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# Stable isotope evidence of diet breadth expansion and regional dietary variation among Middle-to-Late Holocene Hunter-Gatherers of Central California



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## ABSTRACT

Previous stable isotope research on Middle-to-Late Holocene (6600 cal BP-present) hunter-gatherers of Central California has documented significant regional variation in human paleodiets between the San Francisco Bay Area and Central Valley. In general, this geographically-patterned dietary variation tracks greater consumption of marine food resources in the Bay Area and greater consumption of freshwater and terrestrial food resources in the Central Valley. Using stable carbon and nitrogen isotope analysis of bone collagen, stable carbon isotopes of bone bioapatite, and a large series of AMS dates (5420-2975 cal BP), we examine human paleodiets in 238 burials from CA-CCO-548 (Marsh Creek), located near the Sacramento-San Joaquin Delta, in Central California. Isotopic results support archaeofaunal and paleobotanical findings regarding the dietary importance of terrestrial C<sub>3</sub>-resources (e.g., artiodactyls, small seeds, acorns) and freshwater fish. The results further indicate that marine resources were of minor importance and could have been acquired from brackish areas near the mouth of the San Francisco Bay estuary located 9 km away or through trade. Although carbon and nitrogen isotopes of bone collagen suggest continuity in the sources of dietary protein over time. Carbon isotopes of bone bioapatite demonstrate a significant temporal shift toward greater consumption of C<sub>3</sub> plant resources, such as acorns and small seeds, consistent with resource intensification models. The paleodiet of the CA-CCO-548 population is similar to contemporaneous groups located to the south and northwest, but is distinct from groups within the Delta and San Francisco Bay Area.

## 1. Introduction

Prior to Spanish contact in 1769, the hunter-gatherers of Central California inhabited one of the most densely settled landscapes in North America (Cook 1976; Milliken 2006). Past scholars attributed the high population density within the region, in the absence of agriculture, to the natural abundance of wild plant and game resources available to native populations (Baumhoff, 1963; Bean and Lawton, 1976; Kroeber, 1925, 1939). More recently, the notion of resource abundance during the Late Holocene has been challenged by evidence of resource depression in the archaeological record, providing support for resource intensification models (Basgall, 1987; Broughton, 1994a, 1994b). These

models argue for a reduction in foraging efficiency as measured by temporal declines in the relative abundance of higher-ranked, low-cost prey (e.g., artiodactyls) and the increased use of lower-ranked resources, such as small fauna and wild plant foods (Basgall, 1987; Broughton, 1994a, 1994b; Wohlgemuth, 2010a). The Late Holocene archaeological record after 2500 BP is further characterized by larger and more permanent settlements, human population expansion, and intense plant-focused economies (Basgall, 1987; Jones and Klar, 2007; Wohlgemuth, 2010a). Population pressure, increased sedentism, and higher levels of territoriality are important factors in the development of intensive food storage economies, especially those involving storage of nuts and seeds (Cohen, 1981; Keeley, 1988; Kelly, 1992; Testart,

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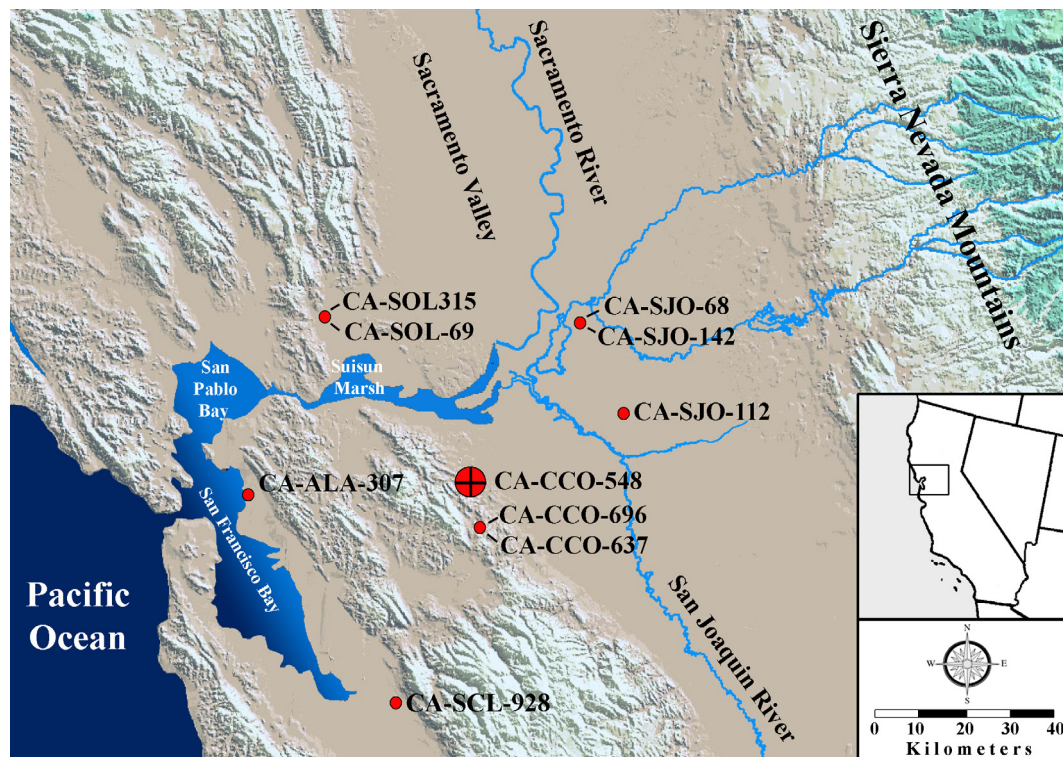


Fig. 1. Map of the archaeological sites discussed in the study.

1982).

Recent excavations at CA-CCO-548 (Marsh Creek site, Pearl Locus) revealed early evidence of sedentary, plant-intensive, foraging economies in Central California (Wiberg, 2010) (Fig. 1). The archaeological deposit contains a large quantity of faunal and archaeobotanical remains, ground stone artifacts, lithics, fire-affected rock, house floors, storage and cooking features, and hundreds of human burials, attesting to intensive occupation for over two millennia (Wiberg, 2010; Stevens et al., 2009). Archaeofaunal studies indicate that freshwater fish (e.g., Sacramento perch, Sacramento suckers), ground squirrels, lagomorphs (hares and rabbits), artiodactyls, and waterfowl were important economic resources (Gobalet, 2004; Gobalet et al., 2010; Valente, 2010). Acorn remains are abundant throughout the occupational sequence, and appear to increase in importance over time relative to small seeds (Wohlgemuth, 2010b).

Here we present stable carbon and nitrogen isotope data from human bone collagen and bone bioapatite from 248 individuals who were buried in the northern part of CA-CCO-548, located along Marsh Creek near the eastern edge of the Central California Delta. AMS dates on 148 burials range between 5420 and 2975 cal B.P., indicating these individuals were interred mainly during the middle part of the Holocene (Eerkens et al., 2013b). Other studies indicate that southern portions of the site continued to be used through the Late Holocene until the time of contact (Burns et al., 2016; Ralston and Fitzgerald, 2014).

We hypothesize that stable carbon and nitrogen isotopes of human bone collagen will reflect a dietary shift from greater consumption of animal proteins (higher trophic level protein sources) towards increased reliance on plant foods (lower trophic level resources), a pattern found in some Late Holocene San Francisco Bay Area locales. We assume that higher trophic-level resources, such as freshwater fish and large terrestrial game, are of higher dietary quality, but when their density is low, are more costly to acquire than vegetal resources. Thus, we hypothesize that human paleodiet will shift over time toward greater reliance on  $C_3$  plant resources, such as acorns and small seeds, providing evidence for intensified use of low-ranked vegetal resources

during the Middle-to-Late Holocene transition. If these expectations are supported, this would suggest reduced foraging efficiency over time (as measured by calories/time), consistent with resource intensification models (Basgall, 1987; Beaton, 1991; Broughton, 1994a, 1994b; Broughton et al., 2010; Wohlgemuth, 2010a).

We then compare stable isotope results from CA-CCO-548 with data from nine other roughly contemporaneous sites in Central California to investigate regional variation in human paleodiet. These sites are located within the eastern and southern reaches of the San Francisco Bay, the Central California Delta, and northwest and the southeast of the Delta (Fig. 1). We hypothesize that San Francisco Bay populations will display elevated stable carbon and nitrogen isotope values, reflecting greater reliance on marine resources, whereas populations from the Delta region will show greater reliance on freshwater and terrestrial  $C_3$  resources. Our analysis provides new perspectives on dietary variability among early sedentary hunter-gatherer populations in Central California.

## 2. Early Period cultures (4950–2450 cal BP)

Two distinct cultural patterns emerged around 5000 years ago in Central California, situated in the Sacramento-San Joaquin Delta region and the San Francisco Bay Area (Gerow and Force, 1968). The Windmill Pattern in the Sacramento-San Joaquin Delta region is defined by extended burials, with individuals placed on their ventral surface (face down) with the head oriented to the west (Beardsley, 1948, 1954; Heizer, 1949; Ragir, 1972). While dorsal (face up) extension is also common, flexed burials and cremations are rare. Grave goods, such as large chipped stone projectile points, quartz crystals, shell beads and shell ornaments, baked clay objects, red ochre, and ground stone artifacts (e.g., mortars, pestles, and charmstones) are common (Heizer, 1949:17–24; Ragir, 1972; Meighan, 1987; Moratto, 1984:203). These mortuary characteristics were used to define the Windmill Pattern, which includes contemporaneous sites in the Central Valley (Moratto, 1984; Schulz, 1981:53). The Early San Francisco Bay Culture (also known as the Lower Berkeley Pattern) in the Bay Area is defined by

flexed burials, with no discernable pattern in cardinal direction, and few grave goods, usually limited to utilitarian items such as ground-stone and bone tools, and commonly associated with red ochre (Gerow and Force, 1968; Moratto, 1984; Milliken et al., 2007; Wallace and Lathrap, 1975). Both the Windmill Pattern and the Early San Francisco Bay Culture are part of what archaeologists generally refer to as the “Early Period” or “Early Horizon” (Beardsley, 1954; Groza et al., 2011; Heizer, 1949; Ragir, 1972).

### 3. CA-CCO-548 (Marsh Creek)

CA-CCO-548 (Marsh Creek site) is southwest of the modern city of Brentwood in Contra Costa County, California. The site measures 200 meters east-west by 150 meters north-south and is situated east of the Diablo Range, adjacent to a perennial waterway known as Marsh Creek, along the western edge of the Sacramento-San Joaquin Delta, in Central California at about 50 m a.s.l. (Fig. 1). Excavations were conducted between 2003 and 2008, as mitigation for a housing development, resulting in the recovery of approximately 480 burials (Wiberg, 2010). At the time of Spanish settlement in 1776, the site was occupied by Bay Miwok (Saclan) speaking peoples, possibly the *Volvon* group (Milliken, 2006). Although there are five burials that date earlier (5420–4038 cal BP), the paper focuses on burials dating to the Central California Taxonomic System’s Early Period that fall between 4000 and 2975 cal B.P.

The archaeofaunal record from CA-CCO-548 indicates that slow-water freshwater fishes were a key dietary resource (Gobalet et al., 2010). Approximately 95 percent of identified fish bones represent Sacramento perch, Sacramento sucker, and species from the cyprinid family, with < 1 percent of the assemblage consisting of anadromous fishes (e.g., salmon and sturgeon). Estuarine and freshwater shellfish were also poorly represented; however, it is possible that these resources were processed where they were acquired, so that only the meat was transported back to the site (Wiberg, 2010). The mammalian bone assemblage was dominated by small-to-medium sized taxa, especially ground squirrels (many with evidence of burning), followed by lagomorphs and carnivores (Valente, 2010). Of particular note is the low abundance of large game (e.g., elk, pronghorn, and mule deer), a pattern that contrasts with other Early Period assemblages from Central California, where these species dominate relative to smaller taxa (Broughton, 1994a; Broughton et al., 2010). The avian assemblage was dominated by anadromous waterfowl and coots, which would have been abundant in the marshlands around the site (Valente, 2010).

CA-CCO-548 lies along an ecotone between the San Francisco Bay estuary and the Sacramento-San Joaquin Delta (Wiberg, 2010:30; Meyer and Rosenthal, 1997). The archaeological context provides an opportunity to explore temporal changes in diet breadth during the transition between the Middle and Late Holocene and to examine regional dietary variation in this transitional zone between the Bay Area and Central Valley. To place CA-CCO-548 into a regional context, we compare stable isotope data from the site with nine roughly contemporaneous sites from Central California, including four Early San Francisco Bay Culture sites (CA-ALA-307; CA-SCL-928; CA-SOL-69; CA-SOL-315), three Windmill Pattern sites from the Sacramento-San Joaquin Delta (CA-SJO-68; CA-SJO-112; SJO-142), and two sites with aspects of both patterns (CA-CCO-637; CA-CCO-696). With the exception of CA-ALA-307 (a San Francisco bayshore site), the comparative sites are located adjacent to fresh- or brackish-water marsh communities, where terrestrial and freshwater resources were locally available. However, individuals from these sites may have had access to marine resources from the San Francisco Bay estuary either through trade or logistical forays.

Although the co-occurrence of diverse burial postures (e.g., flexed vs. extended) at CA-CCO-548 shows little variation through time, burial orientation (e.g., orientation of head with respect to true north) was quadrifid, with modes clearly oriented to the cardinal directions (N,

S, E, W). As well, orientations changed markedly in relative frequency throughout the occupation sequence, as did the spatial location of the active part of the cemetery (Eerkens et al., 2013b). Together the mortuary evidence shows that the cemetery was formed in a deliberate manner, with some burials showing traits similar to Windmill populations from the Central Valley (e.g., extended burials) and others with traits more similar to Lower Berkeley populations (e.g., flexed burials) from the Bay Area. Bennyhoff (1994a, 1994b) interpreted diverse mortuary patterns found within a single site as possible evidence of intermarriage between Lower Berkeley and Windmill peoples, which he referred to as the ‘Meganos Aspect’. Meganos sites are most common in the Delta region, but also appear in areas of the eastern and southern San Francisco Bay Area around 1500 cal BP (Bennyhoff, 1994a, 1994b; Wiberg, 1988; Eerkens et al., 2020). Like at CA-CCO-637 and CA-CCO-696, the mortuary patterns at CA-CCO-548 are intriguing because they suggest cultural interaction between the Bay Area and Central Valley (Wiberg, 2010:336–337).

### 4. Paleodietary reconstruction

Stable carbon and nitrogen isotope analysis has a long history in archaeology, with the first applications in the late 1970s and early 1980s focused on the introduction of maize into the diet of Native Americans in the eastern United States (van der Merwe and Vogel, 1978; Vogel and van der Merwe, 1977), as well as distinguishing between marine and terrestrial diets (Chisholm et al., 1982, 1983; Keegan and DeNiro, 1988; Lovell et al., 1986; Schoeninger et al., 1983; Tauber, 1981; Walker and DeNiro, 1986). Bulk stable carbon and nitrogen isotope analysis of an organism’s tissues reflect the types of food resources consumed during life (DeNiro and Epstein, 1978, 1981; Fry, 2006), and thus provide a means from which to reconstruct various aspects of human paleodiet, such as subsistence transitions, regional dietary patterns, dietary inequality, infant and childhood diets, and the relationship between diet and health (Katzenberg, 2008; Reitsema, 2013; Schwarcz and Schoeninger, 1991).

Stable carbon and nitrogen isotope analysis has been applied in a number of archaeological settings to examine the relative contribution of marine versus terrestrial resources to the diet (Ambrose et al., 1997; Chisholm et al., 1982, 1983; Coltrain, 2009; Coltrain et al., 2004; Keegan and DeNiro, 1988; Richards and Hedges, 1999; Schoeninger et al., 1983; Walker and DeNiro, 1986). Previous isotopic research in California has focused on human paleodiets along the Santa Barbara Channel of southern California (Goldberg, 1993; Harrison and Anne Katzenberg, 2003; Walker and DeNiro, 1986), Monterey Bay (Jones, 1996; Newsome et al., 2004), the San Francisco Bay Area (Bartelink, 2009; Beasley et al., 2013; Gardner et al. 2018), and the Sacramento-San Joaquin Delta region (Bartelink, 2006; Eerkens et al., 2013a).

#### 4.1. Stable carbon and nitrogen isotope analysis

Plant photosynthetic pathways differ based on the mechanism they use to fix atmospheric CO<sub>2</sub>, and reflect adaptive mechanisms for maximizing efficiency of carbon fixation in different environments (Heaton, 1999; O’Leary, 1981, 1988). Thus, stable isotope ratios in plants and the organisms that consume them will vary based on photosynthetic pathway utilized. Most of the earth’s vegetation consists of plants which use a C<sub>3</sub> metabolism, such as trees, shrubs, legumes, and the majority of grasses and tubers found in temperate regions (O’Leary, 1981, 1988). C<sub>3</sub> plants discriminate more against <sup>13</sup>C during carbon fixation compared to plants which use a C<sub>4</sub> metabolism, resulting in plant tissue δ<sup>13</sup>C values that average  $-26.7 \pm 2.7\text{‰}$  (1SD) (n = 370) (Cerling et al., 1998:163). C<sub>4</sub> plants comprise a small amount of the earth’s vegetation, and include tropical grasses found in hot and arid regions, including cultigens such as maize, millet, amaranth, sorghum, and sugarcane. These plants are enriched in <sup>13</sup>C compared to <sup>12</sup>C resulting in higher δ<sup>13</sup>C values that average  $-12.5 \pm 1.1\text{‰}$  (1SD) (n = 455) (Cerling



et al., 1998:163). Crassulacean acid metabolism (CAM) plants include desert adapted cacti and succulents that represent both carbon fixation pathways during photosynthesis, resulting in values that overlap with both  $C_3$  and  $C_4$  plants (O'Leary, 1988). In estuarine ecosystems carbon derives from dissolved bicarbonate, submerged aquatic plants, marine algae, phytoplankton, and freshwater inputs containing organic matter (Fry, 2006). Carbon isotope values from marine ecosystems often overlap with  $C_4$  plants (Ambrose, 1993; Ambrose et al., 1997; Chisholm et al., 1982; Schoeninger and DeNiro, 1984), and are typically 6–7‰ higher relative to organisms from  $C_3$  ecosystems (Schoeninger, 1995).

Dietary analyses using stable carbon isotopes often compare  $\delta^{13}C$  values of collagen with the carbonate in bioapatite to examine the contributions of the protein and non-protein components of the diet. The protein routing model is based on controlled feeding experiments using rodents and pigs, which have demonstrated that bone collagen is synthesized mainly from ingested protein, whereas bioapatite is derived from the whole diet, reflecting all dietary macronutrients (i.e., carbohydrates, lipids, and protein not used by an organism during tissue synthesis) (Ambrose and Norr, 1993; Howland et al., 2003; Passee et al., 2005; Tieszen and Fagre, 1993). Indeed, a study by Fernandes et al. (2012) estimates that 74 percent of the carbon in collagen derives from dietary protein, consistent with the protein routing model. Kellner and Schoeninger (2007), later revised in Frohle et al. (2010), developed a model that uses the isotopic values of carbon from collagen and bioapatite to model the protein and energy sources in the diet. Isotope values plotted against these protein lines provide a more accurate reconstruction of diet as the position along the line indicates the energy source ( $C_3$ ,  $C_4$ , or mixed) based on bioapatite  $\delta^{13}C$  values.

Stable nitrogen isotopes of bone collagen show a 2–4‰ stepwise increase with each trophic level, and thus can be used to reconstruct the food webs of primary producers, herbivores, carnivores, and omnivores within an ecosystem as well as distinguish between terrestrial and marine resource consumers (Ambrose, 1986, 1991; DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Schoeller, 1999; Schoeninger and DeNiro, 1984). Atmospheric  $N_2$  constitutes > 99 percent of exchangeable nitrogen, which is incorporated into most plants through inorganic ammonium and nitrates in soils (Ambrose, 1986, 1991; Heaton, 1987). Terrestrial plants that directly obtain  $N_2$  from the soil have elevated  $\delta^{15}N$  values, whereas leguminous plants have values closer to the standard of AIR (0‰) (Heaton, 1987; Shearer and Kohl, 1986; Virginia and Delwiche, 1982).  $\delta^{15}N$  values in terrestrial plants may be elevated from source values in soils from saline and arid environments, as well as coastal and estuarine margins due to fixation with nitrogen-enriched saline soils and sea spray effects (Ambrose, 1993; Heaton, 1987; Sealy et al., 1987). Plants from marine ecosystems typically have  $\delta^{15}N$  values elevated by ~4‰ compared to terrestrial plants (Ambrose, 1993), and marine vertebrates typically have  $\delta^{15}N$  values that are 6–8‰ higher than terrestrial vertebrates from similar trophic levels (Schoeninger, 1995:85). The higher  $\delta^{15}N$  values in marine ecosystems results from longer food chains with a greater number of trophic levels (Schoeninger, 1995). In human societies,  $\delta^{15}N$  values are often biased towards animal protein consumption since meat is composed of 85 to 90 percent protein, whereas plants contain only 10 to 25 percent protein (Ambrose et al., 2003:219).

#### 4.2. Central California food web

California's Central Valley comprises the Sacramento Valley to the north and the San Joaquin Valley to the south, which is bisected by the Mokelumne River. The Sacramento River and San Joaquin Rivers merge at the Delta in the mid-Central Valley, and drain into the estuary of the San Francisco Bay. Mixing of freshwater and saltwater near the mouth of the estuary creates a brackish, marshy environment with slow moving creeks and sloughs. The area around CA-CCO-548 consists of valley grassland and freshwater marsh, with brackish and riparian woodland habitats in the vicinity (Schoenherr, 1992). The local

environment supported a variety of mammal species, including artiodactyls (pronghorn, mule deer, and elk), lagomorphs (hare, jackrabbit, and cottontail rabbit), ground squirrel, river otter, coyote, raccoon, bobcat, mountain lion, beaver, and grizzly bear (Hildebrandt and Carpenter, 2006; Schoenherr, 1992). Additional fauna included migratory waterfowl (e.g., ducks, geese, and swans), freshwater resident fishes (e.g., minnows and suckers), anadromous fish species (e.g., salmon and sturgeon), and freshwater mussels (Gobalet et al., 2004; Moyle, 2002; Schoenherr, 1992). Insects (e.g., grasshoppers, army worms, yellow jacket larvae) were also present and likely to have served as food resources, but are rarely preserved in the archaeofaunal record (Jacknis, 2004; Lightfoot and Parrish, 2009).

A diversity of wild plant foods was exploited within interior Central California, including plants requiring extensive processing such as acorn, buckeye, and wild cherry (Wohlgeuth, 2004, 2010a:60). Gray pine, sugar pine, bay, hazel, and walnuts were less important resources, but contributed to the diet as supplementary foods. Pine nuts, in particular, are of interest here as pines grow well on hillsides and soils with poor water retention, a process that can lead to a slight enrichment in  $^{13}C$  over  $^{12}C$  compared to plants that are not water-stressed (e.g., Picon et al., 1996; Van de Water et al., 2002). Although pine nuts do not occur locally, they could be acquired through trade with neighboring groups from the Sierra Nevada. Plants yielding small seeds, such as spring-ripening red maids, chia, farewell-to-spring, goosefoot, tarweed, and native barley were important dietary items, as were a variety of terrestrial roots, such as the *Brodiaea* group and the Mariposa lily (Wohlgeuth, 2004, 2010a:60). In general, seeds were harvested in the late spring to early summer, acorns in the fall, and terrestrial roots in late winter to early spring (Bettinger and Wohlgeuth, 2006; Wohlgeuth, 2010a).

In pre-contact Central California, carbon was derived mainly from terrestrial  $C_3$ , marine/estuarine, or freshwater ecosystems (Bartelink, 2006). CAM plants were not locally available, and local  $C_4$  plants, such as saltgrass and cordgrass, would not have been consumed in sufficient quantities to affect stable isotope values. Saltgrass was ashed to create salt as a food flavoring and trade item for some Central California groups (Bettinger and Wohlgeuth, 2006:280; Strike, 1994:53), whereas cordgrass was primarily used as an infant laxative among at least one group in southern California (Strike, 1994:149). Thus, we interpret variation in stable carbon isotope ratios as a measure of the relative importance of marine versus terrestrial resources to the diet.

## 5. Materials and methods

### 5.1. Sex and age

Demographic analysis on the CA-CCO-548 population was performed at San Francisco State University (Griffin et al. 2010). The majority of skeletons were highly fragmented, thus sex and age estimations were conducted using all available indicators, following criteria outlined in Buikstra and Ubelaker (1994). Very few subadults were excavated from the site (~8% of the burial sample), suggesting that infants and children remains did not preserve or were typically buried in a different location than adults (Wiberg, 2010). However, we include a small sample of subadults (n = 10, 5–11 years of age) in the analysis.

### 5.2. Isotope sample preparation

Prior to reburial of all human remains, permission was granted to sample bone from 248 of the 480 excavated burials by the project's Most Likely Descendant (a person assigned by the State of California's Native American Heritage Commission), coauthor Ramona Garibay of both the Bay Miwok and the Ohlone Tribes. Sample selection for stable isotope analysis focused on the more complete burials that showed macroscopic signs of preservation, and preference was given to individuals with available sex and age data. Bone samples were processed

in the Stable Isotope Preparation Laboratory at California State University, Chico. A 2- to 3-gram segment of bone was sampled and then separated for preparation of the collagen and bioapatite fractions. The proteinaceous fraction (hereafter referred to as “collagen”) was extracted by soaking samples in a 0.25 M hydrochloric acid solution until demineralized (Ambrose, 1993; Schwarcz and Schoeninger, 1991). Collagen pseudomorphs were soaked for 24 h in a 0.125 M sodium hydroxide solution to remove humic contaminants. Samples were solubilized in pH  $\approx$  3 water and then freeze-dried in glass scintillation vials. Collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  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 at the University of California Davis. Analytical precision was  $\pm 0.2\text{‰}$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Stable carbon and nitrogen isotopes are reported in permil (‰, parts per thousand) relative to Vienna Pee Dee Belemnite and AIR, respectively (Schoeller, 1999).

To extract bioapatite, bone samples were ground into a powder using a steel mortar and pestle, and sieved through fine mesh screen (200  $\mu\text{m}$ ). Collagen was removed with a 48 h treatment of 1.5 percent sodium hypochlorite solution, replaced once at 24 h using a 0.04 ml solution/mg sample ratio (Koch et al., 1997). Samples were then treated with a 1.0 M acetate-buffered (pH  $\approx$  4.5) acetic acid solution for 24 h (replaced once at 12 h) to remove soluble contaminants using the same solution-to-sample ratio.  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values were simultaneously measured using a GVI Optima Stable Isotope Ratio Mass Spectrometer at the Stable Isotope Laboratory, Department of Earth and Planetary Sciences, University of California, Davis. External precision for  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  is  $\pm 0.07$  and  $\pm 0.04$ , respectively, based on multiple analyses of the calcite standards NBS-19 and UCD-SM92. Stable carbon and oxygen isotopes of bioapatite are reported relative to Vienna Pee Dee Belemnite (Schoeller, 1999).

### 5.3. Evaluation of diagenesis

Collagen integrity was evaluated based on visual examination, percent collagen yield, and atomic C/N ratios (van Klinken, 1999). Collagen yields above 1 percent are considered to yield reliable stable isotope values (Ambrose, 1990; van Klinken, 1999). DeNiro (1985) established that the C/N ratio should be between 2.9 and 3.6 for viable samples. Of the original 248 burials sampled, 198 (79.8%) met these criteria and are considered to have reliable *in vivo* bone collagen values.

Bioapatite sample quality was evaluated using Fourier transform infrared (FTIR) spectroscopy to calculate the carbonate to phosphate ratio (C/P) and infra-red splitting factor (IR-SF), following standard methods, to produce spectra from a KBr pellet using a hydraulic press (Shemesh, 1990; Surovell and Stiner, 2001; Weiner and Bar-Yosef, 1990). Spectra were collected with a Nicolet Magna 500 FTIR analyzer from 4000 to 400  $\text{cm}^{-1}$ . Pearson's *r* correlation test found a strong, negative linear relationship between the IR-SF and C/P ratio ( $r = -0.874$ ,  $p < 0.001$ ;  $r^2 = 0.764$ ), suggesting varying levels of postmortem alteration to bone bioapatite samples. Diagenesis to the mineral fraction results from changes to the lattice structure, causing lower C/P and higher IR-SF calculated from FTIR spectra (Berna et al., 2004; Smith et al., 2007). Studies of modern unaltered bone suggest C/P values below 0.2 and above 0.5 should be considered suspect given that C/P values below 0.1 often demonstrate accelerated collagen loss (Garvie-Lok et al., 2004; Nielsen-Marsh and Hedges, 2000; Smith et al., 2007). Previous studies on archaeological bone samples concluded that samples that contain a measurable amount of extractable collagen tend to have IR-SF values  $< 3.3$ , while samples with no surviving collagen fraction frequently have IR-SF values higher than 3.4, but modern bone can result in values up to 3.5 (Beasley et al., unpublished results; Berna et al., 2004; Smith et al., 2007; Trueman et al., 2008). Based on modern bone studies, bioapatite samples are suspect if IR-SF values  $\geq 3.5$  and C/P values  $\geq 0.5$  or  $\leq 0.15$ . Of the 232 samples with calculated IR-SF and C/P values, 169 samples (72.8%) have IR-SF values  $\geq 3.5$  and 87

(37.5%) have C/P values  $\leq 0.15$ .

However, correlation tests between the IR-SF and bioapatite  $\delta^{13}\text{C}$ , and the C/P ratio and bioapatite  $\delta^{13}\text{C}$  found only a weak relationship (IR-SF:  $r = 0.293$ ,  $p < 0.001$ ,  $r^2 = 0.086$ ; C/P ratio:  $r = -0.210$ ,  $p = 0.001$ ,  $r^2 = 0.044$ ). Although higher crystallinity is significantly correlated with carbonate loss, indicating sample diagenesis, neither indicator is strongly correlated with bioapatite  $\delta^{13}\text{C}$  values. Thus, we assume that the 232 processed bioapatite samples have retained biogenic values.

### 5.4. AMS sample preparation

Chronometric dating of a sub-sample of the burial population was conducted at two AMS laboratories. Beta Analytic Radiocarbon Dating performed AMS dates on human bone from 23 of the burials that were included within our stable isotope sample (Wiberg, 2010). In addition, 125 human bone samples were prepared at the Lawrence Livermore Laboratory's Center for Accelerator Mass Spectrometry. Samples for AMS dating were pre-screened by selecting bones that scored high on a visual quality scale, that had acceptable collagen yields (generally  $> 5\%$ ), and that had atomic C/N ratios between 2.9 and 3.6. A linear mixing model was used to calculate the ‘% marine’ in the sample to adjust the AMS dates. We used a terrestrial end member for  $\text{C}_3$  plants ( $-27.0\% + 5\%$  diet-to-tissue offset =  $-22.0\%$ ) and a marine end member for sea mammals ( $-11.0\% + 5\%$  diet-to-tissue offset =  $-6.0\%$ ), based on the minimum and maximum floral and faunal  $\delta^{13}\text{C}$  values from Central California reported in Bartelink (2006: 141, 147–150). AMS dates were calibrated using the NH Mixed Marine calibration curve in Calib. 6.1.1 (Stuvier and Reimer, 1993), with a reservoir correction ( $\Delta R$ ) of  $365 \pm 50$ . Details on the AMS calibrations are provided in Supplement 1.

## 6. Results

### 6.1. General results

The raw stable isotope values and sample quality indicators for each individual are presented in Supplemental 1. Table 1 presents descriptive statistics for all individuals and summaries by sex. For bone collagen,  $\delta^{13}\text{C}$  values vary from  $-17.6$  to  $-21.8\text{‰}$ , with a mean of  $-19.8 \pm 0.6\text{‰}$  (1 SD). For  $\delta^{15}\text{N}$ , values vary from 7.3 to 13.9‰, with a mean of  $9.5 \pm 1.0\text{‰}$ . In general, the CA-CCO-548 population shows little variation for both collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , suggesting individuals consumed a similar range of food resources. Fig. 2 plots the relationship between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of all individuals, compared with locally available dietary resources, and indicates that the protein component of the diet was derived primarily from terrestrial  $\text{C}_3$  and freshwater fish resources, with a small dietary contribution coming from marine protein. We used a linear mixing model for correcting AMS dates for ‘% marine’ to approximate the relative contributions of terrestrial versus marine protein to the diet. The model provides a mean estimate of  $86 \pm 4$  percent (1 SD; 73–99% min–max) of dietary protein derived from terrestrial  $\text{C}_3$ /freshwater resources versus  $14 \pm 4$  percent (1 SD; 1–27% min–max) of dietary protein derived from marine resources. For bone bioapatite,  $\delta^{13}\text{C}$  values vary from  $-10.3$  to  $-19.5\text{‰}$ , with a mean of  $-14.0 \pm 1.5\text{‰}$  (Table 1). The bioapatite  $\delta^{13}\text{C}$  values indicate that the non-protein component of the diet (carbohydrates and lipids) was depleted in  $^{13}\text{C}$ , consistent with heavy consumption of  $\text{C}_3$  plants (e.g., acorns, small seeds, and geophytes).

Fig. 3 plots the relationship between bioapatite and collagen  $\delta^{13}\text{C}$  using the Froehle et al. (2010) carbon isotope model. Values for most individuals fall on or near the  $\text{C}_3$  protein line. To assess whole diet,  $\delta^{13}\text{C}$  of collagen and bioapatite indicate that the main protein contribution is from  $\text{C}_3$  resources, and most individuals obtained their non-protein energy sources (i.e., carbohydrates and lipids) from terrestrial  $\text{C}_3$  resources. Individuals whose values plot slightly above the  $\text{C}_3$  protein line

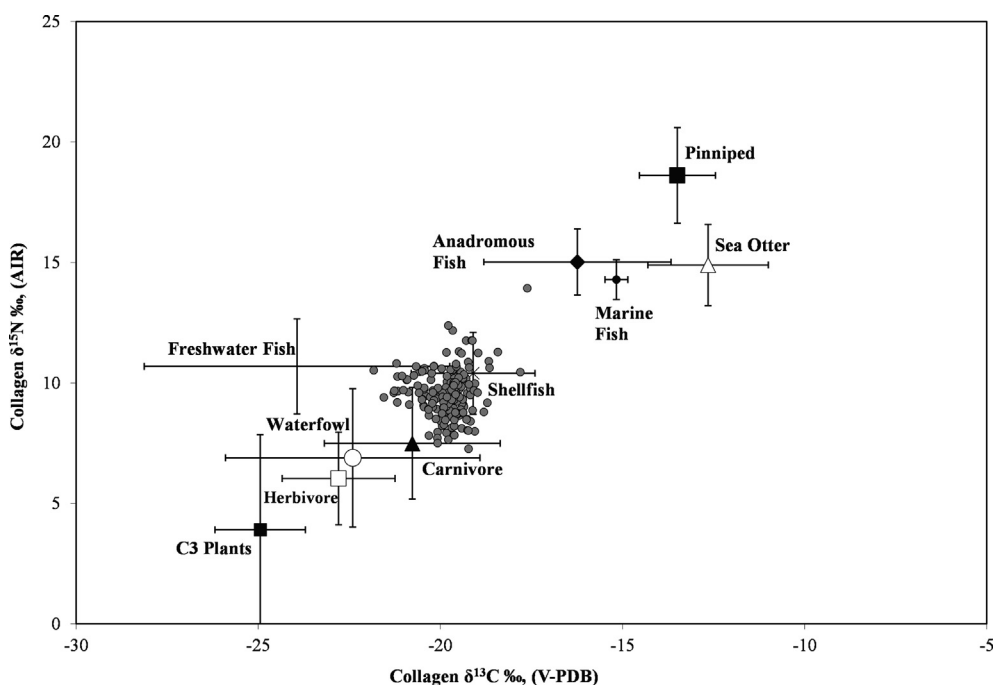
**Table 1**  
Descriptive statistics for isotope and sample quality data in the CA-CCO-548 population.

	$\delta^{13}\text{C}$ Col	$\delta^{15}\text{N}$ Col	C/N Ratio	Col Yield (%)	$\delta^{13}\text{C}$ Apa	$\delta^{18}\text{O}$ Apa	IR-SF	C/P Ratio
Total mean	-19.8	9.5	3.3	5.3	-14.0	-5.6	3.73	0.16
s.d.	0.6	1.0	0.1	4.0	1.5	1.0	0.35	0.05
N	198	198	198	198	233	233	248	248
Female mean	-19.8	9.3	3.3	6.1	-14.2	-5.3	3.6	0.17
s.d.	0.6	1.1	0.1	3.8	1.4	1.0	0.3	0.04
N	69	69	69	69	79	79	80	80
Male mean	-19.8	9.6	3.3	5.3	-14.0	-5.7	3.77	0.16
s.d.	0.5	1.0	0.1	4.4	1.4	1.0	0.36	0.05
N	82	82	82	81	99	99	107	107
Indet. mean	-20.0	9.6	3.3	4.2	-13.8	-5.9	3.8	0.15
s.d.	0.7	0.8	0.1	2.7	1.7	1.1	0.39	0.05
N	40	40	40	41	47	47	53	53

obtained a small amount of dietary protein from marine resources, such as anadromous fish or shellfish.

### 6.2. Temporal comparisons

Fig. 4 plots the relationship between calibrated median probability AMS dates (BP) and collagen  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and bioapatite  $\delta^{13}\text{C}$  values ( $n = 145$ ). The plot does not show a strong linear relationship between AMS dates and collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Fig. 4a–b), suggesting stability in dietary protein sources through time. However, AMS dates do show a moderate positive correlation with bioapatite  $\delta^{13}\text{C}$  values (Pearson's  $r = 0.463$ ,  $p < 0.001$ ,  $r^2 = 0.214$ ). Fig. 4c shows that this relationship is even stronger when the five earliest dates (spanning 5420–4038 cal. B.P.) are excluded (Pearson's  $r = 0.496$ ,  $p < 0.001$ ,  $r^2 = 0.239$ ). These early dates predate the main burial sample by several hundred years. The relationship between AMS and bioapatite  $\delta^{13}\text{C}$  indicates that the non-protein sources of the diet shifted toward more  $^{13}\text{C}$ -depleted vegetal resources between 4000 and 3000 cal. B.P., a pattern that is more pronounced in males than females ( $\sigma$ , Pearson's  $r = 0.543$ ,  $p < 0.001$ ,  $r^2 = 0.311$ ;  $\phi$ , Pearson's  $r = 0.436$ ,  $p < 0.001$ ,  $r^2 = 0.190$ ).



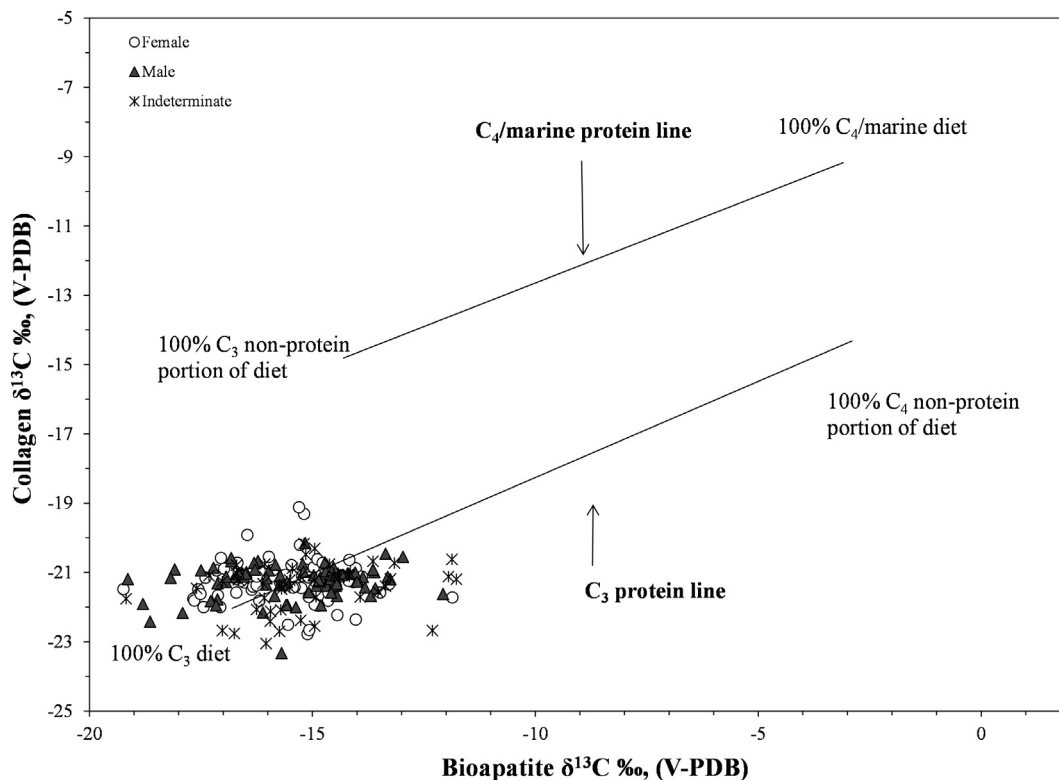
**Fig. 2.** Relationship between the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in prehistoric human bone collagen samples from CA-CCO-548 compared to plant and meat food resource values. Symbols represent sample means and error bars represent 1 SD. We include data from both the San Francisco Bay Area and the Central Valley to include all possible dietary items that could have been exploited by the CA-CCO-548 population. Food web is based on both archaeological and modern fauna (reported in Bartelink, 2006: 141, 147–150 and unpublished data), as well as modern plants. Faunal carbon isotope data have been corrected for diet to tissue offsets ( $-2.4\text{‰}$  for herbivores and waterfowl,  $-3.7\text{‰}$  for fish).

### 6.3. Sex comparisons

Comparisons for all individuals by sex indicate that males and females have very similar dietary isotope values. For example, males and females have identical mean collagen  $\delta^{13}\text{C}$  values, and differ only by  $0.3\text{‰}$  for  $\delta^{15}\text{N}$  and  $0.2\text{‰}$  for bioapatite  $\delta^{13}\text{C}$ . An independent-samples  $t$ -test found a significant difference only for  $\delta^{15}\text{N}$  ( $p = 0.049$ ) suggesting a slight mean difference, in the suite of protein males consumed relative to females.

### 6.4. Regional comparisons

We next compare  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  results from CA-CCO-548 with isotopic data from nine contemporaneous archaeological sites (ca. 4000–2900 cal. B.P.) to provide a regional context for Early Period diets within Central California (Fig. 5a, 5b). These sites are located within a variety of environments, including the San Francisco bayshore and freshwater and brackish marshy areas in and around the Sacramento-San Joaquin Delta (see Fig. 1). Table 2 presents descriptive statistics for each site. While the sites from the California Delta show substantial overlap, CA-ALA-307, located along the eastern margin of the San Francisco Bay, shows significantly elevated  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values



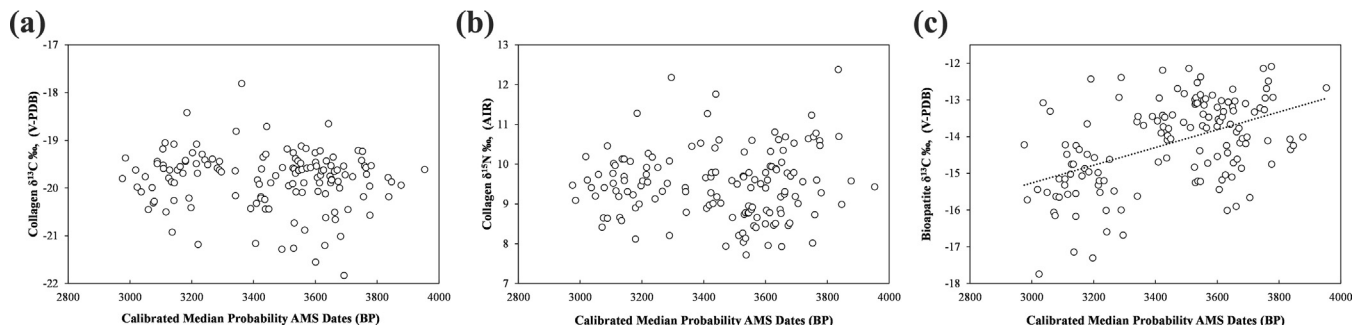
**Fig. 3.** Relationship between bioapatite and collagen  $\delta^{13}\text{C}$  values of CA-CCO-548 samples compared with Froehle et al. (2010) model. To account for atmospheric depletion in  $^{13}\text{C}$  due to fossil fuel burning (i.e., Suess Effect), 1.5‰ was subtracted from each  $\delta^{13}\text{C}$  value to align prehistoric individuals with the modern fauna based model.

reflecting reliance on high trophic level marine protein. An ANOVA test for  $\delta^{13}\text{C}$  only found significant differences for comparisons of CA-CCO-548 with CA-ALA-307 and CA-SJO-112 (Bonferroni,  $p < 0.001$ ); CA-CCO-637, CA-CCO-696, and CA-SCL-928 are excluded due to small sample size.  $\delta^{15}\text{N}$  values are significantly different for comparisons of CA-CCO-548 with CA-ALA-307 and CA-SJO-68 (Bonferroni,  $p < 0.001$ ). A similar dietary difference in whole diet (bioapatite  $\delta^{13}\text{C}$ ) was observed between the eight Delta sites versus the San Francisco bayshore (Fig. 6). Values for most individuals from the Delta fall on or near the  $\text{C}_3$  protein line, overlapping significantly with the CA-CCO-548 population. CA-ALA-307, located on the bayshore, shows substantial marine protein contributions to the diet.

**7. Discussion**

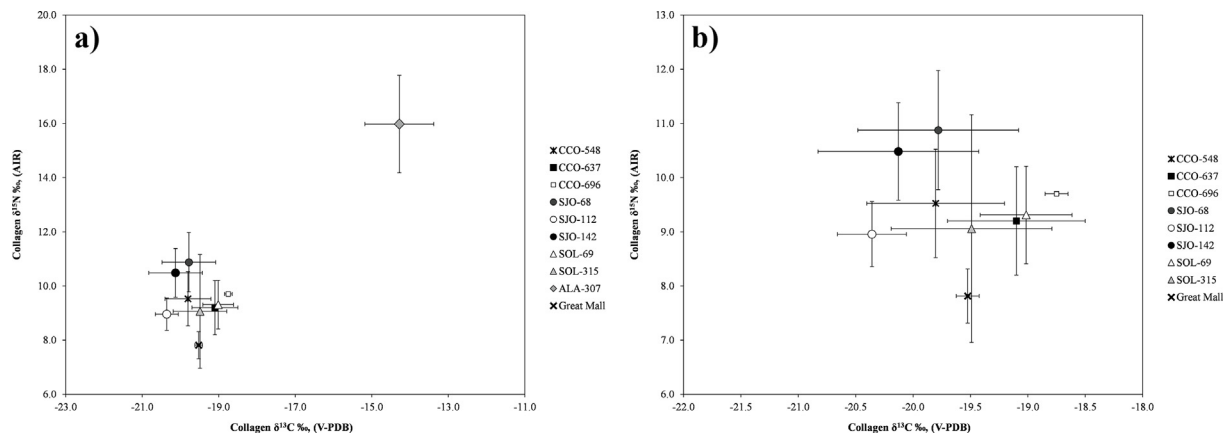
Stable isotope results provide an independent but complementary line of evidence regarding the relative importance of different food resources to the CA-CCO-548 population. Of the various foods available

to site inhabitants, freshwater fish compare most favorably to observed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the human bone collagen. The mean  $\delta^{13}\text{C}$  value for freshwater fish flesh is  $-23.9\text{‰}$ , 4.1‰ lower than the mean value of  $-19.8\text{‰}$  for human bone collagen. However, when accounting for the expected 5‰ diet-to-tissue offset reported in the literature (Ambrose and Norr, 1993; Tieszen and Fagre, 1993), this value is close to the human value. Likewise, the mean  $\delta^{15}\text{N}$  of freshwater fish is 7.7‰, which would appear in human bone collagen as approximately 10.7‰ due to the trophic level effect. Terrestrial herbivore and waterfowl flesh, in contrast, show mean  $\delta^{13}\text{C}$  values around  $-22\text{‰}$ , which would produce human bone collagen values around  $-17\text{‰}$ , slightly higher than even the most elevated  $\delta^{13}\text{C}$  value in the CA-CCO-548 population. Herbivores and waterfowls, with mean  $\delta^{15}\text{N}$  values of 6.0‰ and 6.9‰, respectively, would, however, also produce bone collagen values consistent with much of the population. Finally, estuarine shellfish, with a mean  $\delta^{13}\text{C}$  value of  $-19.1\text{‰}$ , much higher than the  $\delta^{13}\text{C}$  values for the population; the mean  $\delta^{15}\text{N}$  of 10.4‰ for shellfish would, however, be



**Fig. 4.** Plot of the relationship between calibrated median probability AMS dates in BP and (a) collagen  $\delta^{13}\text{C}$  values; (b)  $\delta^{15}\text{N}$  values; and (c) bioapatite  $\delta^{13}\text{C}$  values. Bioapatite  $\delta^{13}\text{C}$  values regressed against AMS dates (Pearson’s  $r = 0.496$ ,  $p < 0.001$ ,  $r^2 = 0.239$ ;  $n = 140$ ). Five dates  $> 4000$  B.P. excluded.





**Fig. 5.** a) Relationship between the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in prehistoric human bone collagen samples from CA-CCO-548 compared to other Central California sites. Symbols represent sample means and error bars represent 1 SD. b) Relationship between the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in prehistoric human bone collagen samples from CA-CCO-548 compared to other interior Central California sites (Note: site CA-ALA-307 is excluded from the plot to better visualize the patterns among the other sites).

**Table 2**

Comparison of stable isotope values from Early Period sites in Central California (Collagen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ).

Site		$\delta^{13}\text{C}$ Col	$\delta^{15}\text{N}$ Col	$\delta^{13}\text{C}$ Apa
CA-CCO-548	Total mean	-19.8	9.5	-14.0
	s.d.	0.6	1.0	1.5
	N	198	198	233
CA-CCO-637 <sup>a</sup>	Total mean	-19.1	9.2	-13.4
	s.d.	0.4	0.7	0.1
	N	3	3	2
CA-CCO-696 <sup>b</sup>	Total mean	-18.7	9.7	-13.3
	s.d.	0.1	0.0	1.4
	N	2	2	2
CA-SJO-68 <sup>c</sup>	Total mean	-19.8	10.9	-14.0
	s.d.	0.7	1.1	1.1
	N	13	13	13
CA-SJO-112 <sup>d</sup>	Total mean	-20.4	9.0	-12.4
	s.d.	0.3	0.6	1.2
	N	23	23	23
CA-SJO-142 <sup>c</sup>	Total mean	-20.1	10.5	-12.0
	s.d.	0.7	0.9	0.8
	N	10	10	10
CA-SOL-69 <sup>e</sup>	Total mean	-19.0	9.3	
	s.d.	0.4	0.9	
	N	6	6	
CA-SOL-315 <sup>e</sup>	Total mean	-19.5	9.1	-11.3
	s.d.	0.7	2.1	0.5
	N	17	17	14
CA-SCL-928 <sup>a</sup>	Total mean	-19.5	7.8	
	s.d.	0.1	0.5	
	N	3	3	
CA-ALA-307 <sup>c</sup>	Total mean	-14.3	16.0	-11.1
	s.d.	0.9	1.8	0.8
	N	18	18	18
All Sites	Total mean	-19.5	9.9	-13.6
	s.d.	1.5	1.9	1.7
	N	293	293	315

<sup>a</sup>CA-CCO-637, CA-SCL-928 (unpublished data produced by Eerkens); <sup>b</sup>CA-CCO-696 (Ralston et al., 2016); <sup>c</sup>CA-ALA-307, CA-SJO-68, CA-SJO-142 (Bartelink, 2006); <sup>d</sup>CA-SJO-112 (Barton et al., 2020); <sup>e</sup>CA-SOL-69, CA-SOL-315 (Eerkens et al., 2020)

consistent with the values for much of the CA-CCO-548 population. The linear mixing model estimated that protein from freshwater and  $\text{C}_3$  ecosystems contributed, on average, 86% to the overall diet (min-max, 73–99%) of the CA-CCO-548 population. Thus, marine food consumption is predicted to be fairly low, on average 14 percent of the protein budget (min-max, 1–27%). Most marine resources, such as shellfish and sea mammals, could not have been acquired within the vicinity of the site, but could have been obtained through trade with groups to the north or through logistical forays. For instance, marine resources could have been acquired 9 km to the north of CA-CCO-548, near the mouth of the San Francisco Bay estuary, where fresh water from the Delta mixes with ocean water. Salmon and sturgeon may have been available seasonally in more local water courses, but bones of these fishes are rare in the CA-CCO-548 deposit, comprising < 1% of identified fish bone (Wiberg 2010).

The bioapatite  $\delta^{13}\text{C}$  values further indicate that the non-protein component of the diet (i.e., carbohydrates and lipids) was depleted in  $^{13}\text{C}$ , consistent with heavy consumption of  $\text{C}_3$  plants (e.g., acorns, small seeds, and geophytes). This is especially evident in the bioapatite versus collagen  $\delta^{13}\text{C}$  plot in Fig. 6, which shows that both protein and non-protein sources largely derive from  $\text{C}_3$  and freshwater ecosystems. Furthermore, the data are consistent with general patterns identified at CA-CCO-548, such as the increase over time in acorn macrobotanical remains, and the abundance of groundstone (Wohlge-muth, 2010b; Stevens et al., 2009). Archaeobotanical analyses at CA-CCO-548 found that acorns were present throughout the temporal span of the site, but their use increased over time relative to small seeds (Wohlge-muth, 2010b). Surprisingly, higher-ranked plant resources, such as roots and tubers, were poorly represented at the site, despite their ubiquity in other interior Central California sites (Wohlge-muth, 2004, 2010a, 2010b).

The stable isotope results provide some support for resource intensification models in Central California. No significant diachronic change was found for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  within the site, suggesting continuity in the sources of dietary protein over time while the site was occupied. However, bioapatite  $\delta^{13}\text{C}$  does show a moderate correlation with time, indicating that the diet shifted toward greater reliance on  $^{13}\text{C}$ -depleted  $\text{C}_3$  plant resources by 3000 cal BP. This finding is consistent with macrobotanical analyses at the site that suggest an increased reliance on acorns and especially small seeds during occupation of the site, and decreased quantities of pine nuts. Although the data show significant inter-individual variation, this result provides support for the hypothesis that the CA-CCO-548 population increasingly intensified wild plant food exploitation over time.

Comparisons by sex indicated few differences in mean stable isotope values, although males had slightly elevated  $\delta^{15}\text{N}$  values (+0.3‰)

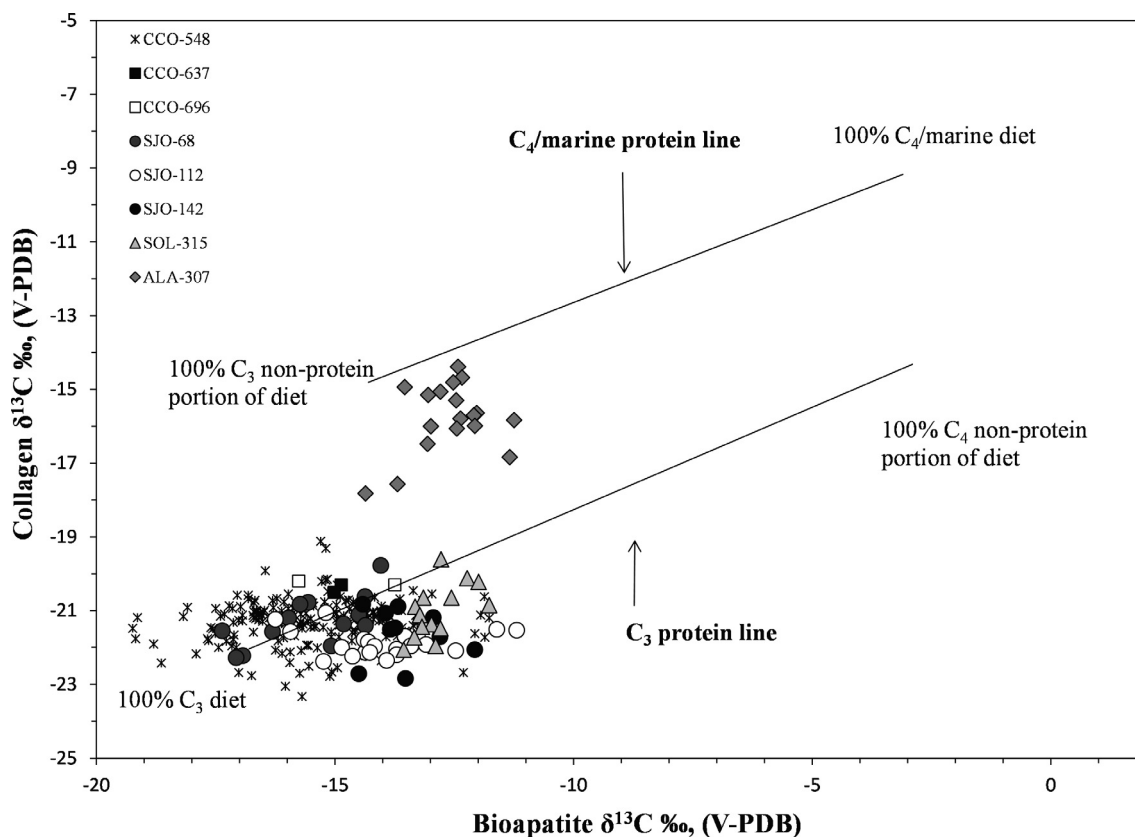


Fig. 6. Inter-site comparison of stable carbon isotopes of apatite and collagen for the CA-CCO-548. Note: Bioapatite data was not available for CA-SOL-69 and CA-SCL-928.

relative to females. In a previous study of the CA-CCO-548 population, sex differences were identified in dentinal collagen samples that corresponded to infant and early childhood diets (Eerkens and Bartelink, 2013). These patterns were interpreted as possible differences in parental investment, whereby male children were provisioned with higher trophic level foods than female children, or as a reflection of a sexual division of labor, where male children started accompanying their fathers and other men in hunting and fishing activities at an early age, and young females accompanied their mothers and other women on gathering forays. Self-provisioning was also considered as a possibility based on evidence from modern foraging societies that indicates children are capable of providing a portion of their own daily caloric requirements (Blurton Jones et al., 1997; Bird and Bliege-Bird, 2005; Bock, 2005; Hawkes et al., 1995). Sex differences in  $\delta^{15}\text{N}$  were most pronounced prior to weaning (2.5‰) and by 7–9 years of age, drop to 1.8‰ (Eerkens and Bartelink, 2013). Thus, it is interesting that by adulthood, human bone collagen differs only by 0.3‰. In light of the more pronounced differences in dentinal collagen during infancy and childhood, this small difference may in fact reflect slightly greater consumption of a higher trophic level food resource by male children, such as freshwater fish; however, it is also possible that bulk isotope analysis of bone collagen may include part of an adolescent dietary signature based on the slow turnover of cortical bone (Hedges et al., 2007; Hill, 1998; Tsutaya and Yoneda, 2013).

Comparisons of the CA-CCO-548 population with the nine other Early Period sites (ca. 4000–2900 B.P.) from Central California reveals broadly similar diets, with the exception of CA-ALA-307, which shows significantly elevated  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values reflecting a high trophic level marine emphasis, and CA-SCL-928, located just southeast of San Francisco Bay, which shows very low  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, indicating a greater emphasis on low-trophic level,  $\text{C}_3$ -based terrestrial resources.

Stable isotope data from sites within the Delta (CA-SJO-68, CA-SJO-

142, CA-SJO-112), and sites located to the northwest (CA-SOL-69, CA-SOL-315) and the southwest of the Delta (CA-CCO-637, CA-CCO-696), fall within the lower left corner of Fig. 5a, and reflect diets focused on protein from  $\text{C}_3$  terrestrial resources and freshwater fish. In particular, CA-SJO-68 and CA-SJO-142, located within the Delta, show the most elevated  $\delta^{15}\text{N}$  values, suggesting greater consumption of higher trophic level resources, such as freshwater fish. For the remaining sites (CA-CCO-548, CA-CCO-637, CA-CCO-696; CA-SOL-69, CA-SOL-315; CA-SJO-112), there is significant overlap in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. For the CCO and SOL sites in particular, lower  $\delta^{13}\text{C}$  and higher  $\delta^{15}\text{N}$  values suggest greater contribution from brackish water protein resources (see Eerkens et al., 2013a). Thus, consistent with our hypothesis, human paleodiets from these sites fall in between the freshwater environment of the Delta and the marine-oriented San Francisco Bay estuary. The bioapatite-collagen model confirms these patterns, and clearly indicates that non-protein resources derived largely from  $\text{C}_3$  resources. Our findings provide new perspectives on dietary variability during the Middle Holocene, and shed light on dietary patterns during the emergence of the sedentary hunter-gatherer populations of Central California.

## 8. Conclusion

This study used  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of bone collagen,  $\delta^{13}\text{C}$  of bone bioapatite, and a large series of AMS dates to investigate dietary variation in a large burial sample from CA-CCO-548 (Marsh Creek), a Middle Holocene site located near the Sacramento-San Joaquin Delta in Central California. Human bone collagen results reveal that freshwater fish, terrestrial game, and wild  $\text{C}_3$  plants (e.g., acorns and small seeds) were the most important food resources, consistent with archaeofaunal and paleobotanical findings from the site. The results further confirm that marine resources were of minor dietary importance and could have

been acquired from brackish areas near the mouth of the San Francisco Bay estuary located 9 km away or through trade. Contrary to our expectation,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values do not show significant temporal change between 5420 and 2975 cal BP, and instead reflect long-term stability in dietary protein sources. However, human bone bioapatite  $\delta^{13}\text{C}$  values show a significant temporal shift over time, reflecting greater reliance on  $\text{C}_3$  plant resources, such as acorns and small seeds. This increased reliance on  $\text{C}_3$  plant resources provides support for resource intensification models, which predict higher investment in acorn storage economies, seed harvesting, and greater exploitation of lower-ranked protein resources. Comparisons by sex indicated few differences in mean stable isotope values, which contrasts with previously published data from dental serial samples from the site that showed greater sex differences in childhood diet. Human paleodiets at CA-CCO-548 are broadly similar to contemporaneous groups located to the south and northwest, but are distinct from groups within the Delta and San Francisco Bay Area. These results contribute to the growing picture of dietary diversity across the Middle and Late Holocene, a period marked by significant population growth and increased sedentism.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jasrep.2019.102182>.

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