Linear Enamel Hypoplasia in Permanent Dentition of Children in the Late Archaic and the Late Prehistoric River Valley

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ABSTRACT The intensification of agriculture is often correlated with an increase in physiological stress, but this relationship is not always clear and needs to be examined in biocultural context. This project compares the timing and duration of stress events of foragers (4000-3000 B.P.) with those of agriculturalists (A.D. 1000-1500) by analyzing linear enamel hypoplasia (LEH) on the permanent anterior teeth of 40 children from Late Archaic and Late Prehistoric Ohio River Valley. Scanning electron microscopy was used to create photomontages of the tooth surfaces. Prevalence, frequency, and duration of LEH were compared between samples using Fisher's exact tests and pairwise ANOVA. Results indicate that agriculturalist children endured the highest prevalence and frequency of stress events; although, forager children endured longer durations of stress events. Variation in stress experiences may be attributed to the nutritional transition to maize consumption and food storage during the Late Prehistoric period. However, a period of increased conflict, population aggregation, and political shifts from interaction with Mississippians are also discussed as contributing factors.

The intensification of agriculture often corresponds to an increase in skeletal indicators of physiological stress and growth disruption relative to hunter-gatherers in similar environments (Cohen and Armelagos, 1984; Cohen, 1989; Steckel and Rose, 2002; Larsen, 2006; Ungar et al., 2017). For example, at Dickson Mounds, Illinois, multiple indicators show increasing levels of nutritional stress and infectious disease with the rise of maize agriculture (Goodman and Armelagos, 1988; Kent 1986). However, this trend is not universal (e.g. Winterhalder and Kennett, 2006). Hutchinson and Larsen (1988) find that stress experiences among Native Americans of St. Catherines Island, Georgia increase in duration over time, despite similarities to previous agriculturalists in maize consumption. The authors argue this is likely due to disease and social changes introduced by the arrival of Spanish missionaries. Therefore, generalizations about the well-being of archaeological populations are not synonymous with their subsistence strategies. Life course hypotheses regarding response to early life stressors are dependent on accurate documentation of duration of stress episodes and the ages at which they occurred in order to better contextualize interpretations (Temple et al., 2013; Temple, 2014). Hutchinson and Larsen (1988) showed that information about stress episode duration could alter interpretations of the relationship between subsistence strategy and physiological stress.

Physiological stress during development results in disruption of enamel formation (Hillson, 1992), creating enamel defects that are permanently archived in the tooth crown. Bioarchaeologists consider these defects to be non-specific indicators of stress attributable to causes including psychological factors, fevers, malnutrition, infection, and trauma (Armelagos et al., 2009; Roberts and Manchester, 2005). While the crown of a tooth is forming, enamel matrix is secreted continuously by ameloblasts in successive layers starting at the crown of the tooth. Successive growth layers are differentiated by striae of Retzius (Hillson, 1996). Striae are temporally divided by cross-striations, which represent a circadian rhythm of growth, each accounting for 24 hours of growth (Fitzgerald, 1995; Hillson, 2005; Lacruz et al., 2012; Reid and Dean, 2006). Therefore, when cross striations are counted between striae of Retzius, the timing in days, or periodicity of the striae, can be determined. Externally, these striae correspond to circumferential structures that are visible on the surface of the tooth, known as perikymata (Antoine and Hillson, 2016; Hillson, 1996; Fitzgerald, 1995). Therefore, as enamel is laid down in successive layers, each perikyma represents a

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period of development in days, as reflected by the periodicity, or cross-striation count.

When a systemic stress event occurs, energy is diverted away from ameloblasts, causing a deficit in matrix secretion and the enamel volume, interrupting the normal distribution of perikymata (Hillson, 1992). The result is an increased distance between successive perikymata. These physiological disruptions during growth produce localized defects on the enamel surface, which can occur as pits, furrows, or planes (Hillson, 2005). Linear enamel hypoplasia (LEH) is the most recognizable and commonly reported form of enamel defect (Hillson and Bond, 1997; Ten Cate, 1994), and takes the form of a horizontal line or linear array of pits on the enamel surface (Goodman and Rose, 1990; Hillson, 1996). The width of LEH is the result of the number of affected perikymata; therefore, representing a quantification of the duration of stress events (Antoine and Hillson, 2016; Hubbard et al., 2009). The appearance of LEH is further influenced by its location on the tooth surface since perikymata are more widely spaced near the occlusal/apical surface and more densely packed at the cervix. This means that LEH near the crown cervix of an incisor, for example, will appear narrower compared to its matching defect on a canine, which will appear in the middle of the crown. Matched defects will often appear at different locations when using two different teeth due to differences in the timing of enamel development. Regardless of their locations on two or more teeth, defects that form in response to the same systemic stress are composed of the same number of striae of Retzius and perikymata (Antoine and Hillson, 2016; Hillson and Bond, 1996).

The occlusal wall of LEH represents the period during which the stress event occurred, while the cervical wall represents the period of recovery (Guatelli-Steinberg, 2008; Hillson and Bond, 1997). The association of LEH with perikymata thus correlates with the chronological development of the tooth enamel. In tooth cusps, striae of Retzius dome over each other and do not outcrop on the surface. As compared to posterior teeth, anterior teeth are the most useful to examine for LEH because more of their enamel surface is covered by perikymata (Goodman and Armelagos, 1985; Hillson and Bond, 1997; Guatelli-Steinberg et al., 2012).

This study reconstructs patterns of growth disruption of permanent teeth of subadults using incremental microstructures to examine LEH prevalence, frequency, duration, and age of occurrence among temporally distinct populations with different subsistence strategies (foraging and agricultural) within the prehistoric Ohio Valley. Linear enamel hypoplasia prevalence, frequency, and duration measure different aspects of the stress experience. Prevalence indicates the proportion of people in a sample who were affected by a physiological growth disturbance during childhood. Frequency reflects the average number of growth disruptions that any one person in the sample is likely to have experienced. Lastly, LEH duration is an indication of how long an individual was affected by a single growth disruption. In other words, LEH duration reflects the amount of time until that person began recovering from such an incident. Therefore, discordance of LEH prevalence, frequency, and stress episode duration is possible, though not always expected.

With the advent of agriculture in North America, maize became an important subsistence staple, although it varied regionally in the rate of adoption (e.g. Cassidy, 1984; Cook and Schurr, 2009; Goodman and Rose, 1984; Sciulli and Oberly, 2002). Isotopic analysis from numerous sites within the Ohio River Valley indicate rapid incorporation of maize in the diet in the Late Prehistoric period (1100 – 400 B.P.) with intensified agriculture (Greenlee, 2002). Although maize meets daily caloric requirements, it is a poor source of amino acids and protein (Spielmann and Angstadt-Leto, 1996; Whitney and Rolfes, 2011). The phytates and plant proteins in maize inhibit iron absorption, and niacin in maize binds to glucose molecules, decreasing their bioavailability (Baynes and Brothwell, 1990; MacKay et al., 2012). The nutritive value of maize is also altered during food processing further removing important minerals and fiber depending on the processing protocols (Rylander, 1994). If absorption or consumption of iron and other nutrients is low, anemia could result, leading to susceptibility of disease and infection (Dubos, 1965; Scrimshaw, 1964; 2003).

It is often assumed that maize gruel was introduced to infants around six months of age in Late Prehistoric populations, when growth needs begin to exceed the nutrients supplied in breast milk (Wright, 1997). Even though breast milk was likely still a significant component of an infant's diet during this time, maize gruel may not have been sufficient in providing supplementary nutrients. Therefore, throughout the weaning process, LEH may represent stresses initially caused by nutritional deficiency (Blakey et al., 1994).

Bone remodeling is also influenced by nutritional stresses and disease (Frost, 2003), and an increase in remodeling per unit area can indicate greater health risk (Cho and Stout, 2003). Nutritional problems are synergistically bound to the frequency of infection since ubiquitous pathogens can become increasingly virulent under the influence of decreased host resistance due to poor nutrition (Dubos, 1965; Scrimshaw, 1964; 2003). Greater remodeling rates in maize agriculturalists compared to foragers have been recorded from Lower Illinois River Valley sites and other locations (Stout, 1983; Stout and Lueck, 1995; Stout and Teitelbaum, 1976). These greater rates may reflect nutritional stress of a low protein maize diet.

In the Ohio River Valley, Perzigian et al. (1984) and

Cassidy (1984) studied stress indicators and dietary transitions, respectively, from Archaic (6000 – 3000 B.P.) to Late Prehistoric (1100 – 400 B.P.) periods. Dental caries and attrition show the greatest difference in populations through time in the Ohio Valley (Sciulli and Oberly, 2002). Maize agriculturalists exhibit higher frequencies of dental caries and increased frequencies of pathological conditions such as periapical lesions and antemortem tooth loss (Sciulli and Oberly, 2002). Although these conditions are a byproduct of age, the earlier appearance and elevated frequencies of dental caries among agriculturalists has been largely attributed to the increased consumption of dietary carbohydrates (Larsen, 2006; Selwitz et al., 2007).

Materials

The sample for this study consists of photomontages of cast replicas of the immature (i.e. incomplete enamel growth) permanent anterior dentition of 40 subadults from three archaeological assemblages: the Duff, Buffalo, and SunWatch sites (Figure 1). The histologically determined ages at death of individuals in the sample range from 2.23 – 10.06 years (Duff), 1.57 – 6.45 years (Buffalo), and 1.09 – 7.15 years (SunWatch) (calculated in Blatt, 2013). Poor preservation of subadult remains in the archaeological record in this area contributed to the limited sample size. Sites chosen for study also display a significant degree of genetic homogeneity (Sciulli, 1990; Sciulli and Oberly, 2002) thereby reducing the potential for variability in the data due to genetic factors. Therefore, we assume that any discrepancies in dental growth and development among the present samples are attributable to individual variation and/or environmental influences. In addition to the age distribution of well-preserved

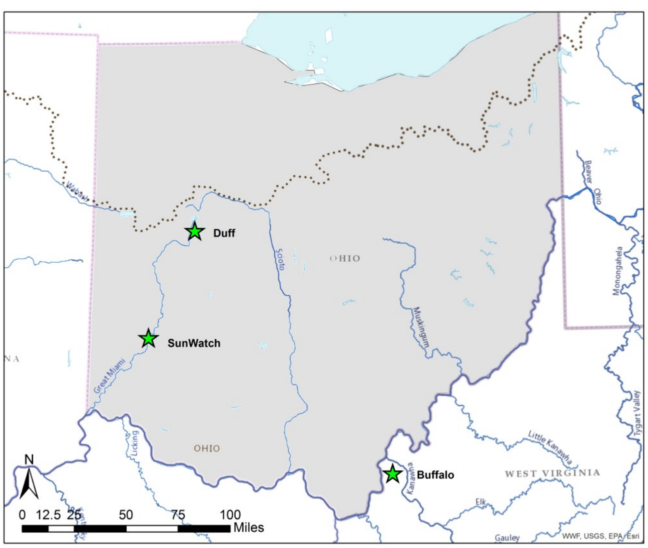

Figure 1. Map of Ohio showing the locations of the sites used in this study.

subadults, the three sites were selected for their regional consistency and the well-studied archaeological and biological contexts they provide.

Given the body of research on the increased disease load that is often associated with the rise of maize agriculture (e.g. Cohen and Armelagos, 1984; Larsen, 2006), we first predicted that the agricultural samples in this study would exhibit a statistically significant increase in physiological stress as measured by a higher prevalence and frequency of LEH compared to the foraging sample. Second, we predicted that these stress episodes would last longer among the agriculturalists due to the assumed persistence of pathogens among a large, sedentary population. Third, we hypothesized that agriculturalist children would exhibit the earliest age of first LEH occurrence because a weaning diet supplemented by maize porridge is more likely to lead to malnutrition and systemic stress earlier in development than a foraging diet that includes a broader spectrum of foods and essential nutrients (Ungar et al., 2017). Overall, the objectives of this study were to assess change across time in the stress experiences that impacted prehistoric children of the Ohio River Valley in association with subsistence and social transitions.

A total of 31 individuals, of 40 originally sampled, had adequately developed and preserved enamel microstructures for the purposes of this study. Individuals were included if they had at least 40% of the enamel present on their incisors (Blatt, 2013). Subadults under the age of two years were excluded from analyses for this reason (Reid and Dean, 2000). Deciduous teeth were excluded since they do not consistently display perikymata. We excluded teeth with taphonomic changes and/or excessive wear to limit error in perikyma counts that would skew chronological assessment.

Duff Site (n=9)

The Duff site (22LO111), located in the mid-western area of Ohio (see Figure 1), consists of a relatively large and complete cemetery (Sciulli and Aument, 1987). Radiocarbon dates of 2,950 ± 155 years B.P. and 3100 ± 40 years B.P. from skeletal material indicate that the site was used for a short period of time during the terminal Late Archaic (ca. 3000 – 2500 B.P.) (Sciulli and Aument, 1987; Sciulli, 1990). During this time, populations lived in small scattered groups at seasonal habitation sites subsisting through hunting and gathering (Parmalee, 1969). The dietary breadth of the Duff people centered on diverse faunal assemblages, nut harvesting, wild plants, and riverine resources (Emerson et al., 2009; Parmalee, 1969). Domesticates, such as maize, were neither produced nor consumed during this period, as supported by isotopic and archaeological evidence (Stothers and Bechtel, 1987). The migratory nature of hunting and gathering populations allowed for the occupation of seasonal habitation sites, reducing the risk of resource depletion and nutritional deficiency. Sciulli and Aument (1987) report high young adult mortality, and low infant mortality among individuals at the Duff site. Warming from the Hypsithermal Period is believed to have decreased the carrying capacity in the region during the latter part of the Late Archaic, but populations appear to remain stable (Ford, 1977).

Buffalo (n=18)

The Buffalo site (46PU31) is located along the banks of the Kanawha River in Buffalo, West Virginia (see Figure 1). It was hastily excavated between 1963-1965 (Hanson, 1975), during which some burials were avoided, crushed, and displaced by bulldozers (Drooker, 2000). The site consisted of permanent settlements inhabited year-round, spanning several temporal/cultural traditions from the Archaic to the Late Prehistoric (1000-1500 A.D.) (Hanson, 1975). For the purposes of this study, only the latter period, which encompasses Fort Ancient, will be discussed since sample materials from Buffalo date from this time.

Fort Ancient is considered a regional culture of kin-structured villages that responded similarly to varying degrees of Mississippian influence (Cook, 2008; Cowan, 1986; Griffin, 1943; Henderson and Turnbow, 1987; Turnbow and Sharp, 1988). The Fort Ancient occupation of the site is seen in two overlapping villages and burials clustered in groups near house structures (Hanson, 1975). From the original excavations, very little information is reported about subsistence or social stratification at Buffalo. Many charred hickory nuts and walnuts were recovered from the site, and it appears that deer were brought back to the site for butchering. There is no reported evidence for other plants used at the site; although, numerous remains of mammals and birds were recovered (Hanson, 1975). Nevertheless, it is commonly assumed that the subsistence economy at Buffalo was associated with horticulture or less intensive agriculture (e.g. Blatt, 2013; Hanson, 1975; Sciulli and Oberly, 2002). Isotope data (^{13}C/ ^{12}C) of Mid-Late Prehistoric remains throughout the region indicates that maize was by far the most consumed food by the Fort Ancient people. Late Prehistoric Ohio Valley populations also tended to have shorter stature, a lower life expectancy, and higher infant (1-3 years) mortality than earlier hunter-gatherers (Cassidy, 1972; 1984). Such changes in mortality patterns are also likely related to the overall increase in population size and sedentism from Archaic to late prehistoric populations in the region.

SunWatch (n=13)

Contemporary with Buffalo, SunWatch (33MY57) village is the most extensively excavated and analyzed Fort Ancient site and is the type site of Middle Fort Ancient (A.D. 1200-1400) (Heilman et al., 1988; Henderson and Pollack, 2001). SunWatch is located along the Great Miami River, three miles south of Dayton, Ohio (see Figure 1). The site is comprised of a well-planned circular village, concentric circles of clustered burials, storage pit structures, and houses that were organized outwardly around a red cedar center pole (Heilman et al., 1988). This organization is thought to be part of a solar alignment system, and culminating in a stockade around the periphery (Heilman and Hoefer, 1981). Relative to hunting and gathering groups, this period is marked by increases in sedentary population size, social complexity, regional integration, and extra-regional trade (Dunnell, 1971; Drooker, 1997; Essenpreis, 1978; Griffin, 1943). Subsistence focused on a maize-intense diet. However, supplementary hunting and gathering was still employed (Rossen, 1992). Carbon isotope analysis reveals no difference between males and females in maize consumption. However, nitrogen isotope analysis shows that females ate less meat than males (Conrad, 1988). Agricultural populations tended to have shorter stature, a lower life expectancy, and high infant mortality compared with hunter gatherers (Cassidy, 1984).

Large Fort Ancient sites, like SunWatch, consumed more maize than smaller villages, but the highest carbon isotope levels were most closely associated with sites that had the highest number of Mississippian goods and structures (Cook and Schurr, 2009; Schurr and Schoeninger, 1995). Research on the degree and consistency of Mississippian interaction with Fort Ancient villages is contentious, as some authors conclude that the Fort Ancient development was necessarily stimulated by the Mississippians (Griffin, 1943; Cook, 2012), while others deny any significant influences (Pollack and Henderson, 2000).

There is significant evidence for Mississippian reach at SunWatch (Cook, 2008; 2012). Skeletal trauma and mortuary iconography that have been associated with warfare and peace keeping among Mississippian leaders, such as the inclusion of dog burials and symbolism associated with Mississippian dog soldiers, appear in one quadrant of the site, which is otherwise segmented by kin groups (Cook, 2012). Development of war leadership and presence of foreign soldiers in the village fit well with the escalating conflicts in the greater region (Milner et al., 2001). In a study of intracemetery biological variation using dental metrics, Sciulli and Cook (2016) found that SunWatch consisted of a primary Late Woodland population with some nonlocal Mississippian individuals, who were highly related to the Late Woodland population. The authors suggested that acculturation accounted for Mississippian cultural characteristics in southwestern Ohio or that gene flow, accounting for the genetic similarities between Fort Ancient and Mississippian peoples, occurred before 800 B.P. (Sciulli and Cook, 2016).

During the occupation of SunWatch, Fort Ancient sites were becoming more aggregated, as Mississippian sites became smaller (Drooker, 1997; Cook and Aubry, 2014). It is likely that these Mississippians migrated into the hinterland of Fort Ancient villages, influencing political and sociocultural transitions at villages such as SunWatch (Cook, 2012; Cook and Aubry, 2014). An influx of foreign elite leaders (as evidenced from burial inclusions and comparisons) and reshuffling of populations from patrilocal residence patterns to matrilocal could have significantly increased adult and developmental stress, nutritional stress, and pathogen load from overcrowding, as compared to other sites used in this study (Cook, 2012; Cook and Aubry, 2014).

Methods

High resolution cast replicas were made of the labial surface of anterior teeth. Photomontages were created using a FEI NOVA Nano Scanning Electron Microscope (SEM) 400 at 50 times magnification (Blatt, 2013). Replicas, histological data from sectioned teeth (discussed below), photomontages, and age estimation used in this study originate from the work of Blatt (2013).

Perikymata were identified as regularly spaced grooves running horizontally across the surface. Linear enamel hypoplasia was identified as an accentuated area in which perikymata were spaced more widely relative to those around them (Guatelli-Steinberg, 2008). Accentuation was first identified subjectively relative to adjacent perikymata. ImageJ (Rasband, 1997-2017) was then used to metrically verify the increase in width from scaled images (Figure 2).

From the photomontages available, we were unable to evaluate enamel depressions in order to identify LEH (as in King et al., 2002; Temple, 2014; 2016). Nevertheless, there was limited bias in LEH identification and perikymata counting because most individuals in the sample died prior to cervical enamel formation, which is the area most affected by tightly "packed" perikymata. Because we did not often have perikymata available to count near the cervix, we did not apply Hassett's (2012) method. However, since metric comparison of width variations among adjacent perikyma-

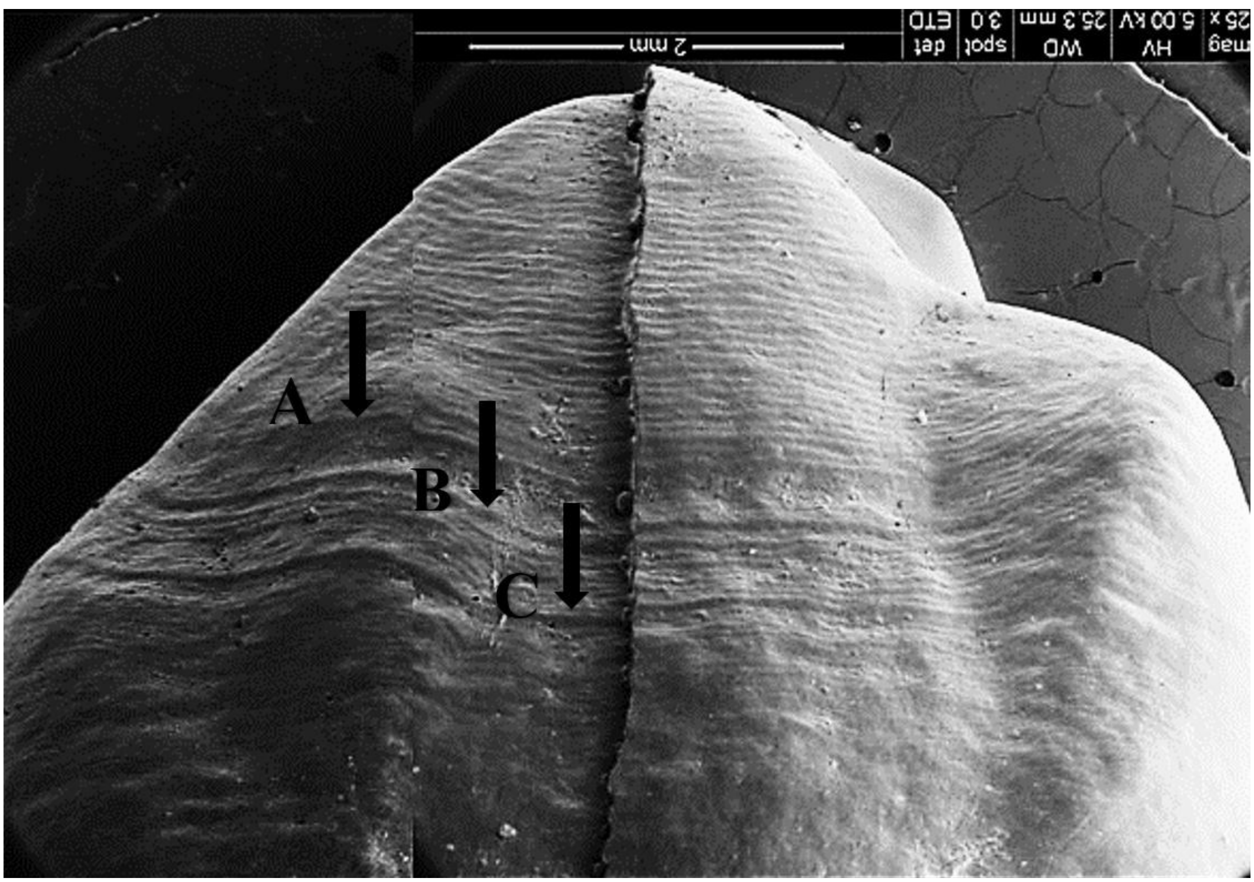

Figure 2. Photomontage example. Lower left canine of individual B6-71 from SunWatch with arrows indicating LEH (and accentuation in the horizontal perikymata). LEH A includes 6 perikymata, LEH B and C both have two perikymata each. Occlusal surface is towards the top. Modified from Blatt (2013).

ta was not performed along the entire enamel surface, LEH prevalence and frequency data represent minima for each sample.

The method of recording LEH used in this study has been applied to other sites in the study region (Martin and Sciulli, 2008; Steckel et al., 2002) and should produce comparable data. Perikymata were counted from the occlusal/apical end until the onset of each accentuation. This procedure eliminated any visual confusion thereby minimizing intra-observer error. Perikymata of each tooth were counted twice at least one week apart and the mean number of perikymata was used as the final count result (Blatt, 2013).

We matched defects between two anterior teeth from each individual in order to eliminate any defects associated with localized trauma. Defects were matched by comparing duration, interval between successive defects, and the histologically derived estimate from enamel microstructures of the age at onset for each hypoplasia on each tooth (Blatt, 2013). Frequency was calculated for each individual by counting the average number of matched LEH occurrences among the anterior teeth. Individuals with two anterior teeth with observable defects were categorized as LEH positive. To calculate prevalence, the number of LEH-positive individuals was divided by the total number of individuals with at least two observable anterior teeth and is presented as a percentage. The interval between successive defects was recorded as the number of perikymata from the beginning of one LEH to the start of the next. Subsequently, perikymata were counted within each accentuation to establish the duration of each LEH event (Hubbard et al., 2009). Duration was expressed as the number of all perikymata affected by a growth disruption.

Although Hillson and Bond (1997) argue that the duration of growth disruption should be evaluated as the number of perikymata in the occlusal wall of a defect, the total number of affected perikymata was used as an indirect indicator of stress duration since the transition between occlusal and cervical walls was often not clear. As such, stress episode duration in this study includes the periods of disruption and recovery (Guatelli-Steinberg et al., 2005). Stress duration was calculated as the total number of perikymata, as defined above, multiplied by the periodicity (Blatt, 2013). Periodicity (number of days represented by each perikyma from each population) was determined in two ways: either by direct counts of cross striations between striae of Retzius, or by measuring the length (in µm) between adjacent striae of Retzius divided by the local mean daily secretion rate (Blatt, 2013). A modal periodicity of eight days (for n = 7 teeth) was determined for the sample through histological analysis by Blatt (2013).

To calculate the ages at which stress events occurred, the total number of perikymata from the occlusal surface of each tooth to the beginning of each identified LEH was counted and multiplied by the periodicity. This total was then added to the initiation times (Reid and Dean, 2006; Blatt, 2013), representing the age at which tooth calcification begins. Subsequently, mean cuspal enamel formation time (determined by Blatt, 2013) was added, representing the time since initiation and first perikyma outcropping, for each specific tooth. The sum of these values and the developmental timing of all perikymata was then subsequently divided by 365.25 to yield age of occurrence in years. The age of occurrence of subsequent defects were calculated by adding the number of days between defects (duration plus interval) to the age of occurrence of the previous defect. Ages ranges at death (with correction factors) for all individuals in this study were previously provided through microstructural and histological analyses by Blatt (2013). It has long been agreed upon and thoroughly tested that microscopic methods provide more accurate age estimates than those using macroscopic methods (FitzGerald and Rose, 2008; Stringer et al., 1990); therefore, many of the estimations normally required for assessment of age and duration of LEH were eliminated.

Since it is unknown if the subsistence economy at Buffalo focused as intensely on maize agriculture as at SunWatch, the samples from these sites are considered separately rather than pooled. To control for possible bias in defect duration comparisons that could have been caused by amount of mineralized enamel available for observation, the samples were separated into two groups: those who died before 3.5 years, and those who died after 3.5 years. Blatt (2013) found that Ohio Valley children completed crown development for all incisors by 3.5 years (ranged 3.13 – 3.5 years). As such, groups were divided by enamel completion such that the younger subadults had at least 40% of enamel visible (Blatt, 2013). Defect prevalence for each site is not affected by this sectioning since all individuals under the age of two were excluded from analyses. Prevalence was compared between samples using a Fisher's exact test in the statistical computing environment R (R Core Team, 2013). Box plots were produced to depict ranges of stress episodes. Stress episode duration and age at first occurrence were compared between the samples using a one-way ANOVA with Tukey's HSD test for each pairwise comparison. Overall, these tested the similarities and differences in the stress experiences of hunter-gatherers relative to agriculturalists in the prehistoric Ohio Valley.

Results

Of the 14 individuals from Buffalo, 12 (85.7%) presented hypoplastic defects. Prevalence among SunWatch individuals was similar; eight of the nine (88.9%) subadults displayed at least one enamel hypoplasia while those from the Duff site exhibited a prevalence of four out of eight (50%). Frequency of LEH for the Buffalo and SunWatch sites was also similar, with 1.8 and 1.89 defects per tooth respectively. The smallest frequency was observed from Duff individuals, with an average of 1.125 defects per tooth. Notably, no more than three defects were matched between anterior teeth on any individual from any site. A summary of the prevalence and frequency results for all subadults are given in Table 1. Despite differences in prevalence, Fisher's exact tests showed that they did not differ significantly between the three sites nor between any two (Table 2).

Table 2. Results of the Fisher's Exact Tests. D = Duff; B = Buffalo; S = SunWatch.

Comparison	X^2	P
B, D, S	4.953	0.119
B, S	0.025	1.000
D, B	1.980	0.131
D, S	1.496	0.131

When individuals were pooled, the duration of stress episodes, given in days representing growth disruption and recovery, varied widely within each group (Table 3, Figure 3). The individuals from the Duff site experienced a longer average duration than those from Buffalo or SunWatch. Duff individuals endured stress episodes for an average of 45.75 days, whereas those from Buffalo and SunWatch endured an average of 34.9 and 25.22 days respectively. Tukey's HSD test suggested subadults from the SunWatch site experienced significantly shorter stress episodes than those from the Duff site (P = 0.021). There was no significant difference between Duff and Buffalo (P = 0.292) nor between SunWatch and Buffalo (P = 0.172). Conversely, when considering the average duration of LEH for each child, instead of average duration in each group, ANOVA showed no significant difference between samples (P = 0.122).

When comparing subadults between sites who died before the age of 3.5 years (see Table 1), Tukey's HSD test showed there was a significant difference in the duration of stress episodes only between Buffalo and SunWatch children (P = 0.03), due to the outlier seen in Figure 4, whose LEH lasted for 98 days. When considering those who died after the age of 3.5 years, duration of stress episodes among Duff children was significantly longer than both Buffalo (P = 0.003) and SunWatch durations (P = 0.018) (Figure 5). Duration of stress episodes between SunWatch and Buffalo children older than 3.5 were not significantly different (P = 0.822).

ANOVA indicates no significant difference in age at first occurrence between the three groups (P = 0.374). The ages at which subadults from all samples experienced their first stress episode began between 0.89 and 2.67 years. Table 3 shows the ages at which each stress episode occurred, and the duration of each event. Age ranges are given for each LEH using the standard deviation values for each tooth, from Blatt (2013). The consistency of multiple LEH occurrences within the second and third years of life among the SunWatch sample prompted an investigation of the interval between LEH. The average times between successive LEH: 115.6 days at Duff; 155.43 days at Buffalo; and 86 days at SunWatch.

Discussion

Although LEH prevalence among the Buffalo and SunWatch children was higher than in the Duff sample, this difference was not statistically significant, suggesting that a similar proportion of individuals were subjected to physiological stress, despite subsist-

Table 1. LEH prevalence and frequency for all individuals in each site

Site	Total Prevalence	Average LEH frequency	Pooled Ages		Ages < 3.5 Years		Age > 3.5 Years	
			Total Observed	Number with Defects	Total Observed	Number with Defects	Total Observed	Number with Defects
Duff	50.00	1.13	8	4	4	3	4	1
Buffalo	85.70	1.80	14	12	7	6	7	6
Sun Watch	88.90	1.89	9	8	7	6	2	2

Table 3. Age at occurrence for each LEH by site given in years, with the duration of each episode given in days. Ranges represent the possible age at which stress episode first began, where individual numbers represent the only viable age at which the episode could have begun, given the age overlap between the observed teeth. Age at death is also presented for each individual.

Site	Individual ID	Age at Death (years)	LEH A Age (years)	LEH A Duration (days)	LEH B Age (years)	LEH B Duration (days)	LEH C Age (years)	LEH C Duration (days)
Duff	B19	10.06	2.67	78	3.03 - 3.12	52	3.36 - 3.49	58
	B37	2.93	1.29	18	1.63 - 1.80	22	-	-
	B45	2.6	1.64	-	-	-	-	-
	B75A	2.93	1.68	32	1.81	58	2.07	48
Buffalo	D10-B24	2.71	2.06-2.11	24	2.31-2.37	72	-	-
	D10-B38	6.12	1.42-1.67	14	-	-	-	-
	D11-B27	2.36	1.27-1.31	28	1.56-1.58	22		
	D12-B9	3.15	2.5 - 2.62	28	3.32 - 3.63	24	-	-
	E7-B12	2.76	2.56	30	2.85	28	-	-
	E8-B18	5.06	1.01	20	1.27	32	-	-
	E8-B76	6.45	0.99 - 1.08	20	1.74	54	2.24 - 2.26	50
	E8-B82	5.06	1.98 - 2.16	14	2.36 - 2.58	24	-	-
	E9-B10	5.81	1.25 - 1.33	22	1.69 - 1.85	26	-	-
	E10-B43A	3.53	1.12 - 1.29	36	1.84	40	2.47	22
	E10-B110	2.91	1.31 - 1.47	40	-	-	-	-
	F10-B20	2.87	0.97	42	1.22	66	1.54	98
SunWatch	B3-74	2.99	1.84 - 2.00	8	-	-	-	-
	B6-71	4.16	1.92	42	2.05	18	2.19 - 2.23	20
	B6-73	2.87	1.38 - 1.47	16	1.45 - 1.57	28	1.54 - 1.65	40
	B6-80	2.67	0.89 - 1.06	8	1.12 - 1.28	24	1.51 - 1.68	42
	B10-80	2.32	1.43 - 1.58	14	-	-	-	-
	B11-76	2.65	1.98 - 2.04	14	2.04 - 2.14	28	2.29	28
	B13-72	7.15	1.56 - 1.84	36	2.39 - 2.56	44	-	-
	B14A-72	2.98	1.85 - 1.88	24	2.14 - 2.24	20	-	-

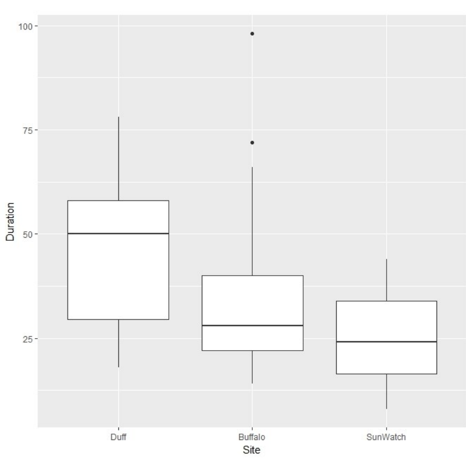

Figure 3. Stress episode duration given in days for all individuals, the whiskers indicate 95% confidence intervals.

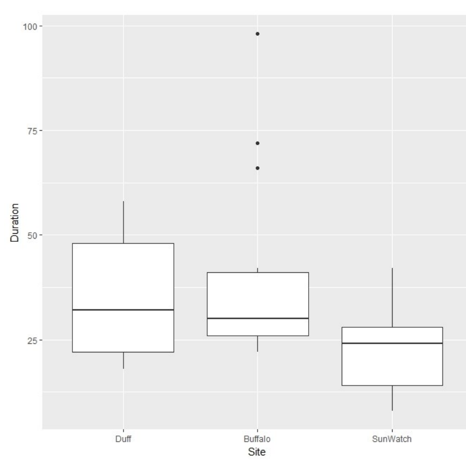

Figure 4. Stress episode duration given in days for individuals who died before the age of 3.5 years, the whiskers indicate 95% confidence intervals.

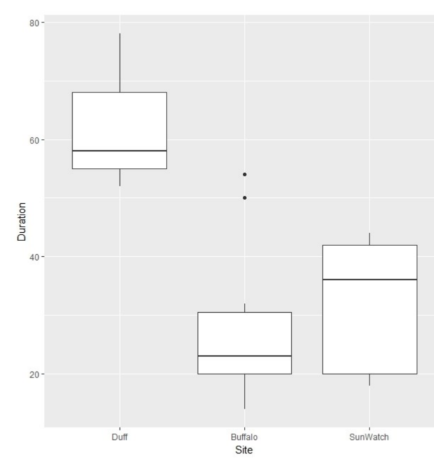

Figure 5. Stress episode duration given in days for individuals who died after the age of 3.5 years, the whiskers indicate 95% confidence intervals.

ence strategy differences and aggregation hazards in Late Prehistory. The average number of defects per tooth was lowest among the Duff sample, supporting the first hypothesis that the agricultural groups experienced a higher frequency of stress episodes. Average duration of LEH defects was significantly different between Duff and SunWatch, where Duff children experienced longer stress episodes than SunWatch children. Here, the duration analysis refutes the second hypothesis because foragers in this study endured longer stress episodes than did maize agriculturalists. These results suggest that Duff children experienced physiological stress less often than those at Buffalo or SunWatch. However, stress episodes lasted longer at Duff than at the other two sites.

Discord between LEH prevalence, frequency, and duration is seen in other studies that use LEH to examine stress differences in archaeological samples. For example, Temple et al. (2013) found that despite greater LEH frequencies among Jomon period foragers from Hokkaido, Japan, they had significantly shorter stress episode durations than Tigara Inupiat from Point Hope, Alaska. In another study, greater stress episode duration was evident in modern Inuit compared to Neandertals from Krapina, despite similar LEH prevalence within each sample (Guatelli-Steinberg et al., 2004). King et al. (2005) found significant differences in LEH prevalence between females from two historic London cemeteries, although there were no significant differences between duration or frequency of stress episode. These studies attribute the differences in stress experiences to seasonal variation in food availability and/or dietary quality between their samples.

The third hypothesis regarding a significant difference in age at first occurrence between agriculturalists and foragers was rejected. This result reflects the susceptibility of children in the first years of life to system stress, regardless of subsistence strategy. Many previous studies have linked the presence of LEH after the first year or so of life to the negative effects of weaning (e.g. Coppa et al., 1995; Corruccini et al., 1985; Ogilvie et al., 1989; Ubelaker, 1992; Webb, 1995). However, since other elements leading to enamel defects include nutritional problems, illness, or poor hygiene, weaning may not be the sole factor leading to growth arrest in enamel during the first year of life (see Blakey et al., 1994; Katzenberg et al., 1996).

Variation between prevalence, frequency, and duration may be attributed to differences in subsistence strategy, dietary breadth, and transitions in population size or Mississippian influences and migration. The dietary breadth of the Duff people (Emerson et al., 2009; Parmalee, 1969) suggests that children had access to foods with sufficient macro- and micronutrients, possibly helping to mitigate continuous nutritional inadequacies, resulting in reduced LEH frequency. However, due to a lack of food surplus and storage (Parmalee, 1969), if climate or seasonal variations occurred causing food depletion or relocation, Duff individuals may have experienced longer episodes of physiological stress. Indeed, climate warming impacted the region during the Late Archaic during the Hypsithermal Period (Ford, 1977), but detailed analysis of climatic changes is lacking in the literature. The increased frequency of stress episodes observed in both SunWatch and Buffalo children supports previous studies suggesting an increase in chronic systemic disturbances in Late Prehistoric agricultural populations (e.g. Cassidy, 1984; Cook, 1984; Danforth, 1999; Mummert et al., 2011; Larsen, 2006; Sciulli and Oberly, 2002).

It is common to also compare stature between groups as an indirect indicator of stress. Specifically, numerous archaeological remains have shown a reduction in stature and decline in overall health with the introduction of intensive agriculture (Cook, 1984; Danforth, 1999; Mummert et al., 2011; Larsen, 2006; Scuilli and Oberly, 2002; Temple, 2010). However, trends in stature reduction and the foraging-agriculture transition are not universal. Many studies have shown a decrease in juvenile stature with the rise of agriculture (e.g. Mummert et al., 2011), while others show no change, an increase, or an increase in regional variability in stature (e.g. Steckel and Rose, 2002; Temple, 2011). Sites from the Lower Illinois River Valley do not indicate adult stature changes through time or subsistence transitions, even though there is evidence of reduced juvenile stature (Cook, 1984). Children from all sites in this study have also been shown to have achieved a lower percentage of attained growth progression of femur length at each age relative to the mean attained growth achieved by the children from the Denver Growth Study and Columbus Morgue samples (Maresh, 1955; Sciulli, 1994; Sciulli and Blatt, 2008). Additionally, significant differences ($P = 0.001$) were reported between skeletal age and dental age between the children in these three sites (Blatt, 2013), indicating compromised physiological growth despite biological age.

SunWatch results also support indications of systemic early life or prenatal stresses reported from enamel defects of deciduous teeth (Martin and Sciulli, 2008). While decreased host resistance is likely to have contributed to the increase in LEH frequency in the Buffalo and SunWatch subadults, a diminished quality of diet is unlikely to be the only cause of an increase in frequency of stress episodes. Although an understanding of the extent of Mississippian influence at Fort Ancient sites, like SunWatch, is currently evolving, patterns of increased conflict and migration in the region are serious means of stress-inducing events to consider when comparing growth disruption through time.

Intensive agriculture at SunWatch allowed for population growth and sedentism, especially for the influx of Mississippian migration, which afforded ample opportunity for the introduction and perpetuation of novel pathogens (Goodman et al., 1984; Larsen, 1995). Increased maintenance of diseases and other pathogens relative to hunting and gathering groups may be a cause of the shortened intervals between successive stress episodes in the SunWatch children. In contrast to Duff individuals, the analysis of stress episode duration in this study suggests SunWatch children experienced shorter episodes of physiological stress.

There are some limitations and future explorations that should be considered within the framework of this project. First, the most evident issue is the small sample size. Juvenile remains from the archaeological record are often sparse and generally poorly preserved when compared to adult remains. It was difficult to obtain a sizeable sample, even from multiple sites. Compounding the problem, the sample was further reduced by omitting some individuals due to the presence of only one anterior tooth where at least two were needed to be considered for analysis.

Additionally, as mentioned above, enamel deposition is not linear in the permanent dentition (FitzGerald, 1995; Hillson and Bond, 1997; Reid and Dean 2000). The beginning of a hypoplastic defect is typically identified as the point where a perikyma is more widely spaced than its occlusal neighbors. Yet, the spacing between each successive perikyma can be variable through different parts of the same tooth since perikymata are packed more tightly in some areas as compared to others (Schwartz et al., 2001). In order to *accurately* calibrate the spacing between perikymata across differently packed areas, the mean distance and standard deviation would ideally be calculated based on the nearest neighbors (Hassett, 2012). However, a reliable and definitive study of dental enamel via microscopic analysis requires expensive analytical tools that are impractical in many field and laboratory situations compared. For many researchers, it is more reasonable, instead, to assess interobserver reliability of dentition that had limited variation in perikymata "packing", as in this study.

Finally, it is noteworthy that enamel hypoplasias can be observed only on those individuals who survived the stress events, and what cannot be known is if the children died during another episode, or due to some other cause. Markers of disease or deprivation in human remains represent only a fraction of the health disruptions in a population since most diseases will not leave lesions on bone, or will abate or kill their hosts before being expressed on skeletal tissue (Wood et al., 1992).

Conclusions

The results of this study indicate observable differences in LEH frequency and duration between the Duff, Buffalo, and SunWatch subadults. Our first hypothesis was confirmed; the agricultural samples reflected an increase in physiological stress through higher LEH frequencies and prevalence than those observed in the foraging sample (see Table 1). Contrary to the second hypothesis, the stress experiences of Duff children lasted longer than those of SunWatch or Buffalo, possibly due to differences in resource storage in cases of resource depletion or climate variations. In this case, SunWatch children relied on protein-deficient maize in a populated environment ripe for spreading pathogens, whereas Duff children had access to a more balanced diet in a sparsely populated environment.

An alternative explanation for the differences in the health experiences of hunter-gatherers and agriculturalists is that Late Archaic (Duff) populations did not possess the techniques, practices, or support for sick individuals. In the case of the Duff population, in which groups were initially becoming larger and less mobile, a sedentary lifestyle was probably in its early stages of development, which would have increased the propensity for pathogens to spread (Sciulli and Oberly, 2002). With that in mind, individuals inflicted with a potentially life-threatening illness may have been more likely to die in a short time, leaving skeletons, or their teeth, unmarked by acute effects of illness.

There is a trend for hunting and gathering populations in the Eastern Woodlands to exhibit fewer pathological conditions on their skeletons and teeth as compared to sedentary populations who assumedly had established techniques and support for ill people (Sciulli and Oberly, 2002; Steckel et al., 2002). On average, in the former populations, individuals with obvious effects of disease did not live as long. Conversely, in groups with well-developed care practices, like that at SunWatch, individuals would have had a better chance of surviving acute phases of disease. As such, these populations would have had longer lives (Sciulli and Oberly, 2002). Therefore, if chronic phases of diseases were to develop, they would manifest in their skeletal remains.

Expansion of this project should explore these trends by supplementing this analysis with the inclusion of adult skeletal materials to assess health within the life course approach (Agarwal, 2016). Ideally, the childhood stress experiences archived in LEH should be examined in individuals who survived childhood in order to assess potential links between childhood stress and health in later life. A greater understanding of the long-term impact of subsistence and social transitions in the Ohio River Valley would also benefit from comparison of adult attained stature with frequency of LEH observed in adults. The assumption being that increased LEH is correlated with having shorter adult stature and shorter lives as the result of childhood stress.

Temple (2014) assessed future risk of stress from early life stress. Such patterns have been demonstrated in recent studies (Amoroso et al., 2014; Temple, 2014; Watts, 2013) but samples from North America have not yet been applied to this model to aid in this complicated discourse. Nevertheless, the study of stress patterns in children is important for narrating the life course of young shortened lives.

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