

# **An Exploration of the Effects of Taphonomy on Isotope Ratios of Human Hair**

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## **ABSTRACT**

Isotope analyses of human remains have been conducted with growing frequency over the past thirty years in anthropology, in both archaeological and forensic contexts. Analyses of isotope ratios of elements such as carbon, nitrogen, hydrogen, oxygen, and strontium from teeth, bones, and hair have provided information regarding individual diet and geographic movement during different life stages. Hair grows at a predictable rate and provides a serial recording of diet and travel history for the weeks and months just prior to death. What has not been systematically studied is whether postmortem decompositional changes to the body have an effect upon isotope ratios in hair. Hair has been used in conjunction with tap water to create isoscapes, or isotope mapping models, that allow prediction of geographic history from analysis of the isotope ratios of hair. These models have been developed using clean modern samples from salons and do not reflect the typical condition of hair found in archaeological or forensic contexts. If the isotope ratios of human hair are to be used reliably in both archaeological and forensic settings, it is essential to understand the effects of taphonomy on isotope signatures, and whether these signatures persist and reflect those seen during life.

This research was conducted at the Anthropology Research Facility in Knoxville, Tennessee, an outdoor laboratory for the study of human decomposition. Body donors with known residence histories ( $n=44$ ) plus two additional donors at the Forensic Anthropology Research Facility in San Marcos, Texas, were enrolled in the study, and carbon, nitrogen, hydrogen, oxygen, and strontium isotopes from human hair samples of these donors were analyzed. Postmortem exposure times for the study ranged from 22

days to more than three years. Results of the study revealed that carbon and nitrogen isotope ratios in human hair, commonly used to make dietary inferences, undergo little change over time and are more reliable than hydrogen, oxygen, and strontium isotope ratios, which are impacted by the depositional environment. This study revealed that isotope ratios of human hair can change postmortem and are influenced by geographic placement location, surface or burial placement, and duration of exposure.

## **PREFACE**

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# **CHAPTER ONE**

## **INTRODUCTION**

Anthropologists have used isotope analyses to address a variety of questions in archaeology related to environmental reconstructions, artifact characterization, migration studies, faunal analysis, and the introduction of domesticated plants such as maize (Schwarcz and Schoeninger, 1991; Katzenburg, 2000; Andrus, 2011; West, 2013). More recently, anthropologists have used isotope analysis methods in forensic contexts to aid with identification of human remains (Benson et al., 2006; Rauch et al., 2007; Aggarwal et al., 2008; Meier-Auenstein and Fraser, 2008; Ehleringer et al., 2010). Stemming from the fields of geology and ecology, biogeochemistry techniques are ideal for the examination of human-environment interactions and can provide valuable information linking people to their local surroundings by yielding clues about diet and geographic location. Whether this information is being used to elucidate trends in ancient lifeways of a population or an attempt to identify an unknown individual in contemporary forensic casework, it is clear that isotope analyses can reveal otherwise unknown behaviors, such as dietary habits and travel history.

When examining human remains, a major consideration for anthropologists is the preservation of the remains. Bones, teeth, and hair are recovered from forensic and archaeological sites in various states of preservation. Taphonomy refers to anything that happens to a body between death and recovery, and can include processes of decomposition, animal activity, plant growth, and climatic activity (Haglund and Sorg, 2006). Taphonomic processes can physically and chemically alter remains, affecting the

results of scientific analyses. This dissertation examines the effects of taphonomy upon isotope ratios of human remains, specifically human hair.

Isotopes are defined as “forms of the same element that differ in the number of neutrons in the nucleus” (Fry, 2006:13). Isotopes of the same element have the same atomic number, but due to the differing number of neutrons, they have different atomic masses. This difference in atomic mass leads to subtle differences in reaction rates when compounds undergo chemical reactions. If the reaction does not go to completion, the isotopic composition of the product may differ from that of the reactant. Thus, measuring the product can tell you something about the chemical process that made it. Carbon, nitrogen, hydrogen, and oxygen are the most abundant elements in living organisms and are most commonly used for isotope analyses of human tissues (Fry, 2006; Hoefs, 2009).

Carbon and nitrogen isotope ratios provide information about an individual’s diet. Carbon isotope ratios, reported as  $\delta^{13}\text{C}$  values, reflect the type of plants consumed by an individual. This is possible because of different photosynthetic pathways known as  $\text{C}_3$ ,  $\text{C}_4$ , and CAM photosynthesis. Plants in the  $\text{C}_3$  group, such as wheat, potatoes, and rice, are characterized by lower  $\delta^{13}\text{C}$  values than  $\text{C}_4$  plants, which include corn and sugarcane (van der Merwe, 1982; MacFadden et al., 1999; Sage et al., 2012). These plant carbon isotope signatures are incorporated into the tissues of organisms that consume them, as well as consumers who eat those organisms. Nitrogen isotope ratios are reported as  $\delta^{15}\text{N}$  values and reflect trophic level within a food web, with values increasing approximately 3-5‰ with each level, from plants, to herbivores, omnivores, and carnivores (Ambrose

and Norr, 1993; O'Connell and Hedges, 1999a; Petzke et al., 2005; Hedges and Reynard, 2007).

Hydrogen, reported as  $\delta^2\text{H}$ , and oxygen, reported as  $\delta^{18}\text{O}$ , are controlled by the hydrologic cycle and are reflective of latitude and altitude (Dansgaard, 1964; Keeling, 1995; Gat, 1996). Isotope ratios of water change as it evaporates from the ocean to clouds, and as it falls in the form of precipitation over land. The lighter isotopes of hydrogen and oxygen are preferentially included in water in the vapor form, and the heavier isotopes of hydrogen and oxygen are the first to be lost as precipitation. This means that the isotopic composition of rainfall is isotopically lighter as one moves from the equator – where the highest heat flux to the Earth is – to the poles. This precipitation supplies available drinking water and is incorporated into human tissues via water and food consumption (Kendall and Coplen, 2001; Sponheimer and Lee-Thorp, 1999). Location, specifically latitude and elevation, affects  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values; therefore geographic location is reflected in these values. Because of the critical nature of understanding hydrology for agriculture, weather, and climate prediction, there have been monitoring networks for decades that provide highly accurate values for  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  around the world.

Another element that is useful for geographic provenience is strontium, reported as  $^{87}\text{Sr}/^{86}\text{Sr}$ . Strontium is less abundant than carbon, nitrogen, hydrogen, or oxygen in the tissues of living organisms. It is incorporated into tissues directly from the environment by consuming water and food with local strontium signatures, and  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios reflect the underlying soil derived from weathered bedrock of geographic locations. Through

natural erosion, weathering, and fluvial processes, the underlying bedrock is modified and incorporated into local soils and waterways (Blum et al., 1993; Crowley et al., 2017), and therefore into the tissues of humans eating and drinking food and water from those specific areas (Beard and Johnson, 2000; Price et al., 2000).

There are different growth rates for different types of tissue, and the type of tissue analyzed reflects different periods of an individual's life. Bone tissue, for example, turns over at a rate of 5-10 years, depending upon the specific skeletal element (Robling and Stout, 2008; Hedges et al., 2007; Beard and Johnson, 2000; Branca et al., 1992). Tooth enamel is formed in utero and early childhood through adolescence and undergoes little change throughout life (Dolphin et al., 2005; Hillson 1996). Tooth enamel, therefore, represents diet and travel history of infancy through childhood. Hair grows at a rate of approximately one centimeter per month (Robbins, 2012). Hence, an overall life history of diet and geographic travel history of childhood (assessed through tooth enamel), later life (5-10 years prior to death reflected by bone), and weeks to months prior to death (recorded in hair) can be constructed through isotope analysis.

Human hair has been used in archaeological contexts to provide clues regarding individual life histories (White et al., 2009; Williams and Katzenberg, 2012; Sharp et al., 2003). More recently, isotopic analyses of human hair have been utilized in contemporary forensic applications for the identification of human remains (Aggarwal et al., 2008; Benson et al., 2006; Meier-Augenstein and Fraser, 2008). Hair provides a serial record along the length of the strand and can be analyzed in segments, making it useful for both archaeological and forensic investigations regarding the time shortly before death.

Despite the historic use of isotopic analyses in bioarchaeology and the increasing use of isotopes in forensic identification of unknown human remains, little is known about the effects of taphonomic processes on isotope ratios, particularly in hair. Human remains of forensic anthropological significance are often exposed to outdoor environments for extended periods of time in intimate contact with rain, soil, and decomposition fluids. In contrast, the models upon which measured isotope ratios are interpreted have been developed using freshly cut hair from salons or living individuals (Ehleringer et al., 2008; Thompson et al., 2010; Nash et al., 2012). These models are from single-collection contemporary contexts in which the hair has been washed and is in relatively pristine condition. The samples have not been exposed to long-term precipitation, temperature fluctuations, or other environmental factors, as would be common for archaeological or forensic samples. Additionally, there are no pre-exposure comparative samples for archaeological and forensic samples, and therefore no definitive means of determining whether there have been any changes to the isotopic compositions of the hair samples from death to recovery of the remains. A better understanding of how taphonomic processes affect ratios of tissues is necessary if this type of analysis is to be reliably used in forensic anthropology.

The specific goal of this project is to determine whether human hair retains antemortem isotopic signatures throughout the process of decomposition. A collaborative project between researchers at Arizona State University, the University of Tennessee, and Texas State University at San Marcos was established. Additional collaborative support was provided by IsoForensics and the University of Utah. In order to observe the effects

of decomposition upon isotope ratios of hair, a two-phase study was designed and carried out at the University of Tennessee Anthropology Research Facility (ARF). The first laboratory of its kind in the world, the ARF provides an opportunity to observe human decomposition in a natural outdoor environment. The Forensic Anthropology Center (FAC) body donation program supports research at the ARF by providing body donors with self-reported life histories, including lifetime residence history. Upon death, body donors are received through an intake process, and information such as cadaver height and weight is recorded. Biological samples of blood, hair, and fingernails are collected from each donor and curated for research. In order to evaluate the effects different geologic and climatic environments may have upon isotope ratios, the Forensic Anthropology Research Facility (FARF) in San Marcos, Texas was used. The Forensic Anthropology Center at Texas State (FACTS) in San Marcos, Texas, has a body donation program similar to that of the FAC. By utilizing two decomposition research facilities with differing geology and climate, the relationship between changes in isotopic composition of hair and depositional environments could be more robustly evaluated. This research explored the extent to which isotope ratios of the most commonly utilized elements in human dietary and geographic studies,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$ , are retained throughout decomposition by comparing the intake hair samples, which reflect compositions closest to antemortem signatures, with those collected after outdoor decomposition over varying time periods.

## Research Hypotheses

To date, no systematic research has been conducted on human remains of known residence history in order to explore the effects that taphonomic processes related to disposition of remains (surface versus buried), decomposition/decay, and environment (weather/temperature) have upon isotope ratios of human hair. With the growing interest in quantifiable means of human identification and the potential for isotopic analyses to inform forensic investigations (Holland et al., 2012; Regan, 2006), it is vital that the taphonomy of isotope ratios be understood. The following hypotheses will address the goals of this study:

1. Post-decomposition isotope values for carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) will not be significantly different from those of hair samples at intake. Both carbon and nitrogen are structurally incorporated into hair, and no prior research suggests that environmental exchange of these isotope ratios occurs in hair samples. Should significant differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values be observed between intake and post-exposure hair samples, the number of days of exposure, location of deposition (Tennessee or Texas), placement condition (surface or burial), soil sample data, and precipitation data will be considered as variables affecting these changes.
2. Hydrogen ( $\delta^2\text{H}$ ) and Oxygen ( $\delta^{18}\text{O}$ ) values of post-decomposition hair samples will differ from intake values. Although hydrogen and oxygen are also structurally incorporated in the keratin proteins of hair, there is a large offset between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values in hair and in the environment, particularly rainfall. In addition, it is known that a significant portion of

the hydrogen is exchangeable with local humidity, so it is expected that exchange may occur between hair and depositional environment, and post-exposure samples will change in the direction of environmental sample values. Should significant differences in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values be observed between intake and post-exposure hair samples, number of days of exposure, location of deposition (Tennessee or Texas), placement condition (surface or burial), and precipitation data will be considered as variables affecting these changes.

3. Strontium ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) ratios from paired intake and post-decomposition hair samples will differ, and post-decomposition samples will reflect the values of the depositional environment. The abundance of  $^{87}\text{Sr}/^{86}\text{Sr}$  in the natural environment is very high relative to the low levels present in human hair. In addition, strontium is not part of the structural formula for the main components of hair. For this reason, it is expected that post-exposure hair samples will change in the direction of values from environmental samples, especially bioavailable leaches of soil samples.
4. Oxygen ( $\delta^{18}\text{O}$ ) and strontium ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) values from both intake and post-exposure samples will reflect last known geographic residence when applied to a GIS model using United States tap water and bedrock data.

### **Research Design**

The study was conducted in two phases. During Phase I of the study, a survey of the ARF was conducted to collect a total of 38 hair mats from donors that had been



decomposing for various lengths of time. These hair mats, along with paired hair samples from the same donor collected during intake, were analyzed for stable isotopes of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^2\text{H}$ , and  $\delta^{18}\text{O}$  at IsoForensics in Salt Lake City, Utah, through a research-in-residence program. Ten of these hair sample pairs were also analyzed for  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios at the University of Utah's Department of Geology in Salt Lake City, Utah. Differences between isotopic composition of intake samples and decomposed samples were observed, leading to a more detailed prospective time resolution study of these changes. Phase I of the study addressed Hypotheses 1, 2, and 3 by assessing whether changes were observed between intake and post-exposure isotope ratios. The results of this phase of the study provided a baseline dataset of observations from hair collected between 22 and 1140 days of exposure. A longitudinal study to better understand the relationship between isotope signatures of postmortem hair and time, environment, and placement condition was conducted during Phase II.

Phase II of the study followed eight donors through the decomposition process for a period of one year to determine if a more fine-scale timeline of isotopic changes, if they occurred, could be established. To address the variables of placement location, placement condition, and local environment in Hypotheses 1-3, six donors were placed at the ARF, with three placed on the surface and three placed in shallow burials. In both archaeological and forensic contexts, human remains are often recovered from burial conditions. Hair that has been recovered with these remains may be analyzed for isotope ratios. Burial placements in Phase II allowed the immediate local microenvironment to be evaluated as a possible variable affecting isotopic composition over time. Because burial

placements were not exposed to precipitation in the same way as surface placements, and burial placements were completely covered with soil, they presented an opportunity to examine environmental variables such as soil and precipitation in both surface and shallow burial contexts. An additional two donors from the Forensic Anthropology Center at Texas State (FACTS) body donation program were placed on the ground surface at the Forensic Anthropology Research Facility (FARF) in San Marcos, Texas. The FARF is a human decomposition research facility in a different climatic environment and was selected as an additional placement site in order to determine the effects of a more arid climate with different underlying geology, which are possible factors affecting isotope values from hair. Hair samples were collected from each of the eight donors over a period of one year as part of a National Institute of Justice (NIJ) funded project (NIJ 2014-DN-BX-K002). To address Hypotheses 1 and 2, isotope analyses for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^2\text{H}$ , and  $\delta^{18}\text{O}$  were completed by the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah in Salt Lake City, Utah, and  $^{87}\text{Sr}/^{86}\text{Sr}$  analyses were conducted at the Keck Laboratory at Arizona State University in Tempe, Arizona to address Hypothesis 3. These serial samples were compared to assess temporal trends in isotope changes in hair throughout decomposition.

For the eight donors in Phase II,  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  values were used along with United States tap water, precipitation, and bedrock data to create predictions for region of origin to test Hypothesis 4. The intake sample values were used to create one predictive map, and the final recovery samples that were collected after one year of exposure were used to create a second map for comparison to determine if the changes seen in  $\delta^{18}\text{O}$  and

$^{87}\text{Sr}/^{86}\text{Sr}$  values would change the predicted region of origin. These maps were also compared to the last known residences of the donors to assess the accuracy for predicting region of origin with these models specifically created using tap water and human hair.

### **Significance of Research**

This study represents the first of its kind to understand how isotope ratios of human hair can change over time throughout decomposition. Bioarchaeologists who incorporate isotopes into studies of ancient populations will benefit from a greater understanding of how their interpretations from isotope analyses may be impacted by changes to tissue isotope ratios over time. Forensic practitioners who rely on isotopes in the absence of other leads during investigations must understand the strengths and limitations of isotopic hair analyses so that they pursue reliable leads for identification of unknown individuals. Previous research models were created using samples that do not directly reflect the conditions in which forensic samples are collected. This project used samples from known individuals collected before and after decomposition, providing a more realistic application of isotope analysis and the opportunity for ground-truthing of results.

### **Summary**

This project examines the effects of taphonomic processes on the isotope ratios of human hair. Chapter Two provides a summary of relevant background information, detailing the biogeochemical processes that influence isotope signatures in humans and their environments, as well as the ways in which anthropologists have used isotopic analyses in bioarchaeology and forensic applications. Chapter Three is a detailed

description of the experimental design and analytical methods of both phases of the study and is organized according to each isotope group and laboratory procedures for the respective analyses. Chapter Four reports the results of Phase I and Phase II of the study. Maps predicting geographic origins of Phase II donors are also presented in this chapter. Chapter Five provides a discussion of these results and their implications for using isotope ratios of human hair in anthropological applications. Chapter Six concludes with final remarks and suggested considerations for practitioners, as well as possible future research.

## **CHAPTER TWO**

### **BACKGROUND**

#### **Taphonomy of Hair**

Taphonomy refers to anything that happens to a body between death and recovery, and can include processes of decomposition, animal activity, plant growth, and climatic activity (Haglund and Sorg, 2006). Within both archaeology and forensic anthropology, taphonomy is used to expand understanding of decay processes and the persistence of tissues and artifacts over extended time scales (Janaway et al., 2009). One of the main areas of research needed is how taphonomy affects the isotope ratios of hair, as no prior research on the effects of decomposition upon isotopes in human hair has been done. This study evaluated the effects of taphonomy upon hair related to placement condition (surface or burial), placement location in two different geologic and climatic environments, and duration of exposure. Human remains of forensic significance are often exposed to outdoor environments for extended periods of time, while the models upon which isotopic analyses are interpreted have been developed using hair from salons (Ehleringer et al., 2008; Tipple, 2015). These models are from single-collection contemporary contexts in which the hair has been washed and is in relatively pristine condition. The samples have not been exposed to precipitation, temperature fluctuations, or other environmental factors, unlike both archaeological and forensic contexts. Additionally, in forensic and archaeological contexts, there are no pre-exposure comparative samples, and therefore no definitive means of determining whether there have been any changes to the isotopic compositions of the hair samples. Although a solid

foundation of studying taphonomic processes in visual, microscopic, and biologic degradation of hair has been established (DeGaetano et al., 1992; Chang et al., 2005; Wilson et al., 2007a and 2007b; Wilson, 2008; Wilson et al., 2010; Tridico et al., 2014; Mazzealli et al., 2015), a better understanding of how taphonomic processes affect tissue isotope ratios is needed (von Holstein et al., 2014).

Because of the historic use of tooth and bone samples to investigate prehistoric populations, much work has been done in order to understand the taphonomic processes that affect bone and tooth preservation over time (Hedges and Millard, 1995; Nielsen-Marsh and Hedges, 2000a and 2000b; Hedges, 2002; Chiaradia et al., 2003; Stathopoulou et al., 2008; Scharlotta et al., 2013; Dudás et al., 2016). Less is known about how decomposition processes affect hair both physically and chemically. Hair keratin is composed of amino acids with a relatively high proportion of the sulfur-containing amino acid cysteine. It is the covalent disulfide bonds of cysteine that provide the structural stability of hair keratin (Block and Bolling, 1939; Wilson, 2008). Because keratin is a relatively robust protein and is resistant to digestion by proteolytic enzymes (Block and Bolling, 1939), hair can persist over long periods of time, resulting in frequent recovery at forensic scenes (Beard and Johnson, 2000; Meier-Augenstein and Fraser, 2008; Ehleringer et al., 2008), as well as ancient archaeological sites (Macko et al., 1999a and 1999b; Sharp et al., 2003; Chang et al., 2005; Thompson et al., 2014; Wilson et al., 2007b).

Hair is recovered with human remains in a variety of contexts and conditions. In archaeological contexts, hair has been recovered from permafrost, as was the case with

the “Ice Man,” discovered in 1991 in the Italian Alps and dated to 5200 years before present (Macko et al., 1999). This hair was analyzed for carbon and nitrogen isotope ratios to investigate diet. Hair has also been recovered from arid conditions associated with mummified remains in locations such as Peru (Knudson et al., 2007; White et al., 2009) and Egypt (Macko et al., 1999b). Hair hundreds to thousands of years old persists over time, as is evidenced by its recovery from archaeological sites, but the degree of preservation is variable.

The primary degrader of keratin is fungi (Wilson, 2008). Keratin is a protein that very few microorganisms can break down. Fungi that digest keratin are called keratinolytic fungi, and they can be found in different types of soil in many environments. Hence, the deposition environment can play a significant role in the amount of degradation seen in hair (Wilson, 2008). When hair has been degraded by fungal activity, oval lesions described as “fungal tunneling” can be observed along the hair shaft (DeGaetano et al., 1992). Physical changes to the hair can be observed through visual and microscopic inspection (Wilson et al., 2004; Wilson et al., 2010), but without comparative samples, the degree to which chemical signatures change remains unclear. One area of focus for research of this subject is the preservation of DNA in archaeological hair samples (Gilbert et al., 2006). Successful extraction of mitochondrial DNA (mtDNA) from bison hair over 64,000 years old provides evidence that under optimal conditions hair preservation over time can provide biochemical information similar to that of skeletal remains (Gilbert et al., 2004).

While isotope analyses have been conducted on hair from archaeological and forensic contexts, and the results have been interpreted for both diet and geographic provenance predictions, these studies do not allow for pre- and post-exposure comparisons from known individuals. Alteration to the hair after death, through exposure to taphonomic processes, must be understood in order to confidently rely upon postmortem isotope analyses for the reconstruction of life history. The current study provides an opportunity to assess the effects of taphonomic processes upon the isotope ratios of human hair of known individuals. By having individual body donors placed in surface and burial conditions, as well as at two different outdoor decomposition facilities, this study evaluated whether changes to isotope ratios of human hair occur over time and what factors may contribute to those changes.

During life, hair preserves isotopic signatures that reflect local environment. These signatures have been presumed to persist after death, making hair useful for archaeological and forensic endeavors. The following section provides a summary of isotope systems commonly used in the analysis of human remains, how these isotopes are controlled in the environment, and what type of information each can provide.

### **Isotopes**

Isotopes are “forms of the same element that differ in the number of neutrons in the nucleus” (Fry, 2006:13). Isotopes of an element have the same atomic number, as the number of protons defines an element’s atomic number. Due to the differing number of neutrons, isotopes have different atomic masses – the sum of the number of protons and neutrons. Because different isotopes have different atomic masses, they have different



vibrational frequencies when chemically bound. The bonding energy for any particular chemical bond between two atoms depends on the atomic masses of the two atoms. Hence, the activation energy to form or break a chemical bond also is contingent upon the atomic masses of the atoms participating in the reaction. Because the chemical reaction rates depend on the atomic masses, this results in different abundances of the isotopic forms of these elements after chemical reactions. For most elements, the isotope with the fewest neutrons (i.e., “the lightest”) is typically the most abundant in nature, as is the case for elements such as carbon ( $^{12}\text{C}$ ), nitrogen ( $^{15}\text{N}$ ), hydrogen ( $^1\text{H}$ ), and oxygen ( $^{16}\text{O}$ ). These elements are of particular interest as they are the dominant constituents of biological molecules including proteins, carbohydrates, lipids and nucleic acids.

Isotope abundances are measured using an isotope ratio mass spectrometer (IRMS, for carbon, nitrogen, hydrogen, and oxygen) or a multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS, for strontium). The IRMS system measures the ratio of two isotopes of the same element in pure gas forms (e.g.,  $\text{H}_2$ ,  $\text{CO}_2$ , etc.), for example the ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$ , and that ratio is compared to the ratio of an internationally recognized reference standard.

For carbon isotopes, the standard is Vienna Pee Dee Belemnite (VPDB). This standard is no longer available, so measurements are typically made against pure gases that have been calibrated relative to the internationally-agreed upon standard. Standards for isotopes commonly measured in biological remains are listed in Table 1. The isotope value is reported as “delta notation” or the difference between the measured sample ratio

Table 1. Isotopes and natural abundances of carbon, nitrogen, hydrogen, oxygen, and strontium with international measurement standards.

| Element | Isotope          | Abundance % | International Measurement Standard |
|---------|------------------|-------------|------------------------------------|
| C       | <sup>12</sup> C  | 99.89       | VPDB <sup>1</sup>                  |
|         | <sup>13</sup> C  | 1.11        |                                    |
| N       | <sup>14</sup> N  | 99.64       | Atmospheric Air                    |
|         | <sup>15</sup> N  | 0.46        |                                    |
| H       | <sup>1</sup> H   | 99.98       | VSMOW <sup>2</sup>                 |
|         | <sup>2</sup> H   | 0.02        |                                    |
| O       | <sup>16</sup> O  | 99.76       | VSMOW <sup>2</sup> (in water)      |
|         | <sup>17</sup> O  | 0.04        | VPDB <sup>1</sup> (in carbonates)  |
|         | <sup>18</sup> O  | 0.20        |                                    |
| Sr      | <sup>84</sup> Sr | 0.56        | SRM 987 <sup>3</sup>               |
|         | <sup>86</sup> Sr | 9.86        |                                    |
|         | <sup>87</sup> Sr | 7.00        |                                    |
|         | <sup>88</sup> Sr | 82.58       |                                    |

<sup>1</sup> Vienna Pee Dee Belemnite, <sup>2</sup>Vienna Standard Mean Ocean Water, <sup>3</sup>NIST Strontium Carbonate Isotopic Standard

(R<sub>sample</sub>) and the standard ratio (R<sub>standard</sub>). This isotope value is denoted by the symbol “ $\delta$ ” and the units are “per mil,” indicated by the symbol “‰” (Fry, 2006: 22).

The general equation for this is expressed as the following (Brand and Coplen, 2012):

$$\delta \text{ element (‰)} = (R_{\text{sample}} / R_{\text{standard}}) - 1$$

Chemical reactions are affected by the atomic electron configuration, so isotopes of the same element have similar properties and behave correspondingly during chemical reactions (Schwarcz and Schoeninger, 1991). However, reactions involving different isotopes occur at different rates due to the differences in atomic mass. This results in variations in the ratios of isotopes of the same element in somewhat predictable ways (van der Merwe 1982). The larger the relative variation in mass between the isotopes of

an element, the larger the isotopic range in nature tends to be. For instance, the relative mass difference in hydrogen between  $^1\text{H}$  and  $^2\text{H}$  is 100%, and the natural range of variation is  $\sim 300\%$ . In contrast, the relative mass difference between  $^{12}\text{C}$  and  $^{13}\text{C}$  is 8.3%, and the range of natural variation in carbon is  $\sim 45\%$ .

Isotopes that do not change over time through radioactive decay are stable isotopes (Hoefs, 2009). Stable isotopes can be classified as radiogenic if they are products of the decay of radioactive isotopes (Schwarcz and Schoeninger, 1991), although “stable isotopes” is frequently used in ecology and anthropology to refer to mass-dependent isotope variation. Generally, the analysis of stable isotopes is used to discern chemical processes, while radiogenic isotope analyses are associated with discerning source. For example, stable isotopes of carbon relate to the photosynthetic process within plants, whereas radiogenic isotopes of strontium are used to determine the parent material and geological source of a rock sample.

Differing relative isotope abundances can be observed within organisms and in localized environments. These differences are due to fractionations that occur during phase changes and transport, where the subtle variations in the masses of the nuclei of molecules cause particular isotopes to be concentrated in specific phases (Hoefs, 2009). Fractionation is the process by which weaker chemical bonds are broken and lighter isotopes are preferentially lost through these broken bonds. This is the unequal partitioning of isotopes between two substances, or even two types of tissues (Hoefs, 2009). Fractionation is very common with temperature, humidity, altitude, underlying geology, and proximity to the ocean all impacting isotopic abundances of particular

elements. An understanding of fractionation processes is required to effectively utilize stable isotope analyses to understand biological and chemical processes acting upon organisms.

## **Isotope Systems and Local Environments**

Isotope analyses are ideally suited for studying biological and environmental interactions, as the local environmental conditions impact isotope ratios of materials and organisms within that ecosystem (Koch, 1998). Dietary and water resources within an ecosystem are some of the strongest drivers of isotope ratios in biological organisms. Consumption of available plants and animals, along with drinking water, has a direct impact upon the isotopic composition of the body of the consumer. Elements commonly used to investigate these relationships between geography and the organisms that live there include carbon, nitrogen, hydrogen, oxygen, and strontium. The following section provides an overview of these isotopes and how environmental interactions affect isotope values of these elements in living organisms.

### **Carbon Isotopes**

The carbon cycle represents the exchange of carbon dioxide (CO<sub>2</sub>) between the atmosphere, the ocean surface, and land ecosystems (Fry 2006: 45). Carbon isotopes are transferred from atmospheric CO<sub>2</sub> to plants through photosynthesis. The three different photosynthetic pathways (referred to as C<sub>3</sub>, C<sub>4</sub>, or CAM photosynthesis) impart different carbon isotope signatures. Hence, carbon isotopes can provide information about the types of plants consumed directly, as well as those consumed by other food sources throughout the food web (Meier-Augenstein, 2006). The difference in photosynthetic

processes results in differing isotopic values of carbon in C<sub>3</sub> and C<sub>4</sub> plants. CAM plants have  $\delta^{13}\text{C}$  values similar to C<sub>4</sub> plants, but there are virtually no CAM plants consumed in large quantities by modern human populations – e.g., pineapple, agave. C<sub>3</sub> plants exhibit carbon isotope ratios between -35‰ to -20‰, C<sub>4</sub> plants have values at -16‰ and -9‰, and CAM plants have intermediate values overlapping with C<sub>4</sub> plants between -12‰ and -23‰ (van de Merwe, 1982; Hayes, 2001). For example, the carbon uptake of C<sub>3</sub> plants such as rice, wheat, and barley, is more than five times greater than that of C<sub>4</sub> plants like corn, millet, and sugar cane (van der Merwe 1982; MacFadden et al., 1999). C<sub>3</sub> plants are depleted in  $^{13}\text{C}$  compared to  $^{12}\text{C}$ , and this difference is translated into the tissues of the organisms that consume them. Because there is little to no overlap in the ranges between the carbon ratios of C<sub>3</sub> and C<sub>4</sub> plants, carbon isotope ratios are an ideal analytical tool to assess the representation of these different types of plants in diet.

Within human populations, the plants consumed can reflect cultural and individual preferences, as well as availability related to climate and agricultural practices. For example, people who consume primarily rice-based proteins in their diets will have very different carbon isotope signatures than those who eat primarily corn and sugar products. Rice is a C<sub>3</sub> plant and has a general range of -26.3‰ to -29.7‰, while corn and sugar are C<sub>4</sub> plants with mean values of -14.0‰ for corn and -11.2‰ for sugar (Hart et al., 2003; Rodushkin et al., 2011). Schoeninger and DeNiro (1984) note that mixtures of plants in the diet, as well as marine food sources can impact an individual's overall  $\delta^{13}\text{C}$  values. The significant impact of marine food sources on an individual's carbon isotopic signature is due to the 7‰ difference between seawater bicarbonate and atmospheric CO<sub>2</sub>

(Craig, 1953). Most contemporary human diets consist of a combination of C<sub>3</sub> and C<sub>4</sub> plants, as well as marine food sources, so consideration must be paid to cultural preferences, individual preferences, and availability of these dietary components when interpreting diet.

### **Nitrogen Isotopes**

The nitrogen cycle is complex, and involves both biological and physical processes that occur in terrestrial, marine, and atmospheric environments (Högberg, 1997; Evans, 2001; Amundson et al., 2003; Fry 2006; Szpak, 2014). Nitrogen isotopes ( $\delta^{15}\text{N}$ ) in living organisms are affected by diet. Consumers generally have more positive nitrogen isotope values than primary producers, providing an indication for trophic position in food webs (Schoeninger and DeNiro, 1984; Ambrose and Norr, 1993).  $\delta^{15}\text{N}$  values of organisms typically increase 3-5‰ with each trophic level (Petzke et al., 2005; O'Connell et al., 2012). Plants, for example, have  $\delta^{15}\text{N}$  values between 0‰ and 5‰, and secondary or tertiary consumers have values averaging 9‰ or more (Ambrose and Norr, 1993; Hedges and Reynard, 2007). Generally, nitrogen is fixed into plants from N<sub>2</sub> in the atmosphere, though not all plants are nitrogen fixers (Fry, 2006). Nitrogen can also be incorporated into plants through soil additives like fertilizers (Choi et al., 2003; Bateman et al., 2005; Choi et al., 2006). Nitrogen incorporated into animal tissues comes from protein in the diet. The  $\delta^{15}\text{N}$  of animal tissues reflect the entire diet (both plants and animals consumed), but meat  $\delta^{15}\text{N}$  values will be dominantly reflected in the signature because of the high protein content, and plants will play only a minor role in the  $\delta^{15}\text{N}$  signature of an individual (Ambrose and Norr, 1993; Sponheimer et al., 2003). In

humans,  $\delta^{15}\text{N}$  values will vary most between individuals who are vegans and those who consume meat or other animal products (e.g., dairy, eggs) as part of their diets (Petzke et al., 2005). Organic food production also impacts  $\delta^{15}\text{N}$  values, as methods of crop fertilization produce different isotopic signatures (Choi et al., 2003; Bateman et al., 2005; Choi et al., 2006). An individual consuming a strictly organic diet could therefore show differences in  $\delta^{15}\text{N}$  signatures, as well. Marine foods tend to be isotopically heavier than land-based foods, so significant marine food consumption will also impact an individual's  $\delta^{15}\text{N}$  value (Hueselmann et al., 2013; Nash et al., 2012). In addition, metabolic states including pregnancy and starvation can also impact  $\delta^{15}\text{N}$  values (Fuller et al., 2004 and 2005; Mekota et al., 2006; DeLuca et al., 2012).

### **Hydrogen and Oxygen Isotopes**

Hydrogen ( $\delta^2\text{H}$ ) and oxygen ( $\delta^{18}\text{O}$ ) isotopes in the environment are controlled by the hydrologic cycle. Condensation and evaporation are two processes that influence isotopic values of water (Dansgaard, 1964; Gat, 1996). Water containing the lighter isotopes is preferentially evaporated, leaving behind the heavier isotopes in the originating pool. This principle applies as water moves through the hydrologic cycle, with clouds forming over the sea and moving inland and across continents. Here, the heavier isotopes are preferentially condensed and fall as precipitation, whereas the lighter isotopes stay in the vapor form. Factors such as climate, altitude, and proximity to the coast all influence the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values in precipitation, and thus available drinking water (Dansgaard, 1964; Bowen and Revenaugh, 2003; West et al., 2010; Kennedy et al., 2011).

These isotopes consumed from drinking water are preserved in an organism's tissues and reflect the environment in which the organism lives (Kendall and Coplen, 2001; Sponheimer and Lee-Thorp, 1999). When considering the expected  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values from human tissues, diet, drinking water, and atmospheric  $\text{O}_2$  from breathing must all be considered as contributing sources, with isotopic composition from food and water having the greatest influence (Wong et al., 1988; Sharp et al., 2003; Ehleringer et al., 2008; Kennedy et al., 2011; O'Grady et al., 2012; Podlesak et al., 2012).

### **Strontium Isotopes**

Strontium is an element with a similar chemical behavior to calcium, and its isotopes are influenced by the underlying bedrock geology of a geographic location. Generally speaking, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio varies among different types of rocks.  $^{87}\text{Sr}$  is formed from rubidium-87 ( $^{87}\text{Rb}$ ) through radiogenic processes, so older rocks (i.e., more time to decay) and rock types with higher rubidium-to-strontium ratios have higher  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios than relatively younger rocks (Price et al., 2013; Crowley et al., 2017). The age of the bedrock, as well as the type of bedrock underlying local soil, affects strontium isotope ratios. For example, older soils are more enriched in  $^{87}\text{Sr}$  than younger soils (Beard and Johnson, 2000). The underlying bedrock is incorporated into local soils and water sources through erosion and fluvial processes, creating unique isotope signatures for that geographic area. Plants that grow in the local soil and animals that eat the plants and drink the water then incorporate those signatures into their tissues. Any tissue that is relatively high in calcium such as bones and teeth, will also have relatively high concentrations of strontium. The decay constant for  $^{87}\text{Rb}$  to  $^{87}\text{Sr}$  is quite long (48.8



billion years) compared to human timescales, so in forensic and anthropological applications, the amount of radioactive decay they undergo is negligible (Minster et al., 1982).

The two primary sources of isotopically distinct strontium for humans are marine and terrestrial sources. Marine foods commonly share the same isotope value of seawater (Veizer, 1989). Terrestrial food sources vary according to local levels of strontium, thus reflecting the local environment. It is assumed that the  $^{87}\text{Sr}/^{86}\text{Sr}$  isotopic composition of plants and animals match their nutritional intake (Price et al., 2013). Hence, due to its relationship with geographic location, strontium is a powerful element for predicting geographic origins.

The biological processes that incorporate these isotopes from local environments into human tissues must be understood in order to translate analyses into meaningful information about the individual's diet and travel history. The next section provides an overview of human hair formation and growth, and how isotopes from food, water, and the environment are incorporated into the structure of hair.

### **Biology of Hair**

Human hair is composed of a protective outer layer known as the cuticle. The cuticle is made up of overlapping cells, which occur in a regular pattern along the length of the hair (Knecht, 2012). The inner structure of a hair is called the medulla. Hair is primarily composed of the structural protein keratin (Robbins, 2012). Keratin is made up of amino acids, which are derived from dietary proteins or produced within the human body (Block and Bolling, 1939; Rogers et al., 1997; Thompson et al., 2010). The link

between dietary input and hair isotopic signature makes hair a useful tool for investigating an individual's recent life history.

Human hair exhibits three discrete phases of growth and grows at an estimated rate of about 1 cm per month (Robbins, 2012). Hair is formed within a follicle, which has its own vascularization and innervations, connecting hair directly to the body's physiological processes (Wilson and Tobin, 2010). Hair growth occurs when cells form the hardened keratin that emerges from the scalp. This active period of hair growth is called the anagen phase. An individual hair follicle can stay in the anagen phase for up to ten years. Catagen, a resting period for the follicle, is the next phase. Catagen phase can last approximately three weeks. It is followed by the telogen phase, during which the follicle is resorbed. Telogen phase can last three months. Finally, hair is naturally shed from the follicle in the exogen phase (Kempson and Lombi, 2011; Robbins, 2012). Because a single individual has hair in various growth stages at any given time, human hair samples can be highly variable between individual strands (Remien et al., 2014). At any given time, though, an estimated 85% of hair is in the growth phase (Kempson and Lombi, 2011).

Food and water are significant contributors to the isotopic composition of hair (Ehleringer et al., 2008; Podlesak et al., 2012). An individual's unique body chemistry is recorded during keratinization, developing a chronological record of the individual's diet, drinking water, and environment. Once hair is formed, it does not undergo additional biogenic changes, potentially making it a reliable recorder of dietary and geographic travel history of an individual (Fraser and Meier-Augenstein, 2007; Ehleringer et al.,

2008; Thompson et al., 2010). Keratin is a protein, and the formation of protein incorporates hydrogen and oxygen largely from drinking water (Sharp et al., 2003; Ehleringer et al., 2008; Podlesak et al., 2012). This process occurs when hydrogen and oxygen from ingested proteins chemically react through exchange with the hydrogen and oxygen already in the body water pool. Body water is made up of food waters, drinking water, metabolized food, and diatomic oxygen from food as it is broken down into amino acids (Thompson et al., 2010). The greatest contribution of hydrogen and oxygen isotopes in the structural proteins of human hair is believed to come from drinking water (Ehleringer et al., 2008). Understanding the relationships between element compositions from diet and the formation of keratin in hair creates the opportunity to link environment to the biochemistry of hair composition, and it is this connection that has led to the development of the models of water isotope ratios from local environments in order to predict the isotope ratios found in human hair (Ehleringer et al., 2008; Bowen et al., 2009; Thompson et al., 2010; Podlesak et al., 2012).

In the same way hydrogen and oxygen are incorporated into keratin through ingested food and drinking water, carbon and nitrogen from diet are incorporated into hair. Amino acids derived from food and *in vivo* synthesized amino acids that make up the keratin structural protein of hair reflect contributions of plants and animal proteins to the individual diet (O'Connell and Hedges, 1999a and 1999b; O'Connell et al., 2001). While  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values are not used in the isotope modeling described above, dietary preferences can be combined with geographic data to make additional inferences based

upon regional food preferences (Regan, 2006; Bartelink et al., 2014; Hülsemann et al., 2015).

Strontium is a non-structural element incorporated into hair through both endogenous and exogenous sources. Both sources must be considered when interpreting  $^{87}\text{Sr}/^{86}\text{Sr}$  values of human hair (Kempson et al., 2006; Tipple et al., 2013). The endogenous source of strontium in the hair is from food and water and reflects the environment of those food and water sources. Exogenous strontium in hair comes from external sources, such as bathing water, or in the case of hair recovered with human remains from outdoor scenes, soil contaminants (Kempson and Skinner, 2005; Tipple et al., 2013). The endogenous signature reflects the dietary contributions of  $^{87}\text{Sr}/^{86}\text{Sr}$ , which would more closely represent a timeline of geographic travel history, but it has proven difficult to separate the endogenous and exogenous strontium in hair samples for analysis (Tipple et al., 2013). This means that if someone spends a relatively short time in a geographic environment with a different  $^{87}\text{Sr}/^{86}\text{Sr}$  signature than what has been recorded in hair from prior long-term travel history, the exogenous contributions could override the endogenous signature. Simply put, if an individual from the Midwestern United States spends a week in Florida and frequently washes his or her hair in local tap water, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of a hair sample from that individual may more closely resemble that of a Florida resident than that of a Midwestern resident. Great care must be taken during sample cleaning in order to remove exogenous strontium from hair, and it is unclear from previous research (Kempson et al., 2006; Tipple et al., 2013) how much contaminants from deposition environments, such as soil and precipitation, can impact strontium

isotope ratios in human hair (von Holstein et al., 2014). Sampling hair from an outdoor decomposition environment, as well as surrounding soil matter from the deposition site, can provide valuable insights regarding the degree to which  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of postmortem hair are affected by the environment. Nonetheless, strontium isotope ratios of human remains can be compared to bedrock maps to make predictions about geographic provenance (Beard and Johnson, 2000; West et al., 2009; Voerkelius et al., 2010; Chesson et al., 2012). While these bedrock maps do not provide direct references for the biologically available strontium isotopic compositions in a region, they allow reasonable inferences to be made about corresponding regional values, and can therefore increase the resolution of geographic predictions, particularly when paired with those made from oxygen values (Schwarcz et al., 2010; Tipple, 2015; Chau et al., 2017).

Hair serves as a linear recorder and grows at a predictable rate, so it can provide useful information about unknown individuals regarding the weeks and months prior to death, including recent travel history. Isotope ratios of hair can potentially distinguish a geographically stationary individual from a transient individual and can estimate whether an individual was local to the area in which the remains were recovered, or whether that individual may have recently arrived from another place (Meier-Augenstein and Fraser, 2008; Font et al., 2015; Aggarwal et al., 2008; Beard and Johnson 2000; Kamenov et al 2014; Chau et al., 2017). In the absence of other leads, geographic travel history can provide powerful clues to assist in the identification of unknown individuals. In order to use the isotope ratios of hair to make these predictions, mapping models of tap water and geology have been created to spatially relate isotope values to geographic locations.

These isotope maps, or “isoscapes,” are used in conjunction with isotope values from tissues, such as hair, to predict geographic provenance.

### **Isoscapes**

The term “isoscape” (a combination of ‘isotope’ and ‘landscape’) refers to models that spatially relate isotope values onto maps generated using geographic information systems (GIS) (West et al., 2010). For forensic applications discussed later in this chapter, an isoscape model of United States tap water was used to make predictions about geographic travel history from isotopes in human hair (Bowen and Revenaugh, 2003; Bowen, 2017; Bowen et al., 2016; Ehleringer et al., 2008; Podlesak et al., 2012).

Bowen and colleagues (2007) demonstrated spatially patterned variability in tap water collected across the United States. These patterns in the isotope values of tap water generally relate to processes of the hydrologic cycle that influence  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  isotopes of precipitation. The isotopic composition of precipitation varies across the world, with areas in low latitudes and along the coast having higher  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values (Dansgaard, 1964; Bowen and Revenaugh, 2003). Models that express this spatial variation in precipitation isotope values are useful, as they provided a baseline for the development of body water models that can translate isotope ratios of human tissues into predicted drinking water. They also provide comparative values that are spatially correlated and can be used to predict the geographic travel history of individuals by relating isotope ratios of body tissues to the isotope values on the predictive model maps and estimating possible regions of similarity. The error rates for the tap water isoscape models are reported as 12% for  $\delta^2\text{H}$  values and 1.8% for  $\delta^{18}\text{O}$  values (Bowen et al., 2007).

These predictive models are used in conjunction with body water models that examine how food and drinking water exert primary control over the  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$  values of human hair. Ehleringer and colleagues (2008) developed a model to correlate  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of tap water to  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of hair samples regionally collected from hair salons. These hair samples were presumed to come from local residents and reflect local isotope signatures, but this information is ultimately unknown, as the hair samples were not linked to known individuals. Additionally, the hair samples had not been exposed to an outdoor or decomposition environment for prolonged periods of time, as is the case with archaeological or forensic hair samples. The current study provides an opportunity to apply the model to known individuals and determine whether post-exposure changes in isotope ratios, should they occur, affect predictions from the model.

Throughout the development and use of these models, another area of concern has been that of homogenization of the modern American diet. With produce, meat, and processed foods shipped to local grocery stores from all over the world, as well as the mass distribution and use of bottled water, there is reason to question whether local environment can be reflected in isotope values from human tissues. Chesson and colleagues (2010) evaluated the differences between a supermarket diet and a fast food diet by comparing isotope ratios of similar meals from the two different sources. There were no significant differences between overall meals prepared from locally purchased supermarket ingredients and meals purchased from a fast food chain, and ultimately both meals had  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values consistent with local water sources. However, when

broken down into sources, the isotope values of proteins (from beef in this study) represented a local signature but those from carbohydrates (potatoes and bread in this study) did not. This suggested that models such as that from Ehleringer and colleagues (2008), which incorporates isotopes from food sources may be enhanced by accounting for the relative contributions of those sources that reflect local signatures and those that do not. The body water model developed by Podlesak and colleagues (2012) does just that.

Bottled water from locations around the world was tested in a separate study by Bowen and colleagues (2005). Their study revealed that bottled water isotope ratios were comparable to those of the naturally available local water sources. Most bottled water is derived and bottled from regional sources. It is reasonable to infer that bottled water is not an isotopically different component of diet from that of locally available drinking (tap) water. While more work needs to be done to understand the amount of human variation between individuals in terms of isotope ratios, the models available are a reasonable starting point for understanding the potential sources of variation in living individuals.

Given the strong and direct connections between biogeochemical signatures of local environments and the people who interact with them, it is not surprising that isotope analyses are a tool used by anthropologists. Specifically, anthropologists can make predictions about past behaviors, such as diet and travel history, through isotopic measurements of body tissues with high survivability over time, such as bones, teeth, and



hair. The following section provides a summary of the use of isotopes in archaeological research and forensic anthropology casework.

### **Anthropology and Isotopes**

Temporal chronologies of an individual's life can be developed by comparison of isotope values between human tissues of different developmental periods and turnover rates. The choice of tissue to be analyzed is dictated by the time period of an individual's life that is of most interest. Multiple tissues together can provide a record of life history movement from childhood to adulthood and the time shortly before death. For example, the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of dental enamel provides a permanent record of diet and geographic location at the time of formation from infancy to adolescence (Dolphin et al., 2005; Hillson, 1996), while the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of bone reflects dietary intake over the last several years of life (Beard and Johnson, 2000; Branca et al., 1992; Meier-Augenstein and Fraser, 2008). Understanding the rate of elemental turnover in bone is important for isotopic interpretation, as it is variable in different parts of the skeleton, largely based upon the size, composition, and functional demands activity places upon those bones. The femur, for instance, experiences turnover roughly every ten years, while the ribs remodel at a rate of approximately five years (Robling and Stout, 2008). Hair, in contrast to bone, is composed of keratin, which is produced at a predictable rate and can provide a recent incremental growth record and information on the diet and mobility information related to the time shortly before death (Ehleringer et al., 2008; Font et al., 2012; Vautour et al., 2015; Mant et al., 2016). Bones, teeth, and hair have been used by anthropologists

for analysis of both ancient and modern human remains in order to better understand individual lifeways and enhance the biological profile.

### **Archaeology**

Archaeologists use geochemical analyses in a variety of ways, from characterization of artifacts (Mason et al., 2016) to paleoenvironment and paleoclimate reconstructions (Koch, 1998; Goldberg et al., 2001; Reitz and Shackley, 2012). Isotopes are often used in the analysis of floral and faunal remains from archaeological sites in order to better understand seasonal patterns, mobility, the availability of resources, and agricultural practices (Price et al., 1994; Price et al., 2000; Roberts et al., 2016).

Bioarchaeologists have used isotope analyses of human remains to reconstruct prehistoric lifeways, including diet and mobility (Katzenburg, 2000). The following review will focus upon the use of isotope analyses as related to human remains, specifically as they apply to diet and migration in the ancient record.

Bioarchaeologists have used isotope signatures to provide insight into behaviors that are otherwise invisible in the archaeological record. Migration patterns (Knudson et al., 2012; Price et al., 2000; Price et al., 1994), eating behaviors (Buikstra and Milner, 1991; Macko et al., 1991a and 1991b; O'Connell and Hedges, 1999a; Knudson et al., 2007), utilization of landscape and resources (Schoeninger, 1995; Britton et al., 2011; Knudson and Buikstra, 2007), and geographic origins (Müller et al., 2003; Knudson et al., 2005; Knudson et al., 2009) can be explored through isotopic analyses.

The carbon isotope ratios of archaeological human remains provide dietary information and have been used to determine when maize agriculture was adopted by

populations within North America (Vogel and van der Merwe, 1977; DeNiro and Epstein, 1978; Price et al., 1985; Schwarcz and Schoeninger, 1991). Through the analysis of  $\delta^{13}\text{C}$  values of human remains, archaeologists were able to detect population patterns and shifts from diets composed of  $\text{C}_3$  plants to  $\text{C}_4$  plants, primarily maize. In addition to dietary shifts, adoption of maize is also indicative of behavioral shifts from hunting and gathering to more sedentary lifestyles.

Schoeninger (2009) combined  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data from multiple archaeological studies in order to compare the varied subsistence strategies associated with the adoption of maize agriculture. Her study combined  $\delta^{13}\text{C}$  values of human bone apatite and bone collagen across multiple North American sites, as well as  $\delta^{15}\text{N}$  values of human bone collagen to demonstrate that there was not a singular dietary shift with the introduction of maize agriculture that was universally expressed across all regions. Recall that maize is a  $\text{C}_4$  plant and associated with higher  $\delta^{13}\text{C}$  values. Agriculture sites in Ontario and at Grasshopper Pueblo that were evaluated for the study seemed to rely heavily upon maize as a primary nutritional source, which was likely supplemented with freshwater fish and waterfowl (Katzenberg, 1989; Olsen, 1990; Yerkes, 2005). The  $\delta^{13}\text{C}$  values indicated a strong  $\text{C}_4$  component in the diet and the results were consistent for all individuals from both sites. In contrast, Schoeninger (2009) presented data from two American Bottom Upland sites (Emerson, 2002; Yerkes, 2005) that suggest a more varied subsistence strategy between the two sites. One small group of individuals had  $\delta^{13}\text{C}$  values consistent with a  $\text{C}_3$  diet of wild nuts and plants supplemented with deer and squirrel, as indicated by faunal remains and  $\delta^{15}\text{N}$  values. Another group from less than 25 miles away had  $\delta^{13}\text{C}$

values consistent with the C<sub>4</sub> maize signature and supplemented with fish, indicated by faunal remains and  $\delta^{15}\text{N}$  values. In isolation, each of these archaeological sites represents a single population for a moment in time. By combining data from multiple studies, Schoeninger (2009) demonstrated that adoption of maize agriculture did not produce an instant shift to reliance solely upon crop cultivation and more sedentary behaviors. Subsistence strategies were more complex and likely related to the availability of secure lands and risk management. This study demonstrated the utility of isotope analyses in archaeological investigations in food sources, not just for determining the introduction of maize into the diet, but also for the interpretation of behaviors and subsistence strategies that were not universal, even within the same geographic region.

An understanding of human migration can also be explored through the carbon and nitrogen isotopic signatures of archaeological remains. Seasonal movement in populations from coastal areas to inland areas are evident in  $\delta^{13}\text{C}$  values of individuals from those populations, which are markedly different from those of people who inhabit the same geographic area consistently over a span of time (Sealy and van der Merwe, 1985; Sealy and van der Merwe, 1986).  $\delta^{13}\text{C}$  values of teeth and bones have been used in determining whether individuals can be considered as part of a local community or non-local (Knudson et al., 2012; O'Connell and Hedges, 1999b; Price et al., 2000; Sandford, 1993).

Knudson and colleagues (2012) used isotopes from bones, teeth, and hair from a single individual in order to reconstruct diet and mobility of the individual, an adult male found interred along a route connecting the North Chilean coast to the Loa River Valley.

This individual was found in isolation, and the archaeological questions posed about his life history were also intended to better understand the prehistoric use of the route he was traveling. Radiocarbon dating placed the individual in the Late Formative Period at around 73-330 AD (Cases et al., 2008). Artifacts associated with the individual pointed to both coastal and inland traditions, suggesting a relationship between this individual and the two groups. Isotopic analysis was performed in order to provide additional information about diet and travel history of the individual over his lifetime.

$\delta^{13}\text{C}$  data from bone collagen from this individual was consistent with a mixture of  $\text{C}_3$  and  $\text{C}_4$  plants and also likely included a substantial amount of marine food sources.  $\delta^{15}\text{N}$  data also indicated that he consumed marine food sources, particularly high-trophic level marine protein, over the last 10–30 years of his life (Knudson et al., 2012). Recall that bone provides information about the time period of its formation and turnover. For this study, fragments from a rib and the tibia were used. Tooth enamel was also used for this study, reflecting early childhood diet in  $\delta^{13}\text{C}$  values from carbonate analysis. These values were consistent with the consumption of a mixture of  $\text{C}_3$  and  $\text{C}_4$  plants, as well as marine resources during the first years of life (Knudson, 2012).  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from hair analysis of this individual were intermediate, implying that this individual repeatedly switched between higher and lower trophic-level resources, which were interpreted as the individual traveling back and forth from inland to the coast. This was further reinforced by segmented analysis of hair, which revealed distinct patterns of dietary changes from marine to terrestrial food sources, indicating travel between coastal and inland areas in the last months of life. Because hair grows in a predictable manner,

segmented analysis allowed for the interpretation of roughly one-month intervals of the individual's life just prior to death. Knudson and colleagues (2012) provided with their study a wonderful example of how an individual's diet and mobility in childhood, mid-life, and months prior to death can be predicted from isotope analysis of teeth, bones, and hair. Archaeological studies such as this allow not only insight into individual life histories, but also allow researchers to make inferences about the ways in which ancient people were moving across the landscape and between different locales.

Archaeological studies have used isotopes to provide individual and combined population data to learn more about ancient mobility and diet. These studies have allowed the presentation of more comprehensive pictures of the past and have introduced biogeochemical analyses into the anthropologist's tool kit. More recently, forensic anthropologists have begun adding isotope analyses to biological profiles of unknown human remains in order to provide additional individualizing information in the absence of other leads. The following section presents a review of the use of isotopes in forensic investigations and the utility of isotope analysis for identification of unknown human remains.

### **Forensic Anthropology**

Over the past few decades, isotope analysis has shown potential to assist with the identification of unknown human remains in forensic investigations (Benson et al., 2006; Rauch et al., 2007; Aggarwal et al., 2008; Juarez, 2008; Meier-Auenstein and Fraser, 2008;; McLean et al., 2013; 2014; Font et al., 2012). There are approximately 13,500 unidentified individuals in the National Missing and Unidentified Persons System

(NAMUS) ([www.identifyus.org](http://www.identifyus.org)). Investigators turn to medical examiners, anthropologists, and other forensic experts for as much information as they are able to provide. Isotopic analyses of human remains can provide additional details beyond the skeletal biological profile regarding an individual's geographic travel or residence history. Isotopic analysis does not provide outright identification of an individual, but it can provide new leads and refine search areas when the origin and identity of the deceased individual is unknown. This information adds another layer to the investigation and has resulted in the identification of unknown individuals in several cases (Cerling et al., 2003; Ehleringer et al., 2008; Meier-Augenstein and Fraser, 2008; Font et al., 2012).

Just as archaeologists can discern details of life histories from ancient human remains, forensic anthropologists can build similar profiles for unknown modern individuals. As with the archaeological examples, hair, teeth, and bone provide information about diet and geographic residence for variable times prior to the death of an individual. For example, investigators may be interested in knowing if an individual has been in a specific geographic area for an extended period of time, or whether they were transient and passing through at the time of death. In this case, teeth and bone could provide childhood and late life information, and analysis of hair could provide important clues regarding more recent travel history of the unknown individual. The final months of life are often of the most interest to investigators; therefore, human hair can provide valuable insights into the diet and geographic movement of an individual just prior to death. Hair is frequently recovered during forensic investigations, and the relatively small sample size needed for isotopic analysis makes hair a preferred tissue for forensic

sampling (Ehleringer et al., 2008). In addition, hair analysis is non-destructive relative to the skeleton and dentition – an important consideration in forensic cases.

One such case is that of “Saltair Sally,” a cold case of an unidentified female found near Salt Lake City, Utah. The skeletal remains were discovered in 2000 and, with no DNA matches in the missing persons database and no other leads to establish identity of the individual, the case went unsolved until new information was discovered during isotope analysis of a hair sample recovered with the skeletal remains.  $\delta^{18}\text{O}$  values of the hair, which were measured and tested in increments along the length of the strands, revealed that the individual had been traveling periodically. She was mobile rather than residing in one location. Three distinct geographic locations were revealed when the  $\delta^{18}\text{O}$  values were paired with models from oxygen isotopes in drinking water (Bowen et al., 2007; Remien et al., 2014). By using isoscapes, as discussed above, biogeochemists were able to produce useful new leads in the case and ultimately led to the identification of the unknown remains as Nikole Bakoles, a young mother who had been missing since early 2000 (Ehleringer et al., 2010).

Another forensic case that utilized isotopes from bone, hair, and nails to provide leads for identification of unknown remains was from Dublin, Ireland (Meier-Augenstein and Fraser, 2008). In March 2005, a mutilated torso and dismembered limbs of a male were discovered. Fingerprints and DNA were recovered but did not match anyone in the database. In an effort to gain additional information for the investigation, isotope analysis was used.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from hair and nails indicated a diet comparable to samples obtained from local individuals. Segmented analysis of hair and nails provided



information for an estimated seven (7) months prior to death, and the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  indicated that the individual had lived in or around Dublin or an area with comparable isotope signatures.

Because the individual was of African ancestry, it was suspected he immigrated to Dublin from elsewhere. Bone apatite from a femur sample was analyzed for  $\delta^{18}\text{O}$ , and the results were compared to global precipitation data. The results pointed to only five regions across the world that have comparable  $\delta^{18}\text{O}$  values; the Horn of Africa region was decided to be the most likely point of origin for the individual based upon precipitation and biological profile data. Based upon bone turnover rates and analysis of inner and outer portions of the femur, it was estimated that he left the region approximately 6.3 years prior to death. Based upon the travel history generated by stable isotopes, investigators were led to a possible identification, which was confirmed with DNA testing of a family member. The deceased was a 39-year-old male who had immigrated to Ireland from Kenya seven years before his death, just as the isotope data had suggested. Once an identification was made, suspects in the murder were arrested and ultimately convicted (Meier-Augenstein and Fraser, 2008).

In addition to forensic cold cases, isotopes have been used to assist with identification of the remains of United States military personnel (Beard and Johnson, 2000; Regan, 2006; Holland et al., 2012; Bartelink et al., 2014). By using  $\delta^{13}\text{C}$  from bones and teeth, forensic anthropologists at the Defense POW/MIA Accounting Agency (DPAA) have been able to distinguish the remains of U.S. service members from those of Asian origin, which is valuable in contexts of commingling (Bartelink et al., 2014).

Isotope analysis of a single tooth was used to aid in the identification of a United States airman (Holland et al., 2012).

A significant number of unidentified human remains in the United States are believed to be those of undocumented border crossers (UBCs) (Eschbach et al., 2003; Anderson, 2008; Juarez, 2008). Anderson (2008) notes that some of the challenges facing identification are the rapid decomposition of remains in the high heat of the desert, scavenger activity damaging identifying characteristics such as facial features, and the inability of family members to come to the U.S. to assist with identification. Additionally, many UBCs do not have antemortem medical or dental records for comparison, and frequently do not carry identification. All of these challenges are relevant only if medical examiners and forensic anthropologists have some idea of where the individual may have started their journey. Isotope analysis in order to estimate region of origin is a potentially powerful tool for aiding in the identification of UBCs (Juarez, 2008).

Forensic identification of unknown human remains relies upon interdisciplinary work of law enforcement, medical examiners, anthropologists, and many others. Isotope analysis of human remains can assist with these investigations by providing additional layers of information including dietary and geographic histories. Because hair provides the most recent history prior to death, it is especially valuable for identification. In order to use human hair from forensic contexts, though, it is necessary to understand the effects of taphonomic processes upon isotope values. In addition, there has been a great deal of concern about the scientific rigor of forensic science used in the courtroom (National

Research Council, 2009; Mnookin et al., 2011). This study addresses these questions in order to better understand the utility of isotope analysis of human hair for identification of unknown remains.

### **Summary**

Isotope ratios of carbon, nitrogen, hydrogen, oxygen, and strontium in the environment are controlled by natural processes. These isotope ratios are incorporated into human tissues via drinking water and food and provide a means to biochemically link individuals to their geographic environments and diets. Isotope analysis of human remains has historic roots in archaeological interpretations of diet and mobility patterns. Through the analysis of bones, teeth, hair, and nails for carbon and nitrogen isotopes, researchers have estimated the relative contributions of C<sub>3</sub> and C<sub>4</sub> plants (via  $\delta^{13}\text{C}$ ), as well as fish and terrestrial animal protein sources (via  $\delta^{15}\text{N}$ ) to individual diets. More recently,  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$ , have been used in order to better understand how prehistoric people were moving geographically across various regions during their lifetimes.

Borrowing from bioarchaeological applications of isotope analysis, forensic anthropologists are now incorporating the use of isotopes into the biological profile of unknown human remains in order to provide additional information about the individual that can assist with identification. As the development and turnover time for various body tissues differs, the time period of life represented by each tissue time is different. Teeth provide information about infancy and childhood, bones about the last five to ten years of life, and hair can provide information about the weeks and months prior to death. Hair

acts as a serial recorder of dietary and geographic history for the most recent time period and is therefore especially useful in reconstructing information about the individual in the time just before death.

Using what is known about the natural cycles that influence isotope values and what is known about how isotopes from the environment are incorporated into human tissues through drinking water and food, biogeochemists have created predictive models that can use environmental processes, such as precipitation, to predict the isotope values of drinking water. These models can be used along with the isotope values from human hair to predict geographic travel histories of unknown individuals, thereby adding another layer of information to the investigation.

The use of these models is increasing in popularity in forensic investigations to identify unknown human remains. The models for translating isotopes from human hair, however, have been developed using samples primarily from unknown individuals and from sources such as salons, which do not provide a means for comparing known information to results, and also do not reflect the conditions in which forensic hair samples are typically found. Forensic samples are typically exposed to soil and precipitation in outdoor environment for prolonged time periods, in contrast to clean hair from salons. This study addresses questions regarding the preservation of isotope signatures in human hair throughout the decomposition process. Building upon previous research that assumes the preservation of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope values in human hair throughout decomposition, this study compared isotope ratios of pre-exposure and post-decomposition hair samples to determine whether the values

changed over time. The next chapter provides a description of the laboratories, materials, and methods used to test the hypotheses outlined in Chapter One to determine whether isotopic signatures of hair change throughout decomposition.

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

The purpose of this chapter is to provide a detailed description of the research design and sampling strategy employed to test Hypotheses 1- 4 as outlined in Chapter 1. Anthropology laboratory research facilities and body donation programs are described first, followed by the overall research design. Phase I sampling strategy and analysis methods are discussed next, followed by sampling and analysis methods for Phase II.

#### **Anthropology Laboratory Locations**

Two anthropology laboratories for the study of human decomposition were utilized in this study. The following section outlines the details of these two facilities, including operation procedures, body donation programs, geography, geology, and climatic environments.

##### **Anthropology Research Facility, Knoxville, Tennessee**

(35°56'26" N, 83°56'36" W)

The Anthropology Research Facility (ARF) is an outdoor laboratory for the study of human decomposition in a natural environment. Established in 1981 by Dr. William M. Bass, the ARF was the first laboratory of its kind and has been used for a variety of research and training projects over the last 35 years. The systematic study of postmortem changes to human remains was first conducted at the ARF, and this foundational research continues to direct questions about human decomposition.

The ARF is located near the University of Tennessee Medical Center, and is

bordered by asphalt parking lots to the south and west, and by the Tennessee River to the north. A wooden privacy fence and additional chain link fence delineates the east border of the property and surrounds the entire three acres of the ARF. The bedrock lithology of the ARF is defined by its location on the Newala Formation of the Lower Ordovician Knox group to the south and the Middle Ordovician Chapman Ridge sandstone to the north (Cattermole, 1958). Corresponding local soil includes clay and silty clay soil upon limestone and clay loam upon calcareous sandstone with subordinate shale. The estimated  $^{87}\text{Sr}/^{86}\text{Sr}$  value of these marine geologic units was between 0.7088 and 0.7092 (McArthur et al., 2001).

The climatic conditions at the ARF are generally temperate and moist, with high rainfall. The mean annual high temperature in Knoxville, TN, is  $20.7^{\circ}\text{C}$  ( $69.3^{\circ}\text{F}$ ) and the mean annual low temperature is  $9.8^{\circ}\text{C}$  ( $49.7^{\circ}\text{F}$ ), with an overall mean temperature of  $15.3^{\circ}\text{C}$  ( $59.5^{\circ}\text{F}$ ). Annual precipitation averages 1224 mm (48.2 in) ([www.usclimatedata.com](http://www.usclimatedata.com)). The annual  $\delta^{18}\text{O}$  of precipitation at ARF is estimated to be  $-6.3 \pm 0.5\text{‰}$  (VSMOW, 95% C.I.) and the  $\delta^2\text{H}$  is  $-39 \pm 6\text{‰}$  (VSMOW, 95% C.I.) (Bowen, 2017; Bowen and Revenaugh, 2003).

In order to conduct research on human decomposition, the ARF is supported by a body donation program. There are currently over 4,000 pre-registered donors (Steadman personal communication, 2017). As part of the registration process, the donors complete questionnaires that provide the opportunity to self-report individual life histories, including basic information like age, sex, ancestry, stature, and weight. More detailed information includes current and past medical conditions, occupation, and lifetime

residence history. This information is kept as part of the donor's individual record and is available to researchers as anonymized data.

Upon death, donors are transported to the William M. Bass Forensic Anthropology Building, which is located adjacent to the ARF. Each donor is processed through the body donation program's intake procedures. Intake includes the collection of biological samples of hair, blood, and fingernails, which are curated and available for research. Intake samples of hair for each individual are divided into two aliquots of approximately 100 hairs each, with one aliquot curated in frozen storage at -20°C, and the other at room temperature. Additional data collected as part of the intake process includes cadaver height, weight, and photographs of the donor. Once the intake process is completed, donors are stored in the body cooler or a freezer until placement at the ARF. Daily photographs are taken until decomposition changes are no longer observed. Once the donor is decomposed and skeletonized, the remains are recovered as part of student or law enforcement training. The skeletal remains are taken to the William M. Bass Forensic Anthropology Building where they are cleaned. The cleaned skeletons are then inventoried and accessioned into the William M. Bass Skeletal Collection, where they are curated for skeletal research.

### **Forensic Anthropology Research Facility, San Marcos, TX**

(29°56'18" N, 98°0'30" W)

A second human decomposition facility was used for this project in order to provide contrasting macroenvironments. The Forensic Anthropology Research Facility (FARF) is located on 26 acres of Freeman Ranch in San Marcos, Texas. The climatic



conditions at the FARF are warmer and more arid than those in Tennessee. The mean annual temperature is 20.2°C (68.5°F). The average high temperature in San Marcos, TX, is 26.4°C (79.6°F) and the average annual low temperature is 14°C (57.3°F). Annual precipitation averages 908 mm (35.8 in) ([www.usclimatedata.com](http://www.usclimatedata.com)).

The underlying bedrock of the FARF is made up of mudstones, limestone, and minor cherts from the Lower Cretaceous period. The  $^{87}\text{Sr}/^{86}\text{Sr}$  value of these marine rocks was between 0.7072 and 0.7074 (McArthur et al., 2001). The  $^{87}\text{Sr}/^{86}\text{Sr}$  values of FARF differ from those at the ARF and therefore provide an opportunity to evaluate the effects of the decomposition environment upon the values from human remains over time. The mean annual  $\delta^{18}\text{O}$  of precipitation at FARF is estimated to be  $-6.4 \pm 0.6\text{‰}$  (VSMOW, 95% C.I.) and the  $\delta^2\text{H}$  is  $-43 \pm 6\text{‰}$  (VSMOW, 95% C.I.) (Bowen, 2017; Bowen and Revenaugh, 2003).

Body donation and intake procedures at the FARF are similar to those at the ARF, as are recovery procedures. Biological samples, metric data, and photographs are taken of each donor at intake and are curated for research. Life history information, including lifetime residence history is also available for each donor.

## **Research Design**

This study was conducted in two phases. The first phase of the study (Phase I Hair Mat Study) examined whether the isotopic compositions ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$ ) of human hair changed over time during decomposition, addressing Hypotheses 1, 2, and 3. Samples were collected by surveying the ARF in July 2015 for available hair mats that could be correlated to a specific donor. Each hair mat sample was compared to

the hair sample collected at the time of intake from the same donor to determine if there were differences in isotopic compositions between the two time periods (intake vs. post-decomposition). Hair samples were analyzed as part of a Research-in-Residence program at IsoForensics, Inc. and the University of Utah, in Salt Lake City, Utah.

In order to better understand how decomposition processes influence changes in the isotopic compositions of hair over time and address the specific variables of exposure duration, placement environment, and placement condition presented in Hypotheses 1-3, six donors from the University of Tennessee body donation program were enrolled in the second phase (Phase II Serial Sampling) of the study. Hair samples were collected from each of the donors at specified times during decomposition over the course of a one-year period. An additional two donors from the Forensic Anthropology Center at Texas State (FACTS) body donation program were placed at the Forensic Anthropology Research Facility (FARF) in San Marcos, Texas, so that the effects of two differing climatic and geological environments could be observed. Phase II of the study was conducted as part of a larger NIH-funded project (NIJ 2014-DN-BX-K002) in collaboration with Dr. Gwyneth Gordon of Arizona State University.

The remainder of this chapter provides a detailed description of the materials and methods used for both phases of this study. Because multiple laboratories, each with slightly different protocols, were utilized for hair sample preparation and analysis, detailed descriptions for each phase of the study are provided below. Differences in laboratory protocols are procedural only and do not impact the interpretation of the results as presented in Chapters Four and Five.

## **Phase I: Hair Mat Study**

### **Donor Enrollment**

Thirty-eight (38) donors, 25 females and 13 males, from the University of Tennessee body donation program were enrolled in the Phase I Hair Mat Study portion of this project. A survey of the ARF was conducted in July 2015, and donors were selected for this study based upon the presence of hair mats that could be definitively associated with specific donors at the ARF. An effort was made to represent the various stages of decomposition, from fresh to skeletonized, as well as a broad time range of exposure of 22 to 1140 days, indicated by number of days since placement at the ARF (Table 2). For each of the enrolled donors, intake samples were obtained from the curated collection so that corresponding intake and post-exposure hair samples from each donor could be analyzed.

## **Isotope Analysis**

### **Sample Cleaning**

Hair samples were collected and wrapped in aluminum foil prior to preparation and stored at  $-20^{\circ}\text{C}$ . Each hair sample of approximately 25 strands was mechanically cleaned to remove any debris from the surface. Hair was gently washed by soaking in 50 mL beakers of deionized water. For samples with excessive soil and debris present, mild dish detergent was applied to the hair sample and then rinsed away with deionized water before the soaking step. Water-washed hair was soaked for five minutes in a 2:1 chloroform:methanol solution to remove surficial contaminants, particularly lipids. The supernatant was discarded and the procedure was repeated three times.

Table 2. Individual donor information for Phase I hair mat study

| Anonymized Donor Number | Age at Death (years) | Sex    | Ancestry | Residence at Time of Death | Days of Exposure |
|-------------------------|----------------------|--------|----------|----------------------------|------------------|
| 732                     | 65                   | Male   | White    | Rockford, TN               | 373              |
| 733                     | 40                   | Female | White    | Fayetteville, TN           | 385              |
| 735                     | 74                   | Female | White    | Knoxville, TN              | 1038             |
| 736                     | 38                   | Male   | White    | Elgin, SC                  | 143              |
| 738                     | 50                   | Male   | White    | Rock Hill, SC              | 385              |
| 739                     | 65                   | Male   | White    | Maryville, TN              | 77               |
| 741                     | 62                   | Male   | White    | Bybee, TN                  | 465              |
| 742                     | 79                   | Female | White    | Chattanooga, TN            | 443              |
| 744                     | 77                   | Female | White    | Fayetteville, GA           | 734              |
| 746                     | 62                   | Female | White    | Columbus, IN               | 398              |
| 748                     | 58                   | Female | White    | Cosby, TN                  | 52               |
| 749                     | 66                   | Female | White    | Knoxville, TN              | 825              |
| 750                     | 72                   | Female | White    | Maryville, TN              | 871              |
| 752                     | 62                   | Female | White    | Elizabethton, TN           | 1003             |
| 753                     | 78                   | Female | White    | Milledgeville, TN          | 395              |
| 754                     | 37                   | Male   | White    | Marshall, MO               | 419              |
| 755                     | 83                   | Female | White    | Kingston, TN               | 62               |
| 756                     | 75                   | Male   | White    | Drane Hill, AL             | 77               |
| 757                     | 56                   | Female | White    | Vinemont, AL               | 569              |
| 758                     | 81                   | Male   | White    | Cleveland, TN              | 199              |
| 759                     | 65                   | Male   | White    | Landrum, SC                | 445              |
| 760                     | 70                   | Female | White    | Douglasville, GA           | 22               |
| 761                     | 87                   | Male   | White    | Sandy Springs, MD          | 83               |
| 767                     | 78                   | Male   | White    | Dunlap, TN                 | 727              |
| 769                     | 56                   | Female | White    | Cartersville, GA           | 380              |
| 743                     | 67                   | Female | White    | Birchwood, TN              | 478              |
| 744                     | 77                   | Female | White    | LaFayette, GA              | 734              |
| 768                     | 76                   | Female | White    | Frankfort, KY              | 127              |
| 762                     | 78                   | Male   | White    | Knoxville, TN              | 169              |
| 763                     | 82                   | Female | White    | Pleasant Hill, TN          | 175              |
| 764                     | 94                   | Female | White    | Harriman, TN               | 169              |
| 765                     | 53                   | Female | White    | Ooltewah, TN               | 310              |
| 751                     | 72                   | Female | White    | Vonore, TN                 | 952              |
| 734                     | 43                   | Female | White    | Knoxville, TN              | 1140             |
| 737                     | 62                   | Male   | White    | Knoxville, TN              | 1021             |
| 745                     | 53                   | Female | White    | Oxford, NY                 | 445              |
| 747                     | 82                   | Female | White    | Lenoir City, TN            | 169              |
| 770                     | 21                   | Female | White    | Mount Laurel, NJ           | 485              |

Solvent-cleaned hair was allowed to air dry in a hood. Dry hair was stored in manila coin envelopes until additional analysis-specific preparation was performed (Fraser et al., 2008).

### **Nitrogen and Carbon Analyses**

Clean, dry hair was mechanically cut into approximately 0.25 cm pieces using a new, clean razor blade, then weighed and encapsulated for bulk stable isotope analysis. Samples weighed for nitrogen and carbon isotope analyses were loaded ( $0.5 \text{ mg} \pm 10\%$ ) into  $3.5 \times 5 \text{ mm}$  tin capsules (Costech Analytical Technologies, Inc.; Valencia, California, USA). A set of three reference materials was weighed for analysis alongside samples, with at least four replicates of each reference material per analytical sequence. Weighed, encapsulated samples and reference materials were analyzed for nitrogen and carbon stable isotope ratios using a Thermo Finnigan™ MAT 253 isotope ratio mass spectrometer (Bremen, Germany) with a Costech elemental analyzer (ECS 4010 EA) attached via a Thermo ConFlo IV interface. Samples and reference materials were introduced to the elemental analyzer via a zero-blank autosampler. These stable isotope analyses were completed at IsoForensics, Inc. in Salt Lake City, Utah, USA.

Measured stable isotope ratios of samples were corrected for memory, time, and area effects, if necessary, then “stretch-shift” normalized to international isotope scales using two previously characterized powdered keratin (horn) reference materials, DS (assigned  $\delta^{15}\text{N} = +1.38\text{‰}$ ,  $\delta^{13}\text{C} = -23.12\text{‰}$ ) and ORX (assigned  $\delta^{15}\text{N} = +10.69\text{‰}$ ,  $\delta^{13}\text{C} = -11.39\text{‰}$ ). Quality control was verified using a third powdered keratin reference material, POW (long term  $\delta^{15}\text{N} = +5.95\text{‰}$ ,  $\delta^{13}\text{C} = -24.03\text{‰}$ ).

## Hydrogen and Oxygen Analyses

Prior to encapsulation, clean, dry hair samples were allowed to equilibrate with ambient laboratory atmosphere for at least 48 hours. Keratin reference materials (DS, ORX, and POW) were equilibrated alongside hair samples (Wassenaar and Hobson, 2003; Chesson et al., 2009). Equilibrated hair was mechanically cut into approximately 0.25 cm pieces using a clean razor blade, then weighed and encapsulated for bulk stable isotope analysis. Samples weighed for hydrogen and oxygen isotope analysis were loaded ( $0.15 \text{ mg} \pm 10\%$ ) into  $3.5 \times 5 \text{ mm}$  silver capsules (Costech Analytical Technologies, Inc.; Valencia, California, USA). DS, ORX, and POW were weighed for analysis alongside samples, with at least four replicates of each reference material per analytical sequence. Encapsulated samples and reference materials were stored in 96-well plastic trays under vacuum for a minimum of five days prior to isotope analysis, to remove sorbed water. Sample and reference materials were analyzed for hydrogen and oxygen stable isotope ratios using a Thermo Finnigan™ MAT 253 isotope ratio mass spectrometer with a Thermo high-temperature conversion elemental analyzer (TC/EA) attached via a ConFlo IV interface. Samples were introduced to the TC/EA via a zero-blank autosampler, where samples and reference materials were converted into  $\text{H}_2$  and  $\text{CO}$  gases at  $1400^\circ\text{C}$  in a glassy carbon reactor. These stable isotope analyses were completed at IsoForensics, Inc. in Salt Lake City, Utah, USA.

Measured stable isotope ratios of samples were corrected for memory, time, and area effects, if necessary, then “stretch-shift” normalized to international isotope scales using DS (assigned  $\delta^2\text{H} = -174.1\text{‰}$ ,  $\delta^{18}\text{O} = +6.02\text{‰}$ ) and ORX (assigned  $\delta^2\text{H} = -35.4\text{‰}$ ,  $\delta^{18}\text{O} = +25.09\text{‰}$ ). Quality control was verified using POW (long term  $\delta^2\text{H} = -101.1\text{‰}$ ,

$\delta^{18}\text{O} = +12.43\text{‰}$ ). The  $\delta^2\text{H}$  values of hair samples reported in this study represent the intrinsic, or “non-exchangeable,” fraction of H atoms, assuming a similar fraction of labile H atoms was present in hair samples as found previously for DS, ORX, and POW standards, which are all keratinous materials ( $\sim 10\%$ ).

### **Strontium Analyses**

Strontium analysis of twenty hair samples, representing 10 pairs, was completed at the University of Utah in the Department of Geology and Geophysics. The ten pairs of hair samples were selected to cover a range of outdoor exposure durations. These hair samples underwent additional preparation in a clean laboratory as presented below to remove additional contaminants imbedded in the hair structure. All water used in cleaning was campus deionized water (typically  $\sim 3\text{ M}\Omega$  resistance) that was further purified through a four-stage ion exchange filtration system to  $18\text{ M}\Omega$  and piped throughout the laboratory. All chemistry was conducted with water that went through an additional Millipore brand Milli-Q<sup>®</sup> Gradient point-of-use filtration unit (hereafter referred to as “ $18\text{ M}\Omega$  water”).

50 mg samples of hair were weighed and placed in acid-leached 15-mL tubes.  $18\text{ M}\Omega$  water was added to the tubes, which were sonicated (B3500-MT ultrasonic cleaner; VWR) for 2 minutes to remove coarse contaminants. Tubes were centrifuged for 1 minute and the supernatant was decanted and discarded. 3 mL of acetone was added to each sample, which was then sonicated for 10 minutes. Following sonication, samples were centrifuged. After 1 minute of centrifuging, acetone was decanted and retained in a separate acid-leached 15-mL tube. The hair samples were then treated with acetone a

second time. After the second acetone rinse was completed, and the acetone was decanted into the separate leachate collection tube, 3 mL of 0.1 mol kg<sup>-1</sup> hydrochloric acid (HCl) was added to each hair sample. The hair samples were sonicated 10 minutes and centrifuged 1 minute. The HCl was retained in the leachate collection tube along with the previously collected acetone and 18 MΩ water aliquots. These tubes were retained and stored for possible future residue analysis. Tubes containing hair samples were drained of any remaining liquid and placed uncapped, loosely covered with a cleanroom wipe cover to prevent contamination, in a drying oven at 60°C overnight.

Once completely dry, each solvent-cleaned sample was placed in a 10-mL Teflon digestion vessel. Samples were digested in 2 mL of 16 mol kg<sup>-1</sup> ultrapure nitric acid (HNO<sub>3</sub>) using an Ethos EZ SK-10 High Pressure Rotor microwave digestion system (Milestone, Inc.; Shelton, Connecticut, USA). The digestion method was as follows: 13.3°C min<sup>-1</sup> ramp to 200°C, followed by a 15-minute isothermal period at 200°C, and a 60-minute cool down to room temperature. Certified reference materials TORT-2 Lobster Hepatopancreas Reference Material for Trace Metals (National Research Council Canada; Ottawa, Ontario, Canada) and Human Hair No. 13 (National Institute for Environmental Studies; Tsukuba, Ibaraki, Japan) were digested with hair samples; method blanks were also included. Once cooled to room temperature, the digest solutions were transferred to 2-mL acid-leached centrifuge tubes for storage.

To determine strontium abundances, a 200-μL aliquot of the primary digest was transferred to an acid-leached 15-mL centrifuge tube. 20 μL of a 1 mg kg<sup>-1</sup> indium internal standard was added to the secondary aliquot and diluted to 2 mL with 2.4%



(V/V)  $\text{HNO}_3$ . The remaining digest solutions were reserved for strontium isotope analysis. Abundance measurements were performed at the ICP MS Metals Lab in the Department of Geology and Geophysics at the University of Utah, Salt Lake City, Utah, USA. Strontium was measured via inductively coupled plasma mass spectrometry (ICP-MS), using an Agilent 7500ce mass spectrometer with an octopole reaction system (Agilent Technologies; Santa Clara, California, USA).

Concentrations of strontium in hair sample digests were determined to be very low. In order to insure the best data quality, manual digestion and column chemistry were performed for sample purification. The reserved digests were transferred to rounded interior Teflon containers and placed uncapped on a hot plate set to  $200^\circ\text{C}$  in a laminar flow hood. Once sample digests were dried down to a miniscule droplet,  $400\ \mu\text{L}$  of  $4\ \text{mol kg}^{-1}$  trace metal grade  $\text{HNO}_3$  was added. Strontium in the digests was isolated by pipetting the solution into a Teflon column packed with  $100\text{-}150\ \mu\text{m}$  crown ether strontium resin (Eichrom Technologies; Lisle, Illinois, USA). Prior to beginning, columns were cleaned with  $18\ \text{M}\Omega$  water and conditioned by loading 8 column volumes ( $50\ \mu\text{L}$  each) of  $4\ \text{mol kg}^{-1}$  trace metal grade  $\text{HNO}_3$  applied to the column,  $100\ \mu\text{L}$  at a time.

After column conditioning, sample digests were loaded into the columns as  $100\ \mu\text{L}$  aliquots, with a total of  $400\ \mu\text{L}$  for each sample. Following sample loading,  $300\ \mu\text{L}$  of  $4\ \text{mol kg}^{-1}$  trace metal grade  $\text{HNO}_3$  was added in  $100\ \mu\text{L}$  volumes to clean the resin matrix. To elute the strontium from the resin,  $400\ \mu\text{L}$  of  $0.1\%$   $\text{HNO}_3$  diluted with  $18\ \text{M}\Omega$  water was used to elute the strontium from the resin. The collected strontium sample was

transferred to a 2-mL conical interior Teflon container and 16  $\mu\text{L}$  of concentrated trace metal grade  $\text{HNO}_3$  was added to acidify the solution (approximately 5%  $\text{HNO}_3$ ). Samples were then analyzed at the Strontium Isotope Geochemistry Laboratory in the Department of Geology and Geophysics at the University of Utah using a Thermo Scientific™ Neptune Plus™ high resolution multicollector inductively coupled plasma mass spectrometer (Bremen, Germany). An SRM 987 standard at 50 ppb concentration was run in parallel with samples due to low strontium concentrations in the samples.

### **Phase II: Serial Samples**

The purpose of this portion of the study is to provide longitudinal isotope data from hair samples collected from the same donors over a period of one year. This phase of the study utilized two facilities (ARF and FARF) with different geologic and climatic environments in order to evaluate the variables of exposure time, placement location, and placement condition as proposed in Hypotheses 1- 3. The  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  values from intake and final post-exposure samples from the donors in Phase II were used to create predictive maps for geolocation in order to address Hypothesis 4.

### **Donor Enrollment**

Eight (8) additional donors, six (6) from the FAC body donation program and two (2) from the FACTS body donation program, were enrolled in Phase II of the study, which followed the donors over a period of one year. Donors were selected based upon arrival at the respective facilities during the project start period and whether the donor had head hair one (1) inch in length or longer and had at least two teeth. Individual details about each of the donors can be found in Table 3.

Table 3. Individual donor information for Phase II serial study

| Subject Number | Age at Death (years) | Sex    | Ancestry | Residence at Time of Death | Facility of Placement |
|----------------|----------------------|--------|----------|----------------------------|-----------------------|
| Surface 1      | 50                   | Male   | White    | Rock Hill, SC              | ARF                   |
| Surface 2      | 40                   | Female | White    | Fayetteville, TN           | ARF                   |
| Surface 3      | 56                   | Female | White    | Cartersville, GA           | ARF                   |
| Surface 4      | 83                   | Male   | White    | Austin, TX                 | FARF                  |
| Surface 5      | 97                   | Female | White    | Austin, TX                 | FARF                  |
| Burial 1       | 73                   | Male   | White    | Roswell, GA                | ARF                   |
| Burial 2       | 63                   | Female | White    | LaFollette, TN             | ARF                   |
| Burial 3       | 38                   | Male   | White    | Lenoir City, TN            | ARF                   |

Three (3) of the donors from the FAC program were placed supine in shallow burials at the ARF. Three (3) FAC donors were placed upon the surface at the ARF and the two (2) FACTS donors were placed upon the surface at the FARF. These surface placements were in a supine position and covered with wood and wire mesh cages to discourage animal scavenging. Placements at both facilities occurred in summer 2015. Samples of hair were taken upon intake of the remains and at timed intervals as follows: surface placements, days 1-7 (encompassing the first 2,500 Accumulate Degree Hours (ADH)), day 60, day 90, day 180, and day 360. Buried remains had hair samples collected at placement and at recovery, with one burial sampled at approximately 100 days after placement. Daily photographs of the surface remains were also maintained throughout the first month of the study and monthly thereafter. Soil, precipitation, and tap water samples were collected from the sampling sites as part of the collaborative project. These sample data are included but were not analyzed by the researcher as part of this

project.

### **Sample Cleaning**

Each hair sample from the eight serial donors ( $n=6$  ARF,  $n=2$  FARF) of approximately 100 strands was mechanically cleaned to remove any debris from the surface. Hair was gently washed by sonicating for 10 minutes in 50 mL beakers of Milli-Q<sup>®</sup> water. The water was discarded after sonication. Hair was then sonicated for 10 minutes in a 2:1 chloroform:methanol solution to remove surficial contaminants and lipids. The supernatant was discarded and the chloroform:methanol procedure was repeated until the solution was clear. Cleaned hair was allowed to dry in a laminar flow hood. Clean, dry hair was stored in paper coin envelopes until additional analysis-specific preparation was performed.

### **Nitrogen and Carbon Analyses**

Clean, dry hair was milled into a fine powder using a liquid nitrogen ball mill (6775 Freezer/Mill, SPEX Sample Prep; Metuchen, NJ). The powder was stored in a desiccator, weighed and encapsulated. Samples weighed for nitrogen and carbon isotope analysis were loaded ( $0.5 \text{ mg} \pm 10\%$ ) into  $3.5 \times 5 \text{ mm}$  tin capsules (Costech Analytical Technologies, Inc.; Valencia, California, USA). Weighed, encapsulated samples were sent to the Stable Isotope Facility for Environmental Research (SIRFER) at the University of Utah in Salt Lake City, Utah, for analysis.

Measured stable isotope ratios of samples were corrected for memory, time, and area effects, if necessary, then “stretch-shift” normalized to international isotope scales using two previously characterized powdered keratin (horn) reference materials, DS

(assigned  $\delta^{15}\text{N} = +1.38\text{‰}$ ,  $\delta^{13}\text{C} = -23.12\text{‰}$ ) and ORX (assigned  $\delta^{15}\text{N} = +10.69\text{‰}$ ,  $\delta^{13}\text{C} = -11.39\text{‰}$ ). Quality control was verified using a third powdered keratin reference material, POW (long term  $\delta^{15}\text{N} = +5.95\text{‰}$ ,  $\delta^{13}\text{C} = -24.03\text{‰}$ ).

In addition, four blind reference materials were encapsulated and shipped as unknowns in parallel with samples. This allowed comparison of accuracy and precision of analyses of certified standards to include sample preparation at the Arizona State University laboratory. These included USGS 42 (certified values of  $\delta^{15}\text{N} = +8.05 \pm 0.10\text{‰}$ ,  $\delta^{13}\text{C} = -21.09 \pm 0.10\text{‰}$ ), USGS 43 (certified values of  $\delta^{15}\text{N} = +8.44 \pm 0.10\text{‰}$ ,  $\delta^{13}\text{C} = -21.28 \pm 0.10\text{‰}$ ), as well as two in-house ASU hair standards. These standards collected from salon cuttings were designed to be more similar in isotope values to the North American demographic population at ARF and FARF. ASU H-Std 1 and H-Std 2 were sent to several laboratories for comparison. ASU H-Std 1 has a value of  $\delta^{15}\text{N} = +9.36 \pm 0.08\text{‰}$ ,  $\delta^{13}\text{C} = -18.06 \pm 0.04\text{‰}$ ,  $n = 4$  laboratories), and ASU H-Std 2 has a value of  $\delta^{15}\text{N} = +9.20 \pm 0.18\text{‰}$ ,  $\delta^{13}\text{C} = -16.78 \pm 0.05\text{‰}$ ,  $n = 4$  laboratories).

### **Hydrogen and Oxygen Analyses**

Hair was milled into a fine powder using a liquid nitrogen ball mill (6775 Freezer/Mill, SPEX Sample Prep; Metuchen, NJ). The powder was weighed and shipped in small glass vials to SIRFER at the University of Utah in Salt Lake City, Utah. Prior to encapsulation, samples were allowed to equilibrate with ambient laboratory atmosphere for 48 hours. After equilibration, hair was weighed and encapsulated for bulk analysis (Wassenaar and Hobson, 2003; Chesson et al., 2009). Oxygen and hydrogen samples ( $0.15\text{mg} \pm 10\%$ ) were loaded into 3.5 x 5 mm silver capsules (Costech Analytical

Technologies, Inc.; Valencia, CA, USA). Laboratory reference materials (keratin: DS, ORX, and POW) and USGS 42 and USGS43 were weighed into silver capsules at similar masses to the hair samples. Samples and reference materials were stored in 96-well plastic trays under vacuum for a minimum of 5 days prior to analysis. All samples were analyzed on a ThermoFinnigan MAT 253 isotope ratio mass spectrometer. Samples were introduced to the instrument via a zero-blank autosampler attached to a high temperature conversion elemental analyzer (TC/EA). Reference materials DS and ORX were used for normalization (assigned  $\delta^{18}\text{O} = 6.02\text{‰}$  and  $25.09\text{‰}$ , respectively) while POW was used for quality assurance (long-term mean  $\delta^{18}\text{O} = 12.44\text{‰}$ ,  $2\sigma = 0.54\text{‰}$ ,  $n = 335$ ).

### **Strontium Analysis**

Strontium and trace element analyses of serial samples were completed at the W.M. Keck Foundation Laboratory for Environmental Biogeochemistry at Arizona State University. Additional procedures were completed in a trace-metal clean lab, with all chemical procedures performed in ULPA-filtered Class 10 laminar-air flow exhaust hoods. The hoods are housed in a positively-pressurized clean laboratory with ULPA-filtered air supply routinely maintained at Class 10,000 conditions. This lab was built with minimal metal; all workstations, cabinetry and wall coverings are polypropylene and other corrosion-resistant materials. An adjacent laboratory space maintained at Class 100,000 conditions provides space for acid-cleaning of all reagent vessels and laboratory consumables.

Centrifuge tubes were trace metal grade. All other plastic consumables were cleaned by overnight soaking in a ~1% solution of Micro-90 detergent in 18 M $\Omega$  water.

They were thoroughly rinsed with 18 M $\Omega$  water and soaked for at least three days in 20% by volume reagent grade nitric acid. They were rinsed with 18 M $\Omega$  water and soaked for at least three days in 20% by volume reagent grade hydrochloric acid. They were rinsed at least three times in 18 M $\Omega$  water and dried under ULPA-filtered air in a specially designated hood before being stored in closed plastic bins. Teflon vials (Savillex Corporation, Eden Prairie, MN) are reused after cleaning. After use, vials were rinsed with 18 M $\Omega$  water and labels and any organic material was removed with acetone and a Kimwipe. They were soaked at least overnight in a ~1% solution of Micro-90 detergent in 18 M $\Omega$  water. They were then heated to sub-boiling in 8 mol kg<sup>-1</sup> reagent grade nitric acid for 24 hours, rinsed three times in 18 M $\Omega$  water, heated to sub-boiling in 6 mol kg<sup>-1</sup> reagent grade hydrochloric acid for 24 hours, rinsed three times in 18 M $\Omega$  water and heated overnight in 18 M $\Omega$  water. They were rinsed three times in 18 M $\Omega$  water and dried under ULPA-filtered air in a specially designated hood. In addition, for sensitive blank analyses or small samples, vials had an additional stage of heating with 6 mol kg<sup>-1</sup> trace metal grade hydrochloric acid on a hot plate for at least an hour before rinsing three times with 18 M $\Omega$  water.

All chemical reagents used in chemical processing were at least trace metal grade or better. Nitric acid was either BDH Aristar High-Purity Plus or 70% ACS grade nitric acid additionally purified through a dedicated Savillex DST-1000 Acid Purification System (Savillex Corp., Eden Prairie, MN). Hydrochloric acid was either 36.5-38% ACS grade nitric acid additionally purified through a dedicated Savillex DST-1000 Acid Purification System (Savillex Corp., Eden Prairie, MN). Hydrofluoric acid was Fisher

Chemical TraceMetal™ Grade. Hydrogen peroxide was VWR brand BDH Aristar® Ultra 30% hydrogen peroxide.

50 mg samples of hair were weighed and placed in acid-leached round-interior Teflon vials. 3mL of 0.1M HCl was added to each vial, which was sonicated for 10 minutes. The process was repeated a total of three times and the resulting leachates were combined for each sample. In addition to the leachate, the residual solid was analyzed as well as a separate aliquot of bulk hair (Tipple et al., 2013).

Samples were submerged in 3 mL of concentrated HNO<sub>3</sub>, capped, and heated on a hot plate overnight. The leachates were uncapped and dried the following day. After drying, 1 mL of concentrated HNO<sub>3</sub> and 100 µL H<sub>2</sub>O<sub>2</sub> were added to each sample. The vials were capped and heated on a hot plate overnight. The samples were uncapped and dried the following day. This process was completed a total of four times, or until the organics were sufficiently digested, as indicated by visual inspection of the surface tension of sample. Samples requiring additional purification were treated with a solution of 250 µL HNO<sub>3</sub> and 750µL 0.1M HCl; a 1:3 ratio of nitric to hydrochloric acid, also known as *aqua regia*, is particularly effective at degrading organic matter. Once digestion was completed, samples were dried down and then reconstituted in 1 mL of 2M HNO<sub>3</sub>. A 100 µL (~10%, gravimetrically determined precisely) aliquot of each sample stock solution was diluted by mass to 3.5 mL using 0.32M HNO<sub>3</sub>. This solution was analyzed for elemental concentrations on the Thermo Fisher Scientific iCAP Q quadrupole inductively-couple plasma mass spectrometry (Q-ICP-MS) with Collision Cell Technology (CCT) option. During each measurement session the tuning parameters were



optimized to maximize sensitivity, signal stability and minimize oxide and doubly charged ion production; this included tuning, mass calibration, cross-calibration and performance reports using the instrument manufacturer specified multi-element solutions. Due to the iCAP Q's improved sensitivity combined with low oxide production ratio compared to previous instrument models, all analytes were run in Kinetic Energy Discrimination (KED) mode. Typical instrument tuning parameters are listed in Appendix I.

An internal standard solution of 200 ppb Sc, Ge, Y, In, and Bi was introduced to all blanks, standards and samples by a Y-connection prior to the nebulizer. Corrections for instrumental drift in sensitivity during the course of the run was made by interpolating between internal standard elements. Standard solutions were multi-element solutions that were custom designed to have similar element ratios to natural samples such as soils. Samples were diluted to fall within the linear calibration curve; if an important element was more than 20% outside of the calibrated range, the sample was re-diluted and reanalyzed. Check standards, designed to be similar in concentration to samples, and blanks were analyzed every six samples; typical precision of check standards was better than 2% over the course of a run, and long-term reproducibility of check standards was better than 5% for most elements. When possible, multiple isotopes for elements were measured to look for potential interferences; the concentration for the isotope with the best detection limit and reproducibility and accuracy for the check standard was used in reporting.

Each batch of samples analyzed also had certified reference materials processed in parallel. For hair residue and leachates, we used an in-house standard from a Phoenix, Arizona, area salon that appeared to be from a single individual based on appearance; no additional information was available. The leaching process requires hair that is not powdered, and there is no internationally available material to our knowledge. For bulk hair, IAEA 086, a powdered hair standard with recommended values for Hg, Fe, Zn and methylmercury was analyzed.

Concentration data from this analysis was used to calculate the required volume of stock solution containing a maximum of 100  $\mu\text{g}$  of Ca for each sample. 100  $\mu\text{g}$  of Ca is the maximum amount of solute that can be added to a 1 mL ion exchange column prior to non-quantitative recovery (Romaniello et al., 2015). Samples with  $< 0.5$  ng of lead were processed through manual ion exchange chromatography micro-columns to purify and separate lead for isotopic measurement. Those results will be presented elsewhere. If samples had sufficient lead and strontium, separate aliquots were processed for each isotopic measurement. If the amount of sample was minimal ( $\text{Pb} < 0.5$  ng,  $\text{Sr} < 5$  ng), then the sample was processed first for Pb, and then for Sr. Lead isotopes are highly sensitive to potential contamination; samples varied widely in their Sr/Pb ratios, so there was concern that processing for strontium first could introduce cross-contamination if samples were first processed through the Prepfast automated column chromatography setup.

The desired stock solution was increased to the final volume of 1mL with titrated 2M  $\text{HNO}_3$ . The automated Prepfast-MC ion exchange chromatography system

(Elemental Scientific, Incorporated, Omaha, NE) was utilized in the purification, with only slight modifications in sample volumes from Romaniello et al. (2015). Each step with its associated chemical reagents is listed in Table 4.

The nitric acid molarities for this chemistry were titrated to within 0.2 molarity units, due to a steep slope in the distribution coefficients with nitric molarity. Unlike traditional manual chromatography, the column is reused for subsequent samples. In order to prevent memory effects from impacting later samples, safeguards included, a) analysis of at least four method blanks per rack of sixty samples, b) analysis of at least three standards with very different isotopic compositions per rack of sixty samples, c) triplicate purification and analysis of 5-10% of samples in randomized order, and d)

Table 4. Ion exchange purification of strontium and calcium (1 mL Eichrom Sr-Ca resin column); modified from Romaniello et al. (2015)

| Step | Purpose               | Volume  | Reagent                                                                          |
|------|-----------------------|---------|----------------------------------------------------------------------------------|
| 1    | Condition column      | 10 mL   | 2 mol kg <sup>-1</sup> HNO <sub>3</sub> + 1% (v/v) H <sub>2</sub> O <sub>2</sub> |
| 2    | Load sample           | 1 mL    | 2 mol kg <sup>-1</sup> HNO <sub>3</sub> + 1% (v/v) H <sub>2</sub> O <sub>2</sub> |
| 3    | Elute matrix elements | 10 mL   | 2 mol kg <sup>-1</sup> HNO <sub>3</sub> + 1% (v/v) H <sub>2</sub> O <sub>2</sub> |
| 4    | Elute Sr and Pb       | 8-10 mL | 6 mol kg <sup>-1</sup> HNO <sub>3</sub>                                          |
| 5    | Elute Ca              | 10 mL   | 12 mol kg <sup>-1</sup> HNO <sub>3</sub>                                         |
| 6    | Elute REEs, Hf, Cd, U | 10 mL   | 1 mol kg <sup>-1</sup> HF                                                        |

replacement of ion exchange resin at least every 200 samples. Method blanks were always less than 110 pg Sr; due to the very low amount of blank material, we were unable to properly characterize the blank isotopically. Hence, we decided to not analyze samples with < 2 ng Sr because we could not accurately assess any potential blank contribution.

Strontium eluates from the Prepfast were in 10 mL of 6 mol kg<sup>-1</sup> HNO<sub>3</sub>. The matrix (steps 2 + 3 in Table 4), strontium (step 4) and calcium (step 5) elutions had a 10% post-chemistry aliquot reserved from each sample to be analyzed for elemental concentrations by Q-ICP-MS. The postchemistry measurements were made in order to determine yield and sample purity from matrix. Yields were particularly important because mass-dependent strontium and calcium isotopes, in addition to radiogenic strontium isotopes, were measured for a subset of samples. Ion exchange processing is well known to produce significant mass-dependent fractionation if recovery is non-quantitative. Poor yield will not impact the accuracy or precision of the radiogenic Sr isotopes, but could bias the mass-dependent isotope values. All Sr yields were determined to be > 88 %, which has been demonstrated to give accurate mass-dependent and radiogenic Sr isotopes (Romaniello et al., 2015). Mass-dependent Sr and Ca data are outside the scope of this study and will be discussed elsewhere.

The remaining 9 mL of each sample Sr eluate were dried down completely and digested overnight in 500 µL concentrated HNO<sub>3</sub> and 200 µL 30% H<sub>2</sub>O<sub>2</sub>. This process was repeated until the desired surface tension was established, for a minimum of twice and a maximum of five times. Once digestion was complete, samples were reconstituted in 0.32 mol kg<sup>-1</sup> HNO<sub>3</sub> for MC-ICP-MS analysis.

Using concentrations measured on the Q-ICP-MS, samples were diluted to 50 ppb strontium, with a minimum 0.5 mL required for analysis. To monitor instrument stability and compensate for instrumental fractionation over the course of analysis, 25 ppb of Zr was added to each sample measured for mass-dependent Sr as well as radiogenic Sr. Zr was not added if only radiogenic Sr was being measured. A portion of the samples (10 %) were run in triplicate in order to calculate instrumental standard deviation and external reproducibility. Measurements of  $^{87}\text{Sr}/^{86}\text{Sr}$  were made using a static multi-collector routine that consisted of 1 block of 60 cycles with an integration time of  $4.194 \text{ sec cycle}^{-1}$  and the same cup configuration as described above. The measured  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were blank-corrected, interference-corrected, and normalized for instrumental mass discrimination using a defined  $^{86}\text{Sr}/^{88}\text{Sr}$  value of 0.1194. Solutions of SRM 987 (National Institute of Standards and Technology; Gaithersburg, MD, USA) of 10 or  $50 \mu\text{g kg}^{-1}$ , with certified  $^{87}\text{Sr}/^{86}\text{Sr}$  value of  $0.71034 \pm 0.00026$ , were analyzed before and after each set of five samples when measuring radiogenic Sr only to verify measurement accuracy and precision. To verify detector linearity, gain calibration was run prior to each measurement session, and a series of standards of varying concentration were measured. If only radiogenic Sr was being measured, the linearity standards were 1, 3, 5, 10 and  $20 \mu\text{g kg}^{-1}$ ; if mass-dependent Sr was also being measured, the linearity standards were 10, 20, 35, 50 and  $65 \mu\text{g kg}^{-1}$ .

The reproducibility of the SRM 987 measurements through the life cycle of the study was  $0.710262 \pm 0.000026$  ( $2\sigma$ ,  $n = 598$ ). The  $^{88}\text{Sr}$  signal intensity and  $^{87}\text{Sr}/^{86}\text{Sr}$  ranges were 0.35 – 40 V and 0.70764 – 0.73544, respectively. Additional standards run in

parallel during this project are detailed in Appendix II.

### **Statistics**

Intake and post-exposure isotope data were compared using paired *t*-tests and plotted with a regression line (Phase I) and independent *t*-tests (Phase II) in SPSS (v.24.0, Armonk, NY, 2016). Phase I and Phase II data were further analyzed using individual growth modeling in SAS (v.9.4, SAS Institute, Cary, NC, 2016) to evaluate the influence of variables including number of days of exposure, placement conditions, and placement location.

Individual growth models are designed for exploring longitudinal data on individuals over time (Singer, 1998). The analysis was conducted using SAS 9.4 Proc Mixed Procedure (SAS Institute, 2016). The model was used to analyze the changes of isotopes affected by the length of exposure in days and exposure methods (surface and burial). The fixed effects were defined as the duration of exposure in days as a continuous variable and the exposure methods as a categorical variable, and their interaction term. The random effects in the model were intercept and the length of exposure. The variables are within-subject, which is each individual donor. This model also controlled for the variation of placement within the four seasons, which were treated as a random blocking factor. Alpha ( $\alpha$ ) was set to 0.05.

The individual growth model was also used to analyze the changes in isotope values affected by the length of exposure and study location (ARF or FARF). The fixed effects were the length of exposure in days as a continuous variable and the location as a categorical variable, and their interaction term. The random effects in the model were

intercept and the length of exposure, which were evaluated within subject. The subject is each individual donor. Alpha ( $\alpha$ ) was set to 0.05.

Kendall's tau coefficient and canonical correlations were also used to evaluate the relationship between isotope systems. If a strong correlation existed between changes across all elements, this would indicate the hair was degrading in bulk. The results of the Kendall's tau only showed a correlation between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ( $p < 0.0001$ ) and  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^2\text{H}$  ( $p = 0.0004$ ). The canonical correlation analysis also revealed a significant relationship between  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^2\text{H}$  ( $p < 0.05$ ). These isotopes systems are independent from one another and controlled by different processes, so it is not advisable to statistically evaluate them as dependent variables. Because the analyses did not reveal a correlation between all isotope ratios, these results are not reported or discussed further in this dissertation.

## **Mapping**

Isotope data from human hair are interpreted with respect to geographic origin by relating the measured isotope ratios to those of drinking water. Precipitation data for the last known residence of each Phase II donor were estimated using the Online Isotopes in Precipitation Calculator (v. 2.2, Bowen, 2017). These estimates were combined with measured isotopes of hair samples and input into a geographic information system (GIS) using ArcMAP (v. 10.4, Redlands, CA). For each of the eight donors from Phase II, isotope ratio maps were generated.  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  values were used from intake samples and final post-exposure samples from each donor. Maps were created by using the measured  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  values from hair samples converted to modeled drinking

water values using models developed by Ehleringer and colleagues (2008) and Podlesak and colleagues (2012). These maps present only those expected geographical regions that are within  $\pm 0.5\%$  of the oxygen isotope ratio measured for the sample and/or  $\pm 0.0001$  of the strontium isotope ratio measured for the sample.

### **Summary**

The Phase I Hair Mat Study was conducted using 38 donors at the ARF in order to determine if there were differences between  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope values from intake and post-exposure hair samples. Phase II Serial Sampling was conducted using six donors at the ARF in Knoxville, Tennessee, and two donors at the FARF in San Marcos, Texas, to further understand the differences observed in Phase I of the study. Phase II followed eight enrolled donors over a period of one year in order to better understand if isotopic changes occur based upon days of exposure, deposition location (Tennessee or Texas), deposition condition (surface or burial), and environmental matrix (soil and precipitation). Phase II results for  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  were used to create maps predicting region of origin, and these results were compared to last known residence of each of the donors. All results from Phase I and Phase II are reported in Chapter Four.



## **CHAPTER FOUR**

### **RESULTS**

The results of the Phase I Hair Mat Study and Phase II Serial Sampling Study are presented here and directly address the research question of whether isotope ratios of human hair change over time when hair is exposed to outdoor decomposition environments. Each isotope analysis of Phase I is addressed first, and  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^2\text{H}$ , and  $\delta^{18}\text{O}$  data are presented for each set of paired intake and post-exposure samples. Ten pairs of samples were also analyzed for  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios. Phase I data are used to evaluate Hypotheses 1-3, specifically whether or not isotopic changes are observed between intake and post-exposure samples. Phase II results are presented thereafter for the longitudinal study of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$  analyses, and address the additional environmental variables of placement condition and placement location outlined in Hypotheses 1-3. Phase II data are also used to address Hypothesis 4 by applying  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  values from hair to statistical geolocation models. These Phase II data are organized by isotope system, as in the Phase I results.

#### **Phase I: Hair Mat Study**

The Phase I study compared 38 paired intake and post-exposure samples to determine if changes in  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$  values of human hair occur during decomposition. This phase of the study examined hair collected from the ARF after as few as 22 days of exposure and up to 1140 days (approximately three years) of exposure.

## Carbon

As discussed in the introduction, carbon isotope ratios of human remains are used to estimate the relative contributions of C<sub>3</sub> and C<sub>4</sub> plants to diet.  $\delta^{13}\text{C}$  values vary with diet, with less negative isotope values typically indicating a greater proportion of corn (maize) and other C<sub>4</sub> plants in the diet.

The  $\delta^{13}\text{C}$  values from intake samples, hair mat samples, and the differences between the two time points are provided in Table 5. Note that a negative difference indicates an increase in  $\delta^{13}\text{C}$  values in the post-exposure samples.

The  $\delta^{13}\text{C}$  values of intake samples averaged  $-16.97 \pm 1.32\text{‰}$  and the post-exposure samples averaged  $-16.98 \pm 1.34\text{‰}$ . The mean for the differences between intake and post-exposure samples is  $+0.01 \pm 0.37\text{‰}$ . The range of isotope differences is between  $+1.24\text{‰}$  to  $-1.10\text{‰}$ .

SPSS (v.24.0, Armonk, NY, 2016) was used to run a paired *t*-test to compare the intake and post-exposure samples, and no significant difference between the two groups was observed ( $p = 0.897$ ). There was no systematic difference in the magnitude or direction of offset with the exposure time or condition (surface placement versus burial), as both increases and decreases of  $\delta^{13}\text{C}$  values were observed between intake and post-exposure hair samples.

Because the values remained relatively consistent between time points, interpretations about diet made from  $\delta^{13}\text{C}$  values of intake samples would not differ from those made using post-exposure sample values.

Table 5.  $\delta^{13}\text{C}$  values of pre-and post-exposure hair samples. Shaded rows indicate burial placements.

| Donor Number | Intake Sample $\delta^{13}\text{C}_{\text{VPDB}}$ (‰) | Post-exposure Sample $\delta^{13}\text{C}_{\text{VPDB}}$ (‰) | Difference Between Samples | Total Days of Exposure |
|--------------|-------------------------------------------------------|--------------------------------------------------------------|----------------------------|------------------------|
| 732          | -17.14                                                | -16.79                                                       | -0.35                      | 373                    |
| 733          | -16.42                                                | -15.97                                                       | -0.45                      | 385                    |
| 735          | -17.99                                                | -17.95                                                       | -0.04                      | 1038                   |
| 736          | -16.57                                                | -16.55                                                       | -0.02                      | 143                    |
| 738          | -12.90                                                | -12.86                                                       | -0.04                      | 385                    |
| 739          | -17.64                                                | -17.64                                                       | 0.00                       | 77                     |
| 741          | -16.75                                                | -16.86                                                       | 0.11                       | 465                    |
| 744          | -18.04                                                | -18.27                                                       | 0.23                       | 734                    |
| 746          | -16.25                                                | -16.32                                                       | 0.07                       | 398                    |
| 748          | -17.60                                                | -17.61                                                       | 0.01                       | 52                     |
| 749          | -14.21                                                | -14.36                                                       | 0.15                       | 825                    |
| 750          | -17.44                                                | -17.87                                                       | 0.43                       | 871                    |
| 752          | -15.34                                                | -16.58                                                       | 1.24                       | 1003                   |
| 753          | -17.23                                                | -16.94                                                       | -0.29                      | 395                    |
| 754          | -16.58                                                | -16.89                                                       | 0.31                       | 419                    |
| 755          | -16.31                                                | -16.38                                                       | 0.07                       | 62                     |
| 756          | -16.81                                                | -17.36                                                       | 0.55                       | 77                     |
| 757          | -16.30                                                | -16.52                                                       | 0.22                       | 569                    |
| 758          | -21.24                                                | -21.31                                                       | 0.07                       | 199                    |
| 759          | -16.36                                                | -16.17                                                       | -0.19                      | 445                    |
| 760          | -17.38                                                | -17.39                                                       | 0.01                       | 22                     |
| 761          | -17.30                                                | -17.17                                                       | -0.13                      | 83                     |
| 767          | -16.15                                                | -16.32                                                       | 0.17                       | 727                    |
| 769          | -16.01                                                | -15.45                                                       | -0.56                      | 380                    |
| 770          | -17.85                                                | -17.51                                                       | -0.34                      | 485                    |
| 743          | -18.16                                                | -17.98                                                       | -0.18                      | 478                    |
| 744          | -18.05                                                | -18.27                                                       | 0.22                       | 734                    |
| 768          | -16.81                                                | -16.87                                                       | 0.06                       | 127                    |
| 762          | -17.92                                                | -17.69                                                       | -0.23                      | 169                    |
| 763          | -17.65                                                | -17.54                                                       | -0.11                      | 175                    |
| 764          | -17.69                                                | -17.80                                                       | 0.11                       | 169                    |
| 765          | -17.59                                                | -16.49                                                       | -1.10                      | 310                    |
| 745          | -17.00                                                | -17.41                                                       | 0.41                       | 445                    |
| 747          | -17.32                                                | -17.46                                                       | 0.14                       | 169                    |
| 742          | -17.36                                                | -17.27                                                       | -0.09                      | 443                    |
| 751          | -17.96                                                | -17.88                                                       | -0.08                      | 952                    |
| 734          | -14.69                                                | -14.20                                                       | -0.49                      | 1140                   |
| 737          | -16.82                                                | -17.23                                                       | 0.41                       | 1021                   |

## Nitrogen

Nitrogen isotope ratios of human remains are used to estimate trophic level, or more broadly the amount and type of protein consumed by an individual. Higher  $\delta^{15}\text{N}$  values suggest a higher trophic level, and shifts in trophic level are typically estimated by differences of about 3-5‰ (Schoeninger and DeNiro, 1984; Sponheimer et al., 2003; Hedges and Reynard, 2007). The  $\delta^{15}\text{N}$  values of intake samples averaged  $8.97 \pm 1.05\text{‰}$  and post-exposure samples averaged  $9.10 \pm 1.03\text{‰}$ . Values for each pair and the differences between them are reported in Table 6. The differences in  $\delta^{15}\text{N}$  values between time points averaged  $+0.12 \pm 0.29\text{‰}$ , while the range was  $-0.46\text{‰}$  to  $+1.14\text{‰}$ . There was no systematic difference in the magnitude or direction of offset with the exposure time or condition (surface placement versus burial). The  $\delta^{15}\text{N}$  values of the paired intake and hair mat samples revealed a significant difference between the two groups (paired  $t$ -test,  $p = 0.013$ ), although the magnitude of the difference was small ( $+0.12\text{‰}$ , on average). Since trophic level interpretations are generally based upon differences of an estimated 3‰, the observed differences would not impact the interpretation of these results in regards to general statements about diet.

## Hydrogen

Hydrogen isotope values are also related to the hydrologic cycle, and the same controls on variation in the  $\delta^{18}\text{O}$  values are also seen in the  $\delta^2\text{H}$  values. Hydrogen isotope intake values are  $-73.5 \pm 5.9\text{‰}$ , while post-exposure values average  $-66.5 \pm 4.6\text{‰}$  (Table 7). While the average difference between intake and post-exposure samples is  $-7.0 \pm 4.9\text{‰}$ , the differences range between a minimum of  $-0.7\text{‰}$  and a maximum of  $-20.4\text{‰}$ .

Table 6.  $\delta^{15}\text{N}$  values of pre-and post-exposure hair samples. Shaded rows indicate burial placements.

| Donor Number | Intake Sample $\delta^{15}\text{N}$ (‰) | Post-exposure Sample $\delta^{15}\text{N}$ (‰) | Difference Between Samples | Total Days of Exposure |
|--------------|-----------------------------------------|------------------------------------------------|----------------------------|------------------------|
| 732          | 9.02                                    | 9.20                                           | 0.18                       | 373                    |
| 733          | 9.66                                    | 9.65                                           | -0.01                      | 385                    |
| 735          | 8.59                                    | 8.66                                           | 0.07                       | 1038                   |
| 736          | 8.41                                    | 8.48                                           | 0.07                       | 143                    |
| 738          | 9.60                                    | 9.57                                           | -0.03                      | 385                    |
| 739          | 8.80                                    | 8.46                                           | -0.34                      | 77                     |
| 741          | 8.67                                    | 9.11                                           | 0.44                       | 465                    |
| 744          | 8.10                                    | 7.85                                           | -0.25                      | 734                    |
| 746          | 12.59                                   | 12.95                                          | 0.36                       | 398                    |
| 748          | 8.63                                    | 8.68                                           | 0.05                       | 52                     |
| 749          | 9.52                                    | 9.81                                           | 0.29                       | 825                    |
| 750          | 9.65                                    | 9.64                                           | -0.01                      | 871                    |
| 752          | 10.15                                   | 9.69                                           | -0.46                      | 1003                   |
| 753          | 8.46                                    | 8.79                                           | 0.33                       | 395                    |
| 754          | 7.45                                    | 8.59                                           | 1.14                       | 419                    |
| 755          | 9.52                                    | 9.36                                           | -0.16                      | 62                     |
| 756          | 9.12                                    | 9.31                                           | 0.19                       | 77                     |
| 757          | 9.21                                    | 9.47                                           | 0.26                       | 569                    |
| 758          | 5.53                                    | 5.81                                           | 0.28                       | 199                    |
| 759          | 9.54                                    | 9.76                                           | 0.22                       | 445                    |
| 760          | 9.45                                    | 9.37                                           | -0.08                      | 22                     |
| 761          | 9.19                                    | 9.22                                           | 0.03                       | 83                     |
| 767          | 8.70                                    | 9.05                                           | 0.35                       | 727                    |
| 769          | 9.21                                    | 9.76                                           | 0.55                       | 380                    |
| 770          | 8.93                                    | 9.14                                           | 0.21                       | 485                    |
| 743          | 9.32                                    | 9.60                                           | 0.28                       | 478                    |
| 744          | 8.02                                    | 7.85                                           | -0.17                      | 734                    |
| 768          | 9.39                                    | 9.20                                           | -0.19                      | 127                    |
| 762          | 8.45                                    | 8.69                                           | 0.24                       | 169                    |
| 763          | 8.58                                    | 8.74                                           | 0.16                       | 175                    |
| 764          | 8.03                                    | 8.15                                           | 0.12                       | 169                    |
| 765          | 8.26                                    | 8.82                                           | 0.56                       | 310                    |
| 745          | 9.16                                    | 9.06                                           | -0.10                      | 445                    |
| 747          | 7.85                                    | 7.68                                           | -0.17                      | 169                    |
| 742          | 9.42                                    | 9.73                                           | 0.31                       | 443                    |
| 751          | 8.71                                    | 8.87                                           | 0.16                       | 952                    |
| 734          | 9.37                                    | 9.39                                           | 0.02                       | 1140                   |
| 737          | 10.64                                   | 10.43                                          | -0.21                      | 1021                   |

Table 7.  $\delta^2\text{H}$  values of pre-and post-exposure hair samples. Shaded rows indicate burial placement.

| Donor Number | Intake Sample $\delta^2\text{H}$ (‰) | Post-exposure Sample $\delta^2\text{H}$ (‰) | Difference Between Samples | Total Days of Exposure |
|--------------|--------------------------------------|---------------------------------------------|----------------------------|------------------------|
| 732          | -82.8                                | -72.8                                       | -10.0                      | 373                    |
| 733          | -73.2                                | -59.8                                       | -13.4                      | 385                    |
| 735          | -72.4                                | -61.9                                       | -10.5                      | 1038                   |
| 736          | -74.3                                | -68.1                                       | -6.2                       | 143                    |
| 738          | -67.9                                | -67.1                                       | -0.7                       | 385                    |
| 739          | -73.2                                | -71.9                                       | -1.3                       | 77                     |
| 741          | -69.4                                | -64.4                                       | -5.0                       | 465                    |
| 744          | -70.4                                | -66.9                                       | -3.5                       | 734                    |
| 746          | -69.9                                | -65.9                                       | -4.0                       | 398                    |
| 748          | -74.4                                | -65.5                                       | -8.9                       | 52                     |
| 749          | -95.6                                | -77.4                                       | -18.2                      | 825                    |
| 750          | -70.2                                | -60.5                                       | -9.7                       | 871                    |
| 752          | -66.7                                | -64.0                                       | -2.7                       | 1003                   |
| 753          | -69.6                                | -68.3                                       | -1.3                       | 395                    |
| 754          | -72.0                                | -66.7                                       | -5.3                       | 419                    |
| 755          | -70.9                                | -66.9                                       | -4.0                       | 62                     |
| 756          | -72.4                                | -69.7                                       | -2.6                       | 77                     |
| 757          | -66.5                                | -61.5                                       | -5.0                       | 569                    |
| 758          | -80.1                                | -76.2                                       | -3.9                       | 199                    |
| 759          | -72.7                                | -60.7                                       | -12.0                      | 445                    |
| 760          | -68.1                                | -60.9                                       | -7.3                       | 22                     |
| 761          | -68.7                                | -65.6                                       | -3.1                       | 83                     |
| 767          | -66.2                                | -60.7                                       | -5.6                       | 727                    |
| 769          | -73.5                                | -63.1                                       | -10.4                      | 380                    |
| 770          | -81.8                                | -69.3                                       | -12.5                      | 485                    |
| 743          | -84.0                                | -63.6                                       | -20.4                      | 478                    |
| 744          | -70.4                                | -66.9                                       | -3.5                       | 734                    |
| 768          | -70.1                                | -68.1                                       | -2.0                       | 127                    |
| 762          | -81.4                                | -76.8                                       | -4.6                       | 169                    |
| 763          | -74.4                                | -67.9                                       | -6.6                       | 175                    |
| 764          | -72.7                                | -72.0                                       | -0.7                       | 169                    |
| 765          | -80.2                                | -67.5                                       | -12.8                      | 310                    |
| 745          | -75.6                                | -62.9                                       | -12.8                      | 445                    |
| 747          | -71.6                                | -69.0                                       | -2.7                       | 169                    |
| 742          | -67.3                                | -61.8                                       | -5.5                       | 443                    |
| 751          | -76.0                                | -67.8                                       | -8.2                       | 952                    |
| 734          | -75.1                                | -60.6                                       | -14.5                      | 1140                   |
| 737          | -70.2                                | -65.0                                       | -5.2                       | 1021                   |

Differences observed in the  $\delta^2\text{H}$  values between intake and post-exposure samples were statistically significant (paired  $t$ -test,  $p < 0.001$ ).

Differences for all pairs trend toward more positive  $\delta^2\text{H}$  values, demonstrating a consistent pattern in the changes observed between intake and post-exposure samples. Note that negative differences indicate an increase in  $\delta^2\text{H}$  values, and all post-exposure samples increased from their paired intake samples. Hydrogen values did exhibit significant differences between intake and post-exposure samples. Both the average intake values and average post-exposure values are greater than the average United States value range of -125 to -77‰ reported by Ehleringer and colleagues (2008). The standard deviation of  $\delta^2\text{H}$  between triplicate samples from the same city in their study was  $\pm 5.2\text{‰}$ . Of the paired values presented here, 19 pairs, or 50% of the data set exhibit differences outside this  $\pm 5.2\text{‰}$  inter-individual variability, meaning the differences are not due to expected variation but are related to changes in  $\delta^2\text{H}$  values that occurred during the exposure period.

## Oxygen

Oxygen isotope ratios from human remains are used for geographic travel history predictions. The  $\delta^{18}\text{O}$  values of intake samples averaged  $13.03 \pm 1.18\text{‰}$  and the post-exposure samples averaged  $13.19 \pm 1.09\text{‰}$ . The average difference is  $+0.16 \pm 0.86\text{‰}$ , while the range is from -1.25‰ to +1.85‰. Values for each pair, and the differences between them, are reported in Table 8.  $\delta^{18}\text{O}$  values from the paired intake samples and hair mat samples were not significantly different (paired  $t$ -test,  $p = 0.267$ ).

Table 8.  $\delta^{18}\text{O}$  isotope values of pre-and post-exposure hair samples. Shaded rows indicate burial placements.

| Donor Number | Intake Sample $\delta^{18}\text{O}$ (‰) | Post-exposure Sample $\delta^{18}\text{O}$ (‰) | Difference Between Samples | Total Days of Exposure |
|--------------|-----------------------------------------|------------------------------------------------|----------------------------|------------------------|
| 732          | 12.44                                   | 13.05                                          | 0.61                       | 373                    |
| 733          | 11.67                                   | 13.14                                          | 1.47                       | 385                    |
| 735          | 13.14                                   | 12.72                                          | -0.42                      | 1038                   |
| 736          | 15.40                                   | 14.69                                          | -0.71                      | 143                    |
| 738          | 11.83                                   | 12.72                                          | 0.89                       | 385                    |
| 739          | 13.69                                   | 13.51                                          | -0.18                      | 77                     |
| 741          | 13.94                                   | 13.06                                          | -0.88                      | 465                    |
| 744          | 12.55                                   | 13.73                                          | 1.18                       | 734                    |
| 746          | 11.63                                   | 12.12                                          | 0.49                       | 398                    |
| 748          | 12.39                                   | 13.05                                          | 0.66                       | 52                     |
| 749          | 12.24                                   | 13.40                                          | 1.16                       | 825                    |
| 750          | 11.40                                   | 13.25                                          | 1.85                       | 871                    |
| 752          | 12.27                                   | 12.38                                          | 0.11                       | 1003                   |
| 753          | 12.37                                   | 12.77                                          | 0.40                       | 395                    |
| 754          | 17.25                                   | 16.22                                          | -1.03                      | 419                    |
| 755          | 12.76                                   | 11.84                                          | -0.92                      | 62                     |
| 756          | 13.91                                   | 14.96                                          | 1.05                       | 77                     |
| 757          | 13.67                                   | 13.75                                          | 0.08                       | 569                    |
| 758          | 12.38                                   | 12.72                                          | 0.34                       | 199                    |
| 759          | 13.91                                   | 12.66                                          | -1.25                      | 445                    |
| 760          | 14.87                                   | 14.55                                          | -0.32                      | 22                     |
| 761          | 13.59                                   | 13.65                                          | 0.06                       | 83                     |
| 767          | 13.10                                   | 12.35                                          | -0.75                      | 727                    |
| 769          | 13.66                                   | 12.59                                          | -1.07                      | 380                    |
| 770          | 12.71                                   | 12.87                                          | 0.16                       | 485                    |
| 743          | 13.03                                   | 13.31                                          | 0.28                       | 478                    |
| 744          | 12.55                                   | 13.73                                          | 1.18                       | 734                    |
| 768          | 14.06                                   | 13.08                                          | -0.98                      | 127                    |
| 762          | 11.61                                   | 10.71                                          | -0.90                      | 169                    |
| 763          | 12.85                                   | 14.41                                          | 1.56                       | 175                    |
| 764          | 12.35                                   | 12.58                                          | 0.23                       | 169                    |
| 765          | 11.21                                   | 10.60                                          | -0.61                      | 310                    |
| 745          | 12.27                                   | 11.85                                          | -0.42                      | 445                    |
| 747          | 13.04                                   | 14.19                                          | 1.15                       | 169                    |
| 742          | 13.61                                   | 14.26                                          | 0.65                       | 443                    |
| 751          | 12.47                                   | 13.53                                          | 1.06                       | 952                    |
| 734          | 13.92                                   | 14.35                                          | 0.43                       | 1140                   |
| 737          | 13.50                                   | 12.84                                          | -0.66                      | 1021                   |



Although there were no significant difference between intake samples and post-exposure samples, it is important to note, that ‘statistically significant’ differences and ‘interpretational significance,’ or the degree of difference that would result in an individual being classified to a different geographic region, are not the same thing. This means there is no ‘magic number’ that indicates whether differences in oxygen values are significant in terms of interpretation for geographic provenance modeling. Ehleringer and colleagues (2008) reported oxygen isotope ratios for hair samples from across the United States ranging between 7.4 and 15.9‰ and the average standard deviation between triplicate samples from the same city was 1.0‰. With exception to Donor 754, all hair samples fell within the previously reported oxygen isotope range for the United States.

Though the average difference between intake and post-exposure samples is under the  $\pm 1\text{‰}$  observed in the Ehleringer et al., 2008 study, there is variability between the magnitude of differences between pairs, and 12 of the 38 pairs, or about 32% of the sample pairs studied, differ by an amount greater than  $\pm 1.0\text{‰}$ . For these pairs, this magnitude of difference could result in a different interpretation of geographic origin for the intake sample value and the post-exposure sample value.

### **Strontium**

Ten pairs of samples were analyzed for  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios, and the results are reported in Table 9. Note that the intake sample from Donor 735 had low strontium concentration, which can impact the accuracy of measured isotope ratios. This sample yielded results relatively consistent with the other samples, though, so results are

Table 9.  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of pre-and post-exposure hair samples (\*indicates low strontium concentration in sample)

| Donor Number | Intake Sample $^{87}\text{Sr}/^{86}\text{Sr}$ | Post-exposure Sample $^{87}\text{Sr}/^{86}\text{Sr}$ | Difference Between Samples | Total Days of Exposure |
|--------------|-----------------------------------------------|------------------------------------------------------|----------------------------|------------------------|
| 735          | 0.70830*                                      | 0.74631                                              | 0.03801                    | 1038                   |
| 745          | 0.70948                                       | 0.73196                                              | 0.02248                    | 445                    |
| 747          | 0.71092                                       | 0.71555                                              | 0.00463                    | 169                    |
| 749          | 0.71078                                       | 0.73208                                              | 0.02130                    | 825                    |
| 770          | 0.70909                                       | 0.72233                                              | 0.01324                    | 485                    |
| 752          | 0.71418                                       | 0.72824                                              | 0.01406                    | 1003                   |
| 753          | 0.71073                                       | 0.74067                                              | 0.02994                    | 395                    |
| 758          | 0.70934                                       | 0.72290                                              | 0.01356                    | 199                    |
| 760          | 0.71616                                       | 0.71407                                              | -0.00209                   | 22                     |
| 761          | 0.70963                                       | 0.72653                                              | 0.01690                    | 83                     |

included in the dataset. Intake values average  $0.71086 \pm 0.00246$ , and post-exposure values average  $0.72806 \pm 0.01021$ . The difference between intake and post-exposure samples is statistically significant (paired *t*-test,  $p < 0.001$ ).

The post-exposure samples show a trend in increasing  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios, with exception of Donor 760. Strontium isotope ratios between the paired samples indicate significant differences that all trend toward higher  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in the post-exposure samples, with differences averaging  $0.01720 \pm 0.01159$ . Beard and Johnson (2000) report that the average ranges of analytical error for the analysis of strontium isotopes are  $\pm 0.00001$  to  $0.00003$ , so differences of the magnitude in these samples is interpreted as a real difference and not within the range of expected analytical error. Only one of the pairs exhibited a lower isotope ratio in the post-exposure sample. Donor 760 showed a

decrease of 0.00209 in the post-exposure sample, which was exposed the least number of days (22) of all the samples.

### **Time**

The changes observed in each isotope system evaluated were plotted with a regression line to determine whether a relationship existed between the changes observed and the number of days of exposure. Scatter plots with regression lines are presented in Figures 1-5. The reported  $R^2$  values indicate there is no statistically significant correlation between the magnitude of changes observed and number of days of exposure. The relationship between time of exposure and changes in isotope ratios over time was further investigated with a more fine-grained analysis of the same donors sampled multiple times over a one-year period in Phase II of the study.

## **Phase II Serial Sampling**

In order to address the environmental variables of Hypotheses 1-3, specifically longitudinal time, placement location, and placement condition, eight (8) additional donors were repeatedly sampled over a period of one year in Phase II of the study to determine if higher frequency sampling for these changes in isotope ratios could constrain the temporal pattern of isotopic variations observed in Phase I. In addition, repeated samples might indicate which parameters are the most important drivers of change. Based upon the results from the paired intake and post-exposure samples in Phase I, it was expected that similar patterns in isotope preservation and change would emerge during Phase II of the study. By following the same donors over a period of one year, it was possible to observe the isotope ratios of hair in surface and burial conditions

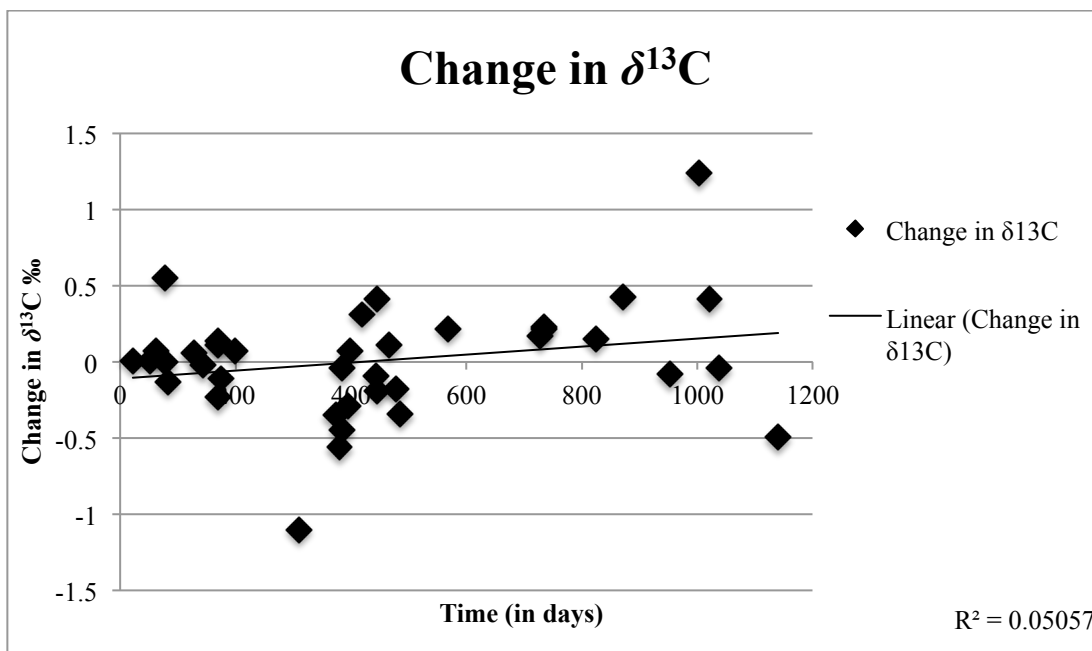


Figure 1. Change in  $\delta^{13}\text{C}$  values over time for Phase I hair samples.  $R^2 = 0.05057$  and indicates no statistically significant relationship between change and exposure time (in days).

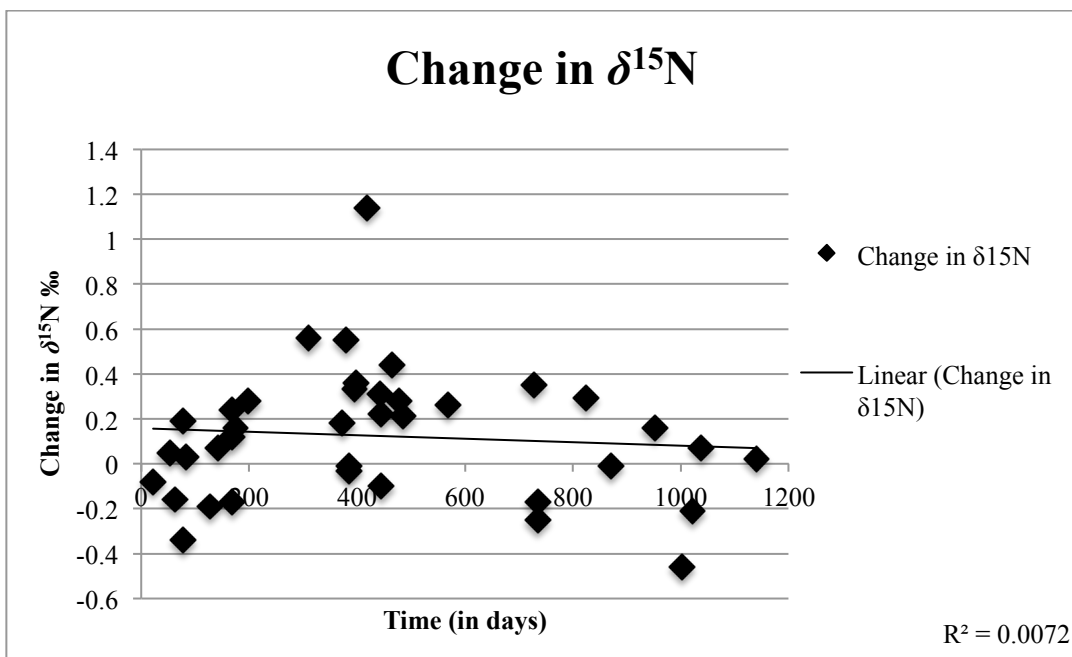


Figure 2. Change in  $\delta^{15}\text{N}$  values over time for Phase I hair samples.  $R^2 = 0.0072$  and indicates no statistically significant relationship between change and exposure time (in days).

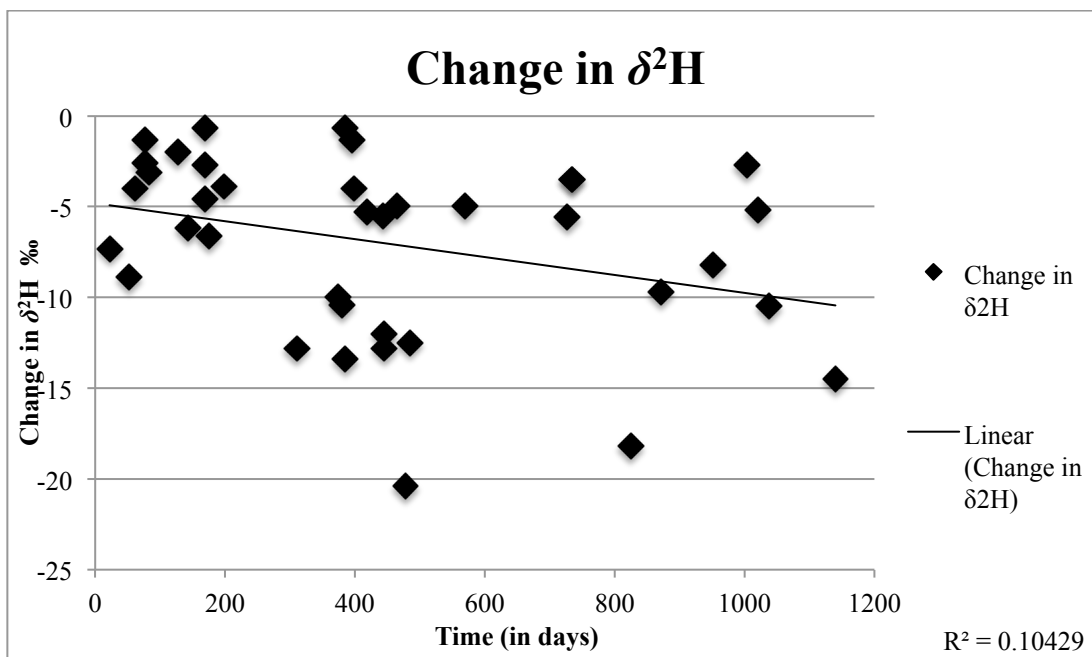


Figure 3. Change in  $\delta^2\text{H}$  values over time for Phase I hair samples.  $R^2 = 0.10429$  and indicates no statistically significant relationship between change and exposure time (in days).

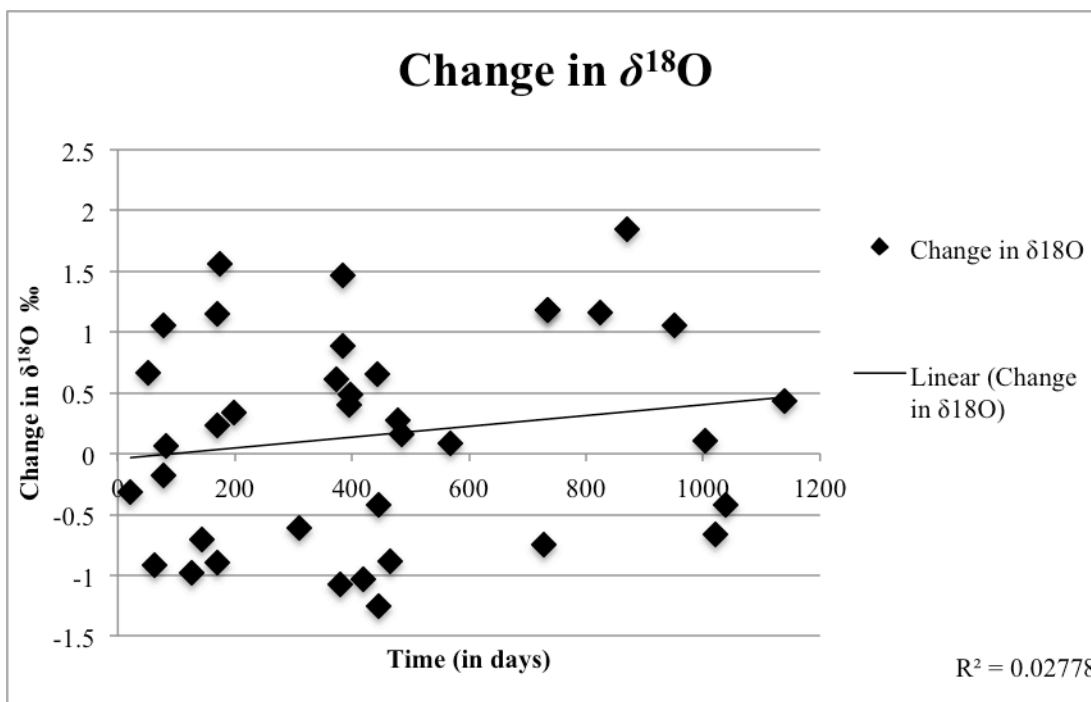


Figure 4. Change in  $\delta^{18}\text{O}$  values over time for Phase I hair samples.  $R^2 = 0.02778$  and indicates no statistically significant relationship between change and exposure time (in days).

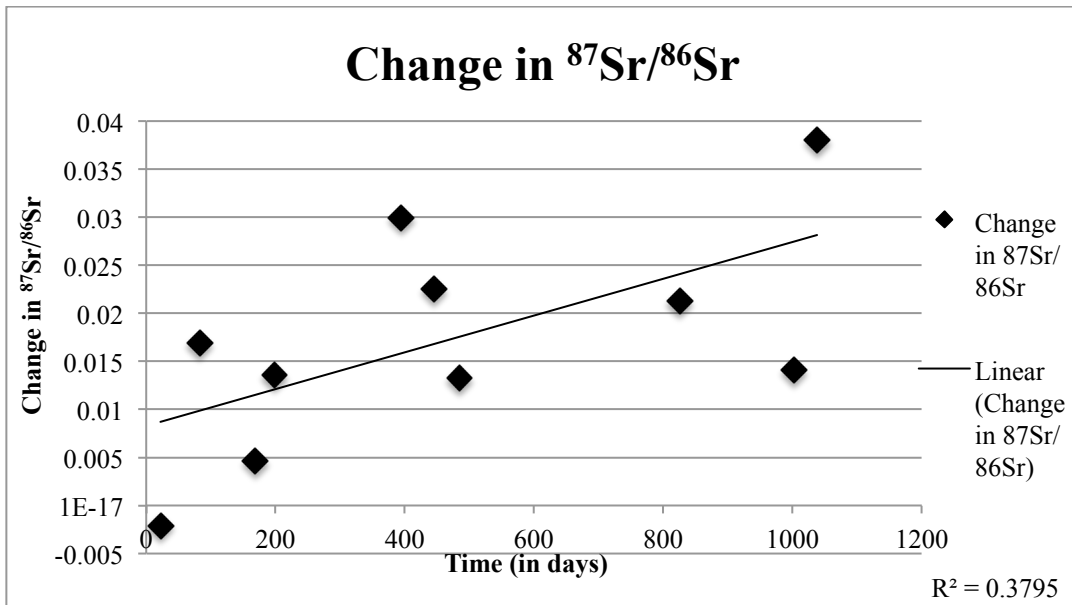


Figure 5. Change in  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios over time for Phase I hair samples.  $R^2 = 0.3795$  and indicates no statistically significant relationship between change and exposure time (in days).



at ARF and surface conditions at FARF for a finer scale analysis.

The five surface donors were sampled daily during the first 10 days of placement, and less frequently thereafter. Surface 1, Surface 2, and Surface 3, as well as Burial 1, Burial 2, and Burial 3 were all placed at the ARF in Knoxville, TN. Surface 4 and Surface 5 were placed at the FARF in San Marcos, TX.  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$  results for each donor at each sampling point are reported below.

### **Carbon**

$\delta^{13}\text{C}$  values for each of the eight donors at each collection interval are reported in Table 10. The overall average  $\delta^{13}\text{C}$  value was  $-15.30 \pm 1.83\text{‰}$ , with individual averages ranging between  $-18.46 \pm 0.18\text{‰}$  and  $-12.64 \pm 0.06\text{‰}$ . Carbon isotope signatures of hair in Phase II followed a similar pattern to the limited temporal variation observed in Phase I. Phase II carbon isotope ratios demonstrated little change over time for each individual. No obvious patterns of change emerge when the data is evaluated overall or by individual donor. Carbon isotope ratios of hair from the burials increased approximately  $0.34 \pm 0.39\text{‰}$ , while carbon isotope ratios of hair from surface donors at ARF changed  $0.00 \pm 0.12\text{‰}$ , and carbon isotope ratios of hair from the surface placements at FARF decreased approximately  $0.08 \pm 0.17\text{‰}$ . This suggests placement location (Tennessee or Texas) and condition (surface or burial) may have a small impact upon the changes seen in  $\delta^{13}\text{C}$  values time. Differences between surface and burial placements ( $p = 0.320$ ) and ARF and FARF placements ( $p = 0.257$ ) were not statistically evident from independent t-tests performed using SPSS (v.24.0, Armonk, NY, 2016). This was further evaluated using

Table 10.  $\delta^{13}\text{C}_{\text{VPDB}}$  values (‰) of hair samples from each donor collected over the entire sampling period.

| <b>DONOR NUMBER</b>                                      | <b>DAYS OF EXPOSURE</b> | <b><math>\delta^{13}\text{C}_{\text{VPDB}}</math> (‰)</b> | <b>Wt %C</b> | <b>%C:%N ratio</b> |
|----------------------------------------------------------|-------------------------|-----------------------------------------------------------|--------------|--------------------|
| Surface 1 (ARF)<br><br>Average and SD<br>-12.64 ± 0.06‰  | 0                       | -12.60                                                    | 43.96        | 2.79               |
|                                                          | 1                       | -12.64                                                    | 43.86        | 2.83               |
|                                                          | 3                       | -12.72                                                    | 43.30        | 2.82               |
|                                                          | 5                       | -12.71                                                    | 43.39        | 2.82               |
|                                                          | 10                      | -12.56                                                    | 43.16        | 2.85               |
|                                                          | 20                      | -12.61                                                    | 42.90        | 2.82               |
|                                                          | 39                      | -12.58                                                    | 44.17        | 2.85               |
|                                                          | 67                      | -12.69                                                    | 42.98        | 2.81               |
|                                                          | 106                     | -12.69                                                    | 43.94        | 2.86               |
|                                                          | 336                     | -12.60                                                    | 43.21        | 2.95               |
| Surface 2 (ARF)<br><br>Average and SD<br>-15.53 ± 0.16‰  | 0                       | -15.49                                                    | 44.41        | 2.80               |
|                                                          | 2                       | -15.60                                                    | 43.02        | 2.85               |
|                                                          | 10                      | -15.67                                                    | 42.62        | 2.77               |
|                                                          | 39                      | -15.48                                                    | 43.81        | 2.74               |
|                                                          | 106                     | -15.75                                                    | 41.58        | 2.83               |
|                                                          | 174                     | -15.30                                                    | 42.40        | 2.92               |
|                                                          | 336                     | -15.40                                                    | 43.05        | 2.94               |
| Surface 3 (ARF)<br><br>Average and SD<br>-15.12 ± 0.10‰  | 0                       | -15.20                                                    | 44.26        | 2.77               |
|                                                          | 1                       | -15.23                                                    | 44.95        | 2.78               |
|                                                          | 2                       | -14.97                                                    | 44.06        | 2.83               |
|                                                          | 5                       | -15.09                                                    | 44.87        | 2.79               |
|                                                          | 34                      | -15.19                                                    | 43.20        | 2.85               |
|                                                          | 101                     | -14.99                                                    | 43.50        | 2.84               |
|                                                          | 169                     | -15.10                                                    | 41.03        | 2.96               |
|                                                          | 331                     | -15.18                                                    | 43.90        | 2.97               |
| Surface 4 (FARF)<br><br>Average and SD<br>-15.07 ± 0.15‰ | 0                       | -15.02                                                    | 44.52        | 2.76               |
|                                                          | 1                       | -15.04                                                    | 42.44        | 2.80               |
|                                                          | 2                       | -15.34                                                    | 41.93        | 2.85               |
|                                                          | 3                       | -14.98                                                    | 42.65        | 2.79               |
|                                                          | 6                       | -14.90                                                    | 42.56        | 2.79               |
|                                                          | 349                     | -15.15                                                    | 40.64        | 2.97               |
| Surface 5 (FARF)<br><br>Average and SD<br>-18.46 ± 0.18‰ | 0                       | -18.38                                                    | 45.72        | 2.79               |
|                                                          | 1                       | -18.29                                                    | 43.45        | 2.76               |
|                                                          | 2                       | -18.53                                                    | 44.29        | 2.80               |
|                                                          | 3                       | -18.33                                                    | 43.44        | 2.77               |
|                                                          | 6                       | -18.44                                                    | 43.99        | 2.78               |
|                                                          | 335                     | -18.78                                                    | 42.21        | 3.00               |
| Burial 1 (ARF)<br>Average and SD<br>-16.51 ± 0.10‰       | 0                       | -16.62                                                    | 45.00        | 2.75               |
|                                                          | 1                       | -16.42                                                    | 45.13        | 2.76               |
|                                                          | 380                     | -16.50                                                    | 42.48        | 2.91               |
| Burial 2 (ARF)<br>Average and SD<br>-16.38 ± 0.08‰       | 0                       | -16.44                                                    | 44.08        | 2.78               |
|                                                          | 380                     | -16.32                                                    | 43.26        | 2.94               |
| Burial 3 (ARF)<br>Average and SD<br>-16.74 ± 0.66‰       | 0                       | -17.20                                                    | 44.32        | 2.79               |
|                                                          | 370                     | -16.27                                                    | 41.92        | 2.93               |

individual growth model statistical modeling, and the results are reported later in this chapter.

### **Nitrogen**

$\delta^{15}\text{N}$  values are reported for each donor in Table 11. Averages for individual donors ranged between  $8.68 \pm 0.28\text{‰}$  and  $9.96 \pm 0.21$  with an overall average  $\delta^{15}\text{N}$  value of  $9.37 \pm 0.59\text{‰}$ . Nitrogen isotope ratios of hair from the serial sampling experiment follow the same trend as the Phase I samples: the magnitude of change is small, though there is a general bias toward increasing values over time. One explanation could be because the lighter nitrogen isotopes are preferentially lost to microbial activity throughout decomposition. Nitrogen isotope ratios follow a similar pattern as carbon, with the ARF burials showing the greatest differences over time, with an average increase of  $0.45 \pm 0.34\text{‰}$ . In comparison, ARF surface donors have an average difference of about  $-0.16 \pm 0.36\text{‰}$ , and FARF surface donors have an average difference of  $-0.24 \pm 0.18\text{‰}$ . No statistical differences between surface and burial placements ( $p = 0.571$ ) and ARF or FARF placements ( $p = 0.448$ ) were observed when the differences were compared with an independent  $t$ -test. There are also no large differences observed between individual donors, indicating a relatively similar diet in terms of amount of protein consumed. As the general interpretation of trophic level from  $\delta^{15}\text{N}$  values is based upon a 3-5‰ difference, each donor would be classified within the same trophic level, whether an intake or post-exposure sample was used. As in Phase I of the study,  $\delta^{15}\text{N}$  values were relatively consistent over the one-year sampling period, and results suggest dietary interpretations from  $\delta^{15}\text{N}$  values of hair are reliable despite decompositional processes.

Table 11.  $\delta^{15}\text{N}_{\text{AIR}}$  values (‰) of hair samples from each donor, collected over the entire sampling period

| DONOR NUMBER     | DAYS OF EXPOSURE | $\delta^{15}\text{N}_{\text{AIR}}$ (‰) | Wt%N  | %C:%N ratio |
|------------------|------------------|----------------------------------------|-------|-------------|
| Surface 1 (ARF)  | 0                | 9.84                                   | 15.75 | 2.79        |
|                  | 1                | 9.81                                   | 15.52 | 2.83        |
| Average and SD   | 3                | 9.67                                   | 15.36 | 2.82        |
| 9.96 ± 0.21‰     | 5                | 9.86                                   | 15.40 | 2.82        |
|                  | 10               | 9.82                                   | 15.17 | 2.85        |
|                  | 20               | 9.74                                   | 15.24 | 2.82        |
|                  | 39               | 9.85                                   | 15.48 | 2.85        |
|                  | 67               | 9.77                                   | 15.29 | 2.81        |
|                  | 106              | 9.83                                   | 15.37 | 2.86        |
|                  | 336              | 10.42                                  | 14.64 | 2.95        |
| Surface 2 (ARF)  | 0                | 9.31                                   | 15.83 | 2.80        |
|                  | 2                | 9.43                                   | 15.10 | 2.85        |
| Average and SD   | 10               | 9.32                                   | 15.37 | 2.77        |
| 9.65 ± 0.44‰     | 39               | 9.24                                   | 15.96 | 2.74        |
|                  | 106              | 9.79                                   | 14.70 | 2.83        |
|                  | 174              | 10.24                                  | 14.52 | 2.92        |
|                  | 336              | 10.24                                  | 14.66 | 2.94        |
| Surface 3 (ARF)  | 0                | 9.34                                   | 15.99 | 2.77        |
|                  | 1                | 9.08                                   | 16.17 | 2.78        |
|                  | 2                | 9.20                                   | 15.57 | 2.83        |
| Average and SD   | 5                | 9.12                                   | 16.09 | 2.79        |
| 9.45 ± 0.35‰     | 34               | 9.41                                   | 15.18 | 2.85        |
|                  | 101              | 9.52                                   | 15.34 | 2.84        |
|                  | 169              | 10.03                                  | 13.86 | 2.96        |
|                  | 331              | 9.91                                   | 14.77 | 2.97        |
| Surface 4 (FARF) | 0                | 9.19                                   | 16.16 | 2.76        |
|                  | 1                | 9.31                                   | 15.15 | 2.80        |
| Average and SD   | 2                | 9.58                                   | 14.71 | 2.85        |
| 9.47 ± 0.23‰     | 3                | 9.48                                   | 15.30 | 2.79        |
|                  | 6                | 9.43                                   | 15.28 | 2.79        |
|                  | 349              | 9.84                                   | 13.69 | 2.97        |
| Surface 5 (FARF) | 0                | 8.13                                   | 16.41 | 2.79        |
|                  | 1                | 8.25                                   | 15.76 | 2.76        |
| Average and SD   | 2                | 8.27                                   | 15.81 | 2.80        |
| 8.39 ± 0.30‰     | 3                | 8.30                                   | 15.68 | 2.77        |
|                  | 6                | 8.42                                   | 15.84 | 2.78        |
|                  | 335              | 8.13                                   | 14.05 | 3.00        |
| Burial 1 (ARF)   | 0                | 8.77                                   | 16.36 | 2.75        |
| Average and SD   | 101              | 8.89                                   | 16.34 | 2.76        |
| 9.12 ± 0.51‰     | 380              | 9.70                                   | 14.59 | 2.91        |
| Burial 2 (ARF)   | 0                | 8.48                                   | 15.87 | 2.78        |
| Average and SD   | 380              | 8.88                                   | 14.73 | 2.94        |
| 8.68 ± 0.28‰     |                  |                                        |       |             |
| Burial 3 (ARF)   | 0                | 8.71                                   | 15.89 | 2.79        |
| Average and SD   | 370              | 9.05                                   | 14.32 | 2.93        |
| 8.88 ± 0.24‰     |                  |                                        |       |             |

## Hydrogen

$\delta^2\text{H}$  values are presented for each donor in Table 12. The overall average  $\delta^2\text{H}$  value was  $-65.1 \pm 4.3\text{‰}$ , with individual averages ranging between  $-74.1 \pm 2.9\text{‰}$  and  $-59.3 \pm 3.4\text{‰}$ . Hydrogen isotope ratios varied more dramatically than any of the other elements, though not in a predictable or systematic way. It has been discussed by previous researchers that hydrogen is highly exchangeable within the environment (Bowen et al., 2005). The results of this study suggest that hydrogen isotope ratio of hair are not useful for predicting geographic travel history, as they can vary dramatically over time within the same individual in an unpredictable way. Oxygen isotope ratios of hair are far more constant over time and should be considered more reliable than hydrogen isotope ratios.

The average  $\delta^2\text{H}$  value decreased by  $1.33 \pm 4.55\text{‰}$  in the ARF surface donors, and increased by  $4.35 \pm 0.47\text{‰}$  in ARF burials. The FARF surface placements had an average  $\delta^2\text{H}$  value increase of about  $2.6 \pm 3.0\text{‰}$ . Independent *t*-tests comparing surface and burial placements ( $p = 0.351$ ) and ARF and FARF placements ( $p = 0.435$ ) did not reveal significant differences.

## Oxygen

$\delta^{18}\text{O}$  values are presented for each donor in Table 13. The overall average  $\delta^{18}\text{O}$  value was  $13.51 \pm 1.08\text{‰}$ , with individual averages ranging between  $12.35 \pm 0.49\text{‰}$  and  $15.07 \pm 0.69\text{‰}$ . The oxygen isotope ratios of hair samples from individual donors throughout the one year sampling period did exhibit more variation than observed in the Phase I samples, but there was no systematic pattern observed: there are both increases and decreases in  $\delta^{18}\text{O}$  values within and between donors over time. Oxygen isotope ratio

Table 12.  $\delta^2\text{H}_{\text{VSMOW}}$  values (‰) of hair samples from each donor, collected over the entire sampling period

| DONOR NUMBER     | DAYS OF EXPOSURE | $\delta^2\text{H}_{\text{VSMOW}}$ (‰) | Wt % H | %O:%H ratio |
|------------------|------------------|---------------------------------------|--------|-------------|
| Surface 1 (ARF)  | 0                | -61.4                                 | 5.58   | 3.85        |
|                  | 1                | -68.7                                 | 5.41   | 3.91        |
|                  | 2                | -66.1                                 | 5.59   | 3.87        |
| Average and SD   | 3                | -64.9                                 | 5.55   | 3.89        |
| -65.3 $\pm$ 1.9‰ | 5                | -67.0                                 | 5.50   | 3.95        |
|                  | 10               | -65.7                                 | 5.57   | 3.95        |
|                  | 20               | -64.9                                 | 5.51   | 3.96        |
|                  | 39               | -64.5                                 | 5.55   | 3.89        |
|                  | 67               | -65.4                                 | 5.36   | 3.94        |
|                  | 174              | -63.2                                 | 5.26   | 3.99        |
|                  | 336              | -66.6                                 | 5.12   | 4.15        |
| Surface 2 (ARF)  | 0                | -64.1                                 | 5.41   | 4.04        |
|                  | 10               | -61.1                                 | 5.14   | 4.15        |
| Average and SD   | 39               | -61.0                                 | 5.27   | 4.06        |
| -59.3 $\pm$ 3.4‰ | 106              | -57.8                                 | 5.27   | 4.17        |
|                  | 174              | -55.8                                 | 5.00   | 4.21        |
|                  | 336              | -55.6                                 | 4.99   | 4.19        |
| Surface 3 (ARF)  | 0                | -60.9                                 | 5.48   | 3.91        |
|                  | 2                | -61.9                                 | 5.42   | 3.98        |
|                  | 5                | -64.5                                 | 5.59   | 3.94        |
| Average and SD   | 34               | -64.1                                 | 5.59   | 3.94        |
| -62.9 $\pm$ 1.7‰ | 101              | -61.1                                 | 5.49   | 3.95        |
|                  | 169              | -65.2                                 | 5.16   | 4.01        |
|                  | 331              | -62.7                                 | 5.15   | 4.06        |
| Surface 4 (FARF) | 0                | -68.6                                 | 6.54   | 3.97        |
| Average and SD   | 1                | -68.5                                 | 5.41   | 3.94        |
| -68.4 $\pm$ 1.3‰ | 2                | -68.2                                 | 4.91   | 3.98        |
|                  | 349              | -65.9                                 | 5.42   | 4.16        |
| Surface 5 (FARF) | 0                | -69.3                                 | 5.55   | 3.86        |
|                  | 1                | -70.1                                 | 5.33   | 3.91        |
| Average and SD   | 2                | -68.1                                 | 5.52   | 3.83        |
| -66.3 $\pm$ 3.4‰ | 3                | -64.5                                 | 5.67   | 3.90        |
|                  | 6                | -65.0                                 | 5.22   | 3.98        |
|                  | 335              | -61.0                                 | 4.70   | 4.25        |
| Burial 1 (ARF)   | 0                | -67.3                                 | 5.53   | 3.93        |
| Average and SD   | 101              | -62.4                                 | 5.40   | 4.00        |
| -64.1 $\pm$ 2.7‰ | 380              | -62.8                                 | 5.18   | 4.10        |
| Burial 2 (ARF)   | 0                | -73.4                                 | 5.50   | 3.90        |
| Average and SD   | 380              | -69.6                                 | 5.08   | 4.10        |
| -71.5 $\pm$ 2.7‰ |                  |                                       |        |             |
| Burial 3 (ARF)   | 0                | -76.2                                 | 5.50   | 3.90        |
| Average and SD   | 370              | -72.0                                 | 4.98   | 4.10        |
| -74.1 $\pm$ 2.9‰ |                  |                                       |        |             |

Table 13.  $\delta^{18}\text{O}_{\text{VSMOW}}$  values (‰) of hair samples from each donor, collected over the entire sampling period

| DONOR NUMBER                                     | DAYS OF EXPOSURE | $\delta^{18}\text{O}_{\text{VSMOW}}$ (‰) | Wt % O | %O:%H ratio |
|--------------------------------------------------|------------------|------------------------------------------|--------|-------------|
| Surface 1<br><br>Average and SD<br>13.20 ± 0.63‰ | 0                | 12.11                                    | 21.47  | 3.85        |
|                                                  | 1                | 12.68                                    | 21.17  | 3.91        |
|                                                  | 2                | 13.37                                    | 21.64  | 3.87        |
|                                                  | 3                | 14.09                                    | 21.58  | 3.89        |
|                                                  | 5                | 12.66                                    | 21.69  | 3.95        |
|                                                  | 10               | 14.03                                    | 21.98  | 3.95        |
|                                                  | 20               | 13.98                                    | 21.80  | 3.96        |
|                                                  | 39               | 13.32                                    | 21.58  | 3.89        |
|                                                  | 67               | 12.90                                    | 21.11  | 3.94        |
|                                                  | 174              | 12.96                                    | 20.98  | 3.99        |
|                                                  | 336              | 13.12                                    | 21.26  | 4.15        |
| Surface 2<br><br>Average and SD<br>12.64 ± 0.91‰ | 0                | 11.53                                    | 21.87  | 4.04        |
|                                                  | 10               | 11.63                                    | 21.31  | 4.15        |
|                                                  | 39               | 12.43                                    | 21.38  | 4.06        |
|                                                  | 106              | 13.48                                    | 21.96  | 4.17        |
|                                                  | 174              | 13.29                                    | 21.08  | 4.21        |
|                                                  | 336              | 13.49                                    | 20.93  | 4.19        |
| Surface 3<br><br>Average and SD<br>13.17 ± 0.74‰ | 0                | 13.54                                    | 21.45  | 3.91        |
|                                                  | 2                | 12.24                                    | 21.60  | 3.98        |
|                                                  | 5                | 14.48                                    | 22.04  | 3.94        |
|                                                  | 34               | 12.50                                    | 21.99  | 3.94        |
|                                                  | 101              | 12.91                                    | 21.68  | 3.95        |
|                                                  | 169              | 13.15                                    | 20.65  | 4.01        |
|                                                  | 331              | 13.35                                    | 20.89  | 4.06        |
| Surface 4<br><br>Average and SD<br>15.07 ± 0.69‰ | 0                | 14.15                                    | 25.97  | 3.97        |
|                                                  | 1                | 15.10                                    | 21.33  | 3.94        |
|                                                  | 2                | 15.81                                    | 19.52  | 3.98        |
|                                                  | 349              | 15.24                                    | 22.55  | 4.16        |
| Surface 5<br><br>Average and SD<br>14.74 ± 0.30‰ | 0                | 15.00                                    | 21.39  | 3.86        |
|                                                  | 1                | 14.69                                    | 20.85  | 3.91        |
|                                                  | 2                | 14.85                                    | 21.17  | 3.83        |
|                                                  | 3                | 14.59                                    | 22.10  | 3.90        |
|                                                  | 6                | 15.07                                    | 20.75  | 3.98        |
|                                                  | 335              | 14.24                                    | 19.97  | 4.25        |
| Burial 1<br><br>Average and SD<br>14.19 ± 0.77‰  | 0                | 14.57                                    | 21.71  | 3.93        |
|                                                  | 101              | 13.30                                    | 21.59  | 4.00        |
|                                                  | 380              | 14.70                                    | 21.24  | 4.10        |
| Burial 2<br><br>Average and SD<br>12.35 ± 0.49‰  | 0                | 12.00                                    | 21.60  | 3.90        |
|                                                  | 380              | 12.70                                    | 20.80  | 4.10        |
| Burial 3<br><br>Average and SD<br>12.42 ± 0.25‰  | 0                | 12.60                                    | 21.60  | 3.90        |
|                                                  | 370              | 12.25                                    | 20.41  | 4.10        |

changes over time occurred in greater amounts in the ARF surface placements. The  $\delta^{18}\text{O}$  values of ARF surface placements had an average increase of  $0.77 \pm 1.02\text{‰}$ , larger than the average increased of about  $0.27 \pm 0.86\text{‰}$  observed for the FARF surface placements. ARF burials had a decreased in  $\delta^{18}\text{O}$  values over time of about  $0.20 \pm 0.83\text{‰}$ . An independent *t*-test comparing differences between placement conditions ( $p = 0.467$ ) and between placement locations ( $p = 0.389$ ) found no significant differences.

Again, it is important to remember that ‘significance,’ while statistically defined, may not reflect the true amount of difference that can impact interpretation and geographic provenance predictions for individuals. The impact of differences in oxygen values on geographic origin predictions will be discussed later in this chapter.

Precipitation is composed of hydrogen and oxygen: and the ARF and FARF differ in estimated precipitation isotope ratios, as seen in Table 14. It is reasonable to infer that precipitation during the sampling period may have had an impact upon isotope ratios of hair over time. Additionally, surface versus burial placement conditions may also impact the changes in  $\delta^2\text{H}$  values over time. This possibility was statistically evaluated using individual growth modeling, and is discussed later in the chapter.

### **Strontium**

$^{87}\text{Sr}/^{86}\text{Sr}$  ratios for each of the eight donors at each collection interval are reported in Table 15. The overall average of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio was  $0.71388 \pm 0.00525$ , with individual averages ranging between  $0.70974 \pm 0.00066$  and  $0.71738 \pm 0.00676$ .  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of hair from the individual donors changed over time with a general trend toward increasing values over time. When the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of hair samples are compared to the  $^{87}\text{Sr}/^{86}\text{Sr}$



ratio of the soil matrix that the samples were exposed to, it appears that the increasing  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio may be influenced by the soil over time (Table 16). The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios cannot reveal whether these increasing values are due to isotopic exchange occurring within the hair that is truly altering the strontium values, or whether the soil matrix is further contaminating the hair over time. Either soil particulates are contaminating the samples, or dissolved soil components are being absorbed by the hair.

The methodology used for sample preparation is the most widely accepted method for minimizing soil contaminants (Tipple et al., 2013). The values reported are for residues whenever possible. The currently accepted preparation protocols, while the best available for avoiding contaminants, are perhaps not sufficient for removing soil contributions from hair samples.

Table 14. Estimated  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values and 95% confidence intervals for precipitation in Knoxville, TN and San Marcos, TX. (Bowen, 2017; Bowen and Revenaugh, 2003)

|                                  | Knoxville, TN | San Marcos, TX |
|----------------------------------|---------------|----------------|
| $\delta^2\text{H}$ (‰, VSMOW)    | -57           | -32            |
| $\delta^2\text{H}$ 95% CI (‰)    | 4             | 14             |
| $\delta^{18}\text{O}$ (‰, VSMOW) | -8.8          | -4.4           |
| $\delta^{18}\text{O}$ 95% CI (‰) | 0.5           | 1.4            |

Table 15.  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of hair samples from each donor, collected over the entire sampling period. Note that the Day 0 sample for Burial 3 did not yield measurable strontium. Asterisks (\*) indicate reporting of bulk samples, as hair digests did not yield sufficient strontium concentrations for measurement. All other reported values are from hair digests.

| DONOR NUMBER          | DAYS OF EXPOSURE | $^{87}\text{Sr}/^{86}\text{Sr}$ |
|-----------------------|------------------|---------------------------------|
| Surface 1 (ARF)       | 0                | 0.70916                         |
|                       | 5                | 0.71172                         |
|                       | 39               | 0.71214                         |
| Average and SD        | 67               | 0.71624                         |
| $0.71343 \pm 0.00326$ | 105              | 0.71184                         |
|                       | 174              | 0.71795                         |
|                       | 336              | 0.71498                         |
| Surface 2 (ARF)       | 0                | 0.70906                         |
|                       | 2                | 0.71056                         |
| Average and SD        | 10               | 0.71511                         |
| $0.71738 \pm 0.00676$ | 106              | 0.72407                         |
|                       | 174              | 0.72109                         |
|                       | 336              | 0.72441                         |
| Surface 3 (ARF)       | 0                | 0.71391                         |
|                       | 1                | 0.71421                         |
| Average and SD        | 5                | 0.71411                         |
| $0.71448 \pm 0.00121$ | 34               | 0.71437                         |
|                       | 101              | 0.71671                         |
|                       | 169              | 0.71284                         |
|                       | 331              | 0.71521                         |
| Surface 4 (FARF)      | 0                | 0.70903                         |
|                       | 1                | *0.70996                        |
| Average and SD        | 2                | *0.71008                        |
| $0.70974 \pm 0.00066$ | 3                | *0.71079                        |
|                       | 6                | 0.70919                         |
|                       | 349              | 0.70940                         |
| Surface 5 (FARF)      | 0                | 0.71004                         |
|                       | 1                | 0.71034                         |
| Average and SD        | 2                | 0.71043                         |
| $0.71056 \pm 0.00047$ | 3                | 0.71036                         |
|                       | 6                | *0.71079                        |
|                       | 335              | 0.71138                         |
| Burial 1 (ARF)        | 0                | 0.71226                         |
| Average and SD        | 101              | 0.71592                         |
| $0.71350 \pm 0.01246$ | 380              | 0.73544                         |
| Burial 2 (ARF)        | 0                | 0.71206                         |
| Average and SD        | 380              | 0.71506                         |
| $0.71350 \pm 0.00220$ |                  |                                 |
| Burial 3 (ARF)        | 370              | 0.71517                         |

Table 16.  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$  values from soil samples taken at placement sites for Phase II Donors.

| Location  | $\delta^{13}\text{C}_{\text{VPDB}} (\text{‰})$ | $\delta^{15}\text{N}_{\text{AIR}} (\text{‰})$ | $^{87}\text{Sr}/^{86}\text{Sr}$ |
|-----------|------------------------------------------------|-----------------------------------------------|---------------------------------|
| Surface 1 | -26.96                                         | 4.75                                          | 0.71291                         |
| Surface 2 | -27.37                                         | 2.38                                          | 0.71276                         |
| Surface 3 | -27.29                                         | 2.76                                          | 0.71241                         |
| Surface 4 | -17.2                                          | 4.66                                          | 0.70906                         |
| Surface 5 | -19.42                                         | 4.36                                          | 0.70942                         |
| Burial 1  | -25.92                                         | 4.36                                          | 0.71231                         |
| Burial 2  | -24.35                                         | 6.43                                          | 0.71511                         |

Though no significant differences were revealed via independent *t*-test for placement condition ( $p = 0.587$ ) or placement location ( $p = 0.249$ ), some general observations were made. The ARF burials saw a greater increase in  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios, with an average of  $0.00995 \pm 0.01147$ , as compared to ARF surface placements, which had an average increase of about  $0.00493 \pm 0.00516$ ; FARF surface placements had an average increase over time of  $0.00074 \pm 0.0005$ . All  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios increased over time, but the largest increase was observed for the ARF burials. As previously discussed, this could be due, in part, to soil contaminants. Burial samples were completely covered in soil for the duration of the project. Average  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of soil at the ARF ( $0.71310 \pm 0.00115$ ) are higher than those from the FARF ( $0.70924 \pm 0.00025$ ), so the degree of change may also be reflective of the difference in soil values seen between the two facilities, with ARF samples increasing more, on average, than FARF samples. Because the offsets between ARF hair and soil samples are larger than the offset between hair and FARF soil samples, it is not surprising that ARF changes are larger.

### Statistical Modeling of Time, Location, and Placement Condition

Independent *t*-tests were used to compare surface and burial differences and ARF and FARF differences, but this comparison of mean differences did not include the variable of exposure time combined with placement condition or location. In order to evaluate the effects of time (in days of exposure), placement location (Tennessee or Texas), and placement conditions (surface or burial), as possible variables affecting isotope values, individual growth modeling was used. This type of analysis is used to explore longitudinal data for individuals over time.

The first model was used to analyze the changes in isotope ratios affected by the duration of exposure in days and placement condition (surface and burial). The results of this analysis are reported in Table 17. The fixed effects were defined as the duration of exposure in days as a continuous variable and placement condition as a categorical variable, and their interaction term. The random effects in the model were intercept and the duration of exposure. They are within-subject variables, and each individual donor is a subject. This model also controlled for the variation of placement within the four seasons, which was treated as a random blocking factor. Both Phase I and Phase II donors were used for this analysis, but FARF donors were excluded, as there were no burial placements there. The results of this individual growth model are summarized in Table 18 and reveal significant effects of exposure duration upon  $\delta^{15}\text{N}$  values ( $p = 0.009$ ),  $\delta^2\text{H}$  values ( $p < 0.001$ ), and  $^{87}\text{Sr}/^{86}\text{Sr}$  ( $p = 0.003$ ). Only  $\delta^2\text{H}$  values are significantly affected by placement condition of surface versus burial. It is somewhat surprising that placement condition does not have a significant effect upon  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios; however, there were

Table 17. Individual growth model evaluating the effects of duration of exposure (in days) and placement condition (surface or burial). ( $\alpha=0.05$ )

| $\delta^{13}\text{C}$           |         |                   |
|---------------------------------|---------|-------------------|
|                                 | F-Value | Pr > F            |
| Days of Exposure                | 2.23    | 0.1434            |
| Surface vs. Burial              | 0.01    | 0.9434            |
| Days*Placement                  | 0.64    | 0.4319            |
| $\delta^{15}\text{N}$           |         |                   |
|                                 | F-Value | Pr > F            |
| Days of Exposure                | 13.11   | <b>0.009</b>      |
| Surface vs. Burial              | 0.12    | 0.7313            |
| Days*Placement                  | 0       | 0.9936            |
| $\delta^2\text{H}$              |         |                   |
|                                 | F-Value | Pr > F            |
| Days of Exposure                | 57.06   | <b>&lt;0.0001</b> |
| Surface vs. Burial              | 4.62    | <b>0.0427</b>     |
| $\delta^{18}\text{O}$           |         |                   |
|                                 | F-Value | Pr > F            |
| Days of Exposure                | 2.10    | 0.1586            |
| Surface vs. Burial              | 0.32    | 0.5800            |
| Days*Placement                  | 0.01    | 0.9477            |
| $^{87}\text{Sr}/^{86}\text{Sr}$ |         |                   |
|                                 | F-Value | Pr > F            |
| Days of Exposure                | 13.15   | <b>0.0031</b>     |
| Surface vs. Burial              | 0.15    | 0.7029            |
| Days*Placement                  | 0.24    | 0.6295            |

Table 18. Summary of individual growth models and statistically significant variables. Note, \*\*\* is  $p < 0.001$ , \*\* is  $0.1 > p > 0.001$ , and \* is  $p < 0.05$ .

| Isotope                         | Factors tested in the model                              |
|---------------------------------|----------------------------------------------------------|
| $\delta^{13}\text{C}$           | Days of Exposure, Surface vs. Burial, DaysPlacement      |
| $\delta^{15}\text{N}$           | Days of Exposure**, Surface vs. Burial, DaysPlacement    |
| $\delta^2\text{H}$              | Days of Exposure***, Surface vs. Burial**, DaysPlacement |
| $\delta^{18}\text{O}$           | Days of Exposure, Surface vs. Burial, DaysPlacement      |
| $^{87}\text{Sr}/^{86}\text{Sr}$ | Days of Exposure**, Surface vs. Burial, DaysPlacement    |

only three donors in burial placements with  $^{87}\text{Sr}/^{86}\text{Sr}$  analyses performed and Burial 3 did not have an intake sample for comparison, so the low sample size may have impacted this portion of the statistical analysis.

A second individual growth model was used to analyze the changes in isotope ratios affected by the duration of exposure and location (i.e., ARF or FARF). Results are reported in Table 19. The fixed effects were the duration of exposure in days as a continuous variable and the location as a categorical variable, and their interaction term. The random effects in the model were intercept and the duration of exposure, and the subject is each individual donor. Surface donors from the Phase II study were used for this analysis. Burial donors were excluded, as there were no burial placements at FARF. The results of the model are summarized in Table 20. In this model,  $\delta^{13}\text{C}$  values show effects from a combination of both days of exposure and location ( $p = 0.002$ ). This means that  $\delta^{13}\text{C}$  values are impacted by the number of days of exposure, but the degree of change is also influenced by the location of the placement.  $\delta^{15}\text{N}$  values are again affected by the exposure time ( $p = 0.002$ ), but are not affected by location.

Table 19. Individual growth model evaluating duration of exposure (in days) and placement location (ARF or FARF). ( $\alpha=0.05$ )

| $\delta^{13}\text{C}$           |         |                   |
|---------------------------------|---------|-------------------|
|                                 | F-Value | Pr > F            |
| Location (ARF or FARF)          | 1.76    | 0.1927            |
| Days of Exposure                | 0.84    | 0.3643            |
| Days*Location                   | 10.93   | <b>0.0021</b>     |
| $\delta^{15}\text{N}$           |         |                   |
|                                 | F-Value | Pr > F            |
| Location (ARF or FARF)          | 1.92    | 0.1769            |
| Days of Exposure                | 50.66   | <b>0.0057</b>     |
| Days*Location                   | 1.77    | 0.1943            |
| $\delta^2\text{H}$              |         |                   |
|                                 | F-Value | Pr > F            |
| Location (ARF or FARF)          | 7.74    | <b>0.0088</b>     |
| Days of Exposure                | 2.30    | 0.2432            |
| Days*Location                   | 0.21    | 0.6141            |
| $\delta^{18}\text{O}$           |         |                   |
|                                 | F-Value | Pr > F            |
| Location (ARF or FARF)          | 55.12   | <b>&lt;0.0001</b> |
| Days of Exposure                | 0.57    | 0.5643            |
| Days*Location                   | 1.48    | 0.2746            |
| $^{87}\text{Sr}/^{86}\text{Sr}$ |         |                   |
|                                 | F-Value | Pr > F            |
| Location (ARF or FARF)          | 8.85    | <b>0.0070</b>     |
| Days of Exposure                | 1.76    | 0.2762            |
| Days*Location                   | 1.46    | 0.2395            |

Table 20. Summary of individual growth models and statistically significant variables. Note, \*\*\* is  $p < 0.001$ , \*\* is  $0.1 > p > 0.001$ , and \* is  $p < 0.05$ .

| Isotope                         | Factors tested in the model                              |
|---------------------------------|----------------------------------------------------------|
| $\delta^{13}\text{C}$           | Location of Placement, Days of Exposure, DaysLocation**  |
| $\delta^{15}\text{N}$           | Location of Placement, Days of Exposure**, DaysLocation  |
| $\delta^2\text{H}$              | Location of Placement**, Days of Exposure, DaysLocation  |
| $\delta^{18}\text{O}$           | Location of Placement***, Days of Exposure, DaysLocation |
| $^{87}\text{Sr}/^{86}\text{Sr}$ | Location of Placement**, Days of Exposure, DaysLocation  |

Not surprisingly, location of placement significantly affects  $\delta^2\text{H}$  values ( $p = 0.0088$ ) and  $\delta^{18}\text{O}$  values ( $p < 0.0001$ ). Both  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  are used for geographic interpretations and directly related to precipitation values. It is reasonable to conclude that the changes seen in  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  would differ between locations because the potential source of those changes (precipitation) differs between the two locations of placement.

$^{87}\text{Sr}/^{86}\text{Sr}$  ratios are significantly affected by location ( $p = 0.0030$ ), but not days of exposure. The soil values of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of soil from ARF and FARF are very different, with ARF soil ( $0.71310 \pm 0.00115$ ) having a much higher average  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio than FARF soil ( $0.70924 \pm 0.00025$ ). If soil contaminants are impacting  $^{87}\text{Sr}/^{86}\text{Sr}$  values from post-exposure hair samples, then location, and thereby the soils to which the donors were exposed in that specific location, would certainly play an important role in the changes seen in  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios.

These statistical models were created to evaluate the variables of time in days of exposure, placement condition of surface or burial, and placement location at ARF or FARF and the influence these variables may have upon the changes seen in isotope ratios



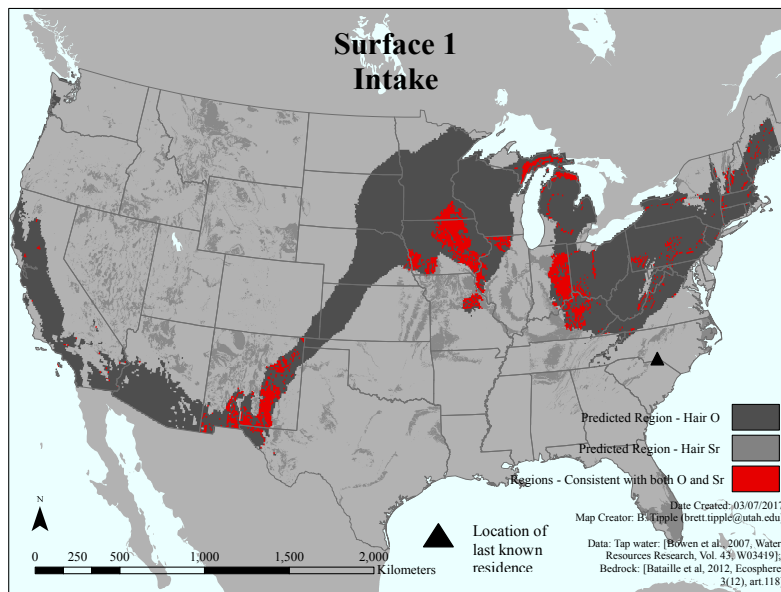
of human hair after environmental exposure. While the models are not comprehensive and do not fully explain the observed changes in isotope ratios over time, they provide a method for considering the possible influences of environmental factors in placement locations. Not surprisingly, the models demonstrate that different isotope systems are impacted by different environmental factors, and each isotope system must be evaluated independently in order to understand the factors that may influence changes in isotope ratios of human hair that has been exposed to an outdoor decomposition environment.

### **Geographic Residence Predictions**

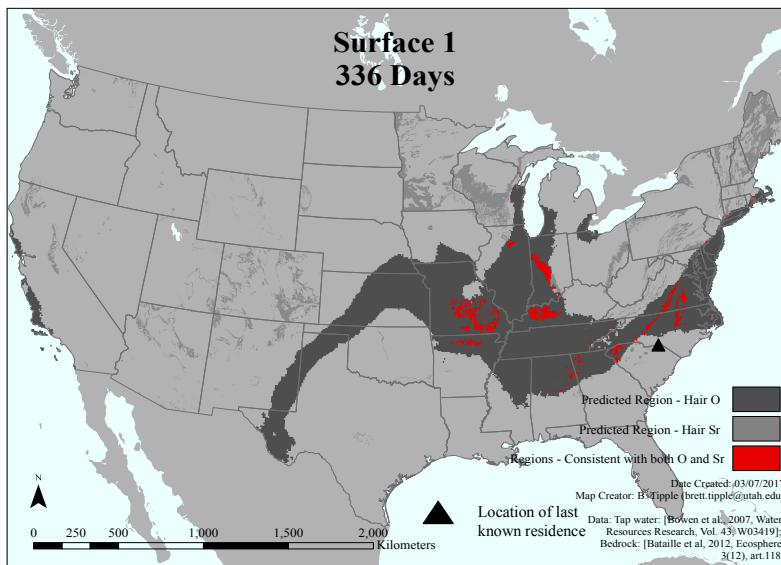
Hypothesis 4 states that it is expected that both intake and post-exposure  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  values will accurately predict last known residence of the donors in Phase II. This hypothesis was tested by applying the oxygen and strontium ratios of hair from each donor to a statistical model. In order to predict geographic travel history, isotope ratio data of human hair were interpreted by relating the measured isotope ratios to those of drinking water. This was accomplished by relating measured isotope ratios of the hair to drinking water into a geographical information system (GIS) using ArcMAP (v. 10.4, Redlands, CA). The isotope ratio maps generated represent only those expected geographical regions that are within  $\pm 0.5\%$  of the oxygen isotope ratio measured for the sample and/or  $\pm 0.0001$  of the strontium isotope ratio measured for the sample. Maps were created by using the known  $\delta^{18}\text{O}$  values of hair samples converted to modeled drinking water values using equations developed by Ehleringer and colleagues (2008) and Podlesak and colleagues (2012). Maps of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were created assuming bedrock and hair have a one-to-one relationship. These drinking water values were

applied to maps from tap water models (Bowen et al., 2007) and strontium bedrock maps (Bataille et al., 2012). Maps representing the intake and post-exposure  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  values of each donor from Phase II of the study are shown below in Figures 6-13. The locations reported fall within a 95% confidence interval of the models used.

When the last known residence was plotted against the predicted regions of origin, 13 of the 16 maps had predicted regions where both the strontium and oxygen isotope ratios fell within 250 km (approximately 155 miles) of the known locations (Table 21). Predicted regions generated using strontium isotope ratios fell within 250 km of the known locations 100% of the time. (Note that Burial 3 did not have  $^{87}\text{Sr}/^{86}\text{Sr}$  data from the intake hair sample.) Predictions based on oxygen isotope ratios were less accurate, only yielding correct predictions for 13 of the 16 maps, or 81% of the time. Ehleringer and colleagues (2008), utilizing  $\delta^{18}\text{O}$  values of human hair collected from salons across the continental USA, stated that, “the model, which predicts hair isotopic composition as a function of drinking water, bulk diet, and dietary protein isotope ratios, explains >85% of the observed variation and reproduces the observed slopes relating the isotopic composition of hair samples to that of local drinking water.” With this in mind, the accuracy of predictions made from measured oxygen isotope ratios in this study is comparable to the variation observed by Ehleringer et al., 2008. When the predicted regions for  $\delta^{18}\text{O}$  values and  $^{87}\text{Sr}/^{86}\text{Sr}$  values were combined, 12 out of 15 maps or 80% of the maps predicted last known residence within 250 kilometers.

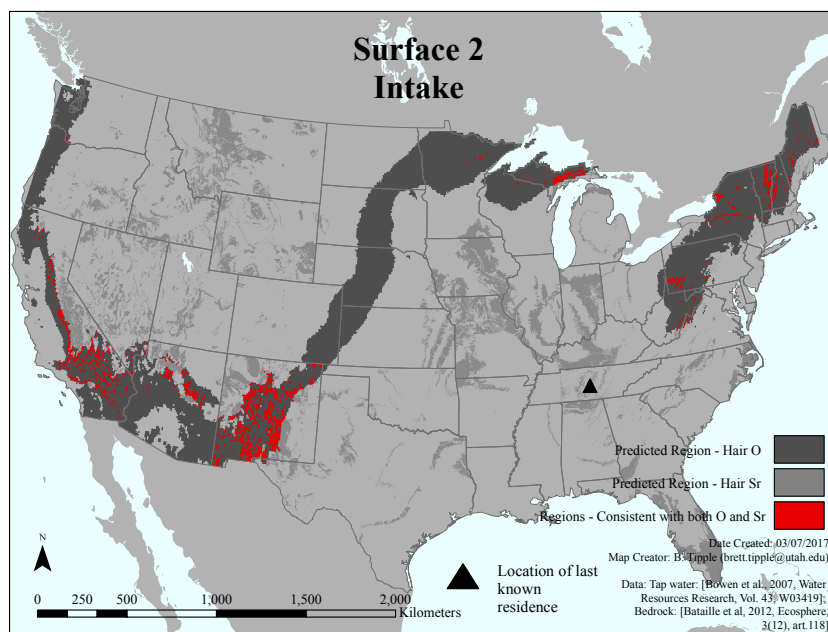


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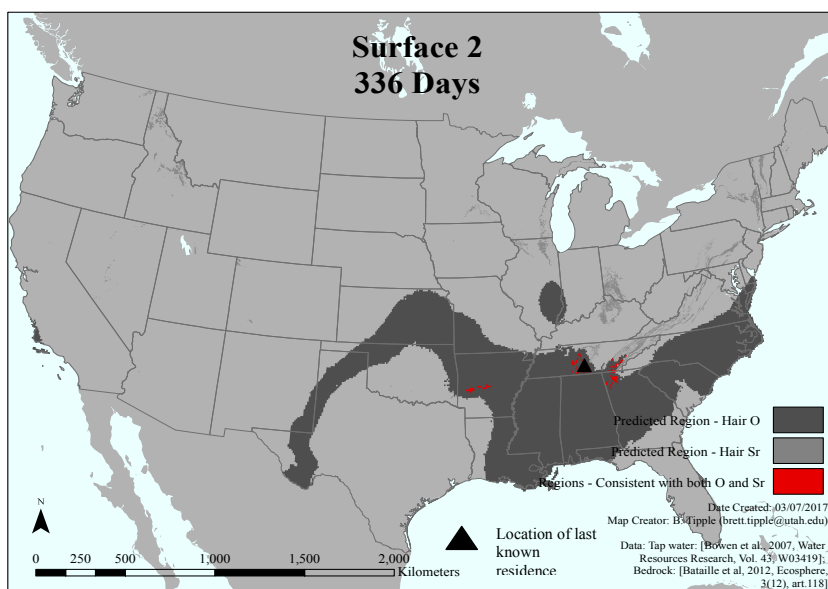


B

Figure 6. Geographic origin predictions from  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  data for Surface 1 Donor. Intake hair sample values are represented by A and post-exposure samples collected on day 336 are represented in B. Last known residence is Rock Hill, SC, designated by the black triangle on the map.

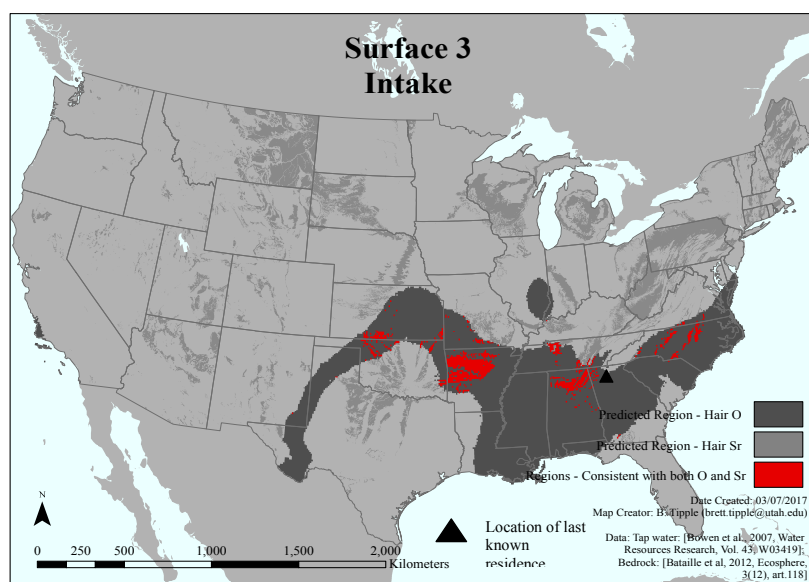


A

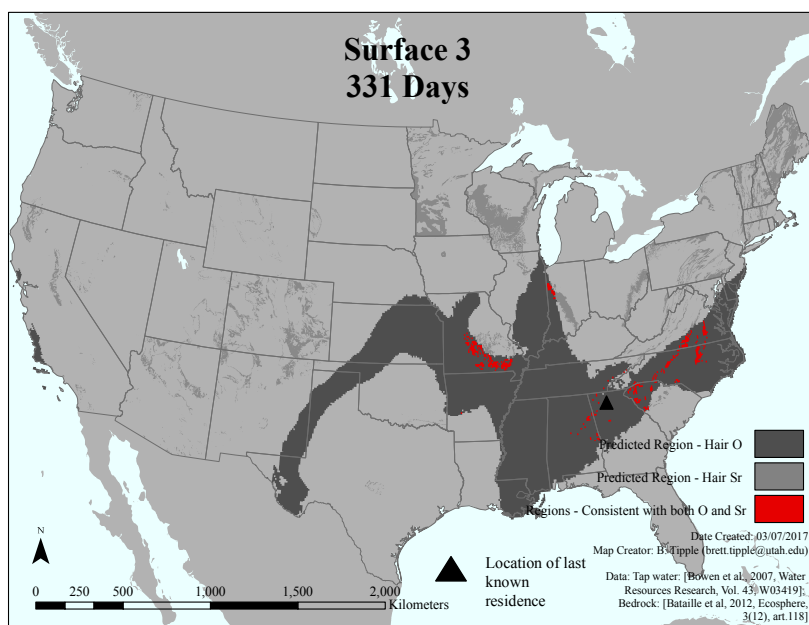


B

Figure 7. Geographic origin predictions from  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  data for Surface 2 Donor. Intake hair sample values are represented by A and post-exposure samples collected on day 336 are represented in B. Last known residence is Fayetteville, TN, designated by the black triangle on the map.

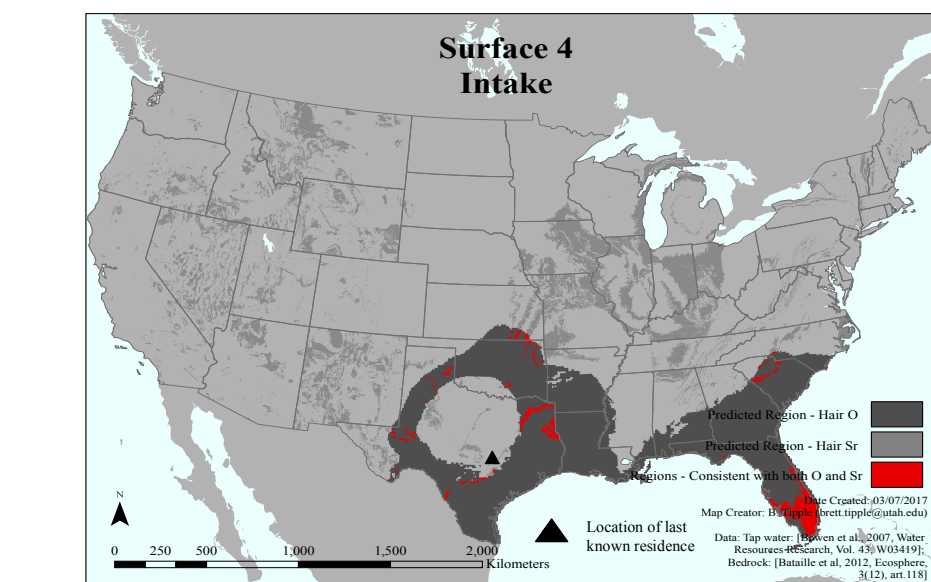


A

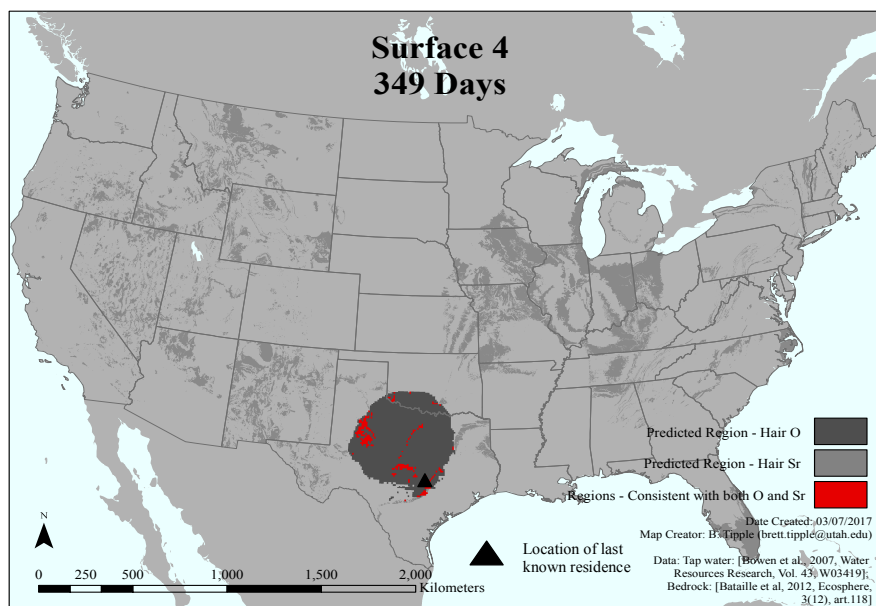


B

Figure 8. Geographic origin predictions from  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  data for Surface 3 Donor. Intake hair sample values are represented by A and post-exposure samples collected on day 331 are represented in B. Last known residence is Cartersville, GA, designated by the black triangle on the map.

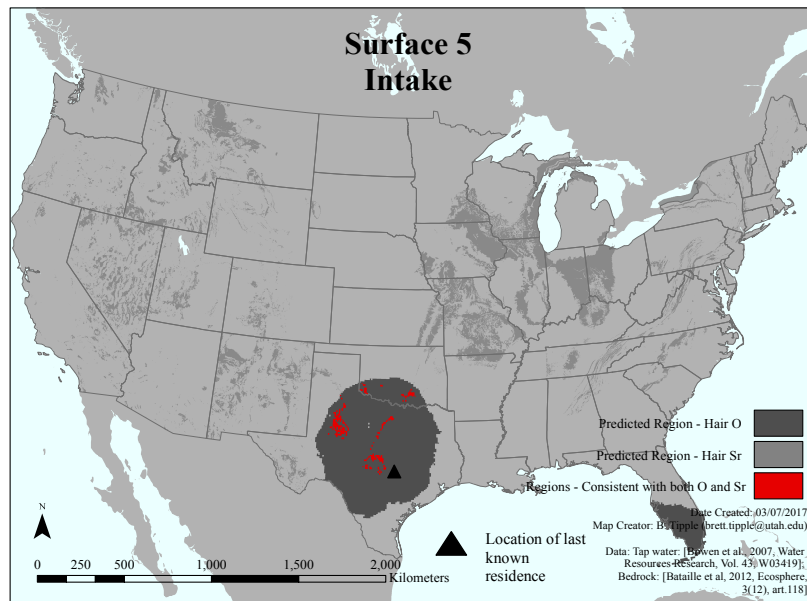


A

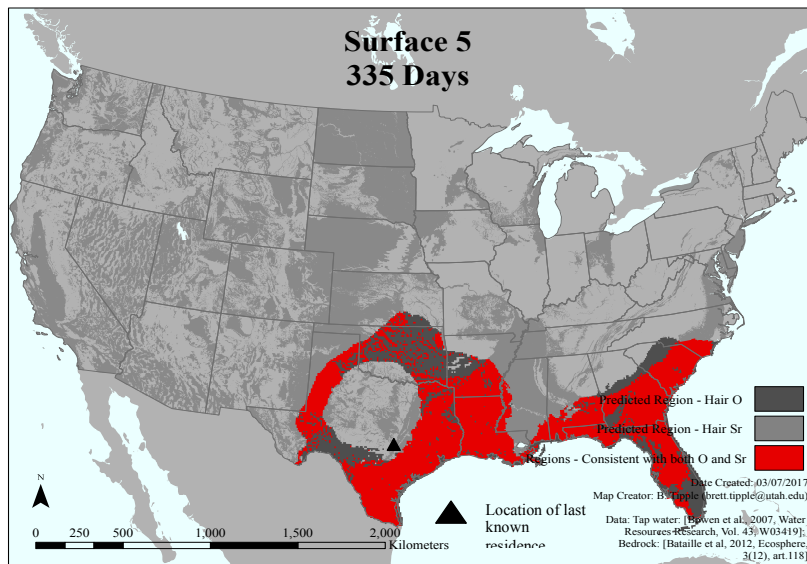


B

Figure 9. Geographic origin predictions from  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  data for Surface 4 Donor. Intake hair sample values are represented by A and post-exposure samples collected on day 349 are represented in B. Last known residence is Austin, TX, designated by the black triangle on the map.

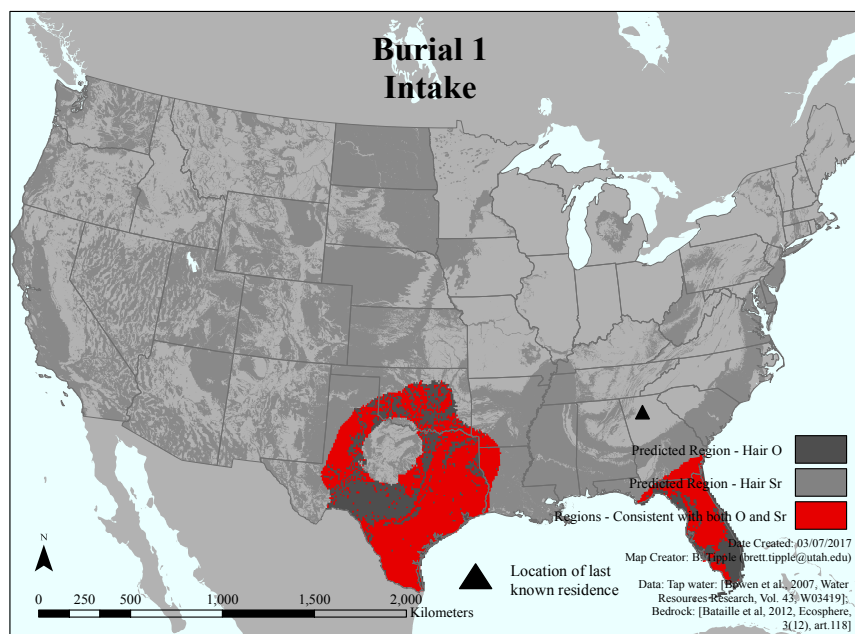


A

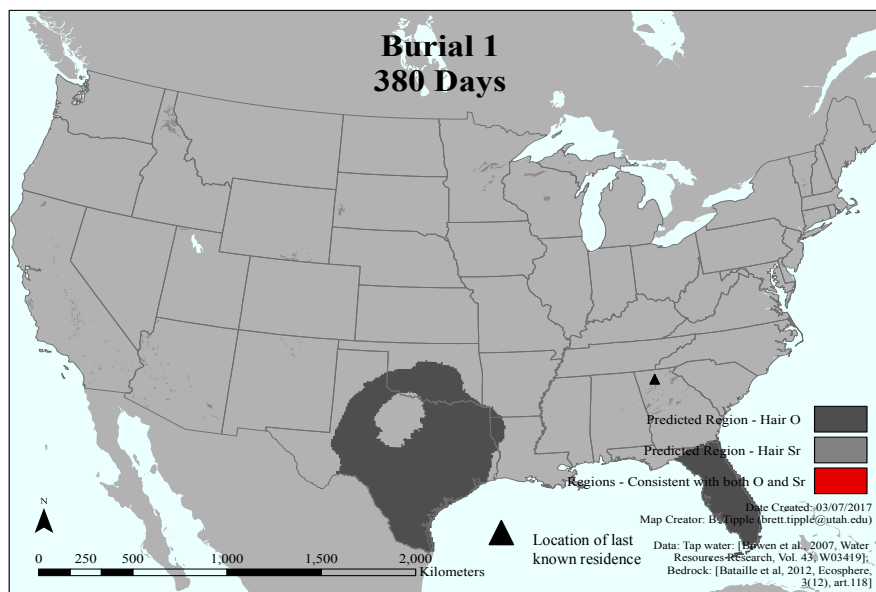


B

Figure 10. Geographic origin predictions from  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  data for Surface 5 Donor. Intake hair sample values are represented by A and post-exposure samples collected on day 335 are represented in B. Last known residence is Austin, TX, designated by the black triangle on the map.



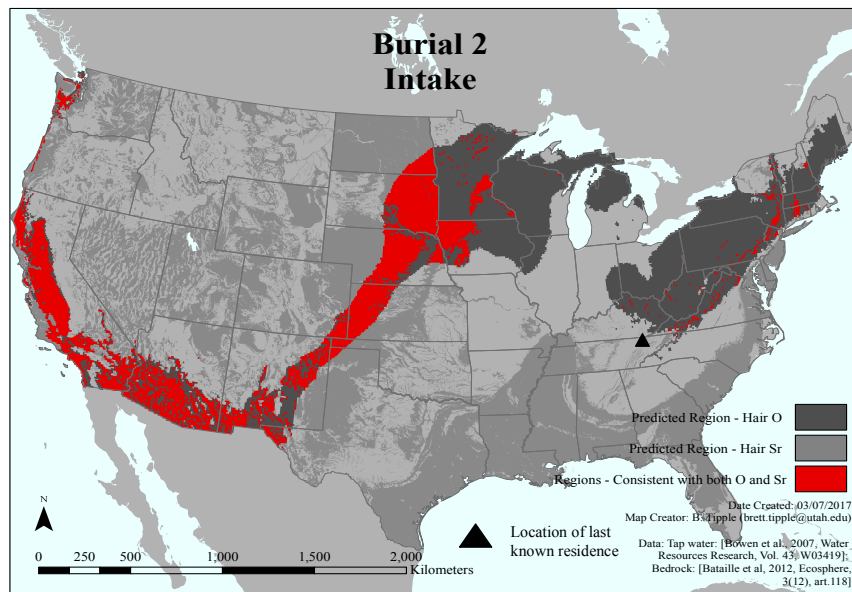
A



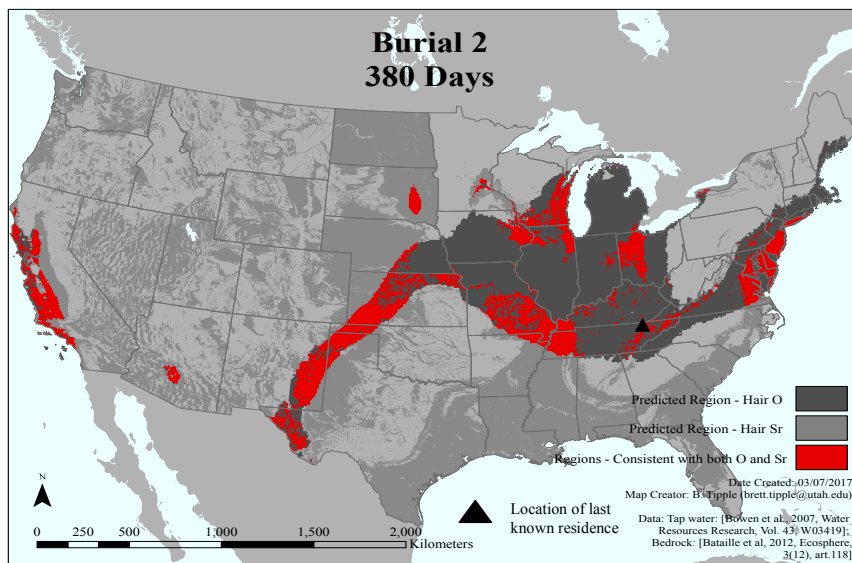
B

Figure 11. Geographic origin predictions from  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  data for Burial 1 Donor. Intake hair sample values are represented by A and post-exposure samples collected on day 380 are represented in B. Last known residence is Roswell, GA, designated by the black triangle on the map.



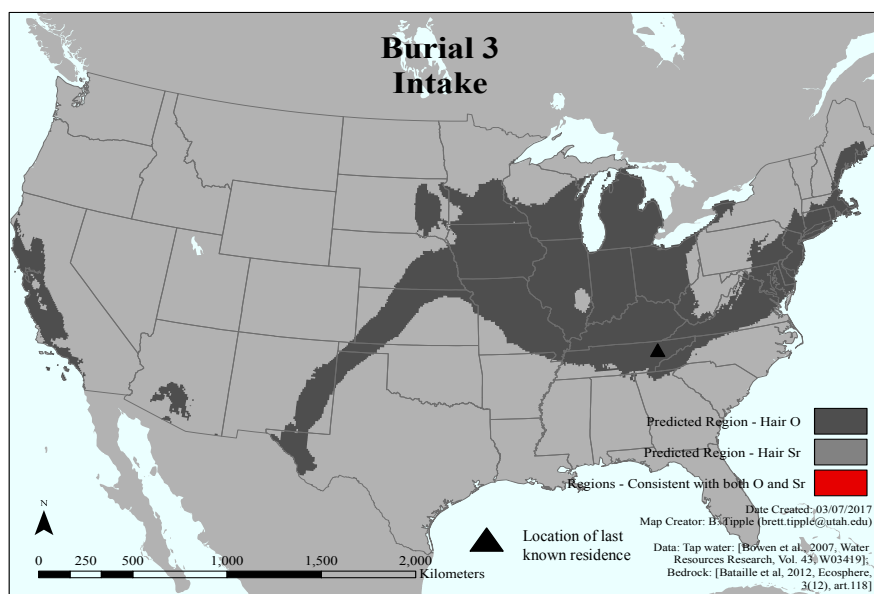


A

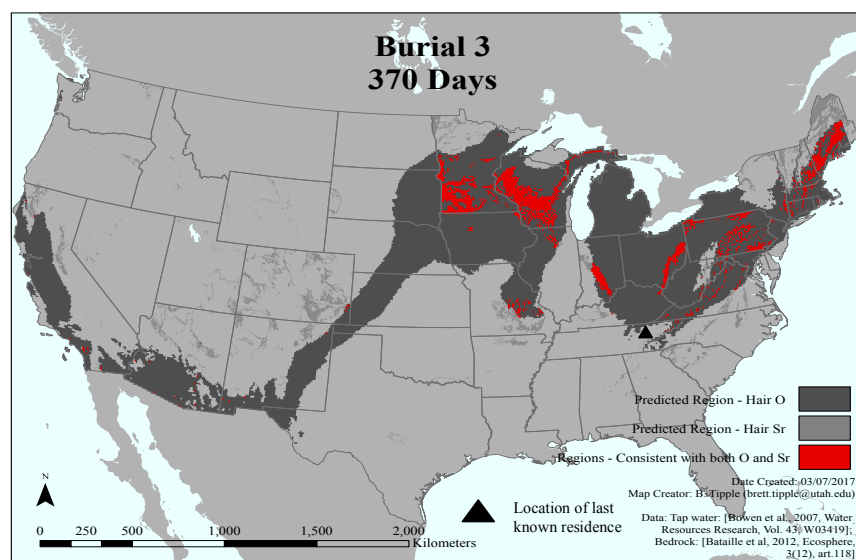


B

Figure 12. Geographic origin predictions from  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  data for Burial 2 Donor. Intake hair sample values are represented by A and post-exposure samples collected on day 380 are represented in B. Last known residence is LaFollette, TN, designated by the black triangle on the map.



A



B

Figure 13. Geographic origin predictions from  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  data for Burial 3 Donor. Intake hair sample values are represented by A and post-exposure samples collected on day 370 are represented in B. Last known residence is Lenoir City, TN, designated by the black triangle on the map. Note that strontium was not available for the intake hair sample.

Table 21. Accuracy of modeling for estimating geographic residence prior to death within 250km (~155mi), indicated by ‘Y.’ Inaccuracy is indicated by ‘N.’ \*\*Burial 3 intake sample did not have sufficient Sr concentration for  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio analysis.

|                  | Intake Sample Values            |                       |      |  | Post-exposure Sample Values     |                       |      |
|------------------|---------------------------------|-----------------------|------|--|---------------------------------|-----------------------|------|
|                  | $^{87}\text{Sr}/^{86}\text{Sr}$ | $\delta^{18}\text{O}$ | Both |  | $^{87}\text{Sr}/^{86}\text{Sr}$ | $\delta^{18}\text{O}$ | Both |
| <b>Surface 1</b> | Y                               | Y                     | Y    |  | Y                               | Y                     | Y    |
| <b>Surface 2</b> | Y                               | N                     | N    |  | Y                               | Y                     | Y    |
| <b>Surface 3</b> | Y                               | Y                     | Y    |  | Y                               | Y                     | Y    |
| <b>Surface 4</b> | Y                               | Y                     | Y    |  | Y                               | Y                     | Y    |
| <b>Surface 5</b> | Y                               | Y                     | Y    |  | Y                               | Y                     | Y    |
| <b>Burial 1</b>  | Y                               | N                     | N    |  | Y                               | N                     | N    |
| <b>Burial 2</b>  | Y                               | Y                     | Y    |  | Y                               | Y                     | Y    |
| <b>Burial 3</b>  | **                              | Y                     | **   |  | Y                               | Y                     | Y    |

Surprisingly, the maps from intake samples overall seem to predict the location of last known residence within 250 km less frequently than those made using the post-exposure sample values. Intake values are assumed to represent the isotope signatures of individuals with the least amount of variation from the values seen in life. It is reasonable to expect then that the predictions made from those values would most accurately represent the last known residence. In this case, however, post-exposure samples, which did exhibit some variation relative to the intake sample signatures, predicted last known residence more often than the pre-exposure intake sample values. This is most likely due to the uncertainty associated with the models and underlying base layers, and not related directly to intake and post-exposure sample variation previously discussed. Generally speaking, though, maps made using oxygen and strontium isotopes from human hair were able to predict last known residence within a 250-kilometer range. It would be advisable to anthropologists using such models to increase the radius of areas of interest in order to be sure the correct geographic area is included in predictive maps.

## Summary

Hypothesis 1 stated that post-decomposition  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of human hair would not be significantly different from those of samples at intake. While the differences in  $\delta^{13}\text{C}$  values were not significant in either phase of the study, supporting this hypothesis, in Phase I of the study  $\delta^{15}\text{N}$  values did show significant differences between intake and post-exposure samples.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of post-exposure samples generally increased from original intake values, but the degree of change seen does not affect dietary interpretations for the individuals, so these isotopes seem to preserve sufficiently within hair over time. Individual growth models suggest that the changes seen in  $\delta^{13}\text{C}$  values are connected with exposure environment and time. The models also reveal that  $\delta^{15}\text{N}$  values are impacted by time of exposure. These models address the effects of the suggested environmental variables as proposed in Hypothesis 1.

Hypothesis 2 stated that  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of post-decomposition hair samples would differ from intake values.  $\delta^{18}\text{O}$  values generally increased over time in both Phase I and Phase II, though Phase II values had a greater increase. Both increases and decreases were observed in both Phase I and Phase II. Neither phase saw statistically significant changes in  $\delta^{18}\text{O}$  values, though. Individual growth models were used to address the environmental variables suggested in Hypothesis 2 and showed a statistical relationship between the placement location (ARF or FARF) and changes to  $\delta^{18}\text{O}$  values, but no significant relationship between exposure time and changes in  $\delta^{18}\text{O}$  values.

$\delta^2\text{H}$  values decreased over time in both Phase I and Phase II samples, and the hydrogen isotope ratios were highly variable in both phases. Individual growth models suggest there is a relationship between  $\delta^2\text{H}$  values and time of exposure, whether an

individual is buried or placed on the surface, and the location of placement (ARF or FARF). Due to the high variability and multiple influences that must be considered, hydrogen isotope ratios of hair should be interpreted cautiously and may not be useful as oxygen isotope ratios of hair for geographic provenance predictions.

Hypothesis 4 predicted  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios from paired intake and post-decomposition hair samples would differ, and post-decomposition samples would reflect the values of the depositional environment. This hypothesis was supported;  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of both Phase I and Phase II samples generally increased over time and appear to be influenced by the soil in the placement location. When compared to values from soils near the area of placement, Phase II samples changed over time to more closely resemble the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the surrounding soil matrix. Individual growth models indicated statistical relationships between  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios and days of exposure, as well as location of placement (ARF or FARF).

The  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  data from Phase II donors were also applied to a geographic prediction model by relating the values from hair samples to tap water values in order to address Hypothesis 4. This hypothesis stated that oxygen and strontium isotope ratios of hair from both intake and post-exposure conditions would reflect last known geographic residence when applied to mapping models using United States tap water and bedrock data. Using this model,  $\delta^{18}\text{O}$  values of both intake and post-exposure samples successfully predicted the last known residence of donors within 250 kilometers 13 out of 16 times, approximately 81% accuracy.  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were used in a geographic provenance model that applied the values to geographic bedrock maps. The

predictions made with  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios predicted the last known residence of Phase II donors within 250 kilometers in all 15 instances, or 100%. When combined,  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  data predicted the last known residence within 250 kilometers 12 out of 15 times, or 80% of the time.

Each isotope system evaluated provides unique information on individual diet and geographic movement at the individual level. These isotope systems should be used to with an understanding of how the tissue isotope ratios may be impacted by time of exposure, location of deposition, and conditions of placement. These and additional considerations are explored in greater detail in Chapter Five.

## CHAPTER FIVE

### DISCUSSION

This study examined the effects of taphonomic processes upon isotope ratios of human hair by comparing pre-exposure samples collected during intake procedures with post-exposure samples that were collected from outdoor anthropology research facilities in Tennessee (ARF) and Texas (FARF). Both surface and burial placements were used in this study in order to evaluate the differential role, if any, of rain and soil on the isotopic changes. Each isotope system evaluated in this study provides a unique, independent piece of information that, when combined with each other and the biological profile, can yield additional layers of detail to assist with identification of unknown remains in forensic contexts (Beard and Johnson, 2000; Meier-Augenstein and Fraser, 2008; Aggarwal et al., 2008; Bartelink et al., 2014; Font et al., 2015; Gentile et al., 2015; McLean et al., 2014), or can build a more robust interpretation of prehistoric life in bioarchaeological studies (Knudson et al., 2007; Knudson et al., 2009; Fuller et al., 2012; Knudson et al., 2012). The following discussion addresses the implications of this study's findings upon the application of isotope analysis of human hair for dietary and geographic provenance predictions in forensic anthropology and bioarchaeology.

#### **Carbon and Nitrogen**

Hypothesis 1 stated that post-decomposition carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope ratios would not be significantly different from those of hair sampled at intake. Consistent with this hypothesis, both Phase I and Phase II  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values remained generally constant, with no significant differences between intake and post-exposure

samples. The small differences observed were not significant: the interpretations of dietary consumption made from intake hair samples would not differ from those made from post-exposure hair measurements.

The individual growth models were used to evaluate effects of duration of exposure, location of placement, and placement condition. The first linear growth model revealed that changes to carbon isotope ratios were impacted by placement location (ARF or FARF) and days of exposure combined, seen in Table 17 ( $p = 0.0021$ ). The reasons for this difference in  $\delta^{13}\text{C}$  values are not clear, as the carbon isotope ratios do not appear to converge on the  $\delta^{13}\text{C}$  values of soil. The mean ARF soil  $\delta^{13}\text{C}$  value was  $-26.38 \pm 1.27\text{‰}$  (Table 16), and the average offset between hair and soil values was  $11.94 \pm 2.49$ . A limitation of the linear growth model to evaluate location (ARF vs. FARF) is that it only uses three ARF and two FARF placements. It is possible that a larger sample size may provide more robust results.

Nitrogen isotope ratios showed significant differences based upon number of days of exposure in both models ( $p = 0.009$  and  $p = 0.0057$ ), indicating a relationship between time and the changes in nitrogen isotope ratios. Differences between intake and post-exposure samples were not in a consistent direction, and both increases and decreases were seen in nitrogen isotope ratios over time (Phase I Table 6, Phase II Table 11).

The nitrogen cycle is complex, with many variables (Szpak, 2014; Amundson et al., 2003). Comparing  $\delta^{15}\text{N}$  values of soil demonstrated a wide range within the ARF Phase II placements (from 2.38 to 6.43‰). The ARF has been used for decomposition research for over thirty years, and nitrogen is closely tied with decomposition processes



(Damann et al., 2015). One possibility is that the variation in the soil was due to the frequency of use and/or the amount of time since another donor was previously placed at the same location. Another possibility is the amount of nonhuman organic material, such as leaf litter, that has been incorporated into the soil samples. The  $\delta^{15}\text{N}$  values of FARF soil samples were much less variable compared to the  $\delta^{15}\text{N}$  values of ARF soils (FARF SD  $\pm 0.21\text{‰}$  vs. ARF SD  $\pm 1.63\text{‰}$ ). An explanation for the difference between the  $\delta^{15}\text{N}$  values of FARF and ARF soils may be due to FARF being a relatively new facility with no repeated human decomposition in the placement areas of soil sampling; in addition, the ARF facility has a deep soil horizon and very different patterns of vegetation. In Phase II of the study,  $\delta^{15}\text{N}$  values from hair increased over time for each donor. It is possible that this increase was attributable to microbial activity, in which the decomposers are preferentially consuming the lighter isotope of nitrogen, thereby increasing the  $\delta^{15}\text{N}$  values of the donor hair (Metcalf et al., 2016).

Metcalf and colleagues (2016) sampled skin and grave soil associated with four human bodies (two spring and two winter placements) over the course of several months. The samples were evaluated for archaeal, bacterial, microbial eukaryotes, and fungal communities in order to characterize the microbial diversity associated with decomposition. They also followed soil pH, ammonium, and nitrate concentrations throughout the study. The study showed large taxonomic changes within the microbial community over a relatively short period of observation, and the changes to soil chemistry provided soil microbial organisms with increased opportunity for nitrogen cycling. Future research could examine isotopic changes in  $\delta^{15}\text{N}$  with a Metcalf et al.

(2016) type of analysis of soil for nitrogen-fixing microbes over time in order to further understand these soil differences and whether they impact changes in nitrogen isotope ratios of human remains. In addition, breaking down soil components into nitrate, nitrite, dissolved and solid nitrogen is likely to illuminate the causes of these variations.

Again, it is important to remember that the differences observed in  $\delta^{15}\text{N}$  values of hair due to exposure would not affect the dietary interpretations made from these  $\delta^{15}\text{N}$  values though these differences were statistically significant. As an example, the greatest difference in  $\delta^{15}\text{N}$  values between intake and post-exposure hair samples was 1.14‰, observed in Phase I Donor 754 (Table 6). A change in trophic level as reflected in  $\delta^{15}\text{N}$  values of bulk hair is expected to be 3-5‰, so a difference of ~1‰ would not classify the individual differently based upon intake or post-exposure values.

## **Diet**

The carbon and nitrogen isotope ratio results from both Phase I and Phase II of the study provide general information about the diets of the donors enrolled in this study. When intake values are plotted together, the diets appear to have a relatively limited variability compared to global dietary patterns, whereby carbon and nitrogen isotope ratios appear to be in similar ranges for all donors. From the Phase I results, Donor 758 has relatively low  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Figure 14). These data are consistent with the individual being vegan with a large consumption of proteins derived from  $\text{C}_3$  plants (Bol and Pflieger, 2002), and it is reasonable to infer that Donor 758 may have been a vegan or vegetarian. No dietary information is available for this donor, an 81-year-old male with a body mass index (BMI) of 31.1, which falls in the obese range (National Heart,

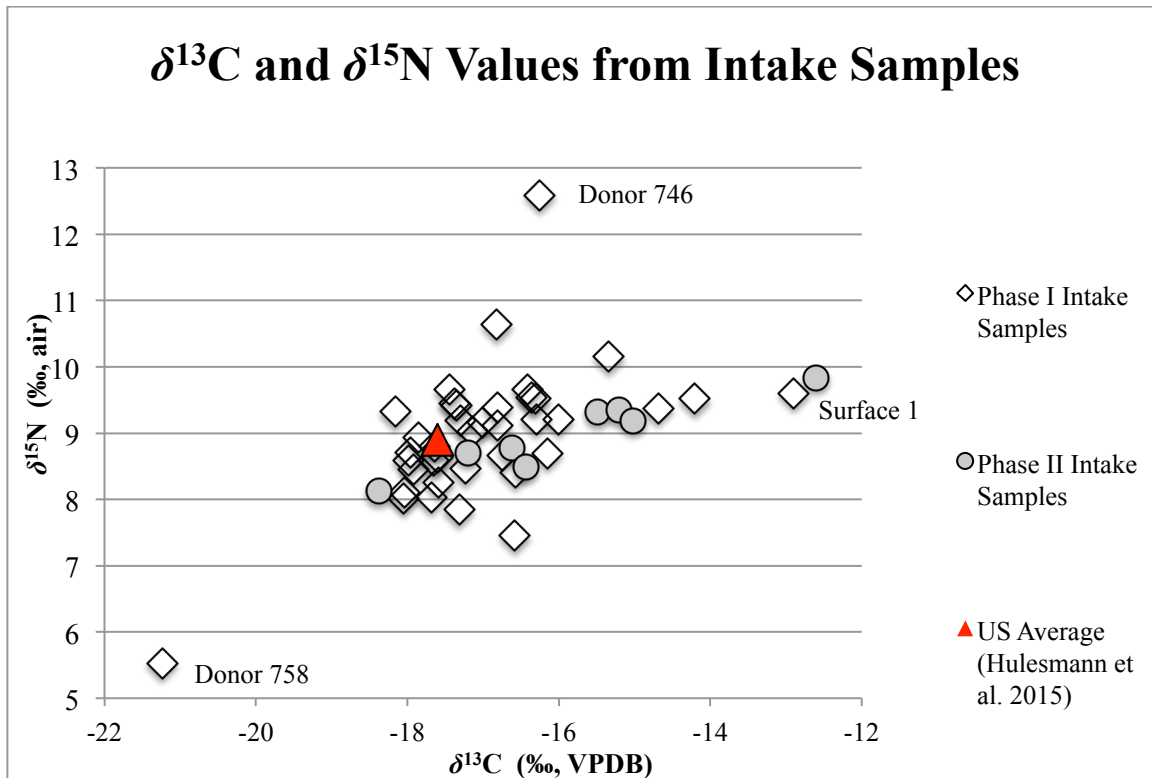


Figure 14. Carbon and nitrogen values of intake hair samples from all Phase I and Phase II donors. United States average values (from Hülsemann et al., 2015) are indicated by the red triangle.

Blood, Lung Institute, 1998).

Overall, the  $\delta^{13}\text{C}$  values for donors in this study were consistent with the reported U.S. averages. Hüsleemann and colleagues reported U.S. hair  $\delta^{13}\text{C}$  values with an average of  $-17.6 \pm 1.2\text{‰}$ , and this study's donors have an average value of  $-16.78 \pm 1.44\text{‰}$ . Expected  $\delta^{13}\text{C}$  values from individuals living in the United States are generally higher than those living in Europe (Figure 15). Valenzuela et al. (2012) reported  $\delta^{13}\text{C}$  averages for areas near the southeastern United States at slightly higher averages (approximately  $-15.7\text{‰}$   $\text{SD} > 0.5\text{‰}$ ). However, the southeastern states in which the donors in this study reported last known residence are not represented in the Valenzuela et al. (2012) sample set. From Phase II, Surface 1 donor has a relatively high  $\delta^{13}\text{C}$  ratio ( $-12.60\text{‰}$  at intake) compared to the other individuals in this study (median value:  $-16.78 \pm 1.44\text{‰}$ ). This is generally interpreted as the individual having a high dietary intake of corn, proteins derived from  $\text{C}_4$  plants, and foods with added sugar. Most donors in this study had final reported residences in the Southern United States, a region that is frequently associated with diets high in processed foods, often indicating high corn and sugar content. The differences in the mean  $\delta^{13}\text{C}$  values of hair from these populations may be due to dietary differences associated with regional and cultural preferences. Otherwise, carbon isotope ratios are fairly similar to one another, with little difference between donors that would differentiate one donor from another in terms of dietary preferences.

The  $\delta^{15}\text{N}$  values of intake samples in this study are indistinguishable from the U.S. averages reported by Hüsleemann and colleagues (2015). Their study reported an

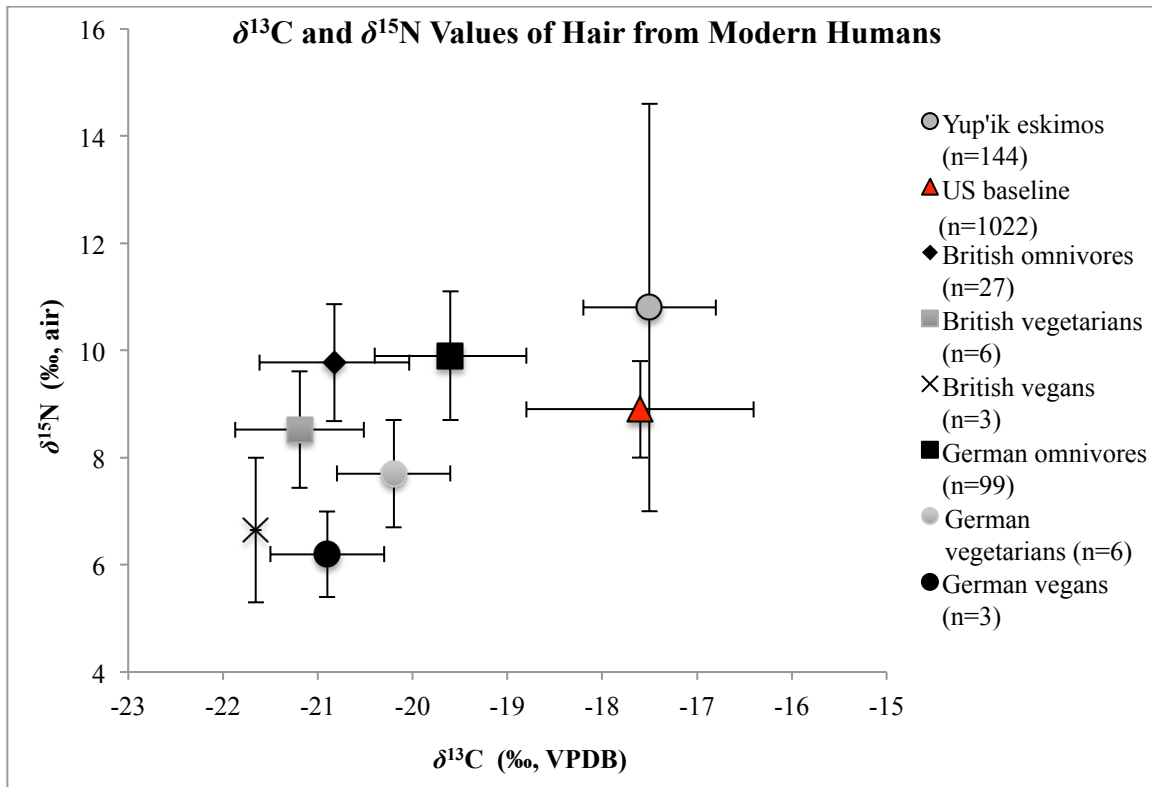


Figure 15. Compiled  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of hair samples from modern humans from Nash et al. (2009), Bol and Pflieger (2002), and Petzke et al. (2005). The US baseline values are from Hülsemann et al. (2015).

average  $\delta^{15}\text{N}$  value of  $8.9 \pm 0.9\text{‰}$ , and values from this study were  $8.9 \pm 1.1\text{‰}$ . When evaluating differences in  $\delta^{15}\text{N}$  values, Donor 746 does have nitrogen isotope ratios higher than other donors (Figure 14), which could indicate 1) relatively more protein consumption (Petzke et al., 2005; O’Connell et al., 2012), 2) a higher proportion of marine protein (Hedges and Reynard, 2007; Huelsemann et al., 2013; Kuhnle et al., 2013), or 3) starvation or wasting (Deschner et al., 2012; Mekota et al., 2006; Petzke et al., 2010; Fuller et al., 2005). A possible explanation is that the higher  $\delta^{15}\text{N}$  value may be related to health. It has been shown that people suffering from eating disorders or severe nutritional deficiencies exhibit higher  $\delta^{15}\text{N}$  values (Mekota et al., 2006). This is attributed to the individual essentially “self-digesting” in the absence of other sources of nutrition (Mekota et al., 2006). The observed isotope data collected from this study cannot reveal the specific dietary details that determine  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of donor hair, but it is worth noting that individuals may be distinct from others from the same regional population. These differences may provide potential clues regarding individual identification in the absence of other leads. A review of the donor’s intake information revealed a calculated body mass index (BMI) of 21.1, which falls into normal range for a 62-year-old female (National Heart, Blood, Lung Institute, 1998). Cause of death was listed as septic shock, which is not indicative of a long-term illness that would be associated with wasting or an eating disorder. No additional health or diet information for this donor was available, but based upon the information provided, it is likely that the high  $\delta^{15}\text{N}$  value of hair for this donor is related to diet, particularly a significant proportion of seafood in the diet, and not illness.

While the scope of the current study provides an examination of the changes seen in isotope ratios from intake and post-exposure hair samples, there is not enough available information about individual dietary preferences to confirm the interpretations made from these isotope data. In order to maximize the potential research opportunities from the William M. Bass Donated Skeletal Collection, as well as those collections from other anthropology research facilities, pre-registration questionnaires that are filled out by future body donors should be expanded to include questions related to diet. Information regarding whether an individual is on a long-term specialized diet, such as a vegan diet, could provide an opportunity to better understand the expected range of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in humans regionally and from across the United States, as represented by the donors to these programs.

### **Hydrogen and Oxygen**

Hypothesis 2 stated that  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of post-decomposition hair samples would differ from intake values. Unlike carbon and nitrogen isotope ratios, the isotope ratios of hydrogen and oxygen in hair samples from both Phase I and Phase II were more variable over time. Hydrogen isotope ratios differed significantly between intake and post-exposure samples in Phase I. While the differences were not statistically significant in Phase II samples, both Phase I and Phase II saw increases in  $\delta^2\text{H}$  values over time. All paired samples had increases in  $\delta^2\text{H}$  values over time with the exception of Surface 1 donor from Phase II of the study. This observation is consistent with hydrogen in hair exchanging with hydrogen from other sources over time, but it is not clear if there is a specific individual source. When evaluated for further statistical relationships between

days of exposure, placement location, and placement condition, using the linear growth models, differences in  $\delta^2\text{H}$  values over time between intake and post-exposure samples showed relationships between the number of days ( $p < 0.001$ ) and placement condition (surface vs. burial,  $p = 0.0427$ ) in the first model and placement location (ARF or FARF,  $p = 0.0088$ ) in the second model.

Precipitation was proposed as a possible source of mixing or exchange in Hypothesis 2. While  $\delta^2\text{H}$  values measured for hair are moving in the direction of precipitation  $\delta^2\text{H}$  values, the magnitude of change over time does not indicate this is a direct source of change. It has been reported that hydrogen atoms of hair are highly exchangeable with local environmental humidity and there are known problems with accurate determinations of  $\delta^2\text{H}$  values due to the significant proportion of the hydrogen that is highly exchangeable (Wassenaar and Hobson, 2000; Wassenaar and Hobson, 2003; Bowen et al., 2005; Chesson et al., 2009; Meier-Augenstein et al., 2011; Qi and Coplen, 2011). This study used best practices for laboratory equilibration and analyzed samples in batch with certified samples analyzed as unknowns. The measured values are confidently reported as accurate representations of the non-exchangeable fraction.

There were both increases and decreases over time for  $\delta^{18}\text{O}$  values from Phase I and Phase II, though the general trend is toward increased values over time. Linear growth models revealed a significant difference between changes in  $\delta^{18}\text{O}$  values from ARF donors and FARF donors. These differences do not seem to be related to estimated  $\delta^{18}\text{O}$  values of precipitation, because the observed changes are moving away from



precipitation  $\delta^{18}\text{O}$  values, not toward them, indicating there is no simple exchange between precipitation oxygen isotopes and oxygen isotopes in the hair.

Understanding the variability in  $\delta^{18}\text{O}$  values of human hair is remains an active area of research. While several studies have attempted to predict  $\delta^{18}\text{O}$  values in hair based upon diet and drinking water contributions into the body water pool (Ehleringer et al., 2008; Kennedy et al., 2011; Podlesak et al., 2012; McLean et al., 2014), these models are still only providing estimates and do not account for other sources of variation that may exist. It is possible that the intra-individual variability of physiological processes, including but not limited to health factors, levels of physical activity, and individual metabolism contribute to differences in  $\delta^{18}\text{O}$  values between individuals. For these reasons, it is understandable that geographic predictions made from  $\delta^{18}\text{O}$  values in hair may not fall within range of the last known residence 100% of the time. In the case of the 16 maps produced for this study (eight from intake and eight from post-exposure values), predictions based on measured hair  $\delta^{18}\text{O}$  values fell within 250 kilometers of the last known residence 13 out of 16 cases (81%).

The variation in  $\delta^{18}\text{O}$  values observed between intake and post-exposure samples from the same donor averaged less than 1‰ for both Phase I and Phase II donors. When considering the source of differences within intake and post-exposure samples, it is also important to remember the sampling methods used. All analyses were conducted using bulk analysis. This means the entire length of the hair was used for analysis. This would likely not make much difference for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, as people generally eat the same types of food wherever they go. Traveling would likely not impact diet in a way

that would dramatically influence the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of an individual. Traveling would, however, potentially impact  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values along the length of the hair. Recall that hair acts as a tape recorder, making a serial record along the length of hair strands (Remien et al., 2014; Thompson et al., 2014). If an individual traveled to locations with different  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values, and stayed long enough for those signals to be incorporated into their hair, the travel movement could impact the variability of isotope ratios measured via bulk sampling. Additionally, scalp hairs are in multiple phases of growth at any given time, and roughly 85% are in the active growth phase of anagen (Kempson and Lombi, 2005; Robbins, 2012). If hair samples included strands from anagen as well as other growth phases, isotope ratios of the hair will represent different temporal periods of a person's life, rather than consistently representing the same period. If the individual has not done any traveling to isotopically distinct areas, this would not impact the interpretation of the data; however, if an individual has traveled to an isotopically distinct region, those related isotope ratios will be represented only in hairs that are in active growth phase during that time. Future studies could incorporate serial sampling in which individual hair strands are evaluated for growth phase and aligned for sampling in 1-centimeter segments along the length of the hair (Remien et al., 2014).

### **Strontium**

Hypothesis 3 stated that  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of paired intake and post-exposure hair samples would differ, and post-decomposition samples would reflect the values of the depositional environment. Both Phase I and Phase II of the study demonstrated that

$^{87}\text{Sr}/^{86}\text{Sr}$  ratios of human hair increased over time, consistent with this hypothesis.

Strontium is abundant in soil. As a soluble element that is mobile in the natural environment, the large amount of strontium in soil as compared to the relatively small amount of strontium in hair means that contamination is possible. While hydrogen and oxygen are even more abundant in rainwater than strontium is in soil, these elements are structurally incorporated into hair. Strontium is more weakly incorporated in hair through absorption, and thus more susceptible to exchange with surrounding matrix. The results of this study suggest that  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of hair are increasing based upon  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the easily soluble component of soil. This either means that there are still solid soil particulates present in the hair when it is analyzed, or that soluble strontium from the soil is being incorporated into the hair over time. Every effort was made to minimize soil contaminants through the most aggressive cleaning protocol currently proposed (Tippie et al., 2013), but there may not be a suitable way to completely remove all exogenous strontium from hair samples. The number of days of placement, as well as the location of placement (ARF or FARF) were significant variables when evaluated using the linear growth models. The soil differences between the two locations (ARF and FARF) are a likely explanation for the differences seen in the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of hair over time, especially if the soil matrix in contact with the hair is contributing to the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the hair through contamination or incorporation. While  $^{87}\text{Sr}/^{86}\text{Sr}$  is clearly affected by taphonomic changes, it still performed well for geographic provenance predictions.

### **Geographic Residence from $\delta^{18}\text{O}$ and $^{87}\text{Sr}/^{86}\text{Sr}$**

Hypothesis 4 stated that  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  values of both intake and post-exposure samples would reflect last known geographic residence when applied to a mapping model using United States tap water and bedrock data.  $\delta^{18}\text{O}$  values of both intake and post-exposure samples successfully predicted the last known residence of donors within 250 kilometers 13 out of 16 times, approximately 81% accuracy. This accuracy may be related to the underrepresentation of the Southeastern United States in the tap water and hair model data set (Bowen et al., 2007; Ehleringer et al., 2008). An important consideration that must be made in the interpretation of geographic provenance is that donor information contains *residence* history and not *travel* history. If a donor traveled in the months or weeks prior to death, isotope ratios representing those regions would be represented in the hair, impacting the results of the analysis, but that information is not available from donor records. This is a limitation to the study, but would be representative of the challenges that face application of isotope analysis in a real-world setting. If investigators were asking family members about their missing loved one, they would ask about residence history but perhaps not travel history, which may be unknown to relatives or not considered pertinent to the investigation. Even so, travel history versus residence history is an important distinction to make when considering isotope analysis for geographic provenance.

While there are still some obvious limitations to the use of  $\delta^{18}\text{O}$  values for predicting geographic information from human hair, the findings of this study support continued efforts to utilize oxygen isotope ratios of hair to provide additional leads in identification efforts for unknown human remains. As tap water maps become more

refined and span larger geographic areas across the world, comparisons with  $\delta^{18}\text{O}$  values from hair can be used to improve the existing models. The identification of remains of undocumented border crossers, particularly in the Southwestern United States, is a growing need that could significantly benefit from advances in geographic modeling of  $\delta^{18}\text{O}$  values of human hair. More work is needed to understand the inter- and intra-individual variability of oxygen isotope ratios of human hair in order to advance the accuracy of this type of analysis.

$^{87}\text{Sr}/^{86}\text{Sr}$  ratios of hair provided predicted regions within 250 kilometers of last known residence in all 15 cases, despite differences in pre- and post-exposure  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios. These predictions were made by comparing the values from hair to geological bedrock maps. An important caveat when comparing strontium isotope ratios to expected values from bedrock is that bedrock maps are not necessarily representative of the bioavailable strontium in a specific area. For example, in an area that has a high amount of dust from excavation or construction activity, the anthropogenic dust may be characterized by significantly different  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios and can change the bioavailable strontium values. Likewise, deep bedrock will have a very different  $^{87}\text{Sr}/^{86}\text{Sr}$  signature than the surrounding environment due to erosion, weathering, and fluvial processes that can affect the surface landscapes. Researchers are actively working to improve the predictive models to reflect bioavailable strontium and move beyond bedrock maps (Bataille et al., 2012; Bataille and Bowen, 2012; Crowley et al., 2017). These new models incorporate terms that estimate bioavailable strontium from bedrock models, and these modifications stand to substantially improve human geolocation through strontium

isotopes of tissues such as hair. As these new and improved models are developed, mapping applications will continue to improve in accuracy for predicting geographic provenance from  $^{87}\text{Sr}/^{86}\text{Sr}$  values from ratios of human remains.

Strontium alone does not provide a narrow geographic range for expected geographic origin. Because predictions are based upon bedrock, which is similar in many places across the United States, the predicted regions are very large and do not necessarily generate any new information for forensic identifications. However, isotope analyses are far more powerful when isotope systems that are impacted by different and independent factors are combined. As previously suggested by other researchers (Tipple, 2015; Font et al., 2012), combining  $\delta^{18}\text{O}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  analyses and looking at overlapping predicted regions can generate a much smaller pool of possible locations of geographic origin.

Isoscapes provide a unique visual way of representing isotope data from human tissues to those who are not isotope scientists. Maps are easily understood tools that translate the isotope data into usable information for archaeologists, anthropologists, law enforcement investigators, and other non-experts. Providing these simplified visualizations should be done with caution, though, as the complexities of isotope analyses are often lost when reduced to a map or final report. The uncertainty associated with the models and their predictions is difficult to communicate to the non-expert. For example, the models are often tested against samples of known origins, but the predictions do not “hit” the exact location of known origin. To the non-expert, this translates to model failure, but that is not necessarily the case.

Isoscapes are produced from multiple layers of derived spatial data products, imperfect model parameters, and geostatistical analysis, and developing quantitative measures of the uncertainty of the predictions represents a significant challenge (Bowen and West, 2008). The prediction error is only one component of error associated with isoscapes. The selection of the appropriate base layers and isoscape data by researchers to apply to a specific problem, as well as accurately predicting the relationships between tissue isotopic composition and the environment are also sources of uncertainty that are not easily communicated through confidence intervals or error rates.

The final product maps that were produced for this project appear seamless. However, there are gaps in the data used to produce these maps. Individual communities can have high variability in hydrogen and oxygen values of tap water, for example (Tippie et al., 2017). This missing data and small-scale variability is smoothed out by the large-scale projections of a map spanning the entire United States. A non-expert would not necessarily recognize this caveat, so it is upon the isotope science practitioner presenting the models to more thoroughly explain the uncertainty associated with the predictive models. It is also incumbent upon the scientific and law enforcement communities using these models to begin systematically and rigorously challenging the assumptions of the models in order to determine what variables affect when the models do and do not work.

### **Summary**

The findings of this study suggest that carbon and nitrogen isotope ratios of human hair are relatively consistent throughout decomposition. While differences were

observed between pre- and post-exposure samples, these differences were not of a magnitude that would impact dietary interpretations made from the measured isotope ratios. Hydrogen and oxygen isotope ratios were more variable between intake and post-exposure samples, with  $\delta^2\text{H}$  values significantly increasing over time.  $\delta^{18}\text{O}$  values from both pre- and post-exposure samples were able to predict areas of geographic origin within 250 kilometers of last known residence about 81% of the time.  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of hair increased over time, trending toward values from the bioavailable soil component. Statistical modeling using linear growth models revealed relationships between changes and placement location for  $\delta^2\text{H}$ ,  $\delta^{18}\text{O}$ , and  $^{87}\text{Sr}/^{86}\text{Sr}$ , number of days of exposure for  $\delta^{15}\text{N}$  and  $\delta^2\text{H}$ , placement condition for  $\delta^2\text{H}$ , and a combination of days of exposure and placement conditions for  $\delta^{13}\text{C}$  values. While the sample size is small for this study, these relationships between isotope ratios of human hair and environmental factors such as location, placement condition, and duration of exposure, demonstrate that there are multiple influences upon the isotopic composition of human hair postmortem. Implications of these effects have been discussed in this chapter. The following chapter will provide concluding remarks and future directions of research.



## **CHAPTER SIX**

### **CONCLUSION**

Isotope analysis in anthropology is a valuable interdisciplinary tool for reconstructing individual diet and travel histories from human remains in both archaeological and forensic contexts. Borrowed from geochemistry and ecology, isotope analyses must be used with capabilities and limitations in mind. This study has revealed several important considerations not previously investigated in isotope analyses of human hair.

The research presented here has shown that isotope ratios of hair vary over time periods from under one month to more than three years. While the time periods observed for this study are not representative of archaeological time scales, it is reasonable to conclude that hair preserved in archaeological contexts can be used to make inferences about diet using carbon and nitrogen isotope ratios. These values remained relatively consistent over time in this study, and observed differences between pre- and post-exposure hair samples would not impact the interpretations made about diet.

Hydrogen and oxygen isotope ratios proved more variable, with hydrogen isotope ratios spanning extreme increases and decreases in unpredictable ways with no apparent singular influence. Oxygen isotope ratios varied over time but ultimately remained within a range that allowed reasonably accurate geographic origin predictions to be made using tap water and precipitation modeling data. Whether oxygen isotope ratios in hair persist over archaeologically-relevant time scales is unclear and cannot be determined within the limitations of the current study, but it is recommended that these isotope ratios of hair be

used with caution when making predictions about geographic origins of archaeological remains.

Strontium isotope ratios of human hair seem to be dramatically influenced by external environment, assuming  $^{87}\text{Sr}/^{86}\text{Sr}$  signatures similar to the depositional soil matrix in the case of this study. It is reasonable to infer that human hair exposed to soil and/or precipitation over extended periods of time would assume  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios completely different from the original signatures reflecting the living profile of the individual. When analyzing human hair recovered from contexts exposed to water over extended periods of time,  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios should be used with caution.

It has been assumed that isotope ratios of human remains reveal individual life histories, making them a unique and beneficial tool for anthropologists attempting to reconstruct the living behaviors of individuals. Consideration must be made for the limitations of how specific these inferences can be. For example, the effects of certain health conditions, age, dietary restrictions, or cultural food preferences could all potentially impact isotopic signatures of human remains, including hair. Hair itself can be influenced by texture, chemical treatments, styling products, and even age related changes, which were not evaluated as part of this study. These are all considerations that should be investigated through future research in the area of human hair isotope analysis.

Isotope analysis is largely affected by sample selection. If poorly preserved samples are analyzed, the data is not useful. This study demonstrated that sample quality is important in the analysis of human hair because postmortem samples that have been exposed to outdoor environments can have isotope ratios that do not reflect the living

signatures of the individual. All is not lost if a sample is degraded, though. Recognizing that these changes can affect isotope ratios allows practitioners the opportunity to account for possible biases in the results and find ways to correct for them. Anthropologists have been consumers of isotope data from human remains for many years. It is necessary that as a community, anthropologists become more involved in the sample preparation and analysis. Understanding the way data is produced is the responsibility of the researcher, and the interpretation of isotope analyses of human tissues can benefit from collaborative efforts between isotope scientists and anthropologists to better understand human variation, as well as extrinsic factors that can influence analyses.

In addition to analytical issues, understanding the limitations of the current geographic prediction models is essential if the models are going to be useful in forensic applications. Uncertainty surrounding predictions from isoscape models is influenced by the data sets that are used within the models, as well as decisions made by the researcher regarding the selection of modeling parameters. When non-experts like anthropologists and law enforcement are using these models, it is essential that they consult with isotope science practitioners to achieve the best possible results. It is the responsibility of isotope scientists to be transparent in their methods, as well as the limitations of the current models and their capabilities. This is not to say that these models are not valuable tools for investigators. When used within their current capabilities, isoscapes can provide new information in human identification investigations and have generated leads in cold cases that resulted in identifications of unknown human remains. This study has demonstrated that isotope ratios from human hair predicted last known residence within 250 km 80% of

the time. More outreach on the part of anthropologists using isotopes, as well as isotope scientists, is needed in order to educate forensic pathologists, death investigators, and law enforcement on the capabilities of isotope analysis of human remains.

This study had several limitations. One of these is that the time scale evaluated for isotope changes in hair encompasses a range that is applicable to forensic casework, but it is certainly not applicable to archaeological investigations. Additionally, the sample population is composed primarily of individuals who resided in the southern United States for the final years of their lives. Hair collected from these donors does not reflect the range of isotope ratios that would be expected from a larger sample representing the entire United States. Even greater differences would be expected from a global population. There is more work that needs to be done to understand the inter- and intra-individual variations seen in isotope values from around the world, particularly for  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values.

The individuals represented in this study were all self-identified as white, and a majority were female. More diversity in ancestry, a larger number of males, and a wider range of ages, particularly individuals under 40 years of age, would improve this research. Hair texture, treatment, and color, which are all potentially affected by age, sex, and ancestry, can impact results of isotope analyses, and a diverse sample population would allow these variables to be examined in regards to how taphonomic processes may affect different types of hair. Isotope research will continue to increase our understanding of human variation. Future research to establish the true range of inter-and intra-

individual variation in isotopic signatures is needed in order to improve the utility of this type of analysis for forensic use in human identification.

Phase I of this study, especially strontium isotope analysis, was limited by the amount of hair available from intake samples. For multiple donors, there was not enough hair collected at intake to perform all analyses. The current protocol for intake hair collection at ARF is 100 hairs per donor. As hair color, texture, and length vary by individual, this protocol could be improved by increasing the amount of hair collected to be an estimated mass of 100 mg. This amount of hair would allow analysis of light stable isotopes, as well as radiogenic and trace element analyses. There would be a surplus of sample still available for other analyses not related to isotope analysis, as well. Sample size from intake hair samples was occasionally a limitation in this study, and increasing the amount of hair collected from each donor at intake would improve future study options.

Based upon the results of this study, isotope analysis of human hair shows great promise for providing information about individual diet and geographic travel history. When used with consideration for the effects of time and environmental exposure, human hair isotope ratios can add valuable details to a biological profile. In the absence of other identifying characteristics such as fingerprints or DNA profiles, isotope signatures should be considered as a viable analytical tool. Isotope analysis is not cost-prohibitive and should be considered as an analytical option in forensic human identification efforts. Human hair provides a record of the weeks and months prior to death and should therefore be collected and curated for potential isotope analysis when available. Used in

combination with tooth enamel, which provides information regarding childhood diet and geography, and bone, which can provide information regarding the last five to ten years of life, isotopes from hair can complete a rough timeline of individual life history recorded in human tissues. These individual life histories, when combined with the biological profile, provide a powerful analytical tool for identification of unknown human remains.

This study demonstrated both the potential and limitations of isotope analysis of human hair. Carbon and nitrogen isotope ratios in human hair, commonly used to make dietary inferences, undergo little change over time and are more reliable than hydrogen, oxygen, and strontium isotope ratios, which are impacted by the surrounding depositional environment. This study revealed that isotope ratios of human hair can change postmortem and are influenced by geographic placement location, surface or burial placement, and duration of exposure. Isotope ratios of human hair, despite these postmortem changes, can still provide valuable information regarding geographic travel history. As these postmortem changes are better understood, they can be accounted for in predictive models, thereby improving the accuracy of the models. Isotope analysis of human hair can provide a suite of information about diet and geography. When human hair is analyzed postmortem, it is important to acknowledge that taphonomic processes can impact isotope ratios. Deposition environment, placement conditions, and duration of exposure can all influence isotope ratios of human hair.

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## **APPENDIX**

## **APPENDIX I**

### **INSTRUMENT TUNING PARAMETERS**

Instrument settings

RF power 1550 W

Cool gas flow 14.0 L/min

Auxiliary gas flow 0.8 L/min

Sample gas flow 1.01 L/min

CCT gas flow 4.34 mL/min

CCT gas 99.999% He

Nebulizer: 400 uL/min PFA-ST nebulizer (Elemental Scientific Incorporated, Omaha, NE)

Sample introduction system: Peltier cooler at 2.7°C, peristaltic pump at 10 rpm  
information values for Ca, Cu, Mg, Mn, Sc and Se are also listed, but Sr, Pb and other elements analyzed are not listed. For soil bioavailable leaches, no international standard materials exist. However, 10% of samples were leached, processed and analyzed in triplicate to verify reproducibility. Water samples were analyzed in parallel with SLEW-3, SLRS-4 and SRM 1640a to cover the range of ionic strengths of samples. For teeth and bones, we processed NIST 1400 (bone ash) and CUE-001 (an in-house standard in use by Professor Kelly Knudson's lab for more than ten years).

Additional Standards for Strontium run included a gravimetrically spiked standard to determine accuracy when measuring mass-dependent Sr isotopes ("GravSRM"), with a measured value of  $0.709910 \pm 0.000023$  ( $2\sigma$ ,  $n = 70$ ). A matrix-doped standard (SRM-987

Ca/Sr series) series doped at Ca/Sr ratios of 10, 100, 200, 350 and 500 was also used, and the values were always within error of the standard. An SRM-987 standard with a concentration of only 50% of that of the bracketing standard was doped with Ca to a Ca/Sr ratio of 500 was also included. This standard is designed to verify if poorly concentration matched standards with residual matrix will be reproducible. During this project, this standard gave a value of  $0.710254 \pm 0.000032$  ( $2\sigma$ ,  $n=27$ ). BCR-2 (a basalt available from the USGS) gave a value of  $0.705025 \pm 0.000063$  ( $2\sigma$ ,  $n=3$ ; three replicate chemical purifications). The literature value is  $0.705015 \pm 0.00005$  (Ma et al., 2013; Fantle, 2015).

- CUE-001 (an in-house llama bone used in Professor Kelly Knudson's lab, available upon request) gave a value of  $0.704445 \pm 0.000004$  ( $2\sigma$ ,  $n=3$ , one chemical purification, three replicate analytical measurements). The literature value is  $0.704455 \pm 0.000009$  (Romaniello et al., 2015).

- IAPSO (salinity standard for seawater, available from OSIL Environmental Instruments and Systems, UK) gave a value of  $0.709184 \pm 0.000046$  ( $2\sigma$ ,  $n=15$ ) for 11 separate chemical purification aliquots and  $0.709182 \pm 0.000025$  ( $2\sigma$ ,  $n=18$ ) for replicate analyses of a single chemical purification aliquot. The literature value is  $0.709182 \pm 0.000004$  (Ma et al., 2013).

- NIST 1400 (bone ash) gave a value of  $0.713118 \pm 0.000024$  ( $2\sigma$ ,  $n=10$ ) for 10 independent chemically purified aliquots. The literature value is  $0.713150 \pm 0.000160$  (Galler et al., 2007).

## APPENDIX II

### ADDITIONAL STANDARDS RUN IN PARALLEL

Additional standards run in parallel during this project included

- a gravimetrically spiked standard to determine accuracy when measuring mass-dependent Sr isotopes (“GravSRM”), with a measured value of  $0.709910 \pm 0.000023$  ( $2\sigma$ ,  $n = 70$ )
- a matrix-doped standard (SRM-987 Ca/Sr series) series doped at Ca/Sr ratios of 10, 100, 200, 350 and 500; the values were always within error of the standard
- a standard (SRM-987) with a concentration of only 50% that of the bracketing
- standard and doped with Ca to a Ca/Sr ratio of 500. This standard is designed to verify if poorly concentration matched standards with residual matrix will be reproducible. During this project, this standard gave a value of  $0.710254 \pm 0.000032$  ( $2s$ ,  $n=27$ ).
- BCR-2 (a basalt available from the USGS) gave a value of  $0.705025 \pm 0.000063$  ( $2s$ ,  $n=3$ ; three replicate chemical purifications). The literature value is  $0.705015 \pm 0.00005$  Ma et al (2013) and Fantle (2015).
- CUE-001 (an in-house llama bone used in Professor Kelly Knudson’s lab, available upon request) gave a value of  $0.704445 \pm 0.000004$  ( $2s$ ,  $n=3$ , one chemical purification, three replicate analytical measurements). The

literature value is  $0.704455 \pm 0.000009$  (Romaniello et al., 2015).

- IAPSO (salinity standard for seawater, available from OSIL

Environmental Instruments and Systems, UK) gave a value of  $0.709184$

$\pm 0.000046$  (2s, n=15) for 11 separate chemical purification aliquots and

$0.709182 \pm 0.000025$  (2s, n=18) for replicate analyses of a single chemical purification aliquot. The literature value is  $0.709182 \pm 0.000004$  (Ma et al.,

2013).

- NIST 1400 (bone ash) gave a value of  $0.713118 \pm 0.000024$  (2s, n=10)

for 10 independent chemically purified aliquots. The literature value is

$0.713150 \pm 0.000160$  (Galler et al., 2007)

## **VITA**

Tiffany Bivens Saul is the daughter of Bill and G'nell Bivens. A native of Tennessee, she was born and raised in Cleveland, TN, where she graduated from Bradley Central High School with honors (1998). She received her Bachelor of Science in Anthropology (2002) and Master of Science in Biology (2013) from Middle Tennessee State University in Murfreesboro, TN. Tiffany was accepted into the anthropology doctoral program at the University of Tennessee in Fall 2013, where she has conducted research in collaboration with the Forensic Anthropology Center. She currently resides in Nashville, TN with her husband Joe. She will join Middle Tennessee State University as a Research Assistant Professor in Fall 2017.