AGE OF WEANING AND POST-WEANING CHILDHOOD DIETS IN A LATE HOLOCENE PRECONTACT SITE, CA-SOL-11, NEAR SUISUN MARSH

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We examine age of weaning and childhood diet at a Middle Period site in central California, CA-SOL-11, near Suisun Marsh. Stable isotope analyses of serial samples of permanent first molars record information about the diet of an individual when they were between 0 and 9.5 years of age. Our results show that females were breastfed, on average, slightly longer than males at the site. Because breastfeeding represents a significant time and caloric investment by a mother, this suggests greater parental investment in female offspring relative to males. After weaning, young males gained a greater proportion of protein from higher trophic levels and with greater contribution from brackish or marine environments, which we interpret as a higher quality diet. This suggests either preferential provisioning by parents in males after weaning, or evidence of gendered labor practices and diets beginning in early childhood. We also incorporate new analyses of the amelogenin proteins preserved in enamel, AMELX_HUMAN and AMELY_HUMAN, to estimate the sex of one individual previously identified as male based on osteological markers and two individuals that could not be assigned sex based on osteology.

Humans have a unique ability to transform biological necessities into sociocultural norms with symbolic and behavioral implications. One such necessity, breastfeeding, presents one such example through an examination of bioenergetic trade-offs. Mothers in all societies must make choices about how long to breastfeed a child. Breast milk is a high-calorie and pathogen-free food for a child. On the other hand, breast milk is energetically expensive to produce and lactation inhibits ovulation for a mother. The decision about when to wean a child, thus, must balance the trade-offs of increased health of the offspring and potential long-term returns from offspring care and labor, with short-term increased caloric requirements and reduction in fertility to the mother (Kaplan 1996; Voland 1998). Despite the universality of these trade-offs, different societies are distinct in their practices of weaning and post-weaning provisioning. Within a society, sex-biased weaning practices provide insight to multiple aspects of a population's social structure, such as residence patterns and relative importance placed on gendered labor. If female labor is more important to the family economy, or the society is matrilocal (Eggan 1950; Korotayev 2003; Marlowe 2004), mothers may delay the weaning of females relative to males and may also provision females with higher quality diets after weaning.

Here we reconstruct patterns of weaning and post-weaning provisioning through stable isotope analysis. Stable nitrogen isotopes from first molar serial dentin microsamples enable us to reconstruct age at weaning and the average trophic position of provisioned post-weaning foods. Stable carbon isotopes from the same samples indicate contribution of brackish resources to the diet. We are then able to compare the weaning and dietary patterns of males and females to make inferences on population structure based on the results.

BACKGROUND

CA-SOL-11 (hereafter referred to as SOL-11) is located in Green Valley, California, at an elevation of 15 m above sea level (Figure 1). Green Valley is a stretch of land in central California approximately 2.5 km long, and up to 1.6 km wide. The site is bounded on the north, west, and east by tall foothills (Snoke 1967). Green Valley Creek originates in the north from runoff of several natural streams, coalescing with Cook Canyon Creek before passing approximately 90 meters to the west of SOL-11 (Snoke 1967). The riparian woodland environment is adjacent to grasslands, providing access to large and small game mammals, acorns, buckeye, and small seeds. Freshwater fish were available in Green Valley Creek and brackish resources could be found in Suisun Marsh, an estuary located 10 km south of the site (Eerkens et al. 2013).

Students from the University of California, Davis (UC Davis) field school began excavating the site in late 1964 as part of a graduate student thesis project (Snoke 1967). Using obsidian hydration dating and temporally diagnostic artifacts, Snoke (1967) argued that the site dated to the Middle Period. Nineteen recent radiocarbon dates on human bone collagen (see Eerkens et al. 2013, 2019) confirm occupation from approximately 2,250 to 1,050 cal B.P. (300 BCE to 900 CE). This places the site and associated materials within the second half of the Early/Middle Transition Period through nearly all subphases (M1-M4) of the Middle Period, using the new Scheme D chronology proposed by Groza et al. (2011).

THEORETICAL AND ISOTOPIC ORIENTATION

Intensification of plant-based food resources, most notably small-seeded plants and acorns, occurred during the late Holocene in California (Reddy 2016; Wohlgemuth 1996). At SOL-11, groundstone artifacts used in acorn- and small seed-processing tend to be found with female burials (Snoke 1967). This is in accordance with ethnographic accounts of female-associated labor practices among Native Californians, where women were responsible for most of the processing of plant foods (Jackson 1991; Willoughby 1948). On the other hand, recent studies show that the correlation between the sex of a burial and associated tools that we typically associate with plant versus animal processing is much more nuanced and variable than a simple dichotomy of "groundstone = female" and "flaked stone = male" (Buonasera 2013; Byrd et al. 2017:12-3; McGuire and Hildebrandt 1994).

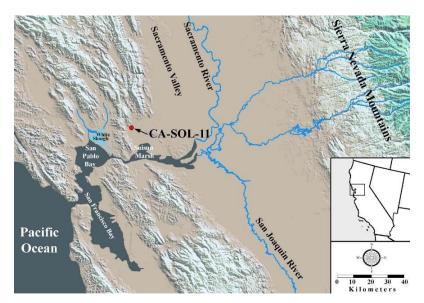


Figure 1. Map of central California showing location of CA-SOL-11.

In any case, as societies depended increasingly on plant foods, there should have been an increase in the demand for processing labor, much of it likely performed by females. Therefore, it has been suggested that with an intensification on plant foods, Native Californian societies should have developed a preference for keeping groups of females together, to facilitate transmission of knowledge about when, where, and how to process plant foods, and likely, access to family-owned gathering patches (Eerkens et al. 2014). Therefore, we predict that female children would have been be favored by mothers, which in turn should be seen by longer periods of dependence on breast milk and higher quality diets after weaning, relative to male children. Some support for this model has been documented in previous studies in California (Eerkens and Bartelink 2013).

We assume high-quality diets are marked by higher $\delta^{15}N$, corresponding to higher trophic level foods. We are unable to make assumptions about quality of diet relating to $\delta^{13}C$; while inhabitants at SOL-11 expended additional calories to attain brackish resources, it is possible that they only did so when terrestrial hunting was insufficient.

We explore early childhood dietary practices at SOL-11 through stable isotope analysis of serial samples of dentin in an early-forming tooth, the permanent first molar. We focus on carbon and nitrogen stable isotopes to identify marine contributions to diet and weaning patterns, respectively. For humans, carbon and nitrogen in bodily tissues are gained entirely through the diet. Hence, measuring these elements in skeletal tissues can serve as tracers of the paleodiet of an individual.

When nitrogen is consumed by an animal, the animal's body preferentially forms waste products such as urea from light nitrogen (14 N), and by extension, assimilates heavy nitrogen (15 N) into bodily proteins such as collagen. This fractionation process on nitrogen concentrates heavier 15 N in bodily tissues relative to dietary 15 N. As a result, δ^{15} N in body tissues increases with each trophic level in ecological systems (Minagawa and Wada 1984).

Plants uptake inorganic carbon dioxide from the atmosphere through photosynthesis to produce either a 3- or 4-carbon intermediate molecule, termed C3 and C4, respectively, before forming glucose. C3 plants, primarily trees, woody bushes, and temperate grasses (e.g., wheat, barley, rice), preferentially assimilate 12 C into tissues (Farquhar et al. 1989). By contrast, C4 plants, primarily tropical grasses and cereals, such as maize, sorghum, and millet, preferentially utilize 13 C (Farquhar et al. 1989). Enrichment of 13 C also occurs in marine resources (Schoeninger and DeNiro 1984). In precontact California, most plants consumed were C3 plants (Bartelink 2006), thus enrichment of carbon (δ^{13} C) is typically an indicator of increased marine contribution to diet. Brackish resources appear isotopically intermediate between terrestrial C3 and marine resources.

While bone is constantly remodeling throughout an individual's life (Hedges et al. 2007; Manolagas 2000), effectively erasing isotopic signatures from early periods in life, dental tissues (enamel, dentin, cementum) grow in sequential layers that do not remodel (Hillson 1996). Permanent first molars, as used in this study, begin forming just prior to birth (median age = 0 years) and continue until 9-10 years old (median age = 9.5 years; Hillson 1996). The microsampling method allows for fine temporal resolution on childhood diet by reconstructing diet in approximately 0.5 to 1-year intervals. This information allows us to trace the diet of a child over the first decade of life, including the major transition from breast milk to solid foods (i.e., weaning), as well as the general composition of post-weaning foods. Because sections can be aged, we can create something of a dietary life history across these years at half- to one-year intervals.

METHODS

This study uses serial dental collagen microsampling to reconstruct diet following procedures outlined in Eerkens et al. (2011, 2013), methods that build on Fuller et al. (2003). Prior to sectioning, each first molar had enamel, cementum, and any visible soil or other adhering material removed using a handheld drill. Each tooth was then sonicated in deionized (DI) water to remove exogenous materials and placed in 0.5M hydrochloric acid for demineralization. Hydrochloric acid was replaced every few days until the tooth was spongy in texture, typically between 10 and 30 days. Once a tooth was demineralized, it was then cut into

horizontal 1-2 mm layers perpendicular to the root axis using a scalpel, producing between 6 and 17 slices depending on tooth length. Each layer was then placed in 0.125M sodium hydroxide for 24 hrs to remove any residual soil humic acids. Upon rinsing with deionized water, each dentin layer was solubilized at 60-70°C in low pH water. After 1-7 days, solubilized collagen in the aqueous layers were separated from any inorganic solids and placed in a freezer. Finally, each frozen sample of solubilized collagen was freeze-dried to remove water and isolate the solid collagen. Carbon and nitrogen isotopic ratios were determined by continuous-flow mass spectrometry at the Stable Isotope Facility at UC Davis. If one serial sample did not produce enough collagen (1 mg) for an accurate result, sequential layers were combined and treated as one data point in figures and statistical analysis. Atomic C/N ratios were used to evaluate collagen sample quality (DeNiro 1985; van Klinken 1999). Samples that fell outside the range of acceptable C/N were eliminated from the analysis.

Prior to sectioning, each tooth was measured from the occlusal surface to the dentinoenamel junction (DEJ), cementoenamel junction (CEJ), and apical root tip. To reconstruct the age at which each layer was formed, we used modern data to approximate that, for first molars, the DEJ was formed at age 0, CEJ at age 2.75, and apical root tip at age 9.5 (Hillson 1996). Since dentin in grows in parabolic layers from the tooth crown towards the apical root tip (Hillson 1996), our straight slices cut across the parabolic layers and each slice represents a range of time of tooth formation with some temporal overlap. While smoothing out some of the diachronic variation, this method still records the evolution of diet over controlled amounts of time.

We manually assigned age at weaning for each burial by visual analysis of dietary curves. Weaning is a process in which the child consumes decreasing amounts of breast milk, and therefore shows an isotopic pattern of decreasing trophic level. Early in life, nitrogen values start out high, corresponding to a time of exclusive breastfeeding, because the infant consuming tissues from the mother (i.e., breast milk) is one full trophic level above the diet of the mother. $\delta^{15}N$ then decreases to a relative minimum, a point at which little to no breast milk is being consumed. After weaning, $\delta^{15}N$ typically increases and stabilizes in early childhood.

Sex and age were assigned using traditional osteological markers (Buikstra and Ubelaker 1994; Phenice 1969; White and Folkens 2005). For three individuals, we applied a new independent sex estimation technique that relies on proteomic signatures preserved in enamel (see Parker et al. 2019). Enamel from DD-9-1 Individual 1 (estimated as male by osteology), CC-9-3 (indeterminate for sex by osteology), and E-9-1 (indeterminate for sex by osteology), was submitted for proteomic analysis. The proteomic approach relies on the detection of fragments of amelogenin proteins, or peptides. Amelogenin is essential in the mineralization of enamel (Fincham et al. 1991; Salido et al. 1992). A distinctive form of the gene resides on both the X and Y chromosomes. As a result, females have only AMELX_HUMAN proteins, and males a particular mixture of AMELX_HUMAN and AMELY_HUMAN (hereafter, simply AMELX and AMELY).

Laboratory methods for the proteomic analysis of amelogenin are detailed in Parker et al. (2019). Briefly, proteins are extracted by incubating ~20 mg pulverized tooth enamel in 1.2 M HCl for 60 min. Following reduction and alkylation, enamel proteins are digested with trypsin. Digested extracts are desalted and concentrated with Zip-Tips®. Liquid chromatography-tandem mass spectrometry of peptides is accomplished with a Thermo Scientific Q-Extractive Plus Orbitrap mass spectrometer equipped with a Proxeon Easy-nLC II HPLC (Thermo Scientific) and Proxeon nanospray source. Spectral data are analyzed using PEAKSTM (10.0) software. After filtering out homologous peptide sequences, the total ion current (TIC) of all unique AMELX and AMELY peptides is combined and compared. AMELY signals provide evidence of a male individual. In the absence of AMELY peptides, female probability, P(F), is assigned based on the combined intensity of AMELX peptides. In particular, large quantities of AMELX peptides in the absence of any AMELY peptides, correlates to higher P(F) values.

RESULTS

Proteomic analyses confirmed that the individual identified as a male based on osteological signatures (DD-9-1, Ind 1) was indeed a male, through the presence of significant amounts of AMELY HUMAN

Table 1. Proteomic Sex Estimation for Three Individuals Based on Normalized Combined Intensity of AMELX and AMELY Peptides in Tooth Enamel.

| Lab No. | BURIAL | Тоотн | ENAMEL (MG) | (AMELX+1) /MG | (AMELY+1) /MG | Pr(F) | SEX ESTIMATE |
|---------|-----------|-------|-------------|------------------|------------------|-------|-----------------|
| TYB_64 | E-9-1 | ULM1 | 21.5 | 4.60E+08 | 4.65E-02 | 0.89 | F |
| TYB_66 | CC-9-3 | URM1 | 21.2 | 8.09E+07 | 4.96E+06 | 0 | M |
| TYB_68 | DD-9-1, 1 | LLM1 | 21.1 | 4.58E+08 | 4.64E+07 | 0 | M |

peptides in his enamel. Additionally, CC-9-3 was determined to be male due to the presence of AMELY_HUMAN in his enamel, and E-9-1 was determined to be female (with probability p = 0.89) due to the lack of AMELY_HUMAN and the presence only of AMELX_HUMAN in her enamel. Sex identifications are reported in Table 1, and are carried through in the analyses reported below.

All nine individuals produced some collagenous material from the serial sampling described above. Using serial dentin microsampling for carbon and nitrogen stable isotopes, we were able to reconstruct the early diets of all individuals from SOL-11. Table 2 provides median age, δ^{13} C, δ^{15} N, and the C/N value for each serial sample in the study. DeNiro (1985) found that collagen samples with C/N ratios between 2.9 and 3.6 generally produced reliable isotopic values. Table 2 shows that some of the SOL-11 first molar samples fall below this threshold. We highlight those with grey cells in Table 2, and treat them with caution in our interpretations.

For each individual, a childhood dietary curve was constructed using the R package ggplot2 (R Core Team 2013; Wickham 2016). Figures 2 and 3 show two such curves for δ^{15} N, for Burials E-9-1 and E-8-3. These curves plot δ^{15} N against the median age of a serial section. The weaning process is marked by a decrease in δ^{15} N, usually between 2-4‰, between ages 1 and 5 years, consistent with a decrease in trophic position, or a transition from breast milk to solid foods (Eerkens et al. 2011). The red dashed lines in Figures 2 and 3 show the bone collagen δ^{15} N previously reported in Eerkens et al. (2013), which represents the diet closer to the time of death, typically the adult diet. Previous studies in precontact Californian societies show that δ^{15} N typically drops across the first several serial sections, before settling to a minimum (Eerkens and Bartelink 2013; Greenwald et al. 2016). We interpret this minimum as the completion of the weaning process. δ^{15} N associated with this weaning minimum is usually 1-2‰ below δ^{15} N of that person's adult bone collagen. δ^{15} N then typically rises again after weaning, through age 9 years. Similar dietary curves were constructed for all nine individuals at SOL-11 that were included in this study.

The δ^{15} N curve for Burial E-9-1 from SOL-11 is more typical of an early childhood dietary trajectory (cf. Eerkens and Bartelink 2013; Eerkens et al. 2018, 2019). Nitrogen enrichment of this individual, a female, decreases from 10.6‰ at 0.5 years to a minimum of 7.7‰ at 4.3 years. Cessation of breastfeeding occurred somewhere between the absolute low of 7.7‰ and the point directly before it (3.3 years), and so we report the median age of weaning to be 3.8 years.

However, children in ancient societies practiced a wide range of dietary patterns, and we also present one such divergent curve from SOL-11 for Burial E-8-3. This individual shows a slightly unusual signature wherein $\delta^{15}N$ seems to increase across the earliest sections, before dropping suddenly between ages 2.9 and 3.8 years (marking completion of weaning), but still above the values seen in adult bone collagen. $\delta^{15}N$ dips slightly after weaning before increasing again in the final serial sample at around age 8 years.

Finally, Figure 4 shows a dietary curve for an individual of indeterminate sex (DD-9-1, Ind 2), for whom we were unable to determine an age of weaning. High occlusal wear removed much of the coronal dentin, removing the earliest growing dentin. The dietary signature only begins at age 2.1 years. The slight drop in $\delta^{15}N$ between 2.1 and 2.6 years of age is likely to represent the final stages of weaning, but lacking earlier forming dentin, it is difficult to determine if this decrease actually represents weaning or simply dietary variation in $\delta^{15}N$.

Table 2. Stable Isotope Data from Serial Samples of First Molars of Nine Individuals at CA-SOL-11.

| BURIAL/SAMPLE | MEDIAN AGE | Δ ¹³ C | Δ^{15} N | C/N | BURIAL/SAMPLE | MEDIAN AGE | Δ ¹³ C | Δ^{15} N | C/N |
|----------------|---------------|-------------------|-----------------|-----|-----------------|---------------|-------------------|-----------------|-----|
| E-9-1-I | 0.5 | -19.8 | 10.6 | 3.2 | EE-9-1-JK | 1.2 | -19.1 | 11.1 | 3.2 |
| E-9-1-H | 1.4 | -20.2 | 9.1 | 3.2 | EE-9-1-HI | 2.5 | -19.3 | 10.0 | 3.1 |
| E-9-1-G | 2.4 | -20.1 | 8.7 | 3.2 | EE-9-1-FG | 3.9 | -18.1 | 9.8 | 2.6 |
| E-9-1-F | 3.3 | -20.0 | 8.5 | 3.2 | EE-9-1-DE | 5.7 | -19.0 | 11.1 | 3.2 |
| E-9-1-E | 4.3 | -20.0 | 7.7 | 3.2 | EE-9-1-C | 6.6 | -19.3 | 9.2 | 3.2 |
| E-9-1-D | 5.2 | -19.4 | 8.4 | 3.2 | EE-9-1-B | 7.5 | -18.6 | 13.1 | 3.3 |
| E-9-1-C | 6.2 | -19.6 | 8.2 | 3.2 | EE-9-1-A | 8.4 | -18.7 | 14.3 | 3.8 |
| E-9-1-AB | 8.1 | -19.5 | 9.0 | 3.2 | HH-2-1-Q | 0.4 | -18.3 | 9.0 | 3.0 |
| BB-1-1-F | 2.9 | -19.5 | 7.7 | 3.3 | HH-2-1-OP | 1.2 | -18.9 | 7.6 | 3.0 |
| BB-1-1-E | 3.9 | -19.2 | 7.9 | 3.3 | HH-2-1-MN | 2.0 | -18.3 | 11.1 | 3.0 |
| BB-1-1-D | 4.9 | -19.0 | 7.7 | 3.0 | HH-2-1-L | 2.8 | -20.1 | 6.2 | 3.0 |
| BB-1-1-C | 5.9 | -19.3 | 7.2 | 3.3 | НН-2-1-ЈК | 3.4 | -19.5 | 7.6 | 3.1 |
| BB-1-1-B | 6.9 | -19.2 | 7.4 | 3.3 | НН-2-1-НІ | 4.4 | -18.4 | 8.8 | 3.0 |
| BB-1-1-A | 7.9 | -19.2 | 7.9 | 3.3 | HH-2-1-G | 5.1 | -18.5 | 9.2 | 3.2 |
| Е-8-3-Н | 1.1 | -19.1 | 11.3 | 3.2 | HH-2-1-EF | 5.9 | -18.1 | 9.5 | 3.3 |
| E-8-3-G | 2.0 | -19.1 | 11.6 | 3.2 | HH-2-1-D | 6.6 | -18.2 | 10.1 | 2.9 |
| E-8-3-F | 2.9 | -18.4 | 12.0 | 3.2 | НН-2-1-С | 7.2 | -18.6 | 9.9 | 3.2 |
| E-8-3-E | 3.8 | -18.9 | 10.3 | 3.2 | HH-2-1-AB | 8.1 | -17.9 | 10.5 | 3.2 |
| E-8-3-D | 4.7 | -18.9 | 10.6 | 3.2 | CC-9-3-I | 1.4 | -18.3 | 9.8 | 2.9 |
| E-8-3-C | 5.7 | -19.1 | 10.3 | 3.2 | СС-9-3-Н | 2.2 | -18.4 | 9.4 | 2.8 |
| E-8-3-B | 6.6 | -19.1 | 9.6 | 3.2 | CC-9-3-FG | 3.5 | -18.7 | 8.6 | 2.9 |
| E-8-3-A | 8.0 | -19.2 | 10.4 | 3.3 | СС-9-3-Е | 5.0 | -19.4 | 8.8 | 3.2 |
| DD-9-1 IND 1-K | 0.4 | -19.1 | 10.0 | 3.2 | CC-9-3-CD | 6.2 | -19.4 | 8.6 | 3.2 |
| DD-9-1 IND 1-J | 1.0 | -19.0 | 9.7 | 3.3 | CC-9-3-BA | 7.5 | -19.4 | 9.7 | 3.4 |
| DD-9-1 IND 1-I | 1.6 | -18.9 | 9.0 | 3.2 | DD-9-1 IND 2-L | 2.1 | -19.3 | 10.3 | 3.2 |
| DD-9-1 IND 1-H | 2.1 | -19.1 | 9.0 | 3.2 | DD-9-1 IND 2-K | 2.6 | -19.7 | 9.9 | 3.3 |
| DD-9-1 IND 1-G | 2.8 | -19.0 | 8.6 | 3.2 | DD-9-1 IND 2-J | 3.2 | -19.6 | 10.5 | 3.2 |
| DD-9-1 IND 1-F | 3.6 | -18.9 | 8.4 | 3.2 | DD-9-1 IND 2-I | 3.7 | -19.7 | 10.5 | 3.2 |
| DD-9-1 IND 1-E | 4.4 | -19.0 | 8.4 | 3.2 | DD-9-1 IND 2-H | 4.3 | -19.3 | 10.5 | 3.3 |
| DD-9-1 IND 1-D | 5.1 | -19.0 | 11.4 | 3.2 | DD-9-1 IND 2-G | 4.8 | -19.2 | 10.9 | 3.3 |
| DD-9-1 IND 1-C | 5.9 | -18.8 | 9.1 | 3.2 | DD-9-1 IND 2-F | 5.4 | -19.1 | 10.5 | 3.2 |
| DD-9-1 IND 1-B | 6.7 | -18.9 | 8.5 | 3.2 | DD-9-1 IND 2-E | 5.9 | -19.2 | 10.8 | 3.2 |
| DD-9-1 IND 1-A | 7.6 | -18.7 | 12.1 | 3.2 | DD-9-1 IND 2-CD | 6.8 | -18.5 | 12.4 | 3.3 |
| E-8-2-M | 0.3 | -19.2 | 9.9 | 2.9 | DD-9-1 IND 2-B | 7.6 | -18.7 | 12.5 | 3.3 |
| E-8-2-L | 1.0 | -19.2 | 9.4 | 2.8 | DD-9-1 IND 2-A | 8.4 | -17.7 | 13.8 | 3.3 |
| E-8-2-J | 2.4 | -18.8 | 8.8 | 2.9 |] | | <u> </u> | | |
| E-8-2-HI | 3.4 | -19.0 | 9.4 | 2.8 | | | | | |
| E-8-2-G | 4.4 | -19.0 | 9.8 | 2.9 | | | | | |
| E-8-2-F | 5.1 | -18.5 | 9.0 | 2.7 | [| | | | |
| E-8-2-DE | 6.1 | -18.9 | 9.0 | 2.8 | ļ | | | | |
| E-8-2-C | 7.1 | -18.7 | 10.8 | 3.2 | Į | | | | |
| E-8-2-AB | 8.5 | -19.2 | 9.3 | 3.0 | | | | | |

 $Notes: \ Samples \ with \ low \ C/N \ values \ are \ highlighted \ in \ gray. \ Data \ from \ these \ samples \ should \ be \ treated \ with \ caution.$

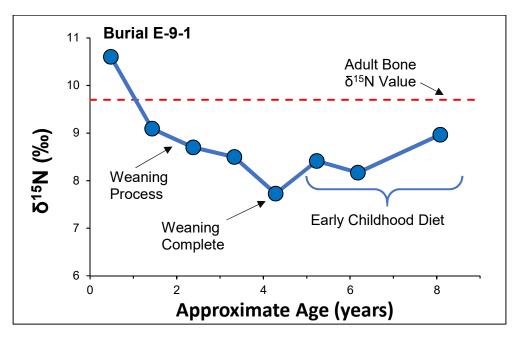


Figure 2. Dietary curve of Burial E-9-1, a female who died in childhood (approximately 10-14 yrs). The dietary curve displays a clear weaning process between ages 0 and 4.3 years of age (median age of weaning = 3.8 years). Red dotted line plots δ^{15} N in her bone collagen reflecting adult diet.

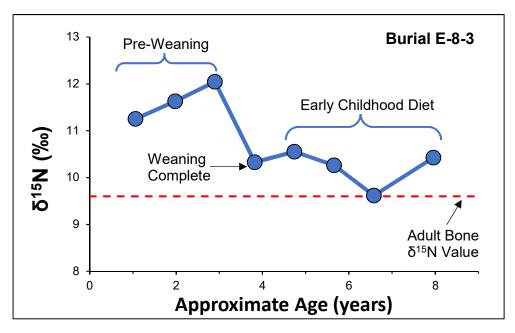


Figure 3. Dietary curve of burial E-8-3, a female who died in older adult years. The curve shows an uncommon pattern with increasing $\delta^{15}N$ between 1.1 and 2.9 years, followed by the expected drop consistent with weaning between age 2.9 and 3.8 years (median = 3.4 years). Red dotted line plots $\delta^{15}N$ in her bone collagen reflecting adult diet.

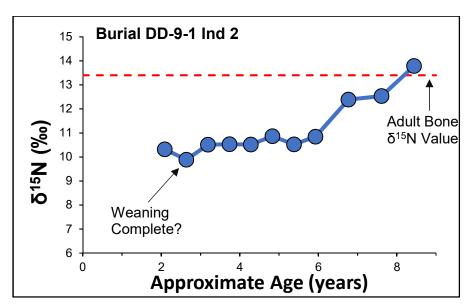


Figure 4. Dietary curve of burial DD-9-1, Individual 2, a person of indeterminate sex, who died in older adult years. The curve does not show a clear weaning pattern due to heavy occlusal wear removing early growing dentin. The slight drop in $\delta^{15}N$ between 2.1 and 2.6 years may represent the final stages of weaning. Red dotted line plots $\delta^{15}N$ in bone collagen reflecting adult diet.

Table 3 provides demographic information, direct bone collagen radiocarbon date (when available), estimated age at weaning, and summary isotopic values at three key points in life, completion of weaning, between 5 and 7 years, and between 7 and 9 years. In the sections below, we discuss results for weaning and early childhood diets separately.

Weaning Age

Figure 5 shows box-and-whisker plots for the age at weaning for females (left) and males (right) at SOL-11. Whiskers represent the maximum and minimum values, thick vertical lines represent medians, and symbols plot statistical means. The figure shows that females in our sample were weaned at an average age of 3.6, about 19 months later than males, who were weaned on average at age 2.0 years. Males also have a wider range of observed weaning ages, although we acknowledge that more males were included in this sample. We estimate the single burial of indeterminate sex in our sample (DD-9-1, Ind 2) was weaned sometime before age 2.6 years, but we are missing too much of the tooth crown to make a confident determination. We note that this is close to the male mean, and likewise the average δ^{15} N values in the age range of 5-9 years for DD-9-1, Ind 2 are similar to that of other males. Thus, we suspect DD-9-1, Ind 2 may be a male based on the isotopic signatures, but lack enough enamel to test the sex using the proteomic method described above, and did not have enough of the post-cranial skeleton to estimate sex.

Post-Weaning Diet

Following previous studies, we divide early childhood diet into four phases: pre-weaning, diet at weaning, diet between the ages of 5 and 7 years, and diet between the ages of 7 and 9 years (Eerkens and Bartelink 2013). We calculated average δ^{13} C and δ^{15} N at each of these points in time (Figure 6), as well as one standard deviation above and below this mean value.

Table 3. Summary Information from Serial Sampling of First Molars from CA-SOL-11, Including Estimated Age at Weaning, Diet at the Time of Weaning, Average Diet at 5-7 Years, and Average Diet at 7-9 Years.

| Burial | SEX | AGE AT DEATH | AGE AT WEANING (YEARS) | POST-WEANING MINIMUM | | 5-7 YEARS | | 7-9 YEARS | | 14C |
|-----------|--------|-----------------|------------------------------|-------------------------|----------------|-------------------|----------------|-------------------|----------------|----------------|
| | | | | δ ¹³ C | $\delta^{15}N$ | δ ¹³ C | $\delta^{15}N$ | δ ¹³ C | $\delta^{15}N$ | AGE B.P. |
| E-9-1 | female | adolescent | 3.8 | -20.0 | 7.7 | -19.5 | 8.3 | -19.5 | 9.0 | |
| BB-1-1 | female | young adult | n/a* | n/a | n/a | -19.2 | 7.3 | -19.2 | 7.1 | $2,220 \pm 30$ |
| E-8-3 | female | old adult | 3.4 | -18.9 | 10.3 | -19.1 | 9.9 | -19.2 | 10.4 | $2,330 \pm 20$ |
| DD-9-1 I1 | male | young adult | 1.3 | -18.9 | 9.0 | -18.9 | 9.7 | -18.7 | 12.1 | $2,050 \pm 20$ |
| E-8-2 | male | young adult | 1.7 | -18.8 | 8.8 | -18.7 | 9.0 | -19.0 | 10.1 | $1,390 \pm 25$ |
| EE-9-1 I1 | male | old adult | 1.9 | -18.1 | 9.8 | -19.1 | 10.2 | -18.7 | 13.7 | $1,850 \pm 40$ |
| HH-2-1 | male | adult | 2.4 | -20.1 | 6.2 | -18.3 | 9.6 | -18.3 | 10.2 | $1,990 \pm 25$ |
| CC-9-3 | male | adolescent | 2.0 | -18.7 | 8.6 | -19.4 | 8.7 | -19.4 | 9.7 | |
| DD-9-1 I2 | indet | old adult | <2.6** | -19.7 | 9.9 | -19.0 | 11.3 | -18.2 | 13.2 | $1,720 \pm 25$ |

Notes: Burial HH-2-1 exhibits a secondary drop in $\delta^{15}N$ at 2.1 years that may alternatively represent the age of weaning; *= does not display a complete weaning signature because much of the early growing dentin had been removed due to occlusal wear; Indet = indeterminate.

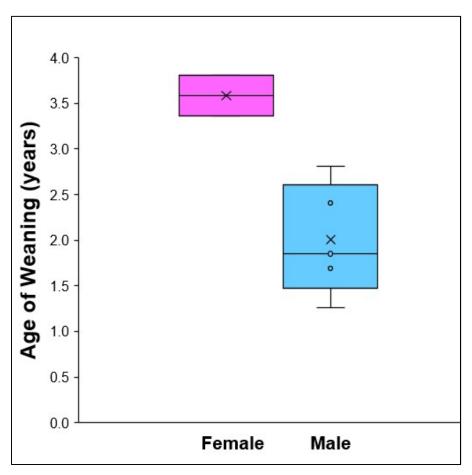


Figure 5. Box and whisker plot of age of weaning, by sex, at SOL-11.

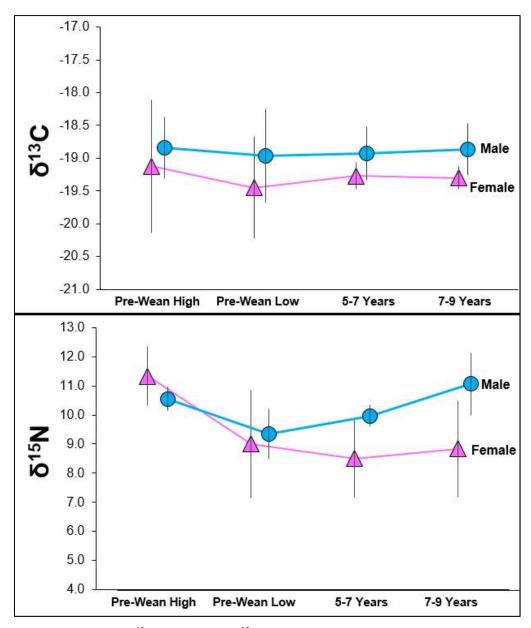


Figure 6. Average $\delta^{13}C$ (upper) and $\delta^{15}N$ (lower) across childhood life history stages, by sex, for individuals from SOL-11.

Figure 6 shows that males are initially roughly equal to females in $\delta^{15}N$ at the pre-weaning and weaning stages. However, beginning by age 5-7 years, a shift is evident wherein males become elevated over females. Although there is much greater overlap, males are also slightly higher, on average, in $\delta^{13}C$ across all childhood life stages. This even holds, surprisingly, pre-weaning periods, although the pre-weaning samples also have the greatest degree of overlap between males and females (as indicated by the vertical bars showing 1 standard deviation above and below the mean value). This suggests boys were eating more brackish foods higher trophic-level foods than girls, especially by age 5-7 years. The male-female dietary difference is most pronounced in the 7-9 age group. While females change only marginally in $\delta^{15}N$ from 5-7 to 7-9 years, males show large increases across these age categories. On average, females are enriched in $\delta^{15}N$ by about 0.3% from early to later childhood, while males increase by 1.8‰.

DISCUSSION

Sex-biased dietary differences, both pre- and post-weaning, appear in the sample of individuals included in this study from SOL-11. The differences are not statistically significant due to low sample size, yet some apparent patterns support our original hypothesis. First, we observe a near 19-month extension in breastfeeding of females compared to males. Since breastfeeding has significant benefits to the child, this finding is consistent with our original hypothesis of a preference for female children and the increased input of labor to the household that they bring.

Second, after weaning, males consume more animal proteins and brackish resources compared to females. Assuming that meat represents a higher quality source of dietary protein, this suggests greater investment in male offspring relative to females. This does not agree with our hypothesis of female preference. We believe one of two factors could account for this overall pattern.

It is possible that parents initially invested more in girls, ensuring that they survive past the age of weaning, in the SOL-11 case, approximately 3.6 years. This window of time (0-4 years) is known to be a difficult one in pre-modern societies where infant mortality rates were especially high. Such a strategy would result in higher mortality rates among male infants and would ensure that more girls would reach late childhood and adolescent years. Once children were past this particularly vulnerable age, however, parents may have switched their preference to provisioning males with a higher quality diet. Such an overall childrearing strategy may have led to a sex imbalance in the society, with more young adult females of marriageable age relative to males.

Alternatively, the differences observed could be due to sex-biased differences in how children learned to become effective hunters and gatherers. If girls began to learn gathering skills with their mothers at a young age, and boys began a similar process to learn hunting skills with their fathers, these activities could take boys and girls to different parts of the landscape during the latter stages of weaning. Effective gathering often requires years of training to learn where the best patches are located, which plants are poisonous, how to locate plants with underground storage organs, and when is the proper time of year to gather various foods, among other information. Girls may have started this process at a young age, 2-3 years, accompanying their mothers (and other female relatives) during gathering bouts and learning these important life skills. During these bouts, mothers could more easily supplement the diets of girls with additional breast milk, extending the weaning process over a longer period of time.

On the other hand, the teaching of boys may have taken them with their fathers (or other male relatives) and away from their mothers, leading to earlier weaning. During such hunting bouts, young boys may have started consuming greater proportions of meat at a younger age (5-7 years), leading to differences between the diets of girls and boys. This may be particularly so in the case of game that was killed at some distance from the village, in which case field butchering of the carcass may have resulted in consumption of some of the lower utility body parts (e.g., heads, organs) in the field.

This second hypothesis may also have involved childhood significant independence. Modern ethnographic accounts note the importance of self-foraging for young children, typically ages 5-9 years (Bird and Bird 2000, 2005). Childhood foraging is predicted when children have access to foods requiring little to no processing and sessile prey (Bird and Bird 2000, 2005; Greenwald et al. 2016). Indeed, there is archaeological evidence of many easily accessible food resources in SOL-11 middens such as the pits of fruits and gastropod shells (Snoke 1967). At least one previous study identified isotopic signatures of childhood foraging in prehistoric central California, interpreting a period of lower δ^{15} N (typically 0.75-1.5‰ lower) within ages 5-9 as indicative of independent foraging (Greenwald et al. 2016). This study found a slightly greater range in the decrease, from 1.1-2.9‰, with one exceptional decrease of 4.8‰. Based on these studies, the temporary post-weaning decreases in δ^{15} N at SOL-11 may represent signals of greater independent foraging behavior, especially for boys.

If independent foraging occurred in mixed groups of males and females, as is reported from modern ethnographies (Hawkes et al. 1995; Bird and Bird 2005), there should be no sex-biased differences in childhood diet. However, this study finds a larger sex-biased difference, suggesting that if independent foraging took place, it must have occurred in separate groups for boys and girls.

CONCLUSION

Using stable isotope analyses on nine first molars, we were able to reconstruct early dietary life histories in a Middle Period central California site. Females in our sample were breastfed longer than males. Upon weaning, males in our sample consumed higher quality diets, composed of more animal meat and more brackish resources (e.g., fish, shellfish). Thresholds of statistical significance were not reached by any metric we analyzed due to low sample size.

Observed patterns of breastfeeding indicate relatively higher value on female offspring, though early childhood dietary composition may suggest greater investment in male offspring. After weaning, young males in this sample are enriched in both ¹⁵N and ¹³C, indicating a diet comprised of relatively more animal proteins and brackish foods. Since we assume that diets enriched in ¹⁵N are higher quality, this contradicts our hypothesis of female preference for children after weaning, if indeed it is parents who were primarily provisioning children.

While it is possible that parents were explicitly provisioning high- or low-quality meals to offspring based on sex, we suspect that the second explanation is more likely, that is, that children were settling into their adult gendered labor and societal roles from ages 5-9 years. As a result, children were spending most of their time around and sharing food with people of the same sex (either adult or children, or both). For the young females, this meant time foraging with mothers and consuming more plant-based calories (and extended weaning as discussed above). For males, gathering and fishing from the edge of Suisun Marsh and hunting would lead to a diet with both higher average trophic position and higher quantities of food with marine-derived carbon and nitrogen.

Early engendering allowed for longer learning periods of labor roles and potential for greater future labor output, a result that we also predict from differential investment in one sex over another. It is therefore difficult to determine net benefits to either sex, although we predict higher numbers of adolescent and young adult females relative to males. To resolve lingering questions, future studies can correlate isotopic results from first, second, and third molars to understand individual diets throughout time with respect to sex. As well, sex-biased differences in osteoarthritis and other skeletal signatures could indicate different activities for males and females. We hope future studies will seek to resolve the different scenarios that could account for the different diets in young males and females we documented at SOL-11.

ACKNOWLEDGEMENTS

Funding for this research was provided, in part, by an NSF grant awarded to Glendon Parker and Jelmer Eerkens (#BCS-1825022) and by a grant to Jelmer Eerkens (#BCS-1318532). We thank the Museum of Anthropology at UC Davis for providing access to the collection.

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