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# Paleoecology and Land-Use of Quaternary Megafauna from Saltville, Virginia

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# Paleoecology and Land-Use of Quaternary Megafauna from Saltville, Virginia

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by
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# ABSTRACT

## Paleoecology and Land-Use of Quaternary Megafauna from Saltville, Virginia

by

Emily Michelle Bruff Simpson

Land-use, feeding habits, and response to seasonality by Quaternary megaherbivores in Saltville, Virginia, is poorly understood. Stable isotope analyses of serially sampled *Bootherium* and *Equus* enamel from Saltville were used to explore seasonally calibrated ($\delta^{18}O$) patterns in megaherbivore diet ($\delta^{13}C$) and land-use ($^{87}Sr/^{86}Sr$). Overall, this dataset suggests an open forest with relatively constant temperatures year round that were similar to modern conditions. Neither *Bootherium* or *Equus* individuals spent a significant amount of time at higher elevations in the nearby Blue Ridge geologic province. These analyses provide a unique perspective on long-term response of large mammals to climatic shifts and their impact on local ecosystems. Results are also relevant to modern conservation decisions, especially those surrounding highland grassy balds in the Appalachians, and their maintenance by grazing pressure.

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# CHAPTER 1

# INTRODUCTION

Paleontological investigations follow a sequence; initially, researchers describe the flora, fauna, and geology of the site, and later can use this knowledge to describe evolutionary changes, climate and ecotone shifts, and differences in the ecological niches of the taxa that are present. The archeology and paleontology of Saltville, Virginia (Figure 1.1), has been investigated sporadically since 1787 (McDonald 1984).

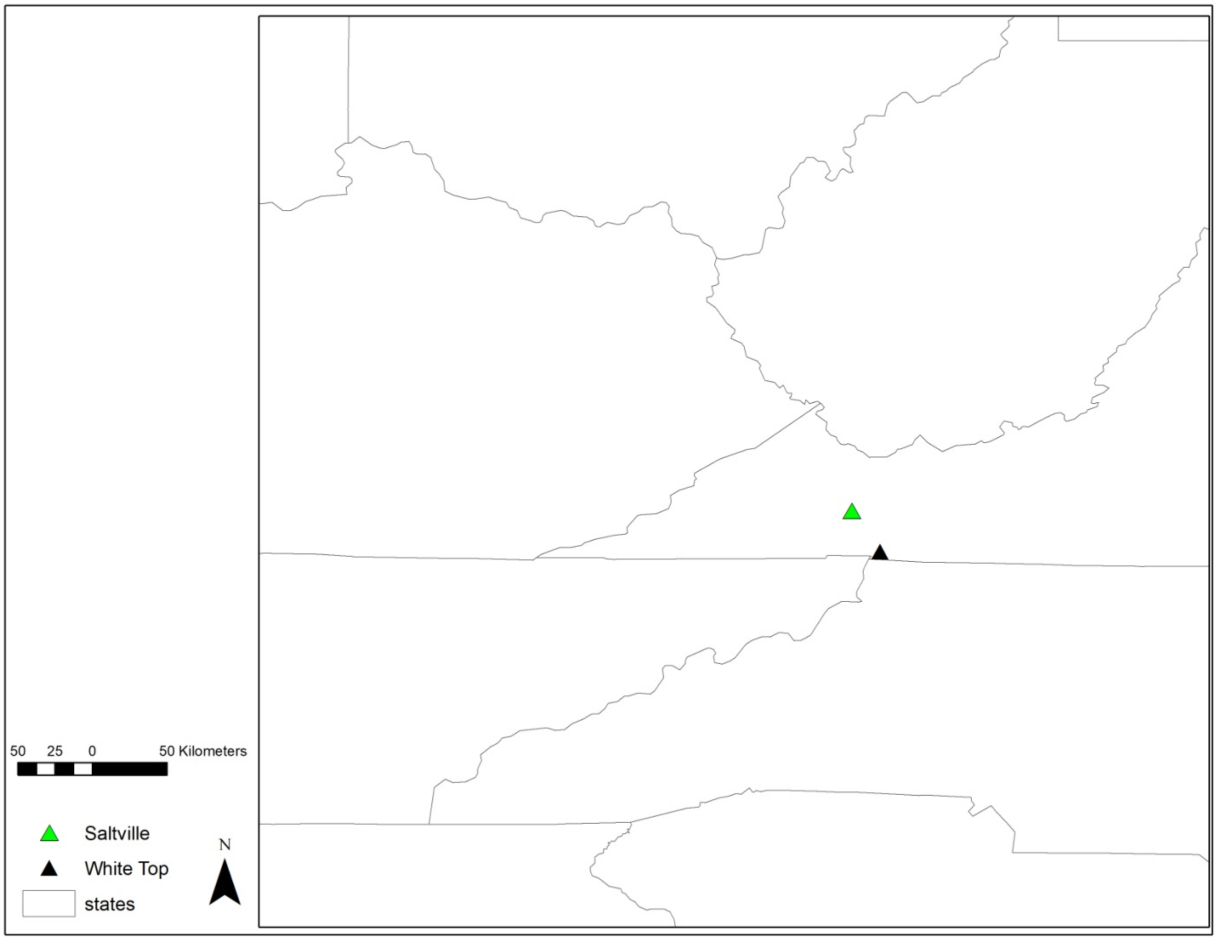


Figure 1.1: Location of Saltville, Virginia and Whitetop Mountain in southwestern Virginia

Through time, excavations have become more systematic, and scientific analyses continue to increase the amount of information that can be gleaned from paleontological materials and their surrounding matrix. The conditions of the surrounding landscape during the Pleistocene are unclear. Quaternary floral and faunal communities have been described as a disharmonious with species from both boreal and warm environments (Delcourt and Delcourt 1986; Ray et al. 1967) and likely represent an environment with no modern analog (Delcourt and Delcourt 1986; Holman 1986). In addition, Weigl and Knowles (1995) used the paleontological assemblage from Saltville as support for their hypothesis that Pleistocene megafauna were key to the maintenance of higher elevation, grass-dominated bald environments below the Holocene tree line.

In recent decades, stable isotopes have become an important component of the paleoecological toolbox. With the aid of modern analogues, precision instruments, and careful consideration of taphonomy, it is possible to reconstruct diets and trace individual movements of extinct taxa. This study focuses on incrementally forming tissues (i.e., enamel) from late Quaternary *Bootherium* and *Equus* found in Saltville, Virginia. It explores the implications of stable carbon (van der Merwe 1982; Ambrose 1981), oxygen (Dansgaard 1964), and strontium isotopes (Capo et al. 1997) for seasonality in feeding ecology and landscape use, with the goal of better understanding the local-scale Quaternary paleoecology and testing the Weigl and Knowles (1995) hypothesis of grassy bald origins and maintenance. Remains of proboscideans (Silverstein 2017)*, Bootherium,* and *Equus,* are all reported from Saltville excavations (Schubert and Wallace 2009; McDonald 1984; McDonald and Bartlett 1983; Ray et al. 1967). I use isotopic analyses to study a limited number of individuals of these large herbivores, in order to understand the ecological niche space of extinct Quaternary megafauna, and to study seasonal variation in land-use and animal diet. Key questions that will be addressed are:

1. What type of environment occurred in the Saltville valley during the late Pleistocene?
2. How well do previously modeled isoscapes represent variation in $^{87}Sr/^{86}Sr$ at a local scale?
3. How extreme were seasonal conditions experienced by the Saltville megafauna?
4. How did seasonality in temperature affect the diet of Saltville megaherbivores?
5. How did local seasonal shifts in temperature impact movement of Saltville megafauna?
6. Were megaherbivores from Saltville valley spending a significant amount of time at higher elevations?
7. Were large herbivores imposing top-down grazing pressure in highland areas such as Whitetop Mountain, supporting the hypothesis for megafaunal maintenance of grassy balds in the Southern Appalachians?

## Site Background

### Geologic Setting

McDonald (2000) suggested based on reviewing geoarchaeological studies in the eastern part of the Saltville valley that three major periods of valley formation (McDonald 2000: Table 1). Around 27,000 years ago a braided river cut through the valley (McDonald 2000). This stream strengthened, leaving behind clasts and reworked fossils of larger sizes before being diverted and blocked 13,000 years ago in an impoundment referred to as Lake Totten (McDonald 2000). More vertebrate fossils were deposited in the lake sediments. The valley remained predominantly lacustrine and marsh land until it was drained for human use in the 1840's

(McDonald 1984; McDonald 1985; McDonald 2000). Delcourt and Delcourt (1986) hypothesize these alluvial changes played an important role in changing faunal and floral elements in the Saltville valley. Environmental shifts from salty marsh to a mosaic of mixed boreal forest and grasslands, with a later transition from pine to hardwood mesic forests (Delcourt and Delcourt 1986). The alluvial history of the valley (McDonald 1984; McDonald 1985; McDonald 2000) is likely related to the climate impacts of the Laurentide ice sheet around 900 km to the north (Dyke et al. 2002). At the last glacial maximum (LGM) 21,000 years ago (Dyke et al. 2002; Mix et al. 2001) harsh, boreal climatic conditions would have existed at Saltville. As the continental glaciers retreated after the LGM milder, landscapes would have become warmer, especially at the transition from the Pleistocene to the Holocene, 13,000-8,000 years ago (Dyke and Prest 1987). Rapid environmental shifts occurring as a response to relatively quick climatic changes have been suggested to be the cause of seemingly mixed floras and fauna in the fossil record, described at Saltville as "disharmonious" (Russell et al. 2009; Ray et al. 1967); either a mild mean annual temperature with little seasonal variation allowed for the co-occurrence of organisms in communities that no longer exist (Semken et al. 2010) or, on the scale of decades and centuries, normal succession of species may not have been able to keep up and reach equilibrium at the rate of climatic change, leading to compositions of species that do not coexist in the modern day (Mann et al. 2019).

### Previous Research on the Saltville Fauna

Excavations at Saltville, Virginia, have produced the remains of the megafauna *Mammut, Mammuthus, Bootherium, Equus, Rangifer, Megalonyx, Arctodus,* and possibly *Cervalces*. A variety of herpetofauna has also been reported from the excavations and contemporary cave sites surrounding the valley (Eshelman and Grady 1986; Holman 1986; Schubert and Wallace 2009) and in some areas, potential evidence of an early human presence (McCary 1986; McDonald 2000; McDonald and Weiderheld 2009). The locality has been variously interpreted as a saltwater marsh (McDonald 1985), or as a mosaic of wooded areas inter-mixed with grasslands (Holman 1986; Ray et al. 1967; Russell et al. 2009). These descriptions support a hypothesis put forward by Holman (1986) regarding an environment in a climate with less seasonally variable temperatures, leading to an ecosystem that supported taxa from both northern and southern extremes. Herpetofauna from Saltville and nearby, co-eval assemblages include four species of turtles and several lizards constrained by reproductive limits to warmer environments (Holman 1986). This contrasts with the boreal affinities *Mammuthus, Rangifer,* and *Cervalces* (Holman 1986), and boreal mixed forest flora described by Delcourt and Delcourt (1986). Thus, multiple studies describe the site as having a "disharmonious fauna," that is, a community of species expected to be found in different climates and ecosystems (Eshelman and Grady 1986, Holman 1986). This may be due to misidentification of some taxa, the presence of a refugia for species during climatic shifts, or the requirements that may have existed in ancient communities different from those today (Eshelman and Grady 1986, Gonzales and Grimm 2009). It could also simply be due to the time-averaging of shifting ecosystems, representing variable ecosystems over time, or lower seasonal extremes in temperature (Semken et al 2010). Direct-dated specimens are needed to confirm contemporaneous fauna, and thus to confirm whether they are 'disharmonious' (Semken et al. 2010) or time-averaged.

### Previous Paleoenvironmental Interpretations

Ray et al. (1967) suggest that the Saltville valley consisted of a landscape spotted with marshes, ponds, and prairies based on the wide variety of pollen. Their results describe a mix of local grass and herbaceous pollen, with a less significant but still high concentration of pine and spruce pollen indicating nearby forests. Ground cover to surface pollen transfer functions (Davis 1983) suggest the 27% Poaceae pollen recovered from the nasal cavity of a Saltville *Bootherium* skull (USNM 23264) (Ray et al. 1967) indicates that this family made up ~19% of the local groundcover. Other families represented by Saltville pollen either over- (e.g., *Pinus*) or under-predict ground cover (Davis 1983). This interpretation was supported by plant macrofossils from the same stratigraphic layer (McDonald and Bartlett 1983), suggesting that pollen from within this skull represented a local record (McDonald and Bartlett 1983) rather than one derived from an earlier time Ray et al. (1967). McDonald and Bartlett (1983) describe flora predating USNM 23264 (Ray et al. 1967) as representing an entirely open environment but with herbaceous ground cover. Their tree pollen abundance data (reported in Table 1.1), particularly of *Quercus, Pinus, Picea, Betula,* and *Abies,* is comparable to environments north of the Great Lakes (Whitmore et al. 2005), which in the modern day is generally deciduous to hardwood forest, slightly south of boreal environments (Whitmore et al. 2005).

Table 1.1. Tree pollen abundance reported by Ray et al. (1967)

| Tree Pollen Taxa | Abundance reported by Ray et al. (1967) |
|---|---|
| *Pinus* | 53% |
| *Picea* | 42% |
| *Abies* | 2.7% |
| *Quercus* | 1% |
| *Betula* | 0.5% |

Delcourt and Delcourt (1986) suggest the dominance of dry, thin-soiled glades with sediment constantly being deposited in the Saltville valley until 16,500 years BP, followed by a mosaic of boreal forests and open grasslands until 12,500 years BP. During this time, they describe mesic forests and the presence of *Pinus,* as well as a variety of other hardwoods and conifers, with more moist valleys containing a patchwork of *Picea* and open grasslands. However, they also report an increase in grasslands in the surrounding southern Appalachians. Since 10,000 years BP, the valley has remained a mixed forest composed of northern, cold climate hardwoods and conifers, but with *Pinus, Picea, Betula,* and *Abies* increasing in abundance and *Quercus* and *Tsuga* decreasing. They did not report on the abundance of grasses (Delcourt and Delcourt 1986).

France et al. (2007) extracted collagen from sub-fossil bones for stable isotope analyses in order to reconstruct trophic levels and the contribution of C3 and C4 plants to the diets of the animals in the Saltville assemblage. According to France et al. (2007), bone collagen from *Bootherium, Mammut, Mammuthus, Rangifer, Megalonyx, Cervalces,* and *Equus,* indicate that all individuals were herbivorous with $\delta^{13}C_{collagen}$ ranging from -19.9‰ to -22.7‰. These values indicate a C3-dominated environment with an open canopy, though they also suggest some degree of niche partitioning between taxa as values vary significantly with species (France et al. 2007). However, the carbon isotope values reported by France et al. (2007) suggest up to 20% C4 cover (using corrected values from France et al. (2007) as end members in a mixing model between pure C3 and C4 diets).

## Environmental Shifts and a Hypothesized Ecological Relict

In the southern Appalachian mountains surrounding Saltville, open environments at high elevations but below the treeline are known as grassy balds (Harshberger 1903). These grassy balds are dominated by *Danthonia compressa,* a species selected for by grazing (Clay 1983), are home to a variety of endemic and disjunct populations such as *Prenanthes roanensis* (Roan Mountain rattlesnakeroot)and *Sibbaldiopsis tridentata*(three-leafed cinquefoil) (Wells 1936; White et al. 1984; Weigl and Knowles 2014), species that grow on a variety of bedrock substrates and soils, at varied temperatures and altitudes, and receive a high amount of precipitation. Balds range in altitude from 4500 to 6000 feet, and do not seem to be related to the type of forest adjacent to them (Mark 1958). Historically, some grassy balds have shrunk, as unimpeded forest succession resulted in encroachment by trees and woody shrubs (Lindsay and Bratton 1979; Gilbert 1954). Many of them no longer maintain their original flora, and only those that are under active management (including grazing and mowing) resemble their relict state (Lindsay and Bratton 1979b). Crawford and Kennedy (2009) tracked this succession at the grassy bald on Craggy Gardens, North Carolina, by studying *Quercus* tree rings. They found that encroachment by trees began on the bald after the 1930's, when Europeans stopped using the area for grazing, and ceased in 1980 with new conservation efforts (Crawford and Kennedy 2009).

The bald on Whitetop Mountain in Jefferson National Forest, VA, has captured the interest of the public and the forest service. Although it was never clear-cut, the mountain, was grazed by cattle until the 1960's, and is a local center for hiking, music, and tourism (Robertson and Hull 2001). The U.S. Forest Service attempted to further develop the area by building parking areas and buildings, until the decision was made to prioritize protecting the area through education and traffic reduction (Robertson and Hull 2001). White et al. (1984) described the species richness on the bald on Whitetop Mountain; despite being among the smallest balds in the region, it had an unexpectedly high species richness. Among the species present were one reported northern disjunct (*Sibbaldiopsis tridentata*) and several Southern Appalachian endemics.

Assuming that the grassy bald on Whitetop Mountain formed during the Last Glacial Maximum as a result of harsher climatic conditions, Weigl and Knowles (1995) suggest that the mountain's close proximity to Saltville (<35 km)supports the hypothesis that megafaunal grazing maintained the grassy balds as the glaciers retreated. This would have resulted in an herbivore-plant trophic cascade that is no longer extant (Weigl and Knowles 1995; Weigl and Knowles 2014). They declined as horses, and deer became the dominant herbivores on historic landscapes, joined by goats, and a reintroduced population of horses after European contact. The average body size of historic herbivores was much smaller than Pleistocene megaherbivores, therefore, they were not able to completely maintain a grassland and the region became a forest-grassland mosaic. The residual impact of megafaunal grazing may have maintained the balds until early historic settlement, although these environments may already have been on the decline (Weigl and Knowles 2014; Weigl and Knowles 1995; Delcourt and Delcourt 1986).

This origin and maintenance scenario is not universally agreed upon, however (Weigl and Knowles 1995; Weigl and Knowles 2014; Lindsay and Bratton 1979). Researchers have suggested that, if the balds date to the LGM, northern disjunct populations should be phylogenetically different (Weigl and Knowles 2014; Weigl and Knowles 1995). However, phylogenetics of populations of *Sibbaldiopsis tridentata* suggest that the northern and southern populations have not been isolated for a long enough period of time to diverge genetically (Bresowar and Walker 2011), and radiocarbon dating of the soils on some balds suggests that some of the balds less than 5000 years old (Knoepp et al. 1998). However, these ecosystems normally succumb to succession without extra selective pressures (Simon 2011; Bond 2010). Anthropogenic or natural fire has been suggested as a maintaining force (Lambert 1958; Clements 1936; Wells 1936), but the grassy balds are not characteristic of fire dependent vegetation communities (Lindsay and Bratton 1979; Gershmehl 1970). Gates (1941) proposed that invasion by gall wasps limited tree encroachment. Other conservation biologists have suggested that they were created by relatively recent (modern) deforestation (Gershmehl 1970; Lambert 1958; Clements 1936; Wells 1936 )or agricultural grazing (Delcourt and Delcourt 1986). Some researchers suggest the presence of balds is due to environmental conditions such as exposure to harsh winters (Harshberger 1903) and wind (Brown 1941), or simply their proximity to the forest edge (Harshberger 1903). Harshberger (1903) and Baskin and Baskin (1988) also suggested that increased access to sunlight prevented encroachment of shade tolerant trees. At ecotonal edges, Billings and Mark (1957)describe harsher, open environments such as balds preventing encroachment by other species.

Aikens and Roach (2014) examined the relationship between bald size and edge effects on the abundance and productivity of *Prenanthes roanensis*, a northern disjunct species. They found that productivity, measured by the number of buds on the plants, decreased towards the edge of a bald (Aikens and Roach 2014). They also found no evidence of decline on balds that have continued to be grazed, with larger population sizes on older balds (Aikens and Roach 2014). However, using MAXENT, Simon (2013) was only able to predict the location of grassy balds 83% of the time using environmental factors in Jefferson National Forest (Simon 2013). This success rate decreased to 30%in Washington National Forest (Simon 2011).

The interpretation of the grassy balds as being maintained by megaherbivores, especially in relationship to Saltville, is salient to ongoing conversations about the conservation of southern Appalachian grassland systems (e.g., Ohwaki et al. 2018; Miller et al. 2017; Bakker et al. 2015).

## The Impact of Megaherbivores on Modern Ecosystems

Modern megaherbivores are known to impose top-down grazing pressures that determine the composition of plant communities, often keeping environments open that would otherwise undergo forest succession (Bond 2010). Here, I discuss the impacts of modern megaherbivores on their surroundings, and topographic limits on their distribution.

Weigl and Knowles (1995) directly reference the presence of Quaternary proboscideans in Saltville paleontological deposits, as exerting top-down pressure strong enough to maintain the grassy bald on Whitetop Mountain. Modern elephants are dependent on water; in part because their large body size requires evaporative cooling to prevent overheating. Dunkin et al. (2013) measured skin temperature and water loss in trained elephants across a temperature gradient in arid regions. They found increased amount of water loss in individuals from increasingly warm environments to make up for heat resulting in their metabolism. (Dunkin et al. 2013). Elevation is a secondary factor in determining elephant distribution (Ngene et al. 2009). Modern populations in the Marsbit protected area are found in forested environments at elevations greater than 1000 meters. They occupy highland areas during the dry season, only venturing to lower shrublands during the wet season (Ngene et al. 2009). Elephants are also limited by food. Sinclair et al. (2010) reported a 30% death rate during a season when water was available but food was scarce. Codron et al. (2011) observed in elephants in Kruger National Park, South Africa, that individual diets did not reflect natural abundance and that elephants engaged in selective feeding, and that they moved to do so.

However, these factors affecting the distribution of elephants may be more important in arid regions, not in humid areas of eastern North America during the late Quaternary. It is unlikely that *Mammuthus* were able to navigate slopes steeper than 25 degrees (Agenbroad 2003). Modern proboscideans are also known to avoid slopes, or find less direct ways to climb them, especially during wet seasons when they may become slippery (Ngene et al. 2009).

Modern elephant disturbance limits the recruitment of trees and tends towards the maintenance of an open environment (Sinclair et al. 2010; Pringle et al. 2008; Skarpe et al. 2004); they also debark trees with their tusks, leaving them susceptible to disease (Buechner and Dawkins 1961). Mammoths and mastodons may have been capable of similar impacts in the past. Pringle et al. (2008) reported that debarked, dead trees killed by elephants were preferred small reptile habitats. The increase in open areas and decrease in woodlands also led to a decrease in avian diversity; however, the increase in edge environment also changed communities in a way that encouraged migrant bird species to take refuge en route to their destination (Herremans 1995). A recent increase in African elephant populations has led to more open vegetation communities, soil changes, and shifts in microbial diversity (Skarpe et al. 2004). Elephant populations are a disturbance agent across their modern range (Buss 1961).

Feral and wild *Equus* populations predominantly feed on grasses throughout the year. They may graze at higher elevations during warm seasons, although they have not been observed ascending slopes greater than 45 degrees (Kissel 1996). Though *Bootherium* is an extinct taxon, some bovids (e.g., *Ovibos*) will ascend to higher elevations with steeper, south facing slopes during times with high snow accumulation to forage where the snow is shallower (Robus 1981). However, they do not seem to be limited by slope (Robus 1981). Movement of large African herbivores is also limited by the presence of elephants (Ferry et al. 2016). Ungulates in Hwange National Park, Zimbabwe, avoid elephants at water sources during the early dry season until water availability reaches a certain point of scarcity. Neither modern musk oxen nor *Equus* are a reliable proxy for Pleistocene species, and modern elephants do not live in an environment analogous to Virginia in the Pleistocene; however, recognizing modern limitations on their behaviors may help us understand their extinct relatives.

The extinction of primary consumers can, but does not always, lead to the change of a plant community. Koerner et al.(2014) experimentally excluded large herbivores from sites in the Konza Prairie Biological Station, Kansas, USA, and in Kruger National Park in South Africa. Both of these experimental sites are in mesic savanna grasslands under periodic fire regimes. After seven years, sites at the Konza Prairie Biological Station exhibited a decrease in species richness and were more affected by fire. However, at Kruger National Park, little change was seen as a result of the exclusion of grazing. Koerner et al. (2014) propose that this is due to more diverse large herbivore diets at Kruger, and that the loss of herbivores did not impact specific plant species, but rather had an impact on the entire vegetative community. In Tanzania, another area with differential grazing, the experimental removal of grazing, removal of individual plant species, and the suppression of fire, protection from grazing had the greatest amount of change to the floral community (Belsky 1992).

Jones et al. (2014) suggested that the main driving force of large mammal dispersal and migration is resource availability, particularly food. They designed an experiment to explore factors influencing movement in modern herbivores. They found that individuals whose resources were supplemented still migrated at the same time as other individuals in the population, but within a more limited range. They also found that migratory range increased with the age of individuals. Interestingly, many species have both a migratory and resident population (Jones et al. 2014). Predation may also be a secondary factor in seasonal movement of some species (Avgar et al. 2014). Theoretically, migration is evolutionarily advantageous, as long as the cost is low enough that it allows for the optimization of resources (Jones et al. 2014; Avgar et al. 2014; Bohrer et al. 2014). Thermoregulation, avoidance of other individuals, and even parasite avoidance have also been observed in large mammal species as factors encouraging seasonal movements, but these are less common and specific to certain taxa (Avgar et al. 2014; Bohrer et al 2014). Plumb et al. (2009) studied the migration behavior of *Bison bison*. Once known for their large numbers and extensive range, the herd in Yellowstone is now severely limited and protected within the park boundaries. Some individuals were found to be leaving the park during the winter. Concern was raised that the population with the park had exceeded carrying capacity, and that dispersal was being observed rather than habitual migration to the lowlands (Plumb et al. 2009). Plumb et al. (2009) found that they were not travelling due to a lack of resources within the park, implying that the regular annual migration would happen regardless of population size or resource availability.

Despite these observations, the environment proposed to have been at Saltville, Virginia, would have been unlike those modern megaherbivores are found in, and thus have been described as disharmonious, or non-analog, faunas. In addition, the boreal pollen suggests a very different environment than the herpetofauna present (Delcourt and Delcourt 1986; Holman 1986). These communities no longer exist, and may actually be the result of taphonomic conditions leading to time-averaging, the realized range of modern animals not extending to their full potential range in the modern day (Schubert 2003), or even radiocarbon plateaus representing individuals that appear to be contemporaneous but are not (Semken et al. 2010). However, finding a mix of boreal and southern resident species together in Pleistocene deposits is not uncommon; one such case is reported by Schubert (2003) in the Ozarks, and interpreted as being the result of cooler, more mesic climate with less seasonal variation in temperature and a mosaic of habitats (Hibbard 1960). It is suggested that the area neither saw conditions extreme enough to drive away the northern nor southern species, allowing for an overlap in range not seen in the modern day (Schubert 2003). However, fossils must be dated, and shown to be contemporaneous, to be confirmed a part of non-analog communities. Radiocarbon dating has been used to determine that, at least in some places, these communities existed and are not artifacts of time-averaging (Stafford et al. 1999). An alternative possibility, rather than overlapping ranges of disharmonious fauna resulting from a more "equable" environment (Hibbard 1960), is that rapidly changing climatic conditions throughout the Pleistocene maintained a patchwork of ecosystems, or a "plaids" environment rather than the predominantly latitudinal gradient of "stripes" of ecosystems that exist today. This may have maintained communities that were unable to adapt to constantly changing conditions, with the climate changing faster than succession of plant communities could occur and reach "equilibrium" (Mann et al. 2019). Previous researchers have suggested that the late Quaternary landscape in Saltville has no modern analogue. Saltville's vertebrate and pollen communities have been suggested to be another example of the result of this pattern (Holman 1986; Delcourt and Delcourt 1986) prior to shifting towards more homogenized, less mosaic environment(Delcourt and Delcourt 1986; Owen-Smith 1989).

## Stable Isotope Ecology and Paleoecology

Isotopic analyses of fossils from Quaternary contexts may help us understand land-use of megaherbivores, and help to understand past regional ecosystems. Determining whether large herbivores historically created significant top-down pressure on an ecosystem is necessary to conservation biologists (Bond et al. 2010).

Britton et al. (2009) used serial tooth enamel samples of $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ in *Rangifer tarandus* molars to study seasonal water budget and land-use. *R. tarandus* are known for long-distance migratory behavior (Bergerud 1990; Fancy et al. 1989). Most individuals studied by Britton et al. (2009) were drinking from a similar water source. Strontium isotope ratios

($^{87}Sr/^{86}Sr$) showed potential for studying seasonal movements in both modern and fossil caribou populations (Britton et al. 2011; Britton et al. 2009), as well as Holocene *Bison* from the Great Plains, USA (Widga et al. 2010), and fossil and modern proboscideans (Kennedy et al. 2011; Barnett-Johnson et al. 2008; Hoppe et al. 1999).

Holocene *Bison* migration patterns have also been studied with the use of serial sampling of strontium isotopes in tooth enamel (Widga et al. 2010). Previously, historians had hypothesized that large mammals, such as *Bison*, engaged in regular seasonal migrations (Curtis 2007; Garrettson 1938), and that these movements are restricted in modern populations due to smaller herd sizes, agricultural activities, and settlement. In this case, researchers found that cyclic seasonal movements during the Holocene were less than fifty kilometers, though there was up to five hundred kilometers of drift over multiple years (Widga et al. 2010).

In another study, Hoppe et al. (1999) studied proboscideans in Florida using $^{87}Sr/^{86}Sr$ in bulk samples tooth enamel. While this does not reveal sub-annual movement patterns, it potentially reveals the region where an individual spent most of its time while the tooth was forming. Samples from this study were compared to local water, rock, and invertebrates to construct a regional isoscape. With the exception of one local individual, most *Mammut* had isotope signatures that were more elevated than the Florida baseline, indicating they spent a significant amount of time outside the state (Hoppe et al. 1999). Later, Hoppe and Koch (2007) found that *Mammuthus* from this region were generally more constrained in movement. A dated series of mastodons suggested that *Mammut* were migrating increasing distances through time, adjusting to changes in vegetation cover throughout the Wisconsinan glacial period (Hoppe and Koch 2007).

In contrast, Baumann and Crowley (2014) used $^{87}Sr/^{86}Sr$, $\delta^{13}C$, and $\delta^{18}O$ to study diet and movement in extinct proboscideans. They found that, of twelve midwestern mastodons and mammoths, only one was migratory. Most individuals included in their study were not feeding outside of the local area. In addition, they found dietary differences between mammoths and mastodons, with *Mammuthus* eating a more C3 diet than *Mammut.*

Dispersal patterns in ancient populations can sometimes provide a proxy for studying large mammal's interactions with their environment prior to significant human impact. In many cases*,* scientists have observed long migrations in large mammals becoming more infrequent. Jones (2014) suggests that behaviors are altered by anthropogenic changes the previous season, including roads, fences, and other structures that may block movement, as well as well-intentioned management strategies such as supplementing resources. Structures may stop movements, or interrupt them, while supplementing food may actually decrease distance travelled by a population from one year to the next. In order to study migration in populations of extinct animals requires a method independent of information gathered from modern observations (Britton et al. 2011). As studying past landscape-use can be independent of human impact and allows for observations of changes with climate and time, such work provides a context to studies focused on the responses of modern species to ecological changes. This information is important to conservation efforts by providing a control for studies on human impact.

## Stable Isotope Methods

Stable isotopes from fossil bones and teeth are useful in informing paleoecologists about animal diets, water budgets, and movement patterns throughout their lives (Passey and Cerling 2002; Koch 1994; Koch 2007;Sharp and Cerling 1998). Understanding how teeth incorporate a dietary isotopic signature is essential, as is understanding how these signals can be altered, post-mortem. Some authors have suggested using incrementally forming dentin (Fisher et al. 2003; Fox and Fisher 2001), or the innermost layer of the enamel (Metcalfe et al. 2011; Passey and Cerling 2002; Trayler et al. 2015). Internal enamel is also the first layer of the tooth to completely mineralize (Blumenthal et al. 2014), so sampling only this internal layer will reduce the amount of time averaged in the dietary signal (Hoppe 2004; Metcalfe et al. 2011).

Enamel is often preferred for these studies for its resistance to diagenesis. It has a low organic content (Hillson 2005) consisting predominantly of hydroxylapatite [$Ca_5(PO_4)(OH)$] at the time of eruption (Hoppe et al. 2004). It also has a low porosity, and is slow to dissolve (Ayliffe et al. 1994), and has a structure of large crystal that replaces a more organic matrix as it matures (Hoppe et al. 2004; Driessens 1980); therefore, it is generally less susceptible to diagenesis than other biological tissues (Sharp et al. 2000; Wang and Cerling 1994).

Enamel also provides a record of conditions experienced by an animal at the time of mineralization (Zazzo et al. 2012), such as diet and movement patterns. While some time averaging from sampled enamel is inevitable, this is dependent on the growth rate of the animal and the sampled tissue. The oldest forming enamel is located at the occlusal surface and mineralization proceeds rootward (Passey and Cerling 2002), ending at the base of the crown (Hillson 2005; Hoppe et al. 2004). Sampling high-crowned, fast growing teeth reduces time averaging (Zazzo et al. 2012). As it forms incrementally, these data can be seasonally calibrated.

Due to the chronology of tooth formation and mineralization, isotopic analyses can address ecological questions at multiple temporal scales. Bulk sampling of enamel, collected parallel to the growth axis of a tooth with the intention of obtaining an average value from the entire time interval of tooth formation, represents annual to decadal scales (Hoppe et al. 1999;

Koch et al. 1998). $\delta^{13}C$ and $\delta^{18}O$ of bulk enamel carbonate samples may represent an individual's diet and water budget, respectively; $^{87}Sr/^{86}Sr$ values may represent where an individual was spending the most time during the formation of the tooth (Hoppe et al.1999; Hoppe 2004). On the other hand, serial sampling of enamel answers questions about seasonal land-use and feeding. Samples collected along the growth axis of a tooth at regular intervals can provide seasonally calibrated information (Copeland et al. 2016; Passey and Cerling 2002;Widga et al. 2010). Serial isotope samples have also been used to refine enamel growth rates (Feranec 2003). Researchers generally try to use late erupting teeth that mineralized post-weaning, with as little wear as possible, to maximize the length of the series (Zazzo et al. 2012).

Passey and Cerling (2002) examined enamel growth in *Hippopotamus amphibious* canines, which grow indeterminately. They sampled the innermost layer of enamel to about half the depth of the enamel along the canines, and found that 75% of the tooth composition could be attributed to average dietary isotope values at the time of deposition, rather than initial tooth formation. They suggested that the growth rate in organisms with continuously growing teeth is constant, but cautioned against the use of this assumptions in organisms with finite growth (Passey and Cerling 2002).For example, Bendrey et al. (2014) showed by measuring horse teeth and observing cyclic values of $\delta^{18}O$ that apposition and mineralization of enamel slows with age in horse teeth, and is not constant. They also found that third molars grow slower than earlier erupting molars. However, the rate of mineralization has been estimated for *Equus* to be about 22 mm/year (Higgins and MacFadden 2004), with the first molar erupting from 0.5 to 23 months, the second molar at 7-37 months, and the third molar at 21-55 months (Hoppe et al. 2004).

In *Ovibos,* the modern musk ox, molars mineralize at differing rates. First molars begin forming at birth, erupt at 6 months and finishes mineralizing at 18 months. The second molar erupts at about 18 months, though it finishes maturing at three years. The third molar erupts at four years, and is considered mature at five years (Allen 1913).

## Stable Carbon Isotopes

Fractionation of $^{13}C/^{12}C$ is caused by different plant photosynthetic pathways, resulting in more negative values in C3 plants than in C4. C3 plants, including cool season grasses, shrubs, and trees, have $\delta^{13}C$ values below -20‰ (Cerling and Harris 1999; Koch et al. 1991). Recycling of $CO_2$ in the understory of closed canopy forests (Farrior et al. 2013, van der Merwe and Medina 1991), further depletes $\delta^{13}C$ taken up by C3 plants through the "canopy effect." Thus C3 plants in these contexts often exhibit $\delta^{13}C$ values below -27‰ (Drucker et al. 2008), which can be detected in enamel (Cerling and Harris 1999). C4 plants include warm season grasses and have $\delta^{13}C$ values greater than -12‰ (Cerling and Harris 1999; Koch et al. 1991; van der Merwe 1982). CAM (Crassulacian Acid Metabolism) plants, such as succulents, alternate between C3 and C4 cycles depending on the time of day. They have $\delta^{13}C$ values intermediate between C3 and C4 plants. CAM plants are predominantly found in dry environments (Cernusak et al. 2013).

Stable carbon isotope values are recorded in herbivore enamel, and reflect animal diets (DeNiro and Epstein 1978; Park and Epstein 1960; van der Merwe 1982). DeNiro and Epstein (1978) demonstrated a relationship between diet and $\delta^{13}C$ in mice. Although they cautioned against using fossil bone carbonate (DeNiro and Epstein 1978), the carbonate fraction of dense, mammalian, tooth enamel is often more resistant to diagenetic changes (Sharp et al. 2000). Cerling and Harris (1999) used enamel to determine diet and habitat in modern herbivores, classifying each as a browser, hyperbrowser, grazer, or hypergrazer. They were able to determine which individuals belonged to each dietary group. $\delta^{13}C$ is depleted when taken up into enamel by 14.1‰ in large ruminant mammals (Cerling and Harris 1999). For example, browsers will have enamel values averaging -8‰ (van der Merwe 1982). When corrected for known fractionation between diet-enamel in ungulates, this value is below -22‰ (van der Merwe 1982; Koch et al. 1994; Cerling et al. 1997; Koch et al 2007). This type of analysis has illuminated interspecies variation in diet (e.g., Trayler et al. 2015; Feranec et al. 2008; Feranec and MacFadden 2000), intraspecies preferences for browsing versus grazing (van der Merwe 1982), the emergence of grasslands through time and associated niches (e.g., Boardman and Secord 2013), and, when paired with stable oxygen or strontium isotope analyses, examining seasonal changes in habitat (e.g., Yann and Desantis 2014; Widga et al. 2010; Hoppe et al. 2004).

Stable carbon isotopes were used in an early study by Chisholm et al. (1986) seeking to describe seasonal movements in archeological populations of *Bison* in the Northern Plains. They expected to see a relatively constant diet (as expressed in bone collagen $\delta^{13}C$ ) in individuals from different regions if they were moving seasonally between localities. However, these values were not constant and were interpreted as evidence that bison did not follow a detectable seasonal migration pattern (Chisholm et al. 1986).

Stable carbon isotopes also fractionate between trophic levels, but only by ~1‰ (France et al. 2007; McNulty et al. 2002).

### Stable Oxygen Isotopes

Stable oxygen isotopes ($\delta^{18}O$) reflect the effects of differential evaporation on the proportion $^{18}O$ and $^{16}O$ in surface water or water vapor (Cappa et al. 2003; Rozanski 1992; Merlivat and Jouzel 1979).The lighter oxygen isotope ($^{16}O$), preferentially evaporates, leaving behind heavier $^{18}O$ and increasing the ratio $^{18}O/^{16}O$.

$\delta^{18}O$ reflects a broad-scale relationship between water temperature and precipitation (Rozanski 1992), as well as aridity (Merlivat and Jouzel 1979). Cappa et al. (2003) looked at fractionation of oxygen isotopes by evaporation, stressing that surface cooling prevents the relationship between evaporation and $\delta^{18}$O from being linear. Variation related to temperature and seasonality is strongest at northern latitudes (Bowen et al. 2005), and predicting any particular climatic condition from them, especially when taken from biological tissue, is complicated (Hedges et al. 2004; Pryor et al. 2014). Begin et al. (2015) found a correlation between precipitation and $\delta^{18}$O in wood confirmed by tree rings; however, the relationship was weak, with an R^2 of 0.42. Pryor et al. (2014) examined $\delta^{18}$O in enamel of large mammals where it correlated to mean annual temperature in some regions. They found a large margin of error and stressed the need for region-specific calibration (Pryor et al. 2014).

Stable oxygen isotopes have been used to examine climate changes in geologic time. Fricke et al. (2010) interpreted low $\delta^{18}$O values from shells and carbonate minerals recovered from terrestrial aquatic environments in Utah and Alberta as evidence of increased, long-term monsoonal activity during the Cretaceous across western North America. A weak relationship between precipitation and $\delta^{18}$O has also been detected at a regional scale in Quaternary cave faunas (Huertas et al. 1997).

Shifts in local water $\delta^{18}$O will be recorded by large herbivores. Passey and Cerling (2002) modeled enamel formation assuming a constant rate of growth. They suggest that shifts in $\delta^{13}$C and $\delta^{18}$O can be detected using serial sampling enamel at regular intervals, and roughly record water-use habits and seasonal changes in precipitation (Passey and Cerling 2002), and potentially whether an individual occupied a mesic or arid environment (Trayler et al. 2015; Dansgaard 1964).

The ability to detect seasonality in animal tissue was tested by Koch et al. (1986) in the incremental growth bands of mastodon tusks, noting that variation in $\delta^{18}$Oof mammalian teeth should reflect temperature-driven seasonal changes in ingested water. Incremental growth structures in mastodon tusk dentin, known as seasonal growth in modern elephants, had detectable changes in $\delta^{18}O$, thus showing that these values reflect seasonality. However, due to potential movements of the animals, these values may not reflect the full magnitude of seasonality in a given location (Koch et al. 1986).

Fricke and O'Neil (1996) examined oxygen isotopes from premolars and molars in bison and sheep teeth to determine the growth rates of these two hypsodont grazers and found that regularly sampling the highest crowned, last forming teeth (particularly third molars) allowed them to calculate taxon-specific growth rates from the cyclic nature of the oxygen isotopic values (Fricke and O'Neil 1996).

Sharp and Cerling (1998) showed seasonal changes in diet ($\delta^{13}C$) can be correlated with changing $\delta^{18}O$ values. They found less variation in an enamel $\delta^{18}O$ series than would be expected if enamel $\delta^{18}O$ were a direct measure of seasonality in precipitation. This suggested that the individuals were drinking from pond or lake where amplitude in seasonal $\delta^{18}O$ was reduced . They also found variation in $\delta^{13}C$ values, and though these stayed within the range of C3 plants, they noted a seasonal variation in $\delta^{13}C$ (Sharp and Cerling 1998). The ability to record changes in precipitation and seasonal shifts in diet was also demonstrated in *Bison* and *Equus* (Higgins and MacFadden 2004).

Regional variation in $\delta^{18}O$, as reported by Bowen et al. (2005), can also be used as a proxy for individual movement. Hobson and Bowen (2004) measured $\delta^{18}O$ and deuterium ($\delta D$) in bird feathers shed at breeding grounds. They found that these values allowed them to correctly infer where the birds molted (Hobson and Bowen 2004). $\delta^{18}O$ has also been used to establish provenance in humans as there is a relationship between the $\delta^{18}$O of serial hair samples to latitude (Ehleringer et al. 2008).

The ability to use oxygen isotopes as environmental indicators is variable by region (Hedges et al. 2004; Pryor et al. 2014), and $\delta^{18}$O is not only affected by local precipitation and temperature, but also by regional patterns in these factors (West et al. 2006). The mean annual $\delta^{18}$O isoscape, a map of variation in sampled surface material, was recreated from data from Bowen et al. (2005) using Arcmap 10.5.1 (Figure 1.1).

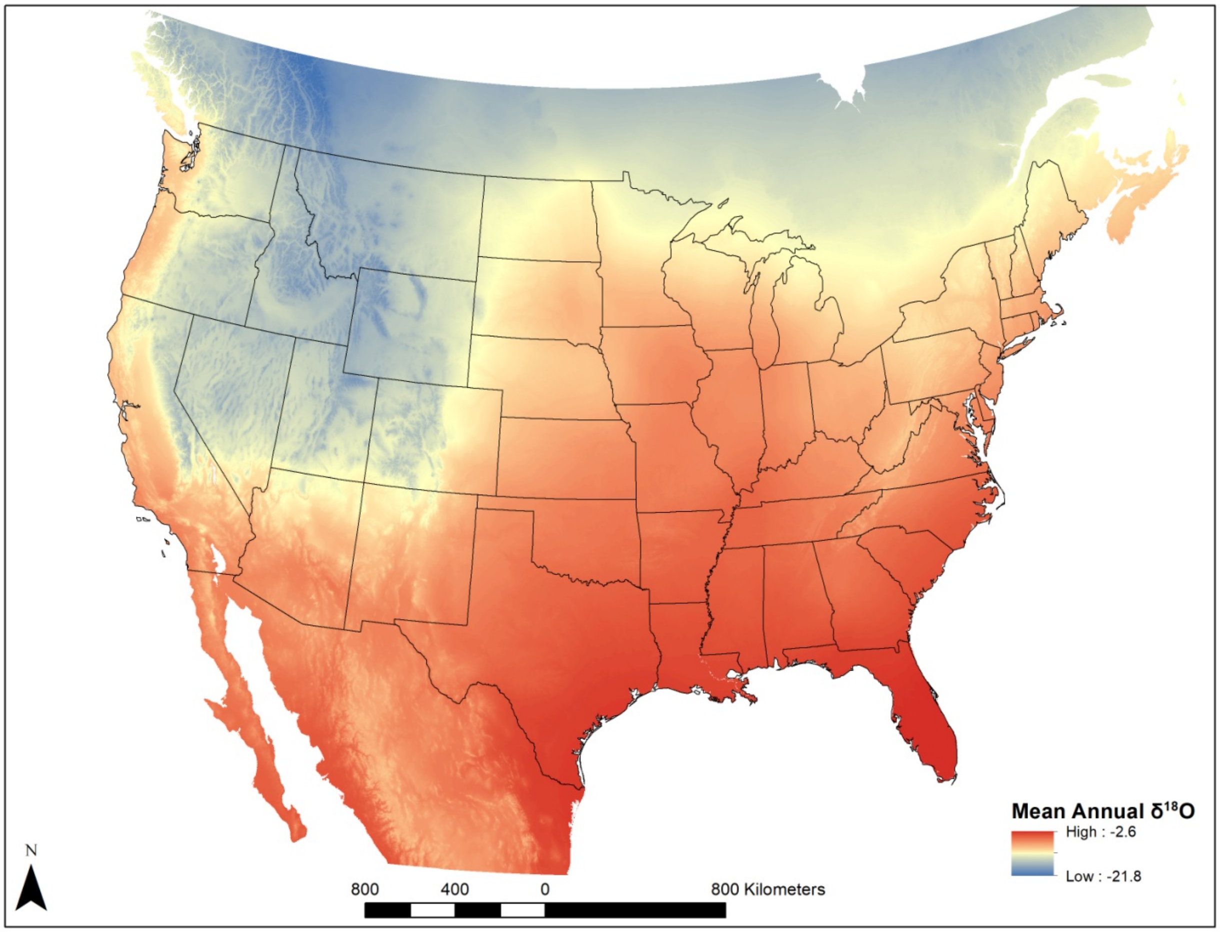


Figure 1.2. Mean Annual $\delta^{18}$O based on precipitation, recreated from Bowen et al. (2005)

As the scope of the current study relates to landscape use during a significantly cooler time in Earth's history (Dyke et al. 1987), the$\delta^{18}$O isoscape created by Bowen et al. (2005) does not reveal useful predictions for animal movement. However,$\delta^{18}O$ will still reflect seasonal changes in temperature. In addition, comparison to isoscape maps by Bowen et al. (2005) will allow for climatic comparisons, if only on a coarse, latitudinal scale.

### Strontium Isotopes

Though stable oxygen isotopes and deuterium have been used to track animal movements (Hobson et al. 2009), their correlation with climatic factors (Bowen et al. 2005) complicates using these proxies to reconstruct ancient behavior and land-use, as climates have changed. To study ancient provenance, a proxy that is independent of climate is needed. Strontium isotope ratios ($^{87}Sr/^{86}Sr$) have also been used to establish provenance, and are related to underlying surface geology (Graustein 1989; Hobson et al. 2009). Strontium occurs naturally in four isotopes of the Earth's crust, two of which are $^{87}Sr$ and $^{86}Sr$, which have abundances of 7.04% and 9.87% respectively (Capo 1998). $^{87}Rb$ has a half life of $5.0x10^{10}$ years, and decays to $^{87}Sr$ (Graustein 1989). Precambrian rocks tend to have elevated $^{87}Sr/^{86}Sr$ of greater than 0.7100. Phanerozoic limestones and dolomites generally have lower ratios, between 0.7060 and 0.7090, with marine sediments being below 0.7050 (Capo 1998). First order variation in the distribution of $^{87}Sr/^{86}Sr$ on a landscape is related to underlying geologic formations (Widga et al. 2017). The $^{87}Sr/^{86}Sr$ of surface sediments are related to age and weathering history of the soil parent material. Soil parent material from older rocks tends to be elevated in $^{87}Sr/^{86}Sr$. However, $^{87}Sr/^{86}Sr$ also varies within geologic units (Widga et al. 2017; Bataille et al. 2012; Bataille and Bowen 2012). Second order variation occurs within a geologic unit(Widga et al. 2017). A reference $^{87}Sr/^{86}Sr$ isoscape can be used, like the $\delta^{18}O$ isoscape, for studies of provenance. Herbivores foraging across this isoscape will exhibit Sr isotope ratios characteristic of vegetation, which reflects the $^{87}Sr/^{86}Sr$ values of the underlying soil parent material. There is no trophic-level fractionation of $^{87}Sr/^{86}Sr$ (Beard and Johnson 2000; van der Merwe 1990).

Bataille et al. (2012) attempted to model bioavailable $^{87}Sr/^{86}Sr$(soluble and able to be taken up by organisms) in the Caribbean. They tested the strength of the model by comparing it to samples taken from the area. When only bedrock was considered, predictive power was low. However, when weathering, aerosol inputs, and local volcanic activity was also considered, more variation was explained. Weathering, depending on the locality, accounted for anywhere from 50 to 90% of local $^{87}Sr/^{86}$ variation (Bataille et al. 2012).

In the Midcontinent of North America, Widga et al. (2017) modeled variation in bioavailable $^{87}Sr/^{86}Sr$. Here, second order variation in surface $^{87}Sr/^{86}Sr$ values is determined by the source of allochthonous deposits (e.g., loess, alluvium, glaciogenic sediments) and subsequent geomorphological history. As expected, areas dominated by Precambrian rock units at the surface exhibited more radiogenic values than Phanerozoic limestones, loess, or alluvium; however, ratios were also elevated in glacial debris containing clasts of Precambrian-aged bedrock transported from the Canadian shield.

Similarly, in the Netherlands, the isoscape created by Kootker et al. (2016) reflected that most of the variation in the $^{87}Sr/^{86}Sr$ was due to the surface sediments rather than bedrock, including material reworked by glaciers, peat, loess, and marine and river sediments (Kootker et al. 2016). Therefore, anytime an isoscape is created, the regional history of the geology has to be taken into account.

In New Mexico, 75% of $^{87}Sr/^{86}Sr$ found in trees could be attributed to atmospheric strontium, with the rest attributed to surface-weathered deposits making up soil parent material (Graustein and Armstrong 1983). However, more recent studies using bedrock values can be used in place of wood samples, and that this variation is enough to determine provenance of archeological samples (Reynolds et al. 2005; Hodell et al. 2004; English et al. 2001). This is especially important when working in regions that are no longer forested.

To account for second order variation like that seen in these studies, Bataille and Bowen (2012) attempted to model this variation continent-wide at a finer scale than had previously been done based on bedrock, weathering, and surface water. The surface water model is derived from the weathering and bedrock models, assuming these to be the source of $^{87}Sr/^{86}Sr$. The models created by Bataille and Bowen (2012) were analyzed to by Crowley et al. (2015) for their effectiveness predicting measured surface strontium isotope ratios. The models were particularly strong at predicting $^{87}Sr/^{86}Sr$ in soil samples, predicting to within 1% based on water models 97.9% of the time. Small mammal skeletal material was predicted to this level of confidence 93.8% of the time, especially in regions with lower amounts of complexity, and ratios in vegetation were predicted 84.6% of the time. Some of this variation from the model may be accounted for by varying root lengths and poorly understood geomorphic histories that affect both the values in a researcher's model and the values found in vertebrate tissue. Vertebrate tissues may also average $^{87}Sr/^{86}Sr$ from across a landscape (Crowley et al. 2015).

Strontium isotope ratios have been successfully used to determine provenance of vertebrate tissues, as it is not fractionated when taken up from soils into biological tissue or through consumption by herbivores (Graustein 1989; Hobson et al. 2009; Flockhart et al. 2015). Flockhart et al. (2015) traced bioavailable strontium through weathered bedrock, milkweed, and butterflies in a controlled greenhouse environment, and found no fractionation between source, plants, and primary consumer. They suggest that this is due to the similarity in atomic mass between strontium and calcium; strontium is taken up by biological tissue following the same pathway as calcium would be when it is available (Flockhart et al. 2015; Widga et al. 2010).

Beard and Johnson (2000) also found a correlation between birthplace and $^{87}Sr/^{86}Sr$ in poached deer. Van der Merwe et al. (1990) successfully used $^{87}Sr/^{86}Sr$ to determine the provenance of elephant bones and ivory by comparing samples from biological tissues to the regional geology, specifically, the differences between areas dominated by highly radiogenic granites contrasted to less radiogenic basalts. Using strontium modeled from water rather than bedrock, Barnett-Johnson et al. (2008) traced the birthplace and movements of salmon using otoliths and successfully classified fish to their birthplace 90% of the time. This was repeated by Kennedy et al. (2011) with skeletal material; they reported that 70% of strontium in calcified tissue originated from food sources.

Because the $^{87}Sr/^{86}Sr$ of soil parent material is related to underlying geology, it is potentially an important tracer in inferring movement, dispersal and migration in ancient vertebrates (Copeland et al. 2016; Hoppe et al. 1999; Widga et al. 2010). If multiple animals are present, it can even be determined whether the animals had travelled together in a herd or family group (Britton et al. 2011;Widga et al. 2010; Fenner 2008). Copeland et al. (2016), for example, studied seasonal east-west and north-south movements in *Equus* in South Africa during the Middle Stone Age. They found that migration east-west was seasonal, potentially following productivity in different areas resulting from different amounts of precipitation. They also excluded north-south movement across the mountains. Hoppe et al. (1999) studied Pleistocene movements of proboscideans across Florida and Georgia, supporting long range movements in *Mammut.* Widga et al. (2010) performed a similar study using serial samples of Holocene *Bison*, and found a smaller degree of seasonal movement than expected. The wide scope of questions that can be answered with this method is only beginning to be explored as Sr isoscapes continue to be refined (Widga et al., 2017; Copeland et al. 2016; Crowley et al. 2015; Bataille and Bowen 2012; Koch et al. 2009; Koch 2007).

# CHAPTER 2

# METHODS

Isotopes from fossilized bones and teeth allow paleontologists to study landscape use by ancient organisms. To answer questions about seasonal use of resources by megaherbivores molars from individuals of *Equus*, *Bootherium,* and *Mammuthus* excavated from Saltville, Virginia are sampled.

In this study, enamel was sampled because of its resistance to diagenesis (Ayliffe et al. 1994) and incremental formation (Zazzo et al. 2012). Samples were taken from fossil teeth serially, rather than in bulk, in order to trace seasonal changes in land-use. Some amount of time-averaging is unavoidable, but for consistency samples were taken parallel to the growth axis of the tooth, and through the entire thickness of the enamel. *Equus* and *Bootherium* molars were sampled using a 1.5 mm carbide drill bit at least once every 3 mm from the base to the crown along the buccal side of the metacone or entocone. About 10 mg of sample were taken from each sampling location with powder from each drilled hole being a single homogenized sample (Widga et al. 2010; Bendrey et al. 2014; Balasse and Ambrose 2005). These homogenized samples were split into two 5 mg samples. Samples are preferentially taken from late erupting upper molars (e.g., M3) (Fricke and O'Neil 1996; Widga et al. 2010).

Powdered enamel samples were weighed and pretreated before being sent to other labs for isotopic analysis. Samples intended for $\delta^{13}C$ and $\delta^{18}O$ analyses were treated with dilute 2.5% NaOCl for 24 hours to remove organic contaminants, rinsed (3x) to neutrality with de-ionized water. This was followed by a four-hour leach in 0.1 M acetic acid to remove adsorbed carbonates. These were again rinsed (4x) with de-ionized water and allowed to dry prior to being sent for isotopic analyses (Widga et al. 2010; modified from Koch et al. 1997).

Enamel carbonate samples were sent to the Iowa State University Stable isotope laboratory (Ames, IA) to be analyzed for $\delta^{13}C$ and $\delta^{18}O$. Samples were measured via a Finnigan MAT Delta Plus XL mass spectrometer in continuous flow mode connected to a Gas Bench with a CombiPAL autosampler. Reference samples (NBS-18, NBS-19) were used for isotopic corrections, and to assign the data to the appropriate isotopic scale. Corrections were done using a regression method. $\delta^{13}C$ results were reported with an analytical uncertainty of 0.03‰ , while$\delta^{18}O$ had an analytical uncertainty of 0.11‰. Stable carbon isotope ratios are reported in relationship to the Peedee belemnite (VPDB) standard as a fraction of these standards per thousand (per mil, ‰). Calcium carbonate from these $^{13}C/^{12}C$ found in modern carbonates. The assumption is that labs have all used samples with the same values to calibrate instruments (Gonfiantini et al. 1995; Barrendsen, G. W., Deevey, E. S., and Gralenski, L. J. 1957). $\delta^{13}C$ is reported in relationship to this standard as follows:

$$\delta^{13}C = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard} - 1] \times 1000$$

Stable carbon isotope ratios of tooth enamel are corrected -14.1 per mil, following Cerling and Harris (1999) to reflect dietary input. They were then converted to an estimate of %C4 following Post (2002), using the equation:

$$\%C4 = [\delta^{13}C_{diet-}(-26.5)]/[-12.7-(-26.5)]*100$$

$\delta^{18}O$ are reported relative to the PDB standard (VPDB) in per mil. $\delta^{18}O$ is defined as follows:

$$\delta^{18}O = [(^{18}O/^{16}O)_{sample}/(^{18}O/^{16}O)_{standard} - 1] \times 1000$$

In this study, $\delta^{18}O$ values are converted to VSMOW prior to other calculations and discussion following:

$$\delta^{18}O_{VSMOW}=1.03086(\delta^{18}O_{PDB})+30.86$$ (Gonfiantini et al. 1995)

Bryant et al. (1996) demonstrated that carbonate and phosphate can be used to study ingested water. Fox and Fisher (2001) established a correlation between carbonate and phosphate derived stable oxygen ratios in proboscideans, allowing for the conversion of carbonate values to phosphate followed by determining the value of ingested water:

$\delta^{18}O_{phosphate} = (\delta^{18}O_{carbonate}/1.106)-4.7288$

Where $\delta^{18}O_{carbonate}$ is the value of $\delta^{18}O$ derived from organic carbonates, or in the case of this study, carbonate from enamel.

$\delta^{18}O$ values from large herbivores, though they have a direct relationship to ingested water, do not reflect them exactly. Thus, they have to first be converted from values sampled in tissue to the environmental ratios that those values reflect. Daux et al. (2008) expressed the relationship between ingested water and $\delta^{18}O$ in human enamel phosphate, as follows:

$\delta^{18}O_{body\ water} = (1.54 * \delta^{18}O_{phosphate})-33.72$

Where $\delta^{18}O_{body\ water}$ is body water, and $\delta^{18}O_{phosphate}$ is the value from enamel phosphate.

Treated samples were selected from the location of the minimum and maximum $\delta^{18}O$ values in each series for analysis of $^{87}Sr/^{86}Sr$. A comparative surface was created to analyze these values. Grass samples were ashed at 450° Celsius. Ashed grasses and enamel samples were dissolved in 3.5 N HNO3 and eluted through Eichrom Sr-spec ion-exchange columns at the University of Kansas Isotope Geochemistry Laboratory (Lawrence, KS). The purified Sr was then analyzed for $^{87}Sr/^{86}Sr$ ratios on a Thermal Ionization Mass Spectrometer (TIMS), an automated VG Sector or VG Sector 54. Isotope ratios were adjusted to correspond to a value of 0.71250 on NBS-987 for $^{87}Sr/^{86}Sr$. Over 120 runs of NBS 987 were made along with the samples. Standard deviation of results is about 11 ppm on ratio, and standard error is miniscule. We also assumed a value of 86Sr/88Sr of 0.1194 to correct for fractionation.

## Creating a Strontium Isoscape

An isoscape of the region was developed by collecting twelve grass samples from a 350 km$^2$ area surrounding Saltville, Virginia. County and city boundaries were downloaded from the Library of Virginia. All geologic formations encompassing 10% or more of the project area are included. ArcMap 10.5.1 was used to determine randomly select sample sites within ten feet of roads for accessibility. The geologic formations shapefile for Virginia and DEM raster data were downloaded from United States Geological Survey. The DEM file, used for slope and elevation contour layers, was downloaded at the scale of ⅓ arc second, to determine sampling locations with a maximum slope of 25 degrees. The roads shapefile was downloaded from Virginia Department of Transportation; all samples were taken within ten feet of roads for accessibility. A raster layer was created to represent these parameters; points for sample localities were then randomly selected using the Random sample tool in ArcMap 10.5.1.

Grass samples were collected as close to selected localities as conditions allowed. In addition, two samples were taken from near excavation sites in the valley; one sample was taken from the bald of Whitetop Mountain in Jefferson National Forest. Nine samples were taken from valley localities with slopes below 25 degrees. The group including the Juniata, Reedsville, Trenton, and Eggleston Formations were sampled twice, although one formation (the

Moccasin or Bays Formation through Blackford Formation was inaccessible. The mean of these two values, as well for the samples taken from the Saltville excavation sites, was used to represent these formations in the created isoscape. Grass samples were ashed at 450° Celsius for one hour before being sent to the TIMS (Thermal Ionization Mass Spectrometry) Laboratory at the University of Kansas for $^{87}$Sr/$^{86}$Sr analysis.

This isoscape was also compared to a slope map to analyze how much bioavailable strontium would have been available to megaherbivores, particularly *Equus* and proboscideans, as well as what areas individuals would have been able to access.

## Statistical Analysis

Stable oxygen, carbon, and strontium values were compared statistically using SPSS 25 using a non-parametric Kruskal-Wallis test to determine for variation in isotope ratios between individuals and taxa. In addition to being analyzed as individuals samples, serial samples from each individual were averaged together and compared to Saltville collagen $\delta^{13}C$ data from France et al. (2007). Carbon isotope ratios were converted to dietary values by correcting -3.5‰ following DeNiro and Epstein (1978), and compared using SPSS 25. Eleven grass samples from the vicinity of Saltville, VA were compared to strontium isoscapes derived from bedrock, surface water, and weathering models developed by Bataille and Bowen (2012) using a related-samples Wilcoxon signed rank tests to test whether the my isoscape differed significantly from published models. $^{87}Sr/^{86}Sr$ values from herbivore enamel were then compared to these grass values to discuss provenance.

# CHAPTER 3

# RESULTS

Raw data from serially sampled enamel, as well as corrected values, are reported in Appendix A. Stable carbon isotopes in serially sampled enamel from *Equus* and *Bootherium* are significantly different between the two taxa ($p\text{-value}<0.05$), as well as between individuals ($p\text{-value}<0.05$). $\delta^{13}C$ is more constrained in *Equus,* ranging only from -26‰ to -28‰, and is more negative than that from most *Bootherium* samples. All stable carbon isotope values are between -23‰ and -28‰, except the youngest forming enamel sample from *Bootherium* VMNH 2304 which has a value of -30.0‰. The largest percent of C4 consumed by an individual is 20.6‰, found in a sample from *Bootherium* VMNH 2304. All *Equus* samples show a diet of 100% C3 plants. Descriptive statistics for $\delta^{13}C$ are in table 3.1.

Table 3.1. Summary of Saltville *Equus* and *Bootherium* Stable Carbon Isotope samples

| Catalog Number | Lab Number | Taxon | Mean $\delta^{13}C_{enamel}$ | Range $\delta^{13}C_{enamel}$ | Range $\delta^{13}C_{enamel}$ | Mean %C4 | Range %C4 |
|---|---|---|---|---|---|---|---|
| VMNH 2304 | ETSU-2018-14 | *Bootherium* | -26.3 | -23.7 to -30.0 | 6.4 | 1.6 | -25.5 to 20.6 |
| VMNH 120141 | ETSU-2018-15 | *Bootherium* | -26.7 | -25.9 to -27.6 | 1.7 | -1.7 | -8.2 to 4.5 |
| VMNH-2392 | ETSU-2018-16 | *Bootherium* | -25.5 | -24.9 to -26.1 | 1.2 | 7.2 | 3.0 to 11.6 |
| VMNH-2321 | ETSU-2018-17 | *Bootherium* | -25.8 | -25.1 to -26.1 | 1.0 | 5.2 | 2.8 to 10.0 |
|  | All | *Bootherium* | -26.1 | -23.7 to -30.0 | 6.4 | 3.1 | -25.5 to 20.6 |
| VMNH 120142 | ETSU-2018-18 | *Equus* | -27.5 | -26.7 to -28.0 | 1.3 | -7.1 | -10.6 to -1.2 |
| VMNH 2350 | ETSU-2018-19 | *Equus* | -27.4 | -27.3 to -27.5 | 0.2 | -6.6 | -7.5 to -5.9 |
|  | All | *Equus* | -27.5 | -26.7 to -28.0 | 1.3 | -6.9 | -10.6 to -1.2 |
|  | All | *Equus & Bootherium* | -26.5 | -23.7 to -30.0 | 6.4 | 0.1 | -25.5 to 20.6 |

$\delta^{18}O_{enamel}$ was significantly different across all individuals (*p-value*<0.05) and across taxa (*p-value*<0.05). The mean of all samples for $\delta^{18}O_{body\ water}$ is -5.2‰, which is comparable to the mean annual surface water values found in the southern Appalachians today (Appendix A) (Bowen et al. 2005). These are more constrained for *Equus,* ranging from -5.0‰ to -8.2‰, in contrast to *Bootherium*, which ranges from -14.1‰ to 2.0‰. No strong correlation was found between $\delta^{18}O$ and $\delta^{13}C$; the $R^2$ for *Equus* and *Bootherium* is 0.296. Individually, *Equus* has an $R^2$ of 0.158, and *Bootherium* has an $R^2$ of 0.179. Descriptive statistics for $\delta^{18}O$ can be found in table 3.2.

Table 3.2: Summary of Saltville *Equus* and *Bootherium* Stable Oxygen Isotope samples

| Lab Number | Taxon | Mean $\delta^{18}O_{enamel}$ | Range $\delta^{18}O_{enamel}$ |
|---|---|---|---|
| ETSU-2018-14 | *Bootherium* | -3.0 | -14.1 to 1.4 |
| ETSU-2018-15 | *Bootherium* | -6.0 | -8.5 to -3.0 |
| ETSU-2018-16 | *Bootherium* | -2.7 | -4.6 to -1.1 |
| ETSU-2018-17 | *Bootherium* | -5.5 | -8.6 to -3.6 |
| All | *Bootherium* | -4.4 | -14.1 to 1.4 |
| ETSU-2018-18 | *Equus* | -7.0 | -8.2 to -5.1 |
| ETSU-2018-19 | *Equus* | -6.7 | -7.6 to -5.7 |
| All | *Equus* | -6.9 | -8.2 to -5.1 |
| All | *Equus & Bootherium* | -5.2 | -1.4 |

Vegetation was sampled for $^{87}Sr/^{86}Sr$ from nine nearby locations to represent local geological variability. These include, one surface vegetation sample from Whitetop Mountain and two samples near Saltville excavation sites, SV5/7 and SV10. Of the sampled localities, $^{87}Sr/^{86}Sr$ is most elevated on Whitetop Mountain (0.7203), which was sampled from the Mount

Rogers Formation, the oldest group sampled, and lower in the valley (0.7094-0.7139), sampled from the Juniata, Reedsville, Trenton, and Eggleston Formations, the Rome Formation, the Elbrook formation, the Maccrady Shale, the Nolichucky and Honaker Formations, the Knox group, the Beekmantown group, and undivided Silurian formations. The samples taken at the Saltville excavation sites were sampled from sediments within the area of the Greenbrier Limestone, and had a value of 0.7139 (USGS). Values from grass samples used to create an isoscape are found in Appendix C. The local isoscape created in this study is in Figure 3.1, with Figure 3.2 showing the isoscape in the area immediately surrounding the excavation sites.

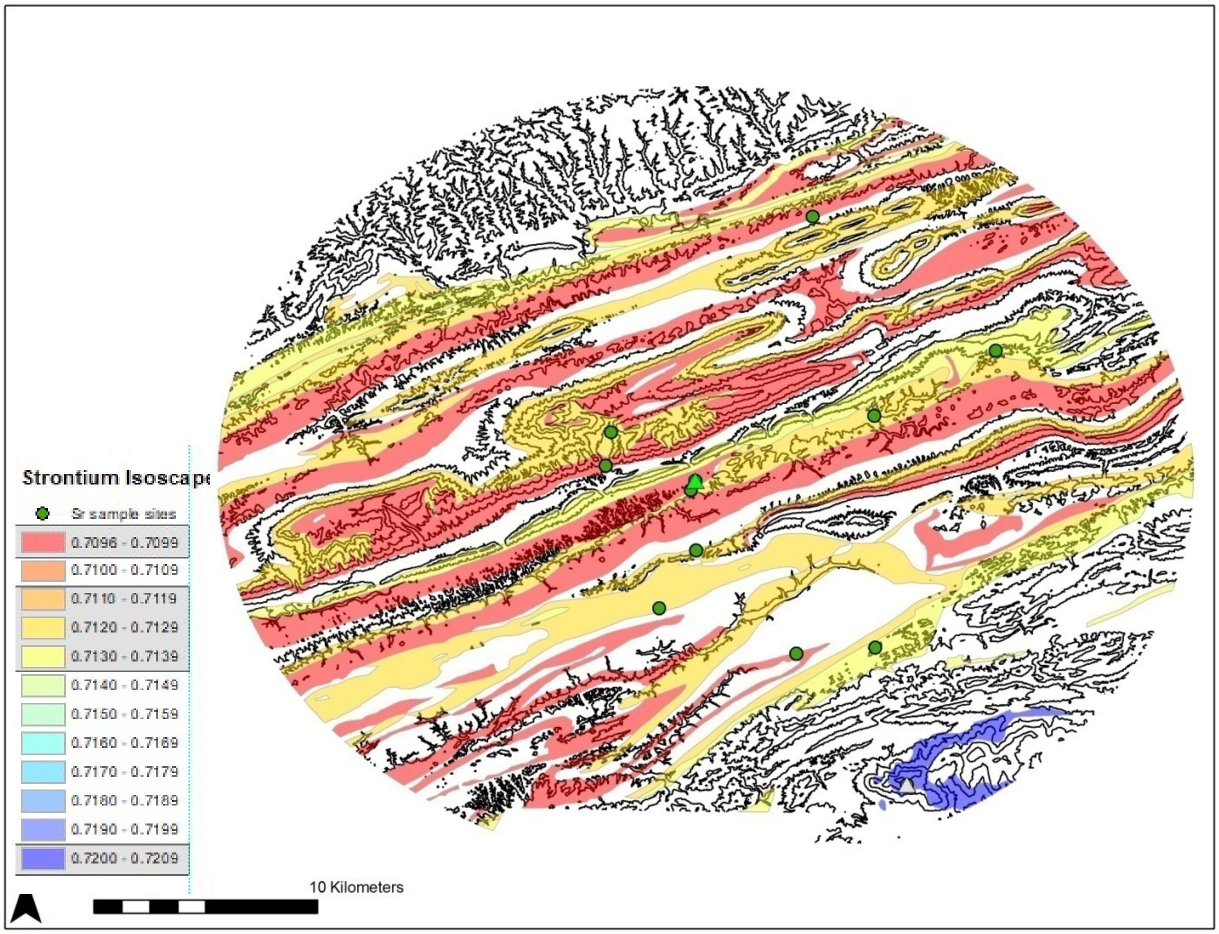


Figure 3.1. Strontium Isoscape created from sampled grasses. The bands represent $^{87}$Sr/$^{86}$Sr values sampled within each geologic formation, with the more elevated values found at Whitetop

Mountain in blue. The base map is a contour map representing elevation. Saltville is represented by the green triangle, and other sampled sites are represented by green circles.

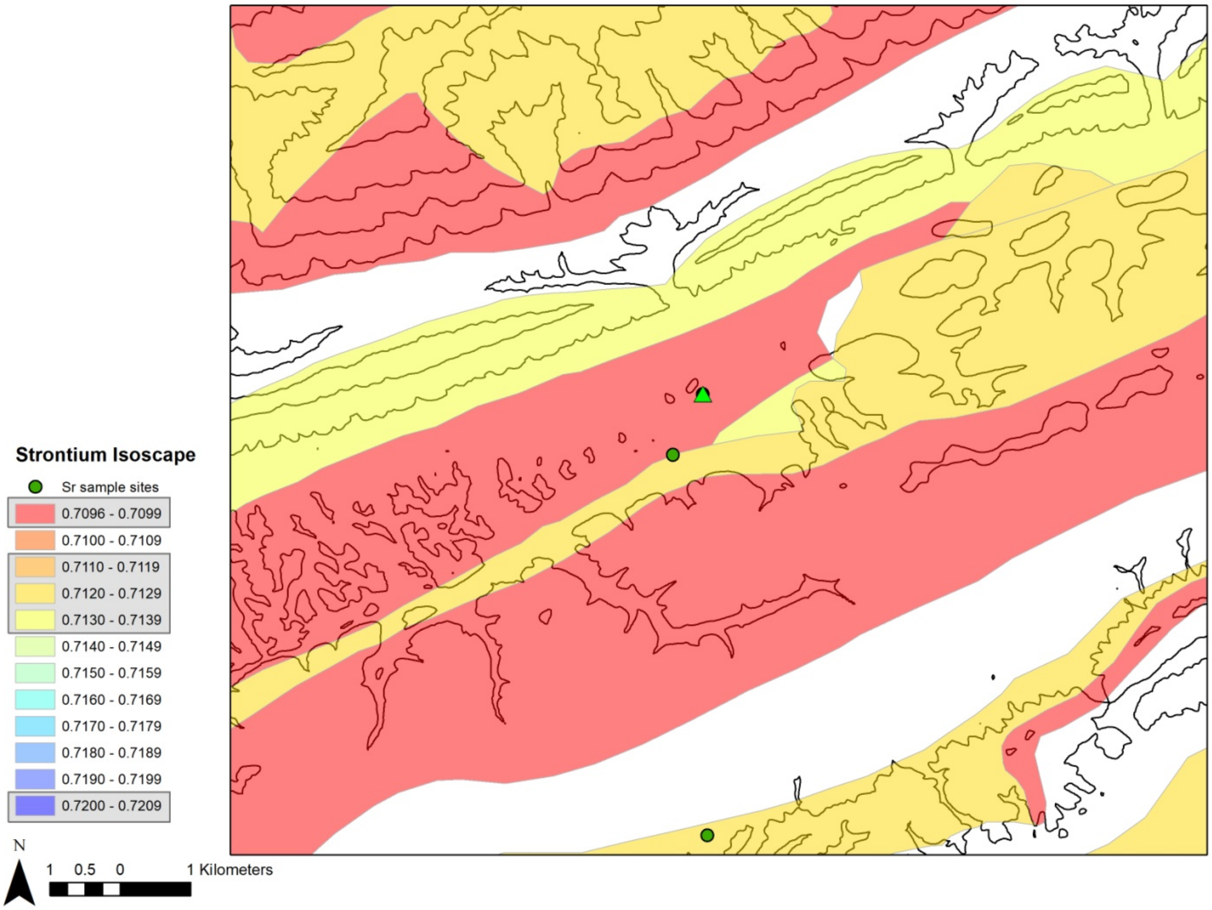


Figure 3.2. Local $^{87}Sr/^{86}Sr$ isoscape in Saltville, Virginia

This local-scale, surface vegetation dataset is compared to published, continental-scale strontium isoscapes derived from bedrock, weathering, and surface water models (Bataille and Bowen 2012). The dataset reported here did not vary significantly from Bataille and Bowen's (2012) bedrock weathering (*p-value*=0.077) model (Figure 3.3), nor was it different from the local water and run-off model derived from the former two (*p-value*=0.752) (Figure 3.4). However, it did vary significantly from the model based on bedrock age (*p-value*=0.008) (Figure 3.5). Strontium isotope ratios vary the most from previous models at higher elevations, although as a whole my isoscape is closest to values predicted based on surface water (Bataille and Bowen 2012).

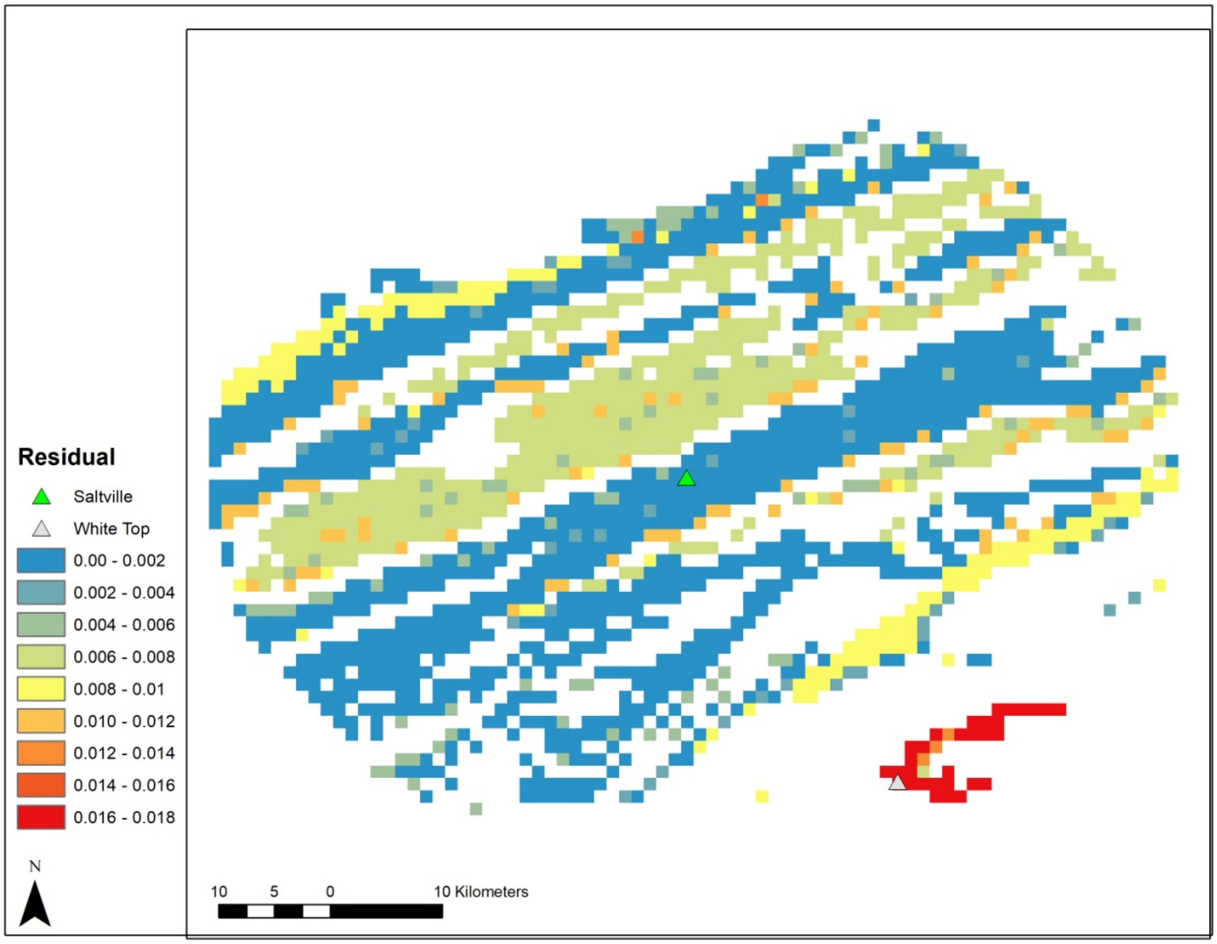


Figure 3.3. Weathered Residual Map, showing the difference between the isoscape modeled by Bataille and Bowen (2012) and the local isoscape shown in figure 3.1

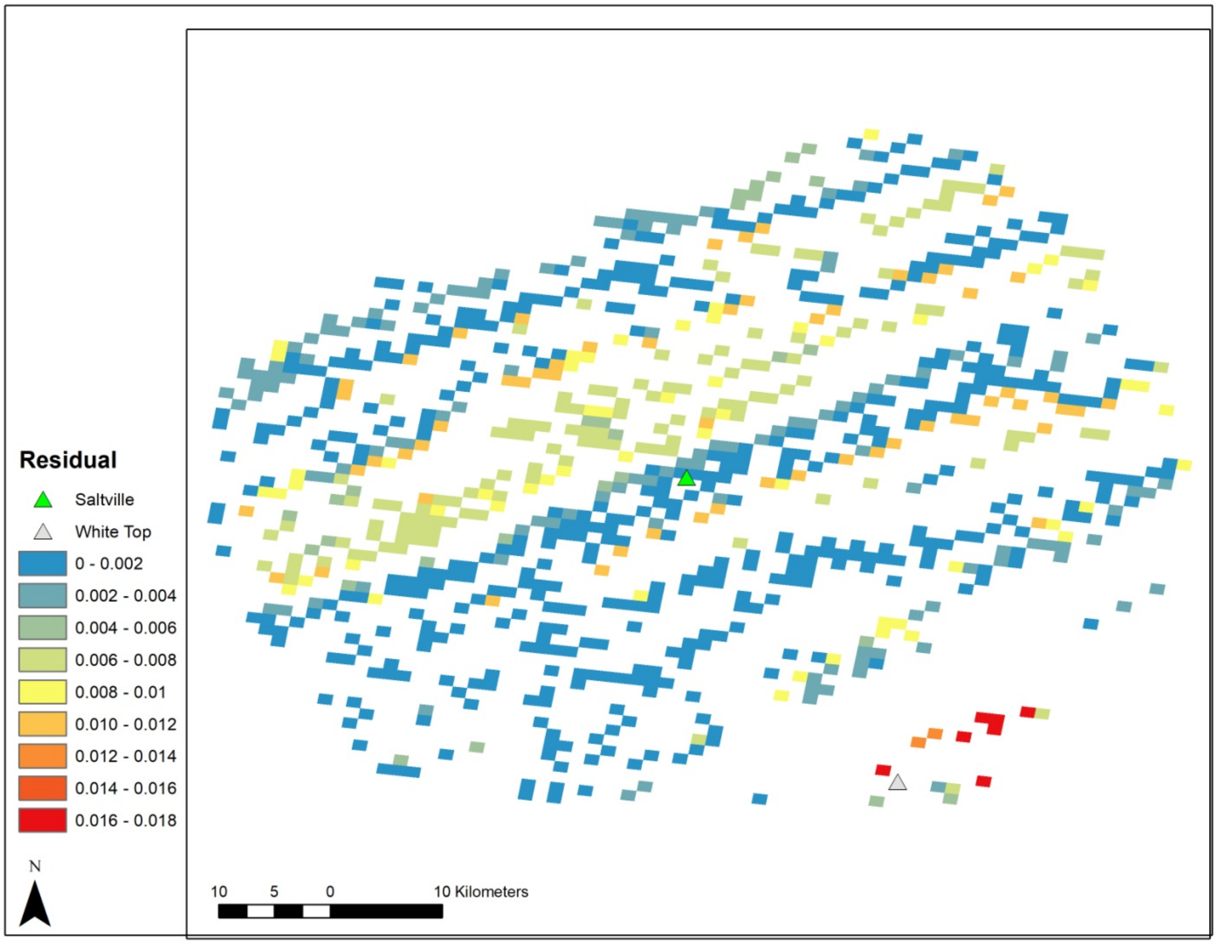


Figure 3.4. Surface Water Residual Map, showing the difference between the isoscape modeled by Bataille and Bowen (2012) and the local isoscape shown in figure 3.1

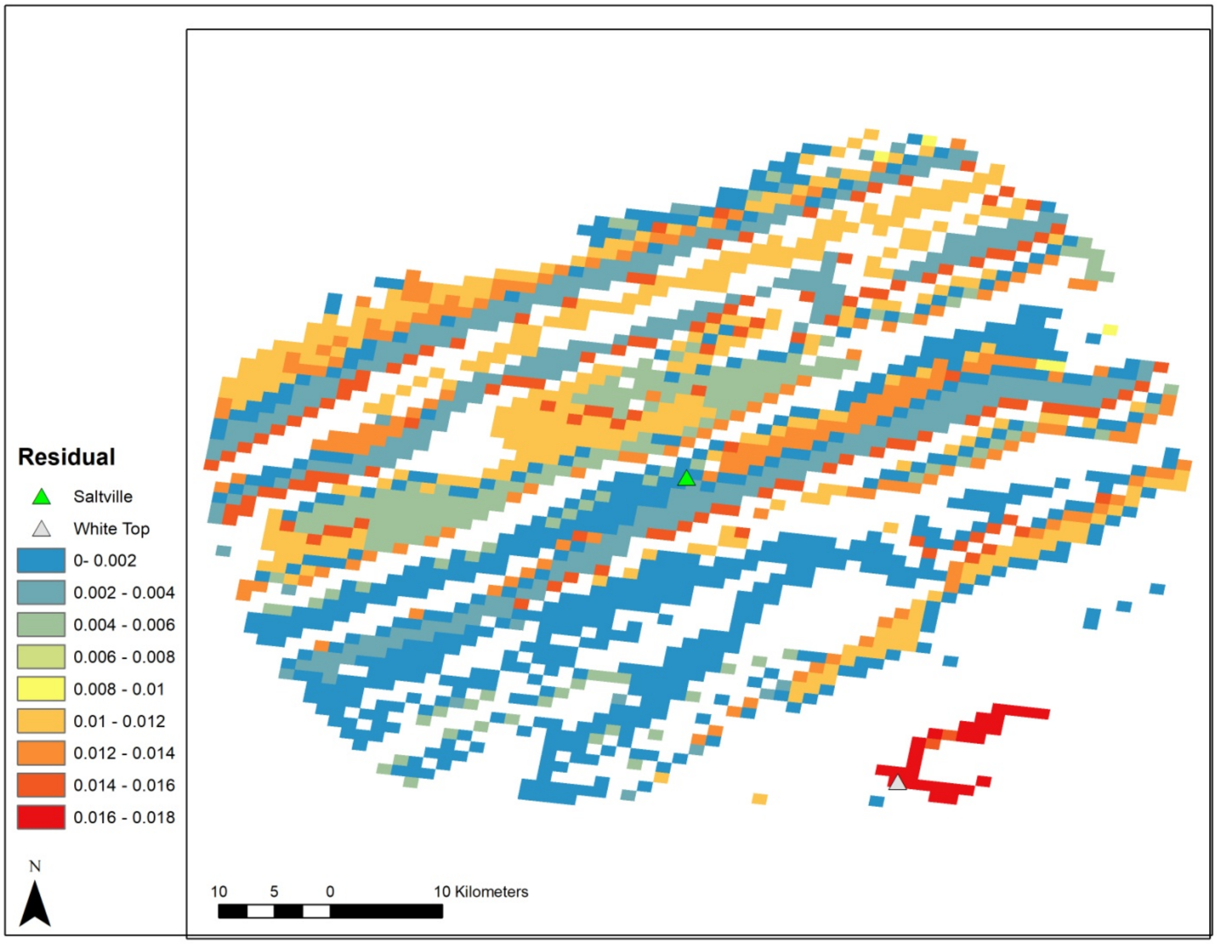


Figure 3.5. Bedrock Residual Map, showing the difference between the isoscape modeled by Bataille and Bowen (2012) and the local isoscape shown in figure 3.1

Tooth enamel $^{87}Sr/^{86}Sr$ values vary significantly between taxa (p= 0.034). However, these values do not vary significantly across individuals within taxa (p=0.220). Neither *Bootherium* nor *Equus* spent significant amounts of time at higher elevations since all values are below 0.7200.

# CHAPTER 4

# DISCUSSION

All samples analyzed in this study have $\delta^{13}C$ values below -20‰, indicating a C3 diet in a mostly closed canopy environment. *Bootherium* tends to have less negative, more variable $\delta^{13}C$ values than *Equus,* and an even greater amount of intra-tooth amplitude in $\delta^{18}O$. *Bootherium* could have had some C4 plants in its diet, or these animals may have simply occupied a different C3 dietary niche. $\delta^{13}C$ and $\delta^{18}O$ vary significantly between teeth, and *Equus* is significantly different in both values from *Bootherium.* Variation in $\delta^{13}C$ is represented in Figure 4.1, and variation in $\delta^{18}O$ is represented in Figure 4.2.However, shifts in $\delta^{13}C$ do not correlate strongly with variation in $\delta^{18}O$, which suggests a lack of seasonality in dietary input($R^2$=0.297) (Figure 4.3).

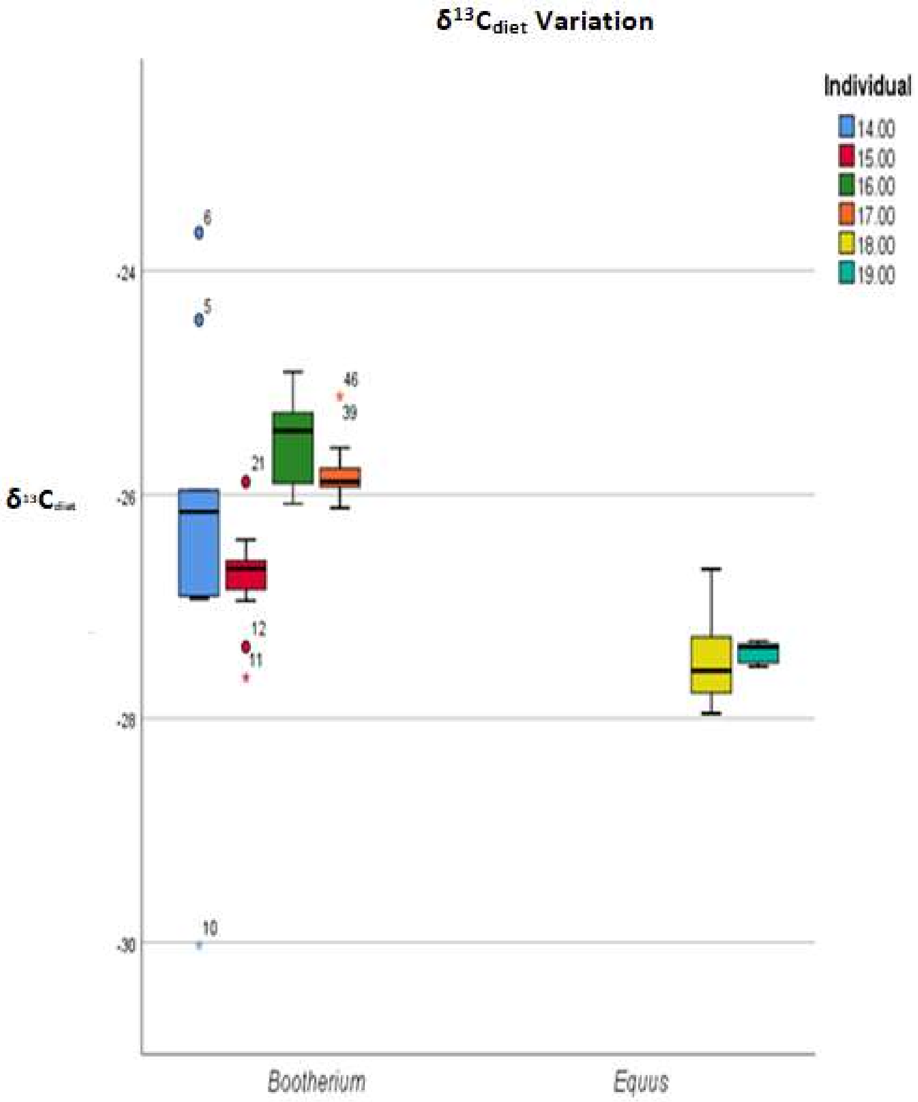


Figure 4.1. Variability in $\delta^{13}C_{diet}$ of Saltville megaherbivores. Outliers are represented by numbers. Point 5 represents, ETSU-2018-14-6, point 6 represents ETSU-2018-14-7, and point 10 represents sample ETSU-2018-14-11. Point 11 represents ETSU-2018-15-2, point 12 represents ETSU-2018-15-3, and point 21 represents ETSU-2018-15-12. Point 39 represents ETSU-2018-17-7, and point 46 represents ETSU-2018-17-14

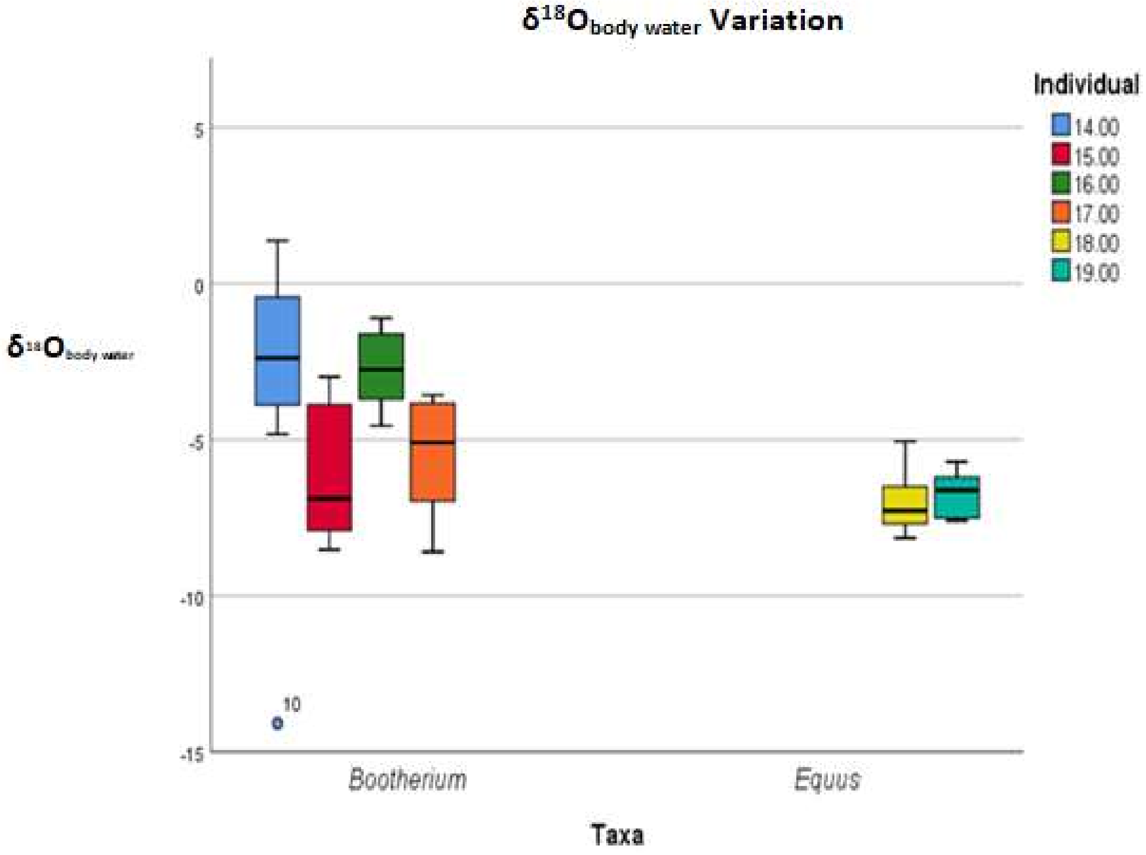


Figure 4.2. Variation in $\delta^{18}O_{\text{body water}}$ in Saltville megaherbivores. Point 10 represents ETSU-2018-14-11

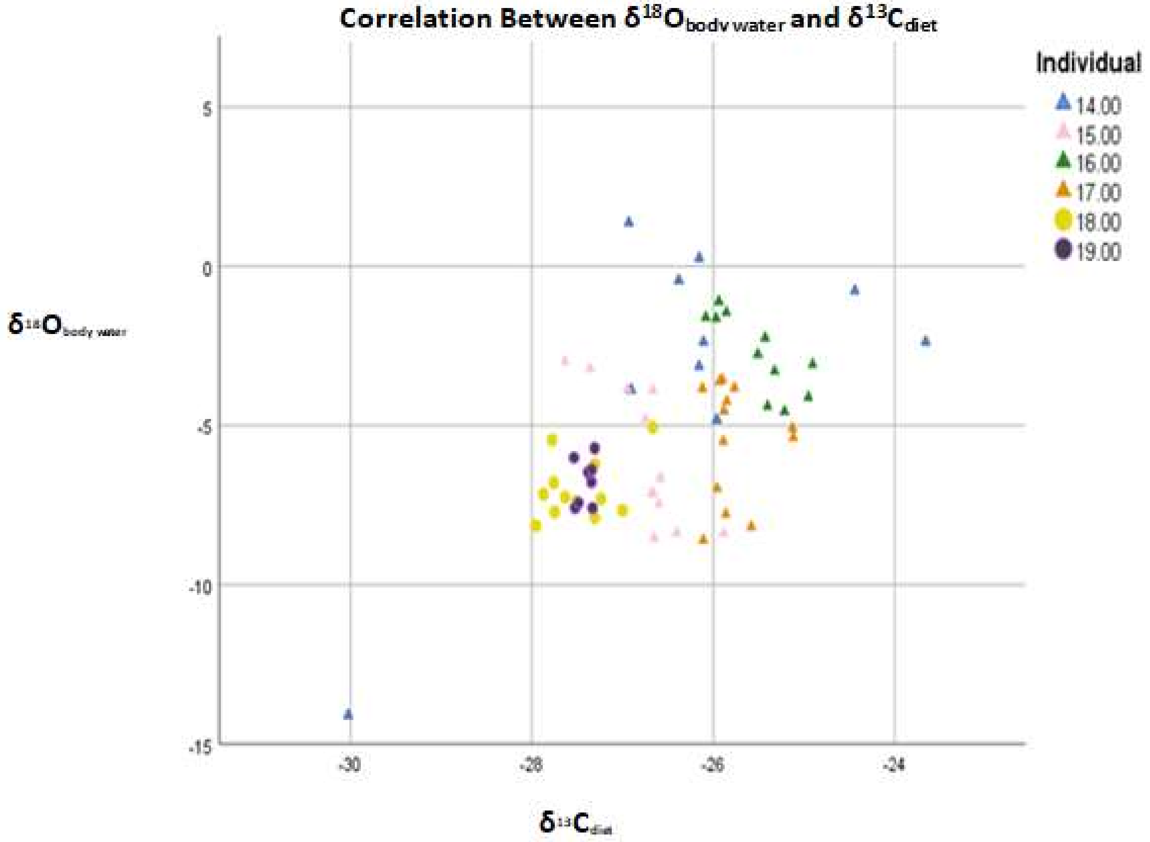


Figure 4.3. Correlation Between $\delta^{13}C_{diet}$ and $\delta^{18}O_{body\ water}$. *Bootherium* is represented by triangles, *Equus* is represented by circles

The mean $\delta^{18}O_{body\ water}$ value for all sampled teeth are comparable to modern values of $\delta^{18}O$ modeled by Bowen et al. (2005) in the southern Appalachian region (Figure 4.4). The mean $\delta^{18}O$for all samples, 5.2‰, suggests a mean annual temperature (MAT) between 15 and 25 degrees Celsius, based on the relationship between $\delta^{18}O_{body\ water}$ and MAT described by Pryor et al. (2014). This relatively warm environment is consistent with that proposed based on the herpetofauna (Holman 1986); however, this is warmer than expected based on the pollen record (Ray et al. 1986; Delcourt and Delcourt 1986), which suggested a taiga or steppe environment. The difference in amplitude between *Equus* and *Bootherium* suggests the use of different water sources. Small order streams will exhibit greater seasonal variability in $\delta^{18}O$ due to changing $\delta^{18}O$ in precipitation (Coplen and Kendall 2000). In contrast, more constrained values in *Equus* may indicate use of water sources lower in the drainage such as large lakes and rivers (Coplen and Kendall 2000). However, less variation in *Equus* teeth may also be the result of sample size or a slower mineralization rate than *Bootherium* (Hoppe et al. 2004). It is also possible that some of the variation is the result of the springs responsible for the source water in Saltville; water from the springs may not reflect precipitation or other surface water. Therefore, the significantly different $\delta^{18}O$ values may indicate a different water source.

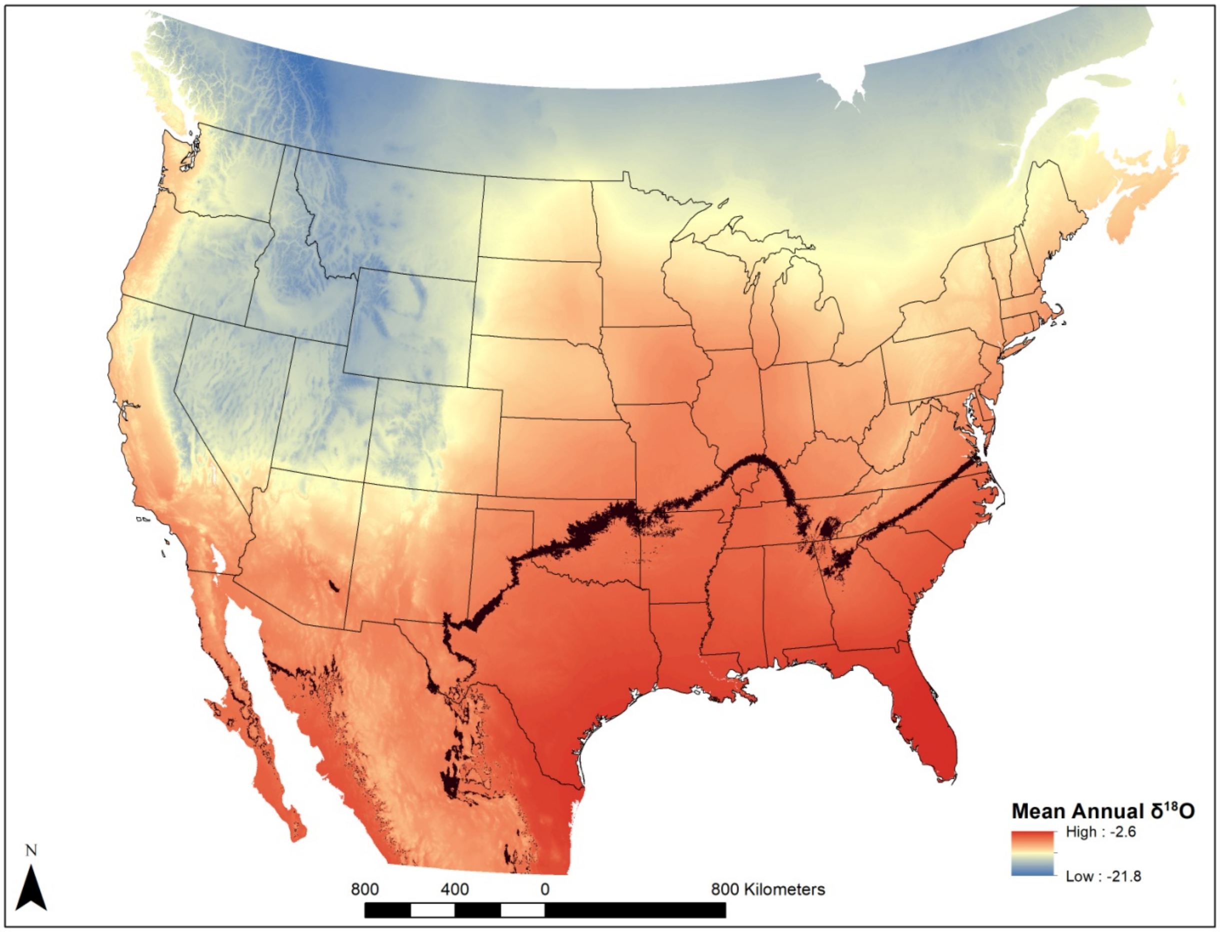


Figure 4.4. Modern mean annual $\delta^{18}O$ isoscape based on precipitation (Bowen 2005) with mean $\delta^{18}O$ of all Saltville samples

The mean $\delta^{13}C_{enamel}$ for each tooth series was compared to results from France et al. (2007) corrected to $\delta^{13}C_{diet}$. France et al. (2007) used collagen from samples of powdered bone material from individuals from Saltville, Virginia. Prior to analysis, collagen values were converted to dietary values, following DeNiro and Epstein (1978). This conversion can be found in Appendix B. The original study reported a general absence of C4 grasses, and suggested that species that would have grazed on C4 plants in other places were able to be sustained by herbaceous C3 plants in the absence of C4 grasses. Within the combined data, these values were found to be significantly different across species, with $p=0.017$. *Equus* stable carbon values were still constrained to between -26‰ and -28‰, despite the addition of another sampled individual. *Equus* samples were more negative than all other taxa. In contrast, *Bootherium* $\delta^{13}C$ ranged from -24‰ to -27‰, with a greater first and fourth interquartile range than *Equus. Bootherium* also overlaps with the $\delta^{13}C$ of reported proboscideans, *Megalonyx*, *Rangifer* and *Cervalces.*

Most sampled teeth from *Bootherium* and *Equus* show little cyclic variation in $\delta^{18}O$ that would suggest seasonality, and little correlation between $\delta^{18}O_{water}$ and $\delta^{13}C_{diet}$. This is especially true of *Equus*. Even compared to previously published (France et al. 2007) collagen samples , *Equus* is on average more negative in $\delta^{13}C$, and more constrained than other taxa (Figure 4.5). However, they are not negative enough to indicate a closed canopy. If there was noticeable seasonality during the time these individuals were alive, this homogenous dietary may indicate regular movement to maintain access to a preferred food source. This has been suggested in other studies, including Chisholm et al. (1986). They proposed that $\delta^{13}C$ should be constant in *Bison* from different localities if a population was moving between sites with the intent of maintaining a constant diet throughout seasons. However, $\delta^{13}C$ varied across sites; therefore, they inferred no seasonal movement between sites.

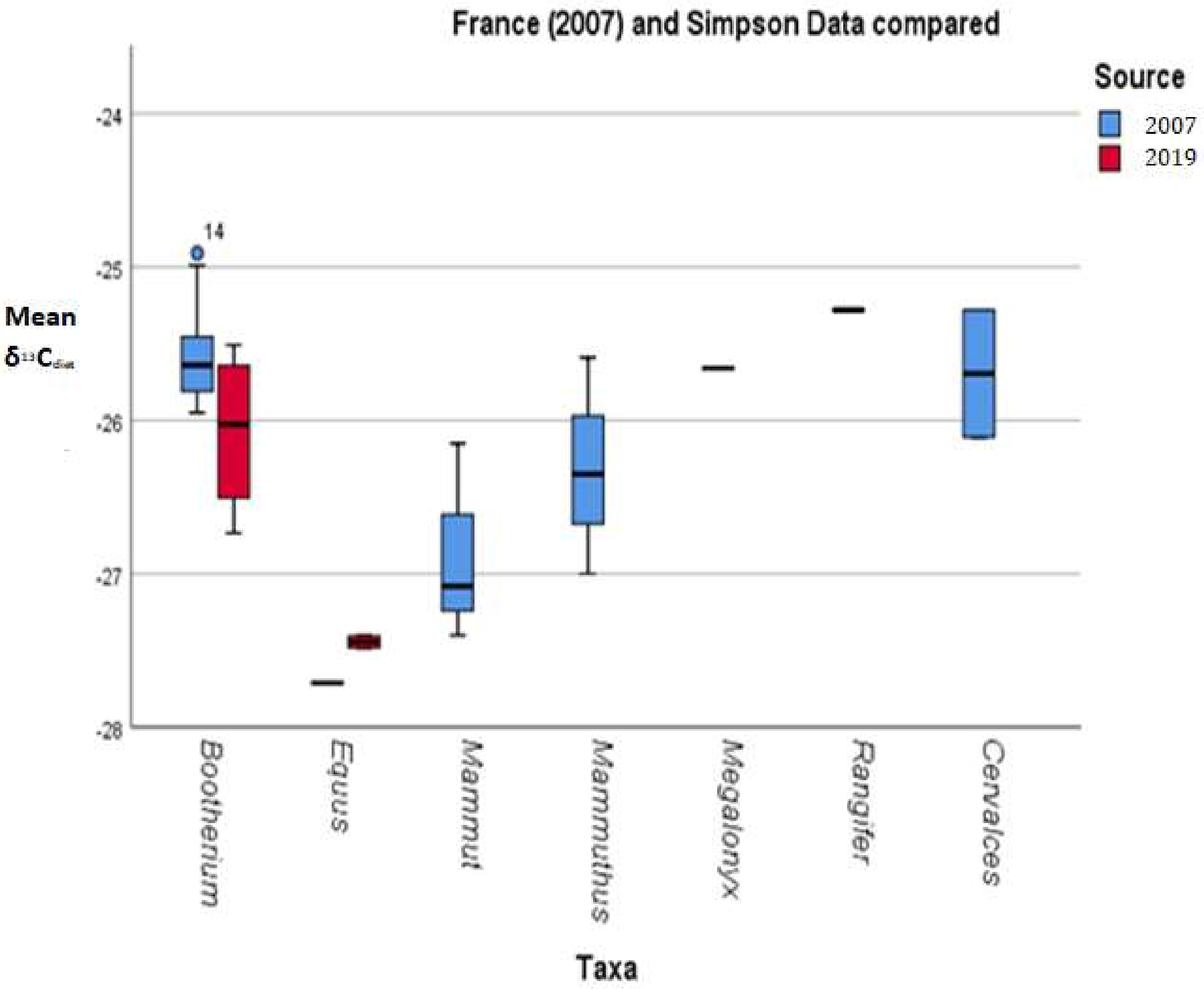


Figure 4.5. Variability in $\delta^{13}C_{diet}$ among multiple Saltville taxa, combined dataset of France et al. (2007) and this study. Outlier 14 represents the mean of samples from ETSU-2018-14

Stable carbon isotope values in *Bootherium* range from -23‰ to -30.0‰, indicating a variable diet that, at their least negative, may include up to 21% C4 plants. More typical are $\delta^{13}C$ values that indicate a completelyC3 diet (<-26.5‰), or even significant time spent in a closed canopy environment (<-28‰). Both extremes are present in a down-tooth series from an upper first molar, ETSU-2018-14, with the less negative values being from near the middle of the tooth and the most negative values being from the latest forming sample, representing mineralization at around 18 months (Allen 1913). The later forming sample is associated with the most negative $\delta^{18}O$ value within the tooth, suggesting that it represents a cooler season. This sample also is associated with the most negative $\delta^{13}C$, which is the only sample in the study that represents feeding in a closed canopy forest environment. The elevated $\delta^{13}C$, with the increase in the amount of C4 potentially in the diet, is associated with an elevated $\delta^{18}O$, or warmer temperature. This individual exhibits a greater amplitude of variation in $\delta^{18}O$ than other individuals; little seasonal variation is observed within other sampled teeth. This might be the result of the use of different water sources, with this individual drinking water from a small, more evaporative source and other individuals drinking from deeper, more averaged sources less prone to seasonal variation. Sampled values of stable carbon and oxygen isotopes are plotted, showing possible cyclic variation in ETSU -2018-14 in Figure 4.6 and little variation in other sampled *Bootherium* molars (Figure 4.7) and the sampled *Equus* molar (Figure 4.8). This tooth could also be from an individual that is not contemporary with other individuals, and might be from a time period of greater seasonal extremes in the $\delta^{18}O$, as opposed to the warmer, milder conditions that may have existed at interglacials (Weigl and Knowles 2014). The most negative, and therefore coldest, $\delta^{18}O$ values suggest tooth formation under colder conditions than those from other samples. However, like other individuals sampled in this study, enamel sampled from this *Bootherium* did not have elevated $^{87}Sr/^{86}Sr$ and was not spending a significant amount of time feeding at higher elevations; different land-use was not the cause of this pattern.

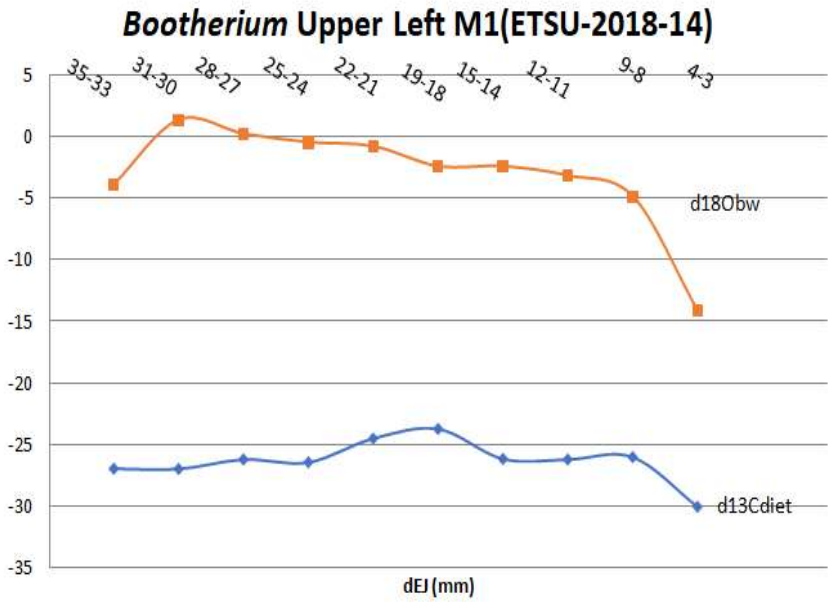
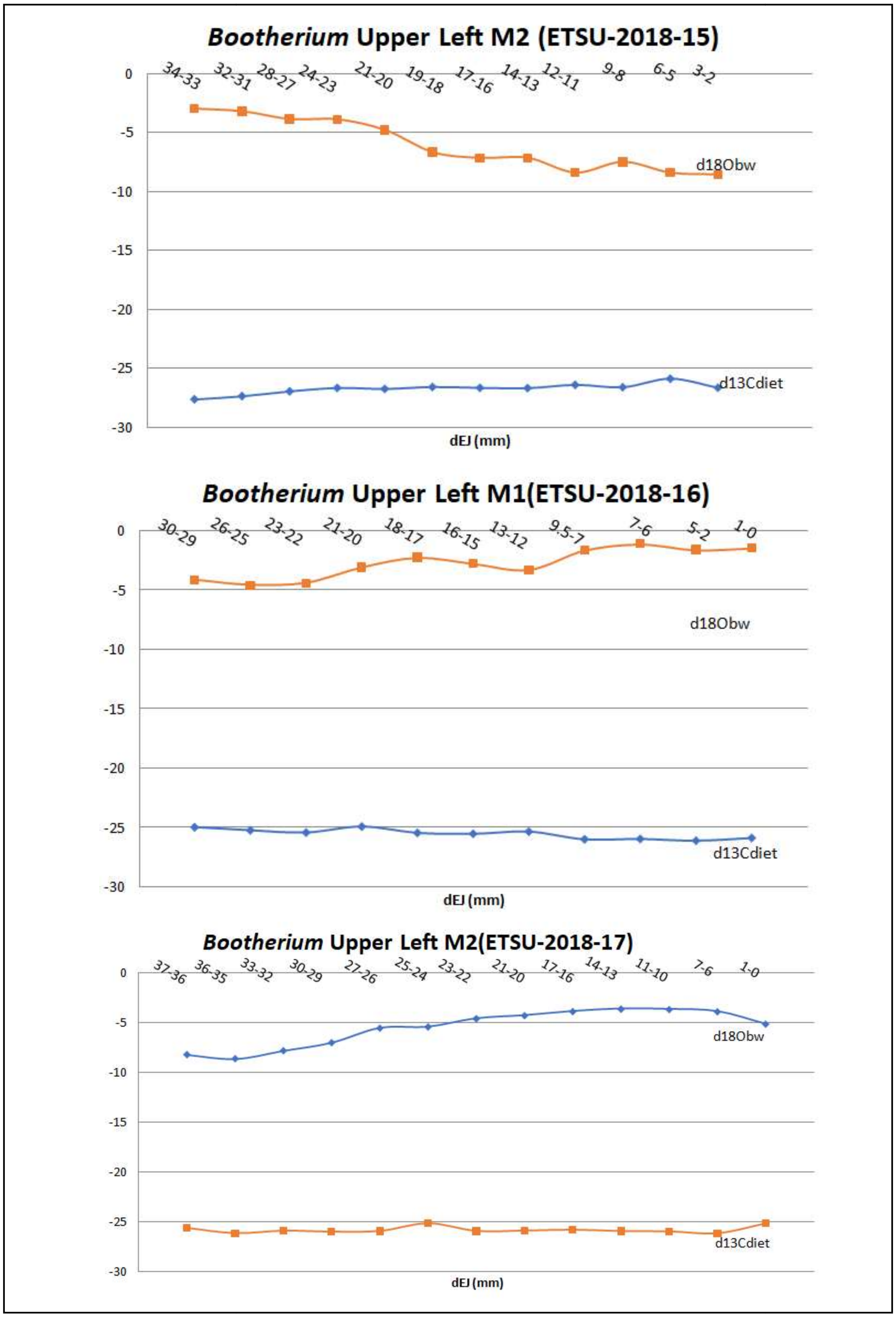


Figure 4.6: Sampled values from ETSU-2018-14


***Bootherium* Upper Left M2 (ETSU-2018-15)**

***Bootherium* Upper Left M1(ETSU-2018-16)**

***Bootherium* Upper Left M2(ETSU-2018-17)**

Figure 4.7: *Bootherium* sample values showing no seasonal variation

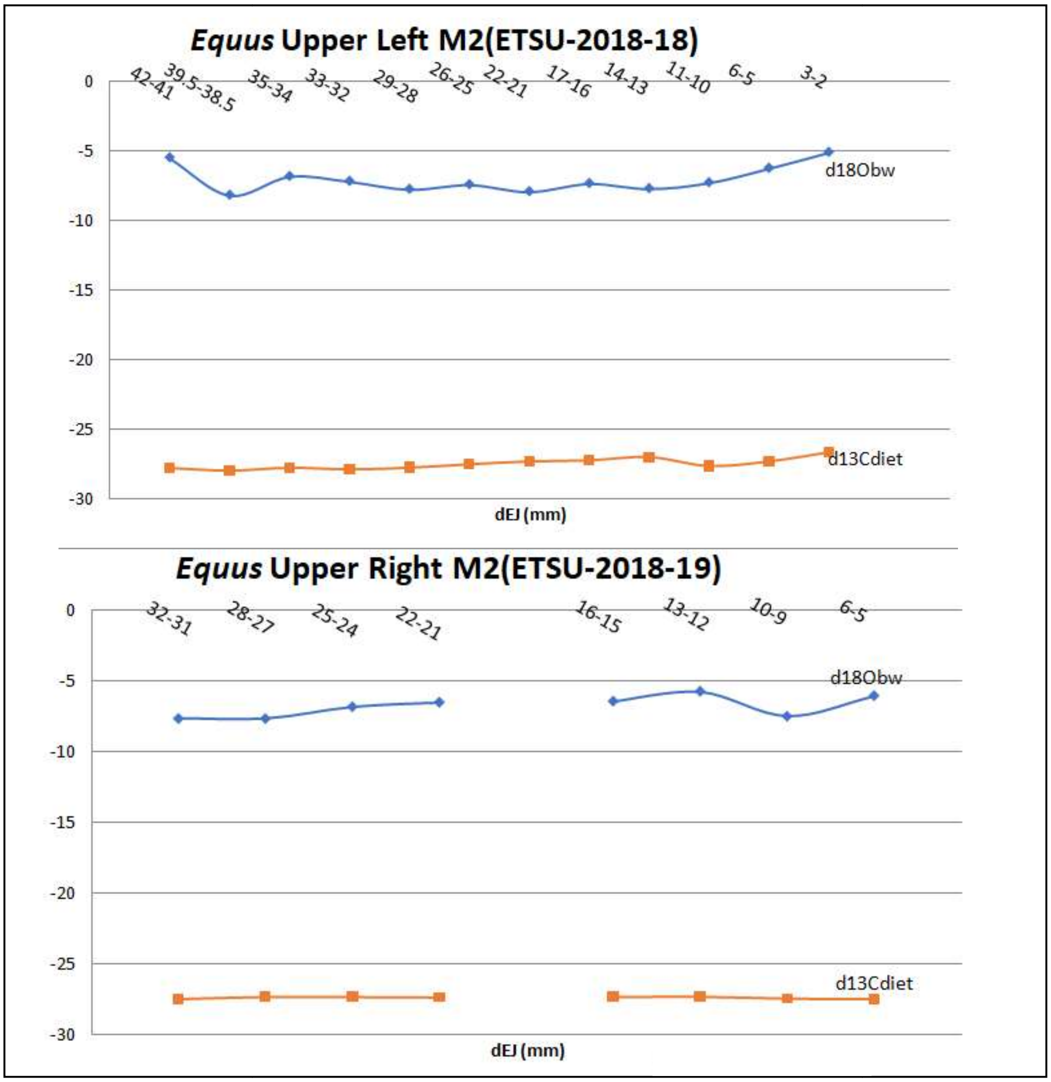


Figure 4.8: *Equus* sampled values showing little seasonal variation

The highest $^{87}$Sr/$^{86}$Sr values were found at higher elevations on Whitetop Mountain, and were more depleted at lower elevations, and were most similar to values modeled by Bataille and Bowen (2012) based on weathering and surface water. $^{87}$Sr/$^{86}$Sr from enamel samples indicate that no individuals were spending a significant amount of time feeding in areas with elevated $^{87}$Sr/$^{86}$Sr; therefore, it is unlikely that *Bootherium* and *Equus* individuals were feeding on Whitetop Mountain or other locations within the Blue Ridge geologic province with more ancient bedrock.

Weigl and Knowles (1995) suggested that the grassy balds, particularly the one on Whitetop Mountain, were formed by harsh, cold conditions during glacial maximums and maintained through milder interglacial periods by large herbivore grazing pressure. The $\delta^{18}O$ amplitude of down-tooth serial samples in most of the individuals analyzed in this study was minimal suggesting that these animals lived during a period of reduced seasonality during an inter-stadial or interglacial period, or relied on buffered sources of water. Therefore, it is unlikely that megaherbivore grazing at higher elevations during warmer periods was significant enough to maintain grassy balds after glacial retreats.

In addition, the location of the bald at Whitetop Mountain is too steep for *Equus* (Kissel 1996) and proboscideans (Ngene et al. 2009; Agenbroad 2003) to ascend directly from the Saltville valley. Ngene et al. (2009) suggested that modern large herbivores also avoid steep slopes more frequently during wetter months; in a mesic environment such as the Pleistocene southern Appalachians, this may have been especially important. A non-direct route to the Whitetop Mountain bald is possible in areas of lower slopes, so physically it is possible for large mammals to feed at higher areas, but navigation to this area would have been neither easy nor direct. *Bootherium* would not have been limited by slope if its behavior was similar to modern musk oxen. Limited access to low-slope routes, and the lack of elevated $^{87}Sr/^{86}Sr$ in both Equus and Bootherium enamel makes it unlikely that individuals were spending, or able to spend, a significant amount of time at higher elevations in the Blue Ridge Mountains.

Strontium isotopes from Saltville *Bootherium* and *Equus* teeth did not indicate that any individual was spending significant amounts of time at higher elevations. This study offers no support for megafaunal maintenance of highland balds during the late Pleistocene. Perhaps instead, they were maintained by the same “plaids” environment that Mann et al. (2019); rapidly changing environmental gradients prevented communities from reaching an equilibrium, leading to several non-analog ecosystems that would not exist in the modern day. They suggested that this created a mosaic environment, patches of ecosystems that did not follow succession in the same way as modern communities that have time to readjust to shifts in conditions. Perhaps these changes maintained a mosaic environment in the case of Whitetop mountain; the variation in glacial extent and eventually the retreat would have altered local climate (Dyke and Prest 1987) faster than succession of local plant communities occur (Mann et al. 2019). When conditions became more stable, those that were not disturbed by anthropogenic causes, such as human induced fires or agricultural grazing, succession may have led to observed declines.

# CHAPTER 5

# CONCLUSIONS

The $\delta^{13}C_{diet}$ of Saltville herbivores indicates the presence of a mosaic environment dominated by C3 cover and limited closed canopy, during the lifetimes of sampled individuals from Saltville, Virginia. There were likely closed canopy forests somewhere nearby; however, this was not the primary niche of individuals included in this study. Assuming that $\delta^{18}O$ is predominantly affected by precipitation, the limited amplitude seen in most sampled individuals suggests that Saltville, Virginia, was surrounded by a warm, mesic environment, possibly with lower extremes in seasonal variation in temperature. The mean $\delta^{18}O$ is consistent with surface water values expected for an environment comparable to the region in the modern day, or even south of this (based on Bowen et al. 2005), indicating a mean annual temperature potentially warmer than today. It is possible that these individuals lived prior to, or after, the LGM, when temperatures were warmer. If $\delta^{18}O$ represents seasonality, then diet ($\delta^{13}C$) and land use ($^{87}Sr/^{86}Sr$) did not vary throughout the year. Despite the lack of seasonality, the warmer climate does not support the hypothesis that there was a more equable environment that was able to support a disharmonious fauna.

Taxa sampled in this study may have experienced limited seasonal variation in temperature given the low $\delta^{18}O$ amplitude, with the exception of VMNH 2304. This individual may not have been contemporary with the others. It is also possible that the $\delta^{18}O$ signal from mean annual temperature is dampened by a buffered water source. Larger bodies of water average seasonal variation in $\delta^{18}O$ more than smaller bodies of water (Coplen and Kendall 2000). If VMNH 2304 were contemporary with other sampled individuals, it could have been drinking from a smaller stream, and thus $\delta^{18}O$ was less dampened.

The low amplitude in $\delta^{18}O$ of VMNH 2304 could also be attributed to utilization of a groundwater source. Springs and seeps are present in modern day Saltville, and may have also been available to the individuals sampled in this study. Stuart-Williams and Schwarcz (1996) sampled groundwater to determine sources of dampening of seasonal variation in $\delta^{18}O$ values from beaver teeth. They found values more elevated than predicted surface water in the same areas; a value of -5.8‰ was observed in northern Missouri. Rather than the Saltville mean of -5.3‰ representing surface water in a relatively warm area, it may be equivalent to groundwater values found in the modern day at latitudes further north, suggesting an environment that may have been able to support the floral community reported by Ray et al. (1967). This would support a more "equable," cooler environment that would be needed for a disharmonious flora and fauna (Semken et al. 2010; Hibbard 1960).

Chronology is important to context when discussing potentially disharmonious faunas (Semken et al. 2010). To test whether the fauna found Saltville, Virginia, represents a non-analog, disharmonious environment, it needs to be determined whether sampled teeth are contemporaneous. Teeth may be from different times, and reworked from another source (McDonald and Bartlett 2000). If it is determined that they are contemporaneous, it would either suggest a more "equable," although warmer than expected, with one individual potentially being from elsewhere, or that most individuals were drinking from a large water source, with one individual drinking from a smaller source. If the samples are not contemporaneous, the sample is likely time-averaged.

Previously modeled isoscapes based on weathering and surface water and run-off are strong predictors of local $^{87}Sr/^{86}Sr$ in the valleys. However, no models were strong predictors of $^{87}Sr/^{86}Sr$ values at higher elevations. At this time, it is unknown why $^{87}Sr/^{86}Sr$ values are depleted compared to those of the original source rock. $^{87}Sr/^{86}Sr$ sampled from enamel, compared to this local isoscape, indicate that individuals were not spending a significant amount of time in regions more radiogenic than the valley itself; therefore it is not likely that they maintained nearby grassy balds environments. There were also no values below 0.7010; however, more depleted values make up a large portion of the valley in the modern day, particularly the excavation sites themselves (Figure 3.2). This could be the result of the teeth having been reworked with the gravel layer, but it is also possible that sediments with $^{87}Sr/^{86}Sr$ depleted below 0.7010 may not have been available to be incorporated by plants and animals during the Quaternary. However, there is no evidence for the large herbivores were spending a significant amount of time feeding on the grassy balds, and thus depending on the age of the sampled specimens, there does not seem to be any support for the idea that they were responsible for the maintenance of these environments.

## Further Work

To better understand the context that individuals in this study fit into, radiocarbon dating will need to be done to correlate them with glacial extent, as well as to give ecological context to the samples. Studying the fluvial history of the valley may lend data to whether specimens could have been transported from elsewhere. The spring water should also be sampled and compared to modern surface water. In addition, further spatial context derived from expanding the isoscape and increasing the concentration of samples within the current study area. In particular, other mountains in the region and the sides of Whitetop mountain should be further sampled, especially the side of the mountain with a lower slope that was previously outside of the defined radius for scope of this study, to better understand local patterns. The increased sampling of additional taxa will also provide a better idea of landscape use of the entire community of large vertebrates, especially proboscideans. A mammoth enamel ridge plate (VMNH 2277) will be sampled at intervals of about 3 mm, representing approximately 1 year per sample. Bulk samples will also be taken from three *Mammut* molars (ETMNH 19334, ETMNH 19335, and ETMNH 19307). This will allow for specific testing of the hypothesis that proboscideans maintained the balds initially after the retreat of the glaciers (Weigl and Knowles 1995).

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# APPENDICES

## Appendix A

### Raw Data and Corrected Values from Serially Sampled Enamel

| Catalog Number | Taxa | Tooth ID | Sample ID | Distance from DEJ (mm) | $\delta^{13}C_{enamel}$ | $\delta^{13}C_{diet}$ | %C4 | $\delta^{18}O_{enamel}$ | $\delta^{18}O_{bw}$ | $^{87}Sr/^{86}Sr$ |
|---|---|---|---|---|---|---|---|---|---|---|
| VMNH 2304 | *Bootherium* | $LM^1$ | ETSU-2018-14-2 | 33-35 | -12.8 | -26.9 | -2.9 | -4.1 | -3.9 | |
| | | | ETSU-2018-14-3 | 30-31 | -12.8 | -26.9 | -2.1 | -0.4 | 1.4 | |
| | | | ETSU-2018-14-4 | 27-28 | -12.1 | -26.2 | 2.5 | -1.2 | 0.3 | |
| | | | ETSU-2018-14-5 | 24-25 | -12.3 | -26.4 | 0.8 | -1.7 | -0.4 | |
| | | | ETSU-2018-14-6 | 21-22 | -10.3 | -24.4 | 14.9 | -1.9 | -0.8 | |
| | | | ETSU-2018-14-7 | 18-19 | -9.6 | -23.7 | 20.6 | -3.0 | -2.4 | 0.7104 |
| | | | ETSU-2018-14-8 | 14-15 | -12.0 | -26.1 | 2.8 | -3.0 | -2.4 | |
| | | | ETSU-2018-14-9 | 11-12 | -12.1 | -26.2 | 2.5 | -3.6 | -3.1 | |
| | | | ETSU-2018-14-10 | 8-9 | -11.9 | -26.0 | 3.9 | -4.7 | -4.8 | |
| | | | ETSU-2018-14-11 | 3-4 | -15.9 | -30.0 | -25.5 | -11.1 | -14.1 | 0.7101 |
| VMNH 120141 | *Bootherium* | $LM^2$ | ETSU-2018-15-2 | 33-34 | -13.5 | -27.6 | -8.2 | -3.5 | -3.0 | 0.7103 |
| | | | ETSU-2018-15-3 | 31-32 | -13.3 | -27.4 | -6.2 | -3.6 | -3.2 | |
| | | | ETSU-2018-15-4 | 27-28 | -12.8 | -26.9 | -3.3 | -4.1 | -3.9 | |
| | | | ETSU-2018-15-5 | 23-24 | -12.6 | -26.7 | -1.2 | -4.1 | -3.9 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | ETSU-2018-15-6 | 20-21 | -12.6 | -26.7 | -1.8 | -4.7 | -4.8 | |
| | | | ETSU-2018-15-7 | 18-19 | -12.5 | -26.6 | -0.6 | -6.0 | -6.7 | 0.7110 |
| | | | ETSU-2018-15-8 | 16-17 | -12.6 | -26.7 | -1.1 | -6.3 | -7.1 | |
| | | | ETSU-2018-15-9 | 13-14 | -12.6 | -26.7 | -1.3 | -6.3 | -7.1 | |
| | | | ETSU-2018-15-10 | 11-12 | -12.3 | -26.4 | 0.7 | -7.2 | -8.3 | |
| | | | ETSU-2018-15-11 | 8-9 | -12.5 | -26.6 | -0.7 | -6.6 | -7.5 | |
| | | | ETSU-2018-15-12 | 5-6 | -11.8 | -25.9 | 4.4 | -7.2 | -8.4 | |
| | | | ETSU-2018-15-13 | 2-3 | -12.6 | -26.7 | -1.1 | -7.3 | -8.5 | 0.7100 |
| VMNH-2392 | *Bootherium* | LM[1] | ETSU-2018-16-2 | 29-30 | -10.9 | -25.0 | 11.2 | -4.2 | -4.1 | |
| | | | ETSU-2018-16-3 | 25-26 | -11.1 | -25.2 | 9.3 | -4.5 | -4.6 | 0.7104 |
| | | | ETSU-2018-16-4 | 22-23 | -11.3 | -25.4 | 8.0 | -4.4 | -4.4 | |
| | | | ETSU-2018-16-5 | 20-21 | -10.8 | -24.9 | 11.6 | -3.5 | -3.1 | |
| | | | ETSU-2018-16-6 | 17-18 | -11.3 | -25.4 | 7.7 | -2.9 | -2.3 | |
| | | | ETSU-2018-16-7 | 15-16 | -11.4 | -25.5 | 7.2 | -3.3 | -2.8 | |
| | | | ETSU-2018-16-8 | 12-13 | -11.2 | -25.3 | 8.5 | -3.7 | -3.3 | |
| | | | ETSU-2018-16-9/10 | 7-9.5 | -11.9 | -26.0 | 3.8 | -2.5 | -1.6 | |
| | | | ETSU-2018-16-11 | 6-7 | -11.8 | -25.9 | 4.1 | -2.4 | -1.1 | 0.7111 |
| | | | ETSU- | 2-5 | -12.0 | -26.1 | 3.0 | -2.5 | -1.6 | |

|  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  | 2018-16-12/13 |  |  |  |  |  |  |  |
|  |  |  | ETSU-2018-16-14 | 0-1 | -11.8 | -25.9 | 4.7 | -2.4 | -1.4 |  |
| VMNH-2321 | *Bootherium* | LM$^2$ | ETSU-2018-17-2 | 36-37 | -11.5 | -25.6 | 6.7 | -7.1 | -8.2 |  |
|  |  |  | ETSU-2018-17-3 | 35-36 | -12.0 | -26.1 | 2.8 | -7.4 | -8.6 | 0.7103 |
|  |  |  | ETSU-2018-17-4 | 32-33 | -11.8 | -25.9 | 4.6 | -6.8 | -7.8 |  |
|  |  |  | ETSU-2018-17-5 | 29-30 | -11.9 | -26.0 | 3.9 | -6.2 | -7.0 |  |
|  |  |  | ETSU-2018-17-6 | 26-27 | -11.8 | -25.9 | 4.4 | -5.2 | 5.5 |  |
|  |  |  | ETSU-2018-17-7 | 24-25 | -11.0 | -25.1 | 10.0 | -5.1 | -5.4 | 0.7108 |
|  |  |  | ETSU-2018-17-8 | 22-23 | -11.8 | -25.9 | 4.5 | -4.5 | -4.5 |  |
|  |  |  | ETSU-2018-17-9 | 20-21 | -11.8 | -25.9 | 4.7 | -4.3 | -4.2 |  |
|  |  |  | ETSU-2018-17-10 | 16-17 | -11.7 | -25.8 | 5.3 | -4.0 | -3.8 |  |
|  |  |  | ETSU-2018-17-11 | 13-14 | -11.8 | -25.9 | 4.3 | -3.9 | -3.6 |  |
|  |  |  | ETSU-2018-17-12 | 10-11 | -11.8 | -25.9 | 4.1 | -3.9 | -3.6 |  |
|  |  |  | ETSU-2018-17-13 | 6-7 | -12.0 | -26.1 | 2.8 | -4.0 | -3.8 |  |
|  |  |  | ETSU-2018-17-14 | 0-1 | -11.0 | -25.1 | 9.9 | -4.9 | -5.1 |  |
| VMNH 120142 | *Equus* | LM$^2$ | ETSU-2018-18-2 | 41-42 | -13.7 | -27.8 | -9.3 | -5.2 | -5.5 |  |
|  |  |  | ETSU-2018-18-3 | 38.5-39.5 | -13.9 | -28.0 | -10.6 | -7.0 | -8.1 | 0.7118 |
|  |  |  | ETSU-2018-18-4 | 34-35 | -13.7 | -27.8 | -9.1 | -6.1 | -6.8 |  |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | ETSU-2018-18-5 | 32-33 | -13.8 | -27.9 | -9.9 | -6.4 | -7.2 | |
| | | | ETSU-2018-18-6 | 28-29 | -13.7 | -27.8 | -9.1 | -6.8 | -7.7 | |
| | | | ETSU-2018-18-7 | 25-26 | -13.4 | -27.5 | -7.3 | -6.5 | -7.4 | |
| | | | ETSU-2018-18-8 | 21-22 | -13.2 | -27.3 | -5.9 | -6.9 | -7.9 | |
| | | | ETSU-2018-18-9 | 16-17 | -13.1 | -27.2 | -5.3 | -6.5 | -7.3 | |
| | | | ETSU-2018-18-10 | 13-14 | -12.9 | -27.0 | -3.6 | -6.7 | -7.7 | |
| | | | ETSU-2018-18-11 | 10-11 | -13.5 | -27.6 | -8.2 | -6.4 | -7.2 | |
| | | | ETSU-2018-18-12 | 5-6 | -13.2 | -27.3 | -5.8 | -5.7 | -6.2 | |
| | | | ETSU-2018-18-13 | 2-3 | -12.6 | -26.7 | -1.2 | -4.9 | -5.1 | 0.7112 |
| VMNH 2350 | *Equus* | $RM^2$ | ETSU-2018-19-2 | 31-32 | -13.4 | -27.5 | -7.4 | -6.7 | -7.6 | |
| | | | ETSU-2018-19-3 | 27-28 | -13.2 | -27.3 | -6.0 | -6.7 | -7.6 | |
| | | | ETSU-2018-19-4 | 24-25 | -13.2 | -27.3 | -6.1 | -6.1 | -6.8 | |
| | | | ETSU-2018-19-5 | 21-22 | -13.3 | -27.4 | -6.4 | -5.9 | -6.5 | |
| | | | ETSU-2018-19-6* | 18-19 | | | | | | |
| | | | ETSU-2018-19-7 | 15-16 | -13.3 | -27.3 | -6.1 | -5.8 | -6.4 | |
| | | | ETSU-2018-19-8* | 12-13 | -13.3 | -27.3 | -5.9 | -5.4 | -5.7 | |
| | | | ETSU-2018-19-9 | 9-10 | -13.5 | -27.5 | -7.1 | -6.6 | -7.4 | |
| | | | ETSU-2018-19-10 | 5-6 | -13.5 | -27.5 | -7.5 | -5.6 | -6.0 | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | ETSU-2018-19-11* | 0-1 | | | | | | |
| *Values were not returned | | | | | | | | | | |

# Appendix B

France et al. (2007) $\delta^{13}C_{collagen}$ dataset. $\delta^{13}C_{diet}$ is calculated using $\delta^{13}C_{diet} = \delta^{13}C_{collagen} - 5$ following DeNiro and Epstein (1978)

| Taxa | Specimen number | $\delta^{13}C_{collagen}$ ‰ PDB (France et al. 2007) | $\delta^{13}C_{diet}$‰ PDB |
|---|---|---|---|
| *Bootherium (N=13)* | Musk ox A | -19.99 | -24.99 |
| | Musk ox B | -20.48 | -25.48 |
| | Musk ox C | -20.95 | -25.95 |
| | Musk ox D | -20.4 | -25.4 |
| | Musk ox E | -20.61 | -25.61 |
| | Musk ox F | -20.94 | -25.94 |
| | Musk ox G | -20.46 | -25.46 |
| | Musk ox H | -19.91 | -24.91 |
| | Musk ox I | -20.67 | -25.67 |
| | Musk ox J | -20.64 | -25.64 |
| | Musk ox K | -20.71 | -25.71 |
| | Musk ox L | -20.81 | -25.81 |
| | Musk ox N | -20.90 | -25.90 |
| *Equus (N=1)* | Horse | -22.71 | -27.71 |
| *Mammut (N=3)* | Mastodon A | -21.15 | -26.15 |
| | Mastodon B | -22.4 | -27.4 |
| | Mastodon C | -22.08 | -27.08 |
| *Mammuthus (N=3)* | Mammoth A | -20.59 | -25.59 |
| | Mammoth B | -21.35 | -26.35 |
| | Mammoth C | -22.00 | -27.00 |
| *Megalonyx (N=1)* | Megalonyx | -20.66 | -25.66 |
| *Rangifer (N=1)* | Caribou | -20.28 | -25.28 |
| *Cervalces (N=2)* | Deer A | -21.11 | -26.11 |

| | | | |
|---|---|---|---|
| | Deer B | -20.28 | -25.28 |

# Appendix C

## Strontium Values for Local Isoscape

| Sample | Latitude | Longitude | Geologic Formation | Geologic Age | Sampled 87Sr/86Sr | Modeled Sr (Bedrock) * | Modeled Sr (surface water)* | Modeled Sr (weathered) * |
|---|---|---|---|---|---|---|---|---|
| SV-2018-5/7 (Saltville) | 36.8719 | -81.774 | Greenbrier Limestone | Mississippian | 0.7094 | 0.7095 | 0.7096 | 0.7089 |
| SV-2018-McDonald | 36.8796 | -81.7702 | Greenbrier Limestone | Mississippian | 0.7097 | 0.7091 | 0.7096 | 0.7089 |
| SV-2018-7 | 37.092 | -81.676 | Knox Group | Cambrian/ Ordovician | 0.7098 | 0.7183 | 0.7098 | 0.7203 |
| SV-2018-9 | 36.89183 | -81.8426 | Undivided Silurian | Silurian | 0.7099 | 0.7143 | 0.7099 | 0.7166 |
| SV-2018-10 | 36.93204 | -81.6263 | Nolichucky and Honaker Formations | Cambrian | 0.7120 | 0.7250 | 0.7120 | 0.7123 |
| SV-2018-11b | 36.98439 | -81.5284 | Maccrady Shale and Price Formations | Mississippian | 0.7139 | 0.7190 | 0.7139 | 0.7123 |
| SV-2018-19b | 36.91861 | -81.8382 | Juniata, Reedsville, Trenton, and Eggleston Formations | Ordovician | 0.7134 | 0.7240 | 0.7125 | 0.7203 |
| SV-2018-20 | 36.82339 | -81.7696 | Juniata, Reedsville, Trenton, and Eggleston Formations | Ordovician | 0.7116 | 0.7240 | 0.7125 | 0.7203 |
| SV-2018-21 | 36.777 | -81.7993 | Elbrook Formation | Cambrian | 0.7122 | 0.7113 | 0.7122 | 0.7113 |
| SV-2018-22 | 36.7453 | -81.6235 | Rome Formation | Cambrian | 0.7138 | 0.7243 | 0.7138 | 0.7219 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| SV-2018-23b | 36.74025 | -81.6891 | Beekman-town Group | Ordovi-cian | 0.7098 | 0.7183 | 0.7098 | 0.7203 |
| SV-2018-Whitetop | 36.6347 | -81.6002 | Mount Rogers Formation | Protero-zoic | 0.7203 | 0.7385 | 0.7203 | 0.7385 |

*from Bataille and Bowen (2012)

# VITA

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