A DEVELOPMENTAL ORIGIN OF INFECTIOUS DISEASE? AN INVESTIGATION OF THE INFLUENCE OF EARLY CHILDHOOD STRESS ON INFECTIOUS DISEASE MORTALITY IN 18TH-19TH CENTURY ITALY

by

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(Under the Direction of Laurie J. Reitsema)

ABSTRACT

The developmental origins of health and disease (DOHaD) framework proposes that early childhood stress reduces fertility and increases adult mortality risk, particularly from metabolic disorders. Much research focuses on the correlation between early childhood stress and adulthood metabolic disorders, but the link between early childhood stress and infectious disease mortality is understudied. This dissertation uses human skeletal remains to study the impact of early childhood stress on infectious disease mortality by comparing 18th-19th-century Italian catastrophic and attritional populations.

Two cholera populations, Alia and Benabbio, are compared with two attritional populations from Badia Pozzeveri and Pieve dei Monti di Villa. This dissertation employs multiple methods to reconstruct early childhood stress events, specifically paleopathological and stature analysis, dental histology, and stable carbon and nitrogen isotope ratio analysis. Cribra orbitalia, porotic hyperostosis, and linear enamel hypoplasia prevalence are used as indicators of early childhood stress. Periostitis prevalence is used as an indicator of heterogeneity in adult environmental stress. Dental histology is used to reconstruct the timing of stress events in early

childhood. Weaning is a stressful period in early childhood where infants are first exposed to food-borne environmental pathogens. Age-at-weaning completion is reconstructed using stable nitrogen isotope analysis.

Sicilians exhibit a significantly lower prevalence of cribra orbitalia and significantly shorter stature compared to the Tuscans. Significantly lower rates of cribra orbitalia among Sicilians is either a result of the 1832 pandemic being less selective than later pandemics or consumption of more terrestrial protein among Sicilians. Shorter stature among Sicilians is consistent with secular trends in stature. Age-at-first defect did not differ significantly between the sites. All sites are found to exhibit an age-at-weaning completion around 3 - 4 years of age. No differences in age-at-weaning completion are found among the populations.

This research finds no relationship between early childhood stress markers and mortality from infectious disease. Instead, this research demonstrates the centrality of cultural context and methodological approach in the interpretation of skeletal remains using the DOHaD framework.

INDEX WORDS: Bioarchaeology, Osteology, Stable isotope analysis, Dental histology, the Developmental Origins of Health and Disease (DOHaD), Italy, 18th-19th Century, Sicily, Tuscany, Stature, Paleopathology, Infectious disease

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DEDICATION

I dedicate this work to my uncle, Kelly Larand Smith, who whispered “genetic engineering” into my ear as a child and, in some small part, inspired my career in biological sciences. This might not be exactly what you were thinking, but I think you would have been proud, nonetheless.

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CHAPTER 1

INTRODUCTION

1.1 Research description

This dissertation research investigates the effects of early childhood stress on infectious disease mortality using bioarchaeological methods. Specifically, I compare stress markers from human skeletal remains derived from cholera victims and attritional populations from 18th-19th-century Italy. Much research finds *in utero* and early childhood stressors contribute the development of metabolic disorders, such as cardiovascular disease or type II diabetes (Barker and Osmond, 1986; Barker, 1995; Barker, 1997; Barker, 1999; Barker, 2004; Barker et al., 1990; Barker et al., 1995; Barker et al., 1992; Barker et al., 1989a; Barker et al., 1989b; Barker et al., 1993; De Boo and Harding, 2006; Hales et al., 1991; McCance et al., 1994; McDade et al., 2001a; McDade et al., 2001b; Osmond et al., 1993; Rasmussen, 2001). Though some evidence exists to suggest that stress *in utero* and early childhood also alters immune function and contributes to mortality from infectious disease (Barker et al., 1989b; DeWitte, 2012; McDade et al., 2001a; McDade et al., 2001b; Moore et al., 1999; Moore et al., 1997; Watts, 1969), other studies find no relationship between stress in early life and infectious disease mortality (Barker et al., 1989b; Moore et al., 2001). This dissertation contributes to this literature by investigating the impact of early childhood stress on mortality from infectious disease.

Recurrent childhood episodes of stress, defined as any environmental or psychological factor causing a stress response, may contribute to infectious disease mortality in adulthood, and thus represent a heterogeneous hidden trait that contributed to infectious disease mortality

profiles. Heterogeneity in frailty, or the unknown phenotypic variation in disease frailty, is a major issue in bioarchaeological research because factors that influence mortality may not be readily observable in skeletal populations (Cohen, 1994; Wood and Milner, 1994; Wood et al., 1992). Assessing heterogeneity in frailty in skeletal populations is a recurrent research theme in bioarchaeological research (Armelagos and Van Gerven, 2003; Byers, 1994; Cohen, 1994; DeWitte and Stojanowski, 2015; Goodman, 1993; Soltysiak, 2015; Wilson, 2014; Wood and Milner, 1994; Wood et al., 1992; Wright, 2003). Bioarchaeologists have linked previous stress episodes to lower age-at-death profiles (Armelagos and Van Gerven, 2003; DeWitte and Bekvalac, 2010; DeWitte and Wood, 2008; Temple et al., 2012).

This dissertation investigates if early childhood stress affects infectious disease mortality in adulthood by comparing skeletal remains from catastrophic populations, specifically cholera victims, to contemporaneous, attritional populations from 18th-19th-century Italy. Cholera is a water-borne bacterial disease that causes rapid dehydration, diarrhea, and sometimes results in death within a week of the onset of symptoms (Barnet, 1972; Kotar and Gessler, 2014; Morris, 2011). The context of the archaeological sites used in this dissertation predates the discovery and wide-spread use of antibiotics to treat cholera, meaning that those that died from cholera infection were the most susceptible and frailest individuals (Rosenberg, 1966; Thomas, 2015). Since cholera victims typically die within a week of exposure, cholera does not produce skeletal lesions that are indicative of cholera infection. Any pathological lesions on cholera victims are a result of previous health insults and thus may be indicative of hidden heterogeneity in risk of death.

The present research has three main goals: (1) determine if early childhood stress and/or chronic stress are significantly associated with mortality from cholera, (2) determine if

periodicity of stress events in early life is significantly associated with cholera mortality and (3) determine if age-at-weaning differs significantly between catastrophic and attritional populations. The following sections provides a detailed overview of the project's cultural context, methodological approach, intellectual significance, and hypotheses.

1.2 Cultural context

The Italian peninsula was divided into multiple smaller states during much of the 18th and 19th centuries. The majority of these states were ruled by other European nations, including Austria, France, and Spain. Successive wars reshaped the Italian political landscape many times throughout the 18th and early 19th centuries. Revolutionaries during the 19th century were inspired by the idea of Italian greatness exemplified by ancient Rome. The Second War of Italian Independence began in 1859 and concluded with unification of the Italian states, with the exception of the Rome and the Papal States. The newly formed Kingdom of Italy was ruled by King Emmanuel II of Piedmont. In 1870, the unification of Italy was complete with the annexation of Lazio.

The majority of the population of Italy during the 18th – 19th century were poor and undernourished (Leoni, 1991). The successive wars and foreign occupation produced a nation that was economically disadvantaged compared to other European nations during the modern era. Approximately 60% of the population of Italy was economically dependent on agriculture in 1870, and industrialization did not take hold in Italy until the 1890s (Duggan, 2014; Esposto, 1992; Profumieri, 2009). The life expectancy in 1861 in Piedmont was 34.5 years and the infant mortality rate was 23.2% (Profumieri, 2009). Diseases such as pellagra, tuberculosis, malaria, and cholera were the main causes of death (Gentilcore, 2014; Ginnaio, 2011; Profumieri, 2009). During the 19th century, successive waves of cholera spread across Europe, resulting in hundreds

of thousands of deaths from the disease (Morris, 1976; Pollitzer, 1954; Retief and Cilliers, 1999; Rosenberg, 1966; Sack et al., 2004; Snowden, 1995; Thomas, 2015). The social, biological, and economic environment in Italy was continuously stressful throughout the 18th and 19th centuries.

Four sites from central and southern Italy are analyzed in this dissertation; two sites where cholera victims were interred and two contemporaneous attritional sites. The historic documents, osteological analysis, and funerary archaeology from Alia (Sicily) and Benabbio (Tuscany) indicate that the individuals buried at these sites are from the 1837 and 1855 cholera epidemics, respectively (Baldino, 2011; Bigazzi, 1999; Bigazzi et al., 2002; Chiarelli et al., 2002; Fornaciari, 2015; Fornaciari et al., 2010; Fornaciari and Coschino, 2012; Guccione, 1991; Guccione, 2002). The two contemporaneous attritional sites at Badia Pozzeveri (Tuscany) and Pieve dei Monti di Villa (Tuscany) were used as community graveyards during the 18th-19th centuries (2018; Rezza, 2009; Santiago-Rodriguez et al., 2019).

Alia is a rural comune of Palermo on the island of Sicily in the western Madonie mountain range (Guccione, 2002). This small city of ~4000 inhabitants is surrounded by fertile agricultural lands (Guccione, 1991; Guccione, 2002). In 1837, approximately 300-400 individuals from Alia died of cholera and were interred outside of town in a cave known as camposanto vecchio (Bigazzi, 1999; Chiarelli et al., 2002). These victims of cholera were rediscovered during wall renovations in 1995 and excavated by the University of Palermo between 1996 and 2002 (Chiarelli, 2002; Guccione, 1991). Previous osteological analysis of the remains in Alia suggests that a minimum of 296 individuals were buried in a mass grave within the camposanto vecchio, which concurs with historic records from the parish of Saint Anna that records 306 cholera victims from 1837 (Bigazzi et al., 2002).

Benabbio is a central Italian village located along the Lima Torrent in Tuscany. The church of San Michele, where 46 cholera victims were buried in 1855, is located outside the main village of Benabbio. The church of San Michele includes a plaque installed soon after the epidemic commemorating the victims of the 1855 cholera epidemic in Benabbio (Laganà, 2007). Parish records and burial style also confirm the burial of cholera victims at the church (Fornaciari et al., 2010). Some graves outside the church included 2 individuals per grave and the graves were covered in lime, a cultural practice during the 18th-19th century used to prevent the spread of infection (Fornaciari et al., 2010; Morris, 1976). Forty-one individuals were buried outside of the church, which coincides with the number of individuals who reportedly died of cholera in Benabbio during the 1855 epidemic (Fornaciari et al., 2010).

Pieve dei Monti di Villa (abbreviated "Monti di Villa") is located across the Lima Torrent from Benabbio in the Bagni di Lucca region of Tuscany. The parish of San Giovanni Battista is located in the center of the village and served as a burial place until 1871 (Rezza, 2009). Approximately 15 individuals were recovered from the 19th-century layers of the graveyard. Individuals from this site will be used as a contemporaneous attritional population to compare to the cholera burials from Alia and Benabbio.

Badia Pozzeveri is located along the Via Francigena, a medieval pilgrimage route, near Altopascio, Italy. The church of San Pietro is a medieval church in Badia Pozzeveri built during the 11th century surrounded by a graveyard that was in use from the medieval period to the 19th century. The church and the surrounding graveyard was excavated by the University of Pisa from 2013 to present (2018; Gibbons, 2013). Though some lime-covered graves were discovered at Badia Pozzeveri, the majority of the burials at this site are attritional burials from the medieval and modern periods (Gibbons, 2013). For this study, we selected individuals from the 18th and

19th-century layers that exhibit typical burial patterns consistent with attritional burials, including single burials with careful placement of the body and no lime covering.

1.3 Methods

The methodological approach to this research is designed to derive the most information from the skeletal remains examined; paleopathological analysis, stature analysis, dental histology, and incremental stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis are used to ascertain the presence of early childhood stressors.

The pathological markers of interest for this dissertation include porotic hyperostosis, cribra orbitalia, linear enamel hypoplasia, and periostitis. Porotic hyperostosis, cribra orbitalia, and linear enamel hypoplasia are all indicative of early childhood stress events, whereas periostitis is indicative of stress near the time of death (FitzGerald, 1998; Goodman and Rose, 1990; Hillson, 2014; King et al., 2005; Massier et al., 1941; McIlvaine, 2015; Mensforth et al., 1978; Stuart-Macadam, 1985; Stuart-Macadam, 1989; Stuart-Macadam, 1992; Waldron, 2009; Walker et al., 2009). I use periostitis in this dissertation to ascertain if heterogeneity in adulthood stressors contributed to mortality from infectious disease. Stature is frequently used as an indicator of overall health in bioarchaeological research because growth stunting usually occurs as a result of disruption of metabolic homeostasis during bone growth (Haviland, 1967; Steckel, 1995).

Dental histology creates a timeline of stress events during early childhood. Histological markers of stress manifest on tooth enamel as accentuated striae of Retzius. Tooth growth is highly regulated genetically, thus the distance between accentuated striae of Retzius and morphological features of teeth (crown apex, cemento-enamel junction) suggest the timing of stress events (Goodman and Rose, 1990; Hillson, 2014).

Collagen extracted from dentin is sampled incrementally and tested for $\delta^{13}C$ and $\delta^{15}N$ values to analyze weaning patterns in early childhood (Beaumont et al., 2014; Beaumont et al., 2015; Eerkens et al., 2011; Fogel et al., 1989; Fuller et al., 2006; Fuller et al., 2003; King et al., 2018; Richards et al., 2002; Sandberg et al., 2014). Weaning is an important period of stress in early childhood as infants transition from exclusively consuming breastmilk to consuming solid foods (Deoni et al., 2013; Horta et al., 2013; Rice and Barone, 2000). Infants consuming breastmilk exhibit higher $\delta^{13}C$ and $\delta^{15}N$ values by approximately 1‰ and 1-3‰ compared to their mother, respectively. The $\delta^{13}C$ and $\delta^{15}N$ values decrease to their mother's levels as solid foods are introduced. Thus incremental sampling of $\delta^{13}C$ and $\delta^{15}N$ values can be used to estimate weaning onset and completion (Beaumont and Montgomery, 2015; Eerkens et al., 2011; Fuller et al., 2006; Fuller et al., 2003; Henderson et al., 2014; Katzenberg et al., 1993; King et al., 2018; Pfeiffer et al., 2017; Wright and Schwarcz, 1999).

1.4 Significance of research

This research contributes to our understanding of human developmental plasticity, human adaptation, and the DOHaD hypothesis. Within the DOHaD framework, this study specifically tests the predictive-adaptive and plasticity/constraint hypotheses as explanations for stress in early life influences later-life health and mortality. Briefly, the predictive-adaptive hypothesis predicts early life stress conveys later life health advantages through a process of adaptability, whereas the plasticity/constraint hypothesis predicts early life stress is linked to adverse later life health effects due to limited energy available to adjust to stress throughout the lifespan (Bogin et al., 2007; Gluckman et al., 2005a; Gluckman et al., 2005b; Kuzawa and Pike, 2005; Kuzawa and Quinn, 2009; Worthman and Kuzara, 2005). The analysis of skeletal remains is advantageous in several regards. First, skeletal populations across deep time represent a wider breadth of human

variation compared to living human populations. Second, longitudinal studies generally are required to analyze the effects of early childhood stress on adult mortality outcomes in living populations, which may require several decades of tracking participants. Research using skeletal populations can be completed more quickly than longitudinal studies on living populations and generally does not require IRB approval. Finally, the historical context from which these skeletal remains are derived occurred before the wide-spread use of antibiotics to treat bacterial diseases. This research differs from previous research on the DOHaD hypothesis by focusing on infectious disease mortality as the main potential outcome of early childhood stress, an outcome that is currently understudied in the DOHaD literature.

In recent years, bioarchaeologists have increasingly engaged with the DOHaD hypothesis using archaeologically-derived skeletal populations (Armelagos et al., 2009; Beaumont and Montgomery, 2015; Beaumont et al., 2015; Blevins, 2015; Boldsen, 2007; DeWitte and Hughes-Morey, 2012; Gowland, 2015; Reitsema et al., 2016; Temple, 2014). However, bioarchaeological research using the DOHaD hypothesis has produced inconsistent results (Temple, 2019). Temple (2019: 35) suggests that the conflicting results of these tests demonstrate that cultural and environmental context, timing of insult, and physiological outcomes at later stages of life should all be considered as potential confounding factors in future research. The present study employs multiple methodological approaches that can contribute to the conversation about the applicability of certain methodologies for DOHaD research.

Despite the development of antibiotic treatments, cholera is considered an emerging infectious disease with 1,227,391 cases and 5,634 deaths reported globally in 2017 (WHO, 2018). The seventh, ongoing pandemic of cholera began in 1961 when the *V. cholerae* O1 El Tor biotype emerged in Asia and spread to Africa, Europe, Oceania and the Americas (Kaper et al.,

1995; Nations and Monte, 1996). Cholera is endemic in areas with high rates of poverty, insufficient sanitation, limited healthcare access, and illiteracy because these structural issues facilitate transmission of the disease (Briggs, 1999; Nations and Monte, 1996). Research into the contributing factors that lead to cholera epidemics, such as early childhood stress and nutritional deprivation in early life, is crucial for creating effective intervention strategies to reduce the global burden of cholera. Additionally, such research may underscore the importance of supplemental nutrition programs, such as Women, Infants, and Children (WIC) and the Supplemental Nutritional Assistance Program (SNAP), in preventing the spread of infectious disease.

1.5 Research questions and hypotheses

This dissertation investigates the effects of early childhood stress on infectious disease mortality, specifically in the context of cholera mortality in 18th-19th-century Italy. The main research question that is addressed by this dissertation is: "Are skeletal markers of early childhood stress indicative of increased susceptibility to death from infectious disease?" To answer this question, this dissertation tests the following hypotheses, which are numbered according to the dissertation chapter that correlates with the hypothesis (Chapters 5, 6 or 7):

1.5.1 Hypothesis 5.1: cholera mortality is associated with later weaning completion

Weaning is a stressful period during early childhood as children transition from exclusive breastfeeding to consumption of weanling foods, which exposes weaning infants to new pathogens and potentially contaminated food while simultaneously reducing the protective benefits of breastmilk (Horta et al., 2013; Lönnerdal, 2003; Motarjemi et al., 1993). Industrialization generally caused a decrease in breastfeeding length as a result of increased pressure for mothers to return to work (Riordan and Countryman, 1980; Wickes, 1953a; Wickes,

1953b). Italy did not experience the rapid industrialization that occurred in western Europe during much of the 19th century (Duggan, 2014; Hearder, 1990) and therefore did not experience the demographic transition towards shorter periods of breastfeeding. Historically, prolonged periods of exclusive breastfeeding beyond age 2 are associated with inadequate access to food in Europe (Wickes, 1953a). Consequently, I hypothesize that cholera victims experienced prolonged periods of breastfeeding due to inadequate access to food and were more susceptible to infectious disease in adulthood.

1.5.2 Hypothesis 6.1: cholera mortality is associated with a higher prevalence of pathological markers

Previous bioarchaeological research testing these hypotheses supports the plasticity/constraint hypothesis, specifically that earlier mortality is associated with more early life stress events (Armelagos et al., 2009; DeWitte, 2012; DeWitte and Wood, 2008; Temple, 2014). Research in living populations found evidence that early life and chronic stress is detrimental to immune function (Barker et al., 1989b; McDade et al., 2001b; Moore et al., 1999; Moore et al., 1997; Vaupel et al., 1979). For these reasons, I anticipate that cholera victims experienced a higher prevalence of pathological markers associated with stress in early life and adulthood compared to the attritional population, which would support the plasticity/constraint hypothesis.

1.5.3 Hypothesis 6.2: cholera mortality is associated with growth stunting

Stature is frequently used in bioarchaeological research as an indicator of overall population health (Haviland, 1967; Steckel, 1995). Growth stunting occurs due to nutritional insufficiency, either because of inadequate access to food or because of disease (Golden, 1994). Smaller bodies are advantageous in stressful environments because they require less energy to

maintain compared to larger bodies (Charnov, 1991; Hawkes, 2006). I anticipate that cholera victims experienced stressful environments in early childhood which led to smaller overall stature in cholera victims compared to the attritional populations.

1.5.4 Hypothesis 7.1: cholera mortality is associated with earlier and more stress events

In a similar study, Temple (2014) finds that the periodicity of linear enamel hypoplasias among Jomon foragers was significantly associated with earlier mortality outcomes. Specifically, he finds a statistically significant positive relationship between age-at-first defect formation and age-at-death. Blakely and Armelagos (1985) also find differences between early onset of stress events and mortality in early childhood. These studies suggest that the timing of stress events may be of critical importance for later life health outcomes. For this reason, I hypothesize that cholera victims experienced earlier, and more stress events compared to the attritional populations.

1.6 Chapter objectives

Chapter 2 reviews the cultural context of 19th-century Italy with further consideration of cholera epidemics and the study sites. Cultural context is an important consideration when working with human skeletal remains because culture and biology have a synergistic relationship. The first section of this chapter describes the geography of Italy and defines the regions of Italy that will be referenced later in the chapter. I next discuss the political history of Italy throughout the 18th and 19th centuries. I then provide a review of the history of cholera during the 19th century and conclude the chapter with a description and history of each site used in this dissertation.

Chapter 3 provides the theoretical framework for the dissertation by reviewing the literature regarding the developmental origin of health and disease (DOHaD) hypothesis. I start

by reviewing the life history theory, a theoretical framework within biological anthropology that focuses on understanding how the timing of life history events varies between and within species, and what factors influence the variation in timing of life cycle events. I discuss the evidence for the DOHaD in epidemiology and biological anthropology, specifically the evidence for the fetal origins of metabolic disorders. I discuss the differences between the plasticity/constraint and predictive-adaptive hypotheses and review the research on the relationship between early childhood stress and infectious disease mortality. I conclude this chapter by discussing previous research within bioarchaeology regarding the DOHaD. The next chapter expands on Chapter 3 by discussing the methodological approaches in bioarchaeology that reconstruct early childhood stress.

Chapter 4 reviews methodological approaches to the DOHaD utilized in bioarchaeological research to establish the framework for interpreting skeletal lesions as evidence of early childhood stress. This chapter begins with a review of bone structure and plasticity to illustrate the benefits and limitations of bioarchaeological research. I then review the paleopathological markers applicable to identifying early childhood stress in skeletal populations. I specifically review pathological lesions, specifically indicators of anemia, dental pathologies, linear enamel hypoplasias, and accentuated striate of Retzius. The final section of this chapter reviews the stable isotope analysis approaches used to analyze early childhood stress, specifically stable carbon, nitrogen, barium/calcium isotope analysis. The next three chapters are article-style chapters that include the results and corresponding discussion for each methodology employed in this dissertation research.

Chapter 5 discusses the results of the incremental stable carbon and nitrogen isotope analysis. We find that age-at-weaning completion is similar across all sites (~3 - 4 years) and

approximately 40% of individuals did not exhibit visible weaning curves. This chapter discusses historical explanations for the regional variation in $\delta^{15}N$ and $\delta^{13}C$. The results of this chapter suggest that there is no association between early childhood stress and infectious disease mortality.

Chapter 6 discusses the results of the paleopathological analysis and stature analysis. We find significant differences in cribra orbitalia prevalence between cholera and attritional populations, but these differences are a result of regional variation in paleopathology frequencies. We also find significant differences in stature between Sicilian and Tuscan sites that are associated with secular trends in stature during the 19th century.

Chapter 7 discusses the dental histology results, specifically discussing the results of the stress chronologies of cholera and attritional populations. We find that site-based differences in age-at-defect formation suggests that the sites are too different to pool into cholera and attritional populations. Instead, site-based differences in stress chronologies are likely a result of idiosyncratic differences in child rearing and variation in distance to trade networks.

Chapter 8 synthesizes of the findings of the paleopathological, stature, dental histology and incremental stable carbon and nitrogen isotope analyses. We find no evidence that early childhood stress is associated with infectious disease mortality in adulthood. Instead, this dissertation highlights how cultural variation, even interregional cultural variation, can impact the results of bioarchaeological research.

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CHAPTER 2:

LITERATURE REVIEW: CHOLERA IN THE 19TH-CENTURY ITALIAN LANDSCAPE

2.1 Introduction

Homo sapiens are social organisms whose biological characteristics are influenced greatly by their cultural context. Bioarchaeological research requires that biological analyses of human skeletal remains be interpreted with consideration for their larger cultural, political, and economic contexts. The present research project engages with cultural contexts from the modern period (1500-1945 CE) Italy, but also requires consideration of the epidemiological history of cholera in Europe during the 19th century. Modern Italian history is characterized by occupation by foreign powers, continuous wars, and declining economic conditions that led to a widening socioeconomic gap between the wealthy and the poor (Duggan, 2014; Hearder, 1990). Italians generally lived in poverty with little access to proper nutrition or sanitation, which made these populations susceptible to infectious diseases. A lack of knowledge about the etiology or effective treatment strategies for cholera also facilitated the spread of the disease throughout the 19th century.

The purpose of this chapter is to provide cultural context for the skeletal remains used in this dissertation. First, I describe the environmental context of 19th century Italy by discussing the geography and regions of Italy. Second, I review the political and economic history of Italy to illustrate how the 18th and 19th centuries in Italy were characterized by occupation by foreign powers, warfare, and economic depression. This section also serves to illustrate how the economic transition from agriculture to industrialization in Italy occurred slowly compared to

other parts of Europe. The transition from agricultural subsistence to industrialization influences secular trends in life history events, particularly weaning and breastfeeding patterns (Popkin, 1994; Wickes, 1953a; Wickes, 1953b). Industrialization in western Europe and North America caused a secular trend towards shorter periods of breastfeeding and earlier weaning (Wickes, 1953b). The result of Italy's slow progression towards industrialization is that the weaning practices of 18th-19th-century Italians should be more similar to weaning and breastfeeding patterns during the renaissance period, when weaning occurred later in childhood.

This chapter also provides the history of cholera during the 19th century to illustrate how the cultural, political, and economic circumstances in Italy intersect with the multiple pandemics of cholera that spread throughout Europe during the 19th century. Since this dissertation analyzes how early childhood stress influences mortality from cholera infection, effective intervention strategies would be a confounding factor to consider. In Europe, however, effective strategies to combat cholera did not exist until the 1890s. This section on cholera history will demonstrate that 19th-century Europe was not equipped with effective treatment strategies or sanitary standards to stop the spread of cholera.

Finally, the last section in the chapter discusses the cultural context of each site analyzed in this dissertation (Table 2.1). Historical and cultural information, previous archaeological analysis, and evidence of cholera infection, where applicable, are discussed for each site.

2.2 The Italian landscape

Italy comprises the Italian peninsula and the islands of Sardinia and Sicily located in southern Europe. The Italian mainland is bordered on the east by the Adriatic Sea, on the south by the Ionic sea, and on the west by the Tyrrhenian Sea. Italians colloquially divide Italy into three main regions: *settentrione* (north), *centro* (central), and *meridione* or *mezzogiorno* (south).

Italy is further subdivided into 20 geographic regions. The northern regions are Liguria, Lombardy, Piedmont, Valle d'Aosta, Emilia-Romagna, Fruili-Venezia Giulia, Trentino-Alto Adige, and Veneto. The central regions are Tuscany, Umbria, Lazio, and Marche. The southern regions include Abruzzo, Basilicata, Calabria, Campania, Molise, Puglia, Sardinia, and Sicily.

In northern Italy, there are seven main regions: Liguria, which comprises the western coast of northern Italy and borders France. Piedmont is north of Liguria and borders both Switzerland and France. Piedmont is a mountainous region surrounded by the Alps on its western and northern borders. Piedmont surrounds the region of Aosta Valley, which borders France to the west and Switzerland to the north. Lombardy is east of Piedmont and is characterized by the Alps to the north and the plains of the Po Valley to the south. The economic capital of Italy, Milan, is located in Lombardy. To the east of Lombardy are three more regions; Trentino-Alto Adige, Veneto, and Friuli Venezia Giulia. Trentino-Alto Adige is a mountainous region that shares its northern border with Austria and its southeastern border with Veneto. Veneto is located on the northeastern coast of Italy and is most famous for the city of Venice along this coastline. Friuli-Venezia Giulia is located northeast of Veneto and shares its northern border with Austria and Slovenia. Emilia-Romagna is south of Lombardy and Veneto and shares a western border with Liguria and Piedmont. The capital of Emilia-Romagna is Bologna.

The geography of northern Italy is comprised of valleys and plains surrounded by mountain ranges that extend across the northern and western borders of Italy. Italy is divided from the rest of Europe by the Alps. The Alps separate Italy from France, Switzerland, Austria and Slovenia along its northern border. The Alps border the Po Plain, which extends from Piedmont eastwardly to the Adriatic coastline. The Po Plain surrounds the Po River, the largest river in Italy, which originates in the western Piedmont Alps. The Po River flows eastwardly

across the Po Plain and terminates in the Adriatic Sea near Venice. The Po Plain can be divided into two subregions: the northern *alta* (highland) and the southern *bassa* (lowland) (Muscio, 2002). The *alta* region of the Po valley is hilly and dry, and consequently less suited for agricultural activities compared to the *bassa* region of the Po valley. The *bassa* region of the Po valley is flat and rich with alluvial soil deposits from the Po River and its tributaries (Muscio, 2002).

Central Italy is divided into four regions: Tuscany, Umbria, Lazio, and Marche. Tuscany is located along the western coast of Italy south of Emilia-Romagna and Liguria. The capital of Tuscany is the city of Florence. South of Tuscany along the western coastline is Lazio, the capital of which is Rome. Lazio also encompasses the independent city-state of Vatican City, the religious center for Catholicism. Inland from Lazio and Tuscany is Umbria. Marche is east of Umbria along with Adriatic coast.

The geography of central Italy is composed of diverse topography and ecology which can be divided into four main geographic structures: the Anti-Apennines, the Pre-Apennines, the Apennines, and the eastern lowlands (Barker, 1981). The Anti-Apennines are the coastal plains and uplands that are surrounded by the Apennine Mountain range on the southern coast of Italy. The Anti-Apennine coastal plain stretches the length of the Italian peninsula with limestone headlands dispersed throughout the plains (Barker, 1981; Mussi and Peresani, 2011). The coastal plain is historically a marshy environment surrounded by uplands composed of sandstones and clay. The Anti-Apennines are home to several important features, specifically the Tiber River, which divides the northernmost Maremma plain from the southern Pontine plain. The Tiber River stretches from the central Apennines to the Tyrrhenian Sea, crossing through modern-day Rome (Mazzini et al., 2011).

The Pre-Apennines are a chain of hills composed of sandstones and clays in the north and limestone in the south. These hills stretch the length of the peninsula just west of the Apennine Mountain chain and rise approximately 1,000 feet above sea-level in modern times (Barker, 1981). The Apennine Mountains begin near the coast of Italy north of Arno River and curve south down the length of the peninsula. The northernmost section of the Apennine Mountains is composed of limestone and dolomite (Barker, 1981). The rest of the Apennine hills are composed of clays, shales, and limestone. The eastern lowlands run parallel to the main Apennine mountain range down the length of the peninsula. They are characterized by a band of marls, clays, and sandstone directly adjacent to the Apennine mountain range, with sand and clay deposits further east (Barker, 1981).

The *meridionale* regions include Abruzzo, Basilicata, Calabria, Campania, Molise, Puglia, Sardinia, and Sicily. Abruzzo is north east of Lazio and south of Marche along the eastern coast of the peninsula. South of Abruzzo is Molise and Puglia, which compose the rest of the eastern coast of Italy along the Adriatic Sea. South of Lazio along the Tyrrhenian coast of Italy is Campania, Basilicata, and Calabria. Sicily is located across the Strait of Messina opposite Calabria. Sicily is the largest island in the Mediterranean Sea with an area of 9,925 square miles (Domenico, 2002a). Sicily borders the Tyrrhenian Sea to the North, the Mediterranean Sea to the southwest, and the Ionian Sea to the east. Northwest of Sicily and south of the French island of Corsica is the second-largest island in the Mediterranean Sea, Sardinia.

The geography of Italy is varied across the peninsula. The *settrionale* region of Italy is characterized by mountainous and hilly regions with some large plains, notably the Po plain. The *centrale* and *meridionale* regions of Italy, including Sicily and Sardinia, are mainly mountainous and hilly. The variation in geography and resources across the peninsula, as well as its strategic

position in the central Mediterranean, made Italy a desirable territory for much of Italy's modern history.

2.3 The unification of Italy in the modern period

At the beginning of the modern period (1600 – 1900 CE), Italy was a collection of disparate states, many of which were occupied by other European nations, including Austria and France. Though Italian unification was romanticized in many parts of Italy, unification was a slow process that required the removal of foreign rule through force. The cultural context of the modern period in Italy was characterized by near continuous wars of conquest, slow economic expansion, and deep social and economic divisions (Duggan, 2014; Hearder, 1990). This chapter section briefly reviews the political and economic history of the Italy during the modern era to provide the cultural context of the four archaeological sites which are the focus of this research.

In the beginning of the modern era, Italy was divided into several small states, only a handful of which were independent (Figure 2.1). In the North, the Duchy of Milan was controlled by Spain, and Venice, Florence, Genoa, and Piedmont were independent. The Papal States were ruled by the Pope in Central Italy. The Kingdoms of Naples, Sardinia, and Sicily were ruled by Spain.

The population in Italy had steadily increased throughout the 15th and 16th centuries after the Black Death had decimated Europe's population in the 14th century (Duggan, 2014). With the increase in population came an increase in economic output, which led to relative stability of the Italian states during the 15th century. However, the beginning of the 17th century saw an economic decline as the merchant class in Italy began to invest in land instead of trade (Duggan, 2014). In the South, absentee ownership of large estates resulted in lower agricultural yields, which resulted in an increase in banditry and difficulty dealing with disasters (Duggan, 2014). In

1630, the plague once again spread into Italy and killed a third to a half of the inhabitants of Milan, Verona, Florence and Venice (Duggan, 2014).

The economy in Italy continued to decline throughout the 17th century while wealthy European monarchs vied for control over the Italian peninsula. The Dukes of Savoy fought Spain and France for Monferrat and Mantua. Venice fought France, Spain, and Savoy for the Austrian Hapsburgs in 1615 and 1620 (Duggan, 2014). In 1635, France, Savoy, Parma, and Mantua fought as allies against Spanish rule. A byproduct of these wars in Italy was an increase in taxation, which further depressed the economy (Duggan, 2014). The government tried to raise funds for these wars by selling off land, monopolies, titles, and privileges, which led to the return of feudalism, especially in Southern Italy (Duggan, 2014). The Italian nobles, often in concert with foreign overlords, ruled much of the peninsula while the middle class dwindled. The result of this widening gap of economic and political power between the nobles and the commoners resulted in revolts (Duggan, 2014). In 1647, the poor of Naples and Messina revolted in response to poor harvests and high taxation. In Palermo, the poor forced out the nobles by taking to the streets. Spain, which ruled over the Kingdom of Naples and Sicily, suppressed these revolts (Duggan, 2014). By the end of the 1600s, people in Italy were impoverished and the economy unbalanced (Duggan, 2014).

The 18th century in Italy was characterized by several wars of succession that divided the Italian States among the major ruling powers (Noether, 1986). Spain remained the dominant force by the beginning of the 18th century, ruling Milan, Sardinia, Naples, Sicily, and parts of Tuscany. However, the expansive empire of Spain was economically unsustainable, and when Charles II, the last of the Spanish Hapsburgs, died in 1700 war broke out between France, England, Spain, and Austria to decide who would rule over the Spanish Empire. Throughout the

18th century, the borders of the many Italian states were drawn and re-drawn as the fight for control of Spain continued across the European continent. In 1707, Austria acquired Lombardy, and by 1713, a series of treaties that ended the War of Spanish Succession re-drew the map of Italy again. Austria acquired the Duchy of Mantua and England acquired Alessandra, Valenza, the Lamellia, and Sicily. In 1718, the English traded Sicily to Spain in exchange for Sardinia (Domenico, 2002a). Sicily was later acquired by Austria in 1720 (Domenico, 2002a). In 1733, the War of Polish Succession erupted as France and Spain moved against Austria to control the throne of Poland. In 1727, the Grand Duchy of Tuscany went to the House of Lorraine after the death of the last Medici heir, Gain Gastone (Domenico, 2002b). During the war, King Charles III of Spain acquired Naples and became the King of Naples and Sicily. The war of Polish Succession ended in 1748 with the Treaty of Vienna. Spain retained Naples and Sicily, and the Duchy of Parma went to Austria. In 1740, the War of Austrian Succession began as European powers tried to prevent the ascension of Archduchess Maria Theresa to the throne of Austria. In 1748, the Treaty of Aix-la-Chapelle ended the war and again divided Italy among the victors. Modena was acquired by the Austrians and Parma, Piacenza, and Gustella went to the Bourbons as a result of the War of Austrian Succession. Thereafter, the borders of Italy were stable until the late 18th century (Noether, 1986).

Italy's continued economic decline was the outcome of the successive wars in the early 18th century. In Lombardy, half of the agricultural land was owned by nobility, and 61% of the land in Agro Romano belonged to a third of the population (Duggan, 2014). In the Kingdom of Naples, 40-50% of the land belonged to either the Church or the nobility, and 2/3 of taxes collected came from those owning a fourth of the total wealth (Duggan, 2014). Urbanization and agriculture expanded in Italy towards the end of the 18th century (Bellotti et al., 2007).

Enlightenment philosophy was influential in Italian politics during the 18th century, but governmental reform was unevenly implemented across the peninsula. Reforms were implemented in parts of northern and central Italy, but Southern Italy remained entrenched in feudalism. Naples, under the rule of the Bourbons, continued feudal economic and social practices, leaving the country's economy vulnerable. In 1792, Paris annexed Savoy and in 1796, Napoleon Bonaparte drove the Austrians from Lombardy to Veneto (Duggan, 2014). Napoleon ended his Italian campaign with the Treaty of Campoformio in 1797, leaving Venice to the Austrians and establishing two sister republics in northern Italy that were divided by the Po River: the Cisalpine Republic to the south and the Transpadane Republic to the north (Duggan, 2014; Hearder, 1990). France was driven back over the Alps by coalition forces in 1799 (Duggan, 2014; Hearder, 1990).

French rule in northern Italy led to the abolition of feudalism, but the revolutionary government of France demanded war contributions in the form of taxes, which drove a wedge between the government and the poor (Duggan, 2014). From 1797 to 1799, the revolutionary government passed laws to end labor dues, tithes, and other residual feudal policies (Duggan, 2014). In 1800, Napoleon expanded again into Italy to reassert French rule; the Cisalpine Republic was restored and renamed the Italian republic (Duggan, 2014). Tuscany became known as the Kingdom of Etruria from 1801 to 1807. Piedmont, Liguria, Parma, Umbria, and Lazio were annexed by France (Duggan, 2014). The Kingdom of Italy was established in 1805 in Piedmont (Duggan, 2014). Naples was conquered by France in 1806 and King Ferdinand's court fled to Sicily (Duggan, 2014). In the South, Sicily was largely occupied by a British garrison and Sardinia was independent (Duggan, 2014).

Napoleon's rule was mainly characterized by intensive centralization of the government, with many of the governmental policies coming directly from Paris (Duggan, 2014). Unlike in France, Napoleon recruited the Italian nobility and did not suppress the aristocracy, which led to less redistribution of wealth in Italy compared to France (Duggan, 2014). The communal lands, such as church property and old manors, were bought up by the Italian nobility, whereas in France, these properties were mainly sold to the peasantry (Duggan, 2014). Napoleonic rule, specifically the lack of progressive policies and the exploitation of Italy's resources to fund French ambitions, led to the emergence of a nationalistic movement in Italy that glorified the Italian past, especially the Roman empire (Duggan, 2014; Hearder, 1990; Noether, 1986).

In 1812, Sicily abolished feudalism while under British rule (Domenico, 2002a). The government intended to sell off the communal properties to both nobles and peasants, but most of the lands were sold to the landlords (Sereni, 1997a). The selling of these communal properties, therefore, did not benefit Sicilian agriculture or the poor, so the social divisions that were established under feudalism largely remained intact (Duggan, 2014; Sereni, 1997a).

Napoleonic rule collapsed in 1813-14, which had severe economic implications for the Italian peninsula. The collapse led to high taxes and rising prices, which caused popular discontent among the populace (Duggan, 2014). Austria replaced France as the dominant power in Italy and restored several anti-Revolutionary rulers to their former positions. Vittorio Emanuele I, the successor of King Charles Emmanuel IV of Sardinia, returned to Turin with the help of Austrian armies, and King Ferdinand IV was restored as King of Naples and Sicily (Duggan, 2014; Hearder, 1990). Vittorio Emanuele I reinstated Roman law and restored aristocratic privileges after removing the French appointees. In December 1816, King Ferdinand IV abolished the Sicilian constitution and combined the Kingdoms of Naples and Sicily to form

the Kingdom of Two Sicilies (Domenico, 2002a). He named himself King Ferdinand I of the Kingdom of Two Sicilies (Domenico, 2002a).

The reinstatement of Austrian rule, along with high taxes and restricted trade, led to political dissent in Lombardy, Veneto, and Sicily. The early half of the 19th century saw an economic slump throughout Europe which led to stagnation in Italian agriculture. In response to this stagnation, landowners intensified labor demands to increase profits, farmers were destitute, and crime and banditry increased as the economy suffered. The population of Italy also experienced an increase from 18 million in 1800 to 22 million by 1840 (Duggan, 2014). This increase in population was restricted to the countryside because pandemic waves of cholera throughout the 19th century caused a reduction in population in urban centers throughout Italy.

In 1830, the Second French revolution occurred in Paris and inspired a series of uprisings in Central Italy the following year. These uprisings failed mainly due to lack of public support. Throughout the 1830s, a nationalistic movement, later known as the *Risorgimento* or 'the resurgence/rebirth', emerged in Italy romanticizing Italian history and culture, and specifically embracing themes of freedom and patriotism (Duggan, 2014; Hearder, 1990; Noether, 1986). The liberal democratic movement, led by Giuseppe Mazzini, embraced this nationalistic cultural shift with the goal of unifying the Italian states. Mazzini, along with Giuseppe Garibaldi, led several revolts against foreign rule during the 19th century. Giuseppe Garibaldi was a young sailor from Nice who became an accomplished general and popular figurehead for the Mazzini-led liberal movement in Italy (Duggan, 2014).

In 1843 and 1844, revolts broke out in Romagna and Calabria. In 1848, a revolt broke out in Palermo and spread to the mainland, which resulted in King Ferdinand II approving a constitution (Domenico, 2002a). A revolt broke out against Austrian rule in Vienna and spread to

Milan in March 1848. Tuscany revolted against the House of Lorraine, which ruled the Grand Duchy, and established a republic in 1848 (Domenico, 2002b). Mazzini congregated his followers in Rome to overtake the city and establish a republic. Mazzini's roman republic held the city until the French invaded in July 1849. Austrian forces overtook Venice by the end of August 1849 and reinstalled the House of Lorraine in Tuscany in 1849 (Domenico, 2002b). Though Venice and Rome inevitably lost against foreign adversaries, their defiance inspired the cause for Italian independence.

During the latter half of the 19th century, Italy's economy was divided between the prosperous North and the stagnant South. In the north, railways were expanded, and agricultural wealth increased (Duggan, 2014; Sereni, 1997b). The railway system expanded from 6,000 km of tracks in 1870 to 16,000 km by 1890 (Sereni, 1997b). The expansion of the railway system in northern Italy reshaped the agricultural landscape; Tuscany and Liguria experienced a decline in olive oil production while tree and shrub cultivation expanded in Southern Italy (Sereni, 1997b). Grain production, especially corn, expanded in the North of Italy (Sereni, 1997b). Overall, the cost of war and reshaping of the Italian economy under unification caused an economic downturn.

Austria ruled the Italian duchies in the North, the Pope ruled much of central Italy including Rome, and King Ferdinand II, grandson of King Ferdinand I, ruled in the South (Figure 2.2). Vittorio Emanuele II, a distant cousin to Vittorio Emanuele I, took the throne of Piedmont in 1849 after the death of his father, King Carlo Alberto. Vittorio Emanuele II kept the constitution established under his father's rule in place. The Piedmontese government embraced economic liberalism, and instituted commercial treaties with England, France, and Austria, introduced free-trade tariffs, and encouraged banking and railway development. Piedmont also courted war with

Austria in an alliance with France. France signed an armistice with Austria in 1859, which allowed Austria to retain Veneto and Piedmont to acquire Lombardy. The war against Austria sparked patriotic uprisings in Italy; Bologna, Perugia, and other cities within the Papal states rebelled against the Pope.

After the War of 1859, Emperor Napoleon III of France made an armistice with Austria, acquiring Nice and Savoy. Peasant uprisings in Sicily against high taxes and oppressive landowners propelled Mazzini's followers to aid in an uprising against King Ferdinand II. The former followers of Mazzini aligned themselves with Vittorio Emanuele II, and Giuseppe Garibaldi led an expedition with the aid of Piedmont into Sicily (Domenico, 2002a; Hearder, 1990). In 1860, after a successful expedition that 'liberated' Sicily and Naples from the rule of King Ferdinand II, a vote was held to determine whether Sicily wanted to unite with Piedmont under Vittorio Emanuele II (Domenico, 2002a). Sicily voted overwhelmingly in favor of joining the alliance, though irregularities occurred on a grand scale during the voting process. The final vote count was 432,053 in favor and 667 opposed (Duggan, 2014). Vittorio Emanuele II became the King of Italy in 1861, with only the Papal states and Veneto remaining independent of the newly unified Italy. In February 1861, the first parliament of the new Italian republic was held in Turin, the capital of the Kingdom of Italy and the Piedmontese constitution was extended to all of Italy.

The unification of the Italy was similar to occupation by foreign forces and many parts of Italy suffered both economically and politically under this new regime. The new tariffs under Piedemontese rule destroyed several industries in Naples, including textiles and engineering factories. High taxes and increasing public debt as a result of the war for Unification led to economic decline. Private wealth was also a criterion for political participation: Only men over

the age of 24 who paid more than 40 lire per year in taxes could vote – less than 2% of the entire population of Italy (Duggan, 2014). During the 1860s, the Piedmontese government attempted to march on Rome several times but were unsuccessful until 1870. In 1866, war with Austria led to the Piedmontese government diverting occupying troops from Sicily to aid in the war effort. Uprisings in Palermo occurred in response to the worsening economic conditions under Piedmontese rule and diminished Piedmontese presence in the region, but these uprisings were quelled by Piedmontese forces. For several decades after the unification of Italy, the government of Piedmont was primarily run by northerners, leaving southerners to feel excluded from the political process. In 1887, Francesco Crispi, a Sicilian, was the first southerner to become Prime Minister of the Kingdom of Italy. By the early 20th century, Southerners were admitted into the civil service.

In the 1870s, 60% of the population in Italy was economically dependent on agriculture (Duggan, 2014). More than half of the grain grown in Italy was consumed by those who made it, and 75% of the expenditures of commoners was for food (Collantes, 2006; Duggan, 2014). Most commoner families lived in poor conditions (Leoni, 1991). In the Po valley in northern Italy, a typical home was a single room with a beaten earth floor that was shared among family members, and sometimes with livestock, such as an ox or mule (Duggan, 2014). In 1881, the census recorded approximately 100,000 people inhabiting subterranean homes, including necropolises, caves, and grottos, and children often had to work in factories instead of attending school. The infant mortality rate also was very high, with 350 infant deaths per thousand births reported in Veneto by the mid-19th century (Dalla-Zuanna and Rosina, 2011). The life expectancy in 1861 was 35.4 years with a 23.2% infant mortality rate in Piedmont, among the richest regions in the newly unified Kingdom of Italy (Profumieri, 2009). The main causes of

death were infectious disease and disease related to poor nutrition or unsanitary living conditions, such as pellagra (vitamin B12 deficiency), tuberculosis, and malaria (Gentilcore, 2014; Ginnaio, 2011; Profumieri, 2009).

The modern political and economic history of Italy demonstrates how the Italian peninsula was frequently occupied by foreign powers that sought to exploit Italian resources instead of implementing revolutionary policies granted to their homelands. The exploitation of resources in Italy, along with continued warring over which of the European powers would be allowed to rule over those resources, led to slow modernization of the Italian peninsula, economic decline, and sharp socioeconomic divisions. Most of the population of Italy was poor, undernourished, and lived in unsanitary conditions (Leoni, 1991).

2.4 A brief history of cholera in 19th-century Italy

Cholera is caused by the bacterium *Vibrio cholerae*, a gram-negative bacterium endemic to coastal and estuarine waters throughout the world (Sack et al., 2004; Thomas, 2015). Only some strains of *Vibrio cholerae* infect humans and cause the disease known as cholera. Specifically, there are two virulence factors required for a bacterium to cause cholera: the cholera toxin (CTX) and the toxin coregulated pilus (TCP) (Kaper et al., 1995; Thomas, 2015). The TCP is the receptor for the cholera toxin and is required to cause infection.

Cholera is typically spread through the consumption of contaminated water or food. Once inside the digestive tract, the bacterium multiplies and releases the cholera toxin, which causes portals in the cell membrane of the large intestine to semi-permanently remain open (Ali et al., 2012; Kaper et al., 1995; Kotar and Gessler, 2014; Sack et al., 2004). These open portals cause the expulsion of electrolytes and water from the surrounding tissues, resulting in rapid dehydration of the victim. Symptoms of cholera infection include vomiting, weakened pulse,

hallow cheeks, dry tongue, excessive thirst, muscle cramps, lethargy/unusual sleepiness or tiredness, glassy or sunken-in eyes, kidney failure/low urine output, and rice-water diarrhea (Barnet, 1972; Kotar and Gessler, 2014).

Victims of cholera, if untreated, will usually die within a week of exposure, but some die within 24 hours (Briggs, 1999). In the most severe cases, victims die within 6-8 hours of exhibiting diarrhea (Morris, 2011). Though severe cases of cholera can cause rapid death in its victims, only about 2-11% of infected persons exhibit this most extreme form of the disease (Morris, 2011). Approximately 59–75 % of infected individuals are asymptomatic, and the remainder of infected persons exhibit mild or moderate symptoms of the disease. Prior to the advent of oral rehydration solution in the 1830s, the fatality rate for cholera was between 30–60% of afflicted individuals (Kotar and Gessler, 2014; Lima, 1994).

Despite emerging as a pandemic threat only in the late 18th and early 19th centuries, cholera is a disease of ancient origins. Cholera was endemic in south Asia for several centuries prior to the spread of the disease to the rest of the world. Ancient Chinese, Hindu and Greek texts refer to a diarrheal disease that is likely cholera (Kotar and Gessler, 2014; Retief and Cilliers, 1999; Thomas, 2015). The next recorded instance of cholera occurred in 1543, when a Portuguese historian, Gaspar Correa, described a cholera outbreak near the Ganges delta in India. Beginning in the 19th century, cholera began to spread beyond the Indian subcontinent throughout the world in pandemic waves. During the 19th century, there were a total of six pandemic waves of cholera that spread into Eastern and Western Asia, Europe, the Americas, and Africa.

The first pandemic wave of cholera (1817-1824) started in Jessore, India near Calcutta at a gathering of pilgrims celebrating the Hindu Festival of Mela (Thomas, 2015). Though cholera

was endemic in India for several centuries prior to the outbreak, the likely reasons for the spread of cholera outside of the Indian subcontinent during the first pandemic are two-fold; First, the eruption of Mount Tambora in Indonesia in April 1815 caused disruptions in the monsoonal cycle and resulted in an overall cooling of the global temperatures (Morris, 1976; Thomas, 2015). The cooling climate and disruption in weather patterns caused famine and mass migration across Asia and Europe, which may have increased the spread of the cholera beyond the Indian subcontinent. Second, the British government, which occupied India during the 19th century, excavated several river systems and changed India's irrigation system, which caused the disruption of river sediment and altered soil composition. British rule also had another effect on the spread of cholera: British troops regularly rotated between military tours in India and return trips to England, increasing in the movement of both people and goods between the Indian subcontinent and other parts of the world (Morris, 1976; Thomas, 2015). No increase in trade or population density between 1810-1830 otherwise could have contributed to the spread of cholera (Morris, 1976).

During the first pandemic, cholera spread rapidly from India to China, Persia, Java, and the Middle East between 1818-1819 (Morris, 1976). In 1823, cholera reached Astrakhan, Russia, and then was dormant for several years (Morris, 1976).

Between 1818 and 1822, 19,494 of 83,366 members of the British army stationed in India contracted cholera and approximately 5% died of the disease (Kotar and Gessler, 2014; Morris, 1976). The overall death rate among the soldiers for all causes was 5.7% (Morris, 1976). On Java, approximately 100,000 people died of cholera (Kotar and Gessler, 2014).

During the first pandemic, the etiology of cholera was unknown, which resulted in ineffective policies to prevent the spread of the disease. The early theories about cholera's

etiology can generally be divided into two main hypotheses: miasma and contagion. Miasmists argued that cholera was spread by miasma, or "bad air", whereas contagionists argued that the disease was spread by contact with contaminated people or objects (Kotar and Gessler, 2014; Morris, 1976; Thomas, 2015). Health officials throughout Europe, when hearing about the emergence of the 'blue death' ravaging other nearby cities, proposed to prevent the spread of infection by quarantining infected ships and/or cargo, or burning specific goods believed to be capable of spreading the disease (Morris, 1976). These measures generally did not prevent the spread of disease because, much like today, the spread of cholera is usually due to structural problems relating to sanitation (Briggs, 1999). The waterborne theory for cholera etiology did not emerge until 1855 and was not widely accepted until the end of the 19th century.

The second pandemic began in India in 1826 at the Kumbh Mela Festival in Haridwar and spread rapidly to other parts of the world (Morris, 1976). This second cholera pandemic (1826-1831) reached into China, Persia, the Middle East, and then into Astrakhan and Volga in southern Russia (Kotar and Gessler, 2014; Morris, 1976; Sack et al., 2004; Thomas, 2015). In Astrakhan, cholera killed approximately 21,000 people in July 1830 (Kotar and Gessler, 2014). Cholera spread into Orenburg, Russia in 1829 and reached Moscow in September 1830 (Morris, 1976). In 1831, cholera had advanced into Poland, and the spread of the disease was exacerbated by the Polish War (Morris, 1976). Cholera spread into Berlin and Vienna, and then into France, Britain, and the Americas (Kotar and Gessler, 2014). Cholera spread to Madagascar, the Middle East, and Egypt (Kotar and Gessler, 2014).

In Britain, the Board of Health attempted to prevent the spread of cholera by closing Britain's ports to all ships from Russia, the Baltic, the Kattegat, and the Elbe, which, unsurprisingly, halted British commerce and trade (Morris, 1976). The quarantine was very

unpopular and caused an economic downturn in Britain (Morris, 1976). In February 1832, cholera was discovered in London, and by summer the regulations were abandoned because cholera was already in England (Morris, 1976). Cholera killed 32,000 in Britain between 1831-1832 (Morris, 1976). Many of the victims of cholera were poor, but some were middle-class. Nonetheless, the poor were blamed for the epidemic of cholera and this inequality was a major source of tension because the upper and middle classes anticipated that cholera would disrupt the power structure in Britain (Morris, 1976). The tension between classes over the rising cholera epidemic made treatment for those afflicted difficult; cholera hospitals were stigmatized and people generally neither wanted cholera hospitals constructed near where they lived nor did they want to go there to seek treatment for the disease (Morris, 1976).

One tactic employed in Britain to prevent the spread of disease was to prepare the bodies of cholera victims for funerary rites quickly and bury them as soon as possible after death. In Britain, cholera burials were regulated thusly: bodies of cholera victims were wrapped in cloth saturated in pitch or coal tar, carried to the gravesite with as few people as possible attending the burial, and an open-air service was performed for the dead (Morris, 1976). Additionally, at St. Anne's Middlesex, bodies also were buried in coffins filled with lime to prevent the spread of disease (Morris 1976: 105).

The third cholera pandemic (1846-1860) took a similar route to the second cholera pandemic, but also reached of Central and South America (Evans, 1988). The third cholera pandemic is notable because as a result of this epidemic thinking around the etiology of cholera shifted slightly. In 1855, Dr. John Snow, a London physician and the father of modern epidemiology, performed his famous Broad Street water pump analysis of the 1855 cholera outbreak in London (Rosenberg, 1966; Thomas, 2015). He noticed that only people who received

water from the Broad Street water pump caught cholera, whereas people working in factories and other businesses near the water pump using water from a different source did not contract the disease. A local workhouse and a brewery within walking distance from the pump were among the businesses unaffected by cholera; they either had their own water source or used a different pump (Thomas, 2015). Workers in factories that used the Broad Street pump contracted the disease. Based on this evidence, Snow concluded that the disease likely was neither a contagion nor a result of miasma, but instead a waterborne illness. Despite publishing his work before his death in 1858, the waterborne theory for cholera etiology was not accepted widely until 1883, when Robert Koch isolated the *Vibrio cholerae* bacterium and identified it as the pathogen causing cholera (Thomas, 2015).

The fourth cholera pandemic (1863-1875) began in the Bay of Bengal and spread along the same trade routes and harbors as in previous epidemics (Kotar and Gessler, 2014; Thomas, 2015). In 1864, cholera cases were reported in Aden on the southern coast of the Red Sea. The following year, cholera had spread northward to Mecca where it killed 15,000 Muslim pilgrims (Thomas, 2015). In 1865, cholera reached Constantinople, and from there it spread into Europe to Naples (Kotar and Gessler, 2014; Thomas, 2015). By 1866, cholera once again reached the Americas (Kotar and Gessler, 2014). The spread of cholera during this outbreak was exacerbated by the adoption of steamboats by the East India Company and the expansion of the British Empire, which increased the movement of people and goods between Calcutta and London (Thomas, 2015).

In 1881, the fifth cholera pandemic (1881 -1896) again saw cholera spread throughout the world, reaching Singapore, Java, Spain, and Mexico (Kotar and Gessler, 2014). In 1881, 60,000 people died of cholera in Egypt (Snowden, 1995). In May 1883, cholera reached Shanghai,

China, and in June it reached Saluzzo, Piedmont, Italy (Kotar and Gessler, 2014). In July cholera had a small flare up in Marseilles, France, resulting in seven cases of cholera and two fatalities (Kotar and Gessler, 2014; Snowden, 1995). In 1884, French troops transported between from Vietnam and France brought the cholera to France in full force: In 1884, Marseille experienced a cholera epidemic that caused 1,781 deaths, 26% of which were Piedmontese construction workers from Italy (Snowden, 1995). The surviving Italians fled Marseille for Italy, spreading cholera down the Italian peninsula to La Spezia and Naples (Snowden, 1995). Naples experienced 7,143 deaths and 14,233 cases of cholera, resulting in 447.6 deaths/10,000 inhabitants, the highest rate of death among major cities in Italy during this pandemic (Snowden, 1995). The first recorded case of cholera in Nagasaki, Japan occurred on August 19, 1885 (Kotar and Gessler, 2014). In 1886, cholera reached Buenos Aires, and in 1889, Baghdad also experienced a cholera epidemic (Kotar and Gessler, 2014). The cholera pandemic was so pervasive that it reached Honolulu, HI in 1895 (Kotar and Gessler, 2014).

The sixth and final cholera pandemic (1899-1947) lasted for almost forty years. As with previous pandemics, the outbreak began in 1899 in India and spread to other countries along trade routes. From 1899 to 1900, outbreaks of cholera occurred in Calcutta and Bombay and spread to Afghanistan and the Persian Gulf to the west, and Burma and Singapore to the east (Pollitzer, 1954). Cholera was carried by sea from Madras to Mecca, and then to Egypt, where cholera killed 34,000 victims in Asyut within three months of the first case (Pollitzer, 1954). Cholera lingered in the Middle East for several years until spreading to St. Petersburg, Russia in 1908. In 1910, cholera was responsible for over 230,000 cases and approximately 110,000 deaths in Russia (Pollitzer, 1954). Cholera remained in Russia throughout the next 15 years, with notable increases in cases during 1915 (66,455 cases), 1918 (41,586 cases), 1920 (29,615 cases),

1921 (207,389 cases, and 1922 (44,049 cases) (Pollitzer, 1954). Cholera was reported in the Italian regions of Apulia and Naples between 1909-1911, with cholera manifesting throughout the Italian peninsula and Sicily in 1911 (Pollitzer, 1954). Cholera also emerged in Hungary between 1909-1913, but only sporadic cases of cholera were reported in western Europe and the Americas during this outbreak because of improved sanitation standards (Kotar and Gessler, 2014).

2.5 Site descriptions

Four sites from Central and Southern Italy are examined in this dissertation (Figure 2.3). These sites are selected because of their regional and temporal proximity to one another, and because they are clearly linked to either a cholera epidemic or attritional burial site. The first two sites, Alia and Benabbio are sites where cholera victims from epidemic waves of cholera during 1832 and 1855, respectively, were buried. Characteristics of both cemeteries support the idea that they were catastrophic burial sites: they were not generally used by the local population for burials, were located outside of the main town centers, and included atypical burial patterns for the cultural and temporal context (Baldino, 2011; Bigazzi et al., 2002; Fornaciari, 2015a; Fornaciari et al., 2010; Fornaciari and Coschino, 2012). Additionally, historic documents and other research supports the claim that these are a catastrophic assemblage related to pandemic waves of cholera during the 19th century. The other two sites, Pieve dei Monti di Villa and Badia Pozzeveri include cemeteries from the 18th to 19th century that were used as general burial sites. Both cemeteries surround churches in use during the 18th and 19th centuries. The burials were carefully placed in the ground, usually in a coffin (Rezza, 2009). The following sections will further detail the history, historic documents, and previous archaeological research associated with each site.

2.5.1 Alia (PA)

Alia is a small, rural *comune* in the Palermo (PA) province on the island of Sicily with approximately 4,000 inhabitants in the 19th century (Guccione, 2002). The village of Alia is situated atop a slope approximately 700 meters in elevation in central Sicily in the western Madonie mountain range. The township of Alia emerged in the historic record in the 1200s CE, though there are earlier mentions of a settlement in the area. The main village is surrounded by the fertile hills of the Sicilian countryside in which a variety of agricultural activities occur, including animal husbandry and crop cultivation. Historically, Alia produced wheat, almonds, and beans, and bred cattle, horses, and sheep, along with some limited beekeeping (Guccione, 1991; Guccione, 2002).

During the cholera epidemic of 1837, approximately 300-400 individuals from Alia perished and were buried in a cave approximately 1 km outside of the town center (Bigazzi, 1999; Chiarelli et al., 2002). Within the cave, known as camposanto vecchio (translated: 'old cemetery'), the cholera victims were buried together in a mass grave. The remains of cholera victims were rediscovered in 1995 during the renovation of the wall encasing the tomb (Chiarelli et al., 2002; Guccione, 2002). Researchers from the University of Palermo and other universities excavated the remains between 1996 and 2000. A book published in 2002 entitled "Alia: antropologia di una communità dell'entroterro siciliano" (translated: "Alia: anthropology of a community of the Sicilian hinterland") summarizes the results of demographic, genetic, and historical analysis of the site. Dr. Renzo Bigazzi published his dissertation research in 1999 entitled "Analisi microevolutive sulla struttura biologica e demografica della popolazione di Alia del XIX secolo" (translated: "Microevolutionary analysis of the biological and demographic structure of the population of Alia during the 19th century") which primarily focused on the

demographic structure of the cholera victims and genetic linkages to living descendants in modern day Alia. The osteological analysis from these two sources finds that there were approximately ~300 individuals buried in the cave.

Since cholera does not leave any skeletal markers that are disease-specific, historical documentation and archaeological evidence are the two main lines of evidence to support the interpretation that the individuals from camposanto vecchio are cholera victims. The government of Alia curates a repository of historical documents about births and deaths at the municipal police station. These historic documents include births and deaths from the early 1800s, but when I visited Alia in the summer of 2017, the death records for the year 1837 were missing. Giuccione (2002) implies that the records from 1837 were present during the late 90s, so they may have been misplaced during my visit to the archive in 2017. The parish of Sant'Anna, however, gave me access to a copy of the parochial records of deaths in Alia during 1837 that specifically discusses the cholera epidemic of that year (Figure 2.4). The parochial document, written in Latin, describes the emergence of cholera in Alia on the 7th of July 1837 and lists the names of the deceased victims of cholera, by date, during July 1837.

In addition to these parochial records, the burial style of the remains from camposanto vecchio indicates they form a catastrophic assemblage (Figure 2.5) (Chiarelli, 2002). The bodies were buried in a mass grave outside of the main village of Alia and according to Bigazzi et al. (2002), the skeletal material from Camposanto vecchio was covered with lime, a common cultural practice in Europe to prevent the spread of disease (Morris, 1976). Giuccione (2002) suggests that deceased *aliese* were typically buried near the Cappella dei Santuzzi, in sanctified ground, instead of the camposanto vecchio. Additionally, Giuccione (2002) describes how, like many other cities in Europe, Alia was ill-equipped to handle the 1837 virulent wave of cholera

because the town lacked sewers and water systems. Osteological analysis also confirms that these individuals were likely cholera victims from 1837. The minimum number of individuals for camposanto vecchio, as calculated by Bigazzi (2002), is 296, which is close to, but does not exceed, the 306 cholera victims recorded in the parish records.

Several obstacles were overcome to include this site in this analysis. Individuals were buried in a mass grave inside a cave; thus, dentitions and bones from the multiple individuals in this collection are disarticulated. Disarticulated skeletons cannot be analyzed using the same approaches as articulated ones; in general, the researchers cannot fully analyze the role of demographics (sex, age, etc.) because each bone must be considered alone, and only the demographic information derived from that individual bone can be used in any subsequent analysis. For this reason, paleopathological analysis is restricted to crania, mandibles, right femurs, and right tibiae for this site only. Teeth and bone samples were taken only from the same element, either a mandible or a cranium. Stature for this population is analyzed only from a population perspective, and not divided based on sex or age since these traits cannot be accurately derived from individual femura or tibiae alone.

2.5.2 Benabbio (LU)

Benabbio is a central Italian village located north-east of Lucca (LU) in the Val di Lima region of Tuscany. The village of Benabbio is located in the Serchio Valley south of the Lima Torrent, a main tributary for the Serchio River. The Serchio Valley is a mountainous region of central Italy characterized by steep mountain slopes covered in woody forests. The modern village of Benabbio is located approximately 200 m south-west of the church of San Michele, where approximately 46 cholera victims out of approximately 900 total inhabitants were buried during the cholera epidemic of 1855 (Figure 2.6) (Fornaciari et al., 2010). The church of San Michele is located at the apex of

a mountain and is surrounded by medieval fortifications that are the remnants of an 11th-century castle. The medieval castle was first mentioned in papers from the Archbishop of Lucca between the 10th and 11th centuries (Baldino, 2011). The location of the castle at the apex of the mountain and the fortification of the castle with a 60 cm-wide wall demonstrates that the castle served as a military fortification to protect the local lord and his lands (Fornaciari et al., 2010). In 1334, the Rossi, Lords of Parma and the Royal Vicars of the city of Lucca, ordered the destruction of the castle surrounding the church of San Michele (Baldino, 2011; Fornaciari et al., 2010). During the 17th century, the church was renamed Benabbio (Baldino, 2011; Laganà, 2007). In 1357, a rector from the parish of Santa Maria Assunta located within the town of Benabbio oversaw the religious functions of the church of San Michele. During the medieval period the church grounds were used as a cemetery, but eventually fell out of favor as a burial site because of a diminishing population in Benabbio (Baldino, 2011). By the beginning of the 19th century, the church was the only structure of the castle still standing (Laganà, 2007).

The church of San Michele was excavated by the University of Pisa's field school between 2007 and 2013. Bone collagen stable carbon and nitrogen isotope ratio analysis and paleopathological analysis of the skeletal collection from Benabbio were conducted by researchers at the University of Pisa (Baldino, 2011; Fornaciari, 2015b; Fornaciari et al., 2012). Professor Gino Fornaciari published stable carbon and nitrogen isotope analysis from rib collagen from this site, finding that inhabitants from Benabbio consumed an omnivorous diet with moderate animal protein (Fornaciari, 2008; Fornaciari, 2015b). The osteological analysis for the cholera victims from Benabbio were studied by Barbara Baldino (2011) for her dissertation on funerary archaeology in Val di Lima. Barbara Baldino used a set of standards for osteological analysis that

are different from those used in the present study. For this study, I re-assessed the skeletal remains using the Global History of Health Codebook standards for data consistency.

Much like Alia, the evidence to support the presence of cholera burials at the church of San Michele is based on historic documents, archaeological data, and osteological analysis. Soon after the 1855 cholera epidemic in Benabbio, a plaque was installed on the walls of the church of San Michele as a memorial to those who died during the epidemic (Figure 2.7) (Laganà, 2007). Additionally, the names of the 44 Benabbio cholera victims were recorded in parochial records from August thru October 1855 (Table 2.2) and a note from the parish priest indicates that the majority of victims dying of cholera were buried inside the castle (Laganà, 2007). The two victims who were buried elsewhere in the village were Francesco Antonio Cianelli and Momerto Contrucci. Archaeologically, the location and burial style of the graves at the church of San Michele is consistent with a catastrophic burial. The bodies were buried in the grounds around the church, within the walls of the castle, which is approximately a 20-minute walk from the main village. This location was advantageous for burial of cholera victims because it was sanctified ground but sufficiently far away from the village to minimize the spread of infection. The bodies were placed in deep graves without coffins, often with more than one individual occupying a single grave (Figure 2.8). Burying more than one body in each grave indicates that these individuals were buried quickly to avoid contaminating the village. Additionally, the burials at Benabbio were covered with lime, which was a common cultural practice to prevent the spread of infection (Fornaciari et al., 2010). Osteologically, the number of bodies recovered from the 19th-century layers outside the church (n = 41) is consistent with the historic documents that indicate 44 individuals died of cholera in 1855 (Fornaciari et al., 2010).

2.5.3 Pieve dei Monti di Villa (LU)

Pieve dei Monti di Villa, Italy is a village located in the Bagni di Lucca region of Tuscany, approximately 12 km north-west of the church of San Michele. Pieve dei Monti di Villa (abbreviated 'Monti di Villa') is situated on a mountain across the Lima Torrent from Benabbio. Monti di Villa houses a medieval parish church, the parish of San Giovanni Battista, which dates to as early as 772 CE (Rezza, 2009). The parish church in Pieve dei Monti di Villa originally was named for Santa Giulia di Controne. Between 772 and 1014, the patron saint of the church gradually switched from Santa Giulia to San Giovanni Battista as it is known today. During this time period, the church was regularly used as a site for both baptisms and burials. The deceased were interred in the area surrounding the church. The church is located within the main town center of the modern village of Monti Di Villa, and consequently was used continuously by the villagers at least until the early 18th century as a religious building with burial services.

The parish of San Giovanni Battista was excavated by the University of Pisa between 2002 and 2006 (Rezza, 2009). Two main areas surrounding the church were excavated: a section along the walls of the church that was used between 1807 and 1870, before the creation of a new cemetery in the village in 1871; and a second area located behind the tribune of the church that dates to the medieval period. The skeletal material from the parish of San Giovanni Battista was previously studied by the Division of Paleopathology at the University of Pisa by Dr. Angelica Vitiello, and Dr. Silvia Rezza, and bone collagen stable carbon and nitrogen isotopes analyzed by Professor Gino Fornaciari (Fornaciari, 2008; Fornaciari, 2015b; Rezza, 2009). For this project, 10 individuals from the 19th-century layers of the site were analyzed for paleopathological markers as a comparative, contemporaneous population for the cholera victims at Alia and Benabbio. The site was re-assessed for pathological and demographic markers using the Global History of Health

Codebook to standardize the comparison among the sites (Steckel et al., 2006). The excavation of the site is featured on the University of Pisa's Division of Paleopathology website and in two issues of Archaeologia Postmedievale (Fornaciari, 2002; Fornaciari, 2004; Fornaciari, 2006).

2.5.4 Badia Pozzeveri (LU)

Badia Pozzeveri is a borough of Altopascio (LU), Italy, located 21 km east of the city of Lucca. The church of San Pietro is an 11th-century church within Badia Pozzeveri along the Via Francigena, an important historic pilgrimage route that runs from Canterbury Cathedral, England to Rome, Italy. The first mention of the church of San Pietro occurred in September 1056 CE when four parcels of land were donated to the church (Seghieri, 1978). In 1103 CE, the church was converted to a monastery with an abbot and 12 resident monks, which required expanding the church's structure toward the south and adding a courtyard and living quarters (Seghieri, 1978). During the 12th century, the monastery expanded, and the monks formed a dedicated leprosarium. In 1325, the monastery walls were damaged after the monks sided with Lucca against Castruccio Castracani of Florence during the Battle of Altopascio. The monastery was later abandoned, but the *chiesa* was used continuously throughout the medieval period and into the 19th century. The medieval burials are located along the side of the church and in front of the medieval facade of the church, which extends 6 meters in front of the modern facade of the church. The front section of the medieval church collapsed in the 17th century, and the collapsed section was used as a graveyard during the 18th century based on burial style and grave goods.

The church of San Pietro was excavated by the University of Pisa from 2013 to the present and served as an international field school in collaboration with the Ohio State University from 2013-2016. The excavation is thoroughly documented on the University of Pisa's Division of Paleopathology website and in Science (2018; Gibbons, 2013). The University of Pisa is

completing osteological analysis of the skeletal remains from the site. Santiago-Rodriguez et al. (2019) recently published an article studying the dental calculus microbiome of the skeletal remains from Badia Pozzeveri. They found several pathogenic species of bacteria, but no *Vibrio cholerae*. For this research, individuals from the 18th- and 19th-century layers whose burial patterns were consistent with general, non-catastrophic burials were selected for analysis in this dissertation. These individuals provide a contemporaneous, comparative sample to contrast with the cholera burials from Alia and Benabbio.

2.6 Conclusion

The concurrent histories of Italy and cholera illustrate the social, cultural, and technological conditions leading to the spread of cholera in six pandemic waves throughout the 19th century. Life for the average Italian in much of 19th-century Italy was politically oppressive, both before and after unification in 1871. While the bourgeoisie and aristocrats were mainly unaffected by political developments in Italy the lower classes, which comprised the majority of the population of Italy, typically lived in unsanitary conditions, often sharing a residence with livestock. In addition to unsanitary conditions, poverty also perpetuated undernutrition among most Italians, which depressed their immune systems and increased their susceptibility to infectious and environmental diseases, such as pellagra, malaria, and cholera. Though the etiology of cholera was discovered by Dr. John Snow in the 1850s, this discovery was largely ignored until the late 19th century, perpetuating ineffective and sometimes dangerous treatments for the disease. The widespread poverty, continuous movement of people due to war and trade, and lack of knowledge about the etiology of cholera created ideal conditions for the disease to spread throughout Italy and the whole of Europe.

Italians lived in a resource scarce and unstable environment for much of the 19th century, but historical analysis alone cannot fully illuminate why certain individuals died and others survived. Illuminating the factors that contribute to the hidden heterogeneity in risk of death requires further analysis.

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Table 2.1: Italian Modern Period Timeline 1527 – 1882.

Date (CE)	Events
1527	Rome is sacked by German and Spanish troops
1559	Peace of Cateau-Cambrésis establishes Spanish control of Italy
1647 – 1648	Naples and Palermo revolt against Spanish rule
1701 – 1714	War of Spanish Succession results in Austrian Habsburgs rule of Italy
1733	War of Polish Succession
1734	Charles of Bourbon becomes king of Naples and Sicily
1740	Emperor Charles VI of Austria dies, starting the War of Austrian Succession
1748	War of the Austrian Succession ended with confirmation of Austrian Habsburg dominance of Italy
1763 – 1764	Famine strikes central Italy, including Naples, Florence, and Rome.
1796	Napoleon Bonaparte invades Italy and defeats Austrians
1805	Napoleon crowned king of Italy in Milan
1808	French occupation of Rome
1814 – 1815	End of the Napoleon era
1816	Kingdom of Two Sicilies Established
1817 – 1824	First cholera pandemic
1820 – 1821	Revolutions break out in Naples, Palermo, and Piedmont
1826 - 1831	Second cholera pandemic
1831	Revolutions in the Duchy of Modena and other Papal states
1846 – 1862	Third cholera pandemic
1848	Revolutions break out across Italy
1859-1860	Second War of Italian Independence
1861	Kingdom of Italy is formally reconstituted with the ascension of Emmanuel II as King of Italy
1862	Garibaldi attempts invasion of Rome from Sicily
1863 – 1875	Fourth cholera pandemic
1866	Italy enters the Austro-Prussian War and is defeated by the Austrians
1870	The Italian Government annexes Lazio
1873-1896	European-wide Great Depression leads to drop in agricultural prices, widespread poverty, and displacement in rural Italy
1881 – 1896	Fifth cholera pandemic
1882	Italy joins the Triple Alliance with Germany and Austria-Hungary
1899 – 1947	Sixth cholera pandemic

Derived from the chronology in Duggan (2014), Hearder (1990), and Thomas (2015)

Figure 2.1: Map of Italy at the end of the 16th century, by C. S. Hammond and CO, N.Y.

Figure 2.2: Map of Italy during unification 1859 - 1924, by C. S. Hammond and CO, N.Y.

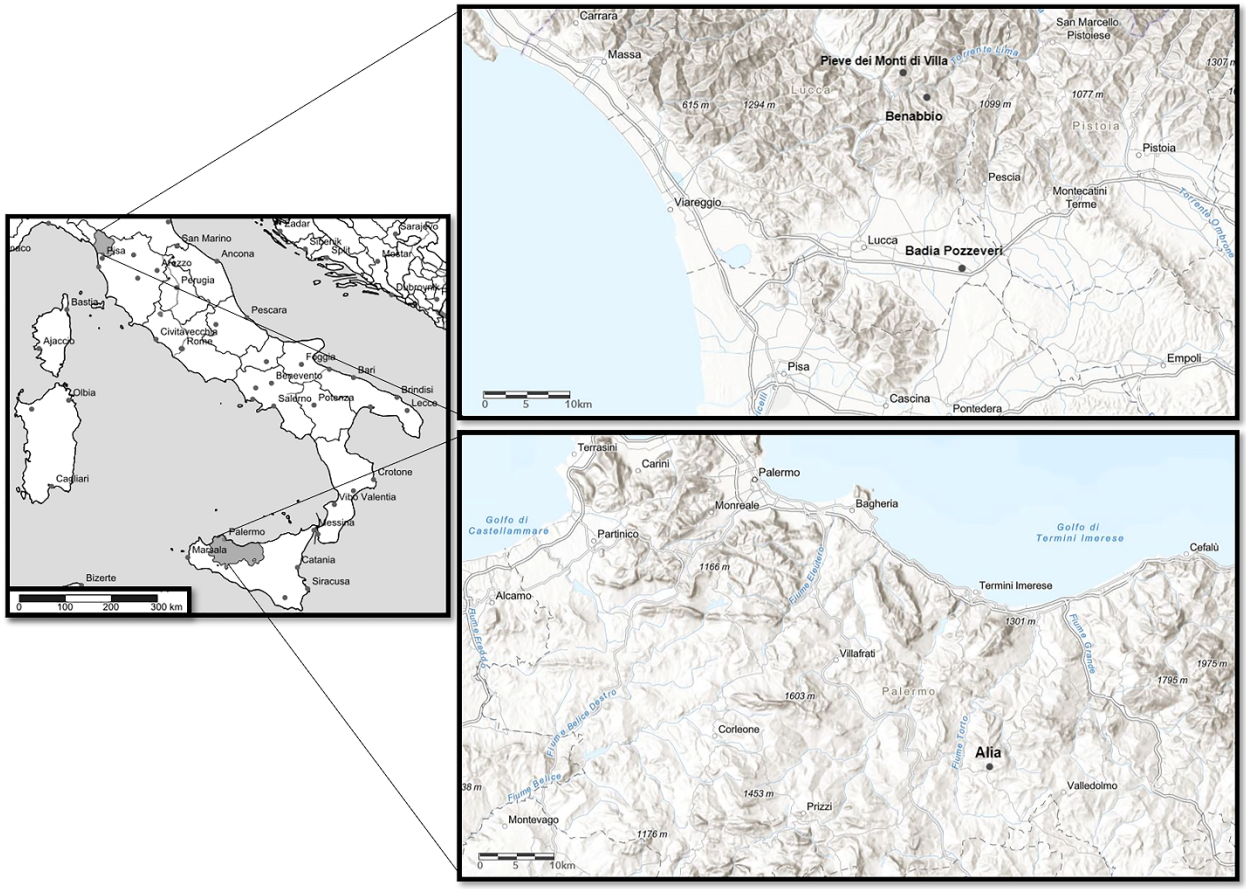

Figure 2.3: Map of Archaeological Sites: Alia, Benabbio, Badia Pozzeveri, and Pieve dei Monti di Villa.

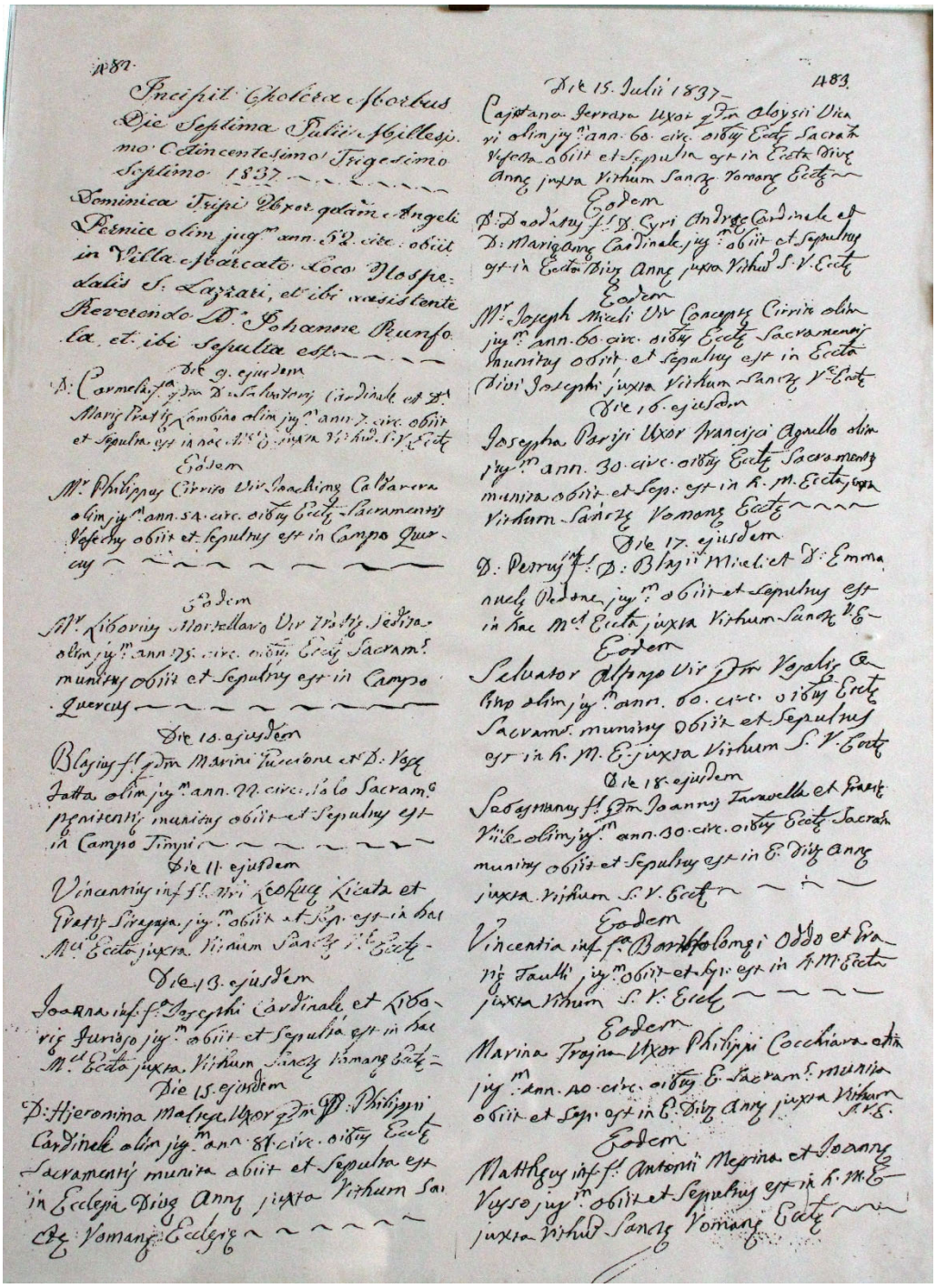

Figure 2.4: Parochial document listing the names of the cholera victims from 1832 in Alia, Italy on the island of Sicily from the parish of Saint Anna. The first four lines of the document roughly translates to "Cholera morbus started on the 7th of July of 1837".

Figure 2.5: Cave entrance to camposanto vecchio located in Alia, Italy on the island of Sicily where ~300 cholera victims were buried in 1837. The plaque on the cave wall translates to “In memory of the cholera epidemic of 1837 that decimated the population of Alia, September 18, 1997, The Municipal Administration”. Image date: July 5, 2017.

Figure 2.6: The church of San Michele located in Benabbio, Italy. The medieval church dates back to the 11th century and is the burial site of the cholera victims from Benabbio in 1855. Image date: July 11, 2015.

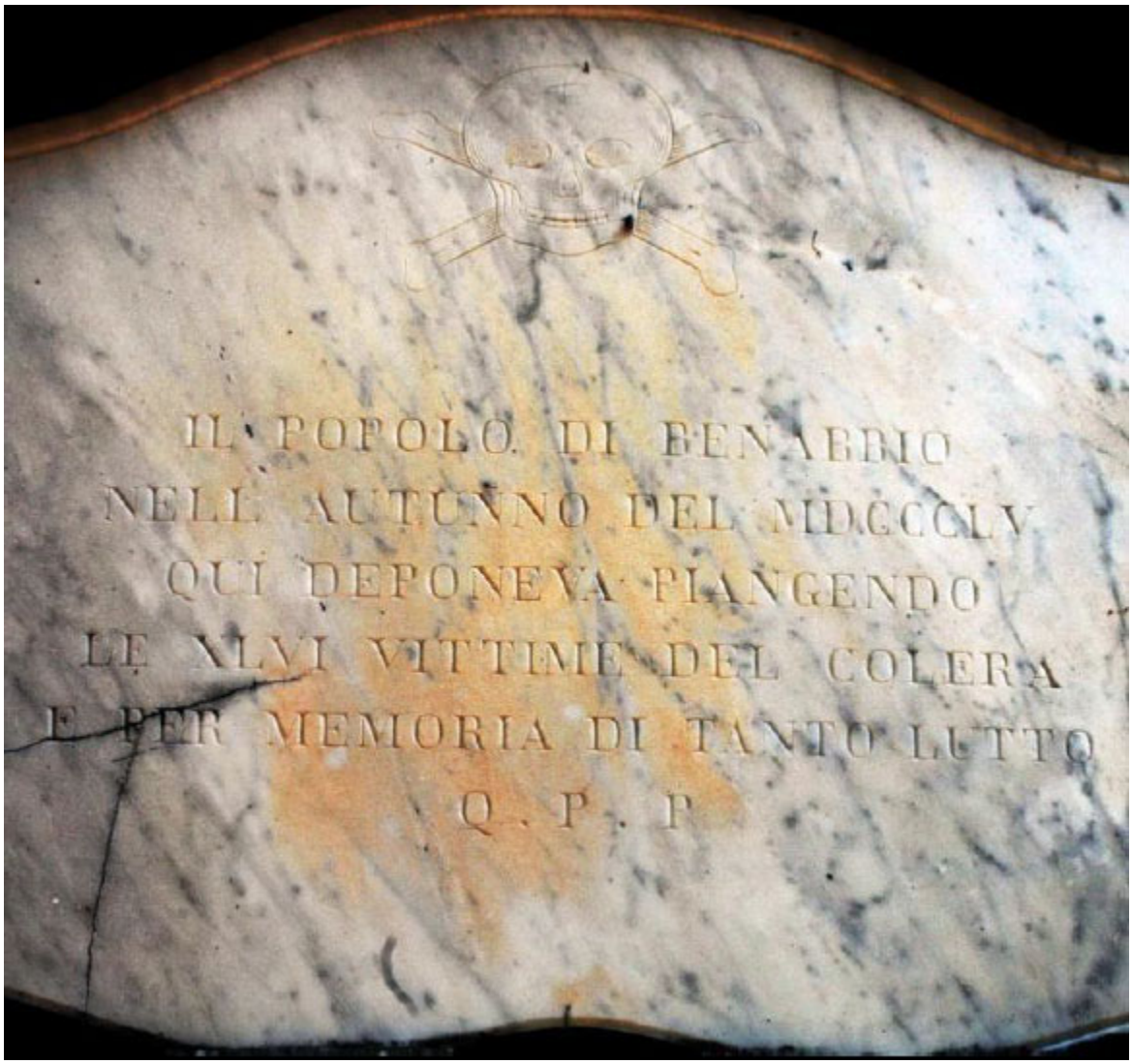

Figure 2.7: Plaque commemorating the internment of the victims of cholera during the 1855 epidemic in Benabbio. Translation: “The people of Benabbio in the fall of 1855 sadly lay here the 46 victims of cholera and the memory of so much mourning.” Image courtesy of A. Fornaciari.

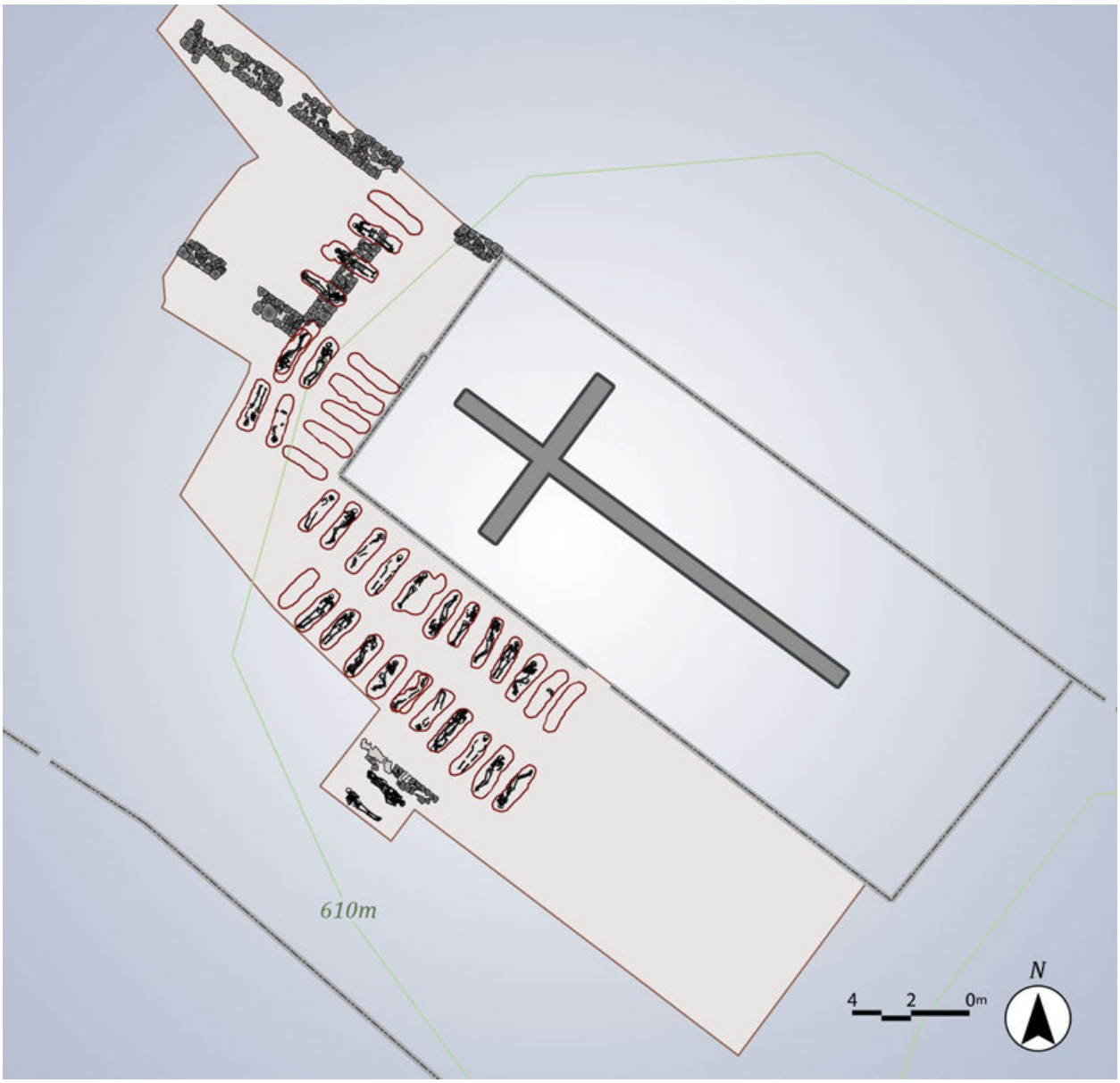

Figure 2.8: Map of the burials at the church of San Michele in Benabbio, Italy. Source: Francesco Coschino, paleopatologia.it.

Table 2.2: List of cholera victims from Benabbio derived from parochial records (APB, Morti D5, cc. 36v – 39r). Age-at-death in parentheses. Source: Baldino (2011).

Maria Angela Franceschi ved. Benedetti (66)	Francesco Marchi (45)
Lucia Contrucci in Giuliani (24)	Maria Assunta Rocchi (11)
Antonio Allegrini (70)	Chiara Michelini in Giusti (62)
Angelo Pierotti (32)	Giovanni Marchi (51)
Lucia Cianelli ved. Bertolini (57)	Nicolao Pierotti (57)
Antonio Contrucci (46)	Giovanni Marchi (87)
Ugo Magnani (7)	Maria Benedetti Rocchi (3)
Assunta Fiorini in Marchi (61)	Giuseppe Magnani (68)
Nicolao Tofani (56)	Francesca Tofani ved. Bertolini (66)
Maria Anna Giuliani in Contrucci (56)	Anna Contrucci in Rocchi (37)
Stella Pierotti ved. Lucchesi (64)	Giovanni Franceschi (49)
Giovanni Marchi (32)	Luigi Franceschi (74)
Francesca Cianelli ved. Magnani (49)	Domenica Contrucci in Giusti (49)
Antonia Fiorini ved. Cianelli (85)	Pietro Franceschi (60)
Giuseppe Rocchi (64)	Marco Antonio Pierotti (78)
Quirico Giuliani (60)	Sebastiano Marchi (60)
Maria Anna Pierotti ved. Betti (68)	Andrea Marchi (9)
Colomba Cianelli ved. Cianelli (71)	Giuliano Marchi (39)
Francesco Pierotti (61)	Martino Franceschi (77)
Rocco Cianelli (72)	Antonio Cianelli (44)
Luca Magnani (29)	Francesco Antonio Cianelli*
Rosa Pierotti (19)	Momerto Contrucci*
Luigi Marchi (57)	Maddalena Contrucci (57)
Bartolomeo Emidio Tofani (3)	**Not buried at the castle cemetery*

CHAPTER 3

LITERATURE REVIEW: BIOARCHEOLOGICAL APPROACHES TO THE DEVELOPMENTAL ORIGINS OF ADULT HEALTH AND DISEASE HYPOTHESIS

3.1 Introduction

The developmental origins of adult health and disease (DOHaD) hypothesis has emerged in the fields of biological anthropology, public health, and epidemiology as a major theoretical framework over the past 40 years (Barker and Osmond, 1986; De Boo and Harding, 2006; Hales and Barker, 2001). Traditionally, living subjects and historical records analysis have been used to study the DOHaD hypothesis. However, research on historical and archaeological populations have incorporated the DOHaD theoretical framework (see Armelagos et al. 2009; Boldsen 2007; Temple 2014).

Analysis of the effects of early childhood stress on skeletal populations complements studies of living populations. Historical and archaeological skeletal populations expand both the time depth and breadth of human variation that is available for analysis. Skeletal remains contain information about health, disease, and stress across the lifespan. Bioarchaeological approaches also are useful because analyses can be completed within a matter of years instead of decades as with longitudinal studies.

The purpose of this chapter is to review the history of the DOHaD hypothesis and life history theory, and to demonstrate the potential of bioarchaeological research to address this hypothesis. The DOHaD hypothesis directly links early childhood stress to adult mortality outcomes, and therefore is the main interpretive framework for this dissertation. This chapter

outlines the evidence for a link between early childhood stress and adult mortality outcomes by reviewing the malnutrition-infection complex, the DOHaD hypothesis, and life history theory. Since human skeletal remains are analyzed in this dissertation, this chapter justifies the use of archaeologically-derived skeletal material by reviewing the literature on the DOHaD hypothesis in biological anthropology and addressing the application of bioarchaeological approaches to study the DOHaD hypothesis, the predictive-adaptive hypothesis, and plasticity/constraint hypothesis.

3.2 Nutrition and immunity

The relationship between nutrition and immunity has been studied extensively since the 1960s, and much of this research has focused on the synergistic relationship between nutrition and immunity, dubbed the 'malnutrition-infection complex' (Chandra, 1983; Chandra, 1993; Keusch, 2003; Scrimshaw, 2003; Scrimshaw and Sangiovanni, 1997; Scrimshaw et al., 1968). The 'malnutrition-infection complex' describes how dietary habits and the nutritional environment are synergistically related to health and immunity (Black et al., 2008; Chandra, 1993; Keusch and Farthing, 1986; Scrimshaw et al., 1968). The immune system requires specific nutrients to function properly. Insufficient nutrition can reduce the immune system's ability to produce antibodies, decrease macrophage activity, or reduce the inflammatory response, causing an overall reduced ability to respond to foreign invaders (Chandra, 1993; Scrimshaw et al., 1968). In turn, infection and disease can influence the ability of the body to absorb nutrients, making them increasingly more susceptible to future disease.

Protein and iron deficiencies are particularly impactful on the immune system in children (Scrimshaw et al., 1968). With regards to protein deficiency, research shows that effects of this type of nutritional inadequacy include decreased serum immunoglobulin, thymic function,

antibody formation, and interferon (Aref et al., 1970; Chandra et al., 1982; Mugerwa, 1971; Reddy et al., 1977; Watts, 1969). Animal protein is a particularly important source of nutrition as it is rich in vitamin B12, which is required for proper antibody formation and leukocyte production (Scrimshaw and Sangiovanni, 1997). Individuals who have died with signs of *kwashiorkor*, or severe protein deficiency, have reduced thymus weights compared to non-malnourished individuals (Watts, 1969).

Iron is an important nutrient that is required for the proper functioning of many bodily organs and iron deficiency is one of the most widespread nutritional deficiencies in the world today. Iron deficiency impairs immune function by reducing phagocyte activity, reducing response to lymphocyte stimulation, reducing natural killer cell production, and depressing cutaneous hypersensitivity (Scrimshaw and Sangiovanni, 1997). Opportunistic infections can occur more frequently in malnourished individuals, and response to immunization is mediated by nutritional status of the host (Chandra, 1993).

Infections and disease cause nutritional deficiencies because of side effects of infection and increased nutritional requirements to sufficiently fight the bodily invaders. Several studies have demonstrated that individuals experiencing chronic infections lose weight while experiencing the disease, and take several weeks to return to their pre-infection weight (Scrimshaw, 1991). Nitrogen loss is also observed among individuals suffering from disease due to catabolism of tissues to produce sufficient energy to fight the infection (Keusch and Farthing, 1986; Scrimshaw, 1991). Intestinal infections also have been linked to reduced protein and fat absorption and increased morbidity and mortality (Scrimshaw, 1991).

3.3 Life history theory

Life history theory is a theoretical framework used to interpret differences among species in terms of the timing of major life events (e.g. sexual maturity; menopause) and the allocation of energy during these stages (e.g. growth of body systems, reproduction and fertility) (Bogin, 2009; Hawkes et al., 1998; Kaplan, 2000). Life history theory emerged from evolutionary ecology and demography. The analysis of life history traits allows ecologists to understand the evolutionary adaptations of organisms in each environment. Life history theory is a relevant framework for anthropological research because the human species has an unusually longer life history compared to most mammals (Bogin, 2009; Hawkes et al., 1998). Life history theory has been used to study differences between humans and non-human primates, to explain the longevity of humans, to estimate the life cycles of extinct primate and hominin species, and to explain demographic transitions, among other research topics (Charnov, 1991; Hawkes et al., 1998; Kaplan, 2000; Popkin, 1994).

Life history theory assumes that energy throughout one's lifespan is a finite resource, and that excessive investment in one period of life can restrict the energy available in later stages of life (Bogin et al., 2007; Charnov, 1991; McDade, 2003; Worthman and Kuzara, 2005). Generally, energy can be invested in growth, maintenance, or reproduction, and excessive investment of energy into one of these stages over the other produces a trade-off. Trade-offs are an important theoretical concept in life history because they can affect the life course of organisms. For example, intensive energy investment in early childhood may allow an organism to survive to adulthood but could affect the quantity or quality of offspring produced by the organism (Charnov, 1991). Excessive energetic investment in growth, maintenance, or reproduction comes at the cost of the ability to invest in these traits later in life.

This approach is derived from the research of E. L. Charnov, who observed that the relationship between certain history traits (average adult life span, age-at-first reproduction, etc.) are invariant among species of the same taxonomic category (mammals, birds, and fish), meaning that these relationships do not change even as the numeric value of the traits themselves change (Charnov, 1991; Charnov, 2002). Charnov proposed using those invariant relationships that are also dimensionless to classify life histories. Since these dimensionless relationships are invariants across species, Charnov hypothesized that dimensionless numbers that characterize trade-offs are also invariants across species. He postulated that trade-offs vary between taxa more than they vary within taxa, and trade-offs, therefore, could be used to estimate the plasticity of life histories among modern and ancient human populations (Charnov, 2002; Hawkes, 2006). Life history theory, particularly the concepts of trade-offs and limited energy stores, is a foundational theoretical framework for the DOHaD hypothesis.

3.4 Evidence for the DOHaD hypothesis

The DOHaD hypothesis is a culmination of 40 years of research into the effects of early childhood stress on adult health and disease. The DOHaD hypothesis posits that *in utero* and early childhood stress is a predisposing factor for early mortality, primarily from metabolic disease (Barker, 1997; Gluckman et al., 2005a; Kuzawa, 2005; Worthman and Kuzara, 2005). The DOHaD hypothesis first emerged from the work of Barker and Osmond in the early 1980's. Barker and Osmond's (1986) first study analyzes census records from the early 1920s and 1970s and finds a correlation with low birth weight and cardiovascular disease mortality. Early childhood and *in utero* stress has been linked subsequently to increased blood pressure (Barker et al., 1990; Barker et al., 1989a; Campbell et al., 1996; Law et al., 1991), glucose tolerance (Desai et al., 1995; Hales and Barker, 1992; Hales and Barker, 2001; Hales et al., 1991), and

cardiovascular disease (Barker et al., 1989a; Barker et al., 1989b; Barker et al., 1993; Osmond et al., 1993).

Due to its association with cardiovascular disease, the effect of *in utero* and early childhood stress on hypertension in adulthood became a focus of many studies after the late 80's. Barker et al. (1989) compared rates of hypertension from a 1970 cohort and 1949 cohort to their birth records, which included data on blood pressure, mother's weight, etc. They found that in areas where cardiovascular disease mortality is higher, 10-year-olds tended to be shorter and have higher pulse rates than those living in other areas and their mothers also tended to be shorter and have high diastolic blood pressure. Barker et al. (1990) analyzed the systolic and diastolic blood pressure of 449 men and women born between 1935 - 1943 at age 46-54 and found that blood pressure was strongly related to placental weight and birth weight. These findings were independent of high body mass and alcohol consumption. Law et al. (1991) analyzed children's systolic blood pressure at age 4 and found that systolic blood pressure at age 4 was inversely related to birth weight, and positively related to placental weight. Their sample included 405 mothers and children (205 boys and 200 girls). Their results are independent of gestation and imply an association with fetal growth. These studies demonstrate that there is a correlation with rates of hypertension in adulthood, or even later in childhood, and birth weight, placental weight, and mother's blood pressure.

Multiple research studies published during the early 90's though the turn of the century found significant correlations between *in utero* and early childhood stress, and the progression of type II diabetes in adulthood. Hales et al. (1991) analyze the glucose tolerance of 845 men born in Hertfordshire from 1911 onwards. The census information for those individuals included birth weight and weight at 1 year of age. They collected the fasting plasma glucose, insulin and

proinsulin concentrations of these individuals at age 64 and found that those with impaired glucose tolerance had lower birth weights and a lower weight at 1 year than those without impaired glucose. These trends were independent of current body mass. Hales and Barker (1992; 2001) found that *in utero* and early childhood stress is related to type II diabetes in adulthood. Earlier research into the causes of type II diabetes found that adults who developed type II diabetes in later life were more likely to have had mothers who were overweight (Neel, 1962). The 'thrifty genotype hypothesis' emerged from this research, and posited that overweight mothers had genes that coded for more 'thrifty' metabolisms, which were more insulin resistant and stored fat more efficiently. Hales and Barker (1992) posited the 'thrifty phenotype hypothesis', which exchanges genes for epigenetic 'programming' of the fetus as the mechanism for the increased insulin resistance among children of overweight mothers. Under this hypothesis, the overweight mother's physiology pre-programmed the fetus to be more insensitive to insulin, which led to the development of type II diabetes in later life. Hales and Barker justify their hypothesis with several observations: 1. Low-birth weight is associated with the development of type II diabetes in adulthood, which implies that poor maternal nutrition is influential in the development of type II diabetes; and 2. Gestational diabetes occurrence does not explain why low-birth-weight infants are more likely to develop type-II diabetes in adulthood. Hales and Barker (1992) hypothesize that insufficient amino acids *in utero* and in early infancy could lead to reduced production or function of beta cells, which are required to process glucose in the bloodstream. McCance et al. (1994) analyzed the relationship between birth weight and type-II diabetes in adulthood among Pima Indians from the Gila River Indian community in Arizona. They found a U-shaped relationship between birth weight and type-II diabetes in adulthood, such that the lowest and highest birth weight infants were most at risk for

developing type-II diabetes as adults (McCance et al., 1994). When they removed the infants whose mothers experienced gestational diabetes during pregnancy, they found that infants with low birth weights were most likely to develop diabetes as adults. They suggest that, considering the high rates of infant mortality among low-birth weight infants, a genetic predisposition to insulin resistance is an adaptive survival strategy for low-birth weight infants.

Many of the early studies on the DOHaD hypothesis focused on discerning the relationship between cardiovascular disease and biological demographics (weight, placental weight, etc.) at birth. Barker and Osmond (1989) compared historic records of neonatal deaths and post-neonatal deaths from 1911 – 1925 in London to death certificates in England and Wales between 1968 – 1978. They found that mortality from stroke, high blood pressure, bronchitis, and ischemic heart disease correlate with high rates of post-natal and neonatal mortality between 1911 – 1925. They suggested that poor intrauterine environments may result in failure of long-term 'programming' of lipid metabolism, resulting in higher risk of developing metabolic disorder in adulthood. Barker et al. (1993) followed a group of 1586 men born between 1907 – 1924 at Jessop Hospital. They compared data collected at birth (weight, head circumferences, etc.) to their standardized mortality ratios (SMRs) for cardiovascular disease, and found that SMRs for cardiovascular disease were lower among those who weighted 5.5 lbs. or less at birth (SMR = 119) compared to those who weighed more than 8.5 lbs. at birth (SMR = 74) (Barker et al., 1993). They found no correlation with cardiovascular disease and gestation length. They suggested that this correlation may be due to reduced fetal growth *in utero*, which led to physiological changes (impaired glucose tolerance, raised plasma fibrinogen and factor VII concentrations, etc.) that predisposed these individuals to cardiovascular disease in adulthood. Osmond et al. (1993) analyzed the SMRs for cardiovascular disease of 5585 women and 10141 men born between

1911 – 1930. They found that the SMR for cardiovascular disease in women (70) and men (77) were similar, and that birth weight had a similar negative correlation with SMRs for cardiovascular disease in both men and women. SMRs for cardiovascular disease fell with increasing birth weight among both men and women. This study is the first to demonstrate that early growth may be linked to cardiovascular disease mortality among women. Osmond et al. (1993) asserted that the results of this study and previous studies indicate that 'programming' occurs in early life and growth disruptions in early life predispose individuals to cardiovascular disease in adulthood. Fetal 'programming' is justified by 5 observations: first, associations between early childhood stress and cardiovascular disease have been observed in multiple different populations; second, these associations are independent of social class at birth, social class as an adult, cigarette smoking, obesity, and alcohol consumption; third, birth weight is independent of social class at birth or in adulthood among the Hertfordshire cohort; fourth, the association between cardiovascular disease and birth weight is specific to cardiovascular disease; fifth, this correlation is strong and graded. They posit that undernourished babies have disturbed glucose-insulin metabolism, which leads to insulin resistance in adulthood (Osmond et al., 1993).

More recent research on the DOHaD hypothesis analyzes intergenerational increased risk of metabolic disease and epigenetic effects. Recent studies into intergenerational effects assume that the intra-uterine environment provides environmental signaling, which will alter the epigenome of the fetus to be more adapted to the current environment (Kuzawa, 2005; Kuzawa, 2008). Environmental signaling in early life may have benefits in later life if the fetus is able to physiologically adjust to the external environment *in utero*. However, the correlation with birthweight and metabolic problems suggests that the flow of nutrients between fetus and mother provides a signal of the nutritional experience over several generations. The intergenerational

effect of epigenetic information tempers the ability of the fetus to adjust to abrupt changes in the environment by prioritizing the collective environments experienced by recent female ancestors (Kuzawa, 2005). The tempering of the current environmental signal by intergenerational epigenetic effects is known as 'intergenerational phenotypic inertia' (Kuzawa, 2005; Kuzawa, 2008). Intergenerational phenotypic inertia is hypothesized to act as an evolutionary force on a slightly faster timescale compared to natural selection. Intergenerational phenotypic inertia is adaptive because it minimizes growth response to short-term fluctuations in environment, and can facilitate adaptation to gradual environmental changes (Kuzawa, 2005). Considering humans have unusually long life expectancies compared to many mammals, possessing genetic and epigenetic markers that prioritize the gradual changes in environments instead of seasonal or novel environmental shifts would be evolutionary advantageous for humans (Kuzawa, 2008).

Though evidence for the DOHaD hypothesis is substantial, researchers have critiqued the DOHaD hypothesis for a variety of reasons. Though much research exists to support the claim that early childhood experiences influence metabolic disease risk, much of this research is based on the claim that external environmental signaling occurs *in utero* via a currently unknown mechanism (Kuzawa, 2005). Epigenetic modifications to the genome are heritable, but these types of modifications occur during gamete formation, long before conception, much less after the embryo has begun to divide. The mechanisms or pathways for transmission of epigenetic information after the first cell division of an embryo are not currently known. De Boo and Harding (2006) provide a systematic critique the DOHaD hypothesis. They state that many of the studies linking birth weight to metabolic disease risk have sampling bias because the sample sizes are too small, and that studies with smaller sample size showed a stronger link between birth weight and metabolic disease mortality compared to studies with larger sample sizes.

Additionally, studies that analyze cardiovascular disease risk in twins find that these individuals do not have a higher risk of disease compared to a general population (De Boo and Harding, 2006). Twins would theoretically have a higher risk of cardiovascular disease compared to the general populace because twins weigh less at birth compared to singleton babies. Low birth weight may also be influenced by gestational length rather than fetal growth. Premature babies tend to have an increased risk of several metabolic disorders, including cardiovascular disease, hypertension, and insulin resistance, and many of the earlier studies of the DOHaD hypothesis have limited data on gestational length (De Boo and Harding, 2006).

3.5 Critical windows of developmental plasticity

A critical window of developmental plasticity is defined as "a period of important structural and functional changes during normal organ development when exposure to certain environmental changes may initiate life-time consequences" (Aguayo-Mazzucato et al., 2006). In developmental biology and psychology, research investigating critical period of developmental plasticity have found time-specific critical windows for brain and language development. Research into critical windows for organs associated with metabolic disorder, such as the pancreas or the thymus, has yet to find time-specific critical periods for these organs in humans for several specific reasons: 1. Psychological and neurological disorders, in many cases, are more noticeable and manifest quickly compared to the subtle changes in physiology that produce metabolic disorder; 2. Research investigating critical windows for metabolism-related organs typically uses animal models to avoid ethical concerns related to human testing and costly longitudinal studies.

Research on the DOHaD hypothesis provides an indirect measurement of the critical windows associated with metabolic disease through the use of retrospective studies (Vickers,

2011). Though much of the research using the DOHaD hypothesis focuses on *in utero* stressors by using birth-related measurements (Armelagos et al., 2009; Barker and Osmond, 1986; Barker et al., 1990; Barker et al., 1989b; Barker et al., 1993; Law et al., 1991; McCance et al., 1994; Moore et al., 1999; Moore et al., 1997; Osmond et al., 1993), several other studies find an association between post-natal metrics and adult mortality (Barker et al., 1989b; Boldsen, 2007; DeWitte, 2012; DeWitte and Wood, 2008; Hales et al., 1991; McDade et al., 2001a; McDade et al., 2001b; Temple et al., 2012; Watts, 1969). Research using animal models also suggests that there are post-natal critical windows of development for the pancreas (Aguayo-Mazzucato et al., 2006), but the extent to which these windows map on to humans is currently unknown.

3.6 Mechanisms for developmental plasticity

Over the past several decades, research has focused on elucidating the mechanisms behind the correlation between adult metabolic disorders and early childhood stress. The hypothalamic-pituitary-adrenal axis (HPA axis) regulates multiple bodily processes including immune responses, energy storage, food intake and body weight (Dallman et al., 1993; Sapolsky, 1998; Tempel and Leibowitz, 1994). The HPA axis regulates the action of cortisol, a hormone associated with the body's stress response and directly affects food intake and body weight in addition to several other bodily systems (Sapolsky, 1998). Increasing the exposure of infants to glucocorticoids is hypothesized to permanently reduce the number of glucocorticoid receptors in the hypothalamus, causing a permanent upregulation of the HPA axis that leads to metabolic syndrome later in life (Seckl, 2004).

Epigenetic inheritance systems are another promising mechanism to explain the correlation between poor nutritional environments *in utero* or early childhood and metabolic disorder in adulthood. Epigenetics is the inheritance of non-genetic information from parent to offspring,

and specifically non-genetic mechanisms that control the expression of genes. DNA methylation, chromatin marking, and small interfering RNAs (siRNAs) are some examples of known epigenetic mechanisms (Jablonka, 2005). DNA methylation occurs when a methyl group (CH_3) is added to a nucleotide, usually a cytosine. The methylated state of a nucleotide usually interferes with the transcription process, reducing the production of the coded protein. Methylated DNA transfers across generations because DNA replication is semi-conservative and leaves the original methylated parent strand of DNA intact during gamete formation. Gene expression can also be regulated by the action of histones. Histones are proteins around which DNA is wrapped during replication. Histones allow for the DNA to be tightly packed into chromosomes for meiosis or mitosis. Four histones with tightly wrapped DNA form a nucleosome, and nucleosomes can twist around one another to form chromatin fiber. DNA that is packed into chromatin fiber cannot undergo transcription, and thus the expression of genes is regulated by chromatin. Small interference RNAs (siRNAs) are short sequences of RNA that inhibit gene expression after transcription. Small interference RNAs are manufactured as normal mRNA sequences but are cut into siRNAs by the enzyme Dicer prior to translation. These siRNAs then bind to an RNA-induced silencing complex (RISC), which binds to and destroys complementary mRNA. This process effectively silences genes by preventing translation of mRNA sequences. Epigenetic mechanisms also explain why many metabolic diseases appear in multiple generations within families. DNA methylation and chromatin marking are epigenetic mechanisms passed down through multiple generations, affecting the risk of disease despite environmental change (Kuzawa, 2008; Kuzawa and Thayer, 2011).

3.7 The plasticity/constraint and predictive-adaptive hypotheses

Two hypotheses have emerged from the DOHaD and life history literature that predict different mortality outcomes associated with early childhood stress. The plasticity/constraint hypothesis, which posits that early childhood stressors produce trade-offs such that stressed individuals will invest their energy in survival in the short term at the expense of reduced energetic ability to adapt to stressors later-in-life (Bogin et al., 2007; Temple, 2014; Worthman and Kuzara, 2005). The plasticity/constraint hypothesis is derived from life history theory, which posits that there is a limited lifetime energy supply to invest in growth, reproduction, or maintenance. Trade-offs occur because of intensive energetic investment in one stage at the expense of investment in later stages. An individual's biological plasticity is constrained by their limited lifetime store of energy, and depletion of this energy in early life results in a reduced capacity for investment later in life.

The predictive-adaptive hypothesis, which stems from the thrifty phenotype hypothesis and fetal programming hypothesis, posits that early childhood stressors will produce a physiological reaction designed to buffer the organism from future stress events (Barker and Osmond, 1986; De Boo and Harding, 2006; Ellison, 2005; Hales and Barker, 2001; Kuzawa and Quinn, 2009). Environments of scarcity in childhood "program" individuals to be adapted to scarce environments in adulthood. If there is a mismatch between a resource-poor childhood and a resource-rich adulthood, metabolic syndromes that would otherwise be advantageous in scarce environments become chronic health problems. Examples of metabolic disease that may be advantageous in resource-poor environments include cardiovascular disease and type II diabetes. Type II diabetes in adulthood manifests physiologically as insulin resistance and glucose intolerance. Researchers have found evidence that malnutrition in early childhood permanently

alters the function of the pancreas, the organ responsible for producing insulin, and results in reduced production of insulin (Hales and Barker, 2001). Alteration to pancreatic function occurs to preserve the growth of more vital organs, such as the brain. A reduced insulin response would not affect an individual that continued to live in a nutritionally poor environment. Increased fat stores and reduced insulin sensitivity among hunter-gatherers would have been evolutionarily advantageous during times of scarcity and the metabolic disorders associated with these traits would only manifest if these individuals lived very long lives (Gluckman et al., 2005b). Insulin-resistance becomes more problematic if the adult environment is calorically dense, resulting in weight gain and metabolic syndrome. Under the predictive-adaptive hypothesis, metabolic syndromes are the product of a mismatch between the *in-utero* environment of scarcity and post-birth environments of nutritional abundance, as often found in modern industrialized societies.

The fundamental difference between the plasticity/constraint and predictive-adaptive hypotheses is that the predictive-adaptive hypothesis assumes a mismatch between the *in utero* and adult nutritional environments (Armelagos et al., 2009). Under the predictive-adaptive hypothesis, early childhood stress due to nutritional scarcity could result in evolutionarily advantageous phenotypic changes in adulthood, if the environment in adulthood remains consistent throughout the lifespan. Only in situations where the adult environment is more nutritionally dense than the *in utero* and early childhood environment do the phenotypic alterations to accommodate the stressful childhood environment result in the development of metabolic syndrome.

This critical difference between the plasticity/constraint and predictive-adaptive hypotheses provides an important opportunity for bioarchaeology to contribute to the conversation about life history theory and the DOHaD hypothesis (Temple, 2019). Since the predictive-adaptive

hypothesis assumes a mismatch between early childhood environment and adult environment, research attempting to compare these hypotheses should seek out populations where the environment is relatively constant. Under constant environmental conditions, the predictive-adaptive hypothesis should predict a longer lifespan for those who experienced stress in early childhood compared to those who did not experience early childhood stress because these physiological alternations would be advantageous in adulthood. The plasticity/constraint hypothesis, in contrast, would predict higher rates of mortality and lower age-at-deaths for individuals who experienced early childhood stress. Studies using these theories have traditionally focused on living populations. However, living populations can be problematic for studying these models because of the spread of globalization and diffusion of the western diet throughout the world. Western foods tend to be inexpensive and high in calories, and the spread of the western diet can cause a mismatch between early childhood environment and adult nutritional environment, particularly among marginalized groups with inconsistent access to food.

Bioarchaeological research has the potential to significantly contribute to the literature on the plasticity/constraint and predictive-adaptive hypotheses. Human bone and dentition contain a variety of information about health throughout the lifespan. Stable carbon and nitrogen isotope analysis of bone and dentin can be used to construct dietary patterns from multiple periods during life, which can be used to understanding how dietary patterns throughout one's life can influence mortality risk in adulthood (Beaumont et al., 2015). Bioarchaeological approaches can provide both time-depth and appropriate cultural context to test theories that require specific environmental conditions, such as an environment of constant scarcity in the case of the predictive-adaptive hypothesis.

3.8 The fetal origins of infectious disease mortality

Though most research on the DOHaD hypothesis has focused on the relationship between early childhood stress and adult metabolic disorders, decidedly less research has investigated the effects of early childhood stress on susceptibility to infectious disease. Approaches to infectious disease mortality using the DOHaD hypothesis have focused on the effects of early childhood stress on immune system function (McDade et al., 2001a; McDade et al., 2001b). Though much of this research finds a significant relationship between early childhood stress and infectious disease mortality, not all studies conclusively demonstrate this relationship.

The effects of *in utero* and early childhood stress on immune function have previously been studied in living human populations (McDade et al., 2001a; McDade et al., 2001b; Moore et al., 1999; Moore et al., 1997; Moore et al., 2001). Moore et al. (1997, 1999) investigated the relationship between birth season (dry or wet) and early mortality in Rural Gambia, and found that among individuals who lived to age 15, those that were born during the dry season and consequently experienced more pre- and post-natal nutritional stress are more likely to die early in life, particularly from infectious diseases. They hypothesize that early childhood stress can influence development of the immune system such that stressed children are more likely to be susceptible to infectious disease as adults. However, a later study found no difference in immune function between children born in the dry and wet season (Moore et al., 2001). McDade et al. (2001a) analyzes the immunocompetence of 103 Filipino adolescents between the ages of 14 to 15 and found that individuals who experienced nutritional inadequacy in early childhood exhibited lower probabilities of mounting an antibody response to the typhoid vaccine compared to those with adequate nutrition in early childhood. McDade et al. (2001b) analyzes the relationship between thymic function and early childhood undernutrition and find that prenatal

undernutrition was significantly associated with reduced thymopoietin production. Thymopoietin affects early T-cell differentiation and regulates the function of mature T-cells. This research demonstrates the existence of a positive relationship between early childhood stress and adult infectious disease susceptibility, but further research is needed to understand this relationship.

3.9 Bioarchaeological approaches to the DOHaD hypothesis

Researchers increasingly use bioarchaeology to study human life history (Armelagos et al., 2009; Blevins, 2015; Boldsen, 2007; DeWitte and Hughes-Morey, 2012; DeWitte and Wood, 2008; Gowland, 2015; Lea et al., 2017; Reitsema et al., 2016; Temple, 2014; Temple, 2019). During the annual meeting of the American Association of Physical Anthropologists in 2016, researchers were invited to present in a podium session entitled "Early-life stress in the past: bioarchaeological approaches to the evolution, ecology, and cultural contingences of human life history". This session focused on how bioarchaeology can contribute to the discussion of the life-long effects of early childhood stress. These publications and presentations demonstrate an increasing interest among bioarchaeologists to engage with the DOHaD hypothesis, though the results of these studies may not always be consistent.

Boldsen (2007) analyzes linear enamel hypoplasia (LEH) prevalence between adults and children from the medieval village of Tirup, Denmark. He finds a statistically significant relationship between LEH formation and mortality in adults. However, he finds a declining risk of death associated with increasing prevalence of LEH among children between the ages of 2/3 – 5 years. He hypothesizes that the inverse relationship between LEH and risk of death among young children may be a consequence of maturation: children who died early in life may not show signs of a health episode because they died too soon. Children who survived a health

episode developed LEH as they matured. The results from Boldsen (2007) demonstrate a causal, but indirect, relationship between early childhood stress and adult mortality.

DeWitte and Wood (2008) analyze human remains from East Smithfield, a 14th-century Black Death cemetery, and Denmark, a non-epidemic cemetery, to determine whether the Black Death was an indiscriminate killer or was more likely to be fatal to individuals who were already unhealthy. They find significant mortality differentials between the two cemeteries, indicating that the Black Death was highly selective for individuals who had a history of health issues. In another study, DeWitte and Hughes-Morley (2012) analyze the East Smithfield Cemetery, and a comparative collection to determine if stature is associated with increased risk of mortality. Using hazard analysis, they find that shorter individuals were more susceptible to the black death and hypothesize that shorter individuals had innately compromised immune systems and shorter individuals experienced malnutrition during development, leading to permanent stunting and susceptibility to disease.

Armelagos and Goodman (2009) analyze several previous studies to assess whether archaeological evidence exists for the DOHaD hypothesis. They find a positive correlation between linear enamel hypoplasias (LEHs) and age-at-death and conclude that the earlier age-at-death experienced by individuals with a high frequency of LEHs is confirmation of the DOHaD hypothesis in archaeological contexts. Beaumont et al. (2015) use incremental stable isotope analysis to examine the intergenerational underpinnings of childhood mortality, finding that subadults from modern and historic contexts who died before tooth formation was complete were born to mothers who were likely nutritionally deprived during pregnancy. Beaumont et al. (2015) demonstrate that stress during pregnancy can result in a higher mortality risk after birth. Both articles have one main research design flaw for testing the DOHaD hypothesis; the authors only

analyze subadult teeth, which biases the sample towards only individuals who died in early childhood. Thus, their conclusions may not be representative of the experience of individuals who died in adulthood.

Blevins (2015) uses the DOHaD hypothesis to analyze the relationship between early childhood stress and mortality from infectious disease. She analyzes 250 individuals from the 20th-century Coimbra Identified Skeletal Collection from Coimbra, Portugal for non-specific indicators of stress and stature. She compares individuals with tuberculosis as the documented cause of death to those with a non-infectious cause of death and finds no statistically significant differences in growth and stress. Her research indicates that tuberculosis mortality was not affected by early childhood stress.

Temple (2014) used bioarchaeological methods to test the validity of the plasticity/constraint and predictive-adaptive hypotheses. Temple (2014) analyzes the perikymata depth and occurrence of LEHs among Late/Final Jomon period foragers (4000 to 2300 BP), and finds a statistically significant positive correlation between age-at-first defect formation and age-at-death. Investment in survival during early childhood decreased survival time at other later-in-life, which supports the plasticity/constraint hypothesis. However, analyzing perikymata depth requires well-preserved, unworn teeth, which biases the sample towards only those individuals who died in childhood and early adulthood. The oldest individual from this study had an estimated age-at-death of 24.9 years. Though Temple (2014) demonstrates that early childhood stressors cause increased risk of mortality in adulthood, the bias in their sample and sample size (32 individuals) demonstrates a need for further investigation of the DOHaD hypothesis using bioarchaeological methods.

Reitsema et al. (2016) analyze dietary and weaning differences between "subadults who lived" (adults) versus "subadults who died" (subadults) from a skeletal collection from medieval Trino Vercellese, Italy. They analyze carbon and nitrogen isotope signatures from bone collagen of 41 subadults who died between the ages 6 to 14.5 years old. Reitsema et al. (2016) find that the average age-at-weaning for the studied population was approximately 4 years of age, with considerable variation in weaning overall. Individuals under the age of 4 years old show high variation in nitrogen isotope values, which the authors attribute to their premature mortality. They find that "subadults who died" appear to have had diets comprised of less animal protein compared to "subadults that lived", suggesting that animal protein may have contributed to a medieval subadult's ability to survive to adulthood.

3.10 Conclusion

This chapter reviews the relevant literature associated with the DOHaD hypothesis, specifically focusing on how the DOHaD hypotheses informs the plasticity/constraint and predictive-adaptive hypotheses. This chapter also addresses how bioarchaeological research has contribute to elucidating the relationship between *in utero*/early childhood stress and adult mortality outcomes.

Though the DOHaD hypothesis emerged almost 40 years ago, research into this phenomena are ongoing in both living and skeletal human populations. Though the effect of early childhood stress has been observed in living and deceased populations, some researchers have found no association between early childhood stress and adult mortality. Furthermore, though some research exists to suggest that early childhood stress affects immune function and, theoretically, adult infectious disease mortality, the link between infectious disease mortality and early childhood stress is understudied at the time of this dissertation. This dissertation research

contributes to this body of literature by investigating the relationship between early childhood stress and infectious disease mortality.

Chapter 4 provides a justification for the methodological techniques utilized in this dissertation by reviewing the methodological approaches in bioarchaeology that are used to study the DOHaD hypothesis in skeletal assemblages.

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CHAPTER 4

LITERATURE REVIEW: METHODOLOGICAL APPROACHES TO THE DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE IN BIOARCHAEOLOGY

4.1 Introduction

As discussed in Chapter 2, the developmental origins of health and disease (DOHaD) hypothesizes that *in utero* and early childhood stress are contributing factors to the development and progression of adult metabolic disorders (Barker and Osmond, 1986; Barker, 2004). The DOHaD hypothesis has been studied over the past 40 years in biological anthropology and epidemiology (Barker and Osmond, 1986; Barker, 1995; Barker, 1997; Barker, 2004; Barker et al., 1990; Barker et al., 1992; De Boo and Harding, 2006; Hales and Barker, 2001; Moore et al., 1997; Prentice and Moore, 2005). More recently, DOHaD hypothesis has become a topic of interest in bioarchaeology, the study of human remains in archaeological contexts (Armelagos et al., 2009; Blevins, 2015; DeWitte and Hughes-Morey, 2012; DeWitte and Wood, 2008; Temple, 2014). Bioarchaeological research has the potential to provide archaeological evidence for the DOHaD hypothesis that includes a more culturally and genetically diverse sampling of humanity than studies of living humans alone (Larsen, 2015).

In addition to their advantages, methodological approaches in bioarchaeology have limitations that also must be considered when conducting research using the DOHaD hypothesis (Temple, 2019). This paper will review the methodological approaches in bioarchaeology that are applicable to understanding the DOHaD hypothesis in archaeological populations. The purpose of this chapter is to provide relevant background information about the methodological

techniques utilized in this dissertation. First, I provide a brief overview of bone growth and plasticity to demonstrate how osseous remains are influenced by life history events. I then provide a brief review of the paleopathological markers analyzed in this dissertation. Finally, I discuss the theoretical underpinnings of stable carbon and nitrogen isotope analysis to reconstruct early childhood diet and weaning. Because this research addresses heterogeneity in frailty, I also provide a brief review of the osteological paradox (Wood et al., 1992). The osteological paradox is an important critique of paleopathological analysis that has shaped the field of bioarchaeology (Armelagos and Van Gerven, 2003; Byers, 1994; Cohen, 1994; DeWitte and Stojanowski, 2015; Goodman, 1993; Soltysiak, 2015; Wilson, 2014; Wood and Milner, 1994; Wright, 2003). Researchers have suggested that investigating how frailty is related to death is a useful approach to address the paradox in bioarchaeological research, which is a focus of this dissertation (DeWitte and Stojanowski, 2015; Wood et al., 1992; Wright, 2003).

4.1.1 Bone Structure and Plasticity

Bone is a dynamic tissue in the body that changes and remodels in response to physical injury, mechanical stressors, nutritional stressors, and infectious disease throughout one's life (Jurmain et al., 2012; Roberts, 2011; Ubelaker, 1999; Waldron, 2009; White and Folkens, 2012). Bone has three primary functions in the body (White and Folkens, 2012). First, the medullary cavity within bones is the main site of production for red and white blood cells Second, bone serves as a reservoir for fat and mineral storage, including calcium. When calcium is needed elsewhere in the body, specialized bone cells can dissolve parts of the mineral component of bone to release calcium into the blood stream. Third, bones provide a mechanical framework that supports muscles, tendons, and ligaments and allows for bodily movement. As an essential component of mechanical movement, bone must be rigid enough to support and protect tissue

and organs, while simultaneously being malleable enough to not break under the mechanical forces imposed by movement and gravity.

Bone is a composite material composed of the inorganic mineral (55-75%) embedded in the organic protein matrix composed primarily of collagen (90%) (Schwarcz and Schoeninger, 1991). The remaining 10% of the organic component of bone is composed of non-collagen proteins (~5%) and lipids and carbohydrates (~5%) (Schwarcz and Schoeninger, 1991). The inorganic component of bone is composed of a crystalline lattice of carbonate-hydroxyl apatite of various compositions, the majority of which is composed of hydroxyapatite.

There are three main types of bone: trabecular (cancellous), cortical, and woven. Woven bone is a temporary, coarsely bundled bone structure found only in newborns and in rapidly healing regions of the skeleton if subjected to trauma (Currey, 2006). Woven bone is gradually replaced with cortical and trabecular bone early in ontogenetic growth (White and Folkens, 2012). Cortical bone and trabecular bone are the mature forms of bone. Cortical bone is the compact and dense bone that covers the surface of all bones (Currey, 2006). Cortical bone is characterized by the presence of Haversian systems, which allow for the penetration of blood and nerves into the bone structure (White and Folkens, 2012). Trabecular bone is composed of thin, bony projections called trabeculae. This spongy type of bone provides support while allowing bone to be lightweight. Additionally, trabecular bone is generally found intersecting red or yellow blood marrow, where red blood cells are produced, or fat is stored. Unlike cortical bone, trabecular bone lacks Haversian systems (White and Folkens, 2012).

Modeling of bones occurs when the bones initially form, whereas remodeling occurs when the bones are re-shaped in response to environmental or mechanical stressors. Bones are modeled through either endochondrial ossification or intramembraneous ossification (Shapiro,

2008). Endochondrial ossification occurs when bones form on a cartilaginous precursor, as with long bones (Shapiro, 2008). Intramembraneous ossification occurs when bone grows within an embryonic connective tissue membrane (Shapiro, 2008). Flat bones, such as cranial bones, grow through intramembraneous ossification. Both bone modeling and remodeling occurs because of the action of specialized cells, specifically osteoblasts and osteoclasts (White and Folkens, 2012). Osteoblasts are responsible for the formation of new bone mineral. They lay down a bone precursor called osteoid that becomes mineralized after deposition. Osteoclasts, on the other hand, are responsible for the resorption of bone mineral. Osteoclastic activity can be triggered due to mechanical stressors or to release important mineral elements stored in bone, including calcium.

Bone remodeling occurs throughout one's lifetime. During ontogeny, woven bone is laid down through either intramembranous ossification or endochondral ossification (White and Folkens, 2012). Most cranial bones are formed by intramembranous ossification, where bone is appositionally ossified between two embryonic connective tissue membranes (White and Folkens, 2012). In contrast, most bones in the skeleton are formed through endochondral ossification (White and Folkens, 2012). *In utero*, long bones exist in the body mainly as a cartilaginous precursor. The cartilaginous precursor becomes ossified such that ossification occurs at the diaphysis (primary center of ossification) and expands appositionally towards the epiphyses (secondary centers of ossification) (White and Folkens, 2012). The growth plate between the metaphysis and the epiphysis allows for the continued growth of the bone throughout childhood (White and Folkens, 2012). When growth is complete, the diaphysis and epiphysis fuse and the growth plate disappears through remodeling.

4.2 Early childhood paleopathology

For the purposes of the DoHaD hypothesis, skeletal markers of pathological conditions in early childhood are of interest because their prevalence can be used to assess the severity and frequency of stress events during childhood (Armelagos et al., 2009; Blevins, 2015; Boldsen, 2007; DeWitte, 2012; DeWitte and Wood, 2008; Temple et al., 2012). When analyzing adult individuals, pathological conditions of adulthood are also of interest because they may be indicative of previous stress events not related to the cause of death that were potentially exacerbated by childhood stress events (Cohen, 1994; Wood and Milner, 1994; Wood et al., 1992). Over the next subsections, I will discuss specific child and adult pathological conditions that leave skeletal lesions that could be applied to the study of the DOHaD hypothesis in bioarchaeology. Specifically, this section provides a literature review of the methodology employed to test hypotheses 6.1 (cholera mortality is associated with a higher prevalence of pathological markers), 6.2 (cholera mortality is associated with growth stunting), and 7.1 (cholera mortality is associated with earlier and more stress events).

4.3 Pathological skeletal lesions of childhood

Though bones remodel throughout the lifespan, some pathological conditions in childhood can leave lasting bony impressions on the skeletal remains of adults (Armelagos et al., 2009; Boldsen, 2007; Goodman and Rose, 1990; Goodman and Rose, 1991). This section will discuss several pathological markers that can be used to assess the childhood health of adult skeletal remains, including pathological markers of anemia, nutritional insufficiency, growth stunting, and dental pathologies. This section will also cover skeletal lesions associated with adult health, or health near the time of death, that can be used to assess the susceptibility of adults to stress or infection other than the main cause of death.

4.3.1 Indicators of anemia

Haemolytic anemia as a result of diet or disease can manifest on the skeleton as porotic lesions on various parts of the body, particularly the thin bones of the cranium (Waldron, 2009). Haemolytic anemia causes blood cells in the body to be destroyed, which results in an increased demand for blood cell production on the body. Rapid turnover of red blood cells can cause expansion of the haemopoietic bone marrow throughout the body (Waldron, 2009). Larger bones, such as the vertebrae or the long bones, may not typically manifest porotic lesions due to bone marrow expansion, but the thin bones of the cranium are more affected by the expansion. Thin bones are more likely to show pathological porosity because of haemolytic anemia. For example, sickle cell anemia and thalassemia are examples of haemolytic anemias caused by genetic disorders (Waldron, 2009).

Iron-deficiency is another cause of anemia that potentially results in porotic lesions (McIlvaine, 2015; Stuart-Macadam, 1992; Walker et al., 2009). Dietary iron deficiency is a generally accepted cause of porotic lesions related to anemia, though this interpretation is controversial (McIlvaine, 2015; Oxenham and Cavill, 2010; Stuart-Macadam, 1992; Walker et al., 2009). Iron deficiency anemia is arguably not associated with reduced red blood cell count and thus no hyperplasia of the marrow cavity occurs that results in porotic lesions (Walker et al., 2009). However, low iron in the bloodstream could result in increased susceptibility to helminth infections, which can result in reduced blood cell count and marrow hyperplasia (Stuart-Macadam, 1992). Other researchers have argued that hyperplasia of the bone marrow occurs independent of red blood cell count, and instead is a result of defective erythroblast development (Orazi et al., 2006; Oxenham and Cavill, 2010).

Porotic hyperostosis and cribra orbitalia are two paleopathological conditions that indicate anemia or blood disorders in early childhood. Porotic hyperostosis manifests on the parietal bones of the cranium (see Figure 4.1) and cribra orbitalia manifests on the surface of the eye orbits. Both are associated with thinning of the cortical layer of bone and thickening of the diploë within these layers (Stuart-Macadam, 1985; Stuart-Macadam, 1989). Due to the remodeling of the cranial bones throughout life, cribra orbitalia and porotic hyperostosis may have manifested in early life but may not be visible in individuals that died in adulthood. Cribra orbitalia and porotic hyperostosis manifest most frequently in children between the ages of 6 months and 2 years, though young adults may also manifest these lesions if remodeling has not obliterated the lesion (Lallo et al., 1977; Stuart-Macadam, 1989). For this dissertation, cribra orbitalia and porotic hyperostosis are used as indicators of early childhood stress.

4.3.2 Indicators of growth stunting

Stature estimation is a useful metric to estimate the overall health of populations (Haviland, 1967; Steckel, 1995). Adult stature is influenced by genetics and environmental conditions, in addition to overall health, but population-level analyses of stature obscure these individual differences (Roberts and Manchester, 2005; Steckel, 1995). Linear growth stunting occurs because of chronic disease or malnutrition during the formative years of childhood (Golden, 1994). Children that are under significant stress may reduce the energy invested in growing to increase the chances of survival, resulting in below-average height in adulthood. From an evolutionary perspective, an overall smaller body size would be advantageous while in an environment that is deficient in nutrition or with high rates of disease because less energy would be required to maintain a smaller body compared to a larger body (Charnov, 1991; Hawkes, 2006). Growth stunting can result in a permanent reduction in body size, though catch-

up growth in later adolescence may cause some individuals who were malnourished or experiencing periods of disease to grow to their full potential (Golden, 1994).

Stature estimation among skeletal samples is generally done by one of two methods: an anatomical approach that considers multiple indicators of stature and a regression approach that uses linear regression equations to calculate stature based on long bone measurements. The anatomical method was developed in the mid-20th century and recently updated (Fully, 1956; Raxter et al., 2007; Raxter et al., 2008). The anatomical technique produces more accurate stature estimates compared to regression technique but requires complete skeletons. The regression technique only requires the presence of at least one long bone, usually a femur, tibia, or humerus, to estimate stature. Regression equations must be derived from a genetically and temporally similar population to the population of study to ensure accurate estimates of terminal height and control for temporal and genetic trends in stature (Vercellotti et al., 2009). Though less precise than the anatomical methods, the regression method is useful for skeletal populations where not all bones are present.

Regression equations are used in this dissertation because, as stated in Chapter 4, a disarticulated skeletal collection from Alia is used in this analysis. Since the skeletal collection from Alia is disarticulated, the anatomical method for stature estimation is not possible for this collection. To keep the methodology for stature estimation consistent across the sites, a regression equation is used to estimate and compare stature between the sites.

4.3.3 Dental pathologies

Though bone remodels constantly throughout the lifespan, teeth are not subject to the continuous remodeling that occurs in the rest of the body. Tooth formation begins *in utero* and continues until ~14 years of age. Once formed, tooth enamel does not remodel and disruptions in

enamel growth leave permanent pathological markers that serve as a reservoir of information about stress in early childhood.

Teeth start to form at the cusp of the crown at the enamel-dentin junction (EDJ) and concludes at the apex of the root. Specialized cells called ameloblasts lay down successive, incremental layers of tooth enamel. Enamel is composed of interlocking prism rods that are produced by ameloblasts and grow outwards from the EDJ to the outer surface of the tooth. Prism rods undulate relative to the plane of secretion and regular undulations down the sides of the crown, known as Hunter-Schreger bands (Hillson, 2014b). Ameloblasts produce daily layers of enamel that form microscopic structures known as prism cross-striations (see Figure 4.2) (Goodman and Rose, 1990; Hillson, 2014b). Every 8–10 days, ameloblasts complete the formation of an enamel layer, known as a stria of Retzius, that forms parallel to the EDJ (Goodman and Rose, 1990; Goodman and Rose, 1991; Hillson, 2014b; Schwartz and Dean, 2008). Perikymata form on the external surface of the tooth as ameloblast activity ceases and tooth formation is complete. Perikymata are separated by buttressing on the surface of the tooth as a result of the terminal end of a striae of Retzius.

4.3.3.1 Linear enamel hypoplasias

Hypoplasias are defects that manifest on the surface of teeth where enamel formation was disrupted in response to stress (Hillson, 2014a). Like other bones, tooth growth can be disrupted if the body is under a sufficient level of stress, which can be caused by nutritional, psychological, or traumatic stress events (Goodman and Rose, 1991). Tooth growth disruption results in decreased ameloblast activity, which reduces the length of prism rods and ultimately results in a depression on the external surface of the tooth (Sarnat and Schour, 1941). Because hypoplasias

can be caused by multiple different stressors, hypoplasias are classified as a non-specific pathology.

There are three main types of hypoplasias: furrow-form, plane-form, and pit-form. Furrow-form defects are narrow lines that form on the surface of teeth due to variation in the spacing of perikymata (Hillson, 2014a). Linear enamel hypoplasias (LEHs) are a furrow-form defect that manifests as linear mesial-distal bands of enamel depression on the surface of teeth (see Figure 4.3) (Hillson, 2014a). LEHs can occur during extended periods of stress that range from weeks to months (FitzGerald, 1998; Shellis, 1998). A plane-form defect has a similar appearance as a furrow-form defect, but with incomplete enamel formation on the surface of the tooth such that enamel microstructures known as Tome's process pits are exposed on the tooth's surface (Boyde, 1970; Hillson, 2014a). Plane-form defects form along the plane of a single accentuated striae of Retzius over a wider area than furrow-form defects (Hillson, 2014a). Plane-form defects occur as a result of abrupt cessation of ameloblast activity. Pit-form defects are discontinuous pits that occur on the occlusal surface of teeth. Much like plane-form defects, pit form defects occur along a single accentuated striae of Retzius as a result of sudden cessation of ameloblast activity (Hillson, 2014a). Pit-form defects are distinct from plane-form or furrow-form defects in that exposure of the accentuated striae of Retzius is discontinuous around the crown's surface, whereas plane-form and furrow-form defects are continuous around the circumference of the tooth (Hillson, 2014a).

4.3.3.2 Accentuated striae of Retzius

The microscopic examination of tooth enamel thin-sections, known as dental histology, is a useful technique for estimating periods of stress during childhood (FitzGerald et al., 2006; Goodman and Rose, 1990; Hillson, 2014a; King et al., 2005). Metabolic disruptions during tooth

development manifest as accentuate striae of Retzius, or Wilson bands, which appear as darkened striae of Retzius in cross-section (Hillson, 2014a). Due to the periodicity of enamel formation and the tight genetic regulation of tooth formation, the analysis of histological features of enamel can produce a timeline of stress events early in life. The age at which the individual experienced stress can be estimated by measuring the average width of a normal striae of Retzius, which has a periodicity of 8-10 days, and the length between accentuated striae of Retzius relative to the EDJ. Additionally, prevalence of accentuated striae of Retzius is frequently used to estimate the level of stress experienced during early childhood (Armelagos, 2010; FitzGerald et al., 2006; Rose et al., 1978; Wright, 1990).

4.4 Stable isotope analysis

Stable carbon and nitrogen isotope analysis is a widely-used method in anthropology to reconstruct past life ways, diet, and migration (Beaumont et al., 2013a; Beaumont et al., 2013b; Beaumont and Montgomery, 2016; Beaumont et al., 2015; Fuller et al., 2005; Fuller et al., 2004; Fuller et al., 2003; Henderson et al., 2014; Jay et al., 2008; Katzenberg et al., 1993; Mekota et al., 2006; Nitsch et al., 2011; Petzke et al., 2005; Prowse et al., 2008; Reitsema, 2013; Reitsema et al., 2013; Reitsema and Vercellotti, 2012; Reitsema et al., 2016b; Richards et al., 2002; Sandberg et al., 2014; Tafuri et al., 2009; Tsutaya and Yoneda, 2015). Stable carbon isotope ratios from bone collagen chiefly reflect the photosynthetic pathway (C_3, C_4 or CAM) of plants consumed, but also overrepresent dietary protein sources (Ambrose and Norr, 1993; O'Leary and O'Leary, 1988; Tieszen and Fagre, 1993). Nitrogen isotopes chiefly reflect the trophic position of dietary protein sources (Macko et al., 1986; Minagawa and Wada, 1984). Carbon and nitrogen isotope analysis also can be used to reconstruct onset-of-weaning and age-at-weaning, respectively (Beaumont et al., 2014; Beaumont et al., 2015; Eerkens et al., 2011; Fogel et al.,

1989; Fuller et al., 2006; Fuller et al., 2003; Richards et al., 2002; Sandberg et al., 2014). This section will review how stable carbon and nitrogen isotopes are used in bioarchaeology and provide justification for the methodology to test hypothesis 5.1 (cholera mortality is associated later weaning completion).

Weaning is a particularly stressful period in early childhood; weaning too early or too late can be detrimental to growth and development (Deoni et al., 2013; Horta et al., 2013; Rice and Barone, 2000). For these reasons, the reconstruction of weaning patterns in the past is particularly applicable to understanding early childhood stress in ancient populations. The next section will review the theory and application of stable isotope analysis of carbon, nitrogen, and oxygen isotopes to the reconstruction of weaning patterns.

4.4.1 Stable carbon isotope analysis

Photosynthesis is the process of converting electromagnetic energy in the form of photons into biochemical energy in the form of glucose (Gest, 2005). Essentially, plants produce sugars from carbon dioxide, sunlight, and water. Not all plants use the same photosynthetic pathways to produce glucose, and different metabolic pathways produce different carbon isotope signatures however (O'Leary and O'Leary, 1988). Plants use three types of metabolic pathways: C_3 (Calvin-Benson cycle), C_4, and Crassulacean acid metabolism (CAM) (O'Leary and O'Leary, 1988). C_3 plants are the most common type of plants found in temperate climates. Some examples of C_3 plants include barley, rice, and wheat. C_4 plants are grasses that grow in more arid conditions compared to C_3 plants, with important examples of C_4 plants being maize (corn), sorghum, and millet (Downton, 1975). CAM plants, such as cacti and pineapples, are adapted for arid conditions and conserve water by alternating between the C_3 and C_4 pathways (Kluge and

Ting, 1978). Since these plants utilize different metabolic pathways, each metabolic pathway exhibits different rates of discrimination against carbon-13.

In C_3 plants, stomata on the leaves remain open, allowing water to evaporate and atmospheric carbon dioxide to enter the plant. Once carbon enters the undergo the Calvin-Benson cycle, during which the enzyme ribulose biphosphate carboxylase (RuBisCo) discriminates against ^{13}C when fixing carbon dioxide from the atmosphere (O'Leary and O'Leary, 1988). The result of this discrimination against carbon-13 is an average $\delta^{13}C$ for C_3 plants of -26‰, with a range of -22 to -34‰ (Troughton, 1971).

C_4 plants uptake carbon dioxide indiscriminately through the action of the enzyme phosphoenolphyruvate (PEP) carboxylase prior to entering the Calvin-Benson cycle within a bundle sheath cell. Carbon dioxide is transported into the bundle sheath cell by PEP indiscriminately, and once inside, the carbon dioxide is trapped. The indiscriminate uptake of carbon dioxide by PEP reduces the effects of RuBisCo's selective processing of light carbon isotopes within the bundle sheath cell. RuBisCo processes the available carbon dioxide within the bundle sheath cell (O'Leary and O'Leary, 1988). The result is a considerably higher average $\delta^{13}C$ compared to C_3 plants; the average $\delta^{13}C$ for C_4 plans is -12.5‰ with a range of -9 to -16‰ (Troughton, 1971).

CAM plants alternate between C_4 and C_3 pathways, utilizing the C_4 pathway during the day, and the C_3 pathway during the night (Kluge and Ting, 1978). The C_4 pathway is energetically expensive, but useful for conserving water in warm, arid conditions. The C_3 pathway is energetically less expensive, but not particularly useful for conserving water because the stomata are continuously open. Thus, CAM plants alternate between the two on a diurnal cycle in order to optimize water conservation during the day and minimize energy expenditure

during the evening. During the night, CAM plants open their stomata and absorb carbon dioxide until the morning, when they close their stomata. The trapped carbon dioxide is then taken up by PEP carboxylase in bundle sheath cells and undergoes C_4 photosynthesis (O'Leary and O'Leary, 1988). In the evening, the stomata open again, and CAM plants undergo direct C_3 photosynthesis. CAM plants exhibit a $\delta^{13}C$ between -10 to -20‰ (O'Leary and O'Leary, 1988).

The difference in carbon isotope ratios between mother and child is approximately +1‰ instead of ~+3 - 6‰ for stable nitrogen isotopes, making the weaning curve derived from stable carbon isotope data more difficult to construct than one derived from stable nitrogen isotopes (Eerkens et al., 2011; Fuller et al., 2006; Fuller et al., 2003; Katzenberg et al., 1993; Wright and Schwarcz, 1999). $\delta^{13}C_{collagen}$ overrepresents the protein component of the diet because dietary protein is routed to collagen production (Ambrose and Norr, 1993; Tieszen and Fagre, 1993). During weaning, the transition from the high-protein diet of breastmilk to low-protein weanling foods would result in a decrease in $\delta^{13}C$ (Krueger and Sullivan, 1984; Wickes, 1953). Some research suggests that carbon isotope analysis can uncover different information about weaning compared to nitrogen isotopes, however. Fuller et al. (2006) hypothesizes that stable carbon isotope ratios are influenced more heavily by the introduction of weanling foods compared to stable nitrogen isotopes because weanling foods are high in carbon and low in protein compared to breastmilk. The high carbohydrate content of weanling foods causes more dramatic changes in carbon isotope ratios than in nitrogen isotope ratios when consumed (Fuller et al., 2003). Stable carbon isotope analysis therefore may reflect the initiation of weaning and could be used to determine the timing of the onset of weaning, whereas stable nitrogen isotope ratios are used more commonly to determine when weaning has ceased.

4.4.2 Stable nitrogen isotope analysis

Nitrogen isotopes exist in two forms, ^{15}N and ^{14}N. ^{15}N has a relative natural abundance of 0.36%, whereas ^{14}N has a relative natural abundance of 99.68% (Schwarcz and Schoeninger, 1991). The international standard for nitrogen isotope analysis is Ambient Inhalable Reservoir (AIR) (Peterson and Fry, 1987). Atmospheric nitrogen is the major reservoir of nitrogen isotopes in the biosphere. Nitrogen is cycled through the biosphere by the action of plants, bacteria, and fungi, which can fix nitrogen by splitting the N_2 molecule and converting to a metabolizable form. The process by which N_2 is converted to ammonium, and subsequently converted to nitrites and nitrates is called ammonification.

The $\delta^{15}N$ values of animals generally reflect their trophic position within the food web (DeNiro and Epstein, 1981). Nitrogen is obtained directly from food sources consumed and is expelled from the body chiefly through the process of urination. Lighter ^{14}N isotopes are preferentially excreted from the body in urine, causing a concentration of heavy nitrogen isotopes within the body (Whitehead, 1970). This process results in an observable diet-tissue space, in which animals higher on the food chain exhibit higher nitrogen values. The value for nitrogen-15 enrichment per trophic position in humans is not currently known but is assumed to be broadly similar to mammals (Hedges and Reynard, 2007). Each trophic position exhibits a ~3‰ offset, though several studies have found offsets that range from 2 - 6‰ (Ambrose, 2000; DeNiro and Epstein, 1981; Hare et al., 1991; Howland et al., 2003; Minagawa and Wada, 1984; O'Connell et al., 2012; Schoeninger and DeNiro, 1984).

Due to this trophic level effect, $\delta^{15}N$ values generally reflect dietary protein (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). Additionally, because terrestrial food webs tend to be much shorter compared to marine resources, nitrogen isotope values often can be used to distinguish marine- and terrestrial- based diets. Marine vertebrates tend to have $\delta^{15}N$ values that

are +6 to +8‰ more positive than terrestrial vertebrates, reflecting a longer marine food web (Schoeninger, 1995; Schoeninger and DeNiro, 1984).

Nitrogen isotopes typically are interpreted to reflect the cessation of weaning, whereas carbon isotopes are interpreted to reflect the introduction of solid foods, or the initiation of weaning (Fuller et al., 2003). Carbon isotopes also have been used in conjunction with nitrogen isotopes to assess intra-population dietary differences in weaning practices among socially-stratified populations in the past (Beaumont et al., 2014; Beaumont et al., 2015; Eerkens et al., 2011; Fogel et al., 1989; Fuller et al., 2006; Fuller et al., 2003; Humphrey, 2014; Katzenberg et al., 1993; Moggi-Cecchi et al., 1994; Nitsch et al., 2011; Pearson et al., 2010; Reitsema, 2012; Reitsema et al., 2016a; Reitsema and Vercellotti, 2012; Richards et al., 2002; Sandberg et al., 2014; Tsutaya and Yoneda, 2013; Tsutaya and Yoneda, 2015). Age-at-weaning has been reconstructed using subadult rib collagen (Fogel et al., 1989; Giuffra and Fornaciari, 2013; Jay et al., 2008; Katzenberg et al., 1993; Nitsch et al., 2011; Pearson et al., 2010; Prowse et al., 2008; Tsutaya and Yoneda, 2013), hair and nail samples (de Luca et al., 2012; Fuller et al., 2006), and tooth dentin (Beaumont et al., 2015; Eerkens et al., 2011; Fuller et al., 2003; Henderson et al., 2014; King et al., 2018; Pfeiffer et al., 2017; Wright and Schwarcz, 1999). Figure 4.4 illustrates how $\delta^{15}N$ and $\delta^{13}C$ change as an infant transitions from exclusive breastfeeding to consumption of solid food. Since early childhood stress is an influential factor in mortality (see Chapter 3), the timeline for weaning milestones may be important factors that influence mortality patterns in the past.

4.5 Considerations for the osteological paradox

The osteological paradox is a critique of paleopathology and paleodemography that illustrates major problems encountered when analyzing human skeletal remains to infer ‘health’

in the past. Wood et al. (1992) outlined three main problems with paleopathological analysis, specifically hidden heterogeneity in frailty, selective mortality, and demographic non-stationarity. Hidden heterogeneity in frailty occurs when individuals within a population are unequally susceptible to disease and stress, and thus have an increased risk of mortality compared to others in their population (Wood et al., 1992). Selective mortality refers to the fact that skeletal assemblages are composed of deceased individuals, and the deceased chiefly represent the frailest individuals of a given age group (Wood et al., 1992). Therefore, the deceased represent a biased sample that does not reflect the living population. Demographic non-stationarity occurs when a population has unequal birth and death rates, either as a result of migration, fertility, or mortality (Bocquet-Appel and Masset, 1982; Wood et al., 1992). Variations in fertility are highly influential on age-at-death assemblages, with rapidly growing populations with high fertility generally exhibiting higher rates of infant mortality, which skews the average age-at-death (Buikstra et al., 1986). A low average age-at-death may be interpreted to mean that individuals are dying younger, and not that a population is growing rapidly due to increased fertility.

Wood et al.'s (1992) overarching criticism of paleopathology and paleodemography is that, in certain situations, subpopulations with no pathological markers may not represent the 'healthiest' individuals within a given population. For example, a population with a low prevalence of pathological markers may have not experienced prior health insults that were severe enough to produce bony lesions, which caused them to succumb to death when they did experience stress. On the other hand, a population with skeletal lesions may have experienced several episodes of stress throughout their lives but were able to survive these stress episodes and died from some other cause later in life. Thus, the interpretation of pathological markers may be

paradoxical in that individuals with the most pathological markers, under some circumstances, may be 'healthier' than individuals with no pathological markers.

The osteological paradox is an important critique that has been widely discussed in bioarchaeology since the publication of Wood et al.'s (1992) article (Armelagos and Van Gerven, 2003; Byers, 1994; Cohen, 1994; DeWitte and Stojanowski, 2015; Goodman, 1993; Soltysiak, 2015; Wilson, 2014; Wood and Milner, 1994; Wright, 2003). In response to this critique, many researchers have suggested that research assessing the underlying causes of heterogeneity in frailty and research into how frailty is related to death would be useful research avenues to address the paradox (DeWitte and Stojanowski, 2015; Wood et al., 1992; Wright, 2003). By investigating the role of early childhood stress on infectious disease mortality, this dissertation avoids some of the problems outlined by the osteological paradox. By focusing on pathological markers that are specific to the early childhood period, a time period that adults survived, this research contributes to the literature on the osteological paradox by testing the premise that prior stress events result in increased frailty.

Wood's critique is reiterated in a more recent critique of bioarchaeological approaches to the DOHaD hypothesis. Temple (2019) highlights the limitations of bioarchaeological methods in analyzing life history tradeoffs, and particularly emphasizes that bioarchaeologists should acknowledge that our research is focused on individuals at the time of death. He advocates for a contextualized approach to bioarchaeological research that acknowledges the social and ecological mechanisms that explain diversity in life history traits and the methodological limitations of life history reconstruction using skeletal assemblages. This dissertation considers this critique by discussing methodological limitations and emphasizing the importance of cultural context on interpretation.

4.7 Conclusion

The DOHaD hypothesis provides a theoretical framework for bioarchaeologists to understand the influence of early childhood stress on adult mortality (Armelagos et al., 2009; Blevins, 2015; Boldsen, 2007; DeWitte and Hughes-Morey, 2012; DeWitte and Wood, 2008; Reitsema et al., 2016b). There are several methodological approaches that are reviewed in this chapter that address early childhood experiences in skeletal samples, including paleopathological analyses, dental anthropology, and stable isotope analysis. The osteological paradox has been proposed as a limitation of bioarchaeological methods in the study of health from skeletal samples (Wood and Milner, 1994; Wood et al., 1992), and should be carefully considered when addressing the DOHaD hypothesis (Temple, 2019).

The subsequent three chapters explore how and if stress during early childhood affects the mortality outcomes for cholera victims. Chapter 5 explores how age-at-weaning affects mortality from cholera. Chapter 6 compares paleopathological markers of early childhood stress, particularly linear enamel hypoplasia, cribra orbitalia, and porotic hyperostosis, between cholera victims and general populations. Chapter 7 uses dental histology to compare the timing of stress events from 0.5 - 11 years of age between cholera victims and a general population. Chapter 8 synthesizes the results of chapters 5 - 7 and includes a discussion of the limitations for interpretation of the data from this research project.

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Figure 4.1: Porotic hyperostosis is manifested as the porotic surface of the parietal bones from individual US1362 from Benabbio, Italy.

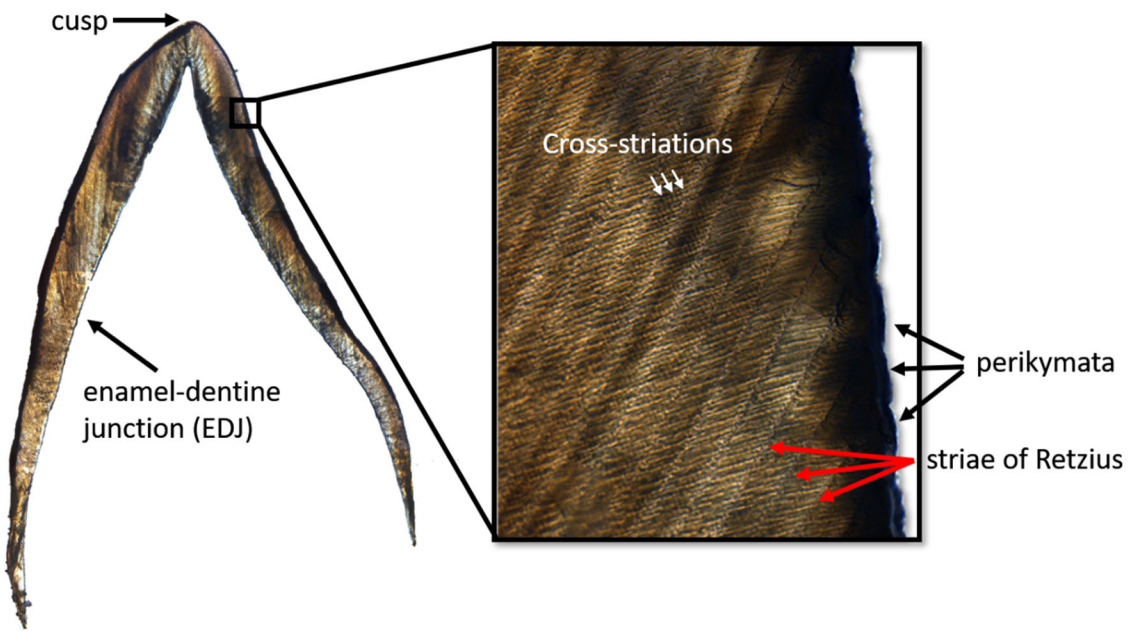

Figure 4.2: Image of enamel microstructures associated with enamel growth and formation.

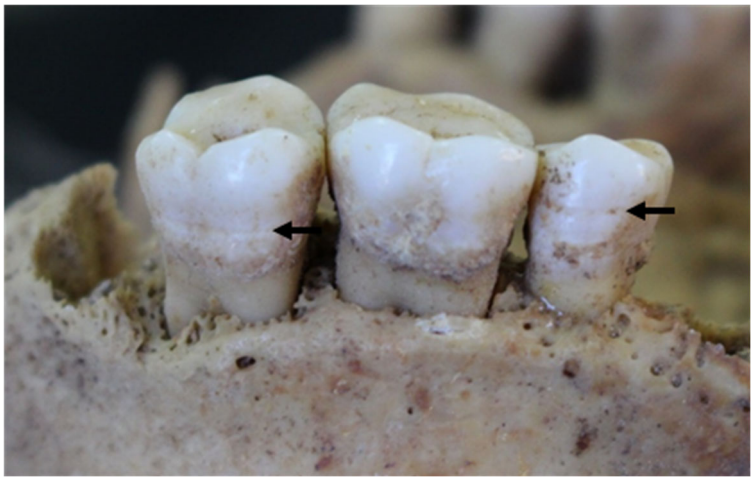

Figure 4.3: Image of the maxilla of individual A67 from camposanto vecchio, Alia, Sicily. Arrows indicate the presence of a linear enamel hypoplasia.

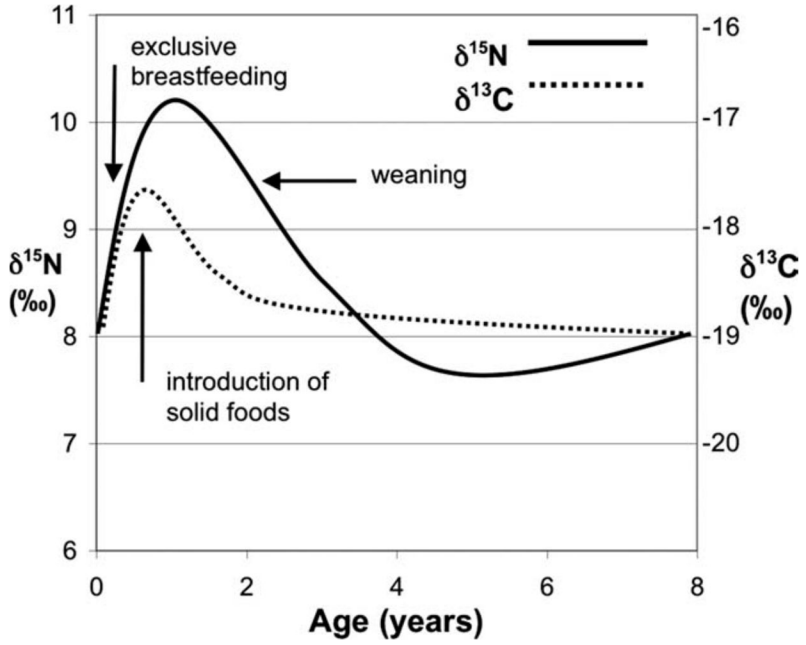

Figure 4.4: Illustration of the relationship between $\delta^{15}N$ and $\delta^{13}C$ during weaning (Nitsch et al., 2011).

CHAPTER 5

EXPLORING THE EFFECTS OF WEANING AGE ON ADULT INFECTIOUS DISEASE MORTALITY[1]

[1] Smith Dobbs A , Reitsema LJ, Sineo L, and Fornaciari A. *To be submitted to the American Journal of Human Biology*.

5.1 Abstract

The developmental origins of health and disease (DOHaD) hypothesis focuses on how early childhood and *in utero* stress affect mortality from metabolic disorder. The role of early childhood stress in mortality from infectious disease is understudied, however. Stressors in early childhood that weaken the immune system may result in increased susceptibility to infectious disease in adulthood. Weaning is one of the earliest periods of significant stress in early childhood. The research presented here investigates the effects of weaning on the cholera mortality among 18th-19th-century Italian populations.

The results of serial dentin stable carbon and nitrogen isotope analyses from canines are compared between catastrophic and attritional populations. Canines are sectioned serially in 1.5–2 mm increments from crown to apex, and $\delta^{15}N_{dentin}$ and $\delta^{13}C_{dentin}$ from these increments are used to compare weaning patterns.

Catastrophic and attritional populations exhibit similar age-at-weaning completion (~4 years). No statistically significant differences in weaning patterns between catastrophic and attritional populations are found. Approximately 40% of the samples lack an elevation in $\delta^{15}N$ during infancy.

The results of this study find that age-at-weaning completion is not a predisposing factor in cholera mortality in adulthood in this sample. Age-at-weaning completion may not be significantly associated with infectious disease mortality because infants that survive to adulthood may have adapted to consuming potentially contaminated weanling foods . Individuals without visible weaning curves may represent infants who were not breastfed or who received supplementary foods since birth.

5.2 Introduction

The developmental origins of health and disease (DOHaD) hypothesis posits that *in utero* and early childhood stress increase susceptibility to metabolic disorders in adulthood (Barker and Osmond, 1986; Barker, 1995; Barker, 1997; Barker, 2004; Gluckman et al., 2005; Kuzawa, 2005; Worthman and Kuzara, 2005). A significant body of knowledge exists that establishes a link between early childhood stress and adult metabolic disorders, including heart disease (Barker et al., 1989a; Barker et al., 1989b; Barker et al., 1993; Osmond et al., 1993), type II diabetes (Desai et al., 1995; Hales and Barker, 1992; Hales and Barker, 2001; Hales et al., 1991; Lithell et al., 1996) and increased blood pressure (Barker et al., 1990; Barker et al., 1989a; Campbell et al., 1996; Law et al., 1991). Researchers hypothesize that early childhood stress triggers a physiological response that primes or 'programs' the child's body to adapt to an environment of scarcity, leading to metabolic malfunction if the adult environment is calorie dense. Though a connection between early childhood stress and metabolic disorder in adulthood is clear, the impact of early childhood stress on infectious disease mortality is understudied and inconclusive.

Cholera is a diarrheal disease caused by the bacterium *Vibrio cholerae*. Cholera is endemic in coastal and estuarine waters throughout the world, but generally causes disease only in areas of the world with inadequate sanitation facilities and access to clean water (Ali et al., 2012; Briggs, 1999; Sack et al., 2004). The cholera bacterium causes infection by releasing a toxin that semi-permanently opens portals in the cell membrane of the large intestines, causing the expulsion of water from the surrounding tissues (Ali et al., 2012; Kaper et al., 1995; Kotar and Gessler, 2014; Sack et al., 2004). Victims of cholera rapidly become dehydrated and death

can occur within 6-8 hours of exhibiting symptoms. During the 18^{th} – 19^{th} century, fatality rates for cholera were between 30-60% (Kotar and Gessler, 2014; Lima, 1994).

Evidence for the link between early childhood stress and immunity is inconsistent. McDade et al. (2001a) found that undernourished individuals who were given the typhoid vaccine exhibited lower probabilities of mounting an adequate immune response to the typhoid vaccine. McDade et al. (2001b) found that prenatal undernutrition was associated significantly with reduced thymopoietin production, which affects the development and maturation of T-cells. Moore et al. (1997, 1999) analyzed the relationship between mortality and season of birth in Gambian children. They found that children born in the hungry season who also survived to age 15 suffered an increased risk of premature death compared to children born during the dry season. They also observed that infectious disease was the main cause of death among children who were born during the hungry season and died after age 15, suggesting that stress in early childhood may cause reduced immune function later in life. Moore et al. (2001) later found no difference in immune function between children born in the wet and dry seasons. Blevins (2015) compared skeletal evidence of early childhood stress and growth stunting between tuberculosis victims and a general population. She found no statistical differences between these populations suggesting that early childhood stress does not influence susceptibility to infectious disease mortality. More research is required to discern the effects of early childhood stress and childhood nutrition on mortality from various infectious diseases.

Breastfeeding has a significant biological impact on the health of children because breastmilk confers protective immunological benefits (Abuidhail et al., 2019; Cunningham, 1995; Neville et al., 2012), reduces gastrointestinal diseases (Cunningham, 1995; Neville et al., 2012), reduces inflammation (Walker, 2010), and promotes a healthy gut microbiome (Kau et al.,

2011; Martin and Sela, 2012; Zivkovic et al., 2011). Breastfeeding practices are typically poorly documented in the historical record (Baxter, 2005). Research on the archaeology of childhood in bioarchaeology has increased in recent years, however (Eerkens et al., 2011; Fogel et al., 1989; Fuller et al., 2006; Fuller et al., 2003; Henderson et al., 2014; Mays et al., 2017). Integrative historical and archaeological approaches to early childhood experiences are needed to contextualize breastfeeding in the past and discern the role of cultural factors in biological outcomes.

Weaning can be reconstructed in archaeological populations using stable carbon and nitrogen isotope analysis (Beaumont et al., 2014; Beaumont et al., 2015; Eerkens et al., 2011; Fogel et al., 1989; Fuller et al., 2006; Fuller et al., 2003; Humphrey, 2014; Katzenberg et al., 1993; Moggi-Cecchi et al., 1994; Nitsch et al., 2011; Pearson et al., 2010; Reitsema, 2012; Reitsema et al., 2016; Richards et al., 2002; Sandberg et al., 2014; Tsutaya and Yoneda, 2013; Tsutaya and Yoneda, 2015). Nitrogen isotope ratios in human bone collagen generally exhibit +3‰ offset per trophic position, though this offset can range from +1 to +6‰ (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; O'Connell et al., 2012). Breastfeeding enriches ^{15}N in infants compared to their mother because infants are consuming a diet that is one trophic position above their mothers (Fogel et al., 1989; Wright and Schwarcz, 1999). Stable carbon isotope ratios also exhibit a slight trophic effect with an offset of +1‰ (Fuller et al., 2003). Carbon isotope ratios typically decline earlier and faster than nitrogen isotope ratios. The cause of the earlier decline is likely that $\delta^{13}C$ signals the introduction of solid foods into the diet at the initiation of weaning (Fuller et al., 2006). Carbon isotope ratios are hypothesized to decline faster than $\delta^{15}N$ values because weanling foods generally are higher in carbohydrates and lower in protein compared to breastmilk. Carbon isotope values are more influenced by the

carbohydrate component of the diet, whereas the $\delta^{15}N$ values are derived primarily from dietary protein (Fuller et al., 2003). Breastmilk is rich in dietary protein, which means that the $\delta^{15}N$ of weaning infants primarily will be derived from breastmilk protein during the weaning process, whereas the $\delta^{13}C$ will be influenced more by weanling foods (Fuller et al., 2006).

This present study uses stable carbon and nitrogen isotope analysis to test the hypothesis that age-at-weaning differed between 19th-century cholera and attritional populations. Weaning is a period of significant stress in early childhood. The process of weaning exposes infants to new pathogens while simultaneously reducing infants exposure to the antimicrobial benefits of breastmilk (Lönnerdal, 2003). In historic contexts, weanling foods may have been nutritionally deficient or contaminated from unsanitary preparation, increasing the risk for diarrheal disease and malnutrition in weaning infants (Horta et al., 2013; Motarjemi et al., 1993). The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) recommends that mothers exclusively breastfeed at least until 6 months of age (WHO, 2009). Since early life is a critical period for brain development, cessation of breastfeeding before 6 months potentially could be detrimental to brain growth, particularly if weanling foods are nutritionally insufficient (Deoni et al., 2013; Horta et al., 2013; Rice and Barone, 2000). Prolonged periods of exclusive breastfeeding, usually beyond 8 months of age, are historically a result of inadequate access to food (Wickes, 1953a). Research on living populations has found several reasons why women might prolong breastfeeding. Some contemporary studies suggest that women prolong breastfeeding when the child is sick (Caulfield et al., 1996; Simondon et al., 2001), while others find that women stop breastfeeding when the child is sick to prevent the mother from transferring 'inadequate' breastmilk to the child (Deeney and Harris-Fry, 2020). Termination of

breastfeeding also may be due to the need to breastfeed another child (Martin, 2001; Simondon et al., 2001).

In the present study, we define age-at-weaning as the complete cessation of breastfeeding when the child exclusively consumes solid food. In the present study, we estimate the age-at-weaning completion using increments of dentin in teeth that grew during childhood of cholera victims and a contemporaneous, attritional population to determine if age-at-weaning is a factor in later life mortality risk from infectious disease. Tooth dentin is used because unlike other types of bone, tooth dentin does not remodel once formed, and excepting secondary dentin, does not form after childhood, and thus captures dietary data at the specific time period in which the dentin was formed. We hypothesize that cholera victims experienced extended periods of exclusive breastfeeding that resulted in decreased immune response compared to the contemporaneous, attritional population.

This research explores the relationship between weaning age and mortality from infectious disease, with four main goals. First, we consider the effect of cultural context on weaning practices by determining if any site- and sex-based differences in age-at-weaning completion exist in the study sample. Second, we determine if those who weaned earlier experienced differential mortality outcomes than those who weaned later in childhood. Third, we determine if age-at-weaning correlates with age-at-death. Finally, we determine if there are statistically significant differences between catastrophic cholera samples and attritional samples with regards to weaning patterns.

5.3 Materials and methods

5.3.1 Skeletal Samples

Skeletons included in this study come from four 18-19th-century cemeteries in Italy (Fig. 5.1), including two cemeteries containing known cholera victims. Camposanto vecchio, located in Alia (PA; Sicily), Italy, and the San Michele of Benabbio (LU; Tuscany), Italy are cemeteries where cholera victims from the 1837 and 1855 cholera pandemics, respectively, were interred. The church of San Pietro in Badia Pozzeveri (LU; Tuscany), Italy and the parish of San Giovanni Battista in Pieve dei Monti di Villa (LU; Tuscany), Italy are two 18th-19th-centuries burial grounds. Table 5.1 summarizes the archaeological sites analyzed in this study.

Camposanto vecchio is a small, cavernous tomb located outside the rural village of Alia inside the Madonie mountain range in the interior of Sicily. In 1837, approximately 300 cholera victims were interred inside the cave. The remains of the cholera victims were discovered in the spring of 1995 while the wall enclosing the tomb was being renovated, and the tomb was excavated by the University of Palermo between 1996-2000 (Chiarelli, 2002; Guccione, 1991). Parochial records from the Parish of Saint Anna in the center of Alia record 306 names of cholera victims that died in the cholera epidemic of 1837. The skeletal analysis conducted by the University of Palermo found that the minimum number of individuals for this site is 296 (Bigazzi, 1999; Bigazzi et al., 2002). Additionally, the burials inside the cave at Camposanto vecchio were covered in lime, a cultural practice during the 19th century that was thought to limit the spread of cholera (Fornaciari et al., 2010; Morris, 1976)

In 1855, the space surrounding the church of San Michele was used to inter 46 cholera victims from the village of Benabbio, Italy. The village of Benabbio is located in the Val di Lima region of Tuscany near the city of Lucca. The church of San Michele sits atop a hill in the

Serchio Valley and is approximately 200 m north-east of the center of Benabbio. Originally, it was the church of the medieval castle of Benabbio that dates to the 13th century. By the 15th century, the castle was abandoned, and the church was not used regularly for burials. The Division of Paleopathology of the University of Pisa excavated the area around the church in the years 2007-2010. The burial location and typology suggest that the 19th-century burials were catastrophic; the burials were in narrow ditches that contained more than one individual in some cases (Fornaciari, 2017; Fornaciari et al., 2010). Similar to the burials at Alia, some bodies from Benabbio were covered with lime. An epigraph naming the 46 cholera victims of 1855 was installed on the exterior of the church of San Michele soon after they were buried (Laganà, 2007). The names also were recorded in parochial records, and a note from a parish priest indicates that 44 of these individuals were buried outside the church of San Michele (Baldino, 2011; Laganà, 2007).

The church of San Pietro, located in Badia Pozzeveri along the Via Francigena pilgrimage route, is an 11th-century church with burials that date from the late medieval period to the contemporary age (Fornaciari et al., 2016). The site was first excavated by the University of Pisa and the Ohio State University in 2011, and excavations continue to the present day. A handful of 19th-century catastrophic burials encased in lime have been found at Badia Pozzeveri, but no other evidence to date has linked these burials to cholera epidemics (Santiago-Rodriguez et al., 2019). For this study, we chose individuals that date to the 18th-19th century whose burial patterns are consistent with non-catastrophic burials, including burial placement within the normal cemetery space, absence of lime within the burial plot, careful placement of the body in a supine position with the arms folded over the chest or waist, and the presence of typical grave goods, such as a rosary or devotional medallion.

The church of San Giovanni Battista, located in Pieve dei Monti di Villa, Italy included a cemetery that was used between 1807 and 1870 before a new cemetery was constructed for the village in 1871 (Rezza, 2009). The church of San Giovanni Battista is located within the town center of Pieve dei Monti di Villa, which is located in the Serchio valley. The University of Pisa excavated the cemetery surrounding the church of San Giovanni Battista between 2002 and 2006, and the skeletal remains were subsequently analyzed by the Division of Paleopathology at the University of Pisa (Fornaciari, 2008; Fornaciari, 2015; Rezza, 2009). Seven out of 10 total individuals recovered from the 19th-century layers possessed canines that were analyzed for histological analysis in this study.

5.3.2 Stable carbon and nitrogen isotope analysis

To reconstruct weaning patterns, one canine tooth was taken from Alia (n=31), Benabbio (n=14), Badia Pozzeveri (n=16) and Pieve dei Monti di Villa (n=7). Males comprise 55.9% of the total sample (n = 38) from all sites. Females comprise 35.3% of the total sample (n = 24) and 8.8% of the sample are of indeterminate sex (n = 6). Those of indeterminate sex were excluded from sex-based statistical analyses due to low sample size. Canine carbon and nitrogen isotope analysis has been used previously to assess weaning patterns among archaeological populations (Fuller et al., 2003; Sandberg et al., 2014). We assigned age estimates to each tooth using a modified version of the method outlined in Beaumont and Montgomery (2015). Tooth crowns and roots were sectioned separately as described below. Crown and root increments were assigned ages using the standards outlined in AlQahtani et al. (2010). For maxillary canines, the crown is formed between 0.6 years (Ci) to 5.5 years (Cc) of age and root forms between 5.5 years (Cc) to 14.5 years (Ac) of age. Mandibular canine crowns form between 0.9 years (Ci) to 5.5 years (Cc) of age and roots form between 5.5 years (Cc) to 13 years (Ac) of age. Dentin

grows incrementally from the dentin enamel junction (DEJ), which results in an oblique angle of growth along the long axis of the tooth (Dean and Scandrett, 1995; Hillson, 2014; Tang et al., 2015). Since tooth formation occurs obliquely, horizontal sampling of tooth dentin cuts across different tooth formation stages. We acknowledge that increment formation ages are not precise due to horizonal sampling, but instead represent an estimate of the increment formation age. In this study, increments are cut at regular intervals along the length of the tooth. Increment formation ages are estimated for each increment using the protocol outlined in Beaumont and Montgomery (2015). In samples where there is a minimum ^{15}N elevation of 0.5‰ in the earliest increments, age-at-weaning is estimated by averaging the ages of the final increment with elevated ^{15}N and the subsequent increment.

When multiple canines were present, the least-worn canine was chosen for analysis. In some cases, the least-worn canine exhibited dentin exposure as a result of tooth wear. To determine how many increments were lost due to tooth wear, we explored whether canines in these study samples without dentinal wear show a relationship between width and height, such that dentin width can be used to estimate dentin height. Calipers were used to measure the width of the dentin at the cementum enamel junction (CEJ) and the height of the dentin from the apex to the CEJ using thin sections of the teeth, which are the subject of other research (Smith et al., In prep). Linear regression equations are derived from mandibular ($R^2 = 0.2288$, $p = 0.006$) and maxillary ($R^2 = 0.01647$, $p = 0.6761$) dentin width and height of unworn canines, and this equation is used to estimate the number of increments missing in worn teeth (Blom et al., 2015; Plavcan et al., 2009). Each 1.5 mm of missing dentin is assumed to correspond to 1 missing increment.

Thin sectioning is achieved using a modified version of Method 1 from Beaumont (2013). The crown was separated from the root using a Dremel® saw, and the crown of the tooth was embedded in resin using the Streurs Epo-Fix epoxy resin kit. The embedded crown was cut into 1.5 - 2 mm incremental sections using a Beuhler Isomet diamond-blade microtome saw. The collagen extraction procedure is adapted from Eerkens (2011) and Henderson (2014). Thin sections were removed from the epoxy and the roots and thin sections were demineralized in 2 mL of 0.5M HCl solutions with replacement once every two days. The tooth sections were then treated for humic contamination by soaking the collagen in 0.125 M NaOH for 20 hours. Secondary dentin was removed for all tooth sections when present. Roots were then sectioned into 1 - 2 mm increments using a scalpel, and the tooth sections were dissolved in 10^{-3}M HCl solution (pH = 3) at 80°C for approximately 24 - 36 hours. The samples were then freeze dried, homogenized, and 500 μg of each sample was weighed into tin capsules on a microbalance for analysis using an Elemental Analyzer coupled to a stable isotope ratio mass spectrometer at the University of Georgia's Center for Applied Isotope Studies. Samples were excluded from the analysis if they exhibited C:N ratios outside of the recommended 2.9 - 3.6 suggested by Ambrose (1993) and van Klinken (1999).

5.3.3 Statistical analysis

All statistical analyses are performed using the statistical platform R (v 3.5.3). To account for unequal sample sizes between the catastrophic and attritional populations, non-parametric analyses are used to statistically assess these populations. A Welch's two-sample t-test is used to determine if there are statistically significant differences in age-at-weaning completion between cholera victims and attritional populations. Sites are compared using a non-parametric Kruskal-Wallis H test and a Dunn Multiple Comparisons test. Welch Two-Sample t-tests are used to

establish any sex-based differences in the sample. A Kaplan-Meier survival curve is used to determine the effects of earlier vs. later weaning on adult mortality outcomes. A Spearman's Rank correlation coefficient is used to determine if age-at-death correlates with age-at-weaning.

5.4 Results

Differences exist in both $\delta^{15}N_{dentin}$ and $\delta^{13}C_{dentin}$ that correspond with diet across early life for the catastrophic and attritional populations. Summary statistics for $\delta^{15}N_{dentin}$ and $\delta^{13}C_{dentin}$ are provided in table 5.2 for the crown increment, the increment at the CEJ, and the final increment from the apex of the root. When analyzed collectively, each crown increment shows higher $\delta^{15}N$ values compared to the $\delta^{15}N$ values of the CEJ and apex increments for all sites. The $\delta^{13}C$ values for the CEJ and apex segments are consistently higher than the crown segment among cholera victims and the general population. When analyzed individually, 71.6% of cholera profiles exhibit a higher $\delta^{13}C$ in early childhood compared to late childhood. The standard deviations for the isotope reference materials used in this analysis are as follows; 1577c (δN_{air} SD = 0.14‰, δC_{VPDB} SD = 0.05‰), 1577c-b (δN_{air} SD = 0.09‰, δC_{VPDB} SD = 0.06‰), spinach (δN_{air} SD = 0.24‰, δC_{VPDB} SD = 0.13‰).

Twenty-eight of the isotope profiles (42.6%) did not exhibit a visible weaning curve. Forty-four percent of cholera victims lacked a visible weaning signal, similar to the attritional population at 39%. Because tooth wear obliterates the earliest-forming enamel, and potentially, the earliest-forming dentin if wear is severe, and because the earliest-forming tissue is likely to be the most enriched in ^{15}N due to a trophic effect concomitant with breastfeeding, we examined whether the specimens lacking expected weaning curves are also the individuals with the most worn teeth. The 28 isotope profiles without visible weaning signals are not associated with tooth wear; only 23.1% of worn teeth exhibit no visible weaning signal.

Twenty-four cholera victims and 14 individuals from the attritional population exhibit visible weaning curves (see Figure 5.2). No statistically significant differences in survival time between individuals with visible weaning curves and those without are observed the study population ($p = 0.88$). The 28 individuals without visible weaning curves are excluded from the weaning age statistical analyses because weaning age could not be estimated for those individuals, leaving a sample size of 38 individuals. Cholera victims have an average age-at-weaning completion of approximately 4.1 years old and the attritional sample has an average age-at-weaning completion of 3.9 years. There are no statistically significant differences in age-of-weaning completion between cholera victims and the attritional sample ($t = 0.21381$, $df = 18.588$, $p = 0.833$). Additionally, no site-based differences in age-at-weaning completion are found in our sample ($\chi^2 = 2.5482$, $df = 3$, $p = 0.4666$).

Though statistically significant differences in $\delta^{15}N$ and $\delta^{13}C$ exist between the sites, no statistically significant differences in age-at-weaning completion between the sites. Samples from Alia and Badia Pozzeveri exhibit similar median $\delta^{15}N$ values, with samples from Alia exhibiting a median $\delta^{15}N$ value of 10.3‰ and samples from Badia Pozzeveri exhibiting a median $\delta^{15}N$ value of 10.8‰. Benabbio has a median $\delta^{15}N$ value of 7.1‰ and samples from Monti di Villa exhibit a median $\delta^{15}N$ value of 7.6‰. Benabbio teeth exhibits the lowest median $\delta^{13}C$ value, at -19.9‰, followed by Alia teeth with a median $\delta^{13}C$ value of -18.5‰ and Pieve dei Monti di Villa teeth with a median $\delta^{13}C$ value of -18.4‰. Badia Pozzeveri teeth exhibits the highest median $\delta^{13}C$ value at -16.0‰. When comparing the $\delta^{15}N$ values between each site, the sites are found to exhibit significantly different medians ($\chi^2 = 223.2$, $p < 0.01$) and variances ($K^2 = 65.9$, $p < 0.01$). A post-hoc Dunn test reveals that the two pairings of Alia and Badia Pozzeveri, and Benabbio and Pieve dei Monti di Villa exhibit statistically similar $\delta^{15}N$ values,

but all other pairings are significantly different from one another ($p < 0.01$). When the same statistical procedure is applied to the $\delta^{13}C$ values, the teeth from these sites exhibit statistically different medians ($\chi^2 = 186.8$, $p < 0.01$) and variances ($K^2 = 455.4$, $p < 0.01$). Alia and Pieve dei Monti di Villa exhibit statistically similar $\delta^{13}C$ values, but all other pairings exhibit significantly different carbon values ($p < 0.05$). No statistically significant differences are found between the sites in terms of average age-at-weaning ($\chi^2 = 2.582$, $df = 3$, $p = 0.4666$). No statistically significant differences in age-at-weaning exist between males and females ($t = 1.866$, $df = 26.272$, $p = 0.07$).

The Kaplan-Meier survival analysis finds no differences in age-at-weaning completion between cholera victims and attritional populations. Individuals are grouped based on the whether they exhibit above average or below average age-at-weaning, or if they did not exhibit weaning curves (Figure 5.3). No statistically significant differences are found between individuals with above average age-at-weaning, below average age-at-weaning, or those that do not exhibit weaning curves ($p = 0.57$). Though individuals with below average age-at-weaning exhibit consistently lower survival probability compared to individuals with above average age-at-weaning, this difference is not statistically significant ($p = 0.22$). No statistically significant differences are found when comparing above and below average age-at-weaning among cholera victims only ($p = 0.5$) and the attritional sample only ($p = 0.22$).

For those with visible weaning curves, the average age-at-death is 37.2 ± 13.8 years, and the average age-at-weaning completion is 4.1 ± 1.3 years. The results of the Spearman's Rank Correlation test show that there is no correlation between age-at-death and age-at-weaning in this sample ($S = 6343.5$, $p = 0.1388$). When excluding the attritional population, cholera victims did not exhibit a correlation between age-at-weaning and age-at-death ($S = 2120.1$, $p = 0.7165$). The

attritional sample also did not exhibit statistically significant difference between these two variables ($S = 170.94$, $p = 0.06$).

5.5 Discussion

The $\delta^{15}N$ and $\delta^{13}C$ values from all sites suggest a mixed terrestrial and marine diet that includes primarily C_3 plants. This result is consistent with previously published bone collagen stable carbon and nitrogen isotope data for Benabbio ($\delta^{15}N$: 8.3±1.0‰, $\delta^{13}C$: -19.8 ±1.3‰) and Pieve dei Monti di Villa ($\delta^{15}N$: 7.8±1.3‰, $\delta^{13}C$: -18.8 ±0.9‰) which found that individuals from these sites consumed an omnivorous diet with moderate animal protein consumption (Fornaciari, 2015).

Cholera victims are found to exhibit a slightly older age-at-weaning completion compared to the attritional population. Cholera victims completed weaning around 4.1 years of age, whereas the attritional population weaned at age 3.9 years. This difference was not statistically significant, and there is considerable overlap between the ages of weaning completion among the cholera victims and the attritional population. This result suggests that the duration of weaning does not contribute to adult mortality from infectious disease.

The results of the Kaplan-Meier survival analysis suggest that those who weaned early consistently exhibit an earlier age-at-death, but this difference is not statistically significant. Therefore, the age-at-weaning did not affect overall mortality in these populations.

Weanling foods during the 19th century were often contaminated with bacteria or parasites (Wickes, 1953a; Wickes, 1953b). Infants that survived to adulthood, like those in our study sample, may have survived infancy by adapting to consuming potentially contaminated weanling food, imbuing them with a better ability to adapt to later life environmental challenges. The timing of weaning completion may not influence infectious disease mortality in adulthood

among infants with extended periods of breastfeeding because these infants may have been exposed to potentially contaminated solid foods for several years before the complete cessation of breastfeeding. Infants who experienced a long period of breastfeeding with food supplementation, over the course of years, would adapt to the constant exposure to pathogens and have built an immunity towards those pathogens by the time weaning completed. The age-at-weaning completion therefore may not represent a sufficiently stressful period in early childhood to produce the physiological changes required to increase susceptibility to disease in adulthood.

No site-based differences are found in age-at-weaning completion, suggesting that age-at-weaning completion does not influence mortality from infectious disease. Furthermore, no differences in weaning age between the sites suggest that weaning practices in 19th-century rural Italy were similar across the peninsula. Site-based statistically significant differences of dentin $\delta^{15}N$ and $\delta^{13}C$ values suggest that there was regional variation in diet, however. This result suggests that the duration of weaning does not contribute to adult mortality from infectious disease and that age-at-weaning completion is similar across the 19th-century Italian landscape, despite variation in dietary composition across sites.

The association between site location and carbon isotope ratios illuminates several aspects of 19th-century Italy's weaning and dietary practices. First, teeth from Alia and Pieve dei Monti Di Villa exhibit less variation in $\delta^{13}C$ compared to those from Benabbio and Badia Pozzeveri during late childhood. Alia and Pieve dei Monti di Villa likely had less access to the variety of foods available during childhood in Benabbio and Badia Pozzeveri. Of all the sites, Badia Pozzeveri exhibits the most variation compared to the other sites, which is likely due to its location in a plain along the trade between Lucca and the Arno River Valley, the principle trade route in Northern Tuscany (Morelli, 2007). The movement of peoples across the landscape in

Badia Pozzeveri may have resulted in more types of foods entering the local food economy, which could have caused a more varied late childhood diet compared to other sites. The elevation in average $\delta^{13}C$ among the residents of Badia Pozzeveri compared to all other sites could be a product of maize (*Zea mays*) cultivated and exchanged across the Lucca Plain. Maize was not widely grown in many parts of Italy during the 18th and 19th centuries, with the exception of the Po Plain in Northern Italy and other smaller flat areas, such as the Lucca Plain (Gentilcore, 2014; Ginnaio, 2011; Pazzagli, 1998). Maize was commonly used to make polenta throughout the 19th century in Northern Italy (Gentilcore, 2014; Ginnaio, 2011).

Many of the individuals in this study did not exhibit elevated $\delta^{15}N$ that might be associated with breastfeeding, which presents an interpretive dilemma for weaning reconstruction. Several other studies also find no visible elevation in $\delta^{15}N$ among serial sections of human dentin (Beaumont et al., 2013a; Eerkens et al., 2011; Henderson et al., 2014; King et al., 2018; Pfeiffer et al., 2017). The lack of visible elevation in $\delta^{15}N$ in early life has several potential interpretations. Individuals without visible elevation in $\delta^{15}N$ during infancy and early childhood could be individuals who were not breastfed or who consumed supplemental foods during infancy. Animal milk, especially cow's milk, were alternative sources of nutrition for infants whose mothers could not breastfeed during the 19th century (Stevens et al., 2009; Wickes, 1953a; Wickes, 1953b). When babies did not thrive on breastfeeding or cow's milk alone, infant diets were supplemented with pap (bread soaked in milk) and panada (broth containing cooked cereals) (Radbill, 1981; Wickes, 1953a). The consumption of cow's milk instead of mother's milk would not enrich ^{15}N in infant tissues relative their mother's $\delta^{15}N$. Lactose malabsorption among Italian children may have contributed to the use of alternative weanling foods early in life. Italian children, in particular, have a higher prevalence of lactose malabsorption compared

to those in other parts of Europe: the rates of lactose malabsorption in Italy range from 19% to 71% (Burgio et al., 1984; Cavalli-Sforza et al., 1987). Infants also may experience lactose intolerance in early life, either due to a temporary allergy, cow's milk allergy, or congenital lactase deficiency (Heine et al., 2017). Infants with mutations that cause congenital lactase deficiency are found in Central Europe, including Italy (Torniainen et al., 2009). Infant distress when consuming breastmilk or cow's milk may have accelerated the weaning process and encouraged infant consumption of non-dairy alternative to breastmilk.

It is also possible that those individuals that lack visible weaning curves could have experienced elevated levels of stress during early childhood that obscured the weaning signal. Nutritional deprivation is linked to an elevation in $\delta^{15}N$ levels (Deschner et al., 2012; Fuller et al., 2005; Hobson et al., 1993). During nutritional deprivation, nitrogen is hypothesized to be reused in the body through the processes of deamination and transamination of amino acids (Hobson et al., 1993). These processes concentrate ^{15}N the tissues of the body, resulting in elevated $\delta^{15}N$ levels. It is possible that individuals without visible weaning curves experienced periods of nutritional deprivation that elevated their $\delta^{15}N$ levels in early childhood. These individuals therefore could have completed weaning around the same time as those with visible weaning signals, but do not exhibit similar isotopic weaning profiles due to a stress-induced elevation in ^{15}N around the time of weaning completion.

Another potential explanation is that these individuals were weaned before 6 months of age and this analysis did not capture the weaning period for these individuals. Historical records suggest that breastfeeding during the 19th century lasted beyond the first year of life, within the developmental ages for human canines. During the 19th century, doctors recommended that women breastfeed exclusively until at least 18 months of age and recommended that weaning

later was preferable to weaning early (Wickes, 1953a). Records dating to the 15th century suggest that weaning was completed between 3 - 4 years (Fulminante, 2015). Stable isotope research, too, suggests that during the medieval period, people in the Eastern Mediterranean region of Europe had an extended period of breastfeeding, with weaning occurring between 3 – 4 years of age, compared to people in continental Europe (Bourbou et al., 2013; Dittmann and Grupe, 2000; Fulminante, 2015; Herrscher, 2003; Turner et al., 2007). Other research using urban and/or high-status remains finds that weaning occurred between 2-3 years of age in 17th-19th-century Italy (Giuffra and Fornaciari, 2013; Moggi-Cecchi et al., 1994). People in rural parts of modern Italy may have experienced extended breastfeeding compared to their urban counterparts as a result of reduced access to food and lower pressures on women to work outside of the home (Wickes, 1953a; Wickes, 1953b). Industrialization in Italy began in the late 19th century and peaked in the early 20th century (Ciccarelli and Fenoaltea, 2013; Federico and Vasta, 2010). Mothers in rural Italy during the late 18th- mid-19th centuries did not experience the same societal pressures to reduce the duration of breastfeeding to work in factories as in continental Europe where the economy was more industrialized (Wickes, 1953b). Thus, an extended period of breastfeeding might have been common in rural Italy during the 18th-19th centuries.

The majority of individuals without visible weaning curves did not exhibit tooth wear (n = 24). However, some individuals did have no visible weaning curve and tooth wear (n = 4). Age-at-death did not differ significantly between these two groups ($W = 55$, $p = 0.7748$), suggesting that they are similar enough to be pooled. To test if the individuals without visible weaning curves exhibit a significantly different age-at-death compared to the groups with visible weaning curves, we re-analyzed the age-at-weaning statistical comparisons including the individuals without visible weaning curves. Individuals without weaning curves are assigned an

age-at-weaning of 0 years. Age-at-weaning did not differ significantly between catastrophic or attritional populations ($t = -0.25188$, $df = 41.234$, $p = 0.8024$), nor between the sites ($\chi^2 = 1.4258$, $df = 3$, $p = 0.6995$), with the inclusion of these individuals. No statistically significant differences in survival time are found between individuals without visible weaning curves and individuals with below median age-at-weaning ($p = 0.6$) or above median age-at-weaning ($p = 0.61$), suggesting that these individuals exhibit a similar age-at-death compared to the rest of the population. When the individuals with no visible weaning curve are grouped with the below median age-at-weaning individuals, no statistically significant differences in survival time are found compared to individuals with above median age-at-weaning ($p = 0.38$).

Using $\delta^{15}N$ to estimate weaning patterns has several methodological limitations that may explain why some individuals do not exhibit elevated $\delta^{15}N$ in early childhood (see Reynard and Tuross 2015). During the early post-weaning period, infant $\delta^{15}N$ dips below mother's $\delta^{15}N$ value (Beaumont et al., 2013b; Eerkens et al., 2011; Henderson et al., 2014; Katzenberg et al., 1996; Richards et al., 2002; Sandberg et al., 2014). One explanation for the 'dips' in nitrogen in late weaning is that rapid growth during early childhood produces a state of positive nitrogen balance, where more nitrogen is used for tissue growth and maintenance and less nitrogen is excreted as waste, resulting in lower $\delta^{15}N$ values (Deschner et al., 2012; Mekota et al., 2006). However, growth velocity only explains some of the variation in infant $\delta^{15}N$ during the post-weaning nitrogen 'dip (Reitsema and Muir, 2015). Diet may be responsible for at least some of the variation in post-weaning $\delta^{15}N$ values (Reitsema and Muir, 2015). The extent to which growth velocity is related to $\delta^{15}N$ values in early childhood is not known, thus a positive nitrogen balance due to rapid growth could obscure the weaning signal in at least some of our sample. In addition to growth velocity, little is known about the effects of gut microbiota on stable carbon

and nitrogen isotope values (Reynard and Tuross, 2015). The gut microbiota develops over the first three years of life and have been found to influence urease gene representation and the metabolism of amino acids (Yatsunenko et al., 2012). Alteration to the gut microbiome in early life may influence $\delta^{15}N$ value due to the role of the gut microbiome in nitrogen metabolism. Controlled studies on living humans that explore the effects the gut microbiome, and growth velocity on $\delta^{15}N$ profiles would be required to definitively state that the lack of $\delta^{15}N$ elevation in early childhood is a result of alternative or supplementary foods during infancy.

5.6 Conclusion

This study finds no differences in weaning patterns between catastrophic and attritional samples. This suggests that the timing of weaning completion is not related to infectious disease mortality from cholera in adulthood. Weaning during the 18th-19th century in rural Italy was complete around 4 years of age at all sites suggesting that weaning practices are similar across the 19th-century Italian landscape, despite regional dietary variation. Age-at-weaning completion may not represent a sufficiently stressful period in early development to affect immune function later in life, particularly if there is an extended period of weaning, because infants may have adapted to a combined diet of breastmilk and potentially contaminated solid foods for an extended period of time prior to the cessation of breastfeeding. The onset of weaning, as opposed to the completion of weaning, may represent a more critical period of early childhood stress that should be investigated in future research.

This study demonstrates the importance of considering cultural context and methodological limitations when approaching the DOHaD using skeletal remains (Temple, 2019). A lack of visible weaning curves for many of the samples is a major methodological limitation in using dentin stable carbon and nitrogen isotope analysis to reconstruct aging

methods. We find that approximately 40% of all samples lack an elevation in $\delta^{15}N$ during infancy, suggesting that these individuals may have not been breastfed, consumed supplementary foods from birth, or experienced stress that affected their nitrogen metabolism. Isotope profiles without visible elevation in $\delta^{15}N$ are frequently found in other studies (Beaumont et al., 2013a; Eerkens et al., 2011; Henderson et al., 2014; King et al., 2018; Pfeiffer et al., 2017), and their inclusion in the interpretation of the sites will provide a more complete understanding of the variation in infant feeding practices throughout history. Further research is needed to discern if that these 'flat' weaning profiles represent individuals who were not breastfed or nutritionally deprived.

This study finds no relationship between infectious disease mortality and age-at-weaning completion, which is consistent with the findings of Blevins (2015) and (Moore 2001), but contrasts with other research that found that skeletal markers are associated with earlier death (Boldsen, 2007; DeWitte, 2012; DeWitte and Wood, 2008; Temple et al., 2012) and that alteration of immune function is associated with early childhood stress (McDade et al., 2001a; McDade et al., 2001b). Extended periods of weaning may not result in altered immune function that increases susceptibility to infectious disease in adulthood because of adaptation to potentially contaminated weanling foods. Future research should consider analyzing other aspects of weaning, such as the onset of weaning or length of the weaning period.

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Table 5.1: Archaeological sites information

Location	Site Name	Burial style	Citations	N
Alia	Camposanto vecchio	Catastrophic (cholera)	Chiarelli et al. 2002 Bigazzi 1999 Guccione 1991	31
Benabbio	Church of San Michele	Catastrophic (cholera)	Baldino 2011 Laganà 2007 Fornaciari 2010 Bini 2010	14
Badia Pozzeveri	Church of San Pietro	Attritional	Santiago-Rodriguez et al. 2019 Gibbons 2013	16
Pieve dei Monti di Villa	Parish of San Giovanni Battisa	Attritional	Rezza 2009 Fornaciari, G. 2006, 2008, 2015 Fornaciari, A. 2004, 2002	7

Table 5.2: Summary statistics for $\delta^{15}N_{dentin}$ and $\delta^{13}C_{dentin}$ for permanent canines.

			$\delta^{15}N$ (‰)			$\delta^{13}C$ (‰)		
	N		Crown	CEJ	Apex	Crown	CEJ	Apex
Cholera Victims								
Alia	31	Mean	10.8	10.1	10.5	-18.7	-18.5	-18.5
		s.d.	0.9	0.6	0.7	0.4	0.2	0.4
Benabbio	14	Mean	8.1	7.1	7.2	-20.4	-19.6	-19.5
		s.d.	0.8	0.8	1.4	0.6	1.0	0.7
TOTALS	45	Mean	10.1	9.2	9.4	-19.2	-18.7	-18.8
		s.d.	1.5	1.5	1.8	0.9	0.7	0.7
Attritional Populations								
Badia Pozzeveri	16	Mean	11.5	10.0	10.2	-16.6	-16.0	-15.9
		s.d.	1.6	1.6	1.6	2.1	2.5	2.8
Monti di Villa	7	Mean	8.0	7.3	7.4	-18.8	-18.3	-18.6
		s.d.	1.2	0.9	1.0	0.5	0.7	0.6
TOTALS	23	Mean	10.5	9.2	9.3	-17.2	-16.7	-16.8
		s.d.	2.2	1.9	2.0	2.0	2.4	2.6

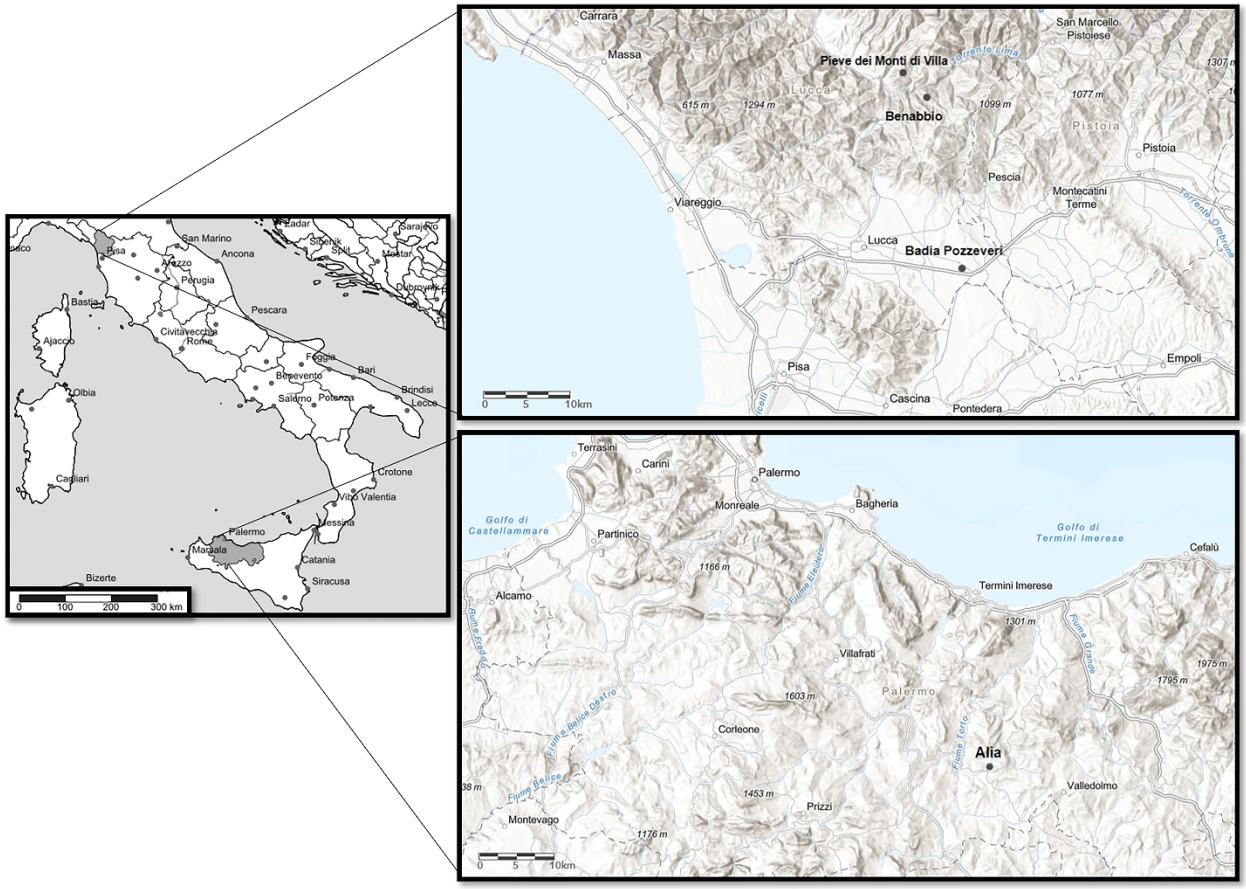

Figure 5.1: Map of archaeological sites: Alia, Benabbio, Badia Pozzeveri and Pieve dei Monti di Villa.

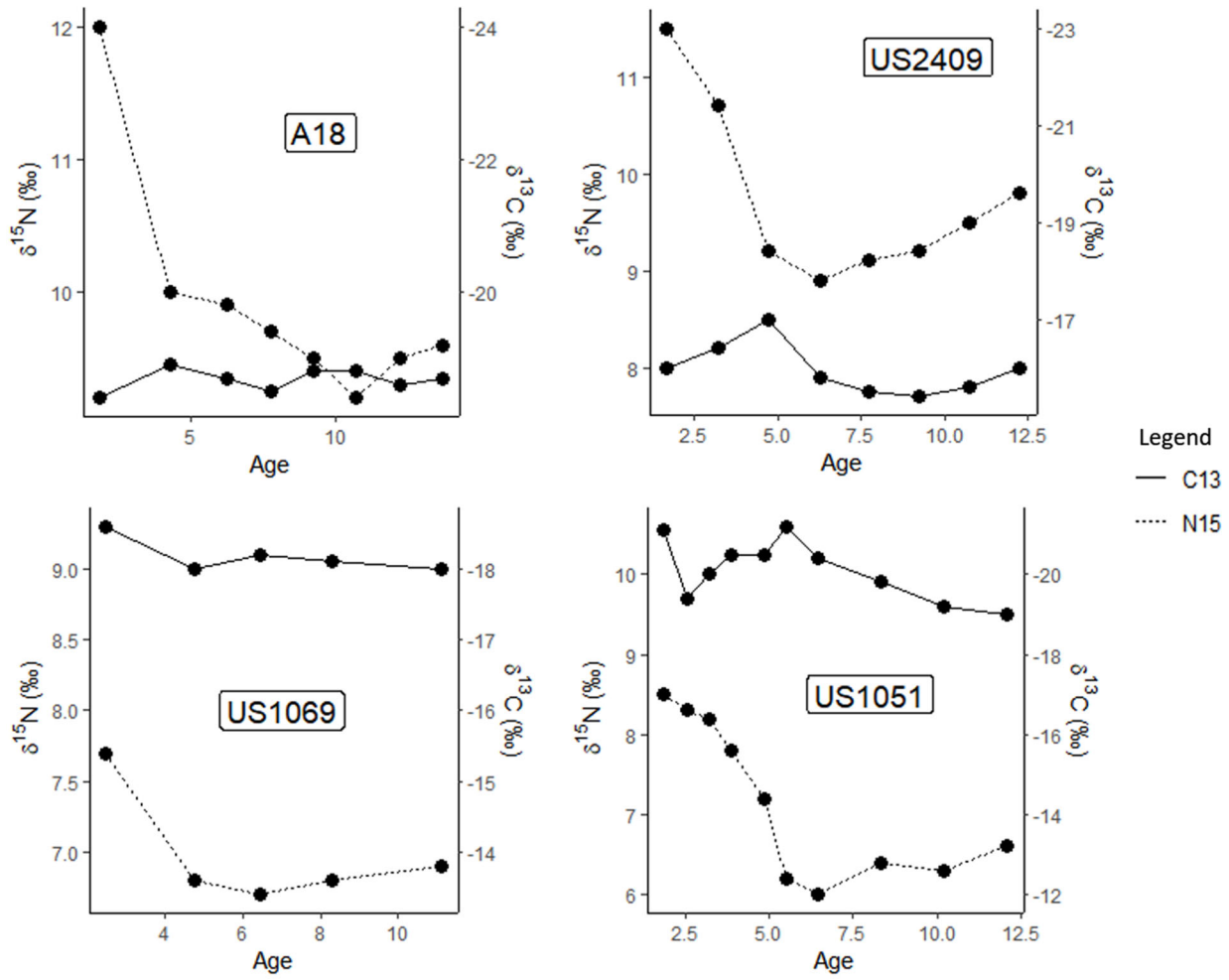

Figure 5.2: Representative weaning profiles derived from incremental dentin carbon and nitrogen isotope from Alia (A18), Badia Pozzeveri (US2409), Benabbio (US1051), and Pieve dei Monti di Villa (US1069).

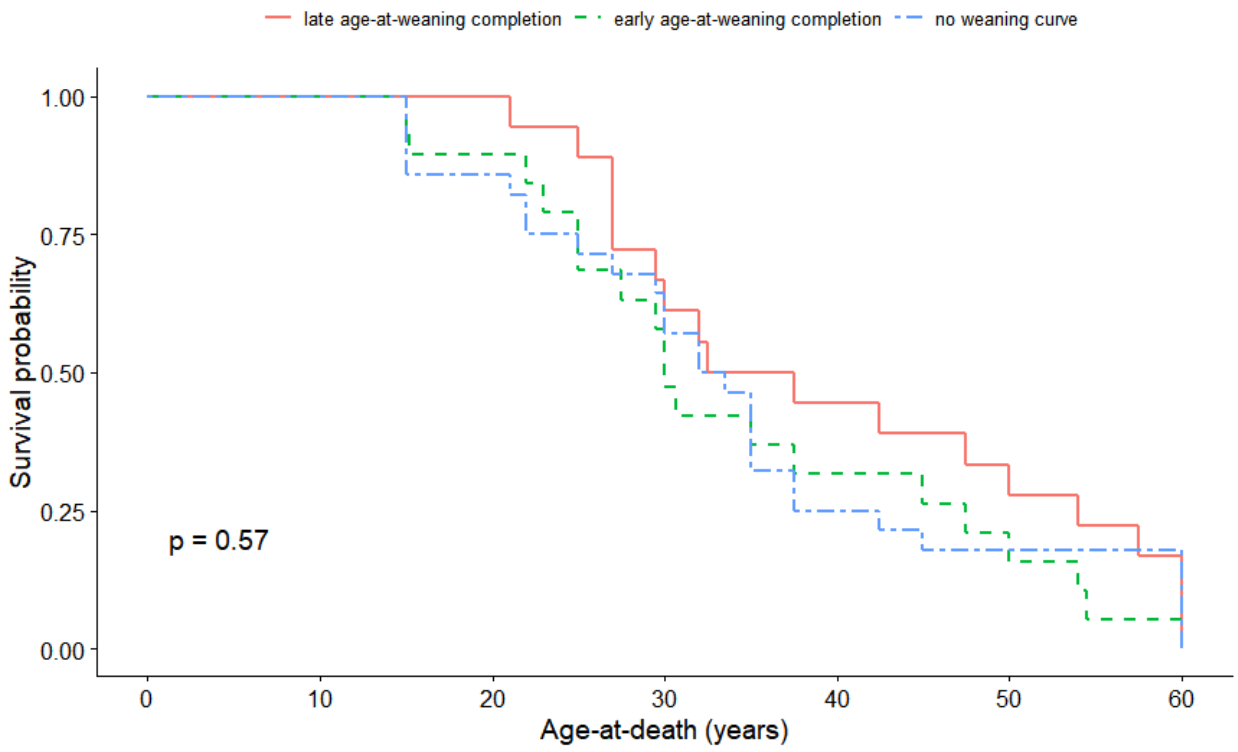

Figure 5.3: Kaplan-Meier survival curve comparing the populations with an early vs. late age-at-weaning completion, and individuals with no weaning curves.

CHAPTER 6

INFECTIOUS DISEASE MORTALITY AND THE DOHAD HYPOTHESIS: DOES EARLY CHILDHOOD STRESS AFFECT MORTALITY FROM INFECTIOUS DISEASE?[2]

[2] Smith Dobbs A, Reitsema LJ, Sineo L, and Fornaciari A. *To be submitted to the International Journal of Osteoarchaeology.*

6.1 Abstract

The plasticity/constraint and predictive-adaptive hypotheses under the developmental origins of health and disease (DOHaD) framework predict conflicting adult mortality outcomes for individuals who experience stress during early childhood. The plasticity/constraint hypothesis posits that energetic investment in survival in early childhood reduces the ability to adapt to later-life stress events, predisposing individuals who experience stress in early childhood to die younger. The predictive-adaptive hypothesis posits that early childhood stress causes physiological changes to the body that allow for increased survival later in life in a similarly stressful environment. This study evaluates these hypotheses in the context of 19th-century Italy by comparing pathological markers (porotic hyperostosis, cribra orbitalia, linear enamel hypoplasia, periostitis, stature) in cholera victims and contemporaneous, attritional populations. Paleopathology prevalence and stature estimation are used to assess the overall health of four populations from 19th-century Italy. We use chi-square tests, survivorship analysis, and odds ratios to compare these pathological markers between cholera victims and attritional populations. Our results find that cholera victims exhibit a significantly lower prevalence of cribra orbitalia and shorter stature compared to the attritional population, but these differences are a result of regional variation in paleopathology prevalence. Sicilians experienced lower rates of cribra orbitalia and shorter stature compared to Tuscans, which is a result of historical and secular trends. This research highlights how cultural context influences and limits the interpretation of bioarchaeological research on the DOHaD hypothesis.

6.2 Introduction

The developmental origins of health and disease (DOHaD) hypothesis posits that early childhood stress affects later-life health outcomes. The DOHaD hypothesis has been studied for

the past 40 years and the majority of this research has focused on the effects of *in utero* and early childhood stress on mortality from metabolic disease in adulthood, including high blood pressure (Barker et al., 1990; Barker et al., 1989a; Campbell et al., 1996; Law et al., 1991), glucose tolerance (Desai et al., 1995; Hales and Barker, 1992; Hales and Barker, 2001; Hales et al., 1991), and cardiovascular disease (Barker et al., 1989a; Barker et al., 1989b; Barker et al., 1993; Osmond et al., 1993). The DOHaD hypothesis has been applied widely in biological anthropology as a framework for understanding the effects of early childhood stress on metabolic disease mortality, but fewer studies have focused on infectious disease mortality or immune function (Blevins, 2015; McDade et al., 2001a; McDade et al., 2001b; Moore et al., 1999; Moore et al., 1997; Moore et al., 2001). The results of the studies that have focused on infectious disease has been mixed; the research of McDade et al. (2001a, 2001b) and Moore (1997) suggest that early childhood stress influences immune function, which leads to an increased risk of death from infectious disease later in life. In contrast, Moore et al. (2001) and Blevins (2015) find no relationship between skeletal markers of early childhood stress and infectious disease mortality.

In recent years, two theoretical models have emerged under the framework of DOHaD: the plasticity/constraint and the predictive/adaptive models (Temple, 2014; Temple, 2019). The plasticity/constraint hypothesis is derived from life history theory, which assumes each organism possesses a limited supply of energy for growth, maintenance, and reproduction (Charnov, 1991). The plasticity/constraint model posits that stress during early childhood reduces available energy for investment in later-life stages, leading to early mortality and reduced reproductive capacity in adulthood (Bogin et al., 2007; Temple, 2014; Worthman and Kuzara, 2005). In contrast, the predictive-adaptive hypothesis posits that early childhood stress produces developmental adaptations that buffer the organism from later-life stress events (Barker and

Osmond, 1986; De Boo and Harding, 2006; Ellison, 2005; Hales and Barker, 2001; Kuzawa and Quinn, 2009). Under this hypothesis, stressful early-life environments lead to developmental adaptations (decreased insulin sensitivity, etc.) that (1) adapt for future environments of scarcity or stress and (2) "program" the body to adapt better to scarce or stressful environments. The result of this programming increases the longevity of the individual, given a continuous scarce environment throughout the lifespan.

In a continuously scarce environment, including many historical contexts, these two models predict opposite mortality outcomes (Temple, 2019; Temple et al., 2012). Research exists that supports both the plasticity/constraint (Armelagos et al., 2009; Boldsen, 2007; DeWitte, 2012; DeWitte and Wood, 2008; Temple et al., 2012; Worthman and Kuzara, 2005) and predictive adaptive models (Barker and Osmond, 1986; De Boo and Harding, 2006; Ellison, 2005; Hales and Barker, 2001; Kuzawa and Quinn, 2009). For example, the timing of early childhood stress markers is positively associated with age-at-death among Jomon foragers, lending support to the plasticity/constraint model (Temple, 2014). In living populations, birth weight has been associated with impaired glucose tolerance and increased fat storage, which would be advantageous in a scarce environment (Hales et al., 1991; McCance et al., 1994). Temple (2019) suggests that these conflicting results may be attributable partially to differences in context and methodological approach. Specifically, Temple (2019) advocates for a contextually driven approach to bioarchaeological research that considers that the cultural and environmental context produces variable skeletal responses to early life trade-offs. This study explores how stress in early childhood affects mortality from infectious disease in the context of 18th- to 19th-century Italy, while emphasizing the influence of context and methods on data interpretation.

Bioarchaeological approaches to the DOHaD hypothesis allow for these hypotheses to be tested with both greater time depth and a greater breadth of human variation than would be possible for research using living subjects (Larsen, 2015). In the present study, we investigate how early childhood stress affects mortality from infectious disease by comparing skeletal markers of stress (cribra orbitalia, porotic hyperostosis, enamel defects, and growth stunting) among cholera victims from 18th- to 19th-century Italy to a contemporaneous, attritional population. In addition to directly addressing the DOHaD hypothesis, the present study also considers how heterogeneity in adult environment affects mortality from infectious disease in the context of 19th-century Italy. Specifically, we test the following hypotheses: (1) cholera victims have a statistically significant higher prevalence of paleopathological markers (porotic hyperostosis, cribra orbitalia, linear enamel hypoplasia (LEH) and periostitis) compared to contemporaneous, attritional populations, (2) the presence of pathological markers is associated with lower rates of overall survivorship when all populations are pooled, and (3) cholera victims exhibit significantly shorter stature compared to the attritional populations.

6.2.1 Cholera etiology and history

Cholera is a disease caused by the bacterium *Vibrio cholerae* and spread through contact with contaminated water sources. Cholera is a re-emerging infectious disease that has caused outbreaks throughout the world in recent decades, particularly in Haiti, South America, Africa, and the Middle East (Ali et al., 2012; Briggs, 1999; Kaper et al., 1995). The *Vibrio* bacterium infects a host by entering the intestines and releasing the cholera toxin (CT) which causes portals in the intestinal wall to become permanently open, causing the expulsion of water and electrolytes from the body into the intestines (Ali et al., 2012; Kaper et al., 1995). Unless treated with rehydration solution or antibiotics, victims of cholera typically die quickly from the

disease due to rapid dehydration; in severe cases, death can occur between 24 hours – 1 week after exposure (Ali et al., 2012; Briggs, 1999). Cholera is endemic in countries without sufficient sanitation standards and high rates of poverty and/or warfare (Briggs, 1999; Marwick, 1992; WHO, 2018). Recent research finds that individuals with blood type O are more likely to die from cholera infection than other blood types (Kuhlmann et al., 2016).

Several pandemic waves of cholera spread throughout Europe during the 19th century. The first pandemic (1816 - 1823) wave of cholera began in India in 1816 and spread into parts of east Asia and northern Africa before reaching Anatolia and the Caucasus (Evans, 1988; Morris, 2011). The second pandemic of cholera (1829 - 1932) started in India and spread throughout Europe and into the Americas. The second pandemic spread into Europe via Russia and across northern Europe. This strain of cholera was more virulent than previous strains and caused a massive loss of life among afflicted populations. Later, the pandemic spread into the Middle East and across the Mediterranean (Kotar and Gessler, 2014). The third cholera epidemic (1841 – 1859) spread into Europe and North America, while also reaching Central and South America. Three more waves of cholera spread throughout Europe before the end of the 19th century.

Since victims of cholera may die within 24 hours of exposure, cholera does not produce any skeletal lesions indicating the presence of disease. Any bony lesions on cholera victims represent past health insults, instead of disease markers related to death. Cholera burials for the present study were identified through a combination of historic documentation, burial style, and archaeological evidence (Baldino, 2011; Bigazzi, 1999; Chiarelli et al., 2002; Fornaciari, 2015a).

6.3 Materials and methods

6.3.1 Sample selection

Four 19th-century sites from Italy are selected for use in this study (Figure 6.1). The cholera victims are derived from sites located in Alia (n = 108), a small rural village in Sicily, and Benabbio (n = 46), a rural village located in Tuscany. Badia Pozzeveri (n = 37), and Pieve dei Monti di Villa (n = 17) are contemporaneous attritional populations from the 18th-19th-century Italy. While catastrophic, atypical burials have been discovered at Badia Pozzeveri (Gibbons, 2013), only the skeletons from typical, attritional burials from this site are included in this study.

Camposanto vecchio is a graveyard on the outskirts of Alia, a small, rural town on the mountainous interior of Sicily. Camposanto vecchio is located inside of a small cave outside of Alia where approximately 300 cholera victims from the epidemic of 1837 were interred (Bigazzi, 1999; Chiarelli et al., 2002). These remains were discovered in the spring of 1995 during the renovation of the wall that encased the tomb (Chiarelli et al., 2002; Guccione, 1991). This site was excavated and analyzed by researchers from the University of Palermo and many other universities between 1996 - 2000 (Chiarelli et al., 2002). The bodies at camposanto vecchio were buried collectively in a mass grave and covered in lime, a common cultural practice during the 19th century to prevent the spread of disease (Bigazzi et al., 2002; Morris, 1976). Osteological analysis and historic documentation indicates that all individuals interred at camposanto vecchio were cholera victims from the 1837 epidemic; the minimum number of individuals buried at camposanto vecchio is 296 and the number of cholera victims recorded in parish documents is 306 individuals (Bigazzi et al., 2002).

The church of San Michele (Benabbio) was the burial site of approximately 40 individuals from the cholera epidemic of 1855 in Benabbio, Tuscany. The village of Benabbio is located within the Val di Lima region of the Serchio Valley near the Lima Torrent, a main tributary for the Serchio River. The Serchio Valley is a mountainous region situated between the Pizzorne plateau to the south and the Tuscan-Emilian Apennine mountains to the north. The Val di Lima of Lucca is characterized by steep mountain slopes covered in forest that are divided by the Lima Torrent.

The church of San Michele is located at the apex of the mountain south of the Lima Torrent approximately 200 meters north-east of the village of Benabbio (Fornaciari et al., 2010). The church of San Michele was originally built in the 11th century as part of a medieval castle that was enclosed in a fortification wall. In 1334 CE, the main defensive structures of the castle were destroyed by invading Parmesan armies, causing the castle to lose its military function (Fornaciari et al., 2010). Between August 26th and October 21st, 1855, forty-six individuals from the village of Benabbio died of cholera and were buried next to the church of San Michele. Though the church was not in use during the 19th century, the cholera dead were buried in the church to avoid contamination and spread of disease because the church was located away from the village center. The arrangement of graves outside of the church of San Michele suggest a hasty burial (Fornaciari et al., 2010). The graves were narrow ditches that usually contain more than one individual. The bodies were placed in the grave on their side instead laying on their back which would be typical for a non-epidemic burial in 19th-century Italy. The bodies likely were wrapped in shrouds before burial, and there is no indication that coffins were used to bury these individuals. The church of San Michele was excavated by the University of Pisa between 2007 and 2013, and several articles have been published about the excavation project (Baldino,

2011; Bini et al., 2010; Fornaciari, 2015a; Fornaciari et al., 2010; Fornaciari and Coschino, 2012; Fornaciari, 2015b; Fornaciari et al., 2012).

Badia Pozzeveri's graveyard is located outside of the village of Altopascio, Italy, near the city of Lucca in Tuscany. The graveyard surrounds the 11th-century medieval church, the church of San Pietro, which has been excavated by the University of Pisa since 2013. The church of San Pietro is located along the Via Francigena, an important historic pilgrimage route that runs from Canterberry Cathedral in the United Kingdom to Rome, Italy. The church of San Pietro at Badia Pozzeveri has a long history that begins in the 11th-century and ends at the beginning of the 19th-century when it was abandoned (Seghieri, 1978). Historic deed transfers between the 10th- and 11th-centuries suggest the existence of the village of Badia Pozzeveri, but the first mention of the church of San Pietro di Pozzeveri occurred in September 1056 CE when four parcels of land were donated to the church (Seghieri, 1978). In 1103 CE, the church was converted to a monastery with an abbot and 12 resident monks, which included expanding the church's structure toward the south, including ranges that enclosed a courtyard that served as living quarters (Seghieri, 1978). A handful of burials at Badia Pozzeveri have burial patterns consistent with catastrophic burial, including non-traditional body placement and lime covered graves (Gibbons, 2013). Only burials that were consistent with traditional 18th-19th-century burial customs are included in this study.

Pieve dei Monti di Villa (abbreviated 'Monti di Villa') is a village located in the Bagni di Lucca region of Tuscany, approximately 12 km north-west of the church of San Michele in Benabbio. The parish of San Giovanni Battista of Monti di Villa was excavated by the University of Pisa starting in 2004 under the supervision of Professor Gino Fornaciari (Rezza, 2009). The skeletal material from the parish of San Giovanni Battista was previously studied by the Division

of Paleopathology at the University of Pisa by Dr. Angelica Vitiello, and Dr. Silvia Rezza (Rezza, 2009). Historical records indicate that the parish of San Giovanni Battista dates to as early as 772 CE (Rezza, 2009). The church was consistently used for baptisms and burials from the early medieval period until at least the mid-19th century. A new cemetery for the village was created in 1871 (Rezza, 2009). For the present research, burials that date between 1808 and 1870 are included as an attritional, comparative population.

6.3.2 Data collection

Pathological conditions are assessed using the standards outlined by Steckel et al. (2006). Generally, each pathological marker is given a score that ranged from 0 – 5 such that 0 indicates that the element is absent for observation, 1 indicates the absence of pathology, and any score above 2 indicates the presence and severity of the pathology. In this study, we report the prevalence of cribra orbitalia, porotic hyperostosis, LEH, and periostitis because these pathological conditions are most commonly found among our study groups. Stature is analyzed as an indicator of chronic stress during childhood.

LEH are linear defects on the surface teeth that develop during tooth formation in early childhood in response to non-specific stressors over a period of weeks to months (FitzGerald, 1998; Hillson, 2014; King et al., 2005). LEH forms on the crown of the tooth during tooth development, which is highly genetically regulated and forms at specific times in childhood, generally beginning at birth for central incisors and ending around 7.5 years old for M2s (Goodman and Rose, 1990; Massier et al., 1941).

Porotic hyperostosis and cribra orbitalia are porotic lesions on the cranium that develop as a result of expansion of the diploë of the cranium that either is caused by anemia, vitamin B12 deficiency, or helminth infections (McIlvaine, 2015; Stuart-Macadam, 1985; Stuart-Macadam,

1989; Stuart-Macadam, 1992; Walker et al., 2009). Active lesions of both porotic hyperostosis and cribra orbitalia usually are found in childhood, and healed lesions more frequently are found in adulthood, so porotic hyperostosis and cribra orbitalia generally reflect episodes of childhood anemia (Stuart-Macadam, 1985; Stuart-Macadam, 1992).

Periostitis is a non-specific indicator of infectious disease that results in the inflammation of the periosteum (Waldron, 2009). The inflamed periosteum causes abnormal mineralized deposits on the surface of the bone. Periostitis can occur at any time during one's lifespan and therefore is used as an indicator of heterogeneous risks in health due to environmental conditions (Mensforth et al., 1978). In the present study, we assess presence and severity of periostitis on the long bones, mainly the femur and tibia. Since the samples from Alia are disarticulated, we assess presence and severity of periostitis on the left tibia for this assemblage.

We use regression equations derived from Hauser et al. (2005) to calculate stature for these populations. Though the anatomical method for stature estimation is more accurate, we chose to use regression equations to calculate stature because of the fragmentary nature of the skeletal collections used in this study and because one population (Alia) is a disarticulated collection that could not be assessed for stature using the anatomical method (Fully, 1956; Raxter et al., 2007; Raxter et al., 2008). The regression equations from Hauser et al. (2005) are derived from a modern Polish population, which is geographically and temporally approximate to modern Italy. Male stature is calculated using the following equation:

$$2.90 \times \text{Maximum Length of Femur} + 37.596$$

Female stature is calculated using the following equation:

$$2.59 \times \text{Maximum Length of Femur} + 50.815$$

Age estimation is calculated using dental development (Ubelaker, 1999), dental wear (Smith, 1991), cranial suture closure (Lovejoy et al., 1985a), epiphyseal plate closure (Haas et al., 1994), pubic symphysis (Brooks and Suchey, 1990; Haas et al., 1994), and the auricular surface (Lovejoy et al., 1985b). Sex is estimated using cranial and pelvic features using the standards outlined in Steckel et al. (2006).

6.3.3 Statistical methods

All statistical analyses for this research are performed using the statistical software platform R (version 3.3.3). Pearson's Chi-squared tests with Yates continuity correction test the significance of the difference in prevalence between the cholera victims and attritional populations. In cases where differences between groups are found, a logistic regression analysis is used to determine if the presence of the skeletal lesion of interest is associated with death from cholera, controlling for age, sex, and site. Survival analysis and Welch Two Sample t-tests are employed to determine how survivorship is affected by the presence or absence of pathological markers. Survival curves are used in epidemiological studies to estimate the probability of survival at specific age intervals and how the probability of survival changes with increasing age. A Welch Two Sample t-test is used to compare stature between cholera and the attritional populations.

6.4 Results

The median age-at-death for cholera victims is 32.5 years and the median age-at-death for the attritional population is 37.5 years. When the data is grouped by site, Alia ($\overline{x}$ = 29.5 years) exhibits the lowest age-at-death compared to Benabbio ($\overline{x}$ = 52.3 years), Monti di Villa ($\overline{x}$ = 45.0 years), and Badia Pozzeveri ($\overline{x}$ = 36.3 years).

At Alia, 8 out of 108 individuals (7.4%) exhibit cribra orbitalia, whereas 11 out of 26 individuals (42.3%) from Benabbio exhibit cribra orbitalia. At Badia Pozzeveri, 5 out of 21 total individuals (23.8%) exhibit cribra orbitalia, and 6 out of 9 individuals (66.7%) from Monti di Villa exhibit cribra orbitalia. Cribra orbitalia presence is significantly lower among cholera victims compared to the attritional populations in this sample ($\chi^2 = 6.857$, df = 1, $p = 0.009$), but when the Sicilian site is compared to the Tuscan sites, the prevalence of cribra orbitalia in the Sicilian site (Alia) is significantly different from the Tuscan sites ($\chi^2 = 31.071$, df = 1, $p < 0.001$). Alia also exhibits statistically significant differences in cribra orbitalia prevalence compared to Benabbio alone ($\chi^2 = 18.205$, df = 1, $p < 0.001$). The three sites from Tuscany do not exhibit statistically significant differences in cribra orbitalia prevalence ($\chi^2 = 5.0372$, df = 1, $p = 0.08$). Due to low sample size and low statistical power, the logistic regression analysis for cribra orbitalia is excluded from the results of this study.

No statistically significant differences between Alia and Benabbio are found for all other pathological markers (porotic hyperostosis, periostitis, and LEH), so these sites are grouped for comparison to the attritional populations. Porotic hyperostosis is slightly less prevalent among cholera victims, whereas periostitis and LEH are more prevalent among cholera victims. These relationships are not statistically significant, however. Table 6.1 summarizes the prevalence for cribra orbitalia, porotic hyperostosis, and LEH for catastrophic and attritional individuals. Table 6.2 summarizes the prevalence of pathological markers when the sites are analyzed separately.

The average age of death is significantly older for those individuals with periostitis ($\overline{x}$ = 47.47 years) compared to individuals that did not have periostitis ($\overline{x}$ = 39.94 years) (95% CI [-13.89, -1.18], t = -2.362, df = 74.826, p = 0.02). The average age of death does not differ when comparing present/absent groups for cribra orbitalia, porotic hyperostosis, and LEH. The average

age-at-death for cholera ($\overline{x}$ = 34.96 years) versus attritional ($\overline{x}$ = 37.54 years) populations do not differ significantly. The survival curves for cribra orbitalia, porotic hyperostosis, periostitis, and LEH are summarized in Figure 6.2.

To test if there are differences in stature between the individual sites, an analysis of variance (ANOVA) is conducted to compare stature between Alia, Pieve dei Monti di Villa, Benabbio, and Badia Pozzeveri (Figure 6.3). The ANOVA is also statistically significant ($df = 3$, $f = 21.59$, $p < 0.001$), and a Tukey Honestly Significant Difference (HSD) reveals that the stature of the samples from Alia are significantly different from the stature of individuals from Benabbio (95% CI [5.79, 13.72], $p < 0.001$) and Badia Pozzeveri (95% CI [4.95, 13.03], $p < 0.001$).

6.5 Discussion

Differences in paleopathology prevalence between Tuscan and Sicilian sites suggests that regional variation may be a better explanation for the significant differences between cholera victims and attritional populations than the predictive/adaptive or plasticity constraint hypotheses. The stature the cholera victims from Alia is significantly shorter compared to the cholera victims in Benabbio, whereas the individuals from Tuscany exhibit statistically similar statures. These results are consistent with historic documents indicating that regional differences in stature existed during the 19th century such that southern Italians exhibited an overall shorter stature compared to northern/central Italians (Arcaleni, 2006; Martínez-Carrión and María-Dolores, 2017). Regional differences in stature during the modern period in Italy correlate with disparities in living conditions and economic growth.

Cribra orbitalia prevalence also varies among the four sites in this study. Alia exhibits a significantly lower prevalence of cribra orbitalia compared to the Benabbio. The population at Benabbio is older (m = 52.25 years) compared to Alia (m = 29.5 years), and thus the low

prevalence of cribra orbitalia observed at Alia cannot be attributed to bone remodeling of cribra lesions. There are several possible explanations to explain why Sicilians appear to have a significantly lower prevalence of cribra orbitalia compared to Tuscans during the 19th century. First, it is possible that the cholera pandemic of 1832 in Alia was less selective than the pandemic experienced by the population of Benabbio in 1855. We would expect that the Alia population would exhibit a lower prevalence of pathological markers overall compared to later pandemics if the 1832 pandemic was less selective than later epidemics. The rates of paleopathology are comparable between all sites in this study, however. Another possible explanation is that dietary differences exist between the two sites such that the population from Alia derived a higher proportion of their diet from terrestrial protein sources compared to the population from Benabbio. Previously published data finds that the population from Benabbio exhibits a bone collagen $\delta^{15}N$ of 8.3±1.0‰ and $\delta^{13}C$ of -19.8 ±1.3‰ (Fornaciari, 2015b). Unpublished data from Alia finds that the bone collagen $\delta^{15}N$ are around 10.2‰ and $\delta^{13}C$ around -18.6 ‰ (Walker, 2019; Walker et al., 2019). The low nitrogen values from Benabbio compared to Alia could be indicative that the individuals from Benabbio were consuming less terrestrial protein compared to those from Alia, and thus were at a higher risk for developing anemia. Researchers have questioned whether iron-deficiency anemia can result in marrow hypertrophy that produces cribra orbitalia and porotic hyperostosis lesions, however (McIlvaine, 2015; Oxenham and Cavill, 2010; Walker et al., 2009). Walker et al. (2009) posits that iron-deficiency anemia could not cause cribra lesions because iron-deficiency results in a reduction in red blood cell production which prevents the hemopoietic marrow expansion that produces cribra lesions. Oxenham and Cavill (2010), however, asserts that iron deficiency anemia results in an increase

in ineffective erythropoiesis and consequently results in bone marrow expansion. So, it is possible that diet may not be a factor in the development of cribra orbitalia.

6.6 Conclusion

This study finds no association between early childhood stress and infectious disease mortality. Previous studies have found either no association between early childhood stress and infectious disease mortality (Blevins, 2015), or excess mortality associated with cribra lesions (DeWitte and Wood, 2008). Though we find significant differences in cribra orbitalia prevalence between catastrophic and attritional populations, site-based variation in cribra orbitalia prevalence suggests that these differences are attributable to variation in cultural context. This study also finds that cholera victims exhibit significantly shorter stature compared to the attritional population, which is consistent with previous bioarchaeological research (DeWitte and Hughes-Morey, 2012). This result, however, is also consistent with secular trends in stature across the Italian peninsula during the 19th century. We attribute differences in average stature among the people at the central and southern sites to be a result of the geographic trends in stature during the 19th century.

This research highlights the importance of considering cultural context in interpreting bioarchaeological data using the DOHaD hypothesis (Temple, 2019). Temple (2019) discusses how cultural context and methodological approach influence the interpretation of bioarchaeological data on the effects of early childhood stress on adult mortality outcomes, which may produce the conflicting interpretations (Blevins, 2015; DeWitte and Hughes-Morey, 2012; McDade et al., 2001a; McDade et al., 2001b; Moore et al., 1999; Moore et al., 1997; Moore et al., 2001). In the present study, site-based variation explains any differences we find between catastrophic and attritional populations. This study demonstrates that bioarchaeologists

should consider historical or environmental trends in paleopathology frequencies when comparing multiple sites and consider the limitations in methodological approach.

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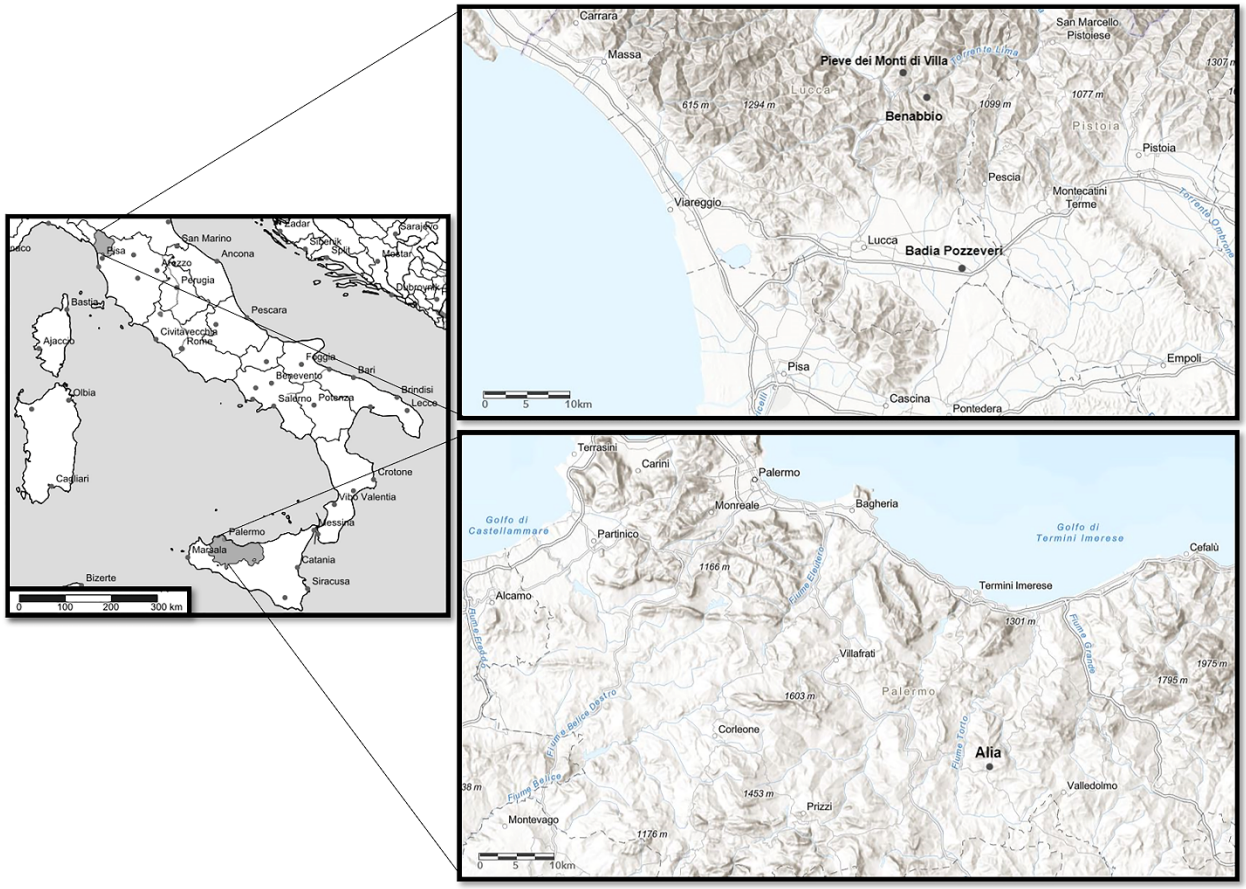

Figure 6.1: Map of archaeological sites: Alia, Benabbio, Badia Pozzeveri and Pieve dei Monti di Villa.

Table 6.1: Prevalence of pathological markers comparing cholera victims and attritional population

Pathology	Cholera		Attritional	
	n	%	n	%
Cribra Orbitalia	19	14.2	11	36.7
Porotic Hyperostosis	55	38.2	17	39.5
Linear Enamel Hypoplasia	56	76.7	25	65.8
Periostitis	121	43.8	16	33.3

Table 6.2: Prevalence of pathological markers between sites.

Pathology	Alia		Benabbio		Badia Pozzeveri		Monti di Villa	
	n	%	n	%	n	%	n	%
Cribra Orbitalia	8	7.4	11	42.3	5	23.8	6	66.7
Porotic Hyperostosis	41	38.3	14	37.8	9	28.1	8	72.7
Linear Enamel Hypoplasia	30	75	26	78.8	22	68.8	3	50.0
Periostitis	107	45.5	14	34.2	9	25.0	7	58.3

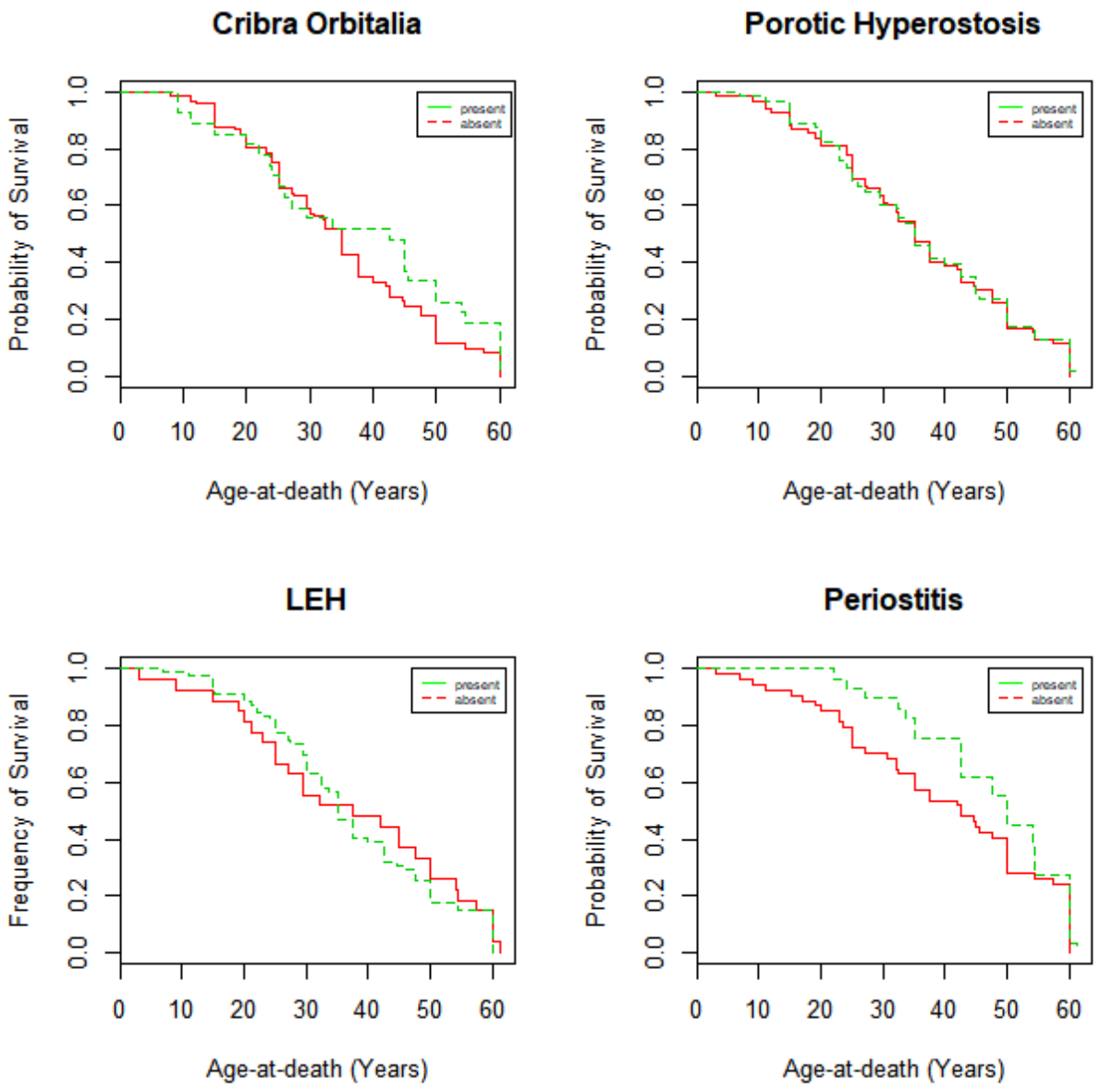

Figure 6.2: Survival curves for cribra orbitalia, porotic hyperostosis, linear enamel hypoplasia, and periostitis.

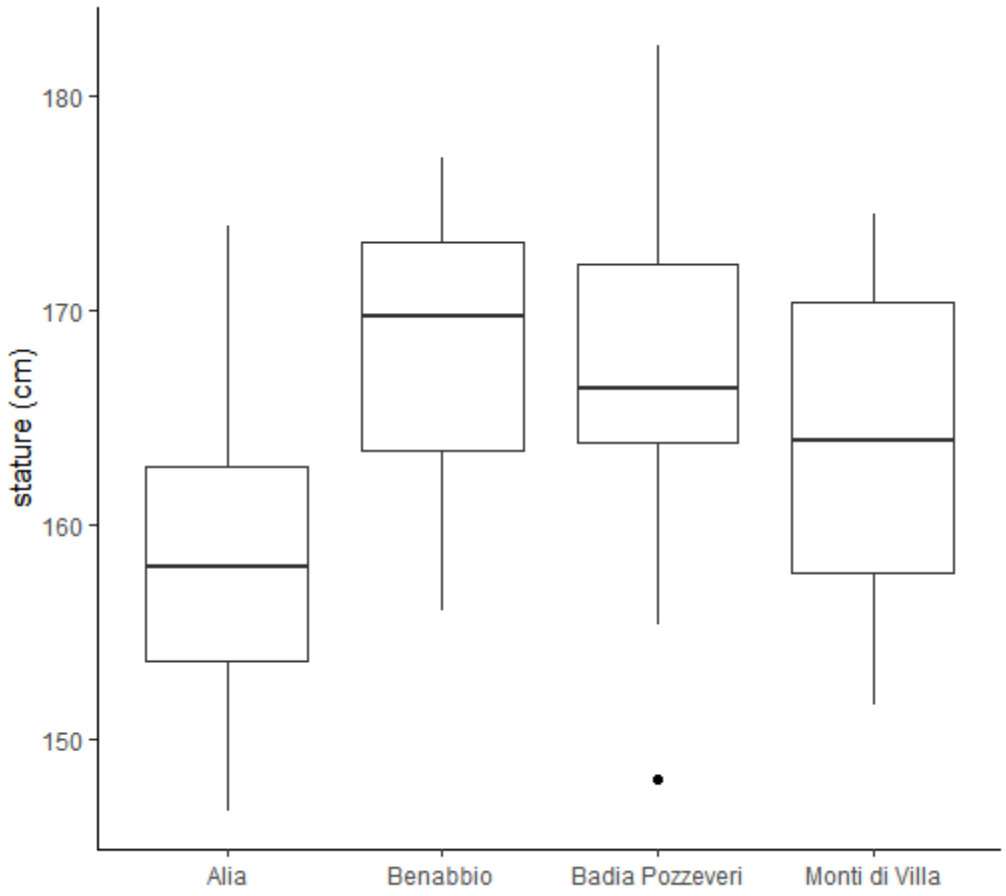

Figure 6.3: Boxplot of the estimated stature of each site. The stature of individuals from Alia is statistically different from Badia Pozzeveri (95% CI [4.95, 13.03], $p < 0.001$) and Benabbio (95% CI [5.79, 13.72], $p < 0.001$).

CHAPTER 7

ADULT INFECTIOUS DISEASE MORTALITY AND EARLY CHILDHOOD STRESS: COMPARING THE TIMING AND FREQUENCY OF ACCENTUATED STRIAE BETWEEN CATASTROPHIC AND ATTRITIONAL POPULATIONS[3]

[3] Smith Dobbs A, Reitsema LJ, Sineo L, and Fornaciari A. *To be submitted to the American Journal of Physical Anthropology*

7.1 Abstract

Stress events in the first years of life cause physiological alterations that affect adult health outcomes. Though the effects of early life childhood stress on adult metabolic syndrome are well researched, less research has focused on the effects of early childhood stress on infectious disease mortality in adulthood. Catastrophic burial populations that resulted from pandemic waves of cholera during the 19th century are compared to contemporaneous attritional populations to investigate the relationship between stress in early childhood and adult infectious disease mortality. Specifically, this research investigates if the timing and frequency of accentuated striae of Retzius are associated with infectious disease mortality in adulthood.

Canines from 46 individuals from 18th-19th-century catastrophic and attritional populations are assessed for the presence, periodicity, and timing of accentuated striae of Retzius. Age-at-first defect, age-at-defect formation, number of accentuated striae, and periodicity are compared between the populations. Regression analysis is performed to determine if any significant relationships exist between periodicity, age-at-death, and number of accentuated striae relative to age-at-first defect.

Significant differences are found between each site with regards to age-at-defect formation, so subsequently each site is statistically analyzed individually instead of pooled. Sites exhibit a similar number of accentuated striae of Retzius, suggesting that the stress burdens are similar across all sites. Interregional differences in periodicity and age-at-first defect are found between the sites, implying that the timing of stress events varied across the Italian landscape. No significant relationships are found between age-at-death and age-at-first defect for any site. This research finds that cultural context is highly influential in the interpretation of bioarchaeological data for the DOHaD hypothesis.

7.2 Introduction

The developmental origins of adult health and disease hypothesis (DOHaD) posits that stress *in utero* and early childhood causes physiological changes that result in increased susceptibility to mortality in adulthood (Barker and Osmond, 1986; Barker, 2004). The DOHaD hypothesis traditionally focuses on the emergence of chronic adult metabolic conditions, including heart disease and type II diabetes, as responses to stressful conditions in early childhood (Barker, 1995; Barker, 1997; Barker et al., 1990; Barker et al., 1992; Barker et al., 1989; Desai et al., 1995). Only a few studies focus on the impact of early childhood stress on immune system function in adulthood and these often yield conflicting results (Blevins, 2015; McDade et al., 2001a; McDade et al., 2001b; Moore et al., 1999; Moore et al., 1997; Moore et al., 2001). In living populations, early childhood stress is associated with low thymus weights (McDade et al., 2001b; Watts, 1969), ineffective immune responses (McDade et al., 2001a), and decreases in the function of lymphocytes (Chandra, 1993). Some research using skeletal assemblages finds that some markers of early childhood stress are associated with infectious disease mortality (DeWitte, 2012; DeWitte and Wood, 2008), whereas similar research finds no association (Blevins, 2015). The present research uses dental histology to compare the timing and frequency of early childhood stress markers of populations who experienced cholera with those that did not (referred to here as attritional populations). The purpose of this research is to determine if the timing and frequency of accentuated striae of Retzius are associated with infectious disease mortality.

Two main theoretical models have emerged within the DOHaD framework that present opposing outcomes given specific conditions. The plasticity/constraint model assumes that each living organism has a limited supply of energy to invest in growth, maintenance, or reproduction.

Trade-offs occur as a result of intensive energetic investment during periods of stress, with maintenance prioritized at the expense of growth or reproduction. Under this model, early childhood stress triggers a trade-off between survival in the short-term and later-life adaptability (Bogin et al., 2007; Temple, 2014; Worthman and Kuzara, 2005). The result of this trade-off is an overall higher risk of death in adulthood among individuals who experienced stress in early childhood.

The predictive-adaptive model, which emerged from the thrifty phenotype and fetal programming hypotheses, describes how early childhood stress results in physiological changes that buffer the organism from stress events in the future (Barker and Osmond, 1986; De Boo and Harding, 2006; Ellison, 2005; Hales and Barker, 2001; Kuzawa and Quinn, 2009). For example, childhood nutritional deprivation may cause physiological changes that result in an increased capacity to store fats. If the environment remains consistently scarce throughout the organism's lifetime, then the increased capacity to store fats is advantageous because the organism is buffered from famine. Mismatches between the environment experienced *in utero* or during early childhood and those encountered in adulthood can result in early childhood physiological adaptations that are maladaptive in adulthood, however. If deprivation in early childhood leads to increased fat storage, and the adult environment is calorie-rich, then the individual will likely manifest a metabolic disorder, such as obesity, type II diabetes, or heart disease. If the environment remains persistently stressful, then early childhood stress will actually confer a survival advantage in adulthood because the bodies of individuals stressed during childhood will be adapted to a stressful environment.

These two models appear to predict divergent health outcomes in adulthood. The predictive-adaptive hypothesis predicts a survival advantage in adulthood for those who

experience early childhood stress given a consistently stressful environment. The plasticity/constraint hypothesis predicts reduced survivorship among adults who experienced early childhood stress (Temple, 2014; Temple, 2019). Bioarchaeological research testing the DOHaD hypothesis has yet to reach a consensus on either the predictive-adaptive or plasticity/constraint hypotheses. Several bioarchaeological studies find evidence that early childhood stress reduces overall mortality (Armelagos et al., 2009; Beaumont et al., 2015; Temple, 2014), and other studies demonstrate the same link between early childhood stress and infectious disease mortality risk (DeWitte and Hughes-Morey, 2012; DeWitte and Wood, 2008). Other studies support the hypothesis that early childhood stress decreases overall mortality risk (Boldsen, 2007). Temple (2019), in his review of bioarchaeological studies that used the DOHaD theoretical framework, suggests that environmental factors, cultural context, and methodological approach are the main factors producing inconsistencies in the results of these studies. The research presented here explores how regional and inter-regional differences in the timing and frequency of early childhood stress influences the interpretation of bioarchaeological data by exploring site-based variation within our sample.

Dental histology is a bioarchaeological method used to analyze periods of stress in early childhood (FitzGerald et al., 2006; Fitzgerald and Saunders, 2005; Rose et al., 1978; Żądzińska et al., 2015). Tooth enamel grows incrementally at a regular periodicity starting from the tooth crown and concluding at the apex of the root. Every 8-10 days, a new layer of enamel forms parallel to the enamel-dentin junction (EDJ) called a stria of Retzius (Goodman and Rose, 1990; Goodman and Rose, 1991; Hillson, 2014; Schwartz and Dean, 2008). Enamel, once formed, does not remodel, and thus preserves a record of stress events occurring *in utero* or very early childhood. Metabolic disturbances during tooth formation disrupts enamel formation and may

cause a discontinuity in the enamel that appears as a brown or accentuated striae of Retzius (Hillson, 2014; Rose et al., 1978; Wilson and Shroff, 1970). Accentuated striae usually form independently of surface enamel defects, such as linear enamel hypoplasia, and generally are interpreted as evidence of short-term stress (Witzel et al., 2008). Since enamel formation is tightly genetically regulated and tooth formation ages are well understood, the age at which the defect formed can be estimated (Goodman and Rose, 1990; Goodman et al., 1988). A chronology of stress events in early life can be reconstructed using adult teeth by measuring the distance between successive accentuated striae (Armelagos, 2010; FitzGerald et al., 2006; Goodman et al., 1988; Rose et al., 1978; Wright, 1990).

In the present study, age-at-defect formation, age-at-first defect, and periodicity of accentuated striae are used to examine timing of stress events. Age-at-defect formation describes the approximate age at which the stress experienced by the individual was sufficient enough to cause enamel growth disruption. In dental anthropology, age of defect formation is used to reconstruct the chronology of stress events that occurred during the time of tooth formation (Dobney and Ervynck, 2000; Temple, 2016). Age-at-first defect is age at which the first defect for an individual is observed. Among Jomon foragers, linear enamel hypoplasia age-at-first defect is positively and significantly associated with age-at-death. Periodicity describes the time interval between successive occurrences of accentuated striae. Periodicity of accentuated striae illustrates how frequently individuals experienced stress episodes (King et al., 2002; King et al., 2005; Temple, 2014; Temple, 2016).

The research presented here uses catastrophic and attritional populations from 18th-19th-century Italy. At the beginning of the 18th century, Italy was fractured into several small states, some of them controlled by foreign occupants (Duggan, 2014; Hearder, 1990). Throughout the

18th and 19th century, Italy was subject to successive wars that had parts of Italy traded between more dominant nations, specifically France, Britain, Austria, and Spain, with only certain parts of Northern Italy remaining independent (Domenico, 2002a; Domenico, 2002b; Duggan, 2014; Hearder, 1990; Noether, 1986). Italian unification was achieved in 1861 under the rule of Vittorio Emanuele II, King of Sardinia-Piedmont. Rome was later added to the new Italian state in 1871. Unlike Western Europe, Italy did not experience significant industrialization until the late 19th century. The Italian economy relied heavily on agriculture, and the majority of the populace lived in poor conditions with high infant mortality and a life expectancy around age 35 years (Collantes, 2006; Dalla-Zuanna and Rosina, 2011; Duggan, 2014; Leoni, 1991; Profumieri, 2009).

Cholera was also a concern for Italy with several pandemic waves of cholera spreading across Europe throughout the 19th century (Sack et al., 2004; Thomas, 2015). Cholera is caused by the bacterium *Vibrio cholerae* which infects victims through contaminated water or food (Sack et al., 2004; Thomas, 2015). Cholera causes rapid dehydration which can result in death in 6-8 hours in severe cases (Morris, 2011). Cholera victims are used here because cholera does not produce pathological skeletal markers, thus all pathological conditions in the skeletal remains of cholera victims are the result of previous stress events. All skeletal markers in cholera victims can be attributed to prior stressors and not to the cause of death.

To assess the association between the timing of stress events in childhood and adult infectious disease mortality, this study has two main research objectives: First, we determine if there are statistically significant differences in the prevalence or timing of stress events between the sites by comparing the number of accentuated striae, age-at-first defect, age-at-defect formation, and periodicity between the sample populations. Second, we determine if age-at-first

defect formation exhibits a statistically significant relationship with age-at-death, periodicity, and number of accentuated striae. More broadly, this research establishes the association between the timing and frequency of accentuated striae of Retzius and infectious disease mortality.

7.3 Materials and methods

Four Italian sites are included in this study: Alia, Benabbio, Pieve dei Monti di Villa, and Badia Pozzeveri. Alia and Benabbio are sites where cholera victims dying in 1832 and 1855, respectively, were interred. Badia Pozzeveri and Pieve dei Monti di Villa are comparative cemeteries that date to the 18th-19th century where our comparative, attritional populations were interred. Figure 7.1 maps the location of each site.

Alia is a small inland village in the province of Palermo on the island of Sicily. Alia is located within the Madonie mountain range and the settlement dates to the 1200s. During the cholera epidemic of 1837, approximately 300-400 individuals from Alia died of cholera and were buried in a mass grave known as camposanto vecchio (Bigazzi et al., 2002; Chiarelli et al., 2002; Guccione, 2002). Camposanto vecchio is located outside the main village inside a cave. The cholera victims were exhumed in 1995 during a renovation of the tomb wall and the remains were examined at the University of Palermo and other European universities between 1996-2000 (Chiarelli, 2002; Guccione, 1991).

Archaeological evidence and historic documents confirm that the remains recovered from the camposanto vecchio were cholera victims from the cholera epidemic of 1837. A parochial document from the Parish of Saint Anna in Alia describes the beginning of the epidemic on July 7, 1837 and lists the names of those who died from the epidemic by date (1837). The minimum number of individuals in the mass grave is 296 individuals, a number corroborated by parish records, which lists 306 Alia cholera victims in 1837 (Bigazzi et al., 2002). Mortuary analysis of

the mass grave also indicates that these individuals died during a catastrophic event. The mass burial of individuals outside the main village suggests that several hundred people died within a short time period. The distance from the main village implies that the cause of death was an infectious agent and that bodies were not carefully buried within the village to prevent the spread of disease.

Benabbio is a Tuscan village situated south of the Lima torrent in the Serchio valley. Approximately 200 m north-east of the modern-day village of Benabbio sits the church of San Michele where cholera victims from the cholera pandemic of 1855 were buried. Archaeological and historic evidence supports the argument that the individuals buried outside the church of San Michele were victims of the 1855 cholera epidemic. Similar to Alia, the remains were interred outside of the main village, an approximately 20-minute walk uphill from the village center. Burial of these individuals was haphazard, with several individuals sharing a commoner grave plot, suggesting that these bodies were interred hastily. Additionally, these burials were covered in lime, a common 19th-century burial practice thought to prevent the spread of infection or disease (Fornaciari et al., 2010; Morris, 1976). The church was excavated by the University of Pisa between 2007 and 2013, during which time they uncovered the remains of 41 individuals in the 19th-century layer, which is consistent the Benabbio parochial records (Baldino, 2011; Fornaciari et al., 2010).

Badia Pozzeveri, one of the attritional populations, is from a borough of the village of Altopascio approximately 20 km east of the city of Lucca. Within Badia Pozzeveri is an 11th-century church, the church of San Pietro, surrounded by a cemetery that was used by the community between the 11th century and the beginning of the 19th century. The church's facade is approximately 6 m from the buried medieval church's facade. During the 17th century, the roof

of the medieval church collapsed, and the collapsed section of the church was converted into a cemetery during the 18th-19th centuries (2018; Seghieri, 1978). The medieval burials at this site were located in front of and along the sides of the medieval facade, whereas the 18th-19th-century burials are mainly located under the floor of the medieval church. Badia Pozzeveri was excavated by the University of Pisa beginning in 2013 (Gibbons, 2013).

Pieve dei Monti di Villa, the other attritional population, is from a village located across the Lima Torrent from Benabbio in the Tuscan Serchio Valley. The parish of San Giovanni Battista is a small parish church in Pieve dei Monti di Villa where 10 individuals were buried in the 18th-19th century. The church is located within the main village and was used continuously from its conception in 772 CE into the early 18th century as church and cemetery (Rezza, 2009). The Pieve was excavated by the University of Pisa between 2002 and 2006 and analyzed for paleopathology and stable carbon and nitrogen isotope ratios by the university's researchers (Fornaciari, 2002; Fornaciari, 2004; Fornaciari, 2006; Rezza, 2009).

7.3.1 Methods

Fifty-four canines from catastrophic populations, Alia (n = 32) and Benabbio (n = 22) are analyzed for this project. Twenty-five canines from the attritional populations, Badia Pozzeveri (n = 16) and Pieve dei Monti di Villa (n = 9), are analyzed for comparison. In total, 44 canines from males, 27 from females, and 8 from individuals of indeterminate sex are sampled. Canines are chosen for this analysis because they are more hypoplastic than other teeth and thus are more likely to manifest enamel defects in response to environmental stressors (Goodman and Armelagos, 1985; Goodman et al., 1980). Canines were selected for inclusion in this study if their tooth wear was minimal. The canines are cut using a Dremel® to remove the root approximately 1 mm below the cementoenamel junction. The tooth crown is embedded in epoxy

resin using the Streurs Epo-Fix Epoxy kit. After embedding, the tooth crown is thin-sectioned using the Buehler Isomet 11-1800 diamond-blade microtome saw. The thin sections are polished using the Hillquist thin section lapidary machine polisher and acid-etched with 1M HCl. Using an Olympus BX50 microscope. Measurements of the thin sections are captured using the VisionGauge® software.

Accentuated striae of Retzius are defined as darkened striae spanning 75% of the length visible from the EDJ to the crown surface (Goodman and Rose, 1991). The presence and number of accentuated striae per thin section are recorded. The summary statistics for number of individuals and accentuated striae per site is presented in Table 7.1. Where applicable, the defect age is estimated for each accentuated striae by dividing the distance of the accentuated stria from the cemento-enamel junction (CEJ) by the average width of two striae of Retzius. This calculation produces the number of striae of Retzius between the accentuated striae and the CEJ, which is multiplied by the average periodicity of accentuated striae (~8-10 days) to find the number of days from the formation of the accentuated striae to the completion of crown formation (Goodman and Rose, 1990; Goodman and Rose, 1991; Hillson, 2014; Schwartz and Dean, 2008). Canine crowns are complete between 4 - 5.4 years of age, and so an average age of 4.4 years is used to estimate defect age for the present study (Anderson et al., 1976; Daito et al., 1990; Fanning and Brown, 1971; Haavikko, 1970; Liversidge, 1995; Moorrees et al., 1963; Nielsen and Ravn, 1976; Trodden, 1982). Periodicity is calculated by subtracting the age of defect formation between successive accentuated striae. For example, if defects occur at 2.4 and 2.6 years, then the periodicity would be 0.2 years.

Several statistical analyses are used to compare the number of accentuated striae, age-at-defect formation, age-at-first defect, and periodicity between sites. Pearson's Chi Square Test is

used to assess differences between groups using accentuated striae count data. For age-at-defect formation, age-at-first defect, and periodicity, histograms and Shapiro-Wilks tests are used to assess the normality of subgroups. Age-at-defect formation is used to determine if the sites are sufficiently similar to pool. If the subgroups are normally distributed, a parametric Pearson's T-test or ANOVA is used to compare groups. Non-normally distributed groups are compared using a Kruskal-Wallis Test. A parametric Tukey test or non-parametric Dunn Test with Benjamini-Hochberg method is used if the results of the ANOVA or Kruskall-Wallis test is statistically significant. A multivariate linear regression is used to determine if age-at-death is related to age-at-first defect, periodicity, and accentuated striae count. The threshold for statistical significance is $p < 0.05$ for all statistical analyses.

7.4 Results

The number of accentuated striae did not differ significantly between the sites ($\chi^2 = 4.2213$, df = 3, $p = 0.2385$). The number of accentuated striae is proportional to the number of individuals analyzed. Alia had both the highest number of individuals (n = 32) and the highest number accentuated striae (n = 206), and Monti di Villa had the lowest number of observed accentuated striae (n = 42), and the lowest number of individuals observed (n= 9).

Age-at-defect formation for individuals from Alia ($W = 0.98077$, $p = 0.007$), Benabbio ($W = 0.94235$, $p < 0.001$), Badia Pozzeveri ($W = 0.95257$, $p < 0.001$), and Monti di Villa ($W = 0.93406$, $p = 0.020$) is non-normally distributed. Figure 7.2 summarizes the statistics for age-at-defect formation between the sites. Individuals from Benabbio, Alia, and Badia Pozzeveri exhibit median age-at-defect formations between 2.265 – 2.720 years, whereas Badia Pozzeveri exhibits a lower median age-at-defect formation of 1.785 years (Figure 7.2). Individuals from both Alia and Benabbio are negatively skewed for age-at-defect formation, whereas individuals from Badia

Pozzeveri and Monti di Villa are positively skewed. A Kruskal-Wallis test reveals statistically significant differences between the sites ($\chi^2 = 43.784$, df = 3, $p < 0.001$). Individuals from Alia have a significantly lower median age-at-defect formation compared to individuals from Benabbio (W = 8138, p = 0.0178). Individuals from Badia Pozzeveri and Pieve dei Monti di Villa also have a significantly different median age-at-defect formation from one another (W = 4098.5, $p < 0.001$). Statistically significant differences in age-at-defect formation suggest that these sites are not similar enough to pool. Instead, the sites are analyzed separately for all subsequent statistical analyses.

Age-at-first defect did not differ significantly between sites. Age-at-first defect is normally distributed for all sites and the variances between sites are not statistically significant ($K^2 = 2.9326$, df = 3, p = 0.4021). Table 7.4 summarizes the statistics for age-at-first defect for each site. The mean age-at-first defect is similar across the sites, with individuals from Badia Pozzeveri exhibiting the highest mean age-at-first defect around age 1.9 years, and Monti di Villa exhibiting the lowest mean age-at-first defect around 1.2 years. Boxplots age-at-first defect for each site are found in Figure 7.3. A One-way ANOVA demonstrates that age-at-first defect does not differ significantly between individuals from each site (F = 2.638, df = 3, p = 0.0584).

Periodicity differs significantly between the sites. The summary statistics for periodicity is presented in Table 7.5. Periodicity is determined to be non-normally distributed for Alia (W = 0.56757, $p < 0.001$), Benabbio (W = 0.57936, $p < 0.001$), Badia Pozzeveri (W = 0.5103, $p < 0.001$), and Monti di Villa (W = 0.73815, $p < 0.001$). Individuals from Badia Pozzeveri and Alia exhibit the highest median periodicities at 0.185 years and 0.110 years, respectively. Individuals from Benabbio and Monti di Villa have median periodicities between 0.08 and 0.06 years, respectively. Several outliers for periodicity exist for each population (Figure 7.4). Individuals

from each site differ significantly from one another for periodicity of accentuated striae (χ^2 = 29.392, df = 3, $p < 0.001$). A post-hoc Dunn test reveals that each population differs significantly from one another for periodicity, with the exception of individuals from Alia and Benabbio (Table 7.6).

Age-at-first defect is associated with average periodicity or number of accentuated striae, depending on the site. Tables 7.7, 7.8, 7.9, and 7.10 summarize the results of the multiple linear regression for Alia (adjusted $R^2 = 0.1696$), Benabbio (adjusted $R^2 = 0.3791$), Monti di Villa (adjusted $R^2 = 0.8484$), and Badia Pozzeveri (adjusted $R^2 = -0.03162$), respectively. A significant negative association between age-at-first defect and periodicity is found among the individuals from Alia ($\beta = -0.53919$, $p = 0.0219$). A significant negative association is found between age-at-first defect and number of accentuated striae among the individuals from Benabbio ($\beta = -0.06287$, $p = 0.0495$) and Monti di Villa ($\beta = -0.193306$, $p = 0.0312$). No significant relationships between variables are found among the individuals from Badia Pozzeveri.

7.5 Discussion

No differences are found in the number of accentuated striae between the sites, suggesting that the stress load for each site is similar. Differences in age-at-defect formation between the sites suggest that regional and interregional variation exists in the timing of stress events, however. Earlier stress events were more common at Badia Pozzeveri ($\tilde{x}$ = 1.785 years), whereas Benabbio ($\tilde{x}$ = 2.720 years), Alia ($\tilde{x}$ = 2.385 years), and Monti di Villa ($\tilde{x}$ = 2.265 years) exhibit a tendency towards later stress events. Furthermore, stress events occurred closer together at Benabbio and Monti di Villa (low periodicity), whereas stress events were spread out more across childhood at Badia and Alia. Interregional differences in the timing of stress suggest that

the stress experiences of individuals from these sites likely was influenced by idiosyncratic variation in cultural context between the sites.

Statistically significant differences in age-at-defect formation also may be associated with idiosyncratic variation in weaning practices. The introduction of solid food in early life is a stressful period during development because of increased exposure to potentially contaminated solid foods and reduced exposure to the microbial and immunologic benefits of breastmilk (Riordan and Countryman, 1980; Wickes, 1953a; Wickes, 1953b). This exposure increases the stress load on infants undergoing weaning and may contribute to the formation of accentuated striae. Alia, Benabbio, and Pieve dei Monti di Villa exhibit similar median age-at-defect formation (2.3 – 2.7 years), whereas Badia Pozzeveri's median age-at-defect formation is around 1.8 years. This difference is statistically different from the other groups, suggesting that these differences may be a result of idiosyncratic differences among the populations at each location. Since Badia Pozzeveri is located in Tuscany near both Benabbio and Pieve dei Monti di Villa, the difference in median age-at-defect formation is not a result of regional variation in cultural child-rearing practices, however.

Periodicity of successive accentuated striae is also significantly greater for individuals from Badia Pozzeveri compared to all other sites. This result suggests that individuals from Badia Pozzeveri experienced a tendency towards less frequent stress events compared to individuals from the other populations. Individuals from Badia Pozzeveri also has a statistically significant earlier age-at-defect formation compared to all other sites. One possible explanation for this result is Badia Pozzeveri's proximity to trade networks. Badia Pozzeveri sits along the Via Francigena, a medieval pilgrimage route that was in use during the 19th century. Inhabitants of Badia Pozzeveri may have experienced increased economic stability compared to other rural

towns due to the movement of people along the Via Francigena. Children from Badia Pozzeveri may have experienced less frequent stress episodes as a result of a more stable and diverse food supply.

We find no relationship between age-at-death and age-at-first defect among our sample populations, suggesting that earlier stress events are not associated with mortality outcomes for either cholera or attritional populations. Among individuals from Alia, we find shorter periodicity is associated significantly with earlier age-at-first defect. Lower numbers of accentuated striae are significantly associated with age-at-first defect among individuals from both Monti di Villa and Benabbio. These results suggest that interregional variation in stress experiences existed across the Italian landscape during the 19th century.

Some researchers have found evidence that early childhood stress is associated with earlier age-at-death among archaeological populations (Boldsen, 2007; Garland et al., 2016; Temple, 2014) but this result is not consistent (Blevins, 2015). Temple (2019) suggests that differences in cultural context may affect the results of DOHaD research in bioarchaeology. The cultural context of 19th century Italy is characterized by war, poverty and economic instability (Domenico, 2002a; Domenico, 2002b; Duggan, 2014; Hearder, 1990). The research presented here demonstrates that the stress experiences of children varied between and within regions of Italy during the 19th century, likely as a result of proximity to trade networks.

7.6 Conclusion

This research finds no significant differences between the number of accentuated striae between the sites, suggesting that the stress burden at each site was similar. However, the timing and frequency of stress events varied among the sites. Additionally, differences in the age-at-defect formation between the three sites from Tuscany suggest that inter-regional variation in the

stress experiences existed in Italy during the 18th-19th century. These differences suggest that cultural context may be a significant, influential factor when attempting to link early childhood experiences to adult mortality outcomes.

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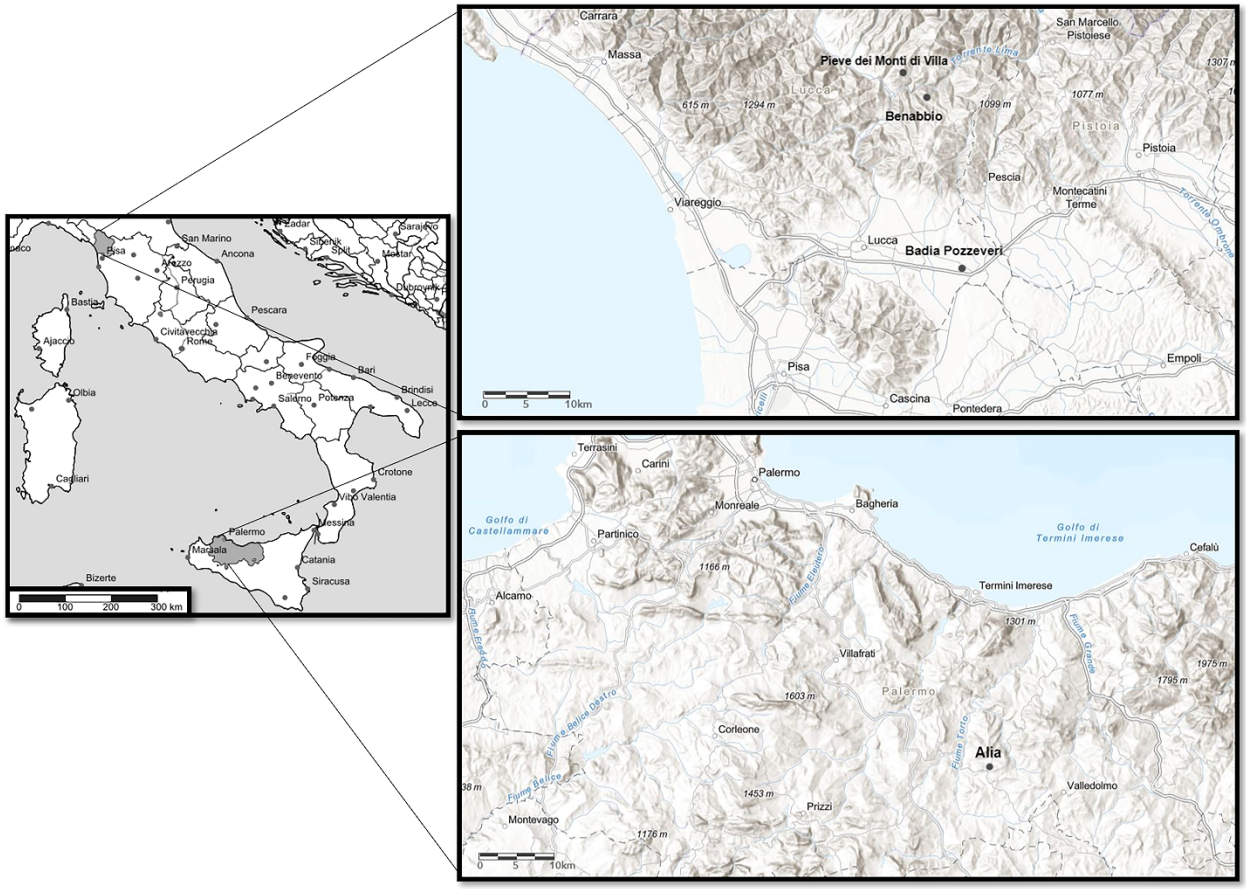

Figure 7.1: Map of archaeological sites: Alia, Benabbio, Badia Pozzeveri and Pieve dei Monti di Villa.

Table 7.1: Number of individuals and number of accentuated striae observed by site

	N Individuals	N Accentuated Striae
Alia	32	206
Benabbio	22	125
LUBP	16	140
PMV	9	42

Table 7.2: Age-at-defect formation summary statistics by site

Site	Min	Median	Mean	Max	Skewness
Alia	0.220	2.385	2.332	4.110	-0.31
Benabbio	0.390	2.720	2.554	3.920	-0.66
Badia Pozzeveri	0.420	1.785	1.824	4.020	0.42
Monti di Villa	1.240	2.265	2.480	3.890	0.27

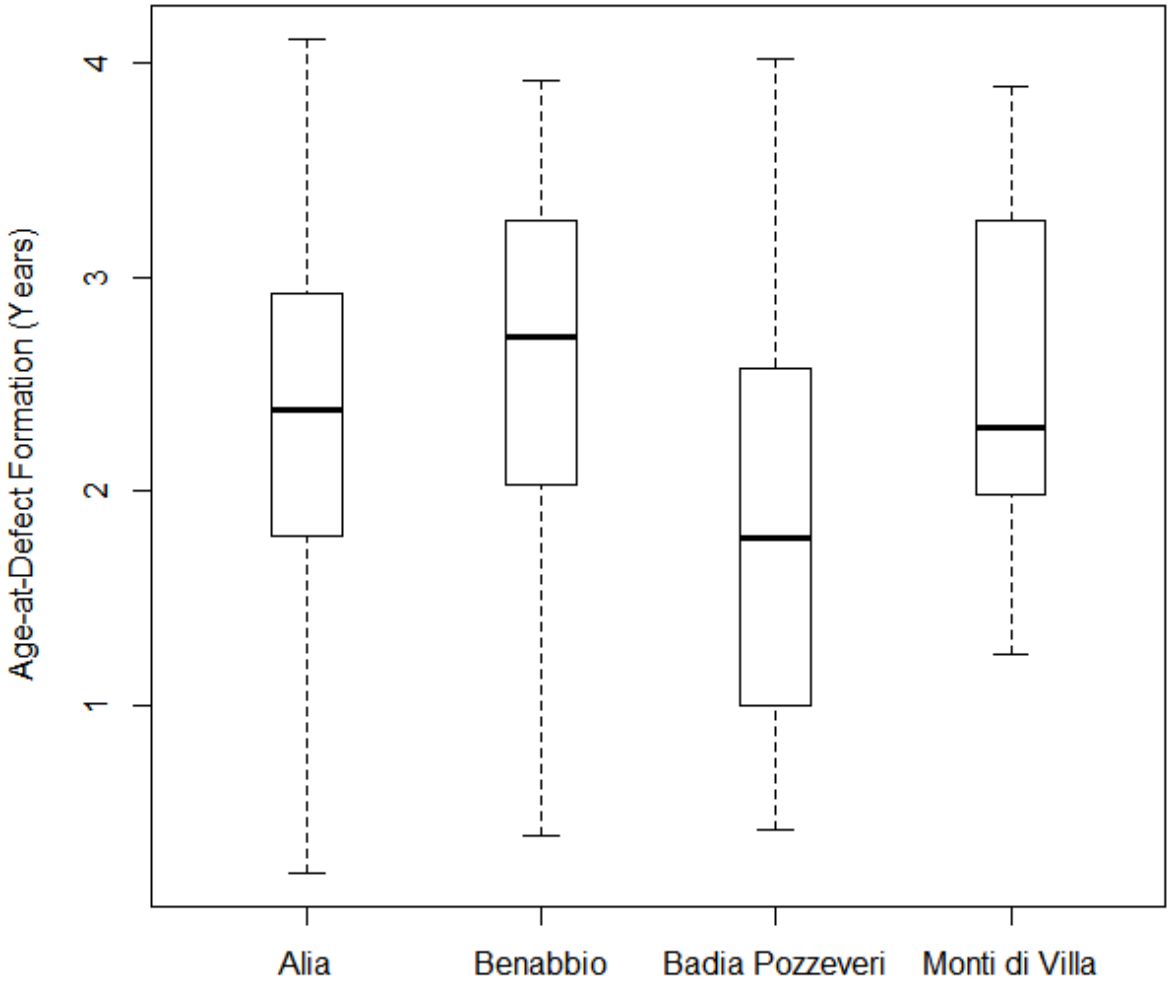

Figure 7.2: Boxplot for age-at-defect formation by site. Statistically significant differences in median age-at-defect formation are found between the cholera sites (Alia and Benabbio) ($W = 8138$, $p = 0.0178$) and the attritional sites (Badia Pozzeveri and Monti di Villa) ($W = 4098.5$, $p < 0.001$).

Table 7.4: Age-at-first defect summary statistics

Site	Min	Median	Mean	Max	Skewness
Alia	0.220	1.660	1.521	3.100	-0.132
Benabbio	0.390	1.920	1.784	2.900	-0.444
Badia Pozzeveri	1.240	1.735	1.923	3.480	0.984
Monti di Villa	0.420	0.920	1.163	2.050	0.147

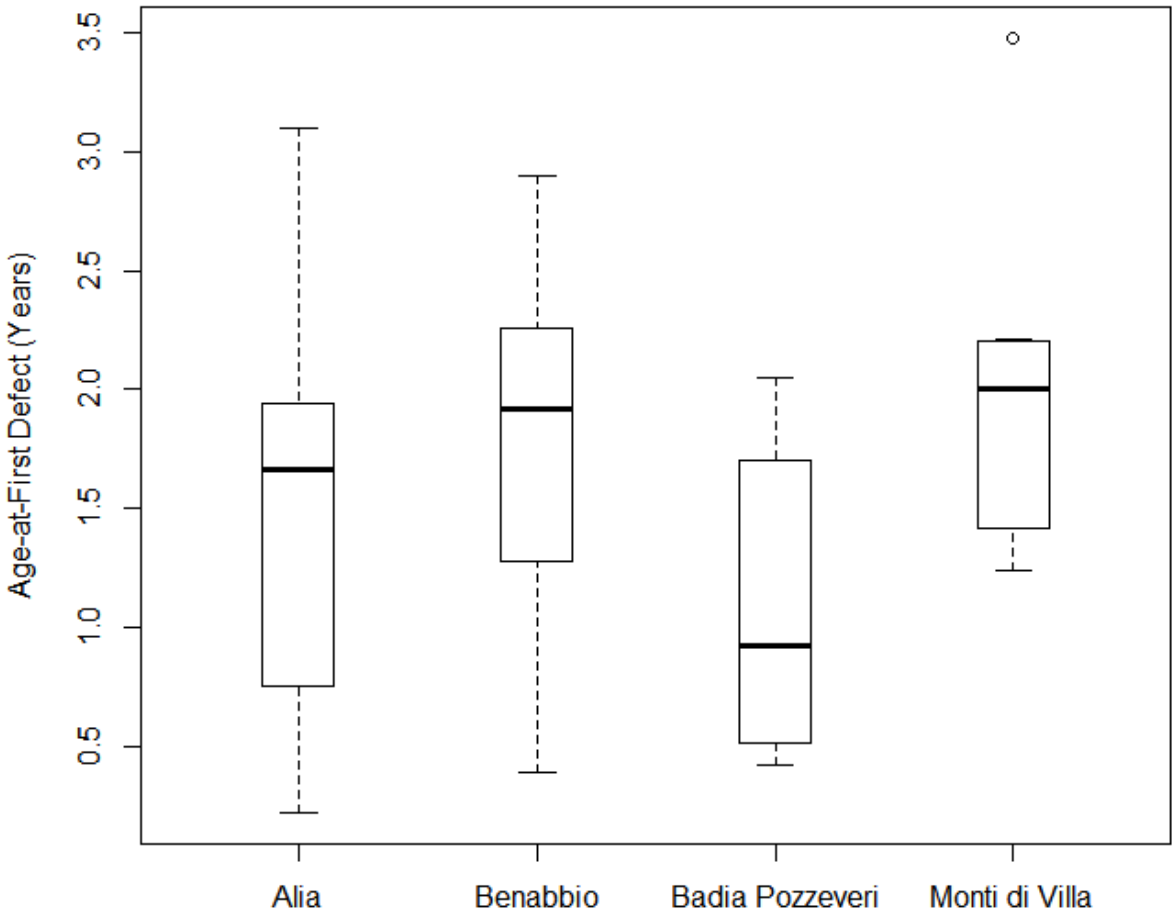

Figure 7.3: Boxplot for age-at-first defect by site. No statistically significant differences are found between individuals from each site for age-at-first defect ($F = 2.638$, $df = 3$, $p = 0.0584$).

Table 7.5: Periodicity summary statistics by site					
Site	Min	Median	Mean	Max	Skewness
Alia	0.010	0.110	0.261	2.910	3.70
Benabbio	0.010	0.080	0.207	1.980	3.23
Badia Pozzeveri	0.020	0.185	0.327	1.540	2.03
Monti di Villa	0.010	0.060	0.153	2.300	4.74

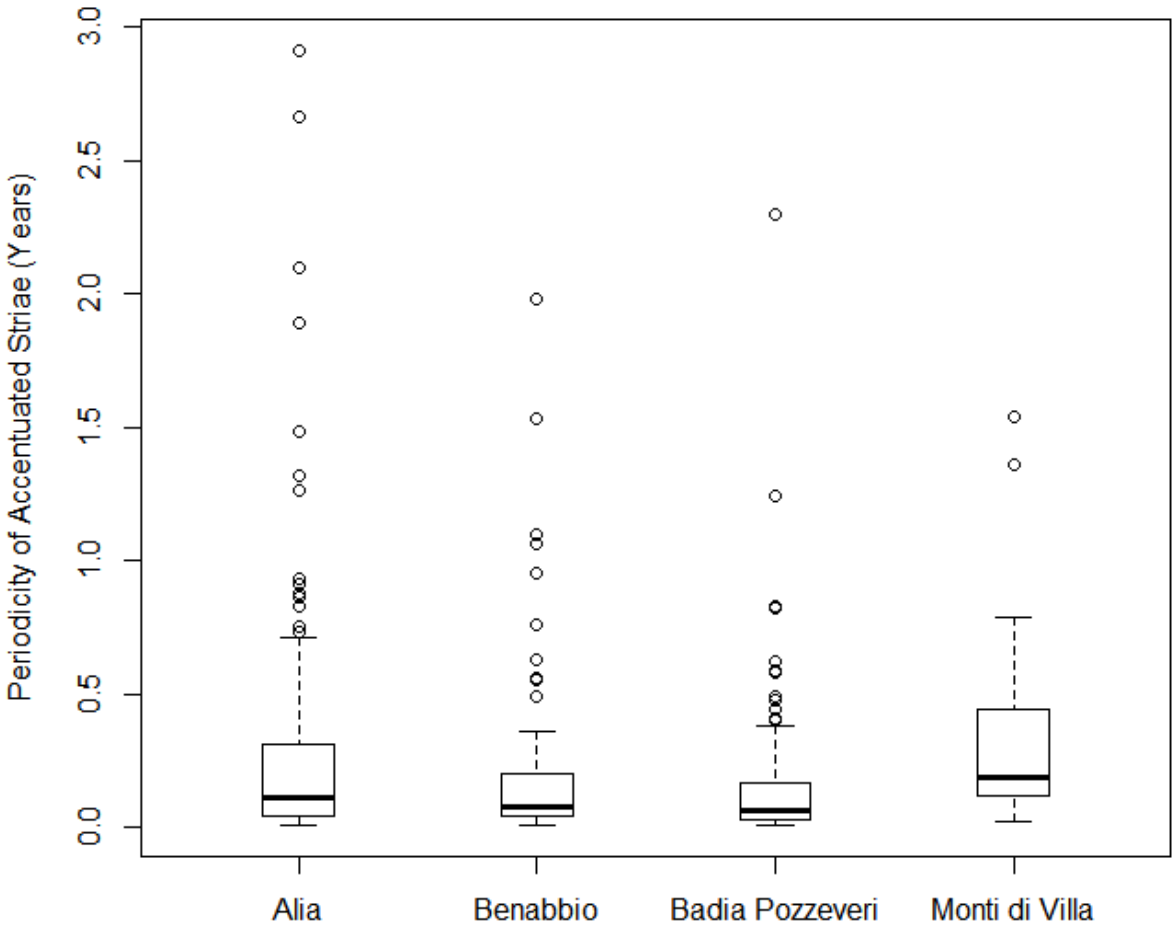

Figure 7.4: Boxplot for periodicity of accentuated striae by site. Periodicity differs significantly between each population ($\chi^2 = 29.392$, df = 3, $p < 0.001$).

Table 7.6: Post-hoc Dunn Test with Benjamini-Hochberg method for Periodicity

	Z	p-value (unadjusted)	p-value (adjusted)
Alia - Benabbio	1.213	0.225	0.225
Alia - Badia Pozzeveri	3.934	< 0.001**	< 0.001**
Benabbio - Badia Pozzeveri	2.131	0.033*	0.0397*
Alia - Pieve dei Monti di Villa	-2.555	0.011*	0.016*
Benabbio - Pieve dei Monti di Villa	-3.152	0.002*	0.003*
Badia Pozzeveri - Pieve dei Monti di Villa	-4.859	< 0.001**	< 0.001**

*p<0.05

**p<0.001

Table 7.7: Multiple Linear Regression for periodicity, age-at-death, and number of accentuated striae relative to age-at-first defect for the population from Alia

Factor	Beta	p-value
Periodicity	-0.53919	0.02193*
Age-at-Death	-0.02202	0.2083
Number of Accentuated Striae	-0.02516	0.5259

*$p<0.05$
**$p<0.001$

Table 7.8: Multiple Linear Regression for periodicity, age-at-death, and number of accentuated striae relative to age-at-first defect for the population from Benabbio

Factor	Beta	p-value
Periodicity	-1.44781	0.0597
Age-at-Death	0.02149	0.4410
Number of Accentuated Striae	-0.06287	0.0495*

*$p<0.05$
**$p<0.001$

Table 7.9: Multiple Linear Regression for periodicity, age-at-death, and number of accentuated striae relative to age-at-first defect for the population from Monti di Villa

Factor	Beta	p-value
Periodicity	0.266243	0.5759
Age-at-Death	-0.008196	0.3214
Number of Accentuated Striae	-0.193306	0.0312*

*p<0.05
**p<0.001

Table 7.10: Multiple Linear Regression for periodicity, age-at-death, and number of accentuated striae relative to age-at-first defect for the population from Badia Pozzeveri

Factor	Beta	p-value
Periodicity	1.01829	0.5762
Age-at-Death	-0.01245	0.4213
Number of Accentuated Striae	-0.03097	0.3456

*$p<0.05$
**$p<0.001$

CHAPTER 8

CONCLUSION

8.1 Introduction

This dissertation research applied the developmental origins of health and disease (DOHaD) theoretical framework to bioarchaeological research by investigating the effects of early childhood stress on adult infectious disease mortality. Specifically, this dissertation compared markers of early childhood stress and chronic stress between 18th-19th-century cholera victims and contemporaneous, attritional populations. This project had three main objectives: (1) determine if early childhood stress and/or chronic stress are associated significantly with mortality from cholera, (2) determine if the timing of stress events in early life is associated significantly with cholera death and (3) determine if age-at-weaning completion differs significantly between catastrophic and attritional populations. I hypothesized that cholera victims likely experienced earlier and more stress events compared to the attritional population, following patterns established in previous bioarchaeological research supporting the plasticity/constraint hypothesis (Armelagos et al., 2009; DeWitte, 2012; DeWitte and Wood, 2008; Temple, 2014). I also hypothesized that cholera victims would exhibit prolonged periods of exclusive breastfeeding, which would be indicative of an inadequate nutritional environment (Riordan and Countryman, 1980; Wickes, 1953a).

This chapter summarizes and synthesizes the main results of this work (see Table 8.1). First, I discuss how the results support or falsify the hypotheses outlined in the introduction. I

then discuss the theoretical contribution of this dissertation. I conclude with a discussion of the limitations and future directions of this research project.

8.2 Age-at-weaning completion

Chapter 5 examined possible relationships between age-at-weaning and susceptibility to cholera death later in life by comparing carbon and nitrogen isotopic evidence for weaning between catastrophic and attritional populations. Stable carbon and nitrogen isotopes from dentin are sampled from canines from the catastrophic and attritional populations. Canines form between the ages of 0.6 to 14.5 years and are used to estimate weaning patterns among archaeological populations (AlQahtani et al., 2010; Fuller et al., 2003; Sandberg et al., 2014). The major findings of this analysis are that (1) 42.6% of the isotope profiles did not exhibit a visible weaning curves; (2) cholera victims exhibit a slightly older age-at-weaning completion (4.1 years) compared to the attritional population (3.9 years), but this difference is not statistically significant ($t = 0.21381$, $df = 18.588$, $p = 0.833$); (3) age-at-weaning completion did not differ significantly between the sites; (4) there are statistical differences in the median and variance of carbon and nitrogen isotope ratios among the four sites analyzed.

I previously hypothesized that cholera mortality was associated with later age-at-weaning completion. Based on the results of this research, cholera victims exhibit a slightly later age-at-weaning completion compared to the attritional population, but the difference in age-at-weaning is not statistically significant. Therefore, cholera mortality is not significantly associated with later age-at-weaning completion. The results of this study suggest that age-at-weaning completion may not be an important factor for the development of immunological responses to early childhood stress. Infants that survived childhood, like our sample population, may be adapted to consuming weanling foods by the time weaning completed. Onset of weaning may be

a more important factor to consider for future research because the onset of weaning is when infants initially are exposed to pathogens from weanling foods and simultaneously deprived of the protective benefits of breastmilk.

The research instead demonstrates how diet varied across the 19th-century Italian landscape. Badia Pozzeveri exhibits the highest median and largest variation in carbon isotope ratios compared to all other sites, which is attributed to the location of the borough along the Via Francigena, a medieval pilgrimage route. Proximity to the pilgrimage route may have granted the citizens of Badia Pozzeveri access to trade networks that were less accessible to the citizens of Benabbio, Monti di Villa, and Alia. During the 19th century, maize was grown in the Po Plain region of northern Italy and may have been exchanged along the Via Francigena and contributed to the elevation in carbon isotope ratios among the individuals from Badia Pozzeveri (Gentilcore, 2014; Ginnaio, 2011).

This research additionally illuminated an interpretive dilemma for weaning reconstruction using archaeological samples. Similar to other isotopic weaning studies, many of the teeth in this study did not exhibit an elevation in $\delta^{15}N$ associated with weaning (Beaumont et al., 2013; Eerkens et al., 2011; Henderson et al., 2014; King et al., 2018; Pfeiffer et al., 2017). Several interpretations are possible for the lack of elevation in $\delta^{15}N$ in early life, specifically that (1) babies without visible weaning curves were never breastfed, (2) babies were weaned before 6 months of age when canines begin to develop, (3) infant growth resulted in a positive nitrogen balance, which lowered $\delta^{15}N$ values, and (4) stress during the weaning period obscured the weaning signal (Deschner et al., 2012; Mekota et al., 2006; Reitsema and Muir, 2015). Weaning before 6 months of age in Italy is unlikely considering historical accounts of breastfeeding practices and other archaeological research on breastfeeding during the 19th century (Bourbou et

al., 2013; Dittmann and Grupe, 2000; Federico and Vasta, 2010; Fulminante, 2015; Giuffra and Fornaciari, 2013; Herrscher, 2003; Moggi-Cecchi et al., 1994; Turner et al., 2007). Growth velocity is also not a likely explanation because growth velocity may not explain all of the variation in infant $\delta^{15}N$ during the post-weaning period (Dailey-Chwalibog et al., 2020; Reitsema and Muir, 2015). Individuals without visible weaning curves likely represent infants who were not breastfed and instead subsisted on supplementary food from birth, though it is possible that some of these individuals weaned early or experienced stress during weaning (Stevens et al., 2009; Wickes, 1953a; Wickes, 1953b).

8.3 Pathological markers and growth stunting

Chapter 6 examines the relationship between early childhood stress and infectious disease mortality by comparing the paleopathological markers and stature of the catastrophic and attritional populations. Porotic hyperostosis, cribra orbitalia, linear enamel hypoplasia (LEH) and growth stunting are pathological markers that form during childhood (FitzGerald, 1998; Hillson, 2014a; King et al., 2005; McIlvaine, 2015; Stuart-Macadam, 1985; Stuart-Macadam, 1989; Stuart-Macadam, 1992; Walker et al., 2009). Periostitis is a pathological marker that forms near the time of death and is used to assess heterogeneity in adulthood stressors that may contribute to mortality risk (Mensforth et al., 1978; Waldron, 2009). Linear regression equations from Hauser et al. (2005) are used to estimate stature.

I hypothesized that cholera mortality is associated with a higher prevalence of pathological markers and shorter stature compared to the attritional population. The results of this study found that the sites do not differ significantly in prevalence of pathological markers, with the exception of cribra orbitalia. Cribra orbitalia is statistically less prevalent among Sicilians compared to the Tuscans ($\chi^2 = 31.071$, df = 1, $p < 0.001$). Alia exhibits the lowest

prevalence of cribra orbitalia (7.4%), followed by Badia Pozzeveri (23.8%), Benabbio (42.3%) and Monti di Villa (66.7%). Differences in cribra orbitalia prevalence between the sites may be a result of (1) the 1832 pandemic being less selective compared to the 1855 pandemic and (2) Sicilians may have consumed more terrestrial protein compared to Tuscans during the 18^{th} – 19^{th} century.

The stature of cholera victims is significantly lower compared to the attritional population (95% CI [-9.65, -2.98], t = -3.8296, df = 39.096, p = 0.0005). However, when the sites are assessed individually, the stature of individuals from Alia is significantly lower than both Benabbio (95% CI [5.79, 13.72], $p < 0.001$) and Badia Pozzeveri (95% CI [4.95, 13.03], $p < 0.001$), which is consistent with secular trends in stature analysis across the Italian landscape. During the 19th century, southern Italians exhibit an overall shorter stature compared to northern and central Italians, but these regional differences are associated with economic disparities (Arcaleni, 2006; Martínez-Carrión and María-Dolores, 2017).

This study finds no support for the hypothesis that early childhood stress is associated with infectious disease mortality. Instead, this study highlights how cultural context influences the interpretation of bioarchaeological data using the DOHaD hypothesis. The variation in stature and paleopathological marker prevalence in this sample is indicative of regional variation in the stress experiences of Italians during the 18^{th}-19^{th} century.

8.4 Dental histology

Chapter 7 compares the timing of stress events between catastrophic and attritional populations using histological analysis of tooth enamel. Disturbances in metabolism in early childhood can disrupt enamel formation and result in the formation of accentuated striae of Retzius (Hillson, 2014b; Rose et al., 1978; Wilson and Shroff, 1970). Accentuated striae

represent a period of short-term stress that lasted from a few days to a few weeks (Witzel et al., 2008). Since teeth form at regular intervals that are genetically constrained, the age-of-formation of accentuated striae can be estimated by measuring the distance between accentuated striae and anatomical features of the tooth (crown apex, cemento-enamel junction, etc.) (Armelagos, 2010; FitzGerald et al., 2006; Goodman and Rose, 1990; Goodman and Rose, 1991; Hillson, 2014b; Rose et al., 1978; Schwartz and Dean, 2008; Wright, 1990). In this study, the timing of stress events was estimated by analyzing canine thin sections from catastrophic and attritional populations.

Age-at-defect formation is compared between the sites to assess if they are similar enough to pool into catastrophic and attritional groups. Statistically significant differences in age-at-first defect are found between the Badia Pozzeveri and Monti di Villa ($W = 4098.5$, $p < 0.001$), Alia and Benabbio ($\chi^2 = 43.784$, $df = 3$, $p < 0.001$), suggesting the sites are too dissimilar to pool. The sites are assessed statistically without pooling for all subsequent analyses.

Age-at-first defect does not differ significantly between the sites. Periodicity, the average interval between accentuated striae, differs significantly between the sites ($\chi^2 = 29.392$, $df = 3$, $p < 0.001$). The results of multivariate linear regression find that periodicity and number of accentuated striae is associated with the age-at-first defect, depending on the site. A significant negative association between age-at-first defect and periodicity is found among individuals from Alia ($\beta = -0.53919$, $p = 0.0219$). Benabbio ($\beta = -0.06287$, $p = 0.0495$) and Monti di Villa ($\beta = -0.193306$, $p = 0.0312$) exhibit a statistically significant negative association between age-at-first defect and number of accentuated striae. Age-at-death is not significantly associated with age-at-first defect in this sample.

I hypothesized that cholera mortality is associated with earlier and more frequent stress events. Regional variation in the timing of stress events made comparing the cholera and attritional populations untenable. The site-based variation in the timing of stress events may be a result of idiosyncratic differences in the weaning practices and proximity to trade networks. This research demonstrates that regional variation is an influential factor on the timing of stress events in early childhood.

8.5 Theoretical implications

Though this dissertation sought to investigate how early childhood stress affects infectious disease mortality, cholera and attritional populations do not exhibit significant differences in paleopathology, dental histology, and weaning patterns, suggesting that early childhood stress does not affect infectious disease mortality. Significant evidence, including mechanisms, supports the DOHaD hypothesis in the context of metabolic disorders (Barker, 1997; Gluckman et al., 2005; Kuzawa, 2005; Worthman and Kuzara, 2005). Metabolic disorders arise in adulthood in modern, industrialized contexts due to a mismatch between the early childhood environment of scarcity and the calorie rich environment of adulthood. The underlying mechanism behind this biological response is that an environment of scarcity in early childhood produces physiological responses that would be adaptive in an adulthood environment of scarcity, such as increased fat storage, but become maladaptive in a calorie-rich environment. Though there is some evidence that the immune system is impaired as a result of early childhood stress (DeWitte and Wood, 2008; McDade et al., 2001a; McDade et al., 2001b; Moore et al., 1999; Moore et al., 1997; Watts, 1969), the reason other studies find no relationship between early childhood stress and infectious disease mortality could be that there are no known adaptive

mechanisms that would produce physiological pathways between the immune system and early childhood stress.

Temple (2019) suggests that cultural context and methodological approach may influence the results of DOHaD research in bioarchaeology. This dissertation finds regional and interregional differences between the sample populations, suggesting that cultural context in this sample varied across the Italian landscape. For example, cribra orbitalia rates in Alia are significantly lower than those found in any sites in Tuscany, which is potentially attributable to dietary differences between the sites. Cultural context, including the quality and quantity of diet, breastfeeding practices, and cultural expectations of children, may explain the results of this dissertation. Site-based differences in the nutritional environment and breastfeeding practices, including when weaning typically began and what types of foods were used as supplemental food, could explain some of the differences in early childhood stress markers observed between the sites. The cultural expectations of children, specifically information like children's contribution to the local labor market and the daily activities of children, would be useful information for interpreting the stress experiences of children from these sites. These contextual differences between sites are difficult to reconstruct with historic records because the experiences of children often are overlooked in the historic record.

Bioarchaeologists interested in DOHaD hypothesis testing may need to carefully consider which specific metrics may be the most relevant to the adult mortality outcome of interest (Temple, 2019). This dissertation finds that weaning completion is not a relevant metric in adult infectious disease mortality in our sample populations. Instead, onset of weaning may be a more important metric because it represents the time when the infants' digestive system is first exposed to weanling foods and disease-inducing microorganisms, whereas the completion of

weaning occurs after the infant has been exposed to disease-inducing agents for a period of months to years. Though weaning studies have been criticized for not specifying what specific part of the weaning process they are analyzing (Dettwyler and Fishman, 1992), many weaning studies in bioarchaeology are still not specific about what part of the weaning process they are reconstructing (Reynard and Tuross, 2015). Furthermore, bioarchaeologists reconstructing weaning patterns generally focus on when weaning has completed (King et al., 2018; Pfeiffer et al., 2017), likely due to the imprecision of currently available weaning reconstruction methods. Specifically, nitrogen may decline in the early increments of dentin thin sections and may level off within one or two increments. Completion of weaning is easier to assess using nitrogen isotope analysis because nitrogen values level off over several increments.

The timing of stress events may be critical to understanding how these the predictive/adaptive and plasticity/constraint hypotheses overlap. Markers of stress that develop over a long period (e.g. stature) or have unspecific times when they emerge (e.g. cribra orbitalia) may not be as useful for DOHaD analysis as metrics that can be associated with specific ages of formation. Stature, though widely used to assess the health of populations, is also associated with secular trends in adult height that may or may not be related to early childhood stress (Arcaleni, 2006). In the context of this dissertation, we found significant differences in stature between individuals from Sicily and Tuscany, which is likely a result of secular trends in stature across the Italian peninsula.

Time-specific stress events in early childhood may be critical to understanding how early childhood stressors affect adult mortality outcomes. Humans may experience a critical period of developmental plasticity in early childhood when stress events produce adaptive physiological changes through epigenetic mechanisms. Stress events outside the critical window are

detrimental to adult health outcomes because developmental adaptation is no longer possible (Aguayo-Mazzucato et al., 2006). Furthermore, the duration and severity of stress may affect mortality outcomes. In this dissertation, interregional differences in periodicity and age-at-first defect of accentuated striae are found, suggesting that the stress experiences of children varied within and between regions during the 18th-19th centuries in Italy. No consistent pattern in age-at-first defect is observed between cholera and attritional populations, suggesting that short-term early stress events did not affect adult infectious disease mortality. Other studies have found that linear enamel hypoplasia (LEH) is associated with earlier death (Armelagos et al., 2009; Boldsen, 2007; Temple, 2014). However, LEH is not directly comparable to accentuated striae of Retzius because LEH represent stress over a period of weeks to months (FitzGerald, 1998; Shellis, 1998), whereas accentuated striae form during of short-term stress events (Larsen, 2015). It may be reasonable to deduce from these studies that long-term stress events may be more impactful on adult mortality outcomes than short-term stress events.

Site-based and regional differences are consistently observed in the data sets explored in this dissertation. Consequently, this dissertation does not find evidence to support either the predictive-adaptive or plasticity/constraint models. A main issue with attempting to discern the plasticity/constraint and predictive adaptive models using skeletal remains is establishing that individuals in historic populations experienced constantly stressful environments. The plasticity/constraint model is derived from research that analyzed metabolic disorders that are a result of an environment of nutritional abundance (Barker, 1995; Barker, 1997; Barker, 2004; Hales and Barker, 1992; Hales and Barker, 2001; Hales et al., 1991). Bioarchaeologists therefore must establish that their study populations experienced constant nutritional stress for the effects of the plasticity/constraint hypothesis to be discernable. As of the publication of this dissertation,

the methods employed by bioarchaeologists cannot specifically establish nutritional insufficiency in skeletal populations. The majority of stress indicators on skeletal remains are non-specific, meaning they could be a result of nutritional, psychological, or physical stress. Stable isotope techniques and dental microwear analysis are employed by bioarchaeologists to reconstruct the types of food consumed in the past, but these techniques cannot provide us with information about the quantity of food consumed. Nutritional deprivation is associated with an elevation in $\delta^{15}N$ (Fuller et al., 2005; Fuller et al., 2004; Hatch et al., 2006; Hobson et al., 1993; Mekota et al., 2006), but this effect is difficult to discern from nitrogen elevation due to consuming high nitrogen foods. Some types of stress may have lasting health effects (psychological, injury), but other types of stress may be more quickly impactful (nutritional).

This dissertation addresses questions of human biological plasticity through life history perspective. No relationship between early childhood stress and infectious disease mortality is found, suggesting that the stress environment at the time of cholera infection is more influential than previous stress episodes with regards to infectious disease mortality.

8.6 Limitations and future directions

There are several limitations to this research project. First, the sample size for many of the sites is small. This research was limited by the available skeletal material from the 18th and 19th centuries in Italy, which is understudied in Italian archaeology. The small sample sizes are managed in this research through the use of non-parametric statistical analysis, which do not assume normal distributions and have a higher threshold for statistical significance.

Another major limitation for this research is the precision of current sampling methods for dentin increments. Current methods for sampling tooth dentin allow for increment sizes between 1 - 2 mm, which yields approximately 5 - 12 increments per tooth (Beaumont et al.,

2014). Small dentin increments vary in the quality of collagen that can be extracted from them. Increments that are too small may produce unusable or unreliable data. Considering the high percentage of samples that produced no visible weaning curves, more precision in the sampling methods for incremental carbon and nitrogen analysis would yield better age estimates for weaning. The tooth grows obliquely, so even a thin slice crosscuts several growth lines.

Future research for this project includes analyzing tooth enamel for Ba/Ca isotope ratios to analyze weaning patterns. Barium/calcium isotope analysis is typically done using laser ablation, which requires a smaller sample size than collagen extraction and thus can produce more precise and numerous increments. The late 19th century also corresponds to the end of the Little Ice Age, a period of climate change that caused harsher winters in Europe and North America. Oxygen isotope analysis of human enamel could also be used to analyze the effects of climate change on these populations. To overcome the issues of regional and environmental variation, a meta-analysis of previous bioarchaeological research on the effects of early childhood stress events on adult infectious disease mortality should be conducted. A meta-analysis of all available data would provide a more complete understanding of how the plasticity/constraint and predictive adaptive hypotheses interact and intersect during early childhood.

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Table 8.1 Summary of results

Chapter	Metric	Periodicity of stress	Timing of Stress	Result
5	Age-at-weaning completion	Months to years	Infancy to early childhood	No significant difference.
6	Cribra orbitalia	Chronic	Infancy to late childhood	Sicilians exhibit a significantly lower prevalence of CO compared to Tuscans.
	Porotic hyperostosis	Chronic	Infancy to late childhood	No significant difference.
	Linear enamel hypoplasia	Weeks to months	*In utero* to late childhood	No significant difference.
	Periostitis	Chronic	Infancy to adulthood	No significant difference.
	Stature	Chronic	Infancy to late childhood	Sicilians exhibit a significantly shorter stature compared to Tuscans.
7	Age-at-defect formation	Days to weeks	0.6 to 4.4 years	Sites differ significantly.
	Age-at-first defect	Days to weeks	0.6 to 4.4 years	Significant negative association with periodicity among individuals from Alia Significant negative association with number of accentuated striae among individuals from Benabbio and Monti di Villa

APPENDIX A:

STABLE CARBON AND NITROGEN ISOTOPE RAW DATA

Site	Sample and section #	Tooth	$\delta^{15}N$	Amt % N	$\delta^{13}C$	Amt % C	C:N
Alia	A10-1	C’	15.4	10.1	-19.6	45.1	3.4
	A10-2	C’	15.2	9.6	-19.6	45.6	3.5
	A10-3	C’	16.6	9.7	-18.6	46	3.2
	A10-4	C’	16.0	9.3	-18.6	44.4	3.2
	A10-5	C’	15.9	9.3	-18.6	45.1	3.3
	A10-6	C’	15.8	9.7	-18.2	44.3	3.3
	A10-7	C’	15.7	9.8	-18.5	43.2	3.2
	A117-1	C’	14.6	11.1	-18.4	41.2	3.3
	A117-2	C’	13.9	10.6	-19.6	42.5	3.6
	A117-3	C’	14.3	10.5	-19.2	41.9	3.4
	A117-4	C’	13.9	9.6	-18.8	39.7	3.3
	A117-5	C’	8.6	9.7	-18.6	24.4	3.3
	A117-6	C’	15.5	9.3	-18.3	43.5	3.3
	A117-7	C’	14.9	9.9	-18.3	41.9	3.3
	A117-8	C’	14.2	10.2	-18.2	39.9	3.3
	A117-9	C’	10.7	10.6	-18.2	29.5	3.2
	A14/ “74”-1	C,	15.2	10.5	-18.3	40.9	3.1
	A14/ “74”-2	C,	15.2	10.9	-18.6	41.5	3.2
	A14/ “74”-3	C,	15.6	10.7	-18.5	42.9	3.2
	A14/ “74”-4	C,	16.0	10.5	-18.3	43.3	3.2
	A14/ “74”-5	C,	16.3	10.0	-18.3	44.5	3.2
	A14/ “74”-6	C,	15.1	10.0	-18	40.9	3.2
	A14/ “74”-7	C,	15.5	10.6	-18.1	42.7	3.2
	A17-1	C,	15.1	10.1	-18.7	42	3.3
	A17-2	C,	15.2	9.8	-18.8	42.6	3.3
	A17-3	C,	15.3	10.3	-18.5	42.9	3.3
	A17-4	C,	15.3	10.1	-18.5	42.7	3.3
	A17-5	C,	15.4	10.4	-18.6	43	3.3
	A17-6	C,	15.1	10.7	-18.3	42.4	3.3
	A18-1	C’	15.1	12.0	-18.4	41.6	3.2
	A18-2	C’	15.4	10.0	-18.9	43.2	3.3
	A18-3	C’	15.1	9.9	-18.7	42.6	3.3
	A18-4	C’	13.3	9.7	-18.5	37.3	3.3
	A18-5	C’	15.7	9.5	-18.8	43.8	3.2

Site	Sample and section #	Tooth	$\delta^{15}N$	Amt % N	$\delta^{13}C$	Amt % C	C:N
	A18-6	C’	15.7	9.2	-18.8	43.9	3.3
	A18-7	C’	15.6	9.5	-18.6	43.3	3.3
	A18-8	C’	15.3	9.6	-18.7	41.2	3.1
	A38-1	C’	15.3	10.1	-18.7	42.1	3.2
	A38-2	C’	13.5	9.8	-18.6	37.6	3.2
	A38-3	C’	15.2	8.7	-18.6	42.3	3.3
	A38-4	C’	15.2	8.8	-18.6	42.4	3.3
	A38-5	C’	13.8	8.6	-18.2	38.2	3.2
	A38-6	C’	9.0	10.6	-20.3	27.6	3.6
	A40-1	C,	14.4	8.6	-18.9	40.5	3.3
	A40-2	C,	14.9	8.3	-19.1	42.3	3.3
	A40-3	C,	12.8	8.1	-19.4	37.3	3.4
	A40-4	C,	15.6	8.7	-18.5	44.1	3.3
	A40-5	C,	14.2	8.7	-18.6	40	3.3
	A40-6	C,	15.0	9.4	-18.6	42.1	3.3
	A40-7	C,	14.8	10.5	-18.4	41.7	3.3
	A40-8	C,	14.7	10.8	-18.3	40.9	3.2
	A42-1	C’	14.0	12.0	-18.9	40.9	3.4
	A42-2	C’	15.0	10.7	-18.4	42.1	3.3
	A42-3	C’	14.4	10.6	-19.1	41.9	3.4
	A42-4	C’	15.0	9.9	-18.3	42.3	3.3
	A42-5	C’	13.6	10.1	-18.3	38.5	3.3
	A42-6	C’	15.1	10.3	-18.2	42.4	3.3
	A42-7	C’	14.6	9.8	-18.4	41	3.3
	A48-1	C’	16.8	12.1	-18.6	46.4	3.2
	A48-2	C’	16.4	10.7	-19	45.5	3.2
	A48-3	C’	16.0	9.4	-18.7	44.7	3.3
	A48-4	C’	16.2	9.4	-18.8	45	3.2
	A48-5	C’	16.3	9.8	-18.5	45.5	3.3
	A48-6	C’	16.5	10.3	-18.6	46.1	3.3
	A48-7	C’	16.3	10.4	-18.9	45.3	3.2
	A52-1	C’	14.7	9.7	-20.4	45.6	3.6
	A52-2	C’	15.5	10.8	-18.8	43.7	3.3
	A52-3	C’	15.7	10.4	-18.2	43.6	3.2
	A52-4	C’	15.6	10.5	-18.2	43.4	3.2
	A52-5	C’	15.8	10.9	-18.1	43.6	3.2
	A52-6	C’	15.5	11.1	-18.1	42.9	3.2
	A52-7	C’	15.2	11.1	-18.2	42.1	3.2
	A81-1	C’	15.8	11.6	-18.6	44.2	3.3
	A81-2	C’	15.5	11.0	-18.5	43.4	3.3
	A81-3	C’	15.9	10.1	-18.4	44.8	3.3

Site	Sample and section #	Tooth	$\delta^{15}N$	Amt % N	$\delta^{13}C$	Amt % C	C:N
	A81-4	C’	16.2	10.0	-18.3	45.6	3.3
	A81-5	C’	16.1	9.3	-18.1	45.2	3.3
	A81-6	C’	15.9	9.5	-18.1	44.5	3.3
	A81-7	C’	15.9	10.0	-18.5	44.9	3.3
	A85-1	C’	16.2	9.5	-18.6	45	3.2
	A85-2	C’	16.0	9.2	-18.8	44.6	3.3
	A85-3	C’	16.3	9.8	-18.4	45.6	3.3
	A85-4	C’	16.2	9.6	-18.2	45.6	3.3
	A85-5	C’	16.2	9.9	-18.2	45.1	3.3
	A85-6	C’	16.1	10.0	-18.1	45	3.3
	A85-7	C’	16.1	9.4	-18.5	44.9	3.3
	A90-1	C’	16.6	10.6	-18.5	46	3.2
	A90-2	C’	16.0	10.3	-18.6	44.7	3.3
	A90-3	C’	16.2	10.3	-18.3	45.6	3.3
	A90-4	C’	16.4	10.0	-18.6	46	3.3
	A90-5	C’	16.2	10.0	-18.4	45.4	3.3
	A90-6	C’	16.1	10.3	-18.3	45.2	3.3
	A90-7	C’	15.9	11.2	-18.5	44.7	3.3
	A99-1	C’	15.8	10.3	-19	44.5	3.3
	A99-2	C’	21.0	10.8	-18.5	59	3.3
	A99-3	C’	15.8	10.3	-18.7	44.3	3.3
	A99-4	C’	14.0	10.4	-18.3	39.3	3.3
	A99-5	C’	17.7	10.5	-18.4	49.3	3.3
	A99-6	C’	18.2	10.6	-18.8	50.6	3.2
	A99-7	C’	12.3	10.6	-18.8	34.2	3.3
	M1-1	C,	15.2	10.3	-18.9	42	3.2
	M1-2	C,	15.3	10.5	-18.3	42	3.2
	M1-3	C,	14.9	10.5	-19	42.4	3.3
	M1-4	C,	15.2	10.5	-18.5	42.9	3.3
	M1-5	C,	15.7	10.8	-18.7	44.4	3.3
	M1-6	C,	15.9	11.3	-18.6	44.4	3.3
	M1-7	C,	15.7	11.6	-18.6	44	3.3
	M1-8	C,	15.0	11.5	-18.2	41.1	3.2
	M1-9	C,	14.1	11.3	-18.5	39	3.2
	M12-1	C,	15.1	10.6	-18.9	41.8	3.2
	M12-2	C,	14.8	10.7	-18.3	42.5	3.3
	M12-3	C,	14.1	9.7	-18.4	41	3.4
	M12-4	C,	13.4	10.1	-18.5	40.7	3.5
	M15-1	C,	15.2	11.0	-18.5	42.5	3.3
	M15-2	C,	11.7	10.1	-18.6	32.8	3.3
	M15-3	C,	15.0	9.9	-19.1	43	3.4

Site	Sample and section #	Tooth	$\delta^{15}N$	Amt % N	$\delta^{13}C$	Amt % C	C:N
	M15-4	C,	15.3	9.5	-18.4	43.1	3.3
	M15-5	C,	15.4	9.2	-18.3	43.7	3.3
	M15-6	C,	15.2	9.3	-18.5	42.7	3.3
	M15-7	C,	15.4	9.5	-18.2	43.1	3.3
	M15-8	C,	15.0	10.0	-18.1	42	3.3
	M17-1	C,	14.9	10.0	-18.6	43.4	3.3
	M17-2	C,	15.1	10.0	-18.6	43.5	3.3
	M17-3	C,	15.5	9.4	-18.3	43.9	3.3
	M17-4	C,	15.7	9.1	-18.1	42.9	3.3
	M17-5	C,	15.4	9.5	-18.2	43.4	3.3
	M17-6	C,	15.4	10.2	-18.3	43.1	3.3
	M17-7	C,	15.4	11.6	-18.6	43.4	3.3
	M19-1	C,	17.2	12.6	-18	47.4	3.2
	M19-2	C,	16.9	10.7	-18.1	47	3.2
	M19-3	C,	15.7	10.5	-18.5	42.9	3.2
	M19-4	C,	15.9	10.5	-18.3	43.5	3.2
	M19-5	C,	14.4	10.1	-18.2	38.8	3.1
	M19-6	C,	15.9	10.1	-18.3	43.6	3.2
	M19-7	C,	15.6	10.3	-18.3	43	3.2
	M19-8	C,	16.1	10.5	-18.3	43.6	3.2
	M20-1	C,	15.4	10.9	-18.8	43	3.3
	M20-2	C,	15.4	10.9	-19.1	43.6	3.3
	M20-3	C,	14.3	10.0	-20.2	44.6	3.6
	M20-4	C,	15.0	10.4	-18.6	43.6	3.4
	M20-5	C,	14.5	11.0	-18.8	44.1	3.6
	M21-1	C,	15.1	11.0	-19.1	40.1	3.1
	M21-2	C,	15.2	10.5	-18.7	42.6	3.3
	M21-3	C,	15.4	10.3	-18.4	43.3	3.3
	M21-4	C,	14.0	10.4	-18.5	38.7	3.2
	M5-1	C,	15.8	11.2	-18.7	43.9	3.2
	M5-2	C,	15.0	10.0	-19	41.6	3.2
	M5-3	C,	15.3	10.6	-18.9	43.8	3.3
	M5-4	C,	15.5	11.0	-18.6	42.8	3.2
	M5-5	C,	15.7	11.2	-18.5	43.4	3.2
	M5-6	C,	15.4	11.5	-18.3	42.7	3.2
	M5-7	C,	15.6	11.8	-18.6	43.4	3.3
	M6-1	C,	15.9	12.3	-18.4	44.1	3.2
	M6-2	C,	15.7	11.4	-18.3	44	3.3
	M6-3	C,	15.4	11.7	-18.7	43.8	3.3
	M6-4	C,	16.3	11.2	-18.1	45	3.2
	M6-5	C,	16.2	11.1	-17.7	45	3.2

Site	Sample and section #	Tooth	$\delta^{15}N$	Amt % N	$\delta^{13}C$	Amt % C	C:N
	M6-6	C,	16.0	10.9	-17.9	44.7	3.2
	M6-7	C,	16.0	10.7	-18.2	44.4	3.2
	M6-8	C,	15.9	10.8	-18.4	44.2	3.2
	M7-1	C,	15.9	10.4	-18.5	44.5	3.3
	M7-2	C,	16.3	10.6	-18.7	45.1	3.2
	M7-3	C,	15.7	10.3	-18.8	43.9	3.2
	M7-4	C,	15.6	8.6	-18.6	43	3.2
	M7-5	C,	16.1	10.0	-18.5	44.9	3.3
	M7-6	C,	16.1	9.8	-18.7	44.8	3.2
	M7-7	C,	16.1	10.4	-18.7	44.8	3.2
	M7-8	C,	15.9	10.0	-18.4	44.4	3.3
	M7-9	C,	15.4	9.8	-18.6	42.9	3.2
	M10-1	C,	16.7	11.2	-18.4	47	3.3
	M10-2	C,	15.7	10.4	-18.7	43.9	3.3
	M10-3	C,	16.0	9.8	-18.6	44.7	3.3
	M10-4	C,	16.7	9.7	-18.5	46.8	3.3
	M10-5	C,	15.7	9.3	-18.5	44	3.3
	M10-6	C,	15.5	9.4	-18.4	43.4	3.3
	M11-1	C,	14.0	10.8	-18.7	38.9	3.2
	M11-2	C,	13.4	10.9	-18.9	38.3	3.3
	M11-3	C,	11.1	10.5	-18.6	31.2	3.3
	M11-4	C,	15.0	9.9	-18.4	42.2	3.3
	M11-5	C,	15.1	10.2	-18.3	42.3	3.3
	M11-6	C,	15.0	10.4	-18.5	42.2	3.3
	M13-1	C,	14.4	11.4	-20.1	44.7	3.6
	M13-2	C,	15.0	9.6	-20	46.6	3.6
	M13-3	C,	15.8	9.2	-18.5	44.8	3.3
	M13-4	C,	14.4	8.5	-18.6	40.5	3.3
	M13-5	C,	15.7	9.0	-18.7	44	3.3
	M13-6	C,	15.8	9.7	-18.7	44.8	3.3
	M18/51-1	C,	15.5	11.4	-18.9	44.5	3.4
	M18/51-2	C,	15.7	10.3	-18.7	44.5	3.3
	M18/51-3	C,	13.9	10.7	-18.6	39	3.3
	M18/51-4	C,	16.4	9.9	-18.2	45.8	3.3
	M18/51-5	C,	13.4	10.0	-18.2	37.6	3.3
	M18/51-6	C,	15.5	10.1	-18.2	43.4	3.3
	M18/51-7	C,	16.3	10.1	-18.3	45.5	3.3
	M3-1	C,	14.2	11.0	-18.7	39.8	3.3
	M3-2	C,	14.6	9.5	-19.3	41.1	3.3
	M3-3	C,	14.2	9.0	-19.3	41.4	3.4
	M3-4	C,	15.2	9.8	-18.9	43.1	3.3

Site	Sample and section #	Tooth	$\delta^{15}N$	Amt % N	$\delta^{13}C$	Amt % C	C:N
	M3-5	C,	15.1	10.0	-18.9	42.7	3.3
	M3-6	C,	14.8	9.3	-18.5	41.7	3.3
	M3-7	C,	14.6	10.3	-18.5	40.9	3.3
	M4-1	C,	16.0	11.5	-18.6	44.5	3.2
	M4-2	C,	15.6	11.0	-18.6	43.5	3.3
	M4-3	C,	16.3	11.1	-18.4	45.6	3.3
	M4-4	C,	16.1	10.9	-18.5	45	3.3
	M4-5	C,	16.2	11.5	-18.3	45.2	3.3
	M4-6	C,	16.3	12.2	-18.3	45.6	3.3
	MAN9-1	C,	13.3	11.4	-18.5	37.3	3.3
	MAN9-2	C,	14.7	10.4	-19	42.5	3.4
	MAN9-3	C,	9.8	10.5	-18.2	27.6	3.3
	MAN9-4	C,	15.7	10.1	-18	44.3	3.3
	MAN9-5	C,	15.4	10.2	-18.1	43.2	3.3
	MAN9-6	C,	15.3	10.4	-18.1	43.1	3.3
	MAN9-7	C,	14.9	10.7	-18.1	41.8	3.3
	MAN9-8	C,	14.7	11.1	-18.1	41	3.3
Benabbio	US1047-1	C,	16.1	8.1	-20.2	42.9	3.1
	US1047-2	C,	14.8	6.7	-21	41.7	3.3
	US1047-3	C,	14.8	6.8	-21.2	44	3.5
	US1047-4	C,	14.7	6.4	-20.8	43.3	3.4
	US1047-5	C,	15.8	6.7	-19.9	41.9	3.1
	US1047-6	C,	15.5	6.5	-19.9	40.9	3.1
	US1047-7	C,	15.3	6.9	-19.9	40	3.1
	US1051-1	C,	14.4	8.5	-21.1	44.4	3.6
	US1051-2	C,	15.4	8.3	-19.4	43.3	3.3
	US1051-3	C,	15.2	8.2	-20	43.4	3.3
	US1051-4	C,	15.7	7.8	-20.5	45.5	3.4
	US1051-5	C,	15.2	7.2	-20.5	44.1	3.4
	US1051-6	C,	15.0	6.2	-21.2	44.6	3.5
	US1051-7	C,	16.1	6.0	-20.4	44.4	3.2
	US1051-8	C,	15.7	6.4	-19.8	43.5	3.2
	US1051-9	C,	15.3	6.3	-19.2	42.2	3.2
	US1051-10	C,	15.6	6.6	-19	43.5	3.2
	US1120-1	C,	16.0	8.9	-19.6	43.9	3.2
	US1120-2	C,	15.0	7.9	-21.5	44.5	3.5
	US1120-3	C,	15.8	7.9	-20.8	44	3.3
	US1120-4	C,	15.9	7.2	-19.9	44.4	3.3
	US1120-5	C,	15.7	6.7	-20.8	44.6	3.3
	US1120-6	C,	15.8	6.9	-20.6	44.8	3.3
	US1120-7	C,	16.7	7.1	-19.6	45.3	3.2
	US1120-8	C,	15.6	7.9	-19.1	42.7	3.2

Site	Sample and section #	Tooth	$\delta^{15}N$	Amt % N	$\delta^{13}C$	Amt % C	C:N
	US1132-1	C’	15.0	6.9	-20.6	45.1	3.5
	US1132-2	C’	14.5	6.4	-20.9	42.8	3.5
	US1132-3	C’	16.6	6.4	-20	45.1	3.2
	US1132-4	C’	16.5	6.3	-19.8	44.4	3.1
	US1132-5	C’	16.3	6.9	-19.5	43.8	3.1
	US1159-1	C’	15.7	8.8	-20.3	43.1	3.2
	US1159-2	C’	15.5	7.8	-20.5	43.2	3.3
	US1159-3	C’	16.4	7.4	-17.4	44.4	3.2
	US1159-4	C’	16.5	7.6	-17.4	44.5	3.2
	US1159-5	C’	16.7	7.6	-18.5	45	3.1
	US1200-1	C,	15.4	8.7	-20.2	42	3.2
	US1200-2	C,	15.9	7.8	-20.5	43.1	3.2
	US1200-3	C,	15.2	7.4	-20.9	44.1	3.4
	US1200-4	C,	14.6	7.1	-20	43	3.4
	US1200-5	C,	17.1	7.0	-18.7	45.5	3.1
	US1200-6	C,	16.7	6.5	-19.6	44.8	3.1
	US1200-7	C,	23.2	6.6	-20.1	61.7	3.1
	US1324-1	C’	14.7	8.3	-20.2	44.3	3.5
	US1324-2	C’	16.1	7.6	-19.5	43.9	3.2
	US1324-3	C’	16.2	7.9	-18.7	44.2	3.2
	US1324-4	C’	15.6	8.1	-19.1	42.2	3.2
	US1324-5	C’	10.4	7.9	-19.7	28.2	3.2
	US1324-6	C’	15.4	7.2	-20	41.5	3.1
	US1329-2	C,	15.5	7.1	-19	41.2	3.1
	US1329-3	C,	14.2	6.9	-18.9	38.3	3.1
	US1329-4	C,	14.8	7.1	-19.4	39.9	3.1
	US1329-5	C,	15.2	7.5	-18.5	39.8	3.1
	US1376-1	C,	14.5	7.3	-19.6	41.2	3.3
	US1376-2	C,	14.6	7.0	-19.1	39.7	3.2
	US1376-3	C,	14.9	7.0	-18.1	39.4	3.1
	US1376-4	C,	13.9	7.1	-18.9	38.2	3.2
	US1377-1	C’	14.6	7.1	-20.5	42.2	3.4
	US1377-2	C’	14.1	7.0	-20.3	40	3.3
	US1377-3	C’	17.9	5.3	-20.3	44.7	2.9
	US1414-1	C,	14.1	9.6	-21.5	43.5	3.6
	US1414-2	C,	14.2	9.1	-19.7	37.2	3.1

Site	Sample and section #	Tooth	$\delta^{15}N$	Amt % N	$\delta^{13}C$	Amt % C	C:N
	US1414-3	C,	11.4	8.6	-19.9	30.4	3.1
	US1414-4	C,	12.5	9.1	-20	33.1	3.1
	US1414-5	C,	15.2	10.5	-19.4	40.8	3.1
	US1419-1	C,	15.8	6.2	-19.6	43.2	3.2
	US1419-2	C,	15.2	5.6	-19.9	41.8	3.2
	US1419-3	C,	15.2	5.3	-20.4	42.4	3.2
	US1419-4	C,	15.0	5.6	-20.5	41.9	3.3
	US1494-1	C,	15.1	7.9	-19.9	42.2	3.3
	US1494-2	C,	15.2	6.8	-18.9	42.5	3.3
	US1494-3	C,	15.1	6.5	-19.5	42.1	3.3
	US1494-4	C,	15.2	6.4	-19.3	42.6	3.3
	US1809-1	C,	14.9	7.1	-20.7	42.4	3.3
	US1809-2	C,	14.8	7.2	-19.8	43.4	3.4
	US1809-3	C,	15.3	6.9	-19.7	43.4	3.3
	US1809-4	C,	14.9	6.4	-20	42	3.3
	US1809-5	C,	14.4	6.6	-20.1	42.9	3.5
	US1809-6	C,	15.3	6.2	-19.2	40.4	3.1
Badia Pozzeveri	US2108-1	C’	13.8	11.0	-14.8	37.4	3.2
	US2108-2	C’	14.5	11.5	-12.9	40.1	3.2
	US2108-3	C’	14.6	10.5	-12.5	40.1	3.2
	US2108-4	C’	13.8	9.4	-14.7	38.5	3.3
	US2108-5	C’	14.5	11.2	-11.7	39.4	3.2
	US2108-6	C’	15.0	11.1	-11.6	40.8	3.2
	US2108-7	C’	14.5	11.2	-12.2	39.4	3.2
	US2167-1	C,	17.5	12.3	-14.9	49.6	3.3
	US2167-2	C,	16.0	10.8	-15.8	44.6	3.2
	US2167-3	C,	16.0	11.6	-15	44.2	3.2
	US2167-4	C,	12.8	12.1	-15.7	39.5	3.6
	US2167-5	C,	16.4	11.6	-15	44.8	3.2
	US2167-6	C,	16.1	11.2	-15.8	44.4	3.2
	US2167-7	C,	15.3	11.3	-14.9	41.8	3.2
	US2167-8	C,	16.2	11.9	-13.9	44.3	3.2
	US2237-1	C,	16.4	13.1	-13.5	44.5	3.2
	US2237-2	C,	16.1	11.9	-15	44.6	3.2
	US2237-3	C,	15.3	12.0	-14.4	44.5	3.4
	US2237-4	C,	16.0	12.1	-11.4	44.7	3.3
	US2237-5	C,	15.4	11.7	-12.5	43.4	3.3
	US2237-6	C,	15.2	12.0	-11.3	42.6	3.3

Site	Sample and section #	Tooth	$\delta^{15}N$	Amt % N	$\delta^{13}C$	Amt % C	C:N
	US2325-1	C,	15.4	11.9	-14.6	41.5	3.1
	US2325-2	C,	16.4	12.0	-14.3	44.8	3.2
	US2325-3	C,	16.2	11.2	-15.2	44.4	3.2
	US2325-4	C,	15.8	11.1	-14.8	44.8	3.3
	US2325-5	C,	16.2	11.6	-12.8	44.7	3.2
	US2325-6	C,	16.0	11.6	-11.5	44.3	3.2
	US2325-7	C,	15.5	11.8	-11.9	42.3	3.2
	US2383-1	C’	12.8	14.1	-15.5	38	3.5
	US2383-2	C’	16.1	11.9	-15.6	44.3	3.2
	US2383-3	C’	16.1	11.7	-15.2	44.6	3.2
	US2383-4	C’	16.3	10.8	-14.7	44.8	3.2
	US2383-5	C’	16.3	10.4	-15.3	45.5	3.3
	US2383-6	C’	13.3	10.3	-15.2	36.5	3.2
	US2383-7	C’	14.1	11.9	-16	39.7	3.3
	US2409-1	C,	15.7	11.5	-16	43.4	3.2
	US2409-2	C,	15.9	10.7	-16.4	44.3	3.2
	US2409-3	C,	15.5	9.2	-17	45.3	3.4
	US2409-4	C,	16.2	8.9	-15.8	45.2	3.3
	US2409-5	C,	16.1	9.1	-15.5	44.6	3.2
	US2409-6	C,	15.8	9.2	-15.4	44.4	3.3
	US2409-7	C,	15.6	9.5	-15.6	43.5	3.3
	US2409-8	C,	15.1	9.8	-16	41.5	3.2
	US2415-1	C,	13.8	6.9	-18.2	38	3.2
	US2415-2	C,	12.5	7.0	-18.1	33.7	3.1
	US2415-3	C,	14.0	8.3	-18.8	38.1	3.2
	US2613-1	C,	15.3	11.0	-16	44.4	3.4
	US2613-2	C,	15.9	10.5	-16.9	45.3	3.3
	US2613-3	C,	16.3	10.7	-14.1	44.8	3.2
	US2613-4	C,	16.4	10.8	-13.6	45	3.2
	US2613-5	C,	16.6	10.7	-13.6	45.5	3.2
	US2623-1	C,	15.4	9.3	-19.8	42.6	3.2
	US2623-2	C,	15.4	9.0	-20.5	43.8	3.3
	US2623-3	C,	14.8	8.6	-20.1	43.6	3.4
	US2623-4	C,	15.5	8.1	-19.7	43.8	3.3
	US2623-5	C,	14.9	8.2	-19.6	41.9	3.3
	US2623-6	C,	15.4	7.5	-19.7	40.4	3.1
	US2776-1	C,	15.7	9.9	-18.6	43.5	3.2
	US2776-2	C,	15.4	9.1	-18.6	43.2	3.3
	US2776-3	C,	15.3	8.2	-18.8	44.2	3.4
	US2776-4	C,	15.8	7.9	-18.7	45.3	3.3
	US2776-5	C,	15.5	8.0	-18.9	44.1	3.3

Site	Sample and section #	Tooth	$\delta^{15}N$	Amt % N	$\delta^{13}C$	Amt % C	C:N
	US2776-6	C,	15.6	7.6	-18.9	44	3.3
	US3287-1	C,	15.5	13.3	15.1	-15.1	3.2
	US3287-2	C,	15.0	11.9	41.2	-14.7	3.2
	US3287-3	C,	13.4	11.1	37.2	-15.4	3.2
	US3287-4	C,	12.0	11.2	36.6	-17.4	3.5
	US3287-5	C,	14.1	11.2	39.4	-16.1	3.3
	US3391-1	C,	16.3	11.9	-14.9	44.6	3.2
	US3391-2	C,	15.8	11.8	-15.7	45	3.3
	US3391-3	C,	16.1	11.2	-16.2	44.3	3.2
	US3391-4	C,	16.2	11.5	-15.2	44.2	3.2
	US3391-5	C,	16	11.3	-14.5	43.8	3.2
	US3397-1	C,	14.8	9.9	-18.3	40.9	3.2
	US3397-2	C,	14.7	9.4	-17.7	41.3	3.3
	US3397-3	C,	13.1	9.7	-18.3	39.5	3.5
	US3397-4	C,	15.5	9.7	-17	42.1	3.2
	US3397-5	C,	12.8	9.2	-18.1	34.9	3.2
	US3397-6	C,	13.7	9.1	-17.9	37.9	3.2
	US3397-7	C,	13.4	9.2	-17.8	35.7	3.1
	US3546-1	C’	15.8	9.4	-18.9	43.8	3.2
	US3546-2	C’	16.1	9.5	-19	45.1	3.3
	US3546-3	C’	16.2	9.1	-18.2	44.6	3.2
	US3546-4	C’	16.1	9.0	-17.7	43.3	3.1
	US3546-5	C’	16.3	8.9	-18.3	44.2	3.2
	US3546-6	C’	16.0	9.1	-18.2	43	3.1
	US3552-1	C,	13.9	13.8	-18.7	37.6	3.2
	US3552-2	C,	14.1	12.1	-19.3	38.8	3.2
	US3552-3	C,	14.3	11.3	-18.8	39.7	3.2
	US3552-4	C,	13.6	11.2	-18.1	39.1	3.4
	US3552-5	C,	15.0	11.0	-17.2	41.1	3.2
	US3552-6	C,	14.9	11.1	-16.8	41.3	3.2
	US3552-7	C,	14.8	11.2	-16.8	40.1	3.2
	US3552-8	C,	14.9	11.3	-16.7	41.1	3.2
	US3555-1	C,	16.1	9.5	-19.3	43.9	3.2
	US3555-2	C,	16.6	9.1	-19.4	45.6	3.2
	US3555-3	C,	15.4	8.7	-19.7	45.6	3.5
	US3555-4	C,	16.1	8.1	-19	44.7	3.2
	US3555-5	C,	16.0	8.8	-19.3	44.8	3.3
	US3555-6	C,	15.8	9.1	-18.3	43.8	3.2
	US3555-7	C,	24.1	8.9	-18.3	66.7	3.2
Pieve dei Monti di Villa	US1057-1	C’	14.9	8.7	-19.2	41.4	3.2

Site	Sample and section #	Tooth	$\delta^{15}N$	Amt % N	$\delta^{13}C$	Amt % C	C:N
	US1057-2	C’	16.1	7.6	-19	45.2	3.3
	US1057-3	C’	16.0	7.9	-18.3	44.7	3.3
	US1057-4	C’	13.7	7.1	-19.2	38	3.2
	US1060-1	C,	14.2	8.6	-19.3	40.2	3.3
	US1060-2	C,	16.1	9.0	-17.2	42.4	3.1
	US1060-3	C,	14.8	8.6	-16.9	39.2	3.1
	US1060-4	C,	15.2	8.5	-18	39.4	3.0
	US1060-5	C,	14.6	8.1	-19.4	39.3	3.1
	US1066-1	C’	15.5	8.5	-18.7	40.6	3.1
	US1066-2	C’	14.8	8.7	-18.6	39.2	3.1
	US1066-3	C’	14.5	9.0	-18.3	37.7	3.0
	US1066-4	C’	15.2	9.3	-17.7	39.8	3.1
	US1067-1	C,	16.6	9.6	-19.1	45.5	3.2
	US1067-2	C,	12.8	7.2	-19.3	38.4	3.5
	US1067-3	C,	15.7	6.7	-18.7	42.8	3.2
	US1067-4	C,	15.2	7.4	-17	41.5	3.2
	US1067-5	C,	14.2	6.6	-18.6	38.3	3.1
	US1069-1	C,	13.4	7.7	-18.6	37.7	3.3
	US1069-2	C,	16.5	6.8	-18	44.3	3.1
	US1069-3	C,	14.6	6.7	-18.2	39.3	3.2
	US1069-4	C,	14.5	6.8	-18.1	38.9	3.1
	US1069-6	C,	14.0	6.9	-18	37.2	3.1
	US1072-1	C,	15.6	7.1	-18.1	44.1	3.3
	US1072-2	C,	14.5	6.4	-18.2	42.7	3.4
	US1072-3	C,	15.4	6.8	-18	44	3.3
	US1072-4	C,	15.0	7.0	-18.3	43.1	3.4
	US1072-5	C,	15.1	7.2	-18.4	43.3	3.4
	US1072-6	C,	14.7	7.6	-17.5	41.7	3.3
	US1072-7	C,	13.8	7.5	-18.7	39.1	3.3
	US1087-1	C,	15.0	6.4	-18.5	41.6	3.2
	US1087-2	C,	15.0	6.3	-18.9	40.9	3.2
	US1087-3	C,	14.8	6.3	-18.6	40.7	3.2

APPENDIX B:

DENTAL HISTOLOGY RAW DATA

Sample	Site	Sex	Age	# of Accentuated Striae	Defect Number	Age of Defect Formation	Periodicity
M12	Alia	M		5	1	1.86	---
					2	2.53	0.67
					3	2.56	0.03
					4	2.59	0.03
					5	3.85	1.26
M14/74	Alia	F	15	0	---	---	---
M15	Alia	M	27	4	---	---	---
M19	Alia	F	22	2	1	0.22	---
					2	2.88	2.66
M17	Alia	F	42.5	5	1	0.78	---
					2	0.84	0.06
					3	1.31	0.47
					4	1.35	0.04
					5	2.67	1.32
M5	Alia	M	30	2	1	1.78	---
					2	1.82	0.04
M20	Alia	F	37.5	10	1	1.58	---
					2	1.61	0.03
					3	1.65	0.04
					4	1.69	0.04
					5	1.72	0.03
					6	1.79	0.07
					7	1.86	0.07
					8	2.15	0.29
					9	2.63	0.48
					10	2.65	0.02
M21	Alia	F	27.5	8	1	1.64	---
					2	2.02	0.38
					3	2.71	0.69
					4	2.92	0.21
					5	3.01	0.09
					6	3.32	0.31
					7	3.65	0.33
					8	3.71	0.06
A52	Alia	M	15	10	1	1.9	---
					2	2.19	0.29
					3	2.74	0.55
					4	2.81	0.07
					5	2.85	0.04
					6	2.86	0.01
					7	3.08	0.22
					8	3.46	0.38
					9	3.75	0.29
					10	3.79	0.04

Sample	Site	Sex	Age	# of Accentuated Striae	Defect Number	Age of Defect Formation	Periodicity
M6	Alia	M	21	15	1	2.33	---
					2	2.37	0.04
					3	2.5	0.13
					4	2.58	0.08
					5	2.72	0.14
					6	2.8	0.08
					7	2.85	0.05
					8	3.05	0.2
					9	3.07	0.02
					10	3.22	0.15
					11	3.26	0.04
					12	3.29	0.03
					13	3.34	0.05
					14	3.42	0.08
					15	3.52	0.1
M7	Alia	M	35	4	1	0.73	---
					2	2.83	2.1
					3	2.95	0.12
					4	3.34	0.39
A48	Alia	M	15	13	1	2.1	---
					2	2.22	0.12
					3	2.24	0.02
					4	2.28	0.04
					5	2.3	0.02
					6	2.35	0.05
					7	2.4	0.05
					8	2.44	0.04
					9	2.52	0.08
					10	2.53	0.01
					11	2.59	0.06
					12	2.61	0.02
					13	3.54	0.93
A90	Alia	M	35	1	1	2.03	---
A85	Alia	I	15	17	1	0.66	---
					2	0.69	0.03
					3	0.79	0.1
					4	1.08	0.29
					5	1.11	0.03
					6	1.18	0.07
					7	1.56	0.38
					8	1.71	0.15
					9	1.89	0.18
					10	2.03	0.14
					11	2.08	0.05
					12	2.16	0.08
					13	2.42	0.26
					14	2.45	0.03
					15	2.48	0.03
					16	2.6	0.12
					17	3.08	0.48
A10	Alia	M	45	11	1	1.92	---
					2	2.15	0.23
					3	2.31	0.16
					4	2.34	0.03

Sample	Site	Sex	Age	# of Accentuated Striae	Defect Number	Age of Defect Formation	Periodicity
					5	2.38	0.04
					6	2.51	0.13
					7	2.59	0.08
					8	2.73	0.14
					9	3.27	0.54
					10	3.62	0.35
					11	3.65	0.03
MAN4	Alia	M	30	2	1	1.2	---
					2	4.11	2.91
MAN10	Alia	F		5	1	0.54	---
					2	0.66	0.12
					3	0.73	0.07
					4	0.78	0.05
					5	0.8	0.02
MAN13	Alia	F	25	2	1	1.96	
					2	2.37	0.41
MAN81/51	Alia	I	27	0	---	---	---
A81	Alia	M	25	6	1	0.42	---
					2	0.74	0.32
					3	0.83	0.09
					4	2.31	1.48
					5	2.37	0.06
					6	3.08	0.71
MAN3	Alia	F	29.5	6	1	1.68	---
					2	2.41	0.73
					3	2.53	0.12
					4	2.56	0.03
					5	3.01	0.45
					6	3.59	0.58
MAN11	Alia	F	29.5	13	1	1.9	---
					2	1.93	0.03
					3	2.06	0.13
					4	2.08	0.02
					5	2.14	0.06
					6	2.78	0.64
					7	2.86	0.08
					8	2.87	0.01
					9	2.9	0.03
					10	2.95	0.05
					11	2.98	0.03
					12	3.06	0.08
					13	3.58	0.52
MAN9	Alia	M	29.5	10	1	0.51	---
					2	0.91	0.4
					3	1.22	0.31
					4	1.31	0.09
					5	1.43	0.12
					6	1.45	0.02
					7	1.77	0.32
					8	2.3	0.53
					9	2.42	0.12
					10	3.17	0.75
A40	Alia	M	35	6	1	1.31	---
					2	2.17	0.86

Sample	Site	Sex	Age	# of Accentuated Striae	Defect Number	Age of Defect Formation	Periodicity
					3	2.29	0.12
					4	2.6	0.31
					5	3.51	0.91
					6	3.57	0.06
A117	Alia	I	30	5	1	0.6	---
					2	0.62	0.02
					3	0.84	0.22
					4	1.72	0.88
					5	2.35	0.63
M2	Alia	I	29.5	8	1	2.43	---
					2	2.61	0.18
					3	2.87	0.26
					4	3.14	0.27
					5	3.29	0.15
					6	3.53	0.24
					7	3.79	0.26
					8	3.9	0.11
A42	Alia	F	35	8	1	1.93	---
					2	2.04	0.11
					3	2.08	0.04
					4	2.34	0.26
					5	2.36	0.02
					6	2.5	0.14
					7	2.61	0.11
					8	3.14	0.53
A38	Alia	M	30	10	1	1.53	---
					2	1.57	0.04
					3	1.71	0.14
					4	1.77	0.06
					5	1.82	0.05
					6	1.93	0.11
					7	1.96	0.03
					8	1.98	0.02
					9	2.39	0.41
					10	3.22	0.83
A18	Alia	M	25	5	1	2.37	---
					2	2.48	0.11
					3	2.52	0.04
					4	2.74	0.22
					5	2.92	0.18
A99	Alia	F	15	7	1	3.1	---
					2	3.12	0.02
					3	3.18	0.06
					4	3.32	0.14
					5	3.44	0.12
					6	3.47	0.03
					7	3.72	0.25
M1	Alia	M	30	6	1	1.57	---
					2	1.6	0.03
					3	1.68	0.08
					4	2.01	0.33
					5	2.07	0.06
					6	3.96	1.89

Sample	Site	Sex	Age	# of Accentuated Striae	Defect Number	Age of Defect Formation	Periodicity
US1047	Benabbio	M	57.5	4	1	1.88	---
					2	2.83	0.95
					3	3.32	0.49
					4	3.35	0.03
US1046	Benabbio	M	47.5	0	0	---	---
US1051	Benabbio	M	50	1	---	---	---
US1132	Benabbio	M	60	13	---	---	---
US1159	Benabbio	F	60	4	1	1.96	---
					2	3.49	1.53
					3	3.64	0.15
					4	3.72	0.08
US1194	Benabbio	I		10	1	1.53	---
					2	1.64	0.11
					3	1.7	0.06
					4	2.05	0.35
					5	2.09	0.04
					6	2.13	0.04
					7	2.27	0.14
					8	3.37	1.1
					9	3.72	0.35
					10	3.88	0.16
US1200	Benabbio	M	47.5	5	1	1.03	---
					2	1.07	0.04
					3	1.39	0.32
					4	1.75	0.36
					5	2.51	0.76
US1355	Benabbio	F	54.5	6	1	2.64	---
					2	2.68	0.04
					3	2.86	0.18
					4	2.99	0.13
					5	3.22	0.23
					6	3.54	0.32
US1052	Benabbio	F	44.5	3	1	0.78	---
					2	1.33	0.55
					3	3.31	1.98
US1362	Benabbio	M	50	0	---	---	---
US1371	Benabbio	M	50	2	1	1.96	---
					2	2.03	0.07
US1470	Benabbio	F	24	1	---	---	---
US1329	Benabbio	I		1	1	2.27	---
US1376	Benabbio	M	33.5	1	---	---	---
US1413	Benabbio	M	42.5	0	---	---	---
US1414	Benabbio	M	42.5	8	1	1.82	---

Sample	Site	Sex	Age	# of Accentuated Striae	Defect Number	Age of Defect Formation	Periodicity
					2	1.9	0.08
					3	1.91	0.01
					4	2.03	0.12
					5	2.59	0.56
					6	2.61	0.02
					7	2.76	0.15
					8	3.1	0.34
US1054	Benabbio	F	19	8	---	---	---
US1377	Benabbio	F	60	34	1	0.39	---
					2	0.46	0.07
					3	0.52	0.06
					4	0.62	0.1
					5	0.73	0.11
					6	0.75	0.02
					7	0.95	0.2
					8	1.16	0.21
					9	1.21	0.05
					10	2.27	1.06
					11	2.29	0.02
					12	2.31	0.02
					13	2.37	0.06
					14	2.38	0.01
					15	2.41	0.03
					16	2.47	0.06
					17	2.51	0.04
					18	2.55	0.04
					19	2.72	0.17
					20	2.77	0.05
					21	2.82	0.05
					22	2.86	0.04
					23	2.91	0.05
					24	2.99	0.08
					25	3.02	0.03
					26	3.15	0.13
					27	3.17	0.02
					28	3.19	0.02
					29	3.22	0.03
					30	3.24	0.02
					31	3.54	0.3
					32	3.55	0.01
					33	3.7	0.15
					34	3.74	0.04
US1419	Benabbio	F	60	13	1	2.9	---

Sample	Site	Sex	Age	# of Accentuated Striae	Defect Number	Age of Defect Formation	Periodicity
					2	2.94	0.04
					3	2.98	0.04
					4	2.99	0.01
					5	3.22	0.23
					6	3.28	0.06
					7	3.33	0.05
					8	3.34	0.01
					9	3.43	0.09
					10	3.6	0.17
					11	3.72	0.12
					12	3.78	0.06
					13	3.92	0.14
US1494	Benabbio	F	35	7	1	2.25	---
					2	2.4	0.15
					3	2.52	0.12
					4	3.15	0.63
					5	3.26	0.11
					6	3.53	0.27
					7	3.61	0.08
US1809	Benabbio	M	60	0	---	---	---
US3352	Badia Pozzeveri	M	47.5	24	1	0.51	---
					2	0.79	0.28
					3	0.88	0.09
					4	0.96	0.08
					5	0.99	0.03
					6	1.01	0.02
					7	1.37	0.36
					8	1.39	0.02
					9	1.42	0.03
					10	1.61	0.19
					11	1.67	0.06
					12	1.74	0.07
					13	1.79	0.05
					14	1.81	0.02
					15	1.84	0.03
					16	1.89	0.05
					17	1.97	0.08
					18	2	0.03
					19	2.03	0.03
					20	2.05	0.02
					21	2.18	0.13
					22	2.36	0.18
					23	2.53	0.17

Sample	Site	Sex	Age	# of Accentuated Striae	Defect Number	Age of Defect Formation	Periodicity
					24	2.89	0.36
US3397	Badia Pozzeveri	F	27	10	1	1.45	---
					2	1.46	0.01
					3	2.08	0.62
					4	2.66	0.58
					5	2.92	0.26
					6	2.95	0.03
					7	2.98	0.03
					8	3.38	0.4
					9	3.54	0.16
					10	4.02	0.48
US2415	Badia Pozzeveri	M	32	15	1	0.42	---
					2	0.48	0.06
					3	0.54	0.06
					4	0.63	0.09
					5	0.93	0.3
					6	0.94	0.01
					7	0.96	0.02
					8	0.99	0.03
					9	1.03	0.04
					10	1.07	0.04
					11	1.09	0.02
					12	1.12	0.03
					13	1.14	0.02
					14	1.16	0.02
					15	1.78	0.62
US3287	Badia Pozzeveri	M	60	7	1	0.92	---
					2	0.97	0.05
					3	1.1	0.13
					4	1.37	0.27
					5	1.68	0.31
					6	2.92	1.24
					7	2.94	0.02
US2108	Badia Pozzeveri	M	25	0	---	---	---
US2383	Badia Pozzeveri	F	30.7	23	1	1.78	---
					2	1.86	0.08
					3	1.92	0.06
					4	1.94	0.02
					5	1.98	0.04
					6	2	0.02
					7	2.03	0.03
					8	2.05	0.02
					9	2.33	0.28

Sample	Site	Sex	Age	# of Accentuated Striae	Defect Number	Age of Defect Formation	Periodicity
					10	2.56	0.23
					11	2.59	0.03
					12	2.61	0.02
					13	2.65	0.04
					14	2.71	0.06
					15	2.78	0.07
					16	3.05	0.27
					17	3.26	0.21
					18	3.33	0.07
					19	3.34	0.01
					20	3.36	0.02
					21	3.61	0.25
					22	3.84	0.23
					23	3.91	0.07
US2613	Badia Pozzeveri	M	50	5	1	1.69	---
					2	2.18	0.49
					3	2.2	0.02
					4	2.26	0.06
					5	2.29	0.03
US2167	Badia Pozzeveri	M	37.5	5	1	0.69	---
					2	0.77	0.08
					3	0.8	0.03
					4	0.9	0.1
					5	0.91	0.01
US2325	Badia Pozzeveri	M	23	8	1	1.99	---
					2	2.4	0.41
					3	2.41	0.01
					4	2.46	0.05
					5	2.57	0.11
					6	2.59	0.02
					7	2.71	0.12
					8	2.73	0.02
US2237	Badia Pozzeveri	M	15.2	0	---	---	---
US2409	Badia Pozzeveri	F	32	15	1	0.5	---
					2	0.55	0.05
					3	0.63	0.08
					4	0.69	0.06
					5	0.73	0.04
					6	0.76	0.03
					7	0.78	0.02
					8	0.81	0.03
					9	0.91	0.1
					10	0.93	0.02

Sample	Site	Sex	Age	# of Accentuated Striae	Defect Number	Age of Defect Formation	Periodicity
					11	0.99	0.06
					12	1.06	0.07
					13	1.14	0.08
					14	3.44	2.3
					15	3.47	0.03
US3391	Badia Pozzeveri	M	60	12	1	0.92	---
					2	0.97	0.05
					3	1.03	0.06
					4	1.05	0.02
					5	1.08	0.03
					6	1.21	0.13
					7	1.24	0.03
					8	1.25	0.01
					9	1.32	0.07
					10	1.34	0.02
					11	1.36	0.02
					12	1.37	0.01
US3546	Badia Pozzeveri	M	32.5	5	1	2.05	---
					2	2.49	0.44
					3	2.87	0.38
					4	2.89	0.02
					5	3.72	0.83
US3555	Badia Pozzeveri	I	37.5	0	---	---	---
US2776	Badia Pozzeveri	M	54.5	6	1	1.7	---
					2	1.98	0.28
					3	2.57	0.59
					4	2.6	0.03
					5	2.63	0.03
					6	2.69	0.06
US2623	Badia Pozzeveri	M		5	1	0.5	---
					2	0.67	0.17
					3	1.49	0.82
					4	1.82	0.33
					5	1.87	0.05
US1060	Monti di Villa	M	22	8	1	1.37	---
					2	1.49	0.12
					3	2.16	0.67
					4	2.54	0.38
					5	2.64	0.1
					6	2.68	0.04
					7	3.17	0.49
					8	3.36	0.19
US1066	Monti di Villa	F	37.5	3	1	2	---

Sample	Site	Sex	Age	# of Accentuated Striae	Defect Number	Age of Defect Formation	Periodicity
					2	2.13	0.13
					3	2.46	0.33
US1067	Monti di Villa	I		12	1	1.24	---
					2	1.85	0.61
					3	1.93	0.08
					4	1.97	0.04
					5	2.1	0.13
					6	2.3	0.2
					7	2.48	0.18
					8	3.24	0.76
					9	3.37	0.13
					10	3.54	0.17
					11	3.77	0.23
					12	3.89	0.12
US1069	Monti di Villa	F	54	6	1	1.47	---
					2	1.57	0.1
					3	1.7	0.13
					4	1.98	0.28
					5	2.15	0.17
					6	3.69	1.54
US1093	Monti di Villa	M	50	1	1	3.48	---
US1087	Monti di Villa	M	45	3	1	2.21	---
					2	2.23	0.02
					3	2.49	0.26
US1057	Monti di Villa	F	60	6	1	1.41	---
					2	1.48	0.07
					3	2.03	0.55
					4	2.47	0.44
					5	3.26	0.79
					6	3.46	0.2
US1072	Monti di Villa	F	54	3	1	2.2	---
					2	3.56	1.36
					3	3.65	0.09
US1087	Monti di Villa	M	45	0	---	---	---