Assessment of Age at Weaning for Post-Contact Maya of Tipu, Belize, Using Stable Carbon, Nitrogen, and Oxygen Isotope Ratios

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ASSESSMENT OF AGE AT WEANING FOR POST-CONTACT MAYA OF TIPU, BELIZE, USING STABLE CARBON, NITROGEN, AND OXYGEN ISOTOPE RATIOS

A Thesis
Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Arts in The Department of Geography & Anthropology

by
Chaney Elizabeth Hiers
B.S., Clemson University, 2013
May 2015
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Abstract

Age at weaning was assessed for a post-Spanish contact Maya population, the Tipu, by sampling 25 individuals (20 subadults less than seven years of age and five females from 18 to 45 years of age). Whole ribs, for younger subadults, and rib fragments, for older subadults and adults, were sampled for stable nitrogen, carbon, and oxygen isotope ratios. Five lines of evidence were used to assess age at weaning: stable carbon composition from collagen ($\delta^{13}\text{C}_{\text{col}}$), stable nitrogen composition from collagen ($\delta^{15}\text{N}_{\text{col}}$), stable carbon composition from apatite ($\delta^{13}\text{C}_{\text{ap}}$), difference in collagen and apatite composition ($\Delta^{13}\text{C}_{\text{ap-co}}$), and stable oxygen composition from apatite ($\delta^{18}\text{O}_{\text{ap}}$).

The subadults interpreted as breastfeeding at time of death were those that displayed the trophic level effect: $\delta^{15}\text{N}_{\text{col}}$ elevated 2‰ to 3‰ above the stable nitrogen composition of the adult females and $\delta^{13}\text{C}_{\text{col}}$ elevated ~1‰ above the stable carbon composition of the adult females. For this study, three subadults less than one year, one aged one to two years, and one aged two to four years displayed the trophic level effect. The other lines of evidence (i.e., $\delta^{13}\text{C}_{\text{ap}}$, $\Delta^{13}\text{C}_{\text{ap-co}}$, and $\delta^{18}\text{O}_{\text{ap}}$) suggest individuals less than one year of age consumed a diet of breast milk, while other subadults consumed a diet equivalent to the mother's before two to four years of age. Breast milk and maize have similar carbon content, and therefore, the $\delta^{13}\text{C}_{\text{ap}}$ varied little between the subadult categories and the adult females. The difference between collagen and apatite ($\Delta^{13}\text{C}_{\text{ap-co}}$) increased as maize consumption in the subadult diet increased and breast milk consumption decreased. Lastly, $\delta^{18}\text{O}_{\text{ap}}$ decreased as the subadults increased their consumption of environmental water. Though ethnohistoric data suggest that individuals at Tipu were weaned between three and four years of age, data from this
study suggests that individuals at Tipu may have been fully weaned by two to three years of age.
Chapter One: Introduction

Archaeological and ethnographic data suggest that the lifeways of the Maya at Tipu, Belize, were profoundly altered by contact with the Spanish in AD 1544 (Jacobi, 2000: 18).

Prior to contact, the traditional culture of the Maya at Tipu thrived despite the collapse of the political and economic systems in the 10th century (Cohen et al., 1994; Graham et al., 1989). However, after the Spanish arrived in Cuba, approximately 27 years before contact with the Tipu, the spread of disease had greatly reduced the population (Jacobi, 2000). After approximately 160 years of alternating between Maya and Spanish control, the site of Tipu was abandoned in AD 1707 (Jones, 1989: 14). The history of the Maya at Tipu is inferred through ethnohistorical accounts from the Spanish and through the archaeological record (Jacobi, 2000; Cohen et al., 1994). According to the archaeological and bioarchaeological records, the diet for adults at Tipu did not alter after Spanish contact (Jacobi, 2000; Graham et al., 1989). However, the influence of the Spanish on subadult diet and more specifically the age at weaning has not been examined.

Stable isotope analyses have been increasingly used in the last forty years to assess the dietary habits of previous populations as well as to assess other characteristics such as age at weaning or population migration (Katzenberg et al., 1993; Herring et al., 1998; Katzenberg, 2007; Hobson and Wassenarr, 2008; Lewis, 2007). The weaning process is challenging for infants; if weaned too early, the consequences can be fatal (Jones, 1989: 414). The stable isotope ratios used to assess age at weaning in archaeological populations are carbon (δ^{13}C), nitrogen (δ^{15}N), and oxygen (δ^{18}O); the former two are analyzed from bone collagen and tooth dentin; the latter, oxygen, along with carbon, is analyzed from bone apatite or tooth enamel. Assessment of age at weaning from bone collagen is primarily based
on trophic level differences between adult females and their nursing infants (Fogel et al., 1989; Bourdou, 2013: 3904).

Ethnohistoric records from Diego de Landa, a friar brought to the Yucatán in AD 1549, indicate that, before Spanish contact, Maya children were weaned between three and four years of age (Landa, 1941: 125; Williams et al., 2005). Records indicate that the demands on women of Tipu significantly increased after Spanish conquest (Clendinnen, 1982), a fact that might have impacted the age at which infants were weaned. Modern Maya women in the agricultural village of Xculoc begin the weaning process around six months of age with the children typically weaned by two (Kramer, 2005: 80).

The purpose of the present study is to research the impact of contact on the age at weaning for the Maya at Tipu. The aim of this project fits within the first of three goals for the skeletal material from Tipu established by Cohen and colleagues (1989). These goals are 1) to assess the health of the Tipu, 2) to note the degree of trauma and pathologies associated with the population, and 3) to understand the overall composition of the burials excavated from the chapel at Tipu. Although age at weaning for a population is not a direct measure of health, the process of weaning often is considered in bioarchaeological research to be a primary etiology for skeletal pathological lesions that form during development (e.g., Harris lines, enamel hypoplasias, and cranial porosities) (Lewis, 2007: 103; Alcorn and Goodman, 1985). Therefore, determining whether or not the age at weaning changed after contact will provide new insight for interpreting patterns of such skeletal lesions not only for the Tipu, but for other post-contact Maya populations. In addition, the duration a subadult breast feeds can have an effect on the development of the infant’s immune system. Breast milk is a source of protection for the infant’s immune system from pathogens that the child is exposed to
during the early stages of life (Jay, 2009: 166). Therefore, the timing of the beginning of breastfeeding plays a significant factor in the rates of mortality and morbidity for the population (Katzenburg et al., 1996).

For this study, carbon and nitrogen isotopes obtained from rib collagen and carbon and oxygen isotopes from rib apatite were assessed in a sample of 25 individuals (including five adult females and 20 subadults aged birth to six years). The expectation for interpretation is that the unweaned subadults would exhibit $\delta^{15}$N and $\delta^{13}$C values that are ~2‰ to 3‰ and ~1‰ higher, respectively, compared to the adults (Bourbou et al., 2013); the $\delta^{15}$N and $\delta^{13}$C values of weaned subadults would be more comparable to adults. The following chapters will review the archaeological and bioarchaeological literature on the Maya at Tipu, the literature for using isotope ratios to assess age at weaning in archaeological populations, and the materials, methods, results, and conclusions of this study.
Chapter Two: Literature Review

History of the Tipu at the Time of Spanish Contact

Tipu, Belize, is home to one of three Maya towns in southeastern Yucatán most influenced by the Spanish (Jones, 1989: 5). The Spanish first contacted the Tipu in AD 1543–1544; however, the Spanish influence over the Maya began with the spread of diseases around AD 1517 (Jacobi, 2000: 17; Cohen et al., 1994: 122). Before the Spanish introduced diseases into Cuba in AD 1517, there were approximately 800,000 Maya residing in the southern lowlands. When the Spanish arrived in AD 1544, the population was reduced to 250,000 Maya (Cohen et al., 1994: 122). Not only did the Spanish introduce disease to the Maya, but repeated epidemics occurred, along with famine due to tropical storms and drought, in the 16th and 17th centuries (Farriss, 1984: 60-61; Cohen et al., 1994: 122).

Tipu was occupied from the Preclassic through the Classic periods (2000 BC–AD 900); however, most artifacts recovered from Tipu date to the Middle and Late Postclassic periods and provide information on the site before Spanish contact (AD 900–1525) (Jones, 1989: 13). Upon Spanish contact in AD 1544, members of the Franciscan Order traveled to Tipu to convert the Maya to Catholicism (Jones, 1989; Jacobi, 2000: 6). From that time until the early 1700s when Tipu was abandoned, the Itza Maya used the town of Tipu as a shield against the expansion of Catholicism into their territories (Jones, 1989: 5). Ethnohistoric documents confirm that, while Catholicism influenced their way of life, Maya traditions were still followed by residents of Tipu for some time (Jacobi, 2000: 17).

Between AD 1544 and AD 1707, both the Maya and a small Spanish regime called the Villa of Bacalar governed Tipu intermittently (Jones, 1989: 5). In this alternating period between Maya and Spanish rule, the population at Tipu fluctuated primarily due to individuals fleeing to live in other areas, a common Maya response to crisis (Jones, 1989:
In AD 1623, the population at Tipu declined to a minimum of thirty individuals; the population then peaked in AD 1643 with 1,100 (Jones, 1989: 115). During this twenty-year period, residents were required to pay tribute, were taxed, and forced to labor under state law, therefore they arduously resisted Spanish influence (Jones, 1989: 5; Farriss, 1984: 47). Inevitably, as time passed, with more Spanish influence the Maya indigenous ways of life, specifically secular and religious ways of life, gradually disappeared (Jacobi, 2000: 18). In AD 1707, the residents of Tipu were displaced from their land by the Spanish and moved to Lake Peten Itza (Jones, 1989: 14).

**Diet at Tipu and the Effect of the Spanish Contact**

Ethnohistoric data suggesting that maize was a significant staple in the Maya diet from the pre-Classic period to the Historic period was confirmed by isotopic studies on human skeletal remains recovered from various sites (White and Schwarcz 1989; Lentz, 1999; White et al., 2006). After direct contact with the Spanish, archaeological evidence indicates that maize remained a significant component of the diet at Tipu, along with squash (Jacobi, 2000:18; Graham et al., 1989: 1255). In addition, faunal remains suggest that animals, birds, and fish were staples of the post-contact diet (Jacobi, 2000:18; Graham et al., 1989:1255).

The main staples of Maya diet were maize, beans, and squash with the form of agricultural system dependent on population size. Before European contact in the Maya lowlands, the milpa (field), or swidden agriculture was the prevailing form of subsistence practiced by the Maya until AD 250 (Farriss, 1984: 125; White and Schwarcz, 1989). During this period, a milpa was cleared and used only for a few seasons, then reforested for soil
nutrient recovery (Farriss, 1984: 125). As the population grew during the Preclassic and Classic periods, milpa agriculture could no longer sustain the people. Therefore, three additional types of agricultural systems were developed: terracing, elevated fields, and a hydraulic system (Farriss, 1984: 128). When the population declined during the Postclassic period, the Maya abandoned the more labor-intensive form of agriculture and resumed using milpa agriculture (Farriss, 1984: 128).

Each of the three stable crops contributed different nutrients to the diet of the Maya. Maize served as the main source of carbohydrates with beans and squash providing a plant source of protein (Gerry, 1993: 141; Lentz, 1999). Maize and beans provided the essential amino acids required for daily function (Lentz, 1999). Vitamins A and B were obtained from squash. In addition, squash served as a good source of potassium and calcium (Lentz, 1999). Foods (e.g. squash, sweet potatoes, guavas, palm nuts, and papaya) consumed by the Maya that provided essential nutrients required otherwise by fats, oils, fruits, meat, and vegetables (Gerry, 1993: 141).

Although plants served as a primary source of caloric intake for the Maya, animals were a significant factor in the diets of the Maya including the diet at Tipu (Emery, 1999). The three main animals consumed by the Maya were domesticated dogs, white-tailed deer, and collared peccaries (Gerry, 1993: 141; Freiwald, 2010). Access to these animals was generally easy. Domesticated dogs were raised in an enclosed area using human food waste. In addition, the Maya had access to white-tailed deer and peccaries because these animals were often found grazing in the maize fields (Gerry, 1993: 148). Other meat sources for the Maya were nine-banded armadillo, turkeys, and turtles (Gerry, 1993: 149).
From the Middle Postclassic to the Colonial period at Tipu, the reliance on armadillos and white-tailed deer in the diet increased along with small game, such as agouti and pacas, that served to replace the brocket deer, a main staple prior to contact (Emery, 1999: 70). Fish and various species of birds (e.g. turkeys and curassows) also became an important staple at Tipu post-Spanish contact (Emery, 1999: 72). The study of Lentz (1999) reports a switch from fats of New World animals to that of Old World domesticated animals in post-contact Maya diets.

At Lamanai, a site known for a strong affiliation with Tipu, both in degree of contact with the Spanish and in geographical proximity, a shift in δ¹³C values was found throughout the site’s history. From the Preclassic to the Terminal Classic period, the δ¹³C values decreased, but the values increased during the Postclassic and Historic periods. Wright and White (1996) suggest several explanations for this pattern, but attribute the change to an increased reliance on maize. Other explanations for this shift include a heavier reliance on marine fish in place of freshwater fish and a reliance on self-domesticated wild game, such as turkeys, that fed primarily on maize (Wright and White, 1996: 177). Nitrogen stable isotope ratios did not fluctuate greatly during any of the time periods, indicating that the consumption of protein sources was not affected by Spanish contact (White and Schwarcz, 1989). The stability of the nitrogen isotope ratio suggests that the source protein was sustained through occupation at Lamanai. That protein was reported to be from herbivores, with occasional contributions of protein from fish and turtles (White and Schwarcz, 1989: 467).

Bone chemistry coupled with floral and faunal analyses from sites in the Belize River Valley, including Tipu, suggest two characteristics of the diet that are different compared to
other Maya sites. First, for the Belize River Valley maize was a less prominent component of
the diet comprising less than half the diet consumed than that observed at other Maya sites
(Freiwald, 2010). Second, the diet within the Belize River Valley was more diverse, and
varied by age, sex, and status of the individual (Freiwald, 2010).

The Tipu Maya did not alter the diet in a discernable matter with contact; however,
other aspects of life did change, especially for women. Before contact the men residing at
Tipu were responsible for producing all products to pay tribute to the lords (Graham et al.,
1989). However, after Spanish contact, the role of women in economic activities
substantially increased (Clendinnen, 1982: 432). The task of making cotton cloth, which was
previously reserved for a woman’s personal use, was deemed mandatory by the Spanish. Not
only was this task now required of women but the quantity and the size of the cloth to be
produced (known as mantas) was increased (Clendinnen, 1982: 432). In addition to the
increased demand for mantas, women were responsible for producing cotton mantles
(textiles) and tending to chickens (Clendinnen, 1982: 433; Thompson, 1974: 39).

Excavation of Tipu

Tipu is located in a valley of the Dzuluinicob province, on the west bank of the Macal
River, near the Belize River in the Yucatán Peninsula, and served as the primary town in the
province (Graham, 2011; Jones, 1989: 285; Jones et al., 1986: 40; Emery, 1999). Today, the
archaeological site is known as Negroman, and consists of plots of land used for cattle
ranching (Graham, 2011; Jones, 1989: 286). In 1978, Grant Jones was inspired by Eric
Thompson’s dedication to Colonial Maya populations and used his research to find Tipu
Through the use of ethnohistorical documents, the \textit{visita} mission, or Christian outpost, was found at Negroman (Graham, 2011; Jones, 1989; Jacobi, 2000: 12).

Tipu was almost exclusively occupied by the Maya except for a circuit-riding Spanish priest that visited Tipu at the \textit{visita} mission (Cohen et al., 1997: 80). A \textit{ramada} chapel, or a rectangular church of simple design, was constructed due to Spanish influence between AD 1564–AD 1569 (Andrews, 1991; Emery, 1999: 76). Although the \textit{ramada} chapel is the specific style of church excavated at Tipu, the terms \textit{ramada} chapel, chapel, and church are used synonymously to describe the building throughout the literature on Tipu (Jacobi, 2000: 10; Andrews 1991). The \textit{ramada} chapel at Tipu is believed to have been in use from AD 1568–AD 1638 (Jacobi 2000: 14).

Excavations of the village began in the early 1980s and were conducted by Grant Jones and Robert Kautz (Graham, 2011; Cohen et al., 1997: 79; Jacobi, 2000: 13). During the first year of excavation, the \textit{ramada} chapel was discovered (Jacobi, 2000: 14). Additional excavations of the chapel were conducted from 1984 to 1987 under the direction of Mark Cohen, Elizabeth Graham, and Sharon Bennett; these later excavations yielded the skeletal population examined in this study (Graham 2011). Approximately 600 individuals were excavated within the church and from the areas around the church.

Graham also excavated burials from houses located in close proximity to the church (Cohen et al., 1997: 80). However, these individuals were not interred using Christian burial practices, but instead were buried using traditional Maya burial practices (i.e., flexed or seated with grave goods) (Cohen et al., 1997: 80). The majority of the individuals interred at Tipu (those buried within or around the \textit{ramada} chapel) were buried in an extended supine position with the traditional Christian orientation of the head lying to the west and the feet to
Almost every individual was buried in a shroud, which was suggested by the position of the feet and presence of pins found with the skeletons. One exception to the shroud burials was that of the eldest man in the cemetery who was buried in a coffin (Cohen et al., 1997: 80). Only one female was interred with a censer produced by the Maya, which, thereby, provided additional evidence of Spanish influence at Tipu because Christians do not inter the dead with ceremonial artifacts (Cohen et al., 1997: 80). Individuals of lower status were buried outside of the church with the status of the individual increasing with proximity to the altar inside of the church (Jacobi, 1997: 141). Inside the church, the higher status individuals’ graves were more evenly spaced while the lower status individuals’ were located in close proximity to one another (Cohen et al., 1997: 80).

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of Individuals Excavated</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Wall</td>
<td>47</td>
</tr>
<tr>
<td>West Wall</td>
<td>156</td>
</tr>
<tr>
<td>South Wall</td>
<td>116</td>
</tr>
<tr>
<td>Total Outside Chapel</td>
<td>319</td>
</tr>
<tr>
<td>Total Inside Chapel</td>
<td>266</td>
</tr>
</tbody>
</table>

When the chapel was excavated, burials were found inside and outside of all four walls except for the east wall (Table 2.1). The cemetery was divided into five sections to complete excavations (Jacobi, 1997: 140). These sections included two areas inside of the church and three areas outside of the church. The inside of the church was divided into front and back portions from which a total of 266 burials were excavated (Jacobi, 1997: 141). Outside of the church, areas to the north, west, and south were excavated and yielded 319 individuals. Specifically, 47 burials were removed from north of the church, 156 burials were removed from the west, and 116 burials were removed from the south (Jacobi, 1997: 141). In
total, 585 burials were excavated from the church and surrounding cemetery by Cohen and his students during several excavation seasons (Graham, 2011: 191). Of this number, 270 were complete skeletons found in their primary burial locations; approximately 100 individuals were primary burials where a portion of the skeleton had been removed from the burial by looters (Graham, 2011). The remaining 300 individuals were from disturbed primary graves and were found commingled in multiple areas around the chapel (Cohen et al., 1997: 80).

**Demographic Profile of the Tipu Maya Collection**

The bones associated with each skeleton excavated from within and outside of the church generally were well preserved compared to other Maya sites. The most intact skeletal elements include the teeth and long bones. The cranial vaults were not as well preserved and preservation of most facial bones was rare (Cohen et al., 1997: 80).

Demographic analyses were conducted under the supervision of Mark Cohen of the State University of New York at Plattsburgh, which was the first home to the Tipu skeletal collection (Graham, 2011; Jacobi, 2000: 88). Age assessments were completed for 492 of 585 individuals (Graham, 2011). Age was assessed for juveniles using tooth development, tooth eruption, fusion or nonfusion of the epiphyses, and the length of the long bone diaphyses (Jacobi, 2000: 89). To determine age for the adults, the auricular surface and pubic symphysis of the os coxae were used, along with dental attrition, cranial suture closure, and cementum annulation (Jacobi 2000, 89). Based on these analyses, Cohen determined that 220 individuals are less than or equal to 18 years of age and 272 are adults greater than or equal to 19 years of age (Jacobi, 2000: 89).
The individuals in the collection are not evenly distributed across all age groups; most individuals are between the ages of two and 40 (Jacobi, 2000: 16; Cohen et al., 1994: 124, 125). The two predominant beliefs for why subadults less than two years of age are under-represented in the skeletal population are 1) difficulty in recovering small items that are not as well preserved, and 2) religious practices that precluded a non-baptized infant from being buried at the chapel (Cohen et al. 1994: 125). Four competing hypotheses currently exist to account for the lack of adults 40 years and older in the population (Cohen et al., 1994: 126). Three of these hypotheses, which correspond well with the dates the cemetery was used, include 1) an epidemic that caused individuals to die young but would have left no evidence on the skeleton; 2) a lack of techniques to assess age in older individuals; or 3) immigration of many younger individuals into Tipu who subsequently died soon after their arrival (Cohen et al., 1994: 126).

Sex was assessed using the os coxae and skull by a team of individuals under the direction of Mark Cohen (Jacobi, 2000: 86). Individuals who displayed a mixture of both male and female nonmetric traits and metric data were assigned as probable female or probable male based on the category that had the most support (Jacobi, 2000: 86). If an individual showed an equal distribution of male and female traits, the individual’s sex was recorded as unknown (Jacobi, 2000, 86). The 585 individuals are comprised of 176 adult males, 119 adult females, and 41 adults and 239 juveniles for whom sex could not be assessed (Jacobi, 2000: 86).

The large sample size and excellent preservation of the skeletal elements excavated at Tipu have served as the basis for many bioarchaeological analyses which have contributed to
understanding the diet, health, and lifestyle of the population of Tipu. Several of these studies are reviewed below.

**Select Bioarchaeological Analyses of the Maya at Tipu**

Based on a sample of 255 individuals, including 149 males and 106 females, Danforth and colleagues (1997) assessed health in the population by examining multiple indicators of childhood and adult stress in addition to changes in reproductive behavior. They examined five indicators of childhood stress: stature, linear enamel hypoplasias, Wilson bands, porotic hyperostosis, and cribra orbitalia. Stature was calculated by applying Genoves’ (1969) formula using femoral and tibial lengths; analyses indicated that the Maya at Tipu were of similar height to the Maya from the Peten region (Danforth et al., 1997: 16). Macroscopic techniques were used to assess the number of enamel hypoplasias on the incisors and canines. Males had an average of 1.56 enamel hypoplasias per incisor and 1.92 per canine, while females displayed only 1.15 and 1.47 for incisors and canines, respectively (Danforth et al., 1997: 16). Wilson bands had a similar distribution between the sexes, with males having .44 per canine and females, .14 per canine. The frequencies of both enamel hypoplasias and Wilson bands were significantly lower in females than in males (Danforth et al., 1997: 16). Porotic hyperostosis and cribra orbitalia also were more commonly observed in men, with only porotic hyperostosis showing a significant statistical difference between the two sexes ($X^2 = 4.55, p < .05$) (Danforth et al., 1997: 16). The results of these analyses indicated that the men had more pathologies present on the skeleton than women, which the authors believed implies that women had healthier childhoods than men (Danforth et al., 1997: 18).
In the same study, Danforth and colleagues (1997) assessed adult health for the Tipu by examining teeth for dental caries and the long bones for periosteal lesions. Based on the analysis of teeth, they found the prevalence of caries was 0.92 per individual in females, which is significantly higher than the 0.71 observed for males. Alternatively, periosteal lesions of the tibiae and fibulae were more frequently observed in males than in females (Danforth et al., 1997: 18). Furthermore, by examining pits of parturition, the authors concluded that females were not bearing children in their mid-adolescent years as suggested by Landa (1941) but rather in their early 20s (Danforth et al., 1997: 20).

Based on their results, Danforth and colleagues (1997: 20) concluded that, although the Maya at Tipu were adapting to Spanish influence, the pathological profile for the population suggests that the people were biologically stressed. This conclusion supports ethnohistoric data that indicate the economic roles of women in Maya culture changed after contact and may have negatively impacted their health (Danforth et al., 1997: 14-15, 20). New responsibilities or roles for women included embroidering cloth, an activity that requires repetitive motion and may have led to activity-induced diseases (such as arthritis).

The study of Jacobi (1997) sought to understand the influence of geography, time period, and kin relationships on the Tipu through the analysis of expressed genotypes observed on teeth. Both metric and nonmetric data were recorded for the teeth to create a list of traits that would characterize an individual as a Tipu Maya (Jacobi, 1997). If unique traits could be attributed to this population, then questions regarding the migration of the people to and from the site, in addition to the possibility of interbreeding between Maya and Spanish, could be answered (Jacobi, 1997). The maxillary and mandibular dentitions were examined for nonmetric traits following standards established by the Arizona State University Dental
laboratory; metric analyses included measurements of the buccolinguial and mesiodistal
distances of the maxillary and mandibular teeth (Jacobi, 1997). Nonmetric trait analyses
indicate that 98% of the skeletons examined displayed shoveling of the central incisor, 63%
displayed double shoveling of the central incisor, 46% had a Carabelli’s cusp on the first
molar, 46% had a canine distal accessory ridge, and hypocones on the maxillary molars show
a prevalence of 99% for M\(^1\), 83% for M\(^2\), and 47% for M\(^3\) (Jacobi, 1997: 142). Other
nonmetric traits commonly observed among the Tipu were a groove on the lateral incisor,
labial curvature of the labial surface of the central incisor, enamel extensions on the second
molars, and tuberculum dentale on both incisors and canines (Jacobi, 1997: 141).

For the metric component of Jacobi’s research (1997), the statistical tests indicated
that there was no difference between the individuals buried inside versus outside the church
in mesiodistal and buccolinguial measurements; however, when a discriminant function
analysis was applied to examine the differences between males and females, the tooth
measurements were found to differ significantly between the sexes (Jacobi, 1997).

Additionally, that author concluded that none of the burials at the site belonged to individuals
of Spanish descent based on the dental nonmetric and metric traits established in this study
(Jacobi, 1997: 148). Furthermore, using the nonmetric traits, the author concluded that the
Tipu did not differ from other surrounding Maya populations (Jacobi, 1997: 148).

A second study examining enamel hypoplasias to assess the health of the Tipu after
contact was conducted by Harvey (2011) using a different subset of approximately 320
subadult and adult skeletons. The teeth of preference for this analysis were the maxillary
right central incisor and the mandibular right canine (Harvey, 2011: 66). In this study, a
description of the enamel hypoplasia was recorded using the methodology suggested in
Of the 325 individuals examined, approximately 73% had canines and 74% had incisors present for analysis (Harvey, 2011: 70). When canines were examined, 87% displayed at least one enamel hypoplasia, while 13% did not have any. Additionally, the frequency of enamel hypoplasias on the central incisors was lower, with approximately 79% displaying one lesion and 21% not showing any (Harvey, 2011: 70). To document the consequences to health with Spanish contact, Harvey (2011) compared two age groups: young adults (18–35 years of age) and older adults (35+ years of age). Her study found that the number of affected canines in young adults (of whom 90% displayed at least one lesion) was significantly greater than that of older adults (of whom 68% displayed at least one lesion). When sex differences in the frequency of enamel hypoplasias were compared, 90% of females had at least one defect present on the canine, while only 86% of males had one defect present on the canine (Harvey, 2011: 71). When incisors were analyzed, the age patterns were similar to those exhibited in the canines (Harvey, 2011: 72). When comparing adults to subadults, approximately 84% of subadults had at least one lesion present on the incisor, while approximately 78% of adults had at least one lesion present (Harvey, 2011: 71). When sex was assessed among adults, the number of affected central incisors from females was greater than the number of affected incisors for males (Harvey, 2011: 72). Therefore, Harvey (2011) found that females experienced more stress during infancy (from birth to three years) than males, which was assessed based on the frequency of hypoplasias.
The difference in the number of defects between the sexes was attributed to the Maya following a patrilineal system, whereby young males were less stressed than young females (Herndon, 1994; Harvey, 2011: 89). The data from Tipu were compared to other Post-contact Maya sites (specifically, Lamanai and Campeche). The frequency of defects was greater for the Tipu than the other pre-contact populations, a fact which suggested the population at Tipu was more stressed at the time of Spanish occupation than before contact (Harvey, 2011: 89).

The study of Murphy (2012) examined enamel hypoplasias and periostitis of long bones to determine if there was a statistically significant correlation between the two pathologies for the Tipu. The lesions present on the radii, ulnae, humerii, fibulae, tibiae, and femora were scored mild, moderate, or severe (Murphy 2012: 30). Based on a sample of 111 individuals, which included both sexes and multiple ages, approximately 54% had at least one long bone displaying signs of periosteal reactions. Of the 54%, approximately 62% were males, 59% were females, and 6% were juveniles (Murphy, 2012: 35). The frequency of periosteal reactions by age groups was 71% of the individuals over 30 years, 60% of young adults (i.e., between 18-30 years), and 25% of juveniles (Murphy, 2012: 35).

In addition to analyzing the long bones, a dental inventory was completed for each individual along with the number of enamel hypoplasias present on each tooth (Murphy, 2012: 30). At least one enamel hypoplasia was found in approximately 40% of the skeletons examined (Murphy, 2012: 35). Of those, approximately 40% were males, 44% were females, and 33% were juveniles (Murphy, 2012: 37). By age, the young adults had the highest percentage (47%) of individuals with enamel hypoplasias, while only 21% of juveniles and 46% of older adults were affected (Murphy, 2012: 37). Multiple statistical tests were
conducted to determine if differences existed between enamel hypoplasias and periosteal reactions in general, as well as differences by sex, by age, and by element. Results indicate that only the differences between males and females were significant (Murphy, 2012: 39).

To assess Spanish influence on Maya social structure, Noldner (2013) examined the cross-section morphology of long bones and the development of entheses from five pre-contact Maya populations as well as from post-contact Tipu skeletons. The post-contact individuals at Tipu were buried both within and outside of the churches (Noldner, 2013: 1). The specific placement of individuals around the church not only confirmed that the Spanish successfully converted the Maya to Christianity in this region, but suggested that a hierarchical social structure was in place (Noldner, 2013:1). The study sought to corroborate that a new, hierarchical social structure was established after contact by examining skeletal markers of activity.

Using the 3D surface scans of upper limb entheses, Noldner (2013) examined differences in enthesal morphology between the Tipu and pre-contact elite and non-elite Maya (Noldner, 2013: 72, 103, 154). Specifically, non-elite samples were examined from Caves Branch Rockshelter and Actun Uayazba Kab. Non-elite status at these sites was ascribed based on the type of grave goods, the presence or absence of cranial or dental modifications, and site structure (Noldner, 2013). The elite samples were from Baking Pot, Cahal Pech, and Je’reftheel (Noldner, 2013: 83). Elite status at these sites was ascribed based on the presence of grave goods, architecture, and placement of body (Noldner, 2013: 82). To assess social status of individuals buried at Tipu, interred both inside and outside the church, enthesis development on Tipu skeletons was compared to enthesis development on skeletons from the sites listed above. From these data, Noldner inferred that differences in enthesal
morphology and, thus, activity patterns, did not exist between females buried inside the church and those buried outside the church at Tipu (Noldner, 2013: 105). The data did not find significant differences in activity levels for men (Noldner, 2013: 103, 154). In addition, the data from that study provided some evidence that the tasks of men and women showed specialization (Noldner, 2013: 108). However, the data did not support distinct activity differences nor a differentiation of tasks between elite and non-elite Tipuans (Noldner, 2013).

Although both sexes at Tipu participated in agricultural activities, even with evidence of sexual dimorphism on the skeletons, the cross-sectional area of the long bones was similar between the sexes (Noldner, 2013: 156, 161). Additionally, there was no difference in upper-limb use based on enthesis development for individuals buried inside or outside of the church (Noldner, 2013: 108, 156). Overall, the data from that study suggest that the colonization of Tipu by the Spanish led to some form of social stratification that was different from the social structure present in the pre-contact period (Noldner, 2013: 171). Although Noldner concludes that Spanish contact led to changes in social stratification, no other studies have supported her conclusion. The Tipu were not buried with grave goods with the exception of one female who was buried with a censor, and the placement of burials could be due to the dates the Tipuans were buried (Cohen et al., 1997). In addition, numerous studies have confirmed that the health of the Tipu was not adversely affected with contact (Danforth et al., 1997; Harvey, 2011; Danforth 1991; Armstrong 1989; Cohen et al., 1989).

In summary, previous bioarchaeological research has generated much information about the impact of the Spanish on the Maya living at Tipu. However, one area that has not been examined is age at weaning. The increased economic demands placed on women after contact not only would have negatively impacted their health, but could have decreased the
time they were able to devote to infant and childcare thus, would have affected the age at weaning. Therefore, assessing age at weaning from archaeological populations can be determined using analyses of stable isotope ratios from bone collagen, tooth dentine, apatite and tooth enamel. The following sections review stable isotope analysis, their use in determining age at weaning, and previous isotopic research that has been conducted on archaeological populations.

**Stable Isotope Analyses: Definition, History, and Theory**

Stable isotopes are defined as variant forms of an element with the same atomic number, but with different atomic masses (Abrams and Wong, 2003: 5). The difference in atomic mass is due to variation in the number of neutrons in the nucleus; the number of protons remains the same. By 2008, 120 elements had been identified with approximately 3100 nuclides, or isotopes of those elements (Fry, 2006: 7). Two hundred eighty three stable isotopes have been identified; these stable isotopes are useful for research because they do not decay with time like unstable, or radioactive isotopes (Fry, 2006: 8; Katzenberg, 2007: 415). When a stable isotope analysis is conducted for oxygen, nitrogen, and carbon the results are interpreted based on comparison to an international standard. Vienna Pee Dee Belemnite (VPDB) is a carbonate standard for oxygen and carbon, whereas Standard Mean Ocean Water (SMOW) is the water standard of comparison for oxygen phosphates in bone (Faure, 1987). The standard for nitrogen is atmospheric N$_2$ (Faure, 1987; Ambrose, 1990: 435). Results of stable isotope analyses are reported as a ratio of the less abundant stable isotope to the more abundant stable isotope (Mckinney et al., 1950). The ratio of stable elements is reported based on the equation:
\[ \delta^P X = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000 \]

where \( P \) = heavy isotope mass, \( X \) = element, and \( R = \) ratio of heavy to light isotope for element (Fry, 2006).

Stable isotope analyses of lighter elements (carbon, nitrogen, oxygen, and sulfur) have been used in several disciplines to answer numerous types of research questions (Table 2.1) (e.g. Hobson and Wassenarr, 2008; Schoeninger and DeNiro, 1984; Sponheimer and Lee-Thorp, 1999; Richards and Hedges, 1999; Larsen, 1997: 270). By the mid-1970s, anthropologists became one of the later groups of researchers to incorporate stable isotopes into their research. However, chemists, physicists, and biologists had conducted stable isotope studies since the mid-1900s (Katzenberg, 2007: 414). The first element archaeologists used was carbon in the mid-1970s to reconstruct paleodiet (Katzenberg, 2007: 415). Subsequently, other elements (i.e., nitrogen, oxygen, strontium, and sulfur) were analyzed to draw inferences not only about diet, but about migration patterns and weaning practices (DeNiro and Epstein 1978, 1981; Katzenberg, 2007; Schoeninger and DeNiro, 1984).

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<th>Table 2.2. Light Stable Isotopes Used in Anthropological Research</th>
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Before one can begin to understand how isotope ratios are determined, the basic concept of mass balance as applied to stable isotopes must be understood (Fry, 2006: 194). For isotopic studies, mass balance is the notion that the masses and isotopes entering a reaction must equal the masses and isotopes resulting from the reaction (Fry, 2006: 194). Fractionation of isotopes occurs at both the atomic and macroscopic levels and is observed in closed systems (i.e., a sealed vessel) and open systems (e.g., those with continuous flow of inputs and outputs) (Fry, 2006: 196). Fractionation is defined as the difference in reaction rates for heavy versus light isotopes (Fry, 2006: 211). Chemical bonds with heavier stable isotope require more energy than lighter isotopes to form and to break bonds due to the additional neutrons and mass in the heavier isotope (Fry, 2006: 199). Different amounts of energy are required to break the bonds of heavier and lighter isotopes; therefore, the rate of bond breakage is different for each isotope, thus causes isotope fractionation or difference in the ratio of the heavier to the lighter isotope (Fry, 2006: 201). Through the process of fractionation, differences in stable isotope ratios are produced, thus allowing anthropologists to answer questions concerning diet and migration. The difference in isotopic composition between a person’s diet and a person’s tissue is reported as an average of 3‰ (parts per mil) higher (Deniro and Epstein, 1978; Schoeninger and Moore, 1992: 258). Therefore, isotopic composition of a tissue yields information on the types of plants (terrestrial vs. aquatic) and animals (terrestrial vs. aquatic) consumed at a site, in addition to, the geographic areas were the resources were extracted (Schoeninger and Moore, 1992: 258).

**Nitrogen Stable Isotopes**

Nitrogen has two stable isotopes used to assess diet in archaeological populations: the lighter stable isotope $^{14}\text{N}$, and the heavier stable isotope $^{15}\text{N}$. Most nitrogen on Earth is
present in the atmosphere or in the oceans, therefore, nitrogen isotopes are used to distinguish land versus marine for animal and plant origins (Larsen, 1997: 283). The difference in the ratio of nitrogen ($\delta^{15}N$) between marine and terrestrial plants is on average 4‰ with most plants ranging from 2‰ to 5‰ (Wright and White, 1996). The isotope ratio is higher in marine plants than in terrestrial plants (Schoeninger and DeNiro, 1984: 625). Additionally, plants that fix nitrogen through the soil will have higher ratios than plants that fix nitrogen from the air (Larsen, 1997: 283). $\delta^{15}N$ values are higher in marine vertebrates than in vertebrates that live on land (Schoeninger and DeNiro, 1984). Other factors to consider when analyzing nitrogen isotopes are the climate of the environment where the isotopes were extracted, the evaporation rate for that particular location, and the nutrients present in the soil (Larsen, 1997: 283).

Not only can nitrogen isotopes be used to assess the proportion of marine versus terrestrial food sources in an individual’s diet, but they can be used to assess age at weaning for juveniles in a population by sampling bone collagen and tooth dentin (Larsen, 1997: 284). Age at weaning is determined based on the trophic-level effect, which states that the diet of a breastfeeding subadult is isotopically heavier, depending on the stable isotope, compared to the diet of his or her mother. For subadults who are breastfed, the nitrogen isotope ratios are higher by 2‰ to 3‰ compared to adults, because breastfed infants feed on a higher trophic level (Schurr, 1997: 920; Fogel et al.; 1989). As a subadult is weaned, the nitrogen isotope ratios become similar to those of adults in the same population (Schurr, 1997: 920; Larsen, 1997: 284). Since weaning is a gradual process, the $\delta^{15}N$ values in subadults undergoing the process of weaning are expected to steadily decrease (Larsen, 1997: 284).
**Carbon Stable Isotopes**

Similar to nitrogen, carbon has two stable isotopes: $^{12}\text{C}$ and $^{13}\text{C}$. The majority of carbon present on Earth is stored in the oceans and is not used in biological processes. However, some of the carbon used in biological processes is incorporated into the plant via photosynthesis. The ratio of $^{12}\text{C}$ to $^{13}\text{C}$ in plants ($\delta^{13}\text{C}$) is determined by photosynthetic pathways used: CAM (crassulacean acid metabolism), C3, or C4. The type of photosynthesis used by plants can be determined by carbon stable isotope ratio analysis thus the carbon ratios can be used to infer the plants consumed in a diet (Dupras and Tocheri, 2007: 63; Ambrose and Norr, 1993: 2).

The climate in the area in which a plant is found may determine the type of photosynthesis used by the plant. Wheat, barley, rice, fruits, vegetables, and trees are all examples of C3 plants. C3 use the Benson-Calvin cycle and have $\delta^{13}\text{C}$ values ranging from $-22\%$ to $-33\%$ VPDB, with a mean of approximately $-27\%$ VPDB (Smith and Epstein, 1971: 64; Friewald, 2010; Wright and White, 1996). The Hatch-Slack cycle is the metabolic pathway used by C4 plants and include maize or corn, sorghum, various forms of millets, and sugar cane; their $\delta^{13}\text{C}$ values range from $-16\%$ to $-9\%$ VPDB, with a mean of approximately $-13\%$ VPDB (Smith and Epstein 1971: 64; Freiwald, 2010; Wright and White, 1996). Because $\delta^{13}\text{C}$ is higher in C4 than C3 plants, the isotope ratios reflect the type of plant(s) consumed by the population (Dupras and Tocheri, 2007: 64). Plants that use CAM include succulents and cacti; their $\delta^{13}\text{C}$ values depend on the environment and can resemble C4, C3, or a combination of both (Larsen, 1997: 272; Ambrose and Norr, 1993: 3).

One method to assess a population’s diet is based on the analysis of stable carbon isotope ratios from bone collagen and apatite, as well as from tooth dentin and enamel.
(Larsen, 1997: 272; Dupras and Tocheri, 2007: 63). A difference of $\sim +5\%$ is observed in the $\delta^{13}C$ values between plants and human collagen; a difference of $\sim +11\%$ to $12\%$ is observed in the $\delta^{13}C$ values between plants and human bone and enamel carbonate (Dupras and Tocheri, 2007: 64). Therefore, a diet consisting mainly of C4 plants should have $\delta^{13}C$ collagen values of $\sim –8\%$ (i.e., $–13\%$ to $+5\%$) and tooth enamel and bone carbonate values of $\sim –1\%$ (i.e., $–13\%$ to $+12\%$) (Dupras and Tocheri, 2007: 64). If a human consumes mainly C3 plants, he or she has collagen values of $\sim –19\%$ and tooth enamel or bone carbonate values of $\sim –12\%$ (Dupras and Tocheri, 2007: 64).

Bone collagen can be used to distinguish marine and terrestrial plant species in the diet (Larsen, 1997: 272). Marine plants display a narrower range of C3 and C4 values than the commonly observed values for terrestrial plants (Larsen, 1997: 272). Additionally, marine animals that consume marine plants display $\delta^{13}C$ values that are lower than terrestrial animals predominantly consuming C3 plants, and values are lower than animals predominantly consuming C4 plants (Larsen, 1997: 272; Schoeninger and DeNiro, 1984).

In addition to using stable carbon isotopes to understand the types of plants consumed by a population, age at weaning can be assessed using carbon isotope ratios through the trophic-level effect. The enrichment of carbon values observed for infants who are breastfeeding is $\sim 1\%$ higher than that of the mother. Carbon isotope ratios, unlike nitrogen stable isotope ratios, can be determined from bone collagen and bone apatite. Although carbon from both sources of human material yield different information with regard to adult and subadult diet, both are important for reconstructing the diet of a past population.
**Difference in $\delta^{13}$C from Collagen and Apatite**

Collagen is the main organic portion of bone, which is comprised of essential amino acids that are incorporated into the body via dietary proteins. Previous research has shown that most human tissues, on average, have relatively more $^{13}$C with $\delta^{13}$C values ranging from +5.1‰ to +6.1‰ compared to those of the animal tissues consumed in the diet (Lee-Thorp et al., 1989; Van Der Merwe and Vogel 1978; Chisholm et al., 1982). More specifically, bone collagen from humans is approximately 3‰ to 6‰ higher $\delta^{13}$C compared to the collagen from the animals that are consumed (Lee-Thorp et al., 1989).

The inorganic portion of bone is known as hydroxyapatite (or apatite); it consists predominantly of carbonate ions, which are incorporated into bone and tooth enamel through blood bicarbonate (Lee-Thorp et al., 1989: 586). Through cellular metabolism of energy substrates (e.g., ATP) blood bicarbonate is produced (Ambrose and Norr, 1993). Carbonate is extracted from apatite for stable isotope analysis, and is either absorbed by bone or is exchanged with other ions via chemical processes into bone (Ambrose and Norr, 1993). Carbon is incorporated in blood bicarbonate and therefore, bone bicarbonate, via intake of CO$_2$ gas through respiration (Ambrose and Norr, 1993).

Stable carbon isotope ratios differ between collagen and apatite and this difference is known as apatite-collagen spacing (Lee-Thorp et al., 1989: 587). The difference between isotopic values is determined by the tropic level (herbivory or carnivory) at which the animal is feeding (Lee-Thorp et al., 1989: 588). Two formulae were derived by Sullivan and Kreuger (1981) to model the relationship between apatite and collagen ratios for varying trophic levels. For herbivores, Sullivan and Kreuger (1981) report that the $\delta^{13}$C for apatite is $+7‰$ higher compared to the $\delta^{13}$C for collagen; for carnivores, $\delta^{13}$C for apatite is $+3‰$ higher.
compared to the $\delta^{13}C$ for collagen (Sullivan and Kreuger, 1981). Lee-Thorp and colleagues (1989) confirmed the apatite-collagen spacing observed in $\delta^{13}C$ values by conducting carbon stable isotope analysis of both apatite and collagen from faunal remains.

Apatite from bone and teeth preserves better than collagen; therefore, it is considered ideal for isotope analysis (Lee-Thorp et al., 1989: 586). In addition to better preservation, the $\delta^{13}C$ values of apatite, which range from $+9.6\%$ to $+13.0\%$, are higher compared to those of collagen (Lee-Thorp et al., 1989; DeNiro and Epstein, 1978; Sullivan and Kreuger, 1981). The difference between the expected value for apatite, which represents long-term diet, and for collagen, which represents the diet of the last ten years, allows a bioarchaeologist to draw inferences about the type of plants consumed by the population.

Collagen is a reflection of the protein component of a human’s diet, whereas apatite is a reflection of all components of the diet. Ambrose and Norr (1993) conducted a study alternating the proportion of proteins, carbohydrates, and lipids in the diet of rats to study carbon routing. Because rats have similar digestive physiology to humans, results from studying rats can serve as a model for human collagen routing (Ambrose and Norr, 1993). The results of this study suggest that carbon isotopic composition from rat collagen was a result dietary protein, and not from carbohydrates and lipids. This conclusion was based on the wide range, $1\%$ to $6\%$, of carbon isotopic values for each treatment when sampled from collagen. However, the range of stable carbon values was much narrower, only $1.8\%$, when rat carbonate was sampled for analysis. Hence, the authors concluded that apatite was routing carbon from lipids and carbohydrates when protein was low in the diet (Ambrose and Norr, 1993). Therefore, when assessing diet of a past population, collagen serves as a reflection of the protein component of the diet, while apatite reflects whole diet composition.
**Oxygen Stable Isotopes**

Oxygen has three stable isotopes: $^{16}\text{O}$, $^{17}\text{O}$, and $^{18}\text{O}$ (Fry, 2006). Oxygen is incorporated into the body by drinking water, eating food, and breathing oxygen into the body cavity. Oxygen then enters into the blood stream via diffusion into hemoglobin and, eventually, is incorporated into bone. In the early 1970s, paleoclimatologists used oxygen ratios extracted from mammal bone to assess climate, which paralleled the beginning of stable isotope analyses in anthropological research (Longinelli, 1984).

In anthropological studies, oxygen isotope ratios ($\delta^{18}\text{O}$) are helpful in identifying the climate of the region in which an individual resided most of his or her life; they can also suggest the age at which infants were weaned in a population (Larsen, 1997: 289; Dupras and Tocheri, 2007: 64). Oxygen isotopes can be sampled from either bone apatite or tooth apatite from enamel (Larsen, 1997: 289). However, enamel is the preferred tissue to sample because bone is a dynamic tissue that remodels throughout an individual’s life, while enamel is static (Dupras and Tocheri, 2007: 64). Research indicates that apatite from tooth enamel provides a more accurate estimate of age at weaning than bone because the crown forms in the early stages of development when a child is obtaining nutrients from breast milk (Dupras and Tocheri, 2007: 64). $\delta^{18}\text{O}$ values in breastfed individuals are higher compared to the values of adults in the population, which are believed to represent the $\delta^{18}\text{O}$ value of the local drinking water (Dupras and Tocheri, 2007: 64). Once weaning has occurred, the $\delta^{18}\text{O}$ values in subadults will gradually approach that of the adults (i.e., drinking water) (Dupras and Tocheri, 2007: 64).
Collagen: Testing for Postmortem Alterations and Quality of Preservation

Diagenesis describes postmortem changes related to the exchange of carbonate present in bone with minerals and ions from the surrounding environment (Lee-Thorp et al., 1989: 586). Collagen breakdown is due to alpha-chains breaking, which then leads to the unwinding of other peptide structures, the destruction of the collagen triple helix, and the loss of collagen to the surrounding environment (Collins et al., 1993).

The degree of degradation determines whether or not the collagen and apatite are suitable for stable isotope analysis (Klinken, 1999: 687; Tycott, 1996: 136). The most common method for assessing the preservation of collagen in archaeological bone sample is by examining the C:N ratio, which is calculated by running the sample through a continuous-flow carbon-nitrogen analyzer mass spectrometer (CN-MS) (Ambrose, 1990; Stafford et al., 1991; Klinken, 1999: 689). The acceptable C:N ratio per sample is between 3.1 and 3.5 (Klinken, 1999: 690). Methods which currently are used to test for preservation of apatite include measuring the loss of sample during preparation, measuring CO$_2$ yield during mass spectrometry, and Fourier transform infrared spectrometry (Tycott, 1996: 136).

A chemical indicator of collagen preservation in bone is the weight percentage expressed in milligrams per gram, which is a measure of collagen yield (Klinken, 1999: 689). When bone is interred, the weight percentage (“wt %”) steadily decreases from the 22 wt % collagen found in fresh bone (Klinken, 1999: 689). The temperature of the surrounding environment is a large determinant of the rate collagen is lost from bone (Klinken, 1999: 689). Once collagen reaches 0.5 wt %, the contamination cannot be removed from the bone (Klinken, 1999: 689). In addition to testing the weight percentage for collagen in general, a weight percentage for carbon and nitrogen within the collagen sample can be tested using a
CN-MS (Klinken, 1999, 689). The acceptable value for the carbon percentage of collagen is 30 wt % and the acceptable value for nitrogen is between 11% and 16 wt % (Klinken, 1999: 690).

Among other factors, temperature is a significant determinant of the preservation of collagen in bone (Klinken, 1999: 688). For example, in remains from subtropical moist areas, collagen preservation is expected to be lower than in bones found in cooler climates (Klinken, 1999: 688). Often, low percent collagen values, high C:N ratios, relatively lower δ^{13}C, and relatively higher δ^{15}N values all can indicate that the sample is low in collagen (Klinken, 1999: 690). However, when poor collagen preservation is suspected, a larger sample of bone can sometimes lessen the possibility of obtaining a C:N ratio that is too low.

Age at Weaning and Stable Isotopes

After mammals give birth and the offspring have detached from the placenta, lactation begins so that the offspring can gain nutrition along with immunity to pathogens (Humphrey, 2010: 453). At the completion or gradual reduction of lactation, the child is weaned from the breast milk of the mother (Humphrey, 2010: 453). To determine the best age to wean a child, Humphrey (2010) states that the costs of lactation versus the nutritional benefit to the young must be weighed against one another. The benefits of breastfeeding to the young are nutritional security and immunity to pathogens to which the mother has been exposed throughout her lifetime (Humphrey, 2010: 454). The costs to the mother include additional energy needed to produce the milk and the inhibition of conception until the offspring is weaned (Humphrey, 2010: 454). Weighing the costs and benefits and taking
cultural practices into consideration, the mother decides the best time to begin weaning a child.

There are four time periods of nutritional uptake before an individual is completely dependent on solid food. According to Humphrey (2010: 458), these stages include placental nutrition, exclusive suckling, mixed feeding, and fully weaned. During each of these phases, distinct isotopic signatures are left in developing bone and teeth (Humphrey, 2010: 458).

Previous research on determining age at weaning from archaeological populations have examined isotopic ratios of carbon and nitrogen from bone collagen or tooth dentin, as well as isotopic ratios of carbon and oxygen from bone apatite or tooth enamel (Schurr, 1997; Richards et al., 2002; Wright and Schwarcz, 1998; Wright and Schwarcz, 1999).

The three major macronutrients consumed during food intake are carbohydrates, proteins, and lipids. Breast milk is rich in carbohydrates and lipids, but is less rich in proteins (Whitney and Hamilton, 1984). Therefore, breast milk is enriched in the heavier carbon isotope, which then are incorporated into bone collagen and apatite, and, thus, are useful for assessing age at weaning. Maize, a C4 plant often cited as the primary weaning food used by the Maya (Landa, 1941), is high in carbohydrates, contains no lipids, and contains less protein than breast milk (Williams et al., 2005). The breast milk of animals was tested and found to reflect the whole diet; therefore, the carbon isotopic values from apatite should not vary much between the mother and infant during the weaning process (Williams et al., 2005). However, the carbon isotopic values from collagen should become lower with the weaning process because breast milk contains more protein, which is richer in carbon atoms than maize (Williams et al., 2005). Additionally, the collagen-apatite spacing should increase throughout the weaning process as the proportion of breast milk to maize changes in the
subadult’s diet (i.e., he or she progressively consumes less breast milk and more maize). Therefore, the age at weaning for a population can be determined based on the carbon stable isotope ratio from collagen ($\delta^{13}C_{\text{col}}$) and from apatite ($\delta^{13}C_{\text{ap}}$), as well as on the difference between the two stable isotope ratios ($\Delta^{13}C_{\text{ap-col}}$).

**Previous Isotopic Studies Examining Age at Weaning For Human Skeletal Material**

Analyses of carbon, nitrogen, and oxygen stable isotopes have been used to assess age at weaning for both prehistoric and historic populations for more than twenty years (Fogel et al., 1989; Schurr, 1997; Herring et al., 1998; Wright and Schwarcz, 1998; Wright and Schwarcz, 1999; Richards et al., 2002; Williams et al., 2005; Fuller et al., 2006b; Burt 2013; Bourbou, 2013). The more common methods cited for assessing age at weaning include sampling collagen from ribs and femora along with tooth dentin and enamel. Several previous studies using archaeological populations are summarized here.

Katzenberg and colleagues (1993) sampled human bone from the MacPherson site located in Ontario, Canada, for stable carbon and nitrogen isotopes to assess differences in diet by age and sex for a prehistoric maize horticulturalist population. A second aim of their research was to correlate dental disease and diet (Katzenberg et al., 1993: 268). Samples for analysis were taken from the ribs of fourteen subadults and fifteen adults consisting of nine females and six males (Katzenberg et al., 1993: 272). When $\delta^{13}C$ values were assessed by age, younger individuals’ values were more varied and more positive than those of older individuals (Katzenberg et al., 1993: 273). No statistically significant differences in $\delta^{13}C$ or $\delta^{15}N$ were found when the data were analyzed by sex (Katzenberg et al., 1993: 273). However, a highly statistically significant result ($p = 0.0001$) was found between infants aged
from birth to two and subadults aged over two (Katzenberg et al., 1993: 274). Therefore, the authors suggested an age relationship is present between isotopic values, but a sex relationship is not supported (Katzenberg et al., 1993: 278). Two subadults had circular caries, which are caused by infection due to a high carbohydrate (maize) weaning diet, and which suggested that weaning stress was present in this population (Katzenberg et al., 1993: 277). Hence, the authors suggested that the infants probably were unable to overcome episodes of infection during development, which resulted in stress (Katzenberg et al., 1993: 277). Based on the results of this study and on preceding work that used stable isotopes to assess infant diet, the groundwork was laid for the next decade of using stable isotopes to assess changes in the age at weaning, and in health for both mothers and infants due to stresses caused by European contact.

In one such study published in 1997, nitrogen isotopes were used to estimate age at weaning for a population in the lower Ohio River Valley (Schurr, 1997). The population is from the Middle Mississippian Angel site, which dates to AD 1300–1450. The femora of 23 juveniles and 46 adults were sampled for nitrogen isotopes. Based on the samples obtained, that author was able to confirm previous data which suggested δ\(^{15}\)N levels are higher while a child is breastfeeding, then decline once weaning begins. The author estimated age at weaning for this agriculturalist community to be between one and three years of age (Schurr, 1997: 923).

To assess age at weaning for the St. Thomas’ Anglican churchyard in Ontario, Canada, rib collagen was sampled from 60 subadults aged between birth and three years (Herring et al., 1998: 431). In addition to a stable isotope analysis, a biometric analysis was used to determine if infants were breastfed, and if so, the age at which the infants were
weaned (Herring et al., 1998: 436). In a biometric analysis, the cumulative infant mortality rate per thousand infants is plotted against the age at death in months of the infants (Herring et al., 1998). Based on this method, the authors found that infants were introduced to non-breast milk foods between five to six months of age (Herring et al., 1998). A plot of $\delta^{15}$N values using a distance weighted least squares curve was used to assess age at weaning (Herring et al., 1998). The curve suggested that infants were still consuming a large portion of breast milk by age two but after that age, the $\delta^{15}$N declined significantly (Herring et al., 1998). Based on both analyses, those authors concluded that a large proportion of women breastfed their infants and, by five months of age the infant’s diet was supplemented with food other than breast milk (Herring et al., 1998: 436). The stable nitrogen isotopes indicate that by 14 months of age infants were no longer receiving breast milk leading the authors to conclude that weaning was approximately a nine-month process for these 19th century Anglo-Saxons (Herring et al., 1998: 436).

Wright and Schwarcz (1998) assessed age at weaning using stable carbon and oxygen isotopes from tooth enamel carbonate. In that study, the first molars, premolars, and third molars were sampled for a total of 104 teeth from 41 skeletons found in Kaminaljuyú. Those authors found that the premolars had higher $\delta^{13}$C values compared to other tooth forms, indicating that the diet of two to six year olds (i.e., the age at which the premolar crowns formed) contained more maize than the diet of individuals less than two years (Wright and Schwarcz, 1998: 10). The first molars and premolars showed little difference in $\delta^{18}$O values, whereas the values from third molars varied more. The authors attributed this variation to individuals consuming breast milk until age six (Wright and Schwarcz, 1998: 14). However, there were a few samples in that study where the $\delta^{18}$O values between first molars and
premolars are different, suggesting a diet containing more ground water and less breast milk was being consumed after age two (Wright and Schwarcz, 1998: 14). Those authors concluded that the consumption of breast milk continued from ages two to six years, which correlates with the ethnohistoric data that indicate children were weaned at approximately four years of age (Wright and Schwarcz, 1998: 15).

Wright and Schwarcz (1999) conducted a second study assessing age at weaning in the same population using dentine collagen instead of enamel carbonate to obtain δ¹³C and δ¹⁵N values. Fifty-two additional samples were taken from premolars, first molars, and second molars. The δ¹⁵N values sampled from dentine collagen were significantly lower in the premolars than in the first molars (Wright and Schwarcz, 1999: 1165). Based on the declining δ¹⁵N values with age obtained from dentine, breast milk was the contributor of protein from birth to approximately two years, and solid foods were the main contributor of protein after two years (Wright and Schwarcz, 1999: 1167). When the data from the two studies are combined, the first molars had higher δ¹⁵N and δ¹⁸O values, while the premolars had higher δ¹³C values (Wright and Schwarcz, 1999: 1165). Therefore, when the subadults’ first molars were developing from birth to three years, the stable nitrogen and oxygen ratios were higher compared to that of later developing teeth. However, once the premolars began to develop, between two and six years of age, the nitrogen and oxygen values declined, but the carbon values increased. The authors attribute this increase to the incorporation of maize into the diet while the premolars were developing. The δ¹⁵N values for that study were higher by only ~1‰ compared to the ~2‰ to 3‰ expected. Therefore, those authors concluded that by age one, after the first molars were fully developed, but before age two, solid foods were introduced into the infant’s diet.
Richards and colleagues (2002) sampled ribs from 71 individuals aged 20 years or younger, and from 28 adults that were older than 20 years, from the Medieval Wharram Percy site. In addition, 37 tooth samples were collected, which included 22 deciduous second molars from individuals aged one and one-half to 11 years, and seven third molars and eight canines from adults (Richards et al., 2002: 206). Historical records from the medieval period suggest that infants were weaned before their second year of life (Richards et al., 2002: 209). The $\delta^{13}C$ and $\delta^{15}N$ values obtained from the rib data in this study indicate that the age at weaning for the Wharram Percy population is consistent with the medical literature (Richards et al., 2002: 209). Specifically, $\delta^{15}N$ values from infants decreased to adult values after age two. These data suggest that the infants were weaned at two years of age instead of before one year of age (Richards et al., 2002: 210). Conversely, results from the dental data were not consistent with the age at weaning suggested in the literature. In fact, no changes in $\delta^{15}N$ values were detected until after 11 years of age (Richards et al., 2002: 208). The discrepancy in the data is often seen in subadults between three to nine years of age. According to those authors, one unlikely explanation is a result of new dentine formation as the tooth develops or to the replacement of collagen, deposited during breastfeeding, with dentine (Richards et al., 2002: 209). The authors believe the most probable explanation for the discrepancy is due to changing $\delta^{15}N$ values as subadults wean from their mothers’ breast milk (Richards et al., 2002: 209).

In 2006, Fuller and colleagues assessed age at weaning in Late to Sub-Roman Britain using both stable carbon and nitrogen isotopes (Fuller et al., 2006b: 45). In this study, approximately 80 rib and femora fragments were sampled from individuals aged from birth to 45 years (Fuller et al., 2006b: 47). Similar previous studies, the carbon and nitrogen
isotopic values were higher in younger individuals (suggesting they were not yet weaned), but were equivalent to those of reproductive females in the older subadults (Fuller et al., 2006b: 47). The carbon isotopic values for individuals between two and 12 years of age were lower than those for adults, which implied different food sources for adults than that for weaned subadults (Fuller et al., 2006b: 47). In that study, the authors concluded that age at weaning was between two and four years (Fuller et al., 2006b: 48).

In a more recent study, Burt (2013) extracted rib collagen from 62 individuals (51 juveniles and 11 adults) to analyze carbon and nitrogen isotope ratios to assess the age of weaning for individuals from the Fishergate House cemetery in York, United Kingdom. Individuals dating to the mid-14th and mid-15th centuries from birth to five or six years of age, and females who were of age to reproduce (18-35), were sampled in an attempt to reconstruct subadult diet (Burt, 2013: 407). Based on the isotope analysis, that study showed that age at weaning was between one and one-half to two years of age; this conclusion was signaled by the decrease in $\delta^{13}$C and $\delta^{15}$N isotope values in juveniles older than two years of age (Burt, 2013: 413).

Bourbou (2013) conducted a study to assess age at weaning for a Greek Byzantine sample of juveniles from multiple sites; her analyses were based on bone samples from 61 individuals less than 13 years of age. Based on ethnographic data, the age at weaning for the Greek Byzantine population was thought to be later than that suggested by isotope analysis from other sites, and occurred at four years of age instead of two (Bourbou, 2013: 3903). The goal of Bourbou’s study was to determine whether or not the age at weaning suggested by ethnographic data was consistent with the age suggested by stable carbon and nitrogen isotopic analyses. Because the data collected represent more than one population, the stable
isotopic values from the juveniles were compared to the mean stable isotopic values for adult females from the juveniles’ sites (Bourbou, 2013: 3906). Although the juvenile data do not show the typical elevation above those of the mothers’ (i.e., the females’), Bourbou (2013) still was able to assess age at weaning for the Greek Byzantine population. The trend observed from both stable carbon and nitrogen isotopes is that, before age three, the isotopic values are higher when compared to the adult females. However, after age three, the values fall within the range of the mothers’ values (Bourbou, 2013: 3908). Therefore, results from that study support the ethnographic data, suggesting that infants were fully weaned at four years of age (Bourbou, 2013: 3908).

One last study assessed age at weaning in two Maya populations and provides data for developing the hypotheses, as well as the analytical methods, used in the current research. Williams and colleagues (2005) assessed age at weaning for two Postclassic Maya populations using five isotopic values: stable carbon isotopes from collagen and apatite, the difference between carbon values of collagen and apatite, stable nitrogen isotopes from collagen, and stable oxygen isotopes from apatite. The two populations examined in that study were from the Marco Gonzalez site (occupied from 100 BC–AD 1350) and the San Pedro site (occupied from AD 1440–AD 1650) (Williams et al., 2005: 782). A total of 67 individuals from both sites were sampled to conduct the stable isotope analyses. The ethnographic data, which suggest age at weaning occurred between three and four years of age for both sites, is supported by the stable isotope analysis (Williams et al., 2005: 788). Similar to results from previous studies, the stable nitrogen isotopic values at these sites were enriched for infants by 2‰ to 4‰, the carbon stable isotopic values were higher by 1‰, and
the oxygen stable isotopic values were higher for infants than for the mothers (Williams et al., 2005).

Summary

As the review of the studies above illustrates, age at weaning can be assessed from both prehistoric and historic populations in both temperate and tropical regions utilizing a variety of methods. In the current study, carbon, nitrogen, and oxygen isotopes obtained from rib collagen and apatite were used to assess age at weaning for the Maya population living at Tipu.
Chapter Three: Materials and Methods

To assess age at weaning for the Tipu population, I examined stable carbon and nitrogen isotope ratios from bone collagen, and stable carbon and oxygen isotope ratios from bone apatite. I analyzed adult and subadult skeletons from the Tipu Maya collection, which currently is curated under the care of Dr. Marie Danforth at the Physical Anthropology Laboratory, University of Southern Mississippi. Prior to beginning this research, I received permission to perform destructive analyses on the collection from the Archaeology Institute of Belize. To select my samples, Dr. Danforth provided me with a demographic profile of the collection. I traveled to the University of Southern Mississippi where I was able to examine the preservation of the remains and to choose the individuals for inclusion in this study.

For stable isotope sampling, I chose rib and rib fragments from a total of 25 individuals, including 20 subadults of varying age groups and five adult females of reproductive age (18-45 years of age). The specific age categories and the number of individuals in each group are presented in Table 3.1.

<table>
<thead>
<tr>
<th>Age Category</th>
<th>Number of Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 year old</td>
<td>4</td>
</tr>
<tr>
<td>1-2 years old</td>
<td>4</td>
</tr>
<tr>
<td>2-4 years old</td>
<td>4</td>
</tr>
<tr>
<td>3-5 years old</td>
<td>3</td>
</tr>
<tr>
<td>4-6 years old</td>
<td>3</td>
</tr>
<tr>
<td>5-7 years old</td>
<td>2</td>
</tr>
<tr>
<td>Adult Females</td>
<td>5</td>
</tr>
</tbody>
</table>

Approximately one to three grams of bone were collected from each individual (Appendix A). Whenever possible, adult and older subadult samples were selected from ribs that were already fragmented; entire ribs were required for the younger subadult samples. Each sample was stored in a paper bag and labeled with an identification number.
For sample preparation, I traveled to the University of Florida (UF) Department of Anthropology Bone Chemistry Lab, where I worked under the direction of Dr. John Krigbaum. Samples were processed according to the laboratory’s established protocols for cleaning bones, and for preparing collagen and apatite samples. The methods are provided in Appendices C and D, respectively (Ambrose, 1990). However, the protocol included scraping the bone to remove surface contaminants, splitting the samples to remove trabeculae, grinding the bone into powder, and processing the powder to extract apatite and collagen isomorphs. Once the samples were prepared, I took them to the UF Department of Geological Sciences Light Stable Isotope Mass Spec Lab for analysis. Dr. Jason Curtis ran the prepared bone apatite samples through a Finnigan MAT 252 Isotope Ratio Mass Spectrometer (IRMS) and the prepared bone collagen samples were run through a Thermo Delta V Advantage IRMS. Results of these analyses were forwarded to me by Dr. Krigbaum. Data I received for each sample included the percent collagen yield, the C:N ratios to assess diagenesis, the precision of the USGS40 standards (δ¹⁵N = 0.19 and δ¹³C = 0.09) and NBS-19 standards (δ¹³C = 0.026 and δ¹⁸O = 0.045). The values for δ¹⁵N_{col}, δ¹³C_{col}, δ¹³C_{ap}, and δ¹⁸O_{ap} were calculated by Dr. Krigbaum, and from these data, I was able to calculate the Δ¹³C_{ap-col}.

Before age at weaning was assessed, two questions were formulated to guide me through statistical analyses as follows:

1. Is there a significant difference (p < 0.05) among the stable isotope means (δ¹⁵N_{col}, δ¹³C_{col}, δ¹³C_{ap}, Δ¹³C_{ap-col} and δ¹⁸O_{ap}) for the four subadult categories (<1 year old, 1-2 years old, 2-4 years old, and 4-6 years old)?
H1: There is a statistically significant difference among the stable isotope means ($\delta^{15}N_{col}$, $\delta^{13}C_{col}$, $\delta^{13}C_{ap}$, $\Delta^{13}C_{ap-col}$ and $\delta^{18}O_{ap}$) for the four subadult categories.

H0: There is no statistically significant difference among the stable isotope means ($\delta^{15}N_{col}$, $\delta^{13}C_{col}$, $\delta^{13}C_{ap}$, $\Delta^{13}C_{ap-col}$ and $\delta^{18}O_{ap}$) for the four subadult categories.

2. Are there significant differences ($p < 0.05$) between the stable isotope means ($\delta^{15}N_{col}$, $\delta^{13}C_{col}$, $\delta^{13}C_{ap}$, $\Delta^{13}C_{ap-col}$ and $\delta^{18}O_{ap}$) for the adult females and each subadult group (<1 year old, 1–2 years old, 2–4 years old, and 4–6 years old)?

H1: There is a statistically significant difference between the stable isotope means for the subadult categories and the adult females.

H0: There is no statistically significant difference between the stable isotope means per ratio tested for the subadult categories and the adult females.

Multiple statistical analyses were conducted on the data to address the questions above and to assess age at weaning. First, descriptive statistics including the mean and standard error of the mean were calculated for each stable isotope ratio, and for the apatite collagen spacing, for all age categories. Second, one-way ANOVA tests were used to assess variation in isotope ratios among several of the subadult age groups. The age categories I compared statistically were those with non-overlapping ranges: <1 year old, 1–2 years old, 2–4 years old, and 4–6 years old. Specifically, for these tests, the null hypothesis was that
there would not be a statistically significant difference among the means for the subadult categories. The alternative hypothesis was that there would be a statistically significant difference among the four subadult category means. Third, confidence intervals (CI) at one sigma (62% confidence) and two-sigma (95% confidence) were calculated for all age groups to determine the probability that, if resampling were to occur for this population, the new mean would fall within the range for this sample. The CIs for the four subadult categories listed above then were compared against the CI for the adult females at both sigma levels. Fourth, paired t-tests were used to assess differences in the means between each of the four, non-overlapping subadult age groups and the adult females. For these tests, specifically, the null hypothesis was that there would not be a statistically significant difference between the adult female mean and each of the means for the four subadult categories. The alternative hypothesis was that there would be a statistically significant difference between the means of the subadult categories and the adult female mean. Fifth, $\delta^{15}\text{N}$ values for the adult mean and for all subadults were plotted against the $\delta^{13}\text{C}_{\text{col}}$ values to help determine the age at which individuals in the population were weaned. For all statistical analyses, if the p-value was less than 0.05, the values of each group were considered to be statistically different.

**Expectations for Interpreting Data**

All of the above information was used to suggest the age at which Tipu subadults were weaned. For subadults, a diet consisting solely of breast milk is indicated by the following: a $\delta^{13}\text{C}_{\text{col}}$ value ~1‰ higher than the female mean and a $\delta^{15}\text{N}_{\text{col}}$ value ~2‰ to 3‰ higher than the female mean. A subadult whose values are comparable to those obtained for the adults would be considered fully weaned. Individuals whose values fell between these
parameters would be interpreted as going through the process of weaning. Because both breast milk and maize are rich in carbohydrates, and less rich in lipids and proteins, the carbon composition is similar. Therefore, the $\delta^{13}C_{ap}$ values should vary little during the weaning process. The collagen-apatite spacing ($\Delta^{13}C_{ap-coal}$) should increase during the weaning process because breast milk consumption decreases (and breast milk is more rich in protein than maize). The oxygen isotopic values are expected to decrease through the weaning process as the subadult consumes less breast milk and more foods, which have incorporated water from the environment.
Chapter Four: Results

Results of the analyses assessing bone quality (diagenesis and collagen preservation) are presented in Table 4.1. All of the C:N ratios fall between 3.1–3.5, indicating diagensis did not significantly alter the preservation of bone collagen for each sample. Therefore, all samples were eligible for data analysis. All of the nitrogen weight percents are between approximately 11% and 16%, and all of the carbon weight percents are above 30%. Thus, all 25 samples are viable and were included in statistical analyses.

Table 4.1. Bone Quality Indicators for Sample by Burial Number and Age

<table>
<thead>
<tr>
<th>Burial</th>
<th>Age (years)</th>
<th>C:N Ratio</th>
<th>wt %N</th>
<th>wt %C</th>
</tr>
</thead>
<tbody>
<tr>
<td>449</td>
<td>0.5-1</td>
<td>3.2</td>
<td>14.94</td>
<td>41.26</td>
</tr>
<tr>
<td>399</td>
<td>0.5-1</td>
<td>3.2</td>
<td>15.49</td>
<td>42.86</td>
</tr>
<tr>
<td>447</td>
<td>0.5-1.5</td>
<td>3.2</td>
<td>15.00</td>
<td>41.67</td>
</tr>
<tr>
<td>433</td>
<td>0.8-1</td>
<td>3.3</td>
<td>14.69</td>
<td>41.02</td>
</tr>
<tr>
<td>346</td>
<td>1-3</td>
<td>3.3</td>
<td>11.59</td>
<td>32.30</td>
</tr>
<tr>
<td>454</td>
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<td>15.09</td>
<td>41.96</td>
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<tr>
<td>481</td>
<td>1-2</td>
<td>3.2</td>
<td>13.20</td>
<td>36.30</td>
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<tr>
<td>435</td>
<td>1-2</td>
<td>3.2</td>
<td>14.81</td>
<td>41.43</td>
</tr>
<tr>
<td>431</td>
<td>2-4</td>
<td>3.2</td>
<td>15.16</td>
<td>42.12</td>
</tr>
<tr>
<td>69B</td>
<td>2-4</td>
<td>3.2</td>
<td>15.49</td>
<td>43.01</td>
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<tr>
<td>167</td>
<td>2-4</td>
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<td>14.85</td>
<td>41.13</td>
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<tr>
<td>237</td>
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<td>3.2</td>
<td>12.81</td>
<td>35.11</td>
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<tr>
<td>140</td>
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<td>15.14</td>
<td>42.13</td>
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<td>3-5</td>
<td>3.2</td>
<td>15.33</td>
<td>42.04</td>
</tr>
<tr>
<td>253B</td>
<td>3-5</td>
<td>3.2</td>
<td>15.35</td>
<td>41.93</td>
</tr>
<tr>
<td>361</td>
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<td>5-7</td>
<td>3.2</td>
<td>15.39</td>
<td>42.03</td>
</tr>
<tr>
<td>181</td>
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<td>3.2</td>
<td>11.25</td>
<td>31.22</td>
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<td>193</td>
<td>20-25</td>
<td>3.2</td>
<td>13.74</td>
<td>37.72</td>
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<tr>
<td>128</td>
<td>25-30</td>
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<td>507</td>
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<td>3.2</td>
<td>15.32</td>
<td>41.94</td>
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<tr>
<td>136</td>
<td>20-30</td>
<td>3.3</td>
<td>10.86</td>
<td>30.57</td>
</tr>
</tbody>
</table>
For each burial number, the estimated age at death, along with the stable nitrogen and carbon isotopic values from collagen (δ^{15}N_{col} and δ^{13}C_{col}), the stable carbon and oxygen isotopic values from apatite (δ^{13}C_{ap} and δ^{18}O_{ap}), and the change in carbon abundance between apatite and collagen (Δ^{13}C_{ap-col}), are listed in Table 4.2.

Table 4.2. Isotope Ratios for Sample by Burial Number and Age

<table>
<thead>
<tr>
<th>Burial</th>
<th>Age (years)</th>
<th>δ^{15}N_{col} ‰</th>
<th>δ^{13}C_{col} ‰</th>
<th>δ^{13}C_{ap} ‰</th>
<th>Δ^{13}C_{ap-col} ‰</th>
<th>δ^{18}O_{ap} ‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>449</td>
<td>0.5-1</td>
<td>10.5</td>
<td>-7.8</td>
<td>-7.2</td>
<td>0.6</td>
<td>-2.6</td>
</tr>
<tr>
<td>399</td>
<td>0.5-1</td>
<td>9.9</td>
<td>-9.1</td>
<td>-5.8</td>
<td>3.2</td>
<td>-2.5</td>
</tr>
<tr>
<td>447</td>
<td>0.5-1.5</td>
<td>11.9</td>
<td>-7.5</td>
<td>-4.8</td>
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<tr>
<td>433</td>
<td>0.8-1</td>
<td>11.0</td>
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<td>-2.7</td>
</tr>
<tr>
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<td>-7.0</td>
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<td>-3.1</td>
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<tr>
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<td>-2.5</td>
</tr>
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<td>-4.4</td>
<td>5.5</td>
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<td>1-2</td>
<td>11.1</td>
<td>-7.6</td>
<td>-5.7</td>
<td>1.9</td>
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<td>2-4</td>
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<td>-5.7</td>
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<td>-2.6</td>
</tr>
<tr>
<td>69B</td>
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<td>-5.7</td>
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<td>-6.9</td>
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<td>-2.9</td>
</tr>
<tr>
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<td>-6.4</td>
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<td>-3.5</td>
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<td>-5.5</td>
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<tr>
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<td>-2.0</td>
</tr>
<tr>
<td>361</td>
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<td>-9.5</td>
<td>-5.6</td>
<td>3.9</td>
<td>-3.2</td>
</tr>
<tr>
<td>411</td>
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<td>-10.3</td>
<td>-5.4</td>
<td>4.9</td>
<td>-3.1</td>
</tr>
<tr>
<td>473</td>
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<td>-6.0</td>
<td>4.4</td>
<td>-2.7</td>
</tr>
<tr>
<td>434</td>
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<td>9.1</td>
<td>-10.3</td>
<td>-4.9</td>
<td>5.4</td>
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<td>-10.5</td>
<td>-6.1</td>
<td>4.4</td>
<td>-2.6</td>
</tr>
<tr>
<td>193</td>
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<td>-3.9</td>
<td>5.9</td>
<td>-2.7</td>
</tr>
<tr>
<td>128</td>
<td>25-30</td>
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<td>-9.5</td>
<td>-6.1</td>
<td>3.4</td>
<td>-3.6</td>
</tr>
<tr>
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<td>-9.3</td>
<td>-6.9</td>
<td>2.4</td>
<td>-3.3</td>
</tr>
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<td>-10.4</td>
<td>-7.7</td>
<td>2.7</td>
<td>-3.0</td>
</tr>
<tr>
<td>136</td>
<td>20-30</td>
<td>8.9</td>
<td>-10.6</td>
<td>-4.6</td>
<td>6.0</td>
<td>-3.5</td>
</tr>
</tbody>
</table>
The mean and standard error were calculated for each age category and are presented in Table 4.3. The mean $\delta^{15}N_{\text{col}}$ for individuals less than one year of age is exactly 2‰ higher than the mean adult female value, and the mean $\delta^{13}C_{\text{col}}$ for subadults less than one year of age is $\sim 2$‰ higher than that of the mean adult female (Table 4.3). The difference in $\delta^{13}C_{\text{ap}}$ values between each subadult category and the mean adult females varied between 0.1‰ to 0.6‰. The smallest difference in $\Delta^{13}C_{\text{ap}}$-col is observed in the individuals less than one year of age; this difference generally increases with age. The maximum mean for $\Delta^{13}C_{\text{ap}}$-col (4.9) was observed for individuals five to seven years of age. All mean $\delta^{18}O_{\text{ap}}$ are elevated above the adult female mean.

Table 4.3. Mean and Standard Error of the Mean for Age Categories

<table>
<thead>
<tr>
<th>Age Category</th>
<th>$\delta^{15}N_{\text{col}}$ %</th>
<th>$\delta^{13}C_{\text{col}}$ %</th>
<th>$\delta^{13}C_{\text{ap}}$ %</th>
<th>$\Delta^{13}C_{\text{ap}}$-col %</th>
<th>$\delta^{18}O_{\text{ap}}$ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 yr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>10.8</td>
<td>-8.1</td>
<td>-5.9</td>
<td>2.3</td>
<td>-2.5</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.4</td>
<td>0.3</td>
<td>0.5</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>1–2 yrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.3</td>
<td>-9.6</td>
<td>-5.7</td>
<td>3.9</td>
<td>-2.7</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.6</td>
<td>0.7</td>
<td>0.5</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>2–4 yrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>10.2</td>
<td>-9.5</td>
<td>-6.2</td>
<td>3.3</td>
<td>-3.1</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.9</td>
<td>0.3</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>3–5 yrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.0</td>
<td>-10.0</td>
<td>-5.9</td>
<td>4.2</td>
<td>-2.8</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.5</td>
<td>0.6</td>
<td>0.2</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>4–6 yrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.7</td>
<td>-10.1</td>
<td>-5.7</td>
<td>4.4</td>
<td>-3.0</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.1</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>5–7 yrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.9</td>
<td>-10.4</td>
<td>-5.5</td>
<td>4.9</td>
<td>-3.0</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.2</td>
<td>0.1</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>18–45 yrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.8</td>
<td>-9.8</td>
<td>-6.1</td>
<td>3.6</td>
<td>-3.2</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.1</td>
<td>0.2</td>
<td>0.7</td>
<td>0.7</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Table 4.4 shows results from the ANOVA tests assessing whether variation in isotopic values and in apatite-collagen spacing among the selected subadult age categories was significant. None of the differences are significant.

<table>
<thead>
<tr>
<th></th>
<th>Statistical Test: One-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta^{15}N_{col} %$</td>
</tr>
<tr>
<td>F value</td>
<td>F=2.214</td>
</tr>
<tr>
<td>p value</td>
<td>p=0.155</td>
</tr>
</tbody>
</table>

Figures 4.1 through 4.5 depict the mean and standard error bars at 62% confidence intervals (CIs) for each isotopic value and the apatite-collagen spacing for the adults and selected subadult age categories. While non-overlapping groups may indicate statistically significant variation, overlap does not necessarily indicate the results are not statistically significant. Therefore, t-tests were run to determine if the variation between each group was significant. Results of the t-tests are reported in Table 4.5.

<table>
<thead>
<tr>
<th>Age Category</th>
<th>Statistical Test: T-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta^{15}N_{col} %$</td>
</tr>
<tr>
<td>&lt;1 year old</td>
<td>p=0.001</td>
</tr>
<tr>
<td></td>
<td>t=14.3</td>
</tr>
<tr>
<td>1–2 years</td>
<td>p=0.357</td>
</tr>
<tr>
<td></td>
<td>t=-27.3</td>
</tr>
<tr>
<td>2–4 years</td>
<td>p=0.116</td>
</tr>
<tr>
<td></td>
<td>t=14.3</td>
</tr>
<tr>
<td>4–6 years</td>
<td>p=0.713</td>
</tr>
<tr>
<td></td>
<td>t=-35.7</td>
</tr>
</tbody>
</table>
For $\delta^{15}\text{N}_{\text{col}}$, (Figure 4.1), with a 62% CI, the standard error of the mean bars for both the four to six year olds and the one to two year olds overlap with the adults. Overlap was not observed between the adults and individuals less than one year old, and between adults and individuals aged two to four years. With a 95% CI, all subadult categories overlapped with the adults, except for individuals less than one year of age. When these values were tested statistically, only the difference between adults and subadults less than one year was significant ($p=0.001$); differences between adult females and other age groups were not significant (one to two years of age ($p=0.357$), two to four years of age ($p=0.116$), four to six years of age ($p=0.713$)).

Figure 4.1. Age category versus mean $\delta^{15}\text{N}_{\text{col}}$ with standard error of the mean.
For $\delta^{13}C_{Col}$ (Figure 4.2), with a 62% CI, the standard error of the mean bars overlap between the adults and the four to six year olds, two to four year olds, and one to two year olds. Overlap was not observed between the error bars for the adult females and for individuals less than one year of age. With a 95% CI, all subadult categories overlap with the adults, except for individuals less than one year of age. A statistically significant difference exists between the adult female mean and the subadults less than one year old ($p=0.004$), but a significant difference was not found between the adult female mean and individuals one to two years of age ($p=0.649$), two to four years of age ($p=0.317$), or four to six years of age ($p=0.723$).

![Figure 4.2. Age category versus mean $\delta^{13}C_{Col}$ with standard error of the mean.](image)

For $\delta^{13}C_{ap}$ (Figure 4.3), with both the 62% and 95% CIs, the standard error of the mean bars overlap between the adult female category and all subadult age categories. No statistical differences were found between the adult females and subadults for any category.
(less than one year old (p=0.970), one to two years old (p=0.925), two to four years old (p=0.702), or four to six years old (p=0.861)).

![Figure 4.3](image)

**Figure 4.3.** Age category versus mean δ¹³C with standard error of the mean.

For Δ¹³C₀₋ᵥ (Figure 4.4), with a 62% CI, the standard error of the mean bars overlap between the adult female category and the subadults aged four to six years, two to four years, and one to two years. With a 95% CI, the adult female error bars overlap with the error bars for the infants less than one year of age. No statistically significant differences were found between adult females and any of the subadult groups (less than one year [p=0.115], one to two years [p=0.840], two to four years [p=0.428], or four to six years [p=0.773]).
For $\delta^{18}\text{O}_{\text{ap}}$ (Figure 4.5), with a 62% CI, the standard error of the mean bars overlap between the adult female category and the subadults ages four to six years and two to four years. Overlap was not observed between the adult females and the two younger age categories. With a 95% CI, the error bars overlap between the adult female category and all subadults except those less than one year of age. A statistically significant difference was found between the adult females and individuals less than one (p=0.011) and one to two years of age (p=0.049); no significant difference was found between the adult females and the older subadult (two to four years (p=0.738), four to six years (p=0.408)).
Lastly, Figure 4.6 shows the plot of the values of $\delta^{15}$N versus $\delta^{13}$C for each subadult with respect to the mean of the adult females’ isotopic values. The subadults in the oval are subadults whose stable carbon and nitrogen isotopic values displayed the trophic level effect (i.e., all five individuals’ values are elevated above the female mean by at least 1‰ for $\delta^{13}$C and 2‰ for $\delta^{15}$N). For $\delta^{13}$C, the subadults’ breastfeeding displayed a 2‰ elevation above the adult females, whereas the expected 2‰ to 3‰ elevation above the mother’s isotopic values was observed for $\delta^{15}$N. Three of these individuals are subadults less than one year old at death, one was between one and two years old, and one was between two and four years old.
Figure 4.6. $\delta^{13}$C and $\delta^{15}$N values (in parts per mil) for the twenty subadults and five adult females of reproductive age. Black rectangles show the expected trophic level effect for breastfeeding subadults.
Chapter Five: Discussion

The current study examined age at weaning using stable isotopic analyses from a subset of 20 subadults less than seven years of age and five females of reproductive age from the Maya site of Tipu in Belize. Rib fragments were sampled and analyzed for stable carbon, nitrogen, and oxygen isotope ratios. Statistical analyses were used to assess variation among different age categories of subadults and between adult females and each age category of subadult. The following chapter discusses the results above and their implication for the age at weaning for subadults at Tipu post Spanish contact. Additionally, data from this study are considered with respect to other bioarchaeological data from Tipu and to isotope data on age at weaning for other Maya sites.

Stable Nitrogen Values from Collagen

No statistical difference among the subadult categories was observed for δ¹⁵N values according to the one-way ANOVA test; however, results from the t-test suggest that a significant difference existed between the value for adults and for individuals less than one year of age. The mean for subadults less than one year was 10.8‰, which was the highest δ¹⁵N value of the six subadult age categories. This mean was exactly 2‰ higher, or one trophic level above, the mean for the adult females (8.8‰). This difference suggests that the individuals aged less than one year generally were still breastfeeding at the time of death. However, when the δ¹⁵N value from each subadult in this age category is examined individually, two individuals (449 and 399) do not meet the 2‰ to 3‰ standard indicative of breastfeeding. Instead, these individuals are enriched 1.1‰ and 1.7‰, respectively, above the mean for females. These lower values suggest that these two individuals had begun
weaning from the mother’s breast milk before death. Of the remaining two individuals in this age category, one (447) has a δ¹⁵N 3.1‰ higher than the female mean, which is elevated above the expected range for infants feeding on a mother’s breast milk. Stable nitrogen isotopic values elevated above one trophic level suggest either that the isotopic signature of breastfeeding had not yet been incorporated into the skeleton of the infant before death, or that breastfeeding had not begun before death. Using infant fingernail clippings, Fogel and colleagues (1989), found that stable nitrogen isotopes take three to five months to incorporate into the nails. Similarly, Herrscher (2003) found that the diet of an infant is incorporated into his or her bones and teeth between three to eight months of age. Thus, the isotopic value for infant 447 (aged between six months and one and one-half years) may indicate an age closer to the lower end of the age range. The fourth individual (Burial 433) has a stable nitrogen isotopic value elevated 2.2‰ above the female mean; this value suggests this infant, aged between approximately ten months to one year of age, was breastfeeding at time of death.

The means for the other five age categories steadily decline as age increases, with the exception of the individuals two to four years old. The average isotopic value for this age category is 10.2‰, which is 1.1‰ higher than the mean of the one to two years old (9.3‰). By examining the individual values, however, it is evident that the disparity arises from one individual (Burial 237) with an anomalously high value (12.3‰). This value is elevated 3.5‰ above the female mean, which indicates this individual was still breastfeeding at the time of death. Two of the remaining three individuals in this age category have δ¹⁵N values that follow the expected trend for the weaning process; the third individual has a slightly elevated value (Burial 431 at 10.4‰). Otherwise, the δ¹⁵N means continue to decline with
age; the 5-7 year olds have an average $\delta^{15}\text{N}$ value of 8.9‰, which is only 0.1‰ above the mean female value.

The plot of the confidence intervals in Figure 4.1 depict, and statistical analyses comparing the means between the various subadult age groups and the adults confirm, that only the youngest age group has a significantly different $\delta^{15}\text{N}$ value. These data suggest that the diet of the individuals in the youngest age group was different from the adults and other subadult groups. Specifically, based on $\delta^{15}\text{N}$, the individuals who died at less than one year of age were breastfeeding, while subadults older than one year (with the exception of Burial 237) had begun the process of weaning and were consuming other foods in addition to breast milk.

**Stable Carbon Values from Collagen**

Similar to the results from nitrogen, no significant difference was found among the four subadult categories for $\delta^{13}\text{C}_{\text{col}}$ using the one-way ANOVA. However, the t-test results supported a significant difference between the adults and individuals less than one year of age. When $\delta^{13}\text{C}_{\text{col}}$ values were examined individually, three subadults in the youngest age category were enriched by over 1.5‰ compared to the adults (the highest, Burial 447, was elevated by 2.3‰); the fourth individual (Burial 399) was elevated only by 0.8‰. As noted above, this individual also had a nitrogen value that was below the expected level for full breastfeeding. The $\delta^{13}\text{C}_{\text{col}}$ value confirms that this individual had begun the process of weaning prior to death.

As with nitrogen, the plot of the confidence intervals for carbon (from collagen) depicted in Figure 4.2, and the statistical analyses comparing the means between the various
subadult age groups and the adults, all confirm that only the youngest age group had a significantly different $\delta^{13}C_{\text{col}}$ value. Again, these data suggest that the diet of the individuals in the youngest age group was different from the adults and other subadult groups.

The steady decrease in $\delta^{13}C_{\text{col}}$ values with age shows that, once weaning began around one year of age, the diet was increasingly supplemented with other foods until the process was complete, perhaps between the ages of two and three. Additionally, the individuals in the sample between three and seven years of age had average isotopic values slightly below the mean female value (ranging from 0.2‰ below the mean in the three to five year olds to 0.6‰ in the five to seven year olds). These data suggest that the types of plants consumed by the adult females and young children may have differed. According to Tozzer (1941), the main weaning food of the Maya was maize. The high $\delta^{13}C_{\text{col}}$ values of the adults and the subadults found in this study are consistent with a diet containing maize.

The ~2‰ elevation in the $\delta^{13}C_{\text{col}}$ values, as depicted in Figure 4.6, did not follow the predicted ~1‰ elevation as cited in previous literature. The additional 1‰ elevation is attributed to the sensitive nature of $\delta^{13}C$ values with fluctuations in the diet of an individual (Dupras 2010; Fuller et al. 2006a). Therefore, the $\delta^{13}C_{\text{col}}$ values were elevated due to the changing sources of carbon in the diets of the mothers, which resulted in a broad range of carbon isotopic values for the mothers (Dupras 2010; Fuller et al. 2006a).

**Stable Carbon Values from Apatite**

The mean $\delta^{13}C_{\text{ap}}$ values for each of the six subadult age categories were elevated above the mean for the adult females by 0.1‰ to 0.6‰. None of these differences (either among the age groups (ANOVA) or between the adults and subadults (t-tests) were
significant. The minimal variation in $\delta^{13}C_{ap}$ values is expected because the carbon isotope signature of breast milk and weaning foods is similar. Maize is high in carbohydrates, which is a macronutrient with high carbon content, few lipids, and little protein (DeNiro and Epstein, 1978). Breast milk is high in lipids and carbohydrates, which both contain carbon atoms (DeNiro and Epstein, 1978). Carbon from bone apatite is incorporated from the whole diet. If one’s diet consists solely of breast milk (e.g., individuals less than one year old), and the isotope signature of breast milk is similar to the primary food for the adults or to the weaning food for the other subadults (i.e., maize), then only slight differences should be apparent among the age groups. The results from the Tipu are consistent with this expectation.

**Change in Stable Carbon Values from Apatite and Collagen**

The subadults less than one year of age display the smallest difference (2.3‰) in stable carbon isotopic values between collagen and apatite ($\Delta^{13}C_{ap-col}$), while the individuals’ five to seven years of age display the largest difference (4.9‰). However, neither the ANOVA, nor the t-test found statistically significant differences among any of the subadult categories, or between the subadult and adult values. Since apatite reflects the whole diet of the individual and collagen reflects the protein portion of diet, subadults feeding only on breast milk are expected to have a smaller $\Delta^{13}C_{ap-col}$ than those whose diet is supplemented with other foods. In this study, the $\Delta^{13}C_{ap-col}$ increases with advancing age, which supports the idea that different foods were increasingly incorporated into subadult diet with age. Interestingly, the $\Delta^{13}C_{ap-col}$ for the one to two year category is higher than that of the two to four year category. This anomaly may indicate that the primary dietary transition from breast
milk to solid foods took place between one and two. After this age, breast milk was only nominally included in the diet.

**Stable Oxygen Values from Apatite**

The mean $\delta^{18}$O$_{ap}$ values for each of the six subadult age categories were elevated above the mean for the adult females by 0.1‰ to 0.7‰; the youngest subadult age group, < 1 year of age, showed the greatest difference. The ANOVA test indicated that there are no significant differences among the subadult categories; t-tests indicate that a statistically significant difference exists between adults and subadults in the two younger age categories.

Stable oxygen isotopes are incorporated into the biological tissues via drinking water, food, and breathing. Breastfed infants are expected to have $\delta^{18}$O$_{ap}$ values that are higher compared to the adults; weaned children will have values similar to those of the adults. The values of children in the process of weaning should show values intermediate between the two. Thus, oxygen data from this study indicate that the younger age groups (which show the greatest variation to the adults) were still breastfeeding (i.e., those less than one year), but were beginning the weaning process between ages one and two. The older subadults, whose values are similar to the adults, would be fully weaned and were consuming water directly from the environment. The anomalously low value in the three to five year age group derives from two individuals (Burials 253B and 140); perhaps these individuals were experiencing some physiological stress (e.g. diarrhea) that not only led to the depletion of oxygen in their system, but ultimately led to their deaths.
Validity of Ethnohistorical Data

Ethnohistoric data recorded by Landa (1941) suggest that subadults were weaned between three and four years of age at Tipu; however, the results from this study suggests that weaning began around age one and most likely ended by two to three years of age. Williams and colleagues (2005), who sampled two Postclassic sites spanning occupation from 100 BC to AD 1650, found that subadults from one to three years of age met the expectations for breastfeeding and subadults between three and four years of age were weaned. At Tipu, the site was occupied from 2000 BC to AD 1707, but the population sampled for this study was interred between AD 1568 and AD 1638. Following the guidelines Williams and colleagues used to assess age at weaning in their study, the individuals in the Tipu population who met the same criteria for breastfeeding were less than one year of age, with weaned individuals between two to three years of age. The discrepancy between the two studies can be attributed to several reasons: 1) the difference in sample size (n=25 individuals for this study and n=67 for Williams and colleagues’ (2005) study), 2) the disparity in the date ranges of the samples used (the Tipu collection dates to a narrower time frame (approximately 70 years); the collections used by Williams et al. span 1,750 years), or 3) the Tipu actually were weaning their children earlier than Maya living in other regions (perhaps because increased demands on women by the Spanish led to less time dedicated to subadult breastfeeding). The first two reasons are methodological. Because a larger sample size would decrease the weight of one individual’s isotopic values on the mean of the age category, additional sampling of the Tipu Maya would eliminate the first suggestion from consideration. Similarly, examining Williams and colleagues’ data in narrower time spans might result in a different age at weaning for the Maya. The third possibility, that weaning
began at an earlier age because of additional demands the Spanish placed on women of Tipu, can be examined more closely by considering other bioarchaeological data from the Tipu.

Although one individual in my sample, aged between two and four years, was breastfeeding, other individuals in younger age categories were weaned before four years of age (specifically, Burials 346, 454, and 481). Previous studies using bioarchaeological indicators also questioned the validity of the reported age at weaning for the Tipu Maya. For example, Danforth and colleagues (1997), examining pits of parturition, found a discrepancy between their results and data recorded by Landa. Specifically, Landa suggested that females were bearing children in their mid-adolescent years, but Danforth and colleagues (1997) found that females were giving birth in their early 20s. In another study, Danforth (1989) found that the frequencies of stria of Retzius (which peaked around two to two and a half years of age) and of Wilson bands increased in individuals two to four years of age; however, by age four the frequencies of the tooth pathologies dropped significantly. Based on these data, Danforth (1989) suggested that the Tipu Maya more likely were weaned around two to three years of age.

In addition to bioarchaeological research, other ethnographic research disagreed with Landa’s reported age at weaning. A study by Benedict and Steggerda (1937) was the first to question the reported age at weaning for Maya subadults after Spanish conquest; they found that two to three years was the weaning age for the Maya in the early 1900s (reported in Wright, 1997).
Explanation for Anomalies in Data

When the trophic level effect is applied to each sample from the Tipu collection, five individuals meet the criteria for breastfeeding, but the individuals are not categorized into the same age category. The reported ages for the individuals are three less than one year of age, one is one to two years of age, and one is two to four years of age. One explanation for the variation in age categories of the breastfeeding subadults is that the age estimation for these individuals may be incorrect. Subadult age assessments were based on multiple indicators, including tooth development, fusion of epiphyses in the os coxae, and length of long bones. However, individuals vary in the timing of epiphyseal closure and long bone lengths can be impacted by nutritional or other metabolic insults; thus, age estimates based on these methods may be skewed. Additionally, if the experience levels of the individuals who performed the age assessments varied, this, too, could have resulted in slightly skewed age estimates.

In addition, the age assessments for the Tipu skeletal collection are relatively broad compared to the age ranges of individuals in the study by Williams and colleagues (2005). The age ranges for the Tipu, especially the younger age category, are varied. The variation in estimates, both within Tipu, and between Tipu and other sites, likely is due to differential preservation in the elements available for assessing age for each individual. Nevertheless, these categorizations may be impacting the results of this study. For example, if the age ranges for the Tipu had been narrower, the subadult groups might have included individuals assigned to a younger or older group and, therefore, would have affected the mean stable isotopic values for the subadult categories. If the age ranges could be narrowed for the individuals sampled, a more accurate age at weaning potentially could be specified for the
subadults in the population. A revision in the age assessments for Tipu might yield results consistent with Williams and colleagues (2005) or, alternatively, they might further support the conclusion that age at weaning for the Tipu was earlier than reported by Landa. Finally, another consideration for the varying age ranges for the four breastfeeding individuals in the sample is that breastfeeding, or the timing of weaning was individually decided by the mother based on the weight of the costs to herself compared to the benefit received by the infant received, as suggested by Humphrey (2010).

An additional explanation to account for anomalies in the data is a result of three problems – demographic nonstationarity, selective mortality, and hidden heterogeneity – that are commonly addressed in bioarchaeological research and collectively known as the osteological paradox (Wood et al., 1992). These three problems address the notion that the population sampled was predisposed to conditions of biological stress, including possible diseases, that would have resulted in the early death of the individuals. Various skeletal elements can be examined in an attempt to determine the pathology (or condition), which could have resulted in the death of the individual. However, ascribing the cause of the pathology is difficult because multiple stressors leave the same (or similar) lesions on bone. Therefore, determining the source of lesions on bone is challenging, it is also important for reconstructing the health profile of a population and for drawing inferences about the level of stress experienced by individuals within the population.

The pathological profile of the Tipu, for both men and women, suggests that the population was stressed during Spanish contact. Ethnohistoric data suggests that Spanish contact resulted in greater stress on the women at Tipu than on the men due to the increase in their economic responsibilities. Bioarchaeological research on the topic is unclear. For
example, using enamel hypoplasias, Harvey (2011) found that females experienced greater stress than males during the period from birth to three years; however, Danforth and colleagues (1997) found that women from Tipu generally had healthier childhoods than men based on a statistically significant difference in the frequency of dental caries and Wilson bands. Ultimately, Danforth and colleagues concluded that, while the conditions under Spanish rule were stressful, the Tipu were successful in adapting to the stressful environment (Danforth et al., 1997). This conclusion was based on the fact that the overall frequency of enamel hypoplasias was not high, and, in addition, the adult skeletons showed little signs of infection and trauma (Danforth et al., 1997). Therefore, the suggestion that Tipu subadults experienced enough stress from Spanish contact to contribute to an early death is difficult to support. Although the females at Tipu had increased responsibility under Spanish rule, the frequency of pathologies from birth throughout early childhood seems to suggest adequate maternal care (Danforth et al., 1997).

Finally, if subadults were weaned too early, the subadult would have been more susceptible to bacterial infections. The mother’s breast milk contains lactoferrin, which contains a receptor for iron that ultimately prevents bacteria from obtaining iron for proliferation (Whitney and Hamilton, 1984). Antibodies are in the mother’s breast milk, which help prevent the subadult from experiencing frequent episodes of diarrhea and from contracting polio (Whitney and Hamilton, 1984). Breast milk contains important sources of hormones, lipids, and enzymes (Whitney and Hamilton, 1984). Breast milk protects the infant from obesity and allergies later in development (Whitney and Hamilton, 1984). According to Danforth and colleagues (1997), the frequencies of porotic hyperostosis and cribra orbitalia for the Tipu are low. Therefore, the number of individuals with anemia in the
population was lower at Tipu than other Maya populations (Tancah, Altar de Sacrificios, Barton Ramie, and Cuello). One cause of anemia is a diet deficient in iron; therefore, the subadults at Tipu were receiving adequate iron supplies in their diets. However, one explanation for those individuals with porotic hyperostosis and cribra orbitalia could be that the subadults were weaned too early from breast milk, and the weaning food was deficient in iron.

**Method Considerations**

In my study, bone collagen and bone apatite were sampled for a total of four isotope measurements ($\delta^{15}N_{col}$, $\delta^{13}C_{col}$, $\delta^{13}C_{ap}$, and $\delta^{18}O_{ap}$), and the collagen-apatite difference was calculated by subtracting the value of $\delta^{13}C_{ap}$ from $\delta^{13}C_{col}$ (i.e., $\Delta^{13}C_{ap-col}$). Therefore, I used five lines of evidence to evaluate the age subadults were weaned at Tipu. However, previous studies assessing age at weaning from archaeological populations more frequently have sampled tissues for collagen (carbon and nitrogen), but occasionally, bioapatite, or both (to examine $\Delta^{13}C_{ap-col}$) (Katzenberg et al., 1993; Wright and Schwarcz, 1998; Richards et al., 2002; Harrison and Katzenberg, 2003; Bourbou et al., 2013; Buhay et al., 2013; Burt, 2013). Although the data obtained from the analyses of apatite (i.e., carbon, oxygen, and the apatite-collagen difference), do not have an equivalent standard for interpretation as do the data obtained from collagen (i.e., the trophic level effect), the former three lines of evidence do show differences among the adult and subadult age groups in this study. Thus, these data provided further evidence not only for age at weaning, but also for revealing subtle or gradual changes in subadult diet. Whereas the assessment of nitrogen and carbon from collagen may continue to be a preferred method for assessing age at weaning from
archaeological populations due to the clear standards for interpretation, the analyses of isotopes from apatite can contribute additional information on subadult diet. Furthermore, the process of extracting apatite is less time consuming than for collagen; therefore, these analyses can be conducted without adding a substantial amount of time to the research. In conclusion, the analyses of five lines of evidence provide a comprehensive assessment for estimating age at weaning for an archaeological population.
Chapter Six: Conclusion

To contribute to existing knowledge of Spanish influence on the Tipu Maya, age at weaning was assessed using stable isotope ratios of carbon, nitrogen, and oxygen, and apatite collagen spacing, derived from rib collagen and apatite in a sample of 20 subadults and five females of reproductive age. Diego de Landa, a circuit-riding priest at the time of Spanish contact, recorded the age at weaning between three to four years (Landa, 1941). Today, Maya women wean their children between one to two years of age (Kramer, 2005). Based on the subset of the population sampled from Tipu, data from this study generally suggest that the weaning process began around one year of age, with the introduction of the primary weaning food, maize. However, the subadults were consuming a diet similar to the adult diet by two to three years of age.

Whereas one statistical analysis (ANOVA) show that differences in isotopic values among the different subadult age groups were not significant, the differences between the adult females and the youngest age group (i.e., infants less than one year) were significant in three out of five isotope parameters (e.g., for $\delta^{15}\text{N}_{\text{col}}$, $\delta^{13}\text{C}_{\text{col}}$, and $\delta^{18}\text{O}_{\text{ap}}$). In addition, the trophic level effect was evident in the nitrogen values (~2‰ to 3‰ difference), and the carbon values derived from collagen (those elevated ~2‰ above the female mean), in five subadults. However, these five individuals were categorized into three different age groups. This fact may indicate there was variation among the Tipu in the age at which mothers began weaning their infants.

The high $\delta^{13}\text{C}_{\text{col}}$ for all individuals indicate that maize, or some other C4 plant, was a primary food source for the Tipu. The lack of variation in $\delta^{13}\text{C}_{\text{ap}}$ and the $\Delta^{13}\text{C}_{\text{ap-col}}$ values indicate that maize (or some other C4 plant) was a weaning food (based on the similarity in
the isotope signatures for breast milk and maize). These findings are consistent with the 
archaeological and ethnohistoric records.

The results of this study suggest the age at which infants were weaned may have been 
earlier than three to four years as recorded in the enthnohistoric data. Among the Tipu, data 
suggest that the only individuals consuming solely breast milk were less than one year of age, 
with the exception of one child who was between one and two years, and one child aged 
between two and four years. As subadult age increases, the trend in data is for $\delta^{15}N_{col}$ values 
to increase and for $\delta^{13}C_{col}$ values to decrease. Therefore, my hypothesis that Spanish contact 
impacted the age at which infants at Tipu were weaned appears to be supported. Additional 
research on the Tipu, as well as on other pre- and post-contact Maya populations, could help 
to confirm the results of this study as well as refine the age at which weaning was completed 
among the Maya.
Literature Cited


### Appendix A: Weight (g) of Rib Fragments

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<th>Letter</th>
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<th>Burial No.</th>
<th>Weight</th>
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<tr>
<td>B</td>
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<td>447</td>
<td>1.12 g</td>
</tr>
<tr>
<td>C</td>
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<td>1.23 g</td>
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<td>D</td>
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<tr>
<td>E</td>
<td>1-2</td>
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Appendix B: Photographs of Tipu Sample

Age Category: <1 year of age

Burial Number: 399

Burial Number: 447

Burial Number: 433

Burial Number: 449
Age Category: 1-2 years of age

Burial Number: 346

Burial Number: 454

Burial Number: 481

Burial Number: 435
Age Category: 2-4 years of age

Burial Number: 431

Burial Number: 69B

Burial Number: 167

Burial Number: 237
Age Category: 3-5 years of age

Burial Number: 140

Burial Number: 343

Burial Number: 253B
Age Category: 4-6 years of age

Burial Number: 361

Burial Number: 411

Burial Number: 473
Age Category: 5-7 years of age

Burial Number: 434

Burial Number: 181
Age Category: Adult Females (18-45 years of age)

Burial Number: 193

Burial Number: 128

Burial Number: 514

Burial Number: 507

Burial Number: 136
Appendix C: Bone Preparation for Stable Isotope Analysis: Cleaning, Sonication, and Bone Grinding

Procedures for cleaning, sonicating, and grinding bone for isotope analysis are modified from Ambrose 1990. I conducted all procedures under the direction of Dr. John Krigbaum during the weeks of July 14–25, 2014. The general protocol is listed below.

1. Photographed rib fragments associated with each individual in my sample (N=25).
2. Examined each rib for contaminants.
3. Removed the contaminants from bone using a scalpel.
4. Split rib fragments in half using a scalpel and scraped the trabecular bone from the inside of the fragment with the scalpel.
5. Placed fragments into a beaker for sonification.
6. Added distilled water (dH₂O) to each beaker.
7. Placed samples into sonicator basin and sonicated for approximately 10 minutes.
8. Decanted dH2O and placed rib fragments onto paper towels to air-dry overnight.

9. Crushed the bone with mortar and pestle.
10. Sieved for the apatite fraction (0.25 mm) and collagen fraction (0.5 mm).
11. Placed the two fractions into separately labeled vials.
Appendix D: Bone Collagen and Bone Apatite Extraction

1. Labeled and weighed 50 plastic test tubes and glass vials to determine percent collagen yield and percent apatite yield after extraction.

Collagen Extraction

1. Weighed out 250 mg of collagen fraction and placed the sample into the test tube.
2. Demineralized bone by adding 12 ml of 0.2 M hydrochloric acid (HCl) to each test tube.
3. Placed test tube into a holder with the cap unscrewed for 24 hours.
4. Replaced HCl in each vial for 4 consecutive days. The samples were first centrifuged for approximately 10 minutes, the HCl was decanted, and fresh 0.2 M HCl was added to the test tubes.
5. Left tubes, with loose caps, for an additional 24 hours until pseudomorphs were present in the test tubes.
6. Rinsed sample to a neutral pH (pH=7) by centrifuging the samples for 10 minutes, decanting the acid, and adding dH2O until the sample was neutral.
7. Added approximately 12 ml of sodium hydroxide (NaOH) to the test tubes for 15 hours.
8. Rinsed the samples to neutral pH again as described above.
9. Added approximately 10 ml of 0.001 M HCl to each test tube and placed into a glass vial.
10. Placed samples into a 95°C oven for 4 hours.
11. Added 100 µl of 1 M HCl to vial and placed back into the oven for an additional 4 hours.
12. Transferred contents from the vials into the test tube and centrifuged for approximately 15 minutes.
13. Transferred solution in suspension back into the glass vial and placed into an oven at 65°C until the solution was condensed to 2 ml. The samples were then allowed to cool on a countertop and placed into the freezer.
14. Removed the samples from the freezer and placed samples into the freeze dryer for 2 days.
15. Removed tubes from the freeze dryer and weighted sample to calculate percent collagen yield.
16. Loaded sample into tins for mass spectrometer.

**Apatite Extraction**

1. Weighed out 50 mg of apatite fraction and placed the samples into a test tube
2. Added approximately 12 ml of 50% sodium hypochlorite (NaOCl) and mixed the contents of the test tube.
3. Placed the test tube into a test tube holder and left for 20 hours to dissolve organic components of bone.
4. Rinsed samples to neutral pH by centrifuging the samples for approximately 10 minutes, decanting the sodium hypochlorite, and adding dH₂O until the sample was neutral.
5. Added approximately 12 ml of 0.2 M Acetic acid (C₂H₄O₂), mixed, and left 16 hours to remove non-biogenic carbonates.
6. Rinsed samples to neutral pH by centrifuging the samples for approximately 10 minutes, decanting the acetic acid, and adding dH₂O until the sample was neutral.
7. Discarded dH₂O and placed samples into the freezer.
8. Removed the samples from the freezer and placed samples into the freeze dryer for 2 days.
9. Removed tubes from the freeze dryer and weighed to calculate percent apatite yield.
10. Loaded sample into tins for mass spectrometer.
Vita

Chaney Elizabeth Hiers was born in Savannah, Georgia, in March 1991. In May 2009, she graduated Valedictorian from Wade Hampton High School in Varnville, South Carolina. After graduation, she attended Clemson University as a Coca-Cola Scholar where she graduated *cum laude* with a Bachelor of Science in Biological Sciences and a minor in anthropology. She began the master’s program at Louisiana State University in the Fall 2013 with an emphasis on forensic anthropology and expects to graduate in May 2015 with a Master of Arts in Anthropology.