Reconstructing Childhood Diet using Dentine Microsamples from Skeletal Remains from Kenchreai and Isthmia, Greece

by

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Abstract

An understudied topic in bioarchaeology is that of childhood diet after weaning. Palaeodietary reconstructions have typically focused on examining breastfeeding and weaning or adult diets. This study uses stable isotope analysis of dentine microsamples to examine the diets of juveniles at the Greek sites of Kenchreai and Isthmia (Late Roman Period). Results from prior research conducted on bone collagen showed elevated $\delta^{13}C$ and $\delta^{15}N$ values in adults at one of these sites, which is suggestive of a significant contribution of marine resources to the diet. However, this elevation is only seen in adults, whereas the values of the juvenile remains suggest that children consumed a different diet that included less fish. The current study seeks to further investigate this dietary difference by conducting stable isotope analysis on dentine collagen. Five premolars from Kenchreai and two premolars from Isthmia were selected for this study and produced a total of fifty-two dentine microsamples. These microsamples provide dietary information for small periods of time within the time frame of premolar formation and completion. Premolars were chosen for this study to target the ages after breastfeeding and weaning at approximately 2.5 years, up until the completion of premolar formation at 14.5 years.

The results of this study agree with the data from the bone collagen analysis, which indicates that children consumed less fish. However, microsampling also allowed for intra-individual dietary trends to be identified. The isotope profiles of three Kenchreai individuals demonstrate a subtle rise in $\delta^{13}C$ and $\delta^{15}N$ values in the microsamples that represent the end of tooth formation. This may reflect a shift to a more adult-like diet that incorporated some marine resources, which is consistent with evidence from the historical record that adult roles and responsibilities began to be adopted around this age.

Preface

This thesis is an original work by Helena D. Ramsaroop. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, project name "Reconstructing Childhood Diet using Dentine Microsamples from Skeletal Remains from Kenchreai and Isthmia, Greece," No. 76773, December 14, 2017.

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Chapter 1: Introduction

Much has been revealed about the rich cultural history of Greece through studies of ancient literature and classical archaeology. However, there are always new things to discover when taking an anthropological approach to research. This can reveal information about the social, cultural and biological aspects of society and the individuals within it. One strength of bioarchaeology is its ability to examine life at an individual level, as well as a population level. Skeletal remains provide insight into the biological wellbeing of people and communities and can inform bioarchaeologists about potential disease or trauma suffered throughout life and the food that was being consumed. A broad topic that is still relatively understudied in bioarchaeology is that of childhood diet. There are numerous bioarchaeological studies that focus on weaning and breastfeeding, which provides insight into the diet and health of infants. On the other end of the spectrum are studies that tell of diets consumed by adults. But what about children who survived past infancy and into older childhood, and even into the teenage years? What was life like for those individuals? Older children and teenagers are underrepresented in palaeodietary studies, in part because until recently there were few methods capable of providing dietary information at a fine enough resolution to address changes over this age range.

Recent developments in stable isotope methodology and technology have allowed for these questions to be addressed more effectively. Theoretically, childhood diet should be easy to investigate isotopically. Teeth develop at known rates and contain structures that form incrementally. These structures are laid down during early development in childhood and complete at known times. Given that the permanent dentition is completely formed within the teenage or early adult years (AlQahtani, Hector, and Liversidge 2010), they contain information from the early years of life. Teeth do not continue to grow or alter themselves after this stage,

and therefore they will always carry this information from childhood. Recent developments in dietary stable isotope studies include dentine microsampling: sectioning dentine into 1mm increments for stable isotope analysis instead of using a bulk dentine or bone sample to reconstruct an individual's diet. The stable carbon and nitrogen isotope values of these incremental sections serve as records of dietary stable isotope values during the process of tooth formation. Thus, taking dentine microsamples of teeth that begin to form after the typical age of weaning can provide dietary information for an individual's childhood after infancy. The benefits of this method include the ability to look at changing diets over an individual's childhood, and the fact that the childhood diet of individuals who survived to adulthood can be examined because teeth do not remodel.

Dentine microsampling can also be used to further examine questions initially raised from stable isotope analysis of bone collagen. A prior bone collagen study of individuals from the Roman-era Greek sites of Kenchreai and Isthmia showed elevated $\delta^{13}C$ and $\delta^{15}N$ values when compared to most other Greek sites, which is suggestive of a greater importance placed on marine foods in the diet (Garvie-Lok 2010). While this is true for the adult remains at the site, isotopic analysis of the few subadult remains analyzed suggested that children and teenagers consumed a different diet that included less fish (Garvie-Lok 2010). The purpose of the current study is to follow up the results from the Kenchreai bone collagen analysis with a microsampling study on the dentine collagen to confirm and further investigate this difference. It also conducts a preliminary analysis of two teeth from nearby Late Roman Isthmia to see whether a full dentine study of that site would be useful.

Chapter 1 presents an overview of the study. Chapter 2 provides insight from historical sources about aspects of life in Roman Greece relevant to the study. A brief timeline of Greek

history in this period is provided and childhood is defined in terms of concepts used in the Greco-Roman period and in bioarchaeological terms. The role of children in society is considered. Finally, a review of available dietary resources in the region and the typical diets of Greeks in this period is presented.

Chapter 3 offers a detailed explanation of the methodology of stable isotope analysis of carbon and nitrogen, and how this allows for the study of ancient diets. An emphasis is placed on identifying marine resource consumption from stable isotope values. The process of dentine microsampling is introduced in this chapter with an explanation of how it advances stable isotope analysis. A brief overview of palaeodietary studies conducted in Greece, focusing on identifying marine resource consumption, is also provided.

Chapter 4 explores the excavation history of the sites of Kenchreai and Isthmia and highlights previous studies conducted at the sites. The individuals studied in the present thesis are introduced. Information on these individuals obtained from previous work is presented, including information from the skeletal analysis such as age and sex, as well as information regarding the burial contexts. The previous isotopic work conducted on bone collagen (Garvie-Lok 2010) is also discussed.

Chapter 5 discusses the process of tooth formation and provides background knowledge that aids in the understanding of the microsampling process. An in-depth discussion of dentine is included. Methods of sample preparation and analysis are outlined in this chapter with an assessment of sample preservation and decay. The discussion of dentine microsampling from Chapter 3 continues here with an explanation of how to estimate the formation age of each microsample of dentine. The chapter concludes with a discussion of the isotopic expectations for the dentine collagen values relative to the previous bone collagen values.

Chapter 6 contains the results of the dentine collagen isotope analysis. Two figures are provided for each individual, one that displays $\delta^{13}C$ values and another for $\delta^{15}N$, both of which are plotted by the age of each dentine segment. The calculated age estimates of each dentine microsample are listed along with the isotopic information.

Chapter 7 comprises the interpretations of the results presented in the previous chapter. Results are compared to the predictions from Chapter 5. New figures are presented here that display the juvenile dentine stable isotope values with the associated bone collagen value for the individual in question. A brief discussion comparing the results to the earlier bone collagen analyses of Kenchreai and Isthmia remains is presented, and a comparison of these sites to some other sites in Roman Greece is made.

Chapter 8 presents the conclusions of the study. Here it is concluded that there is a difference in the diets of children and adults, with children consuming less marine resources. This chapter offers ideas for potential areas of future work that uses dentine microsampling, and for future stable isotope work in Greece.

Chapter 2: Historical and Cultural Background

This chapter presents a brief historical background on Greece in the Roman era. The problem of defining childhood, and who would be considered a child in a bioarchaeological sense and in the ancient Greek and Roman contexts, is considered. Notions of how to feed and care for children are discussed and the roles of children in society are addressed. A review of available food sources in Corinth is included. Evidence for the symbolic and nutritional importance of fish and its availability is considered. This chapter also discusses how bioarchaeology can contribute to knowledge of life in Roman Greece and add to what is known from historical sources, and what contributions have already been made.

2.1. Roman Greece

Rome began its rise to power in the 4th century B.C.E., and by the middle of the 3rd century B.C.E. it dominated most of the Italian Peninsula (Kelly 2006, 4). Rome's conquest occurred at a gradual and steady pace, as it formed alliances with nearby populations and took control of Southern Italy and neighbouring nations (Kelly 2006, 4). This was achieved through strategically planned political and economic moves by the Roman military.

Between the 4th and 3rd centuries B.C.E., the Roman Republic participated in many wars and treaties that revolved around freeing cities from other political powers. An example of this is seen in Rome's conflict with Carthage, a city in North Africa that was a dominant power in the western Mediterranean (Kelly 2006, 4). A series of three wars, known as the Punic Wars, were fought between Rome and Carthage (Waterfield 2014). This eventually resulted in the defeat of Carthage in the Third Punic War, which ended in 146 B.C.E. when the city was destroyed by the Roman military (Kelly 2006, 6). Rome was also in constant conflict with Macedonia, another significant political power of the time. One of the most prominent political figures during the

conflicts with Macedonia was Titus Quinctius Flamininus, who became a Roman consul in 205 B.C.E. and eventually became a commander of the Roman military (Waterfield 2014, 73). He played a huge role in the expansion of Rome's power in the 3rd and 2nd centuries B.C.E. Flamininus' goal was to seize control of Greece, which had been under the rule of Macedonia since the 4th century B.C.E. (Waterfield 2014, 74). Flamininus was in constant conflict with Philip V of Macedon over Greece, and this led to the Roman Republic engaging with Macedonia in the Macedonian Wars over conflicts regarding Greek territories (Waterfield 2014). After the Second Macedonian war in 196 B.C.E. Flamininus made a proclamation at the Isthmian Games, declaring that Greece was now free from Macedonian rule; however it was now under Roman influence (Waterfield 2014, 85). This event is referred to as the Isthmian Declaration. Rome received some resistance from groups such as the Aetolians, and it would take several more decades before Rome politically dominated Greece. The destruction of Corinth in 146 B.C.E. finalized Roman domination in the region (Garland 2008). This caused some major changes: Greece was now governed by Rome, cities and urban settlements gradually expanded, and elite members of Greek society gained positions in Roman political organizations (Woolf 1994, 125). The Roman period continued until the eventual transition into the Byzantine era with the Empire's adoption of Christianity in the 4th century C.E. (Laes 2011, 2).

For both Greece and Rome, Rome's conquest brought a period of transition that resulted in a mosaic of Greek and Roman culture. When Rome seized control of the region, many Greek populations in the East were able to retain their cultural identity and language, largely remaining Greek within a Roman society (Laes 2011; Woolf 1994). A large amount of overlap existed between Greeks and Romans, as there was no single description or definition of Greek or Roman identity (Woolf 1994). A common language, religion, and traditions were important to the

Greeks; Romans on the other hand considered material culture and morality to be of greater importance in defining who they were (Woolf 1994, 130). Although individual identities varied, Greeks adopted Roman traditions and Romans adopted Greek traditions. This includes Romans being influenced by Greek scholars, and Greeks partaking in Roman symposia and spectacula (Woolf 1994, 130).

2.2 Defining Childhood

Our knowledge of childhood in Greece and Rome stems from historical writings, inscriptions on epitaphs, papyri which recorded activities in daily life, and iconography.[1] These sources depict perceptions of childhood that differ from modern Western views. The concepts of the human life course and stages of childhood were written about extensively by Greek and Roman scholars. Ancient authors described in detail how children should be educated and what they should eat. More often than not, opinions differed between individual scholars and authors, which makes it difficult to study ancient attitudes toward children in a holistic manner.

2.2.1 Definitions of Childhood in Bioarchaeology

As discussed by Halcrow and Tayles (2008), concepts of age in bioarchaeology differ from social and cultural definitions of childhood, and both must be considered when conducting the bioarchaeology of juvenile individuals. In bioarchaeology, the terms chronological age and biological age are both used.[2] Bioarchaeologists use the term chronological age to represent age as the amount of time a person has been alive, although this does not always align with the amount of growth and development a person has experienced. Biological age is defined based on skeletal and dental evidence that places individuals into a given estimated age range based on

[1] Information in this paragraph was taken from Laes (2011).
[2] Unless otherwise cited, this section on bioarchaeology contains information from Scheuer and Black (2004).

their progress in growth and development. Methods of skeletal age estimation include assessing epiphyseal development and fusion (which occurs within specific time ranges for different bones) and the size and morphology of bones; dental age can be estimated from the formation and eruption of teeth, which also occur within known time ranges. These methods are used when studying the skeletal remains of children and are one way of grouping individuals of similar developmental stages.

Table 2. 1 Definitions of terms used by bioarchaeologists to describe different stages in childhood (Scheuer and Black 2004, 6).

Category	Definition
Perinate	Around the time of birth
Neonate	First weeks after birth
Infant	Birth to the end of the first year
Early childhood	To the end of the fifth year, often pre-school period
Late childhood	About six years to puberty
Puberty	A physiological term describing the beginning of secondary sexual change at about 10-14 years in girls and 12-16 years in boys
Adolescence	Used by some authors interchangeably with puberty and by others as referring to the period of behavioural and psychological change accompanying puberty

Descriptions of childhood in this thesis refer to skeletal and dental ages and not the social categories of ages used by Greeks and Romans, unless otherwise specified. Table 2.1 contains the definitions of general stages of childhood used to refer to individuals in the thesis study sample, as defined by some behavioural biologists and reviewed by Scheuer and Black (2004). The developmental periods of the teeth studied in this thesis (2.5 through 14.5 years) span the categories of early childhood, late childhood and puberty. The term 'juvenile' is a general term used to refer to individuals who are not yet adults, based on bone fusion and tooth eruption. 'Juveniles' will be used as a general term in this thesis to refer to all individuals who were not

yet skeletally mature. ‘Childhood’ and ‘children’ will be used to encompass individuals from the infant to late childhood categories in the thesis study sample.

2.2.2 Definitions of Childhood in Greece and Rome

Before written records began, some of the earliest indications of how children were seen in Greece derive from art. A noticeable shift in depictions of children in artwork occurs throughout time: while Geometric and Archaic period works portray children as miniature adults, works of the Classical period show anatomically correct depictions (Neils and Oakley 2003, 3). In written records, ages were defined not so much by years, but rather by the stages when certain abilities were gained or tasks could be accomplished. According to Golden (2003, 15) Plato and Aristotle treated childhood as having five stages: 1) from birth until the child was weaned and began talking, at roughly two years; 2) a period up to the age of roughly three to five years when children became more active; 3) a period up to the age of roughly six or seven when children became more social and made friends; 4) schooling, which lasted until puberty at roughly age 14; and 5) adolescence. However, these ideas were not universally held in Greece, and concepts of childhood varied between regions such as in Athens and Sparta, where different ages were deemed appropriate for the marriage of girls (Golden 2003, 19). It is important to recognize that generalizations about childhood (or any other aspect of culture) in ancient Greece are formed based on evidence that may actually be regionally specific, and that it can be difficult to identify whether ideas were widespread (Golden 2003, 21).

Some researchers suggest that stages of childhood can be identified in Greek artwork on the basis of how children and their development are represented in vase paintings, figurines, sculptures (Neils and Oakley 2003, 2). For example, Beaumont (2003) suggests a set of perceived stages of childhood based on an analysis of Athenian vase paintings. From these

works, four stages are distinguished in the appearance of children: 1) infancy, where children are often portrayed as babies or very young (up to approximately 3 years old) and for which no gender distinction is possible, 2) young childhood, defined as when gender distinctions start to be made at about 3 to 7 years and when the child receives an education, 3) older childhood up to the onset of puberty, and 4) adolescence, corresponding to the time between puberty and legal suffrage (males) or marriage (females) (Beaumont 2003, 75-76).

As children grew older, the evidence suggests gendered differences in the age at which childhood was seen to end and adulthood begin. Greek girls were typically married between the ages of 13 and 15; at this point they would leave the fairly sheltered lives of their childhood and assume an adult role (Foley 2003, 135). In contrast, boys became adults around ages 17-18, when they began participating in politics (Neils 2003, 143). As a child reached these significant points in their lives, religious festivals and rituals would be held in celebration (Neils 2003, 145). Such events were important to how Greek children and their families experienced the life course, as they marked transitions into adulthood.

Similar ideas were shared by Romans. The ages at which major developments in growth or ages at which children could partake in social or educational activities are considered important in definitions of childhood described by ancient texts.[3] The development of the deciduous and permanent dentition were deemed noteworthy points of reference. Teething often caused discomfort in infants, and conditions such as fevers, convulsions and diarrhea may have affected the child's health. It was regarded as a milestone after which the next stage of childhood began. Another stage of development often described includes learning how to crawl, walk and speak. The lives of older children varied by class; while sons began to participate in agricultural

[3] Information in this paragraph was taken from Baker (2018) and Rawson (2003).

tasks from an early age, those of craftsmen might be apprenticed by their early teens and those of more affluent households would be educated (Saller 2011, 125). Given that women were normally expected to handle the domestic labor of the home (Saller 2011) and that these tasks must have been learned in girlhood, the daily lives of children would also have varied by gender. The beginning of puberty marked a transitional phase to adulthood, with menstruation beginning in girls and voices changing in boys. Children were considered minors in law before this age, defined as roughly 12 years old for girls and 14 for boys (Saller 2011, 120). However, individuals were not considered to have fully reached adulthood until they married and/or birthed their first child (girls) or became politically active (boys). The age at which individuals reached this milestone also varied by gender, with girls typically marrying and birthing their first child around 13-15 years, and boys becoming involved in politics in the later teenage years or early twenties. Gendered differences appear to have been less important in the ages of the earlier stages of life and to have become more significant at puberty.

The stages of childhood designated in Greek and Roman ancient works often describe similar points in life but vary in the language and terms used to describe them. Some systems might deem certain events more important than others, and others might include more life events, creating a system with more stages. For example, the Hippocratic writers, physicians who contributed medical writings to the *Hippocratic Corpus*, describe a system in which key stages of childhood were defined not based on physical or social milestones but in seven year spans, reflecting beliefs regarding the importance of the number seven (Baker 2018, 81). Similarly, ages were not rigidly defined by numbers but instead reflected "individual development and perceived capacity to determine fitness for responsibilities," (Rawson 2003, 135). As well, some Roman poets and scholars described four or five stages of a human life, not just stages of childhood

(Baker 2018,81). The existence of multiple systems of categorizing the growth and development of children reflects an interest in the development of children in society. Despite differences in these systems, the common stages of infancy, childhood and adolescence are typically included. These three stages would include children from birth up to approximately 14 years. By age 14, children would have passed through periods in which they experienced teething, learned how to walk and speak, were schooled and gained responsibilities in the household, and were socialized and prepared for adult life by learning about and taking part in important festivals or rituals.

2.3 Greco-Roman Views of Health

Like concepts of childhood, views of health also varied. Guidelines for living a healthy life and for taking care of the body were based on an ancient idea that the body needed to maintain a state of balance between heat, cold, dryness, and moistness, which are associated with the Greek humours and temperaments (Baker 2018, 77). This notion is relevant to discussions of diet, because foods were also seen as having their own humoural qualities and thus played an important role in maintaining a person's proper balance (Donahue 2016, 619). There were also ideas that children born at specific times of year were bad omens, and less likely to survive (Baker 2018, 80). This idea of balance, along with beliefs regarding specific numbers and important days, governed many of the decisions made about infants and also influenced the notions of the stages of childhood discussed above (Baker 2018; Rawson 2003).

2.4 Greco-Roman Guidelines for Caring for Children

Instructions for taking care of children are described in accounts from Greek physicians (Rawson 2003). This includes guidelines for how to clean and swaddle an infant, what they should be fed and by what age certain foods should be introduced, as well as instructions for selecting a wet nurse and food a woman should consume to provide the best breast milk.

One of the most detailed and useful set of instructions on feeding children is that in Soranus' *Gynecology* (Soranus 1991). Soranus describes the changing foods that should be given to newborns and infants as they grow. Food was not given to a newborn for up to two days after birth to let the body rest and digest the remaining maternal nutrients in its system. After this time, moderately boiled honey could be fed to the infant. Breast milk from the infant's mother was not allowed to be consumed for twenty days because a woman's milk was considered to be inadequate and unpleasant immediately after birth. Instead, milk was to come from a woman who was able to serve as a wet nurse. Selecting a healthy wet nurse, one who herself ate the proper foods to ensure the proper balance in her body and milk, is also a topic described in detail by Soranus. At around six months, soft foods were to be introduced into the diet to begin the process of weaning. Recommended first foods included bread crumbs softened with honey water or milk, sweet wine, honey wine, soup made from spelt (an ancient type of wheat), a moist porridge, and an egg that can be sipped. Soft breads flavoured with wine were also acceptable on occasion. Weaning progressed by including more of the foods listed above into the diet and decreasing the amount of milk given. This occurred as the teeth erupted, allowing for solid foods to be chewed.

These ideas continued well into the Byzantine age, where physicians like Oribasius derived their own set of ideas about caring for infants (Lascaratos and Poulakou-Rebelakou 2003). Most of the information about breastfeeding, weaning and nutrition introduced by Greek philosophers and doctors was carried into this period, although the timing of the introduction of certain foods differed. Instructions for wet nurses to produce breast milk of sufficient quality and the diet prescribed to infants were similar. Human breast milk was considered the ideal infant food, but substitution with animal milk occurred in some situations. This may have placed

children at risk to develop infectious diseases, nutritional deficiencies, and parasite infestations because animal milk does not strengthen the immune system like human milk and has a different nutritional profile (Lewis 2007; Redfern and Gowland 2012, 126).

Detailed recommendations for children's diets tend to stop after the weaning stage, although dietary suggestions for various illnesses are still made (Donahue 2016). Some classes of foods were thought of as typical or atypical of childhood. Sugary foods such as cakes, sweets and some fruits were commonly associated with Roman children (Laes 2018). In contrast, it was recommended that foods that are bitter or sour not be fed to children, as they might harm the child (Baker 2018, 88). As members of the household with relatively little power or agency, though, we might also suspect that they may have eaten less of some expensive or prestigious foods.

Some scholars have ideas about the relationship between food and identity in the period. Penniman (2017) argues that food influenced the cultural practices, societal ideas and traditions that created identity in the Greco-Roman world. Essentially, the author argues that there were numerous texts describing what children and wet nurses should eat in order to grow into well-formed adults because of the idea that specific food needed to be consumed to become physically and mentally healthy (Penniman 2017). This idea is related to controlling the Greek humours and pleasing the soul (Penniman 2017, 26). It was thought that the best way to achieve pleasing the soul was through food: not only through a child's diet, but through the food consumed by the parents before the child is conceived, which is what the infant's soul is created from (Penniman 2017, 27). It was believed that "proper nourishment ensures the proper formation of one's own soul as well as the soul of one's offspring"(Penniman 2017, 28). Some philosophers also believed that diet influenced brain development and some food could mould smarter individuals

(Penniman 2017, 33). Thus, careful consideration was put into what foods should be consumed to become healthy, and it was the duty of the parents and wet nurse to fulfill this. Perhaps this idea also plays into the objective of surviving through childhood diseases and poor sanitation conditions (Donahue 2016). With the combination of malnutrition, poor sanitation and poor standards of health, there was reason to be concerned over the health of children because of high mortality rates of children within the first ten years of life (Garland 2008). By modern Western standards, Roman children were constantly sick and suffering from diseases that were often incurable (Bradley 2005). Penniman argues that a child's susceptibility to sickness and death reflected back on the Roman Empire, making the Empire vulnerable itself since children were the individuals who would carry on Roman family traditions (Penniman 2017, 42).

2.5 Fish consumption in the Greco-Roman Period

In Roman times, fish was considered to be a high-status item.[4] Certain types of marine fish such as sea bass, sea bream and gilthead, and seafood such as oysters were favoured by wealthy individuals and were considered to be elite food items. Marine fish and seafood were commonly featured at grand banquets and feasts hosted by the elite members of society. The majority of historical sources that discuss seafood do not provide details about what was consumed by the average person (McConnan Borstad, Garvie-Lok, and Katsonopoulou 2018; Corbier 1999; Talbot 2003). While the role of fish consumption in the diet of everyday people is not widely discussed in historical documents, data acquired from archaeological excavations have uncovered the remains of fish, mollusks, and shellfish at Greek and Roman sites (Mylona 2008). In addition to fresh fish, processed fish products were also widely available to both Greeks and Romans. An example would be *garum*, a fermented fish sauce used as a replacement

[4] Unless otherwise cited, knowledge of fish in the Roman world was acquired from Marzano (2018).

for salt in cooking. As well, preserved fish were commonly available and affordable (Bourbou et al. 2011; Maniatis 2000). A variety of types of fish was available at markets, but historical sources are unclear about which fish were favoured by the wealthy and what was available for the poor, as fish have been described as both a food item for those who are poverty stricken and those who are elite (McConnan Borstad, Garvie-Lok, and Katsonopoulou 2018; Mylona 2008). References to children consuming fish are rare in historical documents and in the guidelines provided by physicians like Soranus. Given the elite connotations of many marine items and the tendency for them to be served at special banquets or feasts, it is reasonable to suggest that children may have eaten less fish than adults.

2.6 Diet at Isthmia

Information from different sites and time periods of the Greek countryside have been compiled to discuss diet at Isthmia. Rife (2012) considers the data presented by Brothwell and Brothwell (1998) on general food use in antiquity, Garvie-Lok (2001) for an overview of diet in the Roman, Byzantine, Frankish and Ottoman eras, and information on Byzantine nutrition from Kaplan (1992), Kislinger (1999), Dagron (2002) and Lefort (2002). Synthesizing this information allows Rife (2012) to speculate on the types of resources that would have been available in Isthmia during the Roman period. Despite temporal and geographical differences between the sources listed above, the same natural resources may have been available given that climate and terrain conditions remain unchanged (Rife 2012, 295). Similar resources would presumably be available at Kenchreai given the proximity of the two sites (see Chapter 4 for a discussion of the location and relationships of Kenchreai, Isthmia, and Corinth). The diet at Isthmia is described as: “A mixed agricultural diet combining meats, fruits, vegetables, cereals and dairy. The residents would have eaten both soft, sticky, oily foods (fleshy fruits and

vegetables, meat, honey, dairy), and hard, rough, fibrous foods (raw plants, roots and stalks, nuts and seeds, milled grain, occasional fruit or vegetable casings, husks or even bone)" (Rife 2012, 295). Items such as meat from cow, pig, sheep, goat, chickens and fish were available in the region during the Late Roman and Early Byzantine Periods, as were products like milk, honey and grains (Rife 2012, 294). This is likely not an exhaustive list of every food item that was available and consumed at Isthmia, but it provides enough detail to gain an idea of what was being consumed.

2.7 Contributions of Bioarchaeology

In addition to historical source, bioarchaeology has made significant contributions to the understanding of life in Roman Greece, and specifically to the study of children in this period. As reviewed in Redfern and Gowland (2012), the application of bioarchaeological methods has allowed for studies of burial practices (such as in Rife et al. 2007; McKinley 2000), trauma and possible child abuse (see Wheeler et al. 2013), and mobility and the identification of non-local individuals (e.g. Prowse et al. 2007), to name a few examples. Relevant to the present thesis are studies that use stable isotope analysis to examine breastfeeding and weaning in Greece in various eras (Bourbou et al. 2013; Bourbou and Garvie-Lok 2015; Kwok, Garvie-Lok, and Katzenberg 2018) and in the Roman Empire (Dupras, Schwarcz, and Fairgrieve 2001; Dupras and Tocheri 2007; Prowse et al. 2008). Dietary reconstructions using stable isotope analysis (discussed in Chapter 3) have also provided information on dietary trends in Greece during various eras (such as Iezzi 2015; Panagiotopoulou and Papathanasiou 2015; Papathanasiou 2015; Vika 2015; Triantaphyllou et al. 2008; Kwok and Keenleyside 2015; Richards and Hedges 2008).

Other studies have focused on health differences between urban and rural settlements. Similar diseases and conditions will affect these populations, but there is typically a higher prevalence in urban populations with greater population sizes and poor living conditions (Lewis 2002; Redfern 2003). For example, the results of diachronic studies suggest that childhood health declined in England after the Roman conquest as a result of larger population sizes and a shift towards more urban living (Redfern and DeWitte 2011). This increase in population may have caused infectious diseases to become more widespread among at risk individuals such as children and the elderly (Gowland and Redfern 2010). Similar events likely occurred when the Romans conquered Greece, with increases in population (Woolf 1994) and an increase in mortality rates of children (Bradley 2005).

Donahue (2016) reviews palaeopathological studies and describes how they have contributed to our understanding of nutrition in Greece and Rome. Studies that are relevant to the current thesis include work on some Roman and Greek skeletal samples that identified high frequencies of dental caries and calculus, suggesting a diet high in carbohydrates and low in hard fibrous foods (Bonfiglioli, Brasili, and Belcastro 2003; Tritsaroli 2014). Studies of stature and growth are also important as they examine the impact of stressors such as malnutrition and infection on the average height of a population (Donahue 2016, 627). One such study found adult male stature in a series of Roman skeletal samples to exceed stature in many later cemetery groups, an example of how growth studies can provide unexpected insights about ancient standards of nutrition (Kron 2005). Other studies have identified the prevalence of nutritional deficiencies using paleopathology and relate this to evidence from stable isotope analysis or historical sources. An example is Bisel and Bisel's (2002) study of remains from Herculaneum,

which discussed the prevalence of anemia at the site in relation to evidence for key foods in the Roman diet.

Combining bioarchaeological evidence with that from historical sources such as artwork, medical texts and everyday documents creates a more complete picture of what life was like for the individuals being studied. As reviewed by both Redfern and Gowland (2012) and Donahue (2016), bioarchaeology can aid in developing our understanding of childhood in the past by providing insights into the societal conditions into which infants were born and how they were cared for. Stable isotope analysis, reviewed in the following chapter, can make a major contribution to these efforts.

2.8 Summary

This chapter has provided discussions on perceptions of Greek and Roman children and the importance of diet in Greek and Roman society. From the discussions above, it is evident that many aspects of children's lives were laid out for them before they were born, down to the food they would eat and the roles they would take on as adults. For girls, this meant getting married and having children, and for boys this meant becoming politically active. For both sexes these milestones would have occurred at younger ages than we expect today. Before this point, children were seen as progressing through a series of stages in which they gradually developed the understanding and skills of adults. In addition to outlining these stages of childhood, this chapter has presented discussions of resources consumed by children and the importance and availability of fish to Greek diets; some comments on dietary items likely available at Isthmia has also been presented. These topics will be referred to later in Chapter 7 when discussing the isotope results of the sample from Kenchreai and Isthmia.

Chapter 3: Stable Isotope Analysis

This chapter offers essential information about stable isotope analysis and summarizes the processes for understanding stable carbon and nitrogen isotopes in archaeology. Topics such as obtaining samples from bone collagen and dentine collagen, stable carbon and nitrogen isotope ecology, and the effects of breastfeeding and stress are briefly discussed. Emphasis is placed on detecting the signals from the consumption of marine resources in the past as it relates to the current study of Kenchreai and Isthmia. A brief review of dietary stable isotope studies in Greece is presented. The method of dentine microsampling is also introduced here. The development of the method and its applications to isotope analysis are discussed in this chapter, while details regarding tooth formation, dentine mineralization and how to take microsamples and estimate their formation ages are discussed in Chapter 5.

3.1 Fundamentals of Stable Isotope Analysis

Isotopes are variants of an element, and an element can have multiple isotopes.[5] The atoms of the isotopes of a given element all have the same number of protons but vary in the number of neutrons they contain, affecting their mass. Although multiple isotopes can exist for a single element, their chemical traits are the same because they all share the same number of protons. The most frequently examined isotopes in archaeology are those of the elements carbon and nitrogen, used in palaeodietary reconstructions, and strontium and oxygen, used to examine migration (Price 2015, 71). These isotopes leave signatures in the human body through the foods and water consumed over an individual's lifetime (Price 2015, 71). When an element has multiple isotopes, archaeologists can examine the ratios between these isotopes. A stable isotope ratio compares the amounts of different isotopes of an element that are present in a biological

[5] Unless otherwise cited, the information in this summary was taken from Sharp (2017).

specimen. This is measured using a mass spectrometer, alongside a standard reference material for the same element. For light elements such as carbon and nitrogen, this is reported as a delta (δ) value that is measured in per mil (‰). The delta value expresses the departure in the isotopic composition of a sample from that of a standard reference material. It is calculated as follows:

$$\delta = \left(\frac{R_x - R_{standard}}{R_{standard}}\right) \times 1000$$

In this calculation, R_x refers to the ratio of the heavier to the lighter isotope in the sample and $R_{standard}$ refers to the ratio of the heavier to the lighter isotope in the standard. The standard is defined as zero. Absolute differences in stable isotope composition within most natural systems are low, so the departure is multiplied by 1000 for ease of writing and mathematical operations and expressed per mil (‰). Delta values that fall below zero indicate that there is proportionately less of the heavier isotope relative to the standard, whereas values above zero indicate that more of the heavier isotope is present (Pate 1994, 172). The mass of an isotope influences how well it can form bonds with other elements. This contributes to what is known as fractionation – minor changes to the isotopic composition of a substance that take place during chemical and physical processes. The impact of fractionation varies between isotopes and between tissues within an organism.

Isotopic information can be gained from the analysis of human bones, teeth, hair, and nails. Each tissue has the ability to retain isotopic information associated with specific periods of life and lifestyle choices related to diet or mobility (Price 2015, 72). Bones and teeth are the materials relevant to the current study. They both contain collagen, the protein that is extracted and analyzed in archaeological stable isotope studies (Lee-Thorp 2008, 926). Bone and teeth differ in the information they retain, which is dependent on their organic and inorganic makeup and turnover rate. Collagen in bone and dentine is useful for studying ancient diets because it

averages dietary information over varying periods of time, and is also usually well preserved in archaeological remains, which makes it an invaluable source of information (Bocherens and Drucker 2003).

Bone is an active material; it responds to everyday stress and strain, age and growth-related processes, diseases, and other issues that may affect the integrity of a body (Price 2015, 72). Bone maintenance occurs in a similar fashion to bone formation, with bone cells known as osteoblasts and osteoclasts working to create new tissue and break down old tissue, respectively (Scheuer and Black 2004, 35). This process is referred to as bone turnover. When osteoclasts resorb bone matrix, the isotopic information retained in that matrix is lost. Thus, the information that is gathered from bone comes from the matrix that has formed in more recent years of life. Turnover rates vary depending on the structure of a bone. Turnover in cortical bone takes approximately 25 years, whereas it takes significantly less time in trabecular bone (about 3-4 years) (Olsen et al. 2014; Hedges et al. 2007). However, bone turnover rates can also be influenced by the size and thickness of a bone (Fahy et al. 2017, 11), or by the presence of pathological conditions (Katzenberg and Lovell 1999; Olsen et al. 2014).

In contrast, teeth provide an opportunity to analyze information from an individual's childhood. The formation of the permanent dentition is completed before adulthood. The chemical composition of teeth remains unchanged from childhood and thus reflects information from the early years of life (Price 2015, 72). These signatures can be obtained from tooth enamel and dentine. These tissues form in an incremental manner and can be used to track and identify chemical changes at specific time periods during formation (Humphrey 2016, 502). Dental enamel is more commonly used in strontium isotope studies to answer questions about mobility, whereas dentine records stable carbon and nitrogen isotope signatures and is better suited for use

in palaeodietary analyses (Montgomery 2010; Chenery et al. 2010; Redfern et al. 2016; Kinaston and Buckley 2017; Dean 2017). A more detailed discussion of the processes involved in the formation of enamel and dentine is provided in Chapter 5.

While stable isotope analysis is a useful method, it brings with it numerous challenges. This includes issues of interpretation, how accurately the stable isotope composition of various tissues reflects the diet, and how reliable these analyses are when diagenesis can cause archaeological tissues to break down (Ambrose 1990; van Klinken 1999; Lee-Thorp 2008). Researchers have attempted to solve these issues (see for example Hedges and Reynard 2007; Lee-Thorp 2008). Ultimately, the best way to address these challenges is to acknowledge that issues exist and explain what parameters have been set in place in attempts to account for them.

3.2 Stable Carbon Isotopes and Diet

Carbon has two stable isotopes – ^{12}C and ^{13}C. The standard format for reporting stable carbon isotope ratios is $\delta^{13}C‰$ (Pate 1994). The international standard initially used to report stable carbon isotope values was PeeDee Belemnite (PDB) but this resource has been exhausted (Hoefs 2009, 49). New standards have been formulated that are calibrated to be isotopically identical to the original; the current international standard is Vienna PeeDee Belemnite (VPDB) (Hoefs 2009; Sharp 2017). Because the original PDB standard was a marine limestone with a higher $^{13}C/^{12}C$ ratio than most biological substances, the $\delta^{13}C$ values seen in ecosystems tend to be negative (Price 2015, 73).

The presence and movement of carbon on earth is a result of the carbon cycle. The primary carbon reservoirs include the Earth's mantle, the oceans, and the atmosphere. Movement of carbon occurs between these reservoirs, which usually remain in equilibrium (Sharp 2017, 7-2). This includes fluxes of CO_2 that occurs between plant decomposition and plant respiration,

the atmosphere and surface ocean, as well as between surface and deep oceans. The only exception to these balanced fluctuations occurred two centuries ago when fossil fuels were being burned at an exponential rate that had not been previously seen (Sharp 2017, 7-2). This hugely impacted global $\delta^{13}C$ values of atmospheric CO_2 and caused a decrease in its overall $\delta^{13}C$ value from a preindustrial value of -6.7‰ to its current value of -8.3‰ (Sharp 2017, 7-11).

Photosynthesis is the avenue through which atmospheric carbon is incorporated into plants. During this process plants generate energy and obtain carbon from atmospheric CO_2 to use in tissue growth and maintenance. There are different pathways through which photosynthesis can occur, and these differ depending on the physiology of the plant (Bender 1971, 1243). Terrestrial plants typically follow a C_3 or C_4 pathway, and less commonly operate under a pathway known as CAM (Crassulacean acid metabolism) (van der Merwe 1982, 596). The C_3 and C_4 pathways are named for the three or four molecule carbon compound they initially produce during the fixation of CO_2 (Hoefs 2009, 51). The most common pathway is C_3, which is used by trees and shrubs as well as most cultivars. Maize, millet, and sugarcane are the only important C_4 cultivars, and are examples of the smaller group of warm weather adapted plants that use the C_4 pathway (Pate 1994, 172). CAM plants are found in arid conditions and include desert plants such as cacti (van der Merwe 1982, 597). These pathways show distinct stable carbon isotope signals which allow them to be differentiated from each other, which permits researchers to detect their presence in human and animal diets (Bender 1971; van der Merwe 1982, 596). C_3 plants typically show values ranging from -33‰ to -23‰, with C_4 plants ranging from -16‰ to -9‰ (Sharp 2017, 7-6).

Stable carbon isotope values also vary between land and aquatic plants due to differences in atmospheric and aquatic sources of carbon (Pate 1994, 174). Marine plants typically have

higher $\delta^{13}C$ values than most C_3 terrestrial plants, but some C_4 and CAM plants have values similar to marine grasses and algae (Pate 1994, 174). This similarity can create difficulty in distinguishing between the two sets of resources in some environments. Having an understanding of available resources in a given location will aid in determining whether the signals reflect consumption of marine foods or C_4 resources.

It is understood that animal tissues reflect the isotopic values of the food they ingest, and that they show a small whole-body $\delta^{13}C$ elevation of about 1‰ relative to the food they consume (DeNiro and Epstein 1978, 499). This is referred to as a trophic level effect (Lee-Thorp 2008, 928). In addition, $\delta^{13}C$ variation exists between different tissues within an organism. Different tissues are synthesized through different paths and from different substrates, resulting in different typical $\delta^{13}C$ offsets from the diet for each tissue (Lee-Thorp 2008, 928). The offset in $\delta^{13}C$ values between human tissues such as bone collagen and apatite can provide additional dietary information. For instance, $\delta^{13}C$ values of bone collagen are not an equal reflection of all carbon consumed in the entire diet. Research conducted on this topic has shown that resources contributing protein to the diet are largely reflected in collagen $\delta^{13}C$ values, whereas non-protein sources such as lipids and carbohydrates contribute less to the collagen $\delta^{13}C$ value (Ambrose and Norr 1993, 27). This even occurs in situations where low levels of dietary protein are consumed, although in this case lipids and carbohydrates will influence collagen $\delta^{13}C$ more than they do when protein consumption is high (Ambrose and Norr 1993, 31). Thus, one must consider the contributions of protein, lipids and carbohydrates to fully understand how much each resource contributes to an overall $\delta^{13}C$ value. Various models have been developed in attempts to quantify $\delta^{13}C$ offsets between collagen and diets of varying composition and quality, some of which promise more accurate dietary reconstructions because they account for these varying offsets

between tissues and diet (Kellner and Schoeninger 2007, 1122). However, these models still have difficulty differentiating between resources that produce similar isotope values, such as with protein gained from marine resources as opposed to C_4 plants (Kellner and Schoeninger 2007, 1122). Thus, although they do increase the precision of dietary reconstructions, some uncertainty is inevitable.

3.3 Stable Nitrogen Isotopes and Diet

Nitrogen has two stable isotope isotopes, ^{14}N and ^{15}N, and the standard reference material is atmospheric N_2 (AIR), set at 0‰ (Sharp 2017, 9-1). Unlike atmospheric CO_2, the stable isotope signature of atmospheric N_2 has remained constant, experiencing no major fluctuations throughout history. The nitrogen cycle offers a few pathways for nitrogen fixation (in which N_2 is transformed into nitrogen compounds that can be used by most organisms) and denitrification (the breakdown of nitrogen compounds to produce N_2), and different chemical compounds of nitrogen are created in this process.

Plants obtain nitrogen either directly, from symbiotic nitrogen fixing bacteria, or from soil nitrogen compounds produced by bacteria living in the soil (Price 2015, 75). While most soils usually have $\delta^{15}N$ values between 2‰ and 5‰, there are various external factors that affect these values that are complicated in nature (Kendall 1998, 535). The amount of fractionation that occurs as soil nitrogen compounds are taken up by a plant is dependent on the amount of nitrogen present in the soil – there is little to no fractionation in nitrogen-limited soils, as opposed to a higher fractionation of several per mil for nutrient-rich soils (Sharp 2017, 9-7). These values are also dependent on the type of plant, as fungi and trees will differ (Sharp 2017, 9-7). Some plants, notably the legumes, are capable of nitrogen fixation thanks to their symbiotic bacteria (DeNiro and Epstein 1981, 346). This should be considered in dietary reconstructions

because the $\delta^{15}N$ values of nitrogen fixing plants have a tendency to be around 0‰, lower than plants incapable of nitrogen fixation (DeNiro and Epstein 1981, 346).

Similarly to carbon, the stable nitrogen isotope values of animals are a reflection of the foods they consume (Bocherens and Drucker 2003). Moreover, different tissues within a single organism may have varying $\delta^{15}N$ values (DeNiro and Epstein 1981, 344). Animals' tissues are typically enriched in ^{15}N relative to the items in their diet (DeNiro and Epstein 1981, 343). A trophic level effect is seen as one moves up a food chain – $\delta^{15}N$ values will increase between plants and herbivores, herbivores and carnivores, and prey and predators (Bocherens and Drucker 2003, 47). This shift is typically +2 to +6‰ (Lee-Thorp 2008, 928). The same patterns of enrichment seen in modern fauna are seen in archaeological fauna (Bocherens and Drucker 2003), and they are also recognizable in archaeological humans (e.g. Fogel et al. 1997) and modern humans (e.g. Fuller, et al. 2006) relative to their diets. Trophic level effects can also be seen during breastfeeding, and the magnitude of nitrogen trophic level effects appears to be altered by stress and growth. Therefore, all three of these are worth discussing in detail.

3.3.1 Breastfeeding

The $\delta^{15}N$ trophic level effect is a phenomenon that is also visible between human mothers and infants, where the infant has values higher than the mother during breastfeeding because it is consuming her milk, which has a $\delta^{15}N$ value similar to her tissues (Fuller et al. 2006, 280). For that reason, stable nitrogen isotope values of nursing infants are approximately 2-3‰ higher than that of their mother (Fuller et al. 2006, 280). Once weaning begins, infant $\delta^{15}N$ values start to decline because other foods with lower $\delta^{15}N$ values are consumed along with breastmilk; at the end of the weaning process breastmilk consumption ceases and the infants' $\delta^{15}N$ values will fall within 1‰ of their mother (Fogel et al. 1997, 279). To date, stable isotope studies of children in

archaeological populations have placed an emphasis on detecting breastfeeding and weaning signals (Dupras and Tocheri 2007; Dupras, Schwarcz, and Fairgrieve 2001; Prowse et al. 2008; Fuller, Fuller, et al. 2006; Fuller, Molleson, et al. 2006; Mays, Richards, and Fuller 2002; Bourbou et al. 2013; Tsutaya et al. 2015, 2016). Studies of older children and teenagers are rare but recent developments in stable isotope methodologies has led to an increase in these studies (Beaumont et al. 2013; Eerkens, Berget, and Bartelink 2011). This is further discussed in section 3.5 below.

3.3.2 Nitrogen Isotopes, Growth and Stress

Studies have been conducted to investigate a possible link between growth and stress as possible factors affecting $\delta^{15}N$ values. Ambrose and DeNiro (1987) first observed a pattern of $\delta^{15}N$ elevation in herbivores that experience drought in comparison to species that are not as susceptible to drought. Similar studies followed, and the phenomenon of unexpectedly high $\delta^{15}N$ values was also studied by researchers implementing fasting experiments on birds to investigate whether nutritional stress can cause elevated $\delta^{15}N$ values (Hobson, Alisauskas, and Clark 1993). This $\delta^{15}N$ enrichment was identified in different tissues of individual birds, but an understanding of dietary patterns and physiology of individuals was needed (Hobson, Alisauskas, and Clark 1993).

The effect of stress on $\delta^{15}N$ can be seen in cases of protein malnutrition or general undernutrition, causing tissue $\delta^{15}N$ values to increase as a result of the human body reusing its own tissues (Katzenberg 2008; Doi, Akamatsu, and González 2017). Essentially this recycling of tissue nitrogen applies the trophic level effect a second time, with the result that the tissues the recovered nitrogen is deposited in show increased $\delta^{15}N$ values. An important example of this is demonstrated in a dentine microsampling study conducted on individuals from a workhouse

cemetery who experienced the Great Irish Famine of the 19th century (Beaumont and Montgomery 2016). Periods of stress before admission to the workhouse are evident from a rise in $\delta^{15}N$ with a stable or decreasing $\delta^{13}C$ value, followed by a rise in $\delta^{13}C$ that reflects a switch from consumption of the typical potato-based diet of the region to a government relief diet including maize (Beaumont and Montgomery 2016, 13). Once maize is introduced to the diet, $\delta^{15}N$ values return to more typical levels, suggesting that nutritional stress was reduced (Beaumont and Montgomery 2016, 18). The use of dentine microsampling in this study is an example of the advantages of the technique discussed below; it allows for specific periods of stress to be identified throughout childhood, rather than relying on the long-term dietary average isotope signature that is represented by an individual's bone collagen.

Another potential influence on $\delta^{15}N$ values is growth. The results of some modern laboratory and clinical studies (e.g. Fuller et al. 2004; Webb et al. 2016) suggest that during periods of rapid growth, tissue $\delta^{15}N$ elevation above the diet may decrease, likely because more dietary nitrogen is being used in tissue synthesis and less ^{15}N-depleted nitrogen is being excreted. In some archaeological studies including multiple age groups (e.g. Richards, Mays, and Fuller 2002), a dip in $\delta^{15}N$ relative to adult values is seen in middle childhood after weaning, but it is difficult to determine whether this might be due to an isotopic effect of growth or to a distinctive diet in this period of childhood. Waters-Rist and Katzenberg (2010) investigated the possible link between a decrease in $\delta^{15}N$ during growth and archaeological collagen $\delta^{15}N$ values. They conducted three studies using sub-adult long bones from a historical cemetery in Ontario. Each study focused on a different aspect of bone growth. This included comparing $\delta^{15}N$ values for diaphyses, metaphyses, and epiphyses; comparing metaphyses that have a faster growth rate to those that are slower, and comparing $\delta^{15}N$ values in bones are unfused versus those that are fused

(Waters-Rist and Katzenberg 2010). The results of each study showed no statistical significance between $\delta^{15}N$ values, suggesting no relationship between collagen $\delta^{15}N$ values and growth rate. This is in agreement with the large body of published archaeological stable isotope data in which juvenile collagen $\delta^{15}N$ values appear to solely reflect shifts in diet (see Waters-Rist and Katzenberg 2010 for review). However, the existence of a relationship in various tissues of modern organisms is evident from the information reviewed above. Further work must be done to increase our understanding of this possible relationship.

3.4 Carbon, Nitrogen, and Marine Resources

As indicated by the information above, stable isotope values have the potential to indicate a specific food group or contributor to a diet. Knowing this information allows bioarchaeologists to better understand the contributions of particular foods, including marine resources, to the diet.

An early study by Schoeninger and DeNiro (1984) examined fish and terrestrial animals of varying positions in the food chain to investigate differences in stable carbon and nitrogen isotope values. Their results indicate that $\delta^{15}N$ values from most marine species are higher relative to land animals (Schoeninger and DeNiro 1984, 633). Likewise, the $\delta^{13}C$ values of marine organisms have a tendency to be higher than terrestrial animals. The results of this study fall in line with a previous study on carbon isotopes in marine versus terrestrial organisms and the humans that consume them. This work suggests that a diet consisting entirely of marine foods will produce human collagen $\delta^{13}C$ values around -13‰, whereas diets consisting entirely of terrestrial foods will produce human collagen $\delta^{13}C$ values of approximately -20‰ (Chisholm, Nelson, and Schwarcz 1982; Schoeninger and DeNiro 1984). This becomes more complicated when mixed diets are considered. In these situations, stable isotope values depend both on the particular types of marine or terrestrial resources being consumed and on the size of the

contributions of marine and terrestrial food to the diet (Richards and Hedges 1999, 721). A caveat to this is that marine values can be similar to those of C_4 plants (Chisholm, Nelson, and Schwarcz 1982, 1132). Additional information such as location and available resources in the area, along with the $\delta^{15}N$ values of humans and of available dietary items, must be considered to prevent confusion.

It is important to consider fish and other marine organisms as a dietary item in geographic locations where access to marine resources is clear. For an area such as Greece, where land is surrounded by water, one would think fish played a large role in the ancient diet. Previous analysis of bone collagen from Kenchreai suggests that fish contributed to the diets of adults at the site, despite no fish bones being recovered (see Chapter 4 for further discussion). While this situation is similar across other Greek sites, there are also sites where isotope signals indicate the contrary – that fish was not consumed despite historical evidence proving otherwise. This is true for most Greek sites studied using stable isotope analysis (see Papathanasiou and Richards 2015). Although fish remains are usually absent in the archaeological record, they are constantly mentioned in historical sources and recent literature that discuss dietary trends throughout ancient Greece (Lascaratos and Poulakou-Rebelakou 2003; Dalby 1996; Garnsey 1999). One explanation for the infrequent recovery of archaeological fish remains is that in comparison to mammal bones, fish bones do not preserve as well because of their bone structure (Szpak 2011, 3367). Taphonomic factors such as burial conditions and the effects of scavengers may also influence preservation (Dufour, Bocherens, and Mariotti 1999, 617).

In an important study, Vika and Theodoropoulou (2012) scrutinize the lack of stable isotope evidence for fish consumption by considering the variation seen in Aegean fish stable isotope values. Archaeological fish remains were collected from six sites in Greece dating to

different time periods. Collagen was extracted from these samples for stable carbon and nitrogen isotope analysis. The authors made a point of selecting fish from different habitats and salinity of water, including marine, euryhaline and freshwater environments, because they are representative of the various types of fish found in the Aegean (Vika and Theodoropoulou 2012, 1619). The results of their analysis indicate variation within and between fish groups from similar habitats and salinity, with a range of $\delta^{13}C$ values between -19.2‰ and -10.11‰, and between 6.1‰ and 11.61‰ for $\delta^{15}N$ values (Vika and Theodoropoulou 2012, 1623). In some cases, values for freshwater and marine fish are more similar than had been anticipated (Vika and Theodoropoulou 2012, 1623). This is the first study of its kind in Greece and it demonstrates another challenge associated with considering marine resources in isotopic analyses. The results of this study indicate that Aegean fish have such wide isotopic variation that their dietary impact may have been missed in some previous studies. However, a broad comparison of these fish values to values for archaeological domesticates show that there is still an overall clear offset between terrestrial and marine resources in Greece, with the latter showing higher $\delta^{13}C$ and $\delta^{15}N$ values (e.g. Bourbou and Garvie-Lok 2015). Thus, significant marine resource consumption should typically be reflected by higher human $\delta^{13}C$ and $\delta^{15}N$ values. However, the isotope values of fish are dependent on the size of the fish and their trophic level position. Low trophic level fish, such as sardines and anchovies, produce $\delta^{13}C$ and $\delta^{15}N$ values not far above to those seen in C_3-consuming terrestrial fauna, and thus may be hard to distinguish from them in the diet (Papathanasiou 2015, 47). This offers one possible explanation to the absence of fish seen isotopically – marine resources were being consumed but because of their low trophic level their stable isotope values are indiscernible from other resources, at least when consumed in modest amounts (Garvie-Lok 2001; Lagia, Petroutsa, and Manolis 2007; Papathanasiou 2015). Another

potential explanation is that fish was only consumed for short intervals of time throughout the year, not enough to alter stable isotope values. For example, Byzantine populations fasted multiple times a year and had to refrain from eating meat, but fish and seafood were never restricted (Bourbou 2013, 217). While we know that marine resources were encouraged to be consumed at these times, it is impossible to gain a good understanding of how strictly fasting rules were followed (which may vary between individuals, families and populations), whether individuals could afford to buy fish, and whether fish and other forms of seafood were consistently available year-round (Bourbou 2013, 225).

3.5 Dentine Microsampling

As previously discussed, dentine is an important resource for isotopic studies. Human teeth complete formation within childhood and do not remodel throughout life (Lee-Thorp 2008, 927). However, it is possible for secondary dentine to form later in life as a reaction to tooth wear that exposes the pulp cavity (Hillson 1996, 194). This topic is discussed further in Chapter 5. By comparing isotopic data obtained from bone which provides information on the more recent years of life to data from dental tissues identifying data from the early years of life, researchers can compare adult and childhood values. Dentine microsampling takes this a step further because it provides an opportunity to examine small segments of dentine. Given the incremental nature of dentine growth, each segment represents a short period of time within the known time of tooth formation, thus allowing for the potential identification of dietary change or periods of nutritional stress during the juvenile years of life (Beaumont et al. 2013, 277).

The first archaeological studies to use dentine microsampling applied the method to animal teeth. Koch, Fisher, and Dettman (1989) examined the dentine in the tusks of Pleistocene proboscideans (mammoths and mastodons) because their tusks can be used to understand

seasonality. This study looked for variation along the tusks that could correspond to seasonal changes in $\delta^{18}O$ isotope values of drinking water. The authors concluded that this variation was present, and that regular dark bands of dentine that appear in the tusks represent dentine growth during the winter months (Koch, Fisher, and Dettman 1989, 518). A similar study by Hobson and Sease (1998) examined the dentine of sea lion teeth and was able to identify variation in isotopic signals between tooth annuli. Their findings suggest that the examination of diet through annuli is more informative than analyzing a bulk tooth sample that will provide an average value for the dietary signal over more of the animal's life (Hobson and Sease 1998, 121-122).

Another study sampled modern bovine tooth dentine from molars with the intention of being able to detect changes in diet through isotopic analysis (Balasse et al. 2001). The animals were fed a controlled but changing diet for their entire life. Most of the molars were sectioned into 4mm segments, with two molars being sliced into 8mm segments. The analysis of the dentine isotope values showed that dentine stable isotope values allow for the detection of dietary changes, and the estimation of ages at which these changes occurred (Balasse et al. 2001, 243).

The studies presented above highlight the uses of dentine microsampling and its development as an avenue of study for animal teeth. Studies on human teeth followed soon after. Fuller, Richards, and Mays (2003) obtained segments of dentine from deciduous and permanent teeth from individuals from the medieval site of Wharram Percy, Yorkshire, UK. The purpose of this study was to explore new ways of examining breastfeeding and weaning, as up to that point, previous studies had analyzed bone collagen from ribs. The authors wanted a more precise method that may be able to reveal time frames for specific events that occur during breastfeeding. To achieve this goal, teeth were separated into 3-4 segments, depending on the

development and preservation of the tooth (Fuller, Richards, and Mays 2003, 1674). Bone collagen from ribs was also analyzed to compare the two sources of data. The authors identified a pattern of $\delta^{13}C$ and $\delta^{15}N$ elevation in the dentine values consistent with expected changes during breastfeeding and weaning based on earlier bone collagen studies of the same group (Fuller, Richards, and Mays 2003, 1683). This demonstrates the potential of dentine stable isotope analysis for weaning studies. The authors also pointed out the fact that using bone collagen from juvenile individuals to detect breastfeeding patterns has a major disadvantage compared to using dentine: it represents feeding patterns of those who did not survive, and therefore may not accurately represent the diet of those who reached adulthood (Fuller, Richards, and Mays 2003, 1677). Analyzing dentine collagen from the permanent dentition allows archaeologists to explore breastfeeding histories of individuals who did survive past infancy and childhood and into adulthood.

As more researchers began to use dentine microsampling, the time resolution of the microsamples has improved. Fuller, Richards, and Mays (2003) could only obtain three or four segments because their analytical equipment required relatively large samples. A similar study by Holt (2009) obtained up to four samples from a single tooth. After no major developments in techniques for several years, Eerkens, Berget, and Bartelink (2011) published a paper advocating for microsampling, applying the same basic method and principles but aiming to take 5-10 sections per tooth. This work took advantage of the fact that improvements had been made to mass spectrometers allowing for the analysis of smaller samples. While this approach allows for a more detailed examination of diet over an individual's childhood, the authors recognized that overlap exists between sections due to the way dentine is formed (Eerkens, Berget, and Bartelink 2011, 3107). This topic is elaborated upon in Chapter 5. Their results verified that each dentine

segment examined had a different pattern of stable nitrogen and carbon isotope values, representing a different stage in life, and that changes between segments followed those expected for breastfeeding and weaning (Eerkens, Berget, and Bartelink 2011). This study demonstrated that serial sections of dentine can be used to examine specific time frames within an individual's life, and that it is possible to identify dietary changes between these sections.

Developing methods for higher time resolution in microsampling methods is important because it "offers the opportunity to investigate childhood dietary variation in individuals who survived childhood" (Beaumont et al. 2013, 278). This is significant because it allows for dietary changes to be examined within an individual, and for comparison of dietary choices from their childhood and adulthood. This is not possible for individuals who die before reaching adulthood. Comparing juvenile and adult values also provides a solution for one of the problems described in discussions of the osteological paradox: the fact that the remains of juveniles represent individuals who died before reaching adulthood and may not be representative of the entire population (Wood et al. 1992). Furthermore, diets of contemporary individuals can be compared, allowing for potential analysis across a population, as well as comparisons across sites of similar time periods and cultural groups. Beaumont and colleagues (2013) advanced this methodology. The authors presented two methods of dentine microsampling, with the aim of identifying nutritional stress during childhood through the examination of adult remains. Method one involves embedding a tooth in plaster prior to sectioning, which allowed very accurately measured increments of dentine to be sliced from the tooth with a thin sectioning saw; it was determined that 1mm sections were sufficient in providing enough collagen for analysis (Beaumont et al. 2013, 284). The second method they propose involves demineralizing a tooth prior to sectioning. Instead of a saw, this method uses a scalpel. Each method has an advantage –

method one allows for more accurate measurements but loses zones of dentine between sections because of the kerf taken by the rotating saw; method two allows for more dentine to remain intact and for the potential to create slices that follow tooth morphology, but measurements may be less accurate (Beaumont et al. 2013, 292). Method two of Beaumont et al. (2013) is currently a popular method for studies of human dentine (Henderson, Lee-Thorp, and Loe 2014; van der Sluis, Reimer, and Lynnerup 2015; Kwok 2015).

A different approach has been developed where a micro coring tool or "cardpunch" is used to obtain microsamples of 0.75mm to 1mm to examine early stages of life, and target the microsamples as pre- or post-birth based on their position relative to the neonatal line (Burt and Garvie-Lok 2013). The method was successful in detecting signals from fetal life, infancy and weaning (Burt and Garvie-Lok 2013, 3863). This method has been successfully applied in studies seeking to examine neonatal and infant life in the past (Burt and Amin 2014; Burt 2015), but is too laborious to be practical for looking at later-forming teeth, where there is no neonatal line to be concerned with and slices of dentine work just as well (Garvie-Lok 2018b).

Beaumont and Montgomery (2015) have built on Beaumont et al. (2013) by presenting a method of estimating the age represented by each dentine section. Essentially, dentine formation is displayed on a diagram plotted against the ages at which key moments in growth (crown initiation, crown completion and root completion) are likely to occur based on studies of tooth formation in modern children. Diagrams and calculations are given for estimating the age of dentine sections for every deciduous and permanent tooth (Beaumont and Montgomery 2015). Further details on this method, which was chosen for use in this study, are provided in Chapter 5. These authors successfully apply the microsampling and age estimation methodologies to a skeletal sample linked to the Great Irish Famine of the 19th century (Beaumont and Montgomery

2016). The technique allowed dietary shifts linked to the onset of the famine and the provision of famine relief to be identified. The work is a good example of how well time resolution has increased, showing that researchers are now capable of discussing dietary changes in relation to the approximate ages at which they occurred, providing information about past lives.

3.6 Palaeodietary Studies of Ancient Greece

Before stable isotope analysis was applied to dietary reconstruction, past Greek diets were reconstructed using other archaeological evidence (including faunal and botanical remains) and written historical sources. Most of what is known about historical period diets was gathered from written accounts and artwork. With the increasing popularity of stable isotope analysis to study diet in the last two decades, bioarchaeologists have expanded our knowledge by adding to what we know and confirming or falsifying what was suspected based on other evidence. A large number of isotopic studies have been published for the Greek world (Papathanasiou and Richards, 2015). This brief review focuses specifically on evidence for the role of marine resources in the diet. Studies more relevant to the topic of childhood diet (especially (Kwok, Garvie-Lok, and Katzenberg 2018) are not covered here but are considered in chapter 7.

Vika (2011) reconstructs the diet of populations from the prehistoric, Classical, and Hellenistic periods buried in Ancient Thebes. Overall commonalities in diet are identified between these populations despite the large time span covered. The stable isotope results show a C_3 plant-based diet with some contributions of terrestrial animals, a consistent pattern seen in Greece since the Bronze Age (Vika 2011, 1159). This general isotopic pattern is commonly seen in all eras of Greece, with C_3 cereals, and terrestrial animals such as sheep, goat, cow, pig, and dog being the most significant contributors to diet, while fish appears to have rarely been consumed (Vika 2011, 1157).

A study by Lagia (2015) examines the diet of eighty individuals from the Classical, Hellenistic and Imperial Roman periods, who are buried in the Kerameikos and Plateia Kotzia cemeteries in Athens. Stable isotope analyses of bone collagen revealed a diet largely dependent on terrestrial C_3 resources and a large contribution from terrestrial animal products (Lagia 2015, 125). Contributions made by marine resources were largely unidentifiable in this sample, though Lagia (2015, 126) argues that this may in part be due to the consumption of low trophic level marine resources that are difficult to distinguish isotopically from terrestrial animal products. Another study by McConnan Borstad et al. (2018) examined dietary changes over time in twenty-four individuals from the Hellenistic, Roman, and Byzantine periods of the site of Helike. Stable isotope analysis indicates modest marine resource consumption in individuals from the Hellenistic period; Byzantine individuals have values indicating less marine use, and Roman period individuals demonstrate a diet consistent with complete reliance on C_3 terrestrial resources and terrestrial animals (McConnan Borstad, Garvie-Lok, and Katsonopoulou 2018, 8). It is evident that fish was accessible, but this access to marine resources was not taken advantage of in every era (McConnan Borstad, Garvie-Lok, and Katsonopoulou 2018). The studies by Lagia (2015) and McConnan Borstad et al. (2018) thus show a pattern similar to that seen by Vika (2011) at Thebes: across the Classical through Roman eras there is surprisingly little evidence of marine resource consumption. Even at coastal sites like Helike, fish may have been accessible but that does not mean it was always consumed.

Various studies have been conducted on populations from the Byzantine Empire, which followed Roman Greece. Bourbou and colleagues (2011) examine the stable isotope results of eight Greek Byzantine populations from different periods of the Byzantine era. The authors found that a similar diet was likely consumed at all of these sites despite their differences in

geography and time. They also make note of how previous isotope work conducted on prehistoric Greek sites provide little or no evidence for marine resource consumption (Bourbou et al. 2011, 569). While written sources often mention fish in terms of guidelines for fishing, the medical benefits of consuming fish and fish preparation, these sources lack information on the significance of fish in the Byzantine diet (Bourbou et al. 2011, 571). This is common in all eras throughout Ancient Greece. There are literary accounts of fish consumption but this is not reflected in stable isotope analyses that have been conducted on populations from the Neolithic (Papathanasiou 2015), Bronze Age (Triantaphyllou et al. 2008; Papathanasiou 2015; Vika 2015), select Mycenaean populations (Iezzi 2015), the Geometric Period (Panagiotopoulou and Papathanasiou 2015), the Greco-Roman Period (Kwok and Keenleyside 2015) and the Byzantine Period (Bourbou et al. 2011).

For all of the populations in the studies mentioned above, the isotope evidence does agree that C_3 terrestrial resources and terrestrial animals were the main contributors of dietary protein in the Ancient Greek diet (Papathanasiou and Richards 2015, 199; Triantaphyllou et al. 2008, 3030). This agrees with the data presented in Vika (2011). While most Greek sites have either a lack of archaeological fish remains and/or isotopic evidence that does not suggest marine resource consumption, there are cases where the isotopic results do indicate higher levels of marine consumption, such as with studies conducted at Mycenae Grave Circles A and B (Richards and Hedges 2008). However, this specific example is of an elite burial group which is not typical of archaeological populations in Ancient Greece.

It is important to note that dairy products and eggs show stable isotope values that are indistinguishable from the meat of the domesticates that produced them (Papathanasiou and Richards 2015). High $\delta^{15}N$ values suggesting considerable animal protein consumption are seen

in the Byzantine era; given that animals were a valuable resource, these are most likely explained by dairy and egg consumption (Bourbou and Garvie-Lok 2015, 188).

While it is always preferable to compare the stable isotopes of a human population to those of the resources that population could access, some researchers have attempted to use the research reviewed above to suggest the $\delta^{13}C$ and $\delta^{15}N$ values typical of certain Greek diets. For $\delta^{13}C$, they have suggested that Greek individuals depending on marine resources should have values of approximately -12‰, roughly 10‰ higher than individuals who have a largely C_3-based terrestrial diet, who should show values around -19‰ (Richards 2015, 21; Papathanasiou and Richards 2015, 197). For $\delta^{15}N$, they have suggested that values of 10‰ or higher indicate marine resource consumption (Richards 2015, 21; Papathanasiou and Richards 2015, 199). These researchers argue that instances where $\delta^{13}C$ values are elevated above -19‰ without associated elevation in $\delta^{15}N$ are likely indicative of the consumption of C_4 plants such as millet, rather than marine resources (Papathanasiou and Richards 2015, 197).

3.7 Summary

This chapter discusses fundamental information that is necessary for understanding stable isotope analysis, specifically for the elements carbon and nitrogen. Topics such as breastfeeding, and growth and stress are briefly discussed. An emphasis is placed on identifying and recognizing marine resource consumption through stable isotope values in archaeological populations because this is one of the main objectives of the thesis. The method of dentine microsampling is introduced in this chapter, and developments of the method are explored in Chapter 5. A brief review of some isotopic analyses previously conducted in Ancient Greece is provided.

Chapter 4: Site and Sample

This chapter explores the history and archaeology of the site of Kenchreai, Greece. It explains the history of excavation at the site and outlines the research that has been conducted to date. Burial styles, grave conditions, and taphonomy are discussed, with an emphasis on the tombs from which the remains in this study originate. A brief summary of the history of Isthmia and results of the osteological analysis of the remains from this site are included as well. The bone collagen isotope results that were presented in Garvie-Lok (2010) are discussed for both sites. An overview of the faunal remains used as reference values in this study is provided.

4.1 The Roman Empire: Corinth, Kenchreai and Isthmia

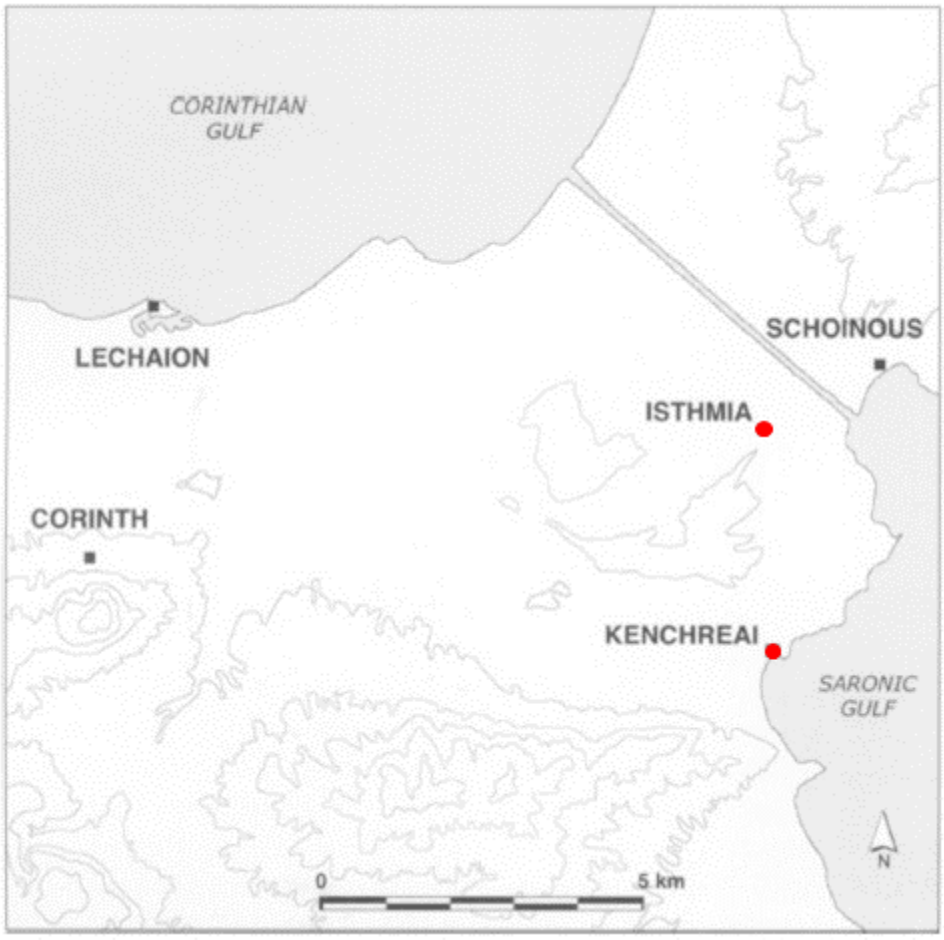
Corinth was an important location for military and economic endeavours in ancient Greece (Rothaus 1995, 293). It was also a central point of access for travel between Central and Southern Greece (ASCSA 1969, 2). Two harbours aided in these ventures: Lechaion, situated on the Gulf of Corinth, and Kenchreai, on the Saronic Gulf (ASCSA 1969, 1). The city of Isthmia was also integral for Corinth because its geographic location provided a military advantage over invaders from the north (Rife 2012, 121). While Corinth and Isthmia were connected by traffic, military and trade, they operated on different levels in terms of population size, with Corinth being more urban and Isthmia being more rural (Rife 2012, 50). Lechaion, Kenchreai, and Isthmia were under Corinth's political control, and together these locations worked to serve the Roman Empire.

4.2 Kenchreai

The site of Kenchreai was a significant location within Roman Greece. It was occupied by Romans during the 1st – 3rd centuries AD, referred to as the Early Roman Period, and the 5th – 7th centuries AD, referred to as the Late Roman and Early Byzantine Periods (Rife et al. 2007).

Archaeological evidence of material from the Classical and Hellenistic Periods is sparse (Rife et al. 2007).

Figure 4. 1 Map illustrating the location of Kenchreai and Isthmia on the Isthmus of Corinth (modified from Rife et al. 2007, 144).



The site sits on the eastern shore of the Isthmus of Corinth, near the Saronic Gulf of the Aegean Sea (Ubelaker and Rife 2008, 97). A major harbour was located in the town. This harbour allowed Kenchreai to play an active role in supporting commercial endeavours, mobility, and interaction within the empire (Rife et al. 2007, 143). Despite this, the town was not under its own administration as it was governed by the city of Corinth (Rife et al. 2007, 143).

4.2.1 Excavations at Kenchreai

The site has been studied infrequently by archaeologists since 1907. Sarris and colleagues (2007) mention that looting has been an issue over the course of excavations at Kenchreai. Nevertheless, many artifacts and human remains were undisturbed by looters, allowing archaeologists to gain a rather complete picture of life at the site.

Preliminary archaeological work at Kenchreai began in 1963 (Scranton and Ramage 1963). The aim of this field season was to determine the extent of the site. Four main areas were studied. The main focus of the work was to examine the architecture of the structures on land and those which now sit below sea level as a result of changing terrain. Researchers identified the remains of columns and buildings located around the harbour, some of which date to the early Byzantine period (Scranton and Ramage 1963). The walls of potential commercial buildings were recognized as well. Archaeological evidence was found for the Roman and early Christian periods. Notable finds from this season include over one thousand coins, many Roman lamps, glass, and large amounts of Roman pottery (Scranton and Ramage 1963).

Further excavations took place from 1963 to 1968, which continued to examine the harbour and the archaeological remains now located below sea level. Findings from the excavation season in 1965 uncovered artifacts such as pottery and figurines that date back to the 6th century B.C (Scranton 1965). Other excavation seasons examined a room that appears to have been destroyed by an earthquake (Scranton 1979), although information about this supposed earthquake is contested (Rothaus, Reinhardt, and Noller 2008). The room in question contained pieces of a glass opus sectile mosaic. Material made from carved ivory and bone was also identified here, along with architectural tools and unfinished structures. Scranton (1979) explains

that these findings are important in understanding the proposed A.D. 375 earthquake and the history of the site.

Numerous volumes have been published from the original excavations which provide detail of the materials found at Kenchreai. This includes information on the landscape and architectural remains (Scranton, Shaw, and Ibrahim 1978), coins (Hohlfelder 1978), ceramics (Adamsheck 1979), lamps (Williams 1981), the aforementioned glass opus sectile mosaic (Scranton, Ibrahim, and Brill 1976), and the items manufactured from ivory, bone and wood (Stern et al. 2007).

4.2.2 The Kenchreai Cemetery Project

Under the direction of Dr. Joseph Rife, another archaeological study was conducted in Kenchreai from 2002 to 2006. This investigation, known as The Kenchreai Cemetery Project (KCP) aimed to examine the main cemetery of the town and study two significant elements of life at Kenchreai: "commercial prosperity and the formation of a local elite; and cultural diversity with prevalent eastern influences" (Rife et al. 2007, 148). This notion of cultural diversity at Kenchreai is taken from the written records; philosophers such as Apuleius and Favorinus described the town as being inhabited by a large and diverse population (Rife et al. 2007, 176).

KCP investigated the major cemetery of Kenchreai, which is located north of the harbour on the Koutsongila ridge (Rife et al. 2007). Other cemeteries were discovered in Kenchreai but were not a focus of this study. However, it was found that artifacts and mortuary practices in graves located south and west of the harbour emulate those of the north cemetery (Rife et al. 2007). Based on this, Rife (2007, 146) states that the graves found in the north cemetery can be considered characteristic of mortuary practices in Roman Kenchreai.

The KCP study was an interdisciplinary effort. Various methods were used to record information about the geography, culture, and skeletal remains of the north cemetery. This was all in an attempt to understand the ritual process of burial at Kenchreai (Rife et al. 2007). The extensive investigation conducted by Rife and associates (2007) has allowed for a holistic understanding of the mortuary behaviour associated with this ancient cemetery. This includes examining the process of burial and meaningful aspects of life that occurred before, during and after burial rituals (Rife et al. 2007, 146). As a result of this unique approach, researchers at Kenchreai were able to examine how material culture and space aid in ritual practices and portraying an individual's identity in Kenchreai (Rife et al. 2007, 146).

One particular avenue of study that was helpful in the KCP is the use of mapping technology. Studying the surrounding space of the burials, including the orientation of graves, their position on the Koutsongila ridge, and their relationship with the surrounding environment supported one of the main goals of KCP. Applying mapping and surveying techniques to this project allowed the researchers to speculate about access to geological resources, the transformation of the ancient coastline, architectural features and other uses for the ridge after Roman occupation (Sarris et al. 2007). With this information researchers were able to create a reconstruction of the ridge. Sarris and associates (2007) were also able to identify burials that remained undisturbed by looters, as well as examine architectural features. They say that the ridge was an ideal location for graves and walls, because of its sturdy geological properties (Sarris et al. 2007, 20). The authors state that the construction of the tombs was meticulously planned by those who built them, as evidenced by the skillful use of the natural environment and the local stratigraphy (Sarris et al. 2007, 20). Thus, there was an awareness of local geography in creating these tombs.

Studies of Kenchreai are ongoing. Further excavations of the cemetery and residence located on the Koutsongila Ridge occurred from 2007-2009 under the co-direction of Joseph Rife and Elena Korka, and additional excavations began in 2014 (Rife and Heath 2013).

4.2.3 Kenchreai: The Burial Contexts

KCP discovered 30 chamber tombs and 28 distinct cist graves cut into the bedrock of the Koutsongila ridge (Ubelaker and Rife 2008). Ubelaker and Rife (2008) describe the chamber tombs themselves as being well preserved even though they were in use for centuries. The authors report that a small number of epitaphs were discovered, which inform researchers that tombs were used to bury generations of families and free persons (Ubelaker and Rife 2008, 98). This claim is supported by the demographic profile of the skeletal evidence, which reveals individuals of varying age, sex, and apparent status. The chamber tombs contained two modes of burial – loculi and niches. The loculi are long and narrow sections located in the lower chamber walls, which is where some individuals were inhumed, whereas niches were located in the upper portion of the chamber walls and hold urns containing cremated remains (Ubelaker and Rife 2008, 101). According to the archaeologists, few niches actually contained urns or cremated remains. Urns were commonly reused, and the original cremated remains were often emptied into the niche or onto the chamber floor (Ubelaker and Rife 2008, 101).

While the tombs were created in the Early Roman Period, there is evidence suggesting the tombs were reused ca. 4th to 7th centuries A.D., during the Late Roman Period. Individuals laid to rest in loculi would be covered with a shallow layer of dirt before the loculi were sealed with a slab of stone (Ubelaker and Rife 2008, 101). Evidence for the cemetery's use in the Late Roman Period stems from bodies being placed over the stone tiles that cover the loculi. The archaeologists of KCP say that it is difficult to say whether the addition of these remains affected

the remains interred during the Early Roman Period. Moreover, Kenchreai has a known history of looting (Ubelaker and Rife 2008, 101). Looters who entered the tombs in search of lavish artifacts would have potentially removed the tiles and moved the skeletal remains during their search, resulting in fragmented and commingled remains (Ubelaker and Rife 2008, 101). Taphonomic processes such as water dripping into the chambers and the growth of pine roots, as well as rodent and snail nests, have impacted the graves by moving bones and causing damage (Ubelaker and Rife 2008, 101). It is possible that a combination of factors such as tomb reuse, looting, and taphonomic processes have led to the commingling and destruction of some of the Early Roman Period remains. The authors are certain that commingling did not occur between individual loculi and niches, only within them (Ubelaker and Rife 2008, 101). Thus, we can assume that all skeletal remains uncovered in a single niche or loculi were interred there during the Early or Late Roman Period, indicating the regular use of the loculi throughout the Roman occupation of the site. Additionally, the authors suggest that there is little chance that remains were commingled during Roman times, and that the commingling is primarily a result of looters displacing tiles and burials in more recent history, in combination with taphonomic processes (Ubelaker and Rife 2008, 101).

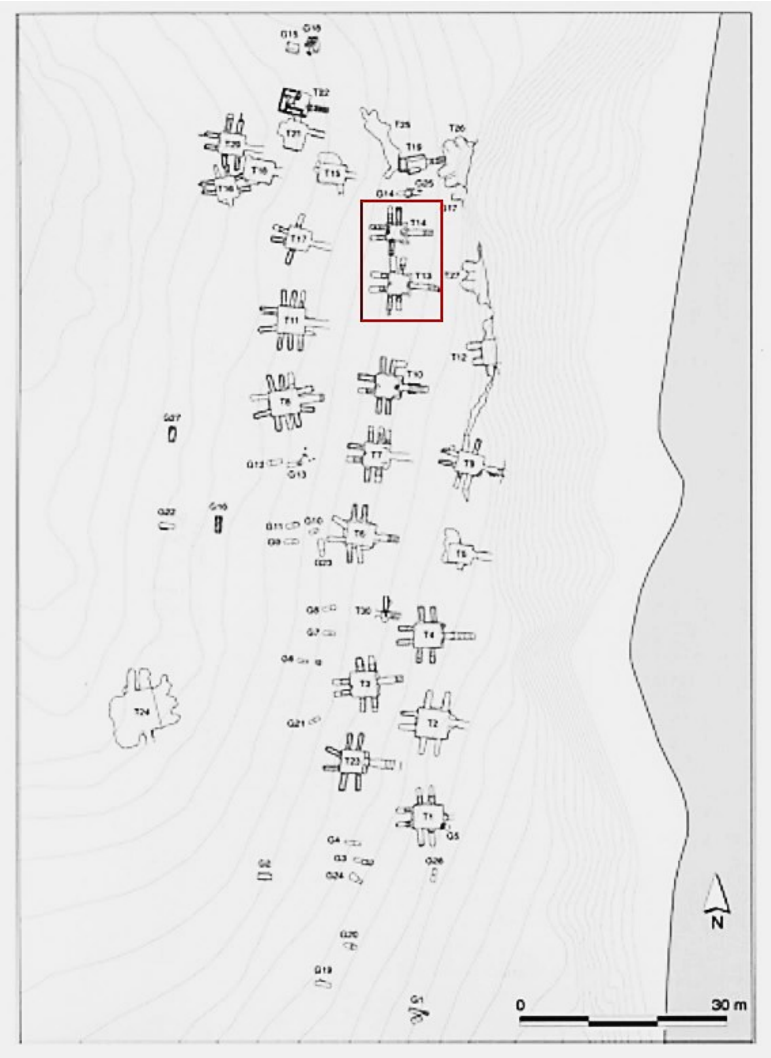
Based on the monumental style, decorations and prominent location of the graves, we can interpret that they were built for elite families (Rife et al. 2007, 175). The reconstruction of the Koutsongila coastline made it possible for researchers to note that the tombs are in close proximity to the sea, and thus would have been visible to ships sailing into the harbour from the northeast and east (Rife et al. 2007; Sarris et al. 2007). As well, tombs would have been visible to those traveling on the nearby road. This form of display of monumental tombs indicates a stratified community given that the monuments use a large area of space in a highly visible

location to demonstrate social identity (Rife et al. 2007, 151). This conveys a message to both locals and foreigners of wealth and status. Moreover, it may convey information about family values and the importance of commemoration (Closterman 2007, 635). This is a phenomenon that appears at other Greek and Roman sites, where tombs may be built along a roadside (Rife et al. 2007, 151). The Kerameikos cemetery in Athens is a notable example (Stupperich 1994; Closterman 2007). There is no information pertaining to the status of the individuals from the Late Roman Period, or any signs pointing to familial relations to the individuals from the Early Roman Period burials.

4.2.4 Kenchreai Tombs 13 & 14

The skeletal remains examined in this study originate from two of the thirty tombs: Tomb 13 and Tomb 14. They are highlighted in Figure 4.2. Ubelaker and Rife (2008) discuss these tombs specifically because they are severely commingled. Different approaches were taken to examine commingling in each tomb. In Tomb 13, two loculi were chosen, one of which was Loculus I where SKEC-02 was buried. The distribution of human remains over the stone covers in Tomb 13 was examined spatially (Jaishankar and Rife 2004). This was in an attempt to recognize whether the bones had been moved from their original burial positions, which were broadly identified based on this distribution and using knowledge of common burial practices in this era (Ubelaker and Rife 2008, 103). Given that many small bones were recovered in this context, the researchers determined that the commingled remains do not come from a secondary deposit and that there are remains from a minimum of three adults and one subadult in Loculus I (Ubelaker and Rife 2008, 102).

Figure 4. 2 Map of burials on the Koutsongila Ridge at Kenchreai (modified from Rife et al. 2007).



It should be noted that when choosing samples for this project, the identification of one of the individuals was uncertain. This is a result of the commingled state of these graves. For this individual, there is no way to associate the chosen tooth to one individual or the other. This is the case for the tooth possibly attributed to SKEC-18, which may actually be from SKEC-19. In the present study, this individual will be referred as SKEC 18/19. The original bioarchaeological analysis estimated the ages of both SKEC-18 and SKEC-19 as adults. Because the identity of this individual is uncertain, their isotope values will be considered for each of the possible identities. The individuals from Kenchreai are listed in table 4.1 below.

Table 4. 1 Contextual information for human remains from Kenchreai (Garvie-Lok 2010).

Individual	Grave	Sex Category	Age Category
SKEC-02	Tomb 13, Loculus I cist (T13-032)	Female	Adult (20-25 years)
SKEC-05	Tomb 13, Loculus III (T13-035)	Unknown	Juvenile (5-7 years)
SKEC-09	Tomb 14 Loculus I cist (T14-037)	Unknown	Adult
SKEC-11	Tomb 14, Loculus II cist (T14-039)	Unknown	Adult
SKEC-17	Tomb 14, Loculus III cist (T14-041)	Unknown	Juvenile (approximately 12 years)
SKEC-18 or 19	Tomb 14 Loculus IV cist (T14-042)	Unknown	Adult

4.2.5 Osteological Analyses at Kenchreai

Little information has been published on the osteological analysis of human remains uncovered at Kenchreai. Poor preservation and the fragmentary nature of the remains has prevented much analysis of skeletal morphology and paleopathology, while also hindering the ability to examine age-related changes and dental pathologies (Rife et al. 2007, 159). The available information reveals minor signs of disease and trauma for the Kenchreai population in the forms of fractured bones and periostitis (Rife et al. 2007, 159).

Three of the Kenchreai teeth selected for this study exhibit some dental calculus, and one showed minor signs of dental wear. Otherwise, the Kenchreai teeth showed no signs of pathology. This reflects selection criteria during initial sampling in the field; teeth that were heavily worn, carious or affected by hypoplasia were rejected(Garvie-Lok 2018a).

4.3 Isthmia

The director of KCP, Joseph Rife, conducted a similar study at the neighbouring site of Isthmia. In Rife (2012) he extensively details the work conducted at this site, including osteological analyses, paleopathological reports as well as cultural and historical insights on funerary treatment. Four phases of occupation have been identified at the site based on differences in dates of the graves. The remains date from the Early Roman Period up to the Early Byzantine Period. Phase I and Phase II-III at Isthmia are identified as The Roman Sanctuary (mid 1st to late 4th centuries) and the Late Roman Fortifications, respectively. The two individuals included in the present study date to the early phase. Given the large span of time for which Isthmia was in use, the aim of the Isthmia project was to study how the lives of these Greek individuals changed over these time periods (Rife 2012, 5). Given that occupation at Isthmia spanned centuries, Rife (2012, 5) was able to examine the transformation of society, the rise and fall of religious practices, changing military strategies, patterns in economy and settlement, and external environmental factors, such as exposure to disease and natural disasters.

Similar to Kenchreai, burial practices at Isthmia included the use of cist graves. Some graves also included multiple individuals, and there is evidence that burials were reused. This is indicated by burial practices that deviate from the norm, including unusual orientations of the human remains, incomplete skeletons, and damaged remains (Rife 2012, 15). Rife (2012) states that there are burials at Kenchreai which have a likeness to those at Isthmia. Both sites exhibit multiple interments within a grave, which likely indicate familial groups being buried together. There is more concrete evidence for this at Kenchreai based on the discovery of the inscriptions present on the exterior of some tombs.

4.3.1 Osteological Analyses at Isthmia

While little is known about the oral health of the population at Kenchreai, there is an abundance of health information about the Isthmia population. An overview of health information is presented alongside an overview of general diet for Isthmia. A variety of sticky, oily and coarse foods were available in this region, all of which play a role in the development of dental calculus, periodontal disease and the deterioration of teeth (Rife 2012, 295). No archaeological evidence was uncovered that could elaborate on oral hygiene practices (Rife 2012, 295). Aside from dental diseases, the most common paleopathological conditions found at Isthmia are arthritic degeneration of the shoulders and vertebrae, fractures, and cribra orbitalia (Rife 2012, 368). Less common conditions include various forms of congenital disorders, joint disease, and infectious diseases such as periostitis (Rife 2012, 368). Paleopathological conditions were noted on the two individuals from Isthmia used in this study, and that information is listed in Table 4.2. Subsistence practices and settlement behaviours may have influenced overall health at Isthmia. In general, malnutrition and infection resulting from subsistence habits and factors associated with urban settlements were a common occurrence among children at Isthmia throughout the Late Roman to Early Byzantine periods (Rife 2012, 311).

Table 4.2: Contextual information for the human remains from Isthmia (Rife 2012, 369-370).

Individual	Grave	Sex Category	Age Category	Paleopathology	Dental Conditions
Isthmia 67-001D	North-east Gate Grave 1	Female	Adult (20-21 years)	Lipping, vertebra	None-slight wear; slight-medium calculus (10 teeth); antemortem tooth loss (1 tooth)
Isthmia 69-004E	North-east Gate Grave 4	Female	Adult (35-44 years)	Rotator cuff disease, right shoulder; porosity, left clavicle	Linear enamel hypoplasia (2 teeth); slight-moderate wear; slight calculus (4 teeth); 1 carious tooth, periodontitis and periapical granuloma (2 teeth); antemortem tooth loss (1 tooth)

4.4 Isotopic Analysis of Bone Collagen at Kenchreai and Isthmia

The results of previous stable isotope work conducted on the human remains from Kenchreai and Isthmia are reported in Garvie-Lok (2010). The author discusses patterns in the isotope results from the two sites. The results suggest that the diet of each population was different, although they both show signals indicative of consuming diets that include terrestrial and marine resources. However, $\delta^{15}N$ values for individuals from Kenchreai are mostly higher than those for the population from Isthmia. Garvie-Lok (2010) states that this likely means marine resources contributed to the diet of those from Kenchreai more so than those at Isthmia. Figures 4.3 and 4.4 display the isotope data obtained from bone collagen.

Garvie-Lok (2010) interprets patterns within the populations as well. In examining the diets of infants and children, it is noted that children show different stable isotope values when compared to the adults. More specifically, the isotope results from older children at Kenchreai show a marked difference in both $\delta^{13}C$ and $\delta^{15}N$, having lower values than the adults for both isotopes (Garvie-Lok 2010, 5). Their values are typical of individuals for which marine resources are not a large part of the diet. According to Garvie-Lok (2010), this observation is in agreement with the literature, which often describes fish as a food source for high-status individuals. Therefore, older children may have consumed less fish than adults.

Figure 4. 3 $\delta^{13}C$ and $\delta^{15}N$ obtained from bone collagen from individuals at Kenchreai (Garvie-Lok 2010).

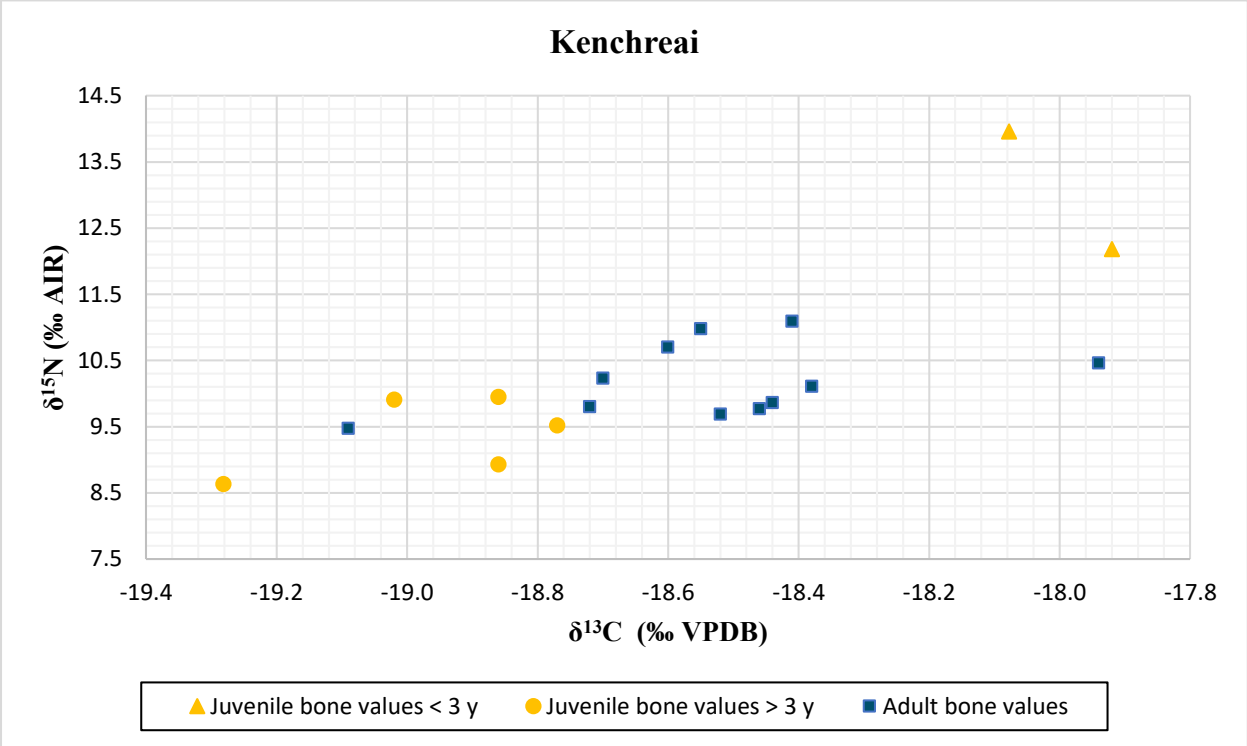
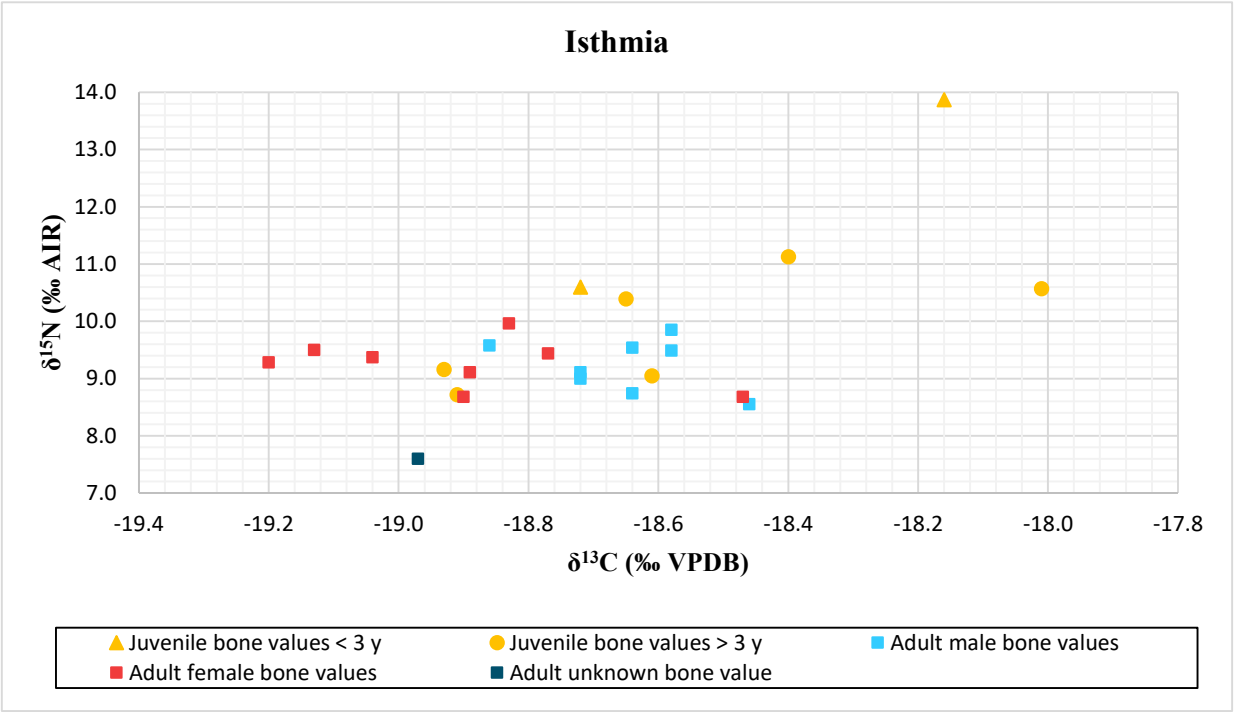


Figure 4. 4 $\delta^{13}C$ and $\delta^{15}N$ obtained from bone collagen from individuals at Isthmia (Garvie-Lok 2010).



The aforementioned study also includes a comparison of the coastal sites of Kenchreai and Isthmia to the Late Roman inland sites of Nemea (ca. 5th – 6th c. C.E.) and Stymphalos (ca. 4th – 6th c. C.E.), both of which had a diet almost exclusively of land-based resources. When comparing Isthmia to these two sites, a clear elevation is seen in $\delta^{13}C$ and $\delta^{15}N$ values at Isthmia although some overlap exists, suggesting that the population at Isthmia ate mostly land-based resources but incorporated marine sources into their diet (Garvie-Lok 2010, 3). In contrast, Kenchreai displays higher $\delta^{13}C$ and $\delta^{15}N$ values, showing little overlap with the populations from Nemea and Stymphalos. This may indicate that Kenchreai placed a higher emphasis on including marine foods into the diet. Overall, the stable isotope values provide a clear example of dietary differences between inland and coastal populations.

4.5 Faunal Comparative Values

There are no faunal remains that have been recovered from Kenchreai or Isthmia that could be used in this study. More specifically, there were no fish bones recovered. Animal remains are frequently unavailable at Greek archaeological sites where human remains are studied through stable isotope analysis because so many of these studies use human remains from contexts that typically don't contain animal remains, such as cemeteries (Richards 2015, 21). Instead, terrestrial and marine isotope values originating from faunal remains from comparable sites and time periods in Greece are used as a comparative dataset. Time periods that fall outside of the Early and Late Roman periods are accepted in this study following the logic that consumable resources tend to stay the same through time in environments that remain relatively unchanged (Rife 2012, 295). The remains of sheep/goat, pig and cow were available at each site, with the addition of dog remains at Helike. It was possible to obtain isotope values for

marine resources from a couple of studies, but this information is still limited temporally and by species.

Bourbou and colleagues (2011) examined human remains from eight Byzantine period archaeological sites in Greece in order to reconstruct marine resource consumption and other aspects of Byzantine diets. That study included comparative land fauna values from a number of archaeological sites, as well as stable isotope values from modern Aegean fish purchased at a market in Athens, as a comparison for the human bone collagen values (Bourbou et al. 2011, 573). A subset of this faunal data – all of the fish values, as well as archaeological fauna from Corinth and Athens – was selected to provide a faunal comparative sample for this study. These values are displayed in Table 4.3. Also included in Table 4.3 are two archaeological fish samples recovered from post-medieval deposits at the site of Zaraka, located in the inland Peloponnese approximately 30 km from Corinth (Bourbou et al. 2011; Pennycook 2008).

Another relevant study used stable isotope analysis to answer questions regarding changes in marine resource use over time at Helike, a Peloponnesian coastal site about 100 km from Corinth (McConnan Borstad, Garvie-Lok, and Katsonopoulou 2018). Included in that investigation is a small sample of Hellenistic era faunal remains from Helike, as well as a number of faunal remains dating to Roman period Corinth. This faunal information is also used as a reference point in the current study for the values of terrestrial animals and is presented in Table. 4.4.

Noticing a gap in the literature, Vika and Theodoropoulou (2012) set out to study the stable carbon and nitrogen isotope values of fish remains from archaeological sites in Greece. The authors collected fish samples from four sites in Northern Greece (Dispilio, Archontiko, Toumba Thessalonikis, and Karabournaki), and two sites on the Aegean islands (Maroulas-

Kythnos and the Cave of Cyclops-Youra). These sites are prehistoric and range from the Mesolithic to the Early Iron Age. A subset of the stable isotope values from that study (omitting freshwater fish) was chosen for use in this study and is presented in Table 4.5. Although these fish come from sites much older than Isthmia and Kenchreai, they represent the largest currently available database of preindustrial Aegean fish values, and are used here for that reason.

While marine isotope values vary by species, in general there is a tendency for marine resources to show higher $\delta^{13}C$ and $\delta^{15}N$ values. This trend is illustrated in Figure 4.5 which displays all of the comparative terrestrial faunal values to marine values that are used in the current study. Most of the stable carbon and nitrogen values for the fish samples are noticeably elevated and distinct from those of the terrestrial animals. Although the variability of marine resources according to location and species can make analyses challenging (Vika and Theodoropoulou 2012, 1625), these general differences still allow the signals of land and marine resources to be distinguished.

Table 4. 3 Faunal information from Athens (6th-18th c AD) and Corinth (12th-13th) for terrestrial animals, and isotope values for modern Aegean fish (Bourbou et al. 2011).[6]

	Animal	$\delta^{13}C$	$\delta^{15}N$
Athens	Sheep/Goat	-19.9	3.3
	Sheep/Goat	-19.8	11.7
	Sheep/Goat	-19.2	3.9
	Sheep/Goat	-20.1	8.0
	Sheep/Goat	-19.9	9.3
	Sheep/Goat	-19.7	3.9
	Sheep/Goat	-19.5	4.2
	Sheep/Goat	-19.4	5.6
	Sheep/Goat	-19.0	7.3
	Sheep/Goat	-19.9	3.7
	Pig	-20.9	6.4
	Pig	-20.3	4.5
	Pig	-18.9	9.1
	Pig	-19.6	4.0
	Pig	-20.8	6.4
	Pig	-21.1	6.2
	Cow	-21.1	4.6
	Cow	-19.9	6.3
	Cow	-19.9	6.7
	Cow	-19.9	6.4
Corinth	Sheep/Goat	-19.8	5.3
	Sheep/Goat	-19.8	3.5
	Sheep/Goat	-19.9	4.0
	Sheep/Goat	-19.1	3.1
	Pig	-19.6	3.6
	Cow	-20.4	4.2
Modern Aegean Fish	Gilt-head sea bream	-17.9	9.7
	White sea bream	-12.1	10.6
	Red mullet	-15.4	8.5
	Horse mackerel	-15.5	8.1
	Sardine*	-16.4	6.7
	Anchovy*	-17.5	6.3
Archaeological Fish	sea bream	-12.1	7.5
	tuna	-10.4	9.6

[6] *Indicates that these are the mean values for four individuals per species.

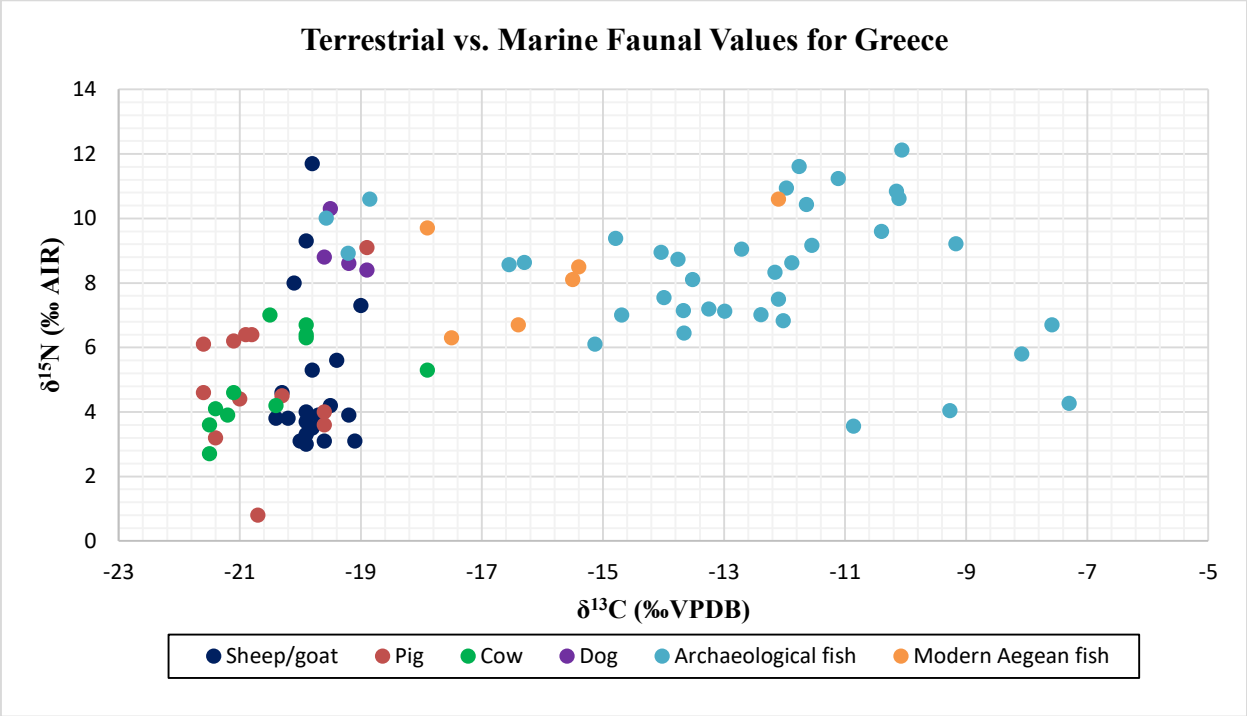
Table 4. 4 Isotope values for terrestrial animals from Helike (Hellenistic period) and Corinth (Roman period) (McConnan Borstad, Garvie-Lok, and Katsonopoulou 2018).

	Animal	**$\delta^{13}C$**	**$\delta^{15}N$**
Helike	Sheep/Goat	-20.4	3.8
	Pig	-21.0	4.4
	Cow	-20.5	7.0
	Cow	-21.2	3.9
Corinth	Sheep/Goat	-19.9	3.0
	Sheep/Goat	-19.6	3.1
	Sheep/Goat	-20.0	3.1
	Sheep/Goat	-20.2	3.8
	Sheep/Goat	-20.3	4.6
	Pig	-21.6	4.6
	Pig	-21.6	6.1
	Pig	-20.7	0.8
	Pig	-21.4	3.2
	Cow	-21.5	3.6
	Cow	-21.4	4.1
	Cow	-21.5	2.7
	Cow	-17.9	5.3
	Dog	-19.2	8.6
	Dog	-19.5	10.3
	Dog	-19.6	8.8
	Dog	-18.9	8.4

Table 4. 5 Isotope values for marine resources from prehistoric sites in Greece (Vika and Theodoropoulou 2012).

Sample	Site	Family	Water	$\delta^{13}C$	$\delta^{15}N$
S-EVA-12794	Youra	Mugilidae	E	-10.86	3.56
S-EVA-12806	Youra	Mugilidae	E	-9.27	4.04
S-EVA-12800	Youra	Mugilidae	E	-12.99	7.12
S-EVA-12803	Youra	Serranidae	M	-14.04	8.95
S-EVA-12804	Youra	Scorpaenidae	M	-11.55	9.16
S-EVA-12807	Youra	Scorpaenidae	M	-13.76	8.73
S-EVA-12808	Youra	Serranidae	M	-14.69	7.00
S-EVA-12809	Youra	Sparidae	M	-13.25	7.19
S-EVA-12810	Youra	Sparidae	M	-13.52	8.10
S-EVA-12795	Youra	Serranidae	M	-12.71	9.05
S-EVA-12798	Youra	Serranidae	M	-19.21	8.92
S-EVA-12799	Youra	Thunnidae	M	-12.39	7.01
S-EVA-12801	Youra	Thunnidae	M	-13.66	6.44
S-EVA-12802	Youra	Sparidae	M	-13.99	7.54
S-EVA-12768	Archontiko	Sparidae	E	-19.57	10.01
S-EVA-12765	Archontiko	Sparidae	E	-14.79	9.38
S-EVA-12764	Archontiko	Sparidae	E	-18.85	10.60
S-EVA-12766	Archontiko	Sparidae	E	-16.30	8.63
S-EVA-12758	Toumba	Sparidae	M	-9.17	9.21
S-EVA-12762	Toumba	Thunnidae	M	-12.02	6.83
S-EVA-12761	Toumba	Moronidae	E	-11.64	10.43
S-EVA-12755	Toumba	Sciaenidae	E	-10.06	12.12
S-EVA-12760	Toumba	Mugilidae	E	-12.16	8.33
S-EVA-12756	Toumba	Mugilidae	E	-10.15	10.84
S-EVA-12759	Toumba	Sparidae	E	-8.08	5.80
S-EVA-12754	Toumba	Sparidae	E	-7.58	6.70
S-EVA-13836	Karabournaki	Mugilidae	E	-7.30	4.27
S-EVA-13843	Karabournaki	Sparidae	E	-11.97	10.94
S-EVA-13835	Karabournaki	Thunnidae	M	-13.67	7.14
S-EVA-13837	Karabournaki	Scombridae	M	-16.55	8.57
S-EVA-13838	Karabournaki	Sparidae	M	-10.11	10.62
S-EVA-13839	Karabournaki	Scombridae	M	-15.13	6.10
S-EVA-13840	Karabournaki	Sparidae	M	-11.76	11.61
S-EVA-13841	Karabournaki	Sparidae	M	-11.11	11.24
S-EVA-13842	Karabournaki	Thunnidae	M	-11.88	8.62

Figure 4. 5 $\delta^{13}C$ and $\delta^{15}N$ obtained from terrestrial and marine faunal remains in Greece.



4.6 Summary

This chapter provides an overview of the history of Roman Kenchreai and the details of past archaeological work conducted at the site. It discusses Kenchreai's relationship to the contemporary site of Isthmia, and their connections to the larger city of Corinth. Together these sites worked to serve the Roman Empire and functioned as points of exchange.

More specifically, contextual information regarding burials and the condition of the human remains has been presented. Factors affecting sample preservation and identification have been explained alongside how they influence the current study. All information that is known regarding the health of the selected individuals is presented here. Stable isotope values for the previous bone collagen analysis are briefly discussed, and the comparative faunal values for terrestrial and marine resources from other Greek sites are included.

Chapter 5: Methods

Essential information regarding tooth formation and the mineralization of dentine is outlined below. This knowledge is important for understanding how the process of dentine microsampling works and why it is a significant development in stable isotope analysis. The development of dentine microsampling as a means of isotope analysis was briefly discussed in Chapter 3. The method of estimating age from microsamples is elaborated upon in more detail here. Details of sample preparation and analysis for the present study are discussed. An assessment of preservation and suitability for analysis is provided for the Kenchreai and Isthmia samples.

5.1 Tooth Formation

To examine the chemical signatures of an individual's dentition, archaeologists can examine both dentine and enamel. To explain how these structures retain chemical information, one can look to their formation process. The two most significant precursor tissues from which teeth are formed are epithelium and mesenchyme. While these tissues are responsible for creating different parts of a tooth, they eventually coalesce to form a complete tooth. This process occurs within three main stages of tooth development.[7] Teeth begin to form in the early period of juvenile skeletal development, with deciduous tooth formation initiating at approximately 4-5 weeks in utero with the appearance of the dental laminae. Following the formation of the dental laminae from epithelium, portions of epithelium pierce the surrounding mesenchyme. These portions of epithelium become the tooth buds, and this is known as the bud stage. The surrounding mesenchyme consolidates with the bud to form the enamel organ, which

[7] Unless otherwise noted, the information in this overview of dental development was taken from Scheuer and Black (2004) and Hillson (1996).

forms from the lamina, and the dental papilla, which is formed from mesenchyme. This is followed by the cap stage, where the dental papilla is surrounded by the enamel organ. An additional thin layer of mesenchyme envelops the enamel organ to create the dental follicle. Together, the dental papilla, enamel organ, and dental follicle make up the tooth germ, which is the precursor of a tooth. Each part of the tooth germ will give rise to different dental structures. The dental papilla will create the dentine and pulp cavity, enamel is formed from the enamel organ, and the dental follicle produces the cementum and periodontal ligament. The final stage in tooth formation is the bell stage, wherein the enamel organ operates to form the tooth cusps. Alongside the contact area where enamel organ and dental papilla meet, the enamel and dentine secreting cells are formed. During mineralization, this area becomes the enamel-dentine junction (EDJ). The enamel and dentine cells formed at this location are the ameloblasts and odontoblasts, respectively. The protein portions of the enamel and dentine matrices are secreted from their respective cells, starting from the cusp tips of the crown and continuing to the root apex. The concluding step in this process is mineralization. The initial protein portion of the secreted dentine matrix, known as predentine, is mineralized to create its final form (Tang, Le Cabec, and Antoine 2015, 206). Odontoblasts continue to exist around the pulp cavity ready to deposit secondary or tertiary dentine in the event of damage to the tooth (Humphrey 2016, 501). This allows some repair, but in a reduced capacity relative to bone as the process of repair is much slower and more limited (Dean 2017, 558). Mineralization is slower for enamel. The primary enamel matrix contains mineral crystals. As the developing matrix passes from its initial secretory stage to the maturation stage, its proteins degrade and the crystals expand into the spaces they leave. These crystals are the final form of enamel. Because the ameloblasts cease to

exist when development is complete, enamel is unable to repair itself in any capacity (Dean 2017, 558).

5.1.1 Dentine Formation

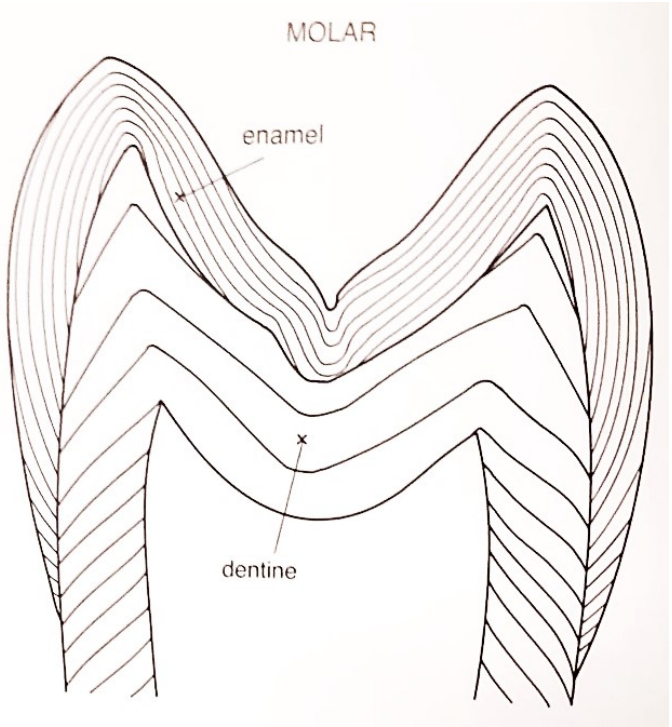
As a tooth develops, dentine is laid down in a systematic matter from the cusp tips of the crown to the apex of the root (Hillson 1996, 121). There are two steps in the creation of dentine. First, the odontoblasts produce a matrix of predentine, and then the odontoblasts mineralize the matrix (Scheuer and Black 2004). This process continues as a tooth erupts, and the timing of eruption differs depending on the tooth.

Although enamel and dentine incorporate the same hydroxyapatite mineral, they differ greatly in their structure, crystal size and organic content (Montgomery 2010, 329). This corresponds to the functional difference between the tissues and explains why dentine is more similar to cortical bone than enamel (Montgomery 2010, 329). This also explains why bone and dentine collagen are ideal for examining carbon and nitrogen isotope signatures while enamel is more suited to exploring strontium and oxygen content. As a result, each structure preserves different information regarding life (Dean 2017, 560). Given these differences, researchers cannot expect dentine and enamel to share reactions to external forces such as taphonomic or diagenetic processes (Montgomery 2010, 329).

It is possible for additional dentine to form later in life after initial tooth formation. While the dentine that forms during infancy and the juvenile period is known as primary dentine, these additional forms are known as secondary and tertiary dentine. Secondary dentine forms within the pulp cavity after tooth formation is complete (Humphrey 2016, 501). It grows within the pulp cavity as a response to exposure of the interior of the tooth as a result of tooth wear (Hillson 1996, 194). Tertiary dentine is formed as a countermeasure against severe damage to a tooth,

such as the damage produced by carious lesions (Humphrey 2016, 501). It is unknown how the inclusion of secondary or tertiary dentine may affect the results of stable isotope analysis, although researchers have speculated that it would obscure any juvenile values with an adult signal (van der Sluis, Reimer, and Lynnerup 2015, 669). It should not be a concern for the Kenchreai and Isthmia samples because none of the teeth selected for this study had severe wear or signs of caries. The crown of SKEC-02 did break apart during initial documentation, resulting in the exposure of the dentine, but this occurred postmortem in the lab. Thus it would not affect the original chemical signature of the tooth although it is a sign to be alert for diagenetic alteration.

Figure 5. 1 An illustration of tooth formation depicting incremental growth of dentine as it develops from crown to root (Hillson 1996, 120).



As illustrated in Figure 5.1, dentine is not precise in its horizontal formation as it is laid down incrementally at increasing angles as formation proceeds into the root. It is difficult to account for this uneven formation during sampling. While it is possible to sample following the

growth lines of dentine using microscopic methods (see Chapter 3), that was outside of the scope of the present study. Increments of dentine were sliced horizontally. Using this method creates some overlap between sequential sections of dentine in terms of the developmental periods they cover, as some of the angled layers will fall into multiple sections. However, in other studies this has not been considered to be a huge concern because it is expected and accounted for. Essentially the overlap should mute signs of dietary shifting over time, but not eliminate them completely (Eerkens, Berget, and Bartelink 2011; Scharlotta et al. 2018). This overlapping period of time is minor and has been estimated to be approximately 1-2 months (Scharlotta et al. 2018, 3).

5.1.2 The Permanent Dentition

Formation of the permanent dentition takes place in the same manner as the deciduous teeth. Initiation times depend on tooth type and range from 3 months (± 1.5 months) to 8.5 years (± 6 months) (AlQahtani, Hector, and Liversidge 2010, 485). Permanent teeth are fully formed and erupted in the teenage years or in early adulthood. The third permanent molar is the final tooth that forms; it typically reaches completion between 16 and 23 years (AlQahtani, Hector, and Liversidge 2010, 485). As a result, the dentine and enamel of the dentition as a whole serve as a record of chemical information from an individual's infancy, childhood and adolescence. Stable isotope analysis of these structures reveals information about diet in this period (see Chapter 3 for a more thorough discussion).

Because the timing of formation of the permanent teeth varies by tooth class, it is important to choose the proper tooth for a given problem. Permanent premolars were selected in the initial sampling at Kenchreai and Isthmia that led to the current study because the long-term research plan was to look at childhood diet and mobility (Garvie-Lok 2018a). The permanent

premolars begin to form around 2.5 years (AlQahtani, Hector, and Liversidge 2010, 485). Based on studies of Roman and Late Roman archaeological samples from Greece (Kwok, Garvie-Lok, and Katzenberg 2018), Egypt (Dupras, Schwarcz, and Fairgrieve 2001; Dupras and Tocheri 2007) and Italy (Prowse et al. 2008), by this age a child is likely to have been fully weaned or close to the completion of weaning. Studying premolars therefore allows us to look past the breastfeeding stage of infancy. The permanent premolars are fully formed and erupted by around 12.5-14.5 years (AlQahtani, Hector, and Liversidge 2010, 485). Thus, these teeth retain chemical information from approximately 2.5-14.5 years of an individual's life, allowing for the examination of diet from this period in childhood. Not only does this allow for the study of diet from juvenile remains, but it also allows for the study of childhood diet using adult remains because the tooth stops recording information when a tooth is fully developed and retains this information unless the tooth is lost to wear or disease. We can examine the same stage of life in individuals who died at different ages, given that they survived past the target age (Beaumont et al. 2013, 278). Therefore, individuals who survived past childhood can be examined alongside those who did not.

5.2 Initial Assessment of Sample Preservation

Before analysis, it is essential to inspect the appearance of the whole tooth for signs of degradation or pathology that might lead to insufficient results. Overall preservation of the six Kenchreai tooth samples is adequate, as defined by Kwok (2015). Four of the teeth were complete. One of them (SKEC-02) was cracked longitudinally from crown to root, with four additional minor cracks in the enamel. Unfortunately, the cracks affected the integrity of the tooth and resulted in two segments of crown breaking off during initial inspection and photography, prior to lab work. This exposed the interior of the tooth. SKEC-18/19 also had four

minor cracks in the tooth crown but remained intact. The root of SKEC-05 was still developing but the crown appeared to be in good condition. The root of SKEC-17 appears to have broken off post-mortem, but the crown has reached the age of crown completion and was well preserved.

Kenchreai individuals SKEC-18/19 and SKEC-17 exhibited slight calculus, but this was not significant enough to retain and thus was removed by sonication in the initial phase of lab preparations. SKEC-11 had a substantial amount of calculus on the crown that was removed with a dental pick and placed in its own microvial that has been stored with the other half of the tooth not used in this analysis. Slight discolouration was present on the roots of SKEC-09 and SKEC-11. The crown and root of SKEC-18/19 were slightly discoloured as well. This discolouration was nothing unusual from what should be expected for the given burial and post-depositional contexts. Photographs of each tooth are included in Appendix 1.

Aside from root etching on both Isthmia samples and minor discolouration on Isthmia 69-004E, these samples are in relatively good condition. Tooth samples from Kenchreai were limited and it was initially unclear how well preserved some of their dentine would be. Including two teeth from Isthmia in this study provided an expanded sample and allowed assessment of preservation of the teeth from that site in order to evaluate feasibility of a full dentine microsampling study involving Isthmia. The fact that Isthmia is a neighboring site and that the populations have been studied together previously was another good reason to include a select few individuals from Isthmia. This provides additional comparative isotopic information for the study of the Kenchreai individuals. It also provides an opportunity to look at dietary habits in the region, rather than examining diet at a single site.

Dental wear was scored for all samples. Following the standards of Buikstra and Ubelaker (1994), the samples showed the following scores: SKEC-05 and SKEC-17 were scored

as a 1 (no wear or slight polishing); SKEC-02, SKEC-09 and Isthmia 67-001D were scored as a 2 (slight evidence of smoothing); SKEC-11 and SKEC-18/19 were scored as a 3 (slight flattening is apparent and small patches of dentine are becoming exposed); and Isthmia 69-004E has a score of 4 (cusps have flattened and a large dentine patch is exposed).

5.3 Review of Quality Control Indicators used for Collagen

It is important to consider that isotope ratios obtained from stable isotope analysis may not be an exact match to the values that existed when the individual was alive. From the moment a specimen is buried it has the potential to go through a number of changes, most of which are dependent on the post-depositional environment. The presence of microbes, changes in temperature, and the progression of time can alter the chemical composition of bones and teeth (Lee-Thorp 2008, 929). Fortunately, it is possible to detect these changes. During and after analysis there are several pieces of information a researcher can use to assess the quality of collagen in samples. This includes sample appearance, collagen yield, carbon and nitrogen collagen concentrations, and atomic C/N ratios (Ambrose 1990; van Klinken 1999; DeNiro 1985; Schoeninger et al. 1989).

When evaluating sample appearance, the ideal archaeological sample will produce white or pale-colored gelatin; samples of lesser quality may produce a substance that ranges from yellow to brown in colour (Ambrose 1990, 436). The Kenchreai and Isthmia samples were examined for this quality indicator using visual inspection.

Yield is another basic and commonly used indicator of quality in archaeological collagen. Collagen yield is an expression of how much collagen is in a bone or dentine sample. It is calculated by comparing the weight of the sample before demineralization to the weight of the sample after gelatinization, which is expressed as a weight percentage (van Klinken 1999, 689).

Collagen yields of modern fresh bone are approximately 22% collagen; as bone or dentine degrades its collagen content decreases, with very low collagen yields (0.5% to 1.0%) considered to indicate insufficient quality for analysis (Ambrose 1990; van Klinken 1999, 689). Collagen yield could not be calculated for the current study because it is impossible to determine the initial dry weight of each dentine segment in isolation from the rest of the tooth. As discussed in detail below, demineralization for the method used in this study starts with an entire tooth half, including the enamel, providing no opportunity to measure initial segment dry weights. This problem is common to most dentine microsampling methods; various strategies for determining collagen yield have been attempted in other studies, but have encountered problems and limitations that make them unfeasible (Kwok 2015).

Examining the collagen concentrations of carbon and nitrogen in a sample can identify the presence of extraneous materials. These measures are expressed as weight %C and weight %N of the prepared gelatin, and are determined during mass spectrometry. Typical values of well preserved and uncontaminated collagen are approximately 34.8 ± 8.8 weight %C and 11-16 weight %N (van Klinken 1999, 691). Values that fall outside of these ranges may indicate that the sample contains contaminants or inorganic materials that could alter the isotopic composition of the sample (van Klinken 1999, 691).

Contamination can also be identified by measuring the atomic carbon to nitrogen ratio of the collagen sample (DeNiro 1985; Ambrose 1990). Acceptable C/N atomic ratios fall into the range of 2.9-3.6, a range produced by examining the ratios of modern bone samples (DeNiro 1985; Schoeninger et al. 1989; Ambrose 1990). The sample C/N atomic ratio is derived mathematically from the %C and %N values generated during mass spectrometric analysis.

All of the indicators discussed here should always be examined together when possible because their values and overall patterning provide a good method of assessing the integrity of archaeological samples. While diagenesis of archaeological collagen is inevitable, these indicators allow researchers to identify and avoid degraded samples, and to base their reconstructions on dependable material.

5.4 Sample Preparation and Analysis

Before sample preparation occurred, photographs were taken of each tooth. Additionally, the following measurements were taken: total height (apical tip to occlusal tip), buccal crown height, lingual crown height, crown width, and crown depth. These data are presented in Appendix 2.

Sample preparation took place in the Bioarchaeology Laboratory in the Department of Anthropology at the University of Alberta. The eight teeth were cleaned using sonication as well as abrasion with a Dremel grinder to remove the exterior surface of cementum. This removes any surface that may have come into contact with contaminants from the burial environment and post-depositional environment. Teeth were sectioned longitudinally down the center using a vise and a Dremel saw. For most of the teeth, these longitudinal sections were precise, but in the case of SKEC-09, the tooth split into two root segments and two crown segments. Associated root and crown segments were stored together and the better-preserved segments were used in this study. In the case of SKEC-02, which was partially broken prior to sonication, a scalpel was used to split the tooth in half as it was already partially split longitudinally.

Samples were demineralized using 0.1M hydrochloric acid (HCl) for two days before switching to 0.3M HCl to speed up the mineralization process (Brown et al. 1988). The HCl solutions were changed daily until demineralization was complete. All samples were

demineralized within three weeks. Once complete, samples were rinsed in single distilled water (dH_2O) and soaked in dH_2O overnight. After this, they were treated with 0.125M sodium hydroxide (NaOH) for six hours. Then they were rinsed in water three times and soaked in dH_2O overnight. They were rinsed again the next day and left to soak for another 24 hours. Once all samples were treated with NaOH and rinsed in dH_2O, the microsampling process could begin. Demineralized teeth were sliced into 1mm sections as presented in method two of Beaumont and associates (2013). Larger increments were noted when taken, usually when it was too difficult to slice a smaller section because it was challenging to control the movement of the tooth while slicing. Notes were made for the sections that contain the cemento-enamel junction (CEJ). A total of sixty-one microsamples were obtained.

Sectioning was followed by gelatinization, a frequently used step in the preparation of archaeological collagen (Longin 1971; Beaumont and Montgomery 2015; Eerkens, Berget, and Bartelink 2011; Guiry, Hepburn, and Richards 2016; Henderson, Lee-Thorp, and Loe 2014; Sandberg et al. 2014). Microvials were filled with a pH3 water solution, which was created by mixing single distilled water with HCl. Following this step, the microvials were first placed in flotation devices and subsequently in a hot water bath at 80°C. They were left for 48 hours to gelatinize the samples. Samples were removed from the bath and centrifuged for 15 minutes at 21°C at 6500 rpm using an Eppendorf 5430R machine. This process separated the collagen from any contaminants or waste that remained in the vial. The solubilized collagen of each sample was transferred to a new vial.

In preparation for freeze-drying, the microvials were opened and covered with a small piece of parafilm. The parafilm was pierced with a small hole and the lids of the microvials remained open. Microsamples were freeze-dried by Alvin Kwan in a Labconco freeze dry system

at -44°C in the Biogeochemical Analytical Service Laboratory (BASL) of the Department of Biological Sciences at the University of Alberta. During this process, some of the samples overflowed onto the parafilm. This issue was easily fixed because no contamination between different individuals occurred. However, there was some overlap between microsamples within individuals. This was the case for Kenchreai individuals SKEC-05 and SKEC-11. This is not a concern because stable isotope values of sequential increments of dentine are likely to overlap (Eerkens, Berget, and Bartelink 2011, 3107).

The fifty-two samples that produced sufficient collagen were packed into tin capsules at the BASL. Approximately 1mg of collagen was packed for the majority of the samples. Four samples produced less than 1mg of collagen and for these, the entire amount of collagen was used for analysis. Packed samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ in the BASL, using a EuroVector Vario Pyrocube elemental analyzer coupled to an Isoprime Vision continuous-flow isotope ratio mass spectrometer. The $\delta^{15}N$ and $\delta^{13}C$ ratios (‰) were determined using the following equation:

$$\delta R‰ = (R_{sample}/R_{standard})\text{-}1) \times 1000$$

Here, R_{sample} was the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample, and $R_{standard}$ is the reference value of the international standards Vienna Pee Dee Belemnite (VPDB) for carbon and AIR for nitrogen.

5.5 Age Estimation Method for Dentine Microsamples

A study by Beaumont and Montgomery (2015) presents a method of designating ages to subsections of dentine. This study offers a more refined method of assigning ages to dentine where previous studies assigned ages based on conversions of tooth measurements and dentine mineralization rates (Eerkens, Berget, and Bartelink 2011; Beaumont et al. 2013; Montgomery et

al. 2013). Beaumont and Montgomery (2015) use diagrams to illustrate tooth formation from initiation to completion, calibrated to the ages at which each stage in tooth development is likely to occur. Doing so allows each microsample of dentine to be aligned with an age in years. This also allows for the comparison of sections from different teeth at the same point in life, as well as the comparison of dietary isotope information between populations and sites (Beaumont and Montgomery 2015). Various age estimation methods have been developed for teeth. The authors base their age information on the Queen Mary University of London Atlas of Human Tooth Development and Eruption (AlQahtani, Hector, and Liversidge 2010). For consistency, this age estimation method is used in the present study. This method is also appropriate for the study sample as it was developed using current standards on a well-documented sample which includes individuals of European ancestry. The ages at which the crown begins to form, the crown formation is complete, and the root apex closes are taken from the London Atlas and used in this illustrative method. The development of each tooth is plotted on a diagram corresponding to the ages at which parts of a tooth develop until the entire tooth is complete. To calculate the length of time it takes for a tooth to form, one can subtract the crown initiation age from the root closure age (Beaumont and Montgomery 2015, 410). Knowing this information allows for the calculation of the age of each microsample. A microsample of the tooth will represent the length of time of formation divided by the total number of sections sampled for a given tooth. Thus, each 1mm section would represent the same amount of time over the course of tooth formation. Because the segments collected are converted to estimated ages based on the tooth's total formation time rather than its total length, this method automatically corrects for tooth size variation between individuals. As well, sections that are less or greater than 1mm can be estimated by altering this formula. This is advantageous in situations where larger sections are

taken as a result of poor preservation (see further discussion in Chapter 6 and Appendices 3 and 4). The diagrams are also a valuable tool if a researcher seeks to target a specific time period. With the diagrams, it is possible to visualize what teeth are forming in a given period, and which tooth would best offer the information for their research purpose. The diagrams from Beaumont and Montgomery (2015) used to estimate the ages of the Kenchreai and Isthmia samples are displayed in the following section.

5.6 Age Estimations of Dentine Increments for Kenchreai and Isthmia Individuals

Only premolars were selected for the Kenchreai and Isthmia samples. As illustrated in Figures 5.2 and 5.4, the upper and lower first premolars begin to form at approximately 2.5 years of age and complete formation around 13.5 years. Therefore, the formation time for these premolars is 11.0 years. This length of time will be used to calculate the amounts of time that each increment of dentine represents. The premolars from individuals SKEC-05 and SKEC-18/19 from Kenchreai were identified as upper first premolars.

While the formation times of the upper and lower first premolars align, the second premolars differ slightly. Figure 5.3 displays the upper second premolar, which is initiated at approximately 3.5 years of age. Tooth formation is complete around 14.5 years. Given this information, time of formation is 11.0 years. The lower second premolar begins to form earlier at 2.5 years of age, as seen in Figure 5.4. However, it also completes formation at approximately 14.5 years. Formation time for the lower second premolar is 12.0 years. The tooth from Kenchreai individual SKEC-11 was identified as an upper second premolar. Teeth from individuals SKEC-02, SKEC-09 and SKEC-17 were identified as lower second premolars.

Figure 5. 2 Age estimation diagram for PM[1]. Created by Beaumont and Montgomery (2015).

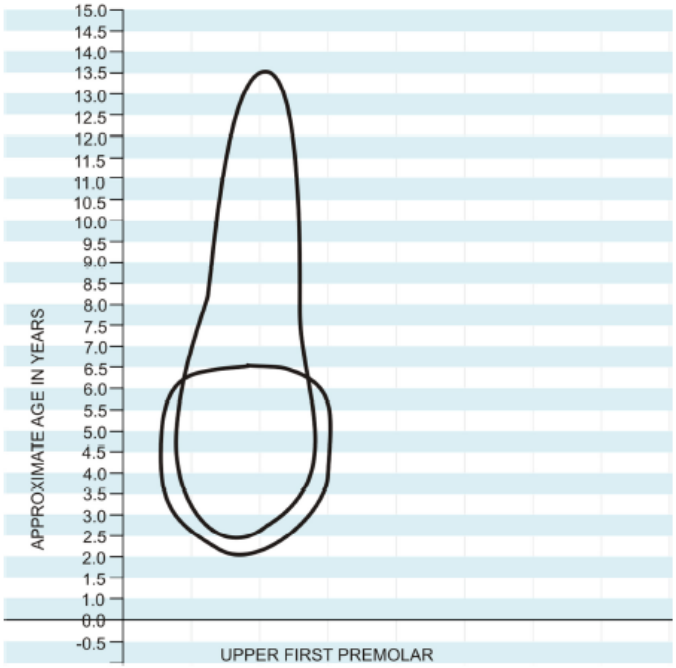


Figure 5. 3 Age estimation diagram for PM[2]. Created by Beaumont and Montgomery (2015).

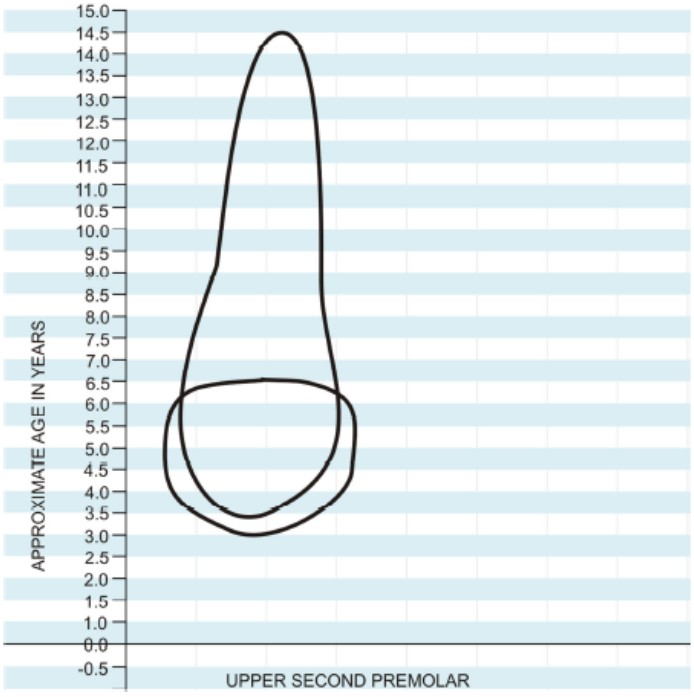


Figure 5. 4 Age estimation diagram for PM_1. Created by Beaumont and Montgomery (2015).

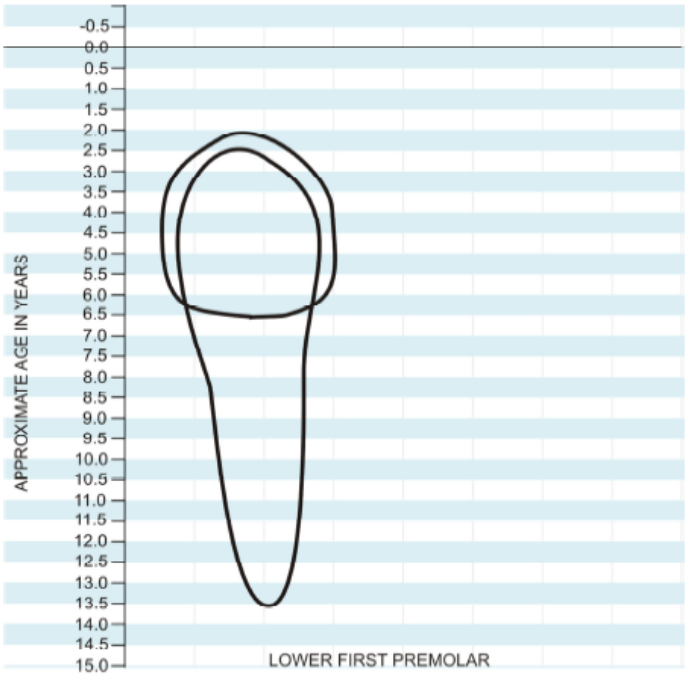
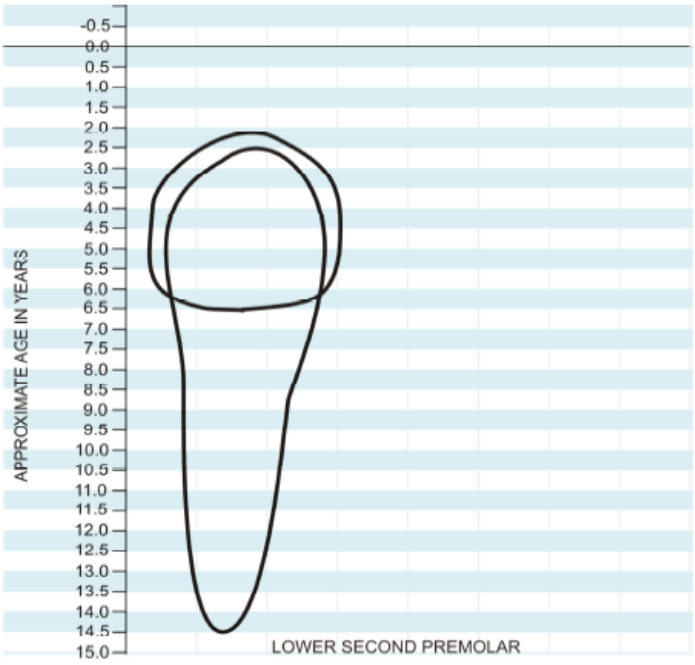


Figure 5. 5 Age estimation diagram for PM_2. Created by Beaumont and Montgomery (2015).



5.7 Expectations for Sample Stable Isotope Values

Previous dietary information reconstructed using bone collagen show that there is an interesting contrast between the diet of older juveniles and adults at Kenchreai. This data indicates that the $\delta^{13}C$ values of older children are distinctly lower than the adult values (Garvie-Lok 2010). Adult individuals at both Isthmia and Kenchreai show evidence of marine resource consumption, with a greater emphasis placed on fish at Kenchreai (Garvie-Lok 2010). It is suspected that the patterning seen in juveniles and adults at Kenchreai is indicative of differences in marine resource consumption. Thus, the expectation is that dentine stable isotope values will be more similar to juvenile bone collagen values than to adult bone collagen values. If other patterns are found in the dental dietary data, factors such as growth and stress will be considered.

5.8 Summary

This chapter has explored the basics of tooth formation, with an emphasis on dentine formation. The quality control indicators used to assess the Kenchreai and Isthmia samples are presented here and sample preservation is briefly discussed. The procedure for processing the samples for analysis is outlined here. The discussion of dentine microsampling is continued in this chapter by explaining how age estimates are calculated for each tooth.

Chapter 6: Results

This chapter presents the data obtained from the stable isotope analysis of the human dentine samples. The raw data for Kenchreai are listed in Tables 6.1 and 6.2 and those for Isthmia in Tables 6.3 and 6.4. Both of these tables include the %C, %N and C/N atomic ratio data, as well as the age estimate for each section. Sample quality and preservation are briefly discussed. Two isotope graphs are presented for each individual, one of which offers the stable carbon isotope values and the other the stable nitrogen isotope values. Both isotopes are plotted using the corresponding age estimate of each dentine segment. A brief commentary about the processes of sectioning the dentine and calculating the ages is included, and the estimates are presented in Tables 6.2 and 6.4.

6.1 Kenchreai

The isotope data produced from the Kenchreai individuals are presented in Tables 6.1 and 6.2. Overall, two microsamples out of thirty-six from Kenchreai failed to produce collagen after freeze drying. These samples are SKEC-09crown and SKEC-11G. Both left a glass-like appearance along the walls of their respective microvials and nothing more. In the case of SKEC-09, this reflects poor overall preservation of the tooth. During the demineralization phase of lab work, this tooth demineralized much more quickly than the other samples. It also broke in the initial stage of lab work when it was sectioned in half longitudinally, creating two fragments of the crown and two fragments of the root. One crown and root fragment were selected and demineralized separately. Demineralization progressed quickly for both fragments with the root disintegrating after five days in HCl. While the root had disintegrated, the quick progression of the crown resulted in a small ambiguous sample, and it was unclear which end represented the crown cusps. Since the cusps could not be identified it is unknown which end of the

demineralized tooth formed first and thus, an age estimate could not be correctly provided for any sections taken from this tooth. To get at least some dietary information for SKEC-09, this was submitted as a bulk sample. Since it did not produce collagen there is, unfortunately, no dietary information for SKEC-09. The brittle original texture, rapid demineralization and failure to produce collagen after gelatinization are consistent with poor collagen preservation in this individual. In the case of SKEC-11G, only one segment from the tooth failed to produce collagen. This may reflect patchy diagenesis affecting only some parts of the dentine, a known pattern in some archaeological skeletal material (Child 1995). These samples are included in Table 6.1 but are marked as *n.d.*

Another issue occurred while sectioning the tooth of SKEC-05. This tooth broke in a way that the pulp cavity separated from the outer layer of the tooth. The pulp cavity was difficult to section and so it was submitted as a bulk sample instead. Two sections were obtained from the outer layer. Since the bulk sample represents the entire tooth, its estimated age overlaps with the estimated age of completion of segment B. However, this is not illustrated in Figures 6.3 and 6.4 because ages are plotted as the midpoint between age of section initiation and completion. No other issues were encountered while sectioning teeth from the individuals from Kenchreai. Thus all thirty-four microsamples from the Kenchreai individuals that produced collagen were accepted for further consideration.

Collagen samples were submitted to the Biogeochemical Laboratory for analysis. The reported precision of the results (based on repeat analyses of internal standards) was ±0.1‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{15}N$ (Kwan 2018). Sample quality was assessed by examining the %C and %N values alongside the C/N atomic ratio of each sample. As discussed in Chapter 5 and shown in Table 6.1, %C and %N fall into the acceptable range of values as reported by van Klinken

(1999, 691). Atomic C/N ratios are also within the acceptable range of 2.9-3.6 for modern samples (Ambrose 1990, 440). In contrast to bone collagen analysis, where collagen yield is a final standard measure of quality, collagen yield cannot be calculated for the method used in this thesis because the initial dry weight of the dentine cannot be obtained in isolation from the tooth's enamel. The %C, %N and atomic C/N values indicate that the collagen isolated from the Kenchreai samples is of sufficient quality for analysis and that levels of contamination are low; thus all stable isotope values were accepted for further consideration.

Age estimations were produced following the procedure outlined in Chapter 5. In most instances, 1mm samples were obtained. However, situations arose where larger segments of 2-3 mm were acquired. This includes cases where it was difficult to control the movement of the tooth during sectioning, or where cracks in the tooth prevented accurate 1mm measurements to be taken. As a result, variable data sets are available for each individual, some of which have only a few data points recording the diet of isolated points in childhood, whereas others have a range of data points representing a more full record of childhood diet. Table 6.2 displays the estimated ages of initiation and completion for each dentine segment. Refer to Appendix 3 for detailed age estimation calculations.

Table 6. 1 Kenchreai preservation indicator and stable isotope values.[8]

Sample ID	%C	%N	C/N (atomic)	$\delta^{13}C$ (‰ VPDB)	$\delta^{15}N$ (‰ AIR)
SKEC-02A	43.5	16.1	3.15	-19.6	8.0
SKEC-02B	43.7	16.1	3.16	-19.7	8.7
SKEC-02C	43.8	16.0	3.18	-19.6	8.9
SKEC-02D	44.3	16.2	3.20	-19.6	8.0
SKEC-02E	44.5	16.2	3.20	-19.7	7.4
SKEC-02F	44.4	16.2	3.19	-19.7	7.5
SKEC-02G	44.1	16.0	3.22	-19.5	8.2
SKEC-02H	44.8	16.0	3.27	-19.3	9.4
SKEC-05bulk	44.0	16.2	3.17	-18.9	10.3
SKEC-05A	43.3	16.1	3.15	-19.0	10.2
SKEC-05B	42.9	15.5	3.24	-18.9	10.1
SKEC-09 crown	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
SKEC-11A	44.7	16.5	3.16	-19.2	9.8
SKEC-11B	44.3	16.4	3.16	-19.2	9.5
SKEC-11C	44.3	16.4	3.15	-19.3	9.4
SKEC-11D	44.4	16.3	3.19	-19.1	9.5
SKEC-11E	44.6	16.2	3.21	-19.0	9.4
SKEC-11F	44.0	16.1	3.19	-18.9	9.4
SKEC-11G	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
SKEC-11H	45.0	16.4	3.20	-18.9	9.9
SKEC-17A	43.5	15.9	3.19	-19.2	9.8
SKEC-17B	43.3	15.8	3.20	-19.4	9.8
SKEC-17C	43.9	16.0	3.19	-19.3	10.1
SKEC-17D	41.4	15.0	3.21	-19.5	9.9
SKEC-18/19A	44.1	16.2	3.17	-19.2	9.4
SKEC-18/19B	44.3	16.3	3.17	-19.1	9.2
SKEC-18/19C	44.4	16.3	3.18	-19.2	8.9
SKEC-18/19D	44.0	16.1	3.18	-19.1	9.0
SKEC-18/19E	44.1	16.1	3.20	-19.1	8.9
SKEC-18/19F	43.5	16.5	3.08	-19.4	9.6
SKEC-18/19G	43.7	16.0	3.19	-19.2	9.6
SKEC-18/19H	44.2	16.2	3.19	-19.0	10.1
SKEC-18/19I	43.9	16.1	3.18	-18.9	10.1
SKEC-18/19J	43.3	15.9	3.17	-18.9	10.1
SKEC-18/19K	43.0	15.8	3.18	-19.0	10.6
SKEC-18/19L	42.7	15.4	3.24	-18.7	10.8

[8] Segments marked with "*n.d.*" did not produce collagen after freeze-drying.

Table 6. 2 Kenchreai stable isotope values and estimated ages.

Sample ID	$\delta^{13}C$ (‰ VPDB)	$\delta^{15}N$ (‰ AIR)	Estimated Initiation Age (years)	Estimated Completion Age (years)
SKEC-02A	-19.6	8.0	2.5	3.4
SKEC-02B	-19.7	8.7	3.4	4.2
SKEC-02C	-19.6	8.9	4.2	5.9
SKEC-02D	-19.6	8.0	5.9	7.6
SKEC-02E	-19.7	7.4	7.6	9.4
SKEC-02F	-19.7	7.5	9.4	11.1
SKEC-02G	-19.5	8.2	11.1	12.8
SKEC-02H	-19.3	9.4	12.8	14.5
SKEC-05bulk	-18.9	10.3	2.5	7.0
SKEC-05A	-19.0	10.2	2.5	5.5
SKEC-05B	-18.9	10.1	5.5	7.0
SKEC-11A	-19.2	9.8	3.5	6.5
SKEC-11B	-19.2	9.5	6.5	7.5
SKEC-11C	-19.3	9.4	7.5	8.5
SKEC-11D	-19.1	9.5	8.5	9.5
SKEC-11E	-19.0	9.4	9.5	10.5
SKEC-11F	-18.9	9.4	10.5	11.5
SKEC-11G	*n.d.*	*n.d.*	11.5	12.5
SKEC-11H	-18.9	9.9	12.5	14.5
SKEC-17A	-19.2	9.8	2.5	3.75
SKEC-17B	-19.4	9.8	3.75	5
SKEC-17C	-19.3	10.1	5	6.25
SKEC-17D	-19.5	9.9	6.25	7.5
SKEC-18/19A	-19.2	9.4	2.5	3.3
SKEC-18/19B	-19.1	9.2	3.3	4.2
SKEC-18/19C	-19.2	8.9	4.2	5.0
SKEC-18/19D	-19.1	9.0	5.0	5.9
SKEC-18/19E	-19.1	8.9	5.9	6.7
SKEC-18/19F	-19.4	9.6	6.7	8.4
SKEC-18/19G	-19.2	9.6	8.4	9.3
SKEC-18/19H	-19.0	10.1	9.3	10.1
SKEC-18/19I	-18.9	10.1	10.1	11.0
SKEC-18/19J	-18.9	10.1	11.0	11.8
SKEC-18/19K	-19.0	10.6	11.8	12.7
SKEC-18/19L	-18.7	10.8	12.7	13.5

6.1.1. SKEC-02

Figure 6. 1 $\delta^{13}C$ values obtained from the dentine segments of SKEC-02 plotted against the estimated age of formation for the segment.[9]

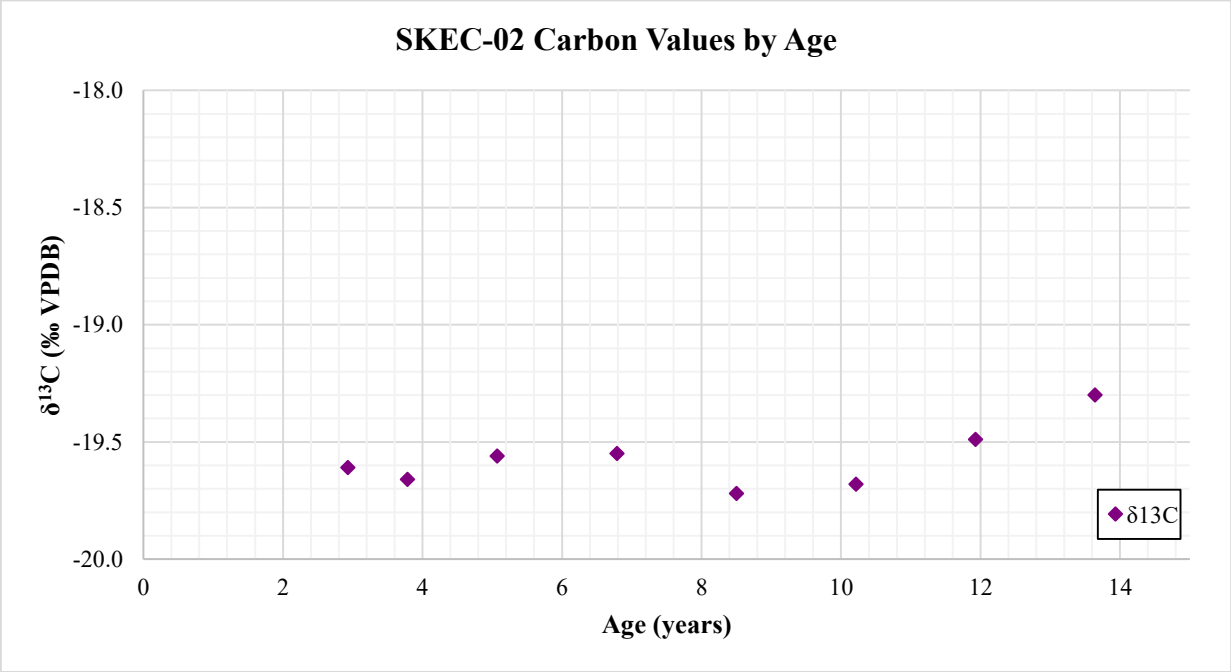
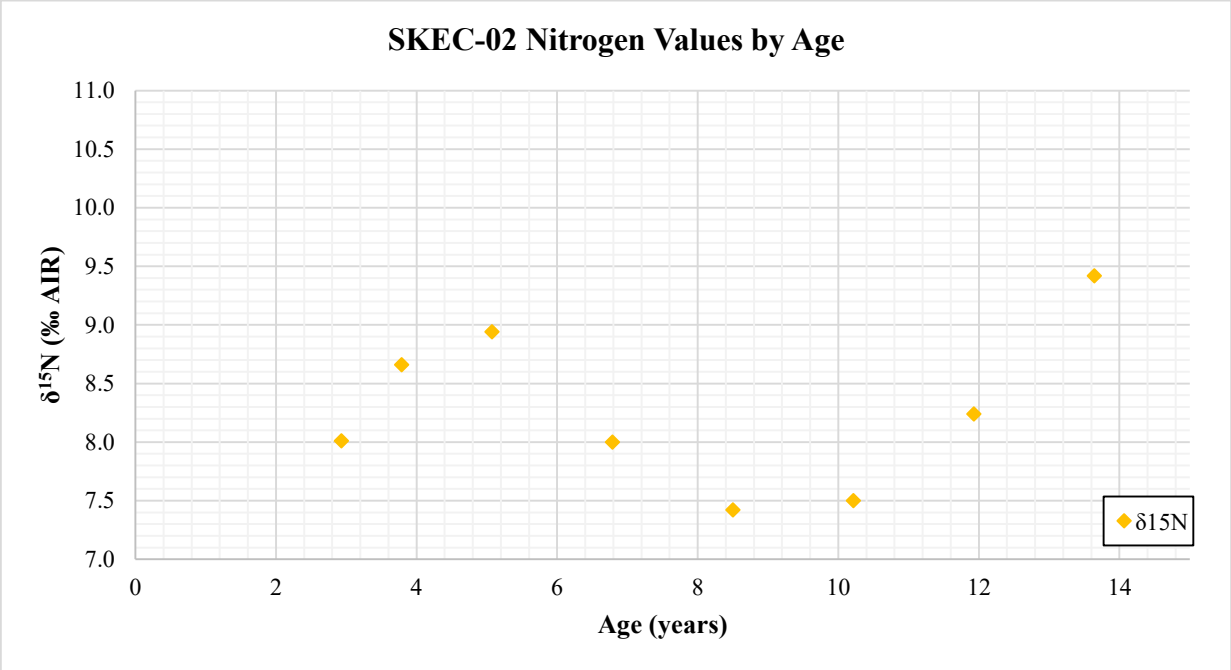


Figure 6. 2 $\delta^{15}N$ values obtained from the dentine segments of SKEC-02 plotted against the estimated age of formation for the segment.



[9] In this and all other figures in this chapter, the estimated age of formation used to plot each segment's value is the midpoint between the estimated ages of initiation and completion.

6.1.2 SKEC-05

Figure 6. 3 $\delta^{13}C$ values obtained from the dentine segments of SKEC-05 plotted against the estimated age of formation for the segment.[10]

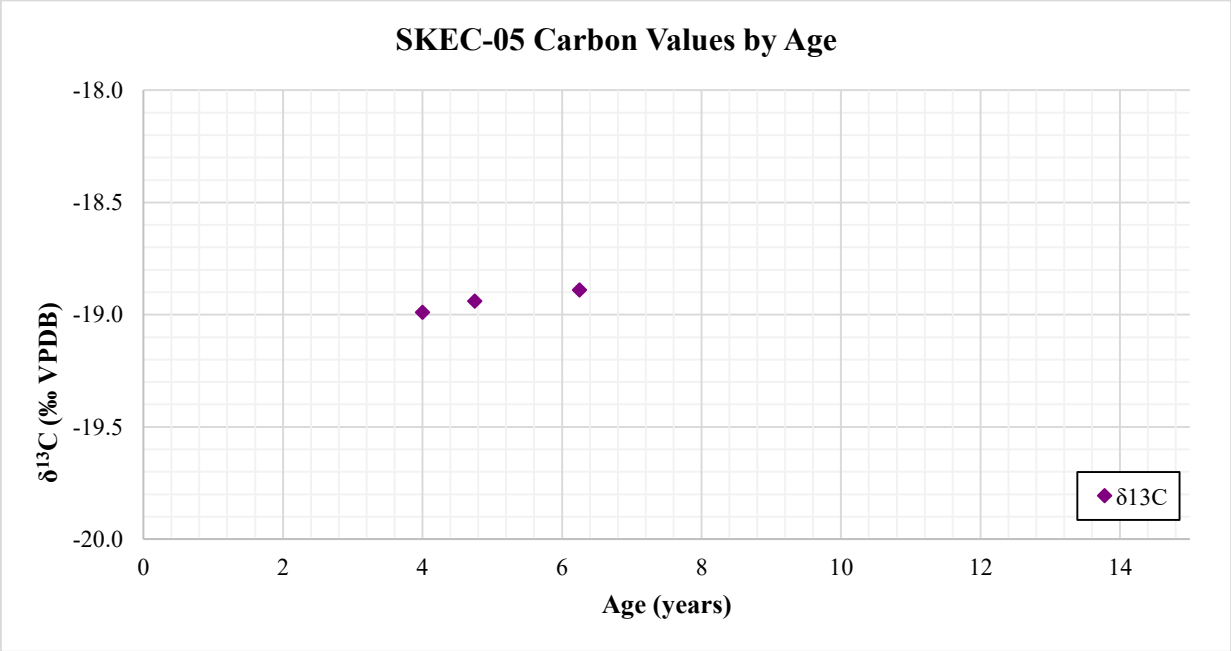
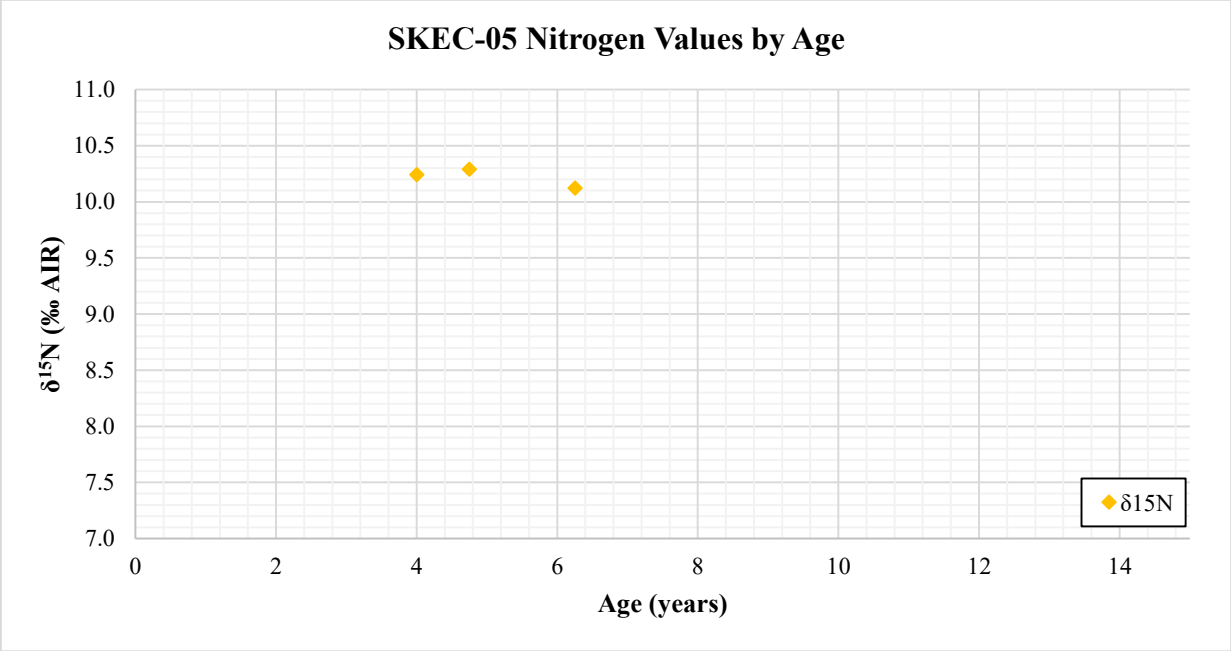


Figure 6. 4 $\delta^{15}N$ values obtained from the dentine segments of SKEC-05 plotted against the estimated age of formation for the segment.



[10] This individual has two isotope values that represent the same age because the bulk sample of the pulp cavity of the tooth represents the same age as the estimated age of completion for this tooth. However, they do not appear this way on the graph because ages are plotted as the midpoint between age of initiation and completion. Refer to Appendix 3 for details.

6.1.3 SKEC-11

Figure 6. 5 $\delta^{13}C$ values obtained from the dentine segments of SKEC-11 plotted against the estimated age of formation for the segment.

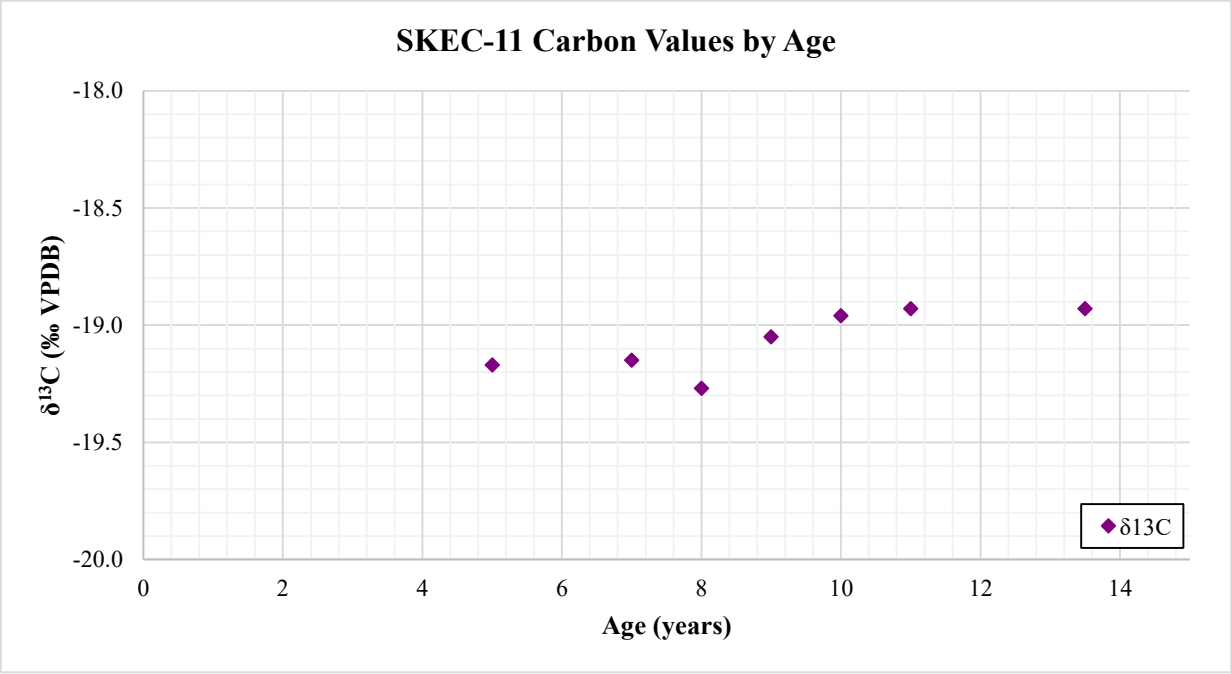
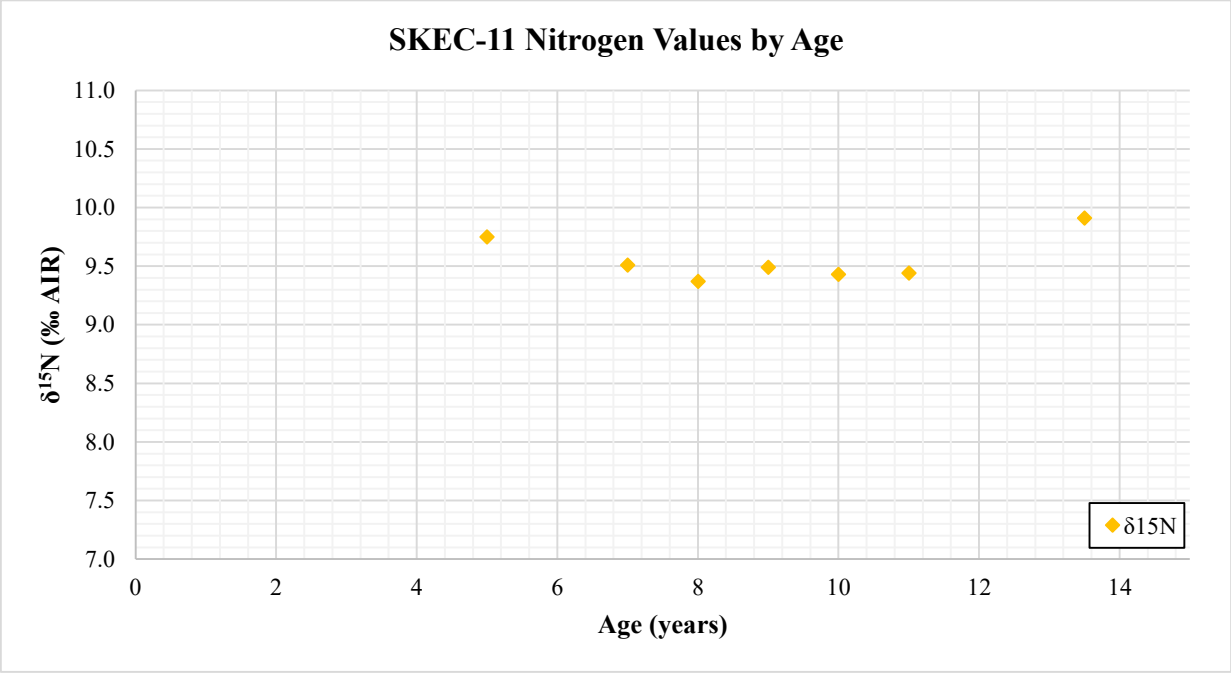


Figure 6. 6 $\delta^{15}N$ values obtained from the dentine segments of SKEC-11 plotted against the estimated age of formation for the segment.



6.1.4 SKEC-17

Figure 6. 7 $\delta^{13}C$ values obtained from the dentine segments of SKEC-17 plotted against the estimated age of formation for the segment.

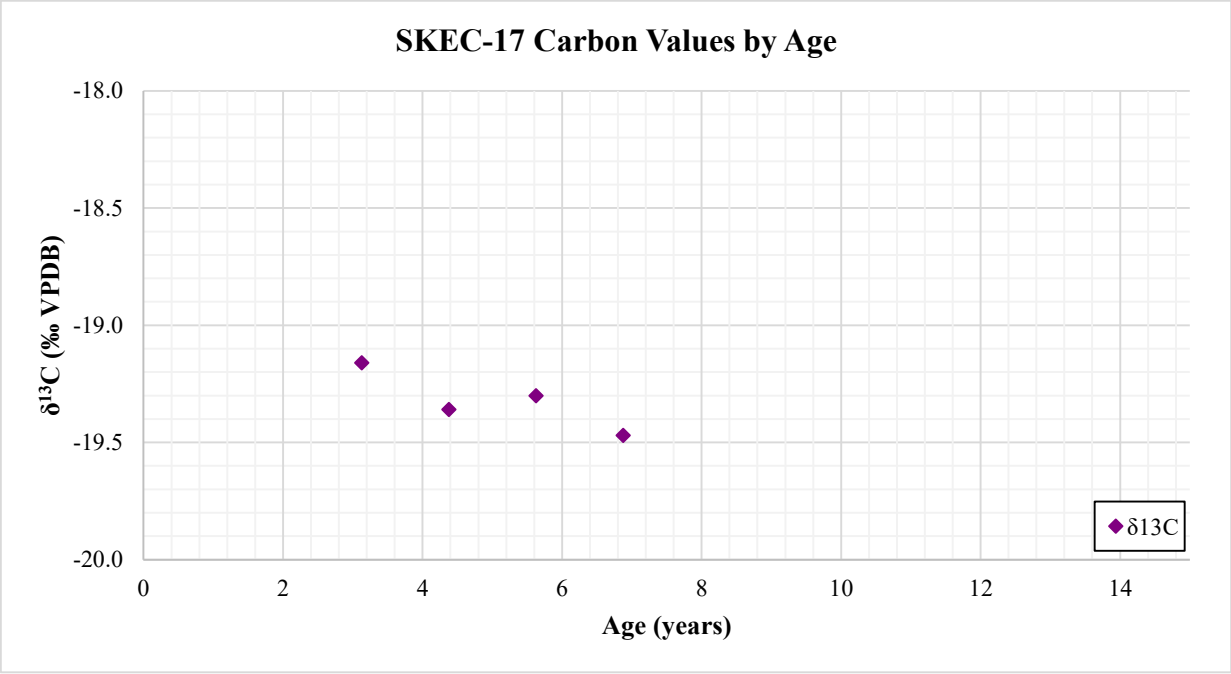
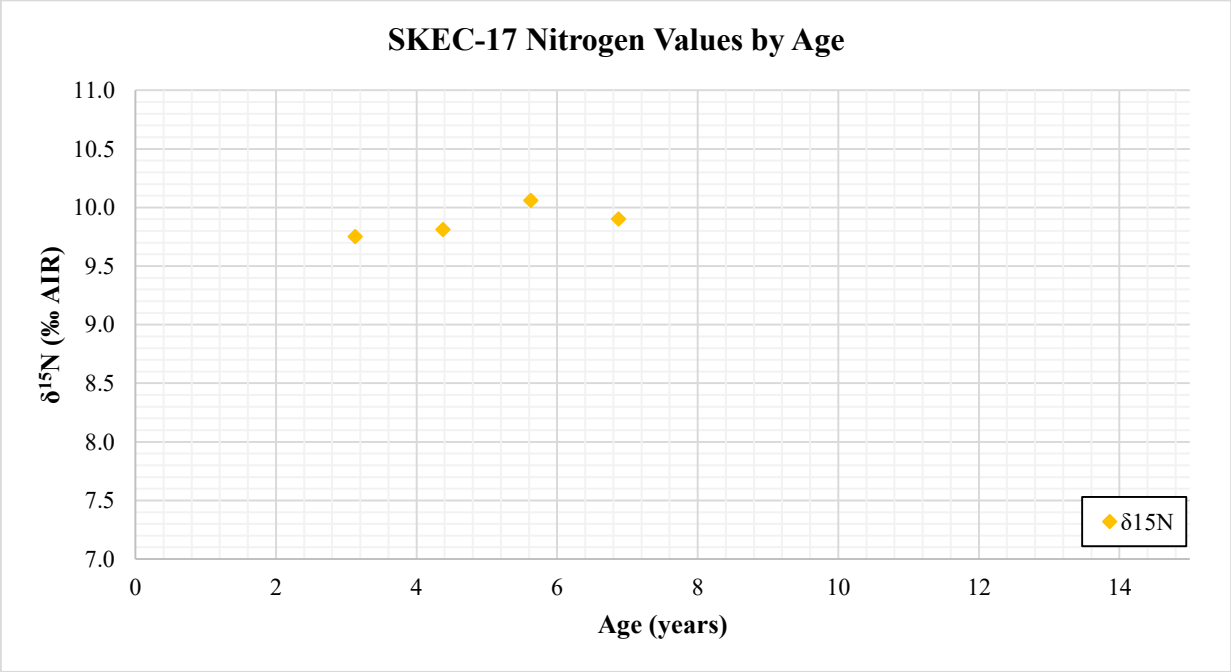


Figure 6. 8 $\delta^{15}N$ values obtained from the dentine segments of SKEC-17 plotted against the estimated age of formation for the segment.



6.1.5 SKEC-18/19

Figure 6. 9 $\delta^{13}C$ values obtained from the dentine segments of SKEC-18/19 plotted against the estimated age of formation for the segment.

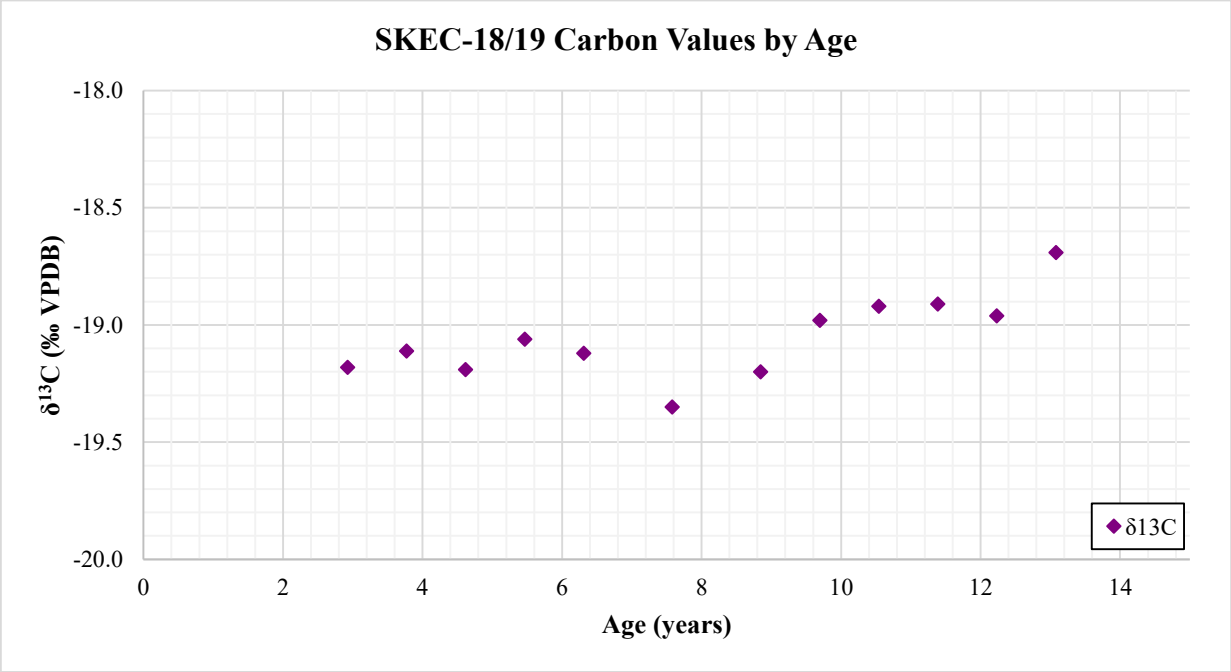
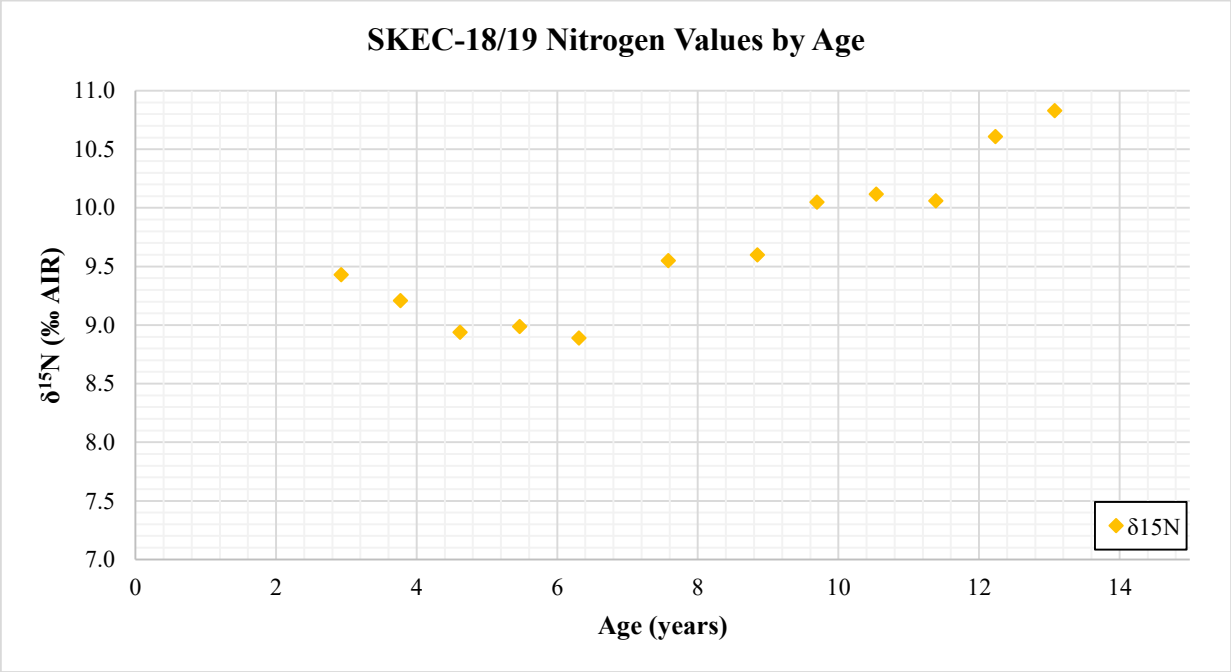


Figure 6.10 $\delta^{15}N$ values obtained from the dentine segments of SKEC-18/19 plotted against the estimated age of formation for the segment.



6.2 Isthmia

Seven microsamples between the two teeth used from the Isthmia individuals failed to produce collagen after freeze drying. These samples are listed as *n.d.* in the tables below. As with SKEC-11, failure of only some microsamples to produce collagen may reflect patchy diagenesis affecting only some parts of the dentine. The Isthmia samples were analyzed in the same equipment runs as the Kenchreai samples, so the analytical precision is the same: ±0.1‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{15}N$ (Kwan 2018). Sample quality for Isthmia was considered using the same preservation indicators discussed for Kenchreai in section 6.1 above. The carbon content for segment F of Isthmia 67-001D was outside of the acceptable range for this study and was excluded because of possible contamination. This follows observations on bone collagen quality indicators made by van Klinken (1999, 691) where he suggested that %C values higher than 34.8±8.8% are indicative of contamination of additional organic carbon. This is the only sample to be excluded from the study according to these preservation indicators. The raw data obtained from the isotope analysis of the Isthmia individuals are displayed in Tables 6.3 and 6.4.

Segments of 1mm were more consistently obtained for the Isthmia teeth. Fewer issues were encountered during the slicing of the Isthmia samples because they were complete teeth of firmer texture, and thus were easier to control while slicing. While multiple isotope values are available for each individual, the seven microsamples that did not produce collagen after freeze drying have left gaps in the dietary timeline of the Isthmia individuals. Although this is unfortunate, there is sufficient information from the other microsamples to comment on dietary changes during childhood for these individuals. Refer to Appendix 4 for the detailed age estimation calculations.

Table 6. 3 Isthmia preservation indicator and stable isotope values.[11]

Sample ID	%C	%N	C/N (atomic)	$\delta^{13}C$ (‰ VPDB)	$\delta^{15}N$ (‰ AIR)
Isthmia 67001D-A	43.5	16.0	3.18	-19.3	10.6
Isthmia 67001D-B	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Isthmia 67001D-C	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Isthmia 67001D-D	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Isthmia 67001D-E	44.8	16.3	3.22	-19.3	10.0
Isthmia 67001D-F	58.9	21.3	3.22	-19.0	9.8
Isthmia 67001D-G	44.0	16.0	3.22	-19.0	10.0
Isthmia 67001D-H	43.9	15.9	3.22	-18.9	10.4
Isthmia 67001D-I	44.3	16.1	3.20	-18.8	10.9
Isthmia 67001D-J	43.5	15.8	3.21	-19.0	10.3
Isthmia 67001D-K	44.3	16.0	3.22	-18.9	9.8
Isthmia 67001D-L	43.4	15.7	3.22	-18.9	9.9
Isthmia 69004E-A	43.5	15.9	3.19	-19.2	9.3
Isthmia 69004E-B	44.1	16.2	3.17	-19.0	9.1
Isthmia 69004E-C	44.0	16.1	3.19	-19.1	9.2
Isthmia 69004E-D	44.0	16.4	3.13	-19.1	9.6
Isthmia 69004E-E	44.3	16.3	3.18	-19.1	9.2
Isthmia 69004E-F	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Isthmia 69004E-G	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Isthmia 69004E-H	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Isthmia 69004E-I	44.0	16.1	3.19	-18.8	9.3
Isthmia 69004E-J	43.8	16.0	3.19	-18.9	9.3
Isthmia 69004E-K	43.0	15.8	3.17	-19.0	9.0
Isthmia 69004E-L	43.1	15.8	3.19	-19.0	9.3
Isthmia 69004E-M	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*

[11] Segments marked with *n.d.* did not produce collagen after freeze-drying and thus were excluded from the study. Isthmia 67-001F was omitted from further consideration because of its high %C value.

Table 6. 4 Isthmia stable isotope values and estimated ages.

Sample ID	$\delta^{13}C$ (‰ VPDB)	$\delta^{15}N$ (‰ AIR)	Estimated Initiation Age (years)	Estimated Completion Age (years)
Isthmia 67001D-A	-19.3	10.6	3.5	4.4
Isthmia 67001D-B	*n.d.*	*n.d.*	4.4	5.3
Isthmia 67001D-C	*n.d.*	*n.d.*	5.3	6.3
Isthmia 67001D-D	*n.d.*	*n.d.*	6.3	7.2
Isthmia 67001D-E	-19.3	10.0	7.2	8.1
Isthmia 67001D-G	-19.0	10.0	9.0	9.9
Isthmia 67001D-H	-18.9	10.4	9.9	10.8
Isthmia 67001D-I	-18.8	10.9	10.8	11.8
Isthmia 67001D-J	-19.0	10.3	11.8	12.7
Isthmia 67001D-K	-18.9	9.8	12.7	13.6
Isthmia 67001D-L	-18.9	9.9	13.6	14.5
Isthmia 69004E-A	-19.2	9.3	2.5	3.3
Isthmia 69004E-B	-19.0	9.1	3.3	4.2
Isthmia 69004E-C	-19.1	9.2	4.2	5.0
Isthmia 69004E-D	-19.1	9.6	5.0	5.9
Isthmia 69004E-E	-19.1	9.2	5.9	6.7
Isthmia 69004E-F	*n.d.*	*n.d.*	6.7	7.6
Isthmia 69004E-G	*n.d.*	*n.d.*	7.6	8.4
Isthmia 69004E-H	*n.d.*	*n.d.*	8.4	9.3
Isthmia 69004E-I	-18.8	9.3	9.3	10.1
Isthmia 69004E-J	-18.9	9.3	10.1	11.0
Isthmia 69004E-K	-19.0	9.0	11.0	11.8
Isthmia 69004E-L	-19.0	9.3	11.8	12.7
Isthmia 69004E-M	*n.d.*	*n.d.*	12.7	13.5

6.2.1 Isthmia 67-001D

Figure 6.11 $\delta^{13}C$ values obtained from the dentine segments of Isthmia 67-001D plotted against the estimated age of formation for the segment.

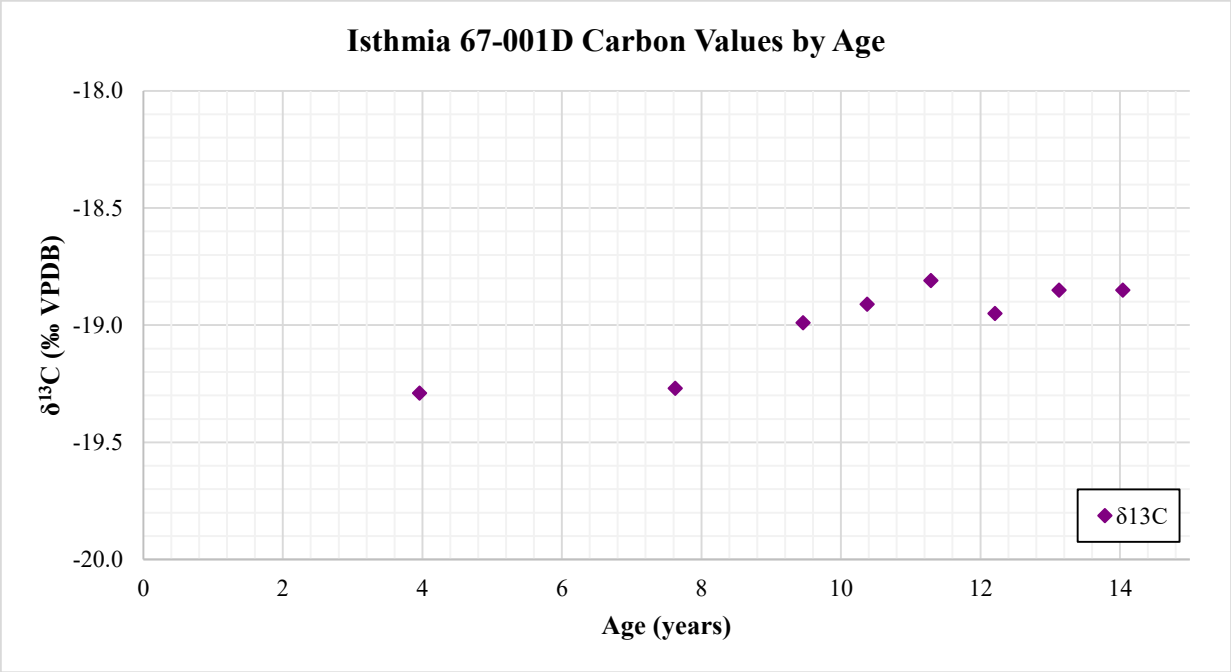
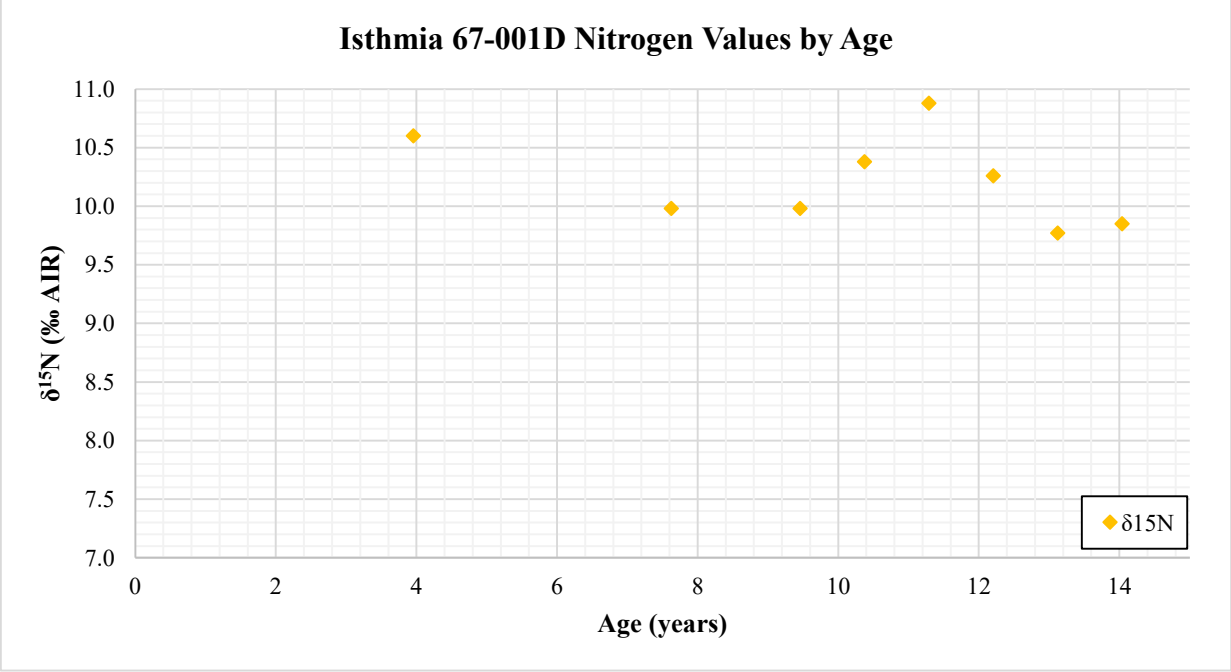


Figure 6. 12 $\delta^{15}N$ values obtained from the dentine segments of Isthmia 67-001D plotted against the estimated age of formation for the segment.



6.2.2 Isthmia 69-004E

Figure 6.13 $\delta^{13}C$ values obtained from the dentine segments of Isthmia 69-004E plotted against the estimated age of formation for the segment.

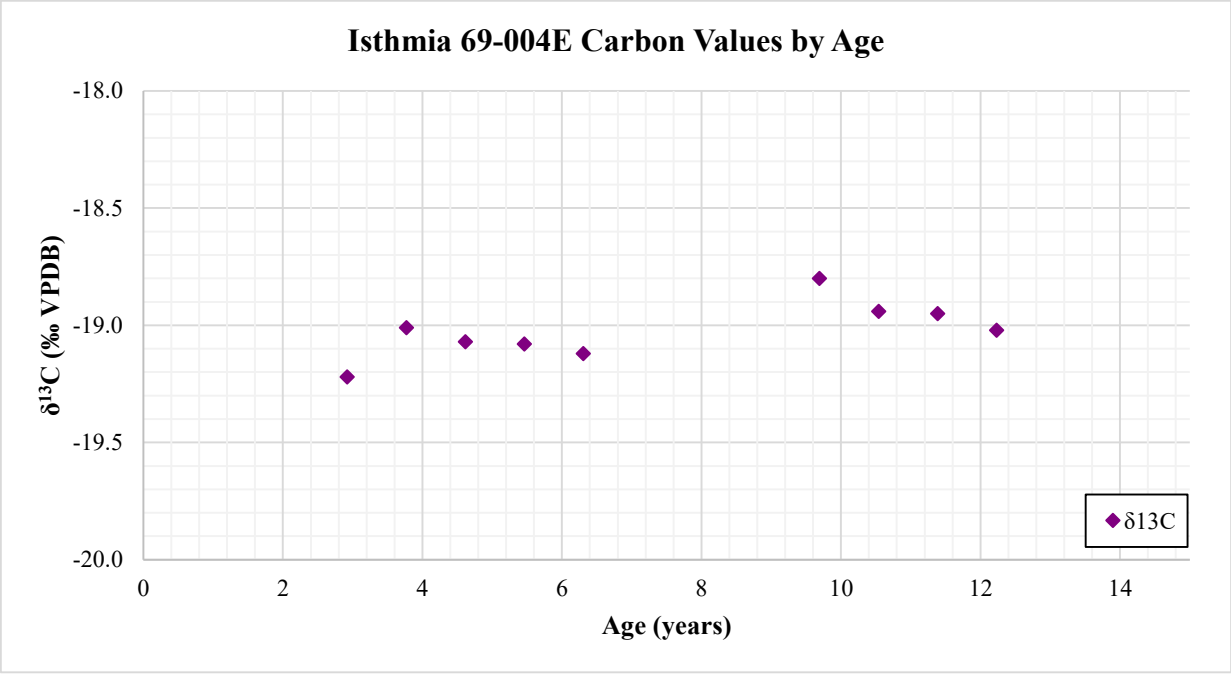
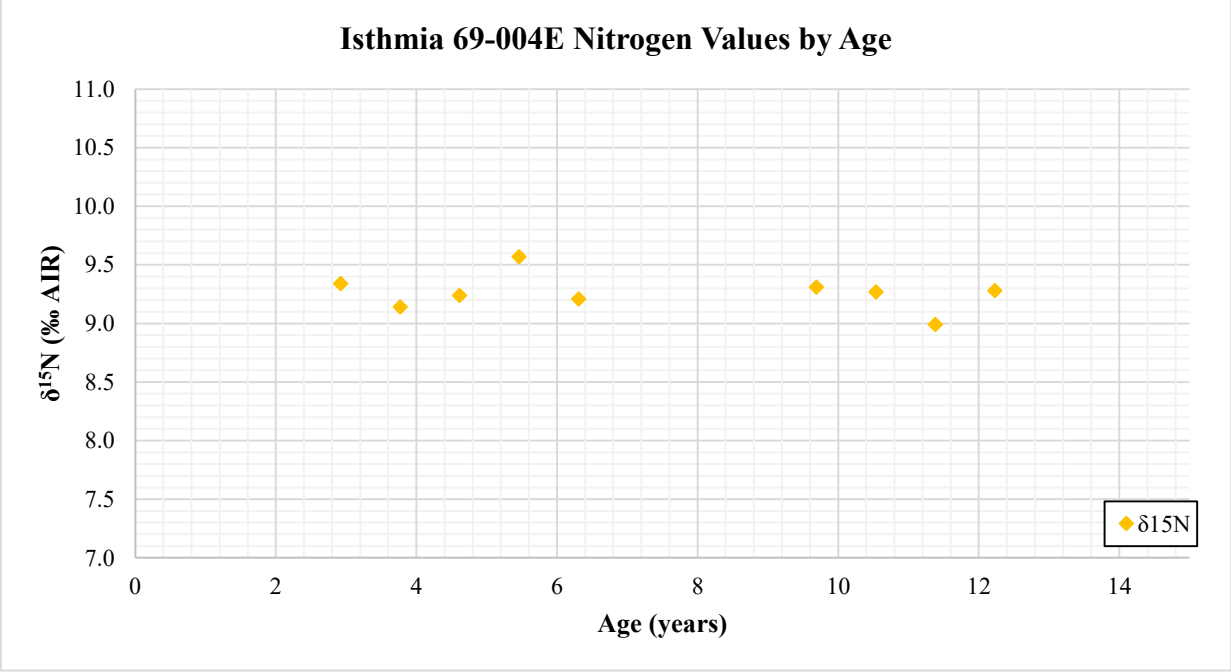


Figure 6. 14 $\delta^{15}N$ values obtained from the dentine segments of Isthmia 69-004E plotted against the estimated age of formation for the segment.



6.3 Summary

This chapter presents the data obtained from the stable isotope analysis of dentine microsamples. Reasons for excluding specific data are defined. Graphs displaying the carbon and nitrogen isotope values are shown in comparison to the age of each dentine increment. Difficulties encountered in the process of obtaining the dentine segments are briefly discussed and the age estimates are provided. Although poor preservation of some teeth complicated the analysis, partial or complete series of dentine values were obtained for all but one of the teeth. These values will be interpreted in the following chapter.

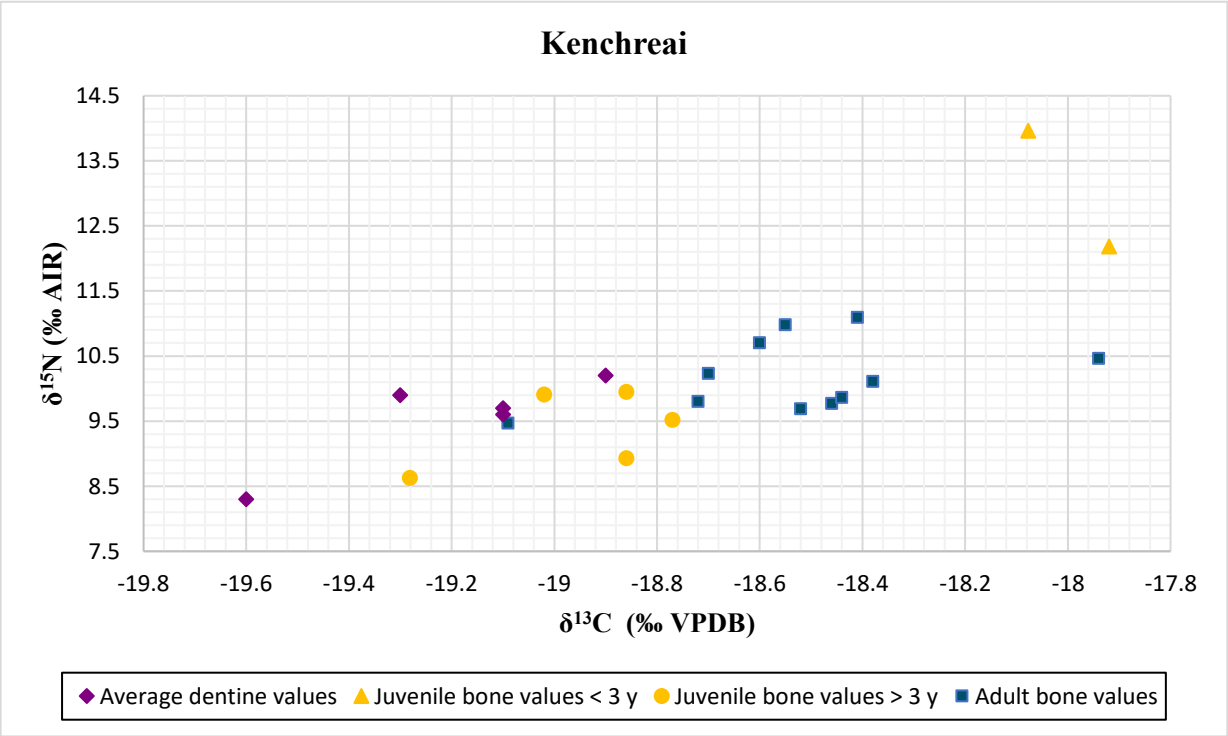
Chapter 7: Dietary and Cultural Interpretations

Interpretations of the data presented in Chapter 6 are discussed below. In-depth discussions of inter- and intra-individual dietary trends for the Kenchreai and Isthmia samples are provided. The graphs displaying the results of the dentine $\delta^{13}C$ and $\delta^{15}N$ stable isotope analyses are presented once more with the addition of adult or juvenile bone collagen stable isotope values from Garvie-Lok (2010) for the same individuals in the study sample. A discussion of general trends at each site is also included along with figures illustrating all juvenile and adult $\delta^{13}C$ and $\delta^{15}N$ isotopic data at each site. The inclusion of marine resources to the diet of each individual is speculated upon.

7.1 Kenchreai

When discussing the Kenchreai values, it is easiest to begin with a general comparison to the bone collagen values previously obtained for the site. For this purpose, average dentine values for $\delta^{13}C$ and $\delta^{15}N$ were calculated for each of the Kenchreai individuals and added to the group of juvenile bone values for the site (see Figure 7.1). This effectively creates a larger juvenile sample, expanding the sample size from seven to twelve individuals by adding the average childhood values for five of the adults at the site. When this is done it can be seen that in general, the dentine collagen stable isotope values are consistent with the bone collagen stable isotope values of children over three years for both carbon and nitrogen. This makes sense, as the formation time of the teeth used (starting at 2.5 years) roughly agrees with that age range. In comparison to the wider Kenchreai sample of bone collagen stable isotope values from adult remains, most of the juvenile bone and dentine stable isotope values are lower for both carbon and nitrogen. The exceptions are the high bone collagen values of two infants (aged <6 months and ~1.5 years), which are consistent with breastfeeding (Garvie-Lok 2010, 4).

Figure 7. 1 Comparison of all $\delta^{13}C$ and $\delta^{15}N$ values for juveniles and adults at Kenchreai.



A basic statistical analysis was used to determine the significance of these age differences. Given the small sample size, unequal variance *t*-tests were performed on the sample. This type of analysis is appropriate for small sample sizes for which no assumptions are made about equal variances (Ruxton 2006; de Winter 2013). The two juveniles younger than 3 years of age whose elevated bone collagen isotope values are indicative of nursing were omitted because including them would defeat the purpose of the *t*-tests, which is to confirm an isotopic difference between adults and older children.

Two-tailed unequal variance *t*-tests were conducted for $\delta^{13}C$ and for $\delta^{15}N$ in juveniles versus adults. Critical values of $p = 0.05$ were used. The results indicate a significant difference in $\delta^{13}C$ values ($t = -4.715$, p-value = 0.00015) and $\delta^{15}N$ values ($t = -2.832$, p-value = 0.011) between juveniles and adults at Kenchreai. Table 7.1 displays general mean/range statistics for Kenchreai and Table 7.2 contains the results of the *t*-tests. As discussed below in section 7.3, this supports

the idea that juvenile and adult diets at Kenchreai did differ, although the small differences in mean $\delta^{13}C$ and $\delta^{15}N$ values suggest that the dietary differences were subtle.

Table 7. 1 General mean/range statistics for $\delta^{13}C$ and $\delta^{15}N$ for juveniles and adults at Kenchreai. "Juveniles" includes the average dentine values and excludes individuals below 3 years of age.

Age Group	n	Mean/range of $\delta^{13}C$ (‰)	Mean/range of $\delta^{15}N$ (‰)
Juveniles	10	-19.1 ± 0.26	9.5 ± 0.63
Adults	11	-18.5 ± 0.28	10.2 ± 0.54

Table 7. 2 Results of unequal variance *t*-tests (two-tailed) for the Kenchreai sample.

Test	*t*-value	*p*-value
Comparison of $\delta^{13}C$ values for juveniles and adults	-4.715	0.00015
Comparison of $\delta^{15}N$ values for juveniles and adults	-2.832	0.011

The adult mean $\delta^{13}C$ and $\delta^{15}N$ values are elevated in comparison to the juvenile mean values. The $\delta^{13}C$ and $\delta^{15}N$ values typical of marine resource consumption provided by Papathanasiou and Richards (2015) suggest that $\delta^{13}C$ values of approximately -12‰ and $\delta^{15}N$ values of roughly 10‰ would be indicative of full marine resource dependence (see Chapter 3 for further discussion). The adult mean $\delta^{15}N$ value falls into this range, but the $\delta^{13}C$ value does not, although it does fall above the value they suggest is typical of a diet based only on land resources. This suggests a mixed diet based primarily on land resources but with the addition of a significant amount of marine protein. The Kenchreai adult means fall close to the means for Grave Circle A at Mycenae ($\delta^{13}C$ = -18.6 ± 0.5‰, $\delta^{15}N$ = 10.5 ± 1.1‰), which have also been interpreted as indicating a mixed diet with significant marine consumption (Richards and Hedges 2008; Papathanasiou and Richards 2015). In contrast, both $\delta^{13}C$ and $\delta^{15}N$ mean values for juveniles fall below the values suggested by Papathanasiou and Richards (2015) to indicate significant marine resource use. This suggests that juveniles were consuming fewer marine resources, which is also suggested by the individual childhood dietary profiles created with dentine stable isotope values.

7.1.1 SKEC-02

Figures 7.2 and 7.3 display the $\delta^{13}C$ and $\delta^{15}N$ stable isotope values obtained from dentine compared to the adult stable isotope values obtained from the same individual's bone collagen. This tooth produced eight microsamples that provide data from the ages of approximately 2.5 to 14.5 years. The $\delta^{13}C$ values are relatively steady, with values ranging from -19.7‰ to -19.3‰. The lowest value of -19.7‰ appears at around age 4, and again between the ages of 7 and 12, where there is a slight decrease in $\delta^{13}C$. The final segment of the tooth that represents the completion of tooth formation has the highest $\delta^{13}C$ value of -19.3‰. For this individual, the adult bone collagen $\delta^{13}C$ value is -18.6‰, up to 1.1‰ higher than the dentine $\delta^{13}C$ values. While the adult $\delta^{13}C$ value is elevated above the juvenile signal, it is apparent from Figure 7.2 that the final few dentine values of this tooth are increasing, potentially moving toward a diet that is typical of the adults at Kenchreai.

Changes in $\delta^{15}N$ are slightly more dramatic, with values ranging from 7.4‰ to 9.4‰. The lowest value of 7.4‰ occurs around 8 years of age after an initial rise to 8.9‰ from age 2.5 to age 5. SKEC-02 is not the only Kenchreai individual who experiences a $\delta^{15}N$ decrease in middle childhood (discussed further below). This decrease occurs around the same time as the decrease in $\delta^{13}C$ values. The $\delta^{15}N$ values increase again between ages 10 and 14, with the highest value being produced by the last microsample. As with the $\delta^{13}C$ values, $\delta^{15}N$ values are increasing toward the adult $\delta^{15}N$ bone value of 10.7‰. Together, the $\delta^{13}C$ and $\delta^{15}N$ dentine values do not indicate clear marine resource consumption as defined by the guidelines used by Papathanasiou and Richards (2015). However, it appears that there is likely a transition late in tooth development towards a diet similar to the adults at the site.

Figure 7. 2 $\delta^{13}C$ values obtained from the dentine segments of SKEC-02 plotted against the estimated age of formation for the segment.[12] The $\delta^{13}C$ adult bone collagen value for SKEC-02 is also plotted.[13]

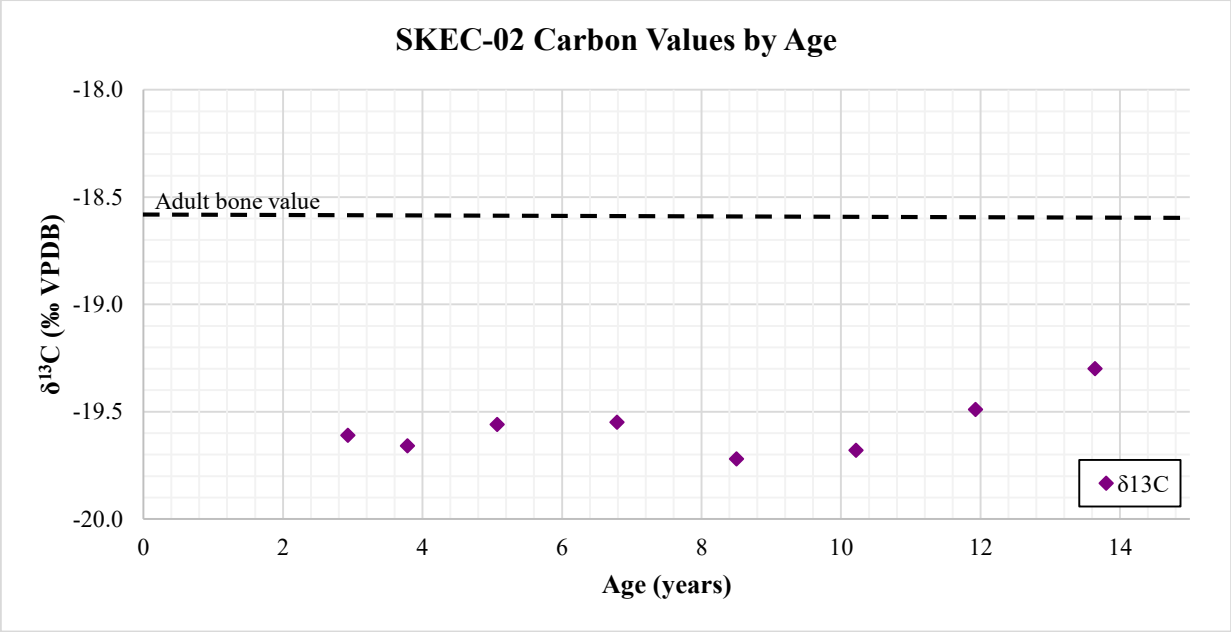
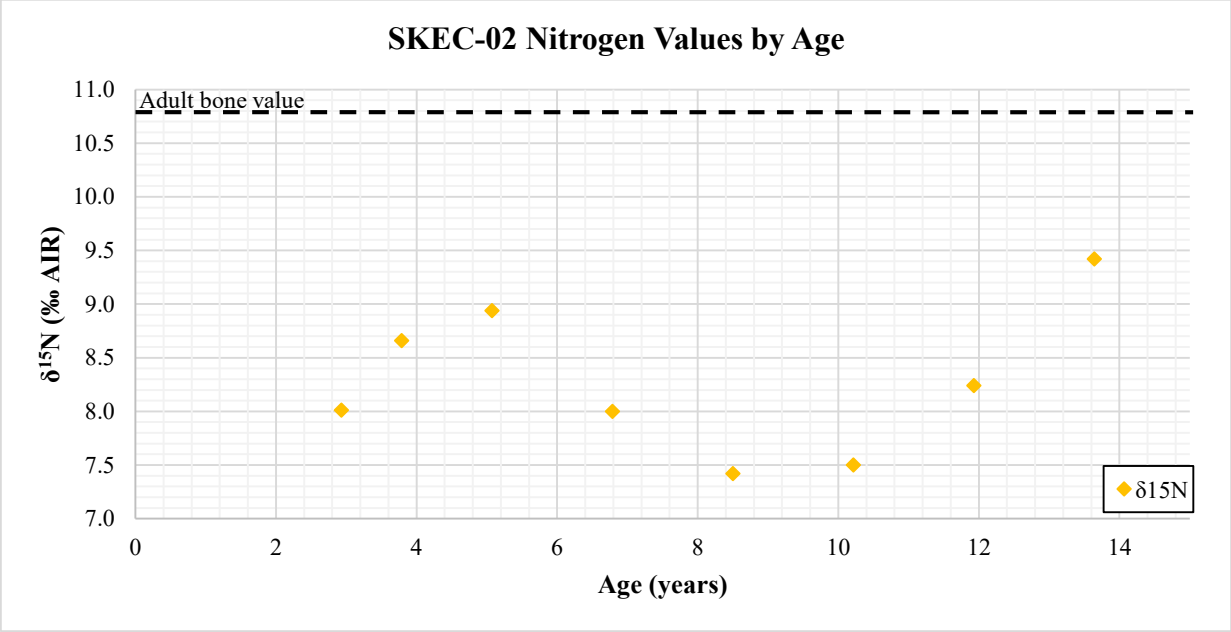


Figure 7. 3 $\delta^{15}N$ values obtained from the dentine segments of SKEC-02 plotted against the estimated age of formation for the segment. The $\delta^{15}N$ adult bone collagen value for SKEC-02 is also plotted.



[12] In this and all other figures in this chapter, the estimated age of formation used to plot each segment's value is the midpoint between the estimated ages of initiation and completion.
[13] In this and all other figures in this chapter, the $\delta^{13}C$ and $\delta^{15}N$ adult or older juvenile bone collagen values are from Garvie-Lok (2010).

7.1.2 SKEC-05

SKEC-05 produced three microsamples that offer dietary information from the ages of approximately 2.5 to 7 years. The bulk sample of the pulp cavity was not sectioned further and therefore represents the entire tooth. Thus the three microsamples do not represent as wide an age range as did those of SKEC-02, which might be why all microsample stable isotope values for this tooth are nearly identical, as seen in Figures 7.4 and 7.5. Osteological analyses estimated this individual to be five to seven years old. Thus, the bone collagen values for SKEC-05 are also representative of early childhood.

There is little variation within the dentine $\delta^{13}C$ values for this individual, which range from -19.0‰ to -18.9‰. This agrees with the juvenile bone collagen $\delta^{13}C$ value, which was also -18.9‰. The $\delta^{15}N$ values range from 10.1‰ to 10.3‰, which are more than 1‰ higher than the juvenile bone value of 8.9‰. This seems odd considering that both bone and dentine collagen values should represent childhood diet up to approximately 7 years of age. A possible explanation could be a slower turnover time in the bone from which the sample was taken, a humeral shaft (Garvie-Lok 2010). The stable isotope values of the bone collagen sample may be reflecting dietary values for a larger time span than the dentine samples. Unfortunately this suggestion is difficult to test because of the poor preservation of the tooth; estimating the formation age of the bulk sample was difficult, and only two further samples could be taken. A clearer idea of dietary change within this individual over the 2.5 to 7 year age range would have been easier to see if the tooth had been better preserved. Since bone and dentine collagen values do not match, accurate comments about fish consumption cannot be made for this individual.

Figure 7. 4 $\delta^{13}C$ values obtained from the dentine segments of SKEC-05 plotted against the estimated age of formation for the segment. The $\delta^{13}C$ juvenile bone collagen value for SKEC-05 is also plotted.

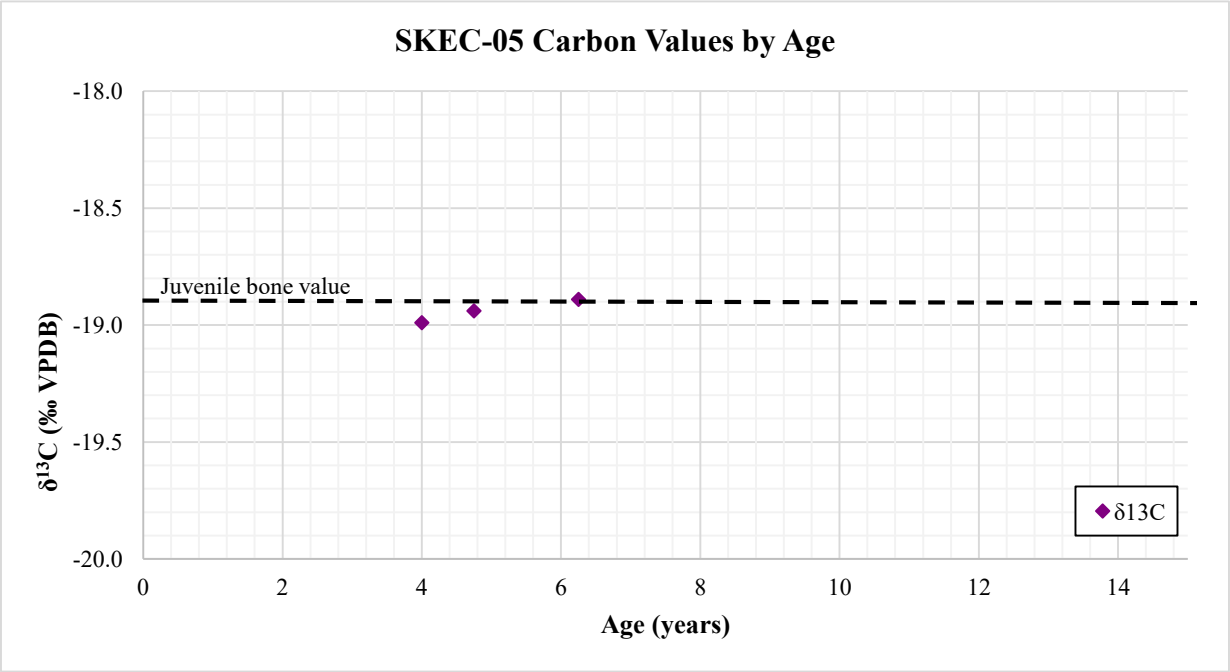
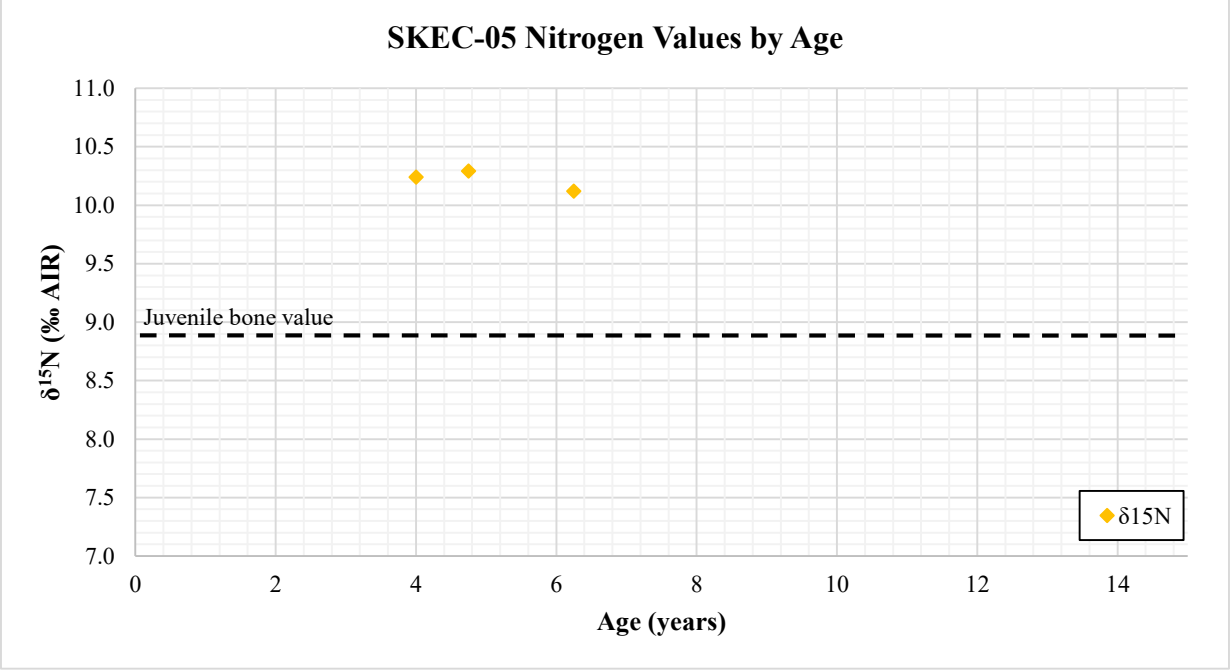


Figure 7. 5 $\delta^{15}N$ values obtained from the dentine segments of SKEC-05 plotted against the estimated age of formation for the segment. The $\delta^{15}N$ juvenile bone collagen value for SKEC-05 is also plotted.



7.1.3 SKEC-11

Seven microsamples of SKEC-11 produced $\delta^{13}C$ and $\delta^{15}N$ stable isotope values that represent dietary trends from approximately 3.5 to 14.5 years of age. There is little variation between these values. The $\delta^{13}C$ values fall between -19.3‰ to -18.9‰, as seen in Figure 7.6. The lowest $\delta^{13}C$ value occurs around 8 years of age, after which values increase. This is a similar pattern to that observed in SKEC-02. The final two segments of SKEC-11 produce the highest $\delta^{13}C$ values. These appear to be rising toward the adult bone collagen value of -18.4‰.

Figure 7.7 shows the $\delta^{15}N$ values. These also show little variation, and range from 9.4‰ to 9.9‰, with the highest value produced by the final segment of the tooth. As with the $\delta^{13}C$ values, the lowest $\delta^{15}N$ value is produced by the segment for which the age is estimated to be 8 years. Values are steady from about 7 to 11 years. Unfortunately the microsample that would represent age 12 did not produce collagen. As a result, it is unclear how quickly the $\delta^{15}N$ values increased to the final segment value of 9.9‰. The adult bone collagen value is 10.1‰, which is close to this final dentine $\delta^{15}N$ value.

Although not reflected by the $\delta^{13}C$ dentine values, it is possible that SKEC-11 was consuming marine resources to cause this slight $\delta^{15}N$ elevation. An alternative explanation is increasing consumption of meat from terrestrial animals such as sheep, goat or pig. As seen in Figure 7.17 below, juveniles from Kenchreai fall into an isotopic space consistent with terrestrial fauna use.

Figure 7. 6 $\delta^{13}C$ values obtained from the dentine segments of SKEC-11 plotted against the estimated age of formation for the segment. The $\delta^{13}C$ adult bone collagen value for SKEC-11 is also plotted.

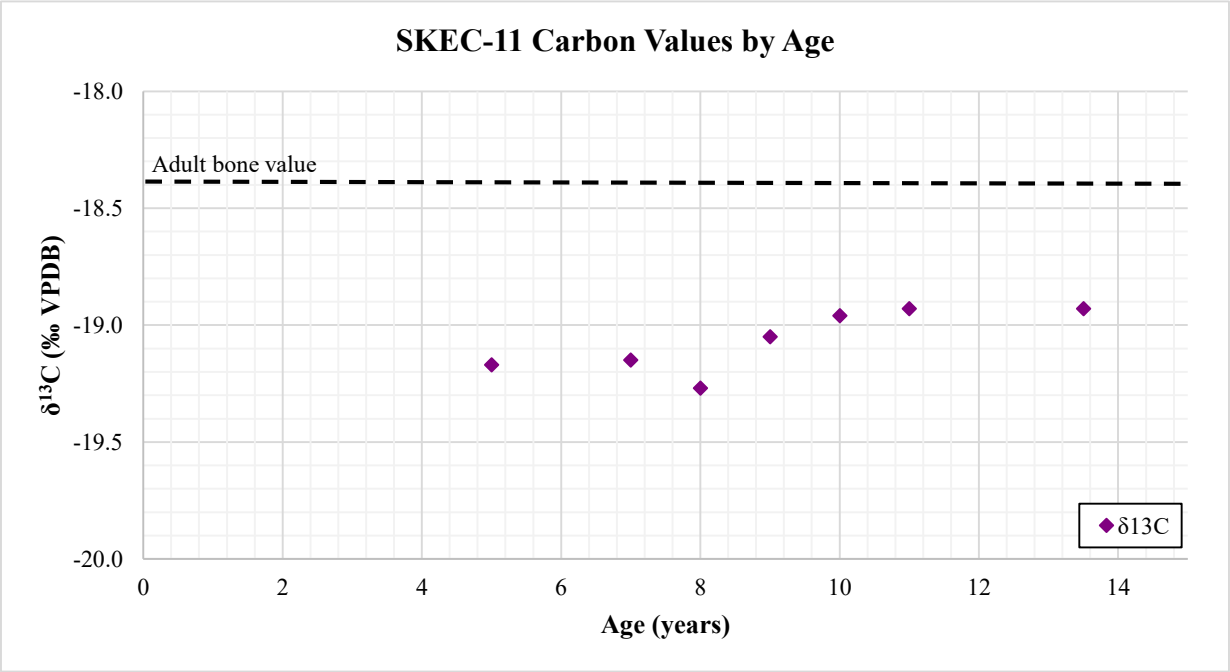
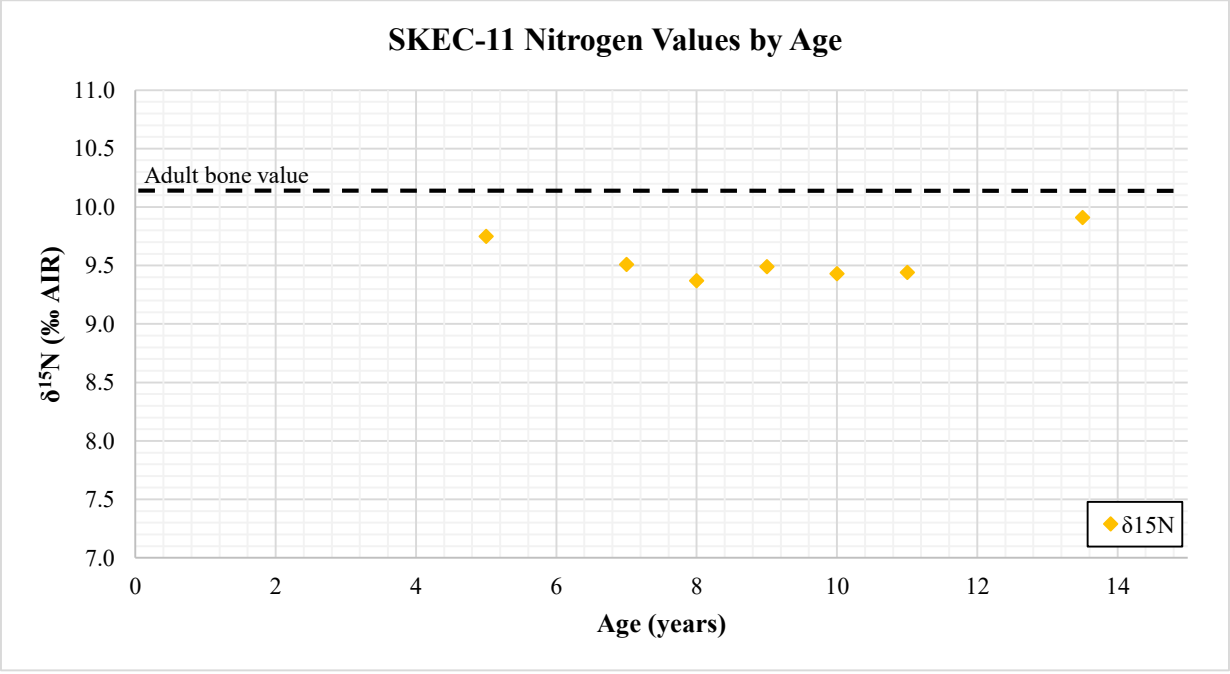


Figure 7. 7 $\delta^{15}N$ values obtained from the dentine segments of SKEC-11 plotted against the estimated age of formation for the segment. The $\delta^{15}N$ adult bone collagen value for SKEC-11 is also plotted.



7.1.4 SKEC-17

The tooth of SKEC-17 was broken and only produced four microsamples that represent the approximate ages of 2.5 to 7.5 years. As displayed in Figure 7.8, $\delta^{13}C$ values range from -19.5‰ to -19.2‰. The highest value comes from the first microsample of the tooth, representing stable isotope values from when this tooth began to develop. The lowest comes from the final segment which occurs around 7 years of age, around the same age where SKEC-02 and SKEC-11 experience a similar decrease in values. From the skeletal age assessment, SKEC-17 is estimated to be approximately 12 years old. The juvenile bone collagen $\delta^{13}C$ value is -19.0‰, which is similar to the values obtained from dentine.

As shown in Figure 7.9, the $\delta^{15}N$ values range from 9.8‰ to 10.1‰ and overlap with SKEC-17's bone collagen value, which is 9.9‰. Segment C (formation age 5.0 – 6.25 years) shows elevation above the bone collagen value, although this is slight. Because the bone collagen value of SKEC-17 does not represent an adult diet, it is also interesting to compare the dentine values of this individual to the Kenchreai adult mean values. Both $\delta^{13}C$ and $\delta^{15}N$ values of the final tooth segment fall below the adult mean $\delta^{13}C$ and $\delta^{15}N$ values, as does SKEC-17's bone collagen value. All of this would be consistent with the idea that marine resources contributed less to the diet of SKEC-17 than to adult diets.

Figure 7. 8 $\delta^{13}C$ values obtained from the dentine segments of SKEC-17 plotted against the estimated age of formation for the segment. The $\delta^{13}C$ juvenile bone collagen value for SKEC-17 is also plotted.

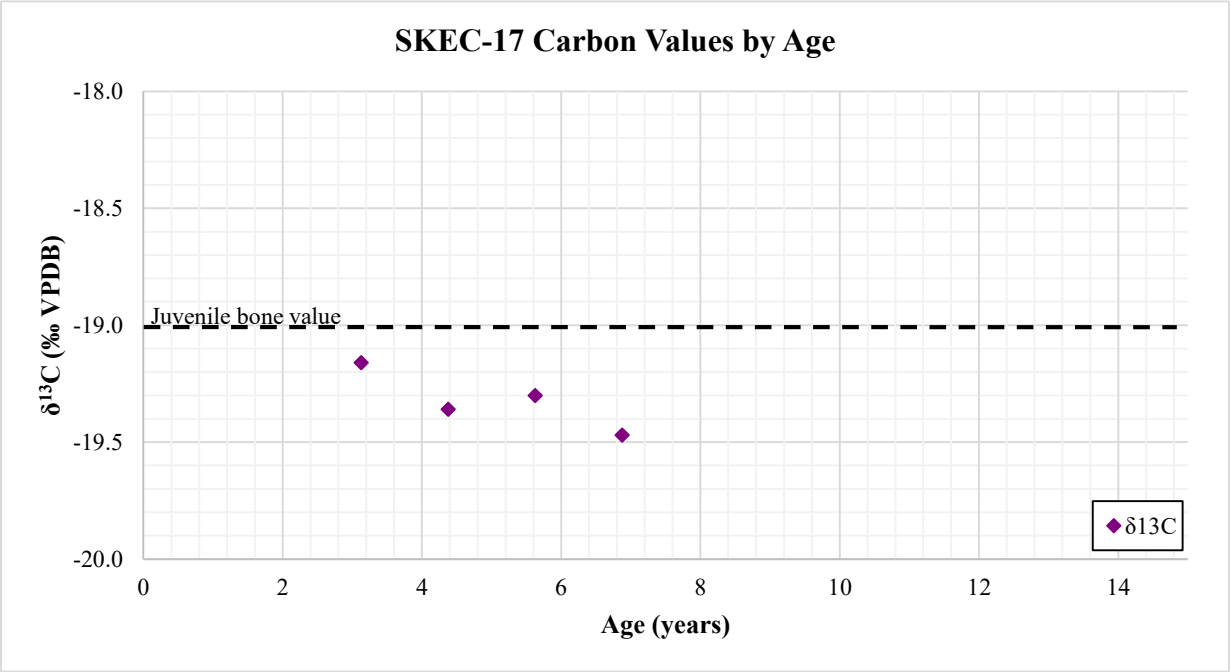
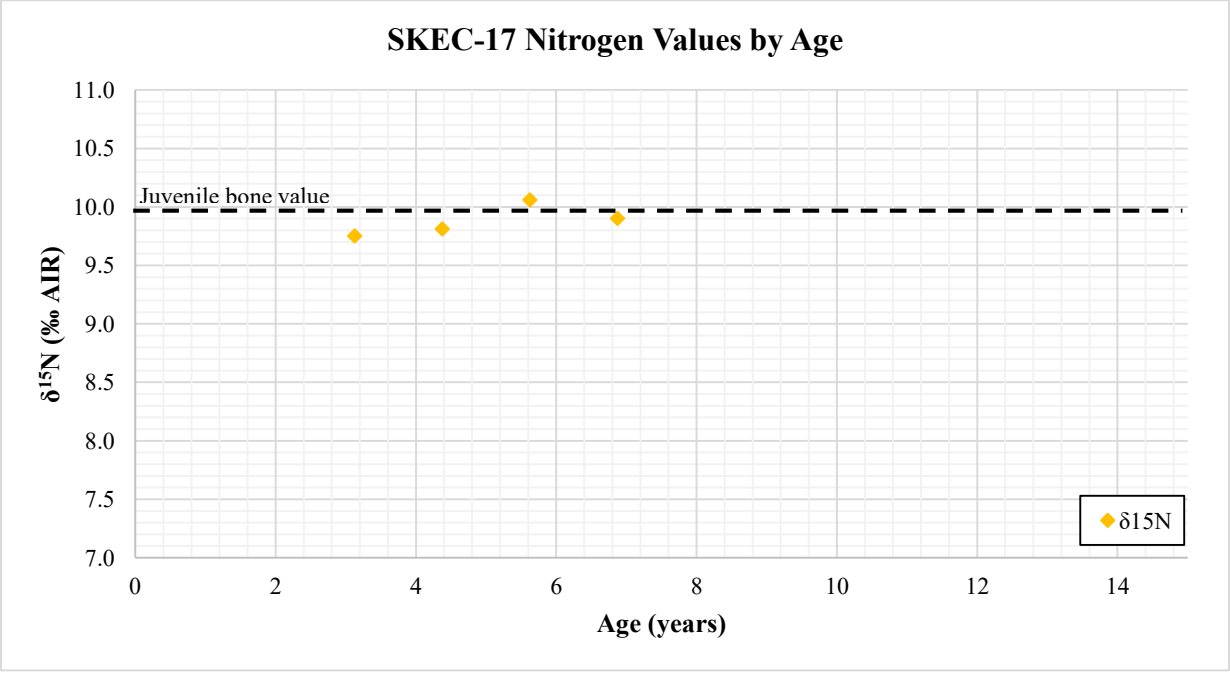


Figure 7. 9 $\delta^{15}N$ values obtained from the dentine segments of SKEC-17 plotted against the estimated age of formation for the segment. The $\delta^{15}N$ juvenile bone collagen value for SKEC-17 is also plotted.



7.1.5 SKEC-18/19

Twelve microsamples were obtained from SKEC-18/19 that map the development of the entire tooth from initiation at 2.5 years to completion at 13.5 years. As seen in Figure 7.10, $\delta^{13}C$ values range from -19.4‰ to -18.7‰. This individual experiences a dip in $\delta^{13}C$ to -19.4‰ at approximately 7-8 years old, as with some other Kenchreai individuals mentioned above. This is the lowest $\delta^{13}C$ value from the dentine microsamples. Two possible adult bone collagen values appear in Figures 7.10 and 7.11 because the tooth could not be definitively matched to one individual as a result of the severely commingled condition of the skeletal remains (discussed in Chapter 4). The highest value is produced by the final segment of the tooth, which overlaps with the adult bone value of SKEC-19 at -18.7‰. SKEC-18 has a slightly higher adult $\delta^{13}C$ value of -18.5‰. Compared to either of these bone collagen values, the dentine $\delta^{13}C$ values suggest less fish consumption during childhood.

The $\delta^{15}N$ values are displayed in Figure 7.11. Values range from 8.9‰ to 10.8‰, and generally increases with age. The $\delta^{15}N$ values also show a decrease in early childhood occurring between 4 and 7 years of age; this recalls the mid-childhood dip seen in the values of other Kenchreai individuals, though it starts earlier than in those individuals and also earlier than the decrease that occurs in SKEC-18/19's $\delta^{13}C$ values. The later dentine $\delta^{15}N$ values of this individual rise above the adult bone collagen values for SKEC-18 and SKEC-19, both of which fall at 9.8‰. The highest dentine value is elevated above the rest at 10.8‰, which is a full 1‰ above the adult bone $\delta^{15}N$ collagen value. The increase in $\delta^{13}C$ and $\delta^{15}N$ values from the later ages of childhood is consistent with increasing marine resource consumption, indeed more than is suggested by this individual's adult bone $\delta^{15}N$ value.

Figure 7. 10 $\delta^{13}C$ values obtained from the dentine segments of SKEC-18/19 plotted against the estimated age of formation for the segment. The $\delta^{13}C$ adult bone collagen values for SKEC-18 and SKEC-19 are also plotted.

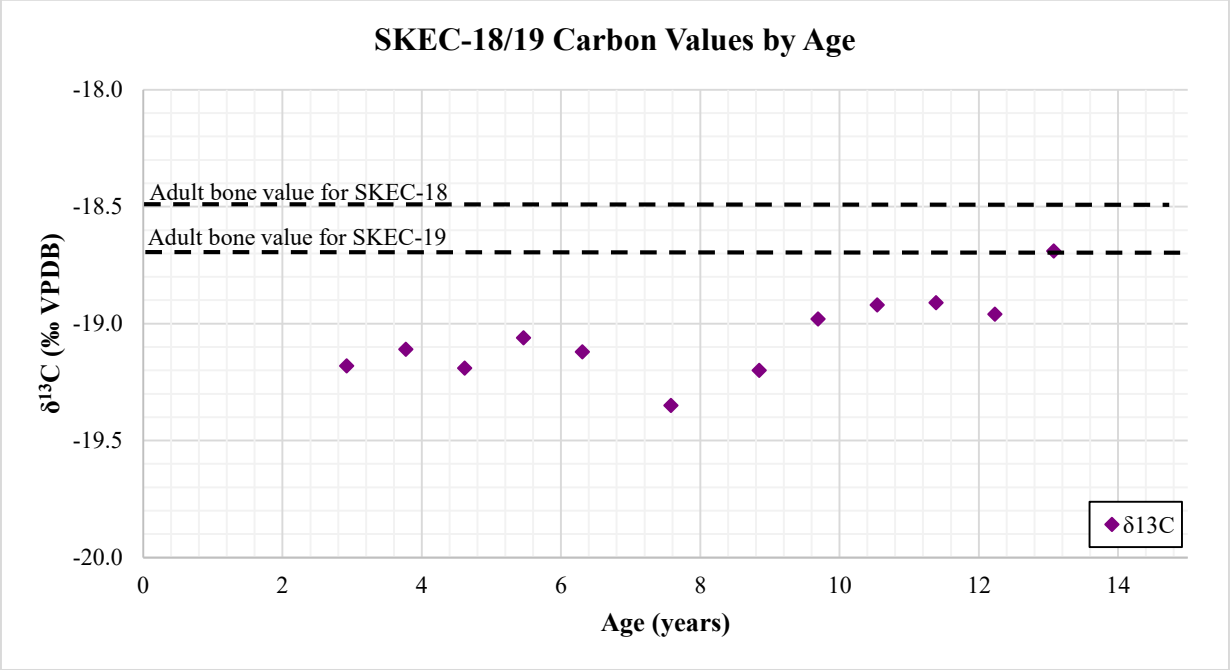
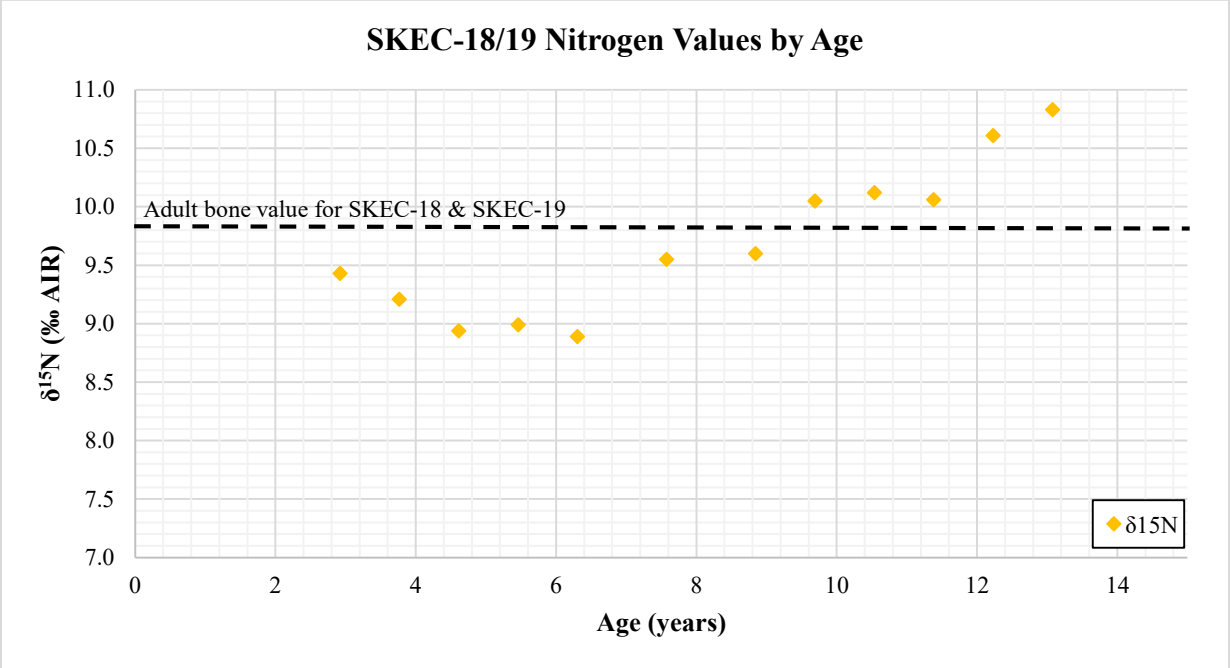


Figure 7. 11 $\delta^{15}N$ values obtained from the dentine segments of SKEC-18/19 plotted against the estimated age of formation for the segment. The $\delta^{15}N$ adult bone collagen value for SKEC-18 and SKEC-19 is also plotted.



7.1.6 Dietary Trends at Kenchreai

Overall, it appears that juveniles at Kenchreai had a different diet from adults that included less marine resources. Some of the $\delta^{15}N$ values obtained from dentine microsamples are similar to adult values, possibly indicating an increase in fish consumption in later childhood. In general, $\delta^{13}C$ dentine values fall below -18.5‰. Looking at Figure 7.1, it is apparent that all Kenchreai samples, both dentine and bone, have $\delta^{13}C$ values between -19.6‰ and -17.9‰. According to Papathanasiou and Richards (2015), $\delta^{13}C$ values in this range indicate a diet primarily based on terrestrial C_3 plants and/or C_3-consuming terrestrial animals.[14] At the high end of the range, some addition of marine resources to this diet is likely. While $\delta^{13}C$ elevation can also indicate the presence of some C_4 plants in the diet, the patterning seen in $\delta^{15}N$ indicates that marine resource use is more likely the case.

The $\delta^{15}N$ values of many of the Kenchreai adults fall above 10‰. This is also true for some of the dentine microsamples. For teeth that yielded dentine values spanning most of the formation period, the stable isotope values tend to increase as the child gets older, and the highest value usually corresponds with the root apex of the tooth. This increase brings the juvenile stable isotope values closer to the adult stable isotope values. There are a few exceptions where the juvenile dentine values match or are higher than the adult values. Elevation above 10‰, if combined with a $\delta^{13}C$ value above -19.0‰, is suggested by Papathanasiou and Richards (2015) to indicate some consumption of fish. Examining the $\delta^{13}C$ and $\delta^{15}N$ values together thus reveals that adults at Kenchreai likely consumed a mixed diet that included both C_3 terrestrial resources, meat from terrestrial animals and fish. This pattern is seen more in juveniles as they get older.

[14] The Kenchreai dentine and collagen values will be compared to the faunal resource values from chapter 4 in section 7.3.

Elevated $\delta^{15}N$ may also be indicative of stress. It is known that individuals considered to be of elite status in the local community were interred in this cemetery (Rife et al. 2007; see also discussion of burial contexts in Chapter 4). There is no information regarding paleopathology of the individuals recovered from Kenchreai. In his discussion of the palaeopathology of Isthmia Rife (2012, 311) notes that both infectious disease and malnutrition potentially impacted children in the Corinthian countryside. Children that experienced multiple stress incidents manifested this in high rates of dental defects (Rife 2012, 311). While there is no evidence to support or disprove stress or malnutrition at Kenchreai, comparison to adult values and associated $\delta^{13}C$ increases make the most probable explanation for rising $\delta^{15}N$ values in late childhood the inclusion of more marine resources to the diet.

Another factor that may influence $\delta^{15}N$ values is growth. As discussed in Chapter 3, growth has been proposed as a possible explanation for decreases in $\delta^{15}N$ values occurring around mid-childhood in some archaeological samples. A decrease of this sort is seen in SKEC-02, SKEC-11, and SKEC-18/19. This could be an isotopic effect of growth, or it could indicate a distinctive diet for children of approximately 6-8 years of age. The link between $\delta^{15}N$ and growth in archaeological populations is still debated. Moreover, these three Kenchreai individuals also experience a decrease in $\delta^{13}C$ around mid-childhood. A $\delta^{13}C$ effect of this sort has not been proposed for growth, making the argument for a specific mid-childhood diet somewhat more plausible. Clearly, though, further research must be done to improve our understanding of childhood changes in $\delta^{13}C$ and $\delta^{15}N$ values.

In most of the individual profiles discussed here, the differences in stable isotope values are small. Variations in $\delta^{15}N$ between dentine microsamples are less than 2‰; differences in $\delta^{13}C$ are less than 1‰. While these may not seem like important differences, we should recall

that subtle variations in diet are to be expected for individuals within a single community and even a single family. If we ignore variations of this magnitude, we are likely to ignore much within-population dietary variation. The results obtained here also show that the isotopic composition of a tooth is more complicated and nuanced than the information provided by a single bulk dentine sample. The complexity of diet cannot be examined to its fullest extent when only bulk samples are analyzed and average values are obtained. For lifetime diet, every individual can be seen in terms of a series of stable isotope values, rather than a single stable isotope signal. This is evident from the isotope profiles above. The microsampling technique has allowed for a greater understanding of how diet can change within an individual over their life. It has also allowed for general patterns to be identified in juveniles, and has revealed that individual variation from this pattern may exist. Microsamples from three of the Kenchreai individuals generally show a shift toward a more adult-like diet as they grow, but SKEC-05 and SKEC-17 do not show this pattern. This may reflect an unknown dietary, health or nutritional factor that influenced their stable isotope values. Data from the dentine stable isotope study has expanded our knowledge of diet at Kenchreai at the site level, but also at the individual level, where juvenile and adult diet can be examined within an individual.

7.2 Isthmia

As with Kenchreai, a brief discussion of the bone collagen values is necessary before discussing individual isotope profiles. Overall, the dentine collagen stable isotope values for the individuals from Isthmia fall into the range of values provided by the analysis of bone collagen. In comparison to the adult bone collagen values, the dentine stable isotope values are either in range or slightly lower than the adult values. In contrast to most of the Kenchreai juveniles,

signals showing the highest elevation were not produced from the last segment of the Isthmia teeth.

The same statistical analysis described in section 7.1 was used for the Isthmia individuals. The average dentine values for $\delta^{13}C$ and $\delta^{15}N$ were calculated for each of the Isthmia individuals and added to the group of bone values for the site (seen in Figure 7.12). Similar to Kenchreai, Isthmia also has two juveniles younger than 3 years of age whose bone collagen values show clear nursing signals (Garvie-Lok 2010, 4). They are excluded from this analysis. As for Kenchreai, a critical value of p =0.05 was used in the testing. Unlike Kenchreai, the results indicate that the $\delta^{13}C$ (t = 0.741, p-value = 0.48) and $\delta^{15}N$ (t = 1.988, p-value = 0.075) stable isotope values of adults and juveniles are not significantly different. Table 7.3 displays general mean/range statistics for the Isthmia sample and Table 7.4 contains the results of the t-tests.

Figure 7. 12 Comparison of all $\delta^{13}C$ and $\delta^{15}N$ values for juveniles and adults at Isthmia.

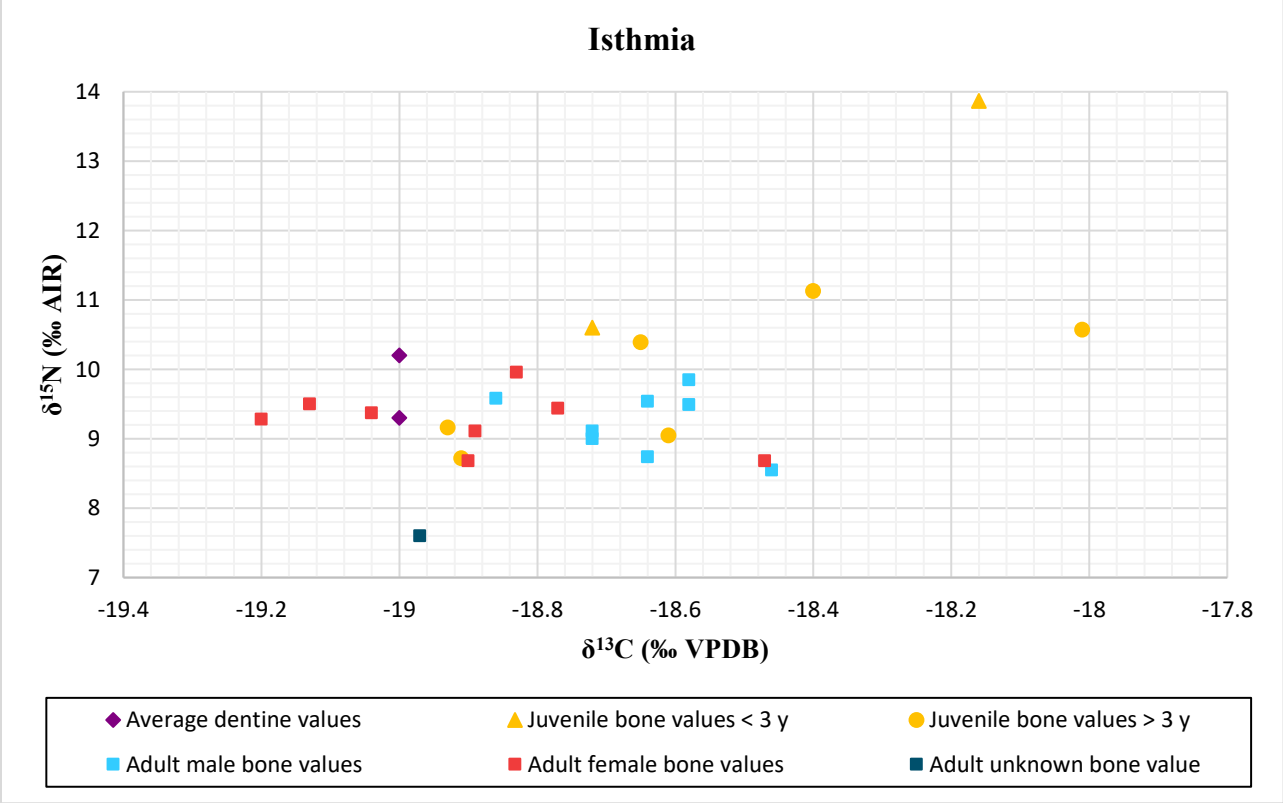


Table 7. 3 General mean/range statistics for $\delta^{13}C$ and $\delta^{15}N$ for juveniles and adults at Isthmia. "Juveniles" includes the average dentine values and excludes individuals below 3 years of age.

Age Group	n	Mean/range of $\delta^{13}C$ (‰)	Mean/range of $\delta^{15}N$ (‰)
Juveniles	8	-18.7 ± 0.35	9.8 ± 0.87
Adults	17	-18.8 ± 0.22	9.1 ± 0.57

Table 7. 4 Results of unequal variance *t*-tests (two-tailed) for the Isthmia sample.

Test	*t*-value	*p*-value
Comparison of $\delta^{13}C$ values for juveniles and adults	0.741	0.48
Comparison of $\delta^{15}N$ values for juveniles and adults	1.988	0.075

There is little variation between the adult mean $\delta^{13}C$ and the juvenile mean $\delta^{13}C$ values. Slightly more variation exists in the $\delta^{15}N$ values with juveniles having a higher mean than adults. This reflects the inclusion of bone collagen values for three juveniles aged around 3 years at death. These three juveniles show higher $\delta^{15}N$ values than older juveniles and adults, and are likely still displaying some nursing elevation. The isotope profiles of the two Isthmia individuals and a discussion of their values in comparison to the adult values are provided below.

7.2.1 Isthmia 67-001D

Twelve microsamples were sectioned from this tooth but three of them failed to produce collagen. An additional sample was rejected from this study because of potential contamination (see discussion in Chapter 6). These samples were not localized to one part of the tooth, so data from the entire tooth representing ages 3.5 to 14.5 are still available. The stable isotope values of the remaining eight microsamples are illustrated in Figures 7.13 and 7.14 below. The $\delta^{13}C$ values range from -19.3‰ to -18.8‰ and generally increase as this individual gets older. The dentine segments that formed after approximately nine years of age match or surpass the adult bone value. The $\delta^{15}N$ values range from 9.8‰ to 10.9‰, and they are all elevated above the adult bone value. Segment I represents diet around age 11, and it has the highest $\delta^{15}N$ value of 10.9‰.

The $\delta^{15}N$ values appear to decrease in early childhood, increase around age 11, and then decrease again. According to Garvie-Lok (2010), adults at Isthmia had a largely C_3 terrestrial based diet, but also consumed some marine resources. The older juvenile bone values at Isthmia suggest low fish consumption relative to the adults and minor age and sex based dietary differences (Garvie-Lok 2010). Perhaps this individual consumed a childhood diet that included more marine resources than their adult diet. Higher consumption of other animal-derived foods such as domesticate meat or dairy seems more likely, though, since this would cause elevated $\delta^{15}N$ values in the absence of $\delta^{13}C$ elevation, which is the pattern seen in Isthmia 67-0001D's dentine. Alternatively, they may have experienced at least one episode of childhood stress that elevated their childhood $\delta^{15}N$ values. The pathological information presented in the osteological report shows no signs of a major childhood stress episode or infectious disease in this individual, although the estimated age at death is young, at 20-21 years (Rife 2012, 370). From the available evidence it is difficult to say why the juvenile $\delta^{15}N$ values are elevated above the adult bone collagen value. It is not unreasonable, though, to suggest that they potentially experienced childhood stress which may have been a factor in their death in early adulthood.

Figure 7. 13 $\delta^{13}C$ values obtained from the dentine segments of Isthmia 67-001D plotted against the estimated age of formation for the segment. The $\delta^{13}C$ adult bone collagen value for Isthmia 67-001D is also plotted.

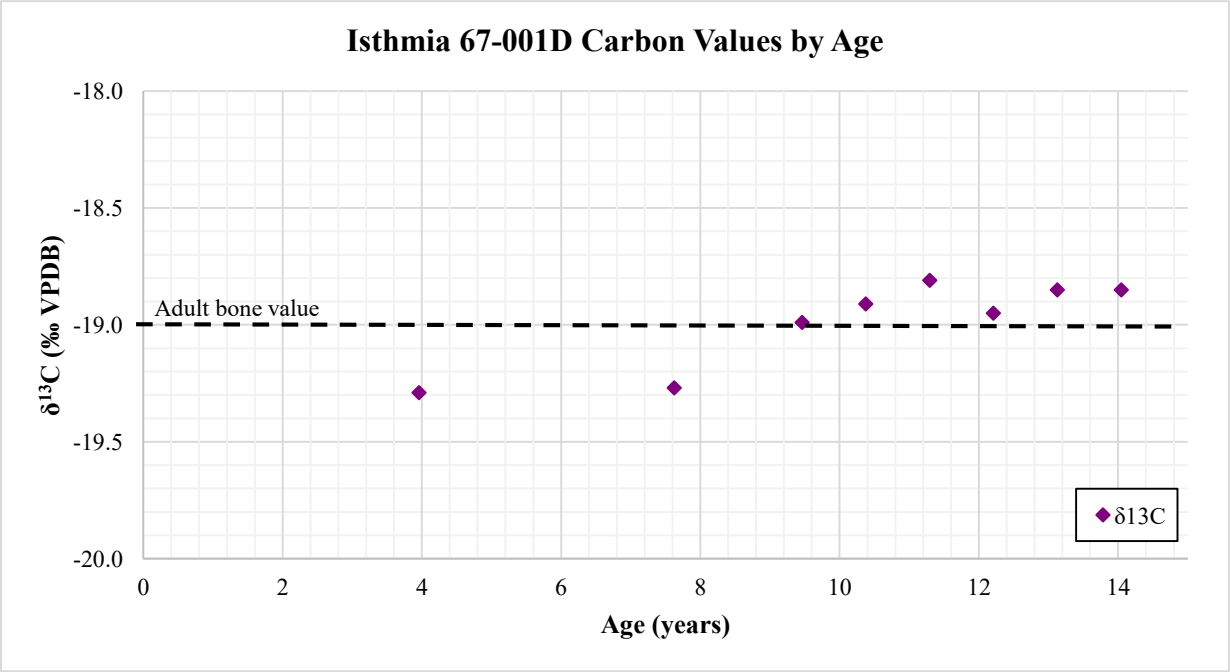
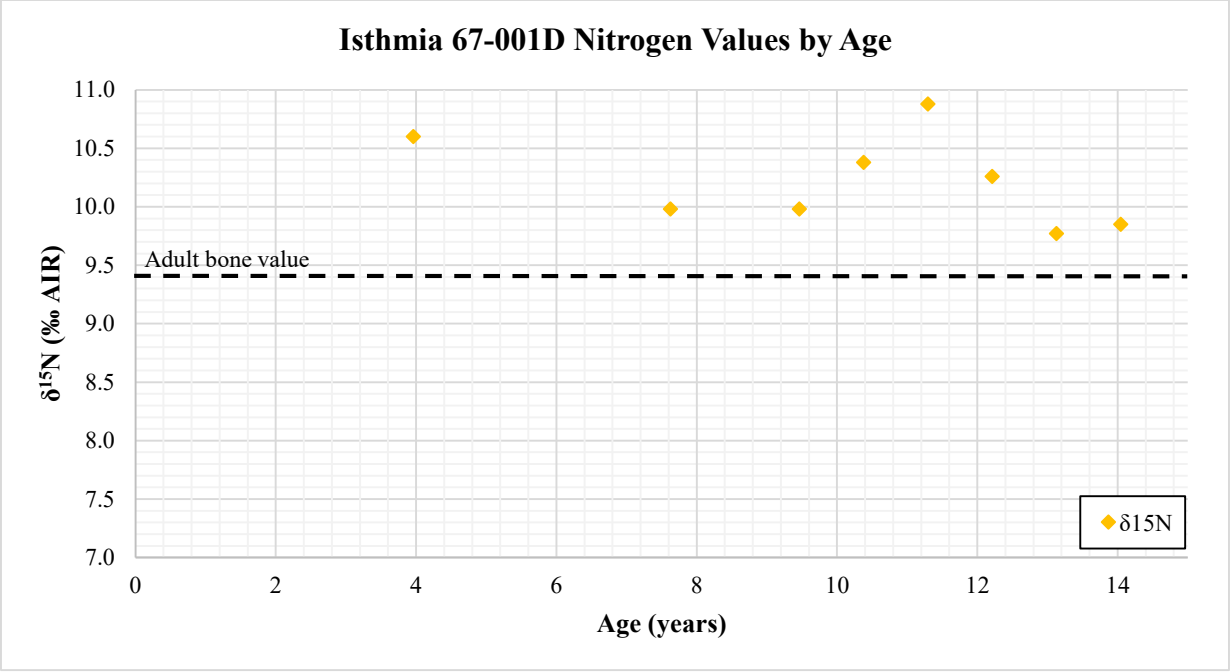


Figure 7. 14 $\delta^{15}N$ values obtained from the dentine segments of Isthmia 67-001D plotted against the estimated age of formation for the segment. The $\delta^{15}N$ adult bone collagen value for Isthmia 67-001D is also plotted.



7.2.2 Isthmia 69-004E

Thirteen microsamples were taken from this tooth, four of which did not produce collagen. Similar to the other Isthmia tooth, the failed microsamples were not localized to one area of the tooth, thus data over the entire course of tooth formation from age 2.5 to 13.5 years is available. This individual does not have associated stable isotope values from bone collagen. Instead, the values are compared to the mean $\delta^{13}C$ and $\delta^{15}N$ values for adults at Isthmia. The $\delta^{13}C$ values of Isthmia 69-004E range from -19.2‰ to -18.8‰. Unlike many other samples in this study, the highest $\delta^{13}C$ value does not occur in the final segment of the tooth. However, the segment containing the root tip did not produce collagen so we cannot say for sure whether values increased toward or above the mean adult value. Based on the values of the three microsamples preceding the root tip, dietary $\delta^{13}C$ was actually decreasing at this time.

The $\delta^{15}N$ values range from 9.0‰ to 9.6‰, with the lowest value occurring at approximately 11 years of age, and the highest around 5-6 years. The $\delta^{13}C$ and $\delta^{15}N$ values for this individual do not reflect the same pattern. For example, where $\delta^{13}C$ increases around age 4, $\delta^{15}N$ decreases. However, there is no overall pattern of opposition in $\delta^{13}C$ and $\delta^{15}N$ changes; instead both measures fluctuate close to the adult means for the site. In comparison to the adult mean values, all but one $\delta^{13}C$ value show a depression in this individual, whereas most $\delta^{15}N$ values are slightly elevated above the adult mean. Using the criteria suggested by Papathansiou and Richards (2015), this individual shows $\delta^{13}C$ and $\delta^{15}N$ values suggestive of a C_3 terrestrial diet with little or no marine resource use.

Figure 7. 15 $\delta^{13}C$ values obtained from the dentine segments of Isthmia 69-004E plotted against the estimated age of formation for the segment. The mean $\delta^{13}C$ adult bone collagen value is also plotted.

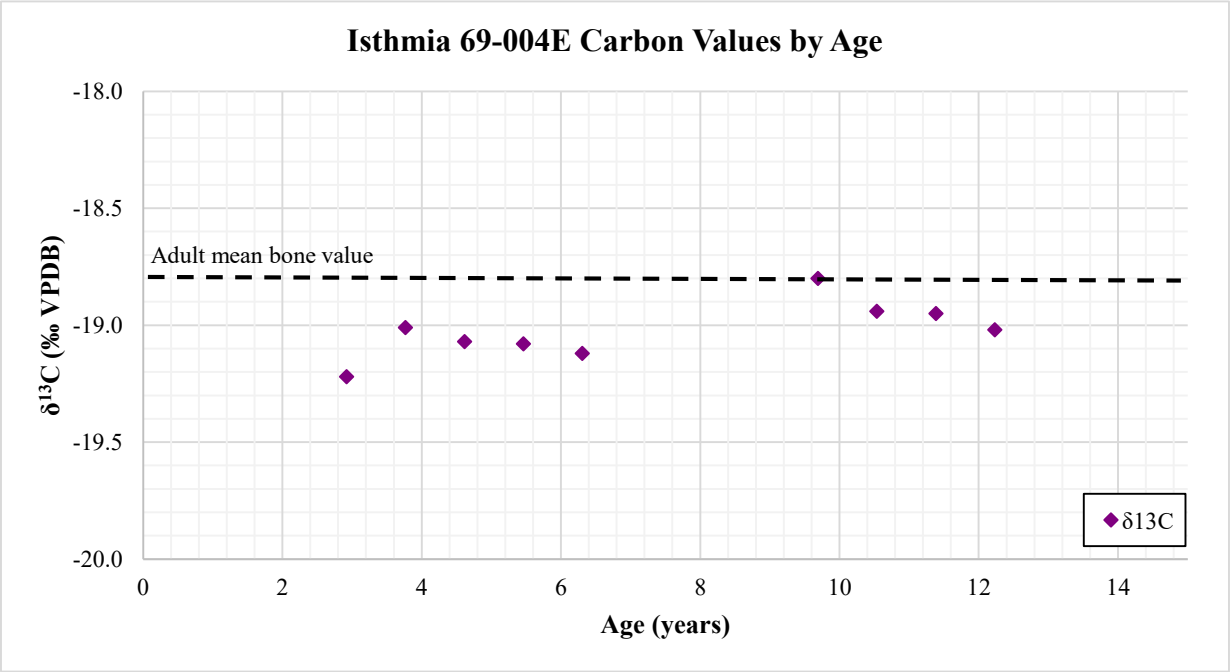
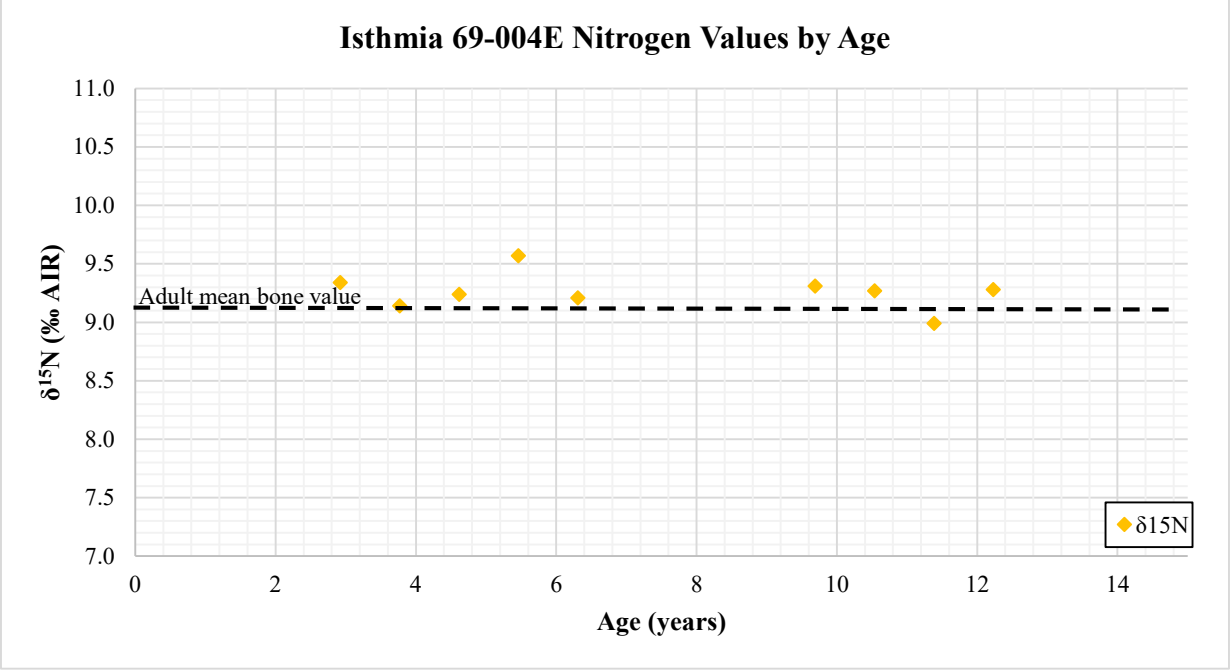


Figure 7. 16 $\delta^{15}N$ values obtained from the dentine segments of Isthmia 69-004E plotted against the estimated age of formation for the segment. The mean $\delta^{15}N$ adult bone collagen value is also plotted.



7.2.3 Dietary Trends at Isthmia

Though the dentine study on Isthmia is preliminary, some general comments can be made. Figure 7.12 presents all of the juvenile and adult dietary data obtained from the Isthmia sample. Overall, $\delta^{13}C$ values are consistent with a C_3 terrestrial diet; $\delta^{15}N$ potentially indicates modest fish consumption but not to the same degree as seen at Kenchreai. This suggests that neither juveniles nor adults were consuming a significant amount of marine resources, which is also suggested by the childhood dietary profiles created with dentine stable isotope values. There is little variation between the values of Isthmia 69-004E and the adult mean values, suggesting small dietary differences. This is also seen in the $\delta^{13}C$ values of Isthmia 67-001D. While it is possible that Isthmia 67-001D consumed a different diet as a child than as an adult and that caused the elevation in their dentine $\delta^{15}N$ values, the possibility of stress cannot be ruled out.

Dentine microsampling has allowed for an in-depth look at childhood diet at Isthmia. This is especially important in cases where childhood dietary isotope values are higher than the adult bone collagen values, demonstrating that diet is more than can be represented by a bulk sample or a single bone collagen value. Identifying these dietary differences allows us to speculate on individual life histories and factors that may have influenced dietary choices.

7.3 Dietary Trends at Kenchreai and Isthmia

The dietary trends at Kenchreai and Isthmia agree with the observations of Garvie-Lok (2010). As can be seen from the isotope profiles presented above, it appears that children were generally eating less marine resources than adults at Kenchreai, whereas any age differences at Isthmia would have been modest. However, a few of the dentine segments analysed in this study produce stable isotope values that are considered to be consistent with marine resource consumption (Papathanasiou and Richards 2015).

In comparison to the faunal values discussed in Chapter 4 (displayed again in Figures 7.17 and 7.18), all dentine $\delta^{13}C$ values from Kenchreai and Isthmia fall fairly close to the values of terrestrial fauna, namely sheep/goat, cow and pig. This suggests a diet dominated by these terrestrial resources, although some marine consumption is possible. Most of the values for archaeological fish provided in Chapter 4 have markedly higher $\delta^{15}N$ values than the land fauna; the majority of the fish samples fall above 6.0‰, whereas most of the land fauna fall below that value. Most of the human collagen and dentine $\delta^{15}N$ values fall above 9.0‰, and many are above 10‰. This $\delta^{15}N$ patterning more strongly suggests a marine component to the diet, as the human $\delta^{15}N$ values are elevated more than a trophic level above most of the land fauna. Thus, overall, the Isthmia and Kenchreai bone and dentine collagen suggests a land-dominated diet supplemented with marine resources, whether the values are compared to the overall guidelines suggested by Papathanasiou and Richards (2015) or to the faunal values reviewed in Chapter 4. Note that even at the expanded scale used to depict them along with the faunal values, a clear difference between Kenchreai juveniles and adults can still be seen (Figure 7.17). In contrast, Isthmia juvenile and adults overlap (Figure 7.18).

Figure 7. 17 All Kenchreai individuals above 3 years of age compared to terrestrial and marine faunal values. The faunal data are those presented in Figure 4.3, but are broadly grouped (terrestrial vs. marine).

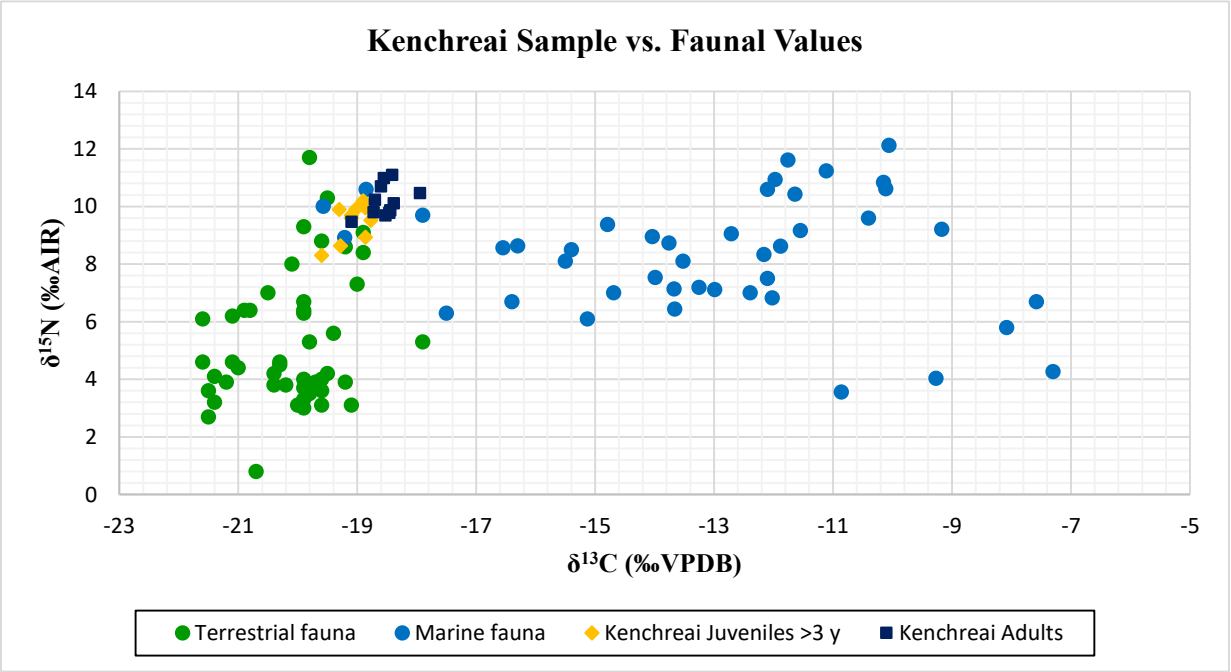
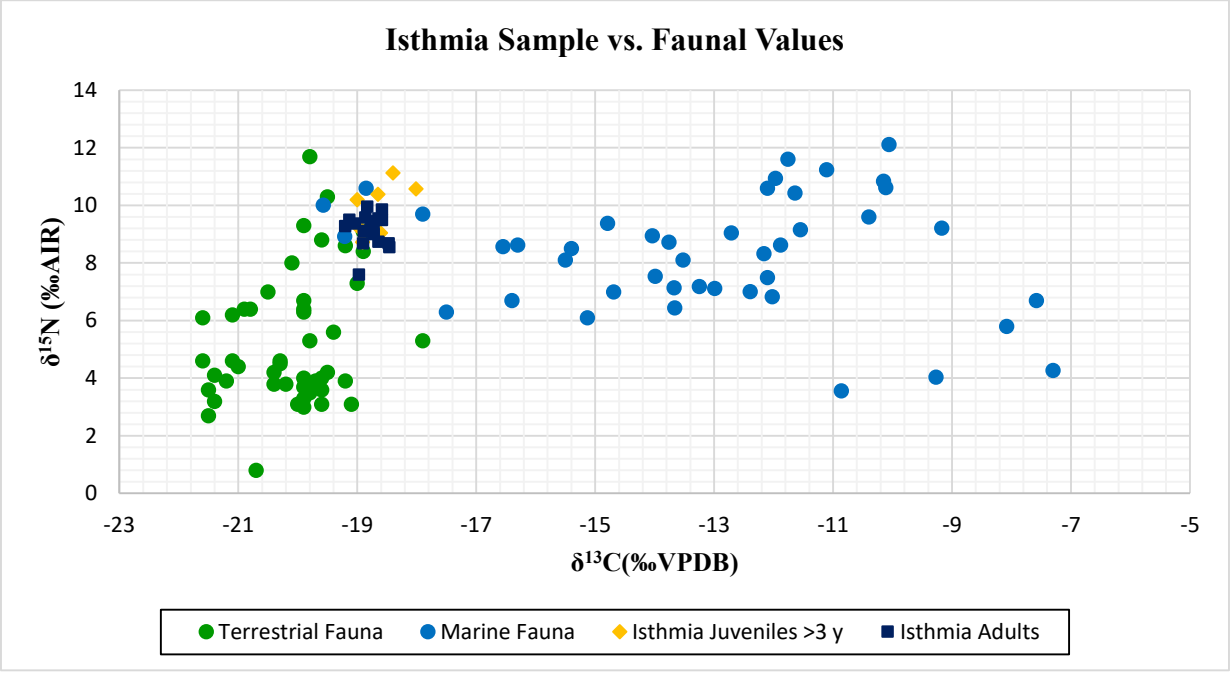


Figure 7. 18 All Isthmia individuals above 3 years of age compared to terrestrial and marine faunal values. The faunal data are those presented in Figure 4.3, but are broadly grouped (terrestrial vs. marine).



Within this overall pattern, the individual dietary profiles show variability. The profiles of SKEC-02, SKEC-11, and SKEC-18/19 show $\delta^{13}C$ and $\delta^{15}N$ values that reflect a shift towards

a diet that included more marine resources – so, more adult-like – toward the end of the tooth's formation period. In this case, it seems that individual dietary histories are consistent with the expected shift from a childhood to an adulthood diet. Isthmia 67-001D also shows a rise for $\delta^{13}C$ toward the end of formation, when it exceeds the bone collagen value, but not for its $\delta^{15}N$ values, which rise and fall over the tooth's formation period but remain consistently above the adult bone collagen value. In a case like this, idiosyncratic dietary changes or the effects of stress might explain the patterning seen. SKEC-05, SKEC-17 and Isthmia 69-004E have $\delta^{15}N$ values that closely match the adult mean $\delta^{15}N$ values for their sites. Isthmia 69-004E has $\delta^{13}C$ values that reach the adult mean, but then the values of the last few microsamples slightly decrease. The $\delta^{13}C$ values of SKEC-05 and SKEC-17 lie below the adult mean; in the case of SKEC-17, the four available $\delta^{13}C$ values, which cover the ages of roughly 3 to 7 years, are clearly decreasing over this time. In these cases, it does not seem that a childhood diet with less fish was being consumed, though there does seem to be dietary variation over the childhood period and between childhood and adulthood.

Adults at Kenchreai were consuming more fish than adults at Isthmia, and more than juveniles at both sites. This suggestion had been made after the initial bone collagen study (Garvie-Lok 2010, 5). Most dentine values agree with this idea, but they add to this by demonstrating subtle dietary differences in some individuals that occurred as the child grew, with the values increasing toward adult values. This dietary difference may be a result of fish being primarily consumed by individuals of high status (Garvie-Lok 2010, 5). Kenchreai was likely an elite community (Rife 2007), so most elite adults would have access to a high-status commodity such as fish. While children may have consumed less fish, it is clear that not all Kenchreai children had the same diet. There are also changes in diet within an individual. This is perhaps a

reflection of status or sex based dietary differences, or different ideas of what is appropriate to feed a child, which may have varied between families.

The stable isotope results are consistent with information about childhood diet presented in Chapter 2. Marine resources were likely a food consumed by high-status individuals. According to Marzano (2018), fish and seafood were popular items to serve at feasts and banquets. These events would have been attended by elite members of society, which would likely exclude most children. The contributions of marine resources to the diet of the average person is unknown as this information is largely unavailable from historical sources. Moreover, historical sources rarely mention children consuming fish. However, the isotope profiles of three Kenchreai individuals demonstrate a rise in $\delta^{15}N$ which more closely resembles their adult bone collagen $\delta^{15}N$ values. The estimated ages at which this rise begins (about 10 years in SKEC-02, 14 years in SKEC-11, and 12 years in SKEC-18/19) are similar to the life stage where children were beginning to gain more adult responsibilities, which was around the onset of puberty (12 to 14 years). This dietary shift toward a more adult-like $\delta^{15}N$ value may reflect the inclusion of more adult foods, such as fish, in the diet, which could be associated with puberty and the transition to adulthood.

Rife (2012, 295) characterizes the diet of Isthmian individuals as including a mix of meats, fruits, vegetables, cereals and dairy products. It is likely that the Kenchreai diet also included some of these elements as they would likely have similar resources given that Kenchreai and Isthmia are in close proximity to each other. Given that Kenchreai was likely an elite community, this population may have had more access to fish than individuals at Isthmia (Rife 2007; Garvie-Lok 2010).

Overall dietary trends can be highlighted when Kenchreai and Isthmia, both of which are coastal sites, are compared to the inland Late Roman sites of Nemea and Stymphalos, previously mentioned in Chapter 4. Nemea and Stymphalos show stable isotope values that are clearly indicative of a C_3 terrestrial diet with no contributions from marine resources (Garvie-Lok 2010, 2013) Values for Nemea are $\delta^{13}C$ = -19.0±0.7‰, $\delta^{15}N$ = 8.5±0.6‰ (n=20, all individuals over 3 years). Values for Stymphalos are $\delta^{13}C$ = -19.2±0.5‰, $\delta^{15}N$ = 8.7±0.6‰ (n=21, all individuals over 3 years). As mentioned in Chapter 4, a clear elevation is seen in $\delta^{13}C$ and $\delta^{15}N$ values at Kenchreai over Nemea and Stymphalos. The $\delta^{13}C$ and $\delta^{15}N$ values of the Isthmia population are elevated relative to the inland sites as well, but there is also overlap with the inland populations. From this information it can be inferred that of the four groups, marine resources were the most important to the diet of the Kenchreai population, and that the Isthmia population incorporated more fish into their diet than the inland groups while still placing a larger emphasis on terrestrial animal and plant foods than Kenchreai.[15] The addition of further juvenile values strengthens this interpretation, as the dentine collagen values fit into the range of juvenile values for each site.

Another study on childhood diet using dentine microsampling was conducted by Kwok and colleagues (2018) on teeth from the same Nemea burial group that yielded the bone collagen samples discussed above. Dentine microsampling was used to examine infant feeding practices in the community by profiling the early diets of twenty-six adults from the site. In contrast to the present thesis, Kwok and colleagues (2018) focus on values from dentine microsamples covering childhood from birth up to 6 years of age to specifically examine breastfeeding and weaning and to identify potential sex-based differences in weaning age. The results of the study suggest that most infants, whether male or female, were weaned by about 2.5 years of age (Kwok, Garvie-

[15] This information on general site patterns was obtained from Garvie-Lok (2010).

Lok, and Katzenberg 2018). This is consistent with the dentine profiles from the Isthmia and Kenchreai premolars. None of these show clear signs of nursing elevation in their earliest segments, suggesting that here too weaning was typically complete by about 2.5 years when the premolars started to form. However, it stands in contrast with the bone collagen $\delta^{15}N$ values from Isthmia, which, as discussed above, seem to show nursing elevation up to the age of 3 years. This may reflect differences between nursing ages based on bone collagen values, which are expected to show some turnover lag, and ages reconstructed by dentine analysis. After weaning, the Nemea isotope profiles show similar slight variations in $\delta^{13}C$ and $\delta^{15}N$ over an individual's childhood to those described for the Isthmia and Kenchreai isotope profiles above. Post-weaning (ages 5.0-5.9 years) dentine values at Nemea were -19.3±0.5‰ for $\delta^{13}C$ and 8.8±1.0‰ for $\delta^{15}N$ (Kwok, Garvie-Lok, and Katzenberg 2018), suggesting that children were eating a terrestrial diet, which agrees with the adult data from Nemea discussed above (Garvie-Lok 2010). The benefits of dentine microsampling are also demonstrated by the Nemea study, as weaning age estimates from dentine show improved accuracy from values that would be obtained by bulk samples (Kwok, Garvie-Lok, and Katzenberg 2018).

The benefits of dentine microsampling are also clearly shown by the present thesis study. Because of this technique, the Kenchreai juvenile sample has now been expanded from seven to twelve individuals. A closer examination of childhood diet was made possible, and a greater understanding of dietary change within childhood has been obtained. While variations in $\delta^{13}C$ and $\delta^{15}N$ values are small, they illustrate that diet is more complex than the information that is gained from only using a single bulk sample or bone collagen value. Nuance has been given to the life history of each individual in this study that is otherwise lost.

7.4 Summary

The results of the study have been interpreted to judge whether children at Kenchreai and Isthmia consumed as much fish as adults at each site. The results indicate that while fish was likely consumed in the later years of childhood, it was not a significant contributor to childhood diet as it is to adult diet. The topics of growth and stress were considered when $\delta^{13}C$ and $\delta^{15}N$ values differed from what was expected. All possible factors that may influence stable isotope data were considered to provide a comprehensive interpretation of each individual. Inter- and intra-individual patterns are identified and general site trends are explored. The data is also briefly compared to the inland sites of Nemea and Stymphalos. The importance of dentine microsampling is reiterated here.

Chapter 8: Conclusions

Using dentine microsampling analysis, collagen $\delta^{13}C$ and $\delta^{15}N$ values were generated for five premolars from Kenchreai and two premolars from Isthmia. These were plotted against the estimated formation age of each microsample and (where available) against that individual's associated adult or juvenile bone collagen value, creating isotopic profiles that were used to examine juvenile diets. The resulting individual dietary profiles provided insights into Late Roman childhood diets, including information on age differences in marine resource consumption and inter-individual dietary variation. The purpose of this final chapter is to summarize the core findings of the thesis, to discuss their relevance to wider research on Roman era diets and to propose some directions for further research.

8.1 The Diet of Juveniles at Kenchreai and Isthmia

The purpose of this study was to follow up the results from the Kenchreai and Isthmia bone collagen analyses with a microsampling study on dentine collagen to investigate an apparent difference in diet between juveniles and adults. The overall findings of this research shows that juveniles at Kenchreai likely consumed a different diet from adults that included less marine resources. Differences between juveniles and adults are not apparent at Isthmia, though this evaluation might change if the rest of the teeth available for Isthmia were studied.

At Kenchreai, using average dentine values from the five teeth studied added five juvenile values to the human collagen stable isotope data set for the site. This confirmed the impression from the initial bone collagen study that marine resources played a larger role in adult diets. Fish may have been a contributor to the diets of juveniles at Kenchreai, but the stable isotope profiles presented in Chapter 7 indicate that it was not as important a dietary item for them as it was for adults at the site. Perhaps fish was considered to be a delicacy for elites living

in Kenchreai. Chapter 2 mentions that fish and seafood were items often served at elaborate feasts and banquets, which would have been hosted and attended by elite members of society. Presumably, most children would not attend these events. Alternatively, this may reflect a distinction between the consumption of food that was considered to be just for children versus food that only adults consumed. The foods often associated with a weaning diet are grain based, and older children were commonly associated with sweets and cakes. It is possible that this is the dietary difference visualized in the isotope profiles. However, within this general patterning the individual stable isotope profiles varied. In three of the Kenchreai individuals, a shift to a more adult-like diet can be seen toward the end of the isotope profiles. These shifts occur between the estimated ages of 10 and 14 years. These profiles illustrate a rise in $\delta^{13}C$ and $\delta^{15}N$ during what we would call late childhood. This may reflect a change in individual dietary preference, with children wanting to consume more adult foods as they grow. It may also reflect variations in family values, with parents expecting children to consume more adult foods as they reached certain ages. The ages of these dietary shifts occur in the stages of childhood in Roman Greece (as discussed in Chapter 2) where children started to gain responsibilities and were at the onset of puberty. A change in diet at this point in childhood is perhaps associated with puberty – maybe certain dietary foods were expected to be consumed to assist the growing body during the physical transition to early adulthood. In the other two Kenchreai teeth and the two teeth from Isthmia, different patterns are seen. This includes one tooth (Isthmia 67-001D) whose $\delta^{15}N$ values may reflect childhood stress. These individual differences are significant not only because they have interesting implications for life at Roman Kenchreai and Isthmia, but also because they illustrate the broader point that childhood diet is more nuanced and complicated than the stable isotope information gained from a single bulk sample or bone collagen value might suggest.

Slight variations in diet within individuals have been identified over the course of their childhoods. This has great implications for the bioarchaeology of children in general, as it provides a more accurate representation of diet and allows for dietary changes to be identified within and between individuals, offering us the opportunity to move beyond research at the group or population level to a more nuanced consideration of individual lives.

8.2 Future Work

One clear possibility for future work is a microsampling study on more of the Isthmia remains. There are more teeth from the Isthmia population available for study. Though several microsamples from the Isthmia teeth used in the current thesis study did not produce collagen, clear isotopic profiles covering most of the formation period were still recovered for both teeth, suggesting that it would be worth microsampling the dentine of the other available teeth. The remaining Isthmia teeth are in adequate condition, much like the ones used in this thesis. Thus, even if this problem continues and some microsamples do not produce collagen, it is likely that the teeth will still provide more dietary information than what we currently have for the individuals in question. It would also be interesting to see whether other Isthmia individuals show potential signs of childhood stress through their $\delta^{15}N$ values, as was the case for Isthmia 67-001D.

Only half of a tooth from each individual was sectioned and used in this study. The other half portions remain in storage and are available for use in future analyses. A possible avenue of research for both Kenchreai and Isthmia would be a stable isotope study on migration. This would allow for the potential identification of any non-local individuals at these sites. This brings up the possibility that the intra-individual dietary differences seen in juvenile and adult values may be a result of growing up in different communities or regions, where they may have

had access to different dietary resources. This possibility was not discussed in the thesis because it cannot be tested with the available data. It would be possible to explore this idea with stable isotope analyses of strontium and oxygen on the remaining partial tooth samples. If the results of the study on migration do not indicate the presence of non-local individuals, that would strengthen the interpretations made in this thesis about marine resource consumption and stress.

In addition to specific studies at Kenchreai and Isthmia, further palaeodietary work that uses dentine microsampling would be beneficial in other Greek archaeological populations. Dentine microsampling offers a solution to the problem of low recovery rates of juvenile skeletal remains at Greek archaeological sites. In the present study, dentine microsampling expanded the set of juvenile dietary values at Kenchreai from seven to twelve individuals, confirming patterning suggested by the initial bone collagen data. Applying the microsampling method to more archaeological sites in Greece will not only increase the number of juveniles represented in a sample, but it will offer a more in-depth look at dietary changes over the childhood period. The benefits of this would include an improved ability to study weaning practices, which are currently poorly understood at most sites due to low sample sizes of juvenile remains (Bourbou et al. 2013). As well, stable isotope analysis of dentine microsamples provides an opportunity to examine juvenile diets after the periods of breastfeeding and weaning. Stable isotope studies often focus either on these topics, or on adult diet. Little consideration is put into the diets of older juveniles and teenagers, in part because it was previously difficult to target diet in this period and possibly in part because of lower research interest in diet at these ages. Now that dentine microsampling offers an improved method of examining this period in an individual's life, more studies can be conducted to increase our understanding of dietary variation within and between individuals and populations during this life stage.

8.3 Summary

This chapter summarizes the main findings of the thesis study, which has examined marine resource consumption in juveniles from the sites of Kenchreai and Isthmia. The $\delta^{13}C$ and $\delta^{15}N$ values obtained in this study have confirmed that juveniles were in fact consuming less fish than adults. Possible reasons for dietary differences are provided. Moreover, the results demonstrate it is possible to detect small dietary changes within individuals. The successful application of the dentine microsampling technique has allowed for the identification of subtle intra- and inter-individual dietary differences in this sample. It also demonstrates the potential for applying this method to other studies, especially in archaeological sites where juvenile skeletal remains are infrequently recovered. Suggestions for future work on the Isthmia remains and for studies using dentine microsampling have been offered.

The results of this study expanded the sample of juvenile remains at Kenchreai and Isthmia. It also provided multiple dietary signals for each individual in the sample, adding more nuance to the previous bone collagen isotope results. This has led to a greater understanding of individual life histories at each site, which are more complex than most stable isotope studies are able to illustrate from a single bone or dentine collagen value.

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Appendix 1: Photographs of Teeth Examined from Kenchreai and Isthmia

Figure A.1.1: Macroscopic view of SKEC-02.

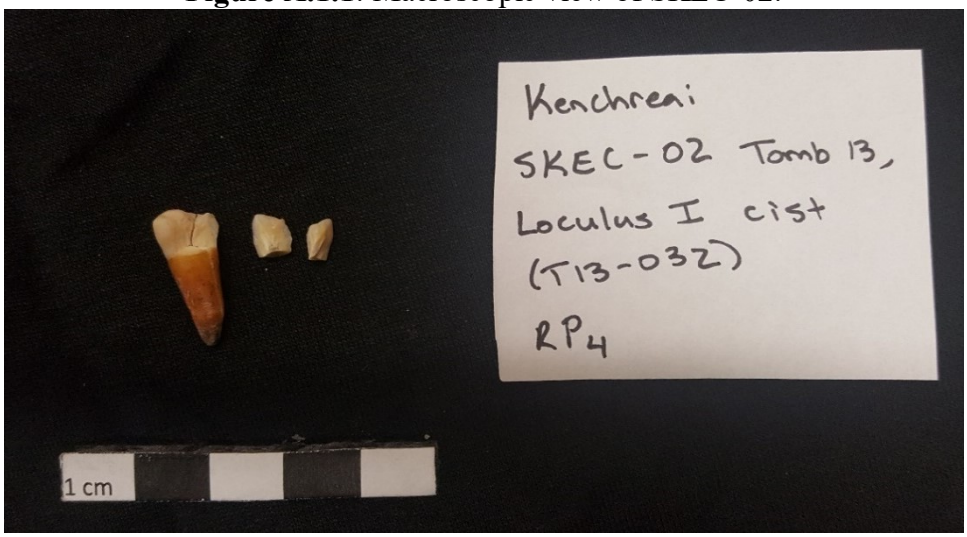

Figure A.1.2: Occlusal view of SKEC-02, showing the crown segments that broke during lab preparations.

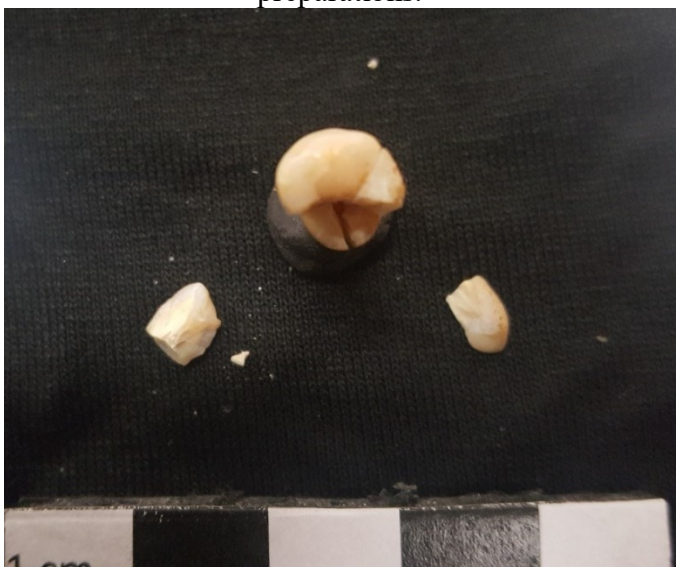

Figure A.1.3: Macroscopic view of SKEC-05.

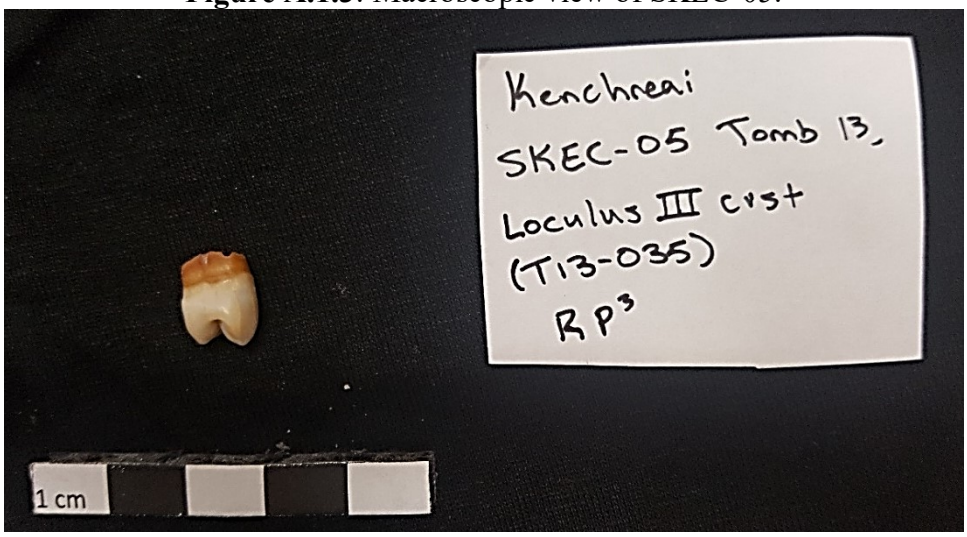

Figure A.1.4: A close-up of SKEC-05, showing the developing root.

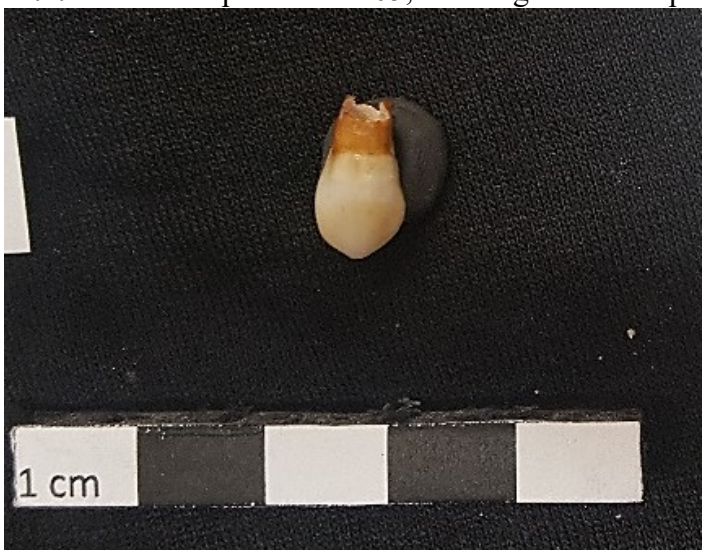

Figure A.1.5: Macroscopic view of SKEC-09.

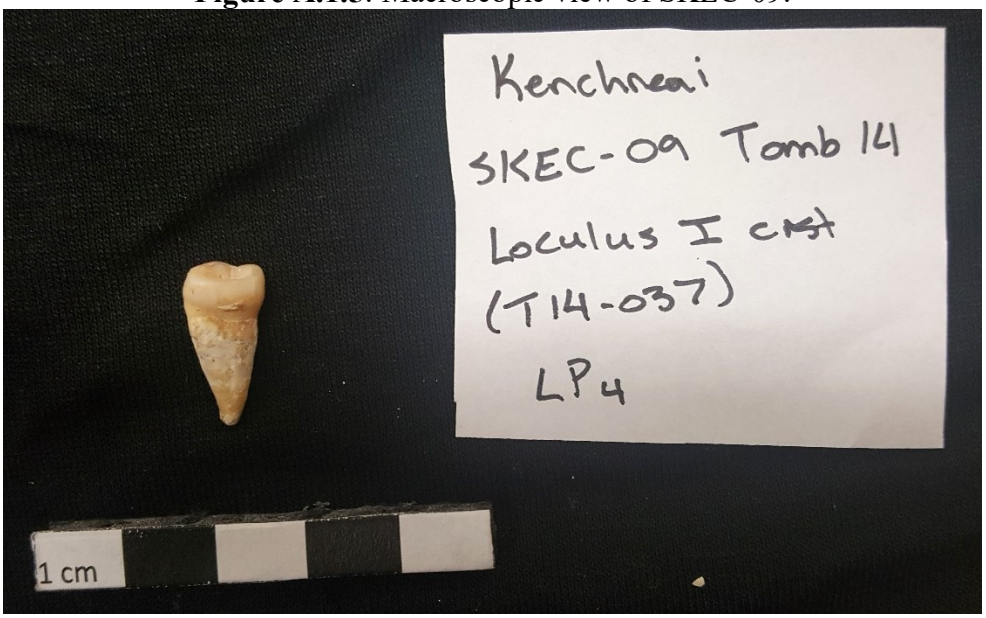

Figure A.1.6: Macroscopic view of SKEC-11.

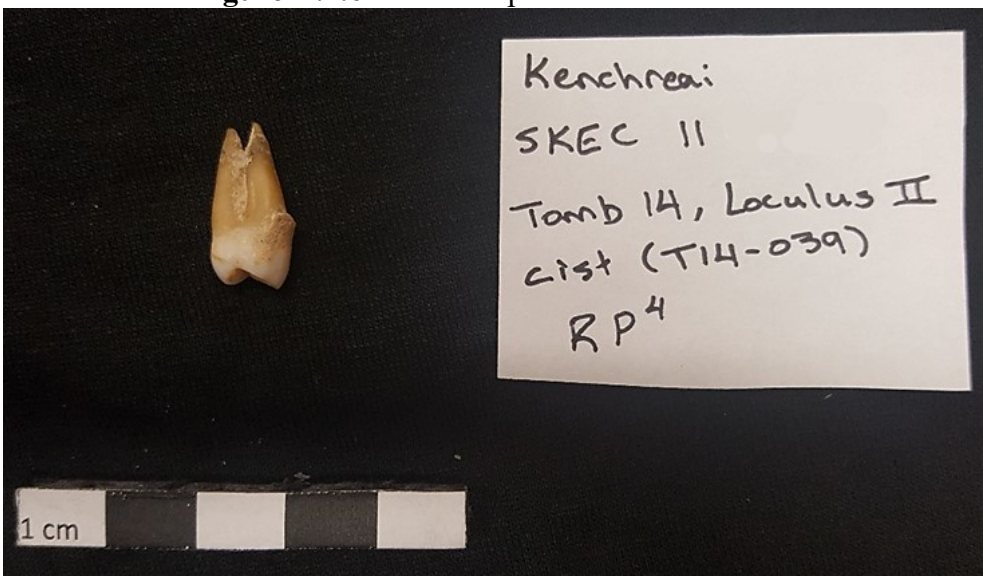

Figure A.1.7: Close-up of the calculus present on the buccal side of the tooth of SKEC-11.

Figure A.1.8: Macroscopic view of SKEC-17.

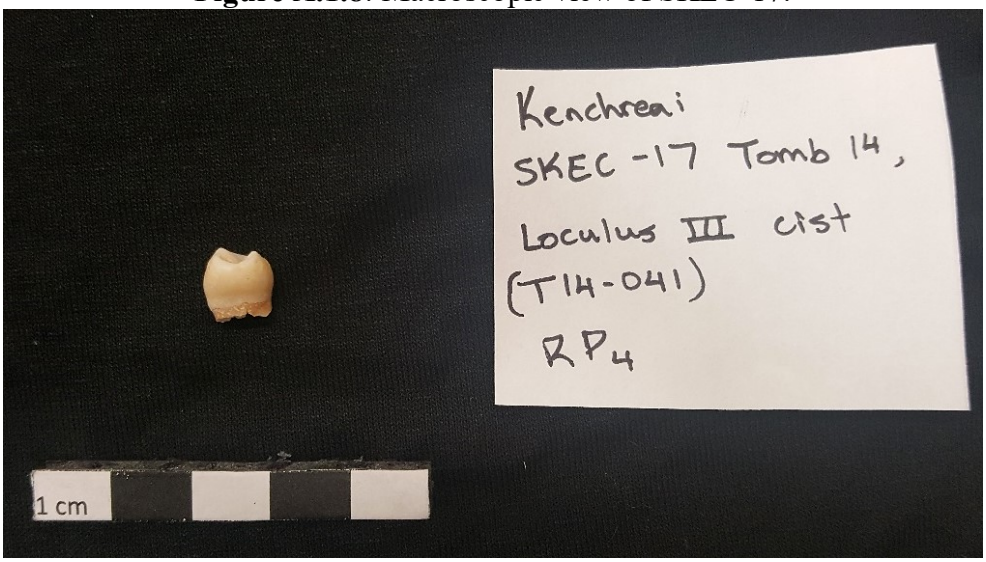

Figure A.1.9: Distal view of the broken root of SKEC-17.

Figure A.1.10: Lingual view of the broken root of SKEC-17.

Figure A.1.11: Macroscopic view of the mesial side of SKEC-18/19.

Figure A.1.12: Occlusal view of SKEC-18/19 with cracks visible in the crown.

Figure A.1.13: Macroscopic view of Isthmia 67-001D.

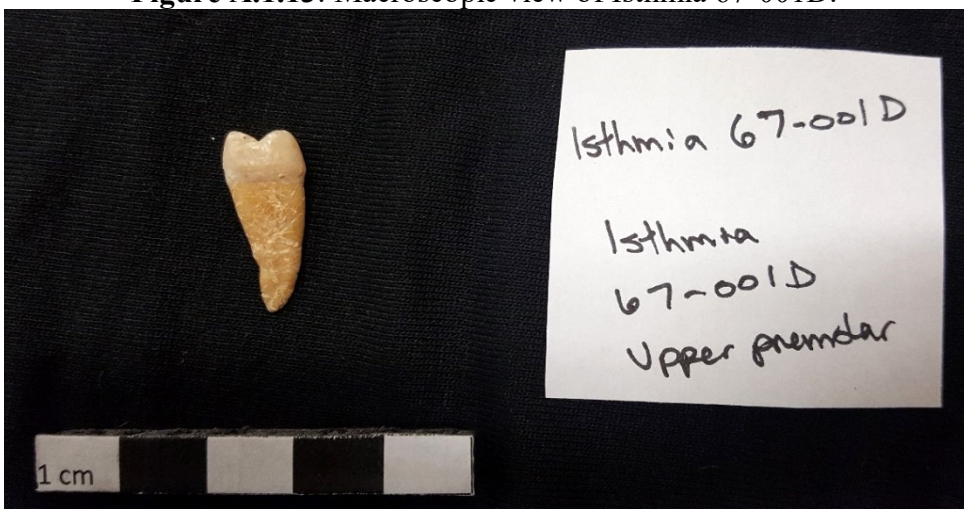

Figure A.1.14: Buccal view of Isthmia 69-004E.

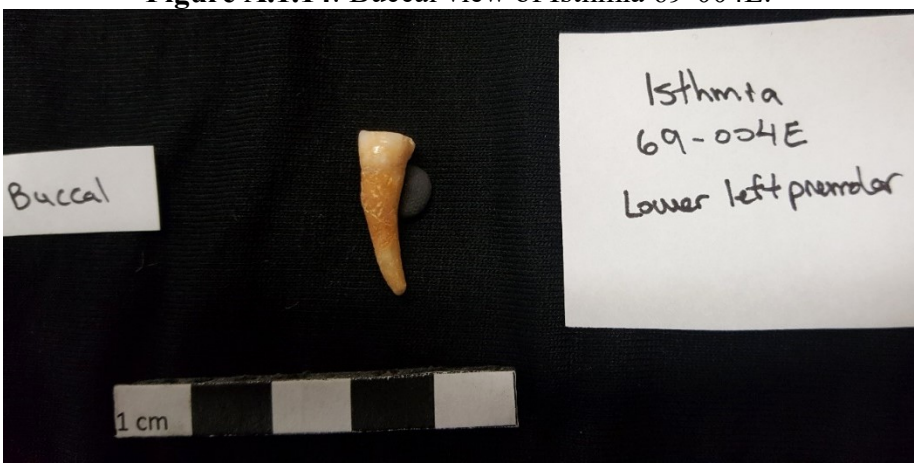

Figure A.1.15: Occlusal view of Isthmia 69-004E.

Appendix 2: Tooth Measurements of Kenchreai and Isthmia Sample

Site	Individual	Tooth	Total height - apical tip to occlusal tip (mm)	Buccal crown height (mm)	Lingual crown height (mm)	Crown width (mm)	Crown depth (mm)
Kenchreai	SKEC-02	RPM_2	18.17	5.66	5.87	7.70	6.95
	SKEC-05	RPM^1	4.16	8.78	7.14	8.59	5.95
	SKEC-09	LPM_2	17.47	4.94	5.09	7.91	6.51
	SKEC-11	RPM^2	18.53	4.21*	4.70	8.44	5.88
	SKEC-17	RPM_2	2.24	7.68	6.25	7.89	6.64
	SKEC-18/19	RPM^1	21.28	7.35	5.58	8.49	6.78
Isthmia	67-001D	PM^2	20.71	6.19	6.11	8.67	6.33
	69-004E	LPM_1	20.41	4.82	3.81	7.11	5.63

*SKEC-11 had a significant amount of calculus on the buccal crown. Buccal crown height was 8.05mm including the calculus. The calculus was measured to be 3.84mm. 8.05-3.84 = 4.21 mm.

Appendix 3: Kenchreai Age Estimates

SKEC-02

Known information:

This tooth is a PM_2

Crown initiation begins at 2.5 years

Root apex closure occurs at 14.5 years

Total formation time = 12 years

Calculation for amount of time a 1mm segment represents:

There are two 0.5mm sections and six 1mm segments. This equals seven 1mm segments.

12/7 = 1.7143

Therefore, each 1mm segment represents 1.7143 years.

Calculation for amount of time a 0.5mm segment represents:

1.7143/2 = 0.8572

Therefore, each 0.5mm segment represents 0.8572 years.

Age estimate calculation of each segment:

Segment ID	Calculation	Estimated Initiation Age (years)	Estimated Completion Age (years)
A	2.5+0.8572	2.5	3.4
B	3.3572+0.8572	3.4	4.2
C	4.2144+1.7143	4.2	5.9
D	5.9287+1.7143	5.9	7.6
E	7.643+1.7143	7.6	9.4
F	9.3573+1.7143	9.4	11.1
G	11.0716+1.7143	11.1	12.8
H	12.785+1.7143	12.8	14.5

SKEC-05
Known information:

This tooth is a PM[1]

Crown initiation begins at 2.5 years

Root apex closure occurs at 13.5 years

Total formation time = 11 years

Lab notes:

Pulp cavity of the tooth separated from the outer layer. Therefore, the pulp cavity represents the entire tooth and should not be counted as a segment in the age calculation. Based on skeletal age estimates of this individual, SKEC-05 is approximately 5-7 years old. The age of the core is approximately 7 years.

Given that this tooth is still developing, the known formation "end" time is 7 years.

Formation time: 7 – 2.5 = 4.5 years

Calculation for amount of time a 1mm segment represents:

For the outer layer of this tooth, there is one 2mm segment and one 1mm segment. This equals three 1mm segments.

4.5/3 = 1.5

Therefore, each 1mm segment represents 1.5 years.

Age estimate calculation of each segment:

Segment ID	Calculation	Estimated Initiation Age (years)	Estimated Completion Age (years)
A	2.5+3	2.5	5.5
B	5.5+2.5	5.5	7
Bulk	-	-	7

SKEC-11
Known information:

This tooth is a PM^2

Crown initiation begins at 3.5 years

Root apex closure occurs at 14.5 years

Total formation time = 11 years

Calculation for amount of time a 1mm segment represents:

There is one 3mm segment, one 2mm segment and six 1mm segments. This equals eleven 1mm segments.

11/11 = 1

Therefore, each 1mm segment represents 1 year.

Age estimate calculation of each segment:

Segment ID	Calculation	Estimated Initiation Age (years)	Estimated Completion Age (years)
A	3.5+3	3.5	6.5
B	6.5+1	6.5	7.5
C	7.5+1	7.5	8.5
D	8.5+1	8.5	9.5
E	9.5+1	9.5	10.5
F	10.5+1	10.5	11.5
G	11.5+1	11.5	12.5
H	12.5+2	12.5	14.5

SKEC-17
Known information:

This tooth is a PM_2

Crown initiation begins at 2.5 years

Root apex closure occurs at 14.5 years

Total formation time = 12 years

Lab notes:

Tooth is broken, root appears to have snapped off. However, tooth has reached the age of crown completion, which is a fixed point in tooth development. Crown completion occurs at approximately 6.5-7 years. Segment D occurs after crown formation is complete and contains part of the root, which is partially visible before the broken edge. According to the diagrams by Beaumont and Montgomery (2015), this places the broken edge of the root at approximately 7.5 years.

The known formation "end" time is 7.5 years.

Formation time: 7.5 – 2.5 = 5 years

Calculation for amount of time a 1mm segment represents:

There are four 1mm segments.

5/4 = 1.25

Therefore, each 1mm segment represents 1.25 years.

Age estimate calculation of each segment:

Segment ID	Calculation	Estimated Initiation Age (years)	Estimated Completion Age (years)
A	2.5+1.25	2.5	3.75
B	3.75+1.25	3.75	5
C	5+1.25	5	6.25
D	6.25+1.25	6.25	7.5

SKEC-18/19
Known information:

This tooth is a PM^1

Crown initiation begins at 2.5 years

Root apex closure occurs at 13.5 years

Total formation time = 11 years

Calculation for amount of time a 1mm segment represents:

There is one 2mm segment and eleven 1mm segments. This equals thirteen 1mm segments.

11/13 = 0.8462

Therefore, each 1mm segment represents 0.8462 years.

Age estimate calculation of each segment:

Segment ID	Calculation	Estimated Initiation Age (years)	Estimated Completion Age (years)
A	2.5+0.8462	2.5	3.3
B	3.3462+0.8462	3.3	4.2
C	4.1924+0.8462	4.2	5.0
D	5.0386+0.8462	5.0	5.9
E	5.8848+0.8462	5.9	6.7
F	6.731+1.6924	6.7	8.4
G	8.4234+0.8462	8.4	9.3
H	9.2696+0.8462	9.3	10.1
I	10.1158+0.8462	10.1	11.0
J	10.962+0.8462	11.0	11.8
K	11.8082+0.8462	11.8	12.7
L	12.6544+0.8462	12.7	13.5

Appendix 4: Isthmia Age Estimates

Isthmia 67-001D

Known information:

This tooth is a PM^2

Crown initiation begins at 3.5 years

Root apex closure occurs at 14.5 years

Total formation time = 11 years

Calculation for amount of time a 1mm segment represents:

There are twelve 1mm segments.

11/12 = 0.9167

Therefore, each 1mm segment represents 0.9167 years.

Age estimate calculation of each segment:

Segment ID	Calculation	Estimated Initiation Age (years)	Estimated Completion Age (years)
A	3.5+0.9167	3.5	4.4
B	4.4167+0.9167	4.4	5.3
C	5.3334+0.9167	5.3	6.3
D	6.2501+0.9167	6.3	7.2
E	7.1668+0.9167	7.2	8.1
F	8.0835+0.9167	8.1	9.0
G	9.0002+0.9167	9.0	9.9
H	9.9169+0.9167	9.9	10.8
I	10.8336+0.9167	10.8	11.8
J	11.7503+0.9167	11.8	12.7
K	12.667+0.9167	12.7	13.6
L	13.5837+0.9167	13.6	14.5

Isthmia 69-004E
Known information:

This tooth is a PM_1

Crown initiation begins at 2.5 years

Root apex closure occurs at 13.5 years

Total formation time = 11 years

Calculation for amount of time a 1mm segment represents:

There are thirteen 1mm segments.

11/13 = 0.8462

Therefore, each 1mm segment represents 0.8462 years.

Age estimate calculation of each segment:

Segment ID	Calculation	Estimated Initiation Age (years)	Estimated Completion Age (years)
A	2.5+0.8462	2.5	3.3
B	3.3462+0.8462	3.3	4.2
C	4.1924+0.8462	4.2	5.0
D	5.0386+0.8462	5.0	5.9
E	5.8848+0.8462	5.9	6.7
F	6.731+0.8462	6.7	7.6
G	7.5772+0.8462	7.6	8.4
H	8.4234+0.8462	8.4	9.3
I	9.2696+0.8462	9.3	10.1
J	10.1158+0.8462	10.1	11.0
K	10.962+0.8462	11.0	11.8
L	11.8082+0.8462	11.8	12.7
M	12.6544+0.8462	12.7	13.5