



Exploring the effects of weaning age on adult infectious disease mortality among 18th–19th century Italians

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Abstract

Objectives: The developmental origins of health and disease (DOHaD) hypothesis describes how early childhood stress affects morbidity and mortality later in life. The role of early childhood stress in mortality from infectious disease is understudied. 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 potential periods of significant stress in early childhood. This research investigates the effect of weaning after ~6 months of age on cholera mortality among 18th–19th-century Italian populations by determining if earlier breastfeeding cessation is associated with earlier mortality, analyzing childhood dietary variation and physiological stress markers, and determining if age-at-weaning completion differs between catastrophic and attritional populations.

Methods: Serial dentin stable carbon and nitrogen isotope analyses from canines are compared between catastrophic ($n = 45$) and attritional groups ($n = 23$). Canines are sectioned serially from crown to apex, and the increment's $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ are used to estimate age-at-weaning completion.

Results: Catastrophic and attritional groups exhibit similar age-at-weaning completion (~2.8 years). Seventy-four percent of individuals lack elevated $\delta^{15}\text{N}$ values in dentin that formed during infancy.

Conclusions: Age-at-weaning completion was 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 weaning completion likely occurred after infants had adapted to consuming contaminated weaning foods. Individuals without detectable weaning curves may represent infants who received supplementary foods since birth or were weaned before the age of 6–9 months.

1 | INTRODUCTION

The developmental origins of health and disease (DOHaD) hypothesis posit that in utero and early childhood stress increase susceptibility to adult non-communicable disorders (Barker, 1995, 1997, 2004;

Barker & Osmond, 1986; Gluckman et al., 2005; Kuzawa, 2005; Worthman & Kuzara, 2005). A significant body of knowledge establishes a link between early childhood stress and adult non-communicable disorders, including heart disease (Barker et al., 1993; Barker, Osmond, Golding, et al., 1989; Barker, Osmond, &



Law, 1989; Osmond et al., 1993), type II diabetes (Desai et al., 1995; Hales et al., 1991; Hales & Barker, 1992, 2001; Lithell et al., 1996) and increased blood pressure (Barker et al., 1990; Barker, Osmond, Golding, et al., 1989; Campbell et al., 1996; Law et al., 1991). Researchers hypothesize that early childhood stress triggers a physiological response that causes metabolic dysregulation that eventually leads to premature death and reduced fertility in adulthood. Research also suggests that immune-related physiological changes occur in response to early childhood stress, including dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis (Fowden et al., 2005; Merlot et al., 2008), inhibition of macrophage functionality (Fonseca et al., 2002; Neto et al., 2001), and decreased cell cytokine response (Coe et al., 2002).

Though evidence exists that links early childhood stress to diminished immunological responses, research investigating the relationship between early childhood stress and infectious disease later in life has yielded inconsistent results. 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 vaccine. McDade et al. (2001b) found that prenatal undernutrition was significantly associated 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 survived to age 15 suffered an increased risk of premature death due to infectious disease compared to children born during the dry season, suggesting that stress in early childhood may have caused reduced immune function later in life. However, Moore et al. (2001) later found no difference in immune function—as measured by vaccine response, delayed-type hypersensitivity, and mucosal immunity—between children born in the wet and dry seasons. However, Moore et al. (2001) observed reduced survivability in adulthood due to all causes among those born during the hungry season, which they attributed to an immunologic memory defect or early immunosenescence. 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 groups suggesting that early childhood stress did 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 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; Gollwitzer & Marsland, 2015; Neville

et al., 2012), reduces gastrointestinal diseases (Cunningham, 1995; Neville et al., 2012), reduces inflammation (Walker, 2010), and promotes a healthy gut microbiome (de Agueero et al., 2016; Kau et al., 2011; Martin & Sela, 2012; Zivkovic et al., 2011). Much research converges on 6 months of exclusive breastfeeding as the optimal amount of time prior to introducing other foods to an infant's diet, with ample evidence of immediate and delayed detriments to health among individuals commencing supplemental feeding prior to this age (Kramer & Kakuma, 2012; WHO, 2009). More debatable is the impact of supplemental feeding later in infancy and childhood (e.g., after the first 6 months).

Breastfeeding practices are typically poorly documented in the historical record (Baxter, 2005). However, the reconstruction of infant feeding practices has become an important topic of interest in bioarchaeological studies in recent years (Eerkens et al., 2011; Fogel et al., 1989; Fuller et al., 2003; Fuller et al., 2006; Henderson et al., 2014; Mays et al., 2017). Among bioarchaeologists, the introduction of complementary foods is traditionally viewed as a period of significant stress in early childhood. Complementary breastfeeding exposes infants to new pathogens while simultaneously the immunological benefits of breastfeeding decline with fewer feedings (Lönnerdal, 2003). Prior to the early 20th century, weaning foods may have been nutritionally deficient or contaminated from unsanitary preparation, increasing the risk of diarrheal disease and malnutrition in weaning infants (Horta et al., 2013; Motarjemi et al., 1993). However, the period of complementary breastfeeding may also be beneficial for the infant (Kendall et al., 2021). During complementary feeding, breastmilk continues to provide the infant with a sterile source of nutrients and calories while continuing to promote the growth of beneficial gut microbiota and a healthy immune system (de Agueero et al., 2016; Macpherson et al., 2017; Martin & Sela, 2012). Complementary breastfeeding has been associated with lower rates of diarrheal disease when compared to children who were not breast-fed (Betrán et al., 2001; Lamberti et al., 2011; Wright et al., 2017). The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) recommend that mothers exclusively breastfeed until at least 6 months of age (WHO, 2009). However, complementary feeding can occur over a period of several years, particularly in pre-industrial contexts where women often did not face the need to return to work outside of the household soon after giving birth (Sellen, 2009; Wickes, 1953a).

Weaning can be reconstructed in archeological 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., 2003, 2006;

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 & Yoneda, 2013, 2015). Nitrogen isotope ratios in human bone collagen generally exhibit a +3‰ offset per trophic position, though this offset can range from +1‰ to +6‰ (DeNiro & Epstein, 1981; Minagawa & Wada, 1984; O'Connell et al., 2012). Exclusively breastfeeding infants exhibit a higher $\delta^{15}\text{N}$ because they are feeding one trophic level above their mother (Fogel et al., 1989; Wright & Schwarcz, 1999). Stable carbon isotope ratios also exhibit a slight trophic effect with an offset of approximately +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 $\delta^{13}\text{C}$ signals the introduction of solid foods into the diet at the initiation of weaning because weaning foods generally contain more carbon and less nitrogen than breastmilk (Fuller et al., 2006). The nitrogen incorporated into tissues is derived mainly from protein sources, whereas the carbon in tissues is derived from protein, carbohydrate and lipid sources (Ambrose & Norr, 1993; DeNiro & Epstein, 1981; Fernandes et al., 2012; Minagawa & Wada, 1984; Tieszen & Fagre, 1993).

Physiological stress, particularly nutritional deprivation, is also associated with an elevation of ^{15}N (Deschner et al., 2012; Fuller et al., 2005; Hobson et al., 1993), which may obscure the weaning signal in dentine isotope profiles. Nutritional deprivation can cause the catabolism of tissues, which consequently concentrates ^{15}N in the body since the lighter ^{14}N isotope is preferentially excreted. The result of this process is an elevation in ^{15}N in tissues formed during the period of stress. Recent research suggests that isotope profiles where carbon and nitrogen isotopes covary are associated with breastfeeding, whereas nitrogen isotope profiles that do not covary with carbon isotope profiles likely indicate periods of physiological stress (Beaumont et al., 2018).

This study uses stable carbon and nitrogen isotope analysis of tooth dentine to test the hypothesis that age-at-weaning completion differed between 18th and 19th-century catastrophic and attritional groups from Italy. Catastrophic burials are defined here as burials that result from a catastrophic event, such as a pandemic. Attritional burials represent the gradual accumulated deaths from a population over time (Margerison & Knüsel, 2002). The catastrophic groups used in this study are burials associated with the cholera pandemics during the 19th-century. Currently, no research has explored the relationship between weaning age and cholera mortality. 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 & Gessler, 2014; Sack et al., 2004). Victims of cholera rapidly become dehydrated, and death can occur within 6–8 h of exhibiting symptoms. During the 19th century, fatality rates for cholera were between 30% and 60% (Kotar & Gessler, 2014; Lima, 1994). In non-endemic populations, cholera disease frequencies are evenly distributed among all age and sex categories (Kaper et al., 1995). Cholera victims with type-O blood experience more severe symptoms than other blood groups, likely due to an enhanced cellular response to the cholera toxin (Glass et al., 1985; Kuhlmann et al., 2016).

Here, we define age-at-weaning completion as the complete cessation of complementary breastfeeding such that the child begins to exclusively consume solid food and stops consuming breast milk. We estimate the age-at-weaning completion using serial increments of tooth dentin from canines. Tooth dentin is used because it does not remodel once formed and, excepting secondary dentin, does not form after childhood. Thus, dentin captures dietary data at the specific time period in which it was formed. This research uses tooth dentin from canines, which typically form between 6 and 9 months of age. Though canines form late in infancy, age-at-weaning completion should be captured in canine dentin because the complementary feeding period for infants in 18th–19th century Italy likely occurred over several years since Italy did not experience significant industrialization until the late 19th-century (Duggan, 2014; Esposto, 1992; Profumieri, 2009).

There are three main goals of this research. First, we determine if earlier breastfeeding cessation is associated with differential mortality outcomes compared to later breastfeeding cessation. Second, we analyze the changes in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ profiles to determine if individuals experienced dietary variability and/or physiological stress during childhood. Finally, we determine if age-at-weaning completion differed between catastrophic and attritional samples.

2 | MATERIALS AND METHODS

2.1 | Skeletal samples

Skeletons included in this study come from four 18- to 19th-century cemeteries in Italy (Figure 1), including two cemeteries containing known cholera victims.

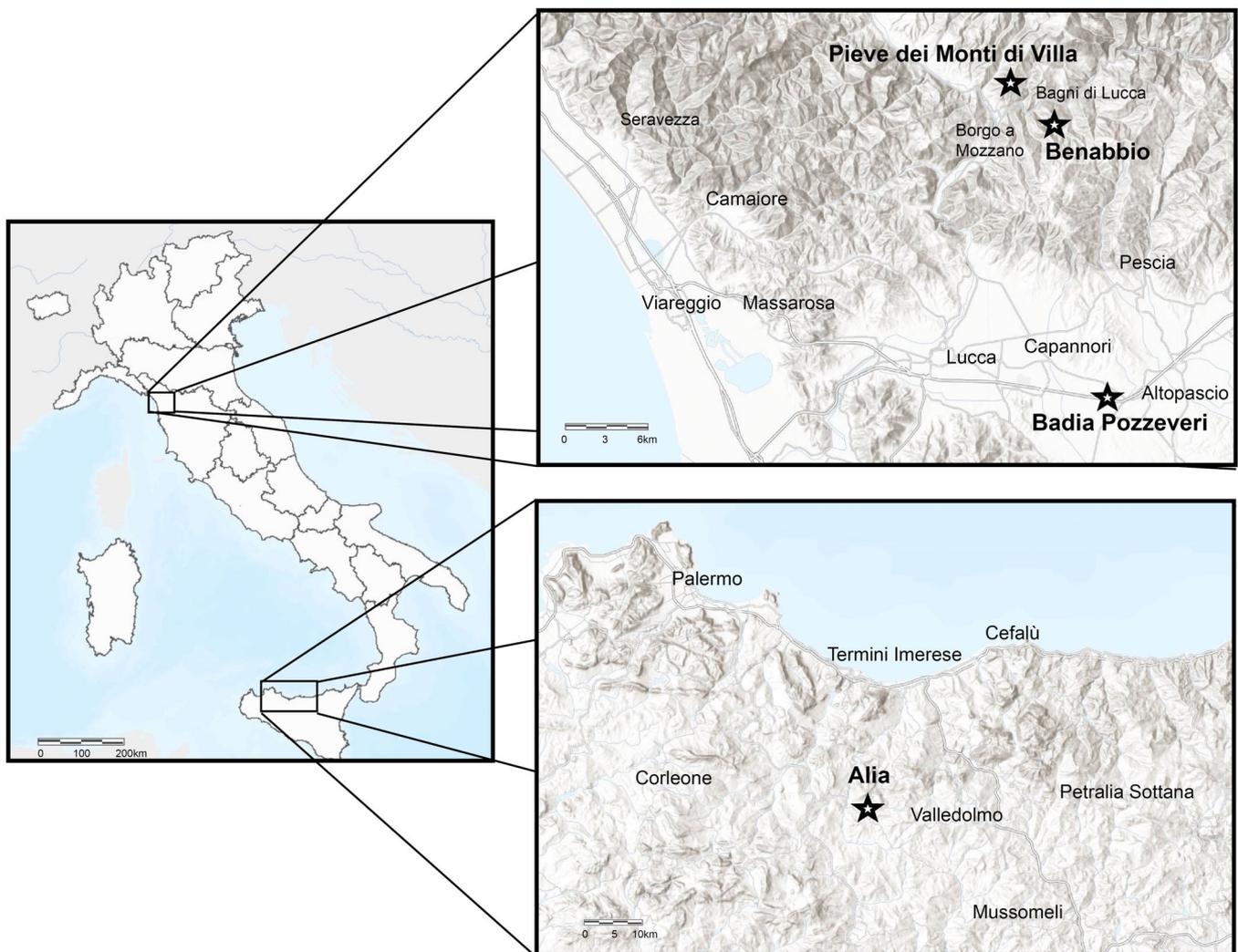


FIGURE 1 Map of archeological sites: Alia, Benabbio, Badia Pozzeveri and Pieve di Monti di Villa

Camposanto vecchio, located in Alia (PA; Sicily) and the San Michele of Benabbio (LU; Tuscany) are cemeteries where cholera victims from the 1837 and 1855 pandemics, respectively, were interred. The church of San Pietro in Badia Pozzeveri (LU; Tuscany) and the parish of San Giovanni Battista in Pieve di Monti di Villa (LU; Tuscany) are two 18th–19th-centuries burial grounds containing individuals who died from various unknown causes.

Camposanto vecchio is a 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 and 2000 (Chiarelli, 2002; Guccione, 1991). Since this site contained a mass burial, the remains from Camposanto

vecchio were disarticulated. Parochial records from the Parish of Saint Anna in the center of Alia record 306 names of cholera victims that died in the 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 between 2007 and 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 19th century (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 has linked these burials to cholera epidemics (Santiago-Rodriguez et al., 2019). For this study, we chose individuals that date to the 18th and 19th century whose burial patterns are consistent with non-catastrophic burials, including grave 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 di Monti di Villa, Italy included a cemetery that was used between 1807 and 1870 (Rezza, 2009). The church of San Giovanni Battista is located within the village center of Pieve di Monti di Villa, which is located in the Serchio valley. The University of Pisa excavated the 19th-century 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, 2015; Rezza, 2009).

2.2 | Stable carbon and nitrogen isotope analysis

To reconstruct weaning patterns, one canine tooth was taken from each individual from Alia ($n = 31$), Benabbio

($n = 14$), Badia Pozzeveri ($n = 16$) and Pieve di Monti di Villa ($n = 7$). Of the total sample, 55.9% ($n = 38$) of individuals are male, 35.3% ($n = 24$) are female, and 8.8% ($n = 6$) are of indeterminate sex. Those of indeterminate sex were excluded from sex-based statistical analyses. Since individuals from Alia are disarticulated and aggregated, social status was not able to be reconstructed or inferred from these remains. The archeological context and historic documents related to Benabbio and Pieve di Monti di Villa suggest that all individuals from these sites were of low social status (Baldino, 2011; Rezza, 2009). Individuals from Badia Pozzeveri were included in this study if their burial context was consistent with a low social status, such as a non-prestigious burial location and a lack of grave goods within the burial.

Age-at-death estimates were based on dental development (Ubelaker, 1999), cranial suture closure (Lovejoy, Meindl, Mensforth, & Barton, 1985), tooth wear (Smith, 1991), epiphyseal fusion (Buikstra & Ubelaker, 1994), auricular surface morphology (Lovejoy, Meindl, Pryzbeck, & Mensforth, 1985), and pubic symphysis morphology (Brooks & Suchey, 1990; Buikstra & Ubelaker, 1994). The presence of paleopathological markers was assessed using the standards outlined in the Global History of Health Codebook (Steckel et al., 2006). Age-at-death estimates typically produce upper and lower limits on the age. The average of the upper and lower limits of the age-at-death estimate was used to perform statistical procedures, including the survival analysis. Only adult individuals (>15 years) were included in the study. Individuals in the 60+ category were assigned the age of 60 years. Sex was estimated using the standards outlined in Steckel et al. (2006).

Age estimates were assigned to each tooth increment 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 based on tooth development data derived from Western European populations (AlQahtani et al., 2010). For maxillary canines, the crown forms between 0.6 years (approximately 7 months) (Ci) to 5.5 years (Cc) of age and the root forms between 5.5 years (Cc) and 14.5 years (Ac) of age. Mandibular canine crowns form between 0.9 years (approximately 11 months) (Ci) and 5.5 years (Cc) of age and the roots form between 5.5 years (Cc) and 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 & 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 horizontal sampling, but instead represent an estimate of the increment formation age. Age-at-weaning completion was estimated when isotope profiles exhibited both covariation between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in the earliest increments (per Beaumont et al., 2018) and a minimum ^{15}N elevation of 0.5‰ in the earliest compared to later increments. For the purposes of this analysis, the age associated with the increment after the final increment with elevated ^{15}N is considered the age-at-weaning completion. Fluctuations in ^{15}N in later increments, after the initial drop in ^{15}N , are not considered when estimating age-at-weaning completion because these changes may be a result of dietary or physiological changes associated with later childhood.

Thin sectioning is achieved using a modified version of Method 1 from Beaumont et al. (2013b). The crown was separated from the root by cutting horizontally at the cemento-enamel junction using a Dremel[®] saw. The crown of the tooth was embedded in resin using the Streurs Epo-Fix epoxy resin kit. The embedded crown was bisected and then horizontally sectioned into 1.5–2 mm increments using a Beuhler Isomet diamond-blade microtome saw. The collagen extraction procedure is adapted from Eerkens et al. (2011) and Henderson et al. (2014). Thin sections were removed from the epoxy and the roots and thin sections were demineralized in 2 ml of 0.5 M HCl solution with replacement once every 2 days. The samples were then treated for humic contamination by soaking the collagen in 0.125 M NaOH for 20 h. Secondary dentin was removed for all tooth sections when present. After demineralization, the roots were horizontally 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 h. The samples were then freeze-dried, homogenized, and 500 µg of each sample was weighed into tin capsules on a microbalance for analysis using a Carlo-Erba NA-series elemental analyzer coupled to Thermo Delta V IRMS system at the University of Georgia's Center for Applied Isotope Studies. Samples were excluded from the subsequent analysis if they exhibited C:N ratios outside of the recommended 2.9–3.6 suggested by DeNiro (1985). The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of duplicate reference standards (1577c, 1577c-b, and spinach) were found to have a standard deviation of 0.16‰ and 0.08‰, respectively. The analytical error was 0.28‰.

2.3 | Statistical analysis

Statistical analyses are performed using the platform R (v3.5.3). To account for unequal sample sizes between the catastrophic and attritional groups, non-parametric

analyses are used. Since non-parametric analyses generally utilize medians to compare the central tendencies of groups, medians are the primary statistic discussed in the results section. Means or averages are discussed when parametric analysis are used to compare groups. 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 groups. 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 investigate 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. χ^2 tests are used to compare the prevalence of pathological markers between different subgroups.

3 | RESULTS

Summary statistics for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ are provided in Table 1 for individual increments at the crown, the CEJ, and the apex of the root. When analyzed collectively, the crown increment had higher mean $\delta^{15}\text{N}$ values compared to the CEJ and apex increments for all sites and between catastrophic and attritional groups. The $\delta^{13}\text{C}$ values for the CEJ and apex segments are consistently higher than the crown segment among all sites. The same pattern in $\delta^{13}\text{C}$ values is observable when comparing catastrophic and the attritional groups. The majority of cholera victim's profiles (71.6%) and slightly more than half of the attritional profiles (54.5%) exhibit lower $\delta^{13}\text{C}$ values in early childhood (crown dentine increments) compared to late childhood (CEJ and apex dentine increments). The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for each increment are available in Table S1.

Table 2 summarizes the results of the weaning age estimation analysis. Eighteen of the isotope profiles (26.5%) exhibited a detectable weaning curve. Twenty-nine percent of cholera victims and 22% of the attritional group exhibited a detectable weaning signal. All detectable weaning curves were found among coronal dentine increments. Since severe tooth wear obliterates the earliest-forming dentin, we examined if worn teeth are associated with undetectable weaning curves. Of the 18 individuals with worn teeth, only one exhibits a detectable weaning curve. Of the 47 individuals with no tooth wear, 17 exhibit a detectable weaning curve. The results of a χ^2 test suggest that tooth wear is significantly associated with the lack of a detectable weaning curve ($\chi^2 = 5.83$, $df = 1$, $p = .016$). Since tooth wear was

TABLE 1 Summary statistics for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ from the crown, CEJ, and apex increments of permanent canines

	N		$\delta^{15}\text{N}$ (‰)			$\delta^{13}\text{C}$ (‰)		
			Crown ^a	CEJ ^b	Apex ^c	Crown ^a	CEJ ^b	Apex ^c
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
Combined (Catastrophic)	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
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
Combined (Attritional)	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

^aCalculated using the crown increment closest to the occlusal surface.

^bCalculated using the increment closest to the CEJ.

^cCalculated using the apex increment closest to the root apex.

significantly associated with the lack of a detectable weaning curve, the only worn tooth with a detectable weaning curve (US1132) was excluded from subsequent analyses.

Twelve cholera victims (26.7%) and five attritional individuals (21.7%) exhibit detectable weaning curves (see Figure 2). The 51 individuals without detectable weaning curves are excluded from the weaning age statistical analyses because weaning age could not be estimated, leaving a sample size of 30 individuals. Cholera victims have an average age-at-weaning completion of approximately 2.7 years and the attritional sample has an average age-at-weaning completion of 3.2 years. There are no statistically significant differences in age-of-weaning completion between cholera victims and the attritional sample ($t = -1.23$, $df = 7.68$, $p = .25$). Additionally, no site-based differences in age-at-weaning completion are found in our sample ($\chi^2 = 2.13$, $df = 2$, $p = .35$). No statistically significant differences in age-at-weaning exist between males and females ($t = -1.29$, $df = 6.76$, $p = .24$).

Statistically significant differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ exist between the sites. Individuals from Alia and Badia Pozzeveri exhibit similar median $\delta^{15}\text{N}$ values around 10.5‰. Individuals from Benabbio and Pieve di Monti di Villa exhibit median $\delta^{15}\text{N}$ values of 7.2‰ and 7.6‰, respectively. Individuals from Benabbio exhibit the lowest median $\delta^{13}\text{C}$, at -19.9‰, followed by individuals from Alia with a median $\delta^{13}\text{C}$ of -18.6‰ and individuals from Pieve di Monti di Villa with a median $\delta^{13}\text{C}$ of

-18.4‰. Individuals from Badia Pozzeveri exhibit the highest median $\delta^{13}\text{C}$ at -16.3‰. The sites have significantly different median $\delta^{15}\text{N}$ values ($\chi^2 = 223.2$, $p < .01$) with a post hoc Dunn test showing that the two pairings of Alia and Badia Pozzeveri, and Benabbio and Pieve di Monti di Villa, exhibit statistically similar median $\delta^{15}\text{N}$ values, but all other pairings are significantly different from one another ($p < 0.01$). These sites also exhibit statistically different median $\delta^{13}\text{C}$ values ($\chi^2 = 186.8$, $p < .01$). Individuals from Alia and Pieve di Monti di Villa exhibit statistically similar median $\delta^{13}\text{C}$ values, but all other pairings exhibit significantly different median $\delta^{13}\text{C}$ values ($p < .05$).

The average age-at-weaning for all individuals with weaning curves is 2.8 years. The Kaplan–Meier survival analysis shows that statistically significant differences exist between individuals with above average (≥ 2.8 years [$n = 9$]; late age-at-weaning), below average age-at-weaning (< 2.8 years [$n = 8$]; early age-at-weaning), and individuals that did not exhibit weaning curves ($n = 51$; $p = .027$; Figure 3). Individuals with below average age-at-weaning exhibit a statistically significant lower survival probability compared to individuals with above average age-at-weaning or no weaning curve.

Individuals with and without detectable weaning curves were compared with regards to paleopathological lesion frequency to determine if any other stress-related factors may explain differences in these groups. No differences were found between individuals with detectable weaning curves and those without with regards to rates

TABLE 2 Summary of sample demographics and weaning analysis

Site	ID#	Sex	Age-at-death	Tooth sampled	Dentin exposed	Weaning age estimate (years)	Weaning curve detectable
Alia	A10	M	45	C'	No	3.1	Yes
Alia	A117	I	30	C'	No	1.8	Yes
Alia	A14	F	15	C,	No		No
Alia	A17	F	22	C,	No		No
Alia	A18	M	25	C'	No	3.1	Yes
Alia	A38	M	30	C'	No		No
Alia	A40	M	35	C,	No		No
Alia	A42	F	35	C'	No		No
Alia	A48	M	15	C'	No	2.2	Yes
Alia	A52	M	15	C'	No		No
Alia	A81	M	25	C'	No		No
Alia	A85	I	15	C'	Yes		No
Alia	A90	M	35	C'	Yes		No
Alia	A99	F	15	C'	No		No
Alia	M1	M	30	C,	No		No
Alia	M15	M	27	C,	No	2.1	Yes
Alia	M17	F	42.5	C,	Yes		No
Alia	M19	F	22	C,	No	2.1	Yes
Alia	M20	F	37.5	C,	No	4.0	Yes
Alia	M21	F	27.5	C,	No		No
Alia	M5	M	30	C,	No		No
Alia	M6	M	21	C,	Yes		No
Alia	M7	M	35	C,	No		No
Alia	M10	F		C,	No		No
Alia	M11	F	29.5	C,	No		No
Alia	M13	F	25	C,	Yes		No
Alia	M18/51	I	27	C,	No		No
Alia	M3	F	29.5	C,	No	2.4	Yes
Alia	M4	M	30	C,	No	4.0	Yes
Alia	MAN9	M	29.5	C,	No	2.0	Yes
Alia	M12	M	21	C,	No		No
Benabbio	US1047	M	57.5	C,	Yes		No
Benabbio	US1051	M	50	C,	Yes		No
Benabbio	US1120	M	27	C,	No	1.6	Yes
Benabbio	US1132	M	60	C'	Yes	3.6	Yes
Benabbio	US1159	F	60	C'	No	3.5	Yes
Benabbio	US1200	M	47.5	C,	Yes		No
Benabbio	US1324	F	60	C'	Yes		No
Benabbio	US1329	I		C,	No		No
Benabbio	US1376	M	33.5	C,	Yes		No
Benabbio	US1377	F	60	C'	No		No
Benabbio	US1414	M	42.5	C,	Yes		No

TABLE 2 (Continued)

Site	ID#	Sex	Age-at-death	Tooth sampled	Dentin exposed	Weaning age estimate (years)	Weaning curve detectable
Benabbio	US1419	F	60	C,	No		No
Benabbio	US1494	F	35	C,	No		No
Benabbio	US1809	M	60	C,	No		No
Badia Pozzeveri	US2108	M	25	C'	No		No
Badia Pozzeveri	US2167	M	37.5	C,	Yes		No
Badia Pozzeveri	US2237	M	15.2	C,	No	2.1	Yes
Badia Pozzeveri	US2325	M	23	C,	No	3.2	Yes
Badia Pozzeveri	US2383	F	30.7	C'	Yes		No
Badia Pozzeveri	US2409	F	32	C,	No	4.0	Yes
Badia Pozzeveri	US2415	M	32	C,	No		No
Badia Pozzeveri	US2613	M	50	C,	No	2.7	Yes
Badia Pozzeveri	US2623	M	32	C,	No		No
Badia Pozzeveri	US2776	M	54.5	C,	Yes		No
Badia Pozzeveri	US3287	M	60	C,	Yes		No
Badia Pozzeveri	US3391	M	60	C,	Yes		No
Badia Pozzeveri	US3397	F	27	C,	No		No
Badia Pozzeveri	US3546	M	32.5	C'	No		No
Badia Pozzeveri	US3552	M	47.5	C,	No		No
Badia Pozzeveri	US3555	I	37.5	C,	No	4.0	Yes
Monti Di Villa	US1057	F	60	C'	Yes		No
Monti Di Villa	US1060	M	22	C,	No		No
Monti Di Villa	US1066	F	37.5	C'	No		No
Monti Di Villa	US1067	I		C,	No		No
Monti Di Villa	US1069	F	54	C,	Yes		No
Monti Di Villa	US1072	F	54	C,	Yes		No
Monti Di Villa	US1087	M	45	C,	Yes		No

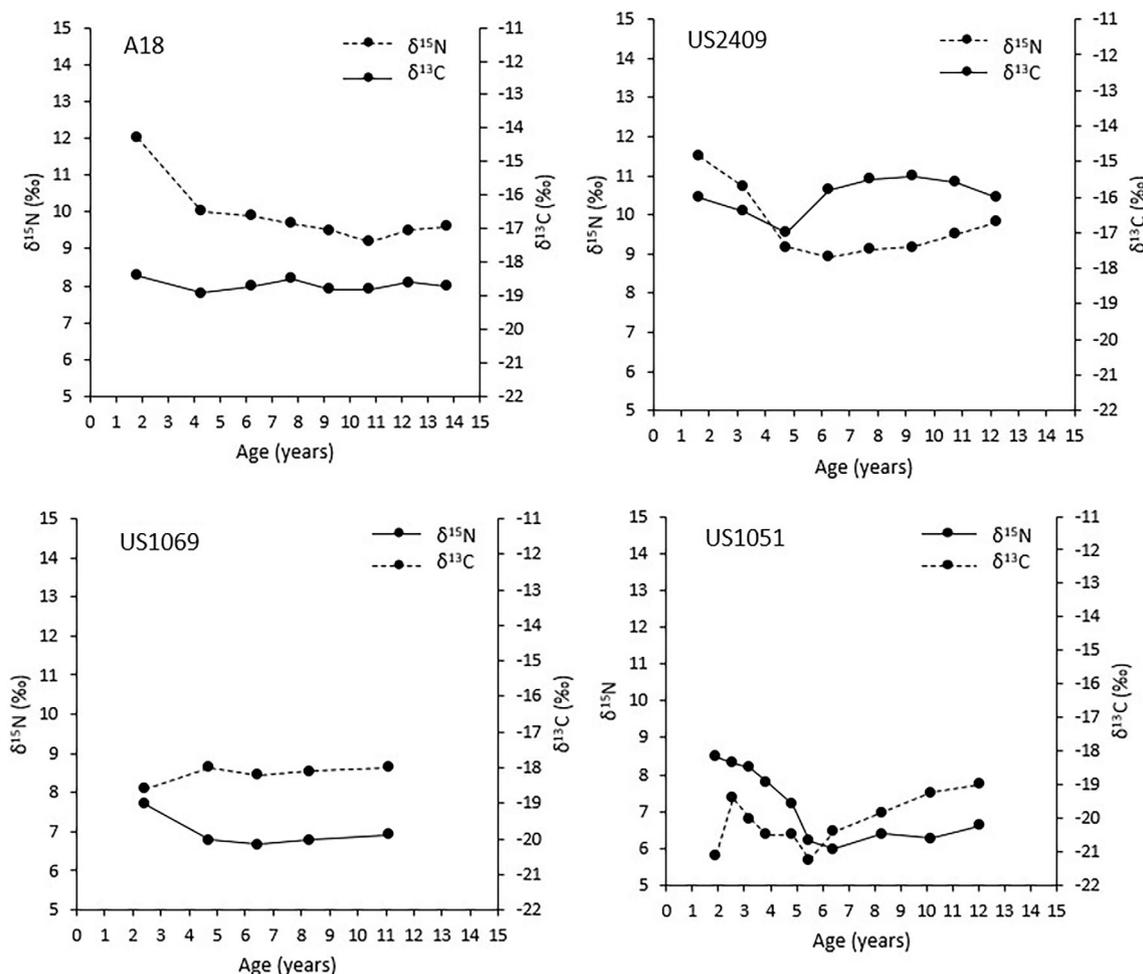


FIGURE 2 Plots showing incremental dentin carbon and nitrogen isotope values from Alia (A18), Badia Pozzeveri (US2409), Benabbio (US1051), and Pieve di Monti di Villa (US1069) against age-of-formation of dentin increment. A18 and US2409 show covariation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in the earliest increments, whereas US1069 and US1051 show no covariation

of cribra orbitalia ($\chi^2 = 0.88$, $df = 1$, $p = .35$), porotic hyperostosis ($\chi^2 = 2.37$, $df = 1$, $p = .12$), carious lesions ($\chi^2 = 0.84$, $df = 1$, $p = .36$), abscesses ($\chi^2 = 0.36$, $df = 1$, $p = .55$), linear enamel hypoplasia ($\chi^2 = 0$, $df = 1$, $p = 1$), or scurvy ($\chi^2 = 0$, $df = 1$, $p = 1$).

For those with detectable weaning curves, the average age-at-death is 30.0 ± 14.5 years, and the average age-at-weaning completion is 2.7 ± 0.9 years. The results of Spearman's Rank Correlation test show that there is a correlation between age-at-death and age-at-weaning in this sample ($S = 397$, $p = .04$). Cholera victims ($S = 144$, $p = .10$) and the attritional group ($S = 12.818$, $p = .55$) do not exhibit a correlation between age-at-weaning and age-at-death.

Several weaning profiles showed an increase in nitrogen isotope ratios in late childhood (age 9+) that did not covary with the carbon isotope profiles. A chi-square test reveals that the number of individuals with late childhood elevations in $\delta^{15}\text{N}$ differs significantly between the

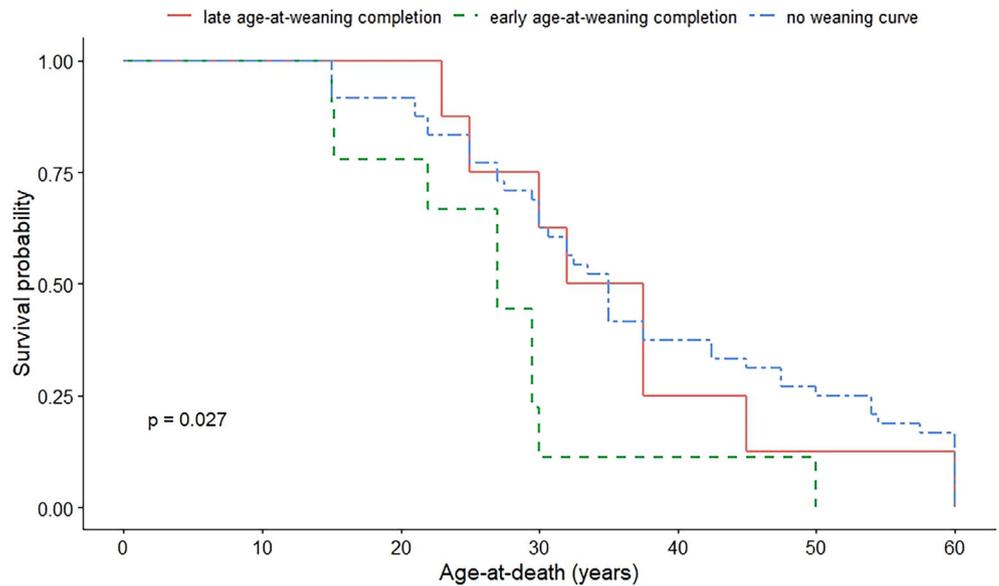
sites ($\chi^2 = 12.77$, $df = 3$, $p = .005$). The majority of profiles with elevated nitrogen isotopes in late childhood come from Alia ($n = 14$), followed by Badia Pozzeveri ($n = 2$), and Benabbio ($n = 1$). No individuals from Monti di Villa showed late childhood elevations in nitrogen isotopes. The number of individuals with elevated $\delta^{15}\text{N}$ in late childhood did not differ significantly between the sexes ($\chi^2 = 0.75$, $df = 2$, $p = .69$).

4 | DISCUSSION

4.1 | Regional and interregional dietary variation

The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values from all sites suggest a mixed terrestrial and marine diet that included primarily C_3 plants. This result is consistent with previously published bone collagen $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ data for Benabbio ($n = 41$,

FIGURE 3 Kaplan–Meier survival curve comparing the groups with an early versus late age-at-weaning completion, and individuals with no weaning curve



$\delta^{15}\text{N}$ (\bar{x}): $8.3\text{‰} \pm 1.0\text{‰}$, $\delta^{13}\text{C}$ (\bar{x}): $-19.8\text{‰} \pm 1.3\text{‰}$) and Pieve di Monti di Villa ($n = 26$, $\delta^{15}\text{N}$ (\bar{x}): $7.8\text{‰} \pm 1.3\text{‰}$, $\delta^{13}\text{C}$ (\bar{x}): $-18.8\text{‰} \pm 0.9\text{‰}$) which found that individuals consumed an omnivorous diet with moderate animal protein consumption (Fornaciari, 2015). Individuals from Alia and Pieve di Monti di Villa exhibited less variation in $\delta^{13}\text{C}$ compared to those from Benabbio and Badia Pozzeveri during late childhood, suggesting that individuals from Alia and Pieve di Monti di Villa likely had a less varied diet during late childhood.

Individuals from Alia exhibited a higher median $\delta^{15}\text{N}$ value compared to those from Benabbio and Monti di Villa, suggesting that the diets of these individuals contained more marine food sources. Sicilian cuisine typically emphasizes fish consumption (Domenico, 2002), and Alia is located approximately 40 km from the northern coast of Sicily. Though individuals from Alia kept domesticated animals, including cattle, horses, and sheep (Chiarelli et al., 2002), marine protein sources were likely also included in their diet because of trade with coastal villages. Though Sicily is mainly known for their cultivation of durham wheat, millet has also been cultivated in Sicily since the 7th century AD (Chiarelli et al., 2002; Ramsay & Wilson, 2013; Vetro, 1992). Individuals from Alia exhibited a median $\delta^{13}\text{C}$ of -18.5‰ , suggesting that their diet included both millet and wheat in addition to other fruits and vegetables (Ramsay & Wilson, 2013; Vetro, 1992).

Badia Pozzeveri's location may explain why individuals from this site exhibited the most variation in $\delta^{13}\text{C}$ and a relatively high median $\delta^{15}\text{N}$ value. Badia Pozzeveri is located approximately 40 km from the Tyrrhenian Sea along the Via Francigena trade route between Lucca and the Arno River Valley, the principal trade route in

Northern Tuscany (Morelli, 2007). The movement of people 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 median $\delta^{15}\text{N}$ value at Badia Pozzeveri was similar to that of Alia, indicating that individuals at this site consumed some marine-based protein sources. The higher average $\delta^{13}\text{C}$ among the residents of Badia Pozzeveri could be a product of maize (*Zea mays*) cultivation and exchange across the Lucca Plain. The only areas of 18th- to 19th-century Italy that regularly cultivated maize were the Po Plain in Northern Italy and other smaller flat areas, such as the Lucca Plain, where it was commonly used to make polenta (Gentilcore, 2014; Ginnaio, 2011; Pazzagli, 1998). Millet was also commonly consumed in Northern Italy from the medieval period through the 19th century (Betri, 1998; Reitsema & Vercellotti, 2012). Millet flour was generally baked into a bread, and sometimes mixed with corn and rye flour before baking (Betri, 1998). Both corn and millet could have been traded along the Via Francigena and contributed to higher $\delta^{13}\text{C}$ values among individuals from Badia Pozzeveri. Unlike Badia Pozzeveri, Pieve dei Monti di Villa and Benabbio are more rural and not located near any major trade routes, which may explain why they do not exhibit the elevation in $\delta^{13}\text{C}$ found among individuals from Badia Pozzeveri.

4.2 | Physiological stress in childhood

Several isotope profiles exhibited an elevation in $\delta^{15}\text{N}$ in late childhood (~ 9 years) that did not covary with $\delta^{13}\text{C}$, suggesting that the elevation in nitrogen isotope values



could be the result of physiological stress in late childhood (Beaumont et al., 2018). Most of these profiles belonged to individuals from Alia. Previous research indicated $\delta^{15}\text{N}$ ratios become higher in tissues among individuals experiencing wasting and illness (Fuller et al., 2005; Hobson et al., 1993; Mekota et al., 2006). All sites exhibit a similar prevalence of paleopathological indicators such as periostitis, porotic hyperostosis, and linear enamel hypoplasia. However, Alia exhibited a significantly lower prevalence of cribra orbitalia compared to all other sites, suggesting that subadults from Alia experienced less stress compared to subadults from other sites. However, the low prevalence of cribra orbitalia at Alia was surprising because they have the lowest average age-at-death compared to all other sites. Cribra orbitalia is a disease of childhood and its lesions can be obliterated due to bone remodeling over time, so a group with a low average age-at-death is likely to exhibit more cribra orbitalia compared to an older, but geographically and culturally similar, group. An alternative explanation is that individuals from Alia died before manifesting cribra lesions. However, only adults (>15 years old) were included in this analysis, and cribra lesions typically form in early childhood (Stuart-Macadam, 1985). Considering that individuals from Alia had the lowest age-at-death among all the groups, it is possible that Sicilians experienced relatively low stress in childhood (<9 years) compared to the other groups, but relatively more stress compared to the other groups in late childhood (>9–10 years) and adulthood (>15 years).

4.3 | Weaning completion and mortality outcomes

Individuals with below average (<2.8 years) or above average (≥ 2.8 years) age-at-weaning completion exhibited different survivability, suggesting that age-at-weaning completion may affect overall mortality in these groups. Specifically, individuals with a younger age-at-weaning completion exhibited an earlier age-at-death compared to individuals with an older age-at-weaning or those with no detectable weaning curve. These results suggest that weaning earlier than 2.8 years during the mid-19th century in Italy resulted in an increased mortality risk in adulthood. Furthermore, cholera victims also exhibit a statistically significant positive correlation between age-at-death and age-at-weaning, whereas no relationship between these variables is observed among the attritional group. However, these results are based on age-at-death estimates from osseous remains of adults. The methods used to estimate age-at-death in adults over the age of 35 years are generally less precise than age

estimates for younger individuals. It is possible that the survivorship analysis may be inaccurate due to the imprecision of age-at-death estimations for adults. Further research with a larger, contemporaneous skeletal population would be required to confirm or refute these results.

Cholera victims exhibited a slightly younger age-at-weaning completion around 2.7 years of age compared to the attritional group, which exhibited a weaning age around 3.2 years. This difference was not statistically significant, and there was considerable overlap between the ages of weaning completion among the cholera victims and the attritional group. This result suggests that the duration of weaning did not contribute to adult mortality from cholera. Therefore, the age-at-weaning completion did not affect overall infectious disease mortality in these groups.

Though some evidence exists to link early childhood stress impaired immune function, including reduced thymic function and reduced vaccine response (McDade et al., 2001a, 2001b), no research has detailed an epigenetic pathway that links early childhood stress to reduced immune function later in life. It is possible that no pathway exists, or if one does exist, the alterations to the immune system may not be permanent or long-term. Furthermore, if subtle differences in immune system exist among those who experience significant early childhood stress, then they may not be detectable in skeletal samples considering that most infectious disease does not leave skeletal markers. A major limitation of the present study is the small sample size of the attritional group ($n = 5$) included in the age-at-weaning estimation. A larger sample of culturally and temporally similar populations may be able to better discern the relationship between early childhood stress and infectious disease mortality.

4.4 | Weaning practices in 18th–19th century Italy

Weanling foods during the 19th century were often contaminated with bacteria or parasites (Wickes, 1953a, 1953b). Infants that survived to adulthood, like those in our study sample, may have survived infancy by adapting to consuming contaminated food, imbuing them with a better ability to adapt to later life environmental challenges. Most individuals in our study were fed a combination of breastmilk and complementary food until ~ 2.8 years of age. Infants who experienced a long period of breastfeeding with food supplementation, over the course of years, would adapt to the exposure to pathogens and have built an immunity toward those pathogens by

the time complementary feeding ended. 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 were found in age-at-weaning completion, suggesting weaning practices in 19th-century rural Italy were similar across the peninsula. Site-based statistically significant differences of dentin $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values suggest that there was regional variation in diet, however. This result suggests that the timing of the cessation of complementary breastfeeding does not contribute to adult mortality from infectious disease and that age-at-weaning completion was similar across the 19th-century Italian landscape, despite variation in dietary composition across sites.

4.5 | Explanations for the lack of detectable weaning curves

Over half of the individuals in this study did not exhibit isotope profiles that might be associated with breastfeeding, which presents an interpretive dilemma for weaning reconstruction. Several other studies also found no elevation in $\delta^{15}\text{N}$ among serial sections of human dentin (Beaumont et al., 2018; Beaumont, Geber, et al., 2013; Eerkens et al., 2011; Henderson et al., 2014; King et al., 2018; Pfeiffer et al., 2017). The lack of elevated $\delta^{15}\text{N}$ values in early life has several potential interpretations.

Individuals without elevated $\delta^{15}\text{N}$ values during infancy and early childhood could be individuals who were not breastfed. 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, 1953b). When babies did not thrive on breastmilk 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 breastmilk would not enrich infant tissues with ^{15}N relative their mother.

Another potential explanation is that these individuals completely ceased breastfeeding before 6 months of age and this analysis did not capture their weaning period. This explanation conflicts with the available research on breastfeeding from 19th-century Italy in bioarchaeology. Stable isotope research suggests that infants in the Eastern Mediterranean region of Europe experienced several years of complementary breastfeeding during the medieval period, with the cessation of breastfeeding occurring between 3 and 4 years of age

(Bourbou et al., 2013; Dittmann & Grupe, 2000; Fulminante, 2015; Herrscher, 2003; Turner et al., 2007). Other research using remains of urban and/or high-status individuals found that the completion of breastfeeding occurred between 2 and 3 years of age in 17th–19th-century Italy (Giuffra & Fornaciari, 2013; Moggi-Cecchi et al., 1994).

The historical context of these sites also suggests that complementary feeding likely occurred over a period of several years. People from these rural sites in Italy may have experienced years of complementary breastfeeding as a result of reduced access to food and lower pressures on women to work outside of the home (Wickes, 1953a, 1953b). Industrialization in Italy began in the late 19th century and peaked in the early 20th century (Ciccarelli & Fenoaltea, 2013; Federico & Vasta, 2010). Mothers in rural Italy during the late 18th to 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).

Individuals without detectable weaning curves also experienced similar rates of paleopathological markers compared to those with detectable weaning curves. If individuals without detectable weaning curves are interpreted as individuals who weaned before 6 or 9 months of age or did not breastfeed, then the lack of differences in pathological markers compared to those with detectable weaning curves suggests that no breastfeeding/breastfeeding before 6–9 months of age was not associated with an increase in susceptibility to other pathological conditions in childhood and adulthood.

Severe tooth wear can cause loss of the earliest-formed dentin, which is most likely to be enriched in ^{15}N due to breastfeeding. Though most without detectable weaning curves also did not exhibit tooth wear ($n = 34$; 66.7%), only one individual with tooth wear exhibited a detectable weaning curve (5%), indicating the lack of weaning curves for some individuals may be the result of dentin lost through wear. The results of this study find that 94.4% of canines with tooth wear did not exhibit a detectable weaning curve, suggesting that teeth with severe wear should be excluded from weaning reconstruction studies.

There are other physiological factors influencing $\delta^{15}\text{N}$ ratios that complicate isotopic interpretations of weaning and could blur the appearance of weaning curves. These include growth velocity and the gut microbiome (see Reynard & Tuross, 2015). During the early post-weaning period, infant $\delta^{15}\text{N}$ often falls below mother's $\delta^{15}\text{N}$ 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 isotope values in the post-weaning period 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 $\delta^{15}\text{N}$ values (Deschner et al., 2012; Mekota et al., 2006). However, the effects of growth velocity on nitrogen isotope ratios are not detectable when comparing fast- and slow-growing bone collagen tissues (Waters-Rist & Katzenberg, 2010). Additionally, growth velocity only explains some of the variation in infant $\delta^{15}\text{N}$ during the post-weaning nitrogen “dip” (Reitsemá & Muir, 2015).

Little is known about the effects of gut microbiota on stable carbon and nitrogen isotope values (Reynard & Tuross, 2015). The gut microbiota plays a role in nitrogen metabolism during the first 3 years of life and influences urease gene expression and the metabolism of amino acids (Yatsunenko et al., 2012). Consequently, alteration to the gut microbiome in early life may affect $\delta^{15}\text{N}$ values. Controlled studies on living humans that explore the effects of the gut microbiome and growth velocity on $\delta^{15}\text{N}$ profiles would be required to definitively state that the lack of $\delta^{15}\text{N}$ elevation in early childhood is a result of alternative or supplementary foods during infancy.

5 | CONCLUSIONS

The first main goal of this study was to determine if earlier breastfeeding cessation is associated with differential mortality outcomes compared to later breastfeeding cessation. This study found no relationship between adult infectious disease mortality and early childhood stress, which is consistent with the findings of Blevins (2015) and (Moore et al., 2001), but contrasts with other research that found that the presence of skeletal markers (linear enamel hypoplasia, calculus, cribra orbitalia, etc.) were associated with earlier death (Boldsen, 2007; DeWitte, 2012; DeWitte & Wood, 2008; Temple et al., 2012), and that the alteration of immune function was associated with early childhood stress (McDade et al., 2001a, 2001b). Complementary breastfeeding in 18th–19th century rural Italy was complete around 2.8 years of age at all sites, suggesting that weaning practices were similar across the landscape. Age-at-weaning completion may not have been a sufficiently stressful period in early development to affect immune function later in life. Infants may have adapted to a combined diet of breastmilk and contaminated solid foods for an extended period of time prior to the cessation of breastfeeding. Furthermore, no known physiological pathways are known that link early childhood stress to immune dysregulation, suggesting the need for further research

into the pathways by which immune function may be influenced by early childhood stress. Future research should consider analyzing other aspects of weaning, such as the onset of weaning or length of the weaning period.

The second main goal of this research was to determine if individuals experienced dietary variability and/or physiological stress during childhood. This study found that diet during childhood varied both regionally and inter-regionally across the 18th- to 19th-century Italian landscape. Dietary variation between the four archeological sites was explained through location and access to trade networks. Benabbio (Tuscany) and Monti di Villa (Tuscany) exhibited similar dietary landscapes in early childhood, with individuals consuming mostly moderate animal protein. Badia Pozzeveri (Tuscany) exhibited the most dietary variation, likely due to its location along the Via Francigena trade route. This variation suggests that the culinary landscape in Badia Pozzeveri was varied, and likely contained millet and corn that was imported from Northern Italy. Individuals from Alia (Sicily) likely consumed a mixture of terrestrial and marine proteins along with a mixed C_3/C_4 diet. Furthermore, several individuals, the majority of which were from Alia, exhibited a rise in $\delta^{15}\text{N}$ values beginning around age 9 that did not covary with $\delta^{13}\text{C}$. Despite individuals from Alia exhibiting a lower rate of cribra orbitalia and similar rates of other paleopathological lesions compared to Tuscans, this rise in $\delta^{15}\text{N}$ was likely a result of an increase in physiological stress in late childhood. This suggests that Sicilians in the 19th century experienced a more stressful environment compared to Tuscans from late childhood through early adulthood.

The third main goal of this research was to determine if age-at-weaning completion differed between the catastrophic and attritional groups. Though cholera victims exhibited a slightly younger age-at-weaning completion (~ 2.7 years) compared to the attritional group (~ 3.2 years), this difference was not statistically significant. The overlap in age-at-weaning completion between catastrophic and attritional groups suggests that the timing of weaning cessation did not contribute to cholera mortality.

More than half of the samples lacked elevated $\delta^{15}\text{N}$ values during infancy, suggesting that these individuals may have been fully weaned before 6 or 9 months old, consumed supplementary foods from birth, or experienced factors that affected their nitrogen metabolism (rapid growth and the gut microbiome). Individuals with and without detectable weaning curves did not have significantly different rates of paleopathological markers, suggesting that they did not experience more or less stress compared to the other groups. The majority of worn teeth that were analyzed for weaning profiles did

not exhibit a detectable weaning pattern, suggesting that worn teeth should be excluded from future weaning reconstruction analyses. Isotope profiles without detectable elevation in $\delta^{15}\text{N}$ 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 would provide a more complete understanding of the variation in infant feeding practices throughout history. Further research is needed to discern if individuals without detectable weaning profiles represent individuals who were not breastfed or weaned early.

AUTHOR CONTRIBUTIONS

April K. Smith: Conceptualization (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); writing – original draft (equal). **Laurie Reitsema:** Conceptualization (equal); formal analysis (equal); funding acquisition (equal); supervision (equal); writing – review and editing (equal). **Antonio Fornaciari:** Resources (equal); supervision (equal); writing – review and editing (equal). **Luca Sineo:** Resources (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports these findings are available from the corresponding author upon reasonable request.

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