Technical Note: A Linear Model for Predicting $\delta^{13}C_{protein}$

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ABSTRACT **Objective:** Development of a model for the prediction of $\delta^{13}C_{protein}$ from $\delta^{13}C_{collagen}$ and $\Delta^{13}C_{ap\text{-}co}$. Model-generated values could, in turn, serve as "consumer" inputs for multisource mixture modeling of paleodiet. **Methods:** Linear regression analysis of previously published controlled diet data facilitated the development of a mathematical model for predicting $\delta^{13}C_{protein}$ (and an experimentally generated error term) from isotopic data routinely generated during the analysis of osseous remains ($\delta^{13}C_{co}$ and $\Delta^{13}C_{ap\text{-}co}$). **Results:** Regression analysis resulted in a two-term linear model ($\delta^{13}C_{protein}$ (%) = (0.78 × $\delta^{13}C_{co}$) − (0.58× $\Delta^{13}C_{ap\text{-}co}$) − 4.7), possessing a high *R*-value of 0.93 ($r^2 = 0.86$, $P < 0.01$), and experimentally generated error terms of ±1.9% for any predicted individual value of $\delta^{13}C_{protein}$. This model was tested using isotopic data from Formative Period individuals from northern Chile's Atacama Desert. **Conclusions:** The model presented here appears to hold significant potential for the prediction of the carbon isotope signature of dietary protein using only such data as is routinely generated in the course of stable isotope analysis of human osseous remains. These predicted values are ideal for use in multisource mixture modeling of dietary protein source contribution. Am J Phys Anthropol 157:694–703, 2015.

Recent advances in multisource mixture modeling of stable isotope systems hold considerable potential for bioarchaeologists interested in the reconstruction of paleodiet. To make such modeling applications useful, however, the user must possess the ability to gauge tissue-specific source-consumer fractionation for all isotope systems of interest. These methods, in point of fact, demand quantification of trophic enrichment factors (TEF)/fractionation/discrimination offsets in order for their bioarchaeological promise to be fully realized. While several recent studies have addressed and attempted to quantify the diet-collagen or protein-collagen offset for nitrogen isotope systematics ($\delta^{15}N$) (e.g. Hedges and Reynard, 2007; O'Connell et al., 2012), far less work (Froehle et al., 2010) has been done to assess such offsets for carbon stable isotopes ($\delta^{13}C$).

Here, employing a corpus of previously published data from controlled diet experiments with rats, mice, and pigs, we present a multiple (two variable) linear model by which the $\delta^{13}C$ value (plus/minus an experimentally generated error term) of dietary protein ($\delta^{13}C_{protein}$) can be accurately predicted from isotopic data routinely generated during the analysis of osseous remains ($\delta^{13}C_{co}$ and $\Delta^{13}C_{ap\text{-}co}$). By combining the output of this regression with (one of the several) proposed fractionation offsets between dietary protein and bone collagen for $\delta^{15}N$, one is able to take advantage of multisource mixture modeling software, and thus develop quantitative and probabilistic models of paleodietary mixtures.

After presenting this multiple linear regression model for predicting $\delta^{13}C_{protein}$, we demonstrate its utility by means of a Markov Chain Monte Carlo simulation [using the software package MixSIAR (Stock and Semmens, 2013)] of the diet of individuals drawn from our ongoing research into the Formative Period of the Atacama Desert of northern Chile. The method detailed here permits us to move beyond relative statements of dietary contribution to probabilistic and uncertainty integrated quantification of dietary inputs on an individual-by-individual basis (Semmens et al., 2009).

THE NEED FOR QUANTIFICATION IN ISOTOPIC PALEODIETARY STUDIES

The past 30 years have witnessed an explosive increase in the use of stable isotope analysis in bioarchaeology (see Pestle et al., 2014: Fig. 1). Indeed, stable isotope analysis has now become a standard technique used in the reconstruction of ancient humazn paleodiet, paleomobility, and paleoclimate. And yet, with limited recent exceptions (e.g. Ambrose et al., 2003; Kellner and Schoeninger, 2007; Arcini et al., ; Coltrain and Janetski, 2013; Colonese et al., 2014; Fernandes et al., 2012a, 2014; Ugan and Coltrain, 2012), the paleodietary reconstruction facilitated by this analysis has been limited to relative statements about source contributions (e.g. more of food *X*, less of food *Y*). Such relative statements are

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made even more contingent when different classes of food are isotopically similar and/or when one accounts for the various sources of uncertainty (e.g. measurement error, source variability, differential fractionation). However, various tools have existed for the last 20 to 25 years that enable a greater degree of quantification of food class contribution, thereby, potentially at least, advancing the fidelity of bioarchaeological reconstructions of paleodiet.

Linear mixing models, which permit the quantification of source contribution for $n+1$ sources (where n represents the number of isotope systems [elements] under consideration), have been used in stable isotope ecology for over a decade (Schwarcz, 1991; Phillips, 2001a,2001b). More than 10 years have passed since the introduction of IsoSource, the first widely available multisource mixture modeling software for stable isotope analysis (Phillips and Gregg, 2003). This software, and other similar models (Lubetkin and Simenstad, 2004), produced nondeterministic solutions for scenarios wherein more than $n+1$ sources (classes of food) are present, and in some cases began to confront issues of uncertainty in such reconstruction (Phillips 2001a,2001b, 2005). It is only in the past five years, however, that the introduction of Bayesian modeling has cemented the potential for bioarchaeologists to apply modeling to their datasets. In 2008, Moore and Semmens (2008) first proposed MIXSIR, "a Bayesian-mixing model that estimates probability distributions of source contributions to a mixture while explicitly accounting for uncertainty associated with multiple sources, fractionation and isotope signatures" (Moore and Semmens, 2008; p 470). SIAR (Parnell et al., 2010), IsotopeR (Hopkins and Ferguson, 2012), and, most recently, MixSIAR (Stock and Semmens, 2013) were all built upon the foundation laid by MIXSIR. It is MixSIAR, which runs within the R environment (R Core Team, 2014), with which we are most familiar, and which we employ below in our case study. Regardless of the specific package employed, it is evident that these Bayesian techniques have great potential for reconstructions of paleodiet as they "offer a powerful means to interpret data because they can incorporate prior information, integrate across sources of uncertainty and explicitly compare the strength of support for competing models or parameter values" (Moore and Semmens, 2008; p 471).

The use of such models is, however, discouraged unless, "there is very strong evidence that no sources are missing and that TEFs are estimated correctly" (Parnell et al., 2013; p 396). Thus it is that in the case of bioarchaeology, the primary driver of the lack of quantification in stable isotopic reconstructions of paleodiet is not a dearth of tools with which to quantify source contributions, but rather our inability to determine, with any fidelity, consumer (human) isotope signatures that accurately account for TEF/fractionation between foodstuffs and consumer tissues. While the dynamics of $\Delta^{15}N_{protein\text{-}collagen}$ have been extensively studied (DeNiro and Epstein, 1981; Schoeninger and DeNiro, 1984; Schoeninger, 1985; Hare et al., 1991; Schoeller, 1999; Ambrose, 2000; Howland et al., 2003; Sponheimer et al., 2003; Hedges and Reynard, 2007; Warinner and Tuross, 2009; O'Connell et al., 2012), and fractionation offsets of varying magnitude have been proposed, our inability to determine $\Delta^{13}C_{protein\text{-}collagen}$ has, to date, held us back. The present work aims to remedy that gap in our knowledge.

MATERIALS AND METHODS

Initially, we compiled (Table 1) isotopic data derived from several published controlled diet experiments on rodents (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Jim et al., 2004) and pigs (Hare et al., 1991; Howland et al., 2003; Warinner and Tuross, 2009). These studies provide detail on dietary (whole diet and dietary protein) macronutrient proportion and carbon isotope compositions as well as the $\delta^{13}C$ signatures of consumer bone collagen and, with the exception of Hare et al. (1991), bone hydroxyapatite (in which case, during analysis, the data points with missing value were omitted listwise). These experimental data are indispensible for the construction of a model such as that proposed here in that we have no such comparable dataset (with isotope values for both dietary inputs and skeletal biomolecules) for humans (see below for a discussion of the limitations of using rodent and pig data in the construction of models to be applied to humans). While previous studies (e.g. Kellner and Schoeninger, 2007; Froehle et al., 2010; Fernandes et al., 2012b) have analyzed the same dataset for various purposes, none has done so with the goal of providing a predictive model by which dietary isotope values might be generated.

The corpus of isotopic data was then analyzed using a forward stepwise linear model employing the Akaike Information Criterion with small sample size correction (AICc). This analysis was conducted using SPSS v.20 (IBM, NY). As the dependent variable of interest at present is the carbon isotope composition of dietary protein ($\delta^{13}C_{protein}$), $\delta^{13}C_{co}$ and $\Delta^{13}C_{ap\text{-}co}$ were selected as explanatory/predictor variables for the linear model. This decision was made on the basis that while $\delta^{13}C_{diet}$ and $\delta^{13}C_{ap}$ are strongly correlated ($r = 0.93$, $P < 0.01$), $\delta^{13}C_{protein}$ was much more strongly correlated with $\delta^{13}C_{co}$ ($r = 0.87$, $P < 0.01$) and $\Delta^{13}C_{ap\text{-}co}$ ($r = -0.64$, $P < 0.01$) than was $\delta^{13}C_{ap}$ (0.34, $P = 0.05$). Moreover, the inclusion of the $\delta^{13}C_{ap}$ term did not improve the predictive power of the model beyond that obtained with the tow-term solution detailed below.

The result of this analysis is an experimentally derived two-term regression (with individual and mean 95% confidence intervals) by which $\delta^{13}C_{protein}$ can be predicted from the routinely generated $\delta^{13}C_{co}$ and $\Delta^{13}C_{ap\text{-}co}$ terms. This step, when combined with the application of one of several previously proposed values for $\Delta^{15}N_{protein\text{-}collagen}$, facilitates the use of Bayesian mixture models. Such modeling enables a far greater degree of specificity about source contribution than previous simple regression models (Kellner and Schoeninger, 2007; Froehle et al., 2012), with which one could only specify, in broad terms, the type of macronutrient mixture (e.g. C_3 protein and C_3 carbohydrates or marine protein and C_4 carbohydrates) that might produce a given set of consumer isotope values.

RESULTS

Linear modeling of the isotopic data derived from the rodent and pig controlled diet experiments produced the following regression formula (Fig. 1):

$$\delta^{13}C_{protein}(\%) = \left(0.78\times\delta^{13}C_{co}\right) - \left(0.58\times\Delta^{13}C_{ap\text{-}co}\right) - 4.7$$

This regression possesses a very high R-value of 0.93 ($r^2 = 0.86$, $P < 0.01$) and a small AICc of 40.2. The r^2

TABLE 1. Animal controlled diet isotope data (corrected by 1.5% to account for depletion of $\delta^{13}C$ from burning of fossil fuels in order to make values directly comparable to ancient values) used in generation of linear model plus predicted $\delta^{13}C_{protein}$ values and model residuals

Source	Diet/ID	% Protein	$\delta^{13}C_{protein}$	$\delta^{13}C_{diet}$	$\delta^{13}C_{co}$	$\delta^{13}C_{ap}$	$\Delta^{13}C_{ap\text{-}co}$	Predicted $\delta^{13}C_{protein}$	Residual
				Rodents					
Ambrose and Norr (1993)	1A	20	−24.8	−23.7	−19.9	−14.2	5.7	−23.4	−1.4
	2B	5	−12.7	−22.8	−13.2	−11.9	1.3	−15.7	3
	3C	5	−24.8	−10.6	−12.2	−1.4	10.8	−20.3	−4.5
	4D	70	−12.7	−15.6	−8.2	−6.2	2.0	−12.2	−0.5
	5E	70	−24.8	−21.0	−19.2	−12.0	7.2	−23.7	−1.1
	6F	20	−23.8	−13.1	−14.5	−3.7	10.8	−22.1	−1.7
	12,13G	20	−24.8	−13.2	−15.4	−4.1	11.3	−23.1	−1.7
Tieszen and Fagre (1993)	1	18.2	−23.2	−24.1	−20.4	−15.3	5.1	−23.4	0.2
	2	18.1	−24.6	−20.6	−20.0	−14.8	5.2	−23.2	−1.4
	3	18.1	−19.6	−15.9	−14.1	−5.4	8.7	−20.6	1
	4	18.4	−23.2	−23.1	−20.2	−13.7	6.5	−24.1	0.9
	5	18.4	−15.8	−21.7	−14.3	−12.8	1.5	−16.6	0.8
	6	36.1	−12.2	−18.4	−10.3	−7.3	3.0	−14.4	2.2
	7	4.7	−19.2	−21.5	−14.0	−12.7	1.3	−16.3	−2.9
	8	12	−12.1	−10.3	−8.3	−1.6	6.7	−15	2.9
Jim et al. (2004)	d2a4	20	−23.0	−23.4	−18.4	−13.0	5.4	−22.1	−0.9
	d4h	20	−13.1	−10.7	−6.4	−1.6	4.8	−12.4	−0.7
	d5i	20	−13.1	−20.8	−10.8	−11.5	−0.7	−12.7	−0.4
	d6j2	20	−16.3	−21.8	−13.0	−11.9	1.1	−15.4	−0.9
	d7k2	20	−16.3	−11.4	−8.2	−2.0	6.2	−14.6	−1.7
	d8l2	20	−16.3	−16.8	−10.7	−7.1	3.6	−15	−1.3
				Swine					
Hare et al. (1991)	C_3	–	−25.9	−23.8	−22.4	–	–	–	–
	C_4	–	−11.7	−10.9	−7.7	–	–	–	–
Howland et al. (2003)	3	20	−23.3	−23.2	−19.1	−12.7	6.4	−23.2	−0.1
	4	20	−22.0	−19.0	−17.2	−10.3	6.9	−22	0.0
	5	20	−20.7	−16.5	−15.4	−7.5	7.9	−21.2	0.5
	6	20	−19.3	−14.4	−13.9	−4.8	9.1	−20.7	1.4
	8	20	−24.0	−24.2	−18.1	−12.1	6.0	−22.2	−1.8
	10	20	−14.9	−14.0	−10.0	−2.8	7.2	−16.6	1.7
Warinner and Tuross (2009)	2/nix	13	−22.3	−21.5	−17.0	−9.1	7.9	−22.4	0.1
	3/nix	13	−22.3	−21.5	−17.9	−9.1	8.8	−23.6	1.3
	4/nix	13	−22.3	−21.5	−17.4	−10.0	7.4	−22.4	0.1
	5/nix	13	−22.3	−21.5	−17.4	−9.3	8.1	−22.8	0.5
	6/nix	13	−22.3	−21.5	−17.2	−9.1	8.1	−22.7	0.4
	7/nix	13	−21.7	−20.7	−17.1	−8.5	8.6	−22.9	1.2
	8/raw	13	−21.7	−20.7	−18.3	−9.8	8.5	−23.8	2.1
	9/raw	13	−21.7	−20.7	−17.2	−7.8	9.4	−23.4	1.7
	10/raw	13	−21.7	−20.7	−17.2	−8.7	8.5	−22.9	1.2

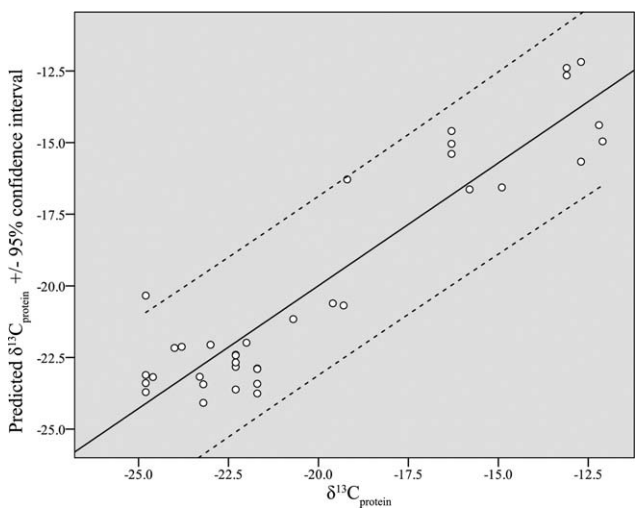

Fig. 1. Scatterplot of actual $\delta^{13}C_{protein}$ and predicted $\delta^{13}C_{protein}$ featuring linear regression (solid line, $r^2 = 0.86$, $P < 0.01$) and 95% confidence intervals (dashed lines).

value of the two-term regression is far higher than that obtained through the use of $\delta^{13}C_{co}$ alone ($r^2 = 0.76$, this study, $r^2 = 0.65$, Kellner and Schoeninger, 2007; p 1121). The linear modeling employed in the present analysis also allows for determination of the regression's 95% confidence interval, defined by the following formulae:

$$\text{Lower bound, } \delta^{13}C_{protein}(\%) = \left(0.62\times\delta^{13}C_{co}\right) - \left(0.78\times\Delta^{13}C_{ap\text{-}co}\right) - 7.0$$

$$\text{Upper bound, } \delta^{13}C_{protein}(\%) = \left(0.93\times\delta^{13}C_{co}\right) - \left(0.37\times\Delta^{13}C_{ap\text{-}co}\right) - 2.3$$

More importantly, however, as regards individual predictions (and the generation of individual error terms), the 95% prediction intervals for individual values ranged from −3.7% to +3.7%, with a standard deviation of ±1.9% for any predicted individual value of $\delta^{13}C_{protein}$ and a standard error of the estimate of ±1.7%.

Residuals about the regression line averaged 0.0 ± 1.6% and had a range of −4.4% to +3.0% (Table 1, Fig. 2). These residuals are approximately normally

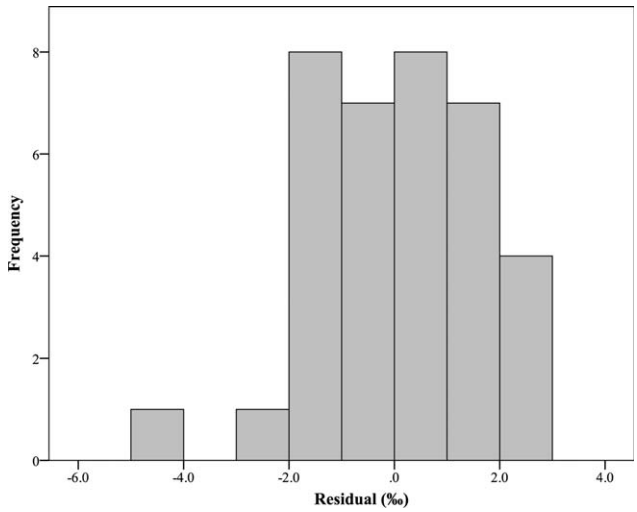

Fig. 2. Histogram of model residuals (predicted $\delta^{13}C_{protein}$ vs. actual $\delta^{13}C_{protein}$). Shapiro-Wilk test ($W = 0.98$, $df = 36$, $P = 0.73$).

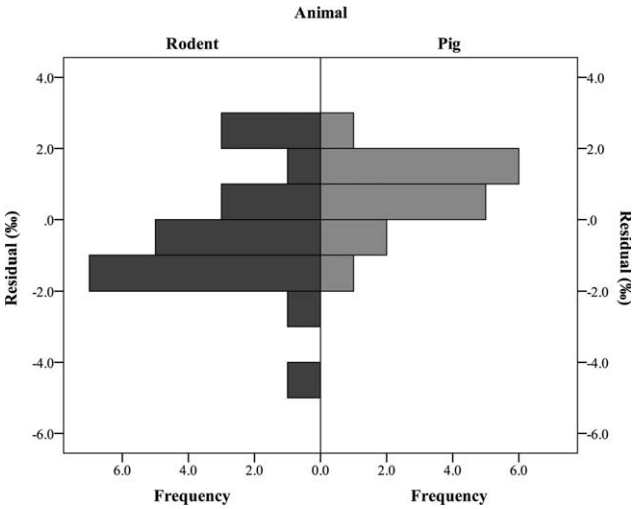

Fig. 3. Population pyramid comparing model residuals (predicted $\delta^{13}C_{protein}$ vs. actual $\delta^{13}C_{protein}$) by taxon. The distributions of the residuals for the two taxa are significantly different as judged by a two-sample Kolmogorov-Smirnov test ($D = 1.8$, $n = 36$, $P < 0.01$).

distributed, as demonstrated by the results of a Shapiro-Wilk test ($W = 0.98$, $df = 36$, $P = 0.73$). An attempt to identify factors correlated with the regression residuals identified only two significant correlations. First, there was a weak positive correlation ($r = 0.38$, $P = 0.02$) between the residual values and $\delta^{13}C_{protein}$, meaning that higher $\delta^{13}C_{protein}$ values were correlated with larger positive residuals. Second, there was a moderate negative correlation ($r = -0.46$, $P < 0.01$) between the residual values and $\Delta^{13}C_{diet\text{-}protein}$, such that diets in which dietary protein was depleted in ^{13}C relative to whole diet produced more negative residuals, and vice versa. This latter relationship was presaged by the findings of Froehle et al., 2010, and in particular their Figure 3a, in which the direction and magnitude of $\Delta^{13}C_{diet\text{-}protein}$ was identified as being similarly correlated with $\Delta^{13}C_{collagen\text{-}diet}$. Neither of these correlations

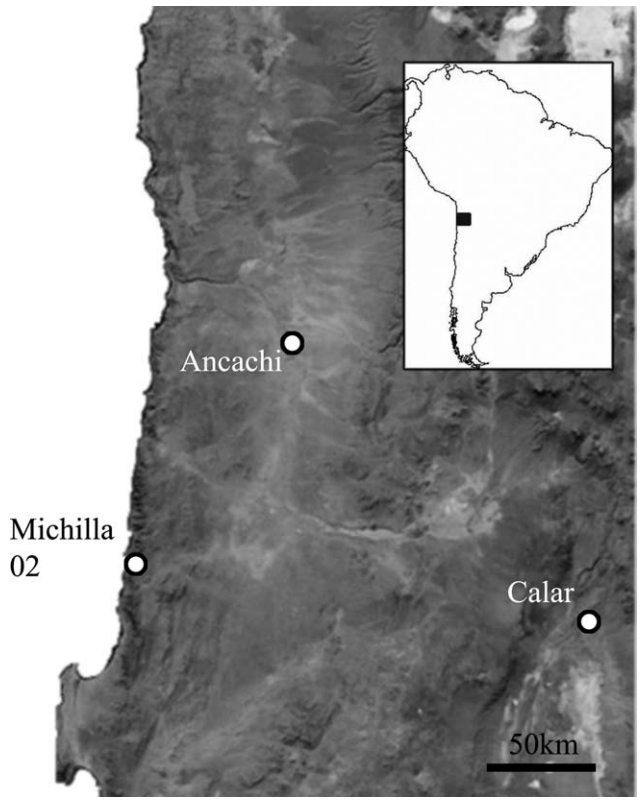

Fig. 4. Map of northern Chile with locations mentioned in text labeled.

is particularly troubling, as would have been, for example, strong correlations with percent dietary protein or particular isotopic combinations (e.g. ^{13}C enriched dietary protein and depleted whole diet). Instead, the model would seem to produce accurate predictive values for $\delta^{13}C_{protein}$ across a broad range of dietary combinations, in terms of both macronutrient and isotopic composition.

Given recent debates about the suitability and comparability of different animal taxa (rodents and pigs) as isotopic proxies for ancient humans (Warinner and Tuross, 2009; Froehle et al., 2010), residuals about the line were also considered and analyzed by taxon (pigs versus rodents). As displayed in Figure 3, residuals for pigs ($0.7 \pm 1.0\%$) were significantly more positive ($t = -2.2$, $df = 34$, $P = 0.03$) than those observed in rodents ($-0.5 \pm 1.8\%$). Moreover, the distributions of the residuals for the two taxa are significantly different as judged by a two-sample Kolmogorov-Smirnov test ($D = 1.8$, $n = 36$, $P < 0.01$). The potential implications of these findings are discussed below.

It should be noted that the model we propose makes no allowance for the potential isotopic effects engendered by differences in body size between the taxa used to construct the model and humans. While Passey et al. (2005) found differences in offsets between the carbon isotope signature of diet and hydroxyapatite (enamel) correlated with differences in body size and/or digestive physiology, a previous meta-analysis of much of the same experimental data used in the present work found that, "body size shows no effect on $\Delta^{13}C_{diet\ protein\text{-}collagen}$ within the sample. The average value for the large mammals is not significantly different from that in the small mammals,

TABLE 2. Chemical, elemental, and isotopic data (both observed and predicted) for individuals included in case study

Site	Michilla 02	Ancachi	Calar
Individual	22-01	UR-3	3055
Collagen yield (wt%)	11.9	18.4	17.8
wt% C	41.7	39.6	41.6
wt% N	15.4	13.6	15
Atomic C:N	3.2	3.4	3.2
$\delta^{13}C_{co\text{-}PDB}$ (%)	−11.0	−14.8	−15.7
$\delta^{15}N_{co\text{-}AIR}$ (%)	23.1	20.4	10.7
$\delta^{13}C_{ap\text{-}PDB}$ (%)	−8.7	−11.6	−11.0
$\Delta^{13}C_{ap\text{-}co}$ (%)	2.2	3.2	4.8
Calculated $\delta^{13}C_{protein} \pm 1.9\%$	−14.5	−18.0	−19.6
Simulation 1: Calculated $\delta^{15}N_{protein} \pm 1.2\%$	19.5	16.8	7.1
Simulation 2: Calculated $\delta^{15}N_{protein} \pm 1\%$	17.1	14.4	4.7

Collagen and apatite extraction followed protocols detailed in Pestle (2010) and Pestle and Colvard (2012). Note different $\delta^{15}N$ fractionation values used in simulation 1 versus simulation 2.

and the ranges overlap when the low protein values are removed" (Kellner and Schoeninger, 2007; p 1121).

CASE STUDY

To demonstrate the efficacy of this model, we employed it and a Bayesian mixing model (MixSIAR) to reconstruct the diets of three individuals drawn from ongoing research into the Formative Period societies of northern Chile's Atacama Desert. These three individuals would be thought, on the basis of site location (Fig. 4), clear (and well-preserved) zooarchaeological evidence, and a preliminary examination of their isotopic values (Table 2), to exemplify three rather different dietary strategies. Individuals from Michilla 02, a coastal site, would be expected to have relied primarily on marine protein sources whereas individuals resident at Calar, located over 200 km from the coast, likely had a terrestrially (C_3) focused protein diet. Finally, as Ancachi appears to have been a coastal trading outpost on the lower reaches of the Loa River, individuals residing there may well have consumed a mixture of marine and terrestrial proteins. Employing the calculated values for trophic enrichment presented here and MixSIAR, a publically available open-source Bayesian mixture modeling software, we are able to model, probabilistically, the contribution of four different classes of proteinaceous foodstuffs (marine mammals, marine finfish, marine invertebrates, and terrestrial mammals) to the diets of individuals from each of these sites.

As shown in Table 2, the first step was to determine the isotopic signature (both $\delta^{13}C$ and $\delta^{15}N$) for the dietary protein mixture consumed by these three individuals. To account for different reconstructions of $\delta^{15}N$ trophic level enrichment proposed by different authors, we carried out two distinct simulations. In both instances, for $\delta^{13}C$ we applied the calculated output of the regression formula derived from the experimental animal data, while we used an offset of $3.6 \pm 1.2\%$ (based on omnivorous mammal values derived from DeNiro and Epstein (1981); Hare et al., (1991); Ambrose (2000); Howland (2003); Sponheimer et al., (2003); and Warinner and Tuross (2010)) for $\delta^{15}N$ in Simulation 1 and $6 \pm 1\%$ [based on the recent results of O'Connell et al. (2012)] for Simulation 2. These values were provided as inputs for the MixSIAR "consumer" file. Trophic enrichment factors, having been accounted for in the first step, were set at 0%, while their associated standard deviations (1.9% for $\delta^{13}C$ in both simulations, 1.2% and 1% for $\delta^{15}N$ in simulations 1 and 2, respectively) were provided in the MixSIAR "discrimination" file (MixSIAR applies the discrimination values and standard deviations to the food sources rather than consumers in order to allow for differential fractionation of different food sources). Finally, the means and standard deviations of the carbon and nitrogen isotopic values of the four principal (archaeologically attested) proteinaceous food groupings: terrestrial mammals, marine mammals, marine invertebrates, and marine finfish, were input in the MixSIAR "sources" file (with details on the foodweb provided in Table 3). As discussed more fully below, the proposed model cannot differentiate between animal- and plant-derived proteins, and the lack of the inclusion of the latter as a potential source of dietary protein is, in the present case, a testament to a lack of archaeological data on high protein plants that would have been accessible to the individuals under analysis rather than any statement about its presumed unimportance.

TABLE 3. Isotopic data for proteinaceous foodweb categories provided as source inputs for MixSIAR model

Category	n	$\delta^{13}C_{co\text{-}PDB}$ (%)	$\delta^{15}N_{co\text{-}AIR}$ (%)
Marine fauna			
Finfish	49	-15.9 ± 1.7	19.9 ± 2.1
Invertebrates	31	-14.2 ± 3.6	16.4 ± 1.9
Mammals	5	-14.8 ± 0.1	21.5 ± 3.4
Terrestrial fauna			
Mammals	31	-19.3 ± 4.5	6.9 ± 1.6

Markov Chain Monte Carlo options were set as follows: number of chains (3), chain length (50,000), burn-in (25,000), thin (25). Both process and residual error were included, thereby accounting for both estimated uncertainty in source and discrimination values (process error) and unknown sources of error (residual error) (Stock and Semmens, 2013). We chose not to include measurement error (instrumental uncertainty) as a source of error in the present model, as the small magnitude of this uncertainty (commonly cited as 0.1% for $\delta^{13}C$ and 0.2% for $\delta^{15}N$) is far exceeded by the uncertainties of the modeled trophic enrichment factors. Standard error propagation techniques reduce their impact to a negligible 0.01% and 0.04%, respectively, as compared to trophic enrichment uncertainties in excess of 1% for both $\delta^{13}C$ and $\delta^{15}N$. Intra-laboratory variability in isotopic measurements (Pestle et al., 2014) was also excluded from consideration as an error source, as all human samples were analyzed in the same laboratory, allowing for direct comparability of measured values.

Modeled dietary contributions of the four specified food sources are provided in Figures 5 and 6 (means) and Tables 4 and 5 (5th, 50th [median], and 95th percentiles, as well as means and standard deviations). Recall that in the case of modeling of this sort, the 50th percentile (median) value represents the most likely contribution of that foodstuff, as based on the model's thousands of simulations, whereas the 5th and 95th percentile values define the confidence interval for the estimate. As with any multisource mixture modeling, while the measures of central tendency for each source

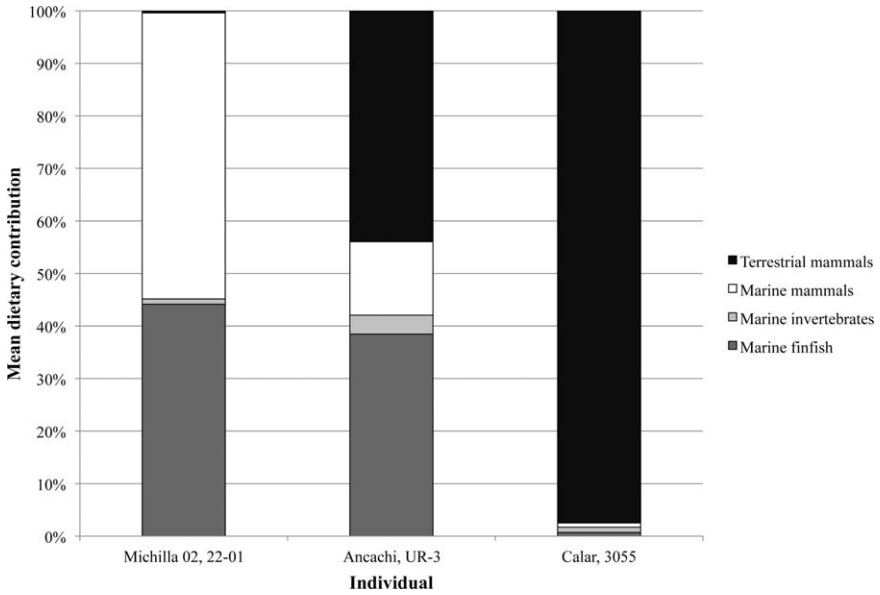

Fig. 5. Results of simulation 1. Stacked column plot showing mean dietary contributions of various dietary protein sources to the diet of individuals analyzed and discussed in case study. Note that mean values are used in this graphical presentation as they sum to 100% whereas, in the case of the simulated mixture models, median need not do so.

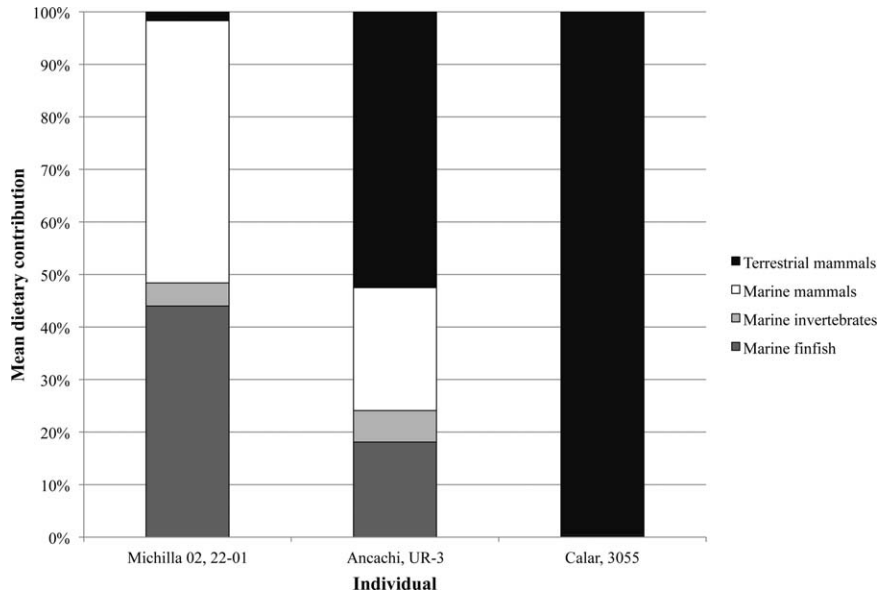

Fig. 6. Results of simulation 2. Stacked column plot showing mean dietary contributions of various dietary protein sources to the diet of individuals analyzed and discussed in case study. Note that mean values are used in this graphical presentation as they sum to 100% whereas, in the case of the simulated mixture models, median need not do so.

are of interest, several other factors (e.g. small ranges, low maxima [either standard deviation or 5th to 95th percentile], and high minima) are more telling. Using these indicators, we can state with a demonstrable degree of certainty that, for instance, marine mammals (simulation 1: $54.5 \pm 17.2\%$, simulation 2: $49.09 \pm 17.1\%$) and marine finfish (simulation 1: $44.2 \pm 17.2\%$, simulation 2: $44.0 \pm 15.8\%$) were the major contributors to the protein diet of the Michilla Norte 02 individual (22-01), while marine invertebrates (simulation 1: $1.0 \pm 2.3\%$, simulation 2: $4.4 \pm 7.0\%$) and terrestrial mammals (simulation 1: $0.4 \pm 0.9\%$, simulation 2: $1.7 \pm 2.6\%$) played little, if any, role.

Similar statements of order and limiting/bounding can be made for the other individuals analyzed. By these methods, we can state, with probabilistic certainty, that Calar 3055 consumed, at a minimum (5th percentile), 96.3% (simulation 1) or 99.5% (simulation 2) terrestrial protein. Finally, based on simulation 1, Ancachi UR-3 would appear to have consumed a minimum of 21.1% (and most likely 46.5%) marine finfish, despite residing some 65 km from the sea. Obviously, the results of

TABLE 4. Results of simulation 1

Site	Michilla 02	Ancachi	Calar
Individual	22-01	UR-3	3055
Marine finfish			
5th percentile (%)	32.2	21.1	0.0
50th percentile (%)	43.5	46.5	0.0
95th percentile (%)	72.9	64.3	4.5
Mean (%)	44.2	38.5	0.7
SD (%)	17.2	21.8	1.7
Marine invertebrates			
5th percentile (%)	0.0	0.0	0.0
50th percentile (%)	0.0	0.2	0.0
95th percentile (%)	5.4	20.0	6.3
Mean (%)	1.0	3.6	1.0
SD (%)	2.3	8.8	2.7
Marine mammals			
5th percentile (%)	42.9	0.0	0.0
50th percentile (%)	55.2	0.9	0.0
95th percentile (%)	82.4	55.7	4.7
Mean (%)	54.5	14.0	0.8
SD (%)	17.2	20.4	2.0
Terrestrial mammals			
5th percentile (%)	0.0	39.1	96.3
50th percentile (%)	0.0	44.1	99.3
95th percentile (%)	2.0	56.2	100.0
Mean (%)	0.4	43.9	97.5
SD (%)	0.9	7.6	3.8

Modeled dietary contributions (mean, standard deviation, 5th, 50th, and 95th percentile) for four potential sources of dietary protein.

TABLE 5. Results of simulation 2

Site	Michilla 02	Ancachi	Calar
Individual	22-01	UR-3	3055
Marine finfish			
5th percentile (%)	34.5	0.1	0.0
50th percentile (%)	44.8	10.8	0.0
95th percentile (%)	68.3	50.5	1.1
Mean (%)	44.0	18.1	0.2
SD (%)	15.8	19.0	0.6
Marine invertebrates			
5th percentile (%)	0.0	0.0	0.0
50th percentile (%)	0.8	0.1	0.0
95th percentile (%)	19.7	38.4	1.1
Mean (%)	4.4	6.0	0.2
SD (%)	7.0	12.9	0.8
Marine mammals			
5th percentile (%)	38.4	0.2	0.0
50th percentile (%)	49.2	16.9	0.0
95th percentile (%)	79.7	60.8	1.1
Mean (%)	49.9	23.4	0.2
SD (%)	17.1	23.2	0.8
Terrestrial mammals			
5th percentile (%)	0.0	46.5	99.5
50th percentile (%)	0.3	52.8	99.9
95th percentile (%)	7.4	67.5	100.0
Mean (%)	1.7	52.5	99.4
SD (%)	2.6	9.7	1.4

Modeled dietary contributions (mean, standard deviation, 5th, 50th, and 95th percentile) for four potential sources of dietary protein.

simulation 2, which had a much larger $\Delta^{15}N_{protein\text{-}collagen}$ offset reduces that estimate, although over 25% of that individual's dietary protein intake was still marine based (10.8% marine finfish plus 16.9% marine mammals).

The results obtained confirm the general assumptions about dietary makeup at the different sites while also providing a far greater degree of specificity about source contribution. If combined with other quantitative measures (e.g. radiocarbon dates), these quantitative estimates of dietary contribution could permit further modeling and statistical evaluation (for example, the study of temporal trends or correlations) and thereby allow higher fidelity reconstruction of foodways in the Formative Period Atacama, or wherever applied.

DISCUSSION AND CONCLUSION

While previous studies have quantified, with some notable disagreement, $\Delta^{15}N_{protein\text{-}collagen}$ (DeNiro and Epstein, 1981; Schoeninger and DeNiro, 1984; Schoeninger, 1985; Hare et al., 1991; Schoeller, 1999; Ambrose, 2000; Sponheimer et al., 2003; Howland et al., 2003; Hedges and Reynard, 2007; Warinner and Tuross, 2010; O'Connell et al., 2012), and $\Delta^{13}C_{diet\text{-}apatite}$ (Vogel and Van Der Merwe, 1977; Krueger and Sullivan, 1984; Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Bocherens et al., 1995; Howland et al., 2003; Jim et al., 2004; Kellner and Schoeninger, 2007), no agreed-upon method yet exists for determining $\Delta^{13}C_{protein\text{-}collagen}$. Even those recent studies that have made use of Bayesian mixture modeling methods of the sort employed here (Arcini et al., 2012; Fernandes et al., 2012a, 2014; Ugan and Coltrain, 2012; Coltrain and Janetski, 2013; Colonese et al., 2014), have not provided an experimentally derived means by which to calculate this value. The method shown above represents a first step towards that goal. This model is not without its limitations, and here we consider how a range of potentially problematic issues might bear on the model presented herein.

Warinner and Tuross (2009) argued that isotopic data from rodents and pigs were not directly comparable, but Froehle et al. (2010) countered that differences between those species stemmed from differences in protein sources and not from metabolic differences between animals. Based on the re-analysis of Froehle et al. (2010), and especially their finding that the observed taxonomic differences stem from differences in dietary inputs rather than underlying physiology, we were confident that data derived from both rodents and pigs could be included in the construction of the model. The significantly more positive residuals we observed for swine versus rodents could thus result from underlying differences in dietary protein carbon isotope composition (which averaged $-21.2 \pm 3.3\%$ for pigs vs. $-18.9 \pm 5.0\%$ for rodents), although that difference in dietary protein was not significant ($t = 1.6$, $df = 36$, $P = 0.12$). While it is undeniable that the proposed model predicts for pigs (at any given value for $\delta^{13}C_{protein}$ input) a higher-than-observed value and for rodents a value lower than that measured in that individual's dietary protein there is, nonetheless, substantial overlap in the range of observed residual values. This is the case despite there being at least two major differences between the experimental diets and animals used in the construction of this model.

First, it has been observed that the rodents employed in the studies referenced here consumed highly unnatural and simplified diets of refined food pellets whereas the pigs were fed more natural diets containing whole food items. The respective quality, texture, and

digestibility of their diets might have been expected to significantly affect digestion and isotopic incorporation. Second, the training set data of this model were derived from taxa with distinct digestive anatomies and physiologies. Both rodents and pigs have proportionally larger hindguts and exhibit a greater capacity for hindgut fermentation than humans, as is evidenced by their fully formed caeca, but rodents, unlike pigs, are coprophagic, re-ingesting their fecal pellets so as to extract maximum attainable nutrition from their diet (Chivers and Langer, 1994). And yet, as the findings of others (e.g. Kellner and Schoeninger, 2007 or Froehle et al., 2010) whose work relied on the same datasets, confirm, it does not appear that these rather major underlying differences (in both dietary composition and digestive physiology) in model-building data sources unduly impacts the utility of the proposed model.

The magnitude of average taxon-specific prediction errors (as gauged by predicted-observed $\delta^{13}C_{protein}$ residuals) fall near or below the Minimum Meaningful Difference (MMD) value for $\delta^{13}C$ analysis of bone collagen (0.6%) and hydroxyapatite (1.2%) presented elsewhere (Pestle et al., 2014). Indeed, this finding, in combination with the results of others (Kellner and Schoeninger, 2007, Froehle et al., 2010), gives us an indication of the limits of our ability to detect, using isotopic methodologies, the effects of differences in digestive physiology (e.g. ruminants versus hindgut fermenters) and dietary makeup (natural versus processed diets) that potentially inform diet-tissue offsets. If nothing else, this finding makes it quite clear that more experimental studies using animal models fed more natural/representative diets are needed, both in general, and in order to validate the use of these earlier studies in building models applied to ancient human cultures. Moreover, as humans (the taxon whose diet we are ultimately interested in modeling), unlike the taxa employed in model building, have only a vestigial caecum and rely more on endogenous enzymes, rather than fermentation, to facilitate food breakdown, it would be desirable to better elucidate the effects of these apparently important differences in digestive physiology on diet-tissue isotope dynamics.

Also of concern, Kellner and Schoeninger (2007) suggested that differential proportions of protein in different individuals' diets might adversely affect the accuracy of models such as that proposed here. However, we found no significant correlation ($r = -0.08$, $P = 0.63$) between protein amount (% protein in Table 1) and residuals about the regression line. This finding indicates that differences in dietary macronutrient composition are not significantly determinative of the model's predicted values. Over the observed range of dietary protein contributions (4.7–70.0%, as based on source experimental diet data), this model would appear to be equally robust.

Findings of both Froehle et al. (2012) and Fernandes et al. (2012b) amply illustrate the influence of carbon derived from both dietary protein and whole diet on $\delta^{13}C_{co}$. Thus, any model that might attempt to predict $\delta^{13}C_{protein}$ without taking into account, in some form, the contribution of carbon derived from whole diet or dietary energy could produce erroneous values. The model we propose, through the incorporation of the $\Delta^{13}C_{ap\text{-}co}$ term, accounts for the experimentally-determined impact that $\delta^{13}C_{energy}$ values could have on the prediction of $\delta^{13}C_{protein}$ (as the $\Delta^{13}C_{ap\text{-}co}$ value tracks differences in the isotope values of dietary energy and protein). It is interesting to note that in terms of predictor importance, the $\delta^{13}C_{co}$ term of our regression is roughly four times (0.8 vs. 0.2) more important in estimating a consumer's $\delta^{13}C_{protein}$ value than the individual's $\Delta^{13}C_{ap\text{-}co}$ signature. It is likely not coincidental that this difference in relative importance comes close, at least, to previous estimations (e.g. Froehle et al., 2010; Fernandes et al., 2012b) that dietary protein contributes at least three-fifths of the carbon atoms to collagen, while accounting for the other two-fifths derived from dietary energy. The proposed model thus accounts for the influence of whole diet/dietary energy when attempting to calculate dietary protein carbon isotope values from measured skeletal isotope values.

Finally, while Harrison and Katzenberg (2003) have demonstrated that animal protein likely contributes more heavily to collagen synthesis than plant protein (because animal proteins contain a higher proportion of the indispensable amino acids than plant proteins), it should be acknowledged that the proposed model is not capable of discriminating between these disparate (floral versus faunal) protein sources. As such, any application of this model to regions/settings possessing protein-rich plant foods, or those examples where recourse to high protein plants was made in order to compensate for a lack of access to animal-derived protein, should be certain to include these plants in their protein foodweb values, otherwise they risk fundamentally misestimating protein source contributions.

It goes without saying, perhaps, that it is our sincere hope that the model presented here might be tested broadly on a variety of archaeological populations, and refined accordingly. It is particularly important, however, that it be evaluated using populations or individuals for whom diet is known, rather than inferred, as is the case in the presented case study. Such an application would provide the only means by which the models accuracy and precision could be assessed in full.

Bearing these points in mind, the model presented above would appear to hold significant potential for the prediction of the carbon isotope signature of dietary protein using only such data as is routinely generated in the course of stable isotope analysis of human osseous remains. The generation of individual $\delta^{13}C_{protein}$ values (plus experimentally generated error terms) is ideal for use in multisource mixture modeling of dietary protein source contribution, and raises intriguing possibilities for moving beyond relative statements of dietary contribution to probabilistic and uncertainty integrated quantification of dietary inputs.

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LITERATURE CITED

Ambrose SH. 2000. Controlled diet and climate experiments on nitrogen isotope ratios of rats. In: Ambrose SH, Katzenberg MA, editors. Biogeochemical approaches to paleodietary analysis. New York: Kluwer Academic. p 243–259.

Ambrose SH, Buikstra JE, Krueger HW. 2003. Status and gender differences in diet at mound 72, cahokia, revealed

by isotopic analysis of bone. J Anthropol Archaeol 22:217–226.

Ambrose SH, Norr L. 1993. 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, editors. Prehistoric human bone: archaeology at the molecular level. New York: Springer-Verlag. p 1–37.

Arcini C, Ahlström T, Tagesson G. 2012. Variations in diet and stature: are they linked? Bioarchaeology and paleodietary bayesian mixing models from Linköping, Sweden. Int J Osteoarchaeol 24:543–556.

Bocherens H, Drucker D. 2003. Trophic level isotopic enrichment of carbon and nitrogen in bone collagen: case studies from recent and ancient terrestrial ecosystems. Int J Osteoarchaeol 46–53. 53.

Bocherens H, Fogel ML, Tuross N, Zeder M. 1995. Trophic structure and climatic information from isotopic signatures in pleistocene cave fauna of Southern England. J Archaeol Sci 22:327–340.

Chivers DJ, Langer P, editors. 1994. The digestive system in mammals: food, form, and function. New York: Cambridge University Press.

Colonese AC, Collins M, Lucquin A, Eustace M, Hancock Y, Ponzoni RdAR Mora A, Smith C, DeBlasis P, Figuti L, Wesolowski V, Plens CR, Eggers S, de Farias E, Gledhill DS, Craig AOE. 2014. Long-term resilience of late holocene coastal subsistence system in Southeastern South America. PLoS One 9:e107034.

Coltrain JB, Janetski JC. 2013. The stable and radio-isotope chemistry of Southeastern Utah basketmaker II burials: dietary analysis using the linear mixing model SISUS, age and sex patterning, geolocation and temporal patterning. J Archaeol Sci 40:4711–4730.

DeNiro MJ, Epstein S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351.

Fernandes R, Millard AR, Brabec M, Nadeau M-J, Grootes P. 2014. Food reconstruction using isotopic transferred signals (FRUITS): a Bayesian model for diet reconstruction. PLoS One 9:e87436-

Fernandes R, Nadeau M-J, Grootes PM. 2012b. Macronutrient-based model for dietary carbon routing in bone collagen and bioapatite. Archaeol Anthropol Sci 4:291–301.

Fernandes R, Rinne C, Grootes PM, Nadeau M-J. 2012a. Revisting the chronology of northern German monumentality sites: preliminary results. Frühe Monumentalität Und Soziale Differenzierung 2:87–103.

Froehle AW, Kellner CM, Schoeninger MJ. 2010. Focus: effect of diet and protein source on carbon stable isotope ratios in collagen: follow up to Warinner and Tuross (2009). J Archaeol Sci 37:2662–2670.

Froehle AW, Kellner CM, Schoeninger MJ. 2012. Multivariate carbon and nitrogen stable isotope model for the reconstruction of prehistoric human diet. Am J Phys Anthropol 147: 352–369.

Hare PE, Fogel ML, Stafford TW, Mitchell AD, Hoering TC. 1991. The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. J Archaeol Sci 18:277–292.

Harrison RG, Katzenberg MA. 2003. Paleodiet studies using stable carbon isotopes from bone apatite and collagen: examples from Southern Ontario and San Nicolas Island, California. J Anthropol Archaeol 22:227–244.

Hedges REM, Reynard LM. 2007. Nitrogen isotopes and the trophic level of humans in archaeology. J Archaeol Sci 34: 1240–1251.

Hopkins JB, Ferguson JM. 2012. Estimating the diets of animals using stable isotopes and a comprehensive bayesian mixing model. PLoS One 7:e28478.

Howland MR, Corr LT, Young SMM, Jones V, Jim S, Van Der Merwe NJ, Mitchell AD, Evershed RP. 2003. Expression of the dietary isotope signal in the compound-specific ^{13}C values of pig bone lipids and amino acids. Int J Osteoarchaeol 13:54–65.

Jim S, Ambrose SH, Evershed RP. 2004. Stable carbon isotopic evidence for differences in the dietary origin of bone cholesterol, collagen and apatite: implications for their use in palaeodietary reconstruction. Geochim Cosmochim Acta 68: 61–72.

Kellner CM, Schoeninger MJ. 2007. A simple carbon isotope model for reconstructing prehistoric human diet. Am J Phys Anthropol 133:1112–1127.

Krueger HW, Sullivan CH. 1984. Models for carbon isotope fractionation between diet and bone. In: Turnlund J, Johnson PE, editors. Stable isotopes in nutrition. Washington, DC: American Chemical Society p 205–222.

Lubetkin SC, Simenstad CA. 2004. Multisource mixing models to quantify food web sources and pathways. J Appl Ecol 41: 996–1008.

Moore JW, Semmens BX. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecol Lett 11:470–480.

O'Connell T, Kneale CJ, Tasevska N, Kuhnle GGC. 2012. The Diet-body offest in human nitrogen isotopic values: a controlled dietary study. Am J Phys Anthropol 149:426–434.

Parnell AC, Inger R, Bearhop S, Jackson AL. 2010. Source partitioning using stable isotopes: coping with too much variation. PLoS One 5:e9672.

Parnell AC, Phillips DL, Bearhop S, Semmens BX, Ward EJ, Moore JW, Jackson AL, Grey J, Kelly DJ, Inger R. 2013. Bayesian stable isotope mixing models. Environmetrics 24: 387–399.

Passey BH, Robinson TF, Ayliffe LK, Cerling TE, Sponheimer M, Dearing MD, Roeder BL, Ehleringer JR. 2005. Carbon isotope fractionation between diet, breath CO_2, and bioapatite in different mammals. J Archaeol Sci 32:1459–1470.

Pestle WJ. 2010. Diet and society in Prehistoric Puerto Rico, an isotopic approach. Ph.D. Dissertation. Chicago: University of Illinois.

Pestle WJ, Colvard M. 2012. Bone collagen preservation in the tropics: a case study from ancient Puerto Rico. J Archaeol Sci 39:2079–2090.

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

Phillips DL. 2001a. mixing models in analyses of diet using multiple stable isotopes: a critique. Oecologia 127:166–170.

Phillips DL. 2001b. uncertainty in source partitioning using stable isotopes. Oecologia 127:171–179.

Phillips DL, Gregg JW. 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia 136:261–269.

Phillips DL, Newsome SD, Gregg JW. 2005. Combining sources in stable isotope mixing models: alternative methods. Oecologia 144:520–527.

Schoeller DA. 1999. Isotope fractionation: why aren't we what we eat? J Archaeol Sci 26:667–673.

Schoeninger MJ. 1985. Trophic level effects on $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios in bone collagen and strontium levels in bone mineral. J Hum Evol 14:515–525.

Schoeninger MJ, DeNiro MJ. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochim Cosmochim Acta 48:625–639.

Schwarcz HP. 1991. Some theoretical aspects of isotope paleodiet studies. J Archaeol Sci 18:261–275.

Semmens BX, Ward EJ, Moore JW, Darimont CT. 2009. Quantifying inter- and intra-population niche variability using hierarchical bayesian stable isotope mixing models. PLoS One 4: e6187.

Sponheimer M, Robinson T, Ayliffe L, Roeder B, Hammer J, Passey B, West A, Cerling T, Dearing D. Ehleringer J. 2003. Nitrogen isotopes in mammalian herbivores: hair $\Delta^{15}N$ values from a controlled feeding study. Int J Osteoarchaeol 13:80–87.

Stock BC, Semmens BX. 2013. Mixsiar, version 1.0. Available at: http://Conserver.Iugo-Cafe.Org/User/Brice.Semmens/Mixsiar. Accessed March 14, 2015.

R Core Team. 2014. R: a language and environment for statistical computing, version 3.1.0. Vienna, Austria: R Foundation for Statistical Computing. Available at: http://www.R-Project.Org/. Accessed March 14, 2015.

Tieszen LL, Fagre T. 1993. Effect of diet quality and composition on the isotopic composition of respiratory CO_2, bone collagen, bioapatite, and soft tissues. In: Lambert JB, Grupe G, editors. Prehistoric human bone: archaeology at the molecular level. Berlin: Springer-Verlag. p 121–155.

Ugan A, Coltrain J. 2012. Stable isotopes, diet, and taphonomy: a look at using isotope-based dietary reconstructions to infer differential survivorship in zooarchaeological assemblages. J Archaeol Sci 39:1401–1411.

Vogel JC, Van Der Merwe NJ. 1977. Isotopic evidence for early maize cultivation in New York state. Am Antiq 42:238–242.

Warinner C, Tuross N. 2009. Alkaline cooking and stable isotope Tissuee-diet spacing in swine: archaeological implications. J Archaeol Sci 36:1690–1697.

Warinner C, Tuross N. 2010. Brief communication: Tissue isotopic enrichment associated with growth depression in a pig: Implications for archaeology and ecology. Am J Phys Anthropol 141:486–493.