VALIDATING ISOSCAPING METHODS: A STUDY OF OXYGEN, STRONTIUM, AND SULFUR

by

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DEDICATION

I would like to dedicate this work to my parents, Tracy Tucker, BJ and Abby Tyner. I am so incredibly grateful for the love and support that you all of provided me throughout the years. Thank you for the constant encouragement and the reminders that anything is possible with enough willpower.

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LIST OF ABBREVIATIONS

Abbreviation	Description
FACTS	Forensic Anthropology Center at Texas State
TXSTDSC	Texas State Donated Skeletal Collection
FARF	Forensic Anthropology Research Facility
DPAA	Defense POW/MIA Accounting Agency
IRMS	Isotope Ratio Mass Spectrometer
ICPMS	Inductively-Coupled Plasma Mass Spectrometer
METAL	Metals, Environmental and Terrestrial Analytical Laboratory

ABSTRACT

Isotopic analysis can be a useful tool for forensic anthropologists when trying to identify unknown persons. Specifically, oxygen, strontium, carbon and nitrogen from teeth, bone, and hair have been used to evaluate and predict geographic location and diet of human remains. By using ten individuals from the Texas State Donated Skeletal Collection with known residential histories this study aims to provide insight on the precision and accuracy of this tool. Two samples were taken from each donor, one tooth and one portion of one cortical bone, in order to evaluate childhood and adult geolocations.

Oxygen and strontium samples were prepared for each sample and the resulting values used to create a predictive isoscape. This map was then compared to the reported place of birth and end of life residency locations. Sulfur analysis was also performed on each cortical bone sample to determine if sulfur isotope ratios of human bone may be useful for geolocation purposes. Analysis of the oxygen isotope values were postponed due to the COVID-19 pandemic; however, strontium ratios accurately predicted the location of 60% of the analyzed individuals. The ten donors were separated into inland and coastal populations, but no significant differences were found using sulfur analysis.

I. INTRODUCTION

Statement of Purpose and Problem

Stable isotopes from multiple tissues (i.e., teeth, bones, hair, and nails) have been used in forensic anthropology as a geolocation tool to help narrow down the missing persons list for human remains by predicting the possible geographical region of birth, adult residence, diet, and recent travel history (Bartelink & Chesson, 2019; Benson et al., 2006; Aggarwal et al., 2008; Chesson et al., 2018; Katzenberg, 2008; Fry, 2006; Rauch et al., 2007). However, few studies have tested the validity of using isotopes on a sample with known geographic location data. Therefore, there is a need to test the validity of stable isotopes to narrow geographical location in the United States. The purpose of this thesis is to validate current methods using bone and teeth against a sample with known adult geographic residence using individuals donated to the Forensic Anthropology Center at Texas State (FACTS). The chosen individuals all have detailed, self-reported geographic residences making them ideal for this study. Using a multi-isotope approach, a predictive map, known as an isoscape (West et al., 2008; West et al., 2010; Bowen, 2010), was created to compare individual isotope ratios to previously collected oxygen and strontium isotope data to predict the most likely locations of origin. This prediction was then compared to the self-reported data to test the accuracy of current methods.

This research addresses two main questions:

1. To what extent are isotope mapping methods (isoscapes) accurate predictors of geolocation in the United States?
2. Can sulfur isotope ratios be used to narrow the geolocation? Specifically, can sulfur be used to infer a coastal or inland location prior to death?

Intellectual Merit and Broader Impact

Isoscaping methods are used in multiple areas of study. Archaeologists and paleoanthropologists often use strontium signatures to infer if an individual has moved geographical locations during their lifetime (Beard & Johnson, 2000; Katzenberg, 2008; Schwarcz & Shoeninger, 1991; Price et al., 2000). Understanding how accurate and precise these measurements are could improve future studies, as well as support previous findings. Multi-isotope isoscapes can also be used in modern forensic cases to infer location prior to death, or recent residency to aid in identification of human remains (Benson et al., 2006; Bartelink & Chesson, 2019; Aggarwal et al., 2008; Ehleringer et al., 2010; Juarez, 2008; Katzenberg & Krouse, 1989; Holland et al., 2012; Cerling et al. 2016).

There are no standard methods in place for isotope analyses to determine geographical origins, and forensic anthropologists and archaeologists trying to identify locations of origin for unidentified human remains via isotopes use various methods (Pestle et al., 2014). However, very few of these methods have been validated against a known sample, especially one as well documented as the TXSTDSC. This study aims to allow greater insight into the utility of stable isotope analysis and influence how future researchers approach these analyses as well as if the methods meet the scientific standard for forensic casework. In addition, this study aims to lead to a better understanding what factors most affect sulfur isotope ratios in human tissues. Sulfur has been analyzed alongside carbon and nitrogen to help determine if sulfur can indeed be associated with location or if dietary and pollution factors are too confounding (O'Brien, 2015; Chesson et al., 2018; Nehlich, 2015; Richards et al., 2001).

II. LITERATURE REVIEW

All human beings incorporate elemental isotopes (referred to as isotopes from this point forward) into their tissues from their surrounding environment in multiple ways. Individuals take in isotopes from food sources, water sources, and the air exchanged while breathing. These isotopes are then used within the body and assimilated into an individual's tissues with new cell growth. These tissues and their isotopic signatures can then provide different information about the geographic location of residency as well as travel history (Chesson et al., 2018; Bartelink & Chesson, 2019; Ehleringer et al., 2010; Juarez, 2008; Cerling et al. 2016).

In cases of unidentified remains, the biological profile is useful for a first pass filter, greatly reducing the number of potential matches. Once completed, investigators may still be left with a description that matches a large number of people in the world's population. Residential information can help to further concentrate the search for matching missing persons reports. Using multiple isotopic signatures is the most highly recommended method to indicate residential information as single isotope approaches are often unreliable due to their broad predictions; with a single isotope, the likelihood of multiple locations sharing a signature greatly increases (Bartelink et al., 2014; Bender et al., 2015; Rauch et al., 2007; Chesson et al., 2018; Laffoon et al., 2017; Warner et al., 2018). When more than one isotope is used, it eliminates much of this ambiguity by narrowing these prediction ranges to an overlapping area of interest.

While oxygen and strontium are commonly used in location and migration studies, sulfur is typically used to infer an individual's diet (Chesson et al., 2018; Richards et al., 2001; Nehlich, 2015) but may aid in separating individuals living inland

from those living on the coast (Bartelink & Chesson, 2019; Chesson et al., 2018; Bender et al., 2015; Valenzuela et al., 2011). This study will further explore if sulfur may be used to also infer location in modern humans. Individuals were chosen to represent both inland and coastal populations, then the sulfur ratios for each group were compared along with carbon and nitrogen values.

Elemental Chemistry

Each atom consists of three main particles. Positively charged protons and neutrally charged neutrons are found in the nucleus and add mass to the atom, while negatively charged electrons are found outside of the nucleus in a charged field and have negligible mass. An individual element is described by its atomic number, or total number of protons present. Each element (e.g. oxygen, strontium, and sulfur) has two or more isotope forms. Isotopic forms are created when an element has a different number of neutrons, causing a difference in overall mass. When this mass difference becomes too great, the element begins to decay over time and loses nuclear mass it is then considered unstable or radioactive, as is the case with ^{14}C (Fry, 2006).

If an element does not decay over time, it is considered stable and remains active in an ecosystem in its original form (Hoefs, 2009). In order to conduct isotope analysis, the two most common, naturally occurring isotopes of that element are measured, and a ratio of the two is calculated (see Table 2.1). For example, ^{18}O and ^{16}O are the two most common isotopes of oxygen, and a ratio of these isotopes is derived using a per mil (‰) equation (see below) and is represented as $\delta^{18}O$ (Ehleringer and Rundel, 1989; Brand and Coplen, 2012; West et al., 2006). This delta value in per mil notation will be how each element is represented moving forward. Strontium values are not derived using this

equation and are represented as absolute values.

$$\delta X_{\text{standard}} = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000$$

Table 2.1: Naturally occurring isotope abundances, reproduced from Katzenberg, 2008.

Element	Isotope	Abundance (%)
Hydrogen	^{1}H	99.985
	^{2}H	0.015
Carbon	^{12}C	98.89
	^{13}C	1.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{16}O	99.759
	^{17}O	0.037
	^{18}O	0.204
Sulfur	^{32}S	95.00
	^{33}S	0.76
	^{34}S	4.22
	^{36}S	0.014
Strontium	^{84}Sr	0.56
	^{86}Sr	9.86
	^{87}Sr	7.02
	^{88}Sr	82.56

Tooth Biology and Chemistry

The process of tooth growth and development is well-documented (Gustafson and Koch, 1974; Anderson et al., 1976; Hillson 1996). Because of this known timeline, isotopic data derived from tooth enamel can be used to inform researchers about the location and diet of an individual during their childhood years. Tooth development begins within a space in the jaw known as a tooth crypt where the first portion of the tooth to form is the enamel cap. Enamel is the mineralized portion of the tooth crown that will eventually become exposed to the environment and does not typically remodel once formed. The tooth crown's growth is completed within the crypt, including the inner portions consisting of dentin and the pulp chamber (White, Black, & Folkens, 2012; Hillson, 1996). Each tooth has its own developmental timeline and with the exception of

the third molars, most tooth crowns have developed by the age of 8. Third molars typically complete crown growth between the ages of 12 and 15 (Mincer et al., 1993).

A majority of the mineral component found in enamel is hydroxyapatite which has a chemical formula of $Ca_{10}(PO_4)_6(OH)_2$, this makeup allows for constant substitution. For example, strontium (Sr), though heavier than calcium, has the same chemical charge and size and can easily substitute for calcium in this formula, allowing researchers to derive strontium ($^{87}Sr/^{86}Sr$) ratios (Burton, 2008; Beard & Johnson, 2000). Similarly, a carbonate (CO_3) group can replace the phosphate (PO_4) groups in the formula allowing carbon ($\delta^{13}C$) ratios to be derived (Katzenberg, 2008; Burton, 2008).

Bone Biology and Chemistry

Isotopic analyses focus on cortical bone rather than trabecular bone. Cortical bone is compact and made two main components: hydroxyapatite and collagen (White, Black, & Folkens, 2012). Hydroxyapatite or apatite is the inorganic portion of bone and accounts for approximately 70% of the material in dry bone. The other 30% is made up of organic materials, of which a majority is bone collagen (Katzenberg, 2008). Collagen weaves through the hardened, crystal structure of the apatite allowing enough flexibility that bone can respond to a certain amount of stress without breaking. Unlike tooth enamel, bone will regenerate and remodel itself throughout an individual's lifetime, reflecting the most recent diet and location as it does (Branca et al. 1992; Beard and Johnson, 2000; Hedges et al., 2007; Robling & Stout, 2008). Because of this process, isotopes derived from bone apatite and collagen will reflect the diet and location from the last few years of an individual's life, which is advantageous for forensic cases.

Both bone apatite and bone collagen can be isolated through different methods

(described in detail later). As described above, bone apatite allows for frequent elemental substation and can thus be isolated and used to study the isotope ratios of strontium, lead, and other trace elements (Burton, 2008; Aggarwal et al., 2008). Therefore, isolating bone apatite can allow researchers to derive strontium ($^{87}Sr/^{86}Sr$), carbon ($\delta^{13}C$), and oxygen ($\delta^{18}O$) ratios (Burton, 2008; Beard & Johnson, 2000; Chesson et al., 2018; Katzenberg, 2008; Bataille and Bowen 2012; Coelho et al., 2017; Bataille et al., 2018; Laffoon et al., 2017; Rauch et al. 2007). Though carbon can be derived from bone apatite, it occurs in greater quantities in bone collagen. Bone collagen is a protein built from amino acids either created within the body taken in from an external source (Klepinger, 1984). These amino acids are made up of a multitude of elements, including carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$). Thus, bone collagen is the tissue that is considered the most reflective of an individual's diet (Katzenberg, 2008).

While dietary isotopes (such as carbon and nitrogen) may vary between individuals, even in the same geolocation, oxygen, hydrogen, and strontium are typically varied by region rather than by individual (Bartelink & Chesson, 2019**;** Chesson et al., 2018; Bataille et al., 2018; Ehleringer et al., 2008). Oxygen and hydrogen are elements that can be derived from water sources, while strontium is located in soil and geological formations (Bataille & Bowen 2012; Beard & Johnson, 2000). Because these isotope ratios are spatially correlated, it is possible to create a map of their predicted values based on ground water and rainfall, as well as geological samples. These maps are commonly known as isoscapes (Valenzuela et al. 2011, Bataille et al. 2018, Laffoon et al. 2017, Warner et al. 2018, Wassenaar et a. 2009, West et al. 2008; West et al., 2010).

Isoscapes

An isoscape is the name given to a map representing isotope values for a given region (West et al., 2008; West et al., 2010). Isoscapes are generated by using data collected from throughout the region and predictive modeling to create a gradient in the regions without known data (Bowen, 2010). Several isotopes can be linked to regional values (discussed in detail below), due to their association with location, precipitation patterns, and geological formation (Valenzuela et al., 2011, Bataille et al., 2018, Laffoon et al., 2017, Warner et al., 2018, Wassenaar et al., 2009, West et al., 2008). The isotope values predicted in these maps can be compared to ratio values taken from tissue samples. Similar values may indicate that an individual resided in a particular location or spent some amount of time, such as long-term traveling, in that location (West et al. 2008; West et al., 2006; Meier-Augenstein, 2010).

Hydrogen and Oxygen

Hydrogen (δ^2H) and oxygen (δ^{18}O) are available to humans from many sources. Though hydrogen values can be determined, previous studies have found it highly unreliable for predicting location (Bartelink & Chesson, 2019; Chesson et al., 2018). As such, hydrogen will not be included in this research. The most common sources of δ^{18}O are drinking water or atmospheric oxygen that is inhaled (Ehleringer et al., 2008). Since plants are physiologically taking in water, their tissues are assimilating those signatures and reflecting them. Thus, another source of δ^{18}O can be diet based incorporation. δ^{18}O values are affected by precipitation and environmental conditions (Chesson et al. 2018; Bowen & Revenaugh, 2003), but a general predictive isoscape is available through the University of Utah at waterisotopes.org (Bowen et al. 2007; Bowen et al. 2011).

Oxygen ratios can be derived either from groundwater (Wassenaar et al. 2009,

Bowen et al. 2011) or from tap water (Warner et al. 2018, Bowen et al. 2007). These values have been used to create isoscapes in the past and with predictive modeling and have been found to be sufficient to help identify provenance of human remains from bone and hair samples numerous times (Wassenaar et al., 2009; Warner et al., 2018; Laffoon et al., 2017; Meier-Augenstein & Fraser 2008; Meier-Augenstein, 2010).

Strontium

$^{87}Sr/^{86}Sr$ is the isotope ratio used to infer strontium values of geologic formations. Strontium is an element found in the bedrock and soil of an environment. It is of note that ^{87}Sr can be a produced by radiogenic means through the decay of rubidium (^{87}Rb). Thus, the rubidium content of bedrock can influence the local values of $^{87}Sr/^{86}Sr$ present and should be considered, as should the age of the bedrock being sampled (Chesson et al., 2018; Beard & Johnson, 2000). Strontium values are often incorporated into the tissues of vegetation if other elements are lacking in the soil (namely calcium). Thus, values can be transferred to humans from dietary sources (Blum et al., 1993; Crowley et al., 2017; Chesson et al. 2018; Beard & Johnson, 2000). Strontium can also be found in water sources, as bedrock is eroded, allowing another potential source for human incorporation (Chesson et al., 2018; Chesson et al., 2012; Bataille and Bowen, 2012). Though ^{87}Sr is derived from ^{87}Rb, the rate of decay is not fast enough to change the strontium ratio values within modern remains that are actively being evaluated (Crowley et al., 2017).

As discussed earlier, strontium has a similar ionic size and charge to calcium, allowing it to replace calcium in hydroxyapatite (Burton, 2008). Unlike the other elements being investigated, strontium does not fractionate through the food web. This means that strontium ratios found in human remains should directly reflect the original

source of bedrock (Coelho et al., 2017; Beard & Johnson, 2000). This allows us to evaluate strontium as a true value, rather than utilizing the per mil calculation necessary for other elements. The $^{87}Sr/^{86}Sr$ ratio has been previously sampled and signatures from faunal remains, soil samples, and rocks have been used to create isoscapes (Bataille et al, 2018; Laffoon et al, 2017) using predictive modeling (Bataille et al. 2018, Bataille and Bowen 2012) and have been useful for identification purposes (Bartelink & Chesson, 2019; Laffoon et al., 2017; Rauch et al., 2007; Aggarwal et al., 2008).

Sulfur

Sulfur is present in many forms in the environment including atmospheric sulfur dioxide, terrestrial and marine sulfate, and hydrogen sulfide. Sulfur dioxide can enter the atmosphere by natural means (such as volcanic activity) or as a byproduct of human activity (as in the use of fossil fuels). With many different potential sources, $\delta^{34}S$ signatures are understandably extremely variable (Fry, 2006, Chesson et al., 2018, Bender et al. 2015, Valenzuela et al. 2011; Richards et al., 2001; Nehlich, 2015).

Recently, sulfur has been associated with marine based diets (Bender et al. 2015; Nehlich, 2015) as an increased consumption of marine sulfate, but it has also been associated with geolocation in the past (Valenzuela et al. 2011, Ziegler et al., 2016). Geographic proximity to volcanic activity with increased hydrogen sulfide or a coastline where sulfate might be more prevalent in the sulfur cycle could influence human signatures. In fact, $\delta^{34}S$ in coastal soil often presents with higher values than inland soil (Fry, 2006; Chesson et al., 2018; Nehlich, 2015). Valenzuela et al. created a $\delta^{34}S$ isoscape and suggested that sulfur may be a valid way to trace the origin of human remains (2011). Sulfur's assimilation processes are not well understood, which contributes to the

contradicting explanations among the literature (Bartelink & Chesson, 2019).

Carbon

Before entering the food web in a usable form, inorganic carbon dioxide must be transformed by plants. There are three types of carbon pathways, C_3, C_4, and CAM, each following a different physiological process to "fix" carbon into a usable form during photosynthesis. These pathways create unique $\delta^{13}C$ ratios which can be used to distinguish which types of plants humans and other animals regularly consume (Larsen et al., 2013, Chesson et al., 2018). While CAM plants are not typically a staple food, C_3 and C_4 plants feature prominently in the modern human diet. C_3 plants include rice, wheat, soy, and many fruits and vegetables while C_4 plants include corn, sugarcane, millet and many grass-like species (van der Merwe, 1982; Chesson et al., 2018). By consuming these plants or livestock that have eaten these plants, humans begin to incorporate carbon values into their own tissues; often a mixture of C_3 and C_4 plants.

Nitrogen

Nitrogen, like carbon, enters the food web at a producer level. Plants take in nitrogen from soil through their roots. Several environmental factors influence these initial $\delta^{15}N$ values, namely soil conditions, temperature and precipitation, and the presence or absence of fertilizer (Chesson et al., 2018). Nitrogen fractionates at each trophic level in the food web. As an organism consumes nitrogen, only the heavier isotopes remain in the system while the lighter isotopes are removed as waste. This allows for a positive trend where animals (including humans) have slightly higher $\delta^{15}N$ values the farther up in the food chain they are (Chesson et al., 2018).

Isotope Use in Forensic Anthropology

Although research studies have used known samples to create and validate isoscapes (Laffoon et al., 2017; Warner et al., 2018; Saul, 2017), a majority of cases in a forensic setting may benefit from using isotopes to narrow down identification (Benson et al., 2006; Bartelink & Chesson, 2019; Aggarwal et al., 2008; Ehleringer et al., 2010; Juarez, 2008; Katzenberg & Krouse, 1989; Holland et al., 2012; Cerling et al. 2016). Once residential information is predicted, allowing a more concentrated missing person's search, other methods, such as DNA analysis, may be used to confirm an individual's identity (Bartelink et al., 2014; Meier-Augenstein & Fraser, 2008; Rauch et al., 2007). In these instances, isotopes give an approximate location prior to death allowing law enforcement to compare a smaller pool of possible victims via DNA.

Bartelink et al. (2014) used isotopes to differentiate between United States soldiers and Asian origin soldiers for the Defense POW/MIA Accounting Agency (DPAA). Though both sets of soldiers were in the same region, their diets were significantly different in the years leading up to their war assignment. As isotope values in bone reflect up to the last few years of life, this became a significant way to help identify United States versus Asian origin. Using carbon and nitrogen for diet analysis the researchers were able to delineate between the two groups, then used mitochondrial DNA from a familial database to confirm the identity of three sets of remains, again exhibiting the value of isotopes in forensic anthropology.

Meier-Augenstein & Fraser (2008) similarly used isotope analysis to determine location origins for a murder victim. In this case, isotope ratios from hair showed that the victim had recently moved, as the ratios differed from those found in the bone apatite.

Using this knowledge, the search was narrowed to people who had entered the country recently from one of the predicted regions matched to the bone signatures, and DNA analysis of possible family members was used to confirm the identity of the victim.

However, in cases where DNA analysis and family comparison are unavailable, it is unclear to what extent the methods used for isotope analysis are accurate and reliable. Without such obvious distinctions as dietary difference or a change in residential history, a general isoscape prediction must be consistent. Overlapping isotope values, or similarities between values can make using a single-isotope approach difficult. Most studies state that a more precise analysis can be made by using a multi-isotope approach; this approach is stated to be the ideal option whether using hair, bone, or tooth enamel as a sample source (Bartelink et al., 2014, Bender et al., 2015, Rauch et al., 2007, Chesson et al., 2018, Laffoon et al., 2017, Warner et al., 2018).

Research Statement

The purpose of this project is to test the validity of current stable isotope methods used in forensic anthropology. To do so, this research has used isotope databanks and isoscaping methods available to current forensic anthropologists to create a multi-isotope isoscape of the United States. This research has compared samples from ten individuals in the Texas State University Donated Skeletal Collection (TXSTDSC) with a documented lifetime residence history. Two samples were taken from each donor (tooth enamel and bone) in order to reflect residential history from their childhood as well as their recent past. The isotope ratios derived from these samples were used in combination with the created isoscape to predict the most probable locations; these maps were then compared with the known life histories to evaluate the accuracy of the prediction.

III. MATERIALS AND METHODS

Materials

The Texas State University Donated Skeletal Collection has been used for previous isotope research (Herrmann et al., 2015, Saul, 2017, Gordon et al., 2019). These studies utilized hair (Saul, 2017), bone, and tooth enamel samples (Gordon et al., 2019; Herrmann et al., 2015). More information on previous samples can be found in Appendix Table 1.

Ten individuals from the Texas Stated Donated Skeletal Collection with well-documented geographic residential histories were used. All donors with known geographic residence information were analyzed and those with the most thorough residential history were chosen. Five individuals were chosen based on their close proximity to a saltwater coast (2010.009, 2012.045, 2014.039, 2015.001, and 2016.055), while another five were chosen to represent inland residencies (2009.007, 2011.003, 2011.007, 2012.003, and 2012.035). Table 3.1 details each individual's self-reported residential location for the last 30% of their life. These donor numbers have a corresponding sample ID which will be used for the remainder of this document.

Table 3.1. Individuals from the Texas State University Donated Skeletal Collection sampled for this project.

TXST ID	Sample ID	City, State	Classification	Sample
2009.007	T01-19	Chicago, IL	Inland	Premolar. 7th rib
2010.009	T02-19	Los Angeles, CA	Coastal	Molar, 7th rib
2011.003	T03-19	Memphis, TN	Inland	Premolar, 7th rib
2011.007	T04-19	Homer, NY	Inland	Premolar, 7th rib
2012.003	T05-19	El Paso, TX	Inland	Premolar, 7th rib
2012.035	T06-19	Oklahoma City, OK	Inland	Premolar, 7th rib
2012.045	T07-19	Houston, TX	Coastal	Premolar, 7th rib
2014.039	T08-19	Houston, TX	Coastal	Molar, 7th rib
2015.001	T09-19	Houston, TX	Coastal	Premolar, 7th rib
2016.055	T10-19	Port Orchard, WA	Coastal	Molar, 7th rib

Methods

Sample Collection

Whole teeth were collected from each of the ten donors. The collection of molars was preferred due to the large amount of enamel available; however, if a molar was not available, a premolar was selected (Table 2.1). Before sampling, each tooth was photographed and cast. Then a Dremel tool was used to take a sample of enamel from each tooth, measuring 2.5-3.0 grams in weight. The remainder of the tooth was returned to the TXSTDSC and reassociated with the appropriate donor.

In order to collect samples for apatite and collagen, each donor's ribs were sided and then seriated. The left seventh rib for each individual was taken and a section removed with a Dremel saw. In order to preserve the integrity and length of the element, a small window was cut from the superior portion of the midshaft of the rib. Each section removed weighed approximately 2.0 grams, though variations may have occurred due to individual bone densities. Each of these segments were then cut into two or three smaller fragments for easier transport and analysis.

Sample Preparation

All samples were prepared at Arizona State University's Metals, Environmental and Terrestrial Analytical Laboratory (METAL). Due to the complexity of the preparation process and the numerous methods each sample needed to follow, a flow chart (Figure 3.1) describes each process in detail below.

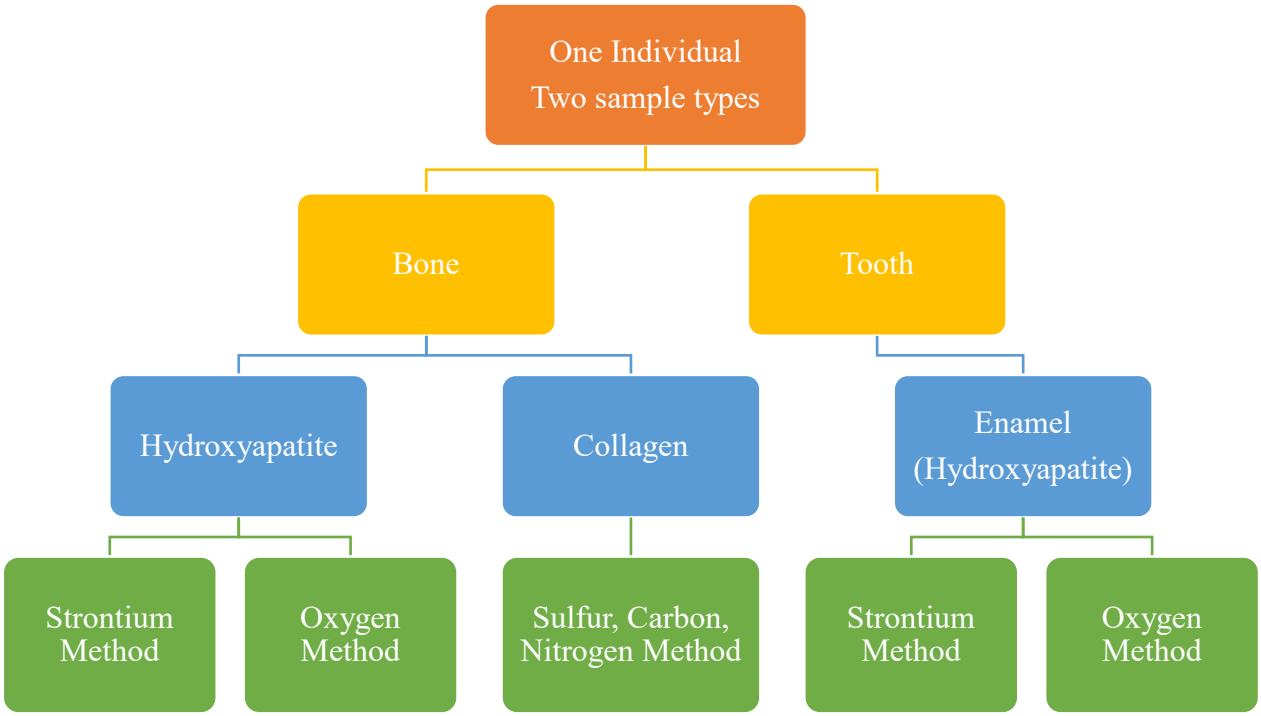

Figure 3.1: A flow chart demonstrating the processes each sample type underwent for analyses. Details of each method can be found below.

Tooth Preparation

Each tooth was rested in 3% hydrogen peroxide (H_2O_2) in order to remove surface contaminants for 48 hours. The teeth were then rinsed with ultra-pure water and brushed lightly with a toothbrush, then left in water to sonicate for 30 minutes. The vibrations caused during sonication were meant to help loosen any remaining surface contaminants before a final rinse. All teeth were then dried for approximately 48 hours at 60° C in a drying oven. Tooth enamel was removed and powdered using a diamond coated, narrow cone Dremel bit with frequent weighing to ensure an adequate amount had been removed. Care was taken to avoid removing dentine, as well as avoiding various fillings and other dental alterations that may contaminate the sample. Powdered enamel was then placed

into a centrifuge tube to be separated for oxygen and strontium analyses as described below.

Bone Apatite Preparation

Rib fragments were initially cleaned with a diamond coated Dremel blade, removing the outer layer of external cortical bone and any remaining trabecular bone. The bone was then powdered using a clean Dremel blade and placed into a centrifuge tube to be weighed and separated for oxygen and strontium analyses.

Bone Collagen Preparation

Rib fragments were cleaned, removing the first layer of cortical bone and remaining trabecular bone. These fragments were then weighed and rinsed with ultra-pure water five times, then left in water to sonicate for 10 minutes before being centrifuged at 4000 RPM for 10 minutes. This rinsing process was then repeated before each sample was placed in pure ethanol (EtOH) and sonicated for 5 minutes, finally being rinsed five more times in ultra-pure water. This chemical cleaning was intended to rid the bone of any surface contaminants that may have remained. The samples were then dried overnight at 60° C in an oven and reweighed after cooling to room temperature.

The bones underwent a demineralization process where each fragment was placed in a 15 mL, acid washed tube and 10 mL of 0.6 M hydrochloric acid (HCl) was added. These samples were stored at 4° C and the HCl changed every 24 hours until the demineralization process was complete. To guarantee the process was complete, each sample was checked for two criteria daily: 1.) the presence of air bubbles on the sample indicates a chemical reaction is still occurring and 2.) when completed, the samples will

be malleable and elastic when manipulated with a probe. The collagen samples were then rinsed five times in ultra-pure water, then stored in water at 4° C.

Elemental Analyses

Oxygen Analysis

The oxygen isotope analysis is performed on the oxygen component of carbonate (CO_3) distributed throughout bone apatite and tooth enamel. An aliquot (targeted to be 20-30 mg with some variation) of each the powdered tooth enamel and rib fragments was placed into 15 mL, acid washed tubes and covered with 10 mL of 30% H_20_2 then left at room temperature for 24 hours. H_2O_2 reacts with the organic materials within the powder, leeching them out into the solution. After the 24-hour waiting period, the samples were centrifuged at 4000 RPM for 10 minutes to force the solid sample to the bottom of the tube for easy discarding of the supernatant. The sample was then rinsed with ultra-pure water, repeating the centrifuge process and discarding the rinse five times. The samples were then placed in an oven at 60* C to be dried.

Once dry, 10 mL of 1M acetic acid buffered with 1M sodium hydroxide was added to each sample. The sodium hydroxide brought the acidity down enough to protect endogenous CO_3 (targeted for analysis) while keeping the solution stable. Any diagenetic CO_3 that may have been introduced to the sample would have been removed during this cleaning and leeched into solution for discard. The acid mixture was removed after centrifuging (4000 RPM, 10 min) at exactly 4 hours in order to prevent the diagenetic CO_3 from re-solidifying. The remaining sample was then rinsed using ultra-pure water and centrifuge five times, then dried at 60° C. This remaining powder was then sent to the University of California at Davis Stable Isotope Facility (SIF) for oxygen analysis on

a GasBench Isotope Ratio Mass Spectrometer. This type of IRMS dissolves the carbonate within the sample into carbon dioxide (CO_2) with anhydrous phosphoric acid. The gaseous CO_2 is then analyzed using a gas chromatography column to calculate the $\delta^{18}O$ values. These methods were modified from Chesson et al. (2019), Crowley and Wheatley (2014), and France and Owsley (2015) by the Gwyn Gordon Group at ASU's METAL.

Strontium Analysis

Strontium is an inorganic component found in hydroxyapatite. The organic components of the apatite were removed by ashing the bone and teeth overnight in a furnace at 800°C. The resulting powder was then weighed and dissolved in 16 M nitric acid (HNO_3). This solution was then diluted with water to create a 5 M stock. The samples were then run on an inductively coupled plasma mass spectrometer (ICPMS) to check for any diagenetic indicators as well as element ratios of calcium and strontium.

Manual columns were cleaned with ultrapure water and loaded with a strontium resin. The resin was then conditioned with 5 M HNO_3, keeping it in a moist state to prevent cracks from forming in the gel. The stock solutions were then loaded into the columns, then rinsed 5 M HNO3. The strontium in the original solution has a high affinity for the resin and remained in the column while the acid rinsed the remaining matrix away. This was then discarded, and ultrapure water was used to elute the strontium, as strontium has a higher affinity for water than the resin. This release was collected and dried down for a short period of time before a nitric acid/hydrogen peroxide solution was added and the containers sealed and placed on a hot plate. This process allowed any remaining organics from the resin or remaining matrix to be oxidized. After oxidation, the samples were dried down once more and reconstituted in 0.32 M HNO_3. A

Multicollector-Inductively Coupled Plasma Mass Spectrometer was then used to determine the $^{87}Sr/^{86}Sr$ ratio. These methods were modified from Romanciello et al. (2015) and Knudson et al. (2010) by the Gwyn Gordon Group at ASU's METAL.

Sulfur, Carbon, and Nitrogen Analyses

Once the demineralization process was completed (samples did not show bubbles and collagen malleable to the touch), the bones were placed into ultrapure water and centrifuged. The water was then discarded and replaced (this will simply be referred to as rinsed from here on), repeating the rinsing process until the pH of the solution post-centrifuge was the same as the initial pH of the ultrapure water, thus showing complete removal of the hydrochloric acid. Once the pH of each sample was stable, a mixture of chloroform, methanol, and ultrapure water was added to remove any lipids, such as grease, which may remain on the bone. This solution was changed every 24 hours until the sample no longer produced an emulsification layer at the top of the tube. The samples were then rinsed and dried at 60° C overnight. Each sample was then placed in 15 mL of 0.125 M sodium hydroxide (NaOH) for 24 hours. This helps to remove any humic substances (such as contamination from soil during placement at FARF). At the end of the 24-hour period, the samples were rinsed five more times then placed in 10mL of 0.03 M hydrochloric acid, then placed in a 70° C oven overnight. The added heat allowed each segment of collagen to solubilize into a liquid, which was then frozen into a solid and freeze dried.

Once freeze dried, the collagen was a powder consistency which was weighted and placed into tin capsules before being sent to the University of California at Davis Stable Isotope Facility (SIF) to undergo sulfur analysis on an Elemental Analysis Isotope

Ratio Mass Spectrometer (EA-IRMS). On this instrument, the samples are combusted and forced to react with tungsten oxide, allowing the powdered collagen to enter a gaseous state. The gasses created are separated and sulfur oxide (SO2) is analyzed by the IRMS determining the $\delta^{34}S$ values. Similarly, N_2 and CO_2 gasses are analyzed for $\delta^{13}C$ and $\delta^{15}N$ values. These methods were modified from France and Owsley (2015) and Marsteller et al. (2016) by the Gwyn Gordon Group at ASU's METAL.

Building an Isoscape: Oxygen and Strontium Predictions

Using standard isoscaping methods (Bowen, 2010; Bataille et al., 2018; Laffoon et al., 2017; Wassenaar et al., 2009; Warner et al., 2018), and existing models, a basemap was created with two layers (Figures 4.1 and 4.2) using ArcGIS software (Esri, 2018). Oxygen data collected by Bowen et al. (2007, 2011) was overlaid with bioavailable strontium data provided by Bataille (personal communication, Bataille et al., 2020). This two-layer base map was then used to evaluate isotope rations from each tooth and bone sample separately, resulting in twenty maps total. Using ArcGIS Pro's classification feature, only areas matching the given strontium value ± 0.001 of the sample were highlighted using the classify feature of ArcGIS Pro (uncertainty determined via personal communication with Bataille, based on map development). This same process was used to highlight matching oxygen values. The reported error rates for the oxygen isoscape are 1.8‰ (Bowen et al., 2007) while the reported Root Mean Squared Error of variability for the strontium isoscape is 0.001 (Bataille et al., 2020, personal communication).

The resulting map was then evaluated for any areas where the two layers intersected were then compared with the self-reported residential histories of the donor. Ratios from the ten tooth samples were compared with geographic information from the

first fifteen years of each individual's life while ratios from the ten bone samples were compared with geographic information from the last twenty years of life. If a majority of the reported city limits fell within the highlighted region, the prediction was considered a success (see Table 4.2).

Coastal versus Inland: Sulfur Evaluation

As this research aims to determine if there is a difference in sulfur ratios between geolocations, these samples were statistically analyzed rather than classified with an isoscape. An Independent Samples T-test was performed to assess if regional difference was present. Following these results, supplementary Pearson correlations were performed between sulfur and carbon, sulfur and nitrogen, and carbon and nitrogen in order to assess dietary influence. To evaluate interpretational rather than statistical significance, the sulfur ratios were used to create a scatter plot (see Figure 4.23).

IV. RESULTS

Question 1: To what extent are isotope mapping methods (isoscapes) accurate predictors of geolocation in the United States?

Oxygen

Oxygen values for each individual are currently unavailable due to the COVID-19 pandemic. The samples are currently in the University of California at Davis Stable Isotope Facility (SIF), which has been under a stay-at-home order since March 19, 2020. They are currently taking steps to reopen and continue processing samples. Once available, the oxygen values will be compared to the existing oxygen isoscape (Figure 4.1).

Figure 4.1: Generated oxygen isoscape for the contiguous United States. This map was created using ArcGIS software and data collected by Bowen et al. (2007) available at http://wateriso.utah.edu/-waterisotopes/pages/data_access/ArcGrids.html.

Strontium

Strontium values for each sample were calculated by ASU's METAL staff after data was collected from the Multicollector-Inductively Coupled Plasma Mass Spectrometer. The sample values were then compared with the previously generated base map of strontium for the United States (Figure 4.2) using the classify feature of ArcGIS.

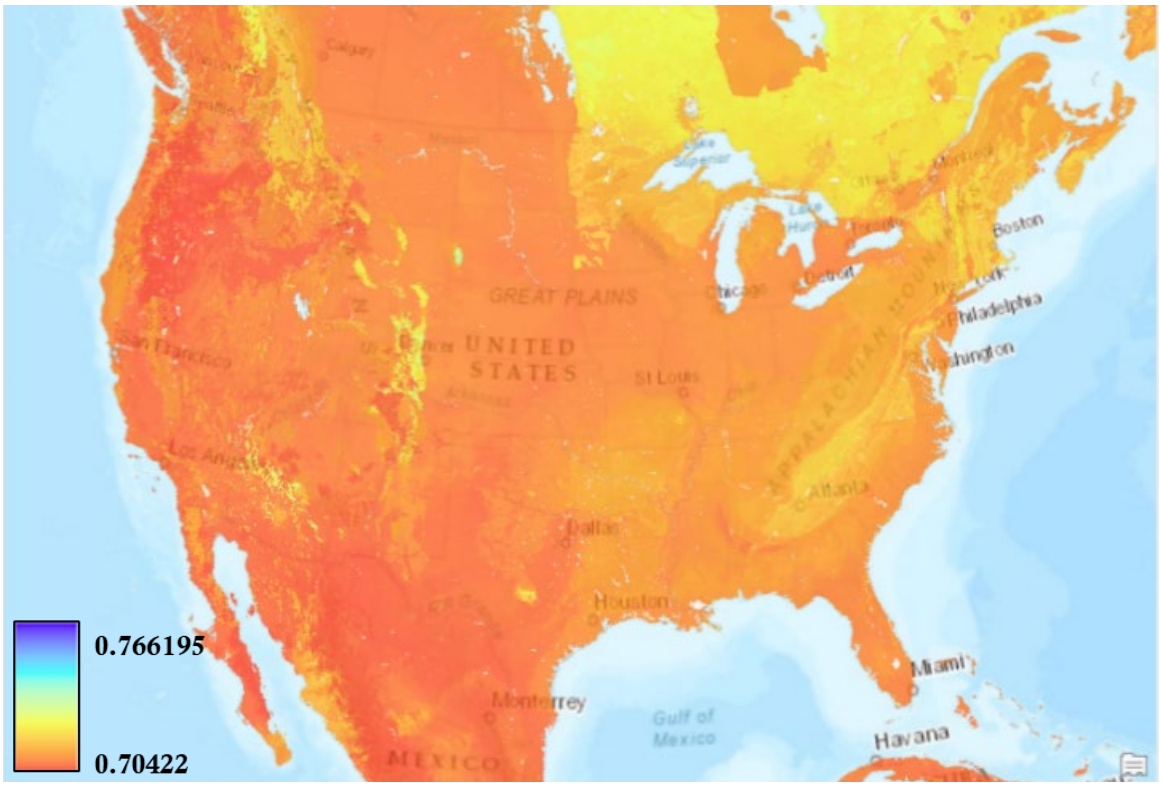

Figure 4.2: Generated strontium isoscape for North America. This map was created using ArcGIS software and data provided by Clément Bataille (personal communication, 2020).

Individual Results

Two samples were taken from each donor as indicated in Table 3.1. These values were used to generate one layer of each sample's dual-element isoscape using the methods described above. While these predictions are not complete without oxygen data, the single layer maps are available below (Figures 4.3-4.22).

Table 4.1: $^{87}Sr/^{86}Sr$ results for each sample.

Sample ID	Sample Type	$^{87}Sr/^{86}Sr$	Reported Location
T01-19a	Enamel	0.70929	Chicago, IL
T01-19b	Cortical Bone	0.70928	Buffalo Grove, IL
T02-19a	Enamel	0.70944	Los Angeles, CA
T02-19b	Cortical Bone	0.70913	Los Angeles, CA
T03-19a	Enamel	0.70960	Locke, TN
T03-19b	Cortical Bone	0.70924	Memphis, TN
T04-19a	Enamel	0.71179	Brohard, WV
T04-19b	Cortical Bone	0.70962	Homer, NY
T05-19a	Enamel	0.70860	San Antonio, TX
T05-19b	Cortical Bone	0.71065	El Paso, TX
T06-19a	Enamel	0.70877	Ft. Leavenworth, KS
T06-19b	Cortical Bone	0.70929	Oklahoma City, OK
T07-19a	Enamel	0.70931	Center, TX
T07-19b	Cortical Bone	0.70876	Houston, TX
T08-19a	Enamel	0.70940	W. Bloomfield, MI
T08-19b	Cortical Bone	0.70870	Houston, TX
T09-19a	Enamel	0.70911	Houston, TX
T09-19b	Cortical Bone	0.70906	Houston, TX
T10-19a	Enamel	0.70985	New York, NY
T10-19b	Cortical Bone	0.70893	Port Orchard, WA

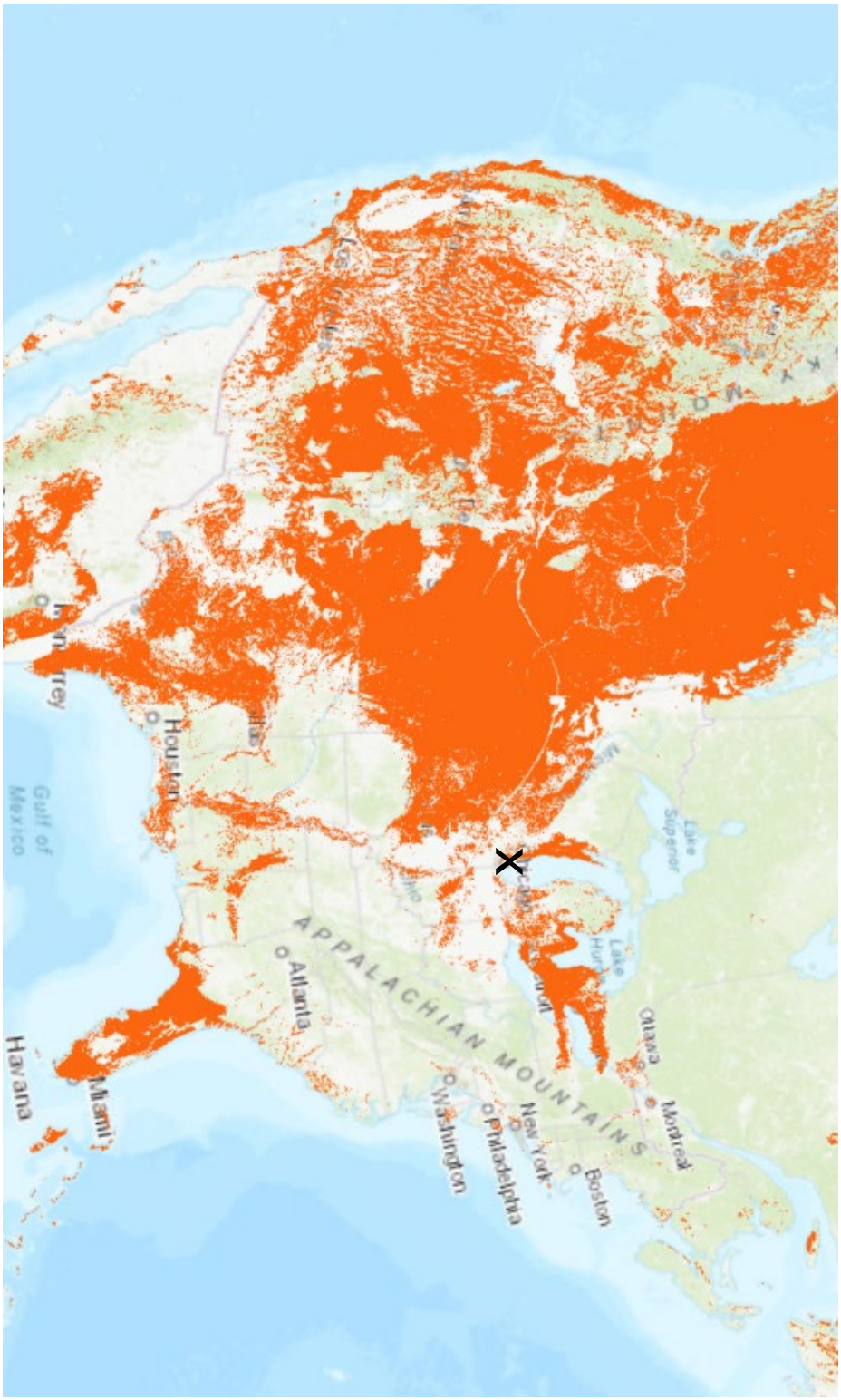

Figure 4.3: The generated strontium isoscape for T01-19a. This individual's place of birth was reported as Chicago, IL, shown by an 'X' on the map above.

Figure 4.4: The generated strontium isoscape for T01-19b. This individual's place of death was reported as Buffalo Grove, IL, shown by an 'X' on the map above.

Figure 4.5: The generated strontium isoscape for T02-19a. This individual's place of birth was reported as Los Angeles, CA, shown by an 'X' on the map above.

Figure 4.6: The generated strontium isoscape for T02-19b. This individual's place of death was reported as Los Angeles, CA, shown by an 'X' on the map above.

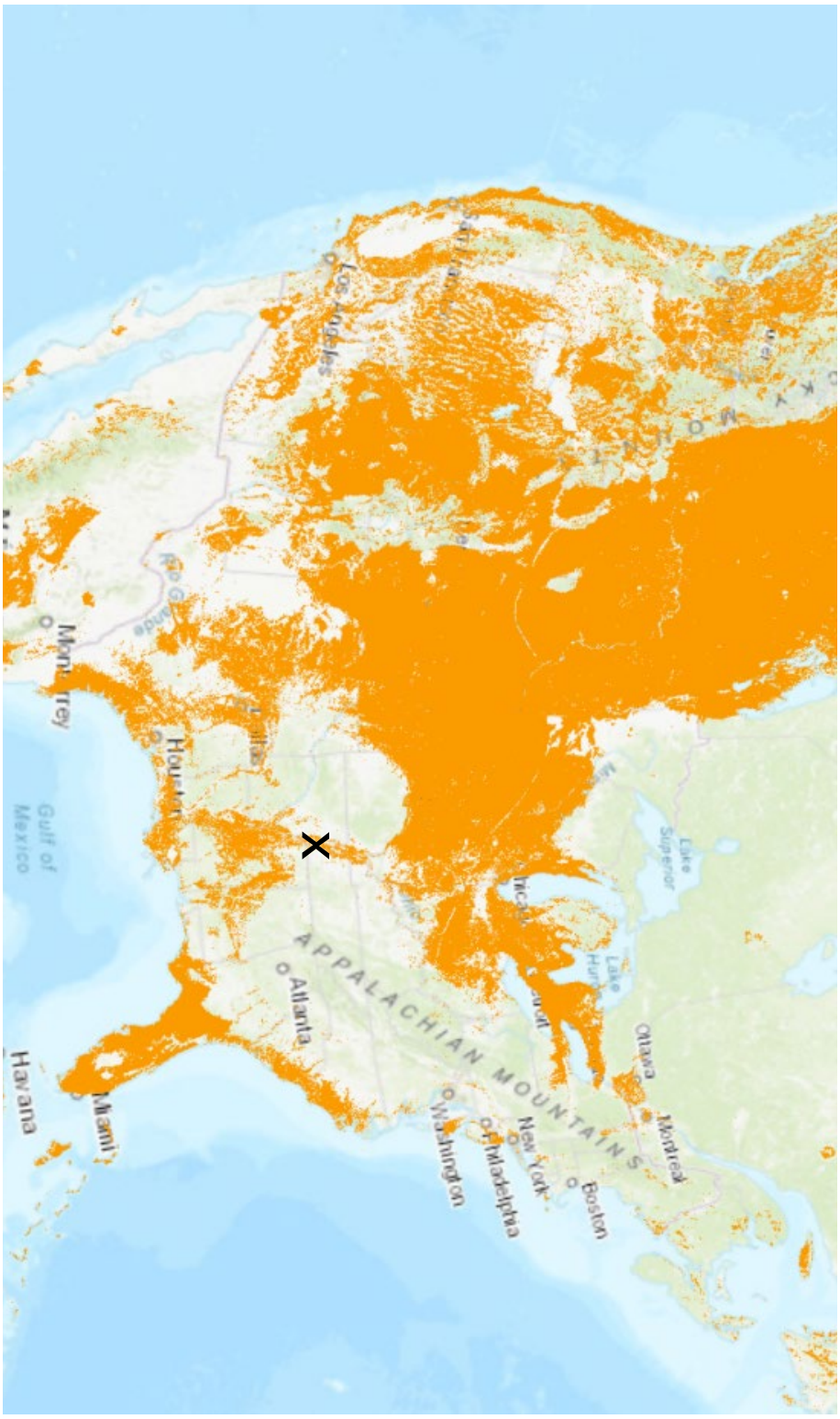

Figure 4.7: The generated strontium isoscape for T03-19a. This individual's place of birth was reported as Locke, TN, shown by an 'X' on the map above.

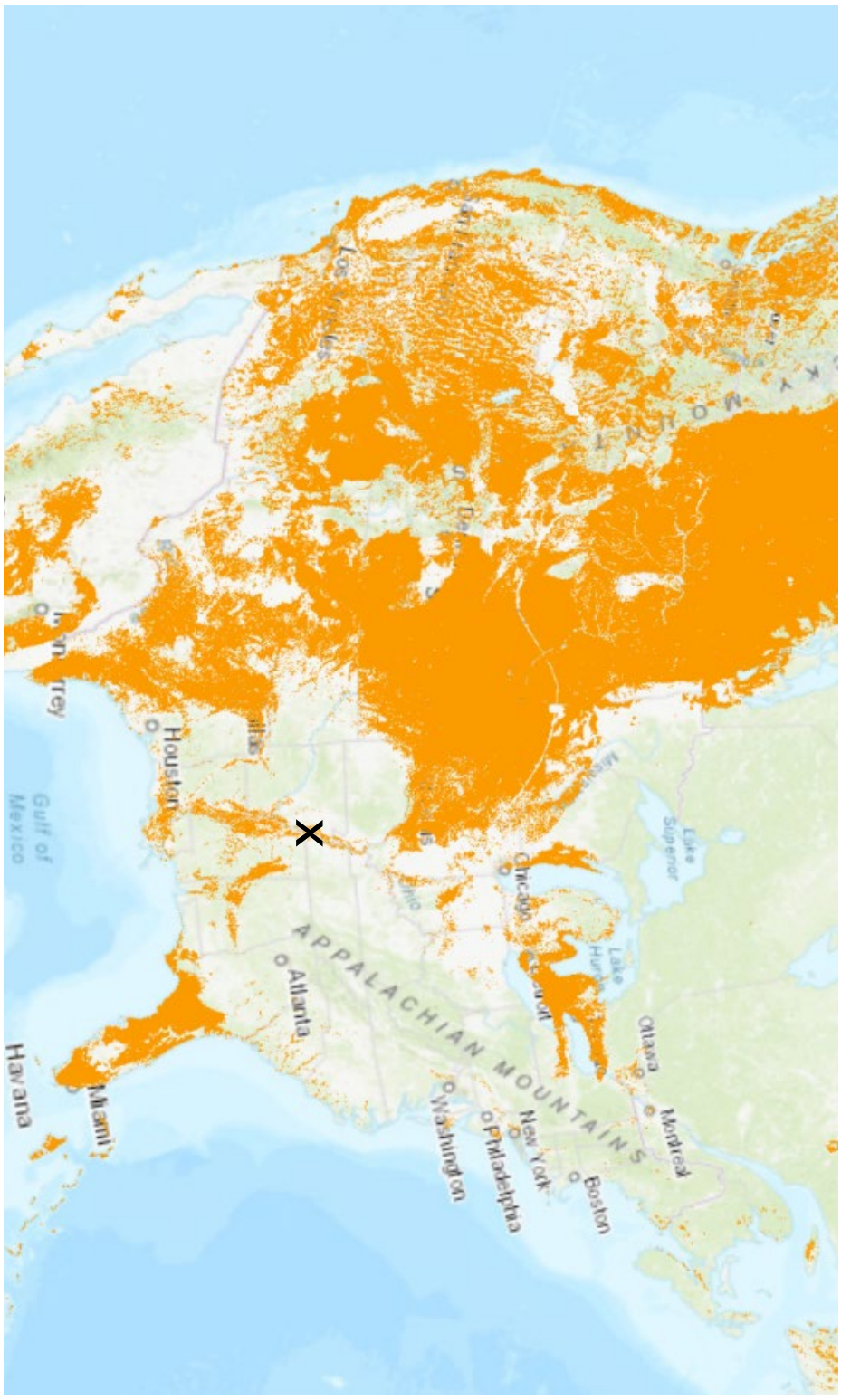

Figure 4.8: The generated strontium isoscape for T03-19b. This individual's place of death was reported as Memphis, TN, shown by an 'X' on the map above.

Figure 4.9: The generated strontium isoscape for T04-19a. This individual's place of birth was reported as Brohard, WV, shown by an 'X' on the map above

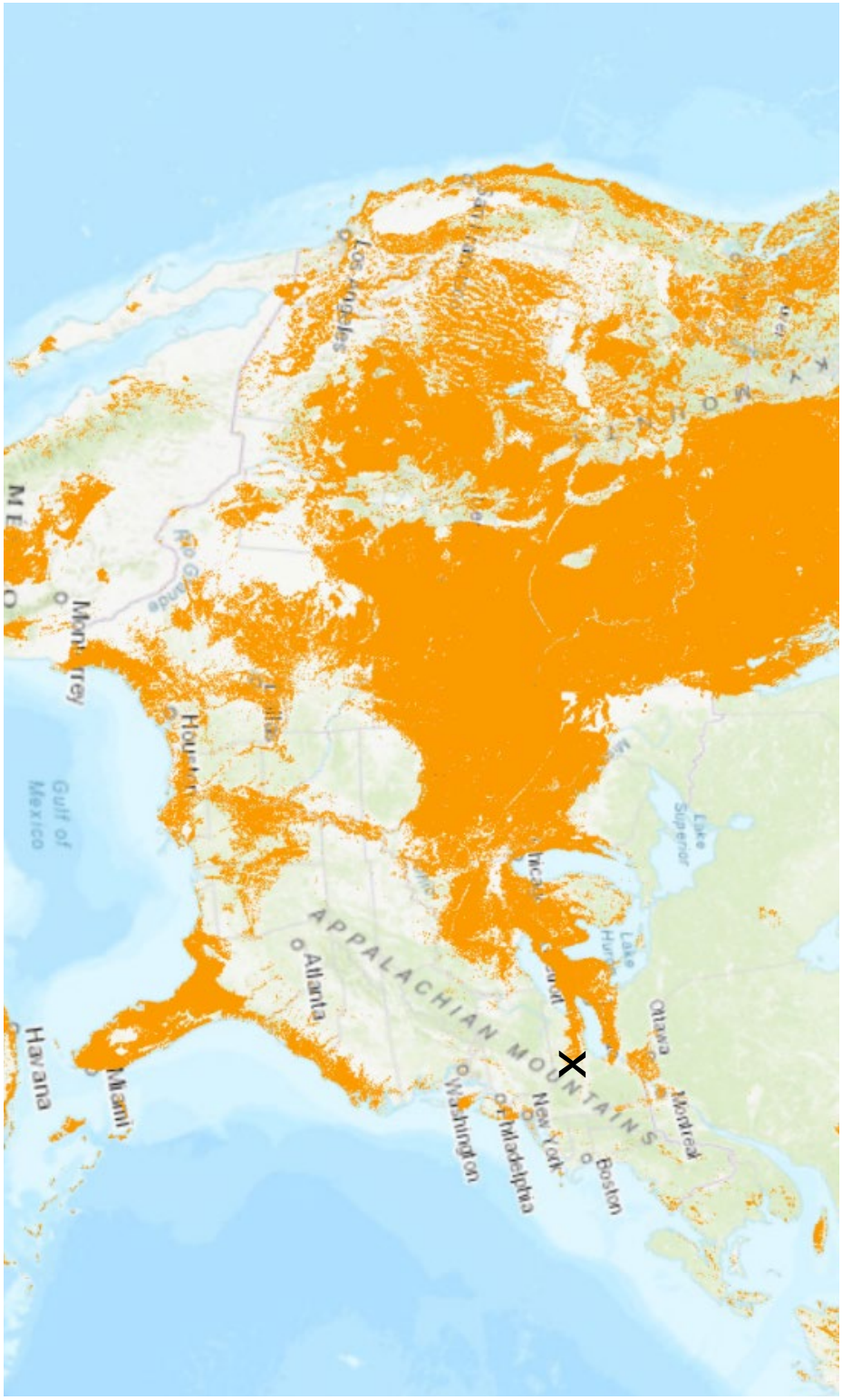

Figure 4.10: The generated strontium isoscape for T04-19b. This individual's place of death was reported as Homer, NY, shown by an 'X' on the map above.

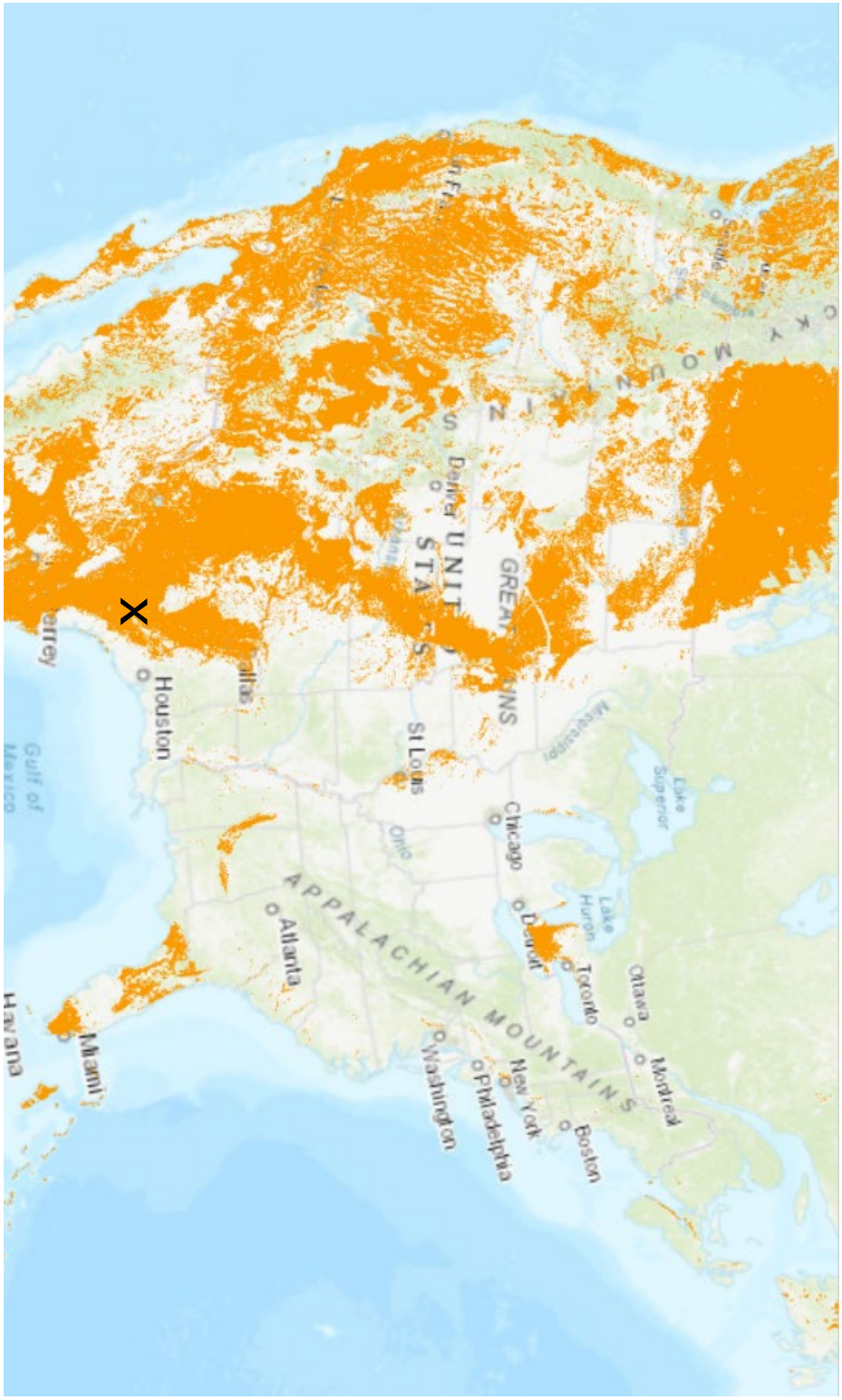

Figure 4.11: The generated strontium isoscape for T05-19a. This individual's place of birth was reported as San Antonio, TX, shown by an 'X' on the map above

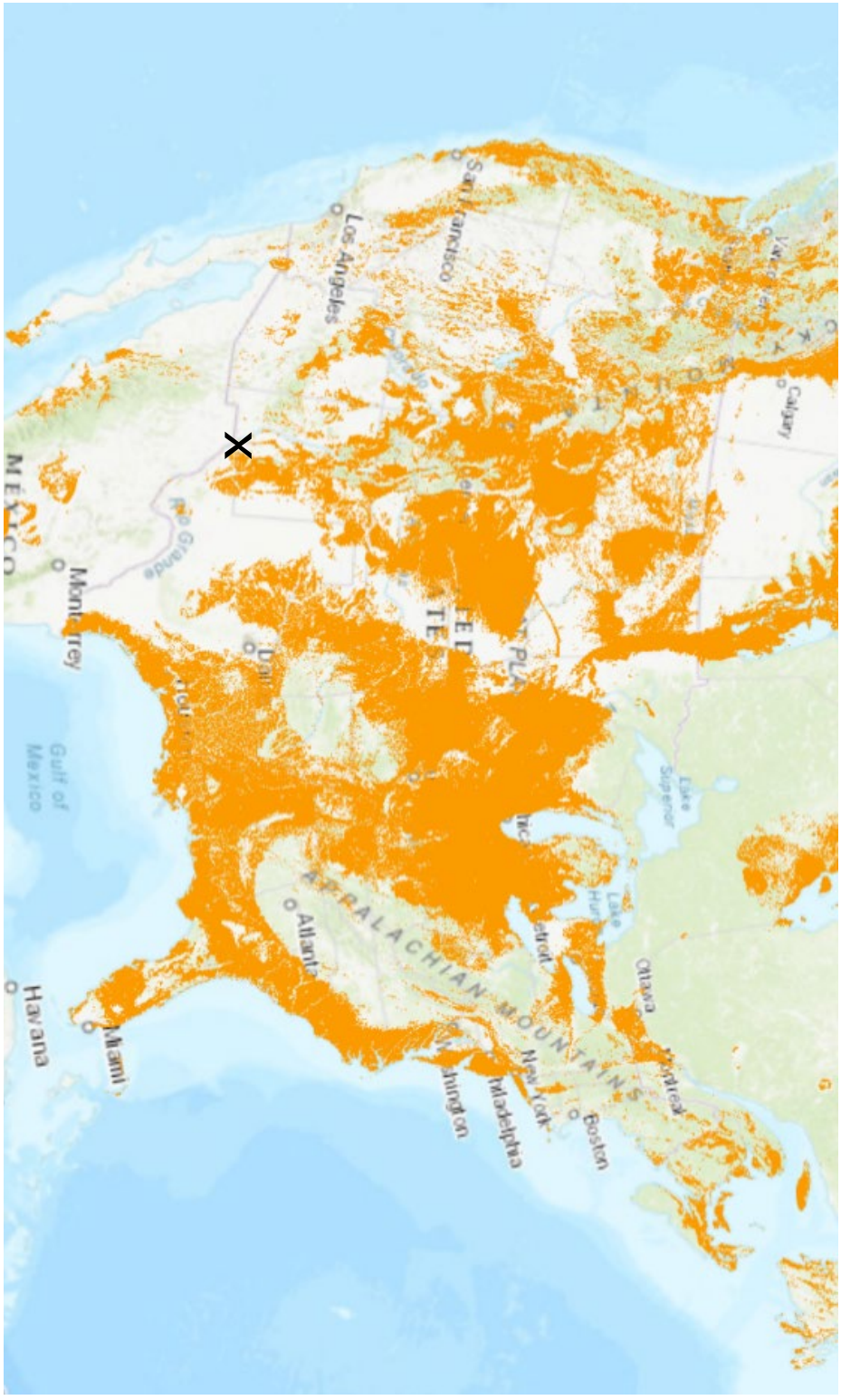

Figure 4.12: The generated strontium isoscape for T05-19b. This individual's place of death was reported as El Paso, TX, shown by an 'X' on the map above.

Figure 4.13: The generated strontium isoscape for T06-19a. This individual's place of birth was reported as Ft. Leavenworth, KS shown by an 'X' on the map above.

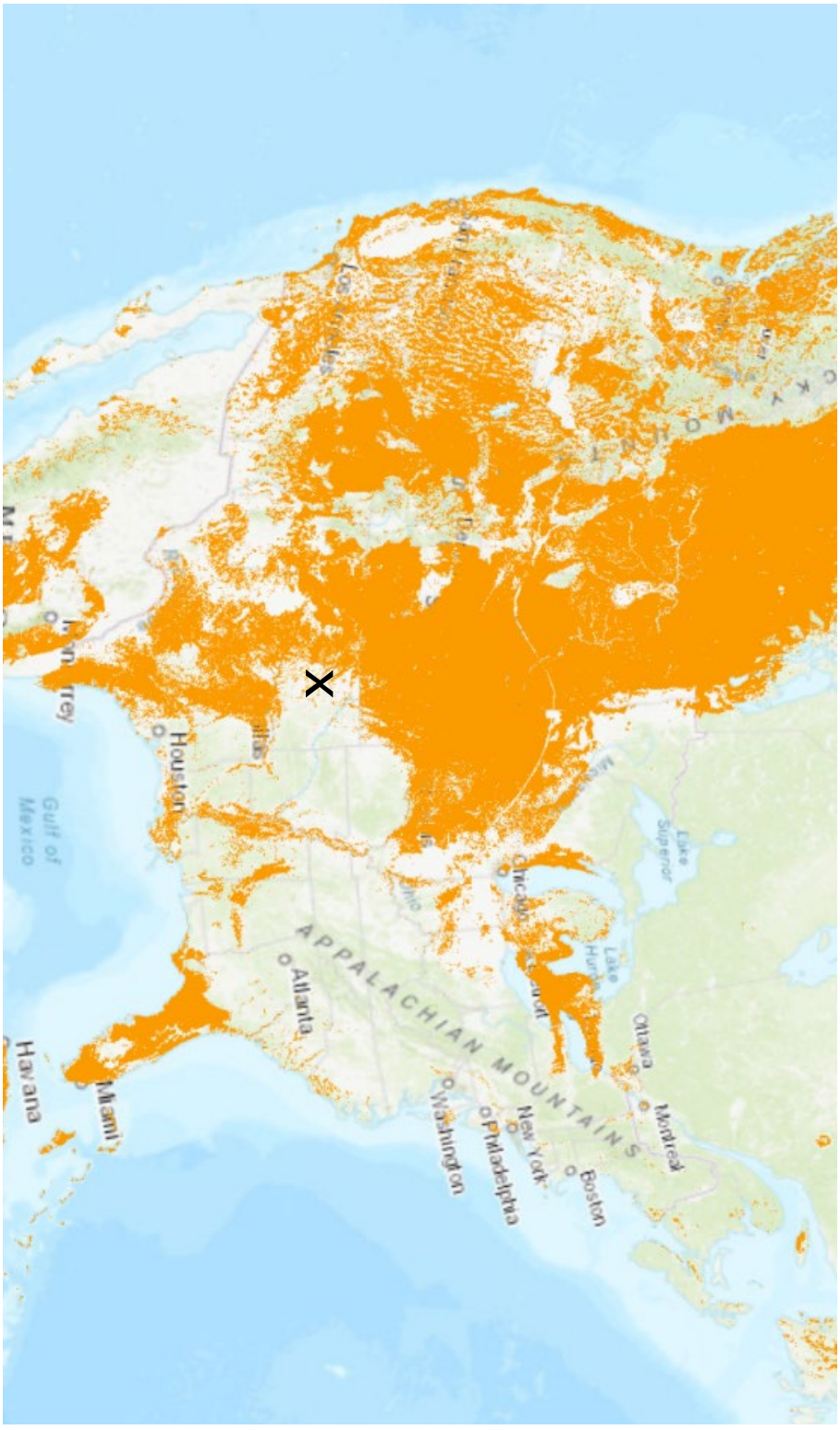

Figure 4.14: The generated strontium isoscape for T06-19b. This individual's place of death was reported as Oklahoma City, OK, shown by an 'X' on the map above.

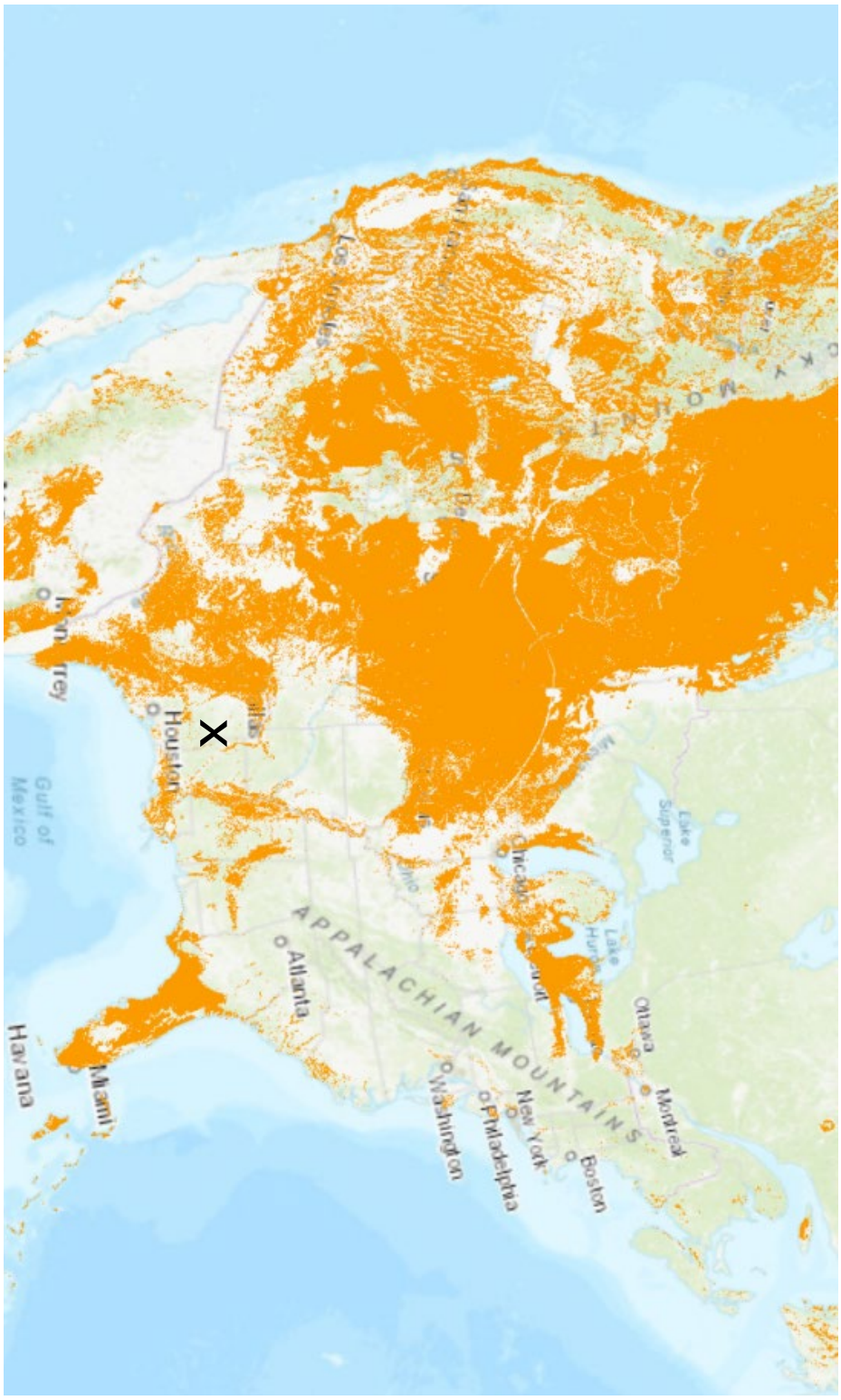

Figure 4.15: The generated strontium isoscape for T07-19a. This individual's place of birth was reported as Center, TX, shown by an 'X' on the map above.

Figure 4.16: A The generated strontium isoscape for T07-19b. This individual's place of death was reported as Houston, TX, shown by an 'X' on the map above.

Figure 4.17: The generated strontium isoscape for T08-19a. This individual's place of birth was reported as W. Bloomfield, MI, shown by an 'X' on the map above.

Figure 4.18: The generated strontium isoscape for T08-19b. This individual's place of death was reported as Houston, TX, shown by an 'X' on the map above.

Figure 4.19: The generated strontium isoscape for T09-19a. This individual's place of birth was reported as Houston, TX, shown by an 'X' on the map above.

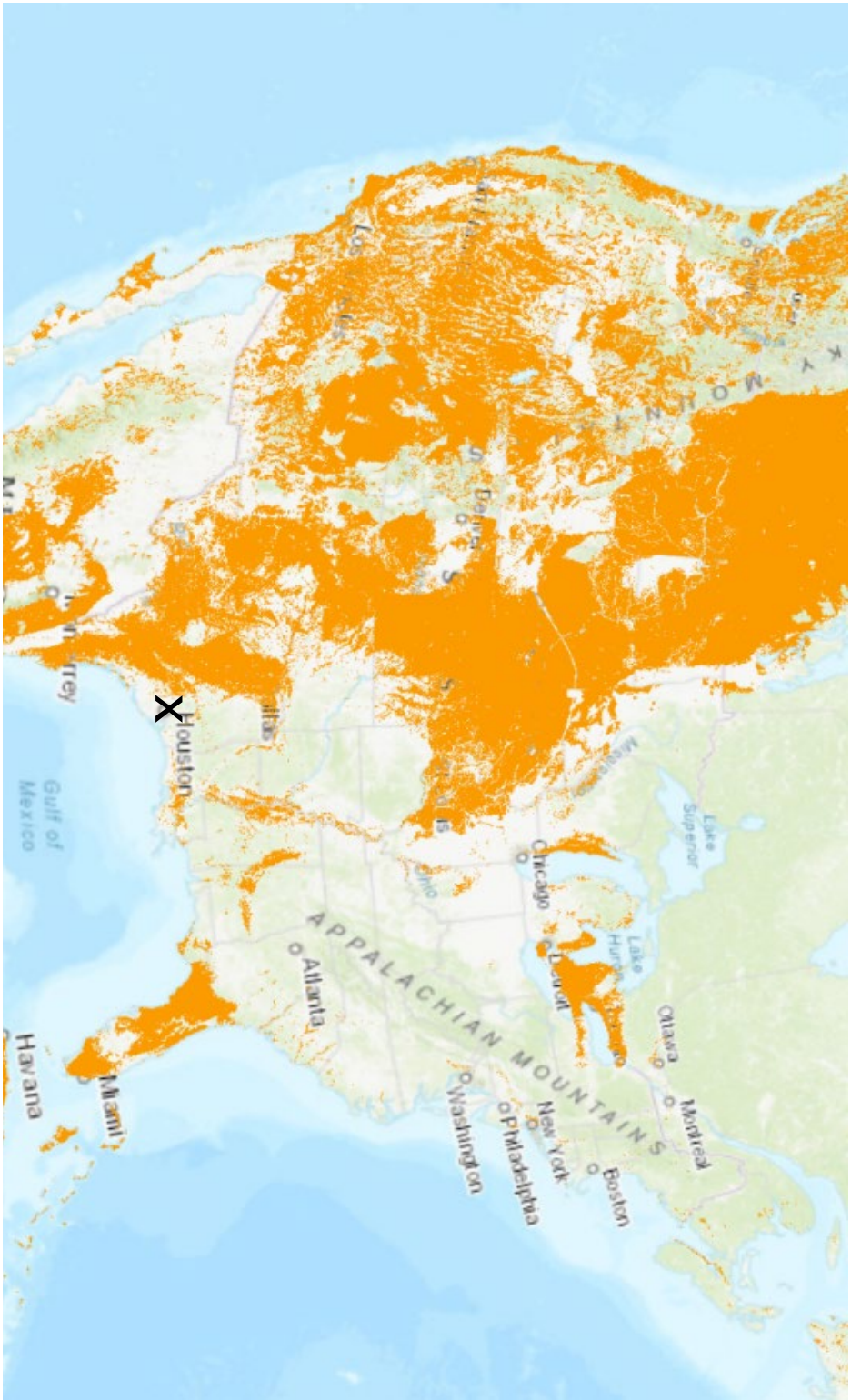

Figure 4.20: The generated strontium isoscape for T09-19b. This individual's place of death was reported as Houston, TX, shown by an 'X' on the map above.

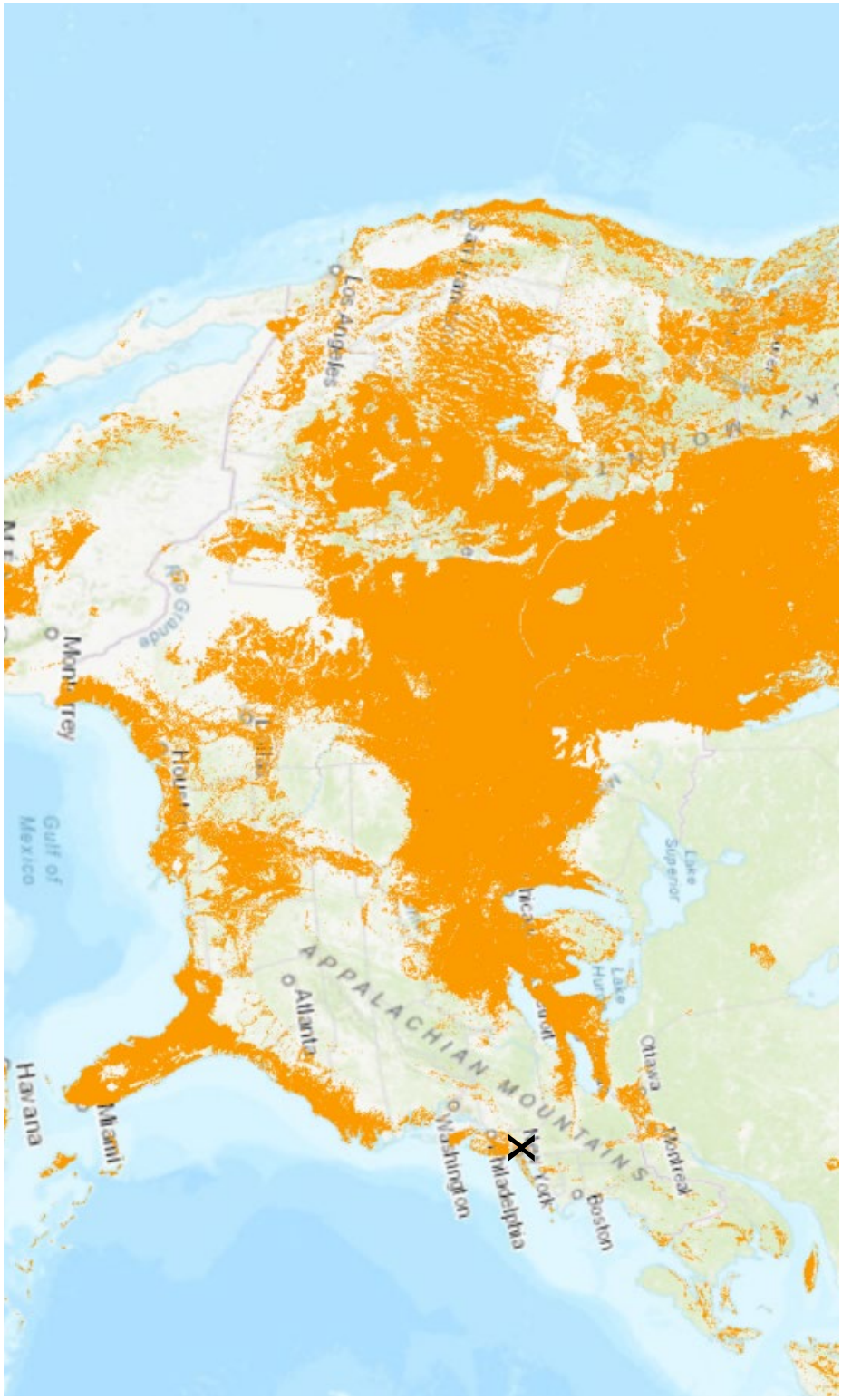

Figure 4.21: The generated strontium isoscape for T10-19a. This individual's place of birth was reported as New York City, NY, shown by an 'X' on the map above.

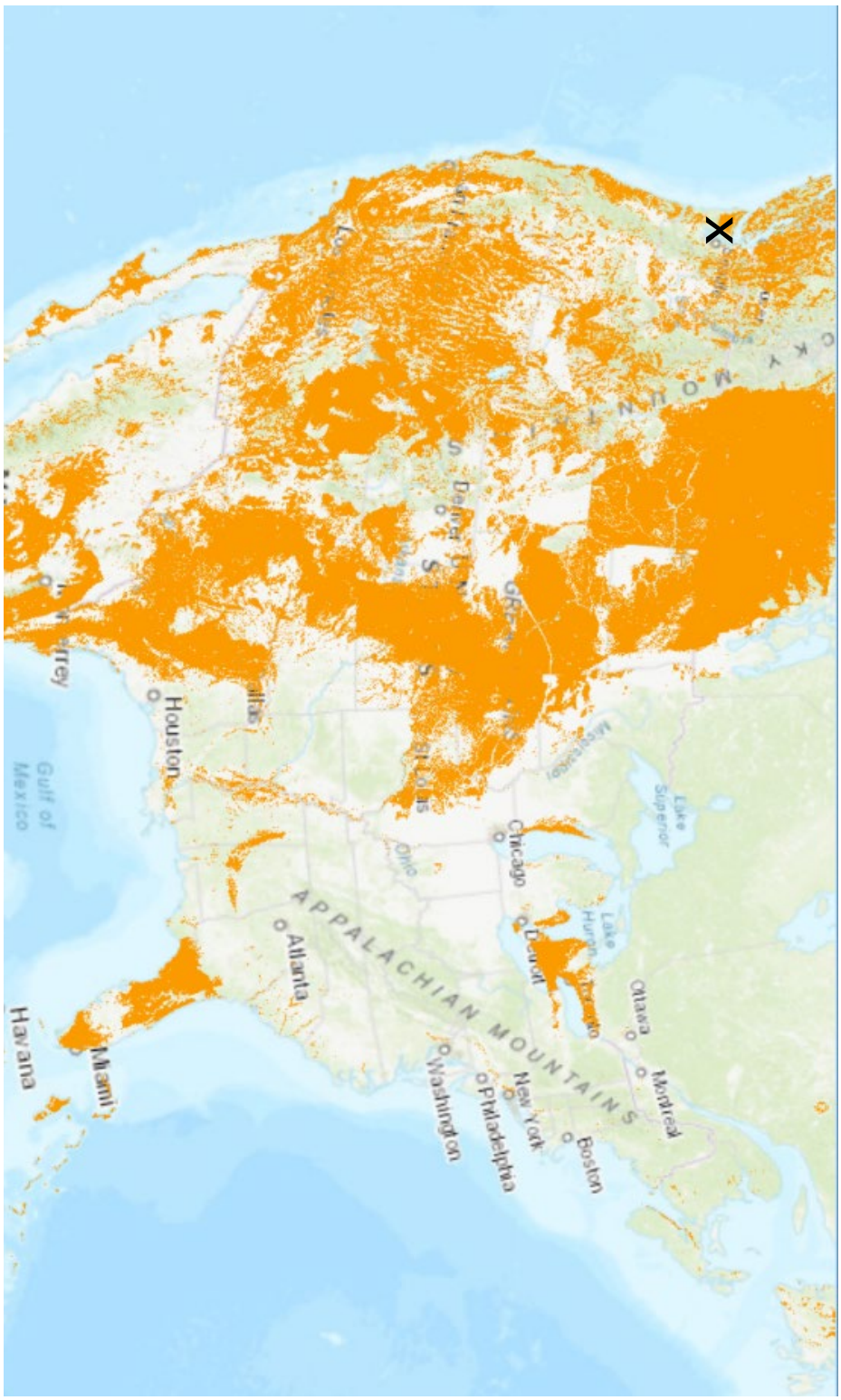

Figure 4.22: The generated strontium isoscape for T10-19b. This individual's place of birth was reported as Port Orchard, WA, shown by an 'X' on the map above.

Table 4.2: Summary of predicted location and the relation to reported location for each sample. This table is incomplete and minimum distance from known location will be calculated once oxygen values are complete.

Sample ID	Reported Location	Strontium Match	Min Distance from known location (miles)
T01-19a	Chicago, IL	Partial	Pending
T01-19b	Buffalo Grove, IL	Partial	Pending
T02-19a	Los Angeles, CA	Partial	Pending
T02-19b	Los Angeles, CA	Yes	Pending
T03-19a	Locke, TN	No	Less than 5 miles
T03-19b	Memphis, TN	Partial	Pending
T04-19a	Brohard, WV	No	Less than 5 miles
T04-19b	Homer, NY	Yes	Pending
T05-19a	San Antonio, TX	Yes	Pending
T05-19b	El Paso, TX	Partial	Pending
T06-19a	Ft. Leavenworth, KS	Partial	Pending
T06-19b	Oklahoma City, OK	Partial	Pending
T07-19a	Center, TX	No	Less than 15 miles
T07-19b	Houston, TX	No	Less than 30 miles
T08-19a	W. Bloomfield, MI	Yes	Pending
T08-19b	Houston, TX	No	Less than 30 miles
T09-19a	Houston, TX	No	Less than 30 miles
T09-19b	Houston, TX	No	Less than 30 miles
T10-19a	Queens, New York, NY	Partial	Pending
T10-19b	Port Orchard, WA	No	Less than 5 miles
Total	N/A	60%	N/A

Question 2: Are sulfur signatures able to be used to narrow the geolocation? Specifically, can sulfur be used to infer a coastal or inland location prior to death?

A T-test was performed for assessment of regional difference and was not found to be significant ($t(8)=2.3060$, $p=0.5275$). A visual representation was also created (Figure 4.23) but there is no visual separation between coastal and inland populations. For dietary analysis, a Pearson correlation test was performed to compare sulfur and carbon, sulfur and nitrogen, and finally carbon and nitrogen. Sulfur and carbon ($r=0.0635$, $p=0.8616$) and sulfur and nitrogen ($r=0.1528$, $p=0.6735$) were not found to be

significantly correlated. Carbon and nitrogen (r=0.6698, p=0.0341) were found to be of significant relation.

Table 4.3: δ^{34}S, δ^{13}C, and δ^{15}N results for each sample.

Individual	δ^{34}S (‰)	δ^{13}C (‰)	δ^{15}N (‰)	Region
T01-19b	2.92	-15.99	10.15	Inland
T02-19b	1.46	-16.47	10.73	Coastal
T03-19b	1.93	-15.35	11.59	Inland
T04-19b	3.48	-16.00	11.06	Inland
T05-19b	2.89	-15.11	11.45	Inland
T06-19b	4.21	-14.95	11.10	Inland
T07-19b	3.30	-16.21	11.02	Coastal
T08-19b	2.21	-16.46	10.88	Coastal
T09-19b	2.67	-14.54	11.33	Coastal
T10-19b	3.91	-16.92	10.50	Coastal

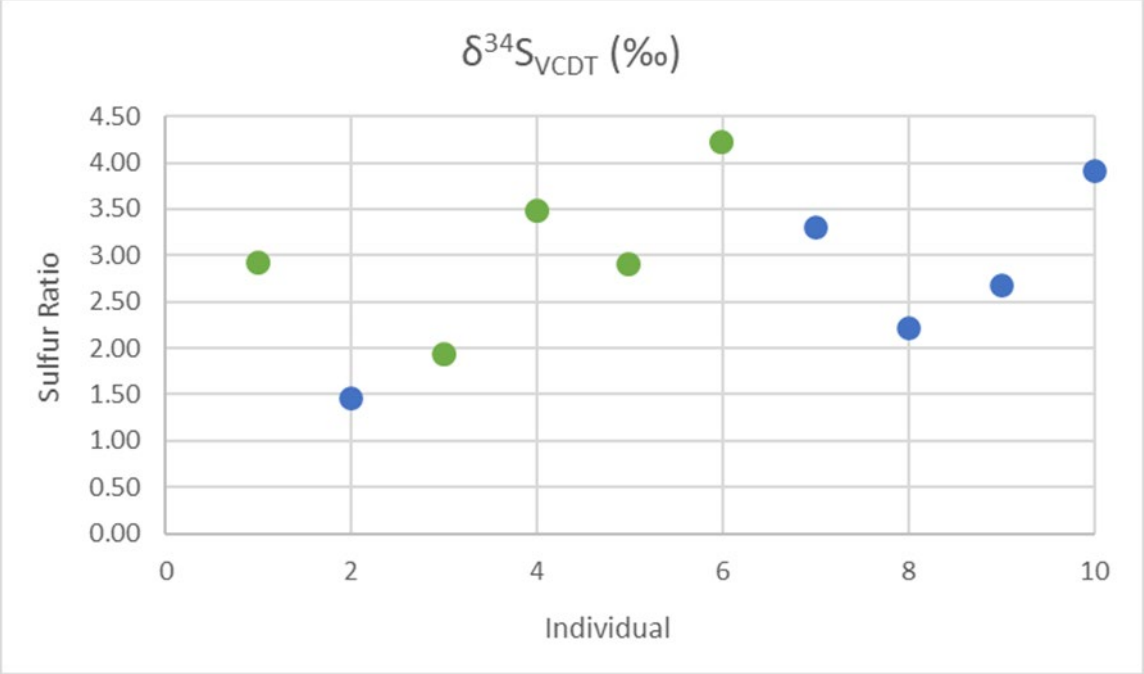

Figure 4.23: A scatter plot created to visually interpret coastal versus inland sulfur values. The coastal population is represented in blue while the inland population is represented in green.

V. DISCUSSION

This research was completed in effort to further the understanding of isotope ratios in human remains. Specifically, in using a modern population with relatively well-documented residential histories the goal of this study was to determine the accuracy of isotopic analyses and their application to Forensic Anthropology. The partial findings of this research do indicate that isotopic analysis is a useful tool to anthropologists but must be utilized with an understanding of the environment and habits of a modern population.

Question 1: To what extent are isotope mapping methods (isoscapes) accurate predictors of geolocation in the United States?

Oxygen

Oxygen values for each individual are currently unavailable due to the COVID-19 pandemic. This lack of data has severely limited the exploration of research question 1. A direct evaluation of location prediction is not currently possible.

Strontium

As previously discussed, $^{87}Sr/^{86}Sr$ ratios in human remains directly reflect the original source of bedrock (Coelho et al., 2017; Beard & Johnson, 2000). This means strontium is an extremely reliable isotope for studying and predicting the geolocation of past and present humans (Bartelink & Chesson, 2019; Chesson et al., 2018; Laffoon et al., 2017; Rauch et al. 2007; Meier-Augenstein, 2010). While the isoscapes exhibited above (Figures 4.3-4.22) do show discrete patterns and differences, even within an individual (Figures 5.1), they hardly allow for a concentrated search. While there are clear differences between maps, each isoscape still covers a large range spanning several states of potential residential matches.

The broad prediction ranges shown above are problematic when the purpose of the analysis is identification. This is a limitation of isotopic research that has been discussed in depth, and it is widely agreed that a multi-isotope approach is more likely to produce a more narrow field of search (Bartelink et al. 2014, Bender et al. 2015, Rauch et al. 2007, Chesson et al. 2018, Laffoon et al. 2017, Warner et al. 2018). The partial results represented in this research emphasize the necessity of a multi-isotope approach, and it was never the intention of this research to use a single isotope method.

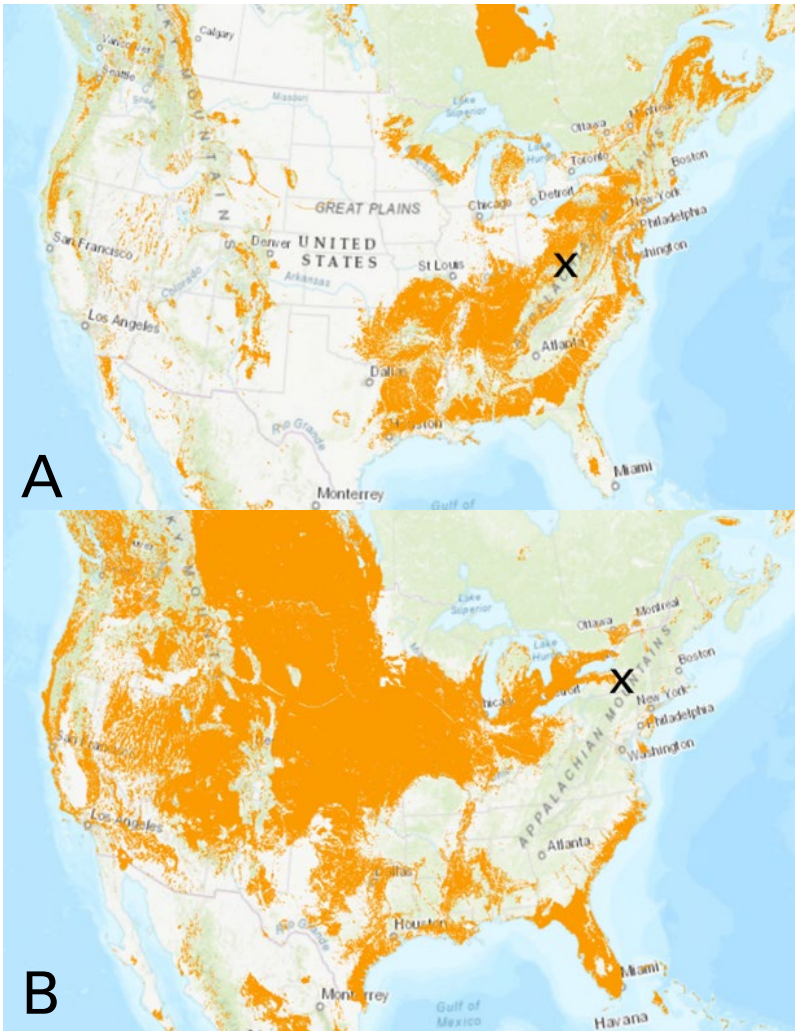

Figure 5.1: Demonstrating discrete patterns within individual T04-19. A) T04-19a with a reported location of Brohard, WV; B) T04-19b with a reported location of Homer, NY. The respective locations are indicated with an 'X' on the map.

However, even with the limitations of using a single isotope, the predictive isoscapes were able to accurately highlight the reported residences of twelve samples. Eight of the twelve are listed in table 4.2 as being a partial match. With these locations, the predictive isoscape highlighted a portion within the city limits (see Figure 5.2). In the case of individual T10-19, their place of birth was reported as Queens, New York City, NY. Interestingly, Queens is one of two New York City boroughs with substantially highlighted regions in the predictive isoscape. The other predicted borough is Brooklyn which shares a border with Queens. In this case, a defined area of prediction could be used to differentiate between areas within a large city.

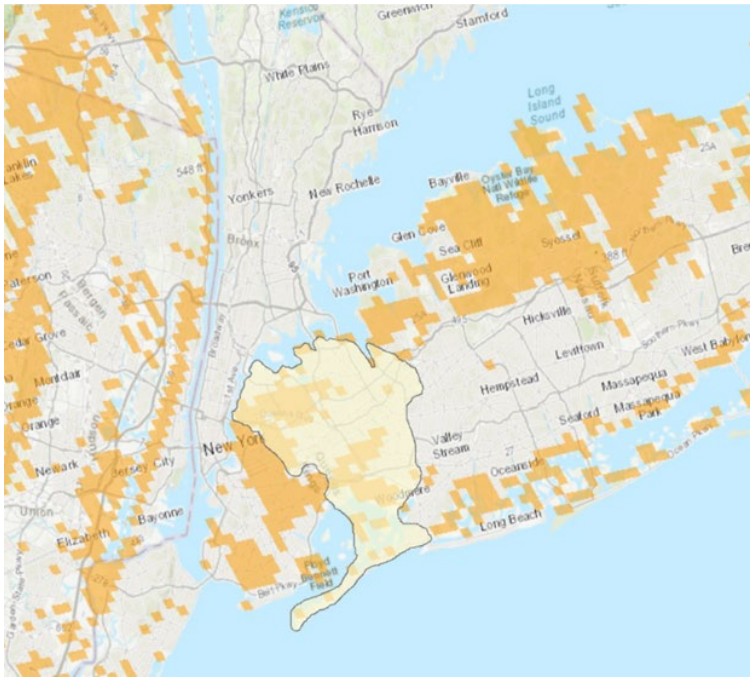

Figure 5.2: Demonstrating discrete patterns within a city limit. Individual T10-19 reported a place of birth as Queens, New York City, NY which is highlighted in yellow. The area of prediction from strontium values is highlighted in orange.

While several reported residencies did not match their respective isoscape, it is important to note that four of the eight were within a fifteen mile radius of the closest

highlighted region, an easy distance for a commute to work or to a grocery store from a rural area.

Each of the four individuals with a reported location of Houston, TX were not accurately placed within the isoscape. Interestingly, all of these individuals exhibit a similar pattern of prediction (Figure 5.3). Each of these individuals fall within a thirty-mile radius of the closest highlighted region, though the more clustered areas fall within a seventy-mile radius. Within this greater radius are Lake Livington and Lake Conroe, both with highlighted regions, to the north of Houston. According to Houston Public Works, Lake Conroe provides 60 million gallons of water per day, while Lake Livington provides 806 million gallons per day to the water supplies of Houston (2020).

In light of these findings, it is important for future investigators to consider the implications of a modern society on isotope ratios. With larger cities sourcing water from nearly 100 miles away, hydrogen, oxygen, and strontium ratios may not reflect a reported location (be it a missing person's address or self-reported information for a donation program). Similarly, many persons in rural communities or large cities often commute for work or regularly travel outside of a five-mile radius of their home. Using isotopic analyses, it is possible that without an extension or buffer of sorts the reported ratios may exclude a potentially correct prediction. Numerous studies have shown that strontium can be useful for estimating geolocation (Bartelink & Chesson, 2019; Katzenberg, 2008, Chesson et al., 2018, Bataille and Bowen, 2012, Saul, 2017, Bataille et al. 2018, Laffoon et al. 2017, Rauch et al. 2007). This research, though incomplete, agrees that strontium should be utilized in combination with other isotopes for predictive modeling of human remains.

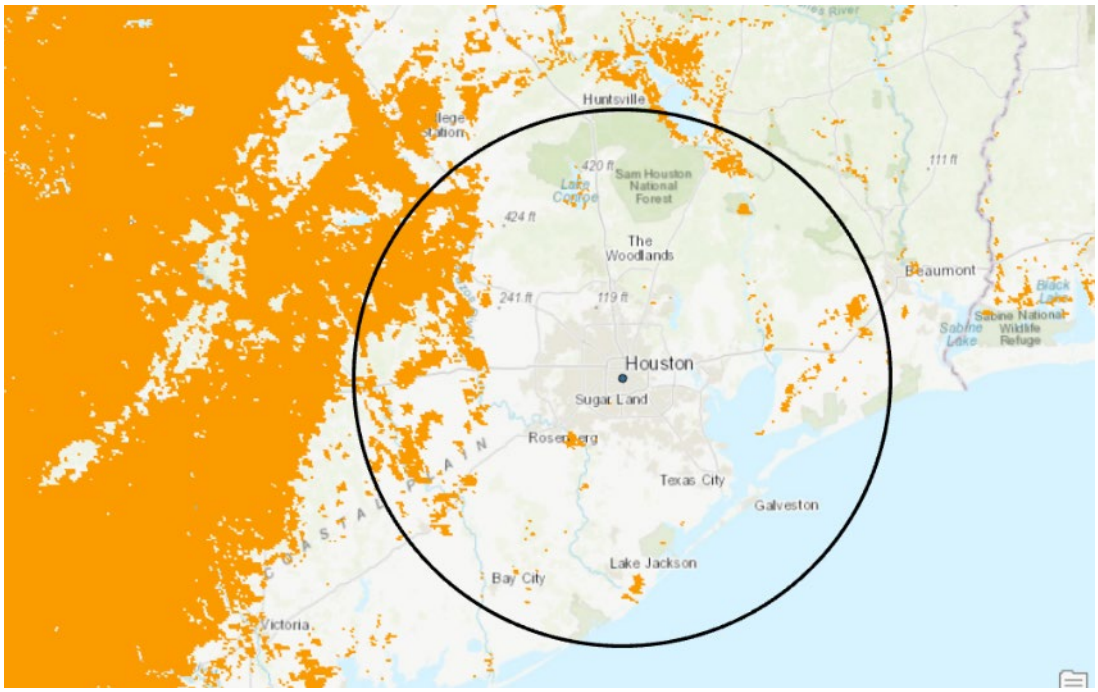

Figure 5.3: Pattern of prediction for T09-19a. Similar to T07-19b, T08-19b, and T09-19b all with a reported location of Houston, TX. The black circle represents a 70 mile radius from the center of Houston.

Question 2: Are sulfur signatures able to be used to narrow the geolocation? Specifically, can sulfur be used to infer a coastal or inland location prior to death?

In this study, sulfur remains ambiguous for narrowing potential geolocation. There are no significant differences between the coastal and inland sample groups, and comparisons between sulfur and the other dietary isotope ratios (δ^{34}S to δ^{13}C and δ^{34}S to δ^{15}N) also did not show significant correlation. Sulfur is a relatively new stable isotope studied in the field of anthropology, as such it is underrepresented in the literature (Meier-Augenstein, 2010). As such, it is still largely unknown what factors influence δ^{34}S intake into human tissues. With a multitude of potential environmental sources, a direct correlation for a single source of δ^{34}S ratios can be difficult to distinguish.

In any given location, the proximity to the coast, increased atmospheric sulfur due to pollution, local geology, etc. could affect an individual's δ^{34}S ratio (Bartelink &

Chesson, 2019; Chesson et al., 2018, Fry, 2006; Richards et al., 2001; Meier-Augenstein, 2010; Nehlich, 2015). It is likely due to these unpredictable and ever-changing circumstances that current literature in addition to this research have contradicting results (Richards et al., 2001,Valenzuela et al., 2011, Bender et al., 2015). The assimilation of sulfur into human tissues should be studied further before using this element predict geolocation. Perhaps using a more quickly turned over tissue such as hair (Valenzuela et al, 2011, Bender et al, 2015) or nails allows for a better representation of geolocation.

Limitations and Future Studies

This research was conducted with a relatively small sample size and self-reported or next-of-kin reported data. Conducting these analyses with more thorough location information such as a zip code or a postal address would have allowed for a more precise review of the generated predictions, specifically, within larger cities that span several miles. The information collected by the Texas State Willed Body Donation Program also does not account for potential travel and/or commuting of an individual or any diet preferences that may have influenced isotope ratios. It is therefore recommended that future paperwork for the TXSTDSC invite potential donors and next-of-kin to share recent travel and regular commutes as well as any changes in diet.

It is also of note that there are no standard preparation methods for samples and cross-study comparisons are difficult as the same source or sample could have various ratios reported through different methods and laboratories (Pestle et al., 2014). While this does not affect directly affect this research, creating more open communication about methods used and defining a standard method for each isotope would greatly benefit future studies in this field.

In addition to these factors, it should be considered that constantly changing environmental and global conditions greatly affect isotopic analyses. The ease of long-distance travel, long commutes, and supermarkets with non-local groceries may all introduce isotope ratios that may not reflect a known residence for an individual (Ehleringer et al. 2008). This may prevent predictive models from accurately placing a missing person, instead possibly making the field of search *too* narrow. This is one of the most limiting factors of predictive isoscaping and should be approached with caution by forensic anthropologists whose goal is identification of an unknown individual.

Finally, as with a majority of scientific fields, isotopic research is constantly growing and evolving. Climatic conditions are currently changing, as are pollution rates, these changes may directly affect isotope ratios such as $\delta^{34}S$ and $\delta^{18}O$. With these shifting environments and new discoveries in the field, it is important to use the most up to date information and recognize the many factors which may influence the results. Forensic Anthropologist who are using isotope analyses to narrow the field possible identification should do so with an understanding that these analyses are greatly impacted by a variety of factors.

APPENDIX

Table 1: A list of donors from the TXSTDSC which have been previously sampled for isotopic analyses.

Grant	Donor ID	Bone Apatite	Bone Collagen	Enamel	Hair
Herrmann et al. 2015	2009.001	--	--	O, Sr	--
Herrmann et al. 2015	2008.002	C, O	C, N	O, Sr	--
Herrmann et al. 2015	2008.003	C, O	C, N	O, Sr	--
Herrmann et al. 2015	2009.003	C, O	C, N	O, Sr	--
Herrmann et al. 2015	2009.004	C, O	C, N	O, Sr	--
Herrmann et al. 2015	2010.004	C, O	C, N	O, Sr	--
Herrmann et al. 2015	2011.004	C, O	C, N	O, Sr	--
Gordon et al. 2019	2015.004	--	--	--	C, N, O, H, Sr
Herrmann et al. 2015	2009.005	--	--	O, Sr	--
Herrmann et al. 2015	2009.006	C, O	--	O, Sr	--
Gordon et al. 2019	2015.006	--	--	--	C, N, O, H, Sr
Herrmann et al. 2015	2009.007	C, O	C, N	O, Sr	--
Herrmann et al. 2015	2010.007	C, O	C, N	O, Sr	--
Herrmann et al. 2015	2009.008	C, O	C, N	O, Sr	--
Herrmann et al. 2015	2010.008	C, O	C, N	O, Sr	--
Herrmann et al. 2015	2009.009	C, O	C, N	O, Sr	--
Herrmann et al. 2015	2010.009	C, O	C, N	O, Sr	--
Herrmann et al. 2015	2010.010	C, O	C, N	O, Sr	--
Gordon et al. 2015	2015.010	--	--	--	C, N, O, H, Sr
Herrmann et al. 2015	2009.011	C, O	C, N	O, Sr	--
Gordon et al. 2019	2015.011	--	--	--	C, N, O, H, Sr
Herrmann et al. 2015	2009.012	C, O	C, N	O, Sr	--
Herrmann et al. 2015	2011.014	C, O	C, N	O, Sr	--
Gordon et al. 2019	2015.014	--	--	--	C, N, O, H, Sr
Herrmann et al. 2015	2011.015	C, O	C, N	O, Sr	--
Gordon et al. 2019	2015.020	--	--	--	C, N, O, H, Sr
Gordon et al. 2019	2015.023	--	--	--	C, N, O, H, Sr
Gordon et al. 2019	2015.025	--	--	--	C, N, O, H, Sr
Gordon et al. 2019	2014.039	--	--	--	C, N, O, H, Sr
Gordon et al. 2019	2015.040	C, O, Sr	--	C, O, Sr	C, N, O, H, Sr
Gordon et al. 2019	2015.043	C, O, Sr	--	C, O, Sr	C, N, O, H, Sr
Gordon et al. 2019	2015.045	C, O, Sr	--	C, O, Sr	C, N, O, H, Sr
Gordon et al. 2019	2015.046	C, O, Sr	--	C, O, Sr	C, N, O, H, Sr
Gordon et al. 2019	2015.051	C, O, Sr	--	C, O, Sr	C, N, O, H, Sr
Gordon et al. 2019	2014.065	--	--	--	C, N, O, H, Sr

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