DIETARY RECONSTRUCTION USING STABLE ISOTOPES AT TWO PRE-CONTACT SITES IN YOLO COUNTY

JELMER W. EERKENS, JENA GOODMAN, AND SUSAN TALCOTT
UNIVERSITY OF CALIFORNIA, DAVIS

We present new stable isotope and radiocarbon data from two pre-contact sites in Yolo County, to examine diachronic change in paleodiet in the southern Sacramento Valley. We examine stable carbon and nitrogen isotope signatures in human bone collagen from four individuals from CA-YOL-171, an Early-period site on Willow Slough in the Yolo Basin, and 12 individuals from YOL-187, a Late-period site on lower Cache Creek. Stable isotope data are consistent with a shift from consumption of higher trophic level foods, including salmon and sturgeon, in the Early period to a greater emphasis on lower trophic level items, such as small freshwater fish and/or plant foods, in the Late period. At the same time, results document significant inter-individual variation within sites, showing that not everybody ate the same suite of foods. The reasons for these inter-individual dietary differences, whether sex-, age-, status-, or idiosyncratic-based, should be the focus of future research.

Archaeologists use a range of techniques to investigate paleodiets, each with advantages and disadvantages. Zooarchaeological and paleobotanical analyses allow archaeologists to determine the species and genera that ancient foragers exploited. However, bones and charred plant remains enter the archaeological record in a range of ways and can be differentially deposited, preserved, and recovered. This makes straightforward links between faunal and floral remains and ancient diet challenging. As well, such materials were typically discarded by a range of individuals in a society and accumulated over long periods of time (e.g., centuries to millennia in some cases). As a result, we are unable to link them to particular individuals of the past.

Stable isotope analyses of human remains, on the other hand, represent diet over much shorter time intervals and allow us to link diet to particular individuals. As a result, we can examine connections between certain aspects of diet and various biosocial conditions, such as age, sex, burial position, and status, as measured by the quantity and types of associated grave goods. However, the level of specificity using stable isotopes in dietary reconstruction is low, and we are only able to determine general categories of diet, such as marine-focused vs. terrestrial-focused, or plant-focused vs. game-focused. When faunal and paleobotanical analyses are combined, we gain the greatest understanding of ancient diets. In this article, we report and discuss bone collagen stable isotope values for a sample of human remains recovered from two sites, YOL-171 and YOL-187, in the lower Sacramento Valley (Figure 1; Table 1).

METHODS AND APPROACH

In dietary studies around the world, carbon isotopes ($^{13}C/^{12}C$, expressed as $\delta^{13}C$ relative to a standard) often provide an estimate of the consumption of C3 vs. C4 plants. The majority of plants around the world are C3 plants, producing a three-carbon molecule during the fixation of atmospheric carbon. This method of photosynthesis discriminates against the heavier ^{13}C, resulting in $\delta^{13}C$ values in plants between -30‰ and -22‰. By contrast, a small number of plants produce a four-carbon molecule (C4) and have tissues with $\delta^{13}C$ values typically between -16‰ and -10‰. While the number of C4 photosynthesizers is low, several important crop plants, such as maize, millet, sugar cane, and sorghum, fall in this category, allowing archaeologists to estimate their importance in local diets (Schwarcz and Schoeninger 1991). In central California, there are few C4 plants, and the majority of those were not important dietary staples (Bartelink 2009).

Carbon enters marine environments mainly through exchange with atmospheric CO_2 and through photosynthesizing phytoplankton. $\delta^{13}C$ values of biologically available carbon in marine environments

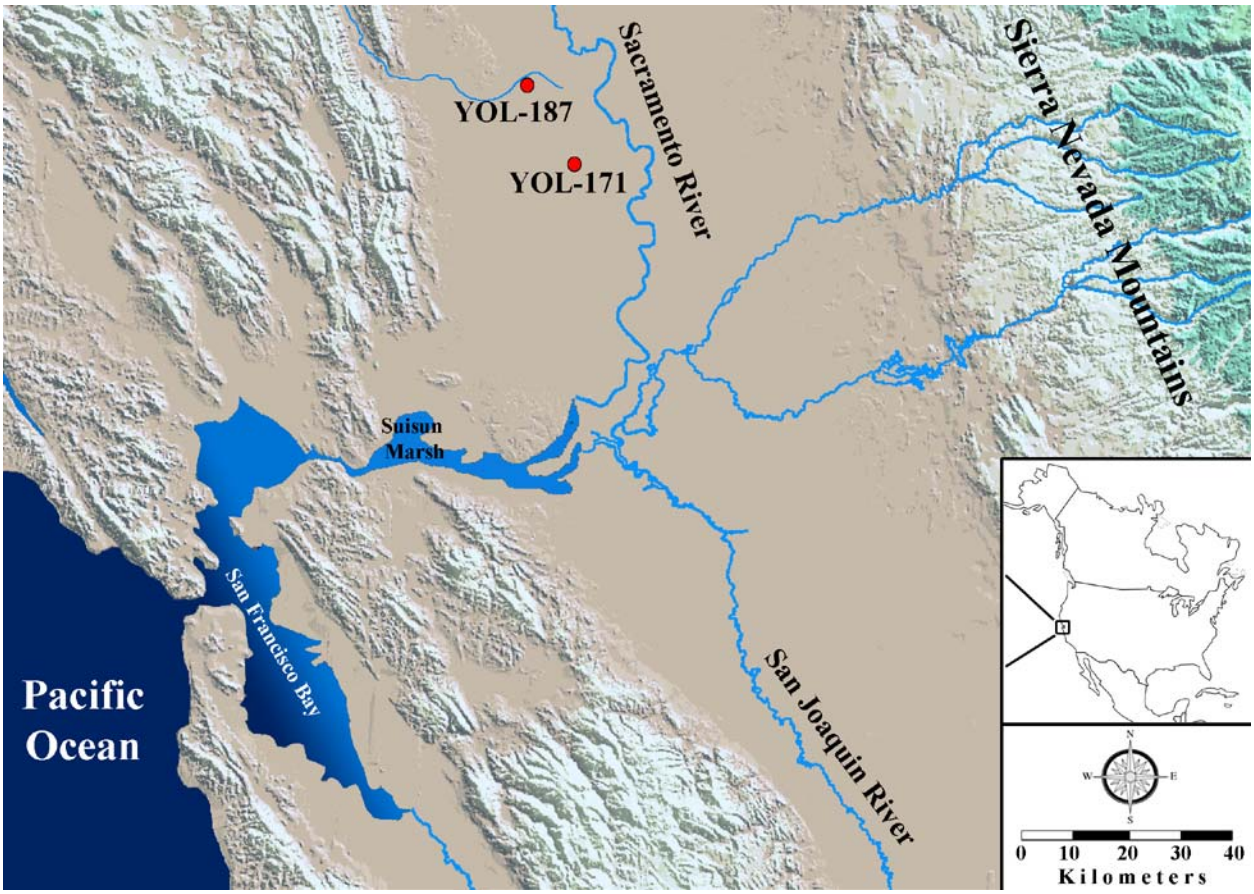

Figure 1. Map of the lower Sacramento Valley and the approximate locations of YOL-171 and YOL-187.

Table 1: Samples included in this study.

Site	Catalog/ Burial #	Age	Sex	Element	Burial Position
YOL-171	Burial 1	Young adult	Indeterminate	R. humerus	Loose flex
YOL-171	Burial 2	Young adult	Male	L. femur	Loose flex
YOL-171	Burial 3	Young adult	Indeterminate	Long bone	Tight flex
YOL-171	419-11	Adult	Indeterminate	Long bone	Indeterminate
YOL-187	Burial 1	Subadult	Indeterminate	L. ulna	--
YOL-187	Burial 2	Adult	Male	R. radius	--
YOL-187	Area C	Indet.	Indeterminate	L. humerus	--
YOL-187	Area E	Young adult	Indeterminate	R. humerus	--
YOL-187	472	Adult	Indeterminate	L. ulna	--
YOL-187	454-17	Adult	Male?	L. femur	--
YOL-187	454-21	Adult	Indeterminate	L. femur	--
YOL-187	459-4	Adult	Indeterminate	R. mandible	--
YOL-187	459-5	Adult	Indeterminate	L. mandible	--
YOL-187	464	Young adult	Male?	L. mandible	--
YOL-187	422	Adult	Indeterminate	Occipital	--
YOL-187	454-6	Adult	Indeterminate	L. mandible	--
YOL-187	468-9	Indeterminate	Indeterminate	L. ulna	--

typically overlap with those of C4 plants. Because C4 plants were generally not consumed in central California, we can use $\delta^{13}C$ as a discriminator of terrestrial- vs. marine-derived carbon, with heavier (less negative) $\delta^{13}C$ indicating a greater contribution of marine organisms to the diet (Bartelink 2009; Schoeninger et al. 1983; Schwarcz and Schoeninger 1991). Brackish water environments, such as Suisun Marsh and the California Delta, will display intermediary values (Eerkens et al. 2013).

While $\delta^{13}C$ reflects marine vs. terrestrial input in central California, nitrogen isotopes ($^{15}N/^{14}N$, expressed as $\delta^{15}N$, again relative to a standard) reflect the general trophic level of consumed foods (Schoeninger and DeNiro 1984). Nitrogen fractionates during the digestion of food, the formation of urea, and the synthesis of new biological tissues in animals, favoring the retention of the heavier ^{15}N for the latter. As a result, $\delta^{15}N$ increases by about 3-4‰ with each trophic level. In terrestrial systems in central California, there are essentially three trophic levels: plants, browsers (vegetarians), and carnivores. By contrast, in aquatic environments there are more trophic levels, resulting in greater enrichment of $\delta^{15}N$ at the top of the food chain (typically large fish, predatory birds, and aquatic mammals).

As omnivores with a range of material technologies to harvest foods, humans can fall in a wide range of trophic level positions, and can gain their food from a wide array of micro-environments, including marine, freshwater, and terrestrial locations. In some highly cooperative groups, humans share food widely, while in others they are more individualistic and only share within family units. In the former, diets will be very similar and inter-individual differences will be minimal, while in the latter we expect much higher inter-individual differences. Stable isotope analyses are particularly informative at highlighting such dietary patterns, which can then be used to reconstruct certain aspects of social structure and organization in ancient societies.

Controlled feeding experiments suggest collagen is synthesized in the body mainly from dietary protein intake. More specifically, Fernandes et al. (2012) estimate that 74 percent of the carbon and nitrogen in bone collagen is routed from dietary protein, with the remainder coming from lipids and carbohydrates. This suggests that isotopic values in collagen reflect mainly, though not exclusively, dietary protein. By contrast, bone bioapatite (the inorganic, mineral component of bone) is synthesized more from the whole diet (i.e., a mix of protein, lipids, and carbohydrates). Comparing isotopic values in collagen with bioapatite allows researchers to parse out the sources of different macronutrients. We plan to analyze bioapatite from these same sites in the future, but focus below only on bone collagen.

To isolate collagen for analysis, we followed a modified Longin procedure (Longin 1971). Approximately 1 g of cortical bone was cleaned of any surface contamination by first drilling exposed surfaces with a diamond bit and then sonicating the sample in deionized H_2O (three five-minute baths, with the dH_2O replaced after each bath). The sample was left in an open container until completely dry, weighed, and demineralized with a solution of 0.5M hydrochloric acid (HCl). HCl was changed every other day until the sample was completely demineralized (up to two weeks). The bone was then washed three times with dH_2O and soaked in 0.125M sodium hydroxide (NaOH) for 24 hours to remove humic acids. The sample was rinsed five times with dH_2O to remove any residual NaOH.

Slightly acidic pH 3 water was added to the vial, and the sample was placed in a 70°C oven for approximately 24 hours to solubilize collagen. If the sample was not completely solubilized, then after centrifuging the sample for three to four minutes, the pH 3 solution was pipetted into a clean vial and the process was repeated, for up to two additional times. The original sample vial was then discarded and the pH 3 solution placed in an oven with no cap, to evaporate the solution until about 3 ml of solution remained. The sample was then placed in a freeze-dryer to remove all remaining water, isolating the collagen fraction.

Collagen total C, total N, $\delta^{13}C$, and $\delta^{15}N$ were measured by continuous-flow mass spectrometry (PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer) at the Stable Isotope Facility, UC Davis. Carbon isotopes ratios, $\delta^{13}C$, are expressed in per-mil notation (parts per thousand) relative to the PeeDee Belemnite standard (arbitrarily set at 0‰), while N isotope ratios, $\delta^{15}N$, are expressed against N_2 in modern atmospheric air (also arbitrarily set to 0‰).

SAMPLES SELECTED

YOL-171, or the Yolo County Landfill Cemetery, is a buried site discovered in 1981 during mechanical excavations associated with expansion of the Yolo County Central Landfill. At a depth of over 2.5 m, the remains of at least four individuals were exposed within a sandy matrix thought to date to the Middle Holocene. The site is currently 10 km from the Sacramento River and 4.5 km from Willow Slough. Either of these watercourses may have run near the site when it was in use; however, no paleoenvironmental analyses were conducted at the time of excavation. Other than removal of the burials and an examination of backhoe spoils piles, which contained artifacts, ecofacts, and human bone, no formal archaeological research occurred at that time. Thus, despite the name, it is unclear if the site is only a cemetery or also includes habitation and other refuse (i.e., midden). Very little is known about the antiquity of the site or the cultural affiliation of the remains and associated artifacts. Many of the human remains and other materials were left in the field. However, samples of bone and some artifacts were retained and curated at the UC Davis Museum of Anthropology, which granted permission to conduct the analyses in this study.

YOL-187, or the Swimming Pool site, is located in the town of Yolo along the lower reaches of Cache Creek. The site was test excavated by Eric Wohlgemuth in the 1990s due to disturbances associated with construction of a swimming pool. Diagnostic artifacts and a radiocarbon date indicate occupation during the Late period, from 500 to 100 cal B.P. At least two burials were recorded and excavated, as well as a large number of disassociated human bones. Samples for radiocarbon and stable isotope analysis were selected from these remains. We tried to select bones to maximize the number individuals represented in our study. This included sampling bone collected from different parts of the site, or selecting bone from repeating elements (e.g., two left mandibles) or elements that clearly represented different individuals (e.g., a bone from a juvenile and a bone from an adult). However, given the level of disturbance at the site, it is possible that some of the bones may represent fragments from the same individual. Permission to conduct DNA and isotope analyses was granted by Mary Norton of Cortina Rancheria, assigned as the Most Likely Descendant (MLD) for the project.

RESULTS

Six radiocarbon dates on human bone collagen were obtained from the two sites. Both the radiocarbon age, in years B.P. (before 1950), and the calibrated age in years before 1950, are reported in Table 2. A linear mixing model developed by Bartelink (2009) was used to estimate the percentage of carbon deriving from marine sources in the collagen samples. A marine reservoir correction of 250 ±40 years was then used to help calibrate the dates, using the online version of Calib 7.1 (http://calib.qub.ac.uk).

Radiocarbon dates confirm that YOL-171 is an older site, dating to the Middle Holocene. Three dates produced nearly identical calibrated ages between 4500 and 4300 cal B.P. (with the date on Burial 1 producing a very minor tail out to 4783 cal B.P.). The three radiocarbon dates from YOL-187 are also in line with estimated ages for that site, showing a Late Holocene age. The three dates produced calibrated age estimates between 517 and 317 cal B.P.

Collagen yields were good for all samples reported in Tables 1 and 2, above 1 percent yield by weight. As well, atomic C/N ratios for the collagen samples are in line with accepted values for well-preserved collagen.

$\delta^{13}C$ and $\delta^{15}N$ values are notably different for the two sites, indicating different diets, especially in the source(s) of dietary protein. Figure 2 plots $\delta^{13}C$ and $\delta^{15}N$ for the 16 individuals from this study, and also includes ellipses for human bone collagen from other central California regions and environments, as reported in Eerkens et al. (2013). The figure shows that, barring one outlier, the YOL-187 individuals fall within the range of foragers at other sites in the Sacramento Valley that are not directly on the Sacramento River (such as YOL-110 and SOL-270; unpublished data in possession of the senior author). The YOL-187 individuals are generally low for both $\delta^{15}N$ and $\delta^{13}C$, indicating a protein source that is low in trophic

Table 2: Results of stable isotope and radiocarbon analyses.

Site	Catalog/ Burial #	$\delta^{13}C$	$\delta^{15}N$	C / N	Estimated Marine Carbon	^{14}C Date B.P.	Calibrated Date Range (2-sigma) B.P.
YOL-171	Burial 1	-18.8	12.1	3.3	20%	4130 ±50	4298-4783
YOL-171	Burial 2	-18.8	10.9	3.2	20%	--	--
YOL-171	Burial 3	-18.5	12.0	3.2	22%	4070 ±25	4290-4511
YOL-171	419-11	-18.3	12.9	3.3	23%	4110 ±30	4309-4526
YOL-187	Burial 1	-19.5	9.3	3.3	16%	--	--
YOL-187	Burial 2	-19.5	10.0	3.3	16%	535 ±20	464-517
YOL-187	Area C	-19.9	9.7	3.3	13%	--	--
YOL-187	Area E	-20.0	9.8	3.3	13%	450 ±25	317-498
YOL-187	472	-19.6	10.5	3.4	15%	--	--
YOL-187	454-17	-19.2	12.4	3.3	18%	--	--
YOL-187	454-21	-19.6	10.7	3.5	15%	--	--
YOL-187	459-4	-19.6	10.2	3.3	15%	--	--
YOL-187	459-5	-19.6	10.2	3.2	15%	--	--
YOL-187	464	-20.0	9.4	3.2	13%	--	--
YOL-187	422	-19.8	10.6	3.5	14%	--	--
YOL-187	454-6	-19.6	10.1	3.4	15%	470 ±20	321-499
YOL-187	468-9	-19.6	10.0	3.2	15%	--	--

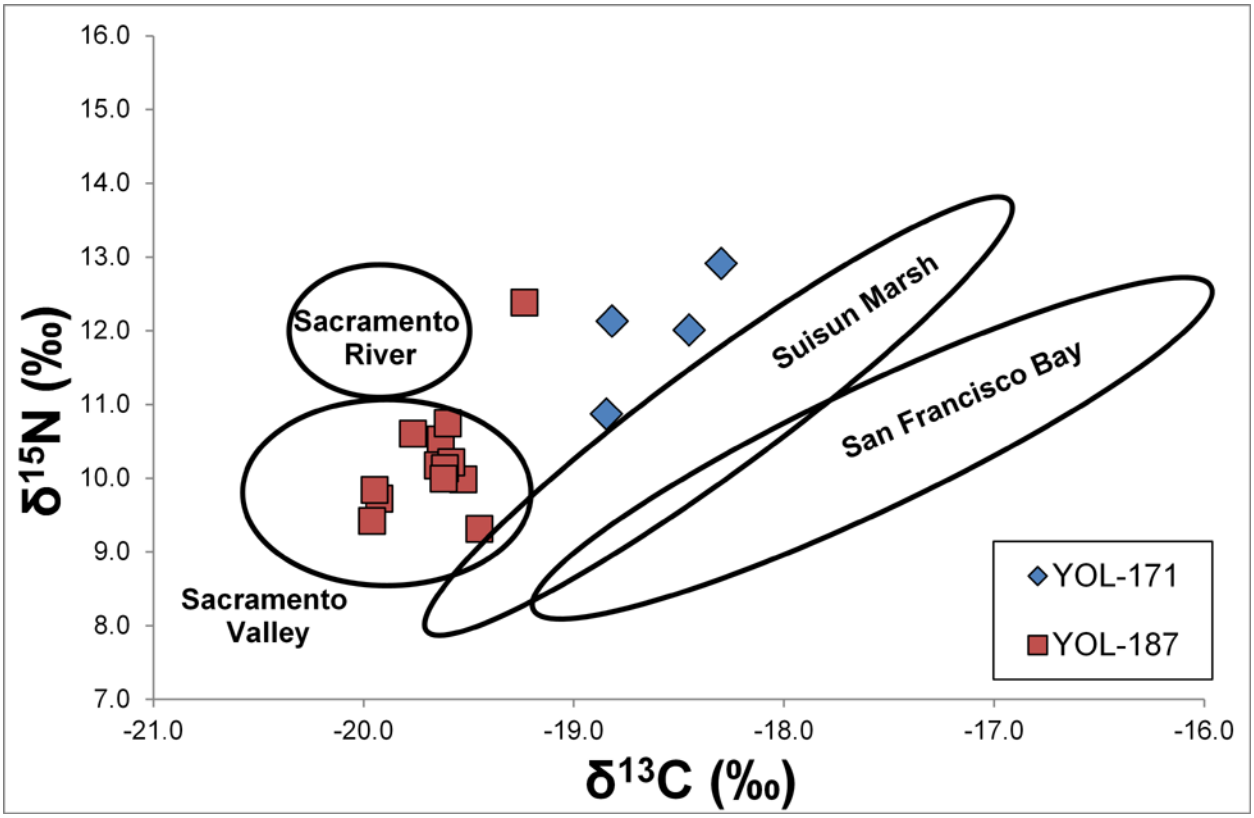

Figure 2. $\delta^{13}C$ and $\delta^{15}N$ for the samples included in this study and in comparison to ellipses representing isotopic range in other central California regions and environments.

level (e.g., more plant-focused than fish- or animal-focused) and with little marine-derived carbon (e.g., very little salmon or sturgeon). The findings conform to general notions about Late-period diets in the Sacramento Valley, which were heavily focused on acorns and small seeds (Wohlgemuth 1996).

By contrast, the four YOL-171 samples fall in a different part of the graph, one that does not overlap with individuals from other regions and environments in central California. The YOL-171 carbon isotopes are slightly enriched (less negative $\delta^{13}C$) compared to Sacramento River foragers, indicating greater input of marine dietary resources, and similar to values observed for foragers exploiting Suisun Marsh. However, nitrogen isotopes are elevated over those previously recorded for Suisun Marsh, indicating exploitation of protein from slightly higher trophic levels. Note also that one of the YOL-187 individuals is close to the group of YOL-171 foragers.

DISCUSSION AND CONCLUCIONS

Taking the two sites together, the data support a model of dietary intensification over time in the region. The isotopic data suggest a shift from higher trophic level prey items in the Middle Holocene to lower trophic level items in the Late Holocene, at least for sources of dietary protein. At the same time, the data also suggest a decrease in the importance of marine-derived protein, presumably salmon and sturgeon. Although we do not have faunal remains from YOL-171, an analysis of fish bone from YOL-187 supports this finding. Gobalet (2004) records only 25 salmon and two sturgeon bones among a sample of 394 identified fish bone from YOL-187, less than 7 percent of all identified fish specimens. Other sites in the Sacramento Valley, especially those farther north, often have much higher percentages of salmon bone (e.g., Hildebrandt and Darcangelo 2008).

This overall dietary shift is also in line with previous analyses of faunal and floral remains in the Sacramento Valley (e.g., Broughton 1994; Wohlgemuth 1996), as well as previous stable isotope analyses on human bone collagen (e.g., Bartelink 2009). These studies document an increasing role of small-bodied game, small fish, acorns, and small seeds in local diets between the Middle and Late Holocene. Such a dietary shift would result in decreases in $\delta^{13}C$ and $\delta^{15}N$ values in human bone collagen, as recorded between the two sites in this study.

The data from YOL-171 suggest exploitation of higher numbers of salmon and/or sturgeon. Currently, salmon and sturgeon do not run in the minor Willow Slough. This suggests either that Willow Slough was larger in the Middle Holocene and did contain such fish, or that individuals buried at the site regularly accessed food resources from the Sacramento River, which, of course, does host such fish. We do not know about residential and/or land-use patterns of people in the Middle Holocene in Yolo County. Thus, while they were buried at YOL-171, they may have resided closer to the Sacramento River, or may have made seasonal movements across the valley floor to and from the Sacramento River. It is also possible that the Sacramento River ran closer to YOL-171 during the Middle Holocene and was readily accessible from the site. Additional paleoenvironmental research is badly needed in this part of the Sacramento Valley to reconstruct the meanderings of the river over time, as well as research on human mobility patterns.

The stable isotope data also point to significant variation in individual diets within the sites. For example, one adult individual from YOL-187, in particular, had a diet that is consistent with consumption of higher trophic level foods, with a larger input of marine carbon. Perhaps this individual and/or his/her family exploited salmon to a greater degree than other individuals at the site, and was perhaps responsible for the majority of the salmon bones that were recovered in the midden. Additional analyses would be necessary to determine this, but the variation in dietary signatures points to an important result, namely, that not everyone at these sites was consuming the same suite of foods. Instead, there is significant dietary diversity between different individuals.

Understanding the factors behind inter-individual dietary differences would be an important contribution to archaeological research in the region. Thus, it is possible that males and females had different diets, that individuals who immigrated to the site (for example, through marriage) had different

dietary preferences, or that status may account for differences in access to some resources (e.g., high-status individuals controlled good fishing spots and access to salmon or sturgeon). Unfortunately, our sample size from these two sites is too small and we do not have the necessary demographic information to explore obvious factors that might account for such inter-individual dietary diversity (e.g., sex, age, status, immigrant status). Future research should seek to address the source of such variation at these and other sites. Such analyses are sure to contribute important information regarding social organization and resource sharing patterns in these pre-contact societies in central California.

ACKNOWLEDGEMENTS

We thank Mary Norton, MLD, for supporting scientific analyses of her ancestors. We hope such information will help tell the individual life stories of people from the past and are of value not just to archaeologists but to Native communities and others as well. We thank the UC Davis Anthropology Museum for providing access to samples from YOL-171 and Joy Matthews and the staff at the UC Davis Stable Isotope Facility for their assistance with the project. Finally, we thank the graduate and undergraduate students at the UC Davis Archaeometry Lab for their various contributions to this project, including Alex Greenwald, Greg Burns, Candice Ralston, Marcos Martinez, and Kelli Sullivan.

REFERENCES CITED

Bartelink, Eric J.
2009 Late Holocene Dietary Change in the San Francisco Bay Area: Stable Isotope Evidence for an Expansion in Diet Breadth. *California Archaeology* 1:227-52.

Broughton, Jack M.
1994 Late Holocene Resource Intensification in the Sacramento Valley, California: The Vertebrate Evidence. *Journal of Archaeological Science* 21:501-514.

Eerkens, J. W., Madeline Mackie, and Eric J. Bartelink
2013 Brackish Water Foraging: Isotopic Landscapes and Dietary Reconstruction in Suisun Marsh, Central California. *Journal of Archaeological Science* 40:3270-3281.

Fernandes, Ricardo, Marie J. Nadeau, and Pieter M. Grootes
2012 Macronutrient-based Model for Dietary Carbon Routing in Bone Collagen and Bioapatite. *Archaeological and Anthropological Sciences* 4:291-301.

Gobalet, Kenneth W.
2004 Using Archaeological Remains to Document Regional Fish Presence in Prehistory: A Central California Case Study. *Transactions of the Western Section of the Wildlife Society* 40:107-113.

Hildebrandt, William R., and Michael J. Darcangelo
2008 *Life on the River: The Archaeology of an Ancient Native American Culture*. Heyday Books, Berkeley, California.

Longin, R.
1971 New Method of Collagen Extraction for Radiocarbon Dating. *Nature* 230:241-242.

Schoeninger, Margaret J., and Michael J. DeNiro
1984 Nitrogen and Carbon Isotopic Composition of Bone Collagen from Marine and Terrestrial Animals. *Geochimica et Cosmochimica Acta* 48:625-639.

Schoeninger, Margaret J., Michael J. DeNiro, and Henrik Tauber
1983 Stable Nitrogen Isotope Ratios of Bone Collagen Reflect Marine and Terrestrial Components of Prehistoric Human Diet. *Science* 220:1381-1383.

Schwarcz, Henry P., and Margaret J. Schoeninger
1991 Stable Isotope Analyses in Human Nutritional Ecology. *Yearbook of Physical Anthropology* 34:283-321.

Wohlgemuth, Eric
1996 Resource Intensification in Prehistoric Central California: Evidence from Archaeobotanical Data. *Journal of California and Great Basin Anthropology* 18:81-103.