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Natural Isoscapes of Louisiana: Stable Isotope Analysis of Oxygen, Carbon, and Strontium

Miley Jackson

Louisiana State University and Agricultural and Mechanical College, mpj6983@gmail.com

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NATURAL ISOSCAPES OF LOUISIANA:
STABLE ISOTOPE ANALYSIS OF OXYGEN, CARBON, AND STRONTIUM

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Arts

in

The Department of Geography and Anthropology

by
Miley Jackson
B.A., University of Louisiana at Lafayette, 2012
B.S., University of Louisiana at Lafayette, 2012
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Abstract

Stable isotope analysis has been used by archaeologists and anthropologists to better understand time, provenience, and diet of past humans, but the utility of stable isotope analysis in modern humans has not been fully explored. Forensic anthropologists today primarily identify human skeletal remains, and stable isotope analysis of bone may narrow down the possible identities of an individual. Determining the natural variability within the state of Louisiana is the first step in determining whether individuals from different areas of the state are distinguishable from one another.

This thesis investigated the natural isotopic variability in Louisiana with respect to oxygen ($\delta^{18}\text{O}$), carbon ($\delta^{13}\text{C}$), and strontium ($\epsilon^{87}\text{Sr}$) by sampling the bones of white tailed deer throughout the state, as well as two raccoons and one fox. The deer revealed distinct value regions for all three isotopes, which were found to be in agreement with available water data for the state. The two raccoons, fox, and deer in comparison against each other revealed measurable differences between natural and urban fauna of the same region, between omnivores and herbivores of the same environment, and between natural and urban omnivores. These differences provide evidence that modern humans should exhibit values different than the natural environment due to a grocery store diet and allow for the extension of the project to investigate the variability among Louisiana cities. It was also determined that Cascade dish detergent does not inhibit stable isotope analysis of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, but more work must be done to understand the interaction of Cascade and $\epsilon^{87}\text{Sr}$.

Chapter 1 Introduction

Stable isotope analysis of human and non-human bone is a technique often used by archaeologists and bioarchaeologists to evaluate the diet of ancient populations. In recent years, such analysis has received the attention of forensic anthropologists who seek additional resources to aid in the identification of persons whose bodies are found in local communities. The current thesis research involves the tracking of oxygen, carbon, and strontium isotopes in modern, non-human animal bone with the goal of establishing a proxy for human comparisons. The Louisiana Repository for Unidentified and Missing Persons Information Program could use such a proxy to aid in the identification of recovered unidentified individuals and to determine the possibility of whether the individual resided in Louisiana.

First, in order to understand how isotopes are incorporated into bone from the environment, isotope mobility and fractionation will be tracked from soils to plants and then to herbivores and omnivores. Following this review, the research will be composed of two major phases.

The first phase focuses on stable isotope analysis of samples of non-human animal bones from various wilderness regions within Louisiana with the goal of establishing the landscape, or isoscape, of Louisiana. Developing this isoscape by establishing the distribution of carbon, oxygen, and strontium isotope ratios will help to determine whether or not there are isotopically-discernible regions within Louisiana. If so, a bone sample of unknown origin should be able to be attributed to a particular region of origin within the state.

White tailed deer, *Odocoileus virginianus*, will be used for the first phase of the research. These naturally-abundant herbivores provide a readily available source of bone due to seasonal hunting. Samples of deer bone for this research were donated by local hunters and collected by local butchers who specialize in processing deer for consumption. Deer in Louisiana are hunted from October to February and only adult deer are permissible to harvest (Thayer 2009). Deer are considered mature around 2.5 years of age and are visibly recognizable by the absence of white spots along their backs (Thayer 2009). All deer used in the current research were harvested during the 2012-2013 hunting season.

The second phase of the research project will include carbon, oxygen, and strontium analysis of bone from urban omnivorous fauna that consume the discarded food remnants of modern humans. Analyzing such fauna will explore the isotopic composition of modern human diets within urban settings. This phase will aid in distinguishing locales within the state where people are consuming from grocery stores that receive the same processed foods.

The first phase of research is encompassed by the thesis presented, and the second phase of research remains to be conducted at a later time. Both phases, wilderness and urban faunal analysis, are vital steps to creating a statute of isotopic comparison for modern humans. Such a statute of comparison has potential for aiding investigators in obtaining identities for the persons who remain unidentified in Louisiana.

Chapter 2 Literature Review

2.1 Isotopes

The elements on the periodic table are discernable by the number of protons in the nucleus of the atom. Isotopes are atoms of the same element possessing variable numbers of neutrons. Some isotopes are stable, meaning the number of protons in the atom will not change with time. Others are radioactive, meaning the nucleus will decay, forming daughter nuclides different from the parent nuclide. Both types of isotopes are useful and provide very different information.

Radioactive isotopes, discovery credited to Marie Curie awarded with the 1911 Nobel Prize (Nobel Foundation 1966), can be naturally occurring, such as carbon-14 (^{14}C), which is a radioactive isotope of carbon. In a living organism, ^{14}C is in equilibrium with the environmental levels of ^{14}C . Upon death, one half of the ^{14}C population will disassociate into Nitrogen-14 (^{14}N), a stable isotope, at a regular rate of 5,730 years (IAEA 2009). The ratio of ^{14}N to the remaining ^{14}C allows for the calculation of time elapsed since the death of the organism. Therefore, ^{14}C is useful in establishing dates for archaeological material. Radiocarbon dating allows archaeologists to reliably date artifacts, reposition events in time, and validate relative dating methods.

Stable isotopes, discovery credited to Harold Urey awarded with the 1934 Nobel Prize (Nobel Foundation 1966), can provide meaningful information to archaeologists and anthropologists. Elemental masses reported on the periodic table are weighted averages of the naturally occurring stable isotopes of each element. One particular isotope occurs in majority, where the other isotopes occur

in low proportions, thus making ratios of stable isotopes useful. Environmental processes such as the water cycle and interactions between food webs can distribute various isotopes in different concentrations, called fractionation. The ratios of isotopes are shown to change between environments, called isoscapes. As organisms consume from their local environment, further fractionation occurs as isotopes are incorporated into living tissues (organs, bones, etc.) or hard parts (shells, claws, etc.). These distributions of stable isotopes make them useful in reconstructing past climates, dietary preferences, as well as deducing migration patterns of organisms in archaeological and modern contexts.

2.1.1 Measuring Isotopes

Stable isotopic analysis is conducted using the principles of mass spectrometry. A mass spectrometer (MS) is an instrument that separates a sample according to mass. These can be compounds, molecules, or isotopes. An MS can be designed to work with different sample types (liquid, solid, etc.). A diagram of an MS can be found in Figure 1.

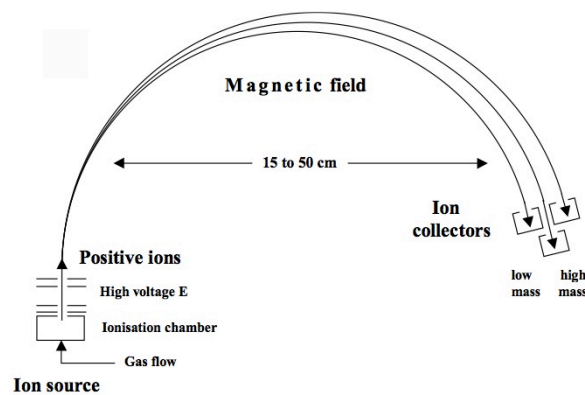


Figure 1: MS Diagram (IAEA Measuring Techniques 2009)

Coupling the MS with an inductively coupled plasma (ICP) will vaporize a liquid sample, ionize it, and then accelerate it to separate the ions according to mass. This instrumentation, ICP-MS, allows for multiple elements to be measured simultaneously without a loss of precision (Dean 2005).

The first step in ICP-MS analysis is the introduction of the sample into the ICP portion of the instrument. The ICP part of the instrument consists of a torch encompassed by a series of copper inductive coils, supplied with an electric current (radio frequency), through which a carrier gas flows. Figure 2 presents a diagram of an ICP.

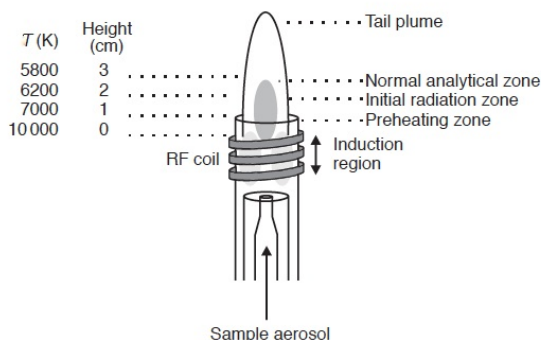


Figure 2: Diagram of ICP instrumentation (Hou 2000)

Carrier gases are inert, meaning that they do not easily react with other atoms; Argon is frequently used as a carrier gas, but other noble gases may also be used. An electrical spark applied to the carrier gas introduces free electrons. A magnetic field generated by the induction coils moves the electrons until they collide with atoms of the carrier gas, thus initiating ionization of atoms and transition into the plasma phase at temperatures 6,000-10,000K (Hou 2000).

A few microliters (μL) of a liquid sample are passed through a nebulizer, introducing the sample into the center of the plasma within the torch, thus becoming

ionized. The known differences in behavior of elements regarding the degree of ionization (amount of electrons lost or gained) allow the elements to be separated through a series of cones and passed into the mass spectrometer portion of the instrument. The differences in mass for each isotope cause them to be deflected by an electromagnet in varying degrees. As a result, the atoms impact different locations within detectors. The relative abundance of each isotope is determined from the amount of strikes recorded at locations within the detectors. The coupling of elemental separation by ICP and isotopic mass separation by MS gives greater precision than MS alone when measuring isotopic ratios in multielemental samples.

Measuring isotope abundances and ratios of isotopes within samples is only useful if results can be compared between samples. Comparison between samples is achieved by calibrating the sample measurements according to a common standard, creating a universal zero. Values are then reported as deviations from the standard rather than absolute values. Although the standards used vary with respect to the element of interest, the equation used to report relative isotope abundances does not (Eq. 1).

$$\delta^{bX} = \left(\frac{\left(\frac{b_X}{a_X} \right)_{\text{sample}}}{\left(\frac{b_X}{a_X} \right)_{\text{standard}}} - 1 \right) * 1000 \quad \text{Eq. 1}$$

“X” represents the element measured; “a” is the mass of the majority isotope measured; and “b” is the mass of the minority isotope measured. The units are parts per thousand (‰), and the symbol δ is pronounced delta (Craig 1961).

Understanding the δ values is vital in understanding isotopic data. A value of $\delta = 0.00$ means there is no significant difference, within analytical precision, between

the sample and the standard. A positive value of δ indicates relatively more of the heavier isotope of bX compared to the standard, whereas a negative value of δ indicates more of the lighter isotope of bX with respect to the standard.

2.1.2 Isotope Fractionation

Isotope fractionation is the natural separation of isotopes by mass through various environmental and metabolic pathways. Relevant pathways for this study include the hydrologic cycle, photosynthesis, and cellular metabolism in large mammals. To understand the details of how oxygen, carbon, and strontium are carried through these pathways, each element is addressed in turn within Section 2.3 - Elements of Analysis.

2.2 Current Applications of Isotopic Analysis

Stable isotope analysis utilizing carbon, oxygen, and strontium has aided many anthropological researchers in a variety of subjects. Stable isotopes have allowed for paleoclimate reconstructions, paleodiet reconstruction, and provenience determinations of human, animal, and plant remains (Cormie 1994, Ezzo 1997, Lee-Throp 1989, Longinelli 1984, Webb 2006). It is possible to use the reconstructed paleoclimate, paleodiet, or the substance provenience to assist in drawing conclusions regarding the cultural aspects of the past, such as the rise of agriculture and migration patterns of past humans and animals. However, isotopic analysis is not limited to prehistory. Work has and is being done to apply isotopic studies in historic and modern contexts. An historic application of isotopic analysis will be presented followed by modern forensic applications.

2.2.1 Historic Application

The first study chosen to exhibit the utility of isotope analysis in an historic context was authored by T. D. Price et al. and published in 2012. Construction activities at Campeche, Mexico, in 2000 revealed a cemetery containing 180 individuals. Historical records revealed that the site was associated with a Spanish colonial town established approximately AD 1540-1541, confirmed with radiocarbon dating (Price et al. 2012).

Sixteen individuals were sampled from tooth and bone for strontium analysis, revealing seven individuals to be native to Campeche and the remaining to have previously resided elsewhere. Bone collagen and apatite samples were taken from 35 individuals, revealing a marked dependence on C4 plants at Campeche. It was observed that individuals labeled as non-local according to strontium data demonstrated significantly lower carbon values than individuals labeled as local, owing to differences in C4 plant dependence at their previous region of residence. Of those carbon based non-locals, there was marked variability of the carbon values, indicating separate regions of origin rather than a group translocation. Oxygen isotope analysis of 64 individuals revealed no differences in isotopic values. This occurrence was interpreted that the individuals who were non-local came from a region that was similar in climate and thus had similar oxygen isotope distribution.

The osteological, isotopic, and historical data are inter-supportive. The historical records indicate a local Maya village, Spanish colonists, and slaves transported from Africa. These records are supported by comparable values of strontium and oxygen from Spain and Africa found within the non-local individuals.

Along with the carbon values, giving further information regarding diet, a more detailed life history for the city of Campeche can be compiled.

2.2.2 Forensic Applications

The first study selected to demonstrate the modern application of isotope analysis in forensic anthropology is a case study where isotopic analysis provided information when other methods could not. Meier-Augenstein and Fraser (2008) conducted a study in Dublin, Ireland, to aid in the identification of dismembered human remains found in 2008. The biological profile of the remains (middle aged, possibly of African ancestry, male) matched numerous missing person profiles, beyond a manageable amount. Nitrogen, hydrogen, carbon, and oxygen isotopic analyses of hair, nail, and bone were performed in hopes of eliminating some of the possible missing person profiles matching the description of the found remains.

Consideration of only the carbon and oxygen data will be presented in respect to the thesis project at hand. The carbon and oxygen values from the unidentified individual were evaluated using an indigenous Dublin individual as a standard for comparison.

The carbon isotopes obtained from the unidentified individual did not show a dependence of C4 plants, making North America and North Africa unlikely places of origin. The inner cortical bone exhibited a different oxygen isotope composition from the outer (older) cortical bone, which was interpreted as a combination of the oxygen content of water from the area in which he previously resided and that of Dublin. The outer cortical bone exhibited oxygen values corresponding to five possible regions worldwide. From the length of time required for bone on the femur to

completely turnover, linear regression suggested that the individual would have had to reside in Dublin for approximately 6.3 years (± 2.9 years) to account for the differences in oxygen content between inner and outer cortical bone.

The combined isotopic and skeletal information resulted in the following profile: male, middle aged, African ancestry, immigrated to Dublin within approximately 4-9 years, and a possibility of originating from five distinctive regions worldwide. This information provided investigating officers with justification for DNA testing of a possible relative to the individual. Ultimately, the DNA results led to the positive identification of the individual, a 39 year-old male who immigrated from Kenya seven years before his death, but isotope analysis provided key information.

A second study, conducted by Van der Peijl et al. (2013), investigates the utility of multi-isotope analysis in third molars. Oxygen and strontium were measured from 30 life-long Netherland residents and from five individuals who were born abroad and moved to the Netherlands during childhood or adulthood. Differences in oxygen values between native individuals and foreign individuals were observed and served to exclude a person of nonlocal origin. The strontium values showed a greater variability between regions of residence, and the combination of oxygen and strontium values provide specific requirements for a person to be considered a Netherland resident.

A third study regarding isotope analysis, Prutsman-Pfeiffer et al. (2013), considers the isotopic composition in bone from 22 deceased, undocumented border crossers from Mexico to the United States (positively identified via DNA analysis) and 38 individuals resident to western and central New York. Thirty-one elements

along with lead were measured in the bone samples. Bones sampled included tibia, femora, and parietal bones. New York parietal/tibia bones from the same individual showed no significant difference in isotopic composition and anatomic location, and the Mexican samples showed a parietal/tibia difference only in respect to manganese. Five elements revealed a significant difference between New York and Mexican samples: aluminum, manganese, tin, zinc, iron, and lead. Lead values for each group clustered around environmental values previously reported for the respective areas, indicating a relationship to local soil.

The studies presented here demonstrate the utility of isotope analysis in characterizing individuals beyond what is physically observable from the skeletal remains. However, these studies use isotopes to distinguish regions of large geographical separation. The project proposed in this thesis addresses the possibility of isotopic distinction within a small geographic area, offering potential means of identifying individuals of separate areas within a region, such as the state of Louisiana. To begin understanding how the present thesis will accomplish such a task, a discussion of the isotopic variation within the environment is presented.

2.3 Elements of Analysis

2.3.1 Oxygen

Oxygen naturally occurs in three isotopes: ^{16}O , ^{17}O , and ^{18}O . The abundance of each isotope is as follows: 99.76% ^{16}O , 0.035% ^{17}O , 0.20% ^{18}O (Nier 1950). The δ values ($\delta^{18}\text{O}$) are reported in respect to ^{16}O because ^{16}O is the most abundant oxygen isotope.

Ocean water became one of the standards for $^{18}\text{O}/^{16}\text{O}$ measurements because the oceans are the main contributor to precipitation (Craig 1961). Standard Mean Ocean Water (SMOW) has a value of $^{18}\text{O}/^{16}\text{O} = 1.008$ (Craig 1961). The modern standard is Vienna Standard Mean Ocean Water (VSMOW) and has a value $\delta^{18}\text{O} = 0.00$ in reference to SMOW (Lin 2010).

Oxygen Fractionation during evaporation (Craig 1965):

Oxygen isotopic fractionation during evaporation is thermodynamic and kinetic. Thermodynamic fractionation occurs because the intermolecular forces of water molecules containing ^{18}O (H_2^{18}O) require more energy to change phase (i.e. evaporate) than water molecules containing ^{16}O (H_2^{16}O). This causes water vapor to be ^{18}O deficient and the liquid water to be ^{18}O enriched. Kinetic fractionation occurs because of the mass difference between ^{18}O and ^{16}O . Increased energy is required to move ^{18}O compared to ^{16}O , causing the same deficiency and enrichment in water vapor and liquid phase water, respectively, as in the thermodynamic effect. Both of these effects are primarily driven by temperature (energy source), where higher temperatures result in more ^{18}O in the vapor phase. Figure 3 provides an illustration of kinetic and thermodynamic effects on oxygen fractionation.

Oxygen composition of rainwater:

The oxygen isotopic composition of rainwater is affected by five discernable factors: the latitudinal effect, continental effect, altitude effect, seasonal effect, and amount effect (IAEA 2009).

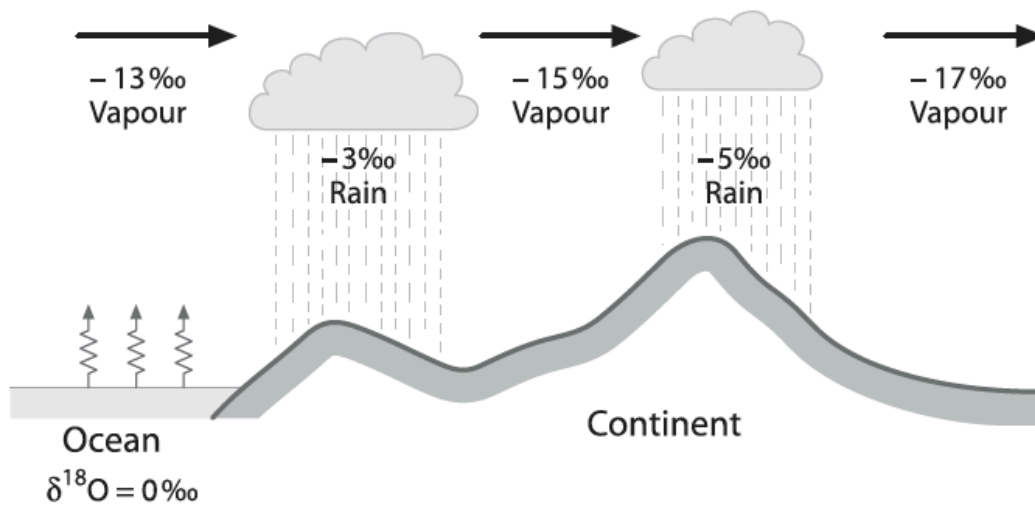


Figure 3: Oxygen Isotope Fractionation in the Atmosphere (Hoefs 2009)

Latitudinal effect: More negative ^{18}O values will be observed at increasing latitudes.

Continental effect: More negative ^{18}O values will be observed for precipitation as moving more inland from any direction.

Altitude effect: With increasing altitude, there will be a decrease in ^{18}O in precipitation.

Seasonal effect: Lower temperatures will result in a decrease of ^{18}O in precipitation.

Amount effect: With heavier rainfall, more negative ^{18}O values will be observed.

The five factors noted above have a greater affect on the isotopic composition of rainwater than the body of water from which the rainwater was derived (Craig 1965). Overall, the composition of rainwater is a reflection of climate and atmospheric processes (Dansgaard 1975; Lawrence 2004). Multiple studies have

been conducted on the oxygen isotope composition of rainwater on a global scale and the distribution of $^{18}\text{O}/^{16}\text{O}$ ratios. Figure 4 provides the global distributions of $\delta^{18}\text{O}$ values recorded in rainwater as reported by Bowen in 2010.

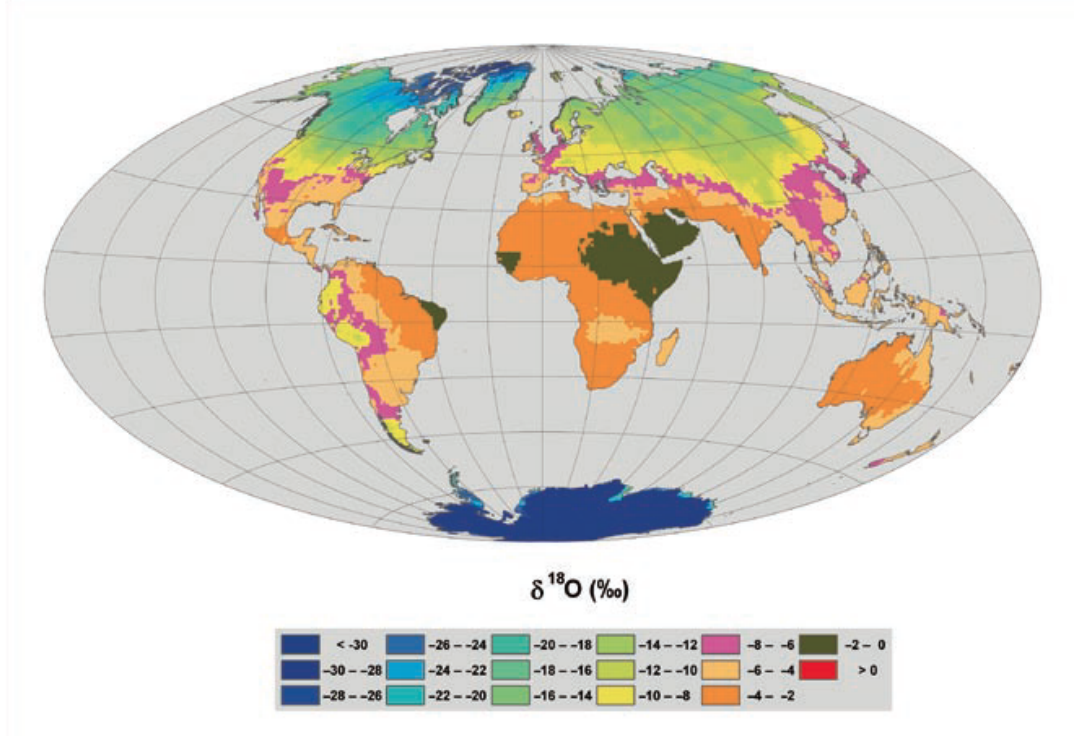


Figure 4: World distribution of $\delta^{18}\text{O}$ in Rainwater (Bowen 2010)

Oxygen content in Leaf Water:

Following the deposition of water in the terrestrial environment, uptake of water by organisms is the next step to incorporation in mammalian bone. The water within a plant has been shown to be isotopically unchanged from the water in the soil (Barbour 2007; Landais 2006). The alteration of ground water within a plant occurs during leaf transpiration of water, resulting in ^{18}O enrichment in the leaf water (Barbour 2004; Webb 2006). Enrichment of leaf water has been documented at 3‰ with a 10% decrease in humidity (Cuntz 2007). Global measurements of leaf water have been recorded, and a map of the distributions is presented in Figure 5.

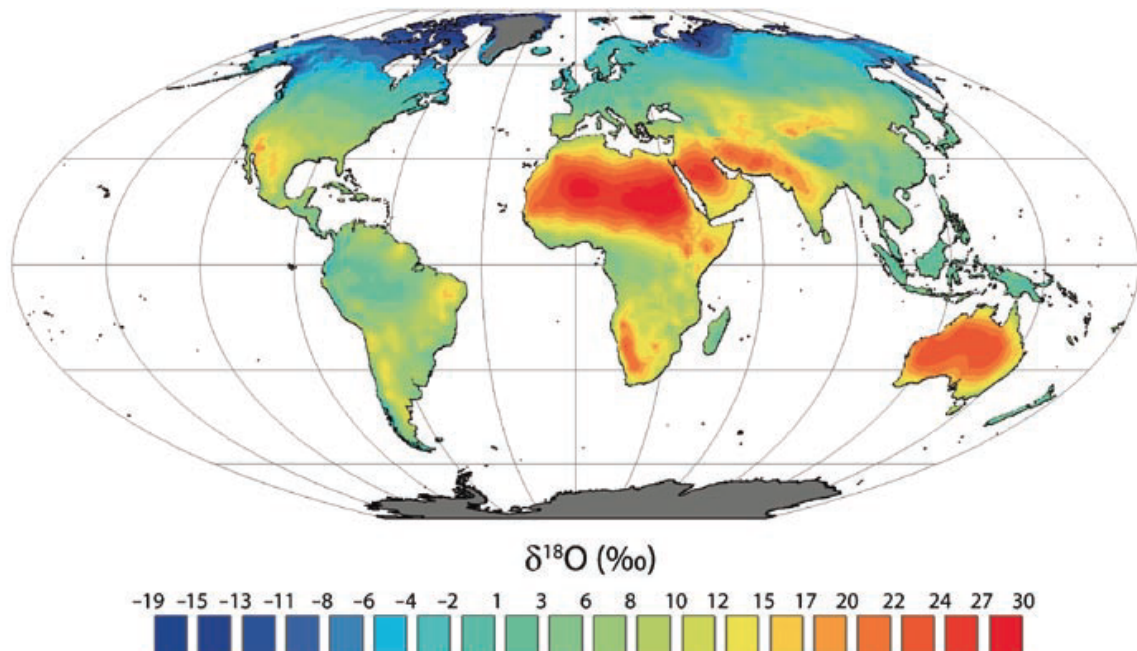


Figure 5: Global Measurement of Leaf $\delta^{18}\text{O}$ (West 2008)

2.3.2 Carbon

Carbon has three natural isotopes (^{12}C , ^{13}C , ^{14}C) occurring in the following abundances: 98.9% ^{12}C , 1.1% ^{13}C , $<1.0 \times 10^{-12}$ ^{14}C (Neir 1950). ^{14}C is a radioactive isotope and the degeneration of this isotope into ^{14}N has become useful for dating archaeological settings. The ratio of ^{13}C to ^{12}C is the measurable isotopic variation in the environment (IAEA 2009). A standard for comparison of $^{13}\text{C}/^{12}\text{C}$ values in carbonates is Vienna Pee Dee Belemnite (VPDB), with a value of +1.95‰ (Craig 1957; Gonfiantini 1984).

Plant Composition of Carbon:

Carbon isotope fractionation through plant matter is a much more regular and stable process than that for oxygen. Common terrestrial plants available for consumption can be divided into two distinctive groups based on photosynthetic pathways: C3 and C4 plants. Only a general outline of how these pathways affect

carbon fractionation will be presented here, but further information can be found in Farquhar 1989, Van Der Merwe 1991, Martin 2012, along with many others, some of which are cited in this literature review and some that are not. Carbon-13 fractionation occurs differently between C3 and C4 plants because of the different photosynthetic pathways. For both pathways, carbon uptake is primarily through the intake of atmospheric carbon dioxide; and so, $\delta^{13}\text{C}$ values are reported with respect to the atmospheric composition of CO_2 .

C3 plants convert CO_2 into a three-carbon compound, 3-phosphoglycerate, as the first step in photosynthesis, therefore termed C3 pathway (Martin 2012). C3 plants comprise 90% of terrestrial plants and include trees, shrubs, and wheat. C3 plants have been documented to deplete $\delta^{13}\text{C}$ as much as -18.5‰ in respect to atmospheric CO_2 (Farquhar 1989).

C4 plants convert CO_2 into a four-carbon compound, phosphoenolpyruvate, during the first step in photosynthesis, therefore termed C4 pathway (Martin 2012). Examples of C4 plants include maize, crabgrass, and sugarcane, which deplete $\delta^{13}\text{C}$ by -4.5‰ (Farquhar 1989).

Factors Affecting Carbon Composition in Plants:

The canopy effect and water stress are two factors influencing the availability of carbon to plants. The canopy effect is dependent upon the thickness of branch cover from taller trees. If the canopy is dense, plants underneath have access to a smaller amount of CO_2 (Van der Merwe 1991). The second effect, water stress, provokes an enrichment in ^{13}C ; C3 plants have shown a 2-4‰ enrichment (Johnson 1989; Tieszen 1991); C4 plants have shown a 0.5‰ enrichment (Ghannoum 2002).

However, ¹³C enrichment due to water stress is dependent upon the species of the plant (Fravolini 2002).

2.3.3 Strontium

Strontium has four naturally occurring stable isotopes (⁸⁴Sr, ⁸⁶Sr, ⁸⁷Sr, and ⁸⁸Sr) with abundances of 0.56% ⁸⁴Sr, 9.86% ⁸⁶Sr, 7.00% ⁸⁷Sr, and 82.58% ⁸⁸Sr (IUPAC 2009). ⁸⁷Sr is a product of the radioactive decay of 87-Rubidium, which creates large variability in the distribution of ⁸⁷Sr today (Pye 2004).

Reporting strontium isotope ratios is often converted into an epsilon notation, shown in Equation 2 (Eq. 2) (Beard 2000).

$$\epsilon^{87}\text{Sr} = \left(\frac{\left(\frac{^{87}\text{Sr}}{^{86}\text{Sr}} \right)_{\text{sample}}}{\left(\frac{^{87}\text{Sr}}{^{86}\text{Sr}} \right)_{\text{standard}}} - 1 \right) * 10,000 \quad \text{Eq. 2}$$

Standard values for comparison of strontium values include but are not limited to the ⁸⁷Sr/⁸⁶Sr of bulk earth, found to be 0.7045, the ⁸⁷Sr/⁸⁶Sr of the natural abundances, or NBS 987 (Beard 2000).

Strontium in Soils:

Strontium values in soils are highly variable between locations and largely reflect the materials from which the soil was derived, underlying geology, and atmospheric conditions (Dasch 1969; Pye 2004). Strontium isotopes are variable between locations, but not necessarily unique to a location. With a particular region of interest, strontium isotopes may confirm or negate the possibility of an organism spending a large amount of time in that particular region. A geologic map of Louisiana is presented in Figure 6 for reference against the predicted strontium

values based on the underlying bedrock of Louisiana presented in Figure 7 and Figure 8 (Chacko 2010; Beard 2000; Bataille 2012).

Because water, not sediment, provides strontium entry to the biological food chain, it can be more beneficial to compare strontium ratios from biological samples to soil leachate samples rather than bulk rock samples (Pye 2004).

Strontium in Water:

Strontium in water is largely derived from the strontium content of the geological area in which the water resides. Research has shown that strontium does not fractionate while traveling through environmental and biological processes, making strontium isotope ratios useful for tracing the location in which an animal consumed food (Aberg 1995; Capo 1998; Pye 2004). Figure 9 presents strontium values predicted for water in the contiguous United States (Bataille 2012).

For this study, the values obtained from Louisiana deer will be taken as representative for the biologically available strontium, most closely related to the strontium in local water values.

2.3.4 Expectations

The literature review presented here is primarily composed of studies that have modeled what the isotopic composition should measure over the United States or the globe. None of the studies here have conducted a localized investigation into isotopic variation within a small area such as an individual state, and have depicted Louisiana as uniform, or near uniform, in respect to isotopic values. Understanding the environmental factors which contribute to isotopic variation and fractionation have led to the expectation of variation within small regions as well as large regions.

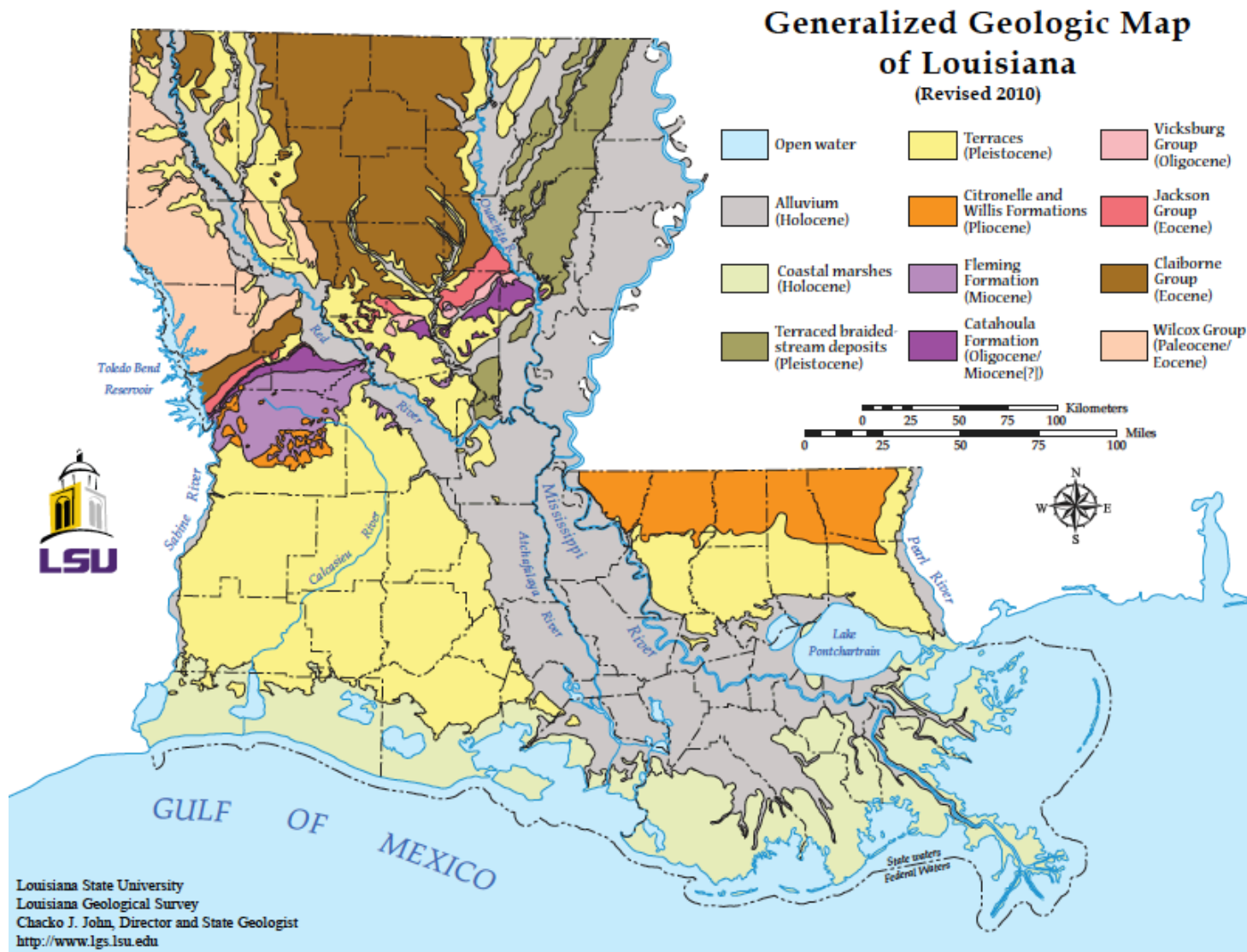


Figure 6: Generalized Geologic Map of Louisiana (Chacko 2010)

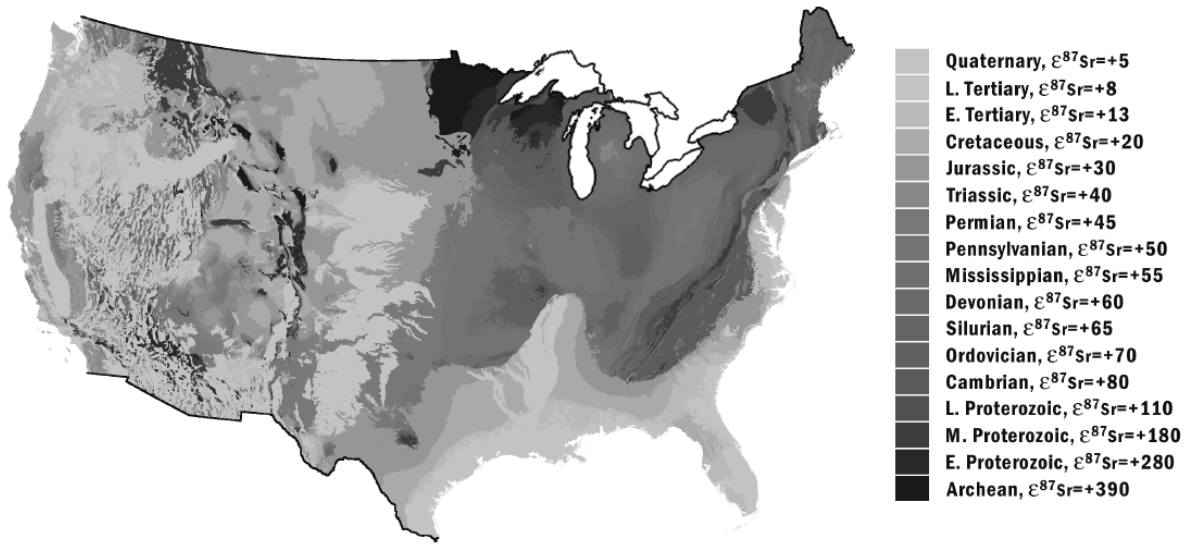


Figure 7: Predicted Sr Geologic Values (Beard 2000)

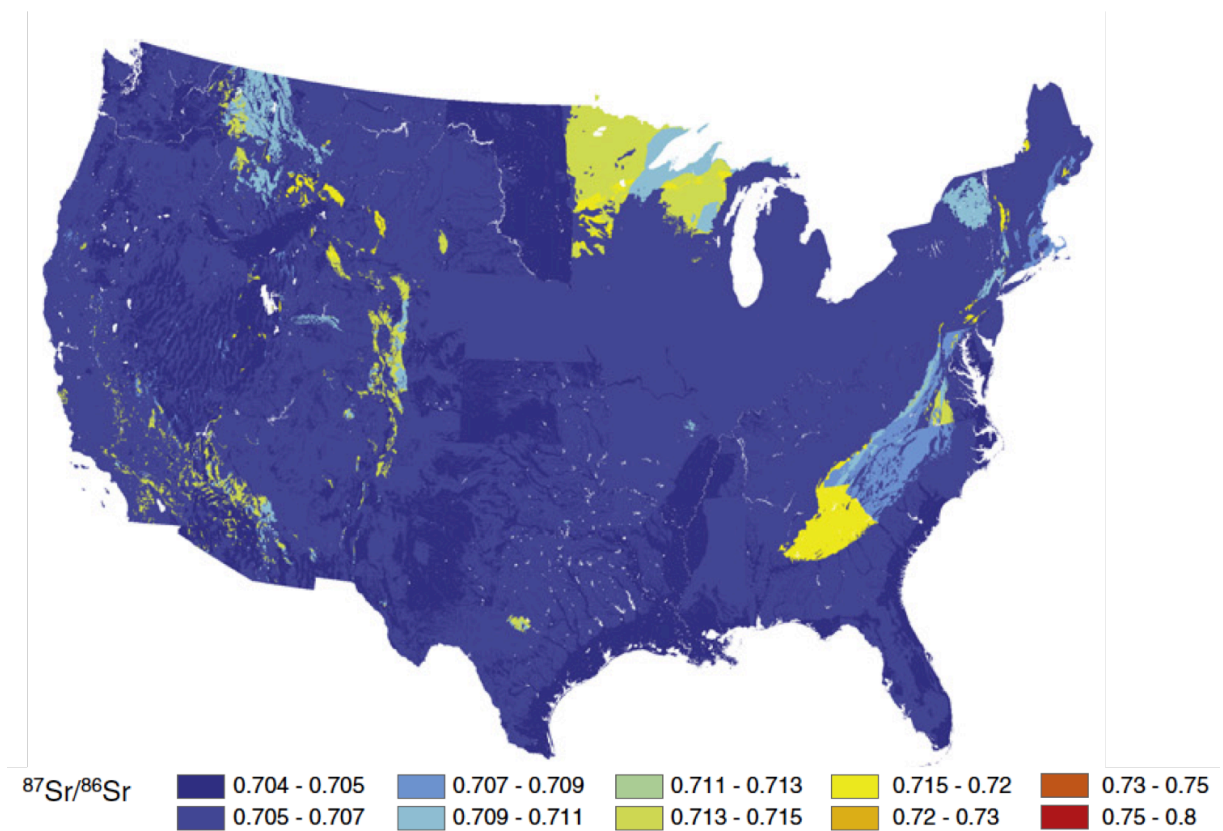


Figure 8: Predicted Sr Geologic Values (Bataille 2012)

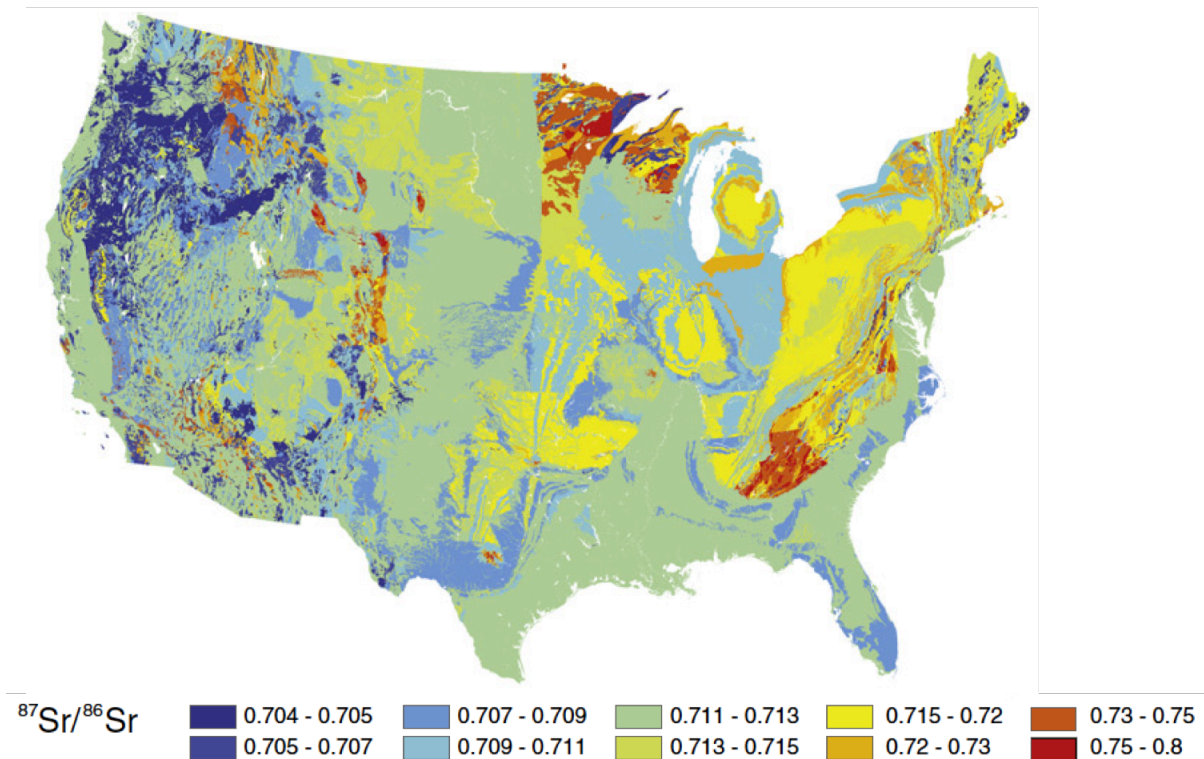


Figure 9: Predicted Sr Values of Local Water (Bataille 2012)

And so, the current thesis presents a study of isotopic variation within the small region of Louisiana.

With our eventual goal of understanding environmental variation in terms of bone samples, an understanding of bone structure and the process of isotope incorporation into bone must first be established.

2.4 Bone Structure and Function

Bone is a complex tissue, serving vertebrates mechanically and biologically. The biological function of bone includes storage of calcium and phosphorous, contributing to the phosphacalcic metabolism of the body, hosting bone marrow, and production of blood corpuscles (Fratzl 2008). Bone functions mechanically by providing muscle attachment sites and supporting internal organs.

Bone formation, osteogenesis, and resorption are in constant flux within the body and are primarily conducted by osteoblasts (bone forming cells) and osteoclasts (bone destroying cells) (Fratzl 2004). Bone is formed using materials consumed by the organism, which carry the isotopic distribution of the local environment. Localized isotopic variations in the environment in which the organism lived and consumed food make attributing an organism to a particular environment possible (Fratzl 2004).

The deer femora used in this study consist of two different structural types: cortical bone and trabecular bone. The cortical bone forms a shaft encasing the bone marrow, where the trabecular bone forms the bone ends (White 1991). Mineralized collagen, a large, insoluble fibrous protein, constitutes the bulk of dense cortical bone, making it very rigid; trabecular bone contains mineralized collagen, but is of porous nature and highly vascularized (Fratzl 2008).

The mineral component of bone is plate-like, of the nano (10^{-9}) scale, and often referred to as a crystallite (Wagner 1998; Weiner 1992). The mineral constituting bone crystallites is a biologically-generated type of apatite known as hydroxyapatite, more commonly referred to as bioapatite. Bioapatite is approximately 70% of bone mass and is responsible for the density of bone (Wagner 1998; Wright 1996). The mineral is of the general formula: $\text{Ca}_{10}(\text{PO}_4)_6(\text{F},\text{OH},\text{Cl})_2$ and is likely to accept chemical substitutions (Wopenka 2005). Substitutions of F^- , Cr^+ , Na^+ , K^+ , Fe^{+2} , Zn^{+2} , Sr^{+2} , Mg^{+2} , citrate, and carbonate (CO_3^{-2}) into the crystal lattice of bioapatite make this mineral more reactive than other minerals in the apatite family (Dorozhkin 2007). The small dimensions, substitution susceptibility,

and constant resorption/deposition are three major features of bioapatite in bone (Dorozhkin 2007).

2.5 Isotope Incorporation into Bone

Phase one of this project, composing a natural isoscape for Louisiana, is being accomplished by the use of white tailed deer (*Odocoileus virginianus*). White tailed deer is a herbivorous species subsisting almost entirely on C3 plants (Bello 2001; Brown 1992; Harlow 1994; Kroll 1994). Consumption of C3 plants incorporates oxygen, carbon, and strontium into the bioapatite of bone.

Deer consume oxygen and carbon through drinking water, respiration, and consuming food. However, the major contribution of oxygen to the body water of deer is from the leaf water of plants consumed by the deer (Cormie 1994; Luz 1990). The body water of deer is the substrate from which mineralized tissues derive their oxygen content, and research has determined that no fractionation occurs during the mineralization process (Fricke 1996; Longinelli 1984; Luz 1984). Therefore, the bioapatite of deer bones should reflect the isotopic ratio of the ingested leaf water (Bryant 1995; Kohn 1996).

Carbon is incorporated into the bioapatite primarily through the diet (Lee-Thorp 2005) after conversion into dissolved inorganic carbon in the forms of CO₂, carbonic acid (H₂CO₃), bicarbonate (HCO⁻³), and carbonate (CO₃⁻²) (Passey 2005). Mammalian body temperature results in approximately a 10‰ enrichment of ¹³C (Lee-Thorp 1989; Lee-Thorp 2005), but the specific amount is dependent upon the species (Ambrose 2003; Kohn 1996; Sullivan 1981).

Strontium incorporation into bioapatite is possible because of the similar size and electronic behavior of strontium and calcium. Sr^{+2} can be substituted for Ca^{+2} within bioapatite, but only 20-24% of ingested strontium is incorporated into bone (Beard 2000). The result is a decrease in amount of strontium with increasing trophic level, but the absorbed strontium does not fractionate through biological processes, leaving the original isotopic proportions intact (Ezzo 1997).

Chapter 3 Materials and Methods

The white tailed deer was selected for study of the Louisiana isoscape because it is a large, herbivorous, and non-migratory natural fauna of Louisiana. Although deer is not the only fauna of this sort, it is most readily available for sampling due to the seasonal hunting of this fauna by Louisiana residents. Samples were obtained through collaboration with butchers in Pine Prairie and Pineville, Louisiana, who were asked to accumulate femora while processing deer for local hunters during the 2012-2013 hunting season. The butchers also agreed to record the location, exact as possible, from which each deer was harvested.

The butchers froze all obtained femora until collection was possible; the femora remained frozen until maceration and disarticulation could be performed. Freezing has been shown to have no effect on bone integrity and has also been shown to have no effect on isotope composition of the bone (Andrus 2002). Only femora were used for this project; however, additional elements (e.g. tibia, metapodials) were sometimes submitted as articulated limbs. The additional elements collected were destroyed, retained for duplicate sample analysis, or donated to other research projects.

3.1 Maceration

Due to the sensitivity of bioapatite (Dorozhkin 2007), no chemical agents were used to assist in the removal of muscular tissue. This was done to avoid any induced digenesis of bioapatite.

Disarticulation and maceration of all deer elements were accomplished with scalpels, scissors, and hemostats. If possible, this process occurred immediately

upon arrival at the lab, but in some cases, it had to be postponed due to lab use, time constraints, or volume of samples. In the case of postponed maceration, the samples remained frozen until a suitable time.

Due to the extensive time required to fully macerate deer femora without warm water or acting chemical agents, a 450 Digital Sonifier fitted with a cell disruptor horn was used to expedite the maceration process (Figure 10). A cell disruptor homogenizes tissue while submerged in water using ultrasonic pulsation. This method does not destroy bone collagen, and research suggests that DNA sampling actually has a better yield after cell disruption compared to other techniques (Branson 2006). Distilled deionized water (DD H₂O) was used to submerge each bone, avoiding contamination from tap water, and fresh DD H₂O was used to clean each bone, avoiding inter-sample contamination.



Figure 10: Digital Sonifier and Cell Disruptor Horn

3.2 Cell Disruption

Test runs using excess deer elements (e.g. tibia) from disarticulated hind limbs, determined the optimal operation parameters to be multiple eight minute intervals at 90% amplitude, removing loosened tissue between intervals.

Additionally, warm water aids tissue removal and has been shown to do so without altering isotopic ratios (Andrus 2002); thus, warming the tissue before subjecting to cell disruption was employed to quicken the maceration process. To quicken the process even further, the distal and proximal ends of each bone were removed with a handheld bone saw (Figure 11).



Figure 11: Removal of Distal and Proximal Ends of a Deer Femur

Removal of the distal and proximal end of each femur allowed for less water to be used, increasing the potency of the cell disruptor, and reduced the amount of tissue to be removed. Once cut, femora were submerged in heated water for approximately 15 minutes, transferred to room temperature water for cell disruption, and then returned to the warmed water for approximately 10 minutes while the cell disruptor cooled. A range of 16 to 48 minutes of cell disruption was required to fully free a bone of all muscular tissue. After a bone was deemed fully clean, it was dried under a fume hood for a minimum of 24 hours, then labeled and stored in a paper bag.

3.3 Femora Distribution, Inclusion, and Testing Parameters

A total of 61 deer femora were collected from the 2012-2013 hunting season. Forty-four deer femora were sampled for isotopic analysis, as well as one fox femur and two raccoon femora (recovered from natural death incidents). Deer femora were excluded from the sampling pool if the epiphyses on either the proximal or distal end of the femur were not fused, the harvest location could not be verified, the harvest location was sufficiently represented, the femur incurred damage from butchering, or the femur was cleaned with assisting agents before the established use of cell disruption for maceration. All deer femora representing different locations were sampled, and, when possible, samples were taken from two or more different deer of the same location/parish.

Two femora from Deer 4 were included for controls to observe any differences incurred from cleaning methods. One femur was cleaned using warm water and cell disruption, while the second femur was cleaned using warm water and Cascade dish detergent as an assisting agent. Of the two raccoons included in the sampling pool, raccoon 1 was cleaned in warm water and Cascade while raccoon 2 was cleaned with only warm water. This was done to assess how differences in cleaning methods could affect thinner cortical bone.

Three deer (18, 19, 20) were obtained from the same location during the same hunting expedition. Deer 18 and 19 were cut prior to cell disruption while deer 20 remained uncut. Deer 18 and 19 will serve as duplicate analyses for the location, and deer 20 will serve as a comparison of femora cut prior to cell disruption and femora cut following cell disruption. It is expected that bone cut prior to cell

disruption will be isotopically unchanged from bone cut after cell disruption, but all three deer were included in the sampling pool to test this expectation.

Although this thesis is focused on establishing the natural isoscape of Louisiana, an initial assessment of the contrast between herbivorous and omnivorous data, as well as natural versus urban faunal data was also undertaken. A fox from Evangeline parish, where multiple deer were collected, was included in the sampling pool for omnivorous versus herbivorous data. Two raccoons, from an urban setting, were also included in the sampling pool for assessment of natural versus urban fauna. It is important to obtain an initial look at these differences in order to determine the feasibility of subsequent projects toward the goal of creating a proxy for modern human samples.

From the collection of 47 femora, 38 different Louisiana locations are represented, 20 Louisiana parishes, and a total of seven states are represented by at least one sample. Table 1 presents the spatial distribution of samples within Louisiana, and Table 2 presents the spatial distribution of samples by state.

Table 1: Distribution of Animal Samples by Parish

Louisiana Parish	Number of Samples	Louisiana Parish	Number of Samples
Allen	1	LaSalle	2
Avoyelles	1	Livingston	1
Beauregard	1	Maddison	1
Catahoula	2	Morehouse	1
Desoto	1	Natchitoches	1
East Baton Rouge	2	Rapides	4
Evangeline	10	Sabine	1
Grant	1	St. Helena	2
Iberia	1	Vernon	2
Iberville	1	Winn	2
		Total	38

Table 2: Distribution of Animal Samples by State

State	Number of Animal Samples
Kansas	1
Louisiana	38
Mississippi	1
Ohio	3
Tennessee	1
Texas	2
West Virginia	1
Grand Total	47

3.4 Sampling Procedures

The femora sampled in this study were prepared in the following manner. The proximal and distal ends of each femur were removed using a handheld bone saw, leaving only the femora shafts for sampling. The femora shafts were wrapped in foil and dried in a Heratherm General Protocol Oven, item number 50125548, at 50°C for 24 hours (Figure 12).



Figure 12: Heratherm General Protocol Oven

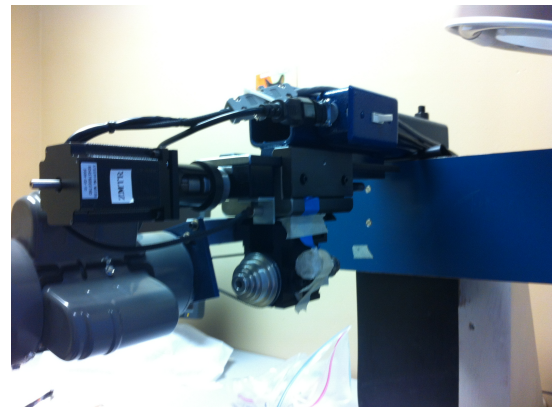


Figure 13: Taig 3000 Micromill 90° Orientation

A sample, amounting to approximately 10 – 25 mg, of bone powder is required for isotope analysis (Tykot 2004; Garvie-Lok 2004). Bone powder was produced using a Taig 3000 micromill at an altitude of 120 mm, under freehand operation, with carbide drill bits of 1.2 mm (HP 4), (Figure 13). The micromill is mounted to a table, 90° to the ground, allowing for generated powder samples to fall into weighing paper held underneath the drill site.

A small area on the anterior distal one-third portion of each femur was prepared for drilling by removal of the surface cortical bone, approximately 0.5 mm, by lightly running the drill bit across the area (Figure 14).

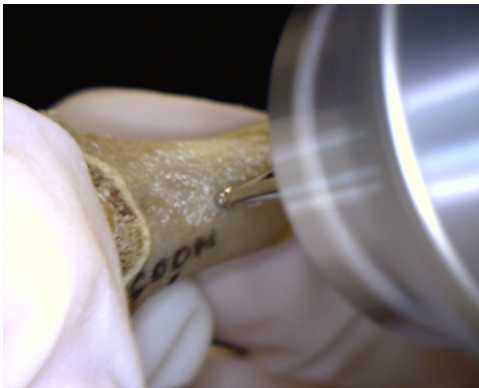


Figure 14: Removing Surface Layer of Drill Site on Shaft of Faunal Femur

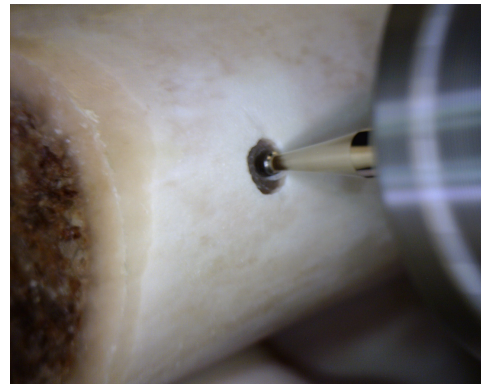


Figure 15: Radial Drilling on Faunal Femoral Shaft

Two separate holes were drilled in each femur to a depth of approximately 1.2 mm. The holes were then expanded radially (Figure 15) until the requisite sample amount was obtained.

One hole was drilled to obtain a sample of 0.02 g for ^{13}C and ^{18}O analysis in the Department of Geological Sciences, The University of Texas at Austin under the direction of research engineer Dorinda Ostermann. The second hole was drilled to obtain 0.01 g for strontium analysis at the High-Precision Mass Spectrometry and

Environment Change Lab (HISPEC), National Taiwan University under the direction of Dr. Shen Chuan-Chou. Figure 16 shows the location and size of the two holes drilled to obtain sample powder.



Figure 16: Two Sampling Holes on the Shaft of a Faunal Femur



Figure 17: (A) Cleaned Femur
(B) Cut Femur
(C) Sampled Femur

The faunal femora have undergone considerable change throughout the collection, cleaning, and sampling process. Figure 17 presents three femora, each at a different stage of processing.

Several procedures were employed in an effort to maintain sample integrity and avoid cross contamination. Gloves were worn, and new drill bits and gloves were used for each femur. The drill bit and bone were cleaned with an air duster to remove any residual powder after removing the surface cortical bone and between drilling each hole. Weighing paper for the scale was changed for each sample collected as an additional measure to avoid cross contamination and ensure the appropriate amount of sample collection. All samples were measured using a Fisher Science Education scale, and then transferred to a labeled micro centrifuge tube for storage.

The obtained bone powder is expected to represent a homogeneous sample of isotopic ratios throughout the life of the animal. The sample masses obtained from the femora can be found in Appendix A.

The samples collected were organized and mailed to the respective labs via the United States Postal Service. The samples were received in Austin within one week of mailing, and received in Taiwan within one month of mailing.

3.5 Analytical Precision

In order to understand the sample data analyzed by The University of Texas at Austin and the HISPEC Lab, how the data are measured and reported in terms of analytical precision and mass spectrometry must be understood.

Analytical precision is a measure of how well an analytical instrument is making repeat measurements, as well as a measure of whether or not measurements of the same sample are alike or different. A sample in a mass spectrometer is measured numerous times, and an average of all measurements for a single sample is reported as the measured value for that sample.

Precision is the long-term reproducibility for that instrument. In order to assess the precision of an instrument, a standard of known value is measured multiple times throughout a sequence of samples. The average and standard deviation of the standard is compared to the known value of standard, to determine accuracy. If the average value of the standard is the same as the known value of the standard, no correction is needed; otherwise, the data are adjusted to the known value of the standard. The standard deviation of the standards is used to determine the analytical precision of the instrument. The precision estimates how much

variability can be expected when measuring the same sample multiple times (Beers 1957). The standard deviation of the standard (σ), or analytical precision, can be used at the 68% ($\pm 1 \sigma$), 95% ($\pm 2 \sigma$), or 99% ($\pm 3 \sigma$) confidence level to determine whether or not two samples are alike or different (Beers 1957). For this reason, high precision instruments are desirable, allowing scientists to detect and measure even minute differences among samples.

The ICP-MS at the Department of Geological Sciences at The University of Texas at Austin used to analyze the bone powder samples for ^{13}C and ^{18}O reported the analytical precision for each element at the time of analysis. The analytical precision for $\delta^{18}\text{O}$ was reported as 0.05‰ and for $\delta^{13}\text{C}$ was reported as 0.07‰.

The High-Precision Mass Spectrometry and Environment Change Lab (HISPEC) at National Taiwan University that analyzed samples for ^{87}Sr used an ICP-MS with the analytical precision reported as 0.000010 at the time of analysis, and when converted to ϵ , as are the sample values, 0.10.

Chapter 4 Results

4.1 Faunal Data

The faunal samples analyzed at The University of Texas at Austin for ^{18}O and ^{13}C were reported as $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values and are presented in Table 3. The ^{87}Sr values for the faunal samples analyzed at National Taiwan University were reported as $^{87/86}\text{Sr}$ values; these values were converted to ϵ notation for data interpretation and both values are presented in Table 3.

Table 3: Faunal $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and $\epsilon^{87}\text{Sr}$ Values
(VPDB Standard for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) (NBS 987 Standard for $\epsilon^{87}\text{Sr}$)

Animal	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\epsilon^{87}\text{Sr}$	$^{87/86}\text{Sr}$ Raw Value	Parish
16	-2.80	-16.44	78.22	0.715844	Allen
12	-3.83	-13.71	-1.28	0.710197	Avoyelles
14	-2.93	-17.35	42.46	0.713304	Beauregard
54	-2.48	-11.70	-2.82	0.710088	Catahoula
55	-2.18	-15.11	-5.85	0.709873	Catahoula
21	-2.81	-14.64	24.06	0.711997	Desoto
Raccoon 1	-5.03	-10.81	-4.46	0.709971	East Baton Rouge
Raccoon 2	-3.74	-13.38	-6.62	0.709817	East Baton Rouge
Fox 1	-4.21	-13.92	61.73	0.714673	Evangeline
2	-3.76	-15.54	56.03	0.714268	Evangeline
3	-4.39	-14.60	72.62	0.715446	Evangeline
4 Cascade	-2.49	-14.92	59.14	0.714664	Evangeline
4 Cell Disruptor	-2.42	-14.91	61.60	0.714489	Evangeline
7	-2.38	-16.07	24.15	0.712003	Evangeline
8	-4.82	-15.00	74.49	0.715579	Evangeline
10	-3.54	-16.23	83.82	0.716242	Evangeline
32	-2.84	-16.52	81.19	0.716055	Evangeline
36	-1.95	-16.29	72.77	0.715457	Evangeline
18	-4.39	-15.88	61.47	0.714654	Gallia County, OH
19	-3.53	-18.11	63.86	0.714824	Gallia County, OH
20	-4.49	-16.15	60.62	0.714594	Gallia County, OH
33	-2.48	-16.25	10.84	0.711058	Grant
46	-5.24	-16.73	48.49	0.713732	Hancock County, WV
60	-3.73	-15.90	-10.94	0.709511	Iberia

(Table 3 continued)

Animal	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\epsilon^{87}\text{Sr}$	$^{87/86}\text{Sr}$ Raw Value	Parish
28	-3.39	-15.59	-4.91	0.709939	Iberville
6	-3.83	-14.75	51.57	0.713951	Jasper County, TX
59	-3.39	-16.13	-11.23	0.709491	Jefferson County, MS
47	-3.13	-11.98	-5.17	0.709920	Kiowa County, KS
30	-3.30	-15.36	55.06	0.714199	LaSalle
53	-3.11	-15.81	40.40	0.713158	LaSalle
61	-4.42	-17.03	36.08	0.712850	Livingston
25	-3.38	-18.76	-7.31	0.709768	Maddison
52	-4.46	-16.39	37.56	0.712956	Meigs County, TN
49	-3.23	-15.98	1.55	0.710398	Morehouse
34	-3.43	-15.80	74.97	0.715613	Natchitoches
37	-1.99	-16.10	71.87	0.715393	Rapides
50	-3.95	-12.56	54.81	0.714181	Rapides
51	-1.94	-17.18	3.78	0.710556	Rapides
57	-2.50	-18.44	-6.81	0.709805	Rapides
45	-2.42	-16.24	-13.99	0.709295	Sabine
27	-2.40	-12.18	-0.73	0.710236	Shelby County, TX
13	-3.64	-14.02	-16.66	0.709105	St. Helena
26	-4.26	-17.68	-30.29	0.708136	St. Helena
15	-2.80	-15.60	56.20	0.714280	Vernon
35	-3.08	-16.86	98.58	0.717290	Vernon
29	-1.99	-16.43	15.73	0.711405	Winn
31	-1.37	-16.46	18.97	0.711635	Winn

Although the main goal of this project was to create an isoscape for Louisiana in terms of $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and $\epsilon^{87}\text{Sr}$, procedural tests were required to justify the manner in which samples were handled, presented in subsequent sections. Afterward, the regional results will be presented, compared to previous data presented in the Literature Review, and the differences among environments and diets will be presented.

4.2 Differences in Cleaning Methods

Forensic anthropologists routinely use chemical agents to aid in cleaning human remains. A variety of products are suitable, in the sense that they do not have an adverse affect on DNA sampling; such chemicals include acetic acid, ammonia, trypsin, and many others (Narwrocki 1997; Garvie-Lok 2004; Li 2011). In order to assess the possible interference of Cascade dish detergent, the agent used at FACES Laboratory, with stable isotope analysis, a simple experiment was conducted.

The experiment conducted was two fold. In order to test the effects of Cascade independent of cortical bone thickness, both raccoon and deer femora were used. Two femora from the same deer, Deer 4, and two raccoon femora, Raccoon 1 and Raccoon 2, were used. Considering the raccoons were of comparable age, collected together from the same site, and are known to have died at the same time, it is feasible that these raccoons had very similar diets. And so, the comparison between these raccoons for the effects of Cascade is valid.

One deer femur and Raccoon 2 were cleaned using the cell disruptor, serving as the control, and the other deer femur and Raccoon 1 were cleaned using warm water and Cascade. All four bones were sampled for $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and $\epsilon^{87}\text{Sr}$ analysis, results are presented in Table 4 and Table 5. Figures 18 - 23 provide a graphical representation of Table 4 and Table 5. The analytical precision for each isotope at the 95% confidence level is represented as error bars in the respective graph.

Table 4: Cascade Affects on Thin Cortical Bone

	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\epsilon^{87}\text{Sr}$
Raccoon 1 Cascade	-5.03	-10.81	-4.46
Raccoon 2 Cell Disruptor	-3.74	-13.38	-6.62
Difference	1.29	2.57	2.16
Analytical Precision	0.05	0.07	0.10

$\delta^{18}\text{O}$ of Raccoons

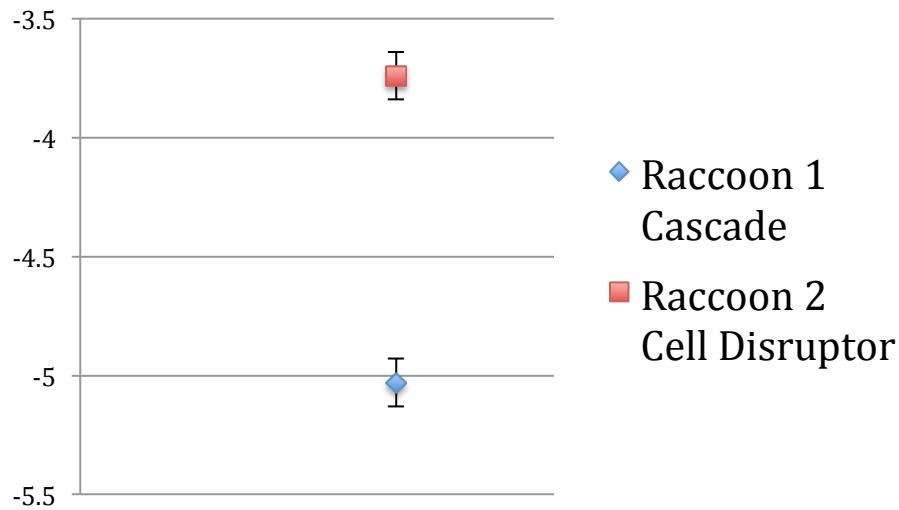


Figure 18: Cascade Affects on $\delta^{18}\text{O}$ of Raccoons

The error bars for $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and $\epsilon^{87}\text{Sr}$ do not overlap for the raccoons, i.e. thin cortical bone. The non-overlapping values between Raccoon 1 and Raccoon 2 indicate that these measurements are analytically different from one another. This difference could possibly be due to dietary differences causing the raccoons to be absolutely different rather than relatively different from the effects of Cascade. These raccoons were thought to be of similar diet, but the abundance of discarded grocery store food available to these raccoons introduces the variable of food preference and the possibility of isotopically different diets, making the determination of Cascade effects on thin cortical bone inconclusive.

$\delta^{13}\text{C}$ of Raccoons

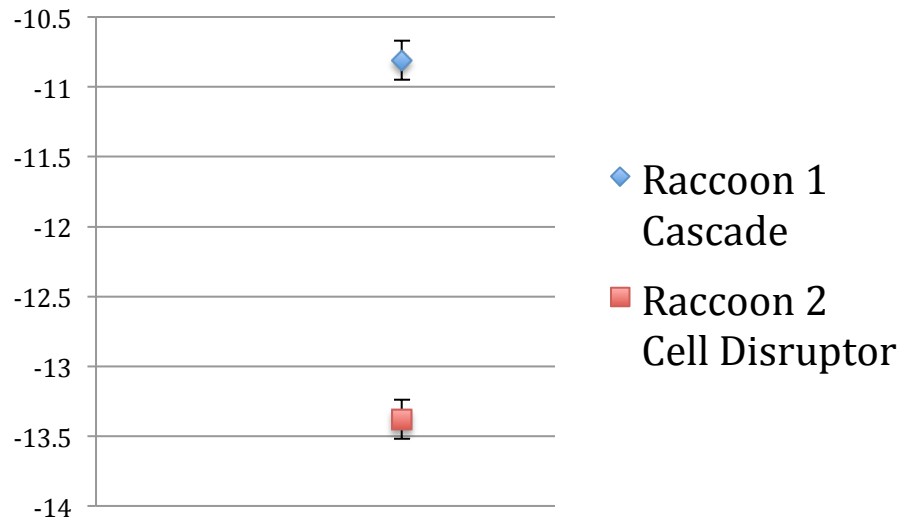


Figure 19: Cascade Affects on $\delta^{13}\text{C}$ of Raccoons

$\epsilon^{87}\text{Sr}$ of Raccoons

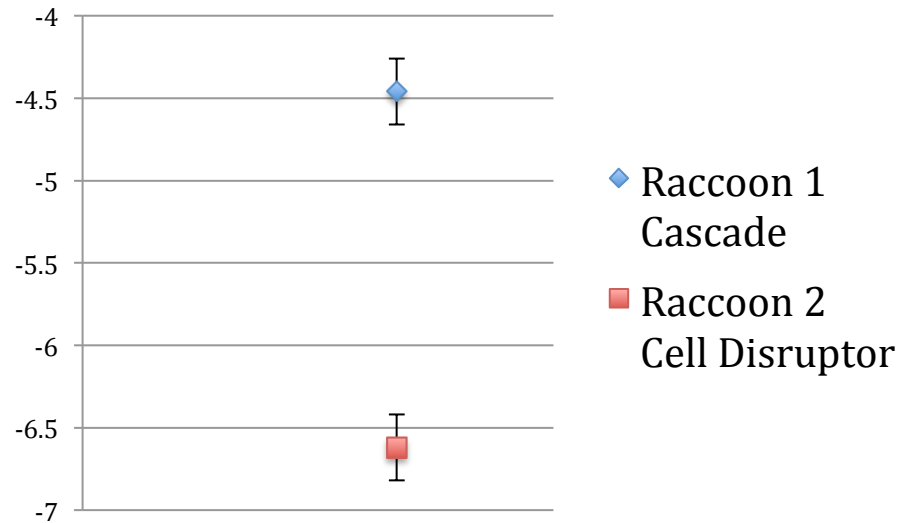


Figure 20: Cascade Affects on $\epsilon^{87}\text{Sr}$ of Raccoons

Table 5: Cascade Affects on Thick Cortical Bone

	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\epsilon^{87}\text{Sr}$
Deer 4 Cascade	-2.49	-14.92	59.14
Deer 4 Cell Disruptor	-2.42	-14.91	61.60
Difference	0.07	0.01	2.46
Analytical Precision	0.05	0.07	0.10

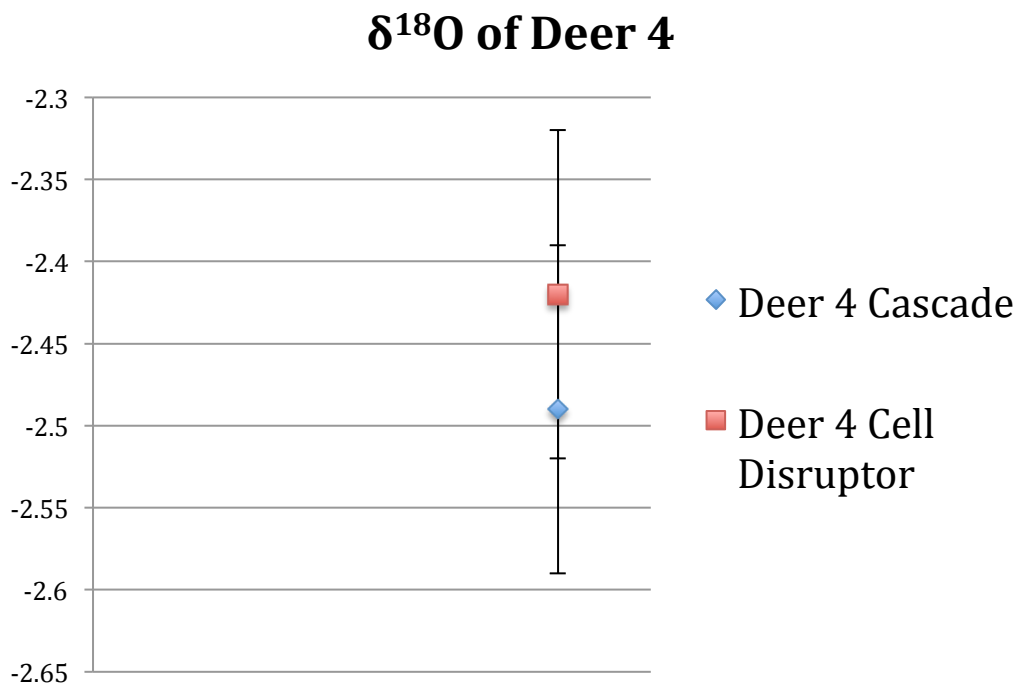


Figure 21: Cascade Affects on $\delta^{18}\text{O}$ of Deer 4

The error bars for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ overlap for Deer 4, i.e. thick cortical bone. The overlapping values between Deer 4 Cascade and Deer 4 Cell Disruptor indicate that these measurements are not analytically different from one another. This leads to the procedural conclusion that Cascade does not significantly alter the isotopic values in thick cortical bone samples in regards to $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$. However, the error bars do not overlap for $\epsilon^{87}\text{Sr}$, indicating that Cascade did have an effect on measuring $^{87/86}\text{Sr}$.

$\delta^{13}\text{C}$ of Deer 4

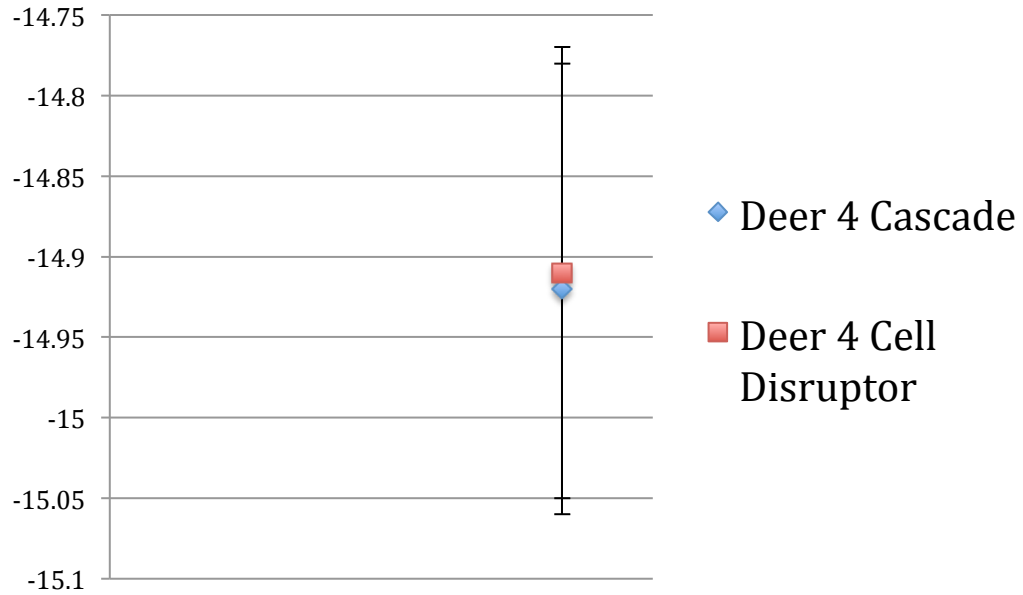


Figure 22: Cascade Affects on $\delta^{13}\text{C}$ of Deer 4

$\epsilon^{87}\text{Sr}$ of Deer 4

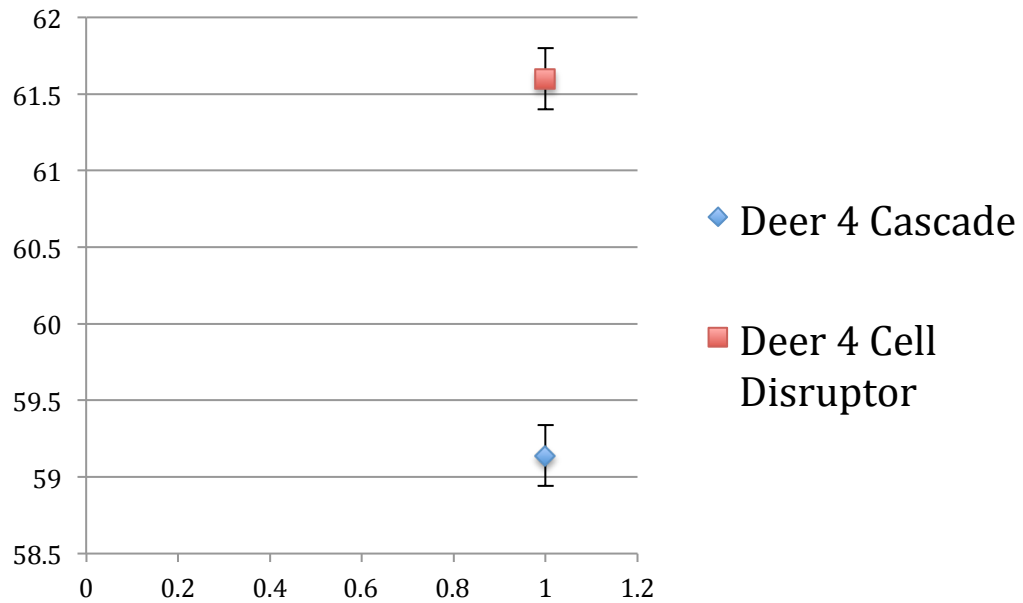


Figure 23: Cascade Affects on $\epsilon^{87}\text{Sr}$ of Deer 4

4.3 Individuals of the Same Urban Environment

The raccoon samples collected offered an opportunity for an initial assessment of similar individuals living in an urban environment. There are many food options within one human household, and therefore many food options within the trashcans of human households. The two raccoons collected can serve as a pilot study of two individuals living in the same household, since they are of comparable age and were collected together from the same death incident. The results in Table 4 show that although these individuals were living in a similar environment, the individuals are different from one another. From these results we can say that individuals of the same household may possess different isotopic values and further investigation is merited to determine the degree of difference among such individuals.

4.4 Difference between Cut and Uncut Femora

The femora in this project were cleaned using ultra sonic cell disruption. The distal and proximal ends of the femora, areas with large, tough muscle attachments, were removed to decrease the time needed to completely free a femur of all tissue. Although all femora used in the study were processed in this manner, one deer femur, Deer 20, was left whole. Deer 20 served as a control to which Deer 18 and Deer 19 can be compared, being harvested at the same time and location. From this comparison, any affect on isotopic values caused by the removal of the distal and proximal ends can be assessed. The comparison of the values for Deer 18, Deer 19, and Deer 20 are presented in Table 6 and Figures 24 - 26.

Table 6: Cut vs. Uncut Femora

	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\epsilon^{87}\text{Sr}$
Deer 18 (Cut)	-4.39	-15.88	61.47
Deer 19 (Cut)	-3.53	-18.11	63.86
Deer 20 (Uncut)	-4.49	-16.15	60.62
Analytical Precision	0.05	0.07	0.10

Cutting Effects on $\delta^{18}\text{O}$ of Deer Femora

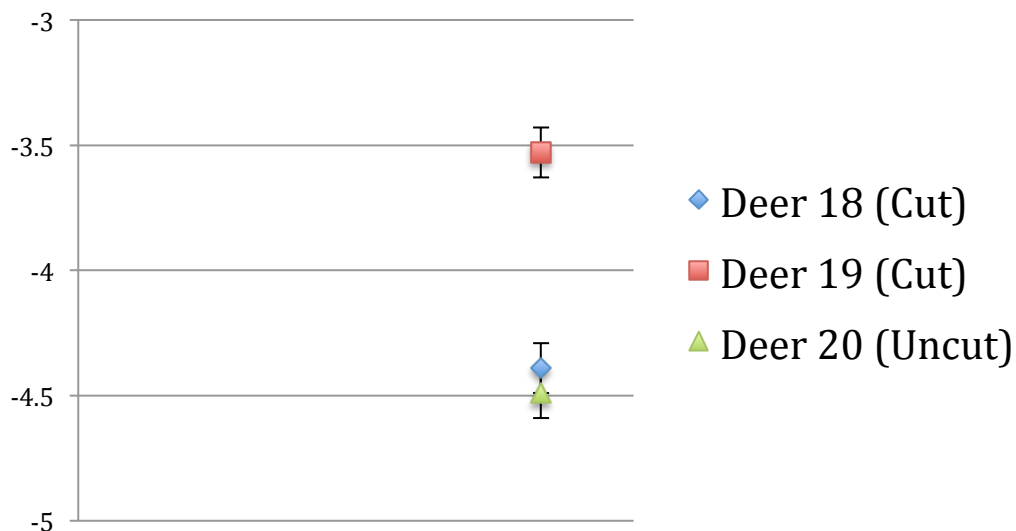


Figure 24: Cutting Effects on $\delta^{18}\text{O}$ of Deer Femora

Figures 24 – 26 show that Deer 18 (Cut) and Deer 20 (Uncut) are more similar in value to each other than to Deer 19 (Cut). This shows that there is more difference among the individuals than there is in the procedural difference of being cut prior or following cell disruption.

4.5 Intrasample Reproducibility

The ability to replicate measurements is important for establishing the reliability of data. To assess the repeatability of measurements within one deer, some samples excised were separated into two aliquots.

Cutting Effects on $\delta^{13}\text{C}$ of Deer Femora

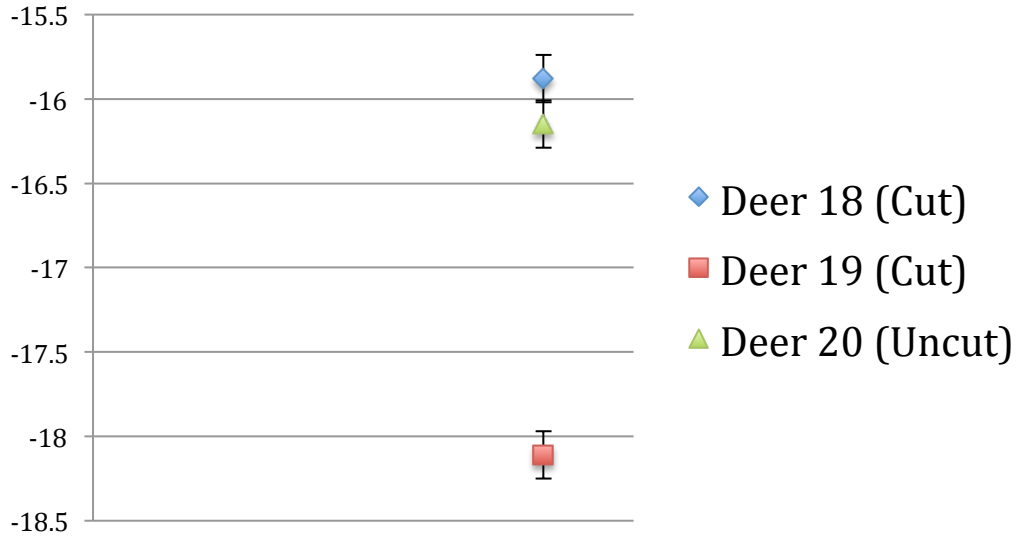


Figure 25: Cutting Effects on $\delta^{13}\text{C}$ of Deer Femora

Cutting Effects on $\epsilon^{87}\text{Sr}$ of Deer Femora

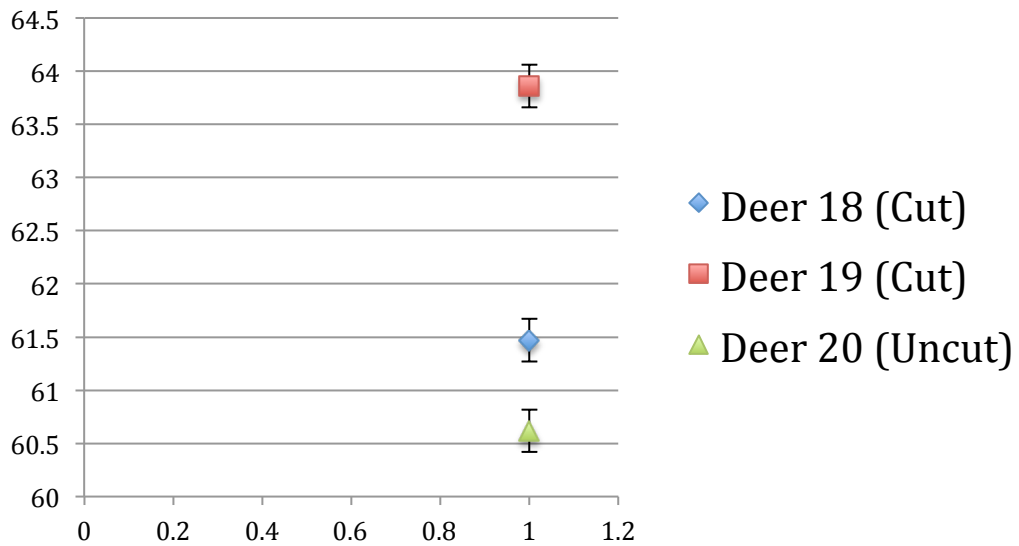


Figure 26: Cutting Effects on $\epsilon^{87}\text{Sr}$ of Deer Femora

For $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, analysis on one aliquot from Deer 55 occurred in August and the second occurred in October; the results are presented in Table 7, Figure 27, and Figure 28. Reproducibility testing for $\epsilon^{87}\text{Sr}$ was conducted on two separate deer, separating each sample into two aliquots. Analyses on both aliquots were done in the same instrument sequence; the results are presented in Table 8 and Figure 29.

Table 7: $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ Reproducibility

Deer 55	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$
August	-1.99	-15.16
October	-2.18	-15.11
Difference	0.19	0.05
Analytical Precision	0.05	0.07

Table 8: $\epsilon^{87}\text{Sr}$ Reproducibility

	Deer 26	Deer 35
$\epsilon^{87}\text{Sr}$	-29.85	99.08
$\epsilon^{87}\text{Sr}$	-30.74	98.09
Difference	0.89	0.99
Analytical Precision	0.10	0.10

$\delta^{18}\text{O}$ Reproducibility of Deer 55

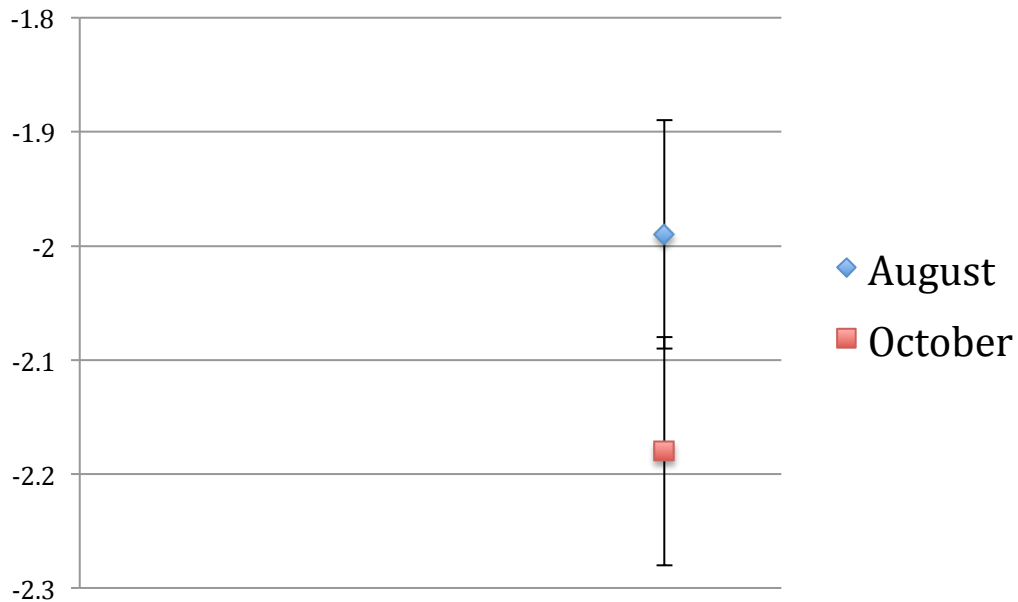


Figure 27: $\delta^{18}\text{O}$ Reproducibility of Deer 55

$\delta^{13}\text{C}$ Reproducibility of Deer 55

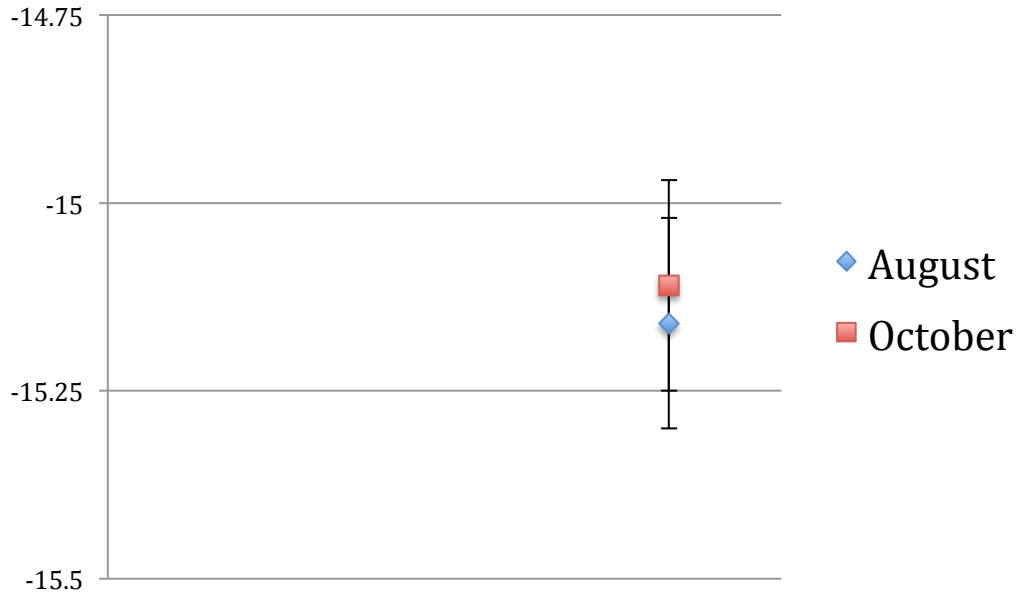


Figure 28: $\delta^{13}\text{C}$ Reproducibility of Deer 55

$\epsilon^{87}\text{Sr}$ Reproducibility of Deer 26 and Deer 35

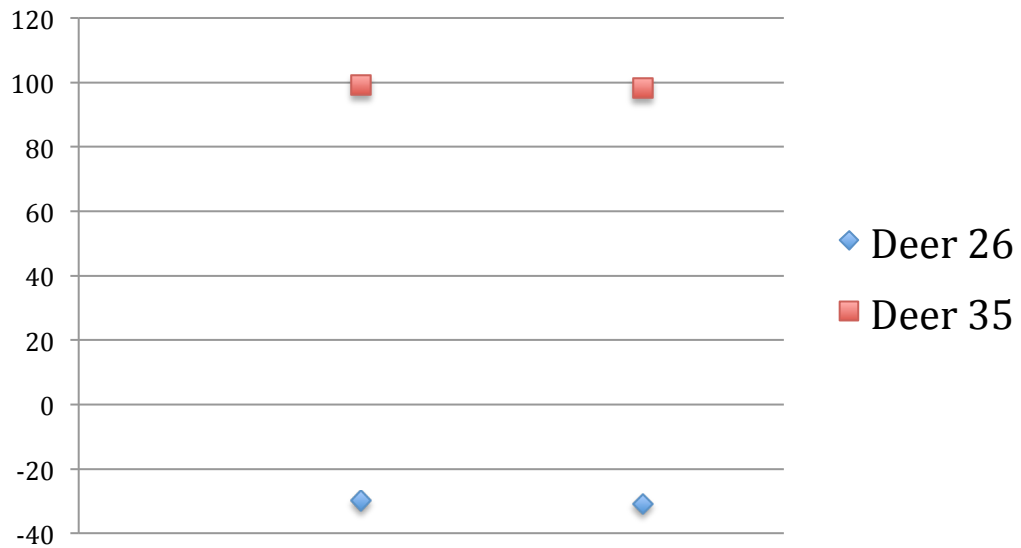


Figure 29: $\epsilon^{87}\text{Sr}$ Reproducibility of Deer 26 and Deer 35

Figures 27 – 29 clearly demonstrate that the $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and $\epsilon^{87}\text{Sr}$ values between samples of the same deer are within analytical precision, even when analyzed in different months. From these data we can infer that multiple analyses from the same bone would result in data sets that are not measurably different.

4.6 Mapping of Louisiana Deer Data

The $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and $\epsilon^{87}\text{Sr}$ values obtained for the faunal samples, Table 3, have been separated into parishes and averaged whenever possible. The averages and standard deviations have been plotted over the respective Louisiana parishes, which are presented as Figures 30 – 32. Only Louisiana deer values were used to make these maps; fox and raccoon samples were not included. Two deer samples were excluded, Deer 4 Cascade and Deer 20. Deer 4 Cascade was not included because Cascade is not consistent with the cleaning methods of the other deer, and Deer 20 was not included because it was not cut for cell disruption processing, differing from the other deer in the study.

4.7 Isotopic Regions of Louisiana

Plotting an average value for each of the 19 parishes has yielded regions of Louisiana with distinctive value ranges for $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and $\epsilon^{87}\text{Sr}$. Before discussing each regional map of isotopic values, the methodology of determining regions will be discussed.

Regions were determined by statistically assessing the differences among parish averages. The standard deviation for each average value gives an assessment of how far the values in the data set are from the average value. Adding and subtracting the standard deviation from the average will give a range in

which the values in the data set lie. The range of values for each parish was determined in this manner, and parishes with overlapping ranges were grouped into the same region. This practice resulted in regions possessing values distinct from other regions.

Regional maps for the faunal data of $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and $\epsilon^{87}\text{Sr}$ are presented in Figures 33 – 35. The value ranges for each region are presented in the corresponding map legend. Some region value ranges do overlap with other regions, but the spatial separation of the similar values necessitated their placement into separate regions. Overlap in values among regions can be attributed to the lack of multiple samples from which to determine an average as well as to the lack of samples from some parishes.

4.8 USGS and Faunal Data Comparison

The USGS (United States Geological Survey) has documented carbon-13 and oxygen-18 concentrations of groundwater in many areas of Louisiana from 1998 to the present, with the exception of one measurement taken in Catahoula Parish in 1983. The retrieved data from the USGS groundwater database for Louisiana included all sites for which analyses had been done and averages were determined by parish; these data can be found in Appendices B and C.

The number of analytical sites within the parish and the number of faunal samples obtained from that parish can be found in Appendices B and C as well. Although all data will be interpreted as averages across parishes, Appendix D presents each faunal sample matched with the closest USGS analytical measurement site.

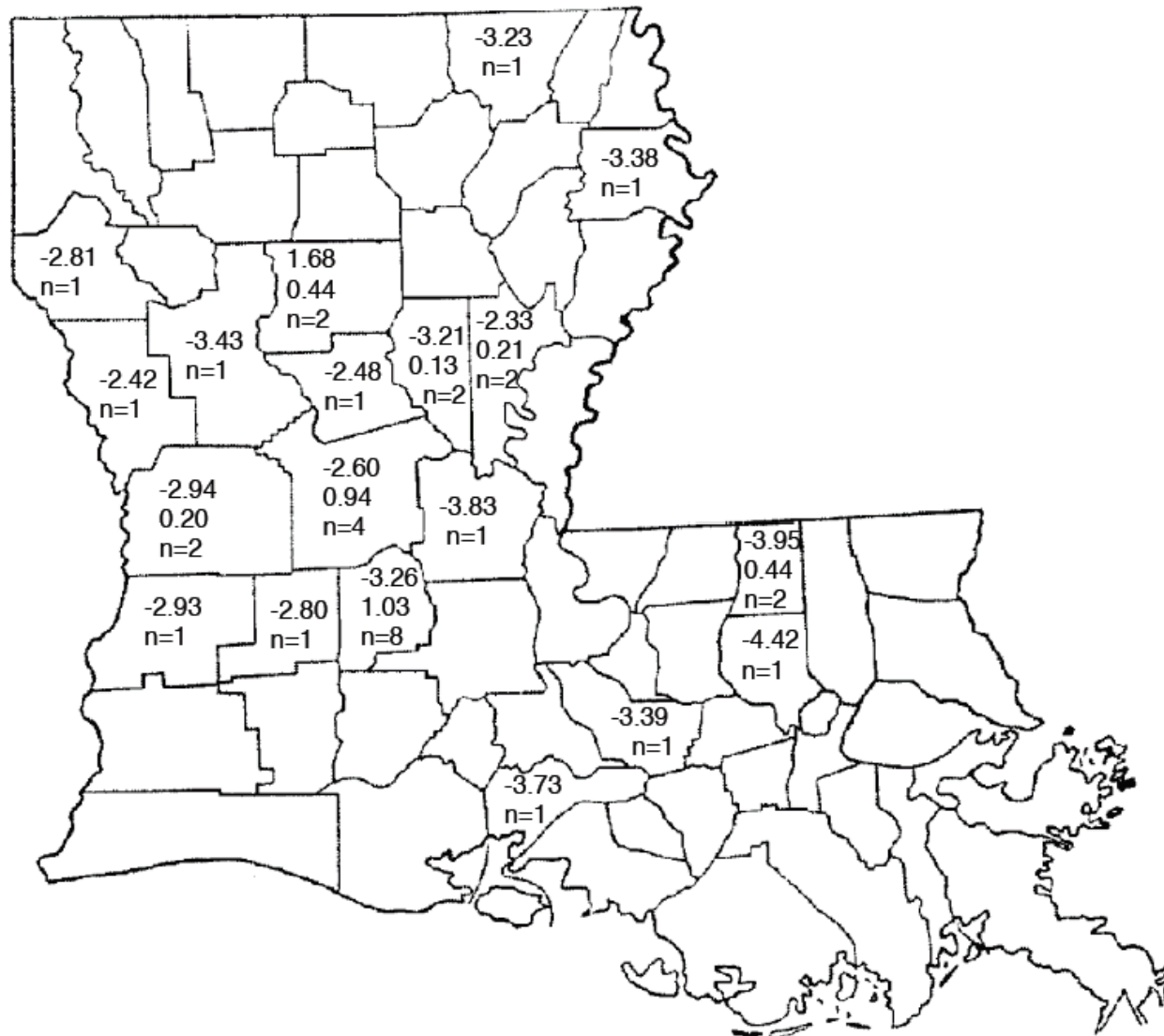


Figure 30: Louisiana Plot of Average $\delta^{18}\text{O}$ Deer Values and Standard Deviation

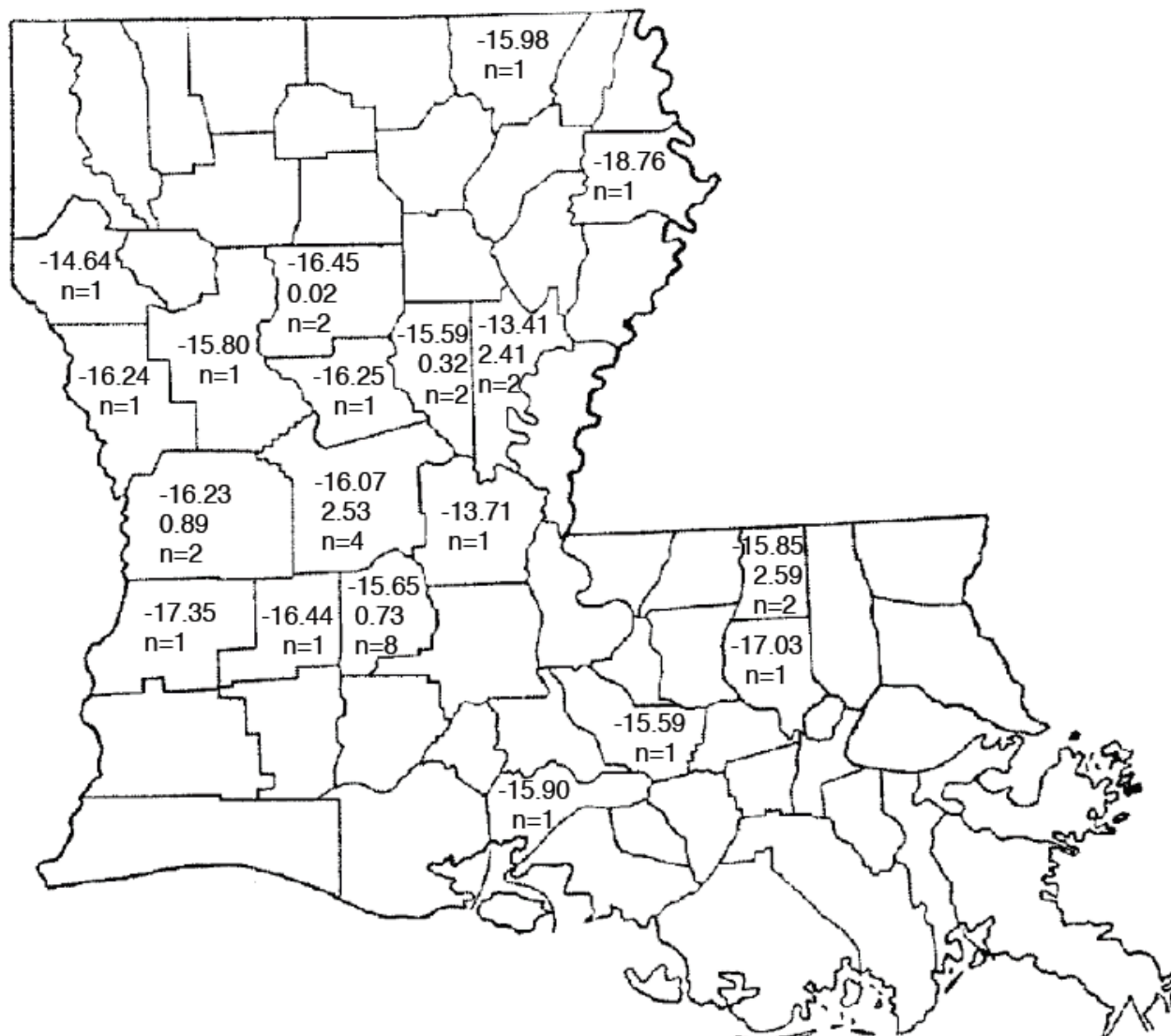


Figure 31: Louisiana Plot of Average $\delta^{13}\text{C}$ Deer Values and Standard Deviation

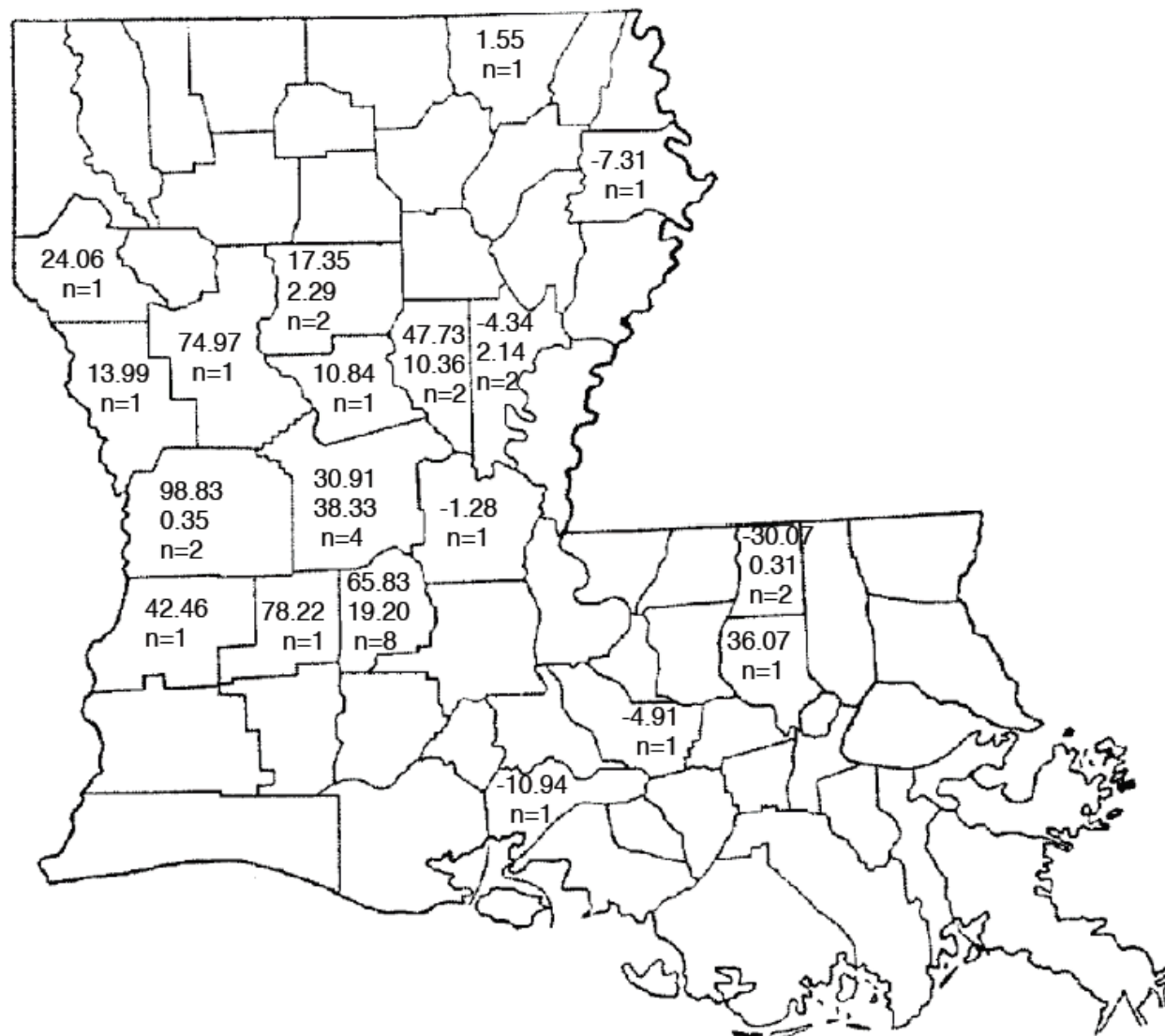


Figure 32: Louisiana Plot of Average $\epsilon^{87}\text{Sr}$ Deer Values and Standard Deviation

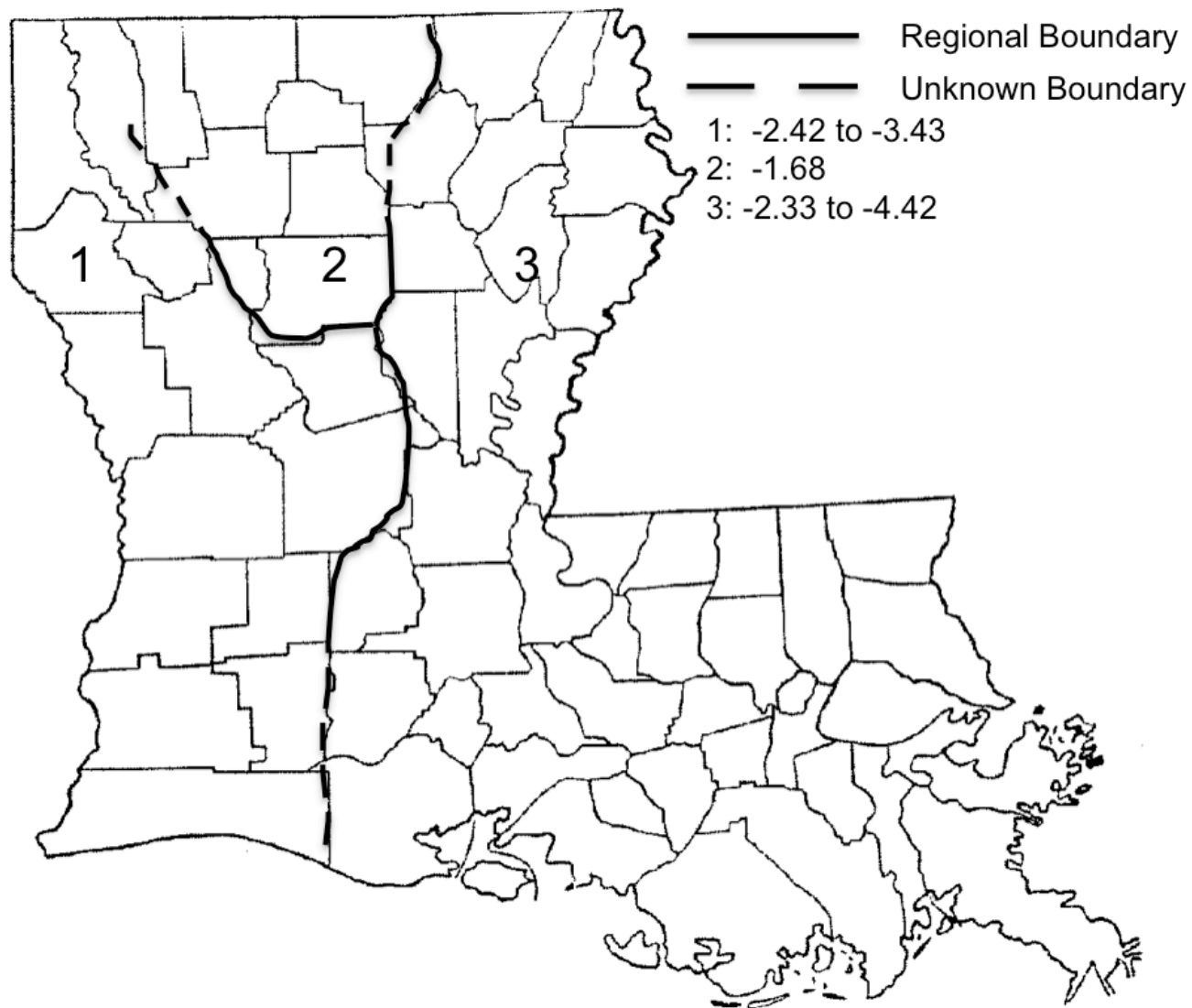


Figure 33: $\delta^{18}\text{O}$ Faunal Regions of Louisiana (VPDB)

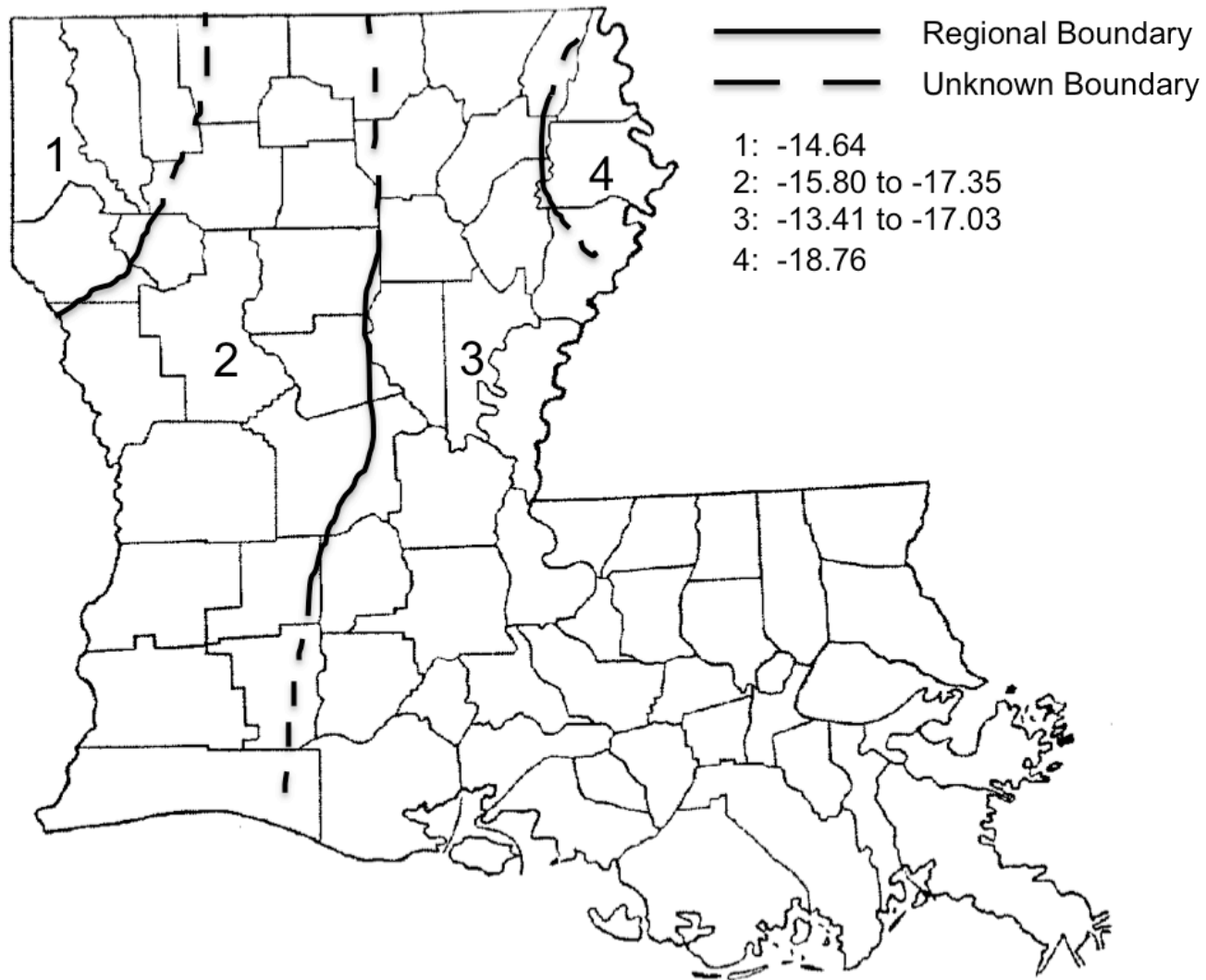


Figure 34: $\delta^{13}\text{C}$ Faunal Regions of Louisiana (VPDB)

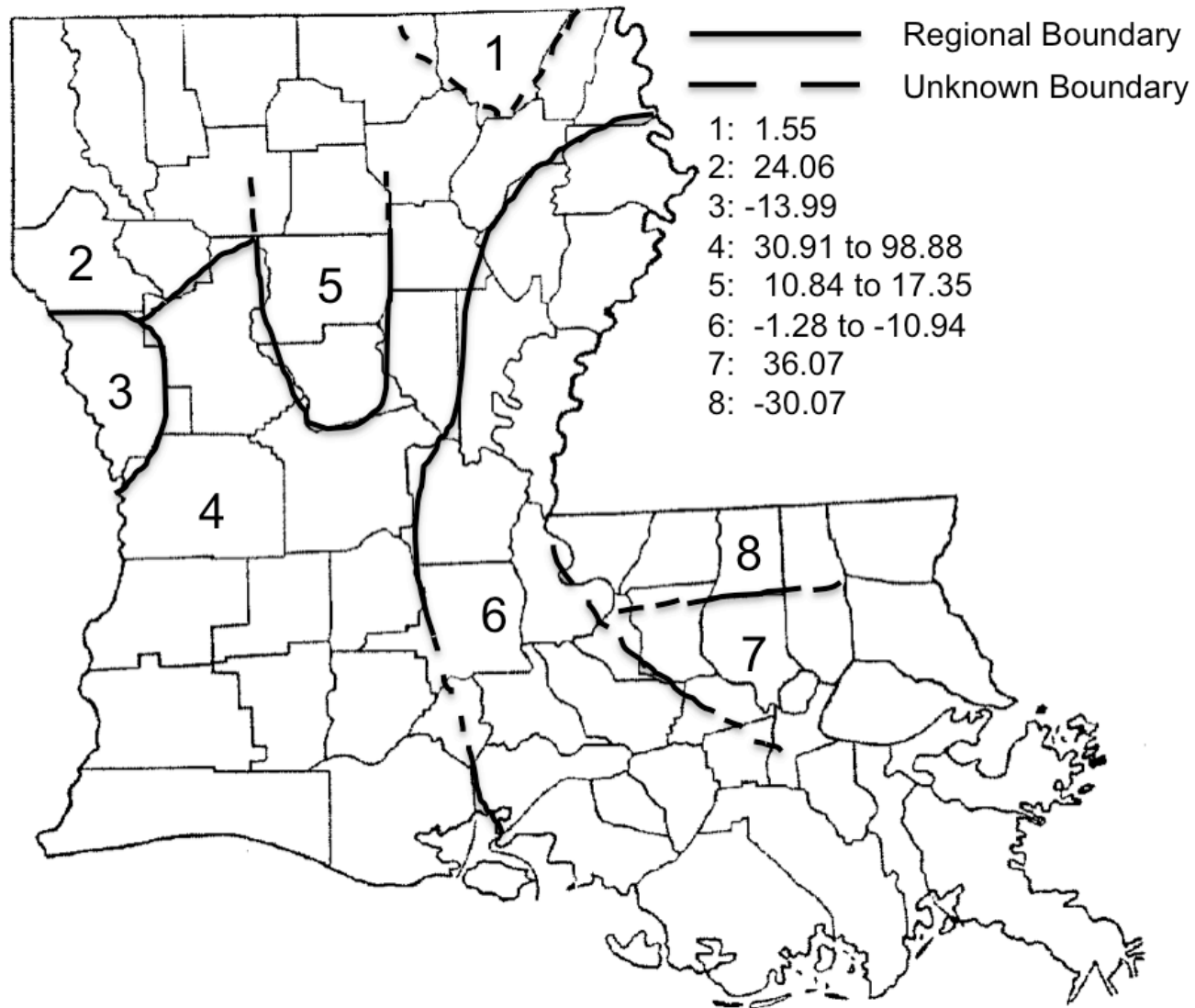


Figure 35: $\epsilon^{87}\text{Sr}$ Faunal Regions of Louisiana (NBS 987)

The USGS data are reported with respect to the VSMOW standard for $\delta^{18}\text{O}$ and VPDB for $\delta^{13}\text{C}$, but the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ faunal values are reported in respect to the VPDB standard. Regional maps were constructed along the same methodology as the regional maps for the faunal data. Agreement among the distinguishable regions according to faunal data and USGS data would negate the necessity of converting VSMOW values into VPDB values for exact comparison of $\delta^{18}\text{O}$. Figure 36 presents the $\delta^{18}\text{O}$ maps made from the USGS data and the deer data.

Consulting these maps in unison, the regional divisions are not contrary between maps. The USGS data were able to separate the state into more regions, but the USGS also had data available for more parishes. The general partition of the state into east and west, along the west bank of the Mississippi River, as in the deer data map, is still evident in the USGS map. The northern pocket of distinct values is also evident in both maps. Further sampling of both water and deer should bring these maps into further agreement.

The $\delta^{13}\text{C}$ faunal data are more difficult to compare to USGS data. The USGS data for $\delta^{13}\text{C}$ are very limited. The regions distinguishable from each data set are compared in Figure 37. The northwestern corner of Louisiana forms a region according to both the USGS and deer data, but the data are too sparse for any further comparison or interpretation.



(A)



(B)

Figure 36: $\delta^{18}\text{O}$ Regional Comparison: (A) USGS (B) Deer



(A)



(B)

Figure 37: $\delta^{13}\text{C}$ Regional Comparison: (A) USGS (B) Deer

4.9 Faunal Strontium and Water Strontium Comparison

Strontium values obtained from the Louisiana deer are a measure of the biologically available strontium in the environment; strontium that has entered the local water supply and is available for consumption. A predictive model of strontium water values for the United States, Figure 9 in section 2.3.3, reports little variability in Louisiana raw strontium values, predicting all of Louisiana to fall within the range of 0.711 – 0.713 (Bataille 2012). The raw values for the deer data are presented in Appendix E, but the values range from 0.708 – 0.717. The values measured in the deer encompass 5 range categories in the predicted model, meaning Louisiana should be colored with five colors rather than one, Figure 9.

4.10 Faunal Data and Geologic Comparison

The geology of Earth impacts the availability and distribution of stable isotopes. The similarity of the regional boundaries formed by the deer data and the geologic regions of Louisiana suggest the reliability of bone in recording stable isotopes.

The impact of geology is mostly reflected in strontium stable isotopes, but the deer data presented in this thesis demonstrate the possibility that oxygen stable isotopes may have some geologic dependence as well. Figure 38 presents the geologic regions of Louisiana as reported by the Louisiana Geologic survey compared to the $\epsilon^{87}\text{Sr}$ regions of the deer data, and Figure 39 presents the $\delta^{18}\text{O}$ deer regions compared to the geologic regions of Louisiana (Chacko 2010).

The map comparison has shown that strontium values in bone are highly dependent on the soils of Louisiana; both maps have very similar regional

boundaries. The $\delta^{18}\text{O}$ regions are in clear accordance with the rivers in Louisiana, but the rivers are also separating different geologic regions of Louisiana. Louisiana geology may have more of an impact on isotopic variation incorporated into the water sources available for animal consumption than previously considered.

4.11 Differences among Natural and Urban Fauna

Assessing the differences between an urban diet and natural diet of the same isotopic region is an important step in assessing the applicability of stable isotope analysis in modern humans. The two urban raccoons were acquired from East Baton Rouge Parish. No deer samples were harvested from East Baton Rouge, but the parish has been attributed to particular regions within the three isoscapes of Louisiana. Table 9 presents the isotopic comparison of urban and natural diets for the region encompassing East Baton Rouge Parish.

Table 9: Urban vs. Natural Diet

	Urban (Raccoons) Average, Standard Deviation	Natural (Deer) Region Range
$\delta^{18}\text{O}$	-4.39, 0.91	-2.33 to -4.42
$\delta^{13}\text{C}$	-12.10, 1.81	-13.41 to -17.03
$\epsilon^{87}\text{Sr}$	-5.54, 1.53	36.07

The average values of the urban raccoons are within the natural regional values for both $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, but the $\epsilon^{87}\text{Sr}$ values are vastly different. Similar $\delta^{18}\text{O}$ values suggest that the water values between an urban and natural environment are not dissimilar for the area around East Baton Rouge Parish. The map of Louisiana deer $\delta^{13}\text{C}$ values includes Baton Rouge in the region of values from -13.41‰ to -17.03‰, but no parish in this region, or any parish on the map presented in Figure 31, has an average deer $\delta^{13}\text{C}$ value of approximately -12.00‰.

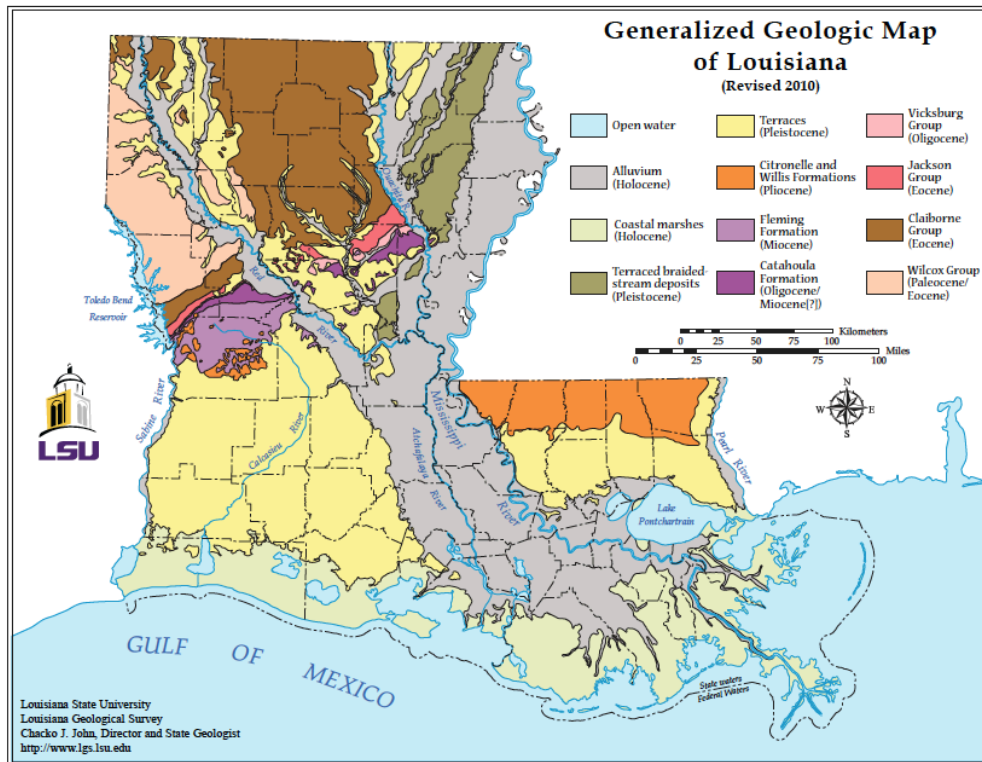
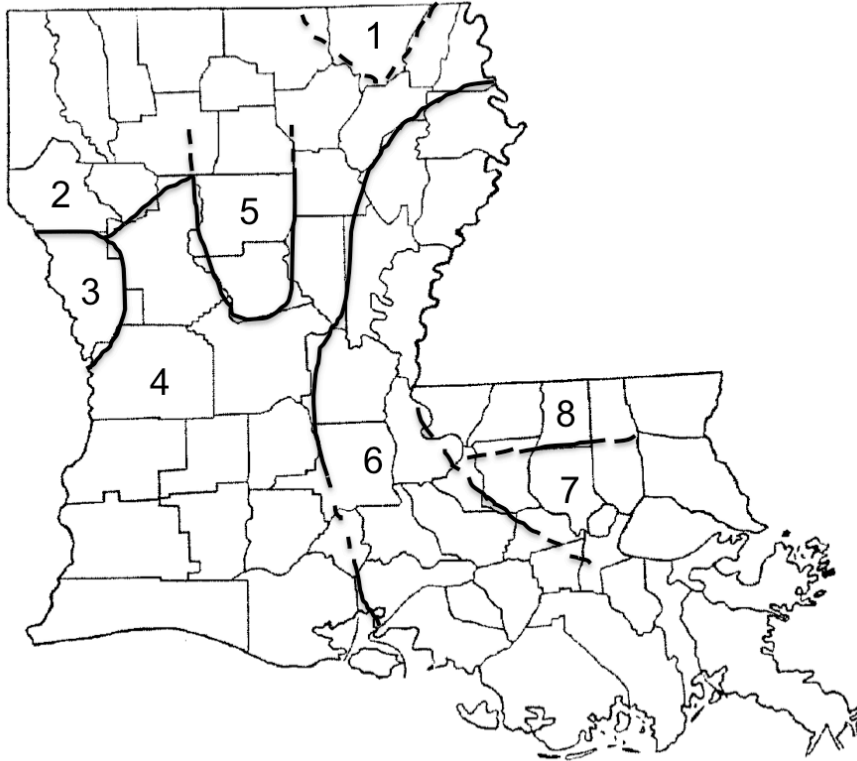


Figure 38: $\epsilon^{87}\text{Sr}$ Deer Data and Louisiana Geology Comparison

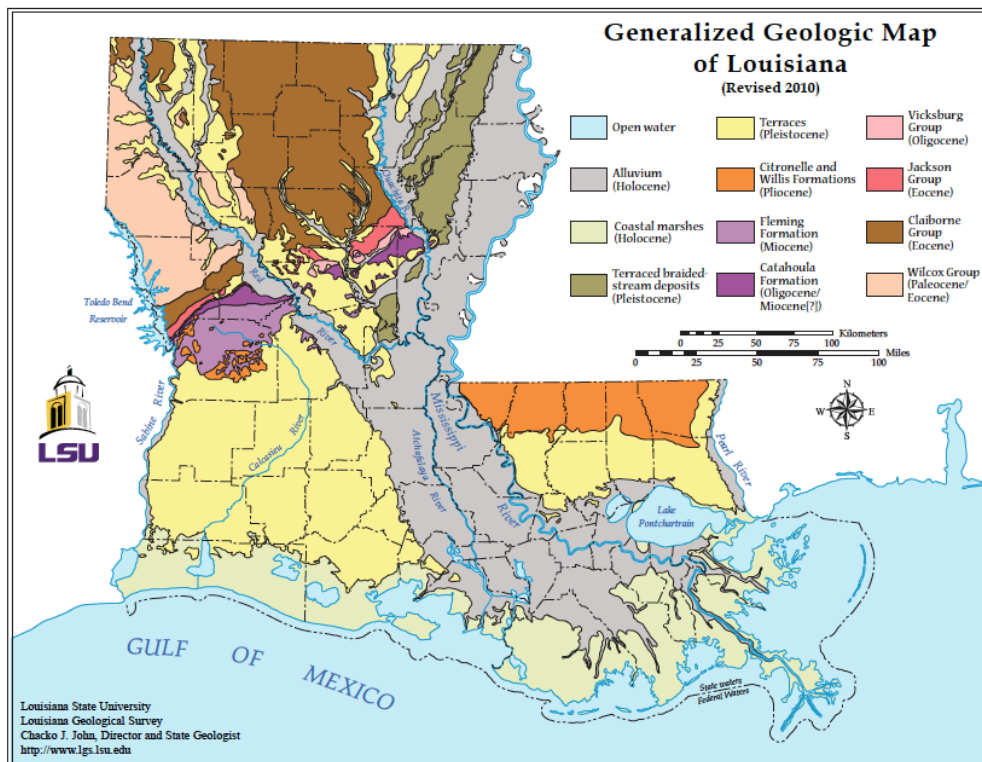


Figure 39: $\delta^{18}\text{O}$ Deer Data and Louisiana Geology Comparison

The standard deviation of the raccoon average does overlap with one value in the region of Louisiana including Baton Rouge, but this is a weak correspondence considering the overwhelming majority of values in the region of approximately -15.00‰. The difference in $\delta^{13}\text{C}$ values between the raccoons and deer can be attributed to differences in the type of vegetation consumed by the animals.

Further confirmation of dietary differences can be seen in the $\epsilon^{87}\text{Sr}$ values. The urban values from the raccoons have an average of -5.54 while the natural average value for the isotopic region is 36.07. This extreme difference suggests that distinguishing urban diets from natural diets is possible.

4.12 Differences among Herbivores and Omnivores

The collection of a fox from Evangeline Parish enabled the comparison of herbivores and omnivores from the same natural environment. Evangeline Parish is represented by 8 deer samples in our data set as well as the one fox. The average and standard deviation of the deer values for the parish are presented in Table 10 along with the fox isotopic values.

Table 10: Omnivore vs. Herbivore

	Omnivore (Fox)	Herbivore (Deer) Average, Standard Deviation
$\delta^{18}\text{O}$	-4.21	-3.26, 1.03
$\delta^{13}\text{C}$	-13.92	-15.65, 0.73
$\epsilon^{87}\text{Sr}$	61.73	65.83, 19.20

The $\delta^{18}\text{O}$ and $\epsilon^{87}\text{Sr}$ values for the fox are within the range of possible values for Evangeline Parish, but the $\delta^{13}\text{C}$ for the fox is not. The similarities between $\delta^{18}\text{O}$ values are expected, these fauna should be using the same water sources. The differences in $\delta^{13}\text{C}$ are also expected with the dietary differences of an omnivore and

an herbivore. The $\epsilon^{87}\text{Sr}$ value similarity indicates that biologically available strontium to these fauna is similar regardless of the omnivorous or herbivorous dietary difference.

4.13 Differences among Natural and Urban Omnivores

The two omnivores sampled here, urban raccoon and natural fox, provide the means to compare omnivores of different environments. The isotopic values for each are presented in Table 11.

Table 11: Natural Omnivore vs. Urban Omnivore

	Natural Omnivore (Fox)	Urban Omnivore (Raccoons) Average, Standard Deviation
$\delta^{18}\text{O}$	-4.21	-4.39, 0.91
$\delta^{13}\text{C}$	-13.92	-12.10, 1.81
$\epsilon^{87}\text{Sr}$	61.73	-5.54, 1.53

The $\delta^{18}\text{O}$ value of the fox and the average value for the raccoons agree with one another, and the values agree with the isotopic region, Region 3 of Figure 33, to which their harvest parishes are located. This agreement supports the expectation that water sources are not significantly different between the natural and urban environments within the same isotopic region.

The raccoon $\delta^{13}\text{C}$ average value and the fox $\delta^{13}\text{C}$ value are different. The fox value being just outside of the value range of the raccoon average, suggesting different foods that are available to omnivores of urban areas are different than the available foods in a natural environment. The $\epsilon^{87}\text{Sr}$ values are extremely different between the fox and the raccoons as well, further evidencing the differences in foods available to urban and natural omnivores.

4.14 Faunal Values by State

Along with the 19 Louisiana Parishes represented by the deer data, six other states are represented by at least one sample. The values for the out-of-state samples are presented in Table 12.

Table 12: Out of State Faunal Samples

State	$\delta^{18}\text{O}$ Sample Values	$\delta^{13}\text{C}$ Sample Values	$\epsilon^{87}\text{Sr}$ Sample Values
Kansas	-3.13	-11.98	-5.17
Mississippi	-3.39	-16.13	-11.23
Ohio (Same County)	-4.39 -3.53	-15.88 -18.11	61.61 63.86
Tennessee	-4.46	-16.39	37.56
Texas (Different Counties)	-2.40 -3.83	-14.75 -12.18	51.57 -0.73
West Virginia	-5.24	-16.73	48.49

In order to contextualize these data, Table 13 presents the possibility of out of state values being confused with Louisiana values. If an isotopic value fits within an isotopic region of Louisiana, a ✓ replaced the value appearing in Table 12, and if the value does not fit within a Louisiana isotopic region, an X replaced the value. The consultation of all three isotopic values, $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and $\epsilon^{87}\text{Sr}$, allowed for the determination if a sample from another state could be mistaken as a sample from Louisiana.

Table 13 clearly demonstrates that Ohio, Tennessee, and Texas could be confused for Louisiana according to isotopic ratios of $\delta^{18}\text{O}$, $\delta^{13}\text{C}$, and $\epsilon^{87}\text{Sr}$ presented in this thesis. Reasons for these overlapping values could be due to similar precipitation patterns, diet of deer, and geologic formations.

Table 13: Possible Confusion among States

State	$\delta^{18}\text{O}$ Values	$\delta^{13}\text{C}$ Values	$\epsilon^{87}\text{Sr}$ Values	Possible Confusion with Louisiana
Kansas	✓	✗	✓	No
Mississippi	✓	✓	✗	No
Ohio	✓ ✓	✓ ✓	✓ ✓	Yes
Tennessee	✓	✓	✓	Yes
Texas	✗ ✓	✓ ✗	✓ ✗	Yes
West Virginia	✗	✓	✓	No

It is expected that further sampling of these isotopes and the inclusion of other isotopes, such as nitrogen and lead, may help to resolve the possible confusion among states. Although Mississippi was not classified as a state of possible confusion, it is reasonable that Mississippi and Texas would have similar values to Louisiana since they are bordering states. A spatial map of faunal sampling in Texas, Louisiana, and Mississippi is presented in Figure 40 to contextualize the sampling distribution among these states.



Figure 40: Faunal Sampling Distribution of Texas, Louisiana, and Mississippi

Chapter 5 Discussion

5.1 Isoscapes of Louisiana

The current literature of modeled isotopic values for the United States and globe expect Louisiana to have uniform, or near uniform, isotopic values in respect to $\delta^{18}\text{O}$ and $\epsilon^{87}\text{Sr}$ (Bowen 2010; Beard 2000; Bataille 2012), but the faunal data collected here do not show the uniformity predicted by the models.

The $\delta^{18}\text{O}$ values have revealed a transition from approximate values of -2‰ to approximate values of -4‰ when moving across Louisiana west to east, while lower values of approximately -1‰ are located in the northern portion of the state.

The $\epsilon^{87}\text{Sr}$ values for Louisiana show more variation than predicted by Beard (2000) or Bataille (2012). The shapes of the value regions are also different, more closely resembling the shape of geologic regions presented by Chacko (2010). The strontium values cannot be summarized as a transition of values, as can be done with the oxygen, but the strontium regions are closely following the geologic formations of Louisiana.

The $\delta^{13}\text{C}$ values do not show a transition or sequential flow of values, but $\delta^{13}\text{C}$ does show definite regions of differing values. Although some overlap is reported for the $\delta^{13}\text{C}$ regions, each region presents a different mode value. Region 1 is mostly represented by values of approximately -14‰, Region 2 approximately -16‰, Region 3 approximately -15‰, and Region 4 approximately -18‰.

5.2 Time Periods of Collection

This study has collected samples from a span of only six months; however, the deer bone has been formed over a period of at least 2 - 2.5 years (time required

for a deer to reach adulthood). Although variation can be seen within this short time interval, large-scale variations occurring over many years cannot be assessed with the current faunal samples. Hurricanes, excessive precipitation, drought, and seasonal flooding can influence the isotopic values available to deer.

In order to show that the deer are directly reflecting environmental isotopic variation during the 2 – 2.5 year period of bone formation, sequential incremental water values are needed for those 2 – 2.5 years. Water values from 2010 to 2013 would reflect the 2 – 2.5 year period for the faunal data collected in this thesis and would provide an accurate evaluation of how deer bone records environmental fluctuations. Water data could not be obtained for that particular time period or for the exact locations of deer harvest, but some water data was obtained from the USGS.

The USGS water data are limited but can be useful. The data were collected sporadically over a large time span. Some areas were sampled in the same month for multiple years; others were sampled in different months for multiple years. Some were sampled for multiple months in the same year, and other areas were only sampled once. For a chronological assessment of the sampling see Appendix F. Despite the sparse and sporadic sampling of Louisiana groundwater, different isotopic regions are discernable.

An isotopic record as long and detailed as possible for Louisiana would be ideal in creating a regional map for which to compare modern humans. For this reason, the USGS data may be useful; it is a long time span but a sporadic chronology. In one respect, the sporadic chronology may not be a hindrance. Being

able to distinguish regions for the state with limited data, from sporadic sampling through time, gives credence to the idea that isotopic regions may be independent of time. It is feasible that the overall value of the region may change over long periods of time, centuries or more, as climates and weather patterns undergo significant change. However, the distinctions between separate regions should be maintained due to the same acting principles that created the distinctive regions.

It is expected that further data collection will help define the boundaries of the distinguishable regions within the state, and that these regions will be maintained despite seasonal or yearly variations in isotopic values.

5.3 Application in Modern Humans

Modern humans in the United States today may or not consume local foods, prefer an herbivorous or omnivorous diet, or move around rather than living in one place for an extended period of time. The maps presented in this thesis are assessments of the natural isoscapes of Louisiana and do not account for the behavioral aspects of diet in modern humans. Some faunal samples collected have allowed for an initial assessment of some of the differences observed between a natural and urban diet, offering some insight into the possibility of distinguishing historic modern humans from contemporary modern humans. Strontium isotopes exhibited the most variability in the state and will be used to discuss the possible distinctions between modern humans.

Two raccoons harvested from East Baton Rouge Parish were urban omnivores. The region of harvest for the raccoons has an $\epsilon^{87}\text{Sr}$ value of 36.07 while the raccoons yielded a value of -5.54. The raccoons, consuming urban food sources

from the trash of modern humans, are definitely distinguishable from the natural consumers of the region of harvest. However, comparison to these values involves a dietary difference, omnivore versus herbivore.

The ideal comparison would be an urban omnivore to a natural omnivore of the same region. The data available for this thesis did not provide the opportunity for that comparison, but a natural omnivore of a different region is available for comparison. The fox harvested from Evangeline parish yielded values consistent with the region from which it was harvested, unlike the raccoons that possessed different values than the region from which it was harvested. This difference suggests that the urban diet in the raccoons contributed more to the difference from the harvest region than the difference between an omnivorous and herbivorous diet. From this interpretation, historic modern humans should more closely reflect the areas in which they lived, and contemporary modern humans should exhibit differences from the natural environment due to urban diets. However, much more work must be done to assess the validity of this hypothesis.

Another obstacle in the application of stable isotope analysis in contemporary modern humans is whether individuals of the same urban environment will reflect similar isotopic values. The difference in isotopic values between the two raccoons, Table 4 and Figures 18 – 20, suggests that individuals of the same neighborhoods or cities may possess differing isotopic values, but sampling of multiple individuals throughout a city is required to investigate this possibility as well as the degree to which these individuals differ. A widespread sampling among urban centers of

Louisiana, as well as other states, must also be done to determine if urban centers are distinguishable from one another.

The ability of strontium to reflect the dietary differences between urban omnivores, natural omnivores, and natural herbivores is a good indication that the dietary differences among early historic modern humans and current modern humans will also be reflected by strontium. The measurable differences between the urban raccoons and the natural fox have provided evidence for phase two of the current thesis, an urban study comparing cities of Louisiana. If differences among natural and urban environments continue to be observed, then the application of stable isotope analysis to modern humans may be useful in a forensic setting.

Modern humans cannot and should not be compared to the isoscapes presented in this thesis. Modern humans are omnivores, and contemporary modern humans consume the majority of their food from grocery store purchases, which may or may not be supplied by local food sources. The isoscapes presented in this thesis are derived from herbivorous fauna, assessing the natural variability in the state of Louisiana. Before historic modern humans can be compared to an isoscape, a more comprehensive study of natural omnivores of Louisiana must be done, and an urban study must be performed to determine the isotopic variability available to contemporary modern humans. If variation does not exist among urban environments, the ability to distinguish between contemporary modern humans of different cities may not be possible.

Whether dealing with the remains of historic or contemporary modern humans, the manner in which the remains are cleaned and sampled may have an

impact on the values obtained during stable isotope analysis. Cascade dish detergent was determined to have no meaningful affect on the stable isotope value of thick cortical bone, in regards to $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, but did have an affect on $\epsilon^{87}\text{Sr}$, the degree of which must be investigated further. These results allow for forensic anthropologists to continue using Cascade in cleaning bone samples as well as permitting bones previously cleaned with Cascade to be sampled, but more work must be done to understand the interaction between ^{87}Sr and Cascade.

Chapter 6 Conclusion

Developing an isoscape, mapping of isotopic values over landscapes, on the scale of individual states can offer criminal investigators another tool in identifying individuals under the classification of Jane or John Doe. The current thesis has shown that provenience determination on a small scale, within the state of Louisiana, is possible and has potential to be applied in modern humans. Prior to application in modern forensic casework, the borders of the natural isotopic regions in the state of Louisiana will need to be refined, long-term sampling chronologies will need to be established, and urban isotopic variability needs to be assessed.

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Appendices

Appendix A: Sample Masses Obtained from Faunal Femoral Shafts

Animal	Sample Mass (g)		Animal	Sample Mass (g)	
	Austin	Taiwan		Austin	Taiwan
Raccoon #1	0.01	0.01	Deer #29	0.02	0.01
Raccoon #2	0.01	0.01	Deer #30	0.02	0.01
Fox #1	0.02	0.01	Deer #31	0.02	0.02
Deer #2	0.03	0.01	Deer #32	0.02	0.01
Deer #3	0.02	0.01	Deer #33	0.02	0.01
Deer #4 CD	0.02	0.01	Deer #34	0.03	0.01
Deer #4 Cascade	0.02	0.01	Deer #35	0.02	0.01
Deer #6	0.02	0.01	Deer #36	0.02	0.02
Deer# 7	0.02	0.02	Deer #37	0.02	0.01
Deer #8	0.02	0.01	Deer #45	0.02	0.01
Deer #10	0.03	0.01	Deer #46	0.02	0.01
Deer #12	0.02	0.01	Deer #47	0.02	0.01
Deer #13	0.02	0.01	Deer #49	0.02	0.01
Deer #14	0.02	0.02	Deer #50	0.02	0.01
Deer #15	0.02	0.01	Deer #51	0.02	0.01
Deer #16	0.02	0.01	Deer #52	0.02	0.01
Deer #18	0.02	0.01	Deer #53	0.02	0.01
Deer #19	0.02	0.01	Deer #54	0.02	0.01
Deer #20	0.02	0.01	Deer #55	0.02	0.01
Deer #21	0.03	0.02	Deer #57	0.02	0.01
Deer #25	0.02	0.01	Deer #59	0.02	0.01
Deer #26	0.02	0.01	Deer #60	0.02	0.01
Deer #27	0.02	0.01	Deer #61	0.02	0.01
Deer #28	0.02	0.02			

Appendix B: USGS Louisiana Site Data $\delta^{18}\text{O}$ (VSMOW)

Parish	Number of Analytical Sites	$\delta^{18}\text{O}$ Range	$\delta^{18}\text{O}$ Average	Number of Faunal Samples
Acadia	4	-1.81, -3.58	-2.81	
Allen	2	-2.68, -4.04	-3.36	1
Beauregard	1		-4.20	1
Bienville	7	-4.60, -4.96	-4.76	
Bossier	3	-4.65, -4.89	-4.76	
Calcasieu	2	-3.88, -3.26	-3.57	
Cameron	1		-2.78	
Catahoula	1		-8.80	2
DeSoto	1		-4.48	1
East Baton Rouge	1		-4.36	
East Carroll	1		-3.66	
East Feliciana	1		-4.17	
Evangeline	4	-2.42, -3.87	-3.12	10
Franklin	2	-4.30, -4, 72	-4.51	
Iberville	1		-6.46	1
Jackson	2	-4.51, -5.00	-4.76	
Jefferson Davis	8	-1.69, 3.90	-3.06	
Lafayette	9	-3.51, -4.22	-3.80	
Lincoln	2	-4.98, -5.08	-5.03	
Madison	1		-4.46	1
Morehouse	3	-3.50, -5.04	-4.52	1
Ouachita	6	-4.47, -5.01	-4.82	
Rapides	1		-4.65	4
St. Landry	2	-3.92, -3.77	-3.85	
St. Mary	1		-2.91	
St. Tammany	1		-4.20	
Tangipahoa	1		-4.43	
Tensas	1		-3.76	
Union	5	-5.03, -5.26	-5.14	
Vermillion	3	-3.16, -3,55	-3.37	
Washington	1		-4.30	
Webster	5	-4.74, -5.46	-5.11	
Winn	4	-4.49, -4.97	-4.81	2

Appendix C: USGS Louisiana Site Data $\delta^{13}\text{C}$ (VPDB)

Parish	Number of Analytical Sites	$\delta^{13}\text{C}$ Range	$\delta^{13}\text{C}$ Average	Number of Faunal Samples
Bienville	7	-14.39, -22.73	-20.11	
Bossier	3	-22.08, -23.96	-22.8	
DeSoto	1		-17.38	1
Jackson	2	-13.37, -14.17	-13.77	
Lincoln	2	-9.66, -14.24	-11.95	
Morehouse	1		-7.95	1
Ouachita	6	-6.36, -12.70	-9.41	
Union	5	-9.58, -15.24	-11.34	
Webster	5	-13.39, -20.82	-16.38	
Winn	4	-6.14, -23.95	-12.3	4

Appendix D: Faunal Samples Matched with USGS Analysis Site

Animal	Parish	Location	USGS Site	USGS Location
Raccoon 1	East Baton Rouge	Baton Rouge, La	EB-1135	Baton Rouge
Raccoon 2	East Baton Rouge	Baton Rouge, La	EB-1135	Baton Rouge
Fox 1	Evangeline	Pine Prairie, La	Ev-5477Z	Ville Platte
2	Evangeline	Lizard Park, La	Ev-5477Z	Ville Platte
3	Evangeline	Crooked Creek, La	Ev-5477Z	Ville Platte
4 Cascade	Evangeline	Evangeline Parish, La	Ev-5486Z	Basile
4 CD	Evangeline	Evangeline Parish	Ev-5486Z	Basile
6	Jasper County	Jasper, TX		
7	Evangeline	Crooked Creek, La	Ev-5477Z	Ville Platte
8	Evangeline	Easton, La	Ev-5477Z	Ville Platte
10	Evangeline	Easton, La	Ev-5477Z	Ville Platte
12	Avoyelles	Lemoine, La		
13	St. Helena	St. Helena, La		
14	Beauregard	Merryville, La	Be-6077Z	Kernan
15	Vernon	Evans, La		
16	Allen	Kinder, La	Al-5477Z	Kinder
18	Gallia County	Gallipolis, Oh		
19	Gallia County	Gallipolis, Oh		
20	Gallia County	Gallipolis, Oh		
21	Desoto	DeSoto Parish, La	Wb-113	Stonewall
25	Maddison	Tallulah, La	Ma-28	Tallulah
26	St. Helena	St. Helena, La		
27	Shelby County	Joaquin, Tx		
28	Iberville	Atchafalaya Basin, La	Ib-60	Plaquemine
29	Winn	Winn Parish, La	W-5135Z	Winn Parish
30	LaSalle	LaSalle Parish, La		
31	Winn	Winn Parish, La	W-5135Z	Winn Parish
32	Evangeline	Beaver Creek, La	Ev-5477Z	Ville Platte

Animal	Parish	Location	USGS Site	USGS Location
33	Grant	Grant Parish, La		
34	Natchitoches	Natchitoches Parish, La		
35	Vernon	Fort Polk, La		
36	Evangeline	Evangeline Parish, La	Ev-5486Z	Basile
37	Rapides	Pineville, La	R-1365	Boyce
45	Sabine	Sabine Parish, La		
46	Hancock County	Weirton, Wv		
47	Kiowa County	Haviland, Ks		
49	Morehouse	Jones, La	Mo-479	Bonita
50	Rapides	Tioga, La	R-1365	Boyce
51	Rapides	Sharp, La	R-1365	Boyce
52	Meigs County	Decatur, Tn		
53	LaSalle	Jena, La		
54	Catahoula	Catahoula Parish, La	Co-111	Jonesville
55	Catahoula	Larto, La	Co-111	Jonesville
57	Rapides	Rapides Parish, La	R-1365	Boyce
59	Jefferson County	Fayette, Ms		
60	Iberia	Loreauville, La		
61	Livingston	Denham Springs, La		

Appendix E: ^{87/86}Sr Raw Values

Animal	^{87/86}Sr Value
Raccoon 1	0.709971
Raccoon 2	0.709817
Fox 1	0.714673
2	0.714268
3	0.715446
4 CD	0.714664
4 Cascade	0.714489
6	0.713951
7	0.712003
8	0.715579
10	0.716242
12	0.710197
13	0.709105
14	0.713304
15	0.714280
16	0.715844
18	0.714654
19	0.714824
20	0.714594
21	0.711997
25	0.709768
26-a	0.708168
26-b	0.708105
27	0.710236
28	0.709939

Animal	^{87/86}Sr Value
29	0.711405
30	0.714199
31	0.711635
32	0.716055
33	0.711058
34	0.715613
35-a	0.717326
35-b	0.717255
36	0.715457
37	0.715393
45	0.709295
46	0.713732
47	0.709920
49	0.710398
50	0.714181
51	0.710556
52	0.712956
53	0.713158
54	0.710088
55	0.709873
57	0.709805
59	0.709491
60	0.709511
61	0.712850

Appendix F: Time Data for USGS Sites

USGS Variation Between Years

Lafayette Parish

	2001	Month	2002	Month	2013	Month
180	-3.91	March	-3.51	July	3.71	July
			-3.53	July		
			-3.87	July		
			-3.70	July		
			-4.22	July		
			-3.89	July		
			-3.86	July		
AVG	-3.91		-3.80		3.71	
St. Dev.			0.24			

Morehouse Parish

	1996	Month	1998	Month	2012	Month
180	-4.93	April	-4.61	May	-5.04	September
			-3.50	May		
AVG	-4.93		-4.06		-5.04	
St. Dev.			0.78			

USGS Variation between Months

Bienville Parish – 2012

180	May	June	Sept.	Oct.
	-4.60	-4.81	-4.60	-4.82
		-4.72	-4.96	
		-4.84		
AVG	-4.60	-4.79	-4.78	-4.82
St. Dev.		0.06	0.25	

13C	May	June	Sept.	Oct.
	-18.16	-14.39	-22.38	-21.95
		-22.50	-18.69	
		-22.73		
AVG	-18.16	-19.87	-20.54	-21.95
St. Dev.		4.75	2.61	

Bossier Parish – 2012

180	June	Sept.
	-4.73	-4.65
	-4.89	
AVG	-4.81	-4.65
St. Dev.	0.11	

13C	June	Sept.
	-23.96	-22.08
	-22.37	
AVG	-23.17	-22.08
St. Dev.	1.12	

Calcasieu Parish – 2001

180	Feb	April
	-3.26	-3.88
AVG	-3.26	-3.88
St. Dev.		

Evangeline Parish – 2001

180	February	March
	-3.68	-2.42
	-2.50	-3.87
AVG	-3.09	-3.15
St. Dev.	0.83	1.03

Jackson Parish – 2012

180	May	Sept.
	-4.51	-5.00
AVG	-4.51	-5.00
St. Dev.		

13C	May	Sept.
	-13.37	-14.17
AVG	-13.37	-14.17
St. Dev.		

Jefferson Parish – 2001

180	February	March
	-1.69	-3.90
	-3.00	
	-3.87	
	-3.79	
	-2.82	
	-2.72	
	-2.67	
AVG	-2.94	-3.90
St. Dev.	0.74	

Lincoln Parish – 2012

180	May	Sept.
	-4.98	-5.08
AVG	-4.98	-5.08
St. Dev.		

13C	May	Sept.
	-9.66	-14.24
AVG	-9.66	-14.24
St. Dev.		

Ouachita 2012

180	May	July	Sept.	Oct.
	-4.84	-4.84	-5.01	-4.76
	-4.47		-5.00	
AVG	-4.66	-4.84	-5.01	-4.76
St. Dev.	0.26		0.01	

13C	May	July	Sept.	Oct.
	-9.22	-10.27	-8.27	-9.61
	-12.70		-6.36	
Avg.	-10.96	-10.27	-7.32	-9.61
St. Dev.	2.46		1.35	

St. Landry Parish – 2001

180	February	April
	-3.92	-3.77
AVG	-3.92	-3.77
St. Dev.		

Union 2012

180	June	Sept.
	-5.26	-5.03
		-5.14
		-5.05
		-5.20
AVG	-5.26	-5.11
St. Dev.		0.08

13C	June	Sept.
	-15.24	-9.88
		-11.29
		-9.58
		-10.71
AVG	-5.24	-10.37
St. Dev.		0.78

Vermillion Parish – 2001

180	February	March
	-3.16	-3.55
	-3.40	
AVG	-3.28	-3.55
St. Dev.	0.17	

Webster 2012

180	June	Sept.	Oct.
	-5.29	-5.46	-4.85
	-5.20		
	-4.74		
AVG	-5.08	-5.46	-4.85
St. Dev.	0.30		

13C	June	Sept.	Oct.
	-13.39	-14.19	-17.99
	-15.52		
	-20.82		
AVG	-16.58	-14.19	-17.99
St. Dev.	3.83		

Winn 2012

180	May	Sept.
	-4.91	-4.88
	-4.49	-4.97
AVG	-4.70	-4.93
St. Dev.	0.30	0.06

13C	May	Sept.
	-7.54	-11.57
	-23.95	-6.14
AVG	-15.75	-8.86
St. Dev.	11.60	3.84

Appendix G: $\delta^{18}\text{O}$ Comparison of Animal Value and USGS Values

Animal	$\delta^{18}\text{O}$ (VPDB)	USGS Match Site $\delta^{18}\text{O}$ (VSMOW)	Parish	USGS $\delta^{18}\text{O}$ Range (VSMOW)	USGS $\delta^{18}\text{O}$ Average (VSMOW)
Raccoon 1	-5.03	-4.36	East Baton Rouge		-4.36
Raccoon 2	-3.74	-4.36	East Baton Rouge		-4.36
Fox 1	-4.21	-2.50	Evangeline	-2.42, -3.87	-3.12
2	-3.76	-2.50	Evangeline	-2.42, -3.87	-3.12
3	-4.39	-2.50	Evangeline	-2.42, -3.87	-3.12
4		-2.42	Evangeline	-2.42, -3.87	-3.12
Cascade	-2.49				
4 CD	-2.42	-2.42	Evangeline	-2.42, -3.87	-3.12
6	-3.83		Jasper County		
7	-2.38	-2.50	Evangeline	-2.42, -3.87	-3.12
8	-4.82	-2.50	Evangeline	-2.42, -3.87	-3.12
10	-3.54	-2.50	Evangeline	-2.42, -3.87	-3.12
12	-3.83		Avoyelles		
13	-3.64		St. Helena		
14	-2.93	-4.20	Beauregard		-4.20
15	-2.80		Vernon		
16	-2.80	-2.68	Allen	-2.68, -4.04	-3.36
18	-4.39		Gallia County		
19	-3.53		Gallia County		
20	-4.49		Gallia County		
21	-2.81	-4.48	Desoto		-4.48
25	-3.38	-4.46	Maddison		-4.46
26	-4.26		St. Helena		
27	-2.40		Shelby County		
28	-3.39	-6.46	Iberville		-6.46
29	-1.99	-4.49	Winn	-4.49, -4.97	-4.81
30	-3.30		LaSalle		
31	-1.37	-4.49	Winn	-4.49, -4.97	-4.81
32	-2.84	-2.50	Evangeline		
33	-2.48		Grant		
34	-3.43		Natchitoches		

Animal	$\delta^{18}\text{O}$ (VPDB)	USGS Match Site $\delta^{18}\text{O}$ (VSMOW)	Parish	USGS $\delta^{18}\text{O}$ Range (VSMOW)	USGS $\delta^{18}\text{O}$ Average (VSMOW)
35	-3.08		Vernon		
36	-1.95	-2.42	Evangeline	-2.42, -3.87	-3.12
37	-1.99	-4.65	Rapides		-4.65
45	-2.42		Sabine		
46	-5.24		Hancock County		
47	-3.13		Kiowa County		
49	-3.23	-3.50	Morehouse	-3.50, -5.04	-4.52
50	-3.95	-4.65	Rapides		-4.65
51	-1.94	-4.65	Rapides		-4.65
52	-4.46		Meigs County		
53	-3.11		LaSalle		
54	-2.48	-8.80	Catahoula		-8.80
55	-2.18	-8.80	Catahoula		-8.80
57	-2.50	-4.65	Rapides		-4.65
59	-3.39		Jefferson County		
60	-3.73		Iberia		
61	-4.42		Livingston		

Appendix H: $\delta^{13}\text{C}$ Comparison of Animal Value and USGS Values

Animal	$\delta^{13}\text{C}$ (VPDB)	Parish	$\delta^{13}\text{C}$ Range USGS (VPDB)	$\delta^{13}\text{C}$ Average USGS (VPDB)
Raccoon 1	-10.81	East Baton Rouge		
Raccoon 2	-13.38	East Baton Rouge		
Fox 1	-13.92	Evangeline		
2	-15.54	Evangeline		
3	-14.60	Evangeline		
4 Cascade	-14.92	Evangeline		
4 CD	-14.91	Evangeline		
6	-14.75	Jasper County		
7	-16.07	Evangeline		
8	-15.00	Evangeline		
10	-16.23	Evangeline		
12	-13.71	Avoyelles		
13	-14.02	St. Helena		
14	-17.35	Beauregard		
15	-15.60	Vernon		
16	-16.44	Allen		
18	-15.88	Gallia County		
19	-18.11	Gallia County		
20	-16.15	Gallia County		
21	-14.64	Desoto		-17.38
25	-18.76	Maddison		
26	-17.68	St. Helena		
27	-12.18	Shelby County		
28	-15.59	Iberville		
29	-16.43	Winn	-6.14, -23.95	-12.3
30	-15.36	LaSalle		
31	-16.46	Winn	-6.14, -23.95	-12.3
32	-16.52	Evangeline		
33	-16.25	Grant		
34	-15.80	Natchitoches		
35	-16.86	Vernon		
36	-16.29	Evangeline		
37	-16.10	Rapides		
45	-16.24	Sabine		

Animal	$\delta^{13}\text{C}$ (VPDB)	Parish	$\delta^{13}\text{C}$ Range USGS (VPDB)	$\delta^{13}\text{C}$ Average USGS (VPDB)
46	-16.73	Hancock County		
47	-11.98	Kiowa County		
49	-15.98	Morehouse		-7.95
50	-12.56	Rapides		
51	-17.18	Rapides		
52	-16.39	Meigs County		
53	-15.81	LaSalle		
54	-11.70	Catahoula		
55	-15.11	Catahoula		
57	-18.44	Rapides		
59	-16.13	Jefferson County		
60	-15.90	Iberia		
61	-17.03	Livingtson		

Appendix I: Faunal Data by Parish for Mapping

Parish	n	18O		13C		87Sr	
		Average	St. Dev.	Average	St. Dev.	Average	St. Dev.
Allen	1	-2.80		-16.44		78.22	
Avoyelles	1	-3.83		-13.71		-1.28	
Beauregard	1	-2.93		-17.35		42.46	
Catahoula	2	-2.33	0.21	-13.41	2.41	-4.34	2.14
Desoto	1	-2.81		-14.64		24.06	
Evangeline	8	-3.26	1.03	-15.65	0.73	65.83	19.20
Grant	1	-2.48		-16.25		10.84	
Iberia	1	-3.73		-15.90		-10.94	
Iberville	1	-3.39		-15.59		-4.91	
LaSalle	2	-3.21	0.13	-15.59	0.32	47.73	10.36
Livingston	1	-4.42		-17.03		36.08	
Maddison	1	-3.38		-18.76		-7.31	
Morehouse	1	-3.23		-15.98		1.55	
Natchitoches	1	-3.43		-15.80		74.97	
Rapides	4	-2.60	0.94	-16.07	2.53	30.91	38.33
Sabine	1	-2.42		-16.24		-13.99	
St. Helena	2	-3.95	0.44	-15.85	2.59	-30.07	0.31
Vernon	2	-2.94	0.20	-16.23	0.89	98.83	0.35
Winn	2	-1.68	0.44	-16.45	0.02	17.35	2.29

The Vita

Miley Jackson, a native of Pine Prairie, Louisiana received two bachelor degrees from the University of Louisiana at Lafayette in 2012. One bachelor's degree is an American Chemical Society Certified Bachelor of Science in Chemistry while the other is a Bachelor of Liberal Arts in Anthropology.

Miley began her undergraduate career at McNeese State University. While there, she conducted research with chemistry professor Dr. Boggavarpu investigating the bonding arrangements of silicon bismuth compounds, resulting in a publication in the Journal of Chemical Physics. After one year at McNeese State University, Miley decided to transfer to the University of Louisiana at Lafayette where she could pursue a degree in anthropology along with chemistry.

During Miley's time at the University of Louisiana at Lafayette, she spent each summer working as an intern with the North Louisiana Criminalistics Laboratory in Alexandria, Louisiana. The internship allowed Miley to receive training in firearm analysis, fingerprint analysis, drug chemistry, arson analysis, DNA analysis, and blood alcohol analysis. Miley also participated in the Louisiana Biomedical Research Program where she conducted stable isotope research with Dr. Bao of Louisiana State University.

After working as a forensic assistant, Miley decided to enter the graduate program at Louisiana State University to pursue training as a forensic anthropologist. As a master's candidate for graduation in May, 2014, Miley intends to find a job at a forensic laboratory where she will work as a forensic analyst.