STABLE ISOTOPE WEANING ANALYSIS USING DENTAL SERIAL SECTIONS

INDIVIDUALS AND VARIATION: STABLE ISOTOPE ANALYSIS OF WEANING USING DENTAL SERIAL SECTIONS.

By
SARAH HOLT, B.A., B.S.

A Thesis
Submitted to the School of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree
Master of Arts
McMaster University

MASTER OF ARTS (2009) McMaster University
(Anthropology) Hamilton, Ontario

TITLE: Individuals and variation: stable isotope analysis of weaning using dental serial sections.

AUTHOR: Sarah Holt, B.A., B.S. (the Ohio State University)

SUPERVISOR: Professor A. Cannon

NUMBER OF PAGES: vii, 57

Abstract

Duration of breastfeeding and the process of weaning in past populations are of interest to anthropologists because of implications for short and long term health effects, fertility rates and birth spacing, and differential resource access in the earliest years of life. Stable isotope analysis of tissue from archaeological populations can create a picture of weaning patterns, providing one lens for assessing child health in past populations and the process has become widely used in the past three decades. The most frequently utilized method for stable isotope analysis of weaning requires a high frequency of child burials from a range of ages to create a population curve that compares the changes in child diet to the adult mean. By approaching weaning through a population aggregate pattern, however, data on individual variation is lost and with it, any patterns in variability. This study uses a Greek sample from Apollonia Pontica in modern Bulgaria to test the usefulness of approaching stable isotope weaning studies from a multi-dimensional perspective. By using dental serial sections from a smaller total sample, individuals show dietary levels dependant on age, revealing both population level patterns of a shared cultural weaning standard as well as variation in the diets of individuals. In addition to bypassing several of the major limitations of population aggregate sampling, the serial sampling method provides comparable conclusions to an independent study while simultaneously providing additional data on variability within household diets obscured by the traditional method.

Acknowledgements

I would first like to thank my supervisors, Aubrey Cannon and Henry Schwarcz, for their time, advice and patience throughout the research and writing process. I am also grateful to Charles FitzGerald for his gift of time and knowledge about all things dental, as well as his keen editing eye. Special thanks goes to Anne Keenleyside for her generous contribution of both samples and data from the Apollonia materials.

Immeasurable gratitude to my husband, Josh Holt, for putting his own career on hold and moving to Canada to see me throughout this process. We took the path less travelled together, and that has made all the difference. I must additionally thank my fellow graduate students for two years of support, encouragement and necessary distractions: Alison Devault, Martyna Janjua, Nadia Densmore, Ani Chenier, Laura Waddell, Katie-Sue Derejko, and Catherine Paterson. To the particularly outstanding Rev. Stacey Hallman, M.A., this thesis would not have been possible without you.

I would like to thank Ann Herring, Janet Padiak and Ellen Badone for guidance when I needed it most. Your compassion and advice is much appreciated.

I would finally like to thank my wise mother, Dawna Lisa Buchanan for her love and encouragement and the rest of my family, innumerable and supportive always.

Table of Contents

List of Figures and Tables

Figures

Tables

Introduction

Purpose of Study

Although dietary stable isotope analysis of dental tissue has become *de rigueur* for establishing weaning timelines in past populations, there is still much room for expansion on the established techniques, particularly in the approach to sampling method. The goal of this research is to provide one multidimensional approach to stable isotope analysis studies of weaning through dental serial section sampling. Specifically, this thesis will utilize human remains from the site of Apollonia Pontica, Bulgaria to: 1) determine a population level pattern of weaning age and duration, 2) examine the weaning experience of individuals who appear to be outliers and 3) test the major assumptions of population aggregate sampling. The strengths and weaknesses of the dental serial sampling approach for other sites will be discussed, as well as the role maternal diet plays in the sub-adult isotope data.

Overview

This study takes deciduous first molars [dm1], deciduous second molars [dm2], and permanent first molars [M1] from eleven individual sub-adults and uses macroscopic seriation of morphological landmarks with established developmental time periods. The sections were analyzed for the dietary isotope signatures of carbon and nitrogen, and charted by individual over time. The overlapping developmental ranges of the sections are used to create a pattern of δ15N and δ13C levels over time and compared with the expected pattern for individuals experiencing weaning.

Using δ15N levels, a population level pattern of introduction of solid foods to the infant around 6 months and cessation of breast milk complete between one and three years of age with a post-weaning diet comparable to known adult levels is visible in the majority of individuals sampled (n=7). Individuals who do not fit this pattern, those who would appear as outliers in population aggregate sampling, are found to have experienced weaning at the same ages, but may also be experiencing a maternal and adult diet unusual to the larger population.

Using δ13C levels, half the sampled subadults (n=5) show the expected pattern of decreased enrichment consistent with introduction of solid foods around age 6 months. The remaining six sub-adults show levels with continued enrichment, stasis, or contrasting patterns between the tooth types. This variation in patterning suggests heterogeneity in the supplementary food given to weaning infants, the most likely options at Apollonia being C3 cereal grains and goat milk. The variation also suggests differences in maternal diet during pregnancy and breastfeeding, visible at the individual level.

Choice of Sample

Apollonia Pontica, a Greek colony located on the Black Sea coast of Bulgaria and dating to the 5^{th} – 2^{nd} centuries BC, provided a unique opportunity to test this sampling

method. Primarily, the site contained a large, well preserved sub-adult burial assemblage. As this method works best with mixed dentition, the particular age subset of 2-8 year old individuals was needed and represented at this site. Additionally, dietary isotopic analysis had been completed on the adult population, and the findings were of a largely homogeneous diet (Keenleyside et al. 2006). This simplified the interpretation of the infant dietary levels and provided a baseline for the expected levels of carbon and nitrogen enrichment in the sub-adult teeth developing after the completion of weaning. Finally, a weaning study using the traditional population aggregate method has been recently completed on the site (Kwok and Keenleyside n.d.), providing results for a true comparison of methodology.

Contents

Chapter 1 addresses background information relevant to this research. A basic explanation of how stable isotope analysis is used to study diet in dental tissues will be presented along with site specific information about Apollonia Pontica. A concise literature review of weaning studies will be discussed with a focus on dietary stable isotope approaches to exploring the weaning process.

Chapter 2 presents the materials and methods used in this study.

Chapter 3 presents the results of the study with brief descriptions of the findings categorized by individual. Population level data are also graphed and summarized.

Chapter 4 provides a discussion of the findings of this study. The population level patterns specific to Apollonia are described and individual variation is discussed in the context of the site. Applications of the dental serial sampling method for other sites are detailed, with limitations and advantages of the method outlined, including the role of maternal diet in sub-adult isotopic studies. In addition, an example of intra-individual variation of a single tooth type is tested.

Chapter 5 concludes the study with a summary and discussion of the broader implications of the method and the results, including directions for further research.

Chapter 1: Background

Weaning Studies

In recent decades the process of weaning in past populations, encompassing the duration of breastfeeding, the introduction of solid foods, and the transitional period of decreased breastfeeding,), have been of keen interest to anthropologists. Initially understood as an event and later conceptualized as a process, the experience of weaning has long-term effects on individual and population level health, fertility, and mortality, all integral concepts in anthropological discourse. Short and long-term health effects of diet in early childhood and demographic reconstruction have been two main areas heavily influenced by weaning studies because infant feeding practices offer one line of evidence to investigate a wide range of questions dealing not only with subadults, but with the adults who care for them as well as the adults they will become.

Many researchers have noted a complex relationship between weaning and infant mortality. In a historical analysis of North American and European populations, Knodel and Kinter (1977) found that the duration of breastfeeding affected both overall rates and age structure of infant mortality. Cultures which breastfed consistently and for longer durations showed lower mortality while those with short or absent breastfeeding periods showed increased infant mortality in the earliest three months of life. The authors stress that because the age distribution of infant mortality is commonly used as a health indicator for the larger population (Sawchuk et al. 2002) an understanding of the infant feeding history affecting the mortality curve is crucial to avoid bias of interpretation.

Goodman and Armelegos (1989) approach weaning as a morbidity event; an early health stressor that affects morbidity and mortality through the rest of life. In this context, they consider weaning age an indicator of health in those less than five years old. Weaning age is conceptualized as a period of culturally induced stress which may leave a recognizable marker on the body in the form of linear enamel hypoplasias in the teeth, Harris lines in the bones, or at the population level through a spike in the mortality curve for infants due to increased percentage of infant deaths during weaning. Archaeological examples of this demographic change in mortality patterns have been discussed at length (Gordon et al. 1963) and a 1981 study by Janowitz et al. showed an association between early cessation of breastfeeding and higher mortality in a modern Egyptian sample.

Katzenberg et al. (1996) emphasize that the duration of breastfeeding and length of the weaning period offers information about a multitude of complicated aspects of mother/child interactions. In addition to health effects on children, lactational amenorrhea, the decrease in maternal fertility during breastfeeding, has potential impact on birth spacing and population growth rates. Knodel (1977) found a decrease in breastfeeding time led to decreased birth spacing in modern populations in Boston, Bavaria, and India which directly accounted for higher population growth and higher infant mortality rates. This relationship between infant feeding practices and fertility is somewhat contentious; Schurr (1997) compared stable nitrogen isotope patterns to demographic fertility modeling and found no simple correlation between weaning and fertility, with bone turnover rates in juveniles and cultural reasons for increased fertility acting as potentially confounding factors.

While researchers have investigated weaning from many approaches, the infant feeding literature has come to stress the concept of a "weaning process" (Dettwyler and Fishman 1992; Herring et al. 1998; Katzenberg et al. 1996; Stuart-Macadam and Dettwyler 1995) because there exists variability within and between individuals throughout the early years of an infant's life. Weaning is not a single event that can be isolated; instead, it can be considered a gradual process that takes place in three phases: breastfeeding, introduction of solid foods, and cessation of breast milk.

Stable Isotope Analysis

Stable isotope analysis of tissue from archaeological populations can create a picture of the weaning process, providing one lens for assessing child health in past populations and it has become widely used in the past twenty years. Through the process of growth and development, carbon and nitrogen signatures affected by nutritional intake are retained in the skeletal and dental collagen of the human body as markers of individual diet. Carbon stable isotopes reflect vegetal dietary intake, distinguishing between the levels of consumption in C3 plants such as wheat and barley, and C4 plants such as maize through a ratio of ^{13}C to ^{12}C notated as $\delta^{13}C$. Individuals consuming a diet high in C4 plants will retain a $\delta^{13}C$ level higher than those consuming a majority of C3 plants. Nitrogen stable isotopes represent the general composition of dietary protein intake and can be used to distinguish between trophic levels of diet, essentially pinpointing the position of the individual in the local food chain. While an individual $\delta^{15}N$ value (the ratio of ^{15}N to ^{14}N) can provide information about the specific food types contributing to protein consumption, in weaning studies the data of interest are the comparison of infant levels to maternal levels and the change in $\delta^{15}N$ through the course of early childhood (e.g. Fuller et al. 2005; Richards et al. 2003).

The widespread use of nitrogen isotope values to determine weaning patterns in archaeological populations is based on a predictable increase in enrichment described by Schoeninger and DeNiro (1984:625) as a "trophic level effect" which shows "a 3‰ enrichment in $\delta^{15}N$ values at each successively higher trophic level". Fogel et al. (1997) sampled fingernails of breastfeeding infants and mothers and compared the $\delta^{15}N$ levels and found that children consuming breast milk retain a $\delta^{15}N$ value of 1-3‰ higher than the mother: the infant is at a higher trophic level than the mother. Enrichment over the mother was seen in infant fingernails during breastfeeding, followed by a decrease in enrichment at the start of weaning.

Elaborating on this process, Fogel, et al. (1997) used two archaeological samples to demonstrate population curves of $\delta^{15}N$ levels showing a discernable decrease in $\delta^{15}N$ over adults interpreted as the weaning age in these prehistoric peoples. Herring et al. (1998) confirmed that stable isotope patterns of $\delta^{15}N$ matched historical records for weaning age in an Ontario, Canada sample, and recent studies have continued to use nitrogen isotope levels to establish weaning age in archaeological populations (Dupras et al. 2001; Fogel et al. 1997; Fuller et al. 2003; Herring et al. 1998; Katzenberg et al. 1996; Katzenberg and Pfeiffer 1995; Prowse et al. 2005; Richards et al. 2003; Schurr 1997; Wright and Schwarcz 1998).

Fuller et al. (2005) followed a set of living mothers and infants from birth to complete cessation of breast milk through sampling of both hair and fingernails. Their results confirm the trophic level effect on $\delta^{15}N$ between infant and mother pairs with a 1-3‰ increase in the babies. Additionally, they found $\delta^{13}C$ enrichment also follows a predictable pattern of enrichment of approximately 1‰ during breastfeeding with a predictable decrease during weaning. By adding $\delta^{13}C$ analysis to the $\delta^{15}N$ patterns, a more complete picture of the transitional food stage after introduction of solid foods and before cessation of breast milk can be seen. However, the $\delta^{13}C$ enrichment decreases more rapidly in the infant than $\delta^{15}N$ when solid foods are introduced, and once enrichment decreased to adult levels, no consistent pattern of $\delta^{13}C$ levels was observed.

Current sampling method

The most frequently used method for stable isotope analysis of weaning creates a pattern of dietary levels across the population and determines mean age of weaning from the aggregate. Population aggregate sampling requires a high frequency of child burials from a range of ages to establish a population curve that compares the changes in child diet to the adult mean (see Figure 1.1). Levels of $\delta^{15}N$ are taken from collagen in rib or femur ends of sub-adult individuals at various ages of death. These are compared to an adult mean to approximate the age at which individuals lose the elevated trophic levels seen during breastfeeding and descend to the lower adult levels of a fully weaned diet (e.g. Dupras et al. 2001; Schurr 1997; Katzenberg and Pfeiffer 1995) (see Figure 1.2).

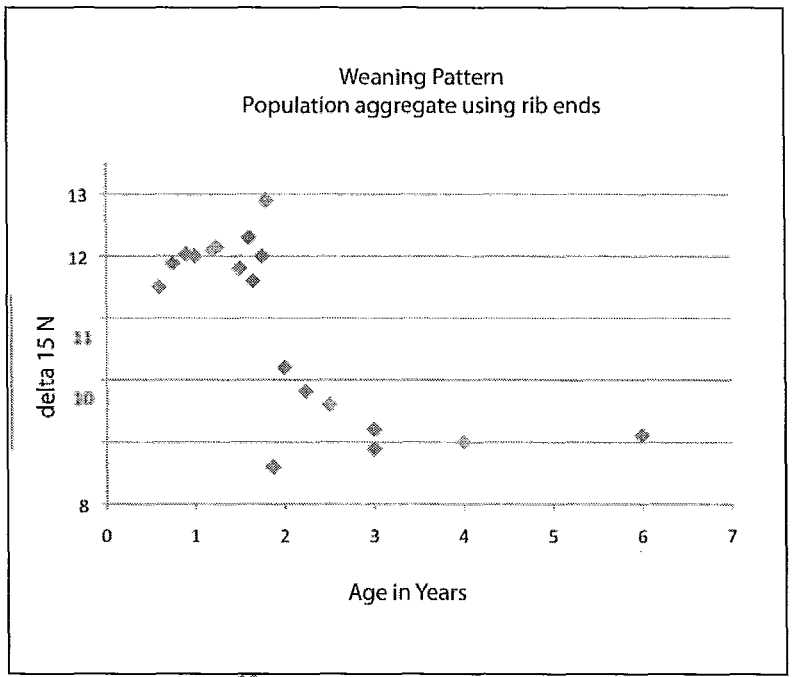


Figure 1.1: Example of $\delta^{15}N$ levels plotted for rib ends (artificial data set).

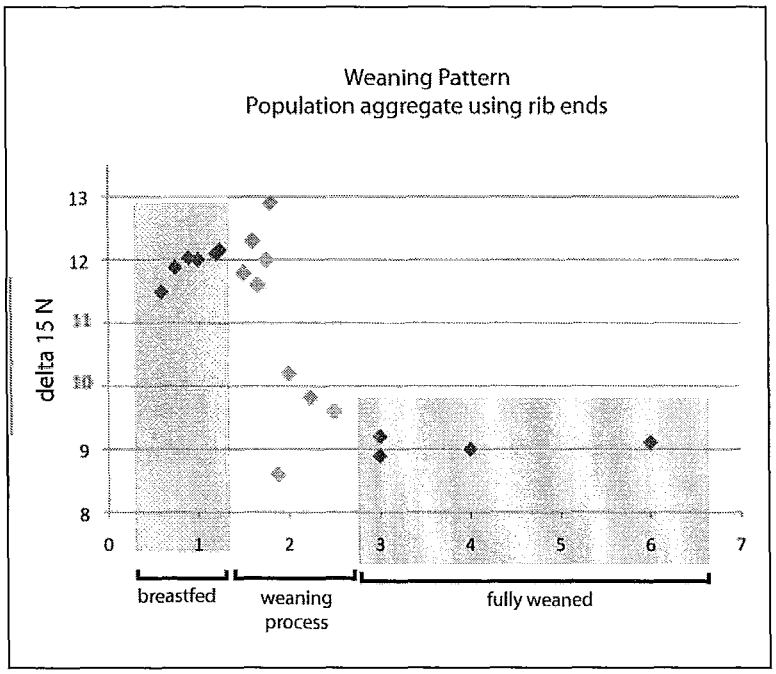


Figure 1.2: Interpretation of aggregate sampling method. Grouped samples represent individuals who died during three phases of diet (artificial data set).

One major limitation on this line of study is the need for a specific range of ages in the sample: high sample sizes of infants and young children are needed to show the ages during which weaning occurred. Due to taphonomic effects and differential burial practices remains of sub-adults are often unavailable in the quantities or age ranges necessary (Saunders and Barrans 1999; Saunders et al. 1995; Molleson, 1991; Saunders and Hoppa 1993;Fry 1999; Guy et al. 1997; Ucko 1969). One way of circumventing this sample limitation is to use adult teeth. Wright and Schwarcz (1999) use an adult sample of enamel and dentine formed during childhood to provide baseline levels of levels of carbon and oxygen in collagen formed during the end of the weaning process, however, while the use of the first permanent molar can confirm the levels of $\delta^{15}N$ and $\delta^{13}C$ in early childhood, and be compared to the adult diet, it does not provide a detailed timeline for introduction of solid foods and cessation of breast milk.

Another limitation of the aggregate method appears in analysis of the results. In order to apply the pattern represented by the burial sample to the full population, several assumptions are made about weaning, here summarized from Fuller et al. (2003).

1: The population had a culturally determined age for weaning which researchers can detect.
2: The weaning period lasted a few months.
3: After weaning, children consume the same diet as adults.

Another common assumption is:

4. All the mothers had isotopically similar diets.

All weaning research is impacted by the "strong cultural influences on infant feeding practices in all societies studied" (Katzenberg et. al. 1997:193). In past populations then, where cultural attitudes to breastfeeding may not be recorded or are otherwise unknown, it is indeed a major assumption that the weaning process is a commonality within the population. For example, figure 1.3 shows two individuals who would appear as outliers and therefore be excluded from the study results. As summarized by Herring and colleagues (1998:435), "we are unable at this time to determine whether the children who show [unusual] $\delta^{15}N$ permil values within the adult range were never breast-fed, were breastfeeding occasionally, or were weaned completely several months prior to their death". One frequent interpretation of outlier weaning values is abnormal feeding patterns due to illness of the child (Kwok and Keenleyside n.d.; Dupras et al. 2001). This inference is subjective, and even with strong supporting palaeopathological and archaeological evidence, questions of interpretation remain: what was the experience of weaning for these individuals? Do outlier values of $\delta^{15}N$ at time of death always indicate an abnormal weaning experience?

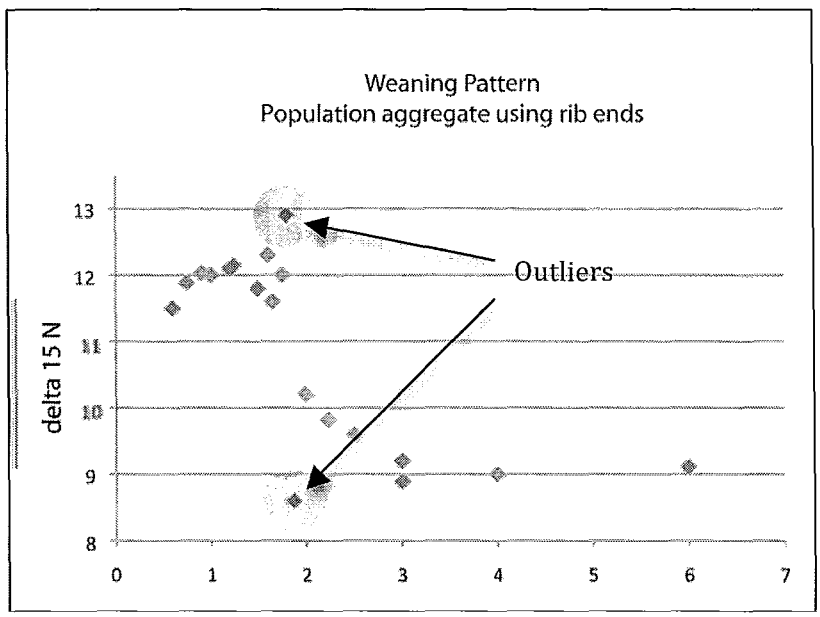


Figure 1.3: Outliers in population aggregate sampling (artificial data set). How do they fit into assumption of shared cultural process of weaning?

By approaching weaning through a population aggregate pattern, data on individual variation are lost and with them, any pattern in variability that might shed light on the homogeneity of the weaning process within a population. If weaning is truly approached as a process, it is through individual change over time that patterns of variation of the process within the population will be visible. Individuals that present as initial outliers may in fact provide more support for the expected pattern than can be seen from a single isotopic level at the time of death. Conversely, individuals who seem to conform to the population level pattern may have an aberrant weaning history that is not reflected in their age-at-death diet. Particularly in sites where groups with differing weaning practices may be overlapping in the death assemblage, weaning age and duration at the individual level can be expected to differ from the population aggregate means. A multidimensional approach that illuminates variation within and between individuals is necessary to observe the effects that cultural influences have on stable isotope signatures during weaning, contributing to the larger dialogue on the homogeneity of diet within a population and across all age groups.

Seriation sampling

Building on the idea of the observable trophic level decrease in $\delta^{15}N$ enrichment during weaning, a number of studies focusing on faunal materials have attempted to assess changes within a single tooth. Balasse (2001) assessed nitrogen isotope signatures in a modern *Bos taurus* sample by seriating both the M1 and M2 from individual cows along developmental growth lines. The results show a change in $\delta^{15}N$ through the full development of the teeth in one-month increments and a plotted curve is consistent with the controlled weaning process. A follow-up study conducted on an archaeological sample from Neolithic France (Balasse and Tresset, 2002) used similar serial sectioning to compare two archeological cow jaws to one modern cow jaw. By plotting the $\delta^{15}N$ decrease across the development of the molar teeth, weaning age of the Neolithic sample was found to take place at a younger age than in the modern bovine.

Similar microsampling of individuals has also shown short-term diet changes in caribou teeth (Drucker et al. 2001) and sea lion tusks (Hobson and Sease 1998). Because these studies were able to employ control of diet in their subjects, they show the feasibility for using seriation to track dietary change over small time increments. It should be noted that these faunal studies refer to this style of microsampling as intra-tooth isotope analysis, while the favored term for human teeth is *dental serial section isotope analysis.*

In 2003, Fuller and colleagues published a preliminary elaboration on the traditional sampling approaches in human populations. Their method used serial sections of multiple deciduous and permanent teeth and compared them to the rib end from the same individual to create an individual specific timeline for weaning. By comparing a group of individual weaning histories, a population pattern was established.

For their sample of infants from Wharram Percy, England, the researchers chose to sample the crown and 2-3 sections of the root (dependant on length) of the dm2, C, and M3 in addition to rib ends. These seriations produced an overlapping timeline from 6

months *in utero* to 20 years of age, limited by the age at death of the individuals. The results showed progressive reduction in levels of both $\delta^{13}C$ and $\delta^{15}N$ in each serial section consistent with the loss of trophic effect of breast milk through the weaning process.

Variation in this pattern was seen in two individuals who displayed a developing root section enriched in $\delta^{15}N$ over the crown section. Fuller et al. explain this variation as "expected if this individual was breast fed until time of death" (2003: 1676). Variation was also seen in two individuals with earliest levels of $\delta^{15}N$ significantly lower than the rest of sample. Fuller et al. interpret these results as individuals who did not breastfeed soon after birth, but later consumed breast milk regularly as isotopic signatures return to expected levels in subsequent sections.

The results of this initial study using serial sectioning reveals a clear pattern of enrichment for both $\delta^{13}C$ and $\delta^{15}N$ values in the dm2, showing a decrease from crown to cervical root to apical root, with the rib end displaying the lowest level of enrichment. The permanent teeth (canines and M3) and rib ends of adult individuals show a less predictable pattern. This is consistent with individual dietary variation through adolescence since the majority of the dental tissue in these teeth is formed after weaning. Because the sections span such large developmental periods in the tooth, and many of the sections are developed after weaning, the time resolution of this study is low, and does not provide an exact timeline for the introduction of transitional foods or cessation of breast milk in the individual diet.

The challenge for continuing research with dental serial sampling in stable isotopic weaning studies is to increase the time resolution for the earlier periods of infancy to more accurately reflect the introduction of solid foods and the cessation of breast milk. Once a multidimensional approach to population patterns is in place, the assumptions for population analysis can be tested and the homogeneity of the weaning experience within a population can be addressed. This study will use the Ancient Greek site of Apollonia Pontica located in the modern city of Sozopol, Bulgaria as an example case for how dental tissue sampling methods can improve the time resolution of the seriations and will provide some interpretations for how outliers in the sample may reflect larger trends or exceptions to the population weaning patterns.

Chapter 2: Materials and Methods

Apollonia Pontica

The sample population is from the ancient Greek colony of Apollonia (5th to 2nd centuries BC), which is located on the western coast of the Black Sea in what is now the Bulgarian city of Sozopol [See Figures 2.1, 2.2]. Founded in the 7th century BC by Greek settlers from Miletus, and continuously occupied for over 600 years, Apollonia was a thriving trade port located on a rocky peninsula surrounded by fertile land (Hermary and Panayotova 2006:56; Nedev and Panayotova 2003:96). The necropolis from which the samples used in this study derive contained mainly pit burials with occasional wooden coffins, stone cists, and tile-lined burials (Nedev and Panayotova 2003).

Apollonian dietary practices included the Athenian tradition of the "Mediterranean triad" of wine, cereals and olive oil (Hoddinott 1975); however, adults in this active port colony appeared to enrich their diets with a variety of grains and marine foods (Keenleyside et al. 2006). Keenleyside and colleagues also found no significant difference in diet by age or sex in the adult diet based on the clustering of isotope values.

The Apollonia population was chosen for this study based on the availability of multiple teeth of mixed dentition from an appropriate age range for weaning assessment; however, homogeneity of adult diet simplifies the analysis of the variation in the transitional weaning foods and diet of childhood discussed in this work. Additionally, an independent study (Kwok and Keenleyside in press using population aggregate methods to assess the weaning patterns at Apollonia provides a blind assessment for methods comparison.

The sub-adults samples used in this study come from five sites within the larger necropolis: 5040, 5094, 5518, 8036, and the main necropolis

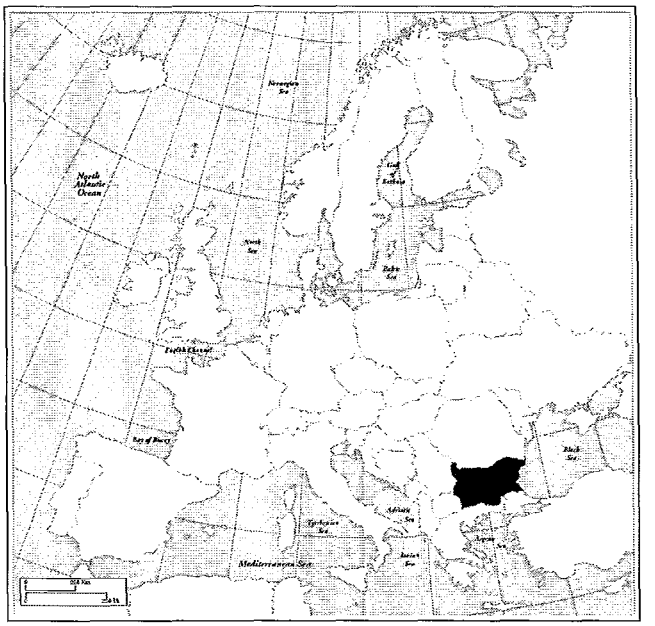


Figure 2.2: Location of Sozopol (Apollonia) and the necropolis (from Keenleyside and Panayotova 2006)

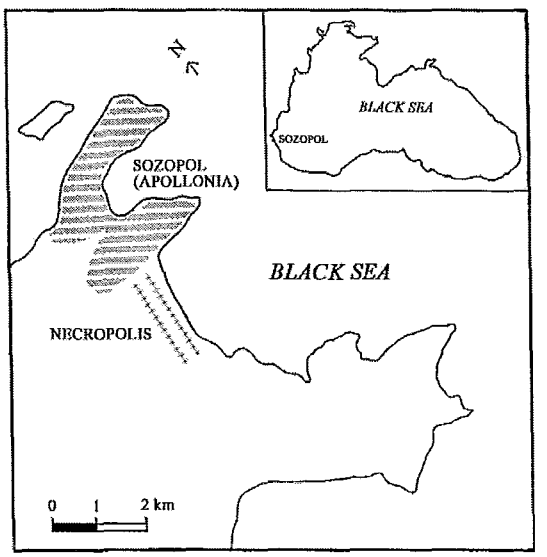


Figure 2.1: Location of Bulgaria and the black sea (Alabama Maps 2009)

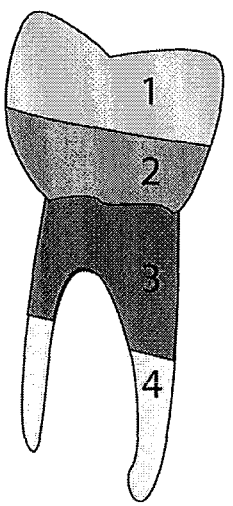
Dental Sampling

Eleven individuals were selected for this study based on availability of mixed dentition of two to three teeth representing the developmental period of approximately 5 months *in utero* to 3 years. Age at death for each individual was determined using the Smith method (1991) for dental development.

Deciduous first molars (n=11), deciduous second molars (n=11) and permanent first molars (n=10) were selected for this study. All teeth studied were free of caries, except 5040-24.2, which contained a small distal interproximal carie that is included in the second section from this tooth. One piece of alveolar bone in which the M1 of individual 295 was embedded was included as a separate section as noted in the data results.

Sectioning

Each tooth was embedded in dental wax and vertically sectioned through the mesial cusps using a Buehler Isomet low speed diamond saw with a 0.3mm kerf. The portion of the tooth containing the distal cusps was then further sectioned horizontally while the other tooth block was retained for future analysis. The tooth portions containing cusps were horizontally divided into a maximum of four serial sections each [see Fig.2.3] determined by macroscopic division into age ranges as defined by Moorrees, Fanning and Hunt (1963) stages and Gustafson and Koch means (1974). The developmental age for each section is presented in Table 2.1. For individuals who had reached only partial development for specific sections, inclusion of the section was based on quantity of developed material, with only samples expected to produce usable amounts of collagen included in the analysis.



1. Crown initiated to Crown half complete (C_i-$C_{½}$)
2. Crown half complete to Crown complete ($C_{½}$- C_c)
3. Root initiated to Root half completed (R_i – $R_{½}$)
4. Root half complete to Root complete ($R_{½}$ - R_c)

	dm^1	dm^2	dm_1	dm_2	M^1	M_1
C_i-$C½$	5 mo *in utero* - birth	6 mo *in utero*- 4 mo	5 mo *in utero*-birth	6 mo *in utero*-4 mo	birth – 8 mo	birth – 8 mo
$C½$-C_c	birth – 6 mo	4 mo – 10 mo	birth- 6 mo	4 mo-10 mo	8 mo- 3 yrs	8 mo-3 yrs
R_i – $R½$	6 mo-10 mo	10 mo-2 years	6 mo- 10 mo	10 mo- 2yrs	3-10 yrs	3-10 yrs
$R½$ - R_c	10 mo- 2.3 years	2-3 yrs	10 mo- 2 yrs	2-3 yrs		

Table 2.1: Serial sections with Gustafson and Koch (1974) mean age ranges.

Enamel and dentine do not grow in easily separable, equal horizontal increments and currently there is no accepted method for practically determining exact age based on dentine apposition; consequently, error in aging each section has been introduced due to the sampling method. Enamel and dentine do not grow in equal horizontal increments and the direction and duration of the mineralization can affect the isotopic levels recorded in the tissue. Enamel and dentine initially form by the apposition of collagen matrix that proceeds out from the EDJ in concentric patterns of three-dimensional growth, which Hillson describes as either overlapping "sleeve-like layers" or "domes" (1996: 121), depending on their position in relation to the cusp. The challenge for serial sectioning studies lays in the necessity of cutting a horizontal section through these growth patterns since each cross section does not represent a truly homogeneous period of growth. Each will incorporate some material from other growth layers [See figure 2.4], and therefore serial isotopic analysis may show a gradual pattern in dietary change, even when the shift itself may have been sudden. In my study, the serial sections are comprised of minimum developmental increments of four months. While there remains unavoidable overlap of growth layers between the serial sections, the larger section size reduces the impact of this bias on each individual sample.

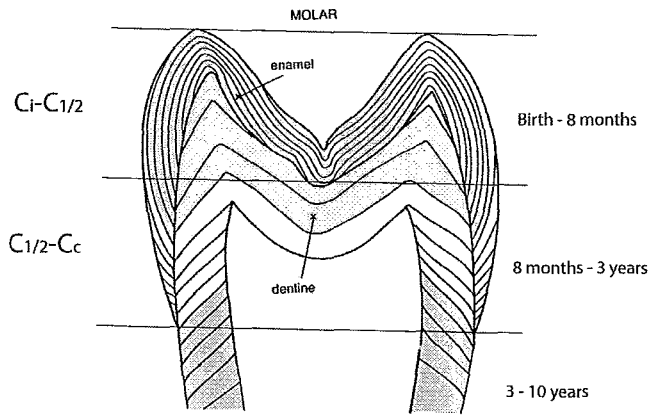


Figure 2.4: Example of growth layer overlap in seriations taken from the M1 (from Hillson 1996).

In general, girls will show advanced dental development over boys, but as archaeological subadult remains are not assigned a sex, there is no way to correct for the range of error in aging introduced by sex. While the Gustafson and Koch mean age ranges have been noted as a subjective age assessment (Hillson 1996), it has the benefit of a combined mean for both boys and girls and is the most appropriate age estimation for this sample. Additionally, since there is no baseline comparison to a neonatal line, a microstructural marker laid down in dental tissues at birth, nor a historical record of birth, these teeth have been assigned developmental ages, which may differ from chronological ages.

Collagen extraction

Tooth samples were prepared for isotopic analysis at the McMaster Geochemistry laboratory, Hamilton, Ontario. Dental wax was removed from the sections in two steps. Initially, wax removal was attempted by heating the samples in distilled water to 90°C and then skimming the melted wax from the top using cotton swabs. This was not effective; therefore, the majority of the wax residue was removed by submersion in an 80°C acetone bath for five minutes, followed by twenty minutes in test tubes of distilled water immersed in a SonicCleaner ultrasonic cleaning bath which provided gentle agitation of the samples to loosen any remaining wax. The sections were then moved to clean test tubes, dried overnight at 60°C and weighed.

Collagen was extracted from the sections following a modified Longin procedure (Chisholm et al. 1983). Each dental section was demineralized in 0.25M hydrochloric acid [HCl] at room temperature for 24 hours and then rinsed with distilled water to restore neutrality. A solution of 0.125M sodium hydroxide [NaOH] was applied for 40 minutes to remove any humic acids contaminating the sample from the burial environment, after which each section was again rinsed in distilled water. The samples were then heated in a 0.05M solution of HCl at 80°C. The collagen took longer than expected to solubilize (five days) and 17 of 105 total sections showed a particulate

suspension. There was no discernable pattern of section, tooth type or individual to explain this particulate, and there does not seem to have been any effect on the final isotopic values. When the collagen was solubilized, the samples were centrifuged, decanted, and dried at 60°C. As the protein content of enamel is negligible, the demineralization process ensures that the enamel is essentially removed from the sections and it is noted that the data therefore reflects the collagen of the remaining dentine, however, the crown samples contain large amounts of enamel relative to dentine and there may be an amelogen signal mixed with the collagen.

The samples were next run on a Thermo-Finnigan DeltaPlus XP coupled to a Costech elemental analyzer. Diagenesis was examined using the C:N ratios which fell within the acceptable range of 2.9 to 3.6 (DeNiro 1987), indicating adequate sample preservation. Four samples were excluded from further analysis: three had elevated C:N ratios (#5040-24.1.4, #5518-12.1.4, #8036-11.2.4) and one (#5518-46.2.2) yielded much higher $\delta^{13}C$ values than the other sections from the same tooth. This latter was likely still contaminated with dental wax (Ambrose, 1990; Ambrose and Norr, 1993).

All results are reported in units of per mil (‰) with respect to the PDB standard for $\delta^{13}C$ and the AIR standard for $\delta^{15}N$. The precision of analyses is estimated to be ± 0.1 ‰ for both isotope ratios. Note, however, that due to the small size of the samples (average size 0.15 mg before demineralization) it was not possible to carry out replicate analyses of these samples.

Limitations

Dental wax contamination was an initial concern in the sectioning of this sample. Although the wax does not permeate the dental tissues and was therefore considered ideal for embedding the teeth during sectioning, it proved to be very difficult to remove from the tiny and delicate serial sections that were created. In particular, the cuspal areas of the teeth retained visible amounts of wax and the concavities of incomplete crowns were packed with wax after the initial removal attempt. The acetone bath and agitation in distilled water removed all visible and tactile residue, but some contamination may have remained, as evidenced by the particulates observed during collagen extraction. In addition to contamination, the wax removal process directly increased loss of sample material from disarticulated cusps and thin, friable root sections through breakage.

Three samples of dental wax established a mean $\delta^{13}C$ value of -28.3‰. Given the population appropriate $\delta^{13}C$ levels found in the dental samples (average = -18.6 ‰), it is reasonable to assume contamination is negligible, as any substantial amount of wax included in the dental tissue would have decreased the $\delta^{13}C$ significantly. The exception, as noted above, was section #5518-46.2.2, which showed a $\delta^{13}C$ level of -24.4, a significantly more negative level than is reasonable for this sample, which seems to indicate wax contamination. This section was excluded from the analysis.

The choice of tooth types used in sampling creates a limitation in the ages available for analysis. The use of mixed dentition allows the researcher to track dietary change over the largest range of ages (approximately five months *in utero* to eight years of age); with the majority of sections representing four, six or 12 months of development useful in assessing chronological change in diet during early childhood. Sections from M1 and the root of dm2 encompass a much longer period of development, thus levels are less specific for ages 18 months to three years. Unfortunately, this is precisely the age range when cessation of breast milk is expected to occur. By using this series of teeth (dm1, dm2, M1) dietary information from between three and ten years of age is available only as an average from this entire period which does not provide time specific information about the dietary levels of childhood and may obscure the end of the weaning process in individuals who have extended breastfeeding or in populations that practice extended weaning periods. Ideally rib ends would be included in the analysis to provide this additional information by contributing a base level of dietary isotopic signatures at or near the time of death.

As discussed by Balasse and Tresset (2002), the greatest limitation of serial sampling of dental tissue is time resolution: the error introduced through serial sectioning by the averaging effect of the horizontal sectioning. Each section is potentially introducing error proportional to the length of developmental time sampled, and the smallest sections (comprised of approximately four developmental months) have the least amount of possible bias. This decrease in bias is effective when the temporal shift is monotonic, as expected in most weaning situations where a gradual increase of solid foods accompanies a relative decrease in breast milk (Fuller et al. 2006). In this situation, the average isotope levels represented in each seriation becomes part of a larger pattern of temporal change that will still be visible across the sections. In cases of abrupt weaning, either culturally or through an unexpected loss of the mother, the resolution of even the smaller serial sections will encompass far too much development to show this step-wise change. The abrupt weaning event will be averaged into the larger pattern, obscuring the trend. In this way, serial sectioning is still affected by the assumption of Fuller et al. (2003), that the process of weaning lasts several months. Although a pattern of decreased enrichment will still be visible in the temporal sequence of the serial sections, the resolution of macroscopic sectioning is not refined enough to capture events that take place over weeks or days.

Additionally, as a function of sectioning, the width of the saw removed material of approximately three millimeters at each sectioning point, representing between three and five percent of the total tooth tissues. Results and analysis treat sections as continuous and do not account for this loss of developmental material as the time resolution is not known precisely and should not affect the pattern of change through time at the individual sub-adult level.

In addressing the bias introduced through developmental structures the importance of patterns in interpretation of stable isotope analysis of dental serial sectioning cannot be stressed enough. As opposed to using a single isotopic signature as a general indicator of diet at time of death, (the method used for analysis of rib ends) serial sectioning of deciduous teeth provides a pattern of change from the maternal baseline diet

to the individual diet at the end of the sample due to death or cessation of tooth development. While a detailed timeline for this dietary change is created, any abrupt change in diet occurring within a section will be obscured within the average; interpretation within the context of each individual's pattern through time as well as within the larger population pattern is crucial to analysis.

Chapter 3: Results

Table of Stable Isotope Data

Individual	Sample	Tooth	δ^{13} C	δ^{15} N	C:N
241	241.1.1	Rdm^1	-18.92	12.56	3.26
	241.1.2	Rdm^1	-18.87	12.26	3.24
	241.1.3	Rdm^1	-18.54	11.38	3.19
	241.1.4	Rdm^1	-18.88	10.62	3.32
	241.2.1	Ldm^2	-19.02	12.28	3.26
	241.2.2	Ldm^2	-18.81	11.79	3.22
	241.2.3	Ldm^2	-18.84	10.47	3.25
	241.3.1	RM_1	-18.8	9.02	3.45
	241.3.2	RM_1	-19.42	9.21	3.35
266	266.1.1	Ldm^1	-18.31	13.79	3.31
	266.1.2	Ldm^1	-18.72	13.15	3.4
	266.1.3	Ldm^1	-17.19	13.66	3.21
	266.2.1	Ldm^2	-18.23	13.1	3.31
	266.2.2	Ldm^2	-18.31	11.96	3.2
	266.2.3	Ldm^2	-18.39	11.29	3.41
	266.4.1	RM^1	-18.61	12.3	3.22
	266.4.2	RM^1	-17.99	10.61	3.27
295	295.1.1	Ldm^2	-18.85	9.87	3.32
	295.1.2	Ldm^2	-18.67	12.24	3.21
	295.1.3	Ldm^2	-18.84	11.8	3.27
	295.2.1	LM^1	-19.37	11.04	3.47
	295.2.2	LM^1	-19.61	10.54	3.41
	295.3	alveolar bone	-19.61	10.24	3.23
314	314.1.1	Ldm_1	-18.29	14.23	3.22
	314.1.2	Ldm_1	-18.05	14.34	3.16
	314.1.3	Ldm_1	-18.1	13.94	3.18

Individual	Sample	Tooth	$\delta^{13}C$	$\delta^{15}N$	C:N
	314.1.4	Ldm_1	-18.09	12.93	3.18
	314.2.1	LM_1	-18.48	14.29	3.19
	314.2.2	LM_1	-18.45	12.83	3.2
	314.2.3	LM_1	-19.34	11.31	3.2
428	428.1.1	RM1	-17.94	13.18	3.19
	428.1.2	RM1	-17.83	12.71	3.23
	428.1.3	RM1	-18.6	11.43	3.28
	428.2.1	Ldm_2	-18.06	12.86	3.2
	428.2.2	Ldm_2	-18.62	11.43	3.21
	428.2.3	Ldm_2	-19.33	10.3	3.24
	428.2.4	Ldm_2	-20.31	10.38	3.61
5040-24	5040-24.1.1	Ldm^1	-17.73	14.86	3.15
	5040-24.1.2	Ldm^1	-18	15.67	3.21
	5040-24.1.3	Ldm^1	-18.52	14.98	3.26
	5040-24.2.1	Ldm_1	-17.76	15.29	3.18
	5040-24.2.2	Ldm_1	-18.1	15.53	3.22
	5040-24.2.3	Ldm_1	-18.3	14.38	3.2
	5040-24.2.4	Ldm_1	-19.32	12.55	3.51
	5040-24.3.1	Rdm_1	-17.74	15.42	3.17
	5040-24.3.2	Rdm_1	-18.25	15.58	3.25
	5040-24.3.3	Rdm_1	-18.75	14.1	3.35
	5040-24.3.4	Rdm_1	-19.23	12.61	3.44
	5040-24.4.1	Ldm^2	-18.99	12.66	3.35
	5040-24.4.2	Ldm^2	-18.32	14.16	3.21
	5040-24.4.3	Ldm^2	-17.96	15.1	3.22
5040-46	5040-46.1.1	Rdm_1	-17.35	16.98	3.15
	5040-46.1.2	Rdm_1	-17.44	16.41	3.2
	5040-46.1.3	Rdm_1	-17	16.05	3.19

Individual	Sample	Tooth	δ^{13} C	δ^{15}N	C:N
	5040-46.1.4	Rdm_1	-17.11	15.98	3.2
	5040-46.2.1	Rdm_2	-17.33	16.47	3.18
	5046-46.2.2	Rdm_2	-17.13	15.69	3.19
	5040-46.2.3	Rdm_2	-16.94	15.68	3.19
	5040-46.3.1	RM_1	-17.23	15.73	3.14
	5040-46.3.2	RM_1	-17.18	15.6	3.2
	5040-46.3.3	RM_1	-17.11	15.13	3.17
	5040-46.3.4	RM_1	-18.22	14.52	3.46
5094-9	5094-9.1.1	Ldm^1	-18.36	12.54	3.2
	5094-9.1.2	Ldm^1	-18.47	12.23	3.22
	5094-9.1.3	Ldm^1	-18.83	11.61	3.22
	5094-9.1.4	Ldm^1	-19.05	11.41	3.26
	5094-9.2.1	Rdm_2	-18.44	12.2	3.17
	5094-9.2.2	Rdm_2	-18.95	11.31	3.19
	5094-9.2.3	Rdm_2	-19.1	11.09	3.18
	5094-9.2.4	Rdm_2	-20.22	10.57	3.47
	5094-9.3.1	RM_1	-18.64	12.05	3.18
	5094-9.3.2	RM_1	-19.35	10.5	3.17
5518-12	5518-12.1.1	Rdm_1	-17.92	12.95	3.17
	5518-12.1.2	Rdm_1	-18.14	10.13	3.4
	5518-12.1.3	Rdm_1	-17.59	12.27	3.17
	5518-12.1.4	Rdm_1	-17.98	11.77	3.28
	5518-12.2.1	Ldm_2	-18.18	12.62	3.2
	5518-12.2.2	Ldm_2	-17.8	12.74	3.16
	5518-12.2.3	Ldm_2	-17.82	11.08	3.18
	5518-12.2.4	Ldm_2	-18.18	8.91	3.5
	5518-12.3.1	Rdm^2	-18.11	10.67	3.38
	5518-12.3.2	Rdm^2	-17.86	12.5	3.19

Individual	Sample	Tooth	$\delta^{13}C$	$\delta^{15}N$	C:N
	5518-12.3.3	Rdm^2	-18.6	10.94	3.4
	5518-12.4.1	RM^1	-17.94	12.84	3.15
	5518-12.4.2	RM^1	-17.86	11.54	3.19
5518-46	5518-46.1.1	Rdm^1	-18.49	12.39	3.23
	5518-46.1.2	Rdm^1	-18.25	12.22	3.17
	5518-46.1.3	Rdm^1	-18.7	11.11	3.19
	5518-46.2.1	Rdm^2	-18.52	11.86	3.22
	5518-46.2.3	Rdm^2	-19.04	10.49	3.22
	5518-46.3.1	RM^1	-18.88	5.37	3.5
	5518-46.3.2	RM^1	-19.38	10.4	3.2
	5518-46.3.3	RM^1	-19.38	9.93	3.21
8036-11	8036-11.1.1	Rdm^1	-16.72	13.7	3.18
	8036-11.1.2	Rdm^1	-17.11	13.76	3.18
	8036-11.1.3	Rdm^1	-16.87	13.16	3.26
	8036-11.1.4	Rdm^1	-16.36	13.61	3.28
	8036-11.2.1	Rdm^2	-17.03	13.7	3.19
	8036-11.2.2	Rdm^2	-17.1	13.62	3.18
	8036-11.2.3	Rdm^2	-15.75	13.2	3.16
	8036-11.3.2	LM^1	-16.17	13.19	3.16
	8036-11.3.3	LM^1	-18.56	11.16	3.19
	8036-11.3.1	LM^1	-17.38	13.34	3.2

Table 3.1: Stable carbon and nitrogen isotope data and C:N ratios - $\delta^{13}C$ (‰ VPDB) $\delta^{15}N$ (‰ , AIR).

Average Isotopic Values

	dm1	$\delta^{13}C$	$\delta^{15}N$	dm2	$\delta^{13}C$	$\delta^{15}N$	M1	$\delta^{13}C$	$\delta^{15}N$
C_i- C½	5 mo *in utero* – birth (n=8)	-17.98	13.72	6 mo *in utero*- 4 mo (n=9)	-18.18	12.82	birth – 8 mo (n=10)	-18.27	12.24
C½- C_c	birth – 6 mo (n=9)	-18.12	13.35	4 mo – 10 mo (n=10)	-18.30	12.60	8 mo- 3 yrs (n=10)	-18.32	11.71
R_i – R½	6 mo- 10 mo (n=9)	-17.93	13.13	10 mo- 2 years (n=10)	-18.18	12.18	3-10 yrs (n=4)	-18.97	10.98
R½ - R_c	10 mo- 2.3 years (n=6)	-17.91	12.72	2-3 yrs (n=3)	-19.57	9.95			
total change		-0.07	-1		-1.39	-2.87		-0.7	-1.26

Table 3.2: Average $\delta^{13}C$ and $\delta^{15}N$ for all individuals by section and tooth type.

Graphs: Change in δ15N and δ13C over time by Individual

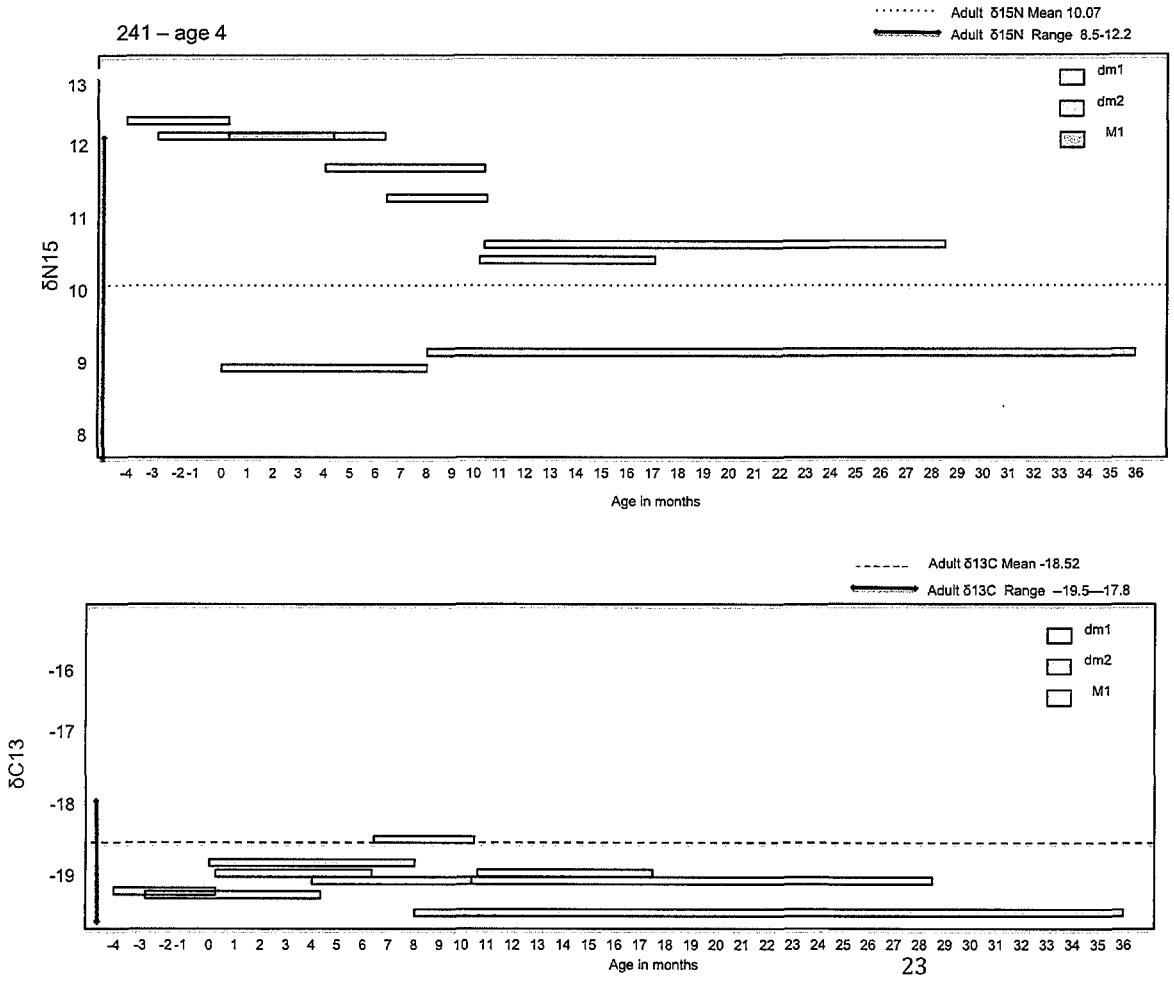


Figure 3.1: Individual dietary timeline for individual 241.

Individual 241 shows initial decrease in $\delta^{15}N$ between age 6-10 months, with total decrease in $\delta^{15}N$ of ~3‰. Individual reaches post-weaning diet within known adult range. M1 presents unusual pattern of increase in enrichment and lower overall levels of $\delta^{15}N$ inconsistent with expected pattern.

Individual 241 shows initial decrease in $\delta^{13}C$ around 4-6 months, with total decrease in $\delta^{13}C$ of ~1‰. Individual reaches post-weaning diet within known adult range. Dm1 and dm2 present pattern of increased enrichment in first three sections inconsistent with expected pattern, however, dm1 and M1 show decreased enrichment between 8 months and 3 years.

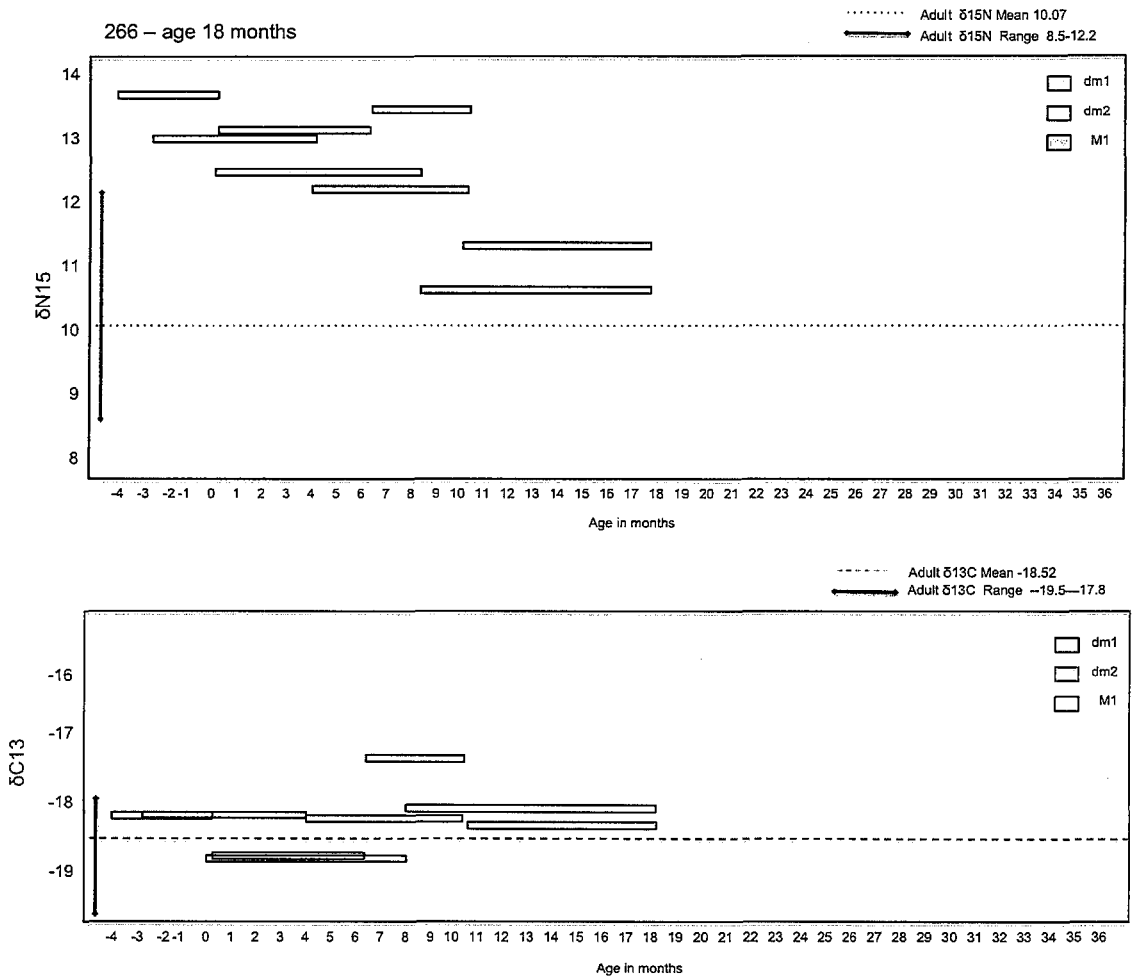


Figure 3.2: Individual dietary timeline for individual 266.

Individual 266 shows initial decrease in $\delta^{15}N$ between birth and 6 months, with total decrease in $\delta^{15}N$ of ~3‰. Individual reaches post-weaning diet within known adult range.

Individual 266 shows initial decrease in $\delta^{13}C$ around 4-6 months, with total decrease in $\delta^{13}C$ of ~1‰. Individual reaches post-weaning $\delta^{13}C$ levels within the known adult range.

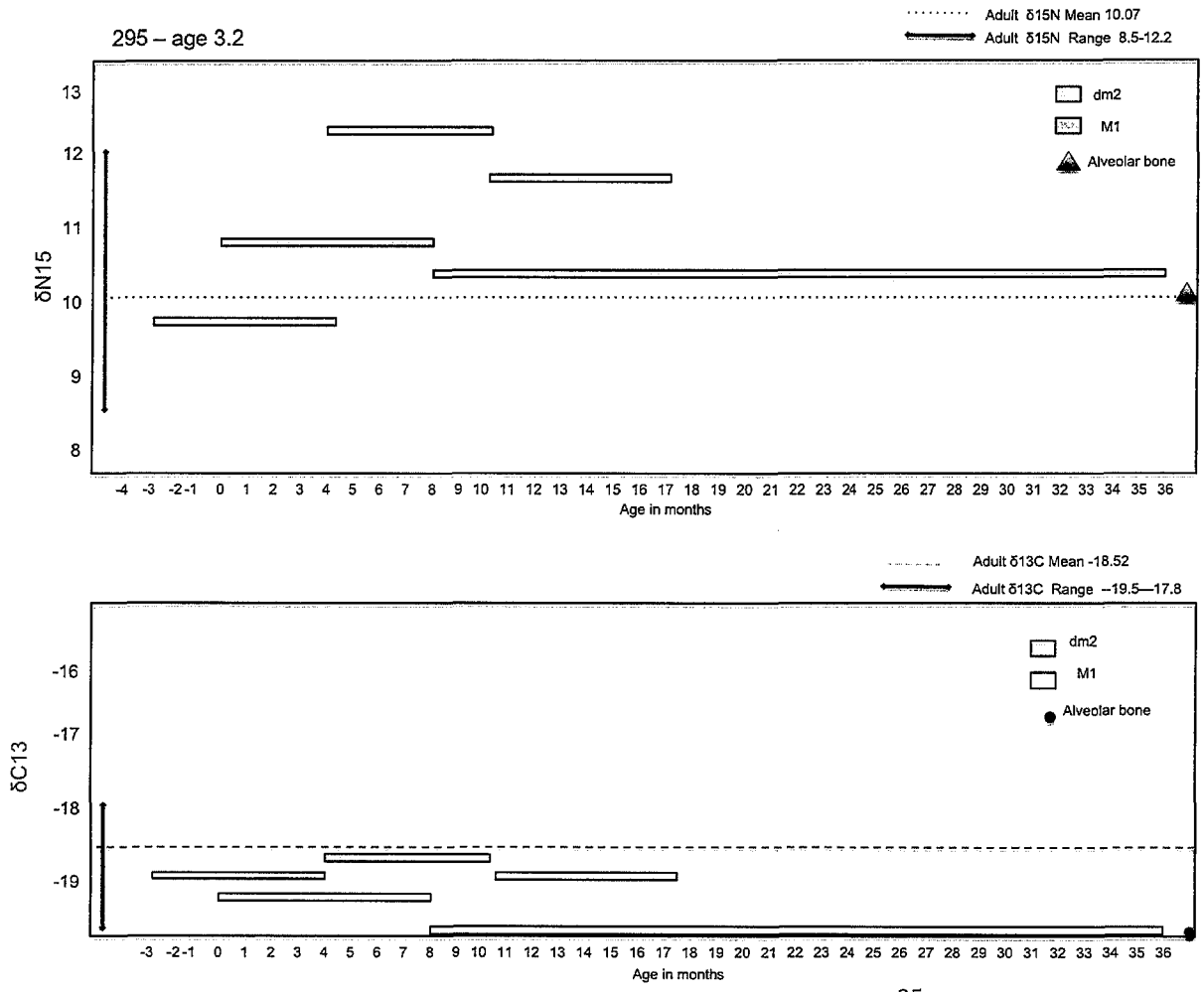


Figure 3.3: Individual dietary timeline for individual 295.

Individual 295 shows an initial decrease in $\delta^{15}N$ between age 8-16 months, with total decrease in $\delta^{15}N$ of ~2.5‰. This individual reaches a post-weaning diet within the known adult range. Dm2 shows unusual pattern of enrichment around four months.

Individual 295 shows initial decrease in $\delta^{13}C$ around 8-10 months, with a total decrease in $\delta^{13}C$ of ~1‰. This individual reaches a post-weaning $\delta^{13}C$ levels within the known adult range. Dm2 shows unusual pattern of enrichment around four months.
$\delta^{15}N$ but not $\delta^{13}C$ of alveolar bone agrees with average adult diet values.

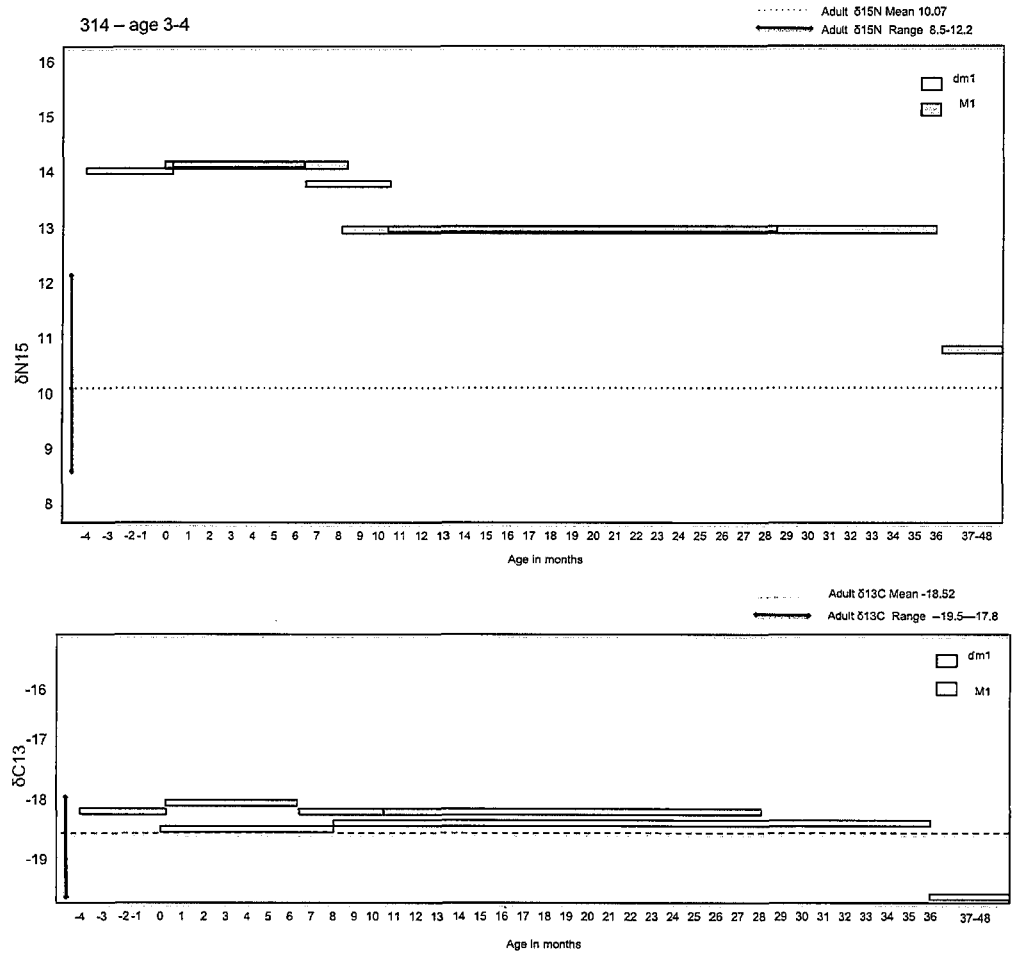


Figure 3.4: Individual dietary timeline for individual 314.

Individual 314 shows an initial decrease in $\delta^{15}N$ between age 6-10 months, with total decrease in $\delta^{15}N$ of ~3‰. This individual reaches post-weaning diet within known adult range. Individual shows unexpected additional decrease in final section of M1.

Individual 314 shows an initial decrease in $\delta^{13}C$ around 6 months, with total decrease in $\delta^{13}C$ of ~2‰. This individual reaches a post-weaning diet within the known adult range. Individual 314 shows an unexpected additional decrease in final section of M1.

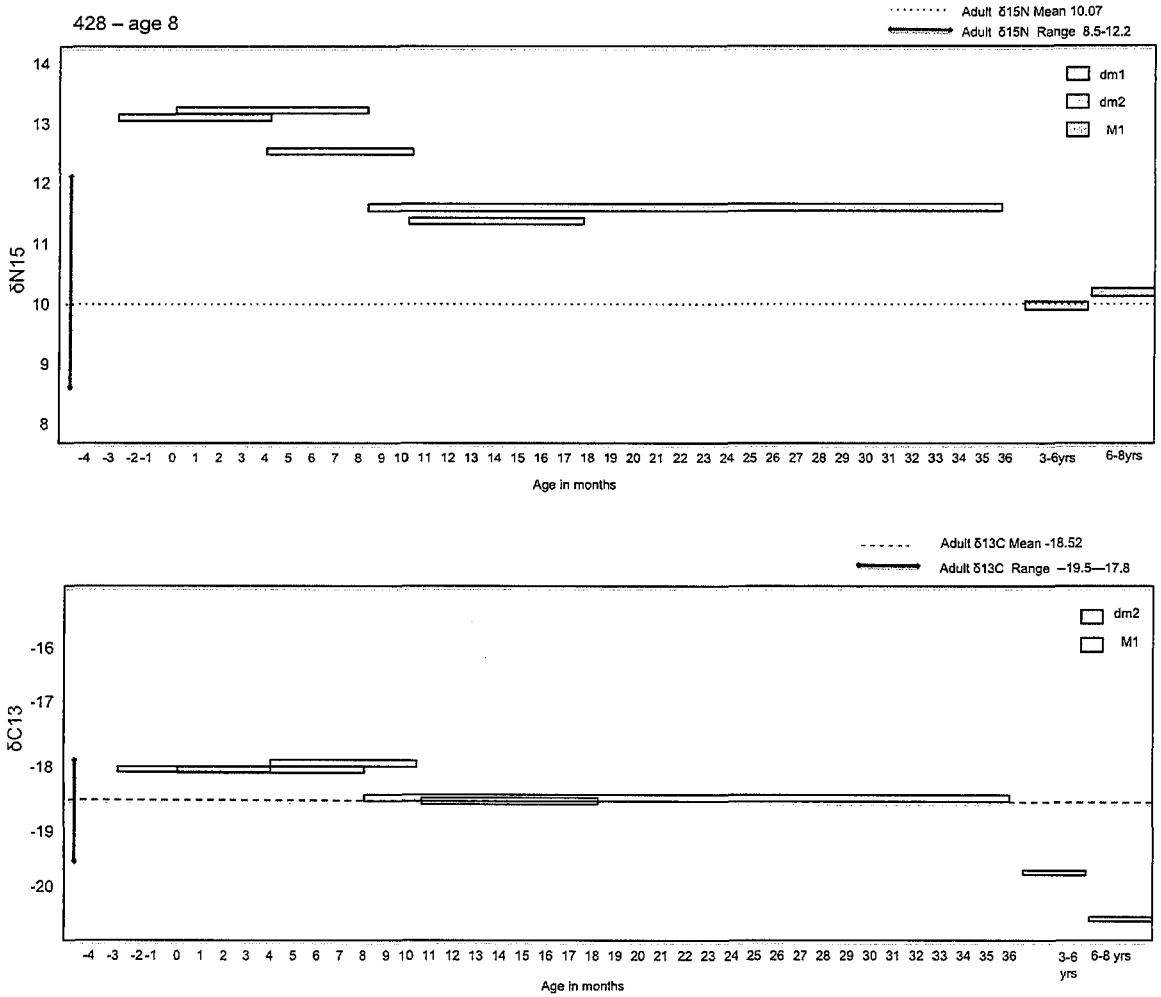


Figure 3.5: Individual dietary timeline for individual 428.

Individual 428 shows initial decrease in $\delta^{15}N$ between age 4-10 months, with total decrease in enrichment of $\delta^{15}N$ of ~3‰. Individual reaches post-weaning diet within known adult range. Individual shows unexpected additional decrease in final sections of M1.

Individual 428 shows initial decrease in $\delta^{13}C$ around 8 months, with total decrease in $\delta^{13}C$ of ~1‰. Individual reaches post-weaning diet within known adult range. Individual shows unexpected additional decrease in final sections of M1.

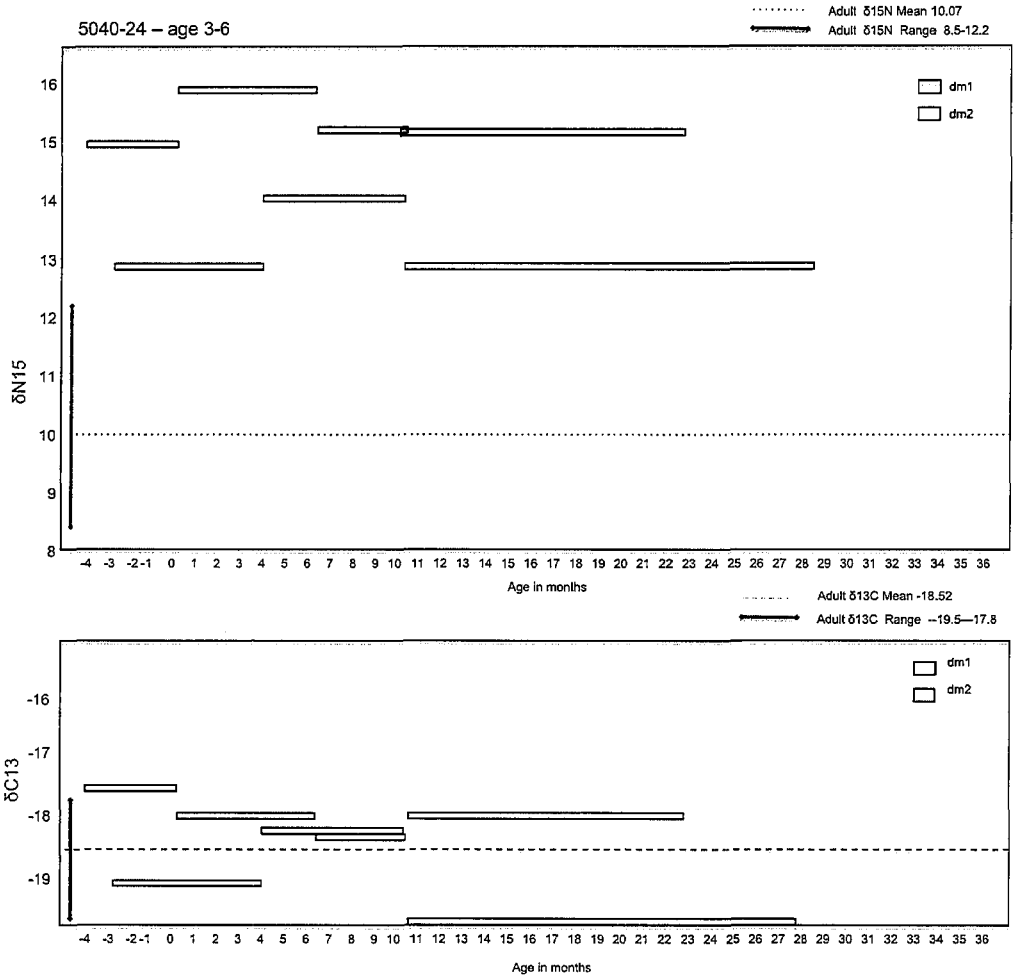


Figure 3.6: Individual dietary timeline for individual 5040-24.

Individual 5040-24 shows an initial decrease in $\delta^{15}N$ between age 6-10 months, with total decrease in $\delta^{15}N$ of ~3‰. This individual does not reach a post-weaning diet within the known adult range and dm2 presents an unusual pattern of increase in enrichment that is inconsistent with the expected pattern.

Individual 5040-24 shows initial decrease in $\delta^{13}C$ around birth to 6 months, with a total decrease in $\delta^{13}C$ of >2‰. This individual reaches a post-weaning diet within the known adult range, however dm2 presents an unusual pattern of increased enrichment in first three sections inconsistent with expected pattern, but consistent with the pattern of enrichment seen in the $\delta^{15}N$ levels.

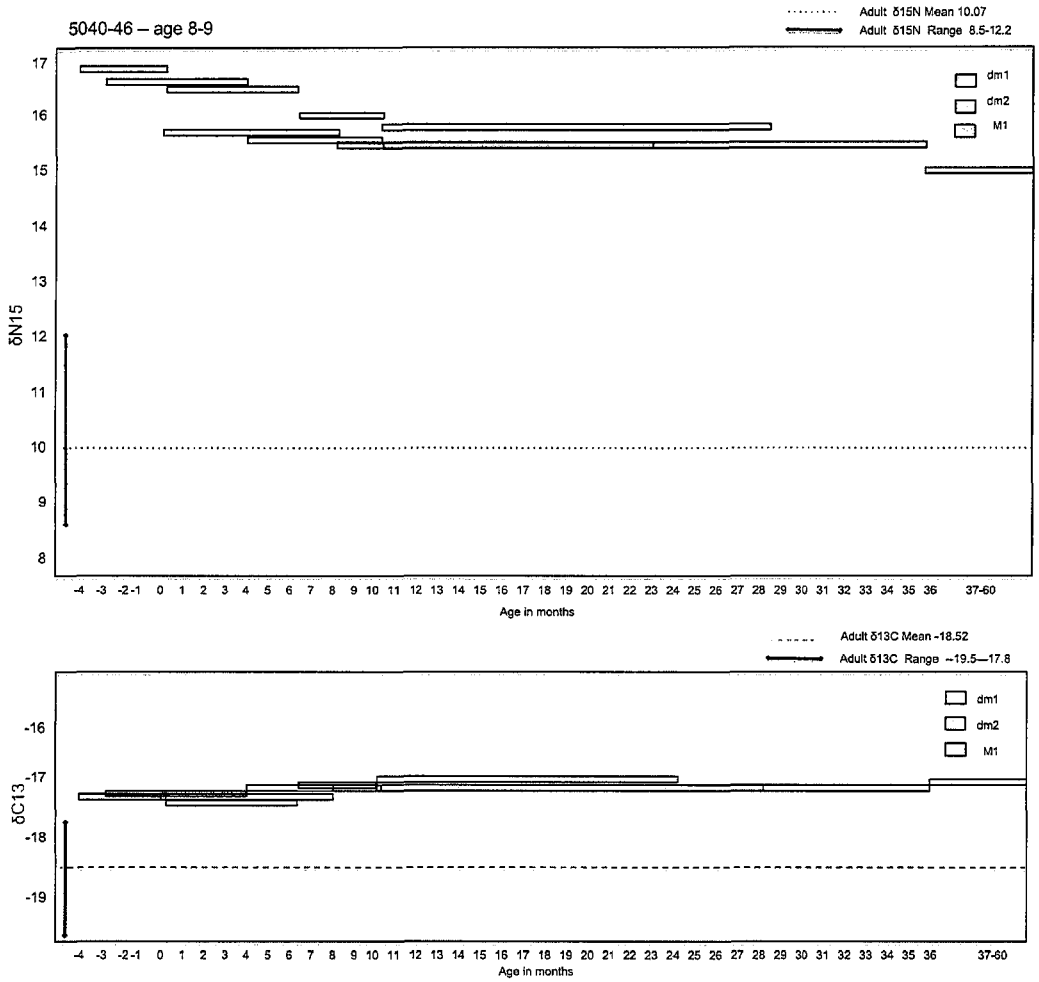


Figure 3.7: Individual dietary timeline for individual 5040-46.

Individual 5040-46 shows an initial decrease in $\delta^{15}N$ between 6-10 months, with a total decrease in $\delta^{15}N$ of ~3‰. This individual does not reach a post-weaning diet within the known adult range. All levels of $\delta^{15}N$ are higher than the expected range.

Individual 5040-46 shows an initial decrease in $\delta^{13}C$ around birth to six months, but displays an overall slight increase in $\delta^{13}C$ of <1‰. This individual does not reach a post-weaning diet within the known adult range.

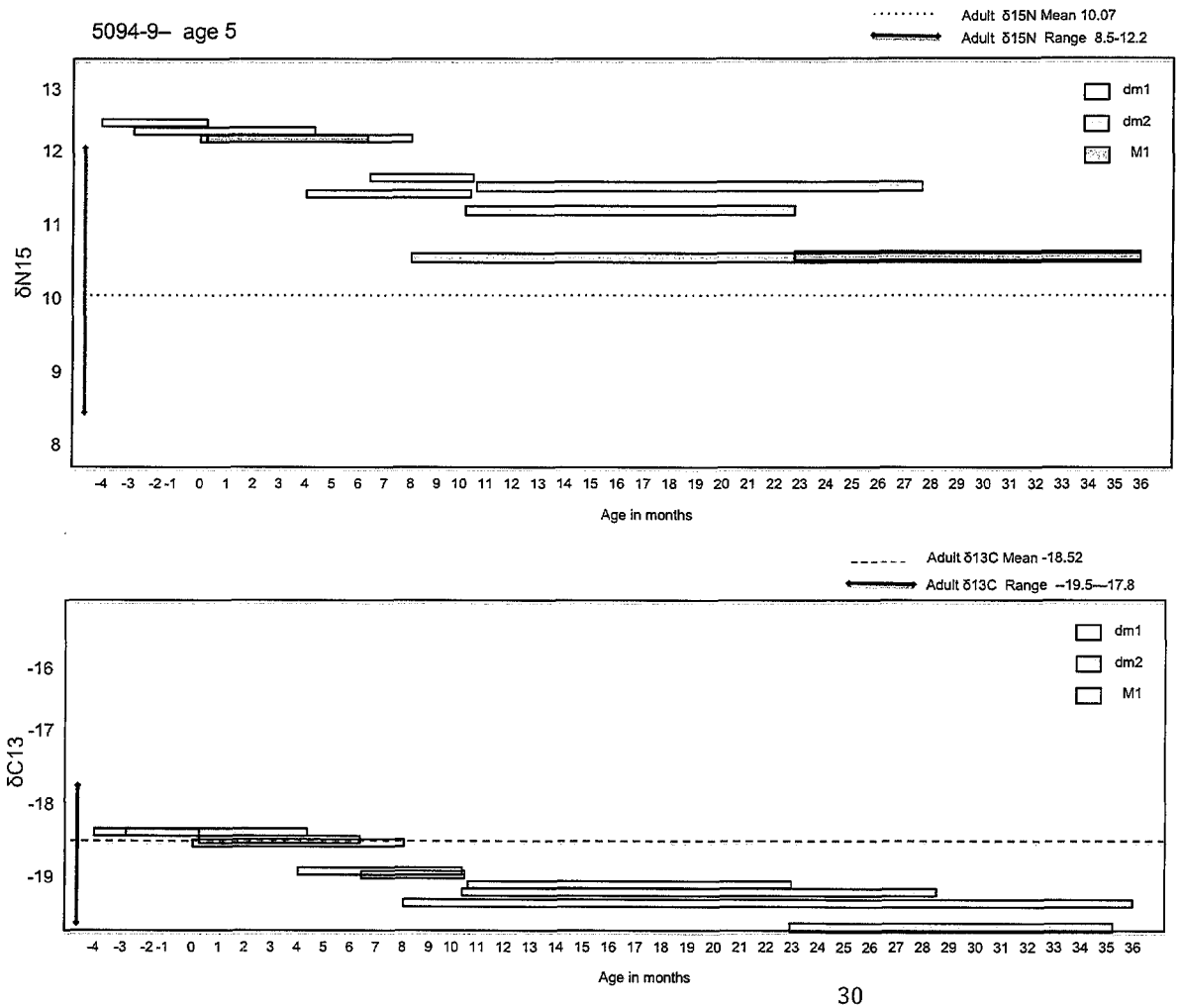


Figure 3.8 Individual dietary timeline for individual 5094-9.

Individual 5094-9 shows an initial decrease in $\delta^{15}N$ between age 4-10 months, with total decrease in $\delta^{15}N$ of ~2‰. This individual reaches a post-weaning diet within the known adult range.

Individual 5094-9 shows an initial decrease in $\delta^{13}C$ around 4-10 months, with total decrease in $\delta^{13}C$ of ~1.5‰. This individual reaches a post-weaning diet within the known adult range.

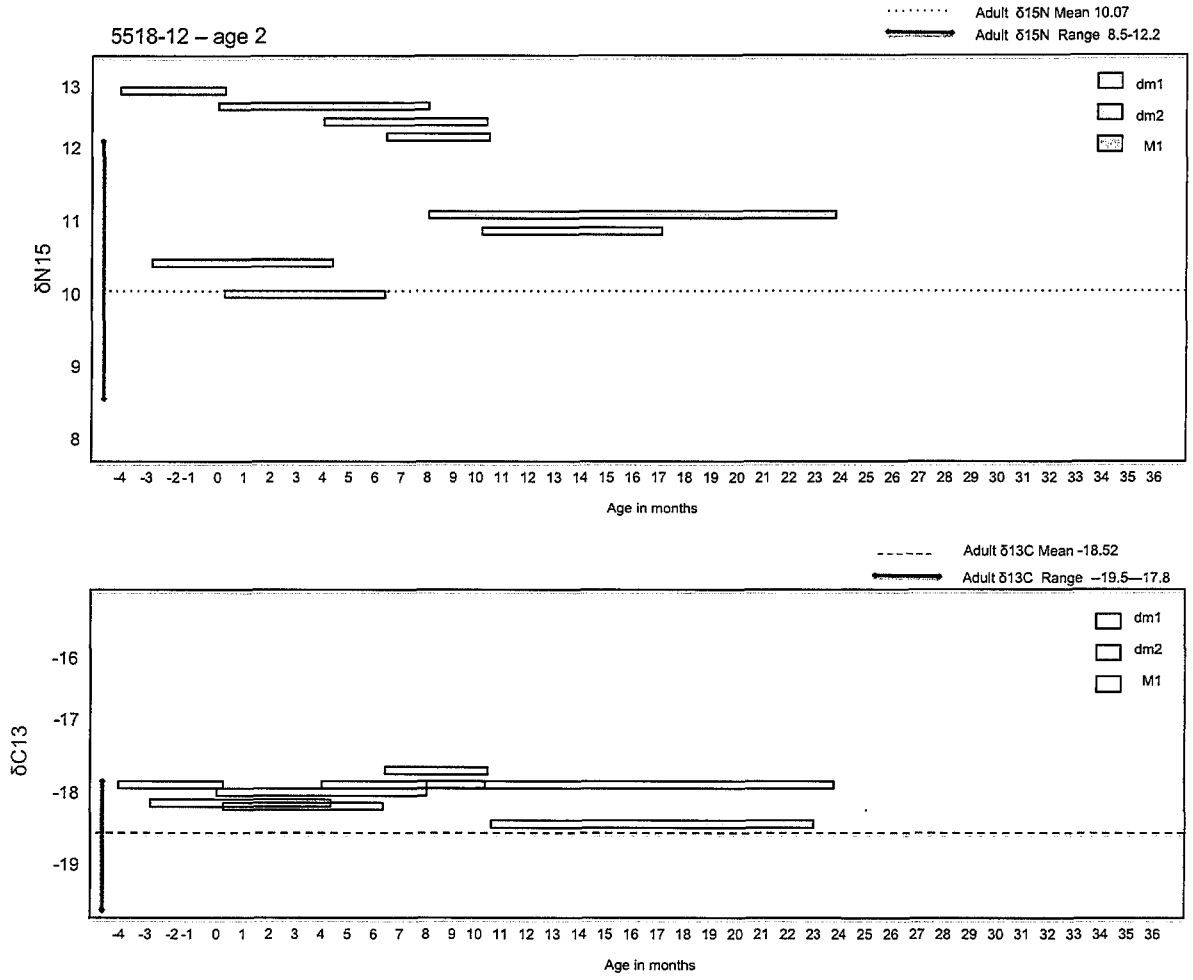


Figure 3.9: Individual dietary timeline for individual 5518-12.

Individual 5518-12 shows an initial decrease in $\delta^{15}N$ between age 4-10 months, with total decrease in $\delta^{15}N$ of ~2‰. This individual reaches a post-weaning diet within the known adult range.

Individual 5518-12 shows an initial decrease in $\delta^{13}C$ around 7-11 months, with total decrease in $\delta^{13}C$ of ~.75‰. This individual reaches a post-weaning diet within the known adult range.

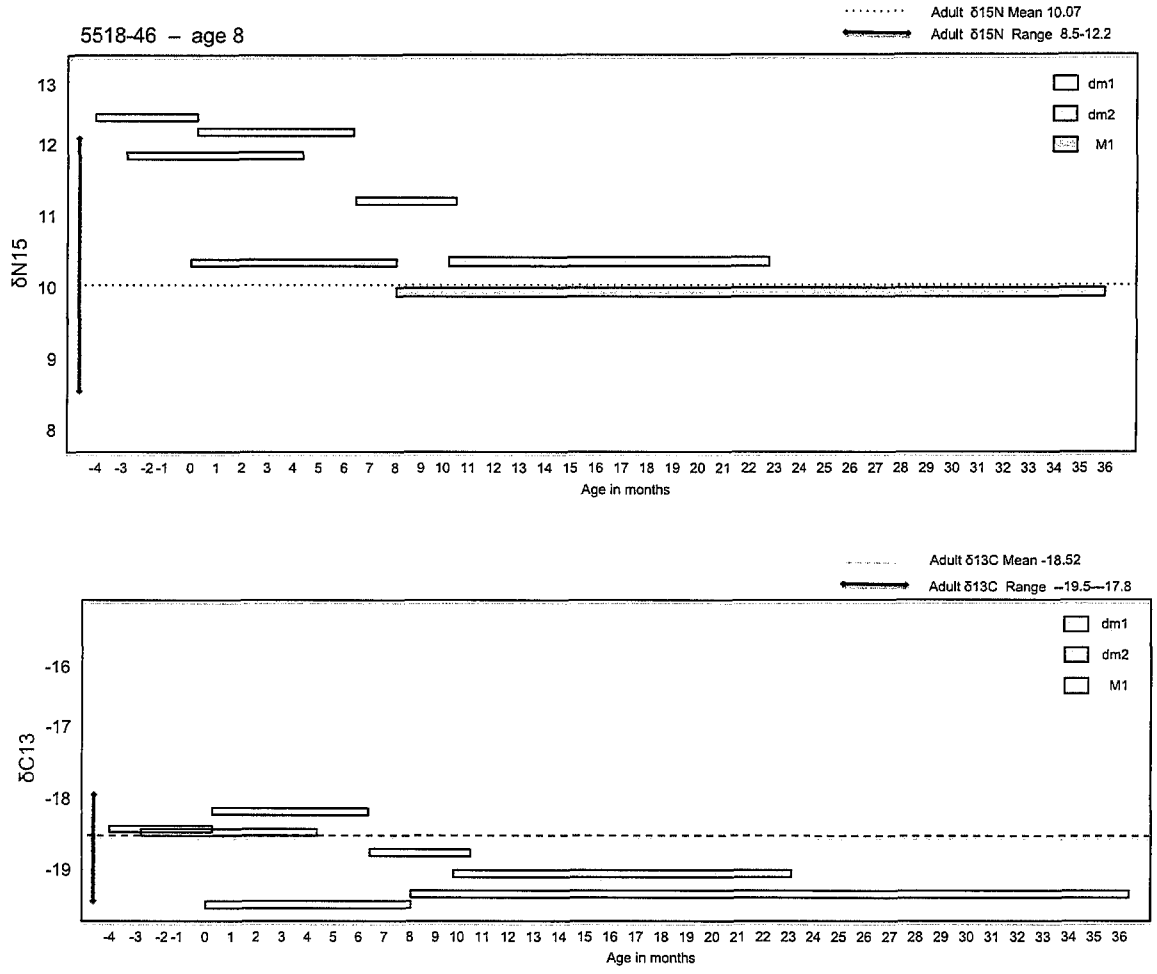


Figure 3.10: Individual dietary timeline for individual 5518-46.

Individual 5518-46 shows an initial decrease in $\delta^{15}N$ between age 6-10 months, with total decrease in $\delta^{15}N$ of ~3‰. This individual reaches a post-weaning diet within the known adult range.

Individual shows an initial decrease in $\delta^{13}C$ around 6-10 months, with total decrease in $\delta^{13}C$ of ~1‰. This individual reaches a post-weaning diet within the known adult range. M1 presents unusual pattern of slightly increased enrichment inconsistent with expected pattern.

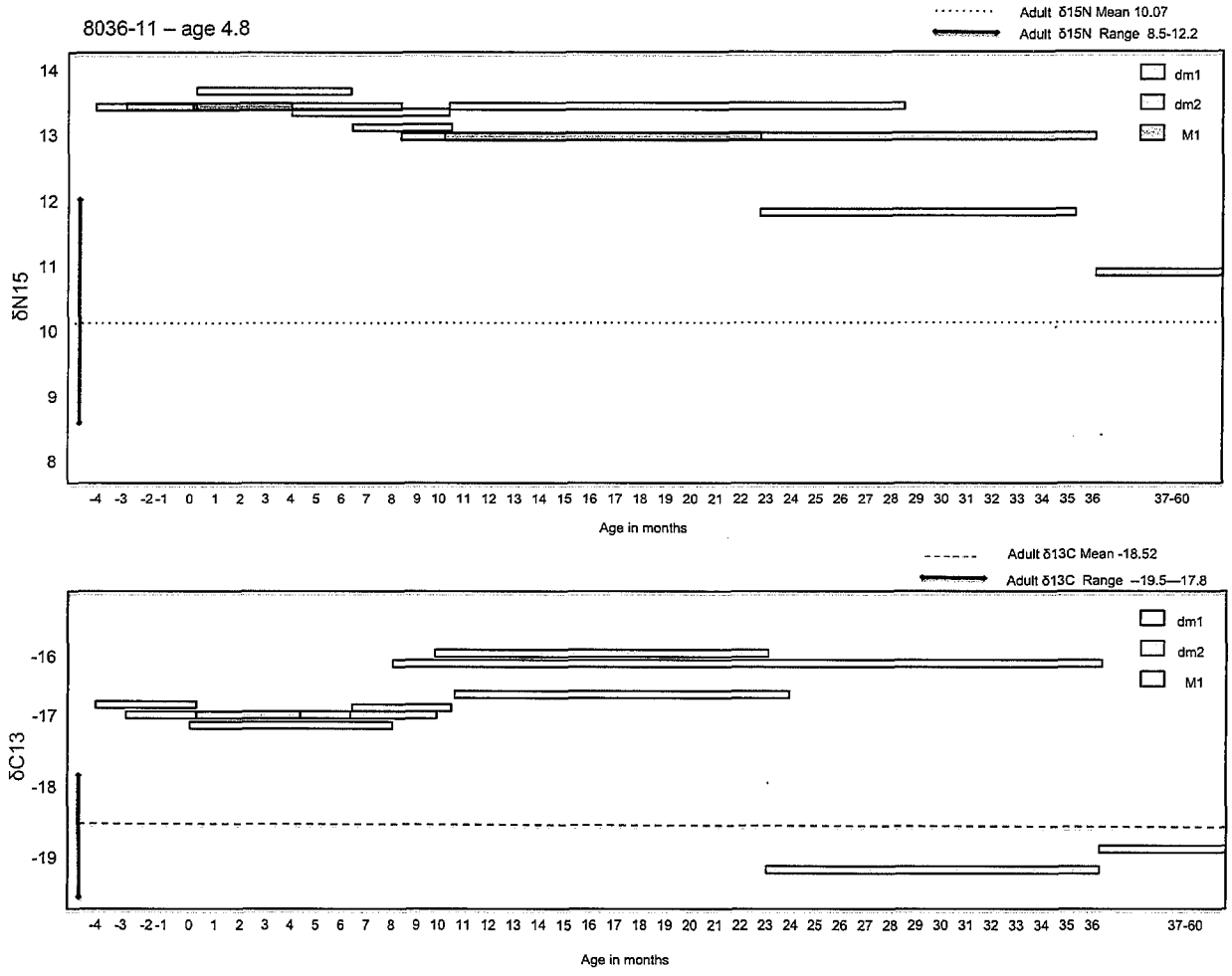


Figure 3.11: Individual dietary timeline for individual 8036-11.

Individual 8036-11 shows an initial decrease in $\delta^{15}N$ between age 6-10 months, with a total decrease in $\delta^{15}N$ of ~3‰. This individual reaches a post-weaning diet within the known adult range.

Individual 8036-11 shows an unexpected increase in $\delta^{13}C$ around 6-10 months, with total increase in $\delta^{13}C$ of ~1‰, followed by a decrease in enrichment around 23 months with a total decrease in enrichment of ~4‰. Although this Individual reaches a post-weaning diet within the known adult range, the elevated levels of $\delta^{13}C$ from 8-23 months are much higher than the expected levels for this population.

$\delta^{13}C$ vs $\delta^{15}N$ Correlations

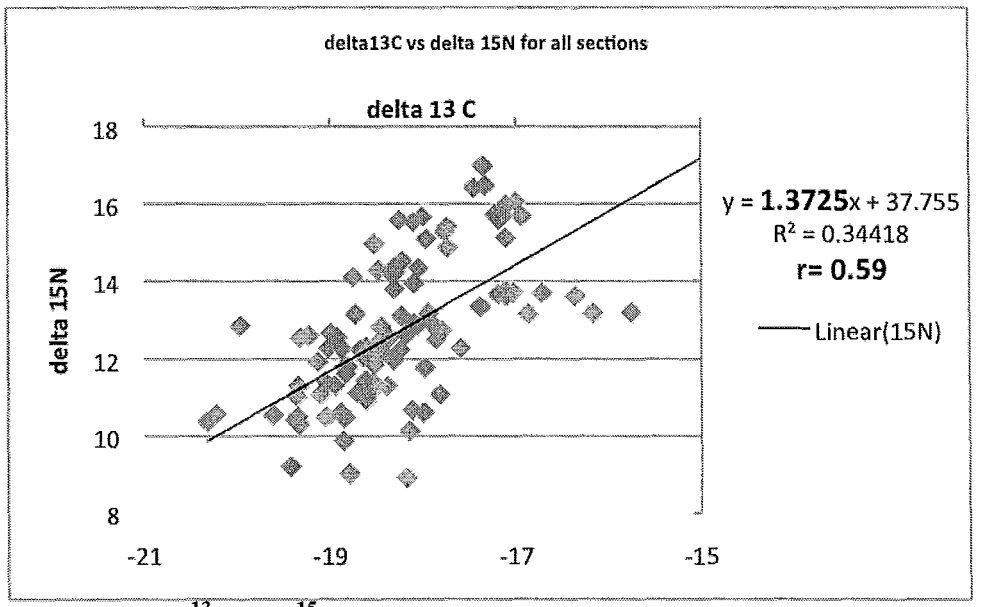


Figure 3.12: $\delta^{13}C$ vs $\delta^{15}N$ for all sections.
$\delta^{13}C$ vs $\delta^{15}N$ plotted for all sections shows weak correlation (r= 0.59), and a slope of 1.4. There is more scatter than expected if weaning process is homogeneous in all individuals.

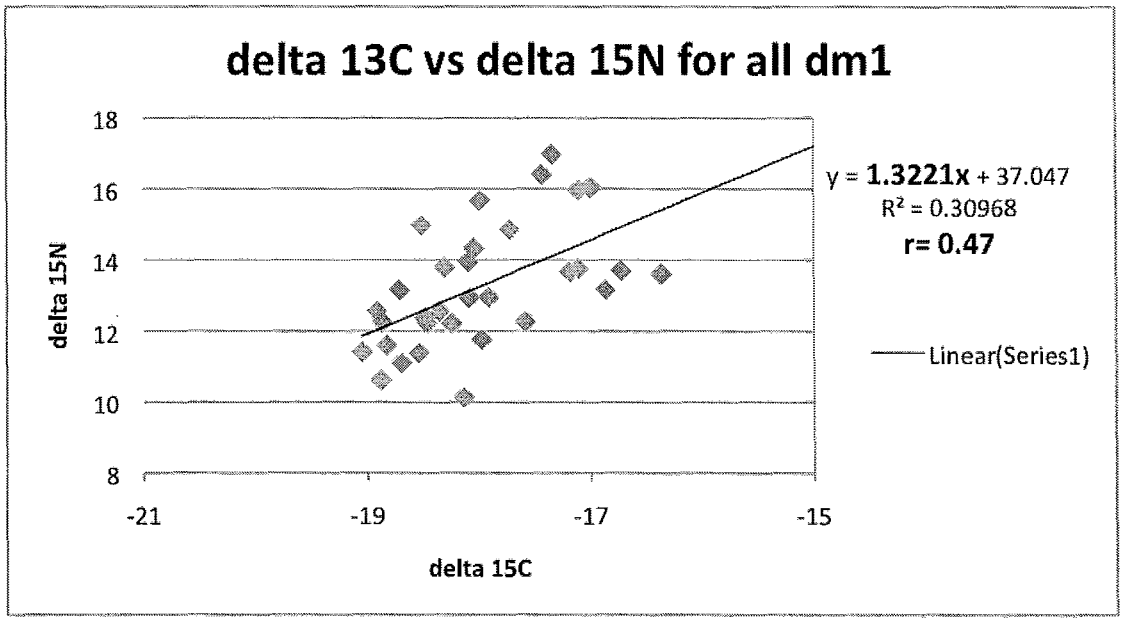


Figure 3.13: $\delta^{13}C$ vs $\delta^{15}N$ for all dm1.

$\delta^{13}C$ vs $\delta^{15}N$ plotted for all dm1 sections shows weak correlation (r= 0.47), and a slope of 1.3. There is more scatter than expected if weaning process is homogeneous in all individuals.

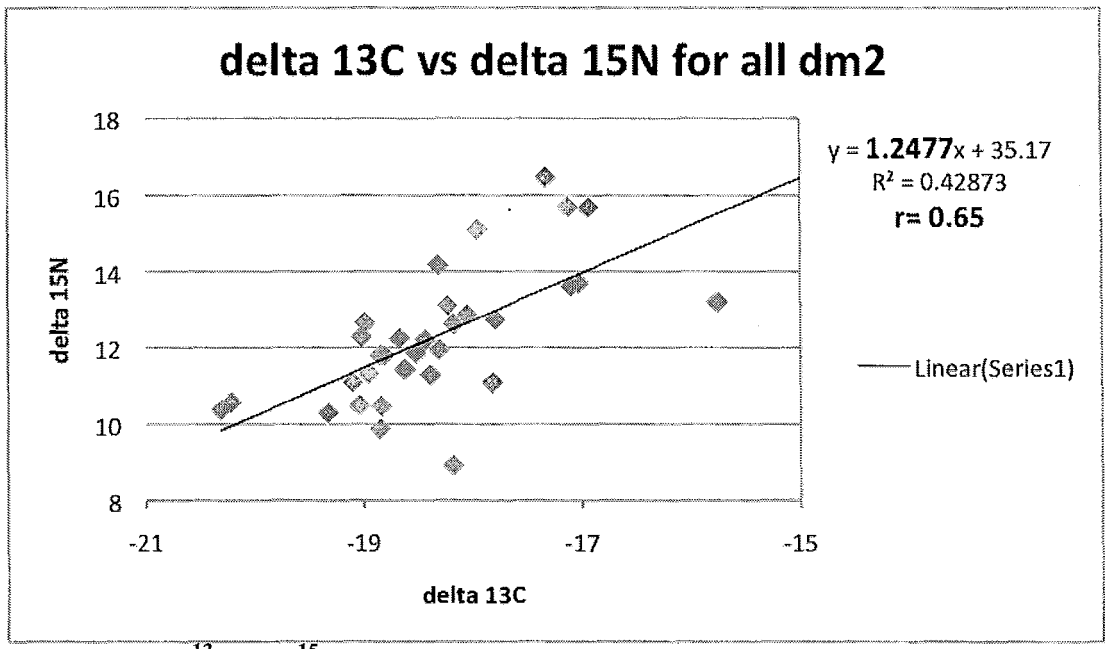


Figure 3.14: $\delta^{13}C$ vs $\delta^{15}N$ for all dm2

$\delta^{13}C$ vs $\delta^{15}N$ plotted for all dm2 sections shows weak correlation (r= 0.65), and a slope of 1.2. There is more scatter than expected if weaning process is homogeneous in all individuals.

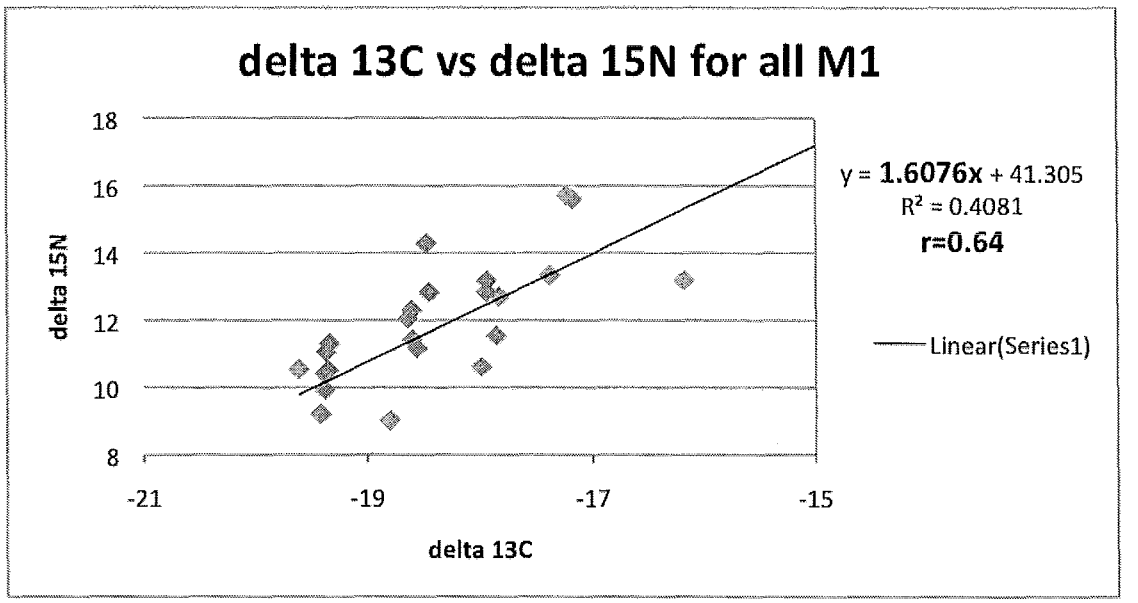


Figure 3.15: $\delta^{13}C$ vs $\delta^{15}N$ for all M1.
$\delta^{13}C$ vs $\delta^{15}N$ plotted for all M1 sections shows weak correlation (r= 0.64), and a slope of 1.6. There is more scatter than expected if weaning process is homogeneous in all individuals.

Figures 3.16-3.40 show $\delta^{13}C$ vs $\delta^{15}N$ plotted for individual teeth where at least three seriations were sampled. Individual slope and correlation of the regression line for each tooth is notated and emergent patterns will be discussed in chapter 4.

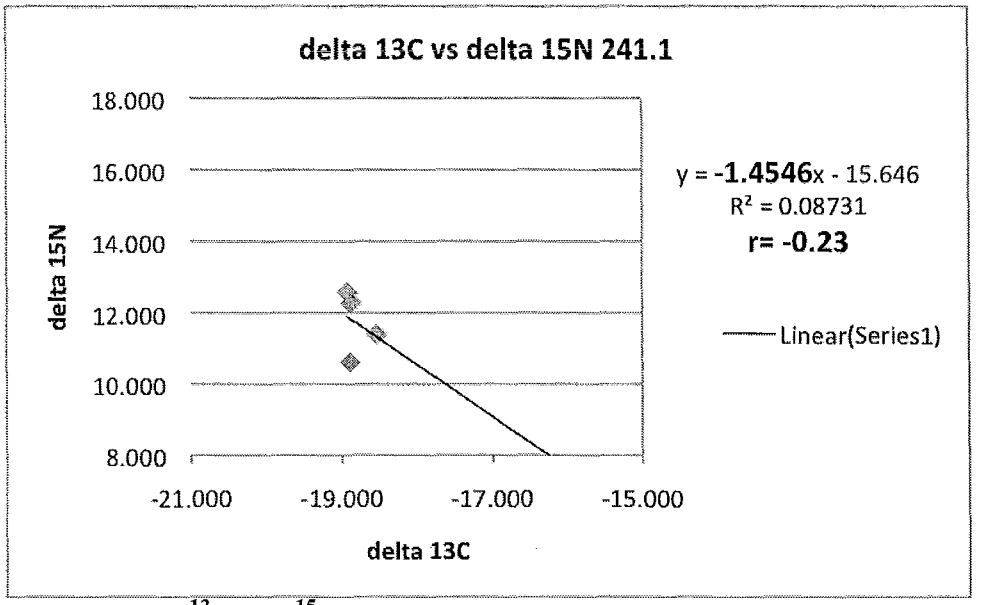


Figure 3.16: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 241.1

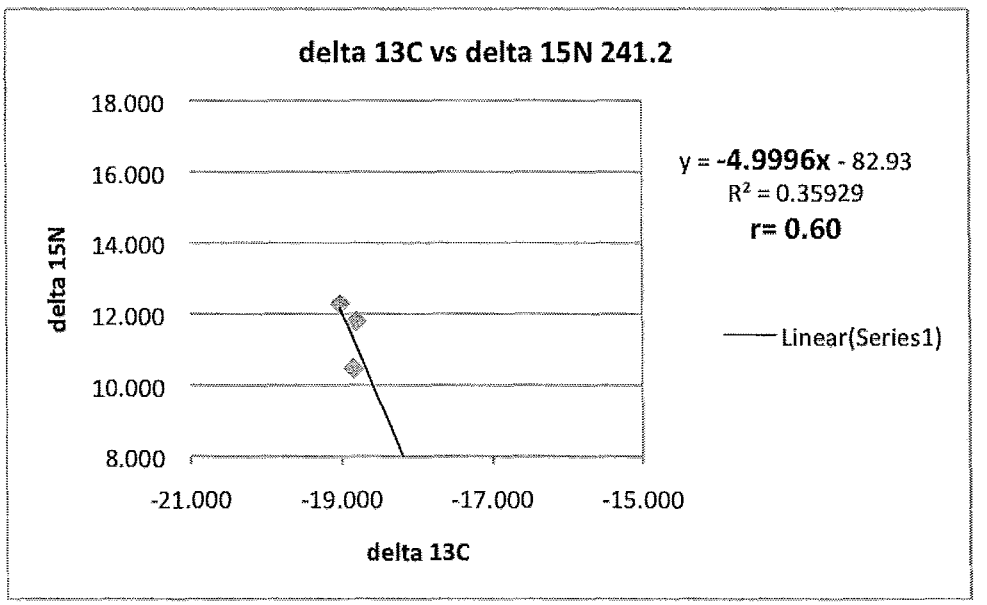


Figure 3.17: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 241.2

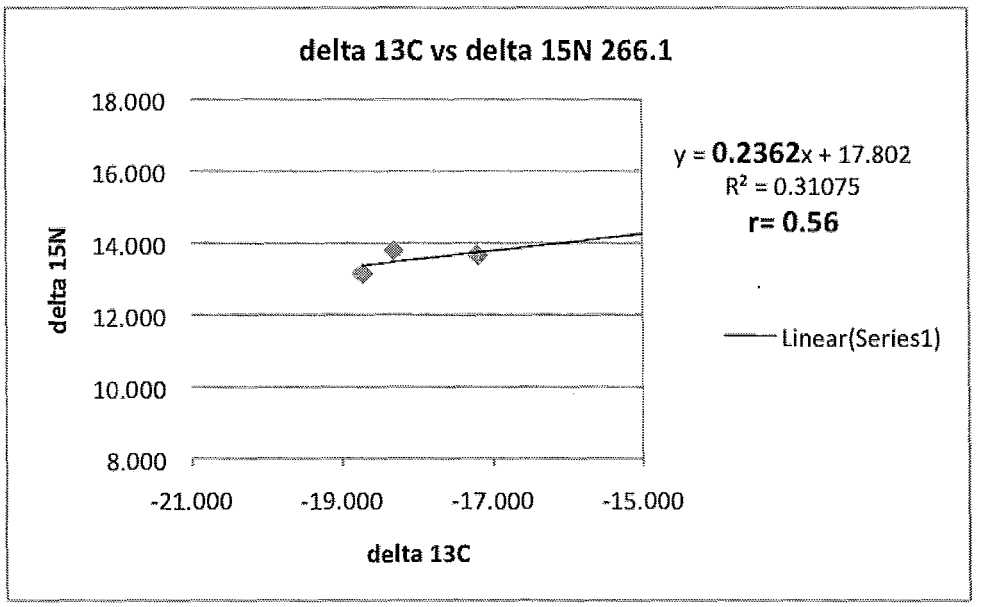


Figure 3.18: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 266.1

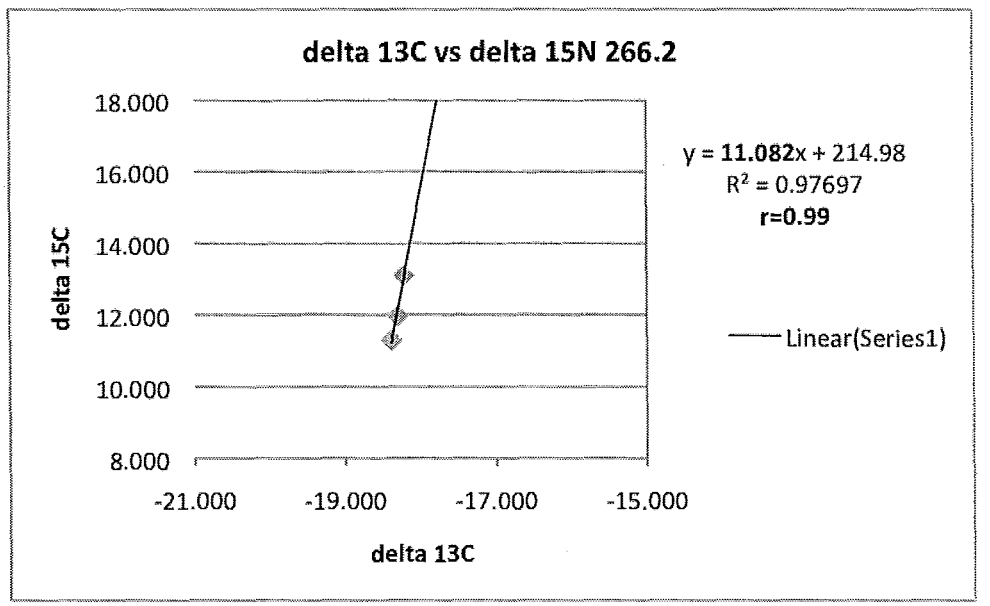


Figure 3.19: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 266.2

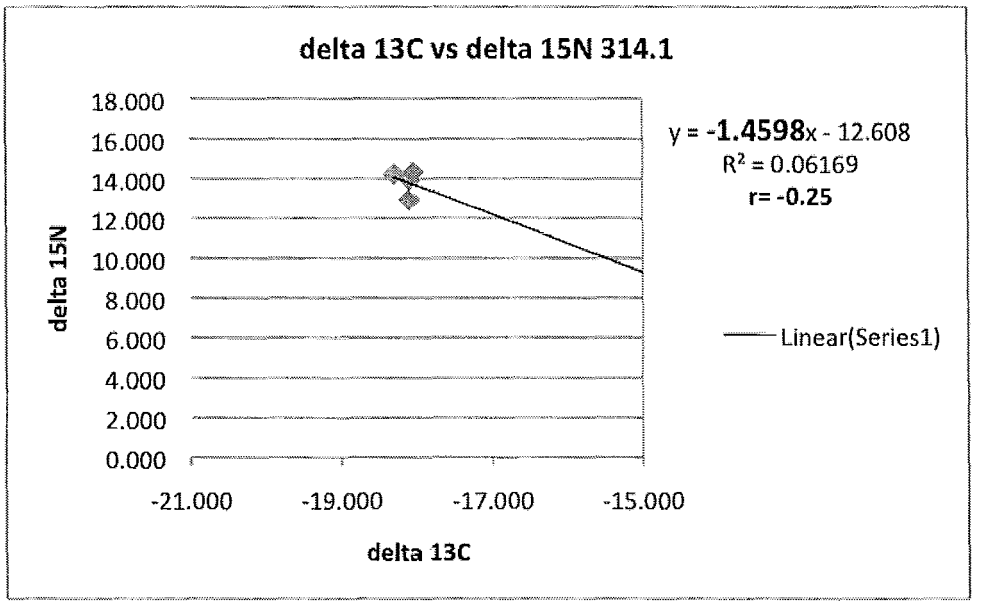


Figure 3.20: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 314.1

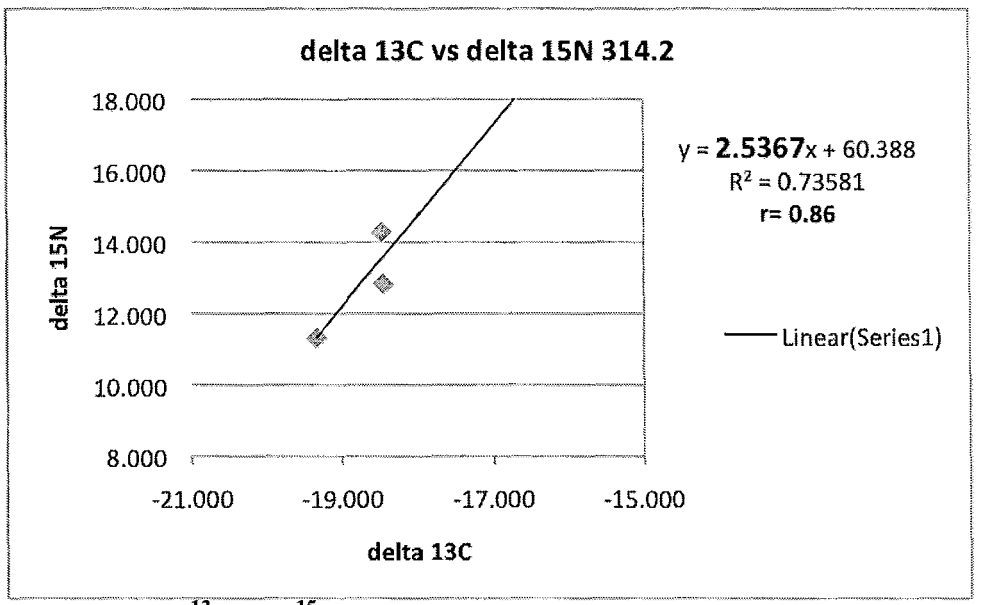


Figure 3.21: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 314.2

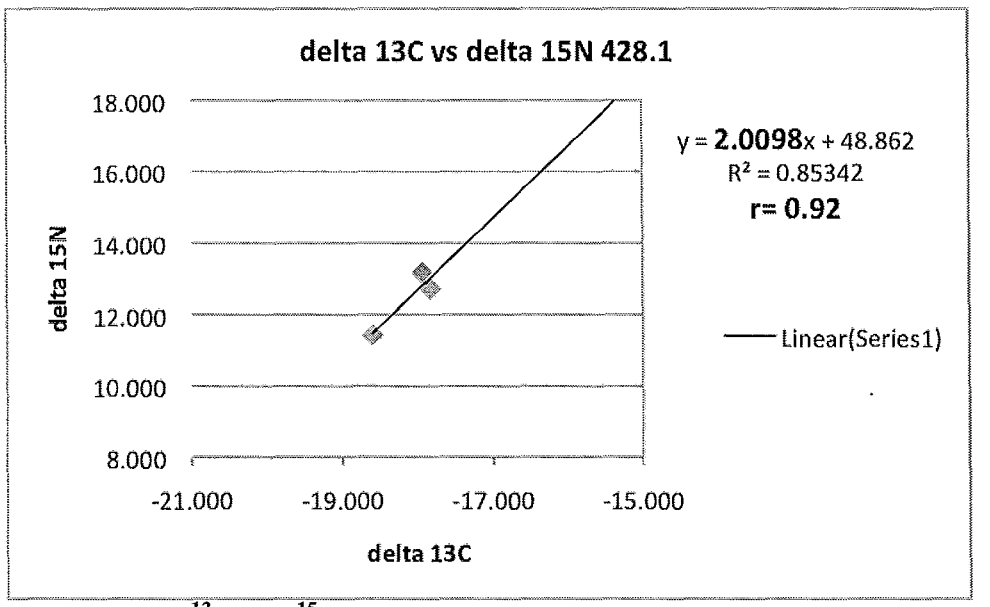


Figure 3.22: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 428.1

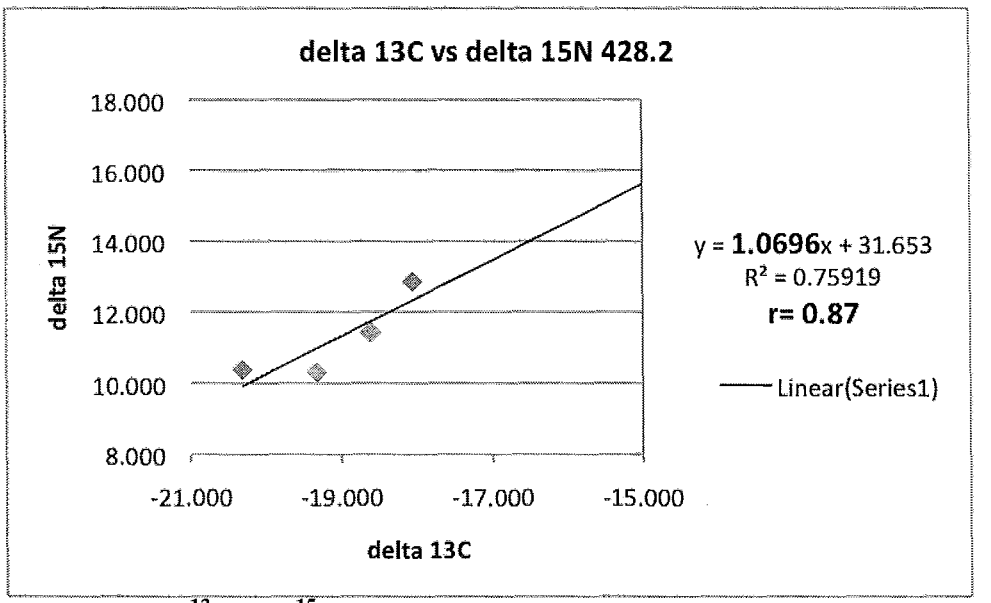


Figure 3.23: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 428.2

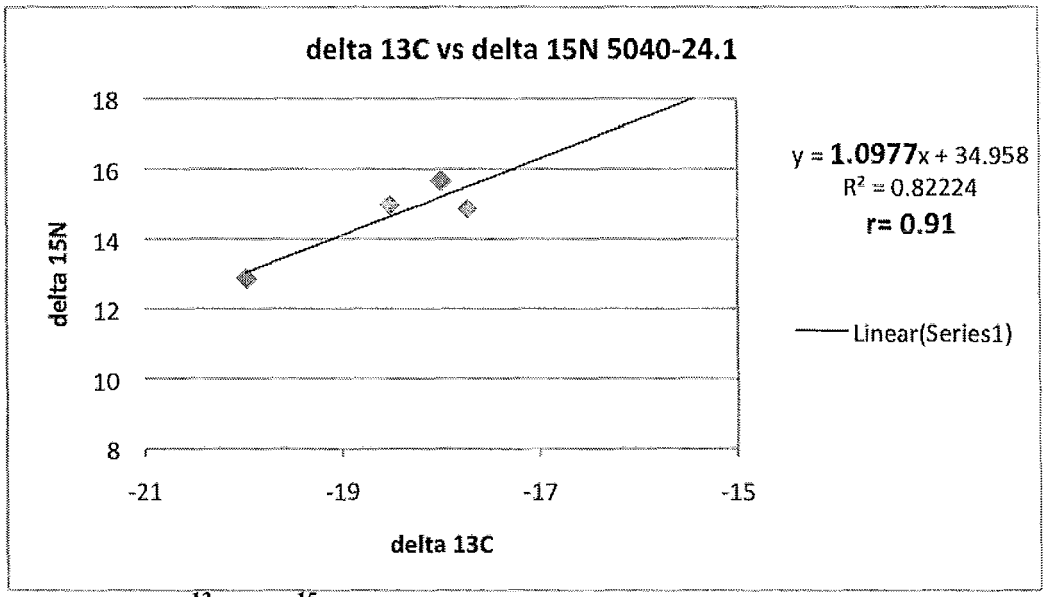


Figure 3.24: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 5040-24.1

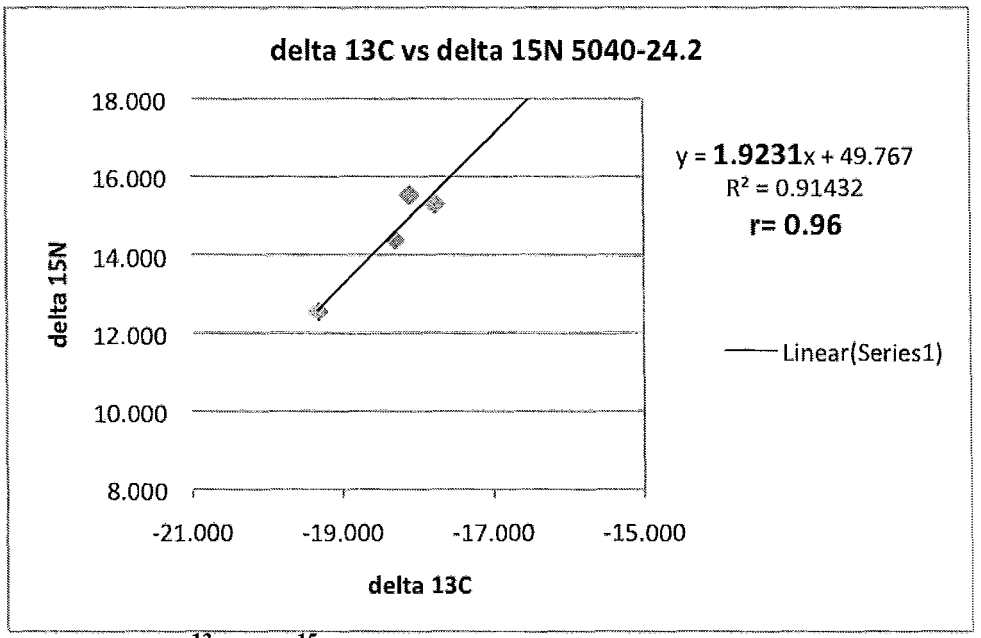


Figure 3.25: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 5040-24.2

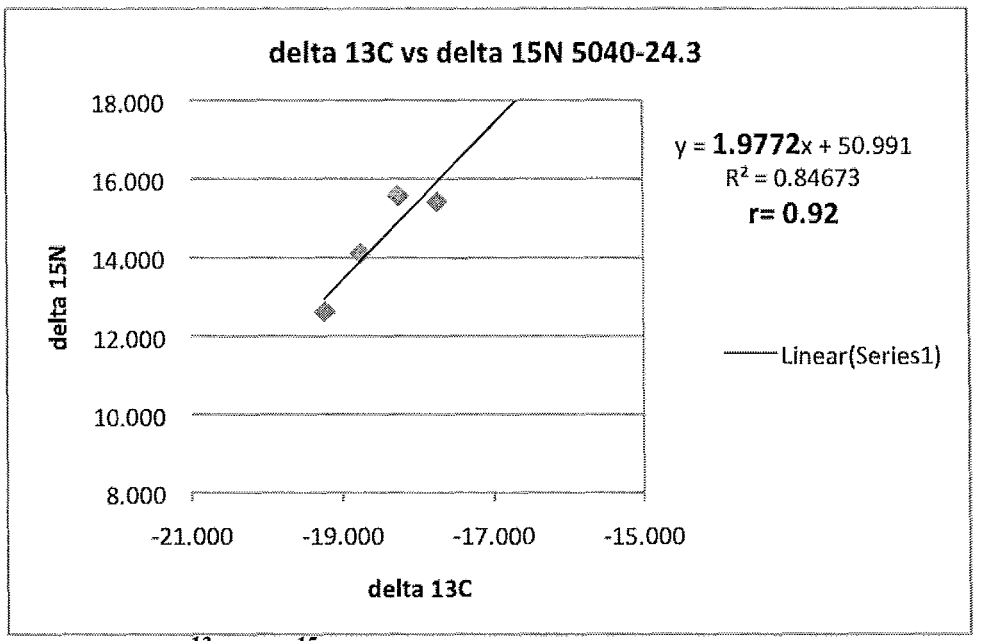


Figure 3.26: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 5040-24.3

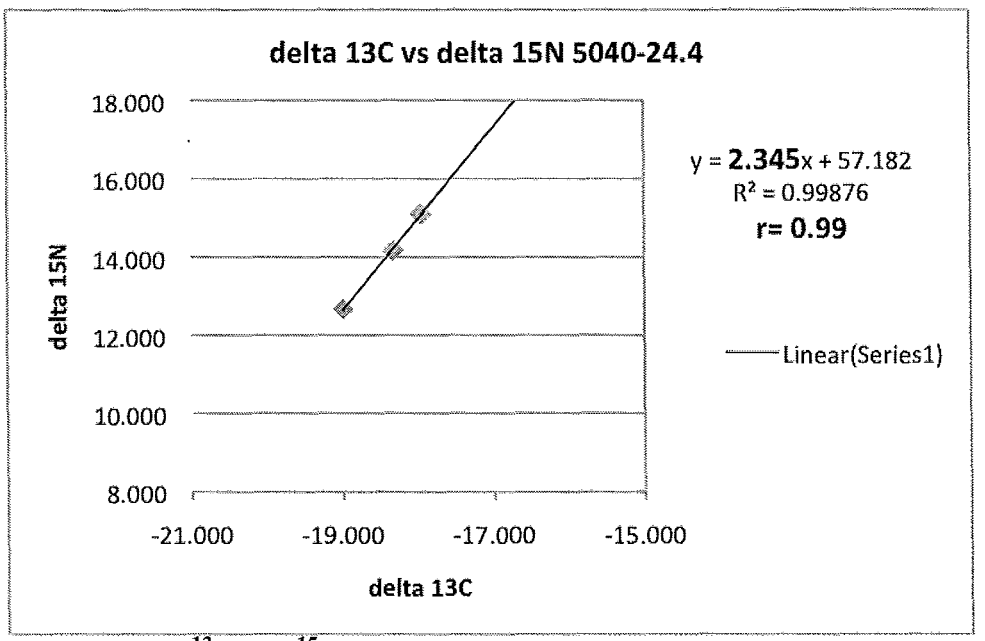


Figure 3.27: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 5040-24.4

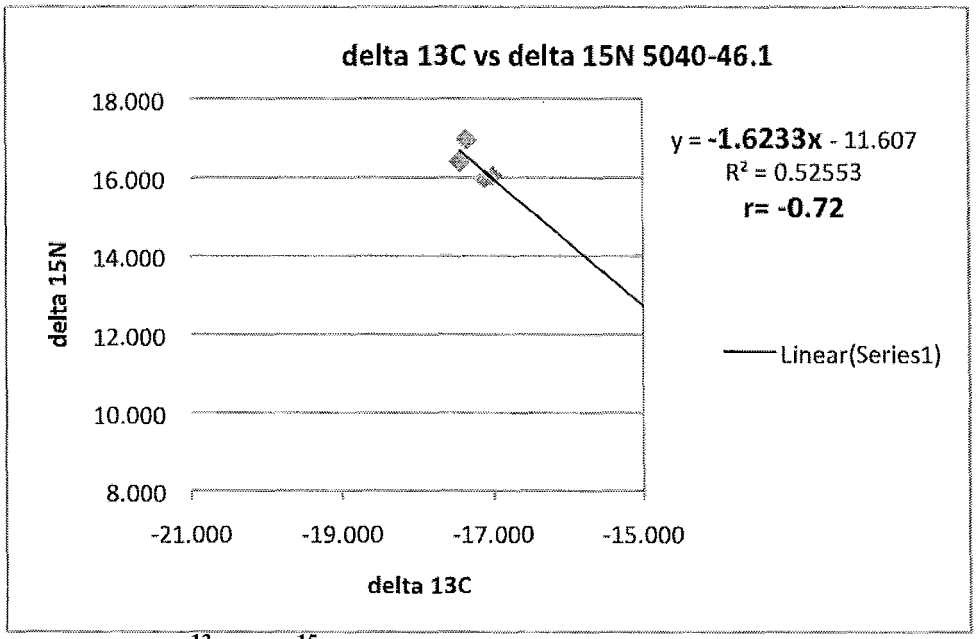


Figure 3.28: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 5040-46.1

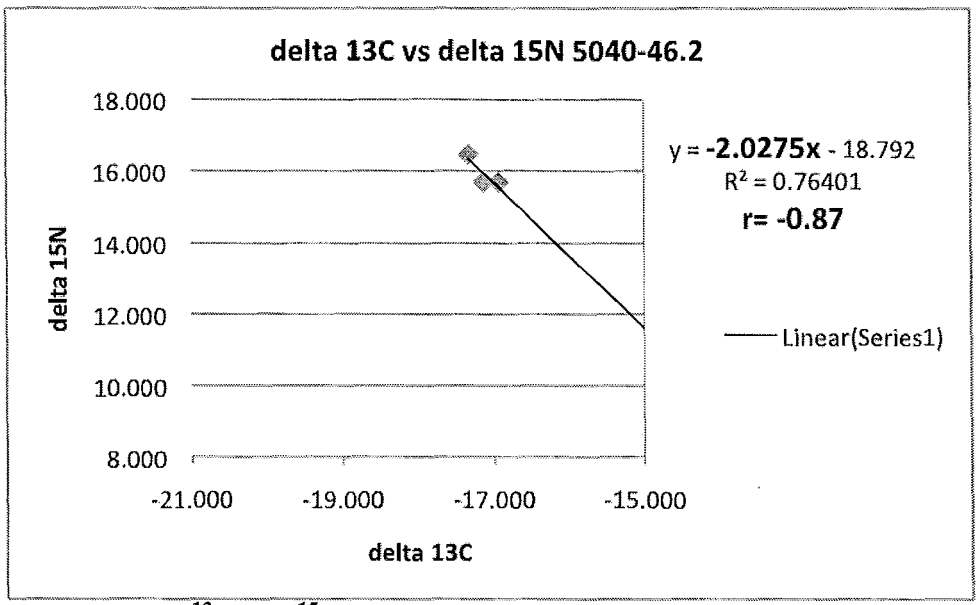


Figure 3.29: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 5040-46.2

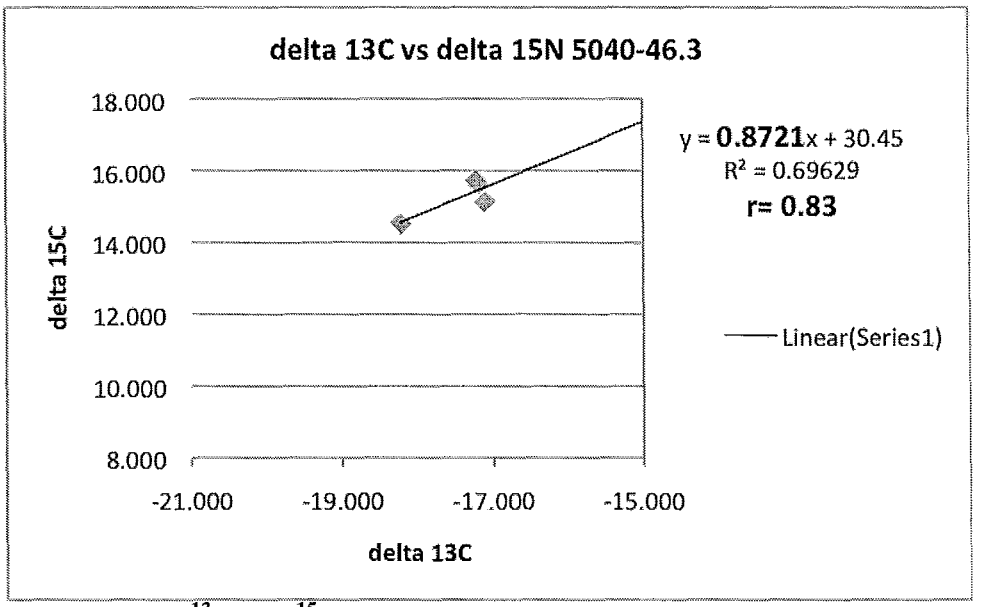


Figure 3.30: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 5040-46.3

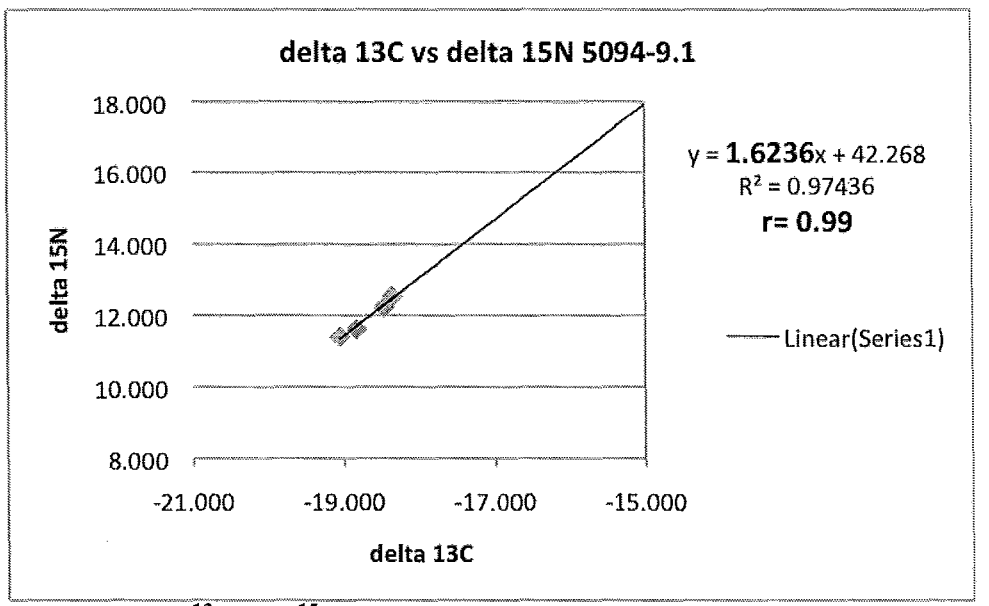


Figure 3.31: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 5094-9.1

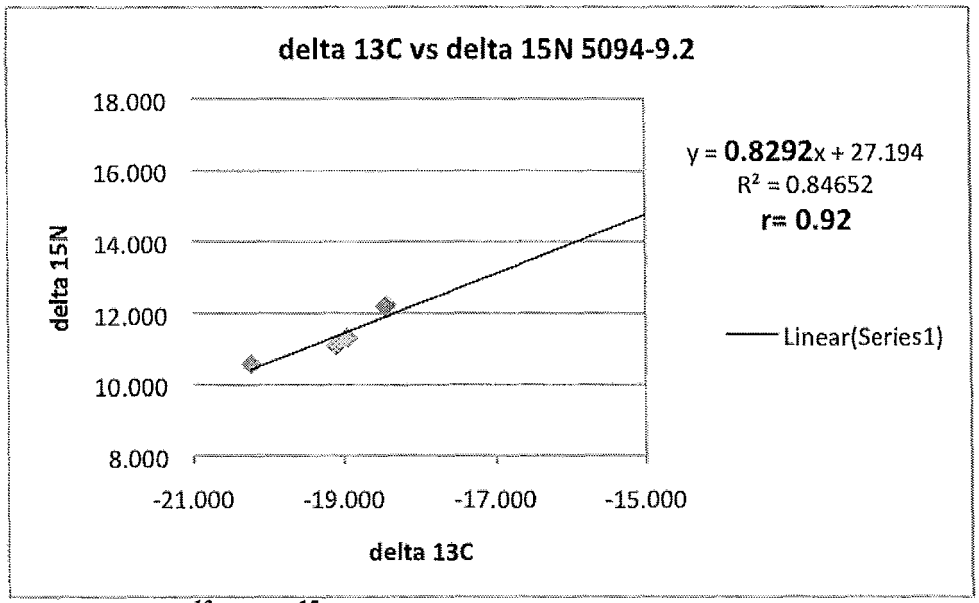


Figure 3.32: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 5094-9.2

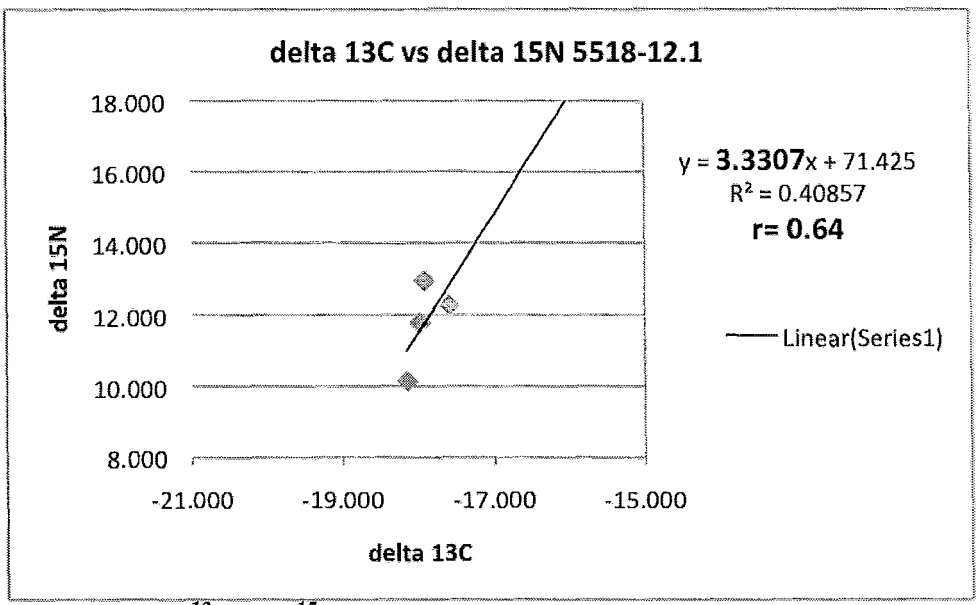


Figure 3.33: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 5518-12.1

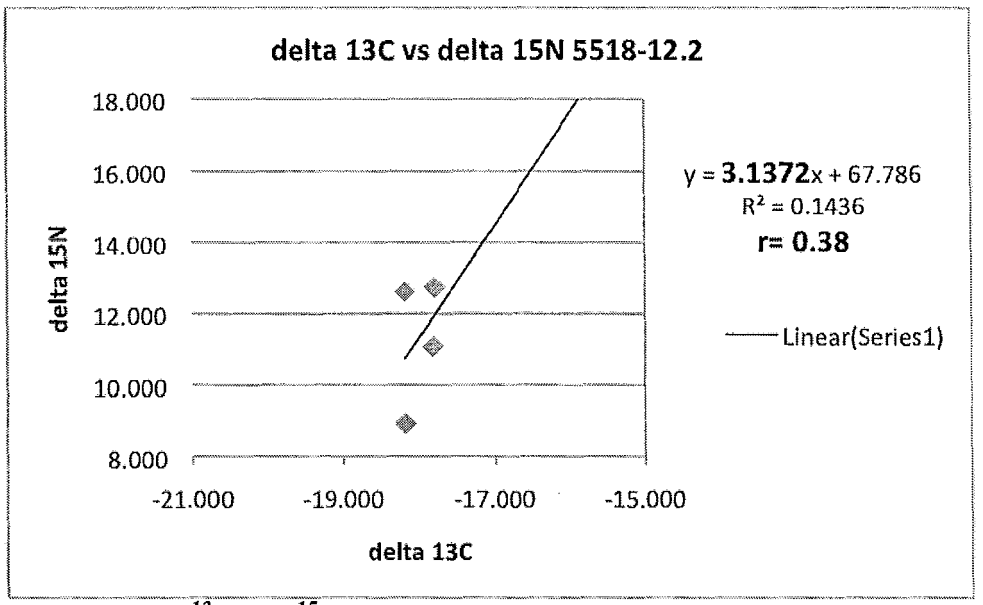


Figure 3.34: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 5518-12.2

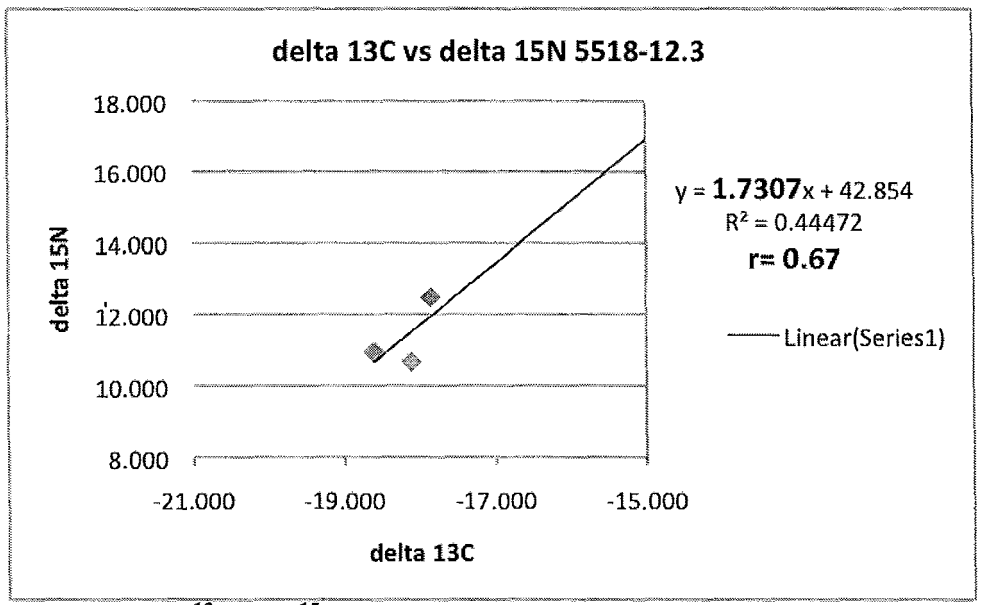


Figure 3.35: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 5518-12.3

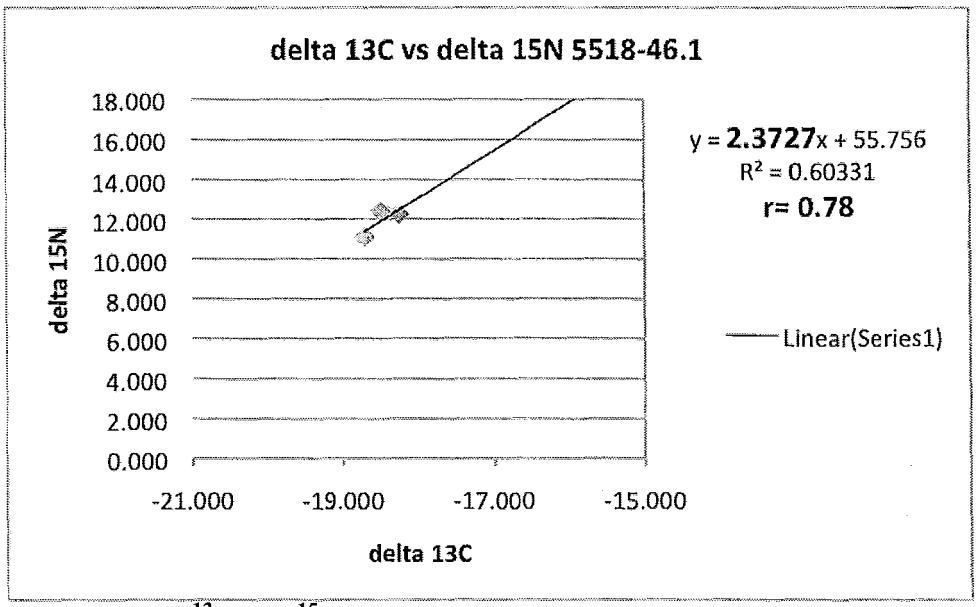


Figure 3.36: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 5518-46.1

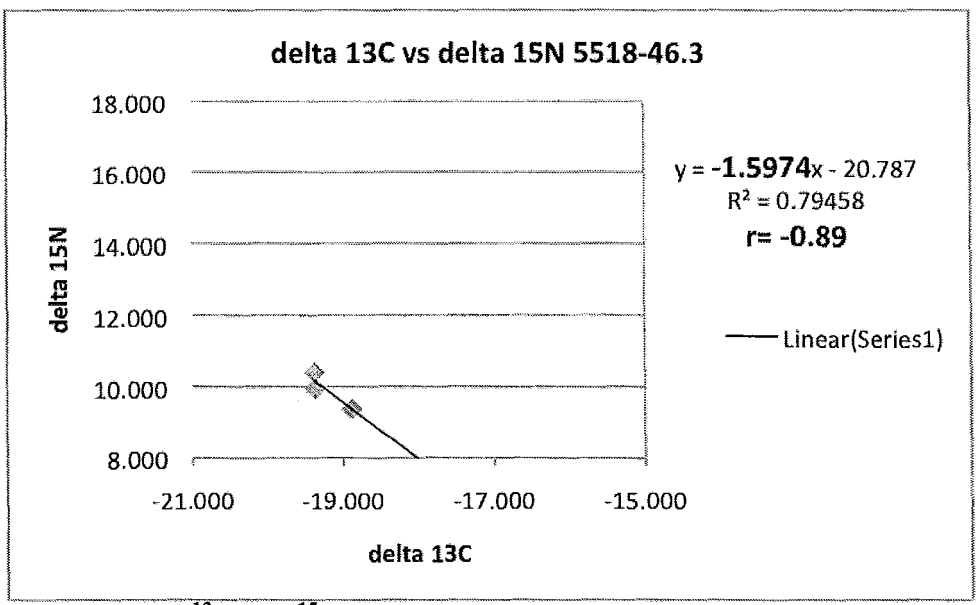


Figure 3.37: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 5518-46.3

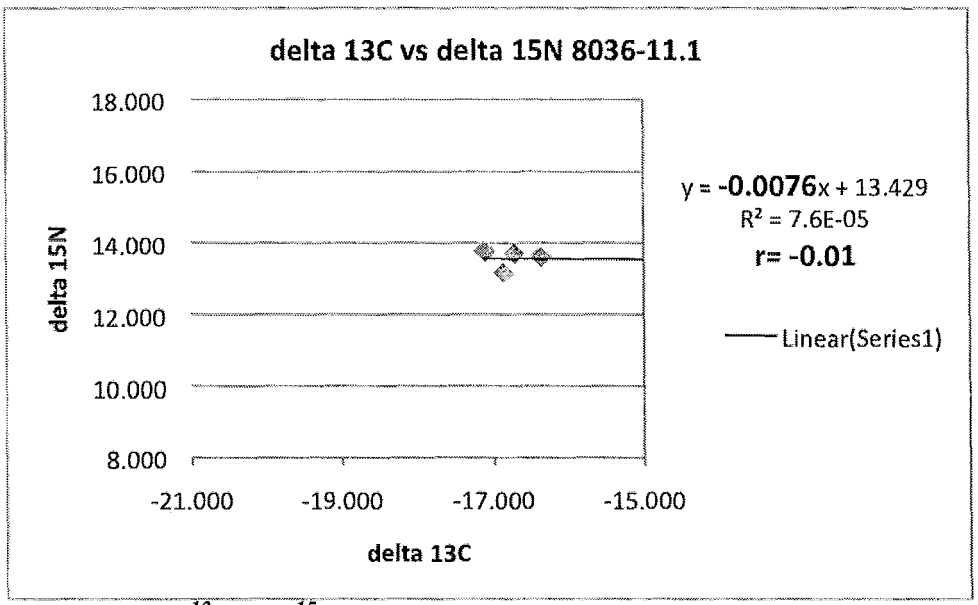


Figure 3.38: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 8036-11.1

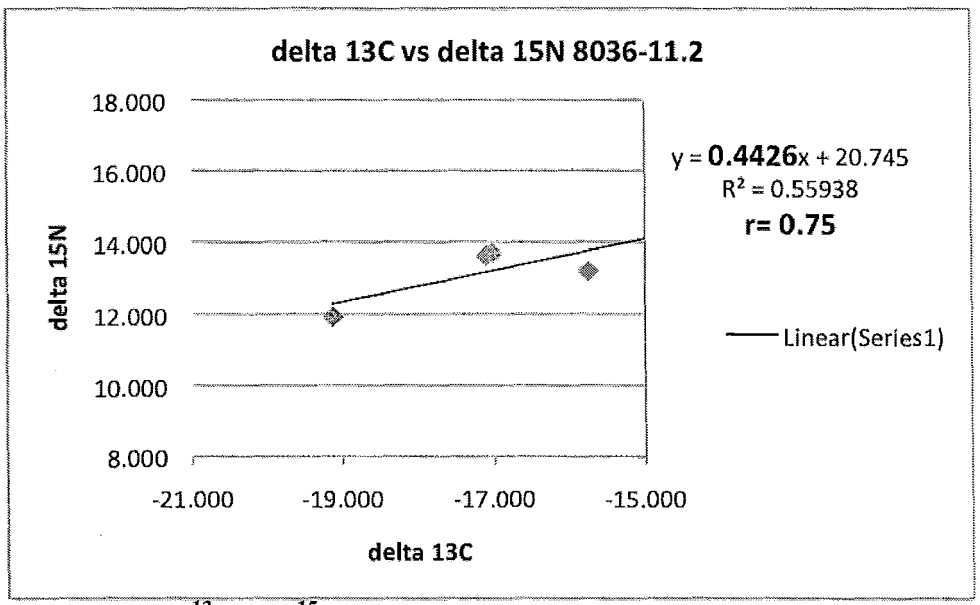


Figure 3.39: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 8036-11.2

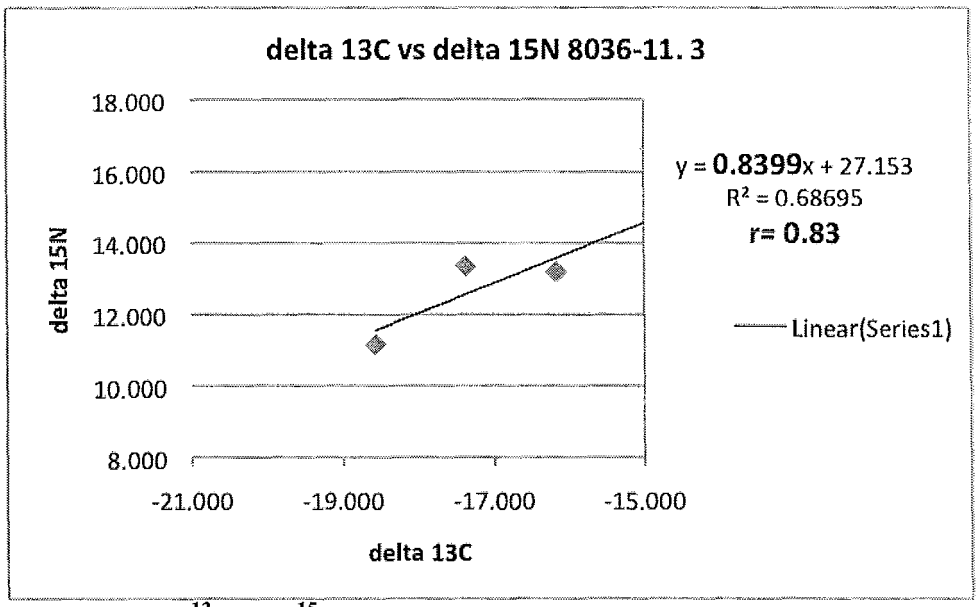


Figure 3.40: $\delta^{13}C$ vs $\delta^{15}N$ for tooth 8036-11.3

Chapter 4: Discussion

Summary of Results

The average values for all sections were δ C of -18.31 and δ N of 12.59, indicating an average diet of early childhood which fits the expected pattern of slightly elevated nitrogen consumption (through breast milk) over the adult mean of δ 15N 10.07 (with a range of 8.5-12.2) and within the adult diet range for δ 13C of -19.5 to -17.8 (with a mean of -18.52).

On average, the change within each tooth type was a good fit for the expected pattern of decreased enrichment in δ 15N of 1-3‰ and decrease in δ 13C of 1‰ [see Figure 4]. The only tooth type that did not fit the expected pattern was dm1, where δ 13C levels decreased by only 0.07‰. This tooth type subsumed the longest period of prenatal and breastfeeding development displayed very high pre-natal $\delta^{15}N$ and showed the most variation between individuals suggesting the maternal diet may have a significant impact on variation in the isotopic levels of the infant. For this analysis, it should be noted that all tooth types have been compiled into single categories, without distinguishing jaws or arches.

The average values for all sections were $\delta^{13}C = -18.31$, $\delta^{15}N = 12.59$, indicating an average diet of early childhood which fits the expected pattern of slightly elevated nitrogen consumption (through breast milk) over the adult mean of $\delta^{15}N = 10.07$ (range of 8.5-12.2) and within the adult diet range for $\delta^{13}C$ of -19.5- -17.8 (mean -18.52).

On average, the change within each tooth type was a good fit for the expected pattern of decreased enrichment in $\delta^{15}N$ of 1-3‰ and decrease in $\delta^{13}C$ of 1‰ [see Figure 3.2]. The only tooth type that did not fit the expected pattern was dm1, where there was no consistent trend in $\delta^{13}C$. This tooth type subsumed the longest period of prenatal and breastfeeding development, displayed very high pre-natal $\delta^{15}N$ and showed the most variation between individuals suggesting the maternal diet may have a significant impact on variation in the isotopic levels of the infant. For this analysis, it should be noted that all tooth types have been combined into single categories, without distinction for jaw or side designation.

Antimere and Jaw Variation

Figure 4.1 shows three deciduous first molars from individual 5040-24. Although there is slight variation among the isotopic signatures from corresponding serial sections in the three teeth, none are larger than 1‰, and each tooth shows nearly the same pattern of enrichment. The variation seen may have resulted from the bias introduced with the macroscopic sectioning technique of overlapping growth layers, or may be due to the slight natural asymmetrical developmental variation found within healthy individuals (DiBennardo and Bailit 1978; Harris and Nweeia 1980; Siegel and Doyle 2005). The analytical methods used in this work would draw the identical conclusion that solid food was introduced to this individual around six months of age and that nitrogen levels had returned to the range of adult levels by age two to three years. Although by no means incontrovertible, this comparison does suggest that the level of variation between jaw and

arch types does not significantly impact the isotopic dietary analysis; therefore, this is why sample sizes are enlarged by aggregating jaw and arch data for each tooth type.

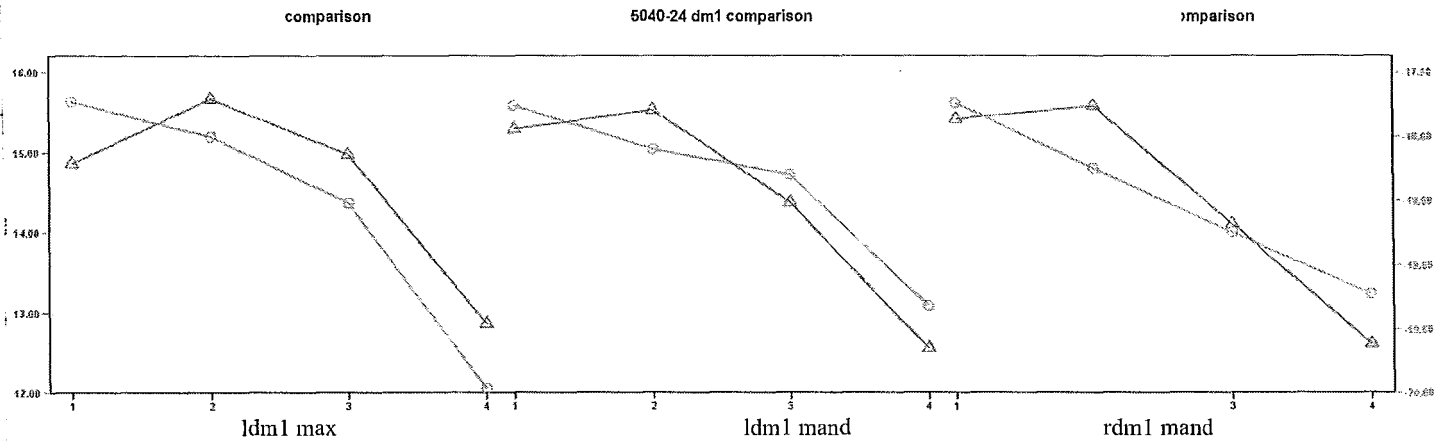


Figure 4.1: Comparison of $\delta^{13}C$ and $\delta^{15}N$ in three deciduous first molars from individual 5040-24.

A comparison of the permanent dentition used in this study (M1) was not possible with this sample as only one M1 was collected per individual; however, it is noted that permanent teeth display higher rates of variation in development between the upper and lower jaws and may, therefore, reflect slightly more divergent isotopic levels between the jaws (Hillson 1996).

Weaning Period at Apollonia – Individual Patterns

Supplementary feeding seems to have begun early in Apollonia, between six and ten months. This is seen in the decreased enrichment of $\delta^{13}C$ in the range of 1-3 ‰ per individual during these months that indicates a loss of the trophic level effect from the mother. Additionally, breast milk consumption seems to have decreased correspondingly, with $\delta^{15}N$ levels generally decreasing in the range of 1-3 ‰ per individual between six and 12 months of age.

Cessation of breastfeeding seems to be complete by age three years. All individuals who survived to this age show the expected pattern of decrease in $\delta^{15}N$ of ~3‰ over the earliest levels of enrichment, indicating a change in diet consistent with completion of weaning. The majority of individuals (n=7) have $\delta^{15}N$ levels approximating the adult diet by the age of 12 months indicating the standard duration of the weaning process was six months. By age 36 months, five individuals have reached the adult mean and eight of ten total surviving children are within the range of adult diets. Because the tooth sections that give data for this age have a poor time resolution compared to sections which developed earlier in life, the precise ages for cessation of

breast milk was not determined, and it is not suggested that weaning continued to age three years for any individual, although weaning was certainly complete by this age.

Two individuals (5040-24, 5040-46) do not decrease to adult levels, although they do show the expected pattern of decreased enrichment of approximately 3‰ during infancy. These individuals show an unusually high $\delta^{15}N$ from birth, and may represent a household with a non-standard diet from the majority population, reflecting an unusual maternal diet or an adult diet out of the sample range.

$\delta^{13}C$ levels in half the individuals (five out of 11) followed the expected decline of enrichment pattern on the scale of 1-2‰. However, the remaining six individuals showed patterns of continued enrichment (5040-46, 8036-11), stasis (314), or showed contrasting patterns between the tooth types (241, 266, 5040-24). These findings suggest that some infants were given ^{13}C-enriched supplementary foods that would maintain or increase the enrichment during weaning, the most likely source at Apollonia being goat or sheep milk and cereal grains; there are faunal remains of goat and sheep at Apollonia (Keenleyside et al. 2006), and historical Greek texts note recommendations for weaning gruels made of milk, honey and cereals such as barley (Brothwell and Brothwell 1969, Brothwell 1998). However, given Fuller and colleagues' 2005 research that showed $\delta^{13}C$ levels to be both unstable and variable by individual during the weaning process, as well as the inconsistent patterning of the Apollonia sample, it is difficult to say with certainty whether animal milk was consumed during weaning on this evidence alone. In a region already known to rely on marine resources, analysis of other isotopes such as strontium and oxygen would be more useful in isolating and identifying the inclusion of specific adult foods into the infant diet.

These results are comparable to another study of this sample using analysis of rib ends (Kwok and Keenleyside in press). They find that "weaning at Apollonia began between six months and one year of age, and was completed between the ages of 2 and 4", consistent with the serial section results.

Serial Sectioning and Outliers

As in weaning studies of other sites (e.g. Herring et al. 1998), Kwok and Keenleyside (in press) found several individuals in the Apollonia sample who did not fit into the expected pattern of isotopic values using the population aggregate method. In addressing three specific outliers with commonly cited unusual values and suggestions for interpretation they raise, data from the dental serial sectioning can provide some insight to the accuracy of these common explanations and how often individuals truly are outside a shared weaning experience in a population.

Early Weaning

Kwok and Keenleyside (in press) interpret a three-month old (366) with a $\delta^{15}N$ signature similar to the adult female mean as having never been breastfed or weaned early. The dental serial sampling shows all outliers in the sample were engaging in same cultural timeline for weaning, with no individuals showing absence of breastfeeding or early weaning near three months. Several individuals in my sample have seriations that show $\delta^{15}N$ levels around three months that are near the adult mean (295, 5518-12, 5518-

46) but in the larger context of all seriations, show conformity to the expected weaning pattern. While my sample did not specifically address any sub-adults who died in infancy, the larger trends for weaning behavior suggest that individual 366 is more likely to represent an isotopic signature from a breastfeeding mother with lower than average levels of $\delta^{15}N$.

Delayed Weaning

Kwok and Keenleyside (in press) also describe several individuals past the expected weaning age who contributed elevated $\delta^{15}N$ signatures and they interpret this as delayed weaning. The dental serial sectioning sample shows two individuals who display elevated levels after completion of weaning process at expected time (5040-24, 5040-46); if only ribs ends had been sampled from these individuals, the interpretation of delayed weaning would have categorized these sub-adults as unusual for the sample, and often the assumption of poor health leading to prolonged breastfeeding accompanies this analysis. However, through dental serial sampling it is apparent that these two sub-adults survived weaning on the normal timeline, and it is the household diet that appears to be the true outlier.

Symbolism of Grave Goods

Through the excavation process, individuals 8036-11 and 204 were associated with a feeding vessel (Kwok and Keenleyside in press). Because population aggregate sampling provided only a single $\delta^{15}N$ value indicative of an adult diet, Kwok and Keenleyside posit several theories for the importance of the vessel: "they had already been weaned, were never breastfed, or were breastfed for only a short duration. It is possible that these individuals had to rely on these vessels for nutrition. Alternatively, they may have been used to feed supplementary fluids when these subadults were neonates, and were buried with them for symbolic reasons". Individual 8036-11 was also included in the dental serial section sample, and presents a pattern of a normal weaning period, but shows an unexpected increase in $\delta^{13}C$ beginning during weaning and continuing until death, supporting the idea that the feeder was in use to provide an unusual post-weaning diet of milk, honey and C3 cereals as discussed earlier.

Homogeneity of Weaning Diet

In order to assess the homogeneity of the weaning diet from another approach, $\delta^{13}C$ is plotted against $\delta^{15}N$ for all samples [Fig. 3.12]. While a slight linear correlation is evident ($r=0.59$), there is significant scatter. If all infants were experiencing a similar change in diet during weaning, the correlation should be much stronger and the noisiness of the data supports Kwok and Keenleyside's (in press) findings that weaning at Apollonia was not an identical process in every home. When $\delta^{13}C$:$\delta^{15}N$ is plotted by tooth type, [Figures 3.13, 3.14, 3.15] the weakest correlation is in dm1 ($r=0.47$), the tooth which incorporates the greatest amount of prenatal and breastfeeding development. This raises the question of the effect of maternal diet in the infant isotopic levels: is the source of variation in the transitional foods presented to the weaning infant, or in the diet of the nursing mother?

Diet of Mothers

While Keenleyside and colleagues (2006) assessed the adult diet at Apollonia and found no significant variation by sex or age, a social grouping not addressed for obvious sampling feasibility, was nursing mothers. In every weaning study, although the isotopic signatures sampled are derived from sub-adult tissue, these levels directly reflect the diets of the mother, presumably each experiencing pregnancy and breast-feeding slightly differently and perhaps altering their normal adult diet as a result. In population aggregate sampling, variation in maternal diet affects the scatter of the C:N groupings as in effect, each sub-adult sampled introduces an additional maternal diet to the equation. Dental serial sampling significantly decreases this source of error; if the infant is assumed to have breastfed from only one source, the sub-adult teeth can be used to infer information about the short-term changes in maternal diet during breastfeeding. Just how consistent was the diet of the mother?

To see the effects of change in the maternal diet, $\delta^{13}C$ vs $\delta^{15}N$ is plotted for individual teeth where at least three sections were sampled [See figures 3.16-3.40]. Several weaning studies have found a ratio of 3:1 change in the nitrogen and carbon levels throughout weaning consistent with the loss of the trophic level in the infant (Prowse et al. 2007; Richards et al. 2006). As long as the diet of the lactating mother remains the same, weaning onto a single transitional diet similar to adult diet will produce a linear relationship with a slope of 3:1 and a strong correlation between $\delta^{15}N$ and $\delta^{13}C$, with scatter expected where there is variation in the maternal diet during these periods. In the Apollonia sample one individual shows a slope approaching 3 (5518-12), although several show very strong correlations between $\delta^{13}C$ and $\delta^{15}N$ (314.2, 428.1, 428.2, 5040-24.1, 5040-24.2, 5040-24.3, 5040-24.4, 5040-46.2, 5094-9.1, 5094-9.2, 5518-46.3). In these individuals with strong correlation, it is likely that the weaning diet remained consistent throughout the transitional food period. In the remaining teeth where the correlation is low and slope is not 3:1, either the weaning food or the maternal diet was varied, while in individuals such as 8036-11, who shows extremely low correlation and variation of slope in all teeth, both variations may be taking place simultaneously. This relationship is complex and may not be fully separable in the current data, however, isotopic compositions of weaning foods are relevant to understanding what happens to collagen δ values during weaning. If protein-deficient cereal foods provided the bulk transitional food diet in Apollonian infants, a slope closer to 1 can be expected, with a proportional increase where more protein rich goat's milk was included in the diet.

Applications for Interpretation of Site Specific Dietary Analysis

The use of dental serial sampling provides an opportunity to test several of the assumptions stated in chapter 1. Assumption 1, which posits a detectable culture specific age for weaning, seems to be valid. The majority of infants follow a similar pattern of dietary supplementation at approximately six months and cessation of breastfeeding by age three years. The additional information provided through the multidimensional approach allows for incorporation of individuals that would previously have been considered outliers. For example, individuals 5040-24 and 5040-46, if using a single

sample level from age at death, would have been interpreted as outliers since these two samples did not conform to the population pattern and showed abnormally high $\delta^{15}N$ beyond normal weaning age. On the other hand, by showing the pattern of change in diet over time for each individual, it becomes clear that both individuals did in fact experience a shared timeline for weaning, with supplementary foods introduced around six months, and were fully weaned by age three. The variation in overall diet represented by their unexpected levels presents avenues for further research into heterogeneity of the population and potential resource disparity between children and adults at Apollonia. Specifically, were these particular families natives of Apollonia or were these individual children connected to Apollonia through the busy trade port as visitors or immigrants? Did they have access to alternative marine resources at the site due to social status or did they reflect unique child feeding or household diet practices their families maintained from another area? The higher levels of $\delta^{15}N$ observed in both individual sub-adults with unusual diets may indicate maternal consumption of more marine resources than the typical Apollonian individual. Stable isotope analysis of strontium may provide answers to these questions.

Assumption 2, that weaning lasts a few months (and is therefore not a single event, but a process), is also supported by serial sampling. Although there may still be a potential for error introduced through the time resolution bias (as discussed in chapter 2), my data shows a duration of decline in isotopic values of about 24 months. Because duration of the transitional food period is of interest in some approaches to infectious disease studies (Goodman and Armelegos 1989; Knodel and Kinter 1977; Sawchuk, et al. 2002), the individual timelines created by dental serial sampling may provide supporting evidence for the introduction of parasites or nutritionally deficient transitional foods. For example, variation in the duration of weaning at Apollonia shows two general patterns: weaning complete by 12 months, and weaning complete by three years. This duality, as well as the lack of a strong linear correlation of $\delta^{13}C$:$\delta^{15}N$ within the tooth types provides support to the finding of Kwok and Keenleyside (in press) that some children at Apollonia were given animal milk as a supplementary food, while others were not.

Assumption 3, that children are weaned onto adult diets, also appears to be validated by my findings. The majority of the sample individuals reaches adult diet levels during the weaning process and follows the pattern of 1-3‰ decline in enrichment from *in utero* levels to what is therefore likely to be maternal levels. The two individuals who remain enriched over the range of Apollonian adults still display this decline in enrichment which indicates that they *were* weaned onto an adult diet, but simply an adult diet that was unusual for the general population. In other samples, dental serial sectioning may show a more pronounced difference in the post-weaning diets of early childhood compared to the range of adult diets. In cultures where children are not full members of society, or are given special status, a unique dietary signature may provide clues to their role in the larger population. In order to interpret unique isotopic signatures, seriation provides the ability to discern whether it is the experience of the weaning process that is unusual, or the diet after weaning. While both options might show identical isotopic levels at the time of death, it is the variation at the individual level compared to population patterns that will allow for nuanced analyses.

Assumption 4 is clearly not satisfied; variation in maternal diet is apparent within and between individuals in the sample, but the present method allows for some analysis to separate the effects of maternal diet from transitional food diet within an individual sub-adult. By addressing this aspect of infant diet, the diet of nursing mothers can be included in the discussion of resource consumption of the larger population. By identifying individuals affected by both maternal dietary changes and variation within the transitional food diet, several common assumptions made about presumed unusual nursing situations can be challenged and refined.

On major concern for all studies using sub-adult samples is whether the burial sample is representative of the larger living population (Saunders and Barrans 1999; Saunders et al. 1995). In dietary analysis, unexpected isotopic levels prior to death can provide evidence for unusual health concerns that may have contributed to early death (Fuller et al. 2003), however, this sample does not provide sufficient data to assess the effect of burial representativeness through the diets of children and infants. The two infants who died during the weaning process (266, 18 months and 5518-12, two years) show no deviation from the expected pattern. At minimum, the use of individuals who survived into early childhood avoids the potential bias of weaning as a direct cause of death (Katzenberg et al. 1996).Of the nine sampled children who lived beyond the weaning period, six had cribra orbitalia lesions, often interpreted as evidence of parasitic disease or iron-deficiency (Stuart-Macadam 1992) so the health of these individuals may have been compromised. Keenleyside and Panayotova (2006) found high incidence (21%) of healed cribra orbitalia lesions in the adults from Apollonia, and Kwok and Keenleyside (in press) support the theory that the early introduction of contaminated milk or cereals to the diet may have caused iron-deficiency anemia in children through parasites and pathological microorganisms. The consumption of iron-inhibiting phytates in goat's milk, cereals and wine during childhood is also discussed as a factor leading to megaloblastic anemia. In this case, the high incidence of cribra orbitalia in this sample may show the effects of the normal population diet, and does not indicate obvious bias in the representativeness of the burial sample for reconstructing the weaning process at Apollonia.

Chapter 5: Conclusion

Summary

The dental serial sampling method outlined in this research provides a more specific timetable for stable isotope weaning studies than the serial sections used by Fuller et al. (2003). The first crown sections of both the dm1 and dm2 establish a baseline for maternal diet because they represent development *in utero,* and this maternal baseline is useful in interpreting dietary levels at completion of weaning. Although the true mother-child pairing is rarely found in archaeological materials, this isotopic signature provides a link between the diets of the infant and the mother, providing information on variation in both.

An additional benefit is the relative permanence of dental tissue sampling. Because the enamel and dentine preserve tissue from earlier stages of development, deciduous tooth microsampling can retrieve isotopic information from infancy in children up to age ten, in effect offering a longitudinal record of development through this period. This method will therefore be useful in samples that do not contain infants at a variety of developmental stages. While Apollonia provided opportunity for a 64 child sample using a population aggregate method (Kwok and Keenleyside in press) a majority of burial samples do not include such high prevalence of infant and child burials that also span the necessary age range.

By creating a population level pattern built from more intensive individual patterns, dental serial sectioning provides an opportunity to test common assumptions about the apparent outliers in a sample. Individuals with single stable isotopic signatures indicative of abnormal weaning patterns, either through early or delayed weaning, can be compared to a site specific context to draw a larger picture of normal variation in weaning timelines and household diet. In addition, archaeological information on goods associated with individual children can be better understood through a clear dietary history.

There are some drawbacks to this method, however; in order to recreate the entire period of development that spans the weaning process, serial sampling requires destructive sampling of at least two teeth per individual (dm2 and M1), although emerging microsampling techniques using laser ablation may be able to produce similar data with less destruction. Additionally, my results show that although neither antimeres, jaw nor side seem to affect the patterns of isotopic levels, each *tooth type* may reflect slightly different levels than others developing coevally. This is likely to arise because although two different tooth types may be developing at the same time, each will be at different stages of developmental maturity. As a result, it is recommended that all three teeth should be sampled to create a picture of trends, as opposed to taking each tooth for an absolute value of isotopic levels. Additionally, the inclusion of rib ends to show levels at the time of death would contribute greater clarity to the cessation of breast milk and a discussion of diet in childhood (after weaning) by providing a comparison of $\delta^{15}N$ to levels at age of death.

Directions for Future Research

Ideally, this research will be replicated on additional sites where population aggregate sample weaning studies are already complete. Comparison of interpretation of outliers with the serial sampling method will lead to a more accurate understanding of variation in the weaning experience between individuals in a population. In new studies, this sampling method can be applied to sites where population aggregate sampling is inappropriate due to limitations such as sample size or ages of subadults in the burial sample. Future studies using dental serial sectioning to approach anthropological weaning reconstruction should attempt to include strontium and oxygen isotopes in the analysis, particularly in regions known to utilize marine resources, as this will provide more information on the specific types of transitional foods being consumed and may provide more information on the variation between diets of breastfeeding mothers and the general adult population.

References

Alabama Maps (Cartographer). (n.d.) *Europe* [Online Image]. Retrieved July 15, 2009, from http://alabamamaps.ua.edu/contemporarymaps/world/europe/europe3.jpg

Ambrose SH. 1990. Preparation and characterization of bone and tooth collagenfor isotopic analysis. J Arch Sci 17:431-451.

Ambrose SH. 1993. Isotopic analysis of paleodiets: methodological and interpretive Considerations. In Sandford MK editor. Investigations of AncientHuman Tissue: Chemical Analyses in Anthropology. Langhorne, PA: Gordon and Breach Science p. 59-130.

Ambrose SH, Norr LE. 1993.Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In Lambert JB, Grupe G editors. Prehistoric Human Bone: Archaeology at the Molecular Level. Berlin: Springer-Verlag p. 1-36.

Balasse M. 2001. Detection of dietary changes by inta-tooth carbon and nitrogen isotopic analysis: an experimental study of dentine collagen of cattle (Bos taurus). Journal of Archaeological Science 28:235–245

Balasse M, Tresset A. 2002. Early weaning of Neolithic domestic cattle (Bercy, France) revealed by intra-tooth variation in nitrogen isotope ratios. Journal of Archaeological Science29: 853–859

Brothwell DR. 1988 Foodstuffs, cooking, and drugs. In Grant M, Kitzinger R editors. Civilization of the Ancient Mediterranean: Greece and Rome. New York: Charles Scribner's Sons, New York p. 247-261.

Brothwell DR, Brothwell P. 1969. Food in Antiquity, A Survey of the Diet of Early Peoples. London: Thames and Hudson.

Chisholm BS, Nelson DE, Schwarcz HP. 1982. Stable carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. Science 216:1131-1132.

Chisholm S, Nelson DE, Hobson K, Schwarcz HP, Knyf M. 1983. Carbon isotope measurement techniques for bone collagen: notes for the archaeologist. J of Arch Sci 10:355-360.

DeNiro MJ. 1987. Stable isotopy and archaeology. Am Sci 75:182-191.

Dettwyler KA and Fishman C. 1992. Infant Feeding Practices and Growth. Annual Rev of Anthropol 21:171-204

Dibennardo R, Bailit HL. 1978. Stress and dental asymmetry in a population of Japanese children. Am J of Phys Anthropol 48(1):89-94.

Drucker D, Bocherens H, Pike-Tay A, Mariotti A. 2001. Isotopic tracking of seasonal dietary change in dentine collagen: preliminary data from modern caribou.Comptes Rendus de l'Académie des Sciences - Series IIA - Earth and Planetary Science. 333(5):303-309.

Dupras TL, Schwarcz HP, Fairgrieve SI. 2001. Infant feeding practices in Roman Egypt. Am J of Phys Anthropol 115:204-212.

Fogel ML, Tuross N, Johnson BJ, Miller GH. 1997. Biogeochemical record of ancient

humans. Organic Chem 27(5-6): 275-287.
Fuller BT, Fuller JL, Harris, DA, Hedges, REM. 2005. Detection of breastfeeding and weaning in modern human infants with carbon and nitrogen stable isotope. Am J of Phys Anthropol 129:2 279-293
Fry SL. 1999. Burial in Medieval Ireland 900-1500: A Review of the Written Sources. Dublin: Four Courts Press.
Goodman AH, Armelagos GJ. 1989. Infant and childhood morbidity and mortality risks in archaeological populations. World Archaeology 21(2):225-243
Gordon JE, Ishwari D, Chitkara MB, Wyon MB. 1963. Weanling Diarrhea. Nutrition Reviews. 48:215-217
Gustafson G, Koch G. 1974. Age estimation up to 16 years of age based on dental development. Odontol Revy 25(3):297-306.
Guy H, Masset C, Baud C. 1997. Infant taphonomy. Inter J of Osteoarch 7:221-229.
Harris EF, Nweeia MT. 1980. Dental Asymmetry as a measure of environmental stress in the Ticuna Indians of Columbia. Am J of Phys Anthropol 52(1):133-142.
Hermary A and Panayotova K. 2006. 52 la nécropole d'apollonia du pont nouvelles découvertes de la mission franco-bulgare. Archeologia 431:52-63.
Herring DA, Saunders SR, Katzenberg MA. 1998. Investigating the weaning process in past populations. Am J of Phys Anthro105(4):425-440.
Hillson S. 1996. Dental Anthropology. Cambridge: Cambridge University Press
Hobson KA, Sease JL. 2006. Stable isotope analysed of tooth annuli reveal temporal dietary records: an example using steller sea lions. Marine Mammal Science 14:116-129.
Hoddinott RF. 1975 Bulgaria in antiquity: an archaeological introduction. London: Benn; Toronto: General Pub. Co.
Janowitz B, Henderson Lewis J, Parnell A, Hefnawi F, Younis MN, Serour GA. 1981. Breast-feeding and child survival in Egypt. J of Bio Sci 13:287-297.
Katzenberg MA, Pfeiffer S. 1995. Nitrogen isotope evidence for weaning age in a nineteenth century Canadian skeletal sample. In Grauer AL editor. Bodies of Evidence: Reconstructing History Through Skeletal Analysis. New York: John Wiley and Sons p. 221-235.
Katzenberg MA, Herring DA, Saunders SR. 1996. Weaning and infant mortality: evaluating the skeletal evidence. Am J of Phys Anthropol 101:177-199.
Keenleyside A, Panayotova K. 2005. A bioarchaeological study of the Greek colonial population of Apollonia Pontica. Archaeologia Bulgarica 9(2):21-38.
Keenleyside A, Panayotova K. 2006. Cribra orbitalia and porotic hyperostosis in a Greek colonial population from the Black Sea. Inter J of Osteoarch 16(5):373-384.
Keenleyside A, Panayotova K, aSchwarcz H. 2006. Stable isotopic evidence of diet in a Greek colonial population from the Black Sea, J of Archaeo Sci 33:1205-1315.
Knodel, J. and Kinter H. 1977. The impact of breastfeeding patterns on the biometric analysis of infant mortality. Demography 14(4):391-409.
Kwok CS, Keenleyside A. n.d. Baby Bones, Food, and Health: Isotopic Evidence for

Infant Feeding Practices in the Greek Colony of Apollonia Pontica (5th – 2nd centuries B.C.)

Molleson T. 1991. Demographic implications of the age structure of early English cemetery samples. Actes des Journees Anthropologiques 5:113-121.

Moorrees FA, Fanning EA, Hunt EE. 1963. Age Variation of Formation Stages for Ten Permanent Teeth.J DENT RES 42: 1490-1505.

Nedev D, and Panayotova K. 2003. Apollonia Pontica (end of the 7th – 1st Centuries BC). In Grammenos D and Petropoulouse E Eds. *Ancient Greek Colonies in the Black Sea* pp. 95-155.

Prowse TL, Saunders SR, Schwarcz HP, Garnsey P,Macchiarelli R, Bondioli L. 2005. Isotopic and dental evidence for infant and young child feeding practices in an imperial Roman skeletal sample.Am J of Phys Anthropol137:294 – 308

Richards MP, Fuller BT, Mays SA. 2003. Stable carbon and nitrogen isotope variation in tooth dentine serial sectionals from Wharram Percy. J of Archaeo Sci 30:1673-1684.

Saunders SR and Barrans L. 1999. What can be done about the infant category in skeletal samples? In Hoppa RD, FitzGerald CM editors. Human Growth in the Past studies from bones and teeth. Cambridge University Press:183-208

Saunders SR, Herring DA, Boyce G. 1995. Can Skeletal Samples accurately represent the living populations they came from? The St. Thomas' Cemetary site, Belleville, Ontario. In Grauer AL editor. Bodies of Evidence: Reconstructing History Through Skeletal Analysis. New York: Wiley Liss p. 69-103.

Saunders SR, Hoppa RD. 1993. Growth deficit in survivors and non-survivors: biological mortality bias in subadult skeletal samples. Yrbk of Phys Anthropol 36:127-151.

Sawchuck LA, Burke SDA, Padiak J. 2002. A matter of privilege: Infant mortality in the Garrison Town of Gibraltar, 1870-1899. J of Fam hist 27(4):399-429.

Schoeninger MJ, DeNiro MJ. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochimica et Cosmochimica Acta 48:625-639.

Schoeninger MJ, DeNiro MJ, TauberH. 1983. Stable nitrogen isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric human diet. Science 220:1381-1383.

Schurr MR. 1997. Stable nitrogen isotopes as evidence for the age of weaning at the Angel Site: a comparison of isotopic and demographic measures of weaning age, J of Archaeo Sci 24:919-927.

Siegel MI, Doyle WJ. 2005. The differential effects of prenatal and postnatal audiogenic stress on fluctuating dental asymmetry. J of Exper Zoo 191(2):211-214.

Smith BH. 1991. Standards of human tooth formation and dental age assessment. In Advances in dental anthropology. New York: Wiley-Liss.

Stuart-Macadam P. 1992. Porotic hyperostosis: A new perspective. Am J of Phys Anthropol 87:39-47

Stuart-Macadam P, Dettweyler K editors. 1995. Breastfeeding: biocultural perspectives. New York: Walter De Gruyter.

Wright LE, Schwarcz HP. 1998. Stable carbon and oxygen isotopes in human tooth enamel: identifying breastfeeding and weaning in prehistory. Am J of Phys Anthropo 106:1-18.

Wright LE, Schwarcz HP. 1999. Correspondence between stable carbon, oxygen and nitrogen isotopes in human tooth enamel and dentine: infant diets at Kaminaljuyu. J of Archaeo Sci 26:1159-1170.

Ucko P.1969. Ethnography and archaeological interpretation of funerary remains. World Archaeology 1(2): 261-280.