CARBON AND NITROGEN ISOTOPE ANALYSIS OF BONE COLLAGEN AT *AYTTAKIŠ ʻÉETE HIRAMWIŠ TRÉPAM-TAK* (CA-ALA-677/H)

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As part of a collaborative project between the Muwekma Ohlone Tribe, Far Western Anthropological Research Group, Caltrans, and University of California, Davis, we report new carbon and nitrogen stable isotope results on bone collagen from Ohlone ancestral remains from Ayttakiš ʻÉete Hiramwiš Trépam-tak (CA-ALA-677/H), a Middle-Late Transition and Late Phase 1 settlement in the Sunol Valley of central California. Stable isotope information from 19 individuals allows for dietary reconstruction on an individual-by-individual basis. We show that diets at the site were dominated by low trophic-level terrestrial foods, or plants, with little input from San Francisco Bay or other aquatic environments. There is marked homogeneity among individuals buried at the site, and little evidence for differences by sex or archaeological time. Slight changes in diets with age are apparent, with males decreasing meat intake with age and females increasing low trophic-level foods with slightly higher $\delta^{13}C$ values, likely a type of plant that grows away from permanent sources of water, such as pine nuts. Together, the data suggest a high degree of food sharing within the site.

Stable isotope analysis of human skeletal materials provides information about past diet (i.e., general categories of what people were eating. Significantly, unlike midden constituents that inform about diet at the scale of a population, stable isotope analysis of human skeletal materials provides paleodietary information at the scale of an individual.

This report describes carbon and nitrogen stable isotope analysis of bone collagen from 19 individuals from archaeological site *Ayttakiš ʻÉete Hiramwiš Trépam-tak* (CA-ALA-677/H). We use carbon isotopes as a tracer of access to bayshore and marine resources, including in interior locations, and to better understand the exploitation of anadromous and brackish water fishes (e.g., salmon, sturgeon). We use nitrogen isotopes as a tracer of trophic position in the local food chain, especially plant versus game versus aquatic foods.

Ayttakiš ʻÉete Hiramwiš Trépam-tak (meaning "Place of Woman Sleeping Under the Pipe" as named by the Muwekma Ohlone Tribe Language Committee) is an ancestral Native American Ohlone settlement situated in the Sunol Valley of the southeast San Francisco Bay region (Figure 1; see Leventhal et al. 2017). Planned Caltrans highway widening construction to improve safety led to a Caltrans funded archaeological mitigation excavation of the site. The Far Western Anthropological Research Group conducted the excavations in partnership with the Muwekma Ohlone Tribe of the San Francisco Bay area, who were appointed Most Likely Descendant for the project by the state's Native American Heritage Commission. The Tribe participated in all aspects of fieldwork and were the primary excavators of all burials. All analytical studies of ancestral remains were reviewed and approved by tribal leadership, and the tribe partnered with the Far Western research team and University of California, Davis (UCD), to conduct these investigations of their ancestors.

Figure 1. Map of central California, with location of Ayttakiš ʻÉete Hiramwiš Trépam-tak.

The site is an extensive (5,200 m^2) sedentary village consisting of a thick deposit of cultural material, along with an associated cemetery (Byrd et al. 2022). Archaeological investigation of 8.1% of the site recovered a wide range of Native American cultural items, including more than 3,300 artifacts, numerous food remains, four features, and 16 burials containing 19 individuals. The site dates from 755 to 515 cal BP based on 25 radiocarbon dates from features, burials, and generalized site deposits. The settlement therefore started in last third of the Middle-Late Transition (MLT) period through a good portion of the Late 1 period. The ancestral burials were dated to a more limited time range (720 to 597 cal BP).

APPROACH

With permission from the Muwekma Ohlone tribal leadership, we extracted and measured carbon and nitrogen isotope ratios from bone collagen. Because dietary protein (versus carbohydrates and fats) is differentially routed to collagen, these analyses inform mainly on the sources of protein in the diets of individuals (Ambrose and Norr 1993; Kellner and Schoeninger 2007; Tieszen and Fagre 1993).

In dietary studies around the world, carbon isotopes ($^{13}C/^{12}C$, expressed as $\delta^{13}C$, see below) often provide an estimate of the consumption of C_3 versus C_4 plants. The majority of plants around the world are C_3 plants, producing a three-carbon molecule during the fixation of atmospheric carbon. This method of photosynthesis discriminates against the heavier ^{13}C, resulting in $\delta^{13}C$ values between -30‰ and -22‰. By contrast, a small number of plants produce a four-carbon molecule (C_4) and produce tissues with $\delta^{13}C$ values typically between -16‰ and -10‰ (i.e., about 12-14‰ higher). While the number of C_4 photosynthesizers is low, several important crop plants (such as maize, millet, sugar cane, and sorghum) fall into this category, allowing archaeologists to estimate their importance in local diets where those crops were grown.

In central California, there are few C_4 plants, and the majority of those were not typically consumed (Bartelink 2006, 2009). However, botanists have documented a pattern whereby there is a small 1–4‰ increase in $\delta^{13}C$ in plants that grow in chronically water-stressed environments (Gebrekirstos et al. 2011; Picon et al. 1996; Van de Water et al. 2002). Oaks tend to grow along more permanent water courses, and as a result, acorns do not show this enrichment. Likewise, the seeds of annual plants, which typically grow after rains when soils are wet, also do not show this ^{13}C enrichment. On the other hand, manzanita and pine trees do often grow in such environments. Isotopic analyses on carbonized acorn versus manzanita and pine nut fragments in archaeological sites in Green Valley, central Sierra Nevada, and the Coast Range of Monterey County confirm these results, with manzanita and pine showing a 1–3‰ increase in $\delta^{13}C$ (Eerkens et al. 2020; Hull et al. 2017). Furthermore, human bone collagen of individuals who lived in these regions in time periods where manzanita and pine nuts are suspected to have been an important source of dietary calories and protein also show a slight enrichment in $\delta^{13}C$.

Carbon enters marine environments mainly through exchange with atmospheric CO_2 and through photosynthesizing phytoplankton. $\delta^{13}C$ values of biologically available carbon in marine environments typically overlap with those of C_4 plants. Because C_4 plants were generally not consumed, we can use $\delta^{13}C$ in central California as a discriminator of terrestrial- versus marine-derived carbon, with higher (less negative) $\delta^{13}C$ values indicating a greater contribution of marine organisms to the diet (Bartelink 2009; Schoeninger et al. 1983; Schwarcz and Schoeninger 1991). Moreover, within the lower $\delta^{13}C$ range, we can further discriminate between acorns (and seeds of annual plants) versus pine nuts (and other water-stressed plant foods).

While $\delta^{13}C$ reflects marine versus terrestrial dietary input in central California, nitrogen isotopes ($^{15}N/^{14}N$, expressed as $\delta^{15}N$; see below) reflect the averaged trophic level of consumed foods. Nitrogen fractionates during the synthesis of biological tissues, favoring the retention of the heavier ^{15}N. As a result, $\delta^{15}N$ increases by about 3–5‰ with each trophic level. In terrestrial systems in central California, there are essentially three trophic levels: plants, vegetarians, and carnivores. By contrast, in aquatic environments there are more trophic levels, resulting in more elevated $\delta^{15}N$ values at the top of the food chain (typically large fish, predatory birds, and aquatic mammals). $\delta^{15}N$ from bone collagen, then, reflects the trophic level of dietary protein.

METHODS

To isolate collagen for this analysis, a modified Longin procedure was followed (Longin 1971). Approximately 1-2 g of cortical bone was cleaned of any surface contamination by first drilling exposed surfaces with a diamond bit and then sonicating the sample in deionized H_2O (three to six five-minute baths, with the dH_2O replaced after each bath). The bone sample was left in an open container until completely dry, then weighed and demineralized with a solution of 0.5M hydrochloric acid (HCl). HCl was changed every other day until the sample was completely demineralized (up to 2 weeks). The bone was then washed five times with dH_2O and soaked in 0.125M sodium hydroxide (NaOH) for 48 hours to remove humic acids. The sample was rinsed five times with dH_2O to remove any residual NaOH.

Slightly acidic pH3 water was added to the vial and the sample was placed in a 70-90°C oven for approximately 24 hours to solubilize collagen. The pH3 solution was then pipetted into a clean vial. If the entire bone sample was not solubilized, this process was repeated up to two additional times. Finally, all extraction vials were freeze dried to remove the water and isolate the collagen fraction.

Collagen $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$) 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 UCD. Carbon isotope ratios, $\delta^{13}C$, are reported expressed in permil notation (parts per thousand) relative to the Pee Dee Belemnite standard (arbitrarily set at 0‰), while N isotope ratios, $\delta^{15}N$, are expressed against N_2 in modern atmospheric air (also arbitrarily set to 0‰).

RESULTS

Table 1 provides a breakdown of the sample by age at death, sex, and time period, along with $\delta^{13}C$ and $\delta^{15}N$. Also shown are %C, %N, and C/N ratios, a measure of collagen sample quality. Samples within the range of 2.9-3.6 for C/N are typically considered of "good" quality in paleodietary results (DeNiro 1985), while %C > 13% and %N > 4% are typically considered acceptable for ancient collagen studies (Ambrose 1990; Guiry and Szpak 2020; van Klinken 1999). Time period was determined using independent radiocarbon ages measured on bone collagen. As shown in Table 1, all collagen samples fall within the acceptable C/N, %C, and %N ranges. The high degree of consistency in these values indicates excellent preservation of the organic component of bone at the site.

Overall, the $\delta^{13}C$ values within the *Ayttakiš 'Éete Hiramwiš Trépam-tak* population indicate that marine and C_4 plant foods were not significant contributors to local diets. The adult average of -19.3‰ indicates that, in general, less than 15% of the total protein budget derived from marine sources (including

Table 1. Results of Stable Isotope Analysis at Ayttakiš ʻÉete Hiramwiš Trépam-tak.

Burial #	Median Age at Death (yrs)	Sex	$\delta^{13}C$	$\delta^{15}N$	%C	%N	C/N	AMS cal BP
1	25-30	M	-19.1	6.8	47%	17%	3.26	669
2	6-10	M	-19.3	7.8	54%	19%	3.23	669
3	20-24	M	-19.0	7.4	46%	16%	3.29	681
4	26-34	F	-18.9	7.0	47%	17%	3.32	718
5A	33-46	F	-19.4	7.0	45%	16%	3.26	720
5B	0.05-0.1	Ind.	-18.3	7.5	40%	14%	3.27	--
6	16-23	M	-19.5	7.8	40%	14%	3.32	683
7	45-59	M	-19.2	7.2	41%	15%	3.25	671
8	5-6	F	-19.7	7.4	45%	16%	3.32	676
9A	50-83	M	-19.4	6.9	39%	14%	3.30	673
9B	35-44	F	-19.3	7.0	42%	15%	3.19	661
10A	18-24	F	-19.6	6.6	40%	14%	3.33	697
10B	0.05-0.1	F	-19.4	7.0	39%	14%	3.24	--
11	33-46	F	-19.2	7.6	47%	17%	3.30	687
12	24-27	M	-19.7	7.5	45%	16%	3.29	683
13	4-5	M	-19.2	8.0	43%	16%	3.17	666
14	45-59	F	-19.2	7.2	43%	15%	3.28	597
15	17-23	M	-19.3	7.0	41%	15%	3.27	722
16	16-20	M	-19.5	7.0	40%	14%	3.28	664

Note: Ind. = Indeterminate.

any inland-caught salmon, sturgeon, and other anadromous fishes). By contrast, research at bayside and coastal sites in central California typically produce bone collagen $\delta^{13}C$ values between -17‰ and -10‰ (Bartelink 2009; Beasley et al. 2013; Diaz et al. 2018; Martinez et al. 2015), or between 30% and 75% marine-derived protein.

COMPARISON TO OTHER CONTEMPORANEOUS REGIONAL SITES

Figure 2 plots the *Ayttakiš ʻÉete Hiramwiš Trépam-tak* population against a sample of individuals from other regional sites (see Bartelink 2006; Eerkens and Bartelink 2019; Eerkens et al. 2015; unpublished data), with labels indicating ecological contexts. As shown, with one slight exception (Burial 5B), the *Ayttakiš ʻÉete Hiramwiš Trépam-tak* individuals plot in the lower left-hand side of the $\delta^{13}C$-$\delta^{15}N$ space, close to sites CA-ALA-554 (in the modern-day city of Pleasanton) and CA-SCL-919 (near the modern-day city of Milpitas). The one exception, Burial 5B (an infant) has slightly elevated $\delta^{13}C$ values compared to other individuals. The unusual diet of this infant may have been a factor in its premature death.

Likewise, the low $\delta^{15}N$ values for adults at *Ayttakiš ʻÉete Hiramwiš Trépam-tak*, averaging 7.1‰, are consistent with a relatively low overall trophic position within the local food web. This suggests high dependence on plant foods at *Ayttakiš ʻÉete Hiramwiš Trépam-tak*. By contrast, sites in the California Delta and along the Sacramento River typically average between 10‰ and 12‰, indicative of greater consumption of fish and/or large game resources. In short, adult dietary protein at *Ayttakiš ʻÉete Hiramwiš Trépam-tak* came mainly from terrestrial food webs, especially plants.

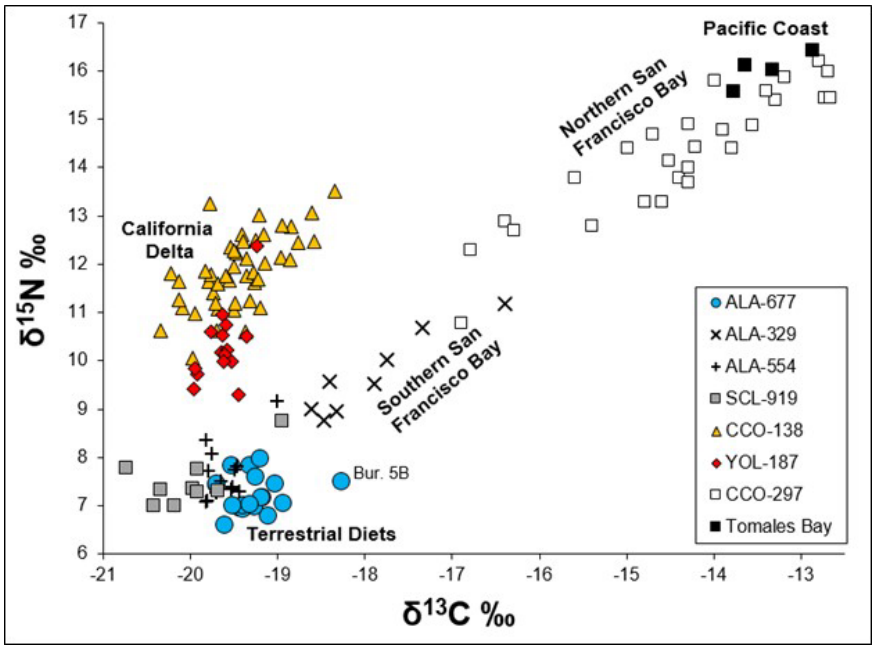

Figure 2. $\delta^{13}C$ versus $\delta^{15}N$ for Ayttakiš ʻÉete Hiramwiš Trépam-tak individuals, relative to a sample of other regional sites of approximately the same time period.

Table 2 shows summary statistics for subgroupings within the population, including adult males, adult females, and subadults. There is very little difference in average values, though subadults have higher inter-individual variation than the other groups. This indicates that individuals who died before reaching adulthood had quite variable diets. It is possible that some of this dietary variation contributed to under-nutrition for certain individuals, which may have played a role in their untimely deaths. By contrast, those who survived into adulthood had quite stable diets in terms of isotopic composition.

Table 2. Average and Standard Deviation in $\delta^{13}C$ and $\delta^{15}N$ for Subgroups within Ayttakiš ʻÉete Hiramwiš Trépam-tak.

Subgroup	**$\delta^{13}C$**		**$\delta^{15}N$**	
	Avg	**Stdev**	**Avg**	**Stdev**
All Adults	-19.3	0.22	7.2	0.34
Adult Male	-19.3	0.24	7.2	0.36
Adult Female	-19.3	0.23	7.1	0.32
All Subadults	-19.2	0.50	7.5	0.41

COMPARISON BY AGE

Given the general isotopic homogeneity of the *Ayttakiš ʻÉete Hiramwiš Trépam-tak* population, as described above, it is not surprising that that there is only slight variation by age category within the sample. Figure 3 plots $\delta^{15}N$ (left) and $\delta^{13}C$ (right) by age at death. As shown, there is higher variation among younger individuals below the age of 30 years, with less variation thereafter, although the sample size is small.

There is some indication that male and female diets changed slightly over the life history of individuals. Again, the sample size is small, but Figure 3 shows that male $\delta^{15}N$ decreases slightly with increasing age ($R^2 = 0.36$), while female $\delta^{13}C$ increases ($R^2 = 0.30$). This may indicate that males decreased intake of large game and increased plant protein consumption over their lifespans. Extreme tooth wear could explain such a shift, making plant gruels an easier source of nutrition compared to meat as an individual aged. By contrast, females seem to have consumed slightly greater amounts of low trophic-level foods (i.e., plants) that were enriched in ^{13}C as they got older. A likely such food for females would be pine nuts.

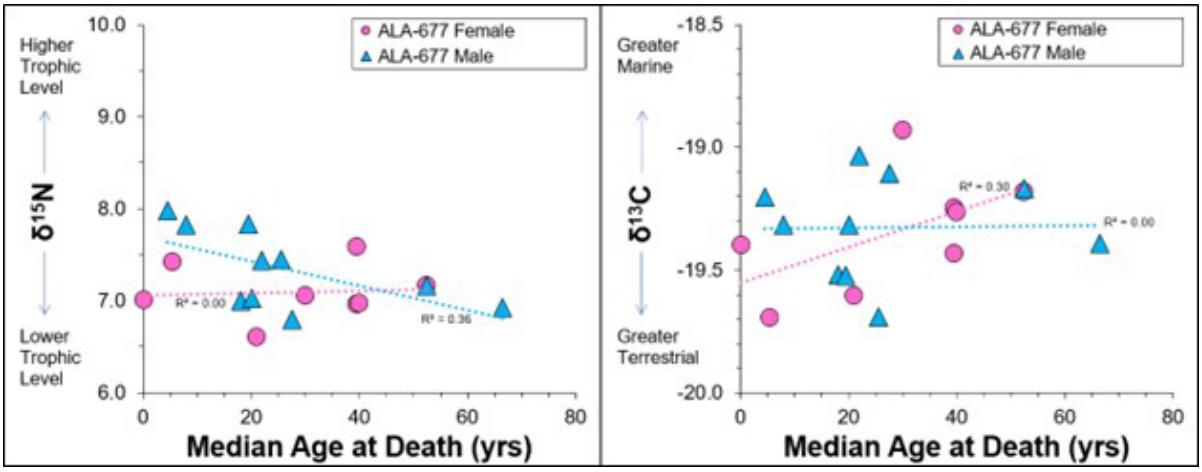

Figure 3. Comparison of $\delta^{15}N$ (left) and $\delta^{13}C$ (right) and against median age at death.

COMPARISON BY ARCHAEOLOGICAL TIME

Finally, Figure 4 examines carbon (right) and nitrogen (left) isotopes over archaeological time, as measured by calibrated median radiocarbon date on bone collagen. The figure does not suggest major changes in diet across the approximately 60 years of site occupation.

CONCLUSIONS

Stable isotope signatures from bone collagen provide insight into inter-individual variation in diet in the Sunol region during the MLT and Late Phase 1 time periods. Compared to other sites in the broader region outside the Sunol area, the *Ayttakiš ʻÉete Hiramwiš Trépam-tak* adults display a distinctive isotopic signature that sets them apart from sites in many other environmental settings in central California. Overall, the signature at *Ayttakiš ʻÉete Hiramwiš Trépam-tak* is consistent with a highly terrestrial diet focused on plant foods (such as acorns) and small amounts of terrestrial game (such as lagomorphs and ungulates), with very little fish or marine shellfish. Despite its proximity, there seems to be only very minor input of San Francisco Bay aquatic foods or anadromous fishes.

Overall, there is very little inter-individual variation within the *Ayttakiš ʻÉete Hiramwiš Trépam-tak* population, indicating a group of individuals eating the same range of foods. The data hint at some slight

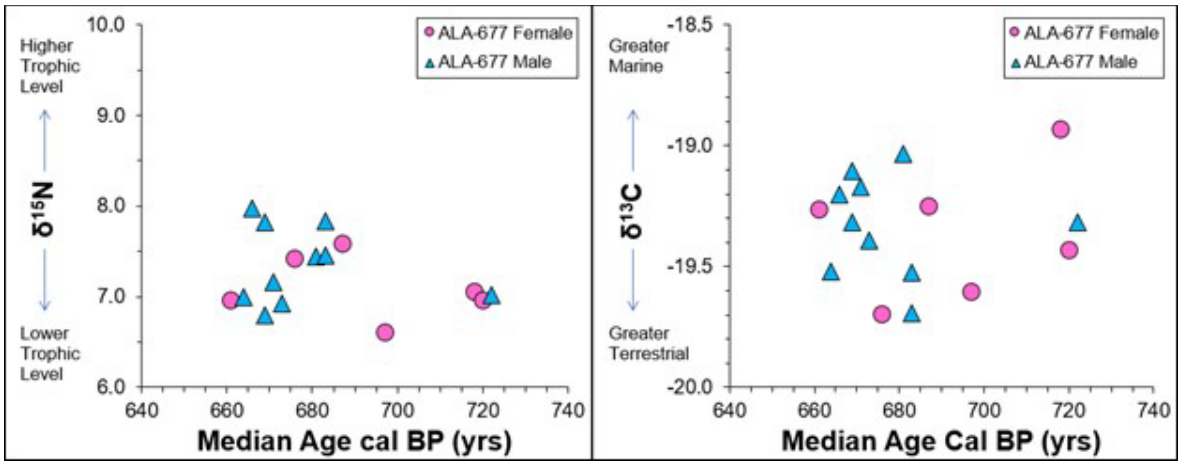

Figure 4. Comparison of $\delta^{15}N$ (left) and $\delta^{13}C$ (right) and against radiocarbon date.

changes over the life cycle in the diets of females, with a shift towards slightly greater quantities of water-stressed plant foods (such as pine nuts) in women over 30 years in age. Other than this slight difference, isotopes reveal that adult males and females had nearly equal diets in terms of isotopic composition. This is consistent with a high degree of intra-group sharing of foods. Likewise, there is no apparent change in diet over the roughly 60 years of time the site was occupied.

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