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Stable isotope indicators of provenance and demographics in 18th and 19th century North Americans

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ABSTRACT

Using stable isotopes to gain insight into individual life histories is a valuable tool for unidentified or incomplete remains lacking historic records. This study analyzed stable carbon, nitrogen, and oxygen isotopes from bones and teeth of 18^{th}–19^{th} century North Americans of known ancestry, social class, and region of origin in an effort to discern qualitative patterns and create a quantitative predictive model of demographic information. The $\delta^{13}C_{collagen}$, $\delta^{13}C_{structural\ carbonate}$, and $\delta^{18}O_{structural\ carbonate}$ values provide the most overall information for detecting demographic differences, with $\delta^{15}N_{collagen}$ and $\delta^{18}O_{phosphate}$ to a lesser degree. Region of origin was the most predictable demographic factor with 82% correct classifications based on a two-variable model using $\delta^{13}C_{collagen}$ and $\delta^{18}O_{meteoric\ water}$ calculated from $\delta^{18}O_{structural\ carbonate}$, which reflects the influence of dominant local vegetation types and local drinking water. Ancestry was correctly identified in 68% of cases using $\delta^{13}C_{collagen}$. Social class was less predictable with correct identification in 60% of cases based on $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values where the upper class was most distinguishable. Isotope patterns observed in ancestry and social class groups are linked to cultural food preferences and food availability. Certain sample sites, such as military burials and urban cemeteries, show a greater range of isotope values suggesting a variety of individual regional origins and cultural backgrounds. Burials of extreme upper or lower class individuals show greater isotopic homogeneity suggesting reliance on localized food sources or cultural preferences for particular dietary choices.

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1. Introduction

Archaeological, anthropological, and forensic studies rely upon basic demographic information such as ancestry, age, social class, and provenance to draw sociological conclusions and interpret the life history of deceased individuals. Morphometric analyses may fail to yield such information from poorly-preserved or incomplete remains and written historical records are often non-existent. This study examines stable carbon, nitrogen, and oxygen isotopes in bones and teeth as qualitative indicators and a quantitative methodology for input into the statistical prediction of demographic information. These isotopes are proxies for residence and dietary habits linked to ancestry or social class (Ambrose, 1993; Koch, 1998; Koch et al., 1994; MacFadden, 2000; Peterson and Fry, 1987). A large sample of North American archeological remains (18^{th}–19^{th} centuries) with known demographics is used as an exemplar for developing a predictive statistical isotope model for regional, ancestry, and social status combinations from remains of indeterminate origin.

1.1. Stable isotope theory and considerations

Bones and teeth consist of organic and mineral components that record isotopic information. Well-preserved bones and tooth dentin contain collagen, a durable protein with carbon and nitrogen isotopes incorporated largely from dietary protein (Ambrose and Norr, 1993; Froehle et al., 2010; Hedges, 2003; Jim et al., 2004; Krueger and Sullivan, 1984; Lee-Thorp et al., 1989; Tieszen and Fagre, 1993). Bone, tooth enamel, and tooth dentin contain phosphate ($-PO_4$) in the mineral hydroxyapatite and structural carbonate ($-CO_3$) substituting for $-PO_4$ and $-OH$ groups in hydroxyapatite, both of which incorporate oxygen isotopes from drinking water (Bryant and Froelich, 1995; Kohn, 1996; Luz and Kolodny, 1985). Structural carbonates also include carbon isotopes incorporated largely through blood dissolved carbonates that reflect carbohydrate and lipid dietary components (Hedges, 2003; Tieszen and Fagre, 1993; Zazzo et al., 2010). Bone collagen and hydroxyapatite remodel throughout life and capture a lifetime

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average of isotopic input (Fogel et al., 1997; Francillon-Vieillot et al., 1990; Katzenberg, 1993; Stenhouse and Baxter, 1979). Teeth remodel very little after formation and capture isotope values of diet and water input during the shorter span of tooth formation only (Carlson, 1990).

Isotope values are reported in standard delta notation:

$$\delta X = \left[\left(R_{\text{sample}} - R_{\text{standard}}\right)\Big/\left(R_{\text{standard}}\right)\right]*1000$$

where X represents the system of interest (i.e. ^{13}C, ^{15}N, ^{18}O), R represents a ratio (i.e. $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$), and units are per mil (‰). The standards are V-PDB, atmospheric air, or V-SMOW for C, N, or O, respectively. This study analyzes carbon and nitrogen isotopes of bone and tooth collagen ($\delta^{13}C_{\text{collagen}}$ and $\delta^{15}N_{\text{collagen}}$), oxygen isotopes in phosphates and structural carbonates ($\delta^{18}O_{\text{phosphate}}$ and $\delta^{18}O_{\text{structural carbonate}}$), and carbon isotopes of structural carbonates ($\delta^{13}C_{\text{structural carbonate}}$).

Carbon isotopes indicate the plant type consumed by an individual. Two photosynthetic pathways common to most plants, C3 (dicots, most trees, shrubs, few grasses, and wheat) and C4 (specific grasses and sedges including corn), produce distinct isotope ranges with the former depleted in ^{13}C (Heaton, 1999; O'Leary, 1988; Smith and Epstein, 1971). Given the fractionation of ~2–5‰ between plants and bone collagen (Balasse et al., 1999; Hedges, 2003; Koch, 1998; Roth and Hobson, 2000; van der Merwe, 1982), humans consuming a strict C3 diet exhibit $\delta^{13}C_{\text{collagen}}$ values of ~-22 to −18‰, while those consuming a strict C4 diet (largely corn-based) exhibit values of ~−11 to −7‰. Given the fractionation of ~12–15‰ between diet and bone structural carbonate in large mammals (Hedges, 2003; Koch, 1998; Kohn and Cerling, 2002; Passey et al., 2005; Zazzo et al., 2010), a strict C3 diet exhibits $\delta^{13}C_{\text{structural carbonate}}$ values of ~−13 to −9‰, while a strict C4 diet exhibits values of ~−1 to +3‰. Most human isotope values fall within these endpoints as people typically consume a mix of plant types and livestock/game with mixed feed. Given that isotopically enriched C4 grasses and corn crops were more common in warmer southern regions, the difference between C3- and C4-based diets translates to the difference between a primarily northern versus southern diet, respectively, for North Americans. Southerners should have enriched $\delta^{13}C$ values since both wild and domesticated animals consume local plants, and corn itself was a staple in southern diets (Pace, 1993). Northern diets, European-style diets of immigrants, or European-style diets consumed as a cultural preference would include less corn and produce $\delta^{13}C$ values in the C3 range.

Nitrogen isotopes reflect trophic position and amount of meat in the diet. Mammals, including humans, fractionate nitrogen between diet and bone collagen, producing a ~3–4‰ stepwise enrichment with trophic level (Bocherens and Drucker, 2003; DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Post, 2002; Schoeninger and DeNiro, 1984; Sutoh et al., 1987). Archaeological North Americans exhibits $\delta^{15}N_{\text{collagen}}$ values of ~+8 to +14‰ indicating a high position in the trophic structure and significant carnivory (Fogel et al., 1997; Katzenberg, 1993; Katzenberg et al., 2000; Raynor and Kennett, 2008; Ubelaker and Owsley, 2003). In a time period when ancestry and social class are intimately linked, it is hypothesized that lower classes and African Americans had less access to meat and relied on less expensive, readily available grains, resulting in depleted $\delta^{15}N_{\text{collagen}}$.

Oxygen isotopes in precipitation and meteoric drinking water have clear regional patterns in North America with enriched values in warmer lower latitudes and depleted values in cooler higher latitudes (Bowen and Wilkinson, 2002; Dutton et al., 2005; Kendall and Coplen, 2001). The $\delta^{18}O$ of local meteoric drinking water correlates with mammalian body water and $\delta^{18}O_{\text{phosphate}}$ with subtle variations in fractionation according to species and climatic variables (Bryant and Froelich, 1995; D'Angela and Longinelli, 1990; Daux et al., 2008; Kohn, 1996; Levinson et al., 1987; Longinelli, 1984; Luz and Kolodny, 1985; Luz et al., 1984). Observed $\delta^{18}O_{\text{phosphate}}$ and $\delta^{18}O_{\text{structural carbonate}}$ therefore serve as a proxy for region of origin with northerners showing more depleted $\delta^{18}O$ values than southerners.

Inherent in the use of any isotope system for investigating demographic information is the assumption that sampled individuals relied upon a fairly local diet and remained regionally stationary. Immigration, imported foods, or extensive cross-regional migrations due to cultural influences or military service could produce a biased indication of provenance. For this reason, archaeological remains from the 18th–19th centuries represent an initial test case insofar as these people were more likely to adhere to these assumptions.

2. Methods and materials

2.1. Samples

Sample localities consist of twelve 18th–19th century North American burial sites (Table 1). Remains were procured through associations with state and federal agencies, universities, contract archaeology programs, and local administrators of churches and cemeteries. All individuals were evaluated by the Smithsonian's National Museum of Natural History Division of Physical Anthropology. All individuals included in this study were adults. Ancestry classifications and sex were assigned based on osteological criteria. Region of origin and social class were assigned based on site locations and historical records.

2.2. Chemical extraction

Solid bone chunks and tooth dentin were isolated for collagen extraction using a rotary tool or bone saw. Bone, tooth dentin, and tooth enamel were powdered for phosphate and structural carbonate analyses using a mortar and pestle.

Collagen was extracted from bones and tooth dentin according to modified methods of Longin (1971). Solid bone and dentin pieces (~200–500 mg) were sonicated in water to remove sediments and labile salts, and demineralized in 0.6 M HCl at 4 °C for 24 h increments (acid replaced daily) until reaction ceased. Samples were rinsed to neutrality before soaking in 0.125 M NaOH for 24 h to remove humic and fulvic acid contaminants. Remaining crude extract was reacted in 0.03 M HCl at 95 °C for 18 h to separate soluble and insoluble phases of collagen. The resulting supernatant was lyophilized to isolate purified collagen extract.

Structural carbonate was extracted from bones, tooth dentin, and tooth enamel by modified methods of Bryant et al. (1996). Approximately 20 mg of powdered material was soaked in 2–3% sodium hypochlorite to remove organic components. Samples were rinsed and soaked in 1 M acetic acid solution buffered with 1 M calcium acetate (pH~4.5) for 4 h to remove secondary carbonate phases. Samples were rinsed to neutrality and dried (60 °C).

Phosphate was isolated from bones, tooth dentin, and tooth enamel using the method of Dettman et al. (2001). Approximately 20 mg of powdered material was soaked in 2 M hydrofluoric acid for 24 h to liberate phosphate ions. The resulting supernatant was isolated, diluted, and neutralized with 20% ammonium hydroxide. Insoluble silver phosphate was precipitated by addition of 2 M silver nitrate, then rinsed and dried (60 °C).

Table 1
Sample localities and demographic information.

Site	Site location	Time period	Origin of individuals[b]	Ancestry	Socio-economic status[c]
A.P. Hill	Ft. A.P. Hill, VA	1780–1830	Southern United States	African American	Slaves
Congressional Cemetery	Washington, DC	1850–1900	Washington, DC and surrounding areas	Caucasian	High status, burial in affluent family tombs
First African Baptist Church (FABC)	Philadelphia, PA	1824–1842	Philadelphia	African American	Free black, possibility of run-away slaves
Ft. Craig-C[a]	New Mexico	1854–1877	Northern United States, probably some European immigrants	Caucasian	Military – Union, enlisted soldiers
Ft. Craig-A[a]	New Mexico	1866–1877	Louisiana, Kentucky, Virginia	African American	Military – Union, enlisted soldiers, some former slaves
Foscue Plantation	North Carolina	1800–1849	Southern United States	Caucasian	High status – plantation family
Glorieta Pass	New Mexico	1862	Texas, a few possible European immigrants	Caucasian	Military – Confederate, mostly enlisted, 1 officer included
Kincheloe Cemetery	Virginia	1830–1860	Southern United States	Caucasian	Middle – high status – plantation family
Parkway Gravel	Delaware	1850–1900	Delaware	African American	Slaves or former slaves
Pettus	Virginia	1700's–1800's	Virginia, possibility of direct African origin	African American	Slaves
Trinity Catholic Church	Georgetown, Washington, DC	1800–1850	Washington, DC and surrounding areas	Caucasian	Middle class
Walton Family Cemetery	Connecticut	1750–1830	Connecticut	Caucasian	Farmers
Woodville Cemetery	Delaware	1790–1850	Delaware	Caucasian	Middle class

[a] The letters "C" and "A" denote Caucasian and African American individuals, respectively.
[b] Statistical classifications: northern (Congressional Cemetery, FABC, Ft. Craig-C, Parkway Gravel, Trinity Catholic Church, Walton Family Cemetery, Woodville Cemetery), southern (A.P. Hill, Foscue Plantation, Glorieta Pass, Kincheloe Cemetery, Pettus).
[c] Statistical classifications: upper class (Congressional Cemetery, Foscue Plantation, Kincheloe Cemetery, 1 Glorieta Pass officer), middle class (Trinity Catholic Church, Walton Family Cemetery, Woodville Cemetery), military (Ft. Craig-A, Ft. Craig-C, Glorieta Pass), lower class (A.P. Hill, FABC, Parkway Gravel, Pettus).

2.3. Mass spectrometry methods

All samples were analyzed on Thermo Delta V Advantage mass spectrometers at the Smithsonian OUSS/MCI Stable Isotope Mass Spectrometry Laboratory. Collagen was weighed into tin cups, combusted in a Costech 4010 Elemental Analyzer, and the resulting N_2 and CO_2 gases measured for $\delta^{15}N_{collagen}$ and $\delta^{13}C_{collagen}$ values. Silver phosphates were weighed into silver cups, thermally decomposed in a Thermo High Temperature Conversion Elemental Analyzer, and the resulting CO gas measured for $\delta^{18}O_{phosphate}$. Structural carbonates were acidified in concentrated phosphoric acid (SG $\geq$ 1.92) at 25 °C for 24 h. The released CO_2 gas was introduced to the mass spectrometer via a GC Pal and Thermo GasBench II system and measured for $\delta^{13}C_{structural\ carbonate}$ and $\delta^{18}O_{structural\ carbonate}$. All samples were calibrated and corrected to international standards. The $\delta^{13}C_{collagen}$, $\delta^{15}N_{collagen}$, $\delta^{13}C_{structural\ carbonate}$, and $\delta^{18}O_{structural\ carbonate}$ values have an error of $\pm 0.2‰$ (1σ); $\delta^{18}O_{phosphate}$ values have an error of $\pm 0.4‰$ (1σ).

2.4. Analysis of diagenesis

Burial introduces potential for diagenetic alteration. Samples included in statistical analyses were selected based on previously determined criteria for well-preserved collagen: a C:N ratio of 2.8–3.6 and weight %N yield of ~11–16% (Ambrose, 1990; Bocherens et al., 1991, 1994, 1996, 1997; DeNiro, 1985; Drucker et al., 2001, 2003; Jorkov et al., 2007; McNulty et al., 2002). Preservation criteria for phosphates and structural carbonates have been studied separately, but are arguably less well established (Iacumin et al., 1996b; Kohn et al., 1999; Michel et al., 1995; Person et al., 1995, 1996; Tuross et al., 1989; Zazzo et al., 2004). Hydroxyapatite grains within bones and teeth are essentially nested within the collagen protein matrix thereby protecting the mineral grains from recrystallization and, presumably, isotope alteration (Nelson et al., 1986; Person et al., 1996; Tütken et al., 2008; Veis, 2003). For this reason the criteria for collagen were used as the deciding factors in determining preservation quality.

2.5. Meteoric water conversion

To facilitate the examination of regional differences, oxygen isotope values were converted to average meteoric water values. While the absolute $\delta^{18}O_{structural\ carbonate}$ and $\delta^{18}O_{phosphate}$ values can be used directly to analyze regional differences statistically, we present our analyses of the converted $\delta^{18}O_{meteoric\ water}$ values to assist future research into the provenance of unknown individuals where a $\delta^{18}O_{meteoric\ water}$ value is critical to assigning region of origin. Several formulas have been published for such conversions; this study utilizes Bryant et al. (1996) to calculate $\delta^{18}O_{body\ water}$ from $\delta^{18}O_{structural\ carbonate}$, and Longinelli (1984) to calculate $\delta^{18}O_{meteoric\ water}$ from $\delta^{18}O_{phosphate}$ and $\delta^{18}O_{body\ water}$. This combination produces $\delta^{18}O_{meteoric\ water}$ values in agreement with $\delta^{18}O_{meteoric\ water}$ ranges in the regions of origin for individuals included herein.

2.6. Statistics

Descriptive statistics were computed on all isotope variables using indicators of sex, ancestry, region, social class and burial site. We tested for equality between body elements (i.e. bone, dentin, enamel) both as repeated measures on the same individual and across differing individuals. Elementary statistics and tests were completed on SPSS ver. 20 (2012). Each variable and combinations satisfied the basic linear model assumptions of normality of population distribution, equality of variances and independence of error distributions. General linear models of ancestry, regional, and social class groups were constructed, tested and compared for predictive ability using multivariate analyses on SAS ver 9.2 (2010) and MathCad (2012). Within a general linear model framework, the usual analysis of variance involves p groups in general. The multivariate extension in vector notation involves 2 or more criterion variables with H_0: $m_1 = m_2 = \ldots m_k$, where the m are the kx1 vectors of means. Since our purposes are predictive we also require that the Mahalanobis' D^2 test rather than the analysis of variance conceptualization of Hotelling's T^2 test, but where the former test statistic

Table 2
Descriptive statistics on totals and test results with isotope variables from three type sources: bone, dentin and enamel.

Type[a]		$\delta^{18}O_{phosphate}$ (‰, VSMOW)	$\delta^{18}O_{meteoric\ water}$[b] (‰, VSMOW)	$\delta^{13}C_{structural\ carbonate}$ (‰, VPDB)	$\delta^{18}O_{structural\ carbonate}$ (‰, VSMOW)	$\delta^{18}O_{meteoric\ water}$[c] (‰, VSMOW)	$\delta^{15}N_{collagen}$ (‰, air)	$\delta^{13}C_{collagen}$ (‰, VPDB)
Bone	*N*	135	135	133	133	133	134	134
	Mean	17.4	−7.9	−9.0	24.8	−3.6	10.8	−14.2
	St Dev	1.1	1.7	2.0	1.6	2.6	0.9	2.4
Dentin	*N*	41	41	39	39	39	41	41
	Mean	19.0	−5.3	−9.1	27.8	1.3	10.7	−14.3
	St Dev	1.2	1.9	2.8	1.8	3.0	1.1	3.3
Enamel	*N*	44	44	40	40	40	–	–
	Mean	19.8	−4.0	−9.2	27.4	0.7	–	–
	St Dev	1.3	2.1	3.1	1.3	2.2	–	–
Total	*N*	220	220	212	212	212	175	175
	Mean	18.1	−6.6	−9.1	25.8	−2.0	10.7	−14.2
	St Dev	1.6	2.4	2.4	2.1	3.4	1.0	2.7
Test of variance equality	*p*[e]	0.224	0.224	<0.0001[d]	0.303	0.303	0.686	0.052
Test of means	*F*; df	90.6; 2217	90.6; 2217	0.179; 2209	78.4; 2209	78.4; 2209	0.339; 1173	0.091; 1173
	p[e]	0.000	0.000	0.836	0.000	0.000	0.561	0.763

[a] All bones and teeth were sampled from adult individuals.
[b] Values are $\delta^{18}O_{meteoric\ water}$ calculated from $\delta^{18}O_{phosphate}$ using the equation of Longinelli (1984).
[c] Values are $\delta^{18}O_{meteoric\ water}$ calculated from $\delta^{18}O_{structural\ carbonate}$ using the equation of Longinelli (1984) and Bryant et al. (1996).
[d] For $\delta^{13}C_{structural\ carbonate}$ because the Levene test for variances was significant, both Welch's ANOVA and weighted least squares were performed and both yielded a non-significant result for difference of the three means.
[e] Values are considered significant if $p < 0.05$.

is a simple linear transformation of the latter. This D^2 test then forms a canonical discrimination model of group difference and is the equivalent, within the GLM framework, of multiple regression with a discrete criterion variable of group membership. Fisher's classification functions were derived after group determination. Since our data set comprised a non-random selection in the sense that cemeteries were predetermined, the exact specification of our models cannot be used for strong inferential statements. The most useful information for future isotopic skeletal identification work is use of the variables that we found to be most powerful predictors of each category of interest. One set of 11 individuals known to have lived in both regions (i.e. Fort Craig African Americans) was left out of modeling efforts and used posteriori only for assignment based upon probability structure.

3. Results

3.1. Diagenesis

Approximately 79% of analyzed samples satisfied preservation criteria (135/179 bone samples, 41/50 dentin, 44/50 enamel) and were included in further analyses (Table 1, Supplementary Information). C:N ratios for well-preserved collagen ranged from 3.1 to 3.6 and wt %N ranged from 6.0 to 18.0%.

3.2. Analysis of total sample

The 220 well-preserved samples were examined for differences between bone, dentin, and enamel (i.e. type) isotope values (Table 2, Supplementary Information). Although results for correlations and means tests are the same for the transformed $\delta^{18}O_{meteoric\ water}$ values and the original $\delta^{18}O_{structural\ carbonate}$ or $\delta^{18}O_{phosphate}$ values, we present them in tables since numerical values are distinct. Figs. 1–3 present $\delta^{15}N_{collagen}$ vs. $\delta^{13}C_{collagen}$, $\delta^{13}C_{structural\ carbonate}$ vs. $\delta^{18}O_{meteoric\ water}$ values converted from $\delta^{18}O_{structural\ carbonate}$ to facilitate examination of regional differences, and $\delta^{18}O_{meteoric\ water}$ values converted from $\delta^{18}O_{phosphate}$, respectively. For $\delta^{13}C_{structural\ carbonate}$ and $\delta^{18}O_{structural\ carbonate}$ the mean values varied significantly by type; for $\delta^{13}C_{structural\ carbonate}$ the variances varied by type. We therefore continued with all predictive statistics, univariate and multivariate analyses on bone only, using dentin and enamel when available for comparative purposes. Males and females were not statistically different for means or variances and were analyzed as one set in further tests.

3.3. Analysis by region

Table 3 provides descriptive statistics by region of origin (i.e. northern or southern). Except for $\delta^{15}N_{collagen}$, univariate regional tests detected significant differences between northern and southern means. After analyzing all possible subsets of variables, a model using $\delta^{13}C_{collagen,}$ the most heavily weighted, and $\delta^{18}O_{meteoric\ water}$ best distinguished regions (Fig. 4A). MANOVA using these two variables showed the centroids for north and south could be separated at the $p < 0.0001$ level. For the original data with both the biased procedure based upon the measurements made on bone and a cross classified leave-one-out criterion we obtained approximately 82% correct regional classification with this two-variable model, with 82% of southern and 81% of northern individuals correctly assigned. When isotope variables from tooth dentin were tested separately, the best model for identification of regional differences was also a two-variable model based upon $\delta^{13}C_{collagen}$ and $\delta^{18}O_{meteroric\ water}$ with approximately equivalent correct classification (85%). After model determination we assigned the 11 African Americans from Ft. Craig of indeterminate regional origin. The assignments with highest probability relative to the model resulted in 3 being assigned as northerners and the remainder as southern.

3.4. Analysis by ancestry

Table 3 includes descriptive statistics and test results for divisions by ancestry (i.e. Caucasian/European or African American). Using variables based on bone, univariate tests on the group means showed significant differences for $\delta^{13}C_{collagen}$, $\delta^{13}C_{structural\ carbonate}$, $\delta^{18}O_{structural\ carbonate}$, and $\delta^{18}O_{meteoric\ water}$. No significant differences were detected for $\delta^{15}N_{collagen}$ or $\delta^{18}O_{phosphate}$ means. The best predictive model used the single variable of $\delta^{13}C_{collagen}$ producing a prediction rate of 68%, with approximately equal ancestry assignment rates by region (Fig. 4B).

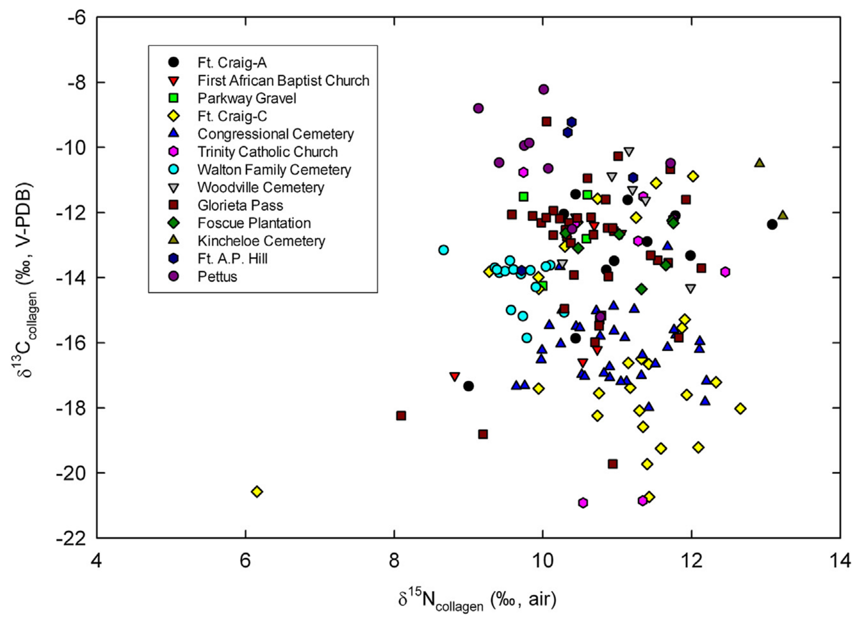

Fig. 1. Carbon and nitrogen isotope values from bone and tooth collagen.

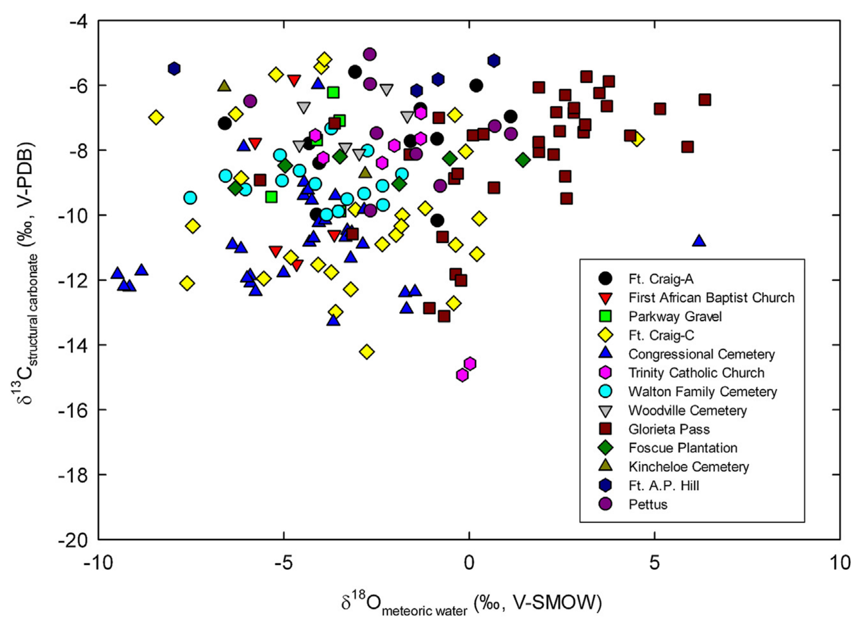

Fig. 2. Carbon and oxygen isotope values from structural carbonates. Oxygen values are converted to meteoric water values.

3.5. Analysis by combined ancestry and regional groupings

Since prediction of ancestry and regional categories were each better than by random chance, we divided the data into simultaneous groupings (i.e. northern Caucasian, southern Caucasian, northern African-American, southern African-American). Descriptive statistics and test results are presented in Table 3. Using variables based upon bone, univariate tests on the group means were significant with each isotope. The best predictive model used $\delta^{13}C_{collagen}$ and $\delta^{18}O_{meteoric\ water}$ with MANOVA tests for these two variables showing significant differences between centroids (Fig. 4C). All 4 groups were pair-wise significantly different with Scheffe's test except for the southern and northern Caucasians. However, we could achieve only 57% total predictability with the lowest correct classification for the southern Caucasians of only 33%. The African-American individuals were classified over 70% correctly regardless of regional group.

3.6. Analysis by social class

Table 3 gives descriptive statistics and test results by approximate socio-economic status (i.e. military, low/slave, middle, and upper class). Significant results for means were obtained except for $\delta^{18}O_{phosphate}$ and the transformed $\delta^{18}O_{meteoric\ water}$. The best predictive model relied upon 4 variables: $\delta^{13}C_{collagen}$, $\delta^{15}N_{collagen}$, $\delta^{18}O_{meteoric\ water}$ (calculated from carbonates), and $\delta^{13}C_{structural\ carbonate}$ (Fig. 4D). However, this model could not pair-wise separate groups of middle class from military, or middle class from slaves.

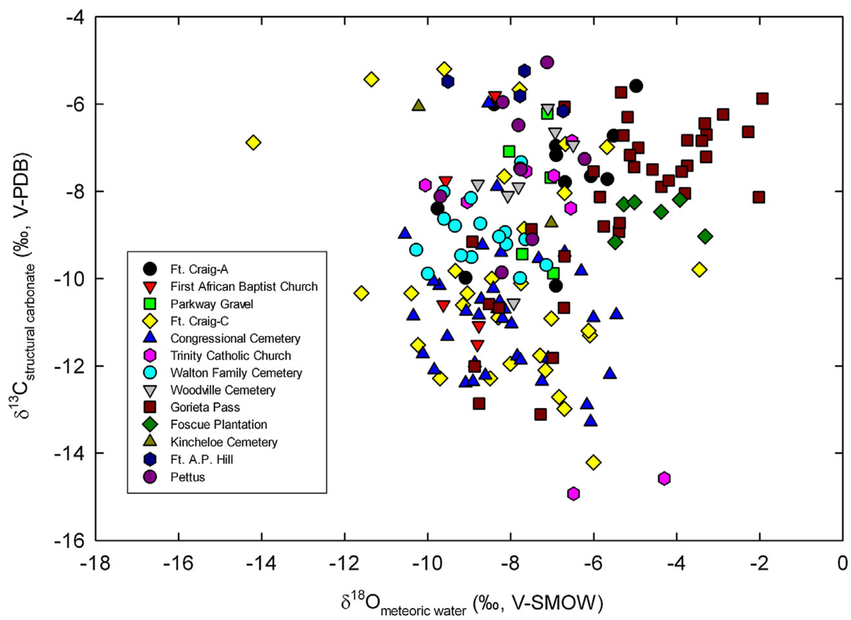

Fig. 3. Oxygen isotope values from phosphates. Oxygen values are converted to meteoric water values and plotted against carbon isotope values from structural carbonates.

Correct classification was approximately 59% slaves, 61% middle class, 68% upper class and 52% military yielding an overall total of 60%.

4. Discussion

4.1. Predictability of demographic factors

The use of carbon, nitrogen, and oxygen isotopes from bones and teeth in our modeling suggests that demographic distinctions are possible. The most notable differences were between regions. Regions were distinguished by $\delta^{18}O$ and $\delta^{13}C_{collagen}$ values, which reflect both latitudinal distributions of $\delta^{18}O$ values in drinking water and $\delta^{13}C$ values in plants. The latitudinal signal from $\delta^{18}O$ was expected, with northerners showing relatively depleted values. The observed regional distinction in $\delta^{13}C_{collagen}$ values (which largely reflect dietary protein) suggests people consumed local meat from animals that digested C3 (northern and southern latitudes) or C4 (present mostly in southern latitudes) plants. The $\delta^{13}C_{structural\ carbonate}$ values are less distinct between regions, although similarly southerners have more enriched average values. This observed $\delta^{13}C_{structural\ carbonate}$ enrichment (which largely reflects dietary carbohydrates and lipids) suggests a greater proportion of corn in the southern diet. Overlapping regional values suggest at least partial consumption of diverse carbohydrates and plant material, possibly from non-local regions. While corn was a more common staple in central and southern regions, apparently trade of crops and growth of localized corn in the north was substantial enough to be reflected in the overall isotope dietary signal. Interestingly, region could be determined with roughly equivalent accuracy using isotopes from bone or teeth. Teeth isotope values do not remodel post-eruption and reflect childhood upbringing. They may represent a more pure test of region of origin whereas adult values from bone, which do remodel continually throughout life, may drift with migration to other localities.

Ancestry groups within our data set were less distinguishable and are likely the result of living environment and available food sources. The strongest predictors of ancestry are $\delta^{13}C_{collagen}$ and $\delta^{18}O$ with African Americans showing more enriched values. The largest influence on these isotopes across ancestry groups is likely the fact that ancestry was strongly linked to region during this time. Secondarily African Americans also showed an enriched $\delta^{13}C_{structural\ carbonate}$ value which may indicate a cultural or economic preference for a corn-based diet.

Social class was the most difficult to distinguish within this data set. The upper class was more easily identified due to considerable overlap in values between middle, military, and lower classes. This difficulty is due in part to the organization of demographic categories within the sites available for this study. Although modeling treats demographic factors as independent, our assigned social classes span the different ancestry categories and regions of origin, thus mingling influences from these factors and increasing the isotope ranges. The observed differences in social class for our data are best distinguished by $\delta^{13}C_{collagen}$, $\delta^{13}C_{structural\ carbonate}$, and $\delta^{15}N_{collagen}$. The majority of lower class individuals are African American and it is likely the $\delta^{13}C$ values differ within social class and ancestry groups for the same reasons. Southern African American slaves, which constitute the majority of values included in the lower class $\delta^{15}N_{collagen}$ average, exhibit depleted $\delta^{15}N_{collagen}$ values ($\leq 1.0‰$) compared to other groups suggesting they had less access to meat protein.

Combining demographic indictors leads to poorer overall prediction. Attempts to classify an individual by combined ancestry and region were less successful than modeling the demographic groups separately. The 4-group model had lower overall correct classifications for Caucasians than African-Americans. Caucasians from two northern sites, Trinity Church and Fort Craig, had large $\delta^{13}C$ and $\delta^{18}O$ ranges overlapping with southern Caucasians. Because of this overlap, a two-part modeling strategy proved more accurate. We recommend assigning separately region of origin with a model using $\delta^{18}O$, and ancestry using $\delta^{13}C$. The $\delta^{15}N$ can then be considered to further isolate levels of social class in the context of hypothesized ancestry and region. This method decreases the variability and increases group identification for individuals of indeterminate demographics.

The best indicators of the demographic classifications considered herein are repeatedly $\delta^{13}C_{collagen}$ and $\delta^{18}O_{structural\ carbonate}$ (and by implication $\delta^{18}O_{meteoric\ water}$). The demographic classifications provided by $\delta^{13}C_{structural\ carbonate}$ and $\delta^{18}O_{phosphate}$ tend to be redundant and mimic $\delta^{13}C_{collagen}$ and $\delta^{18}O_{structural\ carbonate}$ respectively, a correlation that has been noted previously (Ambrose and Norr, 1993; France and Owsley, in press; Iacumin et al., 1996a; Loftus and Sealy, 2012; Martin et al., 2008; Pellegrini et al., 2011). We reinforce the recommendation that isotope analysis of collagen and structural carbonates provides insight into the region, ancestry, or social class of an unknown individual. Analysis of $\delta^{18}O_{phosphate}$ has a larger inherent error compared to $\delta^{18}O_{structural\ carbonate}$ and can be avoided to save sample material, time, and costs.

4.2. Individual site analysis

After examining demographic information in broad groups, trends in individual sites can be considered. The two burial sites connected to Civil War military units and shortly thereafter, Ft. Craig and Glorieta Pass, have isotope values fairly distinct from one another with a select few individuals plotting with the opposing group. These outliers may be due to European immigrants, local militia members, or individuals enlisting with the side opposite their home region. Isotope data can now help researchers reconstruct histories for individuals of unknown origin from Civil War burials, which are still occasionally exposed by erosion, commercial development, or through archaeological investigations. Residency is often assigned to individuals through knowledge of the military units present during battles and their associated city/region of enlistment. Our model can identify those that do not adhere to this assumption and suggest a status of northern, southern, immigrant, or local resident to facilitate reconstruction of life histories.

Congressional Cemetery (Washington, DC) contains high-status individuals from four family tombs in the mid-northern latitudes. They show relatively depleted $\delta^{13}C$ values suggesting a European-style diet heavy in C3 plants, which is consistent with their affluence and ability to afford preferred foods. Notably contrasting sites are the rural Walton Family Cemetery (Connecticut), Woodville Cemetery (Delaware), and Trinity Church Cemetery (Washington, DC), all containing mid-northern latitude Caucasians. The Walton Cemetery represents a northern farm family with average $\delta^{13}C$ values ~2‰ more enriched than Congressional Cemetery families, indicating a larger influence of dietary corn in the former. The Walton group also shows $\delta^{15}N$ values depleted by ~1.2‰ suggesting less protein intake and lower social class compared to Congressional Cemetery. The Walton Family was likely reliant on local produce including corn from family-owned plots while the wealthier Congressional Cemetery individuals selected and could afford a more cosmopolitan diet. The large range of isotope values from Woodville and Trinity Church Cemeteries suggest a population of more diverse regional origin compared to Congressional Cemetery. Trinity Church Cemetery serviced a range of social

Table 3
Descriptive statistics for data grouped by region, ancestry, social status, and regional/ancestry combinations.

Group		$\delta^{18}O_{phosphate}$ (‰, VSMOW)	$\delta^{18}O_{meteoric\ water}$[a] (‰, VSMOW)	$\delta^{13}C_{structural\ carbonate}$ (‰, VPDB)	$\delta^{18}O_{structural\ carbonate}$ (‰, VSMOW)	$\delta^{18}O_{meteoric\ water}$[b] (‰, VSMOW)	$\delta^{15}N_{collagen}$ (‰, air)	$\delta^{13}C_{collagen}$ (‰, VPDB)
Region of origin:	*N*	29	29	28	28	28	28	28
Southern	Mean	18.1	−6.7	−7.6	25.5	−2.4	10.7	−12.0
	St Dev	1.1	1.8	1.5	1.5	2.4	0.9	2.0
	Min	15.8	−10.2	−10.6	22.2	−8.0	9.1	−16.0
	Max	20.3	−3.3	−5.0	27.7	1.1	13.2	−8.2
Region of origin:	*N*	95	95	94	94	94	95	95
Northern	Mean	17.1	−8.3	−9.6	24.5	−4.2	10.7	−14.9
	St Dev	1.0	1.5	1.9	1.5	2.5	0.9	2.2
	Min	13.3	−14.2	−12.9	20.4	−11.0	8.7	−19.7
	Max	20.2	−3.5	−5.2	29.7	4.5	12.7	−10.1
Region of origin:	*F*	21.46	21.46	24.08	10.73	10.73	0.74	42.48
Test of group Means	*p*[c]	<0.0001	<0.0001	<0.0001	<0.001	<0.001	0.11	<0.0001
	df	(1120)	(1120)	(1120)	(1120)	(1120)	(1120)	(1120)
Ancestry:	*N*	33	33	33	33	33	33	33
African American	Mean	17.4	−7.7	−7.7	25.3	−2.8	10.5	−12.5
	St Dev	0.8	1.3	1.8	1.4	2.3	0.9	2.5
	Min	16.1	−9.8	−11.5	22.2	−8.0	8.8	−17.3
	Max	19.2	−5.0	−5.0	27.7	1.1	13.1	−8.2
Ancestry:	*N*	102	102	100	100	100	101	101
Caucasian	Mean	17.3	−7.9	−9.4	24.6	−3.9	10.8	−14.7
	St Dev	1.2	1.8	1.9	1.6	2.6	0.9	2.2
	Min	13.3	−14.2	−12.9	20.4	−11.0	8.7	−19.7
	Max	20.2	−3.3	−5.2	29.7	4.5	13.2	−10.1
Ancestry:	*F*	0.57	0.57	22.67	5.00	5.00	3.00	25.01
Test of group Means	*p*[c]	0.45	0.45	<0.001	0.27	0.27	0.09	<0.0001
	df	(1131)	(1131)	(1131)	(1131)	(1131)	(1131)	(1131)
Region/ancestry combination:	*N*	13	13	13	13	13	13	13
South/African American	Mean	17.4	−7.8	−6.9	25.8	−2.0	10.2	−10.7
	St Dev	0.6	1.0	1.5	1.5	2.6	0.7	2.0
	Min	16.2	−9.7	−9.9	22.2	−8.0	9.1	−15.2
	Max	18.4	−6.2	−5.0	27.7	1.1	11.7	−8.2
Region/ancestry combination:	*N*	9	9	9	9	9	9	9
South/Caucasian	Mean	17.0	−8.3	−8.8	24.3	−4.5	10.3	−14.2
	St Dev	0.6	1.0	2.2	0.5	0.9	0.7	2.2
	Min	16.2	−9.6	−11.5	23.5	−5.8	8.8	−17.0
	Max	17.9	−7.0	−5.8	24.9	−3.5	11.1	−11.5
Region/ancestry combination:	*N*	16	16	15	15	15	15	15
North/African American	Mean	18.7	−5.8	−8.3	25.3	−2.7	11.1	−13.1
	St Dev	1.1	1.8	1.1	1.4	2.4	0.9	1.3
	Min	15.8	−10.2	−10.6	23.0	−6.6	10.1	−16.0

Table 3 (*continued*)

Group		$\delta^{18}O_{phosphate}$ (‰, VSMOW)	$\delta^{18}O_{meteoric\ water}$[a] (‰, VSMOW)	$\delta^{13}C_{structural\ carbonate}$ (‰, VPDB)	$\delta^{18}O_{structural\ carbonate}$ (‰, VSMOW)	$\delta^{18}O_{meteoric\ water}$[b] (‰, VSMOW)	$\delta^{15}N_{collagen}$ (‰, air)	$\delta^{13}C_{collagen}$ (‰, VPDB)
	Max	20.3	−3.3	−6.1	27.2	0.4	13.2	−10.5
Region/ancestry combination:	*N*	86	86	85	85	85	86	86
North/Caucasian	Mean	17.1	−8.3	−9.6	24.5	−4.1	10.8	−15.0
	St Dev	1.0	1.5	1.9	1.6	2.6	0.9	2.2
	Min	13.3	−14.2	−12.9	20.4	−11.0	8.7	−19.7
	Max	20.2	−3.5	−5.2	29.7	4.5	12.7	−10.1
Region/ancestry combination:	*F*	11.97	11.97	10.23	3.77	3.77	3.15	18.19
Test of group means	*p*[c]	<0.0001	<0.0001	<0.0001	0.013	0.013	0.028	<0.0001
	df	(3118)	(3118)	(3118)	(3118)	(3118)	(3118)	(3118)
Social class:	*N*	22	22	22	22	22	22	22
Lower/slave class	Mean	17.2	−8.0	−7.7	25.2	−3.0	10.3	−12.1
	St Dev	0.6	1.0	2.0	1.4	2.4	0.7	2.7
	Min	16.2	−9.7	−11.5	22.2	−8.0	8.8	−17.0
	Max	18.4	−6.2	−5.0	27.7	1.1	11.7	−8.2
Social class:	*N*	28	28	28	28	28	29	29
Middle class	Mean	17.1	−8.3	−8.4	24.8	−3.6	10.3	−13.2
	St Dev	0.7	1.1	1.0	0.9	1.6	0.9	1.4
	Min	15.8	−10.3	−10.0	22.5	−7.5	8.7	−15.9
	Max	18.2	−6.5	−6.1	26.2	−1.3	12.5	−10.1
Social class:	*N*	41	41	41	41	41	41	41
Upper class	Mean	17.3	−7.9	−10.3	−23.9	−5.1	11.1	−15.5
	St Dev	1.1	1.8	1.7	1.6	2.7	0.8	1.7
	Min	15.6	−10.6	−12.9	20.4	−11.0	9.6	−18.0
	Max	20.3	−3.3	−6.0	27.0	0.1	13.2	−10.5
Social class:	*N*	42	42	42	42	42	42	42
Military	Mean	17.6	−7.5	−8.8	25.4	−2.7	11.0	−14.7
	St Dev	1.4	2.2	2.1	1.6	2.6	0.9	2.6
	Min	13.3	−14.2	−12.7	21.9	−8.5	9.0	−19.7
	Max	20.2	−3.5	−5.2	29.7	4.5	13.1	−10.9
Social class:	*F*	1.14	1.14	13.13	7.59	7.59	9.20	13.81
Test of group means	*p*[c]	0.34	0.34	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	df	(3129)	(3129)	(3129)	(3129)	(3129)	(3129)	(3129)

[a] Values are $\delta^{18}O_{meteoric\ water}$ calculated from $\delta^{18}O_{phosphate}$ using the equation of Longinelli (1984).
[b] Values are $\delta^{18}O_{meteoric\ water}$ calculated from $\delta^{18}O_{structural\ carbonate}$ using the equations of Longinelli (1984) and Bryant et al. (1996).
[c] Values are considered significant if $p < 0.05$.

classes that included local individuals and non-locals moving to a forming urban center from other areas.

The Foscue (North Carolina) and Kincheloe (Virginia) sites contain middle-upper class Caucasians from southern plantations and present an interesting contrast to Congressional Cemetery individuals of similar ancestry and class, but different region. All three groups show similarly enriched $\delta^{15}N_{collagen}$ values indicative of higher social class. However, Foscue and Kincheloe individuals show $\delta^{13}C$ enrichment (≥2‰) indicating a greater C4/corn dietary component compared to Congressional Cemetery. This reflects an environmentally influenced dietary preference different from their Congressional Cemetery counterparts who were extremely affluent and urban, and thus dependent on selectively purchased rather than home-grown foods.

Northern African Americans represented in the First African Baptist Church (Pennsylvania) and Parkway Gravel (Delaware) sites present an interesting case study of cultural versus regional influence. The $\delta^{13}C$ values (from protein and carbohydrates) are similar to those from the south, but the depleted $\delta^{18}O$ values (from drinking water) indicate significant time spent in the north. Similarity with southern $\delta^{13}C$ values suggests an African-American food preference for a corn-based diet based upon their cultural upbringing. Given the increasing number and movement of African Americans northward during the 1800's it is possible that these individuals were only one generation removed from slavery and a southern-influenced lifestyle. Compared to other sites, the $\delta^{15}N$ values are relatively low suggesting a lower socio-economic standing indicative of fewer employment opportunities available to African Americans during that time.

The Pettus and Ft. A.P. Hill sites in Virginia represent African American slaves raised in the south as evidenced by their relatively enriched $\delta^{18}O$ values. The $\delta^{15}N$ values are fairly low signifying the expectedly low social status of slave populations. The $\delta^{13}C$ values are the most enriched of all sites indicating a dominantly C4/corn based diet. The similarity of slave diets to that served at the governing plantation is currently debated (Fairbanks, 1984; Samford, 1996). Although this study does not include the concurrent Caucasian upper class from these particular sites, the slave sites can be compared to Foscue and Kincheloe Plantation Caucasians. The latter sites show $\delta^{15}N$ enrichment of ~2‰ and $\delta^{13}C$ depletion of ~2‰ compared to Pettus and A.P. Hill individuals suggesting the diets of the middle to upper class were different. The upper class plantation diet had a greater proportion of meat and C3/wheat carbohydrates than the slaves.

A combined suite of stable isotope values is a useful tool for reconstructing information on archaeological remains of unknown origin. Extrapolating from our predictive modeling to 20th and 21st century humans is beneficial, but involves certain caveats. Modern Americans live a global lifestyle with diets and travel spanning entire continents. As demonstrated by the more mobile group of northern African Americans and the urban cemeteries we examined, the isotope indicators overlap without the control of a local diet and groups are less distinct statistically. Stable isotope values have been used to determine geographic origins of modern humans, but were limited to identifying broad regions only (Alkass et al., 2011; Daeid et al., 2010; Kennedy et al., 2011; Meier-Augenstein and Fraser, 2008). Our study supports that stable isotopes are useful for coarse geographic identifications for

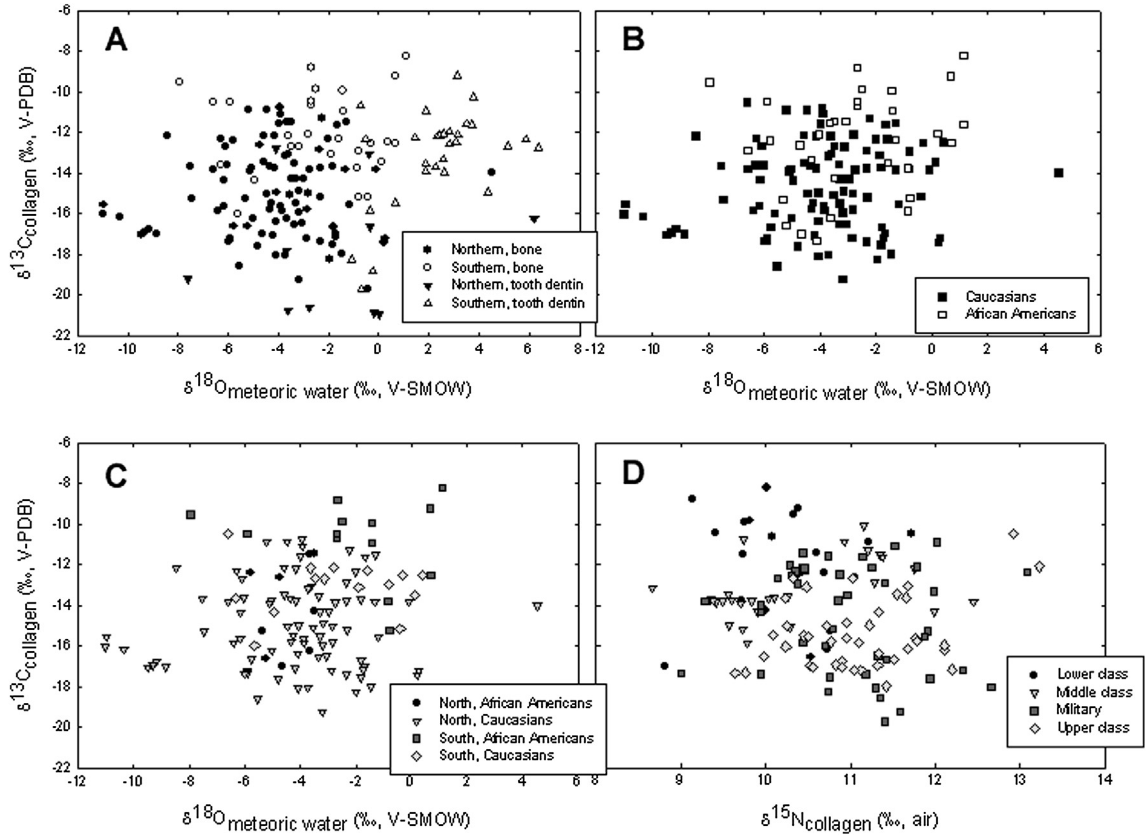

Fig. 4. Data grouped by A) region, B) ancestry, C) regional/ancestry combinations, and D) social class. The best predictive isotopes are shown, except in D where $\delta^{18}O_{meteoric\ water}$ and $\delta^{13}C_{structural\ carbonate}$ were also predictive.

contemporary humans, but cannot currently provide focused geographic and forensic racial determinations.

5. Conclusion

This study suggests that predicting region of origin, ancestry, and social class with stable isotopes is plausible for 18th–19th century North Americans. Carbon and oxygen isotopes in collagen and structural carbonates provide the best predictors of these demographic factors based upon our data. The strongest predictor of region is $\delta^{18}O$; the strongest predictors of ancestry are $\delta^{13}C_{collagen}$ combined with $\delta^{18}O$ to a lesser degree. Social class, an imprecisely controlled variable in this study, was the least predictable with $\delta^{15}N_{collagen}$ and $\delta^{13}C$ providing the strongest distinction. Social class is most distinguishable relative to the lowest class within an area due to regional variation in diet. Regional mobility, immigrants, and cultural preference for certain foods may confound predictions thereby rendering the use of a similar model less tenable for more recent humans who experience greater mobility and dietary variety.

Examination of isotope values from individual sites produced several notable points. Cemeteries from urban areas and military burial sites have a greater range of isotope values, which speaks to a variety of regional origins and cultural backgrounds. Distinct isotopic outliers in military units may represent immigrants or individuals exercising a social choice of alliance in opposition to their home region. Urban cemeteries often include people of different social classes and individuals moving to cities from other regions. Sites containing upper class individuals or the extreme lower classes (such as slaves) are more homogeneous in their isotope values, which may reflect dietary preferences and vastly different economic means for acquiring foods. Upper class southern plantation families and their slaves both depend on local crops and game as evidenced by enriched $\delta^{13}C$ and $\delta^{18}O$ values, although slaves consumed less meat and a greater proportion of corn. Upper class northern individuals tend to show more depleted $\delta^{13}C$ values indicative of a more northern or European-style diet.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jas.2013.10.037.

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