Final Technical Report

"*Isotope Analyses of Hair as a Trace Evidence Tool to Reconstruct Human Movements: Combining Strontium Isotope with Hydrogen/Oxygen Isotope Data*"

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Abstract

Strontium (Sr) isotope ratios of human hair have attracted interest as they potentially record an individual's environment. It has been previously established that the $^{87}Sr/^{86}Sr$ ratios of human tissues composed of hydroxyapatite (e.g., bones and teeth) relate to geography; however, the application of $^{87}Sr/^{86}Sr$ ratios of keratinous human tissues (e.g., hair and fingernails) has been avoided due to low strontium concentrations within these tissues. Recent technological advances have made strontium isotope analysis of keratin-based tissues possible, which have proven useful in reconstructing animal geospatial histories. Human hair is structurally similar to non-human keratinous tissues and thus its $^{87}Sr/^{86}Sr$ value should also record environmental information. However, the Sr abundance and $^{87}Sr/^{86}Sr$ ratio of human hair is influenced by both endogenous (i.e., dietary sources) and exogenous (i.e., external deposition) Sr contributions; thus separating the external Sr environmental signals from the internal Sr dietary indicators is required prior to the application of $^{87}Sr/^{86}Sr$ ratios of human hair for geospatial applications.

The purpose of this research program was to determine if the $^{87}Sr/^{86}Sr$ ratios of human tissues hair related to a geographically controlled variable. Five research objectives were defined to:

1. Develop analytical methodologies in order to analyze the $^{87}Sr/^{86}Sr$ ratio of human hair and establish that $^{87}Sr/^{86}Sr$ variation exists within a population of human hair.
2. Establish protocols for the isolation of the endogenous and exogenous Sr signals within human hair.
3. Constrain endogenous and exogenous Sr sources and isotopic signals to human hair through a controlled study and determine which signal has the greatest potential as a forensic tool to reconstruct an individual's geographic-movement history.
4. Determine the geospatial relationship between the $^{87}Sr/^{86}Sr$ ratios of human hair and a geographically mediated parameter and expand to the city-, region-, and nation-scale.
5. Compare Sr and oxygen (O) isotope ratios along the hair length of known travelers, establish a relationship between both isotope systems, and determine if the $^{87}Sr/^{86}Sr$ ratios reflect known geographic movements in a similar fashion as O isotope ratios.

Hair materials were collected at salons and barbershops with additional samples provided by volunteers with known diets and eating habits. In the first application of its kind, we employed an in-line strontium purification methodology and a multi-collector inductively coupled plasma mass spectrometer to measure the Sr isotope ratio of human hair.

In this program, we successfully measured the $^{87}Sr/^{86}Sr$ ratio of human hair and found that extensive variation existed in the $^{87}Sr/^{86}Sr$ ratio of hair (Objective 1). Hair-cleaning methodologies were established to determine the extent that endogenous and exogenous strontium signals could be isolated from human hair. These results indicated that external environmental Sr (exogenous) signals could be distinguished from the internal dietary Sr (endogenous) signals (Objective 2). Through a dietary questionnaire and study, we

established that the exogenous $^{87}Sr/^{86}Sr$ ratios of human hair related to geography, where the human hair isotope values were primarily influenced by isotope ratio of municipal waters in that an individual bathed (Objective 3). We found $^{87}Sr/^{86}Sr$ ratios of human hair corresponded to the $^{87}Sr/^{86}Sr$ ratios of tap waters available to the individual (Objective 4) and observed large variations in the $^{87}Sr/^{86}Sr$ ratios of tap waters between municipalities in one metropolitan area and between locations across the continental United States. We established the $^{87}Sr/^{86}Sr$ ratios of municipal waters were governed by geography and by the water distribution system in a specific locality and thus developed the foundation for larger geospatial applications of the $^{87}Sr/^{86}Sr$ ratios of human hair. Lastly, we found $^{87}Sr/^{86}Sr$ variation through the transverse cross sections of hair indicating exogenous Sr infiltrated into the hair and suggesting the geospatial relationship between Sr and O isotope ratio may be multifaceted (Objective 5). The conclusions of this program of study show that the $^{87}Sr/^{86}Sr$ ratios of human hair are related to geography and permit the further development of strontium isotope ratios of modern human hair as a forensic tool.

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Executive Summary

PROBLEM: Reconstructing the travel-movement of individuals can be important to many forensic investigations, spanning from homeland security issues at the national level to cold-case investigations at the local level. Analysis of stable isotopes recorded in human scalp hair at natural abundance levels has shown to be a useful tool to law enforcement and has assisted in reconstruction of the recent geographic-movement histories of individuals. This is because hair proteins (i.e., keratin), and the stable isotopes contained within keratin, are recorders of an individual's geographical environment. For example, the oxygen (O) isotope values ($\delta^{18}O$) of human hair keratin have provided novel travel/geographic origin information that helped guide local, national, international criminal investigations. This is due to the well-established relationship between the $\delta^{18}O$ values of human hair and drinking water. Since the $\delta^{18}O$ values of drinking water vary predictably across landscapes, the $\delta^{18}O$ values of human hair correlate to specific geographic regions. These mappable projections of isotope values across landscapes are termed an "isoscape."

Our research firm pioneered the use of $\delta^{18}O$ isoscape projections as a forensic tool for determining human region-of-origin. *While the use of a $\delta^{18}O$ isoscape provides a valuable law enforcement tool for describing geographical spaces where an individual may have originated, the estimated geographical regions can be broad. A complementary isotopic approach using human hair that reflects different geographic information would refine the region-of-origin predictions based on $\delta^{18}O$ hair values alone*.

PURPOSE: Strontium (Sr) isotope ratios ($^{87}Sr/^{86}Sr$) of human hair may be an additional estimator of geographic region independent of O isotopes as Sr is not a structural component of hair and the $^{87}Sr/^{86}Sr$ ratio of a geographic area is largely controlled by regional geology. The $^{87}Sr/^{86}Sr$ ratios of biological materials have been successfully applied to many region-of-origin questions in the fields of archaeology, ecology, food science, and forensic science. When both O and Sr isoscapes are combined and layered, the overlapping areas further constrain the estimated regions from which an individual originated. There are two major questions these developing and complementary technologies can potentially answer for law enforcement and forensic communities:

1) *Is the individual a resident of the region in which the person was found*?
2) *If not, from what regions could the individual have originated?*

The applications of coupled O and Sr isotope analysis of hair are far-reaching considering the ubiquitous occurrence of human hair at many crime scenes. Specifically, the uses of this technology include, but are not limited to, reconstructing travel histories of unidentified murder victims, reconstructing movements of trans-nationals associated with organized crime syndicates and having uncertain origins and travel histories, and investigating the region-of-origin of exploited women and children transported across state and international boundaries.

The purpose of this research program was to determine (a) if the $^{87}Sr/^{86}Sr$ ratios of human hair relate to geography and (b) if the Sr and O isotope ratios of human hair can be linked to refine predicated regions-of-origins of an individual.

RESEARCH DESIGN AND FINDINGS: A series of tasks were identified to complete this program of research. Following task identification, experiments were designed and material collections were made to complete the tasks and meet the research objectives of this program of study.

Task #1. The first task was to develop analytical methodologies to measure the $^{87}Sr/^{86}Sr$ ratio of human hair. In the first application of its kind, we employed an in-line Sr purification methodology and a multi-collector inductively coupled plasma mass spectrometer to measure the Sr isotope ratio of human hair. All Sr isotope measurements were made using a ThermoFisher Scientific Neptune *Plus* multi-collector ICP-MS. Samples were introduced using an online Sr purification method utilizing a peristaltic pump, a pair of 6-way valves, an in-line purification column, and an autosampler. Variable speed settings on the peristaltic pump allowed samples to be rapidly loaded into the purification column where Sr was trapped while all other elements were rinsed away; the column flow was then reversed and purified Sr was eluted into the spray chamber. This analytical system proved stable, reliable, and able to differentiate $^{87}Sr/^{86}Sr$ ratios of sampled materials within the fifth decimal place – significant precision to address all research questions posed in this program of study. This methodology is a significant analytical step forward as it greatly decreases the time needed to make an Sr isotope measurement of hair.

Task #2. Once analytical methodologies were established and in place, we confirmed that $^{87}Sr/^{86}Sr$ variation exists within human hair. Hair materials for this task were collected at a salon in Salt Lake City, Utah. This survey of randomly collected hair materials showed measurable, consistent, and statistically significant variations in the $^{87}Sr/^{86}Sr$ ratio of the hair in a population of individuals from one geographic region. This proof-of-concept experiment allowed us to establish that the $^{87}Sr/^{86}Sr$ ratios of human hair likely records dietary and/or environmental information.

Task #3. There are two distinct sources of Sr that must be considered and separated in order to understand dietary and/or environmental information recorded by the $^{87}Sr/^{86}Sr$ ratios of human hair. Thus, the next task was to determine the extent that endogenous and exogenous Sr pools could be isolated from human hair keratin. The endogenous Sr signal reflects the elemental concentration within hair at formation (i.e., diet), while the exogenous Sr signature represents additional Sr that is incorporated into hair as it grows (i.e., environment). Sr abundance in hair increases along the length of human hair from proximal to distal portions. These Sr increases are due to the incorporation of exogenous Sr onto and/or into the hair cuticle as the hair is exposed to the external environment. This combination of Sr sources (i.e., endogenous and exogenous) complicates the interpretation of $^{87}Sr/^{86}Sr$ ratios of human hair.

To chemically differentiate the endogenous and exogenous Sr isotopic signals to hair, five different hair-cleaning methodologies were selected and applied to samples. In this study, hair materials were collected at a local salon in the Salt Lake City metropolitan area of Utah. We found that replicate applications of an individual treatment removed a

consistent amount of Sr from hair and that replicate analyses showed each treatment altered the Sr isotope ratios of hair consistently. A mass-balance approach was used to demonstrate that Sr was quantitatively removed and was accounted for in either the treated hair or the leachate. We observed that the Sr isotope ratio varied as a function of treatment aggressiveness and was a function of the difference between endogenous and exogenous Sr components. As a result, the isotope ratio of hair and hair leachates treated with the most aggressive cleaning methods reflected the isotope ratios of the endogenous and total exogenous Sr signatures, respectively. The results of this study indicate that external environmental Sr signals can be distinguished from the internal dietary Sr signals and therefore permit the application of Sr isotope ratio analysis of modern human hair for geospatial applications. These experiments laid the foundation for continued development of the geospatial application of the $^{87}Sr/^{86}Sr$ of human hair. The data and results of Tasks #1-3 were published in the journal *Analytica Chimica Acta*.

Task #4. In addition to chemical isolation of the endogenous and exogenous Sr sources, we sought to understand the relative importance of the endogenous and exogenous Sr isotopic signals to the overall $^{87}Sr/^{86}Sr$ ratio of human hair. Further, we sought to determine which Sr source (i.e., endogenous or exogenous) to human hair had the greatest potential as a forensic tool to reconstruct an individual's geographic-movement history. For this task, we designed an experiment to identify the relative importance of Sr sources (e.g., bedrock, dust, food, drinking water, etc.) to the $^{87}Sr/^{86}Sr$ of human hair by collecting hair from volunteers living in metropolitan area of Salt Lake City, Utah. We compiled information from the volunteers regarding sample location, age, sex, ethnicity, and dietary habits with standardized questionnaires. We discovered that the endogenous $^{87}Sr/^{86}Sr$ ratios did not affect the overall $^{87}Sr/^{86}Sr$ ratios of human hair. Nutritional surveys and independent dietary isotope ratios of the hair (e.g., $\delta^{13}C$ and $\delta^{15}N$) supported significant dietary differences between the ethnic groups, potentially suggesting differing endogenous dietary contributions of Sr may be expected. However, we found no relationship between ethnicity and $^{87}Sr/^{86}Sr$ value of hair, indicating the endogenous Sr isotopic signal did not modify the $^{87}Sr/^{86}Sr$ ratios of the hair.

We observed a significant association between $^{87}Sr/^{86}Sr$ value of hair and collection location. As individuals lived within 10 miles of each other and thus were likely exposed to similar bedrock, dust, and environmental contaminates, we had not predicted a relationship between $^{87}Sr/^{86}Sr$ ratios and collection location, due to the close proximity of collection sites. These results indicated another exogenous Sr source was affecting the overall $^{87}Sr/^{86}Sr$ ratios of human hair. Following these findings, tap waters were collected from the sampling locations and surrounding regions; striking linkages between $^{87}Sr/^{86}Sr$ ratios of hair and tap water were observed. This outcome indicated the predominate Sr signal incorporated into the hair was from municipal water and that hair was a sensitive temporal carrier of this environmental information.

To determine the geospatial relationship between the $^{87}Sr/^{86}Sr$ ratios of human hair and municipal water, we sampled hair from individuals living throughout the Salt Lake Valley, Utah and tap water from public buildings throughout the Salt Lake Valley, Utah. From this exercise, we found that individuals with larger $^{87}Sr/^{86}Sr$ ratios resided in neighborhoods that had larger $^{87}Sr/^{86}Sr$ ratios of tap water. These results further

suggested exposure to tap water was the strongest driver of overall $^{87}Sr/^{86}Sr$ ratios of hair. Considering an individual's repeated exposure to municipal waters through regular bathing, tap water-derived Sr may be the most important exogenous Sr source to human hair. From this, we concluded that mapping variation in the $^{87}Sr/^{86}Sr$ ratios of tap waters would provide an ideal base layer to link the $^{87}Sr/^{86}Sr$ ratios of human hair to a geographically defined variable. We plan to submit these findings to *Forensic Science International*.

Task #5. To link the Sr isotope system to the O isotope system, the $\delta^{18}O$ values of human hair and tap waters collected throughout the Salt Lake metropolitan area in Task #4 were measured. We found that the sampled communities had water with distinct $^{87}Sr/^{86}Sr$ ratios and $\delta^{18}O$ values. Our findings suggest that communities and municipalities within the Salt Lake Valley use different and unique combinations of water resources. In turn, the isotopic signals from the unique combinations of water resources used within a community are transferred to the individuals that reside within that particular community. These results indicated that the isotope ratios of human hair isotope are primarily influenced by isotope ratios of municipal waters used by an individual for drinking ($\delta^{18}O$) and bathing ($^{87}Sr/^{86}Sr$). Thus, the distinct combination of Sr and O isotope ratios allow for much greater fidelity in geographic predictions and can be used to nearly a zip code-specific level, at least within the Salt Lake City metropolitan region. These city- and regional-scale linkages between Sr and O isotope ratios of water and hair will be submitted to *Forensic Science International* or another peer-reviewed forensic science journal.

Task #6. As we established the linkages between water and hair Sr and O isotope ratio, our understanding of the temporal and spatial variation in the $^{87}Sr/^{86}Sr$ ratios and $\delta^{18}O$ values of municipal waters was lacking. Communities within the Salt Lake metropolitan area of Utah acquire municipal water from a mix of local groundwater and water from distant regions. Within a community, the proportions of each water source used depend on water ownership by different municipality-specific water utilities. Thus, the Salt Lake Valley was an ideal test region for investigating temporal and spatial isotope patterns as large variations in both the $^{87}Sr/^{86}Sr$ ratios and $\delta^{18}O$ values of municipal waters were expected. To establish the temporal and spatial patterns in the $^{87}Sr/^{86}Sr$ ratios and $\delta^{18}O$ values (as well as $\delta^{2}H$ values) of municipal waters, we sampled ~30 locations across the Salt Lake Valley at four intervals during one calendar year. This research design allowed us to capture isotopic patterns associated with variations in seasonal water usage and changes in municipal water sources. We found that tap water $^{87}Sr/^{86}Sr$ ratios across the Salt Lake Valley varied with water management practice and season. From this, we established the $^{87}Sr/^{86}Sr$ ratios of municipal waters were governed by geography and geology, but most importantly by the water distribution system in a specific locality. This discovery provided a foundation for larger geospatial applications of the $^{87}Sr/^{86}Sr$ ratios and $\delta^{18}O$ values of human hair. The data and results of these experiments will be submitted to the journal *Water Research*.

Task #7. To expand to the linkages between Sr and O isotope ratios of tap water and hair to larger spatial scales, we measured the $^{87}Sr/^{86}Sr$ ratios of hair collected from fifty-six

cities throughout the United States. Sr and O isotope ratios of tap water from these cities had been previously measured (as part of related programs of research) and showed significant isotopic variation and coherent spatial patterns across the continental USA. The $\delta^{18}O$ values of hair had been measured (as part of another project) and were shown to be a function of the $\delta^{18}O$ value of drinking water. As part of this project, the $^{87}Sr/^{86}Sr$ ratios of hair samples from these cities were measured and indicated that tap water and hair $^{87}Sr/^{86}Sr$ ratios were linked; this corroborated the linkages found at the local scale in Tasks #4 and 5. These results further established that the isotope ratios of human hair are primarily influenced by isotope ratios of municipal waters available to an individual for drinking ($\delta^{18}O$) and bathing ($^{87}Sr/^{86}Sr$). The data and results of these experiments will be submitted to the *Proceedings of the National Academy of Sciences of the USA*.

Task #8. Lastly, several experiments have been completed or are in progress to compare the $^{87}Sr/^{86}Sr$ ratios and $\delta^{18}O$ values along the hair length. As part of the experiment to chemically isolate the endogenous from exogenous Sr signal from hair (Task #3), we noted that Sr isotope ratio of hair and leachate varied as a function of treatment aggressiveness. This result indicated that exogenous Sr infiltrated into the hair and suggested linking the geospatial relationships between Sr and O isotope ratio along the length of a hair may be complex. This is because Sr abundance in hair increases (1) along the length of human hair due to the continual incorporation of exogenous Sr and (2) along a transverse cross section of hair due to the initial incorporation of endogenous Sr from diet and the infiltration of exogenous Sr from the environment. In contrast, only lateral variations in the O isotope ratios of hair occur. Thus, linking the laterally varying O isotope signal to the both laterally and transversely varying Sr isotope signal may require a detailed mathematical modeling effort. To test these relationships, hair from a known traveler–a horse that was transported from Brazil to Utah–has been sampled. The $\delta^{18}O$ values of the traveler demonstrated isotopic variations consistent with known dates of travels and locations of travels. While bulk Sr and O isotope analyses of hair have demonstrated that geographic information is recorded in both isotope signals, this "traveler" dataset will determine if paired Sr and O isotope analyses along the of a hair can be used to reconstruction an individuals travel history.

CONCLUSIONS: This program of study has shown that the $^{87}Sr/^{86}Sr$ ratios of human hair clearly relates to geography. We have isolated the exogenous and endogenous Sr isotope signals to hair and shown that the exogenous Sr isotope signal is most informative for forensic questions. In addition, we have determined that the most important exogenous Sr to hair is the Sr contained in bathing water. Our findings permit the development of the application of Sr isotope ratios of modern human hair as a novel forensic tool. We conclude that the combination of $\delta^{18}O$ values and $^{87}Sr/^{86}Sr$ ratios of hair constitute a distinct isotope signature, which may allow for greater specificity for region-of-origin assignment during criminal investigations than the use of one isotope measurement alone. The prediction of spatially explicit patterns in the Sr and O isotope ratios of human hair has many potential implications with respect to human movements and with regard to specifying regions-of-origin in anthropological, archeological, and forensic studies.

Introduction

Reconstructing the travel or movement of individuals is important to criminal investigations. Analysis of stable isotopes recorded in human hair has assisted in reconstruction of the geographic-movement histories of individuals. While this state-of-the-art tool has proven valuable to law enforcement, the geographical regions where an individual traveled or moved predicted from measured isotope values can be spatially large. A novel and complementary analysis and prediction approach using human hair would help refine these region-of-origin estimates.

Strontium (Sr) isotope ($^{87}Sr/^{86}Sr$) ratios within organic materials record the $^{87}Sr/^{86}Sr$ ratios of the environment in which an organism lives and/or migrates through. Thus, $^{87}Sr/^{86}Sr$ ratios are an additional estimator of an organism's geographic region. Environmental $^{87}Sr/^{86}Sr$ ratios vary geographically as a function of age and the Rb/Sr ratio of the rock or soil type, creating a definable and predictable geographic landscape tool for sourcing a specimen [1]. ^{86}Sr (9.87% abundance) is a stable isotope, while ^{87}Sr (7.04%) is radiogenic produced by the beta decay of ^{87}Rb [2].

The $^{87}Sr/^{86}Sr$ ratios of biological materials have been successfully applied to many region-of-origin questions in the fields of archaeology [3-12], ecology [13-15], food science [16-19], and forensic science [20-24]. These studies indicate that the $^{87}Sr/^{86}Sr$ ratio of internal organism tissues reflects the $^{87}Sr/^{86}Sr$ ratios of water and diet during the time period(s) when the biological materials formed. In contrast, exposed tissues (i.e., hair, horns, nails, etc.) record the $^{87}Sr/^{86}Sr$ ratios of contamination from the external environment (i.e., dust).

Sr contained within newly formed hair initially reflects water and food sources of the individual; once the hair segment protrudes from the follicle, the hair $^{87}Sr/^{86}Sr$ ratios changes [25]. As hair is exposed to the external environment and detached from the animal's metabolism, the Sr concentration within hair increases with exposure time, reflecting continuous inputs of dust and aerosol particles into the external hair matrix [25]. This externally-sourced Sr is imbedded within the keratin sheets of hair and not easily removed by bathing or washing [26].

An advantage of using strontium isotope analysis compared to the analysis of the light stable isotopes (such as oxygen) to understand an organism's geographic movement is the negligible or absence of isotopic fractionation from the Sr source into the organic tissue [1]. In others words, the $^{87}Sr/^{86}Sr$ ratios of organic tissues directly reflect the $^{87}Sr/^{86}Sr$ ratios of Sr source(s). The lack of Sr fractionation between tissues and dietary input has been shown in variety of different organic tissues, including antlers [27], teeth and bones [28-35], leaves [36, 37], and feathers [13, 38].

The overall aim of this program of research was to determine whether or not Sr isotope analyses can be used as a diagnostic tool to precisely estimate the recent regions-of-origin of humans through chemical analyses of scalp hair. The overarching goal of this program of research was to build a data product and a model product that would further refine and

constrain the region-of-origin predictions based initially on oxygen (O) isotope ratio analyses. Five research objectives were defined to meet this aim and goal:

1. Develop analytical methodologies in order to analyze the $^{87}Sr/^{86}Sr$ ratio of human hair and establish that $^{87}Sr/^{86}Sr$ variation exists within a population of human hair.
2. Establish protocols for the isolation of the endogenous and exogenous Sr signals within human hair.
3. Constrain endogenous and exogenous Sr sources and isotopic signals to human hair through a controlled study and determine which signal has the greatest potential as a forensic tool to reconstruct an individual's geographic-movement history.
4. Determine the geospatial relationship between the $^{87}Sr/^{86}Sr$ ratios of human hair and a geographically mediated parameter and expand to the city-, region-, and nation-scale.
5. Compare Sr and oxygen (O) isotope ratios along the hair length of known travelers, establish a relationship between both isotope systems, and determine if the $^{87}Sr/^{86}Sr$ ratios reflect known movements in a similar fashion as O isotope ratios.

We accomplished these objectives by designing a series of experiments and data collections to test four specific hypotheses:

Hypothesis 1.
The variations in Sr isotope ratios of human scalp hair reflect source differences in exogenous Sr inputs of specific geographical regions and not variations in the Sr isotope ratios of endogenous inputs.

Hypothesis 2.
Extensive geographic variations in the Sr isotope ratios of hair exist and these variations reflect the known variations in exogenous Sr isotopes across the United States.

Hypothesis 3.
The movement of an individual from one region to another will be reflected in changes in Sr isotopes incorporated exogenously along the length of a hair segment.

Hypothesis 4.
Endogenous and exogenous Sr sources contribute to the latitudinal variations in $^{87}Sr/^{86}Sr$ ratios of hair, but the contribution of endogenous Sr is small relative to exogenous Sr.

Methods

Efforts and Experiments

Effort 1. Measurement of Sr isotope ratios of hair using MC-ICP-MS

Hair samples were selected from a single salon in the metropolitan area of Salt Lake City, Utah to establish that variation existed in human hair Sr isotope ratio. The hair samples were analyzed for Sr concentrations and isotope ratios.

Experiment 1. Investigating the consistency of hair cleaning methods

To test the consistency of different hair cleaning methods, separate aliquots of three randomly selected hair samples were treated with five different cleaning methods for a total of three independent replicates of each cleaning method. The three replicates of treated and untreated hair samples were analyzed for Sr elemental abundances and isotope ratios. The leachates from the three replicates were analyzed for Sr concentration and isotope ratios.

Experiment 2. Investigating the efficiency of hair cleaning methods

The efficiency of different hair cleaning methods to remove Sr was tested using hair samples treated with three select cleaning treatments. Twenty-two hair samples from our collections were selected for this experiment. The treated hair samples and leachates from treatments were analyzed for Sr concentrations and isotope ratios.

Experiment 3. Using diet to understand endogenous and exogenous Sr sources

To assess the relative contributions of endogenous and exogenous Sr to human hair, more than one hundred samples of hair were collected from school children within Salt Lake City, Utah (as part of the *National Children's Study*). Along with hair samples, study volunteers provided dietary information, ethnicity, and basic biological statistics (age, sex, height, weight, BMI, etc.) allowing us to investigate endogenous dietary Sr inputs. The hair samples were analyzed for Sr concentrations and isotope ratios.

Experiment 4. Linking the Sr isotope ratio of hair to geography

Tap waters from public buildings throughout Salt Lake City, Utah were used to test the linkages between the Sr isotope ratio of hair and tap water. Water samples were collected near sample locations used in *Experiment 3* and analyzed for Sr concentrations and isotope ratios.

Experiment 5. Combining Sr and O isotope ratios of hair for geospatial applications on city-level and regional scales

The tap waters and hair samples used in *Experiments 3* and *4* were used to investigate geospatial linkages between the Sr and O isotope ratios. Hair samples collected in *Experiment 3* and water samples collected in *Experiment 4* were analyzed for O isotope ratios.

Experiment 6. Establishing temporal and spatial patterns in the Sr isotope ratios of municipal water supplies

Tap waters from public buildings were collected seasonally throughout Salt Lake Valley, Utah within a single seasonal cycle (i.e., calendar year) to understand the seasonal and

spatial patterns in the Sr isotope ratio of municipal water supplies. Water samples were analyzed for Sr concentrations and isotope ratios.

Experiment 7. Developing linkages between Sr isotope ratios of hair and tap water on the national scale

Analyses of hair specimens collected from regions across the United States that are known to have distinct and different bedrock $^{87}Sr/^{86}Sr$ ratios were used for this experiment. For this study, samples included barbershop hair specimens that had been previously collected from nearly 100 different cities in the contiguous USA. Hair samples were analyzed for Sr concentrations and isotope ratios and compared to previously published dataset of water Sr isotope ratios for these same regions.

Experiment 8. Relating Sr and O isotope ratios along the length of a hair

To assess if Sr isotopes vary along the length of hair and record spatial and temporal movements, hair specimens from a horse with a known travel history was used. The specimen was from an individual known to have traveled between Brazil and Utah. Hair samples were sequenced and analyzed for $\delta^{18}O$ and Sr concentrations and isotope ratios.

Samples

Effort 1, Experiment 1 and 2.

Hair samples used in this study were collected from a single salon in Taylorsville, Utah, USA. Twenty-two locks of hair were collected as clippings from the salon floor and immediately placed into paper envelopes after cutting. Discarded hair on the floor of a barbershop is classified as trash and is not subject to IRB protocol requirements. This sampling protocol explicitly removes potential sampling bias as the barber shop/salon samples incorporate hair from numerous individuals. All hair samples derived from residents of Utah's Salt Lake Valley. No other demographic, dietary, socioeconomic, or travel history information was collected from the individuals. Locks of hair ranged 5-10 cm in length with no notation of original orientation or overall length of the individual's hair. The hair samples varied in color, treatment (dyed, chemically curled, straightened), thickness, and texture ranging from naturally curly to straight.

Experiments 3, 4, and 5.

As part of another study, a hair sample, a self-administered food frequency questionnaire, and a collection of biometrics were obtained from a cross-sectional study of children and adolescents, including participants ranging from 9 to 18 years of age. The study was conducted in four different educational institutions in Salt Lake City, Utah: a public elementary school (grades 5th and 6th), a charter school (grades 6th to 9th), a public high school (10th grade), and a private college. The height and weight of participating individuals were measured at the time of hair collection. Body mass index (BMI) and age-specific BMI percentiles were calculated using the CDC's Children's BMI Tool for

Schools (http://www.cdc.gov). We analyzed 102 hair samples for Sr elemental abundance and of these 69 for Sr isotope ratio.

Experiment 6.

Water samples were collected from public building taps throughout the Salt Lake Valley, Utah. Approximately 30 water samples were collected from fourteen municipalities four times during a seasonal cycle (summer, July 29th; fall, October 23rd; winter, January 17th; spring, April 16th) to capture seasonal variations. If sampling location was closed, a new nearby sampling location (i.e., building) was selected.

Experiment 7.

Hair samples used in Experiment 7 had been previously collected [40]. Briefly, samples were collected in 65 municipalities of the coterminous USA, located in 18 states. We randomly collected discarded hair from the floor of three barbershops in each municipality. Samples were collected during a cross-country sampling effort in summer 2004. Three sets of discarded hair clippings were collected in cities or towns with populations of <100,000 to increase the probability that the hair sample was associated with a local citizen. No information was available regarding the identification and/or origins of the individuals from which discarded hair samples were obtained. We assumed that these individuals were residents of the city in which the hair sample was collected and that they had no dietary differences. Hair samples were collected in paper coin envelopes. Since original collection date, envelopes had been stored at room temperature and in the dark.

Experiment 8.

Hair from a horse that was transported from Brazil to Utah was used in this experiment. In addition, a stable-mate of the transported horse was used as a baseline comparison. Hair strands of hair of >400 cm were collected from both individuals fifteen months after the Brazilian horse had moved.

Hair cleaning

Experiments 1 and 2.

Hair samples were cleaned in a variety of polar and acidic reagents to test which cleaning method was most effective in removing external strontium contamination. To remove lipids, residues, and surface contaminates, subsamples (~50 mg) of each hair sample were cleaned with each of the following treatment solvents: 1) ultrapure water, 2) chloroform:methanol mixture (2:1), 3) 0.1 M HCl, 4) International Atomic Energy Agency (IAEA)-recommended [39] and 5) IAEA-recommended plus 0.1 M HCl. For Treatments 1, 2, and 3, hair subsamples were placed into 15-ml centrifuge tubes with enough solvent to completely cover the hair (~3-5 ml). Centrifuge tubes were placed in an ultrasonic bath and sonicated for 10 min. After sonication, the solute was decanted into another centrifuge tube. This process was repeated a total of three times with all decanted solutes ("leachates") combined into a single centrifuge tube. For Treatments 4

and 5, we followed a modified IAEA hair washing methodology with and without an additional wash of 0.1 M HCl. The IAEA method is a sequential cleaning with acetone, water, and acetone. This protocol calls for the hair to be rinsed successively in acetone, water, and again in acetone for 10 min each. Treatment 4 was a slight modification of this approach as hair was sonicated in each prescribed solvent for 10 min. Treatment 5 was a modification of Treatment 4 in that an additional 10 min sonication in 0.1 M HCl was applied following the last acetone wash. Following each cleaning step in Treatments 4 and 5, the solute was decanted into a centrifuge tube to combine all solutes from each cleaning step.

All acetone (EMD, HPLC-grade), chloroform (EMD, OmniSolv®), and methanol (EMD, OmniSolv®) used in hair cleaning were HPLC grade or higher. The ultrapure water used for sample cleaning and acid dilutions was from a Milli-Q Academic A10® system (EMD Millipore; Billerica, Massachusetts, USA) with a resistivity >18 MΩ. Cleaned hair samples were allowed to dry at room temperature for 72 hrs within a laminar flow hood. Subsamples of each hair sample were also collected and left untreated as a control for cleaning methods.

Experiment 3.

Hair samples were treated using hair cleaning Treatment 2 [chloroform:methanol mixture (2:1)] from *Experiments 1* and *2*.

Experiments 7.

Hair samples were cleaned using hair cleaning Treatment 5 (IAEA-recommended plus 0.1 M HCl) following the findings of *Experiments 1* and *2*.

Experiments 8.

Hair samples were treated using hair cleaning Treatment 2 [chloroform:methanol mixture (2:1)] from *Experiments 1* and *2*.

Water collections

Experiment 6.

Water samples were collected in acid-leached 125-ml low-density polyethylene (LDPE) bottles. Prior to collection, the water tap was opened and allowed to run for 10 sec. Samples were sealed and stored in the dark at 10° C prior to analysis.

Experiment 7.

Water samples used in this experiment had been initially procured for hydrogen and oxygen stable isotope measurements and collected in 1- or 3-dram borosilicate glass vials [40]. Since original collection date, vials had been stored capped and sealed (Parafilm M laboratory film; Pechiney Plastic Packaging, Menasha, Wisconsin, USA) at room

temperature and in the dark. While storage in borosilicate glass is not ideal for trace element analysis due to possible leaching, we found no contamination from the glass in regards to Sr.

Sample preparation for strontium isotope analysis

Hair samples were digested using an Ethos EZ® microwave digestion system (Milestone, Inc.; Shelton, Connecticut, USA). Approximately 50 mg of hair was weighed into a Teflon® digestion microvessel. Two milliliters of concentrated ultrapure concentrated HNO_3 (Aristar® ULTRA; BDH Chemical, Darmstadt, Germany) were added to the microvessel containing the hair and the microvessel was then sealed, submerged in 10 ml of milli-Q water and 50 µl H_2O_2 (30% v/v), and then placed within an outer vessel. The outer vessel was then placed in the digester carousel. Two certified reference materials (TORT-2 Lobster Hepatopancreas Reference Material for Trace Metals from the National Research Council Canada and Human Hair No. 13 from the National Institute for Environmental Studies) and a method blank were digested along with the hair samples following the principle of identical treatment. The microwave program used for hair digestion was 13.3°C/min ramp to 200°C, followed by an isothermal at 200°C for 15 min with a 60 min cool down to room temperature. The microwave was operated at full power (1500 W) for all heating cycles. Once cooled to room temperature, the hair digests were transferred to acid-leached 2-ml snap-cap centrifuge tubes. A 100-µl aliquot of the primary hair digest was transferred to a 15-ml tube and the volume was brought to 10 ml with ultrapure water. A standard solution containing 10 ppb In was added to each sample as an internal concentration standard.

Hair leachates and 10-ml aliquots of all solvents (Milli-Q water, acetone, chloroform, methanol, and 0.1 M HCl) were transferred to acid-leached Teflon® beakers and evaporated on a 50°C Teflon® coated hotplate within a laminar flow hood to concentrate any solutes present. If additional dissolution was needed, samples were refluxed on the hotplate in ultrapure HNO_3 until dissolved. The solutes were rehydrated with concentrated ultrapure HNO_3 (2 ml) and transferred to acid-leached 2-ml snap-cap centrifuge tubes. A 100-µl aliquot of the rehydrated solutes was transferred to a 15-ml tube with 10 ml of ultrapure water. A standard solution containing 10 ppb In was added to each sample as a concentration internal standard.

Strontium abundance and isotope analysis

All strontium elemental abundances were measured via inductively coupled plasma quadrupole-mass spectrometry (ICP-MS) on an Agilent 7500ce instrument (Agilent Technologies; Santa Clara, California, USA) at the ICP-MS Metals Lab in the Department of Geology & Geophysics at the University of Utah, Salt Lake City, Utah, USA. A double-pass spray chamber with perfluoroalcoxy fluorocarbon (PFA) nebulizer (0.1 mL/min), a quartz torch, and nickel cones were used. A calibration solution containing Sr was prepared gravimetrically using a single-element standard (Inorganic Ventures, Inc.; Christiansburg, Virginia, USA). Standard reference solution T-205 (USGS; Reston, Virginia, USA) was measured as an external calibration standard at least

five times within each analytical run. The long-term reproducibility for T-205 and differences relative to the accepted values indicated that the Sr concentrations were accurate within 10 %.

All strontium isotope measurements were made using a Neptune *Plus* multi-collector ICP-MS (ThermoFisher Scientific; Bremen, Germany) housed in the Department of Geology & Geophysics at the University of Utah, Salt Lake City, Utah, USA. Digests were introduced using an online Sr purification method following Mackey and Fernandez [41] and Chesson et al. [42]. This online system automates the purification of Sr by utilizing a peristaltic pump, a pair of 6-way valves, an in-line separation column, and a SC-2 DX autosampler with a FAST2 valve block (Elemental Scientific; Omaha, Nebraska, USA). The in-line separation column was packed with crown ether Sr resin (Eichrom Technologies; Lisle, Illinois, USA). Variable speed settings on the peristaltic pump allowed samples to be rapidly loaded into the purification column where Sr was trapped while all other elements were rinsed away; the column flow was then reversed and purified Sr was eluted into the spray chamber. A timing solution containing 66 ppb Sr was analyzed daily to insure proper chromatography and to assess column chemistry and efficiency. The instrument was operated at an RF power of 1200 W with nickel sampling and skimmer cones (1.1 mm and 0.8 mm apertures, respectively) and was optimized daily for signal intensity and stability. Cool, auxiliary and sample gas flow rates were 16 L/min, 0.85 L/min, and 0.91 L/min, respectively. The instrument was tuned for sensitivity daily with a solution containing 20 ppb Sr. For $^{87}Sr/^{86}Sr$ analysis, a static multi-collector routine was used that consisted of 1 block of 170 cycles with an integration time of 1.032 sec per cycle for an individual analysis. Each analysis was followed by a blank to monitor the efficiency of the crown ether Sr resin column. Sr isotope ratios of samples and references were blank- and interference-corrected and then normalized for instrumental mass discrimination using a defined $^{86}Sr/^{88}Sr$ of 0.1194.

Solutions of the international Sr standard reference material SRM 987 (National Institute of Standards and Technology; Gaithersburg, Maryland, USA) were analyzed with samples. The ratio of samples to standards within a single run was 5:1. Within-run reproducibility of SRM 987 was 0.71030 ± 0.00004 (2σ, n = 92). The long-term mean $^{87}Sr/^{86}Sr$ of SRM 987 analyzed using the automated purification method and MC-ICP-MS at concentrations of hair and leachate measurements was 0.71027 ± 0.00004 (2σ, n = 292).

Sample preparation for hydrogen and oxygen isotope analysis

The hair sample was ground into a homogeneous, fine powder. Because there is partial isotopic exchange of H atoms in keratin with water (either in liquid or vapor form), all samples were analyzed together with hair reference materials for which the δ^2H value of nonexchangeable H atoms had been determined. Both reference material and unknown hairs were allowed to equilibrate with water vapor in the laboratory atmosphere for a minimum of 48 hours before being weighed (150 μg) in silver capsules; weighed samples were stored under vacuum for 7 days before being analyzed. Measured values for the

reference materials were then used to determine the nonexchangeable H isotope ratios of the samples from the measured values and isotope mass balance.

Hydrogen and oxygen isotope analysis

The stable isotope abundances of water samples were analyzed on a Picarro model L1102-i water analyzer. Each sample was analyzed four times (four consecutive replicate injections) alongside a set of three laboratory reference materials, which had previously been calibrated to the VSMOW scale. Sets included two primary laboratory reference materials for slope/intercept normalization and a secondary laboratory reference material for quality assurance and quality control (QA/QC). Using delta notation, stable isotope ratios for oxygen and hydrogen are calculated as: $\delta = [(R_{samp}/R_{std})\text{-}1]$, where R represents the $^{2}H/^{1}H$ or $^{18}O/^{16}O$ abundance ratio, and R_{samp} and R_{std} are the ratios in the sample and standard, respectively. $\delta^{2}H$ and $\delta^{18}O$ values are expressed relative to the standard Vienna Standard Mean Ocean Water (VSMOW).

The stable isotope abundances of hair samples were analyzed on a ThermoFinnigan MAT 253 isotope ratio mass spectrometer. Samples were introduced to the instrument via a zero-blank autosampler attached to a high temperature conversion elemental analyzer (TC/EA). Samples were analyzed alongside sets of reference materials for data normalization. Sets included two primary laboratory reference materials for slope/intercept normalization and a secondary laboratory reference material for QA/QC. $\delta^{2}H$ and $\delta^{18}O$ values are expressed relative to the standard VSMOW.

Statistical analysis

Statistical analysis was completed using JMP® 10 (SAS; Cary, North Carolina, USA) for Mac OS X. The measured strontium concentrations of untreated hair samples and those cleaned with the different treatments were compared to each other (both against untreated and treated hair and between the treatments) using paired two-tail T-tests. P-values were considered significant at the $\alpha = 0.05$ level.

Results

Effort 1.

We were able to successfully prepare and analyze human hair for Sr elemental abundances and isotope ratios. Shown in **Table 1** are the Sr concentrations and isotope ratios of untreated hair samples from a single salon. We found the untreated hair samples from a single salon in the Salt Lake City metro area ranged 0.7 - 45.1 $\mu g\ g^{-1}$ with a mean value of 14 ± 14 $\mu g\ g^{-1}$ (2σ). We found Sr isotope ratios of the untreated subsamples of the hair samples used in this task ranged from 0.70909 to 0.71469, with the three aliquots having a standard deviation of 0.00003 or less (2σ).

Experiment 1.

We used both element abundances and isotope ratios to investigate the consistency of different hair cleaning methods. Shown in **Table 2** are the Sr concentrations of the three hair samples analyzed untreated and after treatment with the five washing methods. We found the Sr concentration ranged 8.3 to 58.9 μg g^{-1} (dry weight) for the untreated hair subsamples, with the unwashed hair subsamples for each of the three hair samples having a mean value of 11.8 ± 4.2 μg g^{-1}, 44.5 ± 18.2 μg g^{-1}, and 45.1 ± 1.0 μg g^{-1}, respectively.

Table 1. Description, strontium content and isotope ratio of untreated human hair samples used in this study.

Serial Number	Color	Texture	Sr (μg g^{-1})			$^{87}Sr/^{86}Sr$		
1	Brown	Curly	11.8	±	0.5	0.71027	±	0.00002
2	Dark Brown	Curly	44.5	±	1.0	0.71328	±	0.00001
3	Light Brown	Straight	45.1	±	0.9	0.71403	±	0.00001
4	Salt/Pepper	Curly	13.5	±	0.2	0.71287	±	0.00001
5	Brown with Grey	Straight	20.2	±	0.4	0.71274	±	0.00001
6	Brown	Straight	7.5	±	0.2	0.71196	±	0.00001
7	Brown	Straight	1.0	±	0.1	0.70909	±	0.00005
8	Salt/Pepper	Straight	4.0	±	0.1	0.71151	±	0.00002
9	Red	Straight	9.9	±	0.1	0.70956	±	0.00001
10	Brown	Straight	7.0	±	0.1	0.71172	±	0.00002
11	Red	Straight	21.3	±	0.3	0.71147	±	0.00001
12	Strawberry Blonde	Straight	7.4	±	0.1	0.71122	±	0.00001
13	Brown	Straight	3.3	±	0.2	0.71244	±	0.00001
14	Brown	Straight	0.8	±	0.7	0.71230	±	0.00003
15	Brown	Straight	17.4	±	0.3	0.71333	±	0.00001
16	Salt/Pepper	Curly	11.5	±	0.2	0.71469	±	0.00001
17	Light Brown	Straight	2.1	±	0.2	0.71393	±	0.00002
18	Reddish Brown	Straight	10.2	±	0.1	0.71042	±	0.00001
19	Dirty Blonde	Straight	4.5	±	0.2	0.71302	±	0.00001
20	Brown with Blonde Streaks (possibly dyed)	Straight	40.7	±	0.2	0.71154	±	0.00001
21	Dark Brown	Straight	0.7	±	0.2	0.71232	±	0.00003
22	Light Brown	Straight	13.5	±	0.1	0.71090	±	0.00001

We found that Treatment 3 removed an average of 93 ± 2 % (2σ) of the Sr (**Figure 1**). Treatments 1, 2, and 4 removed relatively similar amounts of Sr from the hair samples with 11 ± 6 % (2σ), 10 ± 16 %, and 19 ± 7 % of the Sr removed, respectively (**Figure 1**). Treatments 1 and 4 were statistically different ($p = 0.0291$), whereas the differences between Treatments 1 and 2, and Treatments 2 and 4 were not significantly different. Treatment 5 combined the IAEA methodology with a single 0.1 M HCl leach, and as expected, it removed more Sr [57 ± 7 % (2σ)] than the IAEA method (Treatment 4) alone and less Sr than Treatment 3 (**Figure 1**).

Hair subsamples treated with the various cleaning methods produced internally consistent $^{87}Sr/^{86}Sr$ ratios (**Table 4**) with the majority having standard deviations below the ± 0.00004 level. Of the five treatments, Treatment 3 showed the most internal variation with subsamples from two of the three hair samples producing standard deviations greater ± 0.00004 (2σ = 0.00011 and 0.00006). In addition, Treatments 4 and 5 also had one hair subsample with standard deviations greater than ± 0.00004 (2σ = 0.00008 and 0.00008, respectively). Nonetheless, these results indicated that $^{87}Sr/^{86}Sr$ ratios from treated hair subsamples were generally reproducible at ± 0.00004 or better, while treatments that involved weak acidic conditions to leach Sr from hair were ± 0.00011 or better.

Experiment 2.

To investigate the efficiency of hair cleaning methods in removing Sr, we focused on three of the five cleaning methods tested in *Experiment 1*. Shown in **Table 3** are the Sr concentration results of using Treatments 3, 4, and 5 on all twenty-two hair samples collected for this study. For this Experiment, only Treatments 3, 4, and 5 were tested as it was established in Experiment 1 that Treatments 1, 2, and 4 yielded similar Sr concentration results (**Figure 1**). Treatment 4 was selected over Treatments 1 and 2 as it is the hair washing method recommended by the IAEA and has been used extensively in previous studies of other element concentrations. After cleaning, we found hairs washed with Treatment 3 had an average Sr content of 2.8 μg g^{-1} and ranged 0.1 – 14.5 μg g^{-1}; the average and range for hairs washed with Treatment 4 were 14.2 μg g^{-1} and 0.8 - 71.5 μg g^{-1}, respectively, and for hairs washed with Treatment 5 were 6.0 and 0.4 – 22.5 μg g^{-1}, respectively. We found Treatments 3 and 5 removed an average of 62 ± 38 % (2σ) and 46 ± 32 % of the Sr content, while Treatment 4 added an average of 8 ± 57 % Sr to the treated hair (**Table 3**). Of the three cleaning treatments, only hair samples cleaned with Treatments 3 and 5 had significantly different Sr concentrations than the unwashed (control) hair samples ($p = 0.0012$ and 0.0010, respectively). We found the hair samples cleaned with the IAEA-recommended treatment (Treatment 4) did not have significantly different Sr concentrations than the control hair samples ($p = 0.7551$).

Shown in **Table 5** are the $^{87}Sr/^{86}Sr$ ratios of hairs after treatment along with the $^{87}Sr/^{86}Sr$ ratios of the leachates collected during treatment. We found the $^{87}Sr/^{86}Sr$ ratios of treated hair subsamples were not always predictably higher or lower than the untreated hair subsamples.

Table 2. Strontium content of untreated and treated hair samples

Serial Number	Treatment	Replicate A ($\mu g\ g^{-1}$)	Replicate B ($\mu g\ g^{-1}$)	Replicate C ($\mu g\ g^{-1}$)	Average ($\mu g\ g^{-1}$)	SD ($\mu g\ g^{-1}$)	Average Change from Unwashed (%)
1	Untreated (Control)	10.6 ± 0.3	8.3 ± 0.2	16.5 ± 0.4	11.8	4.2	
	1 Water	9.9 ± 0.2	11.2 ± 0.4	12.0 ± 0.3	11.0	1.0	-7
	2 Chloroform:Methanol	12.3 ± 0.3	13.1 ± 0.2	12.6 ± 0.2	12.7	0.4	7
	3 0.1 M HCl	0.6 ± 0.2	1.0 ± 0.2	bdl ± n/a	0.8	0.3	-93
	4 IAEA	10.9 ± 0.2	9.8 ± 0.2	10.9 ± 0.2	10.5	0.6	-11
	5 IAEA + 0.1 M HCl	4.4 ± 0.2	4.8 ± 0.2	4.9 ± 0.2	4.7	0.3	-60
2	Untreated (Control)	58.9 ± 0.7	24.1 ± 0.6	50.5 ± 0.5	44.5	18.2	
	1 Water	40.5 ± 0.7	40.5 ± 0.5	40.6 ± 0.6	40.5	0.1	-9
	2 Chloroform:Methanol	39.5 ± 0.2	38.9 ± 0.2	37.1 ± 0.3	38.5	1.3	-14
	3 0.1 M HCl	2.4 ± 0.2	2.8 ± 0.1	2.4 ± 0.2	2.5	0.2	-94
	4 IAEA	31.4 ± 0.4	35.1 ± 0.2	34.7 ± 0.6	33.7	2.1	-24
	5 IAEA + 0.1 M HCl	23.1 ± 0.1	22.9 ± 0.2	21.4 ± 0.2	22.5	0.9	-49
3	Untreated (Control)	45.7 ± 0.4	44.0 ± 0.6	45.6 ± 0.5	45.1	1.0	
	1 Water	36.2 ± 0.5	36.5 ± 0.3	39.0 ± 0.5	37.3	1.5	-17
	2 Chloroform:Methanol	32.8 ± 0.2	34.0 ± 0.3	34.6 ± 0.2	33.8	0.9	-25
	3 0.1 M HCl	3.4 ± 0.2	3.6 ± 0.2	3.7 ± 0.1	3.6	0.2	-92
	4 IAEA	36.2 ± 0.2	35.7 ± 0.2	35.8 ± 0.2	35.9	0.3	-20
	5 IAEA + 0.1 M HCl	17.3 ± 0.2	17.4 ± 0.2	17.7 ± 0.3	17.4	0.2	-61

*bdl: below detection limit

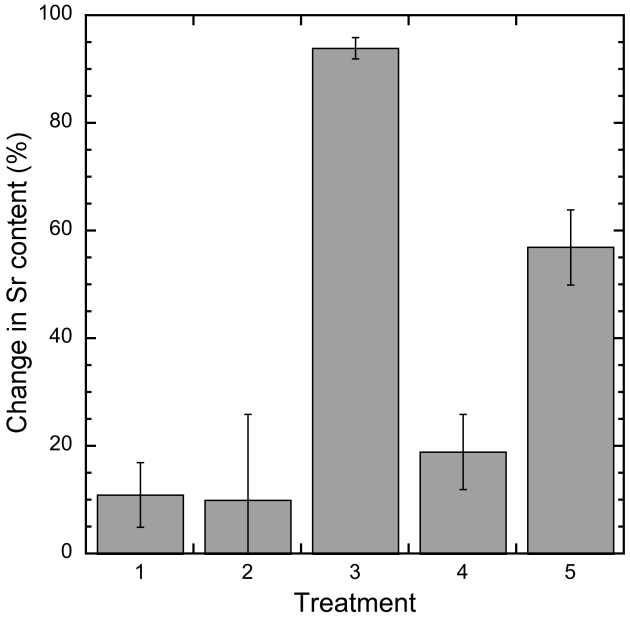

Figure 1. The percent change in the strontium concentration of hair samples after various washing treatments. Percent change was calculated using the difference between the average strontium content in unwashed (control) hairs and hairs treated with the five washing methods used in *Experiment 1*. Error bars show the standard deviation.

Table 3. Strontium content of treated hair samples used in this study and difference between treated and untreated Sr content

	Treatment 3		Treatment 4		Treatment 5	
Serial Number	Sr (µg g^{-1})	% change	Sr (µg g^{-1})	% change	Sr (µg g^{-1})	% change
1	0.9 ± 0.2	-93	10.5 ± 0.3	-11	4.7 ± 0.3	-60
2	2.5 ± 0.3	-94	33.7 ± 0.7	-24	22.5 ± 0.3	-49
3	3.6 ± 0.3	-92	35.9 ± 0.4	-20	17.4 ± 0.4	-61
4	14.5 ± 0.3	7	9.5 ± 0.1	-29	9.6 ± 0.2	-29
5	8.7 ± 0.1	-57	6.6 ± 0.1	-67	3.8 ± 0.1	-81
6	5.3 ± 0.1	-29	6.6 ± 0.2	-12	4.2 ± 0.1	-43
7	0.5 ± 0.1	-51	0.8 ± 0.1	-16	0.9 ± 0.1	-6
8	0.3 ± 0.1	-92	2.7 ± 0.1	-32	0.6 ± 0.1	-86
9	3.8 ± 0.1	-61	7.4 ± 0.5	-26	8.3 ± 0.2	-16
10	3.3 ± 0.1	-54	2.8 ± 0.1	-60	4.4 ± 0.1	-38
11	0.6 ± 0.1	-97	13.7 ± 0.2	-36	0.7 ± 0.1	-97
12	1.7 ± 0.1	-77	19.1 ± 0.2	160	7.0 ± 0.1	-5
13	2.6 ± 0.1	-20	2.2 ± 0.2	-33	4.2 ± 0.2	28
14	0.5 ± 0.2	-33	1.0 ± 0.2	24	0.9 ± 0.2	14
15	2.3 ± 0.2	-87	30.4 ± 0.4	74	19.4 ± 0.2	11
16	bdl ± n/a	n/a	18.2 ± 0.2	58	3.2 ± 0.2	-72
17	0.6 ± 0.3	-73	2.5 ± 0.1	17	1.2 ± 0.3	-45
18	2.0 ± 0.2	-80	20.6 ± 0.2	103	3.0 ± 0.2	-70
19	2.4 ± 0.2	-47	5.1 ± 0.2	14	1.9 ± 0.2	-58
20	1.5 ± 0.2	-96	71.5 ± 0.6	76	13.3 ± 0.2	-67
21	0.8 ± 0.3	10	1.0 ± 0.1	41	0.4 ± 0.4	-50
22	1.0 ± 0.1	-92	10.2 ± 0.1	-24	2.8 ± 0.1	-79
Average	2.8 ± 0.2	-62	14.2 ± 0.2	8	6.1 ± 0.2	-44

Table 4. Strontium isotope ratios of untreated and treated hair samples

Serial Number	Treatment	Replicate A ($^{87}Sr/^{86}Sr$)	Replicate B ($^{87}Sr/^{86}Sr$)	Replicate C ($^{87}Sr/^{86}Sr$)	Replicate Average (SD)	Pooled Leachate ($^{87}Sr/^{86}Sr$)
1	Untreated (Control)	0.71030 ± 0.00002	0.71027 ± 0.00001	0.71023 ± 0.00002	0.71027 ± 0.00003	
	1 Water	0.71032 ± 0.00001	0.71029 ± 0.00001	0.71030 ± 0.00001	0.71031 ± 0.00002	0.71026 ± 0.00002
	2 Chloroform:Methanol	0.71032 ± 0.00001	0.71033 ± 0.00001	0.71030 ± 0.00001	0.71031 ± 0.00002	0.71022 ± 0.00004
	3 0.1 M HCl	0.71064 ± 0.00004	0.71044 ± 0.00003	0.71048 ± 0.00004	0.71052 ± 0.00011	0.71022 ± 0.00002
	4 IAEA	0.71031 ± 0.00001	0.71027 ± 0.00001	0.71030 ± 0.00001	0.71029 ± 0.00002	0.71026 ± 0.00001
	5 IAEA + 0.1 M HCl	0.71037 ± 0.00001	0.71035 ± 0.00002	0.71039 ± 0.00001	0.71037 ± 0.00002	0.71026 ± 0.00001
2	Untreated (Control)	0.71329 ± 0.00001	0.71327 ± 0.00001	0.71328 ± 0.00001	0.71328 ± 0.00001	
	1 Water	0.71317 ± 0.00001	0.71321 ± 0.00001	0.71319 ± 0.00001	0.71319 ± 0.00002	0.71321 ± 0.00001
	2 Chloroform:Methanol	0.71314 ± 0.00001	0.71311 ± 0.00001	0.71315 ± 0.00001	0.71314 ± 0.00002	0.71304 ± 0.00002
	3 0.1 M HCl	0.71333 ± 0.00002	0.71321 ± 0.00001	0.71330 ± 0.00001	0.71328 ± 0.00006	0.71327 ± 0.00004
	4 IAEA	0.71314 ± 0.00002	0.71315 ± 0.00001	0.71312 ± 0.00002	0.71314 ± 0.00002	0.71323 ± 0.00001
	5 IAEA + 0.1 M HCl	0.71339 ± 0.00002	0.71328 ± 0.00001	0.71325 ± 0.00001	0.71331 ± 0.00008	0.71335 ± 0.00001
3	Untreated (Control)	0.71406 ± 0.00001	0.71400 ± 0.00001	0.71402 ± 0.00001	0.71403 ± 0.00003	
	1 Water	0.71379 ± 0.00001	0.71385 ± 0.00002	0.71379 ± 0.00001	0.71381 ± 0.00003	0.71432 ± 0.00001
	2 Chloroform:Methanol	0.71380 ± 0.00002	0.71382 ± 0.00001	0.71386 ± 0.00001	0.71383 ± 0.00003	0.71427 ± 0.00004
	3 0.1 M HCl	0.71276 ± 0.00002	0.71273 ± 0.00002	0.71275 ± 0.00001	0.71275 ± 0.00001	0.71444 ± 0.00002
	4 IAEA	0.71392 ± 0.00001	0.71406 ± 0.00001	0.71393 ± 0.00001	0.71397 ± 0.00008	0.71406 ± 0.00001
	5 IAEA + 0.1 M HCl	0.71350 ± 0.00001	0.71349 ± 0.00001	0.71351 ± 0.00001	0.71350 ± 0.00001	0.71431 ± 0.00001

Table 5. Strontium isotope ratio of treated hair and leachates

Serial Number	Treatment 3		Treatment 4		Treatment 5	
	Hair ($^{87}Sr/^{86}Sr$)	Leachate ($^{87}Sr/^{86}Sr$)	Hair ($^{87}Sr/^{86}Sr$)	Leachate ($^{87}Sr/^{86}Sr$)	Hair ($^{87}Sr/^{86}Sr$)	Leachate ($^{87}Sr/^{86}Sr$)
1	0.71052 ± 0.00004	0.71022 ± 0.00002	0.71029 ± 0.00001	0.71026 ± 0.00001	0.71037 ± 0.00002	0.71026 ± 0.00001
2	0.71328 ± 0.00001	0.71327 ± 0.00004	0.71314 ± 0.00002	0.71323 ± 0.00001	0.71331 ± 0.00001	0.71335 ± 0.00001
3	0.71275 ± 0.00001	0.71444 ± 0.00002	0.71397 ± 0.00001	0.71406 ± 0.00001	0.71350 ± 0.00001	0.71431 ± 0.00001
4	0.71269 ± 0.00001	0.71313 ± 0.00001	0.71273 ± 0.00002	0.71359 ± 0.00002	0.71264 ± 0.00001	0.71324 ± 0.00001
5	0.71257 ± 0.00001	0.71277 ± 0.00001	0.71252 ± 0.00001	0.71343 ± 0.00002	0.71262 ± 0.00003	0.71287 ± 0.00001
6	0.71215 ± 0.00002	0.71170 ± 0.00001	0.71215 ± 0.00001	0.71148 ± 0.00005	0.71218 ± 0.00002	0.71164 ± 0.00002
7	0.70970 ± 0.00006	0.70922 ± 0.00004	0.70931 ± 0.00004	0.71015 ± 0.00011	0.70944 ± 0.00006	0.70884 ± 0.00010
8	0.71129 ± 0.00006	0.71150 ± 0.00001	0.71142 ± 0.00002	0.71136 ± 0.00002	0.71140 ± 0.00004	0.71140 ± 0.00002
9	0.70946 ± 0.00002	0.70950 ± 0.00001	0.70950 ± 0.00001	0.70953 ± 0.00002	0.70949 ± 0.00001	0.70954 ± 0.00001
10	0.71161 ± 0.00002	0.71169 ± 0.00001	0.71166 ± 0.00002	0.71173 ± 0.00002	0.71163 ± 0.00002	0.71172 ± 0.00002
11	0.71148 ± 0.00006	0.71144 ± 0.00001	0.71147 ± 0.00001	0.71147 ± 0.00002	0.71152 ± 0.00004	0.71142 ± 0.00001
12	0.71135 ± 0.00003	0.71118 ± 0.00001	0.71118 ± 0.00001	0.71119 ± 0.00002	0.71130 ± 0.00002	0.71118 ± 0.00001
13	0.71289 ± 0.00002	0.71225 ± 0.00001	0.71280 ± 0.00002	0.71165 ± 0.00002	0.71283 ± 0.00002	0.71193 ± 0.00002
14	0.71219 ± 0.00005	0.71264 ± 0.00009	0.71225 ± 0.00006	0.71287 ± 0.00016	0.71213 ± 0.00006	0.71270 ± 0.00011
15	0.71329 ± 0.00003	0.71335 ± 0.00001	0.71329 ± 0.00001	0.71344 ± 0.00004	0.71329 ± 0.00001	0.71343 ± 0.00001
16	0.71510 ± 0.00019	0.71463 ± 0.00001	0.71459 ± 0.00001	0.71466 ± 0.00006	0.71460 ± 0.00002	0.71465 ± 0.00001
17	bdl ± n/a	0.71432 ± 0.00002	0.71352 ± 0.00002	0.71486 ± 0.00011	0.71355 ± 0.00006	0.71459 ± 0.00002
18	0.71046 ± 0.00003	0.71046 ± 0.00001	0.71043 ± 0.00001	0.71043 ± 0.00002	0.71039 ± 0.00002	0.71038 ± 0.00001
19	0.71336 ± 0.00004	0.71313 ± 0.00002	0.71303 ± 0.00002	0.71309 ± 0.00002	0.71261 ± 0.00004	0.71316 ± 0.00002
20	0.71171 ± 0.00004	0.71151 ± 0.00001	0.71153 ± 0.00001	0.71150 ± 0.00002	0.71161 ± 0.00001	0.71148 ± 0.00001
21	0.71228 ± 0.00004	0.71278 ± 0.00008	0.71188 ± 0.00004	0.71224 ± 0.00010	0.71197 ± 0.00009	0.71230 ± 0.00007
22	0.71018 ± 0.00005	0.71091 ± 0.00001	0.71083 ± 0.00002	0.71106 ± 0.00002	0.71044 ± 0.00002	0.71096 ± 0.00001

*bdl: below detection limit

Experiment 3.

To investigate the role of diet in understanding endogenous and exogenous Sr sources, we used paired hair samples and dietary surveys collected from school-age volunteers. The majority of samples were from female volunteers as these volunteers had more hair and the collected hair samples could be concealed. Hair samples had been previously analyzed for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios, which had been successfully used to investigate different dietary inputs. The $\delta^{13}C$ and $\delta^{15}N$ values of the samples we used in *Experiment 3* ranged from -19.6 to -15.6 ‰ and from 8.1 to 9.6 ‰, respectively, and were related to culturally driven diet differences. As seen in **Figure 2**, both $\delta^{13}C$ ($p = 0.0001$) and $\delta^{15}N$ ($p = 0.0003$) of Caucasians were lower than those of Hispanics. (Only Caucasians and Hispanics were compared due to limited sample density for other ethnicities.) We found Sr concentrations in these hair samples ranged 0.08 – 27.5 μg g^{-1} (**Figure 3**), but there were no distinguishable differences in Sr content between the sexes or ethnicities.

We found that the $^{87}Sr/^{86}Sr$ ratios of hair of used in this experiment ranged from 0.70910 to 0.71509. There was no discernable relationship between $^{87}Sr/^{86}Sr$ and age (**Figure 4a**). In addition, there was no relationship between ethnicity/diet and $^{87}Sr/^{86}Sr$ of hair (**Figure 4b**). While no relationship between $^{87}Sr/^{86}Sr$ value of hair and ethnicity/diet was observed, we did observe a relationship between $^{87}Sr/^{86}Sr$ value of hair and collection location (**Figure 5a**).

Experiment 4.

We collected tap water nearby the hair sampling locations used in *Experiment 3* to more fully investigate the link between Sr isotope ratios of hair and geography (location) (**Figure 6**). The $^{87}Sr/^{86}Sr$ ratios of tap waters from Salt Lake City ranged from 0.70849 to 0.71399 (**Figure 5b**). The overall range of student's hair (measured in *Experiment 3*) and Salt Lake City tap water $^{87}Sr/^{86}Sr$ values were similar.

Experiment 5.

To combine Sr isotope data with O isotope data, we measured the hair samples collected in *Experiment 3* and the water samples collected in *Experiment 4* for $\delta^{18}O$ values. We found Salt Lake City hair samples had an average value of 9.9 ± 0.8 ‰ with four samples were removed as outliers. There was no relationship between $\delta^{18}O$ values of hair and collection location (**Figure 7**). Using these measured $\delta^{18}O$ values of hair and the model developed by Ehleringer et al. [40], we predicted $\delta^{18}O$ values of tap water with a range of -17.0 to -12.5 ‰ (**Figure 8**). Shown in **Figure 8** is the distribution of measured tap water $\delta^{18}O$ values. We found the $\delta^{18}O$ values of Salt Lake Valley tap waters had an average value -16.3 ± 0.2 ‰.

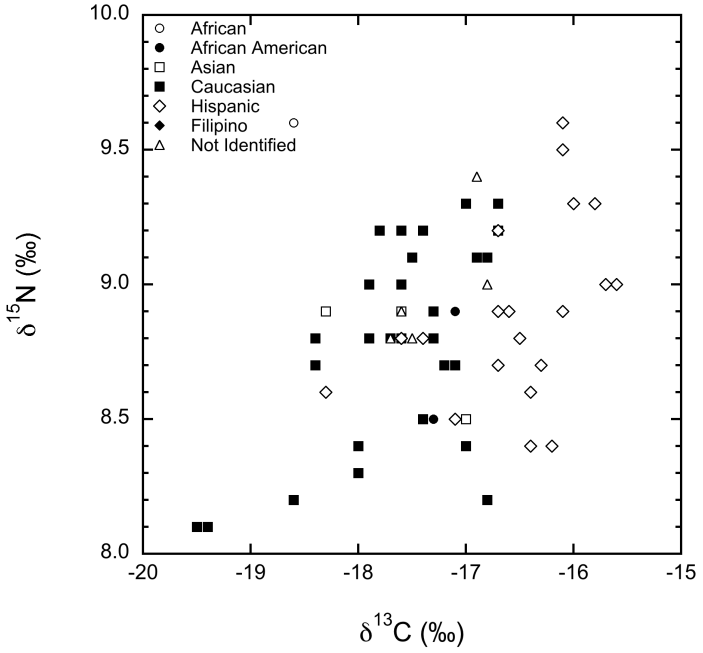

Figure 2. $\delta^{13}C$ and $\delta^{15}N$ values of hair collected in *Experiment 3*. Symbols represent self-assigned ethnicities.

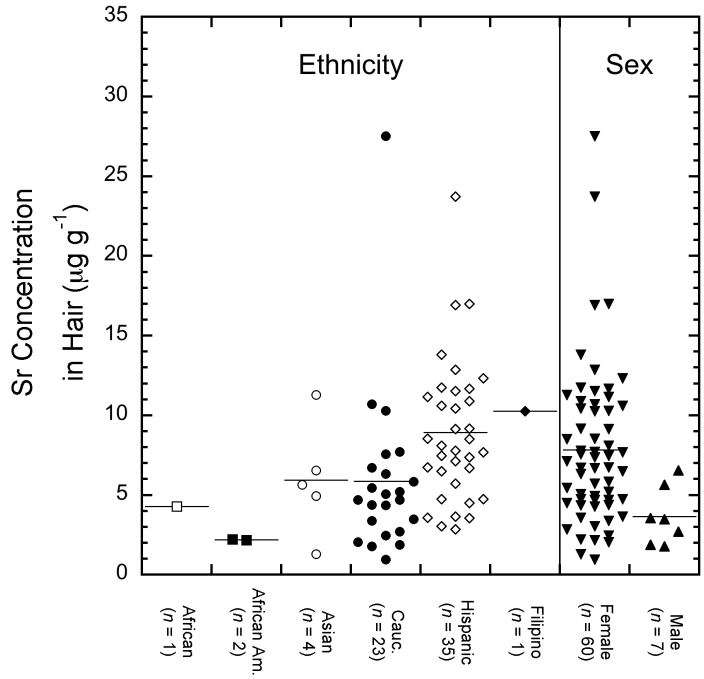

Figure 3. Sr concentration of hair collected in *Experiment 3*, sorted by ethnicity and sex. Lines represent mean of measurements.

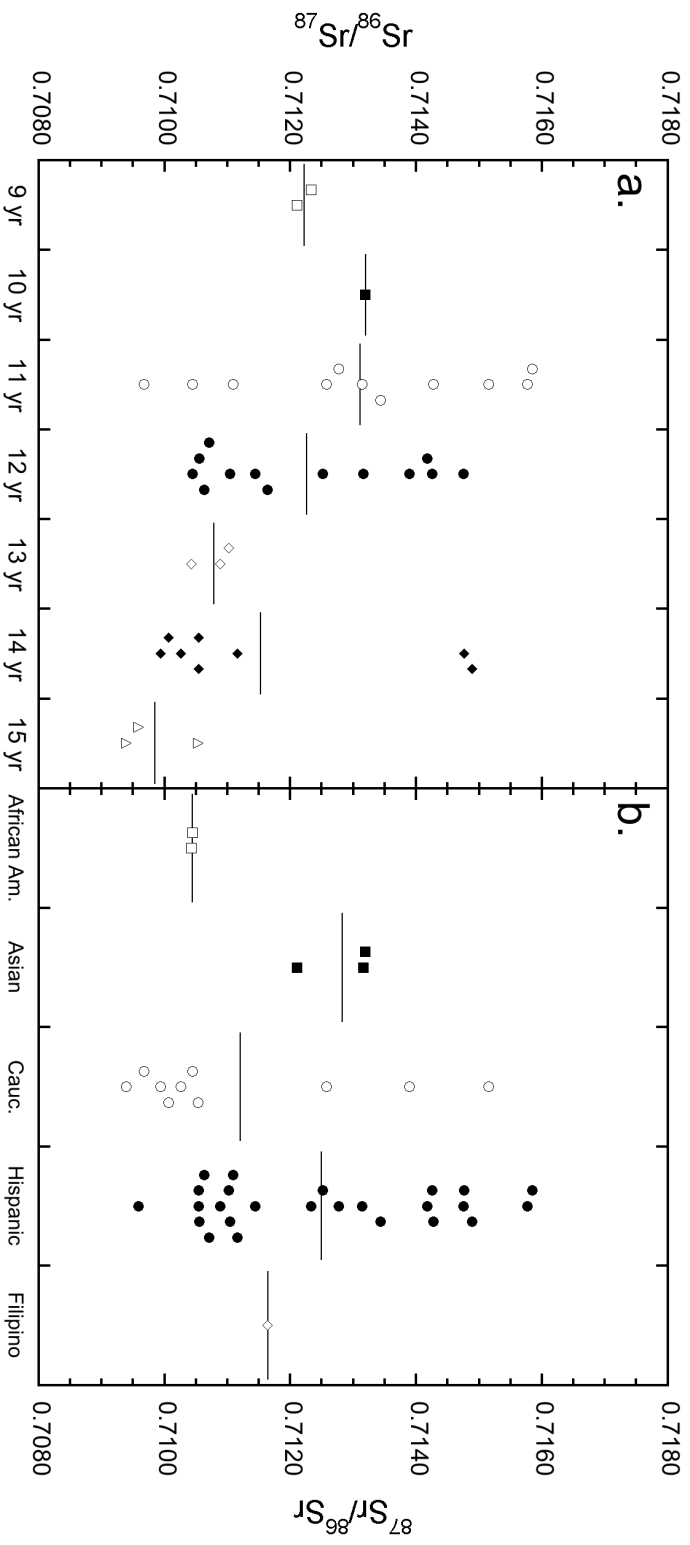

Figure 4. $^{87}Sr/^{86}Sr$ ratios of hair collected in *Experiment 3*, sorted by sex (**a.**) and ethnicity (**b.**). Lines represent mean of measurements.

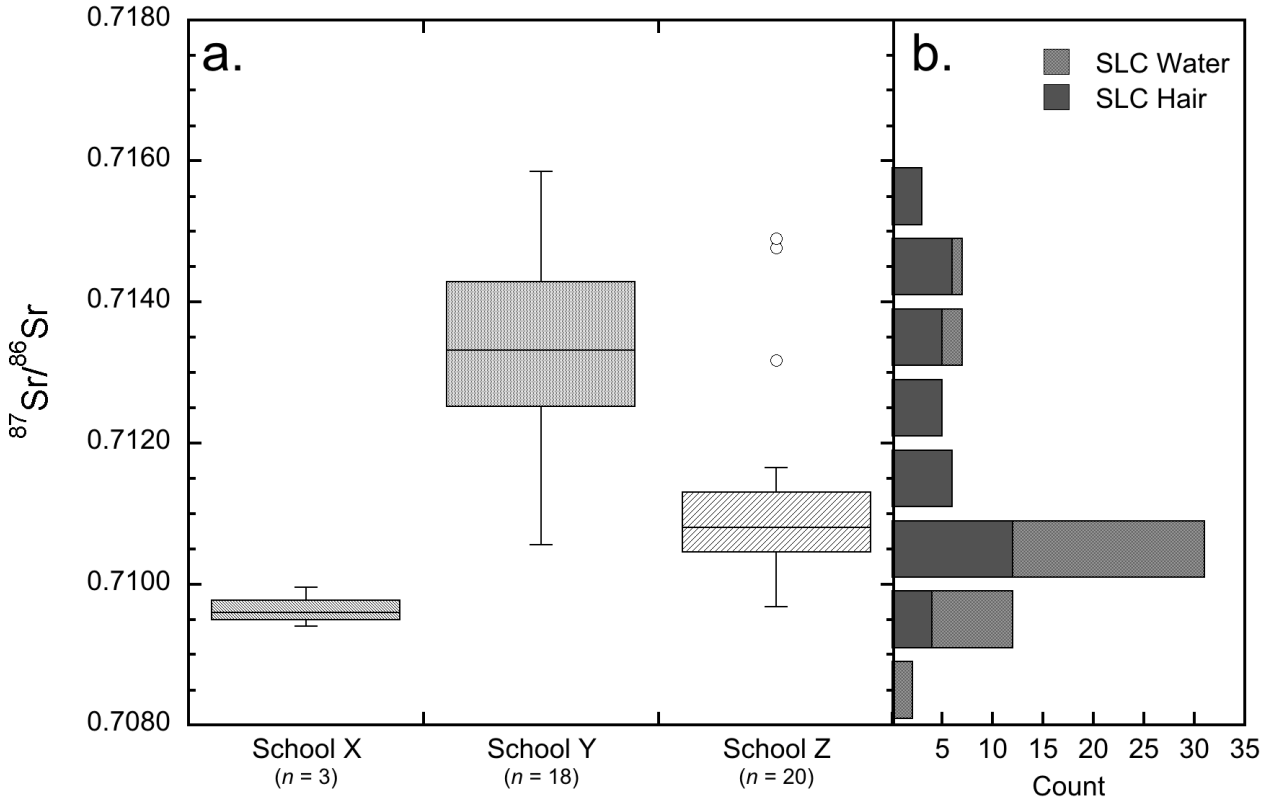

Figure 5a. Box and whisker plot of the $^{87}Sr/^{86}Sr$ ratios of hair used in *Experiment 3* sorted by school (collection location). Top and bottom of box represent 1st and 3rd quartile, and the whiskers indicate the 1.5 interquartile range. Outliers are noted by open circles. (**b.**) Histogram of $^{87}Sr/^{86}Sr$ ratios of hair and water collected in *Experiments 3* and *4*. Data were collected during four sampling campaigns (summer 2012, fall 2012, winter 2012 and spring, 2013) and combined.

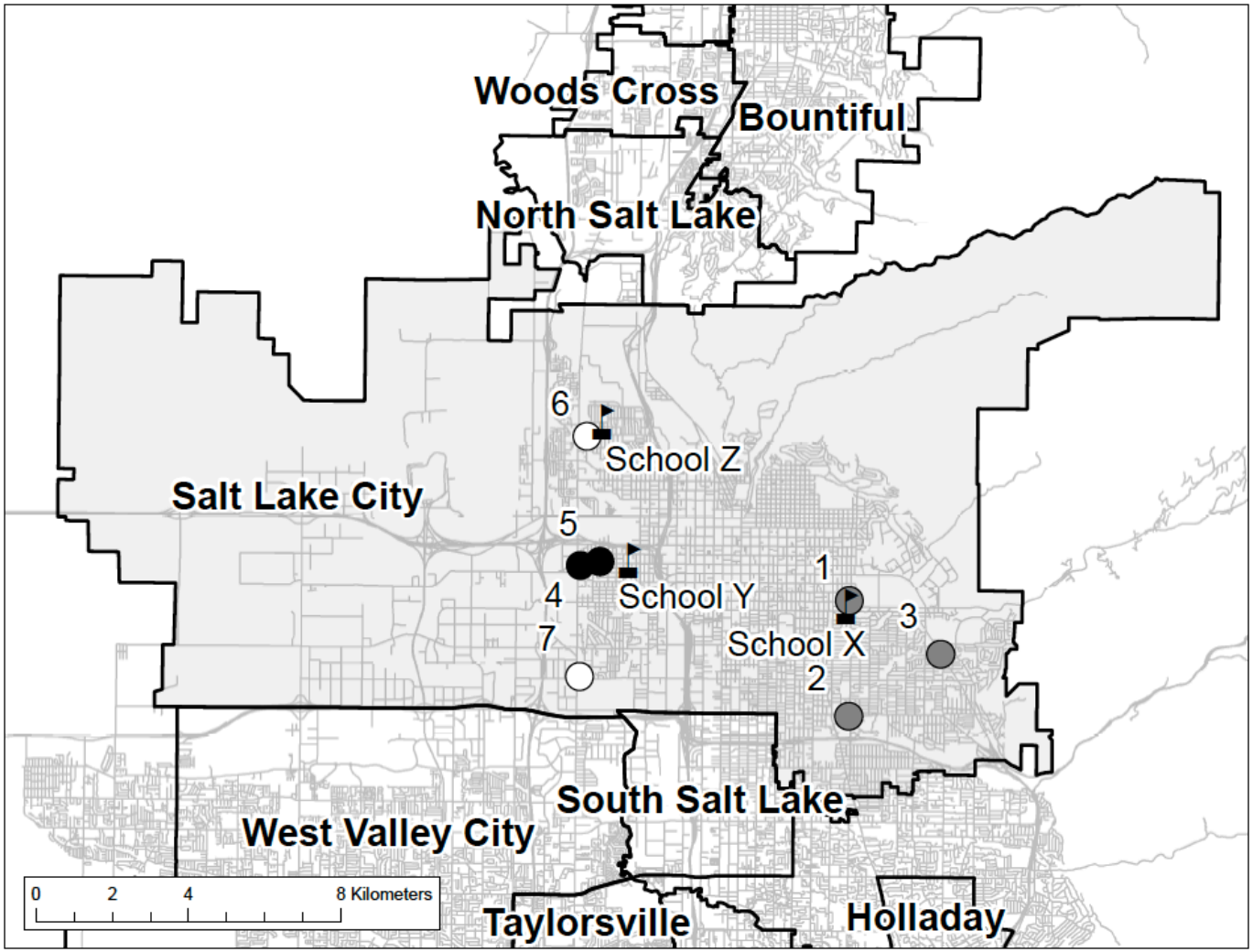

Figure 6. Map of collection sites for hair and water samples from *Experiments 3* and *4*. Sampling locations (schools) are shown with schoolhouse symbol. Dots represent water collection locations. Dot coloring indicates similar isotopic behavior.

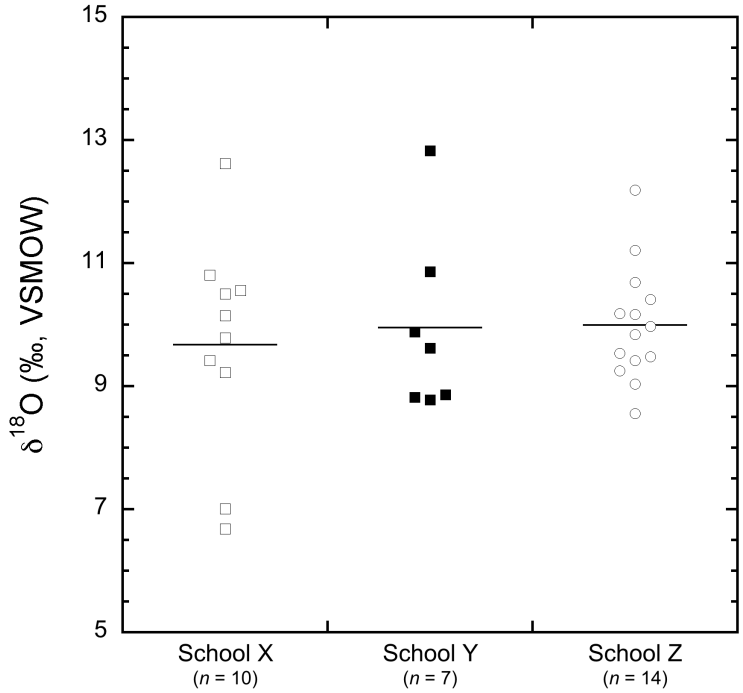

Figure 7. $\delta^{18}O$ values of hair collected in *Experiment 5*, sorted by collection location. Lines represent mean of measurements. All sample were collected in Salt Lake City, Utah.

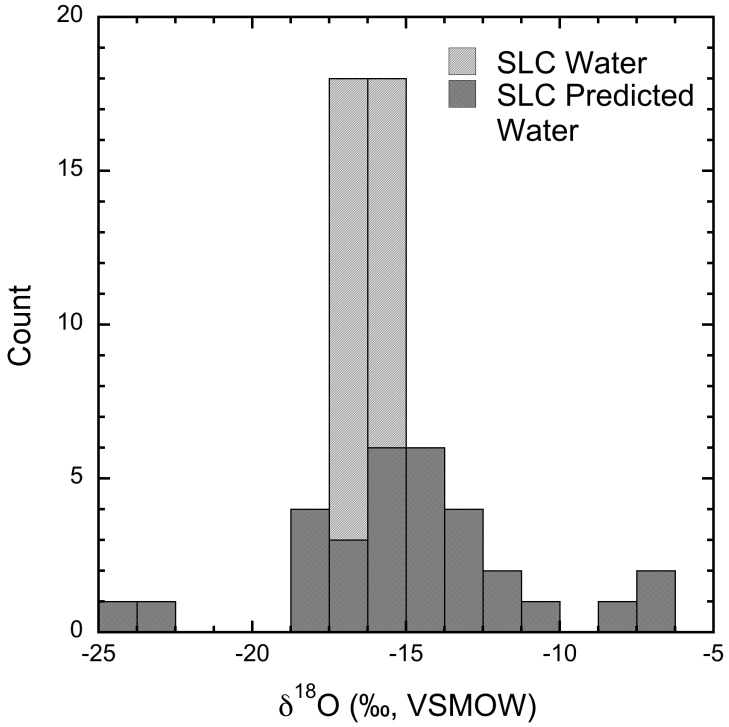

Figure 8. Histogram of $\delta^{18}O$ values of measured (light grey) and predicted (dark grey) Salt Lake City tap water from *Experiment 5*.

Experiment 6.

To capture seasonal variability in $^{87}Sr/^{86}Sr$ values, we collected tap waters in the summer, fall, winter, and spring seasons (July 2012, October 2012, January 2013, and April 2013, respectively) within the Salt Lake City, Utah metropolitan area. We found tap water $^{87}Sr/^{86}Sr$ values ranged from 0.70940 to 0.71227, 0.70956 to 0.71475, and 0.70849 to 0.71343 for the summer, fall, and winter collection intervals, respectively. We noted clear spatial patterns with several regions characterized as having more radiogenic $^{87}Sr/^{86}Sr$ values (**Figures 9a-c**).

Experiment 7.

We expanded our investigation of linkages between Sr isotope ratio and geography from the local/regional scale to the national scale in *Experiment 7*. One hundred nine hair samples and the corresponding leachates were digested for Sr isotope analysis and analyzed for Sr content. Results suggest similar relationships between hair and water $^{87}Sr/^{86}Sr$ ratios as observed in *Experiments 3, 4,* and *6*. We found an average of 0.71061 and 0.71054 with ranges from 0.70760 to 0.71962 and 0.70758 to 071918 for hair and leachate samples, respectively. Generally, hair and leachates were strongly correlated (**Figure 10**). In addition, we identified twenty-one samples with evidence of either movement or changes in water $^{87}Sr/^{86}Sr$ ratios by comparing the isotopic difference between hair and leachate. Hair and leachate pairs with a difference of 0.0004 or more for $^{87}Sr/^{86}Sr$ ratios were removed from the larger population (**Figure 10**). Further, seven samples were removed from the population due to measured variations in water $^{87}Sr/^{86}Sr$ ratios (**Figure 10**).

Experiment 8.

Prior to relating isotope values along the length of a hair, we first studied if transverse isotopic variation across the hair was present. We used the data collected for *Experiments 1* and *2*. We found the $^{87}Sr/^{86}Sr$ ratios of treated hair may or may not vary with increasing aggressiveness of treatment and the removal of increasing amounts of Sr. Shown in **Figure 11** are the Sr isotope ratio profiles of 3 selected hair samples.

To study if travel movements and consequential changes in $^{87}Sr/^{86}Sr$ ratio of water are reflected along the length of a hair and the hair's $^{87}Sr/^{86}Sr$ ratio, we used hair from a horse that was transported from Brazil to Utah. In addition, we compared the transported horse to its stable-mate that did not move from Utah during the interval in that the transported horse moved. We found the horsehair had a Sr content range from 3.3 to 29.6 $\mu g\ g^{-1}$ and 1.5 to 3.8 $\mu g\ g^{-1}$ for the stationary and transported horses, respectively. We observed the concentration of Sr increased along the length of both horsehairs (**Figure 12**). Isotope data will be collected in the coming month.

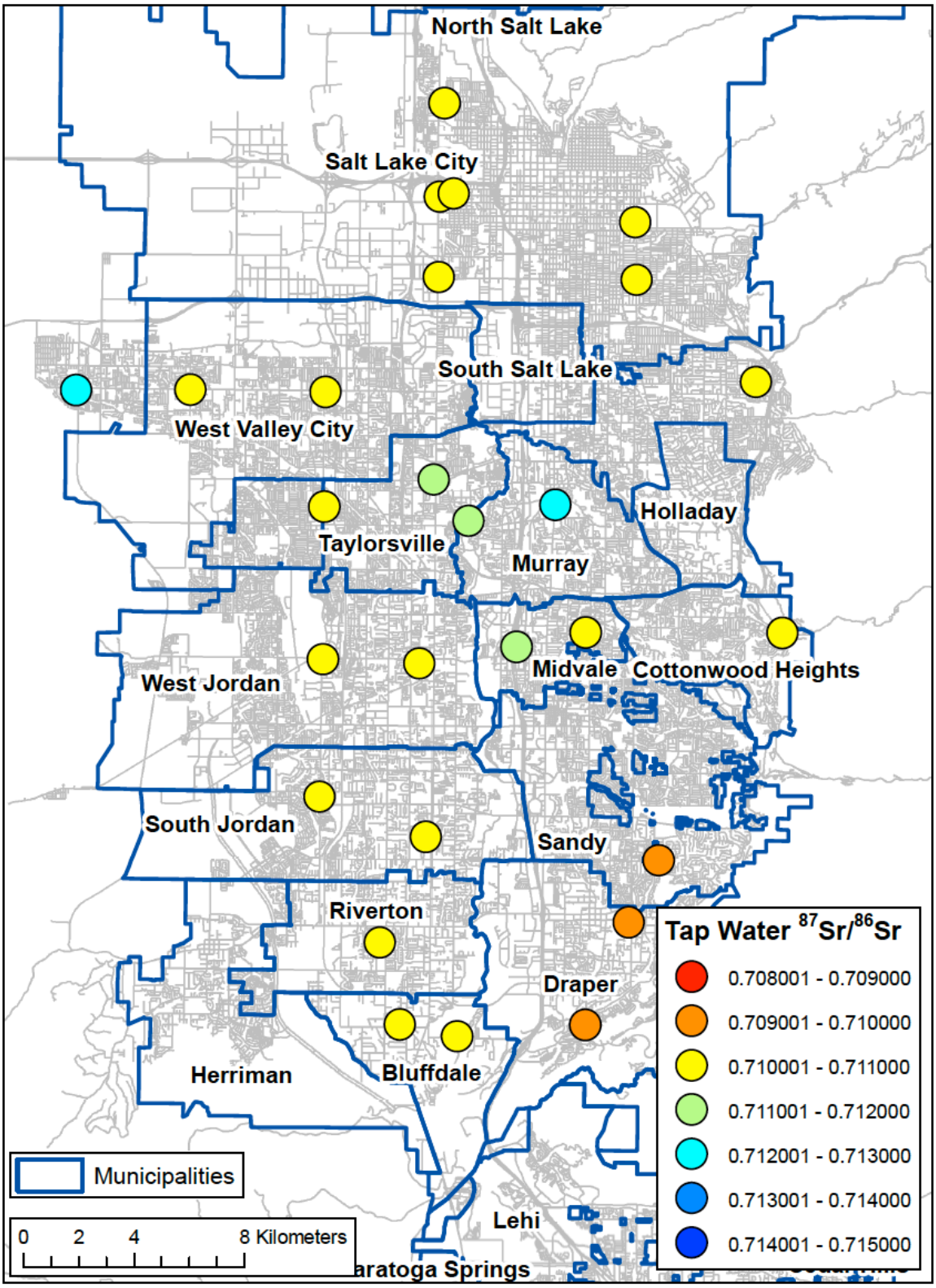

Figure 9a. Spatial projection of the $^{87}Sr/^{86}Sr$ ratio of tap water throughout the Salt Lake Valley, Utah during the summer (June) of 2012.

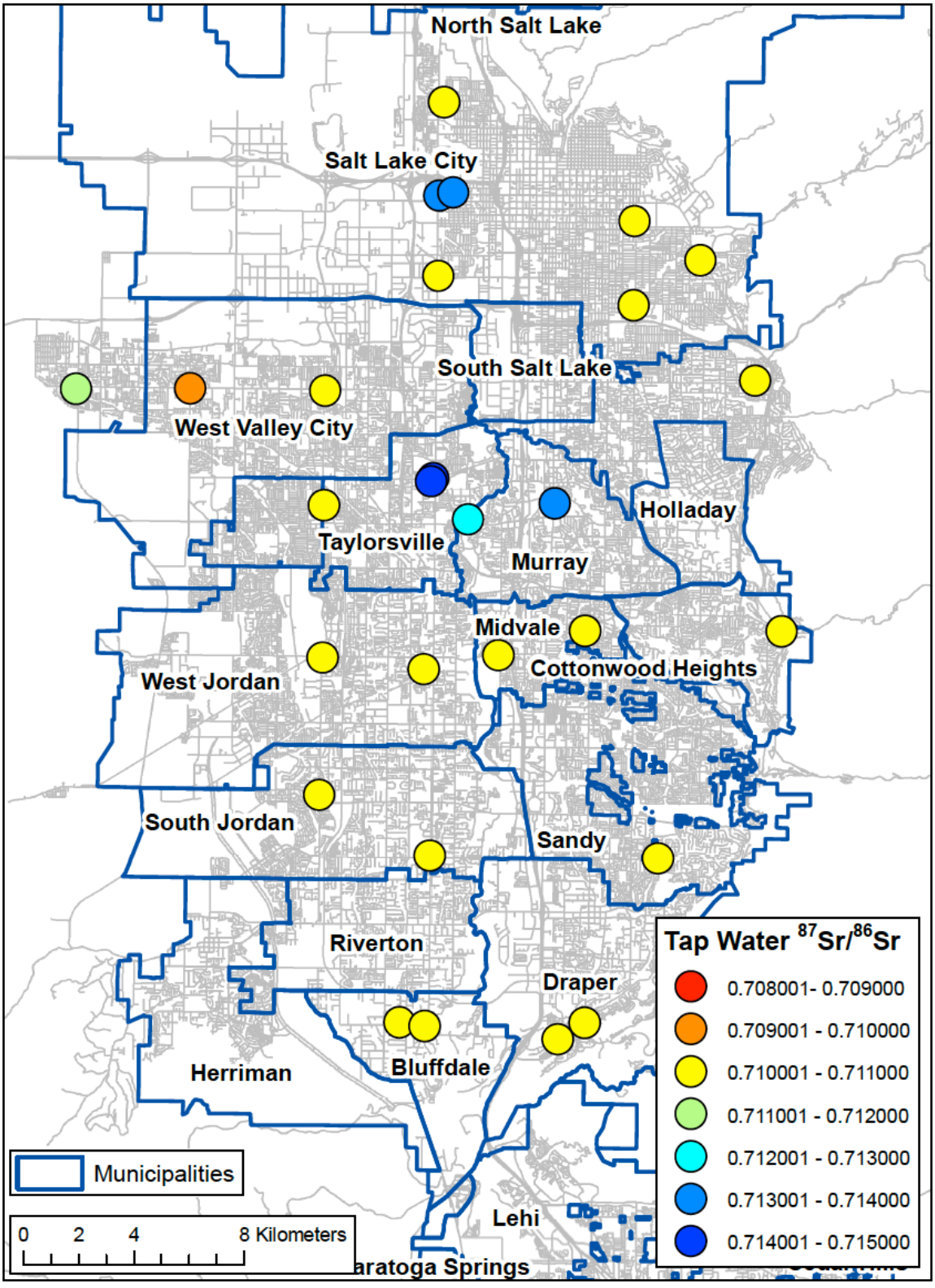

Figure 9b. Spatial projection of the $^{87}Sr/^{86}Sr$ ratio of tap water throughout the Salt Lake Valley, Utah during the fall (October) of 2012.

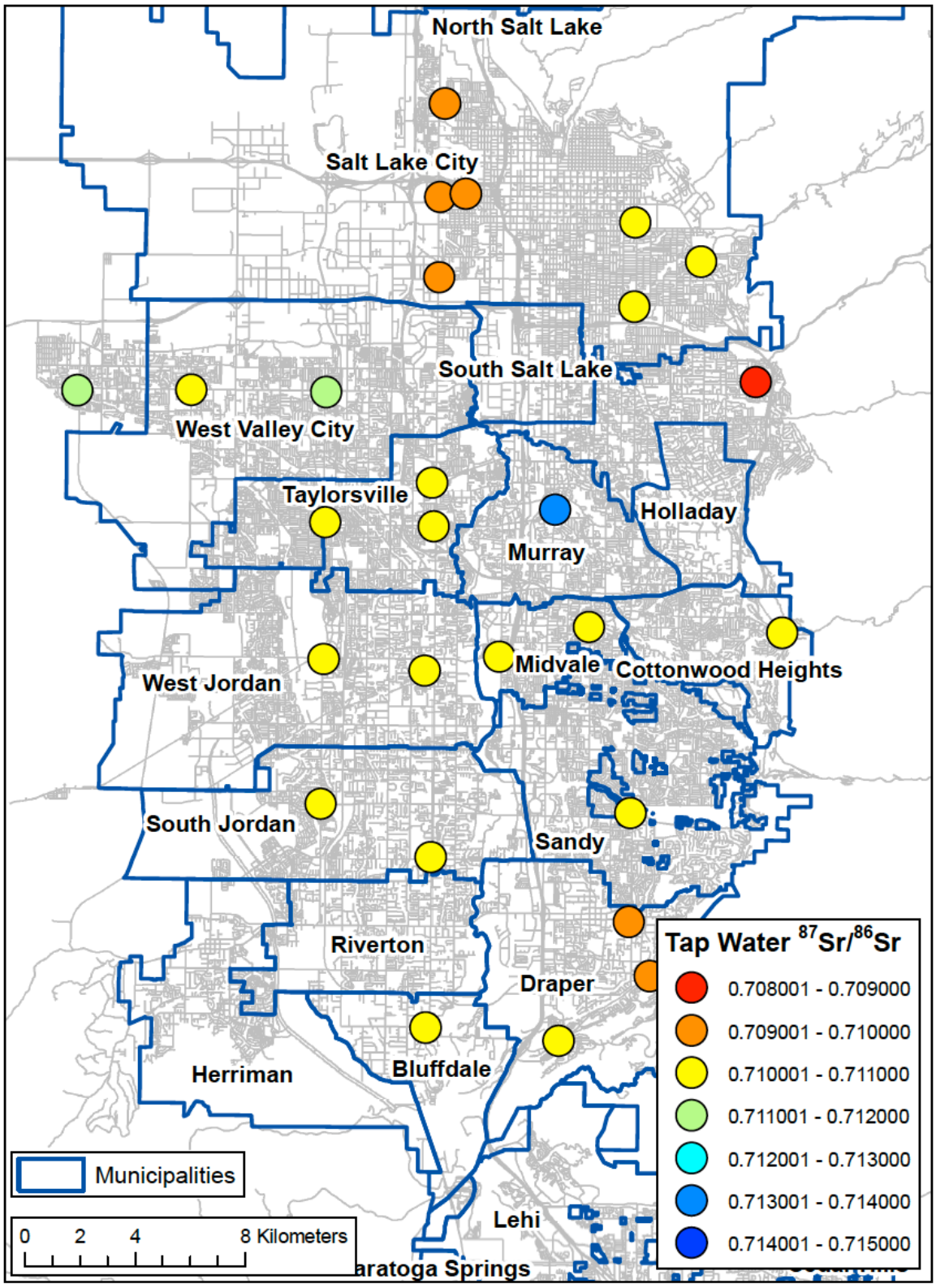

Figure 9c. Spatial projection of the $^{87}Sr/^{86}Sr$ ratio of tap water throughout the Salt Lake Valley, Utah during the winter of 2013.

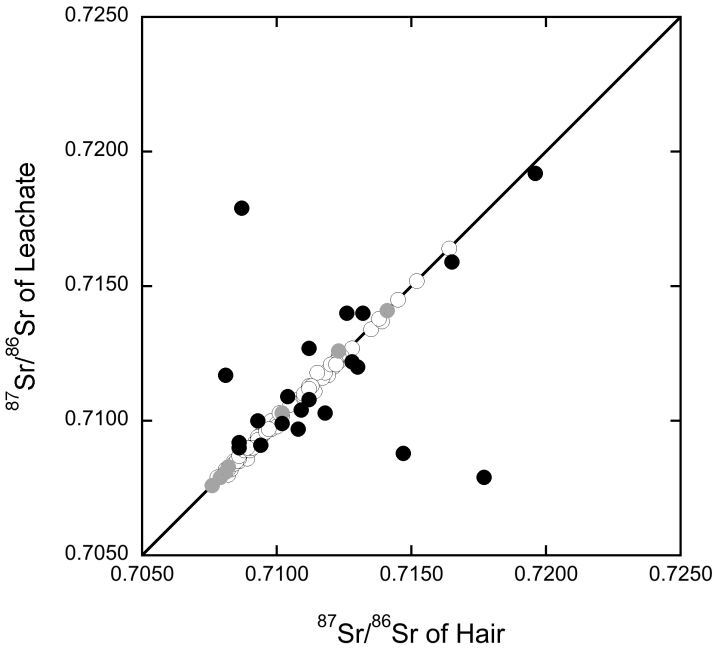

Figure 10. Cross plot of $^{87}Sr/^{86}Sr$ ratio of hair against $^{87}Sr/^{86}Sr$ ratio of leachate. Open symbols represent samples with hair-leachate differences less than 0.0004, black closed symbols represent samples with hair-leachate differences greater than 0.0004, grey closed symbols indicate samples from locations with known variability in water $^{87}Sr/^{86}Sr$ ratio. The 1:1 line is shown.

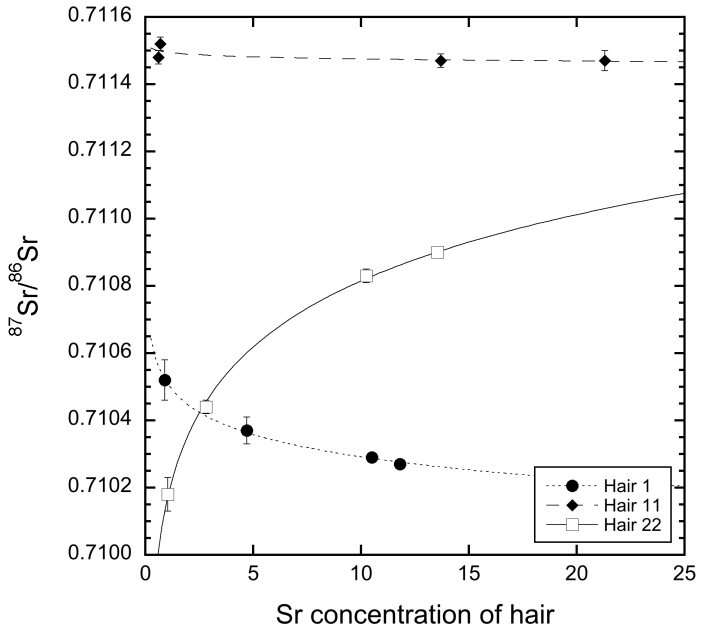

Figure 11. The variation in strontium isotope ratios of treated hair as a function of strontium concentration remaining in the hair. Three strontium isotope-concentration profiles of hair samples are shown, as these profiles are representative of those observed in the larger dataset. Trend lines are shown. Error bars are the standard deviation of the individual strontium isotope analysis.

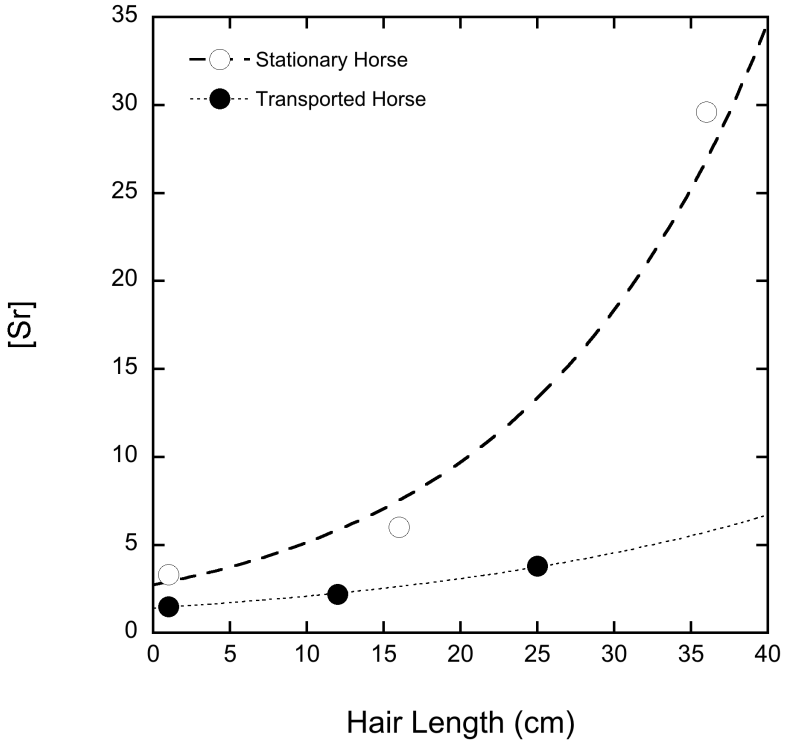

Figure 12. Sr concentration in horsehair along the length. Closed and open symbols represent transported horse and stationary horse, respectively.

Discussion and Conclusions

Experiments 1 and 2.

Our findings from *Experiment 1* and *2* show that regardless of total Sr removed by each washing method, replicate applications of an individual cleaning protocol removed a consistent fraction of Sr from human hair. In all cases except one, the three independent cleanings of subsamples of an individual hair sample yielded standard deviations less than 10 % of the average Sr concentration (**Table 2**). The untreated subsamples of each hair sample generally had a larger standard deviation than the three treated subsamples (**Table 2**). The larger variation in Sr concentration among untreated subsamples relative to treated subsamples was likely due to differential surface contamination on the untreated hair. Thus, we conclude that – regardless of the exact cleaning method used – washing hair prior to Sr analysis consistently removed any surface contamination and a fraction of the total Sr in a hair sample. This consistency provides increased fidelity in the application of any specific cleaning method to isolate Sr fractions within and on the surface of human hair.

We found cleaning method Treatment 3 removed the most Sr content from hair (**Figure 1**) and yielded Sr contents (**Table 3**) most similar to newly erupted human hair (e.g., 0.75 to 1.20 $\mu g\ g^{-1}$) [43, 44]. It has been argued that newly erupted human hair reflects only the endogenous Sr content, as the hair has not been subjected to the exogenous Sr sources [45, 46]. These data suggested that Treatment 3 was the best cleaning method for separating endogenous from exogenous Sr fractions in human hair.

Treating hair with organic solvents and acidic solutions altered the $^{87}Sr/^{86}Sr$ ratios. Differences in the $^{87}Sr/^{86}Sr$ ratios between untreated and treated hair subsamples indicated treatment methods could affect the resultant isotope ratio. We found that in most cases, the differences between the $^{87}Sr/^{86}Sr$ ratios of untreated and treated hairs were distinct within measurement uncertainty (**Tables 4** and **5**), thus prior cleaning method must be considered when comparing hair $^{87}Sr/^{86}Sr$ ratio data.

Finally, we found that Treatment 3 (0.1 M HCl) was the most aggressive washing method tested, leaving the lowest Sr concentrations in the cleaned hair. We conclude that hair cleaned with Treatment 3 is representative of the endogenous Sr signal, while the leachate recovered represents the *integrated* exogenous Sr signal. To constrain the more *recent* exogenous Sr signal, we conclude that the leachates from hair washed with Treatment 5 (IAEA + 0.1 M HCl) are likely more representative of near-term Sr exposure. If a bulk hair Sr signal is needed, we suggest Treatments 1, 2, or 4 are equivalent and analysis of the treated hair sample would capture a combined exogenous and endogenous Sr signal. Thus, to understand dietary Sr inputs, hair treated with Treatment 3 is likely the most useful, and leachates from Treatments 3 and 5 are the most appropriate for geospatial applications and forensics practices.

The findings and conclusions from *Experiments 1* and *2* allowed us to meet Research Objectives 1 and 2. Furthermore, data from *Experiments1* and *2* supported Hypothesis 1.

Experiment 3.

As sources of Sr in hair are both endogenous (i.e., diet, drinking water) and exogenous (i.e., dust, pollution, etc.), in *Experiment 3* we tested if the endogenous contributions of Sr subjugated the resulting isotope values by controlling for age, sex, sample location, ethnicity, and diet. Nutritional surveys and independent investigation of dietary patterns using the carbon and nitrogen stable isotope analysis of hair supported significant dietary differences between the ethnic groups, potentially suggesting differing endogenous dietary contributions of Sr may be expected. However, we found no relationship between ethnicity and $^{87}Sr/^{86}Sr$ value of hair (**Figure 4b**), indicating the endogenous Sr isotopic signal was not large enough to impact the $^{87}Sr/^{86}Sr$ ratios measured for hair.

We observed a significant association between $^{87}Sr/^{86}Sr$ value of hair and collection location in *Experiment 3*. As individuals lived close to each other and thus were likely exposed to similar bedrock, dust, and environmental contaminates, we had not predicted a relationship between $^{87}Sr/^{86}Sr$ ratios and collection location, due to the close proximity of collection sites. From these results, we conclude another endogenous or exogenous Sr source was affecting the overall $^{87}Sr/^{86}Sr$ ratios of human hair. Possible explanations for location-specific differences in $^{87}Sr/^{86}Sr$ values of hair on a local/regional level include: (1) there are subtle variations in dust delivery or pollution to different regions of the city; (2) drinking water may contribute more endogenous Sr to hair than expected; (3) contamination from hair styling products; and/or (4) bathing water may also contribute to the Sr isotope signal in hair.

Previous research supports greater exogenous Sr importance to hair elemental

composition and the individuals used for hair collection in *Experiment 3* all lived within 10 miles of each other and were likely exposed to similar dust environments. We thus considered exposure to bathing/showering water as the most likely exogenous source of Sr to hair. This hypothesis was explored in *Experiment 4*.

The findings and conclusions from *Experiment 3* were used to fulfill Research Objectives 3. Furthermore, *Experiment 3* added further support to Hypothesis 1.

Experiment 4.

Building off of the findings and conclusions of *Experiment 3*, *Experiment 8* (see, **Figure 11**), and previous research, we considered tap water as the likely exogenous source of Sr in human hair through exposure from bathing or showering. Given that Sr content in hair increases with exposure [25], therefore exogenous source must contribute additional Sr relative to endogenous Sr sources. We suggest that environmental contributions of Sr atoms to hair keratin are derived exogenously from bathing water, where mobile Sr atoms are deposited on the surface of hair through repeated washing [25]. Hair is porous due to its inherent structure and response to wetting and heating [47]; thus exogenous Sr atoms from bathing and showering migrate into the hair structure, rather than remaining only at the surface. Considering an individual's repeated exposure to tap waters through regular bathing, tap water-derived Sr may be the most important exogenous Sr source to human hair and a critical parameter to constrain for the increased development and application of $^{87}Sr/^{86}Sr$ ratios of human hair in region-of-origin assessment is the $^{87}Sr/^{86}Sr$ ratios of municipal tap waters.

Shown in **Figure 5b** is a histogram of Salt Lake City tap water and hair $^{87}Sr/^{86}Sr$ ratios. We found individuals with larger $^{87}Sr/^{86}Sr$ ratios lived in locations that had larger $^{87}Sr/^{86}Sr$ ratios of tap water. From this, we conclude exposure to tap water through bathing or showering was the strongest driver of hair $^{87}Sr/^{86}Sr$ ratios in the Salt Lake City, Utah.

The findings and conclusions from *Experiment 4* were used to fulfill Research Objectives 3. Furthermore, *Experiments 4* provided additional support to Hypotheses 1 and 2.

Experiment 5.

In addition to measuring $^{87}Sr/^{86}Sr$ ratios of Salt Lake City tap water and hair, we also analyzed the $^{18}O/^{16}O$ ratios of these materials. This additional analysis provides a foundation for the increased application of the combined $^{87}Sr/^{86}Sr$-$\delta^{18}O$ isoscape development. Here, we found Salt Lake City hair samples had an average $\delta^{18}O$ value of 9.9 ‰ (**Figure 7**).

We found the $\delta^{18}O$ values of Salt Lake City tap waters range had an average value -16.3 ‰ (**Figure 8**). Using the measured $\delta^{18}O$ values of hair, we predicted $\delta^{18}O$ values of tap water with a range of -17.0 to -12.5 ‰ using the model developed by Ehleringer et al. [40]. The variation between measured $\delta^{18}O$ values of water and predicted $\delta^{18}O$ values of

water are likely due to dietary and activity differences between the volunteers. In short, the hair model of Ehleringer et al. predicted Salt Lake City tap water $\delta^{18}O$ values within uncertainty (**Figure 8**).

We found when $^{87}Sr/^{86}Sr$ and $\delta^{18}O$ ratios of hair were paired, the additional variable ($^{87}Sr/^{86}Sr$) distinguished individuals with similar $\delta^{18}O$ values. From this, we conclude that by coupling Sr and O isotope ratios of hair additional information is garnered for region-of-origin assessment.

Experiment 5 was used to develop further research questions and assess the feasibility of Research Objective 5.

Experiment 6.

Considering the relationship between hair $^{87}Sr/^{86}Sr$ ratio and sampling location we observed in *Experiment 3*, we considered tap water as the additional source of Sr to hair and found in *Experiment 4* striking linkages between $^{87}Sr/^{86}Sr$ ratios of hair and tap water indicating the predominate Sr signal incorporated into the hair was from municipal water. While these linkages between the $^{87}Sr/^{86}Sr$ ratios of tap water and hair were clear, we knew little of the spatial and temporal variability of the $^{87}Sr/^{86}Sr$ ratios of tap water within the Salt Lake metropolitan area. Previously, we noted differences in the $^{87}Sr/^{86}Sr$ value of two tap waters from the east and west sides of the Salt Lake Valley. Given this, we undertook a seasonal sampling campaign to collect tap waters throughout the Salt Lake Valley at four intervals during yearly seasonal cycle. The initial sampling took place in June 2012 and was comprised of 27 collection locations from 15 municipalities within the Salt Lake Valley. We found tap water $^{87}Sr/^{86}Sr$ ratios ranged from 0.70940 to 0.71227 for this collection interval with spatial patterns apparent (**Figure 9a**). We found several regions with more radiogenic $^{87}Sr/^{86}Sr$ ratios.

We found tap water $^{87}Sr/^{86}Sr$ ratios for the fall and winter collection interval also had clear spatial patterns (**Figure 9b and c, respectively**). Similar to the June sampling, we found several "hot spots" with more radiogenic $^{87}Sr/^{86}Sr$ ratios. These additional sampling periods captured nearly the entire range of values as observed in $^{87}Sr/^{86}Sr$ ratios of hair from Salt Lake City (**Figure 4**).

In the Salt Lake Valley there are three major source waters (local groundwater, local Wasatch Mountains snowmelt, transported Uinta Mountains waters) and each municipality within the Salt Lake Valley area uses a different combination of these sources as they provide water to their residents. Municipal water sources vary seasonally and their $^{87}Sr/^{86}Sr$ ratios vary according to the source utilized. As an example, approximately 85-90 % of the municipal water used in Salt Lake City derives from surface waters with the other 10-15 % coming from ground water sources [48]. Facility managers often change from surface water sources to ground water sources in the late summer/early fall depending on winter snowpack and reservoir levels [48].

As each water source has a distinct $^{87}Sr/^{86}Sr$ ratio, individuals living in different municipalities can be distinguished from one another based on the $^{87}Sr/^{86}Sr$ ratio of their hair. We found that within a single municipality (Salt Lake City), different areas of the city received isotopically different waters during different times of the year (**Figure 13**). These patterns help explain the data from *Experiments 3* and *4*. The more radiogenic $^{87}Sr/^{86}Sr$ ratios likely derive from ground water sources. Murray City relies exclusively on ground water sources and Murray City waters are consistent with these more radiogenic Salt Lake City waters (**Figure 9a-c**). However, to interpret the $^{87}Sr/^{86}Sr$ ratios of waters and hair at this scale, knowledge of a municipality's water distribution system is required. While differences between municipalities within a single metropolitan area increases the complexity of a interpretation, the added data layer of water distribution system actually provides a new and critical piece of information for region-of-origin assessment and greatly increases the fidelity of predictions.

Experiment 6 will be used to further develop a framework to meet Research Objectives 4 and 5. Furthermore, *Experiment 6* will be used to support Hypotheses 2 and 3.

Experiment 7.

Strontium isotope ratios of tap water have been measured for nearly one hundred cities across the United States [42] and $^{87}Sr/^{86}Sr$ ratios of hair samples from a portion of these cities (**Figure 14**) indicate similar linkages between tap water and hair $^{87}Sr/^{86}Sr$ ratios to what was established in Salt Lake City, Utah (*Experiments 4*, *5*, and *6*). Chesson and others [42] found that the fraction of variation in the $^{87}Sr/^{86}Sr$ ratio of tap water across the United States explained by the $^{87}Sr/^{86}Sr$ of underlying bedrock was very low ($r^2 = 0.10$). Differences between water and bedrock $^{87}Sr/^{86}Sr$ ratios suggested that the many U.S. municipal water systems relied on local groundwater and the transport of water across geologic gradients [42]. This phenomenon was observed on a smaller scale within the Salt Lake Valley metropolitan area in *Experiment 6*. We found similar relationships from the larger spatial dataset used in *Experiment 7*.

Experiments 6 and *7* were used to meet Research Objectives 4 and 5. Furthermore, data collected in *Experiment 7* supported Hypotheses 2 and 3. In total, *Experiments 4, 6,* and *7* suggest that the most critical parameter to constrain for future research and the increased development of $^{87}Sr/^{86}Sr$ ratios of human hair in region-of-origin assessment is mapping the $^{87}Sr/^{86}Sr$ ratios of municipal tap waters.

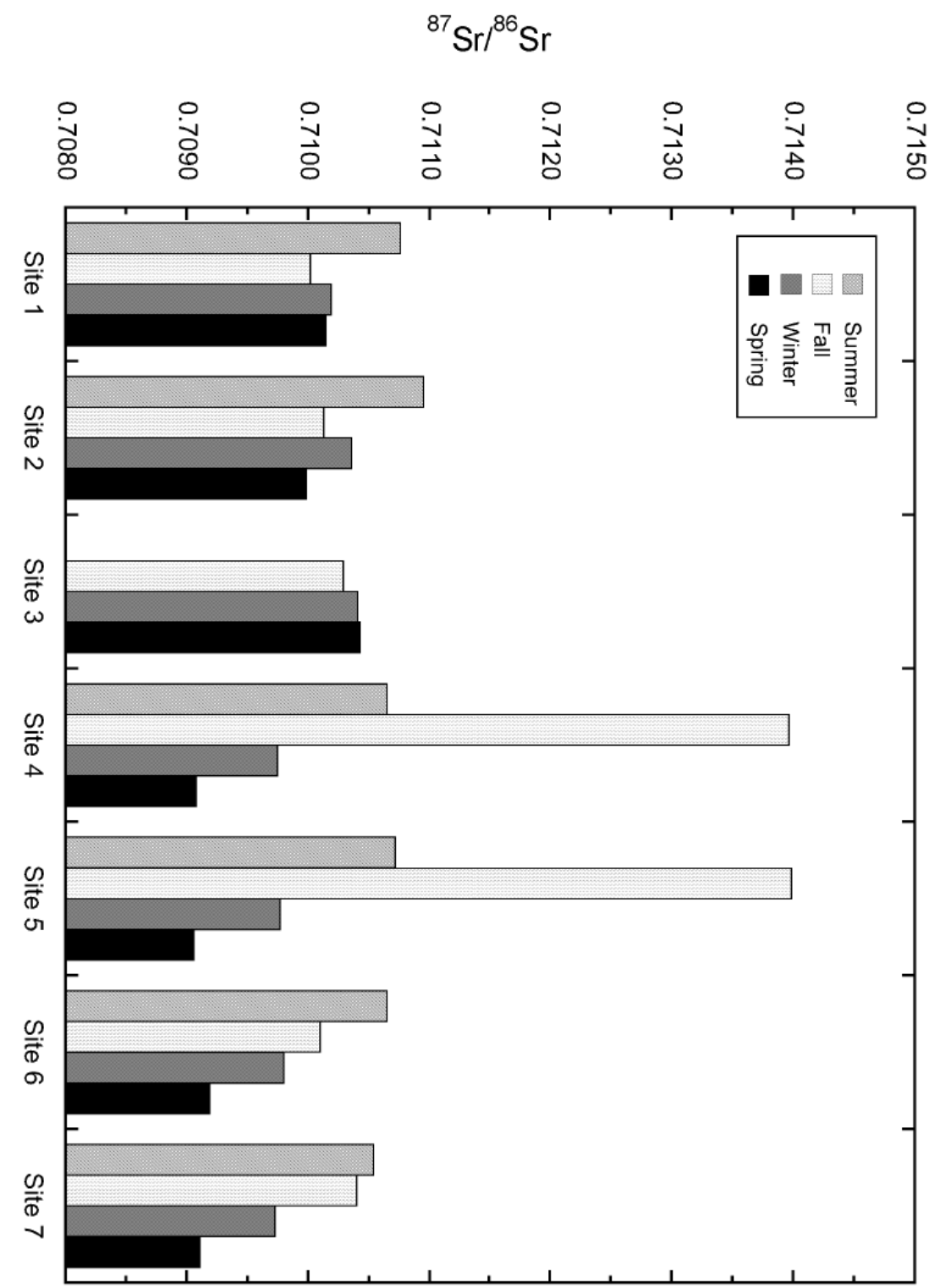

Figure 13. $^{87}Sr/^{86}Sr$ ratios of tap waters from seven locations within Salt Lake City, Utah. Sites correspond to locations on **Figure 6**.

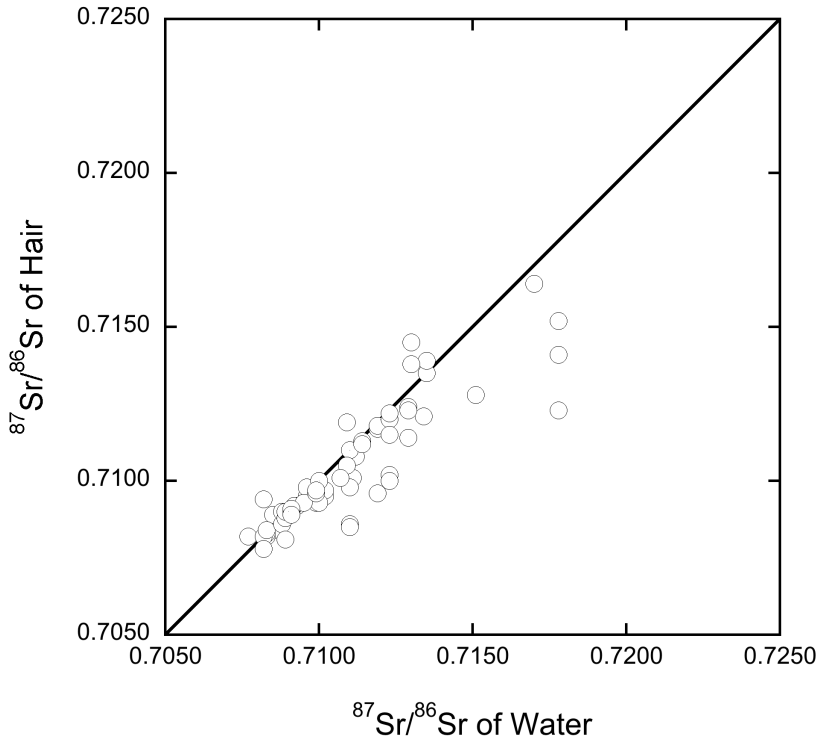

Figure 14. Cross plot of $^{87}Sr/^{86}Sr$ ratio of hair against $^{87}Sr/^{86}Sr$ ratio of waters from 56 U.S. cities. 1:1 line is shown.

Experiment 8.

During the course of *Experiments 1* and *2*, we found the $^{87}Sr/^{86}Sr$ ratios of hairs treated with the various cleaning treatments were not always predictably higher or lower than the untreated hair subsamples. These data indicated that the $^{87}Sr/^{86}Sr$ ratios of hair either remained static or varied with treatment method. The more aggressive the cleaning treatment, the more Sr was removed from deeper layers within the hair keratin matrix, leaving behind Sr only within the interior of the hair. Furthermore, variations in $^{87}Sr/^{86}Sr$ ratios were related to changes in the Sr content in the treated hair samples in some examples (i.e., by leaching external Sr from hair, the overall $^{87}Sr/^{86}Sr$ value of the residual hair could change). If the treatment methods removed Sr uniformly throughout the hair sample, we would expect Sr concentrations to decrease while $^{87}Sr/^{86}Sr$ ratios remained static, regardless of treatment method. We did not observe this in the majority of samples (**Table 5**). These results suggested that there could be differences in the $^{87}Sr/^{86}Sr$ ratios across the transverse cross section profile of a hair.

Our findings from *Experiment 8* indicated that there could be differences in the isotope ratios of Sr across the transverse cross section profile of a hair (**Figure 11**). From this, we conclude that variations in $^{87}Sr/^{86}Sr$ ratios of exogenous Sr sources may be recorded not only along the length of a hair, but also across the cross section of the hair itself.

To expand *Experiment 8*, we used hair from two horses with known travel histories. We found differences in Sr concentration along the length of the hair. These results are consistent with previous studies [38, 43] and reflect the continuous addition of exogenous

Sr. We observed differences in Sr concentration between the two horsehair samples (**Figure 12**). This observation may be related to the color of the hair, where the stationary horse had dark brown/black hair, while the transported horse had white hair. Previous research has demonstrated that trace elements are often lower in light colored hair [43]. Isotope results are forthcoming for these samples.

Experiments 8 will be used to meet Research Objectives 4 and 5 and to add support to Hypotheses 3 and 4.

Description of Deliverables

Progress Reports

Four Semi-Annual Progress Report were delivered via the GMS system for the time periods between:

- Sept 1, 2011-Dec 31, 2011,
- Jan 1, 2012-June 30, 2012,
- July 1, 2012-Dec 31, 2012,
- Jan 1, 2013-June 30, 2013, and
- July 1, 2013-Dec 31, 2013.

Ten Financial Status Reports were also submitted for the time periods between:

- Sept 1, 2011-Sept 31, 2011,
- Oct 1, 2011-Dec 31, 2011,
- Jan 1, 2012-Mar 31, 2012,
- April 1, 2012-June 30, 2012,
- July 1, 2012-Sept 30, 2012,
- Oct 1, 2012-Dec 31, 2012,
- Jan 1, 2013-Mar 31, 2013,
- April 1, 2013-June 30, 2013,
- July 1, 2013-Sept 30, 2013, and
- Oct 1, 2013-Dec 31, 2013.

The PI provided project status reports during eight conference calls with the NIJ project manager on:

- Oct 1, 2012,
- Dec 10, 2012,
- Feb 11, 2013,
- April 15, 2013,
- June 17, 2013,
- August 8, 2013,
- November 12, 2013, and
- December 17, 2013.

Database

All data collected for hair and waters during this work effort have been collated in a relational FileMaker® database. This database includes sample information, collection location, element composition, and a measured stable isotope ratio for the elements H, O, and Sr. The database is stored on IsoForensics' secure fileserver.

Dissemination of Research Findings

Publications

Tipple, B.J., Chau, T., Chesson, L.A., and Ehleringer, J.R., (2013) Isolation of the endogenous and exogenous strontium pools in modern human hair, *Analytica Chimica Acta*, vol. 798, iss. 1, pp. 64-73.

Tipple, B.J., Valenzuela, L.O., Chesson, L.A., and Ehleringer, J.R., (*In Preparation*) The strontium isotope ratios of municipal water are recorded in human hair, *Forensic Science International*.

Tipple, B.J., Valenzuela, L.O., Chesson, L.A., Chau, T., and Ehleringer, J.R., (*In Preparation*) Combining O and Sr isotope landscapes of human hair for geolocation, *Forensic Science International*.

Tipple, B.J., Bowen, G., Chesson, L.A., Valenzuela, L.O., Chau, T., Cerling, T.E., and Ehleringer, J.R., (*In Preparation*) Water management practices are reflected in the strontium isotope ratios of municipal waters, *Water Research*.

Tipple, B.J., Valenzuela, L.O., Chesson, L.A., Chau, T., Cerling, T.E., and Ehleringer, J.R., (*In Preparation*) Linking O and Sr isotope ratios of hair across the United States, *Proceedings of the National Academy of Sciences of the USA.*

Presentations

Oral

Isotope analysis of hair as a trace evidence tool to reconstruct human movements. American Academy of Forensic Sciences – National Institute of Justice Grantee Symposium, Atlanta, GA (February 20, 2012).

Isotope analysis of hair as a trace evidence tool to reconstruct human movements. American Academy of Forensic Sciences – National Institute of Justice Grantee Symposium, Seattle, WA (February 18, 2014).

Internet Webinar

Isotope analysis of hair as a trace evidence tool to reconstruct human movements. three web-based seminars for NIJ Forensic Science R&D Team (March-April, 2012).

Isotope analysis of hair as a trace evidence tool to reconstruct human movements. three web-based seminars for NIJ Forensic Science R&D Team (March-April, 2014).

Poster

The spatial patterns of water management practices are reflected in the strontium isotope ratios of human hair, American Geophysical Union Annual Meeting, San Francisco, California (December, 2012).

Isolation of exogenous strontium in modern human hair and applications of Sr for human geolocation, The Forensic Isotope Ratio Mass Spectrometry Network Conference, Montreal, Quebec (September, 2013).

Combining strontium and oxygen isotope ratios of hair for human provenancing, Geological Society of America Annual Meeting, Denver, Colorado (October, 2013).

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