PALEODIETARY RECONSTRUCTIONS USING STABLE ISOTOPES IN THE DON PEDRO RESERVOIR AREA

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Excavations at three sites in Tuolumne County in the 1970s during the construction of Don Pedro Reservoir recovered human remains representing a minimum of 60 individuals. In collaboration with the Tuolumne Me-wuk, stable isotope analysis has been performed on bone collagen to gather life history information on 53 of these individuals, prior to reburial. This paper focuses on paleodietary reconstructions, in particular differences between males and females, between adults and adolescents, and changes over time. The data also demonstrate a distinctive regional isotopic signature that is unique from other areas in Central California.

A collaborative study between the Tuolumne Me-Wuk and the Department of Anthropology, University of California, Davis (UC Davis), provides new information on paleodiets in the Central Sierra Nevada. Stable isotope analysis enables the reconstruction of dietary signatures at the individual level, which facilitates comparison of paleodiets among individuals within a site, or between populations from different sites (DeNiro and Epstein 1981; Schoeninger and DeNiro 1984; Schoeninger et al. 1983; Schoeninger and Moore 1992). This in turn allows us to test hypotheses about inter-group and intra-group variations in diet within or between geographic regions, for example, between males and females or young and old. When paired with radiocarbon dates, this approach also aids in testing hypotheses about diachronic dietary change. At the same time, stable isotope analysis of human remains is limited in that it cannot identify exact foods consumed (e.g., acorn vs. pine nut), however, it is ideal for distinguishing general categories of food (e.g., marine vs. terrestrial; plant vs. animal).

This study examines dietary signatures in three pre-contact archaeological sites in Tuolumne County, California: CA-TUO-279, CA-TUO-300, and CA-TUO-314. These sites were discovered during archaeological surveys during 1970 when, after three years of preparatory construction activities for the New Don Pedro Reservoir, archaeological reconnaissance funding was made available. Preparatory construction activities for the new reservoir greatly impacted the archaeology of the area, and included subsequent excavation of a number of sites, including the three listed above.

The human skeletal materials included in this study were recovered during excavations in 1970 and 1971. The collection was housed at San Francisco State University's Treganza Museum until 2014, when the skeletal materials were repatriated under the Native American Graves and Repatriation Act (NAGPRA) to the Tuolumne Me-Wuk tribe. Skeletal materials were temporarily transferred to UC Davis to undergo isotope and DNA analyses at the request of the tribe, prior to their planned reburial.

This study reports bone collagen isotope data produced from this collaborative effort. Future studies will focus on DNA and other isotopic data from these collections. This paper, in particular, aims to examine the general dietary signature of each individual within the collection to determine if: 1) there were dietary changes over archaeological time; 2) if there were dietary differences between three sites; and 3) if there were dietary differences between males and females and adults and juveniles within the sites. Additionally, this study compares findings from the three Tuolumne County sites to similar analyses at other sites in Central California to place the results within a broader regional context.

BACKGROUND

Stable isotope reconstruction of paleodiet works best when used in concert with other methods, such as analysis of ethnographic records and faunal and paleobotanical identification. Each approach adds a dimension to what should be expected in the paleodiets of individuals and populations. The bulk of what is currently known of pre-contact diets in the Central Sierra Nevada is from ethnographic accounts and archaeological investigations. The sections below review what is known about native Tuolumne diets from these two sources.

Ethnographic Subsistence Patterns

The ethnography of the Tuolumne Me-Wuk shows that subsistence activities followed a seasonal round, consuming and storing food as it became seasonally available at varying altitudes within the Central Sierra Nevada Mountains and foothills (Barrett and Gifford 1933:136). At the time of Barrett's 1908 ethnography, Tuolumne Me-Wuk territory encompassed the drainages of the Tuolumne and Stanislaus Rivers from their points of origin in the Sierra Nevada in the east, the foothills, to portions of Great Central Valley to the west. There are five major elevation and moisture-dependent biotic zones within these boundaries as one ascends in elevation moving east (Barrett and Gifford 1933:128-135; Storer and Usinger 1963:26-28). The zones, often overlapping at the margins and where ecological conditions are met, offer a wide variety of plant and animal resources, with some species restricted to one zone, some found within two or three, and still others ubiquitous in every zone (Storer and Usinger 1963:26).

Permanent residential settlements, associated with year-round subsistence activities, occurred mainly in three biotic communities below 1,200 meters in elevation: the Lower Sonoran, Upper Sonoran, and the Transition Zone (Barrett and Gifford 1933:128-135). Travel and temporary encampments above 2,000 meters in the Transition, Canadian and Hudsonian zones were a summertime activity; snow no longer acted as an impediment and temperatures in the valleys below rose to lofty heights warranting escape (Barrett and Gifford 1933:128-135; Bennyhoff 1956:26-27).

Marking the western-most periphery of the territorial boundary, the Lower Sonoran Zone ranges in elevation from 15 to 120 meters and is characterized by Me-Wuk exploitation of valley oak acorns (*Quercus lobata*), a variety of grass seeds, antelope (*Antilocapra americana*), jackrabbit (*Lepus californicus*), ducks, geese, grasshoppers and salmon (Barrett and Gifford 1933:135; Storer and Usinger 1963:26).

The foothills and some portions of the Sierra Nevadas are characterized by the Upper Sonoran Zone, which ranges in elevation from 150-1,200 meters (Storer and Usinger 1963:27). This zone is described as being an oak-park woodland and gray pine belt, with some areas as chaparral (Barrett and Gifford 1933:133; Storer and Usinger 1963:27). Major resources utilized in this area were gray pine nuts (*Pinus sabiniana*), the acorns of blue and interior live oak (*Quercus douglasii, Quercus wislizenii*), manzanita berries (*Arctostaphylos manzanita*), buckeye nuts (*Aesculus californica*), deer (*Odocoileus hemionus*), jackrabbit, valley quail (*Callipepla californica*), gray squirrel (*Sciurus griseus*), and California ground squirrel (*Otospermophilus beecheyi*) (Barrett and Gifford 1933:135; Storer and Usinger 1963:32-33).

The gray pine belt gives way to the yellow pine belt in the Transition Zone, which varies in elevation from 365-1,980 meters (Storer and Usinger 1963: 27). Principal resources within this biotic community are the acorns of the black oak (*Quercus kellogii*), sugar pine nuts (*Pinus lambertiana*), a variety of greens, bulbs and tubers, deer, gray squirrel, mountain quail (*Oreortyx pictus*), pigeons (*Columbidae sp.*), trout (*Oncorhynchus sp.*), and chrysalides (Barrett and Gifford 1933:135, 136-139; Storer and Usinger 1963:27).

The Canadian Zone (lower boreal forest) is located at elevations ranging from 1650-2450 meters. It is dominated by lodgepole pine (*Pinus contorta*) and red fir (*Abies magnifica*), with some areas producing a variety of edible greens and herbs. Mountain sheep (*Ovis Canadensis*) and a variety of hares (*Lepus americanus*, *Sylvilagus nuttallii*) and birds inhabited the region (Storer and Usinger 1963: 27-33).

The Hudsonian zone is sub-alpine, ranging in elevation from 2300-3050 meters. It is sparsely forested by whitebark pine (*Pinus albicaulis*), foxtailed pine (*Pinus balfouriana*), and lodgepole pine. Ground cover includes some low growing grasses, sedges, and herbs. Mountain sheep, squirrel (*Urocitellus beldingi*) and marmot (*Marmota flaviventris*) are the predominant animal species (Storer and Usinger 1963: 29-33).

Along with the dominant species listed within each biotic zone, a variety of berries, fruits, seeds, greens, fungi, bulbs and corms were also consumed as some were available in almost any of the biotic zones while others became ripe or ready for picking variably throughout the three main biotic zones (Barrett and Gifford 1933: 151-163).

Breaking the resources down by the season in which they were gathered and utilized begins with the collecting of gray pine nuts during the early fall, while acorns were gathered during the late fall and early winter; both were stored for consumption during the winter and early spring (Barrett and Gifford 1933). Mushrooms, meat, and stored foods typified winter subsistence as people took shelter in permanent winter villages below the snowline. Springtime saw people disperse; those who had wintered in the foothills may have traveled into the plains to hunt antelope while those living in the transition zone would follow the deer to higher elevations (Levy 1978:402). Greens, mostly clovers and bulbs, were gathered in the spring as an additional supplement to meals of stored acorns and pine nuts; animals and insects were also consumed, but to a lesser degree than in winter (Barrett and Gifford 1933). Summer saw groups move about gathering seeds at varying elevations before the late summer burning of certain resource patches (Barrett and Gifford 1933: 136; Levy 1978). Plums and cherries were consumed between the seed and acorn harvests (Barrett and Gifford 1933:136). Meat was consumed year round, with animals being hunted and trapped both communally and individually. Of the terrestrial animals, deer was the most highly prized year round, while gray squirrel and rabbits came in a close second. Salmon are mentioned as being important in the foothills, while trout was favored in the mountains (Barrett and Gifford 1933:137).

There are a few notable differences when diets are parsed by sex, mainly in the form of restrictions placed on women. For example, women who had recently given birth did not eat salt, grease, and meat products, while a new father only observed a salt taboo (Aginsky 1943:436). Likewise, upon puberty, girls were to observe the taboo on meat, and were not to pound acorns, gather, make baskets, or get wood (Aginsky 1943:437-438). A female's menses also brought about a meat and salt taboo to be followed as she began every cycle, and she was restricted from cooking for others (Aginsky 1943:439).

The ethnographic accounts of subsistence activities provide a snapshot of dietary practices during the 1800s and early 1900s and provide a context for understanding paleodiets. Of course, it cannot be assumed that subsistence practices remained unchanged throughout prehistory: populations increased, new technologies were introduced, and the climate shifted, affecting water supplies as well as the flora and fauna found within the region. The archaeological record allows for investigations into the foods being utilized throughout prehistory, evidenced by artifact assemblages, faunal remains, plant remains, and as discussed below, stable isotope signatures.

Evidence from Artifacts and Paleobotanical Remains

Many early archaeological investigations in the Central Sierra Nevada extrapolated ancient subsistence practices based on artifact types found in sites, especially ground stone technologies (Beardsley 1954; Bennyhoff 1956; Hunt 2004; Jackson 1991; Leftwich 2010). Millingstones and handstones are typically assumed to be for seed processing, while mortars and pestles are typically thought to represent nut and acorn processing. As well, bedrock mortars are attributed to the intensified processing of acorns alone.

As such, much emphasis has been placed on determining the timing of acorn use as a staple crop (Basgall 1987).

Artifact assemblages from the New Melones Reservoir area pre-dating 7000 cal BP are characterized by a paucity of ground stone, but do have many projectile points suggesting hunting of large game was important (Moratto 2002:37). From the East Sonora Bypass Project area, handstones and millingstones were recovered from sites dating to 7000-3000 cal BP time frame (Rosenthal 2008:90). This suggests that during this early period of occupation in the Central Sierra Nevada, subsistence activities shifted to include plant foods in need of grinding, although hunting remained important. There is no exact time frame for when mortars and pestles were introduced, however they occur in fewer numbers relative to handstones and milling stones (Rosenthal 2008: 87). The introduction of the bedrock mortar in this region probably occurred somewhere between 1100 and 610 cal BP (Moratto 2002; Rosenthal 2008:87). The persistence of hunting activities continued throughout all periods, with the atlatl and large projectile points being replaced by the bow and arrow around 1000 cal BP (Rosenthal 2008:109).

Recent studies in the Central Sierra Nevada incorporate analysis of charred remains recovered through flotation. These studies show that acorns, gray pine nuts, manzanita, small seeds, and berry pits are found throughout all time periods; however, their relative quantities change through time. Archaeobotanical analyses at six sites in the East Sonora Bypass Project of Tuolumne County indicate that two major plant foods dominate the entire archaeological record from 7000-100 cal BP: gray pine nuts and acorns (Rosenthal 2008:94). Gray pine was found in higher quantities than acorn from 7000-3000 cal BP, with lower quantities of small seeds and manzanita also present. During the period 3000-1100 cal BP, both gray pine and acorn decrease relative to small seeds and other nuts, however, both still remain as the chief species utilized. Wohlgemuth notes that small seed usage appears to be inconsequential in both of these early periods (Rosenthal 2008:95). In the latest period, from 1100-100 cal BP, small seeds and manzanita double in abundance from earlier amounts with a wider variety of species present, including farewell to spring, clover, tule, phacelia, buttercup, goosefoot, brome grass, fiddleneck, blue grass, two species of tarweed, filaree, popcorn flower, rush, plantain, and various seeds from the sunflower family, the bean family, grass family, and purslane family (Rosenthal 2008:99). While thin-shelled pine, thought to be sugar pine, becomes more common in the latest period, acorns and gray pine still dominate, with acorn probably displacing gray pine as the staple food crop. The dominance of acorn was also noted in the ethnographic accounts of Barrett and Gifford (1933), as well as Kroeber (1925) (Rosenthal 2008:96-98).

Unfortunately, faunal remains in sites are often fragmentary and occasionally burnt, only rarely identifiable to genus or species. Excavations at the New Melones project area produced small quantities of identifiable faunal remains, with deer, rabbit and squirrels as the prominent species represented through most of time. However, later components dated to between 700 and 100 cal BP showed additional evidence of the use of various birds, fish, freshwater mussel, turtle, black bear, coyote, tule elk, and antelope (Moratto 2002:42). Faunal remains from the East Sonora Bypass Project are more numerous, with the preponderance of large size mammal, mostly artiodactyl and presumably deer, throughout all time periods (Rosenthal 2008:101). Small to medium mammals, likely rabbits and hare, are the next most common category, although in remarkably lower quantities than the medium/large mammals (Rosenthal 2008:104). Surprisingly, next in order of abundance are charred remains of the western pond turtle, then birds, and small mammal, each representing minimal fractions of the whole data set and showing up in the archaeological record for this particular area during the 3000-100 cal BP range (Rosenthal 2008:104).

Study Sites

Site TUO-279, located on a small peninsula jutting out into the now flooded Tuolumne River, is at an elevation of about 244 meters above sea level, placing the site within the Upper Sonoran Zone (see Figure 1). Primary tree species noted during an ecological survey of the area at the time of excavation include interior live oak, blue oak, gray pine, yellow pine, and buckeye. The ecological survey, conducted

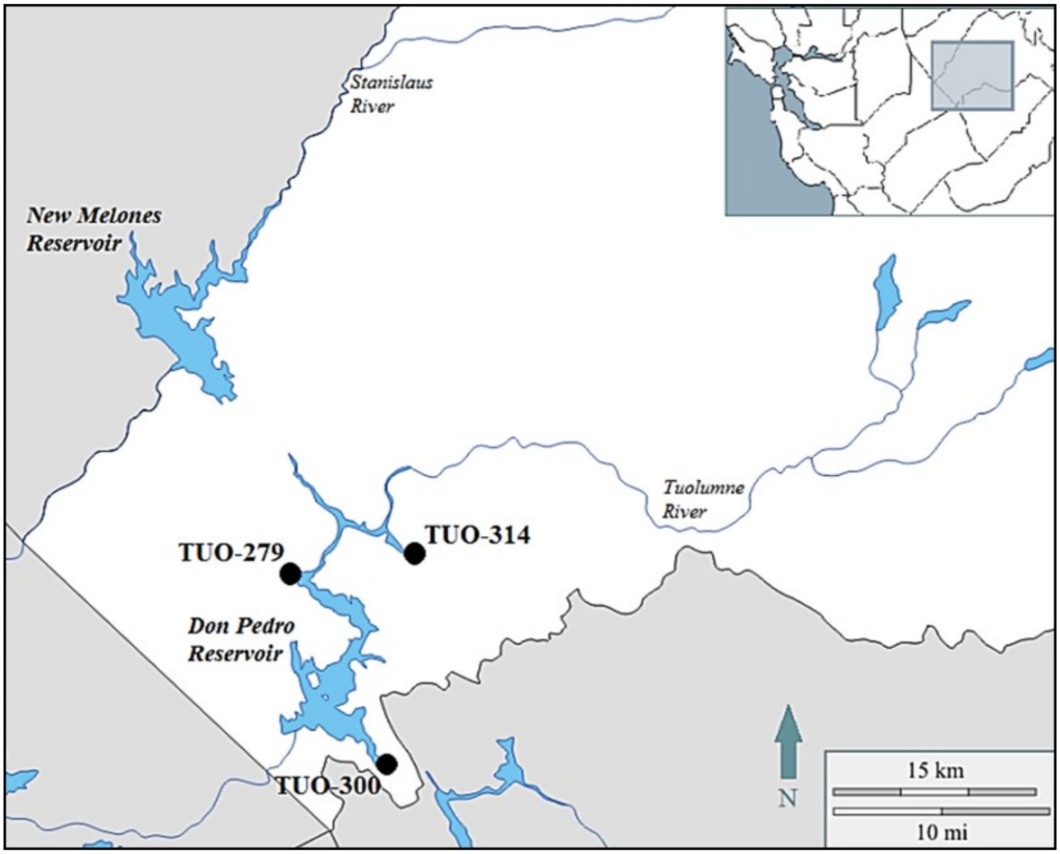

Figure 1. TUO-279, TUO-300, and TUO-314 are shown here in relation to their locations within Tuolumne County and central California.

during late April (1970 or 1971), indicates that a wide variety of resources noted by Barrett and Gifford (1933) as spring time foods were still available. These include mule ears, clover, miner's lettuce, common monkey flower, larkspur, rose lupine, soap root, harvest brodiaea, blue dicks, and white-leaf manzanita (Moratto 1971:49-52). Animal species present during the survey and noted as utilized by the Central Sierra Me-Wuk (Barrett and Gifford 1933) included mule deer, squirrels, scrub jay, and California quail.

Excavations at this site took place from November of 1970 to January 1971. One radiocarbon date was obtained from a charcoal sample recovered from a depth of 55 cm from within one of the units, giving an estimated median age of 350 cal B.P. (295±90 B.P.). Artifacts related to subsistence activities include: one mano, one metate, one edge-pecked cobble, 36 flake tools of obsidian, chert, and quartz, two steatite vessel fragments, five arrow points, and ten dart points (Moratto 1971:45-54). A large number of faunal remains were recovered, however, owing to the poor state of preservation and small fragmentary size of many, very few were identifiable beyond that of mammal. Of those identifiable are one horse molar, one dog or coyote tooth, and a few mule deer. Also present were five pieces of fresh-water mussel (Moratto 1971:61-62). No archaeobotanical remains were recovered.

The excavations at TUO-279 also unearthed three human burials, two of which were completely excavated, while the third was left in the field due to time constraints (Moratto 1971:45). Burial 1 consists of two fragmentary vertebrae, three phalanges, a right malar, and two proximal radial epiphyses. Burial 2 consists of a partial cranium, femora, tibiae, and a pelvis (Moratto 1971:162). Later analysis carried out at San Francisco State University concluded that bones recovered from the two burials represent up to nine individuals. It is from these nine individuals that samples were taken for isotopic analysis.

Site TUO-300, referenced as the Roger Creek Site, is located along Roger Creek, at the southeastern extent of Don Pedro Reservoir (see Figure 1) at an elevation of 260 meters in the Upper Sonoran Zone. Roger Creek, most likely an intermittent stream, drains a small valley which supported a savanna community of gray pine, blue oak, interior live oak, California buckeye and various chaparral species (Moratto 1971:78).

The site, virtually undamaged, was chosen for excavation in order to examine exploitative activities in areas away from the main river and to test a hypothesis concerning changes in settlement pattern development as a function of population increments (Moratto 1971:80). The site measured 40 x 55 meters, with cultural deposits found to depths of 180 cm. The center of the site was marked by a 9-x-8 m house pit. Two radiocarbon dates were obtained from charcoal samples from within midden deposits ranging from 50-70 cm in depth, yielding estimated median ages of 680 cal B.P. (730±95 B.P.) and 960 cal B.P. (1045±90 B.P.). A third radiocarbon date obtained from a carbonized post making up the house structure gave a median age of 950 cal B.P. (1035±90 B.P.) (Moratto 1971: 111).

Subsistence related artifacts recovered from TUO-300 include four metates (two broken), nine fragments of portable mortars, one pestle, two fragments of unmodified river cobble, five intact bifacial manos, 12 mano fragments, 13 oval bifacial manos, four sub-rectangular bifacial manos, one fish spear element, a steatite vessel fragment, a stone atlatl weight fragment, pebble cores/choppers, and a number of projectile points (Moratto 1971:81-88). Other than a few deer teeth and one fish bone, all faunal remains were so weathered that identity beyond that of mammal was impossible. Fresh water mussel was present in small quantities, totaling 143 grams in 107 cubic meters of midden (Moratto 1971:109). All of the artifacts and bone fragments were encrusted with a calcareous material. No archaeobotanical remains were recovered.

A number of human remains were also unearthed during excavations at the site. In total, eight burials were found within the house structure, and 13 burials were recovered from other parts of the site, with many representing more than one individual. In total, 37 individuals represent this burial population and were included in the study.

Site TUO-314 is located along the south bank of Moccasin Creek, about one mile from its confluence with the Tuolumne River (see Figure 1). It was located within the Upper Sonoran vegetation zone, within an oak savanna. The site had been heavily damaged by earthmoving equipment and a trailer park prior to excavations in 1971. It was noted that the remains of hundreds of burials littered the disturbed surface, with cairns, red ochre stains, and concentrations of cremated human bone present (Moratto 1971:129).

Site TUO-314 was marked by a paucity of artifacts related to subsistence activities. Of the 21 artifacts recovered, one projectile point/knife was found, as were seven flake tools. The remaining artifacts were awls, beads, and glass shards. Ash deposits and fire-cracked rock were noted, suggesting cooking activities. The site also contained the remains of a house floor, measuring approximately six meters in diameter, and a cairn. Seventeen bedrock mortars within a granite outcrop were located about 183 meters from the northwest edge of the site. Faunal remains were found within the site perimeter, totaling 98 pieces and weighing 96.83 g. Large mammal remains constitute the largest portion (67 pieces/79.83 g), followed by small amounts of bird bones (13 pieces/6.9 g), small mammal remains (4 pieces/3.4 g), shell (4 pieces/3.2 g), rodent remains (9 pieces/2.4 g), and one canid bone weighing 1.1 grams. Trace amounts of three pieces of carbonized floral remains are also noted within the report (Moratto 1971:131-134). Analysis beyond that mentioned within the report was never undertaken. Fourteen individuals were recovered from the site and are included in this analysis.

Approach

Stable isotope analysis of bone collagen provides information on paleodiet within the last 5 to 15 years of a person's life (Schoeninger and Moore 1992). Controlled dietary analyses among a range of mammal species shows that approximately 75% of bone collagen is routed from dietary protein, with carbohydrates and lipids comprising the remaining 25% (Fernandes et al. 2012; Froehle et al. 2010).

Previous studies show that the ratios of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ can distinguish between different dietary food sources (DeNiro and Epstein 1981; DeNiro 1987; DeNiro and Schoeninger 1983). For example, plants using the C_3, C_4, and CAM (Cerulean Acid Metabolism) photosynthetic pathways fractionate carbon in different ways, leading to distinctive $^{13}C/^{12}C$ signatures (DeNiro 1987). Similarly, carbon enters terrestrial and marine ecosystems in different manners producing unique isotopic signatures (DeNiro 1987). As a result, carbon isotopes can help differentiate human diets high in C_3 (e.g., acorns) vs C_4 (e.g., maize) plants, or marine vs. terrestrial foods (Figure 2). $^{13}C/^{12}C$ ratios are measured against the PeeDee Belemnite (PDB) standard and are noted as $\delta^{13}C$. The PDB is set at 0‰ (parts per thousand, or permil), however it is enriched in ^{13}C relative to most organics. Because of this, $\delta^{13}C$ values are normally negative in relation to the standard.

Nitrogen isotope ratios complement carbon isotopes and provide additional discriminatory information on paleodiets. The ratio of ^{15}N to ^{14}N in bodily tissues is strongly dependent on the trophic level of the consumer, with $^{15}N/^{14}N$ increasing approximately 3‰ with each trophic level (Schoeninger and Moore 1992). Terrestrial environments typically have three trophic levels: plants, herbivores, and carnivores. Aquatic environments typically display many more trophic levels. In this respect, $^{15}N/^{14}N$ in bone collagen can discriminate between a vegetarian and a meat eater, and between a terrestrial and aquatic consumer (see Figure 2). In stable isotope analysis, $^{15}N/^{14}N$ ratios are measured against the standard of atmospheric N_2 (AIR) and are noted as $\delta^{15}N$. The standard is set at 0‰, and it is depleted in ^{15}N relative to most living organisms. As a result, $\delta^{15}N$ values are almost always positive when measured against the standard.

Expectations

California is environmentally diverse, offering different resources in different areas. Previous stable isotope analysis-based studies of paleodiets indicate that there is a patterning to the dietary signatures of humans which correlate with the variable ecologies and environmental conditions in which they live. Analyses of individuals from the Sacramento Valley have different and non-overlapping isotopic signatures than those from San Francisco Bay (Bartelink 2006:166; Bartelink 2009). Likewise, individuals from the Sacramento River Delta differ in their isotopic signatures from those in the Sacramento Valley locations and from those in the Bay (Beasley et al. 2013; Eerkens et al. 2013). We predict that the Tuolumne County sites will exhibit a unique regional signature when compared to other archaeological dietary signatures within Central California.

We anticipate the stable isotope signatures of the individuals to reflect a terrestrial-based diet, rather than one indicative of consumption of marine-based foods. There are two reasons for this. First, these sites are located far inland. While this may seem fairly intuitive, the consumption of anadromous fish is not out of the question because these sites are also in close proximity to a major river (the Tuolumne) and salmon are mentioned by ethnographers as being on the menu (Barrett and Gifford 1933). However, there is a dearth of archaeological evidence for habitual consumption of salmon in this region (Gobalet et al. 2004). Second, based on what is known from both the ethnographic and archaeological records, discussed above, plants and terrestrial fauna are the major contributors to native Tuolumne diets, but the proportions of protein contributions from these two sources remain to be seen.

We also expect that, if the temporal ranges of the sites are large enough, we will see changes in diet through time. If there are changes, they may be in response to either changing ecological conditions or changing cultural behaviors, both of which are often linked in regards to subsistence. We do not anticipate large differences in dietary signatures among the three sites because they are all located within the same biotic community, and as such, behaviors related to subsistence will likely follow along the same pattern in terms of consumption of food resources throughout contemporaneous periods.

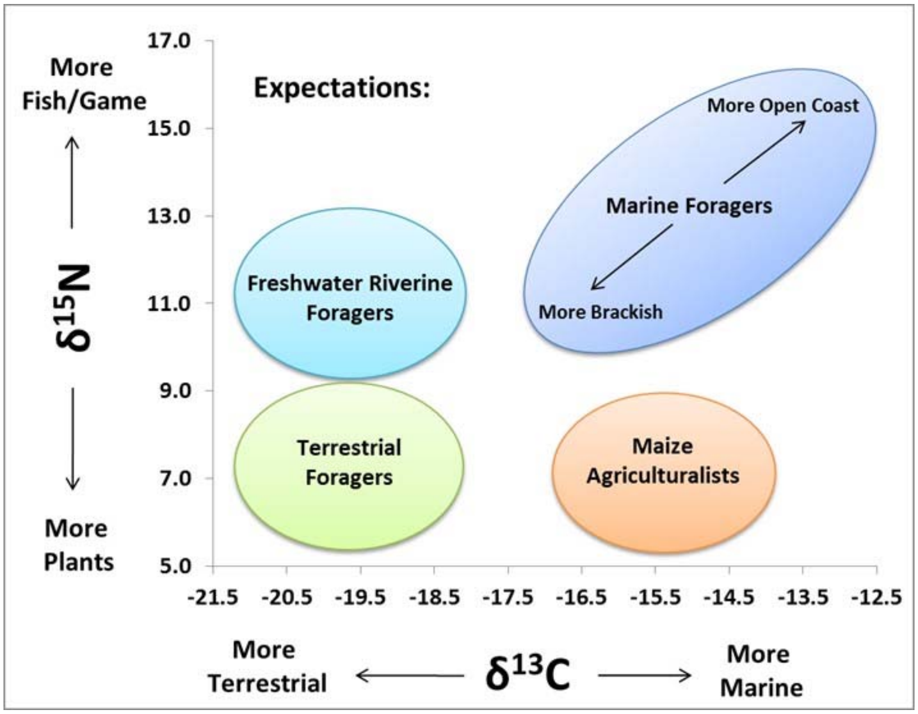

Figure 2. Expected ranges in $\delta^{13}C$ and $\delta^{15}N$ for different foraging niches.

At the same time, there remains the possibility of patterned dietary differences among individuals. Because of the sexual division of labor in hunter-gatherer societies (men hunt, women gather), we might expect to see men enriched in $\delta^{15}N$ compared to women due to differential protein consumption. We may also see a difference between adolescents and adults due to the uncertainties and variation of parental investment in children within hunter-gatherer societies.

MATERIALS AND METHODS

Materials

Samples for this study represent a total of 53 individuals, including 30 adults, ten juveniles, and thirteen of undetermined age. Seven were determined to be from males and eight from females, while 38 individuals could not be sexed. Nine individuals are from TUO-279, including one juvenile and one adult male. Thirty-one individuals are from TUO-300, including eight adult females, four adult males, and five juveniles. Thirteen are from TUO-314, with four juveniles and two adult males. Ribs are the most common element sampled, but the exact bone element sampled varied depending on what was available. Information for sampled individuals is organized in Tables 1, 2, and 3.

Methods

To isolate collagen, we followed a modified Longin procedure (Longin 1971). Approximately one gram of cortical bone from each sample was cleaned by drilling exposed surfaces with a diamond bit and then sonicating in deionized H_2O (three five-minute baths, with the dH_2O replaced after each bath). The samples were left in open containers until completely dry. Clean samples were then weighed and placed into 20 mL scintillation vials. They were demineralized with a solution of 0.5 M hydrochloric acid (HCl). The HCl was changed every other day until the samples were completely demineralized (up to two weeks). The bone was then washed three times with dH_2O and soaked in 0.125 M sodium hydroxide

Table 1. Table of individuals from TUO-279.

SITE	BURIAL/INDIVIDUAL	SEX	AGE AT DEATH	ELEMENTS SAMPLED
TUO-279	B1	Indeterminate	Adult	thoracic vertebra spinous process
TUO-279	B2, Indiv. 1	Male	27-35	left fibula fragment
TUO-279	B2, Indiv. 2	Indeterminate	Adult	left occipital fragment
TUO-279	B 4	Indeterminate	Adult	tibial fragment
TUO-279	Unk1	Indeterminate	Adult	spinous process
TUO-279	Unk2	Indeterminate	Indeterminate	rib fragment
TUO-279	Unk3	Indeterminate	Adult	right third metacarpal
TUO-279	Unk4, Indiv. 1	Indeterminate	Adult	rib fragment
TUO-279	Unk4, Indiv. 2	Indeterminate	Adolescent	spinous process fragment

(NaOH) for 24 hours to remove humic acids. The samples were rinsed five times with dH_2O to remove any residual NaOH.

Slightly acidic pH3 water was added to the vials, and the samples were placed in a 70°C oven for approximately 24 hours to solubilize collagen. If the samples were not completely solubilized, the pH3 solution from each sample was pipetted into clean, weighed vials and the process was repeated until the samples were solubilized. The sample was then placed in a freeze-dryer to remove all remaining water, isolating the collagen fraction.

Collagen total C, total N, δ^{13}C, and δ^{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. For this study, carbon isotopes ratios, δ^{13}C, are expressed in permil notation (parts per thousand) relative to the PDB standard (arbitrarily set at 0‰), while N isotope ratios, δ^{15}N, are expressed against N_2 in modern atmospheric air (also arbitrarily set to 0‰). Seventeen collagen samples (TUO-279 N=6, TUO-300 N=7, TUO-314 N=4) were also submitted for AMS radiocarbon dating.

RESULTS AND DISCUSSION

Table 4 reports the results for δ^{13}C and δ^{15}N for the nine individuals from TUO-279. The δ^{13}C ranges between -18.9‰ and -17.1‰, with a mean of -18.4‰ and standard deviation of 0.43‰. The δ^{15}N ranges between 5.9‰ to 8.1‰, with a mean of 6.8‰ and standard deviation of 0.66‰. The small ranges and standard deviations indicate a fairly homogenous diet. The δ^{13}C and δ^{15}N values allude to a terrestrial based protein source, low in trophic level (likely plants). There is no indication that these individuals were consuming salmon on a regular basis. Based on the low δ^{15}N values, we believe these individuals were obtaining the majority of their protein from pine nuts and acorns, with some lagomorphs and ungulates.

Table 5 reports the results for TUO-300. Two individuals (Unk 14 and Unk 15) returned no collagen, and are excluded from the analysis. The other twenty-nine individuals range in δ^{13}C from -20.1‰ to -18.0‰, with a mean of -19.1‰ and standard deviation of 0.57‰. The δ^{15}N ranges from 6.6‰ to 9.5‰, with a mean of 8.0‰ and standard deviation of 0.8‰. Although variation is slightly higher than TUO-279, these values indicate a fairly homogenous diet based primarily on terrestrial protein sources. It is interesting that the δ^{13}C values are on average more depleted in ^{13}C but ^{15}N enriched relative to individuals from TUO-279.

Table 2. Table of individuals from TUO-300.

Site	Burial/Individual	Sex	Age at Death	Elements Sampled
TUO-300	B 1	Female	Adult	fibula fragment
TUO-300	B 2	Indeterminate	Adult	left zygomatic fragment
TUO-300	B 3	Male	Adult	tibial fragment
TUO-300	B 5	Indeterminate	Adult	tibial fragment
TUO-300	B 6	Indeterminate	Juvenile	cranial fragment
TUO-300	B 8	Female	21-35	mandible fragment
TUO-300	B 9A	Female	Old Adult	mandible fragment
TUO-300	B 9B	Indeterminate	Adult	radius fragment
TUO-300	B10A1	Female	21-35	right mandibular ramus fragment
TUO-300	B10A2	Female	21-35	rib fragment
TUO-300	B 11A	Indeterminate	4-8	long bone fragment
TUO-300	B 13	Indeterminate	5-9	rib fragment
TUO-300	Unk1	Indeterminate	5-9	long bone fragment
TUO-300	Unk2, Indiv. 1	Female	Adult	long bone fragment
TUO-300	Unk3	Indeterminate	Adult	femur fragment
TUO-300	Unk4	Male	Young Adult	cranial fragment
TUO-300	Unk5	Female	Old Adult	left distal femur fragment
TUO-300	Unk6	Indeterminate	Adult	left fibula fragment
TUO-300	Unk7A	Indeterminate	Adult	femur fragment
TUO-300	Unk7B	Indeterminate	4-8	left humerus fragment
TUO-300	Unk8	Indeterminate	Indeterminate	cranial fragment
TUO-300	Unk9	Female	Adult	mandible fragment
TUO-300	Unk11	Male	Adult	temporal fragment
TUO-300	Unk12	Indeterminate	Indeterminate	intermediate hand phalanx fragment
TUO-300	Unk13	Indeterminate	Indeterminate	rib fragment
TUO-300	Unk14	Indeterminate	Indeterminate	right temporal fragment
TUO-300	Unk15	Indeterminate	Indeterminate	hand phalanx fragment
TUO-300	Unk16A	Female	Adult	left 4th metatarsal
TUO-300	Unk16B	Male	Adult	right zygomatic fragment
TUO-300	Unk16C	Indeterminate	Indeterminate	rib fragment
TUO-300	Unk18	Indeterminate	Indeterminate	rib fragment
TUO-300	Unk20	Indeterminate	Indeterminate	right temporal fragment

Table 3. Table of individuals from TUO-314.

Site	Burial/Individual	Sex	Age at Death	Elements Sampled
TUO-314	Burial 2A	Indeterminate	Adult	mandible fragment
TUO-314	Burial 2B	Indeterminate	Young Adult	left mandible fragment
TUO-314	Burial 4A	Indeterminate	Young Adult	left mandible fragment
TUO-314	Burial 4B	Indeterminate	Indeterminate	long bone fragment
TUO-314	Unk1C	Indeterminate	Juvenile	rib fragment
TUO-314	Unk2A	Indeterminate	Juvenile	rib fragment
TUO-314	Unk2B	Indeterminate	Adult	tibial fragment
TUO-314	Unk5A	Indeterminate	Indeterminate	fibula fragment
TUO-314	Unk6A1	Indeterminate	Adult	long bone fragment
TUO-314	Unk6A2	Indeterminate	Adult	rib fragment
TUO-314	Unk7A	Male	Adult	tibial fragment
TUO-314	Unk7B	Indeterminate	Child	tibial fragment
TUO-314	Unk8A1	Male	Adult	long bone fragment
TUO-314	Unk8A2	Male	Adult	temporal fragment
TUO-314	Unk8B	Indeterminate	Juvenile	rib fragment

Table 4. $\delta^{13}C$ and $\delta^{15}N$ values for individuals from TUO-279; radiocarbon dates are reported as median calibrated dates and the range at the two sigma level, in years cal B.P.

Sample ID	Sex	Age at Death	$\delta^{13}C$	$\delta^{15}N$	C/N	% Col. Yield	Median Dates (cal BP)	2σ ^{14}C (cal BP)
TUO-279 B1	Ind.	Adult	-17.74	6.38	3.5	5.4%	950	927-1041
TUO-279 B2 Indiv.1	M	Adult	-18.63	7.16	3.4	3.1%	180	71-290
TUO-279 B2 Indiv.2	Ind.	Adult	-18.37	6.97	3.3	4.4%	1665	1571-1734
TUO-279 B4	Ind.	Adult	-18.63	7.08	3.5	2.3%	1645	1568-1703
TUO-279 Unk1	Ind.	Adult	-18.89	5.89	3.0	10.7%	900	799-933
TUO-279 Unk2	Ind.	Ind.	-18.84	6.47	3.4	2.0%		
TUO-279 Unk3	Ind.	Adult	-18.61	6.92	3.4	27.7%	175	45-301
TUO-279 Unk4 Indiv.1	Ind.	Adult	-17.61	8.14	3.4	13.7%		
TUO-279 Unk4 Indiv.2	Ind.	Juv.	-18.36	5.90	2.9	14.3%		

Note: Ind. means Indeterminate; Age at Death has been simplified here to either Adult, Juv. (Juvenile), or Ind.

Table 6 reports the values for the thirteen individuals from TUO-314. The average $\delta^{13}C$ value for these individuals is -19.08‰, the same as for TUO-300. The $\delta^{13}C$ standard deviation is 0.59, which is again small. For this population, $\delta^{13}C$ ranges between -20.5‰ and -17.9‰, with a mean of -19.1 and a standard deviation of 0.6. $\delta^{15}N$ varies from 3.3‰ to 8.3‰, with a mean of 6.6‰ and a standard deviation of 1.1‰. The individuals from this site are most variable of all three sites, and display a strong signature for a terrestrial based diet.

Table 5. $\delta^{13}C$ and $\delta^{15}N$ values for individuals from TUO-300.

Sample ID	Sex	Age at Death	Δ^{13}C	Δ^{15}N	C/N	% Col. Yield	Median Dates (BP)	2Σ ^{14}C (BP)
TUO-300 B1	F	Adult	-19.05	6.59	3.2	2.9%	800	730-911
TUO-300 B2	Ind.	Adult	-19.88	7.25	3.2	2.8%		
TUO-300 B3	M	Adult	-18.29	9.24	3.3	1.5%	2945	2867-3033
TUO-300 B5	Ind.	Adult	-19.15	7.49	3.2	3.2%	1820	1735-1871
TUO-300 B6	Ind.	Juv.	-18.87	9.07	3.3	7.6%	1665	1571-1734
TUO-300 B8	F	Adult	-18.27	7.79	3.2	3.4%	2730	2517-2752
TUO-300 B9A	F	Adult	-19.35	7.70	3.3	2.5%	390	304-454
TUO-300 B9B	Ind.	Adult	-19.18	6.57	3.4	2.3%	2240	2148-2310
TUO-300 B10A	F	Adult	-19.36	7.58	3.3	3.1%		
TUO-300 B11A	Ind.	Juv.	-19.80	7.55	3.3	3.8%		
TUO-300 B13	Ind.	Juv.	-19.68	6.59	3.2	2.9%		
TUO-300 Unk1	Ind.	Juv.	-19.85	7.87	3.7	2.3%		
TUO-300 **Unk2 Indiv.1**	**F**	**Adult**	**-19.24**	**6.88**	**3.4**	**0.4%**		
TUO-300 **Unk3**	**Ind.**	**Adult**	**-18.34**	**8.63**	**3.4**	**0.3%**		
TUO-300 Unk4	M	Adult	-19.79	8.17	3.5	1.1%		
TUO-300 Unk5	F	Adult	-20.05	9.48	4.1	1.9%		
TUO-300 Unk6	Ind.	Adult	-18.07	8.36	3.2	21.5%		
TUO-300 Unk7A	Ind.	Adult	-18.02	9.16	3.3	1.6%		
TUO-300 Unk7B	Ind.	Juv.	-19.06	8.35	3.3	2.6%		
TUO-300 Unk8	Ind.	Ind.	-19.56	6.83	3.3	3.7%		
TUO-300 Unk9	F	Adult	-19.30	8.21	3.4	5.1%		
TUO-300 Unk11	M	Adult	-18.61	8.08	3.3	3.2%		
TUO-300 Unk12	Ind.	Ind.	-18.45	8.50	3.3	3.7%		
TUO-300 Unk13	Ind.	Ind.	-18.83	8.36	3.4	4.3%		
TUO-300 Unk16A	F	Adult	-18.51	8.49	3.6	2.3%		
TUO-300 Unk16B	M	Adult	-19.64	8.67	3.4	1.9%		
TUO-300 Unk16C	Ind.	Ind.	-18.92	7.23	3.4	3.9%		
TUO-300 Unk18	Ind.	Ind.	-19.36	8.22	3.3	2.7%		
TUO-300 Unk20	Ind.	Ind.	-18.64	8.43	3.3	1.8%		

Note: individuals in bold type had a low percent of collagen yield, and their values are likely not reliable.

As shown in Figure 3, the samples from the three sites generally fall within the range expected of terrestrial foragers in Central California. Site TUO-279 individuals tend to fall on the lower right of this distribution, while TUO-300 is slightly elevated in δ^{15}N.

Table 6. δ¹³C and δ¹⁵N values for individuals from TUO-314.

SAMPLE ID	SEX	AGE AT DEATH	Δ¹³C	Δ¹⁵N	C/N	% COL. YIELD	MEDIAN DATES (BP)	2Σ ¹⁴C (BP)
TUO-314 B2A	Ind.	Adult	-18.72	6.61	3.6	2.0%	2230	2148-2319
TUO-314 B2B	Ind.	Adult	-18.93	6.53	3.5	3.6%	2480	2358-2699
TUO-314 B4A	Ind.	Adult	-19.27	6.42	3.4	2.0%	695	661-735
TUO-314 B4B	Ind.	Ind.	-19.34	6.87	3.6	2.5%	820	735-911
TUO-314 Unk1C	Ind.	Juv.	-18.68	6.90	3.2	9.9%		
TUO-314 Unk2A	Ind.	Juv.	-19.77	5.58	3.2	3.5%		
TUO-314 Unk2B	Ind.	Adult	-18.83	7.14	3.2	4.6%		
TUO-314 Unk5A	Ind.	Ind.	-19.40	7.81	3.2	4.0%		
TUO-314 Unk6A	Ind.	Adult	-18.73	6.73	3.2	4.3%		
TUO-314 Unk7A	M	Adult	-18.90	6.53	3.3	2.0%		
TUO-314 Unk7B	Ind.	Juv.	-20.50	3.30	3.1	4.9%		
TUO-314 Unk8A1	M	Adult	-19.37	6.24	3.2	2.2%		
TUO-314 Unk8A2	Ind.	Adult	-17.85	8.26	3.2	6.8%		

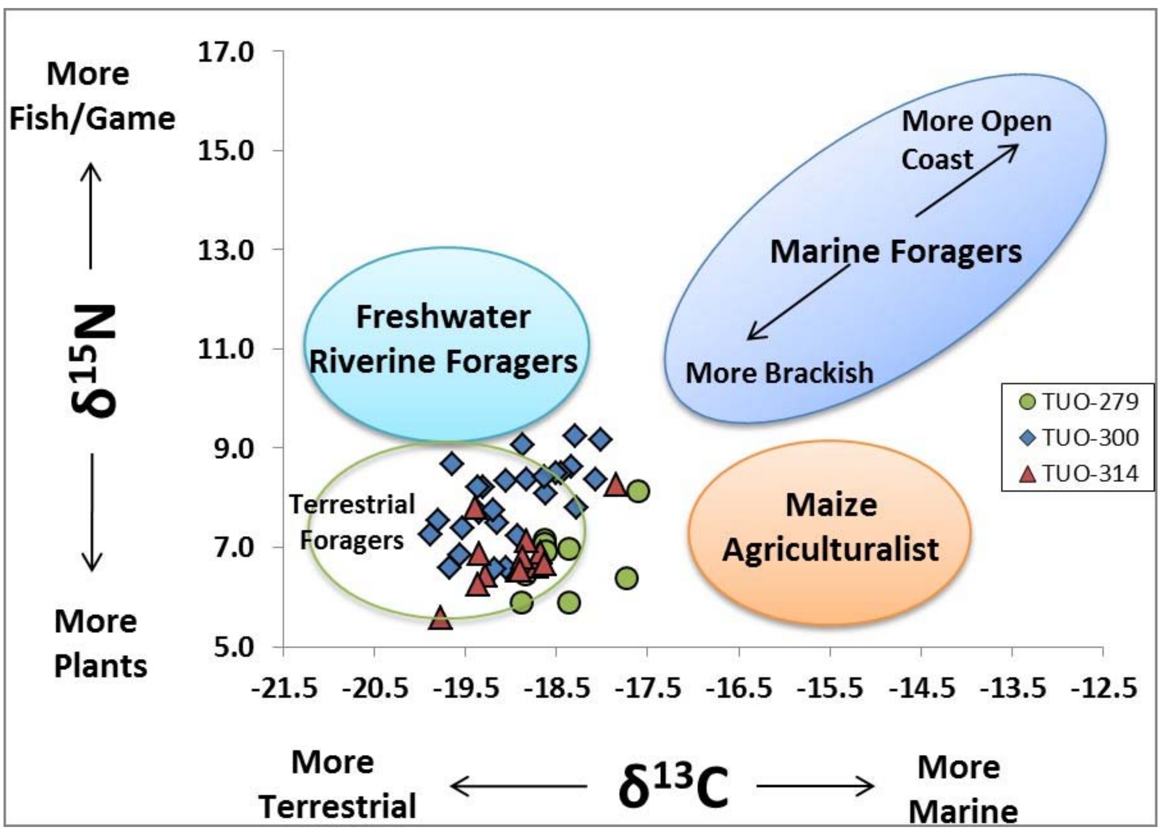

Figure 3. δ¹³C vs. δ¹⁵N for all individuals in this study.

When $\delta^{15}N$ and $\delta^{13}C$ are parsed by sex, we see no difference between the seven males and eight females. These fifteen individuals all fall within the same range, which indicates that both men and women were likely consuming many of the same foods. As well, there is no difference in the diets between adults and juveniles, signifying that juveniles were not fed a specialized diet.

As indicated by the radiocarbon dates, the three sites each have long time spans of occupation. TUO-279 ranges between 1665 and 175 cal B.P., TUO-300 between 2945 and 390 cal B.P., and TUO-314 from 2480 to 695 cal B.P.

When we consider the three sites together and examine temporal changes in $\delta^{13}C$ and $\delta^{15}N$, there is a clustering during the 1150 to 605 cal B.P. period that is most pronounced in $\delta^{15}N$ values (Figures 4 and 5). This corresponds with the Medieval Climatic Anomaly (MCA), a period of drought that affected many regions of the Northern Hemisphere (Schwitalla 2013:8-10). The Sierra Nevada region did not escape this drought, evidenced by tree ring cores and temperature reconstructions (Graumlich 1993). The effects of this drought on hunter-gatherer diets in the Sierra Nevada are less well known. The dietary adaptive response to drought is evidenced here, by the clustering of five individuals between 5.9 and 6.9‰ for $\delta^{15}N$. Overall, there is a trend in declining $\delta^{15}N$ and $\delta^{13}C$ values between 3000 and 1000 cal BP, indicating that the protein sources in the diet were slowly changing to more terrestrial and lower trophic levels.

This is probably indicative of the intensification of plant resources that is pervasive throughout California (Wohlgemuth 1996). This is also correlates with the archaeobotanical and faunal remains found during excavations at New Melones and the East Sonora Bypass Project (Moratto 2002; Rosenthal 2008), which indicate a wider variety of species utilized. Coupled with the archaeological data, we suggest that this pattern is indicative of expanding diet breadth to include more lower-ranked foods, especially acorns, small seeds, and other plant foods. There is a slight rebound for $\delta^{15}N$ in the most recent period, after the MCA, which may correspond with a dietary rebound.

CONCLUSIONS

Isotopic analysis of bone collagen from three sites excavated in the early 1970s in the Don Pedro Reservoir reveals new information on ancient dietary practices of people living at these sites. Radiocarbon dates also reveal new information on the archaeological age of the midden deposits. We highlight three main patterns here that we hope to explore further in future analyses.

First, 17 new radiocarbon dates on bone collagen show that all three sites have a long period of occupation, as measured by human interments. As best as can be determined from the small number of radiocarbon dates, TUO-300 and TUO-314 date from at least 2500 years ago, and TUO-279 from at least 1500 years ago. All three sites seem to have been occupied continuously from then through very recent times.

Second, throughout this temporal window, diets in the region were focused on terrestrial foods, especially plant foods. This finding is consistent with ethnographic studies that document the importance of acorn, small seeds, and gray pine as staples of the diet. It also supports archaeological studies of artifact types and paleobotanical remains that suggest plant foods were important in local economies. However, contrary to some ethnographic reports, salmon does not appear to have been a major contributor of protein in local diets.

Third, taken together, the three sites document a trend of decreasing trophic position during the hotter and drier MCA. Such a decrease in trophic position is consistent with a shift in diet towards an increasing contribution of plant foods in the diet, perhaps as the concentration of terrestrial game or freshwater fish decreased. Further, there appears to be a homogenization (i.e., decreased inter-individual variation) of diets during the MCA. This may indicate a greater degree of intra-community sharing during these more challenging environmental conditions. A slight rebound in the trophic level of local diets after approximately 600 cal BP seems to indicate relaxed environmental conditions, with greater availability of game and/or fish.

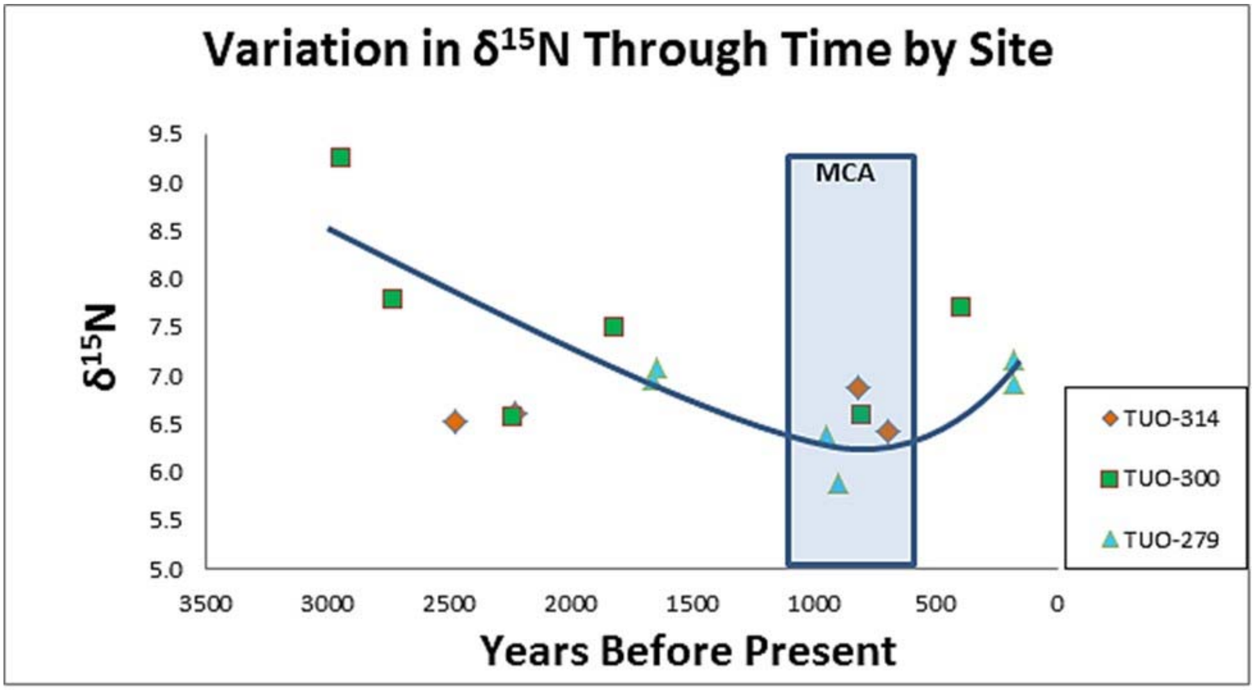

Figure 4: $\delta^{15}N$ vs. Years Before Present highlighting the Medieval Climatic Anomaly

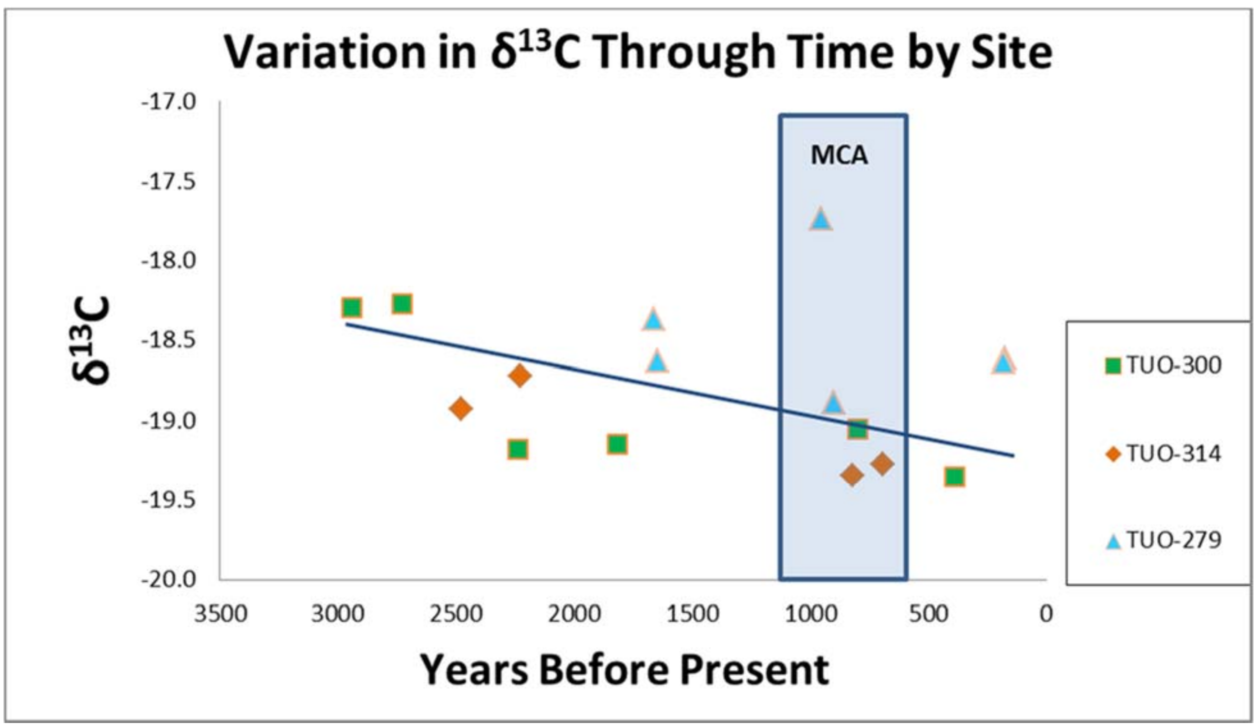

Figure 5: $\delta^{13}C$ vs. Years Before Present highlighting the Medieval Climatic Anomaly.

In sum, a partnership between Tuolumne Me-Wuk and the University of California documents how stable isotope analysis of small fragments of human bone can contribute new knowledge about ancient diets, and human responses to large-scale climatic events such as the MCA. The remains have now been reburied, but these analyses provide a small window into the lives and life histories of individuals from the past. Sharing and retelling those stories keeps their memories alive for people today and others in the future.

ACKNOWLEDGMENTS

Part of the funding for this work comes from a National Science Foundation grant to JWE (BCS-1318532). We thank the Tuolumne Me-Wuk for their support for this project. We also thank the undergraduate and graduate students of the Archaeometry Lab at UC Davis for their help and guidance. Finally, we thank Joy Matthews and the staff at the UC Davis Stable Isotope Facility for their services.

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