



Chronology, duration, and periodicity of linear enamel hypoplasia at the late Iron Age site Non Ban Jak, Thailand: A quantitative microscopic analysis

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ABSTRACT

We provide the first application of a quantitative microscopic approach that does not rely on the presence of perikymata for the identification and comprehensive analysis of linear enamel hypoplasia (LEH) to a large archaeological sample from Southeast Asia. Additionally, we introduce *MicroPolySharp*, a new computer program that automates the assessment of these stress episodes, and we present new dental crown width/height ratios for the region.

Enamel surface depth profiles were measured from epoxy dental replicas generated from 48 individuals using a confocal microscope. The identification and analysis of LEH episodes was undertaken using *MicroPolySharp*, which combines three recently published methods specifically suited to worn archaeological samples. Ten parameters were examined: frequency, prevalence, episode duration, stress duration, recovery duration, age at first onset, age at last onset, total growth disruption, proportion of available enamel affected, and periodicity.

Results revealed a high prevalence of LEH: 97.92 % (47/48) individuals affected. LEH frequency averaged 2.5 episodes per individual (range = 1 to 4 episodes). The chronology of episodes averaged 3.66 years (range = 1.4 to 5.5 years). The age of earliest episode occurrence averaged 3.1 years (range = 1.4 to 4.2 years). While the age of last episode averaged 4.3 years (range = 2.9 to 5.5 years). Duration of growth disruptions (stress + recovery) averaged 103 days (range = 27 to 269 days). Consistent with other studies, the average duration of the stress portion of episodes was 56 days (range = 9 days to 6.4 months) while the duration of the recovery portion averaged 47 days (range = 8 days to 5 months). The total amount of growth disruption for individuals averaged 263 days (range = 87 to 543 days). The proportion of available crown height affected by growth disruption averaged 21 % (range = 6 % to 42 %), while LEH periodicity for the sample averaged 8.6 months (range = 2.4–23 months). Stress and recovery durations, along with age at first episode, were the most sensitive and useful parameters for discerning differences between the subgroups of interest. Finally, the effects of any amount of variable crown height wear within the study group was found to have a significant confounding effect on all aspects of LEH results and interpretation if not properly controlled. Given the high prevalence, all individuals were, for the most part, equally affected. Future analysis of samples from the periods prior to and leading up to the Late Iron Age from nearby sites will be required to provide much needed context to the high levels of systemic stress observed at Non Ban Jak.

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1. Introduction

Over the last two decades, numerous studies have sought to evaluate and/or refine the available methods for identifying and analysing linear enamel hypoplasia (LEH), especially those that rely on high-resolution microscopic approaches (Bocaage & Hillson, 2016; Bocaage, Humphrey, & Hillson, 2010; Cares Henriquez & Oxenham, 2017, 2018, 2020; Gamble & Milne, 2016; Guatelli-Steinberg, 2008; Hassett, 2012; Hubbard, Guatelli-Steinberg, & Sciulli, 2009; Martin, Guatelli-Steinberg, Sciulli, & Walker, 2008; O'Hara, 2017; Reid & Guatelli-Steinberg, 2017; Temple, Nakatsukasa, & McGroarty, 2012). The majority of these have been preliminary demonstrations of techniques using limited sample sizes. While studies have begun to apply these methods to larger samples where hypotheses regarding stress and local ecology might be tested, many others remain limited to employing subjective or macroscopic methods (Dirks, Humphrey, Dean, & Jeffries, 2010; Dirks, Reid, Jolly, Phillips-Conroy, & Brett, 2002; Guatelli-Steinberg, 2003; Guatelli-Steinberg, Larsen, & Hutchinson, 2004; King, Humphrey, & Hillson, 2005; Merrett et al., 2016; Temple, 2014, 2016, 2018, 2020; Temple, McGroarty, Guatelli-Steinberg, Nakatsukasa, & Matsumura, 2013). One of the key reasons for this is that most of the new advancements are related to techniques that require the presence of visible perikymata along the entire tooth surface. However, many archaeological dental samples tend to be affected by labial/buccal wear that obscures their visibility making these samples unsuitable for use with such techniques (Cares Henriquez & Oxenham, 2017; Hubbard et al., 2009). Furthermore, as methods become more advanced, the calculations required become more complex and time consuming, and can be difficult to replicate accurately without considerable training.

The purpose of this study is to provide the first application of a quantitative microscopic approach for the identification and analysis of LEH to a large Southeast Asian late Iron Age archaeological sample from Non Ban Jak (NBJ), Thailand. A further objective is to provide a unique methodology that considers multiple aspects of these growth disruptions beyond individual frequency and prevalence to include chronology (i.e., the developmental age of episodes), episode duration, stress duration, recovery duration, age at first onset, age at last onset, total growth disruption, proportion of available enamel affected, and periodicity. Each of these factors is assessed with respect to biological sex, age-at-death, occupation mound, and mortuary phase, while also accounting for individual tooth crown height wear, which has been found to significantly impact the interpretation of results.

This study makes use of *MicroPolySharp*, a computer program that incorporates recently published methods for the identification and analysis of LEH that are specifically designed for use with archaeological dental samples when perikymata are not clearly visible (Cares Henriquez, 2020). Written in C# (programming language) and compiled using Visual Studio, this end-user-program eliminates the need for researchers to perform complex and time-consuming calculations manually by automating the entire process of identification and analysis of LEH when using microscopic data. The program's usefulness lies in its ability to reduce the time required for the analysis of LEH from 30 to 45 min per tooth, to under one minute for multiple teeth of the same individual. Furthermore, due to the automated nature of the analysis, it not only improves the accuracy of the results by minimising the introduction of user errors but also their replicability and comparability.

2. Materials and method

2.1. Sample

Permission to carry out this research, which is non-destructive and did not require the export of materials from the origin country, was granted by The National Research Council of Thailand (permit numbers 0002/9647; /9582) under the aegis of *The Prehistory of the Upper Mun Valley* project. The sample includes all individuals (n = 48) with an

assessable permanent anterior dentition from Non Ban Jak, a late Iron Age archaeological site located in Northeast Thailand. Teeth that presented with more than 60 % crown height wear, cracks, obscured labial surface, or undetectable cemento-enamel junction (CEJ) were excluded. The sample was made up of 19 females, 23 males, and six indeterminate sex individuals. Sex estimation data for adults was provided by Buckley et al. (2020). Buckley et al. (2020) assigned adults to three relative age categories (young, middle and old) after Buikstra and Ubelaker (1994). These relative age categories have been retained, while they have been renamed using Buikstra and Ubelaker's (1994) age ranges. As such, eight individuals were classed as adolescents aged 15–19 years old, 11 were young adults aged 20–35 years old, 17 were mid-adults aged 35–50 years old, 11 were old adults aged 50+, and one was categorised as an adult with no specific age range. In terms of the physical distribution of the sample, two occupation mounds were identified with eight individuals (four males, and three females) excavated from the East mound, and 40 (19 males, 16 females) from the West mound. In terms of the temporal distribution of the sample (Higham, 2020), six individuals came from mortuary phase (MP)1 (100–540 CE), 14 from MP2 (250–530 CE), 15 from MP3 (350–550 CE), 13 from MP4. There are no radio-carbon dates for MP4 with Ward (2019) suggesting an approximate age of 550–820 CE.

2.2. Data collection

High resolution silicone impressions were produced using *Affinis® Perfect Impressions (Coltene®) Regular Body polyvinylsiloxane*, which was applied to the labial surfaces of the anterior maxillary and mandibular dentition. This was used to create silicone moulds that were then used to create replicas using *Epofix™*, a cold-setting resin based on two fluid epoxy components (*Struers ApS*, Milton, QLD, Australia). Enamel surface depth profiles were measured from these epoxy replicas using an Olympus LEXT OLS5000 laser scanning confocal microscope. Detailed descriptions of the resin replica production methods and enamel surface depth profile construction are provided in Cares Henriquez and Oxenham (2017) and Cares Henriquez and Oxenham (2020), while OLS 5000 microscope settings are presented in Table 1.

2.3. Estimates of crown height wear

While the issue of crown height wear is of fundamental importance to studies of LEH, it is currently the least resolved issue and requires detailed attention. Crown height wear estimation is a crucial step when carrying out an assessment of LEH. This is because for LEH to be useful, beyond simply noting if they are present or absent or stating the number of defects per individual, it is helpful to estimate the chronology of LEH episodes as well as their duration (Cares Henriquez & Oxenham, 2017, 2018, 2020), something that has been of interest for some considerable time (e.g. Hutchinson and Larsen (1988)). To do this accurately, it is necessary to know the relative position of defects from the cemento-enamel junction (CEJ) as well as the amount of time captured by the crown height that is available, and even minimal wear can add uncertainty to estimates of LEH chronology (Guatelli-Steinberg, Buzhilova, &

Table 1
OLS 5000 Extended Topography Scan Settings.

OLS 5000 Microscope Settings
Operation Mode: Default
Objective: 10X
Data Acquisition: 3D > Stitching Function
Data Type = Height > Profile
X resolution (Xcv) = 1.236962
Y resolution (Ycv) = 1.248557
Z resolution (Zcv) = 0.019082
(Resolution unit = micrometer)
Stitching Area: Overlap 10 %

Trinkaus, 2013).

At present, there is no consensus on the best method for estimating crown height wear, and so the approaches employed by studies can vary markedly. Some studies, such as Martin et al. (2008) and Berbesque and Hoover (2018), estimate crown height by reconstructing the original projection of the crown height based on the morphology of unworn specimens. However, especially when working with archaeological specimens where attrition is common, it is not unusual for there to be no individuals with minimally worn teeth in the sample.

An alternative is either to rely on sample / species crown height averages or simply exclude individuals deemed to have more than 10–20 % crown height wear (Cares Henriquez & Oxenham, 2017, 2020; Guatelli-Steinberg, 2003, 2004; Hillson, 1992; Modesto-Mata et al., 2017; Temple, 2007, 2014). However, while this latter option may seem like an optimal solution, it too can be problematic as it can significantly reduce sample sizes and is most likely to exclude older individuals who in general are more likely to be affected by attrition and as such reduce the available age range of the sample. In spite of these issues, including individuals that have more than minimally worn teeth is a worthwhile exercise, especially to ensure that all age at death categories are represented and, therefore, be able to obtain a complete picture of how systemic physiological stress was experienced by the whole population of interest.

However, to do this, accurate estimates of wear are essential. Wear can be a complicated factor. On the one hand, crown height wear (attrition) is likely to have a strong correlation with age at death, especially in archaeological samples. On the other, due to the limited window of time that is captured by dental crowns (that is they have a minimum and maximum age limit), crown height wear can also affect the interpretation of observed patterns when it comes to commonly assessed outcomes such as number of defects, age at first episode and total growth disruption. Consequently, being able to account for the effects of wear on any differential patterns that might be observed is crucial.

2.3.1. Objective crown height wear estimates

In this study, crown height wear estimates are constructed based on the previously published Reid and Dean (2000) deciles, which provide the foundation for many of the processes included in the methods used in this study (Cares Henriquez & Oxenham, 2018, 2020). As such, the reported wear percentage refers to the visible crown height (i.e., 10 % = one decile of crown height wear, 20 % = two deciles of crown height wear), and how much time each of these deciles represents changes between teeth type.

The crown height wear estimates are calculated using a novel approach that relies on crown width/height ratios obtained from unworn teeth in the sample. While this method has not been previously utilised in bioarchaeological studies, it is proposed by a number of researchers in the field of dentistry for estimating crown heights in the context of treatment planning in restorative dentistry, prosthodontics, and periodontal surgery (Garg & Goje, 2018; German, Chu, Furlong, & Patel, 2016; Köseoglu & Yanikoğlu, 2019; Magne, Gallucci, & Belser, 2003; Marcushamer et al., 2011; Sah et al., 2014; Shahid, Alam, & Khamis, 2015; Sitthiphan et al., 2015; Tsukiyama et al., 2012; Volchansky & Cleaton-Jones, 2001). Studies that propose this approach examine the relationship between crown width and crown height (length) from an aesthetics perspective and focus either on clinical crown height or anatomical crown height. The goal of these studies is to create standards for determining optimal cosmetic outcomes based on natural oral aesthetics (Tsukiyama et al., 2012).

To date, anatomical crown width/length ratios have been studied in the maxillary anterior teeth of Central European and Asian individuals. Magne et al. (2003) examined crown width/length ratios using digital images of worn and unworn extracted teeth of Swiss individuals and found that the width/length ratio was 78 % for central incisors, 73 % for lateral incisors, and 73 % for canines. A similar study by Marcushamer

et al. (2011), which followed the same protocol, examined the maxillary anterior crown proportions of Asian individuals from Japan, and found the ratio was 72 % for central incisors, 67 % for lateral incisors, and 67 % for canines.

A number of studies have demonstrated differences in tooth size and morphology both within and between different populations (Lee, 1977; Yaacob, Nambiar, & Naidu, 1996; Younes, al-Shammery, & el-Angbawi, 1988). Therefore, using previously published width/length ratios, Tsukiyama et al. (2012) undertook a comparative analysis of width/length ratios between Asian and Central European (Swiss) individuals. The study found a significant difference in the width/length ratios between these two populations and concluded that Central European individuals had larger width/length ratios than Asian individuals, noting that the maxillary teeth of the latter group were more slender. One of the key findings of these aforementioned studies into anatomical crown/width ratios has been confirmation that incisal wear has no effect on the average value of width within the same tooth group (Magne et al., 2003; Marcushamer et al., 2011; Tsukiyama et al., 2012). Therefore, a normally preserved tooth width together with appropriate ratio values for the sample of interest can be used to estimate the original tooth crown length (Tsukiyama et al., 2012).

Crown height wear was estimated using crown width/height ratios obtained from unworn teeth in the Non Ban Jak sample. The crown width height ratio for three maxillary and mandibular tooth groups (central incisors, lateral incisors, and canines) was calculated following previously published protocols by Magne et al. (2003) and Marcushamer et al. (2011) as $R = W/L$, where W is the mesiodistal crown dimension, L is the incisocervical crown dimension, and R is the width/length ratio. The calculated sample ratios for the Non Ban Jak sample are presented in Table 3. Crown height wear for each individual included in this study was then estimated by using the measured mesiodistal measurement (width) of each tooth and the calculated sample width/ratio for the tooth type to solve for the incisocervical measurement (length/crown height) as $L = W/R$.

2.4. LEH analysis

The analysis of LEH episodes was undertaken using MicroPolySharp, a computer program that combines three recently published methods specifically suited for identifying and analysing LEH in archaeological dental samples that do not have clear or continuous visible perikymata. Fig. 1 shows the program's main screen, which is used to load the microscopically collected data for analysis. The first two methods are the 'Micro Polynomial' method and the 'Common Cycle' approach, which are used for the objective identification of defects along the enamel surface of multiple teeth of the same individual (Cares Henriquez & Oxenham, 2017, 2020). The third is a set of distance-based exponential regression equations used for the objective estimation of LEH episode chronology and episode duration (Cares Henriquez & Oxenham, 2018).

2.4.1. Objective identification of defects and LEH episodes

The identification of LEH episodes followed the protocols described in Cares Henriquez and Oxenham (2020). Defects on individual teeth were identified as depressions along the enamel surface that deviated

Table 2
Equations for LEH Defect Chronology by Cares Henriquez and Oxenham (2018).

Tooth Type	Equation
Upper central incisor	Age ^a = 5e ^{-0.146x}
Upper lateral incisor	Age = 5.1e ^{-0.106x}
Upper canine	Age = 5.3e ^{-0.112x}
Lower central incisor	Age = 3.8e ^{-0.135x}
Lower lateral incisor	Age = 4.2e ^{-0.144x}
Lower canine	Age = 6.2e ^{-0.141x}

a Age in years.

Table 3

Mean values (SD) in mm and range of the width, length, and width/length ratio of the three unworn tooth types of the maxillary and mandibular dentition for Non Ban Jak (Thailand).

TOOTH	n	WIDTH	W/L RATIO
Upper Central Incisor (range)	12	8.20 (0.61) (6.99–8.96)	0.72 (0.04) (0.62–0.78)
Upper Lateral Incisor (range)	9	6.82 (0.96) (5.50–8.63)	0.62 (0.06) (0.56–0.72)
Upper Canine (range)	15	7.96 (0.71) (6.34–8.98)	0.74 (0.05) (0.64–0.81)
Lower Central Incisor (range)	7	5.63 (0.81) (4.83–6.74)	0.62 (0.09) (0.45–0.73)
Lower Lateral Incisor (range)	5	6.23 (0.49) (5.75–6.78)	0.65 (0.07) (0.53–0.72)
Lower Canine (range)	15	6.83 (0.57) (6.02–7.67)	0.61 (0.07) (0.48–0.75)

from a fitted trendline. These defects were then chronologically ‘matched’ with other defects across multiple teeth of the same individual by constructing a common cycle of defects and allowing each data series a maximum offset of +/- 200 days. Here, only those defects that could be chronologically matched were considered as evidence of systemic stress (i.e., LEH episodes). It should be noted that this study differentiates between ‘defects’ and ‘episodes’, with defects referring to those depressions on individual teeth which have not yet been chronologically matched, whereas ‘episodes’ refers to matched defects. For the handful of individuals in the sample that were represented by a single tooth, three distinct data transects were collected and used for matching of defects.

2.4.2. LEH episode chronology

LEH episode chronology is estimated from the constructed common cycle of defects for each individual using distance-based exponential regression equations by Cares Henriquez and Oxenham (2018), which are presented in Table 2. The equations rely on previously published histological data and use the distance of the start of the defect from the CEJ (mm), which has been standardised to a 10 mm tooth, to estimate the age of the individual at the time of defect formation (Cares Henriquez & Oxenham, 2020). In these equations, the standardised distance

of the start of the defect “x” is calculated as:

$$x = \left(d / \frac{l}{1-m} \right) \times 10$$

where the distance of the defect from the CEJ in mm (d), is divided by the observed length of the tooth in mm (l), which itself is divided by $(1-m)$, where (m) is the percentage of tooth that is missing expressed as a decimal. Then, this is multiplied by 10 to produce a distance measurement that has been standardised to a 10 mm tooth.

2.4.3. LEH frequency and prevalence

LEH frequency was calculated as the number of episodes of growth disruption (chronologically matched defects) for each individual. Prevalence of LEH was calculated as the number of individuals with one or more chronologically matched defects divided by the total number of individuals and is presented as a percentage.

2.4.4. LEH episode duration estimates

LEH episode duration estimates, which encompass both the period of illness and recovery, are calculated by subtracting the start age from the end age of each episode that was identified in the common cycle of defects for an individual. This calculation makes use of the same exponential regression equations used for estimating chronology, which converts the episode start and end location from the CEJ (mm) to time (years) (see section 2.4.2) (Cares Henriquez, 2019; Cares Henriquez & Oxenham, 2018, 2020). These estimates are used to calculate average episode durations as well as the amount of total growth disruption experienced (in days) by individuals during the time when teeth were forming.

2.4.5. Total growth disruption estimates

Total growth disruption estimates were calculated as the sum of the duration of all identified LEH episodes (stress + recovery) from the constructed common cycle of defects for the individual. This is a cumulative value comprised of the number and duration of LEH stress events and does not take into account crown height wear.

2.4.6. Estimates of proportion of available crown height affected

The proportion of available crown height affected by growth

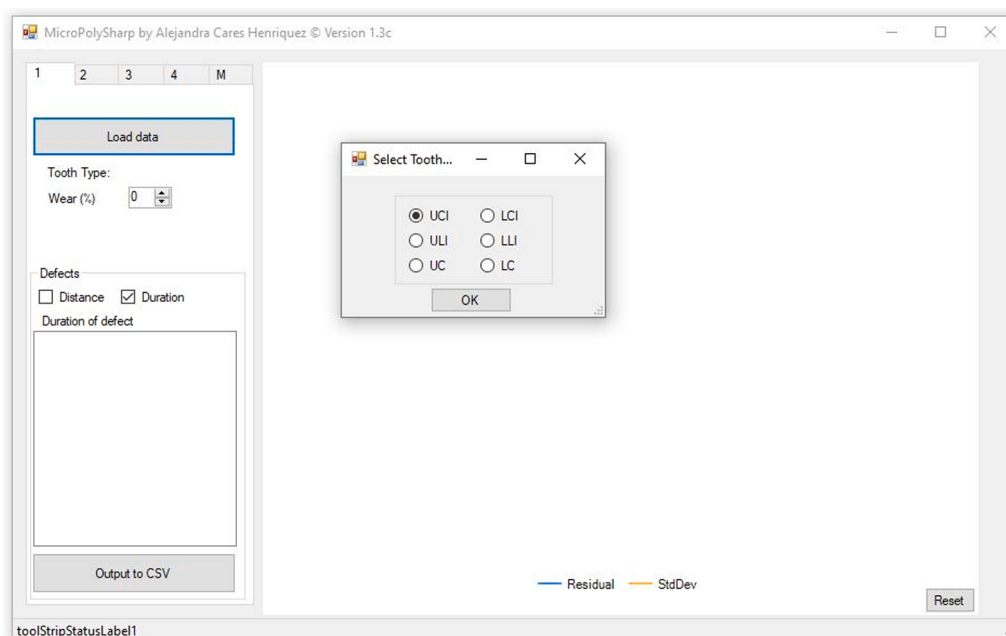


Fig. 1. MicroPoly Sharp V 1.3 –Showing Main Screen – Data Loading and Tooth Selection.

disruption was calculated as the sum of the duration of all identified LEH episodes (stress + recovery) from the constructed common cycle of defects for the individual. This is a cumulative value comprised of the number and duration of LEH stress events. Since this study includes individuals that have up to 60 % crown height wear, this estimate is calculated as a proportion of affected enamel from the available crown height for an individual and is given as a percentage.

2.4.7. Average periodicity estimates

The average periodicity of LEH episodes (interval between successive episodes) experienced by each individual is calculated by subtracting the age at onset or chronology (in years) for each matched stress episode from the age at onset of the previous stress event. These interval values were then averaged and multiplied by 12, to produce an average periodicity for the individual in months.

2.5. Statistical analysis

The distribution of the chronology for all observed LEH episodes, also referred to as age at episode formation or age at onset, was assessed for skewness to ensure that these were normally distributed. Box plots of crown height wear, number of LEH episodes, age at first episode formation, proportion of growth disruption, and average periodicity were constructed to present a visual representation of the distribution between groups by age at death, sex, mortuary phase, and occupation mound. One-way ANOVAs were used to determine if there were any statistically significant differences between the means of subgroups (age at death, sex, and mortuary phase groups). When comparing against groups by occupation mound a Kruskal-Wallis ANOVA test was used instead due to the large difference in subgroup sample sizes. In addition, multiple regressions were undertaken to evaluate any associations between an individuals' number of LEH episodes, age at first episode formation, total disruption, and average periodicity and age at death, sex, mortuary phase, and occupation mound. These excluded any individuals with no matched defects, and individuals from the undetermined age at death category (adult). To control for any potential effects of crown height wear, this was also included as a predictor variable in these regressions. The significance threshold for all analyses was set at 0.05.

3. Results

3.1. Crown Width/Height ratio for Non Ban Jak

Crown width/height ratios for the Non Ban Jak sample were calculated from unworn teeth for the three maxillary and mandibular tooth groups of interest in this study (central incisors, lateral incisors, and canines), and are presented in Table 3.

3.2. LEH at Non Ban Jak

The chronology (age at onset) of all LEH episodes, as well as estimates of periodicity, episode duration (stress + recovery), total disruption duration, and proportion of enamel growth disruption are presented in the [Supplementary Information](#) (SI) (Table A1) alongside demographic information for each examined individual. Here, episodes are those defects which have been chronologically matched across multiple teeth of the same individual, and as such represent the minimum number of LEH episodes that can be confidently attributed to systemic physiological stress (see section 2.4.1). Additional information regarding tooth type, number of teeth used for matching, estimated crown height wear, and common cycle offset are also included in the SI (Table A1) to ensure the results are replicable and verifiable.

To provide an illustration of tooth wear, Fig. 2 shows the dentition of Burial 120, a mid-adult aged male, excavated from the West occupation mound, and associated with Mortuary Phase 3. Fig. 3 shows a typical microscopic enamel surface scan, which presents a 3D surface scan on the top left corner, a coloured surface height topography map on the top right corner, and an enamel surface profile based on where the data transect is chosen from on the bottom. Figs. 4 through to 7 show screenshots of a typical analysis of LEH using MicroPolySharp, again using Burial 120 as an example.

3.2.1. Frequency, prevalence, and distribution

Of the 48 individuals assessed, a total of 119 matched defects were observed. Frequency of LEH at Non Ban Jak, that is the number of matched defects per individual, ranged from 1 to 4 episodes, with an average of 2.5 episodes. The prevalence of LEH, that is the percentage of individuals affected, was 97.92 % (47/48). The observed defects for one individual, B108 a mid-aged male, could not be matched chronologically and so is considered to have zero verifiable episodes of LEH. The chronology for all matched LEH episodes, that is the age at onset (also referred to as age at episode formation), follows a normal distribution (skewness = -0.21, kurtosis = -0.05) (Fig. 8).

When considering other studies that have used microscopic techniques, LEH prevalence at Non Ban Jak is similar to that reported for the Houtaomuga group from Northeast China at 96.8 % (30/31), an inland hunter-gatherer population that relied on a fish and millet agriculture as part of their subsistence strategy (Merrett et al., 2016). A similarly high LEH prevalence was observed by Temple et al. (2013) among the Jomon foragers from Hokkaido, Japan with 100 % (5/5), Jomon foragers from coastal Honshu 100 % (20/20), Tigara Point Hope foragers 80 % (8/10), and by Guatelli-Steinberg et al. (2004) for the Inuit Point Hope sample 100 % (9/9), and Krapina Neanderthal foragers 100 % (11/11) (see Table 4). These exceptionally high levels of systemic stress are in line with observations previously made by Hassett (2012, 2014) and others, which repeatedly demonstrate that microscopic approaches are able to



Fig. 2. Non Ban Jak Burial 120 – Close up of dentition examined (left upper central incisor (21), left upper lateral incisor (22), and left upper canine (23), with estimated 26% crown height wear.

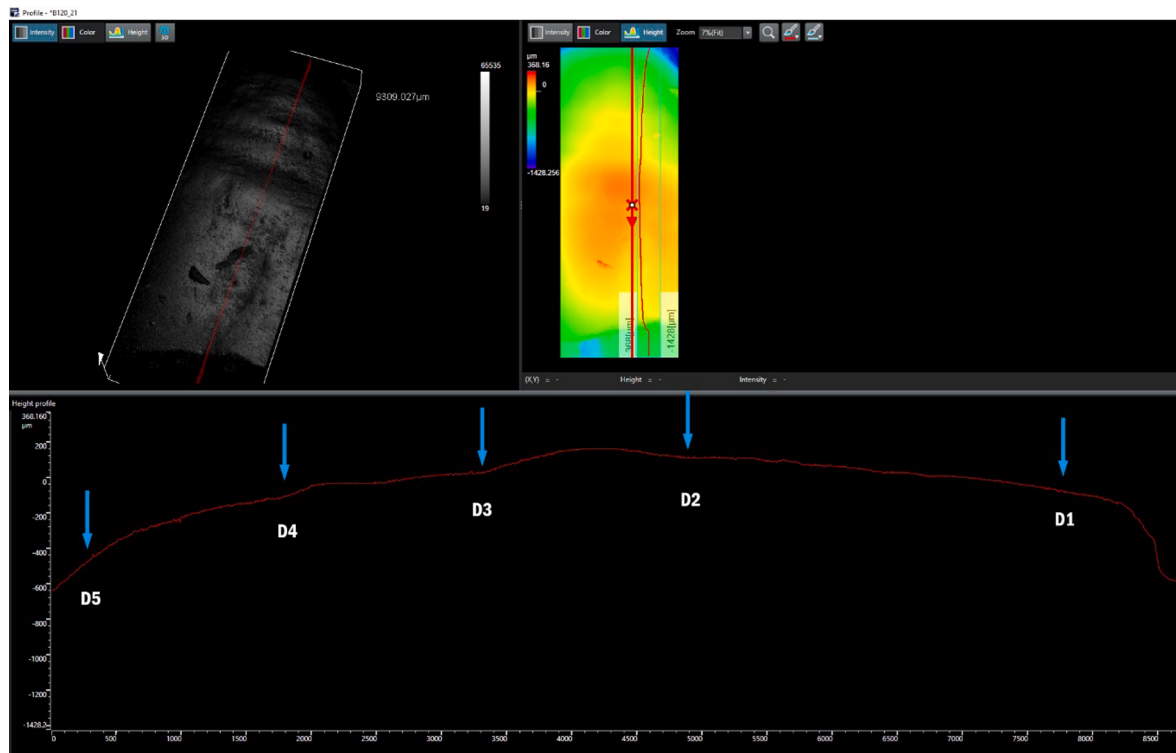


Fig. 3. Microscopic scan of Burial 120 Upper Central Incisor (21), and raw enamel surface profile (data not cleaned), with arrows showing approximate location of individual tooth defects (not yet matched across multiple teeth of the same individual) Note: Top left shows 3D surface of tooth captured using OLS microscope. Top right shows coloured surface height topography (blue being the lowest, orange being the highest point). Bottom shows raw enamel surface profile prior to data cleaning of tooth edge, with approximate location of defects identified on this tooth (also see Fig. 4 where the defects are identified).

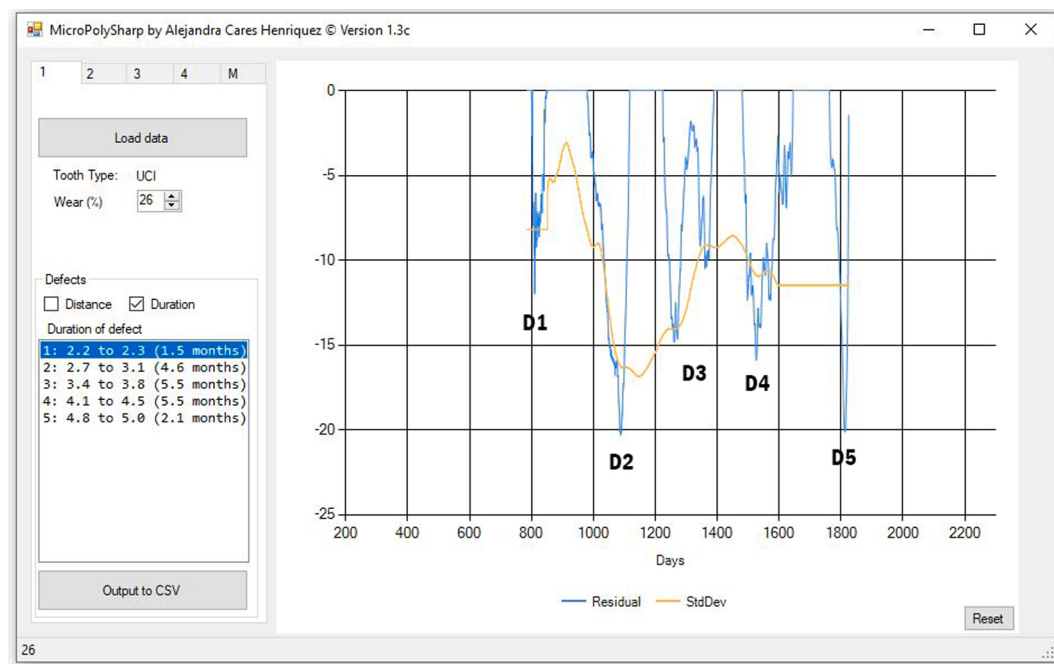


Fig. 4. MicroPolySharp Analysis of Burial 120 – Showing Tooth 1 Upper Central Incisor 21 (D1 = defect 1, etc).

identify more instances of systemic stress (Cares Henriquez & Oxenham, 2017; Guatelli-Steinberg et al., 2004; Temple et al., 2013).

3.2.2. Chronology of LEH episodes

The chronology of episodes ranged from 1.4 to 5.5 years of age, with

a median age of 3.73 years, and an average age of 3.66 years. The age of earliest episode formation ranged from 1.4 to 4.2 years of age, with an average and median age of 3.1 years. Meanwhile, the age of last episode ranged from 2.9 to 5.5 years of age, with an average and median age of 4.3 years.

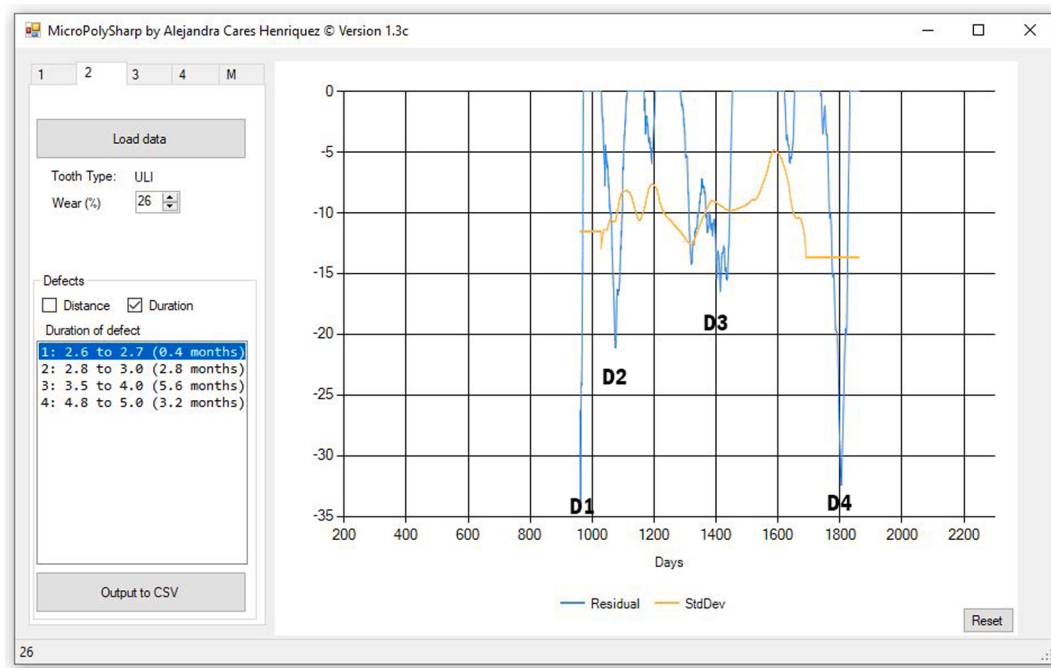


Fig. 5. MicroPolySharp Analysis of Burial 120 - Showing Tooth 2 Upper Lateral Incisor 22.

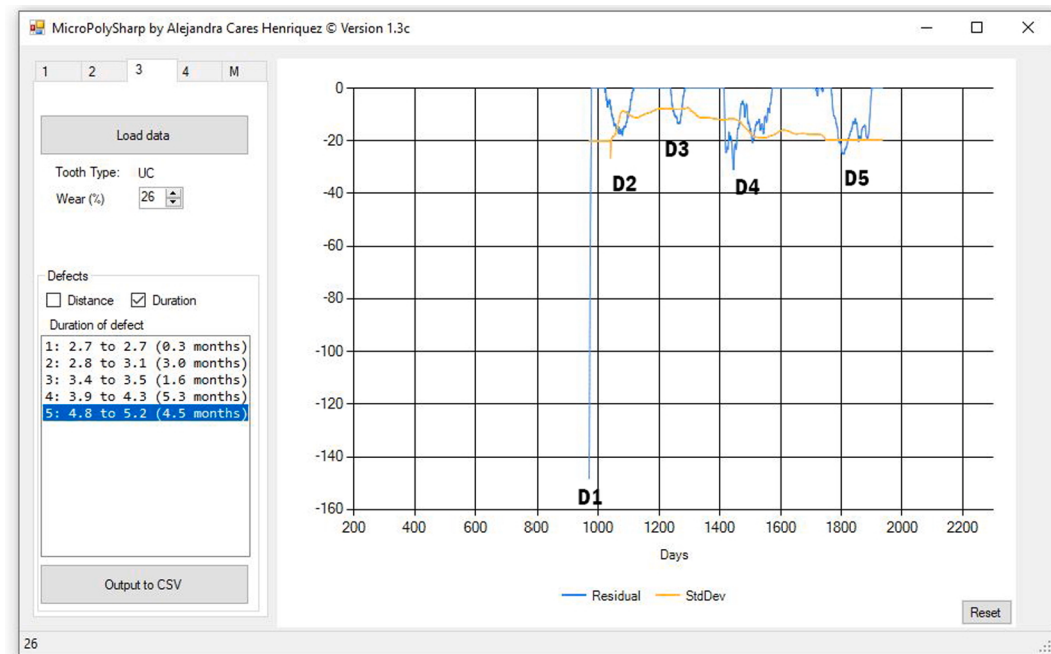


Fig. 6. MicroPolySharp Analysis of Burial 120 - Showing Tooth 3 -Upper Canine 23.

3.2.3. Episode duration (stress + recovery), stress duration, and recovery duration

The duration of individual matched episodes of LEH (stress + recovery) averaged 103 days (3.4 months) and ranged from 27 to 269 days (1–9 months) (see Fig. 9). The average episode duration is comparable to that reported for Inuit foragers of 107 days (range = 56–176 days). On the other hand, the average episode duration was almost twice as long as that reported for Neanderthal foragers of 58 days (range = 8–200 days) (Guatelli-Steinberg et al., 2004).

At Non Ban Jak, stress duration, as represented by the period in the occlusal wall of each hypoplastic defect, averaged 56 days (range =

9–191 days) (see Fig. 9). This average is similar to the 62 days (range = 24–128 days) observed for Inuit foragers, and 53 days (range = 16–96 days) reported for Neanderthal foragers, both of which were populations that lived in extreme arctic and pleniglacial environments. Furthermore, the Non Ban Jak average is almost twice as long as that reported for the inland hunter-gatherer-fisher-millet cultivators from Houtaomuga (23 days), as well as coastal Honshu Jomon (27 days), and Hokkaido Jomon (24 days) populations (Merrett et al., 2016).

The recovery time of individual LEH episodes at Non Ban Jak averaged 47 days (range = 8–151), which was slightly lower than that reported for Inuit foragers (67 days; $N = 5$; range = 32–96 days) and

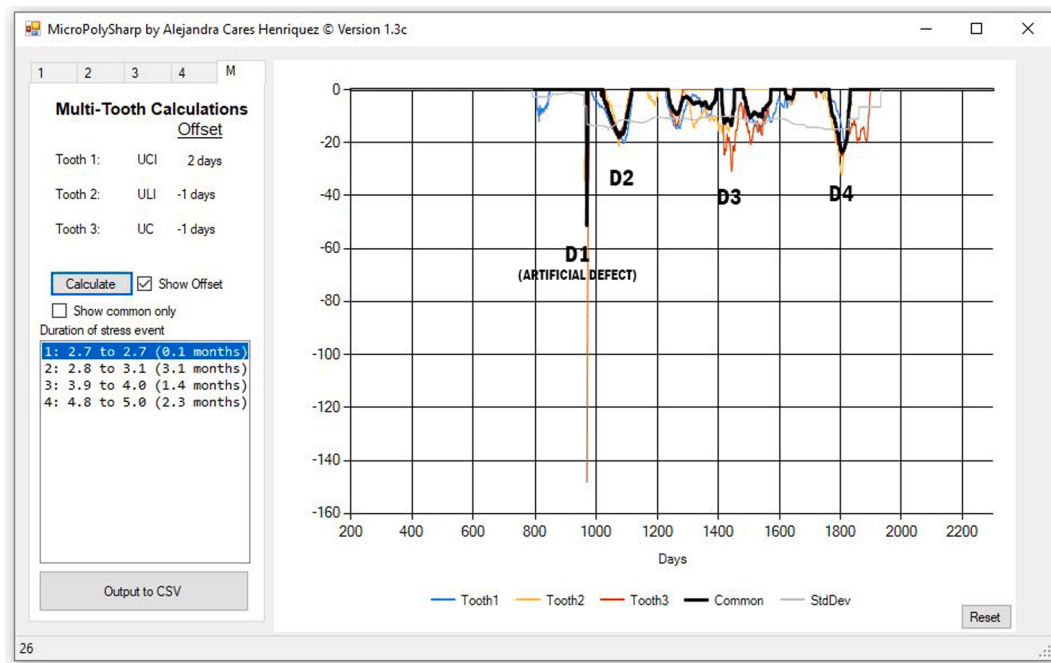


Fig. 7. MicroPolySharp Analysis of Burial 120 - Showing Common Cycle of Defects (matched defects = LEH episodes) Notes: See (Cares Henriquez & Oxenham, 2020) for discussion on artificial defects.

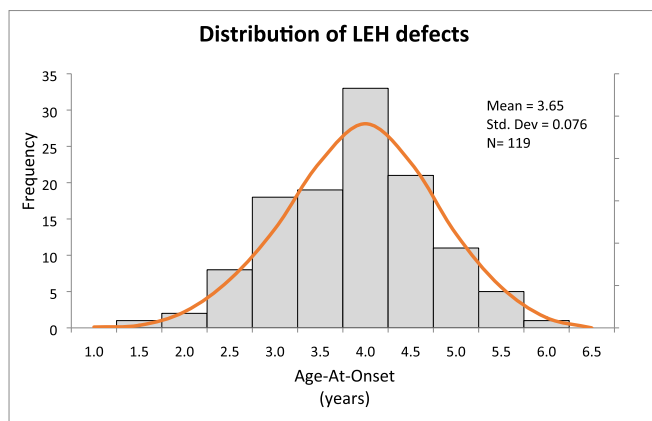


Fig. 8. The Chronological Distribution of all Matched LEH in this Study.

Neanderthal foragers (50 days; $N = 4$; range = 16–104 days) (see Fig. 9). However, it should be noted that the raw data reported for the latter two groups only includes a fraction of all individuals in their respective studies.

3.2.4. Total growth disruption, proportion of available crown height affected, and periodicity

The total amount of growth disruption, calculated as the sum of the duration of all identified stress episodes for a single individual, averaged 263 days (8.7 months) and ranged from 87 to 543 days (3–18 months).

The proportion of available crown height affected by growth disruption averaged 21 % and ranged from 6 % to 42 %, while LEH periodicity for the sample averaged of 0.7 years (8.6 months) and ranged between 0.2 and 1.9 years (2.4–23 months).

3.3. Crown height wear

The distribution of crown height wear was assessed based on age at death, sex, mortuary phase, and occupation mound, and can be

Table 4

Microscopic Studies of LEH - Archaeological Context, Time Period, and LEH Prevalence of HTMG^a, Jomon^b, Tigara^b, Inuit^c, and Neanderthal^c in comparison with Non Ban Jak.

Site / Group	Date	Subsistence	N (Individuals)	LEH Prevalence (%)
Non Ban Jak (S.E. Asia)	50–800 CE	Wet-rice agriculture (increased reliance)	48	98 %
Houtaomuga ^a (N.E. China)	2250–2050 BP	Inland hunter-gatherer, fish, millet agriculture	55	96.8 %
Hokkaido ^b (Jomon)	4000–2300 BP late/final	Foragers/ marine mammals and fish	5	100 %
Coastal Honshu ^b (Jomon)	4000–2300 BP	Foragers / fewer marine mammals and fish than Hokkaido	20	100 %
Tigara ^b (Point Hope)	1300–1700 CE	Arctic whaling culture associated with the Point Hope site complex	10	80 %
Inuit – Alaska ^c (Point Hope)	700–300 BP	Foragers	9	100 %
Neanderthal ^c (Krapina)	130,000 BP ± 10000	Foragers	11	100 %

^a Merrett et al. (2016).

^b Temple et al. (2013).

^c Guatelli-Steinberg et al. (2004).

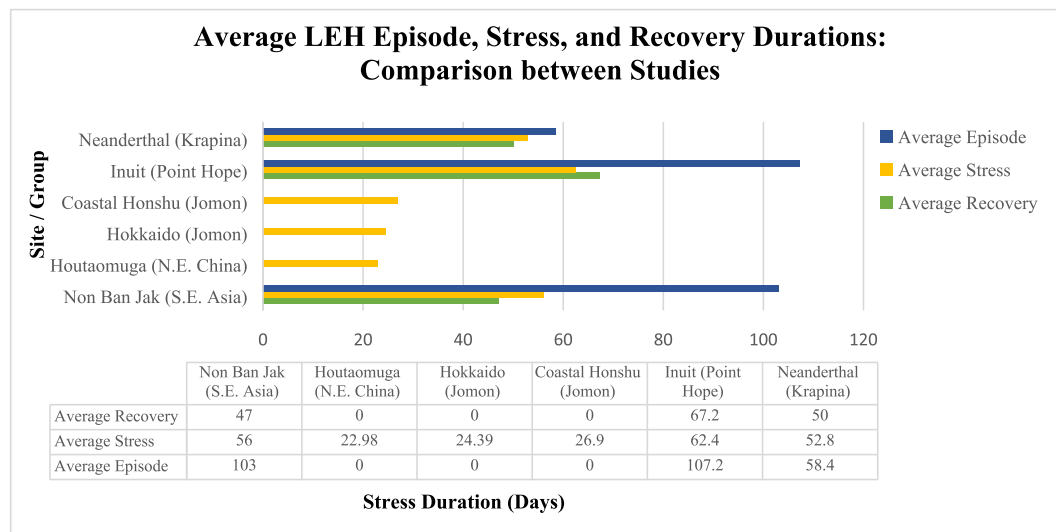


Fig. 9. Average LEH Episode, Stress, and Recovery Durations for Houtaomuga^a, Jomon^b, Inuit^c, and Neanderthal^c in comparison with Non Ban Jak ^aMerrett et al. (2016) ^bTemple et al. (2013) ^cGuatelli-Steinberg et al. (2004)* *Note Neanderthal average episode duration (dark blue) includes seven defects that had three or less perikymata within the entire defect and consequently no distinction is made between the number of perikymata in the occlusal and cervical walls. This results in a much lower than expected episode average, in relation to the average stress and recovery durations.

visualised in the SI (B). The results of a one-way ANOVA showed a statistically significant difference in the average percentage of crown height wear between the age at death groups ($F = 8.249$; $DF = 3$; $P = 0.0002$). No differences were observed by sex ($F = 0.513$; $DF = 2$; $P = 0.602$), mortuary phases ($F = 1.355$; $DF = 3$; $P = 0.269$) or between the mounds ($H = 1.991$; $P = 0.158$). The results of all ANOVA analyses are presented in the SI (Table A2).

A multiple regression was undertaken to investigate any potential correlations between crown height wear and age at death, sex, mortuary phase, and occupation mound. A summary of these and all subsequent multiple regression analyses outcomes are provided in the SI (Table A3). The overall regression model was statistically significant and found to explain 40 % of the variance ($F(4,41) = 6.685$, $P = 0.000$, $R^2 = 0.395$). A significant positive association between crown height wear and age at death was observed ($B = 7.162$, $P = 0.000$). No significant relationships were observed between crown height wear and sex ($B = 1.444$, $P = 0.569$), mortuary phase ($B = -2.402$, $P = 0.158$), or between occupation mound ($B = 5.041$, $P = 0.235$).

3.4. Frequency (number of LEH episodes) by age at death, sex, mortuary phase, occupation mound, and wear

A comparison of the distribution of total number of episodes by age, sex, mortuary phase, and mound are presented in the SI (B). An independent between-groups ANOVA yielded no statistically significant difference in the average number of episodes between the age at death groups ($F = 0.680$; $DF = 3$; $P = 0.569$), sex ($F = 0.687$; $DF = 2$; $P = 0.508$), mortuary phases ($F = 1.393$; $DF = 3$; $P = 0.257$), or between the mounds ($H = 0.088$; $P = 0.717$). The results of all ANOVA analyses are presented in the SI.

A multiple regression was undertaken to investigate whether there was an association between age at death, sex, mortuary phase, occupation mound, or crown height wear and the number of episodes an individual had (see SI). The overall regression model was not statistically significant ($F(5,41) = 1.165$, $P = 0.343$, $R^2 = 0.124$). No significant relationships were observed between number of episodes and any of the variables of interest. The full results of this regression can be found in SI.

3.5. Age at first episode, age at death, sex, mortuary phase, occupation mound, and wear

A comparison of the distribution of age at first episode by age at death, sex, mortuary phase, and mound is presented in the SI (B). The results of a one-way ANOVA indicated a significant statistical difference in the average age at first episode between the age at death groups ($F = 4.03$; $DF = 3$; $P = 0.013$). No significant difference was identified in average age at first episode by sex ($F = 0.44$; $DF = 2$; $P = 0.647$), mortuary phase ($F = 2.719$; $DF = 3$; $P = 0.056$), or by mound ($H = 2.70$, $P = 0.101$).

A multiple regression between age at first episode and age at death, sex, mortuary phase, occupation mound, and crown height wear indicated that the model explained 64 % of the variance ($F(5,40) = 13.922$, $P = 0.000$, $R^2 = 0.635$) (SI). A significant positive association between age at first episode and crown height wear was observed ($B = 0.032$, $P = 0.000$). Additionally, a significant negative association between age at first episode formation and mortuary phase ($B = -0.146$, $P = 0.036$) was also observed. There was no significant relationship between age at first episode and age at death ($B = 0.032$, $P = 0.677$, sex ($B = 0.049$, $P = 0.621$), or occupation mound ($B = 0.240$, $P = 0.159$).

3.6. Total growth disruption by age at death, and sex, mortuary phase, occupation mound, and wear

A comparison of the distribution of total disruption time by age at death and sex is presented in the SI. The results of a one-way ANOVA (see SI) indicated that there was no significant difference in the average total growth disruption by age at death ($F = 2.316$; $DF = 3$; $P = 0.089$), by sex ($F = 0.228$; $DF = 2$; $P = 0.797$), mortuary phase ($F = 0.168$; $DF = 3$; $P = 0.917$), or occupation mound ($H = 1.134$; $P = 0.287$).

A multiple regression was also carried out to investigate further the relationship between total disruption and age at death, sex, mortuary phase, occupation mound, and crown height wear (see SI). The overall regression model was significant ($F(5,40) = 3.463$, $P = 0.011$, $R^2 = 0.302$), and taken as a set, predicts 30 % of the variance in total disruption time. A significant negative association between total disruption time and crown height wear was observed ($B = -3.924$, $P = 0.009$). Again, no association was found between total disruption time and age at death ($B = -10.797$, $P = 0.544$), sex ($B = -17.312$, $P = 0.457$), mortuary phase ($B = -12.676$, $P = 0.422$), or occupation mound ($B =$

-27.941, $P = 0.476$).

3.7. Proportion of available crown height affected by growth disruption by age at death, sex, mortuary phase, and occupation mound

A comparison of the proportion of available crown height affected by age at death, sex, mortuary phase, and occupation mound is presented in the SI (B). The results of a one-way ANOVA indicated no significant statistical difference in the mean proportion of affected enamel by age at death ($F = 1.070$; $DF = 3$; $P = 0.372$), sex ($F = 1.183$; $DF = 2$; $P = 0.316$), mortuary phase ($F = 1.060$; $DF = 3$; $P = 0.376$), or occupation mound ($H = 0.563$; $P = 0.453$).

A multiple regression was undertaken to investigate whether there was an association between the proportion of available enamel and age at death, sex, mortuary phase, or occupation mound. The overall regression model was not statistically significant ($F(4,41) = 0.554$, $P = 0.698$, $R^2 = 0.051$). No significant relationships were observed between proportion of affected enamel and any of the variables of interest. The full results of this regression can be found in the SI.

3.8. Episode duration by age at death, sex, mortuary phase, occupation mound, and wear

The distribution of the average duration of episodes by age at death, sex, mortuary phase, and mound can be visualised in the SI (B). The results of an independent between-groups ANOVA yielded a statistically significant result for difference in average episode duration by age at death ($F = 4.532$; $DF = 3$; $P = 0.008$) (see SI). However, no statistically significant difference in the average episode duration was observed by sex ($F = 0.332$; $DF = 2$; $P = 0.719$), mortuary phase ($F = 0.335$; $DF = 3$; $P = 0.800$), or mound ($H = 0.721$; $P = 0.396$).

A multiple regression between average episode duration and age at death, sex, mortuary phase, occupation mound, and crown height wear was undertaken to investigate any possible associations. The overall regression model was significant ($F(5,41) = 8.206$, $P = 0.000$, $R^2 = 0.500$), with the overall model predicting 50 % of the observed variance in average episode duration (see SI). A significant negative relationship was observed between average episode duration and mortuary phase ($B = -10.615$, $P = 0.021$), as well as with crown height wear ($B = -1.747$, $P = 0.000$). No significant associations were observed between average episode duration and age at death ($B = -4.502$, $P = 0.353$), sex ($B = -4.552$, $P = 0.487$), or mound ($B = -2.918$, $P = 0.791$).

3.9. Stress and recovery duration by age at death, sex, mortuary phase, occupation mound, and wear

The results of an independent between-groups ANOVA yielded a statistically significant difference in the average duration of stress between the age groups ($F = 3.651$; $DF = 3$; $P = 0.020$). However, no statistically significant difference in the average episode duration was observed by sex ($F = 0.271$; $DF = 2$; $P = 0.764$), mortuary phase ($F = 0.122$; $DF = 3$; $P = 0.947$), or mound ($H = 1.068$; $P = 0.302$) (see SI). The distribution of average stress duration by age at death, sex, mortuary phase, and occupation mound is presented in the SI (B).

A multiple regression was undertaken between average stress duration and age at death, sex, mortuary phase, mound, and crown height wear. The overall regression model was significant ($F(5,41) = 4.187$, $P = 0.004$, $R^2 = 0.338$), with the overall model predicting 34 % of the observed variance in average stress duration (see SI). A significant negative relationship was observed between average stress duration and crown height wear ($B = -0.931$, $P = 0.006$). No significant associations were observed between average stress duration and age at death ($B = -3.620$, $P = 0.351$), sex ($B = 2.033$, $P = 0.697$), mortuary phase ($B = -1.702$, $P = 0.631$), or mound ($B = -1.182$, $P = 0.893$).

The results of an independent between-groups ANOVA yielded no statistically significant difference in the average recovery duration

between the age at death groups ($F = 1.844$; $DF = 3$; $P = 0.154$), by sex ($F = 2.703$; $DF = 2$; $P = 0.078$), mortuary phase ($F = 2.435$; $DF = 3$; $P = 0.078$), or occupation mound ($H = 0.982$; $P = 0.322$) (see SI). The distribution of average recovery duration by age at death, sex, mortuary phase, and occupation mound is presented in the SI (B).

A multiple regression between average recovery duration and age at death, sex, mortuary phase, mound, and crown height wear was undertaken to investigate any possible associations (see SI). The overall regression model was significant ($F(5,41) = 6.861$, $P = 0.000$, $R^2 = 0.456$), with the overall model predicting 46 % of the observed variance in average episode duration. A significant negative relationship was observed between average recovery duration and mortuary phase ($B = -8.920$, $P = 0.001$), as well as with crown height wear ($B = -0.818$, $P = 0.001$). No significant associations were observed between average episode duration and age at death ($B = -0.836$, $P = 0.767$), sex ($B = -6.599$, $P = 0.090$), or mound ($B = -1.717$, $P = 0.790$).

3.10. Periodicity by age at death, sex, mortuary phase, and occupation mound

A comparison of the distribution of the average periodicity of individual by age at death, sex, mortuary phase, and mound is presented in the SI (B). The results of an independent between-groups ANOVA yielded no statistically significant difference in the average periodicity between the age groups ($F = 1.089$; $DF = 3$; $P = 0.364$), by sex ($F = 1.639$; $DF = 2$; $P = 0.206$), or between the mortuary phases ($F = 0.939$; $DF = 4$; $P = 0.451$) (see SI). A statistically significant result for difference in average periodicity was observed between the mounds ($H = 6.205$; $P = 0.013$).

A multiple regression between average periodicity and age at death, sex, mortuary phase, mound, and crown height wear was undertaken to investigate whether any of these variables were significant predictors (see SI). The overall regression model was not significant ($F(5,40) = 1.793$, $P = 0.136$, $R^2 = 0.183$). No significant relationships were observed between average periodicity and any of the variables of interest.

4. Discussion

As noted in the Introduction, this is the first large scale microscopic approach to the identification and analysis of LEH in Southeast Asia to date. As these are the first such data generated for the region, excluding basic frequency and prevalence results, they are not comparable to traditional macroscopic studies of LEH (K. M. Domett & O'Reilly, 2009; Douglas, 1996; Oxenham, 2006; Ward et al., 2020). Until more microscopically generated data are available both regionally and globally, for that matter, inter-site and multi-period comparisons are effectively on hold. Notwithstanding, our results can be interpreted through the lens of the archaeological understanding of late Iron Age Thailand.

4.1. Crown width/height ratios

An investigation of crown/width height ratios based on 63 teeth deemed to have minimal wear indicated that the width/length ratio for the maxillary teeth differed slightly from previously published results of the maxillary dentition of Asian (Japanese) and Central European individuals. Compared to previously published ratios, the proportion of maxillary central incisors at Non Ban Jak was the same as that of the Asian (Japanese) sample, while the upper lateral incisors were more slender. The upper canine, on the other hand, was slightly wider on average than both the Japanese and Central European samples, with the Non Ban Jak ratio more closely resembling the European sample. Since ratios of anatomical crown width/height of mandibular teeth have not been previously published, these could not be compared.

4.2. Crown height wear and its effect on the interpretation of LEH results

Due to the inclusion of individuals with crown height wear that ranged from minimal to 60 %, it was imperative to determine the potential effects this threshold might have on the interpretation of LEH results for this study. This was achieved by considering the distribution of crown height wear between the different age at death groups, as well as by sex, mortuary phase, and occupation mounds. A significant difference was observed in the average percentage of crown height wear between the different age at death groups, with a significant positive relationship detected between these two variables. These results support the often observed trend that older individuals, especially in archaeological samples, are more likely to suffer from occlusal or crown height wear (King et al., 2005).

Cursorial secondary tests of the statistical analysis in this study were conducted using two sets of stricter thresholds of crown height wear for the inclusion of individuals to determine if this impact was due primarily to having such a wide range of wear (0–60 %) or whether any amount of wear had the potential to affect the results in the same manner. The first was limited to those individuals with 30 % or less wear, and the second to those with 20 % or less wear. In both instances, the results remained the same, with crown height wear being the primary operating force for observed differences when considering the different variables of interest and the different subgroups.

These secondary tests suggest that even if the amount of accepted crown height wear is reduced, say from 50 % to 30 % or even 20 % (which to put into perspective is the equivalent of 3 mm or 2 mm of enamel wear on a 10 mm tooth), if left unaccounted for, can still mistakenly appear as differences in how subgroups are experiencing LEH. Consequently, simply reducing the threshold of accepted crown height wear is not sufficient to deal with the effects it may have on the results, because it is the inclusion of crown height wear variation, however minor the range, that has the potential to distort the findings. Therefore, it is imperative that studies control for its effect, whether this be achieved by estimating the wear for each individual included in the analysis, as is done in this study, or alternatively, limiting the analysis only to include individuals with the same amount of wear.

4.3. Distribution of LEH episode chronology

The LEH episode chronology for the sample (also sometimes referred to as age at onset or age at defect formation), was found to be normally distributed. This normal distribution is taken as evidence that earlier stress episodes on the incisal third of teeth were not simply missed for individuals with a later age at first episode when that portion of the tooth is available for examination. This is important for two reasons. First, the particular geometry of striae of Retzius (internal incremental layers of enamel) make the identification of LEH defects in the incisal third of teeth more difficult, as these result in LEH defects that are shallower and more poorly defined than on the mid and cervical thirds (Guatelli-Steinberg, Ferrell, & Spence, 2012; Hillson & Bond, 1997; King et al., 2005). Second, the incisal or occlusal third of teeth is most likely to suffer from both crown height and labial surface wear, and generally this is more likely to occur in older individuals (King et al., 2005). While some studies address this latter issue by limiting their samples to include only individuals with minimal attrition (usually 10 % wear), here the threshold is set to include teeth that preserve 60 % or more of crown height. This threshold was chosen to maximise the sample size to obtain a more comprehensive understanding of how systemic stress was experienced by individuals across all the age categories represented in the sample.

LEH episodes extended from 1.4 to 5.5 years of age. Thus, they covered almost the entirety of time that is captured during the development of the anterior dentition and is thus available for observation. The median age of stress events was found to be 3.73 years of age, with an average age of 3.66 years. Quite often, a concentration of LEH found

at this age is associated with the process of weaning, which is understood to begin anywhere from the age of about 6 months with the introduction of supplementary foods and extends until breast-feeding is terminated (Sellen, 2007). However, an increased presentation of stress events at this age may in fact simply be a consequence of tooth crown geometry that results in LEH being more prominent and easier to identify in the intermediate or mid-section of the crown rather than an indication of higher stress burdens (Guatelli-Steinberg et al., 2012; Hillson & Bond, 1997; Temple, 2016). Furthermore, due to the considerable variation in both global and cultural ages embraced for the weaning process, the age at which the process occurs is best identified isotopically from specific skeletal samples rather than based simply on an increased manifestation of these events (Humphrey, 2008).

4.4. Prevalence of LEH at Non Ban Jak

Of the 48 individuals included in this study, 47 exhibited at least one defect that could be matched chronologically across multiple teeth of the same individual and was considered as evidence of systemic stress. While the defects identified on B108 (a mid-aged male) could not be matched chronologically, these could still be the result of systemic stress. Nevertheless, these were discounted from the overall results. As such, the findings from this study suggests not only that the prevalence of LEH at Non Ban Jak was extremely high (97.92 %), but that the proportion of individuals with LEH was higher than the 32/38 (84.2 %) previously reported in a macroscopic analysis of LEH at Non Ban Jak (Ward et al., 2020). When comparing macroscopic and microscopic studies of LEH, it is not unusual for the latter both to identify LEH on most individuals that are assessed as well as to identify more instances of LEH in general (Cares Henriquez & Oxenham, 2017; Hassett, 2014; King et al., 2005; Temple, 2016). Since almost all individuals were affected by LEH, there are no differences in prevalence between any of the four subgroups of interest (i.e., age at death, sex, mortuary phase, and occupation mound).

4.5. Frequency (number of LEH episodes)

The number of episodes per individual was used as a parameter to investigate whether differences were present between various subgroups in this sample. The results suggest the average number of LEH stress events experienced by individuals at Non Ban Jak was similar regardless of age at death, sex, mortuary phase, or occupation mound. Unsurprisingly, no correlation was found between these four factors and the number of stress episodes experienced by an individual.

Some studies have considered the link between number of episodes and age of first episode to investigate the potential of LEH as a predictor of early mortality (Berbesque & Hoover, 2018; Merrett et al., 2016; Temple, 2014). Berbesque and Hoover (2018) observed that individuals with more LEH had an earlier age of first episode, juveniles had the highest incidence of LEH, and that a higher incidence of LEH was associated with earlier age of death. Similarly, Temple (2014) also found that individuals with an earlier age at first episodes had greater amounts of LEH compared with those with a later age at first episode, and that younger individuals had an earlier age at first episode. These correlations were then used as a means for contemplating the results with respect to a predictive response model vs a plasticity/constraint model of human life history, which was possible given that the crown height wear threshold for all individuals was set to less than 90 % (Temple, 2014).

Here, this association is not investigated due to the intricate relationship that exists between the number of LEH and earliest age of first episode, in particular once crown height wear is introduced. Due to the limited window of time that is captured by teeth, these two variables will always be dependent on each other. That is, there is a set amount of space in which LEH defects can occur, and that, together with the gradual change in perikymata spacing, acts as a constraint on the

possible maximum number of LEH that a tooth can capture. This relationship becomes even more complex once variable crown height wear is introduced (discussed in section 4.2), because less wear on a tooth will result in the possibility and higher probability of earlier first episodes remaining on the tooth enamel as a record of stress that was experienced.

Simply put, age at death becomes a predictor of crown height wear, and crown height wear is a predictor of age at earliest episode. Consequently, if wear is not properly accounted for, this then can mistakenly appear as though age at earliest episode is a predictor for age at death.

4.6. Age at first stress episode

A noticeable difference in average age at first episode between the age groups was observed, with older individuals experiencing their first episode at a much later age than those who died at a younger age. However, unlike the findings of other microscopic studies that were not confounded by variable crown height wear within its sample, this was not accompanied by evidence of a significant relationship between age at first episode and age at death to indicate that earlier exposure was correlated with a higher risk of premature mortality (Temple, 2014). A closer investigation of the results suggests that in this study this observed pattern is best explained by the relationship between crown height wear and age at death (see section 4.2 and 4.5), which results in older individuals generally having less enamel available for examination and as such, presenting with their first episode at a slightly later age.

Interestingly, a significant negative association was observed between age at first episode and mortuary phase, suggesting that individuals in the later mortuary phases were experiencing their first episode at a relatively younger age than those from earlier phases. Further analysis confirmed that, unlike other relationships observed in this study, this association was not driven by the effects of crown height wear, noting that no statistically significant difference in the average percentage of crown height wear was observed between the mortuary phases. Given the small subgroup sample sizes, this finding should be interpreted with caution. Nevertheless, this observed relationship is certainly worthy of further investigation when additional samples become available. No other differences or associations were observed between age at first episode and sex, or occupation mound.

4.7. Total growth disruption and proportion of available crown height affected by growth disruptions

The average amount of total growth disruption experienced by older individuals was considerably less than for those in the younger age categories. However, again, when examined more closely, this was the product of significant negative association between total disruption time and crown height wear (see Section 4.2 and 4.5).

Unsurprisingly, given the extremely high levels of LEH prevalence at Non Ban Jak, once growth disruption was controlled for and considered as a proportion of available crown height, there was no difference in the proportion of crown affected by growth disruptions for any of the subgroups of interest.

4.8. Duration of LEH Episodes, Stress, and recovery

The duration of entire LEH episodes (stress + recovery) averaged 3.4 months (103 days) and ranged from approximately just under one month to nine months. A significant difference in the average duration of episodes was observed between individuals by age at death, with younger individuals appearing to experience, on average, lengthier LEH episodes. However, further investigation suggests that this difference is best explained by the effects of crown height wear (see section 4.2 and 4.5).

While the LEH episode average was most similar to that previously observed for Inuit foragers from Point Hope (107 days), the range

captured by both the Krapina Neanderthal (8–200 days) and Inuit foragers (56–176 days) fell within, or close to, that recorded for Non Ban Jak (27–269 days) (Guatelli-Steinberg et al., 2004). However, this episode average is inclusive of both the stress and recovery portion of LEH. Given that the few existing microscopic studies of other regions rely on the presence of perikymata, it is much more likely to see results of LEH duration discussed in terms of just the stress portion of these episodes, that is the period of time captured in the occlusal wall of each defect.

When considering the individual components of LEH episodes, the average duration of stress at Non Ban Jak was 1.9 months (56 days) and ranged from 9 days to 6.4 months. A significant difference in the average stress duration of LEH was observed between individuals by age at death, with younger individuals appearing to experience lengthier LEH stress durations. Again, further investigation confirmed that this was best explained by the effects of crown height wear (see section 4.2 and 4.5).

The average recovery duration of LEH at Non Ban Jak was 1.6 months (47 days) and ranged between 8 days to 5 months, and no differences were observed between the different subgroups of interest. This average was slightly shorter than that reported for Inuit (67 days) and Neanderthal (50 days) foragers.

The results of this study show that individuals at Non Ban Jak were coping with stress events that, on average, lasted almost two months. While this was on par with the duration of stress events observed for Inuit (62 days) and Neanderthals (53 days), both forager groups that lived in extreme cold environments, it is almost twice as long as that experienced by the two forager Jomon and Tigara groups, whose diet was supplemented with marine mammals, fish, and C3 plants (Guatelli-Steinberg et al., 2004; Temple, 2014). Similarly, it was also twice as long as that reported for the Houtaomuga inland hunter-gatherer population, which aside from supplementing their diet with fish, relied on millet agriculture (Merrett et al., 2016).

Archaeological evidence at Non Ban Jak suggests that while rice was a significant component of the diet, this was also supplemented by domestic pig, cattle, and water buffalo, as well as shellfish and fish. Notwithstanding, there appears to be an increased reliance on wet-rice agriculture as part of an adaptive response to climate change (reduced rainfall) in the late Iron Age period at Non Ban Jak (Higham et al., 2020), which may have led to a narrowing of dietary breadth. Communities attempted to counter the increasing aridity through the construction of moats/reservoirs to conserve and distribute water. Subsequently, this also led to a shift from dry rice farming, as evidenced by weed seeds in nearby Ban Non Wat, to the establishment of fixed, irrigated wet-rice fields (Castillo et al., 2018; Higham et al., 2020).

It is worth situating this discussion in the context of the demographic picture at Non Ban Jak. Buckley et al. (2020) note that of a total sample of 195 individuals, 125 were aged less than 15 years old. Using the McFadden and Oxenham (2018) fertility estimation model this equates to a very high total fertility rate of 7.18 children per woman on average. Further, the estimated rate of natural population increase (RNPI) for Non Ban Jak is an exceptionally high 4.84 %, albeit not unexpected given previous modelling of RNPI in ancient Southeast Asia (see (McFadden & Oxenham, 2020)). It would seem reasonable to suggest that substantive changes in subsistence and lifeways in tandem with significantly high rates of fertility and rate of natural population increase may be correlated, if not causally related, with the much longer lasting periods of stress observed at Non Ban Jak, when compared to the two forager Jomon and Houtaomuga groups.

A negative relationship was observed between LEH episode duration and mortuary phase, signalling that, on average, the length of LEH episodes gradually became shorter as time progressed during the 800-year period covered between mortuary phase one and mortuary phase four. Interestingly, when considering the separate components of LEH episodes (stress + recovery), this same pattern was observed for recovery duration, but not for stress duration. This suggests that while the overall

length of LEH episodes and periods of stress remained fairly stable over the Late Iron Age period at Non Ban Jak, as time went by, individuals were experiencing their first stress episode at a younger age, albeit they were starting to recover more quickly from these physiological insults.

A relatively recent study into long bone lengths at Non Ban Jak found that the mean length of long bones for males increased over time (average = 169.6 cm; range: 154.9–180.5 cm), while this measure decreased over time for females, except for the tibia and fibula (average = 157.9 cm; range = 150.7–166.1 cm) (Ward et al., 2020). However, these differences between the sexes were not found to be statistically significant. In fact, preliminary comparison of estimated stature between those reported at Non Ban Jak and other prehistoric samples from the region, found that females at Non Ban Jak were, on average, taller than those from other sites (third tallest of the prehistoric samples from Cambodia and Thailand) and males were found to be the tallest (Buckley et al., 2020). When considered together, these findings suggest that perhaps despite living in an environment that presented with additional exposure to stress, which consequently meant that all members of the community were equally affected, the community was adapting and becoming more resilient over time.

Currently, there is no consensus as to how the many complex changes that were taking place in the Upper Mun River valley during the Iron Age, including changes in diet, migratory patterns, environmental, and socioeconomic changes affected the health status of these populations. A number of studies have observed an increase in the prevalence of infectious diseases, infant mortality, along with evidence of increased levels of non-specific systemic stress, albeit from limited bioarchaeological data (Halcrow, Tayles, & King, 2016; King, Halcrow, Tayles, & Shkrum, 2017). On the other hand, other studies have reported an improvement in health over time, with some complexities, including evidence of a particular deterioration in the final stages of this period (Cekalovic, 2014; Domett & Tayles, 2006; Newton, 2014).

The results of this study, which is focused solely on Non Ban Jak, provides further evidence of the intricate and complex nature of assessing the health outcomes of these past communities, and highlights the trouble with attempting to apply a 'one size fits all' model of health. While the relationships observed at Non Ban Jak certainly allow room for speculation, it should be noted that the subgroup sizes are in fact relatively small. As such, much more data is needed to ascertain whether the ostensible trend of gradual increased levels of stress coupled with what might be interpreted as improved resilience will stand the test of time, both for other samples from these final stages as well as those periods leading to the Iron Age and late Iron Age.

4.9. Periodicity

A modest difference in the average periodicity between the East and West occupation mounds was observed, with individuals in the West mound having, on average, shorter intervals of time between episodes of LEH than those from the East. However, further analysis showed that despite a difference in mean periodicity, occupation mound was not found to be a predictor for periodicity. Given that no other LEH aspects investigated (i.e., number of LEH, age at first episode, growth disruption, or duration) have indicated any differences between the mounds, the observed difference in mean periodicity is likely to be the result of sampling than of any genuine differences due to the substantially different sample size between the East ($n = 8$) and West ($n = 39$) mounds.

5. Limitations

The recently published methods employed in this study have certainly gone some way in helping to address an ever-widening gap between subjective qualitative and objective quantitative approaches for assessing LEH in archaeological samples. Such methods have added to, and improved, the bioarchaeological toolkit available to researchers

thus making it possible to assess the experience of systemic stress in archaeological samples using microscopic methods that do not rely on the presence of visible perikymata. Nevertheless, a key limitation of the approach advocated here is that there are numerous layers of estimation that take place in each of the steps for identifying and then analysing LEH (chronology, duration, and periodicity), precisely due to the method's non reliance on perikymata counts.

Perikymata count approaches also have their own limitations, namely that unless the samples are examined histologically, they too must approximate values for chronology, duration, and periodicity, as they rely on known average perikymata periodicities and will often report their findings based on 8 days, 9 days, and 10-day averages. Perikymata periodicity, or daily secretion rate (i.e., that is the exact number of days of growth enamel that these represent) not only varies between different populations, but also from person to person. This daily secretion rate is only constant for all of the teeth in any given individual (FitzGerald, 1998). In humans, this has been documented to range from 6 to 12 days (Smith et al., 2007), with an average of 8–9 days (Dean & Reid, 2001). As such, rather than placing too much emphasis on minor differences in observed values, the greatest benefit to be gained from results obtained using the methods employed in this study will be for identifying trend patterns and observing general changes over time, and differences between subgroups of interest, and avoiding the trap of false or misplaced precision in the numerical values reported.

6. Conclusion

As has been noted by other researchers, the variables used for ascertaining differences between groups and populations can vary in their degree of usefulness (King et al., 2005). Furthermore, there are also several caveats as to how results are interpreted. Crown height wear, for example, which affects the amount of available enamel surface that is available for examination, was found to have a significant impact on the way results are interpreted.

The findings of this study suggest that simply setting a minimum crown height wear threshold (i.e., less than a certain %) is not sufficient to control for the potential effects this can have on other variables of interest. This is because even a small amount of variation between individuals can create the illusion of differences between subgroups that do not exist. It is suggested that there are three options to avoid this issue: 1) estimate the individual wear for each tooth, as is done in this study; 2) only include individuals with no wear, which can make it difficult to include a full range of ages for the group being studied; and 3) only include individuals that have the same amount of wear.

Additionally, prevalence, that is the percentage of individuals affected by LEH, is often used in macroscopic studies to assess differences between groups (Bennike, Lewis, Schutkowski, & Valentin, 2005; Buzon, 2006; K. M. Domett & O'Reilly, 2009; Novak et al., 2018; Obertová, 2005; Oxenham, 2006; Pechenkina, Benfer, & Zhijun, 2002; Pietruszewsky & Douglas, 2002; Shuler et al., 2012; Stodder, 1997; Temple, 2007, 2010; Ward et al., 2020). However, as microscopic studies are increasingly demonstrating, these differences in prevalence are much more likely to be associated with the resolution of the observations than actual differences within populations that exhibit LEH (Cares Henriquez & Oxenham, 2017; Hassett, 2012, 2014; King et al., 2005; Temple et al., 2012). Frequency is also another variable that is often discussed by macroscopic studies. However, this does not take into account that a large number of short stress events can in fact represent the same amount of total growth disruption as a smaller number of long stress episodes due to the normal variation in spacing of perikymata along the tooth (Hillson & Bond, 1997). Therefore, while in some cases it may be possible to observe a difference in the number of stress episodes between groups, a simple tally of these cannot adequately differentiate the experience of these stress events.

Similar issues can arise when assessing periodicity or the amount of time between stress events. This is because the mean periodicity for an

individual with numerous short stress events can look quite similar to that of an individual with fewer events of extended periods. Consequently, the interpretation of periodicity differences can also be problematic. As such, it is best that results are interpreted alongside other corroborating evidence.

Overall, this study found that the individual components of LEH episode duration, that is stress and recovery durations, along with age at first episode were the most sensitive and useful parameters for discerning differences between the subgroups of interest. In cases of severe systemic stress at a population level, where an extremely high proportion of individuals are affected by LEH, it is not unusual to experience difficulties in identifying differences in how these stresses were experienced (Merrett et al., 2016). Given the extremely high prevalence of LEH at Non Ban Jak, all individuals were, for the most part, equally affected. As such, it was only by exhausting all possible variables of interest that made it possible to delve deep enough to discern subtle differences as well as confirm similar levels of exposure and experiences.

In terms of the available data from Southeast Asia, and more specifically Thailand, Non Ban Jak is a window into the later stages of a long occupation of the region by farmers, which began some 2500 years earlier. Therefore, it is critical that samples from the Neolithic, Bronze Age, and Iron Age, as preserved by human skeletal remains at nearby Ban Non Wat, are also examined at the same resolution as those from Non Ban Jak. Only then, can this begin to shed light into changes in the experience of systemic stress, as evidenced by LEH, prior to and leading into the late Iron Age Period as recorded by Non Ban Jak.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jasrep.2023.103866>.

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