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# Stable isotope analysis of serial samples of third molars as insight into inter- and intra-individual variation in ancient diet

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## ABSTRACT

Identification and analysis of faunal and botanical remains provide important insight into paleodiet, but tend to average dietary behaviors over many individuals and large windows of time. Stable isotope analyses of human bone collagen and apatite, while less specific regarding diet, nicely complement faunal and macrobotanical analyses by giving glimpses into the diets of individuals, but still time-average diet over large windows (ca. 5–20 years). Recent advances in micro-sectioning of teeth provide the opportunity to examine intra-individual changes in diet over small windows of time, as small as 1 year or less. We use stable isotope data from human third molar dentin as a window into examining intra-individual variation in diet at a hunter-gatherer archeological site in Central California (CA-SOL-11). Data show significant dietary variation between individuals, suggesting there were many ways that individuals met basic dietary needs at the site. Data also show that some individuals significantly altered their diet in the course of their lives, with pulses of high brackish-water exploitation lasting between 1 and 4 years. We suggest one of three scenarios to explain these fluctuations: that brackish water foods served as fallback resources, that some individuals shifted their place of residence, or that changing trade relations may have caused fluctuations in consumption of brackish-water foods.

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## 1. Introduction

Reconstructions of paleodiet have traditionally relied on analyses of faunal and/or macrobotanical remains in midden. Attributes of bones and/or botanical remains allow for very detailed identification, often down to the species or genus level, as well as identification of the age and sex of specific prey items (Klein and Cruz-Uribe, 1984; Lyman, 1994; Reitz and Wing, 1999). Such data are very useful for examining a wide range of prehistoric behaviors, such as the micro-environments people exploited to gain their food, how people managed herds of animals, and the seasonality of foraging activities. Data from such analyses are often used to test predictions or models derived from foraging theory, such as the diet breadth model. However, faunal and botanical remains from sites represent accumulations of dietary refuse of groups of people, typically over large windows of time, while foraging decisions are made by individuals over short windows.

Over the last 20 years, stable isotope studies have matured and contributed a new means of reconstructing paleodiet. These analyses have provided new perspectives on many long-standing debates in archeology, such as human evolution and the transition to agriculture. For example, such research has shown that Neanderthals were top-level carnivores in Pleistocene Europe (Richards and Trinkaus, 2009), that there was a significant temporal lag between the first appearance and

later dependence on maize agriculture in North America (Buikstra and Milner 1991; Van der Merwe and Vogel, 1978), and has revealed the timing of pig and dog husbandry in China (Barton et al., 2009).

Many applications of stable isotopes still focus on analysis and comparison of diet among different populations, for example Neanderthals vs. modern humans, or hunter-gatherers vs. agriculturalists. Less often do archeologists focus on intra-population variation, that is, why individuals within a contemporaneous group vary in their isotopic signatures. Yet, such individual-scale analyses are better suited for testing hypotheses drawn from evolutionary theory in anthropology, which focus on the actions of individuals, not groups of people. Even fewer studies in archeology focus on variation in diet over time within particular individuals, though recent studies on hair and teeth have attempted to address this issue (Beaumont et al., 2012; Burt, 2015; Eerkens et al., 2011, 2014a; Fuller et al., 2003; Sharp et al., 2003; Webb et al., 2013).

This study examines variation in stable isotope signatures in a Late Holocene site (CA-SOL-11) in Central California (see Fig. 1). The site is located in an inland setting on a riparian corridor next to oak grasslands. Upland hunting grounds (in the California Coast Range) and a brackish-water marsh (Suisun Marsh) are accessible within 10–15 km. Radiocarbon dates and temporally diagnostic artifacts place the site during what local archeologists refer to locally as the Middle Period, or roughly 2500–1200 cal BP. A previous analysis (Eerkens et al., 2013), focused on stable isotope analysis of bone collagen and apatite of the burial population as a whole. That study demonstrated the distinctive isotopic

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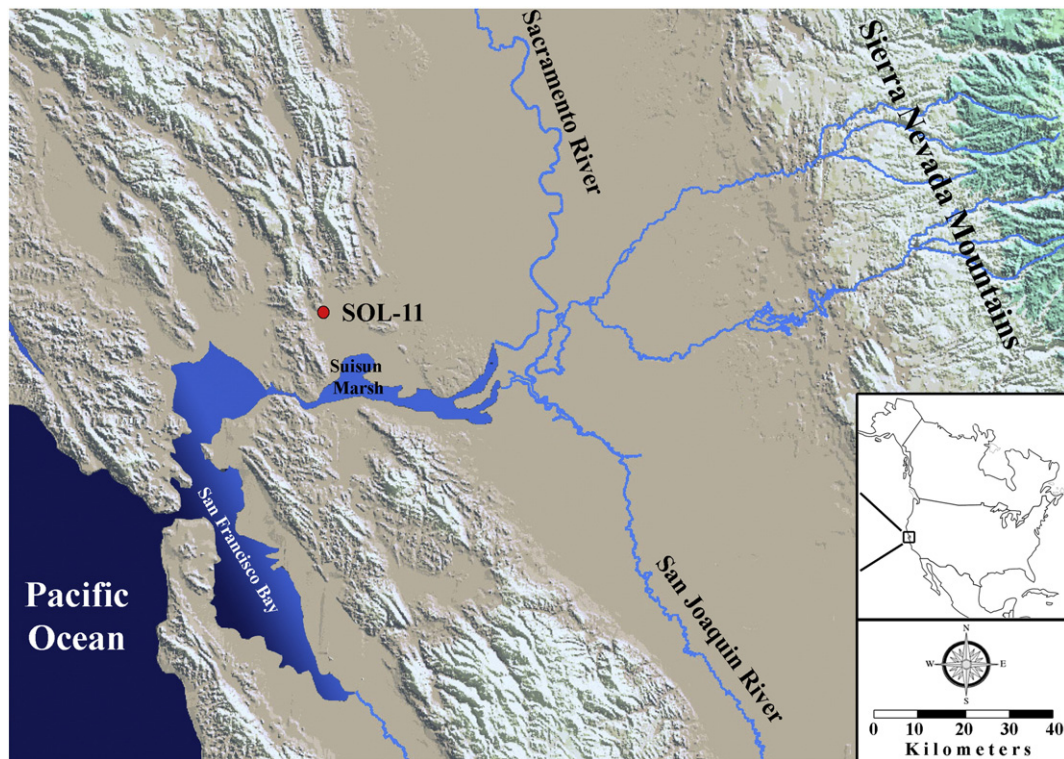


Fig. 1. Map of Central California showing location of CA-SOL-11.

signature of CA-SOL-11 inhabitants relative to other contemporary forager populations living in coastal, riverine, and non-riverine terrestrial environments in Central California. This distinctive signature, at the population level, was attributed to varying degrees of exploitation of foods derived from the brackish-water environment of nearby Suisun Marsh.

This study focuses on intra-individual variation in stable isotopes as a means of examining change in the diet within the life of an individual, and how different individuals from the same site exploited the range of nearby environments for their subsistence base. In particular, we are interested in examining whether brackish-water resources were exploited by everyone or just some individuals, and whether they were consistently exploited during all years or only during certain years. Here, we follow recent discussions of food types, including staple vs. fallback items, recently discussed in the physical anthropology literature (Marshall et al., 2009). The results, we suggest, provide a much more nuanced view of different food resources and how they were used in ancient human populations, and complement faunal and paleobotanical analyses.

### 1.1. Stable isotopes in Central California

In paleodietary studies, carbon isotopes ( $^{13}\text{C}/^{12}\text{C}$ , expressed as  $\delta^{13}\text{C}$ , see below) often provide an estimate of the consumption of  $\text{C}_3$  vs.  $\text{C}_4$  plants. The majority of economically-important plants around the world are  $\text{C}_3$  plants that discriminate against the heavier  $^{13}\text{C}$  during photosynthesis, resulting in  $\delta^{13}\text{C}$  values between  $-30\%$  and  $-22\%$  (Cerling et al., 1998; Ehleringer et al., 1993; Farquhar et al., 1989). By contrast,  $\text{C}_4$  photosynthesis produces tissues with  $\delta^{13}\text{C}$  values typically between  $-16\%$  and  $-10\%$ . While the number of  $\text{C}_4$  photosynthesizers globally is low, several important crop plants, such as maize, millet, sugar cane, and sorghum, fall in this category (Ehleringer et al., 1991; Tipple and Pagani, 2007), allowing archeologists to estimate their importance in local diets. In Central California, there are few native  $\text{C}_4$

plants, and the majority of those were not important sources of food (Bartelink, 2006; Cloern et al., 2002).

Carbon enters marine environments mainly through exchange with atmospheric  $\text{CO}_2$  and is present as dissolved inorganic carbon (DIC). This carbon can then be absorbed into organisms through photosynthesis, especially by phytoplankton (Boutton, 1991), and enters the food chain.  $\delta^{13}\text{C}$  values of biologically available carbon in marine environments is typically enriched relative to terrestrial environments, and largely overlaps with values recorded in  $\text{C}_4$  plants. Thus, rather than a  $\text{C}_3$  vs.  $\text{C}_4$  marker, we can use  $\delta^{13}\text{C}$  in Central California as a discriminator of terrestrial- vs. marine-derived carbon, with heavier (less negative)  $\delta^{13}\text{C}$  indicating a greater contribution of marine organisms to the diet (Bartelink, 2009; Schoeninger et al., 1983; Schwarcz and Schoeninger, 1991).

Nitrogen isotopes ( $^{15}\text{N}/^{14}\text{N}$ , expressed as  $\delta^{15}\text{N}$ , see below) reflect the general trophic level of consumed foods. Nitrogen fractionates during the synthesis of biological tissues, favoring the retention of the heavier  $^{15}\text{N}$ . As a result,  $\delta^{15}\text{N}$  increases by about 3–4‰ with each trophic level. In terrestrial systems in Central California, there are essentially three trophic levels, plants, vegetarians, and carnivores. By contrast, in aquatic environments there are more trophic levels, resulting in greater enrichment of  $^{15}\text{N}$  at the top of the food chain. The latter include large fish, predatory birds, and aquatic mammals.

In humans, consumed protein is differentially routed to collagen (Ambrose and Norr, 1993; Kellner and Schoeninger, 2007; Tieszen and Fagre, 1993). A recent study estimates that 72% of the carbon in collagen comes from dietary protein, with the remaining 28% derived from carbohydrates and lipids (Fernandes et al., 2012). Combined,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of human collagen can be used to discriminate foraging, especially for protein, in different environments. In general, foragers in coastal environments tend to have elevated levels of  $\delta^{13}\text{C}$ . As well, because nitrogen isotopes fractionate with each trophic level and marine environments tend to have more trophic levels,  $\delta^{15}\text{N}$  also tends to be elevated in these settings. By contrast, foragers in terrestrial environments typically display non-overlapping ranges of C and N isotopes.

Where C4 plants dominate local landscapes,  $\delta^{13}\text{C}$  values in collagen can overlap values for coastal foragers, but  $\delta^{15}\text{N}$  will be lower due to the lower trophic level of plants. In landscapes where C3 plants dominate, both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are lower than for coastal foragers. In lacustrine and riverine settings, where there is no marine carbon but there are many trophic levels, foragers display elevated  $\delta^{15}\text{N}$  values but lower  $\delta^{13}\text{C}$  values. Finally, brackish-water environments are intermediate between marine and lacustrine/riverine environments (Eerkens et al., 2013).

An important component of the research below concerns the interpretation of variation in stable isotope signatures. In particular, we need to know if a shift in stable isotope signatures in serial samples represents a behaviorally meaningful shift in diet, or variation due to instrumentation precision and/or collagen extraction methods, or potentially, non-dietary variation within individuals (e.g., changes in metabolism).

Repeated analyses of standards with known isotopic composition at the UC Davis Stable Isotope Facility, run on the same instrument used in this study, show that instrument precision is 0.1‰ for  $\delta^{13}\text{C}$  and 0.2‰ for  $\delta^{15}\text{N}$ . Shifts in stable isotope values less than this may simply due to instrumentation error. However, variation in standards do not take into account variation in sample preparation techniques (i.e., collagen extraction). Using slightly older instrumentation than is used in labs today, DeNiro and Schoeninger (1983) extracted collagen from different bones of the same individual non-human animal that had been fed a monotonous diet, and found variation of between 0.5–1.0‰ for  $\delta^{13}\text{C}$  and 0.6–1.4‰ for  $\delta^{15}\text{N}$ . More recent experiments by Pestle et al. (2014), where multiple labs were sent a single piece of bone from the same animal, and asked to extract five replicates of collagen, reveal slightly lower variation. Eighteen labs, generally using newer mass spectrometer instruments, produced an average range (max–min value) of 0.3‰ for  $\delta^{13}\text{C}$  and 0.4‰ for  $\delta^{15}\text{N}$  for the five replicates.

In this paper we use the more conservative figures of 1.0‰ for  $\delta^{13}\text{C}$  and 1.4‰ for  $\delta^{15}\text{N}$ , as suggested by DeNiro and Schoeninger (1983), as minimum values that reflect a dietary or metabolic change for an individual. Thus, shifts in  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  equal or greater than these values across the serial sections of third molars are interpreted here as behaviorally meaningful changes in either diet or metabolism, though we generally refer to the former. Shifts smaller than these values could represent minor dietary changes, but would require additional analyses and experimentation with control samples to evaluate such an hypothesis.

## 1.2. Dentin serial sampling

Dentinal and enamel tissues in human teeth grow in thin layers over time (Hillson, 1996). Each layer forms over a number of weeks to months as tissues are initially deposited and subsequently mineralized. For dentin, the layers accumulate from the dentin–enamel junction in the crown downwards through the root over time. The age at which a tooth forms varies greatly by tooth, with some beginning growth in utero and finishing within the first year of life (e.g., deciduous teeth), and others beginning and ending much later in life (e.g., third molars). Although mineralization of a particular layer takes several weeks or months, once formed these tissues do not remodel later in life (unlike bone). Because dentin and enamel are synthesized in the human body using biomolecules that are digested from foods, tissues from particular layers in teeth reflect diet during the window of time those layers were forming.

Advances in mass spectrometry now allow for the analysis of small samples. As a result, it is possible to isolate sections of teeth and determine their stable isotope composition (e.g., Beaumont et al., 2012, 2015; Burt, 2015; Eerkens and Bartelink, 2013; Eerkens et al., 2011; Fuller et al., 2003). This facilitates tracing of certain aspects of diet over the window in which that section grew. By analyzing serial samples, it is

possible to reconstruct these aspects of diet across successive windows of time for a particular individual.

Permanent third molars begin formation at the dentin–enamel junction (DEJ) between 7 and 10 years of age, and continue growing through age 18 to 25 years, when the apical root tip is completed (Hillson, 1996). The cementum–enamel junction (CEJ) typically forms between age 12 and 16 years, when the crown is complete. In this paper, we took serial micro-sections of third molars to reconstruct diet across this window of time, using these age-related landmarks to control for time within a tooth. For older individuals, the DEJ was occasionally not present, having been worn away. In those cases, the CEJ along with estimated rates of growth in third molars, were used to establish an internal age.

Sample preparation followed procedures established in Eerkens et al. (2011) for first molars. Teeth were cleaned with a small brush of any adhering soil or other exogenous material, sonicated in deionized water ( $\text{dH}_2\text{O}$ ), and cut in half longitudinally (i.e., crown to root) with a slow-speed diamond-coated saw. All cementum and enamel was removed and the pulp chamber reamed out from one half of the tooth using a hand-held drill. This tooth half was then weighed and demineralized in a solution of .5 M hydrochloric acid (HCl) in a refrigerator set at 5 °C. HCl was changed every other day until the sample was completely demineralized (generally 1–2 weeks).

The tooth was then rinsed with  $\text{dH}_2\text{O}$ , and sliced into parallel serial sections, beginning at the apical root tip and working up towards the crown. Note that these cuts are generally parallel to growth layers within the crown, but cut across diagonal growth layers in the root (see Eerkens et al., 2011). Because layers accumulate in a cone-like manner within the root, we are unable to manually cut cones out of the demineralized root, but must cut horizontally across growth planes. As a result, adjacent serial samples in the root include some material from the same layers of growth (i.e., adjacent sections do not represent mutually exclusive temporal windows). This will cause stable isotope fluctuations to be somewhat smoothed within the root sections, that is, if the isotopic composition of the diet abruptly changed while the root was forming, some of the abruptness would be smoothed out in the resulting serial section isotope data. However, we estimate that every other sample within the root has less than 10% of the same (synchronous) material, by volume. The number of serial sections produced varied slightly by tooth depending on the degree of occlusal wear, and the size, length, and structure of the tooth.

Following demineralization, any secondary dentin that was not reamed out during drilling was also removed. Secondary dentin typically separated from the primary dentin after demineralization. If large enough, this secondary dentin was run as a separate isotopic sample (data not reported here). Following slicing, each serial sample was placed in a separate vial and immersed in .125 M NaOH (sodium hydroxide) for 24 h to remove humic acids. The sample was rinsed with  $\text{dH}_2\text{O}$  to remove any residual NaOH and placed in slightly acidic pH 3 water in an oven set to 70 °C to solubilize collagen. Solubilized collagen was then freeze-dried to remove all remaining water, isolating the collagen fraction.

Between 0.8 and 1.2 mg of collagen was weighed out from each serial section for stable isotope analysis. In some cases, there was not enough collagen from a serial sample, and adjacent sections had to be combined to achieve a total of 1 mg. Carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) for each serial sample was 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, University of California Davis. Carbon isotope ratios,  $\delta^{13}\text{C}$ , are reported expressed in permil notation (parts per thousand) relative to the Pee Dee Belemnite standard (arbitrarily set at 0‰), while N isotope ratios,  $\delta^{15}\text{N}$ , are expressed against  $\text{N}_2$  in modern atmospheric air (also arbitrarily set to 0‰).

Collagen extracted from bone was submitted for radiocarbon dating from 11 of the 12 individuals. The amount of marine carbon in collagen was estimated using a linear mixing model and the measured  $\delta^{13}\text{C}$  in



the bone collagen (see Bartelink, 2006). A marine reservoir correction ( $\Delta R$ ) of  $365 \pm 50$ , established for San Francisco Bay, was used to correct the radiocarbon dates. Bone  $\delta^{13}\text{C}$  was used to estimate percent marine carbon, which was used to help calibrate the radiocarbon age BP (Stuvier and Reimer, 1993).

### 1.3. CA-SOL-11

This analysis is focused on 12 individuals from the archeological site CA-SOL-11, located in Green Valley just outside the modern city of Fairfield, California (see Fig. 1). The site was originally excavated in 1965–66 by the UC Davis Archaeology Field School Class. The only published analysis of these materials is a Master's Thesis written in 1967 by James Snoke that focuses on the artifacts (Snoke, 1967), and our recent analyses of stable isotope data from bone collagen and apatite (Eerkens et al., 2013).

The site is at 15 m elevation above sea level on the east banks of perennial and freshwater Green Valley Creek. This creek and its riparian corridor provided access to a wide range of plant foods, including berries, bulbs, and some seed resources, as well as freshwater fish and shellfish, and possibly, migrating salmon during certain seasons. Adjoining grasslands produce high quantities of small seeds, an important staple in Central California diets (Wohlgenuth, 1996), as well as access to small and large mammals, such as rabbits and deer. Hills rise rapidly to the east, north, and west of the site, and within a day's hike would have provided access to ecozones hosting a range of additional non-riparian terrestrial resources, including hunting grounds for deer, groves of oaks providing acorn (perhaps the most important Central California staple; see Basgall, 1987; Tushingham and Bettinger, 2013), and areas providing manzanita, buckeye, and other plant foods. Although analysis of faunal remains was not the focus of Snoke's research at SOL-11, he notes that elk, deer, and antelope remains were most common among the identifiable faunal remains, especially in the deeper levels of the midden deposit.

Aquatic foods, particularly fish and shellfish, could have been gathered within a day's walk from Suisun Marsh, a brackish water estuary located approximately 15 km to the southeast. Anadromous fish (salmon and sturgeon) would have been available both in Green Valley Creek and Suisun Marsh. Indeed, sturgeon scutes have been recovered during excavations at nearby sites in Green Valley such as SOL-315 and SOL-355 (Wiberg, 1992; Wiberg, 1993). As well, shellfish (e.g., bay mussel; *Mytilus trossulus*) would have been available in Suisun Marsh. Other nearby Middle Period sites such as SOL-355 and SOL-364 do contain significant numbers of Bay mussel (Wiberg, 1993). Snoke (1967:14) noted that shell was prominent in the midden deposit at SOL-11, but did not identify species. He did find that the ratio of shell to bone was higher in the shallower levels of the site, which he interpreted as a shift from greater emphasis on hunting early in time to more shellfishing later in time (we return to this point in the discussion section). Together, the

faunal data indicate exploitation of both terrestrial and estuarine environments.

Many groundstone artifacts were recovered at the site, indicating the importance of plant foods in local diets, but unfortunately, no flotation and/or paleobotanical analyses were undertaken by Snoke to reconstruct plant use. Studies at nearby sites indicate that large numbers of acorn and small grass seeds are commonly recovered in flotation studies, and we assume CA-SOL-11 would have been similar had such studies been conducted.

We selected well-preserved third molars from twelve adult individuals (Table 1) for serial micro-sectioning. For all teeth but one, we were able to extract over a dozen serial samples, with minimum of six serial samples for a more fragmentary tooth and a maximum of 19 for a well-preserved one.

## 2. Results

Collagen yield was greater than 3% by weight for all twelve teeth. In four cases, generally near the apical root, two adjacent serial sections had to be combined to submit enough collagen, and in one case three serial samples had to be combined. In total, 166 collagen samples were submitted from the 12 teeth. Collagen quality for individual serial samples was also evaluated using the atomic C/N ratio (DeNiro, 1985). Only four of the 166 collagen samples were outside the range of acceptable C/N values and were excluded from further analysis. Table 2 lists the remaining 162 isotopic measurements, along with the median estimated age for each serial section using the age-related dental landmarks discussed above.

Overall,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  display a positive linear relationship in the 162 collagen samples ( $R^2 = 0.78$ ;  $p < .0001$ ). This indicates that foragers varied the protein in their diet between two extremes, one with higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and the other with lower  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Figure 2 plots  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for all serial samples, with symbols tracking individuals (females plotted as circles, males as triangles, and indeterminate sex as other symbols). Also plotted are ellipses that capture the range of bone collagen samples from previously analyzed samples from other sites (see Eerkens et al., 2013). The M3 serial samples generally fall within the range of the previously-analyzed bone collagen samples from CA-SOL-11 (Suisun Marsh ellipse). However, the dentinal collagen samples do not overlap with bone collagen samples from foragers living on the Central Coast of California, San Francisco Bay estuary, or along the Sacramento River (ranges of previous analyses shown with ellipses in Fig. 2).

Fig. 2 also highlights the distinctive character of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in most individuals. That is, although there is some overlap, individuals tend to occupy different regions within the isotopic space. This suggests that each person had a slightly different diet, not just on average, but even over smaller windows of time in their teenage years. In other words, individuals tended to consume similar suites of foods

**Table 1**

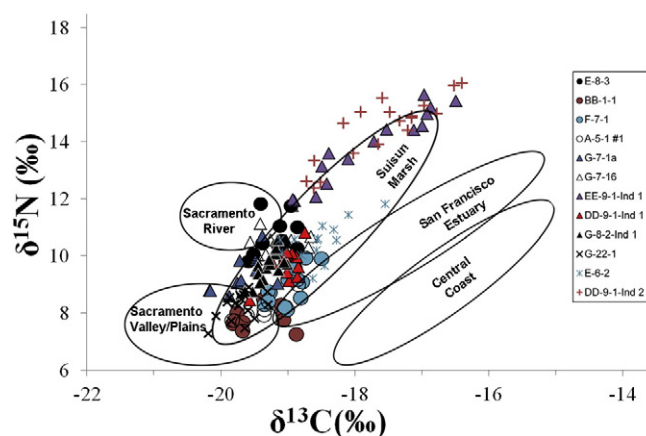
Age and sex of the individuals included in this study, tooth sampled, and information from bone collagen and calibrated radiocarbon dates.

Burial #	Age at death	Sex	Third molar sampled	# of serial sections	Bone collagen $\delta^{13}\text{C}$	Estimated % of marine carbon	$^{14}\text{C}$ age	Calibrated age BP (2-sigma)
E-6-2	13–18	Indet	ULM3	15	−18.6	21%	2130 ± 20	1882–1989
A-5-1 in. 1	20–30	Female	URM3	6	−19.6	15%	2100 ± 15	1897–1990
G-22-1	20+	Indet	LRM3	16	−19.6	15%	2210 ± 25	2001–2144
BB-1-1	35–40	Female	LRM3	13	−19.4	16%	2220 ± 30	1993–2153
DD-9-1 in. 2	40+	Indet	LLM3	19	−17.3	29%	1720 ± 25	1335–1516
G-8-2 in. 1	35–40	Male	LRM3	16	−19.2	18%	2020 ± 15	1784–1891
F-7-1	50+	Female	LRM3	16	−18.4	23%	n/a	
G-7-16	25–40	Male	LLM3	15	−18.4	23%	2120 ± 30	1829–1987
DD-9-1 in. 1	25–40	Male	ULM3	15	−18.4	23%	2050 ± 20	1740–1889
G-7-1a	35–40	Male	LLM3	13	−18.7	21%	2100 ± 20	1828–1969
E-8-3	40–50	Female	LLM3	14	−18.9	19%	2330 ± 20	2147–2306
EE-9-1 in. 1	45–60	Male	LRM3	15	−17.7	27%	1850 ± 40	1418–1692

Notes: ind = Individual #; Indet. = Indeterminate.

**Table 2**  
Results by individual serial section, with estimated median age,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

Median age	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Median age	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	Median age	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
G-7-1a			E-6-2			E-8-3		
9.0	-20.2	8.7	8.9	-18.3	10.5	8.9	-18.9	10.2
10.0	-18.9	10.1	9.8	-18.9	10.5	9.7	-19.1	10.3
10.8	-19.3	9.7	10.6	-18.1	11.4	10.5	-19.6	9.7
11.5	-19.2	9.0	11.6	-19.1	10.0	11.3	-19.4	10.4
12.2	-19.9	8.5	12.6	-18.6	10.5	12.2	-19.1	10.1
13.2	-20.2	8.7	13.3	-18.8	9.9	13.1	-19.5	10.0
14.4	-19.8	9.0	13.9	-18.6	9.1	14.9	-19.1	10.4
15.2	-19.7	9.8	14.8	-18.5	9.6	15.7	-19.2	10.3
16.1	-19.4	10.6	15.7	-18.6	10.1	16.7	-19.1	10.4
17.0	-19.1	10.4	16.4	-18.6	10.5	17.7	-18.9	10.9
17.8	-19.7	9.1	17.4	-18.3	10.8	18.4	-19.1	11.0
18.5	-19.5	9.4	18.4	-17.6	11.7	19.2	-19.0	11.7
19.9	-19.5	9.8	19.7	-19.1	10.5	20.3	-19.4	11.7
DD-9-1-ind 2			F-7-1			G-8-2-ind 1		
8.7	-18.0	13.5	8.8	-19.3	8.6	9.0	-19.5	8.7
9.3	-17.4	14.7	9.5	-19.3	8.7	9.8	-19.7	8.7
9.9	-16.8	14.9	10.2	-19.3	8.3	10.5	-19.3	9.5
10.6	-16.4	16.0	10.9	-19.4	8.2	11.2	-19.5	9.6
11.2	-16.5	15.9	11.6	-19.3	8.3	12.0	-19.5	9.3
11.8	-17.2	14.8	12.3	-19.0	8.1	12.6	-19.4	9.1
12.5	-17.2	14.8	13.0	-19.0	8.0	13.3	-19.2	9.4
13.1	-17.2	14.3	13.7	-19.1	8.1	14.0	-19.4	9.0
13.7	-17.7	13.8	14.4	-18.8	8.4	14.7	-19.2	10.0
14.4	-18.6	12.3	15.4	-18.8	9.0	15.4	-19.2	10.2
15.0	-18.5	12.5	16.5	-19.0	9.1	16.1	-19.2	10.2
15.6	-18.7	12.5	17.2	-18.9	9.1	16.8	-19.2	10.5
16.3	-18.6	13.3	17.9	-18.5	9.8	17.6	-19.0	9.6
16.9	-18.2	14.6	18.6	-18.9	9.5	18.3	-19.1	9.7
17.5	-17.9	15.0	19.3	-18.9	9.7	19.1	-19.3	9.6
18.2	-17.6	15.5	20.3	-18.8	9.8	20.3	-19.3	9.8
19.1	-17.5	15.0						
20.4	-17.0	15.2						
G-7-16			EE-9-1-ind 1			G-22-1		
10.3	-19.5	9.9	9.0	-18.9	11.9	8.9	-19.9	8.3
11.0	-19.5	9.9	9.9	-18.9	11.9	9.6	-20.2	7.2
11.7	-19.4	11.0	10.6	-18.1	13.3	10.3	-19.3	8.2
12.4	-19.6	10.4	11.3	-17.5	14.4	11.0	-19.3	8.7
13.2	-19.3	10.5	12.4	-16.9	15.1	11.7	-19.6	8.0
13.9	-19.6	10.0	13.5	-16.5	15.3	12.4	-19.7	8.4
14.6	-19.3	10.0	14.2	-17.1	14.3	13.1	-19.7	8.5
15.3	-19.0	10.4	14.9	-16.9	14.9	14.7	-19.9	7.6
16.1	-19.0	10.2	15.6	-17.0	14.5	15.6	-19.7	7.4
16.8	-18.7	10.2	16.4	-17.7	13.9	16.3	-19.7	8.2
17.5	-18.7	10.6	17.1	-18.6	12.0	17.0	-19.7	8.6
18.2	-19.0	10.5	17.8	-18.4	12.5	17.8	-19.6	8.8
19.1	-19.0	10.2	18.5	-18.5	13.1	18.6	-19.9	8.5
20.3	-19.0	11.7	19.2	-18.4	13.5	19.3	-20.1	7.8
			20.3	-17.0	15.6	20.3	-19.8	8.2
A-5-1			BB-1-1			DD-9-1-ind 1		
9.1	-19.4	8.0	8.9	-19.8	7.9	9.0	-19.0	9.0
10.6	-18.9	8.9	9.8	-19.4	8.4	10.1	-18.9	9.2
12.3	-19.6	7.8	10.9	-19.8	7.5	11.0	-19.0	9.3
14.0	-19.4	7.8	11.8	-19.8	7.7	11.8	-19.2	9.7
15.6	-19.8	7.7	12.7	-19.7	7.6	12.5	-18.9	9.5
18.7	-19.7	8.7	14.5	-19.1	7.9	13.2	-18.9	9.9
			16.6	-19.1	7.7	13.9	-19.1	9.9
			17.8	-19.7	7.3	14.6	-19.0	9.9
			20.0	-18.9	9.2	15.3	-19.0	10.0
						16.0	-19.1	9.9
						16.7	-19.0	10.1
						17.4	-18.8	10.7
						18.1	-19.1	9.6
						19.4	-19.6	8.4



**Fig. 2.**  $\delta^{13}\text{C}$  vs.  $\delta^{15}\text{N}$  in serial third molar samples from CA-SOL-11.

markedly over time. All three individuals begin the sequence around age 8.5–9 years with relatively low isotopic values. All three then display a notable increase (greater than 1‰) in  $\delta^{13}\text{C}$  vs.  $\delta^{15}\text{N}$ , followed by a decrease between ages 13 and 17 years, and a second increase soon thereafter. This indicates significant changes in the source of dietary protein over time. By contrast, the three individuals in the right-hand column of Fig. 3 (G-8-2-ind 1, DD-9-1-ind 1, and F-7-1) show much less internal variation (less than 1‰ change for  $\delta^{13}\text{C}$ ), indicating a more consistent and stable diet over time.

Table 3 provides summary statistics (average, standard deviation, and range) for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . As well, we have subjectively classified the serial sample patterns into one of two curve types: “Flat” indicating only minor fluctuations over time (<1‰ shift in  $\delta^{13}\text{C}$ ; 4 females, 3 males, 1 indeterminate sex), and “Two peaks” where  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  seem to co-oscillate twice (>1‰ shift in  $\delta^{13}\text{C}$ ; 2 males, 2 indeterminate sex). Curiously, no individual seems to show a single large peak or spike in isotopic values, with DD-9-1 ind#1 (the middle right peak sequence in Fig. 3) coming the closest to such a pattern.

Finally, we note that both individuals who have higher average  $\delta^{13}\text{C}$  values in serial samples (DD-9-1-ind 2, and EE-9-1-ind 1) also display younger calibrated radiocarbon ages. This pattern mimics earlier analyses of radiocarbon dates on bone collagen reported in Eerkens et al. (2013) where burials post-dating 1600 cal BP tend to have higher  $\delta^{13}\text{C}$ .

### 3. Discussion

Our previous stable isotope analyses of bone showed that access to brackish-water resources was heterogeneous for CA-SOL-11 inhabitants (Eerkens et al., 2013). Some individuals consumed significant quantities of resources obtained from Suisun Marsh, enough to notably elevate  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in bone collagen and apatite. Others had only minimal access to these foods, and appear to have gained most of their macronutrients from more terrestrial sources. Continual remodeling of bone means that collagen and apatite represent an average of diet over many years prior to death. Thus, it was unclear from the bone isotope data whether individuals with elevated bone collagen and apatite  $\delta^{13}\text{C}$  consistently consumed brackish-water foods, or only did so periodically.

Stable isotope data from permanent third molars confirm the bone isotope results by suggesting high inter-individual variation, but provide additional detail on intra-individual variation. Like the bone isotopic data, only some individuals have elevated  $\delta^{13}\text{C}$  values in their third molar collagen sections. Further, there is a strong correlation in the third molar and bone collagen  $\delta^{13}\text{C}$  values. Fig. 4 compares average  $\delta^{13}\text{C}$  from the M3 serial sections against bone collagen  $\delta^{13}\text{C}$  for the same individual. The two people who are notably enriched in  $^{13}\text{C}$  in serial tooth samples (EE-9-1-ind 1, and DD-9-1-ind 2) were also enriched in their bone collagen and apatite. Likewise, individuals who were lowest in average M3  $\delta^{13}\text{C}$  were also lowest for bone collagen  $\delta^{13}\text{C}$ .

throughout their second decade of life, but these suites were different from person to person.

Examining serial sections within particular individuals reveals further patterning not evident in Fig. 2. Fig. 3 plots serial samples for six individuals in the study. In the left column are three individuals (G-7-1a, DD-9-1-ind 2, and EE-9-1-ind 1) whose isotopic composition changed

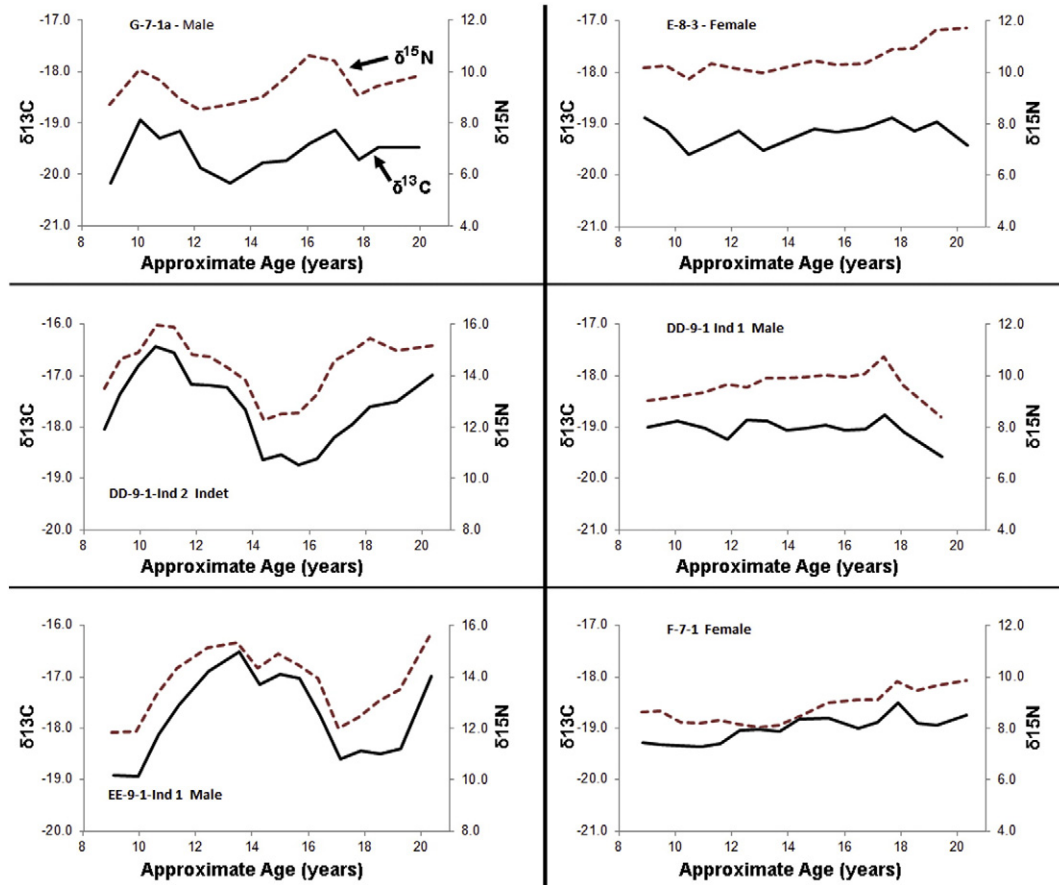


Fig. 3. Serial  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in six individuals from CA-SOL-11 showing variable (left) and stable (right) diets.  $\delta^{15}\text{N}$  shown as dashed and  $\delta^{13}\text{C}$  as solid line.

Together, the bone and tooth  $\delta^{13}\text{C}$  values show that Suisun Marsh was not a foraging ground for all individuals buried at the site, but only for some individuals or families (ca. 15–20% of the population). Assuming that at least some of these individuals were living contemporaneously, this further implies that food was not widely or systematically shared between families and/or individuals at the site, otherwise, inter-individual isotopic variation would have been lower. Instead, it appears that different contemporary individuals and/or families had different dietary patterns.

At the same time, the serial tooth samples reveal fine-scaled temporal data on how different environments were exploited by particular individuals within their teenage years. Those with low bone collagen  $\delta^{13}\text{C}$ , indicating little use of brackish water foods during the last decade of their life, seem to have made only minor or no use of brackish water foods throughout their second decade of life as well. On the other

hand, those who did consume significant quantities of brackish water foods, did so only intermittently during their second decade. The three most notable examples (left pane of Fig. 3) display two visible peaks in  $\delta^{13}\text{C}$ , followed by valleys that are close to the site average. The peaks are enriched in  $\delta^{13}\text{C}$  by 1‰ or more over the valleys. This suggests that these individuals were consuming a diet similar to others from the site during some periods (i.e., the valleys), but during others were consuming much higher quantities of marshland brackish-water foods. Judging by the width of the peaks, these dietary shifts lasted up to four years in time in some cases (EE-9-1-ind 1, and DD-9-1-ind 2), but were shorter, 1–2 years, for others (E-6-2). In short, even for foragers who made use of Suisun Marsh, brackish water foods were not a staple

**Table 3**  
Summary statistics and curve shape for M3 serial samples from CA-SOL-11.

Burial #	Avg. $\delta^{13}\text{C}$	Stdev. $\delta^{13}\text{C}$	Range $\delta^{13}\text{C}$	Avg. $\delta^{15}\text{N}$	Stdev. $\delta^{15}\text{N}$	Range $\delta^{15}\text{N}$	Shape
E-6-2	-18.6	0.42	1.5	10.4	0.68	2.6	Two peaks
A-5-1 in. 1	-19.5	0.35	0.9	8.2	0.52	1.2	Flat
G-22-1	-19.7	0.24	0.9	8.2	0.47	1.6	Flat
BB-1-1	-19.5	0.36	0.9	7.9	0.58	1.9	Flat
DD-9-1 in. 2	-17.6	0.73	2.3	14.4	1.13	3.7	Two peaks
G-8-2 in. 1	-19.3	0.18	0.7	9.6	0.52	1.8	Flat
F-7-1	-19.0	0.25	0.9	8.8	0.64	1.8	Flat
G-7-16	-19.2	0.31	0.9	10.4	0.48	1.8	Flat
DD-9-1 in. 1	-19.0	0.20	0.8	9.66	0.56	2.3	Flat
G-7-1a	-19.6	0.38	1.3	9.44	0.68	2.1	Two peaks
E-8-3	-19.2	0.23	0.7	10.5	0.61	2.0	Flat
EE-9-1 in. 1	-17.8	0.82	2.4	13.7	1.28	3.7	Two peaks

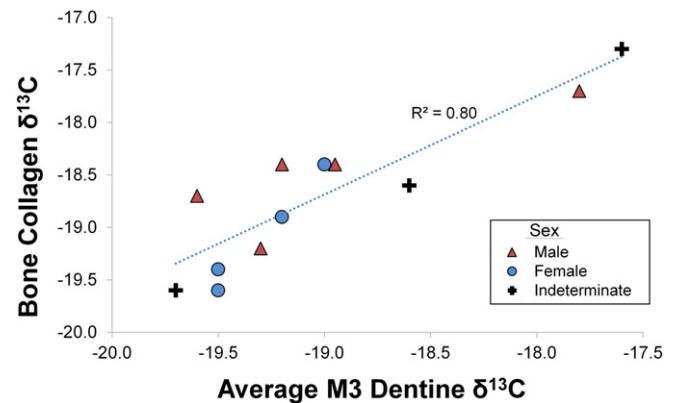


Fig. 4. Comparison of average third molar collagen  $\delta^{13}\text{C}$  and bone collagen  $\delta^{13}\text{C}$ , by sex, at CA-SOL-11.

consistently exploited every year, but were mainly consumed during pulses that varied from one to five years in length.

What could have prompted these pulses of brackish water food consumption for only some individuals? We suggest three possible interpretations. First, it is possible that brackish-water foods were fallback resources (see Marshall et al., 2009), only exploited during periods when other environments closer to the site failed to provide enough food for CA-SOL-11 inhabitants. The estuarine fish and shellfish refuse found at the site (see Snoke, 1967), then, represent the byproduct of such acquisition activities during difficult years. Individuals who lack a brackish-water signal, then, either lived their teenage years entirely during “good” years, where fallback foods were not needed, or relied on non-marsh fallback foods. If this interpretation is correct, it suggests that different families exploited different environments for their fallback foods. In this respect, it is somewhat unusual that the three individuals with the strongest brackish-water signal all have double peaks in  $\delta^{13}\text{C}$  and nobody displays a single (or triple) peak in the serial sections. Additional sampling would help resolve this issue.

Along these lines, it may be significant that individuals with radiocarbon dates younger than 1600 cal BP display the strongest brackish-water signatures, while the majority pre-dating this point in time have more terrestrial carbon signatures. Perhaps climatic variation after 1600 cal BP, was higher in the Green Valley area and required more frequent use of fallback foods, such as shellfish (e.g., Jones et al., 1999). Alternatively, perhaps population density increased markedly after 1600 cal BP causing an imbalance between food resources and population levels, necessitating more intensive exploitation of fallback foods (see Broughton, 1994, 1999; Wohlgemuth, 1996). Indeed, the general increase in  $\delta^{13}\text{C}$  in bone collagen over time may correspond to Snoke's (1967) observation that the deeper midden layers at the site had a higher ratio of bone to shell than the shallower layers. This suggests that diets may have shifted over time at the site to a greater reliance on shellfish and the incorporation of brackish-water fallback foods.

Second, it is possible that the peaks in  $\delta^{13}\text{C}$  represent temporary shifts in the place of residence of individuals, for example, to a location on or very close to Suisun Marsh. Such residential shifts could be a byproduct of resource shortfall, as above (e.g., going to live with extended family or fictive kin during hard years). However, they could also be a byproduct of other factors, such as exogamous marriage patterns. In this respect, it may be significant that two of the four individuals with two peaks in  $\delta^{13}\text{C}$  are male (while the remaining two are indeterminate for sex). The ethnographic literature in California indicates that males commonly performed bride service (e.g., Driver, 1937:89, 128; Gifford, 1926, 1967:30; Kroeber, 1925), which involved an extended stay in the village of their potential bride when they married exogamously. While there, a man would hunt and gather food for his to-be in-laws, to pay off the loss of labor of a daughter, who would ultimately move back to the husband's village as part of a patrilocal post-marital residence system. While the ethnographic record of California may not apply wholesale to archeological cases, it can provide hypotheses for understanding human behaviors. Indeed, bride service and initial matrilineal post-marital residence is documented ethnographically among other modern hunter-gatherer groups as well (e.g., Wood and Marlowe, 2011).

If bride service explains the shifting isotopic values observed in the CA-SOL-11 archeological samples, such service may have involved movements undertaken in two steps. The first step may have been in early teenage years as a trial, perhaps to meet the bride and her family, followed by a return to the man's natal village. The second step may have occurred at the time of marriage (i.e., initial matrilineal residence). Following marriage, and perhaps one or more children, the couple would eventually return to the man's natal village (i.e., CA-SOL-11). If this latter factor accounts for the serial sample pattern, we would expect to find women who display systematically elevated  $\delta^{13}\text{C}$  in M3 serial samples, especially higher in the crown (since they would have been born and living at a village near Suisun Marsh where brackish-water

resources were important throughout their teenage years). Yet, we do not have such evidence among the small sample of females from CA-SOL-11. Additional sampling of females, and the use of other isotopes such as Sr (see Eerkens et al., 2014b; Jorgenson et al., 2009), could help address this issue.

Third, pulses of brackish-water foods could represent episodes of regularized trade of subsistence foods between some inhabitants of CA-SOL-11 and individuals living on or nearly Suisun Marsh. It is possible that CA-SOL-11 inhabitants had access to some natural resource or finished product that they could have traded to people living on Suisun Marsh. For example, Snoke (1967) thought CA-SOL-11 inhabitants manufactured charmstones and bone tools on site. Perhaps some of these items were traded off-site to villages to the south on Suisun Marsh. Likewise, Schmitz (2014) has robust evidence for charmstone manufacture in the Middle Period at nearby CA-SOL-364. Breakdowns or reorganizations in trade relations may have caused large-scale fluctuations in access to brackish-water resources for some, but not all, CA-SOL-11 inhabitants. During periods of more active trade, some individuals may have acquired a brackish-water signature in their third molar serial samples.

#### 4. Conclusions

Stable isotope analysis of bone apatite and collagen can help archeologists evaluate the role of different environments and/or resources in the diets of individuals. However, with only a single estimate of diet over a number of years prior to death, it is difficult to determine if individuals continually or only occasionally accessed such resources. Moreover, such information only reflects diet during the last decade or so of life, and may not be reflective of dietary behaviors over the entire lives of individuals.

Serial sampling of teeth can provide estimates of paleodiet over smaller windows of time, typically 0.5 to 2 years, depending on collagen preservation. For first molars, which grow between age 0 and 9, we can capture important dietary changes early in life, such as weaning (e.g., Eerkens et al., 2011). For third molars, which grow largely during the second decade of life (ages 9–21), we can examine changes in diet as individuals are transitioning into adulthood. In many traditional societies, this will include the years just before and after marriage, which may include post-marital residence shifts, and the birth of children.

At CA-SOL-11, our analyses of serial samples show that brackish-water foods were consumed in significant quantities by only a small segment (ca. 10–15%) of the adult population. Our serial sampling shows that even for these individuals, brackish-water foods were not a staple of the diet, but were accessed in pulses between one and four years in length. Between these pulses, individuals were consuming diets that were similar to other individuals from the site.

While we cannot determine the reasons behind these pulses with confidence, we can say that Suisun Marsh, the most likely source of brackish-water foods, must not have been regular foraging grounds even for these individuals. Instead, it must have served either as an area to gather fallback foods, as a source of items accessed through irregular trade, or may have played a larger dietary role during periodic residence shifts for some individuals only.

The suggestion that Suisun Marsh provided fallback foods is in line with recent stable oxygen isotope analyses of shellfish remains from a contemporaneous and nearby site, CA-SOL-364, which shows that brackish-water shellfish (mussels) were only harvested during wintertime (Eerkens et al., n.d.). There, we suggested that shellfish played the role of fallback food, an easily accessible (i.e., sessile) source of protein to complement the carbohydrate-rich stored plant foods (i.e., acorns and small seeds) that were used for overwintering. We do not have access to shellfish remains from CA-SOL-11, but additional excavations at the site could yield shells amenable for seasonality estimation using stable isotopes, and may show that CA-SOL-11 inhabitants only ate shellfish in wintertime also. Thus, it is possible that the brackish-water



signature at CA-SOL-11 is from a similar wintertime exploitation of mussels, and perhaps fish, as a fallback food, an activity that increased in frequency after 1600 cal BP.

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