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# **RESEARCH REPORT**

# NATIONAL INSTITUTE OF JUSTICE OFFICE OF INVESTIGATIVE AND FORENSIC SCIENCES

REPORT TITLE: Isotopic and Elemental Analysis of the William Bass Donated Skeletal Collection and Other Modern Donated Collections

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# **Abstract**

 Isotopic and elemental characteristics of human bone, teeth, and hair have been demonstrated as useful biomarkers for forensic anthropologists and criminal investigators. These biomarkers trace locations and movements of the individuals, and aid in the identification of human remains. This project analyzed multiple isotopes (carbon, oxygen, hydrogen, strontium, and nitrogen), and trace elements in modern human bone, teeth, and hair from the William Bass Donated Skeletal Collection (WBDSC), the Maxwell Museum Documented Skeletal Collection (UNM), and the Texas State University-San Marcos Forensic Research Facility (TSU-SM). The WBDSC represents the largest modern donated osteological collection in the United States and is located at the University of Tennessee Forensic Anthropology Center. Samples from individuals with self-or family-reported birth locations and movement histories were used for this study. In addition, individuals with unknown natal histories were compared against the known residential history data set. Sample preparation and isotope analyses were conducted at the University of Tennessee Stable Isotope Laboratory, the University of Alabama-Huntsville, the R. Ken Williams '45 Radiogenic Isotope Geosciences Laboratory at Texas A&M University, and the University of Alabama Stable Isotope Laboratory in Tuscaloosa. Trace element analysis was conducted at the Mississippi State University Department of Chemistry Laser Ablation Unit. A total of 290 individuals were sampled for a combination of stable isotope and trace element analyses. The samples included: powdered enamel for strontium analysis, bone collagen extraction for carbon and nitrogen analyses, bone apatite and  $Ag_3PO_4$  enamel precipitate for oxygen analysis, and bulk hair samples for hydrogen analysis. Results from the study indicate that the enamel  $\delta^{18}O$  values from the WBSC collection are overall reflective of individuals' birth locations, whereas hair keratin  $\delta^2$ H values are influenced by individuals' death locations, which is consistent with isotope studies of forensically derived human samples. This suggests that the application of dual isotopes (O and H) provides a clear picture of residential history by spatially locating the beginning (tooth) and the ending (hair) of the individual life journey. Although the correlation coefficient of the enamel  $\delta^{18}O$  values with local water is not as high as the previously reported values, the relationship does follow the trend of earlier results. A potential influence of the elevated  $\delta^{18}$ O values may result from the isotopic pattern of tap water when compared to precipitation. Isotope results were built into a publically available database, Forensic Isotopes Nation Database (FIND), which is accessible to various researchers that can serve as a repository for human derived isotopic information from anatomical collections with residential histories or for resolved forensic cases involving bone, enamel, or hair isotope data. This project has generated a national isotope database derived from the WBDSC and the other donated skeletal collections; provided forensic anthropologists and criminal investigators a comparative isotopic database of known residence from various sampled tissues; and has evaluated the effectiveness of implementing multiple isotopic analyses to estimate movement histories for modern forensic cases.

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# **Executive Summary**

 Isotopic and elemental characteristics of human bone, teeth, and hair have been demonstrated as useful biomarkers for forensic anthropologists and criminal investigators. These biomarkers trace locations and movements of individuals and aid in the identification of human remains. This project analyzed multiple isotopes (carbon, oxygen, hydrogen, strontium, and nitrogen) and trace elements in modern human bone, teeth, and hair from the William Bass Donated Skeletal Collection (WBDSC) at the University of Tennessee-Knoxville (UTK), the Maxwell Museum Documented Skeletal Collection at the University of New Mexico (UNMMM) and the Texas State University-San Marcos (TSU-SM) Forensic Research Facility. The WBDSC represents the largest modern osteological collection in the United States and is located at the University of Tennessee Forensic Anthropology Center. Samples from individuals with self-reported or family-reported origins and movement histories were used for this study. In addition, individuals with unknown natal histories were examined against the known residential history data set. Sample preparation and isotope analyses were conducted at the University of Tennessee Stable Isotope Laboratory, the University of Alabama-Huntsville, the R. Ken Williams '45 Radiogenic Isotope Geosciences Laboratory at Texas A&M University, and the University of Alabama Stable Isotope Laboratory in Tuscaloosa. Trace element analysis was conducted at the Mississippi State University Department of Chemistry Laser Ablation Unit.

 In total, samples from 290 individuals were tested for biogeochemical analysis. The samples included: powdered enamel for strontium analysis, bone collagen extraction for carbon and nitrogen analyses, bone apatite and  $Ag_3PO_4$  enamel precipitate for oxygen analysis, and bulk hair samples for hydrogen analysis.





\* LA-ICP-MS results are preliminary data and are included in FIND as counts per second (cps)

 Multiple constituents including bone collagen, bioapatite (phosphate and carbonate), and hair keratin from 290 donors from WBDSC, UNMMM, and TSU-SM were prepared for  $\delta^{13}C$ ,  $\delta^{18}O$ ,  $\delta^{15}N$ , <sup>87</sup>Sr/<sup>86</sup>Sr, and  $\delta^{2}H$  analysis using refined protocols. The protocols were enhanced by shortening the cycle of each sample preparation period for collagen extraction utilizing a filterbag method assisted by an ultrasonic water bath, and modifying a Thermo TC/EA for improving analysis precision of phosphate  $\delta^{18}O$ . Dental enamel was sampled using a NewWave Micromill and analyzed for  $\delta^{18}$ O of phosphate. The averaged  $\delta^{18}$ O value for a select sample of the dental enamel was  $16.91\pm2.21\%$  VSMOW (n=215) (see Table 5). Our initial correlation analysis of the dental  $\delta^{18}$ O values with meteoric water  $\delta^{18}$ O at birth location (modeled) yielded the equation

 $(\delta^{18}O_{tooth}=0.62*\delta^{18}O_{water}+21.74, r=0.63, n=45)$  (Figure 2) (Herrmann et al., 2010), which was similar to the equation ( $\delta^{18}O_{\text{bone}}=0.64*\delta^{18}O_{\text{water}}+22.37$ , r=0.98) generated by Longineli (1983). With the addition of the new samples from TSU-SM and UNMMM the correlation reduced significantly  $(\delta^{18}O_{tooth}=0.349*\delta^{18}O_{water}+20.164, r=0.36, n=120)$  and appears to vary by collection with TSU-SM and UNMMM showing poor relationships.

Non-exchangeable  $\delta^2$ H of hair keratin was also analyzed using a Thermo TC/EA. The averaged  $\delta^2$ H was -83.35± 6.36‰ VSMOW (n=14). The  $\delta^2$ H values exhibited a positive correlation with the meteoric water  $\delta^{18}$ O at death location (r=0.81), and a negative correlation with altitude (r=-0.73), which is consistent with the isotope "Altitude Effect".

In addition, bioapatite carbonates samples (n=290) were analyzed for  $\delta^{13}$ C and  $\delta^{18}$ O. The averaged value was -9.53±1.28‰ (VPDB) for  $\delta^{13}$ C, and -11.23± 4.00‰ (VPDB) for  $\delta^{18}$ O. These values vary by collection with marked differences between the TSU-SM samples as compared to both UTK and UNMMM for  $\delta^{13}$ C and a significant difference between UTK as compared to both TSU-SM and UNMMM. In addition, the samples show regional differences in the range of  $\delta^{18}$ O values with the UNMMM samples exhibiting a substantial range and the UTK range being far more limited.

Bone collagen samples (n=215) were extracted for  $\delta^{13}$ C and  $\delta^{15}$ N analysis. In addition, N%, C%, N/C ratios were recorded for a subset of these samples (Figure 8). The averaged carbon content in human phalanx (both hand and foot) bone collagen was 32.93±6.87%, 11.31 $\pm$ 3.32% for nitrogen content. The averaged C/N ratio was 3.133 $\pm$ 1.04. The averaged  $\delta^{13}$ C value was -16.32±1.25‰ (VPDB), and 11.24±0.53‰ (AIR) for  $\delta^{15}N$ .

Powdered enamel samples ( $n=57$ ) were collected for  ${}^{87}Sr/{}^{86}Sr$  analysis. Sr results are available for 55 samples and were combined with the results presented by Regan (2006) for military personnel. The <sup>87</sup>Sr/<sup>86</sup>Sr ratios were then converted to epsilon values (Beard and Johnson 2000). The resulting epsilon values were compared to modeled bedrock and modeled drainage and bedrock epsilon values based on Beard and Johnson (2000) and Bataille and Bowen (2012), respectively. In addition, the values were plotted on the available GIS coverages for both models and specific point values based on birth locations were extracted from the raster coverages of these two models. The two extracted values were plotted to assess the relationship of the measured vs. location expected (or modeled) position. Both models provide poor fits to the modeled values. The data does appear to follow the pattern relative to birth location. The plot of ratios organized by value from low to high shows general patterning at the state level.

The data from this study suggests that the dental enamel  $\delta^{18}$ O values from the WBSC collections are overall reflective of individuals' birth locations, whereas hair keratin  $\delta^2$ H values are influenced by individuals' death locations, which is consistent with several other isotopic studies of forensically derived human samples. This suggests that the application of the dual isotopes (O and H) could provide a better picture of residential history by spatially locating the beginning (tooth) and the ending (hair) of the individual life journey. Although the correlation coefficient of the dental  $\delta^{18}$ O with local water is not as high as reported by several other researchers, the relationship, however, does follow the trend of the earlier study. The isotopic pattern of tap water as compared to precipitation could be a potential factor. It is also suspected that the WBDSC does not represent a more geographically heterogeneous sample and it is likely that self- or family-reported residential histories as is the practice at the UTK FAC are more variable.

 This study has implications for law enforcement, practicing forensic geochemists, and forensic anthropologists interested in isotope and trace element research. This study also

provides a large isotope dataset from three donated human skeletal collections currently used as reference samples for active forensic anthropologists. These data enhance our understanding of the isotopic variation in modern humans, specifically modern US residents. The isotopic variation observed in these samples is greater than typical controlled laboratory studies, but the results do conform to current isotopic models, specifically for  $\delta^{18}O$ , and is therefore useful for estimating residential histories. Future isotopic work with unidentified decedents could be linked to their NamUs record and used to provide potential matches within the system based on geographic histories.

 Broader impacts of the study relate to the Forensic Isotopic National Database (FIND). The data generated by this study will be made available to researchers through FIND, where researchers can also submit their own results from forensic casework and modern donated collections. FIND is accessible at http://find.msstate.edu/fmi/webd with a user name and password of FINDUser and FindUSer, respectively. FIND will serve as a repository of forensic isotopic data for human skeletal, dental, and hair studies.

 This project generated a national isotope database derived from the WBDSC and the other donated skeletal collections; provided forensic anthropologists and criminal investigators a comparative isotopic database of known residence from various sampled tissues; and has evaluated the effectiveness of implementing multiple isotope analyses to estimate the movement histories of modern humans for forensic cases.

 The study has also contributed to the training and laboratory experiences of both graduate and undergraduate students at the University of Tennessee- Knoxville, Mississippi State University, University of Alabama-Huntsville, University of New Mexico, and Texas State University-San Marcos. The PIs on the project have also reached out to the medicolegal community to provide these services for a nominal fee during the process of the grant. Dr. Li has processed several bone and enamel samples for  $\delta^{18}$ O as well as carbon and nitrogen. Isotopic analysis is now viewed as an important step in the analysis of unidentified decedents in some agencies.

 As the donated collections at UTK, UNM, and TSSU as well as several new body donation programs across the country grow, it is essential that reliable residential histories be collection from the donors and that isotopic data be collected (specifically adequate hair samples for research requests). These expanding collections combined with recent forensic isotope surveys and recently published isotope data will provide a much better picture of the isotopic and trace element variation across the United States. It is anticipated that this study will provide a foundation for future research with these collections.

#### Literature Cited

- Bataille CP, Bowen GJ. 2012. Mapping 87Sr/ 86Sr variations in bedrock and water for large scale provenance studies. Chemical Geology 304-305:39–52.
- Beard BL, Johnson CM. 2000. Strontium isotope composition of skeletal material can determine the birth place and geographic mobility of humans and animals. J. Forensic Sciences 45(5): 1049-1061.
- Herrmann NP, Li Z-H, and Soto M. 2010. Isotopic evaluation of modern human remains from the University of Tennessee William M. Bass Donated Collection. *American Journal of Physical Anthropology* Supplement 50:127-127.
- Longinelli A. 1984. Oxygen isotopes in mammal bone phosphate: A new tool for paleohydrological and paleoclimatological research? Geochimica et Cosmochimica Acta 48:385-90.

# **Introduction**

 Isotopic and elemental characteristics of human bone, teeth, and hair have been demonstrated as useful biomarkers for forensic anthropologists and criminal investigators. These biomarkers trace locations and movements of individuals and aid in the identification of human remains. This project analyzed multiple isotopes (carbon, oxygen, hydrogen, strontium, and nitrogen) and trace elements in modern human bone, teeth, and hair from the William Bass Donated Skeletal Collection (WBDSC), the Maxwell Museum Documented Skeletal Collection and the Texas State University-San Marcos Forensic Research Facility. The WBDSC represents the largest modern donated osteological collection in the United States and is located at the University of Tennessee Forensic Anthropology Center. Samples from individuals with self- or family-reported birth location and movement histories were used for this study. In addition, individuals with unknown natal histories were examined against the known residential history data set. Sample preparation and analysis was conducted at the University of Tennessee Stable Isotope Laboratory, the University of Alabama-Huntsville, and the University of Alabama Stable Isotope Laboratory in Tuscaloosa. Trace element analysis was conducted at the Mississippi State University Department of Chemistry Laser Ablation Unit. Strontium analysis was performed by Texas A&M University Radiogenic Isotope Geochemistry Laboratory.

 In total, samples from 290 individuals were prepared for analysis. Samples included: powdered enamel samples for strontium analysis, apatite and collagen extractions from bone, Ag3PO4 precipitate from powdered dental enamel, and a limited number of bulk hair samples. Our study indicates that the dental enamel  $\delta^{18}O$  values from the WBSC collections are overall reflective of the individual's birth location, whereas hair keratin  $\delta^2$ H values are influenced by the individual's death location, which is consistent with several other isotopic studies of forensically derived human samples and suggests that the application of dual isotopes (O, H) could provide better constraint on the residential history by pinpointing the beginning (tooth) and the ending (hair) of the individual life journey. Although the correlation coefficient of the dental  $\delta^{18}O$  with local water is not as high as reported by several other researchers, the relationship, however, does follow the trend of the earlier study. This could result from the potential influence of the isotopic pattern of tap water as compared to precipitation. In addition, these results have been built into a basic publically available database, Forensic Isotopes Nation Database (FIND), which is accessible to various researchers and can serve as a repository for human derived isotopic information from anatomical collections with residential histories or for resolved forensic cases involving bone, enamel or hair isotope data. This project has generated a national isotope database derived from the WBDSC and the other donated skeletal collections; provided forensic anthropologists and criminal investigators a comparative isotopic database of known residence from various sampled tissues; and it has evaluated the effectiveness of implementing multiple isotopic analyses to estimate the movement histories for modern forensic cases.

### *Statement of the problem*

This study examines the isotopic compositions of various modern human tissues within three donated human skeletal collections from across the United States. The study attempts to discover better constraints for tracing the birth locations, movement histories, and living environments of modern individuals through the examination of multiple isotope data ( $\delta^{13}C$ ,  $\delta D$ ,  $\delta^{18}O$ , <sup>87</sup>Sr/<sup>86</sup>Sr, and  $\delta^{15}N$ ) and trace elements. The William M. Bass Donated Skeletal Collection (WBDSC), housed at the University of Tennessee (UTK) Forensic Anthropology Center (FAC), served as the primary sample for the project. In addition to WBDSC, numerous tissue samples from the Maxwell Museum Documented Skeletal Collection at University of New Mexico (MMDSC) and the Texas State University-San Marcos Donation Program (TSSM) were obtained and analyzed. The regional variation in birth (including foreign born individuals) and death locations and demographic diversity of these donated skeletal collections provided an ideal opportunity to examine isotope and trace element variation across the United States.

 The goals and objectives of this study were: 1) to build a national database of isotope and trace element profiles for human ossified and soft tissues of known origins derived from WBDSC, MMDSC and TSSM, 2) to provide forensic anthropologists and criminal investigators a comparative database to determine location of residence of unidentified decedents based on the tissue sampled, and 3) to refine laboratory and analytical methods for constraining the locations and movement histories of modern individuals by implementing multiple isotope data. For example, isotope profiles could be highly complicated by individuals who consume global range food products or pre-packaged foods.

Previous studies of isotopic and elemental signatures from human bone, hair, and teeth in a forensic context have typically focused on a limited number of samples or have examined foreign-born individuals. By testing a large number of natural born individuals this study should provide a baseline data set for examining isotopic and elemental variation in these three tissues from a modern US sample. Ideally, this study will allow forensic anthropologists and investigators to identify age-specific residential range for the unidentified decedent based on the isotopic signature of the tissue examined. In total, 290 individuals from these three collections have been examined including 796 individual elemental assays. These results are all included in Forensic Isotopic National Database (FIND, available at http://find.msstate.edu/fmi/webd).

#### *Literature Review of Forensic Applications of Biogeochemistry*

 The identification of unknown persons is of the utmost importance for the forensic anthropologist. Positive identification of human remains is determined when unique characteristics known to exist are established (Ubelaker, 2008), and are typically based on traditional identification methods. These methods include personal documentation, dental records, radiographs, morphology, pathology, and establishing a biological profile. The biological profile is especially important in countries where dental records are not obtainable or when individuals are found skeletonized without documentation. Molecular methods aid in the identification process and may even assist in the arrest of the perpetrator.

 Recent methods that have become increasingly popular in assisting with the identification process are stable isotopes and trace elements. Vast amounts of isotope and trace element data are being collected to demonstrate the validity of the methods. Geochemistry methods have aided in forensic cases relating to environmental accidents, food authenticity, distinguishing drugs, elucidating explosives, geo-referencing materials, and provided supporting evidence for the biological profile (Meier-Augenstein, 2010; Pye and Croft, 2004). Isotopes are an informative tool when DNA is absent, or beneficial when used in conjunction with other reliable identification methods. Isotopes and trace elements are especially important when remains are highly fragmented, as in cases of natural disasters or incineration. Data generated from the WBDSC, MMDSC, and TSSM collections provides further supporting evidence for the application of biogeochemistry in the identification of human remains.

 Unlike DNA 'fingerprinting' that is immutable, stable isotopes and trace elements change over time. This variability is advantageous for compiling a multi-variable stable isotope profile (SIP). Isotopes are extraordinary due to the different numbers of neutrons that reveal information about the local environment and surrounding ecosystems (Fry, 2006). Isotopes essentially divulge the geographic signatures of humans, since isotopes from local drinking water and food sources are incorporated into human enamel, bone hydroxyapatite phosphate and carbonate, hair keratin, and nails. Both 'light' and 'heavy' isotopes are used for diet reconstruction and/or residential history, but 'heavy' isotopes have several advantages. 'Heavy' isotopes barely exhibit any fractionation and can also tolerate high temperatures after incineration (Harbeck et al., 2011). Trace elements also aid in the SIP, by revealing environmental toxin exposure or medical treatments. Some have even suggested that trace elemental compositions may provide individual provenance despite similar diet composition (Burton et al., 2003; Cucina et al., 2011). There are advantages and disadvantages to every method, including biogeochemistry methods. Many have expressed concern over the effect globalization has on modern human signatures. From a review of the literature, we found studies with both positive and negative results, but it appears that despite globalization, modern humans retain the local isotope signature, regardless of the consumption of bottled water and foreign products.

 Previous biogeochemistry studies in forensic anthropology demonstrate the usefulness of stable and radiogenic isotopes and trace elements of modern human remains. FIND is the beginning of standardization in the United States and provides a baseline for narrowing the geographic range for forensic investigators using isotope signatures for the identification process. This review section demonstrates the importance of biogeochemistry in forensic anthropology through the description and recent application of isotopes and trace elements in the field, and is followed by a review of the modern globalization concern.

#### 'Light' Stable Isotopes

 'Light' isotopes are elements on the periodic table with small atomic masses. Carbon (C) and nitrogen (N) were the first 'light' stable isotopes used by anthropologists to study dietary trends of archaeological populations (Katzenberg, 2008). Since the 1970's, the use of 'light' isotopes expanded to include oxygen (O), hydrogen (H), and sulfur (S), enabling studies of residential histories for past and modern skeletal assemblages. Variation in stable isotopes allows researchers to study the amount in certain ecosystems, and therefore human populations residing in specific regions. This variation is caused by kinetic or equilibrium physiochemical processes that fractionate isotopes called isotope fractionation (Meier-Augenstein, 2010). In nature, abundance variation has been well documented for 10 elements having 2 or more stable isotopes, C, N, O, H, and S being five commonly used in anthropology. Therefore, the upper and lower bounds of the atomic weights have been determined in naturally occurring terrestrial environments (Brand and Copeland, 2012). Environmental processes, such as evaporation and condensation that effect the hydrogen cycle, allow these small isotopic signatures to be quantified for O and H. Another important process of stable isotope variation in the environment is isotope mixing. Isotope mixing occurs after the fractionation process, where the fractionated material is transported over large distances mixing with other fractionated or non-fractionated materials. These geochemical processes are the bases behind the use of 'light' stable isotopes for the study of human residential histories.

 Variation of isotopic abundances in the environment is retained in human tissues and has the ability to be quantified for forensic science purposes. 'Light' isotope profiles of C, N, H, and O have been determined using teeth, bones, hair, and nails from modern human remains. In human tissues stable isotope signatures are determined from the ratio of the isotope values ingested by a person during the ages that the skeletal tissues are formed (Wright, 2005). Both collagen and apatite are used for stable isotope analysis depending on the isotope the forensic anthropologist is interested. For instance, collagen is the preferred biochemical fraction when conducting a dietary analysis of C and N, due to the large amount of collagen contained in bone (Ambrose, 1993). Tooth enamel is generally preferred over dentine, because of the hard hydroxyapatite composition of enamel, and its resistance to digenetic alterations from soil and other surrounding materials. There are also advantages to using both bones and teeth for the same forensic case. Human teeth are formed early in development, locking in the isotope signature. Bone, on the other hand, remodels over time, revealing the later isotope signatures of individuals, approximately the last 10-15 years of their life (Katzenberg, 2008). A large number of studies using hair have been generated in recent years, ranging from residential histories of the dead to starvation and malnutrition in the living. Hair strains are less invasive making the method more attractive to forensic investigators (Santamaria-Fernandez et al., 2009). Recent forensic case studies have generated SIPs that aided in the determination of the unknown individual using a combination of tissue samples and multiple 'light' stable isotopes.

#### Selected Isotope Case Studies of Diet

 In forensic cases, studies of diet may be used as an unbiased biomarker to infer information about specific foodstuffs and location. 'Light' isotopes have been used to establish malnourishment and neglect in child abuse cases. For instance, Neuberger et al. (2013) measured daily  $\delta^{13}$ C and  $\delta^{15}$ N values in human hair to reconstruct nutritional histories of deceased individuals. Their study showed that elevated  $\delta^{15}N$  values were associated with very low BMI indicating the catabolism of bodily protein, and changes in  $\delta^{13}$ C values indicative of low energy and the loss of fat deposits. Although carbon and nitrogen stable isotopes are successful in analyzing dietary inputs,  $\delta^{13}C$  and  $\delta^{15}N$  values of hair are not significant for documenting geospatial patterns of human movements. However, data documented from Valenzuela et al. (2011) did suggest that residents of the northeastern continental United States consume a higher  $C_3$  plant based diet, whereas the average U.S. diet is high in  $C_4$  plant based foods.

#### Selected Isotope Case Studies for Residential Histories

 The use of stable hydrogen and oxygen isotopes in forensic anthropology increased over the last decade (Copeland and Qi, 2012). O and H gained popularity due to the fact that they provide insight into human residential history patterns. Hydrogen and oxygen abundances of precipitation and waterways form the basis of isotope hydrology, which is reflected in the tap water consumed by humans across the globe.  $^{18}O$  and  $^{2}H$  or deuterium (D) signatures are wellestablished proxies of climate and source water, and are reliable methods for determining the provenance of human remains. In particular, the use of oxygen isotope composition was proposed as the most dependable method for forensic provenance studies at the 2008 American Academy of Forensic Sciences meeting on new geochemical techniques for geographic and forensic identification. Hydrogen isotopes are also useful proxies depending on the tissue type

sampled for analysis. Although in a pilot study by Holobinko et al. (2011) demonstrated that D in tooth enamel was not a useful proxy for geographic origin. On the other hand, sulfur has been shown to be a useful biomarker for determining the residential histories of modern populations. Valenzuela et al. (2011) found that geospatial patterns of sulfur in the continental United States had detectable regional variations, with lower values in the plains region and higher  $\delta^{34}S$  values closer to the coast. Similar results were observed in European studies of sulfur in lamb, beef, and dairy products (Camin et al., 2007; Perini et al., 2009).

#### 'Heavy' Stable Isotopes

 The application of 'heavy' stable isotopes, such as strontium (Sr), lead (Pb), and neodymium (Nd), have a more recent history in forensic science. Forensic scientists apply these isotopes towards litigation when the identification of source material is warranted. 'Heavy' stable isotopes are used in cases for determining bullet material, soil composition, drug sourcing, or for provenance of unidentified human remains. Forensic geochemistry relies on the subtle differences of the chemical composition and isotope abundances. A particular material is characterized then compared to potential source materials (Aggarwal et al., 2008). In the case of human remains the potential sources are the locations on the earth or the residential history of the individual.

 Large variations of isotopes exist in nature due to the initial abundance of the isotope at the time of the Earth's formation and the change in the amount over time (Pye, 2004). For instance, the isotope <sup>87</sup>Sr results from the radioactive decay of <sup>87</sup>Rb and overtime the ratio of  ${}^{87}$ Sr to the stable isotope  ${}^{86}$ Sr in rock increases with time as a function of the rocks Rb/Sr ratio (Bataille and Bowen, 2012). The strontium signature at the time of the Earth's formation 4.5 billion years ago was calculated as 0.699 and over time the strontium signature has increased with slight variations depending on the parent material of the rock: igneous, metamorphic, or carbonate rich bedrock. Old metamorphic rock has  ${}^{87}Sr/{}^{86}Sr$  ratios near 0.715, where recent volcanic rocks have values approximately 0.704 (Wright, 2005). Variations in the underlying bedrock, due to time, type of parent material, and weathering have contributed to specific geographic strontium signatures which enable the forensic anthropologist to track the movements of humans on the landscape.

 The ability for certain elements to withstand high temperatures is advantageous for the forensic investigator. Fire is often used in attempt to destroy forensic evidence in criminal cases, commonly in attempt to prevent the identification of the victim (Ubelaker, 2009). DNA degrades over time and can only withstand low temperatures. Harbeck et al. (2011) conducted a study to determine when DNA and certain isotopes can be retrieved from burned bone. Stable isotope values of carbon, nitrogen, and oxygen could withstand temperatures up to 200<sup>o</sup>C. However, strontium isotopes were reliable up to temperatures reaching 1000<sup>o</sup>C. This method is accurate and unaltered by cremation environments. This demonstrates the effectiveness of 'heavy' stable isotopes, and the usefulness of Sr for mass disasters and homicide investigations.

 'Heavy' stable isotopes also enable forensic scientists to perform studies without the worry of determining all of the foods consumed by the population. There is no measurable fractionation of Sr isotopes like there are for 'light' stable isotopes. This advantage eases the interpretation of stable isotope results. Although 'heavy' isotopes appear advantages to 'light' stable isotopes, acquiring a combination of results enables a clearer assessment of the unknown individuals in forensic cases.

#### Selected Isotope Case Studies for Residential Histories

 The majority of biogeochemistry research in forensic anthropology applies Sr or a combination of Sr and O, C, and N. An influential forensic biogeochemistry study using Sr was conducted by Juarez (2008) at the University of California, Santa Cruz that examined the remains of undocumented border crossers in the United States. Due to the lack of a welldeveloped missing persons database for Mexican-born individuals, many unidentified immigrants' remains stored in coroners or medical examiners offices in border communities await identification. The Sr research successfully narrowed the search of several individuals down to specific regions within Mexico.

 Outside the United States, stable isotopes have been used in conjunction with other identification methods to identify human remains. For instance, Rauch et al. (2009) used Sr and Pb in a forensic investigation of an individual found near a highway in Germany with gunshot trauma to the cranium, along with trauma fractures on the maxilla and mandible. Odontology results suggested that the individual was from Eastern Europe or the Russian Federation, and the 'heavy' stable isotope data narrowed the birth-location to Romania. Upon further assessment of the Romanian missing persons database, the unknown individual was confirmed by family member DNA. The evidence in this investigation also led to the arrest and confession of the victim's killers. Although isotope research presented in these case studies do not provide a direct 'fingerprint' for identification, the region of origin was narrowed, which allowed the search efforts for missing persons to be centered on smaller geographic areas.

#### Isoscapes

 Isoscapes are maps created to visualize the geographic distributions of stable isotopes. They were first used by geologists interested in the anthropogenic effects of pollutants, and ecologists monitoring the migrations of organisms. In the mid-twentieth century, the International Atomic Energy Agency (IAEA), in cooperation with the World Meteorological Organization, launched a global survey to measure the isotopic composition of precipitation to monitor climate change. This project was known as the Global Network of Isotopes Project (GNIP), and influenced the collection of large climate and hydrology datasets of groundwater isotopes throughout the globe. These early isotope maps pioneered the way for isoscapes used in many scientific disciplines today. For instance, Wassenaar et al. (2009) inspired by the GNIP project, went beyond the scope of the early research to refine  $\delta D$  and  $\delta^{18}O$  of groundwater in Mexico. Their isoscapes were tested in an ecological study of household songbirds to compare the hydrogen isotopes in feathers to the refined groundwater isoscape of Mexico (Hobson et al., 2009). Results from this study suggested that baseline isoscapes are needed for the species of interest.

 In forensic anthropology, baseline isoscapes of tap water, body water, and rainwater have been created for the study of human residential histories. Isoscapes in forensic science are typically generated from the collection of stable isotope data, and modeled using geo-statistical applications, such as ArcGIS. Many of the recent isoscape studies mimic the first national-level survey of tap water ratios by Bowen et al. (2007). For example, the temporal variation of oxygen isotopes  $(\delta^{18}O)$  in drinking water over a two-year period in the United States was conducted by Kennedy et al. (2011) following the methods of this earlier work. The isoscape generated from

the 2011 study applied robust Bayesian statistics that successful linked human hair from an unidentified female to four possible regions in the western United States of America.

 Isoscapes focusing on stable oxygen and hydrogen isotopes have to consider factors affecting the variation of tissue absorption into consideration. One factor influencing isotopic variation is body water. A sophisticated predictive model with equations to estimate the turnover time for non-residents to reach isotopic equilibrium in a new geographic region was estimated by Podlesak et al. (2012). They predicted the variation of stable oxygen and hydrogen in drinking and body water and the time it would take an individual to 'absorb' the geographic signature in the United States. More controlled isotopic research experiments are needed to test predictive models, such as these.

 GIS models have also helped test hypotheses for Sr isoscapes, using Silicate Model Theory. For instance, Bataille and Bowen (2012) applied this theory to model Sr of bedrock and water throughout the continental United States. Large-scale baseline isoscapes are useful for the forensic anthropologist, aiding in the identification of human remains. These large-scale isoscapes are now being refined for individual states in order to pinpoint individuals to more specific locations. For instance, Beasley (2013) presented a recent refinement of stable oxygen isotopes and an isoscape of northern California at the American Academy of Forensic Sciences meeting.

 Isoscapes are a useful resource for comparison when developing a stable isotope profile. Awareness of open source networks, such as http://isomap.org, a web-GIS cyber infrastructure where researchers can analyze, visualize, and share spatiotemporal isotopic models and derived data, has been address by Bowen et al. (2009), and in academic circles. The  $\delta^{18}O$  and  $^{87}Sr/^{86}Sr$ results from this NIJ project may be compiled into isoscapes and shared with the open source isoscape community.

#### Trace Elements

 Trace elements of bone and teeth have aided in the identification of unknown individuals due to unique elemental patterns associated with specific locations. Trace elements have been used to identify Mozart's skull, the last domicile of Ötzi the iceman, and monitor toxic metals in human hair (Stadlbauer et al., 2007; Hoogewerff et al., 2001). Forensic anthropologists use trace elements when human remains are found at crime scenes, mass burials, and mass disasters. Trace element analysis operates similarly to stable isotope analysis, but is complicated by the physiochemical processes of living organisms. Complications affecting the relative elemental abundances found in bones and teeth are trophic-level biopurification of nutrients, metabolic activity, selective diffusion according to element, and physiochemical characteristics, such as the element's charge and the ionic radius (Ezzo, 1994). For instance, Sr and barium (Ba) are positively charged ions with large ionic sizes that are similar to calcium (Ca), therefore Sr and Ba can replace Ca ions in the hydroxyapatite (inorganic portion) of bones and teeth (Burton, 2008). Trace elements are incorporated into human tissues by soil and dust inhalation. Some have suggested, such as the EPA, that certain elements are strongly linked to soil and dust ingestion, making food preparation and cleanliness a factor for certain elemental concentrations, such as Ba (Kohn et al., 2013). Trace element concentrations also depend on the tissue type: enamel, primary dentine, secondary dentine, trabecular bone, or cortical bone. If tissue type is not considered, the interpretation of trace element results may bias the study. If all 'complications' are factored into the concentration values received from the laboratory or mass

spectrometer, trace element analysis is a reliable biogeochemistry method. It can be used for environmental contaminates, exposure biomarkers, health and diet, and residential history. Trace element analysis has also been used to unravel diagenetic processes that occur postmortem (Tütken et al., 2011). There is great potential for trace element analysis in forensic anthropology as an addition to the SIP.

#### Environmental Contaminates as Exposure Biomarkers

 High levels of certain trace elements may aid in the identification of an individual. For instance, if an individual was exposed to environmental contaminates, occupational hazards, elevated elemental signatures may be detected in their bones, teeth, and hair. Anthropogenic emissions or toxic metal pollutants are discharged into soils and aquatic ecosystems in high levels annually. These pollutants result from coal combustion, sewage sludge, agricultural production, and commercial production. Cd, Cu, Pb, Cr, and Zn are found in fertilizers, pigments, lubricants, and chemicals. In the 1980's, approximately 80-90% of the arsenic (As) produced was applied to soil as an agricultural 'organic' pesticide (Nriagu and Pacyna, 1988). Arsenic poisoning may result from working and living downwind from smelters or drinking water from contaminated wells. Geographic regions at risk from well-water arsenic contamination include: Taiwan (southwest coast), South America (Argentina and Chile), India (Bengal), Mexico, Bangladesh, China (Inner Mongolia), Alaska, and some parts of the southwestern United States (Hindmarsh, 2002). Lead has served as a biomarker for many different types of exposure events. High levels of Pb in primary teeth are a well-established indicator of environmental Pb exposure in children and adolescents, which has also been linked to several neurodevelopmental health outcomes (Hare et al., 2011). These are a few examples of how trace element analysis may be used as biomarkers, and assist in the identification of human remains of individuals who once lived in geographic areas where they were exposed to environmental contaminates.

#### Medical Treatments as Exposure Biomarkers

 Elevated levels of trace metals and rare earth elements (REE's) analyzed from human tissue may also result from medical treatments. Throughout history metals were added to medicines for certain illnesses or to treat symptoms. For instance, Mozart stated on his deathbed that he had been poisoned by acqua toffana, a mixture of arsenic and lead oxide, used to treat severe stomach disease and served as a pain killer in the 18th and 19th centuries (Stadlbauer et al., 2007). High concentrations of As and Pb were found in the bones and teeth thought to be his remains. Hair can be used as a biomarker for exposure later in life, and identify any recent medical treatments. Several drugs containing noble metals like platinum (Pt) are the major constituents used in pharmaceutical cancer treatments. Trace element analysis of Pt in cancer patients' hair was monitored throughout the treatment, and raised Pt levels were observed in the hair strain when the individual received a cisplatin treatment (Pozebon et al., 2008). Advancements in laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) eases sample preparation and trace element analysis of teeth and hair. LA-ICP-MS is also a powerful tool for mapping small changes over time, like in the Pozebon et al. (2008) study, and is a less destructive method then the typical MC-ICP-MS or IRMS mass spectrometers. Gadolinium (Gd) is a REE that is toxic and used in small doses as a contrast agent in medical

images, such as MRIs. It was found that cortical bone concentration of Gd in exposed patients was as high as 31nmol/g, which is approximately 1000 times higher than individuals who were not exposed (Darrah, 2008). Another REE used for radionuclide therapy during painful bone metastases is Samarium (Sm) (Darrah, 2011). Gd, Pt, As, Sm and Pb are potentially useful for forensic identification of human remains when medical records are available for comparison.

### Trace Elements as Biomarkers for Residential Histories

 The use of trace elements as biomarkers for determining geographic provenance has been debated. In geochemistry, trace element ratios are used to distinguish geological formations by examining the chemical processes over time and the variation of trace elements from mixing depending on the geologic setting. Since trace element patterns vary according to the upper continental crust and geologic processes, trace elements may be useful for forensic geographic provenance studies. However, trace element incorporation into bone depends on natural abundances, along with dietary and metabolic processes, as discussed above. Despite these biological processes, Burton et al. (2003) argued that different bedrock geology can result in different bone and teeth trace elemental compositions despite similar diets, therefore indicating, to some extent, individual provenance (Cucina et al. 2011). Some have argued against this theory, suggesting that the evidence of different metabolically active tissues have significantly different concentrations of trace elements (Kohn et al., 2013). Elements that are metabolically regulated, such as Sr, Sc, Ti, Co, Fe, and Cr, may not be directly linked to geographic provenance because fractionation of these elements is large. Ba, Pb, Al, REE's, and the transition metals V and Mn are not metabolically regulated and thus may be useful for residential histories. Darrah (2008) specifically noted the success of La/ Dy, Mn/ La, and Al/ La ratios in distinguishing non- locals in a sample population from New York. Overall, the trace element composition retains the elemental signature of the previous domicile for several years following migration, which is useful for residential histories in forensic studies when bones and hair are available.

### The Effects of Globalization on Isotope Signatures

 The association between geography and isotopic signatures is directly linked by diet. Therefore, the concern of how globalization affects the modern human isotope signature is relevant to the forensic investigator. The modern diet is full of fast food, bottled water, imported fruits and vegetables, and foreign beverages. This 'food footprint' is larger than it was a generation ago, justifying the need for further investigations of the isotope global network. Is the isotope signature of modern humans altered due to globalization? Recent studies of fast food, beef, bottled water, and milk suggests that most consumables retain their place-of-origin signature. Therefore despite an increasingly global homogenized diet, studies analyzing hair, nails, and teeth from the United States, Canada, and Europe have found dietary heterogeneity (Valenzuela et al., 2012; Engel, 2010; Ehleringer et al., 2008).

 The fact that food and beverages retain their place-of-origin signature may complicate the isotope signature of modern humans. A large number of useful food and beverage sourcing studies have been conducted outside forensic anthropology. For instance, Chesson et al. (2008) analyzed H, C, N, and O of fast food meals in the United States, and concluded that  $\delta^{18}O$  and  $\delta^{2}H$ of beef exhibited large geographic variation. A more in depth study of restaurant and

supermarket foods, suggested that there was no correlation between carbohydrates, such as potatoes and wheat, and the signature of tap water, whereas a direct link could be established between beef and place of origin (Chesson et al., 2010a). Chesson et al. (2010b) also noted a promising correlation between non-local beverages, such as milk, and the  $\delta^{18}O$  signature of the local tap water supply. Another geographic region where the sourcing of bottled waters has received considerable attention is South Korea. Bong et al. (2009) examined 50 different brands of bottled water available in South Korea, and found only slight correlations between marine and sparkling waters. Strontium stable isotopes have also been tested in Korean bottled waters that suggested Sr as a useful biomarker for bottle d water origin (Kim et al., 2013).

 As seen from the literature review, countless factors complicate the modern human isotope signature. Since some foods retain the place of origin signature, studies of geolocation are still possible in the United States. Individual life histories play a major role in the ability for isotopes to be used to source human migration patterns. Trends drive the American lifestyle, whether the trend is to consume bottled water or eat/shop locally. For instance, the more locallygrown food an individual consumes, the less bias the SIP. Social economic status would also factor into the reliability of the SIP, assuming individuals with limited economic means would have less access foreign products. Also, the  $(\delta^{18}O)$  tap water signature of the region should substantially correlate with the isotope signature of modern humans because tap water is still ingested when cooking.

 Forensic anthropologists rely on comparative skeletal collections to determine age, sex, and ancestry of human remains. Past databases of comparative anatomical skeletal collections were compiled for the purpose of skeletal identification research (Jantz and Moore-Jansen, 1988). In addition to comparative skeletal collections, baseline biogeochemistry results of known individuals amplify the chance for the correct identification of missing and unknown persons. Complimentary methods associated with the standard biological profile are becoming increasingly important for law enforcement officials, and methods borrowed from biogeochemistry provide additional evidence in a court of law. Stable isotopes and trace elements aid in the identification of unknown individuals through the determination of birth and death locations, along with visualizing human movements across the globe. Database expansion supports the method acceptability (Ubelaker, 2008) of isotopes and trace element analysis.

#### Literature Cited

- Aggarwal J, Habicht-Mauche J, Juarez C. 2008. Application of heavy stable isotopes in forensic isotope geochemistry: A review. Applied Geochemistry 23:2658–2666.
- Ambrose SH. 1993. Isotopic analysis of paleodiets: methodological and interpretive considerations. Food and nutrition in history and anthropology 10:59–130.
- Bataille CP, Bowen GJ. 2012. Mapping 87Sr/ 86Sr variations in bedrock and water for large scale provenance studies. Chemical Geology 304-305:39–52.
- Beasley M. 2013. Extending the Biological Profile: Using Stable Isotope Analysis as an Exclusionary Tool in Region-of-Origin Investigations of Unidentified Remains.
- Bong YS, Ryu JS, Lee KS. 2009. Characterizing the origins of bottled water on the South Korean market using chemical and isotopic compositions. Analytica Chimica Acta 631:189–195.
- Bowen GJ, Ehleringer JR, Chesson LA, Stange E, Cerling TE. 2007. Stable isotope ratios of tap water in the contiguous United States. Water Resources Research 43.
- Bowen GJ, West JB, Vaughn BH, Dawson TE, Ehleringer JR, Fogel ML, Hobson K, Hoogewerff J, Kendall C, Lai CT, Miller CC, Noone D, Schwarcz H, Still CJ. 2009. Isoscapes to address largescale earth science challenges. Eos 90:109–110.
- Brand WA, Coplen TB. 2012. Stable isotope deltas: tiny, yet robust signatures in nature. Isotopes in Environmental and Health Studies 48:393–409.
- Burton J. 2008. Bone chemistry and trace element analysis. In: Biological Anthropology of the Human Skeleton, Second Edition. . p 443–460.
- Burton JH, Price TD, Cahue L, Wright LE. 2003. The use of barium and strontium abundances in human skeletal tissues to determine their geographic origins. International Journal of Osteoarchaeology 13:88–95.
- Camin F, Bontempo L, Heinrich K, Horacek M, Kelly SD, Schlicht C, Thomas F, Monahan FJ, Hoogewerff J, Rossmann A. 2007. Multi-element (H, C, N, S) stable isotope characteristics of lamb meat from different European regions. Analytical and bioanalytical chemistry 389:309–320.
- Chesson LA, Podlesak DW, Erkkila BR, Cerling TE, Ehleringer JR. 2010a. Isotopic consequences of consumer food choice: Hydrogen and oxygen stable isotope ratios in foods from fast food restaurants versus supermarkets. Food Chemistry 119:1250–1256.
- Chesson LA, Podłesak DW, Thompson AH, Cerling TE, Ehleringer JR. 2008. Variation of hydrogen, carbon, nitrogen, and oxygen stable isotope ratios in an american diet: Fast food meals. Journal of Agricultural and Food Chemistry 56:4084–4091.
- Chesson LA, Valenzuela LO, O'Grady SP, Cerling TE, Ehleringer JR. 2010b. Hydrogen and Oxygen Stable Isotope Ratios of Milk in the United States. Journal of Agricultural and Food Chemistry 58:2358–2363.
- Coplen TB, Qi H. 2012. USGS42 and USGS43: Human-hair stable hydrogen and oxygen isotopic reference materials and analytical methods for forensic science and implications for published measurement results. Forensic Science International 214:135–141.
- Cucina A, Tiesler V, Sierra Sosa T, Neff H. 2011. Trace-element evidence for foreigners at a Maya port in Northern Yucatan. Journal of Archaeological Science 38:1878–1885.
- Darrah TH. 2008. Inorganic trace element composition of modern human bones: Relation to bone pathology and geographical provenance.
- Darrah TH. 2011. Characterization of Lead, Transition Metal, and Rare Earth Element Composition of Human Bone by ICP-MS and LA-ICP-MS.
- Ehleringer JR, Bowen GJ, Chesson LA, West AG, Podlesak DW, Cerling TE. 2008. Hydrogen and oxygen isotope ratios in human hair are related to geography. Proceedings from the National Academy of Sciences 105:2788–2793.
- Engel N. 2010. Geographic variation of stable isotopes in western Canadians: A potential aid in the forensic identification of human remains.
- Ezzo JA. 1994. Zinc as a paleodietary indicator: An issue of theoretical validity in bone-chemistry analysis. American Antiquity:606–621.
- Fry B. 2006. Stable isotope ecology. Springer. New York, NY.
- Hare D, Austin C, Doble P, Arora M. 2011. Elemental bio-imaging of trace elements in teeth using laser ablation-inductively coupled plasma-mass spectrometry. Journal of dentistry 39:397–403.
- Hindmarsh JT. 2002. Caveats in hair analysis in chronic arsenic poisoning. Clinical Biochemistry 35:1- 11.
- Hobson KA, Van Wilgenburg SL, Larson K, Wassenaar LI. 2009. A feather hydrogen isoscape for Mexico. Journal of Geochemical Exploration 102:63–70.
- Holobinko A, Meier-Augenstein W, Kemp HF, Prowse T, Ford SM. 2011. 2H Stable Isotope Analysis of human tooth enamel: A new tool for forensic human provenancing? Rapid Communications in Mass Spectrometry 25:910–916.
- Hoogewerff J, Papesch W, Kralik M, Berner M, Vroon P, Miesbauer H, Gaber O, Künzel K-H, Kleinjans J. 2001. The last domicile of the Iceman from Hauslabjoch: a geochemical approach using Sr, C and O isotopes and trace element signatures. Journal of Archaeological Science 28:983–989.
- Jantz RL, Moore-Jansen PH. 1987. A data base for forensic anthropology. Final Report to the National Institute of Justice.
- Juarez CA. 2008. Strontium and Geolocation, the Pathway to Identification for Deceased Undocumented Mexican Border-Crossers: A Preliminary Report\*. Journal of Forensic Sciences 53:46–49.
- Katzenberg MA. 2008. Stable isotope analysis: a tool for studying past diet, demography, and life history. Biological Anthropology of the Human Skeleton, Second Edition:411–441.
- Kennedy CD, Bowen GJ, Ehleringer JR. 2011. Temporal variation of oxygen isotope ratios (δ18O) in drinking water: Implications for specifying location of origin with human scalp hair. Forensic Science International 208:156–166.
- Kim G-E, Shin W-J, Ryu J-S, Choi M-S, Lee K-S. 2013. Identification of the origin and water type of various Korean bottled waters using strontium isotopes. Journal of Geochemical Exploration.
- Kohn MJ, Morris J, Olin P. 2012. Trace element concentrations in teeth–a modern Idaho baseline with implications for archaeometry, forensics, and palaeontology. Journal of Archaeological Science.
- Meier-Augenstein W. 2010. Stable Isotope Forensics. John Wiley, Chichester.
- Michaela Harbeck first, Ramona Schleuder, Julius Schneider, Wolfgang W. Schmahl. 2011. Research potential and limitations of trace analyses of cremated remains. Forensic Science International 204:191–200.
- Mützel Rauch E, Lehn C, Peschel O, Hölzl S, Roßmann A. 2009. Assignment of unknown persons to their geographical origin by determination of stable isotopes in hair samples. International Journal of Legal Medincine 123:35–40.
- Neuberger FM, Grupe G, Jopp E, Püschel K, Graw M. 2013. Signs of malnutrition and starvation-Reconstruction of nutritional life histories by serial isotopic analyses of hair. Forensic Science International 226:22–32.
- Nriagu JO, Pacyna JM. 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature 333:134–139.
- Perini M, Camin F, Bontempo L, Rossmann A, Piasentier E. 2009. Multielement (H, C, N, O, S) stable isotope characteristics of lamb meat from different Italian regions. Rapid communications in mass spectrometry 23:2573–2585.
- Podlesak DW, Bowen GJ, O'Grady S, Cerling TE, Ehleringer JR. 2012. δ 2H and δ 18O of human body water: A GIS model to distinguish residents from non-residents in the contiguous USA. Isotopes in Environmental and Health Studies 48:259–279.
- Pozebon D, Dressler VL, Matusch A, Becker JS. 2008. Monitoring of platinum in a single hair by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) after cisplatin treatment for cancer. International Journal of Mass Spectrometry 272:57–62.
- Pye K, Croft DJ. 2004. Forensic geoscience: introduction and overview. Geological Society, London, Special Publications 232:1–5.
- Pye K. 2004. Isotope and trace element analysis of human teeth and bones for forensic purposes. Geological Society Special Publications 232:215–236.
- Santamaria-Fernandez R, Giner Martínez-Sierra J, Marchante-Gayón J, García-Alonso J, Hearn R. 2009. Measurement of longitudinal sulfur isotopic variations by laser ablation MC-ICP-MS in single human hair strands. Analytical and Bioanalytical Chemistry 394:225–233.
- Stadlbauer C, Reiter C, Patzak B, Stingeder G, Prohaska T. 2007. History of individuals of the 18th/19th centuries stored in bones, teeth, and hair analyzed by LA–ICP–MS: a step in attempts to confirm the authenticity of Mozart's skull. Analytical and bioanalytical chemistry 388:593–602.
- Tütken T, Torsten W. Vennemann, Hans-U. Pfretzschner. Nd and Sr isotope compositions in modern and fossil bones – Proxies for vertebrate provenance and taphonomy. Geochimica et Cosmochimica Acta 75:5951–5970.
- Ubelaker DH. 2008. Forensic Anthropology: methodology and diversity of applications. In: Biological Anthropology of the Human Skeleton, Second Edition. . p 41–69.
- Ubelaker DH. 2009. The forensic evaluation of burned skeletal remains: a synthesis. Forensic science international 183:1–5.
- Valenzuela LO, Chesson LA, Bowen GJ, Cerling TE, Ehleringer JR. 2012. Dietary heterogeneity among western industrialized countries reflected in the stable isotope ratios of human hair. PLoS ONE.
- Valenzuela LO, Chesson LA, O'Grady SP, Cerling TE, Ehleringer JR. 2011. Spatial distributions of carbon, nitrogen and sulfur isotope ratios in human hair across the central United States. Rapid Communications in Mass Spectrometry 25:861–868.
- Wassenaar LI, Van Wilgenburg SL, Larson K, Hobson KA. 2009. A groundwater isoscape (δD, δ 18O) for Mexico. Journal of Geochemical Exploration 102:123–136.
- Wiersema J, Love JC, Naul LG. 2009. The influence of the Daubert guidelines on anthropological methods of scientific identification in the medical examiner setting. In: Hard Evidence: Case Studies in Forensic Anthropology, Prentice Hall, New Jersey. p 80–90.
- Wright LE. 2005. Identifying immigrants to Tikal, Guatemala: Defining local variability in strontium isotope ratios of human tooth enamel. Journal of Archaeological Science 32:555–566.

### *Statement of hypothesis or rationale for the research*

Forensic scientists routinely examine animal and human hair, human bone and enamel, and food remains in an effort to determine the origin of these materials. Stable isotope and elemental analysis as an emerging technique has increasingly received attention in criminal investigations and forensic anthropology in recent years (Bol and Pflieger 2002; Petzke et al. 2005; Benson et al. 2006; Schwarcz 2007; Cerling et al. 2007; Ehleringer et al. 2007; Fraser and Meier-Augenstein 2007; Meier-Augenstein and Fraser 2008). The various stable isotopes and elements, and the tissue from which they are derived, provide a wealth of information on the dietary and residential history of an individual (Table 1). The goals of this study are to assess the level of variation in different isotopes for a series of donated skeletal collections from across the United State. Stable C and N isotope analyses of human bone collagen and apatite can allow quantitative estimates of dietary components because bone collagen is disproportionately produced from the protein portion of the diet. Bone apatite on the other hand is deposited from dissolved bicarbonate in the human body system, which is drawn from all dietary components and digested waters (DeNiro and Epstein 1980; Fricke and O'Neil 1996; Muldner and Richard 2005; Hedges et al. 2005). The level of consumption of terrestrial and marine plants and animals will alter the isotopic signature. Higher N and C isotope values are presumed to be associated with a greater intake of animal proteins (Schoeninger et al. 1984), and thus imply higher social status within historical communities.

Stable O, and H isotope analyses of bone and teeth enamel phosphate and carbonate provide environmental water isotope information and diet history, because the  $\delta^{18}O$  values of ingested drinking water are directly related to bone phosphate  $\delta^{18}O$  values (Muldner and Richards 2007). Due to the remarkable geographical differences in  $\delta^{18}$ O of meteoric waters and tap waters, the analysis of  $\delta^{18}O$  can provide information about geographical locations and the movement history of the individuals (Bowen et al., 2007).

The alkaline earth metal element Strontium (Sr) in human and animal bone and tooth minerals is inherited through dietary items which uptake the element from the residual parent material, sea water and/or soil. The biologically-available signature of the organism is proportional to the local Sr level. In addition, Sr isotope compositions vary regionally, and there is little isotopic fractionation observed through trophic level (Beard et al. 2000). Diagenetic alteration of bone can replace strontium with extended exposure to soil or sea water, but these changes typically do not affect enamel. The Strontium isotopes consist of four stable isotopes  $({}^{88}\text{Sr}, {}^{87}\text{Sr}, {}^{86}\text{Sr}$  and  ${}^{84}\text{Sr})$  with natural isotope abundance of 82.53%, 7.04%, 9.87%, and 0.56%, respectively. These distinct isotopes vary geographically and are related to food and water supply source. The Sr isotope ratio  $({}^{87}Sr/{}^{86}Sr)$  in various geological and biological products exhibits geographical characteristics.

Rare earth elements (REE) and other trace elements have proven to be regionally specific and provide evidence of medical treatments and environmental exposure. Darrah (2009) has demonstrated the utility of laser ablation for the examination of Sr, REEs and other trace elements in bone. The level of lead (Pb) within a sample is clearly related to exposure, and these levels can be examined geographically.

### Literature Cited

- Beard, B.L., C.M. Johnson, 2000. Strontium isotope composition of skeletal material can determine the birth place and geographic mobility of humans and animals. J. Forensic Sciences 45(5): 1049-1061.
- Benson, S., C. Lennard, P. Maynard, C. Roux, 2006. Forensic applications of isotope ratio mass spectrometry—A review. Forensic Science International 157: 1-22.
- Bol, Roland, C. Pflieger, 2002. Stable isotope (13C, 15N and 34S) analysis of the hair of modern humans and their domestic animals. Rapid Communications in Mass Spectrometery 16:2195-2200.
- Bowen GJ, Ehleringer JR, Chesson LA, Stange E, and Cerling TE. 2007. Stable isotope ratios of tap water in the contiguous United States. Water Resources Research 43:1–12.
- Cerling, T.E., L.K. Ayliffe, M.D. Dearing, J.R. Ehleringer, B.H.Passey, D.W. Podlesak, A.-M. Torregrossa, A. G. West, 2007. Determining biological tissue turnover using stable isotopes: the reaction progress variable. Oecologia 151: 175-189.
- Darrah TH. 2009. Inorganic trace element composition of modern human bones: Relation to bone pathology and geographical provenance. Unpublished Disertation, University of Rochester.
- DeNiro, M. J. and S. Epstein, 1980. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45: 341-351.
- Ehleringer, J.R., T.E. Cerling, and J.B. West, 2007. Forensic Science Applications of Stable Isotope Ratio Analysis. In: R.D. Blackledge (Ed.), Forensic Analysis on the Cutting Edge: New Methods for Trace Evidence Analysis. Wiley, New York.
- Fraser, I., W. Meier-Augenstein, 2007. Stable ²H isotope analysis of modern-day human hair and nails can aid forensic human identification. Rapid Communications in Mass Spectrometry 21(20):3279- 3285.
- Fricke, H. C., J. R. O'Neil, 1996. Inter- and intra-tooth variation in the oxygen isotope composition of mammalian tooth enamel phosphate: implications for palaeoclimatological and palaeobiological research. Palaeogeography, Palaeoclimatology, Palaeoecology 126:91-99.
- Hedges, R.E.M., R.E. Stevens, and P.L. Koch, 2005. Isotopes in Bones and Teeth. In: M.J. Leng (ed.), Isotopes in Palaeoenvironmental Research. Springer, Netherlands.
- Meier-Augenstein W, and Fraser I. 2008. Forensic isotope analysis leads to identification of a mutilated murder victim. Science and Justice 48:153–159.
- Muldner, G., M.P. Richards, 2005. Fast or feast: reconstructing diet in later medieval England by stable isotope analysis. Journal of Archaeological Science 32: 39-48.
- Muldner, G., M.P. Richards, 2007. Stable isotope evidence for 1500 years of human diet at the city of York, UK. American Journal of Physical Anthropology 133: 682-697.
- Petzke, K.J., H. Boeing, S. Klaus, C.C.Metges, 2005. Carbon and nitrogen stable isotopic composition of hair protein and amino acids can be used as biomarkers for animal-derived dietary protein intake in humans. The Journal of Nutrition 135: 1515-1520.
- Schoeninger, Margaret J. and Michael J. DeNiro, 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochimica et Cosmochimica Acta 48: 625-639.
- Schwarcz, H.P., 2007. Tracing Unidentified Skeletons Using Stable Isotopes. Forensics Magazine June/July (http://www.forensicmag.com/articles.asp?pid=152).



 **Table 2.** Isotope and trace element analysis information matrix.

# **Methods**

The methods for bone collagen extraction, bone apatite preparation, tooth  $Ag_3PO_4$ preparation, and stable isotope analysis are described in detail in this section. Strontium isotope analysis and trace element samples and methods are also discussed below. An aggregate count of the samples used for this report is presented in Table 3. A detailed listing of the samples is presented in Tables 5-11.



**Table 3.** Summary of the estimated sizes and actual sample used for the project.

# *Bone collagen extraction (Filter Bag Method)*

 The filter bag method was developed specifically for this project to increase yield and reduced sample preparation times. Bone samples were limited to middle hand and foot phalanges from the donated collections. Sample selection for UNM and TSU-SM was decided by the collection manager and often their concern was sample size. As a result, UNM submitted middle foot phalanges and UTK and TSU-SM submitted middle hand phalanges.

# Preparation of Bone Samples

Bone was sampled at the University of Alabama-Huntsville in the Stable Isotope Laboratory. Sample batches of sixteen were prepared in 50 ml beakers. Approximately 40 ml of chloroform:acetone solution was added to each beaker, and placed in the sonicator for 3 hours to clean and remove lipids. The chloroform:acetone solution was decanted, and beakers were filled with hot 1000ml of DI water. Samples were sonicated in DI water for approximately 30 minutes, and then drained. Samples were then dried at 50°C overnight. When samples were thoroughly dry, powdered samples were obtained using a Dremel with a bone-cutting bit. The portion of the outer surface of the sample was removed with the Dremel, as well as any medullary bone, and discarded. Drill bits were cleaned with 1M HCL and rinsed with DI water in between samples. Using the bone-cutting bit with the acetate shield in place 1.0 g of bone powder was sampled, and then the powder was sieved using a 75  $\mu$ m screen. Approximately 0.5 g of the  $>75 \mu$ m

powder fraction was used for collagen extraction, and <75 µm powder was reserved for apatite extraction.

#### Preparation of Filter Bags

Teflon fabric was cut into  $3.5$ " ( $\sim$ 9 cm) wide strips, and folded in half, lengthwise, to create a 1.75" wide strip. The fabric was sealed at one end of the strip,  $\sim 1/8$ " from end, using an impulse sealer set at "4". The bags were measured 1" (2.5 cm) from this sealed line and a third line, 0.25" away from the second one was sealed. This step was repeated along the entire strip. The bags were cut between the second and third lines (the ones separated by 0.25") in order open the bags, measuring  $1.5$ " x  $1.75$ " ( $\sim$  4 x 4.5 cm) each. Nylon monofilament line was used to attach labels to the collagen samples. All material, Teflon fabric bags and nylon lines, were placed in a 600ml beakers filled with ~500ml of acetone for sterilization. Beakers were sonicated for 1 hour. Acetone was then decanted and boiled in DI water for 15 minutes. DI water was then drained, and sample bags were dried at 50°C overnight.

Clean filter-bags were weighed, and then 0.5 g of the collagen sample was added to each bag and weighed to calculate the sample weight. Sample labels for each bag were attached to the end of clean nylon lines, and sealed with an impulse sealer. Bags were then placed into previously cleaned and annealed (600°C furnace for 3 hours) 50 ml beakers for collagen extraction.

#### Collagen Extraction Procedure

Collagen was extracted by first adding 50 ml of 0.2 M HCl to each beaker with a prepared filter-bag. Samples were sonicated for 2 hours, and then rinsed with several washes of DI water. Samples were redissolved in 50 ml of 0.125 NaOH, and sonicated for two hours. NaOH solution was decanted and rinsed several times with DI water, and redissolved in 50 ml of  $10^{-3}$  M HCl, neutralize any remaining NaOH. The HCl was replaced with 50 ml of fresh  $10^{-3}$  M HCl, and sonicated for an additional three hours. Collagen samples were then topped off with 10- <sup>3</sup> M HCl, and placed in a 95°C oven, overnight. After 24 hours, the filter-bags were removed from the beakers, and the beakers were returned to the oven for three hours in order for the collagen solution to condense. Collagen solution was transferred into a labeled, pre-weighed 20 ml scintillation vial, and returned to the oven for the solution to condense to 1 ml, and placed in the freezer. Frozen samples were immediately transferred to the freeze-dryer for 24 hours.

#### *Bone apatite preparation*

 Bone powder with particle size <0.25 mm obtained from the collagen sample preparation were used to prepare the bone apatite. Bone powder samples were loaded into 1.7ml microcentrifuge tubes and placed into vials filled with DI water. The samples were dissolved in 1.5ml 5-6% NaOCl sonicated for three hours, decanted, and redissolved in NaOCl for 24 hours. Samples were placed in the centrifuge, decanted, and dissolved in 0.1M acetic for four hours. Bone apatite samples were centrifuged, decanted, and repeated six times. Once the samples were frozen, they were immediately transferred to the vacuum line and dried to  $10^{-3}$  Torr. After reaching the expected vacuum, the samples were ready for stable isotope analysis.

### *Tooth Ag3PO4 preparation*

The protocol for tooth  $Ag_3PO_4$  preparation was adopted from Stephan (2000), with minor modifications that included the application of the ultrasonic water bath, and enhanced NaOCl solution. Enamel was sampled using a NewWave MicroMill to obtain approximately 10 mg of powdered tooth enamel (<0.25 mm). Samples were processed to remove any organic substances by reacting 5.6% NaOCl in 2 ml centrifuge tubes at room temperature with gentle agitation. After 24 hours, the sample solution was centrifuged, dissolved organic substances were removed, and the residue washed 4 to 5 times to neutrality with doubly distilled water. Humic substances were removed by adding 1 ml 0.15 M NaOH for 48 hours with gentle agitation. Samples were rinse 5 or more times for neutrality and to prevent the formation of salt during the acid dissolution. Dry apatite was dissolved in 2 ml HF for 24 hours at room temperature. The solution was centrifuged to separate  $CaF<sub>2</sub>$  and insoluble organic matter from the phosphate solution. The phosphate solution was pipetted into15 ml tubes and neutralized with 3 ml 2 M KOH. The remaining residue was rinsed with 2 ml of doubly distilled water and placed into beakers. Samples were dissolved in 3 ml of a buffered silver ammine solution  $(0.2 M AgNO<sub>3</sub>;$ 1.16 M NH<sub>4</sub>NO<sub>3</sub>; 0.75 ml conc. NH<sub>4</sub>OH) to precipitate  $A\alpha_3PO_4$ , and gradually warmed to 70<sup>o</sup>C for 3 hours, and slowly cooled to room temperature. When the samples were gently heated, the pH of the solution was about 10, and as the ammonia evolved the pH decreased, and  $\text{Ag}_3\text{PO}_4$ began to precipitate. The reaction ends at a pH around 7.0 and the precipitation is quantitative. The crystals were filtered with a  $0.2 \mu$  filter, and washed several times with distilled water, then dried overnight at 50°C.

# $δD, δ<sup>18</sup>O, δ<sup>13</sup>C, and δ<sup>15</sup>N$  Analyses

 Stable isotopic compositions of all samples, except for those from the Maxwell Museum Documented Skeletal Collection and the Texas State University-San Marcos Forensic Research Facility, were analyzed in the Stable Isotope Laboratory in the Department of Earth and Planetary Sciences at UTK or the University of Alabama Tuscaloosa. Two Thermo-Electron isotope ratio mass spectrometers, including a Delta Plus and a Delta Plus XL, were used for these analyses. The Delta Plus is a dual inlet mass spectrometer, coupled with CarboFlo for carbonate analysis. The Delta Plus XL serves as a continuous flow mass spectrometer, coupled with the high Temperature Conversion/Element Analyzer (TC/EA) for analyzing  $\delta D$ , and  $\delta^{18}O$  in hair and phospate, and Costech ECS4010 Elemental Analyzer (EA) for analyzing C%, N%, C/N ratio,  $\delta^{13}$ C and  $\delta^{15}$ N in collagen.  $\delta^{13}$ C and  $\delta^{15}$ N analysis of bone collagen was conducted with the Costec EA 4010-IRMS system. Approximately 1 mg of collagen was needed to generate C%, N%, C/N ratio,  $\delta^{13}$ C and  $\delta^{15}$ N data for each sample. The EA-IRMS system was equipped with a 50-position Zero-Blank autosampler. Four EA standards and six isotope standards were placed among the 50 samples. Quality control was implemented by using a two-endpoint correction algorithm.

 $\delta^{18}$ O analysis of tooth phosphate was conducted with a Thermo-Electron TC/EA-IRMS system. About 200 µg of silver phosphate derived from tooth enamel phosphate was needed to generate enough signal for the  $\delta^{18}$ O measurement for each sample. The TC/EA-IRMS system is equipped with a 50-position Zero-Blank autosampler. To implement quality control, a minimum of eight  $\delta^{18}$ O standards including NIST 120C and IAEA 601 and 602 were placed in each carousal. The two-endpoint correction algorithm was performed for the raw data correction.

 $\delta^{13}$ C and  $\delta^{18}$ O analysis of apatite carbonate from bone was conducted on Finnigan CarboFlo Microvolume-Dual Inlet mass spectrometer. Approximately 70µg carbonate-equivalent of purified sample was needed for each analysis. Eight carbonate standards were included to insure quality control for each run.

 $\delta^2$ H and  $\delta^{18}$ O analysis of hair was conducted with the TC/EA-IRMS. Approximately 100 µg was needed from each sample. Non-exchangeable hydrogen and oxygen isotope standards including BWB, CFS and CHS were placed among the samples for quality control and calibration.

### *⁸⁷Sr/⁸⁶Sr Analysis*

 Strontium isotope analyses were conducted at the R. Ken Williams Radiogenic Isotope Geosciences Laboratory at Texas A&M University, with all sample preparation conducted in a Class 100 clean laboratory environment. For the strontium isotope analysis, approximately 5 to 20 mg of powdered tooth enamel was dissolved in 15 mL Savillex PFA vials using 500 µl of 3N HNO3 and evaporated. The sample was then redissolved in 500 µl of 3N HNO3 and then purified through cation exchange columns filled with Sr exchange resin. The SrSpec resin was cleaned in the column with repeated washes of Milli-Q H2O and then conditioned with 3N HNO3. SrSpec resin was used once for sample elution and then discarded. The dissolved sample was loaded in the column and rinsed with five washes of 300  $\mu$ L of 3N HNO3, and then Sr was eluted with 1 mL of H2O. After the purified samples were dried down, they were redissolved in 2 µl of 0.3N H3PO4 and 2 µl of TaCl5, and loaded on to degassed Re filaments for analysis by a Thermo Scientific Triton thermal ionization mass spectrometry (TIMS).

#### *Trace Element Analysis*

 Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a reliable method used in forensic science for determining trace elements. LA-ICP-MS provides faster analysis times and is virtually non-destructive when compared to the traditional TIMS method (Horstwood et al., 2008). Dentition from two of the donated collections were sampled from the WBDSC  $(n=21)$  and TSUSM  $(n=5)$ . Sample preparation for the teeth was minimal due to the benefits of pre-ablation, which removes contaminates from the surface of the enamel and purges the chamber before the ablation process. The ICP-MS system used was an Elan DRC II coupled to a New Wave Research UP-213 laser ablation system (New Wave Research, Fremont, CA, USA) equipped with Nd:YAG laser emitting a nanosecond laser pulse in the fifth harmonic with a wavelength of 213 nm. ICP-MS was optimized daily with the Elan 6100 DRD set-up solution (Lot # 13-176GSL1). Tuning of the laser ablation unit was achieved using National Institute of Standards and Technology (NIST) standard reference materials SRM 610 and SRM 612. Another advantage of laser ablation is that quantitative analysis using a non-matrix matched calibration with internal standards is possible when no reference standards are available (Castro et al., 2010). Limited standards are available for teeth. NIST 1486 (bone meal) and NIST 1480 (bone ash) are used for bone samples and may be used for teeth, but were not used in this study. Instead optimization was achieved through the techniques mentioned above. Operating conditions for the optimized LA-ICP-MS system are given in table 4. Variable parameters include plasma power, argon gas flow, and lens voltage for the ion beam focusing for the mass spectrometer.

 Enamel bioapatite provides a chronological record of changes in trace elements during the time of the tooth's formation (Balasse, 2002). By sampling enamel over the entire length of the tooth, changes in residential histories or certain biomarkers, such as medical treatments or environmental contaminates, can be observed in elevated REE's or trace metals. Since enamel matrix formation proceeds from the apex toward the cervix of the crown, the laser ablation sampling scheme was set to read trace element variations across the length of the tooth. Linear lines, approximately 400 µm in length, were placed towards the cervix of the crown, along the midline, and towards the apex, and ablated to a depth of 50 $\mu$ m. Intra-tooth sampling is beneficial when using known birth locations of the donated collections in order to determine if the variations are in fact caused by the known factors involved.



**Table 4**. LA-ICP-MS operating conditions.

# Literature Cited

- Balasse M. 2002. Reconstructing dietary and environmental history from enamel isotopic analysis: time resolution of intra-tooth sequential sampling. International Journal of Osteoarchaeology 12:155–165.
- Castro W, Hoogewerff J, Latkoczy C, Almirall JR. 2010. Application of laser ablation (LA-ICP-SF-MS) for the elemental analysis of bone and teeth samples for discrimination purposes. Forensic science international 195:17-27.
- Horstwood MSA, Evans JA, Montgomery J. 2008. Determination of Sr isotopes in calcium phosphates using laser ablation inductively coupled plasma mass spectrometry and their application to archaeological tooth enamel. Geochimica et cosmochimica acta 72:5659-5674.
- Stephan E. 2000. Oxygen Isotope Analysis of Animal Bone Phosphate: Method Refinement, Influence of Consolidants, and Reconstruction of Palaeotemperatures for Holocene Sites. Journal of Archaeological Science 27:523-535.

# **Results**

#### *Statement of Results*

 Multiple constituents including bone collagen, bioapatite (phosphate and carbonate), and hair keratin from 290 donations from WBDSC, UNMMM, and TSU-SM were prepared for  $\delta^{13}C$ ,  $\delta^{18}O$ ,  $\delta^{15}N$ , <sup>87</sup>Sr/<sup>86</sup>Sr, and  $\delta^{2}H$  analysis using refined protocols. The protocols were enhanced by shortening the cycle of each sample preparation period for collagen extraction utilizing a filterbag method assisted by an ultrasonic water bath, and modifying a Thermo TC/EA for improving analysis precision of phosphate  $\delta^{18}O$ . Dental enamel was sampled using a NewWave Micromill and analyzed for  $\delta^{18}$ O of phosphate. The averaged  $\delta^{18}$ O value for a select sample of the dental enamel was  $16.91\pm2.21\%$  VSMOW (n=215) (see Table 5). Our initial correlation analysis of the dental  $\delta^{18}$ O values with meteoric water  $\delta^{18}$ O at birth location (modeled) yielded the equation  $(\delta^{18}O_{\text{tooth}}=0.62*\delta^{18}O_{\text{water}}+21.74, r=0.63, n=45)$  (Figure 2) (Herrmann et al., 2010), which was similar to the equation ( $\delta^{18}O_{\text{bone}}=0.64*\delta^{18}O_{\text{water}}+22.37$ , r=0.98) generated by Longineli (1983). With the addition of the new samples from TSU-SM and UNMMM the correlation reduced significantly  $(\delta^{18}O_{\text{tooth}}=0.349*\delta^{18}O_{\text{water}}+20.164, r=0.36, n=120)$  and appears to vary by collection with TSU-SM and UNMMM showing poor relationships (Figure 3).

Non-exchangeable  $\delta^2$ H of hair keratin was also analyzed using a Thermo TC/EA. The averaged  $\delta^2$ H was -83.35± 6.36‰ VSMOW (n=14) (Table 6). The  $\delta^2$ H values exhibit a positive correlation with the meteoric water  $\delta^{18}$ O at death location (r=0.81) (Figure 4), and a negative correlation with altitude  $(r=-0.73)$  that is consistent with the isotope "Altitude Effect".

In addition, bioapatite carbonates samples (n=290) were analyzed for  $\delta^{13}C$  and  $\delta^{18}O$ . The averaged value was -9.53±1.28‰ (VPDB) for  $\delta^{13}$ C, and -11.23± 4.00‰ (VPDB) for  $\delta^{18}$ O. These values vary by collection with marked differences between the TSU-SM samples as compared to both UTK and UNMMM for  $\delta^{13}$ C and a significant difference between UTK as compared to both TSU-SM and UNMMM. These differences are evident, and are shown in the basic plot of the data shown in Figure 5. In addition, the samples show regional differences in the range of  $\delta^{18}$ O values (Figure 6) with the UNMMM samples exhibiting a substantial range and the UTK range being far more limited. Several of the UNMMM samples' residential histories were limited to only the state for death locations, which resulted in several repeated latitudes and longitudes. Even with this generalizing effect the regional differences in oxygen values are evident in the bone bioapatite samples.

The relationship between bone bioapatite ( $\delta^{18}$ O ‰, VPDB) and tooth enamel phosphate  $(\delta^{18}O \%_{0}, VSMOW)$  requires the conversion of the bioapatite value from VPDB to VSMOW (**http://www.cstl.nist.gov/div837/837.01/outputs/standards/algorithm/background.htm**). The bioapatite values were converted based on the published standards and compared (Figure 7). The tooth enamel phosphate  $\delta^{18}$ O in relation to the bone bioapatite is enriched in  $^{18}$ O by 6.25±1.63 (‰, VSMOW) (n=67) for UTK, -0.82±5.60 (‰, VSMOW) (n=18) for TSU-SM, 1.77±4.08 (‰, VSMOW) (n=86) for UNMMM. The low to inversed enrichment values for TSU-SM and UNMMM suggests a marked migration history for the skeletal collections in the western samples.

Bone collagen samples (n=215) were extracted for  $\delta^{13}$ C and  $\delta^{15}$ N analysis. In addition, N%, C%, N/C ratios were recorded for a subset of these samples (Figure 8). The averaged carbon content in human phalanx (both hand and foot) bone collagen was 32.93±6.87%,

11.31 $\pm$ 3.32% for nitrogen content. The averaged C/N ratio was 3.133 $\pm$ 1.04. The averaged  $\delta^{13}$ C value was -16.32 $\pm$ 1.25‰ (VPDB), and 11.24 $\pm$ 0.53‰ (AIR) for  $\delta^{15}N$ . The latitude range of death locations for the collagen samples is between 29.424 and 44.840; the longitude range extends from -123.230 to -72.502. Significant correlations were observed between C and N isotopes and death locations (Table 10).

Powdered enamel samples ( $n=57$ ) were collected for  ${}^{87}Sr/{}^{86}Sr$  analysis. Sr results are available for 55 samples and were combined with the results presented by Regan (2006) for military personnel. The  ${}^{87}Sr/{}^{86}Sr$  ratios were then converted to epsilon values (Beard and Johnson 2000) using the equation below:

$$
\varepsilon^{87} \text{Sr} = \left( \frac{\left( \frac{^{87} \text{Sr}}{^{86} \text{Sr}} \right)_{\text{measured}}}{\left( \frac{^{87} \text{Sr}}{^{86} \text{Sr}} \right)_{\text{bulkearth}}} - 1 \right) * 10000.
$$

respectively. In addition, the values were plotted on the available GIS coverages for both models The resulting epsilon values were compared to modeled bedrock (A) and modeled drainage and bedrock (B) epsilon values based on Beard and Johnson (2000) and Bataille and Bowen (2012), (Figure 9). Specific point values based on birth locations were extracted from the raster coverages of these two models and the two values were plotted to assess the match of the measured vs. location expected (or modeled) position (Figure 10). Both models provide poor fits to the modeled values. The data does appear to follow the pattern relative to birth location. The plot of ratios organized by value from low to high shows general patterning at the state level (Figure 11).

 The LA-ICP-MS is included as preliminary and should be the focus of future studies with donated human skeletal collections. Figure 12 provides a breakdown of the ratio of lead ( $Pb_{208}$ ) relative to Rubidium  $(Rb_{85})$  to simply see if any individuals appear to have excessive Pb. There are at least two individuals that show excessive lead levels (UT37-07d and UT33-08D).

 All of the assays and residential history information have been combined and added to an open database for researchers to use. The data is available in FIND and can be access from Mississippi State University (http://find.msstate.edu/fmi/webd).

# *Tables*

# Samples Analyzed

Table 5. PO<sub>4</sub> Enamel Samples prepared and processed.









\* sample damaged during processing 

<b>IndividualID</b>	SampleID	SampleID_2	<b>Tissue</b>	Mass (mg)	d2H
UT11-08	11-08	23394	Hair	0.102	$-72.7677$
UT82-08	82-08	23395	Hair	0.106	$-87.3357$
UT29-08	29-08	23396	Hair	0.092	$-86.1753$
UT21-08	21-08	23398	Hair	0.092	$-83.2222$
UT17-08	17-08	23399	Hair	0.102	$-86.1230$
UT64-08	64-08	23402	Hair	0.094	-77.2733
UT15-08	15-08	23403	Hair	0.100	$-86.2161$
UT116-08	116-08	23405	Hair	0.094	$-84.2166$
UT42-08	42-08	23406	Hair	0.102	$-80.3098$
UT05-08	05-08	23407	Hair	0.102	$-97.1124$
UT56-08	56-08	23410	Hair	0.106	$-72.0598$
UT49-08	49-08	23411	Hair	0.094	$-85.0602$
UT56-08	56-08D	23412	Hair	0.092	$-72.0598$
UT38-08	38-08	23413	Hair	0.090	$-86.0547$

**Table 6.** Hair samples prepared and processed.



**Table 7.** Apatite samples prepared and processed.

















**Table 8.** Bone collagen samples prepared and processed.













**Table 8.** Strontium samples analyzed by Texas A&M University Geochemistry Laboratory  $(n=55)$ .





# *Figures*

Figure 1. Filter bag experiment for bone collagen extraction: The distinct advantage of the Filter-Bag method is the limited time required to perform the chemical extraction portion of the procedure, and allows more samples to be processed simultaneously. The red squares represent data from raw bone samples; the blue diamonds are the results from Ambrose method (Ambrose et al., 1990). The triangles are the results from the Filter-Bag method.



**Figure 2.** Preliminary  $\delta^{18}O$  relationship between tooth enamel  $PO_4^{3}$  and local meteoric water at birth location. The local meteoric water  $\delta^{18}$ O values were derived from Bowen, 2006, Earth and Atmospheric Sciences, Purdue University. The yellow dash line represents bone  $PO<sub>4</sub><sup>3-</sup> \delta<sup>18</sup>O$ values from Longinelli, 1984.



**Figure 3.** Final  $\delta^{18}$ O relationship between tooth enamel  $PO_4^{3}$  and local meteoric water at birth location. The local meteoric water  $\delta^{18}O$  values were derived from Bowen, 2006, Earth and Atmospheric Sciences, Purdue University. The dash line represents bone  $PO<sub>4</sub><sup>3</sup>$   $\delta^{18}O$  values from Longinelli, 1984. The three samples are depicted as different symbols.



Figure 4.  $\delta^2$ H relationship between hair keratin non-exchangeable hydrogen isotope and local meteoric water at death location. The local meteoric water d2H values were derived from Bowen, 2006, Earth and Atmospheric Sciences, Purdue University. Non-exchangeable  $\delta^2H$ values were calculated by analyzing BWB, CFS and CHS samples (Wassenaar et al, 2003) (see the insert).



**Figure 5.** Bone apatite  $\delta^{13}$ C values plotted by  $\delta^{18}$ O values with 50% density ellipses for each sample (UTK=blue triangles; TSU-SM=green diamonds; UNMMM=small red circles). The relationship exhibits distinct distributions for the three collections with the UTK sample distinguished from TSU-SM and UNMMM along the  $\delta^{18}O$  axis and the TSU-SM collection distinguished from UTK and UNMMM along the  $\delta^{13}$ C axis.



**Figure 6.** Bone apatite  $\delta^{18}$ O values plotted by latitude and longitude with 50% density spheres for each sample (UTK=blue dot; TSU-SM=green dots; UNMMM=black dots). The relationship exhibits distinct distributions for the three collections. The UTK sample exhibits the highest  $\delta^{18}$ O values. The TSU-SM and UNMMM exhibit similar  $\delta^{18}$ O distributions but the death locations are distinct.



**Figure 7.** δ<sup>18</sup>O relationship between bone apatite (blue line) and tooth enamel phosphate (red line) with divisions by collection. Compared to the tooth enamel phosphate  $\delta^{18}O$ , the bone apatite is enriched in <sup>18</sup>O by  $6.25 \pm 1.63$  (‰, VSMOW) (n=67) for UTK, -0.82 $\pm$ 5.60 (‰, VSMOW) (n=18) for TSU-SM,  $1.77\pm4.08$  (‰, VSMOW) (n=86) for UNMMM. The low to inversed enrichment values for TSU-SM and UNMMM suggesting marked migration into the western samples.



**Figure 8.** Bone collagen C/N ratio (A),  $\delta^{13}$ C values (B),  $\delta^{15}$ N values (C), and relationship between C and N isotopes (D). The C/N ratio averages 3.133±1.04 and the ratios exhibit a nearly constant value for the UTK and TSU-SM collections with considerable variation in the UNMMM collection. This variation may be related to the fact that the UNMMM samples were derived from foot phalanges, which produced low collagen yields. The averaged  $\delta^{13}C = -16.32 \pm 10^{-10}$ 1.250‰. The averaged d15N =  $11.24 \pm 0.53$ ‰. The latitude range of death locations for these samples is between 29.424 and 44.840; -123.230 and -72.502 for longitude range. Significant correlations were observed between C and N isotopes and death locations.

**Table 1.** Correlation matrix of bone collagen results with longitude (death), latitude (death), elevation (death), and age. The results show significant correlations (p<0.05, *shaded*) with nearly all values except latitude.





Figure 9. Enamel Sr (<sup>87</sup>Sr/<sup>86</sup>Sr) epsilon values plotted on the modeled bedrock (A) and modeled drainage and bedrock (B) epsilon values based on Beard and Johnson (2000) and Bataille and Bowen (2012), respectively. Blue dots represent birth locations of UTK or TSU-SM samples.



Figure 10. Enamel Sr ( ${}^{87}Sr/{}^{86}Sr$ ) ratio values plotted by modeled bedrock (A) and modeled drainage and bedrock (B) ratio values based on Beard and Johnson (2000) and Bataille and Bowen (2012), respectively. Dots represent birth locations of UTK or TSU-SM samples and the diagonal line is a one-to-one relationship. The age and drainage model appears to conform to the mean  $87$ Sr/ $86$ Sr values but fall off as the ratios increase.



Figure 11. Enamel Sr ( $87\text{Sr}/86\text{Sr}$ ) ratio values plotted in ascending order by project (Red and Blue – current NIJ project; Green and Black – Regan (2006) study). Regan's data for military personnel is included for comparative purposes in the plot. In general, there is some degree of clustering of values by state.



Figure 12. Preliminary LA-ICP-MS results based on counts per second (cps). The plot depicts Pb208 cps scaled by Rb85 cps. Overall, the values are consistent with the standard (610 or 612) except UT37-07D and UT38-08D.

# **Conclusions**

### *Discussion of findings*

Our study indicates that the dental enamel  $\delta^{18}$ O values from the WBSC collections are overall reflective of the individual's birth location, whereas hair keratin  $\delta^2$ H values are influenced by the individual's death location, which is consistent with several other isotopic studies of forensically derived human samples and suggests that the application of the dual isotopes (O, H) could provide better constrain on the residential history by pinpointing the beginning (tooth) and the ending (hair) of the individual life journey. Although the correlation coefficient of the dental  $\delta^{18}$ O with local water is not as high as reported by several other researchers, the relationship however does follow the trend of Longinelli (1984). This could result from the potential influence of isotopic pattern of tap water as compared to meteoric precipitation, especially with the inclusion of the samples from Texas and New Mexico. It is also suspected that the WBDSC does not represent a geographically heterogeneous sample and it is likely that self- or family-reported residential histories, as is the practice at the UTK FAC, are more variable.

### *Implications for policy and practice*

 This study has implications for law enforcement and practicing forensic geochemists and forensic anthropologists interested in isotope and trace element research. This study has provided a large isotopic dataset from three donated human skeletal collections currently used as reference samples for active forensic anthropologists. These data enhance our understanding of the isotopic variation in modern humans, specifically modern US residents. The isotopic variation we see in these samples is greater than typically observed in more controlled laboratory studies, but the results do conform to current isotopic models, specifically for  $\delta^{18}O$ , and is therefore useful for estimating residential histories. Future isotopic work with unidentified decedents could be linked to their NamUs record and used to provide potential matches within the system based on geographic histories.

 Broad impacts of the study relate to the Forensic Isotopic National Database (FIND). The data generated by this study will be made available to researchers through FIND and researchers can also submit their own results from forensic casework and modern donated collections. FIND will serve as a repository of forensic isotope data for human skeletal, dental, and hair studies. The study has also contributed to the training and laboratory experiences of both graduate and undergraduate students at the University of Tennessee- Knoxville, Mississippi State University, University of Alabama-Huntsville, University of New Mexico, and Texas State University-San Marcos.

 The PIs on the project have also reached out to the medicolegal community to provide these services for a nominal fee during the process of the grant. Dr. Li has processed several bone and enamel samples for  $\delta^{18}O$  as well as carbon and nitrogen. Isotopic analysis is now viewed as an important step in the analysis of unidentified decedents in some agencies.

#### *Implications for further research*

 As the donated collections at UTK, UNM, and TSSU as well as several new body donation programs across the country grow, it is essential that reliable residential histories be collection from the donors and that isotopic data be collected (specifically adequate hair samples for research requests). These expanding collections combined with recent forensic isotope surveys being conducted and recently published will provide a much better picture of the isotopic and trace element variation across the United States. It is anticipated that this study will provide a foundation for future research with these collections.

# **References**

- Aggarwal J, Habicht-Mauche J, and Juarez C. 2008. Application of heavy stable isotopes in forensic isotope geochemistry: A review. Applied Geochemistry 23:2658–2666.
- Ambrose, S. H., 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. Journal of Archaeological Acience 17:431-451.
- Bataille CP, and Bowen GJ. 2012. Mapping 87Sr/ 86Sr variations in bedrock and water for large scale provenance studies. Chemical Geology 304-305:39–52.
- Beard, B.L., C.M. Johnson, 2000. Strontium isotope composition of skeletal material can determine the birth place and geographic mobility of humans and animals. J. Forensic Sciences 45(5): 1049-1061.
- Benson, S., C. Lennard, P. Maynard, C. Roux, 2006. Forensic applications of isotope ratio mass spectrometry—A review. Forensic Science International 157: 1-22.
- Bentley, R. A., 2006. Strontium isotopes from the earth to the Archaeological skeleton: A review. Journal of Archaeological Method and Theory 13: 135-187.
- Bol, Roland, C. Pflieger, 2002. Stable isotope (13C, 15N and 34S) analysis of the hair of modern humans and their domestic animals. Rapid Communications in Mass Spectrometery 16:2195-2200.
- Bong YS, Ryu JS, and Lee KS. 2009. Characterizing the origins of bottled water on the South Korean market using chemical and isotopic compositions. Analytica Chimica Acta 631:189–195.
- Bowen GJ, Ehleringer JR, Chesson LA, Stange E, and Cerling TE. 2007. Stable isotope ratios of tap water in the contiguous United States. Water Resources Research 43:1–12.
- Bowen GJ, Ehleringer JR, Chesson LA, Thompson AH, Podlesak DW, and Cerling TE. 2009a. Dietary and physiological controls on the hydrogen and oxygen isotope ratios of hair from mid-20th century indigenous populations. American Journal of Physical Anthropology 139:494–504.
- Bowen GJ, West JB, Vaughn BH, Dawson TE, Ehleringer JR, Fogel ML, Hobson K, Hoogewerff J, Kendall C, et al. 2009b. Isoscapes to address large-scale earth science challenges. Eos 90:109–110.
- Bowen GJ, and West JB. 2008. Isotope Landscapes for Terrestrial Migration Research. Available from: internal-pdf://Bowen and West 2008-0584965393/Bowen and West 2008.pdf
- Bowen GJ. 2010. Isoscapes: Spatial pattern in isotopic biogeochemistry. Annual Review of Earth and Planetary Sciences 38:161–187.
- Brenčič M, Ferjan T, and Gosar M. 2010. Geochemical survey of Slovenian bottled waters. Journal of Geochemical Exploration 107:400–409.
- Cerling, T.E., L.K. Ayliffe, M.D. Dearing, J.R. Ehleringer, B.H.Passey, D.W. Podlesak, A.-M. Torregrossa, A. G. West, 2007. Determining biological tissue turnover using stable isotopes: the reaction progress variable. Oecologia 151: 175-189
- Chesson LA, Podlesak DW, Erkkila BR, Cerling TE, and Ehleringer JR. 2010a. Isotopic consequences of consumer food choice: Hydrogen and oxygen stable isotope ratios in foods from fast food restaurants versus supermarkets. Food Chemistry 119:1250–1256.
- Chesson LA, Podłesak DW, Thompson AH, Cerling TE, and Ehleringer JR. 2008. Variation of hydrogen, carbon, nitrogen, and oxygen stable isotope ratios in an american diet: Fast food meals. Journal of Agricultural and Food Chemistry 56:4084–4091.
- Chesson LA, Valenzuela LO, Bowen GJ, Cerling TE, and Ehleringer JR. 2011. Consistent predictable patterns in the hydrogen and oxygen stable isotope ratios of animal proteins consumed by modern humans in the USA. Rapid Communications in Mass Spectrometry 25:3713–3722.
- Chesson LA, Valenzuela LO, O'Grady SP, Cerling TE, and Ehleringer JR. 2010b. Hydrogen and Oxygen Stable Isotope Ratios of Milk in the United States. Journal of Agricultural and Food Chemistry 58:2358–2363.
- Chesson LA, Valenzuela LO, Ogrady SP, Cerling TE, and Ehleringer JR. 2010c. Links between purchase location and stable isotope ratios of bottled water, soda, and beer in the united states. Journal of Agricultural and Food Chemistry 58:7311–7316.
- Coplen TB, and Qi H. 2012. USGS42 and USGS43: Human-hair stable hydrogen and oxygen isotopic reference materials and analytical methods for forensic science and implications for published measurement results. Forensic Science International 214:135–141.
- Daeid NN, Buchanan HAS, Savage KA, Fraser JG, and Cresswell SL. 2010. Recent advances in the application of stable isotope ratio analysis in forensic chemistry. Australian Journal of Chemistry  $63:3 - 7$ .
- Dotsika E, Poutoukis D, Raco D, and Psomiadis D. 2010. Stable isotope composition of Hellenic bottled waters. Journal of Geochemical Exploration 107:299–304.
- Darrah TH. 2009. Inorganic trace element composition of modern human bones: Relation to bone pathology and geographical provenance. Unpublished Disertation, University of Rochester.
- DeNiro, M. J. and S. Epstein, 1980. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45: 341-351.
- Ehleringer, J.R., T.E. Cerling, and J.B. West, 2007. Forensic Science Applications of Stable Isotope Ratio Analysis. In: R.D. Blackledge (Ed.), Forensic Analysis on the Cutting Edge: New Methods for Trace Evidence Analysis. Wiley, New York.
- Fraser I, Meier-Augenstein W, and Kalin RM. 2006. The role of stable isotopes in human identification: A longitudinal study into the variability of isotopic signals in human hair and nails. Rapid Communications in Mass Spectrometry 20:1109–1116.
- Fraser, I., W. Meier-Augenstein, 2007. Stable ²H isotope analysis of modern-day human hair and nails can aid forensic human identification. Rapid Communications in Mass Spectrometry 21(20):3279- 3285
- Fraser I, Meier-Augenstein W, and Kalin RM. 2008. Stable Isotope Analysis of Human Hair and Nail Samples: The Effects of Storage on Samples. Journal of Forensic Sciences 53:95–99.
- Fricke, H. C., J. R. O'Neil, 1996. Inter- and intra-tooth variation in the oxygen isotope composition of mammalian tooth enamel phosphate: implications for palaeoclimatological and palaeobiological research. Palaeogeography, Palaeoclimatology, Palaeoecology 126:91-99.
- Gulson B, Jameson CW, and Gillings B. Stable lead isotopes in teeth as indicators of past domicile. Available from: http://library-resources.cqu.edu.au/JFS/PDF/vol\_42/iss\_5/JFS425970787.pdf
- Hedges, R.E.M., R.E. Stevens, and P.L. Koch, 2005. Isotopes in Bones and Teeth. In: M.J. Leng (ed.), Isotopes in Palaeoenvironmental Research. Springer, Netherlands.
- Holobinko A, Meier-Augenstein W, Kemp HF, Prowse T, and Ford SM. 2011. 2H Stable Isotope Analysis of human tooth enamel: A new tool for forensic human provenancing? Rapid Communications in Mass Spectrometry 25:910–916.
- Juarez CA. 2008. Strontium and Geolocation, the Pathway to Identification for Deceased Undocumented Mexican Border-Crossers: A Preliminary Report\*. Journal of Forensic Sciences 53:46–49.
- Katzenberg MA. 2011. Review of: Stable Isotope Forensics: An Introduction to the Forensic Application of Stable Isotope Analysis. Journal of Forensic Sciences 56:1673–1673.
- Kempson IM, and Lombi E. 2011. Hair analysis as a biomonitor for toxicology, disease and health status. Chemical Society Reviews 40:3915–3940.
- Kennedy CD, Bowen GJ, and Ehleringer JR. 2011. Temporal variation of oxygen isotope ratios (δ18O) in drinking water: Implications for specifying location of origin with human scalp hair. Forensic Science International 208:156–166.
- Kim GE, Ryu JS, Shin WJ, Bong YS, Lee KS, and Choi MS. 2012. Chemical and isotopic compositions of bottled waters sold in Korea: Chemical enrichment and isotopic fractionation by desalination. Rapid Communications in Mass Spectrometry 26:25–31.
- De Laeter J. 2010. The Rosetta Stone of isotope science and the uranium/lead system. Mass Spectrometry Reviews 30:757–771.
- Lehn C, Mützel E, and Rossmann A. 2011. Multi-element stable isotope analysis of H, C, N and S in hair and nails of contemporary human remains. International Journal of Legal Medicine 125:695–706.
- Li, Zheng-Hua, Nicholas P. Herrmann, Richard L. Jantz, Miriam Soto, 2013. Isotope forensic evaluation of modern human remains from the University of Tennessee William M. Bass Donated Skeletal Collection. American Academy of Forencis, Washington D.C. Abstract with program, D3.
- Longinelli A. Oxygen isotopes in mammal bone phosphate: A new tool for paleohydrological and paleoclimatological research ? Geochimica et Cosmochimica Acta 1984:48:385-90.
- Meier-Augenstein W, and Fraser I. 2008. Forensic isotope analysis leads to identification of a mutilated murder victim. Science and Justice 48:153–159.
- Michaela Harbeck first, Ramona Schleuder, Julius Schneider, Ingrid Wiechmann, Wolfgang W. Schmahl, and Gisela Grupe. 2011. Research potential and limitations of trace analyses of cremated remains. Forensic Science International 204:191–200.
- Muccio Z, and Jackson GP. 2009. Isotope ratio mass spectrometry. Analyst 134:213–222.
- Muldner, G., M.P. Richards, 2005. Fast or feast: reconstructing diet in later medieval England by stable isotope analysis. Journal of Archaeological Science 32: 39-48.
- Muldner, G., M.P. Richards, 2007. Stable isotope evidence for 1500 years of human diet at the city of York, UK. American Journal of Physical Anthropology 133: 682-697.
- Mützel (Rauch) E, Lehn C, Peschel O, Hölzl S, and Roßmann A. 2009. Assignment of unknown persons to their geographical origin by determination of stable isotopes in hair samples. International Journal of Legal Medincine 123:35–40.
- O'Brien DM, and Wooller MJ. 2007. Tracking human travel using stable oxygen and hydrogen isotope analyses of hair and urine. Rapid Communications in Mass Spectrometry 21:2422–2430.
- O'Connell TC, and Hedges REM. Investigations Into the Effect of Diet on Modern Human Hair Isotopic Values.
- Parks CL. 2009. Oxygen Isotope Analysis of Human Bone and Tooth Enamel: Implications for Forensic Investigations. Available from: internal-pdf://Parks 2009-1896488707/Parks 2009.pdf
- Petzke, K.J., H. Boeing, S. Klaus, C.C.Metges, 2005. Carbon and nitrogen stable isotopic composition of hair protein and amino acids can be used as biomarkers for animal-derived dietary protein intake in humans. The Journal of Nutrition 135: 1515-1520.
- Podlesak DW, Bowen GJ, O'Grady S, Cerling TE, and Ehleringer JR. 2012. δ 2H and δ 18O of human body water: A GIS model to distinguish residents from non-residents in the contiguous USA. Isotopes in Environmental and Health Studies 48:259–279.
- Regan LA. 2006. Isotopic determination of region of origin in modern peoples: Applications for identification of United States war-dead from the Vietnam conflict. Unpublished Dissertation, University of Florida, Department of Anthropology.
- Santamaria-Fernandez R, Giner Martínez-Sierra J, Marchante-Gayón J, García-Alonso J, and Hearn R. 2009. Measurement of longitudinal sulfur isotopic variations by laser ablation MC-ICP-MS in single human hair strands. Analytical and Bioanalytical Chemistry 394:225–233.
- Schoeninger, Margaret J. and Michael J. DeNiro, 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochimica et Cosmochimica Acta 48: 625-639.
- Schwarcz, H.P., 2007. Tracing Unidentified Skeletons Using Stable Isotopes. Forensics Magazine June/July (http://www.forensicmag.com/articles.asp?pid=152) .
- Sealy, J.C., N.J. van der Merwe, A. Sillen, F.J. Kruger, H.W. Krueger, 1991. 87Sr/86Sr as a dietary indicator in modern and Archaeological bone. Jouranl of Archaeological Science 18: 399-416.
- Sillen, A., M. Kavanagh, 1982. Strontium and paleodietary research: A review. Yearbook of Physical Anthropology 25: 67-90.
- Thomas Tütken, Torsten W. Vennemann, and Hans-U. Pfretzschner. Nd and Sr isotope compositions in modern and fossil bones – Proxies for vertebrate provenance and taphonomy. Geochimica et Cosmochimica Acta 75:5951–5970.
- Valenzuela LO, Chesson LA, Bowen GJ, Cerling TE, and Ehleringer JR. 2012. Dietary heterogeneity among western industrialized countries reflected in the stable isotope ratios of human hair. PLoS ONE [Internet] 7. Available from: internal-pdf://Valenzuela et al 2012-2638568196/Valenzuela et al 2012.pdf
- Valenzuela LO, Chesson LA, O'Grady SP, Cerling TE, and Ehleringer JR. 2011. Spatial distributions of carbon, nitrogen and sulfur isotope ratios in human hair across the central United States. Rapid Communications in Mass Spectrometry 25:861–868.
- Wassenaar, L. I., K.A. Hobson, 2003. Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. Isotopes in Environmental and Health Studies. 39 (3):211–217.
- Wassenaar LI, Van Wilgenburg SL, Larson K, and Hobson KA. 2009. A groundwater isoscape (δD, δ 18O) for Mexico. Journal of Geochemical Exploration 102:123–136.
- Yang L. 2009. Accurate and precise determination of isotopic ratios by MC-ICP-MS: A review. Mass Spectrometry Reviews 28:990–1011.

# **Dissemination of Research Findings**

# *Websites:*

http://find.msstate.edu (currently http://find.msstate.edu/fmi/webd) An example screen capture is provided. Access is available with username FINDUser and password FINDUser.



**Figure 13.** Screen capture from FIND.

# *Publications:*

2010 Herrmann NP, Li Z-H, and Soto M.

Isotopic evaluation of modern human remains from the University of Tennessee William M. Bass Donated Collection. *American Journal of Physical Anthropology* Supplement 50:127-127.

# *Papers/Posters Presented:*

2013 Li Z-H, Herrmann NP, Jantz RL, and Soto ME

Isotope Forensic Evaluation of Modern Human Remains From the University of Tennessee William M. Bass Donated Skeletal Collection. Poster presented at the Sixty-fifth Annual Meeting of the American Academy of Forensic Sciences, Washington D.C.

2013 Warner MM, Herrmann NP, Trask W, Li Z-H, and Li Y

Strontium Variation in the William M. Bass and Texas State University Donated Skeletal Collections: A Preliminary Assessment. Paper presented at the Mountain, Swamp, and Beach Regional Forensic Conference, Starkville, MS.

#### 2011 Herrmann NP, Li Z-H, Weinand D, and Soto M

Isotopic and Elemental Analysis of the William Bass Donated Skeletal Collection and Other Modern Donated Collections. "Did Colonel Mustard Really Kill Miss Scarlet in the Library with the Lead Pipe?" Identifying Clues Through NIJ Research. Grantee symposium organized by the National Institute of Justice, Chicago, Illinois. February.

#### 2010 Herrmann NP

Isotopes and Databases: Tools For the Identification of Unknown Forensic Cases. Invited lecture to the Department of Anthropology, Texas State University, San Marcos. February.

#### 2009 Herrmann NP, Li Z-H, Weinand DC, Jantz RL, and Soto ME

Isotopic And Elemental Analysis of the William Bass Donated Skeletal Collection and Other Modern Donated Collections. Invited paper presentation at the National Institute of Justice Forensic Science Research and Development Forensic Anthropology Working Group, Alexandria, Virginia. December.

#### 2009 Herrmann NP

Forensic Anthropology, NamUs and Isotopes. Lecture presented at the Mississippi MS State ME Training Course, Jackson, MS. December.

### 2009 Herrmann NP

Archaeology, Bones, and People: Forensic Anthropology of the Past. Lecture presented as part of Mississippi Archaeology Month at Plymouth Bluff Center, Columbus, MS. October.