Forensic Anthropology Vol. XX, No. XX: 1–10
DOI: 10.5744/fa.2023.0006

TECHNICAL NOTE

Effects of maceration on light stable isotopic ($\delta^{13}C$, $\delta^{18}O$) values of pig (*Sus scrofa*) rib bone carbonate: implications for geolocation estimates of unidentified human remains

Ligia Ceja[a] · Angela Soler[b] · Richard Mortlock[c] · Rhonda Quinn[d]*

ABSTRACT: Geoprofiling isotopic analyses provide investigative leads for unidentified human remains cases by determining possible regions of origin or excluding unlikely residences during life, which in turn can reduce the number of missing persons ~~cases~~ an investigator must consider. However, maceration methods involving heat, bleach, baking soda, and detergents have much potential to significantly change biogenic isotopic values in the structural carbonate phase of bone bioapatite. Here we test the impact of seven maceration methods on $\delta^{13}C$ and $\delta^{18}O$ values of bone carbonate (BC) of pig (*Sus scrofa*) ribs. Four of the seven maceration methods altered pig $\delta^{13}C_{BC}$ values with offsets ranging from 0.4‰ to 1.4‰; this amount of change would not severely impact human diet or geolocation interpretations. Five of the methods significantly decreased pig $\delta^{18}O_{BC}$ values by averages ranging between 1.0‰ and 2.6‰ likely due to the isotopic exchange between bone and heated water. As an illustrative exercise, we compared our study's results to macerated rib $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values of an identified New York City resident previously in the custody of the New York City Office of Chief Medical Examiner (NYC OCME). We suggest that maceration methods, especially those involving heated water, can potentially contribute to erroneous geolocation estimates garnered from rib $\delta^{18}O_{BC}$ values of unidentified individuals.

KEYWORDS: stable isotope analysis; maceration; bone carbonate; geolocation; isotopic offset

1. Introduction

Maceration is a standard procedure conducted by forensic anthropologists prior to analyses of human skeletal remains. Since the earliest cadaveric maceration in the nineteenth century (Ghosh 2015), a variety of methods have been employed including mechanical removal, water submersion, boiling, simmering, and treatments with bleach, detergents, enzymes, and degreasers, depending on the conditions of remains, available facilities, funds, time, and purpose of analyses. Previous studies have compared different maceration methods to determine best practices for maximizing preservation of bone features and surface modifications with the aim of constructing the biological profile, conducting trauma and pathological analyses, and retrieval of nuclear and mitochondrial DNA (Mairs et al. 2004; Rennick et al. 2005; Steadman et al. 2006; Lee et al. 2010; King & Birch, 2015).

Biogeochemical analyses (e.g., radiogenic and stable isotopic analyses, elemental concentration analyses) of bone, enamel, and other tissues are used to complement the biological profile and contribute to the estimations of year ranges of birth and death (Spalding et al. 2005; Ubelaker et al. 2006, 2022; Hodgins 2009; Johnstone-Belford et al. 2022), geographic location of birth and residential mobility (Gulson et al. 1997; Beard and Johnson 2000; Ehleringer et al. 2008), as well as dietary practices (Nardoto et al. 2006; Chesson et al. 2008). Stable and radiogenic isotopes are incorporated into organismal tissues through food and water intake and particle inhalation (see reviews of Koch et al. 1989, 1998). Measured isotopic values (e.g., $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{34}S$, $^{87}Sr/^{86}Sr$, $^{206}Pb/^{207}Pb$) from various human tissues are patterned geographically (Ehleringer et al. 2010; West et al. 2010) and, when used in concert, triangulate potential geographic locations ("geolocations") during the time of tissue formation (Laffoon et al. 2017; Bartelink & Chesson 2019; Kramer et al. 2020). These analyses aid to further individuate unidentified remains, provide investigative leads, and potentially narrow the number of missing persons for consideration

[a]San Diego County Medical Examiner's Office, San Diego, CA, USA
[b]New York City Office of Chief Medical Examiner, Forensic Anthropology Unit, New York, NY, USA
[c]Rutgers University, Earth and Planetary Sciences, Piscataway, NJ, USA
[d]Bowling Green State University, School of Earth, Environment & Society, Bowling Green, OH, USA
*Correspondence to: Rhonda Quinn, Bowling Green State University, School of Earth, Environment & Society, 190 Overman Hall, Bowling Green, OH 43403, USA
E-mail: rlquinn@bgsu.edu

Received 24 April 2023; Revised 25 August 2023; Accepted 5 September 2023

(Rauch et al. 2007; Meier-Augenstein & Fraser, 2008; Kamenov et al. 2014; Remien et al. 2014; Bartelink et al. 2016; Lehn et al. 2015b, 2022).

One commonly used and relatively inexpensive geoprofiling isotopic analysis yields both $\delta^{13}C$ and $\delta^{18}O$ values from the structural carbonate phase of bone bioapatite (denoted here with subscript BC for bone carbonate). $\delta^{13}C$ values ($^{13}C/^{12}C$ ratios expressed in delta notation in parts per thousand, per mil, ‰) of various human tissues reflect those of consumed foods (Katzenberg 2000) during the time of tissue formation and/or remodeling (Hedges et al. 2007) and can be fractionated by physiology, health, and nutritional status (Reitsema 2013). Dietary protein and carbohydrate $\delta^{13}C$ values are differentially routed to organic (e.g., bone collagen, hair keratin) and inorganic (e.g., bone carbonate) phases of human tissues, where $\delta^{13}C_{BC}$ values represent whole-diet $\delta^{13}C$ values (Ambrose & Norr 1993; Kellner & Schoeninger 2007). Culturally influenced dietary practices yield different food $\delta^{13}C$ values, and thus human tissue $\delta^{13}C$ values show broad geographic patterning (Lehn et al. 2015a). For example, extensive use of corn- and sugarcane-based foods, both C_4 plants, in the diets of US residents, influenced by socioeconomic factors of food choice (Ehleringer et al. 2020), typically yields relatively higher $\delta^{13}C$ values compared to those of individuals residing in some European and Asian countries (Valenzuela et al. 2011; Bartelink et al. 2014; Lehn et al. 2015a).

$\delta^{18}O$ values ($^{18}O/^{16}O$ ratios expressed in delta notation in parts per thousand, per mil, ‰), incorporated into organic (e.g., collagen) and inorganic (e.g., bone/enamel phosphate and carbonate) phases of human tissues, reflect consumed $\delta^{18}O$ values from drinking water and water in food as well as from inhaled atmospheric O_2 (Kohn & Cerling 2002). Drinking water $\delta^{18}O$ values are primarily controlled by temperature- and latitude-influenced rainfall and groundwater $\delta^{18}O$ values (Craig 1961; Dansgaard 1964) generally resulting in a north (lower $\delta^{18}O$) to south (higher $\delta^{18}O$) gradient (Dutton et al. 2005; Bowen et al. 2007). $\delta^{18}O_{BC}$ values, as well as those of enamel carbonate and enamel or bone phosphate $\delta^{18}O$ values (denoted with subscript PHOS), are used to infer the $\delta^{18}O$ value of imbibed/ingested water (denoted here as $\delta^{18}O_{IW}$) source regions and thus serve as a proxy of geolocation during bone and enamel formation (Ehleringer et al. 2008; Ammer et al. 2020). Local tap water $\delta^{18}O$ (subscript tap) values are typically used as a proxy for bioavailable drinking water sources (e.g., Ammer et al. 2020).

Biogeochemical methods as applied to human skeletal and dental materials were initially established in archaeological and paleontological contexts and not originally developed to meet the medicolegal standards of forensic sciences (e.g., Christensen & Crowder 2009). Consequently, tests of intra-laboratory isotopic variation (Pestle et al. 2014; Chesson et al. 2019), enhanced measures of quality control (Chesson et al. 2021), and improved reporting (Szpak et al. 2017; Berg et al. 2022) of human tissues are accumulating. Moreover, geoprofiling results of known individuals are utilized to better establish method parameters and gauge analytical resolution and accuracy while considering specific variables of unidentified human remains in forensic contexts (O'Brien & Wooller 2007; Font et al. 2012; Kamenov & Curtis 2017; Mancuso & Ehleringer 2019; Hu et al. 2020; Regan et al. 2020; Quinn et al., 2021). The FIRMS (Forensic Isotope Ratio Mass Spectrometry) Network (https://www.forensic-isotopes.org) was founded to "to develop the scope of stable isotope techniques in forensic applications" and promotes best practices by method validation and inter-laboratory comparison studies (see Dunn & Carter 2018).

In the archaeological and paleontological literature, much work has focused on determining potential changes to biogenic (i.e., formed during life) isotopic values of different tissues and phases within tissue types due to diagenetic (i.e., recrystallization, fossilization) and taphonomic (i.e., burial environment) alterations (Wang & Cerling, 1994; Iacumin et al., 1996; Kohn & Cerling, 2002; Lee-Thorp, 2002). As compared to archaeological and paleontological skeletal and fossil materials, time since death of human remains in forensic contexts is short (<75 years), and thus diagenesis is generally assumed to be negligible. However, experimental studies demonstrate that several taphonomic factors can potentially fractionate biogenic isotopic values of human remains in short time periods, especially those of bone carbonate. For example, cooking and burning significantly change biogenic $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values (DeNiro et al. 1985; Munro et al. 2008; Sarancha et al. 2022). Boiling and simmering increase water $\delta^{18}O$ values as ^{16}O is preferentially removed via evaporative processes (Brettell et al. 2012; Gagnon et al. 2015). These fractionated water $\delta^{18}O$ values can then be incorporated into tissues via ingestion (Tuross et al. 2017). When fleshed and skeletal materials are submerged in boiling or simmering water, $\delta^{13}C_{BC}$ values are largely unchanged; however, $\delta^{18}O_{BC}$ values have been found to significantly decrease, likely due to exchange with the submersion water that has relatively lower $\delta^{18}O$ values than bone (Munro et al. 2007, 2008; Tuross et al. 2017). Various chemical treatment procedures for removing exogenous components from bone prior to mass spectrometry have also been assessed (Koch et al. 1997; Yoder & Bartelink 2010; Crowley & Wheatley 2014). Notably, procedures that utilize bleach significantly change $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values due to bone's high organic content, small crystallites, and large surface areas for bicarbonate adsorption (Koch et al. 1997; Crowley & Wheatley 2014).

Traditional maceration methods involving bleach and heated water similar to chemical treatment procedures or taphonomic alterations are predicted to change biogenic

$\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values. More recent maceration procedures, typically involving heated water, utilize detergents, degreasers, and sodium carbonate, which also have potential to modify $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values via dissolution and recrystallization, and adsorption of secondary materials (Koch et al. 1997; Crowley & Wheatley 2014). Thus, $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ analysis of macerated unidentified human remains has much potential to yield fractionated and thus non-biogenic values, leading to erroneous diet and geolocation estimations. Here we quantified the impact of seven commonly used maceration methods on pig (*Sus scrofa*) rib $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values. We then compared our experimental results to the $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values of a macerated rib segment from a New York City resident who was identified by the New York City Office of Chief Medical Examiner (NYC OCME). Isotopic analyses were originally commissioned by a private isotope testing company in an effort to identify the individual. A subsequent unrelated investigative lead eventually resulted in a DNA-based identification. To maintain confidentiality of the decedent and family, no identifying information is shared.

2. Materials and Methods

2.1. Pig rib bone sample

Pigs (*Sus scrofa*) were selected for this study because the species shares anatomical, physiological, and dietary similarities to humans and are the preferred analogue in forensic research (Healy et al. 2015, Armstrong et al. 2016, Matuszewski et al. 2020). Rib samples were also chosen for the study to skeletally match previously analyzed human rib segments. Ribs are commonly used for isotopic analysis due to availability and their potentially high turnover rates (Parfitt 1983; Pearson & Lieberman 2004). Isotopic analysis of rib bone is estimated to provide geolocation and dietary information ≤10 years prior to the individual's year of death (Cox and Sealy 1995; Lamb et al. 2014). Racks of pig ribs representing five individual pigs were purchased from local butchers in Queens, New York, and show $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values within the range of US residents (Valenzuela et al. 2011; Berg & Kenyhercz 2017) and NYC OCME cases (Quinn et al. 2020).

2.2. Macerated and control rib bone sets

First, rib racks were disarticulated with a scalpel, and individual pig ribs were cut in half to create maceration-control rib bone sets in order to minimize potential intra- and inter-individual $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ differences. Control samples were manually cleaned using a scalpel and forceps. Macerated rib segments underwent one of seven different maceration methods until flesh softened and could be easily removed with a blunt scraping tool. The maceration methods, described in Table 1, were taken from the literature (e.g., Fenton et al. 2003; Steadman et al. 2006) and/or were previously or are currently employed by the NYC OCME. These include the following with abbreviation in parentheses: (1) sodium hypochlorite bath (Bleach), 2) boiled water bath (Boil), (3) simmered water bath (Simmer), (4) dish soap and meat tenderizer (DS+MT), (5) detergent and sodium carbonate (D+SC), (6) detergent, sodium carbonate, and degreasing agent (D+SC+DA), and (7) degreasing detergent (SK). In all maceration methods except Bleach, samples were monitored every hour. Bleach samples were monitored every hour for the first eight hours, then monitored every 12 hours until completion. ◂T1

TABLE 1—*Descriptions and Durations of Maceration Methods Used in This Study*

Maceration Method (abbreviation in text)	Description of Maceration Method	Duration
Sodium hypochlorite bath (Bleach)	Submerged in 10% Clorox bleach solution at room temperature (20°C).	10 days
Boiled water bath (Boil)	Submerged in 10 L of boiling (100°C) tap water.	4 hours
Simmered water bath (Simmer)	Submerged in 10 L of heated (90°C) tap water.	6 hours
Dish soap and meat tenderizer (DS+MT)	Submerged in heated (90°C) solution consisting of 36 cc of Adolph's non-seasoned meat tenderizer, 36 cc of Palmolive dish soap, and 10 L of tap water.	6 hours
Detergent and sodium carbonate (D+SC)	Submerged in heated (90°C) solution containing 20 cc of Alconox detergent, 20 cc of sodium carbonate (Arm and Hammer Washing Soda), and 2 L of tap water.	6 hours
Detergent, sodium carbonate and degreasing agent (D+SC+DA)	D+SC method, rinsed with tap water, submerged in a heated (90°C) solution consisting of 150 mL of liquid household ammonia (Austin's Clear Ammonia) and 2 L of tap water. Following Fenton et al. (2003), samples were monitored in ammonia solution until small lipid globules floated to the surface.	6 hours + 1 hour for degreasing
Degreasing detergent (SK)	Submerged in heated (90°C) solution consisting of 60 mL of Super Kleen (Delta Foremost Chemical Corp.) and 2 L of tap water.	6 hours

—-1
—0
—+1

2.3. Sample preparation and mass spectrometry

Following maceration, powders were drilled from the cortical bone of rib segments with a rotary drill (Foredom series) affixed with a carbide bit. From each of the seven maceration-control sets, samples from three locations along a control rib segment and 10–16 locations along a maceration segment were drilled and collected into sterile polypropylene centrifuge tubes. Bone carbonate was isolated following methods of Koch et al. (1997), a commonly used method by light stable isotopic studies (as reviewed in Crowley & Wheatley, 2014) and reported by the private isotope testing company originally commissioned by the NYC OCME. This entailed weighing 10–15 mg of bone treating with a 30% H_2O_2 for 24 h (0.5 ml/20 mg) to remove organic matter and with a 0.1 M acetic acid buffered with calcium acetate (pH 5, 0.5 ml/20 mg) for 24 h to remove non-structural carbonate. Samples were rinsed five times in Milli-Q ultrapure water after each treatment and dried at room temperature. Powders weighing between 1–2 mg were loaded into Wheaton 1 ml v-vials and sealed. $\delta^{13}C$ and $\delta^{18}O$ were measured using a Multiprep device attached to a Micromass Optima isotope ratio mass spectrometer (IRMS). Samples were reacted in 100% H_3PO_4 at 90°C and the evolved CO_2 was collected in a liquid nitrogen cold finger. Stable isotope values are reported relative to Vienna-Pee Dee Belemnite (V-PDB) through the analysis of an in-house laboratory reference material (RGF1). Analytical precision is 0.05‰ and 0.08‰ for $\delta^{13}C$ and $\delta^{18}O$, respectively, based on replicates of RGF1.

2.4. Analytical standards in forensic isotopic analysis

Forensic isotopic analysis continues to be developed to meet international standards for medicolegal contexts. The FIRMS Network advocates the use of two international calibration standards to generate a stretch-shift or slope-intercept normalization scheme during each run of the mass spectrometer (Dunn et al. 2017; Dunn & Carter 2018). This protocol accounts for potential scale compression (Meier-Augenstein & Shimmelmann 2019) and better enables comparisons of isotopic values amongst different laboratories. Scale compression, as defined by Meier-Augenstein and Shimmelmann (2019) is "the sum of all mass discriminatory effects associated with sample gas transfer to the IRMS, sample gas admission into the ion source of the IRMS and possibly processes inside the ion source itself." Notably, the pig rib maceration experiment and associated $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ analyses, conducted as part of the first author's (LC) thesis project in 2018, followed the protocol described below used by the Stable Isotope Laboratory in the Department of Earth and Planetary Sciences at Rutgers University.

During the pig rib $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ analyses, eight RGF1 standards were run intermittently with every 24 samples and measured over the course of 20 hours, which represents one run of the mass spectrometer. RGF1 is routinely calibrated to NBS-19 to ensure consistency, using 1.95‰ and –2.20‰ for $\delta^{13}C$ and $\delta^{18}O$, respectively, as reported by Coplen (1994). The internal lab reference material is offset from NBS-19 by +0.10 and +0.04‰ for $\delta^{13}C$ and $\delta^{18}O$, respectively. The average standard deviation of RGF1 provides an estimate of external reproducibility and accounts for potential contributions to scale compression, including but not limited to reference gas depletion, changes in source response, and changing temperature and humidity room conditions. The lab analyzes NBS-19 in combination with NBS-18 ($\delta^{13}C$ = –5.01‰ and $\delta^{18}O$ = –23.2‰) to monitor and to correct for changes in source linearity, and their respective $\delta^{13}C$ and $\delta^{18}O$ values bracket the range of pig rib $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values reported in our study. Notably, the analytical precision does not reflect the reproducibility of pig rib $\delta^{13}C$ and $\delta^{18}O$ values in this study, which due to the complex nature of bone remodeling (Hedges et al. 2007) and potential dietary heterogeneity of an omnivorous domestic pig are predicted to show significant intra-individual variation. Since this study utilized only one standard (RGF1) during each run to account for scale compression rather than two as advocated by the FIRMS Network, we offer these results as a proof of concept to further gauge if a comparable study with human skeletal elements is warranted.

2.5. Statistical analyses and conversion equations

We compared the control and maceration pig rib $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values (‰, V-PDB) of each of the seven control-maceration sets with two-tailed t-tests using StatPlus v7 statistical software (AnalystSoft Inc). In order to compare pig rib $\delta^{18}O_{BC}$ values (relative to V-PDB) to local tap water $\delta^{18}O$ values (reported relative to Vienna-Standard Mean Ocean Water, V-SMOW), we utilized the same conversion equations reportedly used by the private isotope testing company at the time of the commissioned work by NYC OCME for the NYC resident's rib bone segment and employed by other forensic geoprofiling studies (e.g., Ammer et al. 2020). The equation $\delta^{18}O_{V\text{-}SMOW} = 1.03091 \times \delta^{18}O_{V\text{-}PDB} + 30.91$ (Coplen et al. 1988) converts the measured bone carbonate reported relative to V-PDB to V-SMOW. The equation $\delta^{18}O_{PHOS} = 0.998 \times \delta^{18}O_{CARB} - 8.5$ (‰, V-SMOW; Iacumin et al. 1996) is utilized to convert carbonate $\delta^{18}O_{BC}$ to phosphate $\delta^{18}O_{PHOS}$. To estimate the $\delta^{18}O_{IW}$ value from the $\delta^{18}O_{PHOS}$ value, the equation $\delta^{18}O_{IW} = 1.54\ (\pm 0.09) \times \delta^{18}O_{PHOS} - 33.72\ (\pm 1.51$; Daux et al. 2008) is used. To estimate the NYC resident's geolocation, the private isotope testing company mapped the calculated $\delta^{18}O_{IW}$ value (±0.5‰) on a modeled US tap water $\delta^{18}O_{tap}$ isoscape (data from Bowen et al. 2007).

-1—
0—
+1—

3. Results

3.1. Within group $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ variation

Separate control and maceration groups displayed intra-individual (i.e., intra-rib) isotopic variation (see standard deviations in Tables 2 and 3). Maximum within group $\delta^{13}C_{BC}$ differences ranged from 0.1‰ to 2.4‰ in the control groups (n = 3) and from 0.5‰ to 3.5‰ in the maceration groups (n = 10 to 16). Maximum within group $\delta^{18}O_{BC}$ differences ranged from 0.2‰ to 3.1‰ in the control groups (n = 3) and from 2.4‰ to 4.0‰ in the maceration groups (n = 10 to 16).

3.2. Maceration vs. control group $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values

The mean $\delta^{13}C_{BC}$ differences of all maceration methods ranged from −1.2‰ to +1.4‰ (Table 2). Mean $\delta^{13}C_{BC}$ differences within maceration-control rib bone sets that reached statistical significance (two-tailed t-test, $p < .05$) included Boil, Simmer, and both detergent-based methods, D+SC and D+SC+DA.

The mean $\delta^{18}O_{BC}$ differences of all maceration methods ranged from +0.2‰ to −2.6‰ (Table 3). Mean $\delta^{18}O_{BC}$ differences within maceration-control rib bone sets that reached statistical significance (two-tailed t-test, $p < .05$) included Boil, Simmer, DS+MT, D+SC, and SK. Six of the seven maceration methods decreased $\delta^{18}O_{BC}$ by more than 1‰, although the D+SC+DA method did not reach statistical significance.

TABLE 2—*$\delta^{13}C_{BC}$ Values (V-PBD, ‰) of the Control-Maceration Group Sets and Results of t-tests*

Maceration Method	Control Group Mean $\delta^{13}C_{BC}$ ±1σ ‰, V-PDB (n)	Maceration Mean $\delta^{13}C_{BC}$ ±1σ ‰, V-PDB (n)	Maceration-Control $\delta^{13}C_{BC}$ Difference ‰, V-PDB	t-test Statistic, p-value
Bleach	−5.7 ± 0.1 (3)	−5.8 ± 0.4 (15)	−0.1	0.3, .764
Boil	−5.0 ± 0.0 (3)	−4.5 ± 0.6 (13)	+0.6	3.1, **.008**
Simmer	−2.6 ± 0.1 (3)	−3.7 ± 1.5 (11)	−1.2	2.6, **.027**
DS+MT	−4.1 ± 1.2 (3)	−4.5 ± 1.0 (16)	−0.4	0.6, .581
D+SC	−3.8 ± 0.1 (3)	−3.5 ± 0.2 (10)	+0.4	4.6, **.006**
D+SC+DA	−6.4 ± 0.3 (3)	−5.0 ± 0.6 (10)	+1.4	5.5, **.001**
SK	−6.0 ± 0.2 (3)	−5.9 ± 0.4 (11)	+0.1	0.4, .671

TABLE 3—*$\delta^{18}O_{BC}$ values (V-PBD, ‰) of the Control-Maceration Group Sets and Results of t-tests*

Maceration Method	Control Group Mean $\delta^{18}O_{BC}$ ±1σ ‰, V-PDB (n)	Maceration Mean $\delta^{18}O_{BC}$ ±1σ ‰, V-PDB (n)	Maceration-Control $\delta^{18}O_{BC}$ Difference ‰, V-PDB	t-test Statistic, p-value
Bleach	−7.2 ± 0.8 (3)	−6.9 ± 0.8 (15)	0.3	0.5, 0.650
Boil	−7.8 ± 0.1 (3)	−9.7 ± 0.7 (13)	−2.0	9.6, **<0.001**
Simmer	−7.5 ± 0.4 (3)	−8.5 ± 0.7 (11)	−1.0	3.2, **0.015**
DS+MT	−6.9 ± 0.9 (3)	−8.7 ± 1.0 (16)	−1.9	3.6, **0.008**
D+SC	−8.0 ± 0.3 (3)	−10.6 ± 0.8 (10)	−2.6	8.2, **<0.001**
D+SC+DA	−6.8 ± 1.6 (3)	−8.0 ± 1.1 (10)	−1.2	1.2, 0.303
SK	−6.3 ± 0.3 (3)	−7.7 ± 0.8 (11)	−1.4	4.5, **0.001**

4. Discussion

4.1. Effects of maceration on pig rib $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values

Statistical comparisons found that some of these maceration methods significantly altered biogenic pig rib $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values; however, the degree of the effects is important to consider. Notably, the intra-individual (i.e., intra-rib) isotopic variation in each control group ranged from 0.2‰ to 3.1‰, which may reflect variability in dietary and drinking water values available to domestic pigs. Generally, people have a wide range of food and beverage options, and those choices appear to manifest as an equal or greater intra-individual isotopic range (Eerkins et al. 2011, 2016; Mauer et al. 2014; Olsen et al. 2014; Fahy et al. 2017; Plomp et al. 2020; Berg et al. 2022). Notably, inter- and intra-laboratory comparisons have found comparable and greater $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ variation even within a single human femoral bone (Pestle et al. 2014; Chesson et al. 2019).

The changes in pig rib $\delta^{13}C_{BC}$ values between the maceration-control sets were modest, non-directional, and within the range of intra-individual variation. However, the majority of maceration methods assessed here, all of which used heated water, significantly decreased pig rib $\delta^{18}O_{BC}$ values by 1.0–2.6‰. Maceration water was derived from NYC tap water, which yields $\delta^{18}O_{V\text{-}SMOW}$ values between −8‰ and −6‰ (Bowen et al. 2007). Pig rib $\delta^{18}O_{BC}$ values converted to $\delta^{18}O_{V\text{-}SMOW}$ are 22.7‰ to 24.4‰. Based on comparable results of the boiling study of Munro et al. (2008), we interpret that the exchange between pig rib bone $\delta^{18}O_{V\text{-}SMOW}$ and the markedly lower $\delta^{18}O_{V\text{-}SMOW}$ of maceration water for four to six hours is the likely cause for the significant decrease with all heated water methods. Sodium carbonate included in two of the maceration methods were predicted to cause partial dissolution and potential reprecipitation of carbonate. However, results did not indicate larger isotopic offsets in the D+SC and D+SC+DA compared to the other maceration-control group sets.

The potential effects of maceration on the accuracy of estimating geolocation and diet from $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values depends on the offset amount within the constructed isoscapes and dietary models. For example, an individual's measured $\delta^{13}C_{BC}$ value can be used to estimate $\delta^{13}C$ of whole diet denoted in $\%C_4$ diet (Schwarcz et al. 1985; Ambrose et al. 1997; Schoeninger 2009). Using a simple linear mixing model (methods reviewed in Quinn 2019), assuming average C_3

(–28‰) and C_4 (–12‰) food endmembers, a shift of 1‰ equates to a ~6% change in $\%C_4$ diet. Statistically significant changes to $\delta^{13}C_{BC}$ values from maceration methods found here occurred in both positive and negative directions but were on average less than or only slightly above 1‰. Consequently, although statistically significant, impacts of maceration to $\delta^{13}C_{BC}$ values shown here would be negligible for modern human dietary reconstructions. That is, a substantial dietary shift possibly due to a major change in geolocation would not be interpreted with a $\delta^{13}C_{BC}$ shift of ±1‰.

On the other hand, due to the limited span of precipitation $\delta^{18}O$ values across the globe (Waterisotopes.org) and within US $\delta^{18}O_{tap}$ values (Bowen et al. 2007; Ehleringer et al. 2008), a >1‰ decrease in a rib $\delta^{18}O_{BC}$ value has the potential to alter the geolocation interpretation of an unidentified individual. For example, annual tap water $\delta^{18}O_{tap}$ values across the US range from –22‰ and +2‰ (V-SMOW) and are depicted in 2‰ intervals to provide general geographic boundaries of the isotopic gradient (Bowen et al. 2007; Cerling et al. 2016). The effect of maceration on a rib bone $\delta^{18}O_{BC}$ value exceeding 1‰ has potential to shift a geolocation estimation into a different interval of the US $\delta^{18}O_{tap}$ isoscape, possibly resulting in the designation of a resident as non-local to the region.

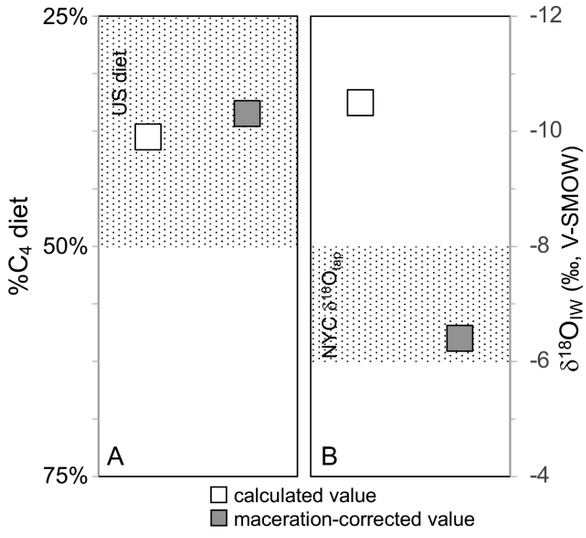

FIGURE 1—*Comparison of Brooklyn resident's rib bone carbonate (A) $\%C_4$ diet and (B) $\delta^{18}O_{IW}$ estimations without (white-filled boxes) and with corrections for maceration effects (gray-filled boxes). Dotted areas in the graphs represent the ranges of NYC $\delta^{18}O_{tap}$ value (Bowen et al. 2007) and typical US diets (Berg & Kenyhercz, 2017).*

4.2. Known New York City resident

We compared the relative effects of one maceration method to the isotopic geoprofiling analyses of a previously sampled New York City resident who was positively identified through other means. One tissue type analyzed by the private isotope testing company originally commissioned by the NYC OCME, the right ninth rib, macerated with the D+SC method, represents the individual's known residence in Brooklyn, approximately ≤10 years prior to death. Table 3 lists the measured $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values and the calculated percentage of C_4 foods in the diet ($\%C_4$) and $\delta^{18}O_{IW}$ value. Figure 1 plots the calculated $\%C_4$ and $\delta^{18}O_{IW}$ values against the maceration-corrected $\%C_4$ and $\delta^{18}O_{IW}$ values for the NYC resident's rib bone. Figure 2 was redrawn after the US $\delta^{18}O_{tap}$ isoscape of Bowen et al. (2007) and the original geolocation map provided to NYC OCME by the private isotope testing company.

The maceration conducted by the NYC OCME Forensic Anthropology Unit for the presented case was the D+SC method, which in this study yielded average $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ offsets of +0.4‰ and –2.6‰, respectively. The estimated percentage of whole diet C_4 foods ($\%C_4$) based on the reported rib $\delta^{13}C_{BC}$ value of –9.9‰ is 38%. Considering an increase of 0.4‰ due to the D+SC maceration method, the individual's calculated $\%C_4$ would shift to 36%, but would not result in a different diet interpretation (Fig. 1a). Both of these estimates are within typical US $\delta^{13}C$-based diets, which range from about 10% to 50% C_4 resources (Valenzuela et al. 2011; Berg & Kenyhercz 2017; Fig. 1a). Geolocation according to the estimated $\delta^{18}O_{IW}$ value, however, was reconstructed in more northern and western US regions than the individual's known residence in Brooklyn (Fig. 2, only northeastern US regions are depicted). Considering a decrease of 2.6‰ due to the D+SC maceration method, the individual's calculated rib $\delta^{18}O_{BC}$ value shifts to −4.5‰ and equates to a $\delta^{18}O_{IW}$ value of −6.4‰ (Table 4; Fig. 1b), which is within the $\delta^{18}O_{tap}$ range (–8.0 to −6.0‰) for NYC (Bowen et al. 2007). The direction and magnitude of the $\delta^{18}O_{BC}$ offset due to the D+SC maceration procedure corresponds with the erroneous geolocation estimation of the NYC resident's known geolocation and the region's $\delta^{18}O_{tap}$ range (Fig. 2).

4.3. Maceration as a potential source of error in geolocation estimations

Based on the results of this study, we suggest that maceration can significantly alter biogenic pig rib $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values. Coupled with the case study of a known NYC resident, we suggest that maceration is a potential source of error in $\delta^{18}O_{BC}$-based geolocation estimations from human rib bones. The measured pig rib $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ offsets found in this study, however, are not intended to serve as correction factors for each reported maceration method, but should serve as a proof of concept, warranting additional research on human remains on the potential impacts of maceration on geoprofiling isotopes. We also advocate for employing the current analytical standards based on the method validation

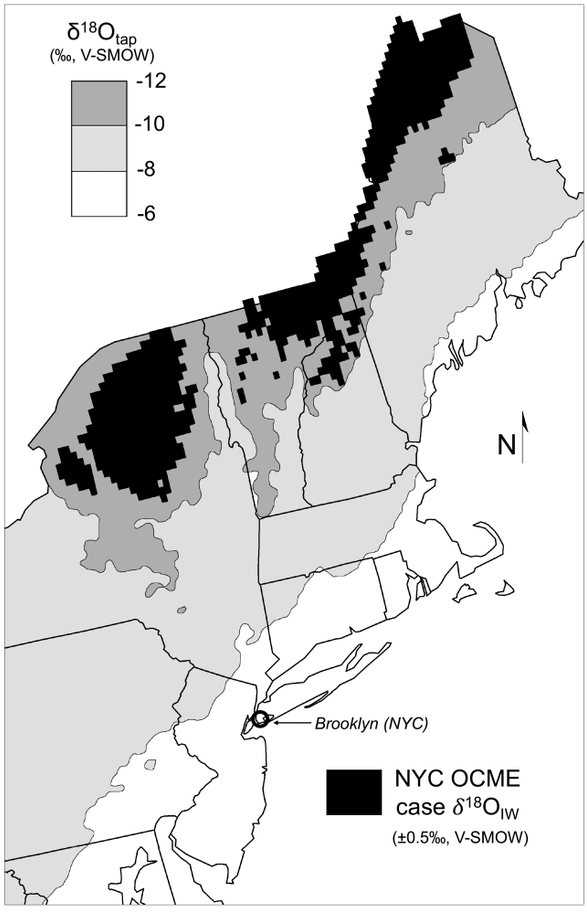

FIGURE 2—*NYC OCME Brooklyn resident's rib bone carbonate $\delta^{18}O_{IW}$-based geolocation estimation (shaded black) commissioned by a private isotope testing company (only northeastern US portion of map is shown here). US $\delta^{18}O_{tap}$ isoscape redrawn from Bowen et al. (2007).*

TABLE 4—*NYC OCME Brooklyn Resident's Measured and Calculated Isotopic Values from the Ninth Rib Segment*

Measured or Calculated Isotope	Value or Range (‰)
Measured $\delta^{13}C_{BC}$ (V-PDB)	–9.9
$\%C_4$ diet estimate	38%
Maceration effect-corrected $\delta^{13}C_{BC}$ (V-PDB)	–10.3
Maceration effect-corrected $\%C_4$ diet estimate	36%
Typical US diet	10–50%
Measured $\delta^{18}O_{BC}$ (V-PDB)	–7.1
Calculated $\delta^{18}O_{BC}$ (V-SMOW)	23.6
Calculated $\delta^{18}O_{IW}$ (V-SMOW)	–10.5
Maceration effect-corrected $\delta^{18}O_{BC}$ (V-PDB)	–4.5
Maceration effect-corrected $\delta^{18}O_{BC}$ (V-SMOW)	26.3
Maceration effect-corrected $\delta^{18}O_{IW}$ (V-SMOW)	–6.4
NYC $\delta^{18}O_{tap}$ (V-SMOW)	–8.0 to –6.0

studies of the FIRMS Network to aid in inter-laboratory comparisons and collaborations. Until correction factors specific to skeletal element, bone type, and isotopic system can be established, we suggest that best practices in forensic anthropology should include consideration of the potential direction and magnitude of $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ offsets when geoprofiling previously macerated remains of an unidentified individual.

5. Conclusion

Isotopic analyses are becoming increasingly widespread in forensic anthropology to enhance the biological profile and aid in identification efforts. Additional experimental studies are required to gauge the accuracy and precision of these methods that consider the particular contexts, procedures, and medicolegal standards of the discipline. Maceration, a standard procedure in forensic anthropology, commonly involves heated water, detergents, degreasers, and/or sodium carbonate. This study demonstrates that maceration methods involving heated water significantly altered biogenic pig cortical rib $\delta^{13}C_{BC}$ and $\delta^{18}O_{BC}$ values. Comparing the isotopic offsets to an identified New York City resident illustrates that maceration may have been one of the factors that contributed to an erroneous geolocation estimation based on the individual's rib $\delta^{18}O_{BC}$ value. Thus, additional research quantifying effects of maceration on human bone $\delta^{18}O_{BC}$ values is warranted. We recommend practitioners should consider the impact of various maceration methods on bone elements typically submitted for isotopic analyses when planning to geoprofile an unidentified individual.

Acknowledgements

The authors thank the Forensic Anthropology Unit at the NYC OCME, especially Bradley Adams. The NYU Skeletal Biology MA program under the direction of Susan Antón and Scott Williams provided invaluable graduate student mentorship and training. Isotopic analyses and laboratory support was generously provided by the Stable Isotope Laboratory under the direction of James Wright in the Department of Earth and Planetary Sciences at Rutgers University. Funding for isotopic analyses through a private contractor was provided to the NYC OCME by the National Institute of Justice (NIJ-2015-4055). Funding was provided to RLQ for student laboratory training and isotopic analyses by the National Science Foundation (BCS-1455274).

References

Ambrose SH, Butler BM, Hanson DB, Hunter-Anderson RL, Krueger HW. Stable isotope analysis of human diet in the Marianas archipelago, Western Pacific. *American Journal of Physical Anthropology.* 1997;104:343–361.

—-1
—0
—+1

Ambrose SH, Norr L. Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In: Lambert JB, Grupe G, eds. *Prehistoric Human Bone: Archaeology at the Molecular Level*. Berlin: Springer-Verlag; 1993:1–37.

Ammer STM, Bartelink EJ, Vollner JM, Anderson BE, Cunha EM. Spatial distributions of oxygen stable isotope ratios in tap water from Mexico for region of origin predictions of unidentified border crossers. *Journal of Forensic Sciences* 2020;65: 1049–1055.

Armstrong P, Nizio KD, Perrault KA, Forbes SL. Establishing the volatile profile of pig carcasses as analogues for human decomposition during the early postmortem period. *Heliyon* 2016;2 e00070.

Bartelink EJ, Berg GE, Beasley MM, Chesson LA. Application of stable isotope forensics for prediction region of origin of human remains from past wars and conflicts. *Annals of Anthropological Practice* 2014;38:124.

Bartelink EJ, Chesson LA. Recent applications of isotope analysis to forensic anthropology. *Forensic Sciences Research* 2019;4:29–44.

Bartelink EJ, MacKinnon AT, Prince-Buitenhuys JR, Tipple BJ, Chesson LA. Stable isotope forensics as an investigative tool in missing persons investigations. In: Morewitz SJ, Sturdy Colls C, eds. *Handbook of Missing Persons*. New York: Springer International Publishing; 2016:443–462.

Beard BL, Johnson CM. Strontium isotope composition of skeletal material can determine the birth place and geographic mobility of humans and animals. *Journal of Forensic Sciences* 2000;45(5):1049–1061.

Berg GE, Chesson LA, Yuryang J, Youngsoon S, Bartelink EJ. A large-scale evaluation of intra-person isotopic variation within human bone collagen and bioapatite. *Forensic Science International* 2022;336:111319.

Berg G, Kenyhercz M. IsoLocate, v1.2. 2017; https://anthropologyapps.shinyapps.io/ IsoLocate.

Bowen GJ, Ehleringer JR, Chesson LA, Stange E, Cerling TE. Stable isotope ratios of tap water in the contiguous USA. *Water Resources Research* 2007;43:W03419.

Brettell R, Montgomery J, Evans J. Brewing and stewing: The effect of culturally mediated behaviour on the oxygen isotope composition of ingested fluids and the implications for human provenance studies. *Journal of Analytical Atomic Spectrometry* 2012;27:778.

Cerling TE, Barnette JE, Bowen GJ, Chesson LA, Ehleringer JR, Remien CH, et al.. Forensic stable isotope biogeochemistry. *Annual Review of Earth and Planetary Sciences* 2016;44:175–206.

Chesson LA, Kenyhercz MW, Regan LA, Berg GE. Addressing data comparability in the creation of combined data sets of bioapatite carbon and oxygen isotopic compositions. *Archaeometry* 2019;61:1193-1206.

Chesson LA, Thompson AH, Podlesak DW, Cerling TE, Ehleringer JR. Variation in hydrogen, carbon, nitrogen, and oxygen stable isotope ratios in the modern American diet: Fast food meals. *J Agric Food Chem* 2008;56:4084–4091.

Christensen AM, Crowder CM. Evidentiary standards for forensic anthropology. *Journal of Forensic Sciences* 2009;54: 1211–1216.

Coplen TB. Normalization of oxygen and hydrogen isotope data. *Chemical Geology* 1988; 7:293–7.

Cox G, Sealy J. Investigating identity and life history: Isotopic analysis and historical documentation of slave skeletons found in the Cape Town foreshore, South Africa. *International Journal of Historical Archaeology* 1997;1:207–224.

Craig H. Isotopic variations in meteoric waters. *Science* 1961;133: 1702–1703.

Crowley BE, Wheatley PV. To bleach or not to bleach? Comparing treatment methods for isolating biogenic carbonate. *Chemical Geology* 2014;381:234–242.

Dansgaard W. Stable Isotopes in Precipitation. *Tellus* 1964;16: 436–468.

Daux V, Lecuyer C, Heran M-A, Amiot R, Simon L, Fourel F, et al. Oxygen isotope fractionation between human phosphate and water revised. *Journal of Human Evolution* 2008;55: 1138–1147.

DeNiro MJ, Schoeninger MJ, Hastorf CA. Effect of heating on the stable carbon and nitrogen isotope ratios of bone collagen. *Journal of Archaeological Science* 1985;12:1–7.

Dunn PJH, Carter JF, eds. *Good practice guide for isotope ratio mass spectrometry*, 2nd ed. Bristol, UK: FIRMS; 2018.

Dunn PJH, Salouros H, Carter JF, Doyle SP. Forensic application of stable isotope delta values: Proposed minimum requirements for method validation. *Rapid Commun Mass Spectrom* 2017;31:1476–1480.

Dutton A, Wilkinson BH, Welker JM, Bowen GJ, Lohmann KC. Spatial distribution and seasonal variation in $^{18}O/^{16}O$ of modern precipitation and river water across the conterminous USA. *Hydrological Processes* 2005;19:4121–4146.

Eerkens JW, Sullivan K, Greenwald AM. Stable isotope analysis of serial samples of third molars as insight into inter- and intra-individual variation in ancient diet. *Journal of Archaeological Science: Reports* 2016;5:656–663.

Eerkens JW, Berget AG, Bartelink EJ. Estimating weaning and early childhood diet from serial micro-samples of dentin collagen. *Journal of Archaeological Science* 2011;38:3101–3111.

Ehleringer JR, Bowen GJ, Chesson LA, West AG, Podlesak DW, Cerling TE. Hydrogen and oxygen isotope ratios in human hair are related to geography. *Proceedings of the National Academy of Sciences USA* 2008;105:2788–2793

Ehleringer JR, Thompson AH, Podlesak DW, Bowen GJ, Chesson LA, Cerling TE, et al. A framework for the incorporation of isotopes and isoscapes in geospatial forensic investigations. In: West JB, Bowen GJ, Dawson TE, Tu KP, eds. *Isoscapes*. New York: Springer; 2010:357–387.

Ehleringer JR, Covarrubias Avalos S, Tipple BJ, Valenzuela LO, Cerling TE. Stable isotopes in hair reveal dietary protein sources with links to socioeconomic status and health. *Proceedings of the National Academy of Sciences USA* 2020;117: 20044–20051.

Fahy GE, Deter C, Pitfield R, Miszkiewicz JJ, Mahoney P. Bone deep: Variation in stable isotope ratios and histomorphometric measurements of bone remodelling within adult humans. *Journal of Archaeological Science.* 2017;87:10–16.

Fenton TW, Birkby WH, Cornelison J. A Fast and Safe Non-Bleaching Method for Forensic Skeletal Preparation. *Journal of Forensic Sciences* 2003;48:274–276.

Font L, van der Peijl G, van Wetten I, Vroon P, van der Wagt B, Davies G. Strontium and lead isotope ratios in human hair: Investigating a potential tool for determining recent human geographical movements. *Journal of Analytical Atomic Spectrometry* 2012;27:719–732.

Gagnon CM, Andrus CFT, Ida J, Richardson N. Local water source variation and experimental Chicha de Maiz brewing: Implications for interpreting human hydroxyapatite $\delta^{18}O$ values in the Andes. *Journal of Archaeological Sciences Reports* 2015;4:174–181.

Ghosh SK. Human cadaveric dissection: a historical account from ancient Greece to the modern era. *Anatomy & Cell Biology* 2015;48:153–69.

-1—
0—
+1—

Gulson BL, Jameson CW, Gillings BR. Stable lead isotopes in teeth as indicators of past domicile-a potential new tool in forensic science? *Journal of Forensic Science* 1997;42:787–791.

Healy CA, Schultz JJ, Parker K, Lowers B. Detecting submerged bodies: Controlled research using side-scan sonar to detect submerged proxy cadavers. *Journal of Forensic Sciences* 2015;60:743–752.

Hedges REM, Clement JG, Thomas DL, O'Connell TC. Collagen turnover in the adult femoral mid-shaft: Modeled from anthropogenic radiocarbon tracer measurements. *American Journal of Physical Anthropology* 2007;133:808–816

Hodgins GWL. Measuring atomic bomb-derived ^{14}C levels in human remains to determine Year of Birth and/or Year of Death. *NIJ Report* 2009;98.

Hu L, Chartrand MMG, St-Jean G, Lopes M, Bataille CP. Assessing the reliability of mobility interpretation from a multi-isotope hair profile on a traveling individual. *Frontiers Ecology Evolution* 2020;8:568943.

Iacumin P, Bocherns H, Mariotti A, Longinelli A. Oxygen isotope analyses of co-existing carbonate and phosphate in biogenic apatite: A way to monitor diagenetic alteration of bone phosphate? *Earth and Planetary Science Letters* 1996;142:1–6.

Kamenov GD, Kimmerle EH, Curtis JH, Norris D. Georeferencing a cold case victim with lead, strontium, carbon, and oxygen isotopes. *Annals of Anthropological Practice* 2014;38: 137–154.

Kamenov GD, Curtis JH. Using carbon, oxygen, strontium, and lead isotopes in modern human teeth for forensic investigations: A critical overview based on data from Bulgaria. *Journal of Forensic Sciences* 2017;62:1452–1459

Kellner CM, Schoeninger MJ. A simple carbon isotope model for reconstructing prehistoric human diet. *American Journal of Physical Anthropology* 2007;133:1112–1127.

Katzenberg MA. Stable isotope analysis: A tool for studying past diet, demography, and life history. In: Katzenberg MA, Saunders SR, eds. *Biological Anthropology of the Human Skeleton*. New York: Wiley-Liss; 2000:305–328

Kim S-T, Coplen TB, Horita J. Normalization of stable iotope data for carbonate

minerals: Implementation of IUPAC guidelines. *Geochim Cosmochim Acta* 2015;158;276–289.

King C, Birch W. Assessment of maceration techniques used to remove soft tissue from bone in cut mark analysis. *Journal of Forensic Sciences* 2015;60:124–135.

Koch PL, Tuross N, Fogel ML. The effects of sample treatment and diagenesis on the isotopic integrity in biogenic hydroxylapatite. *Journal of Archaeological Science* 1997;24: 417–429.

Kohn MJ, Cerling TE. (2002). Stable isotope compositions of biological apatites. In: Kohn MJ, Rakovan J, Hughes JM eds. Phosphates. Geochemical, Geobiological and Materials Importance. *Reviews in Mineralogy and Geochemistry*, v. 48. Mineralogical Society of American, Washington, DC 2002;455–488.

Kramer RT, Bartelink EJ, Herrmann NP, Bataille CP, Spradley K. (2020). Application of stable isotopes and geostatistics to infer geographic origin for deceased undocumented Latin American migrants. In: Parra RC, Zapico SC, Ubelaker DH eds. *Forensic Science and Humanitarian Action: Interacting with the Dead and the Living.* John Wiley and Sons 2020;425–440.

Laffoon JE, Sonnemann TF, Shafie T, Hofman CL, Brandes U, Davies GR. Investigating human geographic origins using dual-isotope ($^{87}Sr/^{86}Sr$, $\delta^{18}O$) assignment approaches. *PlosONE* 2017;12:e0172562.

Lamb AL, Evans JE, Buckley R, Appleby J. Multi-isotope analysis demonstrates significant lifestyle changes in King Richard III. *Journal of Archaeological Science* 2014;50:559–565.

Lee EJ, Luedtke JG, Allison JL, Arber CE, Merriweather DA, Steadman DW. The effects of different maceration techniques on nuclear DNA amplification Using Human Bone. *Journal of Forensic Sciences* 2010;55:1032–1038.

Lee-Thorp JA. Two decades of progress towards understanding fossilization processes and isotopic signals in calcified tissue minerals. *Archaeometry* 2002;44:435–46.

Lehn C, Lihl C, Roßmann A. Change of geographical location from Germany (Bavaria) to USA (Arizona) and its effect on H–C–N–S stable isotopes in human hair. *Isotopes Environment Health. Studies* 2015a;51:68–79.

Lehn C, Mützel E, Rossmann A. Multi-element stable isotope analysis of H, C, N and S in hair and nails of contemporary human remains. *International Journal of Legal Medicine* 2011;125: 695–706.

Lehn C, Rossmann A, Graw M. Provenancing of unidentified corpses by stable isotope techniques-presentation of case studies. *Science and Justice* 2015b;55:72–88.

Lehn C, Rossmann A, Graw M, Davies GR. Identification of a female murder victim found in Burgenland, Austria in 1993. *Forensic Sciences Research* 2022;7:308–318.

Mairs S, Swift B, Rutty GN. Detergent: an alternative approach to traditional bone cleaning methods for forensic practice. *American Journal of Forensic Medicine and Pathology* 2004;25: 276–284.

Mancuso CJ, Ehleringer JR. Resident and nonresident fingernail isotopes reveal diet and travel patterns. *Journal of Forensic Sciences* 2019;64:77–87.

Matuszewski S, Hall MJR, Moreau G, Schoenly KG, Tarone AM, Villet MH. Pigs vs. people: The use of pigs as analogues for humans in forensic entomology and taphonomy research. *International Journal of Legal Medicine* 2020;134:793–810.

Maurer A-F, Person A, Tütken T, Amblard-Pison S, Ségalen L. Bone diagenesis in arid environments: An intra-skeletal approach. *Palaeogeography, Palaeoclimatology, Palaeoecology* 2014;416:17–29.

Meier-Augenstein W, Fraser, I. Forensic isotope analysis leads to identification of a mutilated murder victim. *Science & Justice* 2008;48:153–159.

Meier-Augenstein W, Shimmelmann, A. A guide for proper utilization of stable isotope reference materials. *Isotopes in Environmental and Health Studies* 2019;55:113–128.

Munro LE, Longstaffe FJ, White CD. Effects of heating on the carbon and oxygen-isotope compositions of structural carbonate in bioapatite from modern deer bone. *Palaeogeography, Palaeoclimatology, Palaeoecology* 2008;266:142–150.

Nardoto GB, Silva S, Kendall C, Ehleringer JR, Chesson LA, Ferraz ESB, et al. Geographical patterns of human diet derived from stable-isotope analysis of fingernails. *American Journal of Physical Anthropology* 2006;131:137–146

O'Brien DM, Wooller MJ. Tracking human travel using stable oxygen and hydrogen isotope analyses of hair and urine. *Rapid Communications in Mass Spectrometry* 2007;21:2422–2430.

Olsen KC, White CD, Longstaffe FJ, von Heyking K, McGlynn G, Grupe G, Rühli FJ. Intraskeletal isotopic compositions ($\delta^{13}C$, $\delta^{15}N$) of bone collagen: Nonpathological and pathological variation. *American Journal of Physical Anthropology* 2014;153: 598–604.

Parfitt AM. (1983). The physiologic and clinical significances of bone histomorphometric data. In: Recker RR ed. *Bone Histomorphometry: Techniques and Interpretation*, CRC Press, Boca Raton, Florida, 1983;143–223.

—-1
—0
—+1

Pearson OM, Lieberman DE. The aging of Wolff's "Law": Ontogeny and responses to mechanical loading in cortical bone. *Yearbook of Physical Anthropology* 2004;47:63–99.

Pestle WJ, Crowley BE, Weirauch MT. Quantifying inter-laboratory variability in stable isotope analysis of ancient skeletal remains. *PlosONE* 2014;9:e102844.

Plomp E, von Holstein ICC, Kootker LM, Verdegaal-Warmerdam SJA, Forouzanfar T, Davies GR. Strontium, oxygen, and carbon isotope variation in modern human dental enamel. *American Journal of Physical Anthropology* 2020;172:586–604.

Quinn RL. Isotopic equifinality and rethinking the diet of *Australopithecus anamensis*. *American Journal of Physical Anthropology* 2019; 169:403–421.

Quinn RL, Alesbury H, Ceja L, Soler A, Godfrey L. Food has no borders: Insights from the forensic isotopic profile of a New York City immigrant. *Forensic Anthropology* 2021;4:14–25.

Quinn RL, Alesbury H, Soler A, Ceja L, Godfrey L, Setera JB. Isotopic analyses of New York City's unidentified individuals: differentiating migrants from local residents in a multicultural metropolitan context. *Proceedings of the American Association of Forensic Sciences* 2020;A112.

Rauch E, Rummel S, Lehn C, Büttner A. Origin assignment of unidentified corpses by use of stable isotope ratios of light (bio-) and heavy (geo-) elements—a case report. *Forensic Sci Int* 2007;168:215–218.

Regan LA, Bower NW, Brown SJ, Lundstrom CC, Bartov G, Cooney MD. Forensic isoscapes based on intra-individual temporal variation of $\delta^{18}O$ and $^{206}Pb/^{207}Pb$ in human teeth. *Forensic Science Research* 2021;6:42–52.

Reitsema L. Beyond diet reconstruction: Stable isotope applications to human physiology, health and nutrition. *American Journal of Human Biology* 2013;25:445–456.

Remien CH, Adler FR, Chesson LA, Valenzuela LO, Ehleringer JR, Cerling TE. Deconvolution of isotope signals from bundles of multiple hairs. *Oecologia* 2014;175:781–789.

Rennick SL, Fenton TW, Foran DR. The effects of skeletal preparation techniques on DNA from human and non-human bone. *Journal of Forensic Sciences* 2005;50:1016–1019.

Sarancha JJ, Eerkens J, Hopkins CJ, Goncalves D, Cunha E, Oliveira-Santos I, et al. The effects of burning on isotope ratio values in modern bone: Importance of experimental design for forensic applications. *Forensic Science International* 2020; 337:111370.

Schoeninger MJ. Stable isotope evidence for the adoption of maize agriculture. *Current Anthropology* 2009;50:633–640.

Schwarcz HP, Melbye J, Katzenberg A, Knyf M. Stable isotopes in human skeletons of southern Ontario: Reconstructing paleodiet. *Journal of Archaeological Science* 1985;12:187–206.

Spalding KL, Buchholz BA, Bergman LE, Druid H, Frisen J. Age written in teeth by Nuclear Tests. *Nature* 2005;437:333–334.

Steadman DW, DiAntonio LL, Wilson JJ, Sheridan KE, Tammariello SP. The effects of chemical and heat maceration techniques on the recovery of nuclear and mitochondrial DNA from bone. *Journal of Forensic Sciences* 2006;51:11–17.

Szpak P, Metcalfe JZ, Macdonald RA. Best practices for calibrating and reporting stable isotope measurements in archaeology. *Journal of Archaeological Science: Reports* 2017;13:609–616.

Tuross N, Reynard LM, Harvey E, Coppa A, McCormick M. Human skeletal development and feeding behavior: the impact on oxygen isotopes. *Archaeological Anthropology* 2017;9: 1453–1459.

Ubelaker DH, Buchholtz BA, Stewart JEB. Analysis of artificial radiocarbon in different skeletal and dental tissue types to evaluate date of death. *Journal of Forensic Sciences* 2006; 51:484–488.

Ubelaker DH, Parra RC. Radiocarbon analysis of dental enamel and bone to evaluate date of birth and death: Perspective from the southern hemisphere. *Forensic Science International* 2011; 208:103–107.

Valenzuela LO, Chesson LA, O'Grady SP, Cerling TE, Ehleringer JR. Spatial distributions of carbon, nitrogen and sulfur isotope ratios in human hair across the central United States. *Rapid Communications in Mass Spectrometry* 2011; 25:861–868.

Wang Y, Cerling TE. A model of fossil tooth and bone diagenesis: Implications for paleodiet reconstruction from stable isotopes. *Palaeogeography, Palaeoclimatology, Palaeoecology* 1994; 107:281–289.

Waterisotopes.org. https://wateriso.utah.edu/waterisotopes. Created 2003. Accessed February-March, 2023.

West JB, Bowen GJ, Dawson TE, Tu KP. *Isoscapes*. New York: Springer 2010.

Yoder CJ, Bartelink EJ. Effects of different sample preparation methods on stable carbon and oxygen isotope values of bone apatite: A comparison of two treatment protocols. *Archaeometry* 2009;52:115–130.