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RESEARCH ARTICLE

Interpreting Linear Enamel Hypoplasia at the Amastris (Ağlayan Ağaç) Chapel Cemetery From the Eastern Roman Imperial Period Region of Paphlagonia (Türkiye)

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ABSTRACT

The aim of this study is to evaluate the values of linear enamel hypoplasia (LEH) observed in the permanent teeth of individuals excavated from a chapel site dated to the Late Eastern Roman Period (6th–9th centuries) through analytical methods. The study is based on LEH findings identified in 148 (37.4%) of 396 permanent teeth belonging to 30/54 individuals. LEH measurements were conducted using two well-established methodologies documented in the literature. The prevalence of LEH suggests that the onset of the weaning process in infancy or early childhood coincided with the emergence of disease-inducing factors, estimated to occur between the ages of 3.5 and 5 years. Comparative evaluation of the two methodological approaches revealed consistent and convergent findings. It is hypothesized that a combination of factors including metabolic disorders, pathological conditions, environmental influences, socialization patterns, poor hygiene, nutritional deficiencies, and suboptimal living conditions contributed to heightened disease susceptibility during the weaning phase. This study presents the first LEH dataset from the Western Black Sea (Paphlagonia) region of Türkiye and contextualizes the findings through comparisons with other coastal harbor communities previously examined in a limited capacity. This research focuses on a comprehensive analysis to help create a more chronological and geographical comprehensive LEH dataset of ancient Anatolian societies in later periods.

1 | Introduction

The analysis of osteological remains has become an essential method for reconstructing the biological characteristics and lifestyles of ancient populations. The study of osteological remains plays a crucial role in reconstructing the biological and sociocultural characteristics of ancient populations. Among these, dental remains hold particular significance, as they differ from other skeletal elements in their inability to repair or regenerate once formed (Mann and Hunt 2012; White et al. 2012). This characteristic renders teeth invaluable in bioarchaeological research, as they serve as permanent records of physiological stress experienced during infancy and childhood (Saunders & Barrens, 1999). Consequently, dentition functions as a biological time capsule, preserving crucial insights into early-life conditions in archaeological assemblages (Maclellan 2005).

Dental evidence provides critical information regarding the origins of dietary diversity, extended childhood development, longevity, and other fundamental aspects of ancient human biology, including that of early hominins (Steinberg 2016). Changes in dietary patterns across different societies can provide insights into historical nutritional practices, daily activities, environmental adaptations, health conditions, social structures, and cultural frameworks (Erkman et al. 2022). Furthermore, these dietary transitions present a unique opportunity to investigate physiological stressors and genetic influences from both macroscopic and microscopic perspectives (Larsen 2018).

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One of the most informative indicators of early life stress is enamel hypoplasia, a defect in enamel formation resulting from disruptions during odontogenesis. The presence of enamel hypoplasia in archaeological populations offers valuable insights into the health, nutritional status, and environmental conditions experienced by infants and children in antiquity.

Based on their morphological features, hypoplastic defects are classified as Type 3 (pits), Type 4 (horizontal grooves), Type 5 (vertical grooves), and Type 6 (missing enamel). Type 4 is generally known as linear enamel hypoplasia (LEH), as defined in the anthropological literature (Goodman and Rose 1990).

LEH is a developmental defect of dental enamel, characterized by the incomplete formation of enamel during periods of growth. This condition manifests as transverse bands of reduced enamel thickness on the tooth surface (Kreshover 1960; Goodman et al. 1980). LEH is considered a physiological stress marker, with etiological factors including nutritional deficiencies, physical trauma, infectious diseases, parasitic infestations, and metabolic or genetic disorders during early childhood (Dabrowski et al. 2021). It is widely recognized in the literature as a disruption in ameloblast function, which impairs the process of enamel deposition and results in structural deficiencies in the enamel layer (Wang et al. 2024).

The development of alveolar bone in children from nutritionally disadvantaged populations may also be adversely affected, particularly during periods of reduced retinol levels resulting from inadequate sunlight exposure (Skinner 1986; Skinner and Goodman 1992). Enamel hypoplasia, as a nonspecific marker of metabolic stress, provides valuable insights into episodes of severe malnutrition during infancy and early childhood, especially in relation to the weaning process (Lewis 2007; Goodman et al. 1980). Furthermore, LEH offers critical information on intrauterine and environmental adaptations, reflecting the impact of maternal nutritional deficiencies on the formation of adult dentition (Dabrowski et al. 2021; Wang et al. 2024).

Numerous researchers have compiled LEH datasets from diverse populations across the globe, including communities in North America (Goodman and Rose 1990), Northern Europe (Reid and Dean 2000, 2006), and South Africa (Goodman and Rose 1990; Lewis 2007). In contrast, studies examining stress-related factors in ancient Anatolian populations remain notably limited (Büyükkarakaya 2012, 2015; Erkman et.al, 2022).

This study aims to address three primary research objectives. The first objective is to evaluate health conditions related to early infancy and childhood, and to analyze their environmental implications through the examination of LEH data from the Amastris (Ağlayan Ağaç) population, dated to the Late Eastern Roman Period (LERP) in the Western Black Sea region of Türkiye. To gain a deeper understanding of these environmental effects, the dental pathological data of the Amastris population will also be utilized (Taş and Erkman 2024). The second objective is to contribute to the establishment of a regional LEH dataset for the Western Black Sea by comparing findings from Amastris with LEH data from other understudied coastal harbor communities in ancient Anatolia. The third objective is to evaluate and compare the methodologies developed by Goodman and Rose (1990) and Reid and Dean (2000, 2006) for estimating the biological age at which LEH defects formed. This comparison aims to minimize error margins and enhance the precision of age-at-formation estimates. Moreover, the lack of other forms of biological evidence in archaeological investigations on the historical island highlights the significance of utilizing LEH as a stress marker to illuminate key aspects of life during this period.

2 | Materials and Methods

2.1 | Archaeological Context

In 2016, a rescue excavation was conducted by the Amasra Museum Directorate (Figure 1) at a chapel site (Figure 2), which was discovered by chance during the expansion of a tourist facility in Amasra, a city of contemporary touristic significance. The skeletal materials excavated by the Amasra Museum Directorate were received for study with official permits granted by the Ministry of Culture and Tourism of the Republic of Türkiye. The excavation site derives its name from a 350-year-old cypress tree located near the tourist facility. This tree is known for its unique ability to absorb moisture from the sea and air, releasing it as raindrops during the spring and autumn months. "For this reason, the site is popularly known as the "Ağlayan Ağaç'" (Weeping Tree).

Amasra, located in the historical region of Paphlagonia, was originally established by the Phoenicians approximately 3000 years ago under the name Sesamos. In the 3rd century BCE, the city was renamed Amastris by Queen Amastris of the Persian dynasty (Dökü et al. 2006; Verim 2015). In 395 CE, Amasra came under the control of the Byzantine Empire, remaining within the dominion of the Eastern Roman Empire. Because of its strategically advantageous maritime position, Amasra (Amastris) became one of the most prominent port cities of its era and was among the earliest centers of Christian organization (Çam et al. 2019; Çam 2020).

The chapel was likely established as a religious site to serve the local community. However, it was abandoned for reasons that remain unclear and eventually collapsed, unable to withstand the passage of time. In subsequent periods, a larger religious structure was constructed on the site, though it too was abandoned (Figure 2). The area surrounding and within this new structure was later repurposed as a burial ground, with interments occurring in simple earthen graves. No discernible alignment pattern among the burials has been identified (Şahin 2023). Because of the nature of the excavation, which was carried out as a rescue operation, the archaeological data related to the Amastris site remain limited.

As a prominent Byzantine port city, Amastris played a pivotal role in the early organization of Christianity, as evidenced by the presence of numerous churches and chapels (Dökü et al. 2006). Plaster samples recovered from architectural remains in the region indicate a settlement period spanning the 6th–9th centuries CE (Verim 2015).

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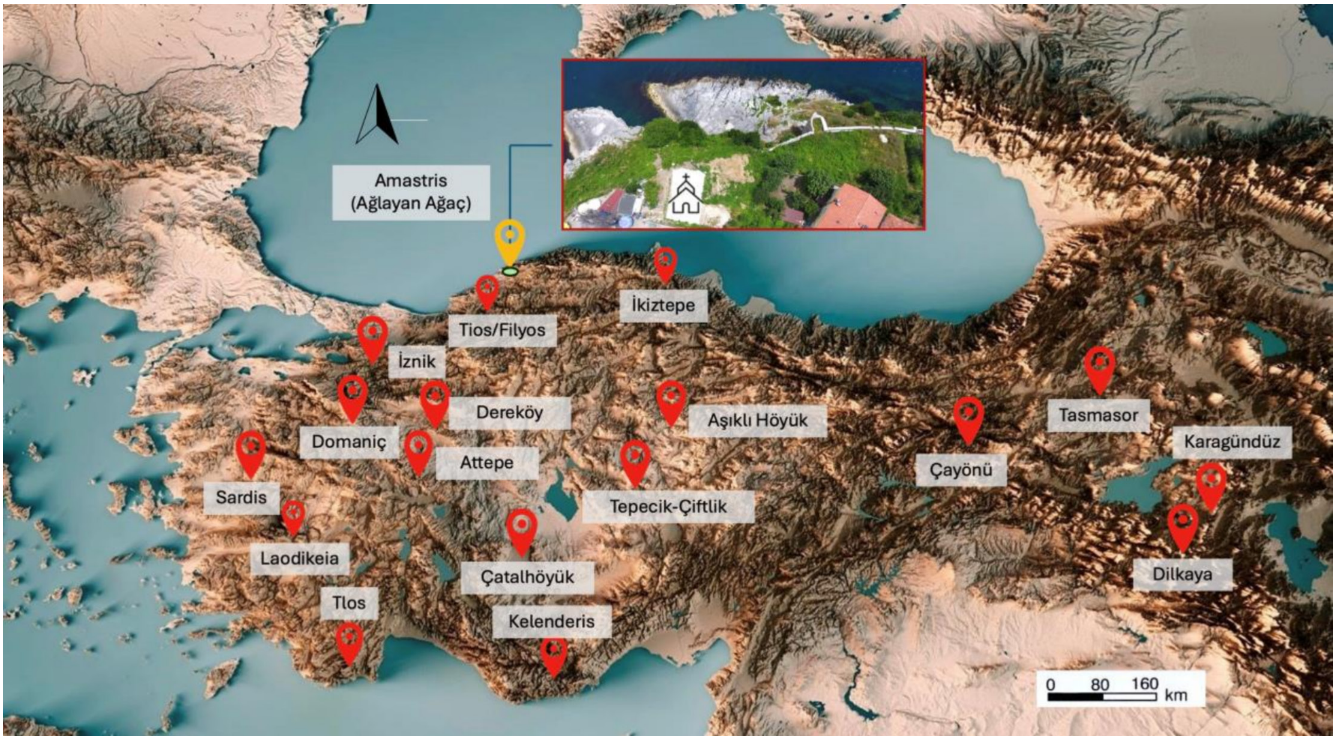

FIGURE 1 | Geographic map showing the location of Amastris and comparative Ancient Anatolian Settlements (Erkman et al. 2025). [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 2 | A view from the (Amastris) Ağlayan Ağaç of Chapel Excavation from the archive. [Colour figure can be viewed at wileyonlinelibrary.com]

2.2 | Method

2.2.1 | Methods for Estimating Age and Sex

In this study, sex estimation of the skeletons was conducted using the criteria outlined by Bass (1987), White et al. (2012), Workshop of European Anthropologists (1980), and İşcan and Steyn (2013), Buikstra and Ubelaker (1994), Krogman and İşcan (1986), and Ubelaker (1978). For age estimation in infants and children, Ubelaker's (1978) tooth eruption chart was employed; for epiphyseal aging in young adults, White-Folkens (1991) was used; age estimation in adults was based on the pubic symphysis method by Todd (1920), White et al. (2012) age-related changes in the auricular surface by Lovejoy et al. (1985), midshaft cross-sections of the clavicle by Kaur and Jit (1990), sternal rib end changes by İşcan and Steyn (2013), and proximal sections of the humerus and femur by Szilvassy and Kritscher (1990). For complex age estimation, the methods described by the Workshop of European Anthropologists (1980) were applied. Previous researchers in ancient Anatolian populations used hypoplasia ratings based on the Brothwell (1981) method; therefore, we followed this method for cross-population comparisons. Age ranges were determined according to Ubelaker (1978), and the Workshop of European Anthropologists (1980), to ensure comparability with previously published studies. Individuals under the age of 18 were classified as children, as their long bone epiphyses had not yet fully fused, and osteometric measurements could not be taken from these bones.

2.2.2 | Methods for Determining LEH

In this study, the chronological onset of enamel hypoplasia was assessed through a comparative analysis of two widely recognized methodologies from the literature (Goodman and Rose 1990; Reid and Dean 2000; Reid and Dean 2006). The band-shaped manifestation of LEH, classified as Type 4 according to the Federation Dentaire Internationale (FDI) index, was systematically recorded in the study documentation (FDI 1982).

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To highlight the stress periods experienced by individuals within this population, the severity of enamel hypoplasia defects was categorized as mild, moderate, or severe, in accordance with the classification criteria established by Brothwell (1981). The initial identification of enamel hypoplasia was carried out through direct visual examination under natural daylight. In cases where lesions were not easily visible, a hand lens was used to aid in the detection of LEH. Teeth exhibiting advanced wear, specifically those with a wear score of 5 or higher, as defined by Bouville (1983), were excluded from the analysis.

For the methodologies proposed by Reid and Dean (2006) and Goodman and Rose (1990), precise measurements of the distance from the enamel surface to the cementoenamel junction (CEJ) were essential. The ages at which stress episodes occurred were determined by measuring the crown height from the CEJ to the identified enamel defect. These measurements were taken using a Mitutoyo digital caliper (Model 500-162-21), with a resolution of 0.01 mm, ensuring maximum accuracy. The presence of LEH in each permanent tooth of individuals from the Amastris population was systematically recorded on the hypoplasia data form. Measurements of LEH were carried out at the Anthropology Department Laboratory of KAEU.

2.2.3 | Statistical Analysis

Within the scope of the study, an independent samples *t*-test was conducted for sex, and an analysis of variance (ANOVA) was performed within the age categories.

3 | Results

3.1 | Demography, Number of Teeth, and Age Ranges of the Amastris Population

Although there were a total of 54 individuals in the cemetery, measurements of 396 permanent teeth from 17 males, 3 females, 7 children, and 3 individuals with undetermined sex, totaling 30 individuals, were suitable for LEH analysis (Table 1). Only the permanent teeth of children with mixed dentition were considered, in addition to postmortem and antemortem losses. This limitation constitutes the restriction of the sample group.

3.2 | Prevalence, Severity, Number of Band LEH, and Number of LEH by Tooth

The overall prevalence of hypoplasia in the permanent dentition of this population was found to be 37.4% (Table 2). Canine teeth exhibited the highest incidence of hypoplasia, with a prevalence rate of 77.1% (Table 2). To investigate the stress periods experienced by this population, both the severity levels and the number of hypoplastic bands were systematically assessed. The severity of hypoplasia was predominantly classified as "mild" (Table 2). The most frequently observed manifestation of LEH corresponded to the first hypoplastic band (Table 3, Figure 4).

TABLE 1 | Distribution of LEH in Amastris population terms of demography.

	Infant and children				Males				Females				Unknown			
Age group	***N***	***n***	**Observed permanent teeth**	**Permanent teeth with LEH**	***N***	***n***	**Observed teeth**	**LEH**	***N***	***n***	**Observed teeth**	**LEH**	***N***	***n***	**Observed teeth**	**LEH**
Infant (0–2.4)	1	—	—	—	—	—	—	—	—	—	—	—	—			
Child (2.5–17.9)	19	7	61	18	—	—	—	—	—	—	—	—	—			
Young adult (18–29.9)	12	6	—	—	2	2	49	16	2	2	34	16	3	2	15	10
Middle adult (30–44.9)	17	15	—	—	16	13	184	68	1	1	25	12	4	1	10	1
Older (45+)	5	2	—	—	2	2	18	7	—	—	—	—	—			
Total	54	30	61	18	20	17	251	91	3	3	59	28	7	3	25	11

Note: N: total number of individuals. *n*: total number of individuals with LEH.

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TABLE 2 | Prevalence of LEH by tooth type and severity of LEH in the Amastris population.

Tooth types	Observed teeth (*n*)	Teeth with LEH (*n*)	Mild LEH		Moderate LEH		Severe LEH		General prevalence rate LEH
			n	**%**	***n***	**%**	***n***	**%**	**%**
I1	42	20	16	80	4	20	—	—	47.6
I2	38	20	16	80	4	20	—	—	52.6
C	57	44	26	50	13	29.5	5	11.3	77.1
PM1	50	25	19	76	6	24	—	—	50
PM2	54	18	10	55.5	7	38.8	1		33.3
M1	70	10	9	90	1	10	—	—	14.2
M2	57	11	7	63.6	3	27.2	1		19.2
M3	28	—	—	—	—	—	—	—	0
Total	396	148	103	69.5	38	25.6	7	9	37.4

Note: n: observed LEH degree (number of teeth).

TABLE 3 | Band counts for LEH in Amastris populations.

Tooth types		1 BAND		2 BAND		3 BAND		4 BAND		5 BAND		6 BAND		7 BAND	
	N	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
I1	42	23	54.8	23	54.8	23	54.8	20	47.6	13	30.9	6	14.3	2	4.8
I2	38	19	50	18	47.4	17	44.7	12	31.5	6	15.8	5	13.2		—
C	57	48	84.2	47	82.4	42	73.7	30	52.6	17	29.8	10	17.5	2	3.5
PM1	50	23	46	20	40	7	14	4	8	1	2		—		—
PM2	54	18	33.3	14	25.9	7	12.9	1	1.8		—		—		—
M1	70	10	14.6	4	5.7		—		—		—		—		—
M2	57	7	12.3	3	5.3	1	1.7	1	1.7		—		—		—
M3	28	—	—		—		—		—		—		—		—
Total	396	148	37.3	129	32.6	97	24.5	68	17	37	9.3	21	5.3	4	1.1

Note: N: total number of teeth; *n*: number of observed bands.

3.3 | LEH Age of Occurrence and Comparison of Methodologies

A comparative analysis of both methodologies reveals that stress indicators in the Amastris population emerge as early as 1 year of age. The highest levels of stress are observed between 3 and 3.49 years, followed by another peak between 4 and 5 years (Figure 3). During the second peak, both the Reid&Dean and Goodman&Rose methods show a declining trend. Furthermore, the mortality ages of infants and children who did not survive within the Amastris community suggest that they were exposed to stress during these same critical age intervals (Figure 3). In the Reid&Dean method, the LEH data recording ends at the age of 6, whereas in the Goodman&Rose method, it ends at the age of 8. Therefore, no numerical records of hypoplasia data are available beyond these ages. However, it can be inferred from the demographic methods that child mortality continued in the population (Figure 3).

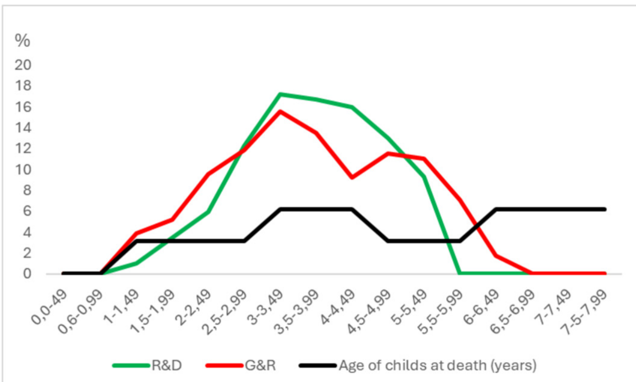

FIGURE 3 | Comparison of R&D and G&R LEH bands in adults with the age of death children. [Colour figure can be viewed at wileyonlinelibrary.com]

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3.4 | Statistical Analysis of R&D and G&R Methods

Descriptive statistics were computed to examine RDMean and GRMean scores across age and sex categories. For the children ($n = 8$), the mean RDMean score was 3.53 (SD = 1.02), with a range from 1.65 to 4.80, while the mean GRMean score was 3.20 (SD = 0.88), with a range from 1.95 to 4.37.

In the 18–30 age group, males ($n = 2$) had a mean RDMean of 3.78 (SD = 0.35) and a GRMean of 3.67 (SD = 0.30). The single female participant in this category had higher scores (RDMean = 4.42, GRMean = 4.43); however, because of the small sample size ($n = 1$), inferential comparisons were not appropriate. Participants with unknown sex in this age group ($n = 2$) had lower mean values (RDMean = 3.18, GRMean = 2.66).

In the 30–45 age group, males ($n = 13$) exhibited relatively high mean scores (RDMean = 4.13, SD = 0.49; GRMean = 4.21, SD = 0.59), while females ($n = 2$) showed slightly lower RDMean (M = 3.94, SD = 0.16) but similar GRMean (M = 4.19, SD = 0.00). Among participants aged 45 and above ($n = 2$), the mean RDMean and GRMean scores were 3.47 (SD = 0.09) and 3.15 (SD = 0.14), respectively (Table 4; Figure 4).

An independent samples *t*-test was conducted to compare RDMean and GRMean scores between male and female participants. No statistically significant differences were found for RDMean, $t(18) = -0.29$, $p = 0.387$, or GRMean, $t(18) = -0.67$, $p = 0.256$. Effect sizes were estimated using Cohen's d, Hedges' g, and Glass's Δ. For RDMean, Cohen's d = −0.18 (95% CI [−1.41, 1.05]); Hedges' g = −0.18; Glass's Δ = −0.29. For GRMean,

TABLE 4 | Statistical comparison of the R&D and G&R.

Age category	Sex	n	Mean	SD	Min	Max	p	Standardizer[a]	Estimate	Cl 95% Lower	Cl 95% Upper
RD											
Children		7	3.5257		1.65	4.80					
Adults	Males	17	4.0129	0.49482	3.40	5.17	0.387	0.48809	−0.183	−1.34	1.004
	Females	3	4.1000	0.29816	3.83	4.42					
GR											
Children		7	3.1971		1.95	4.37					
Adults	Males	17	4.0182	0.63504	2.91	5.20	0.256	0.48809	−0.183	−1.34	1.004
	Females	3	4.2700	0.13856	4.19	4.43					

[a]Independent sample effect sizes Hedges' correction uses the pooled standard deviation, plus a correction factor.

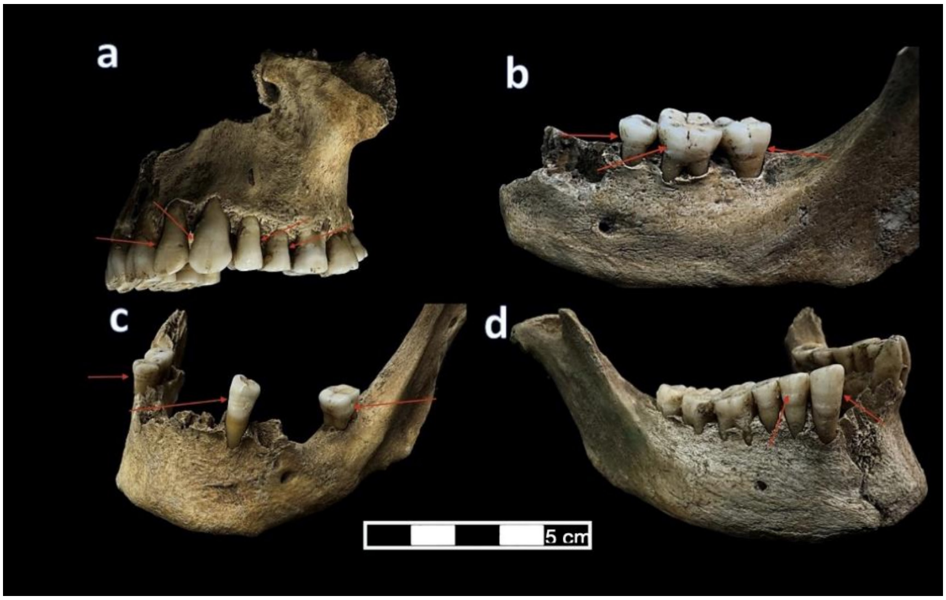

FIGURE 4 | LEH examples (a) Individual no. 24, (b) Individual no. 4, (c) Individual no. 8, (d) Individual no. 6. [Colour figure can be viewed at wileyonlinelibrary.com]

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Cohen's d = −0.42 (95% CI [−1.65, 0.82]); Hedges' g = −0.40; Glass's Δ = −1.82. These effect sizes indicate small to moderate differences, none of which reached statistical significance (Table 4).

A one-way analysis of variance (ANOVA) was performed to compare RDMean and GRMean scores among four groups: male adults, female adults, adults of unknown sex, and children. For RDMean, the group effect was not statistically significant, $F(3, 26) = 2.09$, $p = 0.126$. However, a significant group effect was found for GRMean, $F(3, 26) = 5.22$, $p = 0.006$. The effect size for GRMean, as estimated by eta squared ($\eta^2 = 0.38$), indicated a large effect according to Cohen's (1988) criteria. Complementary measures, including epsilon squared ($\varepsilon^2 = 0.30$) and omega squared ($\omega^2 = 0.30$), confirmed the substantial proportion of variance in GRMean attributable to group membership. For RDMean, eta squared was 0.19, indicating a medium effect, though it was not statistically significant.

4 | Discussion

Understanding the causes of physiological stress, analyzing the lifestyles of ancient societies, and assessing their interactions with the environment are of paramount importance (Ortner 2003). A multitude of factors, including infectious diseases and metabolic disorders, contributed to the high mortality rates observed in ancient Anatolian societies (Büyükkarakaya 2011; Çırak and Çırak 2015; Erkman et al. 2020; Üstündağ 2022). Environmental factors encountered during infancy and childhood, combined with malnutrition, parasitic infections, and communicable diseases, frequently lead to the development of LEH lesions (Goodman et al. 1980). These lesions serve as tangible indicators of physiological stress, manifesting as distinct lines or pits on dental surfaces (Larsen 1997).

The Empire faced numerous political and economic instabilities during the 6th–9th centuries (Xoplaki et al. 2016). Researchers have revealed that palynological data from fruit trees in various regions of Anatolia show a significant decrease in pollen during this period. Palynological and stable isotope data suggest that the cause of this event was moderate drought (Haldon et al. 2014). It is known that a significant portion of the Empire's revenue was derived directly from agricultural products and the taxes imposed based on the quality of the land where these products were cultivated (Haldon 1990). During periods of severe famine, when rainfall was scarce or climatic conditions were harsh, tax reductions were implemented. Researchers have noted that during these famine periods, the Empire provided tax exemptions to the population due to malnutrition (Xoplaki et al. 2016). Climatic changes, which varied across different periods, affected the socioeconomic systems of states whose primary revenue sources were taxes based on agriculture. Therefore, it can be inferred that the Amastris population was also impacted by environmental changes due to climatic adversities (Haldon et al. 2014). The worsening of socioeconomic conditions, as a result of these changes, may have led to nutritional deficiencies and deteriorating health among individuals. However, given the average age of the population, it is evident that these individuals, despite experiencing ecological stress, were able to cope with the stress factors.

The pathological dental findings from the Amastris population offer valuable insights into dietary patterns. In the analysis of the permanent teeth in our population, the prevalence of dental caries is 9.09%, the prevalence of dental calculus is 55.5%, and the prevalence of antemortem tooth loss (AMTL) is 15.4% (Taş and Erkman 2024). Comparative dental pathology analyses with other prominent ancient Anatolian societies, such as Tios/Filyos (Çırak and Çırak 2015), Tlos (Duyar and Atamtürk 2017), Sardis (Eroğlu 1998), and İznik (Erdal 1996) (Figure 1), reveal similar trends. However, elevated rates of dental calculus are observed in societies such as Leodikia (Şimşek 2011), Domaniç (İlbey 2018), Attepe-Dereköy (Erkman et al. 2023), Karagündüz, and Dilkaya (Figure 1) (Gözlük 2004; Erkman,2008; Taş and Erkman 2024). The dietary practices of the Amastris community predominantly centered on unrefined grains, as evidenced by the third and fourth degrees of wear identified using Brothwell's tooth wear classification (Taş and Erkman 2024). Although marine food sources are thought to have been consumed, their dietary contribution remains limited, a trend consistent with other coastal Anatolian settlements (Figure 1).

The occurrence of enamel hypoplasia in the Amastris population is documented at 37.4% (Table 2, Table 5). In contrast, the contemporary Tios community (Çırak and Çırak 2015), located within the same coastal region, exhibits a significantly lower prevalence of 1.6%. Comparative data from other societies reveal rates of 37.6% in Tlos (Duyar and Atamtürk 2017) in the Mediterranean region, 36.8% in İznik (Erdal 1996), near the Sea of Marmara, and 25.7% in the Kelenderis community (Çırak et al. 2013), which dates to the modern period. These variations in stress indicators are closely linked to the distinct lifestyles, subsistence strategies, and nutritional habits of individual societies (Table 5) (Molleson et al. 2005; Büyükkarakaya and Erdal 2006; Büyükkarakaya 2012).

A striking demographic observation emerges from the Amastris population: the infant and child mortality rate is as high as 37.03%. Additionally, 88.89% of deceased infants are categorized within the neonatal group (Şahin 2023). This points to a concerning trend, indicating significant exposure to severe stressors during early life stages (Figure 3). A comprehensive pathological assessment is essential, particularly regarding hypoplasia, which serves as a critical indicator of physiological stress.

It is known from the literature that during periods of low retinol levels due to insufficient sun exposure, the development of the alveolar bone in infants is affected (Skinner 1986; Skinner and Goodman 1992). Considering that changes in climatic conditions influence stress factors, it can be inferred that the generally cloudy and humid weather characteristic of the Western Black Sea coast may have also affected the retinol levels in Amastris individuals during their infancy.

Despite extensive research, there is still no consensus in the literature regarding the presence of porotic lesions in the skeleton, such as cribra orbitalia (CO), porotic hyperostosis (PH), cribra humeralis (CH), or cribra femoralis (CF), which are associated with anemia (Brickley 2024; Moro et al. 2025).

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TABLE 5 | Comparison of LEH frequencies, LEH age at onset, and LEH number of bands with the proportions of other ancient Anatolian human populations.

Population	Period	Researcher	LEH frequencies%	LEH age at onset		LEH number of bands
				GR	RD	
Aşıklı Höyük	Neolitic	Büyükkarakaya and Erdal 2006	8.0	3–3.5	—	2
Çayönü	Neolitic	Büyükkarakaya and Erdal 2006	45.9	1–1.5	—	5
Çatalhöyük	Neolitic	Molleson et al. 2005	12.9	—	—	—
Tepecik Çiftlik	Neolitic	Büyükkarakaya 2014	34	—	—	—
İkiztepe	Early Bronze Age	Büyükkarakaya 2011	43.2	0.01–0.5	1–1.5	7
Laodikeia	Roman	Göksal 2017	11.4	—	—	—
Sardis	(LERP)	Eroğlu 1998	64.5	—	—	—
Tios/Filyos	Byzantium	Çırak and Çırak 2015	1.6	—	—	—
İznik	Late Byzantium	Erdal 1996	36.8	—	—	—
Tlos	Byzantium	Duyar and Atamtürk 2017	37.6	—	—	—
Domaniç	Roman	Gökkurt 2019	17.2	1–1.5	1–1.5	5
Amastris	**(LERP)**	**Present study**	**37.4**	**1**	**1**	**7**
Dilkaya	Medieval	Erkman 2008	13	1.5	—	—
Attepe	(LERP)	Erkman et al. 2023	17.9	—	1–1.5	3
Dereköy	(LERP)	Erkman et al. 2023	23.3	—	1–1.5	3
Karagündüz	Medieval	Gözlük 2004	22,8	—	—	—
Kelenderis	Modern Age	Çırak et al. 2013	25.7	1.5–2	—	—
Tasmasor	Modern Age	Büyükkarakaya 2011	56.7	0.01–0.5	1.5–2	7

Note: GR: Goodman &Rose. RD: Reid &Dean. Only the permanent teeth of adults and children with mixed dentition were considered in all populations.

While anemia (megaloblastic, iron deficiency) remains the primary factor in lesions defined as areas with limited porosity, histological studies suggest additional etiologies such as vitamin deficiencies, parasitic infections, inflammatory responses, osteoporosis, fever, zinc deficiency, birth trauma, prematurity, low birth weight, rickets, infectious diseases, congenital syphilis, and nutritional deficiencies (e.g., scurvy, B12 deficiency, gastrointestinal malabsorption) (Moro et al. 2025; Wapler et al. 2004; Mann and Hunt 2012; Cole and Waldron 2019; O'Donnell et al. 2020; Hengen 1971; Rivera and Mirazón Lahr 2017; Lewis 2018; Kronfield and Schour 1939; Seow 1992; Dolphin and Goodman 2002; Aine et al. 2000; Hillson et al. 1998). There is also potential evidence of marrow hyperplasia in the bone in the literature (Moro et al. 2025). Histological data related to cell types and other parameters are important in determining the cause of acquired anemia (Moro et al. 2025). Recent clinical studies have shown that parasitic infections, which cause severe anemia, disproportionately affect young children and contribute to the development of porotic lesions (Wang et al. 2024). These macroscopic skeletal lesions leave permanent traces and thus provide a valuable dataset for analyzing physiological stress in past populations (Larsen 2015; Büyükkarakaya 2012; Wang et al. 2024).

An analysis of infant and child crania from Amastris reveals a prevalence of PH at 25%, a rate consistent with those found in contemporary populations. However, the prevalence of CO is notably high, reaching 71.43% (Şahin 2023). The pertinent literature has reflected direct relationship between cribra orbital and porotic hyperostosis (Smith-Guzman, 2015; Nunn and Tap, 2000). Although malaria was much more prevalent on Mediterranean Region, some cases were reported in the Black Sea Reagon (Şahin 2003). It is possible that individuals in the Amastris community in the Western Black Sea Region were affected by this condition. When compared with other ancient Anatolian communities, these findings suggest that individuals in early childhood experienced exceptionally high levels of physiological stress. The concurrent occurrence of enamel hypoplasia and CO/PH lesions within the same age cohort further supports this interpretation.

Epidemiological studies conducted in economically disadvantaged societies, including Africa, the Caribbean, and Latin America, have identified pneumonia, measles, malaria, anemia, and HIV/AIDS as primary causes of child mortality, with malnutrition implicated in approximately 60% of these deaths (Wagstaff et al. 2004; Kassebaum et al. 2017). Additionally, the

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use of contaminated water sources significantly exacerbates the transmission of hygiene-related diseases, further contributing to elevated mortality rates (Brockerhoff and Hewett 2000). It is likely that similar patterns existed in ancient societies, where a combination of biocultural and environmental stressors, epidemic diseases, nutritional deficiencies, and chronic illnesses collectively contributed to high rates of early-age mortality (Malis et al. 2024).

Determining the precise etiology of hypoplasia is inherently complex. However, when considered in conjunction with other pathological markers, it provides valuable insights into childhood mortality. Compared with other contemporary Anatolian communities, the Amastris community exhibits a relatively low incidence of periostitis (Şahin 2023). While periostitis was not detected in infants and children, its prevalence among adults underscores its significance in assessing enamel hypoplasia as a marker of physiological stress.

A key factor contributing to the formation of LEH is the weaning process, a pivotal transition from exclusive maternal dependence to exposure to diverse dietary, social, and environmental influences. As breast milk intake decreases, infant immunity becomes compromised, making children more susceptible to pathogens (Maclellan 2005). Bioarchaeological research on the weaning process can provide valuable insights into the lives, strategies, and demographic and social dynamics of past populations (Özdemir et al. 2019). Stable carbon and nitrogen isotope ratio analyses are essential for investigating dietary habits, subsistence practices, social behaviors, and intra-societal changes (Özdemir et al. 2024). Nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope ratios derived from bone collagen or dentin samples of individuals from ancient societies offer tangible biochemical evidence regarding the timing of weaning and dietary practices (Özdemir et al. 2024). These analyses reveal that deviations in isotope ratios are directly related to the cessation of breastfeeding and the introduction of supplementary foods (Fuller et al. 2006; Prowse et al. 2008; Tsutaya and Yoneda 2015; Özdemir 2018; Özdemir et al. 2019; Özdemir et al. 2024). Furthermore, in the evaluation of archaeological populations' dietary habits and weaning processes, trace element ratios, such as Strontium/Calcium (Sr/Ca), are widely used in the literature to reconstruct dietary transitions during infancy (Mays 2003; Özdemir and Erdal 2009; Büyükkarakaya et al. 2017; Kavun et al. 2018).

In numerous studies conducted on the weaning process of ancient Anatolian societies through ($\delta^{15}N$) and ($\delta^{13}C$) analysis, the age of weaning varies. For example, a study conducted at Tepecik-Çiftlik, dating to the Neolithic period (Özdemir et al. 2024), estimated that the weaning process began at 0.2 years and was completed by 1.3 years, indicating a relatively short duration. In contrast, at Çatalhöyük, dating to the same period, the weaning process is reported to have begun at 1 year and concluded between 1.5 and 2 years (Richards et al. 2003). At Aşıklı Höyük, also dating to the Neolithic period, the weaning process began at 1 year and ended at 2 years, while at the Neolithic site of Çayönü Tepesi, this process started at 2 years and continued until 3.5 years. At İkiztepe, dating to the Late Chalcolithic–Early Bronze I, stable isotope and trace element data suggest that the weaning process began around 1 year and was completed between 2 and 3 years (Özdemir et al. 2019). The LEH stress indicators of the Amastris population, which begin at 1 year and reach their highest levels around 3–3.5 years, can be interpreted as a result of the weaning process when considering the isotope analyses of these archaeological populations.

Comparative analyses of LEH formation across Anatolian societies provide valuable insights into childhood stress patterns. The Neolithic Çayönü community exhibited the onset of hypoplasia at 1–1.5 years, with a peak at 4–4.5 years (Büyükkarakaya and Erdal 2006). Similarly, the Aşıklı Mound population showed initial indicators at 3–3.5 years, with a peak at 4.5–5 years. The Ikiztepe community, dating to the Early Bronze Age, demonstrated an earlier onset (1.0–1.49 years) and a peak at 4–4.49 years (Table 5) (Büyükkarakaya 2012). Although some researchers have associated LEH formation with weaning stress, the prevailing evidence suggests that malnutrition and disease were more significant contributors.

In conclusion, the dental and skeletal pathologies observed in the Amastris population highlight the significant physiological stress experienced by its inhabitants, particularly during infancy and early childhood. A multidisciplinary approach that integrates bioarchaeological, isotopic, and epidemiological methodologies is crucial for further elucidating the underlying causes of these stress markers.

5 | Limitations of the Study

A significant limitation is the possible loss of some teeth during the rescue excavation conducted by the Amasra. Although skeletal material from 54 individuals in the community was recovered, LEH values could be calculated from the permanent teeth of only 30 of these individuals. This study's limitations include the smaller than expected sample size, interobserver observational differences, and difficulties in interpreting LEH values. The fact that the excavation site is located within the city walls and the small size of the excavation area may have led to a more unusual gender distribution.

6 | Conclusion

In addition to dental and paleopathological evidence, paleodemographic data suggest that the inhabitants of Amastris may have been adversely affected by political and economic instability, as well as climatic aridity, between the 6th and 9th centuries. Nonetheless, these findings also imply that the population was likely able to adapt to or cope with such stressors. Among the most significant indicators of physiological stress, enamel hypoplasia offers valuable bioarchaeological insights into the health status, nutritional conditions, and environmental challenges experienced by infants and children in Amastris during this period. To establish the most accurate temporal framework for the onset of stress, dental analyses of skeletal remains from the chapel were performed using two distinct methodologies. The graphical analysis demonstrated the effectiveness of both methods, although a six-month discrepancy was observed at the second peak. The identification of two high-frequency stress indicators (Figure 3) suggests that the weaning process in infancy or early childhood likely began during these periods, coinciding

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with heightened exposure to pathogenic factors (Figure 3). The correlation between the age at death and the peaks of total defect numbers suggests that stress in the early period may have been a contributing factor.

Paleodemographic and paleopathological analyses further confirm the presence of CO and PH lesions, which, in conjunction with enamel hypoplasia, highlight the impact of infectious or metabolic disorders, nutritional deficiencies, and adverse living conditions. These findings suggest a strong correlation between physiological stress and environmental hardships within the Amastris community. Comparisons with other ancient Anatolian populations reveal no significant divergence in the timing of stress indicators across these societies. No significant difference was found between male and female individuals in the *t*-test conducted for the RD and GR methods. The one-way analysis of variance (ANOVA) also did not show a significant result between the statistical outcomes of both methods among male adults, female adults, adults with undetermined sex, and children.

A comprehensive evaluation of chemical analyses highlights the pivotal role of complementary foods, including dairy products, cereals, fruits, and vegetables, during the early stages of the weaning process. The continued presence of elevated stress indicators beyond the age of three, following weaning, supports the notion of this dietary transition. Several factors related to weaning, such as digestive difficulties associated with complementary foods, increased environmental exploration, greater exposure to infectious diseases, and pathogen transmission through adult contact, are believed to have contributed to the development of LEH.

The pathological findings suggest that at the time of LEH occurrence, enamel formation in children was complete, signaling their transition towards adulthood. As a result, the prevalence of enamel hypoplasia in the Amastris population likely reflects declining health conditions influenced by environmental stressors, poor hygiene, parasitic infections leading to severe anemia, and potential metabolic or genetic disorders. These factors must be carefully considered when evaluating malnutrition and other physiological stressors to determine the underlying causes of stress indicators in this population.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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