The Use of Isotopes by Forensic Anthropologists and Archaeologists for the Analysis of Human Remains: An Exploratory Study

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A thesis presented to the Honors College of Middle Tennessee State University in partial fulfillment of the requirements for graduation from the University Honors College.

Spring 2024

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Dedicated to my family, my friends, my partner William Childress, and my thesis advisor, Dr. Tiffany Saul. This project would have never been completed without your support.

Acknowledgments

I would like to express my appreciation to my thesis committee chair, Dean John Vile, my thesis advisor, Dr.Tiffany Saul, whom I have had the pleasure of working with throughout my time at MTSU, and Dr. Frank Bailey, who stepped in at the last minute to help my research get approval. I would also like to acknowledge everyone who completed my survey, as they made this report possible.

Abstract

Stable isotope analysis, a common tool in ecology, was applied to bioarchaeology shortly after its inception. In more recent years, stable isotope analysis has been used by forensic anthropologists to help aid in missing persons cases by providing additional leads. With more researchers utilizing this technique, the lack of consistency and standards between the two fields as well as different laboratories have become prevalent. This anonymous survey was designed to help determine those differences in practices to aid in creating a set of standards. It was sent out to many types of researchers, seven of whom responded, who were conducting stable isotope analysis in either archaeology or forensic anthropology. Even with the small number of participants, there were clear similarities and differences between each researcher. These differences help to highlight the growing need for standards among practices in stable isotope analysis.

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Introduction

Anthropologists, simply put, study the cultural and biological processes of humans. This study can be in the context of linguistics, culture, or in this case, forensic anthropology. Archaeologists study people's cultural past, such as where they may have lived, what they may have eaten, where they may have gotten water, and if they ever traveled significantly. Having answers to these questions won't only provide aid in understanding the history of an individual or a civilization, but can also assist in the aforementioned forensic contexts, such as a missing person case. One way that anthropologists and bioarchaeologists have begun to answer these questions is by analyzing isotopes collected from skeletal tissues. Because the isotopes found in these tissues can change based on geography, they can help determine where a person may have lived and died.

Isotopes

Isotopes are variations of an element that differ only in the number of neutrons that reside in the nucleus. They can be seen in all elements of the periodic table and can form artificially by shooting a stable nucleus with charged particles, or they can form naturally and spontaneously when a nucleus goes through radioactive decay (Fry, 2006). Isotopes have been around since the creation of Earth, but most are unstable and

short-lived. However, stable isotopes have survived for billions of years on Earth without rapid decay and can help provide early ecological records. They are also not hazardous to human health in any way (Wada et al, 1995). Most isotopes that are of interest come from hydrogen (H), carbon (C), nitrogen (N), oxygen (O), and sulfur (S), which all have at least two isotopes that interact with organic material. Ecologists have developed an isotope map where they have the heaviest concentration of different isotopes present and isotope patterns. They are also able to study isotopes found in food webs to help determine the diet of populations of different species, including humans. For example, ^{12}C and ^{13}C are found in all plants and do not change as the cells move throughout the food chain. Based on the concentration of the two isotopes it can be determined if a plant uses C4, C3, or CAM processes. Knowing this can determine the environment the plant grew in. These maps and distributions, in recent years, have been able to help anthropologists and archaeologists solve forensic cases and better understand history, respectively. Through different methods, isotopes can be collected from materials such as bones, hair, and teeth and can be compared to isotope maps to help provide identification of remains (Fry, 2006).

Bioarchaeology

Bioarchaeology is the "application of biological anthropological methods to the study of archaeological problems." It could also be defined as "scientific study of human remains from archaeological sites" (Arizona State University). The most commonly used biological remains in bioarchaeology are recovered bone samples and, using isotopes, it can roughly determine the place of origin or migration patterns for the individual, or

reconstruct the history of an entire population (Stodder and Palkovich, 2012). In skeletal samples, specifically their collagen, the organic phase, and the mineral phase (hydroxyapatite or bioapatite) are of interest. All isotopes found in the bioapatite tell a story regarding the diet as a whole, and the oxygen isotopes in the bioapatite can indicate geographically where the water that the individual consumed can be found. Isotopes found in the collagen indicate the isotopes in the protein source consumed by the individual. Different skeletal remains are also able to tell different parts of an individual's story. The bioapatite component of teeth enamel provides a relatively static record of the time the teeth are formed, in childhood and early adolescence, as there is very little change to teeth following this time. All teeth, including permanent ones, often erupt by the age of 13, except for third molars which often erupt between the ages of 18 and 25 (Zimmerman and Jenzer, 2020). With isotopes collected from the bioapatite and collagen from bone samples, a record of a few to several years can be collected. The exact number of years depends on each bone. The femur, for instance, experiences turnover over an extended period of 20 years or more. There are complications to this analysis practice, such as diagenesis, or the breakdown of bone tissue (Chesson et al., 2024).

One of the first examples of isotopes being used in archaeology was in the 1970s by a South African archaeologist, Nikolaas van der Merwe. While excavating in the Iron Age African site Kgopolwe 3, he discovered the remains of a human in an ash pile that he believed did not fit with other remains based on morphology and burial technique. Through isotope analysis, he and his colleagues determined that the remains belonged to a sorghum farmer from a Khoisan village, who happened to die at the site of Kgopolwe 3 (Loftus et al., 2016). Following this, archaeologists have used isotopes in understanding

ancient humans, such as research such as determining the hominin diet. ^{15}N and both ^{12}C and ^{13}C remained present in the remains and were analyzed to understand ecological sources and trophic identification, and were able to be collected from collagen in bones. However, bulk isotopic composition from ancient civilizations has complications due to multiple foods having similar ^{13}C and ^{15}N values, therefore some archaeologists have even begun pulling carbon and nitrogen stable isotopes from present amino acids for analysis (Larsen et al., 2022). There has also been research in understanding nitrogen levels in different trophic levels while dissecting a food web, with indication that there are higher nitrogen isotope compositions present in hot arid environments than in cold and wet ones. The complication with this research is that biological remains being in those environments post-mortem can alter the physiology and lead to misleading results, therefore the researchers were forced to evaluate the food chain as a whole to determine the isotopic composition of the stepwise enrichment between trophic levels (Ambrose, 1995). Lastly, research has been done regarding the $^{87}Sr/^{86}Sr$, Strontium, composition of the prehistoric Andes to determine mobility. Data from both men and women was collected as well as from coastal, Yungas (transitional forests between the other two regions), and highland sites. Strontium is reliable in determining different origins of prehistoric non-native individuals as it can be collected from the hydroxyapatite crystals of bone and tooth enamel and remains relatively unchanged throughout the food chain. So as long as local food is consumed during tissue formation, it can be incredibly beneficial in determining long-term migration or lack thereof. This can be used to try and infer any environmental or cultural changes that may have occurred. The strontium differences that can be collected in both the individual's remains and bedrock are from

the differences in types of water, coastal, flooding, and rain, as well as the differences in the distance of the coast. A general issue with stable isotope analysis in bioarchaeology is that none of these results can be compared to living humans, as it can be with anthropology.

Forensic Anthropology

Forensic anthropology is the study of human skeletal remains, including hair and nails, in collaboration with law enforcement to help with recovery, identification, determining trauma, and estimating the time since the death of the human remains. Forensic anthropology has used stable isotope analysis with greater frequency in recent years to assist in identifying an individual in a criminal investigations context. While the use of isotopes in forensic investigation may be a relatively new practice, it can bring a lot of answers to cases that were otherwise inconclusive. It is important to note that this cannot provide a perfect positive identification that other tests such as DNA could provide. However, similarly to the identifications that can be made in bioarchaeology, diet and geography can often be identified which can give important insight into the individual's life. Bones are the primary source for isotope collection in bioarchaeology, but in forensic anthropology, isotope composition can also be collected through hair and nails. Hair and nails are continuously growing throughout life and can be representative of the few months leading up to an individual's death and are referred to as serial recorders. The isotopes found in the keratin in as little as a centimeter in hair can indicate if the individual traveled or remained static in the months before their death. In the analysis, the carbon and nitrogen isotopes can often be used to determine diet, while the

oxygen isotopes can be used for geographic identification by comparing the oxygen isotopes in the hair to the oxygen isotopes found in the local water sources (Ehleringer et al., 2008). Nails have a similar keratin composition to hair; however, previous research proves that there is a difference in isotope composition, thus there is different information being collected. This difference could be because of the different amino acids found in the nail bed and hair follicle (O'Connell et al., 2001). Although this may appear to be a complication when it pertains to complete analysis, multiple studies have proven the validity of stable isotope analysis through the use of hair, so if present, it is a very reliable resource. When compared to the breakdown of bone tissue, which is seen in forensic science and bioarchaeology but is the primary resource in the latter, keratin is resilient and is often only broken down by fungi, and the isotope composition remains consistent regardless of any environmental or taphonomic changes.

Some ways that isotopes have been used in forensic anthropology have been used in human vs nonhuman skeletal determination and identifying the travel history of unidentified human remains. In 2016, a long bone was discovered at a construction site in San Francisco that was determined to be "possibly human " and "possibly Native American," which would cause issues in continuing construction. However, all initial analyses proved to be inconclusive, but when isotope analysis was performed, the skeletal remains had an elevated amount of $\delta^{13}C$ and $\delta^{15}N$. This isotope composition is caused by a diet of high-trophic level marine protein and is seen in both prehistoric humans from the northern Bay Area and sea mammals of the area, however, the structure of the long bone determined that it was not a sea creature. This analysis helped provide certainty that no crime had been committed recently and also allowed proper identification of Native

American remains. In 2010, a skull of, what was presumed, to be a young Hispanic woman was discovered in south California. There was no complete biological profile, so in 2017 the remains were submitted for isotope analysis to answer the questions "Was this individual from Latin America or were they a U.S.-born Hispanic citizen," as well as any indication of traveling before their death. Analysis from bone and tooth enamel showed a prominent C3/C4 diet, which is more common in the United States as opposed to most Latin countries. There was also present hair that when divided into segments and analyzed showed little change in where water was consumed during the 14 months before her death (Bartelink and Chesson, 2019).

Standards For The Two Analyses

Both the analyses completed by forensic anthropologists and archaeologists result in the identification of diet and location lived. However, the reasoning for determining this information and how it is analyzed differs significantly. The reason archaeologists often make these inferences is that they want to understand history or put remains into a historical context. Forensic anthropologists are more interested in identifying remains in a medicolegal context. There are also differences in the most common material used. In archaeology the most common sources of isotopes are bones. However, bones are not serial recorders and often can tell very little about a few months before death. They are a better representation of a longer compounded period of time. For example, a femur sample could provide an isotope sample from over 25 years, but isn't very specific especially outside of that time frame (Saul et al., 2021). So, compared to hair, there could be a significantly different isotope composition, specifically in carbon and nitrogen

enrichment. If a stable isotope analysis were performed on bone as opposed to hair for a forensic science case, an entirely different geographical region could be concluded. This can lead to a potential misidentification of a victim during a criminal investigation and data that would not be admissible in court. Apart from the material used in the analysis, there are also other differences in methodology that vary between archaeology and anthropology and even just between researchers in the same field. Current isotope researchers have proposed a few questions to help ensure thoughtful quality control. These are

> "(1) How well are the mass spectrometer and associated peripheral(s) operating? (2) Are test results reproducible (over time and between locations)? (3) Can we differentiate between populations that we would like to? (4) How variable are the populations of interest? (5) Have the samples been isotopically altered? (6) How variable are the samples?" (Chesson et. al., 2024).

The second question, "Are test results reproducible (over time and between locations)?" came into use when a research laboratory fractured a ^{14}C-dated ancient human bone, and sections were sent to 20 different research laboratories. There were significant differences in the analysis for collagen and carbonate. It was determined that these differences didn't come from differences in isotope composition, but about half the differences result from sample preparation and the other half of the differences result from analysis. This was exclusively in an archaeological context, but there can still be major impacts with inconsistent results (Pestle et al., 2014). The complication with the third question, "Can we differentiate between populations that we would like to?" is that while there are

isotope databases for human hair or bone collagen accessible online, they often just compile data from samples that are prepared and analyzed at different times and different locations, therefore there won't be accurate ability to compare. Regarding the last few questions, researchers need to be mindful of these factors. Although these questions can give a great guideline, they are not a set of standard practices that everyone is required to adhere to. A set of universal standard procedures must be created that are most beneficial to what is needed to be accomplished by the stable isotope analysis for a forensic context, rather than applying the same method to both contexts. There are recommendations from OSAC and FIRMS, but these are not universally followed. The most accurate standards possible should be used when it could convict an innocent person or misidentify a victim. If standards are created, it is equally important that each isotope researcher in a forensic anthropology context follows proper procedures to ensure consistency in techniques. This not only includes following the QA/QC standards, quality assurance, and quality control, but also adhering to the decisions made by organizations that focus on standardization of scientific practices, such as scientific working groups (SWGs), and the Organization of Scientific Area Committees for Forensic Science (OSACs). This survey was designed to find the differences in current practices and why there are those differences, such as training or lab protocols. Any clear gaps should be apparent and that knowledge can be used to help ensure the data is comparable. This is very important because stable isotope analysis is destructive, so it would be nearly impossible to replace or improve data quality after analysis.

Methods

This was a survey-based project to better understand procedures for stable isotope analysis in archaeological and forensic contexts. Suggestions for questions were made by current practitioners in both archaeological and forensic isotope laboratories. These questions were also influenced by research and good practice recommendations published on stable isotope analysis, such as the FIRMS "Good Practice Guide" for Mass Spectrometry of Stable Isotope Analysis. After extensive preparation and IRB approval, this survey was created using an anonymous Google form. It included questions regarding their practices in their manner of collecting isotope samples from human remains, how the samples are handled and analyzed, and by what guidelines and SOPs they are abiding. The questions were designed to address the most relevant issues as outlined in the references. A business card with the description of the survey and a QR code attached was created for ease of use. The data from these short-answer and multiple-choice survey questions were collected and imported to Google Sheets for easier and still anonymous evaluation. The research was evaluated to determine if the researchers are following similar agreed-upon practices and what gaps are in the research. The untestable value in this research was the assumption that every researcher taking this survey was honest in regards to their practices, and also had a desire for the best and most accurate outcome for their isotope analysis. In any survey, there is a chance someone may alter their answers to what they assume is the correct answer. There is also a risk that researchers would answer the questions quicker, as opposed to the most accurate. These risks were accounted for by making the survey primarily multiple choice with a few short

answers and limiting the number of questions to what seemed necessary. However, these are untestable and unquantifiable values that cannot be asked in a survey, so they must be assumed about the participants.

Results

There were eight responses to the survey. The participant's titles were varied: Postdoctoral Research Scholar, Graduate Student, Graduate Teaching Assistant, Graduate Research Associate, Lab Manager, Undergraduate Research Assistant, Professor, and Assistant Research Professor. While different, they all have lab experience and formal isotope training, except for the Undergraduate Research Assistant.

The Postdoctoral Research Scholar fell outside of the classification options provided in the second question with the options being “Student, Trainee, Technician, Practitioner, or Other.” They have a doctorate, study in the field of anthropology, and have no experience using isotopes in forensic science, but have 10-20 years of experience using isotopes in an archaeological context. They have published peer-reviewed work on isotopes in archaeology. In their research, they collect samples from Tooth Enamel, Tooth Dentine, Bone Collagen, and Bone Bioapatite and prepare and analyze them in a University laboratory. They dust their lab weekly but the lab does not receive cleaning from an outside professional provider. They do not run calibration on their elemental analyzer as they only do sample preparation. They do not use an in-house reference material. In their lab, Carbon (δ13C), Nitrogen (δ15N), Oxygen (δ18O), and strontium are used and prepared, but no analysis takes place there, only sample preparation. No

instrumentation is present in the lab for analysis. The samples are cleaned using one of the following: ultrasonic bath, soaking in ultrapure water, soaking in bleach, and soaking in acetic acid and then being allowed to air dry for 24-48 hours. It is only completed once per sample. For the short answer question, “Describe your data handling practices. For example, how do you correct for memory effects? Time? Area?,” they responded with “We do a series of corrections on the data (haven't seen any memory effects from the mass spec. but we adjust for amp and drift before we calibrate based on 3 known standards).” Lastly, they have 5+ replicates of each reference material per analytical sequence that they measure.

The Graduate Student self-classified as a student out of the options provided. They have a Master’s degree, also study in the field of anthropology, have no experience using isotopes in forensic science but have 5-10 years of experience using isotopes in an archaeological context. They have also published peer-reviewed work on isotopes in archaeology. In their research, they collect samples from the same biological sources as the previous anthropologist and also prepare and analyze them in a University laboratory. The climate of the lab preparation and analysis is “Humid and seasonal.” They are unsure of how often their lab is dusted and the lab does not receive cleaning from an outside professional provider. As the previously mentioned survey participant, they do not run calibration on their elemental analyzer as they only do sample preparation and they do not use an in-house reference material. In their lab, Carbon (δ13C), Nitrogen (δ15N), and Oxygen (δ18O) are used and prepared, but no analysis takes place there, only sample preparation. No instrumentation is present in the lab for analysis. The samples are cleaned using an ultrasonic bath and soaked in hydrogen peroxide then allowed to air dry

in a designated hood for 12-24 hours. It is only completed 2-3 times per sample. For the short answer question, “Describe your data handling practices. For example, how do you correct for memory effects? Time? Area?,” they did not respond. Lastly, they have 1-2 replicates of each reference material per analytical sequence that they measure.

The Graduate Teaching Assistant is also self-classified as a student with a Master’s degree out of the options provided. They study in the field of biological anthropology, and have no experience using isotopes in forensic science but have 1-5 years of experience using isotopes in an archaeological context. They have no published work in stable isotope analysis. In their research, they collect samples from tooth enamel, bone collagen, and plant ash. They also prepare and analyze it in a University laboratory. In response to the climate of the lab preparation and analysis, they stated it was “East Tennessee - mild/moderate climate for light isotope prep (C, N, O), dry desert for Sr prep.” They are unsure of how often their lab is dusted and the lab does not receive cleaning from an outside professional provider. As the previously mentioned survey participants, they do not run calibration on their elemental analyzer as they only do sample preparation and do not use an in-house reference material, however where they send their samples to does have an in-house reference material. They use “NIST 1400 (cow bone ash), JW 1486 (human remains), IAPSO seawater, and tomato leaf ash.” These reference materials are calibrated by comparing them to a confirmed primary or secondary reference material. In their lab, carbon (δ13C), nitrogen (δ15N), oxygen (δ18O), and strontium are used and prepared, but no analysis takes place there, only sample preparation. There is another isotope lab at the laboratory that analyzes all of these except strontium. No instrumentation is present in the lab for analysis. When asked

about how the samples are cleaned, a short-answer response was provided, “Bone samples cleaned with distilled water before demineralization for collagen extraction. Enamel pretreated with sodium hypochlorite and acetic acid to remove organics and exogenous carbonates,” then allowed to dry in an oven for 12-24 hours. It is only completed one time per sample. They do not do replicates of sample measurement for anything, except plant ash for which they do 3 replicates. For the short answer question, “Describe your data handling practices. For example, how do you correct for memory effects? Time? Area?”, they did not respond. Lastly, they have 3-4 replicates of each reference material per analytical sequence that they measure.

The Graduate Research Associate also self-classified as a student with a Master’s degree out of the options provided. They study in the field of forensic science, and have 5-10 years of experience using isotopes in forensic science but have no experience using isotopes in an archaeological context. They have published work in stable isotope analysis in a forensic context. In their research, they collect samples from bone collagen and bone bioapatite. They also prepare and analyze it in a University laboratory. In response to the climate of the lab preparation and analysis, they stated it was a “Subtropical Desert.” Their lab is dusted before and after each analysis and the lab does not receive cleaning from an outside professional provider. When asked to “Please briefly describe the analytical testing routine used”, they were the first to provide a short response. “Background checks prior to analysis. Stability and linearity checks with each instrument run. Data calibration and normalization with each instrument run.” They skipped the question “How often do you run calibration on the elemental analyzer in your research lab?” Their in-house reference materials are “cow bone, cremains, and collagen

supplements" which they calibrate by comparing to a confirmed primary or secondary reference material. In their lab, carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), oxygen ($\delta^{18}O$), sulfur ($\delta^{34}S$), and $^{87}Sr/^{88}Sr$ are utilized and prepped and all but sulfur are analyzed. The laboratory uses EA/IRMS and CO_2 isotopes for analysis. This survey participant was also the first to do an actual full analysis at their lab instead of sending out the prepared samples to other places. The samples are once cleaned with ethanol and soaked in deionized water. The samples are then dried in an oven for 12-24 hours. They responded with "10% of samples are run in triplicate" when asked, "How many replicates of each sample do you measure?" For the short answer question, "Describe your data handling practices. For example, how do you correct for memory effects? Time? Area?", they responded with "Calibration and linearity, linearity curve, and normalization." Lastly, they have 3-4 replicates of each reference material per analytical sequence that they measure.

The Lab Manager self-classified as a practitioner with a Master's degree out of the options provided. They study in the field of biology, and have 20-30 years of experience using isotopes in forensic science, but 10-20 years of experience using isotopes in an archaeological context. They have published work in stable isotope analysis in both a forensic and archaeological context. In their research, they collect samples from bone collagen and tooth enamel. They also prepare and analyze it in an agency or government laboratory. In response to the climate of the lab preparation and analysis, they stated it was "Tropical." Their lab is dusted "regularly" and the lab does receive cleaning from an outside professional provider, this is the first respondent to answer this way. When asked to "Please briefly describe the analytical testing routine

used," they provided the short response, "For EA-IRMS: We check the N2 background prior to an analytical run to ensure it does not exceed a set limit. We run "on-offs" before every analytical sequence and calculate the Std. Dev. for N2, CO2, and SO2 to check stability. We weigh a commercial collagen powder at a variety of masses and analyze it alongside samples to check for (and correct, if needed) linearity effects. We weigh the same commercial collagen powder at a set mass and analyze it throughout a run to check for (and correct, if needed) drift effects." They run calibration on the elemental analyzer after 2-25 samples. Their in-house reference material is "commercial collagen powder" which they do not calibrate. In their lab, carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), oxygen ($\delta^{18}O$), and sulfur ($\delta^{34}S$) are utilized, prepared, and analyzed. The laboratory uses EA/IRMS analysis. When asked about cleaning, they responded with "N/A, as I believe these cleaning options are related to hair samples." This was an interesting response since even those who don't study hair samples have selected a cleaning process. They run 1-2 replicates per sample. For the short answer question, "Describe your data handling practices. For example, how do you correct for memory effects? Time? Area?", they responded with, "We routinely check for (and correct, if needed) linearity and drift effects. We also apply a two-point normalization. Corrections follow: Carter, J. F., & Fry, B. (2013). Ensuring the reliability of stable isotope ratio data—beyond the principle of identical treatment. Analytical and Bioanalytical Chemistry, 405, 2799-2814." They were the only people to cite their reasoning. Lastly, they have 5+ replicates of each reference material per analytical sequence that they measure.

The Undergraduate Research Assistant self classified as a Student without a completed degree. They study in the field of biochemistry and are the only survey

participant to not have any formal isotope training. They have zero years of experience using isotopes in forensic science but 1-5 years of experience using isotopes in an archaeological context. They do not have any published work in stable isotope analysis. Like the last survey participant, they also collect samples from bone collagen and tooth enamel. They also prepare and analyze it in a university laboratory. There may have been confusion regarding the questions about the climate of the workspace, as the response was "Open space with individual work stations allowing for an interactive place with peers and lab mentor." This question should have been worded more clearly if this confusion was possible. Their lab is dusted "After every shift or after switching to preparing a new sample" and the lab does not receive cleaning from an outside professional provider. When asked to "Please briefly describe the analytical testing routine used," they provided the short response, "I have only used Omnic for FTIR analysis by analyzing the heights of specific peaks in processed samples from various sites." They do not run calibration on the elemental analyzer, because they are sample preparation only. They do not have any reference materials. carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and oxygen ($\delta^{18}O$) are utilized and prepared in the lab. The samples are cleaned 2-3 times using an ultrasonic bath and dried in an oven for 3-6 hours. They do not run any replicates per sample. For the short answer question, "Describe your data handling practices. For example, how do you correct for memory effects? Time? Area?", they responded with "Adding acetic acid for tooth enamel." Lastly, they do not have any replicates of each reference material per analytical sequence that they measure, as they do not have any reference material.

The Professor self classified as a practitioner with a doctorate. They study in the field of anthropology and have 5-10 years of experience using isotopes in forensic science and archaeological contexts. They have published work in stable isotope analysis in a forensic context. Unlike the previous survey participants, they use hair samples as well as tooth enamel, bone collagen, and bone bioapatite. They prepare and analyze it in a University laboratory. The self-described climate of the area where sample preparation occurs is "Southern US" and the climate of the area where sample analysis occurs is "West Coast US." Their lab is "thoroughly cleaned before and after sample prep sessions" but the lab does not receive cleaning from an outside professional provider. When asked "Please briefly describe the analytical testing routine used", they did not provide a short response. They do not run calibration on the elemental analyzer, because they are sample preparation only. Their reference material includes cow bone and human hair that they calibrate by comparing to a confirmed primary or secondary reference material. carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), hydrogen ($\delta^{2}H$), oxygen ($\delta^{18}O$), and strontium are prepared in the lab, but not analyzed. The samples are cleaned 2-3 times by soaking in deionized water, soaking in a Chloroform:methanol solution, and by "mechanical cleaning of bone and tooth samples with dremel." They are then air-dried in a hood for 24-48 hours. They do "duplicates of 10% of sample set" and 3-4 replicates of each reference material per analytical sequence.

Lastly, the Assistant Research Professor self classified as someone outside of the provided job titles with a doctorate level degree. The degree is in the field of "Isotope Geochemistry." They have 10-20 years of experience using isotopes in both forensic science and archaeological contexts. They have published work in stable isotope analysis

in both fields as well. In agreement with the previous survey participant, they use hair samples, tooth enamel, bone collagen, and bone bioapatite in their work. They prepare and analyze it in a University laboratory. The self-described climate of the area where sample preparation occurs is “Arid, hot” and the climate of the area where sample analysis occurs is “Arid, hot (some analyses), Coastal, humid (other analyses).” In response to being asked about the dusting schedule about their lab, they stated “The lab - rarely. Sample preparation areas are to be cleaned prior to starting work.” However, it does not receive any outside cleaning. When asked “Please briefly describe the analytical testing routine used”, they responded with

> “d13C and d18O in carbonate: Background gasses are monitored prior to each analytical run. A process blank is analyzed at the beginning and end of each run. Stability is analyzed by measuring a reference gas ten times in triplicate, and an independent secondary standard at the beginning and end of each run. Two normalizing standards are analyzed at the beginning, end, and every 12 samples. Two independent check standards (at least one-matrix matched) are run every 12 samples. Three in-house reference samples are processed in each batch of 49 samples and run as unknowns. If samples are within 10% of the target weight, no linearity correction is made. A linearity series is run to exclude any samples outside of the acceptable range of ion beams.
>
> d13C, d15N in collagen: Blank tins and an acetanilide are run prior to each analytical run, and backgrounds are monitored. Linearity series is analyzed in each run. Two different normalizing standards are run every six samples. An independent check

standard is run every three samples. Three process in-house standards are prepared in parallel with each batch of 49 samples.

For both, precision and accuracy are monitored through use of the Szpak et al spreadsheet.

We also do radiogenic and mass-dependent strontium analyses, and similar levels of background, process blanks, independent check standards, and in-house process standards are used." They do run calibration on the elemental analyzer after every 2-25 samples. Their reference material includes "Bone - cow bone, llama bone, NIST 1400, NIST 1486. " They confirm that they do calibrate their reference material but did not provide a description on how they did so. Carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and oxygen ($\delta^{18}O$) are prepared and analyzed in the lab. The lab uses a EA/IRMS system with CO_2 for analysis. Each sample is cleaned once by soaking in deionized water, soaking in a Chloroform:methanol solution, and through the use of an Ultrasonic Bath. They are then dried in an over for 24-48 hours. In response to replications performed, the researcher responded with "Target is 10% of samples are prepared and measured in triplicate" and 3-4 replicates of each reference material per analytical sequence. They also provided commentary on their data handling process, "The data is first blank-corrected, then corrected for any area effects (linearity). The normalizing standards are compared to the known values to create a calibration curve between the instrument scale and the international scale. Precision and accuracy are monitored using matrix-matched standards prepared in parallel with samples."

Discussion

Even with the smaller data size that is present, there is still evidence of differences in practices between the researchers of the same or different fields. One researcher had experience with isotopes in a strictly forensic context, four had experience with isotopes in a strictly archaeological context and three had experience with both forensic science and archaeology. When comparing the four researchers who had some involvement with forensic science, there were a multitude of similarities and differences. All four had different fields that they majored in and in terms of samples analyzed, no researcher had identical lists. However, samples such as bone collagen were analyzed by each researcher. Three of the four researchers collected samples in a university laboratory but one of the researchers collected samples in a government agency building. None of the climates of the data collections were identical, although the temperatures seemed to all be on the warmer side based on these self-descriptions. Each of these four researchers had different lab cleaning and dusting practices. Three of the four researchers gave in-depth data handling, that did have differences as well as similarities in running background analysis on the machines. All four of the researchers use reference material and every individual list is different but cow bone and bone collagen powder appear in two different lists provided by researchers. The two researchers who provided information on their calibration had the same calibration process, but the other two did not answer this question. Carbon ($\delta^{13}C$), Nitrogen ($\delta^{15}N$), and Oxygen ($\delta^{18}O$) were analyzed by all four of the researchers, but two researchers also analyzed strontium, two analyzed sulfur, and one analyzed hydrogen. Three of the researchers answered questions regarding their sample cleaning practices, and while they all differed, they also all contained deionized water as

part of the process. It is split between all researchers whether they clean their samples once or 2-3 times. In three out of the four researchers who responded, two dried their samples in an oven and one air-dried in a hood. One of the researchers replicated 1-2 samples but the other three replicated 10%. Three of the four also had 3-4 replicates of the reference material. All the researchers who did their own analyses, only three out of all eight, used EA/IRMS, and two of those used CO2.

Now when the additional four researchers are considered, those that only practice stable isotope analysis in the context of archaeology, even more differences become apparent. In just comparison amongst these four, there is more consistency in majors, with three falling under the field of anthropology. There is also more consistency in sample types, except for one researcher who also analyzes plant ash. In similarities to the previously mentioned researchers, a common thread here was bone collagen. They all utilize a university laboratory for sample preparation. Two labs were once again in moderate to warm climates, except for one researcher who did not understand the question being presented and one who chose not to answer. There is little knowledge or consistency when regarding the cleaning practices of the lab of these four researchers. This seems to be consistent with all researchers who took the survey. However, all strictly archaeology researchers do not use any outside cleaning. These researchers also do not do any analysis at their lab, only sample preparation so none of them have any in-house reference materials or calibration. All researchers utilize carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and oxygen ($\delta^{18}O$) isotopes while two of these four also utilize strontium as well. In terms of cleaning the samples, a common practice between each researcher was the utilization of an ultrasonic bath, although each had their own unique practices as well. Ultrasonic

baths were also practiced by two of the researchers who had experience in both fields as well. In terms of drying, two dry in the oven, one air dries in a hood, and one simply air dries. The time differences for these practices range from “3-6 hours” and “24-48 hours.” Each researcher had different answers for their replicates of both samples and reference materials. None of the eight researchers had identical answers to both questions regarding measurement replicates. When comparing just those who use isotope analysis in a forensic context or archaeological context, the biggest differences are with the cleaning of the samples, answers regarding data handling, and all questions regarding the actual analysis of samples, although this is because no strictly archaeological researcher analyzed the samples in their lab.

There are multiple differences between those that use stable isotopes in forensic anthropology compared to an archaeology context, as well there are significant differences between each researcher within the same field. The limitations to these results were that there have only been eight responses to this survey, although this survey will continue to remain open to help gather more knowledge on this topic, some of the questions weren’t properly understood or completed by some of the researchers, and that we did not differentiate practitioners from the same lab. A future goal of this survey would be to survey via interview as opposed to an anonymous survey to avoid these complications.

Conclusion

Based on the results collected, there are differences in the practices conducted. Even in a small sample size, this illustrates a need for a certain set of standards and guidelines. As previously mentioned, there is little way to prove the accuracy of archaeology results and there are less detrimental immediate consequences, it is important to have those consistent standards when applying similar techniques to both forensic anthropology and archaeology. Also, since isotope analysis is a destructive process it is important to have that consistency amongst researchers of the same field, as the process cannot be repeated. This survey could be utilized by those who create the standards for stable isotope analysis to understand the most common practices and where the biggest complications may be in the differences.

Appendix

Survey Questions

An Exploratory Study of How Anthropology and Archaeology Laboratories Conduct Isotope Sample Preparation

This survey is to help me, Emma Lloyd, complete my undergraduate honors thesis for Middle Tennessee State University. These questions will be used to complete an exploratory study to analyze the differences in practices between forensic anthropologist and archaeologists. Please answer all questions to the best of your ability.

This survey has been approved by IRB (IRB-FT2024-161 2/12/24). The only cost is your time, all answers are anonymous, and there is no compensation for completing this survey.

1. What is your position or title?
    a. Short Answer
2. What is your classification?
    a. Student
    b. Trainee
    c. Technician
    d. Practitioner
    e. Other
3. What is your highest degree?
    a. Associate's
    b. Bachelor’s
    c. Master’s
    d. Doctorate
4. What field of study is your degree in?
    a. Short answer
5. Do you have any formal isotope training?
    a. Yes
    b. No

6. How many years have you been utilizing stable isotope analysis in a forensic science context?
    a. None
    b. 1-5
    c. 5-10
    d. 10-20
    e. 20-30
    f. 30+
7. How many years have you been utilizing stable isotope analysis in an archaeological context?
    a. None
    b. 1-5
    c. 5-10
    d. 10-20
    e. 20-30
    f. 30+
8. Have you published peer-reviewed work on isotopes in archaeology or forensic projects?
    a. Yes, forensic projects
    b. Yes, archaeology projects
    c. Yes, both
    d. No
9. What type of samples do you use in your research or practical application? (Select All)
    a. Hair
    b. Nail
    c. Tooth Enamel
    d. Tooth Dentine
    e. Bone Collagen
    f. Bone Bioapatite

g. Other

10. Where do you conduct stable isotope sample preparation?
    a. University laboratory
    b. Private laboratory
    c. Agency or government laboratory
    d. Other…
11. Where do you conduct stable isotope analysis?
    a. University laboratory
    b. Private laboratory
    c. Agency or government laboratory
    d. Other…
12. What is the climate of the area where sample preparation occurs?
    a. Short Answer
13. What is the climate of the area where sample analysis occurs?
    a. Short Answer
14. How often is your lab dusted for cleaning by analysts?
    a. Short Answer
15. Does your lab receive cleaning from an outside professional provider?
    a. Yes
    b. No
16. Please briefly describe the analytical testing routine used. (For example -Do you check background gasses? In what way and how often? Do you check stability? In what way and how often? Do you check linearity? In what way and how often?) If you do not perform analysis in your lab, please respond NA.
    a. Short Answer
17. How often do you run calibration on the elemental analyzer in your research lab?
    a. Not applicable- sample preparation only.
    b. Never
    c. Between every sample
    d. After 2-25 samples

e. After 25-50 samples
f. After 50-100 samples
g. After 100-150 samples
h. After 150-200 samples
i. After 200+ samples

18. Do you have an in-house reference material? If so, what do you use?
    a. Short Answer
19. Do you calibrate your in-house reference material?
    a. Yes
    b. No
20. If so, how do you calibrate your in-house reference material?
    a. Sending reference materials to a secondary lab for confirmation
    b. Comparison to a confirmed primary or secondary reference material
    c. Other:
21. What isotopes are utilized in your work? (Select all that apply)
    a. Carbon (δ13C)
    b. Nitrogen (δ15N)
    c. Hydrogen (δ2H)
    d. Oxygen (δ18O)
    e. Sulfur (δ34S)
    f. Other…
22. What isotopes are analyzed in your facility? (Select all that apply)
    a. Carbon (δ13C)
    b. Nitrogen (δ15N)
    c. Hydrogen (δ2H)
    d. Oxygen (δ18O)
    e. Sulfur (δ34S)
    f. Other…
23. What type of system does your laboratory use for analysis?
    a. LC/CO/IRMS

b. EA/IRMS
c. GC/C/IRMS
d. HTC/IRMS
e. None- Sample preparation only.

24. What isotopes are analyzed in your IRMS?
    a. He2+
    b. H2O
    c. N2+
    d. O2+
    e. Ar+
    f. CO2
    g. None - Sample preparation only
    h. Other…

25. How do you clean your samples prior to analysis? (Select all that apply)
    a. Low-lint laboratory wipes
    b. Soaking in deionized water
    c. Soaking in hydrogen peroxide
    d. Dish detergent
    e. Chloroform:methanol solution
    f. Methanol
    g. Ultrasonic Bath
    h. Other…

26. How many times do you repeat the cleaning process?
    a. Just once
    b. 2-3 times
    c. 4-5 times
    d. 5+ times

27. How do you allow your samples to dry?
    a. Air dry
    b. Air dry in a hood

c. Dry in an oven

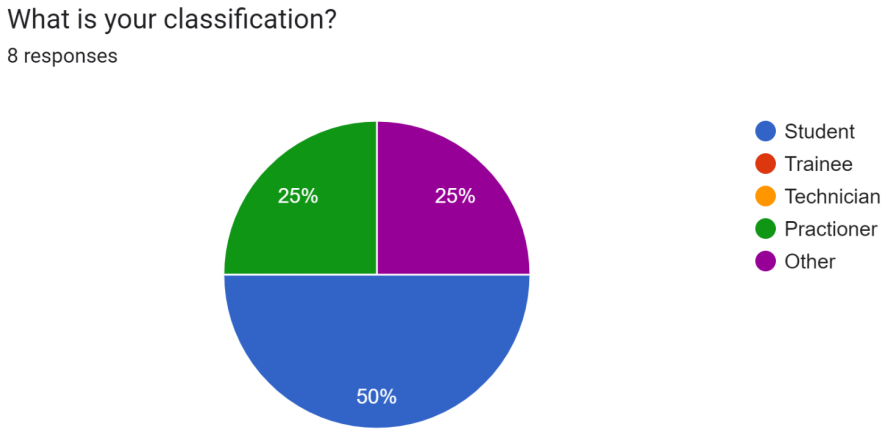
28. How long do you allow your samples to dry?
    a. 0-2 hours
    b. 3-6 hours
    c. 7-12 hours
    d. 12-24 hours
    e. 24-48 hours
    f. 48+ hours
29. How many replicates of each sample do you measure?
    a. None
    b. 1-2
    c. 3-4
    d. 5+
    e. Other…
30. Describe your data handling practices. For example, how do you correct for memory effects? Time? Area?
    a. Short Answer
31. How many replicates of each reference material per analytical sequence do you measure?
    a. None
    b. 1-2
    c. 3-4
    d. 5+
    e. Other…

The results to this study can be found in my Honors Thesis published at Middle Tennessee State University. If you have questions about this research, you may contact me, Emma Lloyd, at ecl3k@mtmail.mtsu.edu. For additional information about giving consent or your rights as a participant in this study, please contact the Middle Tennessee State University (MTSU) Office of Compliance at 615-494-8918 or via email at irb_information@mtsu.edu. (http://www.mtsu.edu/irb)

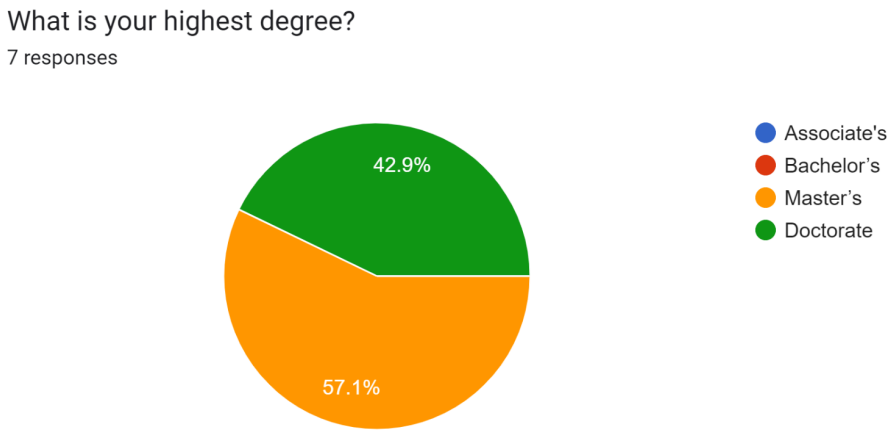
Appendix

Diagrams of Results from Survey on Google Forms

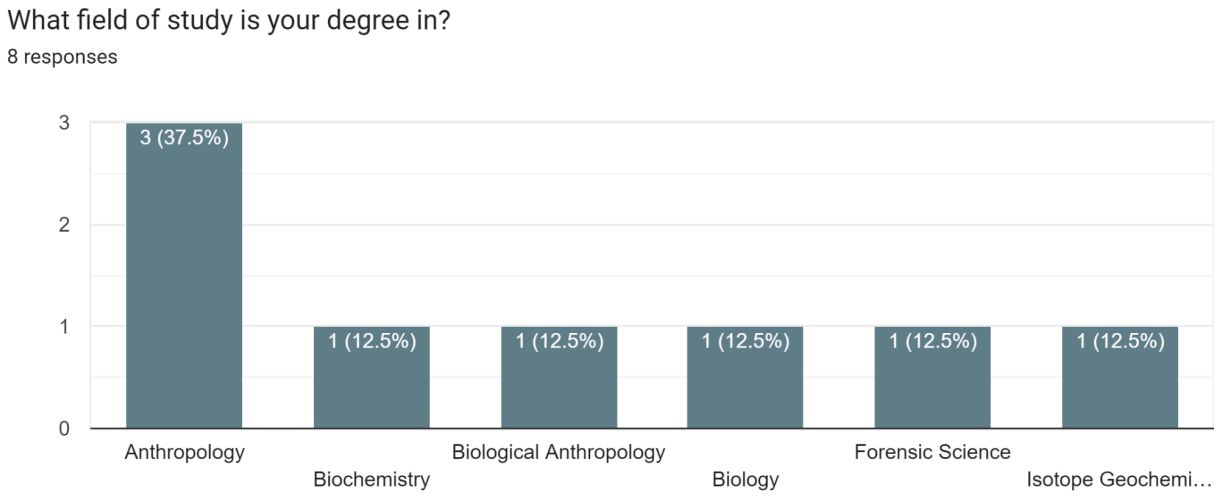
Question 1



Question 2



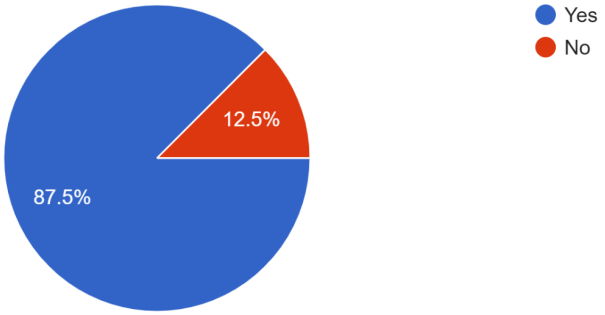
Question 3



Question 4

Do you have any formal isotope training?

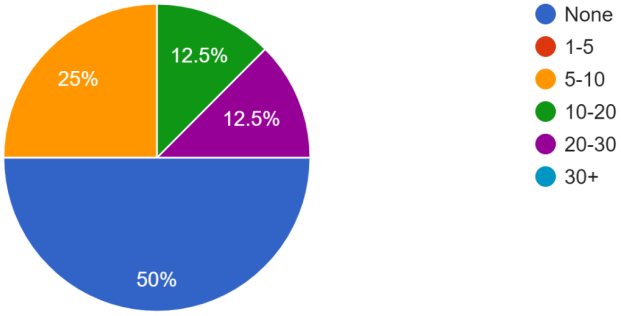
8 responses



Question 5

How many years have you been utilizing stable isotope analysis in a forensic science context?

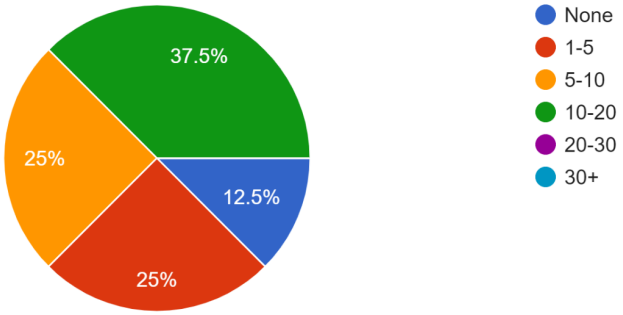
8 responses



Question 6

How many years have you been utilizing stable isotope analysis in an archaeological context?

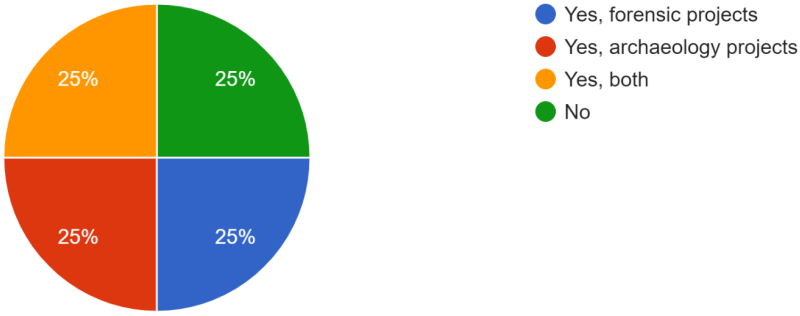
8 responses



Question 7

Have you published peer-reviewed work on isotopes in archaeology or forensic projects?

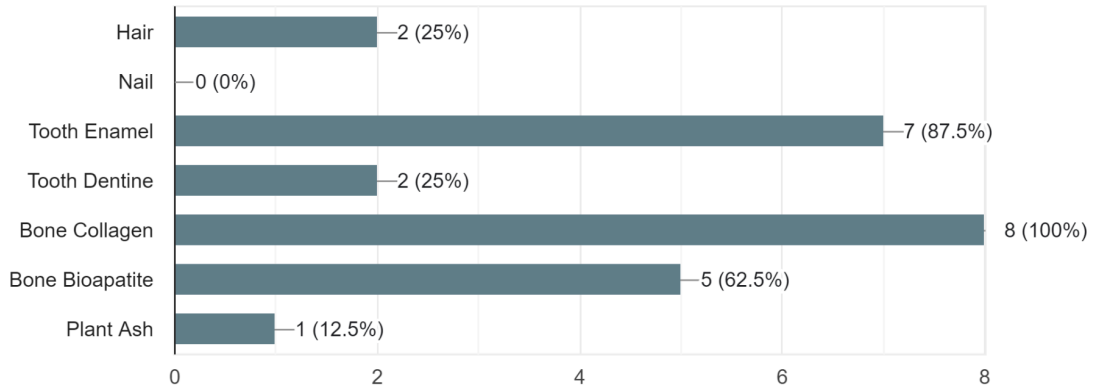
8 responses



Question 8

What type of samples do you use in your research or practical application?

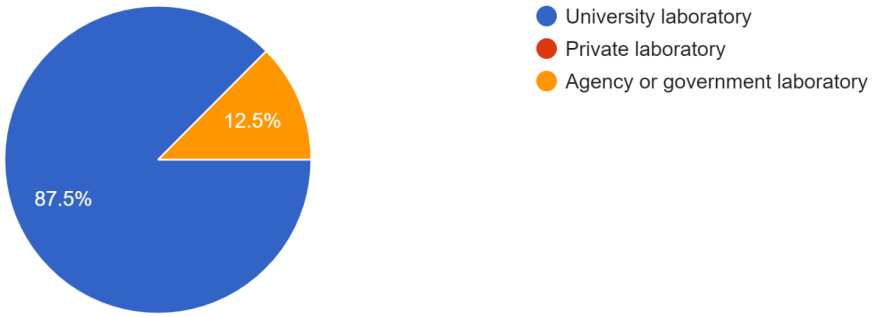
8 responses



Question 9

Where do you conduct stable isotope sample preparation?

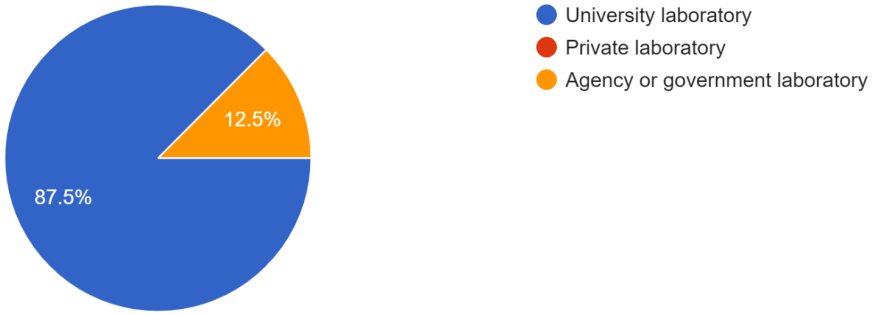
8 responses



Question 10

Where do you conduct stable isotope analysis?

8 responses



Question 11

What is the climate of the area where sample preparation occurs?

7 responses

Humid, seasonal
East Tennessee - mild/moderate climate for light isotope prep (C, N, O), dry desert for Sr prep
Subtropical Desert
Tropical
Open space with individual work stations allowing for an interactive place with peers and lab mentor
southern US
Arid, hot

Question 12

What is the climate of the area where sample analysis occurs?

7 responses

Seasonal

Same as above

Subtropical Desert

Tropical

Same with sample preparation

West coast US

Arid, hot (some analyses), Coastal, humid (other analyses)

Question 13

How often is your lab dusted for cleaning by analysts?

8 responses

I dust weekly

Unsure

Not sure about preps space in Tennessee. Sr prep and analysis took place in a low metal clean lab.

Before and after each analysis.

Regularly

After every shift or after switching to preparing a new sample

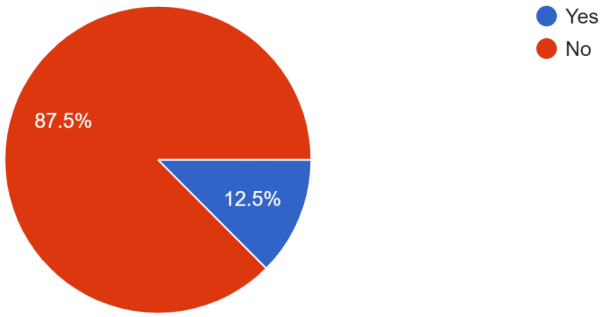
thorough cleaning before and after sample prep sessions

The lab - rarely. Sample preparation areas are to be cleaned prior to starting work.

Question 14

Does your lab receive cleaning from an outside professional provider?

8 responses



Question 15

Please briefly describe the analytical testing routine used.
(For example -
Do you check background gases? In what way and how often?
Do you check stability? In what way and how often?
Do you check linearity? In what way and how often?)
If you do not perform analysis in your lab, please respond NA.

8 responses

NA

NA - Lab Tech in the department with the isotope lab performs this part.

Background checks prior to analysis.
Stability and linearity checks with each instrument run.
Data calibration and normalization with each instrument run.

For EA-IRMS: We check the N2 background prior to an analytical run to ensure it does not exceed a set limit. We run "on-offs" before every analytical sequence and calculate the Std.Dev. for N2, CO2, and SO2 to check stability. We weigh a commercial collagen powder at a variety of masses and analyze it alongside samples to check for (and correct, if needed) linearity effects. We weigh the same commercial collagen powder at a set mass and analyze it throughout a run to check for (and correct, if needed) drift effects.

I have only used Omnic for FTIR analysis by analyzing the heights of specific peaks in processed samples from various sites.

d13C and d18O in carbonate: Background gases are monitored prior to each analytical run. A process blank is analyzed at the beginning and end of each run. Stability is analyzed by measuring a reference gas ten times in triplicate, and an independent secondary standard at the beginning and end of each run. Two normalizing standards are analyzed at the beginning, end, and every 12 samples. Two independent check standards (at least one-matrix matched) are run every 12 samples. Three in-house reference samples are processed in each batch of 49 samples and run as unknowns. If samples are within 10% of the target weight, no linearity correction is made. A linearity series is run to exclude any samples outside of the acceptable range of ion beams.

d13C, d15N in collagen: Blank tins and an acetanilide are run prior to each analytical run, and backgrounds are monitored. Linearity series is analyzed in each run. Two different normalizing standards are run every six samples. An independent check standard is run every three samples. Three process in-house standards are prepared in parallel with each batch of 49 samples.

For both, precision and accuracy are monitored through use of the Szpak et al spreadsheet.

We also do radiogenic and mass-dependent strontium analyses, and similar levels of background, process blanks, independent check standards, and in-house process standards are used.

Question 16

How often do you run calibration on the elemental analyzer in your research lab?

7 responses

Question 17

Do you have an in-house reference material? If so, what do you use?

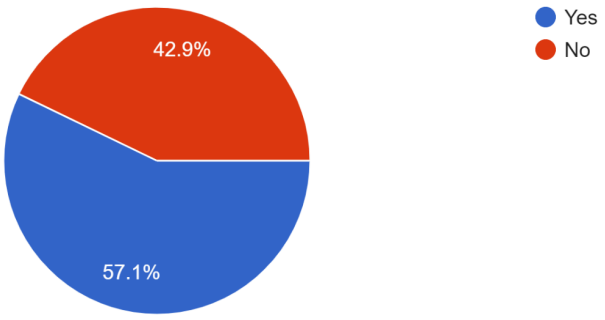
8 responses

NA
Unsure
NA, but ASU lab for Sr has in house reference material. We used NIST 1400 (cow bone ash), JW 1486 (human cremains), IAPSO Seawater, and Tomato Leaf Ash
Yes. Cow bone, cremains, and collagen supplements.
Yes - commercial collagen powder
N/A
yes- cow bone and human hair
Bone - cow bone, llama bone, NIST 1400, NIST 1486.

Question 18

Do you calibrate your in-house reference material?

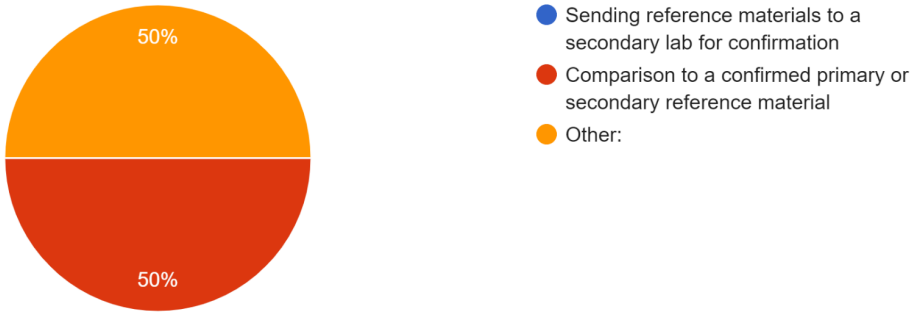
7 responses



Question 19

If so, how do you calibrate your in-house reference material?

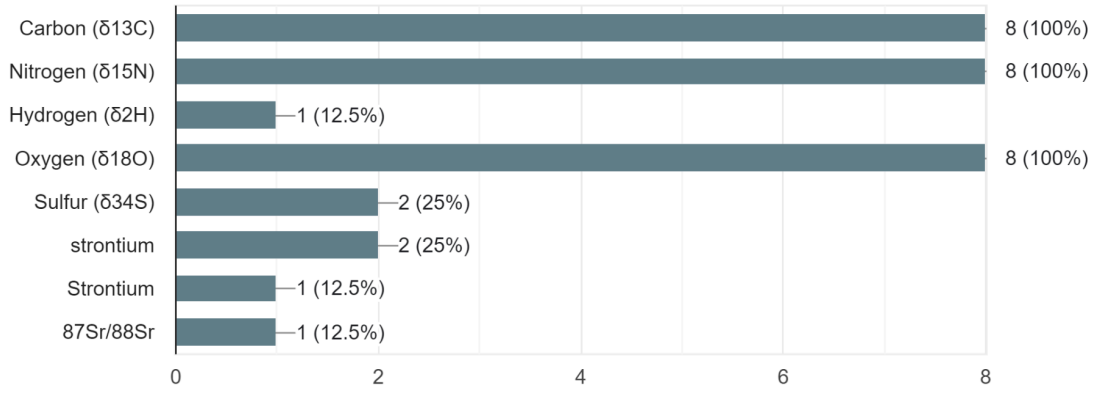
6 responses



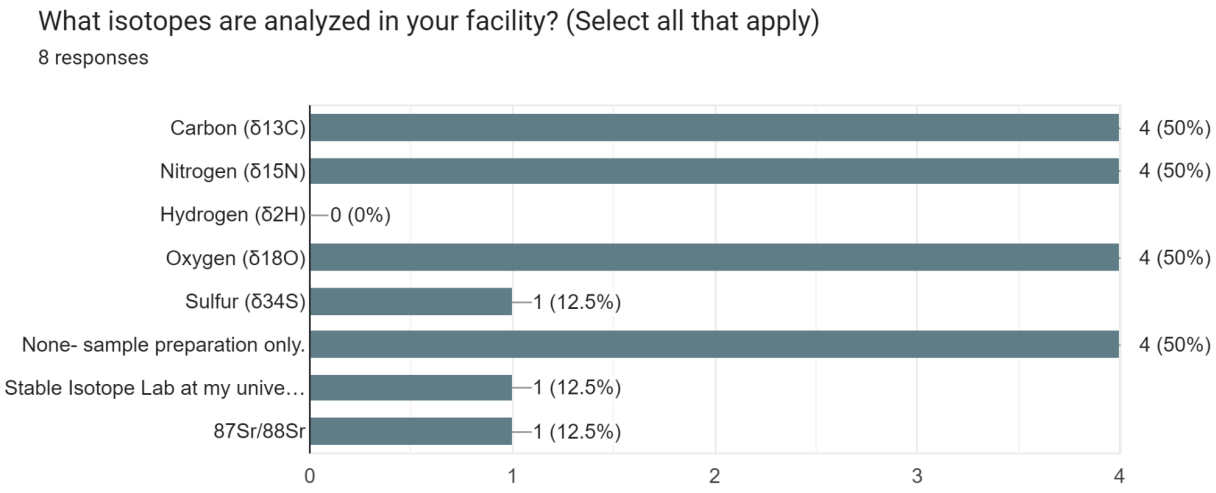
Question 20

What isotopes are utilized in your work? (Select all that apply)

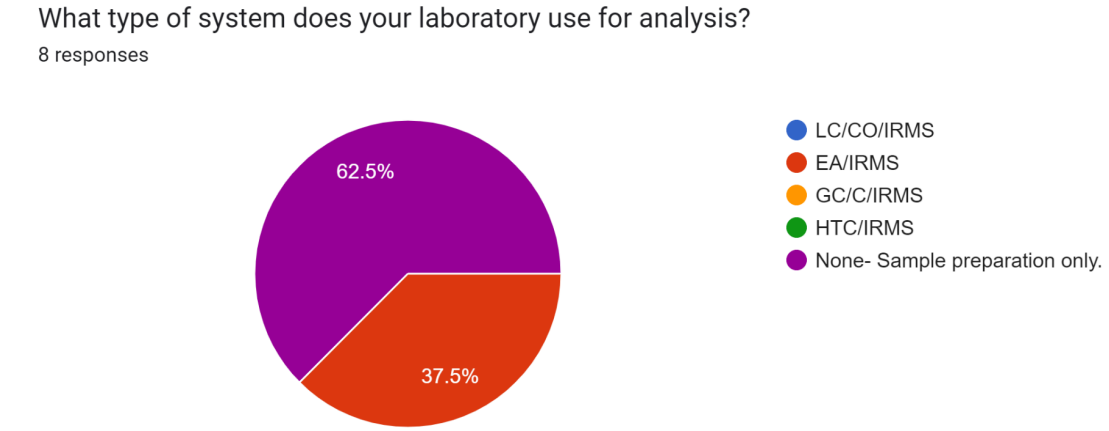
8 responses



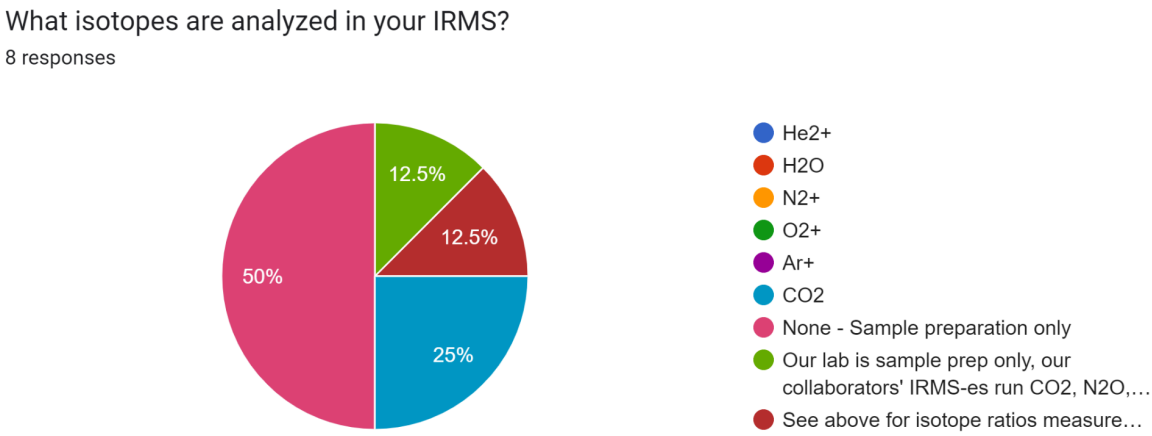
Question 21



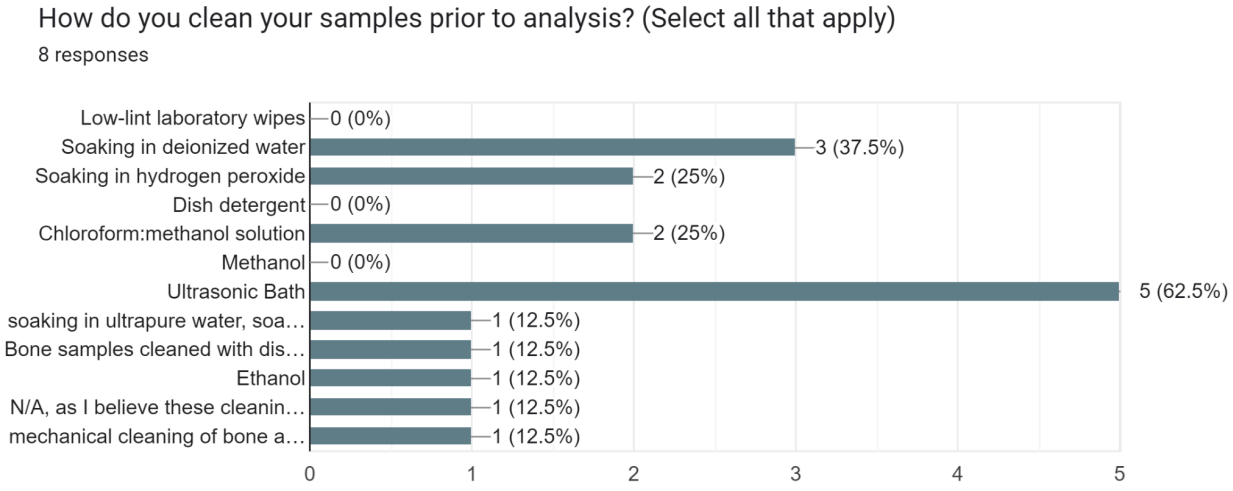
Question 22



Question 23



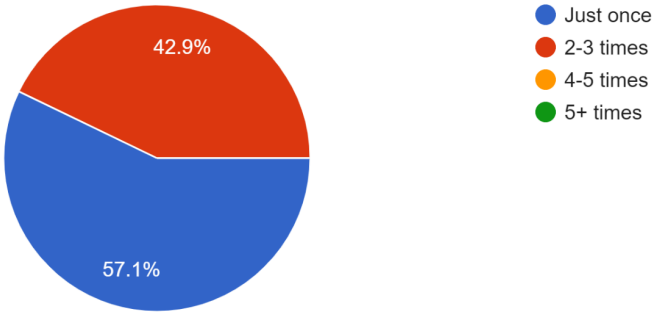
Question 24



Question 25

How many times do you repeat the cleaning process?

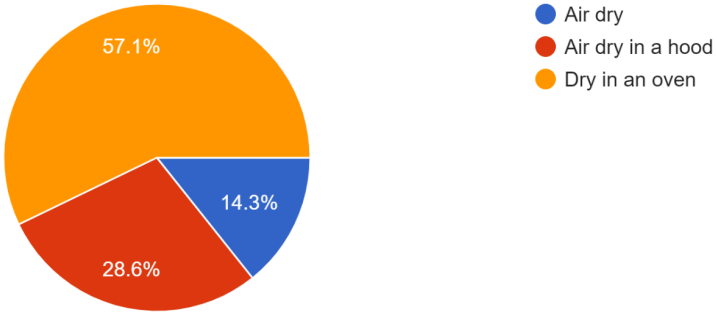
7 responses



Question 26

How do you allow your samples to dry?

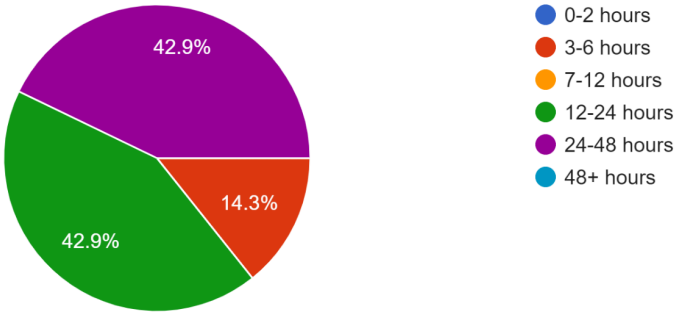
7 responses



Question 27

How long do you allow your samples to dry?

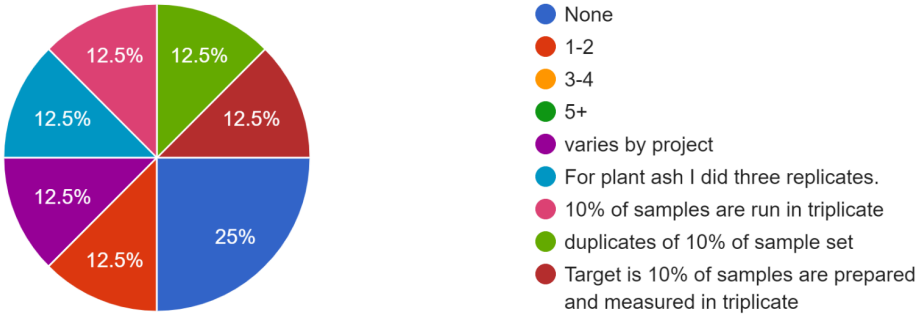
7 responses



Question 28

How many replicates of each sample do you measure?

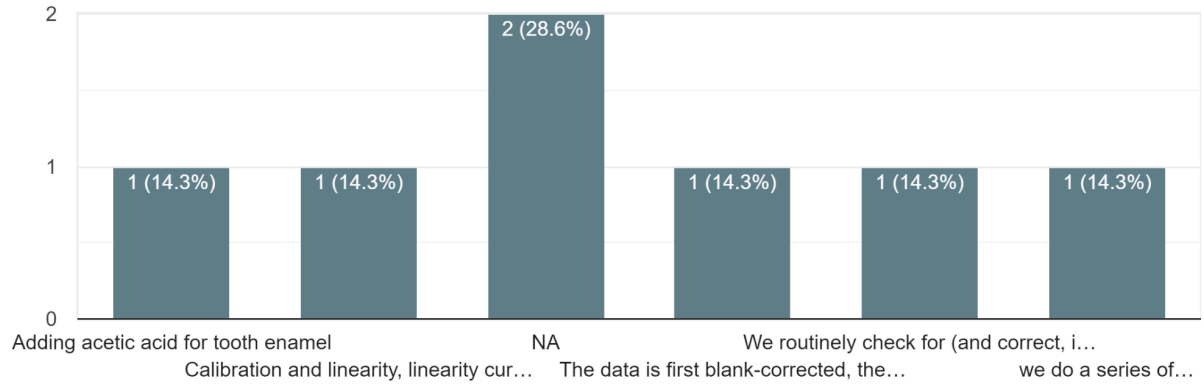
8 responses



Question 29

Describe your data handling practices. For example, how do you correct for memory effects? Time? Area?

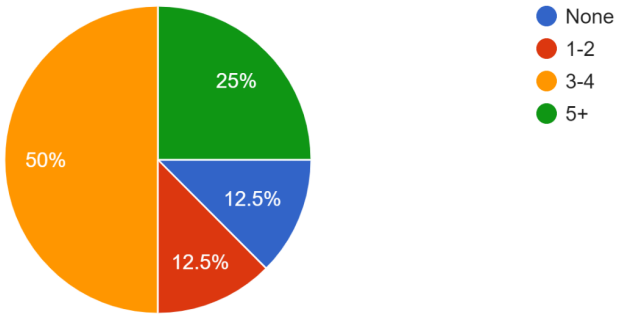
7 responses



Question 30

How many replicates of each reference material per analytical sequence do you measure?

8 responses



Question 31

IRB Approval

Date: 3-18-2024

IRB #: IRB-FY2024-161
Title: An Exploratory Study of How Anthropology and Archaeology Laboratories Conduct Isotope Sample Preparation
Creation Date: 2-6-2024
End Date:
Status: Approved
Principal Investigator: Emma Lloyd
Review Board: MTSU Institutional Review Board
Sponsor:

Study History

Submission Type Initial	**Review Type** Exempt	**Decision** No Human Subjects Research

Key Study Contacts

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