**CHILDHOOD FEEDING PRACTICES IN IRON AGE SOUTH ITALY**

INDIVIDUAL BREASTFEEDING AND WEANING HISTORIES IN IRON AGE SOUTH ITALY USING STABLE ISOTOPE ANALYSIS OF INCREMENTAL DENTINE SECTIONS AND BONE COLLAGEN

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

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**Abstract**

This thesis investigates breastfeeding and weaning patterns in an Iron Age (7th – 4th century BCE) sample of subadults (*n*=12) and adults (*n*=9) buried at the sites of Botromagno, Parco San Stefano and Padreterno in southern Italy. Stable isotope analysis of both human tooth dentine and bone collagen for each subadult, and tooth dentine for adults, was undertaken to create early-life feeding histories. The dentine serial sections were used to determine the onset and completion of weaning for each individual, as well as distinguish general trends in early feeding practices at these Iron Age sites. Results indicate that the average onset of weaning in subadults occurred at 8 ± 3.4 months and weaning was completed by 4 years of age at the latest for all individuals; however, the patterns of breastfeeding and weaning were variable in this sample. This study also explores variation in early childhood diet between survivors and non-survivors (i.e., < 4 years of age). Non-survivors were weaned more rapidly than survivors – possibly contributing to their earlier death – and some non-survivors demonstrated elevated δ15N values that may have been a result of physiological stress. It is, however, difficult to distinguish signals of breastfeeding versus stress in young children who were still likely consuming breast milk. Finally, differences in isotope data between dentine serial sampling and bulk-bone sampling of rib and femoral collagen from the same individuals were investigated. The results show that the combined use of dentine and bone data contribute to more nuanced interpretations of weaning. Further, rib samples represent diet closer to the time of death than femoral samples, as faster bone turnover rate in ribs allow for the incorporation of more recent dietary changes.

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**Declaration of Academic Achievement**

Along with designing the project, I was responsible for compiling a literature review of breastfeeding and weaning analysis in bioarchaeology, as well as, stable isotope analysis and dentine microsampling. I was responsible for the experimental design, data collection and analysis conducted in this study. Dr. Tracy Prowse provided guidance on the theoretical development of this project, access to skeletal material, and composition of the thesis. Mass spectrometry was conducted at the Ján Veizer Stable Isotope Laboratory (formerly G.G. Hatch) at the University of Ottawa.

Using the stable isotope results provided by the Ján Veizer Stable Isotope Laboratory, I analyzed and interpreted the data, as well as wrote the findings. Dr. Tracy Prowse assisted in the statistical methodology and interpretations, while Dr. Megan Brickley and Dr. Tina Moffat provided feedback on the complete version of this thesis.

**Chapter 1: Introduction**

* 1. **Research Overview**

Bioarchaeological analyses of children are useful in understanding diet, disease and health in past populations. They are often used in the exploration of breastfeeding and weaning patterns, providing bioarchaeologists with information on lived experiences of past childhood, and long-term consequences of early diet on growth, health, and survivorship. Research on early feeding practices, specifically patterns of breastfeeding and weaning, has been conducted worldwide on diverse populations, typically through the stable isotope analysis of carbon and nitrogen isotopes in bulk bone collagen (e.g., Nitsch et al. 2011; Herring et al. 1998; Tsutaya & Yoneda 2013; Wright & Schwarcz 1999). In Italy, isotope studies on weaning patterns have focused on the Roman and Medieval periods (e.g. Barbiera & Dalla-Zuanna 2009; Prowse et al. 2008; Moggi-Cecchi et al. 1994) using bone collagen, while the Italian Iron Age (~1st millennium BCE) has been devoid of research pertaining to childhood diet. While bone collagen can provide an average age at which the weaning signal ceases to be present in skeletal remains of a population, it also overlooks inter-individual variation and demonstrates inaccurate onset and completion of weaning. The recent development of incremental dentine sampling techniques developed by Beaumont and colleagues (2013) permits a more time sensitive analysis of dietary change. This thesis presents the first study to investigate early feeding practices in a sample of Iron Age subadults and adults (7th – 4th century BCE), from the sites of Botromagno, Parco San Stefano and Padreterno, in South Italy. Further, this thesis investigates individual breastfeeding and weaning histories using a combination of dentine microsampling and bulk bone methods. As information on childrearing practices during the Italian Iron Age is non-existent, this stable isotope investigation not only provides an insight into possible childhood experiences during this period but also provides a point of comparison for the analysis of later Roman populations and other bioarchaeological studies of weaning in Italy.

The primary objective of this thesis is to determine the onset age of weaning and the age of weaning cessation using stable isotope analysis of serial tooth dentine sections. Using stable carbon and nitrogen values measured within the incremental sections of deciduous and permanent teeth, longitudinal profiles can be created to demonstrate trophic levels shifts in δ15N (~3‰) and δ13C (~1‰) values associated with the consumption and withdrawal of breastmilk, as well as changes in δ13C values associated with the introduction of transitional foods (Fogel et al. 1989; Fuller et al. 2006).

The second objective of this thesis is to investigate variation in weaning practices between children who survived into adulthood and those who did not survive the weaning process. The ‘Osteological Paradox’ by Wood et al. (1992) cautioned bioarchaeologists about the potential biases inherent in archaeological samples when analyzing past health, specifically that skeletal samples are made up of individuals who did not survive. Traditional weaning studies have based their interpretations primarily on subadult bone collagen from skeletal assemblages, excluding those individuals who survived well past the weaning stage. This study addresses this paradox by including early dietary analysis of both subadults (i.e. non-survivors) and adults, thereby highlighting potential differences in weaning patterns that could be tied to survivorship.

An additional goal of this thesis is to evaluate how data from serial dentine sections and bone collagen compare with respect to identifying patterns of breastfeeding and weaning. The time period represented by isotope ratios in bone collagen varies depending on the bones used and the age of the individual as a result of bone growth and remodelling rates; the time represented by serial dentine sections is more precise due to dentine formation and a lack of remodelling. This thesis compares tooth dentine, rib, and long bone, stable isotope values from the same subadult individuals to investigate how well the isotope values in these different tissues correlate.

* 1. **Thesis Structure**

Chapter two is a detailed review of bioarchaeological analysis of breastfeeding and weaning in past and present populations. This chapter begins by outlining the Biocultural model that is used to theoretically frame this research. The concepts of optimal early feeding practices are discussed next, followed by the bioarchaeological approaches that are used to determine weaning patterns in the past. Pre-Roman and Roman childhood feeding strategies are also presented to provide historical context.

Chapter three discusses stable isotope geochemistry and its application in bioarchaeology. First, the chapter provides a comprehensive review of stable isotope analysis, individual applications of carbon, nitrogen and oxygen, as well as the appropriate body tissues that are used for the analysis. Next, the chapter discusses the development of tooth dentine microsampling along with the potential benefits of utilizing tooth dentine as opposed to bone collagen.

Chapter four outlines the materials and methods that are used in this thesis, highlighting bulk bone sampling and dentine serial sampling. A brief history of Iron Age in South Italy is presented, followed by an overview of archaeological excavations at Botromagno, Parco San Stefano and Padreterno. Descriptions of individuals included in this study are also provided, including burial information and a list of dental and skeletal tissues analyzed.

Chapter five presents the stable isotope results. This chapter identifies trends in the dentine isotopic data and compares dentine and bone data from the same individuals. Chapter six interprets these notable trends. Variations in weaning histories within the sample are interpreted, while comparing weaning practices between the sexes, and between survivors and non-survivors. Weaning patterns in this sample are also interpreted alongside historical evidence of Roman weaning practices and bioarchaeological research on early childhood feeding practices. As well, this chapter discusses the benefits of combining dental and bone tissues in stable isotope analysis of dietary change. Limitations of this research are also listed in this section. Finally, chapter seven summarizes the findings of this study and suggests avenues of future research.

**Chapter 2: The Bioarchaeology of Breastfeeding and Weaning**

* 1. **Introduction**

Until the 1990s, children were largely excluded or marginalized from bioarchaeological discourse (Mays et al. 2017). Children were considered unimportant to social life and poor preservation in the archaeological record hindered investigative interests. If they were included in studies, biological anthropologists often used a perspective characterized by Littleton (2011) as the ‘canary in the coalmine’. Past studies would assess the health of past children as a measure of health for an entire population (e.g., Lundberg 1993; Rahkonen et al. 1997). In other words, childhood morbidity and mortality were used as sentinels for overall population health. As two distinctive strands of research converged with archaeology – specifically, historical and feminist discourse (e.g., Pollock 1983; Kamp 2001) – a more considered approach and interest in childhood within archaeology (and later bioarcheology) began to develop. Bioarchaeologists now recognize that the investigation of past childhood is central to a comprehensive study of cross-cultural and temporal variation in past diet, social structures, as well as human biological adaptations to social environments (Katzenberg et al. 1996; Prowse 2011). Current bioarchaeological work focuses exclusively on children in an archaeological context, and tries to model various past childhood experiences (e.g. Lewis 2007; Lewis 2010; Penny-Mason & Gowland 2014; Newman and Gowland 2017; Mays et al. 2017; Beauchesne & Agarwal 2018). A recent journal, first published in 2009, entitled ‘Childhood in the Past’ clearly demonstrates this new interest in exclusively understanding all aspects associated with early diet, development and experiences of children in past populations.

In particular, breastfeeding and weaning practices have become an important area of research providing insight into the long-term consequences of early diet on growth, health, and survivorship. Accordingly, a great wealth of information can be gathered about populations and childhood, present or past, through the study of weaning. This chapter will provide a brief overview of the theoretical framework that is used in the bioarchaeological investigation of breastfeeding and weaning. Next it will discuss juvenile feeding and maternal reproductive ecology, concepts of optimal infant and young child feeding practices, and bioarchaeological methods to studying weaning. Additionally, it will highlight past research on weaning conducted on Roman and pre-Roman skeletal samples, as it is pertinent to this study’s focus on weaning during the Iron Age of southern Italy.

* 1. **Theoretical Framework**

The biocultural model frames the research presented here as it is an integrative approach used to investigate the interaction among biological and sociocultural factors affecting infant and childhood feeding practices (Goodman & Leatherman 1998; Khongsdier 2007; McElroy 1990). Definitions of the biocultural approach have varied over the past several decades, yet it is characterized by a core theme. In general, the biocultural approach emphasizes the reciprocal exchange between biological and cultural aspects of human behaviour and health (Zuckerman & Armelagos 2011). Breastfeeding and weaning are both considered biocultural phenomena as they are biologically necessary processes that are shaped by cultural practices, which can have subsequent physiological and epidemiologic consequences (Fildes, Stuart-Macadam & Dettwyler 1995). More specifically, the act of breastfeeding is an essential stage of early infancy that is associated with multiple physiological developments, such as the maturation of an infant’s immune system, tooth formation, and development of the brain. Yet, cultural aspects such as societal beliefs and practices, socioeconomic status, maternal labour load and so forth, dictate whether the infant is breastfed or not, as well as the age at which breastfeeding and weaning are initiated and completed. Biocultural research focusing on early feeding practices underlines the various cultural and biological influences that shape infant and young child feeding. It can inform us about maternal health, subsistence change, food choice, childcare practices, and differential access to foods as a result of social factors (Moffat & Prowse 2018; Fuller, Richards & Mays 2003; Herring et al. 1998; Wright & Schwarcz 1999). This investigation uses the biocultural model to understand how weaning practices varied in Iron Age Italy (e.g., weaning earlier or later, type of weaning foods, duration of transitional feeding); thereby investigating the cultural and biological interactions that result in the health and well-being of children from this particular time period.

* 1. **Infant and Young Child Feeding Practices (IYCF)**

2.3.1 Age Categories and Terminology

When analyzing past childhood, age categories are often variable between studies and are not necessarily relevant to biological development or social identity (Halcrow & Tayles 2011). The ‘type’ of age category (biological, chronological and social) used in a study depends on the particular research objectives; hence, it is important to outline these objectives as age categories used in bioarchaeological analysis have implications for future interpretation and comparison of these skeletal data among different studies. In this study, age is assigned according to biological range estimates primarily based on tooth formation and eruption and depending on the individual, as well as the skeletal preservation for subadults. Adult age is also assigned using the features of the innominate bone.

The postnatal period includes the following stages: infancy, childhood, juvenile stage, adolescence and adulthood. Based on clinical paediatric medical definitions, some researchers assign the term ‘infant’ to individuals who are younger than one year of age (Forfar et al. 1998, 2003; Scheuer & Black 2000), while others use this term to refer to young children up to three or five years of age (White & Folken 2005). However, Lewis (2007) argues that it is problematic to include children up to five years of age in an “infant” category because it overlooks the major physiological and social developments that occur from birth. In this study, I use the term ‘infant’ to refer to young children under one year of age, while infant and young child feeding (IYCF) covers the period from birth through the completion of weaning and the shift to a post-weaning diet (Sellen 2007). In past studies, ‘subadult,’ ‘juvenile,’ and ‘child’ have all been used interchangeably for individuals who have not yet reached adulthood (e.g. Hoppa 1992; Scheuer & Bowman 1995; Scheuer & Black 2000). Consequently, as the definitions of these terms vary among bioarchaeologists, I use the term ‘subadult’ broadly in reference to both ‘infants’ and ‘young children’ and when generally discussing stages of infancy and childhood (after Halcrow & Tayles 2011). Childhood ends once individuals reach puberty, which starts approximately at 10 to 13 years of age for females and 12 to 16 years for males, during which they are considered adolescents up until 17 years of age (Scheuer & Black 2000).

For this discussion terminology associated with IYCF practices should also be defined. ‘Weaning’ is a process that involves the gradual removal of breast milk and the introduction of ‘complementary foods’ – or non-breast milk foods – with the eventual complete termination of breastfeeding (Herring et al. 1998). This period where nutrition is attained through the combination of breast milk and complementary foods is termed ‘transitional feeding’ (Sellen 2007).

* + 1. Juvenile Feeding and Maternal Reproductive Ecology

Breast milk is the earliest nutrition most infants receive post-birth. It contains various immunological and nutritional properties that are essential for infant survival, growth, and development, especially in the first six months after birth (Kramer & Kakuma 2004). Specifically, breastmilk is known to contain T and B lymphocytes, immunoglobins, antistaphylococcal factor and other resistance factors (Pickering & Ruiz-Palacios 1986; Mestecky et al. 1991). Extensive clinical and epidemiological research demonstrates that infants cannot make efficient use of other foods prior to six months of age; if not exclusively breastfed, infants can suffer deficits and increased morbidity (Dewey et al. 1999, 2001; Kramer et al. 2003). As immunoglobins are highest in colostrum (the first form of milk produced immediately post-birth) and their presence is maintained throughout lactation, this intake of immunological components can be extremely beneficial for infants since their immune systems are still immature. There is also evidence that breastmilk can induce the infant’s immune system to mature more quickly (Newman 1995).

Although the benefits of breastfeeding continue after the first six months, complementary foods are vital to meet an infant’s growing need for energy and nutrition. After six months of age, complementary foods increasingly contribute to the diet, as chewing, tasting and digestive abilities develop. If the transition from exclusive breastfeeding to a mixed-food diet occurs too quickly it can cause the infant to experience weanling diarrhea syndrome and food allergies. This may be because the infant’s immature immune system is forced to cope with food-borne pathogens while risking malnutrition as breastmilk consumption begins to decline (Herring et al. 1998). The frequency of suckling and volume of milk consumed do not necessarily decrease in healthy infants or young children during the weaning process (Sellen 2007); thus, complementary feeding may continue up until three years of age, during which breast milk can remain an important and relatively sterile source of nutrients and passive immunity.

Ethnographic evidence from preindustrial societies reveal that the duration of breastfeeding in modern humans is extremely variable (Sellen & Smay 2001; Kennedy 2005). For instance, among the Ache (an indigenous community in Paraguay), the cessation of weaning begins around two years of age while the women are already two to three months pregnant with their next offspring (Hurtado & Hill 1996). Meanwhile, in the Amele (an Indigenous society from lowland Papua New Guinea), children complete weaning on average around three years of age (Worthman et al. 1993). Researchers have used gestation length, 3:1 weaning/neonate weight ratio, and adult body weight to predict weaning ages for past and present populations (e.g. Harvey & Clutton-Brock 1985; Kennedy 2005). These methods have also been used to infer optimal weaning ages. For instance, according to Charnov and Berrigan (1993), optimal weaning age occurs when an infant reaches 33% of adult body weight; for well-nourished western children the authors suggest that this would occur around 5.8 years of age for girls and 7 years for boys. This however is quite late considering more recent work which suggests that after 6 months of age infants require non-breast milk foods to meet the growing nutritional needs of the child (Sellen 2007). Smith (1992) found a high correlation between weaning age and the timing of permanent first molar eruption, a relationship she characterized as ‘isochronous’, using data from Harvey and Clutton-Brock’s (1985) and Smith’s (1989) studies, which documented life-history patterns across primate species. In accordance with this hypothesis, optimal weaning age was suggested to be around 5.5 to 6 years of age. The range of predicted optimal weaning age in modern humans varies from a minimum of nine months to more than seven years; this wide range of predictions suggest that the factors in determining the initiation of weaning, in humans at least, are not well understood, despite efforts at mathematical reasoning (Kennedy 2005). Weaning age is a complex variable and even though in many cases it would seem highly advantageous for humans to continue nursing, human children are weaned long before the child’s size, dentition, digestive tract, and immune system are mature enough to provide them with an optimum opportunity to survive (Lee et al. 1991).

According to Sellen (2007), the typical age at the end of weaning in approximately 83% of non-industrialized societies is 2.4 to 2.7 years of age, while industrialized societies often wean their children much earlier. Likewise, the process of weaning in some communities can be gradual or (less commonly) abrupt. Nevertheless, the age-related pattern of transitional feeding and the introduction of complementary foods in preindustrial societies resemble modern clinical recommendations for optimal growth and development of infants (Sellen 2007). Clinical recommendations include: (a) initiation of breastfeeding within an hour of birth, (b) a period of exclusive breastfeeding followed by the introduction of nutrient-rich and pathogen-poor complementary foods (food procured in a sanitary environment or foods that are not contaminated) at about 6 months of age; and (c) continued breastfeeding at least until the third year (Sellen 2007). These clinical recommendations do not indicate an upper age limit at which breastfeeding ceases to be of some benefit to children; therefore, it is possible that continued breastfeeding along with complementary feeding might have been a beneficial maternal strategy in the past because of its anti-infective properties and nutritional or physiological benefits to children. Overall, the length of breastfeeding is vital for the survival and health of the infant. Breastfeeding for a short duration can lead to higher rates of infant morbidity and mortality, while longer duration of exclusive breastfeeding may not provide sufficient energy and nutrient requirements (Sellen 2007). This is known as the ‘weanling’s dilemma’, the balance between nutritional requirements and immunological development (Rowland & Barrell 1978).

The process of weaning also has implications for mothers and population fertility. The overall period and frequency of suckling during breastfeeding and the weaning process impacts the length of maternal lactational amenorrhea and the timing of ovulation resumption; along with other factors such as maternal energy flux, contraceptive use, and sexual activity, this can significantly impact birth intervals and overall fertility rates in a community (Sellen & Smay 2001). The interval between births (IBI) reflects the mother’s biological ability to utilize her metabolism and nutritional stores towards herself and an existing infant over that of a developing fetus. Therefore, it is predicted that any intervening factor that may accelerate the process of weaning in preindustrial societies may also increase fertility independently of infant mortality (Sellen & Smay 2001).

**2.4 Bioarchaeological Approaches to Studying Breastfeeding and Weaning**

Past demographic studies have often used the duration of breastfeeding as an important determinant of population fertility (e.g. Bongaarts 1978, 1982; Trussell 1979). As previously mentioned, this is because the suckling stimuli and energetic burden of producing milk during breastfeeding delays the mother’s ovulation; shorter breastfeeding periods result in shorter birth intervals and increased fertility. As a result, the age at the end of weaning has been used by bioarchaeologists as an indicator of fertility in ancient human populations (e.g., Schurr & Powell 2005; Waters-Rist et al. 2011; Tsutaya et al. 2014) and as an indicator of decreased dependency of a child on its mother due to the availability of weaning foods. Buikstra et al. (1986) hypothesized that a shift towards earlier weaning in prehistoric North American populations was correlated with the increased availability of high-carbohydrate resources. These resources were processed into ‘weaning-gruel’, which led to smaller birth intervals and increased fertility, resulting in population growth (Buikstra et al. 1986). In 2001, Sellen and Smay tested the ‘weaning food availability hypothesis’ using available data of 133 distinguishable preindustrial and natural fertility populations extracted from 172 sources. These reports indicated a dependence on starchy staples foods for weaning among predominantly agricultural populations and a variety of feeding styles to suit the perceived needs of young children. Results demonstrated that patterns of weaning were not simply shaped by subsistence practices or the availability of weaning foods; instead, Sellen and Smay (2001) proposed that maternal work patterns and the probability of infection during weaning may account for the changes in weaning practices. McNeilly (2001) further added that changes in female work patterns limit infant access to breastfeeding, thereby resulting in earlier decrease in suckling frequencies and the inability to maintain breastfeeding’s contraceptive effects. The relationship between maternal time allocation to subsistence work and childcare has previously been documented by various researchers (e.g. Popkin 1980; Hurtado et al. 1992; Wandel & Holmboe-Ottesen 1992; Marriot 1996). Similarly, the flux of infectious disease during weaning and subsequent early childhood mortality and increased fertility has also been noted across populations (Rowland et al. 1978; Mock et al. 1993; McDade & Worthman 1998). Schurr and Powell (2005) also investigated weaning onsets and durations before and after the appearance of intensive food production. The authors conducted stable isotope analysis to determine weaning ages and paleodemographic measures of birth rates for four eastern North America sites with differing subsistence practices. Birth rates and weaning behaviour were found to be similar at all four sites indicating that attributing population growth after the appearance of food production as a direct result of earlier weaning is not universally applicable (Schurr & Powell 2005).

According to past demographic studies in Europe and North America, it is challenging to add a third variable, fertility, and study its relationship to breastfeeding and infant mortality in historic and prehistoric contexts (Knodel & Kintner 1977; Wrigley 1977; Kintner 1985; Knodel et al. 1988). This is due to the fact that quantitative information on breastfeeding practices is generally inadequate; hence, it is very difficult to link specific fertility histories of women to the survivorship or mortality of their children (Katzenberg et al. 1996). For some historic populations, IYCF practices and female fertility can be estimated from historic records. Yet, in most cases the necessary information is often unavailable and written documents are of course entirely absent for prehistoric populations. Various methods have been used to infer weaning times in past populations, including non-specific measures of stress (e.g. Goodman & Rose, 1990; Blakey et al. 1994; Larsen, 1995; Tomczyk et al. 2012; Ash et al. 2016) and bone chemistry techniques (e.g. Waters-Rist et al. 2011; Tsutaya & Yoneda 2013; Beaumont et al. 2013; de Armas 2017).

Non-specific stress marker such as dental enamel hypoplasias (DEH), Harris lines, cribra orbitalia and porotic hyperostosis have been used to infer the cessation of weaning (Perry 2005). This is because the process of weaning involves increased interaction between young children and their surroundings, putting them at greater risk for illness and death. They are more susceptible to pathogens present in complementary foods and water, as well as the physiological response to a change in diet (particularly less nutritious foods like alternative milk sources from goats and cows). Furthermore, as mentioned earlier in Section 2.3.2, infants and young children may possess an under-developed immune system due to the decreased consumption of breast milk that provides passive immunity (Larsen 1997). A decline in breast milk consumption can create physical stress during weaning and, if accompanied by any additional acute physiological or even emotional stress experienced during childhood, can result in the slowing and – in dire cases – cessation of normal growth, indicated by DEH and/or Harris lines, in addition to shorter than expected long bones (Perry, 2005). In order for these features to appear in bones and teeth, individuals must also recover from the stressor, at least for a little while after the stress episode.

Dental enamel hypoplasias occur when stress is experienced during enamel deposition in deciduous or permanent teeth, from the second trimester in-utero until approximately 10 years of age. Due to the fact that there are various different stressors that could cause DEH, the exact etiology is hard to determine. Some studies have noted a strong link between socioeconomic, environmental, and emotional stress with DEH development (e.g. Goodman & Rose 1991; Miszkiewicz 2015). Similarly, Harris lines – radiographically visible transverse lines on long bones – develop because of cessation and resumption of bone growth (Perry 2005). These markers can develop as a response to illness or poor health and can remain observable in adult skeletons. However, unlike DEH, Harris lines may be obliterated over time due to bone remodeling. Harris lines can also indicate recovery from a stressor (Papageorgopoulou et al. 2011), thus as a result, Harris lines are not widely used in IYCF studies. There are difficulties with using these osteological indicators to infer infant and young child feeding because they are nonspecific stress markers that can be caused by a variety of stressors including diet, disease, and trauma (Perry 2005).

Still, bioarchaeologists have attempted with varied success to determine whether Harris lines and DEH are weaning-related pathological conditions by identifying the age at which these features develop (e.g. Alfonso et al. 2005; Ash et al. 2016). Environmental stress strongly affects long bone growth making it difficult to determine the age at which Harris lines develop; meanwhile, environmental perturbations usually do not affect the timing of dental enamel deposition allowing researchers to measure the distance of a DEH from the cemento-enamel junction to determine the age at which the stress episodes occur. Accordingly, DEHs can be linked more accurately with a weaning-related stress than Harris lines, yet many associations between weaning and skeletal pathology remain tenuous (Katzenberg et al. 1996).

Blakey and colleagues (1994) discount weaning as a primary cause of DEHs in young children. In enslaved populations in the mid-Atlantic United States, the authors found that DEHs developed after weaning occurred, implying that other stressors related to enslavement caused enamel disruption. Larsen (1997) discovered that while weaning may lead to poor enamel development, the association between DEHs and weaning is occasionally “coincidental rather than real” (49). More recently, Alfonso and colleagues (2005) argued that Harris line formations have often been perceived with a traditional assumption that growth is a continuous process and observable interruptions are pathological. New data indicate that growth is in fact a saltatory process that includes time-constrained growth episodes that occur intermittently, suggesting that Harris lines are by-products of normal growth patterns (Lampl et al. 2001; Lampl & Schoen 2017; Mummert et al. 2018). Alfonso et al. (2005) investigated the validity of enamel hypoplasia and Harris lines as indicators of stress by looking at the degree of concordance in the presence and absence between the skeletal markers during stressful periods. Results from two archaeological skeletal samples from Northern Chile indicated that 63.2% of the population had Harris lines, while 79.4% had enamel hypoplasia (Alfonso et al. 2005). There was no discernable association found between the two indicators of stress (e.g. age or sex related). Instead the authors supported the hypothesis that most Harris lines are the result of normal growth and consequently are not adequate indicators of stress associated with weaning. Similarly, it was suggested by Alfonso et al. (2005) that enamel hypoplasia distribution may be the result of crown morphology causing higher sensitivity and higher visibility of defects formed at a specific age, rather than the sole product of stress.

Ash et al. (2016) analyzed non-specific stress markers (linear enamel hypoplasia, porotic hyperostosis, cribra orbitalia) within five Linearbandkeramik populations from across central Europe in conjunction with published carbon and nitrogen stable isotope results to observe childhood morbidity and mortality as well as social practices related to weaning. Analysis was conducted on a total of 511 skeletons from five Early Neolithic collections (Schwetzingen, Stuttgart-Mühlhausen, Vedrovice, Nitra-Horné Krškany, and Polgár-Ferenci-hát). In contrast to the previously mentioned studies it was found that on average the onset of LEH for all populations fell between two and four years of age, corresponding with stable isotope evidence for weaning after two or three years.

Although some studies (e.g. Lacruz et al. 2005; Tomczyk et al. 2012) have attempted to determine weaning age by using LEH, it can be argued that osteological markers are not sufficient on their own to be used to analyze IYCF practices. Another issue with observing these features in relation to weaning, is that much of the literature on DEH and Harris lines regards weaning as an event (Katzenberg et al. 1996). However, weaning is a process that encompasses the introduction of other foods, decreased dependency on breast-milk and complete cessation of breast-milk consumption. Bioarchaeologists and skeletal biologists have made recent progress in developing different approaches to estimating the timing of breastfeeding, transitional feeding, and termination of weaning. Stable isotope ratios of carbon, nitrogen, and oxygen in skeletal material of subadults and adults have been proven to be a useful approach to track the dietary changes and/or phases of weaning. An overview of stable isotope analysis and its wide range of applications are discussed in Chapter 3.

**2.5 Evidence for Breastfeeding and Weaning in Pre-Roman and Roman Italy**

2.5.1 Archaeological Evidence

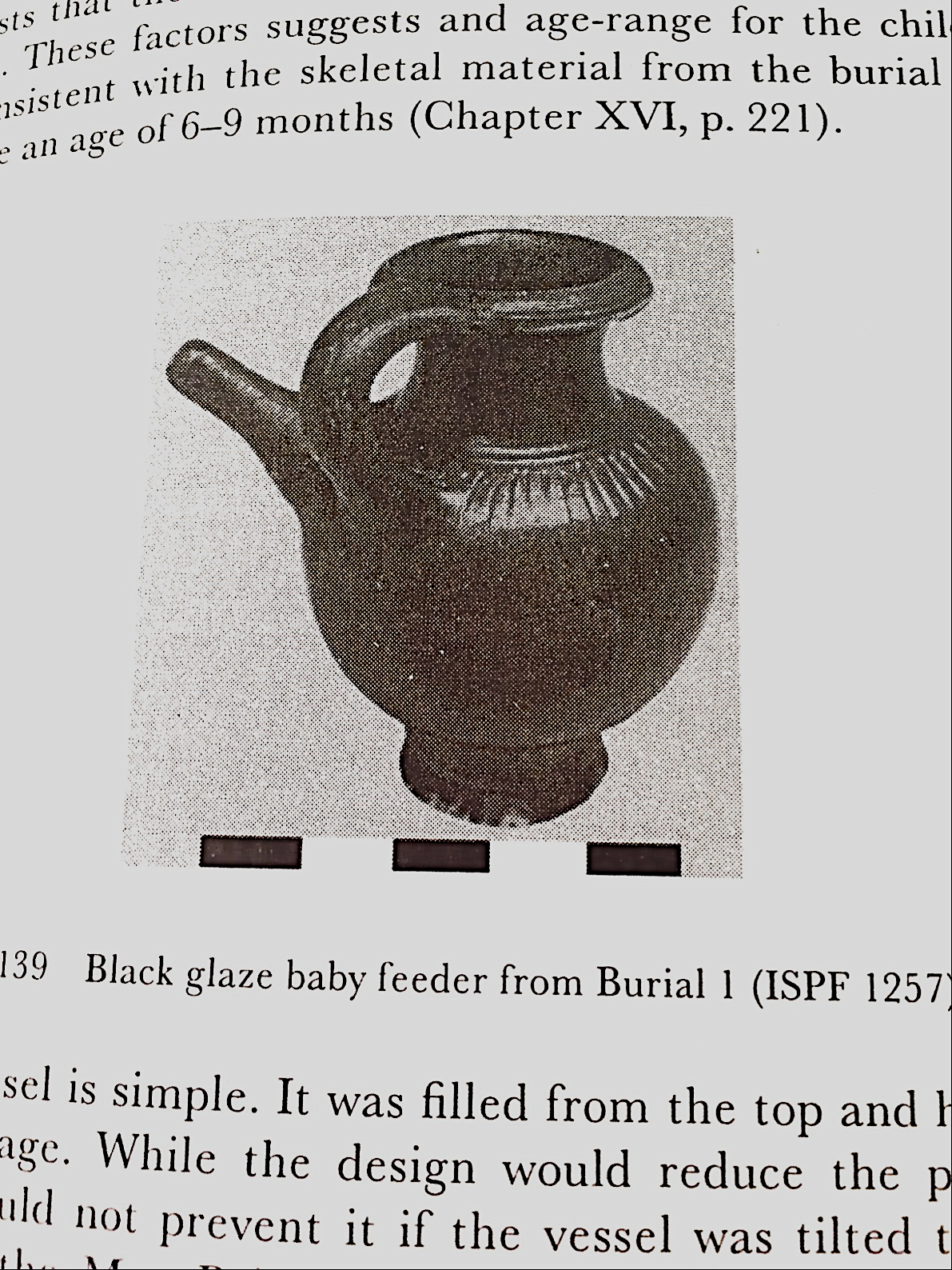
Archaeological artifacts such as grave goods have been used as evidence of infant feeding practices that may have taken place during both the pre-Roman and Roman periods. In her survey of Roman child burial practices in modern Sainte-Barbe quarter of Marseille (1st century BCE – 2nd century CE), Carroll (2018) reports that the majority of Roman child burials (67%) did not have grave goods, but for those that did, 18% were ceramics. Feeding vessels are not common but they have been noted to appear in various geographical sites from the Iron Age to the Roman period. For instance, two *coppi* burials(individuals buried within two semi-cylindrical roof cover tiles) at Nola in the province of Naples (late 4th century BCE) contained a small cup and a feeding bottle (Carroll 2018). Similar styled feeding bottles have been found in the Roman Sainte-Barbe cemetery at Marseille (30 BCE – 300 CE) with two perinatal infants (Moliner et al. 2003). At Lezoux in Gaul, burials of almost a dozen perinatal and young infants were associated with terra sigillata pottery workshops from the 1st – 2nd centuries CE. Some of these infants were buried with grave goods including rings, a lamp, miniature vessels and a feeding bottle – most probably produced at the workshop itself (Alesan et al. 1999). In Britain, at the Roman fort at Reculver, burials (1st – 3rd centuries CE) of eleven infants were discovered, along with these burials a baby feeding bottle was also recovered (Philp 1966). Feeding bottles also accompanied subadults who had not yet been weaned, such as a child between the ages of 1.5 and 2 years found at Roman Rottweil, Germany (1st – 4th centuries CE) (Faber 1998).

Comparable feeding vessels have also been found in association with pre-Roman sites and Greek colony sites in various parts of southern Italy. Historical evidence suggests that Greek colonists in southern Italy were in direct contact with Indigenous Iron Age communities, so much so that there are inferences of cultural interactions and trade with the Greeks (Handberg & Jacobsen 2011; Colivicchi 2011). Ionian cups (from Ionian Greek colonies) were also found in Iron Age settlement of Parco San Stefano (Small 1992) located at Botromagno near Vagnari in southern Italy; thus, it may be possible that Greek styles of infant feeding bottles influenced those found in Iron Age southern Italy. A spouted feeding bottle was found amongst a few objects that were deposited with foetuses and perinatal infants in the Hellenistic well of Athenian agora, indicating that feeding bottles were most likely brought by Greek colonists from Greece (Liston & Rotroff 2013).

At Metaponto, a coastal Greek colony site located along the coast of southern Italy, newborns were buried with numerous ceramic feeding vessels such as cups and feeding bottles, oil flasks (*aryballoi*, *lekythoi*) and terracotta figurines (6th – 3rd centuries BCE) (Carter 1998). Similarly, infant burials at Botromagno (5th to the 3rd centuries BCE) and child graves in the Greek colonies of Himera (Sicily) and Taranto (coastal city in Southern Italy) contained comparable feeding bottles and small cups (Carroll, 2018; Whitehouse et al. 2000). Examples of feeding bottles found at these sites can be seen in Figure 2.1 and 2.2 which present images of feeding bottles found at two infant burials (Tomb 12 and Burial 1) at Botromagno. The bichrome baby feeder shown in Figure 2.1 has a spout and a single handle set at roughly a right angle; this suggests that the vessel was designed to be held by a right-handed child as the size and location of the handle would be uncomfortable for an adult using the vessel to feed a child (Whitehouse et al. 2000). The shape of the vessel is rare and unlike the normal Greek type baby feeders found in southern Italy at the time, such as the black glazed baby feeder in Figure 2.2. Yet, the tongue patterns painted on the vessels do not belong to the traditional Matt-Painted repertoire (commonly found in Iron Age burials) but belong to the peripheral patterns found on Greek figured pottery. The Greek black glazed baby feeder (Figure 2.2) is a simple design with a spout, a single handle set at roughly a right angle and a narrow neck to reduce the risk of spillage. The body chamber is small and the narrow spout indicates that only small volumes of liquid foods could be fed through this vessel. According to Whitehouse and colleagues (2000) since these burials contained a baby feeder, the young children most probably had begun weaning but had not yet outgrown special cups designed to prevent spillage and were probably between 6 – 36 months of age.



**Figure 2.1:** Biochrom**e** feeding bottle from Tomb 12 at Botromagno, 5th century BCE(from Whitehouse et al. 2000, p.181).



**Figure 2.2:** Black glaze baby feeder from Burial 1 at Botromagno, late 4th century BCE (from Whitehouse et al. 2000, p.219).

Greek styled baby feeders found in southern Italy date back to the later 6th century BCE. La Porto (1973) published examples from San Martino (Matera) that date to the late 6th century BCE. Holloway (1970) also published a similar vessel with a short neck and lateral handles from Tomb 24 at Satriano in the Calabria region of southern Italy, dating to the mid 5th century BCE. Tomb 3/1 at Padreterno also contained a baby feeder similar to the black glazed vessel presented in Figure 2.2 dating to first half of the 3rd century CE (AA. VV. 1989 as cited in Whitehouse 2000).

Although these bottles have been argued to be lamp fillers or funerary libation vessels, a Greek terracotta figurine (5th century BCE) illustrates the use and purpose of these vessels (Figure 2.3); the figurine depicts a seated woman holding a spouted cup to the lips of an infant in her lap (Carroll 2018). This interpretation is further supported by the analysis of residues in the feeding bottles at Roman Nijmegen, Aachen and Cologne (1st – 3rd centuries AD) which revealed that they once held milky substances (Huttmann et al. 1989).



**Figure 2.3:** Greek terracotta figurine of the fifth century BC, depicting a mother holding a spouted cup to the lips of a baby on her lap. Drawing by Irene de Luis from Carroll 2018, p.27.

Research on breastfeeding and weaning practices in pre-Roman Italy is very limited. Inferences of IYCF have mainly been drawn from the archaeological finds discussed above and from studies that aimed to explore either population health or diet. For instance, Repetto and colleagues (1988) analyzed a combination of skeletal stress indicators (e.g. dental disease, adult stature reduction, cribra orbitalia, Harris lines) of 11 individuals in a Bronze Age (1700 –1350 BCE) sample from Basilicata, southern Italy, to examine the general health status of the population. The results indicated overall good nutrition with adequate health conditions; however, the authors found an occurrence of enamel hypoplasia in the sample from ages three to four years. They associated this occurrence with nutritional stress linked to delayed completion of weaning as it could produce temporary iron-deficiency due to low iron content of breastmilk (Palkovich 1987). Yet as stated in Section 2.4 current clinical recommendations does not have an upper age limit for breastfeeding, hence, continued breastfeeding will most likely provide children with nutritious benefits rather than cause iron-deficiency.

Another study conducted stable carbon and nitrogen isotope analysis of human remains from four Bronze Age Sites, two from northern Italy and two from southern Italy, to compare diets of these geographically-dispersed yet contemporaneous populations. The human and animal bones from the two sites in northern Italy were significantly enriched in 13C indicating that northern populations were consuming domestic millets (*Panicum miliaceum* and/or *Setaria italica*) (Tafuri et al. 2009). Conversely, the authors found that the samples from the two Bronze Age sites in Southern Italy were significantly depleted in 13C – suggesting that millet was absent from the diet and protein from plants such as wheat and barley largely contributed to the adult diet. Although this research does not explicitly discuss breastfeeding and weaning patterns during this period, the information from this study can be used to understand the general diet that was consumed by Bronze Age populations in Italy; thereby indicating the type of diet that most likely would have been incorporated in the weaning process.

2.5.2 Literary Sources from the Roman Period

In Italy, studies on weaning patterns have focused mainly on Roman and Medieval populations, thus so much more is known about child-rearing practices during these periods than in the pre-Roman period (e.g. Moggi-Cecchi et al. 1994; Prowse et al. 2008; Barbiera & Dalla-Zuanna 2009). The Roman period in particular has been a focus for paleodietary research and, along with the textual and archaeological evidence, the isotopic data are an invaluable addition to our knowledge of food consumed in the past and weaning practices in antiquity.

Historical evidence for attitudes towards breastfeeding and weaning during the Roman period is mainly based on medical texts and wet-nursing contracts. *Gynaecology* by Soranus of Ephesos (early 2nd century CE), *Hygiene* by Galen of Pergamum (late 2nd century CE) and the medical collections of Oribasius of Ephesos (mid-4th century CE) (Raeder 1933) contain the most useful material when analyzing Roman breastfeeding and weaning practices. Soranus practiced medicine in Rome in the early 2nd century CE and had immense influence during the Roman period and the Middle Ages. Of his 20 known publications, which range over a wide field of biological and medical sciences, *Gynaecology* is the one of the most important as it represents ancient gynaecological and obstetrical practices (Temkin & Eastman 1991). Soranus’ work was eventually eclipsed by the work of the Roman physician Galen (130–200 CE). Of Galen’s work, *Hygiene* is still relevant today providing a comprehensive account of preventive medical practices as well as infant and child care. Oribasius (70 – 110 CE) was a prolific writer and Greek physician who compiled medical discoveries of other physicians and covered topics including dietetics, pathology and anatomy.

Soranus believed that during the first two days post-birth, infants were still digesting food acquired in-utero and should not be given additional food (Temkin 1956). After this period, he suggested that infants be given moderately boiled honey to purge the stomach and bowels, and then breastfed. According to Soranus, breastmilk produced during the first 20 days post-birth was unwholesome, since it was considered to be thick and hard to digest; he recommended that children be breastfed by a wet-nurse who had been nursing for two to three months. If a woman who was able to provide milk was not available, then it was suggested that for the first three days the baby was fed honey mixed with goat’s milk, followed by the mother’s milk (although the first portion was discarded). Exclusive breastfeeding was recommended beyond the first 40 days and up until the point the infant’s body became “firm” or after the infant first attempted to sit up, which was considered to happen after six months of age (Temkin 1956). The overall breastfeeding and weaning timetable that was prescribed indicates that transitional feeding could begin after six months of age, and weaning was possibly completed around two to three years of age, but this timetable was likely very flexible (Fildes 1986; Prowse 2011). Poor families who could not afford a wet nurse may have been forced to wean children earlier or may have had to abandon their children to be sold into slavery (Garnsey 1998). The use of artificial nipples was also mentioned in the context of weaning, including vessels that could be used to provide water or diluted wine to thirsty children (Temkin, 1956). This indicates that children may have also been artificially fed milk from animals, such as cows or goats; even though it is possible that this milk could potentially cause dysentery or diarrhea due to high bacterial levels (Allason-Jones 1989). The archaeological evidence of such infant feeding vessels in antiquity is far stronger than that from the literary sources (Fildes 1986), and these vessels have been commonly found in both Greek and Roman cemeteries (as discussed in Section 2.5.1).

Generally, from the works of Soranus and Galen, it can be inferred that weaning food was often composed of semi-liquid cereals but also included bread softened with milk, honey, or wine, as well as porridge, eggs or vegetables and meat for upper-class families. The weaning diet according to Soranus included bread softened with milk, honey diluted with water, soups made from spelt, moist porridges and eggs (Temkin 1956). Meanwhile, Galen suggested that children should be weaned initially with bread, followed by other foods such as vegetables and meat (Green 1951). It is likely that the average Roman family would have been primarily dependent on cereal-based weaning diet as historical accounts identify grain as the base of Roman diet (Garnsey 1998). According to Schmidt and colleagues (2016), information on foods consumed after the cessation of weaning is scarce in ancient literature. References in ancient literature indicate that older children consumed foods that included the shoots of figs, honey, and meat such as pigs (Fildes 1986). This diet would have continued up until the age of 12 for girls and 14 for boys, at which time they were considered to be adults (Allason-Jones 1989). Garnsey (1999) contends that girls and boys would have had a slightly different diet, with girls having had less access to meat and wine. This is due to the fact that patriarchal societies, as Greaco-Roman societies were, often did not generously allocate food for females. Boys likely got more food provisions based on the fact that they made the bulk of productive labour and because they were higher in the hierarchy of power and control. The 4th century physician, Oribasius, wrote about the desire for girls to marry at the age of 18 years (not the beginning of puberty), which he proposed could be achieved by regulating nourishing food (specifically meat) so that girls did not put on weight too quickly and experienced a delay in their ability to have sexual relations and produce children (Garnsey 1999).

Forty wet-nurse contracts on Egyptian papyri from the Roman period (late 1st century CE to early 4th century CE) have also been included in analyses of weaning patterns (Masciadri & Montevecchi 1984). One such nursing contract from 327–32 CE mentions that exclusive breastfeeding should occur for the first 6 months, followed by a transition to cow’s milk for the next 18 months (Dupras et al. 2001). These contracts were used to regulate wet-nurses according to these prescriptive nursing practices; to that extent, they may very closely represent past practices for at least those who used wet-nurse contracts. Yet, there are problems with using these contracts as historical documents as they do not discuss all the contextual information (Prowse et al. 2008). For instance, the contracts map a timeline for breastfeeding but not for weaning and there is no apparent clause preventing the wet-nurse to substitute her milk with other foods during the contract’s validity. The contracts also seem to typically end 6 months after the cessation of breastfeeding (most often around two years) and provide little to no information on the weaning and post-weaning diet. It is important to note that many of these literary sources are biased towards the Roman elite and their food preferences, so relying heavily on literary sources can misconstrue our understanding of Roman diet as well as breastfeeding and weaning practices. Further, these historical sources are considered more prescriptive rather than descriptive of infant feeding practices in the Roman Empire (Garnsey 1999; Dupras et al. 200l; Schmidt et al. 2016).

2.5.3 Stable Isotope Studies

Isotopic studies of bones and teeth from the Roman period have shed more light on past feeding practices and as there are no published studies on breastfeeding and weaning practices in pre-Roman Italian samples this study will be the first. Largely, the isotope data have been roughly consistent with historical accounts of the weaning process from the Roman period falling anywhere between six months up until four years of age.

Prowse and colleagues (2008) examined the patterns of breastfeeding and weaning in a skeletal sample (1st – 2nd centuries CE) from the Imperial Roman necropolis of Isola Sacra (located southwest of Rome) through δ13C and δ15N analysis of rib samples obtained from individuals ranging in age between birth to 13 years. The results revealed that infants began transitional feeding between one to two years and were fully weaned by the age of ~ 2.5 – 3 years. By four to five years of age, δ13C and δ15N values were similar to adult values, that is to say, that any isotopic trace of breastfeeding in the bone was no longer present in individuals over the age of five (Prowse 2008). Fitzgerald et al. (2006) also examined weaning at Isola Sacra (2nd - 3rd centuries CE) by observing Wilson bands in the enamel mantle of deciduous teeth. Wilson bands are enamel defects that arise due to developmental disturbances impacting the production of normal enamel matrix; these defects can be aged based crown development and from incremental growth markers (FitzGerald & Saunders 2005). Results found that almost 40% of the sample (n=127 subadults) had at least one or more Wilson band in at least one of their teeth. It was also noted that Wilson bands largely occurred between six to nine months of age. Correspondingly, Fitzgerald and colleagues (2006) proposed that the occurrence of Wilson bands may be tied to the initiation of weaning as infant vulnerability to growth faltering arises if the supply of breast milk is diminished and inadequate weaning foods are provided which often occurs around nine months of age when there is growth acceleration. The presence of Wilson bands between six to nine months of age on these teeth supports Soranus and Galen’s recommendations that weaning began around 6 months of age. The offset between the weaning age determined by Prowse and colleagues (2008) and Fitzgerald et al. (2006) may be due to a delayed incorporation of dietary elements into ribs as a result of bone turnover rates as well as time-averaging due to bone remodeling. Meanwhile, the weaning event demonstrated by the occurrence of Wilson bands may be more directly recorded in teeth as a bodily response to stress. Still, Wilson bands are similar to other stress indicators as they are non-specific and it is possible that morbidity events other than those associated with the weaning process may be involved.

Rutgers et al. (2009) found that in the late Roman, early Christian Period (4th – 6th century CE), a community from the St. Callixtus catacombs in Rome began transitional feeding of children between two to three years of age, and were completely weaned within approximately 1 year. They conducted stable isotope analysis on twenty-two samples of human bone (femur and foot phalanx) from the Liberian region. Another stable isotope analytical study conducted by Semchuk (2016) using bone rib and femoral fragments for 50 individuals, looked at overall diet at a rural Roman estate in southern Italy, Vagnari (1st – 4th centuries CE). Isotope ratios of individuals from infancy to early childhood exhibited weaning in progress by three years of age, with its completion by five years of age, at the latest. A precise estimate of when complimentary foods were introduced and individuals were weaned off breast milk could not be discerned as many subadults between 0-6 years of age did not have precise age estimates (Semchuk 2016).

Weaning practices outside of Roman Italy have also been investigated. Dupras and colleagues (2001) conducted stable carbon and nitrogen analysis to investigate infant feeding and weaning practices of individuals from the Romano-Christian cemetery (250 CE) in the Dakhleh Oasis, Egypt. After 30 BCE, Egypt was introduced to Roman ideas and practices surrounding IYCF. However, as the Dakhleh Oasis was at the far reaches of the Roman Empire, prior to the study by Dupras and colleagues (2001) it was not clear to what extent early Egyptians adopted Roman child-rearing practices. Stable isotope analysis of 49 rib and humeral samples revealed that supplementary food was introduced around the age of six months and breastfeeding continued up until 3 years of age.

Isotopic studies of bone samples from a Roman era Queenford Farm in Britain (late 4th – mid 6th centuries CE) revealed that weaning completion was a gradual process occurring between the ages of two to four years of age (Fuller et al. 2006; Nehlich et al. 2011). Isotopic analysis of human bone from the Roman Site of Leptiminus (3rd – early 4th centuries CE), located on the eastern coast of Tunisia, suggested that weaning began before the age of two years and was completed by about 3 years of age (Keenleyside et al. 2009). At two Roman-Iron Age sites on the island of Öland, Sweden (0-200 CE), bone collagen and dentine stable isotope analysis demonstrated that there was great variability in young child feeding practices, although solid food was generally introduced around six months and breastfeeding ceased around three to four years of age in the sample analyzed (Howcroft et al. 2012).

Weaning onset in these populations ranges from six months to three years and cessation of breastfeeding from two to four years of age. Despite the variation in weaning ages demonstrated by these geographically separated populations that have their presumed local cultural differences, the majority of their stable isotope results indicate a similar weaning duration ranging from one to two years. This variation amongst temporally similar population groups indicates that the weaning table was quite flexible during the Roman period and may have often deviated from the prescribed feeding practices by Roman physicians.

**2.6 Summary**

Past research has clearly established that the length of exclusive breastfeeding and the weaning process are essential factors for the survival and health of young children. Subsequently, bioarchaeologists have looked at past populations to comprehend how these aspects influence child mortality and shape childhood experience. Studies have shown that during the Roman and pre-Roman period, weaning may have been quite variable but was found by bioarcheologists to generally begin at six months of age and end at approximately three years of age. These finding align with Roman medical writers’ prescriptions described above, and the current clinical recommendations for optimal infant growth and development (Sellen 2007). However, information about weaning patterns during the pre-Roman period is still scarce and most of what we know is derived from archaeological finds and research based on overall population health and dietary behaviours.

**Chapter 3: Stable Isotope Analysis**

**3.1 Introduction**

Stable isotope analysis of skeletal and dental tissues is arguably one of the most direct methods bioarchaeologists use to analyze weaning and infant feeding patterns. As stable isotope values are preserved in bodily tissues, they provide evidence of long-term diet, migration, and episodes of stress over a period of time. This chapter will provide an overview of the biogeochemical background of stable isotope analysis, the wide range of applications and the new developments that are being made within bioarcheology with respect to stable isotope sampling techniques. Further, this chapter will highlight the benefits of using collagen in incremental tooth dentine samples as opposed to bone for stable isotope analysis.

**3.2 Biogeochemical Background**

Isotopes can be either stable or unstable, the difference being that stable isotopes do not undergo radioactive decay, while unstable isotopes decay from one isotope into another (such as 14C into 14 N). For the purpose of investigating past diet and mobility stable isotopes are utilized as they remain unchanged after death of an organism. Essentially, isotopes are atoms of the same element with the same number of protons but different number of neutrons. This difference in the number of neutrons results in varying atomic masses. For example, 12C and 13C are two stable isotopes of the element carbon; 12C has an atomic mass of 12, while 13C has an atomic mass of 13. This leads to what are termed as ‘heavy’ (13C) and ‘light’ (12C) isotopes, with heavy isotopes having more neutrons (Brady 2004). Although these stable isotopes act similarly in chemical reactions, lighter isotopes are preferentially used in these reactions due to their different atomic masses. A lower atomic mass means that biological systems require lesser amounts of energy to use this particular isotope. Moreover, heavier isotopes react more slowly, which leads to the separation, or fractionation, of isotopes during physical or biochemical processes (Lee-Thorp 2008). Fractionation allows for the pathways of isotopes to be traced through a series of reactions, such as the changes from isotopic ratios at the environmental baseline to their incorporation into the tissues of organisms. As a result, the relative abundances of isotopes in various ecosystems differ. These relative abundances are referred to as isotope ratios and are generally expressed in terms of δ (delta) values reported in parts per thousand (‰, or per mil) in relation to a recognized standard:

*Equation 3.1 - Formula to calculate isotope ratios in terms of δ values.*

Where X is the element (e.g., C) and R is the corresponding isotope ratio (e.g. 13C/12C).

Internationally recognized standards have been set in place when determining isotope ratios so that the comparison of δ values can be made between different laboratories. For stable carbon isotopes, Vienna PeeDee Belemnite (VPDB) is the recognized standard calibrated to the same ratio as the calcareous fossil and for stable nitrogen isotopes, atmospheric N2 is used as the reference sample, referred to as AIR.

**3.3 Applications**

The use of stable isotopes is based on the observation that the isotopic composition of animal tissue is a direct reflection of the isotopic composition of the food and water consumed (DeNiro & Epstein 1978, 1981). As food is consumed, it gets broken down into its constituent elements and absorbed by the body; eventually, these elements become incorporated into tissues such as bones and teeth.

Human bones are made of both organic and inorganic components. The organic component of the bone is ~ 90% collagen and small amounts of non-collagenous organics (White & Folkens 2000). Collagen extracted from bone provides both organic carbon and nitrogen; 30 – 40% of modern bone’s dry weight is composed of this organic matrix, meanwhile archaeological samples contain between 0 – 22% (Hoppe et al. 2003; Schwarcz & Schoeninger 2012). The inorganic component of bone is made up of mineral and hydroxyapatite (or bone carbonate), comprising the remaining 60 – 70% of a bone’s dry weight. Bone carbonate provides bioarchaeologists with inorganic carbon as well as oxygen and strontium. Carbonate is more susceptible to diagenesis in comparison to collagen as sedimentary carbonates from the soil leach into the bone, impacting isotope values and reflecting the isotope composition of soil rather than the isotope values representing human diet (Koch et al. 1997). Still, collagen is also susceptible to diagenesis as noted by researchers (e.g., Budd et al. 2000) and assessment of diagenesis is essential when carrying out stable isotope analysis and will be discussed in Section 5.2.

By analyzing the stable isotope values conserved in human bone, bioarcheologists can infer long term diet. More accurately, stable isotopes can be used to indicate the types of food eaten by an individual over a period of time, depending on the bone turnover rate. The stable isotopes of nitrogen (δ15N) and carbon (δ13C) are most often used in biochemical studies of breastfeeding and weaning, although oxygen (δ18O) isotopes have also been employed.

3.3.1 Nitrogen

Nitrogen has two naturally occurring stable isotopes, 14N and 15N, the ratio (15N /14N) of which provides the basis for dietary studies. Stable nitrogen isotope analysis is regularly used in studies that investigate IYCF practices in past populations (e.g. Wright & Schwarcz 1999; Fuller et al. 2006; Tsutaya & Yoneda 2013). The first application of nitrogen stable isotope analysis in a breastfeeding and weaning study was conducted by Fogel and colleagues (1989). By analyzing isotope values in fingernails from modern breastfeeding infants and mothers, the authors demonstrated increased δ15N values for infants in comparison to their mothers while they breastfed, decreased values with the onset of weaning, and similar child/subadult and mother δ15N values shortly after the cessation of weaning. The same study applied this method to an archaeological sample using estimated age-at-death and identified a pattern of high δ15N values among individuals who they hypothesized were breastfeeding and low δ15N values among individuals who were potentially undergoing the weaning process. This study was later confirmed by Fuller and colleagues (2006), who also demonstrated a relationship between breastfeeding infants and maternal δ15N values in a modern study group.

This increase in δ15N values is a result of a trophic level effect, where the δ15N values of an organism are typically 3‰ higher than that of their diet, with successive enrichment effects for higher trophic-level organisms (DeNiro & Epstein 1981). For instance, herbivores will have δ15N values that are 3‰ higher than the plants they eat, and carnivores with have δ15N values that are 3‰ higher than the herbivores they eat (Schoeninger 1985). Infants are enriched in 15N from consuming breastmilk (as their only source of dietary nitrogen is the mother’s milk if they are exclusively breastfed) and exhibit a trophic level effect (ranging from 2 – 4‰) over the δ15N values of their mothers (Fuller et al. 2006). Once the weaning process begins and breast milk is removed from the diet, the δ15N values decrease to mirror the removal of breast milk (as most typical weaning foods contain less 15N than breast milk). A sharp decrease in δ15N values is observed when there is rapid removal of breast milk and a gradual decrease in δ15N values is observed during a more prolonged weaning process. Consequently, change in δ15N values within human remains in relation to age provides evidence for prehistoric and historic weaning behavior (Katzenberg et al. 1993; Schurr 1997).

Using this information, stable isotope analysis has been used to observe weaning patterns for a variety of periods and geographic locations, such as for Iroquoian Ontario (Katzenberg et al. 1993), Medieval England (Richards et al. 2002), 18th and 19th century Britain (Nitsch et al. 2011) Neolithic Turkey (Richards et al. 2003) and during the Roman period (Dupras et al. 2001; Prowse et al. 2008; Rutgers et al. 2009; Semchuk 2016), which I have previously discussed in Chapter 2. These studies investigate breastfeeding and weaning trends by conducting isotopic analysis on bones from large samples with adequate representations of infant and child remains, as δ15N values of these subadult individuals are representative of the weaning age. Researchers create charts that depict the distribution of δ15N data by age, where higher δ15N values among subadults in the first years of life would be indicative of breastfeeding, and the lower values seen in older children would indicate the gradual cessation of breastfeeding and introduction of complementary foods.

There are a number of underlying assumptions implicit in the use of δ15N data in weaning studies. First, it is assumed that the adult female δ15N mean value of the sample is equal to that of breastfeeding mothers, in other words it is assumed that there is very little flexibility in adult female diet (Reynard & Tuross 2015). Another assumption is that the δ15N values from the skeletal remains of young children represent diet at approximately the time of death and that the infants who died are representative of the diet and physiology of the entire population at that age (Beaumont et al. 2013). Bone turnover rates average isotope values obtained over several months and cause a delay in observable shifts in isotopic values, thus bones cannot represent diet at a specific time. Bone turnover rates and the implications of this process on weaning studies is further discussed in Section 3.4. An additional complicating issue is that δ15N in subadult tissues can be impacted by various factors such as maternal diet, stress during fetal development, stress associated with malnutrition or disease, and the physiological demands of growth (Reynard & Tuross 2015). However an approach used to differentiate dietary changes from these factors is to compare stable δ15N with δ13C values. Dietary change is reflected in both nitrogen and carbon isotope values, thus any observable change in δ15N values that is not mirrored in δ13C, would be indicative of other influencers such as stress or growth.

* + 1. Carbon

The first major application of carbon isotopes in archaeology looked at the spread of maize agriculture in North America and the associated implications on diet (e.g., Vogel & van der Merwe 1977; van der Merwe & Vogel 1978). Terrestrial plants differ isotopically as a result of varying photosynthetic pathways and these different pathways cause a difference in the number of carbon atoms fixed by the plants from atmospheric CO2 (Vogel & van der Merwe, 1977). The three pathways are Calvin (C3), Hatch-Slack (C4) and Crassulacean acid metabolism (CAM). C3 and C4 plants fix three and four carbon atoms, respectively, while CAM plants utilize both photosynthetic pathways and subsequently have δ13C values that overlap between both C3 and C4 plants (O’Leary 1988). CAM plants include pineapple, succulents, and other desert plants. The majority of native plants that grow in temperate zones are typically C3 plants, meanwhile C4 plants thrive in hot and arid environments. Some examples of C3 plants include: wheat, barley, oats, rice, and root starches such as potato, manioc and yam, which have δ13C values from -22 to -38‰ but average at -26‰. C4 plants include: maize, millet, and sorghum, which have δ13C values averaging around -13‰ and range from -9 to -21‰. Since the δ13C values of C3 plants differ from those of C4 plants, carbon isotope ratios of human remains can indicate the broad type of diet that was consumed (Katzenberg et al. 1995). Additionally, stable carbon isotope analysis can be used to indicate the amount of marine versus terrestrial foods that was present in an individual’s diet. Marine plants draw their carbon from dissolved bicarbonate (HCO3-) in the ocean, resulting in 13C-enriched tissues over terrestrial C3 plants, hence humans consuming an exclusive marine diet would have higher δ13C values, between -11 to -12‰ (Chisholm et al. 1982; Katzenberg 2008; Lee-Thorp 2008).

Carbon isotope ratios are not often used on their own to understand breastfeeding and weaning due to the fact that carbon has a smaller trophic level effect (i.e., ~1‰) (Richards et al. 2002; Fuller et al. 2003). Accordingly, δ13C values are analyzed along with δ15N data to detect the introduction of complementary foods during the weaning process. Delta13C data can be also used to infer the use of C4-based weaning foods or possibly early introduction of marine foods in the weanling diet, all of which would result in higher δ13C values.

* + 1. Oxygen

Oxygen isotopes (δ18O) are predominantly used to investigate geographic origins and mobility in past human populations rather than diet or breastfeeding. This is because the oxygen in local precipitation largely determines the δ18O values in human tissues and this differs according to latitude, temperature, humidity, and distance from water. However, δ18O can be used to signal exclusively breastfed infants, due to the fact that they have higher δ18O values than adult females, by approximately 2‰ (Moffat & Prowse 2018). As human breast milk is produced from available sources of water, it becomes enriched in 18O over the water consumed by the mother. In addition to documenting this “weaning signal” in archaeological samples (e.g., Wright & Schwarcz 1998; White et al. 2004; Williams et al. 2005; Britton et al. 2015) it has also been used to infer weaning in both extant and extinct animals (e.g., Franz-Odendaal et al. 2003).

* 1. **Sampling for Stable Isotope Analysis in IYCF Archaeological Studies**

Isotopic analysis can essentially be performed on any tissue created through metabolic processes, including blood, hair, skin and fingernails (Brady 2004). Yet the most commonly used materials for isotopic analysis of past human diet are bones and teeth, as other fragile materials preserve poorly. Bone is made up of an organic (collagen) component that principally reflects dietary protein, and an inorganic (hydroxyapatite) component that reflects total diet (protein, carbohydrates, and lipids). Both the organic and inorganic components can be analyzed for dietary reconstruction as both contain carbon, but only collagen contains nitrogen and only hydroxyapatite contains oxygen (Brady 2004). For the study of breastfeeding and weaning, collagen is useful because it has relatively large amounts of nitrogen.

Bone is a dynamic tissue that undergoes continual remodeling to attain and preserve skeletal size, shape and structural integrity as well as regulate mineral homeostasis (Raggatt & Partridge 2010). This constant remodeling creates a lag between the isotopic values in diet and the values observed in bone. It also means that the isotopic values obtained from bone are averages rather than precise measures from a particular time period. Hence, as a breastfeeding infant synthesizes bone collagen over time, the 15N content of new collagen increases due to the breast milk consumption. If breast milk was the only food consumed and nursing continued indefinitely, the δ15N value of bone collagen would eventually approach a higher maximum value (by 2 to 4‰ due to the trophic level effect) in relation to the mother’s diet. As weaning begins, there is some lag observed between the decline in milk consumption and a decline in δ15N values of collagen, perhaps by as much as 1 year, even though bone is remodeled fairly quickly in growing children (Tuross & Fogel 1994; Katzenberg & Pfeiffer 1995; Schurr 1997). For instance, Hedges and Reynard (2007) observed that collagen turnover rate of the femoral mid-shaft in adolescents (10-30% per year for individuals between 1 and 15 years of age) was higher than that of adults (4% per year for females aged 20 years, 3% per year for females aged 80 years, and 1.5 – 3% per year in males of the same ages). Furthermore, rib samples are often used from infants for isotopic work as they have faster bone turnover rate (Wright & Schwarcz 1999). This is due to the fact that ribs have relatively high proportions of cancellous and thin cortical parts, whereas femora have lower proportion of cancellous and thick cortical parts; in other words, the turnover rates are higher in bones with greater surface to volume ratios than those in bones with smaller ratios (Tsutaya & Yoneda 2013). In young juveniles, δ15N and δ13C values from ribs likely reflect diet from a few months prior to death due to rapid bone remodeling (Fuller et al. 2006; Nitsch et al. 2011). While turnover rates in adult bones are difficult to define in terms of a time frame; it is generally understood that they represent the last 10 – 20 years of an individual’s life depending on the type of bone (Sealy et al. 1995; Price et al. 2000).

Sampling different areas of a bone may also impact the isotope values researchers obtain. Bellis et al. (2006) investigated lead (Pb) content in long bones and found that the results were variable and complex. Generally, the authors noted a high Pb content in the center of the bone than at the outer edges. Lead accumulates in bone over many years or decades and variation in Pb content within bone may be due to differential blood supply (trabecular bone in the center is typically more vascularized compared with cortical bone in the peripheral areas and has greater blood capacity) and subsequently may be something to consider when conducting stable isotope analysis using bone.

Presumed nursing infants often demonstrate a nitrogen trophic shift that is lower than the expected 3‰, which in some cases might be due to infants not being breastfed for a variety of reasons as well as variability in the duration of nursing; however it has been hypothesized that growth may be be a factor contributing to lower trophic shifts in nursing infants (Fuller et al. 2006). In the past, researchers investigated nitrogen isotope fractionation during growth to observe the effect of differential amino acid routing towards protein synthesis on δ15N values obtained from bones (Sick et al. 1997; Ponsard & Averbuch 1999; Gaye- Siessegger et al. 2003, 2004). Essentially, it is hypothesized that amino acids that do not undergo transamination or deamination reactions (often because these amino acids contain 15N, the heavier nitrogen isotope) are directly deposited into new tissue during growth, resulting in an overall increase of tissue δ15N (Fuller et al. 2004, 2005; Trueman et al. 2005). In 2010, Waters-Rist and Katzenberg investigated long bones from subadults (n=11) of various ages (ranging from 7 -19 years) from the Uxbridge Ossuary, located northeast of Toronto, Ontario, to compare δ15N obtained from the different areas of growing long bones. The goal of this study was to investigate the relationship between growth and nitrogen isotope fractionation. Bone samples from the metaphyses, diaphyses and epiphyses demonstrated that there was no statistical difference in the mean δ15N ratios of proximal and distal metaphyses and diaphysis, indicating that a nitrogen isotope growth effect was not reflected in nitrogen isotope values of subadult long bones. Waters-Rist and Katzenberg (2010) suggested that this may be a result of long bones growing in circumference (termed appositional growth) through the addition of successive layers of lamellae, which could minimize the variation in metaphyseal and diaphyseal δ15N values. This study also compared δ15N from fast growing metaphyses with slow growing metaphyses, since in long bones proportionally more growth will occur at one of the metaphysis than the other (Scheuer & Black 2000). The results once again indicated that there was no significant difference in the mean δ15N values between the two areas of long bones. The third comparison made was between growing (unfused) and fused bones; results demonstrated that a nitrogen isotope growth effect was either not manifested, or was not detectable in adolescent bone collagen. Based on these observations, Waters-Rist and Katzenberg (2010) proposed that no isotopic difference should be observable between an adult and a growing individual fed on the same food. Nonetheless, isotopic studies investigating paleodiets, especially breastfeeding and weaning, are shifting away from bone and are utilizing other bodily tissues.

Teeth are increasingly being utilized in isotope investigations as tooth enamel (inorganic component) can also be analyzed for carbon and oxygen isotopes. Tooth enamel is made up of 96% hydroxyapatite, with the remaining 4% composed of non-collagenous proteins (Gutiérrez-Salazar & Reyes-Gasga 2003). Enamel of permanent teeth forms in early childhood and undergoes no remodeling during later life, thereby allowing for the study of breastfeeding and weaning practices of individuals who survived into adulthood. Tooth dentine (the organic component) is more like bone, as it is composed of hydroxyapatite and collagen, 70% and 30% by weight, respectively. Yet unlike bone, tooth dentine structure undergoes very little alteration through life and can be used to recover dietary signals from earlier periods of childhood. While bones are constantly remodeling throughout an individual’s life, teeth develop during infancy and childhood and do not remodel. Tooth dentine begins to develop *in utero* and forms through a two-stage process (discussed below). It also makes up the bulk of a tooth, including its roots, and the presence of a collagenous component allows researchers to analyze dentine for carbon and nitrogen isotope ratios. It is generally expected that teeth are less susceptible to diagenesis than bones, due to the absence of a vascular network in tooth dentine and the enamel protecting the crown. Although some studies (e.g., Götherström et al. 2002; Sampietro et al. 2006) support this view, teeth by no means are resistant to the diagenetic changes that impact bone tissues. As tooth enamel is compact and dentine is relatively porous, dentine is more susceptible to diagenetic alteration than enamel. In addition, the high organic content of dentine is also a contributing factor, since it is more easily destroyed during diagenesis and can cause an increase in porosity and fragilities in the structure (Dauphin & Williams 2004). Consequently, assessing diagenesis is essential when carrying out stable isotope analysis regardless of the tissue used. This is further discussed in Section 5.2.

* + 1. Structure of Dentine

Dentine is secreted and mineralized in two phases: odontoblasts secrete an initial dentine matrix (known as predentine), and the deposition of short (20-100 nm) crystals of carbonate hydroxyapatite mineralize the collagen fiber matrix. The rate of initial dentine secretion is relatively consistent; for permanent teeth it is 4-6 mm per day throughout the cuspal areas (Dean & Scandrett 1995). The retreating odontoblasts move away from the enamel/dentine junction (EDJ) creating a layer of newly secreted predentine (Hillson 1996); while the mineralizing front follows the same path 10-20 mm behind. The complete process takes approximately 3-8 days and dentine does not remodel once mineralized. Equally, root dentine is formed from the cement/dentine junction (CDJ) with a rate of 1.3 – 1.5 mm per day (Beaumont et al. 2013). The rate has been noted to change from 1.3 mm per day to match the same rate as the cuspal region within the bulk of the root, then reducing back to 1.3 mm per day as the odontoblasts approach the pulp chamber (Dean & Scandrett 1995). Further, mineralized dentine tubules form a S-shape (Dean & Scandrett 1995).

Incremental dentine sampling follows these known patterns of dentine development and the timing of development for human teeth according to standards outlined by Hillson (1996) and AlQahtani (2012). Since the direction and rate of growth for dentine are well established (Dean & Scadrett 1995), age ranges can be attributed to each sub-sample of dentine from one tooth.

* + 1. Dentine Microsampling

Koch and colleagues (1989) were the first to sample transverse sections of tooth dentine to track changes in isotope ratios during tooth formation. Specifically, Koch et al. (1989) investigated patterns of seasonal mortality using oxygen isotope variation preserved in hydroxyapatite of proboscidean tusks and molars. Other studies have since been performed on faunal teeth to detect isotopic shifts corresponding to dietary changes (e.g., Balasse et al. 2001; Kirsanow et al. 2008; Zazzo et al. 2010; Evacitas et al. 2016). Dentine microsampling has also been used on archaeological populations to observe higher temporal resolution for past infant, childhood and adolescent dietary patterns. For instance, in 2014, Sandberg and colleagues presented intra-tooth stable carbon and nitrogen isotope profiles of dentine collagen from a sample of adults from the Medieval Nubian site of Kulubnarti; they used these profiles to interpret weaning behaviors and dietary history. Similar studies have been conducted to investigate the timing of weaning and diets in post-Medieval London (Henderson et al. 2014), pre-historic California (Eerkens et al. 2011), the Atacama Desert (King et al. 2017) and the Late Antique/Migration period (early 5th century AD) of France (Czermak et al. 2018).

Two main methods are used to obtain serial dentine samples; the first derived from the technique outlined by Fuller et al. (2003), and the second from Burt and Garvie-Lok’s (2013) study. The first and most commonly used microsampling method for human dentine was employed by Fuller and colleagues (2003) and recently revised by Beaumont et al. (2013). This technique is based on slicing incremental thin sections of demineralized human dentine and assigning each sample with an estimated age range based on the direction of dentine growth from the crown towards the root tip, allowing researchers to assign dietary changes with an approximate age. Using the Medieval English site of Wharram Percy (900 – 1500 AD), Fuller and colleagues (2003) applied this microsampling technique to deciduous second molars, permanent canines, and third molars (*n* = 37), to detect evidence of weaning in individual teeth and obtain general intra-individual dietary variation. Researchers who follow Fuller and colleagues’ (2003) method can choose to sample by embedding the root in plaster and slicing it in roughly one-millimeter sections prior to demineralization, or they can sample by partially demineralizing the complete tooth and sectioning the resulting collagenous model using a scalpel (Beaumont et al. 2013). Collagen is then extracted from these thin sections using standard methods of collagen extraction; pure collagen is then run on a mass spectrometer to measure the carbon and nitrogen isotopic ratios within the samples.

Early attempts of human dentine microsampling were able to produce 3 – 4 subsamples which represented an average value of 3 or more years (Fuller et al. 2003). However, Eerkens and colleagues (2011) used these techniques to obtain between five and ten samples from first permanent maxillary molars simply by cutting smaller transverse sections. More recently, this technique has been further refined and has shown that it is possible to get up to 20 samples per tooth, depending on the size and preservation of teeth (Beaumont et al. 2013, 2014; Beaumont & Montgomery 2015). Each advancement of this method is associated with an increase in serial samples taken from a single tooth, which results in a higher resolution and consequently much greater temporal control over diet. The limitation is that increasingly smaller samples are more difficult to analyze on a mass spectrometer as it requires a specific amount of collagen (0.8 – 1 mg) for accurate analysis.

The other less commonly used methodology for microsampling human dentine was first introduced by Burt and Garvie-Lok (2013). Instead of sectioning the whole tooth for analysis, this method uses a cardpunch to sample small cylinders of dentine at predetermined locations. One half of the tooth (with enamel removed) is histologically examined to identify the location of the neonatal line, while the other half of the tooth is demineralized and sampled using the cardpunch at three locations: before the neonatal line, near the neonatal line and at the apex of the root (Burt & Garvie-Lok 2013). The first archaeological application of this method was carried out by Burt in 2015 on a late Medieval sample from the UK. This study demonstrated that children likely began the weaning process around two years of age, but this was not homogenous throughout the sample (Burt 2015). Outside of the analysis of weaning, the data also showed that the diet of weaned children was similar to the diet of older children and adults in the population, contrary to what might have been expected (Burt 2015). Similarly, using a sample of modern deciduous teeth, Burt and Amin (2014) examined variation in infant feeding practices in a modern Canadian sample. The authors were able to demonstrate that this method is capable of picking up breastfeeding signals and can differentiate breastfed from bottle-fed individuals.

The most ideal sampling method would be to sample along the Andresen bands – bands that mark diurnal changes, but it is technically difficult because these are only visible with a microscope and a longitudinal ground section (Beaumont 2013). Additionally, the resulting samples would be too small for current stable isotope analysis tools. The best alternative method has been to follow Fuller and colleague’s (2003) technique of sampling transverse regular or irregular sections from the crown towards the root of the tooth. Although it is recognized that this results in averaging of isotope values since it would include dentine formed at different times, researchers argue that by using the same method for each tooth from each individual in a study, comparison of the dietary and physiological isotopic values at the same developmental stages in each individual can be made (Beaumont 2013).

The issue remains that transverse sectioning does not adequately consider the biological growth patterns of teeth. The directionality of incremental growth layers (dentine tubules) form at oblique lines from the enamel-dentine junction to the pulp chamber; transverse sectioning cuts through the dentine growth layers blending the resulting isotopic sequences and complicating the assignment of chronological age for each sample (Makarewicz & Sealy 2015; Guiry, Hepburn, & Richards 2016). Another issue that has been identified is that tooth formation stages are not equally spaced in time, especially in the crown section (Liversidge 2015), resulting in potential loss of resolution and uncertainty in timing. Addressing these problems has thus far been difficult as these dentine increments are not visible in demineralized teeth. Yet in a recent study by Czermak et al. (2018), microscope images of longitudinal tooth sections were used to take into account the directionality of incremental dentine growth structures during dentine microsampling. Using first permanent molars of 17 adults buried at the Late Antique/Migration Period cemetery of Niedernai, Alsace (France), Czermak and colleagues (2018) sectioned two portions of each tooth. The first set of sections were obtained from the mesial root of the tooth, which were cut into microchunks measuring to ≤1 mm in width, following the natural growth line as closely as possible (using high‐resolution transmission light microscopy images as a reference). The second set of sections were obtained from the central area of the tooth crown by slicing the crown into thin parallel sections of ~0.5 mm thickness from the top towards the pulp chamber. This method provided the authors with 16–20 microsamples from each tooth and subsequently increased temporal resolution, which was useful is detecting a short‐term increase in the otherwise gradually decreasing δ15N trajectories in the crown sections of individuals, indicating a diet temporarily richer in protein or perhaps a short‐term return to breastfeeding.

Something else to consider when sampling dentine are the tissues that are laid down after primary dentine is formed (Beaumont 2013). Secondary dentine is laid down throughout life in permanent teeth after the tooth is fully developed by odontoblasts around the pulp chamber. Similarly, tertiary dentine is produced at sites where there is damage on dentine surface such as wear or caries (Nanci 2003). The impact of these minor tissues on overall dentine isotope profiles are expected to be minor due to the quantity of the secondary dentine in comparison to primary dentine (Beaumont 2013). Research on isotopic variation caused by these tissues is yet to be conducted, but this issue might even be completely avoided by appropriate sample selection and preparation, such as selecting caries-free and unworn teeth or by reaming out the pulp cavity before dentine sampling to remove possible secondary dentine formation (Beaumont 2013).

* + 1. Bone Collagen versus Tooth Dentine

Bone sampling for stable isotope analysis provides a cross-sectional approach to the reconstruction of weaning age in a burial population. With an appropriate sample size, bone sampling allows bioarcheologists to view the entire modal weaning process, from the initial introduction of complementary foods to the complete cessation of breastfeeding. Additionally, bone sample comparisons can be made between populations from different locations and time periods, providing information about variation in breastfeeding practices in relation to environment, culture, history and other factors (Eerkens et al. 2011). However, there are also several disadvantages to using bone collagen for stable isotope analysis. Weaning age is averaged across many individuals in a burial population making it difficult to examine intra-population variation. Grouping large samples to analyze weaning may mask more fine-scaled changes in weaning behavior that may have been a response to short-term social and environmental variations. Hence, dentine is arguably a better tissue to sample for weaning and diet studies as it provides greater temporal resolution and can be used to investigate intra-population variation.

As previously stated in Section 3.4, due to enamel protection and the absence of a vascular network, it is generally expected that dentine is less susceptible to post-mortem changes, suggesting that unlike bone, the isotope values obtained from dentine are less likely to be influenced by post-mortem factors (Pinhasi & Mays 2008). Wood and colleagues (1992) have also stressed that since individuals who experience a rapid death (such as those who die from acute diseases) do not survive long enough to produce skeletal markers indicative of a causative agent, any changes in stable isotope ratios that could result from food deprivation and lead shortly to death would also not be measurable in bone due to long turnover rates and averaging. Dentine microsampling, specifically for young children, can reveal these perimortem changes in stable isotope ratios as it would be recorded in the still-forming dentition and remain unchanged. This also offers the opportunity to compare the dentine profiles of individuals who died during childhood with the profiles of similar-aged children who survived into adulthood.

Recent studies by Beaumont and colleagues (2013, 2014, 2015a, 2016) also support the use of dentine over bone collagen. Beaumont and coworkers’ first dentine microsampling study in 2013 examined four second permanent molars and five first permanent molars from 19th century Lukin Street cemetery in London. This pilot study investigated two approaches for microsampling human tooth dentine (previously discussed under Dentin Microsampling) to obtain an accurately measured sample with minimal loss of tissue and maximum collagen yield. Both methods were able to produce sufficient amount of good-quality collagen and high-resolution intra-dentine profiles over a range of 1 to 13 years of childhood from a single tooth. The subsequent study by Beaumont and colleagues (2014) investigated the reliability of dentine microsampling even in cases of poor dentine preservation. The authors compared dentine profiles obtained from three well-preserved permanent second molars (macroscopically resembling modern unburied teeth) from the Kilkenny Union Workhouse Famine cemetery in Ireland, and three poorly preserved permanent molars teeth (found in burials that were made on chalk, in ditches and in free-draining sands) from the Early Anglo-Saxon Period (5-7th centuries CE) cemetery of West Heslerton, Yorkshire. The results demonstrated that it was possible to achieve reliable dentine data regardless of preservation. Similar to Wood et al. (1992), more recently Beaumont and Montgomery (2016) also emphasized that osteological evidence of malnourishment (e.g. rickets and scurvy) and indicators of systemic stress (e.g. enamel hypoplasia and Harris lines) are only observable in individuals who survive long enough to develop them, leaving out those who succumb to a rapid death; thus, individuals who may have experienced famine and acute diseases associated with famine such as typhus and dysentery will appear healthy. By examining the dentine δ13C and δ15N profiles and rib collagen of workhouse inmates from the Great Irish Famine the authors were able to demonstrate the expected dietary change from C3 potatoes to C4 maize as well as prolonged nutritional and other physiological stress (Beaumont & Montgomery 2016).

In their 2015a study, Beaumont and colleagues also addressed the issue with bone collagen turnover rates. They argued that even for very young juveniles bone takes time to turnover and consequently does not represent the diet and physiology at the time of death. Beaumont et al. (2015a) presented incremental dentine δ15N and δ13C profiles from the permanent and deciduous teeth from 4 archaeological sites in Ireland, England, and Scotland during the Irish Famine. Since individuals from these sites died during tooth formation, the authors were able to compare the average δ15N and δ13C values of bone collagen with the averages obtained from dentine collagen. They found that isotope values in the latter were consistently higher. This may be due to differential growth patterns of bone and teeth as human teeth will continue to grow at the same rate regardless of nutritional status (Elamin & Liversidge 2013), while bone formation may be arrested if nutritional status is poor. If growth is arrested, then bone collagen from that period will no longer be representative of true isotope values, while dentine collagen will continue to form and record more accurate δ15N and δ13C values. Beaumont et al. (2015a) also argued that by analyzing deciduous teeth when available, information on perinatal nutritional status of children can be obtained and combined with information on teeth with different developmental times from the same individuals – allowing for the analysis of up to 20 years of diet and nutrition.

Another issue with bone collagen isotope values is that infants with lower than expected δ15N values are thought to have had little or no access to breastmilk (Jay et al. 2008; Nitsch et al. 2011). Conversely, the δ15N values from bone collagen of a rapidly growing fetus or neonate are related to nitrogen isotope values of the mother which may vary during pregnancy due to changes in diet and physiology (Fuller et al. 2004; Fuller et al. 2006a). These pregnancy δ15N values may not even be the same as the long-term, lifetime averaged bone collagen values obtained from actual or putative mothers or even the wider cemetery sample. By increasing the time resolution of the analyzed skeletal material and using incremental dentine forming in the perinatal and childhood periods of life, the differential roles of diet, stress and maternal health can be observed by noting changes in δ15N values (Beaumont et al. 2015a). Burt and Garvie-Lok (2013) proposed the use of dentine microsampling of deciduous teeth to investigate dentine from postnatal life stages (after the neonatal line) and dentine from the period prior to birth (above the neonatal line). The results from their own study demonstrated a high variability in pre-neonatal values, possibly due to multiple factors such as variation in maternal diet, varying maternal-fetal offset, and variation in maternal δ15N in response to nutritional factors (weight gain and morning sickness) (Fuller et al. 2004, 2005, 2006). Meanwhile, post-natal values were consistent with expected weaning patterns, indicating that nitrogen isotope values of mothers substantially impact the observed δ15N values seen in bodily tissues of infants. Consequently, using dentine serial samples as opposed to bone collagen can allow bioarchaeologists to separate δ15N values influenced by maternal isotope values from true weaning δ15N data.

**3.5 Summary**

Stable nitrogen and carbon isotope analysis are regularly used in studies to investigate breastfeeding and weaning practices in past populations. Bone collagen has often been used to investigate breastfeeding and weaning patterns; however, recent literature has suggested that tooth dentine has more benefits in stable isotope analysis as it is a time-sensitive tissue. More specifically, reconstructions of early diet and life history using an intra-tooth approach can circumvent potential problems associated with mortality bias (which may operate strongly during infancy and childhood), bone turnover rates and rapid death as a result of malnourishment or acute disease. Through sequential dentine sampling, researchers are able to obtain temporal resolution when observing isotopic changes associated with dietary changes, infant-feeding practices and physiological stress in past populations.

**Chapter 4: Materials and Methods**

**4.1 Study Sites**

The territory surrounding Gravina in Puglia in southern Italy has been inhabited since the Paleolithic with some of the oldest settlements found in the districts of Vagnari, Altamura and San Felice, seen in Figure 4.1 (Peruzzi 2016). Various Iron Age (1st millennium BCE) necropoleis have also been identified just outside the modern city of Gravina in Puglia. The number of Iron Age tombs recorded for the entire region is staggering, with estimates ranging from 1200 to 1400 tombs (Peruzzi 2016). However, due to the continuity of occupation and looting in the region (both in antiquity and modern times) a high percentage of tombs have been destroyed. The skeletal samples examined for this study were obtained from three Iron Age sites containing burials: Botromagno (identified in the historical records as *Silvium*), Parco San Stefano, and Padreterno, all located just immediately West of Gravina and separated from the modern town by a ravine (Figure 4.1).



**Figure 4.1:** Map of settlements located in the territory of Gravina in Puglia, south Italy. Modified map from https://www.ed.ac.uk/history-classics-archaeology/classics/research/research-projects/vagnari, accessed May 11, 2019.

* + 1. Historical Background: Iron Age of Southern Italy (~1st millennium BCE)

The beginning of the Iron Age in southern Italy saw a strong continuity of occupation from the Bronze Age (2300 BCE – 950 BCE), indicating that for the first two centuries (10th – 8th centuries BCE) human occupation took place in the same environmental niches as the preceding Bronze Age phases (Yntema 2013). During this period, settlements were highly dispersed along coasts and on gently sloping hills rising over river valleys (Yntema 2013). However, the 8th century BCE saw major changes in settlement patterns in various parts of southeast Italy. Greek settlers began inhabiting areas in southern Italy, Asia Minor (present day Turkey), and the Black Sea region due to overcrowding, famine and political destabilization in mainland Greece (Dunbabin 1979; Cornell 1995).



**Figure 4.2:** Map of Iron Age Communities in Southern Italy. Edited from https://www.calascio.com/culture-and-history/abruzzo-history, accessed May 11, 2019.

By 770 BCE, Greeks were in direct contact with Italy’s Indigenous Iron Age communities, both through trade and extensive Greek colonization of southern Italy. Historical evidence indicates that the Greeks contacted, quarreled, and traded with Italic tribes of southwestern Calabria and Potenza (Samnites, Bruttians, and Lucanians), and the Daunians, Peucetians, and Messapians (collectively known as the Iapygians) of Apulia (in Figure 4.2) (La Torre 2011). Iapygian communities, like those found around Gravina in Puglia, built settlements of small dwellings interlinked with tomb sites and fortified defense positions along the coast and mountainous interior, a common tactic among Iron Age communities at the time (Flecher 2007). Many grave goods recovered from Iapygian tombs support the inference of cultural interaction and trade with the Greeks. Handberg and Jacobsen (2011) note several occurrences from the 8th to mid-7th centuries BCE of slow assimilation of Greek traditions at native Iron Age sites. For example, local matte-painted vessels represent the vast majority of finds at the hilltop Iron Age site of Incoronata (near the southern coast of Italy) and were also discovered at the coastal site of Metaponto (also referred to as Ionian Greek colonies) (Colivicchi 2011). Mixed Graeco-Iapygian ceramic assemblages at both these sites suggest that Metaponto may have been an indigenous settlement prior to the arrival of the Greeks. Mixed ceramic traditions also suggest that local communities profoundly influenced Greek manufacturing traditions, while the Greeks viewed locally procured materials as exotic items fit for both utilitarian and economic use (Dietler 2007). People in the Iron Age settlement of Parco San Stefano also traded with Ionian Greek colonies. This is supported by Ionian style ceramic cups found in association with burials at the site (Small 1992).

Towards the end of the Iron Age (4th century BCE), the region around Gravina in Puglia transitioned into large fortified settlements on hilltops, with smaller communities concentrated around these larger centers. These changes were a result of the geopolitical landscape during the late 4th and early 3rd centuries BCE, which involved several clashes between the expanding Roman Republic and the Samnites of central Italy (Salmon 1955). After the defeat of *Samnium* (328 BCE) and the fall of *Tarantum* in southern Italy (272 BCE), conflict with the Romans over the control of *Magna Graecia* (the general name given to the Greek colonies in the South by the Romans) was imminent (Jeskins 1998). Eventually, pact agreements (*foedera*) between Rome and the Greek city-states (few native Italian communities were involved) gave formal autonomy to the colonies with Roman military garrisons stationed throughout *Magna Graecia* (Gwynn 2013). This Roman subjugation had profound implications for the local Iron Age Lucanian and Iapygian populations. By the early 3rd century BCE there was significant retraction of settlements across Italy, including around Botromagno, as indicated by a decline in the number and density of sites in this region (Small 2002). This period coincides with the defeat of Iapygian tribes by Rome and the reported enslavement of people from ancient *Silvium* (Botromagno) (Small 2002). Alternatively, it may be possible that the rise of other Latin colonies, such as *Paestum* and *Venusia*, attracted many of these inhabitants to relocate closer to economically prosperous cities (La Torre 2011). It also may be possible that the Iron Age settlements struggled after the Roman conquest due to the fact that that they were unable to profit from the new opportunities and were heavily penalized by the Romans for their earlier resistance, especially by the confiscation of public land (Small 1992).

* + 1. Archaeological Sites

The hill of Botromagno is located immediately to the West of Gravina in Puglia, 51 km South-west of Bari and 76 km North-west of Taranto (Figure 4.1). It reaches a high point of 446 m, where the acropolis of the Iron Age settlement formed. The Iron Age site at Botromagno was established around 950 BCE and was among a number of Iron Age settlements constructed in South Italy at this time including, Monte Irsi, Matera, and Altamura, all within a radius of 22 km from Botromagno (Figure 4.1). Occupation was not confined to the plateau, and spread out below the hill between the escarpment and ravine that separates the archaeological sites from the modern city of Gravina in Puglia (Small 1992).

The Iron Age inhabitants of Botromagno were most likely a Peucetian (an Iapygian sub-tribe) community, who were linked with other Peucetian communities for a common religious or military purpose (Peruzzi 2016). The settlement was composed of small, densely concentrated huts. Walls of the houses were likely constructed out of wattle (i.e., weaving thin branches into a woven lattice), and reinforced with clay or mud (daub). Additionally, the floors were underlain with stone rubble and gravel, and then overlaid with clay (Fletcher 2007). Recovered pots and ceramic fragments were impressed and designed with simple geometric patterns. These geometric motifs later evolved into more elaborate forms through contact with Greek artisans who migrated and established permanent colonies along the Ionian coastline by 750 BCE (Fletcher 2007). Further, these hand painted wares dating to the late 8th and first half of the 7th centuries BCE show influences from the Greek late geometric and Protocorinthian decorative motifs (Small 1992). Despite the evidence for sedentary occupation at Botromagno, no burials dating to these earlier phases of occupation were recovered (Peruzzi 2016).

By the 8th century BCE significant cultural transitions occurred including the construction of small cemeteries within the limits of the Iron Age village on Botromagno. Burials took the shape of *groticella* (stone cut tombs), *tegula* burials (large pieces of roof tiles over the burial pits) and simple pit graves, a practice that remained unchanged throughout the greater Apulian region until 350 BCE (Small 1992). Similarly, the burial customs remained consistent overtime with the dead placed in a flexed position and buried with an increasing number of extravagant grave goods (Small 1992). These burials most often contained grave goods such as mixing bowls for wine, smaller cups for drinking as well as bronze and iron fibulae; signifying that the settlement as a whole was flourishing economically (Small 1992). Archaeological site reports by Whitehouse et al. (2000) also suggest that the construction of stone cut sarcophagi may be influenced by Greek styles from Ionian Greeks as Ionian styled cups were also buried with the deceased.

During 4th century BCE, the Iron Age settlement saw a period of great prosperity as the entire plateau of Botromagno and the area West of the ravine seems to have been occupied and large quantities of 4th century pottery were recovered in all the excavated sites (Whitehouse et al. 2000). By the mid - 4th century, fully developed chamber tombs were being made for more lavish burials. They began to include steeply descending entrance corridors or *dromoi* cut into the bedrock, leading to the actual burial chamber. These were intended to be family tombs and to be reused for subsequent burials. Such tombs emphasized the wealth and importance of families as ordinary burials were simultaneously made in the form of simple pits (Small 1992). In addition, a massive stone wall was constructed around the settlement sometime around the late 4th century. Excavations suggest that the wall surrounded the entire perimeter of the site for a length of 3.75 km and stood about 2.7 m high (Whitehouse et al. 2000). The labour necessary to complete the fortification was likely immense as the required stone material equaled to a total of 37,500 blocks (Whitehouse et al. 2000). Walls similar to the one at Botromagno were built around Iron Age communities throughout Apulia and Lucania during this period suggesting a coordinated effort by the local Italic communities to defend against military conflict with the Greeks, Romans, and other Iron Age communities situated in the North (La Torre 2011).

Early archaeological excavations at Botromagno conducted by Joan du Plat Taylor, on behalf of the British School at Rome, found 32 tombs in the 1967 – 68 field seasons (Ward-Perkins et al. 1969; Taylor et al. 1976; Small 1992). Forty-eight tombs were uncovered by the late 1990s from three separate cemeteries located within and on the periphery of the hillside, including the two necropoleis at the base of the hill, known locally as Padreterno and Parco San Stefano (Ward-Perkins et al. 1969; Taylor et al. 1976). The excavations at Botromagno over the decades revealed a substantial number of rock cut chamber tombs, associated artifacts, and a modest settlement composed of small house-dwellings and courtyards. Tombs were seriated by archaeologists relative to their dates of construction, ranging from fossa grave-pit extensions built in the 6th century BCE, to rock cut chamber tombs dating to the 3rd and 2nd centuries BCE (Ward-Perkins et al. 1969). Many of the tombs and sarcophagi contained ceramic vessels and plates, some of Greek origin, that were used to establish the chronology of the cemetery (Small 1992).

Excavations at Padreterno began in the late 1980s and continued until 1999 by the Cooperativa Petra Magna. Over one thousand tombs were found and documented; although there is very little published about this necropolis, we know that approximately 20% of the tombs were very well preserved, and that the excavators were able to recover both the skeletal remains and grave good assemblages (Peruzzi 2016). Similar to the tombs found at Botromagno, Padreterno also had rock-cut chamber tombs. Grave goods and other finds suggest that the cemetery was in use from the 6th to the 4th centuries BCE, prior to a newer cemetery that was built further down South of the site (Ciancio 2008).

Excavations at the cemetery of Parco San Stefano in 1970 revealed partial house plans from the Early Iron Age period (the beginning of the 1st millennium BCE) (Whitehouse et al. 2000). These houses were characterized by walls made of wattle and daub, along with roofs constructed of thatch. The site was later abandoned in the late 7th and 6th centuries BCE, but was used as a cemetery primarily for rock-cut sarcophagus burials. Thereafter, no occupation on the site occurred until the ground was levelled, and Classical period houses were built in between late 4th centuries BCE – early 3th centuries CE (du Plat Taylor et al. 1976). Their clay floors and the associated walking level sealed the areas except for some pithos burials (burials in large storage containers).

**4.2 Sample Collection**

The skeletal material from the sites of Botromagno, Parco San Stefano, and Padreterno were collected by archaeologists during the excavations in the 1960s to 1990s, but the remains were never systematically analyzed. All the skeletal material was housed at the Fondazione Pomarici Santomasi in Gravina in Puglia. In 2012, Dr. Tracy Prowse obtained permission from the Archaeological Superintendency of Puglia to transport the human skeletal material to McMaster University for study, where they are currently curated. Twelve subadult remains from a total of 85 individual (adults and subadults combined) from Botromagno, Parco San Stefano and Padreterno were selected for this study, as only these individuals had teeth which covered early periods of diet, and skeletal elements (specifically those that had both femoral and rib fragments) for stable isotope analysis (Table 4.1). Young children from ages 1 to 10 years were chosen as they experience a major growth spurt, require more nutritious foods, and under unfavourable circumstances are more susceptible to experience compromised growth and health. Accordingly, by restricting the ages of my sample to 1-10 years my research focuses exclusively on feeding patterns during the early phases of infant, and childhood development. Assessment of age was conducted according to the standard methods in human osteology and bioarchaeology from Buikstra and Ubelaker (1994). Age estimations for the 12 subadults were based on the development and eruption of dentition as outlined by Moorrees et al. (1963) and Ubelaker (1989). Sex could not be estimated for subadults as they were too young to have definite cranial or innominate features indicating whether they were male or female. Additionally, most sex estimation methods are sample-specific (e.g., Loth & Henneberg 2001; Sutter 2003), so when these methods are applied to individuals originating from a population that differs, accuracy tends to be lower (Cardoso 2008).

**Table 4.1:** Subadult tooth samples from Botromagno and Parco San Stefano.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample ID** | **Burial Site** | **Age-at-death\*** | **Tooth sample** | **Bone Samples\*\*** |
| G67/I p3 e2 S4 = Tomb 4 | Botromagno | 4 yrs +/- 12 months | lm1 | Und. rib shaft frag.  Und. femoral frag. |
| 22/5/67 Tomba 30 | P. S. Stefano | 8 yrs +/- 24 months | M1 | Und. rib shaft frag.  Und. femoral frag. |
| G67/II P4 S17 Infill 3 | Botromagno | 7-8 yrs +/- 24 months | LM1 | Und. rib shaft frag.  Mid- Shaft Femoral frag. |
| 21/12/71 Tomba di bambino Ind. 1 | Botromagno | 2 yrs +/- 8 months | rm1 | Und. rib shaft frag.  Femoral frag. from the metaphysis region |
| GS70 F25 | Botromagno | 4 yrs +/- 12 months | rm1 | Und. rib shaft frag.  Femoral frag. from the metaphysis region |
| 1968 DB 1 Contents of Sarcophagus | Botromagno | 4-5 yrs | lm1 | Und. rib shaft frag.  Femoral frag. from the metaphysis region |
| G67/I S5 small tomb | Botromagno | 3 yrs +/- 12 months | rm1 | Und. rib shaft frag.  Mid-Shaft Femoral frag. |
| 1967 G67/I SG-Tomb 6(2) Infill 1 | Botromagno | 3 yrs +/- 8 months | lm1 | Und. rib shaft frag.  Und. femoral frag. |
| No Label #2 | P. S. Stefano | 5-6 yrs | lm2 | Not available |
| 1972 Area NA Burial 1 of A-1 | Botromagno | 4 yrs +/- 12 months | lm2 | First rib shaft frag. |
| G67/I P15 S19 | Botromagno | 7-8 yrs +/- 24 months | m1 | Und. rib shaft frag.  Und. femoral frag. |
| G67 Site C RZ3 Pit 3 tomb infill | Botromagno | 9 yrs +/- 24 months | LM1 | Und. rib shaft frag.  Femoral frag. from the metaphysis region |

\*Age-at-death was estimated using dental formation and eruptions charts by Moorrees et al. (1963) and Ubelaker (1989).

\*\* Location of bone sample was variable for each individual due to poor skeletal preservation and fragmentation.

The subadult dental and bone samples used in this study are shown in Table 4.1. Rib and femoral fragments (predominantly the metaphysis area), as well as first and second deciduous molars or first permanent molars were prepared for stable isotope analysis. This means that 10/12 subadult individuals had three sets of isotope data collected: tooth, rib, and femur. Rib collagen represents the isotope values from closer to the time of death, while long bone samples taken from young infants likely contains a mix of collagen from bone deposited in utero and bone deposited after birth, although the metaphyseal ends turn over more rapidly than diaphyseal cortical bone. This is because ribs have a faster bone formation and turnover rate, while long bones have a slower formation and turnover rate, although the rate of bone formation in general is faster in growing infants and children (Sealy et al. 1995). Furthermore, femoral samples from older children may include collagen produced during the period of exclusive breastfeeding and following the introduction of non-breast milk foods, thus, evaluating both rib and femoral fragments gives the opportunity to note variation in nitrogen values at various breastfeeding and weaning stages.

The first deciduous molar begins forming 15 weeks after fertilization and is complete around 2.5 years of age; meanwhile the second deciduous molar begins forming 6 months after fertilization and is complete around 3 years of age (Hillson 1986). The timing of deciduous teeth formation allows researchers to analyze dietary signals from early life stages including fetal life (the maternal diet), breastfeeding, and (depending on weaning age) weaning (Burt & Garvie-Lok 2013). Further, primary dentine is mineralized within 3-4 days of secretion and does not remodel, thus it represents the diet at the time the tooth was growing in contrast to bone collagen, which represents a long-term average of individual’s diet.

Table 4.2 below presents the subadult burial information and associated grave goods. The report on the burial excavations at Parco San Stefano by du Plat Taylor (1976; 1977) does not include a description of ‘Tomba 30’, but all of the burials excavated in Parco San Stefano date between the 7th and 6th centuries BCE. Excavations at Botromagno uncovered a number of infant and child burials distributed throughout the habitation areas reported by Small (1992) and Whitehouse et al. (2000), however not all burials are reported in the publications (i.e., 21/12/71 Tomba di bambino Ind. 1, GS70 F25, 1968 DB 1 Contents of Sarcophagus, 1972 Area NA Burial 1 of A-1 and G67 Site C RZ3 Pit 3 tomb infill). Further, individual identified as ‘No Label #2’ did not have a burial site ID, but was included in boxes with skeletal material from the other Iron Age sites, so it is assumed that it is from this time period.

**Table 4.2:** Subadult burial information.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample ID** | **Burial Site** | **Date cent. BCE** | **Tomb Type** | **Associated Grave goods** |
| G67/I p3 e2 S4 = Tomb 4 | Botromagno | Late Iron Age  (Later 4th) | Rock-cut grotticella | - Large globular stamnos  - Banded ring-handled kantharos |
| 22/5/67 Tomba 30 | P. S. Stefano | Mid-Iron Age  (7th – 6th) | Unknown | Unknown |
| G67/II P4 S17 Infill 3 | Botromagno | Late Iron Age (4th) | Rock-cut pit grave | - Black glazed dish  - 2 Black glazed small jugs  - Black glazed salt cellar  - 4 terracotta figurines of seated women |
| 21/12/71 Tomba di bambino Ind. 1 | Botromagno | Iron Age  (6th – 3rd) | Unknown | Unknown |
| GS70 F25 | Botromagno | Iron Age  (6th – 3rd) | Unknown | Unknown |
| 1968 DB 1 Contents of Sarcophagus | Botromagno | Iron Age (6th – 3rd) | Stone cut sarcophagus | Unknown |
| G67/I S5 small tomb | Botromagno | Late Iron Age (2nd – 1st) | Rock-cut grave | - 5 terracotta figurines of standing women  - Terracotta figurine of seated woman  - Fragments of terracotta figurines |
| 1967 G67/I SG-Tomb 6(2) Infill 1 | Botromagno | Late Iron Age (Later 4th-early 3rd) | Rock-cut grotticella |  |
| No Label #2 | Parco San Stefano | Mid-Iron Age (7th – 6th) | Unknown | Unknown |
| 1972 Area NA Burial 1 of A-1 | Botromagno | Iron Age (6th – 3rd) | Unknown | Unknown |
| G67/I P15 S19 | Botromagno | Iron Age (6th – 3rd) | Stone slab cist grave | No associated pottery |
| G67 Site C RZ3 Pit 3 tomb infill | Botromagno | Iron Age (6th – 3rd) | Pit | Unknown |

Along with the subadult samples, nine archaeological adult teeth (permanent first molars and a first incisor) from the same sites were also prepared for stable isotope analysis (Table 4.3). The adults included in this study were ~ 20 years old at the time of death and represent individuals who survived into adulthood. Adult age estimation was based on methods outlined by Buikstra and Ubelaker (1994), using morphology of the pubic symphysis (after Brooks & Suchey 1990) and when possible, the auricular surface. For those individuals who did not have a well-preserved innominate bone, dentition and epiphyseal union and fusion of primary ossification centers was used (Ubelaker 1989). Sex was estimated using morphological features of the cranium and innominate, such as the greater sciatic notch and pubic symphysis (following Buikstra & Ubelaker 1994). The inclusion of isotope data obtained from the dentine of permanent teeth allows for the comparison of weaning profiles from children who did not survive childhood to those who did survive. Permanent first molars begin to form in-utero at 28-32 weeks after fertilization and are complete by 9-10 years of age (Hillson 1986). Similarly, permanent first incisors begin to form at 0.25-0.3 years of age and are complete by 9 -10 years of age (Hillson 1986). Thus, both these tooth types provide dietary information from earlier life stages.

**Table 4.3:** Adult tooth samples.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample ID** | **Burial Site** | **Age-at-death** | **Sex** | **Tooth Sample** |
| 1972 Grave 2 bottom burial | Botromagno | 21<yrs | Male | LM1 |
| No Label #3 (box 6) | Parco San Stefano | 21 < yrs | Male | RM1 |
| F5 20108 Layer 1047 Skull | Botromagno | 35< yrs | Male | RM1 |
| Tomba 43 | Parco San Stefano | 38.2+/- 10.9 yrs | Female | RM1 |
| Tomba 11 | Parco San Stefano | 35-39 yrs | Male | I1 |
| G67III p.1 e.1 PI 1 Layer 1 pit fill | Botromagno | 16-24 yrs | Female | I1 |
| 1968 GDA 68 R1 F1 L5 | Botromagno | 16 - 20 yrs | Male | LM1 |
| Settore II Tomba 11 Scheletro in Con. | Padreterno | 38.2 ± 10.9 yrs | Female | LM1 |
| 1971 Area NB, Grave 3 | Botromagno | 21< yrs | Unknown | RM1 |

Adult burial information (presented in Table 4.4) was gathered from Whitehouse et al. (2000) and Small (1992), however not all burials were published in these sources. Additionally, No Label #3 (box 6) did not have a burial site ID.

**Table 4.4:** Adult burial information.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sample ID** | **Burial Site\***  **Date cent. BCE** | **Date cent. BCE** | **Tomb Type** | **Associated Grave goods** |
| 1972 Grave 2 bottom burial | Botromagno | Iron Age  (6th – 3rd) | unknown | Unknown |
| No Label #3 (box 6) | P. S. Stefano | Mid-Iron Age  (7th – 6th) | unknown | Unknown |
| F5 20108 Layer 1047 Skull | Botromagno | Iron Age  (6th – 3rd) | unknown | Unknown |
| Tomba 43 | P. S. Stefano | Mid-Iron Age  (7th – 6th) | unknown | Unknown |
| Tomba 11 | P. S. Stefano | Mid-Iron Age  (7th – 6th) | sarcophagus | sherds include an Iron Age geometric handle of a small pot, a staff handle and some plain |
| G67III p.1 e.1 PI 1 Layer 1 pit fill | Botromagno | Iron Age  (6th – 3rd) | Pit | Unknown |
| 1968 GDA 68 R1 F1 L5 | Botromagno | Iron Age  (6th – 3rd) | Fill of Sarcophagus | Unknown |
| Settore II Tomba 11 Scheletro in Con. | Padreterno | Mid-Iron Age  (6th – 4th) | unknown | Unknown |
| 1971 Area NB, Grave 3 | Botromagno | Iron Age  (6th – 3rd) | unknown | Unknown |

* 1. **Stable Isotope Methodology** 
     1. Bone Collagen Stable Isotope Analysis

Bone collagen preparation methods for isotopic analysis were drawn from modified procedures proposed by Longin (1971) and Chisholm and colleagues (1982). If needed the femoral and rib fragments were pulverized with a mortar and pestle. Bone samples were then rinsed with de-ionized water and placed in an ultrasonic bath 3 times for 5 minutes or more if needed (if the water continued to be cloudy after 3 washes). The bone samples were then left in an oven at 60 °C overnight to dry. Once dried, sample weights were measured and recorded.

For bone demineralization, samples were placed in BD Falcon plastic 50 ml centrifuge tubes. The mineral portion of bone was removed by soaking the samples in 0.25 M HCl (hydrochloric acid). The acid was changed daily until the bone mineral dissolved, leaving a pellet composed of organic material, primarily composed of collagen. The remaining bone pellets were washed with de-ionized water 3 times to remove any acid, and then were rinsed with 0.1 NaOH (sodium hydroxide) twice to remove any remaining humic materials; first for 1 minute and then for up to 20-60 minutes to remove any base-soluble contaminants. Following this, the samples were rinsed in de-ionized water 4 times once again to remove any remaining NaOH.

For the hot water extraction, bone pellets were sealed in 50 ml glass test tubes with 0.001 M HCl and placed in the oven overnight at 90 °C. Heating the bone pellet caused the collagen within it to become water soluble. The hot water extracted solution was removed from the oven, and its liquid component was decanted into 50 ml Teflon beakers and placed back in the 90 °C oven. While the liquefied collagen dried, any remaining organic pellets went through the hot water extraction process again to ensure the maximum amount of collagen was extracted.

After the collagen dried, it was liquified with a small amount of de-ionized water and poured into a pre-weighed and pre-labeled ThermoFisher glass vial. The pre-weighed glass vials were placed back in the oven at 80 °C. Once the de-ionized water had evaporated, the solid collagen was weighed and percent yield was calculated using the following equation:

*Equation 4.1 - Formula to calculate bone collagen yield (percent).*

A maximum of 130 mg of collagen was collected from the glass vials and transferred into 2.0 ml micro tubes for mass spectrometry. Mass spectrometry was conducted at the Ján Veizer Stable Isotope Laboratory (formerly G.G. Hatch) at the University of Ottawa using a Thermo Finnigan Delta XP mass spectrometer. The Hatch Lab procedures were as follows. The powdered collagen samples were weighed in tin capsules and loaded into an elemental analyzer linked to an isotopic ratio mass spectrometer (IRMS). In the elemental analyzer the samples were flash combusted at 1800 °C. The resulting carbon dioxide (CO2) and nitrogen (N2) gas products were carried by helium through columns of oxidizing and reducing chemicals optimized for CO2 and N2. A "trap and purge" column separated the CO2 and N2before they were sent to the IRMS interface and IRMS. The IRMS generated the isotope ratios from each sample. Isotope ratios were reported in Delta notation relative to international standards, with δ13C reported as ‰ vs. VPDB (Vienna Pee Dee Belemnite) and δ15N reported as ‰ vs. AIR (Ambient Inhalable Reservoir). G. G. Hatch reports that analytical precision is ± 0.2‰ based on internal laboratory standards. Following mass spectrometry, preservation of bone collagen was evaluated using both percent yield and carbon to nitrogen (C:N) ratios of the samples. A C:N ratio between 2.9 to 3.6 signals that the collagen in bone has not been affected by post-mortem processes (DeNiro, 1985). Ratios of carbon to nitrogen were calculated using Equation 4.2.

*Equation 4.2 - Formula to calculate the carbon to nitrogen ratio in collagen.*

Samples were included in this study if their collagen yields and/or C:N ratios were within the accepted range of 1% (Dobberstein et al. 2009).

* + 1. Dentine Collagen Microsampling

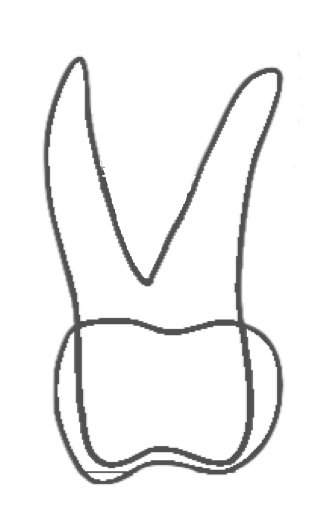
Dentine collagen preparation methods were derived from procedures outlined by Beaumont et al. (2013). Enamel was removed for all tooth samples with a hand held Dremel drill with a diamond burr without breaching the enamel/dentine junction (EDJ). Samples were then rinsed with de-ionized water and placed in an ultrasonic bath 3 times for 5 minutes. After that, the samples were dried in the oven at 60 °C overnight. Teeth were then placed in labeled plastic 50 ml centrifuge tubes for demineralization. Samples were soaked in 0.5M HCl and placed in the refrigerator at 4 °C. Stronger acid was used for teeth in comparison to the bone samples due to the fact that teeth are more densely mineralized. The acid was changed daily until the dentine was demineralized.

To section a demineralized tooth, the complete tooth was first measured with a metal ruler. Using the complete length of the tooth the number of sections were estimated with the minimum width of the sections being 2 mm and maximum being 4 mm in order to obtain the minimum amount of collagen (0.8 mg) required to run through mass spectrometry. Sections were cut using a sharp scalpel on a silicon cutting board starting at the apex and placed in prelabeled and pre-weighed 2.0 ml Axygen microtubes. To each tube and section 0.001 M HCl was added and heated at 70 °C for 24 hours to produce soluble collagen. If there was any remaining organic pellet, the liquid component was decanted into another pre-weighed microtube and dried, while the organic pellet was heated with 0.001 M HCl for another round of heating. Once the dentine collagen was dried, the microtubes were weighed to determine the weight of the collagen sample and percent yield.

*Equation 4.3 - Formula to calculate dentine collagen percent yield.*

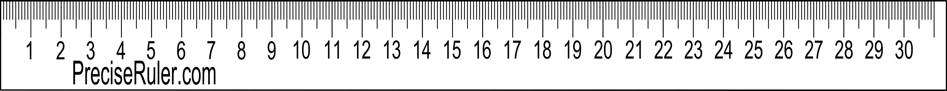
Similar to bone collagen samples, the dentine collagen samples were also sent to the Ján Veizier Isotope Laboratory at the University of Ottawa for mass spectrometry.

The collagen samples obtained from each tooth section were allocated an age range following the method outlined by Beaumont and Montgomery (2015), which was based on the developmental charts produced by the Queen Mary University of London (QMUL) London Atlas (AlQahtani et al. 2010). The total time taken for the tooth to develop was calculated by subtracting the age at cusp initiation from the age at apex closure. This number was then divided by the regularized sections/increments taken from the tooth. If irregular sections were taken, calculations were adjusted to take this into account. In several cases in this study, occlusal wear removed two or more years of growth from the top portion of the tooth, so some samples do not have data for the earliest stages of development. Figure 4.3 serves as a general indication of the timing for dentine development for a mandibular permanent first molar and how age estimation for tooth sections were calculated. It should be noted that these ranges are approximate and are complicated by the differences in development rates between individuals and populations (Beaumont & Montgomery 2015).

****



Length in cm



**Figure 4.3:** Diagram illustrating the ages at which dentine development reach crown initiation, crown completion and apical completion, for upper permanent first molar (demonstrated by the red solid lines) and sectioning of the demineralized mandibular permanent first molar for individual No label #3 (box 6) from this study (shown by the black dashed lines).

**4.4 Statistical Methods for Data Analysis**

Statistical tests were calculated using SPSS 23. Additionally, data were graphed using Excel 2016. The distributions of δ13C and δ15N data were examined using the Kolmogorov-Smirnov and Shapiro-Wilk tests of normality. Statistical tests including ANCOVA and t-tests were used to analyze the isotope data and compare weaning profiles between survivors and non-survivors, adult males and females, as well as to compare rib and femoral isotope values.

**Chapter 5: Results**

**5.1 Introduction**

In this chapter the stable isotope results from incremental dentine sections and bulk bone samples of Iron Age individuals are presented. First, results on sample integrity are presented to show that all samples are free of potential diagenetic alterations. Next, subadult dentine isotope data are presented followed by adult dentine isotope data. Subadult bone collagen isotope data are also presented along with graphs comparing dentine versus bone isotope values for each subadult individual. Lastly, dentine isotope results of individuals who survived the weaning process are compared to individuals identified as ‘non-survivors’ (i.e., individuals who died at age 4 years or younger).

**5.2 Sample Integrity**

As mentioned in Sections 3.3 and 3.4, it is essential to ensure that stable isotope samples have minimal to no diagenetic alternations that would impact interpretations of the data. Three values are used to evaluate the potential degree of diagenetic changes in collagen samples: C:N ratios, individual carbon (%C) and nitrogen (%N) percentages, and collagen yields. The C:N ratios of each tooth section for subadults are presented in Table 5.1, for adults in Table 5.2, and for subadult bone samples in Table 5.3. The range of C:N ratios for all dentine sections is 3.1 - 3.8, with a mean value of 3.2. The range for C:N ratios for the bone collagen samples is 3.2 – 3.7, with a mean value of 3.3. All samples except T17.01 and F4 fall within the accepted range of C:N ratios between 2.9 – 3.6 suggested by DeNiro (1985), indicating that there is likely minimal to no diagenetic impact on isotope values. As T17.01 and F4 have C:N ratios outside of this range (3.8 and 3.7, respectively) they are omitted from further analysis.

The percent C and N values are presented in Table A1 and Table A2, in Appendix A. The range of %C for all dentine sections is 34.5 – 47.5%, indicating that all dentine samples are considered safe from diagenetic alterations as they surpass the minimum expected value of 3% (Ambrose 1993). The range of %C for the bone data is 31.0 – 41.8% fitting with the expected 35% range for carbon (van Klinken 1999). Similarly, the %N ranges for both dentine (12.4 – 16.3%) and bone collagen (8.7 – 15.0%) data fall within the expected ranges of 11 - 16% for nitrogen (van Klinken 1999). Based on the individual carbon and nitrogen percentages, all teeth and bone samples are likely unaffected by diagenetic alterations.

Lastly, the collagen yield within a given sample is another means of assessing the degree of potential diagenetic alteration. Following the steps outlined in Section 4.3, collagen yields were calculated for each tooth sampled (i.e. by adding the individual collagen amounts from each section and dividing that value by the original dry weight of the complete tooth without enamel) and are listed in Table A3, Appendix A. Likewise, collagen yields for bone samples are also listed in Table A4, Appendix A. Collagen yields for tooth samples range from 3.8 – 14.4%, surpassing the minimum 1% requirement for good preservation (Schwarcz & Schoeninger 1991; van Klinken 1999; Dobberstein et al. 2009). Collagen yields for the bone samples range from 2.1 – 38.2%.

The combined analysis of C:N ratios, %C, %N, and collagen yields indicate that all but 2 samples had minimal diagenetic alteration and can be included in further analyses.

**5.3 Stable Isotope Results**

5.3.1 Subadult Serial Dentine Samples

The 62 dentine samples prepared from the 12 subadult Iron Age teeth all yielded sufficient collagen for analysis. Table 5.1 presents the δ13C and δ15N values for all subadult dentine subsamples along with the corresponding estimated age ranges and mean ages. The δ15N values range from 9.2‰ to 16.1‰ with a mean value of 12.2‰. The δ13C values range from -20.0‰ to -17.4‰ with a mean value of -19.0‰.

**Table 5.1**: Stable carbon and nitrogen isotope data for subadult dentine serial samples (n=62).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Skeletal ID** | **Sample ID** | **Age Range (months)** | **Mean Age (years)** | **δ13C**  **(‰, VPDB)** | **δ15N**  **(‰, AIR)** | **C:N** |
| Botromagno  G67/I p3 e2 S4 = Tomb 4 | T1.01 | 2.25 – 11.60 | 0.58 | -18.5 | 12.8 | 3.2 |
| T1.02 | 11.60 – 20.76 | 1.35 | -18.4 | 12.9 | 3.2 |
| T1.03 | 20.80 – 26.80 | 1.98 | -18.7 | 12.2 | 3.2 |
| T1.04 | 26.80 – 32.80 | 2.48 | -18.8 | 11.7 | 3.3 |
| T1.05 | 32.83 – 42.00 | 3.12 | -19.1 | 10.6 | 3.3 |
| Parco San Stefano  22/5/67 Tomba 30 | T2.01 | 18.00 – 23.50 | 1.73 | -18.8 | 12.6 | 3.2 |
| T2.02 | 23.50 – 28.80 | 2.18 | -19.3 | 12.4 | 3.2 |
| T2.03 | 28.80 – 35.40 | 2.68 | -19.5 | 12.5 | 3.2 |
| T2.04 | 35.40 – 42.00 | 3.23 | -19.5 | 12.0 | 3.2 |
| Botromagno  G67/II P4 S17 Infill 3 | T3.01 | 18.00 – 49.50 | 2.81 | -19.8 | 11.4 | 3.2 |
| T3.02 | 49.50 – 48.00 | 4.06 | -19.8 | 10.6 | 3.2 |
| T3.03 | 48.00 – 63.00 | 4.63 | -19.6 | 10.4 | 3.2 |
| T3.04 | 63.00 – 78.00 | 5.88 | -19.5 | 10.3 | 3.2 |
|  |  |  |  |  |  |  |
| Botromagno  21/12/71 Tomba di bambino Ind. 1 | T4.01 | 2.25 – 5.84 | 0.34 | -18.2 | 13.5 | 3.2 |
| T4.02 | 5.84 – 9.42 | 0.64 | -18.2 | 13.4 | 3.2 |
| T4.03 | 9.42 – 12.28 | 0.90 | -19.0 | 12.1 | 3.3 |
| T4.04 | 12.28 – 15.14 | 1.14 | -19.2 | 11.5 | 3.3 |
| T4.05 | 15.14 – 18.00 | 1.38 | -19.4 | 10.9 | 3.3 |
| Botromagno  GS70 F25 | T5.01 | 2.15 – 8.44 | 0.44 | -18.4 | 14.4 | 3.2 |
| T5.02 | 8.44 – 14.73 | 0.97 | -18.7 | 13.1 | 3.2 |
| T5.03 | 14.73 – 19.98 | 1.67 | -19.0 | 12.2 | 3.2 |
| T5.04 | 19.98 – 25.22 | 1.88 | -19.0 | 12.0 | 3.1 |
| T5.05 | 25.22 – 30.47 | 2.32 | -18.9 | 12.2 | 3.1 |
| T5.06 | 30.47 – 35.71 | 2.78 | -19.2 | 11.5 | 3.1 |
| T5.07 | 35.71 – 42.00 | 3.24 | -19.3 | 11.0 | 3.2 |
| Botromagno  1968 DB 1 Contents of Sarcophagus | T6.01 | 2.25 – 7.55 | 0.41 | -18.7 | 15.6 | 3.2 |
| T6.02 | 7.55 – 12.85 | 0.85 | -19.0 | 15.0 | 3.3 |
| T6.03 | 12.85 – 18.15 | 1.29 | -19.0 | 14.2 | 3.2 |
| T6.04 | 18.15 – 23.45 | 1.73 | -19.9 | 13.8 | 3.2 |
| T6.05 | 23.45 – 28.75 | 2.18 | -19.3 | 13.2 | 3.2 |
| Botromagno  G67/I S5 small tomb | T7.01 | 2.25 – 5.84 | 0.34 | -18.4 | 14.2 | 3.1 |
| T7.02 | 5.84 – 9.42 | 0.64 | -18.5 | 14.4 | 3.1 |
| T7.03 | 9.42 – 12.28 | 0.90 | -18.8 | 13.3 | 3.2 |
| T7.04 | 12.28 – 15.14 | 1.14 | -19.1 | 12.7 | 3.2 |
| T7.05 | 15.14 – 18.00 | 1.38 | -19.6 | 12.1 | 3.2 |
| Botromagno  1967 G67/I SG-Tomb 6(2) Infill 1 | T8.01 | 2.25 – 7.93 | 0.42 | -18.7 | 16.1 | 3.2 |
| T8.02 | 7.93 – 13.61 | 0.90 | -18.8 | 15.3 | 3.2 |
| T8.03 | 13.61 – 19.29 | 1.37 | -19.1 | 13.9 | 3.2 |
| T8.04 | 19.29 – 24.97 | 1.84 | -19.1 | 13.5 | 3.2 |
| T8.05 | 24.97 – 30.65 | 2.32 | -20.0 | 13.4 | 3.2 |
| T8.06 | 30.65 – 42.00 | 3.00 | -19.6 | 12.8 | 3.3 |
| Parco San Stefano  No Label #2 | T9.01 | 4.50 – 7.10 | 0.48 | -18.3 | 11.9 | 3.2 |
| T9.02 | 7.10 – 9.50 | 0.69 | -18.8 | 10.5 | 3.2 |
| T9.03 | 9.50 – 11.90 | 0.89 | -19.0 | 9.5 | 3.1 |
| T9.04 | 11.90 – 14.30 | 1.09 | -19.0 | 9.2 | 3.2 |
| T9.05 | 14.30 – 18.00 | 1.35 | -18.9 | 10.1 | 3.2 |
| Botromagno  1972 Area NA Burial 1 of A-1 | T10.01 | 4.50 – 11.60 | 0.67 | -18.4 | 13.7 | 3.2 |
| T10.02 | 11.60 – 18.40 | 1.25 | -19.3 | 11.1 | 3.2 |
| T10.03 | 18.40 – 25.20 | 1.82 | -19.2 | 11.0 | 3.2 |
| T10.04 | 25.20 – 42.00 | 2.80 | -19.6 | 10.0 | 3.4 |
| Botromagno  G67/I P15 S19 | T11.01 | 2.25 – 10.32 | 0.52 | -17.5 | 12.7 | 3.2 |
| T11.02 | 10.32 – 18.60 | 1.20 | -17.4 | 14.0 | 3.2 |
| T11.03 | 18.60 – 30.30 | 2.04 | -18.0 | 13.3 | 3.2 |
| T11.04 | 30.30 – 42.00 | 3.01 | -19.4 | 12.3 | 3.2 |
| Botromagno  G67 Site C RZ3 Pit 3 tomb infill | T12.01 | 18.00 – 28.80 | 1.95 | -19.0 | 11.9 | 3.2 |
| T12.02 | 28.80 – 38.40 | 2.80 | -19.7 | 10.0 | 3.3 |
| T12.03 | 38.40 – 48.00 | 3.60 | -19.7 | 9.8 | 3.3 |
| T12.04 | 48.00 – 63.00 | 4.63 | -19.6 | 9.6 | 3.3 |
| T12.05 | 63.00 – 78.00 | 5.88 | -19.6 | 9.4 | 3.2 |
| T12.06 | 78.00 – 93.00 | 7.13 | -20.0 | 10.2 | 3.3 |
| T12.07 | 93.00 – 108.00 | 8.38 | -19.8 | 10.2 | 3.2 |

Nitrogen and carbon isotope data for each subadult tooth are plotted against the estimated mean age for each section to create isotopic profiles. These charts are presented below in Figures 5.1 – 5.12.

**Figure 5.1:** G67/I p3 e2 S4 = Tomb 4 (T1). Age at death: 4 years ± 12 months.

Based on the serial dentine δ15N and δ13C values from 7 months to 3.1 years, it is likely that this individual (T1) was exclusively breastfed beyond the first year of life (Fig. 5.1). The decline in δ15N and δ13C starting after 1.3 years indicates that weaning began after this age (with a total decrease of 2.3‰). As dentine stable isotope data beyond 3.2 years are unavailable, it is difficult to determine weaning cessation with only deciduous dental tissues, although the overall drop by 2.3‰ is within the expected trophic level decline associated with weaning.



**Figure 5.2**: 22/5/67 Tomba 30 (T2). Age at death: 8 years ± 24 months.

The permanent first molar, T2, represents an eight-year-old subadult (Tomba 30) buried at Parco San Stefano. Due to extensive occlusal dental wear, this tooth only represents stable isotope data from 1.7 to 3.2 years of life. The gradually declining δ15N and δ13C values during these ages indicate that this individual had already begun weaning by 1.7 years (Fig. 5.2). Since stable isotope data preceding 1.7 years are unavailable it is difficult to determine the age at which weaning began and similarly as isotopic data immediately after 3.2 years are unavailable, so it is challenging to identify when weaning ceased. However, because the δ15N values only declined by 0.6‰ over 1.5 years, a much lower decline than the expected 2‰ – 3‰ offset expected with weaning, it can be inferred that the ages captured by this tooth possibly represent the tail-end of weaning. Further, the fact that the last two δ13C values appear to be leveling off supports the hypothesis that weaning may be concluding.



**Figure 5.3:** G67/II P4 S17 Infill 3 (T3). Age at death: 7 – 8 years ± 24 months.

This subadult is represented by T3, a permanent first molar, which provides stable isotope values from ages 2.8 to 5.9 years. Based on the decline in δ15N by 0.8‰ and relative consistency in δ13C values between 2.8 and 4 years, it is likely that this individual was weaned prior to 2.8 years (Fig 5.3). If weaning had occurred between 2.8 and 4.0 years then the decline in δ15N values would also be reflected by a similar, but smaller (i.e., 1‰) decrease in δ13C values due to the trophic level effect in carbon. This is not the case. Furthermore, from 4 to 5.9 years an inverse relationship between δ15N (decrease) and δ13C (increase) values is observed. It is possible that the decreasing δ15N values reflect the burden of depleting dietary protein and accelerated growth experienced during childhood. Simultaneously, stable carbon isotopes values could be revealing dietary change as this individual began to consume a post-weaning diet.

**Figure 5.4:** 21/12/71 Tomba di bambino Ind. 1 (T4). Age at death: 2 years ± 8 months.

Deciduous first molar, T4, represents individual ‘Tomba di bambino Ind. 1’, who was estimated to be two years old at the time of death. From the ages of 0.3 to 0.6 years (4.1 to 7 months of age) the comparatively high δ15N and δ13C values remain stable, indicating that this individual was breastfed during this period (Fig 5.4). Based on the decline in δ15N values by 2.6‰ between 7.7 months to 1.4 years, it can be inferred that the weaning process began after 7.7 months. As later stable isotope values are unavailable, it is not possible to estimate the age when weaning ceased from dental tissues. But again, there is almost a full trophic level drop (~2.5‰) from the first to the last dentine point, so weaning may have been completed by 1.4 years of age. This drop is very similar to T1, which also seems to be indicative of weaning.



**Figure 5.5:** GS70 F25 (T5). Age at death: 4 years ± 12 months.

T5, a deciduous first molar, represents stable isotope data for the individual GS70 F25. The decrease in δ15N by 2.2‰ between 0.48 year (5.3 months) and 1.7 years suggests that weaning began early, before the end of the first year of life (Fig. 5.5). From 1.7 to 2.3 years, δ15N and δ13C values remained relatively unchanged, followed by another decline in both isotopic profiles. This steady phase in stable isotope values may reflect weaning cessation around 2.0 years and the subsequent decline may be a result of a shift in post-weaning diet which is reflected in both nitrogen and carbon isotope values.



**Figure 5.6:** 1968 DB 1 Contents of Sarcophagus (T6). Age at death: 4 – 5 years ± 12 months.

T6, a deciduous first molar, presents stable isotope values for this subadult from 0.4 years (4.9 months) to 2.2 years. The steadily decreasing δ15N and δ13C values between these ages suggest that weaning began around 4.9 months of age (Fig. 5.6). Later phases of life are not recorded by this tooth, thus is it difficult to infer weaning cessation.



**Figure 5.7:** G67/I S5 small tomb (T7). Age at death: 3 years ± 12 months.

T7, a deciduous first molar represents a single individual who was estimated to be 3 years of age at the time of death. The relatively steady δ15N and δ13C values between 0.3 and 0.7 years (4.1 to 7.7 months), followed by a decline in stable isotope values show that this individual was exclusively breastfed up until 7.7 months, and weaning was initiated after this time (indicated by the declining isotope values after this point) (Fig. 5.7). Weaning cessation could not be inferred due to the fact that stable isotope values from deciduous dental tissues past 1.4 years were not available. However, as the total drop in δ15N and δ13C values is consistent with the expected decline (e.g. 3‰ and 1‰, respectively) this child may have been weaned by 1.4 years.

**Figure 5.8:** 1967 G67/I SG-Tomb 6(2) Infill 1 (T8). Age at death: 3 years ± 8 months.

This subadult was estimated to be 3 years of age at the time of death and is represented by the deciduous first molar, T8. Dentine subsamples from this tooth present stable isotope data between 5 months (0.4 year) and 3 years of life. A gradual decline in δ15N by 3.3‰ between these ages is most likely a response to weaning, which began shortly after 5 months and was likely done by 2.5 years of age (Fig. 5.8). The slight drop in δ15N by 3 years, accompanied by a slight increase in δ13C is likely representative of an accelerated growth spurt and depleted 15N-levels along with the consumption of a post-weaning diet.



**Figure 5.9:** No Label #2 (T9). Age at death: 5 – 6 years ± 12 months.

T9, a deciduous second molar, presents stable isotope data for this individual who was estimated to be 5 – 6 years of age at the time of death. The sharp decline in δ15N values by 3‰ after 5.8 months (0.5 year) over the next seven months indicate that this subadult was weaned very rapidly soon after birth (Fig. 5.9). Additionally, the low δ15N values (i.e. < 12‰) displayed by this individual resemble values measured in adult samples at later ages, such as T20 (Botromagno 1968 GDA 68 R1 F1 L5), who at two years of age had a δ15N value of 12.3‰ (Table 5.2). These low δ15N values throughout the early feeding history of this child, could be a reflection of low δ15N maternal values that were incorporated in-utero during early tooth formation or an earlier onset of weaning that began prior to 5.8 months, accompanied by a 15N-depleted supplementary diet.



**Figure 5.10:** 1972 Area NA Burial 1 of A-1 (T10). Age at death: 4 years ± 12 months.

The second deciduous molar, T10, represents this individual who was estimated to be four years of age at the time of death. The high δ15N value at 0.75 year (8 months) indicate that this individual was still breastfeeding at this point, so transitional feeding began after this age (Fig. 5.10). Between one to two years δ15N and δ13C values were both consistent; steady values could be indicative of cessation of weaning. However, as both nitrogen and carbon isotope values continue to decline at 2.8 years, this could instead be a result of weaning cessation or alternatively could be an outcome of accelerated growth (resulting in the depletion of 15N) along with a shift to a post-weaning diet demonstrated by a slight decline in δ13C values.

**Figure 5.11:** G67/I P15 S19 (T11). Age at death: 7 – 8 years ± 24 months.

T11, a deciduous first molar, presents stable isotope data for the individual G67/I P15 S19, who was estimated to be 7 – 8 years of age at the time of death. Elevated δ15N values between 0.5 (6 months) to 1 year, demonstrate an expected 15N-enrichment associated with breastfeeding (Fig 5.11). The decline δ15N values by 1.8‰ between 1 and 3 years indicate that weaning began after the first year, and gradually continued up to the 3rd year. Since a deciduous molar is completely formed by approximately 3 years, weaning cessation could not be identified as δ15N values continued to decrease up until this age (i.e. variability in isotope values continued until 3 years with an absence of plateauing associated with weaning cessation).



**Figure 5.12:** G67 Site C RZ3 Pit 3 tomb infill (T12). Age at death: 9 years ± 24 months.

T12, a permanent first molar, represents this subadult who was estimated to be 9 years of age at the time of death. Due to dental wear on the occlusal surface, isotopic data prior to 2 years could not be examined. From two years of age, there was a 1.9‰ drop in δ15N values, followed by a more gradual decline between 3.6 to 5.9 years (Fig. 5.12). Stable nitrogen isotope values then begin to rise from 5.9 to 7.1 years by 0.8‰ and remain consistent up until 8.4 years. These values indicate that weaning most likely began around two years of age due to the 2‰ – 3‰ offset in δ15N values expected with weaning. Furthermore, it is likely that weaning was complete around 3.6 years of age since δ15N values began to decline more gradually. The depleted 15N-levels and accelerated growth, which was noted in other subadult individuals, could account for the continued decrease in δ15N values. As the δ13C values only increase by 0.2‰ during this period a plant-based diet possibly remained steady. Increase in δ15N values with steady δ13C values from 6 years may be a result of physiological stress.

5.3.2 General Trends in Subadult Dentine Data

From these graphs a general trend of decreasing δ15N values with increasing dentine section age can be discerned, with the exception of T9 (Figure 5.9). The general trend of decreasing δ15N values is consistent with the cessation of exclusive breastfeeding and the beginning of transitional feeding during the weaning process, resulting in lower 15N-enrichment over time. The carbon values were also plotted against mean age in Figures 5.1 – 5.12. Generally, carbon isotope values exhibit a parallel relationship with nitrogen isotope values for all but two individuals (T3 and T12, Figures 5.3 and 5.12). However, the changes in δ13C values for each individual are much smaller (occurring over ~1‰ instead of 0.5 – 2.5‰ as seen in the nitrogen isotope values).

**Figure 5.13:** Comparison of all subadult individual nitrogen histories. Nitrogen isotope values are plotted against the approximate mean ages (in years) for each tooth section.

Figure 5.13 shows the variability between the earliest/youngest nitrogen isotope values measured (ranging over 5‰) for all 12 subadults. This variability may be due to differences in the maternal isotopic signal, which is found in the most occlusal section of dentine (formed *in utero*), while it also may be due to the inclusion of worn teeth that represent later ages (i.e., T2, T3 and T12, with the earliest isotope values measured at 1.73, 2.81 and 1.95 years, respectively) and subsequently demonstrate breastfeeding and weaning periods. Additionally, as mentioned before there is an overall decline in δ15N values over time, although after 3.5 years of age δ15N values available for 2 individuals seem to become less variable. Figure 5.13 also reveals outliers such as T9 who has the lowest starting δ15N value (11.9‰) than other teeth with the earliest nitrogen isotope values measured around ~ 0.4 years or 4.8 months, such as T5, T6, T8 and T11, demonstrating δ15N values of 14.4‰, 15.6‰, 16.1‰ and 12.7‰, respectively. T9 also displays a steep drop over a relatively short period of time, indicating that this person was most likely weaned soon after birth and had a mother with a lower nitrogen isotope signal.

5.3.3 Adult Serial Dentine Samples

Of the 69 incremental samples of adult tooth dentine, only T17.01 had to be omitted due to potential diagenetic changes, indicated by a C:N ratio (3.8) outside the accepted range. Table 5.2 presents the carbon and nitrogen isotope data for all adult tooth samples along with the corresponding age ranges and mean ages. The δ15N values are variable ranging from 7.3‰ to 12.3‰, with a mean value of 9.5‰. The δ13C values range from -21.1‰ to -18.6‰ with a mean value of -19.4‰.

**Table 5.2**: Stable carbon and nitrogen isotope data for adult dentine subsamples (n=69).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Site** | **Skeletal ID** | **Sample ID** | **Age Range (years)** | **Mean Age**  **(years)** | **δ13C (**‰) VPDB | **δ15N (**‰) AIR | **C:N** |
| Botromagno | BOTR1972 Grave 2 bottom burial | T13.01 | 2.00 – 3.40 | 2.70 | -18.9 | 9.6 | 3.2 |
| T13.02 | 3.40 – 4.70 | 4.05 | -19.2 | 8.1 | 3.2 |
| T13.03 | 4.70 – 6.00 | 5.35 | -19.3 | 7.6 | 3.2 |
| T13.04 | 6.00 – 7.30 | 6.65 | -19.3 | 7.4 | 3.2 |
| T13.05 | 7.30 – 8.60 | 7.95 | -19.2 | 7.5 | 3.2 |
| T13.06 | 8.60 – 9.90 | 9.25 | -19.1 | 8.5 | 3.2 |
| T13.07 | 9.90 – 11.20 | 10.55 | -19.2 | 8.5 | 3.2 |
| T13.08 | 11.20 – 12.50 | 11.85 | -19.2 | 9.0 | 3.3 |
| Botromagno | No label #3 (box 6) | T15.01 | 2.00 – 2.90 | 2.45 | -18.7 | 11.2 | 3.2 |
| T15.02 | 2.90 – 4.10 | 3.50 | -19.2 | 9.1 | 3.3 |
| T15.03 | 4.10 – 5.30 | 4.70 | -19.1 | 9.3 | 3.3 |
| T15.04 | 5.30 – 6.50 | 5.90 | -18.9 | 9.4 | 3.2 |
| T15.05 | 6.50 – 7.70 | 7.10 | -18.6 | 9.5 | 3.2 |
| T15.06 | 7.70 – 8.90 | 8.30 | -18.6 | 9.6 | 3.2 |
| T15.07 | 8.90 – 10.10 | 9.50 | -18.9 | 10.1 | 3.2 |
| T15.08 | 10.10 – 11.30 | 10.70 | -18.8 | 10.5 | 3.2 |
| T15.09 | 11.30 – 12.50 | 11.90 | -19.7 | 10.8 | 3.2 |
| Botromagno | BOTR F5 20108 Layer 1047 Skull | T16.01 | 2.00 – 3.60 | 2.80 | -19.9 | 9.0 | 3.2 |
| T16.02 | 3.60 – 5.30 | 4.45 | -20.2 | 8.1 | 3.3 |
| T16.03 | 5.30 – 7.00 | 6.15 | -20.1 | 8.2 | 3.3 |
| T16.04 | 7.00 – 8.10 | 7.55 | -20.0 | 8.6 | 3.2 |
| T16.05 | 8.10 – 9.20 | 8.65 | -20.0 | 8.5 | 3.3 |
| T16.06 | 9.20 – 10.30 | 9.75 | -19.8 | 8.8 | 3.2 |
| T16.07 | 10.30 – 11.40 | 10.85 | -19.9 | 8.8 | 3.2 |
| T16.08 | 11.40 – 12.50 | 11.95 | -20.4 | 9.2 | 3.3 |
| Parco San Stefano | PS Stefano Tomba 43 | T17.02 | 3.75 – 5.50 | 4.63 | -19.0 | 9.7 | 3.3 |
| T17.03 | 5.50 – 7.25 | 6.38 | -18.9 | 9.6 | 3.3 |
| T17.04 | 7.25 – 9.00 | 8.13 | -19.6 | 10.1 | 3.4 |
| T17.05 | 9.00 – 12.5 | 10.75 | -19.2 | 10.3 | 3.2 |
| Parco San Stefano | PS Stefano Tomba 11(?) | T18.01 | 2.50 – 4.16 | 3.33 | -19.9 | 9.8 | 3.2 |
| T18.02 | 4.16 – 5.66 | 4.91 | -19.3 | 9.6 | 3.2 |
| T18.03 | 5.66 – 6.86 | 6.26 | -19.0 | 9.3 | 3.2 |
| T18.04 | 6.86 – 8.06 | 7.46 | -18.8 | 9.5 | 3.2 |
| T18.05 | 8.06 – 9.26 | 8.66 | -18.9 | 9.9 | 3.2 |
| T18.06 | 9.26 – 10.50 | 9.88 | -19.0 | 10.2 | 3.2 |
| T18.07 | 10.50 – 11.66 | 11.08 | -19.3 | 9.9 | 3.2 |
| T18.08 | 11.66 – 13.50 | 12.58 | -19.5 | 10.2 | 3.2 |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| Botromagno | G67III p.1 e.1 PI 1 Layer 1 pit fill | T19.01 | 1.50 – 3.30 | 2.40 | -19.0 | 10.8 | 3.2 |
| T19.02 | 3.30 – 4.50 | 3.90 | -19.1 | 10.8 | 3.2 |
| T19.03 | 4.50 – 5.70 | 5.10 | -19.0 | 10.7 | 3.2 |
| T19.04 | 5.70 – 6.90 | 6.30 | -18.8 | 10.2 | 3.2 |
| T19.05 | 6.90 – 8.10 | 7.50 | -18.8 | 10.1 | 3.2 |
| T19.06 | 8.10 – 9.30 | 8.70 | -18.9 | 10.2 | 3.2 |
| T19.07 | 9.30 – 10.50 | 9.90 | -19.2 | 10.2 | 3.2 |
| T19.08 | 10.50 – 11.70 | 11.10 | -19.3 | 10.3 | 3.2 |
| T19.09 | 11.70 – 13.50 | 12.60 | -19.2 | 10.6 | 3.2 |
| Botromagno | Botr 1968 GDA 68 R1 F1 L5 | T20.01 | 2.00 – 2.90 | 2.45 | -19.0 | 12.3 | 3.2 |
| T20.02 | 2.90 – 4.00 | 3.45 | -19.3 | 10.6 | 3.2 |
| T20.03 | 4.00 – 5.70 | 4.85 | -19.3 | 9.6 | 3.2 |
| T20.04 | 5.70 – 7.40 | 6.55 | -19.3 | 9.7 | 3.2 |
| T20.05 | 7.40 – 9.10 | 8.25 | -19.2 | 10.7 | 3.2 |
| T20.06 | 9.10 – 10.80 | 9.95 | -20.2 | 10.9 | 3.2 |
| T20.07 | 10.80 – 12.50 | 11.65 | -19.4 | 10.1 | 3.2 |
| Padreterno | Padreterno Settore II Tomba 11 Schletro in Conessione | T21.01 | 2.00 – 3.15 | 2.58 | -20.1 | 8.8 | 3.2 |
| T21.02 | 3.15 – 4.32 | 3.74 | -19.8 | 7.7 | 3.3 |
| T21.03 | 4.32 – 5.49 | 4.90 | -19.5 | 8.2 | 3.2 |
| T21.04 | 5.49 – 6.66 | 6.08 | -19.3 | 9.2 | 3.2 |
| T21.05 | 6.66 – 7.83 | 7.25 | -19.2 | 9.7 | 3.2 |
| T21.06 | 7.83 – 9.00 | 8.42 | -19.6 | 10.9 | 3.4 |
| T21.07 | 9.00 – 10.75 | 9.88 | -20.1 | 10.4 | 3.3 |
| T21.08 | 10.75 – 12.50 | 11.63 | -19.2 | 10.8 | 3.2 |
| Botromagno | Botr 1971 Area NB, Grave 3 | T22.01 | 1.50 – 2.67 | 2.09 | -19.4 | 9.4 | 3.2 |
| T22.02 | 2.67 – 3.84 | 3.26 | -19.7 | 8.9 | 3.3 |
| T22.03 | 3.84 – 5.01 | 4.43 | -20.3 | 7.3 | 3.2 |
| T22.04 | 5.01 – 6.76 | 5.89 | -19.8 | 7.4 | 3.3 |
| T22.05 | 6.76 – 8.51 | 7.64 | -20.1 | 7.5 | 3.3 |
| T22.06 | 8.51 – 10.26 | 9.39 | -21.1 | 7.7 | 3.3 |
| T22.07 | 10.26 – 12.01 | 11.14 | -20.2 | 8.0 | 3.3 |

Nitrogen and carbon isotope data for each adult tooth are plotted against the estimated mean age for each section to create isotopic profiles. These charts are presented below in Figure 5.14 – 5.22.

**Figure 5.14:** Botr 1972 Grave 2 bottom burial (T13), male. Age at death: 21 < years.

This adult male is represented by a permanent first molar, T13, and was estimated to be < 21 years of age at the time of death. Dentine data from this tooth provide information on early diet from 2.7 to 11.9 years of age (Fig. 5.14). The relatively low initial δ15N value (9.6‰) measured at 2.7 years is lower than the initial δ15N values ranging from 13 – 16‰ measured for nearly all the subadults (e.g., T4, T6, T8) in this study. The decrease in δ15N values from 2.7 to 5.4 years by ~2.0‰, suggests that this individual may have been weaned up until 5.4 years as the ~2-3‰ is indicative of weaning; yet, as the initial δ15N value is lower than the values noted for subadults in this study, the decline may also be a reflection of accelerated growth resulting in the depletion of 15N-levels. Between five to eight years δ15N and δ13C values remained relatively steady indicating that weaning was likely complete by this time. Following this period, δ15N values were elevated by 1.5‰, while δ13C values remained consistent. This spike in stable nitrogen isotope values could be either a reflection of 15N-enriched diet or more likely due to physiological stress, due to the fact that the δ13C values do not mirror the increase in nitrogen values.



**Figure 5.15:** No label #3 (box 6) (T15), male. Age at death: 21 < years.

This male individual was estimated to be < 20 years of age at the time of death and is represented by a permanent first molar, T15, which provided isotopic values from 2.5 to 11.9 years. Declining δ15N values (by ~2.3‰) between 2.5 years to 3.5 years indicate that weaning began before or around 2.5 years of age and ceased by 3.5 years of age (Fig 5.15). Following this, between 3.5 to 10.7 years of age, the δ15N values uninterruptedly increase by 7‰, while the δ13C values only increased up until 7 years after which they began to gradually decline. This pattern could mean that after 7 years of age the individual likely experienced physiological stress demonstrated by elevated δ15N levels and decreasing δ13C values, and a possible change in plant-based post-weaning diet.



**Figure 5.16:** BOTR F5 20108 Layer 1047 Skull (T16), male. Age at death: 35 < years.

T16, a permanent first molar, represents this male individual who was estimated to be < 35 years of age at the time of death. This tooth presents isotopic information from the early periods of life, between 2.8 and 12 years of age. Declining δ15N values by 0.9‰ from 2.8 to 4.5 years, followed by a 1.1‰ increase, reveals that weaning was likely underway by 2.8 years, and ceased around 4.5 years of age (Fig. 5.16). A consistent increase in both 15N and δ13C values up to around 9.8 years of age, indicates a gradual increase in protein consumption from higher trophic levels. Similar to the previous individual, around 9.8 years of age, δ15N and δ13C values demonstrate an inverse relationship. The continued rise in δ15N values with a decrease in δ13C values suggests physiological stress.



**Figure 5.17:** PS Stefano Tomba 43 (T17), female. Age at death: 38.9 ± 10.9 years.

Permanent first molar, T17, presents early isotopic data for this female individual who was 38.2 years of age at the time death. Based on the steady δ15N and δ13C values measured between 4.6 and 6.4 years, this individual was likely already weaned by 4.6 years of age (Fig. 5.17). Between 6.4 and 8.1 years, δ15N values demonstrate a slight increase, while δ13C drops, with a recovery by 11 years where both isotope values rise. This brief inverse period might be indicative of a catabolic event due to physiological stress.

**Figure 5.18:** PS Stefano Tomba 11(?) (T18), male. Age at death: 35 – 39 years.

T18, a permanent incisor, presents early isotopic data for this male individual who was estimated to be 35 – 50 years of age at death. Decreasing δ15N values between three to six years, accompanied with increasing δ13C values, show that this individual was weaned prior to three years of age (Fig. 5.18). Considering the patterns noted in the individuals from this study, it is highly unlikely that this individual was weaned until 6.3 years of age. Also similar to T13 (Fig. 5.14) the initial δ15N value (9.73‰) is lower than the initial values seen for subadults in this study, hence, the decreasing nitrogen isotope values are most likely associated with the effect of depleting 15N-levels and the added load of accelerated growth. The ultimate rise in δ15N values may be a response to a post-weaning diet that includes higher tropic levels; however, decreasing δ13C values after 7.5 years may suggest a catabolic event such as physiological stress causing the observed elevation in δ15N.



**Figure 5.19:** G67III p.1 e.1 PI 1 Layer 1 pit fill (T19), female. Age at death: 16 – 20 years.

This female individual was 18 to 24 years old at the time of death and is represented by T19, a permanent incisor. Isotopic data between 2.4 to 12.6 years show the least amount of variability in comparison to all other adult individuals in this study. There is a slight decline in δ15N values from 5.1 to 6.3 years by 0.5‰ accompanied by a minor increase in δ13C by 0.2‰ (Fig. 5.19). Due to the overall consistency in the isotope profile of this individual, it is likely that weaning was completed sometime before 2.4 years – prior to available stable isotope data. The slight decline in δ15N values may have been reflective of a brief alteration in protein consumption or possibly a stress episode associated with accelerated growth as seen in other subadult and adult individuals.



**Figure 5.20:** Botr 1968 GDA 68 R1 F1 L5 (T20), male. Age at death: 16 – 20 years.

T20, a permanent first molar, represents this 35 – 49 year old male individual. Declining stable isotope values between 2.5 to 5 years followed by steady isotopic measures suggest that this individual was fully weaned between three to five years of age (Fig. 5.20). Although this appears to be a delayed age for weaning cessation, the drop in δ15N values by 2.7‰ resembles the 2 – 3‰ offset expected with weaning. Furthermore, if these values were associated with physiological or nutritional stress then the decline would only be notable in δ15N values, which is not the case here. Between 6.5 and 8.4 years both δ15N and δ13C increase due to protein consumption from higher trophic level foods, after which δ15N continues to increase while δ13C drops by ~1‰. This is followed by a decline in δ15N and rise in δ13C values. Inverse relationships between stable nitrogen and carbon values are often indicative of physiological stress as presented by other individuals in this study.

**Figure 5.21:** Padreterno Settore II Tomba 11 Schletro in Conessione (T21), female. 38 years ± 10.9 years.

The permanent first molar, T21, presents early stable isotopic data for this female individual who was 40 – 49 years old at the time of death. Declining δ15N values from 2.6 to 3.7 years followed by a gradual increase until 8.4 years, indicate that this adult was fully weaned by 3.7 years of age (Fig. 5.21). The decreasing stable nitrogen values represent the tail-end of the weaning process due to the small offset (1.1‰). Additionally, the rise in δ15N values demonstrates the consumption of a post-weaning diet that remained consistent after 8.4 years of age. As expected for dietary variation, the δ13C values generally mirror the δ15N values, but at a smaller scale (i.e., the trophic level effect for carbon is only ~ 1 ‰).



**Figure 5.22:** Botr 1971 Area NB, Grave 3 (T22), sex unknown. Age at death: 21 < years.

T22, a permanent first molar, represents this individual who was 21 - 22 years of age at the time of death. Decreasing δ15N and δ13C values up until 4.4 years suggest that weaning ceased around this age. As the initial δ15N value (9.42‰) is relatively lower than the initial values noted for subadults, it is also possible that accelerated growth during this period could also have contributed to this decline. Following this, stable nitrogen isotope values steadily increased until the tooth was completely formed; meanwhile δ13C values increased after weaning ceased and then decrease by 1‰ around 6 years of age. These altered δ13C values are most likely linked to variability in a plant-based diet, while protein consumption continued to be a consistent nitrogen source (Fuller et al. 2006).

5.3.4 General Trends of Adult Dentine Data

From the charts, one prominent trend can be discerned. Although there is variability between these individuals, there is repeated evidence of decreased stable nitrogen isotope values associated with the demands of growth and development. This is shadowed by the likely consumption of a post-weaning diet resulting in the gradual increase of δ15N values. The stable carbon isotope data are also presented alongside the nitrogen isotope profiles. Overall, in comparison to nitrogen isotope data there is less variation in carbon isotope histories. Some individuals (T13, T15, T16, T20 and T22) show stable carbon isotope values following a similar pattern to nitrogen isotope values however from 8 – 10.5 years onwards the relationship seems to inverse. Meanwhile, for other individuals (T17, T18, T19) the nitrogen and carbon isotope values share an inverse relationship throughout the time period analyzed. Evidence of catabolic events possibly caused by physiological stress is also noted for six individuals (T13, T15, T16, T17, T18 and T20) through elevated δ15N values countered with decreasing δ13C values. It is important to note that all of these individuals demonstrate isotope values from 2 – 3 years of age and onwards; thus, these carbon values most likely reflect the final stages of the weaning process and a post-weaning diet. Further explanations for all these observed trends will be discussed in the next chapter.

B **Figure 4.5** **a)** Modified diagram from Beaumont and Montgomery (2015a) illustrating the ages at which dentine development reach crown initiation, crown completion and apical completion, for upper permanent first molar. **b)** Sectioning of demineralized mandibular permanent first molar for individual No label #3 (box 6) from this study.

A **Figure 4.5** **a)** Modified diagram from Beaumont and Montgomery (2015a) illustrating the ages at which dentine development reach crown initiation, crown completion and apical completion, for upper permanent first molar. **b)** Sectioning of demineralized mandibular permanent first molar for individual No label #3 (box 6) from this study.

A

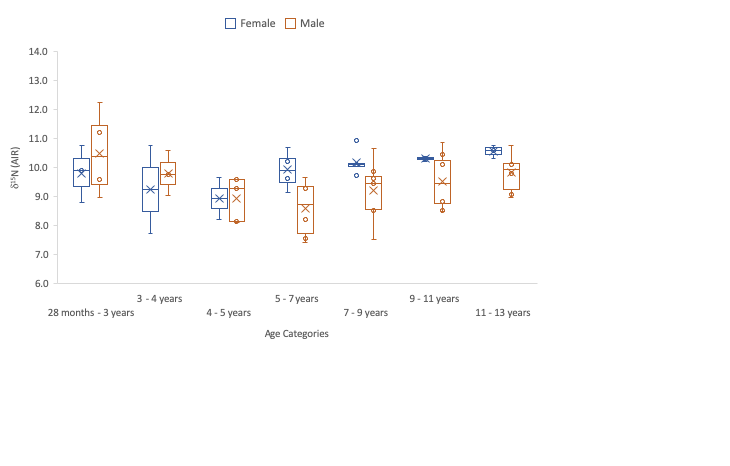
B

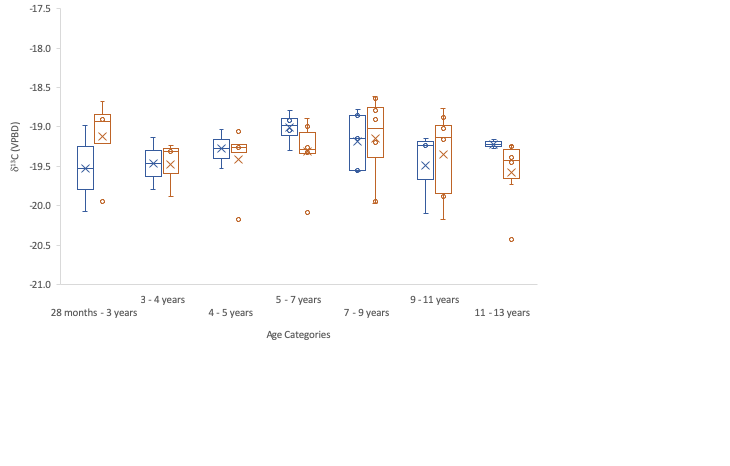
**Figure 5.23:** Comparison of all adult individual nitrogen histories. Group A is characterized by individuals with overall higher nitrogen isotope values and group B is characterized by individuals with overall lower nitrogen values. Nitrogen isotope values are plotted against the approximate mean ages (in years) for each tooth section.

The comparison between all adult individuals in Figure 5.23 demonstrates the variability in the nitrogen isotope values between individuals included in this study. Specifically, two prominent groupings can be identified in this chart. Group A includes T15, T17, T18, T19, T20 and T21and is distinguished by the overall high values of δ15N. Meanwhile, Group B includes T13, T16 and T22 and is characterized by significantly lower δ15N values. Statistical analysis of covariance (refer to Table B1 in Appendix B) demonstrates a significant difference in variance for group A and B (ANCOVA *F = 73.76, p < 0.0001*). Group A includes both males and females from all 3 sites (Botromagno, Parco San Stefano and Padreterno). Meanwhile, group B includes two males and one individual with unknown sex, from the Botromagno site.

**Table 5.4:** Mean stable nitrogen and carbon isotope data for adult males (n=5) and females (n=3).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | 28 months – 3 years | 3 - 4 years | 4 -5 years | 5 -7 years | 7 – 9 years | 9 -11 years | 11 -13 years |
| Male (n=5) | Mean δ15N | 10.5  (n=4) | 9.8  (n=2) | 8.94  (n=5) | 8.6  (n=5) | 9.2  (n=5) | 9.5  (n=5) | 9.8  (n=5) |
| Mean δ13C | -19.1  (n=4) | -19.5  (n=2) | -19.4  (n=5) | -19.3  (n=5) | -19.2  (n=5) | -19.4  (n=5) | -19.6  (n=5) |
| Female  (n=3) | Mean δ15N | 9.8  (n=2) | 9.3  (n=2) | 8.9  (n=2) | 9.9  (n=3) | 10.2  (n=3) | 10.3  (n=3) | 10.6  (n=2) |
| Mean δ13C | -19.5  (n=2) | -19.5  (n=2) | -19.3  (n=2) | -19.0  (n=3) | -19.2  (n=3) | -19.5  (n=3) | -19.2  (n=2) |





**Figure 5.24:** Box and whisker plot comparing the δ15N (top chart) and δ13C (bottom chart) results between males and females for each age category.

Table 5.4 and Figure 5.24 display the stable isotope results for adult males and females. Sample T22 had to be omitted from this analysis due to the poorly preserved skeletal material producing uncertainty of sex. Females demonstrate lower δ15N values from 28 months to 4 years by ~ 0.7‰, which is a relatively small difference and may be the result of measurement error. Conversely, males demonstrate lower δ15N values from 5 years and onwards by ~1‰. Statistical analysis of covariance also found a significant difference in the variance between males and females (in Table B2, Appendix B, ANCOVA *F=4.359, p=0.041*). As this is most likely after weaning, these values may indicate possible differences in dietary protein consumption between males and females. However, it should be noted that as there are only 3 females and 5 males, this sample is not representative of all adults in the Iron Age population, especially for those age groups where there are values for only 2 individuals. The δ13C values between males and females are comparable throughout the age groups (in Table B3, Appendix B, ANCOVA *F=0.296, p=0.588*), suggesting little to no difference in the sources of carbon in the diet. This source was most likely C3 plants, or the consumption of animals (and their by-products) ingesting these plants as the carbon isotope data fall within the associated -22‰ to -16‰ range.

5.3.5 Subadult Bone Collagen

Rib and femur isotope data for the same 11 subadult individuals with dentine data displayed in Section 5.3.1 are presented in the Table 5.3; all samples are included except F4 (femoral sample for 1968 DB 1 Contents of Sarcophagus), which had to be omitted due to possible diagenetic alteration, and only rib values are available and included for 1972 Area NA Burial 1 of A-1. Additionally, individual ‘No Label #2’ is not included in this part of the analysis due to poorly preserved skeletal material. Delta15N values range from 8.7 – 13.7‰ with a mean value of 10.8‰. δ13C values range from -19.8‰ to -18.2‰ with a mean value of -19.3‰.

**Table 5.3:** Stable carbon and nitrogen isotope data of subadult bone samples.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sample/Site ID** | **ID** | **Age (years)** | **δ13C**  **(**‰) VPDB | **δ15N**  **(**‰) AIR | **C:N** |
| Botromagno  21/12/71 Tomba di bambino Ind. 1 (T4) | Femur (F1) | 2.0 | -18.9 | 11.3 | 3.3 |
| Rib (R8) | 2.0 | -18.8 | 11.0 | 3.4 |
| Botromagno  GS70 F25 (T5) | Femur (F2) | 4.0 | -19.0 | 10.6 | 3.6 |
| Rib (R9) | 4.0 | -19.6 | 10.6 | 3.2 |
| Botromagno  G67/I p3 e2 S4 = Tomb 4 (T1) | Femur (F3) | 4.0 | -18.6 | 11.5 | 3.3 |
| Rib (R5) | 4.0 | -19.1 | 9.8 | 3.3 |
| Botromagno  1968 DB 1 Contents of Sarcophagus (T6) | Rib (R10) | 4.5 | -19.5 | 11.7 | 3.2 |
| Botromagno  G67/I S5 small tomb (T7) | Femur (F5) | 3.0 | -19.1 | 12.1 | 3.3 |
| Rib (R11) | 3.0 | -19.4 | 11.5 | 3.2 |
| Botromagno  G67 Site C RZ3 Pit 3 Tomb infill (T12) | Femur (F6) | 9.0 | -19.4 | 9.9 | 3.4 |
| Rib (R6) | 9.0 | -19.8 | 9.6 | 3.3 |
| Parco San Stefano  22/5/67 Tomba 30 (T2) | Femur (F7) | 8.0 | -19.7 | 11.1 | 3.3 |
| Rib (R7) | 8.0 | -19.5 | 11.1 | 3.2 |
| Botromagno  G67/II P4 S17 Infill 3 (T3) | Femur (F8) | 7.5 | -19.6 | 10.2 | 3.4 |
| Rib (R4) | 7.5 | -19.5 | 10.1 | 3.2 |
| Botromagno  G67/I P15 S19 (T11) | Femur (F9) | 7.5 | -19.1 | 8.7 | 3.2 |
| Rib (R3) | 7.5 | -19.5 | 9.0 | 3.2 |
| Botromagno  1967 G67/I SG-Tomb 6(2) Infill 1 (T8) | Femur (F10) | 3.0 | -18.2 | 13.7 | 3.3 |
| Rib (R1) | 3.0 | -19.3 | 12.3 | 3.3 |
| Botromagno  1972 Area NA Burial 1 of A-1(T10) | Rib (R2) | 4.0 | -19.5 | 9.3 | 3.2 |





**Figure 5.25:** Comparison of Subadult rib and femur collagen δ15N and δ13C values for each individual plotted against age at death (n=9). The dotted lines between the rib and femur data points indicate values obtained from one individual.

Figure 5.25 plots δ15N and δ13C values against age at death, and compares rib isotope values with femur isotope values for 9 subadults. It can be seen that the rib δ15N values are consistently lower than femur values. Similarly, rib δ13C values are consistently lower than femur values for all individuals except for F7/R7 (Tomba 30). Another trend noted in these charts is that prior to 5 years of age there is greater disparity between rib and femur nitrogen and carbon isotope values. However, after 5 years of age rib and femur values become very similar. This is most likely due to the differences in rib and femur bone turnover rates, with ribs having a faster turnover rate, so the femur values are showing slightly higher nitrogen because they still have bone formed during earlier stages such as breastfeeding. The difference between rib and femur values may decrease after 5 as a result of a homogenous diet as opposed to the variability seen during the breastfeeding and weaning process. A paired T-test indicated there was no significant statistical difference between rib and femur nitrogen isotope values (refer to Table B4 in Appendix B, *t = -1.869, p = 0.099*). Explanations regarding this trend will be explained in the next chapter.

**5.4 Subadult Serial Dentine vs. Bulk Bone Collagen**

The following series of graphs plot carbon and nitrogen data from dentine, ribs, and femur samples from the same subadult individuals to explore variability between signals obtained from dentine and bones samples.

**Figure 5.26:** G67/I p3 e2 S4=Tomb 4 (T1). Age at death: 4 years ± 12 months.

The deciduous molar (T1), femur (F3), and rib (R5), present stable isotope values for this single four-year old individual. The rib δ15N value (9.8‰) is lower than the δ15N value (10.6‰) of the last serial dentine section, implying that either (1) the individual was weaning up until the time of death or had ceased weaning soon before death, or (2) that the drop in nitrogen reflects a decline associated with growth (Fig. 5.26). In contrast, the femur δ15N value (11.5‰) is higher than both the rib and final dentine section values. Rib and femoral samples incorporate diet consumed over varying lengths of time; a rib represents diet from a few months prior to death, while femoral values represent diet consumed over a longer period prior to death. Consequently, the rib collagen data exhibit stable isotope values that correspond to a period closer to the time of death, while femoral collagen data display stable isotope values that include diet from earlier life stages (such as exclusive breastfeeding), resulting in overall higher δ15N values.

Additionally, the rib δ13C value appears to be consistent with the δ13C value of the last serial dentine section, suggesting that from 3.2 years until the time of death (4 years) there was an insignificant change in plant-based diet. The femur δ13C value is higher by 0.5‰, however, as stated this higher femoral value is likely the result of a slower bone turnover rate, which can reflect food consumed over a longer period of time.

**Figure 5.27:** Parco San Stefano 22/5/67 Tomba 30 (T2). Age at death: 8 years ± 24 months.

Bone data from this subadult (Fig. 5.27) demonstrate a 1.0‰ drop in δ15N values between the last serial dentine section, representing 3.2 years, and 8 years (the estimated age-at-death). As rib and femoral stable isotope data represent differing amounts of time, the fact that both bone samples express comparable δ15N values, may suggest minimal change in dietary protein during the several months leading up to death. Likewise, the rib and femoral δ13C values are similar, with a slight difference of 0.2‰. Since this difference is within the margin of analytical error, it can be assumed that both bone samples as well as the final dentine section demonstrate comparable δ13C values.

Like the previous individual, G67/I p3 e2 S4 = Tomb 4, it is possible that the lower δ15N bone values may be a result of positive nitrogen balance associated with growth. An alternative explanation is that the post-weaning diet was composed of foods with lower δ15N values (like a more terrestrial-based, C3 plant diet).

**Figure 5.28:** G67/II P4 S17 Infill 3 (T3). Age at death: 7 – 8 years ± 24 months.

For Individual T3, both rib and femoral samples provide comparable δ15N and δ13C values in comparison to the last serial dentine section (representing the age 5.9 years), with a discrepancy from each other by 0.1‰ (Fig. 5.28). Based on these values it can be concluded that this individual consumed a relatively consistent diet from 5.9 years up until time of death.

**Figure 5.29:** Gravina Botr 21/12/71 Tomba di bambino Ind. 1 (T4). Age at death: 2 years ± 8 months.

The stable isotope data from the rib and femur samples of T4 present similar δ15N values to the last serial dentine section; yet, the δ13C values appear to be slightly higher (by 0.5‰) (Fig. 5.29). Similarity between nitrogen isotope values between the last dentine and bone samples might be a result of the quicker rate of weaning accompanied by faster overall skeletal bone turnover rates during the first year of childhood. More specifically, since 100 – 200% of the skeleton is expected to be remodelled within the first year of life (ICRP, 1975), and this individual was 2 years old at the time of death, combination of rapid weaning and quicker incorporation of diet by both bone samples may result in similar and more recent rib and femur stable isotope values. This pattern is further discussed in Chapter 6.

**Figure 5.30:** GS70 F25 (T5). Age at death: 4 years ± 12 months.

The stable isotope results from the T5 rib and femoral samples compared to the last serial dentine section deviate slightly from the bone samples, which exhibit a lower δ15N value by 0.4‰ (Fig. 5.30). Similarly, the rib δ13C value is 0.3‰ lower than the δ15N value measured for dentine, while femoral δ13C value is 0.3‰ higher. This variability in stable carbon isotope values could be interpreted as flexibility in plant-based diet after weaning was complete; however, this variation is comparable to the reported analytical error (0.2‰), so this variability is likely not meaningful.

**Figure 5.31**: 1968 DB 1 Contents of Sarcophagus (T6). Age at death: 4 – 5 years ± 12 months.

A femur sample was unavailable for this individual due to poor skeletal preservation, so only rib values were used to interpret diet later in childhood. The rib δ15N value is lower (by 1.5‰) than the last serial dentine sample, while the δ13C values are nearly analogous with only a 0.2‰ difference (within the margin of analytical error) (Fig. 5.31). As rib values represent a period closer to death (4 – 5 years) than the dentine samples, the lower δ15N value and consistent δ13C value may be associated with depleting 15N-diet due to accelerated growth, as opposed to dietary change. If it were dietary change, there would be a comparable shift in the δ13C value, which is not the case for this individual.

**Figure 5.32:** G67/I S5 small tomb (T7). Age at death: 3 years ± 12 months.

R11 and F5 present bone stable isotope values that were used to determine diet later in life, prior to the time of death. In comparison to the last serial dentine section (12.1‰), the femoral δ15N value was exactly equivalent, while the rib stable nitrogen isotope value was slightly lower (11.5‰) (Fig 5.32). Since long bones require a greater amount of time to incorporate changes in diet, similar dentine and femoral isotope values may be a result of averaging and longer bone turnover rate. Conversely, both bone samples present higher δ13C values in comparison to the last serial dentine section. Increased bone δ13C values are most likely a result of dietary change associated with a post-weaning diet. Concurrent decreasing δ15N values are reflective of either positive nitrogen balance or 15N – depleted diet.

**Figure 5.33:** Botr 1967 G67/I SG-Tomb 6(2) Infill 1 (T8). Age at death: 3 years ± 8 months.

Since this individual (T8) died soon after tooth formation, the rib and final dentine section values represent stable isotope data from closer to the time of death. Accordingly, rib values are quite similar to the stable isotope values presented by the last serial dentine section (Fig. 5.33). Femoral stable isotope values are slightly higher than both rib and dentine values; as discussed, this may be because long bones incorporate diet over longer periods of time and thus incorporate elevated stable isotope values associated with earlier dietary phases (i.e. in-utero or exclusive breastfeeding).

**Figure 5.34:** 1972 Area NA Burial 1 of A-1 (T10). Age at death: 4 years ± 12 months.

Only a rib sample was available for analysis of individual (T10). The δ15N value of the rib sample demonstrates a further decline around the time of death while the δ13C value remained constant to the last serial dentine section (Fig. 5.34). By combining both dentine and bone δ15N data, it may appear as though weaning was still underway up until 4 years of age, however the effects of weaning would also be reflected in δ13C values. Since the δ13C value remained constant after three years of age, weaning most likely ceased around the third year and the slightly lower δ15N rib value after this point may be a result of accelerated growth or 15N – depleted diet.

**Figure 5.35:** G67/I P15 S19 (T11). Age at death: 7 – 8 years ± 24 months.

Rib and femur δ15N values for this individual were significantly lower (by 3‰) than the last serial dentine section, while the δ13C values were similar (Fig. 5.35). As exhibited by other subadult individuals in this study, low δ15N values can be reflective of an anabolic state caused by accelerated growth or possibly 15N – depleted post-weaning diet.

**Figure 5.36:** G67 Site C RZ3 Pit 3 tomb infill (T12). Age at death: 9 years ± 24 months.

Rib and femur δ15N values (9.6‰ and 9.9‰, respectively) for this individual (T12) demonstrate lower δ15N values from the last serial dentine section (10.2‰), while the δ13C values for all three tissue samples are relatively alike (Fig. 5.36). The absence of change in stable carbon isotope values accompanied by a variation in nitrogen isotope values is indicative of physiological stress between 7 and 8 years of age (indicated by the increased δ15N values at these ages). However, since femur represents a longer time average, the low value probably reflects diet from earlier life (such as between 3.6 to 5.9 years). On the other hand, rib δ15N does not necessarily ‘pick up’ the increase seen in the last two consecutive dentine samples.

**5.5 Survivors vs. Non – Survivors**

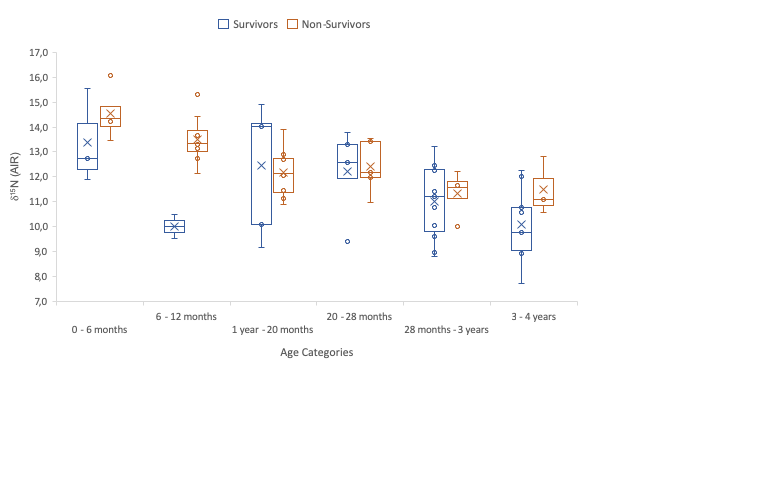
Using Figures 5.13 and 5.23, groups identified as ‘survivors’ and ‘non-survivors’ were categorized. In these figures it can be seen that δ15N values become less variable for each individual after 3.5 years of age. Hence, it is likely that the weaning process for this Iron Age sample concluded around 3.5 years; therefore, individuals who survived past this age were considered survivors, and those who died prior to 3.5 years were considered non-survivors. Individuals who died at 4 years were also categorized as non-survivors since a 6-month period between weaning and death is a relatively short period of time.

**Table 5.5:** Non-survivors and survivors of weaning (n=21).

|  |  |
| --- | --- |
| Non-Survivors (0 – 4 years)  (n=6) | G67/I p3 e2 S4 = Tomb 4 (T1) |
| 21/12/71 Tomba di bambino Ind. 1 (T4) |
| GS70 F25 (T5) |
| G67/I S5 small tomb (T7) |
| 1967 G67/I SG-Tomb 6(2) Infill 1 (T8) |
| 1972 Area NA Burial 1 of A-1 (T10) |
| Survivors (4 < years)  (n=15) | 22/5/67 Tomba 30 (T2) |
| G67/II P4 S17 Infill 3 (T3) |
| 1968 DB 1 Contents of Sarcophagus (T6) |
| No Label #2 (T9) |
| G67/I P15 S19 (T11) |
| G67 Site C RZ3 Pit 3 tomb infill (T12) |
| Botr 1972 Grave 2 bottom burial (T13) |
| No label #3 (box 6) (T15) |
| BOTR F5 20108 Layer 1047 Skull (T16) |
| PS Stefano Tomba 43 (T17) |
| PS Stefano Tomba 11(?) (T18) |
| G67III p.1 e.1 PI 1 Layer 1 pit fill (T19) |
| Botr 1968 GDA 68 R1 F1 L5 (T20) |
| Padreterno Settore II Tomba 11 Scheletro in Conessione (T21) |
| Botr 1971 Area NB, Grave 3 (T22) |

**Table 5.6:** Mean stable nitrogen and carbon isotope data for survivors (> 4 years) and non-survivors (< 4 years) between 0 to 4 years.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | 0 – 6 months | 6 – 12 months | 1 year – 20 months | 20 – 28 months | 28 months – 3 years | 3 - 4 years |
| Non – Survivors (n=6) | Mean δ15N | 14.5  (n=4) | 13.5  (n=6) | 12.2  (n=6) | 12.4  (n=4) | 11.4  (n=4) | 11.5  (n=3) |
| Mean δ13C | -18.4  (n=4) | -18.6  (n=6) | -19.2  (n=6) | -19.0  (n=4) | -19.3  (n=4) | -19.3  (n=3) |
| Survivors  (n=15) | Mean δ15N | 13.4  (n=3) | 10.0  (n=1) | 12.5  (n=3) | 12.2  (n=5) | 11.01  (n=10) | 10.1  (n=9) |
| Mean δ13C | -18.2  (n=3) | -18.9  (n=1) | -18.7  (n=3) | -19.0  (n=5) | -19.4  (n=11) | -19.5  (n=9) |



**Figure 5.37:** Box and whisker plot demonstrating the δ15N results for non-survivors and survivors for each age category.

The graphs and table above demonstrate the differences in the δ15N values for non-survivors and survivors. It can be seen that there is greater variability between non-survivors and survivors within the first year post-birth. Non-survivors show an approximately 1‰ higher δ15N mean value between 0 to 6 months, and 3.5‰ higher mean value than survivors between 6 to 12 months. Analysis of covariance demonstrates that there is a statistically significant difference between survivor and non-survivor nitrogen isotope values (refer to Table B5 in Appendix B, *F=14.691, p = 0.0002*). The issue with the data for the 6-12 month range is that there is one data point representing only one individual and is likely not representative of the entire sample; thus, this should be noted with caution. After 12 months δ15N values between the two groups become less variable. Furthermore, it can be seen that weaning is more gradual for survivors as δ15N values only decrease by ~1‰ from 6 months to 20 months. Meanwhile, non-survivors exhibit a more rapid weaning process as δ15N values decrease by ~1‰ from 6 to 12 months. Carbon isotope values demonstrate less variability between the two groups through the age categories, indicating that the transitional diet was most likely similar between the two groups (ANCOVA, *F=0.398, p =0.529*).

**5.6 Summary**

The stable isotope data presented in this chapter demonstrate that individuals from the three Iron Age sites show shifts in nitrogen and carbon data consistent with breastfeeding and weaning. Subadult dentine data exhibit earlier periods of infant and young child feeding (IYCF), while adult dentine data reveals the later periods of IYCF practices such as post-weaning diet and the eventual consumption of adult diet. The subadult bone data represent the values closer to death and clearly show that the bone samples reflect a post-weaning diet. However, there is a notable difference between isotope values obtained from rib and femoral bone samples. Rib carbon and nitrogen isotope values tend to be lower than femoral samples for younger ages (i.e., < 4 years) – with the exception of T4 – possibly due to a shorter bone turnover rate for ribs and the incorporation of earlier feeding in femoral data.

Lastly, the comparison between survivors and non-survivors reveals a possible difference in weaning patterns. Survivors demonstrate a gradual weaning process, while non-survivors demonstrate higher δ15N values in their first year and a more rapid weaning process. With that said, comparisons between survivors and non-survivors are not reliable as data for certain age categories (e.g. 6-12 months) rely on only one individual. All these trends discussed in the Chapter 5 are further explained by possible scenarios in Chapter 6.

**Chapter 6: Discussion**

**6.1 Introduction**

This chapter combines stable isotope results presented in Chapter 5 with bioarchaeological and historical evidence of breastfeeding and weaning in comparable samples from Italy and other parts of the ancient Mediterranean world. Weaning patterns within the Iron Age samples are outlined first, highlighting the intra-sample heterogeneity, relationship between weaning and mortality, evidence of sex-based variation and potential influence of agricultural practices on stable isotope interpretations. Next, differences between dentine, rib, and femoral stable isotope values are detailed, along with benefits of using a combined approach. Weaning practices at these Iron Age sites are also compared with weaning patterns found at later Roman sites using stable isotope data from this study and previous dietary research. Finally, the limitations of this study are outlined and discussed.

**6.2 Weaning Trends at Botromagno**

6.2.1 Intra-sample variation

Stable isotope results from this study provide insight into the infant and early childhood experience of individuals from southern Italy during the Iron Age. The individual weaning histories discussed in Chapter 5 demonstrate that weaning practices experienced by this group of individuals were not homogenous. This is supported by Figures 5.13 and 5.23 in the Results chapter, which illustrate the scatter in δ15N values between individuals throughout apparent breastfeeding and weaning stages for both subadults and adults. Although the general trend is a decline in δ15N values with age, the onset and cessation of the decline along with the slope are variable. In other words, the onset and cessation of weaning and the length of breastfeeding and weaning were dissimilar throughout the sample.

Numerous studies that have applied dentine microsampling have found a considerable amount of inter-individual variation in early feeding practices. As serial dentine analyses have not been conducted on pre-Roman or Roman populations, comparisons can be made with studies that have focused on geographically or temporally proximate populations. Holt (2009) discovered diverse infant and young child feeding practices amongst a group of subadults (*n =* 11) from the Greek colony at Apollonia Pontica (coast of Bulgaria) dating to the 5th – 2nd centuries BCE, using serial dentine sampling of deciduous first and second molars and permanent first molars. The author found that supplementary feeding began anywhere from six to ten months while weaning was completed on average by 3 years of age, although the precise ages of cessation could not be determined. Similarly, Kwok and colleagues (2018) used serial dentine sections to investigate breastfeeding, weaning and early diet in ancient Greece. Stable isotope analysis of 26 adults from the site of Nemea (5th – 6th centuries CE), demonstrated a range of early feeding practices. Some individuals were breastfed, while others were never breastfed or consumed breast milk for a short period of time; some dentine samples indicated weaning cessation around 2 years of age while others suggested 3 years (Kwok et al. 2018). Eerkens and colleagues (2018) also found inter-individual variability in the length of weaning in the Late Meroitic (1st – 4th century CE) population of Sai Island, Sudan. Using serial dentine samples of eleven adults, the authors found evidence of weaning completion by 2.7 years of age on average with two females demonstrating a later weaning at 3.5 years, while four males indicated an earlier weaning at 2.3 years.

Serial dentine analysis has more commonly been used on 18th and 19th centuries populations (e.g. Beaumont and Montgomery 2015; Beaumont et al. 2014; Henderson et al. 2014), and variation in breastfeeding and weaning patterns have also been noted in these studies. For instance, Smith’s (2018) study on subadults buried in a nineteenth century cemetery in Madrid, Spain, found a modest correlation in δ15N values between all individuals with significant scatter of stable isotope data suggesting flexibility in both breastfeeding and weaning. Similar results were also reported by Henderson and colleagues (2014) who found that δ15N values between individuals were most variable in the first years of life (especially up to 2 years of age) from 18th and 19th century London. Hence, the findings discussed here, along with these other examples, highlight the importance of studying weaning at an individual level as population level analyses may mask these distinct variations.

In this study, δ15N values obtained from the first increment of dentine – closest to the EDJ and representing the earliest stable isotope evidence – for both deciduous (ranging from 11.4 – 16.1‰, with an average value of 13.4 ± 1.5‰) and permanent teeth (ranging from 8.8 – 12.3‰, with an average value of 10.1 ± 1.1‰) reveal variability. Before elaborating on possible explanations for this apparent disparity, it should be noted that the first increment of dentine does not represent the same age range for all individuals. For instance, within subadults, the first increment of dentine sampled for T12 (Fig. 5.12), represents a mean age of 2 years, meanwhile, forT11 (Fig. 5.11) the first increment represents a mean age of 0.5 years. Accordingly, the variability in δ15N values for the first dentine increment is partly due to differences in the mean age associated with the serial sample. Still, individuals with first dentine increment that represent similar mean ages also exhibit heterogeneity in δ15N values. This can be seen between T8 (Fig. 5.8) and T9 (Fig. 5.9) who present first dentine increments associated with mean ages of 5 and 5.8 months, respectively, yet they display δ15N values that have a difference by 4.2‰; indicating that there are other causes for this disparity that are not associated with age. Since the earliest forming dentine in deciduous teeth will include some tissue that was deposited in utero, this variability may reflect a wider range of maternal δ15N values or in other words a wider range of maternal diet. This trend was noted by Beaumont and colleagues (2015b) who also found a wide range of δ15N values measured in the first increment of dentine from both permanent and deciduous teeth interred at the 19th century Lukin Street cemetery in London. For adult teeth in this study, there is a similar variability in the earliest/first δ15N incremental values which represent later childhood diet (~ 2.9 years of age). This variation is not necessarily reflective of maternal diet but of the different phases of transitional feeding along with flexibility in post-weaning diet which is further discussed in Sections 6.2.3 and 6.2.4.

The onset age of weaning also appears to be inconsistent throughout the Iron Age samples analysed in this thesis. As adult teeth (first permanent molars and incisors) exhibit heavy dental wear, the earliest formed dentine could not be retrieved; hence, the age at which weaning began could only be assessed in subadult individuals. Most subadult individuals (n=9) provided earlier isotopic data from within the first year of life. While the individual subadult profiles provide a unique and rich insight into the variation seen in early feeding practices, the data collected here can also be used to examine general trends in weaning. On average, the process of weaning began around 8 ± 3.4 months, but showing a great deal of variability, ranging from 4.9 – 15.5 months.

Likewise, weaning cessation also appears to be flexible in this sample. Since adult teeth present isotopic values associated with later ages (i.e. past 3 years), they can more accurately inform researchers of weaning cessation. This is because the roots of deciduous molars completely form by 3 to 3.5 years of age, at which point most δ15N profiles are still declining. In order to precisely assess the timing of weaning completion, a plateau in δ15N values should be recorded; when dealing with a short time span (i.e. the first 3.5 years of life), weaning would have to be completed well before deciduous teeth are entirely formed, so that plateauing of δ15N values is discernible. Most subadult teeth (n = 10) demonstrate variability in δ15N values between the later/last consecutive dentine sections rather than consistency or plateauing, making it difficult to precisely estimate weaning cessation. Samples in Smith (2018)’s study also demonstrated a lack of consistency in the later consecutive dentine sections leading the author to conclude that individuals either died during the weaning process or were weaned prior to 3 years of age, depending on the magnitude of decrease in both δ13C and δ15N values. With that said, these Iron Age subadults show a weaning associated trophic-level decline (~3‰) in δ15N values, which can be used to estimate weaning completion (King et al. 2017). Based only on stable nitrogen isotope decline the average age of weaning completion is 2.3 ± 0.8 years for subadults, with a range of 1.1 to 3.2 years. Individuals T3 (Figure 5.3) and T12 (Figure 5.12) are exceptions as they present isotopic data representing later stages of life. For T3 weaning was complete prior to 2.8 years, while T12 demonstrates that weaning ceased by 3.6 years. Based on the adult individuals (n = 6), weaning appears to be completed on average by 4 years of age, which is supported by the leveling of δ15N values. From these data it can be seen that adult teeth show later cessation of weaning in comparison to weaning inferred through subadult teeth; this distinction may impact survivorship as prolonged immunological benefits of breastmilk may allow individuals to live into adulthood. However, this difference may also be reflective of the inability of deciduous teeth to record diet past 3 – 3.5 years of age and later stages of weaning.

6.2.2 Relationship between weaning behaviour and mortality

It is well-established that infant and childhood mortality are strongly linked to breastfeeding duration and the quality of supplementary diet, as well as the balance between declining immunological benefits of breastmilk and increasing exposure to environmental pathogens, making weaning a precarious lifecourse transition (Katzenberg et al. 1996). This association between weaning and mortality has led many researchers to investigate past weaning behaviours through stable isotope analysis of bone collagen (e.g., Williams et al. 2005; Clayton et al. 2006; Dupras & Tocheri 2007; Jay et al. 2008; Nitsch et al. 2011). Still, there is a particular risk in reconstructing a population’s weaning behaviour from individuals who died during exclusive breastfeeding or transitional feeding due to selective mortality (Katzenberg et al. 1996). Selective mortality refers to the fact that skeletal samples consist of deceased individuals or individuals who did not survive, ultimately depicting an inaccurate representation of the living population at any given age. This effect is even more heightened when analyzing infants and young children who typically face higher mortality. Thus, dental tissues obtained from adults provide a means to reconstruct weaning behaviours of individuals who survived the process, as tooth enamel and primary dentine do not turn over to reflect dietary or physiological information from later in life (Gage et al. 1989).

Both subadult and adult individuals were grouped as survivors and non-survivors of weaning in Section 5.5 based on weaning completion by 4 years at the latest (as presented by adult teeth). Comparisons between weaning practices of survivors and non-survivors (subadults who were 4 years old or younger at the time of death) in the Iron Age samples revealed that individuals who died during the breastfeeding or weaning process had slightly higher δ15N values (1.0‰) within the first six months of life. There were not enough data for survivors in the 6 to 12-month range, so the 3.5‰ decline noted in this age range is likely due to sample size (see Section 5.5). After the first year, the δ15N values between the two groups become less variable, while, delta 13C values were relatively similar between the two groups throughout the ages.

Higher δ15N values could suggest higher maternal δ15N levels that were incorporated in utero and through exclusive breastfeeding, as it is unlikely that it could indicate a 15N-enriched supplementary diet since infants would need to consume fish to obtain values as high as 14-16‰. Further, the elevated δ15N values and a lack of difference in δ13C values may be an indicator of illness or physiological stress amongst non-survivors. Increase in δ15N values in relation to stress is produced by the body entering a state of tissue breaking or catabolism; without enough dietary protein, the body draws protein from its own tissues, where a second trophic level effect occurs resulting in higher δ15N values. Neuberger and colleagues (2013) proposed an increase by 0.17 – 1.93‰ in δ15N values of human hair, in cases of known malnourishment and starvation. This pattern has also been reaffirmed by incremental analysis of dentine from individuals associated with the 19th century Great Irish Potato Famine (Beaumont & Montgomery 2016). Hence, it is possible that higher stable nitrogen isotope values for non-survivors within the first year of life may suggest a period of stress, although since the first year of life includes a period of breastfeeding, stress cannot be differentiated from breastfeeding signal for young children.

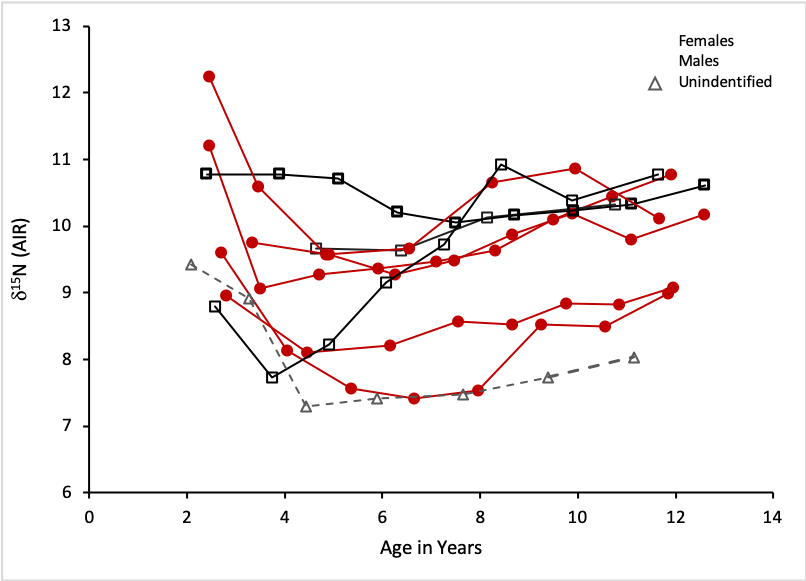
Survivors in this sample appeared to be weaned more gradually as δ15N values decreased by 1‰ over 14 months, while non-survivors exhibited a more rapid weaning through a decrease by 1‰ over only 6 months (refer to Fig 5.37). Breast milk is known to provide a secure, immunologically supportive and easily digested source of nutrition for an infant – at least for the first 6 months of life – while weaning involves gradual or abrupt withdrawal from these benefits. The combination of rapid removal of breast milk from diet (i.e. rapid removal of immunological support) along with exposure to environmental pathogens from the supplementary diet, can be detrimental for infants as their digestive systems may be unable to efficiently digest these foods and their weak immune system would not be able to fight off exposure to food-borne pathogens. Gradual weaning of the survivors (prolonging the immunological benefits of breastfeeding) could have potentially aided these individuals to survive past the weaning phase, although it should be noted that due to the small sample size this needs to be further investigated.

A similar study by Sandberg and colleagues (2014) investigated human remains from Kulubnarti, an early Christian period (550 – 800 CE) Nubian site, to analyze differences in weaning behaviours between non-survivors and survivors (Sandberg et al. 2014). Using rib collagen stable isotope data obtained from a large group of subadults, a profile of isotopic change by age was created for non-survivors. Dentine microsamples from five individuals were also used to create stable isotope profiles for survivors. Analysis of both sources of data demonstrate that the intra-tooth nitrogen isotope profiles all reached low δ15N values and plateau before the rib collagen mean-line, signifying that breastfeeding cessation occurred earlier in the five individuals (survivors) than the average individual who died during the process. The findings of this study are surprising as they not only juxtapose the results found here but contest the general idea that immunological benefits of breastfeeding result in reduced mortality (Knodel & Kintner 1977; Jackson & Nazar 2006). The authors argue that breastmilk alone is insufficient to meet the nutritional requirements of infants beyond 6 months of age, when supplemental foods with adequate nutrition must be included in the infant diet (Sandberg et al 2014). It is possible that earlier weaning in the survivors at Kulubnarti meant that nutritional demands were more adequately met with higher quality complementary foods and that non-survivors relied too heavily on breastmilk past the first 6 months without suitable complementary foods. Something that should be considered in this study is the possibility that rib values may represent delayed isotopic values due to bone turnover rates and that dentine samples probably provide a more accurate representation of diet as isotopic values are recorded (and remain unchanged) as the tooth is formed. This likely means that later weaning cessation interpreted from rib data may simply be a result of delayed incorporation of diet in bone.

Dupras and Tocheri (2007) compared infant weaning patterns at the Roman site of Dakhleh Oasis, Egypt (100 – 450 CE) based on cross-sectional isotopic data and longitudinal profiles created through bulk dentine sampling. In contrast to Sandberg and colleagues’ (2014) work and the results outlined in this study, both survivors and non-survivors were weaned gradually until 3 years of age and supplementary diet was introduced at 6 months, consistent with traditional infant feeding and weaning practices documented by Soranus and Galen (Green 1951; Tempkin 1956).

Interpretations by these studies suggest that a direct relationship between weaning and mortality may not be unequivocal. Factors such as socioeconomic status may impact the quality of life a child may experience and their survival. For example, Lewis (2002), in explanation of the high child mortality at a later period site in mediaeval Britain, argued that many rural and urban children from as young as 7 years were sent to work as apprentices (Cunningham 1995) in conditions which would have been deleterious to their health. While evidence of child labour is not available for the Iron Age period, other causes including exposure to infectious diseases and other pathogens, injuries, malnutrition, and societal practices can lead to childhood mortality.

6.2.3 Sex – based differences in Breastfeeding and Weaning Practices

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**Figure 6.1**: Stable nitrogen isotope histories for adult individuals differentiated by sex.

Variation in breastfeeding and weaning practices was observed in the serial dentine sections of permanent teeth from adult females and males; females (n = 3) displayed lower δ15N values between 28 months and 4 years of age, while males (n = 5) demonstrated lower δ15N values from 5 years of age and onwards (refer to Fig. 6.1). Considering the small sample size to draw definite conclusions, one possible explanation may be that male infants had access to a 15N-enriched supplementary diet whereas female subadults consumed 15N-depleted supplementary foods. Alternatively, low δ15N values during early childhood for females may also be indicative of an early introduction to artificial foods that were a supplement to breastmilk. This would suggest that exclusive and/or prolonged breastfeeding for female infants was less common during the Iron Age. Stable carbon isotope values between males and females were comparable, signifying a similar C3-diet during and after transitional feeding as discussed in Section 5.3.2.

There are no literary sources from the Iron Age of south Italy that can be used to understand these sex-based differences in infant and young child feeding practices. Literary evidence from the later Roman period does indicate differences in diet based on gender; however, it is uncertain how relevant these sources are to the Iron Age populations of Apulia. Past Roman medical writings by Soranus and Galen, outlined the importance of exercising control over the diet of women for physiological and moral reasons, as many of these medical treaties associated dietary constraints with chastity (Gardner 1993; Parker 1998). As men were seen as the active producers of the household and women primarily held domestic duties, females were given less provisions than their male-counterpart (Garnsey 1999). Children were often perceived to be weak with parallel humours to women and were fed a similar diet (Prowse 2011). Between the ages of five and seven years, Roman children began to assume new roles, relationships, and chores that were often differentiated based on sex; these distinctions were extended to diet, which meant male children could have received preferential food allocations (Rawson 2003). Interestingly, although the results from this study align with historical interpretations of male children consuming a more 15N-enriched supplementary diet, females were seen to have slightly elevated δ15N values from the age five and onwards (by ~1‰) suggesting that females may have been fed a more protein rich post-weaning diet. If this pattern was caused due to dietary differences then this signifies that distinctive childrearing methods were practiced in the Iron Age than the reported/expected patterns described for the Roman period.

In a similar study, early feeding histories of males and females from post-Medieval London were examined using serial dentine sections (Henderson et al. 2014). Females on average had slightly lower δ13C values (-18.9 ± 0.9‰, compared to the male average of -18.5 ± 0.8‰) and slightly higher δ15N values (12.1 ± 1.2‰ in comparison to the male average of 11.8 ± 1.3‰) throughout the first eight years of life. According to Henderson and colleagues (2014) these isotopic dissimilarities are unassociated with the rate of dentine formation because these small male to female variances are expressed over multiple years. If this variation was caused by the rate of dentine formation then a more profound difference in stable isotope values between the sexes would be seen; in addition both δ13C and δ15N profiles would express a similar pattern between males and females. Instead, along with possible dietary differences, Henderson and colleagues (2014) propose that physiological factors may also cause disparities in δ13C and δ15N values; for instance, male children are thought to have greater susceptibility to stress and infant mortality. Bouman and coworkers (2005) reported that females have a greater immune response and resistance to pathogens than males due to differing sex hormones. These physiological differences become even more evident under conditions of nutritional or biological stress during childhood. An effect of these differences could be the efficiency of metabolism of limited proteins, with females outcompeting males. Likewise, Teague and colleagues (2013) examined prominence of linear enamel hypoplasia amongst Park Street skeletal sample in London, UK, and found a higher incidence of stress in males than females during the first five years of life. It can also be noted that in Teague et al.’s (2013) investigation, three out of the five males demonstrated possible stress episodes post-weaning (ranging from a minimum of 7 years till a maximum age of 12 years), whereas one out three females demonstrated stress episodes between 8 to 11 years, through the analysis of elevated δ15N levels accompanied by decreasing or consistent δ13C values.

Research on contemporary and historic populations show that male fetuses are more likely to be born pre-term (Ingemarsson 2003), and that mortality among low birth weight infants is higher in males (Stevenson et al. 2000; Bello & Boëtsch 2007). Males also show elevated rates of neonatal deaths due to respiratory distress syndrome (Mage & Donner 2004), Sudden Infant Death Syndrome (SIDS) and infectious disease (Waldron 1987). Hence, another explanation for elevated male δ15N values between 28 months and 4 years of age may be a reflection of weaning associated stress and the inability for male children to cope with depleting immunological benefits and exposure to pathogens. Further, lower male δ15N values from five years may represent the exacerbated combination of already depleted δ15N values, the stress of accelerated growth and the failure to resourcefully metabolize limited proteins. While physiological differences between males and females may be responsible for these patterns, the limited sample size of both males and females in this study should be considered as these patterns cannot adequately be identified without further investigation.

6.2.4 Variation in Isotopic Signals of Adult Permanent Dentition

Disparity in sequential δ15N values was also observed in the teeth from the Iron Age adults, identified as groups A and B (see Fig. 5.23). Group A includes T15, T17, T18, T19, T20 and T21 and is signified by overall higher δ15N values, although T21 shows similar δ15N values to group B during transitional feeding and similar δ15N values to group A post-weaning. Group B includes T13, T16 and T22 and is characterized by statistically lower δ15N values throughout the ages (ANCOVA *F = 73.76, p < 0.0000*1). Group A comprises both males and females from all three Iron Age sites (Botromagno, Parco San Stefano and Padreterno), whereas group B consists of two males and one individual with unknown sex, all from the Botromagno site. Due to a lack of difference in the sex composition of the groups or age-related variance – as both groups include young (~ 20 years) and older individuals (35 years and older) – it is possible that this distinction is a result of socioeconomic status. As complete burial information is unavailable in published excavation reports of Botromagno, this is can only be speculated. Some other reasons could include personal choice, brief shifts in cultural practices or diet and changes in social or environmental conditions.

The absence of written records from the Iron Age period has led researchers to speculate the dietary practices and composition during this pre-Roman phase. Recently, Prowse and colleagues (2017) conducted a preliminary stable isotope analysis of human (n=27, aged 5 years and older) and faunal (n=18) bone collagen from Iron Age samples interred at Botromagno, Parco San Stefano and Padreterno. Stable carbon and nitrogen values obtained from these samples were compared between the three sites, as well as with Roman human and faunal samples found at nearby Roman Vagnari. Collagen data from the Iron Age samples indicated a lack of variability in δ13C values between the three sites, and between the Iron Age sites and Vagnari, suggesting a C3 and terrestrial protein-based diet for all skeletal samples over time; but a significant variability in δ15N values was prominent, with Parco San Stefano and Padreterno having higher δ15N values than Botromagno (Kruskal Wallis test, p<0.05) (Prowse et al. 2017). According to the authors, elevated stable nitrogen values at Parco San Stefano and Padreterno were not caused by fish consumption. The closest water source to the sites, a deep ravine that divides the archaeological site from the modern city of Gravina in Puglia, is a treacherous descent making it difficult to safely obtain fish. Prowse and colleagues (2017) suggested that the disparities in δ15N values between these Iron Age samples may be linked to the practice of manuring – the application of animal waste to replenish the soil and enhance yields – or penning. Manuring or penning cause the soil to be enriched in 15N due to bacterially-mediated reactions where the lighter 14N isotope is preferentially lost (Heaton 1986; Kendall et al. 2007); the residual soil mineral nitrogen pools are then available for plants to utilize. Animals or humans who consume products from manured soil will have higher δ15N values that may be as much as a full trophic level (3‰, or more) higher.

Fraser and coworkers’ (2011) analysis also demonstrates the impact of animal manure application on stable nitrogen isotope values. In this study, changes in δ15N were observed for a broad range of crops (cereals and pulses), under a range of manuring levels/regimes and at a series of locations extending from northwest Europe to the eastern Mediterranean. The results confirmed the potentially radical impact of manuring on δ15N values in cereals, depending on manuring level, but indicated only a slight effect on pulses, which can fix atmospheric nitrogen.

It is possible that manuring was practiced at Padreterno and Parco San Stefano causing isotopic results to demonstrate elevated levels of δ15N values in the teeth of individuals from these sites. Boogart (2004) found that manuring was a common practice in central and southeast Europe (the western loess belt and the Alpine Foreland) from multiple Neolithic sites (5500 – 2200 centuries BCE). The author carried out a series of ecological comparisons between modern weed floras from known crop husbandry regimes, and archaeobotanical samples of arable weed associated with charred crop material from the sites. The results indicated that the cultivation plots tended to be permanent rather than temporary or moving. Further, growing conditions for cereal crops (e.g. einkorn and emmer) were maintained through high inputs of labour, including manuring/middening, tillage and weeding (Boogart 2004).

Archaeological evidence from Botromagno also demonstrated that animals including cattle, swine, and caprines (i.e. sheep and goats) were domesticated and held in farms (Whitehouse 2000). This is further supported by the discovery of farmsteads throughout South Italy, including in the Metapontion countryside where dozens of 6th century BCE farm sites were uncovered (Whitehouse 2000). Along with these farmsteads, nearby necropoleis and sanctuaries were present, indicating that many individuals lived and died near areas where manure may have been utilized and penning was practiced.

In this study, Group A included three individuals (out of a total of six) interred at Parco San Stefano and Padreterno who demonstrated higher δ15N values, while group B only had individuals from Botromagno and demonstrated lower δ15N values, suggesting that manuring and penning practices at Padreterno and Parco San Stefano (the outskirts of the Iron Age settlement) could have caused this disparity. These three individuals in Group A were also included in Prowse and colleagues (2017) analysis and also showed elevated stable nitrogen values in comparison to Botromagno individuals.

Archival records show that most of the land that was not arable in Gravina was used for sheep raising from at least 16th century AD; Whitehouse (2000) suggests that the land around Botromagno was used to produce much the same agricultural products in the Iron Age and Roman periods as stated in archival records, due to the archaeological presence of similar faunal remains. Further, since occupation developed in the open countryside around large settlements such as Botromagno, it can be inferred that Botromagno was functioning as a market centre for foodstuffs produced in the surrounding countryside communities and on the farms (i.e. at Parco San Stefano and Padreterno). Since it is difficult to determine the distribution of animals between these different sites as the distinctions between the areas within a particular period are as great as the variances from one period to another within a particular area, it can only be hypothesized that the differences in Group A and B may be associated with manuring and/or penning.

**6.3 Stable Isotope Analysis of Bulk Bone Collagen and Dentine Microsamples**

A significant statistical difference between rib and femoral values could not be detected, yet Figure 5.6 in the Results chapter shows that rib δ15N and δ13C values were consistently lower than femoral values for all but one subadult individual (T.4). Differences in femoral and rib stable isotope values are most likely due to bone turnover rates as discussed below.

Several studies have used dental tissues in conjunction with bone collagen to obtain profiles of isotopic change for separate individuals (e.g., Richards et al. 2002; Reitsema & Vercellotti 2012). The combined analysis of bone collagen and dentine has been proven to be advantageous because it can avoid interpretations of diet change based on long term stable isotope averages, mortality bias, and provide early childhood as well as dietary information from later life. As previously reviewed, Sandberg and colleagues (2014) compared intra-tooth stable carbon and nitrogen profiles against data obtained from bone collagen analysis of a large sample for the Medieval site of Kulubnarti, Nubia (550 – 800 CE). Bone collagen stable isotope data from previous studies (Turner et al. 2007; Sandberg 2012) were categorized by age-at-death so that the means of each category created a pseudo-longitudinal profile. The comparison between dentine longitudinal profiles and cross-sectional data allowed Sandberg et al. (2014) to investigate variation in the life history trajectories of individuals who survived childhood with those who did not. However, this study overlooked the fact that sample rib means of stable nitrogen isotope values do not directly represent δ15N values at the time of death. Instead, these rib bone stable isotope values are averages that may include high δ15N values from few months prior to death, which may include earlier feeding stages. The turnover rate for adult rib bone is 5% per year, while the turnover rates in younger individuals is much faster; 100 – 200% of the skeleton is expected to be remodelled within the first year of life, while this rate drops by 10% per year by seven years of age (ICRP 1975). Thus, bone remodeling dynamics create a time averaging effect which could differ at various stages of life. The results presented here demonstrate that prior to 5 years of age there was greater disparity between rib and femur nitrogen and carbon isotope values, but after 5 years of age, rib and femur values become very similar. This is likely due to the differences in rib and femur bone turnover rate, with ribs having a faster turnover rate and subsequently incorporating more recent dietary constituent elements. The difference between rib and femur values decrease after 5 years as a result of a potentially homogenous diet as opposed to the variability seen during the breastfeeding and weaning process. Or alternatively, change in the disparity between rib and femur values may be due to the slowing down of bone turnover rates; thereby delaying the incorporation of accurate stable isotope values and resulting in the averaging of values by both rib and femoral bones. The unique case of T4 (see Figure 5.29) demonstrates that both rib and femoral stable nitrogen isotope values were consistent. Since these values were obtained from an individual who died at the age of 2 years, bone remodelling was still at a stage where turnover rates were relatively fast. Faster incorporation of diet by rib and femur means that the bone data is more likely to present similar and more recent isotope values.

Eerkens and collaborators (2011) conducted a comparable analysis and reconstructed the age of weaning for six individuals from the Marsh Creek Site in California by using the combined investigation of dentine and bone collagen data for each individual. Similarly, King and colleagues (2017) examined the weaning process using the combined analysis of incremental dentine sampling and bulk bone isotopic sampling for each infant and child (n=8) from the Atacama Desert, Chile (1700 BC – 1600 CE). According to these researchers, the sampling of a subset of individuals may exaggerate the observable variances between bulk bone and dentinal weaning results due to an overemphasis on idiosyncratic weaning behaviour in the past. Yet in 2015, Burt found that δ15N values between incremental dentine samples and rib samples of Fishergate House individuals were comparable. The breastfeeding patterns indicated by rib and dentine samples were similar with the onset of weaning at 2 years of age. The few fetal rib signals that were available for Fishergate House (Burt 2013) did not differ from the dentine signals that were measured by Burt in 2015, suggesting that both bone and tooth are possibly interchangeable when analyzing weaning patterns at a population level. Nevertheless, King and colleagues (2017) propose that the most useful interpretations of the past might be achieved through an integrative analysis of both methods; conducting a bulk-bone stable isotope analysis of a large number of individuals and collecting dentine microsamples for a few individuals from the same community. The use of both sampling methods (with bulk-bone sampling being the predominant method) will allow broad-scale population behaviour to be interpreted without over-emphasising idiosyncratic individual profiles. Incremental results may then be used to enhance bulk results or paleopathological evidence by allowing description of the variation in weaning practices, and reconstruction of individual stories.

A joint analysis of dentine and bone samples is also useful for a nuanced interpretation of weaning histories and dietary change, especially because it is difficult to assess whether infant or child bone δ15N values reflect the weaning process, times of accelerated or reduced growth, or periods of physiological stress. Incremental techniques, however, have the potential to allow the disentangling of dietary change from these other factors (Beaumont et al. 2015b). Assessment of elevated δ15N values or dissimilar changes in δ13C values in relation to δ15N values can reveal whether isotopic variations relate to stress rather than infant-feeding practices (Beaumont & Montgomery 2016). This means that incremental techniques may prove useful in quantifying the effects of stress on bone values used for cross-sectional sampling. For instance, the dentine isotope data presented in this study provided insight into early childhood feeding patterns. By using this information, bone values for each subadult could be interpreted to be representative of transitional diet, nutritional stress during the post-weaning period or stress associated with accelerated growth. For individual T1 (see Figure 5.26) the comparison between dentine and rib collagen data revealed lower δ15N values for the bone sample, while δ13C values between the two tissues were similar. If declining δ15N values were associated with weaning, then it is expected that δ13C values also decrease (Smith 2018). Yet, as δ13C values were consistent, decreasing δ15N values were interpreted to reflect the ‘double-burden’ of depleting nitrogen (protein) in weaning diet and higher nutrient requirements of growing children; this can ultimately lead to a nitrogen deficit and reduced isotope fractionation, also known as positive nitrogen balance (Schurr 1997). In other words, accelerated growth can cause direct routing of dietary proteins to growing tissues, bypassing the processes that results in the preferential loss of 14N, ultimately leading to lower δ15N values (Schurr 1997). This pattern has been noted by various researchers (Fuller et al. 2004; Eerkens et al. 2011; Beaumont et al. 2012; Henderson et al. 2014). Schurr (1997) found that children aged between 5 – 15 years had lower δ15N values in comparison to adults due to differences in childhood and adult metabolism. Change in bone stable carbon isotopes values as seen for T7 (Figure 5.32) could reveal dietary change as this individual began to consume a post-weaning diet. Beaumont and Montgomery (2016) noted a similar trend in their analysis of dentine δ15N and δ13C profiles of workhouse inmates from the Great Irish Famine of 19th century, where a rise in δ13C was not accompanied by a rise in δ15N. According to the authors, this was most likely due to a change from a predominantly C3 potato-based childhood diet to a diet including C4 maize. Along with a change in δ13C values, this dietary alteration may also cause lower δ15N values due to the consumption of 15N-reduced food (Beaumont & Montgomery 2016), which is also noted in T7.

**6.4 Patterns of Breastfeeding and Weaning in Roman South Italy**

The key findings of this study provide several important avenues to understanding the variability of early childhood feeding practices between Iron Age and Roman populations of Southern Italy. However, the majority of these Roman studies have used bone collagen to interpret breastfeeding and weaning patterns and cannot be directly compared to the dentine results found in the current study. Stable isotope analysis of bone collagen for early childhood diet may not be accurate due to averaged values and delayed incorporation of dietary change. For instance, estimated weaning cessation using bone data in this sample would be after 4 years of age as bone stable nitrogen and carbon values become less variable after this point (although there are no datum points for 5 or 6 years of age). Although estimation based on bone data is much later than the interpreted weaning cessation based on dental analysis (4 years being the latest age), stable isotope analysis of bone collagen from the Roman period provides bioarchaeologists with some context of childrearing practices in antiquity.

The Iron Age sites of Botromagno, Parco San Stefano, Padreterno, and Roman Vagnari represent populations that inhabited the same area in southern Italy at different times in its history. Vagnari is approximately 12 km West of the Iron Age sites that are clustered together outside the city limits of Gravina in Puglia. Semchuk’s (2016) analysis of diet at the rural Roman estate, Vagnari (1st – 4th centuries CE), used long bone data that indicated weaning completion by 5 years of age, at the latest. Subadult δ13C and δ15N bone values at Vagnari also suggest that physician-recommended weaning foods such as semi-liquid cereals were used at this site (Semchuk 2016). Specially, subadults were fed C3 plants and terrestrial proteins since cereals such as wheat were grown at Vagnari and could easily be incorporated in the weanling diet. Milk from cows and sheep could have also been included as subadults from this site exhibit a trophic level effect of +3‰ over the δ15N values of these animals. In Semchuk’s (2016) study mean δ15N and δ13C values were 12.6‰ and -18.0‰, respectively, for infants and young children aged from 0 to 3.9 years. These values closely resemble the bone data means measured in this study for subadults (10.8‰ and -19.3‰, correspondingly), suggesting that individuals during the Iron Age most likely consumed a similar weaning diet to that of Roman people from the same region. Likewise, mean δ15N and δ13C values for children between 4 to 14.9 years were 9.3‰ and -19.0‰ in Semchuk’s (2016) analysis, consistent with the adult dentine means found in this study (9.5‰ and -19.5‰), although a direct comparison of these data must be undertaken with caution due to the large time span between occupation periods.

The weaning timetable presented by Semchuk’s (2016) study only moderately supports previous isotopic research conducted at other Roman sites. For instance, as stated in Section 2.4, Prowse and colleagues (2008) found that weaning began between 1 – 2 years and concluded by 2.5 - 3 years at Isola Sacra (1st – 2nd centuries CE) using rib collagen. At the same site (2nd - 3rd centuries CE) Fitzgerald et al. (2006) used Wilson bands to suggest that weaning began around 6 months of age. Various comparable studies investigating IYCF practices during the Roman period found that weaning occurred between 6 months to 3 years, with some even suggesting that weaning ceased around 4 years of age (Fuller et al. 2006; Nehlich et al. 2011; Keenleyside et al. 2009; Howcroft et al. 2012).

Research on a contemporaneous Egyptian population (100 CE to 450 CE) located to the northwest in the Egyptian Western desert found that transitional feeding ceased at 3 years of age (Dupras & Tocheri 2007). The researchers sampled multiple teeth from each individual (n=102) to create longitudinal profiles of stable carbon and nitrogen isotope data. This method refines the temporal resolution of isotopic values; however similar to bulk bone sampling, values obtained from bulk dentine are averages over the period the tooth was formed.

A more recent study by Eerkens and colleagues (2018) used a dentine microsampling technique to evaluate weaning practices of Kingdom of Mereo from the northern part of Nubia (350 BCE and 350 CE). Based on seven individuals, isotopic results indicated that cessation of weaning occurred between 1.3 to 3.9 years of age. Similar to the results presented here, there was significant intra-sample heterogeneity. Variation within and between these societies can be a result of multiple factors including but not limited to varying local practices and beliefs, subsistence practices, gender roles, female (mother) work-load, introduction of another pregnancy, varying social perceptions of childhood and infancy and socioeconomic status.

**6.5 Research Limitations**

One of the limitations of conducting research on prehistoric populations is that bioarchaeologists do not have any textual evidence to compare with stable isotopic data. The Iron Age in Italy is one of those periods and this thesis is the first study where early childhood feeding is evaluated for a pre-Roman sample. Thus, this analysis aims to present contextual information on weaning practices in Iron Age southern Italy.

Population-level interpretations were further complicated by the relatively limited nature of this sample. Poor preservation of skeletal and dental remains and incomplete excavation records limited the sample size of this study. Still, interpretations made in this analysis provides new insights into the lived experiences of childhood in Iron Age Italy and elucidates some of the IYCF practices of this community.

Incremental sampling has also demonstrated its limitations despite its increasing application in bioarchaeological research and its ability to increase the individual-scale resolution of dietary changes. In this research, serial samples were not mutually exclusive in the time and growth they represented as sections overlap with preceding and subsequent sections due to dentine growth patterns. It can be argued that in the crown this problem is minimized as dentine growth lines are nearly horizontal, while in the root the growth lines are oriented at an oblique angle relative to the pulp cavity. Nonetheless, in this study when observing isotopic change through dentine serial section, it could be done so with caution and with the understanding that the isotopic values are averages and not exactly representative of a particular age.

Since the introduction of dentine microsampling, the potential to obtain smaller samples has grown dramatically. This has allowed researchers to partially address the issue of time averaging by being able to collection up to 20 serial samples for adult teeth (Beaumont et al. 2014) and 14 in deciduous teeth (King et al. 2018). However, the most recent study by Czermak and colleagues (2018) has established the use of microscope images of longitudinal tooth sections using high‐resolution transmission light microscopy to consider the directionality of incremental dentine growth structures during dentine microsampling. With that said, the microsampling technique outlined by Fuller et al. (2003) and Beaumont et al. (2013) is still an important technique in the absence of high‐resolution transmission microscopy images. Transverse sectioning is able to provide general dietary trends by comparing increments of dentine considering dentine accumulates in an accretionary manner from the dentin–enamel junction (DEJ) to the apical root tip (ART) over ontogenetic time. This research averaged five samples per deciduous tooth and eight samples per permanent tooth; in order to ensure that enough collagen was collected from each section, sections were cut between 2 and 3.5 mm in length. The inclusion of teeth with partly developed roots, as well as teeth with excess amounts of dental wear, also limited the number of samples that could be collected. Although this study had fewer subsamples per tooth resulting in limited amount of isotopic data, it ensured adequate carbon and nitrogen in the collagen for isotope analysis.

**6.6 Summary**

Through stable nitrogen and carbon isotope profiles, the breastfeeding and weaning patterns were revealed in this sample of subadults and adults from Botromagno, Parco San Stefano and Padreterno. While each individual presented a unique isotopic profile, a general trend could be noted with the onset of weaning from 8 ± 3.4 months and weaning cessation by 4 years of age at the latest, although completion of weaning appears to have occurred earlier for those individuals who did not survive childhood. Variation in weaning patterns was seen between males and females, possibly due to preferential food allocation for males and physiological differences between the sexes. Meanwhile, weaning variation between survivors and non-survivors was a result of varying lengths of weaning time and possible differential access to protein in transitional diet. Lastly, the combined analysis of serial dentine samples and bulk bone collagen provided a nuanced understanding of feeding trajectories for subadults. Bone values were consistently lower than δ15N and δ13C values presented by dentine as bone samples represented a period later in life, while rib values were generally lower than femoral values as a result of differences in bone turnover rates. Still, the combined analysis of tissues provided more data to evaluate the weaning process or fluctuation in isotopic profiles that could be caused by accelerated or reduced growth, or periods of physiological stress.

**Chapter 7: Conclusion**

**7.1 Summary of Findings**

This thesis is the first to investigate early childhood feeding practices in a sample of subadults and adults interred at three Iron Age sites in South Italy. Stable carbon and nitrogen isotope values obtained from serial dentine sections and bulk bone samples demonstrated great heterogeneity in weaning patterns and diet. Yet, deciduous and permanent teeth of subadults (< 10 years of age) provided the earliest isotopic data from the first year of life allowing for the estimation of an average age of weaning onset, which was calculated to be 8 ± 3.4 months. Weaning cessation amongst subadult individuals was 2.3 ± 0.8 years based on a 2‰ – 3‰ offset in δ15N values; meanwhile, dentine samples obtained from adults (<20 years) demonstrated weaning cessation by 4 years of age determined by plateauing of the isotopic data. Variation between subadults and adults may be a result of permanent teeth providing later ages, therefore later stages/phases of weaning.

The analysis of breastfeeding and weaning of survivors (individuals who survived past the weaning period) and non-survivors (subadults who were 4 years old or younger at the time of death) revealed that those who died early in childhood had higher levels of δ15N during the 0-6 month period (by 1‰). This could be due to physiological stress, or higher maternal δ15N levels. Non-survivors also demonstrated a sudden decrease δ15N over a shorter period of time indicating that they were weaned more rapidly. This could have ultimately caused their early demise as rapid removal of immunological benefits from breastmilk along with exposure to environmental pathogens from the supplementary diet can be detrimental to childhood health. Still there are many other aspects (i.e. maternal labour, socioeconomic status, infectious disease) which can impact mortality and early childhood feeding may not be the only contributor in this sample.

The combined analysis of dentine and bone collagen isotopic data revealed that femur stable isotope values were consistently higher than rib values. This is due to the delayed incorporation of diet in long bones as a result of slower bone turnover rate; yet, after five years of age both bone samples demonstrated similar isotope data possibly due to consistency in post-weaning diet. The overall combination of three different body tissues provided more nuanced interpretation of dietary change and highlighted periods of fluctuating 15N values that were likely a result of accelerated growth or physiological stress rather than transitional feeding.

Results from this thesis are significant as they show the benefits of merging incremental dentine sampling technique with bone isotope data to not only understand relationship between stable isotope values and dietary change, but also the influence of physiological growth and stress on stable isotope profiles. This analysis also emphasizes the importance of investigating early childhood feeding on an individual level along with on a population level as there are multiple factors that shape weaning strategies. Lastly, this thesis provides some pre-Roman context for early childhood feeding as no other research has been conducted on breastfeeding and weaning during this period.

**7.2 Future Directions**

While stable isotope analysis has been the first step towards investigating early childhood feeding in Iron Age South Italy, future research using radiocarbon dating would alleviate some uncertainty regarding intra-sample variation in weaning patterns and diet. Individuals included in this analysis were dated based on past archaeological excavation reports and previously labelled storage boxes in which they were kept; more precise dating could reveal whether significant variances in breastfeeding and weaning patterns between individuals may be a result of temporal differences.

Since this thesis did not include analysis of tooth enamel reserved for each individual, a potential biochemical analysis of peptides in tooth enamel could allow for sex estimation for each individual (Stewart et al. 2017). In the past, sex estimation was limited to adults but more recently the method developed by Stewart et at. (2017) is able to provide a more precise means to estimate sex for adults as well as infant and subadult remains. Sex-based variation in adults was observed in this sample, and historical sources have revealed potential differences in diet between males and females during the succeeding Roman period, thus peptide analysis would enable an efficient assessment of sex-based variation in breastfeeding and weaning practice in Iron Age Italy.

Finally, throughout this thesis it has been recognized that the sample size of adults is limited. It would be beneficial to include more adults (with a sufficient number of both males and females) in future analysis to uncover potential dietary disparities between the sexes. Future research on sex-based weaning practices in Italian Roman populations using peptide analysis and dentine microsampling would also be useful as it would provide comparative data for this study.

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**Appendix A**

Dentine and Bone Collagen Samples Prepared for Mass Spectrometry and Stable Isotope Data

**Table A1:** Carbon and nitrogen percentages, along with C:N ratios for each dentine serial section for subadult and adult teeth.

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample ID** | **C (%)** | **N (%)** | **C:N** |
| T1.01 | 43.3 | 15.8 | 3.2 |
| T1.02 | 44.1 | 16.1 | 3.2 |
| T1.03 | 43.2 | 15.7 | 3.2 |
| T1.04 | 42.6 | 15.3 | 3.3 |
| T1.05 | 41.9 | 15.0 | 3.3 |
| T2.01 | 44.4 | 16.2 | 3.2 |
| T2.02 | 42.5 | 15.6 | 3.2 |
| T2.03 | 42.8 | 15.5 | 3.2 |
| T2.04 | 42.0 | 15.5 | 3.2 |
| T3.01 | 44.7 | 16.3 | 3.2 |
| T3.02 | 43.9 | 16.2 | 3.2 |
| T3.03 | 41.0 | 15.0 | 3.2 |
| T3.04 | 43.9 | 16.1 | 3.2 |
| T4.01 | 36.4 | 13.4 | 3.2 |
| T4.02 | 43.6 | 15.8 | 3.2 |
| T4.03 | 41.4 | 14.5 | 3.3 |
| T4.04 | 40.5 | 14.3 | 3.3 |
| T4.05 | 39.4 | 14.1 | 3.3 |
| T5.01 | 44.2 | 16.2 | 3.2 |
| T5.02 | 43.7 | 16.1 | 3.2 |
| T5.03 | 42.2 | 15.5 | 3.2 |
| T5.04 | 42.0 | 15.6 | 3.1 |
| T5.05 | 43.2 | 16.1 | 3.1 |
| T5.06 | 41.7 | 15.5 | 3.1 |
| T5.07 | 39.5 | 14.6 | 3.2 |
| T6.01 | 43.1 | 15.7 | 3.2 |
| T6.02 | 42.4 | 15.2 | 3.3 |
| T6.03 | 41.5 | 15.1 | 3.2 |
| T6.04 | 41.3 | 14.9 | 3.2 |
| T6.05 | 41.5 | 14.9 | 3.2 |
| T7.01 | 42.8 | 15.9 | 3.1 |
| T7.02 | 43.6 | 16.2 | 3.1 |
| T7.03 | 42.5 | 15.7 | 3.2 |
| T7.04 | 41.8 | 15.3 | 3.2 |
| T7.05 | 40.9 | 14.8 | 3.2 |
| T8.01 | 43.3 | 15.9 | 3.2 |
| T8.02 | 43.1 | 15.7 | 3.2 |
| T8.03 | 41.8 | 15.2 | 3.2 |
| T8.04 | 42.0 | 15.2 | 3.2 |
| T8.05 | 41.5 | 15.0 | 3.2 |
| T8.06 | 41.8 | 14.8 | 3.3 |
| T9.01 | 39.8 | 14.7 | 3.2 |
| T9.02 | 44.0 | 16.3 | 3.2 |
| T9.03 | 43.4 | 16.1 | 3.1 |
| T9.04 | 43.0 | 15.9 | 3.2 |
| T9.05 | 40.9 | 15.0 | 3.2 |
| T10.01 | 41.6 | 15.3 | 3.2 |
| T10.02 | 41.1 | 14.9 | 3.2 |
| T10.03 | 41.6 | 15.1 | 3.2 |
| T10.04 | 40.8 | 14.1 | 3.4 |
| T11.01 | 43.1 | 15.9 | 3.2 |
| T11.02 | 40.8 | 15.1 | 3.2 |
| T11.03 | 42.7 | 15.7 | 3.2 |
| T11.04 | 42.0 | 15.3 | 3.2 |
| T12.01 | 43.5 | 16.1 | 3.2 |
| T12.02 | 44.0 | 15.8 | 3.3 |
| T12.03 | 44.0 | 15.7 | 3.3 |
| T12.04 | 43.6 | 15.6 | 3.3 |
| T12.05 | 42.6 | 15.8 | 3.2 |
| T12.06 | 43.5 | 15.2 | 3.3 |
| T12.07 | 43.1 | 15.6 | 3.2 |
| T13.01 | 43.5 | 15.8 | 3.2 |
| T13.02 | 43.1 | 15.8 | 3.2 |
| T13.03 | 42.4 | 15.5 | 3.2 |
| T13.04 | 42.1 | 15.4 | 3.2 |
| T13.05 | 43.1 | 15.8 | 3.2 |
| T13.06 | 41.6 | 15.2 | 3.2 |
| T13.07 | 41.4 | 15.0 | 3.2 |
| T13.08 | 42.0 | 15.0 | 3.3 |
| T15.01 | 44.2 | 16.0 | 3.2 |
| T15.02 | 44.8 | 16.1 | 3.3 |
| T15.03 | 44.3 | 15.9 | 3.3 |
| T15.04 | 43.0 | 15.7 | 3.2 |
| T15.05 | 44.2 | 16.1 | 3.2 |
| T15.06 | 44.3 | 16.2 | 3.2 |
| T15.07 | 43.8 | 15.9 | 3.2 |
| T15.08 | 43.9 | 16.0 | 3.2 |
| T15.09 | 44.2 | 15.9 | 3.2 |
| T16.01 | 45.0 | 16.3 | 3.2 |
| T16.02 | 35.5 | 12.7 | 3.3 |
| T16.03 | 34.5 | 12.4 | 3.3 |
| T16.04 | 42.2 | 15.2 | 3.2 |
| T16.05 | 43.4 | 15.6 | 3.3 |
| T16.06 | 43.2 | 15.6 | 3.2 |
| T16.07 | 44.3 | 16.0 | 3.2 |
| T16.08 | 41.6 | 14.9 | 3.3 |
| T17.01 | 47.5 | 14.5 | 3.8 |
| T17.02 | 44.7 | 15.6 | 3.3 |
| T17.03 | 42.9 | 15.4 | 3.3 |
| T17.04 | 43.5 | 14.9 | 3.4 |
| T17.05 | 40.7 | 14.8 | 3.2 |
| T18.01 | 43.3 | 15.6 | 3.2 |
| T18.02 | 43.8 | 15.9 | 3.2 |
| T18.03 | 42.8 | 15.6 | 3.2 |
| T18.04 | 43.0 | 15.6 | 3.2 |
| T18.05 | 42.1 | 15.3 | 3.2 |
| T18.06 | 42.0 | 15.2 | 3.2 |
| T18.07 | 42.1 | 15.3 | 3.2 |
| T18.08 | 42.4 | 15.4 | 3.2 |
| T19.01 | 44.5 | 16.1 | 3.2 |
| T19.02 | 44.3 | 16.1 | 3.2 |
| T19.03 | 43.6 | 15.8 | 3.2 |
| T19.04 | 43.9 | 16.0 | 3.2 |
| T19.05 | 43.6 | 16.0 | 3.2 |
| T19.06 | 43.3 | 15.8 | 3.2 |
| T19.07 | 43.6 | 15.9 | 3.2 |
| T19.08 | 41.7 | 15.1 | 3.2 |
| T19.09 | 41.4 | 15.0 | 3.2 |
| T20.01 | 44.2 | 16.1 | 3.2 |
| T20.02 | 44.4 | 16.1 | 3.2 |
| T20.03 | 44.2 | 16.1 | 3.2 |
| T20.04 | 43.6 | 15.9 | 3.2 |
| T20.05 | 43.6 | 15.9 | 3.2 |
| T20.06 | 43.3 | 15.7 | 3.2 |
| T20.07 | 42.2 | 15.3 | 3.2 |
| T21.01 | 44.6 | 16.1 | 3.2 |
| T21.02 | 43.8 | 15.6 | 3.3 |
| T21.03 | 43.5 | 15.7 | 3.2 |
| T21.04 | 39.6 | 14.3 | 3.2 |
| T21.05 | 43.4 | 15.8 | 3.2 |
| T21.06 | 44.7 | 15.4 | 3.4 |
| T21.07 | 43.5 | 15.4 | 3.3 |
| T21.08 | 43.2 | 15.6 | 3.2 |
| T22.01 | 42.2 | 15.3 | 3.2 |
| T22.02 | 41.1 | 14.6 | 3.3 |
| T22.03 | 42.8 | 15.4 | 3.2 |
| T22.04 | 39.4 | 14.1 | 3.3 |
| T22.05 | 40.4 | 14.3 | 3.3 |
| T22.06 | 42.6 | 15.1 | 3.3 |
| T22.07 | 41.6 | 14.6 | 3.3 |
| Mean (n=130) |  |  |  |

**Table A2:** Carbon and nitrogen percentages, along with C:N ratios for each subadult bone sample.

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample ID** | **C (%)** | **N (%)** | **C:N** |
| F1 | 38.5 | 13.4 | 3.3 |
| F2 | 32.1 | 10.4 | 3.6 |
| F3 | 41.5 | 14.9 | 3.3 |
| F4 | 31.0 | 9.9 | 3.7 |
| F5 | 38.9 | 13.7 | 3.3 |
| F6 | 35.4 | 12.0 | 3.4 |
| F7 | 37.2 | 13.0 | 3.3 |
| F8 | 32.1 | 11.1 | 3.4 |
| F9 | 37.5 | 13.6 | 3.2 |
| F10 | 41.7 | 14.8 | 3.3 |
| R1 | 37.2 | 13.3 | 3.3 |
| R2 | 39.7 | 14.4 | 3.2 |
| R3 | 37.8 | 13.8 | 3.2 |
| R4 | 41.1 | 14.8 | 3.2 |
| R5 | 41.3 | 14.8 | 3.3 |
| R6 | 41.8 | 15.0 | 3.3 |
| R7 | 43.8 | 15.9 | 3.2 |
| R8 | 33.9 | 11.6 | 3.4 |
| R9 | 43.4 | 15.7 | 3.2 |
| R10 | 43.3 | 15.7 | 3.2 |
| R11 | 44.4 | 16.0 | 3.2 |
| Mean (n=21) |  |  |  |

**Table A3:** Collagen yields for each tooth collected from each subadult and adult individual.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Site** | **Skeletal ID** | **Sample ID** | **Tooth Type** | **Collagen Yield (%)** |
| Botromagno | G67/I p3 e2 S4 = Tomb 4 | T1 | Decid. molar | 11.5 |
| Parco San Stefano | 22/5/67 Tomba 30 | T2 | Perm. molar | 5.9 |
| Botromagno | G67/II P4 S17 Infill 3 | T3 | Perm. molar | 8.2 |
| Botromagno | 21/12/71 Tomba di bambino Ind. 1 | T4 | Decid. molar | 6.4 |
| Botromagno | GS70 F25 | T5 | Decid. molar | 11.4 |
| Botromagno | 1968 DB 1 Contents of Sarcophagus | T6 | Decid. molar | 4.5 |
| Botromagno | G67/I S5 small tomb | T7 | Decid. molar | 7.0 |
| Botromagno | 1967 G67/I SG-Tomb 6(2) Infill 1 | T8 | Decid. molar | 4.2 |
| Parco San Stefano | No Label #2 | T9 | Decid. molar | 11.0 |
| Botromagno | 1972 Area NA Burial 1 of A-1 | T10 | Decid. molar | 4.9 |
| Botromagno | G67/I P15 S19 | T11 | Decid. molar | 7.0 |
| Botromagno | G67 Site C RZ3 Pit 3 tomb infill | T12 | Perm. molar | 8.7 |
| Botromagno | Botr 1972 Grave 2 bottom burial | T13 | Perm. molar | 8.9 |
| Botromagno | No label #3 (box 6) | T15 | Perm. molar | 13.2 |
| Botromagno | BOTR F5 20108 Layer 1047 Skull | T16 | Perm. molar | 14.4 |
| Parco San Stefano | PS Stefano Tomba 43 | T17 | Perm. molar | 12.0 |
| Parco San Stefano | PS Stefano Tomba 11(?) | T18 | Perm. Incisor | 12.2 |
| Botromagno | G67III p.1 e.1 PI 1 Layer 1 pit fill | T19 | Perm. Incisor | 10.1 |
| Botromagno | Botr 1968 GDA 68 R1 F1 L5 | T20 | Perm. molar | 10.0 |
| Padreterno | Padreterno Settore II Tomba 11 Schletro in Conessione | T21 | Perm. molar | 13.0 |
| Botromagno | Botr 1971 Area NB, Grave 3 | T22 | Perm. molar | 4.0 |

**Table A4:** Collagen yields for all subadult bone samples.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Site** | **Skeletal ID** | **Sample ID** | **Bone Type** | **Collagen Yield (%)** |
| Botromagno | 21/12/71 Tomba di bambino Ind. 1 | F1 | Femur | 4.3 |
| Botromagno | GS70 F25 | F2 | Femur | 11.2 |
| Botromagno | G67/I p3 e2 S4 = Tomb 4 | F3 | Femur | 9.4 |
| Botromagno | 1968 DB 1 Contents of Sarcophagus | F4 | Femur | 8.5 |
| Botromagno | G67/I S5 small tomb | F5 | Femur | 8.1 |
| Botromagno | G67 Site C RZ3 Pit 3 tomb infill | F6 | Femur | 9.0 |
| Parco San Stefano | 22/5/67 Tomba 30 | F7 | Femur | 4.5 |
| Botromagno | G67/II P4 S17 Infill 3 | F8 | Femur | 2.8 |
| Botromagno | G67/I P15 S19 | F9 | Femur | 3.3 |
| Botromagno | 1967 G67/I SG-Tomb 6(2) Infill 1 | F10 | Femur | 10.5 |
| Botromagno | 1967 G67/I SG-Tomb 6(2) Infill 1 | R1 | Rib | 11.0 |
| Botromagno | 1972 Area NA Burial 1 of A-1 | R2 | Rib | 12.6 |
| Botromagno | G67/I P15 S19 | R3 | Rib | 7.8 |
| Botromagno | G67/II P4 S17 Infill 3 | R4 | Rib | 8.2 |
| Botromagno | G67/I p3 e2 S4 = Tomb 4 | R5 | Rib | 10.0 |
| Botromagno | G67 Site C RZ3 Pit 3 tomb infill | R6 | Rib | 14.7 |
| Parco San Stefano | 22/5/67 Tomba 30 | R7 | Rib | 34.8 |
| Botromagno | 21/12/71 Tomba di bambino Ind. 1 | R8 | Rib | 2.1 |
| Botromagno | GS70 F25 | R9 | Rib | 81.8 |
| Botromagno | 1968 DB 1 Contents of Sarcophagus | R10 | Rib | 38.2 |
| Botromagno | G67/I S5 small tomb | R11 | Rib | 44.7 |

**Appendix B**

Statistical Analyses for Variation in Stable Isotope Data

**Table B1**: Results of ANCOVA statistical analysis for adult Group A and B and their differences in stable nitrogen isotope values.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Source** | **Type III Sum of Squares** | **df** | **Mean Square** | **F** | **Sig.** |
| Corrected Model | 42.977a | 2 | 21.5 | 37.039 | 1.8355-11 |
| Intercept | 834.687 | 1 | 834.687 | 1438.735 | 4.621-46 |
| Age Brackets | .173 | 1 | .173 | .298 | .587 |
| Groups | 42.792 | 1 | 42.792 | 73.760 | **2.6402-12** |
| Error | 37.710 | 65 | .580 |  |  |
| Total | 6145.133 | 68 |  |  |  |
| Corrected Total | 80.687 | 67 |  |  |  |

**Table B2**: Results of ANCOVA statistical analysis for adult sexed individuals and their differences in stable nitrogen isotope values.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Source** | **Type III Sum of Squares** | **df** | **Mean Square** | **F** | **Sig.** |
| Corrected Model | 4.620a | 2 | 2.310 | 2.364 | .103 |
| Intercept | 797.760 | 1 | 797.760 | 816.230 | .000 |
| Age Brackets | .422 | 1 | .422 | .431 | .514 |
| Sex | 4.261 | 1 | 4.261 | 4.359 | **.041** |
| Error | 56.688 | 58 | .977 |  |  |
| Total | 5688.445 | 61 |  |  |  |
| Corrected Total | 61.308 | 60 |  |  |  |

**Table B3** : Results of ANCOVA statistical analysis for adult sexed individuals and their differences in stable carbon isotope values.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Source** | **Type III Sum of Squares** | **df** | **Mean Square** | **F** | **Sig.** |
| Corrected Model | .099a | 2 | .050 | .248 | .782 |
| Intercept | 3279.096 | 1 | 3279.096 | 16391.345 | .000 |
| Age Brackets | .037 | 1 | .037 | .187 | .667 |
| Sex | .059 | 1 | .059 | .296 | **.588** |
| Error | 11.603 | 58 | .200 |  |  |
| Total | 22760.620 | 61 |  |  |  |
| Corrected Total | 11.702 | 60 |  |  |  |

**Table B4**: Results from paired t-test statistical analysis for nitrogen isotope values of subadult rib and femoral samples.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Mean** | **Std. Deviation** | **Std. Error Mean** | **Lower** | **Upper** | **t** | **df** | **Sig. (2 -tailed)** |
| Pair 1 – Rib 15N and  Femur 15N | -.44444 | .71351 | .23784 | -.99290 | .10401 | -1.869 | 8 | **.099** |

**Table B5:** Results of ANCOVA statistical analysis for survivors and non-survivors of weaning and their differences in stable nitrogen isotope values.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Source** | **Type III Sum of Squares** | **df** | **Mean Square** | **F** | **Sig.** |
| Corrected Model | 244.520a | 2 | 122.260 | 62.867 | 1.1587-19 |
| Intercept | 4413.960 | 1 | 4413.960 | 2269.697 | 1.9124-82 |
| Age Brackets | 86.532 | 1 | 86.532 | 44.495 | 7.2263-10 |
| Surv. Vs. Non Surv. | 28.571 | 1 | 28.571 | 14.691 | **0.000199** |
| Error | 245.037 | 126 | 1.945 |  |  |
| Total | 15385.296 | 129 |  |  |  |
| Corrected Total | 489.557 | 128 |  |  |  |

**Table B6:** Results of ANCOVA statistical analysis for survivors and non-survivors of weaning and their differences in stable carbon isotope values.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Source** | **Type III Sum of Squares** | **df** | **Mean Square** | **F** | **Sig.** |
| Corrected Model | 8.409a | 2 | 4.204 | 16.673 | .000 |
| Intercept | 9285.491 | 1 | 9285.491 | 36824.119 | .000 |
| Age Brackets | 4.966 | 1 | 4.966 | 19.694 | .000 |
| Surv. Vs. Non Surv. | .100 | 1 | .100 | .398 | **.529** |
| Error | 31.772 | 126 | .252 |  |  |
| Total | 47717.315 | 129 |  |  |  |
| Corrected Tota | 40.180 | 128 |  |  |  |