BREASTFEEDING, WEANING PRACTICES, AND CHILDHOOD DIET IN RURAL ROMAN ITALY

A STABLE ISOTOPE INVESTIGATION OF EARLY LIFE DIET FROM RURAL ROMAN ITALY USING INCREMENTAL DENTINE

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LAY ABSTRACT

In this thesis, I used samples from tooth dentine to analyze longitudinal stable isotope data for breastfeeding, weaning, and post-weaning dietary signals in a sample of 20 individuals from the Roman Imperial estate of Vagnari (1^{st} – 4^{th} c. CE) located in southern Italy. On average, children were weaned by ~3.5 years of age. Despite the similar age-at-weaning across the sample, individuals exhibited different weaning patterns and diversity in early life dietary practices at Vagnari. During and after weaning, the isotope data indicate that children were fed with C_3 plants (e.g., wheat) and terrestrial proteins such as sheep/goat and pig. I analyzed childhood and adult diet by comparing dentine stable isotope data to bone collagen results from a sub-sample of 14 individuals. There was variability between the childhood dentine data and the adult bone collagen data, where individuals appeared to eat more pork and small amounts of fish later in life. This is the first study to explore breastfeeding and weaning practices of rural Roman children in southern Italy using stable isotope analysis of tooth dentine.

ABSTRACT

This thesis examines breastfeeding, weaning, and the post-weaning diets of 18 adults (18y+) and two subadults (aged 10y – 14y) from the rural Imperial Roman (1^{st} – 4^{th} c. CE) site of Vagnari, located in southern Italy. The investigation used a new method to sample dentine sections that accounts for the oblique nature of dentine development and allowed for the assignment of age categories to diagenetically altered teeth without visible dentine lines. The results indicate Vagnari children were weaned by ~3.5y, and that some males appear to have been breastfed longer than females. Despite the similar ages-at-weaning across the sample, the individuals in this study demonstrated a variety of weaning rates (i.e., speeds or paces), post-weaning dietary trends, and changes in diet across the life course. Some individuals (n = 6) appear to have been weaned rapidly, marked by significant removal of breastmilk prior to 2.5y, with small amounts of breast milk remaining in the diet until ~3.5y. Other children (n = 9) were weaned gradually, with slow, consistent removal of breastmilk until as late as 5.0y. Throughout and after the weaning period, children were fed a diet based on C_3 plants and terrestrial proteins such as wheat, goat/sheep, and their by-products. A comparison of early life dentine and adult bone collagen signals for 14 individuals revealed changes in diet with increasing age, in which most people had increased access to higher terrestrial food sources such as pork and/or small amounts of marine food later in life. However, there was notable variation in dietary trends and practices across the sample, suggesting diverse dietary patterns among people from Vagnari.

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ABBREVIATIONS AND SYMBOLS

δ = Lowercase delta, denotes relative amounts of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ from extracted collagen

y = Years of age

‰ = Per mille (i.e., 1 of 1000), used to measure the value of $^{15}N/^{14}N$ ($\delta^{15}N$) or $^{13}C/^{12}C$ ($\delta^{13}C$)

HCl = Hydrochloric Acid

µm = micrometer (1×10^{-6}m)

Dental Notation

Number (e.g., LM_1) = Position in the mouth = maxillary/mandibular, first/second/third (e.g., Mandibular left first molar)

LM^1 = Permanent maxillary left first molar

RM_1 = Permanent mandibular right first molar

RM^1 = Permanent maxillary right first molar

C/c = Canine (permanent/deciduous)

I/i = Incisor (permanent/deciduous)

P/p = Premolar (permanent/deciduous)

DECLARATION OF ACADEMIC ACHIEVEMENT

I declare that I am the sole author of this thesis, working under the supervision of Dr. Tracy Prowse. I conducted a thorough literature review and developed a new method for incremental dentine sectioning. My dental samples were provided by Dr. Tracy Prowse, who also trained me in collagen extraction, statistical analyses, and aided in the theoretical orientation and development of this project. I prepared all samples for stable isotope analysis.

The mass spectrometry for stable isotope analysis was conducted by the Jan Veizer Stable Isotope Laboratory at the University of Ottawa. I interpreted the data with guidance from Dr. Prowse, and wrote all sections of this thesis. Feedback on my thesis was provided by my second committee member, Dr. Megan Brickley and my third reader, Dr. Tina Moffat.

1. INTRODUCTION

1.1 Research Objectives and Thesis Structure

Stable isotope analysis of human remains can provide a wealth of information on the lives of past peoples. Previous explorations of breastfeeding and weaning patterns focussed on the analysis of bone collagen, which provides a single data-point representing an individual's diet over a span of time prior to death. The critique of early breastfeeding and weaning studies was that the analysis of stable isotope data from the bones of infants and children did not accurately represent breastfeeding and weaning patterns in a population, because these were the non-survivors (Beaumont et al., 2013). In recent decades, advancements in the analysis of various human tissues, including dentine collagen (first developed/tested by Fuller et al., 2003), have opened new avenues for research on breastfeeding, weaning, and dietary patterns among individuals who survived weaning. In particular, the analysis of early-forming teeth, such as the first permanent molar, can provide information on early life dietary histories such as breastfeeding, weaning, and early childhood diet (Beaumont et al., 2013).

The objective of this study is to assess intra- and inter-individual variation in dietary patterns during infancy and childhood. This thesis uses a novel method of incremental dentine sectioning for stable isotope analysis (adapted in part from Beaumont et al., 2013) on a sample of individuals interred at a rural Imperial Roman estate (1^{st} – 4^{th} c. CE) in southern Italy. I use stable isotope data from these incremental dentine segments to assess changes in diet over time, employing the life course model and historical evidence to

contextualize observations of early life diet at Vagnari. In this analysis of infant and childhood diet at Vagnari, I address three main questions:

1) At what age were children weaned, and are there any sex-based differences in the age-at-weaning?
2) Are there differences in the rates (i.e., speed, pace) and patterns of breastfeeding and weaning among the sampled individuals?
3) What did people eat throughout the period represented by the tooth dentine, and how do the early life signals compare to the adult signals obtained from bone collagen from the same individuals?

1.2 Definitions and Terminology

Definitions of infancy and childhood are inconsistent in anthropological research. This is because age categories can be incongruent with physical development or personal identity. It is generally accepted that the term 'infant' refers to an individual under 1.0y, but distinct age categories until puberty vary depending on the objective of the study (Halcrow and Tayles, 2008). In this study, I refer to the period between infancy and five years as early childhood, as this is the broad category used in pediatric studies and typically encompasses the entirety of the breastfeeding and weaning period (Job et al., 2019). The term 'subadult' is broadly used in biological anthropology; however, this term can denote a social hierarchy typical of the Western cultural construct of childhood (Sofaer, 2006). Typically, it is used interchangeably with other terms such as 'juvenile' to refer to individuals who have not completed growth and development. I will use the term 'subadult' broadly in reference to

infants and children who have not fully developed their adult dentition. This development is typically complete by 20.5 years of age (AlQahtani et al., 2010).

Weaning is a process marked by the transition from exclusive breastfeeding to the consumption of complementary foods, followed by the complete cessation of breastmilk consumption (Herring et al., 1998; Jay, 2009; Moffat and Prowse, 2018). In this study, I will be referring to the transition from exclusive breastfeeding to the weanling diet (i.e., the introduction of complementary foods) as the 'initiation' or 'commencement' of weaning, whereas the terms 'age-at-weaning' and 'weaning age' will be used interchangeably to denote the complete cessation of breastmilk consumption.

1.3 Theoretical Orientation

The incorporation of the life course approach in the field of bioarchaeology or biological anthropology has allowed researchers to examine specific events throughout an individual's life. This theoretical perspective recognizes that individual agents affect one another, events in the life course act cumulatively, and that the life course itself is dependent on social and historical contexts (Thomas and Znaniecki, 1918; Elder et al., 2003**)**. Life course approaches in bioarchaeology view breastfeeding and weaning as a transition between two life stages (i.e., infancy and childhood), the progress of which can alter the trajectory of the life course itself (Tsutaya and Yoneda, 2015; Cheverko, 2021).

1.3.1 Life Course Approach

The life course perspective was first employed by Thomas and Znaniecki in the early 20th century (1918; cited in Elder et al., 2002). Thomas and Znaniecki (1918) used the approach

to investigate individual and collective life histories, and argued that this neglected aspect of sociological research must be examined through longitudinal studies on various individuals. Their method involved the analysis of life record data from a diverse pool of individuals to consider how past experiences throughout different periods in their lives informed their current lives, and to create a longitudinal record of individual lived experiences. The life course approach was largely ignored for several decades, preventing the exploration of established social pathways of human lives (Volkart, 1951). As a result, there was little interest in tracking life experiences from childhood to senescence, and less so about how early life experiences influenced the course of aging and development within the context of historical and geographic conditions. Longitudinal studies, following Thomas and Znaniecki's (1918) recommendations, were finally conducted in the 1960s, propelled by the need to understand how diverse political and economic conditions in various contexts affected people's lives (Merton, 1968; Elder et al., 2002). Although the term 'life course' is often used interchangeably with 'life span', 'life history' and 'life cycle'; these terms are not synonymous with the life course, and instead refer to separate approaches with different scopes of study.

The life course approach in biological anthropology was first implemented as a conceptual framework to understand the connection between individual lives and the social and biological contexts in which they operate (Sofaer, 2006; Agarwal and Glencross, 2011; Agarwal, 2016; Cheverko, 2021). As Mays et al. state, it "recognizes the cumulative nature of individual biographies" (2017, 42), as well as social conditions (such as social status and gender) that influence aging and the life course overall (Prowse, 2011). This framework

operates on the basis that an individual can possess multiple identities at a single life stage, and that this cumulative identity is fluid throughout the lifespan; as such, the life course approach explicitly investigates the impact of identity and early life experience on later life stages (Agarwal, 2016).

This model has developed in separate yet overlapping disciplines within the social and medical sciences; it was first developed in sociology as a means of understanding how developmental processes, social conditions, and social pathways interact with one another (Elder et al., 2003; Cheverko et al., 2021). Employment of the life course approach depends on five main principles: 1) development and aging are lifelong processes; 2) humans are agents who can construct and alter their life course; 3) individual life courses are guided by time and space; 4) timing of events in the life course influences individual development; and 5) shared relationships influence the development of individual life courses (Elder et al., 2003; Agarwal, 2016; Cheverko, 2021).

In addition to these principles, the life course approach utilizes two concepts: trajectories and plasticity (Thomas and Znaniecki, 1918; Elder, 1985; Elder et al., 2003). A trajectory is a psychological or social pathway over an individual's life span, which is punctuated by specific changes in state, referred to as transitions (Elder, 1985). Transitions can be categorized as age-graded movements into and out of major social roles (or institutions), such as the transition from infancy to childhood vis-à-vis dietary changes during weaning (Brim and Ryff, 1980; Elder, 1985; McLeod and Almazan, 2002). Development (i.e., a series of transitions) alters an individual's trajectory, which is made possible due to the plasticity of the human body (West-Eberhard, 2005; Sofaer, 2006;

Agarwal and Beauchesne, 2011). According to Elder (1985), these effects operate within the larger cultural and environmental conditions and are dependent upon them. Plasticity indicates that an organism is able to change in response to the environment, which is considered permanent, yet not heritable (West-Eberhard, 2005; Halcrow et al., 2020). These concepts and assumptions form an organizational framework that emphasizes the link between social and biological processes, as well as connection between life stages, thereby allowing for the investigation of human experiences in the past. Since its introduction to bioarchaeology, the life course approach has been used to investigate indicators of early life stress in populations as well as gain insight into individual life trajectories (e.g., Gowland, 2015; Agarwal, 2016; Halcrow et al., 2017; Garland et al., 2018; Crowder et al., 2019).

The individuals examined in this thesis all lived past the weaning process, and some even survived into older adulthood (50y+, n = 2). Incremental dentine analysis of the permanent first molar typically produces several isotopic signals representing diet throughout early life (i.e., birth to 8.5y) (AlQahtani et al., 2010), and the dentine signals obtained from this study are compared to the bone collagen results from later in life (in the same individuals) (Semchuk, 2016). This thesis explores the interactions between breastfeeding, weaning, and sex/gender, as well as the relationship between weaning practices, age-at-death, and mortuary treatment (i.e., grave good composition). The life course approach is therefore a useful framework to explore longitudinal changes in diet and any potential connections between early life dietary practices and social and biological circumstances later in life.

1.4 Thesis Structure

Chapter two introduces the principles of stable isotope analysis, the stable isotopes used for analysis of diet, and the applications of this analysis on dentine collagen. Diagenesis is also discussed in this chapter, as are the practical caveats and implications of this type of analysis.

Chapter three discusses the use of bones and teeth in bioarchaeology for the analysis of breastfeeding and weaning patterns. The chapter begins with an overview of the composition of bones and teeth, and a comparison of the different tissues and their use for stable isotope analysis of early life dietary patterns. The chapter concludes with a discussion of the social and biological aspects of breastfeeding and weaning practices, and the caveats and problems with the traditional method of using stable isotope data of non-survivors to assess breastfeeding and weaning in the past.

Chapter four presents the historical background and context of Roman life. This chapter introduces Roman food staples, discusses the relationship between diet, food preparation and nutrition, and identifies key historical differences between Romans of different social statuses and locations. Historical context is also provided on infancy and early childhood in Rome, including gendered differences in infant care, and breastfeeding and weaning practices.

Chapter five introduces Vagnari and provides site information, including archaeological findings from the residential and working areas of the site. The sample collection for this study is covered in this chapter, along with the methodology employed to conduct the stable isotope analysis of incremental dentine.

The results are reported in chapter six; this chapter presents individual early life dietary histories, and examines variation in breastfeeding and weaning practices including weaning age, weaning rates/schedules, age-at-death, age-related dietary trends, and the relationship between weaning practices and mortuary treatment.

Chapter seven interprets the results in the historical and archaeological contexts of rural Imperial Roman estates in southern Italy. Some outliers and notable findings are also discussed, along with an analysis of weaning practices in surrounding sites. The chapter also investigates changes in diet throughout the life course and discusses some limitations of incremental dentine analysis. Finally, the conclusion summarizes the major findings from this study and presents recommendations for future research in this field.

2. STABLE ISOTOPE ANALYSIS OF DENTINE

2.1 Stable Isotope Analysis of Collagen

Stable isotope analysis is a geochemical technique that has been adapted for use in archaeology and biological anthropology. Stable isotopes are atoms with neutron variations in the nuclear structure; for example, the stable isotopes of carbon (C) have 6 protons, but can have 6 or 7 neutrons, which are denoted as ^{12}C and ^{13}C, respectively. These isotopes are integrated into skeletal and dental tissue as constituents of the organic and inorganic matrices at the time of production by osteoblasts and odontoblasts, respectively (DeNiro end Epstein, 1978; van der Merwe and Vogel, 1978). Organisms absorb stable isotopes from food and water consumed and incorporate them into body tissues, including the organic and inorganic components of bone and teeth (Fry and Arnold, 1982; Babraj et al., 2005). The isotopic values recovered from human remains in dietary studies are reflective of the major contributors to the diet of the individual.

Dentine is 60% organic compared to bone that is 30% organic. This thesis focuses on stable isotopes obtained from dentine collagen, which is the most abundant protein in the organic phases of bones and teeth; it constitutes 85-90% of the organic matrix in these tissues (Goldberg et al., 2012; Boskey and Robey, 2013). The two major elemental constituents of collagen are carbon and nitrogen, where carbon represents 35% − 47% of collagen, and nitrogen represents 11% − 17% (Ambrose, 1990; Goldberg et al., 2012; Boskey and Gehron Robey, 2013; Katzenberg and Waters-Rist, 2018).

Isotopic analysis of human skeletal remains first occurred in the late 1970s to detect maize consumption in a population over time (Vogel and van der Merwe, 1977). Stable

isotope analysis of carbon in bone collagen continued and expanded to include the analysis of other isotopes; namely, the analysis of nitrogen and oxygen has become commonplace in the biological anthropological investigation of diet (e.g., Vogel and van der Merwe, 1977; van de Merwe and Vogel, 1978; Schoeninger and DeNiro, 1984; Schwarcz et al., 1985; Schwarcz and Schoeninger, 1991; Katzenberg et al., 1993; Wright and Schwarcz, 1998; Katzenberg and Waters-Rist, 2018). The scope of stable isotope analysis further broadened to include human provenance and migration in the 1980s (e.g., Ericson, 1985; Sealy, 1989).

2.1.1 Carbon

In addition to the two stable isotopes of carbon (^{12}C and ^{13}C) that do not decay over time, there is an unstable carbon isotope, known as the radioisotope ^{14}C, which is often employed in archaeology for dating remains and bone turnover analysis (see section 3.3). Stable isotopes are reported as a ratio of the heavier to lighter isotope (e.g., $^{13}C/^{12}C$) relative to an international standard using the delta notation ($\delta^{13}C$), and the ratio is calculated using this formula (measured in parts per mil, ‰):

Equation 2.1: Calculation used to determine relative amounts of stable isotopes in a sample.

$$\delta R\ ‰ = \left[\frac{R\ sample}{R\ standard} - 1 \right] \times 1000$$

Due to varying carbon fixation pathways in different plant species (i.e., C_3, C_4, CAM), certain plants have different $\delta^{13}C$ signatures. C_3 plants include species of wheat, barley, rice, grasses, trees, and most fruits and vegetables. Species of maize, sorghum,

(some) millet, sugar cane, and tropical grasses are all examples of C_4 plants. CAM plants mostly include cacti and other succulents.

Carbon fixation in C_4 plants is influenced by the plant's mechanism for minimizing water loss (Katzenberg and Waters-Rist, 2018). These types of plants discriminate less against the heavier isotope of carbon (^{13}C) compared to C_3 plants, which employ a different photosynthetic pathway. This results in a non-overlapping isotope signal range for both C_3 and C_4 plants, the detection of which is used to establish the source of carbon in the diet. The $\delta^{13}C$ signature of C_3 plants ranges from −22‰ to −33‰, whereas C_4 plant types produce a $\delta^{13}C$ signature ranging from −16‰ to −19‰ (Smith and Epstein, 1971). CAM plants produce an intermediate $\delta^{13}C$ signature, from −14‰ to−31‰ (Kluge and Ting, 1978). This discrimination against the heavier isotope is referred to as fractionation, and results in a difference in the $\delta^{13}C$ values between the food and the consumer. This applies to the flow of carbon from the atmosphere to plant tissues, as well as from plant tissues to animal tissues (Ambrose and Norr, 1993). The fractionation between carbon in bone collagen and that in the diet is approximately +5‰.

Sources of carbon from different tissue types yield different results; carbon from collagen is representative of ingested protein, whereas the carbon from biological apatite provides information on the whole diet (Krueger and Sullivan, 1984; Ambrose and Norr, 1993; Tieszen and Fagre, 1993). This discrepancy results from the relative composition of collagen and carbonate. Approximately 90% of the isotopic value in collagen is attributable to the presence of essential amino acids; these amino acids are unable to be synthesized by the human body and must be acquired from dietary protein (Hare et al., 1991; Tieszen and

Fagre, 1993). Carbon in bioapatite is incorporated from (blood) serum bicarbonate, which is composed of isotopes from dietary carbohydrates, lipids, and proteins (Krueger and Sullivan, 1984; Lee-Thorp et al., 1989). Analysis of collagen is more specific to breast milk consumption (i.e., a protein-rich diet) since the essential amino acids from digested proteins are preferentially deposited into bone and dental collagen of the nursing infant, bypassing any exchange of stable isotopes in the body (Krueger and Sullivan, 1984; Schwarcz, 1991).

In the case of collagen from bones or teeth, the $\delta^{13}C$ values observed indicate the type of dietary plant contribution or evidence of fish consumption (Schoeninger et al., 1983). More negative values of $\delta^{13}C$ are associated with the consumption of C_3 plants, whereas C_4-dominant diets have less negative values of $\delta^{13}C$ (Katzenberg and Waters-Rist, 2018). In humans consuming a diet consisting primarily of C_3 plants, their average collagen $\delta^{13}C$ values will be approximately -19‰ (Krueger and Sullivan, 1984; Ambrose and Norr, 1993). Humans consuming an exclusively C_4-based diet are expected to have average $\delta^{13}C$ values of ~ -8‰ in collagen (Krueger and Sullivan, 1984; Ambrose and Norr, 1993). A mixed diet would produce intermediate ranges.

Studying changes in $\delta^{13}C$ values can be used to better understand weaning practices, since a small (~1‰) increase in these values is indicative of a small trophic level effect during breastfeeding, as well as a possible shift in values due to the introduction of complementary plant foods during weaning (Wright and Schwarcz, 1998). Fuller and colleagues (2006) demonstrated a ^{13}C enrichment of ~1‰ in exclusively breastfed infants relative to maternal levels, which they attributed to the trophic level effect.

2.1.2 Nitrogen

There are two stable nitrogen isotopes, ^{14}N and ^{15}N, used for stable isotope analysis of diet. Plants can obtain nitrogen through two different mechanisms and this difference in nitrogen acquisition allows certain plants to incorporate it more readily than others (Katzenberg and Waters-Rist, 2018). Legume species (such as chickpeas, lentils, soya beans, and peanuts) have low $\delta^{15}N$ values due to their symbiotic relationship with nitrogen-fixing soil bacteria, *Rhizobium spp.* (Katzenberg and Waters-Rist, 2018), resulting in levels approaching the atmospheric value of 0‰. In contrast, non-leguminous plants (e.g., lettuce, oats, wheat, ryegrass) have higher $\delta^{15}N$ levels as a result of greater enrichment of ^{15}N.

Collagen $\delta^{15}N$ values reflect the protein source that contributed to overall diet. Lower levels of $\delta^{15}N$ are associated with the consumption of plant-based diets, lower trophic level animals (or their by-products), such as terrestrial herbivores or lower trophic species of fish and shellfish (Katzenberg and Waters-Rist, 2018). Nitrogen transfer from one tissue to another is affected by the trophic level effect; this is the process by which ^{15}N is enriched by ~ 3‰ in the consumer relative to diet (Minagawa and Wada, 1984; Schoeninger, 1985). Due to the increased number of trophic levels in aquatic ecosystems (freshwater and marine), consumption of fish will typically be reflected in an increased $\delta^{15}N$ human isotopic signal.

Nitrogen is the most frequently used element in the analysis of breastfeeding and weaning (Tsutaya and Yoneda, 2015). A ground-breaking study by Fogel and colleagues (1989) analysed $\delta^{15}N$ values in the fingernails of breastfeeding mother-infant pairs. They established that nursing infants were enriched in ^{15}N (one of the stable isotopes of nitrogen)

relative to their mothers, demonstrating a trophic level effect resulting from the consumption of the mother's tissue in the form of milk (Fogel et al., 1989). Fuller and colleagues' (2006) analysis of modern mother-infant pairs reports no increase in $\delta^{15}N$ in an exclusively artificially fed infant (via formula), supporting the argument that ^{15}N enrichment during exclusive breastfeeding is indeed due to the trophic level effect as posited by Fogel et al. (1989). These modern studies provided the basis for the stable isotope analysis of weaning practices of past groups (see Chapter 3).

2.2 Nitrogen Balance and Stress

Nitrogen imbalance occurs when the amount of nitrogen ingested does not match the amount excreted; this occurs most during growth, development, and pregnancy, as the body uses the nitrogen to produce protein (Katzenberg and Waters-Rist, 2018). A negative nitrogen balance can result in decreased $\delta^{15}N$ values due to the efficient use of nitrogen in protein production, limiting the processes that produce nitrogen fractionation. This is particularly important to note in the examination of weaning since rapid growth in the early stages in life can consume large amounts of nitrogen to keep up with developmental requirements, so $\delta^{15}N$ values can decrease below the adult female average after completion of weaning.

An increase in $\delta^{15}N$ without a rise in $\delta^{13}C$ may be an indicator of stress, and several studies have investigated this pattern in skeletal samples (e.g., Hobson et al., 1993; White and Armelagos, 1997; Katzenberg and Lovell, 1999; Fuller et al., 2005a; Williams et al., 2011; Wheeler et al., 2013; D'Ortenzio et al., 2015; Nicholls et al., 2020). For example, higher $\delta^{15}N$ values with no change or lower $\delta^{13}C$ values, may be indicative of nutritional

stress from inadequate protein consumption; in this positive nitrogen balance, the body breaks down protein for use in normal functions, while preferentially excreting ^{14}N and retaining ^{15}N, resulting in increased $\delta^{15}N$ values (Hobson et al., 1993; Katzenberg and Lovell, 1999; Fuller et al., 2005a). Water-stress in animals causes an increase of ^{14}N excretion, resulting in enriched ^{15}N in the total body protein (Ambrose and DeNiro, 1986; Heaton et al., 1986; Sealy et al., 1987). Animals and humans living in hot, arid environments are expected to have higher $\delta^{15}N$ signals (White and Schwarcz, 1994). This factor must be taken into consideration when analysing remains of individuals inhabiting various environments.

The introduction of pathogens by way of complementary food consumption during weaning can induce a physiological stress response, resulting in higher $\delta^{15}N$ values (Goodman and Armelagos, 1989). ^{15}N may become enriched as a result of physiological stress induced by various pathological conditions, such as cancer, infectious disease, injury, or pregnancy (White and Armelagos, 1997; Katzenberg and Lovell, 1999; Williams et al., 2011; Wheeler et al., 2013; D'Ortenzio et al., 2015). Due to the multiple factors that can influence nitrogen isotope values, the analysis of both $\delta^{15}N$ *and* $\delta^{13}C$ is necessary to assess impact of stress (either nutritional, physiological, or both), since higher $\delta^{15}N$ values alone cannot discern between breastfeeding and stress signals in dentine.

2.3 Stable Isotope Analysis of Dentine to Detect Breastfeeding and Weaning

2.3.1 Serial/Incremental Sectioning Methods

Incremental analysis of dentine is based on the principle that primary dentine forms at a fixed rate and is unchanged through time; the timing and direction of dentine growth are

established in the literature, and incremental sampling follows this known pattern to produce a high-resolution record of dietary signals (Hillson, 1996). Depending on the tooth type chosen, this analysis can identify both individual and maternal diet and health; analysis of deciduous teeth can provide insight into maternal and fetal health (since deciduous teeth form in utero), while permanent dentition can provide dietary signals of the early life stages of individuals who survived the weaning process and reached adulthood (Kendall et al., 2021). This allows researchers to establish a more accurate depiction of dietary changes in discrete time intervals throughout early life, including weaning patterns and practices. Moreover, if the adult dentition is used, early life diet was definitively not the cause of death, and thus this successful weaning period can be used as a model to explain weaning patterns in the past.

Researchers employed the principles of dental development to track dietary changes over time, with preliminary studies comparing signals obtained from different teeth (e.g., m1 – M1, or M1, M2, M3) (e.g., Fuller et al., 2003, 2004; Dupras and Tocheri, 2007; Sandberg et al., 2014). Fuller and colleagues (2003) were the first to employ serial analysis of three transverse dentine sections of deciduous and permanent teeth in 37 individuals from medieval Yorkshire (10^{th} – 16^{th} c. CE) to study dietary changes occurring early in life (such as weaning). They observed an enrichment of both ^{13}C and ^{15}N, with an average increase in dentine $\delta^{13}C$ relative to rib collagen of +1.2‰ ± 0.4‰, and that $\delta^{15}N$ and $\delta^{13}C$ values in deciduous second molars decreased from crown to root, which was attributed to the introduction of complementary foods during weaning (Fuller et al., 2003). Isotope signals produced from teeth that developed after cessation of breastfeeding (i.e., the third

molar and canine dentine below the CEJ) did not produce any discernible patterns in $\delta^{15}N$ and $\delta^{13}C$ values (Fuller et al., 2003). Fuller and colleagues (2003) reported a low degree of variability in $\delta^{13}C$ values between sections, suggesting a relatively constant source of dietary carbon throughout the individual's life. Conversely, $\delta^{15}N$ values displayed a large degree of variability between tooth sections, indicating variation in protein consumption practices between individuals (i.e., increase vs. decrease in protein consumption throughout life). The permanent canine crown dentine produced an enriched ^{15}N signal in six of eight individuals, reflecting an enrichment effect during breastfeeding, since this portion of the tooth begins forming at five months of age (Fuller et al., 2003). Due to the rate of tooth formation, the signal from each of these three transverse sections represents an extended time span in an individual's life, limiting the temporal resolution of the analysis.

More recently, methodological advancements and improved instrument sensitivity have allowed researchers to analyse much smaller samples with greater precision (e.g., Eerkens et al., 2011; Beaumont et al., 2013, 2014, 2018; Burt and Garvie-Lok, 2013; Beaumont and Montgomery, 2014, 2015; Henderson et al., 2014; Hillson, 2014; Sandberg et al., 2014; van der Sluis et al., 2015; Fernández-Crespo et al., 2018; Czermak et al., 2018, 2019, 2020; Goude et al., 2020; Lee et al., 2020; Ryan et al., 2020; Curtis et al., 2022; Henderson et al., 2022; Ganiatsou et al., 2023). Eerkens and colleagues (2011) examined the permanent first molars of five individuals who lived in California between approximately 4200 BP and 3100 BP. They created 5 – 10 horizontal sections (a thickness of 1mm – 2mm) per tooth for stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$. The reported $\delta^{15}N$ values in the dentine sections corresponding to 0y - 2.0y were ~2‰ – 3‰ above the adult

(rib collagen) signals, and that these values decreased in each subsequent tooth section (Eerkens et al., 2011). Eerkens and colleagues (2011) established that although $\delta^{13}C$ values were less varied throughout the tooth, the value of $\delta^{13}C$ relative to $\delta^{15}N$ displayed positive covariation prior to weaning in four of six individuals, such that both $\delta^{13}C$ and $\delta^{15}N$ decreased in concert. They interpreted the plotted values of the isotopic signals from each dentine section as a "weaning signal" demonstrating the drop in trophic level that occurs as a result of weaning. They estimated the average weaning age in this sample to be approximately 3.6y (Eerkens et al., 2011). Since the researchers made parallel horizontal sections, and because teeth grow in a stacking parabolic manner (see section 3.2), the obtained sections included the $\delta^{13}C$ and $\delta^{15}N$ signals of multiple adjacent age categories. This results in a less accurate age calibration which may not accurately depict dietary history at the correct age.

Beaumont et al. (2013) established an effective method for incremental dentine sampling and demonstrated the ability to detect short-duration events such as weaning. They compared changes in $\delta^{13}C$ and $\delta^{15}N$ values between transverse sections in M1 teeth from individuals from 19th century London (Beaumont et al., 2013). They posited that a 1mm transverse dentine section would represent 200 days of development, indicating that the top section in the crown would produce a dietary signal from the first 9 – 12 months of life (Beaumont et al., 2013). Their method produced up to 18 sections per tooth, with M1 sections representing the dietary signal from the age of six months to 9.5y, yielding a similar (albeit more detailed) longitudinal pattern compared to Eerkens and colleagues (2011).

Burt and Garvie-Lok (2013) employed a new microsampling technique using even smaller dentine sections of modern deciduous teeth to correct for the observed overlap in mineralization lines in serial samples. They first sectioned the teeth longitudinally, then partly demineralized and microsampled the dentine of one half of the tooth using a 0.75mm and 1.00mm punch. Their method, despite using significantly smaller amounts of collagen, demonstrated that microsampling of human deciduous dentine was in fact possible (Burt and Garvie-Lok, 2013). They observed a wide variation in both $\delta^{13}C$ and $\delta^{15}N$ values and a clear distinction in $\delta^{15}N$ between infants who were presumed to be breastfed versus those who were bottle-fed. Variation in $\delta^{13}C$ values was less pronounced compared to $\delta^{15}N$ values, but nonetheless displayed a small trophic level effect in infants with elevated $\delta^{15}N$ (Burt and Garvie-Lok, 2013).

Incremental analysis of transverse dentine sections has become widely applied in stable isotope studies over the past decade (e.g., Beaumont et al., 2014; Henderson et al., 2014; Beaumont and Montgomery, 2015, 2016; King et al., 2017, 2018; Goude et al., 2020). Beaumont and Montgomery (2015) introduced diagram-assisted transverse sectioning as a means of improving the accuracy and time resolution of their analyses. They produced a diagram based on age categories from the London Tooth Atlas (AlQahtani et al., 2010) to provide a guideline for age calibration of serial dentine sections; the chart that they provided indicates that a 1st molar can be segmented into as many as 20 sections, representing an age span from 6 months to 9.5y (Beaumont and Montgomery, 2015). Incremental sampling methods typically utilize evenly spaced sections of the tooth, and the diagrams created by Beaumont and Montgomery (2015, p. 409) allow for a more discrete

and deliberate selection of a particular period of life depending on the section of tooth studied. Brickley and colleagues (2019) created more accurate visual guides by estimating the location and timing of growth lines using a collation of literature on incremental dentine growth and initiation times for tooth mineralization. The diagrams accounted for the oblique nature of dentine line formation by featuring curved dentine lines that more closely align with the stacking parabolic growth patterns of teeth, improving the accuracy of data collected for each age category.

A slightly different incremental sectioning method was employed by Czermak and colleagues (2018), who created ~1.5mm thick 'slices' of demineralized dentine on the longitudinal plane before cutting along visible growth lines with the assistance of a dissecting microscope. This method yielded 16 – 20 microsamples (with a thickness of ≤ 1mm) per tooth, providing a more detailed depiction of infant and early childhood diet in individuals aged 0.5y – 8.0y (Czermak et al., 2018). Czermak et al. (2018) reported high $\delta^{15}N$ values in sections corresponding to the youngest ages that decreased (by a total of 3‰ – 5‰) with age, reaching ranges similar to corresponding bone collagen values. A smaller, but noticeable trophic level effect (0.5‰ – 1.0‰) was observed in the $\delta^{13}C$ values, matching $\delta^{15}N$ patterns in the sample (Czermak et al., 2018). The application of this method yielded similar results in a study analysing $\delta^{13}C$ and $\delta^{15}N$ of M1s and M2s from seven adults aged 20y – 59y, allowing for a comparison of early life dietary patterns between males and females (Fernández-Crespo et al., 2018). Technological advances in recent years have allowed researchers to collect minute levels of demineralized dentine using biopsy

punches as small as 0.35mm in diameter (e.g., Fernández-Crespo et al., 2018; Czermak et al., 2019, 2020; Curtis et al., 2022).

2.3.2 Benefits of Dentine Microsampling

Unlike bone, dental tissue does not remodel after its initial formation, and the rates of dentine formation and mineralization are established in the literature (section 3.2). These unique features allow researchers to determine the ages associated with certain stages of tooth formation. The fixed, chemically stable nature of dental tissues after their initial formation facilitates the maintenance of an unaltered, chronological record of information on infant and early childhood diet, stress, and disease, even after the individual has matured (e.g., Fuller et al., 2003; Beaumont et al., 2013, 2014, 2018; Burt and Garvie-Lok, 2013; Beaumont and Montgomery, 2014, 2015; Henderson et al., 2014; Sandberg et al., 2014; King et al., 2017, 2018; Czermak et al., 2020).

Microsampling is a method of sample collection that relies on the high sensitivity of mass spectrometry equipment due to the small sample volumes obtained; this method mitigates some of the caveats associated with serial sampling, namely that it does not transect as many different mineralisation lines as serial sampling, thereby shortening the time span that the signal represents (Eerkens et al., 2011; Burt and Garvie-Lok, 2013; Beaumont et al., 2013, 2014; Czermak et al., 2018, 2020; Goude et al., 2020;).

This produces a very high-resolution weaning curve with more discrete links to biological age. Moreover, the collection of smaller samples is less destructive, and can increase the amount of research that can be conducted on a single tooth. However, this analysis is limited by both the sensitivity of the machinery used and the integrity of the

samples. Most mass spectrometers can measure samples as small as 0.4mg, but the amount of collagen obtained from each tooth can greatly vary. Low collagen yield from poorly preserved samples necessitates the combination of adjacent dentine sections to obtain sufficient amounts of collagen to produce results. This decreases the total amount of dentine sections (and therefore stable isotope signals) obtained per tooth.

2.4 Diagenesis in Bone and Tooth Collagen

A seminal *Letters to Nature* article by DeNiro (1985) investigated the potential effects of postmortem changes in archaeological faunal bone collagen. He compared the ratio of carbon to nitrogen (C:N) in the extracted collagen relative to the standard range collected from modern samples of 70 species, including humans (DeNiro, 1985). According to the modern data, the expected C:N ratio of unaltered archaeological bone is 2.9 – 3.6. This study was the first to consider post-mortem chemical changes in bone collagen, challenging the implicit assumption of collagen stability made by prior applications of stable isotope analysis (DeNiro, 1985). The C:N ratio in collagen is calculated using this formula (Equation 2.2), which is determined from measuring the respective carbon and nitrogen levels in the sample:

Equation 2.2: Formula used to determine the ratio of carbon to nitrogen recovered from a sample.

$$C{:}N = \%C\ of\ sample \div \%N\ of\ sample \times 14 \div 12$$

The magnitude of the deviation from the acceptable range is reflective of the degree of post-mortem alterations, however, these changes cannot be attributed solely to age of the

sample or depositional environment. DeNiro (1985) emphasized the importance of obtaining C:N ratios prior to data analysis, which can help eliminate heavily altered samples ensuring a more accurate depiction of past diet. However, additional analyses need to be conducted in order to establish presence and extent of diagenesis. During collagen extraction, poor yield and low or skewed %C and %N are indicative diagenetic alteration, which affect the integrity of the results (Ambrose, 1990).

Ambrose (1990) examined extracted bone and tooth collagen of modern and archaeological African human and other mammal samples to determine shared characteristics in diagenetically altered collagen. The variables considered in this study were: total collagen yield (in % of total weight), atomic C:N ratios, and the relative concentrations of carbon and nitrogen in the collagen (Ambrose, 1990). His results suggested that the most obvious indicators of poor collagen preservation were low collagen relative to the total sample weight. Ambrose (1990) reported a range in modern bone collagen of 5.7% – 28.3% by weight, and suggested that well preserved herbivore and human bones have a minimum concentration of 4.0% and 1.8% collagen by weight, respectively (Ambrose, 1990). Well-preserved prehistoric samples had C:N ratios ranging from 2.9 – 3.54; samples with C:N ratios outside of this range had poor collagen yield, indicating significant diagenetic alteration. He determined that the minimum threshold of nitrogen and carbon concentrations for well-preserved bones was 4.8% and 13%, respectively (Ambrose, 1990).

Van Klinken (1999) compared the results from the Ambrose (1990) study to samples from a European population and determined that bone collagen concentration can

be as low as 1.0% in acceptable samples. He suggests that this difference in collagen yield criteria for well-preserved samples is at least in part attributable to the depositional environment. Hot climates and regions with substantial precipitation contribute to faster degradation of collagen relative to temperate and subtropical environments (Ambrose, 1990; van Klinken, 1999). Collagen from the European samples in van Klinken's (1999) study displayed characteristic values in other indicators (i.e., C:N ratio, %C and %N) at lower collagen yields compared to Ambrose's (1990) African samples, perhaps due to the fact that the temperate climate degraded collagen macromolecules to a smaller degree. Van Klinken (1999) determined a slightly narrower range of 3.1 – 3.4 for C:N ratios compared to DeNiro's (1985) original range of 2.9 – 3.6. These results demonstrated that no single indicator is sufficient in determining diagenetic alteration, and instead a combination of C:N ratios, total collagen yield, and concentrations of carbon and nitrogen should all be considered in the estimation of collagen degradation.

A more recent study by Guiry and Szpak (2021) examined the degree to which degradation of a sample can occur while still producing C:N values within the acceptable ranges. They explored the sensitivity of current ancient collagen criteria, and the extent to which diagenesis by endogenous lipid and humic acid contaminants affect archaeological bone in the post-depositional environment. The results suggested that a narrower C:N ratio range of 3.00 – 3.28 in mammals was a more appropriate parameter for acceptably preserved ancient collagen (Guiry and Szpak, 2021). Humic acid contaminants, which come from broken down organic plant matter in the depositional environment, can produce a significant shift in collagen $\delta^{13}C$ (but not $\delta^{15}N$) due to the plant type (C_3 or C_4) of the

surrounding vegetation; for example, individuals consuming primarily C_4 plants or marine sources of food may present a signal skewed towards a C_3 signature due to the fact that their remains were deposited in a C_3 soil environment (Guiry and Szpak, 2021). This is a particularly important consideration when interpreting weaning curves; diagenesis may skew $\delta^{13}C$ values negatively, obscuring the trophic level effect that is otherwise observed in a standard weaning curve. Guiry and Szpak's (2021) results demonstrate that samples with diagenetic alterations show significant correlations between C:N ratios, $\delta^{13}C$, and $\delta^{15}N$.

2.5 Caveats to Stable Isotope Analysis

Stable isotope analysis is a destructive method, which is a major ethical consideration for bioarchaeologists, particularly since archaeological human remains are a rare, finite resource and are often venerated by descendant communities. Moreover, mass spectrometers vary in sensitivity and analytical capacity; some mass spectrometers require larger sample sizes than others, restricting the ability to perform microsampling.

Delta^{15}N is sensitive to environmental changes and is typically found to be elevated in arid regions (Ambrose, 1991). In order to account for this, it is important to reconstruct the isotopic ecology of the region if one is comparing archaeological samples from different regions in weaning studies, which will provide a better depiction of the environmental conditions affecting human diet and isotope signalling. In this study, all samples are coming from the same area in southern Italy (a land-locked site in a region with temperate climate and precipitation), so extreme aridity is not a contributing factor.

Incremental dentine analysis also has notable limitations; application of this method often uses small samples, and despite the increased wealth of information that can be retrieved through the employment of this technique, the limited amount of teeth that most researchers work with may not be considered statistically significant (King et al., 2018). Additionally, the methods for microsampling are delicate and precise in nature, allowing for introduction of human and analytical error (Czermak et al., 2020). Since bone collagen analysis produces a single value (compared to 5 – 20 sections in dentine microsampling), the cost is significantly lower than when performing stable isotope analysis of dentine collagen.

Since many facilities do not possess the technical capability to analyse extremely small samples (e.g., microsamples), adaptations to these methodologies must be made in order to improve temporal resolution within the limits of technology. Image-assisted sectioning, as developed by Czermak et al. (2018), and mapping of growth lines with the help of diagrams (e.g., Beaumont and Montgomery, 2015; Brickley et al., 2019) can allow for the selective sampling of dentine, which is more accurate than transverse sectioning. Both of these methods are feasible and relatively cost-effective, and can allow researchers to improve the temporal resolution of the samples while maintaining sufficient amounts of collagen for mass spectrometry.

2.6 Conclusion

Stable isotope analysis is a useful tool to explore past diet due to its ability to retain dietary signals in bone and tooth collagen long after death. Carbon and nitrogen signals can effectively inform on the dietary choices of the studied individuals to determine the relative

contributions of different food sources (i.e., plants vs. animals) in the overall dietary signal. This tool has been employed by many researchers in the past to establish cross-sectional and longitudinal dietary patterns over time, including in the analysis of breastfeeding and weaning patterns occurring early in life.

Despite the benefits of this type of analysis on skeletal materials, there are considerations that must be taken when employing this methodology, namely that it is a destructive process. Additionally, diagenetic alterations must be assessed prior to interpretation in order to confirm that the post-depositional environment did not inhibit proper investigations of diet.

3. BREASTFEEDING AND WEANING

3.1 Introduction

The human skeleton provides a unique perspective on human experience in the past, as it records evidence of the interaction between biological and cultural influences on the body. Stable isotope analysis has developed rapidly since its introduction to the field of biological anthropology, with investigations of breastfeeding and weaning using stable isotope analysis beginning in the late 1980s. Over the decades, technology and experimental procedures have improved to a degree where researchers are now able to distinguish dietary signals from very short, discrete time periods. This is particularly important in the study of breastfeeding and weaning, a practice that has significant implications for growth, development, health, and mortality of people in the past as well as today. This chapter reviews approaches to studying breastfeeding and weaning in the past through the lens of the life course approach. It will cover the physical properties of teeth and bones, outline tooth development, the advantages and disadvantages of bone versus dentine analyses, discuss previous studies of breastfeeding and weaning in the Roman world, and address limitations of these studies in the context of the bioarchaeology of breastfeeding and weaning.

3.2 Dental Development and Morphology

The study of dental development and morphology has broad applications, ranging from dentistry to forensic sciences and biological anthropology. Dental development follows a relatively consistent temporal pattern across all individuals (AlQahtani et al., 2010; Hillson, 2014). This consistency in dental development allows researchers to establish biological

ages associated with each phase of dental growth and reliably apply them to any study population.

3.2.1 Dentinogenesis

Humans, like most mammals, have two sets of dentition. According to Hillson (2014), the deciduous dentition begins forming at approximately 14 weeks gestational age. Dental development initiates with a bundle of cells referred to as the tooth germ (Schour and Poncher, 1937; Miletich and Sharpe, 2003). There are three major stages of tooth germ development: the bud stage, the cap stage, and the bell stage. Within each tooth germ, there are four components: the enamel organ, the cervical loop, the dental follicle, and the dental papilla (Hillson, 2014). The enamel organ contains cells that are programmed to differentiate into ameloblasts, which are responsible for the formation of the enamel matrix, whereas the cervical loop and dental follicle support and facilitate the normal development of the tooth germ (Hillson, 2014; Miletich and Sharpe, 2003). Hillson (2014) notes that the dental papilla contains the undifferentiated precursors to the internal structures of the tooth, including odontoblasts, which form dentine. According to Chen and colleagues (2022), the differentiation of enamel organ cells into ameloblasts triggers a response in the dental papilla, which signals the differentiation of cells into odontoblasts.

Nanci (2018) describes that odontoblasts produce collagen fibrils and begin to deposit them parallel to the dentinoenamel junction (DEJ), forming a layer of unmineralized mantle dentine, referred to as predentine. These layers grow in a stacking, conical manner, with formation initiating at the highest points of the crown, eventually forming visible layers referred to as mineralisation lines (Schour and Poncer, 1937;

Beaumont et al., 2013). Predentine is an organic tissue composed primarily of type I collagen, and the deposition of predentine by odontoblasts triggers a signalling cascade to initiate enamel matrix formation by ameloblasts (Butler, 1995; Hillson, 2014; Nanci, 2018; Chen et al., 2022).

After the formation of predentine in the anterior portion of the osteoblast, the posterior osteoblast cell portion secretes additional non-collagenous components to facilitate the mineralization of the dentinal matrix (Arana-Chavez and Massa, 2004). Due to the structure and function of osteoblasts, the mineralization of dentine lags behind the deposition of the organic matrix by odontoblasts. Arana-Chavez and Massa (2004) point out that this lag results in approximately 10µm – 40µm of organic predentine separating the odontoblast layer from the mineralization front. The first study to assess the growth rate of dentine and enamel in humans was performed by Schour and Poncher (1937). They performed this by assessing the daily rate of dentine formation in a deciduous central incisor. Their results indicated a daily dentine synthesis rate ranging from 2.5µm/day to 6µm/day (Schour and Poncher, 1937).

Other markers, such as tetracycline, can also be used to determine the rate of human dentine formation. If an infant or child is treated with tetracycline (a commonly prescribed antibiotic), it is deposited into dentine at the time of dentine formation (Egan et al., 1972; Kawasaki et al., 1977; Kawasaki et al., 1980). Kawasaki and colleagues (1980) assessed the rate of dentine growth using tetracycline markers. They established a growth rate of 4µm/day in both unmineralized predentine and mineralized dentine, but found that predentine formation appears to follow a 24-hour cycle, whereas the mineralization process

follows a 12-hour cycle (Kawasaki et al., 1980). These rhythms affect the orientation of the collagen fibres, but a more dramatic, additional shift in collagen fibre orientation occurs on a five-day cycle, which forms the visible growth lines observed under thin-section microscopy (Kawasaki et al., 1980; Nanci, 2018).

Dean and Scandrett (1995) expanded on the initial study by Kawasaki et al. (1980) to assess the daily rate of dentine mineralization in various permanent human teeth. They determined that the rate of dentine mineralization decreases in a linear fashion, slowing as it approaches the pulp chamber. Maximum mineralization rates were reported as follows: the rate of mineralization in permanent incisors was approximately 6µm/day, 5µm/day in canines and premolars, and 3.8µm/day in first permanent molars (Dean and Scandrett, 1995). The rate of mineralization has been found to vary throughout the tooth, following a sigmoidal (i.e., S-shaped) growth curve. Dean and Scandrett (1995) observed slower mineralization in the beginning and end of the development of dentine. In permanent first molars, they determined that initial mineralization rates are slow, ranging from ~1.3µm/day – 1.5µm/day during the first 500µm of mineralization. The rates then rise and reach peak mineralization rates of ~3.8µm/day, and gradually decrease and stabilize at a mineralization rate of ~3 µm/day – 4µm/day until mineralization is complete (Dean and Scandrett, 1995).

3.2.2 Primary, Secondary and Tertiary Dentine

Teeth are mainly comprised of primary dentine. During growth and development, primary dentine is deposited in layers at known rates which allows researchers to measure an isotopic signal for an individual at specific points their life (Dean and Scandrett, 1995). As the individual ages, odontoblasts deposit additional dentine (referred to as secondary

dentine) into the pulp chamber in a process referred to as pulp recession (Nanci, 2018). Secondary dentine is also deposited by odontoblasts at a consistent rate; however, this type of dentine has a drastically reduced rate of production and metabolic activity (~70%) relative to primary dentine (Burke and Samarawickrama, 1995). According to Arora et al. (2016), secondary dentine preferentially deposits onto the floor and roof of the pulp chamber, and this process is referred to as pulp recession. Pulp recession results in a warped, constricted pulp cavity that progresses with advanced age. Despite the similar structures of primary and secondary dentine, secondary dentine has an irregular tubular structure due to dentinal tubule sclerosis (mineral deposition obstructing the dentinal tubules) and constriction within the pulp cavity (Mjör and Karlsen, 1970; Arora et al., 2016). This dentinal senescence results in an altered direction of dentine deposition and a clear delineation between primary and secondary dentine tubules (Mjör and Karlsen, 1970). These differences, however, are typically only visible with the aid of radiographs, microscopy, or histology, which presents a challenge for determining the extent of its presence (Hillson, 2005; Arora et al., 2016; Nanci, 2018).

Arana-Chavez and Massa (2004) report that tertiary dentine, also known as irregular secondary dentine (classified into reactionary and reparative subtypes), is produced as a result of trauma, caries, or attrition. Unlike primary and secondary dentine, which are deposited throughout the dentine-pulp border, tertiary dentine is only deposited by the cells surrounding the affected area through an initiated cell response (Smith et al., 1995; Klinge, 2001). The appearance of tertiary dentine varies depending on the nature of the stimulus and can present with tubules that align with secondary dentine, irregular and sparse tubules,

or have a complete absence of tubules (Smith et al., 1995; Nanci, 2018). Depending on the extent of the cell response to trauma, tertiary dentine can either be readily visible with the naked eye or may require identification via visual magnification or radiography (Hillson, 2005; Towle, 2019). For example, Hillson (2005) reports that extensive occlusal attrition triggers the production of tertiary dentine, which is deposited on the occlusal surface of heavily worn teeth; this results in a striking discoloured appearance of the exposed dentine which can usually be seen with the naked eye. The dark, distinct, colour of tertiary dentine typically presents in patches within the structure of the tooth.

Since secondary and tertiary dentine are deposited later in life, the isotopic signal derived from these tissues is not representative of infant and early childhood diet (Smith et al., 1995; Hillson, 2005). Eerkens and colleagues (2011) suggest that presence of secondary and tertiary dentine has minimal impact on the signal produced by the sample. This argument is based on two main assumptions: (1) there is minimal presence of secondary and tertiary dentine relative to primary dentine (based on visual examination with the naked eye); and (2) secondary dentine is readily deposited throughout the pulp chamber and root canal, and despite the absolute values $\delta^{13}C$ and $\delta^{15}N$ being affected, the relative differences across sections will remain the same, allowing for correct calibration of the weaning curve (Eerkens et al., 2011, p. 3109). Both of these assumptions are inconsistent with the current literature. First, identification of secondary dentine with the naked eye is typically difficult to conduct without magnification (Hillson, 2005; Arora et al., 2016; Nanci, 2018). Second, as mentioned above, Arora et al. (2016) note that secondary dentine is not uniformly distributed within the pulp chamber, and is instead preferentially deposited along the pulp

chamber floor and roof. To mitigate any potential effects of contamination by secondary dentine, researchers often opt to preferentially select teeth that are assumed to have minimal presence of secondary and tertiary dentine (i.e., an intact tooth from a younger individual with no macroscopic indication of dental disease or attrition), or mechanically clear the pulp chamber of the circumpulpal dentine with the assistance of visual magnification and a drill (Beaumont et al., 2013; Sandberg et al., 2014; Beaumont and Montgomery, 2016). Guiry and colleagues (2016) indicate that contamination by secondary and tertiary dentine may incorporate isotopic signals from dentine deposited in later life. This consideration is particularly important to note in older individuals who (typically) have more extensive attrition, damage, and presence of secondary and tertiary dentine relative to younger individuals.

3.2.3 Enamel

Enamel is the hard, mineralized material that protects the dentine structure underneath. Compared to bone, the large, tightly packed crystals of enamel contain little organic material, which aids in the preservation of carbonate and phosphate; these constituents are typically analyzed to obtain information about $\delta^{13}C$ and $\delta^{18}O$ (Wright and Schwarcz, 1998). The mineralized structure of enamel allows for greater preservation of $\delta^{13}C$ and $\delta^{18}O$, as well as a lower likelihood of significant diagenetic alterations. The mineralization rates of enamel are known, and serial sampling of this material can also provide information on weaning through the introduction and timing of complementary foods as indicated by changes in $\delta^{13}C$ and $\delta^{18}O$ (Reade et al., 2015). Since enamel does not contain nitrogen, it

is not typically used in the examination of breastfeeding and weaning. As a result, this thesis focuses on the analysis of tooth dentine rather than enamel.

3.2.4 Use of M1 for Breastfeeding and Weaning Studies

A selection of teeth can be analyzed in concert to create a dietary profile for a time span ranging from fetal development to early adolescence. Prior to incremental sectioning of a single tooth, intra-individual multi-tooth analysis was the primary method of time-contingent dietary observations using teeth (Fuller et al., 2003). Permanent first molars (M1) are typically examined in the investigation of breastfeeding and weaning, since the formation of these teeth begins around the time of birth, with crown completion occurring by 2.5y – 3.0y, and root completion by 9.0y – 10.0y (AlQahtani et al., 2010; Eerkens et al., 2011; Hillson, 2014). The development of this tooth extends the entire range of the breastfeeding and weaning phases, allowing for analysis of the entire process with good resolution. Analysis of permanent first molars allows researchers to examine breastfeeding and weaning patterns in adult remains, which controls for breastfeeding and weaning practices that may have contributed to the early death of the individual. Performing this analysis on adult remains also allows researchers to assess potential gender-based differences in weaning patterns (because sex can be reliably estimated from adult skeletons if preservation is good), allowing for a more nuanced depiction of gender and infant/childhood diet in the past.

3.3 Bone Growth and Development

Bone growth and development is discussed briefly here, because the dentine data produced in this research are compared to previously analyzed bone collagen data (Semchuk, 2016) from the same individuals. Bone is constituted of four major cell types: chondrocytes, osteoblasts, osteocytes, and osteoclasts. These cells are responsible for cartilage production and bone matrix ossification, maintenance, and resorption (Engfeldt, 1958). Osteoblasts are cells that originate from undifferentiated mesenchymal cells at the site of ossification (Engfeldt, 1958; Erlebacher et al., 1995). Depending on the site of ossification, an osteoblast will either deposit bone on a cartilaginous model (referred to as endochondral ossification), or directly onto connective tissue membrane within the mesenchyme itself (intramembranous ossification) (Engfeldt, 1958; Boskey, 1996; White and Folkens, 2005).

Endochondral ossification is the most common bone growth mechanism in the skeletal system, and is responsible for generating most of the bones in the skeleton (White and Folkens, 2005). Longitudinal bone growth occurs through endochondral ossification at the epiphyseal plates of long bones; a calcified matrix of cartilage, produced by chondrocytes, acts as a scaffold for production of bone and bone marrow by osteoblasts (Erlebacher et al., 1995). Intramembranous ossification occurs in the flat bones of the skull, the clavicle, and systemically to add bone to the outer surface of long bones (Erlebacher et al., 1995). This mechanism is responsible for appositional bone growth, which increases shaft diameter in the long bones of a growing individual (White and Folkens, 2005; Setiawati and Rahardjo, 2019).

3.3.1 Bone Remodeling and Turnover

Broader dietary analyses typically employ isotopes extracted from bone collagen (and carbonate) in ribs, long bones, or cranial elements, such as the occipital bone and petrous portion of the temporal bone (e.g., Sealy et al., 1995; Cox and Sealy, 1997; Jørkov et al., 2009; Hedges et al., 2007). Bones remodel over time with varying rates, thus providing a "blurred" image of diet throughout the individual's life (Parfitt, 2002; Hedges, 2007; Tsutaya and Yoneda, 2015). Radiocarbon analysis of tissue from various parts of the musculoskeletal system revealed a time-lag between the exposure to ^{14}C (via CO_2 from the atmosphere) and its incorporation into bone tissue (Broeker et al., 1959; Hedges et al., 2007; Ubelaker and Parra, 2011; Matsubayashi and Tayatsu, 2019). On average, ^{14}C analysis of bone collagen turnover indicates that femoral bone remodelling has a time span of 11 – 25 years, whereas vertebral trabecular bone has a three to five year time-lag between radiocarbon exposure and its incorporation into the tissue (Hedges et al., 2007; Ubelaker and Parra, 2011). This differential delay of ^{14}C incorporation into various bone tissues allowed researchers to estimate the approximate amount of time that is represented by other isotope signals (such as those used for analysis of diet) prior to the death of the individuals.

The rate of bone and collagen turnover is dependent on a multitude of factors, including bone type (e.g., cortical vs. cancellous), age, biological sex, mechanical stress, genetic predisposition, overall health, and sampling location (Libby et al., 1964; Stenhouse and Baxter, 1976; Sealy et al., 1995; Parfitt, 2002; Hedges et al., 2007; Fahy et al., 2017; Matsubayashi and Tayatsu, 2019). Libby and colleagues (1964) determined that cancellous

bone turns over three to ten times faster than cortical bone, with the former turning over approximately 10% per year, and the latter turning over 2.5% per year (Libby et al., 1964).

Bone turnover rates vary throughout the skeleton depending on bone type and location of the sample relative to the marrow and periosteum (Stenhouse and Baxter, 1976; Cox and Sealy, 1997; Jørkov et al., 2009; Fahy et al., 2017; Matsubayashi and Tayatsu, 2019). Ribs reportedly have a higher rate of turnover due to the ratio of trabecular bone to cortical bone and the dynamic nature of the thorax during respiration and movement (Sealy et al., 1995; Skedros et al., 2013). Isotopic signals from ribs are estimated to represent a dietary pattern from the last three to five years of an individual's life (Manolagas, 2000; Hedges et al., 2007). Hedges et al. (2007) reported an annual turnover rate of 1.5% – 4.0% in adult femora due to their thick cortical structure and low surface-to-volume ratio, thus providing a signal for dietary patterns for at least 10 years before the individual's death. Some recent evidence suggests that the difference between femur and rib turnover rates (and time span representation) is negligible (Fahy et al., 2017). Fahy and colleagues (2017) used osteon population density (OPD) as a proxy for bone production and remodeling rates. They did not observe a slower turnover rate in femora relative to ribs, which contradicts the long-standing idea that isotopic signals from the femoral bone reflected a longer-term dietary signal compared to rib bone collagen (Fahy et al., 2017). However, a more recent study conducted by van der Merwe and colleagues (2018) exploring osteomalacia and mineralization defects determined that the highest turnover rates were found in ribs, followed by vertebrae, with long bones having the lowest turnover rates. Bone turnover rates are still an area of uncertainty in osteology and pose a challenge when attempting to

attribute a time span to a bone collagen stable isotope dietary signal. Since the most frequently cited bone turnover rates were reported by Hedges and colleagues (2007), this thesis will be working under the assumption that isotopic signals obtained from femoral collagen represent 11 – 25 years of dietary patterns, whereas the rib collagen can identify diet from three to five years of life.

It is important to note that analysis of bone collagen to study breastfeeding and weaning necessarily requires subadult remains, which are difficult to sex, thereby limiting questions that can be asked about gendered patterns of infant feeding and care (Eerkens et al., 2011). Further, Mays et al. (2017) note that bone collagen isotope studies are by nature a cross-sectional comparison of values from subadults; this type of analysis is not able to differentiate outliers and individuals who have 'average' weaning practices. This can affect the shape of the weaning curve (see Figure 3.1), and create an inaccurate depiction of weaning practices in the past.

The analysis of subadult bone is problematic for three reasons: First, subadult skeletons are relatively rare in the archaeological record. The limited sample numbers often force researchers to compare isotopic signals from individuals who died several centuries apart, but it is not known if weaning patterns remained the same over an extended period of time (Beaumont and Montgomery, 2015). Second, bone collagen analysis using infant and child remains may provide an inaccurate depiction of breastfeeding and weaning patterns due to the lag between dietary intake and incorporation into bone. Finally, subadult remains indicate to bioarchaeologists that there was something that caused the premature death of the individual; however, the cause of death is often unknown. As such, it is difficult

to determine whether or not the death of these individuals is a result of weaning practices or another factor (e.g., infectious disease) (Knodel and Kintner, 1977; Dettwyler and Fishman, 1992; Katzenberg et al., 1996; Tsutaya and Yoneda, 2015).

Ultimately, the analysis of bone is not reflective of the isotopic changes in one individual, but is rather an indicator of general sample-based patterns and trends. This is because a bone sample can only provide one signal for an individual's long-term dietary patterns. Using bone in the study of breastfeeding and weaning produces a cross-sectional view of weaning patterns in a sample comprised of non-surviving individuals of a certain age group.

3.4 Benefit of Dentine Analysis Versus Bone

The strikingly broad range of reported turnover rates of different skeletal elements poses a significant diagnostic challenge in determining the time span represented by the particular sample. Bone is a metabolically active tissue that remodels as an individual grows and ages. Relative to bone, the nature of tooth development and lack of remodeling produces a 'locked in' signal from the time of dentine and enamel synthesis. As a result, teeth are diagnostically superior for determining reliable early life isotope signals to model diet through time at consistent, known rates (Burt and Garvie-Lok, 2013; Sandberg et al., 2014; Beaumont et al., 2018).

Isotopic analysis of incremental dentine is an approach that requires selective sampling of teeth along the known growth lines of the tooth to measure isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ during specific, discrete periods of time (Beaumont et al., 2013). In contrast to bulk bone collagen analysis, incremental dentine analysis allows researchers to view

dietary changes of an individual as they transition from one life stage to another (Mays et al., 2017). Importantly, incremental dentine analysis can be performed on both survivors and non-survivors of the weaning process to provide information about potential dietary differences contributing to earlier mortality. Due to the non-remodeling nature of teeth, incremental analysis provides a specific time frame in which these patterns are observed, which are unaltered by diet later in the individual's life (although secondary and tertiary dentine may form, see section 3.2) (Beaumont et al., 2013; Czermak, 2020).

Incremental dentine can provide a more nuanced view of infant feeding practices in the past by allowing researchers to trace the weaning process on an individual level (e.g., Fuller et al., 2003; Eerkens et al., 2011; Burt and Garvie-Lok, 2013; Beaumont et al., 2013, 2014, 2018; Henderson et al., 2014; Sandberg et al., 2014; van der Sluis et al., 2015; King et al., 2017, 2018; Beaumont and Montgomery, 2015, 2016; Czermak et al., 2018, 2019, 2020; Goude et al., 2020; Ryan et al., 2020; Avery et al., 2021). The comparison of individual stories can disentangle the effects of breastfeeding, weaning, and physiological stress to better understand these individual factors and their interactions.

Beaumont and colleagues (2018) compared results from bone collagen analysis to dentine and found that, compared to bone, dentine indicated changes in isotopic values higher than a typical trophic level shift, and indicate an earlier introduction of complementary foods. They hypothesize that this can be a result of differential growth responses to stress, in which osteoblasts cease to produce collagen while odontoblasts continue to do so. This study also provides credence for the comparison of dentine from

subadult remains to that of adults, which can produce information on dietary factors that may have contributed to morbidity and mortality (Beaumont et al., 2013).

3.5 Breastfeeding, Weaning, and the Life Course

Biological and evolutionary indicators, such as the timing of the eruption of the permanent first molar and development of the immune system, predict that a "natural age of weaning in modern human populations, based on non-human primate patterns, [ranges from] 2.5 and seven years old" (Dettwyler, 1995, p. 39). According to Sellen (2009), primate lactation length is strongly associated to adult female mass, but in humans, breastfeeding practices are flexible and dependent on maternal workload and the availability of cooperative childrearing and feeding. Prolonged breastfeeding is rarely observed in modern human groups, and especially in developed countries; the current recommendations for breastfeeding and weaning timing suggest exclusive breastfeeding for the first six months of age, followed by an 18-month weaning schedule, resulting in a complete cessation of breastfeeding by two years of age (Lawrence and Lawrence, 2021). This discrepancy between the 'natural age' of weaning and current practices can be explained by interlocking biological, environmental, and cultural factors. The comparison of breastfeeding and weaning patterns across communities can inform on numerous aspects of infant and maternal health in addition to diet, such as postpartum maternal fertility and fecundity, infant growth, development, and mortality (Dettwyler and Fishman, 1992; Stuart-Macadam and Dettwyler, 1995; Katzenberg et al., 1996; Taylor et al., 1999; Filteau, 2000; Dettwyler, 2004; Valeggia and Ellison, 2009; Halcrow et al., 2017; Halcrow, 2020).

The weaning process typically occurs in four distinct phases: i) exclusive breastfeeding (EBF), ii) breastfeeding plus infant-specific complementary foods, iii) breastfeeding plus complementary and shared foods from the adult diet, and iv) complete cessation of breastfeeding and complementary foods, shifting to adult diet (Sellen, 2009; Reynard and Tuross, 2015). Mothers contribute their own protein to the production of breast milk. The amino acids from these proteins contain nitrogen, which is further enriched in ^{15}N by the infant upon consumption. As a result of trophic level effects, breastfeeding infants have, on average, $\delta^{15}N$ levels 3‰ – 5‰ higher than those of adult females in the same population (Minagawa and Wada, 1984; Schoeninger, 1985; Fogel et al., 1989; Katzenberg, 1992; Katzenberg et al. 1993; White and Schwarcz, 1994). During the weaning process, as complementary foods are introduced and the consumption of breast milk is reduced, $\delta^{15}N$ levels of the infant decrease until complete cessation of breastfeeding.

This observable pattern, known as the weaning curve (Figure 3.1), can be detected in both longitudinal analysis of modern infant tissues (e.g., nail clippings) (e.g., Fogel et al. 1989; Fuller et al., 2006) and cross-sectional studies of archaeological remains using bone (e.g., Katzenberg et al., 1993; Tuross and Fogel, 1994; Schurr, 1997; Herring et al., 1998; Richards et al., 2002; Fuller et al., 2003; Burt, 2013; King et al., 2018; reviewed by Tsutaya and Yoneda, 2015). The shape of the weaning curve depends on four main factors: (1) the rate of collagen synthesis, (2) the age of weaning onset, (3) the rate of weaning (the speed of total replacement of breastmilk) and (4) the isotopic composition of the weaning and childhood diet (Schurr, 1997). The rate of collagen synthesis is related to the rate of growth of the individual, so faster-growing infants are expected to produce a steeper slope in the

first portion of the weaning curve (Figure 3.1B). The timing of the introduction of complementary weaning foods (with lower $\delta^{15}N$ and $\delta^{13}C$ values than breast milk) affect the rate at which $\delta^{15}N$ changes with age, and the speed at which this occurs affects the curvature and downward slope of the curve (Figure 3.1C). According to Schurr (1997), rapid weaning produces a weaning curve that features an abrupt change in the peak of the

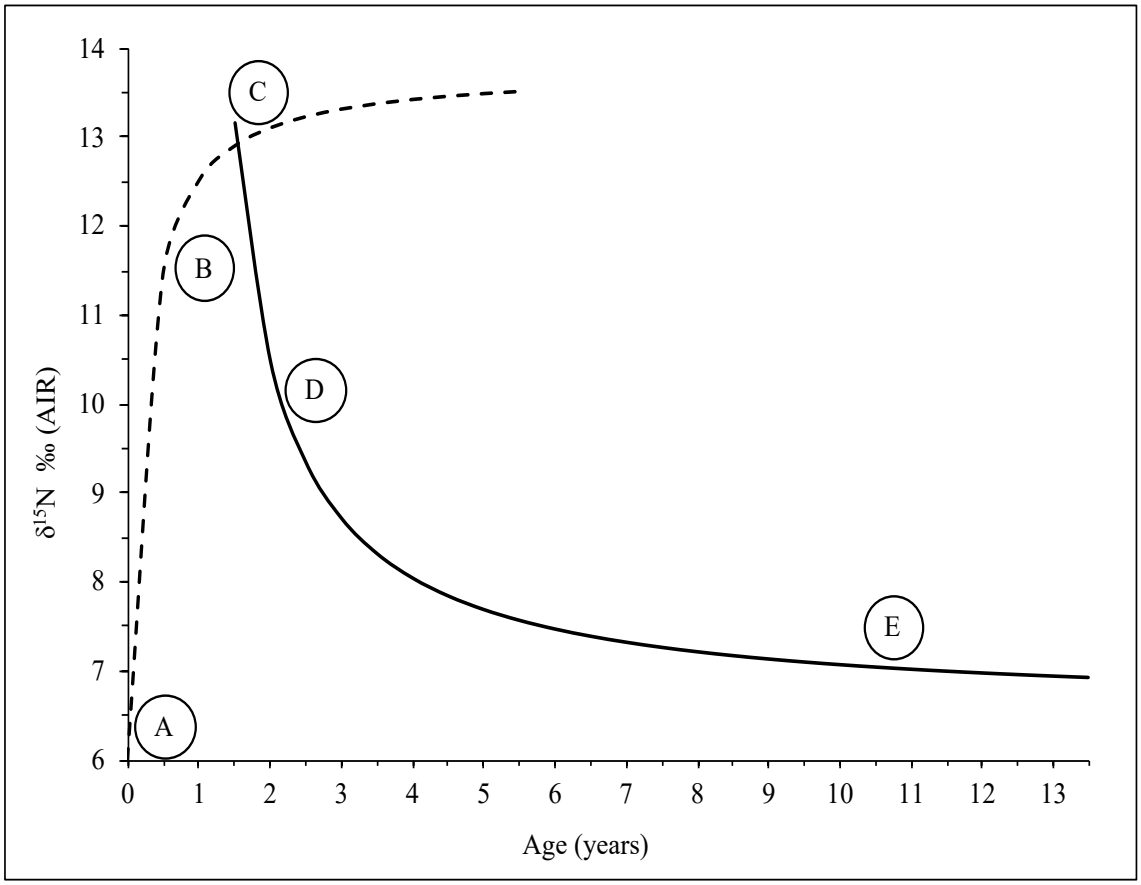

Figure 3.1: Expected weaning curve shape with increasing age in a breastfeeding individual. The dotted line represents the expected signal for exclusive breastfeeding. The solid line represents a weaning signal. A: $\delta^{15}N$ values at birth are expected to be the same as the mother (adult female). B: Exclusive breastfeeding and rapid growth rate at birth result in rapid increase in $\delta^{15}N$. C: Introduction of complementary foods with lower $\delta^{15}N$ signals relative to the mother initiate a decrease in $\delta^{15}N$ values. D: Slope indicates the rate of weaning, with a steeper downward slope indicating rapid removal of breastmilk and a shallow, gentle slope indicating a gradual removal of breastmilk. E: The post-weaning childhood diet. Adapted from Schurr (1997, p. 920).

curve and a steep downward slope (Figure 3.1D). Finally, the isotopic composition of the (post-weaning) childhood diet (Figure 3.1E) determines the equilibrium.

Upon commencement of weaning, the $\delta^{15}N$ values decrease with the decreasing contribution of breastmilk to the total diet. Breastfeeding also produces a trophic level effect for carbon, albeit smaller than nitrogen. Studies indicate that breastfed infants had a ~1‰ increase in $\delta^{13}C$ relative to the mother (Katzenberg et al., 1993; Richards et al., 2002; Fuller et al., 2003, 2006). In contrast to $\delta^{15}N$, the relationship between removal of breastmilk and $\delta^{13}C$ values is less direct, and depends on the weanling diet. Infants and young children relying on a diet based primarily on terrestrial C_3 carbon sources are expected to have more negative $\delta^{13}C$ values (Fuller et al., 2006), whereas contribution of C_4 plants (like millet, Gregoricka and Ullinger, 2022) and aquatic protein sources (e.g., fish, Eerkens and Bartelink, 2013) to the weanling diet would produce $\delta^{13}C$ signals that would either remain stable or increase with decreasing $\delta^{15}N$ values during weaning. This combination of stable isotopes can therefore be analysed to determine the introduction of complimentary foods (through analysis of $\delta^{13}C$) and monitor the duration of breast milk consumption (analysis of $\delta^{15}N$) (Fuller et al., 2006).

3.5.1 Breastfeeding and Weaning Patterns in the Roman World Using Bone Collagen

Most of the studies investigating breastfeeding and weaning practices within the Roman Empire use subadult bone collagen to conduct cross-sectional breastfeeding and weaning studies, thus represent the breastfeeding and weaning patterns of the non-survivors. Since Vagnari is a Roman period site located in southern Italy, this section will focus on publications using bone collagen retrieved from individuals who lived within in the

geographical bounds of the Roman Empire from the 1st – 5th c. CE. Studies examined in this section were selected based on their temporo-spatial proximity to Vagnari.

A total of 12 bone collagen studies fit the criteria of breastfeeding and weaning studies from the Roman Empire that included at least one estimated age-at-weaning (Table 3.1). The sites range in date from the 1st c. BCE until the mid-6th c. CE, but only the samples dated from the 1st – 5th c. CE are included in this analysis. The geographic distribution is also large, including sites from Italy (Prowse et al., 2008; Killgrove and Tykot, 2013; De Angelis et al., 2020a), Egypt (Dupras et al., 2001), Spain (Rissech et al., 2016), Switzerland (Bourbou et al., 2019) and the UK (Fuller et al., 2006; Nehlich et al., 2011; Powell et al., 2014; Redfern et al., 2012, 2018).

There is considerable variation in the reported age of commencement of weaning and transition to post-weaning diet in the bone collagen data; studies by Dupras et al. (2001)

Table 3.1: Studies that conducted stable isotope analysis on bone collagen samples to investigate weaning practices.

Studies Investigating Breastfeeding and Weaning within the Roman Empire (1st c. BCE - 5th c. CE)				
Bulk Bone Collagen Analysis				
Study Authors	**Site/Location**	**Time Period**	**Weaning Start Age (years)**	**Weaning End Age (years)**
Bourbou et al., 2019	Aventicum, Switzerland	1st - 3rd c. CE	0 - 1.5	>3.0
De Angelis et al., 2020	Quarto Cappello del Prete, Italy	1st - 3rd c. CE	-	3.0
Dupras et al., 2001	Kellis 2, Dakhleh Oasis, Egypt	~250 - 450 CE	0.5	~3.0
Fuller et al., 2006	Queenford Farm, UK	Late 4th - Mid 6th c.	-	2.0 - 4.0
Killgrove and Tykot, 2013	Casal Bertone, Italy	2nd - 3rd c. CE	-	>3.0
	Castellaccio Europarco, Italy	1st - 3rd c. CE	-	>3.0
Nehlich et al., 2011	Oxfordshire, UK	1st - 6th c. CE	-	2.0 - 4.0
Powell et al., 2014	London, England	48 - 410 CE	1.0	4.0
Prowse et al., 2008	Isola Sacra, Italy	1st - 3rd c. CE	<1.0	2.0 - 2.5
Redfern et al., 2012	Dorset, England	1st c. BCE - 4th c. CE	0.5	3.0 - 4.0
Redfern et al., 2018	London/Dorset, England	1st c. BCE - 4th c. CE	-	<5.0
Rissech et al., 2016	Carrer Ample 1, Spain	1st - 4th c. CE	-	4.0 - 5.0

and Redfern and colleagues (2012) suggest that exclusive breastfeeding occurred until ~0.5y of age, while others (e.g., Powell et al., 2014; Bourbou et al., 2019) observed exclusive breastfeeding signals until as late as 1.0y – 1.5y of age. The average age for completion of weaning (i.e., complete cessation of breastfeeding, calculated from all ten studies in this category) indicates that children transitioned to a post-weaning diet by ~3.5y.

The weaning patterns using bulk collagen analysis of subadults are briefly addressed below, since their results inform on the early life diet for non-survivors and can be used to explore the relationship between weaning and early life mortality (especially when compared to non-survivors). Prowse and colleagues (2008) examined the rib collagen isotope signals (n = 37) and dental pathology (n = 78) of juveniles aged 1.0y – 12y from the site of Isola Sacra near Rome. Their analysis of deciduous dental health (i.e., caries, dental calculus, abscesses, tooth wear, and antemortem tooth loss) aided in the exploration of complementary foods in weanling diets, since the consumption of soft, sticky foods rich in carbohydrates is associated with an increase in the occurrence of dental pathologies (Powell, 1985). The stable isotope results indicated that individuals under 2.0y had significantly higher $\delta^{15}N$ signals relative to older individuals, with the highest shifts occurring between 1.0y – 2.0y of age (Prowse et al., 2008). Similarly, $\delta^{13}C$ signals were highly variable among individuals aged 1.0y – 2.5y, and were significantly lower in individuals older than 2.0y. These decreases in both $\delta^{15}N$ and $\delta^{13}C$ values after ~2.0y of age are consistent with the initiation of weaning as a child transitions from the high trophic level breastmilk to a lower trophic level post-weaning diet. Dental pathological data indicated an increase in dental caries and occlusal wear, particularly in molars, with

increasing age. These data overall suggest that weaning commenced around 1.0y of age and lasted until complete cessation around 2.5y; dental caries and occlusal wear may indicate the use of soft, carbohydrate-rich complementary foods as well as some grit (resulting in tooth wear), likely from feeding vessels and teething aids (Prowse et al., 2008).

Killgrove and Tykot conducted a study in 2013 to compare dietary differences between urban and suburban cemeteries surrounding Rome. They included the stable isotope findings of three subadults (aged ≤4.0 y), all of whom displayed higher $\delta^{15}N$ values, which ranged from +1‰ – +3‰ relative to the adult female mean (Killgrove and Tykot, 2013). However, among these individuals, only one displayed a signal which indicated a diet based primarily on breastmilk. The $\delta^{15}N$ signals still showed some degree of ^{15}N enrichment between ages 2.0y – 3.0y; based on these results, they estimated that weaning was complete after 3.0y of age.

In a more recent study of diet and weaning from the community of Quatro Capello del Prete, a *suburbium* near Rome, De Angelis and colleagues (2020a) examined the rib collagen of a total of 50 adult and subadult individuals, the majority of whom were aged younger than 6.0y. This site is unique relative to the others since the demographic distribution of the cemetery is heavily skewed towards subadults and females. The analysis was aided by the addition of data previously recovered from Quatro Capello del Prete (De Angelis et al., 2020b), resulting in a total of 71 individuals. The $\delta^{15}N$ signals of individuals aged 0y – 3.0y were distinct from both children aged 3.0y+ as well as adults. Interestingly, the individuals at Quatro Capello del Prete appear to have been weaned later than the ages observed in other Italian bone collagen studies, indicating a complete weaning age of ~3.0y.

It is important to note, however, that the individuals from De Angelis and colleagues' (2020a) study were obtained from a cemetery that consisted mostly of subadults and females.

The early mortality of the individuals analyzed in these bone collagen studies may result in an inaccurate representation of weaning patterns observed in healthy populations, because the weaning pattern cannot be ruled out as the cause of premature mortality. Nonetheless, these data can be compared to the incremental dentine data to elucidate potential dietary differences between survivors and non-survivors.

3.5.2 Breastfeeding and Weaning Using Incremental Dentine Analysis: The Survivors

Despite the growing popularity of incremental dentine studies for the investigation of breastfeeding and weaning, the evidence for breastfeeding and weaning in Imperial Roman populations (particularly in rural and southern European sites) is scant. An exploration of published studies investigating breastfeeding and weaning practices using incremental dentine is provided in Table 3.2. This table includes a small number of studies on Roman populations, plus additional studies from other sites across the world.

To date, only four publications have explored breastfeeding and weaning patterns using incremental dentine analysis of individuals from sites within the Empire (Dupras and Tocheri, 2007; Cocozza et al., 2021; Ganiatsou et al., 2022, 2023). However, all of these studies take place outside of Italy, and only one study (Cocozza et al., 2021) examined a rural population, whereas the rest explored urban and suburban sites such as Thessaloniki in Greece (Ganiatsou et al., 2022, 2023) and the Dakhleh Oasis in Egypt (Dupras and Tocheri, 2007). The studies that explore weaning timelines using incremental dentine on

populations within the Empire suggest that exclusive breastfeeding occurred until 0.5y – 1.0y of age (Cocozza et al., 2021; Ganiatsou et al., 2022), and weaning continued until complete cessation, which in some cases did not occur until age 5.0y (Cocozza et al., 2021). The average ages of weaning for each of the mentioned studies are summarized in Table 3.2.

Table 3.2: Weaning schedules for studies examining breastfeeding and weaning using incremental dentine, separated by the temporo-spatial bounds of the Roman Empire.

Studies Investigating Breastfeeding and Weaning within the Roman Empire (1st c. BCE - 5th c. CE)				
Incremental Dentine Analysis				
Study Authors	**Site/Location**	**Time Period**	**Weaning Start Age (years)**	**Weaning End Age (years)**
Cocozza et al., 2021	Bainesse, UK	1st - 5th c. CE	0.5	2.0 - 5.0
Dupras and Tocheri, 2007	Kellis 2, Dakhleh Oasis, Egypt	~250 - 450 CE	-	3.0
Ganiatsou et al., 2022	Thessaloniki, Greece	168 BCE – 324 CE	1.0	2.0
Ganiatsou et al., 2023	Thessaloniki, Greece	4th c. BCE - 15th c. CE	-	1.0 - 3.0
Incremental Dentine Breastfeeding and Weaning Studies from Outside (Temporo-Spatial) the Roman Empire				
Incremental Dentine Analysis				
Study Authors	**Site/Location**	**Time Period**	**Weaning Start Age (years)**	**Weaning End Age (years)**
Beaumont et al., 2018	Raunds Furnells, England	978 - 1040 CE	0.5 - 2.0	2.5 - 3.0
Burt and Garvie-Lok, 2013	Alberta, Canada	Modern	-	~1.0
Crowder et al., 2019	Transylvania, Romania	4th - 7th c. CE	0.1 - 0.5	3.0
Eerkens et al., 2011	Marsh Creek Site, California	4300 - 3100 BCE	-	3.6
Eerkens et al., 2018	Sai Island, Sudan	1st - 4th c. CE	-	2.7
Fernández-Crespo et al., 2018	Iberia, Spain	3500 - 2900 BCE	~1.0	≤4.0
Fuller et al., 2003	Wharram Percy, England	10th - 16th c. CE	-	~2.0
Goude et al., 2020	Liguria, Italy	Neolithic	~1.0	3.0 - 4.0
Henderson et al., 2014	London, England	18th - 19th c. CE	~0.5	2.0?
King et al., 2017	Atacama Desert, Chile	1700 BCE - 1600 CE	1.6	2.5 - 3.5
King et al., 2018	Atacama Desert, Chile	1700 BCE - 1450 CE	-	1.5 - 3.5
Kwok et al., 2018	Nemea, Greece	5th - 6th c. CE	>1.0	2.6
Salahuddin and Prowse, 2023	Iron Age South Italy	1000 BCE	~0.4	~3.1
Sandberg et al., 2014	Kulubnarti, Nubia	550 - 800 CE	-	2.0
Schmidt et al., 2016	Apollonia Pontica, Bulgaria	Mid 5th - Mid 3rd c. BCE	0.5 - 1.0	3.0
Stantis et al., 2019	Lebanon; Syria	2800 - 1200 BCE	0.5	2.6
Van der Sluis, 2015	Aalborg Cemetery, Denmark	1240 - 1530 CE	-	3.0 - 4.0

The first study to conduct dentine analysis to explore breastfeeding and weaning within the temporo-spatial bounds of the Roman Empire was performed by Dupras and Tocheri (2007) on samples from Kellis 2, in the Dakhleh Oasis, approximately 725km southwest of Cairo. They conducted a multi-tooth analysis (i.e., a comparison of bulk dentine values from multiple teeth belonging to the same individual), comparing 297 deciduous and permanent teeth to examine the weaning histories of 102 subadult and adult individuals. Although the burials are dated from 100 CE – 450 CE, the minimal (and often absent) collection of grave goods indicates Early Christian-style burials. Deciduous teeth were enriched in ^{15}N by 2‰ relative to the permanent teeth. Furthermore, early-forming permanent teeth (i.e., M1s) were also significantly enriched compared to later-forming teeth, but to a smaller degree (0.5‰). The steady, consistent decrease in $\delta^{15}N$ signals with increasing age suggests a gradual replacement of breastmilk with weaning foods, including animal milk (typically from goats and cows). Delta ^{13}C values were also higher in deciduous teeth (by 0.6‰) and displayed a decrease in the root portion of M1 teeth which corresponds to >3.0y of age. The combined results from both $\delta^{15}N$ and $\delta^{13}C$ values indicated a reliance on breastmilk from birth until ~3.0y of age. Dupras and Tocheri (2007) argue that the increase in $\delta^{13}C$ prior to 3.0y of age, followed by an observable decrease, may indicate a contribution of C_4 foods as part of the complementary diet, likely through the consumption of millet-foddered animals or their by-products. It is also possible that the presence of C_4 plant contribution is the result of direct consumption, likely via foods such as millet gruels and porridges.

Cocozza and colleagues (2021) conducted an investigation of weaning patterns of a sample from the rural site of Roman Bainesse, located in the UK. They employed Bayesian modelling to explore the weaning patterns of five individuals aged 15y – 45y. The weaning patterns varied amongst the individuals examined in the study; although weaning consistently began around 0.5y of age, there was considerable variation in the estimated age-at-weaning. Two individuals had an estimated weaning age of 2.0y – 3.0y, whereas three were weaned after 4.0y of age, one of whom had breastmilk in the diet until age 5.0y (Cocozza et al., 2022).

Ganiatsou and colleagues (2022) conducted a stable isotope analysis of breastfeeding and weaning patterns in Imperial Roman Thessaloniki, a provincial capital in northern Greece. Using serial dentine from permanent M1 teeth, they determined the dietary patterns from birth to 7.0y of age for 20 individuals (age-at-death ranged from 15y – 55y). Their results suggested some sex-based differences in weaning practices, where males were weaned earlier (mean = 1.9y) than females (mean = 2.1y), but these differences were not statistically significant. The $\delta^{15}N$ and $\delta^{13}C$ signals in this sample demonstrated a clear trend with age. Delta ^{15}N signals decreased with successive dentine sections, and based on these values, individuals demonstrated distinct dietary phases representing exclusive breastfeeding, weaning, and post-weaning diet (Ganiatsou et al., 2022). Similarly, $\delta^{13}C$ signals also decreased with successive dietary phases, although the values were more variable across the sample. The peak $\delta^{15}N$ value at 0.5y indicates exclusive breastfeeding, and the decrease observed at 1.0y suggests that weaning commenced between these two ages (Ganiatsou et al., 2022). However, their results indicate that for

most of the individuals studied, weaning was fully complete by 2.0y. This weaning schedule closely aligns with the eruption of primary dentition (AlQahtani et al., 2010), as well as Soranus' recommendations for the timing of weaning commencement (Temkin et al., 1991). Based on the $\delta^{15}N$ and $\delta^{13}C$ signals, Ganiatsou and colleagues (2022) posit that weaning foods consisted primarily of C_3 plants and terrestrial proteins, with some potential contribution of marine foods and C_4 plants. This study is one of few to report an exclusive breastfeeding signal, providing crucial information about the beginning of this dietary transition (Ganiatsou et al., 2022).

In a follow-up study using a novel computer program to assist in the reconstruction of weaning patterns and estimation of age-at-weaning, Ganiatsou and colleagues (2023) analyzed the collagen of 31 M^1 teeth. This time, the authors expanded their investigation to explore the weaning patterns of individuals from four different chronological periods: Hellenistic (4th c. BCE – 1st c. BCE), Roman (1st c. BCE – 4th c. CE), Byzantine (4th c. CE – 15th c. CE), and Post-Byzantine (15th c. CE – 16th c. CE). Twenty-six of the individuals included in this study were dated to the Roman period, and their stable isotope signals indicated that the weaning period ranged from 1.0y – 4.0y. The authors compared these signals from the results of their previous study, and determined that there was no significant difference in the weaning age between males and females. With the exception of one individual (weaned at ~4.0y), the typical age-at-weaning was between 2.0y – 3.0y, which is slightly later than the age reported in their previous study (Ganiatsou et al., 2022).

A recent study by Salahuddin and Prowse (2023) used incremental dentine on 12 subadults and eight adults from two Iron Age sites (7th – 4th c. BCE) located ~15km from

the site used in this study. The results of their study showed that weaning began around five months and was completed earlier for subadults (2.0y ± 0.6y) than for adults (3.1y ± 0.3y). Although this site is from an earlier time period, the geographic proximity permits the comparison of breastfeeding and weaning patterns between Iron Age and Roman samples from the same region (discussed further in Chapter 7).

Other incremental dentine explorations of breastfeeding and weaning use isotopic data from sites in the UK (e.g., Fuller et al., 2003; Beaumont et al., 2013, 2014, 2018; Beaumont and Montgomery, 2015), continental Europe (e.g., Schmidt et al., 2015; Van der Sluis, 2015; Czermak et al., 2018, 2019; Fernández-Crespo et al., 2018; Kwok et al., 2018; Crowder et al., 2019; Goude et al., 2020), Africa/Middle East (e.g., Sandberg et al., 2014; Eerkens et al., 2018; Stantis et al., 2019), and the Americas (e.g., Eerkens et al., 2011; King et al., 2017, 2018), spanning time from as early as the mid-5th c. BCE until the 19th c. CE and even the inclusion of some modern samples. It is therefore not surprising that there is a great deal of variation in both the reported starting ages of weaning (from 0.5y – 2.0y of age) and time of complete breastfeeding cessation (from ~1.0y – 4.0y).

3.5.3 Social and Biological Effects of Breastfeeding and Weaning Practices

The study of weaning practices is common as a result of the acknowledgement that it has significant social and biological implications, such as impacts on health, survivorship, reproduction, and population dynamics (Knodel and Kintner, 1977; Dettwyler and Fishman, 1992; Wood et al., 1992; Bentley et al., 1993; Katzenberg et al., 1996; Taylor et al., 1999; Filteau, 2000; Dettwyler, 2004; Jay, 2009; Valeggia and Ellison, 2009). The weaning process can be described as a transition between two life stages: from exclusive

breastfeeding (EBF) to complete cessation of breastfeeding (Dettwyler and Fishman, 1992; Smith et al., 2017; Tsutaya, 2017). Changes in stable isotope values can inform researchers of changes in weaning and subsistence patterns, reveal differential access to food resulting from social or environmental conditions, and challenge existing narratives about differences in weaning practices of people in the past (Mays et al., 2017; King et al., 2018). In particular, infant/early childhood diet and the weaning process greatly influence health, morbidity, and mortality (Katzenberg and Lovell, 1999). Bentley and colleagues (1993) posit that the advent of widespread agricultural practices facilitated an increased population growth and decreased birth spacing attributable to the use of complementary foods in the weaning process. Another major consequence of breastfeeding is suppressed fertility during lactation, so access to suitable complementary foods would facilitate earlier weaning which increases female fecundity (i.e., allowing a shorter time interval between pregnancies) and ultimately results in changes to population dynamics (Taylor et al., 1999; Jay, 2009; Valeggia and Ellison, 2009; King et al., 2018).

Breast milk also provides protection against infectious disease through the transfer of important immune constituents to the infant and minimizes pathogen introduction via artificial feeding (Hahn-Zoric et al., 1990; Katzenberg et al., 1996; Filteau, 2000). Overall, better health and decreased infant mortality rates are attributed to (at least in part) breastfeeding in both developed and developing countries in modern samples (Knodel and Kintner, 1977; Dettwyler and Fishman, 1992; Katzenberg et al., 1996). Breastfeeding and weaning patterns can reflect cultural paradigms surrounding the mother's choice to breastfeed or to opt for artificial feeding; cultural beliefs about the safety and quality of

colostrum and breast milk, as well as external pressures from family and community, greatly influence the infant's breastfeeding and weaning timeline (Dettwyler, 2004).

3.5.4 The Impact of Breastfeeding Patterns and the Biometric Analysis of Infant Mortality

The mortality bias associated with stable isotope analysis of bone collagen may produce results that are not representative of normal infant and early childhood diet. Wood and colleagues (1992) note that cultural customs and ideology may have influenced people to treat sick infants and young children differently by weaning them earlier/later or feeding them a different complementary diet, which may ultimately result in an inaccurate representation of healthy infant and child diet. The biometric analysis of infant mortality was first developed by Bourgeois in 1946. The approach simplified the method of separating infant deaths attributable to congenital or obstetric complications from those attributable to the post-natal environment (i.e., hygiene, nutrition, infectious disease) (Bourgeois, 1946). It is based on the observation that accumulated infant deaths after the first month of life have a positive linear correlation to age. For rural English infants in 1905, the cumulative mortality from the age of one month onwards follows the predicted linear model as posited by Bourgeois (1946). Exceptions to this linear relationship are most commonly expressed as a 'break' in the line, where the slope increases after two to four months after birth. This break was generally considered to be associated with weaning practices and artificial feeding (Pressat, 1972; Knodel and Kintner, 1977). Early work suggested that this 'excessive mortality' was a result of the cessation of breastfeeding. This interpretation was supported by several studies, indicating that (up until recently) artificially fed infants had significantly higher mortality rates compared to breastfed infants

(Wray, 1977; Knodel and Kintner, 1977). The increased mortality upon cessation of breastfeeding due to artificial feeding may be reflected in bone collagen studies that conduct analysis on infant remains. In these cases, bone collagen studies exploring weaning may depict a dietary pattern which resulted in increased mortality and is not representative of the healthy population. Incremental dentine analysis mitigates these concerns and provides information on early life dietary patterns in survivors.

3.6 Conclusion

Early life dietary practices and patterns as transitions can direct life span trajectories and influence individual growth and overall health, and more broadly, fertility and mortality patterns of past groups (Knodel and Kintner, 1977; Wood et al., 1992; Lewis, 2006; Halcrow and Tayles, 2008; Gowland, 2015; Halcrow et al., 2017). Prior analyses of early life diet at Roman sites were typically conducted using bone collagen of subadults that did not survive the weaning process. The analysis of subadult remains may represent an 'unhealthy' weaning pattern, since the cause of death cannot be determined and weaning cannot be ruled out as a contributing factor. Although incremental dentine analysis is increasingly used in studies of breastfeeding and weaning, relatively few weaning studies have analyzed samples from within the Roman Empire. Since this thesis examines a rural Roman estate from southern Italy, it provides key information to better understand the lives of lower classes of rural Romans.

4. ROMAN DIET AND THE LIFE COURSE

4.1 Introduction

Most of what is known about Roman diet and lifestyle is sourced from literary and epigraphic evidence. The writers of texts such as Galen, Cato, Columella, and Soranus wrote extensively on themes of agriculture, health, and diet (Temkin et al., 1991; Garnsey, 1999; Donahue, 2015; Witcher, 2016). Cultural beliefs about gender and infant 'constitution' influenced attitudes towards infant diet and care, including breastfeeding and weaning practices. According to the humoral theory, infants and women had different constitutions (i.e., hot/moist and cold/moist, respectively) relative to each other and other groups including young adults, men, and the elderly (Laes, 2019). The belief in these fundamental biological differences dictated the type of diet prescribed, with the goal of optimizing overall health and longevity (Temkin et al., 1991; Grant, 2000; Donahue, 2015).

Ancient texts and treatises indicate that the typical Roman diet consisted primarily of C_3 (e.g., wheat) and C_4 (e.g., millet) cereals, legumes and pulses, olives, grapes, terrestrial mammals such as pigs/sheep/goats/wild game, avian species (poultry and game fowl), and marine foods such as fish and shellfish (Garnsey, 1999; Erdkamp and Holleran, 2019). However, the exact ratios and contribution of each of these foods, as well as the adherence to the guidelines are unknown. This is particularly true for lower classes and rural populations, since these texts were written primarily for the higher classes of (wealthy, educated) Romans. Social class also dictated the relative access to dietary variety and nutrient-dense foods, whereby elite classes had a more broad, varied diet consisting of several different types of plant and animal products, and lower social classes relied more

on cereals and other plant staples, with less caloric contribution coming from animal products (Garnsey, 1999). Varying degrees of access to certain foods depended on the region and type of settlement occupied. Among 'common' Romans of lower social standing, rural inhabitants had a higher degree of access to dietary variation due to the ability to grow their own food and raise their own animals (see section 4.4.4) (Heinrich, 2019).

Differential preparation of foodstuffs can also result in varying nutritional yields, regardless of the type of food consumed; lower-status individuals often relied on poorer quality foods containing 'contaminants' which hinder optimal absorption of essential nutrients (Heinrich, 2019) (this topic is further discussed in section 4.4.1). Recent studies have examined the Roman diet by using stable isotope analysis, which has provided a more direct line of evidence for examining dietary patterns and practices among various populations in the empire (see reviews by Killgrove, 2014; Bourbou, 2019).

The employment of stable isotope analysis provides an avenue for critically assessing dietary patterns among different segments of ancient society, particularly those who are historically underrepresented in the literature such as poor and/or rural women, infants, and children. This review of possible elements in the Roman diet provides context for understanding the potential foods that were available for the weanling and post-weaning diet of the individuals from Vagnari.

4.2 Plant Sources

4.2.1 Cereals (C_3 Species)

Roman cereal cultivation and consumption were diverse; literary sources suggest that Roman diet was composed of 70% − 80% (of total caloric intake) wheat products, which are C_3 plants (Foxhall and Forbes, 1982; van Limbergen, 2018). Romans had access to a wide variety of C_3 cereals, including two different types of barley (two-row and six-row), five types of wheat species (einkorn, spelt, emmer, bread wheat and hard wheat), oats, rice, rye, and C_4 plants such as common millet, pearl millet, foxtail millet, and sorghum (Heinrich, 2019, p. 103).

Barley was widely used by Romans as both food and for beer production, but the primary use is thought to have been animal fodder combined with grass species (e.g., bitter vetch), grass pea, and grain by-products like cereal chaff (Reed and Roguljić, 2020). Crops that were considered to be less important (but still present) included species of millets, oats, rye, and other cereals (Garnsey, 1999). Other grain crop staples consumed by Romans included pulses, acorn, and sweet chestnut (Heinrich, 2019). Of the cereals available to Romans, the only species that utilize C_4 photosynthetic pathways are millets and sorghum.

The cultivation of choice crops would have been influenced by the growing environment (i.e., amount of rainfall, soil conditions), with a variety of species being grown as a security measure to mitigate shortages and famine in case of crop failure or to fulfill different roles within the broader dietary context (Witcher, 2016; Heinrich, 2019). Garnsey (1999) notes that peasants in the Roman countryside seldom had access to bread wheat

(which was softer and easier to process), often relying on local, 'harder' cereals and legumes.

The considerable consumption of the aforementioned cereals and grains, almost all of which are C_3 plants, would produce low $\delta^{13}C$ and $\delta^{15}N$ values and a 'C_3 plant signal' in the dentine collagen obtained for analysis. Regardless of the species consumed, all of the plants mentioned (save for millet and sorghum) belong to the C_3 plant family and are expected to yield similar $\delta^{13}C$ and $\delta^{15}N$ signals.

4.2.2 Millet (C_4 Species)

Roman consumption of C_4 plants was likely limited to millet, since it is one of few Old World species with a C_4 photosynthetic pathway, with other dietary constituents such as legumes, fruits, olives, and grapes (wine) all belonging to the C_3 category (van Limbergen, 2018). Despite the labour-intensive nature of millet cultivation, harvesting, and processing of millet, there were numerous benefits to growing millet.

Several ancient agricultural writers (e.g., Vergil, Columella, and Pliny the Elder) wrote on the properties of common and Italian millets (Murphy, 2016). They identified them as species best suited for warm weather, but were known to be tolerant of a variety of weather and soil conditions and could be harvested multiple times in a single growing season, making them a hearty supplemental cereal in times of poor soil quality or drought (Spurr, 1983, p. 89; Murphy, 2016). The resilience of millet as a plant can be attributed to the outer husks that protect the seeds. Spurr (1983) argues that the presence of this husk also allows for prolonged storage of the cereal, which can act as an additional security measure in times of food shortage.

Murphy (2016) reports that despite millet being classified as a lower-status food, historical sources indicate that the attitude towards millet was less clear-cut. The presence of millet in the kitchens of elite houses from Pompeii suggests consumption by upper classes as well as by those in lower social strata; although it is possible that slaves or servants prepared these meals for themselves, it is more likely that millet was consumed by the owners of the villas, since servants and slaves typically had segregated cooking facilities (Murphy, 2016). Since millet was likely one of few C_4 plants available to Romans at Vagnari, its contribution to the human diet (either through direct consumption or consumption of animals fed on millet fodder) would have to have been considerable in order to elicit a strong C_4 plant signal, indicated as an increase in $\delta^{13}C$ values with a decrease/no change in $\delta^{15}N$ in human collagen (Katzenberg and Waters-Rist, 2018).

4.2.3 Legumes/Pulses (C_3 Species)

In addition to the original Mediterranean triad of grains, olives and grapes, legumes and pulses were cultivated for both human consumption and their soil-enriching (i.e., nitrogen-fixing) qualities that provided essential nutrients to other crops in rotation (Witcher, 2016). Legumes are a category of plants that include species of beans, lentils, peas, grass peas, chickpeas, fenugreek, vetchlings, white lupine, alfalfa, and bitter vetch, the "dry seeds" of which are referred to as pulses (Heinrich and Hansen, 2019, p.117).

Historical and paleoethnobotanical research indicate that pulses were consumed throughout the Roman Empire, despite the assumption that they were inferior foods consumed only by lower social strata (Heinrich and Hansen, 2019). Pulses and legumes were widely available and were thought to contribute substantially to the total Roman diet;

legumes could be eaten both fresh (as vegetables) and dried (as pulses). Heinrich and Hansen (2019) posit that this consumption pattern allowed for additional harvesting times as well as increased variation and diversification of nutritional intake, since the chemical composition of vegetables differs significantly from the pulses.

Similar to cereals, legumes and pulses available to Romans belong to the C_3 plant category. Regardless of nutritional quality and chemical composition, the stable isotope signals from the consumption of these legumes and pulses would yield similar $\delta^{13}C$ values to each other as well as to other C_3 plant species.

4.2.4 Olives (C_3 Species)

Olive oil (a C_3 plant source) was heavily relied upon by ancient Romans and is thought to have represented up to one third of an individual's overall caloric intake (Hitchner, 2002; Rowan, 2019). The proportion of caloric contribution is unknown, but is estimated to range from 5L to 30L per year (Rowan, 2019). Literary and archaeological evidence indicate a significant reliance on the trade and consumption of olives and olive oil. Rowan (2019) asserts that the olive tree played a significant cultural and economic role as evidenced by the extensive trade and import of olive oil into the Empire. High levels of production and trade allowed for most everyone to have regular access to olive oil for consumption, hygiene, and as a fuel source for lamps.

Olives are relatively well-represented in paleoethnobotanical and archaeological evidence. Recovered plant components and material remains (e.g., dolia and amphorae) used for the processing, storage, and distribution of olive oil (and wine) within the Empire indicate widespread use of these foods (Witcher, 2016). It is unclear how much the rural

population consumed olives, since there is little archaeological and archaeobotanical evidence for olives from rural Roman sites, but consumption may have occurred at sites in olive-growing regions such as southern Italy (Rowan, 2019). Olive oils were one of the few inexpensive sources of fat available to the Romans aside from nuts and oily fish (e.g., sardines, anchovies); although olive oil was the most common and widespread oil, other fats such as sesame, radish, nut, and castor oils as well as animal fat were available to Romans. Since the contribution of olives and olive oil is suggested to have been considerable in ancient Rome, this C_3 plant likely contributed substantially to the $\delta^{13}C$ signals obtained from human collagen samples.

4.2.5 Grapes (C_3 Species)

One of the central beverages in the Roman Empire was wine. Purcell (1985) argues that wine production played a critical role in Roman society and the economic/agricultural activities throughout the Empire. It was available in many different types and preparations, and wines from the entire Mediterranean region were imported into Italy, served in a number of ways (e.g., hot, cold, room-temperature, fortified, diluted). The consumption of wines, especially those with additives of honey or herbs, would have contributed at least in part to overall caloric intake due to the sugar content (Broekaert, 2019). The widespread consumption of wine among Romans would suggest that this C_3 plant would have contributed to the obtained $\delta^{13}C$ values from sampled collagen. The degree of wine consumption in children is poorly understood, but Romans' awareness of water contamination may have resulted in adults giving children small quantities of wine (Laes, 2019). Galen discouraged wine consumption for children, but Laes (2019) suggests that

Galen's mention of it in his writing indicates that this was a common practice. Importantly, the consumption of grapes and raisins by children was also possible (Erdkamp and Holleran, 2019).

4.3 Animal Products

The analysis of faunal evidence can provide insight into the use and treatment of animals within the context of Roman agriculture. Animal products such as meat, fat, milk, cheese, and eggs were generally not considered a major contributor to the diet of Mediterranean people, especially in communities outside of major livestock production areas (Garnsey, 1999; Witcher, 2016; Mackinnon, 2019). Mackinnon (2019) argues that animal products were still culturally significant to Romans, and were often included as an additive into various recipes to enhance flavour and boost nutritional value. Historical evidence suggests that although dairy and eggs were likely available to most people, meat was rarely (if ever) a central component of a dish in poorer households. Roman encyclopaedist Celsus argued that larger domesticated and wild species of quadruped mammals such as cattle, pig, sheep, goat, deer, wild boar, and wild goat were the most nutritious (Mackinnon, 2019). Animal age, size, and cut of meat were also ranked based on nutrition, with larger animals and meats with higher fat content believed to be more nutritious. Preferred cuts of meats from younger animals were reserved for the Roman elite, while tougher cuts of meat from older animals were consumed mostly by the poor. Lower social classes almost certainly consumed animal products in small quantities as a supplement to a primarily vegetarian diet (Mackinnon, 2004). As with other prescriptive texts on diet and health, it is unclear how often these recommendations were followed, especially by poor or rural communities.

Cattle were typically raised as draft animals due to the expensive nature of raising animals of this size, making them an inefficient food source (Garnsey, 1999; MacKinnon, 2010). Witcher (2016) suggests that cattle remains from various sites in Roman Italy are typically from older individuals (aged 3.0y+, with many individuals older than 5.0y), due to a primary function of labour prior to eventual consumption. Sex-based differences in animal remains may further indicate the purpose of the animals at the site. A higher ratio of male:female animals suggest that cattle were used as draft animals rather than for meat and/or milk consumption (Witcher, 2016). Consumption of cattle, including milk and cheese, was not as common as that of sheep and goat (Mackinnon, 2019).

In most faunal assemblages from Roman period sites, over 50% of mammal bones recovered come from pigs, likely representing two breeds (Mackinnon, 2004). Smaller pigs would have been beneficial in free-range environments on family farms, since they were able to forage and eat table scraps, making them easier and cheaper to raise compared to stall-fed pigs selectively bred for meat export (Witcher, 2016). Since the sole purpose of pigs is meat, most animals were slaughtered young, with only 1% of pigs reaching full skeletal maturity. Pork was a common protein source in Italy, since most people raised pigs, especially in rural communities (Mackinnon, 2019). The frequency of pig remains from sites in Italy often outnumber the amount of cattle remains recovered (Mackinnon, 2019).

Romans also raised an abundance of sheep and goats, primarily used for wool, hair, cheese, and skins (Mackinnon, 2004; Witcher, 2016). These remains typically belonged to older individuals; in concert with the observation of an increased female:male ratio, these data suggest a primary function of wool and milk production as opposed to meat (Witcher,

2016). Eggs and meat from poultry and domestic fowl were also popular as indicated by extensive texts on agricultural practices for breeding and raising these animals (Kron, 2014). The consumption of terrestrial herbivores, provided they were fed with similar fodders, would likely result in similar $\delta^{15}N$ and $\delta^{13}C$ values in human collagen. Due to the trophic level effect, individuals consuming any animal-derived products would have higher $\delta^{15}N$ values relative to those with a diet based primarily on the consumption of plants (and especially legumes and pulses, since these have lower $\delta^{15}N$ values relative to animal proteins; Schwarcz et al., 1985). Since pigs are omnivores (and were often fed table scraps; Witcher, 2016), the signals obtained from humans consuming pork products would likely have elevated $\delta^{15}N$ values relative to humans consuming strictly terrestrial herbivores such as sheep, goats, and cattle.

Consumption of fish varied throughout the Empire and depended on proximity to water, social status, and time period (Prowse et al., 2004; Marzano, 2019). Fish and seafood was typically consumed by the wealthy, with large species of fish and various molluscs being sought after by elite classes (Frayn, 1993). A variety of fresh- and saltwater species such as gilthead, sea bream, and bass, as well as mollusc species including oysters and scallops, were available and consumed by upper classes, but the degree of access (which was undoubtably smaller) by lower social classes is unknown (Marzano, 2019). In regions with closer access to fish habitats, ordinary people would have had more access to marine and freshwater proteins relative to regions that were located further inland and away from other major water sources (Prowse et al., 2004; Marzano, 2019). Prowse and colleagues (2004) tested the isotopic signals from garum to determine $\delta^{15}N$ values and the relative

contribution of this fish product to the overall dietary patterns. The garum samples had lower than expected values of $\delta^{15}N$ (mean = 6.5‰), suggesting the use of small, lower-trophic level organisms such as sprat, smelt, and shellfish. These data also suggest that fish sauces such as garum did not contribute significantly to increased levels of $\delta^{15}N$ values in dietary studies (Prowse et al., 2004).

The consumption of both fresh and saltwater fish is associated with elevated $\delta^{15}N$ values relative to both plants and terrestrial animals (Schoeninger et al., 1983; Katzenberg, 1989). Individuals with a diet consisting of high trophic-level aquatic sources are expected to therefore display elevated $\delta^{15}N$ and $\delta^{13}C$ values in their collagen relative to those relying on plant- and terrestrial-based diets.

4.4 Other Aspects of Diet

4.4.1 Diet and Nutrition

The nutritional quality and amount of food in the diet has significant effects on overall health outcomes and experiences throughout the life course (Gowland, 2015). Nutrient absorption is optimized by the proper preparation of a diverse variety of foods. Essential micronutrients must be acquired from diet, since they cannot be synthesized by the human body. These include three major categories: (1) vitamins A, B, C, E; (2) trace elements including copper, zinc, manganese, selenium and iron; and (3) phytochemicals in vegetables (which function as antioxidants) (Opara and Rockway, 2006). Access to this type of nutrient-rich diet was historically restricted to higher-status individuals, with poorer social classes relying on less diverse diets with poorer processing of certain staples such as cereal flours (Garnsey, 1999).

Romans had a holistic attitude towards health, beginning treatment of illnesses primarily with changes in diet prior to other interventions (Temkin et al., 1991; Grant, 2000; Donahue, 2015). Van Limbergen (2018) suggests that as the dominant constituent of the Roman diet, starches and carbohydrates represented up to 2,175 calories for an average person, and 2,775 for active individuals. Wheat and barley are associated with fewer nutritional deficiencies and are more calorically dense relative to other staples such as rice or maize species (Garnsey, 1999). Addition of millet into meals (or when served as a central component of a dish) substantially increased the amount of carbohydrates and volume consumed, improving and prolonging satiety (Murphy, 2016). Cereals are also higher in protein, vitamins B_1, B_3, E, calcium, and iron relative to contemporary root crop staples such as yams (Garnsey, 1999). However, grains have lower levels of lysine (an amino acid most commonly found in animal protein sources) and vitamins A, B_2, C, and D, necessitating variety in the diet to avoid pathological conditions such as iron deficiency anemia and rickets. Grains also provide small amounts of protein and fat in addition to carbohydrates, which may have helped improve the nutrition of people who did not have regular access to meat, fish, eggs, or dairy (Heinrich, 2019).

Wheat bran contains high quantities of essential micronutrients such as iron and zinc, but these are typically bound to phytate, rendering them insoluble and reducing the bioavailability of the minerals to as low as 5% – 10% (Kaul et al., 2018; Lemmens et al., 2018). This low bio-accessibility can result in nutritional deficiencies; currently, women and children in lower- and middle-income countries in particular exhibit a high prevalence of deficiencies in iron and zinc, along with those who rely heavily on monotonous, grain-

based diets (Uvere and Ene-Obong, 2013; Shah et al., 2016; Lemmens et al., 2018). Processing wheat to increase the bioavailability of micronutrients can occur through wheat pearling, germination, fermentation, and low pH hydrothermal processing (De Brier et al., 2015; Lemmens et al., 2018). Bioavailability can also be increased by diversifying the diet to include foods containing phytate enzymes and acids such as animal proteins and fruits and fruit juices containing ascorbic acid (Uvere and Ene-Obong, 2013). However, it is unclear as to whether any or all of these methods were employed in the past. In modern, western contexts where people have access to diverse and nutritious diets, the consumption of whole wheat is recommended as it is associated with a reduced risk of developing cardiovascular disease, obesity, and type II diabetes (Lemmens et al., 2018; Brouns, 2022). However, in individuals who rely mostly on grain-based foods, deficiencies can occur despite the high mineral content present in whole wheat.

Unfortunately, individuals who consumed nutritionally poor diets are not isotopically distinct since the $\delta^{15}N$ and $\delta^{13}C$ signals remain unchanged regardless of the nutritional quality of the diet. This is an important consideration to make, especially when examining the diet of non-survivors who died during the weaning process, since diet cannot be ruled out as a factor contributing to infant/childhood morbidity or mortality. This observation also has important implications for the health of lower classes of Romans with limited access to nutritious, well-prepared ingredients.

4.4.2 Preparation Methods for Grains, Fruit, and Vegetables

Grains may be processed mechanically by grinding or milling, thermally by roasting, boiling or frying, biochemically through the processes of fermentation or germination, or

any combination of these three (Lemmens et al., 2018; Heinrich, 2019). Garnsey (1999) points out that cereals were processed by the Romans in a number of ways, but were most commonly consumed in the form of porridges, breads, and flat-cakes. In addition to these staples, Heinrich (2019) suggests that Romans incorporated cereals in a myriad of recipes, ranging from liquid products such as beer, to gruels, purées, pottages, soups, mashes, and stews. Emmer wheat was most commonly prepared as a bread during the Imperial period; breadmaking required soft (bread) wheat, which was more labour-intensive compared to cereals like durum wheat that were more commonly prepared as porridges or flat breads fried on a griddle (Garnsey, 1999).

Murphy (2016) reports that millet produced breads and porridges which some enjoyed even when other cereal options were available. Recipes consisting primarily of millet, usually combined with olive oil, lard, or milk, were commonly consumed "in the countryside", regardless of crop conditions (Murphy, 2016, p. 68). Grain consumption was emphasized as an important contributor to overall health; wheat also had many medicinal purposes, such as in the production of medical lozenges, and topical 'doughs' and treatments for inflammations, coughs, diarrhea, colic, milk duct clogs, and skin conditions (e.g., sores, birthmarks, leprosies and even bites from venomous animals) (Donahue, 2015). Vegetables were roasted, boiled, or pickled prior to consumption, whereas pulses were often prepared as porridges and stews, or incorporated with other cereal staples (Heinrich and Hansen, 2019). Olives must be processed to be suitable for consumption; the two primary modes of preparation and consumption included table olives and olive oil, with different varieties preferred for different modes of consumption (Rowan, 2019). Olive oil

was produced through the crushing of the fruits by a stone mill (or other crushing device) and compression of the paste to separate out the liquid component, whereas table olives were primarily prepared by brining, pickling, or drying.

4.4.3 Preparation of Animal Products

Although the elite classes had access to an array of animal products from a variety of species, the most common preparations of meat products for ordinary Romans were in the form of blood pudding, sausages, or as mincemeat combined with chopped vegetables obtained from street vendors (Mackinnon, 2019). Preservation of meat products included salting, drying, smoking, pickling, brining, and honey-coating. Mackinnon (2019) reports the most common preparation techniques to be boiling, frying, grilling, roasting, and baking, of which roasting and boiling were considered to be best for digestion. As previously mentioned, the weanling diet was typically plant-based. However, Galen did note that consumption of meat was allowed for infants after bread was introduced (Garnsey, 1999), although the animal type or preparation were not specified.

Preserved fish products in the form of fish sauce (e.g., garum, liquamen, and allex) were common, and were prepared by collecting the brining liquid from salted fish during the preservation process. These sauces were widely exported for use in food and medicine throughout the Empire, particularly in urban environments (Curtis, 1991; Garnsey, 1999; Prowse et al., 2004).

4.4.4 Rural Romans Versus Urban Inhabitants: the Rich and the Poor

It is important to detach social status from the type of settlement that an individual inhabited. Indeed, not everyone who lived in a rural community was poor, and not every urban inhabitant was rich. This intersection of identities complicates our understanding of diet at Vagnari, since the socioeconomic status of those interred in the cemetery is not clear (site information is further discussed in Chapter 5). The following section will address the role of both socioeconomic status and settlement type in an individual's access to different foods and will explore the effects of these factors on the diets and lives of Romans.

Although higher social classes had access to a wide variety of domestic and imported foods, the diet of the lower classes was much more monotonous, consisting primarily of cereals, dry legumes and the addition of other simple and cheap ingredients (Garnsey, 1999). Lower social strata generally had less access to a variety of foods compared to those of higher social standing, but rural people were more likely than the urban poor to consume varied diets (Heinrich, 2019). Those living in rural regions would have had more ability to cultivate their own fruits and vegetables and raise livestock, whereas the metropolitan poor would have had to rely on food available through purchase or charity. Rural communities likely incorporated moderate amounts of pulses and tree-seeds (e.g., acorn and sweet chestnut) into the overall cereal consumption, improving the nutrient profile of the overall diet. Heinrich (2019) posits that lower-status individuals living in rural settings had a more diverse and nutritious diet relative to the urban poor.

It is likely that pulses were consumed in greater quantities in rural communities or higher-status urban inhabitants due to increased access compared to the urban poor

(Heinrich and Hansen, 2019). Fruit trees such as fig and almond were also cultivated, especially in rural settings, since these fruits are calorically dense and could be dried and stored for long periods of time (Jashemski and Meyer, 2002). Country gardens produced vegetables and herbs such as beets, parsnips, leeks, and garlic (Witcher, 2016). Foraging of wild plant species was also common, especially among peasants (Evans, 1980). Species collected included caper, strawberry, almond, asparagus, mulberry, fennel, grass peas, acorn, and blackberry, although the degree of contribution of these plants in the Roman diet is unknown (Witcher, 2016).

Fresh milk from sheep and goats was a popular drink in the countryside, attributable to regular access to milk which would otherwise spoil in transport (Broekaert, 2019). Milk contains essential macro- and micronutrients that are not found in wine, such as fat, vitamins and minerals (Broekaert, 2019). Although fresh fish and game such as deer, hare, and boar were consumed, this is often thought to have been an elite practice, while domestic taxa contributed to the diet of most consumers (Mackinnon, 2019). The degree of access to (and consumption of) meat was unknown for the rural poor; historical texts do not provide evidence of meat rations being given to workers or slaves at Roman villas, but it is possible that occasional consumption occurred (Mackinnon, 2019). What was more likely and frequent was the consumption of milk and cheese, especially in places where large flocks of animals were raised (Mackinnon, 2019).

Patterns of wine consumption in rural populations and among the urban poor are underrepresented in the literature. Since wine was not produced everywhere throughout the Empire, sites that were not near production centers would have relied on a larger variety of

beverages (Broekaert, 2019). There is, however, evidence of wine production and storage at Vagnari (Carroll, 2022). Lower social classes would have had access to fish and seafood through the consumption of smaller, freshwater fish and fish products such as sauces and pastes (Marzano, 2019).

Since Vagnari was a rural site, it is likely that the inhabitants had access to a greater variety of foods relative to lower classes of Romans living in urban areas. Based on a relatively high recovery of common (e.g., ceramic vessels) and 'luxury' (e.g., fine jewelry, bronze vessels) grave goods recovered from the site, Brent and Prowse (2014), suggest that there is little indication that the locals interred at the cemetery were poor or were of low socioeconomic status. Though the socioeconomic status of the individuals from the site is not fully understood, people at Vagnari likely had a relatively diverse diet due to their rural setting, even if they were lower status.

4.5 Roman Infant and Early Childhood Diet and Care

4.5.1 Life Stages and Gendered Differences in Infant Care

Romans used the term *infantia* to refer to the period of life from birth to seven years of age (Harlow and Laurence, 2002). Traditions and ritual practices associated with early life, such as pregnancy, labour, and early childhood suggest gendered differences in attitudes towards male and female infants and children. Harlow and Laurence (2002) indicate that females were thought to mature more rapidly than males, as evidenced by gender-based differences in the timing of important rituals and festivals, which occurred earlier for females relative to males. Diets of women and children were largely ignored in the ancient medical literature (Donahue, 2015). Emphasis was placed on ensuring girls had appropriately timed

pubescence and fertility; their diet typically restricted the consumption of meat, wine, and other 'strong foods'. Since the woman's constitution was thought to be wet and cold, they were recommended to avoid cold, wet foods, including fish and meats high in fat or from new-born animals (Garnsey, 1999). Women who followed these recommendations (either intentionally as a health practice or unintentionally due to decreased access to these foods) would have had lower $\delta^{15}N$ values relative to women who continued consumption of animal products throughout pregnancy. This scenario complicates the establishment of a weaning timeline, since infants with a low-trophic level mother may be mistaken for having been weaned despite presence of breastmilk in the diet, as the values are compared to an adult female mean likely representing females that did consume meat. These texts, however, were written for elite social classes who could afford to employ a wet-nurse or purchase 'healthy' foods for their child; lower-status individuals would likely have fed them with whatever food source was locally available or easily sourced.

4.5.2 Roman Attitudes Towards Breastfeeding and Weaning

The following section will focus on the textual and deciduous dental health evidence regarding Roman infant diet and weaning practices. Men did not play an active role in childbirth and breastfeeding, and as a result, most literary sources written by men do not address these topics (Centlivres Challet, 2017). In the existing evidence, medical writers give instructions, advice, and recipes related to birth and infant diet/breastfeeding (French, 1987; Grant, 2000). Male medical advice included instructions to replace colostrum (which was thought to be indigestible) with other liquids until the "perfected" breast milk came in (Riordan and Wambach, 2010, pp. 53-54). Garnsey (1999) reports that medical texts on

gynaecology recommended that women should not feed their newborn infant for two days, followed by the slow introduction of honey-based liquids before breastfeeding via wet-nurse. It was thought that in the first 20 days post-partum, a woman's milk was unsuitable for infant consumption, and time was needed to be fit for infant consumption. These could be delivered either in the form of porridges, or breads soaked in liquids (e.g., milk, hydromel, and sweetened wines) (Garnsey, 1999). Soranus recommended the use of breast milk over other foods; if the mother was unable (or unwilling) to breastfeed, he suggested hiring a wet nurse, but it is unlikely that common people had access to these services (Temkin et al., 1991; Centlivres Challet, 2017). Due to poor water quality in antiquity, wine was often added to the water and offered to children as a 'safer' alternative (Laes, 2019).

If a child was not thriving, Galen and Soranus recommended extended breastfeeding (Fulmimante, 2015). Simple cereals were suggested as an exclusive diet in the early phases of weaning relatively consistently throughout medical texts (Garnsey, 1999). Commencement of weaning was recommended by Soranus and Galen to occur upon the eruption of the deciduous incisors, which typically occurs around 4.5 – 12 months of age (Temkin et al., 1991; Powell et al., 2014; AlQahtani et al., 2010; Laes, 2019). Analyses of dental health conducted on the deciduous teeth of young children from the Roman site of Isola Sacra demonstrated signs of dental caries associated with the consumption of soft, sticky carbohydrate-rich foods during weaning (Prowse et al., 2008; Prowse, 2011). Complete cessation of breastfeeding was to occur from 2.0y – 3.0y, and historical evidence of wet-nursing contracts recorded on Egyptian papyri (dated from the 1st c. BCE to 4th c.

CE) indicate that most contracts expired when the charge reached 2.0y (Masciadri and Montevecchi, 1984).

Soranus provided some recipes for weanling foods in his chapter on weaning in his medical text, *Gynaecology*; he recommended that breast milk can be supplemented first with bread soaked in hydromel (a fermented diluted honey beverage), milk (species not specified), sweet wine, or honey wine, followed by soups, porridges, and "an egg that can be sipped" (Temkin et al, 1991, p. 117). After bread, Soranus argued that children should be fed soups and moist porridges, whereas Galen asserted that vegetables and meats can be introduced (Garnsey, 1999). Infants that were fed a diet according to Galen, who recommended consumption of meat during weaning, may depict a higher $\delta^{15}N$ value compared to those following the advice of Soranus, who described plant-based complementary foods only.

According to historical texts, milk was strongly associated infancy and early life, and donkey's milk was cited as a supplement for "children who were in need of strengthening" (although goat's milk was almost certainly more common in the Mediterranean region) (Laes, 2019, p. 183). The practice of animal milk consumption around weaning and post-weaning was endorsed by Soranus, who recommended that children be given primarily milk as opposed to wine as a beverage (Laes, 2019). There is some historical evidence of Roman people living in rural communities consuming animal by-products such as eggs and dairy (milk, cheese) (e.g., Garnsey, 1999; Mackinnon, 2019) in early life (section 4.4), but it is uncertain as to how often rural children were given animal milk or other by-products to supplement breast milk or assist with weaning. Some recipes

for complementary foods also feature animal milk, namely goat and cow (Killgrove and Tykot, 2013; MacKinnon, 2019), so it is possible that consumption of these foods occurred at Vagnari during weaning.

4.6 Conclusion

An individual's lived experience throughout the life course is contingent on the environmental, temporal, social, and cultural milieu; individuals of lower social standing or those living in rural communities would have undoubtably different lives and diets compared to urban elites and the urban poor. Similarly, people born and raised in times of famine and food shortage had necessarily different diets, typically with fewer options and less variety compared to those living outside of these times (Garnsey, 1999, p. 34). During crop failure and food shortages, more people may have relied on plants (such as millet) that were more resilient to adverse environmental conditions and drought. Stable isotope analysis of individuals consuming more C_4 plant sources would therefore likely present higher $\delta^{13}C$ values. A C_4-dominant $\delta^{13}C$ signal may also result from the consumption of animal products that were fed on millet-based fodders (Prowse et al., 2004; Killgrove and Tykot, 2013). These factors are important to consider when analyzing diet during childhood and throughout the life course, since they can inform on both individual dietary histories as well as the contemporaneous environmental conditions that shaped the lived experience.

A challenge of stable isotope analysis is that it cannot detect or differentiate between the quality of food being consumed; for example, lower-status individuals who relied mostly on plant-based diets and especially wheat products also typically consumed less finely milled flours that had higher quantities of phytates, inhibiting the absorption of

micronutrients (Garnsey, 1999). Their $\delta^{13}C$ values would be similar to those with more nutritious diets, but the varying nutritional quality would have resulted in different health outcomes. Similarly, poorer individuals (who did not have their own arable land) would likely have relied on smaller quantities of animal products such as dairy, eggs, and cheaper cuts of meat compared to higher status Romans, which would also have prevented the breakdown of phytates in the diet (Garnsey, 1999; Uvere and Ene-Obong, 2013; Mackinnon, 2019). Although individuals of higher social standing likely consumed more varied and nutrient-dense animal products, the observed $\delta^{15}N$ signal would be the same as an individual consuming only milk.

Vagnari is a rural estate, and it is presumed that most of the people interred at the cemetery belonged to lower, working classes of Romans from the surrounding region (refer to Chapter 5 for more information on the site). Children here were therefore raised in a rural setting, perhaps with access to a higher variety of foods compared to their urban counterparts. This chapter has shown the possible diverse sources of diet for children and adults living at Vagnari and provides the historical context to better understand dietary practices in rural Romans.

5. MATERIALS AND METHODS

5.1 Site Information

5.1.1 Vagnari Estate and Cemetery

Vagnari is a 3.5-hectare site located 12km away from the modern city of Gravina in Puglia in southern Italy (Figure 5.1). It is likely located near the Via Appia, a major ancient trading route, which spanned from Rome to the southern Italian coast (Small, 2007; Small and Small, 2011). The site was discovered in the late 1990s by Alastair Small, and has been the subject of an excavation program since 2000. The village, or

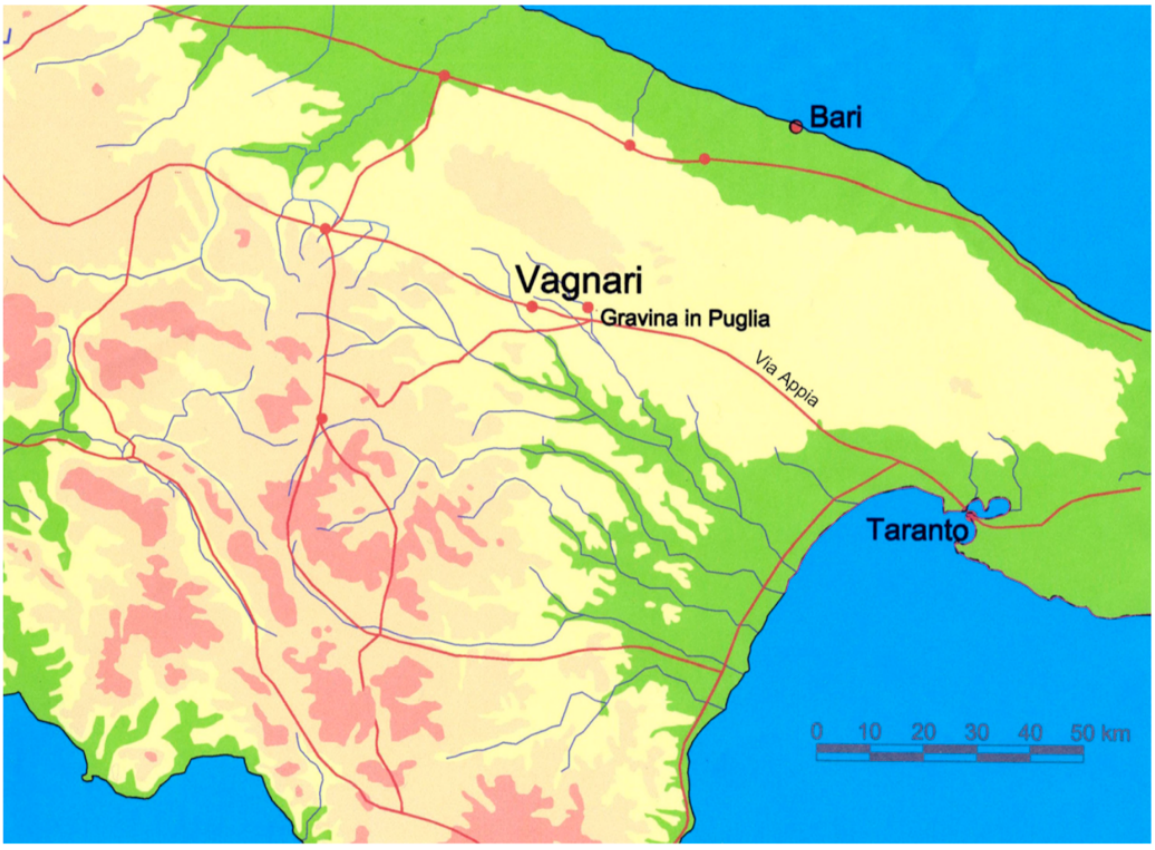

Figure 5.1: Map showing the position of Vagnari relative to modern-day Gravina and the Via Appia trading route. From Prowse and Small, (2008, p. 1). Accessed January 1st, 2023. https://www.fastionline.org/docs/FOLDER-it-2009-131.pdf

'vicus', is located North of a ravine that divides the site, while the cemetery, which was discovered in 2002, is situated to the South (Figure 5.2).

From 2002 to 2019, excavations have unearthed approximately 155 burials over a ~1200m^2 surface, representing all age groups (Ledger et al., 2021). They are labeled according to the feature number in which they were found, in ascending order.

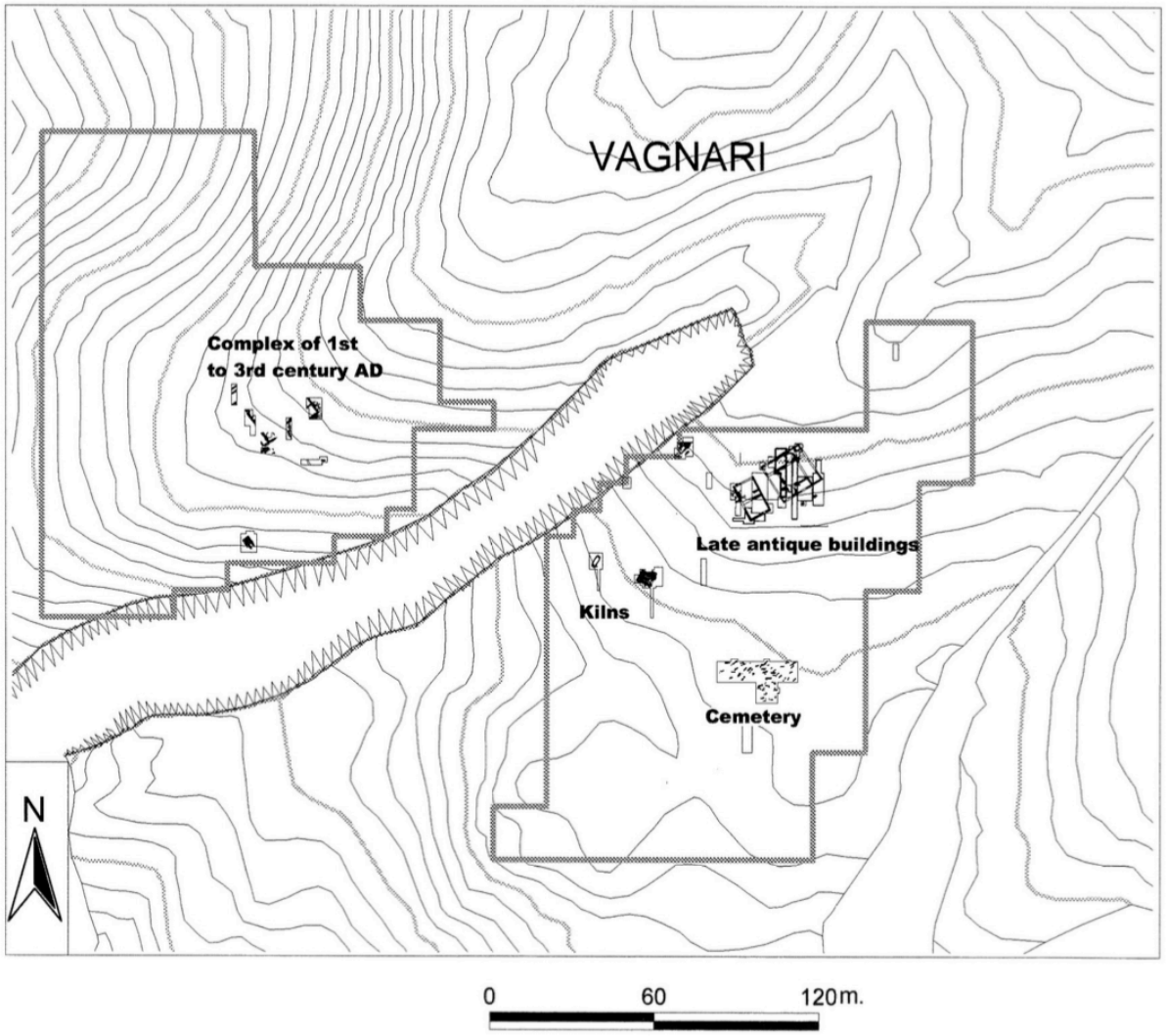

Figure 5.2: View of the Vagnari cemetery showing the divided site. The Vicus is shown on the left (North) and the cemetery is on the right (South) of the ravine. Image sourced from Prowse and Small (2008, p. 2). Accessed January 1st, 2023. https://www.fastionline.org/docs/FOLDER-it-2009-131.pdf.

The burials in the cemetery are mainly dated from the 2nd to 3rd c. CE, with a small number from the 1st and 4th centuries, based on datable grave goods within the burial (e.g., coins, diagnostic pottery) (Prowse, 2011; Brent and Prowse, 2014; Carroll and Prowse, 2014; Prowse et al., 2014). The cemetery does not follow an apparent

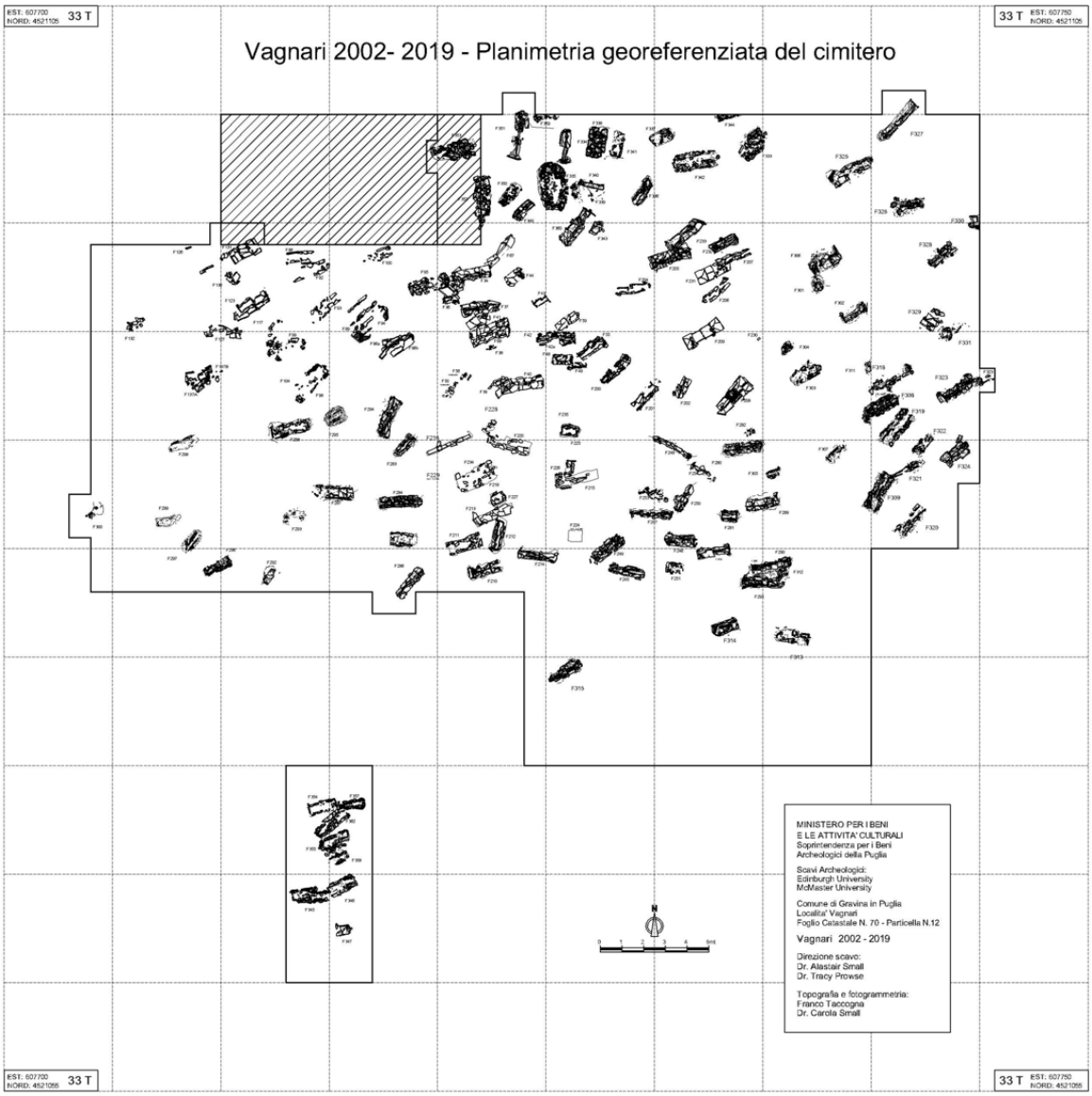

Figure 5.3: Vagnari cemetery plan showing burials excavated from 2002 - 2019. Plan prepared by Franco Taccogna (Gravina in Puglia). Produced with permission from T. Prowse.

chronological organization, with burials from all time periods interspersed throughout the total area of the cemetery (Figure 5.3). Burials are typically one of two types: In 'alla cappuccina' burials, the individual was interred in a shallow grave covered by large roof tiles (tegulae) in an inverted 'V' shape. This is the most commonly used burial type for ordinary individuals (Small, 2007; Prowse and Carroll, 2018). The second type is described as a pit grave, in which an individual was placed in a shallow grave and covered by flat tegulae (Small, 2007, p. 130). Other burial types, such as interred cremains were present, but less commonly found at the site (n = 4) (Prowse, 2022, personal communication).

Grave goods were found in virtually all burials, however differences in quantity, quality, and type of grave goods indicate age- and gender-based differences in status (Brent and Prowse, 2014; Prowse, 2016). Male burials more frequently contained metal implements, whereas females were typically buried with oil lamps (Prowse, 2016). Males on average had more grave goods than females, and older individuals were found with more artifacts compared to younger individuals. The skeletal material at the cemetery is generally poorly preserved, with damage being particularly apparent in infant remains and poor preservation of fragile bone (Prowse and Small, 2008).

5.1.2 Roman Estate Workers at Vagnari

Small (2011) hypothesizes that the vicus was part of an Imperial Roman estate. An Imperial estate was a major production center for various specialized activities that accommodate and serve a large surrounding population (Small, 2011; Rosafio, 2014; Carroll, 2022). Vagnari is located in a region that was first conquered by Romans in the 4th

c. BCE during the Second Samnite War (Rosafio, 2014). This territory (referred to as a saltus) was used for the development of forests and rough grazing, and used for agriculture, pasture, and artisanal work (Small, 2011; Rosafio, 2014). According to Rosafio (2014), ownership of the estate was transferred to the emperor at the beginning of the Principate (27BCE – 284CE), triggering significant growth and development of the site in the 2nd c. CE.

Although the demographic composition of the people of Vagnari is unknown, it is likely that some enslaved individuals were present at the site, as indicated by an inscription of the name of a slave, *Gratus*, on tiles produced at the vicus (Small et al., 2007; Small, 2011; Carroll, 2022). Imported ceramic dolia used for wine production were sent to Vagnari by the emperor; these higher-quality vessels differ from the locally produced dolia typically used by private owners in the region, indicating the Imperial administration of the site (Carroll, 2022; Stirn and Sgouros, 2022). It is likely that Vagnari also employed freedmen and free-born individuals as seasonal workers (such as crop harvesters) or contractors for specialized and semi-specialized labour (Chelotti, 2014; Rosafio, 2014; Carroll, 2022).

Findings from excavations of the vicus indicate a broad economic basis at the site; Vagnari became an established industrial center by the 1st c. CE, and was a hub for crop cultivation, iron-working, pottery making, lead processing, tile manufacturing, with activities expanding to wine production in the 2nd c. CE (Prowse and Carroll, 2017; Carroll, 2019, 2022; Carroll et al., 2021). The vicus was occupied from the 2nd c. BCE to the 5th c. CE, separated into seven distinct phases (Small; 2011; Carroll, 2022). Administrators at Vagnari had a pivotal role in organizing the population and workforce;

since it was located in a large, relatively remote rural area, the site would have encompassed significant political, economic, and cultural roles in the region (Carroll, 2022).

Prior bioarchaeological analyses of the individuals buried at Vagnari have examined diet, migration, and geographic origins throughout the extended history of the site (Prowse et al., 2007; Prowse, 2016; Semchuk, 2016; Emery et al. 2018a, 2018b). The majority (91%) of individuals showed oxygen isotope signals corresponding to the region surrounding the site, suggesting that the estate was operated by the local working class as opposed to imported slave labour (Prowse, 2016; Carroll, 2022). Emery and colleagues (2018a, 2018b) examined the ancient mitochondrial DNA (mtDNA) and $^{87}Sr/^{86}Sr$ (strontium) and $\delta^{18}O$ of ~85 inhabitants from Vagnari. The results from their first study (Emery et al., 2018a) indicated that most of the sample was local to Vagnari (58%), relative to 34% of people who were from the southern Italian peninsula and only 7% from farther away. In a follow-up analysis (Emery et al., 2018b), mtDNA data indicated that despite increased genetic diversity in the population prior to the 1st c. CE, most people analyzed from Vagnari were born and raised in southern Italy. The data suggest that this particular site is associated with rural populations of estate workers outside of the urban centers of the Roman world, and provides critical information on the demographic structure of populations in rural Roman Italy (Emery et al. 2018b).

Carroll (2022) and Stirn and Sgouros (2022) point out that there is no luxury villa or known Imperial residence associated with the Vagnari estate. Despite this, Vagnari is still considered an Imperial estate based on its economy, production, and population dynamics (Small, 2011; Small, 2014b; Carroll, 2022; Stirn and Sgouros, 2022). According

to Carroll (2022), these characteristics distinguish Vagnari from surrounding industrial villages or sites occupied by other Italic peoples. The identity of management of Vagnari is currently unknown; however, as was common in Imperial estates, it is likely that the site operated on a tenancy basis (Small, 2011; Vera, 2014).

Since most of the skeletons found in the cemetery are from the 2nd c. CE (Prowse and Small, 2008; Prowse, 2022), and a significant proportion of the samples in this study are conclusively dated to this time period, I will focus my discussion on archaeological evidence from 'Phase 3' (of seven) of the vicus dated to the same time period, as described by Carroll and colleagues (2022).

5.1.3 Faunal Remains Recovered from the Vicus

Trentacoste (2022) reports that faunal remains were found in every phase of the vicus. Faunal analysis was conducted on grouped faunal materials from Phases 2 – 6 (1st – 3rd c. CE), the results of which will be the focus of this discussion (Trentacoste, 2022). The findings are summarized in Table 5.1.

The most abundant taxa identified from the site were sheep and goats, representing nearly 34% of the total remains recovered. The remains of sheep and goats span from <1.0y of age to >4.0y for older animals; this wide slaughtering window indicates that these animals were likely raised for a variety of purposes, including meat, milk, crop fertilization, and wool production (MacKinnon, 2011; Trentacoste, 2022). Other significant taxa reported by Trentacoste (2022) include pigs with ~30% prevalence and cattle representing just over 13% of the total sample (Table 5.1).

Table 5.1: Recovered faunal remain taxa from Phases 2 – 6 of the Vagnari vicus. Adapted from Trentacoste (2022, p. 177).

Taxon	Number of Identified Specimens	% of Total
Domesticated		
Sheep/Goat	129	33.7
Pig	114	29.8
Cattle	52	13.6
Dog	33	8.6
Chicken	4	1.0
Equid	3	0.8
Wild		
Deer	18	4.7
Large Mammal	7	1.8
Tortoise	6	1.6
Small Rodent	5	1.3
Hare	3	0.8
Stoat/Weasel/Polecat	2	0.5
Rat	2	0.5
Wild Boar	1	0.3
Vole	1	0.3
Bird	1	0.3
Lizard	1	0.3
Medium Mammal	1	0.3
Total	**383**	**100.0**

The bone fusion and dental analyses conducted on the pig remains indicate a strategic culling schedule in which some animals were slaughtered before 18 months of age, and others before 48 months of age (MacKinnon, 2011; Trentacoste, 2022). MacKinnon (2011) and Trentacoste (2022) posit that since the pigs were slaughtered at a

young age, and their primary purpose as livestock is for meat and fat, so it is likely that these animals were bred and consumed at the site. The good health (i.e., absence of disease and age-related degenerative changes) of the pig remains and the high proportion of pigs in the faunal assemblage during Phases 2 – 6 further suggests an Imperial presence at the estate (Trentacoste, 2022). Pigs bred for export at an Imperial estate would have been subject to strict regulations as dictated by Roman legislation; in other words, the health and proportion of the pigs in the recovered faunal remains may indicate swine husbandry and the dietary and economic importance of swine handling at Vagnari (MacKinnon, 2011; Trentacoste, 2022).

Cattle remains typically belonged to animals with a lifespan of 4.0y+; the high proportion of fused cattle bones indicates that cattle played a significant role in agricultural labour (Trentacoste et al., 2021; Trentacoste, 2022). In addition to the livestock, domestic taxa such as dog, chicken, and equid were present, but in much smaller quantities. Among wild taxa, the most commonly found group was deer, but this sample set represented less than 5% of the total remains recovered. Other wild taxa recovered include rodents (such as rats and voles), large mammals (including wild boar), hares, birds, and reptiles (tortoise, lizard), but the prevalence for each taxon is less than 2% (Trentacoste, 2022, p. 177). Animals such as deer and hares were likely hunted or trapped, while other taxa of small mammals and reptiles are thought to be incidental depositions. Trentacoste (2022) suggests that rats would have been present as commensal pests. It must be taken into consideration that the recovered faunal assemblage only represents animals that were brought to Vagnari to die, or the remains of which were brought to Vagnari after death. The true number of

animals that were raised and lived in Vagnari is therefore likely much higher, but is underrepresented due to slaughtering occurring elsewhere (Trentacoste, 2022).

5.1.4 Botanical Evidence from the Vicus

Stirn and Sgourous (2022) report that the most commonly cultivated species at Vagnari were: olive, emmer, einkorn, spelt, free threshing wheat, barley, grass pea, bitter vetch, and stone fruits such as cherries, apricots, and sloe. The botanical finds recovered from Phase 3 (2nd c. CE) are summarized in Table 5.2. The cultivated assemblage is comprised almost entirely of C_3 plants (except for goosefoot, a C_4 species), including grain species such as wheat, barley, einkorn, spelt, legumes such as grass pea and bean, and stone fruits such as apricot and olive. Wild species of grasses and legumes were also present in the assemblage, although in much lower quantities (Table 5.2).

The results represent a diverse assemblage of plants reflecting both internal and external activities occurring at the estate (Stirn and Sgouros, 2022). Plants would have been used internally at Vagnari for food, fodder, or fuel, and for agricultural production and exportation of grain, oil, and/or wine (Stirn and Sgouros, 2022). Grapes were not recovered in Phase 3. This was unexpected, since the presence of dolia at the site is indicative of wine production and storage (Carroll, 2022). This evidence can be explained by poor preservation of plant material or wine processing occurring elsewhere, but this absence warrants further investigation (Margaritis and Jones, 2006; Figueiral et al., 2010; Stirn and Sgouros, 2022). Floor samples from an assumed winery (housing several partially buried dolia placed during Phase 3) contained a mix of cultivated and wild species that are

Table 5.2: Summarized findings of botanical remains from Phase 3 (2nd c. CE) of the vicus. Adapted from Stirn and Sgouros (2022), pp. 199-200.

Plant Species	Number of Identified Specimens	% of Total
Cultivated		
Free Threshing Wheat Grain	7	13.7
General Wheat Grain	6	11.8
Barley	4	7.8
Apricot	4	7.8
Einkorn	3	5.9
Spelt Grain	2	3.9
Glume Wheat Grain	2	3.9
Grass Pea	2	3.9
Olive	2	3.9
Wheat Culm	1	2.0
Bean	1	2.0
Wild		
Goosefoot	4	7.8
Ryegrass	3	5.9
Small-Seeded Legume	2	3.9
Vetch	1	2.0
Canarygrass	1	2.0
Willowherb	1	2.0
Bedstraw	1	2.0
False Cleaver	1	2.0
Restharrow	1	2.0
Geranium	1	2.0
Mallow	1	2.0
Totals	**51**	**100.0**

suggested to have been brought to the room unintentionally. Stirn and Sgouros (2022)

conclude that this room was used primarily for storage of wine and oil.

Relative to earlier phases, the assemblage dating to the 2nd c. CE has an increased proportion of free threshing wheat (surpassing the prevalence of barley); the distribution of plant taxa in Phase 3 compared to Phases 2 and 4 represents a "cleaner [assemblage] with a slightly higher proportion of cereal grain to chaff/weeds" (Stirn and Sgouros, 2022, p. 193). Stirn and Sgouros (2022) suggest that this elevated ratio of grains may indicate an increased scale and intensity of agricultural production in the 2nd c. CE, during the Imperial occupation of the site.

5.1.5 Sex and Age-Based Differences in Diet at Vagnari

Semchuk (2016, p. 108) reported similar dietary signals between adult males and females, indicating a lack of sex-based differences in diet. Access to higher trophic level foods likely changed as the individual aged; isotopic signals of adults demonstrated greater consumption of higher trophic level foods relative to children and adolescents (Semchuk, 2016, p. 88).

Semchuk (2016) analyzed bone collagen from the ribs and femora of 18 subadult (0y – 14.9y) individuals to conduct a small preliminary study on breastfeeding and weaning patterns in the Vagnari sample. She reported a correlation between life stages and variation in diet; bone collagen signals from seven individuals aged 0y - 3.9y years and 11 individuals aged 4.0y – 14.9y indicated that weaning was in progress by 3.0y and $\delta^{15}N$ values gradually declined, approaching the adult female mean by 5.0y at the latest (Semchuk, 2016, pp. 85-87). As mentioned in section 3.3, bone collagen isotopic values represent an average value over relatively long time spans; a signal from a five year-old individual's rib may represent

a dietary signal from ages 2.0y – 5.0y, resulting in poor temporal resolution. This presents a particular challenge when using small sample sizes because there are very few values that can be inputted to establish a weaning curve, decreasing the degree of accuracy of the reported results.

Bone collagen from young individuals may provide an inaccurate representation of the diet of healthy individuals; as previously posited by Wood and colleagues (1992), it is possible that the isotopic signals from young individuals reflects a diet specialized to address an existing illness, or a diet which itself contributed to the premature death of the individual. The use of incremental dentine in this study will narrow the age range represented by each sample, improving the accuracy of the overall weaning curves observed, and more accurately reflecting the dietary patterns of the healthy population. The present thesis will compare incremental dentine stable isotope signals to the bone collagen results obtained from Semchuk (2016), since a majority (n = 14) of the individuals selected for this thesis were also sampled for bone collagen analyses (discussed in section 7.2.5).

5.2 Sample Collection

The samples are housed at McMaster University. Teeth were selected based on the following criteria: (1) The tooth was a permanent first molar (upper/lower/left/right were all included in the selection process), since the development of this tooth spans the entire breastfeeding and weaning period from birth to 8.5y (AlQahtani et al., 2010); (2) The tooth showed minimal wear (i.e., occlusal attrition did not result in the loss of data for younger age categories when breastfeeding/weaning likely occurred); (3) The tooth had enough root material; (4) The tooth had relatively little antemortem damage or presence of carious

lesions; samples in which the carious lesion was too large to drill away or extensive within the tooth structure were excluded from the study; (5) The tooth had experienced little post-mortem damage; severely brittle, fragile, or damaged teeth (missing most of the crown and/or root) were not included in this study. A number of samples (i.e., F126, F206, F286B, F291, F312, F313, F320, F323) were previously used for stable isotope and aDNA analyses, which in some cases resulted in missing (removed) buccal crown cusps and roots. Maxillary teeth with missing buccal roots were included if there was sufficient lingual root present for collagen extraction.

The initial sample selected for this study consisted of 11 females, nine males, and five adults/subadults of unknown sex. Throughout the preparation process, the samples from five individuals were lost due to poor preservation (i.e., they degraded excessively in hydrochloric acid, HCl) and low collagen yield. This resulted in a total of 20 individuals, comprised of nine females, eight males, and three individuals (adult n = 1, and subadult n = 2) of unknown sex (Table 5.3). Age and sex were assessed at the site using methods outlined by Buikstra and Ubelaker (1994). Sex estimation in adults was conducted using a number of methods, including pelvic and cranial morphology and some metric measurements (e.g., humeral head measurements) in cases of poor preservation. Sex was not estimated for subadults and one individual (F212) with poor preservation. Age in adults was assessed using the pubic symphysis, auricular surface, sternal rib end, or cranial suture closure, whereas subadults were aged using dental development and long bone lengths. Due to the poor preservation of many diagnostic skeletal features, some individuals were broadly classed as young adults and adults. For the purposes of this study, adulthood is

Table 5.3: Summary of individuals sampled.

Excavation Year	Individual	Sex	Age	Tooth
2004	F126	M	20y - 25y	LM_1
2004	F127	F	15y - 20y	LM^1
2007	F206	F	50y+	LM_1
2007	F207	M	Young Adult	LM_1
2007	F211	F	< 30y	RM^1
2007	F212	U	Adult	LM_1
2008	F215	F	38.2y ± 10.9y	LM_1
2008	F235	M	~50y ± 12.6y	RM^1
2011	F247	M	Adult	RM^1
2009	F249	M	Adult	LM^1
2012	F286B	U	13y - 14y	RM^1
2013	F291	M	Adult	RM^1
2012	F296	F	25y - 29y	RM^1
2015	F308A	F	Adult	RM^1
2015	F309	M	Young Adult	LM^1
2013	F312	M	Young Adult	RM^1
2015	F313	F	19y - 23y	LM^1
2015	F320	F	38.2y ± 10.9y	RM_1
2016	F323	F	42.5y ± 12.6y	LM_1
2017	F336	U	10y ± 2y	RM^1

associated with the completion of permanent dentition eruption (~20.5y of age, AlQahtani et al., 2010), but individual F127 aged 15y – 20y was also considered an adult, because the presence of sexually dimorphic traits allowed for sex-based comparisons.

5.3 Stable Isotope Methodology

5.3.1 Creating Dentine Cross-Sections

The tooth was ultrasonicated in distilled water three times for five minutes (switching water between washes) and dried before being marked along the highest points of the buccal

crown and the lowest parts of the root using a *Sharpie* pen. The markings were placed in these areas to allow for easy visualization and accurate alignment of the cut during the circular saw step. The marked tooth was embedded into equal parts of *EpoThin Epoxy* resin and hardener and left to cure for 24h. For mandibular molars, the cross-section was marked along the mesio-distal plane, slightly buccal to the midline to collect material from the dentine horns under the occlusal cusps. In maxillary teeth, the cross-section was placed to maximally visualize the buccal roots; M^1 samples with a single remaining lingual root (due to prior analyses, n = 2) were demineralized first and then sectioned to visualize the inner structures of the remaining tooth portion. Once cured, the embedded teeth were cut using a *Buehler IsoMet* Low Speed Precision Cutter with a 3-inch wafer diamond blade to create the tooth cross-section (cutting through the highest portion of the crown and root to maximize the visibility of internal structures) following the protocol outlined in Beaumont et al. (2013).

5.3.2 Dissolving Epoxy

One half of the tooth was used for the analysis, with any remaining material retained for future studies. The tooth sections were placed in 100% acetone in a closed container to dissolve the epoxy for 24h. In some cases, the quantity of the epoxy surrounding the sample resulted in a prolonged dissolving period, up to 72h after initial exposure to acetone. The epoxy adhered strongly to some samples, which necessitated manual removal by carefully scraping the surface of the tooth with metal dental tools. Once the epoxy was dissolved, the sample was placed in distilled water and washed three times in an ultrasonic bath for five minutes. Washed teeth were dried at 60°C for 24h to prepare for enamel removal.

5.3.3 Enamel Removal and Dentine Demineralization

Enamel was carefully removed using a diamond bit tip and handheld Dremel tool. Use of a dissecting microscope for visualizing the enamel-dentine border allowed for effective removal of enamel with minimal dentine damage and loss. Once the enamel was completely removed, the remaining dentine portion of the tooth was soaked in 0.5M HCl, changing the solution daily, until the samples had had demineralized enough to manually incise. Once the samples had sufficiently demineralized, they were removed from the 0.5M HCl, gently tapped dry, and photographed cut-side up next to a 2cm scale.

5.3.4 Calibrating Dentine Line Diagrams

In well-preserved samples, thin section microscopy reveals dentine growth lines that correspond to known rates of formation, whereas diagenetic alterations produce fibre-like patches within the dental structure, obscuring the dentine lines and preventing the use of microscopy for creating accurate sections (Figure 5.4) (D'Ortenzio et al., 2019). Due to limited dentine material, only 12 teeth were prepared for thin-section microscopy. Of these, five teeth had identifiable dentine lines in the crown, but none of the thin sections showed dentine lines in the roots. Thin-sections were examined for the presence secondary dentine, revealing only two samples (F291 and F309) with potential deposition of secondary dentine at the CEJ and root sections, not exceeding more than ~0.5mm in width. No secondary dentine was observed in the crown portion of the tooth, perhaps due to obstruction of dental structures by diagenesis. Diagenetic alterations on the Vagnari samples prevented the use of thin-section microscopy as a guide to create sections along the growth lines or to identify and separate any secondary dentine from the primary dentine formed in early life.

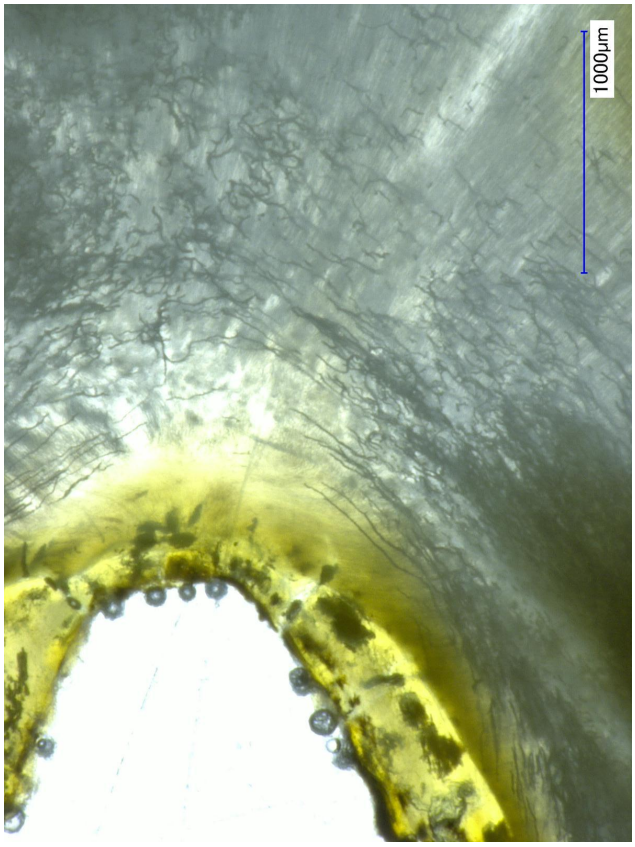

Figure 5.4: 100x Magnified image of F127 (LM^1) dentine thin section. Diagenesis appears as fibre-like structures within the dentine matrix.

In order to mitigate the absence of microscopic images as a visual guide to dentine sectioning, a novel method was devised to estimate the growth lines on the sample. In order to properly correlate a dentine section to a known age category, close-range photographs of the tooth sections were taken, edited to maximize visual contrast, and printed at an enlarged scale (Figure 5.5). The shape and width of sections corresponding to a particular age category was estimated using enlarged maxillary and mandibular M1 diagrams from the Brickley et al. (2019, p. 347, Figure 3b) publication (Figure 5.6). The London Tooth Atlas was employed to determine the age ranges represented by each section; this developmental chart is widely used in biological anthropology (AlQahtani et al., 2010 ,

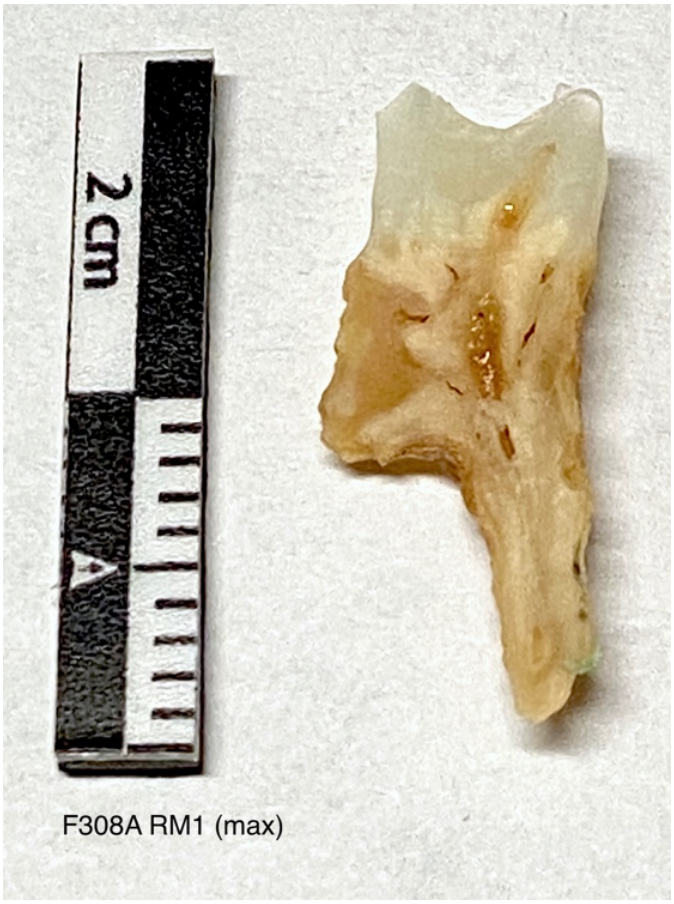

Figure 5.5: Close-range photo of F308A (RM^1). The photo was edited in Preview to enhance the contrast of the internal structures of the tooth.

2014). The diagram was mirrored (showing the dentine lines on both sides) as a visual aid to establish symmetry in morphologically abnormal teeth. The mirrored diagrams were printed and the distance between each growth line was measured using a metric ruler. Points were plotted at peak of the growth line on the pulp horns (oblique points), pulp chamber roof (vertical points), dentine-pulp border (pulp chamber points), and along the external surface of the tooth (lateral landmark points) (Figure 5.7).

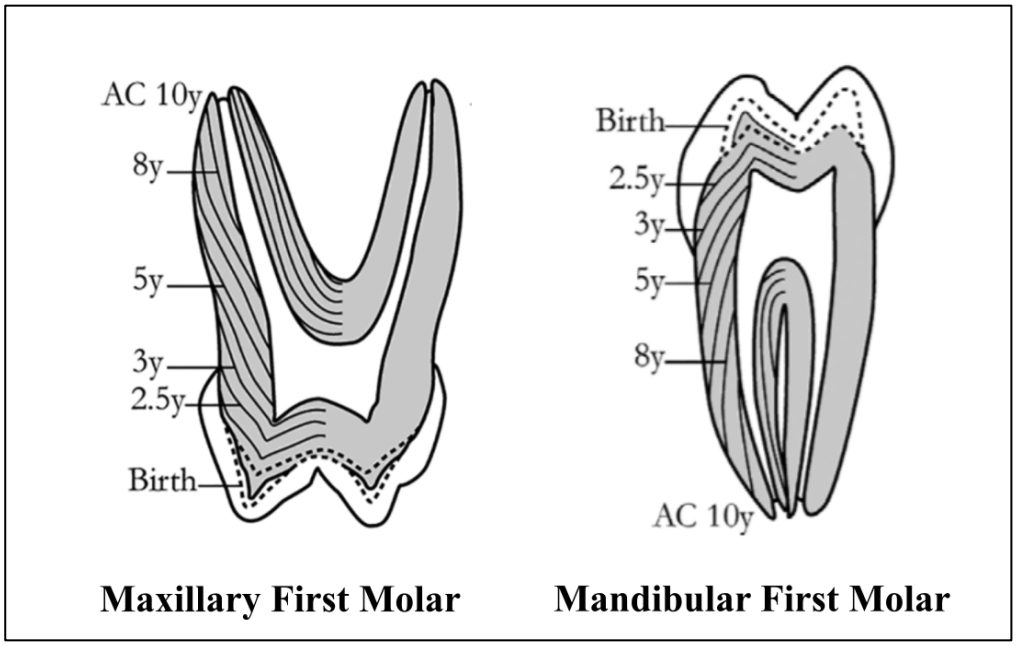

Figure 5.6: Original diagrams showing dentine growth lines in maxillary and mandibular first molars. M^1 from Brickley et al., (2019), p. 347, Figure 3b; M_1 from Brickley et al., (2021), p. 948. [Open Access] DOI: 10.1002/ajpa.23947; 10.1002/ajpa.24292.

5.3.5 Cut Line Mapping

The cut lines were drawn on the sample photo by following these steps: the length of both the diagram and the printed image were measured and a scale was established between the length of the diagram and the length of the image. In cases of damage to the root or occlusal surface, the width of the tooth at the cementoenamel junction (CEJ) was used to establish the scale. This portion of the tooth was least affected by the enamel removal step. Once established, the relative scale was used to measure the width of each section on the diagram and calculate the width of the section on the corresponding image. Landmark points were situated along the vertical axis, oblique axes, the outside surface of the tooth, and pulp chamber walls (Figure 5.7).

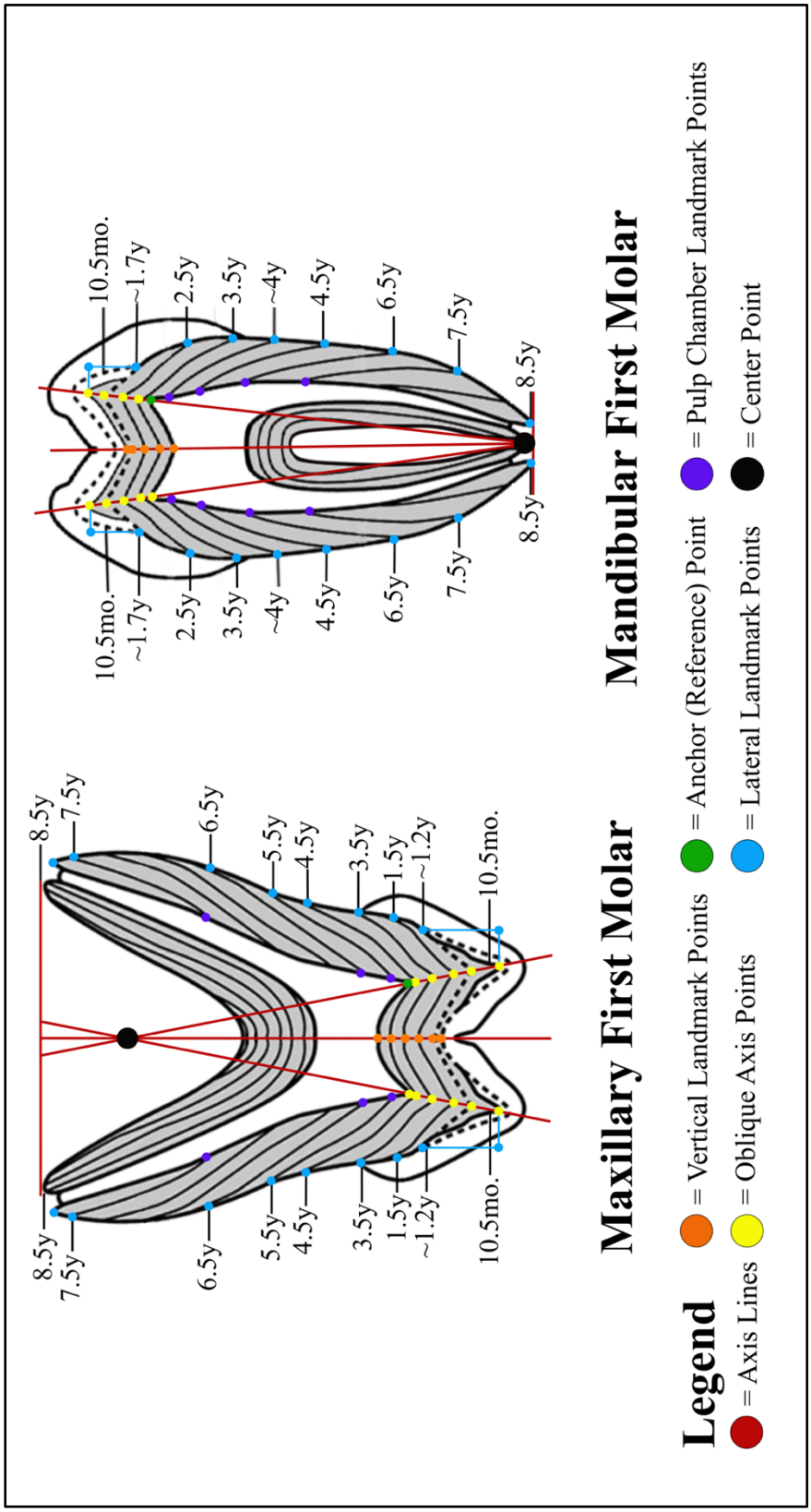

Figure 5.7: Modified cut line diagrams for maxillary and mandibular first molars, showing age ranges according to the London Tooth Atlas. Oblique axes pass through the highest points of the dentine horn, dentine lines, and pulp chamber horn. The location of the axes and landmark points were translated onto magnified photos of sample images as per the described methodology. Original diagrams from Brickley et al., (2019), p. 347, Figure 3b (M^1) and Brickley et al., (2021), p. 948 (M_1).

Most of the samples in this study were morphologically dissimilar to published

diagrams, since they were often narrower, longer, asymmetric or had crooked crowns and/or roots, and frequently had obscured or absent pulp chambers, dentine horns, and other landmark features. In order to estimate the shape and position of each 'dentine line', visibly absent landmarks described above were estimated through the use of vertical, horizontal and oblique axes and reference (anchor) points (Figure 5.7). The morphological variation in the samples forced a necessarily tailored approach to drawing sections on each tooth; landmarks and reference points used (such as the dentine horn, pulp horn, or pulp chamber floor) were dependent on the quality and integrity of the tooth at each region. The best-preserved regions with the most typical morphology were selected as the anchor points for plotting the rest of the landmark points. Landmark points were joined together with curved lines to establish the cut lines and visualize the shape and width of each section. Loss of tissue from the crown or root of some teeth resulted in the absence of isotopic data for certain age categories (Figures 5.8, 5.9). Using the published measured diagrams mitigated any potential errors in age calibration, especially for individuals with missing tooth material and atypical morphology, providing a more accurate estimation of the age range of the section (Figure 5.9).

5.3.6 Tooth Sectioning

Once the images were marked with the cut lines, the tooth was tapped dry and placed on a hard surface, and cuts were made starting at the crown and moving towards the root apex. Using a scalpel and precision tweezers, the sample was held in place and cut along the estimated lines, following the shape of the growth lines indicated on the image as closely

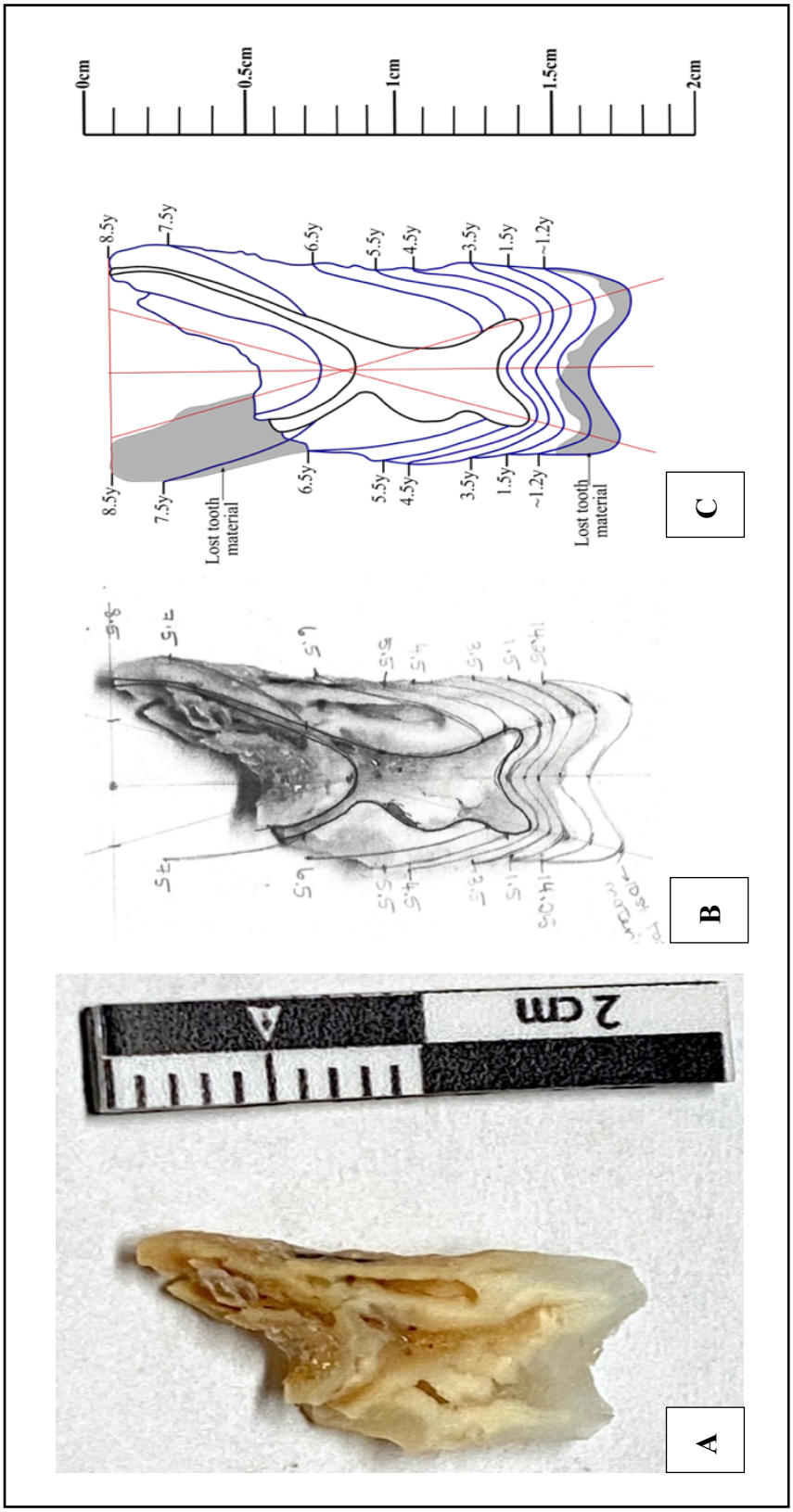

Figure 5.8: Steps taken to estimate cut lines of a maxillary first molar. Reference point chosen = right-hand-side of pulp chamber roof. A: Enlarged photo of F309 LM^1 with 2cm scale. B: Hand-drawn projected cut lines made by following the described methodology. C: Digitized image showing age calibration for each projected cut line. Shaded areas indicate missing dentine. All cuts were performed beginning at the crown moving towards the root.

as possible. Depending on the amount of tooth material present, samples were sectioned to

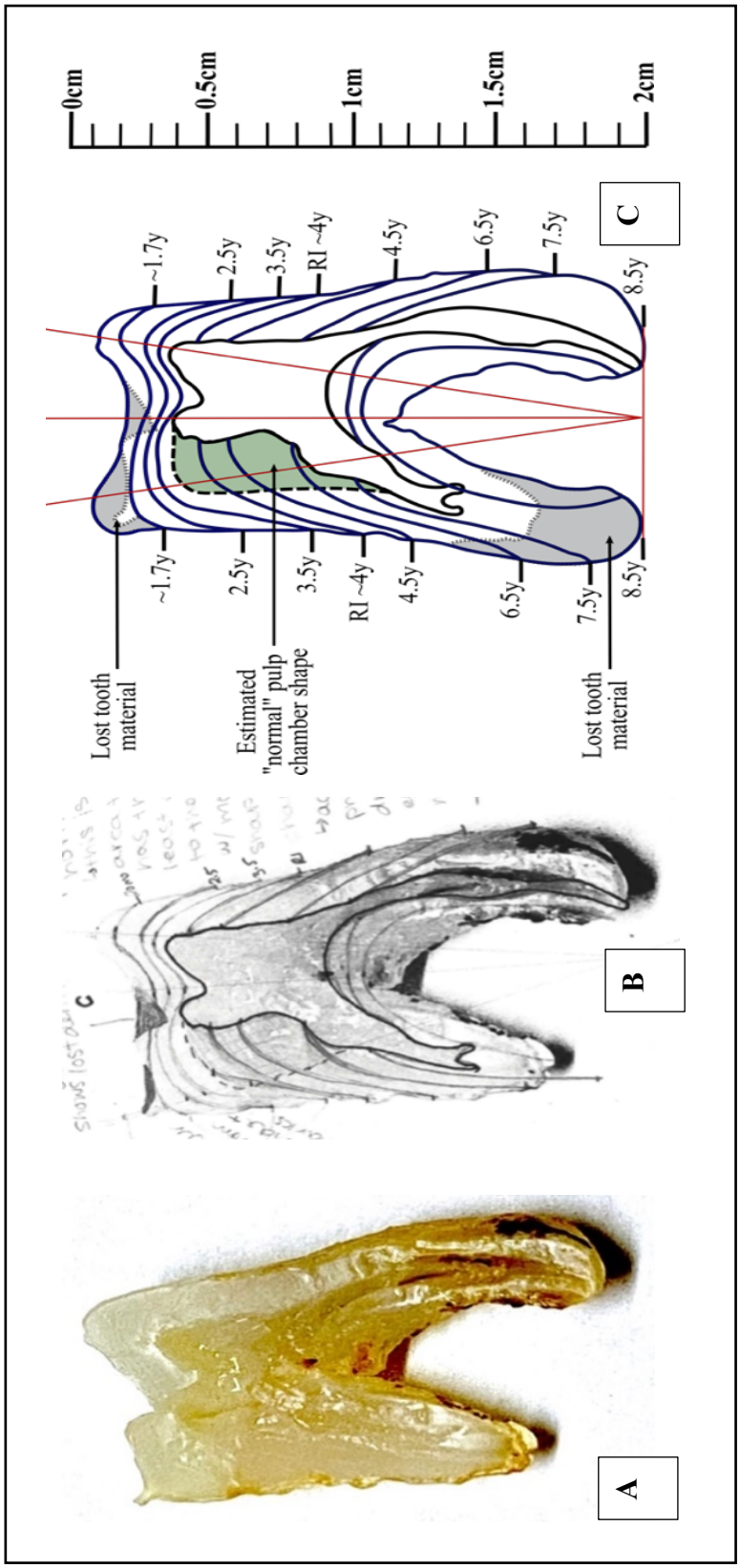

Figure 5.9: Steps taken to create sections of a mandibular molar (with irregular morphology). Reference point chosen = right-hand-side pulp chamber roof and dentine horn. A: Close-range photo of F320 RM_1. B: Hand-drawn cut lines estimated by following the described methodology. C: Digitized image showing age calibration for each projected cut line with 2cm scale. Shaded areas indicate missing dentine (grey) and an estimated "normal" pulp chamber shape (green). All cuts were performed beginning at the crown moving towards the root.

create five – eight segments.

The cut sections were placed in prelabeled and weighed 2ml Eppendorf microtubes, submerged in 0.001M HCl, and heated at 70°C for 24h to extract pure collagen from the sections. Any samples with remaining organic pellet were re-heated at 70°C for additional 24h after decanting and replacing the 0.001 HCl. The supernatant was then dried at 60°C for 24h, rendering the suspended collagen into small flakes to be loaded and sent for mass spectrometry analysis.

5.3.7 Collagen Yield and Sample Integrity

Collagen from each section were weighed and recorded, and the total weight of the sections of each tooth was divided by the total weight of the dentine portion of the tooth prior to demineralization to determine yield.

Equation 5.1: Formula used to determine collagen yield from the tooth dentine

$$\frac{Weight\ of\ Purified\ Collagen\ Sections\ (mg)}{Total\ Dentine\ Weight\ Before\ Demineraliz} \times 100 = \%\ Collagen\ Yield$$

The accuracy of reported results is heavily dependent on the chemical integrity of the sample. Diagenetic alteration plays a significant role in the measured values of C:N ratios, %C, %N, $\delta^{13}C$, and $\delta^{15}N$ (see section 2.4 on diagenesis) (DeNiro, 1985; Ambrose, 1990; Guiry and Szpak, 2020; Guiry and Szpak, 2021). Since a higher ratio of C:N minimally affected the $\delta^{15}N$ signal (the primary stable isotope analyzed in weaning studies) (Guiry and Szpak, 2021), I will be using 2.9 as the lower value and 3.6 as the upper range cut-off for this study.

In addition to the C:N ratio, the collagen yield, %C and %N are important indicators of sample quality; I will be using the more conservative threshold values of 1.8% for collagen yield, %C > 14%, and %N > 4.8%, as recommended by Ambrose (1990).

5.3.8 Mass Spectrometry Analysis

Flakes of purified collagen were scraped from the bottom of the Eppendorf microtubes, weighed to 0.7mg, and loaded into tin capsules for mass spectrometry. Loaded samples were sent to the Ján Veizer Stable Isotope Laboratory at the University of Ottawa for analysis.

5.4 Statistical Analysis Methodology

Statistical tests were performed using IBM SPSS and plotted using Microsoft Excel 2016. The Kolmogrov-Smirnov and Shapiro-Wilk tests were applied to examine the distributions of $\delta^{13}C$ and $\delta^{15}N$. Since the data were normally distributed, the paired t-test (average age, $\delta^{13}C$ and $\delta^{15}N$ values) and Pearson correlation analyses were performed to compare the average $\delta^{13}C$ and $\delta^{15}N$ values to the sex, age category (i.e., dentine section), age-at-weaning, age-at-death, and grave good amounts/diversity. A Welch's t-Test (for unequal sample sizes/variances) was conducted to compare weaning ages between males and females.

6. RESULTS

6.1 Introduction

The dentine collagen results yielded information for several investigative avenues. First, the samples were assessed for integrity and diagenetic alteration. The findings from these analyses are outlined in section 6.2. Incremental dentine sections from the 1st molars were plotted as a 'weaning curve', which depicts temporal changes in $\delta^{15}N$ and $\delta^{13}C$ in early life. Individual weaning histories are discussed in section 6.3. On a sample level, weaning was assessed in sections 6.4 – 6.7 based on rate and duration of the weaning process, sex-based differences, and age-related trends. Section 6.8 compares dietary patterns from dentine results obtained in this study to previously analysed bone collagen results for the same individuals (Semchuk, 2016) to assess diet throughout the life course.

6.2 Sample Integrity Indicators

All available data (n = 130) collected from these individuals is reported in Appendix 1, including information on age, sex, tooth type, burial type, and grave goods, as well as previous bone collagen data from Semchuk (2016) for 14 individuals used in the present study. A total of five samples were lost during sample loading and repackaging. These samples are indicated in yellow. As discussed in Chapter 2, sample integrity can be established by assessing several variables, such as C:N ratios, collagen yield, %C and %N. Sample integrity for all individuals is displayed in Appendix 2. A summary of the sample integrity findings (n = 83 samples, representing 20 individuals) are summarized in table 6.1.

Table 6.1: Summary of sample integrity indicators within the Vagnari sample (n = 83).

Sample Integrity Indicator	Range	Average	Standard Deviation
Collagen Yield	1.9% - 8.8%	6.3%	1.9%
C:N Ratio	3.28 - 3.59	3.42	0.08
%C	15.2% - 58.7%	40.3%	8.2%
%N	5.2% - 20.9%	13.8%	2.9%

6.2.1 C:N Ratios

Of 130 section samples submitted for stable isotope analysis, 41 samples had C:N ratios outside of the acceptable range of 2.9 – 3.6 (highlighted in pink in Appendix 2) (DeNiro, 1985). These sections were excluded from further statistical analyses. The remaining 83 samples had C:N values ranging from 3.28 – 3.59 (± 0.08), meeting the requirements for adequate sample integrity (Table 6.1). The analyses of %C and %N were performed using only samples that met the 2.9 – 3.6 C:N ratio criteria.

6.2.2 %N

The samples included in this study had %N values ranging from 5.2% – 20.9%, with an average of 13.8% ± 2.9% (Appendix 2, Table 6.1). All of the samples within the acceptable C:N range also met the minimum requirements for %N (>4.8%) as outlined by Ambrose (1990).

6.2.3 %C

Among the samples with C:N ratios in the acceptable range (n = 83), the %C obtained from the dentine ranged from 15.2% to 58.7%, surpassing the 14% threshold outlined by Ambrose (1990). The average %C for the sample was 40.3% ± 8.2% (Appendix 2, Table 6.1).

6.2.4 Collagen Yield

The collagen yield ranged from 1.9% (individual F320) to 8.8% (individual F235). All of the teeth used in this study met the minimum collagen yield requirement of >1.8% for adequate sample integrity (Ambrose, 1990). Of the 20 individuals studied, six individuals (F127, F249, F308A, F309, F320, F336) had collagen yields <5%. Van Klinken (1999) defines collagen yields above 5% as indicators of good preservation, therefore the six individuals with lower yields likely experienced mild to moderate diagenetic alteration.

6.3 Individual Weaning Histories

Bone collagen (femur) values for adult females (n = 23) were determined in a previous study by Semchuk (2016). The average adult female $\delta^{15}N$ value was 9.1‰ (range: 7.9‰ - 10.1‰) and the average $\delta^{13}C$ value was -19.5‰ (range: -18.1‰ − -19.9‰) (Semchuk, 2016). These values are used here to represent the expected adult female diet in the Vagnari sample, and are shown as dashed lines in each of the charts. The next sections present the dentine stable isotope data for each individual in the current study. Nitrogen data are in red and carbon data are in green. They have been grouped into three categories, reflecting the patterns observed: (1) early initiation and rapid weaning (n = 7); (2) gradual weaning (n =

7); and (3) insufficient data to determine weaning pattern (n = 6). Table 6.2 presents the dentine data along with Semchuk's (2016) bone collagen data.

Table 6.2: Delta ^{15}N and $\delta^{13}C$ values from dentine and bone collagen. Bone collagen data was collected by Semchuk (2016). Sections excluded due to diagenetic alteration are highlighted in pink. Samples lost during repackaging are shown in yellow. Section codes were assigned to each tooth section corresponding to age. Samples with inadequate amounts of collagen were combined, resulting in a section code with a dash and a midpoint age between the two estimates.

Feature No.	Section Code	Section Age	$\delta^{15}N$ (‰) (AIR)	$\delta^{13}C$ (‰) (V-PDB)	Bone Collagen $\delta^{15}N$ (‰) (AIR)	Bone Collagen $\delta^{13}C$ (‰) (V-PDB)
F126	126.1	1.7	10.2	-20.3	9.1	-19.5
	126.2	2.5	10.2	-19.5		
	126.3	3.5	9.9	-19.2		
	126.4-5	4.5	9.3	-20.6		
	126.6-8	7.5	9.3	-19.8		
F127	127.1	1.2	12.3	-21.3	9.8	-18.4
	127.2	1.5	12.0	-20.1		
	127.3	3.5	-	-		
	127.4	4.5	9.1	-20.1		
	127.5-6	5.5	8.0	-22.6		
	127.7-8	8.0	8.7	-20.1		
F206	206.1-2	2.1	8.7	-19.7	9.3	-19.4
	206.3	3.5	8.3	-20.7		
	206.4	4.0	8.1	-19.9		
	206.5	4.5	8.2	-20.1		
	206.6	6.5	8.7	-19.6		
	206.7	7.5	-	-		
	206.8	8.5	9.3	-20.7		
F207	207.1	1.7	11.4	-19.4	9.6	-19.9
	207.2	2.5	10.6	-19.5		
	207.3	3.5	9.8	-19.8		
	207.4	4.0	-	-		
	207.5	4.5	9.3	-19.6		
	207.6	6.5	9.5	-19.9		
	207.7-8	8.0	9.5	-19.9		
F211	211.1	1.2	10.7	-23.1	9.3	-19.4
	211.2	1.5	10.4	-19.8		
	211.3	3.5	8.6	-19.6		
	211.4	4.5	8.6	-19.5		
	211.5	5.5	8.5	-19.4		
	211.6-7	7.0	8.7	-19.8		
	211.8	8.5	-	-		

Table 6.2 Continued.

Feature No.	Section Code	Section Age	$\delta^{15}N$ (‰) (AIR)	$\delta^{13}C$ (‰) (V-PDB)	Bone Collagen $\delta^{15}N$ (‰) (AIR)	Bone Collagen $\delta^{13}C$ (‰) (V-PDB)
F212	212.1	1.7	12.1	-19.3	N/A	N/A
	212.2	2.5	10.8	-19.8		
	212.3	3.5	10.2	-19.7		
	212.4	4.0	8.9	-19.7		
	212.5	4.5	9.1	-19.8		
	212.6	6.5	8.7	-20.2		
	212.7	7.5	8.7	-21.4		
F215	215.1	1.7	12.3	-19.1	9.6	-19.9
	215.2	2.5	9.1	-19.2		
	215.3	3.5	9.0	-19.7		
	215.4	4.0	8.2	-19.6		
	215.5-6	5.5	8.2	-19.6		
	215.7-8	8.0	8.4	-20.0		
F235	235.1	1.2	8.6	-19.6	9.1	-19.6
	235.2	1.5	8.3	-19.6		
	235.3	3.5	7.9	-19.9		
	235.4	4.5	8.1	-19.5		
	235.5	5.5	8.5	-19.9		
	235.6	6.5	8.6	-20.8		
	235.7	7.5	9.1	-20.8		
	235.8	8.5	9.6	-20.6		
F247	247.1	1.2	9.3	-20.9	9.8	-19.3
	247.2	1.5	9.3	-19.9		
	247.3	3.5	9.1	-20.1		
	247.4	4.5	9.5	-20.0		
	247.5	5.5	9.4	-21.2		
	247.6	6.5	8.7	-23.2		
	247.7-8	8.0	9.9	-20.7		
F249	249.1	1.2	11.7	-19.2	9.7	-19.0
	249.2	1.5	10.5	-20.0		
	249.3-5	4.5	7.2	-25.2		
	249.6-7	7.0	9.8	-20.0		

Table 6.2 Continued.

Feature No.	Section Code	Section Age	$\delta^{15}N$ (‰) (AIR)	$\delta^{13}C$ (‰) (V-PDB)	Bone Collagen $\delta^{15}N$ (‰) (AIR)	Bone Collagen $\delta^{13}C$ (‰) (V-PDB)
F286B	286.1-2	1.4	11.0	-20.1	7.7	-19.5
	286.3	3.5	9.0	-20.3		
	286.4	4.5	7.5	-21.9		
	286.5-6	6.0	7.9	-21.4		
	286.7	7.5	8.5	-21.0		
	286.8	8.5	8.3	-21.8		
F291	291.1	1.2	11.2	-20.0	9.1	-19.5
	291.2	1.5	10.2	-19.9		
	291.3	3.5	8.9	-20.2		
	291.4	4.5	8.6	-20.0		
	291.5	5.5	8.9	-19.7		
	291.6	6.5	9.6	-19.8		
	291.7	7.5	10.3	-19.5		
	291.8	8.5	10.1	-20.5		
F296	296.1-2	1.4	9.7	-20.6	8.2	-19.4
	296.3	3.5	8.5	-19.7		
	296.4	4.5	8.2	-19.8		
	296.5	5.5	7.6	-20.9		
	296.6	6.5	7.6	-20.6		
	296.7-8	8.0	8.2	-20.1		
F308A	308.1-2	1.4	9.6	-22.6	N/A	N/A
	308.3	3.5	9.2	-20.2		
	308.4	4.5	7.4	-20.8		
	308.5-8	7.0	7.7	-21.4		
F309	309.1-2	1.4	13.6	-19.1	N/A	N/A
	309.3	3.5	11.6	-19.7		
	309.4	4.5	9.8	-20.1		
	309.5	5.5	9.4	-20.5		
	309.6-8	7.5	8.1	-21.5		

Table 6.2 Continued.

Feature No.	Section Code	Section Age	$\delta^{15}N$ (‰) (AIR)	$\delta^{13}C$ (‰) (V-PDB)	Bone Collagen $\delta^{15}N$ (‰) (AIR)	Bone Collagen $\delta^{13}C$ (‰) (V-PDB)
F312	312.1	1.2	10.9	-19.9	8.3	-19.5
	312.2	1.5	9.1	-23.4		
	312.3	3.5	8.1	-21.2		
	312.4	4.5	8.9	-20.3		
	312.5	5.5	8.2	-21.1		
	312.6	6.5	-	-		
	312.7	7.5	8.8	-20.0		
	312.8	8.5	8.8	-20.1		
F313	313.1	1.2	9.4	-19.2	N/A	N/A
	313.2	1.5	8.1	-19.9		
	313.3	3.5	7.9	-20.1		
	313.4	4.5	7.7	-19.9		
	313.5	5.0	7.8	-20.0		
	313.6	6.5	7.6	-19.9		
	313.7	7.5	7.9	-20.0		
	313.8	8.5	8.7	-20.7		
F320	320.1-2	2.1	11.1	-19.9	N/A	N/A
	320.3	3.5	9.1	-19.8		
	320.4-6	5.0	8.6	-21.8		
	320.7-8	8.0	7.6	-21.3		
F323	323.1-2	2.1	9.8	-20.4	N/A	N/A
	323.3	3.5	9.4	-20.3		
	323.4	4.0	9.2	-19.5		
	323.5	4.5	8.9	-20.0		
	323.6	6.5	8.5	-20.5		
	323.7	7.5	9.1	-19.5		
	323.8	8.5	9.0	-19.8		
F336	336.1	1.2	12.6	-18.7	N/A	N/A
	336.2	1.5	10.4	-19.1		
	336.3	3.5	9.3	-19.4		
	336.4-5	5.0	8.6	-19.9		
	336.6-8	7.5	8.6	-19.7		

6.3.1 Early Initiation and Rapid Weaning

F215 (Female, 38.2y ± 10.9y)

A weaning curve for F215 was plotted using 6 individual dentine collagen signals (Figure 6.3.A). This tooth had missing dentine due to occlusal dental attrition and enamel removal for a previous study, so earlier age dietary signals are not available. The burial date for this individual is estimated to be 2nd c. CE. Delta ^{15}N decreases from 12.3‰ to 9.1‰ (by 3.2‰) within a span of 11 months, from 1.7y – 2.5y. The $\delta^{15}N$ value approaches the adult female mean (pink dotted line) by 2.5y, and the value decreases only by 0.1‰ at 3.5y, indicating that weaning has finished and the individual was likely transitioned to a childhood diet after

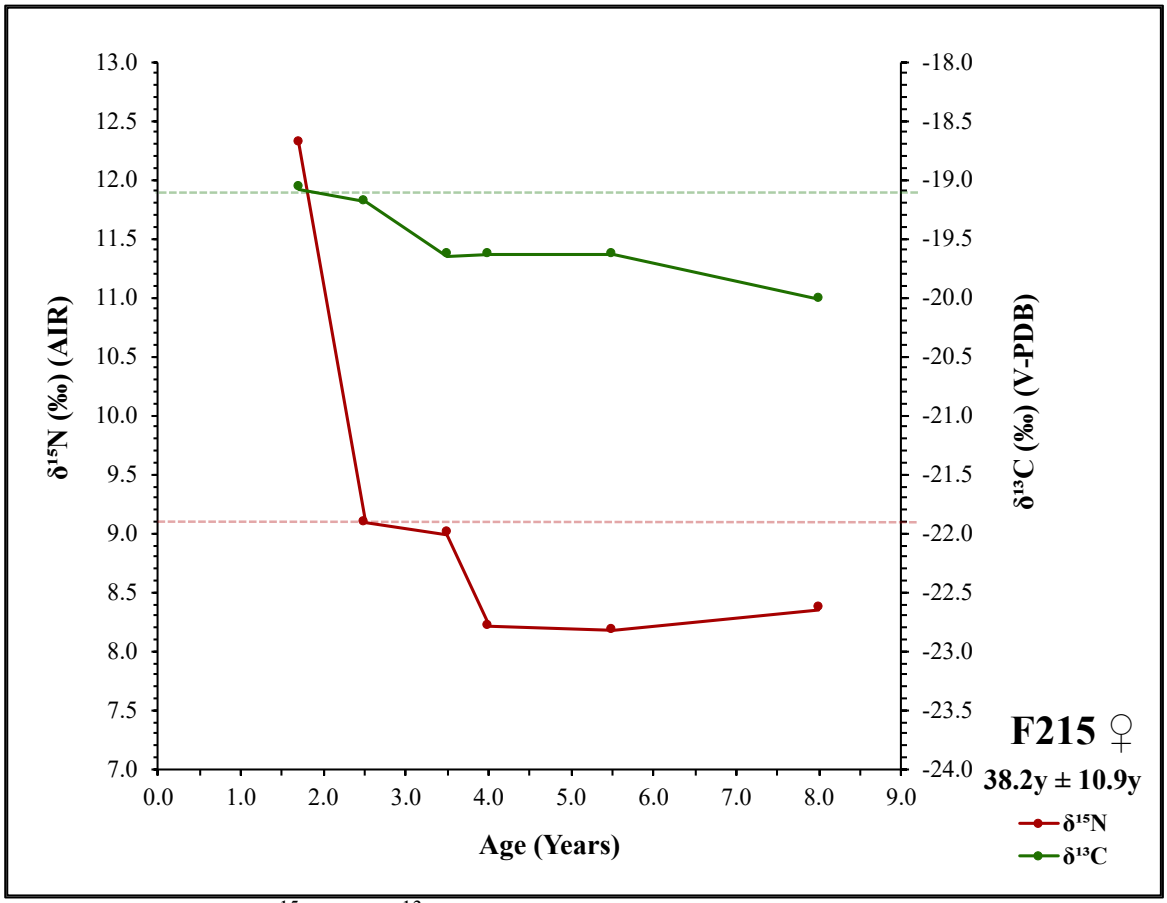

Figure 6.3.A: F215 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female mean (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

a rapid weaning process. The observed drop in $\delta^{15}N$ from 9.0‰ to 8.2‰ between 3.5y and 4.5y may be indicative of a negative nitrogen balance as a result of growth (Katzenberg and Waters-Rist, 2018), since there is no decline in $\delta^{13}C$ during this age.

There is a partial trophic level effect observed in $\delta^{13}C$ (-19.1‰ to -19.7‰) from 1.7y – 3.5y, after which the signal remains stable until 5.5y of age. The adult bone collagen values from this individual were reported to be $\delta^{15}N$ = 9.6‰ and $\delta^{13}C$ = -19.9‰ (Semchuk, 2016). There is a slight difference in the $\delta^{15}N$ and $\delta^{13}C$ signals between the last dentine values at 8.0y (8.4‰ and -20.0‰) and the adult femoral bone collagen values (9.6‰ and -19.9‰). Although the change in $\delta^{13}C$ values is within experimental error, the higher $\delta^{15}N$ value in the femoral bone collagen (by 1.2‰) suggests an introduction of higher trophic level foods later in life.

F313 (Female, 19y – 23y)

Dentine stable isotope data from F313 is plotted in Figure 6.3.B. This individual was interred in a burial dated to the 2nd c. CE. Bone collagen was not analyzed for this individual. Delta ^{15}N demonstrates a 1.4‰ drop from 9.4‰ – 8.1‰ between 1.2y and 1.5y. The $\delta^{15}N$ signal prior to 1.2y is unknown, however the low value at this age category suggests that weaning commenced earlier in life. The pattern suggests that the relatively low $\delta^{15}N$ value at 1.2y is likely not due to a low maternal $\delta^{15}N$ value, and instead reflects diet partway throughout the weaning process which inhibits the observation of a ~3‰ (trophic level effect) drop in $\delta^{15}N$. The observed decrease in $\delta^{15}N$ between 1.2y and 1.5y may indicate that weaning was mostly complete by 1.5y of age. There are slight decreases

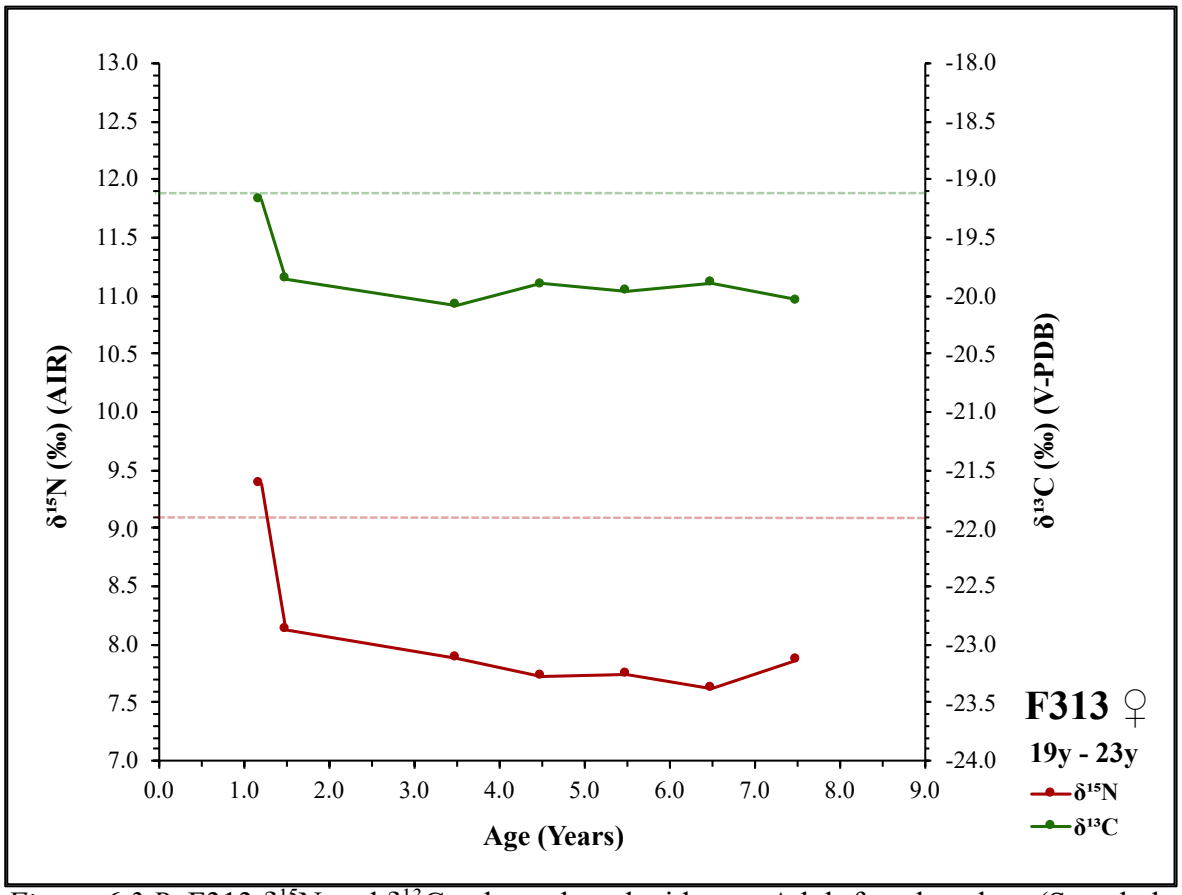

Figure 6.3.B: F313 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female values (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

in both $\delta^{13}C$ and $\delta^{15}N$ from 1.5y – 3.5y, but it appears that weaning was largely done by 1.5 years. Although breast milk was likely still part of the diet at 3.5y (since both $\delta^{13}C$ and $\delta^{15}N$ are decreasing until this age), it likely did not contribute significantly to the diet after 1.5y of age. There is a drop in $\delta^{13}C$ (by ~0.7‰) between 1.2y and 1.5y, suggesting that weaning was still occurring during this time period. Both $\delta^{13}C$ and $\delta^{15}N$ remain relatively stable from 3.5y – 7.5y, suggesting a stable post-weaning and childhood diet.

F323 (Female, 45.2y ± 12.6 y)

The weaning curve associated with F323 is shown in Figure 6.3.C, below. This tooth had some missing dental material due to caries on the lingual dentine horn, resulting in a loss of early life collagen signals prior to 2.1y. The individual associated with F323 was interred in a burial dated to the 2nd c. CE. Bone collagen values for this individual were not analysed, so there is no information on dietary signals from later in life. There is a small decrease (by 1.3‰) in $\delta^{15}N$ values over a period of 4.4 years, between 2.1y and 6.5y, which suggests that the majority of the weaning process and trophic level decline (~1.7‰) in $\delta^{15}N$ occurred prior to 2.1y. After 2.1y, there is no clear, observable trophic level decrease in $\delta^{13}C$, and

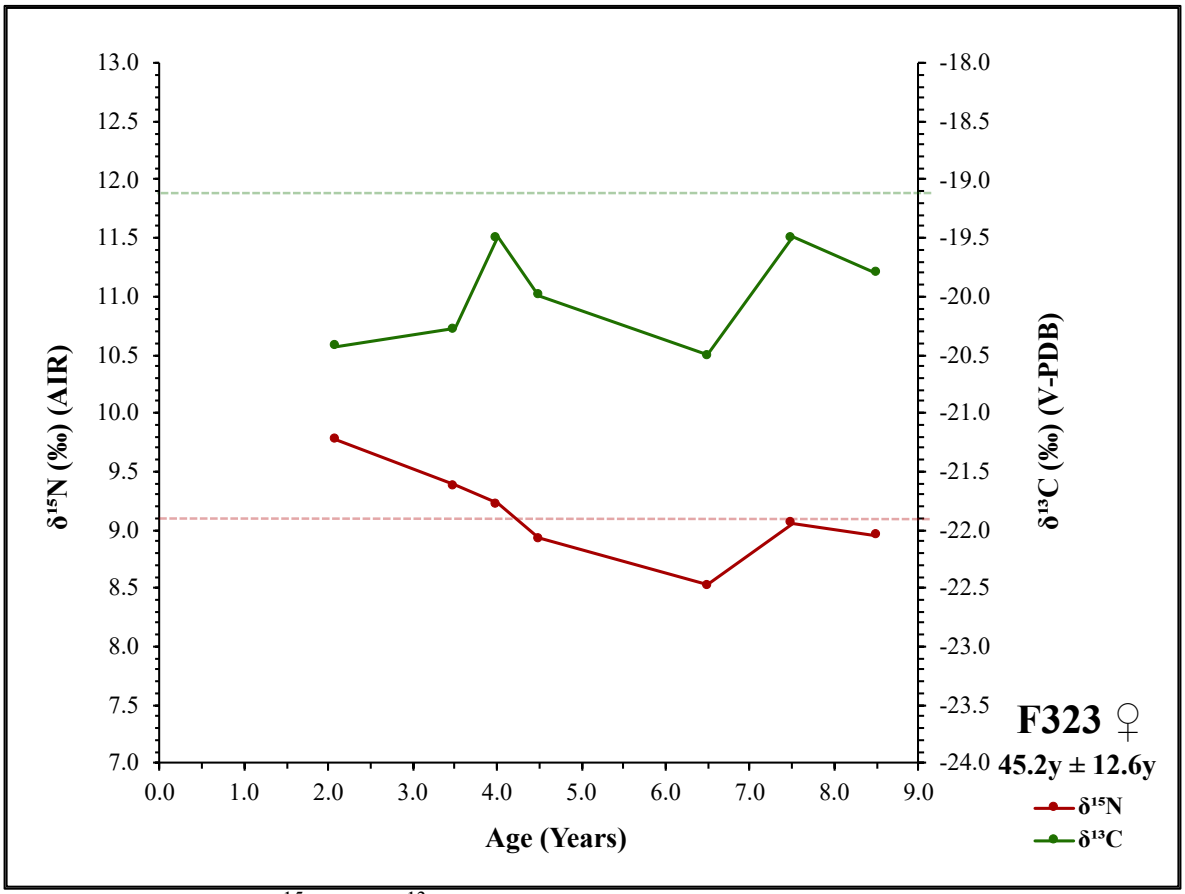

Figure 6.3.C: F323 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female means (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

the slight, gradual decrease in nitrogen over a prolonged period of time suggests that weaning already commenced prior to 2.1y and was nearly complete by this age. The low value at 6.5y may be the result of negative nitrogen balance due to growth. Large differences between $\delta^{13}C$ values at 4.0y, 6.5y, and 7.5y (-19.5‰, -20.5‰, and -19.5‰, respectively) may be indicative of a particular post-weaning/childhood diet in which increased dietary incorporation of C_4 plants occurred at 4.0y and 7.5y.

F206 (Female, Older Adult 50y+)

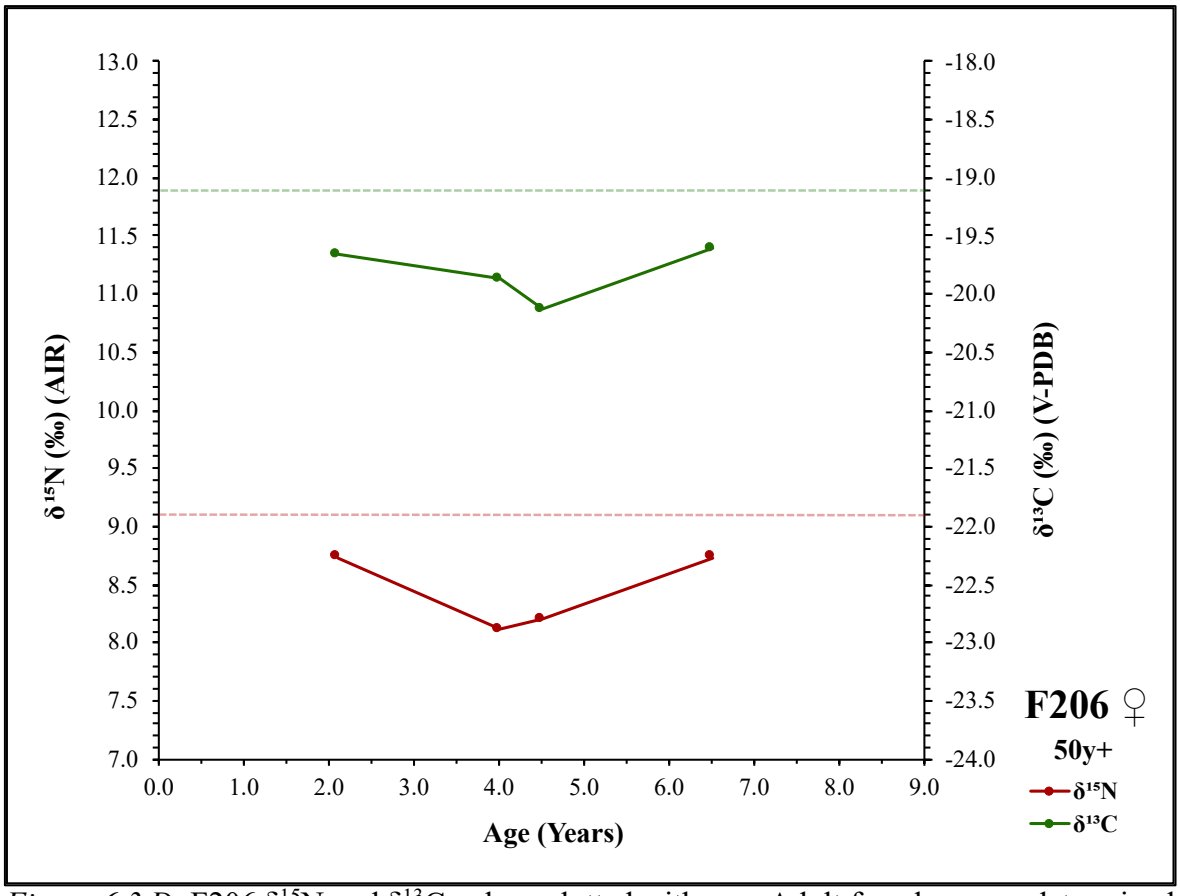

Figure 6.3.D: F206 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female mean, determined by Semchuk (2016) are as follows: $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

Analysis of F206 yielded four data points producing a small weaning curve (Figure 6.3.D). The burial was dated to the first half of the 2nd c. CE. The decrease in $\delta^{15}N$ between 2.1y and 4.0y (from 8.7‰ to 8.1‰) is only 0.6‰, suggesting that although breastmilk consumption was still occurring, a majority of the 2‰ – 3‰ trophic level decrease associated with weaning occurred prior to 2.1y. There was missing dentine from the occlusal surface of this tooth and the collagen yield was low, necessitating the combination of adjacent sections, resulting in a lack of available stable isotope signals earlier in life. It is also possible that these differences are smaller than the full expected trophic level shift for $\delta^{15}N$ and $\delta^{13}C$ (3‰ and 1‰, respectively) because the weaning signals reflect a low maternal $\delta^{15}N$ relative to the adult female average from Vagnari. Nonetheless, the decrease in $\delta^{15}N$ until 4.0y suggests small amounts of breast milk in the diet, since $\delta^{13}C$ is also decreasing.

The adult femur collagen isotope values for this individual were determined to be $\delta^{15}N = 9.3‰$ and $\delta^{13}C = -19.4‰$ (Semchuk, 2016). The dentine $\delta^{13}C$ value at 6.5y (-19.6‰) and the adult bone $\delta^{13}C$ value (-19.4‰) have a relatively small difference (within the analytical error margin of 0.2‰), which suggests an isotopically consistent diet throughout life. However, since there is a large time span between 6.5y and the age-at-death (50y+), more isotopic data is needed to examine diet during the intervening decades of this woman's life. There is a more noticeable difference in $\delta^{15}N$ between 6.5y and the femoral collagen (adult) values, increasing from 8.7‰ at 6.5y to 9.3‰ in adulthood; this difference of 0.6‰ may indicate a slight increase in higher trophic level terrestrial consumption later in life.

F247 (Male, Adult)

Figure 6.3.E depicts the $\delta^{15}N$ and $\delta^{13}C$ values in F247 from ages 1.5y to 4.5y. This individual was recovered from a burial dated from the 1st to 2nd c. CE. The $\delta^{15}N$ signals remain relatively stable from 1.5y – 4.5y, first decreasing by 0.2‰ from 1.5y – 3.5y, and increasing by 0.4‰ from 3.5y – 4.5y. This relative stability suggests that most of the weaning occurred prior to 1.5y. Delta ^{13}C changes by no more than 0.2‰ from 1.5y – 4.5y, suggesting a relatively stable source of carbon during this time. Furthermore, the $\delta^{15}N$ signal at 1.5y is 9.3‰, which approaches the adult female average, suggesting that the

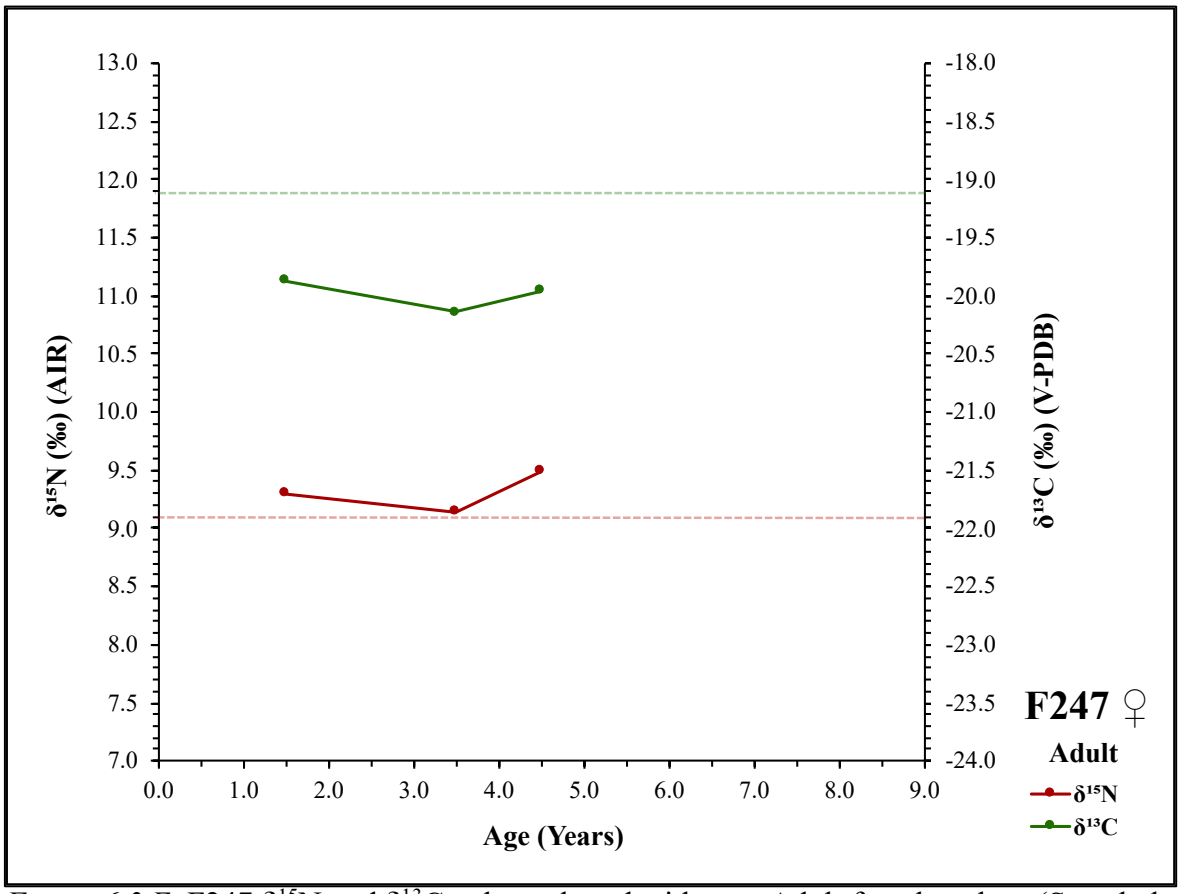

Figure 6.3.E: F247 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female values (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

majority of weaning likely occurred prior to 1.5y. This also supports the hypothesis that weaning was complete quite early in life.

Four dentine sections from F247 had C:N values outside the acceptable range, and so were excluded from the study. However, the $\delta^{15}N$ signal for 1.2y is still low (9.3‰, Table 6.3). Although this study could not accurately detect dietary signals prior to 1.5y of age, the low observed values at 1.5y may indicate either a shortened breastfeeding period with rapid weaning, complete absence of breastfeeding, or a low maternal $\delta^{15}N$ value. There are small differences between the dentine $\delta^{15}N$ value at 4.5y and the femoral bone collagen $\delta^{15}N$ value reported by Semchuk (2016) (9.5‰ and 9.8‰, respectively). There is a 0.7‰ increase between $\delta^{13}C$ signals from dentine at 4.5y (-20.0‰) and the adult bone collagen (-19.3‰). The larger difference in $\delta^{13}C$ relative to $\delta^{15}N$ suggests an increased contribution of C_4 plant sources later in life.

F126 (Male, 20y – 25 y)

The data for F126 are plotted in Figure 6.3.F. Individual F126 has no identified burial date. The first dentine section has an estimated age of 1.7y due to occlusal attrition of dentine. There is an overall decrease of 0.9‰ in $\delta^{15}N$ between 1.7y – 7.5y, which is less than the expected change for an entire trophic level shift (~3‰). Since the $\delta^{15}N$ value at 1.7y is 10.2‰ and remains stable from 1.7y – 2.5y, it is possible that weaning was well underway and the 'flattening' of the weaning curve during this period suggests that weaning may have been nearly complete by 1.7y. At 7.5y, the $\delta^{15}N$ value is 9.3‰, approximating the adult female mean.

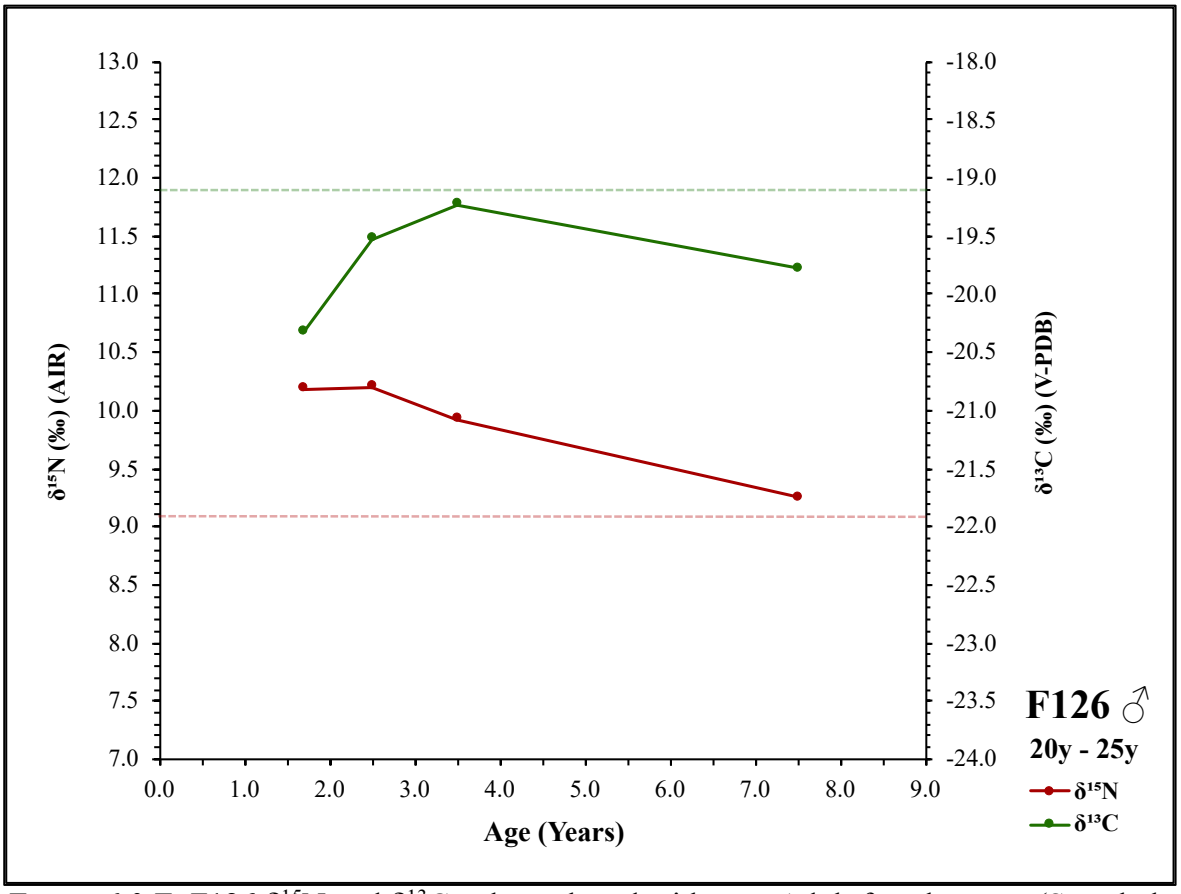

Figure 6.3.F: F126 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female mean (Semchuk, 2016): $\delta^{15}N = 9.0‰ \pm 1.1$ [pink dotted line]; $\delta^{13}C = -19.1‰ \pm 0.5$ [green dotted line].

The $\delta^{13}C$ values from 1.7y – 3.5y increase from -20.3‰ to -19.2‰. Due to the relatively stable $\delta^{15}N$ values, the changes in $\delta^{13}C$ from 1.7y – 3.5y suggest that the early life diet changed, rather than the weaning process continuing until 3.5y of age. The increase in $\delta^{13}C$ may indicate a weanling diet supplemented by C_4 plants. After 3.5y, there is a drop in both nitrogen and carbon, which may reflect a post-weaning dietary shift. The $\delta^{15}N$ and $\delta^{13}C$ signals obtained from the adult bone collagen by Semchuck (2016) for this individual (9.1‰ and -19.5‰, respectively) are similar to the dentine values at 7.5y (9.3‰ and -19.8‰). This minimal difference suggests that the diet likely did not change significantly from the age of 7.5y to the time of death around 20y – 25y.

F235 (Male, 50y ± 12.6y)

Incremental dentine section values for F235 are shown in Figure 6.3.G. The burial date for this individual has not been determined. All nitrogen values are below the adult female average, with values ranging from 8.6‰ – 7.9‰. The carbon shows slight variation, with values ranging from -19.5‰ to -19.9‰ from 1.2y – 5.5y. The lower nitrogen values may indicate that weaning had already initiated prior to 1.2y of age, or that the maternal $\delta^{15}N$ value was initially low, skewing the weaning curve of this individual. Since there is still a slight visible decrease in $\delta^{15}N$ between 1.2y and 3.5y (by 0.7‰), it is likely that weaning continued until 3.5y, but that a majority of weaning (representing ~2.3‰ decrease in $\delta^{15}N$)

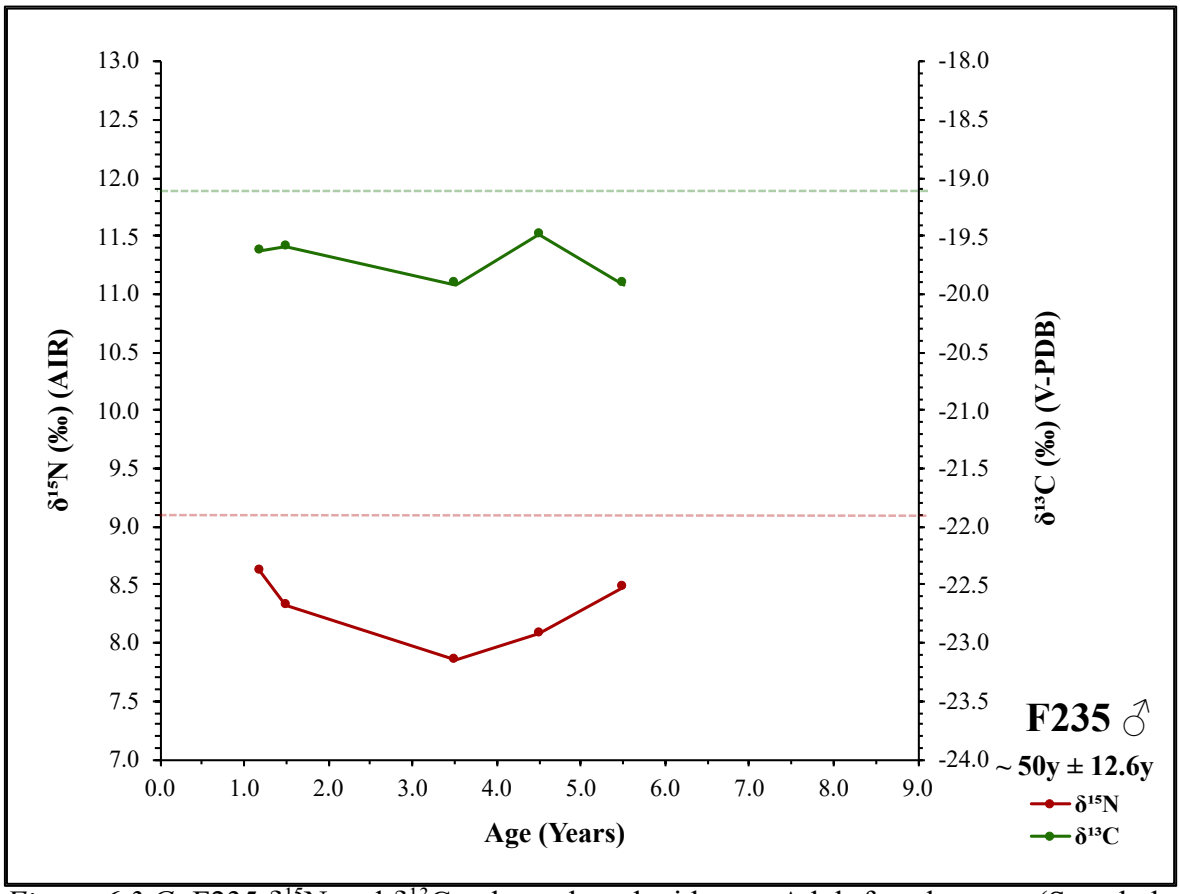

Figure 6.3.G: F235 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female mean (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

took place earlier in life. The $\delta^{15}N$ and $\delta^{13}C$ femoral collagen values (9.1‰ and -19.6‰, respectively) of the individual are higher than the dentine signals at 5.5y (8.5‰ and -19.9‰), suggesting a slightly different diet with increased incorporation of higher trophic level foods after childhood (Semchuk, 2016). Since the difference in $\delta^{13}C$ is only 0.3‰, it is difficult to determine if this difference can be attributed to dietary changes or is largely due to measurement error (± 0.2‰).

6.3.2 Gradual, Prolonged Weaning

F207 (Male, Young Adult)

The weaning curve for F207 is plotted in Figure 6.3.H. The burial date for this individual is unknown. There is a trophic level shift in $\delta^{15}N$ from 11.4‰ – 9.3‰ from age 1.7y – 4.5y, after which $\delta^{15}N$ stabilizes to 9.5‰ and this signal is the same in the last section measured at 8.0y.

The difference in $\delta^{13}C$ is slight, with all values reported being between -19.4‰ and -19.9‰; the $\delta^{13}C$ curve has a similar downward trend to the $\delta^{15}N$ data, displaying a net decrease from 1.7y – 8.0y. The nitrogen signal is indicative of a prolonged weaning process, in which the individual completed weaning by 4.5y and consumed an isotopically stable diet afterwards, up to 8.0y. The low $\delta^{13}C$ after 4.5y suggests that the post-weaning diet was likely a terrestrial, C_3 plant-based diet. The similar $\delta^{15}N$ and $\delta^{13}C$ signals between 8.0y (9.5‰ and -19.9‰) and the adult femoral bone collagen (9.6‰ and -19.9‰) (Semchuk, 2016) indicate an isotopically consistent diet from 8.0y up until the time of death (young adulthood).

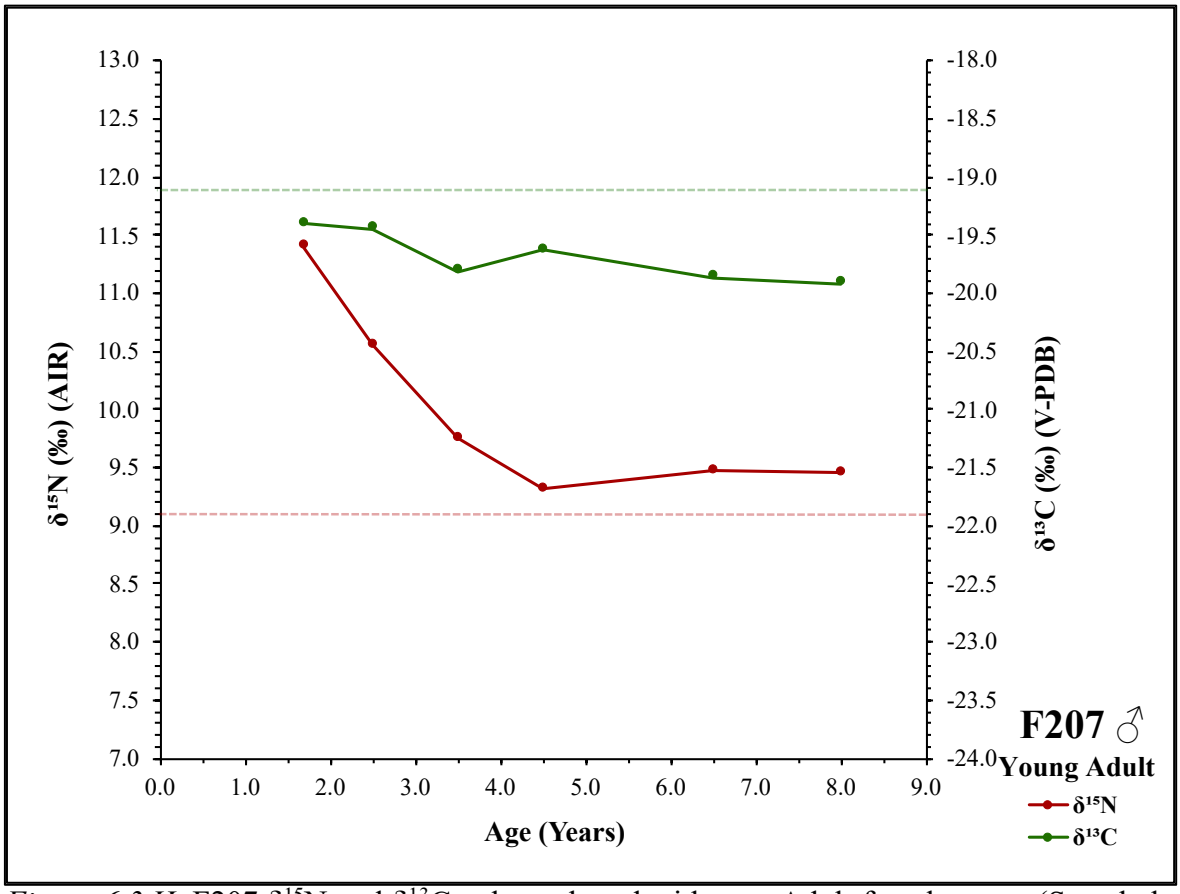

Figure 6.3.H: F207 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female mean (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

F211 Female, Young Adult <30y)

Figure 6.3.I depicts the dentine isotope signals from 1.2y to 7.0y. The burial date for this individual is unknown. A decrease of 2.1‰ in $\delta^{15}N$ is observed from 1.2y – 3.5y, from 10.7‰ – 8.6‰, indicating almost a full trophic level shift. This suggests that weaning was likely underway by 1.2y, with breastmilk gradually removed from the diet until 3.5y, with complete cessation likely occurring around this time. After 3.5y, the $\delta^{15}N$ signal remains relatively stable, ranging from 8.5‰ – 8.7‰. Since this signal is lower than the average adult female value, it is possible that this individual relied more on terrestrial foods early

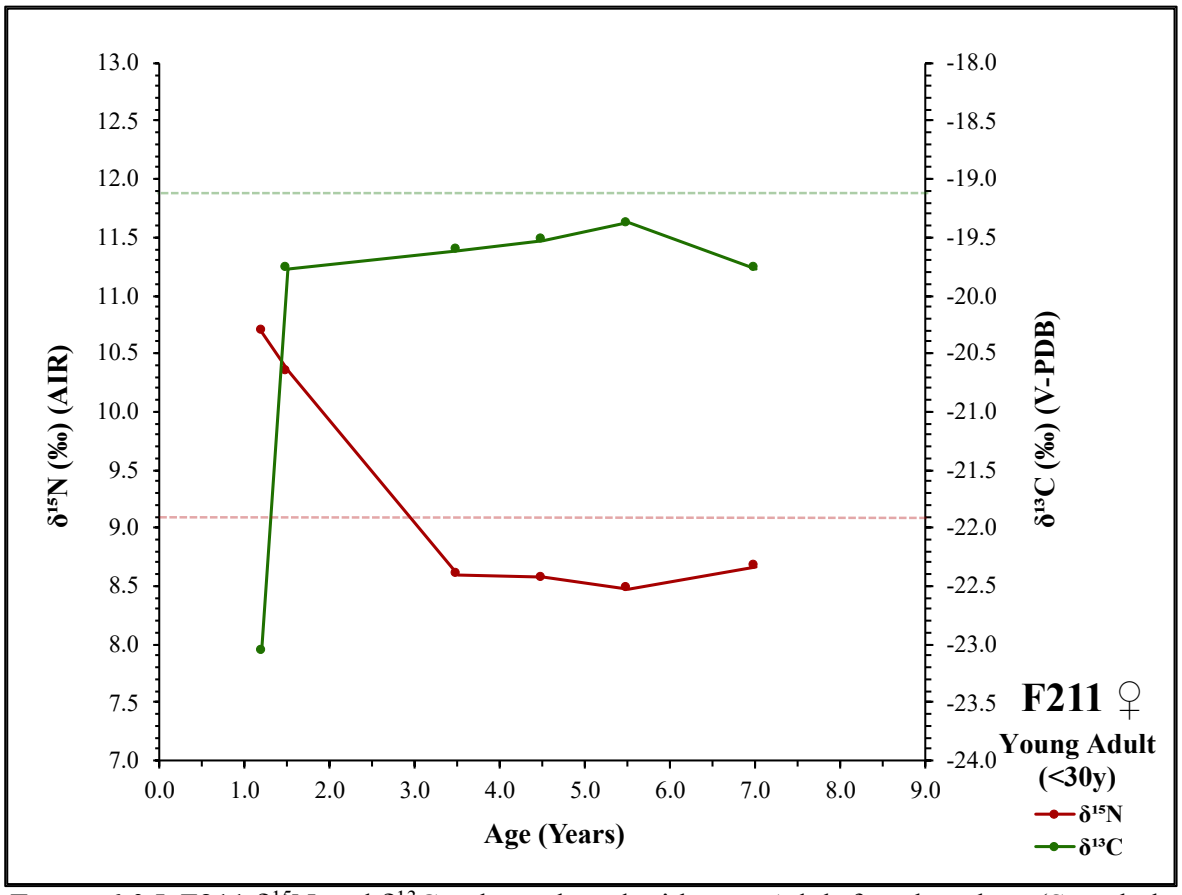

Figure 6.3.I: F211 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female values (Semchuk, 2016): $\delta^{15}N = 9.0‰ \pm 1.1‰$ [pink dotted line]; $\delta^{13}C = -19.1‰ \pm 0.5‰$ [green dotted line].

in life. Even though the $\delta^{15}N$ values are below the adult female average, neither $\delta^{15}N$ or $\delta^{13}C$ signals increase considerably from 3.5y – 7.0y, suggesting that these lower values are not due to negative nitrogen balance.

There is a large difference in $\delta^{13}C$ between 1.2y and 1.5y, from -23.1‰ (which is unusually low compared to others sampled) to -19.8‰, indicating a possible contribution of C_4 plant sources to the weanling diet. There are some slight, but notable differences between dentine $\delta^{15}N$ and $\delta^{13}C$ at 7.0y (8.7‰ and -19.8‰, respectively) and the adult femoral bone collagen values (9.3‰ and -19.4‰). These changes may indicate a slight

increase in the consumption of higher-trophic proteins, such as marine sources, during adulthood.

F212 (Unknown Sex, Adult)

Serial dentine analysis of F212 produced five data points (Figure 6.3.J). The individual associated with F212 has an unknown burial date, and there are no reported bone collagen values. Delta ^{15}N displays a full trophic level shift between 1.5y and 4.0y by 3.2‰ (from 12.1‰ to 8.9‰). Delta ^{13}C values are more stable, decreasing by 0.5‰ from 1.7y – 2.5y, however the values increase and stabilize again from 3.5y onwards. The weaning signal

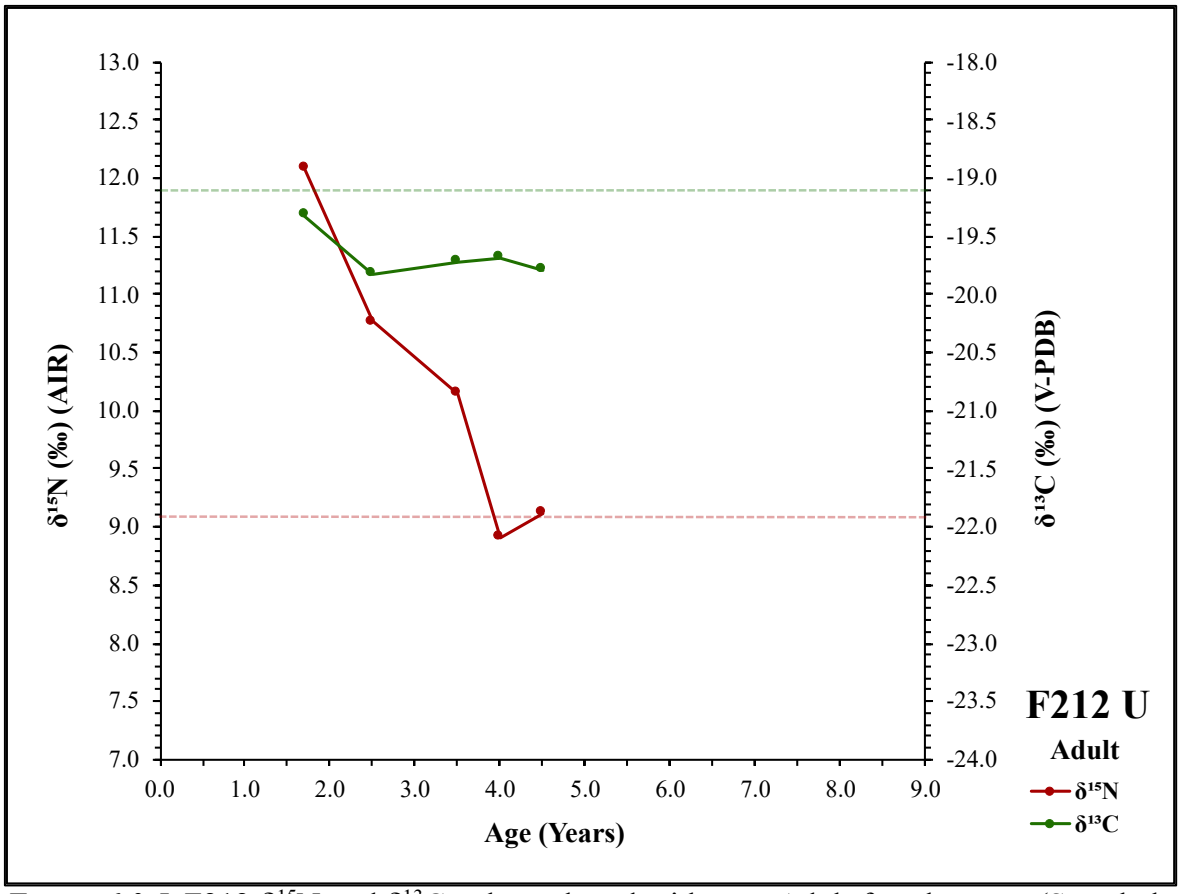

Figure 6.3.J: F212 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female mean (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

suggests a prolonged weaning process that may have begun prior to or around 1.7y, and continued until the completion of weaning, which likely occurred around 4.0y of age. It is also possible that the high $\delta^{15}N$ values at 1.7y represent a higher initial maternal $\delta^{15}N$ signal, but since maternal and/or earlier life signals (e.g., exclusive breastfeeding) are not available, it is not possible to discern whether this is the case. The $\delta^{13}C$ signal changes from 2.5y – 4.5y are within reporting error (± 0.2‰), indicating minimal change in the isotopic composition of dietary plant sources. Since weaning typically causes decreases in both $\delta^{15}N$ and $\delta^{13}C$ values, the relative stability in $\delta^{13}C$ signals from 2.5y – 4.0y suggests a slight contribution of C_4 plants to the weanling diet.

F291 (Male?, Adult)

Figure 6.3.K shows the plotted $\delta^{15}N$ and $\delta^{13}C$ data points for F291. The burial date for this individual has not been determined. There is a decrease in $\delta^{15}N$ by 1.6‰ (from 10.2‰ to 8.6‰) between 1.5y and 4.5y, followed by a consistent increase in $\delta^{15}N$ reaching 10.3‰ by 7.5y. The trend in delta ^{13}C values is similar to nitrogen, decreasing by 0.3‰ (from -19.9‰ to -20.2‰) between 1.5y and 3.5y, followed by a small, but steady increase reaching -19.5 ‰ at 7.5y. The decrease in $\delta^{15}N$ from 1.5y – 4.5y is less than 2‰, suggesting that weaning had commenced prior to 1.5y, but had continued for a prolonged period until complete cessation of weaning at 3.5y – 4.5y, with some contribution of C_4 plants from 3.5y – 4.5y. It is also possible that the lower $\delta^{15}N$ value observed at 4.5y is due to negative nitrogen balance during growth, resulting in a decrease in $\delta^{15}N$ and slight increase in the $\delta^{13}C$ signals from 3.5y – 5.5y.

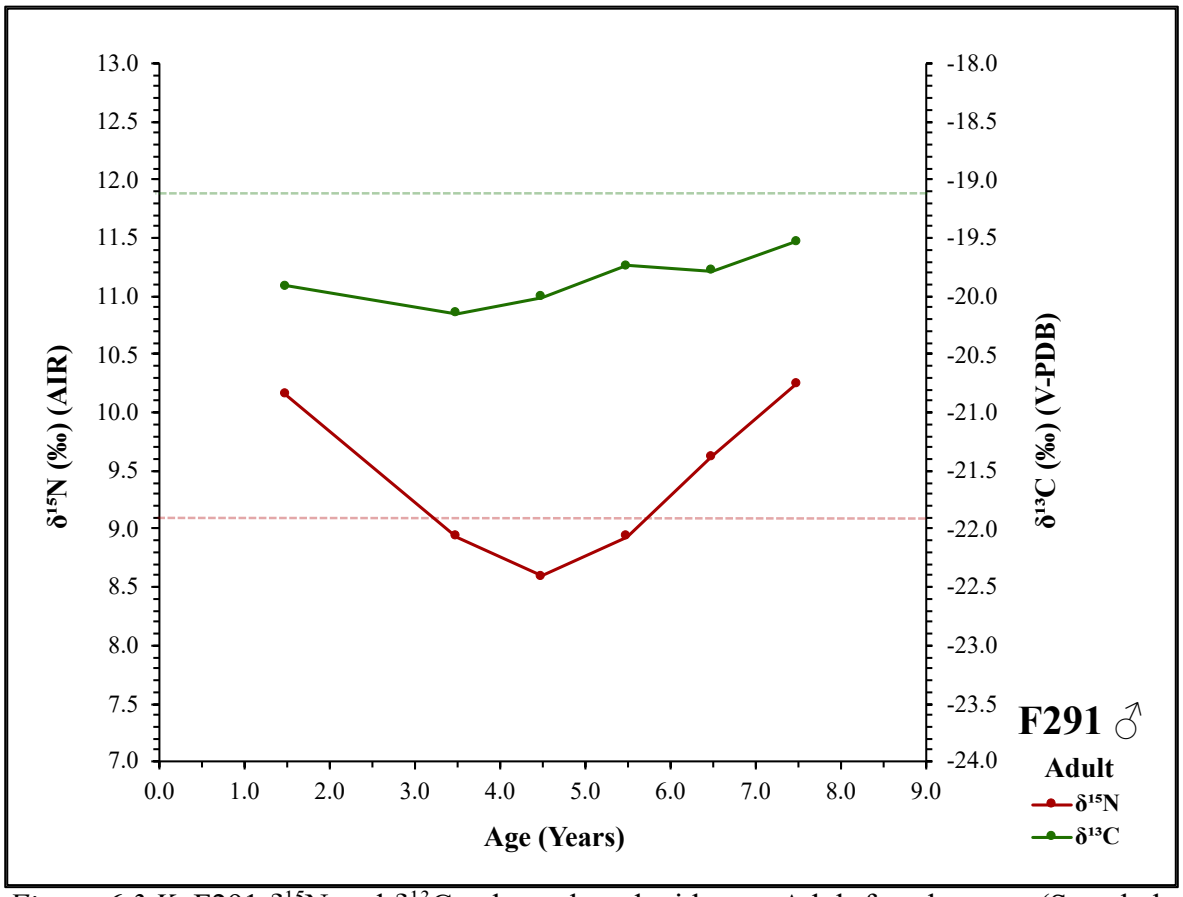

Figure 6.3.K: F291 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female mean (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

The similar weaning curves (except between 3.5y and 4.5y) and increases in both $\delta^{13}C$ and $\delta^{15}N$ suggest that the individual's post-weaning diet had a small contribution of fish and/or marine food sources. The dentine $\delta^{15}N$ values at 7.5y (10.3‰) is higher relative to the adult femoral bone collagen value (9.1‰) whereas the $\delta^{13}C$ values are both -19.5‰ at 7.5y and adulthood (Semchuk, 2016); this difference of 1.2‰ in $\delta^{15}N$ and stability in $\delta^{13}C$ from 7.5y to adulthood may indicate a decrease in higher trophic level sources later in life.

F336 (Unknown Sex, 10.0y ± 2.0y)

Stable isotope data from the dentine collagen collected from F336 is plotted in Figure 6.3.L. The burial associated with F336 was dated to the 2nd c. CE. The bone collagen values for this individual were not analyzed. Delta ^{15}N values decrease by 4.0‰ (from 12.6‰ at 1.2y to 8.6‰ at ages 5.0y and 7.5y). The $\delta^{13}C$ signals decrease steadily (by -1.2‰) from -18.7‰ at 1.2y to -19.9‰ at 5.0y which represents the expected trophic level shift, followed by a small increase to -19.7‰ at 7.5y. Like F309, it is possible that this individual experienced a trophic level shift from high $\delta^{15}N$ food (mother's milk with high $\delta^{15}N$ values) to food grown and consumed at Vagnari (observable in the adult female average from Semchuk,

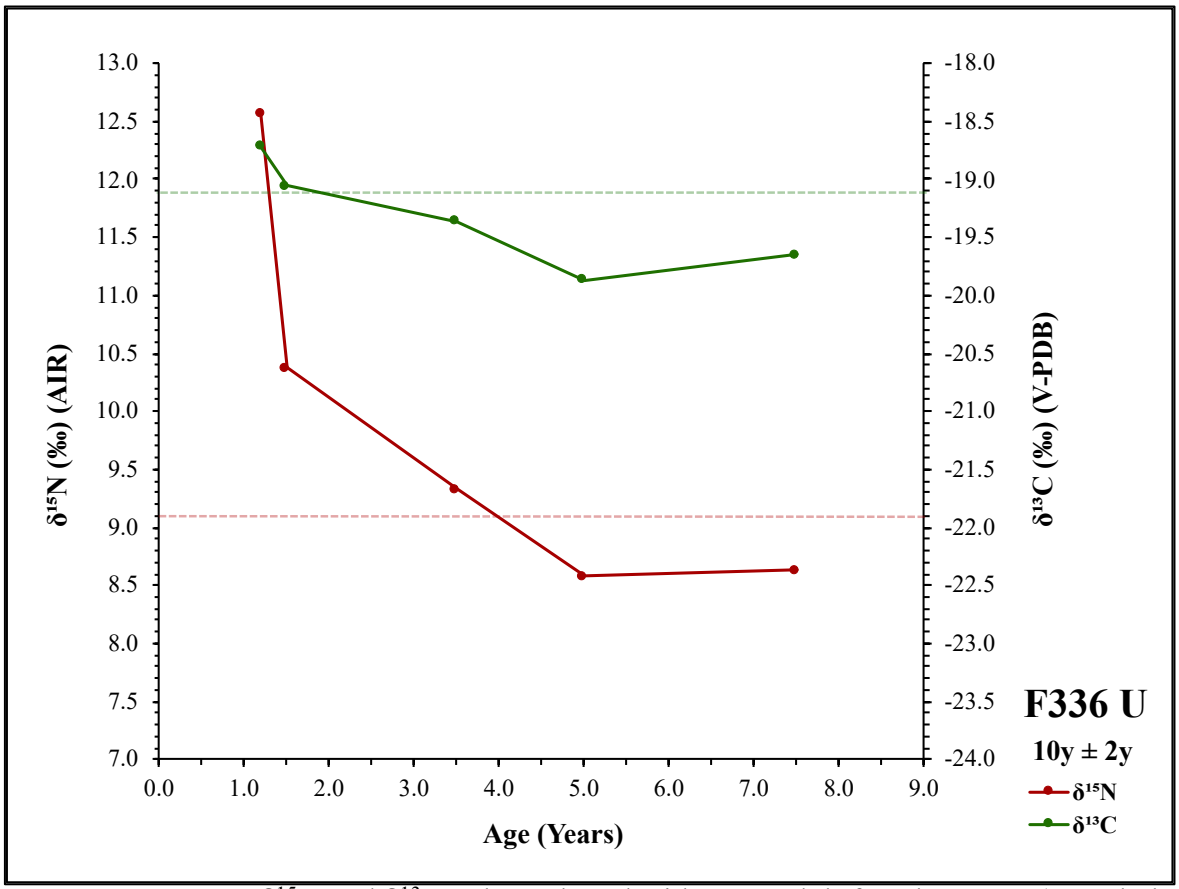

Figure 6.3.L: F336 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female means (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

2016) in addition to the weaning trophic level shift. However, this individual died quite young (ten years old); it is possible that prolonged breastfeeding (until 5.0y) may have been implemented in this individual due to illness. The low $\delta^{15}N$ values at 5.0y may indicate negative nitrogen balance due to growth, but since the $\delta^{13}C$ values also decrease in a similar pattern, the changes are likely due to weaning.

F309 (Male, Young Adult)

The analysis of F309 dentine collagen produced a plot with three data points (Figure 6.3.M). The individual associated with F309 was interred in a burial dated to late 1st/early

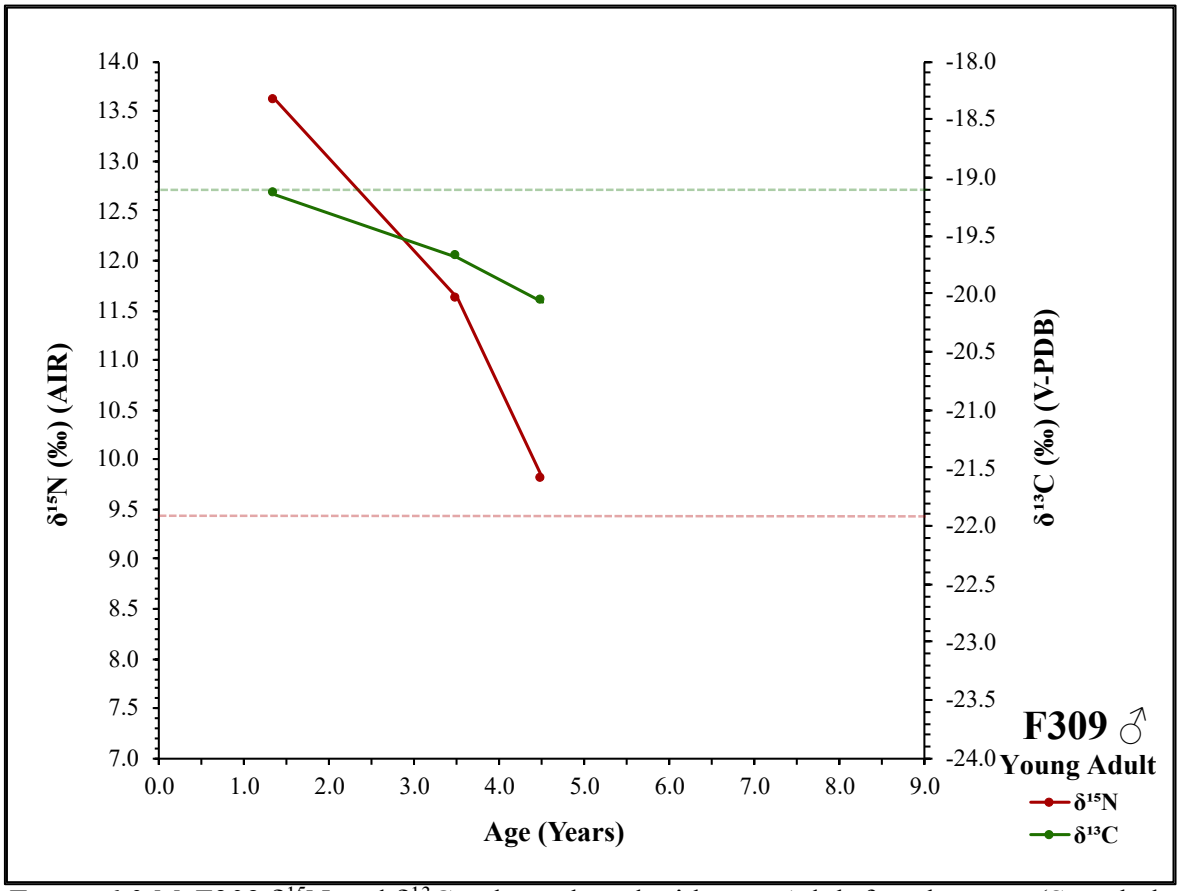

Figure 6.3.M: F309 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female mean (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

2nd c. CE. Bone collagen values for this individual were not reported. Delta ^{15}N signals decrease by 2‰ (from 13.6‰ at 1.4y to 11.6‰ at 3.5y) from 1.4y – 3.5y, and decrease further to 9.8‰ by 4.5y. The $\delta^{13}C$ signals follow a similar pattern to $\delta^{15}N$, demonstrating a trophic level shift of 1‰ (from -19.1‰ at 1.4y to -20.1‰ at 4.5y). The steep decline in $\delta^{15}N$ throughout weaning may result from a two-fold trophic level effect: 1) The trophic level decreases normally with weaning, indicated by a 2‰ – 3‰ decrease in $\delta^{15}N$; 2) This decrease is compounded by a shift from a high $\delta^{15}N$ source (i.e., breast milk) from a mother with higher initial $\delta^{15}N$ values (perhaps due to marine food consumption) to a childhood diet more similar to the adult female average at Vagnari (which is lower than the mother's $\delta^{15}N$). The weaning curve appears to continue decreasing until at least 4.5y, suggesting a prolonged breastfeeding and weaning period.

F320 (Female, 38.2y ± 10.9y)

Figure 6.3.N depicts the changes in dentine collagen $\delta^{15}N$ and $\delta^{13}C$ between 2.1y and 3.5y. The burial associated with F320 is dated to the 2nd c. CE. Bone collagen values were for this individual were not determined. Although the tooth from this individual produced only two usable signals, the difference in $\delta^{15}N$ from 11.1‰ at 2.1y to 9.1‰ at 3.5y demonstrates a trophic level shift of 2‰. The difference in $\delta^{13}C$ values is very slight, increasing from -19.9‰ at 2.1y to -19.8‰ at 2.5y. The $\delta^{15}N$ signals at 2.1y and 3.5y are indicative of ongoing weaning practices. In this individual, weaning likely began prior to 2.1y of age and continued past 3.5y. Since the $\delta^{15}N$ value at 3.5y is similar to the adult female average,

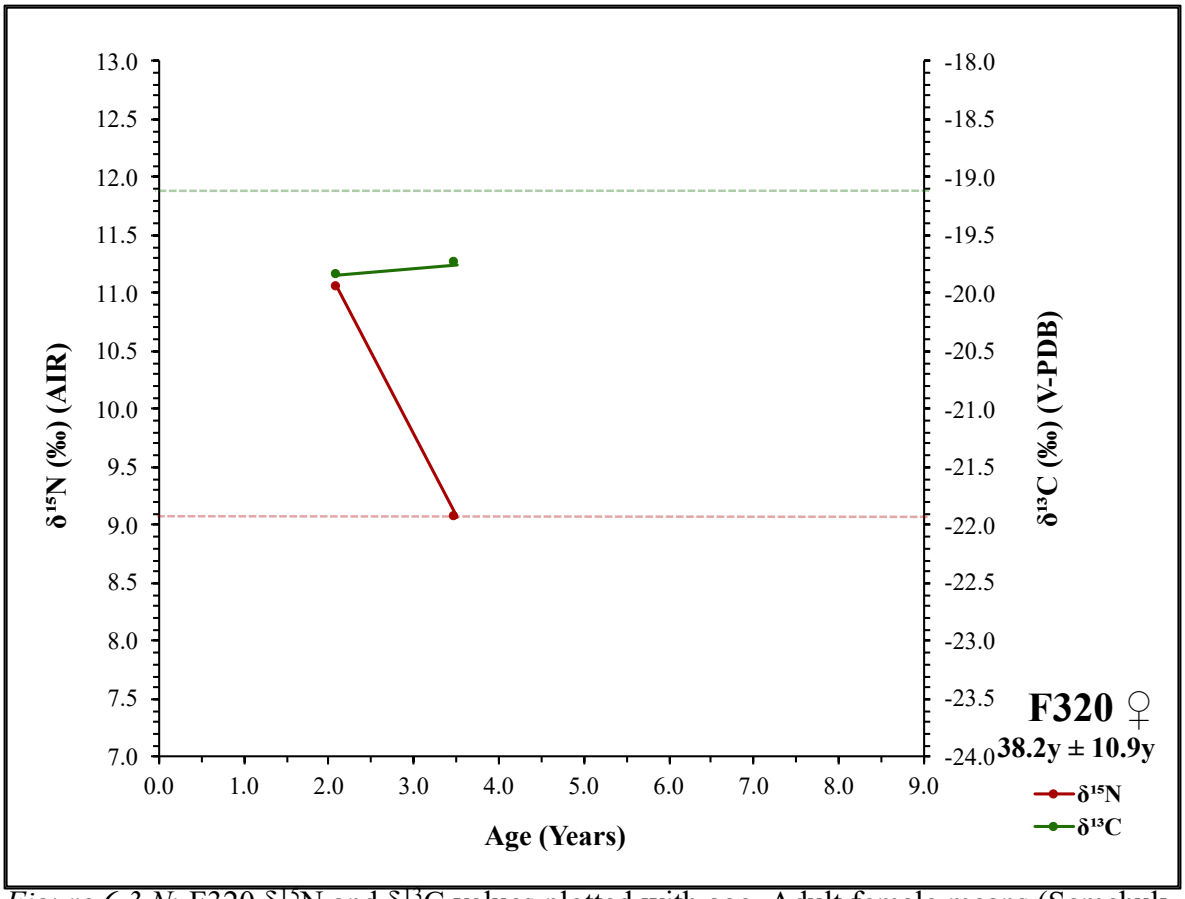

Figure 6.3.N: F320 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female means (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

it is possible that weaning had concluded around 3.5y, but without further data points after 3.5y this is not certain.

6.3.3 Insufficient Data

F127 (Female, 15y - 20y)

The weaning curve from F127 is shown in Figure 6.3.O. This burial is dated to 2nd c. CE. The first two sections of the tooth were excluded from analysis due to diagenesis (i.e., high C:N ratios). The $\delta^{15}N$ values at 4.5y (9.1‰) and 8.0y (8.7‰) are both similar and approach the adult female average at Vagnari, suggesting a relatively stable post-weaning childhood

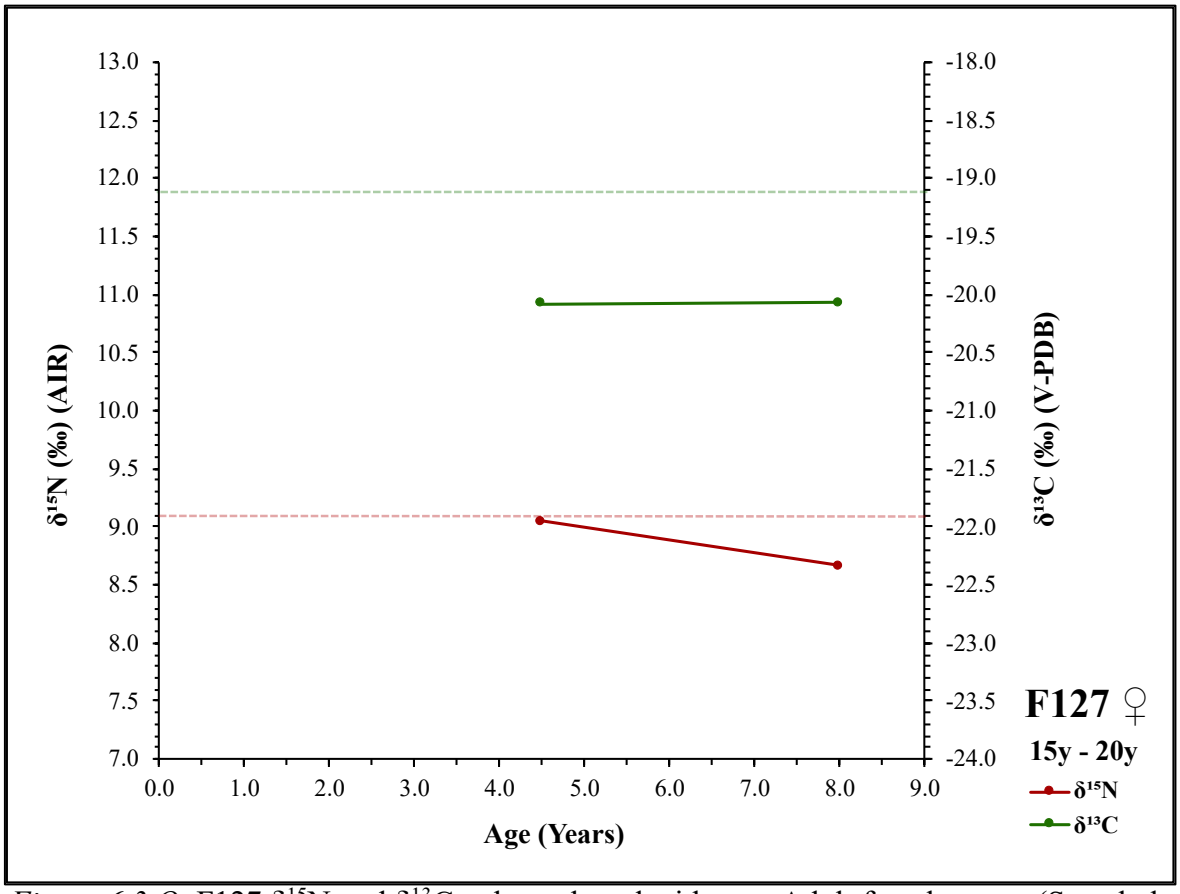

Figure 6.3.O: F127 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female mean (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1 [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5 [green dotted line].

diet. The $\delta^{13}C$ values are stable, -20.1‰ at both 4.5y and 8.0y, which are slightly lower than the female adult average, but not a full trophic level difference when measurement error (± 0.2‰) is taken into account.

The samples that were excluded due to diagenetic alteration (1.2y, 1.5y) had $\delta^{15}N$ signals higher by 2.9‰ – 3.2‰ relative to the value at 4.5y; although these values cannot be included in the analysis, the steep decline from these values to the signal at 4.5y may indicate that the majority of weaning occurred between 1.5y and 4.5y. These results suggest that weaning was completed by 4.5y, and the individual transitioned to a childhood diet that remained relatively stable between 4.5y and 8.0y. Bone collagen values from F127

were reported to be $\delta^{15}N = 9.8‰$, and $\delta^{13}C = -18.4‰$ (Semchuk, 2016). The bone collagen $\delta^{15}N$ and $\delta^{13}C$ values representing diet from later in life are higher than the values at 8.0y (8.7‰ and -20.1‰), which suggests an increased consumption of higher trophic level foods.

F249 (Male?, Adult)

Two collagen data points for F249 are shown in Figure 6.3.P. Two additional data points representing diet at 4.5y and 7.0y were excluded due to C:N ratios being outside the accepted range. The burial date for F249 has not been determined. Between 1.2y and 1.5y, $\delta^{15}N$ decreases from 11.7‰ to 10.5‰ (1.2‰). The $\delta^{15}N$ at 1.5y is still well above the adult female average range of 9.0‰, so it is unlikely that weaning was complete at this age. There is an observable (-0.8‰) difference between $\delta^{13}C$ at 1.2y (-19.2‰) and 1.5y (-20.0‰), just short of a full trophic level effect for carbon. The $\delta^{15}N$ values suggest that weaning was underway during ages 1.2y – 1.5y, but it is likely that weaning commenced prior to 1.2y and had continued past 1.5y, since there was not a full observed trophic level effect in $\delta^{15}N$.

The dentine $\delta^{15}N$ and $\delta^{13}C$ values at 1.5y (10.5‰ and -20.0‰) are observably different from the adult femoral bone collagen values for this individual (9.7‰ and -19.0‰) reported by Semchuk (2016). The higher $\delta^{15}N$ from 1.5y compared to the adult values provide further indication that weaning is still underway at 1.5y of age. The difference in $\delta^{13}C$ from dentine values at 1.5y (-20.0‰) to the adult femoral collagen value (-19.0‰), indicates a relative increase in C_4 plant consumption or increase marine

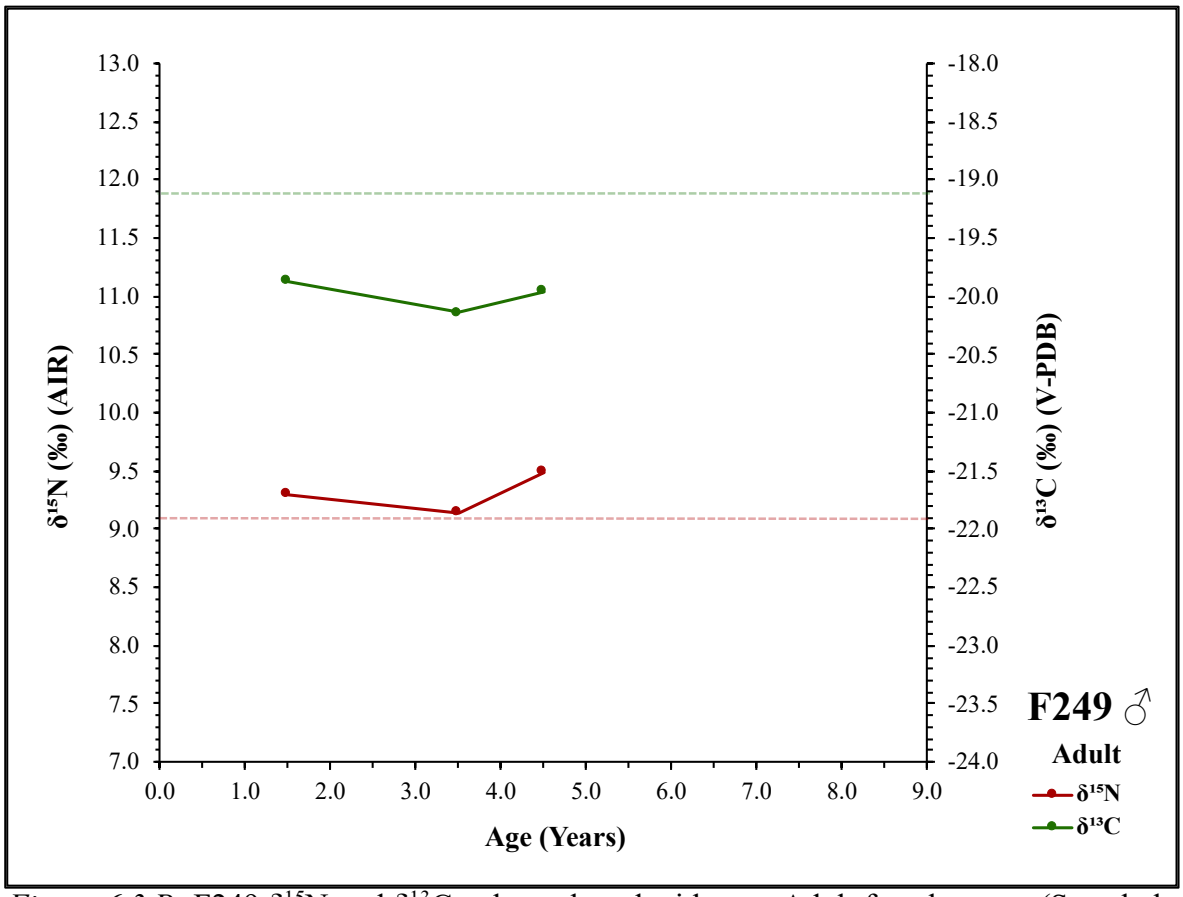

Figure 6.3.P: F249 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female mean (Semchuk, 2016): $\delta^{15}N = 9.0‰ \pm 1.1‰$ [pink dotted line]; $\delta^{13}C = -19.1‰ \pm 0.5‰$ [green dotted line].

consumption in the adult diet. The diet during the large time span between the recovered collagen signals (1.5y from dentine to "adult" bone collagen) may have been dynamic and highly variable, but further isotopic investigation is necessary to explore more nuanced chronological changes.

F286B (Unknown Sex, 13y – 14 y)

The dentine analyzed from F286B yielded two data points for 1.4y and 4.5y (Figure 6.3.Q); four data points for the ages 4.5y – 8.5y could not be included due to diagenetic alteration. F286B was interred in a burial dated to 125 – 150 BCE. There is a decrease in $\delta^{15}N$ by

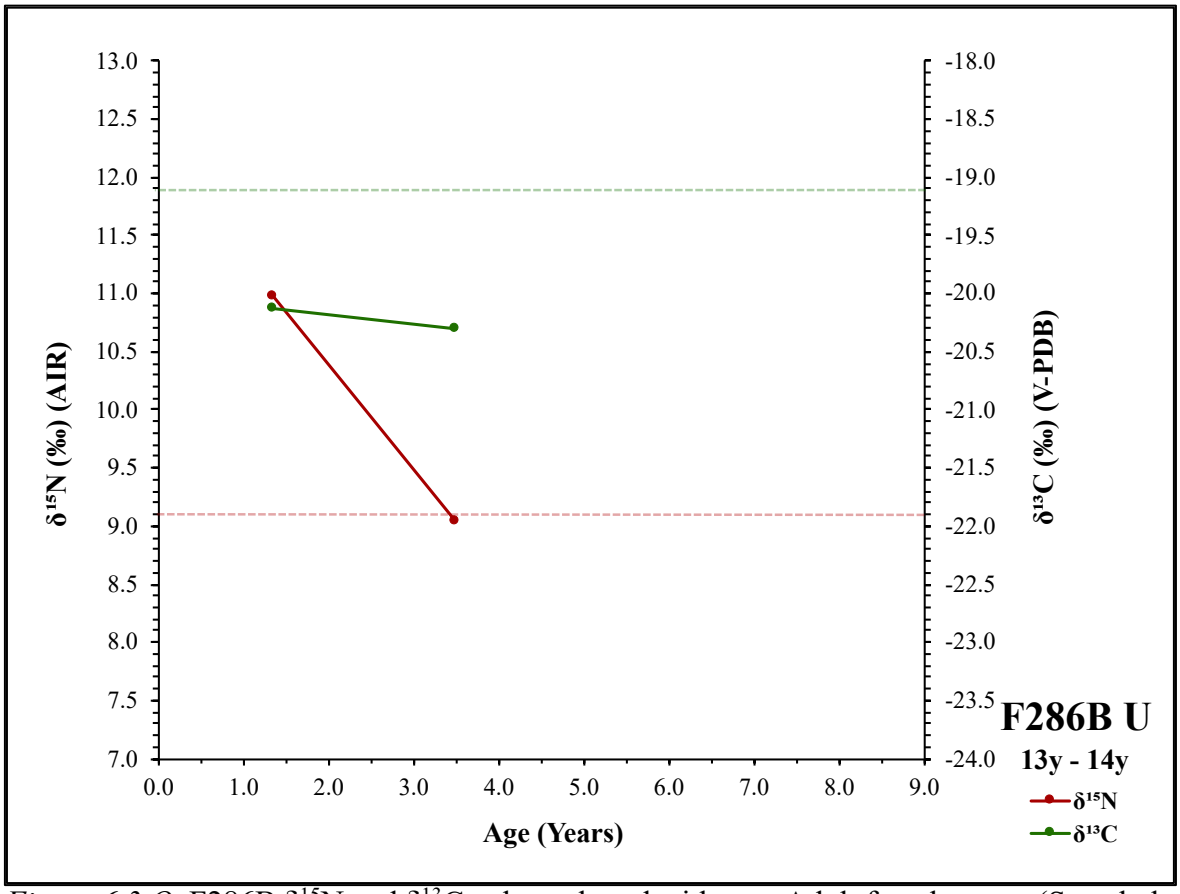

Figure 6.3.Q: F286B $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female mean (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

2.0‰ between 1.4y to 3.5y, (from 11.0‰ to 9.0‰). The clear trophic level shift between 1.4y and 3.5y is indicative of the removal of breastmilk during this time period, but it is possible that weaning commenced prior to 1.4y and continued past 3.5y. The dentine $\delta^{15}N$ signal at 3.5y (9.0‰) is higher than the adult femoral collagen value (7.7‰) (Semchuk, 2016).

The difference in $\delta^{13}C$ is negligible (although lower than the adult female mean), decreasing slightly from -20.1‰ at 1.4y to -20.3‰ at 3.5y. The dentine $\delta^{13}C$ signal at 3.5y (-20.3‰) is lower than the adult femoral collagen value (-19.5‰) (Semchuk, 2016). The 1.3‰ difference in $\delta^{15}N$ may be indicative of a decrease in animal protein consumption

(i.e., a more plant-based diet) or can suggest that this individual was still consuming some amounts of breastmilk at 3.5y, with the femoral collagen signal being more indicative of a post-weaning and childhood diet. This individual died young (13y – 14y), and the slow rate of femoral bone turnover results in a collagen signal can represent up to 11 – 25 years of dietary information preceding death (Hedges et al., 2007). Although bone turnover is faster in subadults, the femoral collagen values may still provide information on diet from relatively early in life. An increase of +0.8‰ in $\delta^{13}C$ from 3.5y to later dietary signals (i.e., from the femoral collagen) may indicate increased incorporation of C_4 plant sources in the post-weaning and childhood diet.

F296 (Female, 25y - 29y)

The weaning curve for F296 is show in in Figure 6.3.R. The burial date for the individual from F296 is unknown. Delta $\delta^{15}N$ from 3.5y – 4.5y decreases by 0.3‰, from 8.5‰ to 8.2‰. Since this change is much smaller than the expected trophic level shift observed during weaning, it is likely that F296 had undergone a majority of the weaning process by 3.5y of age. There is no observable change in $\delta^{15}N$ between dentine values at 4.5y and 8.0y. Delta $\delta^{13}C$ decreases by 0.1‰ from 3.5y to 4.5y (which is within the range of reporting error, 0.2‰), reaching -20.1‰ at 8.0y. The negligible change in both $\delta^{13}C$ and $\delta^{15}N$ suggest that weaning was complete and the diet was relatively stable by 3.5y, with little change throughout childhood. Femoral bone collagen values from the individuals were reported to be $\delta^{15}N$ = 8.2‰, and $\delta^{13}C$ = -19.4‰ (Semchuk, 2016). Dietary stability is reflected in the identical nitrogen values in dentine at 8.0y and adult bone collagen. It is likely that the protein sources remained similar (i.e., terrestrial herbivores) from childhood until the time

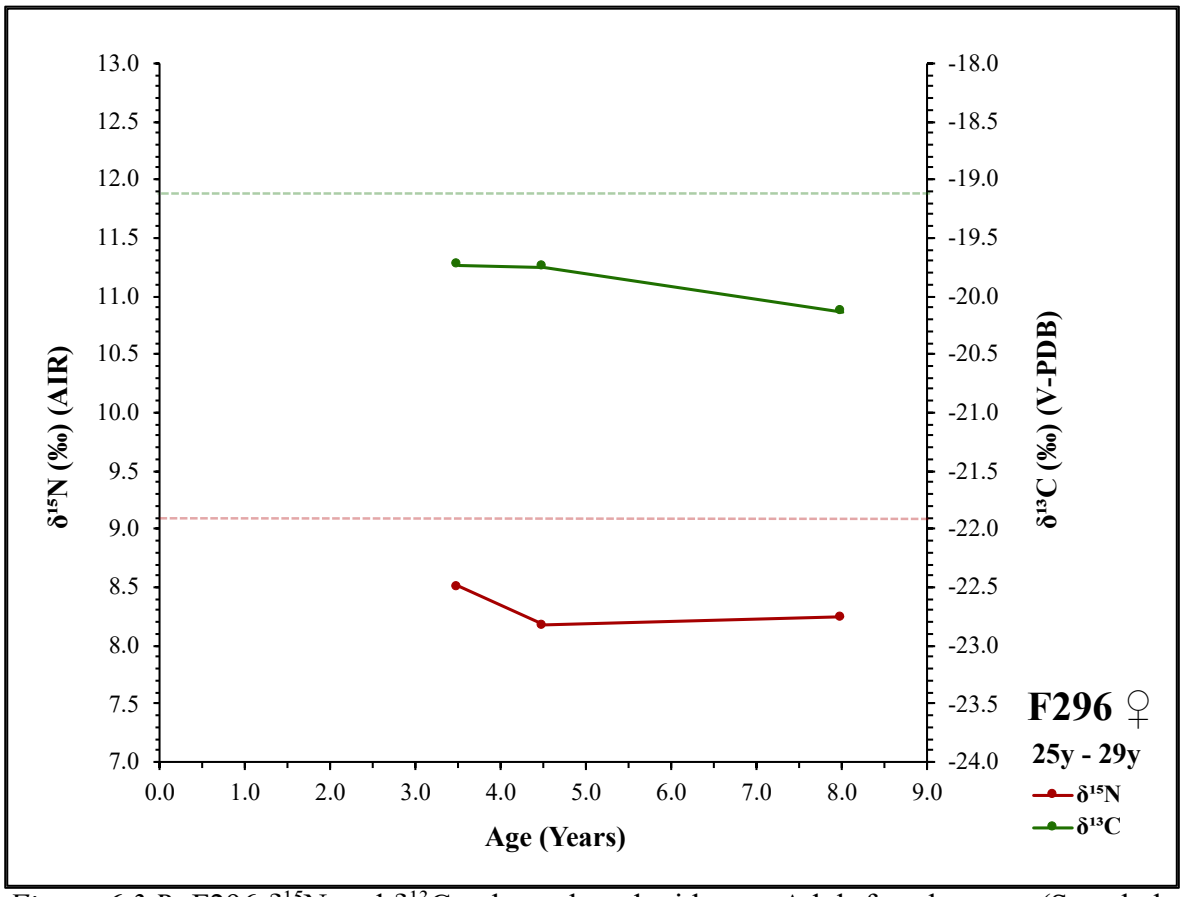

Figure 6.3.R: F296 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female mean (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0. 5‰ [green dotted line].

of death. Delta $\delta^{13}C$ values from femoral collagen were 0.8‰ higher than $\delta^{13}C$ from the dentine collagen at 8.0y, which may indicate the introduction of some C_4 plants later in life.

F308A (Female?, Adult)

Only one dentine section with adequate sample integrity was recovered from F308A, the results of which are plotted on Figure 6.3.S. Little information was recovered from individual F308A; the burial date is unknown, and there were no reported bone collagen values. Diagenetic alteration limited the possibility of investigating weaning patterns in this individual. However, since $\delta^{15}N$ at 3.5y (9.2‰) is similar to the adult female average

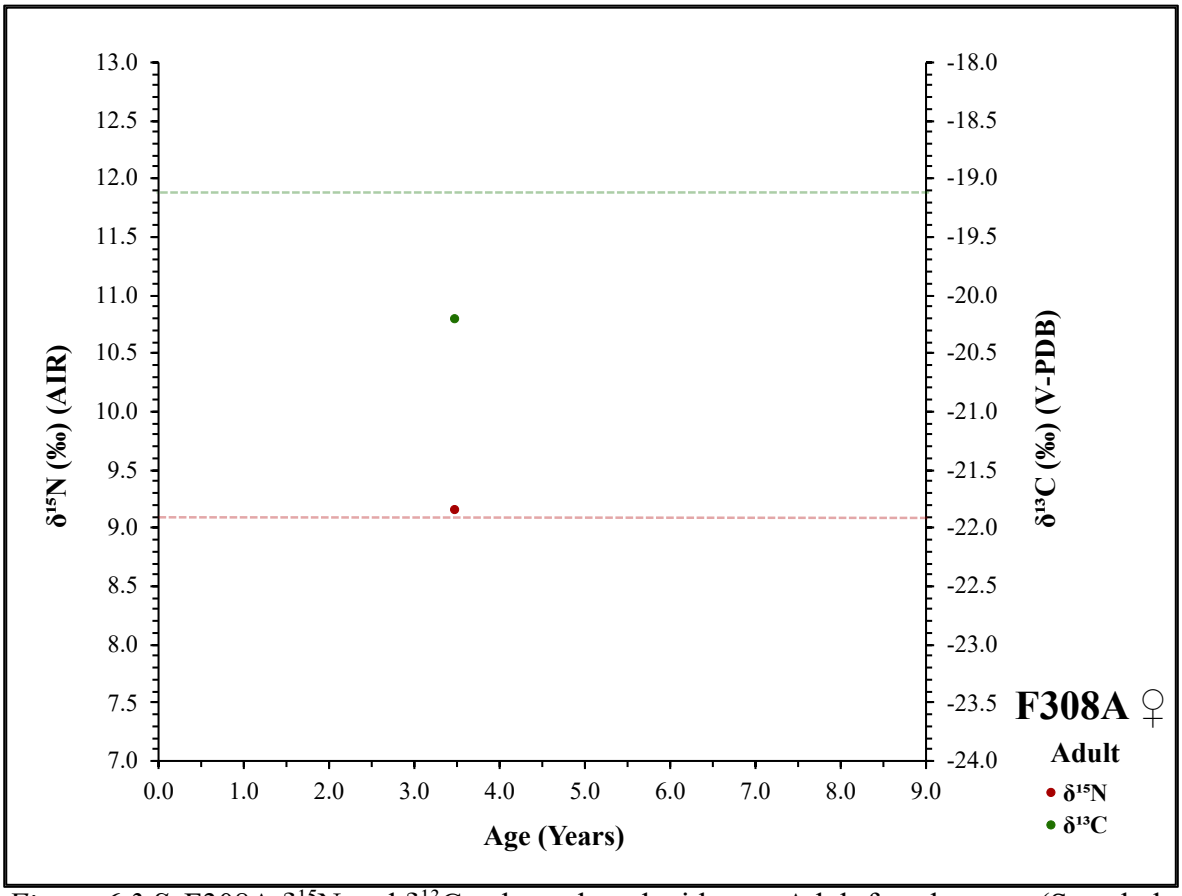

Figure 6.3.S: F308A $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female mean (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

(9.0‰), it is likely that weaning was well underway, and the individual may have been near the end of their weaning period by this age.

F312 (Male, Young Adult)

Delta ^{15}N and $\delta^{13}C$ obtained from dentine sections from F312 are shown in Figure 6.3.T. There is no known burial date for this individual. There is a decrease in $\delta^{15}N$ between 1.2y and 4.5y, from 10.9‰ to 8.9‰ (by 2‰), indicating a trophic level shift associated with the removal of breastmilk from the diet. There is only a 0.1‰ decrease between 4.5y and 7.5y, and no difference in $\delta^{15}N$ between 7.5y and 8.5y, indicating a stable post-weaning and

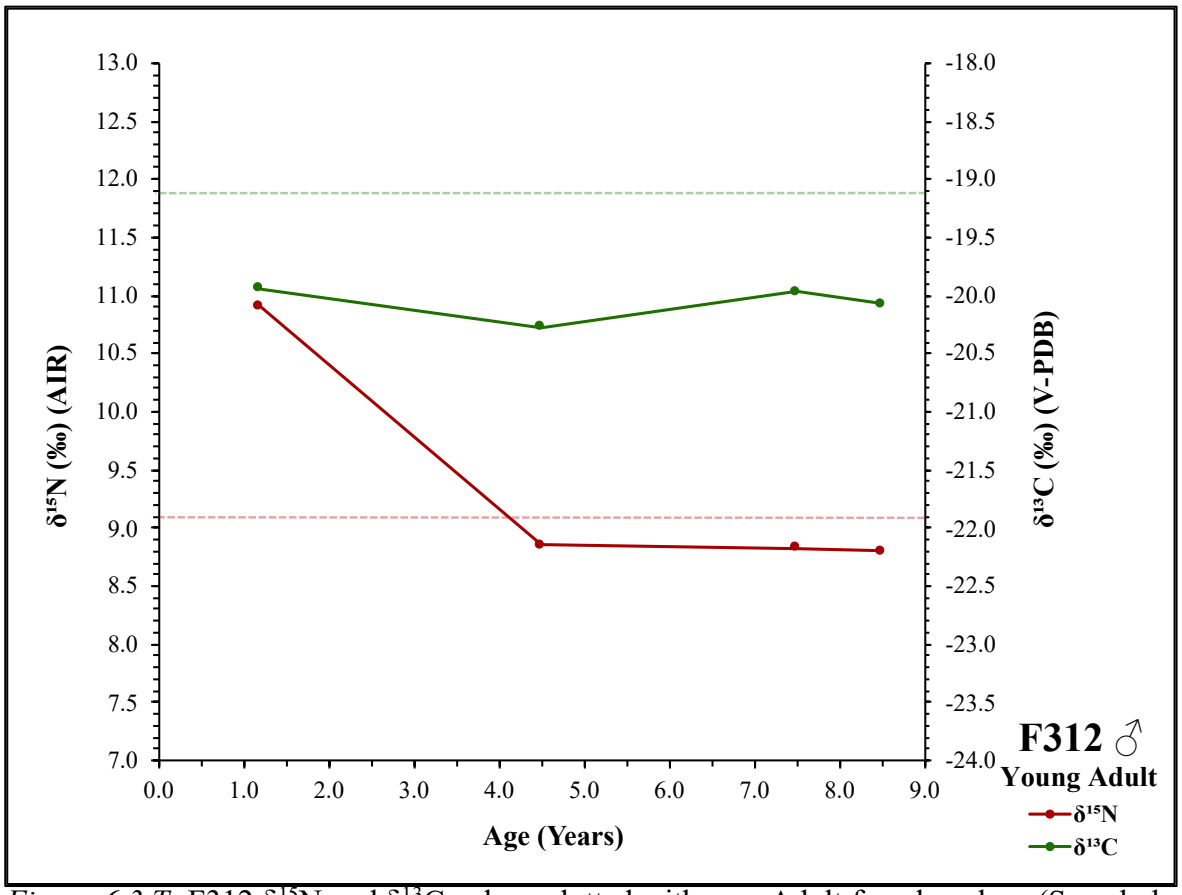

Figure 6.3.T: F312 $\delta^{15}N$ and $\delta^{13}C$ values plotted with age. Adult female values (Semchuk, 2016): $\delta^{15}N$ = 9.0‰ ± 1.1‰ [pink dotted line]; $\delta^{13}C$ = -19.1‰ ± 0.5‰ [green dotted line].

childhood diet. There was a slight difference (-0.4‰) in $\delta^{13}C$ between 1.2y and 4.5y (from -19.9‰ to -20.3‰), followed by relatively stable levels from 7.5y – 8.5y. This signal suggests that the majority of weaning took place between the ages of 1.2y and 4.5y. It is possible that weaning began prior to 1.2y and ended prior to 4.5y, but complete cessation of breastfeeding was conclusively over by 4.5y of age. The intermediate sections (1.5y, 3.5y, 5.5y) were too diagenetically altered to be included in this study, which would have provided a narrower range for breastfeeding and weaning patterns. Dentine $\delta^{15}N$ and $\delta^{13}C$ values at 8.5y (8.8‰ and -20.1‰) are slightly different from the femoral collagen values

reported by Semchuk (2016) (8.3‰ and -19.5‰) representing diet later in life. The small increase in $\delta^{13}C$ (+0.6‰) may indicate an increase in C_4 plant consumption in adulthood, whereas a decrease in $\delta^{15}N$ (-0.5‰) suggests a potential decrease in the consumption of higher-trophic protein sources later in life (i.e., a transition to mostly terrestrial protein sources). However, it is important to note that since these changes are small (all <1‰), the interpretations must be treated with caution.

6.4 General Trends in Dentine Data

The stable isotope results from the dentine sections and bone collagen are summarized in Table 6.2 (section 6.2). Delta $\delta^{13}C$ ranged from -23.1‰ to -18.7‰, with a mean of -19.8‰

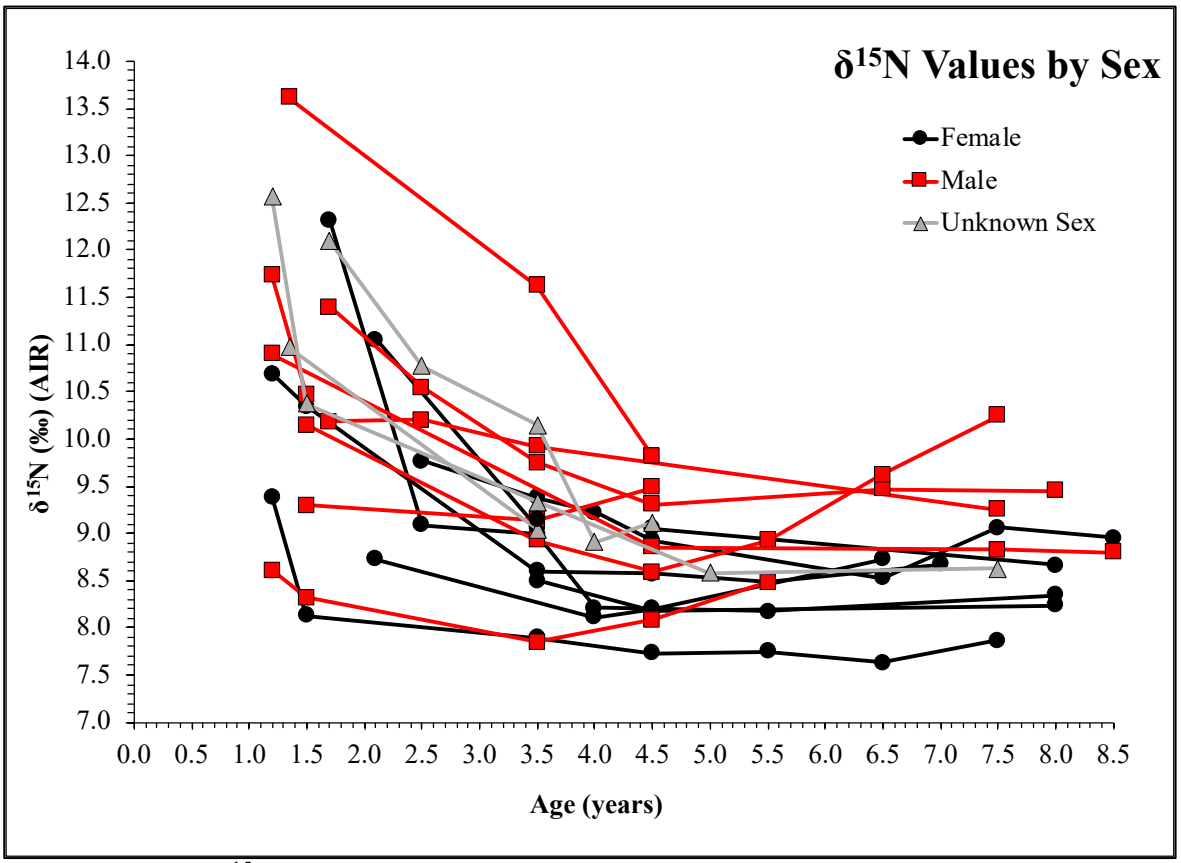

Figure 6.1: Delta^{15}N values plotted with age, separated by sex. Black (dot) = females; red (square) = males; grey (triangle) = Unknown sex.

(± 0.5‰). Delta $\delta^{15}N$ had a mean of 9.4‰ (± 1.2‰), with values ranging from 7.6‰ – 13.6‰. The weaning curves observed in the sample are relatively consistent, showing a general decline in both $\delta^{15}N$ and $\delta^{13}C$ with increasing age (Figures 6.1, 6.2). Discussion on stable isotope signals with respect to age is provided in section 6.7, below. Most individuals appear to have been weaned completely by ~3.5y. There are some observable differences in weaning practices and patterns among the sample, despite the relatively consistent age of complete cessation of breastfeeding. These variations in weaning practices are further discussed in section 6.6. Most individuals appear to have consumed a diet consisting of C_3 plants and terrestrial protein sources.

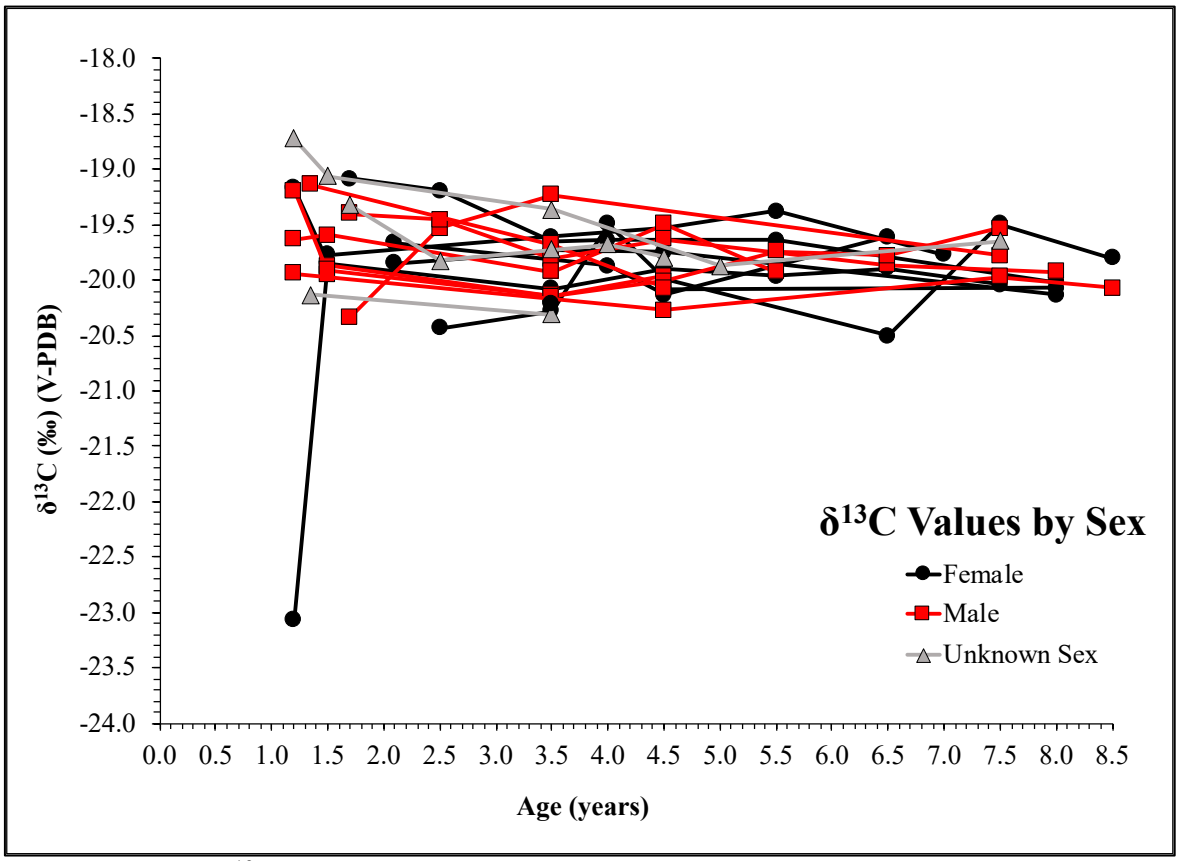

Figure 6.2: Delta^{13}C values plotted with age, separated by sex. Black (dot) = females; red (square) = males; grey (triangle) = Unknown sex.

There is evidence of some C_4 plant consumption as a complementary weaning food in individuals F126, F211, and F323, reflected by the elevated $\delta^{13}C$ signals (with constant or decreasing $\delta^{15}N$) with increasing age. Increasing $\delta^{13}C$ values in concert with increasing $\delta^{15}N$ suggests consumption of higher trophic level foods, such as pork, fish, or other marine resources (Schoeninger et al., 1983). Although terrestrial herbivore faunal remains were more abundant at the site relative to pigs, the expected $\delta^{15}N$ signals in people with exclusive terrestrial herbivore consumption would be lower than the observed values in most of the samples (Table 7.2, faunal isotope signals from the site are further discussed in section 7.2.5). Isotopic signals consistent with considerable fish or pork consumption were observed in the dentine data from only one individual (F291), and therefore fish consumption during weaning and post-weaning was likely not a common practice at Vagnari.

6.5 Patterns of Weaning

As mentioned in section 6.4, there are discernable differences in weaning patterns despite the apparent consistency in complete cessation of breastfeeding throughout the sample. In this study, I have categorized individuals into different categories according to two different observed weaning patterns:

1) Early initiation and rapid weaning: In some individuals (e.g., F126, F206, F215, F235, F247, F313, F323), the majority of weaning occurred rapidly within a compressed time frame (i.e., >50% of the expected 2‰ – 3‰ trophic level decrease in $\delta^{15}N$ prior to 2.5y) before transitioning to a slower 'tapering' weaning process in which breastmilk was

still included in small amounts until the complete cessation of breastfeeding occurring at ~3.5y.

2) Gradual weaning: Other individuals (e.g., F207, F211, F212, F291, F309, F320, F336) demonstrated a more steady, consistent weaning pattern in which the consumption of breastmilk decreased gradually until complete cessation of breastfeeding, also occurring at approximately 3.5y or later. Individuals in this group typically also had prolonged breastfeeding periods, with $\delta^{15}N$ signals continuously decreasing until 4.5y or later (F207, F212, F291, F309, F336 [complete cessation at 5.0y]).
3) Individuals for whom there is not enough data to determine weaning rates: In these cases, there are not enough data points during weaning ages to assess the rate of weaning. Individuals F127, F249, F286B, F296, F308A, and F312 all belonged to this category. These results are summarized in Table 6.3.

6.6 Sex-Based Differences in Weaning Practices

Delta^{15}N values in females range from 7.6‰ – 12.3‰, whereas in males, these values are slightly higher, with a range of 7.9‰ – 13.6‰ (Figure 6.1, Table 6.4). Although the differences are slight, it appears as though some males have slightly elevated $\delta^{15}N$ levels

Table 6.3: Individuals categorized by weaning pattern. Rapid/steep weaning is defined by early commencement of weaning and a rapid decline (resulting in a steep downward slope) in $\delta^{15}N$ values prior to 3.5y. Gradual weaning is defined by a steady decline (resulting in a gentle downward slope) in $\delta^{15}N$ values prior to 3.5y. Individuals lacking information on $\delta^{15}N$ values prior to 3.5y of age were grouped into Insufficient Data.

Weaning Pattern	Individual
Rapid/Steep	F126 | F206 | F215 | F235 | F247 | F313 | F323
Gradual	F207 | F211 | F212 | F291 | F309 | F320 | F336
Insufficient Data	F127 | F249 | F286B | F296 | F308A | F312

relative to females. This may suggest gender-based differences in feeding practices, where males consumed higher amounts of animal proteins. Female $\delta^{13}C$ values range from -23.1‰ to -19.1‰, whereas male $\delta^{13}C$ signals range from -20.3‰ to -19.1‰ (Figure 6.2). Although the minimum value is lower in the female group (-23.1‰), this value comes from a single individual, F211, which skews the results. There is an apparent consistency in plant consumption in the weanling diet among males and females.

Of the seven individuals exhibiting rapid (or abrupt) weaning patterns, four were female (F206, F215, F313, F323) and three (F126, F235, F247) were male. Some breastmilk consumption may have continued until later, such as in F206 until 4.0y – 4.5y, but it is likely that the majority of the expected 2‰ – 3‰ trophic level decrease in $\delta^{15}N$ occurred prior to 2.1y, with breastmilk supplementing early childhood diet in small amounts throughout early childhood. This sample size is quite small, but suggests that rapid weaning was relatively common and likely not related to sex. Prolonged weaning practices

Table 6.4: Changes in $\delta^{15}N$ and $\delta^{13}C$ signals (‰) between the age-at-weaning, the post-weaning diet, and the adult bone collagen data (Semchuk, 2016). The number of data points refers to how many signals are representing the reported values. * = This is the earliest available dentine collagen signal, but it is possible that weaning was complete prior to the assigned age-at-weaning.

Individual	Sex	Weaning Age	$\delta^{15}N$	$\delta^{13}C$	Post-Weaning Age Range	# Data Points	$\delta^{15}N$	$\delta^{13}C$	Age at Death	Bone $\delta^{15}N$	Bone $\delta^{13}C$
F206	F	2.1	8.7	-19.7	4.0 - 6.5	3.0	8.3	-19.9	50y+	9.3	-19.4
F211	F	3.5	8.6	-19.6	4.5 - 7.0	3.0	8.6	-19.6	<30y	9.3	-19.4
F215	F	2.5	9.1	-19.2	3.5 - 8.0	4.0	8.6	-19.7	38.2y	9.6	-19.9
F247	F	3.5	9.1	-20.1	4.5	1.0	9.5	-20.0	Adult	9.8	-19.3
F296	F	3.5	8.5	-19.7	4.5 - 8.0	2.0	8.2	-20.0	25y - 29y	8.2	-19.4
F313	F	3.5	7.9	-20.1	4.5 - 7.5	4.0	7.8	-20.0	19y - 23y	N/A	N/A
F323	F	2.1*	9.8	-20.4	3.5 - 8.5	6.0	9.0	-19.9	19y - 23y	N/A	N/A
F126	M	1.7*	10.2	-20.3	2.5 - 7.5	3.0	9.8	-19.5	20y - 25y	9.1	-19.5
F207	M	4.5	9.3	-19.6	6.5 - 8.0	2.0	9.5	-19.9	Young Adult	9.6	-19.9
F235	M	3.5	7.9	-19.9	4.5 - 5.5	2.0	8.3	-19.7	50y+	9.1	-19.6
F312	M	4.5	8.9	-20.3	7.5 - 8.5	2.0	8.8	-20.1	Young Adult	8.3	-19.5
F212	U	4.0	8.9	-19.7	4.5	1.0	9.1	-19.8	Adult	N/A	N/A
F336	U	5.0	8.6	-19.9	7.5	1.0	8.6	-19.7	10y	N/A	N/A

was apparently equally as common as rapid weaning, and also appears to be relatively equally distributed among males and females, although more males were weaned gradually than females. In this group (n = 7), there were two females (F211, F320), three males (F207, F291, F309), and two individuals of unknown sex (F212, F336) (Tables 6.4, 6.5).

Dentine isotope data allowed for the estimation of an age-at-weaning for 13 of 20 individuals, since not all sampled teeth were able to yield a sufficient amount of datapoints for an accurate assessment of weaning age (Tables 6.4, 6.5). Two individuals, F291 and F309, had multiple possible weaning ages (3.5y – 4.5y and ≥4.5y, respectively), and were excluded from the analysis. Individuals were separated by sex to compare the respective average weaning ages. The female average age-at-weaning was determined to be 3.0y, whereas for males this occurred slightly later, at 3.6y (Table 6.5). The $\delta^{13}C$ and $\delta^{15}N$ between males and females are slightly different, with males having a $\delta^{15}N$ signal that is 0.3‰ higher and a $\delta^{13}C$ signal that is 0.2‰ lower than females. Although it appears as though females were weaned earlier than males, a Welch's t-Test analysis determined that there is no significant relationship between sex and weaning age ($p = 0.23$). Though this type of test is appropriate for assessing unequal sample sizes and does not have a minimum

Table 6.5: Weaning age by sex. Unknown sex = 1 adult (F212), 1 subadult (F336). * = This is the earliest available dentine collagen signal, but it is possible that weaning was complete prior to the assigned age-at-weaning.

Sex	# Samples	Range (years)	Average (years)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Female	7	2.1* - 3.5	3.0	8.8	-19.8
Male	4	1.7* - 4.5	3.6	9.1	-20.0
Unknown	2	4.0 - 5.0	4.5	8.8	-19.8

sample threshold, the number of individuals included in this analysis were quite small (especially males, n = 4) and it is therefore unclear as to whether these results are meaningful.

6.7 Weaning Practices and Mortuary Assemblage

With the exception of F312, all individuals from this study were interred with grave goods (Appendix 1). For the majority of the sample (90%), grave goods consisted of a combination of ceramic fragments/vessels (n = 18), oil lamps ('a perline') (n = 5), and some examples of metal goods such as iron nails or hob nails (n = 13).

Table 6.6: Individuals analyzed to assess age-at-weaning and grave good assemblage. Total number of grave goods refers to the total number items retrieved, counting all items separately. Diversity score refers to the number of different types of artifacts retrieved. Categories are: pottery/ceramics (all pottery sherds = 1); glass (all shards = 1); metal (tools, large nails, etc., = 1 per different item); hobnails (all hobnails = 1); personal items (jewelry, oil lamp; shells = 1 per different item); coins (all coins = 1); miscellaneous (shells, animal bone, stone items, etc., = 1 per different item).

Individual	Sex	Age at Weaning (y)	Total # of Grave Goods	Diversity Score
F126	M	1.7	4.0	3.0
F206	F	2.1	8.0	4.0
F207	M	4.5	5.0	2.0
F211	F	3.5	4.0	1.0
F212	U	4.0	3.0	2.0
F215	F	2.5	6.0	4.0
F235	M	3.5	2.0	2.0
F247	F	3.5	4.0	2.0
F296	F	3.5	11.0	6.0
F312	M	4.5	0.0	0.0
F313	F	3.5	4.0	3.0
F336	U	5.0	7.0	6.0

In order to assess any potential relationship between early life dietary patterns and grave good assemblage at death, the estimated weaning age was compared to the amount and diversity of items recovered from the grave. Since not all individuals had enough isotopic information to estimate an age-at-weaning, this analysis was conducted using 12 individuals (Table 6.6). Grave goods were differentiated by both the total amount of grave goods recovered as well as the diversity score, which is the number of different 'categories' of artifacts retrieved. For example, individual F207 had four different ceramic vessels, and a metal fragment (i.e., five grave good items total). However, since there were only two different 'categories' of items, the diversity score for this grave good assemblage is 2. The categories were divided as follows: pottery/ceramics, glass, metal (+1 per different item), hobnails, personal items (e.g., jewelry, oil lamp, shells, +1 per different item); coins, miscellaneous (shells, animal bone, stone items, +1 per different item).

The age-at-weaning was compared to both the total number of grave and diversity score independently. If both grave goods and early life dietary practices were related to social status (e.g., wealthy individuals were weaned earlier and were interred with a higher variety of grave goods), there would be an apparent correlation between age-at-weaning and grave good amount and/or diversity. Among the 12 individuals included in this analysis, there appeared to be a negative correlation between weaning age and both total grave good number (-0.2) and diversity (-0.1), although neither of these values were statistically significant ($p = 0.08$, and $p = 0.19$, respectively). Given the lack of statistical significance, further analysis must be completed to assess the validity of the observed correlations.

6.8 Age-Related Trends

Age-related trends in $\delta^{15}N$ and $\delta^{13}C$ in the study sample are summarized in Figures 6.3 and 6.4, and Tables 6.7 and 6.8, respectively. Due to the limited amount of isotopic data per age category, assessments on both age and sex would yield too few data points to render meaningful information, so individuals were not separated by sex.

Delta ^{15}N appears highest around 1.4y (12.3‰), with a general downward trend until ~4.0y (8.6‰) (Figure 6.3, Table 6.7). These data demonstrate a general downward trend in $\delta^{15}N$ from 1.2y to ~3.5y – 4.0y, with relative stability (values ranging from 8.4‰ – 8.9‰) from 4.0y onwards. The large range (observed as large error bars) in $\delta^{15}N$ values from ages 1.2y – 3.5y indicate relatively diverse weaning practices at Vagnari, with

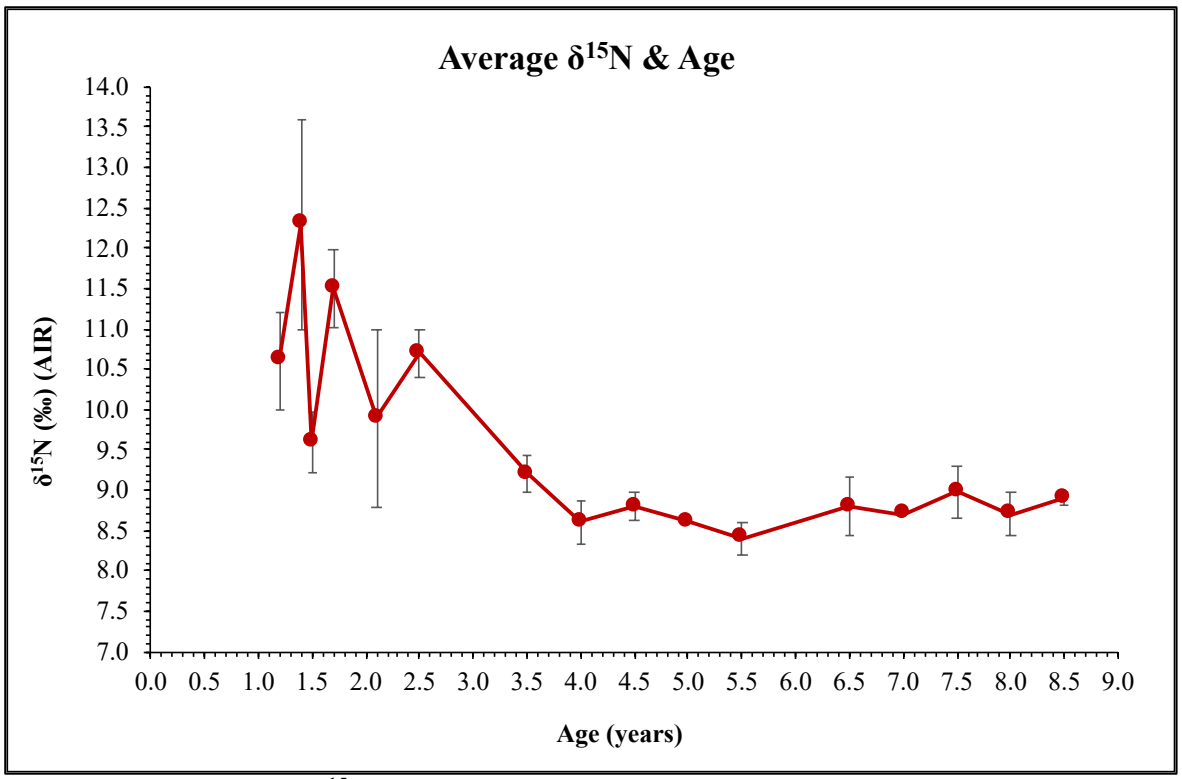

Figure 6.3: Average of $\delta^{15}N$ with increasing age. Note: there is only one sample (each) for 5.0y and 7.0y, resulting in no error bars for these age categories.

Table 6.7: Delta^{15}N levels per age category. Sample Counts refers to the number of samples that contributed to the range, mean, and standard error calculations. Rows highlighted in yellow (i.e., ages 5.0y, 7.0y) have only one datum per category for which statistical analysis could not be performed.

Age Category	Maximum Value (‰)	Minimum Value (‰)	Mean	Standard Error	Sample Counts
1.2	12.6	8.6	10.6	0.6	6
1.4	13.6	10.9	12.3	1.3	2
1.5	10.5	8.1	9.6	0.4	7
1.7	12.3	10.2	11.5	0.5	4
2.1	11.1	8.7	9.9	1.1	2
2.5	10.8	9.1	10.7	0.3	5
3.5	11.6	7.9	9.2	0.2	16
4.0	9.2	8.1	8.6	0.3	4
4.5	9.8	7.7	8.8	0.2	13
5.0	-	-	8.6	-	1
5.5	8.9	7.8	8.4	0.2	5
6.5	9.6	7.6	8.8	0.4	5
7.0	-	-	8.7	-	1
7.5	10.3	7.9	9.0	0.3	6
8.0	9.5	8.2	8.7	0.3	4
8.5	9.0	8.8	8.9	0.1	2

individuals generally completing weaning by 3.5y (Figure 6.3, Table 6.7). The sections representing 1.4y and 2.1y both had only two samples per age category. This may (at least in part) explain the fluctuations in values between age categories prior to 3.5y.

There are no apparent trends in $\delta^{13}C$ with increasing age; average values from 1.2y – 8.5y range from -19.5‰ to -20.0‰ (Figure 6.4, Table 6.8). It appears the plant portion of the diet at Vagnari was primarily composed of C_3 plants with some addition of C_4 plants (especially in individuals F126, F211, and F323). Of the 16 different age categories determined in this study, only two categories had more than ten samples; 3.5y (16 samples), and 4.5y (13 samples) (Tables 6.7, 6.8). There was only one sample each for 5.0y and 7.0y,

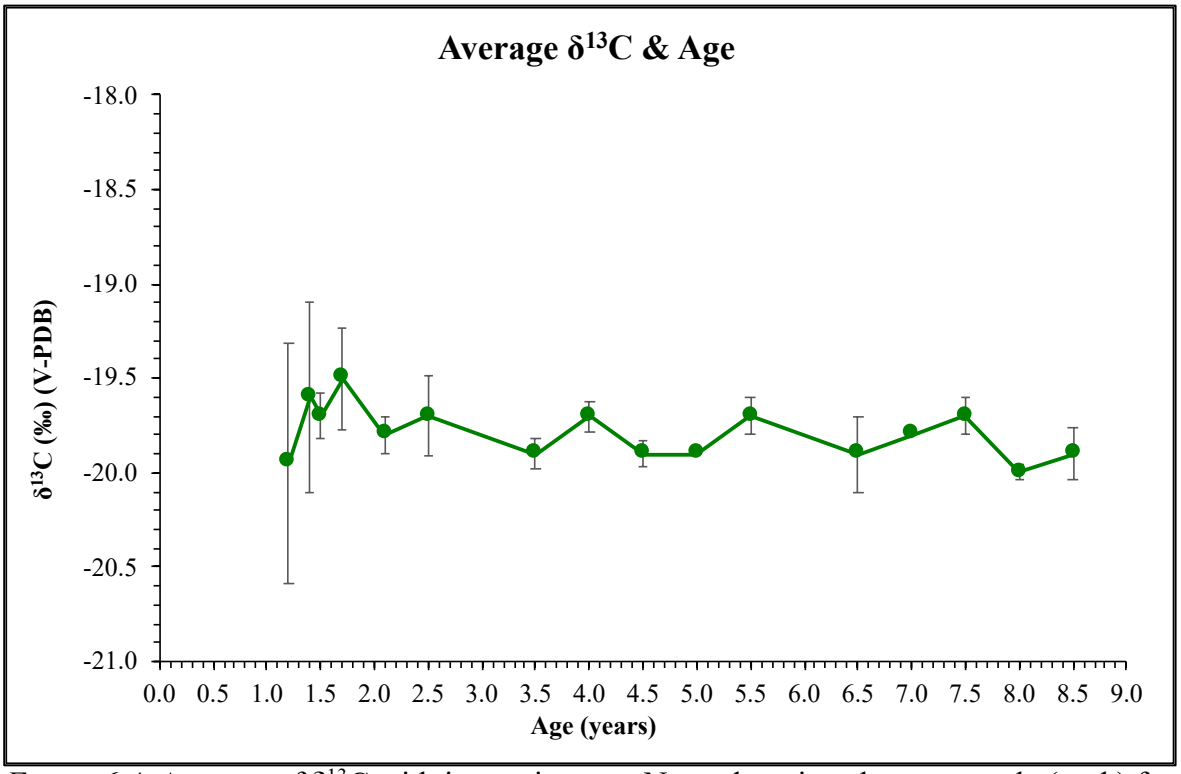

Figure 6.4: Average of $\delta^{13}C$ with increasing age. Note: there is only one sample (each) for 5.0y and 7.0y, resulting in no error bars for these age categories.

providing a single data point per age category. The rest of the age categories had sample sizes ranging from two to seven sections. These sample sizes are important to note, since the observed differences cannot be accurately assessed for significance within the larger sample.

6.8.1 Weaning Practices and Life Expectancy

Even though all the individuals in the present study survived the weaning process, the age-at-death within the sample ranged from as young as 10y (± 2.0y) to older adults aged 50y+. Due to the limited amount of available isotopic dentine data, only seven individuals in this sample had both an estimated age-at-death and an estimated age-at-weaning (F126, F206, F215, F235, F296, F313, F336) (Table 6.9). Individuals who were labeled as 'young adult'

Table 6.8: Delta^{13}C levels per age category. 'Sample Counts' refers to the number of samples that contributed to the range, mean, and standard error calculations. Rows highlighted in yellow (i.e., ages 5.0y, 7.0y) have only one datum per category for which statistical analysis

Age Category	Maximum Value (‰)	Minimum Value (‰)	Mean	Standard Error	Sample Counts
1.2	-18.7	-23.1	-20.0	0.6	6
1.4	-19.1	-20.1	-19.6	0.5	2
1.5	-19.0	-20	-19.7	0.1	7
1.7	-19.1	-20.3	-19.5	0.3	4
2.1	-19.7	-19.9	-19.8	0.1	2
2.5	-19.2	-20.4	-19.7	0.2	5
3.5	-19.2	-20.3	-19.9	0.1	16
4.0	-19.5	-19.9	-19.7	0.1	4
4.5	-19.5	-20.2	-19.9	0.1	13
5.0	-	-	-19.9	-	1
5.5	-19.4	-20	-19.7	0.1	5
6.5	-19.6	-20.5	-19.9	0.2	5
7.0	-	-	-19.8	-	1
7.5	-19.5	-20.4	-19.7	0.1	6
8.0	-19.9	-20.1	-20.0	0.0	4
8.5	-19.8	-20.1	-19.9	0.1	2

or 'adult' were excluded from the analysis. Individuals with a range (e.g., 25y – 29y) were assigned the midpoint age (i.e., 27.5y). Individuals labeled 50y+ were assigned an age of 50y. The average age-at-death for this subsample was 31.3y, and the average age-at-weaning was 3.1y. There was a negative correlation (-0.48) between the estimated age-at-weaning and the estimated age-at-death, although this difference is not significant ($p = 0.27$). If confirmed by future research, this may suggest that individuals weaned later in life on average died earlier than individuals with an earlier age-at-weaning.

6.9 Diet and the Life Course: Dentine to Bone Collagen

Delta ^{15}N and $\delta^{13}C$ signals from different life stages (i.e., once weaned, post-weaning/childhood diet, and adult diet) are compiled in Table 6.10. The weaning age, post-weaning age range, and age-at-death, and the corresponding $\delta^{15}N$ and $\delta^{13}C$ values are shown fully in Table 6.4 (section 6.6), and are condensed in Table 6.10. These signals represent diverse and dynamic dietary practices throughout life at Vagnari. In most samples (70% of individuals represented in Table 6.10), the bone collagen $\delta^{15}N$ signals are higher than the dentine data (age-at-weaning and post-weaning). There were two individuals, F126 and F312, who had a lower $\delta^{15}N$ signal in bone collagen (Semchuk, 2016) relative to the dentine collagen data, suggesting a slightly more terrestrial diet later in life, although these differences are negligible (only -0.7‰ and -0.5‰ from post-weaning to bone collagen, respectively). In some cases (e.g., F206 and F215), the post-weaning $\delta^{15}N$ values are 0.4‰ – 0.5‰ lower than at weaning, and 1.0‰ lower than the bone collagen values collected by Semchuk (2016) (Table 6.10). This slight decrease in $\delta^{15}N$ during post-weaning in these

Table 6.9: Individuals from Vagnari with estimated ages-at-weaning and ages-at-death. Individuals with ambiguous weaning histories and/or ages-at-death (e.g., young adult) were excluded. * = The average age-at-death is not a calculated value and is assigned directly from the estimated range.

Individual	Sex	Age-at-Weaning (years)	Age-at-Death Range	Avg. Age-at-Death (years)
F126	M	1.7	20y - 25y	22.5
F206	F	2.1	50y+	50.0*
F215	F	2.5	38.2y ± 10.9y	38.2
F235	M	3.5	~50y ± 12.6y	50.0
F296	F	3.5	25y - 29y	27.5
F313	F	3.5	19y - 23y	21.0
F336	U	5.0	10y ± 2.0y	10.0

individuals may indicate either a negative nitrogen balance due to growth and development, or a more plant-based diet during the post-weaning years.

Some individuals, such as F235 and F247, have consistent increases in both $\delta^{15}N$ and $\delta^{13}C$ between age categories, which suggests an increasing contribution of higher trophic level resources in the diet with age. The sampled individuals in this analysis demonstrated many unique patterns and changes, highlighting the diversity and dynamic

Table 6.10: $\delta^{15}N$ and $\delta^{13}C$ values obtained from dentine (weaning and post-weaning) and bone (adult) collagen (Semchuk, 2016). Results are reported in ‰.

Individual	Weaned		Post-Weaning		Adult	
	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$
F126	10.2	-20.3	9.8	-19.5	9.1	-19.5
F206	8.7	-19.7	8.3	-19.9	9.3	-19.4
F207	9.3	-19.6	9.5	-19.9	9.6	-19.9
F211	8.6	-19.6	8.6	-19.6	9.3	-19.4
F212	8.9	-19.7	9.1	-19.8	N/A	N/A
F215	9.1	-19.2	8.6	-19.7	9.6	-19.9
F235	7.9	-19.9	8.3	-19.7	9.1	-19.6
F247	9.1	-20.1	9.5	-20.0	9.8	-19.3
F296	8.5	-19.7	8.2	-20.0	8.2	-19.4
F312	8.9	-20.3	8.8	-20.1	8.3	-19.5
F313	7.9	-20.1	7.8	-20.0	N/A	N/A
F323	9.8	-20.4	9.0	-19.9	N/A	N/A
F336	8.6	-19.9	8.6	-19.7	N/A	N/A

Table 6.11: Changes in $\delta^{15}N$ and $\delta^{13}C$ signals through time. Comparison conducted between the section of dentine corresponding to the oldest possible age (i.e., root dentine representing diet from post-weaning/childhood) and bone (i.e., adulthood) collagen values (Semchuk, 2016). N/A = There is no difference between dentine and bone collagen values. * = This dentine signal is from earlier in life and since $\delta^{15}N$ and $\delta^{13}C$ values are elevated relative to the rest of the sample, the signal and is assumed to represent a diet that includes breastmilk.

Dietary Changes (Dentine → Bone)	Individual	Sex	Oldest Dentine Section Age (years)	Age-at-Death	Change in $\delta^{15}N$ (‰)	Change in $\delta^{13}C$ (‰)
Group 1	F126	M	7.5	20y - 25y	N/A	N/A
	F207	M	8.0	Young Adult	N/A	N/A
Group 2	F206	F	6.5	Older Adult (50y+)	+0.6	N/A
	F215	F	8.0	38.2y ± 10.9y	+1.2	N/A
	F235	M	5.5	~50y ± 12.6y	+0.6	N/A
	F291	M?	7.5	Adult	-1.2	N/A
Group 3	F296	F	8.0	25y - 29y	N/A	+0.8
Group 4	F127	F	8.0	15y - 20y	+1.2	-1.7
	F211	F	7.0	Young Adult (<30y)	+0.6	+0.4
	F247	M	4.5	Adult	+0.3	+0.7
	F249	M?	1.5*	Adult	-0.8	+1.0
	F286B	U	3.5	13y - 14y	-1.3	+0.8
	F312	M	8.5	Young Adult	-0.5	+0.6

nature of dietary practices in life. Importantly, despite some variability occurring throughout the life course, the isotopic signals in all of the individuals sampled indicate a diet that is composed primarily C_3 plants and terrestrial proteins, with only some demonstrating a contribution of marine sources and/or C_4 plants.

Bone collagen data was previously collected and reported for 14 individuals in this study by Semchuk (2016). When examining the relative changes in $\delta^{15}N$ and $\delta^{13}C$ signals from infancy and childhood (i.e., the dentine signals) to the older (often adult) signals from bone collagen, four categories emerge: Group 1) No change in $\delta^{15}N$ or $\delta^{13}C$ from dentine values to bone collagen values (or changes are within reporting error); Group 2) Different $\delta^{15}N$ signal from dentine to bone collagen with minimal/no change in $\delta^{13}C$; Group 3) Different $\delta^{13}C$ signal from dentine to bone collagen with minimal/no change in $\delta^{15}N$; and Group 4) Both $\delta^{15}N$ and $\delta^{13}C$ had observable changes when comparing dentine data to bone

collagen. The distribution of individuals within these groups is summarized in Table 6.11. In most cases (F127, F206, F211, F215, F235, F247), bone collagen $\delta^{15}N$ levels were higher than dentine values in the same individual, suggesting an increase in higher trophic level foods with advancing age. The opposite is true in individuals F249, F286B, F291, and F312, which demonstrate a decrease in $\delta^{15}N$ from dentine to bone collagen (Tables 6.1, 6.11). Dentine values of some of these individuals represented diet in early life, presumably during breastfeeding (e.g., F249 at 1.5y), indicating that the difference in $\delta^{15}N$ between the dentine and bone collagen may be attributable solely to the consumption of breastmilk. There is also indication that some individuals decreased their higher-trophic level protein consumption with age; for example, F291's $\delta^{15}N$ dentine signal at 7.5y is a post-weaning dietary signal (10.3‰) and shows no indication of breastmilk in the diet (Figure 6.3.K). This signal is still 1.2‰ higher than the collagen $\delta^{15}N$ value in the adult (9.1‰). The decline in $\delta^{15}N$ from 7.5y to the adult bone collagen value indicates a decrease in the consumption of higher trophic level sources as the individual aged. Delta^{13}C changes from dentine to bone collagen values were observed in seven individuals (F127, F211, 2F47, F249, F286B, F296, F312) (Table 6.11). With the exception of F127 (which has a decrease of 1.7‰ from 8.0y to the adult collagen values), all individuals had higher $\delta^{13}C$ values later in life, indicating greater incorporation of C_4 plants with increasing age (e.g., F249, F286B, F296, F312), or consumption of aquatic food sources if there is an increase in both $\delta^{15}N$ and $\delta^{13}C$ (F211, F247) (Table 6.11).

6.10 Conclusion

The stable isotope analysis conducted on the individuals of this study produced valuable information regarding weaning practices among sample. With 83 viable samples (with adequate sample integrity), weaning signals for 20 individuals were obtained. The individual weaning histories indicate a diverse set of infant feeding and weaning practices, with some individuals weaned quite early (or not breastfed at all) such as F247, and other weaned as late as 5.0y (F336). There appear to be some slight sex-based weaning ages and practices in this sample, however since this initial analysis had small sample sizes, further investigation is required to assess these relationships. Age-based trends of the sampled population indicate that $\delta^{15}N$ decreases with increasing age until ~4.0y, after which the values remain relatively consistent throughout the post-weaning diet. Changes in $\delta^{13}C$ are much less consistent, producing a signal with no clear upward or downward trend.

7. DISCUSSION

7.1 Introduction

This study produced early life dietary data for 20 individuals interred at Vagnari. Despite one individual (F308A) providing only one data point (at 3.5y), the rest of the individuals examined in this study were all able to provide two to seven usable signals per tooth. Most of the individuals in this study (n = 14) were also examined in Semchuk's (2016) analysis of diet at Vagnari using bone collagen. Of the 20 individuals included in this study, there were only six for whom there were no reported bone collagen values: F308A, F309, F313, F320, F323, and F336. The dentine stable isotope results can be effectively compared to the adult bone collagen values to estimate relative (longitudinal) dietary stability throughout the life course; this topic is further discussed in section 7.2.2.

Isotope studies using infant and child bone collagen determine the dietary signal of individuals belonging to a subgroup that is inherently different from the rest of the population; these individuals died prematurely, and the cause of death can seldom be determined. Therefore, it is unclear as to whether their diet may have contributed to their poor health, or if the diet itself was a response or treatment for an infant or child with a pre-existing condition unrelated to weaning. Soranus and Galen both suggested that sickly infants be breastfed for longer than healthy infants (Fulminante, 2015). This prolonged consumption of breastmilk may depict a later-than-normal weaning schedule in bone collagen studies due to the implementation of a specialized diet for sickly/infirm infants. Alternatively, if early weaning occurred and the individual died shortly afterwards, bone collagen studies would indicate that children were weaned earlier than the healthy

population. Dentine analysis allows the researcher to select for 'survivors', that is, those individuals who lived past the age of weaning, which ensures that this transition was not the cause of premature death of the individual. This controls for weaning- or diet-related early life mortality and avoids the grouping of healthy individuals with a typical weaning pattern with those who may have had a weaning pattern intended to treat an illness, or one that resulted in their premature death.

The isotopic data analyzed in the present study demonstrate that weaning patterns and rates among the sampled individuals from Vagnari were variable, but despite this variability, most children were fully weaned by ~3.5y. A comparison of weaning practices from Vagnari to observed patterns from other contemporaneous sites (vis-à-vis incremental dentine analysis) within the Roman Empire is discussed in section 7.3.1.

The post-weaning dietary results (Table 6.10) are consistent with plant and faunal remains recovered at the vicus, as well as historical documents which detail the production and trade of these goods across the empire (Carroll, 2022). What life was like for rural Romans (and especially rural Roman infants and children) is poorly understood, and historical records are often depicted through the lens of an elite Roman writer, who may not have accurately portrayed the true lived experiences of common, rural inhabitants of the Empire.

This study investigates dietary changes throughout the life course and contributes to a growing body of literature on infants and children from antiquity. It is unique in its methodology and study population of individuals from a rural Imperial estate located in southern Italy. A literature search on Roman breastfeeding and weaning practices revealed

that no incremental dentine stable isotope study exploring the breastfeeding and weaning practices of rural populations in Imperial Roman Italy has been published in the English language. Although dentine analysis is a powerful tool, it has limitations that must be taken into account when estimating dietary signals, such as age calibration and interpretive challenges. These are covered in section 7.4.

7.2 Weaning Trends from Vagnari

7.2.1 Variations in Breastfeeding and Weaning Practices

Breastfeeding and weaning practices from this study sample appear to have a wide range of variation in the timing, duration, and final age-at-weaning. In the sample examined in this study broadly, however, it appears as though most individuals were (breastfed and) completely weaned by around age 3.5y (Tables 6.6, 6.7). Among the sampled individuals, there are two distinct weaning patterns: The first identified pattern demonstrates early and rapid removal of breastmilk (representing >50% of the expected full 2‰ – 3‰ trophic-level decrease) from the diet before 2.5y, followed by a smaller, gradual decline in breastmilk consumption until complete cessation at ~3.5y of age (see section 6.5). The second weaning pattern is more gradual, indicating a slower weaning schedule with consistent, moderate removal of breastmilk until complete cessation by at least 3.5y of age or older, with one individual (F336) consuming breastmilk until 5.0y of age.

The earliest dentine signal obtained from this study is 1.2y (from F312, Appendix 1), which is not early enough to be able to estimate the commencement of weaning. Despite permanent first molars forming beginning around the time of birth (Hillson, 2014), many of the samples from Vagnari collected for this study (e.g., F249, F235, F207, F247, F286B,

F206, F211, F215) had some missing tooth material due to attrition and collection of dentine and enamel for prior analyses. The other limiting factor was the amount of collagen required for mass spectrometry analysis. Since the minimum required amount of collagen was 0.7mg and the collagen yield for these samples was relatively low (1.9% – 8.8%, Table 6.1), larger (i.e., wider) sections were required, increasing the age represented by the obtained signal. This resulted in the later age in the first dentine signals obtained in this study relative to other dentine studies using permanent first molars.

None of the weaning signals obtained in this study indicated stable, exclusive breastfeeding, perhaps due to occlusal tooth wear (particularly in older individuals) or insufficient collagen yield in the early life dietary signals. That is, there was no indication of an increase or plateau in the peak $\delta^{15}N$ value prior to its observed decrease. This type of pattern is expected to occur during exclusive breastfeeding as an infant accumulates enriched ^{15}N from breast milk from the time of birth until the onset of weaning, demonstrating a trophic level effect in the infant tissues (Fuller et al., 2006; Humphrey, 2014). So, it is unclear if the signal obtained at 1.2y is representative of an early stage of weaning (just under the peak $\delta^{15}N$ value), or exclusive breastfeeding.

An ‘exclusive breastfeeding’ signal was not reported in a majority of the studies that explore breastfeeding and weaning patterns using incremental dentine analysis (Table 3.2, an exploration of incremental dentine studies is covered in section 7.3). These signals are obtained from individuals with highly intact teeth and unworn dentine horns, indicating a need for teeth with minimal occlusal wear, careful sample preparation, and good collagen

preservation (e.g., Beaumont et al., 2018; Fernández-Crespo et al., 2018; Crowder et al., 2019; Goude et al., 2020; Cocozza et al., 2021; Ganiatsou et al., 2022).

The age of weaning completion in the individuals in this study is later then the recommended prescriptive texts written by Soranus and Galen, both of whom recommended weaning to begin around six months of age and be complete by 2.0y – 3.0y (Fulminante, 2015). Importantly, these texts both stipulate that if the infant is sickly or weak, breastmilk consumption and weaning should be prolonged (i.e., cessation of breastmilk consumption is delayed) until the child is stronger and better capable of digesting solid foods (Temkin et al., 1991; Garnsey, 1999; Fulminante, 2015). This may have been the case for individuals F206, F207, F212, F291, F309, and especially F336, who was breastfed until 5.0y of age (and died around the age of 10y).

Variation in weaning age can occur as a result of parents following instructions about the time of year when to begin weaning; depending on when the infant is born, they may begin the weaning process earlier or later. Soranus, in his second *Gynaecology* book (chapter XXI on how and when to wean), recommended that infants should be weaned in the springtime, since the "unevenness of the climate" that occurs in the autumn can predispose the infant's body to disease in addition to the stress that occurs from the change in dietary habits (Temkin et al., 1991, p. 118).

According to Fulminante (2015), bioarchaeological data collected from throughout the Empire suggests that prescriptive texts of certain authors were preferred in different regions; Soranus, who proposed a weaning schedule beginning at 6 months and ending at ~1.5y – 2.0y (when the child is comfortable digesting solid foods), appears to have been

more popular in the center of the Empire, where earlier weaning is typically observed. Galen, who suggested a gradual weaning schedule (from ~six months to ~3.0y), was apparently more popular in the provinces, suggested by the later weaning ages observed from studies in those regions. Fulminante's (2015) argument is supported by combined bone and dentine collagen data from a variety of Roman period sites, including some near the capital of the Empire such as Isola Sacra (Prowse et al., 2008) and the St. Callistus catacombs in Rome (Rutgers et al., 2009), as well as sites from farther geographic regions, such as Egypt (Dupras et al., 2001; Dupras and Tocheri, 2007), Tunisia (Keenleyside et al., 2009), Sweden (Howcroft et al., 2012), and England (Fuller et al., 2003; 2005b, 2006; Powell et al., 2014). It is important to note that Fulminante's (2015) study incorporated data from both bone and dentine collagen studies. This combination of both survivors and non-survivors may provide an inaccurate depiction of weaning ages and patterns in the healthy population. The estimated weaning patterns at Vagnari relative to other Roman sites are discussed in section 7.3.1.

Although the completion of weaning was delayed in most individuals from Vagnari, both groups of observed weaning patterns and "paces" (i.e., early and rapid weaning; gradual weaning) are consistent with the writings from each author. These findings may suggest mixed preferences in breastfeeding and weaning patterns at Vagnari (likely due to personal inclinations and the health of the infant/child). Notably, the prescriptive historical writings were relatively vague and often used developmental benchmarks as opposed to chronological age. For example, Soranus' recommended timing for the commencement of weaning was upon the eruption of the primary dentition (Temkin et al., 1991; Fulminante,

2015). Since this can occur from 4.5 – 10.5 months of age (AlQahtani et al., 2010), the schedule of the onset of weaning could naturally vary from individual to individual.

7.2.2 Weaning, Survivorship, and the Life Course

Semchuk's (2016) examination of diet at Vagnari also included bone collagen data from seven individuals aged birth – 3.9y, 11 children aged 4.0y – 14.9y, and 23 females and 34 males aged 15y+ (Table 7.1). She determined that for most of the individuals sampled, the $\delta^{15}N$ data indicated that breast milk was still a part of the diet at ~3.0y, and weaning was complete by 5.0y of age. The present study determined that the weaning age in the sample was ~3.5y, but a small group of individuals (F207, F212, F312, and F336) were weaned later, from 4.0y – 5.0y. This suggests that although there were some survivors who were weaned as late as non-survivors, weaning typically ended earlier in the surviving group. Since bone remodels over a prolonged period of time and continuously incorporates dietary signals throughout life, in weaned children, bone turnover may artificially raise the age-at-weaning because it includes signals from earlier in life (prior to the complete replacement of collagen formed during breastfeeding) and may reflect a later completion of weaning.

The prolonged breastfeeding observed in the Vagnari bone collagen may be attributed to the fact that the individuals weaned later were already weaker than the healthy

Table 7.1: Vagnari human bone collagen $\delta^{15}N$ and $\delta^{13}C$ signals obtained from infants/children (unknown sex), and males and females aged 15y+. Adapted from Semchuk (2016, pp. 84-85).

Age Category	Age Range (y)	Sex	# of Samples	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Infancy/Early Childhood	0.0 - 3.9	U	7	12.6	-18.0
Childhood	4.0 - 14.9	U	11	9.3	-19.0
Adolescence/Adulthood	15y+	F	23	9.0	-19.1
Adolescence/Adulthood	15y+	M	34	9.3	-19.2

population (which extended breastfeeding), the consequences of which resulted in their premature death. Early mortality can occur for number of reasons outside of breastfeeding and weaning, such as injury/accident, development of a disease, or in the case of girls/women of reproductive age, death during childbirth. It is also possible that this observation occurred due to the small sample of children aged birth to 3.9y (n = 7) used in Semchuk's (2016) analysis, which was not the main focus of her thesis.

Semchuk's (2016) study found that $\delta^{13}C$ signals collected from bone collagen of individuals younger than 3.0y were higher (i.e., less negative) than $\delta^{13}C$ signals observed in adults (and varied after this age). This is an interesting contrast to findings from this study: only two individuals (F215 at 4.0y and F336 at 5.0y – 7.5y) had $\delta^{13}C$ signals at or above the adult female average at Vagnari (-19.1‰), even with the contribution of breast milk in the diet. The rest of the dentine sections all yielded values below the adult female mean. One possible explanation is that dietary choices that resulted in elevated levels of $\delta^{13}C$ in the bone collagen were in some way connected to the premature death of the non-survivors of Semchuk's (2016) study. For example, the practice of weaning and the consumption of weanling foods with more ^{13}C (e.g., C_4 plants) during this time may have contributed to premature death, or alternatively, certain foods with more positive $\delta^{13}C$ signatures were incorporated into the diet as a reactive measure to treat existing illness. Since bone collagen signals represent diet from a longer time period relative to dentine, it is also likely that these values are related to the incorporation of dietary signals from a longer period of life.

Breast milk consumption in infancy and early childhood is associated with positive future health outcomes; infants with a history of breastfeeding have a reduced risk of a myriad of short- and long-term conditions, such as infectious disease (otic, respiratory, gastrointestinal, epidermal), respiratory conditions (i.e., asthma), metabolic conditions (such as obesity and diabetes), and other serious conditions such as childhood leukemia and sudden infant death syndrome (SIDS) (Ip et al., 2007; Howcroft et al., 2012). However, extended exclusive breastfeeding without adequate supplementation to the diet (using appropriate complementary foods) after six months of age can result in nutritional efficiencies and increased rates of premature mortality (Lawrence and Lawrence, 2021).

A comparison of weaning practices between survivors and non-survivors was conducted by Sandberg and colleagues' (2014) analysis of Medieval Nubian adults using intra-tooth (M1 and canine teeth) dentine signals. Bone collagen data obtained from previous cross-sectional studies using subadult ribs (Turner et al,. 2007; Sandberg et al., 2012) suggested a weaning schedule that began ≤1.0y and was complete at ~5.0y. The intra-tooth data collected from the adults in their study all indicated that weaning was complete prior to this age, suggesting that healthy individuals were weaned earlier than infants and children who died at a young age (Sandberg et al., 2014) (Table 3.2, section 3.5). They posit that this observation may result from the adequate supplementation of nutritious complementary foods to the healthy infant's diet, which more readily met the nutritional requirements necessary for growth and development (and earlier weaning). This is in contrast to non-survivors, who may have suffered from malnutrition/undernutrition as a result of prolonged reliance on exclusive breastfeeding.

Although the study conducted by Sandberg and colleagues (2014) examined individuals from a later time period and a more distant site, the observed relationship between weaning age and mortality is similar to the bone collagen results obtained at Vagnari. This suggests that the relationship between weaning patterns and mortality should be further investigated to determine additional factors contributing to increased mortality rates during this transitional dietary phase.

Importantly, none of the individuals sampled in the present study exhibited signs of elevated $\delta^{15}N$ levels as a result of physiological or nutritional stress. Based on these results, it does not appear that these individuals were deprived of food or exposed enough to major pathogens or chronic infection throughout the weaning process to result in signals indicative of nutritional and/or physiological stress.

7.2.3 Sex-Based Differences in Breastfeeding and Weaning Practices

There are slight differences in weaning rates, ages, and the $\delta^{15}N$ and $\delta^{13}C$ values (at weaning) between males and females (Tables 6.6, 6.7). According to the dentine data, on average, females were weaned slightly earlier (3.0y) than males (3.6y). In females, the weaning age ranged from 2.1y – 3.5y, and in males this range was 1.7y – 4.5y. The earliest possible age for the corresponding individuals (i.e., F323 and F126 respectively) is estimated with existing available isotopic data, although an earlier age-at-weaning is possible for each of these individuals. The upper range for the age-at-weaning for males is also 1.0y later than females, suggesting that there may be a sex-based relationship to weaning practices in which males were weaned later than females.

Three males (F126, F235, F247) were identified to have been weaned rapidly, relative to four females (F206, F215, F313, F323) (Table 6.3). Individuals with a gradual weaning pattern (n = 7) consisted of three males, two females, and two individuals of unknown sex (one adult and one subadult). Although three females and two males (along with one subadult of unknown sex) had insufficient data, the distribution of sex in the known weaning patterns seems to be somewhat skewed, in which slightly more males were weaned gradually and slightly more females were weaned rapidly. More broadly, however, the differences do not indicate a strong sex-based distribution of weaning patterns, possibly due to the small sample size in this study. Given that the number of gradually-weaned individuals (n = 7) is more equal to rapidly-weaned individuals (n = 7), it is likely that both early weaning age and rapid weaning patterns and gradual weaning patterns with a later weaning age were commonly practiced at Vagnari. At this site, the differences in $\delta^{15}N$ and $\delta^{13}C$ values between females and males are small (+0.3‰ and -0.2‰), and suggest a relatively comparable diet at weaning and post-weaning from a stable isotope perspective (Tables 6.6, 6.7).

Though incremental dentine analysis allows researchers to study the differences in weaning schedules related to sex (since the analysis is typically conducted on adults for whom sex can be estimated), this relationship is not often discussed in the literature. Publications that explicitly mention weaning age relative to sex have reported varying relationships between weaning age and sex. In some studies, such as the ones by Eerkens and colleagues (2018) and Ganiatsou and colleagues (2022), females were estimated to have been weaned later than males, whereas others (e.g., Fernández-Crespo et al., 2018;

Kwok et al., 2018) showed no difference in weaning timing between the sexes. This contrasts with the findings from Vagnari, which indicated that some males were breastfed for longer. It is important to note that the only study that is close to Vagnari in time and space is the one conducted by Ganiatsou and colleagues (2022), so the weaning schedules in the other studies may not be as applicable to the present study.

7.2.4 The Weanling Diet at Vagnari

Historical records from the Roman period indicate that common weanling foods typically consisted of soft-prepared cereals such as porridges, which typically made with various types and combinations of C_3 grains, such as wheat, einkorn, spelt, and barley (Garnsey, 1999; Temkin et al., 1991), all of which were recovered from the vicus (Table 5.2) (Stirn and Sgouros, 2022). These domesticated taxa represented 47% of all of the total plant material recovered in the phase representing the 2nd c. CE, indicating considerable reliance on these species at Vagnari. Since these plant foods (i.e., grapes from wine, grains from breads and porridges) are all types of C_3 plants (with the exception of millet, which is not explicitly mentioned in the literature as a weanling food), it is possible that any or all of these complementary foods were consumed at Vagnari, and that these plants contributed to a majority of dietary carbon sources as seen in the strong C_3 signals obtained from the dentine data.

It is not possible to differentiate the consumption of animal meat or by-products isotopically, so the specific weanling diet cannot be discerned. If weanlings were fed exclusively with milk from sheep, goat, or cow with $\delta^{15}N$ isotope signals ranging from 4.0‰ to 4.6‰, they would have an expected $\delta^{15}N$ value of ~7.0‰ – 10.6‰ since the

expected trophic level shift is ~3‰ (DeNiro and Epstein, 1981). Given the range of $\delta^{15}N$ signals obtained from dentine signals at weaning range from 7.9‰ – 10.3‰ (Table 6.10), it is likely that weanlings relied heavily on the consumption of these terrestrial herbivores and/or their by-products, with some individuals consuming some higher trophic sources like pork and/or small amounts of fish. The focus on grains with restriction of meats and marine sources may be apparent in some individuals from the Vagnari sample, such as F206 and F215, who both demonstrated a slight decrease in $\delta^{15}N$ values in the post-weaning period (followed by a slight increase in the bone collagen levels).

The case of F323 is unusual, since there appears to be variability in the post-weaning diet with periods of increased consumption of C_4 plants at ages 4.0y and 7.5y (Figure 6.3.C). The reason behind these changes is unclear; perhaps at these ages there was some shortage of C_3 plants (either in the household or in the community in general) which resulted in an increased contribution of C_4 plants to the diet (directly or indirectly through animal products fed on C_4 plants), or due to changing health or dietary preferences that necessitated a change in eating habits.

The observable increases in $\delta^{13}C$ signals relative to stability or decrease $\delta^{15}N$ signals in the dentine sections of F126 (+0.9‰ increase from 1.7y – 3.5y; Figure 6.3.F) and F211 (+3.3‰ increase from 1.2y – 1.5y; Figure 6.3.I) may indicate the regular incorporation of millet, animal milk, or other animal byproducts throughout the weaning process. Despite the increase in $\delta^{13}C$ in F211, the signal still suggests a strong C_3- based diet. The initial $\delta^{13}C$ signal was particularly low (-23.1‰ at 1.2y) relative to other individuals in this sample, and the increase resulted in a $\delta^{13}C$ value that was well within the C_3 plant range (-

19.8‰). This signal may indicate a low initial (maternal) $\delta^{13}C$ values due to low, or no, consumption of C_4 plantstuffs or perhaps a change in $\delta^{13}C$ signals due to a change from C_3 plants that were grown in environmental conditions that produced lower $\delta^{13}C$ values. Delta ^{13}C signals are thought to vary in vegetation depending on the environmental conditions in which the plants are grown, such as temperature, precipitation, altitude and aridity; however, the exact relationship between these factors is still unclear (Chen et al., 2017).

Direct millet consumption, although typically thought of as a famine food, could also have been implemented for its believed health benefits, or simply for the taste and quality of the grain itself (Murphy, 2016; Donahue, 2015). Nonetheless, the contribution of C_4 plants in either case is not large enough to produce a strong C_4 plant signal, which ranges from -9‰ − -14‰, as opposed to C_3 plants which produce a signal ranging from -20.‰ to -35‰ (Katzenberg and Water-Rist, 2018). While there is evidence of C_4 plant contribution to the diet (e.g., increase in $\delta^{13}C$ with no change in $\delta^{15}N$ from post-weaning to adult values in F296), the post-weaning $\delta^{13}C$ values in all of the samples range from -20.1‰ to -19.5‰ (Table 6.10), which indicates a diet consisting primarily of C_3 plants.

7.2.5 Diet Throughout the Life Course: From Infantia to Juventus

Of the 20 individuals included in the present study, femoral bone collagen isotope values were previously determined for 14 of them: F126, F127, F206, F207, F211, F212, F215, F235, F247, F249, F286B, F291, F296, and F312 (Semchuk, 2016). The bone collagen values obtained from femora represent signals from later in life (i.e., 11 – 25 years up until the age-at-death) relative to dentine (M1 dentine formation initiates at birth and completes by 9y – 10y), allowing for a comparison of dietary patterns at different stages of life

(Hedges et al., 2007; AlQahtani et al., 2010; Hillson, 2014). The temporal gap between the two values, especially in older adults such as F206 and F235 aged ~50y, does not represent diet across the entire lifespan, since the signals representing the intermediate years of the individual's lifespan are not present. Nevertheless, this comparison can produce an avenue to better understand the observed consistencies or changes in one's diet with progressing age more broadly.

The dentine stable isotope values representing diet in early life collected from this study appear to differ only slightly from dietary signals in adults collected from Semchuk's (2016) femoral bone collagen study (see Tables 6.4 and 6.10). Since the consumption of higher trophic-level sources increases both $\delta^{15}N$ and $\delta^{13}C$ signals (Schoeninger et al., 1983), the small offset between some early life $\delta^{15}N$ signals (obtained from dentine) and adult dietary signals (from femoral bone collagen) may be attributed to the changes in consumption of these nitrogen-enriched foods later in life.

There was also some observed variation in $\delta^{13}C$ signals throughout life in this sample. This can indicate either: (1) A changing dependence on C_4-based plant materials with increasing age (e.g., increase in C_4 consumption in F296 from post-weaning to adulthood, Table 6.10); (2) An increase in consumption of higher trophic level foodstuffs with advancing age or (i.e., F291 during the post-weaning period); (3) The contribution of dentine sections which correspond to times of negative nitrogen balance skewed the results to show a smaller degree of nitrogen enrichment than expected.

Considering both $\delta^{15}N$ and $\delta^{13}C$ values together, the most likely scenario is that the increase in higher trophic level foods (e.g., pork) or some marine food consumption in some

adults resulted in the increased nitrogen and carbon signals. These dietary changes with age throughout the life course may be due to an individual's changing dietary preferences, differential access to food sources due to economic reasons, or a change in environment from childhood (where there was greater access to marine sources such as in coastal regions) to adulthood (movement to Vagnari, which is an inland site), likely from the surrounding region (Emery et al., 2018a, 2018b).

There are some outliers, however, who appeared to have lower $\delta^{15}N$ and $\delta^{13}C$ values in their adult femoral collagen relative to the early life post-weaning dentine signals (F126, F286B, F291, F312). An adolescent, F286B (aged 13y – 14y at the time of death), was also unique relative to the rest of the sample. Notably, this individual had the lowest femoral $\delta^{15}N$ value of all of the people in the sample (7.7‰), and was buried earlier (125 – 150 BCE) than the other individuals in this study (shown in Appendix 1). These observations may indicate different dietary preferences over time or may represent a more terrestrial-based 'medicinal' diet for an ill child (Fulminante, 2015).

For six individuals in this sample showing increases in both $\delta^{15}N$ and $\delta^{13}C$ values (Table 6.11), it appears that there is a common driving force behind these elevated signals observed in later in life. These differences suggest that individuals had some access to higher trophic food sources with increasing age such as pork and perhaps small amounts of fish that resulted in an enrichment in both ^{15}N and ^{13}C in their femoral collagen relative to the dentine representing their 'younger selves'. Despite the general pattern of increased trophic level protein consumption with age, some individuals displayed different dietary

trends throughout their life course, demonstrating variable dietary practices at Vagnari (Table 6.11).

Given what is known about the inhabitants of Vagnari and surrounding region, the individuals sampled in this study were likely local, rural, working class Romans (see section 5.1). Garnsey (1999) posited that the lower classes had a primarily plant-based diet, consisting of cereals, legumes, and other accessible ingredients. However, the rural setting likely allowed people from Vagnari to have a more varied diet that included fruits (e.g., apricots) and animal protein from sheep, goat, and pork (Mackinnon, 2011; Trentacoste, 2022), since they were typically estate workers from the surrounding area (Emery et al,. 2018a,b) with the ability to cultivate their own fruits, vegetables, and livestock from personal gardens (Heinrich, 2019; Mackinnon, 2019). People from Vagnari likely also consumed protein from animals such as sheep, goats, and pigs, either through direct meat consumption or the consumption of their milk and cheese (refer to sections 4.4 and 5.1).

Semchuk (2016) analyzed a small sample of faunal remains from the site of Vagnari, including dog, pig, sheep, cow, horse, and unidentified ungulates (hoofed animals, such as deer) (Table 7.2). The $\delta^{15}N$ values of the terrestrial herbivores (i.e., sheep, cow, horse) are low, ranging from 4.0‰ to 4.6‰, and their $\delta^{13}C$ values range from -20.6‰ to -21.1‰, indicating a diet based on C_3 plant material with little to no contribution of C_4

Table 7.2: Faunal bone collagen stable isotope signals from Vagnari. Adapted from Semchuk (2016, p. 78).

Species	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Dog	8.3	-19.6
Pig	7.3	-20.1
Sheep	4.0	-20.6
Cow	4.6	-21.1
Equid	4.3	-20.7
Ungulate 1	7.8	-21.0
Ungulate 2	2.9	-19.6
Ungulate 3	4.0	-21.8

fodders such as millet. The $\delta^{15}N$ values of pigs and dogs are slightly higher (7.3‰ and 8.3‰, respectively), indicating some contribution of terrestrial proteins, which is expected of these omnivorous species who were often fed table scraps (Witcher, 2016). Despite the increased $\delta^{15}N$ observed in pigs, the $\delta^{13}C$ remained similar to herbivores and indicated a diet containing C_3 plant sources. Humans consuming a diet consisting solely of terrestrial herbivore meat or byproducts are expected to have $\delta^{15}N$ values that are ~3‰ higher than the source protein (DeNiro and Epstein, 1981). At Vagnari, this would produce signals ranging from ~7.0‰ – 7.6‰, whereas individuals consuming a diet high in pork would have an expected $\delta^{15}N$ range of ~10.3‰, and even higher if consuming fish or other marine sources.

In the present study, the post-weaning dentine $\delta^{15}N$ values range from 7.8‰ – 9.8‰ (Table 6.10), suggesting that most children typically consumed terrestrial herbivores and their byproducts such as sheep/goat meat, milk, and cheese, with some contribution of higher trophic level protein sources such as pork (and small amounts of aquatic/marine

foods). Some individuals had lower $\delta^{15}N$ values in their post-weaning dentine (F313, $\delta^{15}N$ = 7.8‰) and bone collagen (F286B, $\delta^{15}N$ = 7.7‰) signals, suggesting reliance primarily on terrestrial herbivores. Only one individual, F291, ($\delta^{15}N$ = 10.3‰ at 7.5y) had $\delta^{15}N$ values high enough to indicate a more substantial contribution of higher trophic level foods such as pork and potentially small amounts of marine sources.

Some dentine signals may suggest minor contribution of marine foods to the diet, such as F291 during the post-weaning period, and perhaps F206 and F215 from post-weaning to adulthood. However, none of these increases are more than 1.0‰, suggesting that the contribution of fish and other marine sources was not significant, since high levels of aquatic food consumption generally yield $\delta^{15}N$ values ranging from 17‰ – 20‰ (Schoeninger et al., 1983) (Table 6.10).

Archaeobotanical evidence recovered from Phase 3 (representing occupation during the 2nd c. CE) of the Vagnari vicus indicated the presence of both domestic and wild taxa (Table 5.3) (Stirn and Sgouros, 2022). All of the species, except for goosefoot (n = 1), are C_3 plant species. It is possible that other domestic and wild C_4 plant species such as millet were consumed but not recovered archaeologically, similar to grapes despite the indication of wine production at the vicus (Carroll, 2022). However, the current evidence represented by archaeobotanical finds and isotopic signals recovered from dentine and bone collagen suggest that the diet was based mostly on C_3 plants. It is possible that the observable increase in $\delta^{13}C$ signals in some of the weaning profiles (e.g., F126, F211, and perhaps F323) is attributed to the consumption of animal milk from animals fed on C_4 plants such

as millet. This is unlikely, however, since the existing evidence suggests that there was little contribution of C_4 plants at this site.

7.2.6 Weaning Practices and Grave Good Assemblage

There were some observed negative correlations between the age-at-weaning and grave goods, suggesting that individuals weaned earlier in life had a larger and more diverse grave good collection (Table 6.6). However, further investigation is required to confirm these relationships since the p value for age-at-weaning and total number of grave goods ($p = 0.08$) did not meet the accepted threshold for statistical significance ($p = 0.05$).

It is important to note that some individuals with delayed feeding practices also had impressive grave good collections (e.g., F291, F309, F320, and F336), featuring iron nails, glass fragments, complete ceramic vessels, metal alloy (copper, bronze) fragments/rings, worked stone, a coin, and a shell. Although weaning practices may have reflected aspects of identity in some individuals, the connection between breastfeeding and status cannot be determined from these results. One of the individuals, F336 (Figure 6.3.L), was particularly unusual relative to the rest of the sample. This person was unlike others in their age category, and had an exceptionally late age-at-weaning (5.0y) along with a relatively large grave good collection (n = 7 items). F336, who was only ~10y, belonging to the *pueritia* category (ages 7.0y – 14y) around the time of death, had seven different items found associated with their burial, which is almost double the average number (4.4) found at Vagnari for individuals in this age range (Brent and Prowse, 2014). Although the individual items found associated with F336 were not altogether uncommon (see below), the diverse collection of items is unusual for an individual of this age.

Brent and Prowse (2014) conducted a study to evaluate the grave good distribution at Vagnari. They determined that children aged 0y – 6.0y had an average of 3.3 different items per burial, those aged 7.0y – 14y had 4.4 items per burial, individuals aged 15y – 30y had approximately 6.0 items per burial, and adults older than 30y had an average of 5.6 different items interred with them (Brent and Prowse, 2014). F336 was interred with some typical items such as iron nails (in 44% of all burials), a ceramic lamp (in ~43% of burials), and pottery (in 91% of burials) in also had some higher-value items, such as coins (present in 20% of burials), and unique items such as worked stone items and a shell (Brent and Prowse, 2014). This interesting case warrants further investigation to explore what made this person more 'special' than others at their age, and to better understand the relationship between early life experiences and mortuary treatment.

7.3 Diversity in Breastfeeding and Weaning Practices in the Roman Empire

As mentioned in Chapter 3, only four publications have explored breastfeeding and weaning patterns using incremental dentine analysis of individuals from sites within the empire (Table 3.2) (Dupras and Tocheri, 2007; Cocozza et al., 2021; Ganiatsou et al., 2022, 2023). The studies that explore weaning timelines using incremental dentine on populations within the Empire suggest that exclusive breastfeeding occurred until 0.5y – 1.0y of age (Cocozza et al., 2021; Ganiatsou et al., 2022), and weaning continued until complete cessation, which in some cases did not occur until age 5.0y (Cocozza et al., 2021).

The study by Dupras and Tocheri (2007) on samples from the Dakeleh Oasis, Egypt, estimated the completion of weaning at Kellis 2 around age 3.0y, which is slightly earlier than the findings of the present study (~3.5y). However, there appears to be a higher degree

of C_4 plant contribution in the weaning and post-weaning diets at Kellis relative to the observations from the Vagnari data, perhaps indicating a difference in regional preferences in both the timing of weaning and the foods offered to children during the weaning process. The weanling diet at Kellis was thought to be based primarily on millet gruels and consumption of goat and cow milk (Dupras and Tocheri, 2007). Previous analyses of faunal isotopes from Kellis conducted by Dupras and colleagues (2001) indicate that the goats and cows were fed on millet fodder, reflected in their enriched ^{13}C collagen values, which also increased the values in humans via consumption of goat and cow milk. Despite meeting the requirements for being considered "in the Roman Empire", there is a large geographical distance between Vagnari and the Dakhleh Oasis; this undeniably impacted the respective local weaning patterns due to the differential access to types of complementary foods and/or and the cultural differences between people from southern Italy and central Egypt.

The study by Cocozza and colleagues (2021) on weaning patterns from the rural site of Roman Bainesse, UK, found that weaning began around 0.5y of age, but the age when weaning was complete was variable (ranging from 2.0y – 5.0y). Faunal isotope values from a previous study (Chenery et al., 2011) on diet at Bainesse suggested a diet based primarily on C_3 plants, pork, and some freshwater fish, which appears to be consistent with the reported isotope values from Cocozza and colleagues' (2021) findings. There was an observed increase in $\delta^{15}N$ after age 7.0y, which suggests an increase in animal protein consumption as children aged out of the *infantia* age category.

Age-related differences in animal product consumption are present in some older individuals at Vagnari relative to their subadult signals, and individual F291 even shows

an increase in animal protein (perhaps marine) consumption with increasing post-weaning age (Figure 6.3.K). The results from this study are similar not only in context to Vagnari (i.e., they are rural commercial sites) but also in isotopic signals corresponding to C_3 plant and terrestrial protein consumption. This is the only other study examining incremental dentine data from a rural Roman population (albeit in Britain) associated with a commercial production center. Despite the physical distance between Bainesse and Vagnari, the shared traits of these two sites may indicate some commonalities in the relative access to knowledge and application of written recommendations. However, in contrast to the majority of people from Vagnari, the individuals interred at Bainesse were not buried with grave goods; this difference in funerary practice may indicate a difference in cultural practice (or social status) between Bainesse and Vagnari, which may have extended into breastfeeding and weaning practices.

Incremental dentine data on breastfeeding and weaning patterns in Imperial Roman Thessaloniki, Greece (168BCE – 324CE), indicated that weaning started between 0.5 and 1.0y, with weaning complete by 2.0y (Ganiatsou et al., 2022). The study conducted by Ganiatsou and colleagues (2022) is one of few to report an exclusive breastfeeding signal, providing crucial information about the beginning of this dietary transition (Ganiatsou et al., 2022). There are some similarities between the results from this study and the dentine signals collected from Vagnari despite some considerable differences. Although $\delta^{13}C$ values generally decrease with age and show variability in both sample sets, the individuals from Thessaloniki appear to have been weaned noticeably earlier (~1.5y earlier) than individuals from Vagnari. In a subsequent study, Ganiatsou and colleagues (2023)

determined that there was no significant difference in the weaning age between males and females, and that with the exception of one individual (weaned at ~4.0y), the typical age-at-weaning was between 2.0y – 3.0y. These ages are slightly later than the previous study suggested (Ganiatsou et al., 2022), but are closer to the estimated age-at-weaning at Vagnari (~3.5y).

Salahuddin and Prowse (2023) conducted a multi-tissue analysis (using deciduous and permanent dentine and rib and femoral collagen) of subadult and adult individuals from two Iron Age (4th to 7th c. BCE) sites, Botromagno and Parco San Stefano, located ~15km from Vagnari. In subadult deciduous dentine, they determined that on average, weaning began around 5 months of age and ended at ~1.0y – 2.0y. In survivors, however, weaning ended slightly later, at ~2.0y, leading them to propose that survivors had longer weaning patterns and were weaned later relative to non-survivors (Salahuddin and Prowse, 2023). Salahuddin and Prowse (2023) posit that prolonged consumption of breastmilk may have led to increased chances of survival during this crucial period. This same pattern was observed at Vagnari, which is dated to several centuries later (1st – 3rd c. CE). Based on these findings, it appears as though prolonged weaning practices in this region were practiced from the Iron Age to the Roman era, perhaps due to tradition or the noticed health benefits or the perceived improved survivorship in later-weaned children.

7.4 Limitations of Incremental Dentine Analysis

7.4.1 Age Calibration

Since nitrogen and carbon signals could not be obtained for dentine sections corresponding to ages younger than 1.2y, the beginning of the weaning schedule cannot be determined.

These younger age categories are missing due to dental attrition present in older individuals (e.g., F206) and post-mortem enamel/dentine loss. Other samples in this study were teeth from which enamel was previously removed (e.g., F211, F215, F235, F249, F286B), resulting in a loss of early-life dentine material. This issue also impacted the mapping of the dentine lines, since the outermost layers of dentine may have been removed, the degree of which is unknown. In some cases, sections had to be combined to obtain enough collagen for stable isotope analysis (e.g., F206, F286B, F309, F320, F323). This combination of sections increased the average age represented by the dentine section. These factors resulted in a loss of early-life signals and prevented the observation of an exclusive breastfeeding signal as well as the estimation of the early stages of weaning.

7.4.2 Interpreting Diet from Stable Isotope Results

Stable isotope analysis can inform on the dietary patterns of individuals through the interpretations of $\delta^{15}N$ and $\delta^{13}C$ signals obtained from bone and dentine collagen (Katzenberg and Waters-Rist, 2018). However, it is limited in its ability to estimate the relative contribution of specific foods, such as the proportion of different species of wheat or terrestrial herbivores (e.g., pork vs. sheep/goat). Stable isotope signals cannot inform on the quality of the food either; this is a particularly important topic in the context of weaning.

High quality weaning foods are paramount to the healthy transition from breastmilk to a post-weaning diet, since inadequate nutrition leads to impaired growth and development and increased infant morbidity and mortality (Gowland, 2015). Although $\delta^{15}N$ and $\delta^{13}C$ signals can inform on the potential sources of nutrition (i.e., plant-based, terrestrial, marine-based), they cannot be used to determine the preparation style or

nutritional quality of these foods. The correct preparation of foods is essential to obtaining the maximum nutritional benefits (Uvere and Ene-Obong, 2013; Lemmens et al., 2018; Erdkamp and Holleran, 2019). However, since both groups (upper and lower classes) are consuming the same species of grain, there would be no observable difference in the obtained $\delta^{13}C$ signals. This decreased access to quality complementary foods may have impacted weaning practices and outcomes, forcing individuals to rely on breastmilk for longer relative to other sites (Sandberg et al., 2014). Likewise, infants with a poor-quality weanling diet may have experienced an increased mortality rate despite observing the same weaning schedule and consuming the same types of foods as healthy individuals.

It is possible that infants from Vagnari were fed poorer-quality breads and porridges containing high phytate content, necessitating the prolonged consumption of breastmilk (until ~3.5y) due to the poor nutritional yield and bioavailability of available complementary foods in addition to the existing risks associated with commencement of weaning. Mothers of infants weaned at Vagnari may have decided to prolong breastfeeding to compensate for these negative effects and increase chances of survival into adulthood. However, the increased agricultural production of especially free-threshing wheat, which was easier to process and preferable for bread-making (Šoštarić et al., 2015; Witcher, 2016), at Vagnari during the 2nd c. CE (Stirn and Sgouros, 2022) may have allowed locals to have greater access to cereals which had lower phytate content, which would benefit those relying heavily on grain foods (Uvere and Ene-Obong, 2013; Shah et al., 2016; Lemmens et al., 2018). As a result, the relationship between access to nutritional complementary foods and weaning schedules at Vagnari is unclear.

Historical evidence suggests that people living in rural areas like Vagnari incorporated more pulses, fruits, vegetables, and wild plants in their diet (section 4.4) (Evans, 1980; Jashemski and Meyer, 2002; Heinrich and Hansen, 2019; Witcher, 2016). It is likely that the locals living outside the estate would have had access to a wider variety of foods in their own gardens. The consumption of terrestrial herbivores and omnivores such as pork would be distinct isotopically in the $\delta^{15}N$ signals, but it is unlikely that people were consuming only terrestrial sheep/goat or pork, and would likely have consumed a variety of protein sources and animal products, including species not recovered archaeologically. However, the $\delta^{13}C$ signals in collagen would be more isotopically consistent, since all of the aforementioned foods belonged to a category of either C_3 plants or terrestrial proteins.

Despite some observed variation in diet from weaning to post-weaning to adulthood (Table 6.10), it appears as though most people at Vagnari had a broadly consistent diet throughout life that was based primarily on C_3 plants and terrestrial proteins, with most individuals demonstrating a slight increase in higher trophic terrestrial and marine protein consumption in adulthood and others demonstrating a slight decrease indicative of a more terrestrial, plant-based diet later in life (refer to section 6.9).

The indistinguishable isotopic signals pose a problem when examining diet and its connection to health in past peoples. The caveat is amplified when considering the varying nutritional yields obtained from the same foods prepared in different ways (Heinrich, 2019). This inhibits researchers from ascertaining the direct role of specific foods and dietary practices to overall health. Although there is some variation in $\delta^{15}N$ and $\delta^{13}C$ values with age in the Vagnari sample, the values represent the presence of food types as opposed

to specific species. In other words, there is ambiguity in the stable isotope signals, since stable isotope analysis can only identify foods insofar as their broad category, such as terrestrial proteins, rather than the specific species (i.e., goat, sheep, pork).

Although stable isotope analysis using incremental dentine collagen can depict the nuance necessary to explore changes in diet across an individual's lifespan, it cannot provide detail about the relative diversity and quality of the foods consumed. This hinders researchers' ability to ascertain the relationship between diet, disease, and mortality, particularly during the weaning and post-weaning periods of life.

7.5 Conclusion

The use of incremental dentine for stable isotope analysis of breastfeeding and weaning practices circumvents the inherent issues associated with cross-sectional bone collagen studies (using subadult remains of non-survivors) and can provide a more nuanced view of breastfeeding and weaning practices at Vagnari. While the bone collagen data from Semchuk's (2016) study of individuals who died young at Vagnari rendered a single sample-wide weaning signal, it may inadvertently present results for 'unsuccessful' or 'atypical' weaning practices rather than representing weaning in the healthy population, unlike signals obtained from incremental dentine of survivors from the present study.

Incremental dentine analysis creates an additional method for exploring individual early life histories to better understand variation in feeding practices and potential patterns and connections between these choices and later outcomes in life, such as health, age-at-death, and mortuary treatment. The isotopic data obtained from this study indicated that of the individuals with an identifiable weaning pattern, seven individuals were likely weaned

rapidly, while seven other individuals were weaned gradually. Although it is apparent that various weaning patterns were practiced, it appears as though a majority of children continued to consume small amounts of breast milk until ~3.5y, with the exception of a single individual (F336) who was breastfed until 5.0y of age. This slightly earlier than previous bone collagen findings from the site (Semchuk, 2016), which suggests that non-surviving infants were typically breastfed for longer than survivors.

The collagen signals obtained from this study suggested that males may have more frequently been weaned for longer than females, but the statistical analyses on this small sample indicate no significant difference between weaning ages in males and females. The dietary signals also suggested that infants and children were weaned with C_3 plants and varying amounts of terrestrial proteins, namely sheep, goat, or pork, with some limited consumption of fish or marine food sources.

Post-weaning diet was even more variable, with individuals displaying increases and decreases in both $\delta^{15}N$ and $\delta^{13}C$ with age, suggesting varying contributions of plant and animal consumption throughout the Life Course. Increases in $\delta^{15}N$ were most frequently reported (n = 6, Table 6.11), suggesting an increase in the consumption of higher-trophic food sources such as pork with increasing age. Fewer individuals (n = 4) were found to have decreasing $\delta^{15}N$ values with age, suggesting greater consumption of terrestrial herbivores. Delta^{13}C, with the exception of one individual (F127) increased with age in all individuals. In the individuals with increasing $\delta^{15}N$ values, this would suggest increased consumption of higher-trophic level foods, but in cases where $\delta^{15}N$ remains stable or decreases, changes in $\delta^{13}C$ may be attributable to the varying consumption of C_3

and C_4 plants with age. However, despite changing $\delta^{13}C$ values, there is no strong evidence to suggest consumption of C_4 plants at Vagnari.

The individuals interred at Vagnari follow a weaning pattern that is slightly later than most of the studies explored in this chapter, but is similar to weaning patterns found at rural, provincial sites (e.g., Cocozza et al., 2021). These findings are consistent with the argument that delayed weaning (as recommended by Galen) was more popular and commonly practiced in the Roman provinces, whereas earlier weaning occurred in more often urban and suburban settings (Fulminante, 2015). Individuals from Vagnari were weaned in one of two ways (early/rapid or gradual/delayed), each of which were similar to the timelines recommended by Soranus and Galen, but all of the weaning signals demonstrated some contribution of breastmilk up to ~3.5y of age.

Similar to other sites in the region, there is not a significant sex-based difference in weaning practices, but there may be a relationship between age-at-weaning and age-at-death, and age-at-weaning and grave good diversity. Despite the listed limitations to this method, it is clear that incremental dentine analysis has provided a wealth of information on individual weaning histories and avenues for future research. Advancement of microsampling techniques, which reduces the volume of collagen required for stable isotope analysis, would also increase the amount of available early-life data since the combination of adjacent sections increases the age range represented by the signal (e.g., Czermak et al., 2020). This study contributes to a growing body of work which indicates a wide variety in breastfeeding and weaning practices that depend on many biological, socio-economic, and cultural factors.

8. CONCLUSION

8.1 Summary of Findings

The benefit of using dental tissues for early life dietary history is two-fold: First, dentine forms at known rates that are relatively consistent among all populations and does not remodel after initial formation, which allows researchers to assign a narrower, more specific age to the signal obtained from the dentine section (AlQahtani et al., 2010; Hillson, 2014; Nanci, 2018). Second, analysis of permanent molars can occur in adults, as these teeth are often retained throughout one's life. Since the analysis is conducted on people who lived past the weaning period (i.e., survivors), the data obtained excludes individuals who may have died as a result of weaning practices and early life diet.

The objective of this study was to explore early life dietary history of rural Roman infants and children, and to address three main questions regarding the age-at-weaning completion, weaning patterns/rates, and post-weaning dietary signals throughout the life course (full questions are listed in section 1.1). The stable isotope results from the incremental dentine sections of permanent first molars at Vagnari indicated that on average, children at Vagnari completed weaning around 3.5y, with some observable (but not statistically significant) differences in weaning age between the sexes. Although there appeared to be a relatively similar weaning age among the individuals sampled, there were notable differences in the weaning patterns and rates. Some individuals appeared to have been weaned in a compressed time frame (i.e., >50% of the expected 2‰ – 3‰ trophic level drop in $\delta^{15}N$ associated with weaning prior to 2.5y, with some breast milk remaining

in the diet until ~3.5y), while others demonstrated a more gradual weaning process with a steady decline in $\delta^{15}N$ until complete cessation of breastfeeding.

There appeared to be some differences in weaning practices between the sexes where males were breastfed for longer, although the small sample size hindered the ability to assess true statistical significance. However, despite the differences in the timing of weaning, the $\delta^{15}N$ and $\delta^{13}C$ signals between males and females appeared consistent. The longitudinal analysis of diet in the individuals revealed a variety of dietary patterns from infancy to adulthood as demonstrated by the $\delta^{15}N$ and $\delta^{13}C$ signals representing diet during weaning, the post-weaning childhood period, and adult signals (from Semchuk (2016), n = 14). Interestingly, there was a slight negative correlation (-0.2) between weaning age and grave good assemblage, suggesting that individuals weaned later in life had fewer grave goods, but this was not statistically significant.

The findings from this thesis contribute to a growing body of literature on early life dietary patterns, and is unique in its methodological approach. The protocol for this study was designed to aid in the analysis of teeth that did not have visually identifiable landmarks (i.e., dentine growth lines) and was successful in assigning an age category to a dentine section without the use of thin-section microscopy or other direct visual aids. In addition to the novel procedure, this study is the first to explore the breastfeeding and weaning practices of rural Roman infants and children in southern Italy.

8.2 Future Directions

Recent efforts in breastfeeding and weaning analysis have involved computer programs to aid in the estimation of weaning patterns, such as WARN (Tsutaya, 2020) and WEAN

(Ganiatsou et al., 2023). Both these programs use forms of regression equations which account for the overlap in different age categories represented by the dentine signal obtained from a transverse section. These programs calculate the respective contributions of all age categories represented by the transverse section to estimate the correct age corresponding to a certain dentine section. Tsutaya's (2020) WARN model appears to show substantially younger ages when compared to transverse sections representing ages >3.0y; this may indicate the large margin of error associated with transverse sections corresponding to an age which was estimated by taking an average of ages, since these do not account for the obliqueness of the growth lines present in dentine. Alternatively, the model may not be as accurate when used on this portion of the tooth. This program was designed to calculate the weaning age based on isotopic data of transverse tooth sections, and since this thesis employed a method that sectioned teeth using oblique cut lines, the WARN model was not used on the results.

In the case of Ganiatsou and colleagues' (2023) WEAN program, the model requires very early-life signals (earlier than 1.0y) and teeth with at least five usable signals in order to best estimate the age-at-weaning, and would not have been accurate for the samples obtained from the present study. When these requirements are not met, the model cannot correctly calculate the weaning age and shows obvious inaccuracy in the estimated age-at-weaning. This is particularly problematic in cases where early-life data is missing or in cases of poor preservation of material, such as the remains recovered from Vagnari. The sectioning method employed in this thesis was designed to accommodate the nature of dentine deposition, and aims to minimize the amount of overlap in age categories.

However, the irregular shape of most teeth and the diagenetic alterations of these samples in particular prevented visually-assisted sampling of dentine sections (D'Ortenzio et al., 2019).

Further developments in these computer models may allow for the analysis of breastfeeding and weaning using fewer samples representing later age categories may allow for future analysis using the Vagnari teeth, but presently the limitations of the technology render them unusable for this sample set. Broadly speaking, future investigations of weaning at Vagnari and in rural sites in Roman Italy can build on the research conducted in this study by assessing more survivors to gain a better understanding of weaning patterns in the healthy population and examine potential sex-based differences in weaning ages and practices. Additionally, the link between early life dietary history and mortuary treatment (reflected in the quantity and diversity of grave goods) can be explored further to better understand this relationship.

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APPENDIX 1:
DEMOGRAPHIC, STABLE ISOTOPE, AND MORTUARY CHARACTERISTICS OF SAMPLED INDIVIDUALS

All available data on the individuals studies. Bone collagen data provided by Semchuk (2016). Pink: Samples were excluded due to diagenetic alteration. Yellow = Samples were lost during repackaging and shipment. * = The grave good was recovered from this grave but is thought to be associated with another feature. Section codes with a dash (e.g., 126.4-5) indicates that adjacent sections were combined to obtain sufficient collagen for analysis.

Feature No.	Burial Type	Age	Sex	Grave Goods Recovered	Tooth Sampled	Section Code	Section Age	$\delta^{15}N$ (‰) (AIR)	$\delta^{13}C$ (‰) (V-PDB)	Section Collagen Weight (mg)	C:N Ratio	%C	%N	Total Dentine Weight (mg)	Total Collagen Yield (%)	Bone Collagen $\delta^{15}N$ (‰) (AIR)	Bone Collagen $\delta^{13}C$ (‰) (V-PDB)
F126	Unknown	20y - 25y	M	Iron nail	LM_1	126.1	1.7	10.2	-20.3	3.7	3.56	42.1	13.8	447.0	6.9	9.1	-19.5
				Bronze tube		126.2	2.5	10.2	-19.5	8.8	3.36	43.2	15.0				
				Fragments of pot		126.3	3.5	9.9	-19.2	11.1	3.34	43.5	15.2				
				Fragment of jar rim		126.4-5	4.5	9.3	-20.6	2.4	3.79	45.9	14.1				
						126.6-8	7.5	9.3	-19.8	4.9	3.55	28.5	9.4				
F127	2nd Century CE	15y - 20y	F	Lamp 'a perline'	LM^1	127.1	1.2	12.3	-21.3	4.1	4.07	41.7	11.9	541.3	4.1	9.8	-18.4
						127.2	1.5	12.0	-20.1	1.5	3.67	43.6	13.9				
						127.3	3.5	-	-	3.5	-	-	-				
						127.4	4.5	9.1	-20.1	4.7	3.45	43.0	14.6				
						127.5-6	5.5	8.0	-22.6	4.2	4.56	33.1	8.5				
						127.7-8	8.0	8.7	-20.1	4.1	3.57	19.2	6.3				
F206	First Half of 2nd Century CE	Older Adult (50y+)	F	Stone flake (lithic?)	LM_1	206.1-2	2.1	8.7	-19.7	8.7	3.40	26.6	9.1	698.2	7.9	9.3	-19.4
				Pot fragment		206.3	3.5	8.3	-20.7	5.0	4.00	28.8	8.4				
				Glass fragment*		206.4	4.0	8.1	-19.9	5.4	3.41	26.5	9.1				
				Shallow bowl/plate		206.5	4.5	8.2	-20.1	5.7	3.47	49.4	16.6				
				Iron nail		206.6	6.5	8.7	-19.6	9.8	3.44	41.8	14.2				
				Nail fragment		206.7	7.5	-	-	10.5	-	-	-				
						206.8	8.5	9.3	-20.7	10.4	3.78	18.1	5.6				
F207	Unknown	Young Adult	M	Pot fragment (50+)	LM_1	207.1	1.7	11.4	-19.4	5.5	3.35	49.1	17.1	471.8	7.2	9.6	-19.9
				Rim fragments		207.2	2.5	10.6	-19.5	5.3	3.34	32.1	11.2				
				Pot sherd		207.3	3.5	9.8	-19.8	3.5	3.39	40.1	13.8				
				Metal		207.4	4.0	-	-	4.2	-	-	-				
						207.5	4.5	9.3	-19.6	6.5	3.28	36.1	12.8				
						207.6	6.5	9.5	-19.9	8.0	3.37	56.0	19.4				
						207.7-8	8.0	9.5	-19.9	4.0	3.51	31.9	10.6				
F211	Unknown	Young Adult (<30y)	F	Pot fragment	RM_1	211.1	1.2	10.7	-23.1	1.2	3.40	23.0	7.9	422.2	7.9	9.3	-19.4
				Handle w/ grooves		211.2	1.5	10.4	-19.8	1.5	3.54	44.5	14.6				
				Rim fragment		211.3	3.5	8.6	-19.6	3.5	3.41	15.2	5.2				
				Amphora fragment (x3)		211.4	4.5	8.6	-19.5	4.5	3.35	53.1	18.5				
						211.5	5.5	8.5	-19.4	5.5	3.34	52.1	18.2				
						211.6-7	7.0	8.7	-19.8	9.4	3.47	35.0	11.8				
						211.8	8.5	-	-	4.4	-	-	-				
F212	Unknown	Adult	U	Rim/wall fragments (x3)	RM_1	212.1	1.7	12.1	-19.3	3.4	3.40	44.0	15.1	583.2	6.7	N/A	N/A
				Complete jug w/ handle		212.2	2.5	10.8	-19.8	7.2	3.48	46.0	15.4				
				Iron nail		212.3	3.5	10.2	-19.7	9.3	3.44	45.4	15.4				
						212.4	4.0	8.9	-19.7	5.4	3.36	41.4	14.4				
						212.5	4.5	9.1	-19.8	5.3	3.41	55.6	19.0				
						212.6	6.5	8.7	-20.2	2.7	3.61	45.1	14.6				
						212.7	7.5	8.7	-21.4	5.7	4.10	49.6	14.1				
F215	2nd Century CE (?)	38.2y ± 10.9y	F	Dish	LM_1	215.1	1.7	12.3	-19.1	6.3	3.35	33.0	11.5	509.2	6.0	9.6	-19.9
				Rim of jar		215.2	2.5	9.1	-19.2	3.0	3.34	39.6	13.8				
				Rim/wall sherd		215.3	3.5	9.0	-19.7	4.4	3.42	47.2	16.1				
				Base of closed vessel (x2)		215.4	4.0	8.2	-19.6	4.9	3.36	43.8	15.2				
				Pot sherds/handles		215.5-6	5.5	8.2	-19.6	4.4	3.29	45.4	16.1				
				Hobnails (34)		215.7-8	8.0	8.4	-20.0	7.5	3.44	37.5	12.7				
				Lamp 'a perline'													
				Glass fragment													

Feature No.	Date	Age	Sex	Grave Goods Recovered	Tooth Sampled	Section Code	Section Age	$\delta^{15}N$ (‰) (AIR)	$\delta^{13}C$ (‰) (V-PDB)	Section Collagen Weight (mg)	C:N Ratio	%C	%N	Total Dentine Weight (mg)	Total Collagen Yield (%)	Bone Collagen $\delta^{15}N$ (‰) (AIR)	Bone Collagen $\delta^{13}C$ (‰) (V-PDB)
F235	Unknown	~50y ± 12.6y	M	Part of globular jar	RM[1]	235.1	1.2	8.6	-19.6	11.4	3.45	54.2	18.3	747.7	8.8	9.1	-19.6
				Iron nail		235.2	1.5	8.3	-19.6	7.9	3.46	48.3	16.3				
						235.3	3.5	7.9	-19.9	11.0	3.52	37.8	12.5				
						235.4	4.5	8.1	-19.5	10.9	3.32	33.1	11.6				
						235.5	5.5	8.5	-19.9	12.8	3.44	42.0	14.2				
						235.6	6.5	8.6	-20.8	5.0	3.75	35.7	11.1				
						235.7	7.5	9.1	-20.8	4.7	3.76	32.0	9.9				
						235.8	8.5	9.6	-20.6	1.9	3.61	36.5	11.8				
F247	1st - 2nd Century CE	Adult	M	2 wall sherds; amphora?	RM[1]	247.1	1.2	9.3	-20.9	1.2	3.68	47.9	15.2	308.1	8.2	9.8	-19.3
				3 wall sherds		247.2	1.5	9.3	-19.9	1.5	3.41	41.6	14.2				
				Amphora fragment		247.3	3.5	9.1	-20.1	3.5	3.57	30.3	9.9				
				Animal tooth		247.4	4.5	9.5	-20.0	4.5	3.45	52.1	17.6				
						247.5	5.5	9.4	-21.2	5.5	3.96	47.3	13.9				
						247.6	6.5	8.7	-23.2	6.5	5.00	52.5	12.3				
						247.7-8	8.0	9.9	-20.7	2.7	3.77	40.0	12.4				
F249	Unknown	Adult	M?	Bone pin fragments	LM[1]	249.1	1.2	11.7	-19.2	1.2	3.36	36.1	12.5	244.5	3.8	9.7	-19.0
				Small pot w/handle sherds		249.2	1.5	10.5	-20.0	1.5	3.54	49.5	16.3				
				Silver ring; thin		249.3-5	4.5	7.2	-25.2	2.5	7.36	55.7	8.8				
				Small gold coil w/ head		249.6-7	7.0	9.8	-20.0	2.1	3.63	34.6	11.1				
F286B	125-150 BCE	13y - 14y	U	Pot fragment	RM[1]	286.1-2	1.4	11.0	-20.1	5.3	3.48	36.0	12.1	508.6	5.1	7.7	-19.5
				Amphora neck		286.3	3.5	9.0	-20.3	6.8	3.40	45.8	15.7				
						286.4	4.5	7.5	-21.9	2.9	3.91	43.4	13.0				
						286.5-6	6.0	7.9	-21.4	3.9	3.73	42.6	13.3				
						286.7	7.5	8.5	-21.0	7.5	3.65	47.0	15.0				
						286.8	8.5	8.3	-21.8	8.5	4.05	36.3	10.5				
F291	Unknown	Adult	M?	One-handled cup	RM[1]	291.1	1.2	11.2	-20.0	2.7	3.71	20.8	6.5	610.2	6.9	9.1	-19.5
				Copper alloy ring		291.2	1.5	10.2	-19.9	3.1	3.42	49.3	16.8				
				Hobnails		291.3	3.5	8.9	-20.2	3.1	3.49	38.7	12.9				
				Ceramic fragment		291.4	4.5	8.6	-20.0	8.3	3.45	44.7	15.1				
				Iron nail		291.5	5.5	8.9	-19.7	7.7	3.33	43.7	15.3				
						291.6	6.5	9.6	-19.8	5.6	3.39	42.7	14.7				
						291.7	7.5	10.3	-19.5	6.6	3.32	27.5	9.7				
						291.8	8.5	10.1	-20.5	5.2	3.62	47.2	15.2				
F296	Unknown	25y - 29y	F	Bone pin fragments	RM[1]	296.1-2	1.4	9.7	-20.6	3.5	3.69	41.2	13.0	292.9	7.2	8.2	-19.4
				Ceramic fragments		296.3	3.5	8.5	-19.7	2.6	3.34	39.6	13.8				
				Glass fragments		296.4	4.5	8.2	-19.8	4.8	3.36	32.3	11.2				
				Ceramic vessels (x3)		296.5	5.5	7.6	-20.9	2.8	3.74	52.3	16.3				
				Hobnails		296.6	6.5	7.6	-20.6	3.6	3.63	41.0	13.2				
				Small looped silver		296.7-8	8.0	8.2	-20.1	3.8	3.42	36.5	12.5				
				Chickpea seeds													
F308A	Unknown	Adult	F?	Red slip bowl	RM[1]	308.1-2	1.4	9.6	-22.6	2.8	4.61	40.5	10.3	240.7	4.7	N/A	N/A
				Orange-red vessel		308.3	3.5	9.2	-20.2	3.4	3.55	43.9	14.4				
				Coarse sandy wear fragments		308.4	4.5	7.4	-20.8	2.3	3.74	40.5	12.6				
				Red/brown/orange fragments		308.5-8	7.0	7.7	-21.4	2.7	3.94	42.8	12.7				

Feature No.	Date	Age	Sex	Grave Goods Recovered	Tooth Sampled	Section Code	Section Age	$\delta^{15}N$ (‰) (AIR)	$\delta^{13}C$ (‰) (V-PDB)	Section Collagen Weight (mg)	C:N Ratio	%C	%N	Total Dentine Weight (mg)	Total Collagen Yield (%)	Bone Collagen $\delta^{15}N$ (‰) (AIR)	Bone Collagen $\delta^{13}C$ (‰) (V-PDB)
F309	Late 1st - Early 2nd Century CE	Young Adult	M	Amphora fragment	LM[1]	309.1-2	1.4	13.6	-19.1	2.0	3.42	44.6	15.2	279.6	3.9	N/A	N/A
				Ceramic fragment		309.3	3.5	11.6	-19.7	1.9	3.45	35.1	11.9				
				Glass fragments		309.4	4.5	9.8	-20.1	1.9	3.45	48.5	16.4				
				Iron blade		309.5	5.5	9.4	-20.5	2.6	3.62	37.4	12.0				
				Ceramic bowl (2)		309.6-8	7.5	8.1	-21.5	2.6	4.39	7.1	1.9				
				Bronze alloy ring (2)													
				Iron 'punch'													
F312	Unknown	Young Adult	M	N/A	RM[1]	312.1	1.2	10.9	-19.9	1.2	3.48	31.4	10.5	355.8	8.5	8.3	-19.5
						312.2	1.5	9.1	-23.4	1.5	4.91	34.6	8.2				
						312.3	3.5	8.1	-21.2	3.5	3.72	41.3	12.9				
						312.4	4.5	8.9	-20.3	4.5	3.35	44.3	15.4				
						312.5	5.5	8.2	-21.1	5.5	3.67	31.5	10.0				
						312.6	6.5	-	-	6.5	-	-	-				
						312.7	7.5	8.8	-20.0	7.5	3.28	58.7	20.9				
						312.8	8.5	8.8	-20.1	8.5	3.35	41.4	14.4				
F313	2nd Century CE	19y - 23y	F	Sandy orange vessel	RM[1]	313.1	1.2	9.4	-19.2	6.2	3.31	46.3	16.3	830.5	6.7	N/A	N/A
				Red slip bowl		313.2	1.5	8.1	-19.9	7.2	3.37	45.7	15.8				
				Bent iron nail		313.3	3.5	7.9	-20.1	8.4	3.36	45.0	15.6				
				Lamp 'a perline'		313.4	4.5	7.7	-19.9	10.4	3.42	35.1	12.0				
						313.5	5.0	7.8	-20.0	5.8	3.46	44.5	15.0				
						313.6	6.5	7.6	-19.9	5.8	3.42	40.7	13.9				
						313.7	7.5	7.9	-20.0	7.2	3.45	36.1	12.2				
						313.8	8.5	8.7	-20.7	4.4	3.64	44.2	14.2				
F320	2nd Century CE	38.2y ± 10.9y	F	Glass fragment	RM_1	320.1-2	2.1	11.1	-19.9	3.5	3.46	40.1	13.5	915.4	1.9	N/A	N/A
				Coarse wear jug (1 handle)		320.3	3.5	9.1	-19.8	2.1	3.35	32.1	11.2				
				Metal alloy piece (jewelry?)		320.4-6	5.0	8.6	-21.8	8.7	4.12	37.9	10.7				
				Lamp 'a perline'		320.7-8	8.0	7.6	-21.3	3.2	3.88	29.6	8.9				
				Orange-red bowl													
				Red-orange sherd													
				Iron nail													
F323	2nd Century CE	45.2y ± 12.6y	F	Bronze alloy pot (1 handle)	LM_1	323.1-2	2.1	9.8	-20.4	4.0	3.56	31.0	10.2	752.8	8.3	N/A	N/A
				Ceramic bowl		323.3	3.5	9.4	-20.3	6.3	3.50	46.5	15.5				
				Ceramic fragment		323.4	4.0	9.2	-19.5	10.3	3.30	38.7	13.7				
				Hobnails		323.5	4.5	8.9	-20.0	10.9	3.43	31.3	10.6				
						323.6	6.5	8.5	-20.5	12.5	3.45	45.3	15.3				
						323.7	7.5	9.1	-19.5	13.8	3.32	45.3	15.9				
						323.8	8.5	9.0	-19.8	4.5	3.38	43.5	15.0				
F336	2nd Century CE	10y ± 2y	U	Ceramic vessels (2) & fragments	RM[1]	336.1	1.2	12.6	-18.7	1.4	3.43	43.3	14.7	217.4	4.8	N/A	N/A
				Lamp 'a perline'		336.2	1.5	10.4	-19.1	2.3	3.35	34.7	12.1				
				Iron nail		336.3	3.5	9.3	-19.4	2.6	3.37	34.1	11.8				
				Worked stone with rectangular punched hole		336.4-5	5.0	8.6	-19.9	1.7	3.59	35.2	11.4				
				Bronze coin (M. Aurelius) 2nd C. CE		336.6-8	7.5	8.6	-19.7	2.4	3.56	31.7	10.4				
				Shell (clam?)													

APPENDIX 2:
STABLE ISOTOPE SAMPLE INTEGRITY DATA

Results for the sample integrity indicators for dentine collagen. Samples excluded from the study due to evidence of diagenetic alteration (i.e., C:N ratio is <2.9 or >3.6) are highlighted in pink. Samples lost during the sample preparation process are highlighted in yellow. Sections with inadequate amounts of collagen for mass spectrometry (>2mg) were combined, resulting in section codes with a dash and an estimated age that is at the midpoint between the age estimates for each contributing section.

Feature No.	Tooth Sampled	Section Code	Section Age	$\delta^{15}N$ (‰) (AIR)	$\delta^{13}C$ (‰) (V-PDB)	Section Collagen Weight (mg)	C:N Ratio	%C	%N	Total Dentine Weight (mg)	Total Collagen Yield (%)
F126	LM_1	126.1	1.7	10.2	-20.3	3.7	3.56	42.1	13.8	447.0	6.9
		126.2	2.5	10.2	-19.5	8.8	3.36	43.2	15.0		
		126.3	3.5	9.9	-19.2	11.1	3.34	43.5	15.2		
		126.4-5	4.5	9.3	-20.6	2.4	3.79	45.9	14.1		
		126.6-8	7.5	9.3	-19.8	4.9	3.55	28.5	9.4		
F127	LM^1	127.1	1.2	12.3	-21.3	4.1	4.07	41.7	11.9	541.3	4.1
		127.2	1.5	12.0	-20.1	1.5	3.67	43.6	13.9		
		127.3	3.5	-	-	3.5	-	-	-		
		127.4	4.5	9.1	-20.1	4.7	3.45	43.0	14.6		
		127.5-6	5.5	8.0	-22.6	4.2	4.56	33.1	8.5		
		127.7-8	8.0	8.7	-20.1	4.1	3.57	19.2	6.3		
F206	LM_1	206.1-2	2.1	8.7	-19.7	8.7	3.40	26.6	9.1	698.2	7.9
		206.3	3.5	8.3	-20.7	5.0	4.00	28.8	8.4		
		206.4	4.0	8.1	-19.9	5.4	3.41	26.5	9.1		
		206.5	4.5	8.2	-20.1	5.7	3.47	49.4	16.6		
		206.6	6.5	8.7	-19.6	9.8	3.44	41.8	14.2		
		206.7	7.5	-	-	10.5	-	-	-		
		206.8	8.5	9.3	-20.7	10.4	3.78	18.1	5.6		
F207	LM_1	207.1	1.7	11.4	-19.4	5.5	3.35	49.1	17.1	471.8	7.2
		207.2	2.5	10.6	-19.5	5.3	3.34	32.1	11.2		
		207.3	3.5	9.8	-19.8	3.5	3.39	40.1	13.8		
		207.4	4.0	-	-	4.2	-	-	-		
		207.5	4.5	9.3	-19.6	6.5	3.28	36.1	12.8		
		207.6	6.5	9.5	-19.9	8.0	3.37	56.0	19.4		
		207.7-8	8.0	9.5	-19.9	4.0	3.51	31.9	10.6		
F211	RM_1	211.1	1.2	10.7	-23.1	1.2	3.40	23.0	7.9	422.2	7.9
		211.2	1.5	10.4	-19.8	1.5	3.54	44.5	14.6		
		211.3	3.5	8.6	-19.6	3.5	3.41	15.2	5.2		
		211.4	4.5	8.6	-19.5	4.5	3.35	53.1	18.5		
		211.5	5.5	8.5	-19.4	5.5	3.34	52.1	18.2		
		211.6-7	7.0	8.7	-19.8	9.4	3.47	35.0	11.8		
		211.8	8.5	-	-	4.4	-	-	-		
F212	RM_1	212.1	1.7	12.1	-19.3	3.4	3.40	44.0	15.1	583.2	6.7
		212.2	2.5	10.8	-19.8	7.2	3.48	46.0	15.4		
		212.3	3.5	10.2	-19.7	9.3	3.44	45.4	15.4		
		212.4	4.0	8.9	-19.7	5.4	3.36	41.4	14.4		
		212.5	4.5	9.1	-19.8	5.3	3.41	55.6	19.0		
		212.6	6.5	8.7	-20.2	2.7	3.61	45.1	14.6		
		212.7	7.5	8.7	-21.4	5.7	4.10	49.6	14.1		

Feature No.	Tooth Sampled	Section Code	Section Age	$\delta^{15}N$ (‰) (AIR)	$\delta^{13}C$ (‰) (V-PDB)	Section Collagen Weight (mg)	C:N Ratio	%C	%N	Total Dentine Weight (mg)	Total Collagen Yield (%)
F215	LM_1	215.1	1.7	12.3	-19.1	6.3	3.35	33.0	11.5	509.2	6.0
		215.2	2.5	9.1	-19.2	3.0	3.34	39.6	13.8		
		215.3	3.5	9.0	-19.7	4.4	3.42	47.2	16.1		
		215.4	4.0	8.2	-19.6	4.9	3.36	43.8	15.2		
		215.5-6	5.5	8.2	-19.6	4.4	3.29	45.4	16.1		
		215.7-8	8.0	8.4	-20.0	7.5	3.44	37.5	12.7		
F235	RM^1	235.1	1.2	8.6	-19.6	11.4	3.45	54.2	18.3	747.7	8.8
		235.2	1.5	8.3	-19.6	7.9	3.46	48.3	16.3		
		235.3	3.5	7.9	-19.9	11.0	3.52	37.8	12.5		
		235.4	4.5	8.1	-19.5	10.9	3.32	33.1	11.6		
		235.5	5.5	8.5	-19.9	12.8	3.44	42.0	14.2		
		235.6	6.5	8.6	-20.8	5.0	3.75	35.7	11.1		
		235.7	7.5	9.1	-20.8	4.7	3.76	32.0	9.9		
		235.8	8.5	9.6	-20.6	1.9	3.61	36.5	11.8		
F247	RM^1	247.1	1.2	9.3	-20.9	1.2	3.68	47.9	15.2	308.1	8.2
		247.2	1.5	9.3	-19.9	1.5	3.41	41.6	14.2		
		247.3	3.5	9.1	-20.1	3.5	3.57	30.3	9.9		
		247.4	4.5	9.5	-20.0	4.5	3.45	52.1	17.6		
		247.5	5.5	9.4	-21.2	5.5	3.96	47.3	13.9		
		247.6	6.5	8.7	-23.2	6.5	5.00	52.5	12.3		
		247.7-8	8.0	9.9	-20.7	2.7	3.77	40.0	12.4		
F249	LM^1	249.1	1.2	11.7	-19.2	1.2	3.36	36.1	12.5	244.5	3.8
		249.2	1.5	10.5	-20.0	1.5	3.54	49.5	16.3		
		249.3-5	4.5	7.2	-25.2	2.5	7.36	55.7	8.8		
		249.6-7	7.0	9.8	-20.0	2.1	3.63	34.6	11.1		
F286B	RM^1	286.1-2	1.4	11.0	-20.1	5.3	3.48	36.0	12.1	508.6	5.1
		286.3	3.5	9.0	-20.3	6.8	3.40	45.8	15.7		
		286.4	4.5	7.5	-21.9	2.9	3.91	43.4	13.0		
		286.5-6	6.0	7.9	-21.4	3.9	3.73	42.6	13.3		
		286.7	7.5	8.5	-21.0	7.5	3.65	47.0	15.0		
		286.8	8.5	8.3	-21.8	8.5	4.05	36.3	10.5		
F291	RM^1	291.1	1.2	11.2	-20.0	2.7	3.71	20.8	6.5	610.2	6.9
		291.2	1.5	10.2	-19.9	3.1	3.42	49.3	16.8		
		291.3	3.5	8.9	-20.2	3.1	3.49	38.7	12.9		
		291.4	4.5	8.6	-20.0	8.3	3.45	44.7	15.1		
		291.5	5.5	8.9	-19.7	7.7	3.33	43.7	15.3		
		291.6	6.5	9.6	-19.8	5.6	3.39	42.7	14.7		
		291.7	7.5	10.3	-19.5	6.6	3.32	27.5	9.7		
		291.8	8.5	10.1	-20.5	5.2	3.62	47.2	15.2		
F296	RM^1	296.1-2	1.4	9.7	-20.6	3.5	3.69	41.2	13.0	292.9	7.2
		296.3	3.5	8.5	-19.7	2.6	3.34	39.6	13.8		
		296.4	4.5	8.2	-19.8	4.8	3.36	32.3	11.2		
		296.5	5.5	7.6	-20.9	2.8	3.74	52.3	16.3		
		296.6	6.5	7.6	-20.6	3.6	3.63	41.0	13.2		
		296.7-8	8.0	8.2	-20.1	3.8	3.42	36.5	12.5		

Feature No.	Tooth Sampled	Section Code	Section Age	$\delta^{15}N$ (‰) (AIR)	$\delta^{13}C$ (‰) (V-PDB)	Section Collagen Weight (mg)	C:N Ratio	%C	%N	Total Dentine Weight (mg)	Total Collagen Yield (%)
F308A	RM[1]	308.1-2	1.4	9.6	-22.6	2.8	4.61	40.5	10.3	240.7	4.7
		308.3	3.5	9.2	-20.2	3.4	3.55	43.9	14.4		
		308.4	4.5	7.4	-20.8	2.3	3.74	40.5	12.6		
		308.5-8	7.0	7.7	-21.4	2.7	3.94	42.8	12.7		
F309	LM[1]	309.1-2	1.4	13.6	-19.1	2.0	3.42	44.6	15.2	279.6	3.9
		309.3	3.5	11.6	-19.7	1.9	3.45	35.1	11.9		
		309.4	4.5	9.8	-20.1	1.9	3.45	48.5	16.4		
		309.5	5.5	9.4	-20.5	2.6	3.62	37.4	12.0		
		309.6-8	7.5	8.1	-21.5	2.6	4.39	7.1	1.9		
F312	RM[1]	312.1	1.2	10.9	-19.9	1.2	3.48	31.4	10.5	355.8	8.5
		312.2	1.5	9.1	-23.4	1.5	4.91	34.6	8.2		
		312.3	3.5	8.1	-21.2	3.5	3.72	41.3	12.9		
		312.4	4.5	8.9	-20.3	4.5	3.35	44.3	15.4		
		312.5	5.5	8.2	-21.1	5.5	3.67	31.5	10.0		
		312.6	6.5	-	-	6.5	-	-	-		
		312.7	7.5	8.8	-20.0	7.5	3.28	58.7	20.9		
		312.8	8.5	8.8	-20.1	8.5	3.35	41.4	14.4		
F313	RM[1]	313.1	1.2	9.4	-19.2	6.2	3.31	46.3	16.3	830.5	6.7
		313.2	1.5	8.1	-19.9	7.2	3.37	45.7	15.8		
		313.3	3.5	7.9	-20.1	8.4	3.36	45.0	15.6		
		313.4	4.5	7.7	-19.9	10.4	3.42	35.1	12.0		
		313.5	5.0	7.8	-20.0	5.8	3.46	44.5	15.0		
		313.6	6.5	7.6	-19.9	5.8	3.42	40.7	13.9		
		313.7	7.5	7.9	-20.0	7.2	3.45	36.1	12.2		
		313.8	8.5	8.7	-20.7	4.4	3.64	44.2	14.2		
F320	RM_1	320.1-2	2.1	11.1	-19.9	3.5	3.46	40.1	13.5	915.4	1.9
		320.3	3.5	9.1	-19.8	2.1	3.35	32.1	11.2		
		320.4-6	5.0	8.6	-21.8	8.7	4.12	37.9	10.7		
		320.7-8	8.0	7.6	-21.3	3.2	3.88	29.6	8.9		
F323	LM_1	323.1-2	2.1	9.8	-20.4	4.0	3.56	31.0	10.2	752.8	8.3
		323.3	3.5	9.4	-20.3	6.3	3.50	46.5	15.5		
		323.4	4.0	9.2	-19.5	10.3	3.30	38.7	13.7		
		323.5	4.5	8.9	-20.0	10.9	3.43	31.3	10.6		
		323.6	6.5	8.5	-20.5	12.5	3.45	45.3	15.3		
		323.7	7.5	9.1	-19.5	13.8	3.32	45.3	15.9		
		323.8	8.5	9.0	-19.8	4.5	3.38	43.5	15.0		
F336	RM[1]	336.1	1.2	12.6	-18.7	1.4	3.43	43.3	14.7	217.4	4.8
		336.2	1.5	10.4	-19.1	2.3	3.35	34.7	12.1		
		336.3	3.5	9.3	-19.4	2.6	3.37	34.1	11.8		
		336.4-5	5.0	8.6	-19.9	1.7	3.59	35.2	11.4		
		336.6-8	7.5	8.6	-19.7	2.4	3.56	31.7	10.4		