bison to the landscape will only increase our understanding of the resilient *Bison* species and inform management strategies.

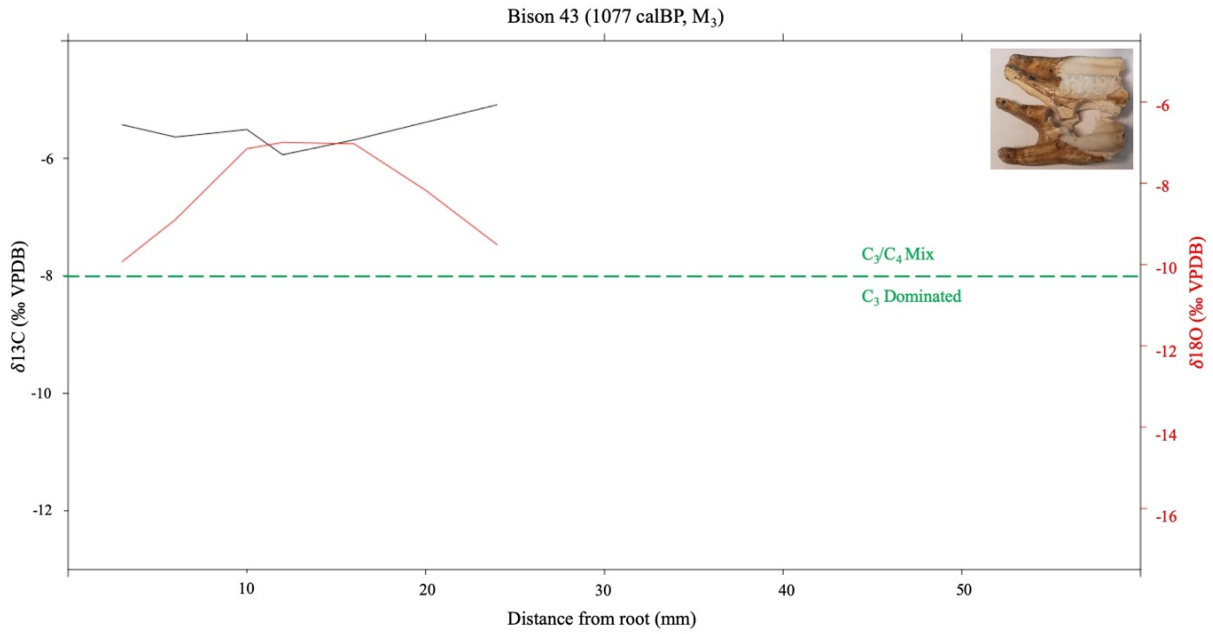


a.

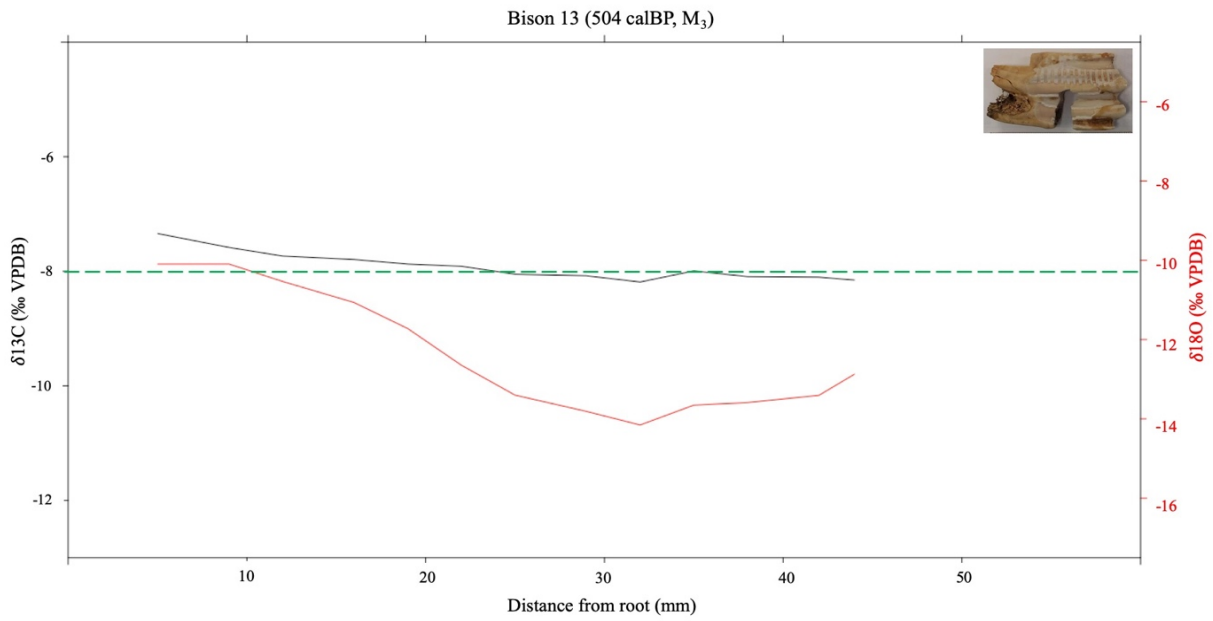


b.

Figure 7. Serially sampled isotopic values for each tooth. Red lines depict oxygen while black lines indicate carbon values. The cutoff for C₃ dominated diets (-8 and below) and a mix of C₃/C₄ (-8 and above) are depicted with a green dashed line. Pictures of the sampled tooth are included as well as whether it was a M₃ (third molar) or M (unspecified M₁ or M₂). M₃ are listed first, from oldest to youngest, then M are listed from oldest to youngest.

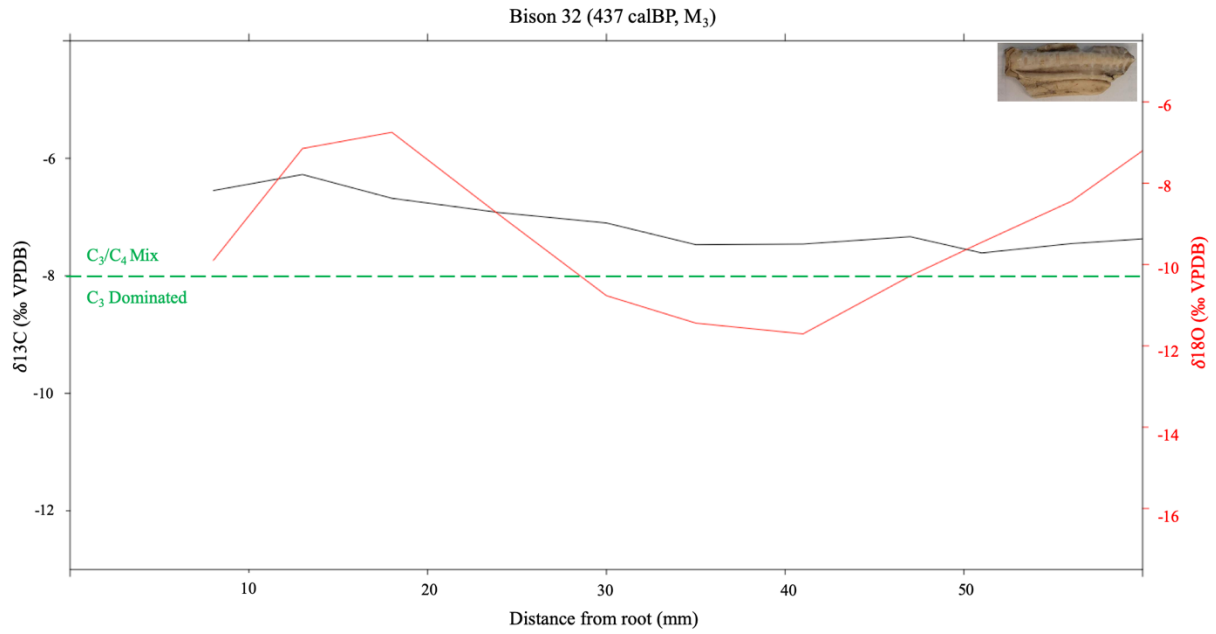


c.

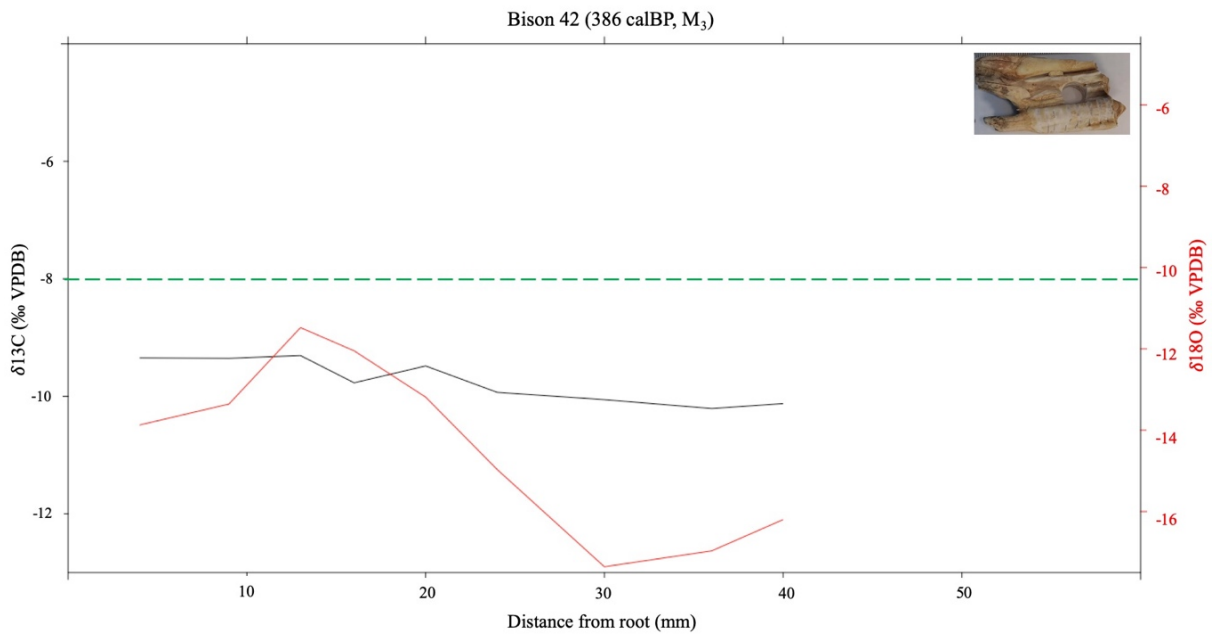


d.

Figure 7 continued.

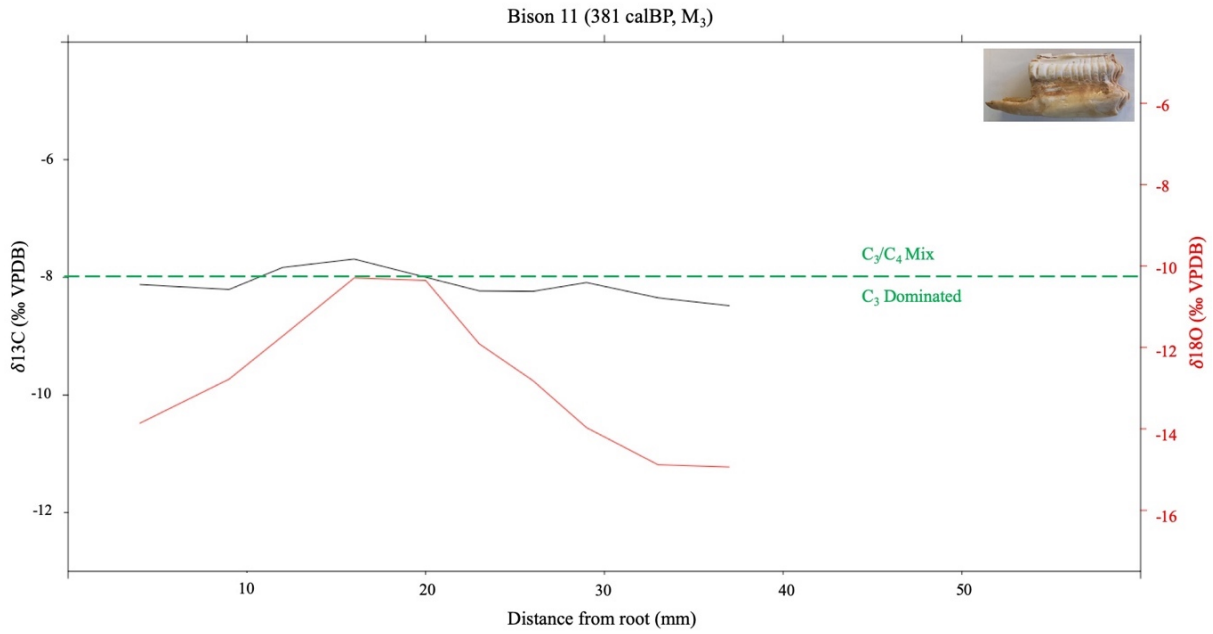


e.

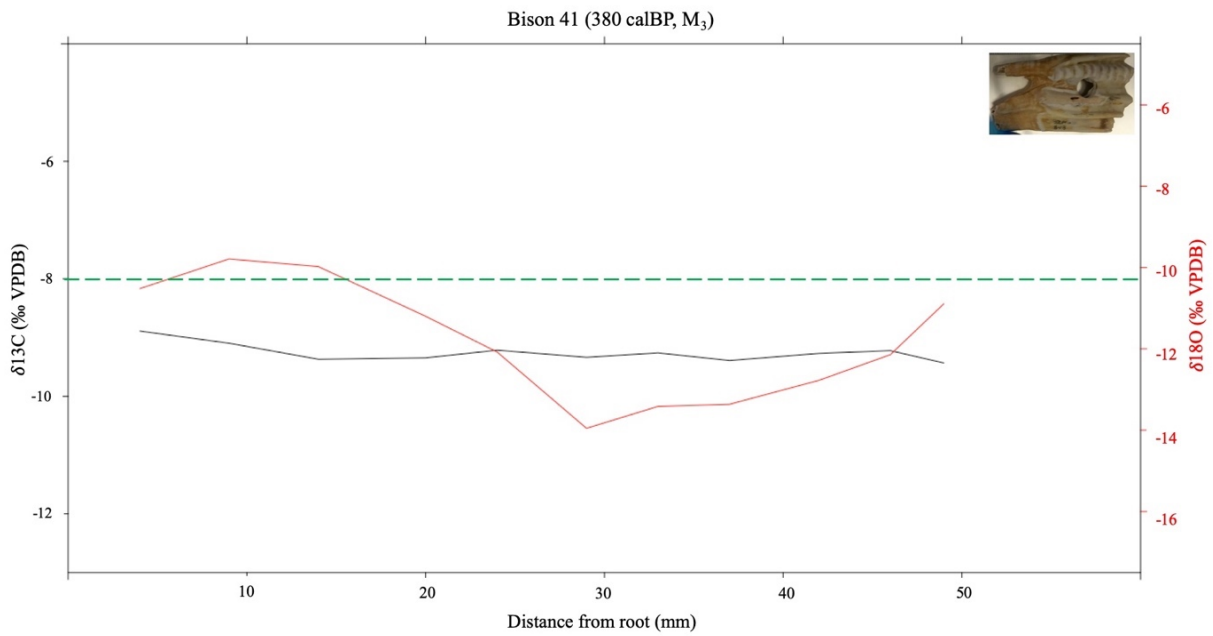


f.

Figure 7 continued.

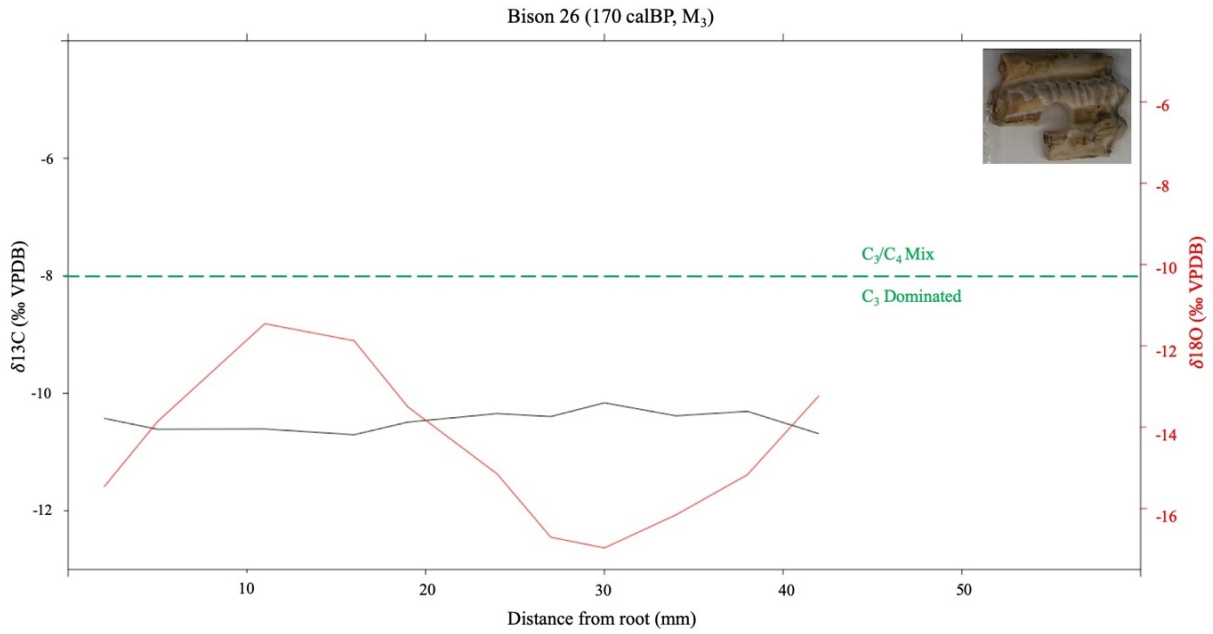


g.

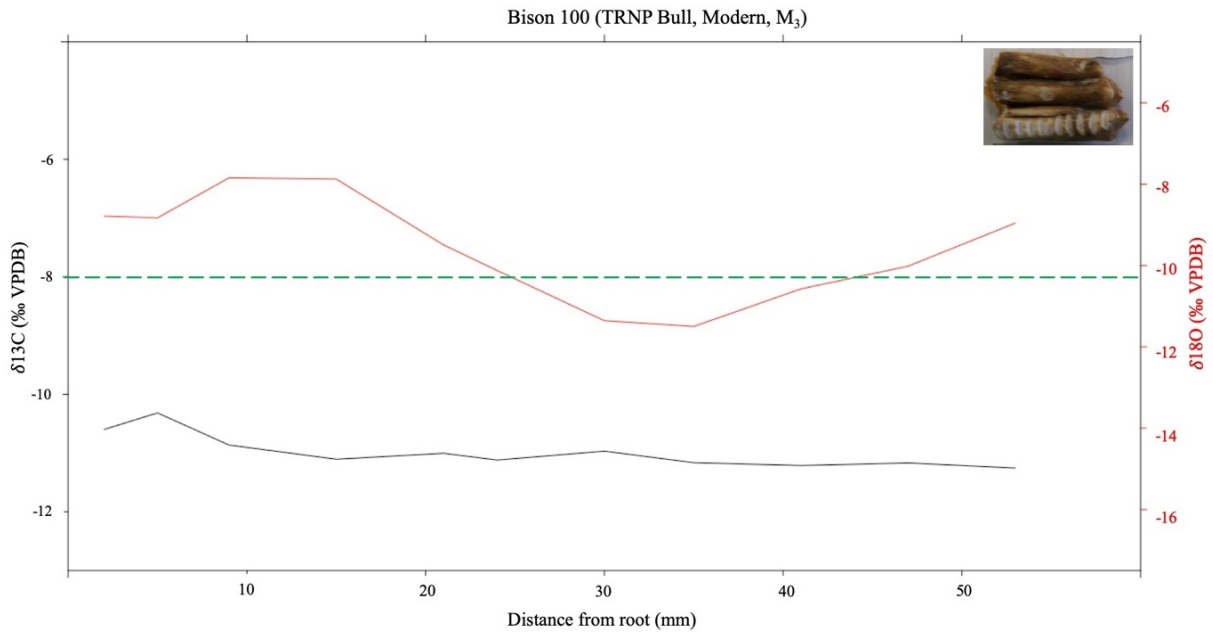


h.

Figure 7 continued.

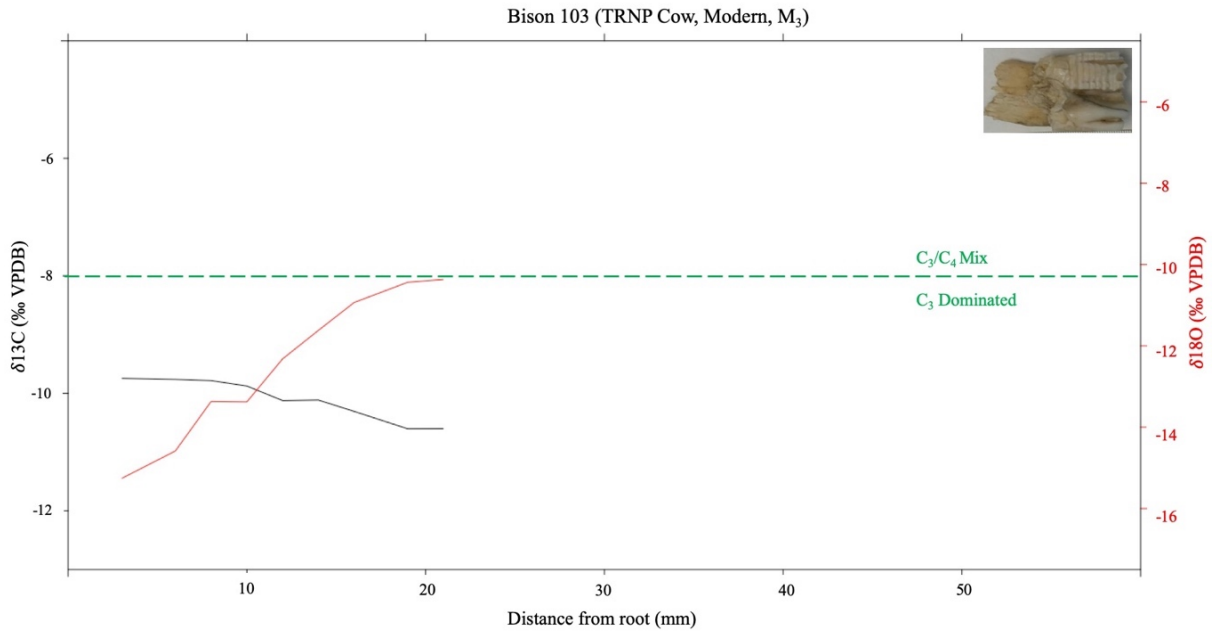


i.

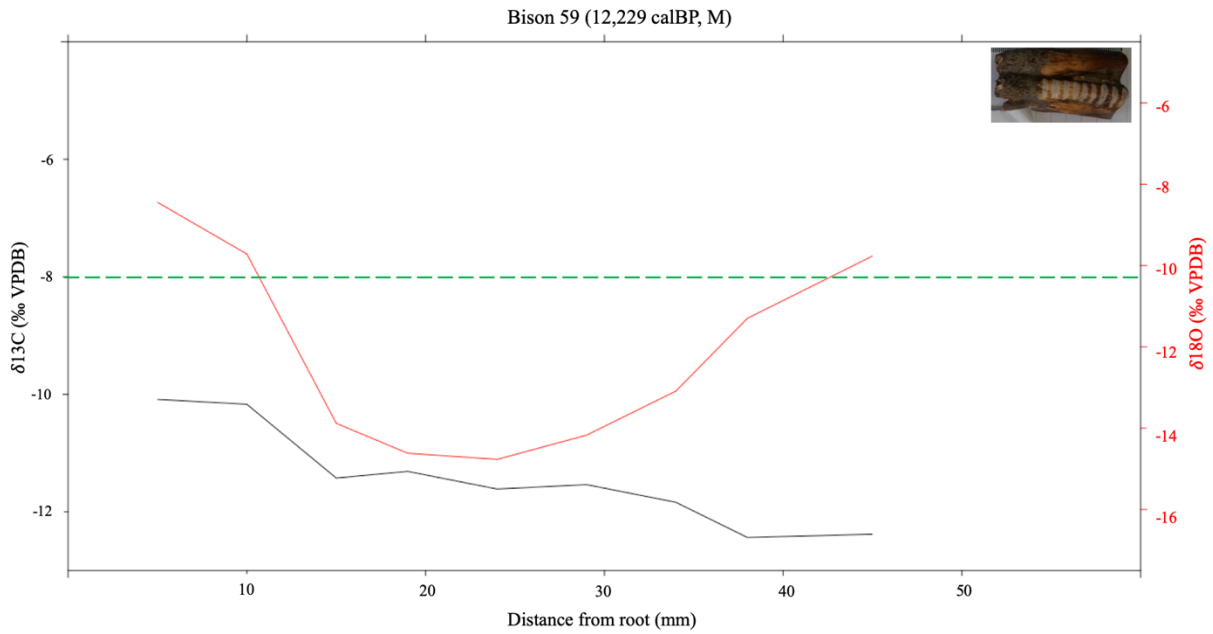


j.

Figure 7 continued.

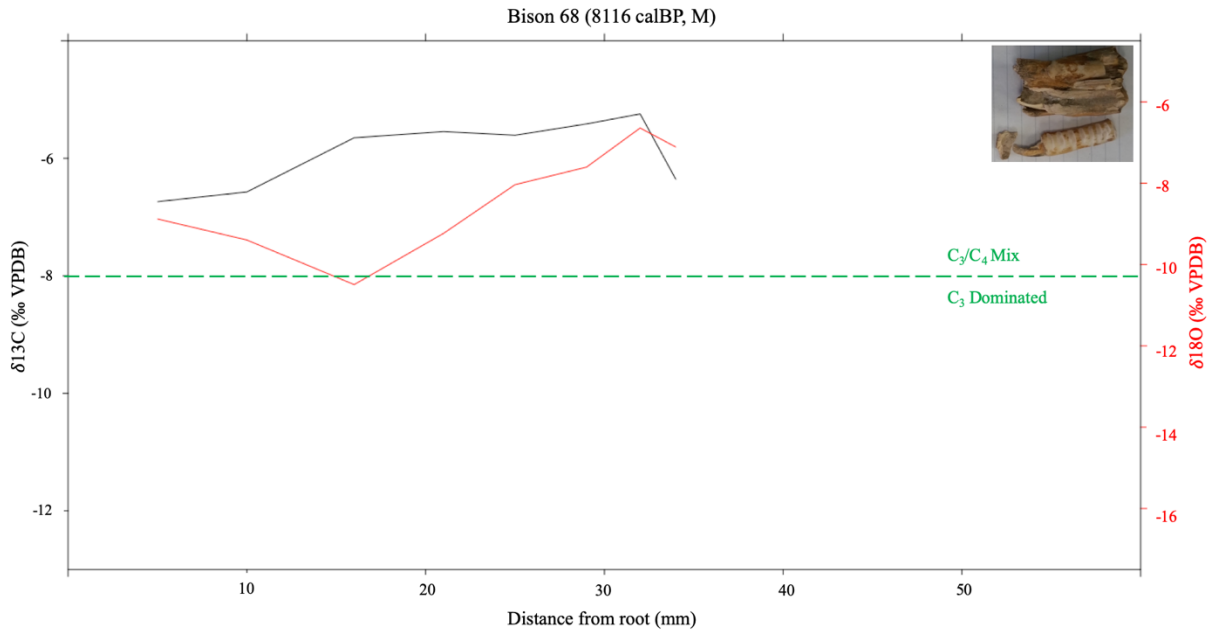


k.

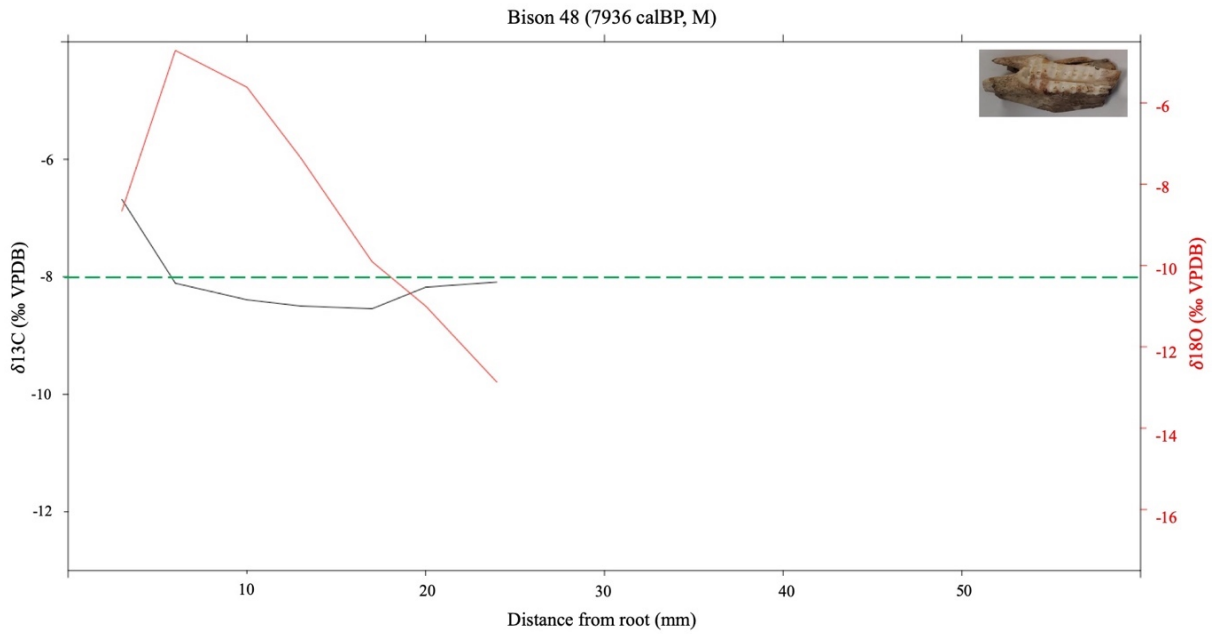


l.

Figure 7 continued.

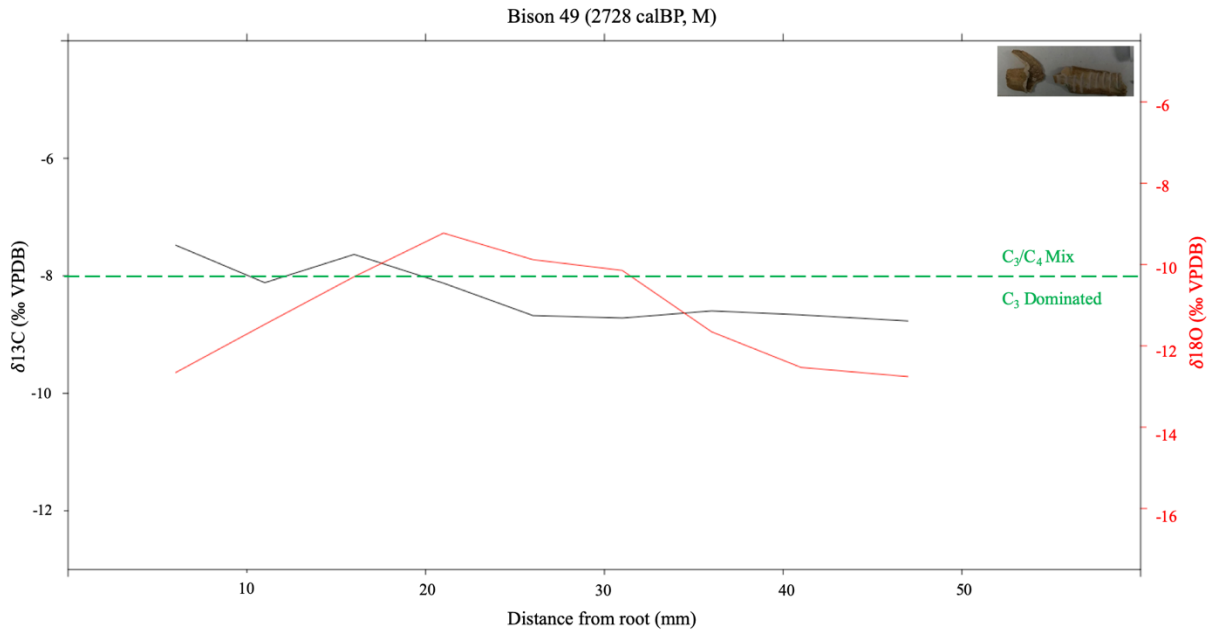


m.

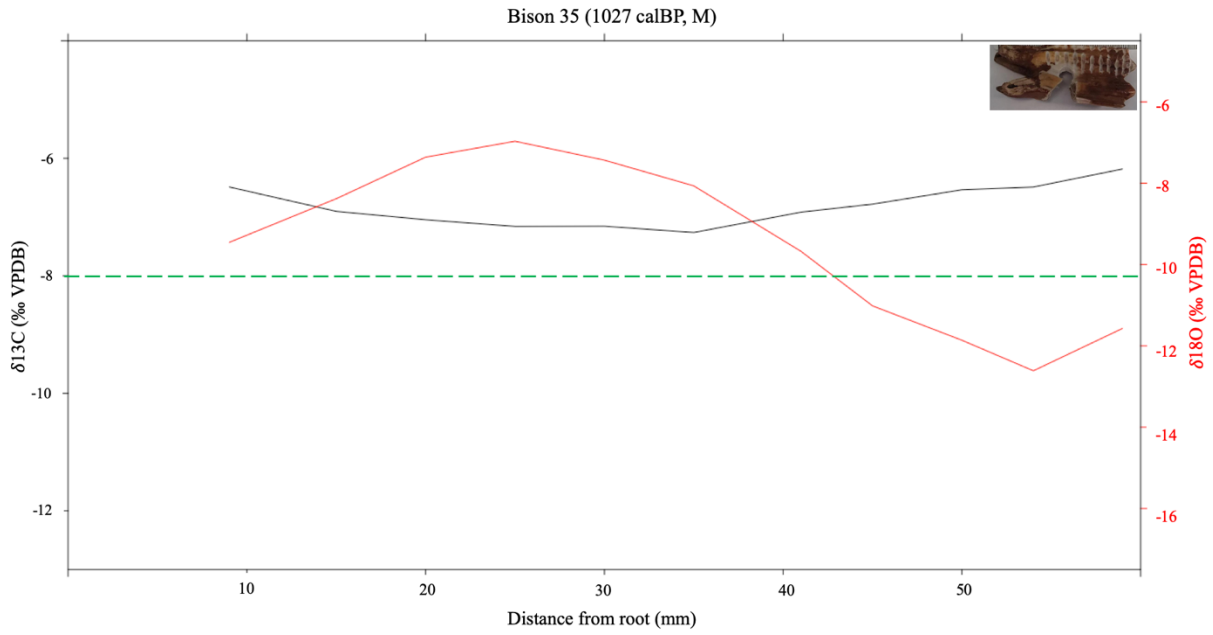


n.

Figure 7 continued.

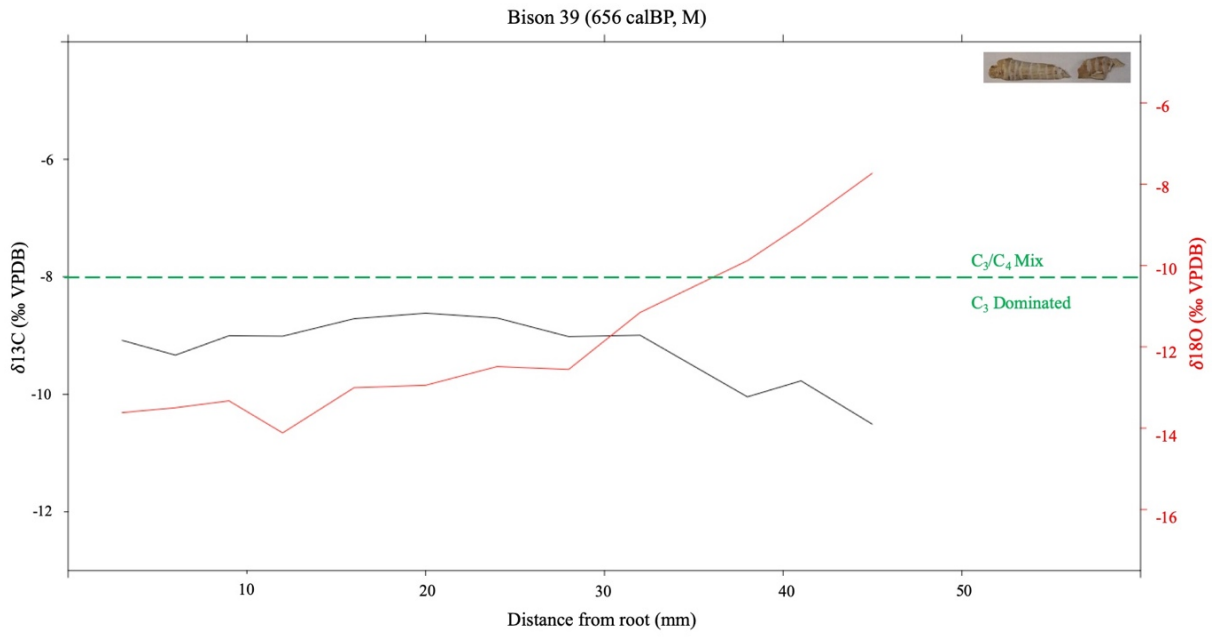


o.

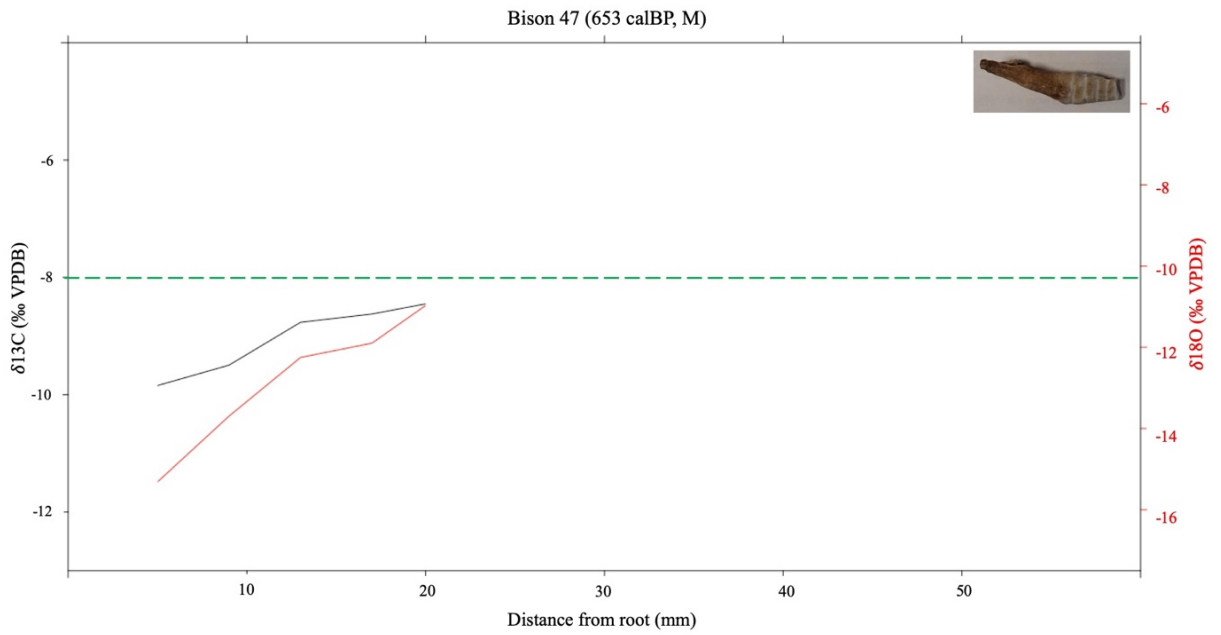


p.

Figure 7 continued.

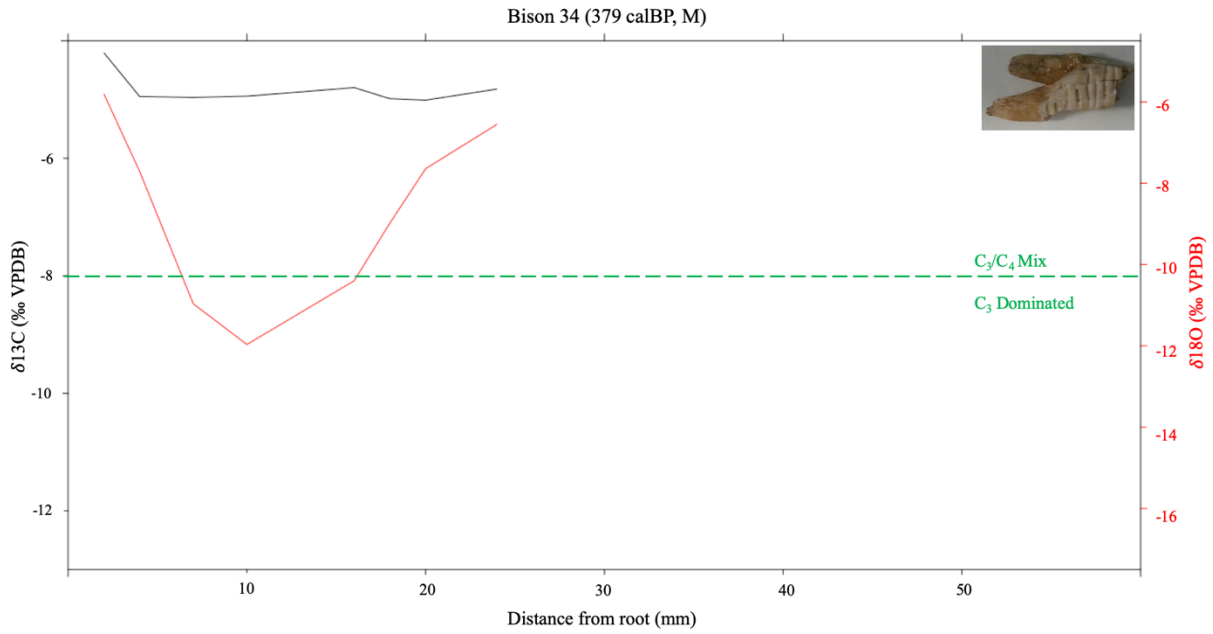


q.

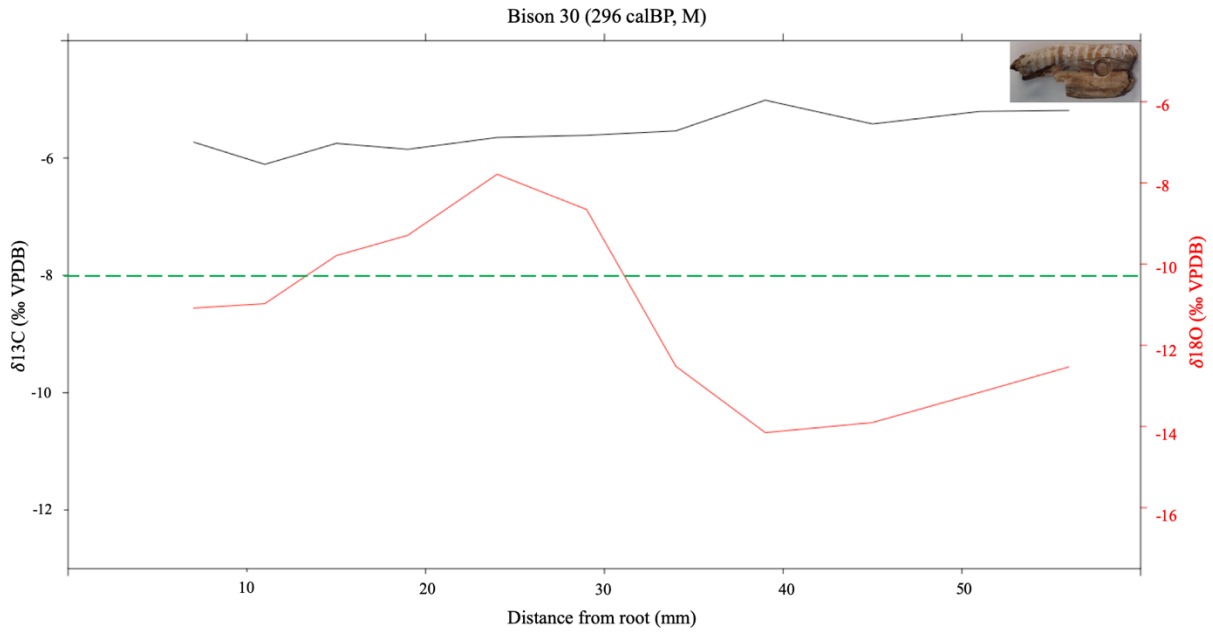


r.

Figure 7 continued.



s.



t.

Figure 7 continued.

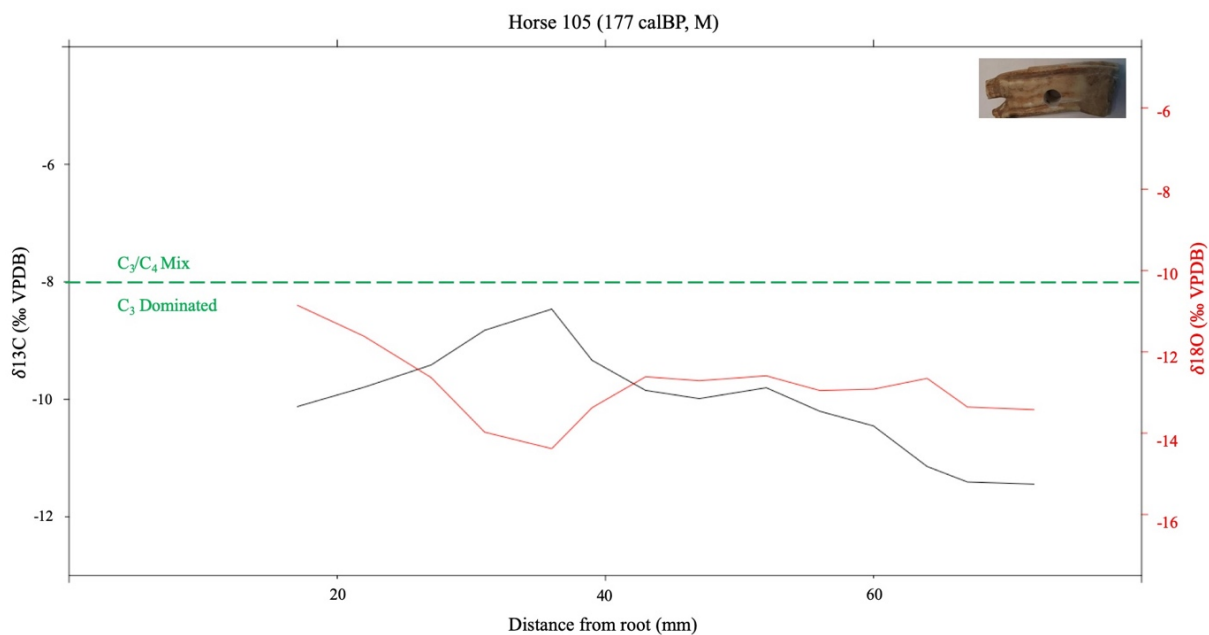


Figure 8. Serial sampled isotopic data for the Late Holocene horse tooth molar (M). Red lines depict oxygen values while black lines indicate carbon values. The cutoff for C₃ dominated diet (-8 and below) and a mix of C₃/C₄ (-8 and above) is depicted with a green dashed line.

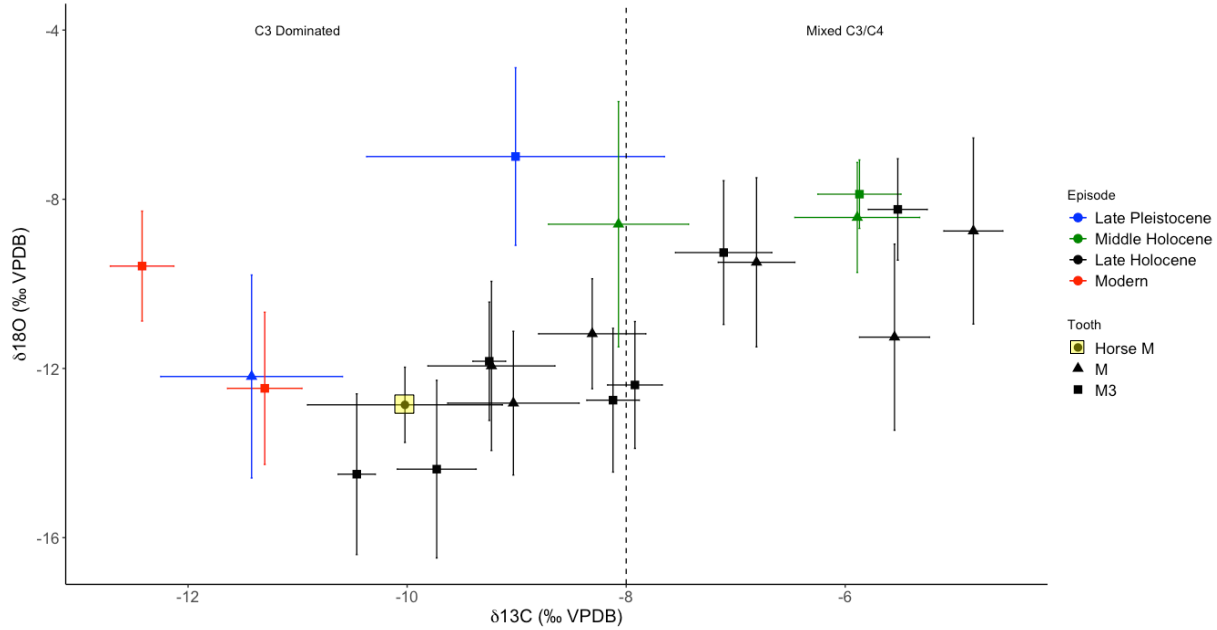


Figure 9. Means and standard deviations for each bison tooth sampled with carbon on the x-axis and oxygen on the y-axis. Colors indicate temporal period and shapes represent the type of tooth, circles for M (unspecified molars M_1 or M_2) and triangles for third molars (M_3). An additional shape symbol (circle with a yellow square to highlight) is added to distinguish the horse tooth.

Table 5. Serial data isotopic (VPDB) values for M (unspecified molars, M_1 or M_2) and M_3 teeth, the calBP age, distance from the root (mm), and temporal episode associated with the sample. LP (Late Pleistocene), MH (Middle Holocene), LH (Late Holocene), and MOD (modern).

ID	Tooth	Distance (mm)	$\delta^{13}C$	$\delta^{18}O$	calBP Age	Episode
62	M3	4	-6.84	-10.12	12431	LP
		8	-7.96	-8.56		
		12	-9.06	-7.60		
		14	-10.09	-5.60		
		18	-10.16	-5.01		
		23	-10.00	-5.03		
67	M3	4	-5.74	-7.96	8107	MH
		7	-5.89	-7.02		
		10	-6.77	-7.00		
		14	-5.87	-6.50		
		18	-5.42	-7.87		
		22	-5.77	-7.85		
		25	-5.84	-7.56		
		28	-6.16	-8.79		
		31	-6.04	-8.54		
		34	-5.69	-8.90		
		37	-5.32	-8.71		

Table 5 continued.

ID	Tooth	Distance (mm)	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	calBP Age	Episode
43	M3	3	-5.43	-9.94	1077	LH
		6	-5.64	-8.90		
		10	-5.51	-7.15		
		12	-5.94	-7.00		
		16	-5.69	-7.03		
		20	-5.39	-8.17		
		24	-5.09	-9.51		
13	M3	5	-7.34	-10.10	504	LH
		9	-7.58	-10.10		
		12	-7.73	-10.53		
		16	-7.79	-11.07		
		19	-7.87	-11.72		
		22	-7.91	-12.65		
		25	-8.05	-13.40		
		29	-8.08	-13.81		
		32	-8.19	-14.15		
		35	-7.99	-13.65		
		38	-8.09	-13.59		
		42	-8.10	-13.41		
		44	-8.15	-12.87		
32	M3	8	-6.55	-9.91	437	LH
		13	-6.28	-7.15		
		18	-6.68	-6.75		
		24	-6.92	-8.78		
		30	-7.10	-10.77		
		35	-7.47	-11.44		
		41	-7.46	-11.71		
		47	-7.33	-10.28		
		51	-7.61	-9.45		
		56	-7.45	-8.45		
		60	-7.37	-7.20		
42	M3	4	-9.35	-13.87	386	LH
		9	-9.35	-13.36		
		13	-9.31	-11.48		
		16	-9.77	-12.05		
		20	-9.48	-13.19		
		24	-9.93	-14.96		
		30	-10.06	-17.36		
		36	-10.21	-16.97		
		40	-10.12	-16.20		

Table 5 continued.

ID	Tooth	Distance (mm)	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	calBP Age	Episode
11	M3	4	-8.12	-13.86	381	LH
		9	-8.21	-12.78		
		12	-7.83	-11.72		
		16	-7.69	-10.29		
		20	-8.00	-10.35		
		23	-8.23	-11.91		
		26	-8.24	-12.81		
		29	-8.09	-13.97		
		33	-8.35	-14.88		
		37	-8.48	-14.94		
41	M3	4	-8.89	-10.52	380	LH
		9	-9.10	-9.79		
		14	-9.37	-9.98		
		20	-9.35	-11.20		
		24	-9.22	-12.08		
		29	-9.34	-13.95		
		33	-9.26	-13.41		
		37	-9.39	-13.36		
		42	-9.27	-12.77		
		46	-9.22	-12.14		
		49	-9.43	-10.89		
26	M3	2	-10.42	-15.47	170	LH
		5	-10.61	-13.86		
		11	-10.61	-11.45		
		16	-10.71	-11.88		
		19	-10.49	-13.50		
		24	-10.34	-15.15		
		27	-10.40	-16.71		
		30	-10.16	-16.97		
		34	-10.38	-16.16		
		38	-10.31	-15.17		
		42	-10.69	-13.23		
59	M	5	-10.09	-8.45	12229	LP
		10	-10.17	-9.72		
		15	-11.43	-13.88		
		19	-11.31	-14.62		
		24	-11.61	-14.77		
		29	-11.54	-14.17		
		34	-11.84	-13.09		
		38	-12.44	-11.30		
		45	-12.38	-9.77		

Table 5 continued.

ID	Tooth	Distance (mm)	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	calBP Age	Episode
68	M	5	-6.74	-8.88	8116	MH
		10	-6.57	-9.40		
		16	-5.65	-10.49		
		21	-5.54	-9.24		
		25	-5.61	-8.04		
		29	-5.42	-7.61		
		32	-5.24	-6.64		
		34	-6.36	-7.11		
48	M	3	-6.68	-8.67	7936	MH
		6	-8.11	-4.71		
		10	-8.39	-5.62		
		13	-8.50	-7.35		
		17	-8.54	-9.90		
		20	-8.18	-11.00		
		24	-8.09	-12.87		
49	M	6	-7.48	-12.66	2728	LH
		11	-8.12	-11.47		
		16	-7.64	-10.30		
		21	-8.13	-9.23		
		26	-8.68	-9.88		
		31	-8.72	-10.15		
		36	-8.60	-11.65		
		41	-8.66	-12.53		
47	-8.77	-12.76				
35	M	9	-6.49	-9.46	1027	LH
		15	-6.90	-8.38		
		20	-7.05	-7.36		
		25	-7.16	-6.97		
		30	-7.16	-7.43		
		35	-7.26	-8.07		
		41	-6.92	-9.68		
		45	-6.78	-11.02		
		50	-6.54	-11.86		
		54	-6.49	-12.61		
59	-6.18	-11.57				

Table 5 continued.

ID	Tooth	Distance (mm)	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	calBP Age	Episode
39	M	3	-9.08	-13.62	656	LH
		6	-9.33	-13.50		
		9	-9.00	-13.33		
		12	-9.01	-14.12		
		16	-8.71	-13.00		
		20	-8.62	-12.94		
		24	-8.70	-12.48		
		28	-9.02	-12.56		
		32	-8.99	-11.15		
		38	-10.04	-9.88		
		41	-9.77	-9.00		
45	-10.51	-7.73				
47	M	5	-9.84	-15.31	653	LH
		9	-9.50	-13.69		
		13	-8.77	-12.25		
		17	-8.62	-11.90		
		20	-8.45	-10.97		
34	M	2	-4.20	-5.80	379	LH
		4	-4.95	-7.71		
		7	-4.96	-10.96		
		10	-4.94	-11.97		
		16	-4.80	-10.40		
		18	-4.98	-8.98		
		20	-5.01	-7.64		
24	-4.82	-6.55				
30	M	7	-5.73	-11.08	296	LH
		11	-6.11	-10.98		
		15	-5.75	-9.79		
		19	-5.85	-9.29		
		24	-5.65	-7.79		
		29	-5.61	-8.65		
		34	-5.54	-12.52		
		39	-5.01	-14.15		
		45	-5.42	-13.90		
		51	-5.21	-13.16		
56	-5.19	-12.53				

Table 5 continued.

ID	Tooth	Distance (mm)	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	calBP Age	Episode
100 (TRNP Bull)	M3	2	-10.60	-8.79	na	MOD
		5	-10.32	-8.83		
		9	-10.86	-7.84		
		15	-11.10	-7.87		
		21	-11.00	-9.49		
		24	-11.12	-10.12		
		30	-10.97	-11.36		
		35	-11.16	-11.50		
		41	-11.21	-10.57		
		47	-11.17	-10.02		
53	-11.26	-8.95				
103 (TRNP Cow)	M3	3	-9.75	-15.26	na	MOD
		6	-9.76	-14.58		
		8	-9.78	-13.37		
		10	-9.88	-13.38		
		12	-10.12	-12.32		
		14	-10.11	-11.62		
		16	-10.31	-10.93		
		19	-10.60	-10.44		
		21	-10.60	-10.37		

Table 6. Serial data values for the Late Holocene (LH) horse M (M_1 or M_2) sample.

ID	Tooth	Distance (mm)	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	calBP Age	Episode
105 (Horse)	M	17	-10.13	-10.85	177	LH
		22	-9.79	-11.62		
		27	-9.42	-12.63		
		31	-8.82	-13.97		
		36	-8.46	-14.38		
		39	-9.34	-13.37		
		43	-9.85	-12.61		
		47	-9.99	-12.71		
		52	-9.80	-12.59		
		56	-10.20	-12.95		
		60	-10.45	-12.92		
		64	-11.14	-12.65		
		67	-11.41	-13.36		
		72	-11.44	-13.42		

Table 7. Summary statistics of carbon and oxygen stable isotopes for enamel sampled from twenty bison and one horse, the archaeological site which they are derived from, and calBP date.

ID	Location	calBP Date Range		calBP median	Temporal Episode	Tooth	n	Distance (mm)	$\delta^{13}\text{C}$ (VPDB)				$\delta^{13}\text{C}$ Amplitude	$\delta^{18}\text{O}$ (VPDB)				$\delta^{18}\text{O}$ Amplitude
		From	To						Mean	Min	Max	SD		Mean	Min	Max	SD	
62	Beacon Island	12547	12140	12431	LP	M3	6	23	-9.01	-10.2	-6.8	1.36	3.4	-6.99	-10.12	-5.01	2.1	5.11
67	Rustad	8178	8036	8107	MH	M3	11	37	-5.87	-6.8	-5.3	0.38	1.5	-7.88	-8.90	-6.50	0.81	2.40
43	Menoken Village	1175	989	1077	LH	M3	7	24	-5.52	-5.9	-5.1	0.27	0.8	-8.24	-9.94	-7.00	1.2	2.94
13	Huff Village	518	487	504	LH	M3	13	44	-7.92	-8.2	-7.3	0.25	0.9	-12.39	-14.16	-10.10	1.5	4.06
32	Larson Village	491	320	437	LH	M3	11	60	-7.11	-7.6	-6.3	0.44	1.3	-9.26	-11.71	-6.75	1.7	4.96
42	White Bison Robe	458	307	386	LH	M3	9	40	-9.73	-10.2	-9.3	0.36	0.9	-14.38	-17.36	-11.48	2.1	5.88
11	White Bison Robe	460	313	381	LH	M3	10	37	-8.12	-8.48	-7.7	0.24	0.78	-12.75	-14.93	-10.30	1.7	4.63
41	White Bison Robe	465	315	380	LH	M3	11	49	-9.25	-9.4	-8.9	0.15	0.5	-11.83	-13.96	-9.79	1.4	4.17
26	Big Hidatsa	290	14	170	LH	M3	11	42	-10.46	-10.71	-10.2	0.17	0.51	-14.50	-16.97	-11.46	1.9	5.51
100	TRNP Bull	na	na	na	MOD	M3	11	53	-12.42	-12.75	-11.8	0.29	0.95	-9.58	-11.50	-7.84	1.3	3.66
103	TRNP Cow	na	na	na	MOD	M3	9	21	-11.3	-12.1	-11.2	0.34	0.9	-12.47	-15.26	-10.37	1.8	4.89
59	Beacon Island	12396	12056	12229	LP	M	9	45	-11.42	-12.4	-10.1	0.83	2.3	-12.19	-14.77	-8.45	2.4	6.32
68	Rustad	8187	8042	8116	MH	M	8	34	-5.89	-6.7	-5.2	0.57	1.5	-8.43	-10.93	-6.64	1.3	4.29
48	Rustad	7972	7860	7936	MH	M	7	24	-8.07	-8.5	-6.7	0.64	1.8	-8.59	-12.87	-4.71	2.9	8.16
49	Alkali Creek	2750	2539	2728	LH	M	9	47	-8.31	-8.8	-7.5	0.49	1.3	-11.18	-12.76	-9.23	1.3	3.53
35	Bundlemaker	1173	979	1027	LH	M	11	59	-6.81	-7.3	-6.2	0.35	1.1	-9.49	-12.61	-6.97	2	5.64
39	Falkirk Bison Kill	673	566	656	LH	M	12	45	-9.23	-10.5	-8.6	0.58	1.9	-11.94	-14.12	-7.73	2	6.39
47	Falkirk Bison Kill	670	565	653	LH	M	5	20	-9.03	-9.8	-8.5	0.6	1.3	-12.82	-15.31	-10.97	1.7	4.34
34	Double Ditch Village	465	315	379	LH	M	8	24	-4.83	-5	-4.2	0.27	0.8	-8.75	-11.97	-5.80	2.2	6.17
30	Falkirk Bison Kill	309	156	296	LH	M	11	56	-5.55	-6.2	-5	0.32	1.2	-11.26	-14.15	-7.79	2.2	6.36
105 (horse)	Medora	289	20	177	LH	M	14	72	-10.02	-11.44	-8.5	0.89	2.94	-12.86	-14.38	-10.85	0.89	3.53

Table 8. Analysis of variance (ANOVA) p-value results for differences between bison carbon and oxygen stable isotopes by temporal groups.

	Isotope	Late Pleistocene	Middle Holocene	Late Holocene
Middle Holocene	$\delta^{13}\text{C}$	< 0.001		
	$\delta^{18}\text{O}$	0.1		
Late Holocene	$\delta^{13}\text{C}$	< 0.001	< 0.001	
	$\delta^{18}\text{O}$	0.17	< 0.001	
Modern	$\delta^{13}\text{C}$	0.99	< 0.001	< 0.001
	$\delta^{18}\text{O}$	0.8	0.003	0.72

CHAPTER IV

MICROFOSSILS IN BISON DENTAL CALCULUS

ABSTRACT

The contents of bison dental calculus can be used to infer paleoecology of ancient bison along with isotopic values. Dental calculus accumulates on the surface of animal teeth and remains well-preserved in a mineralized matrix. Microfossils will become assimilated into the calculus during mastication and can later be extracted for microscopy viewing. The calculus from fifty-four ancient, and three modern bison teeth was extracted, demineralized, and viewed under light microscopy and scanning electron microscopy. Microfossils such as pollen, animal hairs, diatoms, and phytoliths were found. While the utility of this method is limited in making correlations with stable isotopic data due to the difficulty in identifying the objects found, it does give qualitative information about the bison's environment and could lead to new research questions based on the contents found.

INTRODUCTION

Several materials in a bison tooth can be used for isotopic analysis to provide valuable information about ancient bison diet and environment. However, there is another medium present on the surface of bison teeth that allows us to gain additional insight into the time in which the bison lived. Dental calculus (plaque) is a mineralized substance that accumulates on the surface of teeth and remains well-preserved in archeological specimens (Hillson 2005). During

mastication, components from the bison's diet and environment becomes incorporated into the calculus matrix and can later be extracted for microscopic viewing (Middleton and Rovner 1994). In order for the mineralization process to occur, saliva must be present. This is a good indication that the material included in calculus originates when the animal is alive and does not accumulate post-mortem (Middleton and Rovner 1994).

The composition of calculus can vary by species (Middleton and Rovner 1994) as well as the chemical and microbiome makeup of individuals (Power et al. 2014). There is also uncertainty about what materials can be recovered in the extraction process (Weber and Price 2016). In general, pollen grains, diatoms, and starches are well-preserved for viewing (Middleton and Rovner 1994, Cannon 1997, Plumb et al. 2009) and may help parse out the data generated from isotopic analysis. Phytoliths are plant silica bodies that form when monosilicic acid is absorbed by plants through the soil and are also typically abundant in archeological samples.

This method has been predominately used in the study of recreating ancient hominid diet to gain insight into farming and cooking practices (i.e. cooked starch versus raw starch grains) (Henry and Piperno 2008, Piperno and Dillehay 2008, Li et al. 2010, Leonard et al. 2015). In a limited number of studies, the diet of extinct herbivores has been observed to better understand the ecological changes that may have led up to the Pleistocene-Holocene megafaunal extinction event (Boyd 2003, Asevedo et al. 2012). Mastodon (*Mammuth americanum*) specimens from Kansas during the Late Pleistocene revealed a suspicious lack of browse material (trees, shrubs, and other woody vegetation recovered from the calculus matrix (Gobetz et al. 2001). Whether the phytoliths from woody vegetation were inherently less preserved in this context or if mastodons turned to a heavier dependence on grasses when environmental conditions were rapidly changing is unclear (Gobetz et al. 2001). A study on the extinct Pleistocene gomphothere

(*Notiomastodon platensis*) in Brazil found sufficient evidence of browse material incorporated into their diet (Asevedo et al. 2012). Whether these differences are due to the ecology of the herbivores from different regions or if browse material can be less abundant in the archeological record isn't fully understood and supports the notion that more dental calculus data needs to be collected to answer paleoecological questions.

Previous work on one bison from Fawn Creek, Idaho observed microfossils in bison dental calculus and the contents of the infundibulum of bison molars (Cannon 1997). Grass phytoliths found could be categorized as short cell or smooth elongate. Short cell grass phytoliths come in various shapes but are usually symmetrical. Elongate grass phytoliths are plant epidermal cells that are long and slender with tapering edges (Twiss 1987). Short cell phytoliths are found in Festucoid, Chloridoid, and Panicoid tribes of grasses but smooth elongate grass phytoliths are produced by a wide variety of grasses and do not aid in resolving grass composition (Cannon 1997). Pollen from pine and Douglas fir, diatoms (single celled, silica bodied algae), starch, and plant fibers were also extracted from the three bison molars.

In this chapter, the contents of ancient bison dental calculus will be extracted and viewed under light microscopy (LM) and scanning electron microscopy (SEM) to attempt to corroborate information gained from stable isotope analysis and supplement the data with qualitative information from the paleodiet and paleoenvironment of Northern Great Plains bison. The frequency of browse material will be evaluated as well as indications of environmental conditions based on the contents found. It is expected that bison dental calculus will contain pollen from grasses and evergreens, as well as phytoliths from various plants and grasses. Other environmental cues may also be identified. For comparisons to modern counterparts, the calculus from TRNP bison teeth will also be demineralized and viewed.

Two different extraction solutions will be used to demineralize the calculus. All studies mentioned above use hydrochloric acid (HCl) during the demineralization process as outlined by the foundational research of Middleton and Rovner (1994). Tromp et al. (2017) evaluated the utility of ethylenediaminetetraacetic acid (EDTA) to extract microfossils from calculus and suggest it may be a more effective solution to preserve the historical contents. As an added convenience, EDTA is in the protocol for both microscopic observation and DNA extraction, so it can allow for more material to be used for both types of analysis. The extraction of DNA from dental calculus has proven to be a useful medium for collecting environmental DNA (eDNA) and evidence of microbial communities within the oral microbiome (Warinner et al. 2015).

METHODS

Microfossil Extraction

Dental calculus was scraped off of bison teeth using dental picks, a chisel, and various instruments from an archeological tool set. Care was taken to remove large pieces of calculus to minimize surface area exposed to modern elements. If excessive dirt was present on the surface of the tooth and calculus, it was scraped off with a scalpel. The pieces of calculus were weighed and placed in low static plastic 2 mL flip top centrifuge tubes. Larger samples were placed in plastic tubes and split between microscopy and environmental DNA analysis. The microscopy samples were then transferred to the low static 2 mL centrifuge tubes with some designated for light microscopy and some designated for scanning electron microscopy. An initial rinse of approximately 1 mL (or enough to cover the top of the sample) 10% hydrochloric acid (HCl) was used to clean off the exterior of the calculus. This rinse was left on for one minute then pulled off with a pipette. The sample was then rinsed with ~1 mL of deionized (DI) water, agitated, then

the DI water was pipetted off. For samples with a lot of dirt present, the calculus was transferred to a new centrifuge tube and rinsed again with DI water. Once the samples were sufficiently clean on the outer layer, ~1 mL (or enough to cover the top of the sample) of 10% hydrochloric acid HCl was added to the centrifuge tube and allowed to sit until the sample had dissolved. The demineralization process was encouraged by gently probing the samples with a pick periodically. Most samples dissolved within 24 hours but some took up to 72 hours to demineralize. Samples were checked as often as possible to ensure they did not remain in the HCl for long after demineralization. Once demineralized, samples were centrifuged at 13,000 rpm for 7 minutes and the HCl was pipetted off carefully to minimize sample loss. Approximately a 1 mL rinse of DI water was added and the sample was centrifuged again, the water pipetted off and this process was repeated for a second rinse. A small amount of DI water was then added to the light microscopy samples (enough to pipette them onto a glass slide). This procedure was also reproduced with some samples using ethylenediaminetetraacetic acid (EDTA) to compare the outcomes of microfossil viewing with HCl and EDTA extractions. EDTA required more time for demineralization and samples were placed on a shaker on low speed during the demineralization process, but otherwise the extraction procedure was the same.

Microscopy

Light microscopy samples were viewed using a Leica DM750 microscope and a Leica ICC50 camera. Images were captured using Leica Acquire software version 3.4.1. Some light microscopy samples were taken to the Human and Imaging Core Facility of the School of Medicine and Health Sciences at the University of North Dakota (UND) and viewed using a Zeiss LSM Meta Confocal microscope and ZEN software. Samples were viewed under 10x, 20x,

and 40x objectives. Samples for scanning electron microscopy were left to dry slightly and then mounted on aluminum stubs with double sided carbon tape in a positive pressure hood. After properly set, the SEM samples were transferred to the Imaging Core Facility at UND where they were left in a desiccator for at least 24 hours prior to being sputter coated with gold for 20 – 30 seconds (depending on the thickness of the sample, thinner samples were coated for less time). SEM samples were viewed between 5 – 7 kv and 300x to 2000x on a Hitachi 4700 Field Emission SEM.

The risk of incorporation of modern material is high for this extraction process, so precautions were taken to minimize the chance of contamination. Extraction took place in a positive pressure hood and all tools were sterilized after each step. A running tally of microfossils was kept as samples were viewed. Categories for fibrous material (root like structures), elongate grass phytoliths, short grass phytoliths, pollen grains, hair, diatoms, tracheid/vessel elements (water conducting cells in vascular plants), starches, browse, and unidentified were made to organize the observations.

Identification of Microfossils

After microfossils were put into a category, various methods were employed to try to identify the object. Grass phytoliths were characterized using conventions from Twiss et al. (1969). Online databases were also used for identifying plant material. Phytoliths from woody vegetation were recognized using the database from “woodanatomy.ch”. Pollen was narrowed down to the Poaceae family and *Betula* genus using “paldat.org” and “microlabgallery.com”. Other plant components were discerned by searching for microscopic images of various plant parts or images of plants known to be present in the study area to make a visual match.

Chrysophycean cysts were discovered by using a reverse Google image search which led to identified images by Firsova et al. (2011). Other microfossils were identified by reaching out to people knowledgeable in in the appropriate areas. The mite was classified to suborder *Trigynaspida* by fellow graduate student and parasitologist Staci Dreyer. The diatoms were described through an email communication with diatom expert, Dr. Jeffrey Stone, Indiana State University.

RESULTS

Calculus from fifty-four ancient bison teeth and three modern bison teeth was extracted and demineralized (Table 9). Ancient teeth were derived from 14 archeological sites with calBP dates in the Late Holocene (166 – 2728 calBP), except for one tooth from Rustad which is aged in the Middle Holocene (8107 calBP) (Figure 1, Table 9). Twenty-eight samples were split between SEM and light microscopy and 26 samples had the EDTA method applied. There was no observed difference in the preservation of microfossils between the extraction solutions apart from the extraction process taking considerably longer with EDTA. Microfossils found were split into the categories: fibrous root-like material (n=163), elongate grass (n=275), phytolith (n=387), pollen grains (n=268), hair (n=10), diatoms (n=2), tracheids (n=16), starch (n=25), browse (n=8), cysts (n=13), and unidentified (n=4). A total of 1,170 microfossils were observed (Figures 10 – 17, Tables 9, 10).

Taxonomic identifications in ancient material included *Navicula* diatoms, algae from the group *Chrysophyceae* (Figure 10), and pollen from the Poaceae family (Figure 11a; 11d, 11g, 11h). Identifications in modern dental calculus included pollen from the *Betula* (birch) genus (Figure 17e) and a mite which belongs to the diverse suborder of Trigynaspida (Figure 17d).

DISCUSSION

While numerous microfossils were present in the bison's calculus, many were unable to be identified. Fibrous materials likely came from the roots of various plants and elongate grass phytoliths led to no identification due to their presence in many different types of grasses (Cannon 1997). Other phytoliths came from grasses but the only specimens able to be identified with some confidence are from the C₄ subfamilies of Chloridoideae (Figure 13a) and Panicoideae (Figure 17c, in modern bison) grasses (Twiss 1987, Ramsey et al. 2016). Tracheids, elongated cells which transport water in the xylem of vascular cells, were also found in abundance (Figure 14). Tracheids are present in most flowering plants but are not usually seen in grasses.

Several hairs were extracted from the bison calculus (Figure 15). While Figure 15a could potentially be a bison hair, the other three hairs remain unidentified. Figure 15c and 15d have a unicellular medulla comparable to species like cats, mice, and foxes (Hausman 1920). These hairs could have been present in the bison's grazing area and assimilated into the calculus matrix during chewing.

Because bison are one of the few surviving megafauna of the Pleistocene-Holocene transition, paleoecological studies seek to identify the reasons for bison's success. One explanation states that bison were able to switch between grazing and browsing foraging behavior as climatic shifts changed the composition of plant communities. Bison during the Late Pleistocene from different geographical areas within the United States have been shown to incorporate more browse into diet than bison from other temporal periods (Rivals et al. 2007, Coltrain et al. 2004). European bison are thought to have evolved on the grasslands as grazers and then shifted to forested areas and a browsing diet as a result of environmental pressure

(Bocherens et al. 2015). Therefore, a potential utility of observing plant material in ancient bison dental calculus is to determine if there were shifts between grazing and browsing behavior throughout their ecological history.

Browse material was present in bison dental calculus from the present studies' sample assemblage but at a low rate compared to modern bison diet studies, corroborating the idea that browse may be less well-preserved in some contexts (Gobet et al. 2001). Ten percent of bison sampled contained some browse material, not including tracheids, which could also be derived from woody vegetation. A few tree-like (dendriform) phytoliths (Figure 12) were uncovered as well as a pollen grain from the genus of *Pinus* (pine trees) (Figure 11b) and a stemwood phytolith from an evergreen (Figure 12c). Figure 12d was derived from a bison aged 111 calBP in the Sakakawea archeological site while the other two dendriform phytoliths (Figure 12a, 12b) were found in Bison 70 from Alkali Creek. Although Bison 70 was not carbon dated, other bison from Alkali Creek have a calBP date range of 2728 – 1466 (Figure 2, Table 1).

Herbaceous material was recorded more frequently in bison dental calculus samples. Grass pollen was particularly prevalent with 24 bison (44%) having at least one pollen grain (Table 9). Most pollen grains were identified broadly from the Poaceae family based on their dimpled, round appearance and frequent occurrence with air sacs and tails. These components were more visible using light microscopy (Figure 11), however, some pollen air sacs were visible in SEM images (Figure 11e, 11f).

Two forms of algae were observed in the calculus samples, chrysophycean cysts (golden algae) and diatoms. Golden algae are mainly found in freshwater (Tromp 2011) and their abundance in bison dental calculus samples (Figure 10a; 10f) only indicates the availability of water in the bison's habitat. The two diatoms are both identified as biraphid (having two grooves

on the diatom's cell wall) but only one could be narrowed down to the *Navicula* genus (pictured on the right in Figure 10g). Diatoms from the *Navicula* genus are often motile and can be found in freshwater ponds and rivers in North America at various depths (Fritz et al. 1993). The diatoms would have been incorporated into the bison's calculus through drinking.

The modern bison provided a proxy to determine how well ancient material has been preserved within the calculus matrix. Unsurprisingly, the contents of modern bison dental calculus were generally more well preserved than the ancient bison but were not vastly different. Phytoliths in particular were as well preserved in modern bison as they were in the ancient material. Modern bison calculus components consisted of a single pollen granule that belongs to the *Betula* genus (Figure 17e), a member of the subfamily Panicoid grass phytolith (Figure 17c), a vessel element (Figure 17a), a tracheid (Figure 17b), and a Trigynaspida mite (Figure 17d). This mite type is found in soils and under the bark of trees and decaying logs and isn't known to parasitize on mammals, therefore it was likely picked up while grazing or browsing.

Some additional structures are recorded which could not be identified (Figure 16). Figures 16c and 16e resemble seed granules but this could not be confirmed. As more data is collected from the calculus of herbivores, I expect that these pictures will become more useful for future studies.

All of the ancient calculus samples occurred during the Late Holocene except for one bison dated from the Middle Holocene (Bison 67, 8107 calBP). However, the only material found in the Middle Holocene sample was fibrous and root-like, suggesting that the contents of the calculus were less well preserved than Late Holocene bison.

Although it is very difficult to identify microfossils found in bison dental calculus to the species level, information gathered here provides qualitative data to supplement isotopic data.

Researchers are learning more about the diets of the extinct mastodons through the medium of dental calculus and may uncover more localized answers for the cause of their demise. Unlike the mastodon, bison succeeded in surviving the extreme environmental conditions during the terminal Pleistocene. Today, bison face a whole new set of challenges in restricted rangelands and isolated populations. Therefore, employing various methods to assess how bison have adjusted to stark changes in the past will help us to learn what management strategies may be most beneficial in the future.

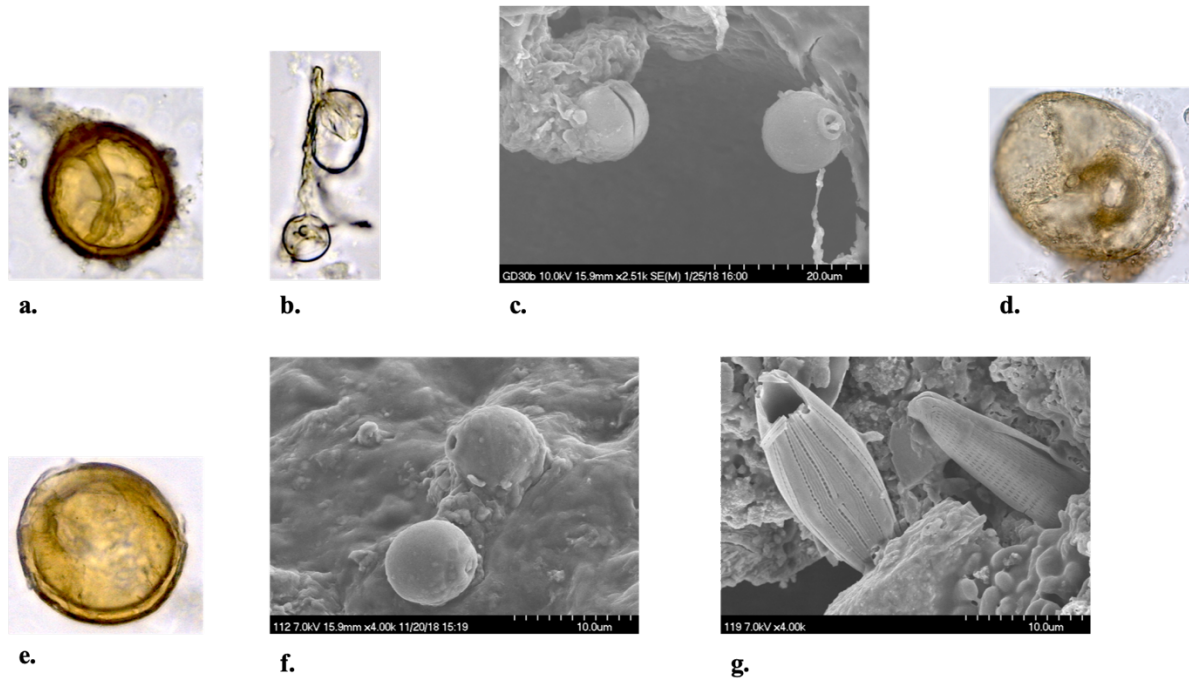


Figure 10. Chrysophycean cysts and diatoms found in bison dental calculus. **a.** cyst, Bison 92, 765 calBP, Menoken Village, **b.** cyst, Bison 95, date na, Menoken Village, **c.** cyst, Bison 30 (SEM), 296 calBP, Falkirk Bison Kill, **d.** cyst, Bison 26, 170 calBP, Big Hidatsa, **e.** cyst, Bison 124, date na, Lower Hidatsa, **f.** cyst, Bison 112 (SEM), date na, Taylor Bluff, **g.** biraphid diatoms, Bison 119, date na, Sakakawea.

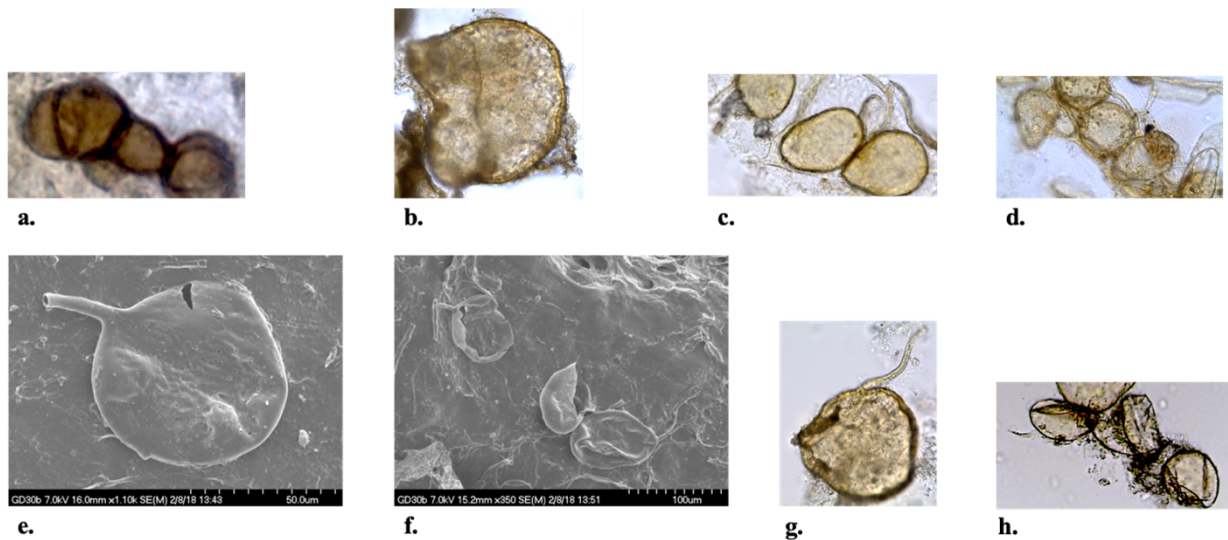


Figure 11. Pollen air sacs, pollen grains, and spores found in bison dental calculus. **a.** pollen or spores, Bison 43, 1077 calBP, Menoken Village **b.** *Pinus* pollen, Bison 74, 1105 calBP, White Bison Robe, **c.** grass pollen, Bison 35, 1027 calBP, Bundlemaker, **d.** grass pollen (LM), **e.** pollen air sac (SEM), and **f.** pollen air sacs (LM), Bison 30, 296 calBP, Falkirk Bison Kill, **g.** grass pollen, Bison 84, date na, Larson Village, **h.** grass pollen, Bison 46, 185 calBP, Forest River.

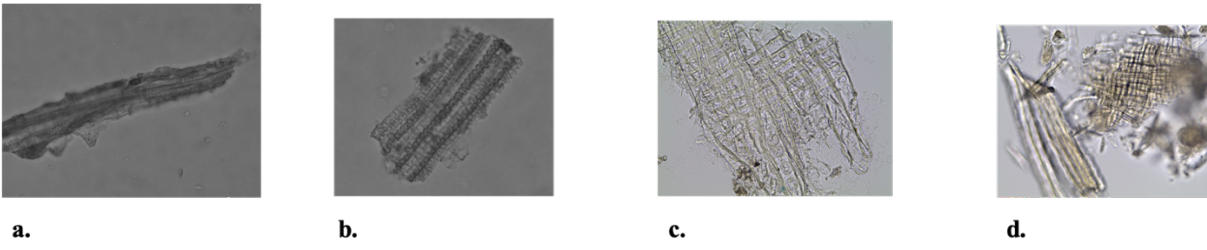


Figure 12. Dendriform (tree-like) phytoliths found in bison dental calculus. **a.** and **b.** dendriform phytoliths, Bison 70, date na, Alkali Creek, **c.** stemwood phytolith, Bison 32, 437 calBP, Larson Village, **d.** dendriform phytolith, Bison 121, 111 calBP, Sakakawea.

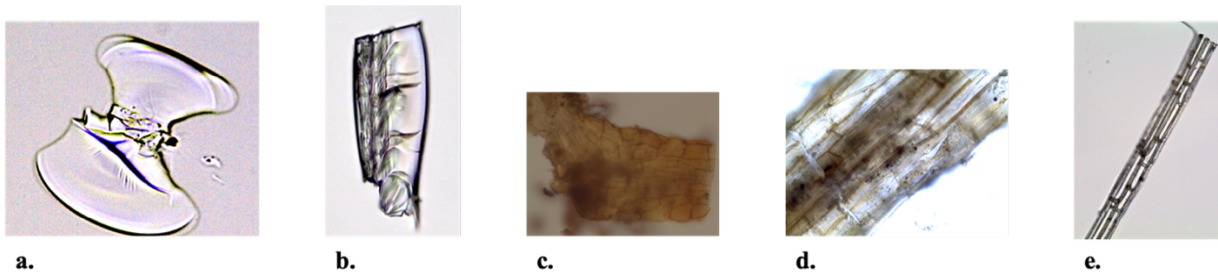


Figure 13. Grass and plant phytoliths found in bison dental calculus. **a.** Chloridoid phytolith, Bison 43, 1077 calBP, Menoken Village, **b.** short grass phytolith, Bison 13, 504 calBP, Huff Village, **c.** plant phytolith cells and **d.** vascular phytolith, Bison 79, date na, Larson Village, **e.** grass phytolith, Bison 112, date na, Taylor Bluff.

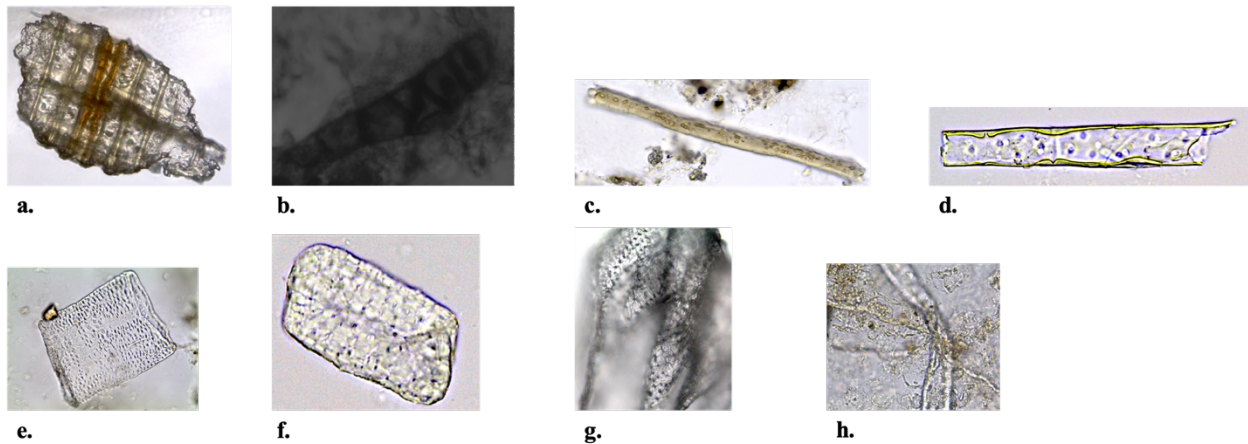


Figure 14. Water conducting plant cells (tracheids and vessel elements) found in bison dental calculus. **a.** tracheid cells, Bison 43, 1077 calBP, Menoken Village, **b.** tracheid cells, Bison 71, date na, White Bison Robe, **c.** tracheid, Bison 13, 504 calBP, Huff Village, **d.** tracheid and **e.** vessel element, Bison 32, 437 calBP, Larson Village, **f.** Bison 84, date na, Larson Village, **g.** vessel element, Bison 26, 170 calBP, Big Hidatsa, **h.** tracheid, Bison 43, 1077 calBP, Menoken Village.

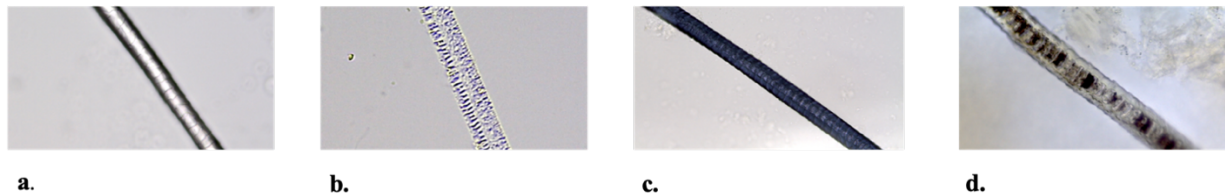


Figure 15. Hairs found in bison dental calculus. **a.** Bison 34, 379 calBP, Double Ditch Village **b.** Bison 26, 170 calBP, Big Hidatsa, **c.** Bison 43, 1077 calBP, Menoken Village, **d.** Bison 47, 653 calBP, Falkirk Bison Kill.

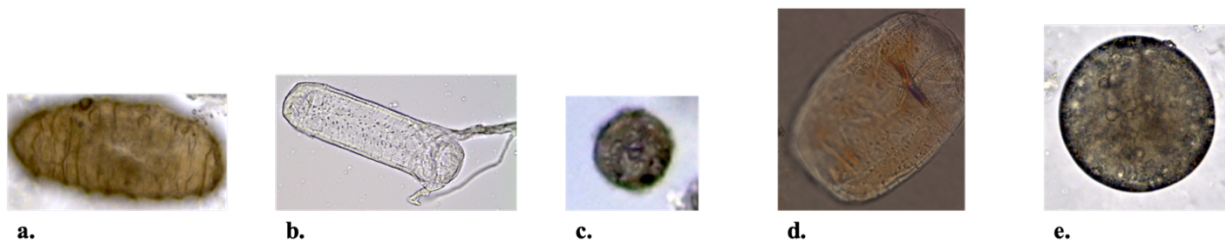
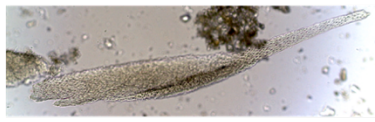
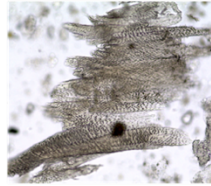


Figure 16. Unidentified microfossils found in bison dental calculus. **a.** oblong shape granule, Bison 92, 765 calBP, Menoken Village, **b.** rod shaped vessel and **c.** round seed-like granule, Bison 84, date na, Larson Village, **d.** oval with red line, Bison 78, date na, Larson Village, **e.** round seed-like granule, Bison 26, 170 calBP, Big Hidatsa.



a.



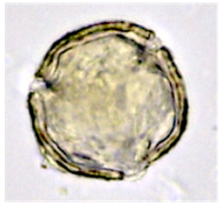
b.



c.



d.



e.

Figure 17. Microfossils found in modern bison dental calculus. **a.** vessel element and **b.** tracheid, Bison 103, TRNP, **c.** Panicoid grass phytolith and **d.** Trigynaspida mite, Bison 104, TRNP, **e.** *Betula* pollen, Bison 104, TRNP.

Table 9. Tally of microfossils observation in bison dental calculus, the age of the sample (if available) location, type of microscopy, type of solution used for extraction, and sample weight (g).

Sample	Age calBP	Episode	Location	Microscopy	Solution	Weight (g)	Fibrous	Elongate Grass Phytoliths	Short Phytoliths	Pollen	Hair	Diatom	Tracheid	Starch/Seed	Browse	Cysts	Unidentified/other	Description of Unidentified
11	381	LH	White Bison	LM	HCL	0.06	0	0	5	0	0	0	0	0	0	0	0	
13	504	LH	Huff Village	LM	HCL	0.27	12	9	16	0	0	0	0	0	0	0	0	
13	504	LH	Huff Village	SEM	HCL	0.25	0	0	0	0	0	0	0	0	0	0	0	
18	166	LH	Sakakwea	LM	HCL	0.44	1	0	9	0	0	0	0	0	0	0	1	oval
18	166	LH	Sakakwea	SEM	HCL	0.21	0	0	0	0	0	0	0	0	0	0	0	
26	170	LH	Big Hidatsa	LM	HCL	0.11	11	8	5	0	0	0	4	1	0	0	0	
26	170	LH	Big Hidatsa	SEM	HCL	0.1	0	1	0	0	0	0	0	0	0	7	0	
26	170	LH	Big Hidatsa	LM	EDTA	0.49	0	7	2	1	0	0	0	0	0	0	0	
26	170	LH	Big Hidatsa	SEM	EDTA	0.08	0	5	1	0	0	0	0	0	0	6	0	
30	296	LH	Falkirk	LM	HCL	1.02	9	18	0	0	0	0	0	0	0	0	0	
30	296	LH	Falkirk	SEM	HCL	0.07	0	0	3	1	0	0	0	0	0	0	0	
30	296	LH	Falkirk	LM	EDTA	0.31	0	0	7	18	0	0	0	0	0	0	1	
30	296	LH	Falkirk	SEM	EDTA	0.13	0	0	5	13	0	0	0	6	0	0	0	
32	437	LH	Larson	LM	HCL	0.48	4	11	52	1	0	0	3	0	1	0	0	
32	437	LH	Larson	SEM	HCL	0.14	0	0	2	1	0	0	3	0	0	0	0	
34	379	LH	Double Ditch	LM	HCL	0.03	0	0	5	0	1	0	0	0	0	0	0	
35	1027	LH	Bundlemaker	LM	HCL	0.37	13	3	3	30	2	0	1	0	0	0	0	
35	1027	LH	Bundlemaker	SEM	HCL	0.33	0	0	7	0	0	0	0	0	0	0	0	
38	167	LH	Taylor Bluff	LM	HCL	0.02	0	0	4	0	0	0	0	0	0	0	0	
41	379	LH	White Bison	LM	HCL	0.04	0	0	3	0	0	0	0	0	0	0	0	
42	386	LH	White Bison	LM	HCL	0.04	0	0	0	0	0	0	0	0	0	0	0	
43	1077	LH	Menoken	LM	HCL	0.87	20	16	31	0	2	0	1	4	1	0	0	
43	1077	LH	Menoken	SEM	HCL	0.13	0	0	3	0	0	0	0	0	0	0	0	
46	185	LH	Forest River	LM	HCL	0.01	0	0	3	5	0	0	0	11	0	0	0	
47	653	LH	Falkirk	LM	HCL	0.12	0	0	2	0	1	0	1	0	0	0	0	
49	2728	LH	Alkali Creek	LM	HCL	0.15	0	0	0	0	0	0	0	0	0	0	0	
52	1533	LH	Alkali Creek	LM	HCL	0.09	0	4	0	0	0	0	0	0	0	0	0	
52	1533	LH	Alkali Creek	LM	EDTA	0	0	3	0	0	0	0	0	0	0	0	0	
67	8107	MH	Rustad	LM	HCL	0.25	12	0	0	0	0	0	0	0	0	0	0	
69	na	LH	Alkali Creek	LM	HCL	0.04	0	6	0	0	0	0	0	0	0	0	0	
70	na	LH	Alkali Creek	LM	HCL	0.06	2	5	28	0	1	0	2	0	0	0	0	
71	na	LH	White Bison	LM	HCL	0.07	7	8	8	4	0	0	0	0	6	0	0	
72	na	LH	White Bison	LM	HCL	0.03	2	1	3	0	0	0	0	0	0	0	0	
73	na	LH	White Bison	LM	HCL	0.08	1	1	3	0	0	0	0	0	0	0	0	
74	1105	LH	White Bison	LM	HCL	0.08	4	11	6	1	0	0	0	0	0	0	0	
75	na	LH	White Bison	LM	HCL	0.02	5	5	16	0	2	0	0	0	0	0	1	round, yellow center
77	383	LH	Larson	LM	HCL	0.25	3	20	17	29	0	0	0	0	0	0	0	
78	na	LH	Larson	LM	HCL	0.15	10	13	37	0	0	0	0	0	0	0	2	ovals, red lines

Table 9 continued.

Sample	Age calBP	Episode	Location	Microscopy	Solution	Weight (g)	Fibrous	Elongate Grass Phytoliths	Short Phytoliths	Pollen	Hair	Diatom	Tracheid	Starch/Seed	Browse	Cysts	Unidentified/other	Description of Unidentified	
79	na	LH	Larson	LM	HCL	0.07	2	4	3	0	0	0	0	0	0	0	0		
80	425	LH	Larson	LM	HCL	0.09	6	0	12	2	0	0	0	0	0	0	0		
83	432	LH	Larson	LM	HCL	0.15	0	6	12	1	0	0	0	0	0	0	0		
84	na	LH	Larson	LM	EDTA	0.05	4	17	10	1	0	0	0	3	0	0	2	rod & round granule	
84	na	LH	Larson	SEM	EDTA	0.05	0	0	0	1	0	0	0	0	0	0	0		
85	na	LH	Larson	LM	EDTA	0.02	1	32	3	0	0	0	0	0	0	0	0		
85	na	LH	Larson	SEM	EDTA	0.03	0	0	0	0	0	0	0	0	0	0	0		
87	na	LH	Larson	LM	EDTA	0.02	1	4	8	0	0	0	0	0	0	0	0		
87	na	LH	Larson	SEM	EDTA	0.04	0	1	0	0	0	0	0	0	0	0	0		
92	765	LH	Menoken	LM	EDTA	0.14	0	7	6	109	0	0	0	0	0	0	0		
92	755	LH	Menoken	SEM	EDTA	0.11	0	0	0	0	0	0	0	0	0	0	0		
94	na	LH	Menoken	LM	EDTA	0.18	0	0	4	0	0	0	0	0	0	0	0		
94	na	LH	Menoken	SEM	EDTA	0.06	0	0	0	0	0	0	0	0	0	0	0		
95	na	LH	Menoken	LM	EDTA	0.01	0	3	4	22	0	0	0	0	0	0	0		
95	na	LH	Menoken	SEM	EDTA	0.02	0	0	0	0	0	0	0	0	0	0	0		
96	na	LH	Menoken	LM	EDTA	0.09	2	4	12	0	0	0	0	0	0	0	0		
96	na	LH	Menoken	SEM	EDTA	0.04	0	0	0	0	0	0	0	0	0	0	0		
106	277	LH	Big Hidatsa	LM	EDTA	x	2	0	0	0	0	0	0	0	0	0	0		
106	277	LH	Big Hidatsa	SEM	EDTA	x	0	0	0	0	0	0	0	0	0	0	0		
110	na	LH	Big Hidatsa	LM	HCL	x	0	0	0	0	0	0	0	0	0	0	0		
112	na	LH	Taylor Bluff	LM	HCL	x	0	0	2	0	0	0	0	0	0	0	0		
112	na	LH	Taylor Bluff	SEM	HCL	x	0	0	0	0	0	0	0	0	0	0	0		
113	na	LH	Sakakwea	SEM	HCL	x	0	0	0	0	0	0	0	0	0	0	0		
113	110	LH	Sakakwea	SEM	HCL	0.66	0	0	0	1	0	0	1	0	0	0	0		
114	na	LH	Sakakwea	LM	HCL	0.11	0	4	0	0	1	0	0	0	0	0	0		
114	na	LH	Sakakwea	SEM	HCL	0.1	0	0	0	1	0	0	0	0	0	0	0		
116	na	LH	Sakakwea	LM	HCL	x	3	0	0	0	0	0	0	0	0	0	0		
116	na	LH	Sakakwea	LM	EDTA	x	0	0	3	3	0	0	0	0	0	0	0		
117	169	LH	Sakakwea	LM	HCL	x	0	4	0	0	0	0	0	0	0	0	0		
118	na	LH	Sakakwea	LM	HCL	x	7	12	4	0	0	0	0	0	0	0	0		
118	na	LH	Sakakwea	SEM	HCL	x	0	2	0	0	0	0	0	0	0	0	0		
119	na	LH	Sakakwea	SEM	Ethanol	0.04	0	0	0	6	0	2	0	0	0	0	0		
121	111	LH	Sakakwea	LM	HCL	x	0	0	0	0	0	0	0	0	5	0	0		
122	169	LH	Sakakwea	LM	HCL	x	0	0	0	0	0	0	0	0	0	0	0		
123	168	LH	Sakakwea	LM	HCL	x	0	0	0	0	0	0	0	0	0	0	0		
123	na	LH	Sakakwea	SEM	HCL	x	0	0	0	1	0	0	0	0	0	0	0		
124	na	LH	Lower Hidatsa	LM	HCL	x	0	2	0	2	0	0	0	0	0	0	0		
125	388	LH	Lower Hidatsa	LM	HCL	x	6	0	5	0	0	0	0	0	0	0	0		
125	388	LH	Lower Hidatsa	SEM	HCL	x	2	1	2	0	0	0	0	0	0	0	0		
126	117	LH	Lower Hidatsa	LM	HCL	x	4	2	0	0	0	0	0	0	0	0	0		

Table 9 continued.

Sample	Age calBP	Episode	Location	Microscopy	Solution	Weight (g)	Fibrous	Elongate Grass Phytoliths	Short Phytoliths	Pollen	Hair	Diatom	Tracheid	Starch/Seed	Browse	Cysts	Unidentified/other	Description of Unidentified
126	117	LH	Lower Hidatsa	SEM	HCL	x	0	0	0	0	0	0	0	0	0	0	0	
127	na	LH	Lower Hidatsa	LM	HCL	x	0	0	0	0	0	0	0	0	0	0	0	
128	na	LH	Lower Hidatsa	LM	HCL	x	0	1	0	0	0	0	0	0	0	0	0	
128	na	LH	Lower Hidatsa	SEM	HCL	x	0	0	0	0	0	0	0	0	0	0	0	
129	na	LH	Lower Hidatsa	LM	HCL	x	0	5	0	0	0	0	0	0	0	0	0	
129	na	LH	Lower Hidatsa	SEM	HCL	x	0	0	0	0	0	0	0	0	0	0	0	
101	0	MOD	TRNP	LM	EDTA	0.01	4	2	5	0	0	0	0	0	0	0	3	round, pollen-like
101	0	MOD	TRNP	SEM	EDTA	0.01	0	1	0	1	0	0	0	0	0	0	0	
103	0	MOD	TRNP	LM	EDTA	0.05	2	4	2	0	0	0	0	0	0	0	0	
104	0	MOD	TRNP	LM	EDTA	0.01	1	2	4	0	0	0	0	0	0	0	1	mite
						n=	163	275	387	255	10	2	16	25	13	13	11	N= 1,070

Table 10. Summary of images of microfossils from Figures 11-18 found in bison dental calculus, their location, magnification, and associated calBP age (if available).

Bison	Figure ID	Magnification	calBP	Site	Description
70	12a	40x	-	Alkali Creek	dendriform phytolith
70	12b	40x	-	Alkali Creek	dendriform phytolith
43	13a	40x	1077	Menoken Village	Chloridoid phytolith
43	15c	40x	1077	Menoken Village	hair
43	14h	40x	1077	Menoken Village	tracheid
43	11a	100x	1077	Menoken Village	pollen or spores
43	14a	40x	1077	Menoken Village	tracheid cells
92	10a	40x	765	Menoken Village	chrysophycean cyst
92	16a	40x	765	Menoken Village	unknown oblong shape
95	10b	40x	-	Menoken Village	chrysophycean cyst
74	111b	40x	1105	White Bison Robe	<i>Pinus</i> pollen
71	14b	40x	-	White Bison Robe	tracheid cells
35	11c	40x	1027	Bundlemaker	grass pollen
47	15d	40x	653	Falkirk Bison Kill	hair
30	11d	40x	296	Falkirk Bison Kill	grass pollen
30	10c	2510x	296	Falkirk Bison Kill	chrysophycean cyst
30	11e	1100x	296	Falkirk Bison Kill	pollen air sac
30	11f	3500x	296	Falkirk Bison Kill	pollen air sacs
13	13b	40x	504	Huff Village	short grass phytolith
13	14c	40x	504	Huff Village	tracheid
32	14d	40x	437	Larson Village	tracheid
32	14e	40x	437	Larson Village	vessel element
32	12c	40x	437	Larson Village	stemwood phytolith
79	13c	20x	-	Larson Village	plant phytolith cells
84	16b	40x	-	Larson Village	unknown, rod shaped vessel
84	14f	40x	-	Larson Village	vessel element
84	16c	10x	-	Larson Village	unknown round granule
84	11g	40x	-	Larson Village	grass pollen
79	13d	40x	-	Larson Village	vascular phytolith
78	16d	40x	-	Larson Village	unknown oval with red line
34	15a	40x	379	Double Ditch Village	hair
46	11h	10x	185	Forest River	grass pollen
26	15b	40x	170	Big Hidatsa	hair
26	16e	40x	170	Big Hidatsa	unknown, round granule
26	14g	40x	170	Big Hidatsa	vessel element
26	10d	40x	170	Big Hidatsa	chrysophycean cyst
121	12d	40x	111	Sakakawea	dendriform phytolith
119	10g	4000x	-	Sakakawea	<i>Navicula</i> diatom (right), biraphid diatom (left)
124	10e	40x	-	Lower Hidatsa	chrysophycean cyst
112	13e	40x	-	Taylor Bluff	grass phytolith
112	10f	4000x	-	Taylor Bluff	chrysophycean cyst
103	17a	10x	Modern, cow	TRNP	vessel element
103	17b	10x	Modern, cow	TRNP	tracheid
104	17c	40x	Modern, cow	TRNP	Panicoid grass phytolith
104	17d	40x	Modern, cow	TRNP	Trigynaspida mite
104	17e	40x	Modern, cow	TRNP	<i>Betula</i> pollen

CONCLUSIONS AND MANAGEMENT IMPLICATIONS

Stable isotopes of carbon, nitrogen, and oxygen in ancient bison bones and teeth describe valuable ecological information about this resilient species over a long period of time. Here, we have documented how the diet of ancient bison has varied in composition from the Late Pleistocene through modern day in the Northern Great Plains region of North America. We can determine the time periods in which bison had a larger breadth of diet than others and when they may have been migrating long distances.

We have seen that bison during the Late Pleistocene consistently have similar isotopic values to modern bison but include more diverse vegetation in their diet. Isotopic signals suggest that bison may have been employing migratory movements during this epoch. Some temporal periods lack sampling points in the data set. Only one bison specimen was available from the Early Holocene and there is a second gap in data shortly after the beginning of the Middle Holocene. Whether the sample site assemblage simply did not represent contexts from this time or if bison were less abundant in the Northern Great Plains is not clear. The reasons for these gaps warrant further investigation into the archeological record as it may reveal interesting insights into the spatiotemporal trends in bison population density over time. Middle Holocene bison exhibit high variability. There are individuals widely apart in isotopic values despite having similar calibrated ages. These wide ranges were recorded in the collagen from Middle Holocene bones as well as the seasonal data obtained from the enamel in Middle Holocene bison's teeth. Large migratory movements may explain the isotopic trends observed in Middle Holocene bison. Late Holocene bison were the most abundant in the sample assemblage. Bison

reached their highest numbers during this temporal period and thrived until their near extirpation in the late 19th century. Moderate variation is recorded in the Late Holocene bison despite the large increase in sample size. This suggests that there may have been an overall shift to less diversity in bison diet as the Earth transitioned from the Middle to Late Holocene epochs. Modern bison exhibit even less variation when compared to their ancient counterparts. Questions still remain as to whether this is a result of restricted rangelands or if bison diet is becoming increasingly narrower as time progresses. Genetic information from bison temporal groups may help uncover more answers to the underlying issues at hand.

All of this information is meant to inform current management for the next generation of bison management. A better understanding of the previous and current plasticity of bison in changing landscapes can help determine what actions should be taken to give bison the best chance of survival as we enter the Anthropocene epoch. The identification of suitable habitat for the restoration of bison is critical to their success as available land diminishes rapidly. Current management strategy is predominately characterized by small bison populations in many areas but perhaps it would be better to secure fewer restoration sites with larger areas of connected habitat.

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